# CHAPTER 10

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

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

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

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

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

## 1. INTRODUCTION

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

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

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

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

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

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

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

## 2. REVISED TAXONOMY

### 2.1 *Cryphonectria*

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

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

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

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

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

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

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

### 2.2 New type for *Cryphonectria*

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

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

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

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

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

**2.3 New genera for *Cryphonectria* spp.**

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

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

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

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

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

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

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

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

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

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

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

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

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

## 3. CONCLUSIONS

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

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

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

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

**ACKNOWLEDGMENTS**

We are grateful to Dr. Scott Enebak who provided us with photographs of chestnut blight. Financial support for the various studies that made this review possible was provided by the National Research Foundation (NRF), members of the Tree Protection Co-operative Programme (TPCP), the THRIP support programme of the Department of Trade and Industry, and the Department of Science and Technology / NRF Centre of Excellence in Tree Health Biotechnology, South Africa.

**REFERENCES**

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

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

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.

Earle FS (1901). Some fungi from Porto Rico. *Muehlenbergia* **1**: 10–17.

Gams W (2005). Report of the Committee for Fungi: 13. *Taxon* **54**: 828–830.

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. Königstein, Germany: Koeltz Scientific Books.

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

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, Glen HF, Wingfield BD, Wingfield MJ (2005c). Proposal to conserve the name *Cryphonectria* (*Diaporthales*) with a conserved type. *Taxon* **54**: 539–540.

Gryzenhout M, Myburg H, Rodas CA, Wingfield BD, Wingfield MJ (2006). *Aurapex penicillata* gen. sp. nov. from native *Miconia theaezans* and *Tibouchina* spp. in Colombia. *Mycologia* **98**: 111–121.

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 (2005a). *Rostraureum tropicale* gen. sp. nov. (*Diaporthales*) associated with dying *Terminalia ivorensis* in Ecuador. *Mycological Research* **109**: 1029–1044.

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

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.

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

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

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

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

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

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

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

Myburg H, Gryzenhout M, Heath RN, Roux J, Wingfield BD, Wingfield MJ (2002). 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 (2003). Conspecificity of *Endothia eugeniae* and *Cryphonectria cubensis*: A re-evaluation based on morphology and DNA sequence data. *Mycoscience* **104**: 187–196.

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

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.

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

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

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

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

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.

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.

Vasilyeva LN (1998). Pyrenomycetidae et Loculoascomycetidae. Plantae non Vasculares, Fungi et Bryopsidae. Orientis extremi Rossica. *Fungi* **4**: 1–418.

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.

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.

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

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.

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

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

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

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

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

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

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

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

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

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

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

**ATCC**, American Type Culture Collection, Manassas, USA;

**CBS**, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands;

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

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

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

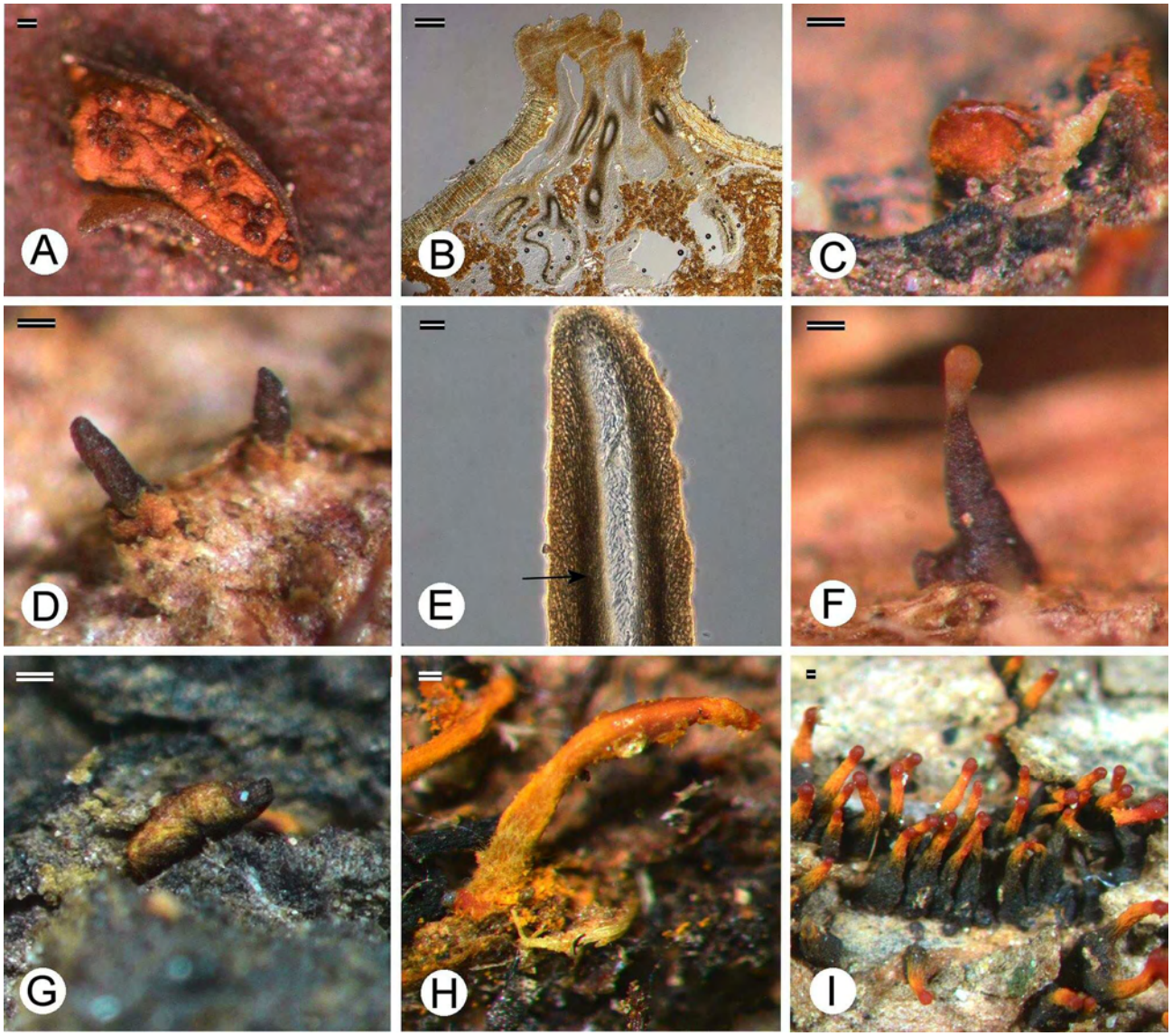


**Fig. 1.** Fruiting structures of *Cryphonectria* and related genera. A. Ascostroma of *Cryphonectria*. B. Longitudinal section through ascostroma of *Cryphonectria*. C. Conidioma of *Cryphonectria*. D. Ascostromata of *Chrysoporthe*. E. Perithecial neck of *Chrysoporthe* (arrow) with brown surrounding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

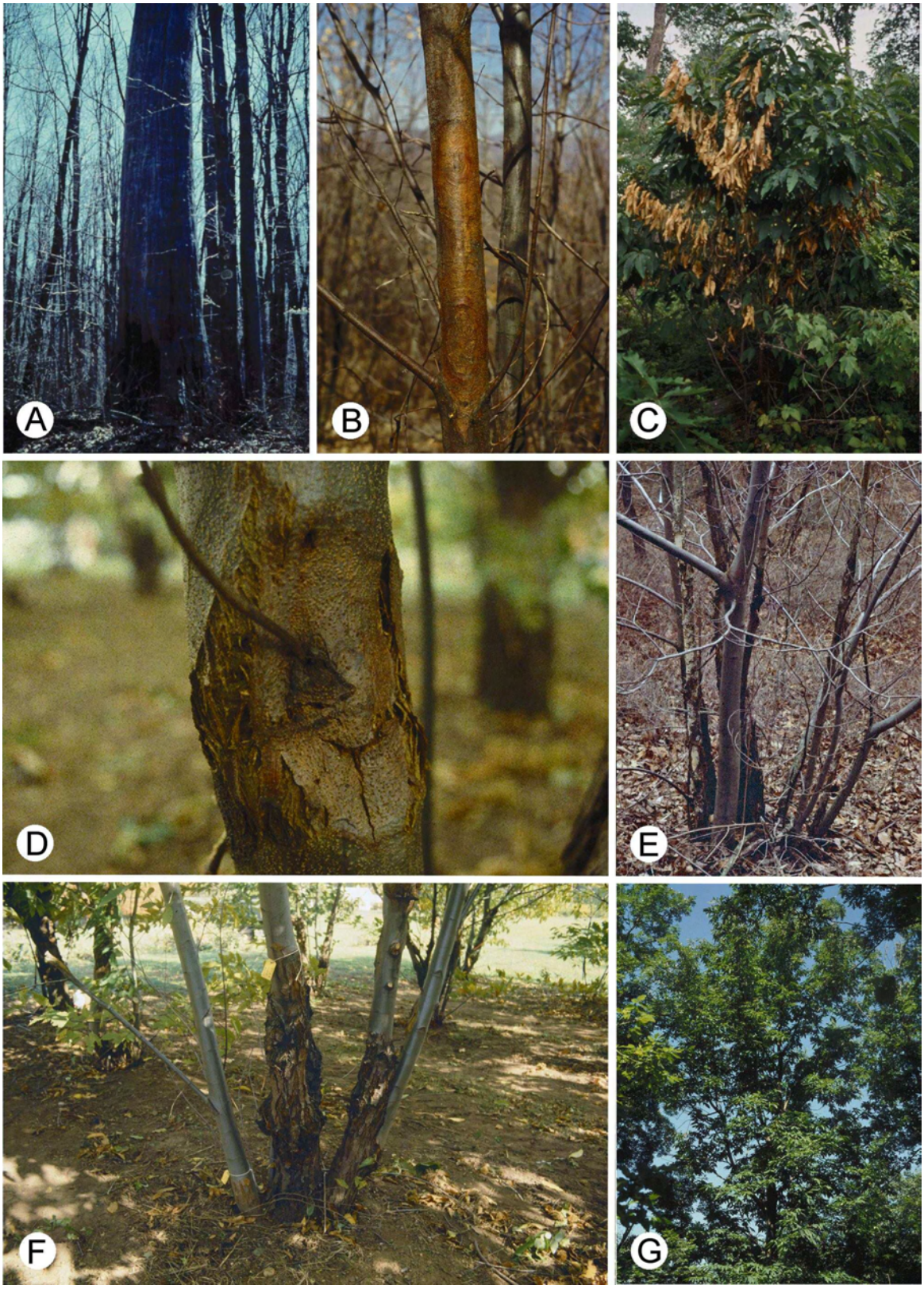


**Fig. 2.** Disease symptoms of chestnut blight caused by *Cryphonectria parasitica*. A. A relic *Castanea dentata* tree still standing long after being killed by chestnut blight after its introduction into the U.S.A. B. Diffuse canker on *Castanea dentata*. C. Die-back of branches with dead leaves still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

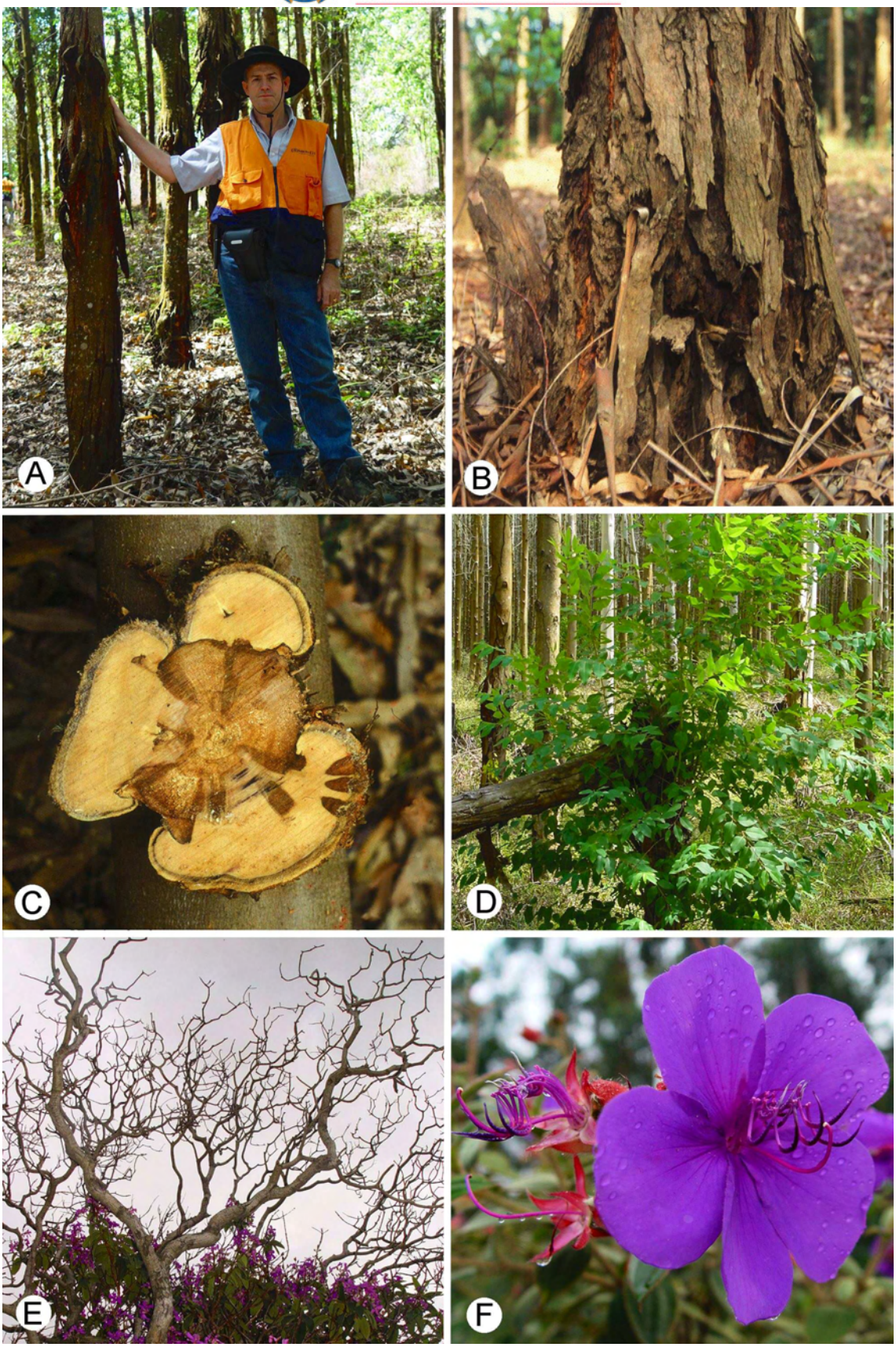


**Fig. 3.** Disease symptoms of Chrysoporthe canker caused by various *Chrysoporthe* species. A–B. Cankers on the trunks and bases of *Eucalyptus* trees. C. Cross section of canker showing killed vascular tissue. D. Stem breakage due to girdling cankers. E. Die-back of *Tibouchina* spp. (F).

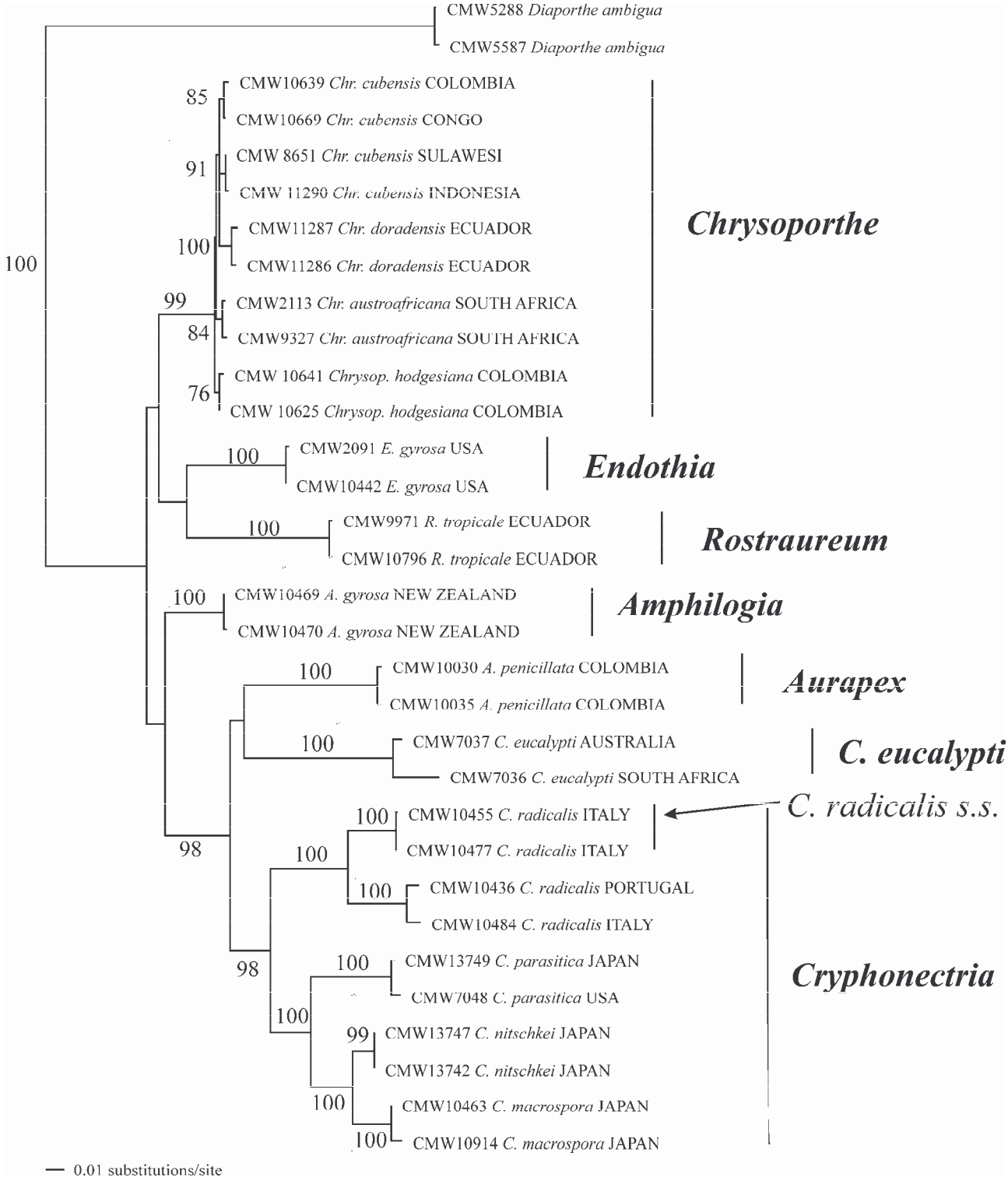


**Fig. 4.** A phylogenetic tree showing the grouping of *Cryphonectria* and related genera. The tree was obtained from a combined DNA sequence dataset of the ITS1, 5.8S rRNA gene and ITS2 regions of the ribosomal operon, and β-tubulin genes respectively. Alignment was obtained using the web interface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