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Taxonomy and phylogeny of *Cryphonectria* and allied genera

by

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DECLARATION

I, the undersigned, hereby declare that the thesis submitted herewith for the degree Ph.D. to the University of Pretoria, contain my own independent work and have hitherto not been submitted for any degree at any other University.

Marieka Gryzenhout

June 2006

"Let him who boasts, boast in the Lord" (1 Cor. 1:31)

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ACKNOWLEDGEMENTS

This Ph. D. thesis is, if I must say so myself, a rather unusual thesis. It is the product of serendipity, six years of work and co-operation between different people. It stretched a lot of boundaries. For me, the journey was worth much more than this thick book, this end-product, you are now holding in your hands. I have learnt much, and with this I do not only mean academically. Numerous times I have been looking forward to this moment where I could pin down in eternal words some of my impressions of the past few years. If the reader would indulge me for spilling a bit of my heart and not having a formal and functional list of thank you's, I would like to share my gratitude for the friendships formed over these years, and for the help I received from various people.

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This thesis is dedicated to my husband and friend, Jean.

SUMMARY

This thesis represents a critical taxonomical review of the fungal genus *Cryphonectria sensu lato*. An appropriate taxonomy for this group is of great importance because it includes many well known tree pathogens such as the chestnut blight fungus *Cryphonectria parasitica* and the *Eucalyptus* canker pathogen *Cryphonectria cubensis*. The many taxonomic changes introduced in studies presented in this thesis have largely arisen as a result of DNA sequence comparisons for *Cryphonectria* spp. that show that *Cryphonectria sensu lato* is comprised of different lineages, strongly supported by robust morphological characteristics. New taxa, of which many are pathogenic, have also been discovered. The expanded number of species of *Cryphonectria* and related genera as well as the consideration of large numbers of isolates has furthermore made it possible to establish a broad view of the group at the super-generic level.

The first part of the thesis deals with studies on *Cryphonectria cubensis*. A new genus *Chrysoporthe* is described for *C. cubensis sensu lato*. Two additional species are also described for phylogenetic sub-clades previously known as *C. cubensis*. These include *Chrysoporthe austroafricana*, representing all isolates from South Africa, and an anamorphic species described in the new genus *Chrysoporthella as Chrysop. hodgesiana*, which is currently only known from Colombia on native *Tibouchina* spp. Isolate collections from several new host genera for *Chr. cubensis* are also characterized. Collections from *Eucalyptus* in Cuba, now representing the epitype of *Chr. cubensis*, also define the type of *Chr. cubensis* as residing in the South American sub-clade. Another new species, *Chrysoporthe inopima* from *Tibouchina*

lepidota in Colombia is described as well as a new species *Chrysoporthe doradensis* for isolates from *Eucalyptus* spp. in Ecuador.

A new family *Cryphonectriaceae* is described in this thesis for *Cryphonectria*, *Chrysoporthe* and *Endothia*. Genera in this family are united by orange stromatic tissue, with the pigments colouring purple in 3% KOH and yellow in lactic acid. The existence of this new family confirms the close relationship of *Cryphonectria* and morphologically similar genera.

A proposal to conserve the name *Cryphonectria* against the new type *C. parasitica* is presented. This is required because *Cryphonectria gyrosa*, the currently accepted type, was erroneously used as type. The conservation of *Cryphonectria* against *C. parasitica* made it possible to describe the new genus *Amphilogia* for *C. gyrosa*. *Amphilogia* also includes a second species from New Zealand described as *Amphilogia major*, although no isolates currently exist for this species.

New genera for existing *Cryphonectria* spp., as well as newly discovered fungi are presented in this thesis. The new genus *Rostraureum* is established for a fungus pathogenic on *Terminalia ivorensis* in Ecuador. This fungus also represents a new species, *Rostraureum tropicale*. *Cryphonectria longirostris*, originating from Puerto Rico, Trinidad and Tabago, is also transferred to *Rostraureum*. A fungus morphologically similar to *Chrysoporthe* on native *Tibouchina*, *Miconia* and exotic *Eucalyptus* spp. in Colombia, is described as *Aurapex penicillata* gen. sp. nov. *Cryphonectria havanensis* is transferred to the new genus *Microthia*. *Cryphonectria coccolobae* also resides in this genus based on morphology, although its phylogenetic relationship to *C. havanensis* could not be confirmed due the absence of isolates. A new fungus was discovered during surveys for *C. coccolobae* on *Coccoloba uvifera* in Florida, which is described in the new genus *Ursicollum* as *U. fallax*. Phylogenetic

analyses in this study also clearly distinguish *Cryphonectria eucalypti* from *Cryphonectria*, and this fungus is thus transferred to the new genus *Holocryphia*.

A minireview is presented at the end of the thesis and discusses the new taxonomic concepts developed for *Cryphonectria* during this thesis, and recent studies by other authors. The review describes how this new taxonomic scheme has changed our view and understanding of the distribution and ecology of *Cryphonectria sensu stricto* from what it has traditionally been seen.

The final part of the thesis is written in the form of a monograph. It contains background information of all the species, including many pathogens, currently known in *Cryphonectria* and allied genera. The majority of these have recently been described, some in this thesis, and this chapter thus contains all recent information pertaining to them. It is intended that this monograph should be useful as a manual, enabling users to work with and isolate these fungi and to identify the different taxa based on morphology and phylogenetic relationships.

The studies presented in this thesis greatly change the taxonomy of *Cryphonectria sensu lato*, which is now seen as representing a large number of genera and species in a new family. Many would argue that *Cryphonectria* is still monophyletic, but the different lineages shown by DNA sequence comparisons are morphologically inordinately diverse, and clearly represent different genera. Studies presented in this thesis further suggest that additional genera await description from diverse geographical areas and ecological niches. The studies presented in this thesis will hopefully provide a foundation against which these new taxa can be compared and will improve our understanding of tree diseases.

PREFACE

Cryphonectria, in the broad sense, includes some of the most important pathogens of trees in the world. *Cryphonectria parasitica*, also known as the chestnut blight pathogen, caused an epidemic in North America that resulted in the death of vast areas of American chestnut (*Castanea dentata*), and it still negatively effects the this tree today. *Cryphonectria cubensis* is one of the most important pathogens of commercially planted *Eucalyptus* trees in tropical and sub-tropical areas of the world and its impact has shaped the composition of the *Eucalyptus* forestry industries, world wide.

The taxonomy of *Cryphonectria* has been seriously considered in the past. These studies were based on morphology and preceded the common application of DNA sequence comparisons. The taxonomy of this group of fungi was also confused because most works treated *Cryphonectria* as a synonym of the morphologically similar *Endothia*. Recent phylogenetic studies have clearly shown that the taxonomy of *Cryphonectria sensu lato* seriously needs to be reassessed.

This Ph. D. thesis is comprised of a suite of studies that reflects a radical revision of the taxonomy of *Cryphonectria* and allied genera. The thesis is presented in two sections. The first Section is comprised of several taxonomic studies presented in the first ten Chapters. These aim to determine the appropriate taxonomic positions of several *Cryphonectria* spp. and of new collections that generally represent newly discovered pathogens causing tree diseases. The second Section of this thesis represents a monograph treated as Chapter 11. In this monograph, all of the newly recognised genera and species are treated in a single document and all relevant literature pertaining to the taxonomy and

ecology of *Cryphonectria* and allied genera are presented. It also provided the opportunity to re-analyse DNA sequence data for all of the fungi in a single treatment and to compare results from past studies.

Chapters 1 to 3 involve taxonomic and ecological studies on *Cryphonectria cubensis*. Chapter 1 presents the description of a new genus *Chrysoporthe* for this fungus. The different phylogenetic sub-clades previously identified within *C. cubensis* based on DNA sequence data, are also studied further to determine whether they represent discrete species or not. A new sub-clade representing isolates from *Eucalyptus* spp. in Ecuador, is characterized in Chapter 2. Chapter 3 includes reports of several new host genera for *Chr. cubensis* and the description of a new species from Colombia, and it encompasses the epitypication of *Chr. cubensis* based on a collection from *Eucalyptus* in Cuba, the type location of *Chr. cubensis.*

Chapter 4 of this thesis presents studies on the family status of *Cryphonectria* and allied genera in the *Diaporthales*. The possible existence of a new family for *Cryphonectria* and *Endothia* had previously been recognized by earlier authors based on DNA sequences of the large subunit of the ribosomal operon. This warranted the description of a new family for *Cryphonectria*, *Chrysoporthe* and *Endothia*.

The studies presented in Chapter 5 reveal that *C. gyrosa* does not represent the true type of *Cryphonectria*. A proposal is consequently made that the name *Cryphonectria* is conserved against a new type, *C. parasitica.* *C. gyrosa* had been shown in previous studies to group in a distinct and undescribed genus including isolates from *Elaeocarpus* spp. in New Zealand. A new genus, *Amphilogia*, is described for this group in Chapter 6. The species, *Amphilogia major*, is also described in this chapter.

Chapters 7 to 10 of the thesis encompass several descriptions of new genera related to *Cryphonectria* that either represent existing *Cryphonectria* spp. or new species. In Chapter 7, a new fungus found on plantation-grown *Terminalia ivorensis* in Ecuador, is characterized. Its relatedness to *Cryphonectria longirostris*, a fungus that resembles it and known from Puerto Rico and Trinidad & Tabago, is also considered. During surveys for *Chr. cubensis* on native *Melastomataceae* in Colombia, a fungus morphologically similar to *Chrysoporthe* was found on native *Tibouchina*, *Miconia* and exotic *Eucalyptus* spp. This fungus is characterized in Chapter 8. Chapter 9 represents taxonomic studies on *Cryphonectria havanensis*, *Cryphonectria coccolobae* and *Cryphonectria eucalypti*. Surveys for *C. coccolobae* on *Coccoloba uvifera* in Florida yielded another fungus, which is also characterized in this chapter.

Studies presented in this thesis and by previous authors, have revealed that a new taxonomic scheme is needed for *Cryphonectria* and allied genera. The various taxonomic changes that are made in studies presented in this thesis impact on the understanding of the relatedness, ecology and pathology pertaining to this important group of fungi. Chapter 10 summarizes the recent changes made to the taxonomy of species broadly recognized as *Cryphonectria* and it treats the ecology, importance and distribution of these fungi.

Chapter 11 of this thesis is presented as a monograph of the newly recognised *Cryphonectriaceae*. Here I review all species that have been described and taxonomic schemes applied in this thesis. Studies by others relevant to the taxonomy of *Cryphonectria* and related fungi are also treated. The monograph provides information on the ecology and diseases caused by the species in the *Cryphonectriaceae*, morphological descriptions and keys, and phylogenetic trees including all known taxa.

The various chapters in this thesis were written as independent papers during the course of approximately six years. All of the papers, with the exception of the monograph, have been published or accepted for publication in recognised mycological journals. These publications represent part of an accumulating resource of taxonomic literature on the *Cryphonectriaceae* that has ultimately required a summary that is presented in a draft monograph in which all genera and species could be treated collectively. It is my hope that these studies will provide a strong foundation for subsequent studies on the taxonomy, ecology and distribution of this important group of fungi.

LIST OF ABBREVIATIONS

AB, AF, AY, DQ = sequence accession numbers for Genebank.

ATCC = American Type Culture Collection, Manassas, VA 20108, USA.

B = Herbarium, Botanischer Garten und Botanisches Museum Berlin-Dahlem, Zentraleinrichtung der Freien Universität Berlin, Berlin, Germany.

BPI = U. S. National Fungus Collections, Systematic Botany and Mycology, Beltsville, USA.

CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

CMW = Culture collection of Michael J. Wingfield, Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

CRY = *Cryphonectria* culture collection of Michael J. Wingfield, Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

CUP = Plant Pathology Herbarium, Cornell University, Ithaca, New York USA.

DAR = Plant Pathology Herbarium, Orange Agricultural Institute, Forest Road, Orange, N.S.W., Australia.

E = from the culture collection of Prof. R. J. Stipes (Department of Plant Pathology, Virginia Polytechnic Institute & State University, Blacksburg, Virginia, USA) now housed in the culture collection (CMW) of FABI.

FLAS = Mycological Herbarium, Department of Plant Pathology, University of Florida, Gainesville, U.S.A.

IMI = Herbarium, CABI Bioscience, Egham, Surrey, U.K.

ITS = Internal transcribed spacer region of the ribosomal operon.

K = Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey, England, U.K.

KB1, CD28, YM2 = isolates used in Liu *et al*. (2003).

KOH = potassium hydroxide.

LSU = Large subunit (28S) of the ribosomal operon.

MAFF = Microorganisms Section, MAFF GENEBANK, National Institute of Agrobiological Sciences (NIAS), MAFF Gene Bank, Ibaraki, Japan.

MEA = malt extract agar

MYA = malt yeast extract agar

NY = William and Lynda Steere Herbarium, New York Botanical Garden, Bronx, New York, USA.

OA = oats agar

PCR = polymerase chain reaction

PDA = potato dextrose agar

PDD = Landcare Research New Zealand Limited, Mt. Albert, Auckland, New Zealand.

PREM = National Collection of Fungi, Pretoria, South Africa.

RFLP = restriction fragment length polymorphism

s. l. = *sensu lato*

s. str. = *sensu stricto*

TFM:FPH = Forestry and Forest Products Research Institute, Danchi-Nai, Ibaraki, Japan, E or Ep refers to an isolate

TrN = Tamura Nei distance model

WA = water agar

PAD = Erbario Patavinum, Centro Interdipartimentale Musei Scientifici, Università degli Studi di Padova**,** Padova, Italy

G = Herbarium, Conservatoire et Jardin botaniques de la Ville de Genève**, Chambésy**/Genève, Switzerland

FH = Farlow Reference Library and Herbarium of Cryptogamic Botany, **Harvard University,** Cambridge, Massachusetts U.S.A.

CHAPTER 1

Chrysoporthe, a new genus to accommodate *Cryphonectria cubensis*

Gryzenhout M, Myburg H, van der Merwe NA, Wingfield BD, Wingfield MJ (2004). *Chrysoporthe*, a new genus to accommodate *Cryphonectria cubensis*. *Studies in Mycology* **50:** 119–142.

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

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

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INTRODUCTION

Cryphonectria cubensis (Bruner) Hodges is a serious and often deadly canker pathogen of commercially grown *Eucalyptus* spp. (Fig. 1A) in plantations (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 (Figs 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*) die-back (Figs 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*) in Brazil (Hodges 1988). The pathogen was recently discovered causing cankers on native *Tibouchina lepidota* (Fig. 1H) and *T. urvilleana* (*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).

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* 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 20 % 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). 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 & Heulsenbeck (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 S1211 and M2095.

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), fifty 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 in 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 measurements 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. 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, 2002b, 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). 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 superficial, fuscous-black, pyriform to globose with attenuated necks (Figs 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 (Figs 4A, 4D), 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 (Figs 4A–C, 4I), 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 24.9–34.03 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.

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 Gryzenh. & M. J. Wingf., **gen. nov.** MycoBank MB500032

Anamorph: *Chrysoporthella* Gryzenh. & 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) Gryzenh. & M. J. Wingf., comb. nov.

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 Gryzenh. & 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) Gryzenh. & 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) Gryzenh. & 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.

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 (Figs 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 (Figs 4D, 4I). *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 (Figs 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 (Figs 4G–L). Conidiomata eustromatic, superficial to slightly immersed, pyriform to clavate, sometimes pulvinate, with one to four attenuated necks per structure (Figs 4G, 4I–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, 9–11). *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*, 1974, C. S. Hodges, IMI 184653; Minas Gerais, Dionisio, *Eucalyptus* sp., 1973, C. S. Hodges, IMI 172718; Minas Gerais, Coronel Fabriciano, *Eucalyptus propinqua*, 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*), 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*, 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*, 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*, 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*, 1980, F. B. Armitage, IMI 249406.

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 Gryzenh. & 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.

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

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, 6B). *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, 6D–E). Conidiomata eustromatic, superficial to slightly immersed, pyriform to clavate, sometimes pulvinate, with one to four attenuated necks per structure (Figs 5G, 6D,

6E), 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). *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.

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.

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 Gryzenh. & 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 basin 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.

Typus: PREM 58022, *Tibouchina semidecandra*, Colombia, Darien, 2001, R. Arbelaez (cultura viva CMW 10641 = CBS 115854).

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.

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*, *Tibouchina urvilleana* and *Tibouchina 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***

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 Histone *H3* genes (Myburg *et al.* 1999, 2002a, 2002b, 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 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* and *M. rubiginosa*, 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 isolation 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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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*	Democratic 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	–	*Chrysoporthe 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, M.J. Wingfield	AF 265653, AF 292034, AF 292037
CMW 9928	–	*Chrysoporthella hodgesiana*	*T. urvilleana*	Colombia	C.A. Rodas, M.J. Wingfield	AF 265654, AF 292035, AF 292038
CMW 9929	–	*Chrysoporthella hodgesiana*	*T. urvilleana*	Colombia	C.A. Rodas, M.J. Wingfield	AF 265656, AF 292036, AF 292039
CMW 10641[b, d]	CBS 115854	*Chrysoporthella hodgesiana*	*Tibouchina semidecandra*	Colombia	R. Arbaleaz	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	*Cryphonectria*	*Quercus* sp.	Japan	T. Kobayashi	AF 452118, AF 525706, AF 525713

		nitschkei				
CMW 10463	CBS 112920	*Cryphonectria 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]	Linked sequence[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,	W. Sinclair	1981

			U.S.A.		
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, F.M. Roberts	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	–	*Eugenia aromatica*	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
Chrysoporthe 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 into *E. grandis* clone	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, R. Heath & L. Lombard	2000
	PREM 57361	–	*T. granulosa*	Durban, South Africa	J. Roux, R. Heath & L. Lombard	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
Cryphonectria parasitica	CUP 2926	CMW 10790	*Castanea dentata*	New York, USA	W.A. Murrill	1907
Cryphonectria nitschkei	TFM: FPH 1045 (holotype)	CMW 10518	*Quercus grosseserrata*	Japan	T. Kobayashi	1954
Cryphonectria 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 blocks.

Group	Isolate number	β-tubulin (Bt1a/1b)										ITS1/5.8S/ITS1				
		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*

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**

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		

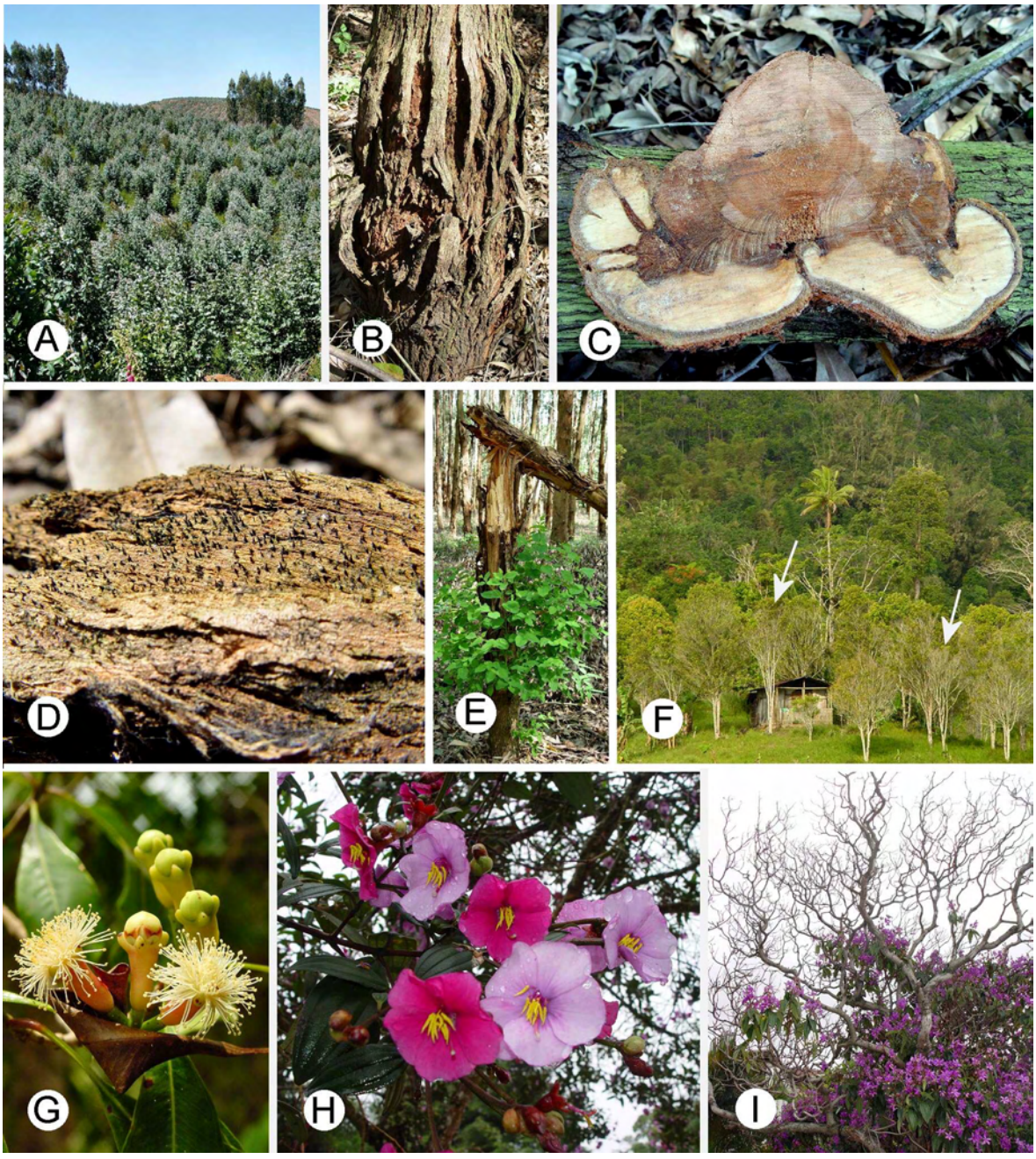


Fig. 1. Hosts and disease symptoms associated with species of *Chrysoporthe*. A. Plantation of *Eucalyptus globulus*. B. Canker caused by *Chrysoporthe cubensis* on the trunk of *Eucalyptus grandis* in Colombia. C. Section through trunk canker caused by *Chr. cubensis* on *E. grandis*. D. Conidiomata 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.

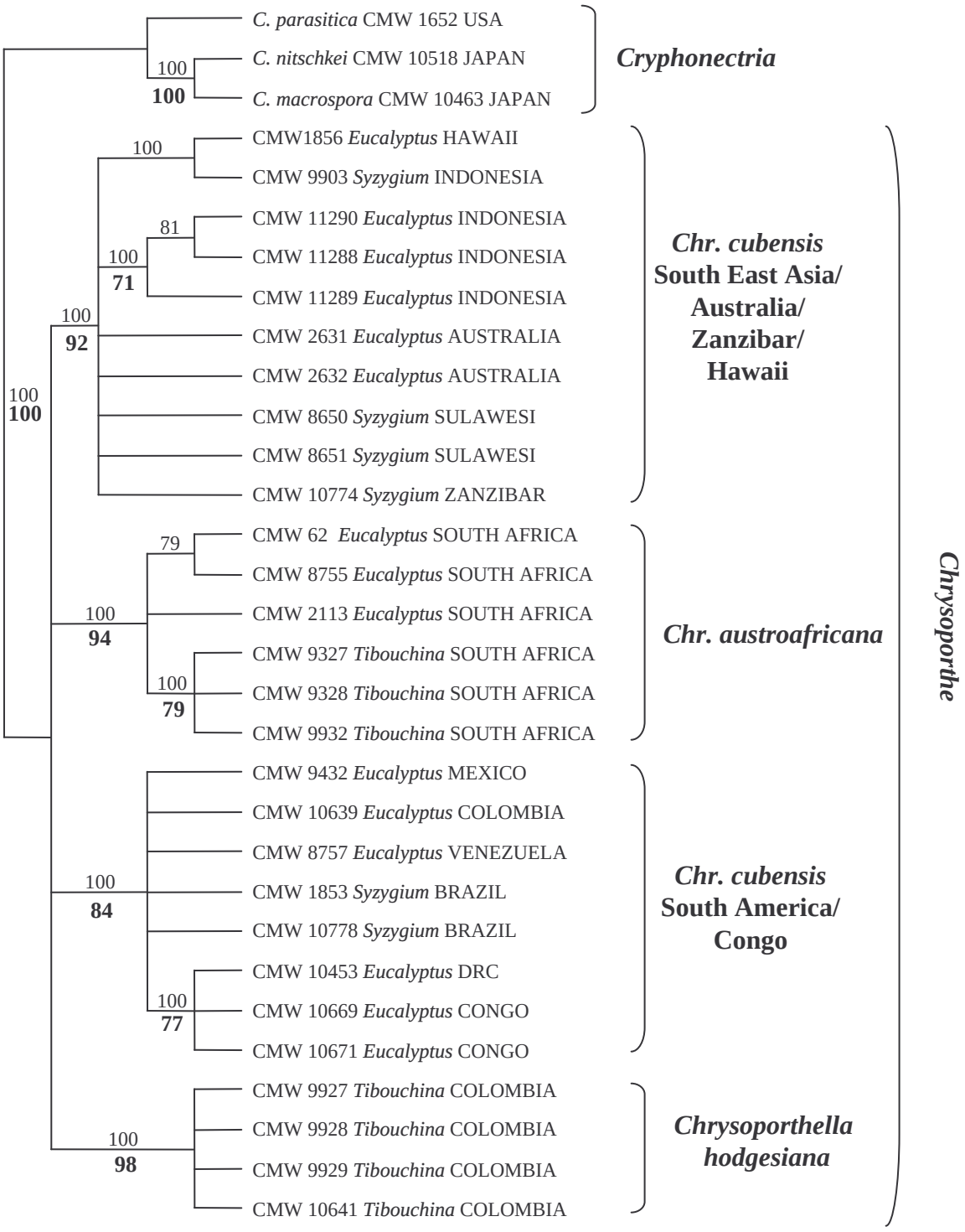


Fig. 2. Majority rule (70 %) consensus tree (tree length = 459 steps, consistency index/CI = 0.939, retention index/RI = 0.951, g1 = –3.6) obtained from 100 trees produced with the TBR algorithm of a heuristic search on a combined data set of ribosomal DNA and β-tubulin gene sequences. Group frequencies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

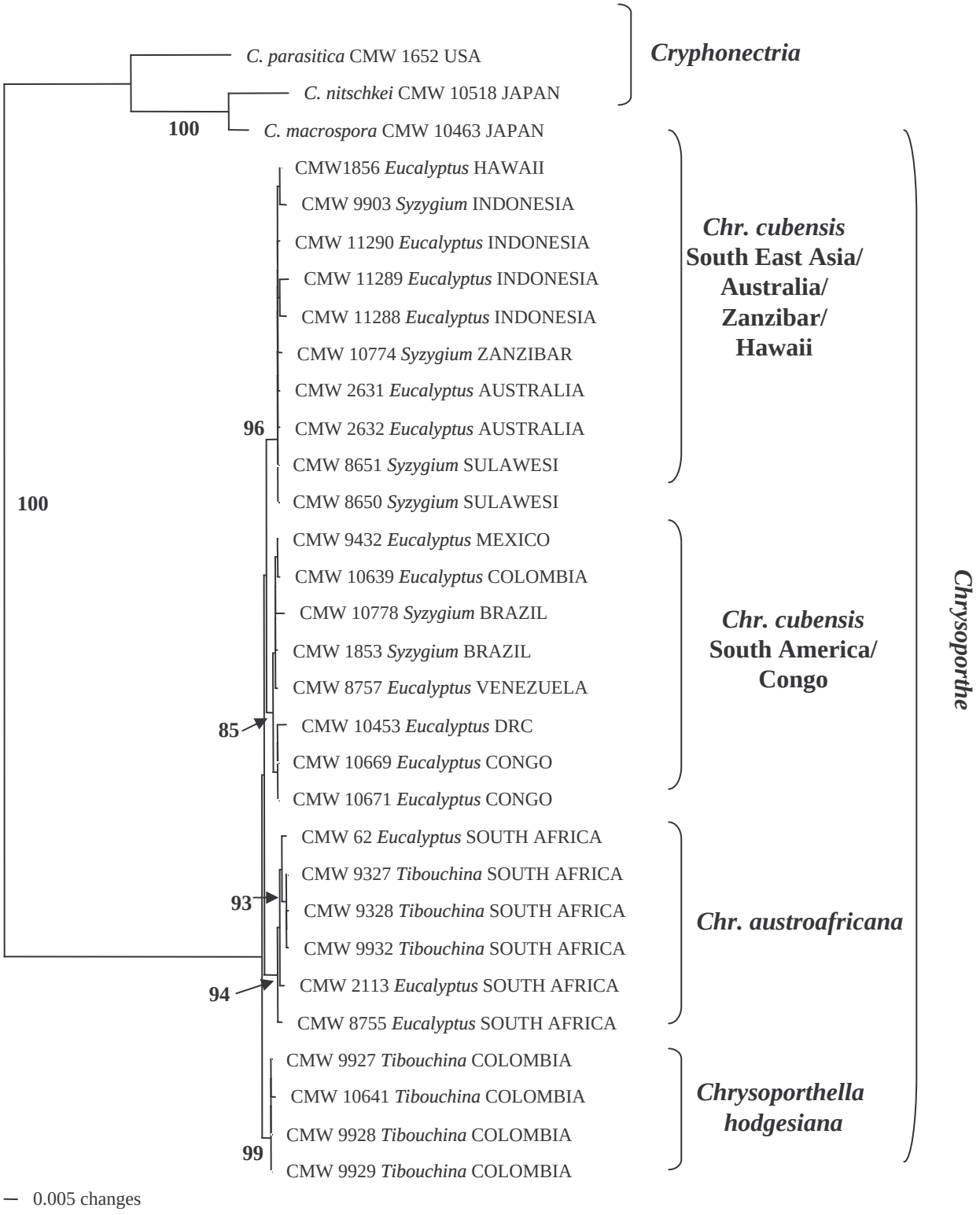


Fig. 3. Distance phylogram obtained with the HKY85 parameter model (G = 0.1717) on the combined data set of ribosomal DNA and β-tubulin gene sequences. Bootstrap values (1000 replicates) of branches are indicated in bold. *Cryphonectria parasitica*, *Cryphonectria nitschkei* and *Cryphonectria macrospora*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

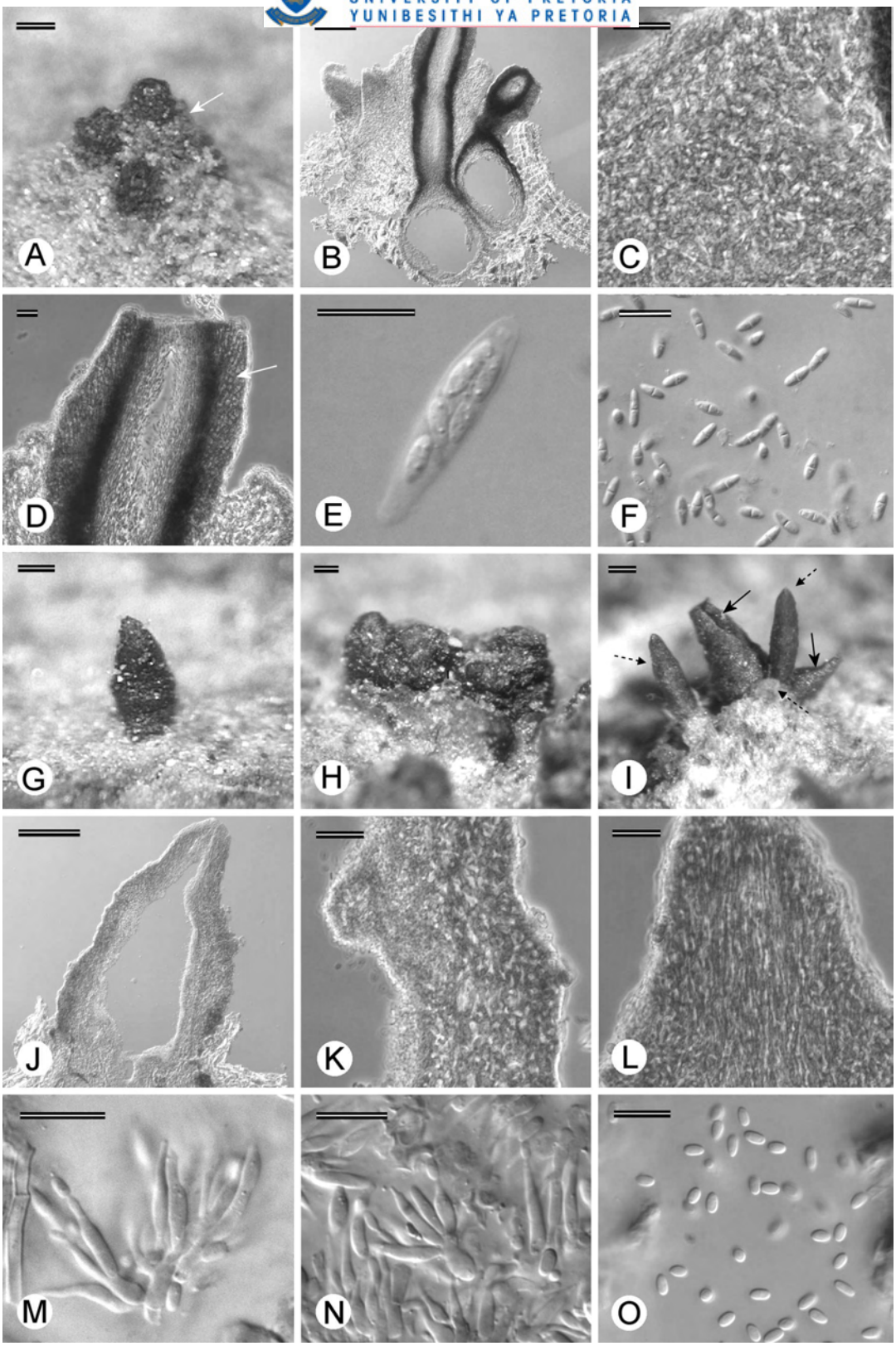


Fig. 4. Micrographs of *Chrysoporthe cubensis*. A. Black perithecial necks and orange stromatic tissue (arrow) of ascostroma on bark. B. Vertical section through ascoma. C. Stromatic tissue of ascostroma. D. Perithecial neck and surrounding tissue (arrow). E. Ascus from specimen PREM 57297 collected 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.

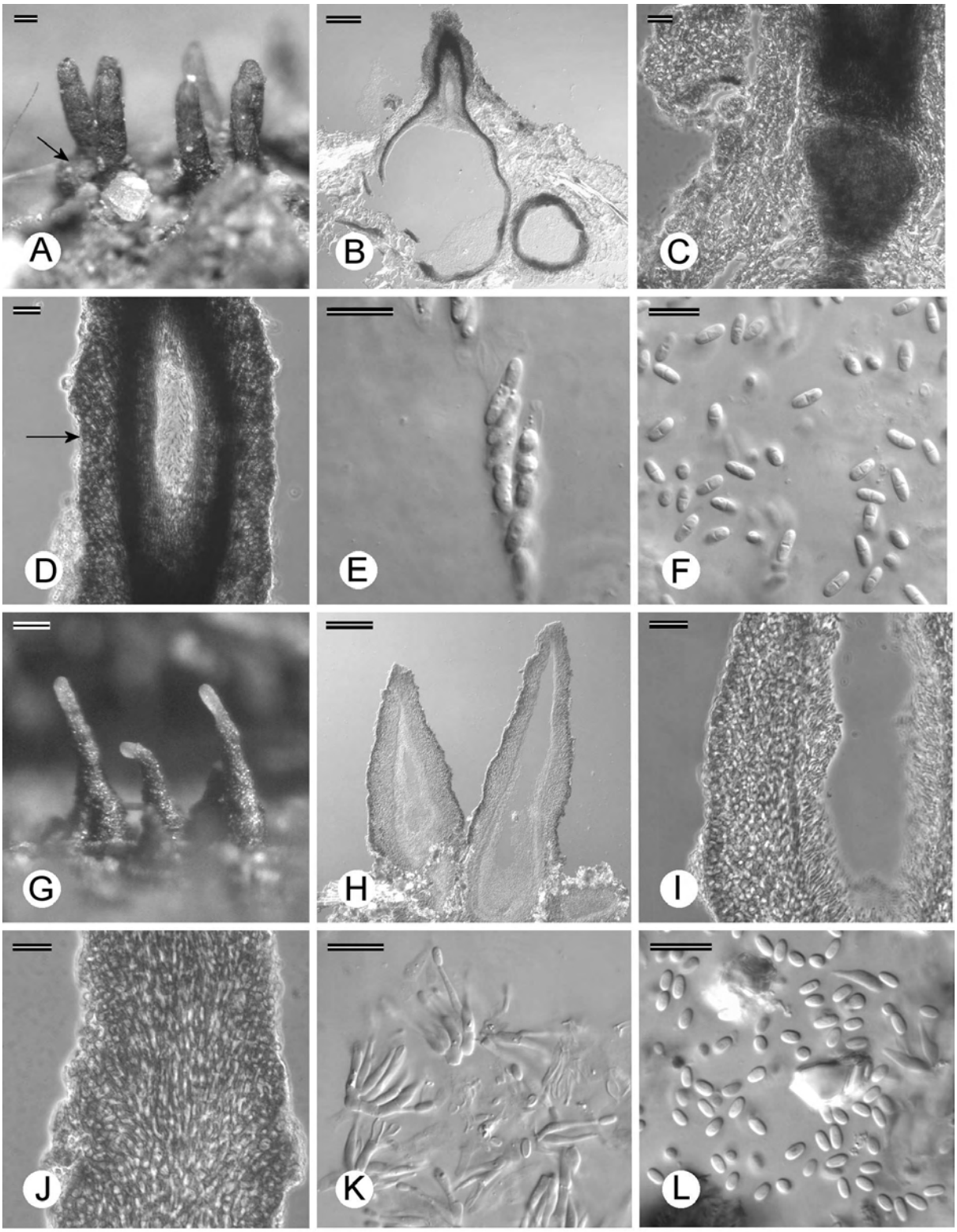


Fig. 5. Micrographs of fruiting structures of *Chrysoporthe austroafricana*. A. Ascostromata on bark showing black perithecial necks and orange stromatic tissue (arrow). B. Vertical section through ascoma. C. Stromatic tissue of ascostroma. D. Perithecial neck and surrounding tissue (arrow). E. Ascus. 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

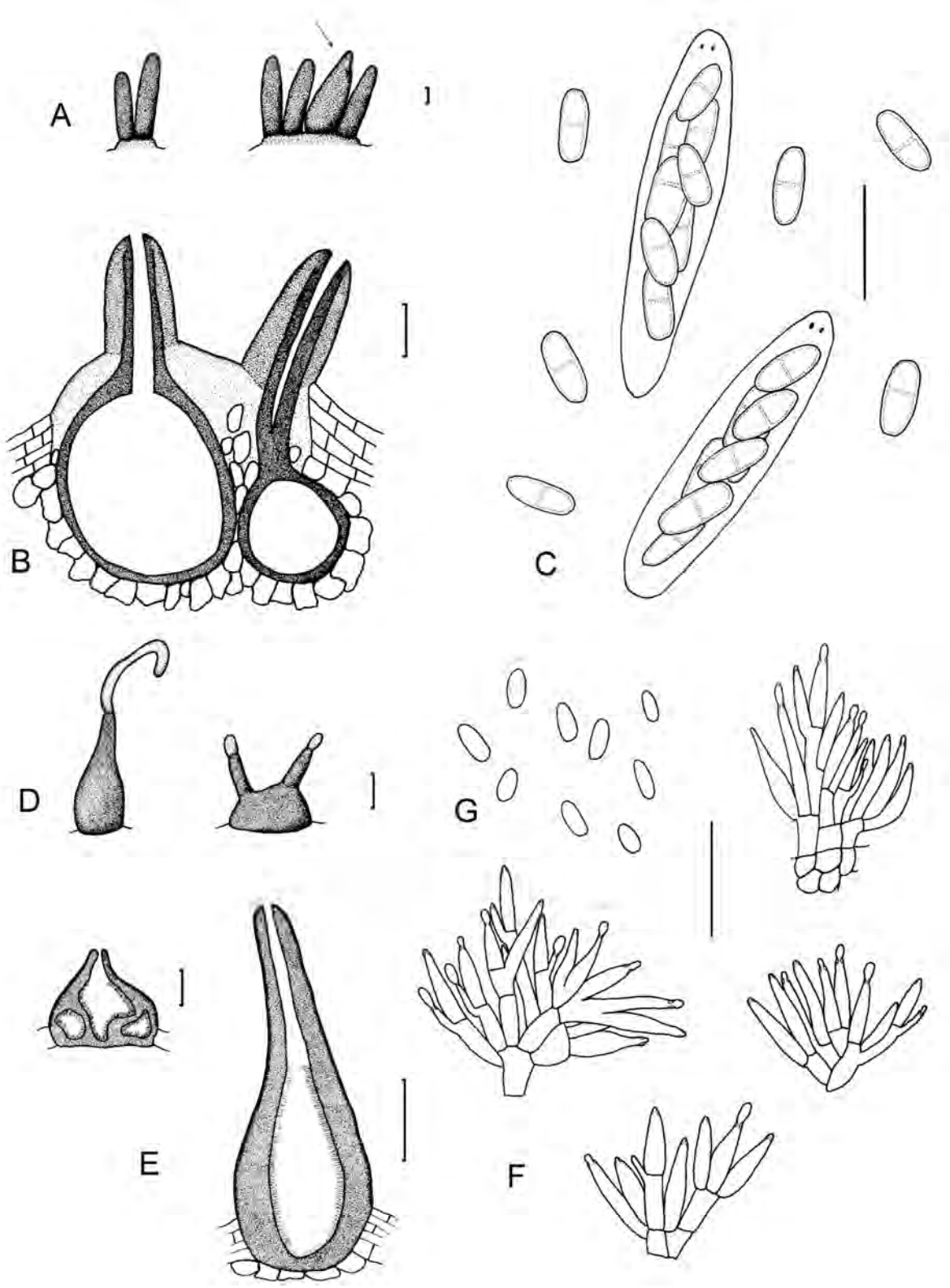


Fig. 6. Line drawings of *Chrysoporthe austroafricana*. A. Shapes of ascomata and conidiomata (indicated with arrow) on bark. B. Section through ascoma. C. Asci and ascospores. D. Shapes of conidiomata. E. Section through conidiomata. F. Conidiophores and conidiogenous cells. G. Conidia. Scale bars A–B,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

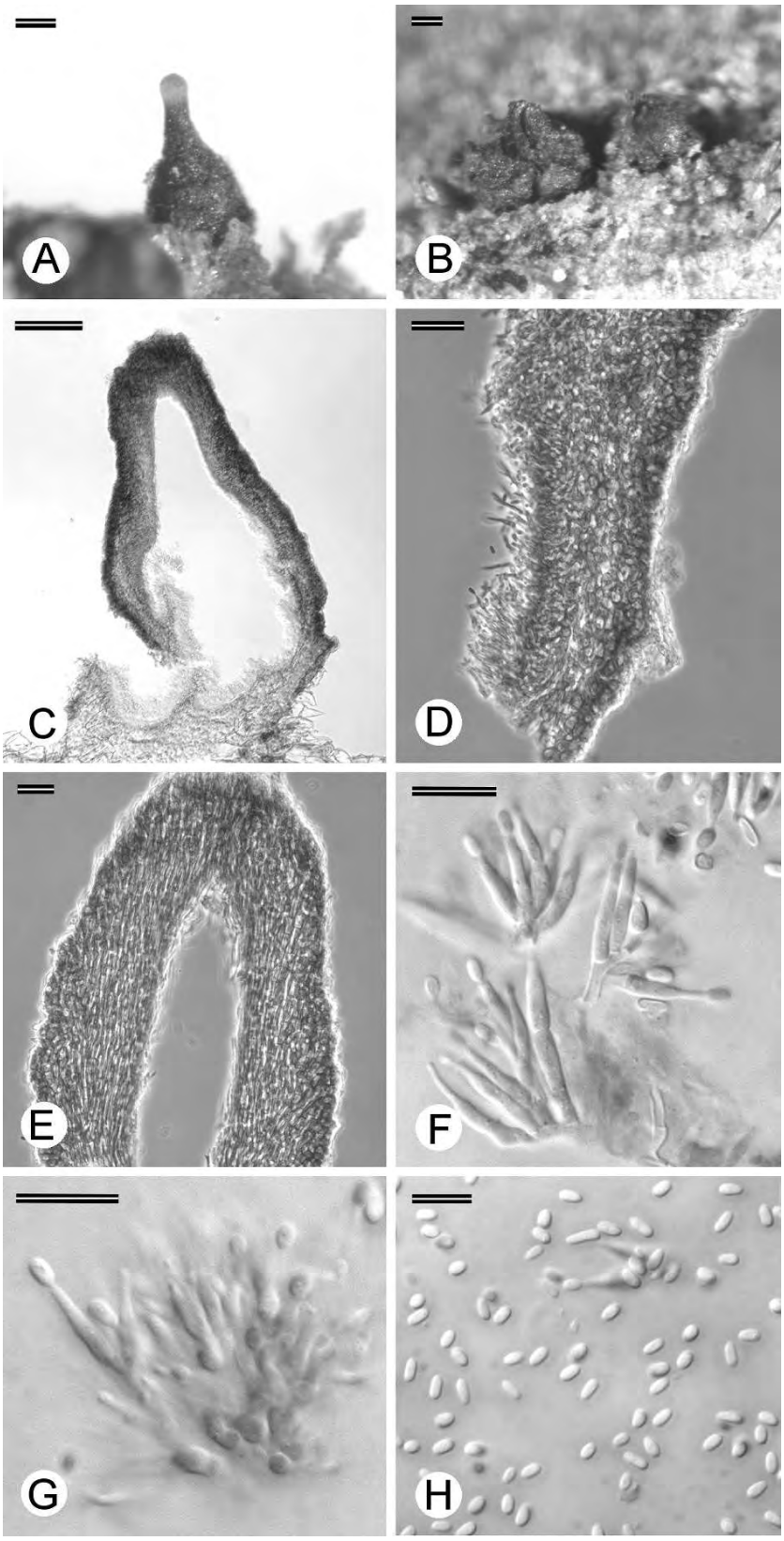


Fig. 7. Micrographs of fruiting structures of *Chrysoporthella hodgesiana*. A–B. Conidiomata of different shapes on bark. C. Vertical section through conidioma. D. Tissue of the conidiomal base. E. Tissue of conidiomal neck. F–G. Conidiophores. H. Conidia. Scale bars A–C = 100 µm; D–E = 20 µm; F–H =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

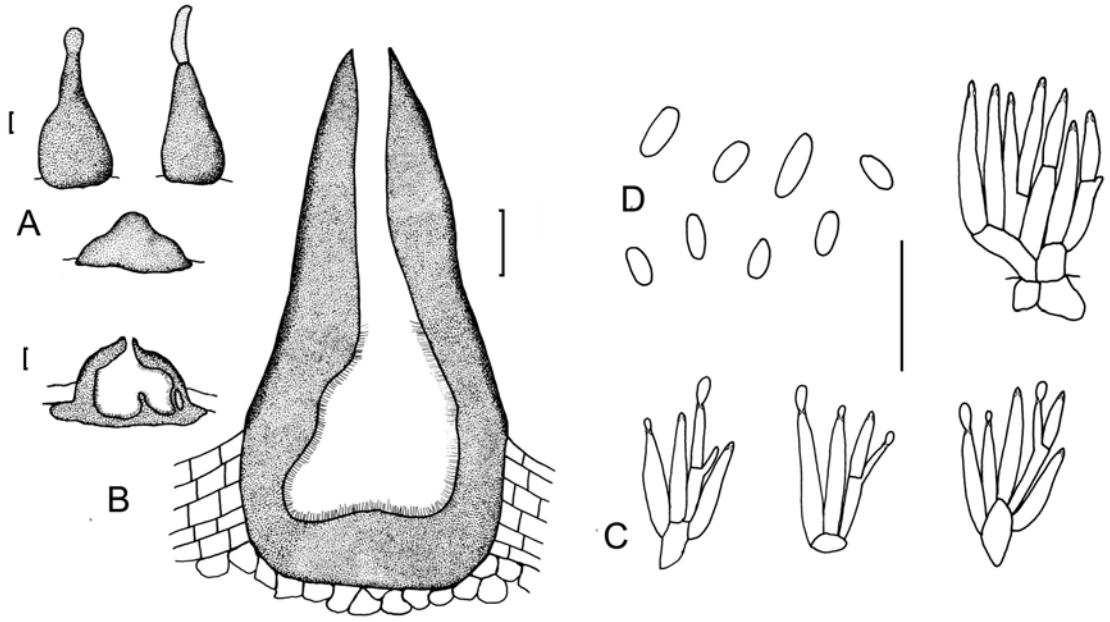
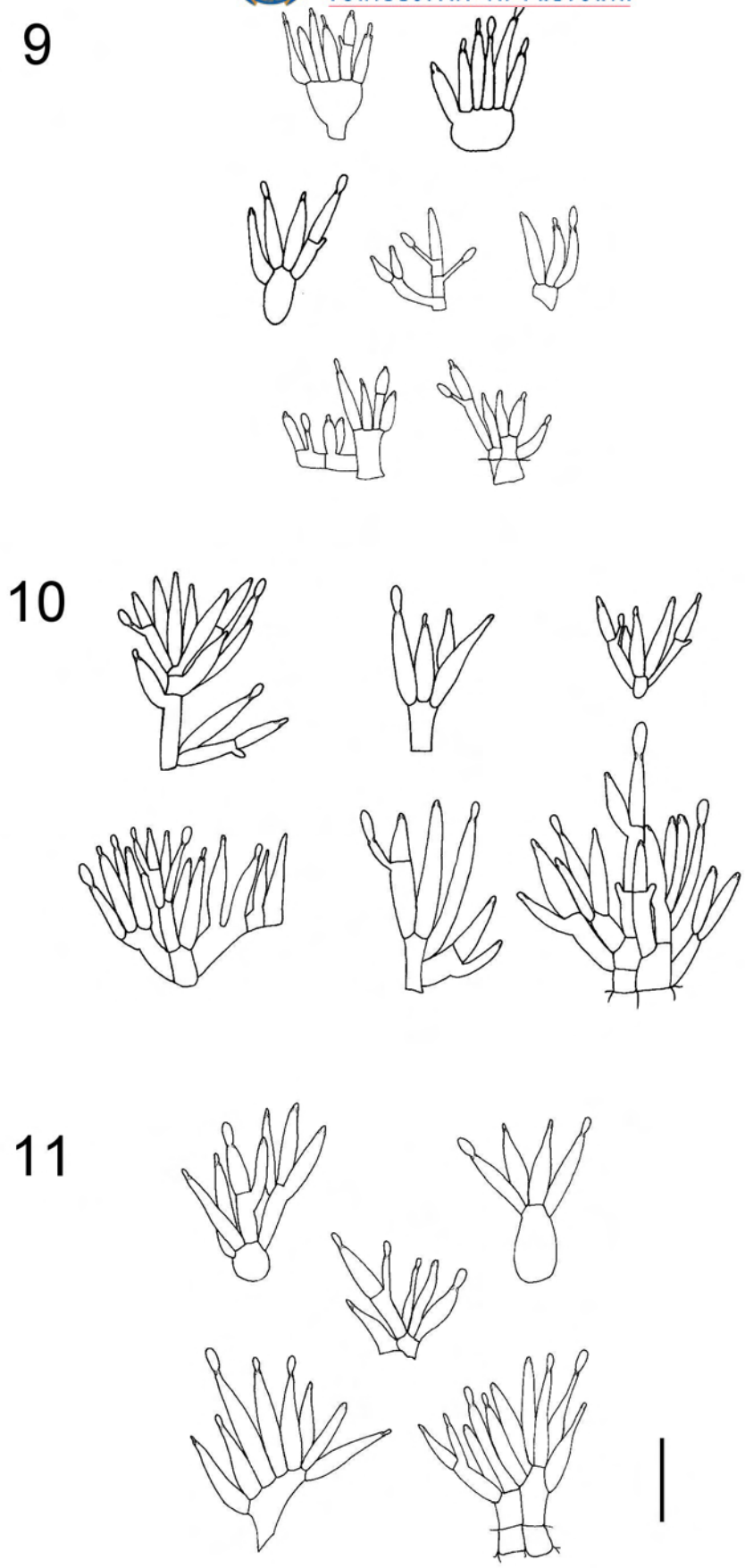


Fig. 8. Line drawings of *Chrysoporthella hodgesiana*. A. Shapes of conidiomata on bark. B. Section through conidiomata. C. Conidiophores and conidiogenous cells. D. Conidia. Scale bars A–B = 100 µm; C–D = 10 µm.



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.

CHAPTER 2

Chrysoporthe doradensis sp. nov. pathogenic to *Eucalyptus* in Ecuador

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Chrysoporthe doradensis sp. nov. pathogenic to *Eucalyptus* in Ecuador

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Abstract: Canker caused by *Chrysoporthe cubensis* is a serious disease of commercially grown *Eucalyptus* in various South American countries. This disease has not previously been recorded from Ecuador. Recent disease surveys in Ecuadorian *Eucalyptus* plantations have led to the discovery of canker symptoms typical of this disease with fruiting bodies resembling *C. cubensis* abundant on diseased tissues. The aim of this study was to characterise the fungus based on morphology and sequences of the ITS1/ITS2 regions of the ribosomal DNA operon and β-tubulin genes. Phylogenetic analyses showed that isolates from Ecuador reside in a clade together with other *Chrysoporthe* spp., but in a clearly distinct group. The distinct phylogenetic position of the Ecuadorian fungus is supported by unique conidial morphology and it is, therefore, described as *Chrysoporthe doradensis* sp. nov. Pathogenicity trials on *Eucalyptus deglupta* showed that the

fungus is highly pathogenic on this commonly planted tree as well as on saplings of *Tibouchina urvilleana*.

Taxonomic novelty: *Chrysoporthe doradensis* Gryzenh. & M. J. Wingf. sp. nov.

Key words: *Chrysoporthe cubensis*, *Chrysoporthe doradensis*, *Diaporthales*, *Eucalyptus*, Ecuador, *Tibouchina*

INTRODUCTION

Chrysoporthe cubensis (Bruner) Gryzenh. & M. J. Wingf., previously known as *Cryphonectria cubensis* (Bruner) Hodges, causes a serious canker disease of *Eucalyptus* trees in plantations. *Chrysoporthe cubensis* is common in the Neotropics where it has been reported from several countries (Bruner 1917, Boerboom & Maas 1970, Hodges *et al*. 1976, 1979, Myburg *et al*. 1999, Van der Merwe *et al*. 2001, Gryzenhout *et al*. 2004/Chapter 1 of this thesis). Girdling cankers on the stems of trees by this pathogen has had a substantial impact on *Eucalyptus* propagation in the tropics and sub-tropics, where it has also greatly influenced plantation practices (Wingfield 2003). The best option for management of this disease has been through breeding and selection of resistant *Eucalyptus* clones, and such programmes have been successfully implemented in various South American countries (Alfenas *et al*. 1983, Wingfield 2003, Rodas *et al*. 2005).

Chrysoporthe is a recently described genus including the fungus previously known as *Cry. cubensis* (Gryzenhout *et al*. 2004). DNA sequence comparisons and

detailed morphological studies have shown that specimens and isolates previously identified as *Cry. cubensis* from various parts of the world, represent at least three species (Myburg *et al.* 2002a, Gryzenhout *et al.* 2004). The fungus now known as *C. cubensis* represents isolates from South and Central America, but also includes isolates from Central Africa, Hawaii, South East Asia and Australia (Gryzenhout *et al.* 2004). In these areas, *C. cubensis* not only occurs on *Eucalyptus* but also on other *Myrtaceae* such as *Syzygium aromaticum* (clove) in Brazil, Zanzibar and Indonesia (Hodges *et al.* 1986, Myburg *et al.* 2003), and *Melastomataceae* such as native *Miconia theaezans* and *Miconia rubiginosa* in Colombia (Rodas *et al.* 2005).

Other than *C. cubensis*, two other species of *Chrysoporthe* are known and one of these occurs in South America together with *C. cubensis*. This species, *Chrysoporthella hodgesiana* Gryzenh. & M. J. Wingf., is recognized as a species of *Chrysoporthe* based on DNA sequence data, but is known only in its asexual state and thus resides in the anamorph genus of *Chrysoporthe*. *Chrysop. hodgesiana* is commonly found in Colombia on native *Tibouchina* spp. (Wingfield *et al.* 2001, Gryzenhout *et al.* 2004) and on *M. theaezans* (Rodas *et al.* 2005). Other than its unique DNA sequences, it can also be distinguished from *C. cubensis* based on its low optimal growth temperature (Gryzenhout *et al.* 2004). Isolates of the fungus previously known as *Cry. cubensis* from South Africa represents the third species that has been provided the name *C. austroafricana* Gryzenh. & M. J. Wingf. This species is defined by ascospores with rounded apices and is currently known only from South Africa (Gryzenhout *et al.* 2004). All three *Chrysoporthe* species are highly pathogenic to *Eucalyptus* spp. (Wingfield *et al.* 1989, Wingfield *et al.* 2001, Wingfield 2003).

Forestry in Ecuador includes the planting of native as well as exotic tree species in plantations. Plantations of *Eucalyptus* are not widespread and little is known regarding the diseases that affect them. Cankers caused by *C. cubensis* are found in neighbouring countries such as Colombia (Van der Merwe *et al*. 2001), but the disease has not been reported from Ecuador. The presence of this disease in Ecuador could have a negative impact on forestry in the country, particularly if susceptible species are planted. This provided the motivation for disease surveys in Ecuadorian *Eucalyptus* plantations and the discovery of a canker disease that forms the basis of this study.

MATERIALS AND METHODS

Symptoms and collection of samples

Eucalyptus grandis and *E. deglupta* trees of various ages between five and 10-years-old were found with stem cankers (Fig. 1A) in plantations near the towns of Buenos Aires. The extent of cankers differed substantially, but in many cases they had girdled and killed trees. Ascostromata and conidiomata were commonly found fruiting around the cankers, and these were collected and transported to the laboratory for further study. Isolates were made, purified and have been lodged in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa (Table 1). Representative isolates have also been deposited in the Centraalbureau voor Schimmelcultures (CBS), Utrecht, Netherlands (Table 1). The original pieces of

bark from which isolates were made were dried and have been deposited in the herbarium of the National Collection of Fungi, Pretoria, South Africa (PREM).

Morphology

Fruiting structures were cut from the bark specimens and examined using the methods outlined in Gryzenhout *et al.* (2004). Fifty ascospores, asci, conidia and conidiophores were measured and are presented as (min–)(average - S.D.) – (average + S.D.)(–max) µm. Only minimum and maximum values arising from the smallest and largest structure were obtained for the eustromata and perithecia. Colours were assigned using the notations of Rayner (1970).

Growth in culture was studied for the isolates (CMW 11286, CMW 11287) from *E. grandis* in Ecuador. This is especially important since *Chrysop. hodgesiana* has been distinguished from *C. cubensis* based on growth characteristics in culture (Gryzenhout *et al.* 2004). Growth of cultures was studied in the dark at temperatures ranging from 15 to 35 ºC, at 5 º intervals. The procedure for assessment of growth in culture was the same as that described by Gryzenhout *et al.* (2004).

DNA sequence comparisons

Sequences were obtained from a number of genic regions of isolates from *E. grandis* in Ecuador. These sequences were compared with those published (Table 1) for *C. cubensis*, *C. austroafricana* and *Chrysop. hodgesiana* from a variety of hosts (Gryzenhout *et al*. 2004, Rodas *et al*. 2005). Isolates of *Rostraureum tropicale* Gryzenh. & M. J. Wingf. were also included (Table 1). This species is closely

related to *Chrysoporthe* and was recently described as a pathogen of *Terminalia ivorensis* and *T. superba* in the same areas of Ecuador where the *Chrysoporthe* sp. was found in this study (Gryzenhout *et al*. 2005/Chapter 7 in this thesis). The *R. tropicale* isolates were included as outgroup taxa, together with the closely related *Cryphonectria parasitica* (Murrill) M. E. Barr, *Cryphonectria macrospora* (Tak. Kobay. & Kaz. Itô) M. E. Barr and *Cryphonectria nitschkei* (G. H. Otth) M. E. Barr (Gryzenhout *et al*. 2004, Myburg *et al*. 2004).

DNA was extracted from mycelium grown in Malt Extract Broth [20 g/L Biolab malt extract] following the protocol described in Myburg *et al*. (1999). The internal transcribed spacer (ITS) regions ITS1 and ITS2, and the conserved 5.8S gene of the ribosomal RNA (rRNA) operon, as well as two regions of the β-tubulin gene, were amplified as described in Myburg *et al*. (1999) and Myburg *et al*. (2002a). PCR products were run on 1% agarose (ethidium bromide-stained) gels, and detected under UV light. The PCR products were purified using a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and sequenced with the same primers that were used to amplify the respective DNA regions. An ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, FS (Perkin-Elmer, Warrington, UK) was used for the sequence reactions on an ABI PRISM 3100™ automated DNA sequencer.

The forward and reverse sequences that were obtained were imported into Sequence Navigator version 1.0.1 software (Perkin-Elmer Applied BioSystems, Foster City, CA). Sequences were manually aligned and inserted, together with those from Rodas *et al.* (2005), in the TreeBASE data matrix (S 1211, M 2095) generated in the study by Gryzenhout *et al*. (2004). Subsequent phylogenetic

analyses were done using PAUP version 4.0b (Swofford 1998). The combinability of the rRNA and β-tubulin gene sequence data sets was determined with a partition homogeneity test (PHT; Farris *et al*. 1994). Parsimony using the heuristic search option with the tree-bisection-reconnection (TBR) branch swapping, MULTREES options (saving all optimal trees) effective and random sequence additions set to 100 was employed to generate trees. During analyses, uninformative sites were excluded and individual CI values were used to reweight base pairs. Distance analyses were also executed using the distance model determined with MODELTEST version 3.5 (Posada & Crandall 1998) to confirm results obtained with parsimony. Thus the Transitional model or TIM (Tavaré 1986) was used (proportion of invariable sites (I) = 0.1840; Base frequency = 0.1952, 0.3262, 0.2408, 0.2379; Rate matrix = 1.0, 3.3491, 1.8115, 1.8115, 5.9357, 1.0). In the heuristic searches, gaps inserted during sequence alignment were treated as fifth character (NEWSTATE), but these were treated as missing data for distance analyses. A 1000 replicate bootstrap analyses was executed to assess the reproducibility levels of the branch nodes of the phylogenetic trees. Individual sequences generated in this study have been deposited in GenBank (Table 1).

Pathogenicity tests

Fifteen one-year-old *E. deglupta* trees were inoculated in November 2001 with isolate CMW 11287 (Table 1) to assess its pathogenicity. The trees were planted on the farm Rio Pitzara near the town of Santa Domingo. An equal number of trees were inoculated with discs from uninoculated MEA plates to serve as negative controls. Inoculation wounds were made with a 10 mm diam punch to remove the

bark and expose the cambium. Agar discs of equal size and bearing the test fungus were inserted into the wounds with the mycelium touching the exposed cambium. Wounds were covered with masking tape to reduce desiccation and contamination. Lesion development was assessed after five weeks by scraping the bark from the lesions and measuring the lesion length. The size of lesions arising from the inoculations was analysed in a Two-Sample t-Test Assuming Equal Variances in Microsoft Excell 2000.

Based on the fact that *Tibouchina* spp. are highly susceptible to *Chrysoporthe* spp. in other countries (Wingfield *et al.* 2001, Myburg *et al*. 2002b, Seixas *et al.* 2004) and are thought to be possible native hosts to *Chrysoporthe* spp. in South America (Gryzenhout *et al*. 2004), susceptibility of these trees, which are native to Ecuador, was also tested. Twenty approximately one-year-old *T. urvilleana* plants grown in pots (Fig. 1D) at a nursery near Buenos Aires, were thus inoculated with the *Chrysoporthe* sp. from *Eucalyptus* in Ecuador. Inoculations were done with isolate CMW 11287 in February 2000 using the same method described above and including the same number of negative controls. Data were analysed in a Two-Sample t-Test Assuming Equal Variances in Microsoft Excell 2000.

RESULTS

DNA sequence comparisons

Amplification products of the ITS1, 5.8S and ITS2 rRNA regions of the ribosomal DNA operon were approximately 600 bp, while those of the two regions of the β-

tubulin genes approximately 550 bp in length. Based on results from the PHT test (P = 0.04), the two data sets did not differ significantly from each other and could thus be combined. The data set consisted of 34 taxa with the *Cry. parasitica*, *Cry. macrospora*, *Cry. nitschkei* and *R. tropicale* isolates as the outgroup (Fig. 2). The ribosomal DNA dataset (553 bp) consisted of 399 constant, 50 variable parsimony-uninformative and 104 variable parsimony-informative characters (g1 = –3.186), while the β-tubulin dataset (896 bp) contained 634 constant, 35 variable parsimony-uninformative and 227 variable parsimony-informative characters (g1 = –2.809). The combined data set contained 1449 sequence characters in total.

Thirty phylogenetic trees were generated from the heuristic search (tree length = 474.5 steps, CI = 0.914, RI = 0.946) and only differed in the length of the branches. These trees and the tree obtained through distance analyses, both displayed the sub-clades observed previously for isolates of *Chrysoporthe* (Myburg *et al.* 2002a, 2003, Gryzenhout *et al.* 2004). These sub-clades included the two in which the morphologically identical isolates of *C. cubensis* reside (bootstrap support = 99 % for the Asian group, 93 % for the South American group), the sub-clade representing *C. austroafricana* (bootstrap support = 97 %) and the sub-clade representing *Chrysop. hodgesiana* (bootstrap support = 89 %). Isolates from Ecuador did not reside in any of the sub-clades representing existing *Chrysoporthe* spp., but formed a distinct sub-clade with a 100 % bootstrap within the greater *Chrysoporthe* clade. This sub-clade was characterised by unique alleles present in all of the isolates (Table 2).

Morphology

Ascostromata and conidiomata (Figs 3–4) of the fungus found on *Eucalyptus grandis* in Ecuador were generally similar to those for the descriptions of *Chrysoporthe* spp. (Myburg *et al.* 2003, Gryzenhout *et al.* 2004). Various morphological features were, however, distinct from those of the described *Chrysoporthe* spp. The most important distinguishing feature of this fungus is its conidial morphology. Conidia of the fungus from Ecuador were larger, (3–)3.5–5(–6) µm in length, variable in shape, cylindrical to oblong to ovoid, and occasionally allantoid (Figs 3K, 4F). Conidia of other *Chrysoporthe* species are oblong to ovoid, and (3–)3.5–4.5(–5) µm in length (Gryzenhout *et al.* 2004). Conidia of the fungus from Ecuador were often also exuded as pale luteous spore drops (Figs 3G, 3H) rather than the typical luteous to orange spore tendrils common to other *Chrysoporthe* species (Gryzenhout *et al.* 2004). Besides pyriform conidiomata, pulvinate conidiomata with short, thin necks (Figs 3G, 4D) were also observed, which differ from those of *C. cubensis* (Gryzenhout *et al.* 2004). No obvious differences in ascomatal structure and colony growth were observed for the fungus from Ecuador and those of other *Chrysoporthe* spp. (Gryzenhout *et al.* 2004).

Taxonomy

Results of the DNA sequence and morphological comparisons clearly showed that the fungus associated with cankers on *Eucalyptus* in Ecuador resides in *Chrysoporthe*. Furthermore, it represents a distinct and undescribed species. The following description is provided for this new species.

Chrysoporthe doradensis Gryzenh. & M. J. Wingf., **sp. nov**. Figs 3–4.

Etymology: after El Dorado, legendary South American city of gold and the golden colour of stromatic tissue of the fungus.

Ascostromata in cortice semi-immersa, collis peritheciorum protrudentibus atrofuscis cylindricis, et textura erumpente ascostromatica limitata aurantiaca visibilibus. *Asci* octospori, fusoidei vel ellipsoidei. *Ascosporae* hyalinae, uniseptatae, fusoideae vel ovales. *Conidiomata* superficialia, pyriformia vel pulvinata, 1 colla in quaque structura, atrofusca. *Basis* stromatis e textura globulosa, 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. *Conidiorum* massa cirrhis vel guttis luteis exudata; conidia hyalina, non septata, oblonga. *Coloniae* in MEA albae cinnamomeo- vel avellaneo-maculatae, celeriter crescentes, temperatura optima 30 ºC.

Ascostromata semi-immersed in bark, recognizable by extending, fuscous-black, cylindrical perithecial necks and in some cases, erumpent, limited ascostromatic tissue appearing orange, 140–380 µm high above the bark, 320-610 µm diam (Figs 3A–B, 4A–B). *Perithecia* valsoid, up to 5 per stroma, bases immersed in the bark, black, globose, 230–400 µm diam, perithecial wall 15–25 µm thick (Figs 3B, 4B). Top of perithecial bases covered with cinnamon to orange, predominantly prosenchymatous, stromatic tissue, which is occasionally visible above the bark surface (Figs 3A–C, 4A–B). *Perithecial necks* black, periphysate, 90–110 µm wide (Figs 3B, 4B). Necks emerging through the bark covered in umber, stromatic tissue of *textura porrecta*, thus appearing fuscous-black, extending necks up to 1680 µm long, 80–140 µm wide (Figs 3D, 4B). *Asci* 8-spored, biseriate, unitunicate, free

when mature, non-stipitate with a non-amyloid refractive ring, fusoid to ellipsoid, (19.5–)21.5–24(–25) × (4–)4.5–6(–7)µm (Figs 3E, 4C). *Ascospores* hyaline, one-septate, fusoid to oval, with tapered apices, (4.5–)5.5–7.5(–8.5) × 2–2.5 µm (Figs 3F, 4C).

Conidiomata eustromatic, superficial to slightly immersed, pyriform to pulvinate, usually with one attenuated neck per structure (Figs 3G–H, 4D–E), fuscous-black, inside umber when young, conidiomatal base above the bark surface 70–300 µm high, 100–290 µm diam, necks up to 300 µm long, 20–90 µm wide. *Conidiomatal locules* with even to convoluted inner surface (Figs 3I, 4E). *Stromatic tissue* of base of *textura globulosa*, the walls of outer cells thickened, neck tissue of *textura porrecta* (Fig. 3J). *Conidiophores* hyaline, with irregular shaped basal cells, (2–)3.5–6(–7.5) × (2–)2.5–4(–5) µm, branched irregularly at the base or above into cylindrical cells, with or without separating septa, total length of conidiophore (9.5–)12.5–18(–22.5) µm (Figs 3K, 4F). *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 3K, 4F). *Conidia* hyaline, non-septate, oblong to ovoid to cylindrical, occasionally allantoid, (3–)3.5–5(–6.5) × 1.5–2(–2.5) µm (Figs 3L, 4F), masses exuded as pale luteous droplets.

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

Substrate: Bark of *Eucalyptus* spp. including *Eucalyptus grandis* and *Eucalyptus deglupta*.

Distribution: Ecuador

Specimens examined: **Ecuador**, Pichincha, Buenos Aires, Buenos Aires nursery, *Eucalyptus grandis*, November 2001, M. J. Wingfield, **holotype designated here** PREM 58581, ex-type cultures CMW 11286 = CBS 115734, CMW 11287 = CBS 115735; Buenos Aires nursery, *Eucalyptus grandis*, July 2004, M. J. Wingfield, PREM 58582; Buenos Aires nursery, ex-type isolate CMW 11287 from *Eucalyptus grandis* inoculated into *Tibouchina urvilleana*, February 2000, M. J. Wingfield, PREM 58583. **South Africa**, Pretoria, ex-type isolates CMW 11286 and CMW 11287 from *Eucalyptus grandis* inoculated into *Eucalyptus grandis* clone ZG14 in the greenhouse, June 2002, M. Gryzenhout & H. Myburg, PREM 58584.

Pathogenicity tests

Inoculations with *C. doradensis* resulted in significant lesion development (avg. 57.5 mm, max. 72 mm) on the *E. deglupta* trees (Fig. 1B). These lesions were girdling trees and were significantly larger ($P < 0.001$) than those in the control inoculations (Fig. 1C). Fruiting structures were produced abundantly on the lesions caused by the test fungus and *C. doradensis* could thus easily be re-isolated from the inoculation wounds.

The inoculations on the *T. urvilleana* trees resulted in extremely long lesions (lesions avg. 111.5 mm, max. 260 mm), often resulting in death of the tree parts above the inoculation points (Figs 1E, 1G) and significantly different ($P < 0.001$)

from those of the control inoculations. As was true on the *E. deglupta* trees, the fungus fruited profusely on the lesions (Fig. 1F) and resulted in girdling of the stems and the production of epicormic shoots below the points of inoculation (Fig. 1G).

DISCUSSION

The stem canker disease caused by the *Chrysoporthe* sp. described in this study appears to be a serious and common disease of *E. grandis* and *E. deglupta* in the lowlands of Ecuador. The climate in this area is tropical and thus very similar to that in other parts of the world where *Chrysoporthe* spp. cause disease. Outward symptoms of the disease are similar to those caused by *C. cubensis*, but morphological and DNA based comparisons have shown clearly that the pathogen represents a distinct species. We have thus provided the name *C. doradensis* for this fungus, which is clearly an important pathogen in Ecuador.

Although peripherally similar to *C. cubensis*, *C. doradensis* could clearly be distinguished from *C. cubensis*. Based on DNA sequences differences, *C. doradensis* grouped separately from *C. cubensis*, *C. austroafricana* and *Chrysop. hodgesiana*. The most pronounced morphological feature supporting this phylogenetic distinction is the variable shape of the conidia of *C. doradensis* that is different from the more uniform oblong to oval conidia of other *Chrysoporthe* spp. In addition, *C. doradensis* produces spore masses of a different colour to those of the other species and *C. cubensis*.

Pathogenicity trials showed that *C. doradensis* is highly pathogenic and results resemble those found with the aggressive pathogen *C. austroafricana* in

South Africa (Van Heerden & Wingfield 2002, Roux *et al*. 2003, Rodas *et al.* 2005). Large lesions were produced by *C. doradensis* on *E. deglupta*, which together with *E. grandis*, is also naturally infected by the fungus in field stands. Although no quantitative data are available, and no *E. grandis* trees were available for inoculation at the time of this study, field observations suggest that *E. grandis* is substantially more susceptible to infection than is *E. deglupta*.

Pathogenicity trials with *C. doradensis* on seedlings of *T. urvilleana* showed that this host is highly susceptible to infection by the pathogen. In the two separate trials involving *E. deglupta* and *T. urvilleana* respectively, lesions on *T. urvilleana* were twice the length of those of *E. deglupta*. Similar results were obtained in pathogenicity trials involving *Chrysop. hodgesiana* (Wingfield *et al*. 2001) and *C. cubensis* (Rodas *et al*. 2005), where the pathogenicity of these species were also compared on *Tibouchina* and *Eucalyptus* species. In these trials, the *Tibouchina* trees were usually considerably more susceptible than the *Eucalyptus* trees, and were often killed during the course of the trials.

The origin of *C. doradensis* is unknown although we suspect that it is native to Ecuador. Despite widespread surveys for *Chrysoporthe* species over the past few years, *C. doradensis* has not been isolated in other parts of South America or the world, indicating that it might be restricted to Ecuador. If *C. doradensis* is native to Ecuador, it would have originated from a host other than *Eucalyptus*, which is a non-native in this country. Other species of *Chrysoporthe* in South and Central America occur on native members of the *Melastomataceae* including species of *Tibouchina* and *Miconia* (Wingfield *et al*. 2001, Gryzenhout *et al*. 2004, Rodas *et al*. 2005). One possibility is that the fungus has originated on native *Melastomataceae* such as

Tibouchina spp. that are common in Ecuador. The fact that *T. urvilleana* was highly susceptible to infection in this study might argue against this genus of tree being a native host. However, *Tibouchina* spp. in Ecuador are never found in the hot humid lowlands but are rather restricted to cool, high altitude forests of Ecuador. Susceptibilty of these trees to *Chrysoporthe* spp. could thus be associated with hot humid climates.

It is not clear whether *C. cubensis* and *Chrysop. hodgesiana* occur in Ecuador. The wide distribution of *C. cubensis* throughout South and Central America would suggest that the pathogen should also occur in this country. *Chrysop. hodgesiana*, however, has been reported only from Colombia (Gryzenhout *et al.* 2004, Rodas *et al.* 2005) and it is unknown whether it occurs elsewhere in South America. Cankers caused by *C. doradensis* are common on *Eucalyptus* spp. in the Ecuadorian lowlands and some of these may be caused by *Chrysoporthe* spp. other than the newly described *C. doradensis*. Identification of these fungi is difficult and demands robusts tests and ideally DNA sequence comparisons. For the present, it is clear that *C. doradensis* is commonly associated with cankers on *Eucalyptus* spp. in Ecuador, but other species of *Chrysoporthe* may also be present. If other species are present, this would potentially complicate disease management strategies.

The relative susceptibility of *Eucalyptus* spp. world wide to canker caused by species of *Chrysoporthe*, has been based on knowledge of the well known *C. cubensis*. The recent discovery that there are various other species related to this fungus, and that these are all pathogenic to *Eucalyptus*, should change this view. While *C. cubensis* might be pathogenic to certain species of *Eucalyptus*, others such

as the new species described in this study, could have a different host range. For example, the fungus causing cankers on *Eucalyptus* in South Africa, now known as *C. austroafricana*, is highly pathogenic and appears to have a rather different biology to *C. cubensis* (Wingfield 2003). These fungi, like the well known and devastating chestnut blight pathogen *Cry. parasitica* (Anagnostakis 1987), are virulent pathogens that can cause substantial damage to trees. Thus great effort should be made to ensure that they are not introduced into new areas of the world, where native *Myrtaceae*, *Melastomataceae*, or possibly hosts in other families, could be highly susceptible to infection by them.

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Table 1. Isolates included in this study. Isolates in bold were sequenced in this study.

Species identity	Isolate number[a]	Alternative isolate number[a]	Host	Origin	Collector	GenBank accession numbers[b]
Chrysoporthe doradensis	**CMW 11286**	CBS 115734	*Eucalyptus grandis*.	Ecuador	M J.Wingfield	AY 214289, AY 214217, AY 214253
	CMW 11287	CBS 115735	*E. grandis*	Ecuador	M.J. Wingfield	AY 214290, AY 214218, AY214254
	CMW 9123	CBS 115717	*Eucalyptus deglupta*	Ecuador	M.J. Wingfield	DQ 224034, DQ 224038, DQ 224039
	CMW 9124	CBS 115716	*E. deglupta*	Ecuador	M.J. Wingfield	DQ 224035, DQ 224040, DQ 224041
	CMW 9125	CBS 115715	*E. deglupta*	Ecuador	M.J. Wingfield	DQ 224036, DQ 224042, DQ 224043
	CMW 9126	CBS 115723	*E. deglupta*.	Ecuador	M.J. Wingfield	DQ 224037, DQ 224044, DQ 224045
Chrysoporthe cubensis	CMW 1856	-	*Eucalyptus* sp.	Kauai, Hawaii	-	AY 083999, AY 084010, AY 084022
	CMW 9903	-	*Syzygium aromaticum*	Kalimantan, Indonesia	C.S. Hodges	AF 292044, AF 273066, AF 273461
	CMW 11290	CBS 115738	*Eucalyptus* sp.	Indonesia	M.J. Wingfield	AY 214304, AY 214232, AY 214268
	CMW 8650	CBS 115719	*S. aromaticum*	Sulawesi, Indonesia	M.J. Wingfield	AY 084001, AY 084013, AY 084024
	CMW 10774	-	*S. aromaticum*	Zanzibar, Tanzania	-	AF 492130, AF 492131, AF 492132
	CMW 2632	-	*Eucalyptus marginata*	Australia	E. Davison	AF 046893, AF 273078, AF 375607
	CMW 10453	CBS 505.63	*Eucalyptus saligna*	Democratic Republic of Congo	-	AY 063476, AY 063478, AY 063480
	CMW 10669	CBS 115751	*Eucalyptus* sp.	Republic of Congo	J. Roux	AF 535122, AF 535124, AF 535126
	CMW 10639	CBS 115747	*E. grandis*	Colombia	C.A. Rodas	AY 263419, AY 263420, AY 263421
	CMW 9993	CBS 115728	*Miconia theaezans*	Colombia	C.A. Rodas	AY 214298, AY 214226, AY 214262
	CMW 10024	CBS 115739	*Miconia rubiginosa*	Colombia	C.A. Rodas	AY 262390, AY 262394, AY 262398
	CMW 8757	-	*Eucalyptus* sp.	Venezuela	M.J. Wingfield	AF 046897, AF 273069, AF 273464
	CMW 1853	-	*S. aromaticum*	Brazil	-	AF 046891, AF 273070, AF 273465
	CMW 10778	CBS 115755	*S. aromaticum*	Brazil	C.S. Hodges	AY 084006, AY 084018, AY 084030

	CMW 9432	CBS 115724	*E. grandis*	Mexico	M.J. Wingfield	AY 692321, AY 692324, AY 692323
Chrysoporthe austroafricana	CMW 2113	CBS 112916	*E. grandis*	South Africa	M.J. Wingfield	AF 046892, AF 273067, AF 273462
	CMW 8755	-	*E. grandis*	South Africa	M.J. Wingfield	AF 292040, AF 273064, AF 273458
	CMW 9327	CBS 115843	*Tibouchina granulosa*	South Africa	M.J. Wingfield	AF 273473, AF 273060, AF 273455
	CMW 9328	-	*T. granulosa*	South Africa	M.J. Wingfield	AF 273474, AF 273061, AF 273456
Chrysoporthella hodgesiana	CMW 9927	-	*Tibouchina urvilleana*	Colombia	C.A. Rodas, M.J. Wingfield	AF 265653, AF 292034, AF 292037
	CMW 9995	CBS 115730	*Tibouchina semidecandra*	Colombia	R Arbelaez	AY 956969, AY 956977, AY 956978
	CMW 10641	CBS 115854	*T. semidecandra*	Colombia	R. Arbaleaz	AY 692322, AY 692326, AY 692325
	CMW 10625	CBS 115744	*M. theaezans*	Colombia	C.A. Rodas	AY 956970, AY 956979, AY 956980
Cryphonectria parasitica	CMW 1652	CBS 112914	*Castanea dentata*	U.S.A.	-	AF 046902, AF 273075, AF 273468
Cryphonectria nitschkei	CMW 13742	MAFF 410570	*Quercus grosseserrata*	Japan	T. Kobayashi	AY 697936, AY 697961, AY 697962
Cryphonectria macrospora	CMW 10463	CBS 112920	*Castanopsis cupsidata*	Japan	T. Kobayashi	AF 368331, AF 368351, AF 368350
Rostraureum tropicale	CMW 9972	-	*Terminalia ivorensis*	Ecuador	M.J. Wingfield	AY 167426, AY 167431, AY 167436
	CMW 10796	CBS 115757	*T. ivorensis*	Ecuador	M.J. Wingfield	AY 167428, AY 167433, AY 167438

[a] CMW = Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; MAFF = Microorganisms Section, MAFF GENEBANK, National Institute of Agrobiological Sciences (NIAS), MAFF Gene Bank, Ibaraki, Japan.

[b] Accession numbers refer to sequence data of the ITS, β-tubulin 1 (primers Bt1a/1b) and β-tubulin 2 (primers Bt2a/2b) regions respectively.

Table 2. Summary of polymorphic nucleotides found within sequences of the ribosomal ITS region and two regions in the β-tubulin genes for *Chrysoporte cubensis*, *Chrysoporte austroafricana*, *Chrysoporte doradensis* and *Chrysoporthella hodgesiana*. Only polymorphic nucleotides shown to occur in all of the isolates in this study or previously (Gryzenhout *et al*. 2004), are shown (in bold typeface), and not alleles that occur in a single or small number of individuals per phylogenetic group. Numerical positions of the nucleotides in the DNA sequence alignments are indicated, and those nucleotides occurring in exons are in bold typeface.

Species	**Isolate numbers**	**β-tubulin (Bt1a/b)**													**ITS1/5/8S/ITS2**				
		141	153	161	162	164	167	185	189	201	209	**258**	**276**	**354**	1040	1326	1333	1334	1359
Chrysoporthe cubensis	All isolates in Asian group	C	C	G	G	A	A	A	T	T	T	T	C	C	A	**G**	**T**	**T**	**G**
	All isolates in South American group	**T**	C	G	**A**	A	A	A	T	T	T	T	**T**	C	A	A	C	-	A
Chrysoporthe austroafricana	CMW 9327, CMW 9328	C	C	G	G	**C**	**C**	A	**C**	**C**	**C**	**C**	C	C	A	A	C	-	A
	CMW 2113	C	C	G	G	**C**	**C**	A	**C**	**C**	**C**	**C**	C	C	A	A	**T**	-	A
	CMW 8755	C	C	G	G	A	A	A	**C**	**C**	**C**	**C**	C	C	A	A	C	-	A
Chrysop. hodgesiana	All isolates	C	C	G	G	A	A	**G**	T	T	T	**C**	**T**	C	**G**	A	C	-	**G**
Chrysoporthe doradensis	All isolates	C	**T**	**A**	G	A	A	A	T	**C**	**C**	T	C	**A**	A	A	C	-	A

Table 2 cont.

Species	**Isolate numbers**	**β-tubulin (Bt2a/b)**								
		539	548	561	586	705	**791**	**812**	**821**	**860**
Chrysoporthe cubensis	All isolates in Asian group	C	G	C	**C**	A	**T**	T	**T**	G
	All isolates in South American group	C	G	C	T	A	C	T	C	G
Chrysoporthe austroafricana	CMW 9327, CMW 9328	**T**	G	C	T	A	C	T	C	G
	CMW 2113	**T**	G	C	T	A	C	T	C	G
	CMW 8755	**T**	G	C	T	A	C	T	C	G
Chrysop. hodgesiana	All isolates	C	**A**	C	T	A	C	**C**	C	**A**
Chrysoporthe doradensis	All isolates	C	G	**T**	**C**	**G**	**T**	T	C	G

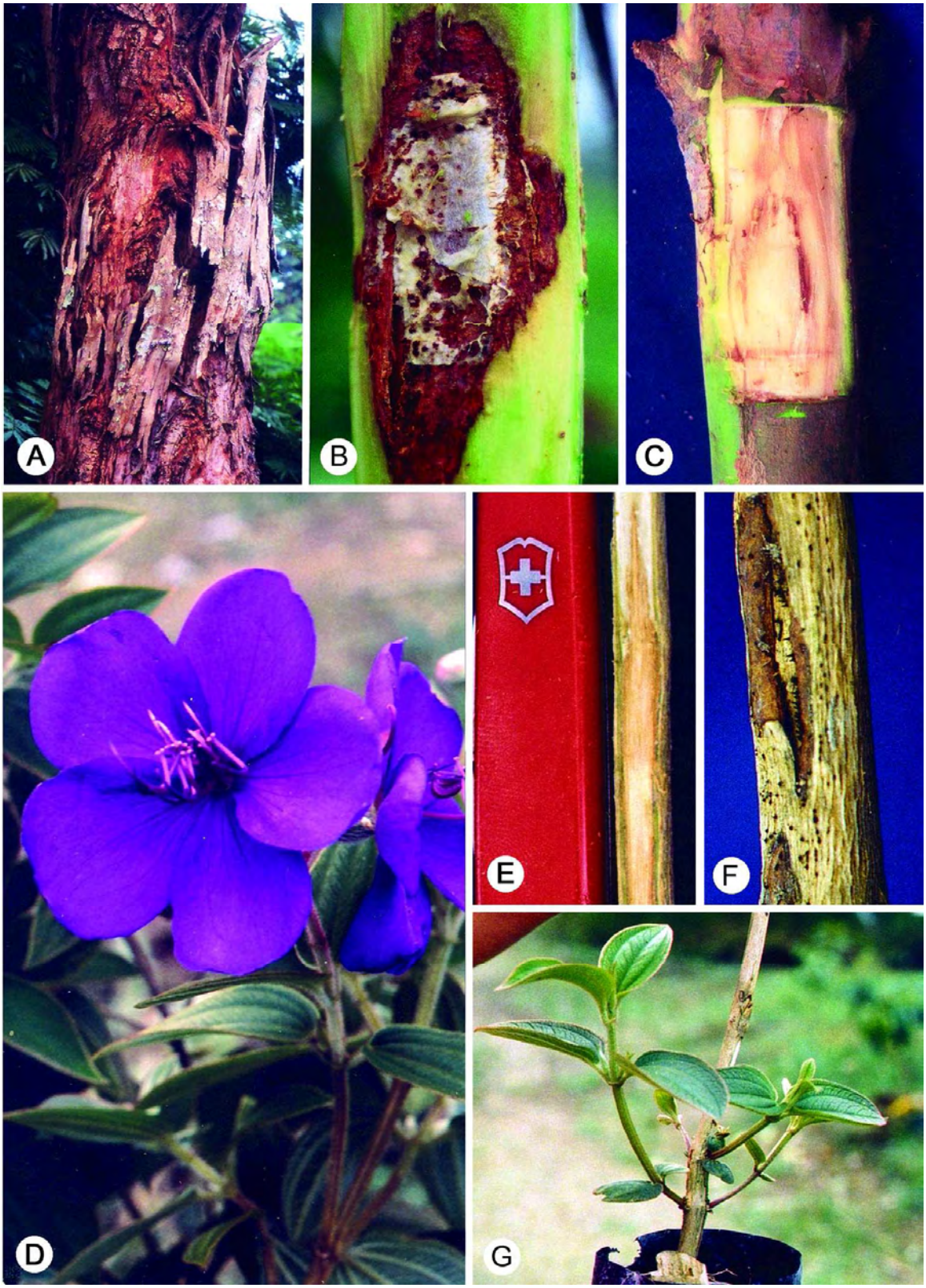

Fig. 1. Symptoms associated with the canker pathogen *Chrysoporthe doradensis* in the field and in inoculation trials. A. Stem canker of a dying *Eucalyptus grandis* tree in the field. B. Lesion on *Eucalyptus deglupta* associated with inoculations. C. Control inoculation on *E. deglupta*. D. *Tibouchina urvilleana*. E. Lesion associated with inoculations on *T. urvilleana*. F. Fruiting structures of *C. doradensis* on *T. urvilleana*. G. Formation of epicormic shoots resulting from inoculations on *T. urvilleana*.

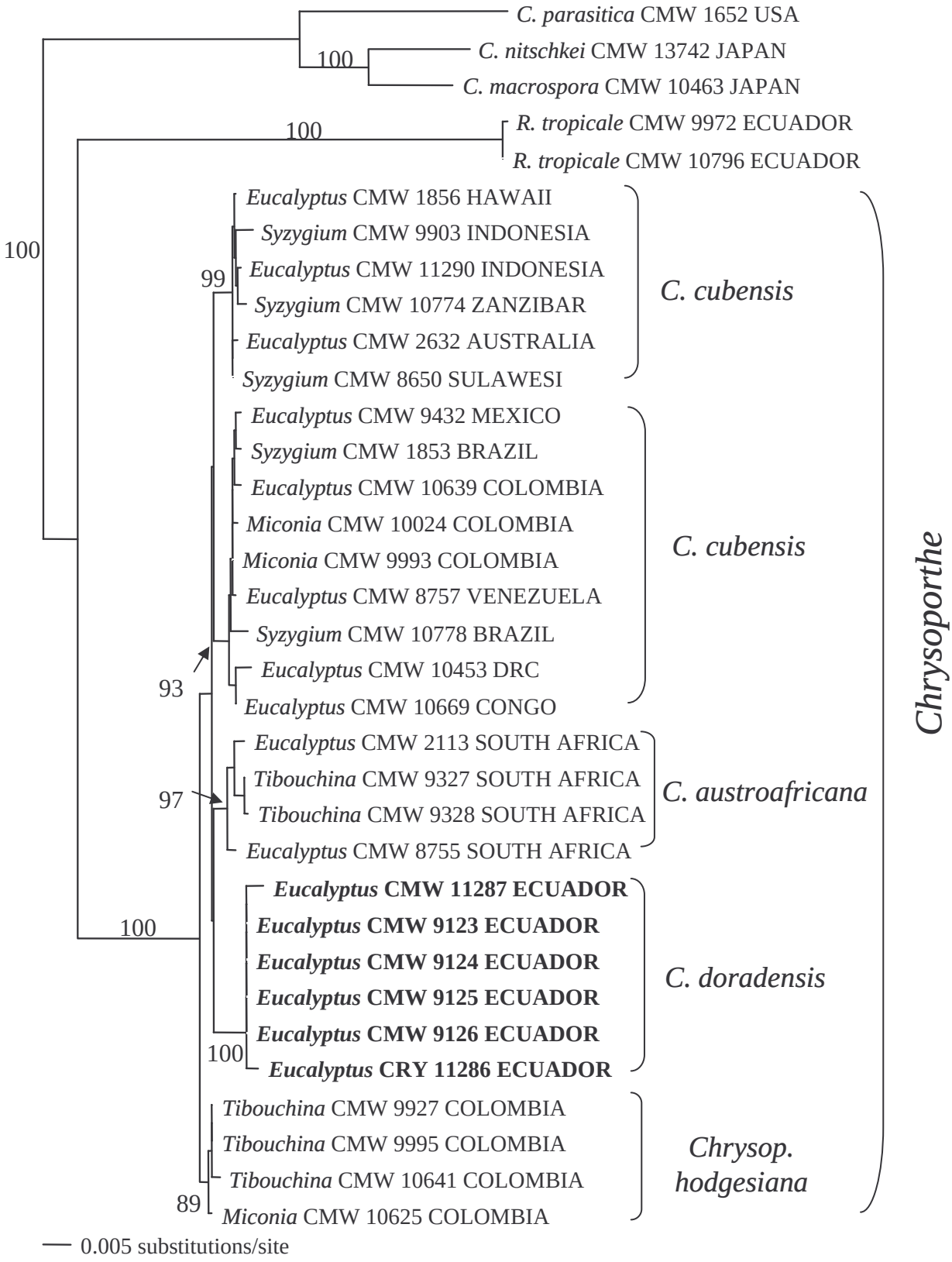

Fig. 2. A phylogram obtained from the combined data set of ribosomal DNA and β-tubulin gene sequences. Distance analyses were done using the Transitional model (proportion of invariable sites (I) = 0.1840; Base frequency = 0.1952, 0.3262, 0.2408, 0.2379; Rate matrix = 1.0, 3.3491, 1.8115, 1.8115, 5.9357, 1.0). Bootstrap values (1000 replicates) of branches are indicated on the branches and isolates representing the new species are in bold type . The outgroups includes *Rostraureum tropicale*, *Cryphonectria parasitica*, *Cryphonectria nitschkei* and *Cryphonectria macrospora.*

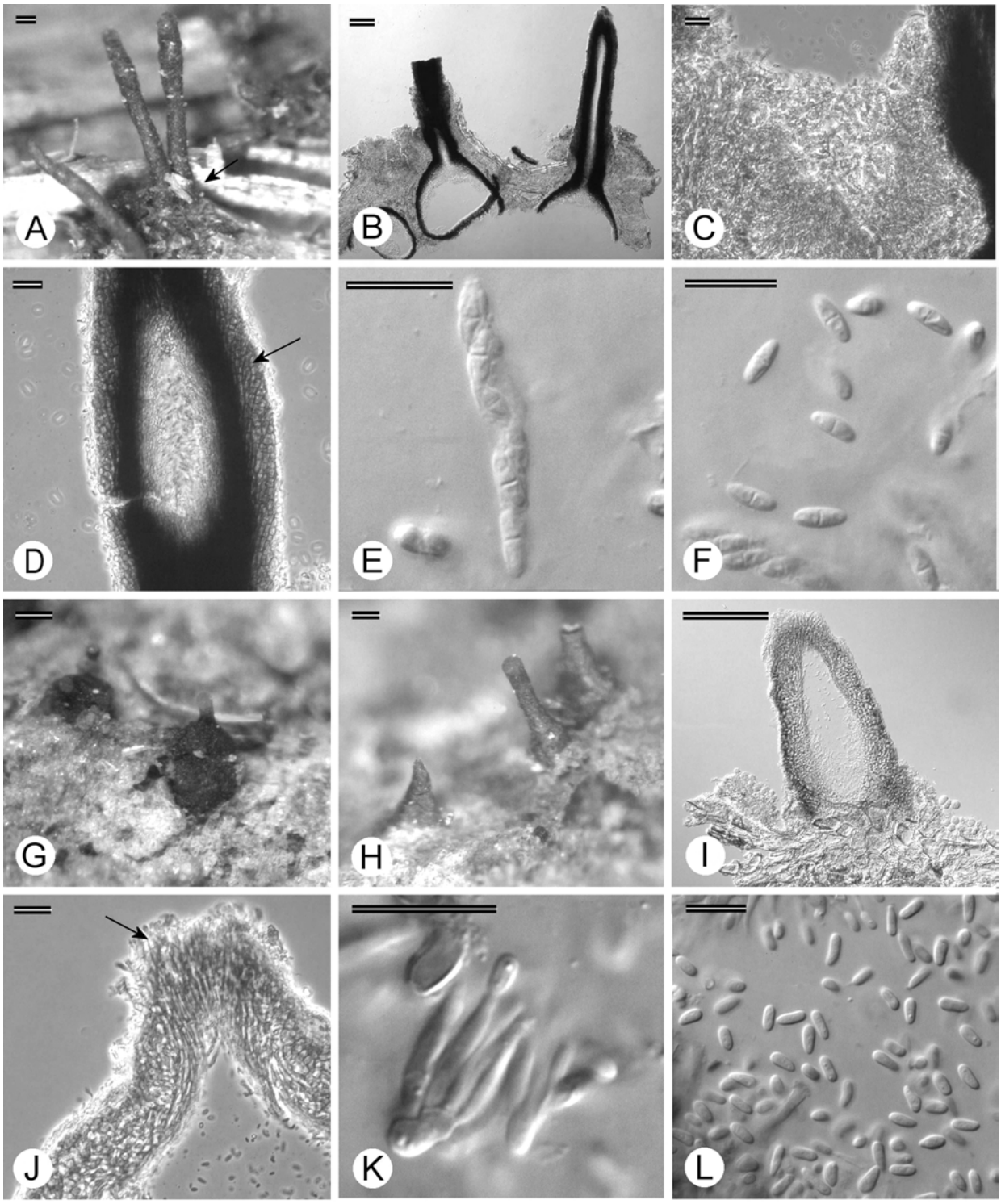

Fig. 3. Micrographs of fruiting structures of *Chrysoporthe doradensis* (from holotype). A. Ascostroma on bark showing black perithecial necks and orange stromatic tissue (arrow). B. Vertical section through ascostroma. C. Stromatic tissue of ascostroma. D. Perithecial neck and surrounding tissue (arrow). E. Ascus. F. Ascospores. G–H. Conidiomata. I. Vertical section through conidiomata. J. Tissue of the conidiomal base and neck (arrow). K. Conidiophores. L. Conidia. Scale bars A–B, G– I = 100 µm; C–D, J = 20 µm; E–F, K–L = 10 µm.

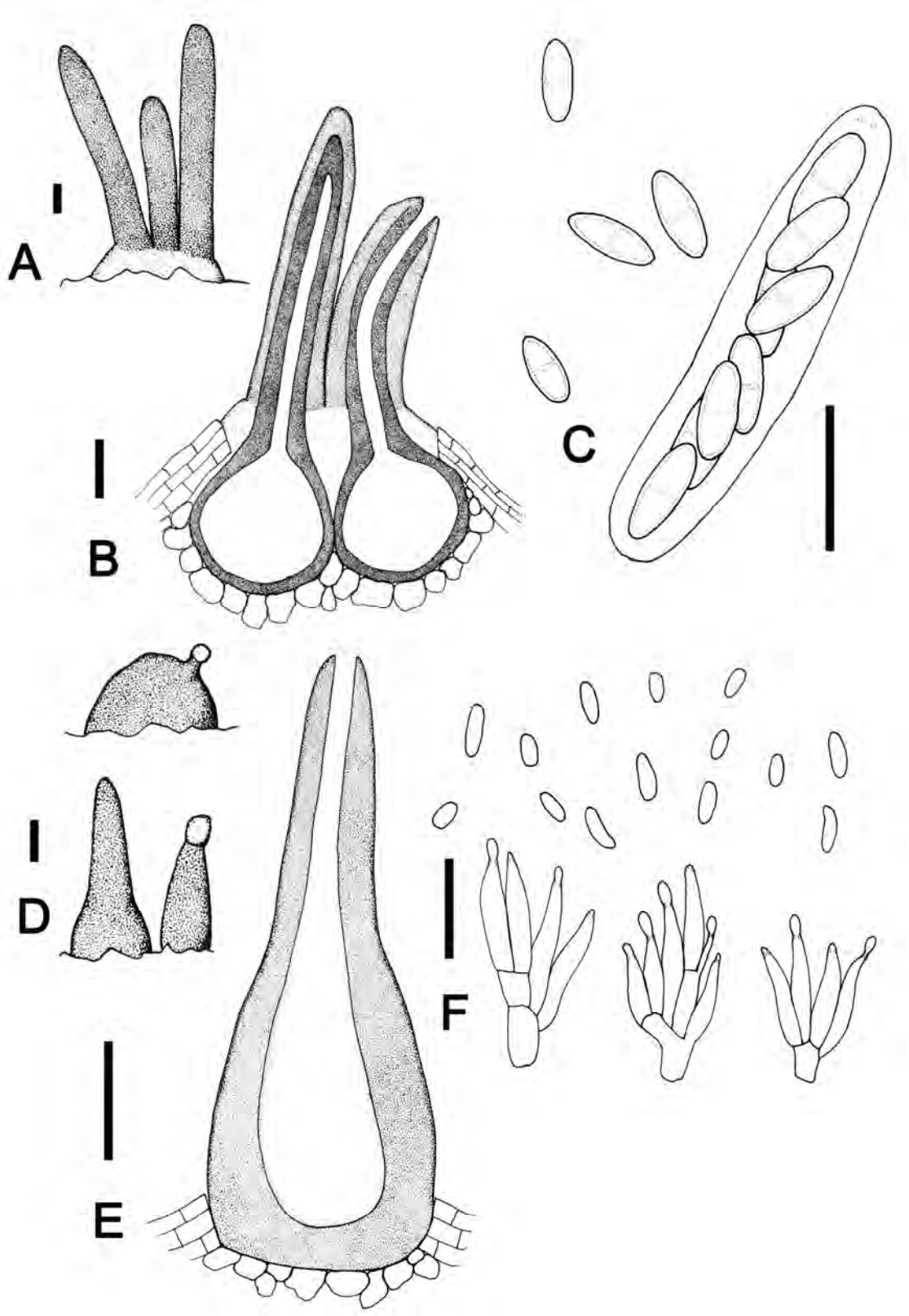

Fig. 4. Schematic drawings of *Chrysoporthe doradensis* (from holotype). A. Ascostroma on bark. B. Section through ascostroma. C. Asci and ascospores. D. Conidiomata on bark. E. Section through conidioma. F. Conidiophores and conidia. Scale bars A–B, D–E = 100 µm; C, F = 10 µm.

CHAPTER 3

Novel hosts of the *Eucalyptus* canker pathogen *Chrysoporthe cubensis* and a new *Chrysoporthe* species from Colombia

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Novel hosts of the *Eucalyptus* canker pathogen *Chrysoporthe cubensis* and a new *Chrysoporthe* species from Colombia

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Abstract. The pathogen *Chrysoporthe cubensis* (formerly *Cryphonectria cubensis*) is best known for the important canker disease that it causes on *Eucalyptus* species. This fungus is also a pathogen of *Syzygium aromaticum* (clove), which is native to Indonesia and, like *Eucalyptus*, in the *Myrtaceae*. Furthermore, *C. cubensis* has been found on *Miconia* spp. native to South America and residing in the *Melastomataceae*. Recent surveys have yielded *C. cubensis* isolates from new hosts, characterized in this study based on DNA sequences for the ITS and β-tubulin gene regions. These hosts include native *Clidemia sericea* and *Rhynchanthera mexicana* (*Melastomataceae*) in Mexico, and non-native *Lagerstroemia indica* (Pride of India, *Lythraceae*) in Cuba. Isolates from these hosts and areas group in the sub-clade of *C. cubensis* accommodating the South American collections of the fungus. This sub-clade also includes isolates recently collected from *Eucalyptus* in Cuba, which are used to

epitypify *C. cubensis*. New host records from South East Asia include exotic *Tibouchina urvilleana* from Singapore and Thailand and native *Melastoma malabathricum* (*Melastomataceae*) in Sumatra, Indonesia. Consistent with their areas of occurrence, isolates from the latter collections group in the Asian sub-clade of *C. cubensis*. DNA sequence comparisons of isolates from *Tibouchina lepidota* in Colombia revealed that they represent a new sub-clade within the greater *Chrysoporthe* clade. Isolates in this clade are described as *Chrysoporthe inopina* sp. nov. nom. prov., based on distinctive morphological differences.

Taxonomic novelty: *Chrysoporthe inopina* Gryzenh. & M. J. Wingf. sp. nov. nom. prov.

Key words: *Chrysoporthe cubensis, Chrysoporthe inopina, Clidemia sericea, Lagerstroemia indica, Melastoma malabathricum, Miconia, Rhynchanthera mexicana, Tibouchina urvilleana*

INTRODUCTION

Chrysoporthe cubensis (Bruner) Gryzenh. & M. J. Wingf., previously known as *Cryphonectria cubensis* (Bruner) Hodges, is a well documented pathogen of various tree species in tropical and sub-tropical areas of the world (Wingfield 2003). On *Eucalyptus* (*Myrtales*, *Myrtaceae*), the fungus causes serious damage, especially in commercial plantations of susceptible species or clones (Hodges *et al.* 1979, Van Heerden *et al.* 2005). The fungus also causes a serious canker disease on *Syzygium aromaticum* (clove, *Myrtaceae*) in Malaysia, Indonesia, Zanzibar and Brazil (Nutman

& Roberts 1952, Reid & Booth 1969, Hodges *et al.* 1986, Gryzenhout *et al.* 2004/Chapter 1 in this thesis). *Chrysoporthe cubensis* has recently been found to cause die-back and cankers on native *Miconia rubiginosa* and *Miconia theaezans* trees (*Myrtales*, *Melastomataceae*), where these trees occur naturally in Colombia (Rodas *et al.* 2005).

In recent years, numerous isolates of *C. cubensis* have been collected from different hosts and parts of the world. Large numbers of these isolates have been characterised based on DNA sequence data for the ITS region of the ribosomal DNA operon, β-tubulin genes and Histone *H3* genes (Myburg *et al.* 1999, Myburg *et al.* 2002a, 2003, Gryzenhout *et al.* 2004). These comparisons have thus shown that isolates of *C. cubensis* group in two well resolved sub-clades, roughly related to geographic distribution, within the greater *Chrysoporthe* clade (Gryzenhout *et al.* 2004). The one sub-clade encompasses isolates mainly from South America, but isolates from the Congo, Republic of the Congo and Cameroon (Africa) also group in this clade (Myburg *et al.* 2003, Roux *et al.* 2003, Nakabonge *et al.* 2006). The second clade contains isolates from many South East Asian countries and Australia (Myburg *et al.* 1999, 2002a), as well as isolates from Zanzibar in Tanzania, Kenya, Mozambique, Malawi (Africa) and Hawaii (U.S.A.) (Myburg *et al.* 2003, Nakabonge *et al.* 2006). Isolates and specimens linked to these two sub-clades are morphologically and phenotypically indistinguishable (Gryzenhout *et al.* 2004).

In addition to the two sub-clades identified for *C. cubensis*, three other phylogenetic sub-clades have also been recognized within *Chrysoporthe,* for isolates previously assigned to *Cry. cubensis* (Myburg *et al.* 1999, 2002a, Wingfield *et al.* 2001, Gryzenhout *et al.* 2004, 2005/Chapter 2 of this thesis). The fungi defining these sub-clades are morphologically distinct from each other and from *C. cubensis* and

have thus been described as novel taxa (Gryzenhout *et al.* 2004, 2005). Collections making up the South African sub-clade have been described as *Chrysoporthe austroafricana* Gryzenh. & M. J. Wingf., recognizable by the rounded apices of the ascospores (Gryzenhout *et al.* 2004). The isolates in the sub-clade from *Tibouchina* trees in Colombia, which do not have sexual structures, have been described in a new anamorph genus for *Chrysoporthe* as *Chrysoporthella hodgesiana* Gryzenh. & M. J. Wingf. (Gryzenhout *et al.* 2004). This fungus can be recognized by its optimal temperature for growth in culture, which is lower than that of other species of *Chrysoporthe*. Isolates from *Eucalyptus* spp. in Ecuador represent the third sub-clade, and this fungus has been described as *Chrysoporthe doradensis* Gryzenh. & M. J. Wingf. based on its variably shaped conidia and pale lutous spore droplets (Gryzenhout *et al.* 2005).

Several hypotheses have been formulated regarding the origin of *C. cubensis*. One of these is that the fungus originated on clove where these trees are native on the Molucca islands of Indonesia (Hodges *et al.* 1986). The fungus could thus have been moved around the world with these trees, when they were planted for the spice trade, and later adapted to infect *Eucalyptus* (Hodges *et al.* 1986). Another view is that *C. cubensis* originated in South and Central America (Wingfield *et al.* 2001). The latter hypothesis is strongly influenced by the wide-spread occurrence of the fungus in various pan-tropical countries and islands of South and Central America and the Caribbean (Gryzenhout *et al.* 2004), its high phenotypic diversity in various South America countries (Van Heerden *et al.* 1997, Van Zyl *et al.* 1998) as well as its discovery on native *Miconia* species in Colombia (Rodas *et al.* 2005). This would be consistent with observations that *C. cubensis* rapidly infected *Eucalyptus* spp. when plantations of these trees were established in South America (Hodges *et al.* 1986,

Seixas *et al*. 2004), or on islands such as Hawaii (Hodges *et al*. 1986). Whether isolates defining the Asian and South American sub-clades of *C. cubensis* represent discrete and cryptic taxa or groups of isolates in the process of speciation is not clear. Population genetic studies are required to resolve this question and thus to more fully understand the probable origin of the fungus.

The hypotheses regarding the origin of *C. cubensis* are based largely on the occurrence of this fungus on hosts other than *Eucalyptus* spp. Although the fungus has been found in Australia where *Eucalyptus* spp. are native, it is not common and it occurs in a mediterranean environment quite atypical for it (Hodges *et al.* 1986, Davison & Coates 1991). Views concerning the origin of *C. cubensis* also rest firmly on our knowledge of the geographical distribution of the pathogen. An expanded view of the hosts and distribution of *C. cubensis* must contribute significantly to a better understanding of the origin of this fungus. We have, therefore, actively collected isolates of *C. cubensis* from trees other than *Eucalyptus* and also from areas where this fungus has not previously been found. In addition, collections have been made in Cuba, the locality of the type specimen, in order to obtain isolates firmly defining this species. The aim of this study was to characterise these isolates, apparently representing new collections of *C. cubensis* from previously unreported hosts and areas, using DNA sequence data and morphological characteristics. An additional aim was to define an epitype for *C. cubensis* and thus to secure the name using DNA sequence data.

MATERIALS & METHODS

Symptoms and collection of samples

Collections used in this study were made in many parts of the world (Fig. 1). In Mexico, fruiting structures of fungi resembling *C. cubensis* were found on *Clidemia sericea* (*Melastomataceae*). These plants are common as weeds and occurred at road sides and in proximity to *Eucalyptus* plantations. Structures of a *Chrysoporthe* sp. were associated with cankers on the stems or at the bases of the stems. Subsequent collections from the same area by Mr. F. Ferreira yielded fruiting structures reminiscent of *C. cubensis* on *Rhynchanthera mexicana* (*Melastomataceae*).

In Cuba, fruiting structures believed to represent *C. cubensis* were common on cankers (Fig. 2A) on *E. grandis*, *E. saligna* and *E. urophylla* trees in Parque Metropolitano (Cerro Municipality. Havana city), Santiago de las Vegas (Boyeros Municipality, Havana city) and La Habano (or Havana city). *Chrysoporthe cubensis* was originally described from this area but there is only one specimen representing the holotype and there are no isolates linked to this important specimen (Gryzenhout *et al.* 2004). Therefore, specimens and isolates were collected from Cuba in order to fortify collections of the fungus and to define an epitype for it. In Cuba, fruiting structures resembling those of *C. cubensis* were also found on the tree *Lagerstroemia indica* (*Myrtales*, *Lythraceae*), commonly known as Pride of India, or crepe myrtle, growing in the gardens of the Institute of Ecology and Systematics, Boyeros Municipality, Havana city (Figs 2E-F).

In North Sumatra, structures resembling a species of *Chrysoporthe* were found on a native *Melastoma* sp. (*Melastoma malabathricum*) also known as the Straits Rhododendron. These trees were part of the natural vegetation around Lake Toba and

in an area where both clove and *Eucalyptus* trees are planted (Figs 2B-C). *Tibouchina urvilleana* plants (Fig. 2D) in Singapore and Thailand were also found bearing fruiting structures similar to those of *Chrysoporthe* spp. This is not a certain host of *C. cubensis* since previous reports of *C. cubensis* on *Tibouchina* spp. (Wingfield *et al.* 2001, Seixas *et al.* 2004) represent *Chrysop. hodgesiana* or are of unconfirmed identity (Gryzenhout *et al.* 2004).

Additional plant material bearing structures resembling those of *Chrysoporthe* spp. and isolates derived from these specimens, were obtained from Colombia. Specimens bearing fruiting structures taken from *T. lepidota* trees were collected at the Libano farm of Smurfit Carton de Colombia, near Pereira, Colombia. This material included teleomorph structures of a fungus resembling a *Chrysoporthe* sp.

Isolations from the fruiting structures on the bark surface of field-collected specimens, were made on malt extract agar MEA [20 g/L malt extract agar (Biolab, Merck, Midrand, South Africa)]. This was done by taking conidial or ascospore drops from the necks of fruiting structures or by exposing the spore mass inside the ascomata and then removing spores from it. The resultant cultures are maintained in the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa, and representative isolates have been deposited with the Centraalbureau voor Schimmelcultures (CBS), Utrecht, Netherlands (Table 1). The original bark specimens connected to the isolations have been deposited in the herbarium of the National Collection of Fungi, Pretoria, South Africa (PREM).

DNA sequence comparisons

Isolates for DNA sequence comparisons were grown in Malt Extract Broth [20 g/L malt extract, Biolab, Midrand, South Africa]. DNA was extracted from mycelium as

described in Myburg *et al.* (1999). 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 as described by Myburg *et al.* (1999). Two regions within the β-tubulin genes were also amplified following the methods of Myburg *et al.* (2002a). Purification of PCR products was done using a QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany).

The purified PCR products were sequenced with the same primers that were used to amplify the respective DNA regions. An ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase (Perkin-Elmer, Warrington, UK) was used to sequence the amplification products on an ABI PRISM 3100™ automated DNA sequencer. The resulting raw nucleotide sequences were edited using Sequence Navigator version 1.0.1 (Perkin-Elmer Applied BioSystems, Inc., Foster City, California, U.S.A.) software.

Sequences were added to the existing dataset (S 1211, M 2095) of Gryzenhout *et al.* (2004) and manually aligned. This dataset (Table 1) thus included isolates of *C. cubensis* from *Eucalyptus* spp. (Myburg *et al.* 2002a, Gryzenhout *et al.* 2004), *S. aromaticum* (Myburg *et al.* 1999, 2003) and *Miconia* spp. (Rodas *et al.* 2005) from different parts of the world; isolates of *C. hodgesiana* from *T. urvilleana* (Wingfield *et al.* 2001, Gryzenhout *et al.* 2004) and *M. theaezans* (Rodas *et al.* 2005); *C. austroafricana* isolates from *Eucalyptus* spp., *T. granulosa* (Myburg *et al.* 2002b, 2002b) and *S. cordatum* (Heath *et al.* 2006); and isolates representing *C. doradensis* (Gryzenhout *et al.* 2005). The outgroup consisted of species of the closely related *Cryphonectria*, namely *Cryphonectria parasitica* (Murrill) M. E. Barr, *Cryphonectria nitschkei* (G. H. Otth) M. E. Barr and *Cryphonectria macrospore* (Tak. Kobay. & Kaz. Itô) M. E. Barr.

Phylogenetic trees were inferred using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford 2002). A 500 replicate partition homogeneity test (PHT) (Farris *et al.* 1994) was applied to the rRNA and β-tubulin gene sequence data sets (after the exclusion of uninformative sites, heuristic search with 100 random sequence additions and tree-bisection-reconnection branch swapping, MULTREES off) to determine whether they could be analysed collectively. A phylogenetic tree was inferred from distance analyses. The correct model for the data sets was found with MODELTEST version 3.5 (Posada & Crandall 1998). The HKY85 model (Hasegawa *et al.* 1985) with the Gamma distribution shape parameter set to 0.1798 (freqA 0.1893, freqC 0.3273, freqG 0.2368, freqT 0.2465, Ti/Tv 2.0064), was shown to be the appropriate model. A 1000 replicate bootstrap analysis was executed to assess the confidence levels of the branch nodes of the phylogenetic tree. The sequence data generated in this study have been deposited in GenBank (Table 1).

Morphology

Fruiting structures were cut from the bark specimens and rehydrated in boiling water for 1 min. The structures, embedded in Leica mountant (Setpoint Premier, Johannesburg, South Africa), were sectioned approximately 12 um thick. The sections were dropped in water, transferred to microscope slides and mounted in 85% lactic acid. Fresh slides from structures were also made with lactic acid or 3% KOH. Twenty measurements of ascospores, asci, conidia and conidiophores were taken from the fresh slides for all specimens, but fifty measurements were taken for the holotype specimen. These are presented as (min–)(average - std. dev.) – (average + std. dev.)(–max) µm. A range of measurements (minimum and maximum) was obtained from at least two structures (representing the smallest and largest) for the anamorph and

teleomorph stromata on the bark, and perithecia representing the midpoint from sections, respectively. Standard colour notations provided by Rayner (1970) were applied.

Growth of isolates CMW 12727 and CMW 12729, which represent the new species from *T. lepidota* in Colombia (Table 1), was compared in culture. Cultures were grown on MEA in 90 mm diam Petri dishes in the dark at temperatures from 15 to 35 ºC at 5 º intervals. Assessment of growth was made as is described in Gryzenhout *et al.* (2004).

RESULTS

DNA sequence comparisons

The PHT analyses showed that the rDNA and β-tubulin sequence data sets did not have any significant conflict (P = 0. 012) and could thus be combined. The dataset consisted of 39 taxa with the aligned ribosomal DNA sequence dataset (538 characters) consisting of 473 constant characters, 20 parsimony-uninformative and 45 parsimony-informative characters (g1 = –3.671). The β-tubulin gene dataset (894 characters) consisted of 716 constant characters, 68 parsimony-uninformative and 110 parsimony-informative characters (g1 = –3.5959). After combination, the data set was comprised of 1432 characters.

The various isolates originating from Mexico, Cuba, Sumatra and Singapore grouped in the two sub-clades representing *C. cubensis*, separately from those representing *C. austroafricana*, *Chrysop. hodgesiana* and *C. doradensis* (Fig. 3). The isolates from *Cli. sericea* (CMW 12471, CMW 13046) and *R. mexicana* (CMW 12734, CMW 12736) in Mexico, grouped in the sub-clade including Mexican and

other South American isolates (bootstrap support 93%). Specifically, the isolates from *E. grandis* (CMW 14394, CMW 14404) and *L. indica* (CMW 16199, CMW 16200) in Cuba, also grouped in the South American sub-clade of *C. cubensis*. The isolates from *T. urvilleana* in Singapore (CMW 12745) and Thailand (CMW 17172, CMW 17178), and *M. malabathricum* (CMW 16192, CMW 18515) in Sumatra grouped in the South East Asian sub-clade (bootstrap support 96%).

Other than the four previously characterised sub-clades in *Chrysoporthe* (Myburg *et al.* 2002a, Gryzenhout *et al.* 2004, 2005), an unexpected sub-clade emerged in the phylogenetic tree (Fig. 3). The clade included isolates representing the new collection (CMW 12727, CMW 12729, CMW 12731) from *T. lepidota* in Colombia (bootstrap support 72%).

Morphology

Fruiting structures produced on specimens from different hosts in Mexico, Cuba, Singapore and Sumatra (Table 2) included both teleomorph and anamorph structures. These structures all resembled features previously described for *C. cubensis* (Bruner 1917, Hodges 1980, Myburg *et al.* 2003, Gryzenhout *et al.* 2004). The morphological characterisation of this material as *C. cubensis* is consistent with the results of the DNA sequence comparisons.

Fruiting structures on specimens collected from *Eucalyptus* spp. in Cuba were similar to those described for *C. cubensis* from other parts of the world. They were also indistinguishable from those of the type specimen (BPI 631857) of *C. cubensis* (Bruner 1917, Gryzenhout *et al.* 2004). The sizes of the asci on the newly collected material from Cuba differed somewhat from those reported by Bruner (1917) for Cuban specimens. They were thus up to 28 µm long, and not up to 34 µm as reported

by Bruner (1917). Gryzenhout *et al.* (2004) also reported that ascus dimensions as reported by Bruner (1917) were inconsistent with those of specimens from other countries, but they could not find asci on the type specimen to confirm the ascus dimensions. The new measurements obtained for Cuban material from *Eucalyptus* in this study are similar to ascus sizes reported by Gryzenhout *et al.* (2004) for specimens connected to *C. cubensis* from South America and South East Asia. The newly confirmed ascus size of (19–)22–26.5(–28) µm for *C. cubensis* represents an additional characteristic to distinguish *C. cubensis* from *C. austroafricana*, which has longer asci [(25–)27–32(–34) µm; Gryzenhout *et al*. 2004).

Fruiting structures on the bark material (PREM 58800) linked to the isolates from *T. lepidota* in Libano, Colombia (CMW 12727, CMW 12729, CMW 12731), which formed a separate sub-clade based on DNA data, could be distinguished from those of existing *Chrysoporthe* species. Asci (Figs 4E, 5C) were longer [(27.5–)29.5–34(–35.5) µm] than those of *C. cubensis* [(19–)22–26.5(–28) µm; Gryzenhout *et al*. (2004)] and *C. doradensis* [(19.5–)21.5–24(–25); Gryzenhout *et al*. (2005)], and corresponded with those of *C. austroafricana* [(25–)27–32(–34) µm; Gryzenhout *et al.* (2004)]. The new species could also be distinguished from *C. austroafricana*, *C. cubensis* and *C. doradensis*, because its ascospores (Figs 4F, 5C) were slightly wider (2.5–3.5 µm) than those of *C. cubensis* [(2–2.5(–3) µm; Gryzenhout *et al*. (2004)], *C. austroafricana* [(2–)2.5 µm; Gryzenhout *et al*. (2004)] and *C. doradensis* [2-2.5 µm; Gryzenhout *et al*. (2005)]. Furthermore, isolates in this group grew optimally at 25 ºC, different to the temperature for optimal growth of 30 ºC for *C. cubensis*, *C. austroafricana* (Gryzenhout *et al*. 2004) and *C. doradensis* (Gryzenhout *et al.* 2005). Conidiomata also had variable shapes, varying from subulate with no attenuated neck, to pyriform with an attenuated neck, to globose (Figs 4G, 5D). These characteristics

distinguished the new species from *Chrysop. hodgesiana*, which is the only other species growing optimally at 25 ºC (Gryzenhout *et al*. 2004). However, since shape and size of the conidiomata can be quite variable between samples (Hodges *et al.* 1986, Rodas *et al.* 2005), the shape of the conidiomata cannot always be used with confidence to distinguish *Chrysop. hodgesiana* from the new species.

Taxonomy

In this study we have characterised collections of *C. cubensis* from *Eucalyptus* spp. from Cuba (PREM 58788–PREM 58791), which is the type locality of *C. cubensis*. Based on morphology, these specimens were similar to other specimens of *C. cubensis*. Isolates connected to these specimens also grouped in the South American sub-clade of *C. cubensis* based on DNA sequence comparisons. In the absence of isolates it has been impossible to tell whether the type specimen of *C. cubensis* from Cuba would group in the South East Asian or South American sub-clade of the fungus (Gryzenhout *et al.* 2004). Certain morphological features such as ascus morphology could also not be studied, due to the poor quality of the type specimen. Epitypification of *C. cubensis* using newly collected Cuban specimens will greatly aid future taxonomic studies of *Chrysoporthe* species. We, therefore, designate specimen PREM 58788 (ex-type culture CMW 14394/CBS 118654, living culture CMW 14404/CBS 118647), which originates from the same locality as the type specimen, as epitype of *C. cubensis*. Additional specimens and linked isolates (Table 2; specimen PREM 58789 = isolate CMW 14378/CBS 118655; PREM 58790 = CMW 14362/CBS 118657; PREM 58791 = CMW 14395/CBS 118648) from Cuba have also been deposited to fortify material defining this species.

Comparisons of specimens from *T. lepidota* collected on the Libano farm in Colombia, showed that this fungus represents a previously undescribed taxon. Although the sub-clade representing this fungus is supported with a relatively low bootstrap value based on the regions of the genome sequenced, the fungus clearly does not group in the sub-clades representing other species. This new species can also be differentiated from other species of *Chrysoporthe* based on morphology. We, therefore, provide the following description for it.

Chrysoporthe inopina Gryzenh. & M. J. Wingf., **sp. nov.**, **nom. prov.** Figs 4-5.

Etymology: Latin, *inopina*, unexpected, referring to the unexpected discovery of this species.

Ascosporae fusoideae vel ovales, utrinque rotundatae, (4.5–)6–7.5(–8) × 2.5–3.5 µm. *Conidiomata* subulata vel pyriformia vel pulvinata.

Ascostromata semi-immersed in bark, recognizable by extending, fuscous-black, cylindrical perithecial necks and in some cases, erumpent, limited ascostromatic tissue appearing orange, 90–540 µm high above the bark, 200–770 µm diam (Figs 4A–B, 5A–B). *Perithecia* valsoid, 1–6 per stroma, bases immersed in the bark, black, globose, 306–390 µm diam, perithecial wall 21–25 µm thick (Figs 4B, 5B). Top of perithecial bases covered with cinnamon to orange, predominantly prosenchymatous, stromatic tissue, which is occasionally visible above the bark surface (Figs 4B–C, 5B). *Perithecial necks* black, periphysate, 77–131 µm wide (Figs 4B, 4D, 5B). Necks emerging through the bark covered in umber, stromatic tissue of *textura porrecta*, thus appearing fuscous-black (Figs 4B, 5A–B), extending necks up to 2330

µm long, 90–180 µm wide. *Asci* 8-spored, biseriate, unitunicate, free when mature, non-stipitate with a non-amyloid refractive ring, fusoid to ellipsoid, (27.5–)29.5–34(–35.5) × (4.5–)5.5–6.5(–7) µm (Figs 4F, 5C). *Ascospores* hyaline, one-septate, fusoid to oval, with rounded apices, (4.5–)6–7.5(–8) × 2.5–3.5 µm (Figs 4E, 5C).

Conidiomata eustromatic, superficial to slightly immersed, subulate to pyriform to pulvinate, with neck attenuated or not, usually with one neck per structure (Figs 4G–H, 5D–E), fuscous-black, inside umber when young, conidiomatal base above the bark surface 100–650 µm high above level of bark, 70–710 µm diam, necks up to 780 µm long, 50–190 µm wide. *Conidiomatal locules* with even to convoluted inner surface, occasionally multilocular, single locule connected to 1 or several necks (Figs 4H, 5E). *Stromatic tissue* of base of *textura globulosa*, walls of outer cells thickened (Fig. 4I), neck tissue of *textura porrecta* (Fig. 4J). *Conidiophores* hyaline, with basal cells of irregular shape and (2.5–)3.5–6(–7) × 2–3.5(–4) µm, branched irregularly at the base or above into cylindrical cells, with or without separating septa, total length of conidiophore (11–)12.5–22.5(–29.5) µm (Figs 4K, 5F). *Conidiogenous cells* phialidic, determinate, apical or lateral on branches beneath a septum, cylindrical to flask-shaped with attenuated apices, (1.5–)2–2.5(–3) µm wide, collarette and periclinal thickening inconspicuous (Figs 4K, 4L, 5F). *Conidia* hyaline, non-septate, oblong, (3–)3.5–4 × (1.5–)2–2.5 µm (Figs 4L, 5F), masses exuded as orange to luteous droplets.

Cultural characteristics: on MEA (CMW 12727, CMW 12729) 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 25 ºC. Cultures rarely sporulating after sub-culturing, teleomorph not produced in culture.

Substrate: Bark of *Tibouchina lepidota.*

Distribution: Colombia

Specimens examined: **Colombia,** Risaralda, Libano farm near Pereira (75° 35′ 49″ W and 40° 43′ 13″ N, 2102 meters above sea level, 3143 mm/y), *Tibouchina lepidota*, Jan. 2003, R. Arbelaez, **holotype** PREM 58800, ex-type culture CMW 12727 = CBS 118659, living cultures CMW 12729 = CBS 118658, CMW 12731 = CBS 118656.

DISCUSSION

Results of this study encompass reports of several new host species for *C. cubensis* from various parts of the world (Fig. 1). Many of these hosts are native to the countries in which they have been found. Thus, isolates of *C. cubensis* residing in the South American sub-clade, originated on native *Cli. sericea* and *R. mexicana* in Mexico. These collections add to those on *Miconia* spp. that have been reported before as native hosts of *C. cubensis* in Colombia (Rodas *et al.* 2005). Isolates from the native melastome, *M. malabathricum*, were shown to group in the South East Asian clade of *C. cubensis*. This represents the first ever collection of *C. cubensis* from a native plant growing in a natural situation in South East Asia. The only other equivalent collection is that of *C. cubensis* from clove collected in Malaysia (Reid & Booth 1969), Sulawesi (Myburg *et al.* 2003) and elsewhere in Indonesia (Hodges *et al*. 1986). Although clove occurs naturally in the Molucca islands, which is relatively close to areas in Indonesia and Malaysia where *C. cubensis* has been found to occur, the trees in the latter areas are planted and not native.

Other hosts of *C. cubensis* reported in this study are exotics in the countries where the fungus was collected. *Lagerstroemia indica* is native to China, but is

planted world-wide as a garden ornamental. In the case of this study, *C. cubensis* was found on this tree in Cuba. Isolates from this collection in Cuba group in the South American sub-clade of *C. cubensis*, which is the same as collections of the fungus now available from *Eucalyptus* in Cuba. The origin of the fungus on *Eucalyptus* and *L. indica* is not known but it probably originated on a native plant in the area. It is, however, also possible that *L. indica* represents a native host of *C. cubensis* in China where the tree is native. If that were the case, isolates of the fungus from China would most probably group in the South East Asian sub-clade. That was also true in previous studies (Myburg *et al.* 1999, Myburg *et al.* 2002a), where an isolate from *E. camaldulensis* in China grouped in the South East Asian clade.

Tibouchina urvilleana is a native of South America, but it is also popular as an ornamental in various countries, because of its conspicuous and attractive flowers. In this study, *C. cubensis* was found on *T. urvilleana* planted as a non-native ornamental in Singapore and Thailand. Isolates from these collections group in the South East Asian clade of *C. cubensis*. The fungus is also known to occur on *Eucalyptus* in Thailand (Old *et al.* 2004). Presumably, the fungus in the area originally came from a native South East Asian plant, possibly a species of *Melastoma* of which there are numerous native species in the area. *Tibouchina* spp. are known to be highly susceptible to infection by *Chrysoporthe* spp. (Wingfield *et al.* 2001, Myburg *et al.* 2002b, Seixas *et al.* 2004) and trees planted as exotics could easily become infected, as appears to have occurred in this situation.

Prior to this study, *Tibouchina* spp. had not been confirmed as hosts of *C. cubensis*. Previous collections from *Tibouchina*, thought to represent *C. cubensis* (Wingfield *et al.* 2001), have recently been shown to represent *Chrysop. hodgesiana* (Gryzenhout *et al.* 2004). The collections of Seixas *et al.* (2004) from *Tibouchina*

were identified based only on morphological characters and before *Chrysoporthe* was described (Gryzenhout *et al.* 2004) It is thus unclear whether these represent *C. cubensis* or one of the related fungi such as *Chrysop. hodgesiana* or *C. inopina*, described in this study. It is, however, likely that *C. cubensis* occurs on native *Tibouchina* spp. in South America due to the susceptibility of these trees to *Chrysoporthe* spp.

The collection of *C. cubensis* from native *M. malabathricum* supports the view that South East Asia could be an area of origin for *C. cubensis.* The fact that the isolates group in the discrete South East Asian clade of the fungus, however, supports the DNA based view that there are two discrete populations of the fungus. Isolates representing both clades have now been found on native plants (species of *Melastoma*, *Miconia*, *Clidemia* and *Rhynchanthera*) and non native plants (*S. aromaticum*, *Eucalyptus*, *Lagerstroemia*, *Tibouchina*) in two geographically distant parts of the world. Either of these areas could represent the area of origin of *C. cubensis*, or the isolates might represent two discrete taxa that have speciated recently.

Ideally, isolates from various hosts should be included in studies at the population level aimed at determining the world-wide population structure and origin of *C. cubensis sensu lato*. Such studies are, however, frustrated by the difficulty of obtaining sufficient numbers of isolates from native hosts in the various countries where they are known to occur. Although there is substantial phylogenetic support for separating the isolates of the two sub-clades of *C. cubensis* into two taxa, these fungi appear to be morphologically identical. They also share hosts in the two regions in which they occur. We thus believe that a population-based species concept is required if these isolates from the two regions are to be treated as discrete taxa. Such studies will be greatly aided by evidence presented in this study that the type

specimen of *C. cubensis* phylogenetically represents the South American form of the fungus. This was previously unknown and the question could only be resolved with collections of *C. cubensis* from Cuba. These specimens and cultures, including an epitype linked to them, should provide a robust basis for future studies.

It is evident from this study that *C. cubensis* is able to infect plants from at least three different families although all of these are in the *Myrtales*. The families include the *Myrtaceae*, *Melastomataceae* and *Lythraceae* and they represent a wide diversity of morphological and physiological characteristics (Dahlgren & Thorne 1984, Conti *et al.* 1997). Even within the families, *C. cubensis* is able to infect plants belonging to different tribes, e.g. the *Syzygieae* and the *Eucalypteae* in the *Myrtaceae* (Wilson *et al.* 2005), and the *Miconieae* and *Melastomeae* in the *Melastomataceae* (Clausing & Renner 2001). Furthermore, artificial inoculations on members of several additional families in the *Myrtales* have shown that they are also susceptible to infection by *Chrysoporthe* spp., although these have not yet been found as natural hosts (Hodges *et al.* 1986, Seixas *et al.* 2004). These hosts include members of the *Rhizophoraceae*, *Combretaceae*, *Onagraceae*, *Punicaceae*, *Sapotaceae* and *Lauraceae*, which represent distinct phylogenetic groups within the *Myrtales* (Conti *et al.* 1996, Conti *et al.* 1997). Only a limited number of genera in each of these families has been tested (Seixas *et al.* 2004), and it is likely that additional hosts of *C. cubensis* will be discovered.

Phylogenetic analyses in this study have revealed an unexpected and closely related new sub-clade in the larger *Chrysoporthe* group. This is the phylogenetic sub-clade accommodating isolates from *T. lepidota* in Colombia. The distinct grouping of the sub-clade from Colombia is clearly supported with morphological characteristics and we were able to describe the new species *C. inopina*. Fruiting structures of *C.*

inopina contained asci longer that those of *C. cubensis*, and ascospores wider than those for all other species of *Chrysoporthe*.

When *C. inopina* was first discovered, our view was that it might represent the teleomorph of *Chrysop. hodgesiana*, or a first report of *C. cubensis* on *Tibouchina* spp. in South America. This is because no teleomorph is known for *Chrysop. hodgesiana*, nor is *Tibouchina* a confirmed host for *C. cubensis* (Gryzenhout *et al.* 2004). DNA sequences of these isolates and morphological comparisons, however, showed clearly that the fungus represents an undescribed taxon. *C. inopina* thus represents a third species of *Chrysoporthe* on native *Melastomataceae* in Colombia, with the other two species *C. cubensis* (Rodas *et al.* 2005) and *Chrysop. hodgesiana* (Gryzenhout *et al.* 2004, Rodas *et al.* 2005). Of these, both *C. inopina* and *Chrysop. hodgesiana* occur on *Tibouchina* spp. and in the same area. It is thus evident that a complex of species, closely related and morphologically similar, occurs on these native trees in South America. Extreme care should thus be taken when collecting and identifying these fungi, especially where work is related to quarantine and disease management, which is clearly important for fungi such as *C. cubensis*.

Chrysoporthe cubensis was previously known almost exclusively as a *Eucalyptus* canker pathogen, but this and other recent studies (Wingfield *et al.* 2001, Myburg *et al.* 2002b, Gryzenhout *et al.* 2004, Rodas *et al.* 2005) have reported on new and diverse hosts for the fungus. These studies have radically changed our perception of the ecology of *C. cubensis*. Furthermore, besides *C. cubensis*, there are many closely related and morphologically similar species that have previously been treated under the older name as representing *Cry. cubensis*. Some of these species in *Chrysoporthe*, such as *C. cubensis*, *C. austroafricana* and *C. doradensis*, are important pathogens of *Eucalyptus*, but all species appear to be pathogens of members

of the *Myrtales*. They should thus be considered as serious potential pathogens that could cause devastating diseases if they were to be accidentally introduced into new areas with large populations of native, susceptible plants. For example, *C. austroafricana*, apparently native to South Africa (Heath *et al.* 2006), causes a serious disease of *Eucalyptus* in that country and represents a significant threat to native forests of *Eucalyptus* in Australia (Roux *et al.* 2003, Wingfield 2003). These relatively unknown and newly discovered fungi deserve more attention from pathologists and quarantine authorities.

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Table 1. Isolates included in this study.

Species identity	Isolate number[a]	Alternative isolate number[a]	Host	Origin	Collector	GenBank accession numbers[b]
Chrysoporthe cubensis	CMW 1856	–	*Eucalyptus* sp.	Kauai, Hawaii	–	AY 083999, AY 084010, AY 084022
	CMW 9903	–	*Syzygium aromaticum*	Kalimantan, Indonesia	C.S. Hodges	AF 292044, AF 273066, AF 273461
	CMW 11289	CBS 115737	*Eucalyptus* sp.	Indonesia	M.J. Wingfield	AY 214303**,** AY 214231, AY 214267
	CMW 8650	CBS 115719	*S. aromaticum*	Sulawesi, Indonesia	M.J. Wingfield	AY 084001, AY 084013, AY 084024
	CMW 10774	–	*S. aromaticum*	Zanzibar, Tanzania	–	AF 492130, AF 492131, AF 492132
	CMW 2632	–	*E. marginata*	Australia	E. Davison	AF 046893, AF 273078, AF 375607
	CMW 12745	CBS 117837	*Tibouchina urvilleana*	Singapore	M.J. Wingfield	DQ368764, DQ368780, DQ368781
	CMW 17172	CBS 118664	*T. urvilleana*	Thailand	M.J. Wingfield	DQ368765, DQ368782, DQ368783
	CMW 17178	CBS 118665	*T. urvilleana*	Thailand	M.J. Wingfield	DQ368766, DQ368784, DQ368785
	CMW 16192	CBS **	*Melastoma malabathricum*	Sumatra	M.J. Wingfield	DQ368767, DQ368786, DQ368787
	CMW 18515	CBS 118651	*M. malabathricum*	Sumatra	M.J. Wingfield	DQ368768, DQ368788, DQ368789
	CMW 10669	CBS 115751	*Eucalyptus* sp.	Republic of Congo	J. Roux	AF 535122, AF 535124, AF 535126
	CMW 1853	–	*S. aromaticum*	Brazil	–	AF 046891, AF 273070, AF 273465
	CMW 10778	CBS 115755	*S. aromaticum*	Brazil	C.S. Hodges	AY 084006, AY 084018, AY 084030
	CMW 9432	CBS 115724	*E. grandis*	Mexico	M.J. Wingfield	AY 692321, AY 692324, AY 692323
	CMW 12734	CBS 115853	*Rhynchanthera*	Mexico	F. Ferreira	DQ368769, DQ368790, DQ368791

			mexicana			
	CMW 12736	CBS 115847	*R. mexicana*	Mexico	F. Ferreira	DQ368770, DQ368792, DQ368793
	CMW 13046	CBS 115762	*Clidemia sericeae*	Mexico	F. Ferreira	DQ368772, DQ368796, DQ368797
	CMW 12471	CBS 115849	*Cli. sericeae*	Mexico	F. Ferreira	DQ368771, DQ368794, DQ368795
	CMW 14394 (ex-epitype culture designated here)	CBS 118654	*E. grandis*	Cuba	M.J. Wingfield	DQ368773, DQ368798, DQ368799
	CMW 14404	CBS 118647	*E. grandis*	Cuba	M.J. Wingfield	DQ368774, DQ368800, DQ368801
	CMW 16199	CBS 118652	*Lagerstroemia indica*	Cuba	M.J. Wingfield	DQ368775, DQ368802, DQ368803
	CMW 16200	CBS 118650	*L. indica*	Cuba	M.J. Wingfield	DQ368776, DQ368804, DQ368805
	CMW 10639	CBS 115747	*E. grandis*	Colombia	C.A. Rodas	AY 263419, AY 263420, AY 263421
	CMW 10026	–	*Miconia rubiginosa*	Colombia	C.A. Rodas	AY 214294, AY 214222, AY 214258
	CMW 10028	–	*M. rubiginosa*	Colombia	C.A. Rodas	AY 214295, AY 214223, AY 214259
	CMW 9980	–	*Miconia theaezans*	Colombia	C.A. Rodas	AY 214297, AY 214225, AY 214261
	CMW 9993	CBS 115728	*M. theaezans*	Colombia	C.A. Rodas	AY 214298, AY 214226, AY 214262
Chrysoporthe austroafricana	CMW 2113	CBS 112916	*E. grandis*	South Africa	M.J. Wingfield	AF 046892, AF 273067, AF 273462
	CMW 9327	CBS 115843	*Tibouchina granulosa*	South Africa	M.J. Wingfield	AF 273473, AF 273060, AF 273455
	CMW 10192	CBS 118649	*Syzygium cordatum*	South Africa	M. Gryzenhout	AY 214299, AY 214227, AY 214263
Chrysoporthella hodgesiana	CMW 10641	CBS 115854	*Tibouchina semidecandra*	Colombia	R. Arbaleaz	AY 692322, AY 692326, AY 692325

	CMW 9995	CBS 115730	*T. semidecandra*	Colombia	R. Arbelaez	AY956969, AY956977, AY956978
	CMW 10625	CBS 115744	*M. theaezans*	Colombia	C.A. Rodas	AY956970, AY956979, AY956980
Chrysoporthe inopina	**CMW 12727** (ex-type culture designated here)	CBS 118659	*Tibouchina lepidota*	Colombia	R. Arbelaez	DQ368777, DQ368806, DQ368807
	CMW 12729	CBS 118658	*T. lepidota*	Colombia	R. Arbelaez	DQ368778, DQ368808, DQ368809
	CMW12731	CBS 118656	*T. lepidota*	Colombia	R. Arbelaez	DQ368779, DQ368810, DQ368811
Chrysoporthe doradensis	CMW 11286	CBS 115734	*E. grandis*	Ecuador	M.J. Wingfield	AY 214289, AY 214217, AY 214253
	CMW 11287	CBS 115735	*E. grandis*	Ecuador	M.J. Wingfield	AY 214290, AY 214218, AY214254
	CMW 9123	CBS 115717	*Eucalyptus deglupta*	Ecuador	M.J. Wingfield	DQ 224034, DQ 224038, DQ 224039
Cryphonectria parasitica	CMW 1652	CBS 112914	*Castanea dentata*	U.S.A.	–	AF 046902, AF 273075, AF 273468
Cryphonectria nitschkei	CMW 10518	CBS 112919	*Quercus sp.*	Japan	T. Kobayashi	AF 452118, AF 525706, AF 525713
Cryphonectria macrospore	CMW 10463	CBS 112920	*Castanopsis cuspidata*	Japan	T. Kobayashi	AF 368331, AF 368351, AF 368350

[a] CMW = Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria; CBS = Centraalbureau voor Schimmelcultures (Utrecht). Isolates in bold were sequenced in this study.

[b] Accession numbers refer to sequence data of the ITS, β-tubulin 1 (primers Bt1a/1b) and β-tubulin 2 (primers Bt2a/2b) regions respectively.

Table 2. Herbarium specimens used in this study.

Species identity	Herbarium number[a]	Linked isolate[a]	Host	Origin	Collector	Date
Chrysoporthe cubensis	PREM 58788 (epitype designated here)	CMW 14394, CMW 14404	*Eucalyptus grandis*	Santiago de las Vegas, Boyeros Municipality, Havana city Cuba	M.J. Wingfield	Jan. 2004
	PREM 58789	CMW 14378	*E. grandis*	60 km west from Havana, Cuba	M.J. Wingfield	Jan. 2004
	PREM 58790	CMW 14362	*E. saligna*	Parque Metropolitano, Cerro Municipality, Havana city.	M.J. Wingfield	Jan. 2004
	PREM 58791	CMW 14395	*Eucalyptus urophylla*	Road to Havana, Cuba	M.J. Wingfield	Jan. 2004
	PREM 58792	CMW 16199	*Lagerstroemia indica*	Havana city, Cuba	M.J. Wingfield	Jan. 2004
	PREM 58793	CMW 12734, CMW 12736	*Rhynchanthera mexicana*	Mexico	F. Ferreira	2002
	PREM 58794	CMW 12734, CMW 12736	*R. mexicana*	Mexico	F. Ferreira	2002

	PREM 58795	CMW 13046	*Clidemia sericea*	Mexico	F. Ferreira	2002
	PREM 58796	CMW 12471	*C. sericea*	Mexico	F. Ferreira	2002
	PREM 58797	CMW 12745	*Tibouchina urvilleana*	Singapore	M.J. Wingfield	Apr. 2003
	PREM 58798	CMW 16192	*Melastoma malabathricum*	Lake Toba, Aek Nauli, Sumatra	M.J. Wingfield	Feb. 2004
	PREM 58799	CMW 18515	*M. malabathricum*	Lake Toba, Sumatra	M.J. Wingfield	May 2005
Chrysoporthe inopina	PREM 58800 (holotype designated here)	CMW 12727, CMW 12729, CMW 12731	*Tibouchina lepidota*	Pereira, Colombia	R. Arbelaez	Jan. 2003

[a] PREM, National Collection of Fungi (Pretoria); CMW = Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria; CMW = Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria.

Fig. 1. Map of world showing the locations of different collections of *Chrysoporthe cubensis* (distinguishing between two sub-clades), *Chrysoporthe austroafricana*, *Chrysoporthe inopina*, *Chrysoporthe doradensis* and *Chrysoporthella hodgesiana*. Only collections verified with DNA sequence data are shown. Different hosts are also shown for collections of *C. cubensis sensu lato*.

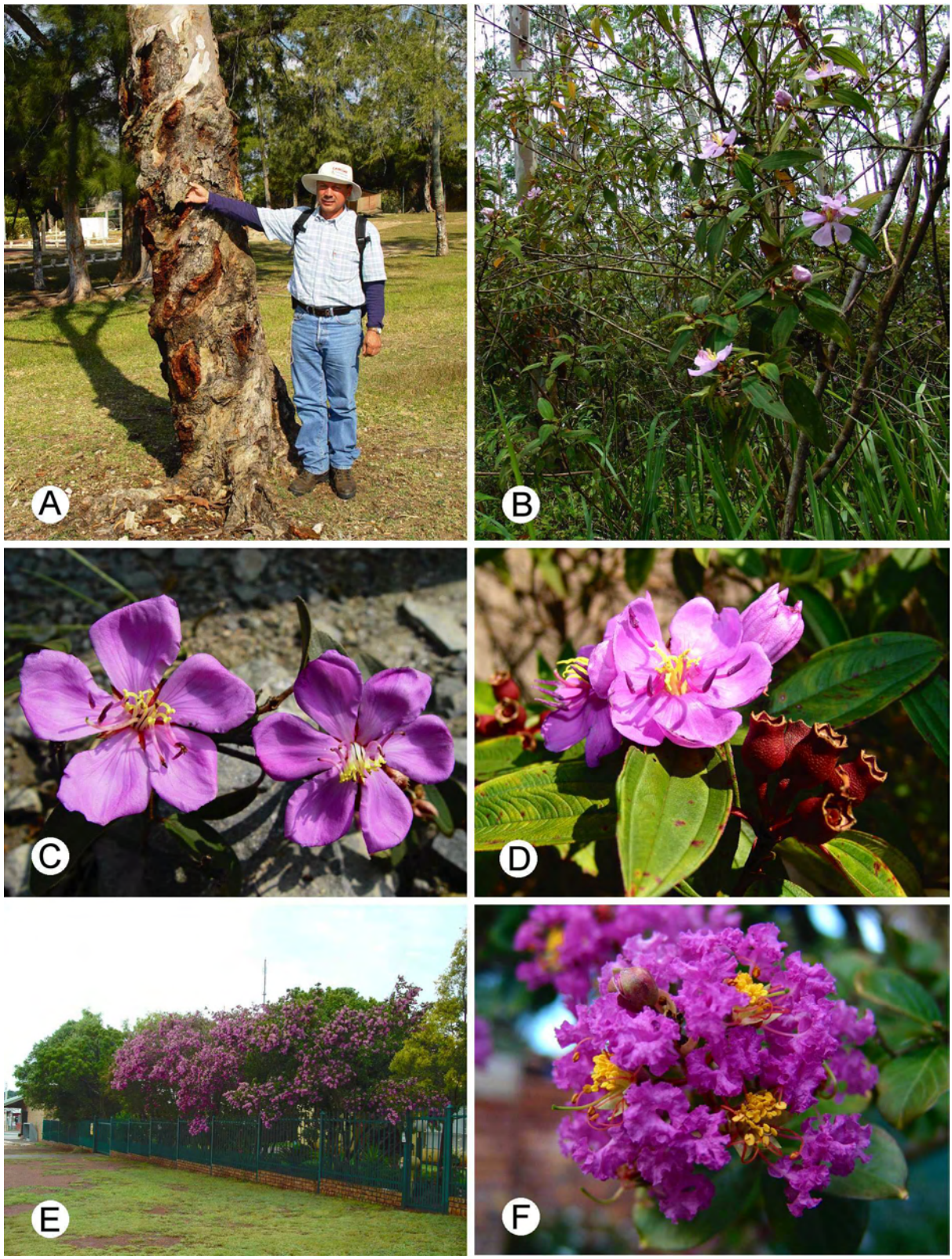

Fig. 2. Different hosts susceptible to *Chrysoporthe cubensis*. A. Basal canker on *Eucalyptus grandis* in Cuba. B. Native *Melastoma malabathricum* trees growing in a plantation in Sumatra. C. Flowers of *M. malabathricum*. D. *Tibouchina urvilleana*. E–F. *Lagerstroemia indica*.

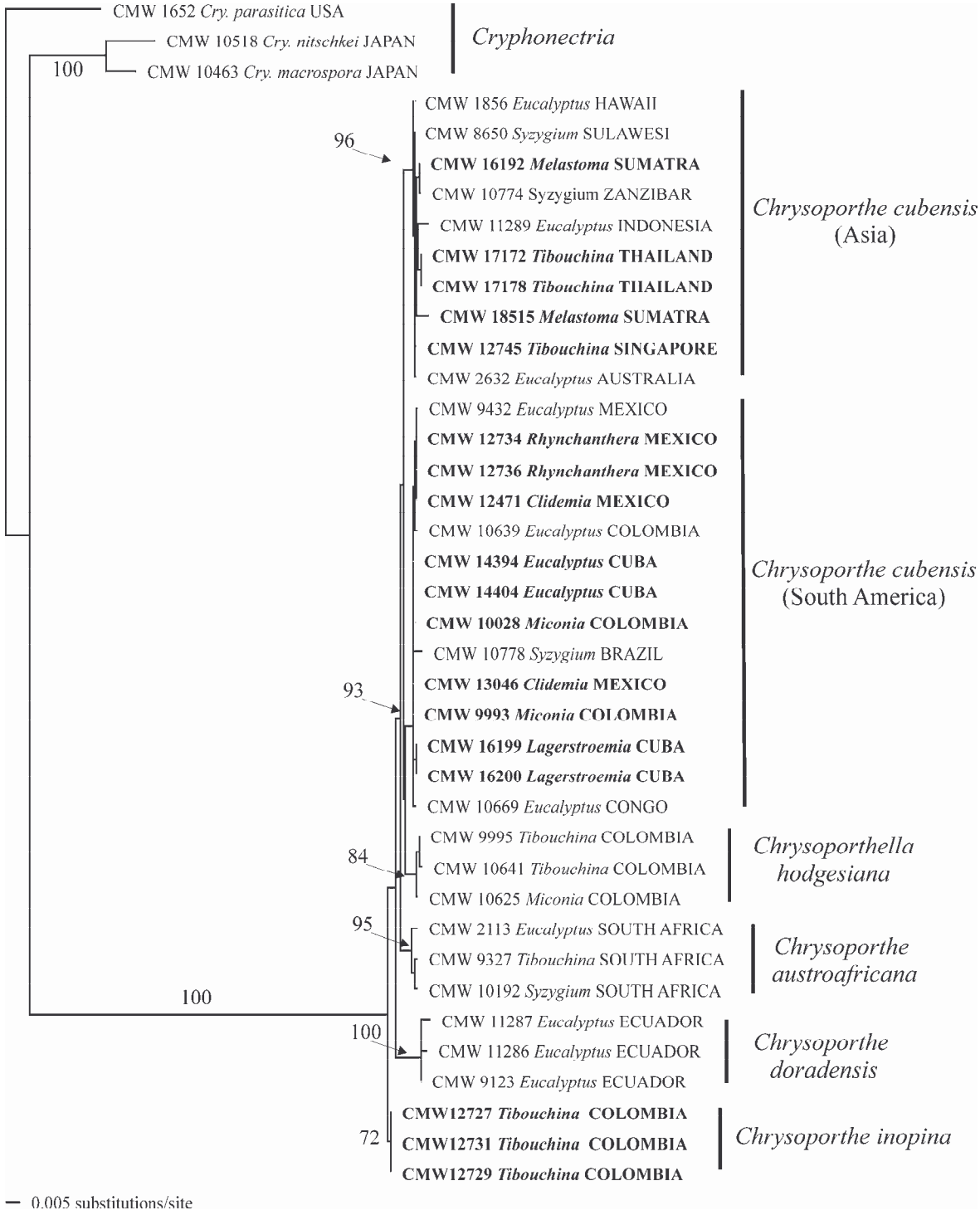

Fig. 3. Phylogram obtained from a combined data set comprising of ribosomal and β-tubulin gene sequences. The phylogram was obtained with distance analyses using the HKY85 parameter model (G = 0.1798, freqA 0.1893, freqC 0.3273, freqG 0.2368, freqT 0.2465, Ti/Tv 2.0064). Confidence levels, determined by a 1000 replicate bootstrap analysis, of the tree branch nodes >70% are indicated. Isolates sequenced in this study are bolded and host species for *Chrysoporthe cubensis* are indicated in italics. *Cryphonectria parastica*, *Cryphonectria nitschkei* and *Cryphonectria macrospora* were defined as an outgroup.

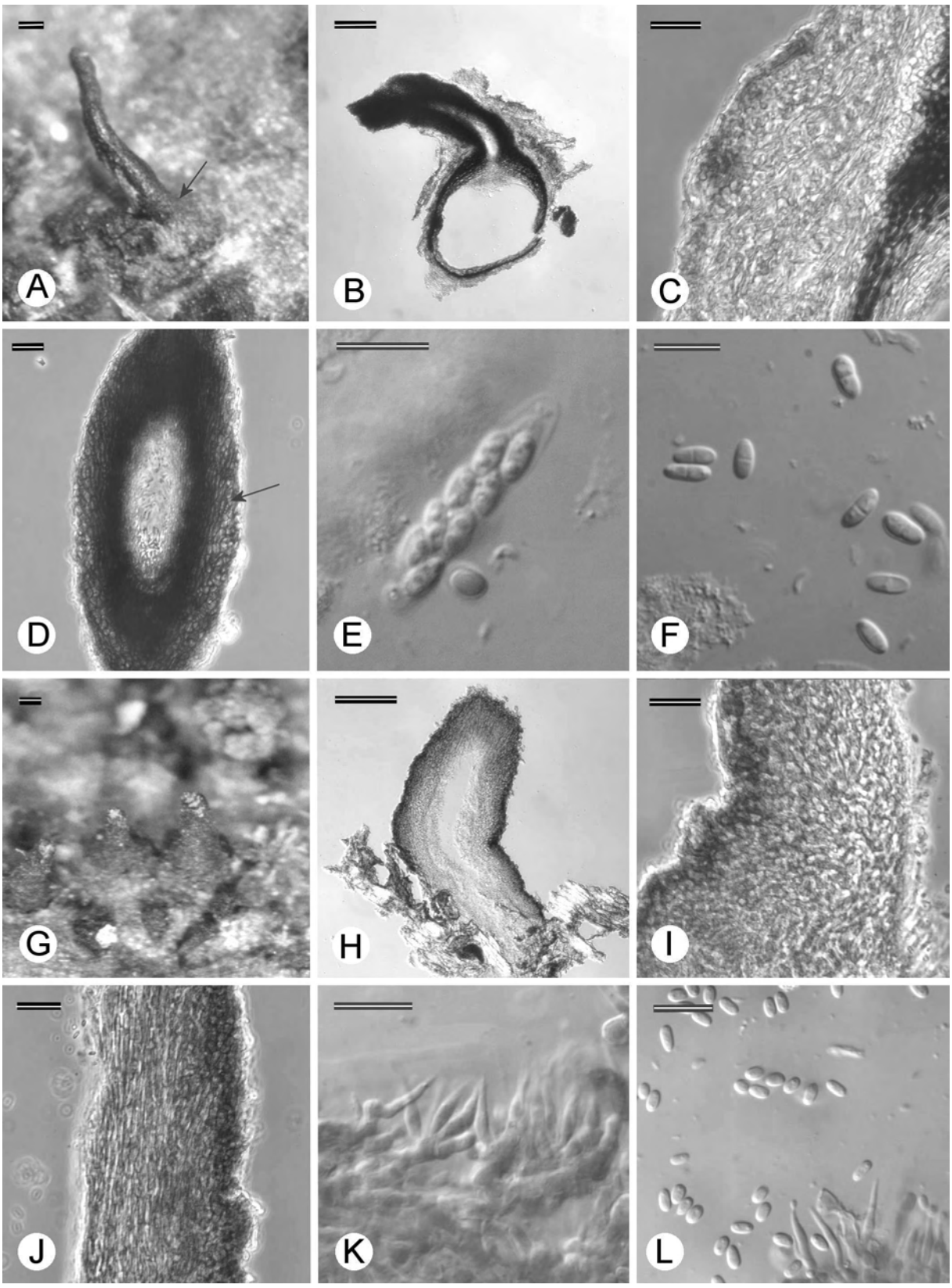

Fig. 4. Light micrographs of *Chrysoporthe inopina* from Colombia (from holotype PREM 58800). A. Black perithecial neck and orange stromatic tissue (arrow) of ascostroma on bark. B. Vertical section through ascoma. C. Stromatic tissue of ascostroma. D. Perithecial neck and surrounding tissue (arrow). E. Ascus. F. Ascospores. G. Conidiomata on bark. H. Vertical section through conidioma. 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.

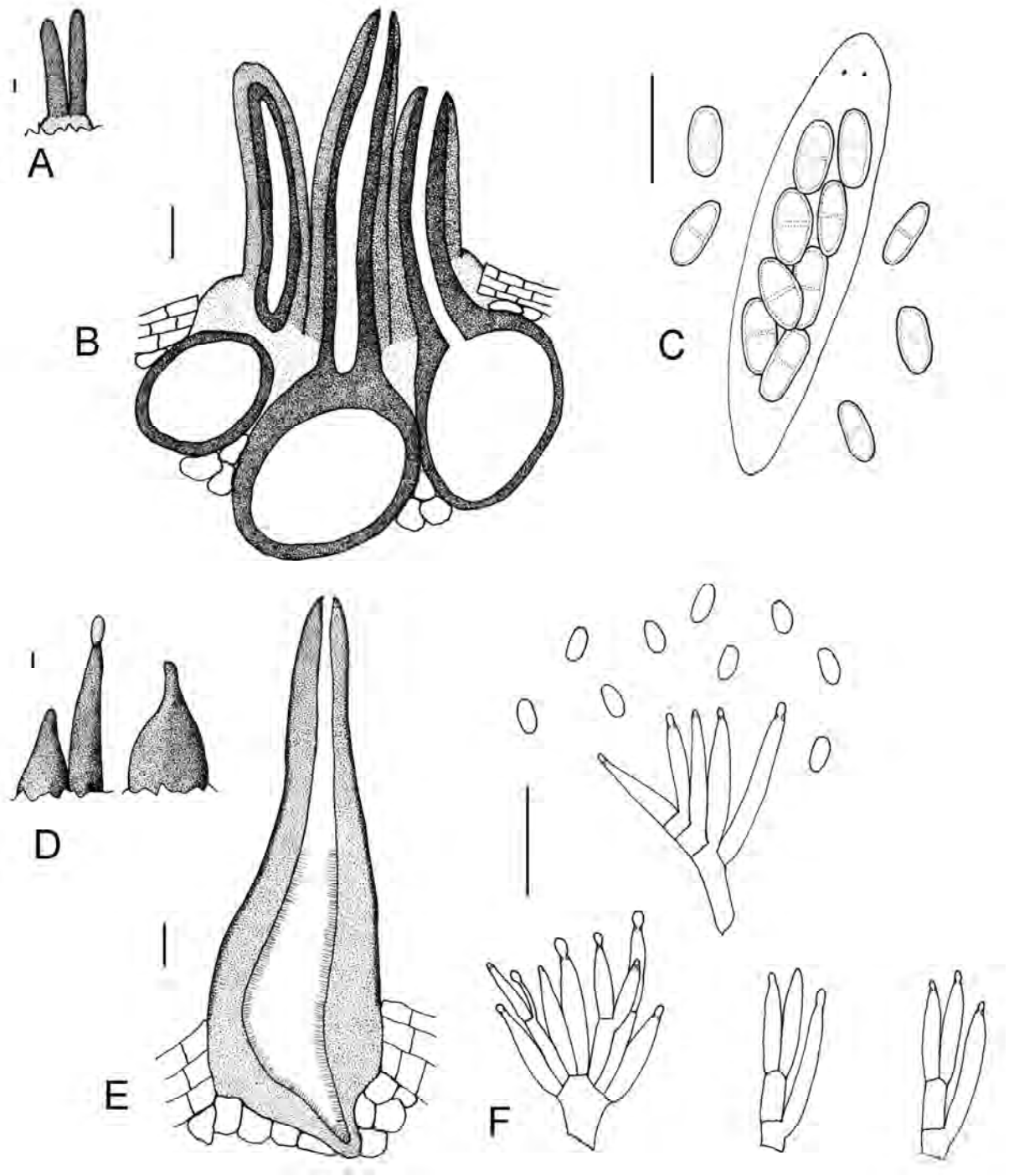

Fig. 5. Line drawings of *Chrysoporthe inopina* (from holotype PREM 58800). A. Shape of ascoma on bark. B. Section through ascoma. C. Asci and ascospores. D. Shapes of conidiomata on bark. E. Section through conidioma. F. Conidiophores, conidiogenous cells and conidia. Scale bars A–B, D–E = 100 µm; C, F = 10 µm.

CHAPTER 4

Cryphonectriaceae (*Diaporthales*), a new family including *Cryphonectria, Endothia, Chrysoporthe* and allied genera

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***Cryphonectriaceae* (*Diaporthales*), a new family including *Cryphonectria, Endothia, Chrysoporthe* and allied genera**

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Abstract. Recent phylogenetic studies on the members of the *Diaporthales* have shown that the order includes a number of distinct phylogenetic groups. These groups represent the *Gnomoniaceae*, *Melanconidaceae*, *Valsaceae*, *Diaporthaceae* and *Togniniaceae*. New groups representing undescribed families have also emerged and they have been referred to as the *Schizoparme*, *Cryphonectria-Endothia* and *Harknessia* complexes. In this study, we define the new family *Cryphonectriaceae* (*Diaporthales*) to accommodate genera in the *Cryphonectria-Endothia* complex. These genera can be distinguished from those in other families or undescribed groups of the *Diaporthales*, by the formation of orange stromatic tissue at some stage of their life cycle, and a purple color reaction in KOH and a yellow reaction in lactic acid associated with pigments in the stromatic tissue or in culture.

Taxonomic novelty: *Cryphonectriaceae* Gryzenh. & M. J. Wingf. fam. nov. nom. prov.

Key words: *Amphilogia*, *Chrysoporthe*, *Cryphonectria*, *Cryphonectriaceae*, *Cryptodiaporthe corni*, *Endothia*, *Rostraureum*

INTRODUCTION

The *Diaporthales* represents a fungal order incorporating approximately 100 genera (Eriksson 2005a). Genera in this order occur on a wide diversity of plant substrates as either saprophytes or parasites (Barr 1978). The parasites include some of the most economically and ecologically important pathogens of trees and agricultural crops. Examples of such a pathogen is *Cryphonectria parasitica* (Murrill) M. E. Barr, which has devastated American chestnut (*Castanea dentata*) populations in North America (Anagnostakis 1987, Heiniger & Rigling 1994), and *Diaporthe phaseolorum* (Cooke & Ellis) Sacc., the causal agent of stem canker of soybeans (Kulik 1984).

Members of the *Diaporthales* are morphologically united by a *Diaporthe*-type centrum (Alexopoulos & Mims 1978, Barr 1978). Morphological characteristics include perithecia with long necks that are located in pseudostromata with no paraphyses, and thick-walled asci that are either evanescent with short stalks or intact (Alexopoulos & Mims 1978, Hawksworth *et al.* 1995). Features such as the presence or absence of stromatic tissue, stromatal tissue type, the position of the perithecia and perithecial beaks relative to the substrate, ascospore shape and ascospore septation have been used to differentiate families and genera in the *Diaporthales* (Barr 1978).

Six families are currently recognised in the *Diaporthales* (Eriksson 2005b). These include the *Diaporthaceae* Höhn. ex Wehm., *Gnomoniaceae* G. Winter, *Melanconidaceae* G. Winter, *Valsaceae* Tul. & C. Tul., *Vialaeaceae* P. F. Cannon and *Togniniaceae* Réblová, L. Mostert, W. Gams & Crous. This classification has largely

emerged from recent DNA sequence comparisons of Castlebury *et al.* (2002), who compared genera representing the families previously recognised in the *Diaporthales.* The *Togniniaceae* is a new family that was described by Réblová *et al.* (2004). The family level status of the *Vialaeaceae*, established by Cannon (Cannon 1995), has not yet been confirmed using DNA sequence data (Castlebury *et al.* 2002).

In addition to the described families, groups not recognised previously were also noted by Castlebury *et al.* (2002). One of these groups includes species of *Schizoparme* Shear and their *Coniella* Höhn. and *Pilidiella* Petr. & Syd. anamorphs, which have been referred to as members of the *Schizoparme* complex (Castlebury *et al.* 2002). The second group included species of *Cryphonectria* (Sacc.) Sacc. and *Endothia* Fr. and this was referred to as the *Cryphonectria-Endothia* complex (Castlebury *et al.* 2002). Species of *Harknessia* M. C. Cooke and allied genera *Dwiroopa* C. V. Subramanian & J. Muthumary and *Apoharknessia* Crous & S. Lee, also formed a group within the *Diaporthales*, although not well supported phylogenetically with the available DNA sequence data (Castlebury *et al.* 2002, Lee *et al.* 2004). This group could not be described as a family since the status of *Wuestneia* Auersw. ex Fuckel as the teleomorph of this coelomycete genus must still be confirmed (Lee *et al.* 2004). Besides these undescribed complexes of species, several species, such as *Greeneria uvicola* (Berk. & M. A. Curtis) Punith., did not group in any of the families or undescribed complexes (Castlebury *et al.* 2002). This suggests that additional groups might emerge in the *Diaporthales* as more species are described or included in phylogenetic comparisons.

Various taxonomic studies considering genus and species delimitation for species of *Cryphonectria* and *Endothia* have been conducted recently (Venter *et al.* 2002, Gryzenhout *et al.* 2004/Chapter 1 in this thesis, Myburg *et al.* 2004a,

Gryzenhout *et al.* 2005a/Chapter 7 in this thesis, 2005b/Chapter 6 in this thesis). These studies have included the recognition of at least four new genera containing species previously placed in *Cryphonectria*. Of these, *Chrysoporthe* Gryzenh. & M. J. Wingf. was described to accommodate the important stem canker pathogen *Cryphonectria cubensis* (Bruner) Hodges and two additional species, *Chrysoporthe austroafricana* Gryzenh. & M. J. Wingf. and the anamorph species *Chrysoporthella hodgesiana* Gryzenh. & M. J. Wingf. (Gryzenhout *et al.* 2004). *Rostraureum* Gryzenh. & M. J. Wingf. was described to include the fungus previously known as *Cryphonectria longirostris* (Earle) Micales & Stipes, and also includes *Rostraureum tropicale* Gryzenh. & M. J. Wingf., a pathogen of *Terminalia ivorensis* A. Chev. trees in Ecuador (Gryzenhout *et al.* 2005a). Another genus, represented by isolates from *Elaeocarpus* spp. in New Zealand and including *Cryphonectria gyrosa* (Berk. & Broome) Sacc., was identified in a study by Myburg *et al.* (2004a) and was subsequently described as *Amphilogia* Gryzenh. & M. J. Wingf. (Gryzenhout *et al.* 2005b). A genus closely related to *Cryphonectria* and *Endothia* and representing isolates from *Syzygium aromaticum* (clove) in Indonesia, was recognised in a study by Myburg *et al.* (2003). This genus was not assigned a name because insufficient herbarium material, linked to isolates, is available.

A collection of isolates representing species of *Cryphonectria* and *Endothia*, as well as those of the newly described genera, has provided the opportunity to substantially expand the LSU DNA sequence data set for the *Cryphonectria-Endothia* complex defined by Castlebury *et al.* (2002). The expanded LSU sequence data set was ultimately used to characterise and describe a family for species and genera in the *Cryphonectria-Endothia* complex of genera. The LSU sequences were also

supplemented with more variable sequences of the ribosomal ITS region and β-tubulin genes, to show infra-familial relationships.

MATERIALS AND METHODS

Isolates studied

Representative isolates for species of *Cryphonectria*, *Endothia*, *Chrysoporthe*, *Amphilogia* and *Rostraureum*, used in previous studies (Myburg *et al.* 2002, 2003, Venter *et al.* 2002, Gryzenhout *et al.* 2004, Myburg *et al.* 2004a, 2004b, Gryzenhout *et al.* 2005a), were included in sequence data analyses (Table 1). Isolates (CMW 10779–10781) that represented the undescribed genus from *S. aromaticum* in Indonesia (Myburg *et al.* 2003), were also included (Table 1).

Isolates of some of the species studied by Castlebury *et al.* (2002) and Zhang & Blackwell (2001) were included. These isolates included *Cryptodiaporthe corni* (Wehm.) Petr. (AR 2814), and isolates of *C. macrospora* (Tak. Kobay. & Kaz. Itô) M. E. Barr (AR 3444) and *C. nitschkei* (G. H. Otth) M. E. Barr (AR 3433) from Siberia, Russia. These were kindly provided for additional analyses by Drs. A. Y. Rossman and L. A. Castlebury (Systematic Botany and Mycology Laboratory, USDA-ARS, Beltsville, Maryland, USA). *Cryptodiaporthe corni* was of interest because it grouped separately from other *Cryptodiaporthe* Petr. species in the *Gnomoniaceae* clade, including the type species *Cryptodiaporthe aesculi* (Fuckel) Petr. (Castlebury *et al.* 2002).

The isolate referred to as *E. eugeniae* (Nutman & F. M. Roberts) J. Reid & C. Booth (CBS 534.82), sequenced by Zhang & Blackwell (2001) and used by Castlebury *et al.* (2002), was acquired from the Centraalbureau voor

Schimmelcultures (CBS), Utrecht, Netherlands. It was necessary to include this isolate because it did not group with the isolate of *Chr. cubensis*, even though it represents a previous synonym of *C. cubensis* (Hodges *et al.* 1986, Micales *et al.* 1987, Myburg *et al.* 2003). The isolate of *C. havanensis* (Bruner) M. E. Barr (CBS 505.63) used in the study of Castlebury *et al.* (2002), has previously been shown (as E40 or CMW 10453) to represent *Chr. cubensis* (Hodges *et al.* 1986, Micales *et al.* 1987, Myburg *et al.* 2004a).

Isolates used in this study (Table 1) are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa. A representative sub-set of these isolates not in other internationally recognised culture collections, is also stored in the culture collection of the Centraalbureau voor Schimmelcutures, Utrecht, Netherlands (Table 1). Background information pertaining to other isolates included in the phylogenetic analyses can be found in the studies of Zhang & Blackwell (2001) and Castlebury *et al.* (2002).

PCR amplification and sequencing

Isolates were grown in Malt Extract Broth [20 g/L Biolab malt extract]. DNA was extracted from the mycelium following the method used by Myburg *et al.* (1999). To characterise the isolates of *Cryptodiaporthe corni* (AR 2814), *C. macrospora* (AR 3444), *C. nitschkei* (AR 3433) and *E. eugeniae* (CBS 534.82), the ITS1, 5.8S and ITS2 regions of the rRNA operon as well as two regions within the β-tubulin gene were amplified using previously described methods (Myburg *et al.* 1999, Myburg *et al.* 2002).

DNA from isolates representing key species of *Cryphonectria*, *Endothia*, *Chrysoporthe*, *Rostraureum*, *Amphilogia* and the undescribed fungi from Indonesia (Table 1), was used to amplify a region of the LSU rDNA gene. Primers pairs ITS3 (White *et al.* 1990) and LR3 (Vilgalys & Hester 1990) were used. The reaction mix used the same reagents and concentrations as those used for the ITS and β-tubulin reactions. PCR conditions were: 95 ºC for 3 min (denature), 30 cycles of 95 ºC for 30 s (denature), 56 ºC for 45 s (anneal), 72 ºC for 1 min (elongation) and a final elongation step of 72 ºC for 4 min. Amplification products were purified using a QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany) and used directly as templates in subsequent sequencing reactions.

Sequencing reactions were as specified by the manufacturers of the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Warrington, United Kingdom). Nucleotide sequence data were generated using an ABI PRISM 3100™ automated DNA sequencer (Perkin-Elmer, Warrington, United Kingdom). The primer pairs used in the respective sequencing reactions were as follows: ITS1 and ITS4 (amplifying the ITS region), Bt1a and Bt1b (amplifying β-tubulin region 1), Bt2a and Bt2b (amplifying β-tubulin region 2), LS1 and LR3 (amplifying LSU rDNA).

The raw sequence data generated for the respective gene regions were edited using Sequence Navigator version 1.0.1 (Perkin-Elmer Applied BioSystems, Inc., Foster City, California) software, exported to PAUP* (Phylogenetic Analysis Using Parsimony) version 4.0b8 (Swofford 1998) and aligned to available sequence data sets. Subsequent phylogenetic analyses were executed using PAUP*.

Analyses of LSU rDNA sequences

The subset of isolates used to generate large subunit ribosomal RNA sequence data for this study included 12 taxa (Table 1). These sequences were aligned with the 650 bp data set of Castlebury *et al.* (2002) obtained from TreeBASE (study accession number S815). This dataset was shown to be sufficient to define the various lineages within the *Diaporthales*, although higher bootstrap values were obtained with a larger dataset (Castlebury *et al.* 2002). Only key taxa representing each lineage and the type species of genera, were retained in the dataset. LSU sequences for additional species from other studies were also added to this database. These included species in the *Schizoparme*-complex derived from Van Niekerk *et al.* (2004); species of *Harknessia*, *Apoharknessia* and *Wuestneia* derived from Lee *et al.* (2004); representatives of the *Togniniaceae* (Réblová *et al.* 2004) and a second *Crypto. corni* isolate sourced from Zhang & Blackwell (2001).

Phylogenetic trees were generated by parsimony and distance analyses. LSU sequence data generated in this study were deposited in GenBank (Table 1) and the datamatrix in TreeBase (SN 2390). Gaps were treated as characters in the parsimony analyses using the NEWSTATE option in PAUP*, and missing in the distance analyses. Parsimony was inferred from TBR swapping algorithms with Multrees inactive and trees randomly added (100 reps). Uninformative characters were excluded and remaining characters were reweighted according to their Consistency Indices (CI) index to reduce the number of trees. A 50% consensus bootstrap analysis was performed with the heuristic search modified by using no branch swapping with the MULTREES option turned off, and only 10 random repeats (Castlebury *et al.* 2002). This was done since bootstrap analysis was inordinately extensive using the parameters defined to generate the trees and could not run to completion (Castlebury

et al. 2002). For the distance analyses, a neighbour-joining tree was generated with the Tamura-Nei (TrNef+I+G) model (Tamura & Nei 1993) with invariable sites (I), Gamma distribution (G) and equal base frequencies was used (I = 0.5726; G = 0.7028; rate matrix 1.0000, 4.2834, 1.0000, 1.0000, 8.9689, 1.0000). These parameters were determined with the program Modeltest version 3.5 (Posada & Crandall 1998).

The probabilities of branches occurring were also tested using Bayesian inference employing the Markov chain Monte Carlo (MCMC) algorithm (Larget & Simon 1999). The program Mr. Bayes vers. 3.1.1 (Huelsenbeck & Ronquist 2001) was used with the following parameters: number of generations = 1000000, sample frequency = 100, number of chains = 4 (1 cold, 3 hot) and a burnin of 1000. Four independent analyses were run, with one of these having 3000000 generations. The likelihood model and settings used were the same as for the distance methods, as determined by Modeltest.

Analyses of ITS rDNA and β-tubulin sequences

The ribosomal DNA (ITS1, 5.8S, ITS2) and β-tubulin sequence data generated in this study were added to already published sequences of other species (Table 1) using the TreeBase sequence matrix (study accession number = S1128, matrix accession number = M1935) from Myburg *et al.* (2004a). Two isolates of *Diaporthe amibigua* Nitschke were used as outgroup, since they are more distantly related members of the same order. The datasets for the two regions of the genome sequenced, were subjected to a partition homogeneity test (Farris *et al.* 1994) to ascertain whether they could be combined in a single sequence dataset in the phylogenetic analyses. Phylogenetic analyses were done using both parsimony and distance methods. All the sequence characters were unordered. Gaps were treated as characters with the

Newstate option in parsimony analyses, and as missing in distance analyses. Parsimony was inferred from heuristic searches, with tree-bisection-reconnection (TBR) and MULTREES options (saving all optimal trees) effective, and trees added randomly (100 repetitions). Uninformative characters were excluded, and remaining characters were re-weighted according to their individual CI to reduce the number of trees.

The distance analysis was done using the Neighbour Joining method and the General Time Reversal model (GTR +I+G) (Rodríguez *et al.* 1990), with G = 1.3390, I = 0.4877, base frequency 0.1929, 0.3348, 0.2315, 0.2409 and rate matrix 0.9638, 2.7348, 1.2919, 1.5236, 3.3995, 1.00. This model was chosen based on likelihood ratio tests performed by Modeltest version 3.5 (Posada & Crandall 1998). The confidence levels of the tree branch nodes were determined by a 1000 replicate bootstrap analysis showing values greater than 70%. Bayesian analyses were made using the same parameters and methodology as those in the LSU analysis, with the exception that the distance settings were those determined for this particular dataset by Modeltest and the long run had 5000000 generations. GenBank accession numbers of sequences generated in this study as well as those from previous phylogenetic studies are listed in Table 1. The resulting dataset and trees have been deposited in TreeBase as SN 2390.

RESULTS

Analyses of LSU rDNA sequences

The LSU sequence data set included 71 taxa, of which *Magnaporthe grisea* (T.T. Herbert) Yaegashi & Udugawa (AB 026819), *Pyricularia grisea* (Cooke) Sacc. (AF

362554), *Gaeumannomyces graminis* (Sacc.) Arx & D. Oliver (AF 362556) and *Gaeumannomyces graminis* (AF 362557) were defined as outgroup taxa. These species do not reside in the *Diaporthales*. The LSU sequence data set consisted of a total of 655 bases of which 468 were constant, 24 were parsimony-uninformative and 163 were parsimony-informative (g1 = –1.1078 after exclusion of uninformative characters). The heuristic search for the MP analyses resulted in 74 trees (tree length = 222.64762 steps, CI = 0.629, RI (Retention Index) = 0.886), which did not differ markedly in the grouping of the major lineages, but differed in branch lengths and the topology within clades. The phylogram obtained with distance analyses (Fig. 2) showed the same lineages, although relationships between the lineages differed. Reasonably high bootstrap values (85%) were obtained for the *Cryphonectria-Endothia* complex in the distance analyses, although bootstrap values were below 50 % for the parsimony analyses. Bayesian analyses showed the same groupings and topology than those obtained in the distance and parsimony analyses with high posterior probability values for the different families (Fig. 2). This included the clade representing the *Cryphonectria-Endothia* complex (posterior probability 74%).

The LSU phylogenetic tree based on our analyses (Fig. 1) was similar to the trees presented by Castlebury *et al.* (2002), although the present study included a substantially greater number of taxa representing the *Cryphonectria-Endothia* complex. These included at least six genera. Inclusion of these additional taxa did not affect the structure of the *Cryphonectria-Endothia* group, which remained a distinct lineage. Other lineages in the phylogram represent the families *Gnomoniaceae*, *Melanconidaceae*, *Valsaceae*, *Diaporthaceae*, *Togniniaceae* and the *Schizoparme* complexes as previously defined (Zhang & Blackwell 2001, Castlebury *et al.* 2002, Réblová *et al.* 2004).

In this study, a core group of *Harknessia* and *Wuestneia* species formed a discrete clade (bootstrap 54%). Species of the closely related genera *Dwiroopa* and *Apoharknessia*, however, did not group in this clade. Some other species such as *G. uvicola, Melanconis desmazieri* Petr. and *Hercospora tiliae* (Pers.) Tul. & C. Tul. retained their groupings separate from the major lineages (Castlebury *et al.* 2002).

Analyses of ITS rDNA and β-tubulin sequences

The dataset consisted of 32 taxa of which the two *D. ambigua* isolates were defined as the outgroup. Results generated with the PHT analyses (P = 0.306) indicated that the rDNA and β-tubulin sequence data sets were significantly congruent and that they could be combined. This is in accordance with the trees of similar topology and having strong support generated from the separate data sets. The aligned ribosomal DNA sequence dataset (566 characters) consisted of 315 constant, 12 parsimony-uninformative and 239 parsimony-informative chararacters (g1 = –0.7185 after exclusion of uninformative characters), and the β-tubulin alignment (955 characters) consisted of 538 constant, 21 parsimony-uninformative and 396 parsimony-informative chararacters (g1 = –0.535 after exclusion of uninformative characters). The combined data set consisted of 1521 characters. The heuristic search resulted in a single most parsimonious tree (tree length = 1223.91668, CI = 0.741, RI = 0.905). Both the distance and Bayesian analyses showed the same grouping of isolates. Exclusion of ambiguously aligned sequences representing the introns in the β-tubulin alignment and the ITS1 regions also resulted in similar trees. The tree obtained using distance analyses was chosen for presentation (Fig. 2).

Phylogenetic analyses based on the ITS region and β-tubulin sequences, showed the same clades as those observed in previous studies (Myburg *et al.* 2003,

2004a, 2004b, Gryzenhout *et al.* 2005a). *Endothia*, *Cryphonectria*, *Chrysoporthe*, *Amphilogia* and *Rostraureum* formed distinct and well-supported clades, while the isolates representing an apparently undescribed genus from clove in Indonesia also formed a discrete group (Fig. 2). The isolate of *E. eugeniae* (CBS 534.82) included in the study of Zhang & Blackwell (2001), grouped in the clade representing this undescribed genus (bootstrap 100%). The isolates of *C. nitschkei* and *C. macrospora* from Russia included in the study of Castlebury *et al.* (2002), grouped with Japanese isolates of *C. nitschkei* in the *Cryphonectria* clade (bootstrap 100%, posterior probability 100%). The isolate of *Crypto. corni* did not reside in any of the clades resulting from the phylogenetic analyses, but grouped closely to them.

Taxonomy

Addition of a more representative taxon set to that analyzed by Castlebury *et al.* (2002) showed that *Cryphonectria*, *Endothia* and closely related genera represent a distinct monophyletic lineage in the *Diaporthales*. This has also been shown based on analyses of a larger LSU sequence data as the one used in this study (Castlebury *et al.* 2002) and it is also supported by easily defined morphological characteristics. These findings provide strong justification for the establishment of a new family in the *Diaporthales*.

Genera in this complex have distinct orange stromatic tissue in the teleomorph state and usually in the anamorph state, which is different from any other species in the *Diaporthales*. Members of this group can also be distinguished from other taxa in the *Diaporthales* by the purple discolouration of the stromatic tissue in 3 % KOH and a yellow colour reaction in lactic acid (Castlebury *et al.* 2002). This discolouration is

due to pigments in the stromatic tissue, often responsible for the orange colour, and that is also produced in culture (Roane 1986, Castlebury *et al.* 2002).

Endothia and *Cryphonectria* represent the oldest and best known names in the group representing these and related fungi. They would thus represent an ideal foundation for a new family name. In the case of *Endothia*, *E. gyrosa*, the type species of the genus, is well characterised morphologically and based on DNA sequences (Shear *et al.* 1917, Roane 1986, Micales & Stipes 1987, Venter *et al.* 2002, Myburg *et al.* 2004a). However, the type specimen of this species is old and only anamorph structures are present on it (Shear *et al.* 1917). An epitype for this species is thus needed (Myburg *et al.* 2004a) and it is not presently available.

The typification of *Cryphonectria* has recently been revised. This was necessary because of nomenclatural problems with *Cryphonectria gyrosa* as type (Myburg *et al.* 2004a, Gryzenhout *et al.* 2005c/Chapter 5 in this thesis), and the fact that this fungus belongs in *Amphilogia* (Myburg *et al.* 2004a, Gryzenhout *et al.* 2005b, 2005c). Hence *Cryphonectria* has been conserved with a new type, *C. parasitica* (Gryzenhout *et al.* 2005c). Due to the importance and notoriety of this fungus, *Cryphonectria* represents an appropriate choice as type for a new family. The following description is thus provided:

Cryphonectriaceae Gryzenh. & M. J. Wingf., **fam. nov., nom. prov.**

Ascostromata subimmersa vel superficialia, textura stromatica aurantiaca, collis peritheciorum cum textura stromatica aurantiaca vel fusconigra tectis. Asci fusoidei. Ascosporae ellipsoideae, fusoideae vel cylindricae, non septatae vel usque ad multiseptatae, hyalinae. Conidiomata eustromatica, subimmersa vel superficialia, aurantiaca vel fusconigra. Cellulae conidiogenae phialidicae. Conidia

perparvula, ovoidea vel cylindrica, non septata, hyalina. Textura stromatica in 3% KOH purpurascit, in acido lactico flavescit.

Ascostromata small to large, erumpent, semi-immersed to superficial, generally with orange stromatic tissue. Perithecia fuscous black to umber, occurring underneath bark surface or superficially in stroma, perithecial necks slender, covered with orange to fuscous-black stromatic tissue. Asci fusoid, aparaphysate, free floating. Ascospores generally ellipsoid to fusoid to cylindrical, aseptate to multiseptate, hyaline. Conidiomata eustromatic, semi-immersed to superficial, pyriform to pulvinate, orange to fuscous black, occasionally occurring in same stroma than perithecia. Conidiogenous cells phialidic, simple or branched. Conidia minute, generally ovoid to cylindrical, aseptate, hyaline. Stromatic tissue colours purple in 3% KOH and yellow in lactic acid.

Typus genus: *Cryphonectria* (Sacc.) Sacc., Syll. Fung. 17: 783. 1905.

DISCUSSION

Results of this study have provided additional evidence to support the establishment of a new family in the *Diaporthales* accommodating species that have previously been treated in the *Cryphonectria-Endothia* complex. Early evidence for the existence of this distinct group was provided in a fundamental study by Castlebury *et al.* (2002), which treated a large number of genera in the *Diaporthales* to delimit family relationships within the order. The aim of the present study was to focus specifically on genera in the *Cryphonectria-Endothia* complex and to include additional isolates, particularly new genera that have recently been assigned to this group. In this way,

we were able to further test the unique nature of the group and to show that it represents a distinct phylogenetic lineage, for which we have now provided family status.

Genera residing in the newly defined *Cryphonectriaceae* can clearly be set aside from other families in the *Diaporthales* based on DNA sequence data, particularly for the LSU region. Their unique nature can also be recognised based on a number of morphological features such as the formation of orange stromata and pigments in the stromatic tissue or in culture (Roane 1986) that can be tested with unique colour reactions in KOH and lactic acid (Castlebury *et al.* 2002). This is similar to the classifications of the *Nectriaceae* within the *Hypocreales*, where one of the distinguishing characteristics of taxa in this family is similar colour reactions in KOH and lactic acid (Rossman *et al.* 1999).

In this study, problems were experienced with low bootstrap support for the *Cryphonectriaceae* in parsimony analyses. Similar low bootstrap support was also shown in parsimony analyses presented by Castlebury *et al.* (2002). These authors, however, showed conclusively that the branch separating the *Cryphonectria-Endothia* complex from the other lineages, was well-supported based on additional distance and Bayesian analyses and a longer DNA sequence dataset. We have confirmed this in our study, where support for the clade representing the *Cryphonectriaceae* was adequately high based on distance and Bayesian analyses.

Analyses of the variable ITS region and β-tubulin genes in the present study have shown that isolates of *Crypto. corni* (AR 2814), *C. macrospora* (AR 3444) and *E. eugeniae* (CBS 534.82) used in the studies of Zhang & Blackwell (2001) and Castlebury *et al.* (2002), represent taxa other than those assigned to them. Thus the *E. eugeniae* isolate was shown to group together with isolates representing an

undescribed genus from clove in Indonesia (Myburg *et al.* 2003). This isolate does not represent *Chr. cubensis*, of which *E. eugeniae* is a synonym to (Hodges *et al.* 1986, Myburg *et al.* 2003). The isolate of *C. macrospora* from Russia represents *C. nitschkei* (Myburg *et al.* 2004b), confirming observations of Vasilyeva (1998) that *C. nitschkei* occurs in Russia, and not only in China and Japan (Myburg *et al.* 2004b).

In the present study, the isolate of *Crypto. corni* treated by Castlebury *et al.* (2002) did not group with any of the isolates of *Cryphonectria*, *Endothia*, *Chrysoporthe*, *Amphilogia* or *Rostraureum*, nor with the isolates from Indonesia that represent an undescribed genus. The fungus does, however, have a position in the *Cryphonectriaceae* based on the LSU sequence data and its orange/yellow stromatic tissue that turns purple in KOH and yellow in lactic acid (Redlin & Rossman 1991, Castlebury *et al.* 2002). This fungus appears to represent an undescribed genus in the *Cryphonectriaceae*, since its morphology does not correspond with any of the genera currently known for this family (Myburg *et al.* 2004a, Gryzenhout *et al.* 2005a, 2005b). For instance, conidiomata of the anamorph, *Myxosporium nitidum* Berk. & Curtis, are fully immersed in the bark and emerge through lenticles as orange, sub-spherical pycnidia (Redlin & Rossman 1991). More detailed studies with additional isolates and specimens of this fungus would be required before a name can be provided for it.

The new family *Cryphonectriaceae* defined in this study includes some of the most serious tree pathogens in the world. Notable examples are the causal agent of chestnut blight *C. parasitica* (Anagnostakis 1987), and *Chr. cubensis*, which is one of the most serious pathogens of plantation-grown *Eucalyptus* spp. (Wingfield 2003). Many other members of the family are also pathogens. For example *R. tropicale* causes cankers on *Terminalia* spp. (Gryzenhout *et al.* 2005a), although it does not

appear to have a large ecological impact. It is likely that additional genera will be discovered that reside in the *Cryphonectriaceae*, as illustrated by the characterisation of the *Crypto. corni* isolate in this study. The description of a new family encompassing *Chrysoporthe*, *Cryphonectria*, *Endothia* and allied genera should facilitate identification and taxonomic studies on these fungi.

ACKNOWLEDGMENTS

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Table 1. Isolates used for the DNA sequence analyses.

Original label name of taxon	Isolate numbers [a]	Additional numbers [a]	Host	Origin	Collector	GenBank Accession numbers [b]	
						ITS, β-tubulin1 and 2	LSU
Chrysoporthe cubensis	CMW 8758	—	*Eucalyptus* sp.	Indonesia	M.J. Wingfield	AF 046898, AF 273068, AF 273463	**AY 194098**
	CMW 2632	—	*Eucalyptus marginata*	Australia	E. Davison	AF 046893, AF 273078, AF 375607	—
	CMW 1853	—	*Syzygium aromaticum*	Brazil	—	AF 036891, AF 273070, AF 273465	—
Cryphonectria havanensis [c]	CMW 10453	E40, CBS 505.63	*Eucalyptus saligna*	Demographic Republic of Congo	—	AY 063476, AY 063478, AY 063480	AF 408339
Chrysoporthe austroafricana	CMW 62	—	*E. grandis*	South Africa	M.J. Wingfield	AF 292041, AF 273063, AF 273458	**AY 194097**
	CMW 2113	CBS 112916	*E. grandis*	South Africa	M.J. Wingfield	AF 046892, AF 273067, AF 273462	—

Chrysoporthella hodgesiana	CMW 9929	—	*Tibouchina urvilleana*	Colombia	C. Rodas, M.J. Wingfield	AF 265656, AF 292036, AF 292039	—
	CMW 10641	CBS 115854	*Tibouchina semidecandra*	Colombia	R. Arbaleaz	AY 692322, AY 692326, AY 692325	—
Rostraureum tropicale	CMW 9972	—	*Terminalia ivorensis*	Ecuador	M.J. Wingfield	AY 167426, AY 167431, AY 167436	**AY 194092**
	CMW 10796	CBS 115757	*T. ivorensis*	Ecuador	M.J. Wingfield	AY 167428, AY 167433, AY 167438	—
Unidentified	CMW 14853	CBS 534.82	*Eugenia aromatica*	Indonesia	S. Mandang	**DQ120759, DQ120763, DQ120764**	AF 277142
	CMW 10780	—	*E. aromatica*	Indonesia	M.J. Wingfield	AY 084008, AY 084020, AY 084032	—
	CMW 10781	CBS 115844	*E. aromatica*	Indonesia	M.J. Wingfield	AY 084009, AY 084021, AY 084033	**AY 194093**
Cryphonectria radicalis	CMW 10436	E14, CBS 165.30	*Quercus suber*	Portugal	B. d'Oliveira	AF 452117, AF 525703, AF 525710	—

	CMW 10455	E42, CBS 238.54	*Q. suber*	Italy	A. Biraghi	AF 452113, AF 525705, AF 525712	**AY 194101**
	CMW 10477	E76, CBS 240.54	*Q. suber*	Italy	A. Biraghi	AF 368328, AF 368347, AF 368347	**AY 194102**
	CMW 10484	E83, CBS 112918	*Q. suber*	Italy	A. Biraghi	AF 368327, AF 368349, AF 368349	—
Cryphonectria parasitica	CMW 7048	E9, ATCC 48198	*Quercus virginiana*	USA	R.J. Stipes	AF 292043, AF 273076, AF 273470	**AY 194100**
	CMW 13749	MAFF 410158, TFM:FPH Ep1	*Castanea mollisima*	Japan	Unknown	AY 697927, AY 697943, AY 697944	—
Cryphonectria nitschkei	CMW 10786		*Quercus* sp.	Japan	M. Milgroom, S. Kaneko	AF 140247, AF 140251, AF 140259	**AY 194099**
	CMW 13742	MAFF 410570, TFM:FPH E19	*Quercus grosseserrata*	Japan	T. Kobayashi	AY 697936, AY 697961, AY 697962	—
	CMW 10527	AR 3433, CBS 109776	*Quercus mongolica*	Russia	L. Vasilyeva	**DQ120761, DQ120767, DQ120768**	AF 408341

Cryphonectria macrospora [c]	CMW 10528	AR 3444, CBS 109764	*Q. mongolica*	Russia	L. Vasilyeva	**DQ120760, DQ120765, DQ120766**	AF 408340
C. macrospora	CMW 10463	E54, CBS 112920	*Castanopsis cuspidate*	Japan	T. Kobayashi	AF 368331, AF 368351, AF 368350	—
	CMW 10914	TFM: FPH E55	*C. cuspidata*	Japan	T. Kobayashi	AY 697942, AY 697973, AY 697974	—
Cryptodiaporthe corni	CMW 10526	AR 2814, CBS 245.90	*Cornus alternifolia*	Maine, USA	S. Redlin	**DQ120762, DQ120769, DQ120770**	AF 408343
Endothia gyrosa	CMW 2091	E13, CBS 112915	*Quercus palustris*	USA	R.J. Stipes	AF 046905, AF 368337, AF 368336	**AY 194114**
	CMW 10442	E27	*Q. palustris*	USA	R.J. Stipes	AF 368326, AF 368339, AF 368338	**AY 194115**
Amphilogia gyrosa	CMW 10469	E67, CBS 112922	*Elaeocarpus dentatus*	New Zealand	G.J. Samuels	AF 452111, AF 525707, AF 525714	**AY 194107**
	CMW 10470	E68, CBS 112923	*E. dentatus*	New Zealand	G.J. Samuels	AF 452112, AF 525708, AF 525715	**AY 194108**

Diaporthe ambigua	CMW 5288	CBS 112900	*Malus domestica*	South Africa	W.A. Smit	AF 543817, AF 543819, AF 543821	—
	CMW 5587	CBS 112901	*M. domestica*	South Africa	W.A. Smit	AF 543818, AF 543820, AF 543822	—

[a] **CMW**, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa; **E**, from the culture collection of Prof. R. J. Stipes (Department of Plant Pathology, Virginia Polytechnic Institute & State University, Blacksburg, Virginia, USA) now housed in the culture collection (CMW) of FABI; **CBS**, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; **MAFF**, Microorganisms Section, MAFF GENEBANK, National Institute of Agrobiological Sciences (NIAS), MAFF Gene Bank, Ibaraki, Japan; **TFM: FPH**, Forestry and Forest Products Research Institute, Danchi-Nai, Ibaraki, Japan, while E or Ep refers to an isolate; **AR**, collection of Dr. A. Rossman, U. S. National Fungus Collections, Systematic Botany and Mycology, Beltsville, USA.

[b] Sequences in bold were derived from cultures in this study. Other sequences were acquired from previous studies as follows: Zhang & Blackwell 2001, Castlebury *et al.* 2002, Myburg *et al.* 2002, Venter *et al.* 2002, Myburg *et al.* 2003, Gryzenhout *et al.* 2004, Myburg *et al.* 2004a, 2004b, Gryzenhout *et al.* 2005a.

[c] The *C. havanensis* isolate represents *Chr. cubensis*, and the *C. macrospora* isolate represents *C. nitschkei*.

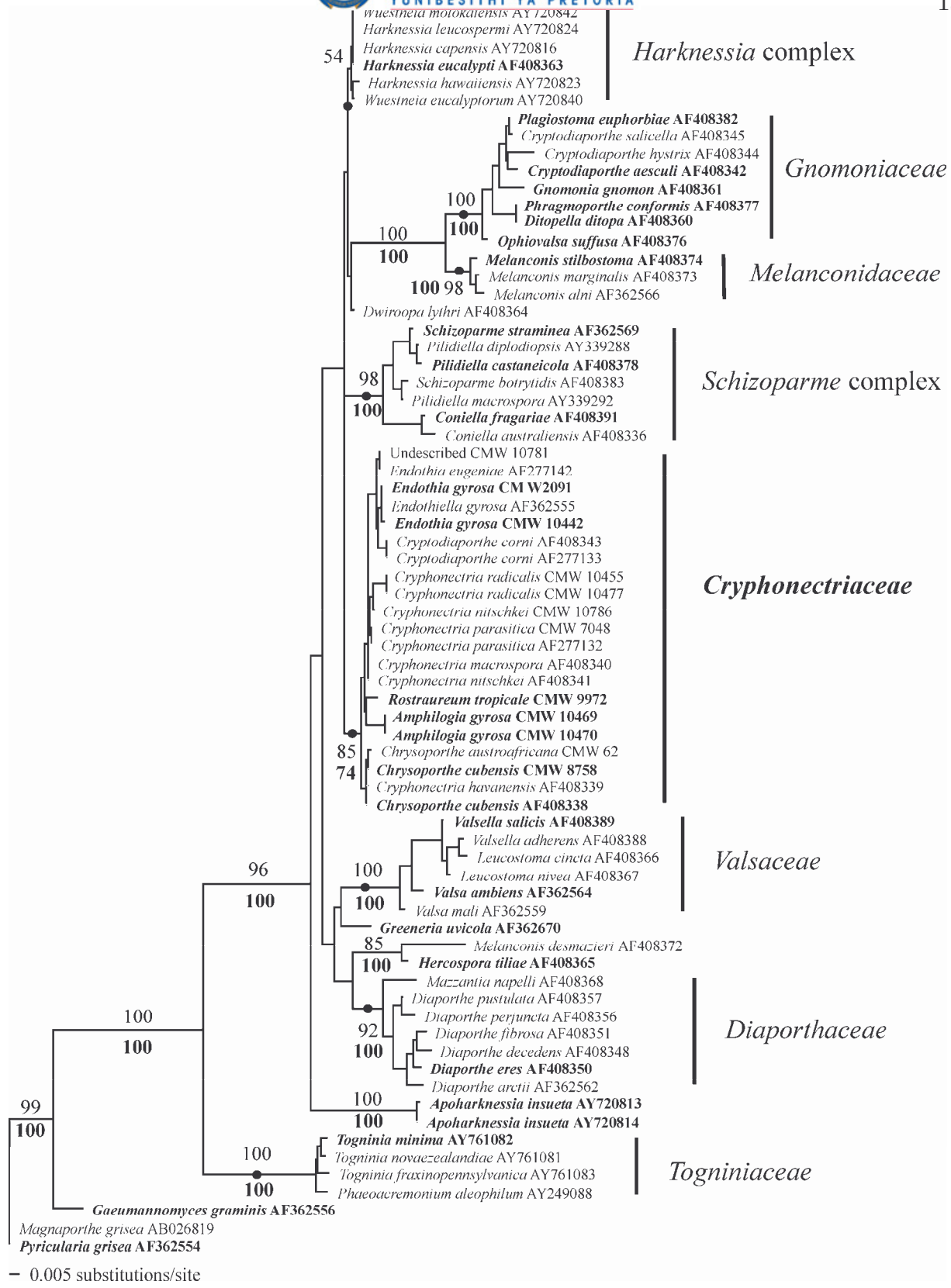

Fig. 1. LSU phylogram based on neighbor-joining analysis of the *Diaporthales*. Taxa in bold represent the type species of the genus. Branches representing families are indicated with dots. Bootstrap values (50%) of only these branches are shown above the branch, with the posterior probabilities given as a percentage in bold typeface. GenBank accession numbers (AB, AF, AY or U) of isolates not sequenced in this study are indicated next to each taxon. The LSU sequence data for *Magnaporthe grisea*, *Pyricularia grisea* and *Gaeumannomyces graminis* generated in the study of Castlebury *et al.* (2002), were used as outgroup taxa to root the LSU phylogenetic tree.

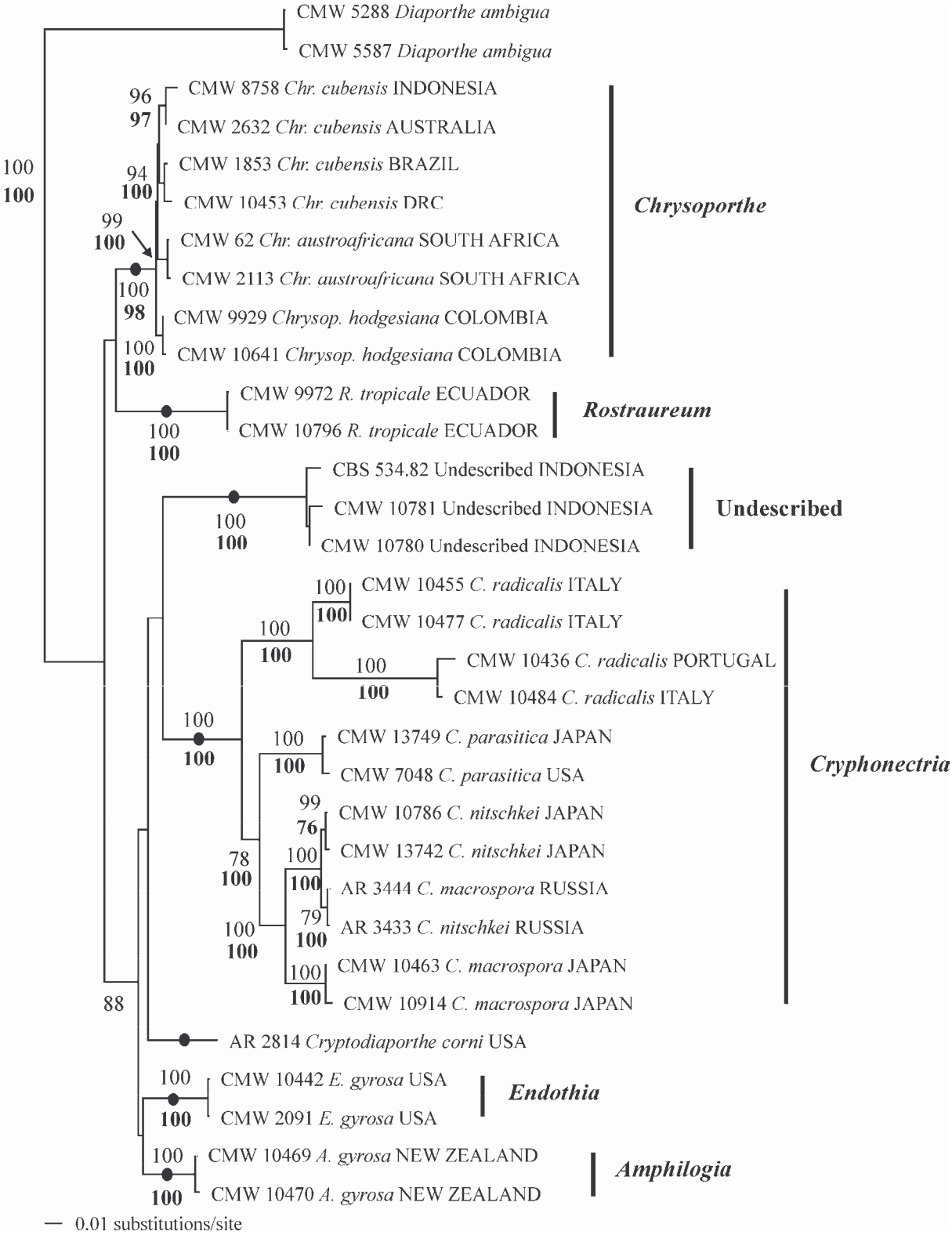

Fig. 2. ITS/β-tubulin phylogram based on neighbor-joining analysis of members of the *Cryphonectriaceae*. Confidence levels of the tree branch nodes are indicated and were determined by a 70% bootstrap analysis (1000 replicates). Posterior probabilities are given as a percentage in bold typeface. Species names in capital letters represent host species. Branches representing genera are accentuated with do[illegible] used as the outgroup.

CHAPTER 5

Proposal to conserve the name *Cryphonectria* (*Diaporthales*) with a conserved type

Gryzenhout M, Glen HF, Wingfield BD, Wingfield MJ (2005). Proposal to conserve the generic name *Cryphonectria* (Sacc.) Sacc. (*Diaporthales*) with a changed type. *Taxon* **54**: 539–540.

(1686) Proposal to conserve the name *Cryphonectria* (*Diaporthales*) with a conserved type

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(1686) ***Cryphonectria*** (Sacc.) Sacc. in Sylloge Fungorum 17: 783. 1905, *nom. cons. prop.*

Typus: *C. parasitica* (Murrill) M. E. Barr (*Diaporthe parasitica* Murrill), *typ. cons. prop.*

The typification of *Cryphonectria* is problematic because the widely accepted choice of *C. gyrosa* (Berk. & Broome) Sacc. as type of the name is not in accord with Art. 7.4 of the ICBN (Greuter *et al.*, Regnum Veg. 138. 2000). *Cryphonectria* was first described as a subgenus of *Nectria* (Fr.) Fr. in 1883 by Saccardo (Syll. Fung. 2: 507. 1883), with two species, *N. abscondita* Sacc. and *N. variicolor* Fuckel, included in this group. Saccardo (in Saccardo & Saccardo, Syll. Fung. 17: 780–781. 1905) raised the subgenus to generic level as *Cryphonectria* (Sacc.) Sacc., including the aforementioned two species as well as *C. gyrosa* (Berk. & Broome) Sacc., *C. moriformis* (Starbäck) Sacc., *C. caraganae* (Höhn.) Sacc. and *C. xanthostroma* (Penz.

& Sacc.) Sacc. Saccardo (l.c., 1905) did not designate a type for *Cryphonectria* but placed *C. gyrosa* first. Von Höhnel (Fragmente zur Mykologie 118: 1479–1481. 1909) designated *C. gyrosa* as the lectotype of *Cryphonectria* specifically because it had been placed first in the list of species recognised by Saccardo & Saccardo (l.c.) ("Als Typus … muß die zuerst angeführte Art … aufgestellt wurden"). This selection is evidently mechanical (Art. 10.5 (b) and *Ex. 7 of the ICBN, Greuter *et al.*, l.c.). Furthermore, it is also incorrect because the species selected was not one of the two original members of *Nectria* subgen. *Cryphonectria* Sacc. When Barr (Mycol. Mem. no. 7: 143. 1978) accepted *C. gyrosa* as the type, she did not treat the two original species of *Nectria* subgen. *Cryphonectria*, namely *N. variicolor* and *N. abscondita*.

Neither of the two original species of *Nectria* subgen. *Cryphonectria* have been examined in recent years. Indeed the type material of *C. abscondita* (Sacc.) Sacc. (PAD, *Wisteria sinensis*) does not contain structures that could be used in morphological studies. The morphology and generic placement of this fungus is thus unknown. Fruiting structures on the type specimens, G 843, FH 843 and B (*Salix triandra*, Oestrich), of *C. variicolor* (Fuckel) Sacc. do not resemble those for *Cryphonectria* species or any other member of the *Diaporthales*, since the ascomata are not stromatic and the perithecia are minute, globose, orange and superficial with striated ascospores. Since the appropriate placement of *C. abscondita* is unknown and *C. variicolor* does not reside in the *Diaporthales*, they are best viewed as taxa of uncertain position and unsuitable as sources of a type. As these are, however, the only candidates for type of *Cryphonectria*, it is, therefore, appropriate (Art. 48 note 2) to propose that the name be conserved with a new type.

Results of a recent taxonomic study (Gryzenhout *et al.*, in Taxon 54: 1009–1021. 2005/ Chapter 6 in this thesis) demonstrate that *C. gyrosa* (Barr, in Mycol.

Mem. no. 7: 143. 1978), widely, though incorrectly, cited as the type of *Cryphonectria*, is generally distinct from most of species currently included in *Cryphonectria*. Furthermore, *C. gyrosa* (K 109807, K 109809, BPI 614797) and its recently recognized allies from New Zealand differ in important characters (cf. Art. 9.17 of the Code) from those in the original description of the genus by Saccardo & Saccardo (l.c.). A separate clade (Myburg *et al.*, in Mycologia: 96: 990–1001. 2004) that includes *C. gyrosa* and a new New Zealand species, is being described separately as a new genus (Gryzenhout *et al.*, l.c.). It would not, therefore, be appropriate to establish *C. gyrosa* as type of *Cryphonectria* by conservation.

By contrast, the proposed type, *C. parasitica* (Murrill) M. E. Barr (in Mycol. Mem. no. 7: 143. 1978) based on *Diaporthe parasitica* Murrill (Torreya 6: 189. 1906), falls within the phylogenetic clade that includes most species of the genus as currently understood (Myburg *et al.*, l.c.). *Cryphonectria parasitica* is one of the most important forest pathogens and has been the subject of intensive studies by scientists including forest pathologists as well as chestnut growers. The name *Cryphonectria* has also been assigned to three important hypoviruses that infect *C. parasitica*, and the condition of reduced virulence caused by these viruses has been most widely studied in *C. parasitica* by virologists and scientists outside plant pathology and mycology. *Cryphonectria parasitica* has been thoroughly characterised based on its phylogenetic relationships and world-wide population structure. Furthermore, ample isolates and herbarium specimens exist for this species, although none of the isolates are directly linked to the type specimen (NY, *Castanea dentata*, Bronx Park, New York, USA, 1905, coll. W. A. Murrill). Its morphological characteristics correspond with those traditionally defined for the genus, and it can thus be chosen instead of one of the alternatives, *C. abscondita* or *C. variicolor*, as type (Art. 10.5). Conservation of

Cryphonectria with *C. parasitica* as type is also strongly justified by the importance of this fungus. Conserving *Cryphonectria* in this way would restrict the usage of the name *Cryphonectria* (Ex. 9 Art. 14.9) to this fungus and close relatives, thus avoiding future changes of its name.

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CHAPTER 6

Amphilogia gen. nov. for *Cryphonectria*-like fungi from *Elaeocarpus* spp. in New Zealand and Sri Lanka

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Amphilogia gen. nov. for *Cryphonectria*-like fungi from *Elaeocarpus* spp. in New Zealand and Sri Lanka

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Abstract. The ascomycete genera *Cryphonectria* and *Endothia* are closely related members of the *Diaporthales*. Recent DNA sequence comparisons have shown that isolates from *Elaeocarpus* spp. in New Zealand, previously identified as *Cryphonectria radicalis* and *Cryphonectria gyrosa*, represent a phylogenetic group distinct from those including other species of *Cryphonectria* and *Endothia*. *Cryphonectria gyrosa* applies to a species that occurs on *Elaeocarpus glandulifer* in Sri Lanka, the same host genus but a different species from which the New Zealand collections were made. The aim of this study was to provide a name for the fungi residing in the phylogenetic group from New Zealand. Morphological characters that define these fungi include superficial conical conidiomata, conidia of variable size and ascospores with one to three septa. These characteristics are not found in other species of *Cryphonectria*. We also recognise a second species in the group from New Zealand that has distinctly larger ascospores. Herbarium specimens of *C. gyrosa* exhibit the same primary characteristics as the specimens from New Zealand and *C. gyrosa* is regarded as conspecific with one of the species in the New Zealand collections. A new

genus, *Amphilogia*, is described for the collections of *C. gyrosa* from Sri Lanka and New Zealand, which also contains the second species from New Zealand, *Amphilogia major* sp. nov.

Taxonomic novelties: *Amphilogia* Gryzenh., Glen & M. J. Wingf. gen. nov., *Amphilogia gyrosa* (Berk. & Broome) Gryzenh., Glen & M. J. Wingf. comb. nov., *Amphilogia major* Gryzenh., Glen & M. J. Wingf. sp. nov.

Key words: *Amphilogia gyrosa*, *Amphilogia major*, *Cryphonectria*, *Cryphonectria gyrosa*, *Diaporthales*, *Elaeocarpus*, New Zealand, Sri Lanka.

INTRODUCTION

The fungal genus *Cryphonectria* (Sacc.) Sacc., as outlined by Barr (1978), includes *Cryphonectria parasitica* (Murrill) M. E. Barr, which is one of the world's most important plant pathogens. This fungus causes the devastating disease known as chestnut blight that completely changed the composition of hardwood forests in the eastern part of Northern America during the first half of the 20th Century (Brewer 1995), and also caused extensive damage in Europe (Anagnostakis 1987, Heiniger & Rigling 1994). Most other species of *Cryphonectria* are either known to be saprobic or their pathogenicity has not been tested. *Cryphonectria radicalis* (Schwein. : Fr.) M. E. Barr occurs in North America, Europe (Shear *et al.* 1917, Roane 1986) and Japan (Kobayashi 1970) primarily on *Fagaceae* (*Fagales*). *Cryphonectria macrospora* (T. Kobay. & Kaz. Itô) M. E. Barr and *Cryphonectria nitschkei* (G. H. Otth) M. E. Barr occur mainly on *Fagaceae* in Japan

(Kobayashi 1970, Roane 1986). *Cryphonectria havanensis* (Bruner) M. E. Barr was first described from *Eucalyptus* spp. in Cuba (Bruner 1916). Reports of this fungus from Japan on *Fagaceae* (Kobayashi 1970, Roane 1986) represent *C. nitschkei*, although it is unclear whether the fungus in Japan and Cuba are the same (Myburg *et al.* 2004a). *Cryphonectria coccolobae* (Vizioli) Micales & Stipes occurs on stems of seagrape (*Coccoloba uvifera* − *Polygonaceae*, *Polygonales*) in the Caribbean (Vizioli 1923).

Other species that have been known in *Cryphonectria* have recently been transferred to new genera. *Cryphonectria longirostris* (Earle) Micales & Stipes is now classified in *Rostraureum* Gryzenh. & M. J. Wingf. (Gryzenhout *et al.* 2005a/Chapter 7 in this thesis). *Chrysoporthe* Gryzenh. & M. J. Wingf. has been described to accommodate *Cryphonectria cubensis* (Bruner) Hodges (Gryzenhout *et al.* 2004/Chapter 1 in this thesis). *Cryphonectria eucalypti* M. Venter & M. J. Wingf. is suspected to be distinct from *Cryphonectria sensu strico* (Myburg *et al.* 2004b) although this question has not been fully resolved.

Endothia Fr. is a genus that resembles *Cryphonectria* morphologically and the taxonomy of these two genera has been confused. Both *Endothia* and *Cryphonectria* have orange, well-developed stromata and similar anamorphs (Shear *et al.* 1917, Kobayashi 1970, Roane 1986), but *Endothia* has aseptate ascospores and large superficial stromata, while *Cryphonectria* has one-septate ascospores and semi-immersed stromata (Barr 1978, Micales & Stipes 1987, Venter *et al.* 2002, Myburg *et al.* 2004b). *Cryphonectria*, established in 1905 (Saccardo & Saccardo 1905), was treated as a synonym of *Endothia* from 1909 onwards (Von Höhnel 1909, Shear *et al.* 1917, Kobayashi 1970). In 1978, Barr suggested that the two genera should be treated as distinct (Barr 1978, Micales & Stipes 1987). Recent phylogenetic studies based on DNA sequence data, have supported this

separation, but they have also shown that these two genera are closely related (Zhang & Blackwell 2001, Castlebury *et al.* 2002, Venter *et al.* 2002, Myburg *et al.* 2004a, 2004b).

Cryphonectria was first described as a subgenus of *Nectria* (Fr.) Fr. (Saccardo 1883), with two species, *Nectria variicolor* Fuckel and *Nectria abscondita* Sacc., listed. *Cryphonectria gyrosa* (Berk. & Broome) Sacc. was listed as *Nectria gyrosa* Berk. & Broome under another subgenus, *Nectria* "subg. *Eunectria* Sacc." (Saccardo 1883). When *Cryphonectria* was elevated to genus level (Saccardo & Saccardo 1905), *Cryphonectria abscondita* Sacc., *Cryphonectria variicolor* (Fuckel) Sacc., *Cryphonectria gyrosa* and three other species, namely *Cryphonectria moriformis* (Starbäck) Sacc., *Cryphonectria caraganae* (Höhn.) Sacc. and *Cryphonectria xanthostroma* (Penz. & Sacc.) Sacc. were included. No type was designated, and the species were listed numerically with *C. gyrosa* placed first in the list (Saccardo & Saccardo 1905). Von Höhnel (1909) reduced *C. gyrosa* (Berk. & Broome) Sacc. to synonymy with *Endothia gyrosa* (Schwein. : Fr.) Fr., the type of *Endothia* (Fries 1849). He also chose *C. gyrosa* as type of *Cryphonectria* because it was listed first, thereby reducing *Cryphonectria* to synonymy with *Endothia*.

The lectotypification of *Cryphonectria* by Von Höhnel (1909) was "based on a largely mechanical method of selection" (Art. 10.5 & *Ex. 7 of the ICBN, Greuter *et al.* 2000), and hence supersedable. Furthermore, it is unacceptable because *C. gyrosa* was not one of the original species of *Nectria* subg. *Cryphonectria* upon which the generic name was based. Von Höhnel's incorrect typification was, however, accepted by Barr (1978), but his synonymy of *C. gyrosa* with *E. gyrosa* was rejected based on differences in ascospore and stromatal morphology between these two genera. It is possible that Von Höhnel based his synonymy, which confirmed observations by Petch (1907), on comparisons of *C. gyrosa* with European *C. radicalis* specimens, a species which was at that time regarded as synonymous with *E. gyrosa*, as summarised by Shear *et al.* (1917). *Cryphonectria gyrosa* has been

erroneously cited as type of the generic name *Cryphonectria.* Since the only valid candidates for type, namely *C. abscondita* and *C. variicolor*, were either unidentifiable or not diaporthalean, conservation of the generic name *Cryphonectria* with *C. parasitica* as type has been proposed (Gryzenhout *et al.* 2005b/Chapter 5 in this thesis).

Cryphonectria gyrosa was first described from Sri Lanka (Berkeley & Broome 1875, Shear *et al.* 1917) and is associated with *Elaeocarpus* spp. Two specimens are connected to the first description of *C. gyrosa* (Berkeley & Broome 1875), but both with hosts unknown. The type specimen (K 109807, originally #638) is from a twig from an unknown locality in Sri Lanka. The second specimen (K 109809, originally #290) was collected at 6000 feet (1850 m) in Nuwara (Mount) Eliya, Sri Lanka. A third collection of specimens (BPI 614797, BPI 614526), believed to represent the same fungus as those connected to the earlier description of *C. gyrosa*, was obtained by Shear *et al.* (1917) when they redescribed this fungus as a new species, *Endothia tropicalis* Shear & N. E. Stevens. The latter species was described to rectify, what the authors believed was an erroneous synonymy with *E. gyrosa* (Shear *et al.* 1917) introduced by Von Höhnel (1909). These specimens (BPI 614526, BPI 614797, both as number 2807) were collected from Hakgala, Sri Lanka, on *Elaeocarpus glandulifer* Mast. and were used as the type specimens for *E. tropicalis* (Shear *et al.* 1917). A report of *C. gyrosa* on *Elaeagnus* (Barr 1978) actually represents *Elaeocarpus* (Myburg *et al.* 2004b). Specimen K 109809 was mentioned by Shear *et al.* (1917) in their description of *E. tropicalis*, but the type specimen, K 109807, of *C. gyrosa* was not considered. There are no cultures linked to the original description of *C. gyrosa* or any more recent collections of the fungus from Sri Lanka.

A recent phylogenetic study including all available isolates of *Endothia* and *Cryphonectria* spp. (Myburg *et al.* 2004b), has shown that, besides the strongly resolved

clades representing *Endothia* and *Cryphonectria*, additional and distinct groups exist. One of these represents species of the newly described genus *Chrysoporthe*, which includes the *Eucalyptus* canker pathogen previously known as *C. cubensis* (Gryzenhout *et al.* 2004). Isolates from *Elaeocarpus* spp. (*Elaeocarpaceae, Oxalidales*) in New Zealand that were labeled as *C. radicalis* and *C. gyrosa* (= *Endothia tropicalis*), respectively, formed the other group.

The aim of this study was to provide a name for specimens linked to isolates from New Zealand, which have been shown to be distinct from *Cryphonectria* based on DNA sequence comparisons (Myburg *et al.* 2004b). The isolates from New Zealand identified as *C. gyrosa* were collected from cankers on the roots of *Elaeocarpus hookerianus* Raoul and *Elaeocarpus dentatus* Vahl (Gilmour 1966, Dingley 1969, Pennycook 1989) that occur on both the North and South Islands of New Zealand (Fig. 1). We have also considered whether specimens labeled as *C. gyrosa* from New Zealand represent the same fungus as that known as *C. gyrosa* from Sri Lanka.

MATERIALS AND METHODS

Morphological comparisons

Herbarium specimens, including fruiting structures linked to isolates from *Elaeocarpus* spp. in New Zealand and recognized by Myburg *et al.* (2004b) as representing a discrete species, were obtained from various herbaria (Table 1). These specimens had been collected from a number of locations in New Zealand (Fig. 1). Cultures are not available for most of these collections. Specimens from Sri Lanka representing *C. gyrosa*, as well as other species of *Cryphonectria* and *Endothia*, were also included (Table 1).

Fruiting structures and surrounding bark were removed from the specimens. These were rehydrated in boiling water for 1 min, mounted in Leica mountant (Setpoint Premier, Johannesburg, South Africa) and sectioned at 12–18 µm thickness, with a Leica CM1100 cryostat (Setpoint Premier) at –20 ºC. The mountant was removed in water and the sections were transferred to lactophenol. Sections of perithecial bases and conidial locules were also made by hand and mounted in lactophenol or 3% KOH for further study. Twenty measurements were taken of conidia, conidiophores, asci and ascospores from each specimen, but fifty measurements were taken from the holotype specimens. Measurements were made using an HRc Axiocam digital camera and Axiovision 3.1 software (Carl Zeiss Ltd., Germany).

Colony growth of isolates CMW 10469 and CMW 10471 (Table 1), identified as *C. radicalis* and *C. gyrosa* respectively, but residing in the unique phylogenetic clade characterised by Myburg *et al.* (2004b), was studied on MEA (20 g/l malt extract, 15 g/L agar [Biolab, Merck, South Africa]). CMW is the culture collection of the Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa, 0002. Growth was observed in the dark at five temperatures ranging from 15 to 35 ºC at 5 ºC intervals. A disc 6 mm diam, taken from actively growing cultures, was placed at the center of four 90 mm plates for each isolate and at each temperature. Two diameter measurements (mm), perpendicular to each other were taken for each plate daily and the means of the eight measurements for each isolate were compared. The experiment was terminated after six days, when the colonies completely covered the plates at the optimum temperature for growth.

RESULTS

Morphological comparisons

Measurement of ascospores on specimens from New Zealand could be used to recognize two distinct species in this study. Herbarium specimens (NY 31874, PDD 32619) from which isolates CMW 10469, CMW 10470 and CMW 10471 originated and that formed the phylogenetic group described by Myburg *et al.* (2004b), have ascospores (9–)9.5–11.5(–12) µm long. The majority of specimens from *Elaeocarpus* spp. in New Zealand (Table 1) have ascospores falling within this range [(7–)8.5–11(–13.5) µm long]. Specimens PDD 20056 and PDD 28490, however, have distinctly longer ascospores [(10.5–)11.5–14(–15.5) µm]. Ascospores of these two specimens often also have one to three septa (Figs 4E, 5C), whereas ascospores for the other specimens have only one or two septa (Figs 2E, 3C). There are no isolates connected to the specimens with larger ascospores and their phylogenetic position cannot be resolved, although they are morphologically similar to those for which isolates are available in other respects than ascospore morphology.

Myburg *et al.* (2004b) previously found that specimens from *E. dentatus* and *E. hookerianus* in New Zealand differ from *Cryphonectria* and *Endothia* spp. Ascospores of the New Zealand specimens have one to three septa in irregular positions (Figs 2E, 3C, 4E, 5C). These are different from ascospores of *Cryphonectria* species that typically have one median septum (Kobayashi 1970, Roane 1986, Myburg *et al.* 2004b). Conidia are often variable in size (Figs 2K–L, 3F, 4K–L, 5F), ranging from 3–12 µm in length, whereas conidia of *Cryphonectria* are generally more uniform in size, ranging from 2–5 µm (Kobayashi 1970, Roane 1986). Conidiomata of the New Zealand specimens are typically superficial, conical to fluted (Figs 2F, 3D, 4F, 5D), although conidial locules can also be observed inside stromata that contain perithecial necks. This is different from

Cryphonectria species, which have semi-immersed, pulvinate conidiomata (Kobayashi 1970, Venter *et al.* 2002, Myburg *et al.* 2004b). Furthermore, ascostromata on the New Zealand specimens are pulvinate and erumpent with perithecia formed in a diatrypoid orientation (Figs 2B, 3B, 4B, 5B). This is more similar to stromata of *Endothia*, but *Endothia* spp. have aseptate, cylindrical ascospores (Shear *et al.* 1917, Kobayashi 1970, Venter *et al.* 2002, Myburg *et al.* 2004b) that can easily be distinguished from those of the structures on *Elaeocarpus* spp.

Careful study of the specimens (K 109807, K 109809) linked to the original description of *C. gyrosa* from Sri Lanka, revealed that the structures originally described for *C. gyrosa* are identical to those on the specimens linked to the description of *E. tropicalis* (BPI 614797, BPI 614256, BPI 797701). The type specimen of *C. gyrosa* (K 109807), however, contains few recognizable structures, and only a few of these structures could be used. Structures on the *C. gyrosa* specimens from Sri Lanka also had a morphology identical to specimens from *Elaeocarpus* spp. in New Zealand, which have previously been assigned the name *C. gyrosa*. Ascospores of the Sri Lankan fungus were generally one-septate, but ascospores with two irregularly spaced septa were found in all three specimens (Figs 6E–F). Ascospores of the *C. gyrosa* specimens from Sri Lanka [(7–)8–9.5(–11.5) µm long] overlapped in size with those of the group from New Zealand with smaller ascospores [(7–)8.5–11(–13.5) µm long]. Specimens BPI 614797 [(4–7(–10) µm long] and K 109809 [4.5–10(–14) µm long], also had conidia (Fig. 6L) that fell within the size range [(3–)4–8.5(–12) µm long] of the specimens for both groups of fungi from New Zealand. Ascostromata on specimens BPI 614797 and K 109807 representing the Sri Lankan fungus (Figs 6A–B), were identical to those of structures on New Zealand specimens, and specimen BPI 614797 contained conical conidiomata (Figs 6G–H) similar to those found on New Zealand specimens. Specimens representing the Sri Lankan fungus could thus not be distinguished

from those originating in New Zealand, connected to isolates that represent a phylogenetic group separate from *Cryphonectria* (Myburg *et al.* 2004b) and with ascospores (7–)8.5–11(–13.5) µm long.

DISCUSSION

Results of this study and Myburg *et al.* (2004b) show that specimens from *Elaeocarpus* spp. in New Zealand, which were previously considered to be *Cryphonectria* spp., are morphologically distinct from other species now classified in *Cryphonectria*. These specimens are connected to isolates that Myburg *et al.* (2004b) showed to be phylogenetically distinct from other *Cryphonectria* spp. Furthermore, our examination of a large collection of specimens from New Zealand indicates that the specimens from New Zealand represent two morphologically related but distinct species. Cultures are available for only one of the species and the phylogenetic relatedness of the two species cannot be considered at this time. However, based on morphology, they can justifiably be treated in the same genus.

The most obvious characteristics defining the two fungi from *Eleaocarpus* in New Zealand as distinct from *Cryphonectria* are ascospores that have up to three septa. This was previously noted for specimen PDD 20056 by Roane (1986). Conidiomata are also different from those found in species of *Cryphonectria* (Shear *et al.* 1917, Micales & Stipes 1987, Myburg *et al.* 2004b). These are typically superficial on the host tissue and are conical to fluted. In contrast, other species of *Cryphonectria* have ascospores with one septum, and conidiomata that are semi-immersed and pulvinate (Myburg *et al.* 2004b).

Specimens of *C. gyrosa* from Sri Lanka, including the type specimen and the type specimen of *E. tropicalis* previously treated as a synonym of *C. gyrosa*, were

indistinguishable from the New Zealand collections from *Elaeocarpus*. They have ascospores of the same size and with one to two septa, although this form of septation has not previously been noted for *C. gyrosa* (Berkeley & Broome 1875, Saccardo & Saccardo 1905, Shear *et al.* 1917, Barr 1978, Roane 1986, Micales & Stipes 1987). Furthermore, conidia are variable in size and fall within the same range as those of specimens from New Zealand. Conidiomata also have a conical to pyriform shape, similar to the New Zealand fungus and different from the pulvinate structures of *Cryphonectria* spp. (Myburg *et al.* 2004b). We conclude that *C. gyrosa sensu stricto* most likely will group in the distinct phylogenetic clade representing the New Zealand specimens as defined by Myburg *et al.* (2004b).

A proposal to conserve the generic name *Cryphonectria* with a conserved type (Gryzenhout *et al.* 2005b), showed that the extensive citation of *C. gyrosa* as type of *Cryphonectria* is contrary to Art. 7.4 of the ICBN (Greuter *et al.* 2000). Since *C. gyrosa* is not eligible as type, it will have no effect on the name *Cryphonectria* if *C. gyrosa* is transferred to another genus. The fungus known as *C. gyrosa* from Sri Lanka and the specimens from *Elaeocarpus* spp. in New Zealand are thus described in a new genus that is closely related to *Cryphonectria*. *Cryphonectria gyrosa* and the specimens with smaller ascospores from New Zealand are treated as one species, the name of which is designated as the type of the new generic name. A second species from *Elaeocarpus* in New Zealand with larger ascospores, is also recognized. A name is provided for this fungus even though cultures and thus DNA sequence data are not available for it. Description of the new genus for the fungus previously known as *C. gyrosa* and the second new species, are provided below. A key facilitating the identification of the new genus and the species residing in it is also provided.

Amphilogia Gryzenh., Glen & M. J. Wingf., **gen. nov.**

Etymology. Greek, *amphi,* on both sides, and *logos*, discussion, thus the Greek personification of disputes; referring to the dispute this genus caused regarding the identity of *Cryphonectria.*

Ascostromata aurantiaca, erumpentia, subimmersa vel superficialia, textura stromatica bene evoluta, pulvinata, collis peritheciorum papillatis vel longis. *Asci* octospori, fusoidei. *Ascosporae* hyalinae, fusoideae vel ellipsoideae, uno vel tribus septis irregulariter dispositis divisae.

Conidiomata aurantiaca, discreta vel super ascostromata, interdum etiam velut loculi in ascostromate videntur, superficialia, conica vel pyriformia vel striata, unilocularia. *Conidiophora* hyalina cum *cellulis conidiogenis* phialidicis apicalibus vel lateralibus in ramis sub septo oriundis. *Conidia* hyalina, aseptata, oblonga vel subfalcata, magnitudine variabili.

Ascostromata orange, erumpent, slightly immersed to superficial, stromatic tissue well-developed, pulvinate with papillate to long orange perithecial necks, perithecia diatrypoid. *Asci* 8-spored, fusoid. *Ascospores* hyaline, fusoid to ellipsoid, containing one to three irregularly spaced septa.

Conidiomata orange, separate or on top of the ascostromata, also evident occasionally as locules inside ascostroma, superficial, conical to pyriform to fluted, unilocular. *Conidiophores* hyaline with phialidic, irregular branching, determinate *conidiogenous cells* that occur apically or laterally on branches beneath a septum. *Conidia* hyaline, non-septate, oblong to slightly curved, of variable size.

Typus: *Amphilogia gyrosa* (Berk. & Broome) Gryzenh., Glen & M. J. Wingf. 2005.

Amphilogia gyrosa (Berk. & Broome) Gryzenh., Glen & M. J. Wingf., **comb. nov.,** Figs 2–3, 6.

≡ *Diatrype gyrosa* Berk. & Broome, J. Linn. Soc. London 14: 124. 1875.

≡ *Nectria gyrosa* Berk. & Broome, J. Linn. Soc. London 15: 86. 1877.

≡ *Cryphonectria gyrosa* (Berk. & Broome) Sacc., Syll. Fung. 17: 784. 1905.

≡ *Endothia gyrosa* (Berk. & Broome) Höhn., Sitzb. Kais. Akad. Wiss. Wien, Math. Naturw. Kl. 118: 1480. 1909, nom. illegit. Art. 53, non (Schwein. : Fr.) Fr.

Holotype. Sri Lanka. 1868 (K 109807).

Epitype. New Zealand. Auckland: Waitakere Ranges, Spragg's Bush, exposed roots on dead tree, 1973, R. E. Beaver (NY 31874, living cultures CMW 10469, CMW 10470 – designated here).

= *E. tropicalis* Shear & N. E. Stevens, U. S. Dept. Agric. Bull. 380: 20-21. 1917.

Holotype: Sri Lanka. Hakgala, *Elaeocarpus glandulifer*, 1913, T. Petch (BPI 614797, BPI 614526).

= *E. havanensis* Bruner, Bull. Govt. For. Exp. Station 226: 140. 1970.

Holotype: Cuba. Santiago de las Vegas, *Eucalyptus* sp., 1916, S. C. Bruner (BPI 614275).

Etymology: Greek, *gyrus*, circle, thus round.

Ascostromata gregarious on bark, often occurring in cracks, often confluent, pulvinate, erumpent, slightly immersed to superficial (Figs 2A–B, 3A–B, 6A–B), 460-500 μm high, 660-950 μm diam, orange, well-developed stromatic tissue (Figs 2C, 6C), prosenchyma at the center, pseudoparenchyma at the edges, orange. Perithecia surrounded with fungal tissue or with bases touching the host tissue, diatrypoid, globose to sub-globose (Figs 2B, 3B, 6B),

340–400 µm diam, walls black, 17–21 µm thick, up to 22 perithecia in a stroma. Perithecial necks periphysate, black, slender (Figs 2B, 3B, 6B), 80–120 µm wide, breaking through the stromatal surface as papillae or long cylindrical beaks covered with orange tissue (Figs 2A–B, 3A–B, 6A), protruding necks up to 440 µm long, 100–200 µm wide. *Asci* (43–)46–52(–55) × (6–)7–8(–9) µm, fusoid, floating freely in the perithecial cavity, stipitate only when immature, unitunicate with non-amyloid, refractive apical ring, 8-spored, biseriate (Figs 2D, 3C). *Ascospores* (9–)9.5–11.5(–12) × (3.5–)4–5(–5.5) µm, oval, hyaline, containing one or two irregularly spaced septa (Figs 2E, 3C, 6E–F).

Conidiomata separate (Figs 2F, 3D, 6G) or above the ascostromata, also appearing as locules inside ascostromata, individual conidiomata unilocular (Figs 2G, 3E, 6H), 400–890 µm high, 100–370 µm diam, orange, superficial, conical to pyriform to fluted, conidiomatal tissue pseudoparenchymatous (Fig. 2H). *Conidiophores* (10.5–)13–19(–24) µm long, branched irregularly, cells delimited by septa or not, hyaline (Figs 2I–J, 3F, 6J–K). *Conidiogenous cells* phialidic, determinate, branches arising beneath a septum, cylindrical to flask-shaped with attenuated apices, (1–)1.5–2.5(–3) µm wide, collarette and periclinal thickening inconspicuous (Figs 2I–J, 3F, 6J–K). *Conidia* (3–)4–8.5(–12) × (1.5–)2–2.5(–3.5) µm, non-septate, oblong to slightly curved, hyaline (Figs 2K–L, 3F, 6L).

Cultural characteristics: Cultures (CMW 10469, CMW 10471) on MEA white when young, often with a luteous center, becoming orange when older, flat and striate with a smooth to sinuous margin, fast growing, covering a 90 mm plate after a minimum of six days; optimum temperature 25–30 ºC.

Substrate: Roots of *Elaeocarpus dentatus*, *E. hookerianus* and *E. glandulifer*.

Distribution: New Zealand, Sri Lanka.

Specimens examined: **Sri Lanka**, 1868, **holotype** K 109807; Nuwara (Mount) Eliya, *Elaeocarpus glandulifer*, G. H. K. Thwaites, K 109809; Hakgala, *Elaeocarpus glandulifer*, 1913, T. Petch, BPI 614797, BPI 614526. **New Zealand,** Auckland, Atanui State Forest, *E. dentatus*, 1973, G. J. Samuels, PDD 32619, living culture CMW 10471; Waitakere Ranges, Spragg's Bush, exposed roots on dead tree, 1973, R. E. Beaver, **epitype designated here** NY 31874, ex-type cultures CMW 10469 = CBS 112922, CMW 10470 = CBS 112923; Waitakere Ranges, *E. dentatus*, 1958, J. M. Dingley, PDD 18377; Titirangi, unidentified living tree, 1973, J. M. Dingley & G. J. Samuels, NY 30873; Waitakere Ranges, Fairy Falls track, *E. dentatus*, 1963, J. M. Dingley, PDD 21944; Waitakere Ranges, Waiatarua, *E. dentatus*, 1963, J. M. Dingley, PDD 25570; Waitakere Ranges, Cutty Grass track, *E. dentatus* root, 1959, S. McBeth, PDD 28497; Waitakere Dam, *E. dentatus*, 1966, J. M. Dingley, PDD 25003; Waitakere Ranges, Upper Piha Valley, *E. dentatus* fallen trunk, 1949, J. M. Dingley, PDD 28485; Upper Piha, *E. dentatus*, 1947, J. M. Dingley, PDD 28482; Waitakere Ranges, Piha, *E. dentatus*, 1948, J. M. Dingley, PDD 28484; Orere, *E. dentatus*, 1963, S. J. Hughes, PDD 20570; Orere, *E. dentatus*, 1953, J. M. Dingley, PDD 28487; Hanua Ranges, *E. dentatus*, 1953, J. M. Dingley, PDD 28488; Hanua Ranges, Moumoukai Valley, *E. dentatus*, 1932, L. M. Cranwell, PDD 3841; Henderson, off Stony Creek, *E. dentatus* root, 1948, J. M. Dingley, PDD 28483; Henderson, Walker's Bush, *E. dentatus*, 1958, S. McBeth, PDD 28494; Henderson Valley, Sharps Bush, *E. dentatus*, 1972, J. M. Dingley, PDD 29819; Northland, Omahuta State Forest, *E. dentatus*, 1963, S. J. Hughes, PDD 21242; Waipoua, *E. dentatus*, 1955, J. M. Dingley, PDD 28492; Coromandel, Camel's Back 1000', *E. dentatus* exposed root, 1934, J. M. Dingley, PDD 28489; Waikato, Taupiri Mt. 900', *E. dentatus*, 1954, J. M. Dingley, PDD 28491; Buller, Orwell Creek, Granville Forest, *E. hookerianus*, 1963, J. M. Dingley, PDD 23365.

Amphilogia major Gryzenh., Glen & M. J. Wingf. **sp. nov.,** Figs 4–5.

Etymology: Latin, *major*, greater, pointing to the ascospores that are larger than those of *A. gyrosa*.

Ascostromata pulvinata vel tuberculata, erumpentia, partim immersa in pulvino stromatico bene evoluto aurantiaco. Perithecia textura stromatica circumdata vel basi hospitem tangentia, globosa vel subglobosa, parietibus nigris. Colla peritheciorum periphysata, nigra, tenuia, per superficiem stromatis ut papillae vel rostra longa cylindrica textura aurantiaca tecta erumpentia. *Asci* fusoidei, solum immaturi stipitati, unitunicati, annulo apicali non amyloideo, refractivo, octospori, biseriati vel uniseriati. *Ascosporae* ovales, hyalinae, uno vel tribus septis irregulariter dispositis divisae.

Conidiomata discreta vel ascostromati insidentia, etiam ut loculi in ascostromate videntur, conidiomata singula unilocularia, aurantiaca, superficialia, conica vel pyriformia. *Conidiophora* irregulariter ramosa, septata an non, hyalina. *Cellulae conidiogenae* phialidicae, cylindricae vel ampulliformes apicibus attenuatis, collari incrassationeque periclinali inconspicuis. *Conidia* non septata, oblonga vel subfalcata, hyalina.

Ascostromata gregarious on bark, often confluent, pulvinate to tuberculate, erumpent, slightly immersed to superficial (Figs 4A–B, 5A–B), 1600-1750 µm high, 1050-3050 µm diam, orange, well-developed stromatic tissue (Figs 4C), prosenchyma at the center, pseudoparenchyma at the edges, orange. Perithecia surrounded with fungal tissue or with bases touching the host tissue, diatrypoid, base globose to sub-globose (Figs 4B, 5B), 330–660 µm diam, walls black, 13–25 µm thick, up to 25 perithecia in a stroma. Perithecial necks periphysate, black, slender (Figs 4B, 5B), 170–260 µm wide, breaking through the stromatal surface as papillae or long cylindrical beaks which are covered with orange tissue (Figs 4A–B, 5A); protruding necks up to 460 µm long, 140–510 µm wide. *Asci* (47–)57.5–77(–87.5) × (7.5–)9–11(–12) µm, fusoid, floating freely in the perithecial cavity, stipitate only when immature, unitunicate with non-amyloid, refractice apical ring, 8-spored, biseriate or

uniseriate (Figs 4D, 5C). *Ascospores* (10.5–)11.5–14(–15.5) × (4.5–)5–6(–6.5) µm, oval, hyaline, containing one to three irregularly spaced septa (Figs 4E, 5C).

Conidiomata separate (Figs 4F, 5D) or on top of ascostromata (Figs 4F, 5A), also appearing as locules inside ascostromata (Figs 4B, 5B), individual conidiomata unilocular (Figs 4G, 5E), 240–820 µm high, 260–500 µm diam, orange, superficial, conical to pyriform, conidiomatal tissue pseudoparenchymatous. *Conidiophores* (4.5–)8.5–19.5(–32.5) µm long, branched irregularly, cells delimited by septa or not, hyaline (Figs 4I–J, 5F). *Conidiogenous cells* phialidic, determinate, apical or lateral on branches arising beneath a septum, cylindrical to flask-shaped with attenuated apices, (1–)1.5–2.5(–3) µm wide, colarette and periclinal thickening inconspicuous (Figs 4I–J, 5F). *Conidia* (3–)3.5–7.5(–12) × (1–)1.5–2(–2.5) µm, non-septate, oblong to slightly curved, hyaline (Figs 4K–L, 5F).

Cultural characteristics: No cultures are available for this fungus.

Substrate: Roots of *Elaeocarpus hookerianus* and *E. dentatus*.

Distribution: New Zealand

Specimens examined: **New Zealand**, Fiordland, Lake Manapouri, *Elaeocarpus hookerianus*, 1948, J. M. Dingley, **holotype** PDD 20056; Westland, Pukekura, *Elaeocarpus dentatus*, 1954, J. M. Dingley, PDD 28490.

The following key summarises the morphological differences between *Amphilogia, Cryphonectria* and *Endothia,* and should serve as an aid in the identification of unknown specimens.

1a. Ascospores aseptate; conidiomata superficial, pulvinate; conidia relatively uniform in size, 3–4 µm long ……..……..…….. ***Endothia***

1b. Ascospores septate ...……...……...…… 2

2a. Conidiomata often superficial, conical to fluted; ascospores 1–3-septate; conidia variable in size, 3–12 µm long ..... ***Amphilogia***

2b. Conidiomata semi-immersed, pulvinate; ascospores always 1-septate; conidia relatively uniform in size, 2–5 µm long ..... ***Cryphonectria***

Amphilogia major can be distinguished from *A. gyrosa* based on features of the teleomorph. Conidiomatal structures do not have explicit diagnostic characteristics. Conidiomata of *A. gyrosa* are often more slender than those of *A. major*, but this feature may be influenced by environmental conditions such as humidity. **The following key is presented to distinguish between the two species:**

1a. Asci up to 55 µm long; ascospores (9–)9.5–11.5(–12) µm long…….. ***A. gyrosa***

1b. Asci longer than 55 µm; ascospores (10.5–)11.5–14(–15.5) µm long…..... ***A. major***

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Table 1. Specimens of *Amphilogia*, *Cryphonectria* and *Endothia* species examined in morphological comparisons.

Identity	Herbarium allocation[a]	Previous labeled name	Host	Origin	Collector	Date
Amphilogia gyrosa	PDD 3841	*Endothia tropicalis*	*Elaeocarpus dentatus*	Moumoukai Valley, Auckland	L. M. Cranwell	1932
	PDD 18377	*E. tropicalis*	*E. dentatus* root	Waitakere Ra., Auckland	J. M. Dingley	1958
	PDD 20570	*E. tropicalis*	*E. dentatus*	Orere, Auckland	S. J. Hughes	1963
	PDD 21242	*E. tropicalis*	*E. dentatus*	Omahuta State Forest, Auckland	S. J. Hughes	1963
	PDD 21944	*E. tropicalis*	*E. dentatus*	Waitakere Ranges, Fairy Falls Track, Auckland	J. M. Dingley	1963
	PDD 23365	*E. tropicalis*	*Elaeocarpus hookerianus*	Granville forest, Westland	J. M. Dingley	1963
	PDD 25003	*E. tropicalis*	*E. dentatus*	Waitakere Dam, Auckland	J. M. Dingley	1966
	PDD 25570	*E. tropicalis*	*E. dentatus*	Waitakere Ranges, Waiatarua, Auckland	J. M. Dingley	1963

PDD 28482	*E. tropicalis*	*E. dentatus*	Waitakere Ranges, Auckland	J. M. Dingley	1947
PDD 28483	*E. tropicalis*	*E. dentatus* root	Henderson, Auckland	J. M. Dingley	1948
PDD 28484	*E. tropicalis*	*E. dentatus*	Piha, Auckland	J. M. Dingley	1948
PDD 28485	*E. tropicalis*	*E. dentatus* fallen trunk	Upper Piha Valley, Auckland	J. M. Dingley	1949
PDD 28486	*E. tropicalis*	*E. dentatus*	Waipoua, Auckland	J. M. Dingley	1949
PDD 28487	*E. tropicalis*	*E. dentatus*	Hunua Ranges, Auckland	J. M. Dingley	1953
PDD 28488	*E. tropicalis*	*E. dentatus*	Hunua Ranges, Auckland	J. M. Dingley	1953
PDD 28489	*E. tropicalis*	*E. dentatus* exposed root	Coromondel Peninsula, Auckland	J. M. Dingley	1954
PDD 28491	*E. tropicalis*	*E. dentatus*	Taupiri Mt., Auckland	J. M. Dingley	1954
PDD 28492	*E. tropicalis*	*E. dentatus*	Waipoua, Auckland	J. M. Dingley	1955
PDD 28494	*E. tropicalis*	*E. dentatus*	Henderson, Auckland	S. McBeth	1958
PDD 28497	*E. tropicalis*	*E. dentatus* root	Waitakere Ranges, Auckland	S. McBeth	1959

	PDD 29819	*E. tropicalis*	*E. dentatus*	Henderson Valley, Waitemata County	J. M. Dingley	1972
	PDD 30873 = NY 30873	*Endothia radicalis*	Exposed roots of unidentified, living tree	Titirangi, Auckland	J. M. Dingley & G. J. Samuels	1973
	PDD 32619[b]	*E. tropicalis*	Exposed *E. dentatus* root	Atuanui State Forest, Auckland	G. J. Samuels	1973
	NY 31874[b]	*E. radicalis*	Exposed roots on dead tree	Waitakere Ranges, Spragg's Bush, Auckland	R. E. Beaver	1973
	BPI 614525	*E. tropicalis*	*E. dentatus*	Omahuta forest, Auckland	S. J. Hughes	1963
	BPI 614524	*E. tropicalis*	*E. dentatus*	Orere, Auckland	S. J. Hughes	1963
	DAOM 93506a	*E. tropicalis*	*E. dentatus*	Omahuta forest, Auckland	S. J. Hughes	1963
Amphilogia major	PDD 20056 (holotype)	*E. tropicalis*	*E. hookerianus*	L. Manapouri, Southland	J. M. Dingley	1948

	PDD 28490	*E. tropicalis*	*E. dentatus*	Pukekura, Westland	J. M. Dingley	1954
Cryphonectria gyrosa [c]	K 109807 (holotype)	*Nectria gyrosa* (#638)	Bark	Sri Lanka	n.a.	1868
	K 109809 [c]	n.a. (#290)	Bark	Nuwara Eliya, Sri Lanka	G. H. K. Thwaites	n.a.
	BPI 614797 [c]	*E. tropicalis*	*Elaeocarpus glandulifer*	Hakgala, Sri Lanka	T. Petch	1913
	BPI 614526 [c]	*E. tropicalis*	*E. glandulifer*	Hakgala, Sri Lanka	T. Petch	1913
	BPI 797701 [c]	*E. tropicalis*	*E. glandulifer* (as *Elaeagnus glandulifer*)	Hakgala, Sri Lanka	n.a.	n.a.
Cryphonectria macrospora	TFM: FPH 1057 (holotype)	*Endothia macrospora*	*Shiia siebordii*	Japan	T. Kobayashi	1954

Cryphonectria nitschkei	TFM: FPH 1045 (holotype)	*Endothia nitschkei*	*Quercus grosseserrata*	Japan	T. Kobayashi	1954
Cryphonectria parasitica	NY (holotype)	*Diaporthe parasitica*	*Castanea dentata*	New York, USA	W. A. Murrill	1905
	CUP 2926	*D. parasitica*	*C. dentate*	New York, USA	W. A. Murrill	1907
	TFM: FPH 629	*Endothia parasitica*	*Castanea crenata*	Koganei, Japan	T. Kobayashi	1953
Cryphonectria radicalis	BPI 797697	*E. radicalis*	*Castanea sativa*	Locarno, Switzerland	n.a.	1862
	BPI 613739	*Endothia fluens*	*C. sativa*	Stresa, Italy	C. L. Shear	1913
Endothia gyrosa	PREM 56218	*E. gyrosa*	*Quercus phellos*	Raleigh, USA	L. Grand	1997

[a] **NY,** William and Lynda Steere Herbarium, New York Botanical Garden, Bronx, New York, USA. **PDD**, Landcare Research New Zealand Limited, Mt. Albert, Auckland, New Zealand. **BPI**, U. S. National Fungus Collections, Systematic Botany and Mycology, Beltsville, USA.

DAOM, National Mycological Herbarium, Eastern Cereal and Oilseed Center (ECORC), Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada. **K**, Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey, U.K. **TFM: FPH**, Forestry and Forest Products Research Institute, Danchi-Nai, Ibaraki, Japan. **CUP,** Plant Pathology Herbarium, Cornell University, Ithaca, New York, USA. **PREM,** National Collection of Fungi, Pretoria, South Africa.

[b] NY 31874 is linked to isolates CMW 10469 and CMW 10470, and PDD 32619 is linked to isolate CMW 10471 (Myburg *et al.* 2004b).

[c] These specimens now represent *A. gyrosa*.

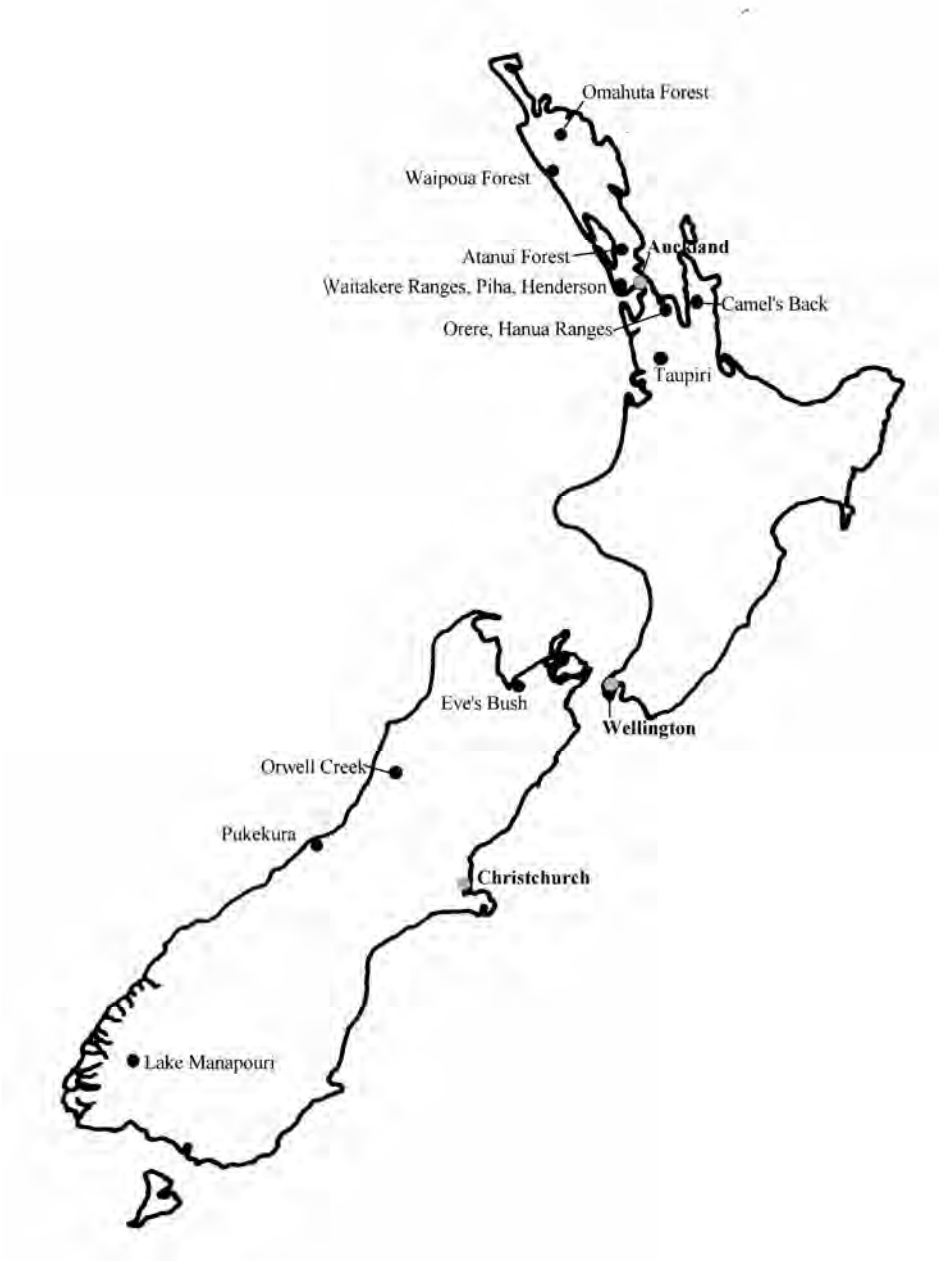

Fig. 1. Map of New Zealand showing the locations of herbarium specimens from *Elaeocarpus* spp.

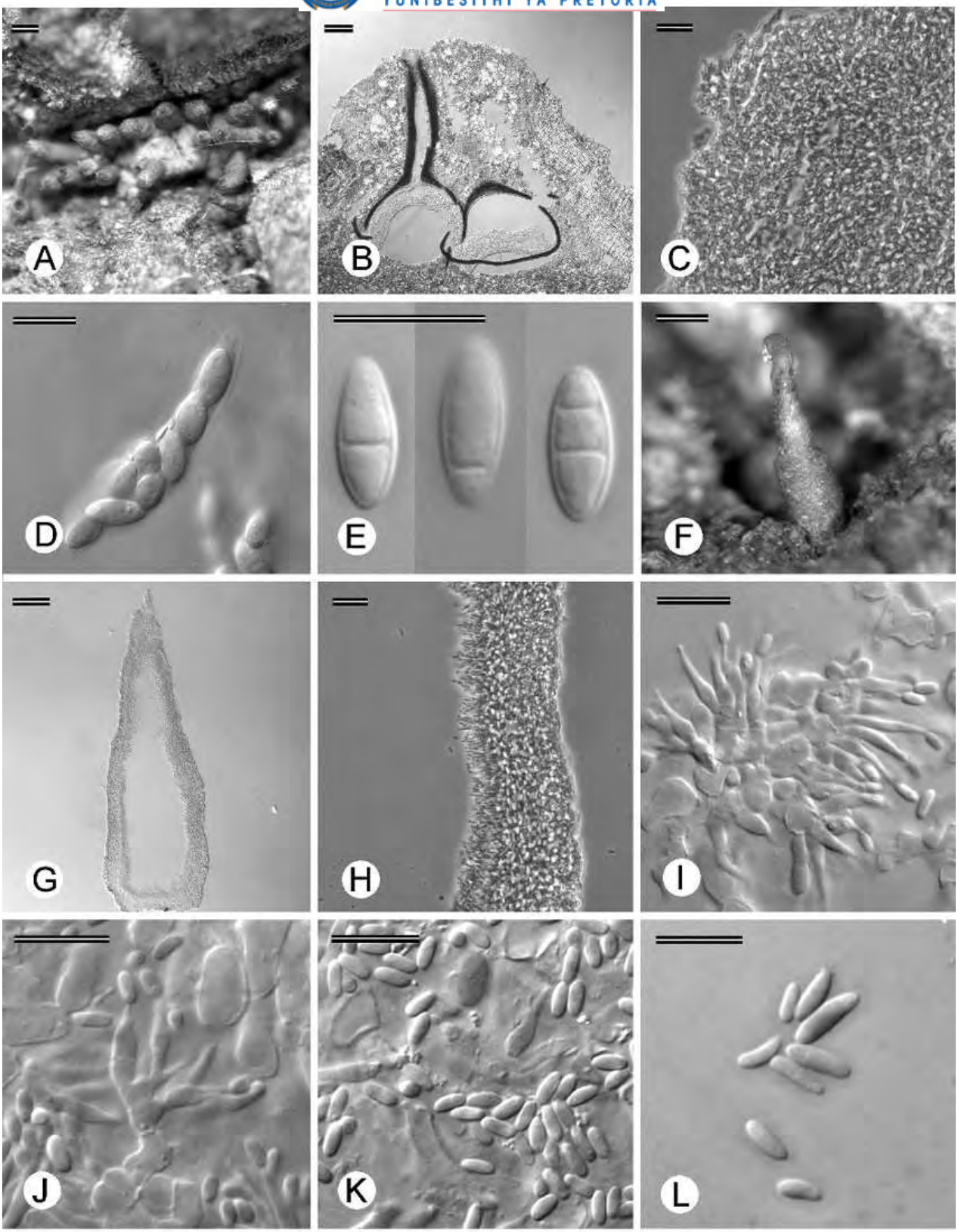

Fig. 2. Micrographs of specimens of *Amphilogia gyrosa* from New Zealand linked to the phylogenetic clade characterised by Myburg *et al.* (2004b). A. Ascostroma on bark with long perithecial necks. B. Vertical section through ascostroma. C. Stromatic tissue of ascostroma. D. Ascus. E. Ascospores with different septation. F. Conidioma on bark. G. Vertical section through conidioma. H. Stromatic tissue of conidioma, longitudinally sectioned. I–J. Conidiophores and conidiogenous cells. K–L. Conidia. Scale bars A, F = 200 µm; B, G = 100 µm; C, H = 20 µm; D–E, I–L = 10 µm.

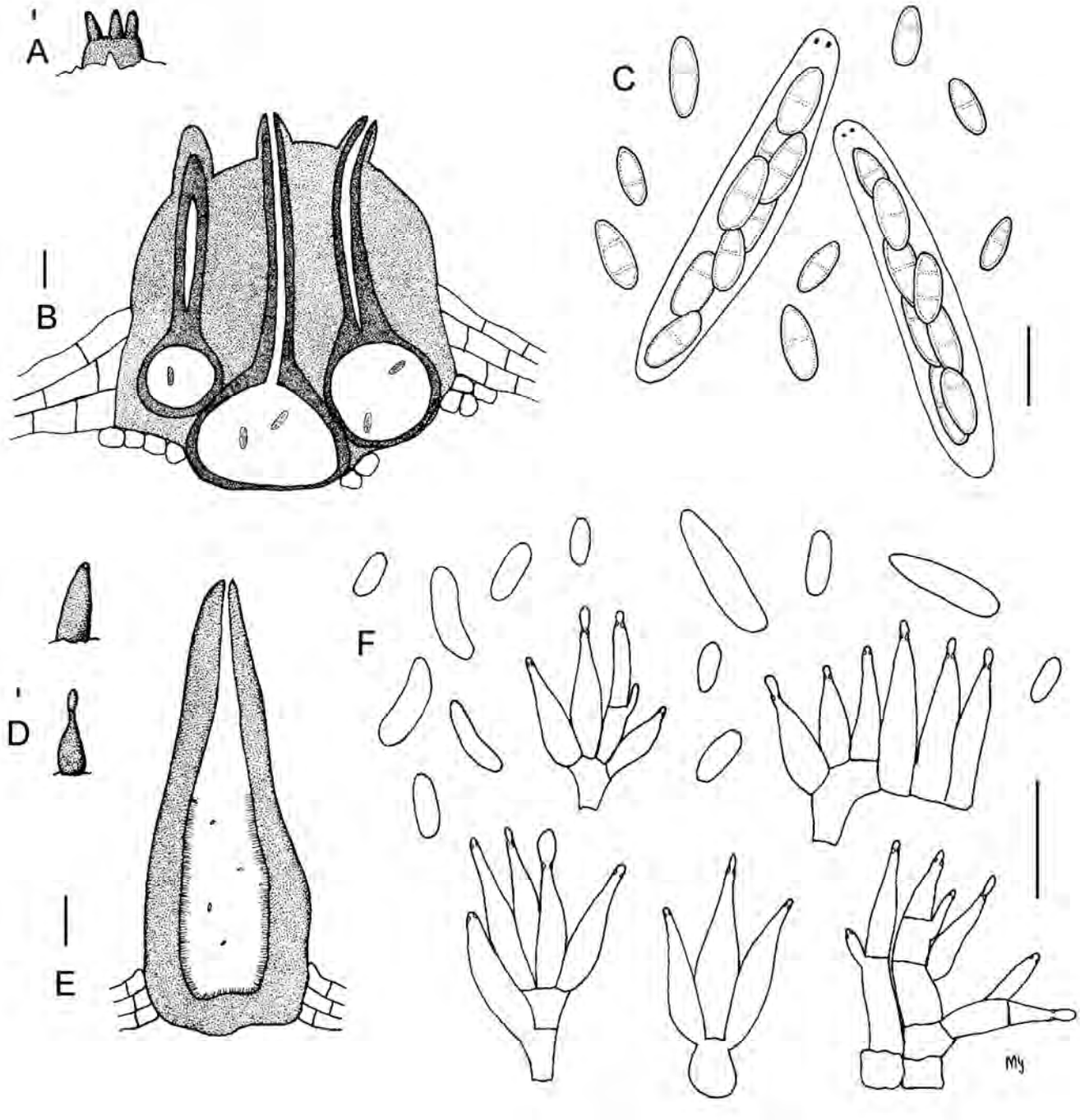

Fig. 3. Line drawings of *Amphilogia gyrosa*. A. Shape of ascostroma on bark. B. Vertical section through ascostroma. C. Asci and ascospores. D. Shapes of conidiomata on bark. E. Vertical section through conidioma. F. Conidiophores, conidiogenous cells and conidia. Scale bars A–B, D–E = 100 µm; C, F = 10 µm.

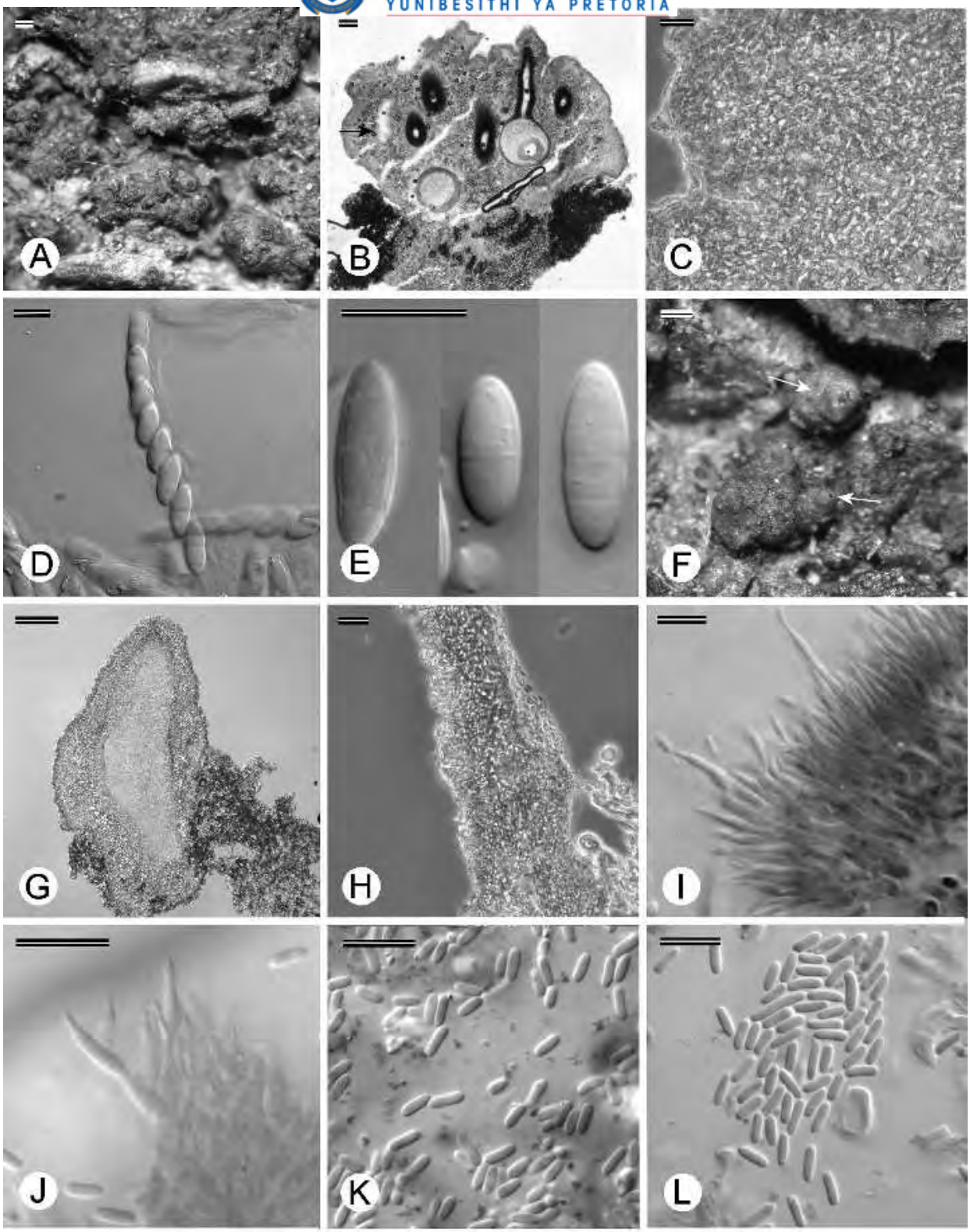

Fig. 4. Micrographs of specimens of *Amphilogia major* from New Zealand. A. Ascostromata on bark with perithecial necks. B. Vertical section through ascostroma, with conidial locule indicated with arrow. C. Stromatic tissue of ascostroma. D. Ascus. E. Ascospores with different septation. F. Conidiomata on bark (arrows). G. Vertical section through conidioma. H. Stromatic tissue of conidioma, longitudinally sectioned. I–J. Conidiophores and conidiogenous cells. K–L. Conidia. Scale bars A–B, F = 200 µm; G = 100 µm; C, H = 20 µm; D–E, I–L = 10 µm.

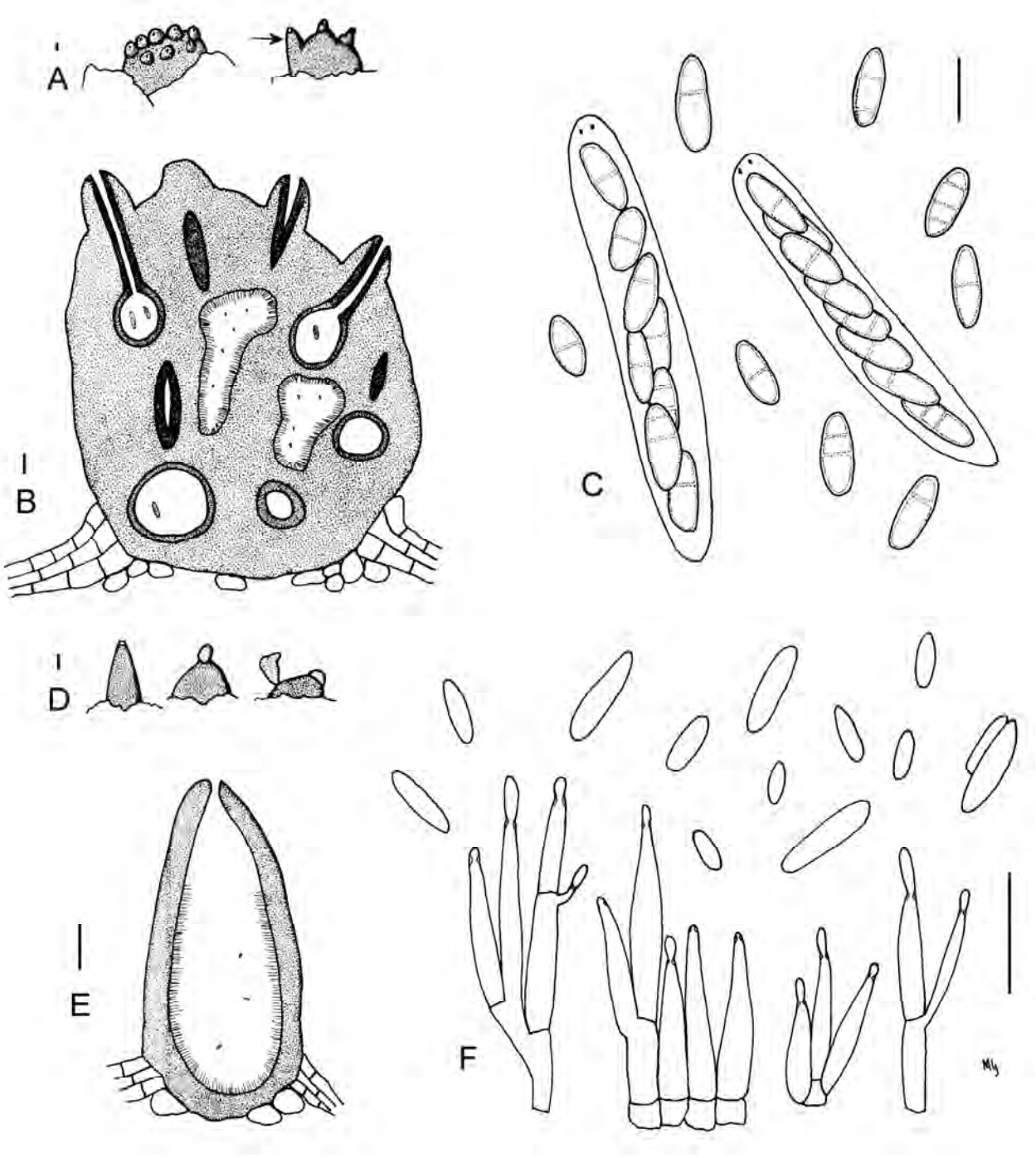

Fig. 5. Line drawings of *Amphilogia major*. A. Shapes of ascostromata on bark with conidioma indicated with arrow. B. Vertical section through ascostroma. C. Asci and ascospores. D. Shapes of conidiomata on bark. E. Vertical section through conidioma. F. Conidiophores, conidiogenous cells and conidia. Scale bars A = 200 µm; B, D–E = 100 µm; C–F = 10 µm.

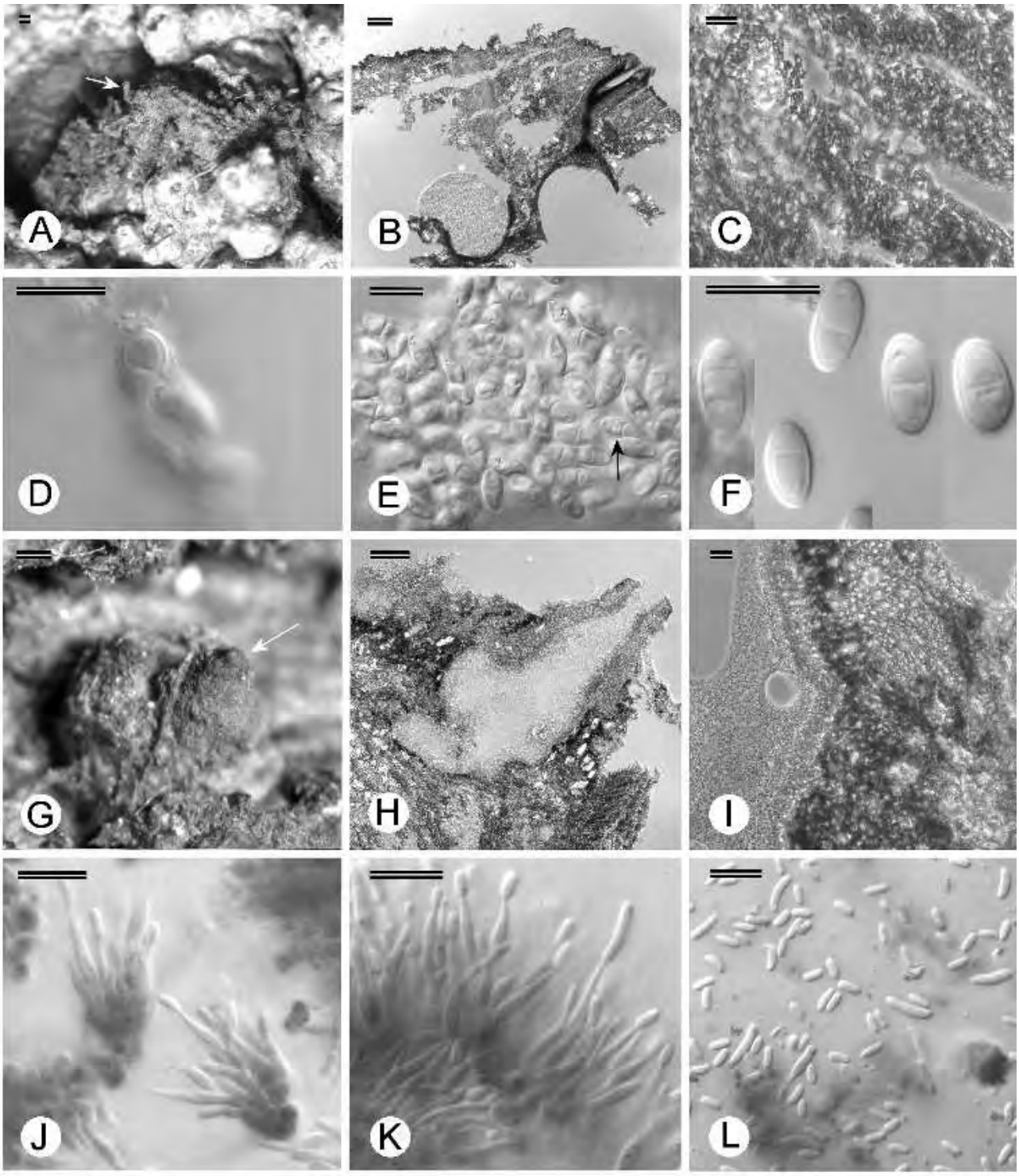

Fig. 6. Micrographs of Sri Lankan specimens of *Amphilogia gyrosa*. A. Ascostroma on bark with long perithecial necks (arrow). B. Vertical section through ascostroma. C. Stromatal tissue of ascostroma. D. Tip of ascus. E–F. Ascospores with different septation. G. Conidioma on bark (arrow). H. Vertical section through conidioma. I. Stromatic tissue of conidioma, longitudinally sectioned. J–K. Conidiophores and conidiogenous cells. L. Conidia. Scale bars A–B, G = 200 µm; H = 100 µm; C, I = 20 µm; D–F, J–L = 10 µm.

CHAPTER 7

Rostraureum tropicale gen. sp. nov. (*Diaporthales*) associated with dying *Terminalia ivorensis* in Ecuador

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***Rostraureum tropicale* gen. sp. nov. (*Diaporthales*) associated with dying *Terminalia ivorensis* in Ecuador**

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Abstract: *Terminalia ivorensis,* a tree of central African origin, is planted in several tropical countries for timber and veneer production. During the course of a recent disease survey, an unknown fungus was found associated with basal cankers on dying *T. ivorensis* in Ecuador. The fungus has orange fruiting structures and septate, fusoid ascospores, similar to those of *Cryphonectria*, a well-known genus of canker pathogens. The aim of this study was to identify the fungus and to assess its pathogenicity. Identification was based on morphological characteristics as well as DNA sequence data. DNA sequence data from the ITS regions of the rDNA operon and two regions of the β-tubulin gene, were compared with published sequences of *Cryphonectria* species and the closely related genera *Endothia* and *Chrysoporthe*. Pathogenicity tests were conducted on *T. superba* saplings. Morphological characterisations revealed that the conidiomata of the fungus from *T. ivorensis,* differed from those typical of *Cryphonectria* in being

superficial and rostrate. Only *Cryphonectria longirostris* was similar to the fungus from *T. ivorensis*, but could be distinguished from it based on conidial size. Phylogenetic analyses showed that the fungus from *T. ivorensis* grouped closely with species of *Cryphonectria*, *Chrysoporthe* and *Endothia*, yet formed a distinct clade. Pathogenicity tests on *T. superba* provided evidence that the fungus is able to cause distinct stem cankers. We conclude that the pathogenic fungus from *T. ivorensis* represents a new genus and new species in the *Diaporthales* and we provide the name *Rostraureum tropicale* for it. The genus is typified by *R. tropicale*. Furthermore, *C. longirostris* is transferred to *Rostraureum*.

Taxonomic novelties: *Rostraureum* Gryzenh. & M. J. Wingf. gen. nov., *Rostraureum tropicale* Gryzenh. & M. J. Wingf. sp. nov., *Rostraureum longirostris* (Earle) Gryzenh. & M. J. Wingf. comb. nov.

Key words: *Cryphonectria*, *Cryphonectria longirostris*, *Diaporthales*, Ecuador, *Rostraureum*, *Terminalia*

INTRODUCTION

Terminalia ivorensis (*Combretaceae*, *Myrtales*) is native to the rainforests of Central Africa (Lamb & Ntima 1971). A similar species, *Terminalia superba*, also occurs in tropical central Africa (Groulez & Wood 1985). Both trees are planted in the tropics as a source of high quality solid timber and veneer. These trees grow rapidly, have straight

stems, are self-pruning and have tended to display a natural resistance to pests and pathogens (Lamb & Ntima 1971, Groulez & Wood 1985).

Few pathogens have been reported from *Terminalia* spp. A *Sphaeronaema* sp. has been associated with die-back of *T. ivorensis* in nurseries (Lamb & Ntima 1971) and an *Endothiella* sp. has also been found on cankers on *T. ivorensis* in Ghana (Ofosu Siedu & Cannon 1976). In Brazil, *Korunomyces terminaliae* Hodges & F.A. Ferreira causes leaf spots on seedlings and young *T. ivorensis* plants (Hodges & Fereira 1981), and *Auerswaldiella parvispora* M. L. Farr causes black blotches on leaves (Farr 1989). Root rot caused by species of *Rosellinia* and *Phytophthora*, leads to die-back of *T. ivorensis* in Panama and Costa Rica (Kapp *et al*. 1997). Some foliage diseases caused by unidentified species of *Cercospora*, *Ramularia*, *Irenina* and *Spaceloma* have been reported from *T. superba* in Africa (Groulez & Wood 1985).

Terminalia ivorensis and *T. superba* are cultivated in Ecuador where both perform well, although *T. ivorensis* trees are prone to unexplained deaths. This study emerged from surveys aimed at gaining an understanding of these deaths. A possible causal agent of basal cankers on dying *T. ivorensis* trees was sought and identified based on morphological characteristics and DNA sequence analyses.

MATERIALS & METHODS

Disease symptoms and specimens

Dead and dying *Terminalia ivorensis* trees were inspected in plantations in the lowland tropics of Ecuador. All trees were mature and ranged in age from 13–15 yrs. Trees appeared to have declined relatively rapidly and diffuse cankers were present in the root

collar region. A fungus with yellow to orange fruiting structures was abundant on the surface of the dead tissue. The fungus was also found on the stumps of recently felled *T. superba,* but these could not be positively connected with a disease problem.

Specimens of the fungus were collected on bark from the surface of cankers and transported to the laboratory. Single conidial and ascospore suspensions were made by suspending spore masses in sterile water, and spreading these onto the surface of malt extract agar [MEA, 20 g/L malt extract agar (Biolab, Merck, Midrand, South Africa)]. Single germ tubes emerging from the spores were transferred to new MEA plates and incubated at 25 °C. Pure cultures have been preserved at 5 °C in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI; University of Pretoria, Pretoria; Table 1), and representative cultures have been deposited in the collection of the Centraalbureau voor Schimmelcultures (CBS; Utrecht). Bark specimens bearing fruiting structures were preserved for morphological comparisons and have been deposited in the herbarium (PREM) of the National Collection of Fungi, Pretoria, South Africa (Table 2).

Morphology

Fruiting structures were cut from the bark and boiled in water for 1 min to rehydrate the cells. The structures were then embedded in Leica mountant and sectioned with a Leica CM1100 cryostat (Setpoint Premier, Johannesburg). Sectioning was carried out at –20 °C. Sections 12–16 µm thick, were dropped in water, transferred to microscope slides and mounted in lactophenol. For the holotype specimen, 50 measurements in lactophenol or 3 % KOH were taken of ascospores, asci, conidia and conidiophores, and are presented

as (min–)(average - S.D.) – (average + S.D.)(–max) µm. A range of measurements was obtained from at least ten structures for the anamorph and teleomorph stromata and perithecia respectively, and at least ten anamorph and teleomorph structures were sectioned to study their internal morphology. Standard colour notations provided by Rayner (1970) were used to describe various elements of the fungus.

The fungus associated with basal cankers on *T. ivorensis* in Ecuador clearly had characteristics similar to those of species of *Cryphonectria* and *Endothia* (shared anamorph: *Endothiella*), and *Chrysoporthe* (anamorph: *Chrysoporthella*). *Chrysoporthe* is a newly described genus accommodating the fungus previously known as *Cryphonectria cubensis* (Bruner) Hodges (Gryzenhout *et al*. 2004/Chapter 1 in this thesis). The fungus from *T. ivorensis* was thus compared with specimens representing species of *Cryphonectria*, *Endothia* and *Chrysoporthe*. One species, *Cryphonectria longirostris* (Earle) Micales & Stipes, was found to be superficially similar to the fungus from *T. ivorensis*. Additional specimens of this fungus were thus included in this study for comparative purposes (Table 2). These specimens originated from dead plant material in Puerto Rico, Trinidad and New Zealand and were obtained from various herbaria. Specimens connected to the *Endothiella* species reported from cankers on *T. ivorensis* in Ghana (Ofosu Siedu & Cannon 1976), as well as another specimen labelled as *C. gyrosa* (Berk. & Broome) Sacc. from *T. ivorensis* in Kenya, were also examined (Table 2).

Growth in culture of isolates CMW 9973 and CMW 10796 (Table 1), was assessed. CMW 10796 originated from the holotype specimen. These studies were conducted on MEA (20 g/L malt extract agar; Biolab, Midrand) as described by Venter *et*

al. (2002). Growth tests were conducted in the dark at temperatures ranging from 15–35 ºC, at 5 ºC intervals.

DNA isolations and PCR amplifications

DNA was isolated from isolates using the method described in Myburg *et al.* (1999). Two β-tubulin gene regions were amplified using the primer pairs Bt1a/Bt1b and Bt2a/Bt2b respectively (Glass & Donaldson 1995). The ITS1 and ITS2, as well as the conserved 5.8S gene of the ribosomal RNA operon, were amplified using primers ITS-1 and ITS-4 (White *et al.* 1990). PCR reactions were done according to Myburg *et al.* (1999) for the ribosomal operon, and Myburg *et al.* (2002b) for the β-tubulin genes. PCR amplifications were performed on a Perkin Elmer GeneAmp PCR System 9700 thermocycler (Perkin-Elmer Applied Biosystems, Foster City, CA). Sizes of PCR products were verified on 1% agarose-ethidium bromide gels using an UV light source.

Sequencing and analysis of sequence data

PCR products were cleaned using a QIAquick PCR Purification kit (Qiagen, Hilden, Germany). These were sequenced in both directions using the same primer pairs that were used in the amplification reactions. Sequencing reactions were conducted using an ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction kit with AmpliTaq® DNA Polymerase, FS (Perkin-Elmer, Warrington, UK). DNA sequences were determined using an ABI PRISM 3100™ automated DNA sequencer.

Sequence Navigator version 1.0.1 (Perkin-Elmer Applied BioSystems) was used to edit the raw sequence data and sequences were manually aligned to already existing

datasets from Myburg *et al.* (2004). Phylogenetic analyses were done using PAUP version 4.0b (Swofford 1998). To test whether the ITS and β-tubulin datasets were homogenous in phylogenetic analyses and thus combinable, a 500 replicate partition homogeneity test (PHT) (Farris *et al*. 1994) were done on the two partitions. Results were confirmed with the Templeton Nonparametric Wilcoxon Signed Ranked test (Kellogg *et al.* 1996). Alignments were analysed using parsimony and heuristic searches with TBR (tree-bisection-reconnection) and MULTREES (saving all optimal trees) options effective, and random additions set to 100. Uninformative sites were excluded and base pairs were reweighted according to their CI. Gaps inserted during manual sequence alignment, were treated as missing in the heuristic searches. Alignments were also subjected to distance analyses and the appropriate distance model for the datasets were determined with MODELTEST version 3.5 (Posada & Crandall 1998). The confidence levels of the branching points were determined by a 70 % bootstrap analysis of 1000 replications (Felsenstein 1985). *Diaporthe ambigua* Nitschke isolates, which also resides in the *Diaporthales* (Castlebury *et al*. 2002), were used as outgroup taxa to root the phylogenetic tree. Sequences were deposited in GenBank and accession numbers are listed in Table 1.

Pathogenicity tests

Pathogenicity of the fungus from *T. ivorensis* could not be tested on *T. ivorensis* due to the fact that trees of this species are rare and difficult to obtain in Ecuador. For this reason, 20 saplings of the related *T. superba* were inoculated in February 2000. An isolate of *Chrysoporthe cubensis* (Bruner) Gryzenh. & M. J. Wingf. (syn. *Cryphonectria*

cubensis (Bruner) Hodges) an important pathogen of *Eucalyptus* spp. (Hodges *et al.* 1976, Sharma *et al.* 1985) and clove (*Syzygium aromaticum,* Hodges *et al.* 1986), was also inoculated onto *T. superba* for comparative purposes.

The saplings for inoculations were approx. 2-yr-old at the time of inoculation. Using a metal punch, approx. 5 mm diam., bark was removed from the stems of trees, approx. 10 cm above ground level. Discs of agar bearing mycelium of the fungus were taken from the actively growing edges of a culture and placed, mycelium side downwards, into the wounds. An equal number of plants were inoculated with sterile agar to serve as controls. Wounds were covered with masking tape to prevent desiccation. Inoculated plants were allowed to grow for six weeks before examination. Masking tape was then removed and lesion lengths were measured. The measurements were subjected to a one-way ANOVA analysis and differences between the inoculation sets were determined using a Bonferroni test (SYSTAT 1996).

RESULTS

Morphology

The fungus from *T. ivorensis* is typically diaporthalean, with periphysate ostiolar canals, no paraphyses present, and unitunicate asci with refractive apical rings (Barr 1978). The orange to yellow fruiting structures are reminiscent of those in *Cryphonectria*, *Chrysoporthe* and *Endothia* (Shear *et al.* 1917, Barr 1978, Micales & Stipes 1987, Gryzenhout *et al*. 2004). The ascospores are one-septate, fusoid to ellipsoid, and similar to those in species of *Cryphonectria* and *Chrysoporthe*, but different from those in *Endothia* (Shear *et al*. 1917, Barr 1978, Micales & Stipes 1987).

Although the stromata are peripherally similar on the bark, the fungus from *T. ivorensis* is distinctly different to species of *Endothia*, *Cryphonectria* and *Chrysoporthe*. The perithecial necks of the fungus from *T. ivorensis* are not lodged within well-developed stromatic tissue, as is found in *Cryphonectria* (Barr 1978, Micales & Stipes 1987, Myburg *et al*. 2004). Instead, the only tissue development, made visible through longitudinal sections, is a sheath of white tissue covered with an orange to luteous-pure yellow layer around the perithecial necks (Figs 1B–C, 2B). In some cases, orange remnant tissue of the anamorph was present on top of the perithecia or orange, rudimentary stromatic tissue occurred between the necks (Figs 1B, 2B). This tissue structure is similar to that found in *Chrysoporthe*, but perithecial necks protruding from the stromatal surfaces in species of *Chrysoporthe* appear fuscous-black (Myburg *et al*. 2003, 2004, Gryzenhout *et al*. 2004), while those in the *Terminalia* fungus are orange. Another distinct difference between *Cryphonectria* and the *Terminalia* fungus is that the anamorph of *Cryphonectria* is usually semi-immersed, pulvinate, convoluted, unilocular to multilocular stromatic (Shear *et al*. 1917, Kobayashi 1970, Myburg *et al*. 2004). The anamorph of the fungus from *T. ivorensis* is superficial to slightly immersed, clavate or rostrate, with long, attenuated necks, unilocular and convoluted at the base (Figs 1G–H, 2D–E). The *Chrysoporthella* anamorph of *Chrysoporthe* has similar conidiomata, but the structures of *Chrysoporthella* are fuscous-black and pyriform (Gryzenhout *et al*. 2004).

One species of *Cryphonectria*, *C. longirostris*, had the same stromatal characteristics as the fungus from *T. ivorensis*. Specimens of *C. longirostris* from Puerto Rico (NY 4340, NY 816, NY 617, NY 266417, NY 6576) had the same orange, superficial, rostrate conidiomata with attenuated necks (Figs 3A, 3E, 4D-E). These occur

singly or on top of the teleomorph stromata (Figs 3A–B, 3E, 4A, 4D–E). The necks of the perithecia are also surrounded with a white sheath of tissue covered with an orange layer (Figs 3C, 4B). Furthermore, perithecia are umber to fulvous in both *C. longirostris* and the fungus from *T. ivorensis*.

Although similar, the fungus from *T. ivorensis* could be distinguished from *C. longirostris* based on a number of morphological characteristics. The conidia of *C. longirostris* (Figs 3L, 4F) are shorter (3–3.5 µm) than those of the fungus from *T. ivorensis* ((3–)3.5–5(–6) µm; Figs 1L, 2F). Although variation associated with different hosts and environments might contribute to the following differences (Shear *et al*. 1917, Hodges *et al*. 1986, Myburg *et al*. 2003), structures of *C. longirostris* are also more complex than those of the fungus from *T. ivorensis*. The pulvinate structures that usually contain the perithecial necks of *C. longirostris*, frequently have strongly convoluted pycnidial locules in the upper parts with extensive tissue development, and with perithecial bases lodged in the bark, in the lower parts. The anamorph structures of the fungus from *T. ivorensis* are less convoluted and perithecia and conidial locules are lodged in little to no stromatic tissue. Conidiomata on the *C. longirostris* specimens were generally larger, bases 600–1300 µm high, 270–880 µm wide, and necks 1011–2050 µm long, than the conidiomata on the specimens from *T. ivorensis*, base 400–600 µm high, 150–500 µm wide, and neck 900–1450 µm long.

The cells giving rise to the conidiophores in the pycnidial cavities of the fungus from *T. ivorensis* and *C. longirostris* frequently contained orange crystals (Figs 3F–G). Thus the linings of the pycnidial locules are bright orange, in comparison to the remainder of the stromatic tissues (Fig. 3F). Other crystals, different in form and colour,

could also be found in the stromatic tissue. This was found to be a variable characteristic and crystals were not present in all specimens.

The fungal structures on the specimen (IMI 187898) from *T. ivorensis* in Ghana, which were connected to the report of Ofosu Siedu & Cannon (1976), were different from those of the fungus on *T. ivorensis* in Ecuador. The specimen from Ghana had orange, pulvinate conidiomata with no elongated necks, and ascomata were clearly stromatic without the characteristic sheath of tissue around the perithecial necks. The specimen (IMI 288729) from *T. ivorensis* in Kenya was also different from the Ecuador samples, since it contained ascostromata without sheaths of tissue around the perithecial necks. These African specimens (IMI 187898, IMI 288729) had uniseptate, fusoid to ellipsoid ascospores and minute, cylindrical conidia and could possibly reside in *Cryphonectria*. Ascospores of the two specimens have overlapping ascospore dimensions, (9.5–)10–11(–12) × (3–)3.5–4(–4.5) µm for IMI 187898, and (8–)8.5–10(–11.5) × (3–)3.5–4 µm for IMI 288729. Spore dimensions in these specimens thus resembled those published for *C. havanensis* (Bruner 1916, Kobayashi 1970, Roane 1986a). However, more extensive comparisons would need to be carried out to verify the identity of these specimens.

A number of specimens labelled *C. longirostris* were examined in this study and clearly do not represent this fungus. Specimen NY 3360 from Trinidad had pulvinate to conical conidiomata with bright orange exteriors, scarlet to rust interiors and the linings of the pycnidial locules were pale luteous. Specimen NY 3098, also from Trinidad, had conical conidiomata with hazel to rust tissue surrounding an orange interior. Ascomata of this specimen were hazel to rust. A specimen from Puerto Rico (NY 511), had

pulvinate, semi-immersed, multilocular conidiomata different from the superficial and clavate conidiomata of *C. longirostris*, with small, orange ascomata. The sheath of tissue around the perithecial necks, characteristic of *C. longirostris*, was also absent. Another specimen from Puerto Rico (NY 1053) had orange, oval, superficial conidiomata. Another *C. longirostris* specimen mentioned by Roane (1986a), PDD 28477 from New Zealand, also lacked the clavate anamorph, but had pulvinate, semi-immersed, multilocular conidiomata. These fungi are not treated further in this study, but we believe that they probably represent undescribed taxa closely related to *Cryphonectria* and its allies.

Sequencing and analysis of sequence data

The datasets consisted of 22 taxa of which the two *D. ambigua* isolates were defined as the outgroup. Results generated with the PHT analyses (P = 0.03) and Templeton Nonparametric Wilcoxon Signed Ranked test indicated that the rDNA and β-tubulin sequence data sets were significantly incongruent and could not be combined as one data set in the phylogenetic analyses. These data sets were consequently analysed separately. The ribosomal DNA sequence alignment consisted of 557 characters of which 339 were constant, 13 were parsimony-uninformative and 205 were parsimony-informative. The dataset showed significant phylogenetic signal (g1 = –1.148). The heuristic search resulted in one most parsimonious tree (tree length = 306, CI = 0.838, RI = 0.912). The Kimura-2 parameter model (Kimura 1980) with a Gamma distribution shape parameter (G) of 0.1979 was used in the distance analyses. The trees obtained with the distance and parsimony analyses showed the same clades of isolates, although the relatedness of these

groups with each other varied. The tree obtained with the distance analyses is shown in Fig. 5.

The β-tubulin DNA sequence alignment contained significant phylogenetic signal (g1 = –0.887) according to Hillis & Huelsenbeck (1992) and included a total of 951 characters of which 562 were constant, 15 were parsimony-uninformative and 374 were parsimony-informative. The heuristic search resulted in one most parsimonious tree (tree length = 659, CI = 0.819, RI = 0.924). MODELTEST indicated that the Tamura-Nei parameter model (Tamura & Nei 1993), with the Gamma distribution shape parameter set to 0.7905 and the proportion of invariable sites (I) as 0.5437, was suitable for the dataset. The tree obtained with parsimony essentially showed the same groupings as the tree obtained with distance analyses, thus only the tree obtained with distance methods was chosen for presentation (Fig. 6).

The phylogenetic trees obtained from the ribosomal DNA and β-tubulin datasets all showed the same number of well-supported clades, although the relationships between clades differed (Figs 5–6). The first clade in the phylogram typified *Chrysoporthe* (bootstrap support 100 % in Fig. 5, 74 % in Fig. 6). This group of fungi has been the subject of intensive study in recent years (Myburg *et al*. 2002a, 2002b, 2003, 2004, Gryzenhout *et al*. 2004). The clade representing the genera *Cryphonectria* (bootstrap support 94 % in Fig. 5, 81 % in Fig. 6) was defined by *Cryphonectria parasitica* (Murrill) M. E. Barr, *Cryphonectria radicalis* (Schwein.: Fr.) Fr., *Cryphonectria nitschkei* (G. H. Otth) M. E. Barr and *Cryphonectria macrospora* (Tak. Kobay. & Kaz. Itô) M. E. Barr. *Endothia gyrosa* (Schwein.: Fr.) Fr. and *Endothia singularis* (Syd. & P. Syd.) Shear & N. E. Stevens represented the genus *Endothia*, although not always forming a well-defined

clade. Myburg *et al.* (2004) previously considered the taxonomy and DNA-based phylogeny of these species.

The unknown fungus isolated from *T. ivorensis* grouped separately from *Chrysoporthe*, *Cryphonectria* and *Endothia* in all analyses based on different areas of the genome (Figs 5–6). Although grouping separately, evolutionary relationships between the different clades differed in the analyses based on ribosomal DNA and β-tubulin genes. This separate grouping is supported by a bootstrap value of 100 % for both areas sequenced, indicating that new genus and species designations should be considered for the fungus from *T. ivorensis*. Regrettably, no isolates representing *Cryphonectria longirostris* or the fungi from *T. ivorensis* in Africa, exist and comparisions with the new fungus are impossible at present.

Pathogenicity

Within six weeks, *T. superba* plants inoculated with the fungus from dying *T. ivorensis* showed well-developed stem cankers (Figs 7A–B). Cankers were 36–84 mm long and were clearly in the process of girdling the stems. ANOVA showed that lesion lengths associated with the inoculated and control plants were significantly different to each other ($P < 0.0001$). The Bonferroni test showed that lesions caused by *Chr. cubensis* (Fig. 7C) were significantly smaller than those caused by the fungus from *T. ivorensis* ($P < 0.0001$). Wounds used to make control inoculations were covered with callus and stem discoloration was equal in length to the size of the original inoculation wound (Fig. 7D).

Taxonomy

Morphological characteristics and phylogenetic data provide good evidence supporting the view that the fungus from *T. ivorensis* represents an undescribed species that should reside in a new genus in the *Diaporthales*. *Cryphonectria longirostris* is similar to this fungus and should be transferred to the new genus as a second species. *Cryphonectria longirostris* can be distinguished from the fungus from *T. ivorensis* by its smaller conidia and larger conidiomata. The fungus from *T. ivorensis* is provided as the type of this new genus, since isolates and DNA sequences are available for this fungus. A more complete and illustrated description of *C. longirostris* is also provided, where features relevant to the new genus in which it now resides, are emphasized.

Rostraureum Gryzenh. & M. J. Wingf., **gen. nov.**

Etymology: Latin, *rostrum* (a beak); and *aureus* (golden) so a golden beak.

Ascostromata flava vel aurantiaca, textura stromatali carenti vel praesenti. Colla *perithecialia* vagina texturae porrectae albae circumcincta, cellulae in superficie exteriori collorum perithecialium aurantiacae vel flavae. *Asci* fusoides. *Ascosporae* fusoides vel ellipsoides, hyalinae, semel septatae. *Conidiomata* clavata, superficialia, unilocularia, luteo-flava vel aurantiaca, collis singulis, binis vel ternis attenuatis. *Conidiophora* hyalina, cellulae basales irregulariter ramosae, phialides cylindricas proferentes, septis divisas an non. *Conidia* cylindrica, hyalina, non septata.

Typus: *Rostraureum tropicale* Gryzenh. & M. J. Wingf. 2005.

Ascostromata erumpent, luteous-pure yellow to orange, consisting of perithecia embedded in bark tissue, with necks erumpent and valsoid, occasionally occurring underneath active pycnidial locules, stromatal tissue absent or present between the necks. *Perithecia* umber to fulvous, bases globose to subglobose, necks periphysate, surrounded by sheath of white *textura porrecta*, cells on outside of erumpent perithecial necks of *textura globulosa* and orange to luteous-pure yellow. *Asci* fusoid, non-stipitate, unitunicate, with non-amyloid refractive apical ring, octasporous. *Ascospores* fusoid to ellipsoid with rounded apices, hyaline, one-septate.

Conidiomata eustromatic, clavate or rostrate, superficial to slightly immersed, unilocular, even to strongly convoluted lining, luteous-pure yellow to orange, one to three attenuated necks, base tissue of *textura epidermoidea*, neck tissue of *textura porrecta* with thickened cells at surface. *Conidiophores* hyaline, consisting of a basal cell, branched irregularly at the base or above into cylindrical cells, delimited by septa or not. *Conidiogenous cells* phialidic, determinate, apical or lateral on branches beneath the septum. *Conidia* cylindrical, hyaline, aseptate.

Rostraureum tropicale Gryzenh. & M. J. Wingf., **sp. nov.,** Figs 1–2

Etymology: Latin, *tropicus* (tropics), refers to the discovery of this fungus in the tropics.

Ascostromata flava vel aurantiaca, textura stromatali inter perithecia in sectionibus plerumque carenti. *Perithecia* valsoidea, umbrina. *Colla perithecialia* umbrina, cellulis vaginae juxta collum perithecii albis, cellulis exterioris luteo-flavis vel aurantiacis. *Asci* fusoidei. *Ascosporae* 8 in quoque asco, hyalinae, fusoideae vel ellipsoideae, semel septatae. *Conidiomata* flava vel aurantiaca, clavata vel rostrata collis

attenuatis vel non, superficialia, unilocularia, perithecia interdum sub conidiomatibus formantia. *Conidiophora* hyalina, cellulae basales irregulariter ramosae, phialides cylindricas proferentes, septis divisas an non, collare et inspissatio periclinalis inconspicuae. *Conidia* cylindrica, non septata, hyalina, guttulae sporarum exsudatae testaceae. In MEA *culturae* opprimuntur; culturae juvenes albae, intus luteae, seniores aurantiacae, crescunt optime ad 25–30 °C, in temperaturis optimis coloniae tegunt 90 mm in 6 diebus.

Ascostromata semi-immersed with pulvinate appearance under dissection microscope (Figs 1A–B, 2A), 544–711 µm wide above bark surface where necks converge, stromatal tissue between perithecia (Fig. 1B) usually absent in sections (Fig. 2B), luteous-pure yellow when young, orange when older. Up to 11 *perithecia*, in valsoid configuration, bases 310–460 µm wide, globose to sub-globose, surrounded by host tissue, umber to fulvous, wall 15–28 µm thick (Figs 1B, 2B). Perithecial necks 45–90 µm wide, periphysate, umber, surrounded by tissue sheath with the cells next to the perithecial neck white, of *textura porrecta*, and the cells on the outer edge of the sheath orange to luteous-pure yellow, of *textura globulosa* (Fig. 1C), neck with surrounding tissue 143–225 µm wide and 250–700 µm long when it emerges above bark surface. *Asci* (23–)27–32(–35.5) × (5.5–)6–7.5(–10.5) µm, fusoid, free when mature, non-stipitate, unitunicate, with non-amyloid apical ring (Figs 1D, 2C). *Ascopores* 8 per ascus, (4–)6–8.5(–9) × 2–3(–3.5) µm, hyaline, fusoid to ellipsoid, sometimes slightly curved, apices rounded, single septum median or off-median (Figs 1E, 2C).

Conidiomata eustromatic, clavate or rostrate with neck attenuated or not (Figs 1G–H, 2D), base 400–600 µm high, 150–500 µm wide, neck 900–1450 µm long, 100–200 µm wide, superficial to slightly immersed, unilocular, even to convoluted lining,

perithecia occasionally forming underneath conidiomata, luteous-pure yellow when young, orange when mature. *Locules* 40–280 µm at widest point, usually single conidial locule in center opening through neck, longitudinal sections at edge of base reveal more than one locule due to convoluted lining (Fig. 1H, 2E). Basal tissue of *textura epidermoidea* (Fig. 1F), tissue at the junction between neck and base of *textura intricata* and neck tissue of *textura porrecta* with thicker cells at edges of neck (Figs 1I). *Conidiophores* hyaline, with a globular to rectangular basal cell, (3–)3.5–6.5(–7) × (2–)2.5–4.5(–6) µm, branched irregularly at the base or above into cylindrical cells, cells delimited by septa or not, total length of conidiophore (12–)15–21(–24.5) µm (Figs 1J–K, 2F). *Conidiogenous cells* phialidic, determinate, apical or lateral on branches beneath the septum, cylindrical to flask-shaped with attenuated apices, 1.5–2(–2.5) µm wide, collarette and periclinal thickening inconspicuous (Figs 1J–K, 2F). *Conidia* (3–)3.5–5(–6) × 1.5–2 µm, cylindrical, aseptate, hyaline, exuded as brick red spore droplets (Figs 1L, 2F).

Cultural characteristics: on MEA suppressed with sparse aerial hyphae when young, remaining suppressed when older, young cultures creamy white with a luteous interior, older cultures are orange to luteous with or without white margins, margins even, conidiomata occasionally produced in mature cultures, optimum growth from 25–30 °C, isolates covering 90 mm plates after 6 days at optimum growth temperatures.

Substrate: Bark of *Terminalia ivorensis* and *T. superba.*

Distribution: Ecuador.

Specimens examined. **Ecuador,** Pichincha, Río Pitzara (0° 15′ 27″ N 79° 7′ 43″ W, 350 meters above sea level), *Terminalia ivorensis*, Nov. 2001, M. J. Wingfield, **holotype** PREM 57519, ex-type cultures CMW 9972, CMW 10796 = CBS 115757; PREM 583301, PREM 583302, PREM 583303, PREM 583304, living culture CMW 9971 from PREM 583301.

Rostraureum longirostris (Earle) Gryzenh. & M. J. Wingf., **comb. nov.,** Figs 3–4

Basionym: *Endothia longirostris* Earle, *Muehlenbergia* **1**: 14 (1901).

≡ *Cryphonectria longirostris* (Earle) Micales & Stipes, Phytopathology 77: 651. 1987.

Ascomata semi-immersed, pulvinate, 700–950 µm wide above bark surface, orange, prosenchymatous stromatal tissue usually present in erumpent part of stromata and containing conidial locules and perithecial necks, perithecial bases at base of structures surrounded by host tissue (Figs 3A–B, 4A–B). Up to 15 *perithecia* per structure, valsoid, bases (250–)285–408(–420) µm wide, globose to sub-globose, umber to fulvous, wall 13–20(–25) µm thick (Figs 3B, 4B). Perithecial necks (50–)52–78(–90) µm wide, periphysate, umber, surrounded by tissue sheath with the cells alongside the perithecial necks white, of *textura porrecta*, and cells at the outer edge of sheath luteous-pure yellow to orange, of *textura globulosa* (Figs 3C); neck with surrounding tissue (140–)156–205(–213) µm wide and (400–)450–600(–650) µm long where they emerge above bark surface (Fig. 3A). No intact *asci* were observed, but according to Earle (1901) asci are spindle-shaped, thin-walled, 25–30 × 6 µm with no paraphyses (Fig. 4C). *Ascopores* 8 per

ascus, (5–)6–7.5(–9) × 2–3(–3.5) µm, fusoid to ellipsoid, apices rounded, hyaline, single septum median or off-median (Figs 3D, 4C).

Conidiomata eustromatic, clavate or rostrate with necks attenuated or not, bases 600–1300 µm high, 270–880 µm wide, necks 1011–2050 µm long, 175–288 µm wide, superficial to slightly immersed, unilocular and convoluted, occurring alone or with teleomorph structures forming below, orange (Figs 3A–B, 3E, 4D–E). Locules 230–1500 µm wide at widest point, usually single pycnidial locule at center, opening through neck, length sections at edges of base reveal more than one locule due to convoluted lining (Figs 3E–F, 4E). Base tissue of *textura epidermoidea* (Fig. 3H), tissue where neck and base join of *textura intricata* (Fig. 3H), neck tissue of *textura porrecta* with thicker cells at edges of neck (Fig. 3I). *Conidiophores* hyaline, with a globular to rectangular basal cell, (2–)3–5(–7.5) × (1.5–)2.5–3.5(–4.5) µm, branched irregularly at the base or above into cylindrical cells, cells delimited by septa or not, total length of conidiophore (13–)15–19.5(–22.5) µm (Figs 3J–K, 4F). *Conidiogenous cells* phialidic, determinate, apical or lateral on branches beneath the septum, cylindrical to flask-shaped with attenuated apices, 1.5–2(–2.5) µm wide, collarette and periclinal thickening inconspicuous (Figs 3J–K, 4F) . *Conidia* 3–3.5 × 1.5 µm, cylindrical, aseptate, hyaline (Figs 3L, 4F), exuded as brick red spore droplets.

Substrate: dead logs and branches

Distribution: Puerto Rico, French Guiana, and Trinidad and Tobago.

Specimens examined: **Puerto Rico**, east of Santurce, bark of fallen tree, 19 Jan. 1900, A. A. Heller, **holotype** NY4340; bark, 24 Jan.–5 Apr. 1923, F. J. Seaver & C. E. Chardon,

NY 617; Naguabo, fallen bark, 25 March 1915, N. Wille, NY 816; Rio Piedras, 18 June 1917, J. A. Stevenson & R. C. Rose, NY 6576. **Trinidad & Tobago,** Ortoire river, Guayaguayare road, bark, 25 March 1921, F. J. Seaver, NY 3320.

DISCUSSION

In this study, we have shown that the fungus associated with basal cankers on *T. ivorensis* in Ecuador represents a new genus and species of *Diaporthales*, for which we have provided the name *R. tropicale*. The decision to place this fungus in a distinct genus is strongly linked to phylogeny based on DNA sequence data. Here, we have shown that isolates of *R. tropicale* formed a clade distinct from species of *Endothia*, *Cryphonectria* and *Chrysoporthe*, the genera that it most closely resembles.

Robust morphological features support the distinct phylogenetic grouping of isolates of *R. tropicale*. The primary distinguishing feature of the genus is the orange, superficial, rostrate, eustromatic conidiomata. This is in contrast to species of *Cryphonectria* that have semi-immersed, pulvinate eustromatic conidiomata (Shear *et al*. 1917, Micales & Stipes 1987, Myburg *et al*. 2004), and *Chrysoporthe* spp., which have superficial, black, pulvinate conidiomata (Hodges 1980, Gryzenhout *et al*. 2004, Myburg *et al*. 2004). Species of *Endothia* has large, pulvinate and superficial conidiomata (Shear *et al*. 1917, Micales & Stipes 1987, Myburg *et al*. 2004).

One species of *Cryphonectria*, *C. longirostris*, exhibits similar characteristics to *R. tropicale.* For this reason, we have transferred *C. longirostris* to the new genus as *R. longirostris*. It is unfortunate that cultures are not available for *C. longirostris* and at the

present time, it is impossible to determine whether our decision to transfer it to *Rostraureum* as a second species, will be supported by phylogenetic data. However, to avoid confusion, we have elected to rely on morphology to support our decision.

The morphology of anamorph, as opposed to teleomorph structures, has recently been shown to provide important taxonomic features in the classification of *Cryphonectria* and *Endothia* (Myburg *et al*. 2004). Thus, species that would have been treated in *Cryphonectria* based on teleomorph morphology, but that have anamorphs different to the pulvinate, semi-immersed, unilocular to multilocular eustromata of *Cryphonectria*, grouped outside the clade representing *Cryphonectria* based on phylogenetic comparisons (Myburg *et al*. 2004). For example, isolates of *Chrysoporthe* with blackened, pyriform eustromatic anamorphs with attenuated necks, and a group of isolates from New Zealand with an orange conical anamorph, grouped outside *Cryphonectria* (Myburg *et al*. 2004). Results of the present study further support the view that anamorph morphology provides a strong indicator of generic status for diaporthalean fungi with orange stromatic tissue.

Observation of various forms of crystals in the stromata and linings of the conidial locules in *R. tropicale* and *R. longirostris*, was an unusual finding in this study. Various pigments have been reported for *Cryphonectria* spp. and these have been clearly summarised by Roane (1986b). These pigments are bisanthraquinones, and include skyrin, skyrinol, oxyskyrin and regulosin (Roane & Stipes 1978, Roane 1986b). A phenolic compound known as endothine red or pigment B, also forms red crystals in the mycelium of some *Endothia* and *Cryphonectria* spp., and imparts a purple colour to growth media (Roane & Stipes 1978, Roane 1986b). Furthermore, species of *Endothia*

and *Cryphonectria* turn 3 % KOH purple and lactic acid yellow (Castlebury *et al*. 2002). It is clear that species of *Cryphonectria* and *Endothia*, and other fungi closely related to them, produce different, brightly coloured pigments in culture and in their fruiting structures. It is possible that these compounds could be linked to the crystals observed in *R. tropicale* and *R. longirostris.*

Various specimens examined in this study could not be identified as belonging to an existing taxon. Herbarium specimens labelled as *C. longirostris* were found to represent at least four different fungi. These fungi all have orange-coloured fruiting structures and conidia or ascospores similar to those of *C. longirostris*. These general characteristics and the Caribbean origin of these specimens undoubtedly led to their identification as *C. longirostris*. The fungi, however, all exhibited unique morphological features different to those that characterise species in existing genera such as *Cryphonectria*, *Rostraureum*, *Chrysoporthe* and *Endothia*. Although the fungi are most probably related to these genera, we expect that they represent undescribed taxa. The acquisition of additional collections and preferably cultures that can be used in DNA sequence comparisons will be useful in providing names for them.

Specimens associated with *T. ivorensis* in Africa represent fungi different from *R. tropicale*. Although the orange stromata found on the African specimens are similar to those of *Rostraureum*, these fungi do not have the typical rostrate conidiomata of *Rostraureum* spp. Characteristics of these specimens resembled those in descriptions of *Cryphonectria havanensis* (Bruner) M. E. Barr, a fungus reported from Cuba and Florida (USA) on various hosts, including *Eucalyptus* spp. (Bruner 1916, Barnard *et al*. 1987). It is, however, also possible that these specimens represent undescribed taxa in

Cryphonectria. The correct identity of these specimens will be difficult to determine in the absence of additional specimens, especially those linked to isolates. Their identification is, however, of considerable interest as they appear to be associated with disease of *Terminalia* spp. in Africa.

The discovery of *R. tropicale* emerged from an interest in dying *T. ivorensis* trees in Ecuador. The fungus is common on basal cankers of dying trees, but we are not convinced that it is the sole cause of tree death. Although we were not able to obtain *T. ivorensis* trees for inoculation in this study, results of inoculations on *T. superba* showed that the fungus is at least, a significant pathogen of this tree after inoculation. We have, however, not found any evidence of natural infections on *T. superba* that led to disease, although intensive surveys have not been undertaken. In the future, we hope to undertake further studies of the death of *T. ivorensis* in Ecuador. It will then hopefully also be possible to obtain trees of this species for inoculation studies with *R. tropicale.*

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Table 1. Isolates of *Rostraureum tropicale*, *Chrysoporthe*, *Cryphonectria* and *Endothia* spp. used for DNA sequence comparisons and growth study.

Culture number [a]	Alternative number[b]	Identification	Host	Origin	Genbank accession number [d]
CMW 8756	–	*Chrysoporthe cubensis*	*Eucalyptus* sp.	Indonesia	AF 046896, AF 273077, AF 285165
CMW 2632	–	*C. cubensis*	*Eucalyptus marginata*	Australia	AF 046893, AF 273078, AF 375607
CMW 8757	–	*C. cubensis*	*Eucalyptus grandis*	Venezuela	AF 046897, AF 273069, AF 273464
CMW 8758	–	*C. cubensis*	*E. grandis*	Venezuela	AF 046898, AF 273068, AF 273463
CMW 1853	–	*C. cubensis*	*Syzygium aromaticum*	Brazil	AF 046891, AF 273070, AF 273465
CMW 8755	–	*Chrysoporthe austroafricana*	*E. grandis*	South Africa	AF 292040, AF 273064, AF 273458
CMW 2113	CBS 112916	*C. austroafricana*	*E. grandis*	South Africa	AF 046892, AF 273067, AF 273462
CMW 7047	ATCC 48197, E5	*Cryphonectria parasitica*	*Quercus virginiana*	MS, USA	AF 368329, AF 273073, AF 273469
CMW 7048	ATCC 48198, E9	*C. parasitica*	*Q. virginiana*	VA, USA	AF 368330, AF 273076, AF 273470
CMW 10477	CBS 240.54, E76	*Cryphonectria radicalis*	*Castanea sativa*	Italy	AF 368328, AF 368347, AF 368346
CMW 10455	CBS 238.54, E42	*C. radicalis*	*C. dentata*	Italy	AF 452113, AF 525705, AF 525712
CMW 10518	CBS 112919, E53	*Cryphonectria nitschkei*	*Quercus* sp.	Japan	AF 452118, AF 525706, AF 525713
CMW 10463	CBS 112920, E54	*Cryphonectria macrospora*	*Castanopsis cupsidata*	Japan	AF 368331, AF 368351, AF 368350

CMW 9971 [c]	CBS 115725	*Rostraureum tropicale*	*Terminalia ivorensis*	Ecuador	AY 167425, AY 167430, AY 167435
CMW 9972 [c]	–	*R. tropicale*	*T. ivorensis*	Ecuador	AY 167426, AY 167431, AY 167436
CMW 9973	CBS 115726	*R. tropicale*	*T. ivorensis*	Ecuador	AY 167427, AY 167432, AY 167437
CMW 9975	CBS 115727	*R. tropicale*	*T. ivorensis*	Ecuador	AY 167429, AY 167434, AY 167439
CMW 10796 [c]	CBS 115757	*R. tropicale*	*T. ivorensis*	Ecuador	AY 167428, AY 167433, AY 167438
CMW 2091	ATCC 48192, E13	*Endothia gyrosa*	*Q. palustris*	VA, USA	AF 368325, AF 368337, AF 368336
CMW 10442	E27	*E. gyrosa*	*Q. palustris*	VA, USA	AF 368326, AF 368339, AF 368338
CMW 10465	CBS 112921, E58	*Endothia singularis*	–	CO, USA	AF 368323, AF 368333, AF 368332
CMW 5288	CBS 112900	*Diaporthe ambigua*	*Malus domestica*	South Africa	AF 543817, AF 543819, AF 543821
CMW 5587	CBS 112901	*D. ambigua*	*M. domestica*	South Africa	AF 543818, AF 543820, AF 543822

[a] Culture collection of the Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria.

Isolates sequenced in this study are in bold. The other sequences were obtained from Myburg *et al.* (1999, 2002b, 2004) and Venter *et al.* (2002).

[b] Numbers preceded with E are designated numbers in the collection of R. Jay Stipes, incorporated in the culture collection of FABI;

ATCC, American Type Culture Collection (Manassas); CBS, Centraalbureau voor Schimmelcultures (Utrecht).

[c] CMW 9972, CMW 10796 were obtained from the holotype specimen PREM 57519; CMW 9971 was obtained from specimen PREM 583301.

[d] Given as sequences from the ITS region, and two regions from the β-tubulin genes amplified with primers 1a/1b and 2a/2b respectively.

Table 2. Specimens used in morphological comparisons.

Reference collection[a]	Identity	Name on specimen	Host	Origin	Date	Collector
PREM 57519 (holotype)[b]	*Rostraureum tropicale*	–	*Terminalia ivorensis*	Ecuador	2001	M.J. Wingfield
PREM 583301[b]	*R. tropicale*	–	*T. ivorensis*	Ecuador	2001	M.J. Wingfield
PREM 583302	*R. tropicale*	–	*T. ivorensis*	Ecuador	2001	M.J. Wingfield
PREM 583303	*R. tropicale*	–	*T. ivorensis*	Ecuador	2001	M.J. Wingfield
PREM 583304	*R. tropicale*	–	*T. ivorensis*	Ecuador	2001	M.J. Wingfield
NY 4340 (holotype) and NY 266417	*Rostraureum longirostris*	*Cryphonectria longirostris*	Fallen tree	Puerto Rico	1900	A. Heller
NY 617	*R. longirostris*	*C. longirostris*	–	Puerto Rico	1923	F.J. Seaver & C.E. Chardon
NY 816	*R. longirostris*	*C. longirostris*	Fallen bark	Puerto Rico	1915	N. Wille
NY 6576	*R. longirostris*	*C. longirostris*	–	Puerto Rico	1917	J.A. Stevenson & R.C. Rose
NY 3320	*R. longirostris*	*C. longirostris*	Bark	Trinidad & Tobago	1921	F.J. Seaver
NY 1053	Unknown	*C. longirostris*	Sticks	Puerto Rico	1923	F.J. Seaver & C.E. Chardon
NY 511	Unknown	*C. longirostris*	unknown	Puerto Rico	1923	F.J. Seaver & C.E. Chardon
NY 3360	Unknown	*C. longirostris*	Forest	Trinidad & Tobago	1921	F.J. Seaver
NY 3098	Unknown	*C. longirostris*	Forest	Trinidad & Tobago	1921	F.J. Seaver
PDD 28477	Unknown	*C. longirostris*	*Coriaria* sp.	New Zealand	1958	J.M. Dingley

IMI 187898	Unknown	*Cryphonectria* sp.	*T. ivorensis*	Ghana	–	P.F. Cannon
IMI 288729	Unknown	*Cryphonectria gyrosa*	*T. ivorensis*	Kenya	1984	D. Pawsey
BPI 631857 (holotype)	*Chrysoporthe cubensis*	*Diaporthe cubensis*	*Eucalyptus botryoides*	Cuba	1916	S.C. Bruner
PREM 57297	*C. cubensis*	*Cryphonectria cubensis*	*Eucalyptus* sp.	Indonesia	2001	M.J. Wingfield
K 109809	*Cryphonectria gyrosa*	Unknown (#290)	Bark	Sri Lanka	–	G.H.K. Thwaites
TFM: FPH 1045 (holotype)	*Cryphonectria nitschkei*	*Endothia nitschkei*	*Quercus grosseserrata*	Japan	1954	T. Kobayashi
CUP 2926	*Cryphonectria parasitica*	*Diaporthe parasitica*	*Castanea dentata*	New York, USA	1907	W.A. Murrill
PREM 56218	*Endothia gyrosa*	*E. gyrosa*	*Q. phellos*	Raleigh, USA	1997	L. Grand

[a] PREM, National Collection of Fungi (Pretoria); NY, New York Botanical Garden (Bronx, New York); PDD, Landcare Research (Mt Albert, Auckland); IMI, CABI Bioscience (Egham, Surrey); BPI, US National Fungus Collections (Beltsville); K, Royal Botanic Gardens (Kew, Surrey); TFM: FPH, Forestry and Forest Products Research Institute (Danchi-Nai, Japan); CUP, Plant Pathology Herbarium (Cornell University, Ithaca, New York).

[b] CMW 9972, CMW 10796 obtained from the holotype specimen PREM 57519; CMW 9971 were obtained from specimen PREM 583301.

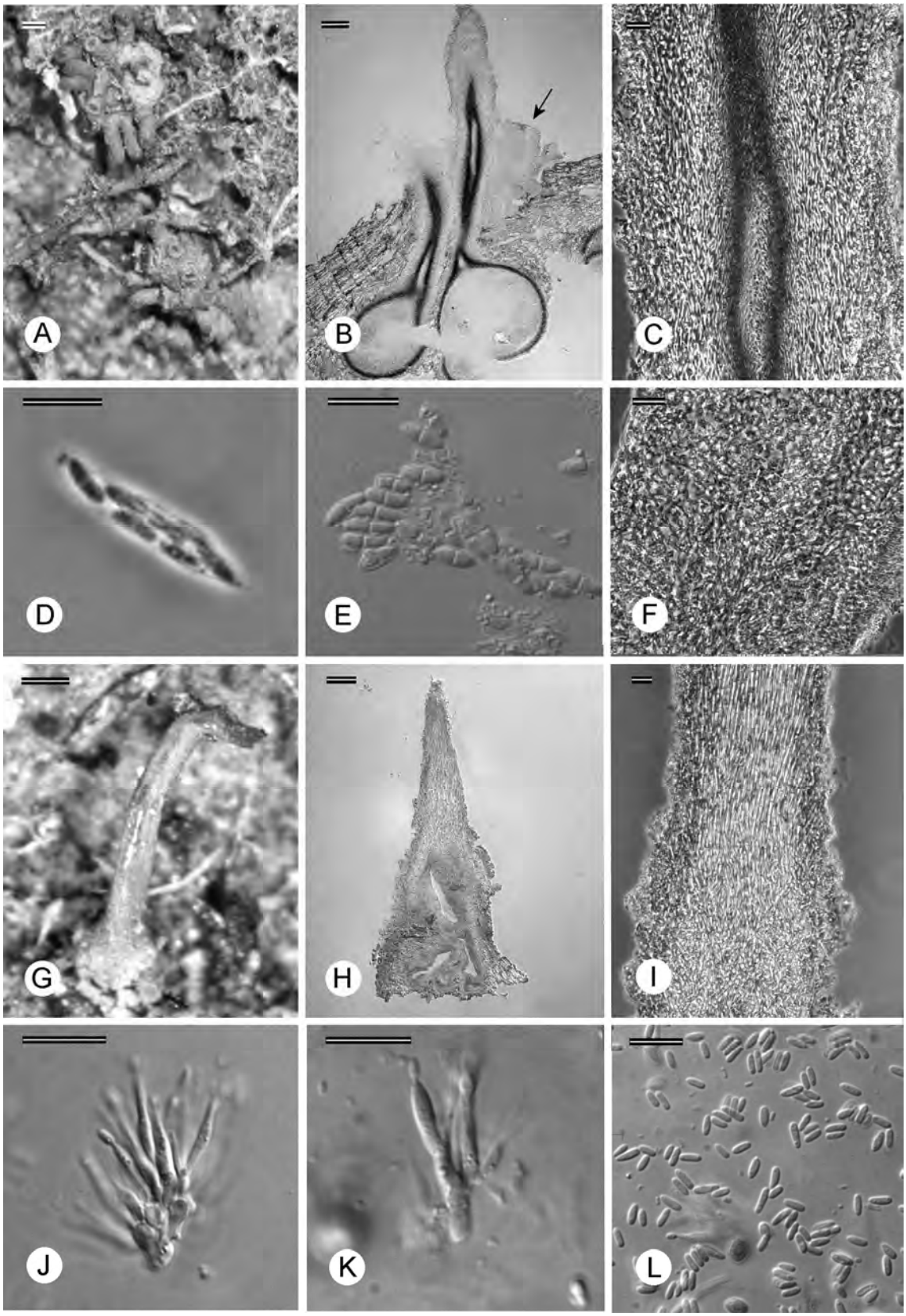

Fig. 1. Fruiting structures of *Rostraureum tropicale* (from holotype PREM 57519). A. Ascomata on bark. B. Longitudinal section through ascoma also showing stromatic tissue (indicated by arrow). C. Tissue of perithecial neck. D. Ascus. E. Ascospores. F. Tissue at base of conidioma. G. Conidioma on bark and sectioned (H). I. Tissue of conidioma where neck begins. J, K. Conidiophores. L. Conidia. Scale bars A, G = 200 µm; B, H = 100 µm; C

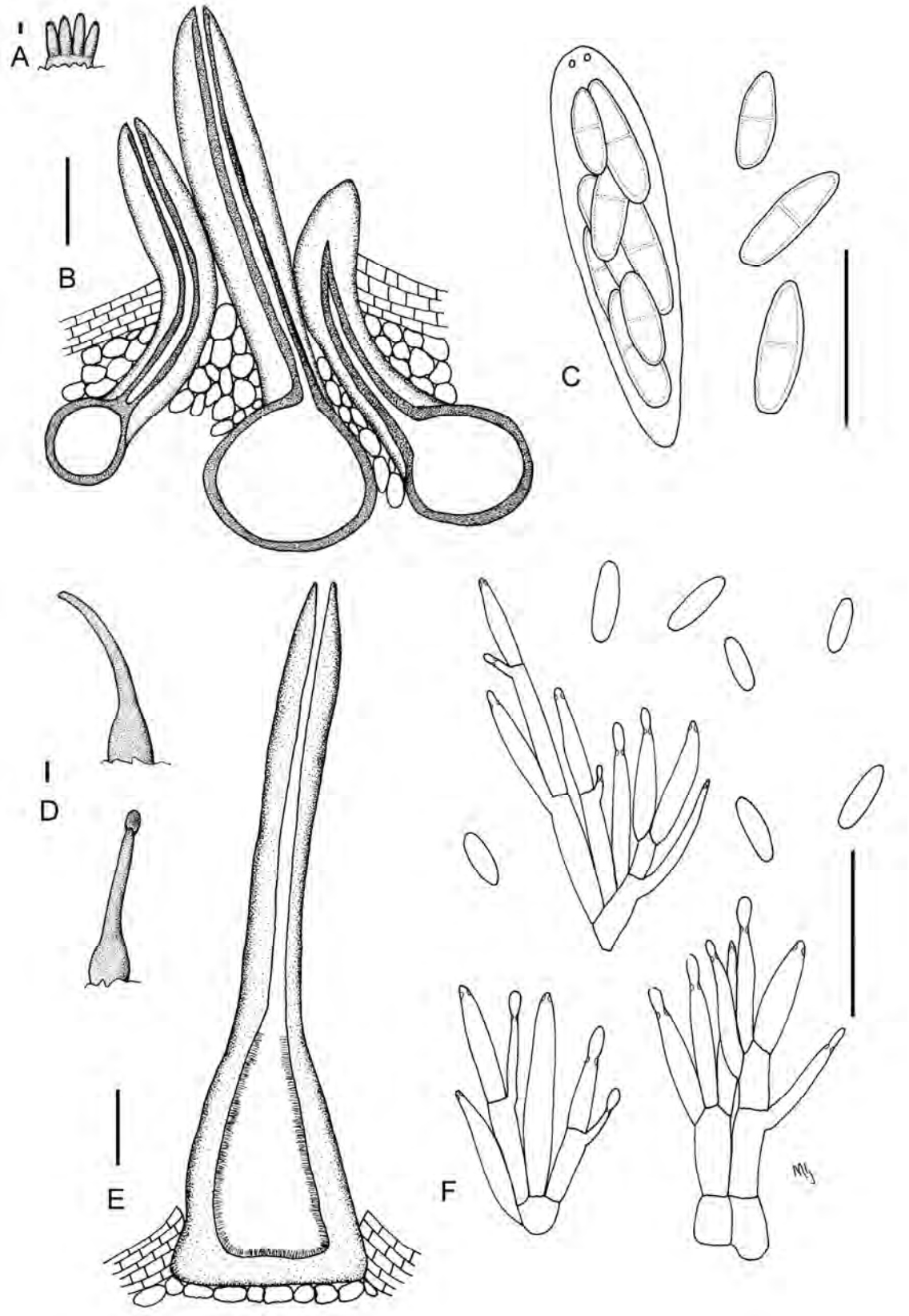

Fig. 2. Schematic drawings of *Rostraureum tropicale* (from holotype PREM 57519). A. Ascomata on bark. B. Section through ascoma. C. Asci and ascospores. D. Conidiomata on bark. E. Section through conidioma. F. Conidiophores, conidiogenous cells and conidia. Scale bars A–B, D–E = 100 µm; C, F

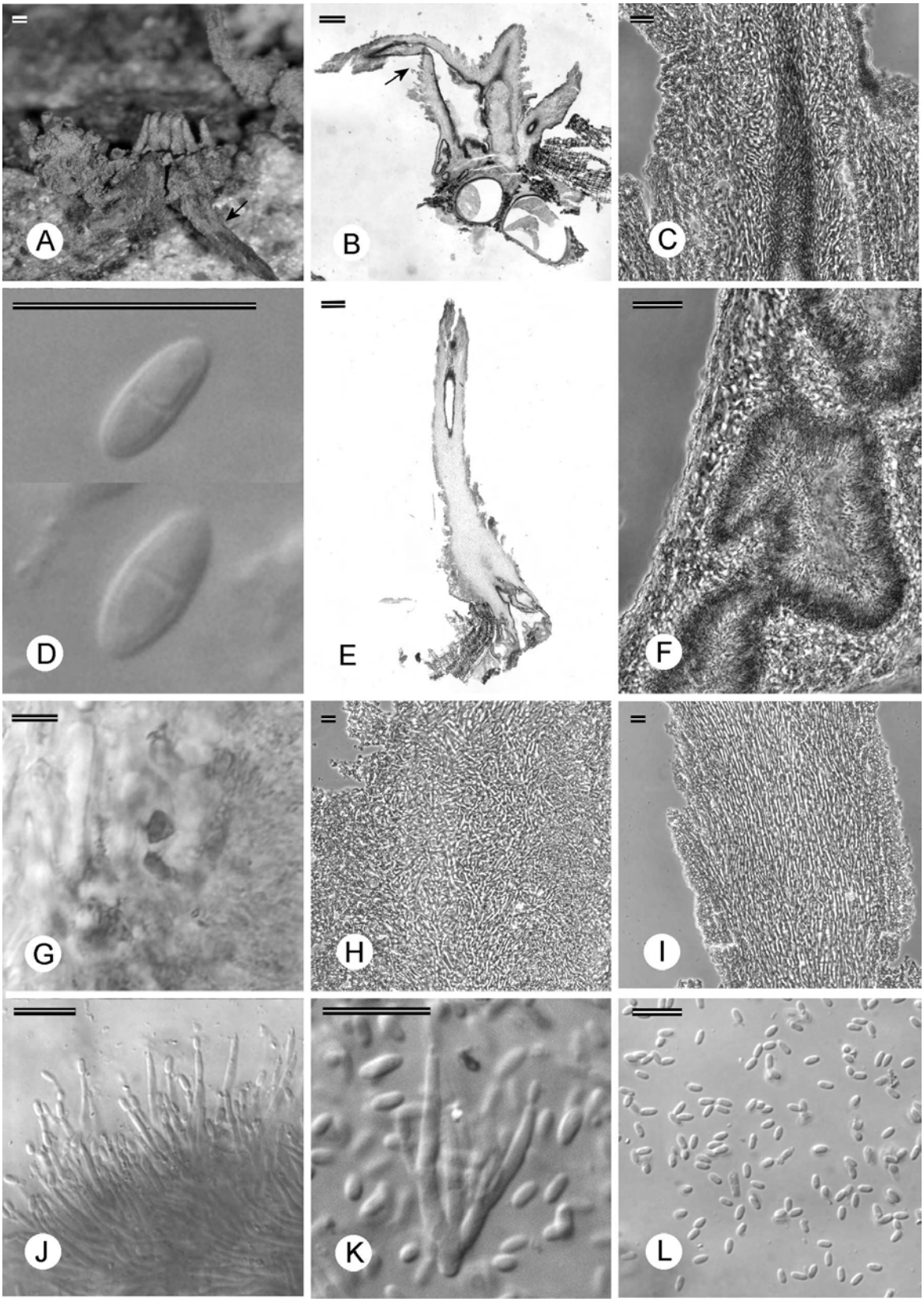

Fig. 3. Fruiting structures of *Rostraureum longirostris* (from holotype NY 4340). A. Ascoma with conidioma attached (indicated with arrow). B. Longitudinal section through ascoma and conidioma (indicated with arrow). C. Tissue of perithecial neck. D. Ascospores. E. Longitudinal section of conidioma. F–G. Crystals in lining of conidial cavity. H. Tissue of conidiomal base where neck begins. I. Tissue of conidiomal neck. J–K. Co rs of A–B, E = 200 µm; C, F, H–I = 20 µm; D, G, J–L

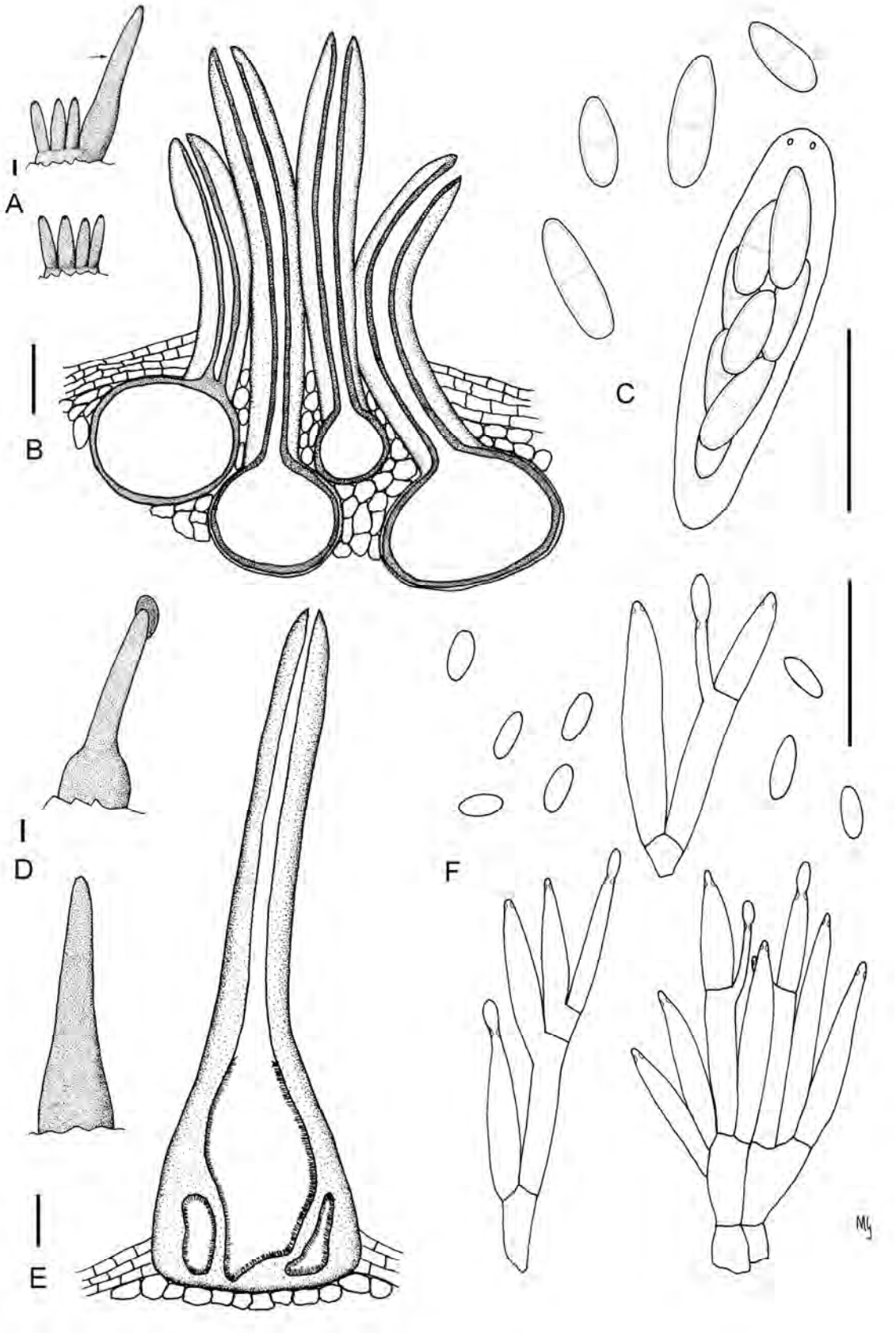

Fig. 4. Schematic drawings of *Rostraureum longirostris* (from holotype NY 4340). A. Ascomata on bark with conidioma (indicated with arrow). B. Section through ascoma. C. Asci and ascospores. D. Conidiomata on bark. E. Section through conidioma. F. Conidiophores, conidiogenous cells and conidia. Scale bars A–B, D–E =

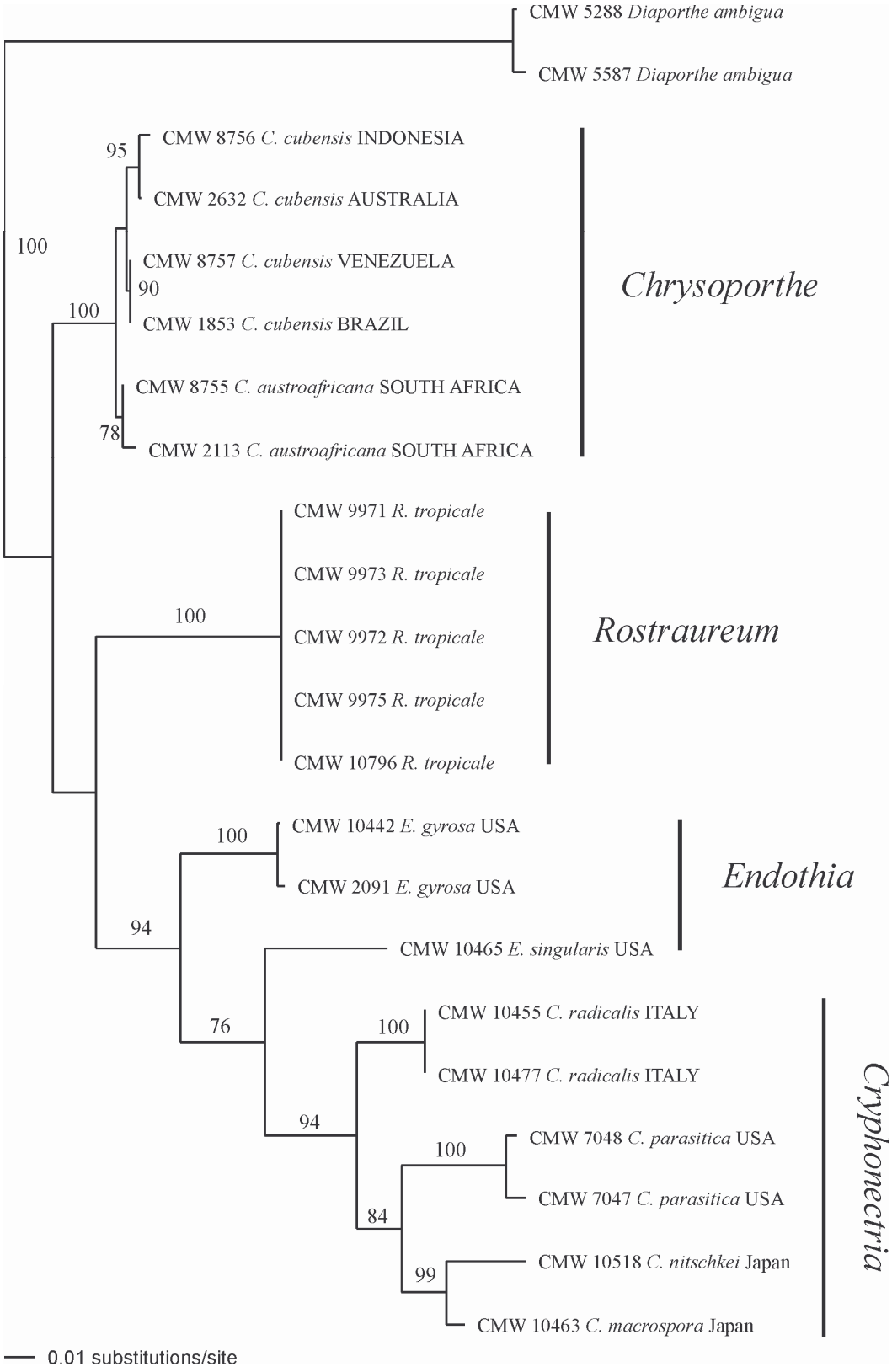

Fig. 5. Distance phylogram showing phylogenetic relationships between *Rostraureum*, *Cryphonectria*, *Chrysoporthe* and *Endothia* spp. based on ITS1/ITS2 DNA sequence of the ribosomal operon. The phylogram was obtained with the Kimura 2 parameter model (G = 0.1979). Bootstrap values greater than 70 % (1000 replicates) are indicated at the branch nodes. The *Diaporthe ambigua* isolates were used as outgroup taxa to root the phylogenetic tree.

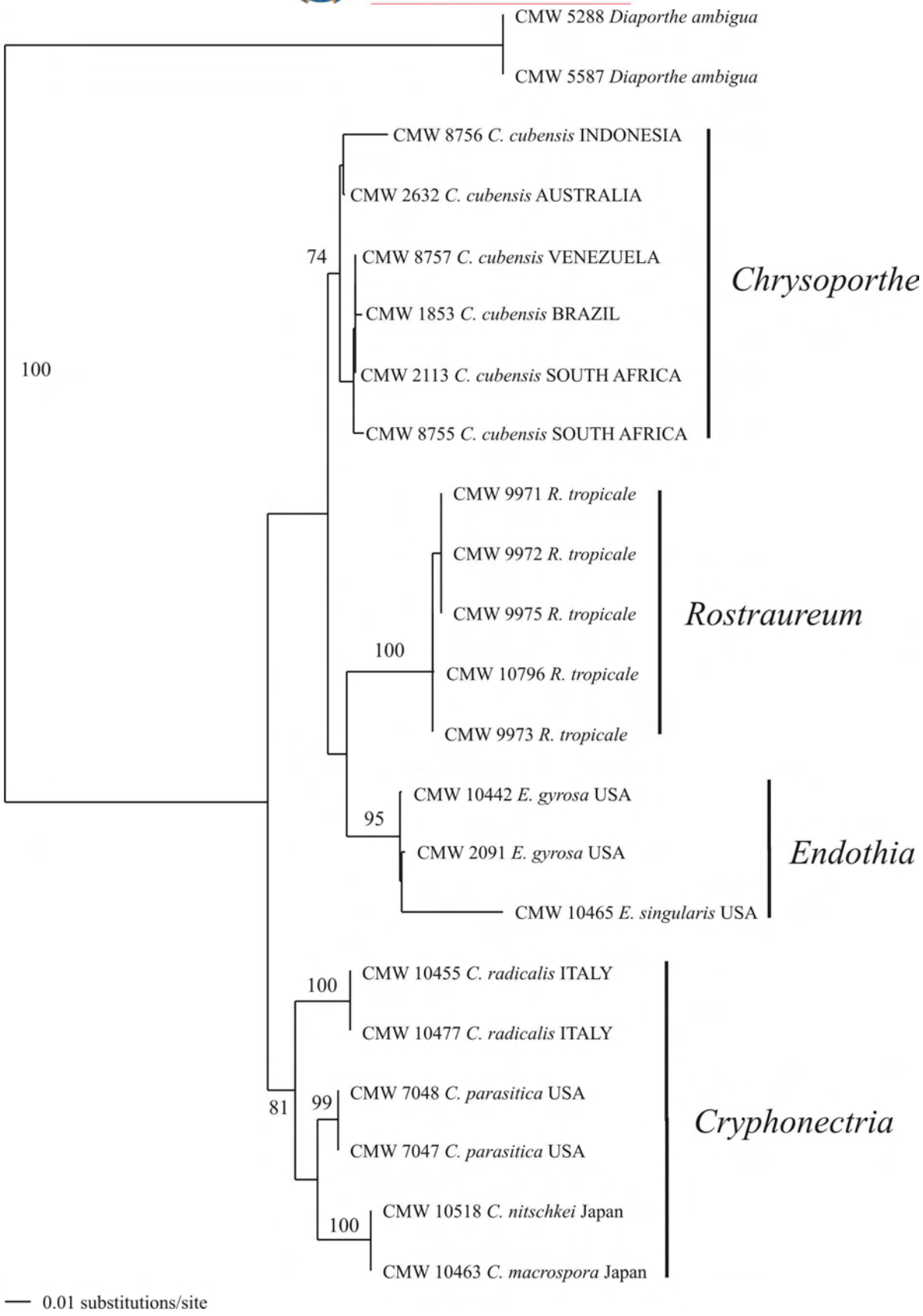

Fig. 6. Distance phylogram showing phylogenetic relationships between *Rostraureum*, *Cryphonectria*, *Chrysoporthe* and *Endothia* spp. based on β-tubulin DNA sequence. The phylogram was obtained with the Tamura Nei parameter model (I = 0.5437, G = 0.7905). Bootstrap values greater than 70 % (1000 replicates) are indicated at the branch nodes. The *Diaporthe ambigua* isolates were used as outgroup taxa to root the phylogenetic tree.

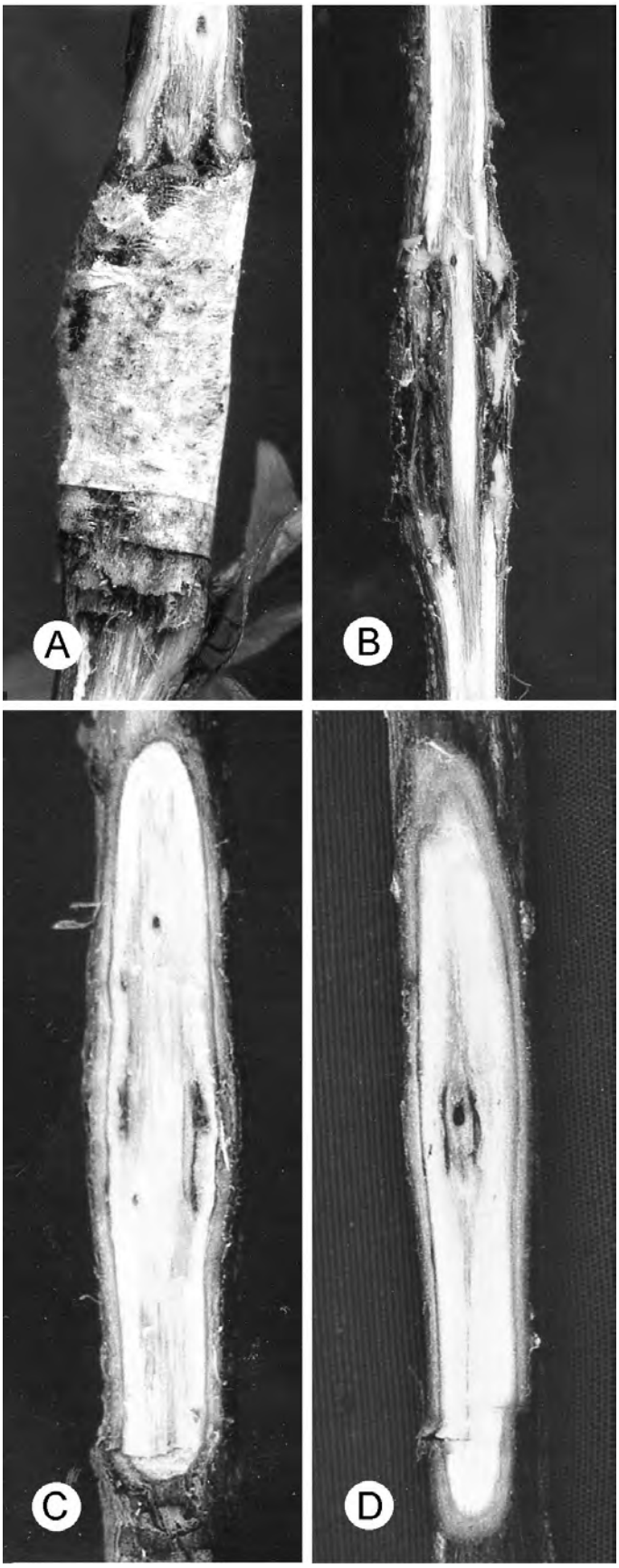

Fig. 7. Lesions associated with inoculation of the newly described *Rostraureum tropicale* and *Chrysoporthe cubensis* on *Terminalia ivorensis* in Ecuador. A. Fruiting structures formed on the canker resulting from inoculation with *R. tropicale*. B. Lesions associated with *R. tropicale*. C. Lesion development associated with *Chr. cubensis*. D. Control inoculation.

CHAPTER 8

Aurapex penicillata gen. sp. nov. from native *Miconia theaezans* and *Tibouchina* spp. in Colombia

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Aurapex penicillata gen. sp. nov. from native _Miconia theaezans_ and _Tibouchina_ spp. in Colombia

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Abstract: Conidiomata of a fungus resembling *Chrysoporthe cubensis*, a serious canker pathogen of *Eucalyptus* spp. (*Myrtaceae, Myrtales*) in tropical and subtropical parts of the world, was found on *Eucalyptus grandis* in Colombia. Fruiting structures of the fungus could be distinguished from those of *C. cubensis* by their distinctly orange conidiomatal necks. This fungus was also found on several plant species native to Colombia including *Tibouchina urvilleana*, *T. lepidota* and *Miconia theaezans* (*Melastomataceae, Myrtales*). Morphological comparisons as well as those based on sequences of the ITS1/ITS2 region of the ribosomal DNA repeat and the β-tubulin gene, were used to characterize this fungus. Its pathogenicity was also assessed on various plants from which it has been collected, either in field or greenhouse trials. Phylogenetic analyses showed that isolates reside in a clade distinct from the four clades accommodating *Chrysoporthe, Cryphonectria*, *Endothia* and *Rostraureum*. Members of this clade are distinguished by the presence of orange

conidiomatal necks with black bases, and a unique internal stromatal structure. No teleomorph has been found for this fungus, for which we have provided the name *Aurapex penicillata* gen. sp. nov. *A. penicillata* produced only small lesions after inoculation on young *T. urvilleana*, *M. theaezans* and *E. grandis* trees, and appears not to be a serious pathogen.

Taxonomical novelties: *Aurapex* Gryzenh. & M. J. Wingf. gen. nov. nom. prov., *Aurapex penicillata* Gryzenh. & M. J. Wingf. sp. nov. nom. prov.

Key words: *Aurapex*, *Chrysoporthe*, Colombia, *Diaporthales*, *Eucalyptus*, *Melastomataceae*

INTRODUCTION

The *Melastomataceae* represents a family of flowering plants common to Neotropical America and Hawaii (Everett 1981). This family resides in the *Myrtales*, which also accommodates the *Myrtaceae* (Conti *et al.* 1996). The *Myrtaceae* includes the genus *Eucalyptus*, many species of which are grown as a source of pulp and timber in plantations around the world (Turnbul 2000).

Chrysoporthe cubensis (Bruner) Gryzenh. & M. J. Wingf. (formerly *Cryphonectria*) is a serious canker pathogen of *Eucalyptus* spp. (Boerboom & Maas 1970, Hodges 1980, Sharma *et al.* 1985, Wingfield 2003) and *Syzygium aromaticum* (clove, also *Myrtaceae*) (Hodges *et al.* 1986), in the tropics and sub-tropics. Intriguingly, this pathogen has recently been shown to cause disease on members of the *Melastomataceae* such as *Miconia theaezans* (niguito) and *Miconia rubiginosa* (mortiño) in Colombia (Rodas *et al.* 2005). A second fungus, *Chrysoporthella*

hodgesiana Gryzenh. & M. J. Wingf., a species of *Chrysoporthe* based on phylogenetic data but known only by its anamorph, also occurs on Colombian *Melastomataceae* such as *M. theaezans* (Rodas *et al.* 2005), *Tibouchina urvilleana*, *Tibouchina lepidota* and *Tibouchina semidecandra* (Gryzenhout *et al.* 2004/Chapter 1 in this thesis). Recognition of *C. cubensis* on hosts residing in the *Melastomataceae* has substantially altered views regarding the origin and distribution of this important tree pathogen (Wingfield *et al.* 2001, Wingfield 2003, Rodas *et al.* 2005).

During the course of surveys in Colombia to assess the occurrence of *Chrysoporthe* spp. on trees other than *Eucalyptus* spp., a fungus similar to *C. cubensis* and *Chrysop. hodgesiana* was found on *Tibouchina* spp. These trees were planted as ornamentals in parks or on farms. The unknown fungus produced only conidiomata that were black and pyriform, in a shape reminiscent of *Chrysoporthe* spp. The fruiting bodies, however, differed from those of *Chrysoporthe* spp. in that the apices of the conidiomatal necks were orange. Subsequent surveys led to the discovery of the fungus on native *M. theaezans* as well as on *Eucalyptus grandis*. The aims of this study were to define the phylogenetic position of this fungus using DNA sequence comparisons, as well as to produce a taxonomic description and generic key. In addition, the pathogenicity of the new fungus was assessed in greenhouse and field inoculation experiments.

MATERIALS AND METHODS

Symptoms and collection of samples

Structures of the unknown fungus were first found in 1996 on *T. urvilleana* and *T. lepidota* in Colombia. These trees were growing in La Culebra park in El Peñol, on a

private farm near Granada (Antioquia Province), and on the Argentina farm of Smurfit Cartón de Colombia near Riosucio (Caldas Province). In a subsequent disease survey in 1998, similar fruiting structures were found on *Miconia theaezans* occurring in native vegetation on La Selva farm near Pereira (Risaralda province). In 2002, this fungus was discovered for the first time on basal cankers on *E. grandis* on the Libano farm near Pereira, as well as on *M. theaezans* at the same location.

The fruiting structures of the unknown fungus occurred around the periphery of cankers on the stems and branches of trees, which occasionally led to branch die-back. In some cases, fruiting structures of *C. cubensis* and *Chrysop. hodgesiana* occurred on the same plant. On *E. grandis*, fruiting structures of the fungus were also found on branches that were in the process of senescence, and the fungus also appeared to colonize branch stubs.

Bark specimens containing conidiomata were collected from cankers and taken to the laboratory for isolation. Single conidial isolates were obtained from spore suspensions on 2 % Malt Extract Agar (MEA) (20 g Biolab Malt Extract, 15 g Biolab Agar, 1 L water, Merck, Midrand, South Africa) and incubated at 25 ºC. The cultures have been maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa, and representative isolates have been deposited with the Centraalbureau voor Schimmelcultures (CBS), Utrecht, Netherlands (Table 1). Original bark specimens from which isolations had been made, were used for morphological characterization and have been deposited in the herbarium of the National Collection of Fungi, Pretoria, South Africa (PREM).

Morphology

Conidiomata were cut from bark specimens, rehydrated for one min in boiling water, and sectioned at –20 ºC with a Leica CM1100 Cryostat after embedding in Leica mountant (Setpoint Premier, Johannesburg, South Africa). Sections approximately 12 µm thick were mounted on microscope slides in lactophenol. Fifty measurements of ascospores, asci, conidia and conidiophores were taken for the holotype specimen and these are presented as (min–)(average – std. dev.) – (average + std. dev.)(–max) µm. Ten structures were sectioned to observe the internal morphology of the fruiting bodies and a range was obtained for the eustromatic bases, necks and conidial locules. Micrographs were taken with a HRc Axiocam digital camera and accompaning Axiovision 3.1 software (Carl Zeiss Ltd., Germany). The color charts of Rayner (1970) were used to define colors of cultures and morphological structures.

Culture morphology of the ex holotype strain CMW 10030 and CMW 11296 (Table 1), was characterized on MEA (20 g/L malt extract agar, Biolab, Merck). These studies were conducted using the technique described by Venter *et al.* (2002). Growth tests were at temperatures ranging from 15 to 35 ºC at 5 º intervals, and cultures were grown in the dark.

DNA sequence comparisons

Representative isolates of the fungus from Colombia were used in the DNA sequence comparisons (Table 1). Sequences of *C. cubensis* isolates from *Miconia* and *Eucalyptus* spp. in Colombia (Gryzenhout *et al.* 2004, Rodas *et al.* 2005), and *Chrysop. hodgesiana* isolates from *Miconia* and *Tibouchina* spp. in Colombia (Wingfield *et al.* 2001, Gryzenhout *et al.* 2004, Rodas *et al.* 2005) were also used. Sequences for isolates of *C. cubensis* from other parts of the world, those of

Chrysoporthe austroafricana Gryzenh. & M. J. Wingf. (Myburg *et al.* 2002a, 2003) and those for recognized members of *Cryphonectria* and *Endothia*, which are closely related to *Chrysoporthe* (Venter *et al.* 2002, Myburg *et al.* 2004a, 2004b), were also included (Table 1). Sequences from the recently described *Rostraureum tropicale* Gryzenh. & M. J. Wingf., a pathogen of *Terminalia* in Ecuador (Gryzenhout *et al.* 2005/Chapter 7 in this thesis), were included in the dataset. *Rostraureum* contains the fungus previously known as *Cryphonectria longirostris* (Earle) Micales & Stipes (Gryzenhout *et al.* 2005). Two *Diaporthe ambigua* Nitschke isolates, which also reside in the *Diaporthales* but have been shown to reside in a different family to that of *Cryphonectria* and related taxa (Castlebury *et al.* 2002), were used as a single outgroup to root the phylogenetic tree generated in this study. The sequence matrix (study accession number = S1128, matrix accession number = M1935) from the study of Myburg *et al.* (2004a) was used as template for the alignment.

DNA was isolated from the fungi as described in Myburg *et al.* (1999). PCR amplification of the ITS1, conserved 5.8S and ITS2 regions of the rRNA operon as well as two regions in the β-tubulin gene, was performed as described in Myburg *et al.* (1999) and Myburg *et al.* (2002a) respectively. Primer pairs ITS1/ITS4 (White *et al.* 1990) were used for the ITS1/ITS2 region, and primer pairs Bt1a/Bt1b and Bt2a/Bt2b respectively (Glass & Donaldson 1995) were used to amplify two β-tubulin gene regions. The PCR products were purified using a QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany). The purified PCR products were sequenced in both directions using the same primers that were used in the amplification reactions. Sequencing reactions were performed using a PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Warrington, U.K.). Nucleotide sequence data were generated with an ABI PRISM 3100™ automated DNA

sequencer. The raw sequence data were manipulated using the Sequence Navigator version 1.0.1 software package (Perkin-Elmer Applied BioSystems, Foster City, California).

Nucleotide sequences were manually aligned by inserting gaps. Gaps were treated as Newstate in the parsimony analyses, and as missing in the distance analyses. Phylogenetic analyses were performed using PAUP* (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford 2002). A Partition Homogeneity Test (Farris *et al.* 1994) was used to determine whether the ribosomal rRNA (ITS1, 5.8S, ITS2) partition and β-tubulin partition could be combined in the phylogenetic analyses. The aligned sequences were analyzed with parsimony by heuristic searches, with the tree-bisection-reconnection (TBR) and MULTREES options (saving all optimal trees) in effect, and sequences added randomly (100 additions). Uninformative sites were excluded and sites were re-weighted according to their individual Consistency Indices (CI) to reduce the number of trees obtained. A distance analysis was also performed using the Tamura-Nei parameter model (Tamura and Nei 1993) with adjusted settings (proportion of invariable site (I) = 0.4334; Gamma distribution (G) = 0.4592; Base frequency 0.2044, 0.3191, 0.2551; Rate matrix 1.00, 2.2953, 1.00, 1.00, 4.5168). This model was chosen as suggested by MODELTEST version 3.5 (Posada & Crandall 1998). Tree branch supports were assessed with a 1000-replicate bootstrap analysis. GenBank accession numbers of sequences generated in this study as well as those from previous phylogenetic studies are listed in Table 1. The DNA sequence alignment has been deposited in TreeBASE (SN 2261).

Pathogenicity tests

Two isolates of the newly described fungus (CMW 10031, CMW 10034) from *M. theaezans* were compared with isolates of *Chrysoporthe* spp. in a contained pathogenicity trial. One *Chrysoporthe* isolate (CMW 2113) represented *C. austroafricana* and has been used in previous pathogenicity tests (Myburg *et al.* 2002b, Van Heerden & Wingfield 2002). The other *Chrysoporthe* isolate (CMW 10639) represented *C. cubensis* from *E. grandis* in Colombia (Gryzenhout *et al.*. 2004).

Pathogenicity of the isolates was compared on each of ten seedlings of *T. urvilleana* and a susceptible *E. grandis* clone (ZG14) in a custom-built phytotron. These trees were approximately 1.5 m tall and 7-mo-old, and they were exposed to natural light conditions and an average daily temperature of ~ 25 ºC. Ten trees were inoculated at a constant height (~ 30 cm above the ground) with sterile water agar (WA, Biolab, Merck) plugs to serve as negative controls. Wounds were made on stems using a cork borer (6 mm diam) to expose the cambium. Discs of the same size were taken from the actively growing edges of colonies and inserted into the wounds with the mycelium facing inwards. Wounds were then covered with plastic film to prevent desiccation and contamination. Trees were inoculated in June 2002 and lesion development was evaluated after 6 wks by measuring lesion lengths below the outer bark.

A field trial to consider the pathogenicity of the fungus under natural conditions was carried out on *M. theaezans* trees and a susceptible clone (018) of *E. grandis* on the Libano farm, Pereira, Risaralda, Colombia. Twenty *E. grandis* and the same number of *M. theaezans* trees were inoculated with isolate CMW 10031 from *M. theaezans* in Colombia. Trees of the *E. grandis* clone were approximately 18-mo-

old (~ 9 m high), while the *M. theaezans* trees formed part of the natural vegetation growing in close proximity to the *E. grandis* trees and were of unknown age (~ 4–6 m high). Ten trees of each species were inoculated with WA as negative controls. Inoculations were carried out in the same way as those described for the phytotron inoculation, except that the inoculation wounds were 4 mm in diam and covered with masking tape. The trees were inoculated in June 2002 and results were evaluated 12 weeks later in September 2002. The lengths of the lesions produced in the cambium were measured and compared after removal of the bark. Data were compared using a one-way Analysis of Variance (ANOVA) computed using the SAS software package, v. 6 (2002). Mean lesion sizes together with 95 % confidence limits were presented graphically.

RESULTS

Morphology

Conidiomata of the fungus on bark specimens of *M. theaezans*, *T. urvilleana* and *E. grandis* were pyriform and superficial with fuscous black bases, and most similar to fruiting structures of *C. cubensis* (Hodges 1980, Myburg *et al.* 2002a, 2003, Gryzenhout *et al.* 2004). These structures were thus different to the anamorph structures of all genera in the *Diaporthales* with orange fruiting structures ie. *Cryphonectria*, *Endothia* and *Rostraureum*, which all have completely orange conidiomata (Myburg *et al.* 2004a, Gryzenhout *et al.* 2005). Conidiomata had fuscous-black, globose bases with long, slender necks (Figs 1A, 2A) that might be confused with those of *C. cubensis* in the absence of the orange neck apices. The conidiomatal base cells were *textura globosa*, umber to sienna when sectioned, with

thick walls, while the inner cells were prosenchymatous (Fig. 1C). This is similar to the basal tissue of conidiomata of *C. cubensis* (Myburg *et al.* 2002a, 2003, Gryzenhout *et al.* 2004). The minute [(2.5–)3–4(–4.5) × 1–1.5(–2) µm], aseptate conidia (Figs 1I, 2C) were also similar to those of *C. cubensis* (Hodges 1980, Myburg *et al.* 2002a, 2003, Gryzenhout *et al.* 2004).

The fungus from *M. theaezans*, *T. urvilleana* and *E. grandis* in Colombia could be distinguished from *C. cubensis* by various unique characteristics of the conidiomata as well as on the basis of cultural morphology (Table 2). Despite having basal tissue similar to that of *C. cubensis*, tissue surrounding the conidiomatal locules is dark, consisting of larger cells than those of the adjacent prosenchyma (Fig. 1C). Neck tissue differed from that found in *C. cubensis* and consisted of square cells at the outer edge, with *textura porrecta* cells at the center, and thinner *textura porrecta* cells lining the ostiolar canals (Figs 1E–1F). The tissue at the tips of the conidiomatal necks was orange and contained orange crystals (Figs 1A, 1G, 2A). Long, sterile hyphae similar to perithecial periphyses occurred in the ostiolar canals (Fig. 1F), but these are absent in *C. cubensis*. Unique protrusions consisting of three to several cell layers were also formed within the conidiomatal locule lining (Figs 1B, 1D, 2B). Cultures of the fungus from *Tibouchina* and *Miconia* spp. had an olivaceous to isabelline interior that differed from the creamy white cultures patched with cinnamon produced by *C. cubensis*.

No ascomata were observed that were known to have been produced by the newly discovered fungus. A few ascomatal structures were observed on specimen PREM 58575 from *M. theaezans*. However, these structures stained brown in 3 % KOH, and not purple as was the case for the conidiomata of the fungus being studied and other members of this group in the *Diaporthales* (Castlebury *et al.* 2002). These ascomata

probably represent a species of *Valsa* co-infecting this particular host, since the ascospores were allantoid and aseptate.

DNA sequence comparisons

The PCR products generated for the ribosomal and two β-tubulin gene regions were between 550 bp and 600 bp in size, respectively. The PHT test (P = 0.182) indicated no significant conflict between the two data sets for these gene regions, which were thus combined in the phylogenetic analyses. There were also no strongly supported conflicts between the trees obtained for the two gene regions separately. The sequence data set included 32 taxa of which the two *D. ambigua* isolates represented a single outgroup taxon. The β-tubulin dataset (total 952 bp including both regions) consisted of 543 constant, 32 variable parsimony-uninformative and 377 variable and parsimony-informative characters (g1 = –0.780817). The ITS dataset (total 573 bp) consisted of 338 constant, 29 variable parsimony-uninformative and 206 variable and parsimony-informative characters (g1 = –0.863275). The combined set amounted to a total of 1525 characters. The heuristic search produced ten trees (tree length = 1095.3 steps, consistency index of = 0.805, retention index of = 0.918) that differed only in branch length for isolates. The tree obtained with distance analyses showed the same clades as the trees obtained with parsimony, one of which was chosen for presentation (Fig. 3). The same groupings of isolates with equally high bootstrap support, were also obtained when ambiguously aligned portions, which mostly represented the introns of the β-tubulin dataset and the ITS1 region, were excluded from the analyses.

The isolates of the anamorphic fungus on *M. theaezans* formed a distinct clade (bootstrap support 100 %) among the other clades in the phylogenetic tree, although it was apparent that some degree of variation existed between isolates, some originating

from the same location but different fruiting structures. The clade representing the anamorphic fungus was most closely related to *Cryphonectria* (Fig. 3). The other clades represented different closely related genera, namely *Cryphonectria*, *Endothia* and *Rostraureum.* Therefore, the anamorphic fungus grouped separately from the isolates of *C. cubensis* and *Chrysop. hodgesiana* from *Miconia* and *Tibouchina* spp. in Colombia, which grouped within the *Chrysoporthe* clade (Fig. 3).

Taxonomy

The fungus found on *M. theaezans*, *T. urvilleana* and *E. grandis* from Colombia has clearly defined morphological features that distinguish it from *Chrysoporthella*, the anamorph genus of *Chrysoporthe*, to which it is morphologically most similar (Table 2). These features are unlike those of any other coelomycete genus. The fuscous black conidiomata are also different from the uniformly orange fruiting structures of *Cryphonectria*, *Endothia* and *Rostraureum*, although the necks of the undescribed fungus are orange. These differences are supported by DNA sequence data showing that isolates of the morphologically distinct fungus from Colombia group separately from those representing *Chrysoporthe*, *Cryphonectria*, *Endothia* and *Rostraureum*.

No teleomorph was found for the fungus considered in this study. The distinct grouping of the fungus from Colombia, however, indicates that the fungus represents a distinct genus, and not the anamorph of an already existing genus. Phylogenetic data present clear evidence of its affinity to members of *Cryphonectria* and allied genera residing in the *Diaporthales.* In the absence of a teleomorph, the fungus from Colombia cannot be described in an ascomycete genus (ICBN, Art. 59.2, Greuter *et al.* 2000). It is thus described as a new species in a new mitosporic genus, and the following description is provided.

Aurapex Gryzenh. & M. J. Wingf., **gen. nov., nom. prov.**

Etymology. Latin, *aureus*, golden, and *apex*, top, refers to the golden colored tips of the conidiomata.

Conidiomata globosa vel pyriformia, basibus fusconigris collis aurantiacis, superficialia. Collum e textura porrecta factum, cellulis parietalibus ostioli gracilioribus, in ora colli quadratis, intra canales ostiolares cum filamentis non septatis. Conidiophorae cylindricae vel ampulliformes, hyalinae. Cellulae conidiogenae phialidicae. Conidia obtusa, hyalina, non septata.

Conidiomata eustromatic, globose to pyriform base with one to several, long, cylindrical to attenuated necks with orange tips, superficial to slightly immersed, fuscous-black. *Tissue* at the edges of conidiomal bases of *textura globulosa*, with elongated cells adjacent to conidial lining and prosenchymatous tissue occurring in the center of the basal tissue. Tissue of necks made up of *textura porrecta* with cells lining the ostiole thinner, cells at edge of necks consisting of square cells. *Conidiophores* cylindrical to flask-shaped, hyaline, occasionally septate with or without lateral branches. *Conidiogenous cells* phialidic. *Conidia* obtuse, hyaline, aseptate.

Typus: *Aurapex penicillata* Gryzenh. & M. J. Wingf., 2006.

Aurapex penicillata Gryzenh. & M. J. Wingf., **sp. nov., nom. prov.** Figs 1–2

Etymology. *penicillus*, a painter's brush, refers to the brush-like protrusions formed by the lining of the conidial locules.

Conidiomata pyriformia cum collis, superficialia, basibus fusconigris collis aurantiacis, textura parietali loculorum prominentia e 3– circiter 15 cellulis formans. Textura colli in ora e cellulis quadratis, intus e textura porecta facta, intra canales ostiolares cum filamentis non septatis. Conidiophorae cylindricae vel ampulliformes apicibus attenuatis, hyalinae. Cellulae conidiogenae phialidicae. Conidia (2.5–)3–4(–4.5) × 1–1.5(–2) µm, obtusa, non septata, hyalina, in forma guttarum sporarum coccinearum exsudata. Coloniae cum hyphis aeriis sparsis, albocremet, intus atro-olivaceae vel isabellinae, celeriter crescentes. temperatura optima 25°C.

Conidiomata single or aggregated, eustromatic, with globose to pyriform bases and attenuated or cylindrical necks, base 120–400 µm high, 300–700 µm wide above bark surface, necks up to ~1800 µm long depending on environmental conditions, 80–225 µm wide, conidiomata superficial to slightly immersed, bases fuscous-black with tips of necks orange (Figs 1A, 1B, 2A, 2B). Unilocular or multilocular (Figs 1B, 2B), locules up to 360 µm diam at widest point, locule lining producing conidiophores forming protrusions consisting of 3 to ~15 cells (Figs 1B, 1D, 2B), locules opening through 1 to 3 necks, each either connected to a single locule or to more than one locule. *Tissue* of base complex with thick-walled cells, *textura globulosa*, umber to sienna at edge, cells around the locules sienna to hazel, larger and more elongated, and almost white prosenchymatous tissue occurring between the edge and the locule (Fig. 1C). Neck tissue consisting of hazel, double-walled, square cells at the edge, with the cells lining the ostiole thinner and those at the center of *textura porrecta* tissue (Figs 1E–1F), long, aseptate filaments, similar to periphyses, occurring inside the ostiolar canals (Fig. 1F), tip of necks of *textura epidermoidea*, containing orange

crystals (Fig. 1G). *Conidiophores* (6–)7.5–13.5(–18.5) × (0.5–)1–1.5(–2) µm, cylindrical or flask-shaped with attenuated apices, occasionally with separating septa and branching, hyaline (Figs 1H, 2C). *Conidiogenous cells* phialidic, determinate, apical or lateral on branches, collarette and periclinal thickening inconspicous (Figs 1H, 2C). *Conidia* (2.5–)3–4(–4.5) × 1–1.5(–2) µm, obtuse, aseptate, hyaline (Figs 1I, 2C), exuded as scarlet spore droplets.

Cultural characteristics: fluffy with few aerial hyphae, creamy white with a dark olivaceous to isabelline interior, margins even, conidiomata occasionally produced in mature cultures, optimum growth at 25 C, isolates covering the surface of 90 mm plates on day 6 at the optimum temperature.

Distribution: Colombia

Substrate: Miconia theaezans, Tibouchina urvilleana, Tibouchina lepidota, Eucalyptus grandis

Specimens examined. **Colombia**, Risaralda, Pereira, Libano farm (75º 35′ 49″ W and 4º 43′ 13″ N, 2102 msal), bark of *Miconia theaezans*, Sept. 2002, C. A. Rodas, **holotype** PREM 57520, ex-type culture CMW 10030 = CBS 115740, additional cultures CMW 10031, CMW 10034, CMW 10035 = CBS 115742; Libano farm, bark of *Eucalyptus grandis*, Sept. 2002, C. A. Rodas, PREM 58578; La Selva farm (75º 35′ 34″ W and 4º 47′ 26″ N, 2048 msal), bark of *Miconia theaezans*, Nov. 1998, C. A. Rodas, PREM 58572; Quindio, Salento, Andes farm (75° 33′ 16″ W and 4° 41′ 08″ N, 2102 masl), bark of *Miconia theaezans*, May 2000, M. J. Wingfield, PREM 58576, living cultures CMW 11296 = CBS 115801; Antioquia, Granada, Granada farm (75° 8′ 10″ W and 6° 6′ 52″ N, 2050 msal), bark of *Tibouchina urvilleana*, Nov. 1998, C. A. Rodas, PREM 58573; Caldas, Riosucio, La Argentina farm (75º 44′ 55″ W and 5º

22′ 25″ N, 2247 msal), bark of *Tibouchina urvilleana*, Nov. 1998, C. A. Rodas, PREM 58574, PREM 58575; Valle, Darien, Cedral farm (76º 26′ 06″ W and 3º 57′ 06″ N, 1825 masl), bark of *Eucalyptus grandis*, Dec 2001, C. A. Rodas, PREM 58577.

Key to *Cryphonectria, Endothia, Chrysoporthella, Rostraureum* and *Aurapex*

1a. Base of anamorph fruiting structures fuscous-black…..…… 2

1b. Anamorph fruiting structures completely orange……...……... 3

2a. Conidiomata uniformly fuscous-black, locule lining even…… ***Chrysoporthella***

2b. Conidiomata fuscous-black but with tip of neck orange, locule lining have brush-like protrusions…..... ***Aurapex***

3a. Conidiomata rostrate, superficial ……...……..... ***Rostraureum***

3b. Conidiomata pulvinate……..……..……... 4

4a. Conidiomata semi-immersed, ascospores uniseptate……...……..... ***Cryphonectria***

4b. Conidiomata superficial, ascospores aseptate……..……...……...…. ***Endothia***

Pathogenicity

Isolates of *A. penicillata* (CMW 10031, CMW 10034) produced small lesions on both *T. urvilleana* and the *E. grandis* clone in the phytotron trial. These lesions did not differ significantly from the control inoculations (Fig. 4). Conidiomata were produced abundantly on the surfaces of the lesions. Lesions associated with *A. penicillata* were significantly smaller ($P < 0.0001$) than those associated with *C. cubensis* and *C. austroafricana* on *E. grandis* and *T. urvilleana* with those of *C. austroafricana* (CMW 2113) on *E. grandis* longest (Fig. 4). The latter isolate was also

the only one observed to girdle inoculated stems resulting in the production of epicormic shoots below the sites of inoculation.

In the Colombian field trial, the isolate of *A. penicillata* (CMW 10031) gave rise to small lesions on *M. theaezans* that did not differ significantly from the control inoculations (Fig. 5). Lesions produced on the *E. grandis* clone did not differ significantly from those on *M. theaezans* (P = 0.0217) and were extremely variable (Fig. 5). Control inoculations on the *E. grandis* clone also gave rise to small lesions, possibly due to endophytes naturally present in the stems of trees (Fig. 5).

DISCUSSION

This study treats the discovery of a new mitosporic genus in the *Diaporthales*, which thus far contains a single species, *Aurapex penicillata*. All indications are that this fungus is native to South America where it occurs naturally on *Miconia* and *Tibouchina* spp. *Aurapex penicillata* is closely related to *Cryphonectria* and *Endothia*, and the recently described genera *Chrysoporthe* (anamorph *Chrysoporthella*) and *Rostraureum*, which contain species previously in *Cryphonectria*. This was established through DNA sequence comparisons of the ITS region of the ribosomal repeat and β-tubulin genes.

The distinction of *A. penicillata* based on DNA sequence comparisons as a genus separate from *Cryphonectria*, *Chrysoporthe*, *Endothia* and *Rostraureum*, and not an anamorph genus of one of the existing genera, is well supported by morphological characteristics. Although the teleomorph is unknown, the morphological characteristics of the asexual state differ substantially from those of its closest relatives. Conidiomata of *A. penicillata* are fuscous-black, superficial and

pyriform with attenuated necks. These resemble the conidiomata of *Chrysoporthella*, the anamorph of *Chrysoporthe* that also has fuscous black conidiomata. *Aurapex* can, however, easily be distinguished from *Chrysoporthella* based on the distinctly orange tips of the necks and by its unique stromatal tissue. This new fungus can also be distinguished from the conidiomata of *Cryphonectria*, *Endothia* and *Rostraureum* spp., which are completely orange in color. These anamorph differences are consistent with those found in previous studies (Venter *et al.* 2002, Myburg *et al.* 2004a, Gryzenhout *et al.* 2005). These studies showed that stromatic and anamorph morphology are the most informative morphological characters that support the different phylogenetic assemblages, even in the absence of sexual structures. Recognition of these phylogenetic assemblages as different genera is strongly supported by the fact that they are morphological very distinct and they would not comfortably reside in a single genus.

Aurapex penicillata could be confused with the serious pathogens *C. cubensis* and *Chrysop. hodgesiana*, which occur on the same hosts and in the same region. This is especially so when the characteristic orange colored necks of *A. penicillata* become dislodged from their conidiomatal bases. Moreover, fruiting structures of these different fungi can occur on the same piece of bark. Of these three fungi, only *C. cubensis* is known to cause serious disease on *Eucalyptus* (Wingfield 2003, Gryzenhout *et al.* 2004) although *Chrysop. hodgesiana* can also infect *Eucalyptus* trees in artificial inoculations (Wingfield *et al.* 2001, Gryzenhout *et al.* 2004).

Aurapex penicillata gave rise to lesions on *E. grandis* in pathogenicity tests, but there was no evidence of significant pathogenicity, at least in comparison to *C. cubensis*. Although *A. penicillata* occurs on *E. grandis* under natural conditions, the pathogen appears to be mainly associated with dead branch stubs. Because of

differences in pathogenicity and importance, it is necessary to identify *C. cubensis*, *Chrysop. hodgesiana* and *A. penicillata* correctly in *Eucalyptus* plantation disease surveys.

Pathogenicity tests conducted in this study should be seen as preliminary as they were limited by the lack of a complete series of known hosts of *A. penicillata*. Our primary objective was to assess pathogenicity, especially given the fact that the highly pathogenic *C. cubensis* has now been found to cause severe disease on *Melastomataceae* native to South America (Rodas *et al.* 2005). Although preliminary, our results show that *A. penicillata* probably poses no threat to *Eucalyptus* or *Melastomatacea.* Furthermore, the common occurrence of the fungus on native *Melastomataceae* in Colombia adds substance to our view that it is native to hosts in that family. Its ability to infect *Eucalyptus* spp. is probably opportunistic and related to the high levels of inoculum on surrounding native vegetation in Colombia.

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Table 1. Isolates of *Chrysoporthe*, *Cryphonectria*, *Endothia*, *Rostraureum* and *Aurapex penicillata* used in this study. Isolates sequenced in this study are in bold.

Identification	Culture no. [a]	Alternative isolate number[a]	Host	Origin	Collector	Genbank Accession no. [b]
Chrysoporthe cubensis	CMW 2632	—	*Eucalyptus marginata*	Australia	E. Davison	AF 046893, AF 273078, AF 375607
	CMW 1856	—	*Eucalyptus* sp.	Kauai, Hawaii	—	AY 083999, AY 084010, AY 084022
	CMW 11290	CBS 115738	*Eucalyptus* sp.	Indonesia	M.J. Wingfield	AY 214304, AY 214232, AY 214268
	CMW 10774	—	*Syzygium aromaticum*	Zanzibar, Tanzania	—	AF 492130, AF 492131, AF 492132
	CMW 9432	CBS 115724	*Eucalyptus grandis*	Mexico	M.J. Wingfield	AY 692321, AY 692324, AY 692323
	CMW 8757	—	*Eucalyptus* sp.	Venezuela	M.J. Wingfield	AF 046897, AF 273069, AF 273464
	CMW 1853	—	*Syzygium aromaticum*	Brazil	—	AF 036891, AF 273070, AF 273465

	CMW 10639	CBS 115747	*E. grandis*	Colombia	C.A. Rodas	AY 263419, AY 263420, AY 263421
	CMW 9993	CBS 115728	*Miconia theaezans*	Colombia	C.A. Rodas	AY 214298, AY 214226, AY 214262
	CMW 10024	CBS 115739	*Miconia rubiginosa*	Colombia	C.A. Rodas	AY 262390, AY 262394, AY 262398
Chrysoporthella hodgesiana	CMW 9927	—	*Tibouchina urvilleana*	Colombia	C.A. Rodas, M.J. Wingfield	AF 265653, AF 292034, AF 292037
	CMW 9995	CBS 115730	*T. urvilleana*	Colombia	R. Arbelaez	AY 956969, AY 956977, AY 956978
	CMW 10625	CBS 115744	*M. theaezans*	Colombia	C.A. Rodas	AY 956970, AY 956979, AY 956980
	CMW 10641	CBS 115854	*Tibouchina semidecandra*	Colombia	R. Arbaleaz	AY 692322, AY 692326, AY 692325
Chrysoporthe austroafricana	CMW 2113	CBS 112916	*E. grandis*	South Africa	M.J. Wingfield	AF 046892, AF 273067, AF 273462
	CMW 8755	—	*E. grandis*	South Africa	M.J. Wingfield	AF 292040, AF 273064, AF 273459
Rostraureum tropicale	CMW 9973	CBS 115726	*Terminalia ivorensis*	Ecuador	M.J. Wingfield	AY 167427, AY 167432, AY 167437
	CMW 9975	CBS 115727	*T. ivorensis*	Ecuador	M.J. Wingfield	AY 167429, AY 167434, AY 167439

	CMW 10796	CBS 115757	*T. ivorensis*	Ecuador	M.J. Wingfield	AY 167428, AY 167433, AY 167438
Aurapex penicillata	**CMW 10030** (ex-type designated here)	CBS 115740	*M. theaezans*	Colombia	C.A. Rodas	AY 214311, AY 214239, AY 214275,
	CMW 10031	—	*M. theaezans*	Colombia	C.A. Rodas	AY 994511, AY 994513, AY 994514
	CMW 10034	—	*M. theaezans*	Colombia	C.A. Rodas	AY 994512, AY 994515, AY 994516
	CMW 10035	CBS 115742	*M. theaezans*	Colombia	C.A. Rodas	AY 214313, AY 214241, AY 214277,
	CMW 11296	CBS 115801	*M. theaezans*	Colombia	C.A. Rodas	AY 214315, AY 214243, AY 214279
Cryphonectria radicalis	CMW 10455	CBS 238.54	*Quercus suber*	Italy	A. Biraghi	AF 452113, AF 525705, AF 525712
Cryphonectria parasitica	CMW 7047	ATCC 48197	*Quercus virginiana*	USA	R.D. Wolfe	AF 368329, AF 273073, AF 273469
Cryphonectria nitschkei	CMW 13742	MAFF 410570	*Quercus grosseserrata*	Japan	T. Kobayashi	AY 697936, AY 697961, AY 697962
Cryphonectria macrospora	CMW 10463	CBS 112920	*Castanopsis cuspidata*	Japan	T. Kobayashi	AF 368331, AF 368351, AF 368350

Endothia gyrosa	CMW 2091	ATCC 48192	*Quercus palustris*	USA	R.J. Stipes	AF 046905, AF 368337, AF 368336
Endothia gyrosa	CMW 10442	—	*Q. palustris*	USA	R.J. Stipes	AF 368326, AF 368339, AF 368338
Diaporthe ambigua	CMW 5288	CBS 112900	*Malus domestica*	South Africa	W.A. Smit	AF 543817, AF 543819, AF 543821
	CMW 5587	CBS 112901	*M. domestica*	South Africa	W.A. Smit	AF 543818, AF 543820, AF 543822

[a] CMW = Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria; CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; ATCC = American Type Culture Collection, Manassas, USA; MAFF = Microorganisms Section, MAFF GENEBANK, National Institute of Agrobiological Sciences (NIAS), MAFF Gene Bank, Ibaraki, Japan.

[b] Given as sequences from the ITS region, and regions from the β-tubulin genes amplified with primers 1a/1b and 2a/2b respectively.

Table 2. Comparison of morphological features distinguishing *Aurapex penicillata* from *Chrysoporthe* spp.

Morphological features	***Chrysoporthe* spp.**[a]	***Aurapex penicillata***
Stromatic tissue	Cells surrounding locule similar to those in center.	Cells surrounding locule darker, larger.
Neck	Uniformly fuscous-black.	Orange apex.
Neck tissue	Cells at edge *textura globulosa*.	Cells at edge square.
Ostiolar canal	Contains no periphyses.	Contains periphyses.
Locule lining	Even to convoluted.	Forms protrusions consisting of three to several cell layers.
Culture morphology	Creamy white with cinnamon patches.	Creamy white with olivaceous to isabelline interior.

[a] According to Myburg *et al.* (2002a, 2003), Gryzenhout *et al.* (2004).

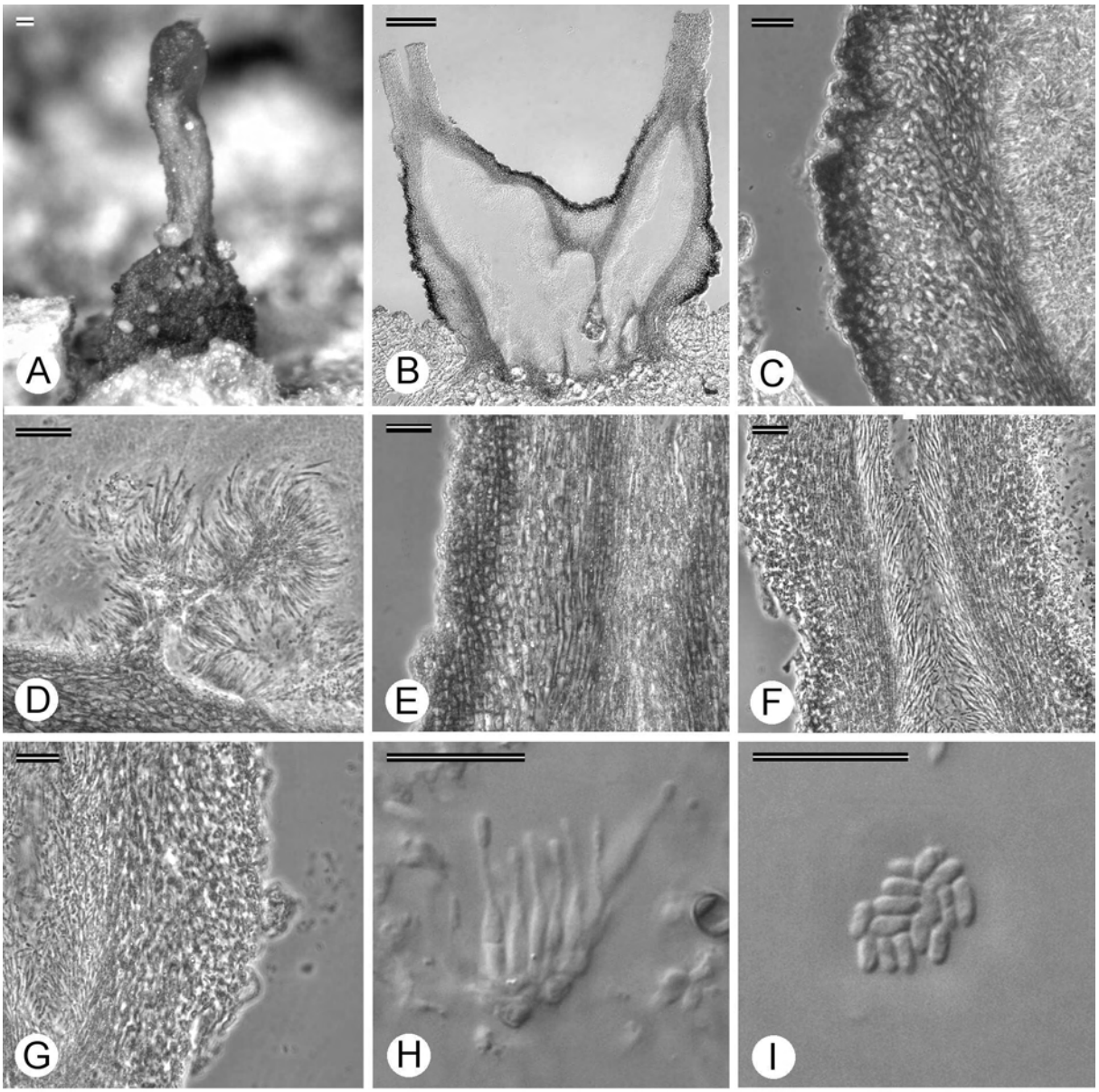

Fig. 1. Fruiting structures of *Aurapex penicillata*. A. Conidiomata on bark and in section (B). C. Tissue at base of conidioma. D. Protrusions in locule lining. E. Tissue of neck and periphyses in ostiolar canal (F). G. Tissue of neck apex. H. Conidiophores. I. Conidia. Scale bars A–B = 100 µm; C–G = 20 µm; H–I = 10 µm.

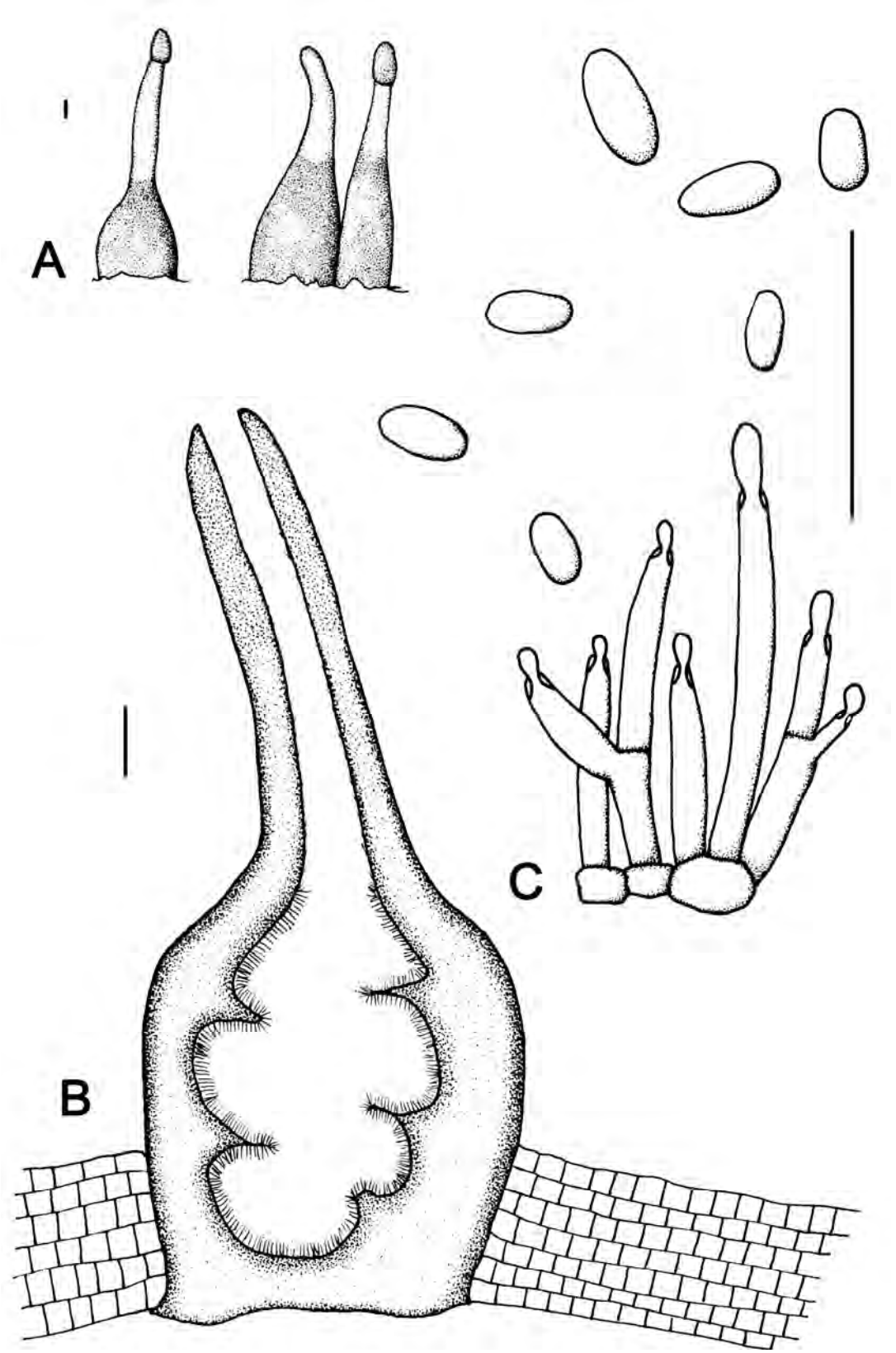

Fig. 2. Schematic drawings of *Aurapex penicillata*. A. Conidiomata on bark. B. Section through conidioma. C. Conidiophores and conidia. Scale bars A–B = 100 µm; C = 10 µm.

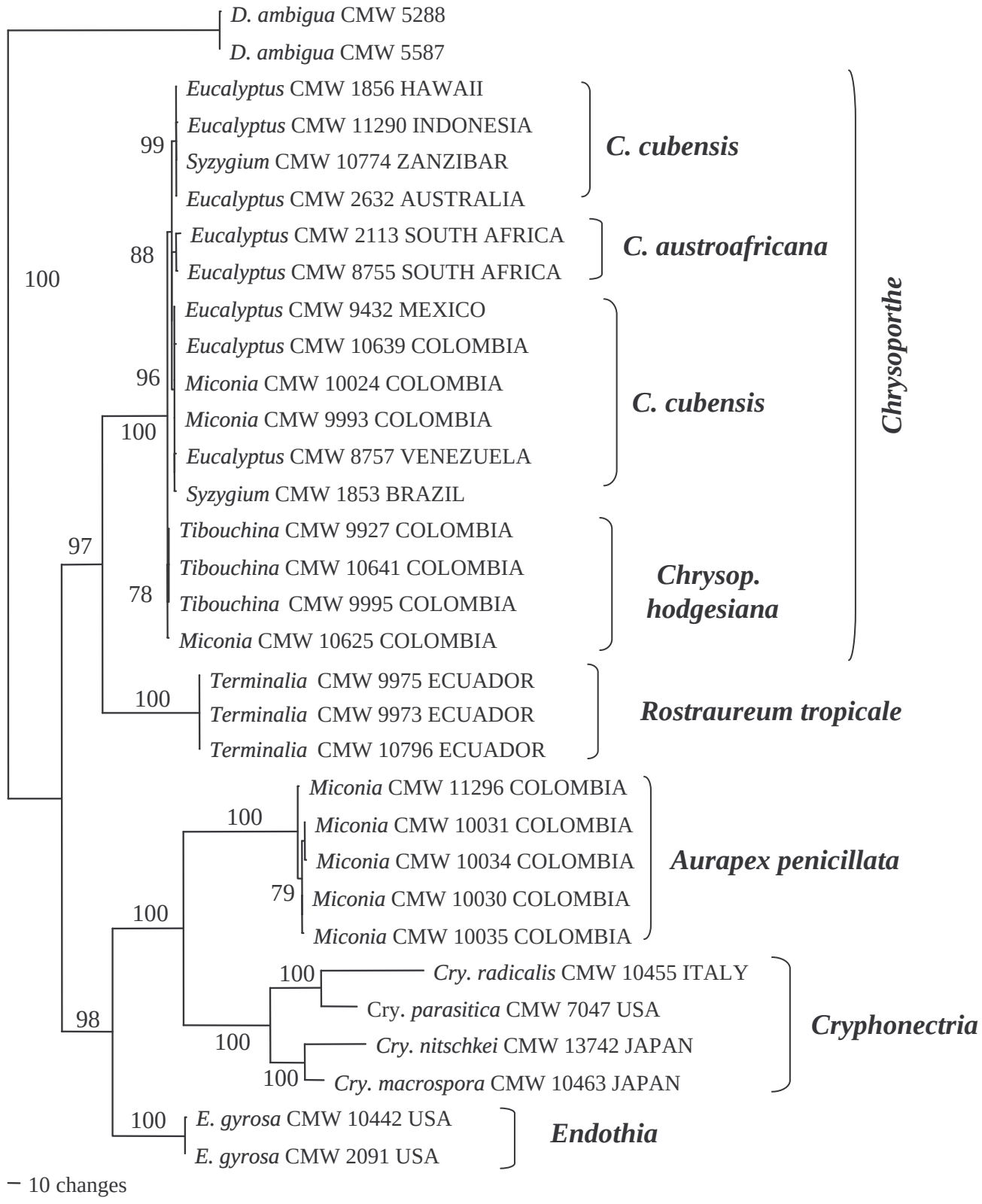

Fig. 3. One of ten most parsimonious trees obtained from a combined data set comprising ribosomal and β-tubulin gene sequences (tree length = 1095.3 steps, consistency index of = 0.805, retention index of = 0.918). Isolates representing the genera *Chrysoporthe*, *Rostraureum*, *Aurapex*, *Cryphonectria* and *Endothia* are represented. Confidence levels >70% determined by a 1000 replicate bootstrap analysis are indicated on the tree branch nodes. Two sequences for *Diaporthe ambigua* were used as an outgroup.

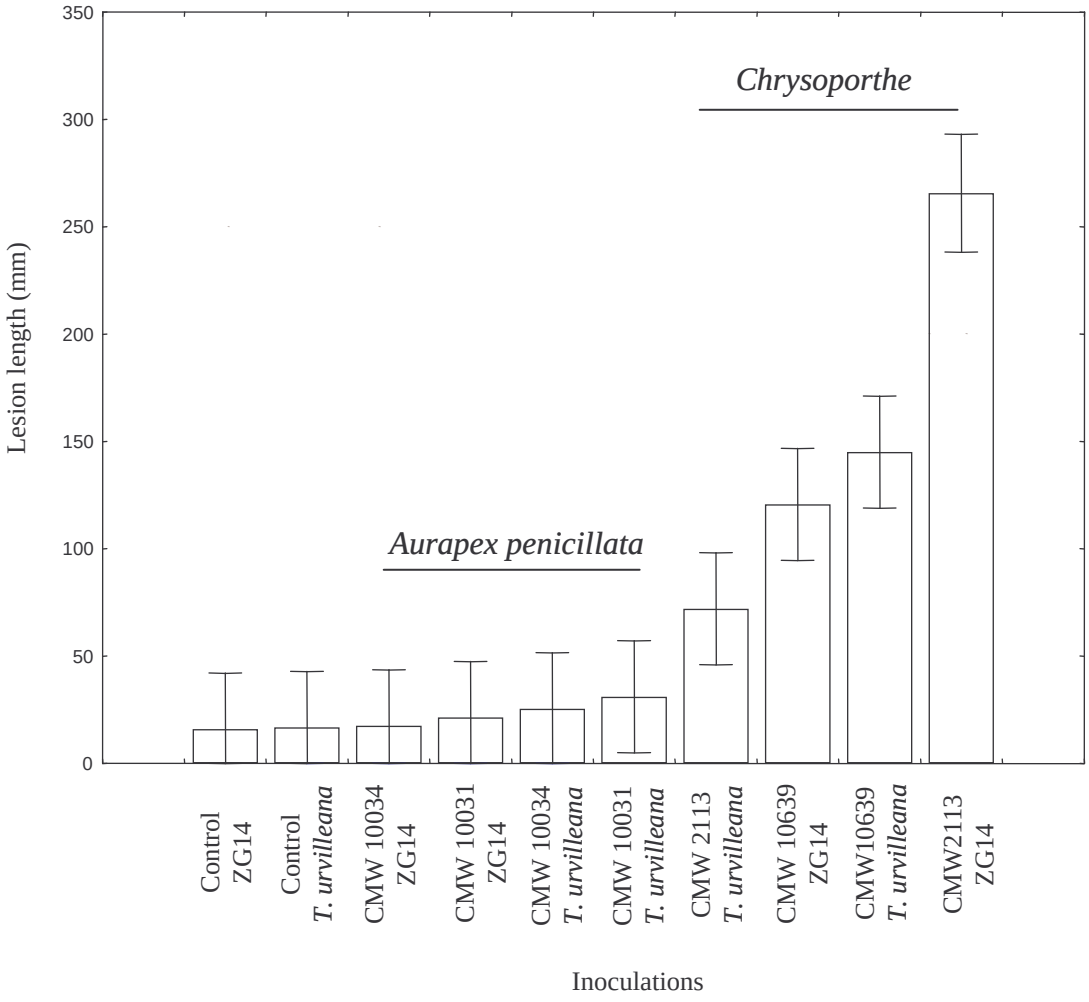

Fig. 4. Mean lesion length in *Tibouchina urvilleana* and a ZG14 clone of *Eucalyptus grandis* resulting from greenhouse inoculations with *Aurapex penicillata* (CMW 10031, CMW 10034), *Chrysoporthe cubensis* (CMW 10639), *Chrysoporthe austroafricana* (CMW 2113) and a negative control. Means are shown with 95% confidence limits.

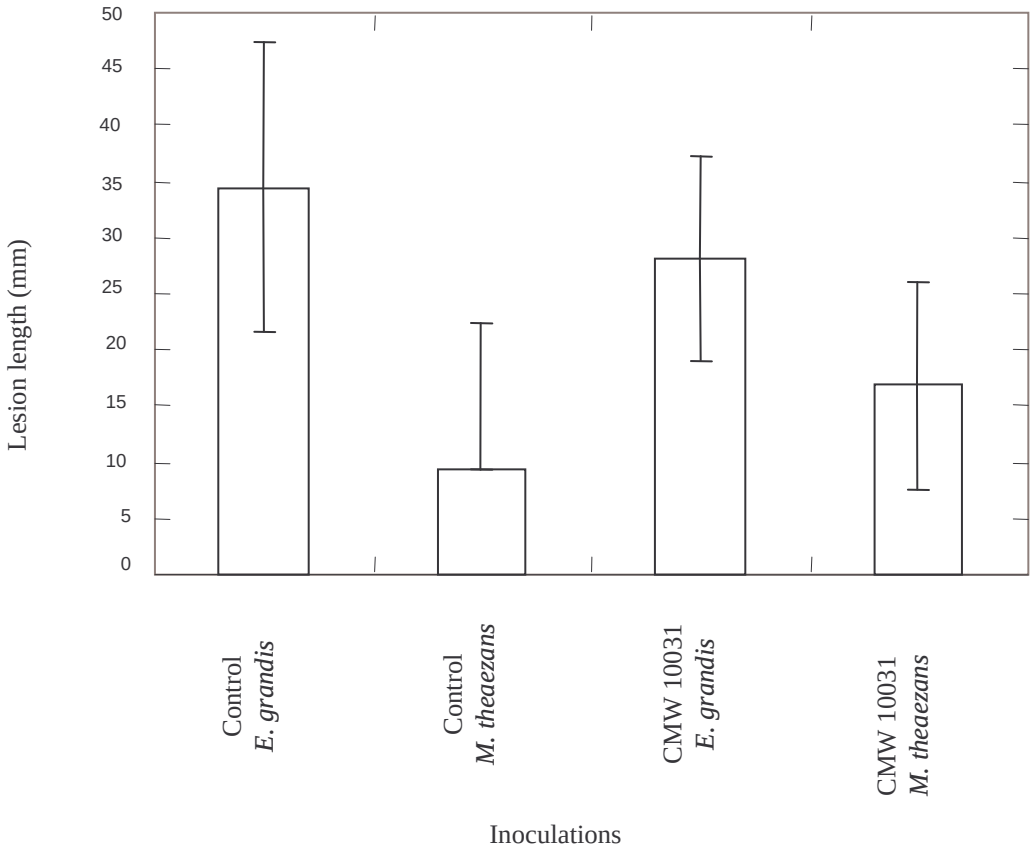

Fig. 5. Comparison of mean lesion length resulting from field inoculations with *Aurapex penicillata* (CMW 10031) and a negative control in *Miconia theaezans* and a susceptible clone (018) of *Eucalyptus grandis*. Means are shown with 95% confidence limits.

CHAPTER 9

Microthia, *Holocryphia* and *Ursicollum*, three new genera on *Eucalyptus* and *Coccoloba* for fungi previously known as *Cryphonectria*

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Microthia, *Holocryphia* and *Ursicollum*, three new genera on *Eucalyptus* and *Coccoloba* for fungi previously known as *Cryphonectria*

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Abstract: *Cryphonectria havanensis* is a fungus associated with *Eucalyptus* species in Cuba and Florida (U.S.A.). Until recently, there have been no living cultures of *C. havanensis* and it has thus not been possible to assess its taxonomic status. Isolates thought to represent this fungus have, however, emerged from surveys of *Eucalyptus* in Mexico and Hawaii (U.S.A.). Results of this study showed that these isolates represent *C. havanensis* but reside in a genus distinct from *Cryphonectria sensu stricto*, which is described here as *Microthia*. Isolates of an unidentified fungus occurring on *Myrica faya* in the Azores and Madeira also grouped in *Microthia* and were identical to other *M. havanensis* isolates. *Cryphonectria coccolobae*, a fungus occurring on sea grape (*Coccoloba uvifera*) in Bermuda and Florida, was found to be morphologically identical to *Microthia* and is transferred to this genus, but as a

distinct species. Surveys for *M. coccolobae* on sea grape in Florida, yielded a second diaporthalean fungus from this host. This fungus is morphologically and phylogenetically distinct from *M. coccolobae* and other closely related taxa and is described as *Ursicollum fallax* gen. et sp. nov. Phylogenetic analyses in this study have also shown that isolates of *C. eucalypti*, a pathogen of *Eucalyptus* in South Africa and Australia, group in a clade separate from all other groups including that representing *Cryphonectria sensu stricto*. This difference is supported by the fact that *Cryphonectria eucalypti* has ascospore septation different to that of all other *Cryphonectria* species. A new genus, *Holocryphia*, is thus erected for *C. eucalypti*.

Taxonomic novelties: *Microthia* Gryzenh. & M. J. Wingf. gen. nov., *Microthia havanensis* (Bruner) Gryzenh. & M. J. Wingf. comb. nov., *Microthia coccolobae* (Vizioli) Gryzenh. & M. J. Wingf. comb. nov., *Holocryphia* Gryzenh. & M. J. Wingf. gen. nov., *Holocryphia eucalypti* (M. Venter & M. J. Wingf.) Gryzenh. & M. J. Wingf. comb. nov., *Ursicollum* Gryzenh. & M. J. Wingf. gen. nov., *Ursicollum fallax* Gryzenh. & M. J. Wingf. sp. nov.

Key words: *Cryphonectria coccolobae, Cryphonectria eucalypti, Cryphonectria havanensis, Diaporthales*, Phylogeny

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INTRODUCTION

Cryphonectria havanensis (Bruner) M.E. Barr was first described from *Eucalyptus* spp. (*Myrtaceae, Myrtales*) in Cuba (Bruner 1916). Bruner (1916) found this fungus on bark of dead, injured or healthy *Eucalyptus* trees, but it did not appear to cause disease. *Cryphonectria havanensis* was also found on dead branches of mango

(*Mangifera indica*, *Anacardiaceae*, *Sapindales*) and avocado (*Persea gratissima*, *Lauraceae*, *Laurales*) lying on the ground in the vicinity of the *Eucalyptus* trees (Bruner 1916). Besides these exotic hosts, fruiting structures of *C. havanensis* were also found on the bark of jobo (*Spondias mombin*, *Anacardiaceae*, *Sapindales*), a plant native to Cuba (Bruner 1916).

Barnard *et al.* (1987) found *C. havanensis* on *Eucalyptus* plantations in Florida. The fungus was, however, reported as *Cryphonectria gyrosa* (Berk. & Broome) Sacc., a name previously used for the species (Kobayashi 1970, Hodges 1980). The identification of the fungus as *C. havanensis* was based on the presence of orange stromata, as well as conidial and ascospore dimensions that resembled those of the type specimen from Cuba. *Chrysoporthe cubensis* (Bruner) Gryzenh. & M.J. Wingf., a fungus previously known as *Cryphonectria cubensis* (Bruner) Hodges (Gryzenhout *et al.* 2004) and a serious pathogen of *Eucalyptus* spp. (Wingfield 2003), was also found in the same plantations (Barnard *et al.* 1987). *Cryphonectria havanensis* was mainly associated with dead coppice shoots in stands of *Eucalyptus grandis* while *Chr. cubensis* was the causal agent of basal cankers and death of coppice shoots (Barnard *et al.* 1987).

Other than reports from tropical or sub-tropical areas of the world such as Cuba and Florida, the name *C. havanensis* has also been used for collections of a fungus from *Eucalyptus globulus* in Japan (Kobayashi & Itô 1956, Kobayashi 1970). The fungus referred to as *C. havanensis* in Japan is also known from other host genera besides *Eucalyptus* (Kobayashi 1970), namely species of *Quercus* (*Fagaceae*, *Fagales*), *Betula* (*Betulaceae*, *Fagales*) and *Pyrus* (*Rosaceae*, *Rosales*). A recent study employing DNA sequence comparisons (Myburg *et al.* 2004a) showed that the fungus referred to as *C. havanensis* in Japan is the same as *Cryphonectria nitschkei*

(G. H. Otth) M.E. Barr. The study by Myburg *et al.* (2004a) did not, however, consider whether *C. nitschkei* is the same as the fungus referred to as *C. havanensis* from Cuba, where *C. havanensis* was originally described (Bruner 1916).

Cryphonectria havanensis and four other fungi in the *Diaporthales* with orange stromatic tissue are known from islands in the Caribbean Sea and Atlantic Ocean (Fig. 1). *Chrysoporthe cubensis* is well-known from several countries in Central and South America (Gryzenhout *et al.* 2004), including Cuba (Bruner 1917) where *C. havanensis* was first discovered. *Cryphonectria coccolobae* (Vizioli) Micales & Stipes occurs as a saprobe on twigs, branches and seeds of *Coccoloba uvifera* (sea grape, *Polygonaceae*, *Polygonales*) from Bermuda (Vizioli 1923) and Florida (Micales & Stipes 1987, Barnard *et al.* 1993). In the Azores and Madeira, an unidentified species of *Cryphonectria* has been associated with cankers on *Myrica faya* (*Myricaceae*, *Myricales*) (Gardner & Hodges 1990, Hodges & Gardner 1992). Another closely related species, *Cryphonectria longirostris* (Earle) Micales & Stipes, occurs in Puerto Rico and Trinidad (Earle 1901, Roane 1986). This fungus is saprobic and has recently been transferred to the new genus *Rostraureum* (Gryzenhout *et al.* 2005a). *Rostraureum* also includes a second new species, *Rostraureum tropicale* Gryzenh. & M.J. Wingf., which is a pathogen of *Terminalia ivorensis* trees in Ecuador (Gryzenhout *et al.* 2005a).

The correct identity of *C. havanensis* and its phylogenetic relationship with species of *Cryphonectria* and closely related genera remained unresolved (Myburg *et al.* 2004b). This is largely due to the absence of isolates that could, with reasonable certainty, be attributed to this species. The same problem was true for *C. coccolobae* (Myburg *et al.* 2004b), which has been suspected to be a synonym of *C. havanensis* (Hodges & Gardner 1990). The relationship between *C. havanensis* and the fungus

attributed to this species from Japan (Myburg *et al.* 2004a) also remains to be resolved.

Recently, fungi closely resembling *C. havanensis* were found on *Eucalyptus* spp. in Mexico and Hawaii, where this fungus had not been known previously. These collections included cultures and specimens on bark and enabled us to reconsider questions relating to the identity and the phylogenetic position of *C. havanensis*.

MATERIALS & METHODS

Symptoms and collection of samples

Fruiting structures thought to represent *C. havanensis* were collected from cankers and dead trees on the stems of *E. grandis* and an unidentified *Eucalyptus* sp. on the island of Kauai (Hawaii, U.S.A.). Fruiting structures of *Chr. cubensis* were also found on the stems of the same *Eucalyptus* spp. in the plantation, but were associated with cankers on living trees. *Chrysoporthe cubensis* was also common on cankered *E. grandis* trees on the island of Hawaii. Specimens of this fungus previously examined from the Hawaiian Islands were all from Kauai (Hodges *et al.* 1979, Myburg *et al.* 2003), and collections made in this study represent the first record of *Chr. cubensis* from the island of Hawaii.

Bark tissue bearing orange fruiting structures resembling *C. havanensis* was also collected from cankers on *E. grandis* in Las Chiapas, Mexico. An additional isolate from Mexico was received from Dr. E.L. Barnard (Florida Division of Forestry, FDACS, Gainesville, Florida). An isolate (ATCC 60862 = CMW 14332) representing *C. havanensis* (collected as *C. gyrosa*) from *Eucalyptus* plantations in Florida, linked to the study of Barnard *et al.* (1987), was acquired from the American

Type Culture Collection (ATCC). Isolates and specimens (Tables 1–2) linked to the report of a *Cryphonectria* species from *M. faya* in the Azores (Gardner & Hodges 1990) were also included in this study. This collection also included authentic isolates (Table 1) of *C. parasitica* from *Castanea sativa* in the Azores (Gardner & Hodges 1990). Unfortunately, no isolates of *C. havanensis* could be obtained from Cuba despite surveys aimed at re-collecting the fungus in that country.

During surveys for *C. coccolobae* on *Co. uvifera* in Florida, a fungus with distinctive orange fruiting structures was found in the vicinity of Fort Lauderdale, Key Biscayne, Dania and Oakland Park (Tables 1–2). This fungus was fruiting profusely on branches and twigs, but was not associated with disease symptoms. It was included in this study to determine whether it represents *C. coccolobae.*

Isolations from fungal structures on bark specimens were made from single conidia and ascospores collected from the apices of pycnidia and perithecia, respectively. The isolates used in this study are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa and representative isolates not originally obtained from internationally recognised collections have been deposited with the Centraalbureau voor Schimmelcultures, Utrecht, Netherlands (Table 1). The original bark specimens from which cultures were made have been deposited in the National Collection of Fungi (PREM), Pretoria, South Africa (Table 2).

DNA sequence comparisons

DNA was extracted from isolates grown in malt extract broth (20 g/L malt extract, Biolab, Midrand, South Africa) as described by Myburg *et al.* (1999). DNA sequences were derived for the internal transcribed spacer (ITS) regions ITS1 and

ITS2, including the conserved 5.8S gene of the ribosomal RNA (rRNA) operon, using primer pair ITS1/ITS4 (White *et al.* 1990), and β-tubulin genes using the primer pairs Bt1a/Bt1b and Bt2a/Bt2b respectively (Glass & Donaldson 1995). For these, the protocols of Myburg *et al.* (1999) and Myburg *et al.* (2002), respectively, were followed. Purification of PCR products for subsequent sequence reactions was done using a QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany). Sequence reactions were performed with the same primers used in the PCR reactions, using the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase (Perkin-Elmer, Warrington, UK). The sequencing reactions were run on an ABI PRISM 3100™ automated DNA sequencer. Nucleotide sequences were analysed using Sequence Navigator v. 1.0.1 (Perkin-Elmer Applied BioSystems, Inc., Foster City, California, U.S.A.) software.

New sequences were submitted to GenBank (Table 1). These also included sequences obtained in this study of additional *Cryphonectria eucalypti* M. Venter & M.J. Wingf. isolates to strengthen the *C. eucalypti* clade presented by Myburg *et al.* (2004b). This fungus is a pathogen of *Eucalyptus* trees in South Africa (Van der Westhuizen *et al.* 1993, Gryzenhout *et al.* 2003) and Australia (Walker *et al.* 1985, Yuan & Mohammed 2000). The sequences were compiled into a matrix using a modified data set (TreeBASE accession numbers S1128, M1935) of Myburg *et al.* (2004b) as a template. Additional sequences from other studies were also added to the data matrix. These included sequences of *Chrysoporthella hodgesiana* Gryzenh. & M.J. Wingf. (Gryzenhout *et al.* 2004, Rodas *et al.* 2005), and those of *Cryphonectria parasitica* (Murrill) M.E. Barr, *Cryphonectria macrospora* (Tak. Kobay. & Kaz. Itô) M.E. Barr and *C. nitschkei* from Japan, including those of isolates referred to as *C. havanensis* (Myburg *et al.* 2004b). Sequences representing *R.*

tropicale (Gryzenhout *et al.* 2005a) and *Amphilogia gyrosa* (Berk. & Broome) Gryzenh. & M.J. Wingf., the new genus that now contains *Cryphonectria gyrosa* (Gryzenhout *et al.* 2005b), were also added. The resultant dataset was deposited with TreeBASE (S***, M***).

The alignment was obtained using the web interface (http://timpani.genome.ad.jp/%7Emafft/server/) of the alignment program MAFFT v. 5.667 (Katoh *et al.* 2002). Phylogenetic analyses were made using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2002). A 500 replicate partition homogeneity test (PHT) was done on the rRNA and β-tubulin gene sequence data sets (after the exclusion of uninformative sites) to determine whether they could be analysed collectively (Farris *et al.* 1994). Phylogenetic analyses included parsimony and distance methods. Maximum parsimony (MP) was inferred using the heuristic search option with the tree-bisection-reconnection (TBR) branch swapping and MULTREES options (saving all optimal trees) effective and a 100 random additions. Gaps inserted during manual sequence alignment were treated as fifth character (NEWSTATE) in the heuristic searches, and missing in distance analyses. Uninformative characters were excluded and remaining characters were reweighted according to the individual Consistency Indices (CI) to reduce the number of trees. For the distance analyses, the correct model for the datasets was found with MODELTEST v. 3.5 (Posada & Crandall 1998). This model was the Tamura-Nei model (TrN+G+I) (Tamura & Nei 1993) with the Gamma distribution shape parameter (G) set to 0.9717 and frequency of invariable sites (I) 0.4643; base frequencies of 0.1903, 0.3411, 0.2301 and 0.2385; and rate matrix of 1, 3.1147, 1, 1, 4.1643, 1. Support for the branch nodes in the various phylogenetic trees was tested with a 1000 replicate bootstrap analysis and is presented as a 70 % majority rule tree.

Morphology

A large number of specimens from different species, hosts and geographical areas were included in the morphological comparisons (Table 2). These included the type specimen of *C. havanensis* (BPI 614275). Conidiomata and ascostromata were cut from bark specimens, rehydrated (1 min) in boiling water and sectioned with a Leica CM1100 cryostat at –20 ºC, 12–14 µm thick. For embedding, Leica mountant (Setpoint Premier, Johannesburg, South Africa) was used, which was dissolved in water after sectioning. Lactic acid (85 %) was used to prepare semi-permanent slides. Hand sections were made with a razor blade to more closely study conidiophore morphology. Fruiting structures were also mounted in 3 % KOH when conidiophores and asci could not easily be observed. Twenty measurements of ascospores, asci, conidia and conidiophores suspended in lactic acid or KOH, were taken for the specimens and these are presented as (min–)(average – std. dev.) – (average + std. dev.)(–max) µm. For the eustromata and perithecia, a size range from the largest and smallest structures was obtained. Colours were assigned to structures using the charts of Rayner (1970).

For growth studies, colony growth was assessed on 90 mm diam plates of MEA (20 g/L malt extract agar, Biolab, Merck, Midrand, South Africa). Four plates were inoculated per isolate. The cultures were grown in the dark at temperatures ranging from 15–35 ºC. Two measurements were taken daily for each plate until the plates were fully covered.

RESULTS

DNA sequence comparisons

The sequence data set consisted of 51 taxa with sequences from two isolates of *Diaporthe ambigua* Nitschke (*Diaporthales*), which reside in a different family in the *Diaporthales* (Castlebury *et al.* 2002), as outgroup. The ribosomal DNA dataset (571 bp) consisted of 335 constant, 10 parsimony-uninformative and 226 parsimony-informative characters (g1 = –0.4143), and the β-tubulin DNA sequence set (966 bp) consisted of 516 constant, 32 parsimony-uninformative and 418 parsimony-informative characters (g1 = –0.3582). Results generated with the PHT analyses (P = 0.004) indicated that trees obtained with the different gene regions were incongruent. This was because the relationship of the *Cryphonectria sensu stricto* clade with the other clades was different in each gene tree. Each tree, however, showed the same clades, which were always highly supported with bootstrap values between 90 and 100%. For this reason we combined the data. The resultant dataset contained 1537 characters.

The heuristic search resulted in six most parsimonious trees (tree length = 1101.9, CI = 0.736, Retention index/RI = 0.943), which differed only in the lengths of the branches. The trees obtained with the distance and parsimony analyses showed identical clades grouping isolates. The same groups of isolates, with high bootstrap values, were obtained when the more variable regions, and thus potentially ambiguously aligned sequences of the introns and ITS1 region, were excluded. The tree obtained with distance analysis based on the complete dataset is presented in Fig. 2.

The isolates thought to represent *C. havanensis* from *E. grandis* in Mexico (CMW 14550, CMW 11297, CMW 11298), Florida (CMW 14332) and Kauai (CMW 10879, CMW 10885), grouped together (Fig. 2) and formed a discrete clade (bootstrap support 100 %) separate from the clades representing species of *Cryphonectria* (Sacc.) Sacc., *Endothia* Fr., *Chrysoporthe* Gryzenh. & M.J. Wingf., *Rostraureum* Gryzenh. & M.J. Wingf. and *Amphilogia* Gryzenh., Glen & M.J. Wingf.. The *C. havanensis* clade (Fig. 2) also included the isolates from *M. faya* in the Azores (CMW 11301) and Madeira (CMW 14551, CMW 11300). *Cryphonectria havanensis* isolates from Kauai grouped separately from *Chr. cubensis* isolates from Kauai (CMW 1856, CMW 11006, CMW 11008) and Hawaii (CMW 10889). The latter isolates all grouped (bootstrap support 100 %) in the South East Asian sub-clade (Myburg *et al.* 2002) of *Chr. cubensis* (Fig. 2).

Isolates from Japan and previously assigned to *C. havanensis* (CMW 10910, CMW 11294), grouped with *C. nitschkei* isolates (CMW 10785, CMW 13747) in the *Cryphonectria* clade (bootstrap support 100%; Fig. 2), as previously reported (Myburg *et al.* 2004a). They thus resided in a clade separate from isolates believed to represent *C. havanensis*. Isolates derived from cankers on *Castanea sativa* (Gardner & Hodges 1990) from the Azores (CMW 14547, CMW 14548) grouped with other *C. parasitica* isolates (CMW 7048, CMW 13749) in the *Cryphonectria* clade (bootstrap support 100 %; Fig. 2).

The *C. eucalypti* isolates formed a discrete clade (bootstrap support 100 %) separate from the clade defining *Cryphonectria s. str.* (Fig. 2). This clade was also separated from the clades representing other genera. The isolates obtained from *Co. uvifera* in Florida also formed a clade distinct from those representing the other

genera (bootstrap support 100 %), and did not group with the isolates representing *C. havanensis*.

Morphology

Fruiting structures on the specimens connected to the isolates from Mexico, Florida and Kauai (Fig. 3) were indistinguishable from those on the type specimen of *C. havanensis* from Cuba. Ascospores [(5.5–)7–9(–10) × (2–)2.5–3(–4) µm], asci [(26.5–)29.5–34.5(–37) × (5–)5.5–7(–8) µm] and conidia [(2.5–)3–4(–5) × 1–1.5 µm] also fell within the range of those reported for the type specimen (Bruner 1916). We are thus confident that the collections from Mexico and Hawaii represent *C. havanensis*, although the phylogenetic relationship between the fungus in Cuba and the isolates from Mexico and Kauai could not be determined due to the lack of isolates from Cuba. Fruiting structures on herbarium specimens of *M. faya* from the Azores and Madeira, linked to isolates (CMW 11300, CMW 11301, CMW 14551) that also grouped with those from Mexico and Kauai, were similar to those from Cuba, Hawaii and Mexico (Fig. 3). A specimen from Puerto Rico (NY 511), annotated as *C. longirostris* but shown by Gryzenhout *et al.* (2005a) not to represent this species, was also morphologically similar to *C. havanensis*.

Clear differences could be seen between the specimens that represent *C. havanensis* (originating from Cuba, Florida, Mexico, Puerto Rico, Kauai, Madeira and the Azores), and those previously labeled as *C. havanensis* from Japan. Non-confluent stromata in the *C. havanensis* specimens were much smaller (200–650 µm diam above level of bark) than those on specimens from Japan (250–1630 µm diam above the level of the bark). Longitudinally sectioned stromata of the *C. havanensis* specimens also tended to be more superficial with reduced tissue development (Fig.

3C, H), while structures on specimens from Japan were distinctly semi-immersed with strongly developed, erumpent tissue. Ascostromata on the *C. havanensis* specimens (Fig. 3A–B) occasionally had long extending perithecial necks (up to 370 µm long) while those from Japan were consistently short (up to 130 µm long). The conidiogenous cells on the *C. havanensis* specimens also had characteristically long, cylindrical conidiophores up to 57 µm long, with the longest of these being sterile, resembling paraphyses (Fig. 3F–K). These structures differed from conidiophores of the Japanese specimens that were up to 29 µm long. Although the structural differences could also be attributed to different hosts, there are also differences, e.g. the presence of paraphyses, that cannot be attributed to hosts. Thus these differences more likely represent robust characteristics to support the distinct phylogenetic grouping (Fig. 2) of specimens representing *C. havanensis s. str.* from those of *Cryphonectria s. str.* and other closely related genera.

Structures of *C. coccolobae* on *Co. uvifera* (Fig. 4) on various specimens were similar to those thought to represent *C. havanensis*. Conidia [(2.5–)3–4.5(–5.5) × 1–1.5 µm] and ascospores [(6.5–)7.5–9(–10.5) × (2.5–)3–3.5(–4) µm] were similar to those of *C. havanensis*, and similar long (up to 62 µm) and cylindrical conidiophores, with the longest sterile, were observed (Fig. 4H–J). A specimen with conidia of (3–)3.5–4.5(–5) × 1–1.5 µm and labeled *C. coccolobae* from bark of *Conocarpus erecta* (CUP 35081) in Bermuda, also contained structures similar to those of the other *C. coccolobae* specimens. Fruiting structures on seed, however, differed from those on bark (Table 2) in being superficial and not semi-immersed.

Asci measured for the different *C. coccolobae* specimens [(32.5–)34.5–39(–41) × (5–)7–9.5(–10.5) µm] were longer and wider than those measured for the majority of *C. havanensis* specimens [(26.5–)29.5–34.5(–37) × (5–)5.5–7(–8) µm].

Ascus size was, however, a variable character since specimen PREM 57518, linked to isolate CMW 11298 grouping with the other *C. havanensis* isolates, had asci of similar size [(31.5–)32–39(–44.5) × (5.5–)6–7.5(–8.5) µm] to those of the *C. coccolobae* specimens and thus longer than the other *C. havanensis* specimens.

The newly collected specimens from *Co. uvifera* in Florida were morphologically different from those representing *C. coccolobae*. Conidiomata were pyriform to rostrate, often having a globose base with a long to tapered cylindrical neck or more than one neck (Figs 5A–D, 6A–B). This was different from conidiomata of *C. coccolobae* which are pulvinate without long necks (Fig. 4E–F). Furthermore, necks of the conidiomata were often covered with short hairs (Fig. 5F). Conidial locules of the Florida specimens (Figs 5D, 6B) also did not contain the long, sterile paraphyses commonly found in locules of *C. coccolobae* (Fig. 4H–J). No teleomorph was observed for the Florida specimens on the bark.

The conidiomata of the Florida specimens did not resemble the anamorphs of *Cryphonectria*, *Endothia*, *Rostraureum*, *Amphilogia* or *Chrysoporthe* although that fungus was closely related to these genera in the DNA sequence comparisons. The conidiomata of the fungus from Florida resembled the rostrate conidiomata of *Rostraureum* (Gryzenhout *et al*. 2005a) most closely, but could be distinguished from *Rostraureum* based on conidiomata that are more pyriform in shape, and with necks more cylindrical. Conidiomata of the newly collected fungus from *Co. uvifera* in Florida also lacked the distinct *textura intricata* tissue at the junction between neck and base in the conidiomata of *Rostraureum* (Gryzenhout *et al.* 2005a). Furthermore, the conidiomatal neck tissue was prosenchymatous (Fig. 5F), and not of *textura porrecta* as is found in *Rostraureum* (Gryzenhout *et al*. 2005a).

One of the specimens labeled as *C. coccolobae* (CUP 34657) contained a fungus morphologically different from *C. coccolobae*, but with orange stromatic tissue. This fungus was erroneously illustrated by Waterston (1947) to represent *C. coccolobae*, an illustration previously used by Seaver & Waterston (1940) in their description of a fungus named *Gnomonia pulcherrima* Seaver & Waterston. These structures occurred on petioles and twigs of *Co. uvifera* from Bermuda (Table 2). The fungus differs from *C. coccolobae* because the perithecial necks extending from the orange stromata are black and not orange as is the case for *C. coccolobae*. Ascospores are also cylindrical, 1–2-septate, guttulate and 11.5–14.5(–16) × (2.5–)3–4(–5) µm. Fruiting structures of this fungus did not colour purple in KOH and yellow in lactic acid, similar to structures of *C. coccolobae*. Previously *G. pulcherrima* was cited as a synonym (Roane 1986) of *C. coccolobae*, but these are clearly distinct fungi.

Taxonomy

Results of the phylogenetic analyses and morphological comparisons have shown clearly that cultures and specimens believed to represent *C. havanensis* do not reside in *Cryphonectria s. str.* but represent a distinct taxonomic group. Based on morphological characteristics, *C. havanensis* most closely resembles *Cryphonectria s. str.*, but it can be distinguished from species in *Cryphonectria s. str.* by its smaller and more superficial stromata, and long paraphyses between the conidiophores. Based on our observations of the material representing *C. havanensis* in this study, we transfer the fungus to a new genus that is closely related to *Cryphonectria*. The following description is provided.

Microthia Gryzenh. & M.J. Wingf., **gen. nov.** MycoBank MB xx.

Etymology: Greek, *micros*, small, and *this*, a heap, thus referring to the small and pulvinate stromata.

Ascostromata subimmersa vel superficialia, pulvinata, aurantiaca. Ascosporae fusoideae vel ellipsoideae, hyalinae, semel septatae. Stromata anamorpha subimmersa vel superficialia, pulvinata, aurantiaca. Conidiophora cylindrica, subcontracta, saepe longa, cellulis longissimis paraphyses fingentibus. Conidia hyalina, cylindrica, non septata.

Ascostromata semi-immersed to superficial, pulvinate, orange, tissue predominantly prosenchymatous but pseudoparenchymatous at edges. *Perithecia* dark-walled, with globose to sub-globose bases and slender periphysate necks that emerge at the stromatal surface as black ostioles in papillae covered with orange stromatal tissue. *Asci* fusiform, floating freely in the perithecial cavity, unitunicate with non-amyloid, refractive apical rings. *Ascospores* fusoid to ellipsoid, hyaline, 1-septate, often with a slight constriction at the septum.

Anamorphic stromata semi-immersed to superficial, pulvinate, orange, uni- to multilocular and convoluted, locules often occurring in the same stroma that contains perithecia. *Conidiophores* cylindrical, slightly tapering, often septate with or without lateral branches beneath the septum, hyaline, often long with longest cells sterile and representing paraphyses, conidiogenous cells phialidic. *Conidia* hyaline, cylindrical, aseptate, expelled through opening at stromatal surface as orange droplets or tendrils.

Typus: *Microthia havanensis* (Bruner) Gryzenh. & M.J. Wingf., comb. nov.

Microthia havanensis (Bruner) Gryzenh. & M.J. Wingf., **comb. nov.** MycoBank MB xx. Fig. 3.

Basionym: *Endothia havanensis* Bruner, Mycologia 8: 241–242. 1916.

≡ *Cryphonectria havanensis* (Bruner) M.E. Barr, Mycologia Mem. 7: 143. 1978.

Specimens examined: **Cuba**, Santiago de las Vegas, *Eucalyptus* sp., 15 Feb. 1916, S.C. Bruner, **holotype** BPI 614275, BPI 614273; *Eucalyptus botryoides*, 25 Mar. 1916, C.L. Shear, BPI 614278; *Spondias* sp., 28 Mar. 1916, C.L. Shear, BPI 614282; Earle' s Herradura, *Spondias myrobalanus*, 5 Apr. 1916, C.L. Shear, BPI 614283, BPI 614284; Santiago de las Vegas, *Mangifera indica*, 6 Apr. 1916, C.L. Shear, BPI 614279, BPI 614280, 26 Mar. 1916, C.L. Shear, BPI 614281. **Mexico**, Las Chiapas, *Eucalyptus saligna*, 26 Feb. 1998, C.S. Hodges, PREM 57518, living culture CMW 11298. **Puerto Rico**, 1923, F.J. Seaver & C.E. Chardon, NY 511. **U.S.A.**, Hawaii, Kauai, *Eucalyptus* sp., Sept. 2002, M.J. Wingfield, PREM 57521, living culture CMW 10879 = CBS 115758, PREM 57522, living culture CMW 10885 = CBS 115760. Florida, Near Palmdale, Glades Co., *Eucalyptus robusta*, 1984, E.L. Barnard & K.M. Old, FLAS 54261, ATCC 60862; *Eucalyptus grandis*, 1984, E.L. Barnard & K.M. Old, FLAS 54263. **Madeira**, Machico, *Myrica faya*, 8 May 2000, C.S. Hodges, PREM 57523, living culture CMW 14551 = CBS 115841. **Azores**, Island of São Miguel, Mosteiro, *M. faya*, C.S. Hodges & D.E. Gardner, PREM 57524, living culture from same locality CMW 11301; Island of Pico, *M. faya*, 30 Jul. 1992, C.S. Hodges & D.E. Gardner, PREM 57525, living culture from same locality CMW 11301; Island of Pico, *M. faya*, 31 May 1985, C.S. Hodges & D.E. Gardner, PREM 58810, living culture from same locality CMW 11301; Island of São Miguel, *M. faya*, 2 Aug. 1992,

C.S. Hodges & D.E. Gardner, PREM 58811, living culture from same locality CMW 11301; Island of Terceiro, *M. faya*, 31 May 1987, C.S. Hodges & D.E. Gardner, PREM 58812, living culture from same locality CMW 11301; Island of Faial, *M. faya*, 27 May 1985, C.S. Hodges, PREM 58813, living culture from same locality CMW 11301.

Notes: Microthia havanensis and *A. gyrosa* have been considered as synonyms when the latter fungus was still known as *C. gyrosa* (Kobayashi 1970, Hodges 1980). *Cryphonectria gyrosa* has also been known as *Endothia tropicalis* Shear & N.E. Stevens during the time that *Cryphonectria* was considered synonymous to *Endothia* (Shear *et al*. 1917, Kobayashi & Itô 1956, Kobayashi 1970, Roane 1986). *Amphilogia gyrosa* is, however, a distinct fungus from *M. havanensis*, as shown clearly in this study.

Specimens of *C. coccolobae* resemble those of *Mi. havanensis* closely and clearly reside in the same genus. Based on the similar spore dimensions, it is also probable that *C. coccolobae* is conspecific with *Mi. havanensis*. However, in the absence of isolates that can be used to confirm the phylogenetic relationship of *C. coccolobae*, we propose that *C. coccolobae* retain its independent taxonomic status for the present. Specimens representing *C. coccolobae* are, however, transferred to *Microthia* since this species clearly does not reside in *Cryphonectria s. str*.

Microthia coccolobae (Vizioli) Gryzenh. & M.J. Wingf., **comb. nov.** MycoBank MB xx. Fig. 4.

Basionym: *Endothia coccolobae* Vizioli, Mycologia 15: 115. 1923 (as *E. coccolobii*).

≡ *Cryphonectria coccolobae* (Vizioli) Micales & Stipes, Phytopathology 77: 651. 1987 (as *C. coccolobii*).

Specimens examined: **Bermuda**, Grape Bay, fruit of *Coccoloba uvifera*, 11 Dec. 1921, H.H. Whetzel, **holotype** CUP 128; Grape Bay, fruit of *Co. uvifera*, 11 Dec. 1921, H.H. Whetzel, **isotypes** BPI 613756, NY 147, other specimen CUP 30512; Elbow Beach, fruit of *Co. uvifera*, 28 Jan. 1926, Whetzel, Seaver & Ogilvie, CUP 34658; South Shore, bark of *Co. uvifera*, 25 Nov. 1940, F.J. Seaver & J.M. Waterston, CUP 57366; Devonshire, *Calophyllum calaba*, 2 Feb. 1926, Seaver, Whetzel & Ogilvie, CUP 35078; Devonshire Bay, *Conocarpus erecta*, 5 Feb. 1926, Seaver, Whetzel & Ogilvie, CUP 35081.

The fungus collected from *Co. uvifera* in Florida as part of this study clearly does not represent *Mi. coccolobae*. DNA sequence and morphological comparisons showed that a new genus should be provided for it and the appropriate description is presented below. No teleomorph could be found on the material, but based on DNA sequence comparisons the fungus clearly belongs to the *Diaporthales* and is closely related to *Cryphonectria* and allied genera. It is, however, described as an anamorphic fungus following Art. 59.2 of the International Code of Botanical Nomenclature (Greuter *et al.* 2000).

Ursicollum Gryzenh. & M.J. Wingf., **gen. nov.** MycoBank MB xx.

Etymology: Latin, *ursus*, a bear, and latin, *collus*, neck. Referring to the hairy neck of the conidioma that reminds of that of a bear.

Conidiomata eustromatica, pyriformia vel rostrata, superficialia, aurantiaca, cum collis uno vel tribus, textura pseudoparenchymatosa sed in collo prosenchymatosa. Conidiophora cylindrica. Conidia cylindrica, hyalina, non septata.

Conidiomata eustromatic, pyriform or rostrate, superficial to slightly immersed in bark, unilocular, internally strongly convoluted, orange, with one to three attenuated or cylindrical necks, tissue pseudoparenchymatous but prosenchymatous in the neck. *Conidiophores* hyaline, delimited by septa or not, cylindrical, conidiogenous cells phialidic, apical or lateral on branches beneath the septum. *Conidia* cylindrical, hyaline, aseptate.

Typus: *Ursicollum fallax* Gryzenh. & M.J. Wingf., sp. nov.

Ursicollum fallax Gryzenh. & M.J. Wingf., **sp. nov.** MycoBank MB xx. Figs 5–6.

Etymology: Latin, *fallax*, false. Refers to the conidiomata that appear to be false ascostromata.

Conidiomata eustromatica, pyriformia vel rostrata, aurantiaca, cum collis attenuatis uno vel tribus, superficialia vel subimmersa. Textura basalis pseudoparenchymatosa, textura collorum prosenchymatosa. Conidiophora cylindrica, apice attenuata an non. Conidia (2.5–)3–4(–5.5) × (1–)1.5(–2) µm, cylindrica, non septata, hyalina.

Conidiomata orange, eustromatic, pyriform to rostrate, with one to three attenuated or cylindrical necks (Figs 5A–B, 6A–B), base 120–400 µm high, 190–550 µm diam, neck up to 400 µm long, 90–180 µm wide, superficial to slightly immersed, unilocular, internally convoluted (Figs 5B–C, 6B). Basal tissue predominantly

pseudoparenchymatous (Fig. 5E), neck tissue prosenchymatous (Fig. 5F). *Conidiophores* hyaline, cylindrical with or without attenuated apex, cells delimited by septa or not, total length of conidiophore (4.5–)5.5–19(–39) µm (Figs 5G–H, 6C). *Conidiogenous cells* phialidic, apical or lateral on branches beneath the septum, cylindrical to flask-shaped with attenuated apices, 1.5–2(–2.5) µm wide, collarette and periclinal thickening inconspicuous (Figs 5G–H, 6C). *Conidia* (2.5–)3–4(–5.5) × (1–)1.5(–2) µm, cylindrical, aseptate, hyaline, exuded as orange droplets (Figs 5I, 6C).

Cultural characteristics: on MEA white, fluffy, margins even, optimum for growth 25–30 °C, isolates covering 90 mm diam plates after 5–6 d at optimum growth temperatures.

Substratum: Bark of *Coccoloba uvifera.*

Distribution: Florida (U.S.A.).

Specimens examined. **U.S.A.**, Florida, Fort Lauderdale, *Coccoloba uvifera*, 8 Mar. 2005, C.S. Hodges, **holotype** PREM 58840, culture ex-type CMW 18119 = CBS 118663; Key Biscayne, *Coccoloba uvifera*, 10 Mar. 2005, C.S. Hodges, PREM 58841, PREM 58842, living cultures CMW 18115 = CBS 118660, CMW 18124 = CBS 118662; Oakland Park, *Coccoloba uvifera*, 11 Mar. 2005, C.S. Hodges, PREM 58843, living culture CMW 18114 = CBS 118661; Dania, *Coccoloba uvifera*, 11 Mar. 2005, C.S. Hodges, PREM 58844, living culture CMW 18110 = CBS **.

Phylogenetic analyses based on the collection of isolates treated in this study and that of Gryzenhout *et al*. (2006), showed that isolates representing *C. eucalypti* from Australia and South Africa form a clade distinct from other species in *Cryphonectria s. str*. This phylogenetic grouping is supported by discrete morphological

characteristics such as aseptate ascospores and small stromata, which are different to those found in *Cryphonectria*. Results of this study provide us with strong justification to erect a new genus for *C. eucalypti*, and a description is provided as follows:

Holocryphia Gryzenh. & M.J. Wingf., **gen. nov.** MycoBank XX.

Etymology: Greek, *holo*, undivided, *crypho*-, secret, referring to undivided ascospores and the semi-immersed nature of the stromata.

Ascostromata subimmersa, pulvinata, aurantiaca. Ascosporae cylindricae, interdum allantoideae, hyalinae, non septatae. Stromata anamorpha subimmersa, pulvinata, aurantiaca. Conidiophora cylindrica, basibus inflatis an non, attenuatae; paraphyses inter conidiophora adsunt. Conidia hyalina, cylindrica, non septata.

Ascostromata semi-immersed, pulvinate, orange, pseudoparenchymatous tissue at the edge of stromata, prosenchymatous tissue in the centre. *Perithecia* dark-walled, with globose to sub-globose bases and slender periphysate necks that emerge at the stromatal surface as black ostioles in papillae covered with orange stromatal tissue. *Asci* fusiform, floating freely in the perithecial cavity, unitunicate with non-amyloid, refractive apical rings. *Ascospores* cylindrical, occasionally allantoid, hyaline, aseptate.

Anamorphic stromata erumpent, semi-immersed, pulvinate, orange, uni- to multilocular and convoluted, locules often occurring in same stroma that contains perithecia. *Conidiophores* cylindrical with or without inflated bases, tapering, often septate with or without lateral branches beneath a septum, hyaline, paraphyses occurring between conidiophores, conidiogenous cells phialidic. *Conidia* hyaline,

cylindrical, aseptate, expelled through an opening at the stromatal surface as orange droplets or tendrils.

Typus: *Holocryphia eucalypti* (M. Venter & M.J. Wingf.) Gryzenh. & M.J. Wingf., comb. nov..

Holocryphia eucalypti (M. Venter & M.J. Wingf.) Gryzenh. & M.J. Wingf., **comb. nov.** MycoBank XX.

Basionym: *Cryphonectria eucalypti* M. Venter & M. J. Wingf., Sydowia 54: 113–115. 2002.

Specimens examined: **South Africa**, Northern Kwazulu-Natal, Mtubatuba, Nyalazi estate, bark of GC747 clone of *Eucalyptus*, 25 Feb. 1998, M. Venter, **holotype**, PREM 56211, ex-type culture CMW 7034; Dukuduku estate, bark of *Eucalyptus grandis*, Oct. 1998, M. Venter, PREM 56214, PREM 56216; KwaMbonambi, Amangwe estate, bark of *E. grandis*, Oct. 1998, M. Venter, **epitype designated here** PREM 56215, living culture CMW 7033 = CBS 115842; Mpumalanga, Sabie, bark of *E. grandis*, Aug. 1998, J. Roux, PREM 56212; Limpopo, Tzaneen, bark of *Eucalyptus saligna*, 6 Feb. 1999, M. Venter, PREM 56305, living culture CMW 7035. **Australia**, Western Australia, Perth, *Eucalyptus globulus*, 1997, M.J. Wingfield, PREM 56217, living culture CMW 7038 = CBS ***.

DISCUSSION

In this study, we describe three new genera that are closely related to *Cryphonectria*. *Microthia* includes the fungi previously known as *C. havanensis* and *C. coccolobae*, while *Holocryphia* represents the *Eucalyptus* pathogen previously known as *C. eucalypti*. *Ursicollum* is a new genus that was discovered on *Co. uvifera* in Florida while attempting to locate fresh specimens of *Mi. coccolobae*. The description of these new genera is justified based primarily on the phylogenetic grouping of the isolates, which are distinct from *Cryphonectria* and other closely related genera such as *Endothia*, *Chrysoporthe* and *Rostraureum.*

Microthia, *Holocryphia* and *Ursicollum* are defined by the following morphological characteristics. The pulvinate and semi-immersed stromata of *Microthia* and *Holocryphia* are similar to those of *Cryphonectria* but are much smaller. Stromata of *Microthia* also tend to be more superficial on the substrate than those found in *Cryphonectria*. Another interesting and unique feature, shared by *Microthia* and *Holocryphia*, is that the conidiomata of both fungi contain exceptionally long cells between the conidiophores. These cells, previously referred to as paraphyses (Venter *et al.* 2002), do not produce conidia. *Microthia* and *Holocryphia* are thus morphologically quite similar but can be distinguished from each other based on ascospore morphology. *Microthia* has single-septate ascospores, while those of *Holocryphia* are aseptate. *Ursicollum* is morphologically distinct from the anamorphs of *Microthia*, *Holocryphia* and other related genera because of its unique orange, pyriform to globose conidiomata with cylindrical to attenuated necks.

Holocryphia eucalypti was previously known as *Endothia gyrosa* (Schwein. : Fr.) Fr. (Venter *et al*. 2002). The fungus was described as a species of *Cryphonectria*

because phylogenetic analyses indicated that isolates of this fungus grouped more closely with *Cryphonectria* than with *Endothia*, the only two genera that it resembled at that time (Venter *et al.* 2001, 2002). This phylogenetic grouping was supported morphologically by the semi-immersed stromata similar to those of *Cryphonectria.* Consequently, the new species was placed in *Cryphonectria,* despite the fact that its single-celled ascospores were different from the two-celled ascospores characteristic of all other *Cryphonectria* species. Phylogenetic studies (Myburg *et al.* 2004b) including more genera and species than those considered by Venter *et al.* (2002) did not provide convincing evidence to separate *H. eucalypti* from other *Cryphonectria* species. It was necessary to include the isolates of additional taxa presented in this study and that of Gryzenhout *et al*. (2006), which are morphologically similar to those of *H. eucalypti*, to reveal the distinction between *H. eucalypti* and species in the *Cryphonectria sensu stricto* clade. The unusual and contradictory fact that *H. eucalypti* (as *C. eucalypti*) had single-celled ascospores different from all species in *Cryphonectria s. str.* with two-celled ascospores, could thus be resolved.

The newly recognised taxonomic position of *Microthia* is well defined because numerous isolates of *Mi. havanensis* could be subjected to DNA sequence comparisons in this study. Although careful examination of the herbarium specimens of *Mi. coccolobae* have led us to suspect that this fungus is a synonym of *Mi. havanensis*, the taxonomic position of the former fungus has yet to be defined precisely. In the past, morphological characteristics such as spore size (Hodges & Gardner 1992), constriction at the ascospore septa and stromatal size (Roane 1986), the length of the perithecial necks (Vizioli 1923, Hodges & Gardner 1992), and the small number of perithecia in the stromata (Vizioli 1923) have been used to distinguish *C. coccolobae* from other species in *Cryphonectria*. These features are,

however, quite variable in specimens. For example, constricted ascospores were seen in specimens of both *Mi. havanensis* and *Mi. coccolobae*, and stromatal morphology varied greatly. Size variation of these characteristics between samples was also observed. For example, asci in specimen PREM 57518 were larger than those in other specimens of *Mi. havanensis.* This was despite the fact that isolate CMW 11298, linked to PREM 57518, grouped with isolates linked to the other specimens of the same species based on DNA sequence data. Another feature that may have convinced previous authors that *Mi. coccolobae* represents a distinct taxon is the superficial fruiting structures on *Co. uvifera* seeds. We believe that this is related to the substrate, since stromatal morphology on the seeds (Vizioli 1923) was superficial, while on bark it is semi-immersed (Micales & Stipes 1987, Gardner & Hodges 1990).

While the morphology of *Mi. coccolobae* and *Mi. havanensis* is very similar, the pathogenicity and ecology of these two species have been reported to be different. In studies to determine the identity of the *Cryphonectria* sp. on *M. faya* (Hodges & Gardner 1992), an isolate of *Mi. coccolobae* from Bermuda failed to colonise freshly-cut branch sections of *M. faya* as successfully as isolates obtained from *M. faya*, which have been shown in this study to represent *Mi. havanensis.* Likewise, the fungus from *M. faya* did not grow in freshly-cut branch sections of *Co. uvifera*, although the *Mi. coccolobae* isolate was able to colonise this substrate. No inoculations were made on living trees of either host (Hodges & Gardner 1992). Reciprocal inoculations on various hosts such as *Co. uvifera*, *Quercus* spp. and *Eucalyptus* spp. with several isolates including *Mi. havanensis* from *Eucalyptus* and *Mi. coccolobae*, showed that the *Mi. coccolobae* isolates alone were able to infect *Co. uvifera* resulting in cankers (Barnard *et al.* 1993). These differences in pathogenicity to *Co. uvifera* may indicate that the two species are distinct, despite their similar

morphology. Another unusual characteristic that distinguishes *Mi. coccolobae* from other closely related fungi is its prolific colonization of fruits of *Co. uvifera*, often while they are still green. In contrast, other species of *Microthia*, *Cryphonectria* and allied genera have been found only on bark. It is for these reasons that we have chosen not to synonymise these species before isolates of *Mi. coccolobae* can be obtained for DNA sequence comparisons.

While searching for fresh material of *C. coccolobae* (now *Mi. coccolobae*) on sea grape in Florida, another morphologically similar fungus, *U. fallax,* was found on this host. This fungus represents a new genus and species, which is closely related to *Cryphonectria* and allied genera, although no teleomorph structures were found for the fungus. Morphological comparisons with *Mi. coccolobae* showed that *U. fallax* is distinctly different from *Mi. coccolobae*. Two closely related and morphologically similar fungi thus occur on *Co. uvifera*, although it could also be possible that previous reports of *Mi. coccolobae* in Florida actually represent *U. fallax*. This will complicate continuing surveys searching for *Mi. coccolobae* on this host in order to obtain isolates for later phylogenetic comparisons.

It has previously been suggested that the fungus referred to as *C. havanensis* in Japan, represents *C. nitschkei* (Myburg *et al.* 2004a). At the time of that study, it was not possible to determine whether *C. nitschkei* was the same as *C. havanensis* in Cuba (Myburg *et al.* 2004a). For the present study, we had at our disposal a substantial collection of isolates linked to additional specimens that we feel confident to have the fungus previously known as *C. havanensis*. We were thus able to conduct morphological and phylogenetic comparisons to show clearly that the type of *Mi. havanensis* represents a fungus different from that of *C. nitschkei* from Japan. The fungus now known as *Mi. havanensis* thus does not occur in Japan.

Microthia havanensis appears to occur saprotrophically on *Eucalyptus* and other hosts. Bruner (1916) described the fungus on dead branches and twigs. Barnard *et al.* (1987) also reported it as a saprotroph on *E. grandis* in Florida, while *Chr. cubensis* was the cause of canker disease in the same plantations. In Mexico and Kauai the fungus was found only on dead, suppressed trees of *Eucalyptus*, and was not associated with cankers. Similarly, although *Mi. havanensis* was associated with cankers on *M. faya* trees in the Azores (Gardner & Hodges 1990), it also occurs on dead trees, and may only play a saprotrophic role on cankers (Hodges & Gardner 1992).

Microthia havanensis frequently occurs on *Eucalyptus* in the same locality as trees infected with *Chr. cubensis*. This is consistent with the fact that both *Chr. cubensis* and *Mi. havanensis* were first described from Cuba in the same locality (Bruner 1916, 1917) and both occurred in the same plantations in Florida (Barnard *et al.* 1987) and Kauai. Clearly the pathogenicity of *Mi. havanensis*, factors that influence its pathogenicity and the ecological relationship between *Mi. havanensis* and *Chr. cubensis*, deserves further consideration.

This study emphasizes the fact that several closely related and morphologically similar fungi, all with orange stromatic tissue, occur on *Eucalyptus* trees worldwide. These fungi previously resided in the single genus *Cryphonectria*, but most have now been transferred to new genera. *Microthia havanensis* and *H. eucalypti* have been newly described in this study. *Cryphonectria nitschkei* occurs on *Eucalyptus* spp. in Japan, and *C. parasitica* and an unknown *Cryphonectria* sp. have also been reported from *Eucalyptus* spp. in Japan (Old & Kobayashi 1988). Lastly, *Chrysoporthe* species, previously treated as the single species *Cryphonectria*

cubensis, also occur on *Eucalyptus* spp. and have been observed in the same geographic regions as *H. eucalypti* and *Mi. havanensis* (Gryzenhout *et al.* 2004).

The various *Cryphonectria* spp. and related fungi occur on *Eucalyptus* spp. in different parts of the world (Fig. 1). Thus *C. nitschkei*, *C. parasitica* and the undescribed *Cryphonectria* sp. on *Eucalyptus* are known from the Far East, *H. eucalypti* occurs in Australia and South Africa, and *Mi. havanensis* is now known from Mexico, Cuba, Puerto Rico, Florida, Hawaii, Azores and Madeira. Furthermore, the different species of *Chrysoporthe* occur in different tropical and sub-tropical countries of the world (Gryzenhout *et al.* 2004). For example, *Chr. austroafricana* occurs specifically in South Africa and *Chr. cubensis* occurs in Hawaii, Central and South America, Central Africa, South East Asia and Australia (Gryzenhout *et al.* 2004).

Cryphonectria, *Chrysoporthe*, *Microthia* and *Holocryphia* differ significantly in their pathogenicity to *Eucalyptus* spp., which is an ecologically important tree that also forms the basis of large forestry industries. *Chrysoporthe* spp. and *H. eucalypti* are considered the most important pathogens in this group. *Mi. havanensis* and the different *Cryphonectria* spp. are mild pathogens or saprophytes. Although the geographical range of *C. nitschkei*, *Mi. havanensis* and *H. eucalypti* is not currently known to overlap (Fig. 1), it is possible that these fungi could be introduced into new areas. It has been hypothesized that *H. eucalypti* has already moved from Australia, where it is presumed to be native due to the widespread occurrence of *H. eucalypti* in native *Eucalyptus* forests in Australia (Walker *et al.* 1985, Old *et al.* 1986), into *Eucalyptus* plantation areas of South Africa (Nakabonge *et al.* 2005). Because of the importance of some of these fungi as pathogens, every effort must be made to identify

collections accurately. This underpins efforts to monitor the spread of diseases and to manage their impact.

The following key is provided to facilitate the distinction between different diaporthalean genera with orange stromatic tissue, some of which occur on *Eucalyptus*:

1a. Conidiomata pyriform to clavate; ascostromata with reduced stromatic tissue ……..…….....……..... 2

1b. Conidiomata pulvinate; ascostromata well-developed…… 4

2a. Conidiomata black; orange ascostroma with black perithecial necks …….. ***Chrysoporthe***

2b. Conidiomata orange…… 3

3a. Conidiomata rostrate with tapered necks; orange stroma with orange perithecial necks ……. ***Rostraureum***

3b. Conidiomata pyriform or rostrate or globose with more cylindrical necks; teleomorph unknown……..…..……...….... ***Ursicollum***

4a. Ascospores septate……... 5

4b. Ascospores aseptate……. 6

5a. Ascostromata large, well-developed, semi-immersed; paraphyses absent in conidial locules ……...…… ***Cryphonectria***

5b. Ascostromata small to medium size, usually superficial; conidial locules containing paraphyses ……***Microthia***

6a. Ascostromata large, well-developed, superficial……...…… ***Endothia***

6b. Ascostromata small to medium size, semi-immersed …….. ***Holocryphia***

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Table 1. Isolates sequenced in this study (in bold) and previously published sequences.

Species identity	Isolate number[a]	Alternative isolate number[a]	Host	Origin	Collector	GenBank accession numbers[b]
Microthia havanensis	**CMW 14332**	ATCC 60862	*Eucalyptus grandis*	Florida (U.S.A.)	E.L. Barnard & K. Old	DQ368734, DQ368739, DQ368740
	CMW 14550	CBS 115855	*Eucalyptus saligna*	Mexico	C.S. Hodges	DQ368735, DQ368741, DQ368742
	CMW 11297	CBS 115765	*Eucalyptus* sp.	Mexico	E.L. Barnard	AY 214319, AY 214247, AY 214283
	CMW 11298	-	*Eucalyptus* sp.	Mexico	C.S. Hodges	AY 214320, AY 214248, AY 214284
	CMW 11301	-	*Myrica faya*	Azores	C.S. Hodges & D.E. Gardner	AY 214323, AY 214251, AY 214287
	CMW 11300	-	*M. faya*	Madeira	C.S. Hodges	AY 214322, AY 214250, AY 214286
	CMW 14551	CBS 115841	*M. faya*	Madeira	C.S. Hodges	DQ368736, DQ368743, DQ368744
	CMW 10879	CBS 115758	*Eucalyptus* sp.	Kauai, Hawaii (U.S.A.)	M.J. Wingfield	DQ368737, DQ368745, DQ368746
	CMW 10885	CBS 115760	*Eucalyptus* sp.	Kauai, Hawaii (U.S.A.)	M.J. Wingfield	DQ368738, DQ368747, DQ368748
Amphilogia gyrosa	CMW 10469	E67, CBS 112922	*Elaeocarpus dentatus*	New Zealand	G. Samuels	AF 452111, AF 525707, AF 525714
	CMW 10470	E68, CBS 112923	*El. dentatus*	New Zealand	G. Samuels	AF 452112, AF 525708, AF 525715
Chrysoporthe cubensis	CMW 10639	CBS 115747	*E. grandis*	Colombia	C.A. Rodas	AY 263419, AY 263420, AY 263421
	CMW 10669	CBS 115751	*Eucalyptus* sp.	Republic of Congo	J. Roux	AF 535122, AF 535124, AF 535126
	CMW 11006	CBS 115732	*Eucalyptus* sp.	Kauai, Hawaii (U.S.A.)	M.J. Wingfield	DQ368719, DQ368723, DQ368724
	CMW 11008	CBS 115733	*Eucalyptus* sp.	Kauai, Hawaii (U.S.A.)	M.J. Wingfield	DQ368718, DQ368721, DQ368722

	CMW 10889	CBS 118666		Hawaii, Hawaii (U.S.A.)	M.J. Wingfield	DQ368720, DQ368725, DQ368726
	CMW 1856	-	*Eucalyptus* sp.	Kauai, Hawaii (U.S.A.)	–	AY 083999, AY 084010, AY 084022
	CMW 8651	CBS 115718	*Syzygium aromaticum*	Sulawesi, Indonesia	M.J. Wingfield	AY 084002, AY 084014, AY 084026
Chrysoporthella hodgesiana	CMW 9994	CBS 115729	*Tibouchina semidecandra*	Colombia	R. Arbelaez	AY 956968, AY 956975, AY 956976
	CMW 10641	CBS 115854	*T. semidecandra*	Colombia	R. Arbaleaz	AY 692322, AY 692326, AY 692325
Chrysoporthe austroafricana	CMW 2113	CBS 112916	*E. grandis*	South Africa	M.J. Wingfield	AF 046892, AF 273067, AF 273462
	CMW 9327	CBS 115843	*Tibouchina granulosa*	South Africa	M.J. Wingfield	AF 273473, AF 273060, AF 273455
Rostraureum tropicale	CMW 9971	CBS 115725	*Terminalia ivorensis*	Ecuador	M.J. Wingfield	AY 167426, AY 167431, AY 167436
	CMW 10796	CBS 115757	*Te. ivorensis*	Ecuador	M.J. Wingfield	AY 167428, AY 167433, AY 167438
Holocryphia eucalypti	CMW 7036	CRY62, CBS***	*Eucalyptus* sp.	South Africa	I. van der Westhuizen	AF 232878, AF 368341, AF 368340
	CMW 7037	CRY45, CBS **	*Eucalyptus delegatensis*	Australia	K.M. Old	AF 232880, AF 368343, AF 368342
	CMW 7038	CRY909, CBS **	*Eucalyptus globulus*	Australia	M.J. Wingfield	AF 232881, AF 368345, AF 368344
	CMW 14545	CRY 103, CBS 115852	*Eucalyptus* sp.	South Africa	I. van der Westhuizen	AF 232877[c], DQ368730, DQ368731
	CMW 14546	CRY 287, CBS 115838	*Eucalyptus* sp.	South Africa	H. Smith	AF 232879[c], DQ368732, DQ368733
	CMW 7033	CBS 115842	*E. grandis*	South Africa	M. Venter	DQ368727, DQ368728, DQ368729
Ursicollum fallax	CMW 18110	CBS **	*Coccoloba uvifera*	Florida (U.S.A.)	C. S. Hodges	-
	CMW 18114	CBS 118661	*Co. uvifera*	Florida (U.S.A.)	C. S. Hodges	-

	CMW 18115	CBS 118660	*Co. uvifera*	Florida (U.S.A.)	C. S. Hodges	DQ368756, DQ368760, DQ368761
	CMW 18119	CBS 118663	*Co. uvifera*	Florida (U.S.A.)	C. S. Hodges	DQ368755, DQ368758, DQ368759
	CMW 18124	CBS 118662	*Co. uvifera*	Florida (U.S.A.)	C. S. Hodges	DQ368757, DQ368762, DQ368763
Cryphonectria parasitica	CMW 13749	MAFF 410158 TFM:FPH Ep1	*Castanea mollisima*	Japan	Unknown	AY 697927, AY 697943, AY 697944
	CMW 7048	ATCC 48198, E9	*Quercus virginiana*	USA	F.F. Lombard	AF 368330, AF 273076, AF 273470
	CMW 14547	CBS 115845	*Castanea sativa*	Azores	D.E. Gardner	DQ368749, DQ368751, DQ368752
	CMW 14548	CBS 115846	*Ca. sativa*	Azores	D.E. Gardner	DQ368750, DQ368753, DQ368754
Cryphonectria radicalis	CMW 10455	CBS 238.54, E42	*Castanea dentata*	Italy	A. Biraghi	AF 452113, AF 525705, AF 525712
	CMW 10477	CBS 240.54, E76	*Quercus suber*	Italy	M. Orsenigo	AF 368328, AF 368347, AF 368346
	CMW 10436	CBS 165.30, E14	*Quercus suber*	Portugal	B. d'Oliviera	AF 452117, AF 525703, AF 525710
	CMW 10484	E83, CBS 112918	*Castanea sativa*	Italy	A. Biraghi	AF 368327, AF 368349, AF 368349
Cryphonectria macrospora	CMW 10463	E54, CBS 112920	*Castanopsis cuspidata*	Japan	T. Kobayashi	AF 368331, AF 368351, AF 368350
	CMW 10914	TFM: FPH E55	*Castanopsis cuspidata*	Japan	T. Kobayashi	AY 697942, AY 697973, AY 697974
Cryphonectria nitschkei	CMW 10785	09494	*Quercus* sp.	China	M. Milgroom & K. Wang	AF 140246, AF 140252, AF 140258
	CMW 13747	MAFF 410569 TFM:FPH E25	*Quercus serrata*	Japan	T. Kobayashi	AY 697937, AY 697963, AY 697964
	CMW 10910[d]	TFM:FPH E11	*Eucalyptus globulus*	Japan	T. Kobayashi	AY 697941, AY 697971, AY 697972
	CMW 11294[d]	TFM:FPH E57	*Quercus grosseserrata*	Japan	T. Kobayashi	AY 214211, AY 214213, AY 214215
Endothia gyrosa	CMW 2091	ATCC 48192, E13	*Quercus palustris*	USA	R.J. Stipes	AF 046905, AF 368337, AF 368336
	CMW 10442	E27	*Q. palustris*	USA	R.J. Stipes	AF 368326, AF 368339, AF

						368338
Diaporthe ambigua	CMW 5288	CBS 112900	*Malus domestica*	South Africa	W.A. Smit	AF 543817, AF 543819, AF 543821
	CMW 5587	CBS 112901	*M. domestica*	South Africa	W.A. Smit	AF 543818, AF 543820, AF 543822

[a] **CMW, CRY** = Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; **ATCC** = American Type Culture Collection, Manassas, USA; **CBS** = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; **TFM:FPH** = Forestry and Forest Products Research Institute, Danchi-Nai, Ibaraki, Japan, E or Ep refers to an isolate; (**09494**) = isolates used in Liu *et al.* (2003); **MAFF** = Microorganisms Section, MAFF GENEBANK, National Institute of Agrobiological Sciences (NIAS), MAFF Gene Bank, Ibaraki, Japan; **E** = from the culture collection of Prof. R.J. Stipes (Department of Plant Pathology, Virginia Polytechnic Institute & State University, Blacksburg, Virginia, U.S.A.) now housed in the culture collection (CMW) of FABI.

[b] Accession numbers refer to sequence data of the ITS, β-tubulin 1 (primers Bt1a/1b) and β-tubulin 2 (primers Bt2a/2b) regions, respectively.

[c] Only the β-tubulin sequences were obtained in this study, while the ITS sequences were obtained from Venter *et al*. (2001).

[d] Previously labelled *Cryphonectria havanensis*.

Table 2. Herbarium specimens examined in this study.

Species identity	Herbarium number[a]	Linked isolate[b]	Host	Origin	Collector	Date
Microthia havanensis	BPI 614275 (holotype)	-	*Eucalyptus* sp.	Santiago de las Vegas, Cuba	S.C. Bruner	15 Feb. 1916
	BPI 614273		*Eucalyptus* sp.	Santiago de las Vegas, Cuba	S.C. Bruner	15 Feb. 1916
	BPI 614278		*Eucalyptus botryoides*	Santiago de las Vegas, Cuba	C.L. Shear	25 Mar. 1916
	BPI 614282	-	*Spondias* sp.	Santiago de las Vegas, Cuba	C.L. Shear	28 Mar. 1916
	BPI 614283	-	*Spondias myrobalanus*	Earle's Herradura, Cuba	C.L. Shear	5 Apr. 1916
	BPI 614284	-	*S. myrobalanus*	Earle's Herradura, Cuba	C.L. Shear	5 Apr. 1916
	BPI 614279	-	*Mangifera indica*	Santiago de las Vegas, Cuba	C.L. Shear	6 Apr. 1916
	BPI 614280	-	*Ma. indica*	Santiago de las Vegas, Cuba	C.L. Shear	Apr. 1916
	BPI 614281	-	*Ma. indica*	Santiago de las Vegas, Cuba	C.L. Shear	26 March 1916
	PREM 57518	CMW 11298	*Eucalyptus saligna*	Las Chiapas, Mexico	C.S. Hodges	26 Feb. 1998
	NY 511	-	Unknown	Puerto Rico	F.J. Seaver & C.E. Chardon	1923
	PREM 57521	CMW 10897	*Eucalyptus* sp.	Kauai, Hawaii (U.S.A.)	M.J. Wingfield	Sep. 2002
	PREM 57522	CMW 10885	*Eucalyptus* sp.	Kauai, Hawaii (U.S.A.)	M.J. Wingfield	Sep. 2002
	FLAS 54261	ATCC 60862	*Eucalyptus robusta*	Near Palmdale, Glades Co., Florida (U.S.A.)	E.L. Barnard & K. Old	1984
	FLAS 54263	-	*Eucalyptus grandis*	Glades Co., Florida (U.S.A.)	E.L. Barnad & K. Old	1984
	PREM 57523	CMW 14551	*Myrica faya*	Machico, Madeira	C.S. Hodges	8 May 2000
	PREM 57524	CMW 11301[c]	*M. faya*	Mosteiro, Island of São Miguel, Azores	C.S. Hodges & D.E. Gardner	
	PREM 57525	CMW 11301[c]	*M. faya*	Island of Pico, Azores	C.S. Hodges & D.E. Gardner	30 Jul. 1992
	PREM 58810	CMW 11301[c]	*M. faya*	Island of Pico, Azores	C.S. Hodges & D.E.	31 May 1985

					Gardner	
	PREM 58811	CMW 11301 [c]	*M. faya*	Island of São Miguel, Azores	C.S. Hodges & D.E. Gardner	2 Aug. 1992
	PREM 58812	CMW 11301 [c]	*M. faya*	Island of Terceiro, Azores	C.S. Hodges & D.E. Gardner	31 May 1987
	PREM 58813	CMW 11301 [c]	*M. faya*	Island of Faial, Azores	C.S. Hodges	27 May 1985
Microthia coccolobae	CUP 128 (holotype)	-	Fruit of *Coccoloba uvifera*	Grape Bay, Bermuda	H.H. Whetzel	11 Dec. 1921
	BPI 613756 (isotype)	-	Fruit of *Co. uvifera*	Grape Bay, Bermuda	H.H. Whetzel	11 Dec. 1921
	NY 147 (isotype)	-	Fruit of *Co. uvifera*	Grape Bay, Bermuda	H.H. Whetzel	11 Dec. 1921
	CUP 30512	-	Fruit of *Co. uvifera*	Grape Bay, Bermuda	H.H. Whetzel	11 Dec. 1921
	CUP 35078	-	*Calophyllum calaba*	Devonshire, Bermuda	Seaver, Whetzel & Ogilvie	2 Feb. 1926
	CUP 57366 (nr. 326)	-	Bark of *Co. uvifera*	South Shore, Bermuda	F.J. Seaver & J.M. Waterston	25 Nov. 1940
	CUP 35081	-	*Conocarpus erecta*	Devonshire Bay, Bermuda	Seaver, Whetzel & Ogilvie	5 Feb. 1926
	CUP 34658	-	Fruit of *Co. uvifera*	Elbow Beach, Bermuda	Whetzel, Seaver & Ogilvie	28 Jan. 1926
Unknown	CUP 34657	-	Petioles of *Co. uvifera*	Hungry Bay, Bermuda	Seaver & Whetzel	14 Jan. 1926
Ursicollum fallax	PREM 58840	CMW 18119	*Co. uvifera*	Fort Lauderdale, Florida (U.S.A.)	C. S. Hodges	Mar. 2005
	PREM 58841	CMW 18124, CMW 18115	*Co. uvifera*	Crandon Park, Key Biscayne, Florida (U.S.A.)	C. S. Hodges	Mar. 2005
	PREM 58842	CMW 18124, CMW 18115	*Co. uvifera*	Key Biscayne, Florida (U.S.A.)	C. S. Hodges	Mar. 2005
	PREM 58843	CMW 18114	*Co. uvifera*	Oakland Park, Florida (U.S.A.)	C. S. Hodges	Mar. 2005
	PREM 58844	CMW 18110	*Co. uvifera*	Oakland Park, Florida (U.S.A.)	C. S. Hodges	Mar. 2005
Holocryphia eucalypti	PREM 56211 (holotype)	CMW 7034	GC747 clone of *Eucalyptus*	Mtubatuba, South Africa	M. Venter	25 Feb. 1998
	PREM 56214	-	*Eucalyptus grandis*	Mtubatuba, South Africa	M. Venter	Oct. 1998
	PREM 56216	-	*Eucalyptus grandis*	Mtubatuba, South Africa	M. Venter	Oct. 1998

	PREM 56215 (epitype designated here)	CMW 7033	*E. grandis*	KwaMbonambi, South Africa	M. Venter	Oct. 1998
	PREM 56212	-	*E. grandis*	Sabie, South Africa	J. Roux	Aug. 1998
	PREM 56305	CMW 7035	*E. saligna*	Tzaneen, South Africa	M. Venter	6 Feb. 1999
	PREM 56217	CMW 7038	*Eucalyptus globulus*	Perth, Australia	M.J. Wingfield	1997
Chrysoporthe cubensis	PREM 58814	CMW 11006, CMW 11008	*Eucalyptus* sp.	Kauai, Hawaii (U.S.A.)	M.J. Wingfield	Sep. 2002
	PREM 58815	CMW 10889	*Eucalyptus* sp.	Hawaii, Hawaii (U.S.A).	M.J. Wingfield	Sep. 2002
Cryphonectria parasitica	CUP 2926	CMW 10790	*Castanea dentata*	New York, U.S.A.	W.A. Murrill	1907
Cryphonectria nitschkei	TFM: FPH 1045 (holotype)	CMW 10518	*Quercus grosseserrata*	Japan	T. Kobayashi	1954
Cryphonectria havanensis [d]	TFM:FPH 633	CMW 10910	*Eucalyptus globulus*	Meguro, Japan	T. Kobayashi	1954
	TFM:FPH 2300	-	*Betula* sp.	Yoshiwara, Japan	Zinno	1963
	TFM:FPH 1270	CMW 13736	*Pyrus sinensis*	Inagi, Japan	T. Kobayashi	1960
	TFM:FPH 1203	-	*Quercus variabilis*	Seto, Japan	T. Kobayashi	1953
	TFM:FPH 1047	-	*Quercus glandulifera*	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; **CUP**, Plant Pathology Herbarium, Plant Pathology Department, Cornell University, Ithaca, New York, U.S.A.; **FLAS**, Mycological Herbarium, Department of Plant Pathology, University of Florida, Gainesville, U.S.A.; **NY**, William and Lynda Steere Herbarium, New York Botanical Garden, Bronx, New York, USA; **TFM: FPH**, Forestry and Forest Products Research Institute, Norin Kenkyu, Danchi-Nai, Ibaraki, Japan.

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

[c] Isolates originating from same locality and host, but are not necessarily linked to specific specimen.

[d] Specimens labeled as *C. havanensis* but actually representing *C. nitschkei*.

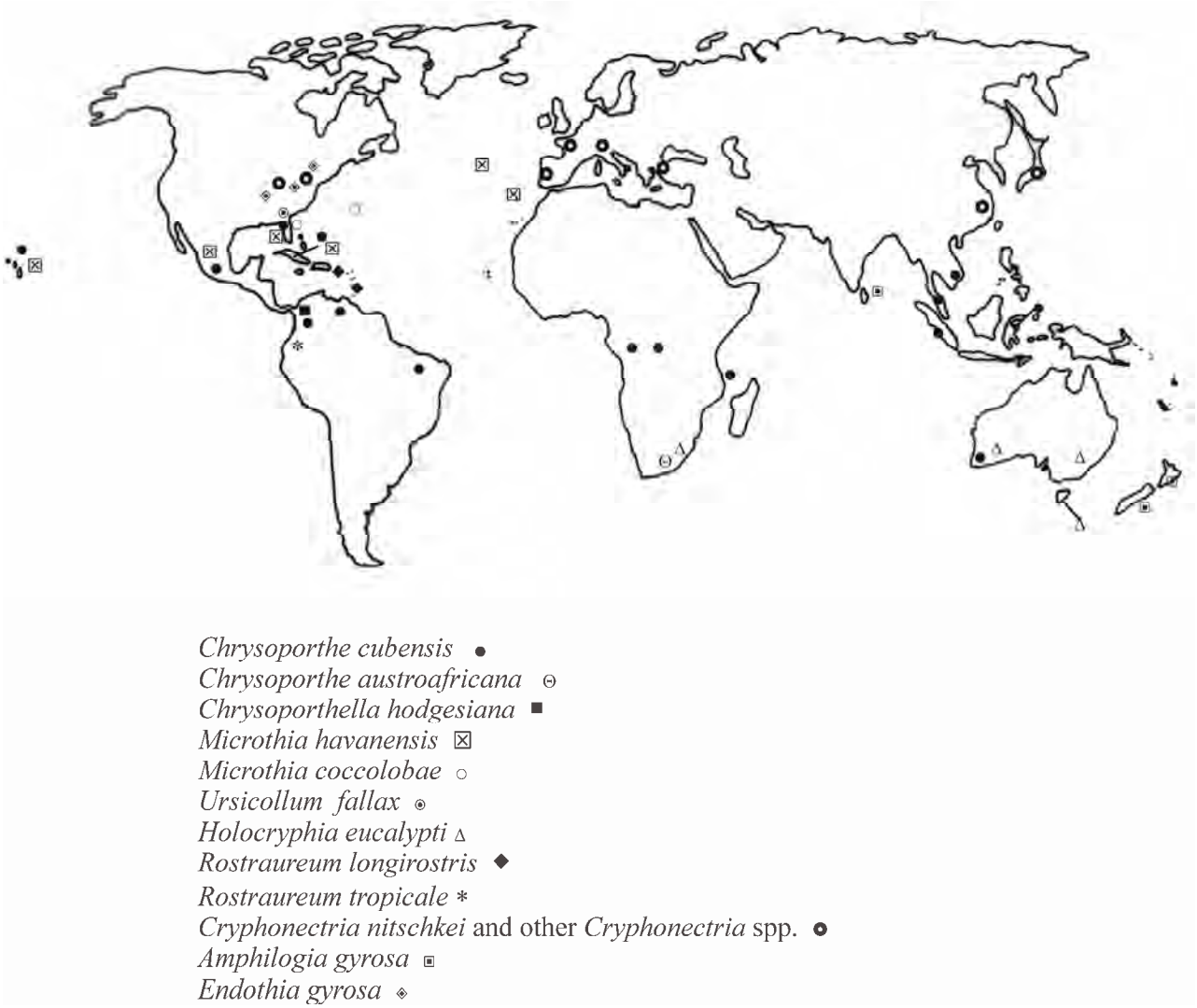

Fig. 1. Map showing the distribution of the various taxa in the *Diaporthales* with orange stromata. Only locations verified with sequence data are shown.

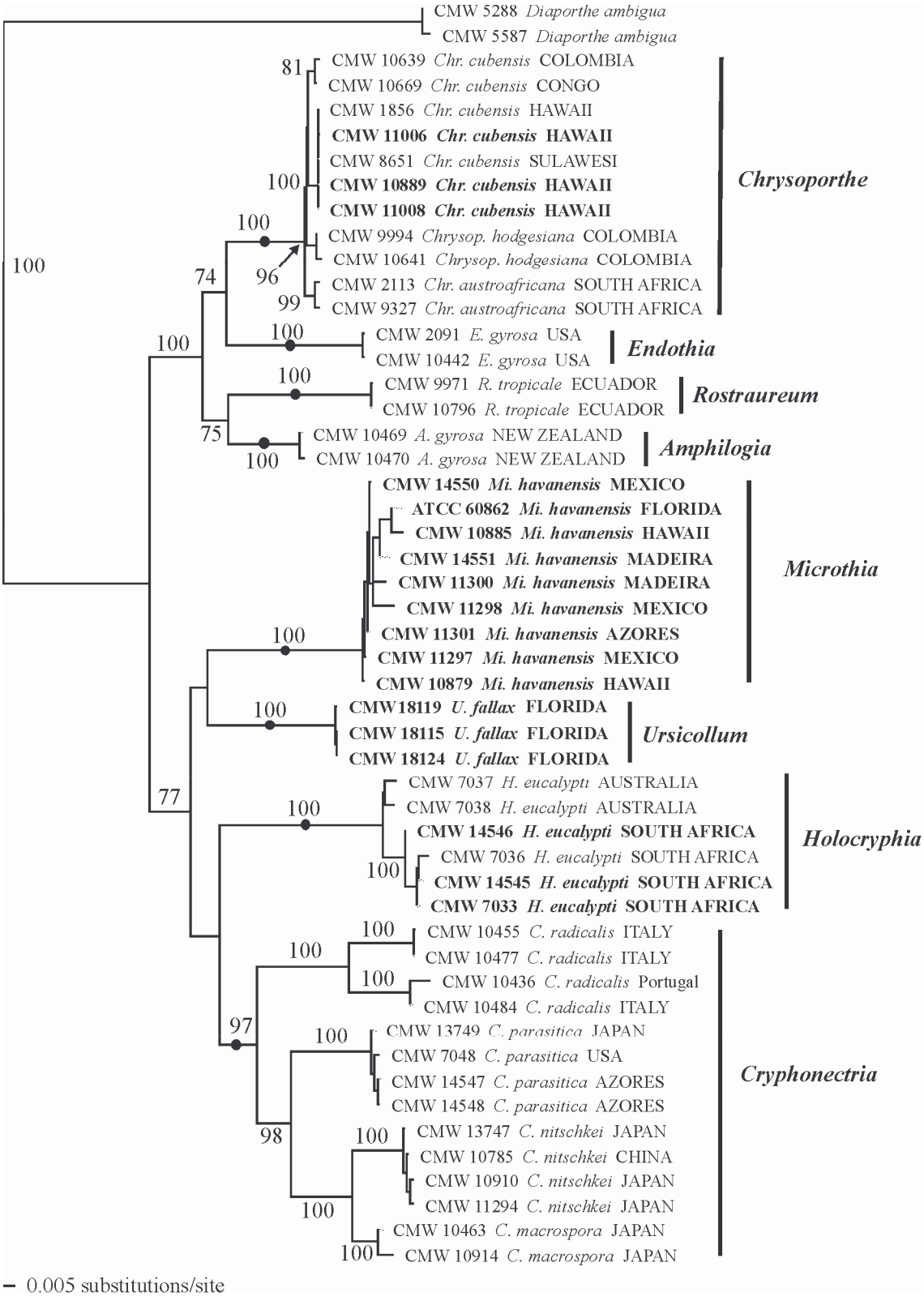

Fig. 2. A phylogenetic tree obtained with distance analyses (TrN+G+I model, G = 0.9717, I = 0.4643, base frequencies 0.1903, 0.3411, 0.2301, 0.2385; rate matrix 1, 3.1147, 1, 1, 4.1643, 1) from a combined DNA sequences dataset of the ITS1, 5.8S rRNA gene and ITS2 regions of the ribosomal operon, and β-tubulin genes. Bootstrap confidence levels (>70%) are indicated on th senting genera are marked with a dot. The outgroup ta

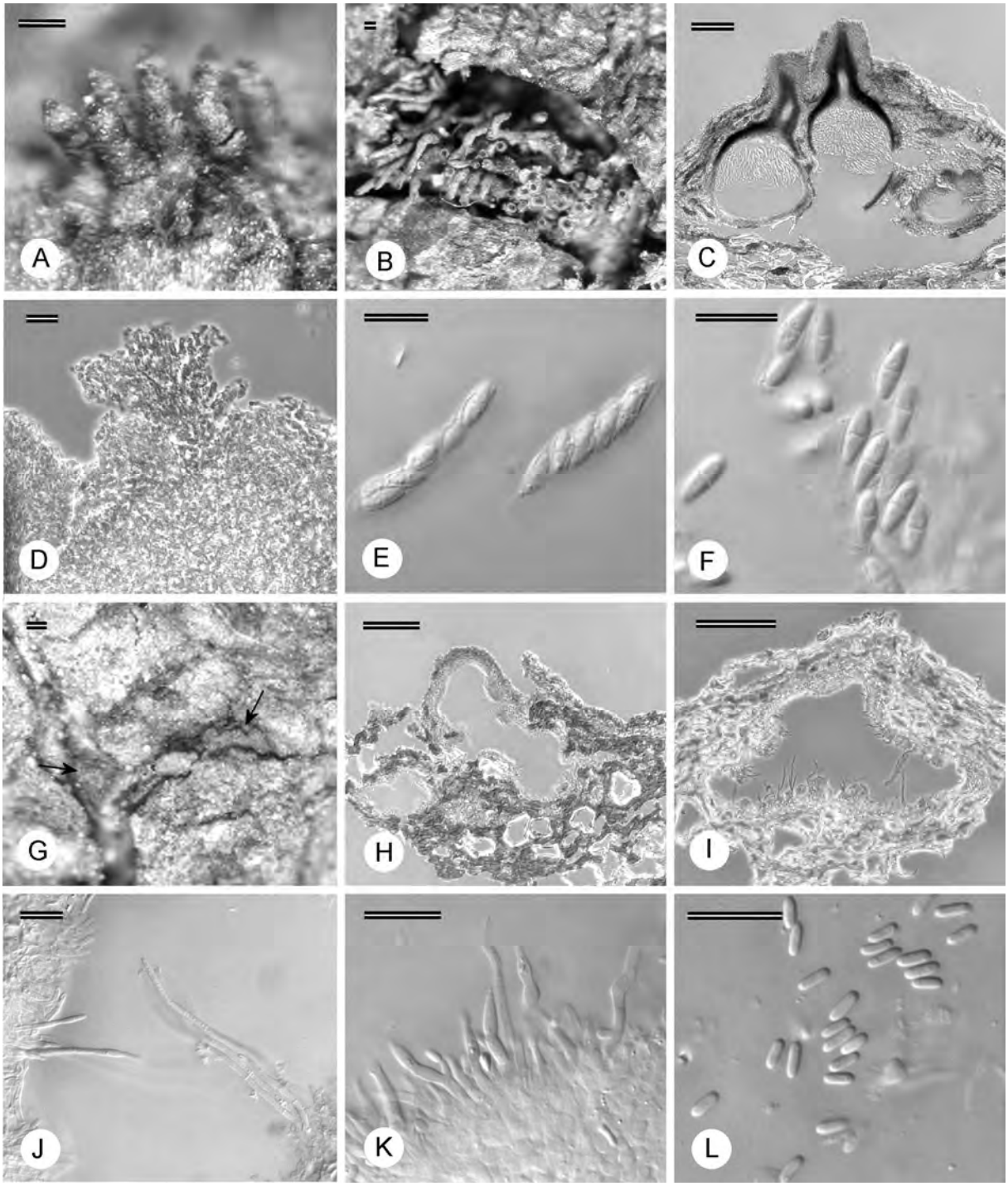

Fig. 3. Fruiting structures of *Microthia havanensis*. A–B. Stereomicrographs of ascomata C. Longitudinal section through ascoma. D. Stromatic tissue. E. Asci. F. Ascospores. G. Conidiomata on bark (arrows). H. Longitudinal section of conidioma. I–J. Long conidiophores and sterile paraphyses. K. Conidiophores. L. Conidia. Scale bars A–C, G–I = 100 µm; D = 20 µm; E–F, J–L = 10 µm.

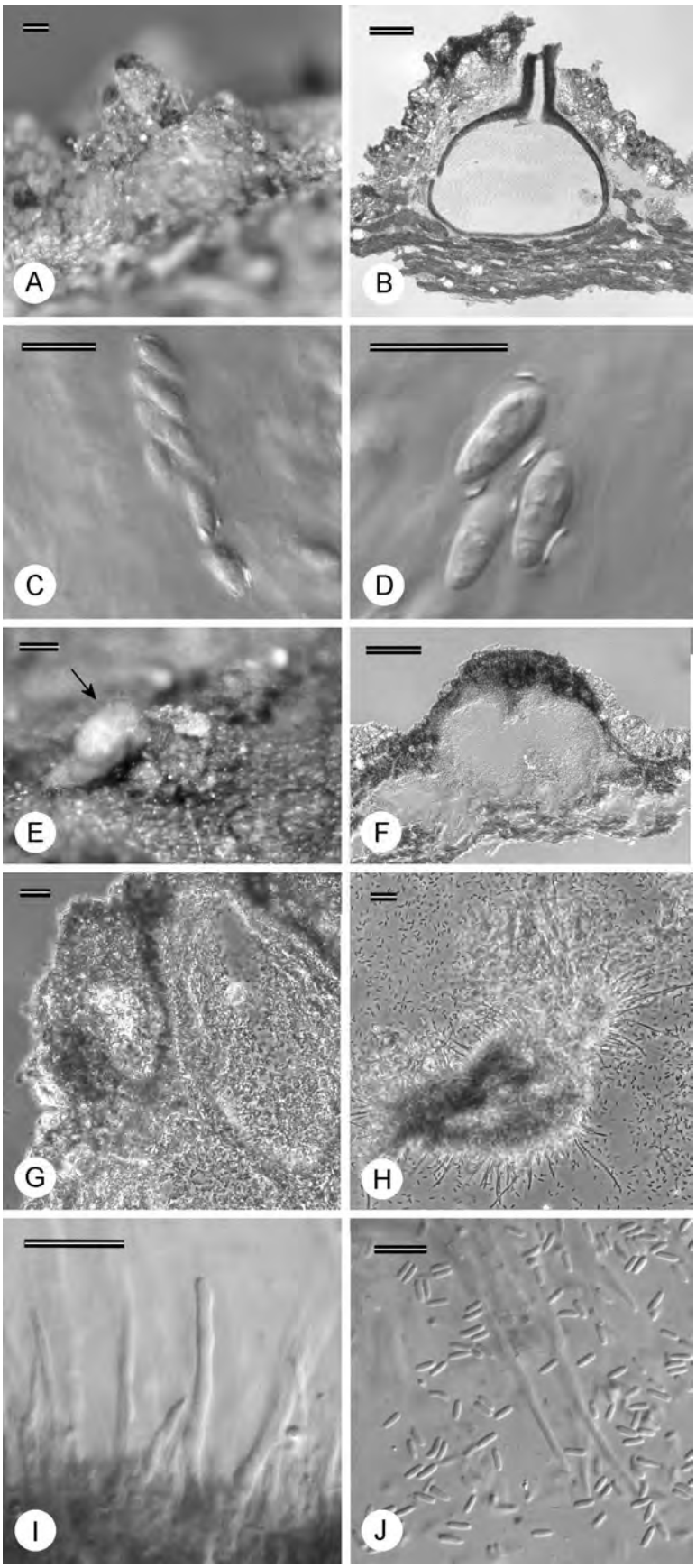

Fig. 4. Fruiting structures of *Microthia coccolobae*. A. Ascoma on bark. B. Longitudinal section through ascoma. C. Ascus. D. Ascospores. E. Conidioma on bark with spore mass (arrow). F. Longitudinal se sue. H. Conidiophores and long paraphyses. I. Conidio Scale bars A–B, E–F = 100 µm; G–H = 20 µm; C–D, I–

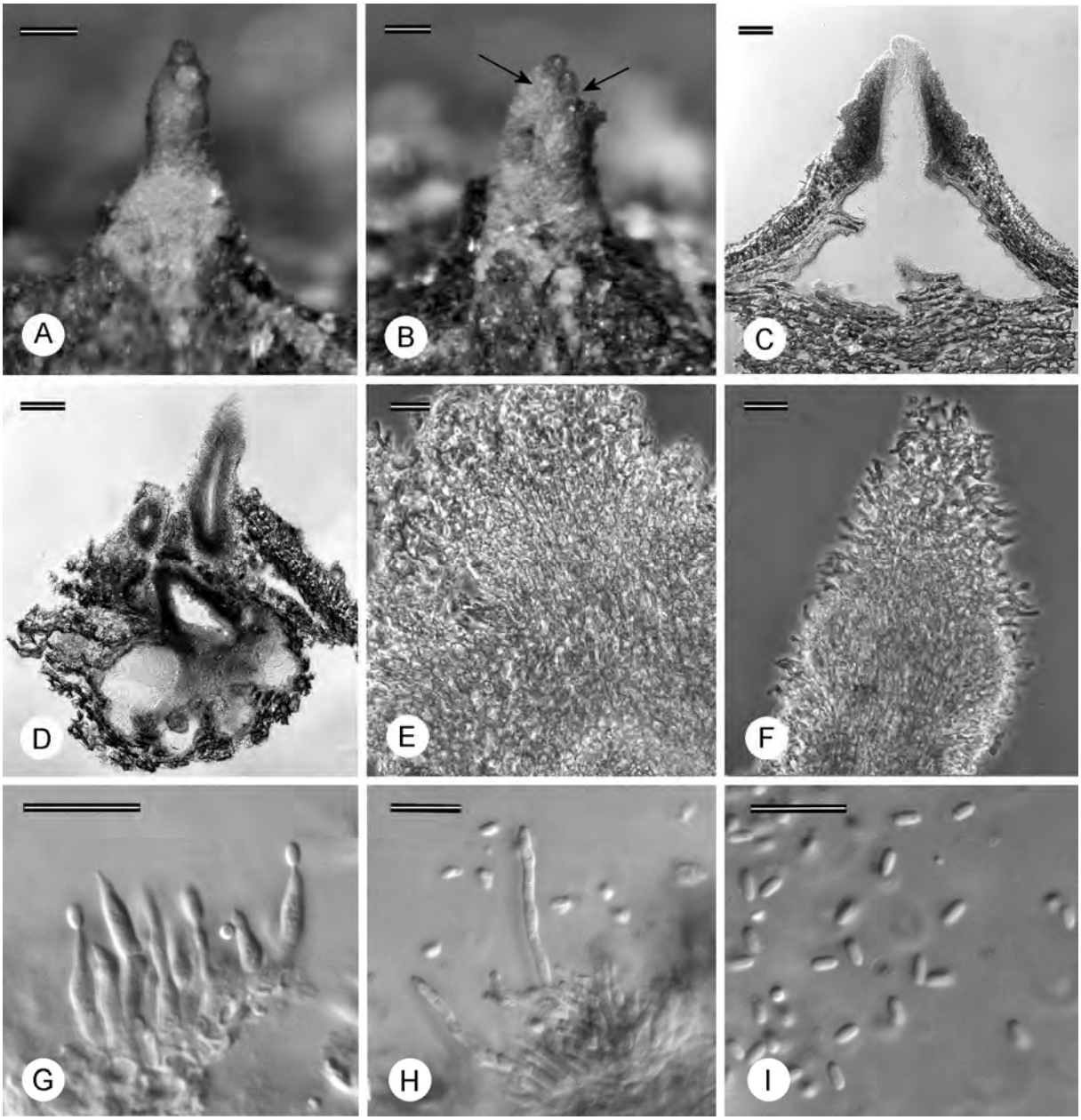

Fig. 5. Fruiting structures of *Ursicollum fallax*. A–B. Conidiomata on bark (necks indicated with arrows). C–D. Longitudinal section through conidioma. E. Tissue at base of conidioma. F. Tissue of neck. G–H. Conidiophores. I. Conidia. Scale bars A–D = 100 µm; E–F = 20 µm; G–I = 10 µm.

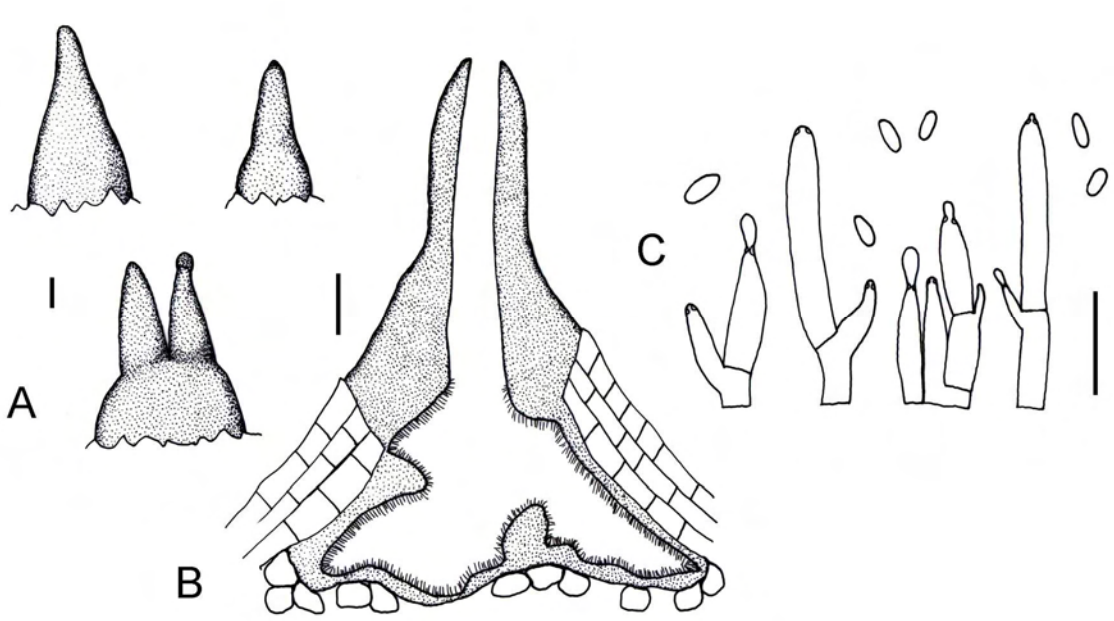

Fig. 6. Line drawings of *Ursicollum fallax*. A. Conidiomata on bark. B. Longitudinal section through conidioma. C. Conidiophores and conidia. Scale bars A–B = 100 µm; C = 10 µm.

CHAPTER 10

New taxonomic concepts for the important forest pathogen *Cryphonectria parasitica* and related fungi

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New taxonomic concepts for the important forest pathogen *Cryphonectria parasitica* and related fungi

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Abstract. Species of *Cryphonectria* include some of the world's most important and devastating tree pathogens. Largely through the application of DNA sequence phylogenies, the taxonomy of these fungi has undergone major changes in recent years. *Cryphonectria*, including the chestnut blight pathogen *Cryphonectria parasitica*, has been restricted to species that have semi-immersed stromata, orange and pulvinate conidiomata, and one-septate ascospores. Other species of *Cryphonectria* with different morphological characteristics have been transferred to new genera that are strongly supported by phylogenetic data. This review represents a summary of the taxonomic changes to species of *Cryphonectria sensu lato*, and we discuss the impact that these changes might have on the understanding of their ecology, pathology and worldwide distribution.

Keywords: *Amphilogia*; *Aurapex*; *Chrysoporthe*; *Cryphonectria*; *Endothia*; *Rostraureum*

1. INTRODUCTION

Species in *Cryphonectria* (*Diaporthales*) are easily recognized on the bark of trees by their large and conspicuous orange stromata (Shear *et al.* 1917, Barr 1978). These stromata (Figs 1A–C) are semi-immersed and erumpent, conidiomata are also orange, pulvinate and stromatic. Species are further identified based on their fusoid to ellipsoid, one-septate ascospores and aseptate, cylindrical conidia (Myburg *et al.* 2004a). *Cryphonectria* spp. occur on a wide variety of woody hosts and they have a world-wide distribution (Table 1), which included the tropics as well as more temperate areas in the past (Shear *et al.* 1917, Kobayashi 1970, Roane 1986a). Many species in this group are economically significant and include some of the world's most serious tree pathogens.

One of the best known species in *Cryphonectria*, *Cryphonectria parasitica* (Murrill) M. E. Barr (Fig. 2). It was introduced into North America where it devastated the American chestnut (*Castanea dentata*, *Fagaceae*, *Fagales*) throughout its natural range (Anagnostakis 1987, Heiniger & Rigling 1994). *Cryphonectria parasitica* was also introduced into Europe, where it resulted in a serious canker disease on *Castanea sativa* or European chestnut (Heiniger & Rigling 1994). Damage caused by this pathogen in Europe has, however, been much less severe than in North America due to reduced virulence imparted by a hypovirus (Heiniger & Rigling 1994). The fungus and its associated viruses have been widely studied due to the significance of *C. parasitica* and the success of the biocontrol brought about by the hypoviruses (Nuss 1992, Hillman & Suzuki 2004, Milgroom & Cortesi 2004).

Cryphonectria represents a relatively small genus of fungi. Until recently, the genus included only 10 species. Besides *C. parasitica*, these included *C. gyrosa* (Berk. & Broome) Sacc., which was the type species, *C. radicalis* (Schwein.: Fr.) M. E. Barr, *C. nitschkei* (G. H. Otth) M. E. Barr, *C. macrospora* (Tak. Kobay. & Kaz. Itô) M. E. Barr, *C. havanensis* (Bruner) M. E. Barr, *C. longirostris* (Earle) Micales & Stipes, *C. coccolobae* (Vizioli) Micales & Stipes, *C. cubensis* (Bruner) Hodges (Micales & Stipes, 1987) and *C. eucalypti* M. Venter & M. J. Wingf. (Venter *et al.* 2002). Other than *C. parasitica*, species such as *C. cubensis* (Fig. 3) and *C. eucalypti* are serious canker pathogens of trees (Old *et al.* 1986, Gryzenhout *et al.* 2003, Wingfield 2003), but the majority of the species are saprophytes on wood (Roane 1986b).

Species of *Endothia* (*Diaporthales*) have commonly been confused with *Cryphonectria*. This is due to their similar orange fruiting structures and a shared anamorph genus, *Endothiella* (Barr 1978). Members of *Cryphonectria* and *Endothia* have also been recorded to share the same hosts and to occur in the same countries. For example, *E. gyrosa* (Schwein.: Fr.) Fr., *E. singularis* (Syd. & P. Syd.) Shear & N. E. Stevens, *C. parasitica* and *C. radicalis* all occur in North America (Table 1) on members of the *Fagaceae* (Roane 1986a). *Cryphonectria* has also been treated as a synonym of *Endothia* for a large portion of its taxonomic history (Shear *et al.* 1917, Kobayashi 1970, Barr 1978, Roane 1986a).

Based on DNA sequence comparisons and phylogenetic analyses, species of *Endothia* can be distinguished (Fig. 4) from those in *Cryphonectria* (Venter *et al.* 2002, Myburg *et al.* 2004a). This is strongly supported by morphological differences such as the aseptate ascospores and superficial stromata, which are characteristic in *Endothia* but

not in *Cryphonectria* (Myburg *et al.* 2004a). Currently, *Endothia* includes only two species, *E. gyrosa* (type) and *E. singularis* (Myburg *et al.* 2004a). A third species with green stromata, *E. viridistroma* Wehm., most likely resides in a genus other than *Endothia* (Myburg *et al.* 2004a).

The application of DNA sequence comparisons revealed the fact that the taxonomy of *Cryphonectria* required revision. For example, *Cryphonectria* was shown not to be monophyletic (Fig. 4), but consisting of many species residing in newly recognised genera (Myburg *et al.* 2004a, Gryzenhout *et al.* 2005a//Chapter 7 in this thesis). These newly recognized groups were supported by morphological features that were inordinantly diverse (Fig. 1) to warrant retaining these taxa in a single genus (Myburg *et al.* 2004a, Gryzenhout *et al.* 2005a). The primary aim of this review is to provide a summary of the recent changes to the taxonomy of species in *Cryphonectria sensu lato*, and to re-evaluate the host range, ecology and distribution of *Cryphonectria* species.

2. REVISED TAXONOMY

2.1 *Cryphonectria*

Phylogenetic comparisons have revealed that isolates labeled as *C. havanensis* in Japan, are identical to those of *C. nitschkei* (Myburg *et al.* 2004b). It has, furthermore, been confirmed (Myburg *et al.* 2004b) that *C. nitschkei* has a wide host range including five plant orders (Table 1). This fungus is restricted to the Far East and is known to occur in Japan, China (Myburg *et al.* 2004b) and Siberia, Russia (Vasilyeva 1998).

European isolates originally labeled as *C. radicalis*, represent two different species of *Cryphonectria* (Myburg *et al.* 2004a, 2004b). This was determined independently based on morphology and DNA sequence comparisons (Fig. 4). *Cryphonectria radicalis*, defined by the type specimen from North America, corresponds morphologically to a phylogenetic group containing isolates from Greece, Italy, Switzerland and Japan (Myburg *et al.* 2004b). This has not been confirmed using phylogenetic analyses, since isolates that can be linked to *C. radicalis* in North America, do not exist. The other species has ascospores longer than those of *C. radicalis sensu stricto*, but specimens had also previously been labeled as *C. radicalis* (Myburg *et al.* 2004b). This species could possibly be linked to a second phylogenetic group consisting of isolates labeled as *C. radicalis* from Italy, France and Portugal, but this is also speculative as there are no isolates linked to herbarium specimens for this species. As the results of the DNA sequence-based and morphological comparisons cannot be linked, this species has not yet been described as a unique taxon.

Additional collections of *C. radicalis sensu lato* are clearly needed to resolve questions regarding its taxonomy. The phylogenetic placement of North American *C. radicalis* isolates has yet to be determined. It is also possible that another species of *Cryphonectria*, similar to *C. radicalis sensu lato* and referred to as *E. radicalis mississippiensis* Shear and N. E. Stevens (Shear *et al.* 1917), occurs in North America (Myburg *et al.* 2004b). Additional collections from Japan will also be required to determine whether the other, undescribed sub-clade encompassing of isolates labelled as *C. radicalis*, co-exists with *C. radicalis* in Japan. This question arose because only a single isolate of *C. radicalis* from Japan, which grouped in the sub-clade representing *C.*

radicalis sensu stricto, has been available for study (Myburg *et al.* 2004b). Isolates of *C. radicalis sensu lato* will, however, be difficult to detect in nature because they are possibly displaced by or their occurrence is masked by *C. parasitica*, especially in Europe and North America (Hoegger *et al.* 2002).

One of the fungi associated with cankers on *Eucalyptus* that has come into consideration during taxonomic studies of *Cryphonectria*, is *C. eucalypti*. This fungus was previously known as *Endothia gyrosa*, which is a well-known associate of stem and branch cankers on native tree species the United States (Stipes & Phipps 1971, Roane *et al.* 1974). Rather unusually, it is also a name that was applied to the causal agent of stem cankers on *Eucalyptus* in Australia (Walker *et al.* 1985, Old *et al.* 1986) and South Africa (Van der Westhuizen *et al.* 1993). One of the reasons that the fungus on *Eucalyptus* was treated as *E. gyrosa*, is that it has orange stromata and aseptate ascospores, which made it similar to *Endothia* spp. (Walker *et al.* 1985). Phylogenetic and morphological studies of isolates from *Eucalyptus* and those of *E. gyrosa* from the U.S.A (Venter *et al.* 2001, 2002) showed that these fungi are not the same, and that the fungus from *Eucalyptus* are closer related to *Cryphonectria*. This led to a name being provided for the fungus on *Eucalyptus* in *Cryphonectria* as *C. eucalypti* (Venter *et al.* 2002).

Although phylogenetic studies have grouped *C. eucalypti* closely with *Cryphonectria*, the fungus is unlike other species of *Cryphonectria*, which all have single septate ascospores (Venter *et al.* 2002, Myburg *et al.* 2004a). *Cryphonectria eucalypti* has aseptate ascospores and thus based on morphological characteristics, has been suspected to represent a distinct genus (Myburg *et al.* 2004a). This hypothesis is confirmed in the new phylogenetic tree presented in the present study (Fig. 4), showing

that *C. eucalypti* groups separately from species in *Cryphonectria sensu stricto*, and a new genus should thus be erected for this species.

2.2 New type for *Cryphonectria*

A group of isolates from *Elaeocarpus* spp. in New Zealand, labeled as *C. gyrosa* and *C. radicalis*, was shown in DNA sequence-based phylogenetic analyses (Fig. 4) to group separately from other *Cryphonectria* spp. (Myburg *et al.* 2004a). Their distinct grouping was defined morphologically by the one to three-septate ascospores, conical and superficial conidiomata (Fig. 1G), and conidia of variable size (Myburg *et al.* 2004a; Gryzenhout *et al.* 2005b/Chapter 6 in this thesis). These isolates are also unique in being associated with root cankers on *Elaeocarpus* spp. in New Zealand (Pennycook 1989).

In resolving the identity of the fungus defined by the isolates from New Zealand, it was realized that the fungus shared the same morphology as specimens of *C. gyrosa* from Sri Lanka (Gryzenhout *et al.* 2005b, 2005c/Chapter 5 in this thesis, Myburg *et al.* 2004a). Because *C. gyrosa* was commonly recognised as the type of *Cryphonectria* (Barr 1978), this implied that isolates residing in the new clade from New Zealand, should have had the name *Cryphonectria*, rather than those of the clade defining currently known *Cryphonectria* spp., including *C. parasitica* (ICBN, Art. 7.2, Greuter *et al.* 2000).

Studies on the type status of *C. gyrosa* showed that *C. gyrosa* was erroneously cited as the type of *Cryphonectria* (Gryzenhout *et al.* 2005c). The error arose because *C. gyrosa* was mechanically selected as type at the time, while the species included in the original sub-genus *Cryphonectria*, namely *C. abscondita* Sacc. and *C. variicolor* Fuckel, were ignored as choice for type (Gryzenhout *et al.* 2005c). This erroneous

lectotypification of *C. gyrosa* and the separate grouping of the isolates similar to *C. gyrosa* from *Cryphonectria* spp., prompted a proposal to conserve the name *Cryphonectria* against a new type (Gryzenhout *et al.* 2005c). The proposal was accepted by the International Association of Plant Taxonomists (IAPT) Nomenclature Committee for Fungi (Gams 2005). The original species *C. variicolor* and *C. abscondita* were not suitable as new types and *C. parasitica* was chosen as the type for the genus. This was justified largely because of its importance as a pathogen and the viruses that have been characterized from it (Gryzenhout *et al.* 2005c).

The clade containing *C. gyrosa* and the isolates from New Zealand could thus be described as a distinct genus with the new name *Amphilogia* (Gryzenhout *et al.* 2005b). *Amphilogia gyrosa* (Berk. & Broome) Gryzenh. & M. J. Wingf. occurs on *Elaeocarpus* spp. in both New Zealand and Sri Lanka . This genus includes a second species, *A. major* Gryzenh. & M. J. Wingf., which also occurs on *Elaeocarpus* spp., but is currently known only from the South Island of New Zealand (Gryzenhout *et al.* 2005b).

2.3 New genera for *Cryphonectria* spp.

Only *C. parasitica*, *C. radicalis sensu lato*, *C. nitschkei* and *C. macrospora* group in the well-supported clade that represents *Cryphonectria* (Fig. 4), and they all have the morphological characteristics that define *Cryphonectria* (Myburg *et al.* 2004a, 2004b). The taxonomic position of *C. havanensis* and *C. coccolobae* remains confused because no isolates exist for these species that can be used in phylogenetic studies based on DNA sequence comparisons (Myburg *et al.* 2004a). The remaining species described in *Cryphonectria* have, however, now been transferred to newly described genera, or will be

transferred soon. These new genera have largely been recognised based on DNA sequence data (Fig. 4). Robust morphological characters also support the phylogenetic grouping of these new genera (Fig. 1).

Chrysoporthe is a new genus that has been described to house isolates of *C. cubensis* from various parts of the world (Gryzenhout *et al.* 2004/Chapter 1 in this thesis). The fungi previously collectively known as *C. cubensis*, are some of the most serious canker pathogens (Wingfield 2003) of commercially grown *Eucalyptus* (*Myrtaceae*, *Myrtales*), and they are also pathogenic to other plant genera in the *Myrtales* (Fig. 3; Table 1). Species in *Chrysoporthe* are all characterized by limited ascostromatic tissue covering the perithecial bases, long perithecial necks covered in black stromatic tissue, and superficial, generally pyriform and fuscous black conidiomata (Figs 1D–F; Myburg *et al.* 2004a, Gryzenhout *et al.* 2004). The anamorph genus *Chrysoporthella*, has been described for asexual structures of *Chrysoporthe* (Gryzenhout *et al.* 2004).

Analyses of DNA sequence data have shown that isolates labeled as *C. cubensis* from different parts of the world, group in five distinct sub-clades (Fig. 4) of *Chrysoporthe* (Gryzenhout *et al.* 2004, Gryzenhout *et al.* 2005d/Chapter 2 in this thesis). These taxa are all pathogens of trees (Fig. 3). Four species (Table 1) could be described based on obvious morphological and ecological characteristics. These species include *Chr. cubensis* (Bruner) Gryzenh. & M. J. Wingf. for the *Eucalyptus* pathogen in South America, South East Asia, Australia and Central Africa (Gryzenhout *et al.* 2004). This species is also able to infect *Syzygium aromaticum* or clove (Hodges *et al.* 1986, Myburg *et al.* 2003) and *Miconia* spp. (Rodas *et al.* 2005). *Chr. austroafricana* Gryzenh. & M. J. Wingf. (Gryzenhout *et al.* 2004) causes cankers on *Eucalyptus* (Wingfield *et al.* 1989),

Tibouchina (Figs 3E–F, Myburg *et al.* 2002) and *Syzygium* spp. (Heath *et al.* 2006) in South Africa. *Chr. doradensis* Gryzenh. & M. J. Wingf. is a newly described species (Gryzenhout *et al.* 2005d) that is pathogenic to *Eucalyptus* spp. in Ecuador. *Chrysoporthella hodgesiana* Gryzenh. & M. J. Wingf., for which the teleomorph has not yet been found (Gryzenhout *et al.* 2004), infects native *Tibouchina* spp. (Wingfield *et al.* 2001, Gryzenhout *et al.* 2004) and *Miconia* spp. (Rodas *et al.* 2005) in Colombia.

Chrysoporthe cubensis isolates reside in two distinct phylogenetic sub-clades (Fig. 4, Gryzenhout *et al.* 2004). The one sub-clade consists of isolates from South and Central America, and Eastern Africa (Gryzenhout *et al.* 2004), while the other sub-clade includes isolates from South East Asian countries, Australia, Tanzania and Hawaii (Myburg *et al.* 2003). Fungi in these two sub-clades are indistinguishable from each other morphologically, although they represent two geographically distinct groups (Gryzenhout *et al.* 2004). Population level techniques will most likely be required to determine whether fungi in these two sub-clades represent distinct species.

A fungus pathogenic to *Terminalia ivorensis* and *Terminalia superba* (*Combretaceae*, *Myrtales*) in Ecuador has recently been discovered and characterized (Fig. 4). This fungus has been described in the new genus *Rostraureum* and it is referred to as *R. tropicale* Gryzenh. & M. J. Wingf. (Gryzenhout *et al.* 2005a). *Rostraureum* (Fig. 1H) is characterized by superficial, orange, rostrate conidiomata with long necks, and semi-immersed ascostromata with little stromatic tissue, except for a white sheath of tissue around the perithecial necks. *Cryphonectria longirostris* also has these characteristics, but can be distinguished from *R. tropicale* based on conidial size (Gryzenhout *et al.* 2005a). For these reasons, *C. longirostris* has been transferred to

Rostraureum (Gryzenhout *et al.* 2005a). In contrast to *R. tropicale*, there is no evidence to suggest that *R. longirostris* (Earle) Gryzenh. & M. J. Wingf. is pathogenic (Earle 1901).

2.4 A newly discovered genus related to *Cryphonectria* and *Chrysoporthe*

Extensive sampling of fungi similar to *Chrysoporthe* and *Cryphonectria* has led to the discovery of a new fungus, *Aurapex penicillata* Gryzenh. & M. J. Wingf. nom. prov. from Colombia. This fungus is associated with canker and die-back symptoms on native *Melastomataceae* (*Tibouchina*, *Miconia*) and *Eucalyptus* trees (Gryzenhout *et al.* 2006/Chapter 8 in this thesis). Morphologically, this fungus is similar to *Chrysoporthella*, with black, superficial and pyriform conidiomata (Fig. 1I). It can, however, be distinguished from *Chrysoporthella* based on the different tissue organization in the two fungi and because the tips of the conidiomatal necks are orange as opposed to necks that are uniformly black in *Chrysoporthella* (Fig. 1F). Phylogenetically, the fungus groups alone but close to *Cryphonectria* and allied genera (Fig. 4). No teleomorph has been found for *A. penicillata*, and it was thus described as the mitotic genus *Aurapex* nom. prov. residing in the *Diaporthales*.

2.5 Position of *Cryphonectria* and allied genera in the *Diaporthales*

Large subunit DNA sequence comparisons of an extensive collection of genera in the *Diaporthales*, have revealed that species of *Cryphonectria*, *Endothia* and *Chrysoporthe* form a well-supported and distinct clade in the order (Castlebury *et al.* 2002). This clade has been referred to as the *Cryphonectria-Endothia* complex. Castlebury *et al.* (2002)

proposed that the unique grouping of these isolates in the *Diaporthales* is supported by morphological features such as stromatic tissue that is orange at some stage in the life cycle of the fungus, and the tissue that turns purple in 3 % KOH and yellow in lactic acid. The unique grouping and morphological characteristics of these genera suggests that *Cryphonectria* and related genera most likely represent a new family in the *Diaporthales*.

3. CONCLUSIONS

Recent taxonomic revisions have restricted the name *Cryphonectria* to only seven species. Other species related to *Cryphonectria* now reside in the three genera *Chrysoporthe*, *Rostraureum* and *Amphilogia*. The species conclusively shown to belong in *Cryphonectria* include *C. parasitica*, which is now also recognized as the type of the genus, *C. nitschkei*, *C. radicalis sensu lato* and *C. macrospora*. In DNA sequence comparisons, these species reside in a distinct clade representing *Cryphonectria sensu stricto*. Of these *Cryphonectria* species, only *C. parasitica* is a serious and primary plant pathogen (Anagnostakis 1987).

Cryphonectria havanensis, *C. coccolobae* and *C. eucalypti* currently retain their position in *Cryphonectria*. However, the taxonomic relationship of *C. havanensis* and *C. coccolobae* with species in the *Cryphonectria* clade must still be clarified. Likewise, *C. eucalypti* should reside in a taxon apart from *Cryphonectria* because of its distinct ascospore morphology (Myburg *et al.* 2004a) and phylogenetic data presented for the first time in this study.

Cryphonectria spp. as they are now recognized, occur in temperate areas of Asia, Europe and North America (Table 1). Only *C. havanensis* and *C. coccolobae* occur in the Caribbean area, while *C. eucalypti* is known from Australia and South Africa (Table 1). *C. parasitica*, *C. radicalis sensu lato*, *C. nitschkei* and *C. macrospora* occur on a wide range of woody plants residing in five plant orders (Table 1). These species are all known from Japan, and it seems probable that this part of the world represents a centre of origin for these fungi. Only *C. radicalis sensu lato* occurs naturally in Europe (Myburg *et al.* 2004b), and additional species remain to be described in this group. *C. radicalis* is also known in the USA, based on herbarium specimens from 1828 (Myburg *et al.* 2004b), although its presence in that country needs to be confirmed based on fresh specimens and DNA sequence comparisons.

During the course of the last decade, wide-ranging changes have been made to the taxonomy of *Cryphonectria* and related genera. These have emerged largely from studies on species such as *Chr. cubensis* that causes a serious canker disease on *Eucalyptus* and related plants (Wingfield 2003). These studies commenced during the time when DNA sequence comparisons and the phylogenetic species concept was emerging as a dominant taxonomic approach, and they would not have been possible without them. Taxonomic studies described in this review provide a framework that should lead to a better understanding of the important tree pathogens residing in this group. They are also likely to lead to the discovery of additional species and to promote a more lucid understanding of the global distribution of invasive or potentially invasive tree pathogens.

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Table 1. Hosts, distribution, representative isolates (ex-type isolates in bold) and representative sequences of *Cryphonectria* spp. and related genera.

Genus	Species	Distribution	Best known host genera	Representative isolates [a]	Representative sequences [b]
Cryphonectria	*C. parasitica*	Japan, China, North America, Europe, Turkey	*Castanea*, *Quercus* (*Fagaceae*, *Fagales*)	CMW7048 = ATCC48198 CMW13749 = MAFF410158	AF368330, AF273076, AF273470 AY697927, AY697943, AY697944
	C. nitschkei	Japan, China, Russia	*Quercus*, *Castanea*, *Castanopsis* (*Fagaceae*, *Fagales*), *Betula*, *Carpinus* (*Betulaceae*, *Fagales*), *Pyrus*, *Prunus* (*Rosaceae*, *Rosales*), *Eucalyptus* (*Myrtaceae*, *Myrtales*),	CMW13742 = MAFF410570 CMW13747 = MAFF410569	AY697936, AY697961, AY697962 AY697937, AY697963,

		Rhus (*Anacardiaceae*, *Sapindales*) and *Larix* (*Pinaceae*, *Pinales*)			AY697964
C. macrospora	Japan	*Castanopsis* (*Fagaceae*, *Fagales)*	CMW10463 CBS112920	=	AF368331, AF368351, AF368350
			CMW10914 TFM: FPH E55	=	AY697942, AY697973, AY697974
C. radicalis sensu lato	Japan, Greece, Italy, Switzerland, France, Portugal	*Quercus*, *Castanea* (*Fagaceae*, *Fagales*), *Carpinus* (*Betulaceae*, *Fagales*)	CMW10455 CBS238.54	=	AF452113, AF525705, AF525712
			CMW10477 CBS240.54	=	AF368328, AF368347, AF368346
			CMW10436	=	AF452117,

			CBS165.30		AF525703, AF525710
			CMW10484	=	AF368327, AF368349, AF368349
			CBS112918		
C. havanensis	Cuba	*Eucalyptus* (*Myrtaceae*, *Myrtales*), *Spondias*, *Mangifera indica* (*Anacardiaceae*, *Sapindales*), *Persea gratissima* (*Lauraceae*, *Laurales*)	n/a		n/a
C. coccolobae	Bermuda, Florida (U.S.A.)	*Coccoloba* (*Polygonaceae*, *Polygonales*)	n/a		n/a
C. eucalypti	Australia, South Africa	*Eucalyptus* (*Myrtaceae*, *Myrtales*)	CMW7036		AF232878, AF368341, AF368340
			CMW7037		AF232880,

						AF368343, AF368342
Endothia	*E. gyrosa*	U.S.A.	*Quercus, Fagus* (*Fagaceae, Fagales*), *Liquidambar* (*Hamamelidaceae, Saxifragales*), *Acer* (*Aceraceae, Sapindales*), *Ilex* (*Aquifoliaceae, Celastrales*), *Vitis* (*Vitaceae, Rhamnales*), *Prunus* (*Rosaceae, Rosales*)	CMW2091 ATCC48192	=	AF046905, AF368337, AF368336
				CMW10442 CBS118850	=	AF368326, AF368339, AF368338
	E. singularis	Colorado (U.S.A.)	*Quercus* (*Fagaceae, Fagales*)	n/a		n/a
Amphilogia	*A. gyrosa*	Sri Lanka, New Zealand	*Elaeocarpus* (*Elaeocarpaceae, Oxalidales*)	**CMW10469 CBS112922**	=	AF452111, AF525707, AF525714
				CMW10740	=	AF452112,

				CBS112923		AF525708, AF525715
	A. major	New Zealand	*Elaeocarpus* (*Elaeocarpaceae*, *Oxalidales*)	n/a		n/a
Chrysoporthe	*Chr. cubensis*	South & Central America, Hawaii, Florida (U.S.A.), South East Asia, Australia, Central Africa	*Eucalyptus*, *Syzygium* (*Myrtaceae*, *Myrtales*), *Miconia* (*Melastomataceae*, *Myrtales*)	CMW10639 CBS115747	=	AY263419, AY263420, AY263421
				CMW10669 CBS115751	=	AF535122, AF535124, AF535126
				CMW8651 CBS115718	=	AY084002, AY084014, AY084026
				CMW11290 CBS115738	=	AY214304, AY214232,

					AY214268
Chr. austroafricana	South Africa	*Eucalyptus*, *Syzygium* (*Myrtaceae*, *Myrtales*), *Tibouchina* (*Melastomataceae*, *Myrtales*)	**CMW2113** **CBS112916**	=	AF046892, AF273067, AF273462
			CMW9327 CBS115843	=	AF273473, AF273060, AF273455
Chr. doradensis	Ecuador	*Eucalyptus* (*Myrtaceae*, *Myrtales*)	**CMW11286** **CBS115734**	=	AY214289, AY214217, AY214253
			CMW11287 **CBS115735**	=	AY214290, AY214218, AY214254
Chrysoporthella hodgesiana	Colombia	*Tibouchina*, *Miconia* (*Melastomataceae*, *Myrtales*)	CMW10625 CBS115744	=	AY956970, AY956979,

						AY956980
				CMW10641	=	AY692322,
				CBS115854		AY692326,
						AY692325
Rostraureum	*R. tropicale*	Ecuador	*Terminalia* (*Combretaceae*, *Myrtales*)	CMW9971	=	AY167425,
				CBS115725		AY167430,
						AY167435
				CMW10796	=	AY167428,
				CBS115757		AY167433,
						AY167438
	R. longirostris	Puerto Rico, Trinidad & Tabago	Unknown hosts, dead wood	n/a		n/a
Aurapex	*A. penicillata*	Colombia	*Miconia*, *Tibouchina* (*Melastomataceae*, *Myrtales*),	**CMW10030** **CBS115740**	=	AY214311, AY214239,

	Eucalyptus (*Myrtaceae*, *Myrtales*)		AY214275
		CMW10035 =	AY214313,
		CBS115742	AY214241,
			AY214277

(Roane 1986[a], Robin & Heiniger 2001, Venter *et al.* 2002, Myburg *et al.* 2003, 2004[a], 2004b, Gryzenhout *et al.* 2004, 2005[a], 2005b, 2005d, 2006, Heath *et al.* 2006)

[a] **CMW**, Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria;

ATCC, American Type Culture Collection, Manassas, USA;

CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands;

TFM:FPH, Forestry and Forest Products Research Institute, Danchi-Nai, Ibaraki, Japan (E or Ep refers to an isolate);

MAFF, Microorganisms Section, MAFF GENEBANK, National Institute of Agrobiological Sciences (NIAS), Ibaraki, Japan.

[b] Accesssion numbers given as sequences from the ITS region, and two regions from the β-tubulin genes amplified with primers 1a/1b and 2a/2b, respectively.

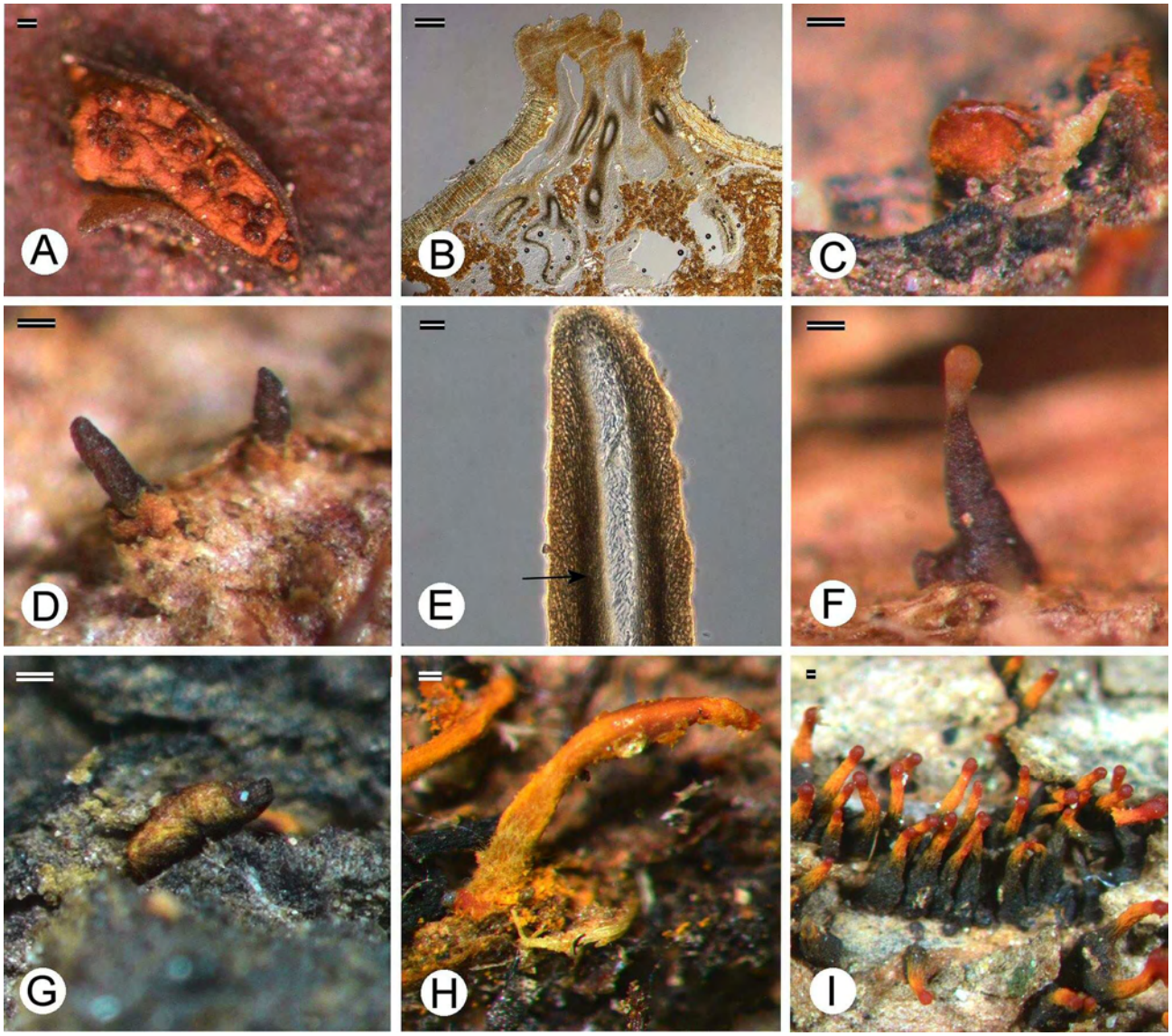

Fig. 1. Fruiting structures of *Cryphonectria* and related genera. A. Ascostroma of *Cryphonectria*. B. Longitudinal section through ascostroma of *Cryphonectria*. C. Conidioma of *Cryphonectria*. D. Ascostromata of *Chrysoporthe*. E. Perithecial neck of *Chrysoporthe* (arrow) with brown surrounding tissue. F. Conidioma of *Chrysoporthe*. G. Conidioma of *Amphilogia*. H. Conidioma of *Rostraureum*. I. Conidiomata of *Aurapex*. Scale bars A–H = 100 µm, I = 200 µm.

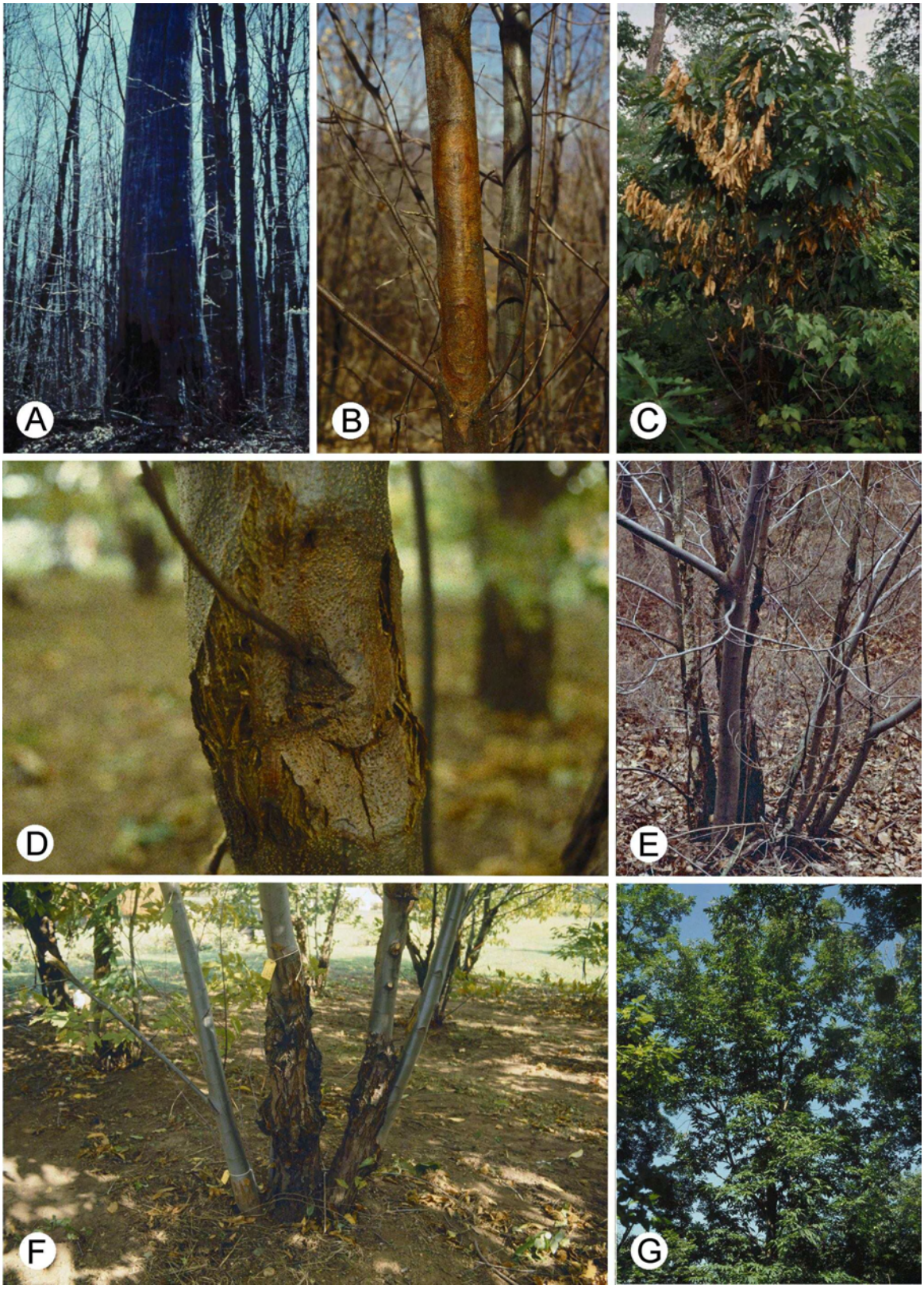

Fig. 2. Disease symptoms of chestnut blight caused by *Cryphonectria parasitica*. A. A relic *Castanea dentata* tree still standing long after being killed by chestnut blight after its introduction into the U.S.A. B. Diffuse canker on *Castanea dentata*. C. Die-back of branches with dead leaves still attached. D. Sunken canker on *Castanea dentata* with fruiting structures visible. E. Numerous stump sprouts from *Castanea dentata* roots of tree killed earlier by chestnut blight. F. Multiple cankers on stump sprouts. G. One of the few large and surviving specimens of *Castanea dentata* in the U.S.A. today.

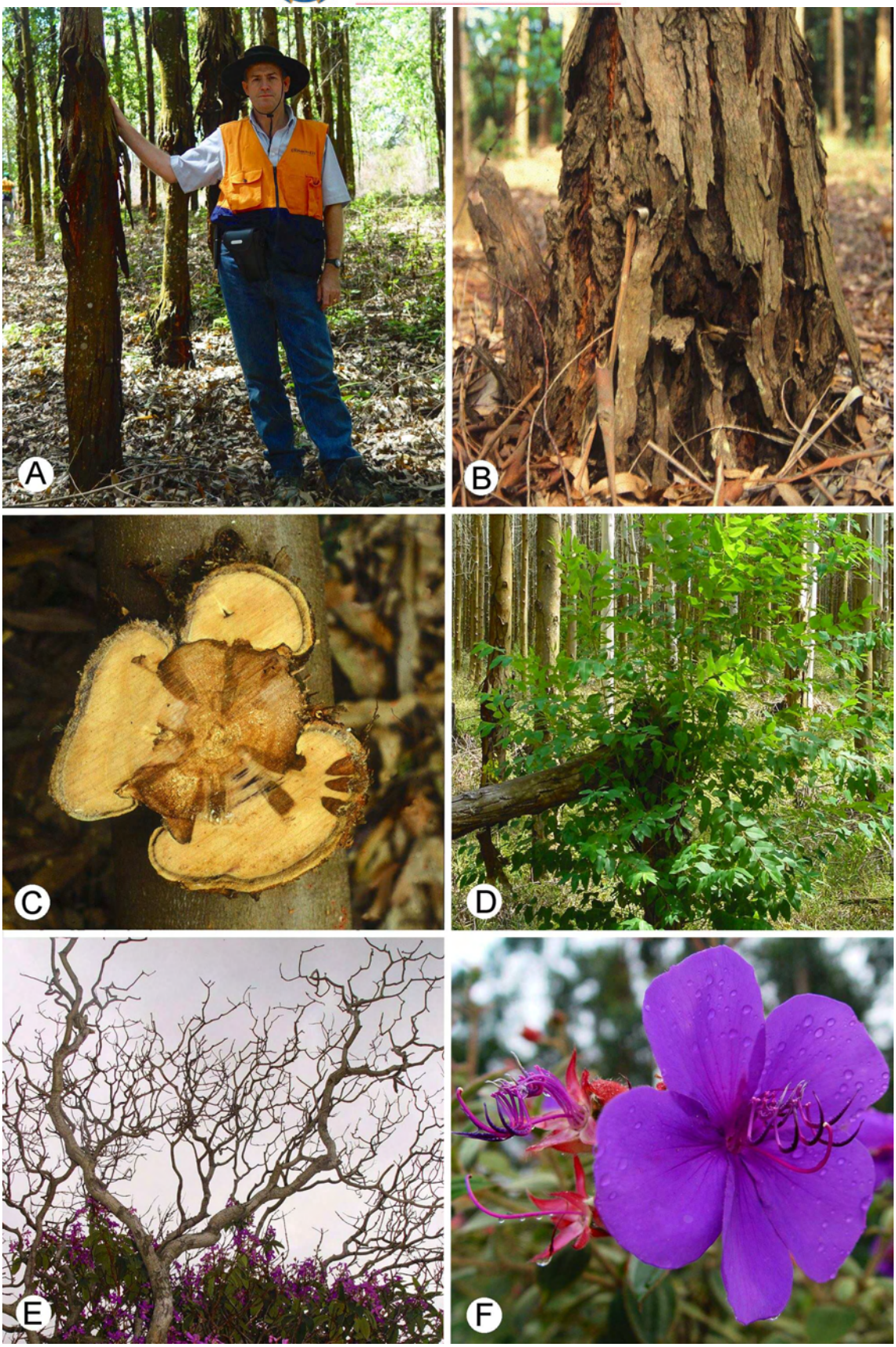

Fig. 3. Disease symptoms of Chrysoporthe canker caused by various *Chrysoporthe* species. A–B. Cankers on the trunks and bases of *Eucalyptus* trees. C. Cross section of canker showing killed vascular tissue. D. Stem breakage due to girdling cankers. E. Die-back of *Tibouchina* spp. (F).

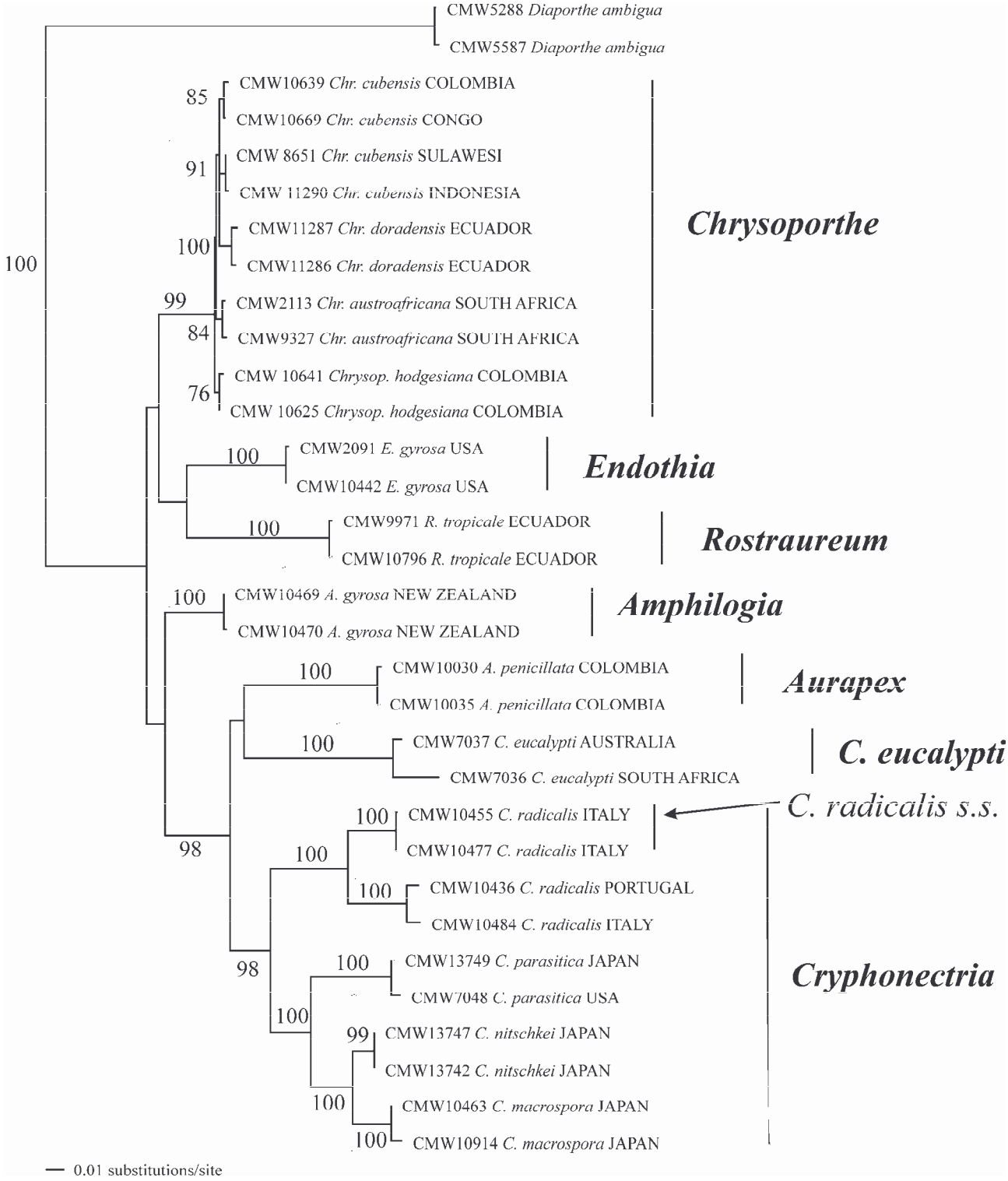

Fig. 4. A phylogenetic tree showing the grouping of *Cryphonectria* and related genera. The tree was obtained from a combined DNA sequence dataset of the ITS1, 5.8S rRNA gene and ITS2 regions of the ribosomal operon, and β-tubulin genes respectively. Alignment was obtained using the web interface (http://timpani.genome.ad.jp/%7Emafft/server/) of the alignment program MAFFT ver. 5.667 (Katoh *et al.* 2002). Distance analyses using the Tamura-Nei model, which was shown by Modeltest to be the appropriate model, were employed. The following parameters were used: G = 0.2301, freqA = 0.1936, freqC = 0.3312, freqG = 0.2287, freqT = 0.2465; rate matrix 1, 3.1964, 1, 1, 3.8818, 1). Bootstrap confidence levels (>70%, 1000 replicates) are indicated on the branches. *Diaporthe ambigua*, another species in the *Diaporthales*, were used as an outgroup taxon.

CONTENTS

Taxonomy, phylogeny and ecology of *Cryphonectria* species and other members of the *Cryphonectriaceae*

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Abstract: The *Cryphonectriaceae* includes some of the most important pathogens of trees and shrubs. Well-known examples are the chestnut blight pathogen, *Cryphonectria parasitica*, and the *Eucalyptus* canker pathogen, *Chrysoporthe cubensis*. The family includes all genera in the *Diaporthales* with orange stromatic tissue that turns purple in KOH and yellow in lactic acid. These include *Cryphonectria*, *Endothia*, *Chrysoporthe*, *Amphilogia*, *Rostraureum*, *Microthia*, *Holocryphia* and *Celoporthe*. The mitotic genera *Aurapex* and *Ursicollum* are also included as they have been shown to reside in the *Cryphonectriaceae* based on DNA sequence data. This monograph presents details of the pathology, ecology and morphological characteristics defining these genera, the majority of which have been described relatively recently. Comprehensive phylogenetic analyses are presented that include all taxa in the *Cryphonectriaceae* for which cultures are available. *Endothiella*, the anamorph genus currently recognized for both *Cryphonectria* and *Endothia*, is specifically assigned to *Cryphonectria*. A new combination, *Cryphonectria japonica*, is also suggested for *Cryphonectria nitschkei*.

Taxonomic novelties: *Cryphonectria japonica* (Tak. Kobay. & Kaz. Itô) Gryzenh. & M.J. Wingf. **comb. nov.,** *Endothiella* Sacc. **emend.** Gryzenh. & M.J. Wingf.

Key words: canker, *Cryphonectriaceae*, *Diaporthales*, forestry, phylogeny

1. INTRODUCTION

The *Cryphonectriaceae* Gryzenh. & M.J. Wingf. nom. prov. represents a group of fungi that include some of the world's most important pathogens of trees. The best known of these pathogens is *Cryphonectria parasitica* (Murrill) M.E. Barr, the causal agent of chestnut blight that has all but destroyed the American chestnut (*Castanea dentata*) in its North American natural habitat. Other important examples of species that cause serious canker diseases on *Eucalyptus* spp., particularly those grown in plantations, are *Chrysoporthe cubensis* (Bruner) Gryzenh. & M.J. Wingf. and *Chrysoporthe austroafricana* Gryzenh. & M.J. Wingf., previously known as *Cryphonectria cubensis* (Bruner) Hodges.

The *Cryphonectriaceae* has recently been erected to contain *Cryphonectria* (Sacc.) Sacc., *Endothia* Fr. and various genera that have been relatively recently described. Many of these new genera include newly discovered taxa or existing species of *Cryphonectria* that have been shown as distinct based on DNA sequence comparisons for a number of variable regions of the genome. These phylogenetic groupings are supported by morphological characteristics not previously recognized to represent generic differences. The numerous taxonomic changes to species in this group have emerged relatively recently (Gryzenhout *et al.* 2006a), and the

Cryphonectriaceae was described in 2006 (Gryzenhout *et al*. 2006b). Ten genera and nine species that are related to *Cryphonectria* and *Endothia* have thus been described, and new hosts and locations have been reported for existing species. This has radically changed aspects of the taxonomy and ecology of *Cryphonectria* and *Endothia* as it was previously understood (Gryzenhout *et al.* 2006a).

The taxonomy of *Cryphonectria* spp. and related fungi has been confused for many years, with genera such as *Endothia* (Shear *et al.* 1917, Kobayashi 1970, Roane 1986a) and *Diaporthe* Nitschke (Bruner 1917) often variably used for them. The taxonomy and identification of this group of fungi is also complicated by the fact that many of the taxa are morphologically similar. Phylogenetic inference was thus largely used to confirm existing species, and also to recognize differences between species and genera. This has been followed by careful morphological examination and in many cases, ecological studies such as pathogenicity tests.

The aim of this monograph is to present a comprehensive taxonomic overview of the genera and species of the *Cryphonectriaceae* as they are now recognized. An attempt is made to integrate the phylogenetic species concepts for these fungi with morphological characteristics and keys are presented with illustrated descriptions for identification. Methods for working with members of the *Cryphonectriaceae* are discussed to aid researchers studying these fungi. Because many species are important pathogens, information pertaining to their ecology, global distribution and control has also been included. It is our hope that this monograph will provide a foundation for future studies of the *Cryphonectriaceae*. This is especially relevant as it is evident that many new taxa remain to be discovered (Gryzenhout *et al.* 2005d) and it is likely that some of these will be important pathogens of global significance.

2. HISTORY OF THE *CRYPHONECTRIACEAE* AND ITS MEMBERS

2.1 Early taxonomic history of *Cryphonectria* and *Endothia*

The oldest genus in the *Cryphonectriaceae* is *Endothia*, which was established in 1849 with *Endothia gyrosa* (Schwein.: Fr.) Fr. as type (Fries 1849). *Cryphonectria* was described as a genus in 1905 (Saccardo & Saccardo 1905). Soon thereafter, *Cryphonectria* was reduced to synonymy with *Endothia* (Von Höhnel 1909). This was because *Cryphonectria gyrosa* (Berk. & Broome) Sacc., lectotypified as type of *Cryphonectria* by Von Höhnel (1909), was synonymised with *E. gyrosa* (Von Höhnel 1909). *Cryphonectria* and *Endothia* were retained as synonyms by subsequent authors, although the species could be divided into groups having either septate or aseptate ascospores (Shear *et al.* 1917, Kobayashi 1970, Roane 1986a).

Barr (1978) separated *Cryphonetria* and *Endothia*. Species with aseptate ascospores were retained in *Endothia* (*Gnomoniaceae* G. Winter) and those with uniseptate ascospores were relegated in *Cryphonectria* (*Valsaceae* Tul. & C. Tul.). This separation of genera was based on the original description of *Cryphonectria* (Saccardo & Saccardo 1905). Treatment of the two genera as different was not supported by Roane (1986a) who believed that the conspicuous orange stromata reflected a single genus. Barr's (1978) treatment of two different genera was, however, relatively widely accepted by other authors due to the distinct morphological differences between the genera (Micales & Stipes 1987) and DNA sequence comparisons (Venter *et al.* 2002, Myburg *et al.* 2004a).

In the classification of Micales & Stipes (1987), nine species of *Cryphonectria* were recognised. These included *C. gyrosa* (type), *C. parasitica*, *Cryphonectria radicalis* (Schwein.: Fr.) M.E. Barr, *Cryphonectria nitschkei* (G.H. Otth) M.E. Barr,

Cryphonectria macrospora (Tak. Kobay. & Kaz. Itô) Barr, *Cryphonectria havanensis* (Bruner) M.E. Barr, *Cryphonectria longirostris* (Earle) Micales & Stipes, *Cryphonectria coccolobae* (Vizioli) Micales & Stipes and *C. cubensis*. A tenth species, *Cryphonectria eucalypti* M. Venter & M.J. Wingf., was later described for a canker pathogen occurring in Australia and South Africa that had been treated as *Endothia gyrosa* (Venter *et al.* 2002). *Endothia* included three species, namely *E. gyrosa* (type), *Endothia singularis* (Syd.) Shear & N.E. Stevens and *Endothia viridistroma* Wehm. (Micales & Stipes 1987).

2.2 Typification of *Cryphonectria*

Cryphonectria gyrosa, a fungus known from *Elaeocarpus* spp. in Sri Lanka (Shear *et al*. 1917), was cited by Barr (1978) and Micales & Stipes (1987) as type of *Cryphonectria*. *C. gyrosa* was, however, not the type of *Cryphonectria* and the misconception had arisen due to the erroneous lectotypification by Von Höhnel (Gryzenhout *et al.* 2005a). Assigning *C. gyrosa* as a lectotype for *Cryphonectria* was not valid because *Cryphonectria variicolor* Fuckel and *Cryphonectria abscondita* Sacc., the original species included in *Cryphonectria* when this genus was treated as a subgenus of *Nectria*, were not considered as candidates for type by Von Höhnel (1909) or Barr (1978). Furthermore, the choice of *C. gyrosa* as type of *Cryphonectria* (Von Höhnel 1909) was mechanical because it was listed first (Gryzenhout *et al.* 2005a).

A new type was needed to conserve the generic name *Cryphonectria*. *Cryphonectria abscondita* and *C. variicolor* were not suitable because the morphology of *C. abscondita* is unknown, and *C. variicolor* does not belong in the *Diaporthales* (Gryzenhout *et al.* 2005a). Gryzenhout *et al.* (2005a) thus made the

successful proposal that *C. parasitica* serve as the type for *Cryphonectria*. This proposal was supported by the fact that the fungus represents *Cryphonectria sensu stricto*, based both on morphology and DNA sequence data. Thus, *Cryphonectria* was conserved for one of the world's most important plant pathogens and one for which a substantial body of literature exists.

Cryphonectria gyrosa was shown to resemble a species in a new genus of fungi on *Elaeocarpus* spp. in New Zealand (Myburg *et al.* 2004a, Gryzenhout *et al.* 2005a, 2005b). Since *C. gyrosa* was shown not to represent the type of *Cryphonectria* and the name *Cryphonectria* was no longer tied to *C. gyrosa* (ICBN, Art. 7.2, Greuter *et al*. 2000), the fungus was transferred to the new genus *Amphilogia* Gryzenh., Glen & M.J. Wingf. This genus also includes the new species *Amphilogia major* Gryzenh., Glen & M.J. Wingf. (Gryzenhout *et al.* 2005b).

2.3 Newly described species and genera

DNA sequence comparisons for members of *Cryphonectria sensu lato* by Myburg *et al.* (2004a) have shown clearly that the group is polyphyletic. Groups defined by phylogenetic differences could be supported by morphological characteristics that are sufficiently different to warrant description of genera in the *Diaporthales* (Myburg *et al.* 2004a). *Cryphonectria parasitica*, *C. nitschkei*, *C. radicalis* and *C. macrospora* were retained in *Cryphonectria s. str.* (Myburg *et al.* 2004a, 2004b), but other species of *Cryphonectria* residing in discrete lineages were assigned to new genera. Besides *Amphilogia*, *Chrysoporthe* Gryzenh. & M.J. Wingf. was described to encorporate *C. cubensis* (Gryzenhout *et al.* 2004), and numerous species have also been described in *Chrysoporthe* (Gryzenhout *et al.* 2004, 2005c, 2006c). *Cryphonectria longirostris* was placed in the new genus *Rostraureum* Gryzenh. & M.J. Wingf., together with

another newly described species, *R. tropicale* Gryzenh. & M.J. Wingf. (Gryzenhout *et al.* 2005d). The new genus *Holocryphia* Gryzenh. & M.J. Wingf. nom. prov. was described for *Cryphonectria eucalypti*, while *Microthia* Gryzenh. & M.J. Wingf. nom. prov. was establised to contain *C. havanensis* and *C. coccolobae* (Gryzenhout *et al.* 2006d). A new genus, *Celoporthe* Nakab., Gryzenh., Jol. Roux & M.J. Wingf. nom. prov., was described to accommodate the new species *Celoporthe dispersa* Nakab., Gryzenh., Jol. Roux & M.J. Wingf. nom. prov. (Nakabonge *et al.* 2006a).

2.4 Description of a new family

Cryphonectria, *Endothia* and the various newly described genera, all have similar morphological characteristics that distinguish them from other genera and families in the *Diaporthales*. In these fungi, stromatic tissue is orange in both the teleomorph and anamorph, or in some cases only in one of these forms (Micales & Stipes 1987, Myburg *et al*. 2004a, Gryzenhout *et al*. 2006b). Furthermore, pigments in the stromatic tissue and mycelium turn purple in KOH and yellow in lactic acid (Anderson 1914, Roane & Stipes 1978, Castlebury *et al.* 2002, Gryzenhout *et al.* 2006b). The close relationship of these genera was confirmed in DNA based comparisons of the large subunit (28S) of the ribosomal genes that showed that *Cryphonectria* and *Endothia* species form a distinct clade in the *Diaporthales* (Castlebury *et al.* 2002). After the addition of more genera to the dataset of Castlebury *et al.* (2002), the new family, the *Cryphonectriaceae*, could be described for *Cryphonectria* and related genera (Gryzenhout *et al.* 2006b).

2.5 Anamorph states

Endothiella Sacc. has commonly been used to refer to the anamorphs of both *Cryphonectria* and *Endothia* (Kobayashi 1970, Barr 1978, Roane 1986a). This tendency most likely stems from the fact that *Endothiella* was the anamorph name applied to both *Cryphonectria* and *Endothia* spp. at the time when these genera were treated as synonyms (Kobayashi 1970, Roane 1986a). Furthermore, when Barr (1978) designated *Endothiella* as the anamorph of *Endothia*, no anamorph genus was designated for *Cryphonectria*. The recent taxonomic revisions to *Cryphonectria* have, however, shown that characteristic morphological differences can often be found in the anamorph (Myburg *et al.* 2004a, Gryzenhout *et al.* 2005d). Hence the use of a single anamorph genus to refer to the asexual states of *Cryphonectria* and *Endothia* is inconsistent with the fact that they reflect discretely different entities.

Endothiella, designated as the anamorph of *Endothia* by Barr (1978), does not represent the anamorph of *Endothia*. *Endothiella gyrosa* Sacc., the type of *Endothiella*, was described for *C. radicalis* (Saccardo 1906) at the time that this species was treated as a synonym of *Endothia gyrosa* (Saccardo 1906, Shear *et al.* 1917). As part of the current study, fruiting structures on the type specimen of *Endothiella gyrosa* (PAD) have also been found to resemble those of the anamorph of *Cryphonectria* (Myburg *et al*. 2004a). This is in contrast to the superficial and large structures of the asexual state of *Endothia* (Venter *et al.* 2002, Myburg *et al*. 2004a). We have chosen not to describe a new anamorph genus for *Endothia* following Art. 59.2 (ICBN, Greuter *et al.* 2000), which recommends that anamorph states should not be unnecassarily described when the anamorph and teleomorph have been connected.

Anamorph structures of genera in the *Cryphonectriaceae* often occur in the absence of teleomorph structures. For example, in Western Australia the *Eucalyptus*

canker pathogen *Holocryphia eucalypti* Gryzenh. & M.J. Wingf. nom. prov (previously *C. eucalypti*), is known only in the asexual state (Walker *et al.* 1985, Davison & Coates 1991). Similarly, the anamorph of *Chr. austroafricana* is by far the most dominant form of the fungus found on *Eucalyptus* spp. in South Africa (Van Heerden & Wingfield 2001). We have, however, not established anamorph genera for the various new genera that we have described in the *Cryphonectriaceae*, following Art. 59.2 (ICBN, Greuter *et al.* 2000), even though the genera are often better known in their anamorph states.

Three new anamorph genera are known in the *Cryphonectriaceae*. *Chrysoporthella* was described for *Chrysoporthe* (Gryzenhout *et al.* 2004). This was necessary for the description of *Chrysoporthella hodgesiana* Gryzenh. & M.J. Wingf., a species for which the teleomorph is unknown (Gryzenhout *et al.* 2004) but where this asexual fungus is clearly a species of *Chrysoporthe* based on DNA sequence comparisons. In such cases, the Code recommends that asexual fungi should not be given teleomorph names where teleomorph states are unknown (ICBN, Art. 59.2, Greuter *et al.* 2000). Two other new genera, *Aurapex* Gryzenh. & M.J. Wingf. nom. prov. and *Ursicollum* Gryzenh. & M.J. Wingf. nom. prov., have also been shown to reside in the *Cryphonectriaceae* based on DNA sequence data (Gryzenhout *et al.* 2006d, 2006e) although no teleomorph have been found for them.

3. DISEASES AND ECOLOGY

Species of the *Cryphonectriaceae* occur on the bark or wood of trees and shrubs in various parts of the world (Fig. 1). Here they exist either as virulent pathogens, facultative parasites or saprophytes. Approximately one species of each of the ten

genera recognized in the *Cryphonectriaceae,* is a pathogen. Some of these pathogens occur on trees growing in their natural habitat, some have been introduced into new environments and others occur on commercially important trees such as those grown in plantations or for their ornamental value (Fig. 1).

The diseases caused by members of the *Cryphonectriaceae* are mostly diffuse cankers. Some are relatively unimportant causing minor branch or stem die-back on trees growing in their natural environments. In contrast, others are of tremendous ecological or economic importance, threatening natural forest stands or commercial enterprises based on plantation forestry. Based on prior experience with diseases such as chestnut blight, it is reasonable to believe that some of the newly recognized canker pathogens in the *Cryphonectriaceae*, even if unimportant, threaten forest ecosystems in countries where they are currently not present. We thus believe that many of these fungi should be afforded significant quarantine importance.

The following section provides information regarding the diseases caused by members of the *Cryphonectriaceae*. In many cases, relatively little is known regarding the ecology or importance of the fungi. This is particularly true for those species that have been recently discovered. In contrast, diseases such as chestnut blight and the *Eucalyptus* canker disease previously ascribed to *C. cubensis*, have been widely studied. In the latter cases, comprehensive treatments have been published previously and only a general overview is presented here.

3.1 *Chestnut blight.*

3.1.1 *The disease.* In its native environment in Japan and China, *C. parasitica* causes mild canker and die-back symptoms on Japanese (*Castanea crenata*) and

Chinese chestnut (*Castanea mollissima*) trees (Fairchild 1913, Shear & Stevens 1913, 1916). This fungus was introduced into the USA approximately one hundred years ago, presumably with Japanese and Chinese chestnut trees (Anagnostakis 1992, 1987). The result was the start of a devastating disease (Figs 2–3) on the American chestnut (*Castanea dentata*), which is commonly referred to as chestnut blight (Merkel 1906, Anagnostakis 1987, Elliston 1981). The devastation caused by the pathogen upon its introduction into North America serves as one of the best known examples of the consequences of introducing a foreign pathogen into a country having large native populations of native trees related to the host of origin (Anagnostakis 1987). Due to the importance and far reaching effects of the disease caused by *C. parasitica*, the fungus and all aspects surrounding it have been intensively studied and it has been treated extensively in a large number of reviews (Shear *et al.* 1917, Boyce 1961, Peace 1962, Elliston 1981, Griffin 1986, Griffin & Elkins 1986, Anagnostakis 1987, Sinclair *et al*. 1987, Newhouse 1990, MacDonald & Fulbright 1991, Nuss 1992, Heiniger & Rigling 1994, Milgroom 1995, Griffin 2000, Dawe & Nuss 2001, Milgroom & Cortesi 2004).

Prior to the onset of chestnut blight, the American chestnut was a dominant tree in the forests of the north eastern United Sates and it was highly valued for its timber, nuts and related products (Griffin 1986, Anagnostakis 1987, Anagnostakis & Hillman 1992, Smith 2000, Youngs 2000). The timber and nuts sustained the livelihoods of many rural people, and the trees were popular as ornamentals in parks and streets (Youngs 2000). Relatively soon after the blight epidemic began, this tree was virtually eliminated from its natural habitat (Elliston 1981, Griffin 1986, Anagnostakis 1987, Heiniger & Rigling 1994) and the composition of hardwood forests on the eastern part of North America was changed irreparably by its demise

(Brewer 1995, Griffin & Elkins 1986). The death of innumerous chestnut trees in forests and urban areas impacted greatly on the lives of large numbers of people, especially those dependent on the trees for income (Youngs 2000).

The American chestnut was saved from total extinction by its ability to produce epicormic shoots below the level of canker formation, although the tree was destroyed in its natural manifestation. Many trees now exist as understory shrubs with shoots coming from the living roots (Anagnostakis 1987, Griffin 2000, Newhouse 1990). To date, the few surviving yet infected *Castanea dentata* trees grow but *C. parasitica* usually prevents trees from maturing to produce seed (Elliston 1981, Anagnostakis 2001, Griffin & Elkins 1986, Smith 2000).

Cankers caused by *C. parasitica* (Griffin 1986, Griffin & Elkins 1986), are typical annual cankers, ellipsoid, and often exposing the cambium (Figs 2–3). They can be sunken, swollen, caloused or superficial, depending on the host. Infection usually is wound-related. Trees affected by the blight can easily be identified because of leaves and flowers that discolour and shrivel without being shed in autumn (Fig. 2D). These are due to a single or several cankers that form below and girdle the branches or main trunks. Orange sexual and asexual fruiting structures can usually be seen on the canker surface. Buff-coloured mycelial fans can also be observed in the outer bark or in the phloem. Cankers on large trees form at the base or higher up on the trunks or branches, but young trees usually have cankers at the bases of their trunks. After infection, a tree can die in one to four years depending on its age. Some trunks survive below the canker and continue to form epicormic shoots, which can again be attacked by the pathogen when they are larger.

In Europe, chestnut blight affects the European chestnut (*Castanea sativa*). *Castanea sativa* is not native to Europe, but was introduced by the Romans from the Black Sea region for timber and food (Anagnostakis 1987, Heiniger & Rigling 1994). These trees are still harvested for their nuts or timber either in natural forests or in orchards (Heiniger & Rigling 1994, Bazzigher & Miller 1991). The first blighted trees in Europe were seen in 1938 in Italy (Biraghi 1946), from where the disease spread throughout Europe (Heiniger & Rigling 1994, Robin & Heiniger 2001). *Cryphonectria parasitica* now occurs from Portugal towards East Europe and so far as Turkey in Asia Minor (Robin & Heiniger 2001).

It is unknown exactly how chestnut blight was introduced into Europe. A probable route of entry could have been through the importation of Asian chestnut species into Europe. Alternatively, the pathogen could have reached Europe through importation of trees or chestnut products from the U.S.A. for breeding purposes (Heiniger & Rigling 1994). Factors that may have contributed to the inadvertent introduction of *C. parasitica* into Europe might be that *C. parasitica* can be an endophyte in *C. sativa* (Bissegger & Sieber 1994), the fungus can also infect nut shells (Jaynes & DePalma 1984), the fungus is commonly present in infected logs and small cankers of grafted plants, and spores may be present on young plants (Heiniger & Rigling 1994).

Disease severity was quite high on *Castanea sativa* and led to tree mortality a few years after the initial detection of the disease in Europe (Heiniger & Rigling 1994). *Castanea sativa* was, however, thought to be slightly more resistant to the blight than *Castanea dentata* (Heiniger & Rigling 1994, Graves 1950) since in Europe, chestnut blight has been substantially less damaging on European chestnut

(*Castanea sativa*) than it has been in North America. Another important factor that has reduced the impact of chestnut blight in Europe was the occurrence of viruses in naturally occurring fungal strains that resulted in reduced virulence (Elliston 1981, Anagnostakis 1987, Heiniger & Rigling 1994). These viruses and their application as biocontrol agents have been intensily studied and are briefly treated below.

3.1.2 *Etiology.* *Cryphonectria parasitica* produces conidia in long, moist tendrils (chirri), while ascospores are usually expelled forcefully (Mickleborough 1909, Elliston 1981, Newhouse 1990). The conidial tendrils are not wind dispersed (Heald *et al.* 1915) but typically dispersed by rain splash over short distances (Elliston 1981, Griffin 1986, Newhouse 1990). Ascospores are dispersed with wind, especially as the bark dries after rain (Anderson 1914, Heald *et al.* 1915, Shear *et al.* 1917, Griffin 1986). It is thus believed that ascospores are responsible for movement of the fungus over long distances (Elliston 1981, Newhouse 1990). Conidia could also play a role in long distance spread, since these structures have been found to survive in soil and could thus be carried with wind-borne dust (Heald & Gardner 1914). Conidia can also be dispersed on the bodies of insects and mites (Craighead 1912, Studhalter & Ruggles 1915, Elliston 1981, Anagnostakis 1982a, Wendt *et al.* 1983, Russin *et al.* 1984, Griffin 1986), birds and mammals (Heald & Studhalter 1914, Scharf & DePalma 1981, Griffin 1986) that come into contact with spore masses on cankers.

3.1.3 *Control.* After the appearance of the disease in the U.S.A., control was attempted by pruning and removal of diseased material, the application of fungicides such as Bordeaux Mixture (Merkel 1905) or clearcutting forests to prevent spread of

the disease (Newhouse 1990). Such efforts could not keep up with the rapid spread of the disease, the large areas affected or the great size of infected trees (Merkel 1905, Newhouse 1990). Given the ineffectiveness of chemical control, the focus of efforts to reduce the impact of this disease has been on breeding for diseases resistance by incorporating the more resistant Asian chestnut species, sylvicutural and general forest management practices and biological control using hypoviruses (Griffin 2000). Other methods, e. g. the application of organic material or soil to cankers and grafts, localized chemical treatment and eradication may serve as methods to control the disease on the small scale (Griffin 1986). The different methods have met with mixed success in the U.S.A. and Europe.

Resistance breeding. In the U.S.A., resistance breeding is largely possible because the Asian species of chestnut, *Castanea crenata* and *Castanea mollissima*, have high levels of resistance to the disease (Hebard 1994). Amongst the different species, *Castanea mollissima* is the most resistant (Fairchild 1913, Graves 1950, Huang *et al*. 1996a). Low levels of resistance have also been found in individual *Castanea dentata* trees (Graves 1950, Clapper 1952, Griffin 1986, Griffin & Elkins 1986, Griffin 2000). Similar breeding programs have also been established in Europe, using resistant *Castanea sativa* trees crossed with *Castanea crenata* and *Castanea mollissima* (Bazzigher 1981, Bazzigher & Miller 1991). The type of resistance, mechanisms, morphological markers for resistance and different programs pursuing this opportunity have been summarized by Griffin (1986).

Many Japanese and Chinese *Castanea* spp. have been imported and planted in North America and have been found to hybridise easily with remaining American chestnuts (Anagnostakis & Hillman 1992). These trees are also used in extensive

back-cross breeding programs with American chestnut trees having low levels of resistance (Burnham 1981), as well as in various other breeding programs (Anagnostakis 1987, Griffin 2000). Resistance of resultant progeny in such programs is tested in pathogenicity trials (Anagnostakis 1991). Recent breeding programs have also been greatly aided through the application of molecular marker-aided selection to reduce the time and improve the efficiency of selecting suitable breeding stock (Bernatzky & Mulcahy 1992, Huang *et al.* 1996b, Kubisiak *et al.* 1997).

Breeding for resistance to chestnut blight presents an option to produce trees that can be planted in parks and gardens. It is, however, not likely to ever restore the American chestnut to its natural environment. This approach to control is also very slow, often resulting in progeny with resistance, but with unsuitable qualities influencing form and vigour that would ensure the survival of the American chestnut in forests (Griffin 2000, Elliston 1981). Resistant hybrids are, however, expected to be planted in the U.S.A. in 2015 (Ronderos 2000).

Biocontrol using hypoviruses. For many years, there appeared to be little hope for the American chestnut with no immediate or permanent control strategies available to stem the spread of chestnut blight. Then, early in the 1950's, the remarkable observation was made that cankers on trees infected by *C. parasitica* in Europe often recovered (Biraghi 1950). Grente (1965) later isolated atypically white strains of *C. parasitica* from these cankers and determined that the strains could be used to heal cankers infected with non-hypovirulent strains. The spontaneous healing of cankers due to these strains and their reduced virulence, was referred to as hypovirulence (Grente 1965). The causal agents of hypovirulence were later found to be double stranded RNA (dsRNA) viruses, which reside in the mycelium of the

pathogen (Day *et al.* 1977). These viruses are now classified in the family *Hypoviridae* (Day *et al.* 1977, Nuss 1992, Hillman *et al*. 2000).

The natural occurrence of hypovirulence in Europe has substantially reduced the impact of chestnut blight, and prevented an epidemic similar to that present in the U.S.A. (Elliston 1981, Anagnostakis 1987, MacDonald & Fulbright 1991, Heiniger & Rigling 1994). The potential of hypovirulence to restore the American chestnut has prompted concerted research on hypovirulence, resulting in a substantial body of literature on this topic. The viruses responsible for this reduced form of virulence have been extensively characterized, and their means of spread and their use as biocontrol agents has been intensely studied (Nuss 1992, Dawe & Nuss 2001, Hillman & Suzuki 2004). Various excellent reviews on hypovirulence in chestnut blight have been published (Elliston 1981, Griffin 1986, Heiniger & Rigling 1994, MacDonald & Fulbright 1991, Nuss 1992, Nuss 1996, Nuss 2000, Dawe & Nuss 2001, Hillman & Suzuki 2004, Milgroom & Cortesi 2004) and details are not repeated here.

Different hypoviruses have been isolated from cultures of *C. parasitica* from Europe, the U.S.A. and Asia. These are known as *Cryphonectria hypovirus* (CHV) 1 (Shapira *et al*. 1991), CHV-2 (Hillman *et al*. 1994), CHV-3 (Smart *et al*. 1999) and CHV-4 (Hillman *et al*. 2000). CHV-1 is the virus associated with hypovirulence in Europe and probably has an Asian origin (Gobbin *et al.* 2003, Liu *et al*. 2003, Peever *et al.* 1998). Interestingly, CHV-1 has also recently been found to naturally infect another *Cryphonectria* species, *C. nitschkei*, in Japan (Liu *et al.* 2003, Myburg *et al.* 2004b). Isolates containing this virus have been released for biocontrol in the USA (Anagnostakis 1990, 2001, Griffin 1999). CHV-2 occurs naturally in the USA,

although to a limited extent (Chung *et al*. 1994, Hillman *et al*. 1994). This virus has also been isolated from isolates in China (Peever *et al*. 1998), although current evidence suggests that it could have been introduced from the U.S.A. (Chung *et al*. 1994, Milgroom & Cortesi 2004). CHV-3 is known only from the U.S.A. (Chung *et al*. 1994, Hillman *et al.* 1994, Hillman *et al*. 1992, Fulbright *et al*. 1983, Peever *et al*. 1997, Melzer & Boland 1999), which also is true for CHV-4, the most common virus (Liu *et al*. 2002, Peever *et al*. 1997).

Amongst the different hypoviruses, CHV-1 has been most extensively associated with hypovirulence, as summarized by Milgroom & Cortesi (2004). The different strains of CHV-1 vary in the extent that they influence the virulence of the fungus (Chen & Nuss 1999, Peever *et al*. 2000). The different strains of CHV-1 and other viruses are often paired with each other in biocontrol inoculations in the U.S.A., e.g. with CHV-1 and CHV-3 most commonly used (Milgroom & Cortesi 2004). CHV-4 has not been used in biological control inoculations since this virus does not affect the virulence of *C. parasitica* (Hillman *et al*. 2000).

Hypoviruses influence *C. parasitica* in several ways, and most of these changes have been characterized in CHV-1 (Milgroom & Cortesi 2004). The most important of these is a reduction in the virulence of infected strains, which results in reduced canker development enabling cankers to heal (Grente 1965, Day *et al.* 1977, Nuss 1992). The viruses also have pronounced effects on isolate morphology, including altered pigmentation, reduced sporulation (Anagnostakis 1982b, Elliston 1985), and reduced oxalate accumulation (Havir & Anagnostakis 1985) as well as laccase production (Rigling *et al.* 1989).

Hypovirulence has been successfully used as a sustainable method to control chestnut blight in Europe (Heiniger & Rigling 1994, Robin *et al.* 2000). This has been possible through the natural spread of the viruses (Heiniger & Rigling 1994), but also by treating cankers with inoculated hypovirulent strains (Turchetti & Maresi 1991, Grente & Berthelay-Sauret 1978, Heiniger & Rigling 1994). This method is especially useful in areas where blight has not yet been reported (Grente & Berthelay-Sauret 1978, Heiniger & Rigling 1994).

The use of hypovirulence as a means of biocontrol of chestnut blight in North America has not been as successful as it has been in Europe. This is because hypovirulence is not easily spread naturally in North America although it can be used effectively to treat cankers (Anagnostakis 1982b, 1987, Milgroom & Cortesi 2004) and can persist in chestnut stands to some extent (Anagnostakis 2001). This largely has to do with the mode of distribution of the hypoviruses. Viruses in an infected strain are transferred to other individuals horizontally by hyphal anastomosis between an infected and uninfected fungal strain, but only when these strains represent the same vegetative compatibility group or VCG (Anagnostakis 1977, Nuss 1996). The population diversity of *C. parasitica* in Europe is relatively low in comparison to the more diverse population in North America (Anagnostakis 1982b, 1987, Anagnostakis *et al.* 1986, Heiniger & Rigling 1994), and viruses are thus not as easily transferred to new strains in the U.S.A. Viruses can also be distributed vertically through conidia (Nuss 1996), but the efficiency of this type of vertical spread is lower in the U.S.A than it is in Europe (Shain & Miller 1992, Peever *et al.* 2000).

In North America, biocontrol through hypovirulence is not a permanent, viable solution for chestnut blight, although constant treatment of individual trees is

usually effective (Milgroom & Cortesi 2004). Besides the problems related to the distribution of the viruses resulting from vegetative compatibility barriers and the large number of different genotypes, other factors also play a role in the sustainability of hypovirulence as an effective biocontrol method (Griffin 2000, Milgroom & Cortesi 2004). The high susceptibility of American chestnuts to the blight, the high inoculum of virulent isolates of the fungus and stresses that the trees must endure in natural forests predisposes these trees to disease (Griffin 2000). Furthermore, superficial cankers can easily turn into more serious cankers, coppice shoots are often killed rapidly and multiple cankers usually form on the trees, giving trees insufficient time to acquire hypovirulent strains (Griffin 2000). Lastly, the different hypoviruses found in North America have different characteristics and distributions than CHV-1 in Europe (Dawe & Nuss 2001).

There is one area of North America where hypovirulence has provided effective biocontrol without continuous human intervention. This is in Michigan where the American chestnut does not occur naturally, but was planted during the early settlements (Brewer 1995, Fulbright *et al.* 1983, MacDonald & Fulbright 1991). Trees inevitably became infected with blight that resulted in mortality. Naturally occurring CHV-3 viruses, however, were later isolated from surviving trees and recovering cankers (Brewer 1982, Fulbright *et al.* 1983). Recovering cankers containing naturally occurring CHV-3 viruses have also been found in Ontario (McKeen 1995, Dunn & Boland 1993, Melzer & Boland 1999, Melzer *et al.* 1997). The reason why chestnut trees were able to recover in Michigan and Ontario is still debatable and it is probably due to a combination of factors related to the occurrence of hypovirulence, the ecology of the disease and the occurrence and ecology of chestnuts in that area (McKeen 1995). The low population diversity of *C. parasitica*

in both Michigan (Liu *et al.* 1996) and Ontario (Melzer & Boland 1999) most likely also has an influence, although it is unclear whether the low diversity is due to the occurrence of hypovirulence, or whether the hypoviruses could more easily spread due to the already existing low diversity (Liu *et al.* 1996).

A significant breakthrough that could enhance the spread of *C. parasitica* hypoviruses has emerged through the production of synthetic RNA transcripts of CHV-1 (Nuss 1992, Dawe & Nuss 2001, Griffin 2000, Milgroom & Cortesi 2004). These virus transcripts have been shown to be able to transfect *C. parasitica* (Choi & Nuss 1992), other species in *Cryphonectria*, as well as species in other related genera such as *E. gyrosa*, *Chr. cubensis* and *Chr. austroafricana*, and to result in similar changes in these isolate as those found in infected *C. parasitica* strains (Chen *et al.* 1994, Van Heerden *et al.* 2001). The most important characteristic of these transcripts is that they are able to be transmitted via the ascospores as well as the conidia of the transfected strain, and were thus thought to be better dispersed under field conditions than would be the case with wild type viruses (Chen *et al.* 1993). Field trials employing one of these transcripts have shown that although the modified viruses were able to survive in the field for two years, they are out competed easily by other virulent strains, partly because of their comparatively low levels of sporulation (Anagnostakis *et al*. 1998). Efforts are ongoing to develop more transgenic transcripts that will be able to successfully persist and spread under field conditions in the U.S.A. (Chen & Nuss 1999, Dawe & Nuss 2001).

Forest management. Forest management and silvicultural practices are crucial to ensure the long-term success of all types of control measures (Griffin 1986, 2000). Site factors are important, i.e. cankers are less severe on trees growing on

mesic sites, or at lower altitudes that has higher temperatures than sites with higher altitudes (Griffin 1986, Griffin *et al*. 1991, 1993, Griffin & Griffin 1995, Griffin 2000). Thinning reduces competition from other hardwoods and improves the overall growth of surviving chestnuts (Griffin *et al.* 1991). Soil fertility and water retention are also important factors that can predispose trees to chestnut blight (Griffin 1986). In European orchards and forests, especially for grafted trees, sanitation is practiced by pruning and removing cankered material from trees, and subsequent treatment of cut surfaces with fungicide (Heiniger & Rigling 1994). Hypovirulent or healing cankers are, however, left in the field to increase the levels of hypovirulent inoculum (Turchetti 1982).

3.1.4 *Population genetics.* The population diversity of *C. parasitica* has been extensively studied (Milgroom 1995, Robin & Heiniger 2001). Phenotypic diversity based on vegetative incompatibility groups has been particularly relevant (Robin & Heiniger 2001) because of the barriers to the spread of viruses imparting hypovirulence (Anagnostakis 1983). Using phenotypic markers such as VCG's, it has been found that the diversity of *C. parasitica* is low in Europe, and higher in the USA (Anagnostakis *et al.* 1986, Milgroom 1995, Liu *et al.* 1996). The phenotypic diversity of *C. parasitica* in Europe has been found to vary considerably between countries and can be used to predict the possible success of biocontrol with hypovirulence (Robin & Heiniger 2001).

Population genetic studies based on genetic markers such as restriction fragment length polymorphisms (RFLPs) and DNA fingerprints have been used to understand the diversity, population structure, geneflow, origin and movement of the

pathogen (Milgroom 1995). These studies have shown that while the populations in North America and Europe are relatively similar in terms of diversity (Milgroom 1995, Milgroom *et al.* 1996), populations in Japan and China are more diverse (Milgroom *et al.* 1992, 1996, Milgroom 1995). The Japanese population shares alleles with the North American and European populations, but has different alleles from the Chinese population (Milgroom 1995, Milgroom *et al.* 1996). These results have thus confirmed that *C. parasitica* was introduced from Japan into North America, corresponding with historical facts that *C. parasitica* is native to China and Japan (Shear & Stevens 1913, 1916) and introduced into North America on nursery stock (Griffin 1986, Anagnostakis 1992). The available data were, however, insufficient to indicate whether *C. parasitica* was introduced into Europe from the U.S.A. or from Japan (Milgroom 1995, Milgroom *et al.* 1996).

The genetic structure of isolates within populations in North America and Europe, as well as other aspects of the population biology of *C. parasitica*, has been intensively treated by Milgroom (1995). Some important aspects concerning the population biology of *C. parasitica* include the following. Other than the population in Michigan (U.S.A.) that is clonal (Liu *et al*. 1996), populations in the U.S.A. consist of recombining genetic neighbourhoods with restricted geneflow (Milgroom 1995). *Cryphonectria parasitica* is homothallic but preferentially outcrossing (Puhulla & Anagnostakis 1971, Milgroom *et al*. 1993), and both selfing and outcrossing occur in the laboratory and in the field (Milgroom *et al*. 1993, Marra & Milgroom 2001, Marra *et al*. 2004). However, self-incompatibility of strains under laboratory conditions also occurs and a bipolar mating system (Marra & Milgroom 2001) has been confirmed by the characterization of two idiomorphs of the MAT genes (McGuire *et al*. 2001,

2004). Self-incompatibility and the ability to self in *C. parasitica* suggest both genetic and environmental control over mating (Marra & Milgroom 2001).

3.2 *Chrysoporthe canker* of *Eucalyptus*

3.2.1 *The disease.* *Eucalyptus* spp. (*Myrtaceae*, *Myrtales*) have been planted around the world as non-natives, primarily for timber, poles, hardboard and pulpwood used in the production of paper (Poynton 1979, Turnbul 2000). The disease Chrysoporthe canker, caused by the fungus previously known as *Cryphonectria cubensis* and hence known as Cryphonectria canker (Hodges 1980, Sharma *et al.* 1985, Hodges *et al*. 1986, Sinclair *et al*. 1987, Wingfield *et al.* 1989, Conradie *et al.* 1990, Old *et al*. 2003, Wingfield 2003), is serious and can be responsible for tree death associated with financial losses in several countries where *Eucalyptus* spp. are planted in fibre and wood farms (Wingfield 2003). In recent taxonomic studies, *C. cubensis* has been transferred to the new genus *Chrysoporthe* (Gryzenhout *et al*. 2004), and numerous new species have also been described in the genus (Gryzenhout *et al.* 2004, 2005c, 2006c). It is now well established that cankers on *Eucalyptus* are caused by at least three of these species of *Chrysoporthe*, i.e. *Chr. cubensis*, *Chr. austroafricana* (Gryzenhout *et al.* 2004) and *Chr. doradensis* Gryzenh. & M.J. Wingf. (Gryzenhout *et al*. 2005c).

All *Chrysoporthe* species causing cankers on *Eucalyptus* spp. occur in tropical and sub-tropical areas (Fig. 1) of the world between the 30° North and South latitudes (Sharma *et al*. 1985). They are predominant in areas with high temperatures and rainfall (Boerboom & Maas 1970, Hodges *et al.* 1976, 1979, Sharma *et al.* 1985). *Chrysoporthe cubensis* occurs in various countries of South and Central America, Florida and Hawaii (U.S.A.), Central Africa, South East Asia and Australia

(Gryzenhout *et al.* 2004, Nakabonge *et al.* 2006b, Roux *et al.* 2005). In contrast, *Chrysoporthe austroafricana* was described based on isolates from South Africa (Gryzenhout *et al.* 2004), but it has recently also been found in Mozambique, Zambia and Malawi (Nakabonge *et al*. 2006b, Roux *et al.* 2005). *Chrysoporthe doradensis* is a recently discovered species currently only known in Ecuador (Gryzenhout *et al.* 2005c).

Chrysoporthe spp. typically kill the cambium of infected trees, thus giving rise to large cankers, usually on the stems (Fig. 4). These cankers will often girdle the stems leading to relatively rapid tree death (Hodges *et al.* 1976, Sharma *et al*. 1985, Florence *et al.* 1986, Wingfield *et al.* 1989). Partially girdled trees typically produce epicormic shoots on the trunks of dying trees (Fig. 4E). Wind and other disturbance in plantations often lead to stem breakage at the sites of cankers on trees (Fig. 4D).

Chrysoporthe cubensis and *Chr. austroafricana* both cause severe canker diseases on their hosts, and there are some important differences in the nature of the cankers that they cause (Myburg *et al.* 1999, 2002a, Gryzenhout *et al.* 2004). *Chr. cubensis* causes cankers both at the bases and higher up on the trunks of trees (Fig. 4), and it infects deeply into the cambium (Hodges *et al.* 1976, Sharma *et al.* 1986, Wingfield 2003). Sexual and asexual structures are also common on cankers (Hodges *et al.* 1979, Sharma *et al.* 1985, Van Heerden *et al.* 1997). In contrast, in South Africa *Chr. austroafricana* most commonly occurs at the bases of trees (Wingfield *et al.* 1989, Myburg *et al.* 2002a) and teleomorph structures of *Chr. austroafricana* are seldom seen (Van Heerden & Wingfield 2001). However, in African countries north of South Africa, both sexual states are common, and cankers higher on the stems can be found (Nakabonge *et al.* 2006b). The pathogen is very aggressive, rapidly colonizing the cambium and girdling trees (Wingfield 2003). *Chr. austroafricana* is

most pathogenic on young trees (Fig. 4C), which can die within as few as two years (Wingfield 2003). In inoculation trials, isolates of *Chr. austroafricana* quickly girdle the cambium while *Chr. cubensis* tends to penetrate deeply, giving rise to relatively long lived stem cankers (Roux *et al.* 2003, Rodas *et al.* 2005, Wingfield 2003).

Chrysoporthe spp. produce spores similar to those of *C. parasitica*. Conidia are expelled in long tendrils when conditions are moist (Fig. 4), while ascospores are dispersed with the wind (Bruner 1917). The means of spore dissemination has not been studied thoroughly for *Chrysoporthe* spp., but spores are most likely distributed in the same manner as those of *C. parasitica*.

3.2.2 *Control.* The high levels of susceptibility to *Chrysoporthe* spp. in some *Eucalyptus* species, limits the planting of susceptible species or genotypes by forestry companies. This can be a serious impediment to the profitability of plantations where planting stock with excellent wood properties must be excluded from forestry programmes. The only effective control strategy to limit these losses and control Chrysoporthe canker is through the costly initiation of breeding and selection programmes for resistance to these pathogens by forestry companies in several countries (Alfenas *et al.* 1983, Sharma *et al*. 1985, Van Heerden & Wingfield 2002, Wingfield 2003, Van Heerden *et al.* 2005). This has been made possible through existing levels (Fig. 5) of resistance in some species (Hodges *et al*. 1976, Alfenas *et al*. 1983, Van Heerden *et al*. 2005), hybridization between species and through the propagation of selected disease-resistant hybrid clones. Brazil was the first country to commence with vegetative propagation of resistance crosses, and this program was the direct result of the devastating disease caused by *Chr. cubensis* (Wingfield 2003). To aid in the selection of resistant genotypes, extensive inoculation trials (Fig. 5) have

been carried out in countries, such as South Africa (Van Heerden *et al*. 2005) where the occurrence and possible future occurrence of *Chr. austroafricana* can also be predicted through computer models (Van Staden *et al*. 2004).

The relative success in using hypovirulence for the biocontrol of *C. parasitica* has raised an interest in the possible use of viruses for the biological control of Chysoporthe canker on *Eucalyptus*. A search for viruses in *Chrysoporthe* has led to some interesting results. The first study on this topic led to the discovery of a double stranded fragment of DNA in isolates of *Chr. cubensis* from *Eucalyptus* in Brazil (Van Zyl *et al.* 1999). The presence of this dsDNA fragment was also shown to be associated with morphological traits in culture such as retarded growth, cultures with irregular margins or those having a distinct white or orange colour (Van Zyl *et al.* 1999). These isolates were also significantly less virulent than uninfected isolates (Van Zyl *et al.* 1999). The dsDNA fragments in South American populations of the fungus have, however, not been studied further.

Surveys for similar dsDNA fragments were conducted in *Chr. austroafricana* populations in South Africa (Van Heerden 2004). These yielded two mitoviruses in a number of isolates, but these viruses did not result in a reduction of virulence in pathogenicity tests (Van Heerden 2004). However, in laboratory experiments, isolates of *Chr. austroafricana* were successfully transfected with a coding strand of one of the hypoviruses transcripts (CHV1-EP713) of *C. parasitica* (Van Heerden *et al.* 2001). Isolates transfected with the hypovirus showed the typical cultural characteristics of hypovirus infection and reduced virulence (Van Heerden *et al.* 2001).

3.2.3 *Hosts other than Eucalyptus*. The *Chrysoporthe* species occurring on *Eucalyptus* also occur on other hosts. Many of these alternative hosts are native to the countries where the fungi occur on non-native trees such as *Eucalyptus* spp. *Chr. cubensis* has caused a serious die-back and canker disease, associated with wounds, on *Syzygium aromaticum* (*Myrtaceae*, *Myrtales*), commonly known as clove (Fig. 6), in Zanzibar, Tanzania (Nutman & Roberts 1952, Hodges *et al.* 1986, Myburg *et al.* 2003). It also occurs on *S. aromaticum* in clove plantation in Brazil (Hodges *et al.* 1986), Indonesia (Hodges *et al.* 1986, Myburg *et al.* 2003) and in Malaysia (Reid & Booth 1969). The latter two areas are close to the Molucca islands (Indonesia) where *S. aromaticum* is native. Reports of the fungus on clove have mainly been under the name *Endothia eugeniae* (Nutman & Roberts) J. Reid & C. Booth, which is an earlier synonym of *Chr. cubensis* (Hodges *et al.* 1986, Micales *et al.* 1987, Myburg *et al.* 2003).

In South America, it was hypothesised that *Chr. cubensis* might be occurring on hosts other than *Eucalyptus* and *S. aromaticum*, because these non-native trees became infected very rapidly after planting (Hodges *et al.* 1986). In surveys of native *Myrtaceae* in Brazil, Hodges *et al*. (1986) found little evidence of infection by *Chrysoporthe* spp., but he did encounter *Chr. cubensis* (Hodges 1988) on *Psidium cattleianum* (strawberry guava, *Myrtaceae*, *Myrtales*). After discovering that species of *Melastomataceae* are highly susceptible to infection by a *Chrysoporthe* sp. (Wingfield *et al.* 2001), extensive surveys of these plants have been undertaken in South and Central America, as well as in other parts of the world. These surveys have led to the discovery of many new hosts for *Chrysoporthe* and also the discovery of various new species of the fungus.

Surveys in Colombia led to the discovery of *Chr. cubensis* on *Miconia rubiginosa* (Fig. 7; commonly known as mortiño) and *Miconia theaezans* (commonly known as niguito) trees (*Melastomataceae*), which are both native to the country (Rodas *et al.* 2005). These plants often also occur as weeds within *Eucalyptus* plantations (Fig. 7C). Similarly, the fungus was isolated from native *Rhynchanthera mexicana* and *Clidemia sericea* (Fig. 7E–F) plants (*Melastomataceae*) in Mexico (Gryzenhout *et al.* 2006c). In Indonesia, it has been found on native *Melastoma melabathricum* (Fig. 6E–F), which also resides in the *Melastomataceae* (Gryzenhout *et al.* 2006c).

Other hosts of *Chrysoporthe* spp. are exotic in the countries where they are grown. These include the South American native *Tibouchina urvilleana* (*Melastomataceae*), also known as the glory tree or *Lassiandra* (Fig. 6G) in Singapore and Thailand. Another recently discovered host is *Lagerstroemia indica* (Fig. 7G–H), known as the Pride of India tree or crepe myrtle (*Lythraceae*, *Myrtales*) in Cuba, but which is a native to China (Gryzenhout *et al.* 2006c). These trees are popular as ornamentals because of their striking flowers. There has also been a report of *Chr. cubensis* on *Tibouchina* trees (Seixas *et al.* 2004) in Brazil, but the identity of the fungus as *Chr. cubensis* on these hosts needs to be verified based on DNA sequence data (Gryzenhout *et al.* 2004).

In addition to host species on which *Chr. cubensis* has been found to occur naturally, a wide range of trees from different families in the *Myrtales* have also been shown as susceptible to the fungus or another *Chrysoporthe* sp. in artificial pathogenicity tests (Hodges *et al.* 1986, Seixas *et al.* 2004). These include the *Rhizophoraceae*, *Combretaceae*, *Onagraceae*, *Punicaceae*, *Sapotaceae* and *Lauraceae*. This could indicate that *Chr. cubensis* is able to infect a much wider

range of plants in the *Myrtales* than is currently understood, although the fungus has yet to be found on these hosts.

Chrysoporthe austroafricana, which is restricted to southern Africa has also been found on hosts other than *Eucalyptus* spp. This fungus is able to infect *Tibouchina granulosa* trees (Fig. 8A–D), commonly planted as an exotic in gardens and streets in South Africa (Myburg *et al.* 2002b). *Psidium guava* (guava) has been shown as susceptible in artificial inoculations, although the fungus has not been found naturally on this non-native invasive weed in South Africa (Swart *et al.* 1991).

A significant recent discovery has been that *Chr. austroafricana* commonly infects native trees belonging to the genus *Syzygium* in South Africa (Heath *et al.* 2006). These include the wide-spread native waterberry tree *Syzygium cordatum* (Fig. 8E–G), and *Syzygium guinuense* (water pear). *Chrysoporthe austroafricana* has also been found on these trees in other African countries such as Mozambique, Malawi and Zambia (Nakabonge *et al*. 2006b). Discovery of *Chr. austroafricana* on native southern African trees provides good evidence that the fungus is probably native to this area and that it has undergone a host shift to infect *Eucalyptus* spp. and *Tibouchina* spp. (Slippers *et al*. 2005).

Chrysoporthe cubensis causes cracking and cankers (Figs 4–7) on branches, die-back symptoms, and it also commonly occurs on branch stubs (Gryzenhout *et al.* 2006c, Rodas *et al.* 2005) on its various woody hosts other than *Eucalyptus*. This is different to the symptoms on *Eucalyptus* where cankers usually occur on the main trunk (Wingfield 2003). The same is true for *Chr. austroafricana* (Fig. 8), which causes severe die-back and cracking on the branches and stems of *T. granulosa* (Myburg *et al.* 2002b), and cankers on branches and main stems, cracked bark and die-back symptoms on *Syzygium* spp. (Heath *et al.* 2006). Tree death has never been

observed for *T. granulosa* (Myburg *et al.* 2002b) and *Syzygium* spp. (Heath *et al.* 2006, Nakabonge *et al*. 2006b).

Only one *Chrysoporthe* sp. causing cankers on non-native *Eucalyptus* spp. grown in plantations is not known on native trees. This is *Chr. doradensis* which is found in Ecuador (Gryzenhout *et al.* 2005c). Given the fact that *Chr. cubensis* occurs on native plants such as members of the *Melastomataceae* in South and Central America, it seems likely that *Chr. doradensis* is native to Ecuador and has moved from native plants to *Eucalyptus*. Many melastomes such as species of *Tibouchina* occur in that country and preliminary pathogenicity tests have shown that this genus is quite susceptible to *C. doradensis* (Gryzenhout *et al.* 2005c). The fact that native trees are not seen to be dying of infection might be explained by the fact that they tend to grow at high altitudes where it is cool, and pathogenicity tests were conducted in the hot and humid lowlands. These are also the areas where the *Eucalyptus* spp. suffer from infection.

3.2.4 *Population genetics*. The origin of *Chr. cubensis* and *Chr. austroafricana* has not been fully resolved, although host range and other studies in recent years have added substantially to our understanding of this question. *Chrysoporthe austroafricana* is thought to be native to southern Africa (Heath *et al.* 2006) since it is not known from any other part of the world (Gryzenhout *et al.* 2004). Its recent discovery on a native host and the low level of susceptibility of that host also supports this view (Heath *et al.* 2006). Other preliminary data that would support this view is that microsatellite markers have been used to show that genetic recombination occurs among isolates of *Chr. austroafricana* on *Eucalyptus* (Van der Merwe 2000). The fact that isolates of the fungus had a low genetic diversity based on vegetative

compatibility studies or VCGs, might have implied a founder effect typical of an introduced pathogen. However, this study was conducted before the fungus was known on a native host and isolates were all from *Eucalyptus*, which could have severely confounded the results. Population studies using isolate collections from non-native *Eucalyptus* and *Tibouchina* trees, and comparing these with isolates from native *S. cordatum* trees are currently being conducted to better understand the origin of *Chr. austroafricana* (Heath *et al*. 2003abstract, ICPP).

The likely center of origin of *Chr. cubensis* is more enigmatic than that of *Chr. austroafricana* and several hypotheses have been proposed. Intriguingly, the fungus has been found in Australia (Davison & Coates 1991) very rarely (Dr. T. Burgess, personal communication), and only on the roots of *Eucalyptus marginata* in Western Australia, which has a mediterranean climate. This is a situation very different to those where it occurs on *Eucalyptus* in other parts of the world. The host is firstly unusual for *Chr. cubensis* and the fungus is best known in areas with a tropical or subtropical climate. Yet there is no question regarding the identity of the fungus, which has been confirmed using isozyme analyses (Davison & Coates 1991) and comparisons of DNA sequence data (Myburg *et al*. 1999). Thus, Australia cannot be excluded for the possible areas of natural occurrence of *Chr. cubensis*.

The first formal hypothesis regarding the origin of *Chr. cubensis* was raised by Hodges *et al.* (1986). Based on the fact that the fungus occurred both on *Eucalyptus* and on *S. aromaticum*, he suggested that it might be native to clove in the Molucca islands and that it could have moved to *Eucalyptus* growing areas via the spice trade. For many years this seemed to be a feasible hypothesis. However, the discovery of the fungus on native plants of particularly the *Myrtales* in South America and Central America led to an alternative hypothesis (Wingfield *et al.* 2001). This was that the

fungus might have jumped from native plants to plantation grown *Eucalyptus* forests in that part of the world.

While *Chr. cubensis* in South and Central America appears to be from native plants in this region, the origin of the fungus in South East Asia remains less clear. Isolates of the fungus reside in two well-defined sub-clades based on DNA sequence data, which clearly divide isolates with a South/Central American origin and an Asia origin (Myburg *et al.* 1999, 2002a, 2003, Gryzenhout *et al.* 2004). This could indicate that *Chr. cubensis* represents geographically linked cryptic species or groups in the process of speciation (Gryzenhout *et al.* 2006c). In contrast to the situation in South and Central America, very little is known regarding potential native hosts of *Chr. cubensis* in South East Asia. Only one native host, *Melastoma melabathricum*, has been found in Sumatra, Indonesia, and it is still unclear whether *Chr. cubensis* occurs on cloves in the Molucca islands, and not only in clove plantations in other areas of Indonesia where it is not strictly native (Gryzenhout *et al*. 2006c). Further surveys and subsequent population genetic studies will be needed to clarify the origin of the fungus in South East Asia.

Thus far, population genetic studies have not elucidated the origin of *Chr. cubensis*. Populations studies based on VCGs showed that populations on *Eucalyptus* from Brazil (Van Zyl *et al.* 1998) and Venezuela (Van Heerden *et al.* 1997) are equally diverse as a population from Indonesia (Van Heerden *et al.* 1997). One of the problems regarding these studies is that they were conducted prior to the knowledge that the fungus occurs on native plants in South and Central America. Thus isolates all originated on *Eucalyptus* where the diversity of the fungus could have been significantly influenced by the ability of isolates to infect this non-native plant. Population studies are currently being conducted on collections of *Chr. cubensis s. l.*

in an attempt to define the origin and intercontinental spread of the pathogen (Van der Merwe *et al.* 2003a abstract icpp). To achieve this, a set of Simple Sequence Repeat (SSR) markers have been developed for use in these studies (Van der Merwe *et al.* 2003b) and these are being applied to large collections of isolates from different hosts and continents.

3.2.5 *Other species in Chrysoporthe*. A number of species of *Chrysoporthe* other than *Chr. cubensis*, *Chr. doradensis* and *Chr. austroafricana*, occur in South America and are not known from non-native *Eucalyptus* spp. or *S. aromaticum.* *Chrysoporthe inopina* is known only from a small collection on *Tibouchina lepidota* in Colombia (Gryzenhout *et al.* 2006c). The anamorphic species connected to *Chrysoporthe*, *Chrysoporthella hodgesiana*, is also known only from Colombia where it occurs on *Tibouchina urvilleana*, *T. lepidota*, *T. semidecandra* and *Miconia theaezans* (Wingfield *et al.* 2001, Gryzenhout *et al.* 2004, Rodas *et al.* 2005). These two species are associated with disease symptoms, such as cankers and die-back, on their host plants but are not considered serious pathogens.

It is possible that *Chrysop. hodgesiana* and *Chr. inopina* have been overlooked on *Eucalyptus* due to the fact that they are morphologically very similar to the more pathogenic species. Pathogenicity trials with *Chrysop. hodgesiana* on *Eucalyptus* have shown that this fungus is highly pathogenic to *Eucalyptus* trees (Wingfield *et al.* 2001). It may thus occur on these trees without being obvious and it certainly should be considered a potential pathogen of commercial significance.

It is evident from studies on native *Melastomataceae* in Colombia that several *Chrysoporthe* species occur on these hosts. These include *Chr. cubensis*, *Chr. inopina* and *Chrysoporthella hodgesiana* (Gryzenhout *et al.* 2004, 2006c). More than

one of these species can also occur on the same tree (Gryzenhout *et al.* 2004, 2006c, Rodas *et al.* 2005), which can lead to confusion. The discovery of *Chr. doradensis* in Eucador (Gryzenhout *et al.* 2005c), which is relatively close to Colombia, also suggests that members of this genus are common in South and Central America. *Chrysoporthe* species thus represent a complex of pathogens on native trees in South and Central America, and it seems likely that additional species will be discovered in pantropical America in the future.

3.3 Canker of *Eucalyptus* caused by *Holocryphia eucalypti*

Holocryphia eucalypti is a canker pathogen of *Eucalyptus* in Australia and South Africa (Walker *et al.* 1985, Old *et al.* 1986, Van der Westhuizen *et al.* 1993, Gryzenhout *et al.* 2003, Old *et al*. 2003). This fungus was first found in Australia in 1982, where it was identified as *Endothia havanensis* (Davison 1982, Davison & Tay 1983). Subsequent reports treated the fungus as *Endothia gyrosa* (Walker *et al.* 1985, Old *et al.* 1986, Yuan & Mohammed 2000). Venter *et al*. (2001, 2002) recognized the distinct differences between this fungus and *E. gyrosa* and described it as the new species *Cryphonectria eucalypti* (Venter *et al.* 2002). More recently, the new genus *Holocryphia* was established for it based on morphological characteristics and DNA sequence comparisons (Gryzenhout *et al.* 2006d).

Holocryphia eucalypti is a wound-associated pathogen (White & Kile 1993) and causes cankers on trunks, cracking of the bark (Fig. 9A–B) and die-back. It has also been reported to be responsible for tree death, especially in young trees (Walker *et al.* 1985, Old *et al.* 1986, Wardlaw 1999, Gryzenhout *et al.* 2003). *Holocryphia eucalypti* is typically an opportunistic pathogen that is most damaging when host trees are suffering from stress conditions such as drought (Old *et al*. 1990, Gryzenhout *et*

al. 2003). It is not generally considered as one of the most serious pathogens of *Eucalyptus* (Old *et al.* 1990, Yuan & Mohammed 2000, Gryzenhout *et al.* 2003), although it has the potential to cause significant losses when environmental conditions favour infection (Wardlaw 1999, Yuan & Mohammed 1999, 2000, Gryzenhout *et al.* 2003). Some *E. grandis* clones have also been shown to be particularly susceptible to infection by the fungus and opportunities exist to avoid disease by planting resistant *Eucalyptus* genotypes (Old *et al*. 1986, Yuan & Mohammed 1999, Gryzenhout *et al*. 2003).

Holocryphia eucalypti is thought to be native to Australia since it commonly occurs on trees in native *Eucalyptus* forests of this country (Davison & Tay 1983, Walker *et al.* 1985, Old *et al.* 1986). In eastern Australia, the fungus commonly produces both the asexual and sexual states on cankers whereas in Western Australia, the sexual state of *H. eucalypti* has never been found (Walter *et al*. 1985, Davison & Coates 1990). It is also believed that *H. eucalypti* was introduced into South Africa (Nakabonge *et al.* 2005). Currently population studies are being conducted to study the relationships of the different populations from Australia and South Africa using microsatellite markers developed for this purpose (Nakabonge *et al.* 2005)

3.4 Canker caused by *Microthia havanensis*

Microthia havanensis is a fungus originally described from *Eucalyptus* trees in Cuba (Bruner 1916), with subsequent reports from Florida (Barnard *et al.* 1987), Mexico and Hawaii (Gryzenhout *et al.* 2006d). The fungus was previously known as *Cryphonectria havanensis* (= *Endothia havanensis* Bruner) (Bruner 1916, Barr 1978, Micales & Stipes 1987) but has also been known under its synonym *C. gyrosa* (= *Endothia tropicalis* Shear & N.E. Stevens) (Kobayashi 1970, Barnard *et al.* 1987).

Most reports of this fungus indicate it does not incite disease although it often occurs on diseased tissue or epicormic shoots (Bruner 1916, Barnard *et al.* 1987) and together with the more aggressive *Chr. cubensis* (Bruner 1916, Barnard *et al*. 1987, Gryzenhout *et al*. 2006d). Inoculation studes are needed to better understand the role of this fungus as a pathogen.

Microthia havanensis can infect trees belonging to various trees belonging to unrelated orders. The fungus has been found associated with cankers on *Myrica faya* (fire tree), where this tree is native in the Azores and Madeira islands (Gardner & Hodges 1990, Gryzenhout *et al.* 2006d). It is most likely that *M. havanensis* has a saprophytic nature on these cankers (Hodges & Gardner 1992, Gryzenhout *et al.* 2006d). There are reports of *M. havanensis* on other trees in Cuba, i.e. *Spondias mombin*, *Mangifera indica* (*Anacardiaceae*, *Sapindales*) and *Persea gratissima* (*Lauraceae*, *Laurales*) (Bruner 1916), but whether these represent the same fungus has yet to be verified.

3.5 Die-back caused by *Aurapex penicillata*

Aurapex penicillata Gryzenh. & M.J. Wingf. is associated with disease symptoms on native *Melastomataceae* in Colombia (Gryzenhout *et al.* 2006e). These native woody shrubs and trees include *Tibouchina urvilleana*, *Tibouchina lepidota* and *Miconia theaezans* (*Melastomataceae*, *Myrtales*). The fungus has been found together with *Chrysoporthe* species on the same tree species and in the same areas, and in some cases, fruiting structures of both genera occur on the same tree (Gryzenhout *et al.* 2006e). *Aurapex penicillata* also occurs on small cankers on *Eucalyptus* (Fig. 9C–E), but was not highly pathogenic on this tree in pathogenicity trials (Gryzenhout *et al.*

2006e). It appears to be mainly associated with dead branch stubs and senescing branches, and as a secondary habitant on lesions caused by other pathogens.

3.6 Cryphonectria gyrosa stem canker

Eucalyptus canker caused by *Chr. cubensis* is well-known in India (Sharma *et al.* 1985, Florence *et al*. 1986). There has, however, been a report from this country of a related fungus that was treated as *Cryphonectria gyrosa* (Sharma *et al*. 1985). This fungus appears also to occur in Vietnam and Indonesia (Old *et al*. 2003) where it infects trees usually more than three years old. Symptoms include cankers, tissue necrosis and splitting of the bark on twigs and branches. Cankers also occur on the trunks and result in girdling and death in *Eucalyptus torrelliana*, which is the most susceptible species. In pathogenicity trials, lesions developed and differences in susceptibility between species were observed (Sharma *et al*. 1985). Despite the seriousness of the cankers on *Eucalyptus torrelliana*, the disease was not considered to be generally important (Sharma *et al*. 1985, Old *et al*. 2003).

The correct identity of the fungus treated as *C. gyrosa* in India, Vietnam and Indonesia, is unknown. Asexual fruiting structures are reported to be orange to red and the teleomorph structures have long slender necks (Sharma *et al*. 1985). No further details regarding the morphology are known to us. It is unlikely that the fungus represents *A. gyrosa*, the new name for *C. gyrosa*, because the teleomorph of *Amphilogia* does not have long, slender necks (Gryzenhout *et al*. 2005b). For the same reason, the fungus also does not resemble *M. havanensis* (= *C. havanensis*) (Gryzenhout *et al.* 2006d), which for some time was treated as a synonym of *C. gyrosa* (Kobayashi 1970, Barr 1978). It is also not likely that the fungus represents *Chr. cubensis* since Sharma *et al*. (1985) and Old *et al*. (2003) were very familiar with

Chr. cubensis, which occurred in the same areas of India, Indonesia and Vietnam. This fungus could represent an undescribed taxon and additional collections including cultures would be required to resolve this intriguing question.

3.7 Cankers on *Eucalyptus* caused by *Cryphonectria* spp.

Some *Cryphonectria* species have been implicated in disease symptoms on *Eucalyptus* spp. in Japan (Old & Kobayashi 1988). The chestnut blight pathogen, *C. parasitica*, has been isolated from canker symptoms on several *Eucalyptus* spp. in this country. Isolates of the fungus were also able to cause significant lesions on five *Eucalyptus* spp. in artificial pathogenicity trials (Old & Kobayashi 1988). Other isolates resembling an unknown anamorph of *Cryphonectria* have also been collected from *Eucalyptus* spp. in the field, although they could not be identified based on morphology. These isolates of the unknown *Cryphonectria* sp. were able to cause lesions on the *Eucalyptus* spp. and on *Castanea crenata*, although they were less pathogenic than *C. parasitica* (Old & Kobayashi 1988). Furthermore, Myburg *et al.* (2004b) reported the occurrence of *Cryphonectria* spp., including *C. nitschkei* and another unidentified species, on *Eucalyptus* in Japan although the pathogenicity of these species on *Eucalyptus* spp. has not been tested. It thus appears that at least three species of *Cryphonectria*, namely *C. parasitica*, *C. nitschkei* and an unidentified species, occur on *Eucalyptus* spp. in Japan.

3.8 Celoporthe canker

A fungus superficially resembling *Chrysoporthe* spp. has recently been found on native *Heteropyxis* and *Syzygium* spp. (*Myrtales*) in South Africa (Nakabonge *et al.* 2006a). This fungus was shown based on DNA sequences and distinct morphological

characteristics to reside in the *Cryphonectriaceae* and to represent a distinct but new genus and species known as *Celoporthe dispersa*. The fungus has high sequence similarity with previously characterized but undescribed isolates (Myburg *et al*. 2003) from clove in Indonesia, but represents a species distinct from that fungus on clove.

Celoporthe dispersa was found associated with canker symptoms on three different tree species, i.e. *Syzygium cordatum*, *Heteropyxis canescens*, both native to South Africa, and the exotic *Tibouchina granulosa* (Nakabonge *et al.* 2006a). In pathogenicity trials, it was more pathogenic on a *Eucalyptus grandis* clone than on seedlings of *Tibouchina granulosa*, although it has not yet been found on *Eucalyptus* spp. in the field. Based on pathogenicity trials *Celo. dispersa* is obviously pathogenic although field observations suggest that it may be a mild or even secondary pathogen. Little is known regarding the distribution or importance of *Celo. dispersa* in South Africa, and such investigations are likely to be complicated by the morphological similarity between *Celo. dispersa* and *Chr. austroafricana*, which share some of the same hosts and often occur on the same tree (Nakabonge *et al*. 2006a).

3.9 Canker of *Terminalia* caused by *Rostraureum tropicale*

Rostraureum tropicale causes cankers on trunks of mature *Terminalia ivorensis* (*Combretaceae*, *Myrtales*) trees in Ecuador where these trees are grown (Fig. 9F–G) in plantations (Gryzenhout *et al.* 2005d). The fungus also caused girdling lesions on *Terminalia superba* saplings in artificial inoculation trials, although it has not been found on this species in the field (Gryzenhout *et al.* 2005d). There have been reports of an orange, *Endothiella*-like fungus on *Terminalia* trees in Central Africa where these trees are native, but these reports are apparently of another undescribed fungus similar to but not the same as *Rostraureum* (Gryzenhout *et al.* 2005d).

3.10 Root cankers on *Eleaocarpus*

Two species of *Ampilogia*, *A. gyrosa* and *A. major*, are associated with cankers on the roots of *Elaeocarpus* spp. in forests of New Zealand (Gilmore 1966, Dingley 1969, Pennycooke 1989, Gryzenhout *et al.* 2005b). These cankers are, however, not serious and do not result in tree death (Gilmore 1966, Dingley 1969, Pennycooke 1989).

3.11 Endothia canker or pin oak blight

Endothia gyrosa causes a disease known as Endothia canker (Fig. 10) in the U.S.A. (Sinclair *et al*. 1987). This disease was a serious problem in the 1970s and 1980s in North America on *Quercus palustris* (*Fagaceae*, *Fagales*) trees, when it was called pin oak blight (Stipes & Phipps 1971, Roane *et al.* 1974). The fungus has been known as a pathogen in North America from the beginning of the 20th Century (Shear *et al.* 1917, Stevens 1917, Weir 1925). The fungus is also pathogenic on various other woody hosts such as *Liquidambar formosana* (Formosan Sweetgum, *Hamamelidaceae*, *Saxifragales*) and *Fagus sylvatica* (European beech, *Fagaceae*) (Snow *et al.* 1974, Sinclair *et al*. 1987, Roane 1986a, Farr *et al*. 1989).

Endothia gyrosa causes cankers on branches (Fig. 9), stems or roots that result in die-back, defoliation and decline of trees (Stipes & Phipps 1971, Roane *et al.* 1974). It is an opportunist and stress-related pathogen (Hunter & Stipes 1978, Appel & Stipes 1984, 1986), and infects through wounds (Stevens 1917, Hunter *et al.* 1976). The disease caused by *E. gyrosa* was ultimately found not to be the primary cause of the pin oak decline of the 1970s and 1980s, which was brought about through a series of abiotic stresses that aggravated cankers caused by the pathogen (Appel & Stipes 1986).

4. PATHOGEN IDENTIFICATION

The pathogens residing in the *Cryphonectriaceae* often share hosts with other fungi of the *Cryphonectriaceae* in the countries or geographical areas in which they are found. These fungi differ in their pathogenicity and they are difficult to distinguish from each other based on morphological characteristics. For example, *Chr. cubensis*, *Chr. inopina*, *Chrysop. hodgesiana* and *A. penicillata* all occur on native *Melastomataceae* in Colombia (Gryzenhout *et al.* 2004, 2006c, 2006e, Rodas *et al.* 2005). Of these species, only *Chr. cubensis* is known as an economically important pathogen on *Eucalyptus*. The other species have all been shown to be able to infect *Eucalyptus* spp. and might also occur on these trees under field conditions. Accurate identification of these fungi is complicated but it is also extremely important when disease problems are being analysed.

The geographical ranges of some important pathogens of trees, such as of *Eucalyptus* spp., can overlap. An important example is the occurrence of *Chr. cubensis* and *Chr. austroafricana* in Africa. These pathogens both cause serious diseases on *Eucalyptus* spp. that are grown in plantations in many African countries (Roux *et al*. 2005). *Chr. cubensis* occurs only on *Eucalyptus* and clove trees in eastern and western Africa (Myburg *et al.* 2003, Roux *et al.* 2003, 2005). Isolates representing the South American sub-clades of the fungus originated from Cameroon, Democratic Republic of Congo and the Republic of Congo (Myburg *et al.* 2003, Roux *et al.* 2003, Nakabonge *et al*. 2006b), while isolates of the Asian sub-clade have been found in Tanzania, Kenya, Malawi and Mozambique (Myburg *et al*. 2003, Nakabonge *et al*. 2006b). *Chrysoporthe austroafricana* occurs on both *Eucalyptus* and native

Syzygium spp., but in South Africa, Mozambique, Malawi and Zambia (Gryzenhout *et al.* 2004, Heath *et al.* 2006, Nakabonge *et al*. 2006b). Previously the geographical range of these two species was not known to overlap, but the study of Nakabonge *et al.* (2006b) has shown that both *Chr. cubensis* and *Chr. austroafricana* occur in Malawi and in Mozambique, although in the latter country their ranges do not overlap. Careful disease monitoring and accurate identificatioons will thus be necessary to monitor the spread of these two pathogens. This is particularly true in Mozambique that is geographically close to South Africa, where in the latter country a large forestry industry exists that is dependant on the planting of *Eucalyptus* spp. bred for resistance to *Chr. austroafricana* (Roux *et al.* 2005, Nakabonge *et al.* 2006b).

The *Cryphonectriaceae* includes some of the most important pathogens of trees in the world. There are, however, a number of species such as *C. radicalis*, *C. nitschkei*, *C. macrospora*, *E. singularis* (Roane *et al.* 1986b) and *R. longirostre* (Gryzenhout *et al.* 2005d) that have not been associated with serious disease problems. These species are generally regarded as saprotrophic. They often co-exist with pathogenic species, and correct identification of these fungi is important. For example, during surveys and isolations for *C. parasitica* in Europe, *C. radicalis* isolates are often inadvertently collected (Hoegger *et al.* 2002, Sotirovski *et al.* 2004). Likewise, *C. parasitica* is native in Japan, where three other *Cryphonectria* spp., i.e. *C. radicalis*, *C. nitschkei* and *C. macrospora*, and the closely related *E. singularis* also occur (Kobayashi & Itô 1956a, Kobayashi 1970). Thus incorrect identifications can easily be made and substantial effort is required to ensure that this does not occur.

Members of the *Cryphonectriaceae* are difficult to identify because many species are morphologically similar. Careful examination and laboratory study is thus needed to derive a correct identification for them (Myburg *et al.* 2004b).

Identification based on morphology is furthermore impeded (Myburg *et al.* 2004b) by the absence of the teleomorph or anamorph structures in some areas and where these states can provide crucial information on the identity of the fungus. Morphological comparisons are also complicated by overlapping size ranges of fruiting structures. In some cases, such as for *Chrysoporthe* spp., identification can often only conclusively be achieved using DNA sequence comparisons (Gryzenhout *et al*. 2004).

The movement of pathogens to areas of the world where they do not occur, must be controlled. Even where species in the *Cryphonectriaceae* are mild or insignificant pathogens in their native surroundings, their impact on trees in new environments and habitats is generally unknown and could be catastrophic. An exmple of this threat is amply found in the case of the chestnut blight epidemic caused by *C. parasitica* in North America. Furthermore, the aggressive *Eucalyptus* pathogen *Chr. austroafricana*, which appears to be native to southern Africa, has been noted (Roux *et al.* 2003, Wingfield 2003, Myburg *et al.* 2004a) as a fungus that should be carefully monitored, and that could seriously threaten native *Myrtaceae* in countries such as Australia, where most *Eucalyptus* spp. are native. This pathogen is also able to infect *Tibouchina* trees (Myburg *et al*. 2003), and thus poses an equally serious threat to native *Melastomataceae* in South and Central America.

Members of the *Cryphonectriaceae* have caused serious disease epidemics, and this provides a good case to regard them as potentially threatening to woody ecosystems, where they do not occur. The fact that many new species have been discovered in recent years also highlights the fact that they can be easily overlooked and that they have been underestimated in terms of their global significance. It is clear that our knowledge regarding the identification of members of the *Cryphonectriaceae* represents a major weakness in forestry quarantine systems and its

capacity to predict serious disease threats. Many hosts of these fungi are also popular ornamental plants enhancing the likelihood that they might be transported between countries, inadvertently carrying pathogenic *Cryphonectriaceae* to new environments.

Fig. 1. World map showing locations of different diseases caused by members of the *Cryphonectriaceae*.

Fig. 2. Chestnut blight. A. Mature European chestnut (*Castanea sativa*) tree. B. Leaves and flowers. C. Nuts. D. Die-back of branches with dead leaves still attached. E. Dying European chestnut tree. F. Patches of dead European chestnut trees. G. Young canker on branch. H. Orange fruiting structures on surface of canker. (Pictures taken by Drs Thomas Kirisits, Erhard Halmschlager and Helmut Höttinger.)

Fig. 3. Biocontrol against chestnut blight using hypovirulence. A. Healed canker on left treated with hypovirulent isolates (one inoculation point indicated with arrow), and treated canker on right still covered with cloth to prevent desiccation. B. Unsuccessfully treated canker with advancing infection point indicated with arrow. C. Lesion formation at different inoculation points of four hypovirulent isolates and one virulent isolate (arrow). D. Isolates of *Cryphonectria parasitica* containing hypoviruses (arrows) and without hypoviruses. E. Naturally healed canker. (Pictures taken by Drs Thomas Kirisits and Karl-Heinz Figl.)

Fig. 4. Disease symptoms associated with *Chrysoporthe* spp. on *Eucalyptus*. A. Cankers on the stems and base of infected trees (B). C. Young tree killed by *Chrysoporthe austroafricana*. D. Stem breakage due to girdling cankers. E. Epicormic shoot formation below level of canker. F. Cross section through stem showing dead cambial tissue. G–H. Fruiting structures on bark with yellow spore tendrils.

Fig. 5. Field trials used to compare susceptibility of *Eucalyptus* clones to Chrysoporte canker caused by *Chr. cubensis* (A) and *Chr. austroafricana* (B–E). A. Clones healthy (left) and naturally infected (right) by *Chr. cubensis*. B. Trial of inoculated trees. C. A resistant clone where the inoculation point has been covered by new host tissue. D–E. Canker developing after inoculation of a susceptible clone.

Fig. 6. Hosts of *Chrysoporthe cubensis* in South East Asia. A. Dying *Syzygium aromaticum* trees in Sulawesi, Indonesia (arrow). B. Die-back of *Syzygium aromaticum* (arrow). C. Flowers of *Syzygium aromaticum* and drying flowers used as spice (D). E. *Melastoma melabathricum* (arrows) growing in *Eucalyptus* plantations. F. *Melastoma melabathricum*. G. *Tibouchina urvilleana*.

Fig. 7. Different hosts of *Chrysoporthe cubensis* in South and Central America. A. *Miconia rubiginosa*. B. Die-back (arrow) of *Miconia rubiginosa* caused by *Chr. cubensis*. C. *Miconia* sp. growing as a weed (arrow) in a plantation. D. Native forest alongside land cleared for planting of *Eucalyptus* plantations. E. *Clidemia sericea*. F. Necrosis (arrow) associated with *Chr. cubensis* on *Clidemia sericea*. G–H. *Lagerstroemia indica*. I. *Tibouchina lepidota*.

Fig. 8. Hosts of *Chrysoporthe austroafricana* in South Africa. A–C. Canker and die-back (arrows) of non-native *Tibouchina granulosa*. D. Flowers of *Tibouchina granulosa*. E–F. Native *Syzygium cordatum*. G. Die-back of *Syzygium cordatum* (arrow).

Fig. 9. A–B. Cracking of bark on *Eucalyptus* spp. associated with cankers caused by *Holocryphia eucalypti*. C–D. Canker and associated necrosis (arrows) of *Eucalyptus grandis* due to infection by several opportunistic pathogens, including *Aurapex penicillata*. E. Fruiting structures of *A. penicillata*. F–G. Dead trees and necrosis on *Terminalia ivorensis* in Ecuador caused by *Rostraureum tropicale*.

Fig. 10. Pin oak blight caused by *Endothia gyrosa* in the United States. A. Healthy pin oak (*Quercus palustris*). B. Pin oak severely damaged with blight. C–E. Cankers on trunk, branch and roots with characteristic orange fruiting structures.

5. WORKING WITH THE CRYPHONECTRIACEAE

5.1 Isolates and specimens

5.1.1 *Observations in the field.* Members of the *Cryphonectriaceae* are usually found on trees in forests and plantations relatively easily. This is because of their conspicuous orange fruiting structures and orange, scarlet or luteous spores that are typically expelled in droplets or tendrils (Figs 2H, 4G–H, 9E, 10C–E). Fruiting structures are bright orange or red when they are young and mature, but they sometimes start to blacken with age or as they dry. Many species in the *Cryphonectriaceae* cause canker symptoms on branches or stems of the plants on which they occur (Figs 2G, 4A–B, 9A–B, 10C–E). This facilitates the search for these fungi that usually fruit abundantly on lesions. Other species are saprophytes on dead bark or wood, which are either attached to the surface of trees or found on the forest floor.

Fruiting structures vary from 0.5 mm to 5 mm in diam and in the field, they are most easily observed with a 10X magnification hand lens. Some of the genera have fuscous black fruiting structures that may be confused with those of other microfungi, especially members of the *Diaporthales*. For example, the black perithecial necks of the teleomorph of *Chrysoporthe* can easily be confused with the teleomorphs of *Diaporthe* and *Valsa* Fr. (Barr 1978). Observations of the structures are usually aided by the conspicuous bright luteous (yellow) or orange spore masses that also tend to impart a yellow or orange colour to the infected bark. Occasionally, orange to yellow mycelium can also be observed underneath the bark surface surrounding the fruiting structures. Ascospore masses are usually a pale luteous (cream) colour.

5.1.2 *Isolations from bark.* Once fruiting structures have been observed, the bark can be collected and transported to the laboratory in order to make isolations. Where there is doubt that the collected fruiting structures represent members of the *Cryphonectriaceae*, the structures can be tested for a purple discolouration in 3% potassium hydroxide (KOH) and a yellow discolouration in lactic acid. This colour reaction is best seen by mounting material on a glass slide and examining it using a light microscope (Fig. 11A–B).

When the fungal material is fresh and fruiting structures contain spore drops made up of conidia or ascospores, these drops can be picked off with a sterile needle or scalpel blade, and transferred to growth medium to obtain pure cultures. Fruiting structures can often be stimulated to expel spores by placing tissue in a moist chamber. This method, however, has the disadvantage that it can induce the growth of opportunistic and rapidly growing hyphomycetes, such as species of *Penicillium* that easily contaminate the samples.

Incubation of specimens in a most chamber is not recommended if the material must later be used for morphological characterisation. This is because asci often do not persist in the spore drops and are thus absent in specimens derived from moist chambers. Likewise conidiophores often disintegrate after the conidia are expelled from structures that have been incubated. Care should also be taken to ensure that the expelled spores are not lost since very few spores remain inside the fruiting structures. Loss of these structures is undesirable as the morphology of the asci, conidiophores and spores is crucial to identify these fungi.

The best method for making isolations is to retrieve spores directly from within fruiting structures. In the case of a teleomorph, ascus and ascospore masses

can be observed as white, slimy or moist masses within the dark perithecial bases. To gain access to the perithecial bases, which are usually located underneath the bark surface, the bark bearing the ascostromal necks must be removed. This is most easily achieved by shaving off the surface of the bark with a sharp blade. For conidiomata, structures can be cut open either horizontally or vertically to expose the spore-containing locules. Conidiomata can be superficial or semi-immersed, and it sometimes will be necessary to cut into the bark to expose the conidial locules containing the luteous conidial mass. When the conidial locules or perithecial bases are dried out, a small drop of water can be applied to them to rehydrate the cells and to more easily pick off any remaining spores.

Single spore isolates are necessary where individual strains are compared in phylogenetic and population genetic studies. Making single spore cultures can also minimise contamination by other fungi, bacteria or yeasts. To obtain single spore isolates, spores obtained from either expelled spore masses or from within the fruiting structures, can be suspended in a small quantity (9 ml) of sterile water. When spore masses are not obvious or where very few spores are present, the entire fruiting structure, punctured or cut in half, can also be suspended in a smaller volume of water to dislodge remaining spores. One millilitre of the spore suspension can then be transferred to growth medium and spread evenly over the surface of the agar using a glass rod with the basal portion bent at a right angle (hockey stick). Alternatively, a drop of the suspension can be placed on the plate and streaked out using an inoculation loop. Often times, serial dilutions are needed to achieve a suspension sufficiently dilute to separate spores on the agar surface, especially for conidial masses that contain very large numbers of spores. After inoculation, agar plates are incubated at 25 ºC to induce germination of the spores, which usually takes 24–36

hours. Single germinating spores or single hyphal strands can then be transferred to clean plates under a dissecting microscope using a sterile needle.

5.1.3 *Growth in culture.* Species of *Cryphonectriaceae* can be cultivated on many types of growth medium (Bruner 1917, Shear *et al.* 1917, Kobayashi 1970, Roane 1986a). These include malt extract agar (MEA, 20g/L malt, 15 g/L agar) and potato dextrose agar (PDA, 20g/L potato dextrose, 15 g/L agar). Sterile twigs of woody plants, placed on water agar (WA, 15 g/L agar), can also be used to induce isolates to sporulate (Bruner 1917, Shear *et al*. 1917). For primary isolations from plant material, agar medium can be supplemented with an antibiotic, e.g. 0.4g/L of Streptomycin sulfate is used to inhibit the growth of contaminating bacteria. Most species belonging to the *Cryphonectriaceae* are fast growing, and will cover a 90 mm diam plate after six days.

For long term storage, malt yeast agar (MYA, 20 g/L malt extract, 15 g/L agar, 2 g/L yeast extract) or oats agar (OA, 50–60 g/L oats, remove solids from autoclaved broth with a cheese cloth or muslim bag and add extract to 17 g/L agar) can be used. Cultures are grown in bottles on agar slants and stored at 5–8 ºC and it is sometimes useful to cover the mycelial surface with sterilised mineral oil. Alternatively, pieces of mycelium-covered agar can be stored in sterilised water. Cultures can also be freeze dried on glass beads or small pieces of wood (R.J. Stipes, pers. comm.) or they can be stored at –70 ºC. Species of *Chrysoporthe* do not survive well in culture and more than one storage method should be used. Regular transferring of cultures to fresh medium also helps to maintain their viability, but copies of the original cultures should be kept.

Anamorph structures can be found in culture, while teleomorph structures are seldom produced on artificial medium. Where these structures form this is typically only be when cultures dry out after they have been left for up to five months (Bruner 1917, Walker *et al.* 1985). Most species, however, loose the ability to sporulate on the growth medium after sub-culturing from the primary isolation plates. Sporulation can be induced by growing cultures on water agar amended with pieces of wood of the host species on which they occur naturally. The fruiting structures produced can be comparable to those formed in nature (Kobayashi 1970), although this have to be established through observation.

The best method to produce fruting structures from cultures of species of *Cryphonectriaceae* is to inoculate cultures into wood, preferably of the original host. Typically, fresh branches or twigs (c. 1-2 cm diam.) are used after sterilizing their suface with alcohol and sealing the cut ends with hot paraffin wax (Hodges *et al.* 1986). A punch or cork borer (5 mm diam or less) is then used to remove the bark which is replaces with a disc of the fungus. Inoculation wounds are then sealed with parafilm or masking tape, and the pieces of wood incubated in a moist chamber for c. 6 days. Often, the fruiting structures that are produced differ from those found on naturally infected tissue, e.g. fruiting structures may be superficial, perithecial necks may be longer, fruiting structures will be covered with hairs, and the shape and size of spores can be variable (Myburg *et al.* 2002b). It is not advisable to use such structures, including structures produced in culture, in taxonomic descriptions unless these structures are first compared with those from naturally infected tissues, and shown to be similar.

5.1.4 *Morphological studies*. The best material to use for characterizing the morphology of *Cryphonectriaceae* is the originally collected specimens on naturally infected bark. For this purpose the original bark material from which cultures have been derived, should always be carefully preserved. Bark specimens must be carefully dried and stored in dry, insect-free containers. Specimens can be prepared for storage by placing them at –20 ºC for 2 days, or –70 ºC for 1 h to kill insects, mites and eggs of these animals. Specimens must then be dried naturally, or using silica gel crystals, or by the application of low heat for a short period of time. Care should be taken not to dry specimens excessively since they become brittle. Specimens can then be placed in envelopes or boxes, while taking care that structures, which often include fragile necks or spore tendrils, are not damaged.

Fruiting structures produced in culture or in artificial inoculations can often exhibit variation in structure, shape and size, although an approximation of the morphology can be obtained (Myburg *et al*. 2002b, 2003). When studying fruiting structures made in culture, these should always be compared with those on natural material to verify that fruiting structures are similar (Kobayashi 1970). In natural bark material, variation in structure can also be observed between specimens from different locations and hosts. For example, fruiting structures of *Chrysoporthe* spp. are more superficial on the bark of *Eucalyptus* spp. than on *Syzygium* spp. (Hodges *et al*. 1986, Myburg *et al*. 2003), and on some samples, pulvinate structures are predominant, while in other specimens, only pyriform structures will occur (Gryzenhout *et al*. 2004).

5.1.5 *Microscopic examination of herbarium specimens*. Morphology of fruiting structures is best observed under a dissection microscope, which can be used to study

both sexual and asexual structures. Wherever possible, it is important to consider both the sexual and asexual states of these fungi, since species can often not be identified based on a single state. To prepare slides for comparisons under a compound microscope, mounting medium such as lactophenol or 85 % lactic acid can be used to prepare semi-permanent preparations, which can be sealed with nail varnish. Temporary preparations in water or 3 % KOH can also be made in combination with semi-permanent preparations.

To study spores, conidial or ascospore masses can be transferred to the mounting medium using the methods described for isolations. Pieces of a fruiting structure, longitudinally hand cut with a razor blade and placed onto a microscope slide to make squash mounts, best reveals asci and conidiophores. It is preferable to make preparations in both lactic acid or lactophenol as well as KOH, since structures that cannot be observed in the former types of mounting medium, can often be studied in the latter. For instance, conidiophores and asci are best studied in KOH, which breaks cells apart to release and distribute these structures. For the purpose of this monograph, all slide preparations were made in lactic acid and KOH.

Preparations of spores, asci and conidiophores together with gross morphology as observed under a dissection microscopes, are often sufficient to make an identification. However, the internal structure and tissue morphology of the ascostromata and conidiomata must also be considered in taxonomic studies. This can only be achieved by producing longitudinal sections either in a crude way through hand cuts using a razor blade, or preferably with a microtome. In preparing this monograph, all sections were made using a Leica CM1100 Cryostat (Setpoint Premier, Johannesburg, South Africa) at –20 ºC and using mounting medium provided by Setpoint Premier.

Fruiting structures are cut from the bark, taking care to remove the entire structure, which can often extend sideways underneath the bark. When structures are cut from old or very dry specimens, removed fruiting structures should be boiled for one min in water to rehydrate the cells (Huhndorf 1991). Sections of structures in this study were 12–16 µm thick depending on the size of the structures and fragility of the stromata. When structures are large or when stromatic tissue is predominantly prosenchymatous and fragile (Table 1), thicker sections may be necessary to avoid shearing of the tissue. In this study, sections were typically transferred to water, which dissolves the mounting medium, and subsequently placed in a drop of mounting medium on a microscope slide with a needle. Alternatively, they were deposited directly on a cover slip that was then placed on a drop of 85 % lactic acid.

5.1.6 *Colony growth.* Comparisons of growth in culture are made at different temperatures under similar conditions to determine the optimal temperature for growth and also to assess colony morphology and colour. Standard colour notations of Rayner (1970) were used to describe colours in this study, although a number of other systems are avalaible for this purpose. To determine the range of temperatures at which species grow, five temperatures are selected that span the suspected optimal temperature for growth of the test fungus. Thus, for tropical and sub-tropical species, growth was assessed from 15 to 35 ºC, while for those species occurring in temperate climates, the range of temperatures considered was from 10 to 30 ºC in this study. To assess growth, two measurements perpendicular to each other were taken of each plate, and four plates were used for each isolate at each temperature. These measurements were then statistically analysed to determine the optimum temperature for growth.

Species of the *Cryphonectriaceae* are usually fast growing and cover a 90 mm diam Petri dish in 4–6 days. Cultures are usually white and fluffy when young, but then turn to colours ranging from olivaceous brown, cinnamon, crimson, orange or luteous (Fig. 12). The pigmented mycelium will also turn purple in 3 % KOH and yellow in lactic acid, similar to the fruiting structures.

The characteristic colours of the fruiting structures and mycelium are due to different pigments produced by the fungi. Four pigments have been identified in studies on different species and these include the bisanthraquinones skyrin, skyrinol, oxyskyrin and regulosin (Roane & Stipes 1978, Roane 1986c). These pigments are found in different combinations in each species (Roane & Stipes 1978). Isolates of *E. gyrosa*, *C. radicalis*, *C. nitschkei* and *E. singularis* also produces a pigment, known as endothine red, which colours (Fig. 12C) the growth medium purple (Roane & Stipes 1978, Shear *et al*. 1917, Roane 1986c). This compound can also be found as red crystals (Fig. 11C) in the mycelium (Hawkins & Stevens 1917, Roane 1986c). Furthermore, Gryzenhout *et al.* (2005d) reported the formation of conspicuous orange crystals inside stromata that lined the conidial locules of *Rostraureum* spp. (Fig. 11I). Similarly, other brightly coloured orange to luteous crystals can be found in the stromatic tissue of other taxa such as *Aurapex* (Fig. 11D–H).

5.2 Phylogenetic circumscription through DNA sequence comparisons

5.2.1 *DNA sequence comparisons*. The use of DNA sequence comparisons has completely changed the taxonomy of *Cryphonectria s. l.* DNA sequences of the variable ITS regions of the ribosomal DNA operon, and parts of the β-tubulin genes, provided early evidence that *Cryphonectria* was polyphyletic (Myburg *et al*. 2004a).

Thus several new genera have been identified that represented species previously treated in *Cryphonectria*, i.e. *Chrysoporthe*, *Rostraureum*, *Amphilogia*, *Microthia* and *Holocryphia*. Likewise new genera such as *Celoporthe* have been discovered (Gryzenhout *et al.* 2004, Myburg *et al.* 2004a, Gryzenhout *et al*. 2005d, 2006d, Nakabonge *et al*. 2006a). The only species that make up the *Cryphonectria s. str.* clade are *C. parasitica*, *C. radicalis*, *C. nitschkei* and *C. macrospora* (Gryzenhout *et al*. 2006a).

DNA sequence comparisons of the same gene regions have been used to characterize newly collected material that did not resemble known species. These invariably represented new species and genera, such as *R. tropicale* (Gryzenhout *et al*. 2005d), *U. fallax* Gryzenh. & M.J. Wingf. (Gryzenhout *et al*. 2006d) and *A. penicillata* (Gryzenhout *et al*. 2006e). This was especially true for species in *Chrysoporthe*, which was previously known under the single species name *C. cubensis* (Gryzenhout *et al*. 2004). Four species of *Chrysoporthe*, i.e. *Chr. austroafricana*, *Chr. inopina*, *Chr. doradensis* and *Chrysop. hodgesiana* (Gryzenhout *et al*. 2004, 2005c, 2006c), have been described in addition to *Chr. cubensis*, and these have primarily been recognized based on DNA sequence data.

Comparisons of sequences of the conserved large subunit (28S) of the ribosomal DNA operon have revealed that *Cryphonectria* and related genera represent a distinct family of fungi that has been described as the *Cryphonectriaceae* in the *Diaporthales* (Castlebury *et al*. 2002, Gryzenhout *et al*. 2006b). This distinct grouping of *Cryphonectria* species and related taxa was unexpected, since these fungi have never been thought to represent a distinct group in the *Diaporthales* (Castlebury *et al*. 2002). Its emergence has clearly been strongly influenced by the discovery of a large number of new genera and species in the family. The *Cryphonectriaceae* also

contains genera and species, such as *Chrysop*. *hodgesiana* (Gryzenhout *et al*. 2004), *Aurapex* (Gryzenhout *et al*. 2006e aur) and *Ursicollum* (Gryzenhout *et al*. 2006d), that have no known teleomorph.

The different phylogenetic assemblages identified as distinct genera, are all well supported by robust morphological characteristics (Myburg *et al*. 2004a, Gryzenhout *et al*. 2005d, 2006d). Indeed, in most cases these genera would not have been recognized as distinct without a clear knowledge of their morphology. Thus, the different phylogenetic clades could also be distinguished from each other based on morphology alone, and this is true even for those represented by a single species such as *Aurapex* (Gryzenhout *et al*. 2006e), *Ursicollum* and *Holocryphia* (Gryzenhout *et al*. 2006d).

The morphological differences between genera and species in the *Cryphonectriaceae* are quite diverse between the different phylogenetic clades for the family. This suggests the phylogenetic clades represent distinct genera and not different species of one genus (Gryzenhout *et al.* 2006e). Many of the clades representing genera, such as *Chrysoporthe* (Gryzenhout *et al.* 2004, 2006c, 2005c), also include different species that can be recognized based on DNA sequences as well as morphological characteristics.

DNA sequence comparisons have provided an important aid to identify species of the *Cryphonectriaceae* that are morphologically similar, such as *Chrysoporthe* spp. These comparisons have also made it possible to identify species for which incomplete collections are available for study. This is often the case where the only feature distinguishing the species is found in either the anamorph or teleomorph (Gryzenhout *et al*. 2004, Myburg *et al*. 2004b). Phylogenetic studies have also been used to decide whether unique morphological features might represent

generic or species differences. For instance, species of *Microthia* could possibly be classified in *Cryphonectria* based on morphology, but sequence comparisons have shown that this fungus resides in a monophyletic clade separate from *Cryphonectria* (Gryzenhout *et al*. 2006d).

DNA sequence comparisons should preferably not be used alone when characterizing species of *Cryphonectriaceae*. This is because many of the newly described genera are monotypic and phylogenetic analyses can suggest that a genus, represented by a single species, is a species of another related genus, while these are in fact morphologically quite distinct. An example of this is *Holocryphia*, which was shown to represent a *Cryphonectria* species based on DNA sequences (Venter *et al*. 2002, Myburg *et al*. 2004a). This species, however, was shown later to represent a distinct genus supported by morphological characteristics unlike those of *Cryphonectria* (Gryzenhout *et al.* 2006d), after inclusion of more taxa in the phylogenetic analyses.

5.2.2 *Restriction Fragment Length Polymorphisms (RFLPs).* RFLPs have been used to a limited extent to distinguish between species in the *Cryphonectriaceae*. Myburg *et al.* (1999) were able to distinguish between *C. parasitica*, *Chr. cubensis* and *E. gyrosa* using restriction enzymes *Alu*I and *Cfo*I on PCR products of the ITS1 and ITS2 region. Likewise, *H. eucalypti* was distinguished from *E. gyrosa* using *Cfo*I and *Eco*RI on PCR products of the same region (Venter *et al.* 2001). These studies, however, included only a limited number of species and it is possible that other species, for which isolates are now available, may have the same restriction patterns. PCR-RFLPs provide a useful and simple tool to separate closely related and morphologically similar species when sequencing is not an option. Now that a large

number of species are recognized in the *Cryphonectriaceae*, it would be useful to consider this technique to separate closely related species that occur in similar areas.

5.2.3. *Methodology*. DNA can usually be easily extracted by any of the well-known methods used for this purpose, and several protocols have been used for the *Cryphonectriaceae* (Myburg *et al.* 1999, Venter *et al.* 2001, Nakabonge *et al.* 2006a). Best results are obtained with young mycelium where no pigments have been produced that can interfere with DNA isolation and amplification. The ITS regions are amplified using the ITS1 and ITS4 primers (White *et al.* 1990) and reaction conditions previously described (Myburg *et al.* 1999, Venter *et al*. 2002, Nakabonge *et al*. 2006a). Two regions of the β-tubulin genes are amplified using primer pairs Bt1a/Bt1b and Bt2a/Bt2b respectively (Glass & Donaldson 1995) following parameters set by Myburg *et al.* (2002a) or Venter et al. (2002). Annealing temperatures for the different species are usually 55 ºC for the ITS region and β-tubulin 1 region (primers Bt1a/b), and either 58 ºC or 65 ºC for the β-tubulin 2 region (primers Bt2a/b). Sequences of the Histone H3 genes have also been used for *Chrysoporthe* species (Myburg *et al.* 2002a) using the primer pair H3 1a/1b (Glass & Donaldson 1995), since the ITS region does not always distinguish between the different sub-clades of this genus (Myburg *et al*. 2002a, Wingfield *et al*. 2001, Gryzenhout *et al*. 2004). The ribosomal DNA large subunit gene has been amplified using the protocols followed by Gryzenhout *et al.* (2006b) and the primer pairs ITS3 (White *et al.* 1990) and LR3 (Vilgalys & Hester 1990) and the same amplification conditions as for other gene regions.

Sequences obtained in various studies considering the taxonomy of species in the *Cryphonectriaceae* have been deposited in Genebank (http://www.ncbi.nlm.nih.gov/)

and their accession numbers have been provided in the individual studies treating species or groups of species. Many of the alignments used in previous studies on the *Cryphonectriacease* can also be obtained from TreeBASE (http://www.treebase.org/), with the study and matrix numbers found in the original publication. These alignments can be used as a template on which to add new sequences. Sequences that are inordinately divergent from existing sequences and representing new genera can be aligned with existing sequences using alignment software. An example of an alignment programme commonly used for this purpose, is MAFFT ver. 5.667 (Katoh *et al*. 2002), which also provides a web interface (http://timpani.genome.ad.jp/%7Emafft/server/) where sequences can easily be aligned on-line without the need of installing the program.

For phylogenetic analyses on the *Cryphonectriaceae*, standard methods inferring the relationships between different isolates have been used. These include parsimony and distance analyses, and full descriptions of parameters used can be found in past studies employing sequence analyses. Different programmes for phylogenetic analyses exist, such as the program PAUP vers. 4.0b10 (Swofford 2002) that has been most extensively used. Other alignment programs freely available from the internet and that can do phylogenetic analyses, are Bioedit (http://www.mbio.ncsu.edu/BioEdit/page2.html) and MEGA (http://www.megasoftware.net/).

Fig. 11. Pigments and different types of crystals found in stromatic tissue of the *Cryphonectriaceae*. A. Yellow discolouration after treatment with lactic acid, and purple discolouration B. caused by 3% KOH in tissue of species in the *Cryphonectriaceae*. C. Red crystals in mycelium associated with endothine red. D. Crystals in stromatic tissue of *Microthia havanensis* and *Cryphonectria radicalis* (E–F). G. Orange crystals in neck and tissue (H) of *Aurapex penicillata*. I. Crystals in the tissue and lining conidial locules of *Rostraureum* spp. Scale bars A–C = 100 µm; D–E, G, I = 20 µm; F, H = 10 µm.

Fig. 12. Variation in cultural characteristics in selected species of the *Cryphonectriaceae* and reflecting different culture morphologies. A–B. *Endothia gyrosa*. C. Purilla purple discolouration of the growth medium. D–H. *Cryphonectria radicalis* showing orange discoloration of the growth medium (F) and sporulation (H). I. *Chrysoporthe cubensis*.

6. IDENTIFICATION OF GENERA AND SPECIES

6.1 Morphological characteristics and terminology

Genera in the *Cryphonectriaceae* are stromatic fungi. This implies that spore-bearing structures are borne inside structures composed of masses of vegetative hyphae (Hawksworth *et al*. 1995). In the case of the teleomorph, the ascus-producing perithecia are found inside the stromatic structures, which are referred to as ascostromata (Table 1, Fig. 13). Conidia are produced by the conidiophores formed within conidiomata that are eustromatic (Table 1, Fig. 13). Conidiomata of some genera in the *Cryphonectriaceae* clearly resemble stromata, but others, for instance those of *Chrysoporthe*, are more similar to pycnidia (a flask-shaped conidioma). However, all conidiomata in the *Cryphonectriaceae* are eustromatic (Myburg *et al*. 2002a) with stromatic tissue development inordinately extensive to represent a pycnidium (Hawksworth *et al*. 1995).

The morphology of the ascostromata and conidiomata are complex due to their stromatic nature, and several morphological characteristics of the stromata are used for identification in addition to spore morphology. Often longitudinal sections are needed to study stromatal structure in addition to squash mounts, which are acceptable for the study of spores, asci and conidiophores. These characteristics are discussed in the following paragraphs, and characteristics and definitions are summarized and illustrated in Table 1 and Figs 13–18.

6.1.1 *Stromata.* Structural morphology of the stroma is an important characteristic used to identity a genus. This mainly includes the relation of the stroma to the host substrate, e.g. whether stromata are semi-immersed or superficial. Host substrate and

environmental conditions such as moisture content (Hodges *et al*. 1986, Shear *et al*. 1917, Walker *et al*. 1985) can, however, influence structure. For example, stromata of *Chrysoporthe* spp. can either be superficial or semi-immersed on different hosts such as *Eucalyptus* and *Syzygium* spp. (Hodges *et al.* 1986, Myburg *et al*. 2003). The morphology of the bark can also influence the form of the stromata and stromata are often confluent (Table 1) in bark fissures, but single and circular on the bark.

The morphology of the stromatic tissue is of greater importance than its structure in identification and this character is in some cases, unique for a genus (Figs 14–15). Tissue morphology is also essentially not influenced by external factors. The morphology of the individual hyphal cells is used to distinguish between different tissue types (Table 1, Figs 14–15) such as pseudoparenchyma, prosenchyma and different *textura* types. The occurrence of host tissue in fungal tissue (Table x) has also been used to define the morphology of the stroma as eustromatic, pseudostromatic, ecto- or entostromatic (Shear *et al*. 1917, Barr 1978, Walker *et al.* 1985, Micales & Stipes 1987).

6.1.2 *Ascostromata.* Although the morphology of the perithecia is uniform in the *Cryphonectriaceae*, morphology of the stromatal tissue surrounding the perithecia is quite variable. The degree of tissue development, e.g. limited or extensive, and relation of the stroma to the host tissue are important characters, as is the manner in which the perithecia are carried within the stroma (Table 1). These features can be influenced by bark morphology and environmental factors, while the morphology of the stroma also influences the orientation of the perithecia. For instance, perithecia will be diatrypoid in robust stromata, but limited stromata that are semi-immersed can

force perithecia into a valsoid orientation (Walker *et al*. 1985, Micales & Stipes 1987, Cannon 1988).

As the perithecial necks extend beyond the stromatal surface, they are covered in a layer of stromatal tissue. This layer can be of different colours and these can be important to distinguish genera (Fig. 13). The ostiolar opening of the true perithecial neck will be visible as a black dot within the extending neck. The length of the extending necks can also be an important character used to distinguish between genera. However, under conditions of high moisture content, considerable variation in length of necks can be found within a species. Neck length is used to indicate differences when environmental effect is considered neglible. For instance, species of *Cryphonectria* usually have necks extending into short papillae on the stromatal surface (Table 1, Fig. 13), while species of *Chrysoporthe* have long, cylindrical perithecial necks (Fig. 13).

6.1.3 *Conidiomata.* Anamorph structures in the different genera of the *Cryphonectriaceae* are very characteristic for each genus. The external shape and colour of conidiomata can almost exclusively be used to identify a genus. These range from orange to fuscous black, with shapes from pulvinate to rostrate (Table 1, Fig. 16). The presence of a neck on conidiomata is also important, since some genera lack necks (Fig. 16). The internal tissue organization of the conidiomata is quite distinctive for the genera, and these can differ from the tissue arrangement of the ascostromata.

Spores are either expelled as drops or tendrils (cirrhi) depending on the size of the opening (Table 1), but this characteristic can also vary within a genus. The internal structure of the conidial locules is quite variable and ranges from single to

multilocular, with even or convoluted linings (Table 1). Single but highly convoluted linings often give the impression of a multilocular stroma where this is sectioned longitudinally and when the different folds of the locules are cut open (Table 1). Cross sections can be made to confirm the number of locules.

6.1.4 *Occurrence of the teleomorph and anamorph*. The teleomorphs and anamorphs of species in the *Cryphonectriaceae* often occur separately on different pieces of bark, or both states can occur together on the same bark. The anamorph and teleomorph can usually be distinguished from each other under a dissecting microscope, although they are often quite similar morphologically (Fig. 13). Both conidia and ascospores can also be produced in a single stroma. For instance, conidial locules can be found in the same stroma that contains perithecia (Fig. 13), and these structures can be formed either before or after the perithecia have developed (Shear *et al*. 1917). In this case, the only external sign that both states occur in a structure, is the production of conidial spore masses together with perithecial necks (Fig. 13), for instance in *Cryphonectria*, *Endothia*, *Microthia*, *Amphilogia* and *Holocryphia*. In other genera, such as *Chrysoporthe* and *Rostraureum*, anamorph structures are morphologically distinct from teleomorph structures. These can thus be distinguished from the sexual structures although occurring on the teleomorph (Fig. 13).

6.1.5 *Spore morphology*. Conidia and ascospores of the *Cryphonectriaceae* are fairly uniform, all being hyaline with no great variation in shape between genera or species. Conidia range from cylindrical to oblong to allantoid, while ascospores are ellipsoidal to cylindrical or allantoid (Fig. 17). Differences in ascospore septation are, however, an important characteristic with ascospores being aseptate, or having one to three

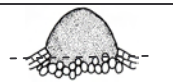
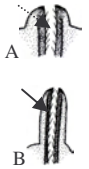
septa in different genera (Fig. 17). The position of the septum can vary within a species, for instance, while it usually occurs at the centre of the spore, it can also be somewhat displaced from the centre (Fig. 17). Some species, especially in *Microthia*, also have slight constrictions at the septa, but this can be variable between specimens. The conidia of all the species in the *Cryphonectriaceae* are aseptate.

6.1.6 *Conidiophores and paraphyses.* Conidiophores in the *Cryphonectriaceae* (Fig. 18) are all hyaline with phialidic conidiogenous cells. The shape of the branching cells is usually cylindrical, with inflated bases or not and apices attenuated or not, while branching is irregular (Fig. 18). Differences between genera occurs mainly in the complexity of branching such as whether the branches radiates from large basal cells or not. Long, cylindrical, sterile cells known as paraphyses (Fig. 18), have also been observed between conidiophores in species of *Microthia* and *Holocryphia*, and can easily be seen at 40X magnification.

6.1.7 *Morphological characteristics used for family, generic and species delimitation.* Certain morphological characteristics found in the *Cryphonectriaceae* are conserved and define the fungi in the *Diaporthales*. These characteristics constitute the *Diaporthe*-type centrum, which includes perithecia with long necks located in pseudostromata, no paraphyses between asci, and asci that are thick-walled and either evanescent or intact (Alexopoulos & Mims 1978, Hawksworth *et al.* 1995). The perithecial walls of *textura epidermoidea*, the periphysate ostiolar canal and beak surface of *textura porrecta* also defines the genera as belonging in the *Diaporthales* (Barr 1978). Asci are usually elliptical, oblong or cylindrical, with a refractive apical ring that is chitinoid and non-amyloid, and they are usually free floating (Barr 1978).

Features that distinguish the *Cryphonectriaceae* from other families of the *Diaporthales* are the orange tissue of the stromata, and the discolouration of the pigments to purple in 3% KOH, and yellow in lactic acid (Roane & Stipes 1978, Roane 1986a, Castlebury *et al.* 2002, Gryzenhout *et al.* 2006b). The degree of development of the stromatic tissue, stromatic tissue type and colour, colour of the extending perithecial necks, colour and shape of the anamorph, and ascospore septation are the main characteristics that define the different genera. Spore shape and size usually distinguish the different species in these genera.

Table 1. Terminology used in this monograph or in other literature (Micales & Stipes 1987, Hawksworth *et al*. 1995).

Morphological characteristic	Terminology	Definition	Illustration
Stroma (pl. -ta)	Eustroma Superficial	Of fungal tissue only Not embedded in host tissue or above level of bark (dotted line)	
	Pseudostroma Ectostroma (A) Entostroma (B) Semi-immersed	Fungal tissue also containing remnants of host tissue Uppermost portion of stroma that breaks through the bark, usually composed of fungal tissue Lower portion of stroma, usually composed of both fungal and host tissue Partially embedded in the host tissue or also below level of bark (dotted line)	
	Prosenchyma	Tissue composed of interwoven, parallel, elongated hyphal cells (more losely arranged than *textura porrecta*)	
	Pseudoparenchyma	Tissue composed of closely packed, isodiametric hyphal cells (smaller cells than *textura globulosa*)	
	Confluent	Continuous or coming together (appear to be a single stroma) as opposed to separate or distinct	
	Erumpent	Bursting through surface of substrate	
Ascostroma (pl. -ta)	Ascostroma Valsoid	A stroma in which asci are produced Perithecial necks convergent	
	Diatrypoid	Perithecial necks separate or parallel	
	Ostiolar canal Periphysate Papilla (A)	Canal (dashed arrow) in neck of perithecium, opens in ostiole Hyphae (arrow) pointing upward inside ostiole of perithecium or pycnidium Small rounded structure where perithecial neck emerge, as opposed to long necks (B)	

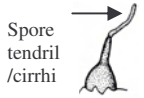
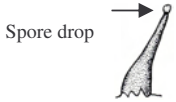
Morphological characteristic	**Terminology**	**Definition**	**Illustration**
Conidioma (pl. -ta)	Locule (arrow)	Cavity inside stroma, in this case in the conidioma, contains conidiophores	
	Single locular	Only one locule	
	Multilocular	More than one locule	
	Convoluted	Folded (sometimes appear multilocular if sections at edge – dashed line)	
`Conidiomatal shape	Pyriform	Pear-shaped	
	Rostrate	Beaked	
	Conical	Almost triangular	
	Subulate	Anvil-shaped	
	Pulvinate	Cushion-shaped	
	Globose	Spherical or almost so	

Fig. 13. Sexual and asexual fruiting structures. A. Ascostroma containing ascus-producing perithecia. B. Conidioma with locules lined with conidiophores. C. Ascostroma also containing conidial locules (arrows). D. Ascostroma cut open and revealing black perithecial bases and necks (arrows). E. External view of ascostroma, indicated by perithecial necks (white arrow), but also containing locules, indicated by conidial spore mass (black arrow). F. Structure consisting of both an ascostroma, indicated by cylindrical necks (black arrows) and conidiomata of different shape (white arrows), where the morphology of these differ. G. Perithecial necks opening as black ostiolesa within orange papillae, or within long orange necks (H). I. Periphysate perithecial neck (arrow). Scale bars A–H = 100 µm; I = 20 µm.

Fig. 14. Schematic drawings of different stromatic tissue types. A. *Textura globulosa*. B. *Textura porrecta*. C. *Textura epidermoidea*. D. *Textura intricata.*

Fig. 15. Characteristic anamorph tissue organization in different genera of the *Cryphonectriaceae*. A. Stromatic tissue of *textura intricata* at the section between the neck and base (A), of *textura porrecta* at the neck (B) and of *textura epidermoidea* at the conidiomal base (C) of *Rostraureum*. D. Stromatic tissue of *textura globulosa* at the conidiomatal base, and of *textura porrecta* at the neck (E) of *Chrysoporthe*. F. Pseudoparenchymatous basal tissue of *Amphilogia*. G. Stromatic tissue of *textura globulosa* at the conidiomal base, and complex tissue at neck (H) of *Aurapex*. I. Pseudoparenchymatous and prosenchymatous (arrow) stromatic tissue found in *Cryphonectria*, *Endothia*, *Microthia* and *Holocryphia*. J. Complex stromatic tissue of the conidiomal base and neck (K) of *Ursicollum*. L. Luteous lining of conidial locules of *Cryphonectria radicalis*. Scale bars = 20 µm.

Fig. 16. Different shapes and colours of conidiomata found in the genera of the *Cryphonectriaceae*. A. Fuscous black, pyriform conidioma of *Chrysoporthe*. B. Fuscous black, globose conidioma of *Chrysoporthe*. C. Fuscous black conidioma of *Celoporthe*. D. Fuscous black conidiomal bases with orange necks of *Aurapex*. E. Orange, rostrate conidioma of *Rostraureum*. F. Orange, pulvinate conidioma of *Cryphonectria*, *Endothia*, *Microthia* and *Holocryphia*. G. Orange, conical conidioma of *Amphilogia*. H–I. Orange conidiomata of *Ursicollum*. Scale bars = 100 µm.

Fig. 17. Schematic drawings of spore morphology. A. Cylindrical. B. Fusoid. C. Oval. D. Ellipsoid. E. Allantoid (curved). F. Comparison of spore shapes depicted in A–D. G. Obtuse (rounded) apex. H. Tapered apex. I. Centered septum. J. Septa at variable positions. K. Oblong. L. Ovoid.

Fig. 18. Conidiophore morphology. A. Morphology of a conidiophore, also showing complex, irregular branching. B. Shapes of conidiogenous cells of different unbranched, unseptated conidiophores. C. Paraphyses between conidiophores. D. Longitudinal section through conidioma with long paraphyses present between conidiophores.

6.2 Identification through phylogenetic analyses

The phylogenetic trees presented in this study include all known taxa in the *Cryphonectriaceae* for which DNA sequences are presently available. Taxa for which there are no isolates or sequences are *M. coccolobae* (Gryzenhout *et al*. 2006d) and *A. major* (Gryzenhout *et al*. 2005b). The trees should make it possible to identify existing species or to characterize new fungi that are discovered in the future. The trees are thus not presented to show evolutionary relationships between the taxa, but to represent sequence templates that can be used for identification.

Two trees are presented based on different areas of the genome. The first tree (Fig. 19) is based on sequences from a subset of the LSU region of the ribosomal DNA operon (Table 2), which is a conserved area typically reflecting generic and family divisions (White *et al*. 1990). LSU sequences are useful when one wishes to determine if the fungus studied is a member of *Cryphonectriaceae*, as presented by Gryzenhout *et al*. (2006b). The second tree (Fig. 20) is based on a combined data set comprised of highly variable sequences of the β-tubulin gene region and ITS regions of the ribosomal DNA operon. Key isolates from previous studies have been used and these are listed in Table 3 together with their Genbank accession numbers. This tree (Fig. 20) can be used to place a fungus in a genus and to determine whether it resembles one of the existing species in the tree. A subset of only the ITS or β-tubulin genes could also be used for this purpose and in this case the β-tubulin gene region shows most variation.

6.2.1 *Materials and methods.* The DNA datasets were compiled from all available sequences (Tables 2–3) and aligned using MAFFT ver. 5.667 (Katoh *et al.* 2002).

The datasets were analysed using PAUP* (Phylogenetic Analysis Using Parsimony) v. 4.0b8 (Swofford 2002). For both datasets, trees generated using distance and parsimony methods were compared with each other. The appropriate distance model that should be used for analyses was determined with MODELTEST v. 3.5 (Posada & Crandall 1998). For parsimony analyses, gaps were treated as NEWSTATE, heuristic searches were done with the TBR swapping algorithm (Multrees active, trees randomly added with 100 reps), uninformative characters were excluded and to obtain fewer trees, the remaining characters were reweighted according to their Consistency Indices (CI).

The strength of the branches was tested using various methods. To determine the support for the individual branches, bootstrap analyses (1000 replicates) were done. Bayesian inference was also employed to test the probabilities of the different branches. For this purpose, the Markov chain Monte Carlo (MCMC) algorithm (Larget & Simon 1999) in the program Mr. Bayes v. 3.1.1 (Huelsenbeck & Ronquist 2001) was used. The following parameters were set: number of generations = 1 000 000, sample frequency = 100, number of chains = 4 (1 cold, 3 hot) and a burnin of 2 000. Four of these analyses were run, as well as an additional long run with the number of generations at 5 000 000.

Differences between the analyses for the different gene regions were as follows:

LSU sequence analysis: The dataset of Gryzenhout *et al*. (2006b) was used as template (TreeBASE SN2390). For the distance analyses, the Tamura-Nei (TrNef+I+G) model (Tamura & Nei 1993) was used with invariable sites (I) = 0.5582, Gamma distribution (G) = 0.6626, equal base frequencies, and rate matrix 1.0000, 4.0778, 1.0000, 1.0000, 8.8778, 1.0000. The same parameters were used for the

Bayesian analyses. Bootstrap analyses (50 % majority rule) were executed for both distance and parsimony methods, but for the parsimony analyses, multrees were inactive, the no branch swapping algorithm was used and only 10 random repeats were done, since the analyses would otherwise not have run to completion.

ITS/β-tubulin sequence analysis: a 500 replicate partition homogeneity test (PHT) was done on the rRNA and β-tubulin gene sequence data sets (after the exclusion of uninformative sites), to determine whether they could be analysed collectively (Farris *et al*. 1994). For the distance analyses, the Tamura-Nei model (TrN+G+I) (Tamura & Nei 1993) was used with G = 0.8312, I = 0.5187, base frequencies 0.1932, 0.3351, 0.2573, 0.2144, and rate matrix 1.0000, 3.1718, 1.0000, 1.0000, 5.6017, 1.0000. The same settings were used in the Bayesian analyses. A 70 % majority rule bootstrap tree was obtained using the same parameters as those for the parsimony analyses.

6.2.2 *Results of the LSU sequence analysis*: The complete dataset comprised of 655 characters and 81 taxa with *Magnaporthe grisea* (T.T. Herbert) Yaegashi & Udugawa, *Pyricularia grisea* (Cooke) Sacc. and *Gaeumannomyces graminis* (Sacc.) Arx & D. Oliver as outgroups. The TreeBASE accession number is SN**. Of the total number of characters, 466 were constant, 26 were uninformative and 163 were informative. The parsimony analyses yielded 18 trees (tree length (TL) = 216.38095, CI = 0.632, retention index (RI) = 0.887) that differed only because of variation inside the different lineages, especially within the *Cryphonectriaceae*. The tree obtained with the distance analysis was essentially the same as those obtained with parsimony. However, with parsimony analysis, the branch supporting the *Cryphonectriaceae* collapsed with the bootstrap analysis, as was also found by Castlebury *et al*. (2002)

and Gryzenhout *et al*. (2006b). Bayesian analyses, however, showed that the *Cryphonectriaceae* clade had a high (89 %) posterior probability.

The distance tree with bootstrap and posterior probability values is shown in Fig. 19. In this tree, the *Cryphonectriaceae* clearly represents a distinct lineage (bootstrap = 87 %, Bayesian posterior probability = 89 %), separate from the other lineages that represented other families in the *Diaporthales*. Many of the branches showing genera within the *Cryphonectriaceae* clade collapsed. This is because the data set on which this tree is based, is inordinately small and conserved to identify genera with certainty.

6.2.3 *Results of the ITS/β-tubulin sequence analysis*: The complete dataset comprised of 44 taxa with two isolates of *Diaporthe ambigua* Nitschke, as outgroup. The ITS dataset comprised of 590 characters (324 constant, 13 uninformative, 253 informative), the β-tubulin set of 1009 characters (531 constant, 26 uninformative, 452 informative) and the combined set amounted to 1599 characters. The dataset has been deposited with TreeBASE as SN**. A *P*-value of 0.036 shows that the two datasets were congruent.

Several trees were obtained from analyses of the ITS and β-tubulin sequence. Analyses of the β-tubulin and ITS data sets separately reflected essentially the same groupings and these are, therefore, not shown. These groups were also the same as those emerging from the analysis of the combined dataset. Because ambiguously aligned sequences in the introns of the β-tubulin genes and the highly variable ITS1 region could influence the groupings of the isolates, these were excluded and analyses were also done only on the more conserved exons and ITS2 region. The resultant tree (data not shown) showed the same well-supported groups as obtained with the tree for

the complete sequence. The trees obtained with distance, parsimony and Bayesian inference on the combined dataset showed the same groups, although in some cases the relationships between the groups differed.

One of the two trees obtained with parsimony analysis on the combined set (TL = 1268.50001, CI = 0.680, RI = 0.887) were chosen for presentation. The two trees differed only in the branch lengths within the *Chrysoporthe* clade. These trees clearly showed the different genera as well supported groups (Fig. 20). A probable undescribed genus is represented by the single isolate of *Cryptodiaporthe corni* (Wehm.) Petr., which have morphological characteristics similar to the *Cryphonectriaceae* and groups separately from other *Cryptodiaporthe* species (Gryzenhout *et al*. 2006b).

An isolate of *E. singularis* has been used in previous studies of groups within the *Cryphonectriaceae* (Venter *et al*. 2002, Myburg *et al.* 2004a). However, inclusion of this isolate usually results in incompatibility of the ITS and β-tubulin data sets because they show different evolutionary relationships (Gryzenhout *et al*. 2005d). The same problem was observed in phylogenetic analyses undertaken for this study (data not shown). The problem arises because the isolate of *E. singularis* in our collection shows an affinity with *E. gyrosa* based on ITS sequences, but the isolate has sequences in the β-tubulin region more similar to those of species in *Cryphonectria s. str.* This results in it grouping separately from any other clade. Because only one isolate representing this species is available and the sequence data for this isolate could not be verified with other representative isolates, it was excluded from our analyses. Where comparisons are desired, the sequences for this isolate can be obtained from Genbank (Myburg *et al*. 2004a).

Table 2. Isolates and DNA sequences used in the LSU sequence analysis.

Identification	Isolate number	Alternative number	Host	Country	*Sequence accession nr.* [a]
Amphilogia gyrosa	CMW10469	CBS112922	*Elaeocarpus dentatus*	New Zealand	AY194107
	CMW10470	CBS112923	*Elaeocarpus dentatus*	New Zealand	AY194108
Aurapex penicillata	CMW10030	CBS115740	*Miconia theaezans*	Colombia	**AY194103**
	CMW10032	–	*Miconia theaezans*	Colombia	**AY194104**
	CMW11295	–	*Miconia theaezans*	Colombia	**AY194089**
	CMW11296	CBS115801	*Miconia theaezans*	Colombia	**AY194090**
Celoporthe sp.	CMW14853	CBS534.82	*Eugenia aromatica*	Indonesia	AF277142
	CMW10781	CBS115844	*Eugenia aromatica*	Indonesia	AY194093
Chrysoporthe austroafricana	CMW62	–	*Eucalyptus grandis*	South Africa	AY194097
Chrysoporthe cubensis	CMW8758	–	*Eucalyptus* sp.	Indonesia	AY194098
	CMW10453	CBS505.63	*Eucalyptus saligna*	Demographic Republic of Congo	AF408339
	–	–	*Eucalyptus urophylla*	Cameroon	AF408338
Cryphonectria parasitica	CMW7048	ATCC48198	*Quercus virginiana*	USA	AY194100
	–	S. Anagnostakis 713	*Castanea* sp.	n/a	AF277132
Cryphonectria nitschkei	CMW10786	–	*Quercus* sp.	Japan	AY194099
	CMW10527	CBS109776	*Quercus mongolica*	Russia	AF408341
	CMW10528	CBS109764	*Quercus mongolica*	Russia	AF408340
Cryphonectria radicalis	CMW10455	CBS238.54	*Quercus suber*	Italy	AY194101

	CMW10477	CBS240.54	*Quercus suber*	Italy	AY194102
Cryptodiaporthe corni	CMW10526	CBS245.90	*Cornus alternifolia*	Maine, U.S.A.	AF408343
	–	ATCC66834	*Cornus alternifolia*	Maine, U.S.A.	AF277133
Endothia gyrosa	CMW2091	ATCC48192	*Quercus palustris*	U.S.A.	AY194114
	CMW10442	CBS118850	*Quercus palustris*	U.S.A.	AY194115
	–	–	*Quercus sp.*	Maryland (U.S.A.)	AF362555
Holocryphia eucalypti	CMW7036	CBS**	*Eucalyptus delegatensis*	Australia	**AY194105**
	CMW7037	–	*Eucalyptus* sp.	South Africa	**AY194106**
Microthia havanensis	CMW11298	–	*Eucalyptus* sp.	Mexico	**AY194091**
	CMW11299	–	*Myrica faya*	Madeira	**AY194087**
	CMW11300	–	*Myrica faya*	Madeira	**AY194088**
Rostraureum tropicale	CMW9972	–	*Terminalia ivorensis*	Ecuador	AY194092
Ursicollum fallax	CMW18119	CBS118663	*Coccoloba uvifera*	Florida (U.S.A.)	****

[a] Isolates in bold were sequenced in this study, while others were obtained from previous studies (Zhang & Blackwell 2001, Castlebury *et al.* 2002, Gryzenhout *et al*. 2006b).

Table 3. Representative isolates (ex-type isolates in bold) and representative sequences of the ITS region and β-tubulin genes for species of *Cryphonectria* and related genera.

Genus	Species	Reference	Representative isolates [a]	Representative sequences [b]
Amphilogia	*A. gyrosa*	Myburg *et al*. 2004a	**CMW10469 = CBS112922**	AF452111, AF525707, AF525714
			CMW10740 = CBS112923	AF452112, AF525708, AF525715
Aurapex	*A. penicillata*	Gryzenhout *et al*. 2006e	**CMW10030 = CBS115740**	AY214311, AY214239, AY214275
			CMW10035 = CBS115742	AY214313, AY214241, AY214277
Celoporthe	*Celoporthe* sp.	Myburg *et al.* 2003	CMW10780	AY084008, AY084020, AY084032
			CMW10781 = CBS115844	AY084009, AY084021, AY084033
	Ce. dispersa	Nakabonge *et al*. 2006a	**CMW9976 = CBS118782**	DQ267130, DQ267136, DQ267142
			CMW9978 = CBS118781	AY214316, DQ267135, DQ267141
Chrysoporthe	*Chr. austroafricana*	Myburg *et al.* 2002a	**CMW2113 = CBS112916**	AF046892, AF273067, AF273462
		Myburg *et al.* 2002b	CMW9327 = CBS115843	AF273473, AF273060, AF273455
	Chr. cubensis	Gryzenhout *et al*. 2004	CMW10639 = CBS115747	AY263419, AY263420, AY263421
		Myburg *et al*. 2003	CMW10669 = CBS115751	AF535122, AF535124, AF535126
			CMW8651 = CBS115718	AY084002, AY084014, AY084026
			CMW11290 = CBS115738	AY214304, AY214232, AY214268
	Chr. doradensis	Gryzenhout *et al.* 2005c	**CMW11286 = CBS115734**	AY214289, AY214217, AY214253
			CMW11287 = CBS115735	AY214290, AY214218, AY214254
	Chr. inopina	Gryzenhout *et al*. 2006c	**CMW12727 = CBS118659**	DQ368777, DQ368806, DQ368807
			CMW12729 = CBS118658	DQ368778, DQ368808, DQ368809
	Chrysoporthella	Gryzenhout *et al*. 2004	CMW10625 = CBS115744	AY956970, AY956979, AY956980
	hodgesiana	Rodas *et al*. 2005	**CMW10641 = CBS115854**	AY692322, AY692326, AY692325
Cryphonectria	*C. parasitica*	Venter *et al.* 2002	CMW7048= ATCC48198	AF368330, AF273076, AF273470

		Myburg *et al*. 2004b	CMW13749 = MAFF410158	AY697927, AY697943, AY697944
	C. macrospora	Myburg *et al*. 2004a	CMW10463 = CBS112920	AF368331, AF368351, AF368350
		Myburg *et al*. 2004b	CMW10914 = TFM: FPH E55	AY697942, AY697973, AY697974
	C. nitschkei	Myburg *et al*. 2004b	CMW13742 = MAFF410570	AY697936, AY697961, AY697962
			CMW13747 = MAFF410569	AY697937, AY697963, AY697964
	C. radicalis s. l.	Venter *et al.* 2002	CMW10455 = CBS238.54	AF452113, AF525705, AF525712
		Myburg *et al*. 2004a	CMW10477 = CBS240.54	AF368328, AF368347, AF368346
			CMW10436 = CBS165.30	AF452117, AF525703, AF525710
			CMW10484 = CBS112918	AF368327, AF368349, AF368349
			CMW13754 = MAFF410152	AY697932, AY697953, AY697954
Cryptodiaporthe corni	*Crypto. corni*	Gryzenhout *et al.* 2006b	CMW10526 = CBS245.90	DQ120762, DQ120769, DQ120770
Endothia	*E. gyrosa*	Venter *et al.* 2002	CMW2091 = ATCC48192	AF046905, AF368337, AF368336
		Myburg *et al*. 2004a	CMW10442 = CBS118850	AF368326, AF368339, AF368338
	E. singularis [c]	Myburg *et al*. 2004a	CMW10465 = CBS112921	AF 368323, AF 368333, AF 368332
Holocryphia	*H. eucalypti*	Venter *et al*. 2002	CMW7036 = CBS***	AF232878, AF368341, AF368340
			CMW7037 = CBS***	AF232880, AF368343, AF368342
Microthia	*M. havanensis*	Gryzenhout *et al*. 2006d	CMW14550 = CBS115855	DQ368735, DQ368741, DQ368742
			CMW11301	AY214323, AY214251, AY214287
Rostraureum	*R. tropicale*	Gryzenhout *et al.* 2005d	CMW9971 = CBS115725	AY167425, AY167430, AY167435
			CMW10796 = CBS115757	AY167428, AY167433, AY167438
Ursicollum	*U. fallax*	Gryzenhout *et al*. 2006d	CMW18115 = CBS118660	DQ368756, DQ368760, DQ368761
			CMW18119 = CBS118663	DQ368755, DQ368758, DQ368759

[a] **CMW**, Research collection of the Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria; **ATCC**, American Type Culture Collection, Manassas, USA; **CBS**, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; **TFM:FPH**, Forestry and Forest Products Research Institute, Danchi-Nai, Ibaraki, Japan (E or Ep refers to an isolate); **MAFF**, Microorganisms Section, MAFF GENEBANK, National Institute of Agrobiological Sciences (NIAS), Ibaraki, Japan. [b] Accesssion numbers given as sequences from the ITS region, and two regions from the β-tubulin genes amplified with primers 1a/1b and 2a/2b, respectively. [c] The isolate of *E. singularis* was not included in the phylogenetic analyses.

Fig. 19. Phylogram based on neighbor-joining analysis of LSU DNA sequences for various genera in the *Diaporthales*. Bootstrap (50% majority rule) and posterior probability values (in bold face) are indicated for the branches representing the different families. *Magnaporthe grisea* (AB026819), *Pyricularia grisea* (AF362554) and *Gaeumannomyces graminis* (AF362557) represent the outgroups. The DNA sequence matrix of Gryzenhout *et al*. (2006b) was used as template, while sequences with AF numbers originated from Zhang & Blackwell (2001) and Castlebury *et al*. (2002).

Fig. 20. Phylogenetic tree obtained with parsimony from a combined DNA sequence dataset of the ITS1, 5.8S rRNA gene and ITS2 regions of the ribosomal operon, and β-tubulin genes. Bootstrap confidence levels (> 70 %) and posterior probabilities (in bold typeface) are indicated on the branches, and those branches representing genera are marked with a dot. *Cryphonectria radicalis s. str.* is shown in a block. The outgroup taxon used in the tree is *Diaporthe ambigua.*

6.3 Dichotomous key to genera

This key is based on characteristics of both the anamorph and teleomorph.

1a. Orange conidiomata ……...…….. 2

1b. Black conidiomata ……...……. 8

2a. Conidiomata pulvinate, ascospores septate or aseptate …… 3

2b. Conidiomata conical or rostrate or pyriform, with or without a neck, ascospores septate …… 6

3a. Ascospores septate ……... 4

3b. Ascospores aseptate …….. 5

4a. Strongly developed, erumpent, semi-immersed stromata, no paraphyses ……..…... ***Cryphonectria*** (p. 454)

4b. Small to medium stromata, semi-immersed to superficial, paraphyses present ……...… ***Microthia*** (p. 479)

5a. Large, erumpent, mostly superificial, strongly developed stromata, numerous conidial locules, no paraphyses in conidial locules .....…..… ***Endothia*** (p. 490)

5b. Small to medium stromata, semi-immersed stromata, few conidial locules of one convoluted locule, paraphyses in conidial locules … ***Holocryphia*** (p. 501)

6a. Conidiomata with necks, ascospores single septate ……...…… 9

6b. Conidiomata conical without attenuated necks, ascospores 1 to 3-septate ...……. ***Amphilogia*** (p. 506)

7a. Conidiomata rostrate, white sheath of tissue surrounding perithecial necks when sectioned longitudinally ……..... ***Rostraureum*** (p. 515)

7b. Conidiomata rostrate to pyriform with large base, neck attenuated or not, teleomorph still unknown ……..……...…….. ***Ursicollum*** (p. 523)

8a. Conidiomata uniformaly black ……. 9

8b. Conidiomata with orange neck ……..…….. ***Aurapex*** (p. 527)

9a. Conidiomata pulvinate to pyriform with attenuated neck, base tissue of *textura globulosa* when sectioned longitudinally, perithecial necks long and covered with dark tissue ……... ***Chrysoporthe*** (p. 532)

9b. Conidiomata pulvinate or conical, occasionally with short necks, base tissue prosenchymatous, perithecial necks short and of same colour than stroma ……. ***Celoporthe*** (p. 562)

6.4 Synoptic key to genera

This synoptic key is useful when certain key characteristics, which are essential when using the dichotomous key, may be missing from a sample. This key should also be useful where certain characters, e.g. stromatal shape, can be interpreted in different ways. Hence some genera are included under more than one characteristic. The synoptic key should also fascilitate identification in the absence of one of the sexual states. As many characters as possible should be used when identifying a specimen with this synoptic key.

STROMATIC CHARACTERISTICS:

Teleomorph colour:

a. Orange stromata: *Cryphonectria*, *Microthia*, *Endothia*, *Holocryphia*, *Amphilogia*, *Rostraureum*, *Chrysoporthe*

b. Stromata not orange: *Chrysoporthe*, *Celoporthe*

c. Orange perithecial necks or having the same colour as the stromata: *Cryphonectria*, *Microthia*, *Endothia*, *Holocryphia*, *Amphilogia*, *Rostraureum*, *Celoporthe*

d. Black perithecial necks or of a different colour than stromata: *Chrysoporthe*

Anamorph colour:

a. Orange conidiomata: *Cryphonectria*, *Microthia*, *Endothia*, *Holocryphia*, *Amphilogia*, *Rostraureum*, *Ursicollum*

c. Black conidiomata: *Aurapex*, *Chrysoporthe*, *Celoporthe*

d. Conidiomata both orange and black: *Aurapex*

Position in bark:

1. Ascostroma:

a. Superficial: *Microthia*, *Endothia*, *Amphilogia*

b. Semi-immersed: *Cryphonectria*, *Microthia, Holocryphia*, *Amphilogia*, *Rostraureum*, *Chrysoporthe*, *Celoporthe*

c. Ascostromata and conidiomata of different shape: *Amphilogia*, *Rostraureum*, *Chrysoporthe*, *Celoporthe*

d. Ascostromata and conidiomata of different colour: *Chrysoporthe*, *Celoporthe*

e. Ascostromata and conidiomata of same shape and colour: *Cryphonectria*, *Microthia*, *Endothia*, *Holocryphia*

2. Conidiomata:

a. Superficical: *Microthia*, *Endothia*, *Amphilogia*, *Rostraureum*, *Ursicollum*, *Aurapex*, *Chrysoporthe*, *Celoporthe*

b. Semi-immersed: *Cryphonectria*, *Microthia, Holocryphia*, *Ursicollum*

Anamorph shape:

a. Pulvinate: *Cryphonectria*, *Microthia*, *Endothia*, *Holocryphia*, *Ursicollum*, *Chrysoporthe*, *Celoporthe*

b. Pyriform: *Amphilogia*, *Ursicollum*, *Aurapex*, *Chrysoporthe*

c. Rostrate: *Rostraureum*, *Ursicollum*

d. Conical or other: *Amphilogia*, *Celoporthe*

e. Without a neck: *Cryphonectria*, *Microthia*, *Endothia*, *Holocryphia*, *Amphilogia*, *Celoporthe*

f. With a neck: *Rostraureum*, *Ursicollum*, *Aurapex*, *Chrysoporthe*, *Celoporthe*

Conidiomatal stromatic tissue:

a. Stromatic tissue similar to teleomorph: *Cryphonectria*, *Microthia*, *Endothia*, *Holocryphia*, *Amphilogia*

b. Stromatic tissue different from teleomorph: *Rostraureum*, *Chrysoporthe*, *Celoporthe*

c. Prosenhyma and pseudoparenchyma: *Cryphonectria*, *Microthia*, *Endothia*, *Holocryphia*, *Amphilogia*, *Ursicollum*, *Celoporthe*

d. Of different textura types (e.g. *textura globulosa* and *textura porrecta*): *Rostraureum*, *Aurapex*, *Chrysoporthe*

e. *Textura intricata* present: *Rostraureum*

Conidial locules:

a. Labyrinthiform or innumerous, small relative to stroma: *Endothia*

b. Locules large relative to stroma, one to few locules: *Cryphonectria*, *Microthia*, *Endothia*, *Holocryphia*, *Amphilogia*, *Rostraureum*, *Ursicollum*, *Chrysoporthe*, *Celoporthe*

c. Locules containing brush-like protrusions: *Aurapex*

d. Paraphyses visible under 40X magnification: *Microthia*, *Holocryphia*

SPORE CHARACTERISTICS:

Ascospore septation

a. Aseptate ascospores: *Endothia*, *Holocryphia*

b. Single septate ascospores: *Cryphonectria*, *Microthia*, *Amphilogia*, *Rostraureum*, *Chrysoporthe*, *Celoporthe*

c. Multiseptate ascospores: *Amphilogia*

Conidial shape

a. Cylindrical: *Cryphonectria*, *Microthia*, *Endothia*, *Holocryphia*, *Amphilogia*, *Rostraureum*, *Aurapex*, *Celoporthe*, *Ursicollum*

b. Oblong: *Chrysoporthe*

c. Spores of variable size: *Amphilogia*

PARAPHYSES AND PERIPHYSES

a. Paraphyses or cylindrical, seemingly sterile cells present: *Microthia*, *Holocryphia*

b. Periphyses in conidiomatal neck: *Aurapex*

6.5 Descriptions

Cryphonectriaceae Gryzenh. & M.J. Wingf., **nom. prov.**, Mycologia 98 (in press). 2006.

Stromata semi-immersed to superficial, with orange stromatic tissue either in the teleomorph and/or the anamorph. Perithecia in the ascostroma fuscous black to umber, globose to triangular, perithecial necks slender, covered with orange to fuscous-black stromatic tissue, periphysate. Asci fusoid, aparaphysate, free floating, stipitate when immature, unitunicate with non-amyloid, refractive apical rings. Ascospores generally ellipsoidal to fusoid to cylindrical, aseptate to multiseptate, hyaline. Conidiomata eustromatic, semi-immersed to superficial, pyriform to pulvinate, orange to fuscous black, occasionally occurring in the same stroma as perithecia. Conidiophores simple or branched, conidiogenous cells phialidic, determinate, apical or lateral on branches, collarette and periclinal thickening inconspicous. Conidia minute, generally ovoid to cylindrical, aseptate, hyaline. Stromatic tissue colours purple in 3 % KOH and yellow in lactic acid.

Typus genus: *Cryphonectria* (Sacc.) Sacc.

Notes: The distinct grouping for what we now recognize as the *Cryphonectriaceae* was first recognized in a phylogenetic study of different families within the *Diaporthales* (Castlebury *et al*. 2002). This study was based on DNA sequence analyses of the LSU region. Isolates of *Cryphonectria*, *Endothia* and *Chrysoporthe* formed a distinct group in the order (Castlebury *et al*. 2002), even after the inclusion of additional genera and species (Gryzenhout *et al*. 2006b). This grouping was

unexpected as *Cryphonectria* and related genera had previously been thought to group in the *Valsaceae* (Hawksworth *et al*. 1995). The characteristic orange stromatic tissue found in many of the genera as well as the tissue pigments that give the distinct colour reactions in KOH and lactic acid represent the most important distinguishing characteristics of this new family.

Cryphonectria (Sacc.) Sacc., Syll. Fung. 17: 783. 1905, *nom. cons.*

Anamorph: Endothiella Sacc.

Etymology: Greek, *crypho-*, secret, meaning hidden *Nectria*

Ascostromata large, pulvinate, erumpent, semi-immersed in bark, orange, upper region eustromatic**,** lower region pseudostromatic, pseudoparenchyma on edge of stroma, prosenchyma in centre. *Perithecia* usually valsoid, fuscous black, embedded beneath surface of bark at base of stromata, necks emerge at stromatal surface as black ostioles covered with orange stromatal tissue to form papillae. *Asci* fusiform to ellipsoidal to sub-clavate. *Ascospores* hyaline, ellipsoidal to fusoid, one median septum.

Conidiomata part of ascostromata as conidial locules or separate structures, orange, pulvinate, semi-immersed, uni- to multilocular, non-ostiolate, with same tissue structure as ascostromata. *Conidiophores* cylindrical or flask-shaped, occasionally with separating septa and branching, *conidiogenous cells* phialidic. *Conidia* minute, hyaline, cylindrical, aseptate, exuded as orange droplets.

Typus: *Cryphonectria parasitica* (Murrill) M.E. Barr.

Notes: *Cryphonectria* and *Endothia* are the best known genera in the *Cryphonectriaceae*. This is because these were the original genera that contained fungi with orange stromata in the *Diaporthales* (Barr 1978). *Cryphonectria* consisted of all of the fungi with single septate ascospores, while *Endothia* consisted of fungi with aseptate ascospores (Barr 1978). The recent taxonomic revisions have divided species of *Cryphonectria* into numerous new genera but restricted species with single-septate ascospores, large and erumpent, semi-immersed stromata and orange, pulvinate conidiomata to *Cryphonectria* (Myburg *et al*. 2004a, Gryzenhout *et al*. 2006a). Species having other characteristics have been transferred to numerous new genera. The species retained in *Cryphonectria* are *C. parasitica*, *C. radicalis*, *C. nitschkei* and *C. macrospora*.

Endothiella Sacc., anam. gen., Ann. Mycol. 4: 273. 1906. **emend.** Gryzenh. & M.J. Wingf.

= *Calopactis* Syd., Ann. Mycol. 10: 82. 1912.

Etymology: dimunitive of *Endothia*

Conidiomata part of ascostromata as conidial locules or separate structures, orange, pulvinate, semi-immersed, uni- to multilocular, non-ostiolate, with same tissue structure as ascostromata. *Conidiophores* cylindrical or flask-shaped, occasionally with separating septa and branching, *conidiogenous cells* phialidic. *Conidia* minute, hyaline, cylindrical, aseptate, exuded as orange droplets.

Typus: Endothiella gyrosa Sacc.

Notes: Endothiella has always been considered the anamorph of *Endothia*, but it has also been used for species of *Cryphonectria* (Kobayashi 1970, Barr 1978, Roane 1986a). This anomaly most likely stems from the long-standing treatment of *Cryphonectria* to *Endothia* as synonyms and the use of *Endothiella* anamorph names for *Cryphonectria* species by Roane (1986a) and Kobayashi (1970). The type of *Endothiella*, *Endothiella gyrosa*, was always thought to be the anamorph of *E. gyrosa*, but *Endothiella gyrosa* was described at a time when *C. radicalis* and *E. gyrosa* was treated as synonyms (Shear *et al*. 1917). Furthermore, the type specimen of *Endothiella gyrosa* (PAD) is of European origin and contains fruiting structures that clearly represent the anamorph of *C. radicalis s. l.* These include conidiomata that do not have labirinthiform conidial locules. The few teleomorph structures present also have 1-septate ascospores [(9–)9.5–11.5(–12.5) ×3.5–4.5 µm]. Therefore, *Endothiella gyrosa* should strictly represent the anamorph of *C. radicalis* and *Endothiella* is thus the anamorph genus for species of *Cryphonectria*.

We have chosen not to describe an anamorph for *Endothia*. This follows the recommendation of the International Code of Botanical Nomenclature (recommendation 59.A.3, Greuter *et al.* 2000) that anamorph names should not unnecessarily be described if the teleomorph is well known. Anamorph names in *Endothiella* have been allocated to *Cryphonectria* spp. in the past (Roane 1986a, Kobayashi 1970) and these are noted in the following species descriptions. However, these names need not be used when referring to the fungus when only anamorph structures are present, as the teleomorph name also represents the holomorph.

Ascospore length can be used with confidence to identify *Cryphonectria* spp. (Kobayashi 1970, Roane 1986a, Myburg *et al*. 2004b). This is largely based on

ascospore length but ascospore width is also an important characteristic for *C. radicalis s. l.* Conidial size is fairly uniform between species and cannot be used to accurately distinguish between the species, except for *C. nitschkei* that has longer conidia.

Table 4 summarises ascospore and conidial sizes for species of *Cryphonectria*. Species are arranged in order of those having the longest to those with the shortest ascospores. The most characteristic sizes are presented in bold typeface. Additional characteristics that are unique to some species are also mentioned. Two morphological groups currently representing *C. radicalis s. l.* are also included in the table.

Table 4. Morphological characteristics distinguishing *Cryphonectria* spp.

Species	Ascospore length	Ascospore width	Conidial length	Other characters
C. macrospora	**14–17**(–19)	(4.5–)**5.5–7**(–8)	3.5–4.5(–5)	Long sterile conidiophores
C. nitschkei	(8–)**9.5–11.5**(–12.5)	(3–)3.5–4.5(–5)	(3–)3.5–**5(–6)**	Occasional clavate conidiogenous cells, colours growth medium purple
C. parasitica	(7.5–)**8–9**(–9.5)	3.5–4(–4.5)	(3–)3.5–4(–4.5)	Mycelial fans in wood
C. radicalis "longer ascospores"	(7–)**8–10**(–12)	(2–)**2.5–3.5**(–4)	(3–)3.5–4(–4.5)	No yellow cell lining around locules
C. radicalis s. str.	(5.5–)**6–7.5**(–8.5)	**2.5–3.5**	(3–)3.5–4(–4.5)	Yellow cell lining around locules; colours growth medium purple

1. ***Cryphonectria parasitica*** (Murrill) M.E. Barr, Mycologia Mem. No. 7: 143. 1978. Fig. 21.

Basionym: Diaporthe parasitica Murrill, Torreya 6: 189. 1906.

Anamorph: *Endothiella parasitica* Roane, Chestnut blight, other *Endothia* diseases, and the genus *Endothia*, APS Press: 38. 1986.

≡ *Valsonectria parasitica* (Murrill) Rehm, Ann. Mycol. 5: 210. 1907.

≡ *Endothia gyrosa* var. *parasitica* (Murrill) Clinton, Science 36: 913. 1912.

≡ *Endothia parasitica* (Murrill) P.J. Anderson & H.W. Anderson, Phytopathology 2: 262. 1912.

≡ *Endothia radicalis* subsp. *parasitica* (Murrill) Orsenigo, Phytopathology Z 18: 215. 1951.

Etymology: Latin, *parasiticus*, parasitic

Ascostromata on host gregarious or single, sometimes confluent, pulvinate, semi-immersed in bark, orange, 200–350 µm high, 300–1200 µm diam, upper region eustromatic**,** lower region pseudostromatic, pseudoparenchyma on edge of stroma, prosenchyma in centre. *Perithecia* usually valsoid, embedded beneath surface of bark at base of stromata, fuscous black, fuscous black, necks emerge at stromatal surface as black ostioles covered with orange stromatal tissue to form papillae extending up to 150 µm above stromatal surface. *Asci* (30–)40–50(–60) × (7–)8(–9) µm, oblong ellipsoidal to sub-clavate (Shear *et al.* 1917, Roane 1986a), 8-spored. *Ascospores* (7.5–)8–9(–9.5) × 3.5–4(–4.5) µm, hyaline, ellipsoidal to fusoid, ends round, one median septum.

Conidiomata part of ascostromata as conidial locules, or separate structures, pulvinate, semi-immersed, orange, uni- to multilocular structures with same tissue

structure as ascostromata, 120–390 µm high, 270–390 µm diam, with locules often convoluted, non-ostiolate. *Conidiophores* (10.5–)12–23(–34) µm long, cylindrical or flask-shaped with attenuated apices, occasionally with separating septa and branching, hyaline, *conidiogenous cells* 1.5–2(–2.5) µm wide. *Conidia* (3–)3.5–4(–4.5) × 1–1.5 µm, hyaline, cylindrical, aseptate, exuded as orange droplets.

Cultural characteristics: Grows optimally at 25 ºC, but growth also proliferous at 20 and 30 ºC (Shear *et al*. 1917). On MEA cultures are white with occasional orange discolouration. Further references to the cultural morphology of *C. parasitica* on different media can be found in Shear *et al.* (1917) and Kobayashi (1970).

Substratum: Bark of *Castanea* and *Quercus* spp. (*Fagaceae*, *Fagales*).

Distribution: Japan, China, North America, Europe, Turkey.

Specimens examined: **U.S.A.**, New York, Bronx Park, *Castanea dentata*, 26 Nov. 1905, W.A. Murrill, **holotype** NY; Bronx Park, *Castanea dentata*, Dec. 1907, W.A. Murrill, CUP 2926. **Japan**, Tokyo, Tsurukawa, *Castanea crenata*, Sep. 1953, T. Kobayashi, TFM:FPH 1326; Tokyo, Koganei, *Castanea crenata*, Sep. 1953, T. Kobayashi, TFM:FPH 629; Chiba, Matsudo, *Castanea crenata*, Jul. 1953, T. Kobayashi, TFM:FPH 608, living culture CMW 10916 = TFM:FPH Ep4; Gifu, Seki, *Castanea crenata*, Oct. 1953, T. Kobayashi, TFM:FPH 600, TFM:FPH 605; Tokyo, Inagi, *Castanea crenata*, May 1953, T. Kobayashi, TFM:FPH 351, living culture CMW 13751 = MAFF 410160; Kanagawa, Yokohama, *Castanea crenata*, 28 Sept. 1953, T. Kobayashi, TFM:FPH 594.

Notes: *Cryphonectria parasitica* has ascospores that are typically 8–9 µm long. This overlaps with ascospore sizes of *C. radicalis s. l.*, but the ascospores of *C. parasitica*

is slightly wider. This fungus also forms unique mycelial fans underneath the bark of infected trees (Shear *et al*. 1917, Griffin & Elkins 1986). These fans are not known for other species (Roane 1986a) and this characteristic fascilitates identification of the fungus in the field.

Cryphonectria parasitica is one of the best known and most intensively studied fungi. This is because it causes chestnut blight that is one of the most important plant diseases in the world. For this reason, it has been chosen to represent the amended type for *Cryphonectria*, after it was shown that the presumed type, *C. gyrosa*, did not represent the genus (Gryzenhout *et al*. 2005a). The name *Cryphonectria* is now firmly tied to *C. parasitica*, which is a well known and commonly encountered fungus for which the name should be unequivocal.

Cryphonectria parasitica has been linked to cankers on *Quercus* spp. as well as on *Castanea* (Peace 1962, Sinclair *et al*. 1987). Besides *Castanea* and *Quercus* spp., Roane (1986a) also mentions numerous other hosts, including *Acer*, *Carpinus*, *Carya*, *Castanopsis*, *Fagus*, *Liriodendron*, *Ostrya* and *Rhus*. It is unclear where these hosts have been found for *C. parasitica*. Due to the morphological similarities between the different species of *Cryphonectria*, and because some of these hosts are also hosts of other *Cryphonectria* species or the morphologically similar *E. gyrosa*, it is likely that fungi on these hosts could have been erroneously identified as *C. parasitica*.

Fig. 21. Fruiting structures of *Cryphonectria parasitica*. A. Ascostroma on bark. B. Longitudinal section through ascostroma. C. Pseudoparenchymatous stromatic tissue. D. Conidioma on bark. E. Longitudinal section through conidioma. F. Prosenchymatous stromatic tissue. G. Ascus. H. Ascospores. I. Conidiophores. J. Conidia. Scale bars A–B, D–E = 200 µm; C, F = 20 µm; G–J = 10 µm.

2. ***Cryphonectria radicalis*** (Schwein.: Fr.) M.E. Barr., Mycologia Mem. No. 7: 144. 1978. Fig. 22.

Basionym: Sphaeria radicalis Schwein.: Fr., Elenchus Fung. 2: 73. 1828.

Anamorph: Endothiella fluens (Sowerby) Tak. Kobay., Bull. Govt. For. Exper. Stat. 226: 136. 1970.

≡ *Valsa radicalis* Ces. & De Not., Comm. Soc. Crittog. Ital. 1: 207. 1863.

≡ *Endothia radicalis* (Schwein.: Fr.) Ces. & De Not., Comm. Soc. Crittog. Ital. 1: 240. 1863.

≡ *Melogramma gyrosum* Tul. & C. Tul., Selecta Fung. Carpol. 2: 87. 1863.

≡ *Endothia gyrosa* (Schwein.: Fr.) Fuckel, Syll. Fung. 1: 601. 1882.

Endothia gyrosa Schwein., Ell. & Ev., No. Amer. Pyren.: 552. 1892.

≡ *Endothia virginiana* P.J. Anderson & H.W. Anderson, Phytopathology 2: 261. 1912.

≡ *Endothia fluens* (Sowerby) Shear & N.E. Stevens, U. S. Dept. Agric. Bull. 380: 16–19. 1917.

≡ *Endothia radicalis* subsp. *aflabellata* Orsenigo, Phytopathology Z 18: 214. 1951.

≡ *Endothia radicalis* (Schwein.: Fr.) Ces. & De Not., Chestnut blight, other *Endothia* diseases, and the genus *Endothia*, APS Press: 36. 1986.

Etymology: Latin, *radicalis*, radical, basal or from a root.

Ascostromata on host gregarious or single, sometimes confluent, pulvinate, semi-immersed in bark, orange, 180–360 µm high, 270–1030 µm diam, upper region eustromatic**,** lower region pseudostromatic, pseudoparenchyma on edge of stroma, prosenchyma in centre. *Perithecia* usually valsoid, embedded beneath surface of bark

at base of stromata, fuscous black, necks emerge at stromatal surface as black ostioles covered with orange stromatal tissue to form papillae extending up to 220 µm above stromatal surface. *Asci* 30–35(–40) × (6–)7(–8) µm long, fusiform or sub-clavate (Shear *et al*. 1917, Roane 1986a), 8-spored. *Ascospores* (5.5–)6–7.5(–8.5) × 2.5–3.5 µm, hyaline, ellipsoidal to fusoid, ends round, one median septum .

Conidiomata part of ascostromata as conidial locules, or separate structures, pulvinate, semi-immersed, uni- to multilocular structures, having the same tissue arrangement as ascostromata, 280–390 µm high, 290–520 µm diam with locules often convoluted, non-ostiolate. Cells surrounding conidial locules forming luteous cell layer in contrast to orange stromatic tissue, pseudoparenchymatous. *Conidiophores* (13–)15.5–31.5(–35.5) µm long, cylindrical or flask-shaped with attenuated apices, occasionally with separating septa and branching, hyaline, *conidiogenous cells* 1.5–2 µm wide. Conidia (3–)3.5–4(–4.5) × 1–1.5 µm, hyaline, cylindrical, aseptate, exuded as orange droplets.

Cultural characteristics: Grows optimally at 25 ºC, but growth also proliferous at 20 ºC (Shear *et al*. 1917). Cultures currently in our possession have been extensively cultured and could not be be used with confidence to determine the true cultural morphology of the species. Reference to cultural characteristics on various media can be found in Shear *et al*. (1917) and Kobayashi (1970).

Substrate: Bark of *Quercus*, *Castanea*, *Fagus* (*Fagaceae*, *Fagales*), *Carpinus*, *Alnus* (*Betulaceae*, *Fagales*).

Distribution: Japan, Greece, Italy, Switzerland, France, Portugal, U.S.A.

Specimens examined: **U.S.A.**, bark of possibly a *Quercus* sp., 1828, L.D. von Schweinitz, **holotype** K 109808; Connellsville, chestnut stump, 1912, P.J. Anderson

and H.W. Anderson, CUP 6178; Glatfelter, *Quercus* sp., 1913, C.L. Shear and N.E. Stevens, NY 1963. **Italy**, Como, 1912, C.L. Shear, BPI 612660; Stresa, *Castanea sativa*, 1913, C.L. Shear, BPI 613739; Rome, *Castanea* sp., 1877, Prof. Liropoli, BPI 797696; Sciolze, 1873, BPI 797698; Como, *Castanea* sp., BPI 797695. **Switzerland**, Etremblieres, *Castanea sativa*, 1913, C.L. Shear, BPI 612672; Locarno, *Castanea sativa*, 1862, BPI 797697; Locarno, *Castanea* sp., 1862, G. de Notaris, BPI 797693; Locarno, *Castanea* sp., 1862, Daldini, BPI 797694. **France**, Bois Bastard PAU 64, *Quercus* sp., Nov. 1991, F. Candoussau, BPI 1112743. **Abkehazia**, *Carpinus betulus*, Woronin, BPI 797692. **Japan**, Tokyo, Meguro, *Quercus variabilis*, Aug. 1953, T. Kobayashi, TFM:FPH 1200; Tokyo, Machida, *Quercus serrata*, Apr. 1954, T. Kobayashi, TFM:FPH 1072; Tokyo, Meguro, *Fagus japonica*, Oct. 1953, T. Kobayashi, TFM:FPH 1202, living culture CMW 13754 = MAFF 410152; Kanagawa, Komayama, *Quercus salicina*, May 1959, T. Kobayashi, TFM:FPH 2483; Shizuoka, Nishina, *Alnus firma*, Jul. 1955, T. Kobayashi, TFM:FPH 601; Tokyo, Asakawa, *Carpinus japonica*, Sept. 1962, T. Kobayashi, TFM:FPH 652.

Notes: Analyses of DNA sequence data have shown clearly that isolates of *C. radicalis* from Europe reside in two distinct sub-clades (Myburg *et al*. 2004a, 2004b). Two groups of *C. radicalis* specimens from Europe that differ in ascospore length [(7–)8–10(–12) µm and (5.5–)6–7.5(–8.5) µm respectively], have also been independantly characterised (Myburg *et al*. 2004b). These morphological groups could not be linked directly to the phylogenetic groups since no isolates were connected to the available specimens. Based on published measurements of Heiniger *et al.* (2002), one sub-clade appears to represent the taxon having ascospores up to 8 µm long (Myburg *et al.* 2004b). This group also corresponds with ascospore

measurements for the type specimen of *C. radicalis* from the U.S.A. This group has tentatively been assigned to *C. radicalis s. str.* (Myburg *et al.* 2004b). The other morphological group with ascospores of 8–10 µm and possibly reflecting the second phylogenetic group will be described when the disparity between the unlinked specimens and isolates can be resolved.

No isolates are linked to the type of *C. radicalis* from North America, and no other isolates exist for this fungus from North America (Myburg *et al*. 2004b). It was, therefore, not possible to confirm that the fungus named *C. radicalis* in Europe definitely represents this species. There are also reports of a second species similar to *C. radicalis*, namely *C. radicalis* var. *mississippiensis* Shear & N.E. Stevens, from the U.S.A. (Shear *et al*. 1917). This species was noticably different in culture from other *C. radicalis* isolates, although no other distint morphological characteristics could be observed (Shear *et al*. 1917). Based on these uncertainties, the description and illustrations presented in this monograph are based only on the type specimen (K 109808) from the U.S.A. This is despite the fact that the specimens from Europe and Japan residing in the *C. radicalis s. str.* group have been listed.

Specimens of *C. radicalis s. l.* can be distinguished from those of other *Cryphonectria* species based on their thinner ascospores, and their respective ascospore lengths. *Cryphonectria radicalis s. str.* also has an interesting morphological feature not present in other species of *Cryphonectria*. The lining of the conidial locules consists of luteous cells that are quite distinctive (Myburg *et al*. 2004b). This luteous lining is present in collections from North America, Japan and Europe that we treat as *C. radicalis sensu strictu*, but it is absent in the the second group occurring in Europe that has longer ascospores (Myburg *et al*. 2004b). This feature may be useful in identifying *C. radicalis* in the absence of the teleomorph,

since conidial sizes of the different *Cryphonectria* spp., especially those of *C. radicalis* and *C. parasitica*, overlap. Lastly, *C. radicalis s. str.* is one of two *Cryphonectria* species that can discolour the agar growth medium purple. The other species with this property is *C. nitschkei* (Roane & Stipes 1978, Shear *et al*. 1917, Roane 1986c). It has not yet been determined whether the other fungus residing in *C. radicalis s. l.* also produces this pigment.

Cryphonectria radicalis and *C. parasitica* often occur together on trees of the same host and in the same areas, therefore *C. radicalis* is often inadvertently isolated instead of *C. parasitica* (Hoegger *et al*. 2002, Sotirovski *et al*. 2004). The two species can, however, be distinguished from each other based on ascospores size, the presence of mycelial fans, discolouration of the growth medium and the luteous lining present in *C. radicalis s. str*. (Table 4). It has been suggested that the more aggressive *C. parasitica* has displaced *C. radicalis* in Europe and especially in North America, where *C. radicalis* was already present before the introduction of *C. parasitica* (Heiniger *et al*. 2002, Myburg *et al*. 2004b). Both species also occur naturally in Japan, where they are most probably native (Myburg *et al.* 2004b). It will, therefore, be difficult to re-collect *C. radicalis* in nature in the United States, although a larger collection of isolates linked to herbarium specimens will be needed to fully resolve the taxonomy of this aggregate of species.

Cryphonectria radicalis, together with *E. gyrosa*, represents one of the oldest species in the *Cryphonectriaceae* and was first described in 1828 from the U.S.A. (Fries 1828), although the anamorph had already been found in 1814 in the United Kingdom (Sowerby 1814). It is, therefore, not surprising that it has frequently been renamed until 1917, when it was placed in *Endothia* (Shear *et al*. 1917) together with *C. parasitica* (as *E. parasitica*). Both species were subsequently moved to

Cryphonectria by Barr (1978). A complete list of synonyms representing the long taxonomic history of *C. radicalis* has been presented by Shear *et al*. (1917), and we have chosen to present only the key references in our synopsis.

Cryphonectria radicalis and *E. gyrosa* both have similar orange stromata and they have consequently been confused with each other or were treated as a single species (Shear *et al*. 1917). It was at this time that *Endothiella gyrosa*, the type of the anamorph genus *Endothiella*, was described (Saccardo 1906). *Endothiella gyrosa* has thus been wrongly assigned to *E. gyrosa* for many years and it should strictly represent the anamorph of *C. radicalis*. This clearly emerges from a careful examination of the type specimen of *Endothiella gyrosa* in the present study.

Fig. 22. Fruiting structures of *Cryphonectria radicalis* (from holotype K109808). A. Ascostroma and conidioma (arrow) on bark. B. Longitudinal section through ascostroma also showing (arrow) conidial locules (from TFM: FPH 652). C. Longitudinal section through conidioma. D. Stromatic tissue. E–F. Luteous lining of conidial locules. G. Ascospores. H. Conidiophores. I. Conidia. Scale bars A–C = 200 µm; D–F = 20 µm; G–I = 10 µm.

3. ***Cryphonectria japonica*** (Tak. Kobay. & Kaz. Itô) Gryzenh. & M.J. Wingf., **comb. nov.** Mycobank MBxxx. Fig. 23.

Basionym: *Endothia japonica* Tak. Kobay. & Kaz. Itô, Ann. Phytop. Soc. Jap. 21: 15. 1956.

Anamorph: *Endothiella japonica* Roane, Chestnut blight, other *Endothia* diseases, and the genus *Endothia*, APS Press: 37. 1986.

≡ *Endothia nitschkei* G.H. Otth, Bull. Govt. For. Exper. Stat. 226: 143–145. 1970, non G.H. Otth.

≡ *Cryphonectria nitschkei* (G.H. Otth) M.E. Barr, Mycologia Mem. 7: 144. 1978.

≡ *Endothia japonica* Tak. Kobay. & Kaz. Itô, Chestnut blight, other *Endothia* diseases, and the genus *Endothia*, APS Press: 37. 1986.

Etymology: Latin, *japonica*, refers to Japan where the fungus was first collected.

Ascostromata on host gregarious or single, sometimes confluent, semi-immersed in bark, pulvinate, spherical to elongated, orange, 230–330 µm high, 250–1630 µm long and 210–1010 µm diam, upper region eustromatic**,** lower region pseudostromatic, pseudoparenchyma on edge of stroma, prosenchyma in centre. *Perithecia* usually valsoid, embedded beneath surface of bark at base of stromata, fuscous black, necks emerge at stromatal surface as black ostioles covered with orange stromatal tissue to form papillae extending up to 130 µm above stromatal surface. *Asci* (42–)44.5–50.5(–56) × 7–8.5(–9.5), fusiform, 8-spored. *Ascospores* (8.5–)9.5–11.5(–12.5) × (3–)3.5–4.5(–5) µm, hyaline, fusiform to oval, ends round, one median septum.

Conidiomata part of ascostromata as conidial locules, or separate structures, pulvinate, semi-immersed, uni- to multilocular structures with same tissue structure, 250–360 µm high, 360–550 µm diam, with locules often convoluted, non-ostiolate. *Conidiophores* (5.5–)7.5–17(–24) µm long, cylindrical or bulbous base, apices attenuated or inflated, cylindrical or flask-shaped, occasionally clavate, with separating septa and branching, hyaline, *conidiogenous cells* (1–)1.5–2 µm wide. Conidia (3.5–)4–5.5(–6.5) × (1–)1.5(–2) µm, hyaline, cylindrical, occasionally slightly curved, aseptate, exuded as orange droplets.

Cultural characteristics: Cultures on MEA fluffy with a smooth to crenate margin, white with orange conidiomata, covering a 90 mm diam plate after minimum of 8 days, optimum temperature for growth 25 ºC. Additional information of cultural characteristics on other media can be found in Kobayashi (1970).

Substrate: Bark of *Quercus*, *Castanea*, *Castanopsis* (*Fagaceae*, *Fagales)*, *Betula*, *Carpinus* (*Betulaceae*, *Fagales*), *Pyrus*, *Prunus* (*Rosaceae*, *Rosales*), *Eucalyptus* (*Myrtaceae*, *Myrtales*), *Rhus* (*Anacardiaceae*, *Sapindales*) and *Larix* (*Pinaceae*, *Pinales*).

Distribution: China, Japan, Siberia (Russia).

Specimens examined: **Japan**, Tokyo, Meguro, *Quercus grosseserrata*, May 1954, T. Kobayashi, **holotype** TFM:FPH 1045; Yamanashi, Narusawa, Mt. Fuji, *Quercus grosseserrata*, May 1954, T. Kobayashi, TFM:FPH 1046; Tokyo, Mt. Ôtake, *Quercus grosseserrata*, May 1954, T. Kobayashi, TFM:FPH 1049, living culture CMW 13744 = MAFF 410568; Nagano, Kano, *Quercus glandulifera*, Oct. 1954, T. Kobayashi, TFM:FPH 1064, living culture CMW 13747 = MAFF 410569; Fukushima, Hanawa, *Rhus javanica*, Oct. 1963, Y. Zinno, TFM:FPH 2225, living culture CMW 13745 =

MAFF 410572; Nagano, Agematsu, *Quercus grosseserrata*, Oct. 1955, T. Kobayashi, TFM:FPH 632, living culture CMW 13742 = MAFF 410570; Nagano, Wada, *Quercus grosseserrata*, Oct. 1955, T. Kobayashi, TFM:FPH 586; Nagano, Nagato, *Quercus grosseserrata*, Oct. 1955, T. Kobayashi, TFM:FPH 570, living culture CMW 13741 = MAFF 410156; Yamanashi, Kobuchizawa, *Castanea crenata*, Apr. 1998, M. Milgroom and S. Kaneko, TFM:FPH 7609, living culture CMW 10786 = KB1; Kyoto, Chudai, *Castanea crenata*, Apr. 1998, M. Milgroom and S. Kaneko, TFM:FPH 7610, living culture CMW 10787 = CD28; Yamada, *Castanea crenata*, 1998, M. Milgroom and S. Kaneko, TFM:FPH 7747, living culture CMW 5877 = YM2. The following specimens were collected as *C. havanensis*: **Japan**, Tokyo, Meguro, *Eucalyptus globulus*, Dec. 1954, T. Kobayashi, TFM:FPH 633, living culture CMW 10910 = TFM:FPH E11; Shizuoka, Yoshiwara, *Betula* sp., Nov. 1963, Zinno, TFM:FPH 2300; Tokyo, Inagi, *Pyrus sinensis*, Feb. 1960, T. Kobayashi, TFM:FPH 1270, living culture CMW 13736 = MAFF 410154; Aichi, Seto, *Quercus variabilis*, Oct. 1953, T. Kobayashi, TFM:FPH 1203; *Quercus glandulifera*, 1954, T. Kobayashi, TFM:FPH 1047.

Notes: *Cryphonectria japonica* has ascospores that are typically 9.5–11.5 µm long. Conidia are also generally longer than those of the other species. Myburg *et al.* (2004b), however, reported considerable variation in conidial size between different specimens. For example, two specimens had conidia up to 7.5 µm long, while the majority of conidia for other specimens were up to 6 µm long. Other morphological characteristics that distinguish *C. japonica* from other species of *Cryphonectria*, are conidiophores that are occasionally clavate in shape, which is different from other species that have only cylindrical to tapered conidiophores. *Cryphonectria japonica*

also colours the growth medium purple, similar to *C. radicalis* (Roane & Stipes 1978).

Cryphonectria japonica was first described by Kobayashi & Itô (1956b) as *Endothia japonica*. However, Kobayashi (1970) reduced it to synonymy with *Endothia nitschkei*, which had been described earlier from *Tilia* in Switzerland (Otth 1868). This was because the sizes of spores in these two fungi overlapped. In her monograph, Roane (1986a) noted that the original specimens and descriptions linked to *E. nitschkei* differ from those of *C. japonica*. For example, in *E. nitschkei* ascospores are not hyaline, conidia are expelled as white tendrils, the fruiting structures do not discolour in lactophenol and perithecia develop within the bark and not in stromata (Roane 1986a). Our examination of the original material (*Tilia*, 1894 Herbier Fuckel, G.H. Otth, NY, nr. 739) revealed that conidia are borne in locules underneath the bark surface and that ascospores are longer [12.5–14(–14.5) × 4.5–5(5.5) µm] than those of *C. japonica*. These two fungi are clearly different and we re-establish the original epithet for the fungus from Japan, in this monograph.

Based on morphological characteristics and DNA sequence comparisons, Myburg *et al*. (2004b) showed that specimens and isolates referred to as *C. havanensis* from various hosts in Japan were identical to those representing *C. japonica*. *Cryphonectria japonica* is also distinct from authentic *C. havanensis*, now *M. havanensis*, occurring in the Caribbean (Gryzenhout *et al.* 2006d). The incorporation of the *C. japonica* specimens and those originally labeled *C. havanensis* under the single name *C. japonica*, has increased the host range of the fungus to span over five orders of plants.

Numerous *Cryphonectria* species occur on *Eucalyptus* trees in Japan (Myburg *et al*. 2004b). For example, Kobayashi & Itô (1956a) originally applied the name *C.*

havanensis to specimens from *Eucalyptus* spp. in that country. Specimens and isolates of a fungus identified as *C. havanensis* isolates from *Eucalyptus* in Japan were, however, later shown to represent *C. japonica* (Myburg *et al*. 2004b). Other *Cryphonectria* spp. occurring on *Eucalyptus* spp. in Japan include *C. parasitica*, an unidentified *Cryphonectria* sp. (Old & Kobayashi 1988) and an unnamed species shown to be unique based on ascospore sizes (Myburg *et al*. 2004b). Of these fungi, *C. parasitica* was shown to be pathogenic on the *Eucalyptus* trees. It is thus clear that additional surveys of *Cryphonectria* spp. on *Eucalyptus* spp. should be conducted in Japan and neighbouring countries to better understand the species occurring on this host.

Fig. 23. Fruiting structures of *Cryphonectria japonica*. A. Ascostroma on bark. B. Longitudinal section through ascostroma. C. Pseudoparenchymatous tissue. D. Conidioma on bark. E. Longitudinal section through conidioma. F. Prosenchymatous tissue. G. Ascus. H. Ascospores. I. Conidiophores. J. Conidia. Scale bars A–B, D–E = 200 µm; C, F = 20 µm; G–J = 10 µm.

4. ***Cryphonectria macrospora*** (Tak. Kobay. & Kaz. Itô) M.E. Barr, Mycologia Mem. 7: 144. Fig. 24.

Basionym: *Endothia macrospora* Tak. Kobay. & Kaz. Itô, Ann. Phytopath. Soc. Japan 21: 152. 1956.

Anamorph: *Endothiella macrospora* Tak. Kobay. & Kaz. Itô, Bull. Govt. For. Exper. Stat. 226: 146. 1970.

Etymology: Latin, *macrospora*, refers to the large ascospores.

Ascostromata on host gregarious or single, sometimes confluent, pulvinate, semi-immersed in bark, orange, 670-730 µm high, 700–940 µm diam, confluent stroma can be up to 5200 µm long, upper region eustromatic**,** lower region pseudostromatic, pseudoparenchyma on edge of stroma, prosenchyma in centre. *Perithecia* usually valsoid, embedded beneath surface of bark at base of stromata, fuscous black, necks emerge at stromatal surface as black ostioles covered with orange stromatal tissue to form papillae or longer necks extending up to 210 µm above stromatal surface. *Asci* (62–)67.2(–73) × (10–)10.9(–11) µm, clavate to oblong-clavate (Kobayashi & Ito 1956b, Kobayashi 1970), 8-spored. *Ascospores* 14–17(–19) × (4.5–)5.5–7(–8) µm, hyaline, ellipsoidal to fusoid, ends round, one median septum.

Conidiomata part of ascostromata as conidial locules, or separate, pulvinate, semi-immersed, uni- to multilocular structures with same tissue structure, 360–400 µm high, 540–630 µm diam, with locules often convoluted, non-ostiolate. *Conidiophores* (9.5–)11.5–21.5(–30) µm long, cylindrical or flask-shaped with attenuated apices, occasionally with separating septa and branching, hyaline, *conidiogenous cells* 1.5–2.5 µm wide, longer cylindrical cells up to 52 µm long present between conidiophores that

appear to be sterile. Conidia 3.5–4.5(–5) × 1–1.5 µm, hyaline, cylindrical, aseptate, exuded as orange droplets.

Cultural characteristics: Cultures currently in our possession have been extensively cultured and could not be be used with confidence to determine the true cultural morphology of the species. Reference to cultural characteristics on various media can be found in Kobayashi (1970).

Substratum: Bark of *Castanopsis cuspidata* var. *sieboldii.*

Distribution: Japan.

Specimens examined: **Japan**, Tokyo, Shinagawa, *Castanopsis cuspidata* var. *sieboldii*, 22 Jun. 1954, T. Kobayashi, **holotype** TFM:FPH 1057, **syntypes** TFM:FPH 1058, TFM:FPH 1060, TFM:FPH 1069, TFM:FPH 1071.

Notes: *Cryphonectria macrospora* has the largest ascospores [(14–17(–19) × (4.5–)5.5–7(–8) µm] of all species in the *Cryphonectriaceae*. Another interesting morphological feature of this fungus is the fact that it has long, cylindrical cells up to 52 µm long between the conidiogenous cells. It is unclear what the function of these cells is. It is possible that they are conidiogenous cells but they have not been seed to produce conidia and could be paraphyses, similar to those found in *Microthia* and *Holocryphia* species.

All specimens of *C. macrospora* are currently known only from a single location and host. It is likely that the fungus occurs more widely in Japan. Surveys in Japan and neighbouring areas on a wide variety of hosts most likely will lead to more specimens of the fungus.

More collecting on different hosts in the Orient and Northern Hemisphere are likely to lead to the discovery of additional *Cryphonectria* spp. and would be taxonomically valuable. This area of the world, in particular the Orient, appears to include the area of origin of *Cryphonectria* species (Gryzenhout *et al*. 2006a). Although surveys have been conducted by Kobayashi and co-workers (Kobayashi & Ito 1956a, 1956b, Kobayashi 1970) in the past, recognition of this area as the likely centre of origin of a group of fungi that include important pathogens would seem worthwhile.

Fig. 24. Fruiting structures of *Cryphonectria macrospora*. A. Ascostroma on bark. B. Conidioma on bark. C. Longitudinal section through ascostroma. D. Pseudoparenchymatous tissue. F. Prosenchymatous tissue. F. Ascospores. G–H. Conidiophores and conidiogenous cells. I. Conidia. Scale bars A–C = 200 µm; D–E = 20 µm; F–I = 10 µm.

Microthia Gryzenh. & M.J. Wingf., **nom. prov.,** Stud. Mycol. 55 (in press). 2006. Mycobank MBxxx.

Etymology: Greek, *micros*, small, and *this*, a heap, referring to the small, pulvinate stromata.

Ascostromata erumpent, semi-immersed to superficial, pulvinate, orange, tissue predominantly prosenchymatous but pseudoparenchymatous at edges. *Perithecia* valsoid to diatrypoid, surrounded by fungal or host tissue, fuscous black, necks emerge at the stromatal surface as black ostioles in papillae covered with orange stromatal tissue. *Asci* fusiform. *Ascospores* fusoid to ellipsoidal, hyaline, 1-septate, often with slight constriction at septum.

Conidiomata often occurring in same stroma that contains perithecia or as separate structures, semi-immersed to superficial, pulvinate, orange, uni- to multilocular and convoluted. *Conidiophores* cylindrical to flask-shaped with tapering apices, often septate with or without lateral branches beneath septum, hyaline, conidiogenous cells phialidic. Long cylindrical cells, or *paraphyses*, occur between conidiophores. *Conidia* hyaline, cylindrical, aseptate, exuded through opening at stromatal surface as orange droplets or tendrils.

Typus: *Microthia havanensis* (Bruner) Gryzenh. & M.J. Wingf.

Notes: *Microthia* was recently described (Gryzenhout *et al.* 2006d) to accommodate the already existing species *C. havanensis* and *C. coccolobae*. *Microthia* was characterised largely due to the availability of new isolates for *C. havanensis* that

could be used in DNA sequence comparisons. These analyses showed that *C. havanensis* groups separately from *Cryphonectria s. str.* Morphological characteristics such as the conspicuous paraphyses and small to medium sized stromata distinguish the genus from *Cryphonectria*, the genus that it resembles most closely (Gryzenhout *et al.* 2006d). This is except for one species of *Cryphonectria*, *C. macrospora*, that also has long sterile cells between the conidiophores. Based on stromatal morphology and the occurrence of paraphyses, *Microthia* is also similar to *Holocryphia* and the fact that it occurs on *Eucalyptus* spp. is also common to both genera (Gryzenhout *et al*. 2006d) They can, however, be distinguished by the aseptate ascospores in *Holocryphia* and the single septate ascospores in *Microthia* (Gryzenhout *et al.* 2006d). The geographical range of occurrence of species in these two genera also does not overlap (Gryzenhout *et al.* 2006d).

Only two species are currently known in *Microthia*. These are *M. havanensis* and *M. coccolobae*. These two species are morphologically indistinguishable, but were found to differ in their ability to infect certain hosts (Gryzenhout *et al*. 2006d). For example, only *M. coccolobae* was able to colonise *Coccoloba uvifera*, while *M. havanensis* grew on twigs of *Myrica faya* and other hosts, but could not infect those of *Coccoloba uvifera* (Hodges & Gardner 1992, Barnard *et al.* 1993). This difference in substrate preference and the lack of isolates to confirm the suspected difference between the two species are the reasons why these two species have not been reduced to synonymy.

5. ***Microthia havanensis*** (Bruner) Gryzenh. & M.J. Wingf., **nom. prov.,** Stud. Mycol. 55 (in press). 2006. Mycobank MBxxx. Fig. 25.

Basionym: *Endothia havanensis* Bruner, Mycologia 8: 241–242. 1916.

≡ *Cryphonectria havanensis* (Bruner) M.E. Barr, Mycologia Mem. 7: 143. 1978.

Etymology: from Havana, Cuba

Ascostromata on host gregarious or single, sometimes confluent, pulvinate, semi-immersed to superficial in bark, orange, 90–400 µm high, 200–650 µm diam, pseudoparenchyma on edge of stroma, prosenchyma in centre. *Perithecia* usually valsoid, embedded in host tissue at base of stromata, fuscous black, necks emerge at stromatal surface as black ostioles covered with orange stromatal tissue to form papillae extending up to 370 µm above stromatal surface. *Asci* normally (26.5–)29.5–34.5(–37) × (5–)5.5–7(–8) µm, could be up to an average of 39 µm (maximum 44.5 µm) for some specimens, fusiform, 8-spored. *Ascospores* (5.5–)7–9(–10) × (2–)2.5–3(–4) µm, hyaline, fusoid, ends round, one median septum, often constricted at septum.

Conidiomata part of ascostromata as conidial locules, or separate structures, pulvinate, semi-immersed to superficial, orange, uni- to multilocular, 280–400 µm high, 130–290 µm diam with locules often convoluted, non-ostiolate. *Conidiophores* (7–)11–22(–31) µm long, cylindrical or flask-shaped with attenuated apices, or not attenuated, occasionally with separating septa and branching, hyaline, *conidiogenous cells* 1.5–2 µm wide. Long cylindrical cells, seemingly sterile and resembling paraphyses, occur between conidiophores, up to 127.5 µm long, these paraphyses

occasionally branch into other sterile, cylindrical cells, or phialides. *Conidia* (2.5–)3–4(–5) × 1–1.5 µm, hyaline, cylindrical, aseptate, exuded as orange droplets.

Cultural characteristics: white and fluffy on MEA, margins even, isolates covering 90 mm diam plates after 7 days at the optimum growth temperature of 25 °C.

Substrate: Bark of *Eucalyptus* spp. (*Myrtaceae*, *Myrtales*), *Myrica faya* (fire tree, *Myricaceae*, *Myricales*). Reported (Bruner 1916) from *Mangifera indica* (mango, *Anacardiaceae*, *Sapindales*), *Persea gratissima* (avocado, *Lauraceae*, *Laurales*), *Spondias mombin* (jobo, *Anacardiaceae*, *Sapindales*).

Distribution: Cuba, Florida and Hawaii (U.S.A.), Puerto Rico, Mexico, Azores, Madeira.

Specimens examined: **Cuba**, Santiago de las Vegas, *Eucalyptus* sp., 15 Feb. 1916, S.C. Bruner, **holotype** BPI 614275, BPI 614273; *Eucalyptus botryoides*, 25 March 1916, C.L. Shear, BPI 614278; *Spondias* sp., 28 March 1916, C.L. Shear, BPI 614282; Earle' s Herradura, *Spondias myrobalanus*, 5 Apr. 1916, C.L. Shear, BPI 614283, BPI 614284; Santiago de las Vegas, *Mangifera indica*, 6 Apr. 1916, C.L. Shear, BPI 614279, BPI 614280, 26 March 1916, C.L. Shear, BPI 614281. **Mexico**, Las Chiapas, *Eucalyptus saligna*, 26 Feb. 1998, C.S. Hodges, PREM 57518, living culture CMW 11298. **Puerto Rico**, 1923, F.J. Seaver & C.E. Chardon, NY 511. **U.S.A.**, Hawaii, Kauai, *Eucalyptus* sp., Sep. 2002, M.J. Wingfield, PREM 57521, living culture CMW 10879 = CBS 115758, PREM 57522, living culture CMW 10885 = CBS 115760. Florida, Near Palmdale, Glades Co., *Eucalyptus robusta*, 1984, E.L. Barnard & K. Old, FLAS 54261, ATCC 60862; *Eucalyptus grandis*, 1984, E.L. Barnard & K. Old, FLAS 54263. **Madeira**, Machico, *Myrica faya*, 8 May 2000, C.S. Hodges, PREM 57523, living culture CMW 14551 = CBS 115841. **Azores**, Island of

São Miguel, Mosteiro, *M. faya,* C.S. Hodges & D.E. Gardner, PREM 57524, living culture from same locality CMW 11301; Island of Pico, *M. faya*, 30 Jul. 1992, C.S. Hodges & D.E. Gardner, PREM 57525, living culture from same locality CMW 11301; Island of Pico, *M. faya*, 31 May 1985, C.S. Hodges & D.E. Gardner, PREM 58810, living culture from same locality CMW 11301; Island of São Miguel, *M. faya*, 2 Aug. 1992, C.S. Hodges & D.E. Gardner, PREM 58811, living culture from same locality CMW 11301; Island of Terceiro, *M. faya*, 31 May 1987, C.S. Hodges & D.E. Gardner, PREM 58812, living culture from same locality CMW 11301; Island of Faial, *M. faya*, 27 May 1985, C.S. Hodges, PREM 58813, living culture from same locality CMW 11301.

Notes: *Microthia havanensis* can infect woody hosts belonging to different orders such as *Eucalyptus* (*Myrtales*) and *Myrica faya* (*Myricales*) (Gryzenhout *et al.* 2006d). Based on available geographical distribution data, this is clearly a tropical to sub-tropical fungus, most likely occurring widely in pantropical America. It is probable that it occurs on native hosts, such as *Spondias mombin*, in these areas although these are currently unknown or unconfirmed. The occurrence of *M. havanensis* on various islands in the Pacific and Atlantic Oceans is also enigmatic, and it would be interesting to know how the fungus has spread to these areas.

There are no living isolates of *M. havanensis* that can be linked to collections of *M. havanensis* from Cuba, the type locality. Fresh collections are needed to confirm that the existing collections from Mexico, Hawaii, the Azores and Madeira are identical to the type and such collections could then serve as an epitype. Furthermore, reports of *M. havanensis* on mango, avocado and *S. mombin* in Cuba

need to be confirmed, and would show conclusively that this fungus occurs on many different and unrelated plants.

Microthia havanensis, under its previous synonyms *C. havanensis* and *E. havanensis* respectively, has been thought (Kobayashi 1970, Hodges 1980) to be synonymous to *C. gyrosa* (syn. *E. tropicalis*). Hence some reports of *M. havanensis*, or of fungi thought to represent this fungus, was under the name *C. gyrosa* (Sharma *et al*. 1985, Barnard *et al*. 1987). Gryzenhout *et al.* (2006d), however, showed with morphological comparisons and DNA sequence comparisons of isolates representing *C. havanensis* and *C. gyrosa*, that the two fungi are distinct and belong in different genera, namely *Microthia* and *Amphilogia* respectively.

Fig. 25. Fruiting structures of *Microthia havanensis*. A. Ascostroma (ascostromatic base indicated with arrow) on bark. B. Longitudinal section through ascostroma. C. Stromatic tissue. D. Conidiomata (black arrows) with spore drop and perithecia (white arrow) on bark. E. Longitudinal section through conidioma. F. Ascus. G. Ascospores. H. Conidial locule with long paraphyses. I. Paraphyses. J–K. Conidiophores. L. Conidia. Scale bars A–B, D–E = 100 µm; C, H = 20 µm; F–G, I–L = 10 µm.

6. ***Microthia coccolobae*** (Vizioli) Gryzenh. & M.J. Wingf., **nom. prov.,** Stud. Mycol. 55 (in press). 2006. Mycobank MBxxx. Fig. 26.

Basionym: *Endothia coccolobae* Vizioli, Mycologia 15: 115. 1923 (as *E. coccolobii*).

≡ *Cryphonectria coccolobae* (Vizioli) Micales & Stipes, Phytopathology 77: 651. 1987 (as *C. coccolobii*).

Etymology: refers to the original host, *Coccoloba*, from which the fungus was described.

Ascostromata on host gregarious or single, sometimes confluent, semi-immersed to superficial in bark, on berries superficial, pulvinate, orange, 130–400 µm high, 200–580 µm diam on seed, but can be up to 780 µm high and 1860 µm diam on bark, pseudoparenchyma on edge of stroma, prosenchyma in centre. *Perithecia* usually valsoid, embedded in host tissue at base of stromata, fuscous black, necks emerge at stromatal surface as black ostioles covered with orange stromatal tissue to form papillae extending up to 240 µm above stromatal surface. *Asci* (32.5–)34.5–39(–41) × (5–)7–9.5(–10.5) µm, fusiform, 8-spored. *Ascospores* (6.5–)7.5–9(–10.5) × (2.5–)3–3.5(–4) µm, hyaline, fusoid, ends round, one median septum, often constricted at septum.

Conidiomata part of ascostromata as conidial locules, or separate structures, pulvinate, semi-immersed to superficial, orange, uni- to multilocular structures, 120–230 µm high, 140–370 µm diam with locules often convoluted, non-ostiolate. *Conidiophores* (5–)7.5–18.5(–30.5) µm long, cylindrical or flask-shaped with attenuated apices, or not attenuated, occasionally with separating septa and branching, hyaline, *conidiogenous cells* 1.5–2(–2.5) µm wide. Long cylindrical cells up to 78 µm long, seemingly sterile and resembling paraphyses, occur between conidiophores,

these paraphyses occasionally branch into other sterile, cylindrical cells, or phialides. Conidia (2.5–)3–4.5(–5.5) × 1–1.5 µm, hyaline, cylindrical, aseptate, exuded as orange droplets.

Substrate: Seed and bark of *Coccoloba uvifera* (sea grape, *Polygonales*, *Polygonaceae*), bark of *Calophyllum calaba* (*Clusiaceae*, *Malpighiales*) and *Conocarpus erecta* (*Combretaceae*, *Myrtales*).

Distribution: Bermuda, Florida (U.S.A.).

Specimens examined: **Bermuda**, Grape Bay, fruit of *Coccoloba uvifera*, 11 Dec. 1921, H.H. Whetzel, **holotype** CUP 128; Grape Bay, fruit of *Co. uvifera*, 11 Dec. 1921, H.H. Whetzel, **isotype** BPI 613756; Grape Bay, fruit of *Co. uvifera*, 11 Dec. 1921, H.H. Whetzel, **isotype** NY 147; Grape Bay, fruit of *Co. uvifera*, 11 Dec. 1921, H.H. Whetzel, CUP 30512; Elbow Beach, fruit of *Co. uvifera*, 28 Jan. 1926, Whetzel, Seaver & Ogilvie, CUP 34658; South Shore, bark of *Co. uvifera*, 25 Nov. 1940, F.J. Seaver & J.M. Waterston, CUP 57366; Devonshire, *Calophyllum calaba*, 2 Feb. 1926, Seaver, Whetzel & Ogilvie, CUP 35078; Devonshire Bay, *Conocarpus erecta*, 5 Feb. 1926, Seaver, Whetzel & Ogilvie, CUP 35081.

Notes: There are no living cultures of *M. coccolobae* and its relationship to *M. havanensis* cannot be confirmed at present. Based on morphological comparisons of herbarium specimens, *M. coccolobae* cannot be distinguished from *M. havanensis* (Gryzenhout *et al*. 2006d). The fungus has, however, not been reduced to synonomy with *M. havanensis* because of reported differences in host specificity between the two species (Barnard *et al.* 1993) and because phylogenetic comparisons cannot be made due to a lack of cultures (Gryzenhout *et al*. 2006d).

The morphology of *M. coccolobae* differs in specimens on different host substrates (Gryzenhout *et al*. 2006d). On seeds of *C. uvifera*, fruiting structures are superficial. In contrast, stomata on the bark of various plants, are semi-immersed and larger.

Recently a new fungus, *U. fallax*, was discovered on bark of *Coccoloba uvifera* in Florida (Gryzenhout *et al*. 2006d). This fungus resembles *M. coccolobae*, but can readily be distinguished from it based on characters of the anamorph stromata. It is possible that previous reports of *M. coccolobae* from Florida represent *U. fallax*, although confirmation of this fact would require additional collections.

Fig. 26. Fruiting structures of *Microthia coccolobae*. A. Ascostroma on bark. B. Conidiomata on bark. C. Longitudinal section through ascostroma and conidiomata (arrows). D. Stromatic tissue. E. Ascus. F. Ascospores. G. Paraphyses. H. Conidiophores. I. Conidia. Scale bars A–C = 100 µm; D = 20 µm; E–I = 10 µm.

Endothia Fr., Sum. Veg. Scand.: 385. 1849.

Etymology: Greek, *endo*, within, *this*, a heap, referring to the perithecia carried within pulvinate stromata.

Ascostromata large, erumpent, pulvinate to clavate, superficial in bark, orange, upper region eustromatic**,** lower region pseudostromatic, pseudoparenchyma on edge of stroma, prosenchyma in centre. *Perithecia* usually diatrypoid, embedded in stromata, fuscous black, necks emerge at stromatal surface as black ostioles covered with orange stromatal tissue to form papillae. *Asci* fusiform. *Ascospores* hyaline, cylindrical, aseptate.

Conidiomata part of ascostromata as conidial locules or separate structures, large, pulvinate, superficial, orange, multilocular, non-ostiolate. *Conidiophores* cylindrical or flask-shaped, occasionally with separating septa and branching, *conidiogenous cells* phialidic. *Conidia* minute, hyaline, cylindrical, aseptate, exuded as orange droplets.

Typus: Endothia gyrosa (Schwein.: Fr.) Fr.

Notes: *Endothia* is the oldest generic name in the *Cryphonectriaceae* (Fries 1849). The genus has accommodated most of the known species of *Endothia* and *Cryphonectria* cited in Micales & Stipes (1987) and at the time when *Cryphonectria* was treated as a synonym of *Endothia* (Shear *et al*. 1917, Kobayashi 1970, Roane 1986a). Currently, only species with aseptate ascospores and large, erumpent and mostly superficial stromata are retained in *Endothia* (Barr 1978, Myburg *et al*. 2004a). The only other genus in the *Cryphonectriaceae* with aseptate ascospores is

Holocryphia, which can be distinguished from *Endothia* by its smaller, semi-immersed stromata and prominent paraphyses (Venter *et al*. 2002).

The only two species accommodated in *Endothia*, *E. gyrosa* and *E. singularis*, can be distinguished based on stromatal size, structure and colour, and mode of conidial dispersal. Their ascospores and conidia are, however, similar in size and shape. **The following table is provided to highlight the differences between the two species:**

	E. gyrosa	*E. singularis* [a]
Stromatal size	1.5–2 × 1.5–3 mm	2–4 × 3–5 mm
Stromatal colour	Orange	"mahogany red" on the outside, "scarlet" within
Texture	Remain intact	Brittle
No. of perithecia	25–50 (Shear *et al*. 1917)	>100 (Shear *et al*. 1917)
Shape of conidial locules	Labyrinthiform	Spherical
Conidial dispersal	Droplets	Disintegration of stroma

[a] According to Shear *et al.* (1917).

7. ***Endothia gyrosa*** (Schwein.: Fr.) Fr., Sum. Veget. Scand.: 385. 1949. Fig. 27.

Basionym: Sphaearia gyrosa Schwein., Syn. Fung. Car. Sup.: 29. 1822.

≡ *Melogramma gyrosum* (Schwein.: Fr.) Tul., Ann. N. Y. Acad. Sci. 1: 185. 1878.

≡ *Endothia gyrosa* (Schwein.: Fr.) Fuckel, Syll. Fung. 1: 601. 1882.

≡ *Endothia gyrosa* (Schwein.) Ell. & Ev., No. Amer. Pyren.: 552. 1892.

≡ *Endothia radicalis* (Schwein.: Fr.) Farl., Science 36: 908. 1912.

≡ *Endothia radicalis* (Schwein.: Fr.) Shear, Phytopathology 2: 211. 1912.

≡ *Endothia radicalis* (Schwein.) Fr., P. J. Anderson & H. W. Anderson, Phytopathology 2: 210. 1912.

Etymology: Greek, *gyros*, circle

Ascostromata on host gregarious or single, sometimes confluent, large, erumpent, pulvinate to clavate, superficial in bark, orange, 900–1800 µm high, 1250–2050 µm diam, upper region eustromatic**,** lower region pseudostromatic, pseudoparenchyma on edge of stroma, prosenchyma in centre. *Perithecia* usually diatrypoid, embedded in stromata at irregular levels, fuscous black, necks emerge at stromatal surface as black ostioles covered with orange stromatal tissue to form papillae extending up to 180 µm above stromatal surface. *Asci* 25–30 × 6–7 µm long, fusiform (Shear *et al.* 1917), 8-spored. *Ascospores* (8.5–)9–11(–11.5) × 1.5–2 µm, hyaline, cylindrical, ends round, aseptate.

Conidiomata part of ascostromata as conidial locules, or separate structures, large, pulvinate, superficial, orange, multilocular structures with same tissue structure, similar in size to ascostromata, locules numerous in labyrinthine pattern, non-ostiolate. *Conidiophores* (4–)5.5–9.5(–12) µm long, cylindrical or flask-shaped with attenuated apices, occasionally with separating septa and branching, hyaline, *conidiogenous cells* 1–1.5(–2) µm wide. *Conidia* (3–)3.5–4(–4.5) × 1–1.5 µm, hyaline, cylindrical, aseptate, exuded as orange to sienna droplets.

Cultural characteristics: cultures on MEA and PDA fuscous black to buff to cinnamon with fluffy or sectored appearance, margins smooth or crenate, often colours growth medium purple, grows optimally at 25 ºC, but growth also proliferous at 20 and 30 ºC (Shear *et al*. 1917). Additional growth characteristics on other growth media are discussed by Shear *et al.* (1917).

Substrate: bark and roots of various *Quercus* spp., *Fagus* spp., *Castanea dentata* (*Fagaceae, Fagales*), *Corylus* sp. (*Betulaceae*, *Fagales*), *Ulmus* sp. (*Ulmaceae*, *Rosales*), *Liquidambar* spp. (*Hamamelidaceae*, *Saxifragales*), *Vitis* sp. (*Vitaceae*, *Rhamnales*), *Acer saccharinum* (*Sapindaceae*, *Sapindales*), *Ilex opaca* (*Aquifoliaceae*, *Aquifoliales*), *Prunus laurocerasus* (*Rosaceae*, *Rosales*).

Distribution: eastern part of North America as far west as Michigan, Kansas, Texas and California.

Specimens examined: **U.S.A**., Raleigh, *Quercus phellos*, 1997, L. Grand, PREM 56218; California, Palo Alto, *Quercus agrifolia*, Jan. 1920, N.E. Stevens, BPI 614211; California, San Rafael Hills, *Quercus agrifolia*, 1924, H.E. Parks, BPI 614513, BPI 614514.

Notes: The type specimen of *E. gyrosa* bears only the anamorph (Shear *et al*. 1917). Although the fungus has been clearly described (Shear *et al*. 1917, Roane 1986a, Venter *et al*. 2002, Myburg *et al*. 2004a), an epitype containing ample teleomorph structures would be useful. *Endothia gyrosa* is fairly common in North America, although samples containing the teleomorph may be more difficult to find.

Endothia gyrosa is the oldest fungus in the *Cryphonectriaceae* besides *C. radicalis*. Because of this, *E. gyrosa* has been frequently moved between genera and has been synonimised to other species, most notably *C. radicalis*. These changes have been summarized well by Shear *et al*. (1917) and the list of synonyms in this study only contains those that reflect the most important taxonomical changes.

Endothiella gyrosa has been used as the anamorph of *E. gyrosa* in the past (Shear *et al*. 1917, Barr 1978, Roane 1986a). In this monograph, we have shown that

Endothiella gyrosa should represent the anamorph of *C. radicalis*, and hence *Endothiella* should be used as the anamorph of *Cryphonectria*. No new anamorph genus will be described for *Endothia* as it has been well-established as a holomorph name.

Endothia gyrosa has been reported from many countries and continents other than North America, where it is best known. A fungus referred to as *E. gyrosa* has been found on a *Quercus* sp. in China (Teng 1934), and *E. gyrosa* has been reported to occur on various *Quercus* spp., *Castanea sativa*, *Castanea crenata* and *Eucalyptus diversicolor* in Portugal, Spain and Italy (Spaulding 1961). Spaulding (1961) also claimed that *E. gyrosa* occurs in Germany, western Europe, Sri Lanka, New Zealand and the Philippines. These reports most likely refer to other fungi that represent species that were treated as synonyms of *E. gyrosa* at that time. Some are most probably also fungi morphological similar to *E. gyrosa* but that had been wrongly identified, especially in the absence of the teleomorph. For example, reports of *E. gyrosa* in Europe most likely represent *C. radicalis* (Myburg *et al*. 2004a, 2004b), and those in New Zealand *A. gyrosa*. Likewise, *H. eucalypti* has also been previously referred to as *E. gyrosa* in Australia and South Africa based on its ascospore morphology (Venter *et al*. 2002). We, however, believe that *E. gyrosa* is a fungus mainly occurring in North America on woody hosts, and most likely does not occur elsewhere in the world.

Examination of specimens from *Liquidambar styraciflua* (BPI 613896, BPI 613897, BPI 613898, BPI 613899) revealed that fruiting structures on this tree were semi-immersed, and were thus quite different from the characteristically superficial structures on *Quercus* and *Castanea*. It may be possible that the bark of *Liquidambar* influences the structure of the fruiting bodies, a phenomenon well-known in the

Cryphonectriaceae (Shear *et al*. 1917, Walker *et al*. 1985, Hodges *et al*. 1986, Micales & Stipes 1987, Cannon 1988). The different morphology on *Liquidambar* may indicate that stromata of *E. gyrosa* are not always superficial, although currently *E. gyrosa* can clearly be distinguished from other *Cryphonectria* spp. on similar *Quercus* and *Castanea* spp. because structures are always superficial on the substrate. It could also be possible that the fungus on *Liquidambar* is different from *E. gyrosa*. Isolates from *Liquidambar* should thus be sought to confirm the identity of the fungus on this tree.

Endothia gyrosa has been reported from many different woody plants including species of *Fagus*, *Liquidambar, Prunus* and *Vitis* (Shear *et al.* 1917, Snow *et al*. 1970, Roane 1986a) and it has an extensive distribution in North America (Shear *et al*. 1917). Although North America has been intensively sampled for members of the *Cryphonectriaceae* since the discovery of chestnut blight, there are cultures of few species other than *C. parasitica*, and surveys have mostly focused on the most common *Fagaceae* such as *Castanea* and *Quercus* spp. Phylogenetic analyses of collections from different hosts may still reveal new fungi.

Fig. 27. Fruiting structures of *Endothia gyrosa*. A. Ascostroma on bark. B. Longitudinal section through ascostroma. C. Stromatic tissue. D. Conidioma on bark. E. Longitudinal section through conidioma. F. Labyrinthiform conidial locules. G. Ascus. H. Ascospores. I. Conidiophores. J. Conidia. Scale bars A–B, D–E = 200 µm; C, F = 20 µm; G–J = 10 µm.

8. ***Endothia singularis*** (Syd.) Shear & N.E. Stevens, U. S. Dept. Agric. Bull. 380: 15–16. 1917. Fig. 28.

Anamorph: *Calopactis singularis* Syd., Ann. Mycol. 10: 82. 1912.

Etymology: Latin, *singularis*, alone, solitary, unique

Ascostromata large, depressed globose, superficial, scattered or gregarious, "mahogany red" on the outside, "scarlet" within, 2000–4000 µm high, 3000–5000 µm diam. *Perithecia* >100, usually diatrypoid, embedded in stromata at irregular levels, base 200–350 µm diam, necks slender and emerge at stromatal surface as depressed conical ostioles. *Asci* 25–35 × 4.5–5.5 µm, oblong cylindrical or subclavate to fusoid. *Ascospores* (7–)7.5–10(–11) × (1.5–)2–2.5(–3) µm, hyaline, cylindrical to allantoid, aseptate.

Conidiomata similar to ascostromata, multilocular structures, numerous spherical locules, non-ostiolate, outer wall coriaceous, becoming brittle. *Conidiophores* 6–8 µm long, subulate, hyaline, *conidiogenous cells* 1 µm wide. *Conidia* 3–4 × 1–1.5 µm, hyaline, ovoid oblong, aseptate, conidia not exuded as droplets but walls of locules disintegrate and outer wall rupture, hence becoming like a powder, only stromatal base still attached to the bark, conidia adhere in a globular mass upon release.

Cultural characteristics: grows optimally at 25 ºC, but growth also proliferous at 20 and 30 ºC (Shear *et al.* 1917). The culture currently in our possession has been extensively cultured and could not be used with confidence to determine the true cultural morphology of the species. Reference to cultural characteristics of *E.*

singularis on various media can be found in Shear *et al*. (1917), while the characteristics of Japanese isolates are discussed by Kobayashi (1970).

Substrate: *Quercus* spp.

Distribution: Colorado, New Mexico.

Specimens examined: **U.S.A.,** Colorado, Palmer Lake, *Quercus gambelii*, Dec. 1911, E. Bethel, **holotype** BPI 614515; Palmer Lake, *Quercus*, Sep. 1912, G.G. Hedgcock & E. Bethel, DAR 11602; Colorado, La Veta, *Quercus leptophylla*, Aug. 1911, E. Bethel, 74908; Colorado, Pagosa Springs, *Quercus funnisonii*, May 1917, G.G. Hedgcock & E. Bethel, DAR 11235.

Notes: Herbarium specimens of this fungus, including the type specimen (BPI 614515), are old and do not contain sufficient intact structures for illustration in this monograph. This is largely because stromata of *E. singularis* are brittle and disintegrate easily (Shear *et al.* 1917). The description and schematic drawing of this fungus presented here are thus largely based on the description and illustrations by Shear *et al*. (1917). Additional specimens and particularly those linked to cultures of this fungus should be sought from the U.S.A., but efforts to collect this fungus are likely to be frustrated by the resemblance of *E. singularis* to *E. gyrosa*.

Only one isolate of *E. singularis* is currently known from the U.S.A. (Myburg *et al*. 2004a). The inclusion of this isolate in phylogenetic analyses, however, always results in discrepancies between the ITS and β-tubulin dataset because the isolate is related to both the *E. gyrosa* and the *Cryphonectria* clade. Based on morphology, *E. singularis* should, however, reside in *Endothia*. Unfortunately, no other isolates are

available to determine if the isolate used by Myburg *et al*. (2004a) truly represents *E. singularis*.

Endothia singularis is the only fungus in the *Cryphonectriaceae* with stromata that disintegrate. This disintegration appears to promote conidial dispersal (Shear *et al*. 1917). This unique characteristic could indicate that the fungus should reside in a genus of its own, as suggested by the separate grouping of the only available isolate of *E. singularis*.

Besides the U.S.A., *E. singularis* is known also from Japan. Specimens from Japan as listed by Kobayashi (1970) and studied in this monograph (TFM:FPH 962, TFM:FPH 1050, TFM:FPH 1199, TFM:FPH 1201, TFM:FPH 1207, TFM:FPH 1208, TFM:FPH 2913), do not have the scarlet interior and brittle nature of North American specimens. Fruiting structures were also semi-immersed in the bark. We believe that the Japanese fungus most likely represents an undescribed species in *Endothia*.

Fig. 28. Schematic drawings of *Endothia singularis.* A. Longitudinal section through ascostroma showing conidial locules (arrow). B. Ascospores. C. Conidia. Scale bars A = 200 µm; B–C= 10 µm.

Holocryphia Gryzenh. & M.J. Wingf., **nom. prov.,** Stud. Mycol. 55 (in press). 2006. Mycobank MBxxx.

Etymology: Greek, *holo*, undivided, *crypho-*, secret, referring to the undivided ascospores and the semi-immersed nature of the stromata.

Ascostromata semi-immersed, pulvinate, orange, pseudoparenchymatous tissue at edge of stromata, prosenchymatous tissue at center. *Perithecia* valsoid, surrounded by host tissue, fuscous black, necks emerge at the stromatal surface as black ostioles in papillae or long necks covered with orange stromatal tissue. *Asci* fusiform. *Ascospores* hyaline, cylindrical, occasionally allantoid, aseptate.

Conidiomata part of ascostromata as locules in same stroma that contains perithecia or separate structures, erumpent, pulvinate, semi-immersed, orange, uni- to multilocular and convoluted, non-ostiolate. *Conidiophores* cylindrical with or without inflated bases, tapering, occasionally with separating septa and branching beneath septum, paraphyses occurring between conidiophores. *Conidia* hyaline, cylindrical, aseptate, exuded through an opening at the stromatal surface as orange droplets or tendrils.

Typus: *Holocryphia eucalypti* (M. Venter & M.J. Wingf.) Gryzenh. & M.J. Wingf.

Notes: *Holocryphia* and *Endothia* are the only genera in the *Cryphonectriaceae* with aseptate ascospores. Their stromatal structures are, however, quite different. Those of *Endothia* are large, erumpent and mostly superficial, while stromata of *Holocryphia* are semi-immersed and smaller, making this fungus more similar to *Cryphonectria* and *Microthia*. *Holocryphia* resembles *Microthia* most closely

because of their similar stromatal size and structure as well as the presence of prominent paraphyses in both genera. These two genera can, however, be distinguished with certainty when teleomorph material is available, since *Microthia* has single septate ascospores.

9. ***Holocryphia eucalypti*** (M. Venter & M.J. Wingf.) Gryzenh. & M.J. Wingf., **nom. prov.,** Stud. Mycol. 55 (in press). 2006. Mycobank MBxxx. Fig. 29.

Basionym: *Cryphonectria eucalypti* M. Venter & M.J. Wingf., Sydowia 54: 113–115. 2002.

Etymology: eucalypti refers to the occurrence of this fungus on *Eucalyptus* spp.

Ascostromata on host gregarious or single, occasionally confluent, pulvinate, semi-immersed in bark, orange, 200–950 µm high, 150–870 µm diam, upper region eustromatic**,** lower region pseudostromatic, pseudoparenchyma on edge of stroma, prosenchyma at centre. *Perithecia* usually valsoid, embedded beneath surface of bark at base of stromata, fuscous black, necks emerging at stromatal surface as black ostioles covered with orange stromatal tissue to form papillae or long necks extending up to 170 µm above stromatal surface. *Asci* (12–)17.5–26.5(–34) × 4.5–7(–9) µm, cylindrical to fusiform, 8-spored. *Ascospores* (4.5–)6–9(–12.5) × (0.5–)1–1.5(–2) µm, hyaline, cylindrical to fusiform, occasionally allantoid, ends round to slightly tapered, aseptate.

Conidiomata part of ascostromata as conidial locules, or separate, pulvinate, semi-immersed, orange, uni- to multilocular structures with same tissue structure and size range as ascostromata, with locules often convoluted, non-ostiolate. *Conidiophores* (4–)8–17.5(–27) µm long, cylindrical or flask-shaped with attenuated

apices, occasionally with separating septa and branching, hyaline, *conidiogenous cells* 1.5–2 µm wide. Cylindrical paraphyses occur between conidiogenous cells, (22–)28.5–50(–71.5) × 1–1.5 µm, slightly tapered towards apex, septate and branching. Conidia (2.5–)3–4(–5) × 0.5–1 µm, hyaline, cylindrical, occasionally allantoid, aseptate, exuded as orange droplets.

Cultural characteristics: cultures on MEA and PDA white, fluffy with smooth margins, sometimes with straw yellow patches, fast growing, covering a 90 mm diam plate after minimum of 9 days at the optimum temperature of 25–30 ºC.

Substrate: *Eucalyptus* spp.

Distribution: South Africa, Tasmania and mainland Australia.

Specimens examined: **South Africa**, Northern Kwazulu/Natal, Mtubatuba, Nyalazi estate, bark of GC747 clone of *Eucalyptus*, 25 Feb. 1998, M. Venter, **holotype**, PREM 56211, ex-type culture CMW 7034; Dukuduku estate, bark of *Eucalyptus grandis*, Oct. 1998, M. Venter, PREM 56214, PREM 56216; KwaMbonambi, Amangwe estate, bark of *Eucalyptus grandis*, Oct. 1998, M. Venter, **epitype designated here** PREM 56215, living culture CMW 7033 = CBS 115842; Mpumalanga, Sabie, bark of *Eucalyptus grandis*, Aug. 1998, J. Roux, PREM 56212; Limpopo, Tzaneen, bark of *Eucalyptus saligna*, 6 Feb. 1999, M. Venter, PREM 56305, living culture CMW 7035. **Australia**, Western Australia, Perth, *Eucalyptus globulus*, 1997, M.J. Wingfield, PREM 56217, living culture CMW 7038 = CBS ***; Albany, Termeil, *Eucalyptus saligna*, 1983, K. Old, DAR 49904, DAR 49909; Currowan, Clyde Mountain, *Eucalyptus saligna*, 1983, K. Old, DAR 49906, DAR 49907; 1984, DAR 49905.

Notes: *Holocryphia eucalypti* was identified as *E. gyrosa* after it was discovered on *Eucalyptus* spp. in Australia (Walker *et al*. 1985) and South Africa (Van der Westhuizen *et al*. 1993). This identification was based on its ascospores that are aseptate and similar in size to those of *E. gyrosa* (Walker *et al*. 1985). Earlier reports of the anamorph in Australia were under the name *E. havanensis* (Davison 1982, Davison & Tay 1983). DNA sequence analyses and RFLP digests, however, clearly showed that isolate collections from Australia and South Africa were different from isolates of authentic *E. gyrosa* from North America (Venter *et al*. 2001, 2002). Phylogenetic analyses also placed the Australian and South Africa isolates in *Cryphonectria* as a distinct species (Venter *et al*. 2002, Myburg *et al*. 2004a). This grouping was supported by the semi-immersed stromatal structure of the fungus, and the fungus from Australia and South Africa were thus described as *C. eucalypti* (Venter *et al.* 2002).

Cryphonectria eucalypti was unusual amongst species of *Cryphonectria* because it has aseptate ascospores. However, subsequent discovery of many new genera in the *Cryphonectriaceae* such as *Microthia* has made it possible to show that isolates of *C. eucalypti* group separately from those representing other species in the *Cryphonectria s. str.* clade (Gryzenhout *et al*. 2006d). The new genus *Holocryphia* was thus established for this fungus.

Holocryphia is best known from Australia (Walker *et al*. 1985, Old *et al*. 1986). In that country it occurs both in the east and the west that are ecologically different areas and well separated from each other by a large area of desert. South Africa is the only other country where *H. eucalypti* is known (Van der Westhuizen *et al*. 1993). It is believed that the fungus was introduced into South Africa from Australia (Nakabonge *et al.* 2005), where it is common on *Eucalyptus* growing in natural forests (Walker *et al*. 1985, Old *et al*. 1986).

Fig. 29. Fruiting structures of *Holocryphia eucalypti*. A. Ascostroma on bark. B. Longitudinal section through ascostroma. C. Stromatic tissue. D. Conidioma on bark and sectioned (E). F. Asci and ascospores. G. Conidiophores and paraphyses (arrow). H. Conidiophores. I. Conidia. Scale bars A–B, D = 200 µm; E = 100 µm; C = 20 µm; F–I = 10 µm.

Amphilogia Gryzenh., Glen & M.J. Wingf., Taxon 54: 1017. 2005.

Etymology: Greek, *amphi,* on both sides, and *logos*, discussion, thus the Greek personification of disputes; refers to the fact that in investigating the taxonomical position of this genus the true identity of *Cryphonectria* was questioned.

Ascostromata erumpent, pulvinate, slightly immersed to superficial, orange. *Perithecia* diatrypoid, surrounded by fungal or host tissue, fuscous black, with papillate to long necks surrounded by orange tissue. *Asci* fusoid. *Ascospores* hyaline, fusoid to ellipsoidal, containing one to three irregularly spaced septa.

Conidiomata separate or on top of the ascostromata, also evident occasionally as locules inside ascostroma, conical to pyriform to fluted, superficial, orange, unilocular, non-ostiolate. *Conidiophores* cylindrical or flask-shaped, occasionally with separating septa and branching, *conidiogenous cells* phialidic. *Conidia* hyaline, oblong to slightly curved, aseptate, of variable size, exuded as orange droplets.

Typus: *Amphilogia gyrosa* (Berk. & Broome) Gryzenh., Glen & M.J. Wingf.

Notes: *Amphilogia* is the only genus that has ascospores with more than one septum. Conidiomata are also unique in being conical without an attenuated neck. The conidia are variable in size and can be up to 12 µm long, but some samples may only contain conidia of the smaller range of spores.

The following key is presented to distinguish between the two species in *Amphilogia.* The key is based on the teleomorph since the anamorph of the two species is not easily distinguishable.

1a. Asci up to 55 µm long; ascospores (9–)9.5–11.5(–12) µm long…….. ***A. gyrosa***

1b. Asci longer than 55 µm; ascospores (10.5–)11.5–14(–15.5) µm long….. ***A. major***

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10. ***Amphilogia gyrosa*** (Berk. & Broome) Gryzenh., Glen & M.J. Wingf., Taxon 54: 1017–1018. 2005. Fig. 30.

Basionym: *Diatrype gyrosa* Berk. & Broome, J. Linn. Soc. London 14: 124. 1875.

≡ *Nectria gyrosa* Berk. & Broome, J. Linn. Soc. London 15: 86. 1877.

≡ *Cryphonectria gyrosa* (Berk. & Broome) Sacc., Syll. Fung. 17: 784. 1905.

≡ *Endothia gyrosa* (Berk. & Broome) Höhn., Sitzb. Kais. Akad. Wiss. Wien, Math. Naturw. Kl. 118: 1480. 1909, nom. illegit. Art. 53, non (Schwein.: Fr.) Fr.

= *Endothia tropicalis* Shear & N.E. Stevens, U. S. Dept. Agric. Bull. 380: 20–21. 1917.

Etymology: Greek, *gyros*, circle, thus round.

Ascostromata gregarious on bark, often occurring confluent in cracks, erumpent, pulvinate, slightly immersed to superficial, 460–500 µm high, 660–950 µm diam, orange, prosenchyma at the center, pseudoparenchyma at the edges. *Perithecia* diatrypoid, surrounded with fungal tissue or with bases touching the host tissue, fuscous black, necks breaking through the stromatal surface as papillae or long cylindrical beaks up to 440 µm long, covered with orange tissue. *Asci* (43–)46–52(–

55) × (6–)7–8(–9) µm, fusoid, 8-spored, biseriate. *Ascospores* (9–)9.5–11.5(–12) × (3.5–)4–5(–5.5) µm, hyaline, oval, ends tapered to round, one or two irregularly spaced septa.

Conidiomata above the ascostromata, also appearing as locules inside ascostromata, or as separate conidiomata, conical to pyriform to fluted, orange, superficial, unilocular, 400–890 µm high, 100–370 µm diam, conidiomatal tissue pseudoparenchymatous. *Conidiophores* (10.5–)13–19(–24) µm long, branched irregularly, cells delimited by septa or not, cylindrical to flask-shaped with attenuated apices, hyaline, *conidiogenous cells* (1–)1.5–2.5(–3) µm wide. *Conidia* (3–)4–8.5(–12) × (1.5–)2–2.5(–3.5) µm, hyaline, oblong to slightly curved, aseptate, exuded as orange droplets.

Cultural characteristics: cultures on MEA white when young, often with a luteous center, becoming orange when older, flat and striate with a smooth to sinuous margin, fast growing, covering a 90 mm diam plate after a minimum of 6 days at the optimum temperature of 25–30 ºC.

Substrate: Roots and bark of *Elaeocarpus dentatus*, *Elaeocarpus hookerianus* and *Elaeocarpus glandulifer* (*Oxalidales*, *Elaeocarpaceae*).

Distribution: New Zealand, Sri Lanka.

Specimens examined: **Sri Lanka**, 1868, **holotype** K 109807; Nuwara (Mount) Eliya, *Elaeocarpus glandulifer*, G.H.K. Thwaites, K 109809; Hakgala, *Elaeocarpus glandulifer*, 1913, T. Petch, BPI 614797, BPI 614526. **New Zealand**, Auckland, Atanui State Forest, *E. dentatus*, 1973, G.J. Samuels, PDD 32619, living culture CMW 10471 = CBS 112924; Waitakere Ranges, Spragg's Bush, exposed roots on dead tree, 1973, R.E. Beaver, **epitype** NY 31874, ex-type cultures CMW 10469 =

CBS 112922, CMW 10470 = CBS 112923; Waitakere Ranges, *E. dentatus*, 1958, J.M. Dingley, PDD 18377; Titirangi, unidentified living tree, 1973, J.M. Dingley & G.J. Samuels, NY 30873; Waitakere Ranges, Fairy Falls track, *E. dentatus*, 1963, J.M. Dingley, PDD 21944; Waitakere Ranges, Waiatarua, *E. dentatus*, 1963, J.M. Dingley, PDD 25570; Waitakere Ranges, Cutty Grass track, *E. dentatus* root, 1959, S. McBeth, PDD 28497; Waitakere Dam, *E. dentatus*, 1966, J.M. Dingley, PDD 25003; Waitakere Ranges, Upper Piha Valley, *E. dentatus* fallen trunk, 1949, J.M. Dingley, PDD 28485; Upper Piha, *E. dentatus*, 1947, J.M. Dingley, PDD 28482; Waitakere Ranges, Piha, *E. dentatus*, 1948, J.M. Dingley, PDD 28484; Orere, *E. dentatus*, 1963, S.J. Hughes, PDD 20570; Orere, *E. dentatus*, 1953, J.M. Dingley, PDD 28487; Hanua Ranges, *E. dentatus*, 1953, J.M. Dingley, PDD 28488; Hanua Ranges, Moumoukai Valley, *E. dentatus*, 1932, L.M. Cranwell, PDD 3841; Henderson, off Stony Creek, *E. dentatus* root, 1948, J.M. Dingley, PDD 28483; Henderson, Walker's Bush, *E. dentatus*, 1958, S. McBeth, PDD 28494; Henderson Valley, Sharps Bush, *E. dentatus*, 1972, J.M. Dingley, PDD 29819; Northland, Omahuta State Forest, *E. dentatus*, 1963, S.J. Hughes, PDD 21242; Waipoua, *E. dentatus*, 1955, J.M. Dingley, PDD 28492; Coromandel, Camel's Back 1000', *E. dentatus* exposed root, 1934, J.M. Dingley, PDD 28489; Waikato, Taupiri Mt. 900', *E. dentatus*, 1954, J.M. Dingley, PDD 28491; Buller, Orwell Creek, Granville Forest, *E. hookerianus*, 1963, J.M. Dingley, PDD 23365.

Notes: Specimens from Sri Lanka, earlier treated as the type of *C. gyrosa* (Barr 1978, Gryzenhout *et al*. 2005a), resemble specimens from *Elaeocarpus* spp. in New Zealand (Gryzenhout *et al*. 2005b). No isolates are, however, connected to the specimens from Sri Lanka and no new isolates exist that validly represent this fungus.

Little is known regarding the distribution and host range of *A. gyrosa* in Sri Lanka. Only two localities of collection are known, namely Hakgala and Nuwara (Mount) Eliya, and no hosts were given for the original collections of this fungus (Berkeley & Broome 1875, 1877). Later collections were from *Elaeocarpus glandulifer* under the name *E. tropicalis* (Shear *et al*. 1917). It would thus be difficult to locate the fungus in Sri Lanka and new collections may not necessarily represent *A. gyrosa*.

Amphilogia gyrosa may be confused with *E. gyrosa* because both species bear the same species epithet. These two fungi are, however, distinct as shown by DNA sequence comparisons and the obvious morphological differences that separate them. The geographical range of these species also does not overlap, with *A. gyrosa* occurring in Sri Lanka and New Zealand, while *E. gyrosa* occurs in North America.

Cryphonectria havanensis (syn. *Endothia havanensis*), now *Mi. havanensis*, has been regarded as a synonym of *A. gyrosa* under its previous names (Kobayashi 1970, Hodges 1980). This was due to the similar ascospore ranges noted for the two fungi (Kobayashi 1970, Hodges 1980). Gryzenhout *et al.* (2006d), however, showed conclusively based on morphology and DNA sequence comparisons that these two fungi are distinct.

Fig. 30. Fruiting structures of *Amphilogia gyrosa*. A. Ascostroma on bark with perithecial necks. B. Longitudinal section through ascostroma. C. Stromatic tissue of ascostroma. D. Conidiomata on bark. E. Longitudinal section through conidioma. F. Stromatic tissue of conidioma. G. Asci. H. Ascospores with different septation. I–J. Conidiophores and conidiogenous cells. K–L. Conidia. Scale bars A–B, D–E = 200 µm; C, F = 20 µm; G–L = 10 µm.

11. ***Amphilogia major*** Gryzenh., Glen & M.J. Wingf., Taxon 54: 1018–1019. 2005. Fig. 31.

Etymology: Latin, *major*, greater, referring to the ascospores and asci that are larger in this species than they are in *A. gyrosa*.

Ascostromata gregarious on bark, often confluent, erumpent, pulvinate to tuberculate, slightly immersed to superficial, orange, 1600-1750 µm high, 1050-3050 µm diam, prosenchyma at the center, pseudoparenchyma at the edges. *Perithecia* diatrypoid, surrounded with fungal tissue or with bases touching the host tissue, fuscous black, necks breaking through the stromatal surface as papillae or long cylindrical beaks up to 460 µm long, which are covered with orange tissue. *Asci* (47–)57.5–77(–87.5) × (7.5–)9–11(–12) µm, fusoid, 8-spored, biseriate or uniseriate. *Ascospores* (10.5–)11.5–14(–15.5) × (4.5–)5–6(–6.5) µm, hyaline, oval, ends tapered to round, containing one to three irregularly spaced septa.

Conidiomata on top of ascostromata, also appearing as locules inside ascostromata or separate or individual conidiomata, conical to pyriform, superficial, orange, unilocular, 240–820 µm high, 260–500 µm diam, conidiomatal tissue pseudoparenchymatous. *Conidiophores* (4.5–)8.5–19.5(–32.5) µm long, branched irregularly, cells delimited by septa or not, hyaline. *Conidiogenous cells* cylindrical to flask-shaped with attenuated apices, (1–)1.5–2.5(–3) µm wide. *Conidia* (3–)3.5–7.5(–12) × (1–)1.5–2(–2.5) µm, hyaline, oblong to slightly curved, aseptate, exuded as orange droplets.

Substrate: Roots of *Elaeocarpus hookerianus* and *Elaeocarpus dentatus* (*Oxalidales*, *Elaeocarpaceae*).

Distribution: New Zealand.

Specimens examined: **New Zealand**, Fiordland, Lake Manapouri, *Elaeocarpus hookerianus*, 1948, J.M. Dingley, **holotype** PDD 20056; Westland, Pukekura, *Elaeocarpus dentatus*, 1954, J.M. Dingley, PDD 28490.

Notes: This species was identified based on its ascospores and asci that are larger than those of *A. gyrosa* (Gryzenhout *et al*. 2005b). Its phylogenetic relationship with *A. gyrosa*, based on DNA sequence comparisons, could not be determined because there are no isolates for it. *Amphilogia major* is only known from two locations on the South Island of New Zealand, while *A. gyrosa* is known from both the North and South Islands of the country (Gryzenhout *et al*. 2005b). Surveys may reveal that *A. major* occurs more widely.

Fig. 31. Fruiting structures of *Amphilogia major*. A. Ascostromata on bark with perithecial necks. B. Longitudinal section through ascostroma, with conidial locules indicated with arrows. C. Stromatic tissue of ascostroma. D. Conidioma on bark (arrow). E. Longitudinal section through conidioma. F. Stromatic tissue of conidioma. G. Asci. H. Ascospores with different septation. I–J. Conidiophores and conidiogenous cells. K–L. Conidia. Scale bars A–B, D–E= 200 µm; C, F = 20 µm; G–L = 10 µm.

Rostraureum Gryzenh. & M.J. Wingf., Mycol. Res. 109: 1039–1040. 2005.

Etymology: Latin, *rostrum*, a beak, and *aureus*, golden, referring to the orange, rostrate conidiomata of the fungus.

Ascostromata erumpent, consisting of perithecia embedded in bark tissue, occasionally occurring underneath active pycnidial locules, stromatal tissue absent or present between the necks, luteous-pure yellow to orange. *Perithecia* valsoid, umber to fulvous, necks erumpent, necks surrounded by sheath of white *textura porrecta*, cells on outside of erumpent perithecial necks of *textura globulosa* and orange to luteous-pure yellow. *Asci* fusoid. *Ascospores* hyaline, fusoid to ellipsoidal with rounded apices, 1-septate.

Conidiomata on top or ascostromata or separate structures, clavate or rostrate, superficial to slightly immersed, unilocular, even to strongly convoluted lining, luteous-pure yellow to orange, one to three attenuated necks, base tissue of *textura epidermoidea*, neck tissue of *textura porrecta* with thickened cells at surface. *Conidiophores* hyaline, consisting of a basal cell, branched irregularly at the base or above into cylindrical cells, delimited by septa or not. *Conidiogenous cells* phialidic. *Conidia* cylindrical, hyaline, aseptate, exuded as orange droplets.

Typus: *Rostraureum tropicale* Gryzenh. & M.J. Wingf.

Notes: *Rostraureum* has 1-septate ascospores and is thus similar to *Cryphonectria*, *Microthia*, *Amphilogia*, *Chrysoporthe* and *Celoporthe*. Species of this genus can, however, easily be distinguished from the other genera because of their uniquely rostrate conidiomata. These conidiomata are also often a bright luteous-pure yellow

(yellow) when young and turn orange with age. Ascostromata superficially resemble those of other species in the *Cryphonectriaceae*, but in longitudinal section perithecial necks are covered with a light-coloured sheath or tissue instead of the orange or fuscous-black tissue for other genera. Two species are known from pan-tropical America, namely *R. tropicale* and *R. longirostre*.

The following key is presented to fascilitate identification of the two species of *Rostraureum*:

1a. Conidiomatal bases generally less than 600 µm long with necks up to 1500 µm long; conidia up to 6 µm long ……..…..…… ***R. tropicale***

1b. Conidiomatal bases generally more than 600 µm long with necks up to 2000 µm long; conidia up to 3.5 µm long ……..…….…….. ***R. longirostre***

12. ***Rostraureum tropicale*** Gryzenh. & M.J. Wingf., Mycol Res. 109: 1040–1041. 2005. Fig. 32.

Etymology: Latin, *tropicus*, tropics, refers to the known occurrence of the fungus in the tropics.

Ascostromata semi-immersed with pulvinate appearance under dissection microscope, 540–720 µm wide above bark surface where necks converge, stromatal tissue between perithecia not conspicuous in sections, luteous-pure yellow when young, orange when older. *Perithecia* valsoid, surrounded by host tissue, umber to fulvous, necks umber, surrounded by tissue sheath with the cells next to the perithecial neck white, of *textura porrecta*, and the cells on the outer edge of the sheath orange to luteous-pure yellow, of *textura globulosa*, neck with surrounding tissue up to 700 µm long when it emerges

above bark surface. *Asci* (23–)27–32(–35.5) × (5.5–)6–7.5(–10.5) µm, fusoid, 8-spored. *Ascopores* (4–)6–8.5(–9) × 2–3(–3.5) µm, hyaline, fusoid to ellipsoidal, sometimes slightly curved, ends rounded, single septum median or off-median.

Conidiomata on top of perithecia or separate structures, clavate or rostrate with neck attenuated or not, superficial to slightly immersed, luteous-pure yellow when young, orange when mature, unilocular, even to convoluted lining, base 400–600 µm high, 150–500 µm wide, neck 900–1450 µm long, 100–200 µm wide. Basal tissue of *textura epidermoidea*, tissue at the junction between neck and base of *textura intricata* and neck tissue of *textura porrecta* with thicker cells at edges of neck. *Conidiophores* hyaline, with a globular to rectangular basal cell that is (3–)3.5–6.5(–7) × (2–)2.5–4.5(–6) µm, branched irregularly at the base or above into cylindrical cells, cells delimited by septa or not, total length of conidiophore (12–)15–21(–24.5) µm. *Conidiogenous cells* cylindrical to flask-shaped with attenuated apices, 1.5–2(–2.5) µm wide. *Conidia* (3–)3.5–5(–6) × 1.5–2 µm, hyaline, cylindrical, aseptate, exuded as brick red spore droplets.

Cultural characteristics: cultures on MEA suppressed with sparse aerial hyphae when young, remaining suppressed when older, young cultures creamy white with a luteous interior, older cultures are orange to luteous with or without white margins, margins even, isolates covering 90 mm diam plates after 6 days at the optimum growth temperatures of 25–30 °C.

Substrate: bark of *Terminalia ivorensis* and *Terminalia superba.*

Distribution: Ecuador.

Specimens examined: **Ecuador**, Pichincha, Río Pitzara (0° 15′ 27″ N 79° 7′ 43″ W, 350 masl), *Terminalia ivorensis*, Nov. 2001, M.J. Wingfield, **holotype** PREM 57519,

ex type cultures CMW 9972, CMW 10796 = CBS 115757; PREM 583301, PREM 583302, PREM 583303, PREM 583304, living culture CMW 9971 from PREM 583301.

Notes: This species was discovered due to its association with dying *Terminalia* trees in Ecuador (Gryzenhout *et al*. 2005d). The other species in *Rostraureum*, *R. longirostre*, is only known as a saprophyte. *Terminalia* trees are native to Africa but planted in South America for timber production. An *Endothiella* species has been reported on *Terminalia* trees in Africa, but was shown not to be *Rostraureum* (Gryzenhout *et al.* 2005d). It thus appears likely that *R. tropicale* was not introduced from Africa into Ecuador on *Terminalia* trees, but probably represent a native fungus that has undergone a host shift to this exotic host (Slippers *et al*. 2005).

Fig. 32. Fruiting structures of *Rostraureum tropicale*. A. Ascostroma on bark. B. Longitudinal section through ascostroma also showing conidioma (indicated by arrow). C. Stromatic tissue of ascostroma. D. Conidioma on bark and sectioned (E). F. Tissue surrounding perithecial neck. G. Tissue at base of conidioma. H. Tissue of conidioma where neck begins. I. Tissue of conidiomatal neck. J. Ascus. K. Ascospores. L. Conidiophore. M. Conidia. Scale bars A–B, D–E= 200 µm; C, F–I = 20 µm; J–M = 10 µm.

13. ***Rostraureum longirostre*** (Earle) Gryzenh. & M.J. Wingf., Mycol. Res. 109: 1041–1042. Fig. 33

Basionym: *Endothia longirostris* Earle, *Muehlenbergia* **1**: 14 (1901).

≡ *Cryphonectria longirostris* (Earle) Micales & Stipes, *Phytopathology* **77**: 651 (1987).

Etymology: Latin, *longus*, long, and *rostrum*, beaked.

Ascostromata semi-immersed, pulvinate, 700–950 µm wide above bark surface, orange, prosenchymatous stromatal tissue usually present in erumpent part of stromata and containing conidial locules and perithecial necks. *Perithecia* valsoid, bases surrounded by host tissue, umber to fulvous, necks umber, surrounded by tissue sheath with the cells alongside the perithecial necks white, of *textura porrecta*, and cells at the outer edge of sheath luteous-pure yellow to orange, of *textura globulosa*; necks with surrounding tissue up to 650 µm long where they emerge above bark surface. *Asci* 25–30 × 6 µm, spindle-shaped, 8-spored (Earle 1901). *Ascopores* (5–)6–7.5(–9) × 2–3(–3.5) µm, hyaline, fusoid to ellipsoidal, ends rounded, single septum median or off-median.

Conidiomata occurring on top of ascostromata or as separate structures, clavate or rostrate with necks attenuated or not, superficial to slightly immersed, orange, unilocular and convoluted, bases 600–1300 µm high, 270–880 µm wide, necks 1010–2050 µm long, 170–290 µm wide. Base tissue of *textura epidermoidea*, tissue where neck and base join of *textura intricata*, neck tissue of *textura porrecta* with thicker cells at edges of neck. *Conidiophores* hyaline, with a globular to rectangular basal cell that is (2–)3–5(–7.5) × (1.5–)2.5–3.5(–4.5) µm, branched irregularly at the base or above into cylindrical cells, cells delimited by septa or not,

total length of conidiophore (13–)15–19.5(–22.5) µm. *Conidiogenous cells* cylindrical to flask-shaped with attenuated apices, 1.5–2(–2.5) µm wide. *Conidia* 3–3.5 × 1.5 µm, hyaline, cylindrical, aseptate, exuded as brick red spore droplets.

Cultural characteristics: no isolates currently exists for this fungus. Shear *et al*. (1917) did growth studies on various media, and reported that cultures are initially white, but turns orange with age.

Substrate: dead logs and branches.

Distribution: Puerto Rico, French Guiana, and Trinidad & Tobago.

Specimens examined: **Puerto Rico,** east of Santurce, bark of fallen tree, 19 Jan. 1900, A.A. Helle, **holotype** NY4340; bark, 24 Jan.–5 Apr. 1923, F.J. Seaver & C.E. Chardon, NY 617; Naguabo, fallen bark, 25 Mar. 1915, N. Wille, NY 816; Rio Piedras, 18 Jun. 1917, J.A. Stevenson & R.C. Rose, NY 6576. **Trinidad & Tobago**, Ortoire river, Guayaguayare road, bark, 25 Mar. 1921, F.J. Seaver, NY 3320.

Notes: No cultures exist for this fungus. *Rostraureum longirostre* was distinguished from *R. tropicale* largely because of its smaller conidia. It occurs on various Caribbean islands and efforts to obtain isolates for this fungus should thus focus in that area. Gryzenhout *et al*. (2005d) also showed that a number of other, morphologically similar but distinct and undescribed fungi also occur in the same areas that could represent new records in the *Cryphonectriaceae*.

Fig. 33. Fruiting structures of *Rostraureum longirostre*. A. Ascostroma on bark. B. Longitudinal section through ascostroma also showing conidioma (indicated with arrow). C. Stromatic tissue of ascostroma. D. Conidioma on bark (indicated with arrow) also showing short perithecial necks. E. Longitudinal section of conidioma. F. Tissue surrounding perithecial neck. G. Tissue at base of conidioma. H. Tissue of conidioma where neck begins. I. Tissue of conidiomatal neck. J. Ascospores. K. Conidiophores. L. Conidia. Scale bars A–B, D–E = 200 µm; C, F–I = 20 µm; J–L = 10 µm.

Ursicollum Gryzenh. & M.J. Wingf., **nom. prov.,** Stud. Mycol. 55 (in press). 2006. Mycobank MBxxx.

Etymology: Latin, *ursus*, a bear, and, *collus*, neck, referring to the hairs on the conidiomatal necks.

Conidiomata pyriform or rostrate, superficial to slightly immersed in bark, unilocular, strongly convoluted lining, orange, one to three attenuated or cylindrical necks, tissue pseudoparenchymatous but prosenchymatous in neck. *Conidiophores* cylindrical, delimited by septa or not, branching, *conidiogenous cells* phialidic. *Conidia* hyaline, cylindrical, aseptate, exuded as orange spore droplets.

Typus: *Ursicollum fallax* Gryzenh. & M.J. Wingf.

Notes: The conidiomata with cylindrical necks on a pulvinate base can easily be confused with ascostromata. Conidiomatal morphology is also variable with conidiomata ranging from rostrate to pyriform to pulvinate with hairy cylindrical necks. These are also the key characteristics distinguishing *Ursicollum* from other genera of the *Cryphonectriaceae*. The rostrate and pyriform forms of the genus resemble conidiomata of *Rostraureum*, but the fungus can be distinguished from species of *Rostraureum* based on the different shape of the conidiogenous cells and different tissue arrangement. No teleomorph has been found for this fungus which is also monotypic, but phylogenetic data show clearly that this genus groups in the *Cryphonectriaceae*.

14. ***Ursicollum fallax*** Gryzenh. & M.J. Wingf., **nom. prov.**, Stud. Mycol. 55 (in press). 2006. Mycobank MBxxx. Fig. 34.

Etymology: Latin, *fallax*, false, referring to the conidiomata that have the appearance of ascostromata.

Conidiomata pyriform to rostrate, superficial to slightly immersed, orange, with one to three attenuated or cylindrical necks, base 120–400 µm high, 190–550 µm diam, neck up to 400 µm long, 90–180 µm wide, unilocular, convoluted lining. Basal *tissue* prosenchymatous at the center and pseudoparenchymatous at the edge, neck tissue prosenchymatous with cells around the ostiolar canal darker. *Conidiophores* (4.5–)5.5–19(–39) µm long, cylindrical with or without attenuated apex, cells delimited by septa or not, cylindrical to flask-shaped with attenuated apices, hyaline, *conidiogenous cells* 1.5–2(–2.5) µm wide. *Conidia* (2.5–)3–4(–5.5) × (1–)1.5(–2) µm, hyaline, cylindrical, aseptate, exuded as orange droplets.

Cultural characteristics: cultures on MEA white, fluffy, margins even, isolates covering 90 mm diam plates after 5–6 days at optimum growth temperatures of 25–30 °C.

Substrate: bark of *Coccoloba uvifera.*

Distribution: Florida (U.S.A.).

Specimens examined: **U.S.A.**, Florida, Fort Lauderdale, *Coccoloba uvifera*, 8 Mar. 2005, C.S. Hodges, **holotype** PREM 58840, ex-type culture CMW 18119 = CBS 118663; Key Biscayne, *Coccoloba uvifera*, 10 Mar. 2005, C.S. Hodges, PREM 58841, PREM 58842, living cultures CMW 18115 = CBS 118660, CMW 18124 = CBS 118662; Oakland Park, *Coccoloba uvifera*, 11 Mar. 2005, C.S. Hodges, PREM

58843, living culture CMW 18114 = CBS 11866; Dania, *Coccoloba uvifera*, 11 Mar. 2005, C.S. Hodges, PREM 58844, living culture CMW 18110 = CBS **.

Notes: In Florida, *U. fallax* occurs on *Coccoloba uvifera* together with *M. coccolobae* (Barnard *et al*. 1993?, Gryzenhout *et al*. 2006d). The two species can easily be distinguished from each other because *M. coccolobae* has pulvinate conidiomata without necks and prominent paraphyses, while *U. fallax* has rostrate to pulvinate conidiomata with necks and no paraphyses (Gryzenhout *et al*. 2006d). However, the teleomorph of *U. fallax* is unknown and it could be that the teleomorphs of *U. fallax* and *M. coccolobae* are morphologically similar. Furthermore, it is also possible that reports of *M. coccolobae* in Florida actually represent *U. fallax*, which would imply that *M. coccolobae* does not occur in that area (Gryzenhout *et al*. 2006d).

Fig. 34. Fruiting structures of *Ursicollum fallax*. A. Conidioma on bark. B–C. Longitudinal sections through conidiomata. D. Tissue in middle and at edge (E) of conidioma. F. Tissue of neck showing darker tissue around ostiolar canal (indicated with arrow). G–H. Conidiophores. I. Conidia. Scale bars A–C = 100 µm; D–F = 20 µm; G–I = 10 µm.

Aurapex Gryzenh. & M.J. Wingf., Mycologia 98 (in press). 2006.

Etymology. Latin, *aureus*, golden, and *apex*, top, referring to the orange necks of the conidiomata.

Conidiomata globose to pyriform fuscous black base with one to several, long, cylindrical to attenuated necks with orange tips, superficial to slightly immersed. *Tissue* of base of *textura globulosa* at edge, elongated cells at conidial lining and prosenchymatous tissue occurring in between, tissue of neck of *textura porrecta* with cells lining the ostiole thinner, cells at edge of neck consisting of square cells. *Conidiophores* cylindrical to flask-shaped, hyaline, occasionally septate with or without lateral branches, *conidiogenous cells* phialidic. *Conidia* hyaline, obtuse, aseptate, exuded as scarlet droplets.

Typus: *Aurapex penicillata* Gryzenh. & M.J. Wingf.

Notes: Fruiting structures of *Aurapex* can easily be distinguished from those of other genera because of the orange neck and fuscous black base, scarlet spore drops, unique tissue arrangement, periphyses in the ostiolar canal and protrusions of the inner surface of the conidial locules. It resembles *Chrysoporthella* most closely because of its fuscous black conidiomata of similar shape. Moreover, when the characteristic orange necks of *Aurapex* break off, the fungus can easily be confused with anamorph structures of *Chrysoporthe* spp. that occur in the same areas and on the same substrates. Detailed microscopic examination will, however, distinguish between *Aurapex* and *Chrysoporthella*.

This genus is monotypic. No teleomorph is known for it although it clearly resides in the *Cryphonectriaceae* based on previous DNA sequence comparisons (Gryzenhout *et al.* 2006e) and those presented in this monograph.

15. ***Aurapex penicillata*** Gryzenh. & M.J. Wingf., Mycologia 98 (in press). 2006. Fig. 35

Etymology. Latin, *penicillum*, a painter's brush, because of the brush-like protrusions formed by the conidial locule lining.

Conidiomata single or aggregated, globose to pyriform base with attenuated or cylindrical necks, base 120–400 µm high, 300–700 µm wide above bark surface, necks up to 1839 µm long depending on environmental conditions, 80–225 µm wide, conidiomata superficial to slightly immersed, bases fuscous-black with tips of necks orange, unilocular or multilocular, locule lining that produces the conidiophores forming protrusions consisting of 3 to c. 15 cells, locules opening through 1 to 3 necks, each either connected to a single locule or to more than one locule. *Tissue* of base complex with cells thick-walled, of *textura globulosa*, umber to sienna at edge, cells around the locules sienna to hazel, larger and more elongated, and almost white prosenchymatous tissue occurring between the edge and the locule, neck tissue consisting of hazel, double-walled, square cells at the edge, of *textura porrecta* tissue within the necks with cells lining the ostiole thinner, long, aseptate filaments, similar to periphyses, occurring inside the ostiolar canals, tip of necks with loosened cells, of *textura epidermoidea*, containing orange crystals. *Conidiophores* (6–)7.5–13.5(–18.5) µm long, occasionally with separating septa and branching, cylindrical or flask-shaped with attenuated apices, hyaline, *conidiogenous cells* (0.5–)1–1.5(–2) µm wide.

Conidia (2.5–)3–4(–4.5) × 1–1.5(–2) µm, hyaline, obtuse, aseptate, exuded as scarlet spore droplets.

Cultural characteristics: cultures on MEA fluffy with few aerial hyphae, creamy white with a dark olivaceous to isabelline interior, margins even, isolates covering the surface of plates after 6 days as the optimum temperature of 25 ºC.

Substratum: *Miconia theaezans, Tibouchina urvilleana, Tibouchina lepidota, Eucalyptus grandis.*

Distribution: Colombia.

Specimens examined: **Colombia**, Risaralda, Pereira, Libano farm (75º 35′ 49″ W and 4º 43′ 13″ N, 2102 msal), *Miconia theaezans*, Sep. 2002, C.A. Rodas, **holotype** PREM 57520, ex-type culture CMW 10030 = CBS 115740, living cultures CMW 10031, CMW 10034, CMW 10035 = CBS 115742; Quindio, Salento, Andes farm (75° 33′ 16″ W and 4° 41′ 08″ N, 2102 masl), *Miconia theaezans*, May 2000, M.J. Wingfield, PREM 58576, living cultures CMW 11296 = CBS 115801; Risaralda, Pereira, La Selva farm (75º 35′ 34″ W and 4º 47′ 26″ N, 2048 msal), *Miconia theaezans*, Nov. 1998, C.A. Rodas, PREM 58572; Libano farm (75º 35′ 49″ W and 4º 43′ 13″ N, 2102 msal), *Eucalyptus grandis*, Sep. 2002, C.A. Rodas, PREM 58578; Antioquia, Granada, Granada farm (75° 8′ 10″ W and 6° 6′ 52″ N, 2050 msal), *Tibouchina urvilleana*, Nov. 1998, C.A. Rodas, PREM 58573; Caldas, Riosucio, La Argentina farm (75º 44′ 55″ W and 5º 22′ 25″ N, 2247 masl), *Tibouchina urvilleana*, Nov. 1998, C.A. Rodas, PREM 58574, PREM 58575; Valle, Darien, Cedral farm (76º 26′ 06″ W and 3º 57′ 06″ N, 1825 masl), *Eucalyptus grandis*, Dec. 2001, C.A. Rodas, PREM 58577.

Notes: *Aurapex penicillata* occurs on the same hosts than *Chr. cubensis*, *Chr. inopina* and *Chrysop. hodgesiana* in Colombia (Rodas *et al*. 2005, Gryzenhout *et al*. 2006e), i.e. species of *Miconia*, *Tibouchina* (*Melastomataceae*) and *Eucalyptus* (*Myrtaceae*). It is more common and abundant on the native melastomes than these *Chrysoporthe* spp. *Aurapex penicillata* can be distinguished from the *Chrysoporthe* spp. based on a number of distinct morphological characteristics (Gryzenhout *et al*. 2006e), most important of which is the orange necks and brick red spore drops.

Fig. 35. Fruiting structures of *Aurapex penicillata*. A. Conidiomata on bark and in section (B). C. Tissue at base of conidioma showing thickened cell walls at edge. D. Darker and lighter (indicated with arrow) inner tissue of base. E. Tissue of neck. F. Tissue of neck apex. G. Periphyses in ostiolar canal. H–I. Protrusions in locule lining. J–K. Conidiophores. L. Conidia. Scale bars A–B = 100 µm; C–F = 20 µm; G–L = 10 µm.

Chrysoporthe Gryzenh. & M.J. Wingf., Stud. Mycol. 50: 129. 2004. MycoBank MB500032

Anamorph: Chrysoporthella Gryzenh. & M.J. Wingf.

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

Ascostromata consisting of black, valsoid perithecia embedded in bark tissue, 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. *Perithecia* valsoid, surrounded by bark tissue, fuscous black, necks long, cylindrical, covered with umber tissue as they protrude through the bark surface, thus appearing fuscous-black. *Asci* fusoid to ellipsoidal. *Ascospores* hyaline, fusoid to oval, with one septum in variable, usually median, position.

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* in the basal region of *textura globulosa* and 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. *Conidia* hyaline, oblong, aseptate, exuded as bright luteous or pale luteous spore tendrils or droplets.

Typus: *Chrysoporthe cubensis* (Bruner) Gryzenh. & M.J. Wingf.

Notes: *Chrysoporthe* can be distinguished from other genera of the *Cryphonectriaceae* by its characteristic fuscous black, pyriform conidiomata that strongly resemble pycnidia on the bark (Myburg *et al*. 2004a, Gryzenhout *et al.* 2004). Only *Celoporthe* and *Aurapex* have similar fuscous black conidiomata, but they can be distinguished from *Chrysoporthe* based on characteristics that have been treated under the sections dealing with these fungi. Furthermore, *Chrysoporthe* spp. are unique because their perithecial necks are covered with dark tissue, and ascostromata are typically orange with long, fuscous black perithecial necks protruding from the stromata (Myburg *et al*. 2004a, Gryzenhout *et al.* 2004). When these necks break off, the orange ascostromata can, however, easily be confused with ascostromata of other genera such as *Microthia* and *Cryphonectria*, which also have single septate ascospores similar to *Chrysoporthe*.

Chrysoporthe cubensis, previously known as *Cryphonectria cubensis*, was recently sub-divided into several species and transferred to the new genus *Chrysoporthe* (Gryzenhout *et al*. 2004). The fungus was first described in *Diaporthe* (Bruner 1917), but it also had characteristics of *Endothia*, the genus with which *Cryphonectria* was synonymous to at that stage. Bruner (1917) used *Diaporthe* for the fungus because of its pyriform, fuscous black conidiomata. The species was transferred to *Cryphonectria* by Hodges (1980), who argued that pathology, cultural characteristics and morphological characters such as a thin layer of orange mycelium tissue was characteristic of *Cryphonectria*. DNA sequence data has, however, revealed that *Cryphonectria cubensis* should reside in a distinct genus (Myburg *et al*. 2004a), leading to the description of *Chrysoporthe* (Gryzenhout *et al.* 2004).

Chrysoporthe cubensis is the best known species in the genus and has been intensively studied during the last two and a half decades (Wingfield 2003). It is thus known from various countries in tropical to sub-tropical America, Africa, Asia and Australia (Gryzenhout *et al*. 2004). Other species of *Chrysoporthe* have only recently been described and are known from areas much more limited than *Chr. cubensis*. *Chrysoporthe austroafricana* is known only from Africa (Gryzenhout *et al*. 2004, Nakabonge *et al.* 2006b), *Chr. inopina* (Gryzenhout *et al*. 2006c) and *Chrysop. hodgesiana* (Gryzenhout *et al*. 2004) are known only from Colombia, and *Chr. doradensis* is known only from Ecuador (Gryzenhout *et al*. 2005c). These species were discovered during surveys on exotic and native trees in the countries in which they occur and through subsequent DNA sequence comparisons.

Chrysoporthe represents a complex of species typically causing stem canker diseases on native trees (Rodas *et al*. 2005, Gryzenhout *et al*. 2006c). For instance, three species are known to occur on native trees in Colombia (Rodas *et al*. 2005, Gryzenhout *et al*. 2006c). These species occur in the same areas and hosts, and may even be found on the same tree. Isolations of these fungi should thus be done systematically and in such a way that isolates can be linked to structures on herbarium material. This is especially important since it is likely that more *Chrysoporthe* species will be discovered in the future.

Chrysoporthella Gryzenh. & M.J. Wingf., anam. gen., Stud. Mycol. 50: 130. 2004. MycoBank MB500033.

Etymology: diminutive of *Chrysoporthe*, used for the anamorph structures that commonly occur independently of the teleomorph.

Conidiomata occurring separately or on top of ascostromata, distinguishable from ascostromata 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. *Conidia* hyaline, oblong, aseptate, masses exuded as bright luteous or pale luteous tendrils or droplets.

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

Notes: *Chrysoporthella* is the only new anamorph genus that has been described for a teleomorph genus in the *Cryphonectriaceae*. Description of this genus was necessary in order to provide a name for *Chrysop. hodgesiana*, a species without a teleomorph (Gryzenhout *et al*. 2004). The anamorphs of other species in *Chrysoporthe* are, however, referred to by the teleomorph name.

The following dichotomous key is based on characteristics of the spores, asci and growth in culture:

1a. Optimal growth at 30 ºC…… 2

1b. Optimal growth at 25 ºC ……...… 4

2a. Ends of ascospores tapered, asci shorter than 28 µm ..…… 3

2b. Ends of ascospores rounded, asci longer than 28 µm …….. ***Chr. austroafricana*** (p. 546)

3a. Conidia oblong, (3–)3.5–4.5(–5) µm long, spore mass bright luteous (yellow) ……..... *Chr. cubensis* (p. 539)

3b. Conidia oblong, ovoid, cylindrical or allantoid, (3–)3.5–5(–6.5) µm long, spore mass pale luteous (cream) ……..…… ***Chr. doradensis*** (p. 551)

4a. Conidiomata generally pulvinate, occasionally with short attenuated neck; teleomorph unknown ……...……..…. ***Chrysop. hodgesiana*** (p. 559)

4b. Conidiomata subulate to pyriform to pulvinate, with neck attenuated or not; ascospores with rounded ends, asci longer than 28 µm …...…… ***Chr. inopina*** (p. 555)

A synoptic key is also presented that should fascilitate identification of *Chrysop. hodgesiana* in the absence of its teleomorph or where evaluating optimal growth temperature is not an assessable technique:

Optimum growth:

a. 25 ºC: *Chr. inopina*, *Chrysop. hodgesiana*

b. 30 ºC: *Chr. cubensis*, *Chr. austroafricana*, *Chr. doradensis*

Ascus size:

a. Asci shorter than 28 µm: *Chr. cubensis*, *Chr. doradensis*

b. Asci longer than 28 µm: *Chr. austroafricana*, *Chr. inopina*

Ascospore morphology:

a. Ends tapered: *Chr. cubensis*, *Chr. doradensis*

b. Ends rounded: *Chr. austroafricana*, *Chr. inopina*

c. Ascospore width less than 3 µm: *Chr. cubensis*, *Chr. austroafricana*, *Chr. doradensis*

d. Ascospore width more than 3 µm: *Chr. inopina*

Conidial morphology:

a. Conidia oblong only: *Chr. cubensis*, *Chr. austroafricana*, *Chr. inopina*, *Chrysop. hodgesiana*

b. Conidia also of other shapes than oblong, such as ovoid, allantoid or cylindrical: *Chr. doradensis*

c. Spore mass bright luteous (yellow): *Chr. cubensis, Chr. austroafricana, Chr. inopina*, *Chrysop. hodgesiana*

d. Spore mass pale luteous (cream): *Chr. doradensis*

Conidiomatal shape:

a. Pyriform to pulvinate, with attenuated necks: *Chr. cubensis, Chr. austroafricana, Chr. doradensis*, *Chr. inopina*, *Chrysop. hodgesiana*

b. Occasionally subulate, without neck attenuated: *Chr. inopina*

Notes: *Chrysoporthe* includes species that are morphologically very similar, with morphological distinction further obscured by variation that occurs between samples

or the absence of an anamorph or teleomorph. Species are differentiated by spore morphology that is subtle and species can often only be conclusively identified based on DNA sequence comparisons. In the latter case, the different species are very closely related and are defined by signature single base pair differences.

Differences between species are mostly in the shape of the ascospore apices and size of the asci. Conidial morphology is only useful to distinguish *Chr. doradensis* from other species. Ascospore and conidial size is relatively uniform throughout the genus, except for subtle differences that can be used in conjunction with other characteristics, such as optimum growth, for identification.

It is difficult to distinguish *Chrysop. hodgesiana* from other species since the teleomorph of this species is unknown and the anamorph structures are similar to those of the other species. The only distinctive difference between *Chrysop. hodgesiana* and other species of *Chrysopothe* is its optimum growth at 25 ºC, similar to that of *Chr. inopina*. *Chrysop. hodgesiana* and *Chr. inopina* can be distinguished because *Chr. inopina* occasionally has conidiomata that are subulate in shape with the necks not attenuated (Gryzenhout *et al*. 2006c). Although the shape of the conidiomata can be quite distinctive for a particular sample, it has been found to vary between samples from different areas. Thus unique shapes may be absent from a sample requiring identification. Since this is the only way to currently distinguish between *Chrysop. hodgesiana* and *Chr. inopina* based on morphology, conidiomatal shape has been included as a character in keys presented in this study despite the fact that this character can be misleading and variable.

Morphology is not always sufficiently reliable to identify the species of *Chrysoporthe*. In some cases identification may be impossible because teleomorph structures are absent. Here, DNA sequence comparisons are recommended to aid in

identifications. For this purpose, the β-tubulin gene region contains sufficient variation, and sequencing of the β-tubulin 2 region can be used for preliminary identification, although additional characters from the ITS region will be useful.

16. ***Chrysoporthe cubensis*** (Bruner) Gryzenh. & M.J. Wingf., Stud. Mycol. 50: 130–133. 2004. MycoBank MB500034. Fig. 36.

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.

Etymology: from Cuba.

Ascostromata 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. *Perithecia* valsoid, bases immersed in bark, fuscous black, 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, necks emerging through bark covered in umber stromatic tissue of *textura porrecta*, appearing fuscous-black, extending necks up to 240 µm long. *Asci* (19–)22–26.5(–28) × (4.5–)5–6.5(–7) µm, fusoid to ellipsoidal, 8-spored. *Ascospores* (5.5–)6.5–7.5(–8) × 2–2.5(–3) µm, hyaline, 1-septate, fusoid to oval, tapered ends, with septum variously placed in the spore but usually central.

Conidiomata occurring separately or on top of an ascostroma, pyriform to clavate, sometimes pulvinate, superficial to slightly immersed, with one to four attenuated necks per structure, 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. *Stromatic tissue* at base of *textura globulosa* with walls of outer cells thickened, and neck cells of *textura porrecta*. *Conidiophores* hyaline, with a globular to rectangular basal cell that is (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. *Conidiogenous cells* cylindrical to flask-shaped with attenuated apices, (1.5–)2–2.5(–3) µm wide. *Conidia* hyaline, oblong, aseptate, (3–)3.5–4.5(–5) × (1.5–)2(–2.5) µm, exuded as bright luteous spore tendrils or droplets.

Cultural characteristics: white with cinnamon to hazel patches on MEA, fluffy, margins smooth, fast-growing, covering a 90 mm diam plate after a minimum of 5 days at the optimum temperature of 30 ºC.

Substrate: Bark of *Eucalyptus* spp., *Syzygium aromaticum* or clove (*Myrtaceae*, *Myrtales*), *Miconia* spp, *Rhynchanthera mexicana*, *Clidemia sericea*, *Melastoma melabathricum*, *Tibouchina urvilleana* (*Melastomataceae*, *Myrtales*). Also reported (Hodges 1988) from *Psidium cattleianum* (*Myrtaceae*).

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), Kenya, Malawi, Mozambique, Indonesia, Malaysia, Singapore, China, India, Australia, Western Samoa.

Specimens examined: **Cuba**, Santiago de las Vegas, *Eucalyptus botryoides*, 1916, C.L. Shear, **holotype** BPI 631857; *Eucalyptus grandis*, Jan. 2004, M.J. Wingfield, **epitype** PREM 58788, ex-type isolate CMW 14394 = CBS 118654, living isolate CMW 14404 = CBS 118647, 60 km east from Habano, *Eucalyptus grandis*, Jan. 2004, M.J. Wingfield, PREM 58789, living culture CMW 14378 = CMW 118655; El Cerro Municipality, *Eucalyptus saligna*, Jan. 2004, M.J. Wingfield, PREM 58790, living culture CMW 14362 = CMW 118657; road to Havana, *Eucalyptus urophylla*, Jan. 2004, M.J. Wingfield, PREM 58791, living culture CMW 14395 = CBS 118648; Habano, *Lagerstroemia indica*, Jan. 2004, M.J. Wingfield, PREM 58792, living culture CMW 16199 = CBS 118652. **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; *Miconia rubiginosa*, 2001, C.A. Rodas, PREM 57517, PREM 58307, PREM 58308, living isolates from same area CMW 9996 = CBS 115731, CMW 10024 = CBS 115739. **Mexico**, Tabasco, *Eucalyptus* sp., 2000, M.J. Wingfield, PREM 57295, PREM 58016, culture from same area CMW 9432 = CBS 115724; unknown, *Rhynchanthera mexicana*, 2002, F. Ferreira, PREM 58793, living cultures CMW 12734, CMW 12736, PREM 58794, living cultures CMW 12734 = CBS 115853, CMW 12736 = CBS 115847; unknown, *Clidemia sericea*, 2002, F. Ferreira, PREM 58795, living culture CMW 13046 = CBS 115762, PREM 58796, living culture CMW 12471 = CBS 115849. **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*, 1974, C.S. Hodges, IMI 184653; Minas Gerais, Dionisio, *Eucalyptus* sp.,

1973, C.S. Hodges, IMI 172718; Minas Gerais, Coronel Fabriciano, *Eucalyptus propinqua*, 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*, 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*, 1978, C.S. Hodges, DAR 35434, culture from same area CMW 1856. **Singapore,** Istana grounds, *Syzygium aromaticum*, 1991, C.P. Yik, dried culture IMI 350626; unknown, *Tibouchina urvilleana*, Apr. 2003, M.J. Wingfield, PREM 58797, living culture CMW 12745 = CBS 117837. **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; Sumatra, Lake Toba, *Melastoma malabathricum*, May 2005, M.J. Wingfield, PREM 58799, living culture CMW 18515 = CBS 118651, Lake Toba, Aek Nauli, *Melastoma malabathricum*, Feb. 2004, M.J. Wingfield, PREM 58798, living culture CMW 16192 = CBS **. **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*, 1980, F.B. Armitage, IMI 249406.

Notes: *Chrysoporthe cubensis* can be distinguished from the other species based on ascospore and ascus morphology. Only *Chr. doradensis* has similar asci and ascospores. These two species can be distinguished from each other based on the uniform conidial morphology and bright luteous conidial masses of *Chr. cubensis*, while *Chr. doradensis* has conidia of variable shape and size, which are discharged in pale luteous drops.

Isolates of *Chr. cubensis* reside in two phylogenetic sub-clades (Gryzenhout *et al*. 2004). One of these includes isolates from South and Central America, and western Africa (Myburg *et al.* 1999, 2002a, 2003, Roux *et al*. 2003, Nakabonge *et al*. 2006b). Those in the other sub-clade are all from South East Asian countries, Australia, Hawaii, and eastern Africa (Myburg *et al.* 1999, 2002a, 2003, Nakabonge *et al*. 2006b). Representative specimens from these different sub-clades are morphologically identical, although they can be distinguished based on DNA

sequence data and their occurrence in different geographical areas of the world (Gryzenhout *et al.* 2004).

Isolates of *Chr. cubensis* in the two sub-clades most likely represent fungi in the process of speciation (Gryzenhout *et al.* 2004, 2006c). Population-level techniques are being applied to resolve the relationship between isolates in the two sub-clades (Van der Merwe *et al.* 2003a, 2003b). If it is shown that the two phylogenetic groups represent distinct species, the name of *Chr. cubensis* will be retained for the sub-clade containing the type from Cuba. No isolates are connected to the type from Cuba, but an epitype from the same area has recently been designated for *Chr. cubensis* (Gryzenhout *et al.* 2006c). Ex-epitype isolates have been shown to reside in the South American sub-clade (Gryzenhout *et al.* 2006c), and this sub-clade would thus represent *Chr. cubensis s. str.*

Until relatively recently, *Chr. cubensis* has been known only to occur on *Eucalyptus* spp. and *S. aromaticum* (clove), both myrtaceous hosts. Surveys conducted during the past decade have led to the discovery of numerous new hosts for this fungus and most of these reside in the *Melastomataceae* (Rodas *et al.* 2005, Gryzenhout *et al*. 2006c). The majority of these new hosts are native to the countries in which they have been found. This radically changed our understanding of the ecology of this well-known *Eucalyptus* pathogen, which was regarded for a long time as a fungus occurring primarily on *Eucalyptus* spp. and *S. aromaticum*. It has lead to new ideas regarding the origin and world-wide movement of the fungus (Gryzenhout *et al*. 2006c).

Fig. 36. Fruiting structures of *Chrysoporthe cubensis*. A. Ascostroma on bark. B. Black perithecial necks and orange stromatic tissue (dash arrow) of ascostroma on bark, with conidioma on top (arrow). C–D. Conidiomata of different shapes on bark. E. Vertical section through ascostroma. F. Vertical section through conidiomata. G. Stromatic tissue of ascostroma. H. Perithecial neck and surrounding tissue (arrow). I. Tissue of the conidiomal base and neck (arrow). J. Ascus. K. Ascospores. L–M. Conidiophores. N. Conidia. Scale bars A–F = 100 µm; G–I = 20 µm; J–N = 10 µm.

17. ***Chrysoporthe austroafricana*** Gryzenh. & M.J. Wingf., Stud. Mycol. 50: 133–135. 2004. MycoBank MB500035. Fig. 37.

Etymology: Latin, *australis*, southern, referring to the southern African occurrence of this fungus.

Ascostromata 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. *Perithecia* valsoid, bases immersed in the bark, fuscous black, 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, necks emerging through the bark covered in umber, stromatic tissue of *textura porrecta*, thus appearing fuscous-black, extending necks up to 1900 µm long. *Asci* (25–)27–32(–34) × (4–)5.5–7(–7.5) µm, fusoid to ellipsoidal, 8-spored. *Ascospores* (5.5–)6–7 × (2–)2.5 µm, hyaline, fusoid to oval, with rounded end, 1-septate with septum variously placed in the spore but usually central.

Conidiomata occurring separately or on top of an ascostroma pyriform to clavate, sometimes pulvinate, superficial to slightly immersed, with one to four attenuated necks per structure, 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. *Stromatic tissue* of base of *textura globulosa*, the walls of outer cells thickened, neck tissue of *textura porrecta*. *Conidiophores* hyaline, with basal cells of irregular shape that is (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. *Conidiogenous cells* cylindrical to flask-shaped with attenuated apices, (1–)1.5–2.5(–3.5) µm wide. *Conidia* 3–4(–4.5) × 1.5–2 µm, hyaline, oblong, aseptate, exuded as bright luteous tendrils or droplets.

Cultural characteristics: white with cinnamon to hazel patches on MEA, fluffy with a smooth margin, fast-growing, covering a 90 mm diam plate after a minimum of 5 days at the optimum temperature of 30 ºC.

Substrate: bark of *Eucalyptus* spp., *Syzygium* spp. (*Myrtaceae*, *Myrtales*) and *Tibouchina granulosa* (*Melastomataceae*, *Myrtales*).

Distribution: South Africa, Mozambique, Malawi and Zambia.

Specimens examined: **South Africa**, KwaZulu-Natal, KwaMbonambi, on *Eucalyptus grandis*, 1989, M.J. Wingfield, **holotype** PREM 58023, ex-type culture CMW 2113 = CBS 112916; *Eucalyptus grandis*, 1986–88, M.J. Wingfield, PREM 49377, PREM 49378, PREM 79379; *Eucalyptus grandis* clone inoculated with isolate CMW 2113 during artificial inoculations, 2003, J. Roux, PREM 58024, living culture CMW 2113 = CBS 112916; 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; Durban, *Tibouchina granulosa*, 2000, J. Roux, R. Heath & L. Lombard, PREM 57360, PREM 57361; KwaZulu/Natal, Sodwana, *Syzygium cordatum*, 2002, R. Heath & J. Roux, PREM 57477, living culture CMW 10046; KwaZulu/Natal, KwaMbonambi, *Syzygium cordatum*, 2002, R. Heath & J. Roux, PREM 57478, living culture CMW 10036; KwaZulu/Natal, Amanzingwenia, *Syzygium cordatum*, 2002, R. Heath & J. Roux, PREM 57479; KwaZulu/Natal, Kosi

Bay, *Syzygium cordatum*, 2002, R. Heath & J. Roux, PREM 57480, living culture CMW 10076; KwaZulu/Natal, St. Lucia, *Syzygium cordatum*, 2001, R. Heath & M. Gryzenhout, PREM 5747; Limpopo, Tzaneen, *Syzygium cordatum*, 2001, R. Heath & M. Gryzenhout, PREM 57474, living culture CMW 9366; Mpumalanga, Hazyview, *Syzygium guineense*, 2001, M. Gryzenhout, PREM 57475, living culture CMW 10192 = CBS 118649.

Notes: *Chrysoporthe austroafricana* and *Chr. cubensis*, the only two species currently known in Africa, both occur on *Eucalyptus* spp. and are important pathogens. The two species can be distinguished from each other by the differences in the shape of the ascospore apices and ascus length. The only species that resembles *Chr. austroafricana*, is *Chr. inopina*, a fungus known from Colombia. *Chr. austroafricana* and *Chr. inopina* can be distinguished based on different optimal growth temperatures, and wider ascospores in *Chr. inopina*.

Although *Chr. austroafricana* is primarily known from South Africa, recent surveys have led to the discovery that this fungus also occurs further north in Africa on both *Eucalyptus* and *Syzygium* trees (Nakabonge *et al*. 2006b, Roux *et al*. 2005). Isolates of *Chr. cubensis* representing both the Asian and South American sub-clades also occurs in various African countries (Myburg *et al*. 2003, Nakabonge *et al*. 2006b). Hence both *Chr. cubensis* and *Chr. austroafricana* are found in Mozambique and Zambia (Nakabonge *et al*. 2006b).

Careful identification is needed when working with samples from the known hosts of *Chr. cubensis* and *Chr. austroafricana* in Africa, as this may be of significant quarantine importance. Ascospore morphology is the only reliable morphological characteristic that can be used to distinguish between these species, but teleomorph

structures are not always available for study. Comparison of DNA sequences for at least one gene region (for example, the β-tubulin 2 region), is typically necessary to confirm identifications. This also ensures that *Chr. cubensis* isolates are characterised in either the South American or Asian sub-clade, as both of these clades occur in different parts of Africa (Myburg *et al*. 2003, Nakabonge *et al*. 2006b).

Fig. 37. Fruiting structures of *Chrysoporthe austroafricana*. A. Ascostroma on bark. B. Conidioma on bark. C. Longitudinal section through ascostroma. D. Longitudinal section through conidiomata. E. Stromatic tissue of ascostroma. F. Perithecial neck and surrounding tissue (arrow). G. Tissue of the conidiomal base. H. Tissue of conidiomal neck. I. Ascus. J. Ascospores. K. Conidiophores. L. Conidia. Scale bars A–D = 100 µm; E–H = 20 µm; I–L = 10 µm.

18. ***Chrysoporthe doradensis*** Gryzenh. & M.J. Wingf., Fungal Diversity 20: 50–53. 2005. Fig. 38.

Etymology: Named for El Dorado, the legendary South American city of gold, referring to the golden colour of the stromatic tissue in this fungus.

Ascostromata semi-immersed in bark, recognizable by extending, fuscous-black, cylindrical perithecial necks and in some cases, erumpent, limited ascostromatic tissue appearing orange, 140–380 µm high above the bark, 320–610 µm diam. *Perithecia* valsoid, bases immersed in the bark, fuscous black, 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, necks emerging through the bark covered in umber, stromatic tissue of *textura porrecta*, thus appearing fuscous-black, extending necks up to 1680 µm long. *Asci* (19.5–)21.5–24(–25) × (4–)4.5–6(–7) µm, fusoid to ellipsoidal, 8-spored. *Ascospores* (4.5–)5.5–7.5(–8.5) × 2–2.5 µm, hyaline, 1-septate, fusoid to oval, with tapered apices.

Conidiomata separate structures or on top of ascostromata, pyriform to pulvinate, superficial to slightly immersed, usually with one attenuated neck per structure, fuscous-black, inside umber when young, conidiomatal base above the bark surface 70–300 µm high above level of bark, 100–290 µm diam, necks up to 300 µm long, 20–90 µm wide, with even to convoluted inner surface. *Stromatic tissue* of base of *textura globulosa*, the walls of outer cells thickened, neck tissue of *textura porrecta*. *Conidiophores* hyaline, with irregular shaped basal cells that is (2–)3.5–6(–7.5) × (2–)2.5–4(–5) µm, branched irregularly at the base or above into cylindrical cells, with or without separating septa, total length of conidiophore (9.5–)12.5–18(–22.5) µm. *Conidiogenous cells* cylindrical to flask-shaped with attenuated apices,

1.5–2(–2.5) µm wide. *Conidia* (3–)3.5–5(–6.5) × 1.5–2(–2.5) µm, hyaline, aseptate, oblong to ovoid to cylindrical, occasionally allantoid, exuded as pale luteous droplets. *Cultural characteristics*: cultures on MEA white with cinnamon to hazel patches, or completely cinnamon to hazel, fluffy with a smooth margin, fast-growing, covering a 90 mm diam plate after a minimum of 4–5 days at the optimum temperature of 30 ºC. *Substrate*: bark of *Eucalyptus* spp., including *Eucalyptus grandis* and *Eucalyptus deglupta*.

Distribution: Ecuador.

Specimens examined: **Ecuador**, Pichincha, Buenos Aires, Buenos Aires nursery, *Eucalyptus grandis*, Nov. 2001, M.J. Wingfield, **holotype** PREM 58581, ex-type cultures CMW 11286 = CBS 115734, CMW 11287 = CBS 115735; Buenos Aires nursery, *Eucalyptus grandis*, Jul. 2004, M.J. Wingfield, PREM 58582; Buenos Aires nursery, ex-type isolate CMW 11287 from *Eucalyptus grandis* inoculated into *Tibouchina urvilleana*, Feb. 2000, M.J. Wingfield, PREM 58583. **South Africa,** Pretoria, ex-type isolates CMW 11286 and CMW 11287 from *Eucalyptus grandis* inoculated into *Eucalyptus grandis* clone ZG14 in the greenhouse, Jun. 2002, M. Gryzenhout & H. Myburg, PREM 58584.

Notes: *Chrysoporthe doradensis* has asci of the same sizes and ascospore apices with the same shape as those of *Chr. cubensis*. However, *Chr. doradensis* is the only species that has conidia of variable shape and pale luteous spore drops. All other species have uniformly oblong conidia that are discharged in bright luteous spore drops or tendrils.

Chrysoporthe doradensis is known only from Ecuador on *Eucalyptus*, and it is also the only *Chrysoporthe* species that has been found in Ecuador (Gryzenhout *et al*. 2005c). Other species such as *Chr. cubensis*, *Chr. inopina* and *Chrysop. hodgesiana* are found in countries neighbouring Ecuador (Gryzenhout *et al.* 2006c). Future surveys should thus focus on determining whether *Chr. doradensis* occurs in the rest of South America, and whether *Chr. cubensis* might be present in Ecuador as both *Chr. doradensis* and *Chr. cubensis* are important pathogens. Since the other *Chrysoporthe* spp. in South America occur on native trees, it is also likely that *Chr. doradensis* occurs on native trees in Ecuador, and surveys should focus on these trees, specifically *Melastomataceae*.

Fig. 38. Fruiting structures of *Chrysoporthe doradensis*. A. Ascostroma on bark showing black perithecial necks and orange stromatic tissue (arrow). B. Conidiomata on bark. C. Longitudinal sections through ascostroma and conidioma (D). E. Stromatic tissue of ascostroma. F. Tissue (arrow) around perithecial neck. G. Tissue of the conidiomal base and neck (H). I. Ascus. J. Ascospores. K. Conidiophores. L. Conidia. Scale bars A–D = 100 µm; E–H = 20 µm; I–L = 10 µm.

19. ***Chrysoporthe inopina*** Gryzenh. & M.J. Wingf., **nom. prov.** Mycol. Res. (in press). Fig. 39.

Etymology: Latin, *inopina*, unexpected, referring to the unexpected appearance of this species.

Ascostromata semi-immersed in bark, recognizable by extending, fuscous-black, cylindrical perithecial necks and in some cases, erumpent, limited ascostromatic tissue appearing orange, 90–540 µm high above the bark, 200–770 µm diam. *Perithecia* valsoid, bases immersed in the bark, fuscous black, top of perithecial bases covered with cinnamon to orange, predominantly prosenchymatous, stromatic tissue, which is occasionally visible above the bark surface, necks emerging through the bark covered in umber, stromatic tissue of *textura porrecta*, thus appearing fuscous-black, extending necks up to 2330 µm long. *Asci* (27.5–)29.5–34(–35.5) × (4.5–)5.5–6.5(–7) µm, fusoid to ellipsoidal, 8-spored. *Ascospores* (4.5–)6–7.5(–8) × 2.5–3.5 µm, hyaline, 1-septate, fusoid to oval, with rounded ends.

Conidiomata separate structures or on top of ascostromata, subulate to pyriform to pulvinate, superficial to slightly immersed, with neck attenuated or not, usually with one neck per structure, fuscous-black, inside umber when young, conidiomatal base above the bark surface 100–650 µm high above level of bark, 70–710 µm diam, necks up to 780 µm long, 50–190 µm wide. *Conidiomatal locules* with even to convoluted inner surface, occasionally multilocular, single locule connected to 1 or several necks. *Stromatic tissue* of base of *textura globulosa*, walls of outer cells thickened, neck tissue of *textura porrecta*. *Conidiophores* hyaline, with basal cells of irregular shape that is (2.5–)3.5–6(–7) × 2–3.5(–4) µm, branched irregularly at the base or above into cylindrical cells, with or without separating septa, total length of

conidiophore (11–)12.5–22.5(–29.5) µm. *Conidiogenous cells* cylindrical to flask-shaped with attenuated apices, (1.5–)2–2.5(–3) µm wide. *Conidia* (3–)3.5–4 × (1.5–)2–2.5 µm, hyaline, oblong, aseptate, exuded as orange to luteous droplets.

Cultural characteristics: cultures 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 5 days at the optimum temperature of 25 ºC.

Substrate: bark of *Tibouchina lepidota.*

Distribution: Colombia.

Specimens examined: **Colombia**, Risaralda, Libano farm near Pereira (750° 35′ 49″ W and 40° 43′ 13″ N, 2102 masl, 3143 mm/y), *Tibouchina lepidota*, 2003, A. Arbelaez, **holotype** PREM 58800, ex-type cultures CMW 12727 = CBS 118659, CMW 12729 = CBS 118658, CMW 12731 = CBS 118656.

Notes: This species has ascospore apices and ascus sizes similar to those of *Chr. austroafricana*. These two species, however, occur on different continents and also have different optimal growth temperatures. *Chr. inopina* occurs on the same hosts and locality as *Chr. cubensis* and *Chrysop. hodgesiana* in Colombia and can easily be confused with them. *Chr. inopina* can be distinguished from *Chr. cubensis* based on its lower optimal growth, different ascospore apex shape and ascus size, and from *Chrysop. hodgesiana* by its uniquely, subulate conidiomata without an attenuated neck. Conidiomatal morphology is, however, a variable character and it may be difficult to determine the correct identity of samples from *Tibouchina* spp. as these trees are confirmed hosts of these *Chrysoporthe* spp.

Chrysoporthe inopina is known from a single collection in Colombia. It is likely that it is native and more widespread on various native *Melastomataceae* in Colombia. Its occurrence on these trees, together with the morphologically similar *Chr. cubensis* and *Chrysop. hodgesiana,* will most likely frustrate collection of additional specimens of this species. Furthermore, it is unknown if this species occurs on *Eucalyptus* and pathogenicity tests are needed to determine its pathogenicity on these trees.

Fig. 39. Fruiting structures of *Chrysoporthe inopina*. A. Ascostroma on bark (orange stromatic tissue indicated with arrow). B. Conidiomata of different shapes on bark. C. Longitudinal section through ascostroma. D. Longitudinal section through conidioma. E. Stromatic tissue of ascostroma. F. Perithecial neck and surrounding tissue (arrow). G. Tissue of the conidiomal base. H. Tissue of conidiomal neck. I. Asci. J. Ascospores. K. Conidiophores. L. Conidia. Scale bars A–D = 100 µm; E–H = 20 µm; I–L = 10 µm.

20. ***Chrysoporthella hodgesiana*** Gryzenh. & M.J. Wingf., Stud. Mycol. 50: 135–136. 2004. MycoBank MB500036. Fig. 40.

Etymology: Latin, named for Dr. Charles S. Hodges in honour of his many years of research on various aspects of the distribution, host range, pathology and taxonomy of *Cryphonectria cubensis*.

Conidiomata superficial to slightly immersed, generally pulvinate, sometimes pyriform, with one to four short attenuated necks per structure, fuscous-black, with an umber interior when young, conidiomatal base above the bark surface 80–310 µm high, 140–640 µm wide, necks up to 380 µm long, 60–170 µm wide. Conidiomatal locules with even to convoluted inner surface, occasionally multilocular, a single locule connected to one or several necks. *Stromatic tissue* at base of *textura globulosa*, the outer cells with thickened walls, neck tissue of *textura porrecta*, with remnant prosenchymatous tissue often found between host cells. *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, cylindrical to flask-shaped with attenuated apices, total length of conidiophore (12–)13–21(–33) µm, *conidiogenous cells* (1.5–)2–2.5 µm wide. *Conidia* (3–)3.5–5(–5.5) × 1.5–2(–2.5) µm, hyaline, oblong, aseptate, exuded as bright luteous tendrils or droplets.

Cultural characteristics: cultures 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 6 days at the optimum temperature of 25 ºC.

Substrate: bark of *Tibouchina semidecandra*, *Tibouchina urvilleana*, *Tibouchina lepidota* and *Miconia theaezans* (*Melastomataceae*, *Myrtales*).

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.

Notes: No teleomorph is known for this species, which is in an anamorph genus specifically described for it. Based on morphology alone, it is difficult to identify this species conclusively because of the variation found in morphological features of the anamorph between hosts and locations. It can be distinguished from the majority of other species based on its optimum growth temperature at 25 ºC (with the other species at 30 ºC) and DNA sequence comparisons. Only *Chr. inopina* has optimal growth at 25 ºC, but it can be distinguished from *Chrysop. hodgesiana* by its different conidiomatal shape. If a teleomorph were to be found for this fungus, it may contain distinct morphological characteristics that could be used for identification. For the present, DNA sequence comparisons will be needed to identify this species conclusively.

Fig. 40. 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. Stromatic tissue in bark. G–H. Conidiophores. I. Conidia. Scale bars A–C = 100 µm; D–F = 20 µm; G–I = 10 µm.

Celoporthe Nakab., Gryzenh., Jol. Roux & M.J. Wingf., **nom. prov.,** Stud. Mycol. 55 (in press) 2006.

Etymology: Latin, *celo*, to hide, referring to the difficulty with which fruiting structures of this fungus are found, and *porthe*, destroyer, referring to the pathogenic nature of the fungus.

Ascostromata 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* fusoid to ellipsoidal. *Ascospores* hyaline, oblong-ellipsoidal, with one median septum.

Conidiomata pulvinate to conical with or without short attenuated necks, superficial, orange to scarlet when young, fuscous black when mature, 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, c*onidiogenous cells* phialidic, apical or lateral on branches beneath the septa. *Conidia* hyaline, oblong to cylindrical to ovoid, occasionally allantoid, aseptate, exuded as bright luteous spore tendrils or droplets.

Typus: *Celoporthe dispersa* Nakab., Gryzenh., Jol. Roux & M.J. Wingf.

Notes: Species of *Celoporthe* are morphologically very similar to *Chrysoporthe* spp. (Nakabonge *et al*. 2006a). Superficially, conidiomata are difficult to distinguish because conidiomata of both genera are black, superficial and can be of similar shape.

Ascostromata are of similar colour, although perithecial necks of *Celoporthe* tend to be shorter and they are not always distinctly fuscous black. Both genera have single septate ascospores. Fruiting structures of *Celoporthe* also frequently occur between those of *Chrysoporthe* on the same piece of bark, and can inadvertently be used to isolate from when *Chrysoporthe* isolates are sought.

Only with thorough microscopical examination and cultural comparisons can these two genera be distinguished from each other (Nakabonge *et al*. 2006a). The main differences between *Celoporthe* and *Chrysoporthe* besides the protruding necks of the ascostromata, are anamorph characteristics (Nakabonge *et al*. 2006a). *Celoporthe* has conidiomata that are pulvinate to conical, occasionally with a short neck, and the stromatic tissue is pseudoparenchymatous. In contrast, conidiomata of *Chrysoporthe* are pulvinate to pyriform with distinct necks, and the stromatic tissue of the base is of *textura globulosa.* Conidia of *Celoporthe* are also cylindrical and not oblong similar to those of *Chrysoporthe*. Cultures of *Chrysoporthe* are characteristically white with cinnamon patches, while those of *Celoporthe* can also be grey and chestnut.

Celoporthe currently contains only a single species, *Ce. dispersa*, which occurs on *Syzygium*, *Tibouchina* and *Heteropyxis* trees in South Africa (Nakabonge *et al*. 2006a). This genus, however, represents a species complex, with another distinct species, identified based on sequence data, present in Indonesia on *S. aromaticum* (Myburg *et al*. 2003). This species could not be described because no fruiting structures on bark material could be confidently linked to the isolates and fruiting structures produced in culture were inordinantly variable to be used in morphological descriptions (Myburg *et al*. 2003). Clearly the taxonomy of *Celoporthe* should be

carefully approached with sequence data paired with morphological studies of herbarium specimens.

21. ***Celoporthe dispersa*** Nakab., Gryzenh., Jol. Roux & M.J. Wingf., **nom. prov.,** Stud. Mycol. 55 (in press). 2006. Fig. 41.

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

Ascostromata semi-immersed in bark, occasionally erumpent, limited, orange to umber ascostromatic tissue that cover the tops of the perithecial bases; ascostromata extending 100–400 µm high above the bark, 320–505 µm diam. Stromatic tissue cinnamon and pseudoparenchymatous at edges, prosenchymatous in centre. *Perithecia* valsoid, bases immersed in the bark, fuscous black, necks 80–100 µm wide, emerging through the stromatal surface, covered in umber stromatic tissue of *textura porrecta* thus appearing umber, extended necks short, up to 50 µm long. *Asci* (19.5–)23.5–29.5(–33.5) × (4.5–)5.5–7(–7.5) µm, fusoid to ellipsoidal, 8-spored. *Ascospores* (4.5–)6–7(–8) × (2–)2.5–3(–3.5) µm, hyaline, oblong-ellipsoidal, with rounded ends, one median septum.

Conidiomata pulvinate to conical without necks, superficial to slightly immersed, occasionally with neck which is slightly attenuated, orange to scarlet when young, fuscous black, conidiomatal bases above the bark surface 300–500 µm high, 200–1000 µm diam. *Conidiomatal locules* with even to convoluted inner surfaces, occasionally multilocular. *Stromatic tissue* pseudoparenchymatous. *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, *conidiogenous cells* cylindrical with attenuated apices, (1.5–)2–3 µm wide. *Conidia* (2.5–)3–4(–5.5) ×

(1–)1.5(–2.5) µm, hyaline, oblong to cylindrical to ovoid, occasionally allantoid, aseptate, exuded as bright luteous tendrils or droplets.

Cultural characteristics: cultures on MEA white with grey patches, becoming umber to hazel to chestnut, fluffy with an uneven margin, fast-growing, covering a 90 mm diam plate in a minimum of 5 days at the optimum temperature of 25 ºC.

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

Distribution: South Africa.

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

Notes: DNA sequences for the ITS region and β-tubulin genes showed that the isolates of *Ce. dispersa* include three sub-clades potentially representing cryptic species (Nakabonge *et al*. 2006a). These sub-clades consist of the ex-type isolates from *Syzygium cordatum* in Tzaneen, isolates from *Heteropyxis canescens* in Lydenburg and isolates from *Tibouchina granulosa* in Durban respectively. No morphological differences could be observed between these groups, but comparisons

were frustrated by the absence of the teleomorph on the samples from Lydenburg and Durban. Additional collections from these hosts will be necessary to clarify the taxonomy of these sub-clades, and more ecological information about these fungi should be gathered.

Fig. 41. Fruiting structures of *Celoporthe dispersa*. A–B. Ascostromata on bark showing ascospore mass (arrow). C. Conidioma on bark. D. Longitudinal section through ascostroma. E. Longitudinal section through conidioma. F. Stromatic tissue of ascostroma. G. Stromatic tissue of conidioma. H. Asci. I. Ascospores. J. Cylindrical conidiophores. K. Conidiophores. L. Conidia. Scale bars A–E = 100 µm; F–G = 20 µm; H–L = 10 µm.

7. SPECIES EXCLUDED OR OF QUESTIONABLE VALIDITY

7.1 Excluded species.

Endothia viridistroma Wehm., Mycologia 28: 38–39. 1936.

Notes: Since *E. viridistroma* has green stromata, with no orange tissue at any stage of its life cycle (Wehmeyer 1936), it is excluded from *Endothia* and the *Cryphonectriaceae*. DNA sequences of an isolate thought to represent *E. viridistroma* showed sequence similarities with *Valsa eucalypticola* Van der Westh. when a BLAST search was performed (Myburg *et al.* 2004a). However, it is unlikely that this species can be in *Valsa* because of its widely erumpent, superficial and large stromata (Wehmeyer 1936, Myburg *et al*. 2004a). It is thus more likely that the isolate sequenced does not truly represent *E. viridistroma*, and isolates of this fungus are thus lacking.

Cryphonectria variicolor (Fuckel) Sacc., Syll. Fung. 17: 781. 1905.

Basionym: *Nectria variicolor* Fuckel, Symb. Mycol.: 181. 1869.

Notes: Ascomata on specimens G 843, FH 843 and B (*Salix triandra*, Oestrich) of this fungus are not stromatic, but only consist of minute, globose, orange and superficial perithecia and striated ascospores (Gryzenhout *et al*. 2005a). These characteristics indicate that this fungus most likely is hypocrealean and does not belong in the *Diaporthales*.

Cryphonectria abscondita (Sacc.) Sacc., Syll. Fung. 17: 781. 1905.

Basionym: *Nectria abscondita* Sacc., Mycologiae Venetae Specimen: 123. 1873.

Notes: Because no fruiting structures are left on the only available specimen (PAD, *Wisteria sinensis*) and the original description is not informative (Gryzenhout *et al*. 2005a), the morphology of this species is unknown and it most likely will never be placed in the correct genus.

Endothia nitschkei G.H. Otth, Naturforsch. Gesellsch. Bern: 48. 1868.

Notes: Kobayashi (1970) synonymised *E. japonica* to *E. nitschkei*, which was already described from Europe (Otth 1868), based on their overlapping ascospores. We have, however, concluded in this monograph that this synonymy is invalid based on our own observations and those of Roane (1986a). Some of the supporting morphological characteristics for this conclusion include conidia expelled as white tendrils, no discolouration in lactophenol (Roane 1986a), conidial locules formed underneath the bark surface and longer ascospores than those of *C. japonica*. Some of these characteristics may indicate that this species does not belong in any of the genera currently in the *Cryphonectriaceae*, and that it may in fact not be in the *Cryphonectriaceae*.

7.2 Species of questionable validity.

Roane (1986a) listed a number of species as doubtful in her monograph as she has not studied the original material. None of these are currently used names in *Endothia*, *Endothiella* or *Cryphonectria* and we have thus not studied these species in this monograph. Future studies should, however, be carried out on these species to validate their position in the *Cryphonectriaceae*.

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LIST OF ABBREVIATIONS

AB, AF, AY, DQ = sequence accession numbers for Genebank.

ATCC = American Type Culture Collection, Manassas, VA 20108, USA.

B = Herbarium, Botanischer Garten und Botanisches Museum Berlin-Dahlem, Zentraleinrichtung der Freien Universität Berlin, Berlin, Germany.

BPI = U. S. National Fungus Collections, Systematic Botany and Mycology, Beltsville, USA.

CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

CMW = Culture collection of Michael J. Wingfield, Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

CRY = *Cryphonectria* culture collection of Michael J. Wingfield, Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

CUP = Plant Pathology Herbarium, Cornell University, Ithaca, New York USA.

DAR = Plant Pathology Herbarium, Orange Agricultural Institute, Forest Road, Orange, N.S.W., Australia.

E = from the culture collection of Prof. R. J. Stipes (Department of Plant Pathology, Virginia Polytechnic Institute & State University, Blacksburg, Virginia, USA) now housed in the culture collection (CMW) of FABI.

FLAS = Mycological Herbarium, Department of Plant Pathology, University of Florida, Gainesville, U.S.A.

IMI = Herbarium, CABI Bioscience, Egham, Surrey, U.K.

ITS = Internal transcribed spacer region of the ribosomal operon.

K = Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey, England, U.K.

KB1, CD28, YM2 = isolates used in Liu *et al*. (2003).

KOH = potassium hydroxide.

LSU = Large subunit (28S) of the ribosomal operon.

MAFF = Microorganisms Section, MAFF GENEBANK, National Institute of Agrobiological Sciences (NIAS), MAFF Gene Bank, Ibaraki, Japan.

MEA = malt extract agar

MYA = malt yeast extract agar

NY = William and Lynda Steere Herbarium, New York Botanical Garden, Bronx, New York, USA.

OA = oats agar

PCR = polymerase chain reaction

PDA = potato dextrose agar

PDD = Landcare Research New Zealand Limited, Mt. Albert, Auckland, New Zealand.

PREM = National Collection of Fungi, Pretoria, South Africa.

RFLP = restriction fragment length polymorphism

s. l. = *sensu lato*

s. str. = *sensu stricto*

TFM:FPH = Forestry and Forest Products Research Institute, Danchi-Nai, Ibaraki, Japan, E or Ep refers to an isolate

TrN = Tamura Nei distance model

WA = water agar

PAD = Erbario Patavinum, Centro Interdipartimentale Musei Scientifici, Università degli Studi di Padova**,** Padova, Italy

G = Herbarium, Conservatoire et Jardin botaniques de la Ville de Genève**, Chambésy**/Genève, Switzerland

FH = Farlow Reference Library and Herbarium of Cryptogamic Botany, **Harvard University,** Cambridge, Massachusetts U.S.A.