## CONTENTS

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

Marieka Gryzenhout[1], Brenda D. Wingfield[2] & Michael J. Wingfield[1]

[1]*Department of Microbiology and Plant Pathology,* [2]*Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa.*

**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 20$^{th}$ 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

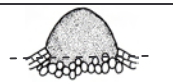
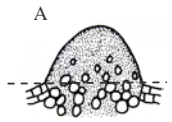
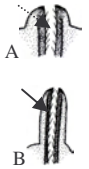
septa in different genera (Fig. 17). The position of the septum can vary within a species, for instance, while it usually occurs at the centre of the spore, it can also be somewhat displaced from the centre (Fig. 17). Some species, especially in *Microthia*, also have slight constrictions at the septa, 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<br>Superficial | Of fungal tissue only<br>Not embedded in host tissue or above level of bark (dotted line) |  |
| | Pseudostroma<br>Ectostroma (A)<br>Entostroma (B)<br>Semi-immersed | Fungal tissue also containing remnants of host tissue<br>Uppermost portion of stroma that breaks through the bark, usually composed of fungal tissue<br>Lower portion of stroma, usually composed of both fungal and host tissue<br>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<br>Valsoid | A stroma in which asci are produced<br>Perithecial necks convergent |  |
| | Diatrypoid | Perithecial necks separate or parallel |  |
| | Ostiolar canal<br>Periphysate<br>Papilla (A) | Canal (dashed arrow) in neck of perithecium, opens in ostiole<br>Hyphae (arrow) pointing upward inside ostiole of perithecium or pycnidium<br>Small rounded structure where perithecial neck emerge, as opposed to long necks (B) |  |

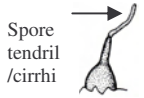
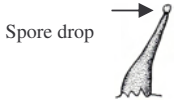
| **Morphological characteristic** | **Terminology** | **Definition** | **Illustration** |
|---|---|---|---|
| Conidioma (pl. -ta) | Locule (arrow) | Cavity inside stroma, in this case in the conidioma, contains conidiophores |  |
| | Single locular | Only one locule | |
| | Multilocular | More than 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***

---

**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*.

**ACKNOWLEDGEMENTS**

All photographs in the monograph were taken by MJW or MG except for the following: photographs of Endothia canker were supplied by Dr R. Jay Stipes (Department of Plant Pathology, Virginia Polytechnic Institute & State University, Blacksburg, U.S.A.); the photograph of Miconia rubiginosa was taken by Mr. Carlos A. Rodas (Smurfit Cartón de Colombia, Investigación Forestal, Colombia); photographs of chestnut blight were sent to us by Drs Thomas Kirisits, Erhard Halmschlager, Karl-Heinz Figl (Institute of Forest Entomology, Forest Pathology and Forest Protection, Department of Forest and Soil Sciences, BOKU – University of Natural Resources and Applied Life Sciences, Vienna) and Helmut Höttinger (Institute of Zoology, Department of Integrative Biology, BOKU – University of Natural Resources and Applied Life Sciences, Vienna); and Ms. Grace Nakabonge (FABI, South Africa) supplied us with some of the morphological photographs of *Celoporthe dispersa.* Some of the LSU sequences used in our analyses were provided to us by Dr. Henrietta Myburg (Plant Sensory Genomics Group, Dept. of Botany, North Carolina State University, U.S.A.) from her Ph. D. dissertation. We are grateful to the curators and staff of all the herbaria and culture collections listed in this monograph, for without the specimens and cultures obtained from them this monograph would not have been possible. We thank Dr. Seonju Lee, FABI, for her helpful suggestions to some parts of the monograph. Dr. H.F. Glen (Natal Herbarium, Durban, South Africa) assisted us with his invaluable nomenclatural expertise in compiling the monograph. We are also deeply grateful to numerous colleagues from various countries who sent us isolates or specimens, or assisted MJW in collecting fungi overseas. Without funding from the National Research Foundation (NRF),

members of the Tree Pathology Co-operative Programme (TPCP), and the THRIP support programme of the Department of Trade and Industry, South Africa, compilation of this monograph would not have been possible.

## REFERENCES

Alexopoulus CJ, Mims CW (1978). *Introductory Mycology. 3rd edition.* John Wiley, New York, U.S.A.

Alfenas AC, Jeng R, Hubbes M (1983). Virulence of *Cryphonectria cubensis* on *Eucalyptus* species differing in resistance. *European Journal of Forest Pathology* **13**: 197–205.

Anagnostakis SL (1977). Vegetative incompatibility in *Endothia parasitica*. *Experimental Mycology* **1**: 306–316.

Anagnostakis SL (1982a). Carpenter ants as carriers of *Endothia parasitica*. In: Proceedings of the *USDA Forest Service American Chestnut Cooperators' Meeting* (Smith HC, MacDonald WL, eds). Morgantown, 5–7 Jan. 1982: 111–113.

Anagnostakis SL (1982b). Biological control of chestnut blight. *Science* **215**: 466–471.

Anagnostakis S (1983). Conversion to curative morphology in *Endothia parasitica* and its restriction by vegetative incompatibiligy. *Mycologia* **75**: 777-780.

Anagnostakis SL (1987). Chestnut blight: the classical problem of an introduced pathogen. *Mycologia* **79:** 23–37.

Anagnostakis SL (1990). Improved chestnut tree condition maintained in two Connecticut plots after treatments with hypovirulent strains of the chestnut blight fungus. *Forestry Sciences* **36**: 113–124.

Anagnostakis SL (1991). Measuring resistance of chestnut trees to chestnut blight. *Canadian Journal of Forestry Research* **22**: 568–571.

Anagnostakis SL (1992). Chestnuts and the introduction of chestnut blight. *Annual Report of the Northern Nut Growers Association* **83**: 39–42.

Anagnostakis SL (2001). American chestnut sprout survival with biological control of the chestnut-blight fungus population. *Forest Ecology Management* **152**: 225–233.

Anagnostakis SL, Chen B, Geletka LM, Nuss DL (1998). Hypovirus transmission to ascospore progeny by field-released transgenic hypovirulent strains of *Cryphonectria parasitica*. *Phytopathology* **88**: 598–604.

Anagnostakis SL, Hau B, Krantz J (1986). Diversity of vegetative compatibility groups of *Cryphonectria parasitica* in Connecticut and Europe. *Plant Disease* **70**: 536-538.

Anagnostakis SL, Hillman B (1992). Evolution of the chestnut tree and its blight. *Arnoldia* **52**: 4–9.

Anderson PJ (1914). The morphology and life history of the chestnut blight fungus. Pennsylvania Chestnut Tree Blight Commission Bulletin 7, Harrisburg, Pennsylvania.

Appel DN, Stipes RJ (1984). Canker expansion on water-stressed pin oaks colonized by *Endothia gyrosa*. *Plant Disease* **68**: 851–853.

Appel DN, Stipes RJ (1986). A description of declining and blighted pin oaks in eastern Virginia. *Journal of Arboriculture* **12**: 155–158.

Barnard EL, Barnard MR, El-Gholl NE, Ash EC (1993). Preliminary investigations of some Florida isolates of *Cryphonectria* and/or *Endothia* spp. from *Castanea*, *Coccoloba*, *Eucalyptus* and *Quercus* spp. Southwide Forest Disease Workshop, Auburn, Alabama, U.S.A., 13–15 January 1993.

Barnard EL, Geary T, English JT, Gilly SP (1987). Basal cankers and coppice failure of *Eucalyptus grandis* in Florida. *Plant Disease* **71**: 358–361.

Barr ME (1978). The Diaporthales in North America with emphasis on *Gnomonia* and its segregates. *Mycologia Memoir* **7**: 1–232.

Bazzigher G (1981). Selection of blight-resistant chestnut trees in Switzerland. *European Journal of Forest Pathology* **11**: 199–207.

Bazzigher G, Miller GA (1991). Blight-resistant chestnut selections of Switzerland: a valuable germ plasm resource. *Plant Disease* 75: 5–9.

Berkeley MJ, Broome CE (1875). Enumeration of the fungi of Ceylon. *Journal of the Linnean Society* **14**: 29–140.

Berkeley MJ, Broome CE (1877). Supplement to the Enumeration of the Fungi of Ceylon. *Journal of the Linnean Society* **15**: 82–86.

Bernatzky R, Mulcahy DL (1992). Marker-aided selection in a backcross breeding program for resistance to chestnut blight in the American chestnut. *Canadian Journal of Forest Research* **22**: 1031–1035.

Biraghi A (1946). Il cancro del castagno causato da *Endothia parasitica*. *Ital. Agric.* **7**: 1–9.

Biraghi A (1950). Caratteri di resistenza in *Castanea sativa* nei confronti di *Endothia parasitica*. *Boll. Staz. Patol. Veg.* **8**: 1–5.

Bissegger M, Sieber TN (1994). Assemblages of endophytic fungi in coppie shoots of *Castanea sativa*. *Mycologia* **86**: 648–655.

Boerboom JHA, Maas PWT (1970). Canker of *Eucalyptus grandis* and *E. saligna* in Surinam caused by *Endothia havanensis*. *Turrialba* **20**: 94–99.

Boyce JS (1961). *Forest Pathology. Third edition*. McGraw-Hill Book Company, New York, U.S.A.

Brewer LG (1982). The distribution of surviving American chestnuts in Michigan. In: Proceedings of the *USDA Forest Service American Chestnut Cooperators' Meeting* (Smith HC, MacDonald WL, eds). Morgantown, 5–7 Jan. 1982: 94–100.

Brewer LG (1995). Ecology of survival and recovery from blight in American chestnut trees [*Castanea dentata* (Marsh.) Borkh.] in Michigan. *Bulletin of the Torrey Botanical Club* **122**: 40–57.

Bruner SC (1916). A new species of *Endothia*. *Mycologia* **8**: 239–242.

Bruner SC (1917). Una enfermedad gangrenosa de los eucaliptos. *Estacion Experimental Agronomica, Santiago de las Vegas, Cuba, Bulletin* **37**: 1–33.

Burnham CR (1981). Blight-resistant American chestnut: There's hope. *Plant Disease***65**: 459–460.

Cannon PF (1988). Proposal to merge the Phyllachorales with the Diaporthales, with a new family structure. *Systema Ascomycetum* **7**: 23-43.

Castlebury LA, Rossman AY, Jaklitsch WJ, Vasilyeva LN (2002). A preliminary overview of the *Diaporthales* based on large subunit nuclear ribosomal DNA sequences. *Mycologia* **94**: 1017–1031.

Chen B, Choi GH, Nuss DL (1993). Mitotic stability and nuclear inheritance of integrated viral cDNA in engineered hypovirulent strains of the chestnut blight fungus. *EMBO Journal* **12**: 2991–2998.

Chen B, Choi GH, Nuss DL (1994). Attenuation of fungal virulence by synthethic infectious hypovirus transcripts. *Science* **264**: 1762–1964.

Chen B, Nuss DL (1999). Infectious cDNA clone of hypovirus CHV1-Euro7: a comparative virology approach to investigate virus-mediated hypovirulence of the chestnut blight fungus *Cryphonectria parasitica*. *Journal of Virology* **73**: 985–992.

Choi GH, Nuss DL (1992). Hypovirulence of chestnut blight fungus conferred by an infectious viral cDNA. *Science* **257**: 800–803.

Chung PH, Bedker PJ, Hillman BI (1994). Diversity of *Cryphonectria parasticia* hypovirulence-associated double-stranded RNAs within a chestnut population in New Jersey. *Phytopathology* **84**: 984–990.

Clapper RB (1952). Relative blight resistance of some chestnut species and hybrids. *Journal of Forestry* **50**: 453–455.

Conradie E, Swart WJ, Wingfield MJ (1990). Cryphonectria canker of *Eucalyptus*, an important disease in plantation forestry in South Africa. *South African Forestry Journal* **152**: 43–49.

Craighead FC (1912). Insects contributing to the control of the chestnut blight disease. *Science* **36**: 825.

Davison EM (1982). *Endothia havanensis* on Jarrah. *Australasian Plant Pathology* **11**: 10–11.

Davison EM, Coates DJ (1991). Identification of *Cryphonectria cubensis* and *Endothia gyrosa* from eucalypts in Western Australia using isozyme analysis. *Australasian Plant Pathology* **20**: 157–160.

Davison EM, Tay FCS (1983). Twig, branch, and upper trunk cankers of *Eucalyptus marginata*. *Plant Disease* **67**: 1285–1287.

Dawe AL, Nuss DL (2001). Hypoviruses and chestnut blight: exploiting viruses to understand and modulate fungal pathogenesis. *Annual Review of Genetics* **35**: 1–29.

Day PR, Dodds JA, Elliston JE, Jaynes RA, Anagnostakis SL (1977). Double-stranded RNA in *Endothia parasitica*. *Phytopathology* **67**: 1393–1396.

Dingley JM (1969). Records of plant diseases in New Zealand. *New Zealand Department of Scientific and Industrial Research, Bulletin* **192**: 120.

Dunn MM, Boland GJ (1993). Hypovirulent isolates of *Cryphonectria parasitica* in southern Ontario. *Canadian Journal of Plant Pathology* **15**: 245–252.

Elliston JE (1981). Hypovirulence and chestnut blight research: fighting disease with disease. *Journal of Forestry* **79**: 657–60.

Elliston JE (1985). Characteristics of dsRNA-free and dsRNA-containing strains of *Endothia parasitica* in relation to hypovirulence. *Phytopathology* **75**: 151–158.

Fairchild D (1913). The discovery of the chestnut bark disease in China. *Science* **38**: 297–299.

Farr DF, Bills GF, Chamuris GP, Rossman AY (1989). *Fungi on plants and plant products in the United States*. APS Press, St. Paul, Minnesota, U.S.A.

Farris JS, Källersjö M, Kluge AG, Bult C (1994). Testing significance of incongruence. *Cladistics* **10**: 315–319.

Florence EJM, Sharma JK, Mohanan C (1986). A stem canker disease of *Eucalyptus* caused by *Cryphonectria cubensis* in Kerala. *Kerala Forestry Research Institute Scientific Paper* **66**: 384–387.

Fries EM (1849). *Summa Vegetabilium Scandinaviae*. Holmiae & Lipsiae, Uppsala: 385–386.

Fries EM (1828). *Elenchus Fungorum v. 2*. Gryphiswaldiae: 73.

Fulbrigth DW, Weidlich WH, Haufler KZ, Thomas CS, Paul CP (1983). Chestnut blight and recovering American chestnut trees in Michigan. *Canadian Journal of Botany* **61**: 3164–3171.

Gardner DE, Hodges CS (1990). Diseases of *Myrica faya* (firetree, Myricaceae) in the Azores, Madeira and the Canary islands. *Plant Pathology* **39:** 326–330.

Gilmour JW (1966). The pathology of forest trees in New Zealand. The fungal, bacterial, and algal pathogens. *New Zealand Forest Research Institute, Technical Paper* **48**.

Glass NL, Donaldson GC (1995). Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* **61**: 1323–1330.

Gobbin D, Hoegger PJ, Heiniger U, Rigling D (2003). Sequence variation and evolution of *Cryphonectria hypovirus* 1 (CHV-1) in Europe. *Virus Research* **97**: 39–46.

Graves AH (1950). Relative blight resistance in species and hybrids of *Castanea*. *Phytopathology* **40**: 1125–1131.

Grente J (1965). Les formes Hypovirulentes d'*Endothia parasitica* et les espoirs de lutte contre le chancre du châtaignier. *Académie d'Agriculture de France, Extrait du Proces-verbal de la Séance* **51**: 1033–1037.

Grente J, Berthelay-Sauret S (1978). Biological control of chestnut blight in France. In: *Proceedings of the American Chestnut Symposium* (MacDonald WL, Cech

FC, Luchok J, Smith HC, eds). West Virginia University Press, Morgantown, West Virigina, U.S.A.: 30–34.

Greuter W, McNeill J, Barrie FR, Burdet HM, Demoulin V, Filgueiras TS, Nicolson DH, Silva PC, Skog JE, Trehane P, Turland NJ, Hawksworth DL (2000). *International Code of Botanical Nomenclature (Saint Louis Code).* XVI International Botanical Congress, St. Louis, Missouri, July–August 2000. Koeltz Scientific Books Königstein, Germany.

Griffin GJ (1986). Chestnut blight and its control. *Horticultural Review* **8**: 291–336.

Griffin GJ (1999). Frequencies and spatial patterns of white hypovirulent and pigmented strains of *Cryphonectria parasitica* within blight-controlled cankers on grafted American chestnut trees 15-16 years after inoculation. *European Journal of Forest Pathology* **29**: 377–390.

Griffin GJ (2000). Bight control and restoration of the American chestnut. *Journal of Forestry* **98**: 22–27.

Griffin GJ, Elkins JR (1986). Chestnut blight. In: *Chestnut blight, other Endothia diseases, and the genus Endothia* (Roane MK, Griffin GJ, Elkins JR, eds). APS Press, St. Paul, Minnesota, U.S.A.: 1–26.

Griffin GJ, Griffin SL (1995). Evaluation of superficial canker instability for hypovirulent *Cryphonectria parasitica* inoculated on American chestnut trees. *European Journal of Forest Pathology* **25**: 351–355.

Griffin GJ, Khan MA, Griffin SL (1993). Superficial canker instability during winter and virulence of *Endothia parasitica* associated with managed forest clearcut and plantation American chestnut trees. *Canadian Journal of Plant Pathology* **15**: 159–167.

Griffin GJ, Smith HC, Dietz A, Elkins JR (1991). Importance of hardwood competition to American chestnut survival, growth, and blight development in forest clearcuts. *Canadian Journal of Botany* **69**: 1804–1809.

Gryzenhout M, Eisenberg BE, Coutinho TA, Wingfield BD, Wingfield MJ (2003). Pathogenicity of *Cryphonectria eucalypti* to *Eucalyptus* clones in South Africa. *Forest Ecology and Managment* **176:** 427–437.

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

Gryzenhout M, Glen HF, Wingfield BD, Wingfield MJ (2005b). *Amphilogia* gen. nov. for *Cryphonectria*-like fungi from *Elaeocarpus* spp. in New Zealand and Sri Lanka. *Taxon* **54**: 1009–1021.

Gryzenhout M, Myburg H, Hodges CS, Wingfield BD, Wingfield MJ (2006d). *Microthia*, *Holocryphia* and *Ursicollum*, three new genera on *Eucalyptus* and *Coccoloba* for fungi previously known as *Cryphonectria*. *Studies in Mycology* **55** (in press).

Gryzenhout M, Myburg H, Rodas CA, Wingfield BD, Wingfield MJ (2006e). *Aurapex penicillata* gen. sp. nov. from native *Miconia theaezans* and *Tibouchina* spp. in Colombia. *Mycologia* **98** (in press).

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.

Gryzenhout M, Myburg H, Wingfield BD, Montenegro F, Wingfield MJ (2005c). *Chrysoporthe doradensis* sp. nov. pathogenic to *Eucalyptus* in Ecuador. *Fungal Diversity* **20**: 39–57.

Gryzenhout M, Myburg H, Wingfield BD, Montenegro F, Wingfield MJ (2005d). *Rostraureum tropicale* gen. sp. nov. (*Diaporthales*) associated with dying *Terminalia ivorensis* in Ecuador. *Mycological Research* **109**: 1029–1044.

Gryzenhout M, Myburg H, Wingfield BD, Wingfield MJ (2006b). *Cryphonectriaceae* (*Diaporthales*), a new family including *Cryphonectria, Endothia*, *Chrysoporthe* and allied genera. *Mycologia* **98** (in press).

Gryzenhout M, Rodas CA, Portales JM, Wingfield BD, Wingfield MJ (2006c). Novel hosts of the *Eucalyptus* canker pathogen *Chrysoporthe cubensis* and a new *Chrysoporthe* species from Colombia. *Mycological Research* (in press).

Gryzenhout M, Wingfield BD, Wingfield MJ (2006a). New taxonomic concepts for the important forest pathogen *Cryphonectria parasitica* and related fungi. *FEMS Microbiology Letters* 258: 161–172.

Havir EA, Anagnostakis SL (1985). Oxaloacetate acetylhydrolase activity in virulent and hypovirulent strains of *Endothia* (*Cryphonectria*) *parasitica*. *Physiological Plant Pathology* **26**: 1–9.

Hawkins LA, Stevens NE (1917). *Endothia* pigments I. *American Journal of Botany* **4**: 336–353.

Hawksworth DL, Kirk PM, Sutton BC, Pegler DN (1995). *Ainsworth & Bisby's Dictionary of the Fungi, 8th edition reprinted.* CAB International, Oxford, United Kingdom.

Heald FD, Gardner MW (1914). Longevity of pycnospores of the chestnut-blight fungus in soil. *Journal of Agricultural Research* **2**: 67–75.

Heald FD, Gardner MW, Studhalter RA (1915). Air and wind dissemination of ascospores of the chestnut-blight fungus. *Journal of Agricultural Research* **3**: 493–526.

Heald FD, Studhalter RA (1914). Birds as carriers of the chestnut-blight fungus. *Journal of Agricultural Research* **2**: 405–422.

Heath RN, Gryzenhout M, Roux J, Wingfield MJ (2006). Discovery of the Cryphonectria canker pathogen on native *Syzygium* species in South Africa. *Plant Disease* **90**: 433–438.

Heath, RN, Roux J, Van der Merwe NA, Wingfield BD, Wingfield MJ (2003). Population structure of *Cryphonectria cubensis* in South Africa. Volume 2-Offered Papers of the 8th International Congress of Plant Pathology, Christchurch, 2-7 February, 2003.

Hebard FV (1994). Inheritance of juvenile leaf and stem morphological traits in crosses of Chinese and American chestnut. *Journal of Heredity* **85**: 440–446.

Heiniger U, Rigling D (1994). Biological control of chestnut blight in Europe. *Annual Review of Phytopathology* **32**: 581–599.

Hillman BI, Fulbright DW, Nuss DL, Van Alfen NK (2000). *Hypoviridae*. In: *Virus Taxonomy: Seventh Report of the International Committee on Taxonomy of Viruses* (Von Regenmortel MHV, Fauquet CM, Bishop DHL, Carstens EB, Estes MK, Lemon SM, Maniloff J, Mayo MA, McGeoch DJ, Pringle CR, Wickner RB, eds.). Academic Press, San Diego: 515–520.

Hillman BI, Halpern BT, Brown MP (1994). A viral dsRNA element of the chestnut blight fungus with a distinct genetic organization. *Virology* **201**: 241–250.

Hillman BI, Suzuki N (2004). Viruses of the chestnut blight fungus, *Cryphonectria parasitica*. *Advances in Virus Research* **63**: 423–472.

Hillman BI, Tian Y, Bedker PJ, Brown MP (1992). A North American hypovirulent isolate of the chestnut blight fungus with European isolate-related dsRNA. *Journal of General Virology* **73**: 681–686.

Hodges CS (1980). The taxonomy of *Diaporthe cubensis*. *Mycologia* **72**: 542–548.

Hodges CS (1988). Preliminary exploration for potential biological control agents for *Psidium cattleianum*. Technical report 66, Cooperative National Park Resources Studies Unit, University of Hawaii at Manoa, Honolulu, Hawaii.

Hodges CS, Alfenas AC, Cordell CE (1986). The conspecificity of *Cryphonectria cubensis* and *Endothia eugeniae*. *Mycologia* **78**: 334–350.

Hodges CS, Gardner DE (1992). Survey for potential biological control agents for *Myrica faya* in the Azores and Madeira islands. Report submitted to the Cooperative National Park Resources Unit, University of Hawaii at Manoa, Department of Botany, Honolulu, Hawaii.

Hodges CS, Geary TF, Cordell CE (1979). The occurrence of *Diaporthe cubensis* on *Eucalyptus* in Florida, Hawaii and Puerto Rico. *Plant Disease Reporter* **63**: 216–220.

Hodges CS, Reis MS, Ferreira FA, Henfling JDM (1976). O cancro do eucalipto causado por *Diaporthe cubensis*. *Fitopatalogia Brasileira* **1**: 129–170.

Hoegger PJ, Rigling D, Holdenrieder O, Heiniger U (2002). *Cryphonectria radicalis*: rediscovery of a lost fungus. *Mycologia* **94:** 105–115.

Huang H, Carey WA, Dane F, Norton JD (1996a). Evaluation of Chinese chestnut cultivars for resistance to *Cryphonectria parasitica*. *Plant Disease* **80**: 45–47.

Huang H, Dane F, Norton JD, Weaver DB, Hebard FV (1996b). Linkage relationships of isozymes and morphological traits in interspecific chestnut crosses. *HortScience* **31**: 419–420.

Huelsenbeck JP, Ronquist F (2001). MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17**: 754–755.

Huhndorf SM (1991). A method for sectioning ascomycete herbarium specimens for light microscopy. *Mycologia* **83**: 520–524.

Hunter PP, Griffin GJ, Stipes RJ (1976). The influence of osmotic water potential on the linear growth of *Endothia* species. *Phytopathology* **66**: 1418-1421.

Hunter PP, Stipes RJ (1978). The effect of month of inoculation with *Endothia gyrosa* on development of pruned branch canker of pin oak (*Quercus palustris*). *Plant Disease Reporter* **62**: 940–944.

Jaynes RA, DePalma NK (1984). Natural infection of nuts of *Castanea dentata* by *Endothia parasitica*. *Phytopathology* **74**: 296–299.

Katoh K, Misawa K, Kuma K, Miyata T (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acid Research* **30**: 3059–3066.

Kobayashi T (1970). Taxonomic studies of Japanese Diaporthaceae with special reference to their life histories. *Bulletin of the Government Forest Experiment Station* **226**: 132–147.

Kobayashi T, Itô K (1956a). Notes on the genus *Endothia* in Japan I. Species of *Endothia* collected in Japan. *Bulletin of the Government Forest Experiment Station* **92**: 81–98.

Kobayashi T, Itô K (1956b). Two new species of *Endothia*. *Annals of the Phytopathological Society of Japan* **21**: 151–152.

Kubisiak TL, Hebard FV, Nelson CD, Zhang J, Bernatzky R, Huang H, Anagnostakis SL, Doudrick RL (1997). Molecular mapping of resistance to blight in an interspecific cross in the genus *Castanea*. *Phytopathology* **878**: 751–759.

Larget B, Simon DL (1999). Markov Chain Monte Carlo Algorithms for the Bayesian Analysis of Phylogenetic Trees. *Molecular Biology & Evolution* **16**: 750–759.

Liu Y-C, Cortesi P, Double ML, MacDonald WL, Milgroom MG (1996). Diversity and multilocus genetic structure in populations of *Cryphonectria parasitica*. *Phytopathology* **86**: 1344–1351.

Liu Y-C, Double ML MacDonald WL, Milgroom MG (2002). Persistence of *Cryphonectria* hypoviruses after their release for biological control of chestnut blight in West Virginia forests. *Forest Pathology* **32**: 345–356.

Liu Y-C, Linder-Basso D, Hillman BI, Kaneko S, Milgroom MG (2003). Evidence for interspecies transmission of viruses in natural populations of filamentous fungi in the genus *Cryphonectria*. *Molecular Ecology* **12**: 1619–1628.

MacDonald WL, Fulbright DW (1991). Biological control of chestnut blight: use and limitations of transmissible hypovirulence. *Plant Disease* **75**: 656–661.

Marra RE, Cortesi P, Bissegger M, Milgroom MG (2004). Mixed mating in natural populations of the chestnut blight fungus, *Cryphonectria parasitica*. *Heredity* **93**: 189–195.

Marra RE, Milgroom MG (2001). The mating system of the fungus *Cryphonectria parasitica*: selfing and self-incompatibility. *Heredity* **86**: 134–143.

McGuire IC, Marra RE, Milgroom ME (2004). Mating-type heterokaryosis and selfing in *Cryphonectria parasitica*. *Fungal Genetics and Biology* **41**: 521–533.

McGuire IC, Marra RE, Turgeon BG, Milgroom MG (2001). Analysis of mating-type genes in the chestnut blight fungus, *Cryphonectria parasitica*. *Fungal Genetics and Biology* **34**: 131–144.

McKeen CD (1995). Chestnut blight in Ontario: past and present status. *Canadian Journal Plant Pathology* **17**: 295–304.

Melzer MS, Boland GJ (1999). CHV3-type dsRNAs and the GH2 genotype in a population of *Cryphonectria parasitica* in Ontario. *Canadian Journal of Plant Pathology* **21**: 248–255.

Melzer MS, Dunn M, Zhou T, Boland GJ (1997). Assessment of hypovirulent isolates of *Cryphonectria parasitica* for potential in biological control of chestnut blight. *Canadian Journal of Plant Pathology* **19**: 69–77.

Merkel HW (1906). A deadly fungus on the American chestnut. *Annual Report of the New York Zoological Society* **10**: 97–103.

Micales JA, Stipes RJ (1987). A reexamination of the fungal genera *Cryphonectria* and *Endothia*. *Phytopathology* **77**: 650–654.

Micales JA, Stipes RJ, Bonde MR (1987). On the conspecificity of *Endothia eugeniae* and *Cryphonectria cubensis*. *Mycologia* **79**: 70–720.

Mickleborough J (1909). *A report on the chestnut tree blight, the fungus* Diaporthe parasitica*, Murrill.* Department of Forestry, Commonwealth of Pennsylvania, Harrisburg, U.S.A.

Milgroom MG (1995). Population biology of the chestnut blight fungus, *Cryphonectria parasitica*. *Canadian Journal of Botany* **73**: S311–S319.

Milgroom MG, Cortesi P (2004). Biological control of chestnut blight with hypovirulence: a critical analysis. *Annual Review of Phytopathology* **42**: 311–338.

Milgroom MG, Lipari SE, Ennos RA, Liu Y-R (1993). Estimation of the outcrossing rate in the chestnut blight fungus, *Cryphonectria parasitica*. *Heredity* **70**: 385–392.

Milgroom MG, Lipari SE, Wang K (1992). Comparison of genetic diversity in the chestnut blight fungus, *Cryphonectria* (*Endothia*) *parasitica*, from China and the U.S. *Mycological Research* **96**: 1114–1120.

Milgroom MG, Wang K, Zhou Y, Lipari SE, Kaneko S (1996). Intercontinental population structure of the chestnut blight fungus, *Cryphonectria parasitica*. *Mycologia* **88**: 179–190.

Myburg H, Gryzenhout M, Heath RN, Roux J, Wingfield BD, Wingfield MJ (2002b). Cryphonectria canker on *Tibouchina* in South Africa. *Mycological Research* **106**: 1299–1306.

Myburg H, Gryzenhout M, Wingfield BD, Milgroom MG, Shigeru K, Wingfield MJ (2004b). DNA sequence data and morphology define *Cryphonectria* species in Europe, China, and Japan. *Canadian Journal of Botany* **82**: 1730–1743.

Myburg H, Gryzenhout M, Wingfield BD, Stipes RJ, Wingfield MJ (2004a). Phylogenetic relationships of *Cryphonectria* and *Endothia* species, based on DNA sequence data and morphology. *Mycologia* **96**: 990–1001.

Myburg H, Gryzenhout M, Wingfield BD, Wingfield MJ (2002a). β-tubulin and Histone *H3* gene sequences distinguish *Cryphonectria cubensis* from South Africa, Asia and South America. *Canadian Journal of Botany* **80**: 590–596.

Myburg H, Gryzenhout M, Wingfield BD, Wingfield MJ (2003). Conspecificity of *Endothia eugeniae* and *Cryphonectria cubensis*: A re-evaluation based on morphology and DNA sequence data. *Mycoscience* **104**: 187–196.

Myburg H, Wingfield BD, Wingfield MJ (1999). Phylogeny of *Cryphonectria cubensis* and allied species inferred from DNA analysis. *Mycologia* **91**: 243–250.

Nakabonge G, Cortinas MN, Roux J, Gryzenhout M, Wingfield BD, Wingfield MJ (2005). Development of polymorphic microsatellite markers for the fungal tree pathogen *Cryphonectria eucalypti. Molecular Ecology Notes* **5**: 558–561.

Nakabonge G, Gryzenhout M, Roux J, Wingfield BD, Wingfield MJ (2006a). *Celoporthe dispersa* gen et sp. nov. from native Myrtales in South Africa. *Studies in Mycology* **55**: 259–271.

Nakabonge G, Roux J, Gryzenhout M, Wingfield MJ (2006b). Distribution of Chrysoporthe canker pathogens on *Eucalyptus* and *Syzygium* species in Eastern and Southern Africa. *Plant Disease* (in press).

Newhouse JR (1990). Chestnut blight. *Scientific American July*: 74–79.

Nuss DL (1992). Biological control of chestnut blight: an example of virus mediated attenuation of fungal pathogenesis. *Microbial Reviews* **56**: 561–576.

Nuss DL (1996). Using hypoviruses to probe and perturb signal transduction processes underlying fungal pathogenesis. *Plant Cell* **8**: 1845–1853.

Nuss DL (2000). Hypovirulence and chestnut blight: from the field to the laboratory and back. In *Fungal Pathology* (Kronstad JH, ed.). Kluwer, The Netherlands: 149–170.

Nutman FJ, Roberts FM (1952). Acute die-back of clove trees in the Zanzibar Protectorate. *Annals of Applied Biology* **39**: 599–607

Old KM, Gibbs R, Craig I, Myers BJ, Yuan ZQ (1990). Effect of drought and defoliation on the susceptibility of eucalypts to cankers caused by *Endothia gyrosa* and *Botryosphaeria ribis*. *Australian Journal of Botany* **38**: 571–581.

Old KM, Kobayashi T (1988). Eucalypts are susceptible to the chestnut blight fungus, *Cryphonectria parasitica*. *Australian Journal of Botany* **36**: 599–603.

Old KM, Murray DIL, Kile GA, Simpson J, Malafant KWJ (1986). The pathology of fungi isolated from eucalypt cankers in south-eastern Australia. *Australian Forestry Research* **16**: 21–36.

Old KM, Wingfield MJ, Yuan ZQ (2003). *A manual of diseases of eucalypts in South-East Asia.* Centre for International Forestry Research, Jakarta, Indonesia.

Otth GH (1868). Sechster Nachtrag zu dem in Nr. 15–23 der Mittheilungen enthalten Verzeichnisse schweizerischer Pilze. Naturforsch. Gesellsch. Bern. Mitteilungen: 48.

Peace TR (1962). *Pathology of trees and shrubs with special reference to Britain*. Oxford University Press, London, U.K.

Peever TL, Liu Y-C, Cortesi P, Milgroom MG (2000). Variation in tolerance and virulence in the chestnut blight fungus-hypovirus interaction. *Applied Environmental Microbiology* **66**: 4863–4869.

Peever TL, Liu Y-C, Milgroom MG (1997). Diversity of hypoviruses and other double-stranded RNAs in *Cryphonectria parasitica* in North America. *Phytopathology* **87**: 1026–1033.

Peever TL, Liu Y-C, Wang K, Hillman BI, Foglia R, Milgroom MG (1998). Incidence and diversity of double-stranded RNAs infecting the chestnut blight

fungus, *Cryphonectria parasitica*, in China and Japan. *Phytopathology* **88**: 811–817.

Pennycook SR (1989). Part II. Fungal plant diseases. In: *Plant Diseases Recorded in New Zealand* 2. Plant Diseases Division, DSIR, Auckland, New Zealand: 117.

Posada D, Crandall KA (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**: 817–818.

Poynton RJ (1979). The Eucalypts. Vol. 2. Department of Forestry, South Africa.

Puhalla JE, Anagnostakis SL (1971). Genetics and nutritional requirements of *Endothia parasitica*. *Phytopathology* **61**: 169–173.

Rayner RW (1970). *A Mycological Colour Chart*. Commonwealth Mycological Institute and British Mycological Society, Kew, Surrey, U.K.

Reid J, Booth C (1969). Some species segregated from the genera *Cryptospora*, *Cryptosporella*, and *Sillia*. *Canadian Journal Botany* **47**: 1055–1060.

Rigling D, Heiniger U, Hohl HR (1989). Reduction of laccase activity in dsRNA-containing hypovirulent strains of *Cryphonectria* (*Endothia*) *parasitica*. *Phytopathology* **79**: 219–223.

Roane MK (1986a). Taxonomy of the genus *Endothia*. In: *Chestnut blight, other Endothia diseases, and the genus Endothia* (Roane MK, Griffin GJ, Elkins JR, eds). APS Press, St. Paul, Minnesota, U.S.A.: 28–39.

Roane MK (1986b). Other diseases caused by *Endothia* species. In: *Chestnut blight, other Endothia diseases, and the genus Endothia* (Roane MK, Griffin GJ, Elkins JR, eds). APS Press, St. Paul, Minnesota, U.S.A.: 27.

Roane MK (1986c). Physiology of *Endothia*. In: *Chestnut blight, other Endothia diseases, and the genus Endothia* (Roane MK, Griffin GJ, Elkins JR, eds). APS Press, St. Paul, Minnesota, U.S.A.: 40–45.

Roane MK, Stipes RJ (1978). Pigments in the fungal genus *Endothia. Virginia Journal of Science* **29**: 137–141.

Roane MK, Stipes RJ, Phipps PM, Miller OK Jr. (1974). *Endothia gyrosa*, causal pathogen of pin oak blight. *Mycologia* **66**: 1042–1047.

Robin C, Anziani C, Cortesi P (2000). Relationship between biological control, incidence of hypovirulence, and diversity of vegetative compatibility types of *Cryphonectria parasitica* in France. *Phytopathology* **90**: 730–737.

Robin C, Heiniger U (2001). Chestnut blight in Europe: diversity of *Cryphonectria parasitica*, hypovirulence and biocontrol. *Forest Snow and Landscape Research* **76**: 361–367.

Rodas CA, Gryzenhout M, Myburg H, Wingfield BD, Wingfield MJ (2005). Discovery of the *Eucalyptus* canker pathogen *Chrysoporthe cubensis* on native *Miconia* (*Melastomataceae*) in Colombia. *Plant Pathology* **54**: 460–470.

Ronderos A (2000). Where giants once stood. The demise of the American chestnut and efforts to bring it back. *Journal of Forestry* **98**: 10–11.

Roux J, Meke G, Kanyi B, Mwangi L, Mbaga A, Hunter GC, Nakabonge G, Heath RN, Wingfield MJ (2005). Diseases of plantation forestry tree species in eastern and southern Africa. *South African Journal of Science* **101**: 409–413.

Roux J, Myburg H, Wingfield BD, Wingfield MJ (2003). Biological and phylogenetic analyses suggest that two *Cryphonectria* spp. cause cankers of *Eucalyptus* in Africa. *Plant Disease* **87**: 1329–1332.

Russin JS, Shain L, Nordin GL (1984). Insects as carriers of virulent and cytoplasmic hypovirulent isolates of the chestnut blight fungus. *Journal of Economic Entomology* **77**: 838–846.

Saccardo PA, Saccardo D (1905). *Sylloge Fungorum* 17: 783–784. Patavii.

Saccardo PA (1906). Annales Mycologici 4 (3): 273.

Scharf CS, DePalma NK (1981). Birds and mammals as vectors of the chestnut blight fungus. *Canadian Journal Zoology* **59**: 1647–1650.

Seisax CDS, Barreto RW, Alfenas AC, Ferreria FA (2004). *Cryphonectria cubensis* on an indigenous host in Brazil: a possible origin for eucalyptus canker disease? *Mycologist* **18**: 39–45.

Shain L, Miller JB (1992). Movement of cytoplasmic hypovirulence agents in chestnut blight cankers. *Canadian Journal of Botany* **70**: 557–561.

Shapira R, Choi GH, Nuss DL (1991). Virus-like genetic organization and expression strategy for a double-stranded RNA genetic element associated with biological control of chestnut blight. *EMBO Journal* **10**: 731–739.

Sharma JK, Mohanan C, Florence EJM (1985). *Disease survey in nurseries and plantations of forest tree species grown in Kerala.* Research Report 36. Kerala Forest Research Institute, Kerala, India.

Shear CL, Stevens NE (1913). The chestnut–blight parasite (*Endothia parasitica*) from China. *Science* **38**: 295–297.

Shear CL, Stevens NE (1916). The discovery of the chestnut blight parasite (*Endothia parasitica*) and other chestnut fungi in Japan. *Science* **43**: 173–176.

Shear CL, Stevens NE, Tiller RJ (1917). *Endothia parasitica* and related species. *United States Department of Agriculture Bulletin* **380**: 1–82.

Sinclair WA, Lyon HH, Johnson WT (1987). Diseases of trees and shrubs. Cornell University Press, Ithaca, U.S.A.

Slippers B, Stenlid J, Wingfield MJ (2005). Emerging pathogens: fungal host jumps following anthropogenic introduction. *Trends in Ecology and Evolution* **20**: 420–421.

Smart CD, Yuan W, Foglia R, Nuss DL, Fulbright DW, Hillman BI (1999). *Cryphonectria hypovirus* 3, a virus species in the family *Hypoviridae* with a single open reading frame. *Virology* **265**: 66–73.

Smith DM (2000). Ill-fated monarch of the eastern hardwood forest. *Journal of Forestry* **98**: 12–15.

Snow GA, Beland JW, Czabator FJ (1974). Formosan sweetgum susceptible to North American *Endothia gyrosa*. *Phytopathology* **64**: 602–605.

Sotirovski K, Papazova-Anakieva I, Grünwald NJ, Milgroom MG (2004). Low diversity of vegetative compatibility types and mating type in *Cryphonectria parasitica* in the southern Balkans. *Plant Pathology* **53**: 325–333.

Sowerby J (1814). Colored figures of English fungi or mushrooms. Sup. London, plate 438.

Spaulding P (1961). *Foreign diseases of forest trees of the world, an annotated list*. Agriculture handbook **197**, United States Department of Agriculture.

Stevens NE (1917). Some factors influencing the prevalence of *Endothia gyrosa*. *Bulletin of the Torrey Botanical Club* **44**: 127–144.

Stipes RJ, Phipps PM (1971). A species of *Endothia* associated with a canker disease of pin oak (*Quercus palustris*) in Virginia. *Plant Disease Reporter* **55**: 467–469.

Studhalter RA, Ruggles AG (1915). Insects as carriers of the chestnut blight fungus. *Dept. of Forestry, Commonwealth of Pennsylvania Bull.* **12**: 1–33.

Swart WJ, Conradie E, Wingfield MJ (1991). *Cryphonectria cubensis*, a potential pathogen of *Psidium guajava* in South Africa. *European Journal of Forest Pathology* **21**: 424–429.

Swofford DL (2002). *PAUP. Phylogenetic Analysis Using Parsimony. Version 4.0b10.* Sinauer Associates, Sunderland, Massachusetts.

Tamura K, Nei M (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology & Evolution* **10**: 512–526.

Teng SC (1934). Notes on Sphaeriales from China. *Sinensia* **4**: 359–433.

Turchetti T (1982). Hypovirulence in chestnut blight (*Endothia parasitica* [Murr.] And.) and some practical aspects in Italy. *European Journal of Forest Pathology* **12**: 414–417.

Turchetti T & Maresi G (1991). Innoculation trials with hypovirulent strains of *Cryphonectria parasitica*. *European Journal of Forest Pathology* **21**: 65–70.

Turnbul JW (2000). Economic and social importance of eucalypts. In: *Diseases and pathogens of eucalypts* (Keane PJ, Kile GA, Podger FD, Brown BN, eds). CSIRO Publishing, Australia: 1–9.

Van der Merwe NA (2000). Genotypic diversity and gametic equilibrium in a South African population of *Cryphonectria cubensis*. In: *Molecular phylogeny and population biology studies on the Eucalyptus canker pathogen Cryphonectria cubensis*. M.Sc. thesis. Department of Genetics, University of Pretoria, South Africa: 148–169.

Van der Merwe NA, Wingfield BD, Wingfield MJ (2003a). DNA data reveal the population structure of *Cryphonectria cubensis*. Volume 2 - Offered Papers of the 8th International Congress of Plant Pathology, Christchurch, 2–7 February, 2003.

Van der Merwe NA, Wingfield BD, Wingfield MJ (2003b). Primers for the amplification of sequence characterized loci in *Cryphonectria cubensis*. *Molecular Ecology Notes* **3:** 494–497.

Van der Westhuizen IP, Wingfield MJ, Kemp GHJ, Swart WJ (1993). First report of the canker pathogen *Endothia gyrosa* on *Eucalyptus* in South Africa. *Plant Pathology* **42**: 661–663.

Van Heerden SW (2004). Molecular characterisation of mitoviruses co-infecting South African siolates of the *Eucalyptus* canker pathogen *Cryphonectria cubensis*. In: *Studies on Cryphonectria cubensis in South Africa with special reference to mycovirus infection.* Ph.D. thesis. Faculty of Natural and Agricultural Science, University of Pretoria, South Africa: 33–76.

Van Heerden SW, Amerson HV, Preisig O, Wingfield BD, Wingfield MJ (2005). Relative pathogenicity of *Cryphonectria cubensis* on *Eucalyptus* clones differing in their resistance to *C. cubensis*. *Plant Disease* **89**: 659–662

Van Heerden SW, Geletka LM, Preisig O, Nuss DL, Wingfield BD, Wingfield MJ (2001). Characterization of South African *Cryphonectria cubensis* isolates infected with a *C. parasitica* hypovirus. *Phytopathology* **91**: 628–632.

Van Heerden SW, Wingfield MJ (2001). Genetic diversity of *Cryphonectria cubensis* isolates in South Africa. *Mycological Research* **105**: 94–99.

Van Heerden SW, Wingfield MJ (2002). Effect of environment on the response of *Eucalyptus* clones to inoculation with *Cryphonectria cubensis*. *Forest Pathology* **32**: 395–402.

Van Heerden SW, Wingfield MJ, Coutinho T, Van Zyl LM, Wright JA (1997). Diversity of *Cryphonectria cubensis* isolates in Venezuela and Indonesia. Proceedings of IUFRO Conference on Silviculture and Improvement of Eucalypts, Salvador, Brasil, August 24–29, 1997. Embrapa, Colombia: 142–146.

Van Staden V, Erasmus BFN, Roux J, Wingfield MJ, Van Jaarsveld AS (2004). Modelling the spatial distribution of two important South African plantation forestry pathogens. *Forest Ecology and Management* **187:** 61–73.

Van Zyl LM, Wingfield MJ, Alfenas AC, Crous PW (1998). Population diversity among Brazilian isolates of *Cryphonectria cubensis*. *Forest Ecology and Management* **112**: 41–47.

Van Zyl LM, Wingfield MJ, Alfenas AC, Crous PW (1999). Hypovirulence detected in Brazilian isolates of *Cryphonectria cubensis*. *Plant Pathology* **48**: 267–272.

Venter M, Myburg H, Wingfield BD, Coutinho TA, Wingfield MJ (2002). A new species of *Cryphonectria* from South Africa and Australia, pathogenic on *Eucalyptus*. *Sydowia* **54**: 98–117.

Venter M, Wingfield MJ, Coutinho TA, Wingfield BD (2001). Molecular characterization of *Endothia gyrosa* isolates from *Eucalyptus* in South Africa and Australia. *Plant Pathology* **50**: 211–217.

Vilgalys R, Hester M (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238–4246.

Von Höhnel F (1909). Fragmente zur Mykologie. IX. Mitteilung, Nr. 407 bis 467. In: *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften. Mathematisch- Naturwissenschaftliche Klasse* 118: 1479–1481.

Walker J, Old KM, Murray DIL (1985). *Endothia gyrosa* on *Eucalyptus* in Australia with notes on some other species of *Endothia* and *Cryphonectria*. *Mycotaxon* **23**: 353–370.

Wardlaw TJ (1999). *Endothia gyrosa* associated with severe stem cankers on plantation grown *Eucalyptus nitens* in Tasmania, Australia. *European Journal of Forest Pathology* **29**: 199–208.

Wehmeyer LE (1936). Cultural studies of three new Pyrenomycetes. *Mycologia* **28**: 35–46.

Weir JR (1925). Notes on the parasitism of *Endothia gyrosa* (Schw.) Fr. *Phytopathology* **15**: 489–491.

Wendt R, Weidhaas J, Griffin GJ, Elkins JR (1983). Association of *Endothia parasitica* with mites isolated from cankers on American chestnut trees. *Plant Disease* **67**: 757–758.

White TJ, Bruns T, Lee S, Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols: a guide to methods and applications* (Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds). Academic Press, San Diego, U.S.A.: 315–322.

White DA, Kile GA (1993). Discolouration and decay from artificial wounds in 20-year-old *Eucalyptus regnans*. *European Journal of Forest Pathology* **23**: 431–440.

Wingfield MJ (2003). 2003 Daniel McAlpine Memorial Lecture. Increasing threat of diseases to exotic plantation forests in the Southern Hemisphere: lessons from Cryphonectria canker. *Australasian Plant Pathology* **23**: 133–139.

Wingfield MJ, Rodas C, Wright J, Myburg H, Venter M, Wingfield BD (2001). First report of Cryphonectria canker on *Tibouchina* in Colombia. *Forest Pathology* **31**: 297–306.

Wingfield MJ, Swart WJ, Abear B (1989). First record of Cryphonectria canker of *Eucalyptus* in South Africa. *Phytophylactica* **21**: 311–313.

Youngs RL (2000). "A right smart little jolt". Loss of the chestnut and a way of life. *Journal of Forestry* **98**: 17–21.

Yuan ZQ, Mohammed C (1999). Pathogenicity of fungi associated with stem cankers of eucalypts in Tasmania, Australia. *Plant Disease* **83**: 1063–1069.

Yuan ZQ, Mohammed C (2000). The pathogenicity of isolates of *Endothia gyrosa* to *Eucalyptus nitens* and *E. globulus*. *Australasian Plant Pathology* **29:** 29–35.

Zhang N, Blackwell M (2001). Molecular phylogeny of dogwood anthracnose fungus (*Discula destructiva*) and the Diaporthales. *Mycologia* **93**: 355–365.

# 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.