FUNGAL DISEASES OF EUCALYPTS IN CHINA

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Fungal diseases of eucalypts in China

By

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(M.Sc Agriculture)

Submitted in partial fulfilment of the requirements for the degree

Philosophiae Doctor

In the Faculty of Natural and Agricultural Sciences, Department of Microbiology and Plant Pathology, Tree Protection Cooperative Programme, Forestry and Agricultural Biotechnology Institute, at the University of Pretoria,
Pretoria, South Africa

November 2010

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Declaration

I, the undersigned, hereby declare that this thesis, submitted herewith for the degree of Philosophiae Doctor to the University of Pretoria, contains my own independent work. This work has hitherto not been submitted for any degree at any other University.

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ShuaiFei Chen

November 2010

This thesis is dedicated to my mother, Wang Lun (April 13, 1956–August 10, 2006)

谨以此论文献给我的母亲：王论（一九五六年三月初三—二零零六年七月十七）

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Acknowledgements

I believe life is a book. We are writing stories for this book everyday. Sometimes the stories are good, but every so often a story with an undesirable plot appears in our book. The most important to me is to always try to write good, positive stories. This is not only because other people around us will be affected by one's "life book", but also because we do not want to regret the path our lives took when we look back upon it.

The past three and a half years during which I conducted research towards obtaining a PhD degree has been one of the most important chapters of my "life book". I have grown as a person and a scientist and was privileged to meet many wonderful people to include in my life story. It is a story about emotion, passion, will-power. It is an incalculable treasure for me. This chapter of my life would not have been possible without the support of many people and organizations and I wish to express my sincere thanks to them:

- For facilities, equipment and funding:

FABI (Forestry and Agricultural Biotechnology Institute), it affected me deeply, providing me with the opportunity to become a scientist in the field of plant pathology, beginning with no previous background in this field, TPCP (Tree Protection Co-operative Programme), UP (University of Pretoria), and CERC (China Eucalypt Research Centre), CFEPP (CERC-FABI Eucalypt Protection Programme), CAF (Chinese Academy of Forestry).

- My supervisors:

Dr. XuDong Zhou (also Prof. at CERC, CAF), who organized the research undertaken for this dissertation and who provided me the opportunity to undertake my PhD studies in South Africa. He introduced me to the FABI family and has been a major driver in building collaboration between FABI and CERC. Thank you for this opportunity and for your guidance and support.

Prof. Jolanda Roux, who introduced me to the world of fungi, and continues to enthuse me with the wonders of fungi. Thank you for guiding me through the writing of my thesis and for being ready to offer your support and help at any time. Your great heart, incredible passion for forest pathology and wide knowledge will affect me forever.

Prof. YaoJian Xie, who was also my MSc supervisor. You are like a father to me, always offering care and support. Thank you for introducing me to FABI. Without your help, this research could not have been conducted.

Prof. Mike Wingfield, who is an inspiration to all his students and colleagues. Thank you for your support and for also helping me to think about the bigger picture and to strive to higher levels. Your passion, wisdom, good heart and wide knowledge will affect me for my whole life.

- Mentors in FABI:

Various people in FABI guided me through the research conducted in this dissertation, helping with laboratory work and with writing of manuscripts. Thank you to each of you:

Dr. Draginja Pavlic for assistance with the Botryosphaeriaceae chapter;

Dr. Lorenzo Lombard with the *Calonectria* chapter;

Dr. Marieka Gryzenhout with the *Chrysoporthe cubensis* and *Celoporthe* chapters;

Dr. Irene Barnes and Mr. Donald Chungu with the *Teratosphaeria zuluensis* chapter;

Prof. Slippers Bernard for comments on the Botryosphaeriaceae chapter;

Dr. Min Lu and Ms. Marelize Van Wyk for showing me the lab technologies.

- FABI friends:

Ladies at the reception desk (Helen Doman, Martha Mahlangu and Rose Visser); ladies in the Culture Collection (Lydia Twala, Maretha Van der Merwe, Marieka Gryzenhout, Martie Van Zyl and Valentina Nkosi); ladies in charge of finances (Eva Muller and Jenny Hale); staff of the sequencing facilities (Gladys Shabangu and Renate Zipfel); my lab mates in TEW lab (Deer Konkarn, Donald Chungu, Elsie Cruywagen, Happy Maleme, Johan Van der Linde, Lenny Mashavha, Linda Ndove and Wilhelm De Beer); the disease clinic teams of 2008 and 2009 (Bernice Porter, Darryl Herron, Donald Chungu, Dr. Didier Begoude, Dr. Guillermo Perez, Izette Greyling, Johan Van der Linde and Michael Mbenoun). Other friends in FABI who assisted me in the lab, shared a joke and encouragement: Albe Van der Merwe, Barry Christie, Dr. Brett Hurley, Dr. ChongXing Zhang, Dr. Eunsung Oh, Dr. Ronald Heath, Dr. Martin Coetzee, Dr. Wubetu Bihon, Fahimeh Jami, Gilbert Kamgan Nkuekam, Heidi Fysh, James Mehl, Prof. Brenda Wingfield, Prof. Emma Steenkamp, Prof. Teresa Coutinho, Magriet Van der Nest, Marcele Vermeulen, Marija Kvas, Markus Wilken, Marthin Tarigan, Matsepo Taole, Tuan Duong, XinTao Mou, and all the other FABIANS.

- Prof. Hennie Groeneveld and Dr. Mike Vander Linde (University of Pretoria) for help with the statistical analyses.
- Dr. Hugh Glen (South African National Biodiversity Institute, South Africa) for the Latin translations for new species.
- Dr. Treena Burgess (Murdoch University, Australia) for providing some isolates used in this thesis.

- Friends out of FABI:

Ming Zhang, who is my best Chinese friend in South Africa. Jeanne Korsman and Yun Chai, who offered lots of supports and help to me. Team mates in the South African Kung-Fu Kitchen soccer club: DeZhi Gao, Dr. HongZe Luo, Fang Wang, HuaNan Gu, George Chen, James Chen, MingXing Zhao, Tao Yuan. Friends in CERC: ChunYan Xie, GuiXiang Zhao, JiaGuang Cao, JieFeng Liu, ShiChao Zhang, WenWei Tan, XiuHua Shang, ZhiHua Wu. Some other friends including ChengSuo Xie, Dr. MingZhou Chen, Dr. XiYin Liang, Dr. XiangTao Zhuan, Hong Xu, Ji Liu, JianHong Li, JianJi An, KeSheng Wang, Prof. XiaoHua Xia, RunZhi Lv, XianMing Ye, XiaoLan Li, YangGen Wei, ZeLei Huang, ZhenShan Luo.

- My family:

My father ChangLiu Chen and my mother Lun Wang, who brought me up and instilled in me the desire to be a good person. My younger sister Fei Chen and my brother-in-law Pei Zhao for your sustained support.

- Everyone who helped make my time in FABI and South Africa a memorable experience! I cannot name all of you, but I know that I appreciate all of you, who helped and have affected me in experiencing this wonderful and colourful world!

Preface

Non-native eucalypt trees are planted widely in South China and are increasing in importance. In the past half Century, particularly the past 20 years, China's eucalypt plantations have been expanding rapidly. Similar to other countries, these plantations are threatened by pests and diseases. However, information pertaining to pests and diseases of eucalypts in China is relatively limited. Studies of plantation health in South China in the past 10 years have reported a number of previously unknown diseases of these trees. This led to the establishment of a eucalypt health programme CFEPP (CERC-FABI Eucalypt Protection Programme, http://www.fabinet.up.ac.za/cfepp) between CERC (China Eucalypt Research Centre, http://www.chinaeuc.com) and FABI (Forestry and Agricultural Biotechnology Institute, http://www.fabinet.up.ac.za) in 2007.

The research in this thesis is aimed at offering a foundation on the most important fungal disease issues of eucalypts in China. Fungal pathogens were characterized using a combination of morphology and DNA sequence data. Where possible, the population diversity of pathogens was investigated to obtain a better idea for future management strategies. All pathogens identified were also used in inoculation experiments to select disease tolerant planting material for the eucalypt industry in South China.

Chapter one provides a summary of the eucalypt plantation industry in Asia and particularly in China, as well as a review of the disease problems of eucalypts in Southeast Asia, India and China. Since the establishment of a plantation forestry industry using eucalypts relies on the importation of seeds into the country, this chapter also reviews the most common routes of movement of plant pathogens. Several examples, particularly pertaining to eucalypt pathogens are discussed. The chapter provides several examples to show the possible origin and population diversity of eucalypt pathogens globally.

Many species in the Cryphonectriaceae cause diseases on trees, including species of *Eucalyptus* and *Syzygium*, both in the plant family Myrtaceae. Stem canker caused by *Chrysoporthe cubensis* in Brazil, is for example, credited for leading to the establishment of clonal forestry worldwide. During disease surveys in South China, symptoms typical of those caused by fungi in the Cryphonectriaceae were observed on both *Eucalyptus* spp. as well as *Syzygium* trees. The aim of the studies in Chapter two was to characterize the fungi collected

from these trees, using multi-gene phylogenies and studies of the morphology of the fruiting bodies of the collected fungi. In order to provide the eucalyptus industry with information regarding the relative susceptibility of currently planted eucalypt clones in China, field inoculation trails were conducted to screen various clones. Cross inoculations of the same isolates onto *S. cumini* were also done to investigate the ability of isolates from the different hosts to infect others in the Myrtaceae.

It has seen a dramatic increase in the number of fungal genera in the Cryphonectriaceae in recent years. Where ten years ago only two genera (*Cryphnoectria* and *Endothia*) were known, fourteen are known today. The advent of DNA sequencing has allowed for the recognition of species despite their having similar morphological features. In Chapter three of this dissertation, species of *Celoporthe* collected from *Eucalyptus* and *Syzygium* trees in South China, are characterized based on morphology and DNA sequence comparisons. The pathogenicity of these species was also evaluated in the greenhouse as well as in field inoculations on several *Eucalyptus* genotypes and *S. cumini*.

Fungi in the Botryosphaeriaceae represent some of the most common and widespread pathogens of trees globally. They often cause disease of trees that are under some form of environmental stress and they have a great capacity to move around the globe, undetected as endophytes. It was thus expecting that during studies that make up this dissertation, a number of isolates of fungi in the Botryosphaeriaceae were obtained. In Chapter four, isolates collected from branch cankers and senescent twigs on several *Eucalyptus* spp. were characterized by PCR-RFLP fingerprinting, morphology and DNA sequence comparisons. The pathogenicity of these fungi was investigated on *Eucalyptus* genotypes, both in the greenhouse and in field inoculation trails.

Cylindrocladium leaf blight (CLB) caused by *Calonectria* spp., is one of the most serious leaf diseases of eucalypts in the world. During disease surveys on *Eucalyptus* plantations in the FuJian Province of Southeast China, symptoms of leaf blight typically caused by *Calonectria* spp. were commonly observed. These fungi were identified and characterised by morphological characteristics, DNA sequence comparisons and sexual compatibility. Field inoculation trails were conducted on two popular Chinese *E. urophylla* × *E. grandis* clones.

Coniothyrium stem canker, caused by the fungi now known as *Teratosphaeria zuluensis* and *T. gauchensis*, is one of the most important diseases of global *Eucalyptus* plantations, particularly in tropical and sub-tropical areas. Where fifteen years ago the disease was known only from South Africa, it is today considered one of the most important constraints globally to the production of sawn timber. In China, this disease was first observed on *Eucalyptus* spp. in 2006. The last chapter of this dissertation reports on studies to determine the current distribution of the disease in South China and with the characterization of isolates collected from diseased trees. Furthermore, the population diversity of *T. zuluensis* on *Eucalyptus* trees in South China was conducted to obtain information on the reproduction and movement of the pathogen in the country. Isolates were collected from three different geographical regions in the GuangDong and GuangXi Provinces and ten microsatellite markers were used to study the population structure of this pathogen.

Research presented in this dissertation provides the most extensive study of fungal pathogens of eucalypts in China conducted to date. It expands the geographic and host range of a number of pathogens and describes the discovery of several previously unknown fungal species that occur on eucalypts in China. Each chapter has been written as a separate entity, and three have been accepted for publication, or appeared in print. It is my hope that the research presented in this thesis will provide a foundation for future studies of eucalypt health in China. It is further anticipated that the results will also assist in the implementation of more robust management strategies to ensure a sustainable industry for the future there.

Chapter 1

Literature Review: Diseases and their importance to eucalypt plantation forestry in China

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ABSTRACT

Eucalyptus spp. are widely planted in many parts of the world as sources of construction timber, fuel wood and for the production of pulp and paper. In China, over the last 60 years, commercial eucalypt plantations have been extensively developed. Like many other countries of the world, the long-term sustainability of these plantations is threatened by pests and diseases. Very limited work has been conducted on diseases and pests of eucalypts in China. Pilot surveys in South China in 2006–2008 identified a number of previously unreported diseases. This has led to the establishment of a eucalypt plantation health programme in the country. This review aims to provide a background for future studies of eucalypt pathogens in China and also in South and Southeast Asia. Furthermore, it should aid in the development of management strategies to reduce their impact. The diseases reported to date in this region are discussed and the most common routes of movement of plantation pathogens are summarized.

1. INTRODUCTION

Eucalypts, including the genera *Eucalyptus* L'Her., *Corymbia* K.D.Hill & L.A.S.Johnson and *Angophora* Cav., represent a unique group of trees including more than 900 species, of which *Eucalyptus* spp. are the most numerous (Brooker 2000; Keane *et al*. 2000; Nicolle 2006). Eucalypts are cultivated to meet a wide variety of needs such as pulp for paper and viscose, fuel wood, charcoal, railway sleepers, fiber board, sawlogs, poles, mining timber, furniture and material for building construction (Eldridge *et al*. 1997; Qi 2002; Mo 2006; Xie 2006; Xu 2006), essential oils for perfumery and medicinal purposes, and bark for tannin production (Qi 2002). They are also planted to prevent soil erosion, to provide shelter and shade as ornamental trees (Qi 2002; Mo 2006; Xie 2006).

Eucalypts, commonly known as gum trees, are mainly endemic to mainland Australia and Tasmania, with a few species indigenous to Indonesia, Papua New Guinea and the Philippines (Keane *et al*. 2000; Qi 2002; Mo 2006; Xiang *et al*. 2006; Xie 2006). They have been introduced into many parts of the world and are widely planted in more than 100 countries, including in areas of the tropics and subtropics of Central and South America, Africa and Asia, semi-arid regions of North Africa, Central Asia and the Middle East, the Mediterranean regions of North Africa and Southern Europe, and cool mountain regions of South America and Africa (Keane *et al*. 2000; Qi 2002; Old *et al*. 2003; Mo 2006; Xie 2006; Iglesias-Trabad & Wilstermann 2008).

Eucalypt plantation areas are growing dramatically, mainly because of the rapid growth of the trees, wide adaptability and the great variety of end uses that they serve. These trees represent one of the most widely planted genera for commercial purposes in the world, with a total of about 20 million hectares (Mha) established in plantations (Iglesias-Trabad & Wilstermann 2008). The three countries with the largest areas planted to eucalypts are India (3.9 Mha), Brazil (3.7 Mha) and China (2.6 Mha) (Iglesias-Trabad & Wilstermann 2008), with extensive commercial plantations also occurring in Australia, Chile, Portugal, South Africa, Spain, Thailand, Uruguay and Vietnam (Turnbull 2000; Old *et al*. 2003; Xie 2006; Xu 2006; Iglesias-Trabad & Wilstermann 2008) to name a few.

Eucalypts have been affected by diseases worldwide, both in the regions where they are native, as well as where they are grown as non-native plantation trees (Sankaran *et al*. 1995b; Keane

et al. 2000; Wingfield *et al*. 2001c, 2008, 2011; Old *et al*. 2003; Dell *et al*. 2008; Zhou *et al*. 2008). Reports of diseases in many countries have increased significantly over the past few decades due to the increased movement and trade in plant products/material between countries (Wingfield *et al*. 2001c, 2008; Old *et al*. 2003). There are also growing examples of fungi from native areas undergoing host jumps to non-native eucalypts, such as the examples of *Puccinia psidii* G. Winter (Eucalyptus rust) and *Chrysoporthe austroafricana* Gryzenh. & M.J. Wingf. (Cryphonectria canker) (Coutinho *et al*. 1998; Wingfield 2003, 2008; Slippers *et al*. 2005). Avoidance of major epidemics of eucalypt diseases requires an increased awareness of the risks from pathogens and a systematic approach to disease management.

Eucalyptus spp. are growing in importance in China (Xie 2006). The aim of this review is to provide a summary of the eucalypt industry in Asia, with a particular focus on China and the disease problems associated with these trees in the sub-region. The possible pathways of introduction of pathogens into and from China are also considered. In this review, the word eucalypts is used to refer to a group of genera including *Eucalyptus* and *Corymbia* where these genera are not referred to more specifically. It is hoped that the reviewed information will provide a foundation for planning future plantation development in China, and more specifically to avoid the ravages of pests and pathogens.

2. EUCALYPTS IN ASIA AND CHINA

2.1. Eucalypts in Asia

In Asia, eucalypts are especially widely planted in the Southern and Southeastern parts of the region (Keane *et al*. 2000; Qi 2002; Iglesias-Trabado & Wilstermann 2008). More than 40% of the world's eucalypt plantations are distributed in China, India, Indonesia, Philippines, Thailand and Vietnam, and especially in China and India (Iglesias-Trabado & Wilstermann 2008). Similar to other regions, they are planted as exotics to provide fibre for the timber and paper industries as well as for fuel, poles (Keane *et al*. 2000; Qi 2002; Old *et al*. 2003; Wingfield *et al*. 2008), small lumber and furniture, essential oils and tannins (Keane *et al*. 2000; Qi 2002; Old *et al*. 2003). Species such as *Eucalyptus camaldulensis* Dehnh., *E. grandis* W.Hill, *E. tereticornis* Sm., *E. urophylla* S.T.Blake, and their hybrids and clones are particularly popular in this region (Qi 2002; Old *et al*. 2003).

2.2. Eucalypts in China

2.2.1. History and current status

Eucalypts were first introduced into China in 1890 as ornamental trees (Qi 2002). They were planted in parks and villages in GuangZhou, Hong Kong and Macao. Typical examples are *E. tereticornis* introduced to GuangXi Province in 1890, *E. rudis* Endl. to FuJian Province in 1894, *E. globulus* Labill. to YunNan Province in 1896, *E. camaldulensis* to SiChuan Province in 1910 and *E. robusta* Sm. to GuangDong and HuNan Provinces in 1916 (Qi 2002, 2006; Qi *et al*. 2006). During the 1940s, the major species planted included, among others, *Corymbia citriodora* (Hook.) K.D.Hill & L.A.S.Johnson (synonym *E. citriodora* Hook.), *E. camaldulensis*, *E. exserta* F.Muell. and *E. globulus* (Qi 2002, 2006; Qi *et al*. 2006). During the period 1951 to 1980, more than 200 species were introduced to Southern China into areas such as the GuangDong, GuangXi, HaiNan, YunNan, as well as FuJian Provinces. Among these, species and hybrids of *C. citriodora*, *E. camaldulensis*, *E. exserta*, *E. globulus*, *E. leizhou* No.1, *E. maidenii* F.Muell., *E. robusta* and *E. tereticornis* were the most widely planted (Qi 2002, 2006; Qi *et al*. 2006).

Since 1980, the development of eucalypt plantations in China has been significantly facilitated by an inter-governmental cooperation agreement between Australia and China. This was achieved through two projects funded by these governments (Qi 2002; Qi *et al*. 2006). One project was between 1981 and 1989, during which 40 eucalypt species were systematically introduced. The second was between 1984 and 1992, with 70 more species introduced into the country (Qi 2002). During this period, species such as *E. camaldulensis*, *E. dunnii* Maiden, *E. grandis*, *E. nitens* Maiden, *E. smithii* R.T.Baker, and *E. urophylla*, were selected and seed orchards established based on provenance and family selections with desirable growth and wood qualities. Hybrid clones, especially those with rapid growth such as *E. grandis* × *E. urophylla*, *E. urophylla* × *E. camaldulensis*, *E. urophylla* × *E. grandis*, *E. urophylla* × *E. tereticornis* were also selected and planted in South China (Qi 2002).

Presently, eucalypt plantations in China have reached the remarkable point of covering of 2.6 Mha (Iglesias-Trabad & Wilstermann 2008). They are distributed over 600 counties in 19 Provinces/Regions throughout China (Qi 2002, 2006) (Fig 1). The major planted areas are in GuangXi, GuangDong, HaiNan, YunNan, as well as FuJian Provinces (Qi 2002, 2006; Qi *et*

al. 2006; Xie 2006; Yang & Peng 2006). In total, over 300 eucalypt species have been introduced into the country, of which more than ten are economically important species that are widely planted (Qi 2002, 2006; Qi *et al*. 2006) (Table 1). Among them, *E. camaldulensis*, *E. dunnii*, *E. globulus*, *E. grandis*, *E. maidenii*, *E. saligna* Sm., *E. tereticornis*, *E. urophylla*, as well as their hybrids and clones are most popular (Qi 2002, 2006; Qi *et al*. 2006; Xie 2006; Xiang *et al*. 2006).

2.2.2. Distribution

Eucalypt trees are widely distributed in China, in different geographic and climatic regions. They are located between 18°20′ N (SanYa, HaiNan Province) to 33°00′ N (HanZhong, ShanXi Province), and 122°19′ E (PuTuo, ZheJiang Province) to 98°44′ E (BaoShan, YunNan Province) (Qi 2002). They also grow in areas with variable altitudes and climatic ranges, from 4 m (eg. Southeast) to 2 400 m (eg. YunGui Plateau) (Qi 2002, 2006). Different species/clones of eucalypts are, therefore, chosen and planted in different areas for best possible site species matching (Table 2, Fig 1) (Qi 2002, 2006; Qi *et al*. 2006).

2.2.3 Future development

Eucalypts represent the most important and fastest-growing trees in South China. They are mainly used for the pulp and paper, essential oil, plywood, fibre wood and furniture industries (Qi 2002; Wen *et al*. 2005; Dai *et al*. 2006; Mo 2006; Xie 2006; Liang 2007; Qian 2007; Yang 2007). Development of eucalypt plantations in Southern China is crucial for the growth and expansion of the modern paper industry in the country (Wen 2006; Xu 2006). Eucalypts have contributed substantially to lessen the shortage of fibre in the country by providing over 30 Mm^3 of wood annually. Furthermore, the Chinese government has committed to the establishment of an additional 40 Mha of plantations by 2020 and eucalypts may contribute to these plantings.

3. EUCALYPT DISEASES IN SOUTHEAST ASIA, INDIA AND CHINA

In some Asian countries, such as China, Indonesia, Thailand and Vietnam, fungal pathogens of eucalypts appeared rapidly after the establishment of large eucalypt planting programs (Wingfield *et al*. 2008). Numerous disease and pest problems have been reported affecting

eucalypt plantations in Asian countries (Tables 3–8). In this section, some of the more damaging/important diseases in the region are discussed.

3.1. Leaf and shoot diseases

3.1.1. Leaf and shoot diseases caused by fungi in the Mycosphaerellaceae and Teratosphaeriaceae

Mycosphaerella leaf blotch (MLB) is one of the best known and most important foliage diseases of eucalypts globally (Crous 1998; Crous *et al*. 2004a, 2006c, 2009a; Hunter *et al*. 2006b). The taxonomy of the fungi involved in causing MLB has in recent years undergone significant change and it is now recognized that they reside in at least two families (Mycosphaerellaceae and Tereatosphaeriaceae) and several genera (Crous *et al*. 2006c, 2007a, 2009b, c). The fungi previously treated in the genus *Mycosphaerella* are now known to include at least 30 form genera that share similar phenotypic characters arising from adaptation to similar niches and convergent evolution (Crous *et al*. 2009b, c). It is currently believed that *Mycosphaerella sensu stricto* should best be limited to taxa with *Ramularia* anamorphs, with 12 other well defined clades in the Mycosphaerellaceae representing *Cercospora*, *Cercosporella*, *Dothistroma*, *Lecanosticta*, *Phaeophleospora*, *Polythrincium*, *Pseudocercospora*, *Ramularia*, *Ramulispora*, *Septoria*, *Sonderhenia* and *Zasmidium* (Crous *et al*. 2007a, 2009b, c). The genus *Teratosphaeria*, with species previously accommodated in *Mycosphaerella*, accommodates taxa with *Kirramyces* anamorph. Eight other clades are currently supported in the Teratosphaeriaceae, including *Baudoinia*, *Capnobotryella*, *Catenulostroma*, *Devriesia*, *Penidiella*, *Phaeothecoidea*, *Readeriella* and *Staninwardia* (Crous *et al*. 2007a, 2009b, c). It is currently estimated that genera in the Mycosphaerellaceae and Teratosphaeriaceae include more than 4 000 species (Aptroot 2006; Crous *et al*. 2007a, 2009b, c), of which more than 100 have been associated with eucalypts (Crous *et al*. 2004a, 2006c, 2007a, b, 2009a, b, c; Hunter *et al*. 2004b, 2006b; Andjic *et al*. 2007a, b, c; Burgess *et al*. 2007b).

MLB disease symptoms on eucalypts include leaf spots, leaf necrosis, premature defoliation and loss of growth (Crous *et al*. 1989a, 1998, 2004a, 2006c; Hunter *et al*. 2004a, b, 2006b; Andjic *et al*. 2007a; Burgess *et al*. 2007b). Approximately 32 species in the Mycosphaerellaceae and Teratosphaeriaceae (Crous *et al*. 1989b, 1995, 2004a, 2006c, 2007b;

Old *et al*. 2003; Hunter *et al*. 2006a, b; Andjic *et al*. 2007a, b, c; Burgess *et al*. 2006a, 2007b; Cheewangkoon *et al*. 2008; Zhou *et al*. 2008) have been reported from South and Southeast Asia (Table 3). Among these species, which include important pathogens, such as *Teratosphaeria destructans* (M.J. Wingf., Crous & T.A. Cout.) M.J. Wingf. & Crous [anamorph: *Kirramyces destructans* (M.J. Wingf., Crous & T.A. Cout.) Andjic & M.J. Wingf.] and *T. suttonii* (Crous & M.J. Wingf.) Crous & U. Braun [anamorph: *K. epicoccoides* (Cooke & Massee) J. Walker, B. Sutton & Pascoe] (Old *et al*. 2003). *T. destructans* is considered one of the the most important leaf pathogens of eucalypts, especially in Asian countries such as China, East Timor, Indonesia, Thailand and Vietnam (Wingfield *et al*. 1996a; Old *et al*. 2003; Burgess *et al*. 2006a; Zhou *et al*. 2008) (Table 3). In China, *T. destructans* is a major pathogen of young *Eucalyptus* leaves and shoots in the nurseries, on mother plants and in plantations, and it is widely dispersed in various geographical regions of China (Dell *et al*. 2008).

Teratosphaeria destructans can cause complete defoliation of eucalypt seedlings and trees (Burgess *et al*. 2006a). The disease has been identified on *E. urophylla* hybrids and an unknown *Eucalyptus* sp. in Australia (Burgess *et al*. 2007a), where it is considered to be native and from where it is believed to have been introduced into Asia (Dell *et al*. 2008). Leaf and shoot disease caused by *T. destructans* is difficult to manage. Fungicides can be useful in nurseries but are not very helpful in plantations (Keane *et al*. 2000). For eucalypt plantations, the only practical control measure for this disease is to select disease resistant species and clones (Old *et al*. 2003; Burgess *et al*. 2006a). Furthermore, quarantine measures to limit the spread of the pathogen to new environments are considered to be very important.

Some important species that cause MLB have not yet been reported in Asia. These include *T. nubilosa* (Cooke) Crous & U. Braun, which has a broad distribution globally and is particularly important on cold-tolerant species such as *E. globulus* and *E. nitens* (Park & Keane 1987; Carnegie *et al*. 1998; Carnegie & Ades 2002; Hunter *et al*. 2004a, 2008). *T. nubilosa* typically infects juvenile leaves of these species and causes defoliation (Park & Keane 1982a; Lundquist & Purnell 1987). Many regions in Asia, such as Central South China and North India, share similar *Eucalyptus* spp. and climates with countries where *T. nubilosa* occurs. This provides strong evidence to suggest that countries of Asia should be concerned about the possible accidental introduction of this pathogen into the region. Continued and

vigilant quarantine measures should be developed and enforced by Asian countries to reduce the possibility of *T. nubilosa* and other leaf blight pathogens of eucalypts reaching them.

3.1.2. Cylindrocladium leaf blight

The genus *Calonectria* (*Ca*.) and its *Cylindrocladium* (*Cy*.) anamorphs includes around 68 species (Crous 2002; Lombard *et al*. 2010a), of which a number have been reported as important pathogens of eucalypts (Booth *et al*. 2000; Crous 2002; Old *et al*. 2003; Rodas *et al*. 2005b; Lombard *et al*. 2004, 2010b). *Calonectria* spp. can affect eucalypts both in nurseries and plantations, causing many different symptoms (Booth *et al*. 2000; Crous 2002; Old *et al*. 2003; Lombard *et al*. 2004, 2010b; Rodas *et al*. 2005b). In plantations, *Calonectria* spp. may lead to leaf spots, leaf and shoot blight (CLB) and complete defoliation of trees (Booth *et al*. 2000; Crous 2002; Old *et al*. 2003; Rodas *et al*. 2005b). In nurseries, cutting rot and seedling blight are commonly associated with eucalypt cuttings and seedlings, resulting in death of plants and considerable losses to production (Crous 2002; Old *et al*. 2003; Lombard *et al*. 2004, 2010b).

CLB represents one of the most devastating leaf diseases of eucalypts, with a world wide distribution (Booth *et al*. 2000; Crous 2002; Old *et al*. 2003; Rodas *et al*. 2005b). It is a major problem on eucalypts growing in the humid areas of Asia (Booth *et al*. 2000; Crous 2002; Old *et al*. 2003). A number of species of *Calonectria* are involved in CLB on eucalypts in Asia (Table 4). The most common of these appears to be *Ca. reteaudii* (Bugnic.) C. Booth, which is responsible for epidemic disease in several countries including India, Laos, Thailand and Vietnam. In some regions of Vietnam, repeated defoliation of susceptible provenances of *Eucalyptus* spp. is caused by this pathogen. This is commonly associated with infection by secondary canker-causing fungi, leading to tree death (Old *et al*. 2003).

Like other foliage diseases, infections by *Calonectria* spp. are difficult to control. In nurseries, fungicides can be effective and rigorous sanitation helps to reduce inoculum levels. The most viable means to manage CLB in eucalypt plantations has been to select resistant species and clones of eucalypts (Old *et al*. 2003).

3.1.3. Quambalaria leaf and shoot blight

The genus *Quambalaria* includes six described species (Simpson 2000; De Beer *et al*. 2006; Paap *et al*. 2008; Cheewangkoon *et al*. 2009). They are *Q. coyrecup* T. Paap, *Q. cyanescens* (de Hoog & G.A. de Vries) Z.W. de Beer, Begerow & R. Bauer, *Q. eucalypti* (M.J. Wingf., Crous & W.J. Swart) J.A. Simpson, *Q. pitereka* (J. Walker & Bertus) J.A. Simpson, *Q. pusilla* (U. Braun & Crous) J.A. Simpson and *Q. simpsonii* Cheew. & Crous (Simpson 2000; De Beer *et al*. 2006; Paap *et al*. 2008; Cheewangkoon *et al*. 2009). Species of *Quambalaria* on eucalypts have been found in Africa, Asia, Australia and South America (Wingfield *et al*. 1993; Simpson 2000; Self *et al*. 2002; De Beer *et al*. 2006; Pegg *et al*. 2005, 2008; Roux *et al*. 2006; Zhou *et al*. 2007b; Paap *et al*. 2008; Pérez *et al*. 2008; Cheewangkoon *et al*. 2009), but they all appear to be of Australian origin.

Disease symptoms associated with infection by *Quambalaria* spp. include leaf lesions and shoot blight, typically characterized by the occurrence of powdery white fungal spore masses on the lesions (Wingfield *et al*. 1993; Simpson 2000; Roux *et al*. 2006; Zhou *et al*. 2007b). In Asia, only *Q. pusilla* and *Q. pitereka* have been reported from eucalypt trees (Braun 1998; Simpson 2000; Roux *et al*. 2006; Zhou *et al*. 2007b) (Table 5). Q. *pusilla* was isolated from leaf spots on *E. camaldulensis* in Thailand (Braun 1998). *Q. pitereka* has been reported from a wide range of eucalypts, including those in the genera *Eucalyptus* and *Corymbia* (Walker & Bertus 1971; Pegg *et al*. 2005, 2008; Zhou *et al*. 2007b). *Q. pitereka* causes leaf blight of *C. citriodora* trees, which are grown in plantations in China (Zhou *et al*. 2007b). This, together with recent research results showing that *Q. pitereka* represents an increasing threat to eucalypt plantations in Australia (Pegg *et al*. 2009), indicates that it could potentially become a more important pathogen in China.

3.1.4. Other leaf diseases

Various minor leaf pathogens have been recorded on eucalypts in Southeast Asia. These include *Aulographina eucalypti* (Cooke & Massee) Arx & E. Müll, *Coniella australiensis* Petr., *C. fragariae* (Oudemans) B. Sutton, *Cryptosporiopsis eucalypti* Sankaran & B. Sutton, *Meliola eucalypti* F. Stevens & Roldan ex Hansf., and *Pilidiella eucalyptorum* Crous & M.J. Wingf. (Old & Yuan 1994; Sankaran *et al*. 1995a; Old *et al*. 2002, 2003; Van Niekerk *et al*. 2004) (Table 5). Limited information is available for these pathogens in Asia and they are considered to be of minor importance (Old *et al*. 2003).

3.2. Wilt diseases

3.2.1. Bacterial wilt

Bacterial wilt of eucalypts, caused by *Ralstonia solanacearum* Smith [synonym *Pseudomonas solanacearum* (Smith) Smith] was not observed in eucalypt plantations until the 1980's when reports were made from China (Cao 1982) and Brazil (Sudo *et al*. 1983). Since then, the disease has been widely reported on eucalypts from Australia (Akiew & Trevorrow 1994), Africa (Coutinho *et al*. 2000; Roux *et al*. 2000a, 2001a) and other countries of Asia (Old *et al*. 2003). The pathogen is a soil-borne bacterium, and disease symptoms develop shortly after planting (Old *et al*. 2003). These include the wilting and death of branches and eventually the entire tree. Internal symptoms include extensive xylem discoloration and black streaks in the discoloured xylem (Old *et al*. 2003). Bacterial exudation is often visible when cutting into the roots/stems of affected trees (Old *et al*. 2003).

In Asia, bacterial wilt has been reported from China, Indonesia, Thailand and Vietnam (Cao 1982; Machmud *et al*.1985; Wu & Liang 1988a; Wang 1992; Pongpanich *et al*. 2000; Thu *et al*. 2000; Old *et al*. 2003) (Table 6). In several affected *E. urophylla* plantations in Northern Vietnam, tree mortality was as high as 30% one year after planting (Old *et al*. 2003). *R. solanacearum* is one of the most important eucalypt pathogens in China (Wu & Liang 1988a; Zhou *et al*. 2008). Disease caused by this pathogen is widely distributed in the GuangDong, GuangXi and HaiNan Provinces of the country (Lai 1990; Li 1992; He 1997; Qi *et al*. 2006). In some areas of China, 30–40% of trees are affected by wilt, with yield losses of 20–60% experienced in stands planted with susceptible clones (Li & Wu 1996; Lin *et al*. 1996; Gan *et al*. 2004). There is strong evidence to suggest that the disease develops on trees that are stressed, particularly those having poor root systems and root knots (Wingfield, unpublished).

Few measures have provided for useful the control of bacterial wilt. Planting of resistant material has provided only a partial solution (Sathyanarayana & Anand 1993; Gan *et al*. 2004). Wu & Liang (1988b) have found that certain provenances of *E. exserta*, *E. grandis* × *E. urophylla* and *E. saligna* in China are the most resistant to infection, whereas *E. grandis* and *E. propinqua* Deane & Maiden are moderately susceptible to *R. solanacearum*. Clones that produce effective root systems and that are not susceptible to knotting tend not to be affected by bacterial wilt (Wingfield, unpublished).

3.2.2. Ceratocystis wilt

Ceratocystis spp. are well known causal agents of canker-stain and wilt diseases and they are amongst the most serious pathogens of woody plants in the world (Kile 1993). Diseases of plantation-grown hardwood tree species caused by *Ceratocystis* spp. have increased in number in the last two decades (Roux & Wingfield 2009). Disease symptoms include stem cankers, rapid wilt and death of the affected trees and wood stain (Roux *et al*. 2000b, 2004; Barnes *et al*. 2003b). Diseases of eucalypts caused by *Ceratocystis* spp. are known from Africa (Roux *et al*. 2000b, 2001b, 2005), Asia (Van Wyk *et al*. 2010) and South America (Barnes *et al*. 2003a; Rodas *et al*. 2008; Van Wyk *et al*. 2009) (Table 6). In Southeast Asia, *C. eucalypticola* prov. nom. (Van Wyk, unpublished), in the *C. fimbriata sensu lato* species complex, was recently found on *Eucalyptus* spp. in Indonesia and Thailand (Van Wyk *et al*. 2010), authough no information is available regarding the impact of this or other *Ceratocystis* diseases of eucalypts in Asia. The report by Van Wyk *et al*. (2010) also does not give any information as to whether *C. eucalypticola* prov. nom. in Asia was associated with disease of these trees or not. However, based on reports of *C. eucalypticola* prov. nom. on eucalypts in the Republic of Congo (Roux *et al*. 2000b), Uganda (Roux *et al*. 2001a) and Uruguay (Barnes *et al*. 2003a), the occurrence of this and other species of *Ceratocystis* in China should be monitored.

3.3. Stem/branch cankers

3.3.1. Botryosphaeriaceae canker

The Botryosphaeriaceae (Botryosphaeriales, Ascomycetes) incorporates a family of fungi that have a cosmopolitan distribution and occur on a wide range of monocotyledonous, dicotyledonous and gymnospermous hosts (Barr 1987; Crous *et al*. 2006b; Slippers & Wingfield 2007). Species in this family are generally regarded as weak, opportunistic pathogens, causing disease symptoms on plants under stressful environmental conditions such as those associated with drought, frost, hail and damage caused by other pathogens and pests (Smith *et al*. 1994; Slippers & Wingfield 2007). They also occur in asymptomatic tissues as endophytes and latent pathogens on a variety of trees, including eucalypts (Smith *et al*. 1996b;

Roux *et al*. 2000a, 2001a; Mohali *et al*. 2007; Pavlic *et al*. 2007, 2008; Slippers & Wingfield 2007; Slippers *et al*. 2009).

Disease symptoms caused by species of Botryosphaeriaceae on eucalypts where they are non-native are typically cankers and die-back followed by kino exudation and in severe cases tree death (Smith *et al*. 1996b; Slippers & Wingfield 2007). They are, therefore, considered to be a significant threat to the production and sustainability of eucalypt plantations globally. At least 23 species of Botryosphaeriaceae have been reported associated with eucalypts in commercially-grown plantations world-wide (Slippers *at al*. 2009). With the application of DNA-based molecular tools, a large number of new/cryptic species have been found on eucalypts, mostly in the genera *Lasiodiplodia*, *Neofusicoccum* and *Pseudofusicoccum* (Burgess *et al*. 2006b; Slippers *et al*. 2009).

In Asia, only four species of Botryosphaeriaceae have been characterized on eucalypts, including *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., *Neofusicoccum mangiferum* (Syd. & P. Syd.) Crous, Slippers & A.J.L. Phillips, *N. parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips, and *N. ribis* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips (Old *et al*. 2003; Slippers *et al*. 2009) (Table 7). Except *N. mangiferum*, which was only identified on eucalypts in China (Slippers *et al*. 2009), *L. theobromae* and *N. parvum* are also known from eucalypts in Africa, Australia and South America, and *N. ribis* from eucalypts in Australia and South America (Slippers *et al*. 2009). The distribution of the Botryosphaeriaceae on eucalypts in Asia is most probably incomplete as studies on these fungi in these regions have been relatively limited.

Although the fungi in the Botryosphaeriaceae are considered as endophytes and mostly stress-associated pathogens, they threaten eucalypt plantations in Southeast Asia as their impact can be significant. In some regions of Western Thailand, where clonal trees are grown on a large scale, *N. ribis* is a pathogen of significance in *E. camaldulensis* plantations (Old *et al*. 2003). In some regions of Southeast Asia, a combination of leaf pathogens and species of Botryosphaeriaceae, such as *L. theobromae*, has resulted in the death of young trees (Old *et al*. 2003). The most effective means of management of Botryosphaeriaceae diseases on eucalypts is to avoid planting trees in situations that are not conducive to growth and also to use the best possible sylvicultural practices. It is also possible to select disease resistant eucalypt

clones/hybrids by extensive breeding trials (Old *et al*. 2003) and to use these for plantation development.

3.3.2. Coniothyrium canker

The causal agent of Coniothyrium stem canker disease was first described and characterized as *Coniothyrium zuluense* M.J. Wingf., Crous & T.A. Cout. and described from *E. grandis* in 1996 in South Africa (Wingfield *et al*. 1996b). It has, however, been shown that more than one species is involved in causing Coniothyrium stem canker and the taxonomy of these fungi has undergone a number of changes in recent years (Cortinas *et al*. 2006b, c; Andjic *et al*. 2007a; Crous *et al*. 2007a, 2009a). It is now recognized that at least two species, *Teratosphaeria zuluensis* (M.J. Wingf., Crous & T.A. Cout.) M.J. Wingf. & Crous and *T. gauchensis* (M.-N. Cortinas, Crous & M.J. Wingf.) M.J. Wingf. & Crous, can cause this important stem canker disease of eucalypts (Cortinas *et al*. 2006c; Crous *et al*. 2009a). The pathogens cause serious stem and trunk canker diseases on eucalypts in many parts of the world (Wingfield *et al*. 1996b; Roux *et al*. 2002, 2005; Van Zyl *et al*. 2002; Gezahgne *et al*. 2003a, 2005; Old *et al*. 2003; Cortinas *et al*. 2006b, c; Crous *et al*. 2009a; Chungu *et al*. 2010b). *T. zuluensis* has been reported from Africa (Wingfield *et al*. 1996b; Van Zyl *et al*. 2002; Cortinas *et al*. 2006c, 2010; Chungu *et al*. 2010b), Asia (Van Zyl *et al*. 2002; Gezahgne *et al*. 2003a; Old *et al*. 2003; Cortinas *et al*. 2006b), Central and South America (Roux *et al*. 2002; Cortinas *et al*. 2006c) and Hawaii (Cortinas *et al*. 2004), while *T. gauchensis* is known from Africa (Gezahgne *et al*. 2005; Cortinas *et al*. 2006c) and South America (Gezahgne *et al*. 2003a; Cortinas *et al*. 2006c).

Symptoms of Coniothyrium canker on eucalypts are typically discrete necrotic lesions on stems and branches. These lesions coalesce to form large, gum-impregnated cankers and malformed stems, and then develop into large girdling cankers that reduce wood quality and may lead to tree death (Wingfield *et al*. 1996b; Roux *et al*. 2002; Van Zyl *et al*. 2002; Old *et al*. 2003; Cortinas *et al*. 2006b). In Asia, Coniothyrium canker has been reported only from China (Cortinas *et al*. 2006b), Thailand (Van Zyl *et al*. 2002) and Vietnam (Gezahgne *et al*. 2003a; Old *et al*. 2003) and is caused by *T. zuluensis* (Table 7), while *T. gauchensis* has never been reported from Asian countries. Except for *E. grandis*, *Eucalyptus* genotypes affected by the Coniothyrium canker pathogens include *E. camaldulensis*, *E. urophylla*, as well as their

hybrids with *E. grandis* (Wingfield *et al*. 1996b; Roux *et al*. 2002; Van Zyl *et al*. 2002; Old *et al*. 2003; Cortinas *et al*. 2006b, 2010; Chungu *et al*. 2010b).

3.3.3. Chrysoporthe canker

The disease now known as Chrysoporthe canker was originally thought to be caused by *Cryphonectria cubensis* (Bruner) Hodges (Boerboom & Maas 1970; Hodges *et al*. 1976), and named Cryphonectria canker (Hodges *et al*. 1976; Sharma *et al*. 1985). Research in the last ten years has shown that several species in the newly described genus, *Chrysoporthe*, cause this stem canker disease on eucalypts (Gryzenhout *et al*. 2009). Species of *Chrysoporthe* infecting eucalypts and causing stem cankers include *Chrysoporthe cubensis* (Bruner) Gryzenh. & M.J. Wingf. (Gryzenhout *et al*. 2004), *Chr. deuterocubensis* Gryzenh. & M.J. Wingf. (Gryzenhout *et al*. 2004; Van Der Merwe *et al*. 2010), *Chr. austroafricana* Gryzenh. & M.J. Wingf. (Gryzenhout *et al*. 2004), *Chr. doradensis* Gryzenh. & M.J. Wingf. (Gryzenhout *et al*. 2005) and *Chr. zambiensis* Chungu, Gryzenh. & Jol. Roux (Chungu *et al*. 2010a).

Stem canker diseases caused by *Chrysoporthe* spp. are considered amongst the most important diseases of plantation-grown *Eucalyptus* spp. in the tropics and sub-tropics (Wingfield 2003; Gryzenhout *et al*. 2009). Infection of susceptible young trees can lead to rapid tree death while cankers weaken the stems of older trees, often resulting in stem breakage (Wingfield *et al*. 1989; Conradie *et al*. 1990; Old *et al*. 2003; Wingfield 2003; Gryzenhout *et al*. 2009). The disease has had a substantial impact on the development of eucalypt forestry in the tropics and Southern Hemisphere and vegetative propagation of these trees emerged from efforts to avoid the disease using resistant hybrid clones (Alfenas *et al*. 1983; Old *et al*. 2003; Wingfield 2003; Van Heerden *et al*. 2005; Gryzenhout *et al*. 2009).

Chrysoporthe stem canker of eucalypts in Asia is caused by the recently described pathogen, *Chr. deuterocubensis*, previously known as the Asian phylogenetic group of *Chr. cubensis* (Gryzenhout *et al*. 2004; Van Der Merwe *et al*. 2010). The pathogen is known from several eucalypt species in a number of regions in South and Southeast Asia (Table 7). Teleomorph and anamorph structures appear on the bark of diseased eucalypts after the trees are infected by *Chr. deuterocubensis* (Old *et al*. 2003; Van Der Merwe *et al*. 2010). Susceptible eucalypts are infected rapidly, especially in areas having high rainfall, humidity and temperatures

throughout the year (Old *et al*. 2003). Except for eucalypts, this pathogen has the ability to infect native Myrtales in Southeast Asia, such as native *Syzygium aromaticum* (L.) Merr. & L.M.Perry (Myrtaceae) in Sulawesi, Indonesia (Hodges *et al*. 1986; Myburg *et al*. 2003) and native *Melastoma malabathricum* L. (Melastomataceae) in Indonesia (Gryzenhout *et al*. 2006). Similar to the situation in other countries (Hodges *et al*. 1976; Alfenas *et al*. 1983; Van Heerden & Wingfield 2002; Wingfield 2003; Van Heerden *et al*. 2005), it should be possible to manage Chrysoporthe canker in Asia through the selection of disease resistant eucalypt genotypes.

3.3.4. Cytospora canker

Although not considered primary pathogens, *Valsa* spp. and their *Cytospora* anamorphs are commonly isolated from *Eucalyptus* spp. in association with stem and branch cankers (Old *et al*. 2003; Adams *et al*. 2005, 2006). These opportunistic pathogens are mainly isolated from trees under severe stress due to drought, or trees planted in wet swampy areas with poor drainage. Mechanical wounds are also common sites of infection (Old *et al*. 1991, 2003; Roux *et al*. 2000a, 2001a). It is assumed that these fungi are endophytes in the bark, xylem and leaves (Chapela 1989; Fisher *et al*. 1993; Adams *et al*. 2006). In Asia, several species of *Cytospora* (*Valsa*) have been identified from eucalypt plantations in India, Indonesia, Thailand and Vietnam (Old *et al*. 2003; Adams *et al*. 2005, 2006) (Table 7). *C. eucalypticola* Van Der Westh. is widely distributed in Southeast Asia (Old *et al*. 2003). This fungus was first reported as a pathogen on *E. saligna* in South Africa, where it was found to cause cankers and death of trees (Van Der Westhuizen 1965a, b). *C. eucalypticola* can affect both mature trees and seedlings (Adams *et al*. 2005) in Southeast Asia and both anamorphs and teleomorphs of this fungus have been found associated with stem cankers on *Eucalyptus* in this region (Old *et al*. 2003).

3.3.5. Pink disease

The causal agent of pink disease, *Erythricium salmonicolor* (Berk. & Broome) Burds. [synonyms *Corticium salmonicolor* Berk. & Broome; *Phanerochaete salmonicolor* (Berk. & Broome) Jülich], has a wide host range including *Eucalyptus* spp. It is widely distributed in the tropics and subtropics (Old *et al*. 2003), but has also been reported from eucalypts in temperate regions (Gezahgne *et al*. 2003b; Roux & Coetzee 2005). This pathogen forms

several distinct types of growth on stems and branches of its hosts, including a thin cobweb, pustule, necator, and a salmon pink crust-like stage consisting of the hymenium of the *Erythricium* teleomorph, which occurs on the underside of the dead and dying branches (Old *et al*. 2003). In Asia, this pathogen had been reported from *Eucalyptus* trees in India, Indonesia, Philippines and Vietnam (Old *et al*. 2003) (Table 7). *E. salmonicolor* is a primary pathogen, and able to invade healthy intact bark and attack the cambium (Seth *et al*. 1978). The disease is prevalent in high-rainfall areas and where the relative humidity exceeds 90% (Old *et al*. 2003; Roux & Coetzee 2005). In Asia, *E. salmonicolor* caused significant damage to eucalypt plantations in Southern India (Seth *et al*. 1978; Sharma *et al*. 1984a).

Pink disease can be controlled successfully by early recognition of the symptoms, followed by the application of suitable fungicides (Old *et al*. 2000, 2003). However, this is neither economical nor practical in eucalypt plantations, which often cover thousands of hectares. Similar to other pathogens, selection and breeding for disease resistant material is the most reliable and economically viable route to reduce the impact of this pathogen (Old *et al*. 2003). In situations where the disease is not serious, pruning out diseased branches can also be useful.

3.4. Root diseases

The only root diseases of eucalypts reported from Asia to date, are those caused by root rot fungi in the genera *Armillaria*, *Ganoderma* and *Phellinus* (Basidiomycotina, Basidiomycetes) (Old *et al*. 2003) (Table 8). They infect woody roots and can gain access to the root collars and stems, leading to heart rot and eventual death of trees (Old *et al*. 2003). In general, root diseases caused by these pathogens have not been serious on *Eucalyptus*, which is a situation very different to *Acacia* spp. where dramatic damage has occurred (Old *et al*. 2000). This is probably due to the fact that eucalypts grow fast and they have roots that penetrate deeply, enabling them to avoid infection.

4. STATUS OF EUCALYPT DISEASES IN CHINA

The development of eucalypt plantations in China is threatened by many factors, including pests and diseases. However, limited research has been conducted on eucalypt health, especially diseases, in the country. Bacterial wilt, caused by *R. solanacearum*, is the best known and studied disease of these trees in China, with extensive attention given to its

management (Cao 1982; Wu & Liang 1988a, b; He 1997; Wang *et al*. 1997; Ran *et al*. 2005; Dell *et al*. 2008; Zhou *et al*. 2008). In addition to this, only limited taxonomic work on a number of important pathogens has been undertaken. This includes the characterisation of the causal agents of stem canker caused by *Chr. deuterocubensis* (previously *Chr. cubensis*) (Sharma *et al*. 1985; Hodges *et al*. 1986; Gryzenhout *et al*. 2004, 2009; Van Der Merwe *et al*. 2010) and *T. zuluensis* (Cortinas *et al*. 2006b; Crous *et al*. 2009a). More recently, three species of '*Mycosphaerella*'*, namely '*M*'. *yunnanensis* Barber, Dell & T.I. Burgess, '*M*'. *marksii* Carnegie & Keane, '*M*'. *crystalline* Crous & M.J. Wingf. (Burgess *et al*. 2007b), two species of *Teratosphaeria*, *T. destructans* and *T. sutton* (Burgess *et al*. 2006a; Andjic *et al*. 2007a; Crous *et al*. 2009a) and *Q. pitereka* (Zhou *et al*. 2007b) were also found and characterised from Chinese eucalypt plantations.

During surveys of eucalypt diseases in China between 2006 and 2008, numerous diseases and pathogens, not previously studied in China, were encountered (Zhou *et al*. 2008). Furthermore, *Ca. pauciramosa* C.L. Schoch & Crous and two newly described species, *Ca. cerciana* L. Lombard, M.J. Wingf. & Crous and *Ca. pseudoreteaudii* L. Lombard, M.J. Wingf. & Crous, were identified from a *Eucalyptus* nursery in South China (Lombard *et al*. 2010b). Available information on eucalypt pathogens reported from China is summarized in Tables 3–8.

Eucalypt trees are becoming increasingly important in China, while research on their health is relatively limited in the country. Such research is essential for the sustainability of the industry. Furthermore, information on the pathways of spread of pathogens and pests into the country is essential in order to reduce the rate of introduction of new pests and pathogens. The following section treats the pathways that are most likely to be important for the movement of new pathogens into China. This also includes a brief treatment of some examples of pathogens already present in the country and how they have most likely moved globally.

5. PATHOGEN PATHWAYS

The first line of defense against disease is the exclusion of potentially damaging pathogens. This can be achieved by banning or controlling the movement of plants, propagation material,

* '*Mycosphaerella*'. The name used when these pathogens were first reported from China. This may not be the correct designation for them since the recent changes in taxonomy of MLB pathogens Crous *et al*. (2009b, c).

pathogens or vectors, into disease-free areas from bio-geographical areas in which the pathogens occur. The boundaries between such areas may be between or within nations (Keane *et al*. 2000). Quarantine measures could play a very important role in restricting the introduction of pathogens into new areas (Evans *et al*. 2000; Gadgil *et al*. 2000; Campbell 2001; Wingfield *et al*. 2001b, c, 2008; Burgess & Wingfield 2002; Mumford 2002; Alpert 2006; Slippers & Wingfield 2007; Roux & Wingfield 2009). These measures are especially important for pathogens that cause diseases on eucalypt trees, which are some of the most widely planted exotic tree species in the world (Keane *et al*. 2000; Wingfield *et al*. 2008).

With the rapid increase of global trade and travel, the spread of plant diseases has increased significantly over the past 100 years (Alpert 2006; Tatem *et al*. 2006a, b; Schwartz *et al*. 2006; Jones *et al*. 2007; Lebarbenchon *et al*. 2008). This is also true for eucalypt plantations as increasing numbers of pests and diseases appear in new areas (Wingfield *et al*. 2001b, c, 2008, 2011). In order to manage the spread of diseases, it is necessary to understand the pathways by which the causal agents move around the world (Wilson *et al*. 2008). This information will not only inform policies regarding quarantine, but will also guide future research to improve the management of these diseases.

Common pathways by which pathogens move include air, soil/growth media, germplasm, wood and wood products, equipment, insects and humans. In the next section focus will be placed on those pathways for which it has been shown that forestry pathogens have moved internationally.

5.1. Air

Pathogens can move over long and short distances in air currents and via wind movement (Aylor 1990, 2003; Brown & Hovmoller 2002; Hau & De Vallavieille-Pope 2006). Rust fungi are particularly well adapted for surviving spread over long distances through air currents (Nagarajan & Singh 1990; Agrios 1997; Brown & Hovmoller 2002; Viljanen-Rollinson & Cromey 2002). These fungi have thick walled spores that can survive the radiation and desiccation encountered during dispersal in air currents (Viljanen-Rollinson & Cromey 2002). This, for example, has been demonstrated for the coffee leaf rust pathogen (*Hemileia vastatrix* Berk. & Broome), which has been dispersed from Angola to Brazil, presumably by wind (Carefoot & Sprott 1967; Bowden *et al*. 1971).

Very little is known regarding the movement of important *Eucalyptus* pathogens via the air. A potential candidate for movement in this manner is the rust pathogen of eucalypts, *Puccinia psidii*, which is native on native Myrtaceae in South and Central America (Winter 1884; Coutinho *et al*. 1998; Tommerup *et al*. 2003; Alfenas *et al*. 2005; Glen *et al*. 2007). Eucalyptus rust has been shown to be capable of being wind-dispersed over large distances (Viljanen-Rollinson & Cromey 2002) and once established in new areas, it is likely to move rapidly via air currents (Grgurinovic *et al*. 2006).

Ascomycete fungi are well adapted to dispersal over shorter distances through wind currents. Many of them actively discharge ascospores from their ascomata (Ingold 1971, 2001; Read 1996; Agrios 1997; Fischer *et al*. 2004). *T. nubilosa* can produce airborne ascospores that are actively discharged from ascomata (Park & Keane 1982a). Thus, *T. nubilosa* spores are spread by the wind to new leaves that then serve as new sources of inoculum (Park 1988). This mode of spread, although slower, and over shorter distances, can lead to the movement of new pathogens into an area and across land borders.

5.2. Soil and media

Soil and media represent important pathways for the spread of plant, including forest, pathogens globally (Keane *et al*. 2000; Wingfield *et al*. 2001c; Allen *et al*. 2005). Soil borne pathogens produce resting structures, such as chlamydospores, to facilitate long term survival in soil (Agrios 1997; Keane *et al*. 2000; Crous 2002; Allen *et al*. 2005). Under suitable conditions, they will germinate and infect a host. Soil, together with pathogens, can move widely with plants, equipment, animals and on the shoes of humans. There are consequently many examples of tree pathogens that have moved with soil. Some of the most intriguing are the mycorrhizal fungi found in plantations that have moved globally with soil (Podger *et al*. 1978; Hood & Sandberg 1987; Morrison *et al*. 1991; Agrios 1997; Jones & Baker 2007). Likewise, there are well-documented examples of *Armillaria* spp. that have moved to new continents with potted plants (Coetzee *et al*. 2001, 2003b).

The spread of soil borne pathogens in infected nursery stock is of especial concern globally. This has been shown to be a major method of the spread of pathogens into new areas (Jones & Baker 2007; Brasier 2005, 2008). A number of nursery diseases of eucalypts are caused by

pathogens that could be transferred to new areas through soil/media or seedlings (Keane *et al*. 2000; Old *et al*. 2003). These include, for example, *Botrytis cinerea* Pers. (Keane *et al*. 2000), species of *Calonectria* (*Cylindrocladium*), *Fusarium*, *Phytophthora* and *Pythium* (Keane *et al*. 2000).

The root pathogen of *Pinus* spp., *Rhizina undulata* Fr., a native of the Northern Hemisphere, is one of the best-known soil-borne pathogens of forest trees (Booth & Gibson 1972) and has been moved to many new environments. It has, thus been spread to numerous countries, including countries in the Southern Hemisphere, such as South Africa, most likely with contaminated soil (Wingfield *et al*. 2001c).

Ralstonia solanacearum, the causal agent of bacterial wilt, is a siol-borne pathogen with one of the widest host ranges of all phytopathogenic bacteria (Allen *et al*. 2005). It is also an important pathogen of eucalypt trees with a global range (Dianese *et al*. 1990; Coutinho *et al*. 2000; Roux *et al*. 2001a; Old *et al*. 2003). As mentioned previously, it is one of the most important pathogens in eucalypt plantations in China (Dell *et al*. 2008; Zhou *et al*. 2008). It has been suggested that it can survive in, and spread between eucalypt plantations with, for example, contaminated soil attached to the hooves of cattle (Coutinho *et al*. 2000).

5.3. Germplasm

One of the most important pathways for forest pathogen spread is by germplasm (Keane *et al*. 2000; Wingfield *et al*. 2001b, c, 2008, 2011; Old *et al*. 2003), including vegetatively propagated trees, seedlings, seeds and pollen, as well as rooted cuttings (Evans *et al*. 2000; Old *et al*. 2003; Wingfield *et al*. 2008). Many pathogens have thus spread with the movement of living plants worldwide (Brasier 2005, 2008; Davison *et al*. 2006). A practice known as ‘plants for planting’, that focuses on the movement of plants to new areas, mainly as ornamentals, will hugely accelerate the movement of plant and particularly tree pathogens (Wingfield *et al*. 2011).

The bacterial wilt pathogen, *R. solanacearum*, can cause significant problems in eucalypt nurseries (Keane *et al*. 2000; Coutinho *et al*. 2000; Old *et al*. 2003). Infected nursery stock has been suggested to be a major source of spread of this pathogen to new eucalypt plantations in South Africa (Coutinho *et al*. 2000). This pathogen has a wide host range and

could subsequently also be spread to new forestry areas through infected agricultural and other crops (Janse *et al*. 2004; Coutinho *et al*. 2005).

A considerable threat is posed by endophytic pathogens that can remain undetected in plants, thus easily spreading between countries (Redlin & Carris 1996; Saikkonen *et al*. 1998; Slippers & Wingfield 2007). The Botryosphaeriaceae is a species-rich family that includes pathogens of a wide variety of trees, including eucalypt species (Slippers & Wingfield 2007). It has been shown that the Botryosphaeriaceae occur as endophytes and latent pathogens, without causing symptoms on eucalypts (Smith *et al*. 1994, 1996a, b; Slippers & Wingfield 2007). In general, quarantine systems are not well suited to deal with latent pathogens, which live endophytically in healthy plant material for an extended period of time (Slippers & Wingfield 2007). A number of recent studies have shown that fungi in the Botryosphaeriaceae have most likely spread around the world by the movement of germplasm (Slippers & Wingfield 2007). Examples include *Neofusicoccum eucalypticola* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips and *N. eucalyptorum* (Crous, H. Sm. ter & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips, endophytes that are seemingly specific to eucalypts and native to Australia, and which have been apparently introduced with the host (germplasm) into Chile and South Africa (Ahumada 2003; Slippers *et al*. 2004a, b; Burgess *et al*. 2006c).

The pine pathogen, *Diplodia pinea* (Desm.) J. Kickx f., a member of the Botryosphaeriaceae, has a global distribution because of its movement with germplasm (Smith *et al*. 1996a, 2000; Burgess *et al*. 2001a, b; Slippers & Wingfield 2007). *Pinus* spp. are non-native in Australasia and South Africa, but they have been established extensively in plantations in these countries. The pathogen, however, is a very common endophyte in tissues of these trees and was introduced from the native ranges of *Pinus* spp. to many countries where these trees are grown in plantations (Smith *et al*. 1996a, 2000; Burgess *et al*. 2001a, b). In fact, *D. pinea* has so effectively moved around the globe that in South Africa its genetic diversity is higher than in some of the studied native populations (Smith *et al*. 2000; Burgess *et al*. 2001a, b, 2004). As *D. pinea* is an asexual fungus, its high genetic diversity in South Africa is thought to be the result of multiple introductions from more than one area of the native range of *D. pinea* (Smith *et al*. 2000; Burgess *et al*. 2001a, b, 2004). *D. pinea* populations in South Africa share vegetative compatibility (VC) groups and multilocus genotypes with populations from Australia and New Zealand, further reflecting the high level of movement of this fungus (Burgess *et al*. 2001a, b, 2004).

Chrysoporthe deuterocubensis and *Chr. cubensis*, best known for the canker disease of *Eucalyptus* spp. (Myrtaceae) they cause in the tropics and Southern hemisphere (Wingfield 2003; Gryzenhout *et al*. 2009; Van Der Merwe *et al*. 2010) occur on numerous plant hosts in the Melostomataceae, Lythraceae and Myrtales in the countries of their most likely origin (Gryzenhout et al., 2009; Van Der Merwe *et al*. 2010). Both these pathogens have been reported from Africa, to which it could have spread either on ornamental plants, such as *Tibouchina* spp., or through the spice trade on, for example, cloves (*Syzygium aromaticum*) and infected non-native *Eucalyptus* spp. (Wingfield 2003; Gryzenhout *et al*. 2004; Nakabonge *et al*. 2006, 2007; Van Der Merwe *et al*. 2010).

Several eucalypt pathogens have been reported from seeds of these trees (Keane *et al*. 2000). These include *Acremomium* spp. (Yuan *et al*. 1997), *Aspergillus* spp. (Chalermpongse 1987), *Coniella* spp. (Yuan *et al*. 1997; Van Niekerk *et al*. 2004), *Cylindrocladium* spp. (Johnson 1985), *Fusarium* spp. (Michail *et al*. 1986), *Pilidiella* spp. (Yuan *et al*. 1997; Van Niekerk *et al*. 2004), and *Puccinia psidii* (Old *et al*. 2003). By using PCR-based DNA diagnostic technology, rust spores of the eucalypt pathogen *P. psidii* have been detected in eucalypt seeds and pollen samples from commercial sources in Brazil (Old *et al*. 2003). *Cylindrocladium* and *Fusarium* species are important nursery pathogens that can attach on the surfaces of seeds, spreading with them and reducing the viability of seeds and/or causing post-germination diseases such as damping off, leaf blight, anthracnose and stem cankers (Johnson 1985; Chalermpongse 1987; Anderson & Miller 1989; Crous 2002; Leslie & Summerell 2006).

Leaf blight/spot pathogens especially species in the Teratosphaeriaceae and Mycosphaerellaceae were found relatively early in the establishment of non-native plantations wherever they were established (Crous 1989a, 1996, 1998; Wingfield *et al*. 2001c). It is most likely that these leaf pathogens were introduced from Australia with germplasm such as with seeds (Keane *et al*. 2000; Zhan *et al*. 2003; Burgess *et al*. 2007b; Hunter *et al*. 2008; Pérez *et al*. 2009).

Species of *Quambalaria*, which cause leaf and shoot blight on eucalypts, are suggested to have spread from Australia to other regions via the trade in seeds (Wingfield *et al*. 1993; Pegg *et al*. 2008, 2009). It has been suggested that Australia is the centre of origin of *Quambalaria*

spp, based on their wide distribution in the country and the length of time that species in this genus has been known in Australia (Pegg *et al*. 2008). The fact that countries that have reported the appearance of *Quambalaria* spp. commonly import seeds from Australia, suggests that the movement of *Quambalaria* spp. has been accelerated by the exchange of seeds (Wingfield *et al*. 1993; Pegg *et al*. 2008, 2009).

5.4. Wood and wood products

The movement of wood and wood products is a major pathway for the global movement of tree pathogens and particularly insect pests (Ridley *et al*. 2000; Campbell *et al*. 2001; Palm & Rossman 2003; Keuran & Allen 2004; Haack 2006; Desprez-Loustau *et al*. 2007; Brasier 2008; Colunga-Garcia *et al*. 2009; Wingfield *et al*. 2011). Especially Ophiostomatoid fungi (such as *Ceratocystis* and *Ophiostoma*) and Botryosphaericeae are well adapted to spread globally via wood and wood products (Gibbs 1993; Wingfield *et al*. 2001c; Slippers & Wingfield 2007; Roux & Wingfield 2009). *Ceratocystis* and *Ophiostoma* species commonly occur under bark flaps and associated with insects on logs (Stone & Simpson 1987; Schowalter & Filip 1993; Wingfield *et al*. 2001c; Roux & Wingfield 2009; Ferreira *et al*. 2010). This makes them particularly well suited to movement with wood products.

Amylostereum areoltum (Chaillet) Boiden, a wood decay fungus, is an obligate symbiont of the wood wasp *Sirex noctilio* Fabricius (Talbot 1977). These two organisms have the potential to cause serious damage to conifer species including *Pinus*, *Picea* and *Pseudotsuga* (Spradberry & Kirk 1978). *S. noctilio*/*A. areolatum* in the Southern Hemisphere was introduced from the Northern Hemisphere and during the last 100 years has spread rapidly in the Southern Hemisphere (Wingfield *et al*, 2001c). In Africa, *S. noctilio* was first reported in imported wood in South Africa at a timber yard in Port Elizabeth in 1962 (Taylor 1962). It has since spread from the Cape Province into the Mpumalanga Province through the movement of infected trees and wood (Hurley *et al*. 2007).

It is important to control the spread of plant pathogens with the movement of wood and wood products. International regulations have thus been promulgated to reduce the chances of pathogens and pests spreading between continents on wood and wood packaging material (Evans 2007; Zahid *et al*. 2008). These include specifications that only debarked timber can

be moved internationally. Furthermore, specifications for the treatment of timber prior to its movement have also been clearly outlined (Evans 2007; Haack 2007, 2008; Zahid *et al*. 2008).

5.5. Insects

Numerous insects are adapted for long distance dispersal in wood and wood products, especially when untreated timber is used, or where timber has not been debarked (Campbell 2001; Chornesky *et al*. 2005; Brockerhoff *et al*. 2006; Haack 2006). A number of fungal tree pathogens have developed close associations with insects (Leach *et al*. 1934; Leach 1940; Moller & Vay 1968; Malloch & Blackwell 1993; Schowalter & Filip 1993; Solheim 1993; Agrios 1997; Jacobs & Wingfield 2001; Wingfield *et al*. 2001d; Zhou *et al*. 2007a; Roux & Wingfield 2009). The best known examples in forestry include fungi in the genera *Ceratocystis* and *Ophiostoma,* which live symbiotically with a number of insects, particularly Coleopteran species including bark and ambrosia beetles, as well as nitidulid beetles (Leach *et al*. 1934; Kile 1993; Rollins *et al*. 2001; Kirisits 2004; Harrington 2005; Heath *et al*. 2009).

The primary means of spread of *Ceratocystis* spp. and some *Ophiostoma* spp. in plantations is via insects that visit wounds on trees. *Ceratocystis* spp. residing in the *C. fimbriata* species complex are pathogens of *Eucalyptus*, which require wounds for infection (Kile 1993; Heath *et al*. 2009; Roux & Wingfield 2009; Van Wyk *et al*. 2010). This relationship represents a co-evolution where the fungi produce fruity odours which attract insects that pick up the sticky *Ceratocystis* spores from infected wounds and transmit these spores to fresh wounds that the insects visit to feed on the sap (Kile 1993; Roux & Wingfield 2009). These fungi are thus very well adapted to move, particularly with the insects that can easily move globally via the trade in forest products.

5.6. Humans

Anthropogenic activities are the main factors involved in the movement of pests and pathogens around the world and they are the main drivers of all the pathways discussed in the previous sections (Ridley *et al.* 2000; Tatem *et al*. 2006a, b; Desprez-Loustau *et al*. 2007; Lebarbenchon *et al*. 2008; Holmes *et al*. 2009; Loo 2009; Wingfield *et al*. 2011). The increase in trade and travel between countries and continents has been given as a major reason for the increase in appearance of new diseases of trees and plants (Campbell *et al*. 2001; Schwartz *et*

al. 2006; Desprez-Loustau *et al*. 2007; Slippers & Wingfield 2007; Brasier 2008; Colunga-Garcia *et al*. 2009). This has lead to an increase in the movement of timber and wood products, seeds and other germplasm (Old *et al*. 2003; Wingfield *et al*. 2001c, 2011; Davison *et al*. 2006; Tatem *et al*. 2006a, b; Loo 2009). In South Africa, for example, it has been shown that the number of pathogens and insect pests appearing in plantations of eucalypts and pines has increased logarithmically over the past century, as the trade and movement of forestry products and seeds have increased (Wingfield *et al*. 2008).

6. EXAMPLES ILLUSTRATING THE ORIGINS AND GLOBAL MOVEMENT OF EUCALYPT PATHOGENS

6.1. The Chrysoporthe canker pathogens *Chrysoporthe cubensis* and *Chr. austroafricana*

The population structure and possible origin of the eucalypt cankers pathogens, *Chr. cubensis* and *Chr. austroafricana*, previously known as *Cryphonectria cubensis,* have been studied in more detail than any other fungal pathogens on eucalypts (Hodges *et al*. 1986; Van Heerden *et al*. 1997; Wingfield *et al*. 1997; Van Zyl *et al*. 1998; Van Heerden & Wingfield 2001; Wingfield 2003; Gryzenhout *et al*. 2004, 2009; Rodas *et al*. 2005a; Heath *et al*. 2006; Nakabonge *et al*. 2006, 2007; Pegg *et al*. 2010; Van Der Merwe *et al*. 2010). Use has been made of VCG studies, phylogenetic and coalescent studies as well as microsatellite analyses to elucidate the phylogenetic and population genetic history of these important canker pathogens (Hodges *et al*. 1986; Van Heerden *et al*. 1997; Wingfield *et al*. 1997; Van Zyl *et al*. 1998; Gryzenhout *et al*. 2004; Nakabonge *et al*. 2007; Van Der Merwe *et al*. 2010). Through a combination of the different molecular markers it is now recognized that what was previously known as *Cry. cubensis* in fact represents three distinct species, *Chr. austroafricana*, *Chr. cubensis* and *Chr. deuterocubensis* (Gryzenhout *et al*. 2004; Van Der Merwe *et al*. 2010). This has significant implications, since all three originate from different parts of the world, with two of them already having spread to other continents (Gryzenhout *et al*. 2009; Van Der Merwe *et al*. 2010).

The pathogen now known as *Chr. austroafricana* was one of the most important pathogens of eucalypts in South Africa (Wingfield *et al*. 1989; Wingfield 2003). Early studies on the pathogen, then known as *Cry. cubensis*, using VCGs, rRNA sequence data and RAPDs of populations collected from South Africa, South America and Southeast Asia suggested that

Cry. cubensis was non-native to South Africa and possibly originated from South America (Wingfield *et al*. 1997). After the clarification of the taxonomy of *Chr. austroafricana* (Gryzenhout *et al*. 2004), recent studies, using modern molecular markers have suggested that *Chr. austroafricana* is native to Africa (Heath *et al*. 2006). This hypothesis is further supported by biological data, including the discovery of *Chr. austroafricana* on native hosts in Southern and Eastern Africa (Heath *et al*. 2006; Nakabonge *et al*. 2006). Furthermore, despite numerous collections from other continents, *Chr. austroafricana* has thus far been found only on the African continent (Heath *et al*. 2006; Nakabonge *et al*. 2006; Gryzenhout *et al*. 2009). It is also not known to cause disease on its native hosts, but is an important pathogen of non-native eucalypts, further supporting evidence that it is native to Africa.

Until very recently, the origin of *Chr. cubensis* was confounded by the fact that this fungus was not taxonomically well defined. Use of coalescent studies have now confirmed that what was previously seen as two phylogenetic clades within *Chr. cubensis* (Myburg *et al*. 1999, 2002; Gryzenhout *et al*. 2004, 2009; Rodas *et al*. 2005a; Nakabonge *et al*. 2006), actually represents two unique species, each with its own geographic origin (Van Der Merwe *et al*. 2010). Various hypotheses were presented to explain the origin of what was then known as *Chr. cubensis* (Wingfield 2003). One view was that the fungus originated on native *S. aromaticum* in Indonesia and spread around the world with the trade in spices (Hodges *et al*. 1986). An alternative hypothesis was that the fungus originated on native plants in South America (Wingfield *et al*. 2001a; Wingfield 2003; Rodas *et al*. 2005a). Population genetic studies on *Chr. cubensis* isolates from South America and *Chr. deuterocubensis* isolates from Southeast Asia using VCGs (Van Heerden *et al*. 1997; Van Zyl *et al*. 1998), have shown that there is either a high level of outcrossing within the populations or that they are well-established native populations in their respective areas (Van Heerden *et al*. 1997; Van Zyl *et al*. 1998).

It is well established that more than one species of *Chrysoporthe* causes disease of eucalypts on the African continent (Roux *et al*. 2003; Nakabonge *et al*. 2006). While *Chr. austroafricana* is native to the continent, it is suggested that *Chr. cubensis* and *Chr. deuterocubensis* were introduced into Africa from South America and Asia respectively (Gryzenhout *et al*. 2004; Nakabonge *et al*. 2006). A recent study by Nakabonge *et al*. (2007) showed a very low diversity for populations of *Chr. deuterocubensis* (then known as *Chr. cubensis*) in Eastern Africa, including Kenya, Malawi and Mozambique. This study was

conducted using five pairs of microsatellite markers previously developed for *Cry. cubensis* (Van Der Merwe *et al*. 2003) and further suggest that *Chr. deuterocubensis* in Eastern Africa probably originated from Asia (Nakabonge *et al*. 2007).

Studies of the eucalypt canker pathogens in the Cryphonectriaceae clearly illustrate the importance of sound taxonomy in understanding the global distribution of forest pathogens. They also illustrate the importance of having a sound knowledge of the biology of the pathogen/s and using appropriate tools to consider the widest possible body of evidence. Thus, via a combination of studies considering the biology, phylogenetics, population genetics and morphology of pathogens, it is possible to define them accurately and then also to manage them appropriately.

6.2. The eucalypt leaf blight pathogen *Teratosphaeria nubilosa*

Teratosphaeria nubilosa (previously known as *Mycosphaerella nubilosa* (Cooke) Hansf.) (Crous *et al*. 2009a, c) is one of the most important foliar pathogens on eucalypt trees. It is one of the causal agents of a disease commonly known as Mycosphaerella leaf blotch (MLB), particularly on cold-tolerant species such as *E. globulus* and *E. nitens* (Park & Keane 1987; Carnegie *et al*. 1998; Carnegie & Ades 2002; Hunter *et al*. 2004a, 2008). This fungus typically infects juvenile leaves and susceptible trees can be severely defoliated (Park & Keane 1982a; Lundquist & Purnell 1987).

Teratosphaeria nubilosa was first described from *Eucalyptus* leaves in Victoria, Australia (Cooke 1891) and subsequently in Tasmania, New South Wales, South and Western Australia (Hansford 1956; Park & Keane 1982a, b; Park 1988; Maxwell *et al*. 2001; Milgate *et al*. 2001; Carnegie 2007a, b). Elsewhere, *T. nubilosa* occurs on the North Island of New Zealand (Dick 1982) and in Ethiopia, Kenya, South Africa, Tanzania and Zambia (Crous *et al*. 2004a, 2006c; Hunter *et al*. 2004a; Gezahgne *et al*. 2006), and was also accidentally introduced into commercial plantations of *E. globulus* in Portugal and Spain (Crous *et al*. 2004a), as well as Brazil and Uruguay (Pérez *et al*. 2009, 2010a).

In recent years, a number of studies have been conducted to better understand the origin and movement of *T. nubilosa*, both internationally and at regional scales (Hunter *et al*. 2008, 2009; Pérez *et al*. 2009, 2010a, b). To achieve this, microsatellite markers were developed by

Hunter *et al*. (2008). In a study of eight *T. nubilosa* populations from New South Wales (Eastern Australia), Portugal, South Africa, Spain, Tanzania and Western Australia, it was shown that both gene and genotypic diversities were highest in New South Wales (Hunter *et al*. 2008). Furthermore, this population had the greatest number of alleles of all the populations studied. This, together with the fact that *T. nubilosa* was originally identified from Victoria in Eastern Australia in the 1800s (Cooke 1891), supports the view that *T. nubilosa* originated from Eastern Australia and was spread around the world to plantation areas where eucalypts are grown (Hunter *et al*. 2008).

Based on gene flow data between Australia and populations from the rest of the world, a number of hypotheses have emerged regarding the international movement of *T. nubilosa*. It seems likely that *T. nubilosa* was first introduced into South Africa from Eastern Australia and that it subsequently spread to other parts of Africa and further into Portugal and Spain (Hunter *et al*. 2008). This route of movement is consistent with the pattern of establishment of *Eucalyptus* plantations in Africa and Europe (Hunter *et al*. 2008). A study of *T. nubilosa* in Uruguay showed that only one multilocus haplotype of this pathogen is present in Uruguay and this is the same haplotype as one previously found in Portugal and Spain, where the population of *T. nubilosa* is also represented by a single clone (Hunter *et al*. 2008, 2009). The results suggest strongly that *T. nubilosa* was recently introduced into Uruguay from Portugal or Spain where its population has a very limited diversity, rather than from Australia (Pérez *et al*. 2009).

6.3. The eucalypt canker pathogen *Teratosphaeria zuluensis*

Teratosphaeria zuluensis (Crous *et al*, 2009a), previously known as *Coniothyrium zuluense* (Wingfield *et al*. 1996b), *Colletogleopsis zuluense* (M.J. Wingf., Crous & T.A. Cout.) M.N. Cortinas, M.J. Wingf. & Crous (Cortinas *et al*. 2006b), *Readeriella zuluensis* (M.J. Wingf., Crous & T.A. Cout.) Crous & U. Braun (Crous *et al*. 2007a) and *Kirramyces zuluensis* (M.J. Wingf., Crous & T.A. Cout.) Andjic & M.J. Wingf. (Andjic *et al*. 2007a), was first observed in the Zululand forestry region of the Kwazulu-Natal Province of South Africa in September 1988, where it occurred on a single *E. grandis* clone (Wingfield *et al*. 1996b). For more than ten years, this disease was known only from South Africa, leading to suggestions that it was native to that country. Presently, *T. zuluensis* has been reported on eucalypt trees in numerous countries including those in Africa (Roux *et al*. 2005; Cortinas *et al*. 2006c; Chungu *et al*.

2010b), Asia (Van Zyl *et al*. 2002; Gezahgne *et al*. 2003a; Old *et al*. 2003; Cortinas *et al*. 2006b) and Central America (Roux *et al*. 2002), with new reports occurring regularly.

The disease caused by *T. zuluensis* is currently one of the most important stem canker diseases of eucalypts, with new reports appearing annually. It was originally hypothesised that *T. zuluensis* originated from South Africa (Wingfield *et al*. 1996b) and spread around the world, possibly with seeds (Gezahgne *et al*. 2005). To investigate this hypothesis and to gain a better understanding of the population diversity of *T. zuluensis*, fifteen pairs of microsatellite markers were designed for this pathogen (Cortinas *et al*. 2006a, 2010). The genetic diversity and population structure of two temporally separated populations of *T. zuluensis* in South Africa were investigated, as well as the population structures of isolates from China and Malawi (Cortinas *et al*. 2010). The results showed that South African populations were not the most diverse among the populations studied and that this country is most likely not the origin of *T. zuluensis*. The majority of individuals from the population in China and Malawi were different from individuals from South Africa. Furthermore, high levels of private alleles were observed in each population, and the amount of gene flow between populations was not significant, suggesting that *T. zuluensis* in South Africa, Malawi and China originated independently by multiple introductions from a source that is yet to be discovered (Cortinas *et al*. 2010).

Comparison of temporally separated populations of *T. zuluensis* in South Africa show interesting results for management of this pathogen in the country. In contrast to what was expected, the genetic diversity of *T. zuluensis* has increased during the period from the first population sampling in 1997 and the one carried out in 2005. It was expected that the genetic diversity of this fungus in South Africa would be reduced as susceptible trees were replaced through tree breeding programmes (Cortinas *et al*. 2010). Some research indicated that the capacity of populations to recover genetic diversity after a reduction in population size is not easily predicted (Young *et al*. 1996; Lowe *et al*. 2005). The result depends on a combination of factors that are frequently unknown, such as the original population size and other parameters related to the life history and reproductive structure of the populations (Young *et al*. 1996; Edwards *et al*. 2005; Lowe *et al*. 2005; Reusch 2006). This might also have occurred with the South African *T. zuluensis* populations, but more research on the population structures within South Africa will clarify this interesting result.

Research results have indicated that sexual recombination is not the predominant form of reproduction in the South African and Malawian *T. zuluensis* populations (Cortinas *et al*. 2010). The fact that evidence for recombination was observed in the Chinese population, which is also the most genetically diverse (Cortinas *et al*. 2010), is enigmatic as this fungus has only recently been observed in that country and on *Eucalyptus*, which is not native to this region. These interesting preliminary results provide intriguing hypotheses that will be tested.

Although the origin of *T. zuluensis* remains unknown, the microsatellite markers developed for the pathogen have already provided valuable and unexpectedinformation regarding this pathogen. In the future, additional population studies of *T. zuluensis* within South Africa, as well as in China and in Malawi will be conducted and these will likely provide insight into the global movement of this important pathogen.

7. CONCLUSIONS

Eucalypt forestry is growing in importance globally. In South China, over 2.6 Mha of eucalypts have been planted mainly to supply national demand for pulp and paper. Eucalypts are not only important to meet the rapid growth of the local economy, but also to protect the ecosystem, especially natural forests, from further logging and deterioration.

Eucalypt plantations are threatened by the increasing numbers of pests and pathogens that are moving globally. In South and Southeast Asia, a large number of eucalypt pests and pathogens have already been identified and their impacts need further comprehensive investigation. However, limited work on eucalypt health has been conducted in China and there is a need to strengthen this field of research to maintain and sustain the rapidly growing eucalypt industry.

The movement of tree pests and pathogens globally has increased with the increase of global trade and human movement. Well-known pathways exist for pathogens to be moved between countries and continents. Any one of these could lead to the introduction of new diseases and pests into China, or the spread of previously unknown pathogens, native to China, to other areas of the world. Global dialogue and sound quarantine measures will help to monitor the status of these pathogens and slow their global movement.

Effective and accurate taxonomy of pathogens is critical to understand fungal pathogens and identify their origins and to define possible strategies for disease management. This is an area that has not been well-developed in the past and incorrect pathogen identification has led to many misconceptions regarding the origins and movement of eucalypt pathogens. The emergence of DNA-based technologies has already improved this situation and this will continue to be increasingly important in the future.

Understanding the possible origin and pathways of movement of pathogens is important in the development of quarantine measures, as well as for disease management. The study of the origin and movement of pathogens has been substantially enhanced by modern molecular techniques, particularly a combination of phylogenetics and population genetic studies. Several markers exist to accomplish these goals for some of the more important eucalypt pathogens and others will need to be developed in the future.

8. OBJECTIVES OF THIS DISSERTATION

Plantations of non-native eucalypt species are being extensively developed in South China to meet the needs of the rapid growth in the national economy especially in the past twenty years. The sustainability of these plantations is increasingly threatened by pests and pathogens. Very limited work on eucalypt health, especially on fungal diseases, has been conducted in the country. Therefore, there is an urgent need to (1) investigate and update the health status of eucalypt plantations in China; (2) provide advice and strategies for pest and diseases management to the forestry industry.

The chapters presented in this thesis focus on fungal diseases on eucalypts in plantations in China. The overall aims are to achieve the following goals using multi-gene DNA sequence analyses, pathogenicity tests, and population biology:

(1) To isolate and identify common fungal pathogens from eucalypt plantations and nurseries in South China, and to describe those new to science;

(2) To test and evaluate the pathogenicity of the selected important fungal pathogens on commercial eucalypt hybrids/clones, and to provide strategies for selection of disease resistant planting stock;

(3) To understand the biology of selected important eucalypt pathogen populations and their structure, and to possibly address questions relating to pathogen origin and movement;

(4) To provide a foundation for eucalypt fungal disease research in China and to promote cooperation between research organizations, governments and industries within the country, and in other parts of the world.

9. REFERENCES

Adams GC, Roux J, Wingfield MJ, 2006. *Cytospora* species (*Ascomycota, Diaporthales, Valsaceae*): introduced and native pathogens of trees in South Africa. *Australasian Plant Pathology* **5**: 521–548.

Adams GC, Wingfield MJ, Common R, Roux J, 2005. Phylogenetic relationships and morphology of *Cytospora* species and related anamorphs (*Ascomycota, Diaporthales, Valsaceae*) from *Eucalyptus*. *Studies in Mycology* **52**: 1–144.

Agrios GN, 1997. *Plant pathology*, 4th edn. Academic Press, San Diego, California.

Ahumada R, 2003. Pathogens in commercial *Eucalyptus* plantations in Chile, with special reference to *Mycosphaerella* and *Botryosphaeria* species. MSc thesis, University of Pretoria. Pretoria, South Africa.

Akiew E, Trevorrow PR, 1994. Management of bacterial wilt of tobacco. In: Hayward AC, Hartman GL (eds), *Bacterial wilt: the disease and it's causative agent, Pseudomonas solanacearum*, CABI, Wallingford, pp. 179–198.

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.

Alfenas AC, Zauza EAV, Wingfield MJ, Roux J, Glen M, 2005. *Heteropyxis* natalensis, a new host of *Puccinia psidii* rust. *Australasian Plant Pathology* **34**: 285–286.

Allen C, Prior P, Hayward AC, 2005. *Bacterial Wilt Disease and the Ralstonia solanacearum Species Complex*. APS Press, St. Paul, Minnesota, USA.

Alpert P, 2006. The advantages and disadvantages of being introduced. *Biological Invasions* **7**: 1523–1534.

Anderson RL, Miller T, 1989. Seed fungi. In: (Technical co-ordinators. Cordell CE, Anderson R L, Hoffard WH, Landis TD, Smith Jr RS and Toko HV), *Forest nursery pests*. Agriculture Handbook No. 680, USDA Forest Service: Washington, DC., pp. 126–127.

Andjic V, Barber PA, Carnegie AJ, Hardy GSJ, Wingfield MJ, Burgess TI, 2007a. Phylogenetic reassessment supports accommodation of *Phaeophleospora* and *Colletogloeopsis* from eucalypts in *Kirramyces*. *Mycological Research* **111**: 1184–1198.

Andjic V, Barber PA, Carnegie AJ, Pegg GS, Hardy GESJ, Wingfield MJ, Burgess TI, 2007b. *Kirramyces viscidus* sp nov., a new eucalypt pathogen from tropical Australia

closely related to the serious leaf pathogen, *Kirramyces destructans*. *Australasian Plant Pathology* **36**: 478–487.

Andjic V, Hardy GES, Cortinas MN, Wingfield MJ, Burgess TI, 2007c. Multiple gene genealogies reveal important relationships between species of *Phaeophleospora* infecting *Eucalyptus* leaves. *FEMS Microbiology Letters* **268**: 22–33.

Aptroot A, 2006. *Mycosphaerella* and its anamorphs: 2. Conspectus of *Mycosphaerella*. *CBS Biodiversity Series* **5**: 1–231.

Aylor DE, 1990. The Role of Intermittent Wind in the Dispersal of Fungal Pathogens. *Annual Review of Phytopathology* **28**: 73–92.

Aylor DE, 2003. Spread of plant disease on a continental scale: role of aerial dispersal of pathogens. *Ecology* **84**: 1989–1997.

Bakshi BK, 1974. Control of root diseases in plantations in reforested stands (with special reference to khair, Sissoo, *Eucalyptus*, ect.). *Indian Forestry* **100**: 77–78.

Bakshi BK, 1976. *Forest pathology: principles and practice in forestry*. Forestry Research institute Press, Dehra Dun.

Barnes I, Roux J, Wingfield BD, Neill MO, Wingfield MJ, 2003a. *Ceratocystis fimbriata* infecting *Eucalyptus grandis* in Uruguay. *Australasian Plant Pathology* **32**: 361–366.

Barnes I, Roux J, Wingfield MJ, Old KM, Dudzinski M, 2003b. *Ceratocystis pirilliformis*, a new species from *Eucalyptus nitens* in Australia. *Mycologia* **95**: 865–871.

Barr ME, 1987. *Prodromus to class Loculoascomycetes*. Amherst. Massachusetts: Hamilton I. Newell, Inc.

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

Booth C, Gibson IAS, 1972. *Rhizina undulata*. CMI Descriptions of Pathogenic Fungi and Bacteria. Surrey (UK): Common wealth Mycological Institute, Kew. Report no. 324.

Booth TH, Jovanovic T, Old KM. Dudzinski MJ, 2000. Climatic mapping to identify high-risk areas for *Cylindrocladium quinqueseptatum* leaf blight on eucalypts in main land South East Asia and around the world. *Environmental Pollution* **108**: 365–372.

Bowden J, Gregory PH, Johnson CG, 1971. Possible Wind Transport of Coffee Leaf Rust across the Atlantic Ocean. *Nature* **229**: 500–501.

Brasier C, 2005. Preventing invasive pathogens: deficiencies in the system. *The Plantsman* **4**: 54–57.

Brasier CM, 2008. The biosecurity threat to the UK and global environment from international trade in plants. *Plant Pathology* **57**: 792–808.

Braun U, 1998. *A monograph of Ramularia, Cercosporella and allied genera (phytopathogenic hyphomycetes)*. IHW-Verlag, Eching.

Brockerhoff EG, Bain J, Kimberley M, Knizek M, 2006. Interception frequency of exotic bark and ambrosia beetles (Coleoptera: Scolytinae) and relationship with establishment in New Zealand and worldwide (1). *Canadian Journal of Forest Research* **36: 2**89–298.

Brooker MIH, 2000. A new classification of the genus *Eucalyptus* L'Her. (Myrtaceae). *Australian Systematic Botany* **13**: 79–148.

Brown JMK, Hovmoller MS, 2002. Aerial Dispersal of Pathogens on the Global and Continental Scales and Its Impact on Plant Disease. *Science* **297**: 537–541.

Burgess TI, Andjic V, Hardy GES, Dell B, Xu DP, 2006a. First report of *Phaeophleospora destructans* in China. *Journal of Tropical Forest Science* **18**: 144–146.

Burgess TI, Andjic V, Wingfield MJ, Hardy GES, 2007a. The eucalypt leaf blight pathogen *Kirramyces destructans* discovered in Australia. *Australasian Plant Disease Notes* **2**: 141–144.

Burgess TI, Barber PA, Mohali S, Pegg G, De Beer ZW, Wingfield MJ, 2006b. Three new *Lasiodiplodia* spp. from the tropics, recognised based on DNA sequence comparisons and morphology. *Mycologia* **98**: 423–435.

Burgess TI, Barber PA, Sufaati S, Xu DP, Hardy GES, Dell B, 2007b. *Mycosphaerella* spp. on *Eucalyptus* in Asia: new species; new hosts and new records. *Fungal Diversity* **24**: 135–157.

Burgess TI, Sakalidis M, Hardy GES, 2006c. Gene flow of the canker pathogen *Botryosphaeria australis* between *Eucalyptus globulus* plantations and native eucalypt forests in Western Australia. *Austral Ecology* **31**: 559–566.

Burgess TI, Wingfield BD, Wingfield MJ, 2001a. Comparison of genotypic diversity in native and introduced populations of *Sphaeropsis sapinea* isolated from *Pinus radiata*. *Mycological Research* **105**: 1333–1339.

Burgess TI, Wingfield MJ, 2002. Quarantine is important in restricting the spread of exotic seed-borne tree pathogens in the southern hemisphere. *International Forestry Review* **4**: 56–64.

Burgess TI, Wingfield MJ, Wingfield BD, 2001b. Simple sequence repeat markers distinguish among morphotypes of *Sphaeropsis sapinea*. *Applied and Environmental Microbiology* **67**: 354–362.

Burgess TI, Wingfield MJ, Wingfield BD, 2004. Global distribution of *Diplodia pinea* genotypes revealed using simple sequence repeat (SSR) markers. *Australasian Plant Pathology* **33**: 513–519.

Campbell FT, 2001. The science of risk assessment for phytosanitary regulation and the impact of changing trad regulations. *BioScience* **51**: 148–153.

Cao JD, 1982. Investigation of bacterial wilt in *Eucalyptus saligna* and *E. grandis* introduced from Brazil. *Guangxi Forestry Science and Technology* **4**: 30–31. (In Chinese).

Carefoot GL, Sprott ER, 1967. *Famine on the wind: man's battle against plant disease*. Rand McNally, Chicago, USA.

Carnegie AJ, 2007a. Forest health condition in New South Wales, Australia, 1996–2005. I. Fungi recorded from eucalypt plantations during forest health surveys. *Australasian Plant Pathology* **36**: 213–224.

Carnegie AJ, 2007b. Forest health condition in New South Wales, Australia, 1996–2005. II. Fungal damage recorded in eucalypt plantations during forest health surveys and their management. *Australasian Plant Pathology* **36**: 225–239.

Carnegie AJ, Ades PK, 2002. The proportion of leaf spots caused by *Mycosphaerella cryptica* and *M. nubilosa* on *Eucalyptus globulus*, *E. nitens* and their F1 hybrids in a family trial in Tasmania, Australia. *Australian Mycologist* **2**: 53–63.

Carnegie AJ, Ades PK, Keane PJ, Smith IW, 1998. *Mycosphaerella* diseases of juvenile foliage in a eucalypt species and provenance trial in Victoria, Australia. *Australian Forestry* **61**: 190–194.

Chalermpongse A, 1987. Current potentially dangerous forest tree diseases in Thailand. *Biotrop special publication* **27**: 77–90.

Chapela IH, 1989. Fungi in healthy stems and branches of American beech and aspen: a comparative study. *New Phytologist* **113**: 65–75.

Cheewangkoon R, Crous PW, Hyde KD, Groenewald JZ, To-anan C, 2008. Species of *Mycosphaerella* and related anamorphs on *Eucalyptus* leaves from Thailand. *Persoonia* **21**: 77– 91.

Cheewangkoon R, Groenewald JZ, Summerell BA, Hyde KD, To-anun C, Crous PW, 2009. Myrtaceae, a cache of fungal biodiversity. *Persoonia* **23**: 55–85.

Chornesky EA, Bartuska AM, Aplet GH, Britton KO, Cummings-Carlson J, Davis FW, Eskow J, Gordon DR, Gottschalk KW, Haack RA, Hansen AJ, Mack RNRN, Rahel FJ, Shannon MA, Wainger LA, Wigley TB, 2005. Science Priorities for Reducing the Threat of Invasive Species to Sustainable Forestry. *BioScience* **55**: 335–348.

Chungu D, Gryzenhout M, Muimba-Kankolongo A, Wingfield MJ, Roux J, 2010a. Taxonomy and pathogenicity of two novel *Chrysoporthe* species from *Eucalyptus grandis* and *Syzygium guineense* in Zambia. *Mycological Progress* **9**: 379–393.

Chungu D, Muimba-Kankolongo A, Wingfield MJ, Roux J, 2010b. Identification of fungal pathogens occurring in eucalypt and pine plantations in Zambia by comparing DNA sequences. *Forestry* **83**: 507–515.

Coetzee MPA, Wingfield BD, Bloomer P, Ridley GS, Wingfield MJ, 2003a. Molecular identification and phylogeny of *Armillaria* isolates from South America and Indo-Malaysia. *Mycologia* **95**: 285–293.

Coetzee MPA, Wingfield BD, Harrington TC, Steimel J, Coutinho TA, Wingfield MJ, 2001. The root rot fungus *Armillaria mellea* introduced into South Africa by early Dutch settlers. *Molecular Ecology* **10**: 387–396.

Coetzee MPA, Wingfield BD, Roux J, Crous PW, Denman S, Wingfield MJ, 2003b. Discovery of two northern hemisphere *Armillaria* species on Proteaceae in South Africa. *Plant Pathology* **52**: 604–612.

Colunga-Garcia M, Haack RA, Adelaja AO, 2009. Freight transportation and the potential for invasions of exotic insects in urban and periurban forests of the United States. *Journal of Economic Entomology* **1**: 237–246.

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

Cooke MC, 1891. Australian fungi. *Grevillea* **19**: 60–62.

Cortinas MN, Barnes I, Wingfield BD, Wingfield MJ, 2006a. Polymorphic microsatellite markers for the *Eucalyptus* fungal pathogen *Colletogloeopsis zuluensis*. *Molecular Ecology Notes* **6**: 780–783.

Cortinas MN, Barnes I, Wingfield MJ, Wingfield BD, 2010. Genetic diversity in the *Eucalyptus* stem pathogen *Teratosphaeria zuluensis*. *Australasian Plant Pathology* **39**: 383–393.

Cortinas MN, Burgess T, Dell B, Xu D, Crous PW, Wingfield BD, Wingfield MJ, 2006b. First record of *Colletogloeopsis zuluense* comb. nov., causing stem canker of *Eucalyptus* in China. *Mycological Research* **110**: 229–236.

Cortinas MN, Crous PW, Wingfield BD, Wingfield MJ, 2006c. Multi-locus gene phylogenies and phenotypic characters distinguish two species within the *Colletogloeopsis*

zuluensis complex associated with *Eucalyptus* stem cankers. *Studies in Mycology* **55**: 135–148.

Cortinas MN, Koch N, Thain J, Wingfield BD, Wingfield MJ, 2004. First record of the *Eucalyptus* stem canker pathogen, *Coniothyrium zuluense* from Hawaii. *Australasian Plant Pathology* **33**: 309–312.

Coutinho TA, 2005. Introduction and Prospectus on the Survival of *Ralstonia solanacearum.* In: Allen C, Prior P, Hayward AC (eds), *Bacterial Wilt Disease and the Ralstonia solanacearum Species Complex*. APS Press, St. Paul, Minnesota, USA, pp. 29–38.

Coutinho TA, Roux J, Riedel KH, Terblanche J, Wingfield MJ, 2000. First report of bacterial wilt caused by *Ralstonia solanacearum* on eucalypts in South Africa. *Forest Pathology* **30**: 205–210.

Coutinho TA, Wingfield MJ, Alfenas AC, Crous PW, 1998. Eucalyptus rust: A disease with the potential for serious international implications. *Plant Disease* **82**: 819–825.

Crous PW, 1998. *Mycosphaerella spp. and their anamorphs associated with Leaf spot diseases of Eucalyptus*. Mycological Memoi no.21. APS Press, St. Paul, Minnesota, USA.

Crous PW, 2002. *Taxonomy and pathology of Cylindrocladium (Calonectria) and allied genera*. APS Press, St. Paul, Minnesota, USA.

Crous PW, Alfenas AC, 1995. *Mycosphaerella gracilis* and other species of M*ycosphaerella* associated with leaf spots of *Eucalyptus* in Indonesia. *Mycologia* **87**: 121–126.

Crous PW, Braun U, Groenewald JZ, 2007a. *Mycosphaerella* is polyphyletic. *Studies in Mycology* **58**: 1–32.

Crous PW, Groenewald JZ, Mansilla JP, Hunter GC, Wingfield MJ, 2004a. Phylogenetic reassessment of *Mycosphaerella* spp. and their anamorphs occurring on *Eucalyptus*. *Studies in Mycology* **50**: 195–214.

Crous PW, Groenewald JZ, Risède J-M, Simoneau P, Hyde KD, 2006a. *Calonectria* species and their *Cylindrocladium* anamorphs: species with clavate vesicles. *Studies in Mycology* **55**: 213–226.

Crous PW, Groenewald JZ, Risède J-M, Simoneau P, Hywel-Jones N, 2004b. *Calonectria* species and their *Cylindrocladium* anamorphs: species with sphaeropedunculate vesicles. *Studies in Mycology* **50**: 415–430.

Crous PW, Groenewald JZ, Summerell BA, Wingfield BD, Wingfield MJ, 2009a. Co-occurring species of *Teratosphaeria* on *Eucalyptus*. *Persoonia* **22**: 38–48.

Crous PW, Hong L, Wingfield BD, Wingfield MJ, 2001. ITS rDNA phylogeny of selected *Mycosphaerella* spp. and their anamorphs accurring on *Myrtaceae*. *Mycologia* **105**: 425–431.

Crous PW, Knox-Davies PS, Wingfield MJ, 1989a. A summary of fungal leaf pathogens of *Eucalyptus* and the diseases they cause in South Africa. *South African Forestry Journal* **149**: 9–16.

Crous PW, Slippers B, Wingfield MJ, Rheeder J, Marasas WFO, Philips AJL, Alves A, Burgess T, Barber P, Groenewald JZ, 2006b. Phylogenetic lineages in the Botryosphaeriaceae. *Studies in Mycology* **55**: 235–253.

Crous PW, Summerell BA, Carnegie AJ, Mohammed C, Himaman W, Groenewald JZ, 2007b. Foliicolous *Mycosphaerella* spp. and their anamorphs on *Corymbia* and *Eucalyptus*. *Fungal Diversity* **26**: 143–185.

Crous PW, Summerell BA, Carnegie AJ, Wingfield MJ, Groenewald JZ, 2009b. Novel species of Mycosphaerellaceae and Teratosphaeriaceae. *Persoonia* **23**: 119–146.

Crous PW, Summerell BA, Carnegie AJ, Wingfield MJ, Hunter GC, Burgess TI, Andjic V, Barber PA, Groenewald JZ, 2009c. Unravelling *Mycosphaerella*: do you believe in genera? *Persoonia* **23**: 99–118.

Crous PW, Wingfield MJ, 1996. Species of *Mycosphaerella* and their anamorphs associated with leaf blotch diseases of *Eucalyptus* in South Africa. *Mycologia* **88**: 441–458.

Crous PW, Wingfield MJ, 1997. New species of *Mycosphaerella* occurring on *Eucalyptus* leaves in Indonesia and Africa. *Canadian Journal of Botany* **75**: 781–790.

Crous PW, Wingfield MJ, Mansilla JP, Alfenas AC, Groenewald JZ, 2006c. Phylogenetic reassessment of *Mycosphaerella* spp. and their anamorphs occurring on *Eucalyptus* II. *Studies in Mycology* **55**: 99–131.

Crous PW, Wingfield MJ, Marasas WFO, Sutton BC, 1989b. *Pseudocercospora eucalyptorum* sp. nov. on *Eucalyptus* leaves. *Mycological Research* **93**: 394–398.

Dai ZM, Liang XZ, Chen F, He BX, Liu YZ, 2006. The developing actuality of *Eucalyptus* in Jiangmen city and discussion on the ecological function of *Eucalyptus*. *Journal of Guangdong Forestry Science and Technology* **22**: 94–97. (In Chinese).

Davison EA, Drenth A, Kumar S, Mack S, Mackie AE, McKirdy S, 2006. Pathogens associated with nursery plants imported into Western Australia. *Australasian Plant Pathology* **35**: 473–475.

De Beer ZW, Begerow D, Bauer R, Pegg GS, Crous PW, Wingfield MJ, 2006. Phylogeny of *Quambalariaceae* fam. nov. including important *Eucalyptus* pathogens from South Africa and Australia. *Studies in Mycology* **55**: 289–298.

Dell B, Hardy G, Burgess T, 2008. Health and nutrition of plantation eucalypts in Asia. *Southern Forests* **70**: 131–138.

Desprez-Loustau ML, Robin C, Buee M, Courtecuisse R, Garbaye J, Suffert F, Sache I, Rizzo D, 2007. The fungal dimension of biological invasions. *Trends in Ecology and Evolution* **22**: 472–480.

Dianese JC, Dristig MCG, Cruz AP, 1990. Susceptibility to wilt associated with *Pseudomonas solanacearum* among six species of *Eucalyptus* growing in equatorial Brazil. *Australasian Plant Pathology* **19**: 71–76.

Dick M, 1982. Leaf inhabiting fungi of eucalypts in New Zealand. *New Zealand Journal of Forestry Science* **12**: 525–537.

Edwards KR, Travis SE, Proffitt CE, 2005. Genetic effects of a large-scale *Spartina alterniflora* (Smooth Cordgrass) dieback and recovery in the Northern Gulf of Mexico. *Estuaries* **28**: 204–214.

Eldridge K, Davidson J, Harwood C, Van Wyk G, 1997. *Eucalypt domestication and breeding*. Oxford, Science Publications, UK.

Evans G, Dempsey S, McLachlan K, Coleman C, McRae C, 2000. *Quarantine risks associated with importation of Eucalyptus seed*. Australian Quarantine and Inspection Service (AQIS), Canbera, Australia.

Evans HF, 2007. ISPM 15 treatments and residual bark: how much bark matters in relation to founder populations of bark and wood boring beetles. In: Evans H, Oszako T (eds), *Alien invasive species and international trade*. Forest Research Institute, Sêkocin Stary, Poland, pp. 149–155.

Ferreira EM, Harrington TC, Thorpe DJ, Alfenas AC, 2010. Genetic diversity and interfertility among highly differentiated populations of *Ceratocystis fimbriata* in Brazil. *Plant Pathology* **59**: 721–735.

Fischer M, Cox J, Davis DJ, Wagner A, Taylor R, Huerta AJ, Money NP, 2004. New information on the mechanism of forcible ascospore discharge from Ascobolus immerses. *Fungal Genetics and Biology* **41**: 698–707.

Fisher PJ, Petrini O, Sutton BC, 1993. A comparative study of fungal endophytes from leaves, xylem and bark of *Eucalyptus nitens* in Australia and England. *Sydowia* **45**: 338–345.

Gadgil PD, Wardlaw TJ, Ferreira FA, Sharma JK, Dick MA, Wingfield MJ, Crous PW, 2000. Management of disease in Eucalypt plantations. In: Keane PJ, Kile GA, Podger FD, Brown BN (eds), *Diseases and Pathogens of Eucalypts*. CSIRO Publishing, Australia, pp. 519−529.

Gan S, Li M, Li F, Wu K, Wu J, Bai J, 2004. Genetic Analysis of Growth and Susceptibility to Bacterial Wilt (*Ralstonia solanacearum*) in *Eucalyptus* by Interspecific Factorial Crossing. *Silvae Genetica* **53**: 5–6.

Gezahgne A, Cortinas MN, Wingfield MJ, Roux J, 2005. Characterisation of the Coniothyrium stem canker pathogen on *Eucalyptus camaldulensis* in Ethiopia. *Australasian Plant Pathology* **34**: 1–6.

Gezahgne A, Roux J, Hunter GC, Wingfield MJ, 2006. *Mycosphaerella* species associated with leaf disease of *Eucalyptus globulus* in Ethiopia. *European Journal of Forest Pathology* **36**: 253–263.

Gezahgne A, Roux J, Thu PQ, Wingfield MJ, 2003a. Coniothyrium stem canker of *Eucalyptus*, new to Argentina and Vietnam. *South African Journal of Science* **99**: 587–588.

Gezahgne A, Roux J, Wingfield MJ, 2003b. First report of pink disease on *Eucalyptus camaldulensis* in Ethiopia. *Plant Pathology* **52**: 402.

Gibbs JN, 1993. The biology of Ophiostomatoid Fungi causing sapstain in trees and freshly cut logs. In: Wingfield MJ, Seifert KA, Webber JA (eds), *Ceratocystis and Ophiostoma*: *Taxonomy*, *Ecology and Pathogenicity*. APS Press, St. Paul, Minnesota, USA, pp. 153–160.

Glen M, Alfenas AC, Zauza EAV, Wingfield MJ, Mohammed C, 2007. *Puccinia psidii*: a threat to the Australian environment and economy. *Australasian Plant Pathology* **36**: 1–16.

Grgurinovic CA, Walsh D, Macbeth F, 2006. Eucalyptus rust caused by *Puccinia psidii* and the threat it poses to Australia. *EPPO Bulletin* **36**: 486–489.

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, 2005. *Chrysoporthe doradensis* sp. nov. pathogenic to *Eucalyptus* in Ecuador. *Fungal Diversity* **20**: 39–57.

Gryzenhout M, Rodas CA, Portales JM, Clegg P, Wingfield BD, Wingfield MJ, 2006. Novel hosts of the *Eucalyptus* canker pathogen *Chrysoporthe cubensis* and a new *Chrysoporthe* species from Colombia. *Mycological Research* **110**: 833–845.

Gryzenhout M, Wingfield BD, Wingfield MJ, 2009. *Taxonomy, phylogeny, and ecology of bark-inhabiting and tree pathogenic fungi in the Cryphonectriaceae*. APS Press, St. Paul, Minnesota, USA.

Haack RA, 2006. Exotic bark- and wood- boring *Coleoptera* in the United States: recent establishments and interceptions. *Canadian Journal of Forestry Research* **36**: 269–288.

Haack RA, Petrice TR, 2008. How risky is bark that is associated with treated wood packing materials? In: Gottschalk KW (ed), *Proceedings, 18th U.S. Department of Agriculture interagency research forum on gypsy moth and other invasive species 2007*. Gen. Tech. Rep. NRS-P-28.

Haack RA, Petrice TR, Nzokou P, 2007. Do bark beetles and wood borers infest lumber following heat treatment? The role of bark. In: Gottschalk KW (ed), *Proceedings, 17th U.S. Department of Agriculture interagency research forum on gypsy moth and other invasive species 2006*. Gen. Tech. Rep. NRS-P-10.

Hansford CG, 1956. Australian Fungi. III. New species and revisions (continued). *Proceedings of the Linnean Society of New South Wales* **81**: 23–51.

Hansford CG, 1962. The Meliolineae supplement. *Sydowia* **16**: 302–323.

Harrington TC, 2005. Ecology and evolution of mycophagous bark beetles and their fungal partners. In: Vega FE (ed), *Insect-Fungal Associations: ecology and evolution*. Oxford University Press, Oxford, UK, pp. 257–291.

Hau B, De Vallavieille-Pope C, 2006. Wind-dispersed diseases. In: Cooke BM, Jones DG, Kaye B (eds), *The Epidemiology of Plant Diseases*, 2nd edn. Springer, Dordrecht, pp. 387–416.

He XY, 1997. Survey of forest bacterial wilt in China. *Forest Pest Disease* **1**: 43–46. (In Chinese).

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

Heath RN, Wingfield MJ, Wingfield BD, Meke G, Mbaga A, Roux J, 2009. *Ceratocystis* species on *Acacia mearnsii* and *Eucalyptus* spp. in eastern and southern Africa including six new species. *Fungal Diversity* **34**: 41–68.

Hodges CS, Alfenas AC, Ferreira FA, 1986. The conspecificity of *Cryphonectria cubensis* and *Endothia eugeniae*. *Mycologia* **78**: 343–350.

Hodges CS, Reis MS, Ferreira FA, Henfling JDM, 1976. O cancro do eucalipto causado por *Diaporthe cubensis*. *Fitopatalogia Brasileira* **1**: 129–167.

Holmes TP, Aukema JE, Holle BV, Liebhold A, Sills E, 2009. Economic Impacts of Invasive Species in Forests Past, Present, and Future. In: The Year in Ecology and Conservation Biology. *Annals of the new york academy of sciences* **1162**: 18–38.

Hood IA, Sandberg CJ, 1987. Occurrence of *Armillaria* rhizomorph populations in the soil beneath indigenous forests in the Bay of Plenty, New Zealand. *New Zealand Journal of Forestry Science* **17**: 83–99.

Hunter GC, Crous PW, Carnegie AJ, Wingfield MJ, 2009. *Teratosphaeria nubilosa*, a serious leaf disease pathogen of *Eucalyptus* spp. in native and introduced areas. *Molecular Plant Pathology* **10**: 1–14.

Hunter GC, Crous PW, Roux J, Wingfield BD, Wingfield MJ, 2004a. Identification of *Mycosphaerella* species associated with *Eucalyptus nitens* leaf defoliation in South Africa. *Australasian Plant Pathology* **33**: 349–355.

Hunter GC, Crous PW, Wingfield BD, Pongpanich K, Wingfield MJ, 2006a. *Pseudocercospora flavomarginata* sp nov., from *Eucalyptus* leaves in Thailand. *Fungal Diversity* **22**: 71–90.

Hunter GC, Roux J, Wingfield BD, Crous PW, Wingfield MJ, 2004b. *Mycosphaerella* species causing leaf disease in South African *Eucalyptus* plantations. *Mycological Research* **108**: 1–10

Hunter GC, Van Der Merwe NA, Burgess TI, Carnegie AJ, Wingfield BD, Crous PW, Wingfield MJ, 2008. Global movement and population biology of *Mycosphaerella nubilosa* infecting leaves of cold-tolerant *Eucalyptus globulus* and *E. nitens*. *Plant Pathology* **57**: 235–242.

Hunter GC, Wingfield BD, Crous PW, Wingfield MJ, 2006b. A multi-gene phylogeny for species of *Mycosphaerella* occurring on *Eucalyptus* leaves. *Studies in Mycology* **55**: 147–161.

Hurley BP, Slippers B, Wingfield MJ, 2007. A comparison of control results for the alien invasive woodwasp, *Sirex noctilio*, in the southern hemisphere. *Agricultural and Forest Entomology* **9**: 159–171.

Iglesias-Trabad G, Wilstermann D, 2008. *Eucalyptus universalis*. Global cultivated eucalypt forest map 2008 Version 1.0.1 in GIT Forestry Consulting's EUCALYPTOLOGICS:

Information resources on *Eucalyptus* cultivation worldwide. Retrieved from http://www.git-forestry.com (March 29th 2009).

Ingold CT, 1971. *Fungal Spores. Their Liberation and Dispersal*. Clarendon Press, Oxford, UK.

Ingold CT, 2001. Turgor and spore release in ascus and basidium. *Mycologist* **15**: 45.

Jacobs K, Wingfield MJ, 2001. *Leptographium species: tree pathogens, insect associates,* and agents of blue-stain. APS Press, St. Paul, Minnesota, USA.

Janse JD, Van Den Beld HE, Elphinstons J, Simpkins S, Tjou-Tam-Sin NNA, Van Vaerenbergh J, 2004. Introduction to Europe of *Ralstonia solanacearum* Biovar 2, Race 3 in *Pelargonium zonale* Cuttings. *Journal of Plant Pathology* **86:** 147–155.

Johnson GI, 1985. Occurrence of *Cylindrocladium crotalariae* on peanut (*Arachis hypogaea*) seed. *Plant Disease* **69**: 434–436.

Jones DR, Baker HA, 2007. Introductions of non-native plant pathogens into Great Britain, 1970–2004. *Plant Pathology* **56**: 891–910.

Keane PJ, Kile GA, Podger FD, Brown BN, 2000. *Diseases and Pathogens of Eucalypts*. CSIRO Publishing, Australia.

Keuran M, Allen E, 2004. Keeping forest pests from moving around the world. *Unasylva* **55**: 29–30.

Kile GA, 1993. Plant diseases caused by species of *Ceratocystis sensu stricto* and *Chalara*. In: Wingfield MJ, Seifert KA, Webber JA (eds), *Ceratocystis and Ophiostoma*: *Taxonomy, Ecology and Pathogenicity*. APS Press, St. Paul, Minnesota, USA, pp. 173–183.

Kirisits T, 2004. Fungal Associates of European Bark Beetles With Special Emphasis on the Ophiostomatoid Fungi. In: Lieutier F, Day KR, Battisti A, Grégoire JC, Evans HF (eds). *Bark and Wood Boring Insects in Living Trees in Europe, a Synthesis*. Kluwer Academic Publishers, Dordrecht, pp. 181–235.

Lai JS, 1990. Integrated management of eucalypt pests and diseases. *Yunnan Forestry* **3**: 20–21. (In Chinese).

Leach JG, 1940. *Insect transmission of plant diseases*. McGraw-Hill Book, New York, UK.

Leach JG, Orr LW, Christensen C, 1934. The interrelationships of bark beetles and blue-staining fungi in felled Norway pine timber. *Journal of Agricultural Research* **49**: 315–341.

Lebarbenchon C, Brown S, Poulin R, Gauthier-clerc M, Thomas F, 2008. Evolution of pathogens in a man-made world. *Molecular Ecology* **17**: 475–484.

Leslie JF, Summerell BA, 2006. *The Fusarium Laboratory Manual*, Blackwell Publishing Ltd.

Li H, Wu XY, 1996. The current status, causes and control of forest diseases in Hainan Province. *Tropical Forestry* **24**: 101–103. (In Chinese).

Li WD, 1992. The occurrence and control of eucalypt bacterial wilt in HaiNan province. *HaiNan Forestry Science & Technology* **3**: 21–22. (In Chinese).

Liang QY, 2007. Scientifically managing Eucalypt plantations to promote sustainable development of GuangDong forestry. *Eucalypts Science & Technology* **24**: 41–43. (In Chinese).

Lin XP, Lin XJ, Wu GJ, Li XX, Cai JX, Xu SC, 1996. The epidemic law of bacterial wilt in *Eucalyptus*. *Journal of Central South Forestry University* **16**: 49–55. (In Chinese).

Lombard L, 2004. Fungal diseases in *Eucalyptus* and *Acacia* nursery in South Africa. MSc thesis, University of Pretoria. Pretoria, South Africa.

Lombard L, Crous PW, Wingfield BD, Wingfield MJ, 2010a. Phylogeny and systematics of the genus *Calonectria*. *Studies in Mycology* **66**: 31–69.

Lombard L, Zhou XD, Crous PW, Wingfield BD, Wingfield MJ, 2010b. *Calonectria* species associated with cutting rot of *Eucalyptus*. *Persoonia* **23**: 41–47.

Loo JA, 2009. Ecological impacts of non-indigenous invasive fungi as forest pathogens. *Biological Invasions* **11**: 81–96.

Lowe AJ, Boshier D, Ward M, Bacles CE, Navarro C, 2005. Genetic resource impacts of habitat loss and degradation; reconciling empirical evidence and predicted theory for neotropical trees. *Heredity* **95**: 255–273.

Lundquist JE, Purnell RC, 1987. Effects of Mycosphaerella leaf spot on growth of *Eucalyptus nitens*. *Plant Disease* **71**: 1025–1029.

Machmud, M, 1985. Bacterial wilt in Indonesia. In: Craswell ET, Pushperajah E (eds), *Bacterial wilt disease in Asia and the South Pacific*, ACIAR Proceedings 13. ACIAR, Canberra, pp. 30–34.

Malloch D, Blackwell M, 1993. Dispersal biology of the Ophiostomatoid fungi. In: Wingfield MJ, Seifert KA, Webber JA (eds), *Ceratocystis and Ophiostoma*: *Taxonomy, Ecology and Pathogenicity*. APS Press, St. Paul, Minnesota, USA, pp. 195–206.

Maxwell A, Hardy GEStJ, Dell B, 2001. First record of *Mycosphaerella nubilosa* in Western Australia. *Australasian Plant Pathology* **30**: 65.

Michail SH, El-Saved AB, Salem MA, 1986. Fusarium post-emergence damping-off of *Eucalyptus* and its control measures in Egypt. *Acta Phytopathologia at Entomologia Hungarica* **21**: 127–133.

Milgate AW, Yuan ZQ, Vaillancourt RE, Mohammed C, 2001. *Mycosphaerella* species occurring on *Eucalyptus globulus* and *Eucalyptus nitens* plantations of Tasmania, Australia. *Forest Pathology* **31**: 53–63.

Mo XY, 2006. *The Silviculture Theory and Method of Eucalypt Plantation*. China Forestry House Press, Beijing, China. (In Chinese).

Mohali S, Slippers B, Wingfield MJ, 2007. Identification of Botryosphaeriaceae species from *Eucalyptus*, *Acacia* and *Pinus* in Venezuela. *Fungal Diversity* **25**: 143–165.

Moller WJ, Vay JE de, 1968. Insect transmission of *Ceratocystis fimbriata* in deciduous fruit orchards. *Phytopathology* **58**: 1499–1508.

Morrison DJ, Williams RE, Whitney RD, 1991. Infection, disease development, diagnosis and detection. In: Shaw CJ, Kile GA (eds), *Armillaria Root Rot Disease*. Agriculture Handbook No. 691. Washington, DC: Forest Service, United States Department of Agriculture, USA, pp. 62–75.

Mumford JD, 2002. Economic issues related to quarantine in international trade. *European Review of Agriculture Economics* **29**: 329–348.

Myburg H, Gryzenhout M, Wingfield BD, Wingfield MJ, 2002. 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* **44**:187–196.

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

Nagarajan S, Singh DV, 1990. Long-Distance Dispersion of Rust Pathogens. *Annual Review of Phytopathology* **28**: 139–153.

Nakabonge G, Gryzenhout M, Wingfield BD, Wingfield MJ, Roux J, 2007. Genetic diversity of *Chrysoporthe cubensis* in eastern and southern Africa. *South African Journal of Science* **103**: 261–264.

Nakabonge G, Roux J, Gryzenhout M, Wingfield MJ, 2006. Distribution of *Chrysoporthe* canker pathogens on *Eucalyptus* and *Syzygium* species in Eastern and Southern Africa. *Plant Disease* **90**: 734–740.

Nicolle D, 2006. A classification and census of regenerative strategies in the eucalypts (*Angophora*, *Corymbia* and *Eucalyptus*—Myrtaceae), with special reference to the obligate seeders. *Australian Journal of Botany* **54**: 391–407.

Old KM, Dudzinski MJ, Pongpanich K, Yuan ZQ, Thu PQ, Nguyen NT, 2002. Cryptosporiopsis leaf and shoot blight of eucalypts. *Australasian Plant Pathology* **31**: 337–344.

Old KM, See LS, Sharma JK, Yuan ZQ, 2000. *A manual of diseases of tropical acacias in Australia, South-East Asia and India*. Centre for International Forestry Research, Indonesia.

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

Old KM, Yuan ZQ, 1994. *Foliar and stem diseases of Eucalyptus in Vietnam and Thailand. Internal report*, CSIRO Division of Forestry and Australian Centre for Agricultural Research, Canberra.

Old KM, Yuan ZQ, Kobayashi T, 1991. A *Valsa* teleomorph for *Cytospora eucalyptocola*. *Mycological Research* **95**: 1253–1256.

Paap T, Burgess TI, McComb JA, Shearer BL, Hardy GE STJ, 2008. *Quambalaria* species, including *Q. coyrecup* sp. nov., implicated in canker and shoot blight diseases causing decline of *Corymbia* species in the southwest of Western Australia. *Mycological Research* **112**: 57–69.

Palm ME, Rossman AY, 2003. Invasion Pathways of Terrestrial Plant-Inhabiting Fungi. In: Ruiz GM, Carlton JT (eds), *Innasive species: vectors and management strategies*. Island Press, pp. 31–43.

Park RF, 1988. Epidemiology of *Mycosphaerella nubilosa* and *M. cryptica* on *Eucalyptus* spp. in South-eastern Australia. *Transactions of the British Mycological Society* **91**: 261–266.

Park RF, Keane PJ, 1982a. Leaf diseases of *Eucalyptus* associated with *Mycosphaerella* species. *Transactions of the British Mycological Society* **79**: 101–115.

Park RF, Keane PJ, 1982b. Three *Mycosphaerella* species form leaf diseases of *Eucalyptus*. *Transactions of the British Mycological Society* **79**: 95–100.

Park RF, Keane PJ, 1987. Spore production by *Mycosphaerella* species causing leaf diseases of *Eucalyptus*. *Transactions of the British Mycological Society* **89**: 461–470.

Pavlic D, Slippers B, Coutinho TA, Wingfield MJ, 2007. Botryosphaeriaceae occurring on native *Syzygium cordatum* in South Africa and their potential threat to *Eucalyptus*. *Plant Pathology* **56**: 624–636.

Pavlic D, Wingfield MJ, Barber P, Slippers B, Hardy GEStJ, Burgess TI, 2008. Seven new species of the Botryosphaeriaceae from baobab and other native trees in Western Australia. *Mycologia* **100**: 851–866.

Pegg GS, Carnegie AJ, Wingfield MJ, Drenth A, 2009. *Quambalaria* species: increasing threat to eucalypt plantations in Australia. *Southern Forests* **71**: 111–114.

Pegg GS, Drenth A, Wingfield MJ, 2005. *Quambalaria pitereka* on spotted gum plantations in Queensland and northern New South Wales, Australia. Proceedings of the XXII IUFRO World Congress, 8-13 August 2005, Brisbane, Australia. *International Forestry Review* **7**: 337.

Pegg GS, Gryzenhout M, O'Dwyer C, Drenth A, Wingfield MJ, 2010. The *Eucalyptus* canker pathogen *Chrysoporthe cubensis* discovered in eastern Australia. *Australasian Plant Pathology* **39**: 343–349.

Pegg GS, O'Dwver C, Carnegie AJ, Burgess TI, Wingfield MJ, Drenth A, 2008. *Quambalaria* species associated with plantation and native eucalypts in Australia. *Plant Pathology* **57**: 702–714.

Pérez CA, De Beer ZW, Altier NA, Wingfield MJ, Blanchette RA, 2008. Discovery of the eucalypt pathogen *Quambalaria eucalypti* infecting a non-*Eucalyptus* host in Uruguay. *Australasian Plant Pathology* **37**: 600–604.

Pérez CA, Wingfield MJ, Altier NA, Blanchette RA, 2009. Mycosphaerellaceae and Teratosphaeriaceae associated with *Eucalyptus* leaf diseases and stem cankers in Uruguay. *Forest Pathology* **39**: 349–360.

Pérez G, Hunter GC, Slippers B, Pérez C, Wingfield BD, Wingfield MJ, 2009. *Teratosphaeria* (*Mycosphaerella*) *nubilosa*, the causal agent of Mycosphaerella leaf disease (MLD), recently introduced into Uruguay. *European Journal of Plant Pathology* **125**: 109–118.

Pérez G, Slippers B, Wingfield BD, Finkenauer E, Wingfield MJ, 2010a. Mycosphaerella leaf disease (MLD) outbreak on *Eucalyptus globulus* in Brazil caused by *Teratosphaeria* (*Mycosphaerella*) *nubilosa*. *Phytopathologia Mediterranea* **48**: 302–306.

Pérez G, Slippers B, Wingfield BD, Hunter GC, Wingfield MJ, 2010b. Micro- and macrospatial scale analyses illustrates mixed mating strategies and extensive geneflow in populations of an invasive haploid pathogen. *Molecular Ecology* **19**: 1801–1813.

Podger FD, Kile GA. Watling R Fryer J, 1978. Spread and effects of *Armillaria luteobubalina* sp. nov. in an Australian *Eucalyptus regnans* plantation. *Transactions of the British Mycological Society* **71**: 77–87.

Pongpanich K, 2000. *Eucalyptus* pathology in Thailand. In: *Eucalypt diseases and their management*. Proceedings of a workshop, Kasetsart University, BANGKOK 6–8 November 2000. ACIAR 9441 Final Report. Attachment 4.

Qi SX, 2002. *Eucalyptus in China*. China Forestry House Press, Beijing, China. (In Chinese).

Qi SX, 2006. The introduction and development situation of *Eucalyptus* in China. *Guangxi Forestry Science* **4**: 250–252. (In Chinese).

Qi SX, Wang HF, Wen YQ, 2006. *The Manual of Eucalyptus Plantation*. China Forestry House Press, Beijing, China. (In Chinese).

Qian GQ, 2007. Ecological problem and development countermeasure of *Eucalyptus*. *Journal of Hunan Forestry Science and Technology* **34**: 67–70. (In Chinese).

Ran LX, Liu CY, Wu GJ, Van Loon LC, Bakker PAHM, 2005. Suppression of bacterial wilt in *Eucalyptus urophylla* by fluorescent *Pseudomonas* spp. in China. *Biological Control* **32**: 111–120.

Read ND, Beckett A, 1996. Ascus and ascospore morphogenesis. *Mycological Research* **100**: 1281–1314.

Redlin SC, Carris LM, 1996. *Endophytic fungi in grasses and woody plants: systematics, ecology, and evolution*. APS Press, St. Paul, Minnesota, USA

Reusch TBH, 2006. Does disturbance enhance genotypic diversity in clonal organisms? A field test in the marine angiosperm *Zostera marina*. *Molecular Ecology* **15**: 277–286.

Ridley GS, Bain J, Bulman LS, Dick MA, Kay MK, 2000. *Threats to New Zealand's indigenous forests from exotic pathogens and pests*. Department of Conservation. Wellington, New Zealand.

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

Rodas CA, Lombard L, Gryzenhout M, Slippers B, Wingfield MJ, 2005b. *Cylindrocladium* blight of *Eucalyptus grandis* in Colombia. *Australasian Plant Pathology* **34**: 143–149.

Rodas CA, Roux J, Van Wyk M, Wingfield BD, Wingfield MJ, 2008. *Ceratocystis neglecta* sp. nov., infecting *Eucalyptus* trees in Colombia. *Fungal Diversity* **28**: 73–84.

Rollins F, Jones KG, Krokene P, Solheim H, Blackwell M, 2001. Phylogeny of Asexual Fungi Associated with Bark and Ambrosia Beetles. *Mycologia* **93**: 991–996.

Roux J, Coetzee MPA, 2005. First report of pink disease on native trees in South Africa and phylogenetic placement of *Erythricium salmonicolor* in the Homobasidiomycetes. *Plant Disease* **89**: 1158–1163.

Roux J, Coutinho TA, Mujuni Byabashaija D, Wingfield MJ, 2001a. Diseases of plantation *Eucalyptus* in Uganda. *South African Journal of Science* **97**: 16–18.

Roux J, Coutinho TA, Wingfield MJ, Bouillet J-P, 2000a. Diseases of plantation *Eucalyptus* in the Republic of Congo. *South African Journal of Science* **96**: 454–456.

Roux J, Harrington TA, Steimel JP, Wingfield MJ, 2001b. Genetic variation in the wattle wilt pathogen *Ceratocystis albofundus*. *Mycoscience* **42**: 327–332.

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

Roux J, Mthalane ZL, De Beer ZW, Eisenberg B, Wingfield MJ, 2006. *Quambalaria* leaf and shoot blight on *Eucalyptus nitens* in South Africa. *Australasian Plant Pathology* **35**: 427–433.

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

Roux J, Van Wyk M, Hatting H, Wingfield MJ, 2004. *Ceratocystis* species infecting stem wounds on *Eucalyptus grandis* in South Africa. *Plant Pathology* **53**: 414–421.

Roux J, Wingfield MJ, 2009. *Ceratocystis* species: emerging pathogens of non-native plantation *Eucalyptus* and *Acacia* species. *Southern Forests* **71**: 115–120.

Roux J, Wingfield MJ, Bouillett JP, Wingfield BD, Alfenas AC, 2000b. A serious new disease of *Eucalyptus* caused by *Ceratocystis fimbriata* in Central Africa. *Forest Pathology* **30**: 175–184.

Roux J, Wingfield MJ, Cibria´n D, 2002. First report of Coniothyrium canker of *Eucalyptus* in Mexico. *Plant Pathology* **51**: 382.

Saikkonen K, Faeth SH, Helander M, Sullivan TJ, 1998. Fungal endophytes: A Continuum of Interactions with Host Plants. *Annual Review of Ecology and Systematics* **29**: 319–343.

Sankaran KV, Sutton BC, Balasundaran M, 1995a. *Cryptosporiopsis eucalypti* sp. nov. causing leaf spots of eucalypts in Australia, India and USA. *Mycological Research* **99**: 827–830.

Sankaran KV, Sutton BC, Minter DW, 1995b. A checklist of fungi recorded on *Eucalyptus*. *Mycological Papers* **170**: 1–376.

Sathyanarayana HV, Anand N, 1993. Developing bacterial wilt resistant F1 hybrids for processing in tomato (*Lycopersicon exculentum*). In: Hartman GL, Hayward AC (eds), *Bacterial Wilt*. ACIAR proceedings, No. 45, pp. 158–162.

Schowalter TD, Filip GM, 1993. *Beetle-pathogen interactions in conifer forests*. Academic Press, San Diego, California, USA.

Schwartz MW, Hoeksema JD, Gehring CA, Johnson NC, Klironomos JN, Abbott LK, Pringle A, 2006. The promise and the potential consequences of the global transport of mycorrhizal fungal inoculum. *Ecology Letters* **9**: 501–515.

Self NM, Aitken EAB, Dale MD, 2002. Susceptibility of provenances of spotted gums to Ramularia shoot blight. *New Zealand Plant Protection* **55**: 68–72.

Seth SK, Bakshi BK, Reddy MAR, Singh Sujan, 1978. Pink disease of *Eucalyptus* in India. *Europen Journal of Forestry Pathology* **8**: 200–216.

Sharma JK, Mohanan C, Florence EJM, 1984a. Outbreak of pink disease caused by *Corticium salmonicolor* in *Eucalyptus grandis*. *Tropical Pest Management* **30**: 253–255.

Sharma JK, Mohanan C, Florence EJM, 1985. Occurrence of *Cryphonectria* canker disease of *Eucalyptus* in Kerala, India. *Annals of Applied Biology* **106**: 265–276.

Sharma JK, Mohanan C, Maria Florence EJ, 1984b. A new stem canker disease of *Eucalyptus* caused by *Botryodiplodia theobromae* in India. *Transactions of the British Mycological Society* **83**: 162–163.

Simpson JA, 2000. *Quambalaria*, a new genus of eucalypt pathogens. *Australian Mycologist* **19**: 57–62.

Slippers B, Burgess T, Pavlic D, Ahumada R, Maleme H, Mohali S, Rodas C, Wingfield MJ, 2009. A diverse assemblage of Botryosphaeriaceae infect *Eucalyptus* in native and non-native environments. *Southern Forests* **71**: 101–110.

Slippers B, Crous PW, Denman S, Coutinho TA, Wingfield BD, Wingfield MJ, 2004a. Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. *Mycologia* **96**: 83–101.

Slippers B, Fourie G, Crous PW, Coutinho TA, Wingfield BD, Carnegie A, Wingfield MJ, 2004b. Speciation and distribution of *Botryosphaeria* spp. on native and introduced *Eucalyptus* trees in Australia and South Africa. *Studies in Mycology* **50**: 343–358.

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

Slippers B, Wingfield MJ, 2007. Botryosphaeriaceae as endophytes and latent pathogens of woody plants: diversity, ecology and impact. *Fungal Biology Reviews* **21**: 90–106.

Smith H, Kemp GHJ, Wingfield MJ, 1994. Canker and die-back of *Eucalyptus* in South Africa caused by *Botryosphaeria dothidea*. *Plant Pathology* **43**: 1031–1034.

Smith H, Wingield MJ, Crous PW, Coutinho TA, 1996a. *Sphaeropsis sapinea* and *Botryosphaeria dothidea* endophytic in *Pinus* spp. and *Eucalyptus* spp. in South Africa. *South African Journal of Botany* **62**: 86–88.

Smith H, Wingield MJ, De Wet J, Coutinho TA, 2000. Genotypic diversity of *Sphaeropsis sapinea* from South Africa and Northern Sumatra. *Plant Disease* **84**: 139–142.

Smith H, Wingfield MJ, Petrini O, 1996b. *Botryosphaeria dothidea* endophytic in *Eucalyptus grandis* and *Eucalyptus nitens* in South Africa. *Forest Ecology and Management* **89**: 189–195.

Solheim H, 1993. Ecological aspects of fungi associated with the spruce beetle *Ips typographus* in Norway. In: Wingfield MJ, Seifert KA, Webber JA (eds), *Ceratocystis and Ophiostoma*: *Taxonomy, Ecology and Pathogenicity*. APS Press, St. Paul, Minnesota, USA, pp. 195–242.

Spadberry JP, Kirk AA, 1978. Aspects of the ecology of siricid woodwasps (Hymenoptera: Siricidae) in Europe, North Africa and Turkey with special reference to the biological control of Sirex noctillio F. in Australia. *Bulletin of Entomological Research* **68**: 341–359.

Stone C, Simpson JA, 1987. Influence of *Ips grandicollis* on the incidence and spread of bluestain fungi in *Pinus elliottii* billets in north-eastern New South Wales. *Australian Forestry* **50**: 86–94.

Sudo S, Oliveira GHN, Pereira AC, 1983. Eucalipto (*Eucalyptus* spp.), bracaatinga (*Mimosa scrabella* Penth.) novas hospedeiras de *Pseudomonas solanacearum* E. F. Smith. *Fitopatologia Brasileira* **8**: 631.

Talbot PHB, 1977. The *Sirex–Amylostereum–Pinus* association. *Annual Review of Phytopathology* **15**: 41–54.

Tatem AJ, Hay SI, Rogers DJ, 2006a. Global traffic and disease vector dispersal. *PNAS* **103**: 6242–6247.

Tatem AJ, Rogers DJ, Hay SI, 2006b. Global Transport Networks and Infectious Disease Spread. *Advances in Parasitology* **62**: 293–343.

Taylor JS, 1962. *Sirex noctilio* F., a recent introduction in South Africa. *Entomologist's Record* **74**: 273–274

Thu PQ, Old KM, Dudzinski MJ, Gibbs RJ, 2000. Results of eucalypt disease surveys in Vietnam. In: *Eucalypt diseases and their management*. Proceedings of a workshop, Kasetsart University, BANGKOK 6–8 November 2000. ACIAR 9441 Final Report. Attachment 4.

Tommerup IC, Alfenas AC, Old KM, 2003. Guava rust in Brazil – a threat to *Eucalyptus* and other Myrtaceae. *New Zealand Journal of Forestry Science* **33**: 420–428.

Turnbull JW, 2000. Economic and social importance of eucalypts. In: Keane PJ, Kile GA, Podger FD, Brown BN (eds), *Diseases and Pathogens of Eucalypts.* SCIRO Publishing, Australia, pp. 1−9.

Van Der Merwe NA, Gryzenhout M, Steenkamp ET, Wingfield BD, Wingfield MJ, 2010. Multigene phylogenetic and population differentiation data confirm the existence of a cryptic species within *Chrysoporthe cubensis*. *Fungal Biology* **114**: 966–979.

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

Van Der Westhuizen GCA, 1965a. A disease of young *Eucalyptus saligna* in Northern Transvaal. *South African Forestry Journal* **54**: 12–16.

Van Der Westhuizen GCA, 1965b. *Cytospora eucalypticola* sp. nov. on *Eucalyptus saligna* from Northern Transvaal. *South African Forestry Journal* **54**: 8–11.

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

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 TA, Van Zyl LM, Wright JA, 1997. Diversity of *Cryphonectria cubensis* isolates in Venezuela and Indonesia In: *Proc. IUFRO*

Conference on Silviculture and Improvement of Eucalypts, Salvador, Bahia, Brazil, pp. 142–146.

Van Niekerk JM, Groenewald JZ, Verkley GJM, Fourie PH, Wingfield MJ, Crous PW, 2004. Systematic reappraisal of *Coniella* and *Pilidiella*, with specific reference to species occurring on *Eucalyptus* and *Vitis*. *Mycological Research* **108**: 283–303.

Van Wyk M, Wingfield BD, Mohali S, Wingfield MJ, 2009. *Ceratocystis fimbriatomima*, a new species in the *C. fimbriata sensu lato* complex isolated from *Eucalyptus* trees in Venezuela. *Fungal Diversity* **34**: 173–183.

Van Wyk M, Wingfield BD, Wingfield MJ, 2010. Global distribution of a *Eucalyptus* pathogen. (Unpublished).

Van Zyl LM, Coutinho TA, Wingfield MJ, Pongpanich K, Wingfield BD, 2002. Morphological and molecular relatedness of geographically diverse isolates of *Coniothyrium zuluense* from South Africa and Thailand. *Mycological Research* **106**: 51–59.

Van Zyl LM, Wingfield MJ, Alfenas CA, Crous PW, 1998. Population diversity among Brazilian isolates of *Cryphonectria cubensis*. *Forestry Ecology Manage* **112**: 41–47.

Viljanen-Rollinson SLH, Cromey MG, 2002. Pathways of entry and spread of rust pathogens: implications for New Zealand's biosecurity. *New Zealand Plant Protection* **55**: 42–48.

Walker J, Bertus AL, 1971. Shoot blight of *Eucalyptus* spp. caused by an undescribed species of *Ramularia*. Proceedings of the Linnean Society of New South Wales, series. 2. **96**: 108–115.

Wang J, Cen BZ, Su H, 1997. A review of studies on bacterial wilt caused by *Pseudomonas solanacearum* in forest trees. *Journal of South China Agricultural University* **18**: 118–121. (In Chinese).

Wang WY, 1992. Survey of *Eucalyptus* diseases in Taiwan. *Bulletin of Taiwan Forestry Research Institute* **7**: 179–194. (In Chinese).

Wen YG, Liu SR, Che F, 2005. The ecological problem and sustainable management of *Eucalyptus* Industrial Plantation. *Journal of Guangxi Sciences Acadamy* **21**: 13–18. (In Chinese).

Wen ZY, 2006. Use a correct way to understand the *Eucalyptus* plantation in southern China. *China Paper Newsletter* **6**: 13–16. (In Chinese).

Wilson JRU, Dormontt EE, Prentis PJ, Lowe AJ, Richardson DM, 2008. Something in the way you move: dispersal pathways affect invasion success. *Trends in Ecology and Evolution* **24**: 136–144.

Wingfield MJ, 2003. Increasing threat of diseases to exotic plantation forests in the southern hemisphere: Lessons from *Cryphonectria* canker. *Australasian Plant Pathology* **32**: 133–139.

Wingfield MJ, Crous PW, Boden D, 1996a. *Kirramyces destructans* sp. nov., a serious leaf pathogen of *Eucalyptus* in Indonesia. *South African Journal of Botany* **62**: 325–327.

Wingfield MJ, Crous PW, Couthinho TA, 1996b. A serious new canker disease of *Eucalyptus* in South Africa caused by a new species of *Coniothyrium*. *Mycopathologia* **136**: 139–145.

Wingfield MJ, Crous PW, Swart WJ, 1993. *Sporothrix eucalypti*, a shoot and leaf pathogen of *Eucalyptus* in South Africa. *Mycopathologia* **123**: 159–164.

Wingfield MJ, Rodas C, Myburg H, Venter M, Wright J, Wingfield BD, 2001a. Cryphonectria canker on *Tibouchina* in Colombia. *Forestry Pathology* **31**: 297–306.

Wingfield MJ, Roux J, Coutinho T, Govender P, Wingfield BD, 2001b. Plantation disease and pest management in the next century. *Southern African Forestry Journal* **190**: 67−71.

Wingfield MJ, Slippers B, Hurley BP, Coutinho TA, Wingfield BD, Roux J, 2008. Eucalypt pests and diseases: growing threats to plantation productivity. *Southern Forests* **70**: 139–144.

Wingfield MJ, Slippers B, Roux J, Wingfield BD, 2001c. Worldwide movement of exotic forest fungi, especially in the Tropics and the Southern hemisphere. *Bioscience* **51**: 134−140.

Wingfield M, Slippers B, Roux J, Wingfield BD, 2011. Fifty years of tree pest and pathogen invasions, increasingly threatening world forests. In: Richardson DM (ed), *Fifty Years of Invasion Ecology: The Legacy of Charles Elton*. Blackwell Publishing Ltd, pp. 89–99.

Wingfield MJ, Slippers B, Zhou XD, de Beer ZW, Govender P, Wingfield BD, 2001d. Global spread of insect-associated fungi on exotic plantation pines. *IUFRO World Series* **11**: 107–114.

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

Wingfield MJ, Van Zyl LM, Van Heerden S, Myburg H, Wingfield BD, 1997. Virulence and the genetic composition of the *Cryphonectria cubensis* Bruner population in South Africa. In: Lieutier F, Mattson WJ, Wagner MR (eds), *Physiology and Genetics of Tree–Phytophage Interactions.* INRA Editions, Gujian, pp. 163–172.

Winter G, 1884. Repertorium. Rabenhorstii fungi europaei et extraeuraopaei. Centuria XXXI et XXXII. *Hedwigia* **23**: 164–175.

Wu QP, Liang ZC, 1988a. Identification and pathogenic tests of the causal organism of the bacterial wilt of *Eucalyptus*. *Journal of South China Agricultural University* **9**: 59–67. (In Chinese).

Wu QP, Liang ZC, 1988b. Selection of species and provenances of *Eucalyptus* for resistance to bacterial wilt. *Journal of South China Agricultural University* **9**: 41–45. (In Chinese).

Xiang DY, Chen JB, YE L, 2006. The development situation, challenges and countermeasure of GuangXi's eucalypt plantation. *GuangXi Forestry Science* **4**: 195–201. (In Chinese).

Xie YJ, 2006. *Eucalypt research at the beginning of 21st century*. China Forestry House Press, Beijing, China. (In Chinese).

Xu DP, 2006. The fast growing and high yield eucalyptus forest is a necessary choice for China's forestry-pulp-paper integration. *China Paup & Paper Industry* **27**: 15–25. (In Chinese).

Yang MS, Peng Y, 2006. Current status and development trend of China's *Eucalyptus* pulp material. *Paper and Paper Making* **25**: 17–20. (In Chinese).

Yang RD, 2007. The growth and ecological effects of artificial multi-storied mixed forest of *Eucalyptus*. *Journal of Fujian forestry science and technology* **34**: 36–40. (In Chinese).

Young A, Boyle T, Brown T, 1996. The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology and Evolution* **11**: 413–418.

Yuan ZQ, Old KM, Midgley SJ, Solomon D, 1997. Mycoflora and pathogenicity of fungi present on stored seeds from provenances of *Eucalyptus pellita*. *Australasian Plant Pathology* **26**: 195–202.

Zahid MI, Grgurinovic CA, Walsh DJ, 2008. Quarantine risks associated with solid wood packaging materials receiving ISPM 15 treatments. *Australian Forestry* **71**: 287–293.

Zhan J, Pettway RE, McDonald BA, 2003. The global genetic structure of the wheat pathogen *Mycosphaerella graminicola* is characterized by high nuclear diversity, low

mitochondrial diversity, regular recombination, and gene flow. *Fungal Genetics and Biology* **38**: 286–297.

Zhou X, Burgess TI, De Beer ZW, Lieutier F, Yart A, Klepzig K, Carnegie A, Portales JM, Wingfield BD, Wingfield MJ, 2007a. High intercontinental migration rates and population admixture in the sapstain fungus *Ophiostoma ips*. *Molecular Ecology* **16**: 89–99.

Zhou XD, De Beer ZW, Xie YJ, Pegg GS, Wingfield MJ, 2007b. DNA-based identification of *Quambalaria piterka* causing severe leaf blight of *Corymbia citriodora* in China. *Fungal Diversity* **25**: 245–254.

Zhou XD, Xie YJ, Chen SF, Wingfield MJ, 2008. Diseases of eucalypt plantations in China: challenges and Opportunities. *Fungal Diversity* **32**: 1–7.

Table 1. Popular eucalypt species planted in China.

Species	**Origin**	**Distribution (Province/Region)**
Eucalyptus urophylla	Archipelago, Eastern Indonesia	GuangDong, GuangXi and HaiNan, etc.
E. grandis	Eastern Australia	SiChuan, some area of GuangDong and GuangXi, etc.
E. camaldulensis	Australia	FuJian, GuangDong, GuangXi, HuNan, ShangHai, SiChuan, YunNan and ZheJiang
E. globulus	New South Wales and Victoria, Australia	GuangXi, GuiZhou, JiangSu, JiangXi, SiChuan and YunNan
E. maidenii	New South Wales and Victoria, Australia	GuangDong, GuangXi and YunNan
E. tereticornis	Australia	FuJian, GuangDong, GuangXi, JiangXi, SiChuan, YunNan and ZheJiang
E. dunnii	Eastern Queensland and New South Wales of Australia	FuJian, HuNan and JiangXi
E. saligna	New South Wales and Queensland, Australia	GuangDong, GuangXi, SiChuan and YunNan
Corymbia citriodora	Queensland, Australia	FuJian, GuangDong, GuangXi, HuNan, JiangXi, SiChuan, YunNan and ZheJiang, etc
C. torelliana	Northern Queensland, Australia	GuangDong, GuangXi and Hong Kong
E. nitens	New South Wales and Victoria, Australia	FuJian, HuNan, JiangXi, SiChuan and YunNan
E. exserta	Northeast Australia	GuangDong and GuangXi
E. pellita	New South Wales and Queensland, Australia	GuangDong and GuangXi
E. botryoides	Southeast Australia	GuangDong and GuangXi
E. robusta	New South Wales and Southern Queensland, Australia	
E. wetarensis	Wetar island of Indonesia	GuangDong, GuangXi and HaiNan, etc

Table 2. Distribution of eucalypt clones/species in different geographic and climatic areas in China.

Area	**Suitable eucalypt species/clones**	**Climate Type**[a]	**Climate characteristics**	**Distribution**	**Development actuality**
A: Low altitude areas of south and Southeast where closer to sea level	*Eucalyptus* Congo No.12, *Eucalyptus urophylla*, *E. urophylla* × *E. camaldulensis*, *E. urophylla* × *E. grandis*, *E. urophylla* × *E. tereticornis*, etc.	Tropical to Southern sub-tropical	High temperatures with high rainfall in summer, minimum temperature above 0˚C, mostly rains in summer	GuangDong, GuangXi, HaiNan, Hong Kong, South YunNan, Southeast FuJian, TaiWan, Macao	The primary eucalypt plantations Area
B: Inland mountainous regions in sub-tropical areas	*E. camaldulensis*, *E. dunnii*, *E. grandis*, *E. grandis* × *E. urophylla*, *E. urophylla* × *E. camaldulensis*, *E. wetarensis*, *E. wetarensis* × *E. camaldulensis*	Middle sub-tropical	High temperatures with high rainfall in summer, the lowest temperature –5˚C, has frost, light snow seldom	Central South HuNan and JiangXi, Central West FuJian, North GuangXi, Northern verge of GuangDong, South ZheJiang	Suitable eucalypt trees can be widely planted
C: High altitude areas of YunNan, GuiZhou, and SiChuan	*E. camaldulensis*, *E. dunnii*, *E. maidenii*, *E. nitens*, *E. smith*	Middle sub-tropical	Cool in summer and warm in winter, rainfall distributed throughout the year, but especially in winter	Central GuiZhou, North YunNan, Southern verge of SiChuan	Suitable eucalypt trees can be widely planted

D: SiChuan Basin	*E. botryoides*, *E. camaldulensis*, *E. grandis*, *E. robusta*	Middle sub-tropical	Temperature is high in summer, and low in winter, the lowest temperature –7˚C, rainfall distributed especially in summer	Central SiChuan (SiChuan Basin)	Suitable eucalypt trees can be widely planted
E: Northern verge	*E. camaldulensis*, *E. dunnii*, *E. urophylla* × *E. camaldulensis*	Northern sub-tropical to temperate	Temperature is high in summer, and low in winter, always have frost and snow in winter	AnHui, JiangSu, North GuiZhou, HuNan, SiChuan and ZheJiang, Northern verge of ShanXi, Southern verge of HuBei	Very small area have been planted, cool resistant breedling is really necessary in this area

[a] The sub-tropical area in China was divided into Southern, Middle and Northern sub-tropical regions by the geographic and climatic differences.

Table 3. Leaf diseases caused by Mycosphaerellaceae and Teratosphaeriaceae reported from eucalypt forestry in Southeast Asia, India and China.

Species	Anamorph	Host	Location	References
'*Mycosphaerella*'[a] *crystallina*	*Pseudocercospora crystallina*	*Eucalyptus urophylla*	**China**	Burgess *et al*. 2007b
'*M*'. *citri*	*Zasmidium citri*	*Eucalyptus* sp.	Thailand	Crous *et al*. 2009c
		E. camaldulensis hybrid	Southeast Vietnam	Burgess *et al*. 2007b
'*M*'. *eucalyptorum*		*Eucalyptus* sp.	Indonesia	Burgess *et al*. 2007b
'*M*'. *gamsii*		*Eucalyptus* sp.	India	Crous *et al*. 2006c
'*M*'. *heimii*	*Pseudocercospora heimii*	*E. camaldulensis*	Thailand	Crous *et al*. 2007b
		Eucalyptus sp.	Vientein, Laos	Cheewangkoon *et al*. 2008
'*M*'. *heimioides*	*P. heimioides*	*E. urophylla*, *Eucalyptus* sp.	Indonesia	Crous *et al*. 1995, 1996, 2001; Burgess *et al*. 2007b
'*M*'. *irregulari*		*Eucalyptus* sp.	Udonthani, Thailand	Cheewangkoon *et al*. 2008
'*M*'. *konae*	*P. konae*	*E. camaldulensis*	Thailand	Crous *et al*. 2007b
'*M*'. *marksii*		*Eucalyptus* sp.	YunNan, **China**	Burgess *et al*. 2007b
		Eucalyptus sp.	Irian Jaya, Indonesia	Burgess *et al*. 2007b
		E. camaldulensis	Vietnam	Old *et al*. 2003
'*M*'. *pseudomarksii*		*Eucalyptus* sp.	Thailand	Cheewangkoon *et al*. 2008
'*M*'. *quasiparkii*		*Eucalyptus* sp.	Udonthani, Thailand	Cheewangkoon *et al*. 2008
'*M*'. *stramenticola*		*E. camaldulensis* hybrid	Southeast Vietnam	Burgess *et al*. 2007b
		E. grandis	Lake Toba, Indonesia	Burgess *et al*. 2007b
'*M*'. *sumatrensis*		*Eucalyptus* sp.	Indonesia	Crous *et al*. 2006c; Burgess *et al*. 2007b
''*M*'. *thailandica*	*P. thailandica*	*E. camaldulensis*	Thailand	Crous *et al*. 2007b
		Eucalyptus sp.	Vientein, Laos	Cheewangkoon *et al*. 2008
'*M*'. *verrucosiafricana*		*Eucalyptus* sp.	Indonesia	Crous *et al*. 2006c
'*M*'. *vietnamensis*		*E. grandis* hybrid;	Southeast Vietnam	Burgess *et al*. 2007b
		E. camaldulensis hybrid		
		Eucalyptus sp.	Vientein, Laos	Cheewangkoon *et al*. 2008
		E. camaldulensis, *Eucalyptus* sp.	Thailand	Cheewangkoon *et al*. 2008
'*M*'. *yunnanensis*		*E. urophylla*	YunNan, **China**	Burgess *et al*. 2007b
Teratosphaeria destructans	*Kirramyces destructans*	*E. grandis*, *Eucalyptus* sp.	Sumatra, Indonesia	Wingfield *et al*. 1996a; Old *et al*. 2003; Andjic *et al*. 2007b; Crous *et al*. 2009c
		E. urophylla	East Timor	Old *et al*. 2003
		E. camaldulensis	Eastern Thailand	Old *et al*. 2003; Andjic *et al*. 2007b

		E. camaldulensis, *E. urophylla*	Vietnam	Old *et al*. 2003; Andjic *et al*. 2007b
		E. urophylla, *Eucalyptus* spp.	GuangZhou, GuangDong, **China**	Burgess *et al*. 2006a
T. obscuris		*Eucalyptus* sp.	Irian Jaya, Indonesia	Burgess *et al*. 2007b
		E. pellita	Southeast Vietnam	Burgess *et al*. 2007b
'*Teratosphaeria*'[b] *suberosa*		*Eucalyptus* sp.	Indonesia	Crous *et al*. 1995, 1996, 2006c
T. suttonii	*K. epicoccoides*	*E. urophylla*	GuangDong, **China**	Burgess *et al*. 2007b
		Eucalyptus spp.	Hong Kong, **China**	Crous & Wingfield 1997
		Eucalyptus spp.	Taiwan, **China**	Crous & Wingfield 1997
		Eucalyptus spp.	India	Crous & Wingfield 1997; Old *et al*. 2003; Andjic *et al*. 2007a
		E. grandis, *Eucalyptus* spp.	Indonesia	Crous & Wingfield 1997; 2009; Old *et al*. 2003; Andjic *et al*. 2007a; Burgess *et al*. 2007b
		Eucalyptus spp.	Philippines	Crous & Wingfield 1997; Old *et al*. 2003
		E. pellita	Vietnam	Crous *et al*. 2009c
	Dissoconium dekkeri	*E. camaldulensis*	Thailand	Crous *et al*. 2009c
	Penidiella eucalypti	*E. camaldulensis*, *Eucalyptus* sp.	Thailand	Cheewangkoon *et al*. 2008
	Pseudocercospora basiramifera	*E. pellita*	Thailand	Crous *et al*. 2001; Hunter *et al*. 2006a, b
	P. chiangmaiensis	*E. camaldulensis*	Chiang Mai, Thailand	Cheewangkoon *et al*. 2008
	P. eucalyptorum	*Eucalyptus* sp.	Taiwan, **China**	Crous *et al*. 1989b
		Eucalyptus sp.	India	Crous *et al*. 1989b
		Eucalyptus sp.	Thailand	Crous *et al*. 1989b
		Eucalyptus sp.	Vietnam	Crous *et al*. 1989b
	P. flavomarginata	*E. camaldulensis*	Thailand	Hunter *et al*. 2006a
	P. gracilis	*E. urophylla*, *Eucalyptus* sp.	Indonesia	Crous *et al*. 1995, 1996, 2006c; Burgess *et al*. 2007b
	P. pseudoeucalyptorum	*Eucalyptus* sp.	YunNan, **China**	Crous *et al*. 2004a
	P. robusta	*E. robusta*	Malaysia	Crous *et al*. 2001, 2006c; Hunter *et al*. 2004b, 2006a, b
	P. schizolobii	*E. camaldulensis*	Thailand	Crous *et al*. 2009c
	Stenella xenoparkii	*Eucalyptus* sp.	Indonesia	Crous *et al*. 2006c; Burgess *et al*. 2007b

[a]'*Mycosphaerella*', the genus was identified as *Mycosphaerella* previously, but the genus name has been changed for many of these species Crous *et al*. (2009b, c).

[b]'*Teratosphaeria*', named in Crous *et al*. (2009b, c).

Table 4. Leaf diseases caused by *Calonectria* spp. reported from eucalypt forestry in Southeast Asia, India and China.

Species	Anamorph	Host	Location	References
Calonectria asiatica	*Cylindrocladium asiaticum*	Soil under *Eucalyptus* spp.	Indonesia	Crous *et al*. 2004b
Ca. brassicae	*Cy. brassicae*	*Eucalyptus* spp.	India	Crous 2002; Lombard *et al*. 2010b
		Soil under *Eucalyptus* spp.	Vietnam	Crous 2002; Lombard *et al*. 2010b
Ca. cerciana		*E. urophylla* × *E. grandis* cutting	GuangDong, **China**	Lombard *et al*. 2010b
Ca. colhounii	*Cy. colhounii*	*Eucalyptus* spp.	India	Crous 2002; Old *et al*. 2003
		Soil under *Eucalyptus* spp.	Indonesia	Old *et al*. 2003
		Soil under *Eucalyptus* spp.	Thailand	Old *et al*. 2003
Ca. curvata	*Cy. curvatum*	*Eucalyptus* spp.	India	Old *et al*. 2003
Ca. eucalypti		*Eucalyptus grandis*	Indonesia	Lombard *et al*. 2010a
Ca. hurae	*Cy. hurae*	*Eucalyptus* spp.	Thailand	Crous 2002; Crous *et al*. 2006a
Ca. ilicicola	*Cy. parasiticum*	*Eucalyptus* spp.	India	Old *et al*. 2003
		Eucalyptus spp.	Malaysia	Old *et al*. 2003
Ca. indusiata	*Cy. theae*	*Eucalyptus* spp.	India	Crous 2002; Crous *et al*. 2006a
		Eucalyptus spp.	Indonesia	Old *et al*. 2003
Ca. kyotensis	*Cy. floridanum*	*Eucalyptus* spp.	India	Crous 2002; Crous *et al*. 2004b
		E. camaldulensis, *E. grandis*, *E. urophylla*	TaiWan, **China**	Wang 1992
Ca. multiseptata	*Cy. multiseptatum*	*E. grandis*	Indonesia	Crous 2002; Crous *et al*. 2006a
Ca. pauciramosa	*Cy. pauciramosum*	*E. urophylla* × *E. grandis* cutting	GuangDong, **China**	Lombard *et al*. 2010b
Ca. pseudoreteaudii		*E. urophylla* × *E. grandis* cutting	GuangDong, **China**	Lombard *et al*. 2010b
Ca. pteridis	*Cy. pteridis*	*Eucalyptus* spp.	India	Crous 2002; Crous *et al*. 2006a
Ca. reteaudii	*Cy. reteaudii*	*Eucalyptus* spp.	India	Old *et al*. 2003
		E. urophylla, *Eucalyptus* spp.	Indonesis	Crous 2002; Lombard *et al*. 2010b
		Eucalyptus spp.	Laos	Old *et al*. 2003
		Eucalyptus spp.	Malaysia	Old *et al*. 2003
		E. grandis, *Eucalyptus* spp.	Thailand	Crous 2002; Crous *et al*. 2006a; Lombard *et al*. 2010b
		E. camaldulensis, *Eucalyptus* spp.	Vietnam	Crous 2002; Crous *et al*. 2004b, 2006a; Lombard *et al*. 2010b
Ca. sulawesiensis		*Eucalyptus* sp.	Sulawesi, Indonesia	Lombard *et al*. 2010a
Ca. sumatrensis	*Cy. sumatrense*	*Eucalyptus* spp. (soil)	Indonesia	Crous *et al*. 2004b

Table 5. Leaf diseases caused by species of *Aulographina*, *Coniella*, *Meliola*, *Pilidiella* and *Quambalaria* reported from eucalypt forestry in Southeast Asia, India and China.

Pathogen	**Host**	**Location**	**References**
Aulographina eucalypti	*Eucalyptus* sp.	Vietnam	Old *et al*. 2003
Coniella australiensis	*Eucalyptus* sp.	India	Old *et al*. 2003
	Eucalyptus sp.	Indonesia	Old *et al*. 2003
	Eucalyptus sp.	Vietnam	Old *et al*. 2003
C. fragariae	*Eucalyptus* sp.	India	Old *et al*. 2003; Van Niekerk *et al*. 2004
	Eucalyptus sp.	Indonesia	Old *et al*. 2003
	Eucalyptus sp.	Vietnam	Old *et al*. 2003
Cryptosporiopsis eucalypti	*Eucalyptus* sp.	India	Sankaran *et al*. 1995a; Old *et al*. 2003
	Eucalyptus sp.	Indonesia	Old & Yuan 1994; Old *et al*. 2002, 2003
	Eucalyptus sp.	Laos	Old & Yuan 1994; Old *et al*. 2002, 2003
	Eucalyptus sp.	Vietnam	Old & Yuan 1994; Old *et al*. 2002, 2003
Meliola eucalypti	*Eucalyptus* sp.	Philippines	Hansford 1956, 1962; Old *et al*. 2003
Pilidiella sp.	*Eucalyptus* sp.	**China**	Zhou *et al*. 2008
Pilidiella eucalyptorum	*Eucalyptus* sp.	Indonesia	Van Niekerk *et al*. 2004
	Eucalyptus sp.	Vietnam	Van Niekerk *et al*. 2004
Quambalaria pitereka	*Corymbia citriodora*	GuangDong, **China**	Zhou *et al*. 2007b
Q. pusilla	*E. camaldulensis*	Thailand	Braun 1998

Table 6. Wilt diseases caused by *Ceratocystis* sp. and *Ralstonia solanacearum* reported from eucalypt forestry in Southeast Asia, India and China.

Pathogen	**Host**	**Location**	**References**
Ceratocystis eucalypicola prov. nom.	*Eucalyptus* sp.	Thailand	Van Wyk *et al*. 2010
	Eucalyptus sp.	Indonesia	Van Wyk *et al*. 2010
Ralstonia solanacearum	*Eucalyptus* spp.	Indonesia	Machmud 1985; Old *et al*. 2003
	E. saligna, *E. grandis*	GuangXi, **China**	Cao 1982; Old *et al*. 2003; Ran *et al*. 2005
	E. saligna, *E. urophylla*, *E. grandis* and their hybrids and clones	GuangDong and HaiNan, **China**	Wu & Liang 1988a; Lai 1990; Li 1992; He 1997; Old *et al*. 2003
	E. camaldulensis	TaiWan, **China**	Wang *et al*. 1992
	Eucalyptus spp.	Thailand	Pongpanich 2000; Old *et al*. 2003
	Eucalyptus spp.	Vietnam	Thu *et al*. 2000; Old *et al*. 2003

Table 7. Stem/branch diseases reported from eucalypt forestry in Southeast Asia, India and China.

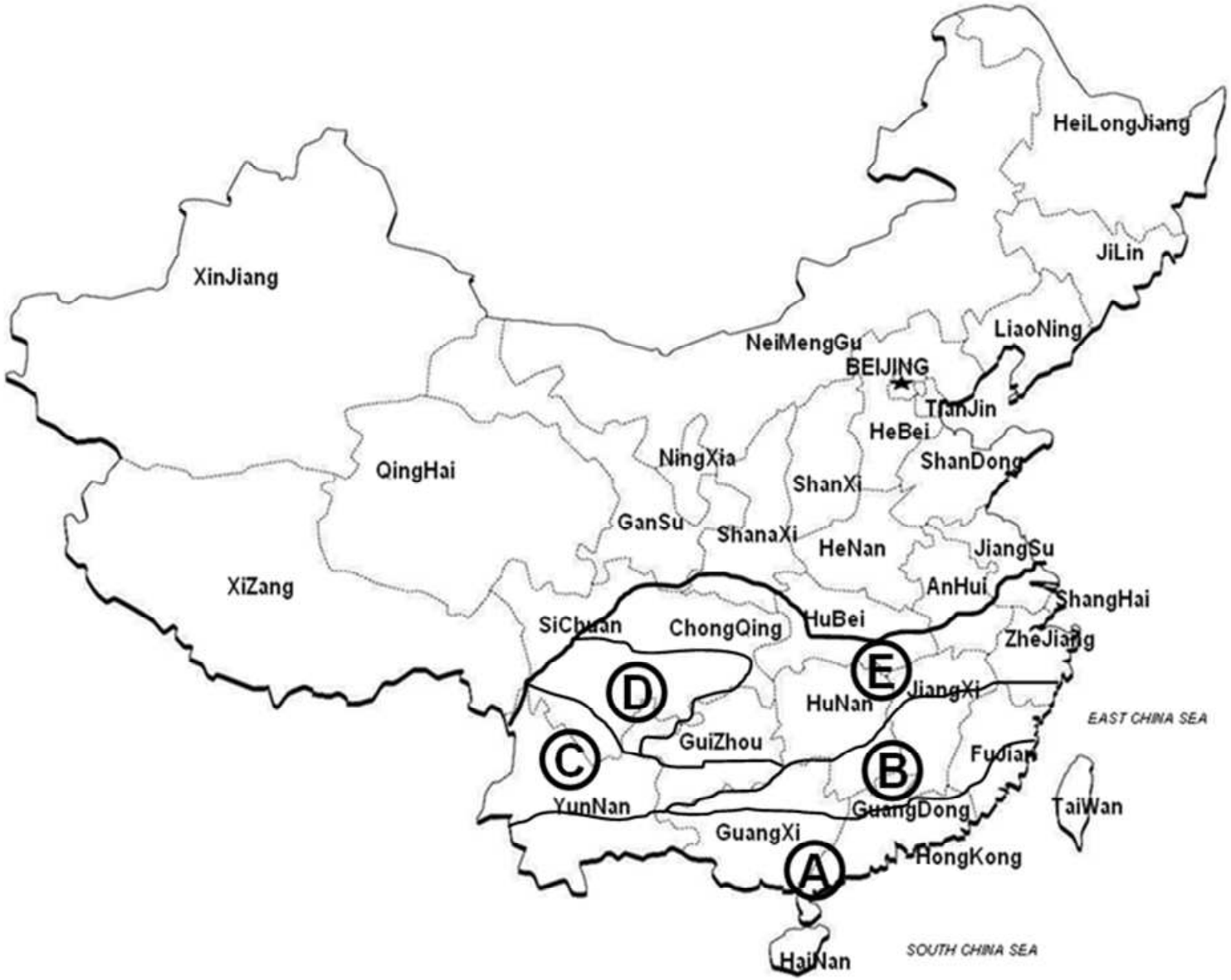
Pathogen	**Host**	**Location**	**References**
Chrysoporthe deuterocubensis	*Eucalyptus* sp.	Hong Kong, **China**	Sharma *et al*. 1985; Hodges *et al*. 1986; Myburg *et al*. 1999, 2002
	E. deglupta	Thailand	Myburg *et al*. 1999
	E. aromatica, *Eucalyptus* sp.	Malaysia	Old *et al*. 2003; Gryzenhout *et al*. 2009
	Eucalyptus sp.	Indonesia	Old *et al*. 2003
	Eucalyptus sp.	Singapore	Gryzenhout *et al*. 2009
	Eucalyptus sp.	Vietnam	Old *et al*. 2003
	Eucalyptus sp.	India	Sharma *et al*. 1985; Hodges *et al*. 1986; Gryzenhout *et al*. 2009
'*Cryphonectria gyrosa*' canker	*E. torrelliana*, *Eucalyptus* sp.	Thailand	Old *et al*. 2003
	E. torrelliana, *Eucalyptus* sp.	Indonesia	Old *et al*. 2003
	E. torrelliana, *E. tereticornis*	India	Sharma *et al*. 1985; Hodges *et al*. 1986; Gryzenhout *et al*. 2009
	E. grandis, *E. deglupta*, *E. alba*	India	Sharma *et al*. 1985; Hodges *et al*. 1986; Gryzenhout *et al*. 2009
Cytospora agarwalii	*Eucalyptus* sp.	Jabalpur, India	Adams *et al*. 2005
C. eucalypticola	*Eucalyptus* sp.	Indonesia	Old *et al*. 2003
	Eucalyptus sp.	Thailand	Old *et al*. 2003
	Eucalyptus sp.	Vietnam	Old *et al*. 2003
Cytospora-like species	*E. urophylla*	Indonesia	Adams *et al*. 2005, 2006
C. putative	*E. camaldulensis*	Thailand	Adams *et al*. 2006
C. valsoidea	*E. grandis*	Sumatra, Indonesia	Adams *et al*. 2005, 2006
Valsa eugeniae	*Eucalyptus* sp.	Suluwesi, Indonesia	Adams *et al*. 2005
V. myrtagena	*E. grandis*	Sibisa, North Sumatra, Indonesia	Adams *et al*. 2005
Erythricium salmonicolor	*Eucalyptus* sp.	India	Seth *et al*. 1978; Old *et al*. 2003
	Eucalyptus sp.	Indonesia	Old *et al*. 2003
	Eucalyptus sp.	Philippines	Old *et al*. 2003
	E. camaldulensis	Vietnam	Old *et al*. 2003
Lasiodiplodia theobromae	*E. camaldulensis*	Thailand	Old *et al*. 2003
	E. grandis, *E. tereticornis*	India	Sharma *et al*. 1984b
Neofusicoccum parvum	*Eucalyptus* sp.	**China**	Slippers *et al*. 2009
N. ribis	*Eucalyptus* sp.	**China**	Slippers *et al*. 2009

N. mangiferum	*Eucalyptus* sp.	**China**	Slippers *et al*. 2009
Teratosphaeria zuluensis	*E. camaldulensis*, *Eucalyptus* sp.	Thailand	Van Zyl *et al*. 2002
	E. urophylla	Vietnam	Gezahgne *et al*. 2003a; Old *et al*. 2003
	E. urophylla	GuangDong, **China**	Cortinas *et al*. 2006b
	E. urophylla × *E. grandis*	GuangXi, **China**	Zhou *et al*. 2008

Table 8. Root diseases reported from eucalypt forestry in Southeast Asia, India and China.

Pathogen	**Host**	**Location**	**References**
Armillaria sp.	*Eucalyptus grandis*	North Sumatra, Indonesia	Coetzee *et al*. 2003a; Old *et al*. 2003
Ganoderma sp.	*Eucalyptus* sp.	India	Bakshi 1974, 1976; Old *et al*. 2003
Ganoderma sp.	*E. pellita*	Sumatra, Indonesia	Old *et al*. 2003

Fig 1. Distribution of eucalypts in different geographic and climatic area in China.



Chapter 2

Identification and pathogenicity of *Chrysoporthe cubensis* on *Eucalyptus* and *Syzygium* spp. in South China

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This chapter has been published as: Chen SF, Gryzenhout M, Roux J, Xie YJ, Wingfield MJ, Zhou XD, 2010. Identification and pathogenicity of *Chrysoporthe cubensis* on *Eucalyptus* and *Syzygium* species in South China. *Plant Disease* **94**: 1143–1150.

ABSTRACT

Chrysoporthe includes important pathogens of plantation-grown *Eucalyptus* spp. and has been reported from several tree genera in the order Myrtales in tropical and subtropical areas of the world. During disease surveys in South China, fruiting structures typical of *Chrysoporthe* spp. were observed on cankers on *Eucalyptus* and *Syzygium cumini* trees. The aim of this study was to confirm the identify the *Chrysoporthe cubensis* infecting the *Eucalyptus* spp. and *S. cumini* and to test the pathogenicity of the fungus. Following glasshouse trials to select virulent isolates, field inoculations were undertaken to screen different commercial *Eucalyptus* genotypes for their susceptibility to the fungus. Isolates were characterized based on their morphology and DNA sequence data for the β-tubulin and internal transcribed spacer regions of the ribosomal DNA. Results showed that the putative pathogen represented the Asian form of *Chr. cubensis,* which occurred on numerous different *Eucalyptus* spp. and hybrid clones as well as *S. cumini*. Field inoculations showed that all six of the *Eucalyptus* genotypes tested are susceptible to infection by *Chr. cubensis*. Significant differences were observed between them, providing prospects to select disease-tolerant planting stock in the future.

keywords: Cryphonectriaceae, fungal pathogens, Myrtales, plantation forestry, tree diseases

1. INTRODUCTION

Stem canker diseases caused by *Chrysoporthe* spp. are considered amongst the most important diseases of plantation-grown *Eucalyptus* spp. in the tropics and sub-tropics (Wingfield 2003; Gryzenhout *et al.* 2009). Infection of susceptible young trees can lead to rapid tree death while cankers weaken the stems of older trees, often resulting in stem breakage (Wingfield *et al.* 1989; Conradie *et al.* 1990; Old *et al.* 2003; Wingfield 2003). The stem canker diseases caused by *Chrysoporthe* spp. have had a substantial impact on the development of *Eucalyptus* forestry in the tropics and Southern Hemisphere (Alfenas *et al.* 1983; Campinhos & Ikemori 1993; Old *et al.* 2003; Wingfield 2003; Van Heerden *et al.* 2005); vegetative propagation of these trees emerged from efforts to avoid the disease using resistant hybrid clones (Wingfield 2003).

It is well established that Chrysoporthe canker, previously known as Cryphonectria canker, of *Eucalyptus* spp. that once was thought to be caused by a single fungus, *Cryphonectria cubensis* (Bruner) Gryzenh. & MJ. Wingf., is caused by a number of different species of *Chrysoporthe* in different parts of the world (Gryzenhout *et al.* 2004, 2009). These include *Chrysoporthe cubensis*, with a wide distribution and representing two distinct phylogenetic lineages, one in South and Central America and a second in Southeast Asia (Gryzenhout *et al.* 2009). Other species of *Chrysoporthe* on *Eucalyptus* are *Chr. austroafricana* Gryzenh. & M.J. Wingf. in Southern Africa (Gryzenhout *et al.* 2004), *Chr. doradensis* Gryzenh. & M.J. Wingf. in Ecuador (Gryzenhout *et al.* 2005), and *Chr. zambiensis* Chungu, Gryzenh. & Jol. Roux in Zambia (Chungu *et al.* 2010).

Apart from *Eucalyptus* spp., *Chrysoporthe* spp. have been shown to be especially prevalent on other tree species in the order Myrtales (Gryzenhout *et al.* 2009). These fungi have been found on *Syzygium* spp. (Myrtaceae) in Africa (Nutman & Roberts 1952; Heath *et al.* 2006; Nakabonge *et al.* 2006), Brazil (Hodges *et al.* 1986), Indonesia (Hodges *et al.* 1986; Myburg *et al.* 2003), and Malaysia (Reid & Booth 1969); on *Tibouchina* spp. (Melastomataceae) in South Africa (Myburg *et al.* 2002a), South America (Wingfield *et al.* 2001; Seixas *et al.* 2004; Gryzenhout *et al.* 2006) and Southeast Asia (Gryzenhout *et al.* 2006); and on *Lagerstroemia indica* (Lythraceae) in Cuba (Gryzenhout *et al.* 2006). *Chr. cubensis* is also known from other trees in the family Melastomataceae, including *Miconia* spp., *Rhynchanthera mexicana* and

Clidemia sericea in Central and South America (Rodas *et al.* 2005; Gryzenhout *et al.* 2006) and *Melastoma melabathricum* in Indonesia (Gryzenhout *et al.* 2006).

Chrysoporthe cubensis has been reported from different hosts in several regions of Southeast Asia. These reports include those from *Eucalyptus* spp. in India (Sharma *et al.* 1985), Indonesia (Old *et al.* 2003), Malaysia (Old *et al.* 2003), Thailand (Myburg *et al.* 1999), Vietnam (Old *et al.* 2003), and Hong Kong, China (Sharma *et al.* 1985; Hodges *et al.* 1986; Myburg *et al.* 1999). The fungus has also been reported from native *Syzygium aromaticum* in Sulawesi, Indonesia (Hodges *et al.* 1986; Myburg *et al.* 2003); from native *M. melabathricum* (Melastomataceae) in Sumatra, Indonesia (Gryzenhout *et al.* 2006); and on non-native *Tibouchina urvilleana* (Melastomataceae) in Singapore and Thailand (Gryzenhout *et al.* 2006).

During the course of the past two decades, China has invested significantly in the establishment of plantations of *Eucalyptus* spp. as a source of pulp for the manufacture of paper, particle board, and plywood, as well as timber for construction. Approximately two million hectares of *Eucalyptus* plantations have already been established in South China (Xie 2006). Similar to the situation in other countries, these trees have rapidly been affected by diseases (Zhou *et al.* 2008). Recent surveys of *Eucalyptus* plantations in South China identified several pathogens affecting these trees, including *Chr. cubensis* associated with a stem canker disease (Zhou *et al.* 2008). Early work in the country was conducted based only on fungal morphology, and there was no extensive survey of the species diversity, host range of the fungus, or a consideration of its pathogenicity. The aim of this study was to survey *Eucalyptus* and related species for the presence of *Chrysoporthe* spp. and to identify these fungi based on DNA sequence data and morphological characters. In addition, the relative susceptibility of commercially planted *Eucalyptus* genotypes to Chrysoporthe canker was evaluated in field inoculation trials.

2. MATERIALS AND METHODS

2.1. Sampling

Eucalyptus plantations in three provinces in South China (Fig 1), where most plantations of these trees have been established, were surveyed for the presence of stem cankers in November and December 2006 and June 2007 as well as between September and November

2008. Where present in these areas, *S. cumini* trees that are related to *Eucalyptus* spp. in the family *Myrtaceae* were also examined for the presence of stem cankers. Sections of bark from the surface of stem cankers bearing fruiting structures resembling *Chrysoporthe* spp., including ascostromata and conidiomata, were collected and transported to the laboratory for isolations. Samples were incubated in moist chambers for 1 to 3 days to induce the production of spores from the fruiting bodies. Single spore tendrils were transferred to 2% malt extract agar (MEA; 20 g of Biolab malt extract, 20 g of Biolab agar, and 1 liter water; Biolab, Merck, Midrand, South Africa) and incubated at 25°C. From the resultant cultures, single hyphal tips were transferred to fresh 2% MEA to obtain pure cultures. All cultures are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa, and the China Eucalypt Research Centre (CERC), Chinese Academy of Forestry (CAF), China.

2.2. Morphology

To study the morphology of the fungi collected, fruiting structures were taken from bark specimens and sectioned using a Leica CM1100 cryostat (Setpoint Technologies) at –20°C following the method described by Gryzenhout *et al.* (2006). Samples from China were compared with those of *Chrysoporthe* spp. (Gryzenhout *et al.* 2004). For measurements, structures from the bark on five trees of *Eucalyptus* clones W5 and U6, respectively, and 10 from *S. cumini* trees from GuangDong Province (Table 1, Fig 1), were selected. Measurements were made from 25 conidiophores, basal cells of conidiophores, conidia, asci, and ascospores. The results are presented as minimum – (mean – standard deviation) – (mean + standard deviation) – maximum. To obtain an indication of the minimum and maximum size ranges of the stromata, measurements were obtained from structures representing the smallest and largest for the anamorph and teleomorph stomata on both *Eucalyptus* and *S. cumini* trees.

2.3. DNA sequence comparisons

Representative isolates collected from different *Eucalyptus* species or clones and *S. cumini* in different geographic regions of South China (Table 1, Fig 1) were selected and used for DNA sequence comparisons. Prior to DNA extraction, isolates were grown in 2% MEA at 25°C for 5 to 7 days. For each isolate, actively growing mycelium from one MEA plate per isolate was

scraped from the surface of the media using a sterile scalpel and transferred to 1.5-µl Eppendorf tubes. DNA was extracted from the mycelium following the method used by Myburg *et al.* (1999). DNA was separated by electrophoresis on a 1% agarose gel, stained with ethidium bromide, and visualized under UV light. Samples were treated with 3 µl of RNase (1 mg/ml) and left overnight at 37°C to degrade RNA.

The internal transcribed spacer (ITS) regions, including the 5.8S ribosomal DNA operon and two regions within the β-tubulin gene region, were amplified using the primer pairs ITS1 and ITS4 (White *et al.* 1990) and βt1a/βt1b and βt2a/βt2b, respectively (Glass & Donaldson 1995). Polymerase chain reaction (PCR) conditions were as outlined by Myburg *et al.* (2002b). PCR products were visualized with UV light on 1% agarose (ethidium bromide-stained) gels. The amplified products were purified using 6% Sephadex G-50 columns (Steinheim, Germany) as described by the manufacturers.

Each PCR product was sequenced in both directions with the same primers that were used for PCR reactions. The ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Applied Biosystems, Foster City, CA) was used to perform the sequencing reactions. The sequence products were purified using 6% Sephadex G-50 columns, whereafter electropherograms were generated on an ABI PRISM 3100 autosequencer (Perkin-Elmer Applied Biosystems). Nucleotide sequences were analyzed using MEGA4 (Tamura *et al.* 2007). All sequences obtained in this study have been deposited in GenBank (Table 1).

Sequences were aligned with the online version of MAFFT v. 5.667 (Katoh *et al.* 2002), using the iterative refinement method (FFT-NS-I settings), and adjusted manually. The sequence data were analyzed using phylogenetic analyses using phylogeny (PAUP; version 4.0b10) (Swofford 2002). The combinability of the ITS and β-tubulin 1 and 2 gene sequence data sets was determined with a partition homogeneity test (PHT) in PAUP (Farris *et al.* 1995; Swofford 2002). Most parsimonious trees were obtained with heuristic searches using stepwise addition and tree bisection and reconstruction (TBR) as the branch-swapping algorithms. All equally parsimonious trees were saved and all branches equal to zero were collapsed. Gaps were treated as the fifth character. A bootstrap analysis (50% majority rule, 1 000 replications) was done to determine the confidence levels of the tree-branching points (Felsenstein 1985). Previously published sequences for *Chr. austroafricana, Chr. cubensis, Chr. doradensis, Chr. hodgesiana* (Gryzenh. & M.J. Wingf.) Gryzenh. & M.J. Wingf. (3,9–

11), and *Chr. inopina* Gryzenh. & M.J. Wingf. were used for comparative purposes. *Cryphonectria parasitica* (Murrill) M.E. Barr (*Cryphonectriaceae*) was used as the outgroup taxon to root the phylogenetic trees (Gryzenhout *et al.* 2009).

2.4. Pathogenicity tests

2.4.1. Glasshouse trials

Six isolates (CMW26888, CMW26891, CMW26892, CMW26895, CMW26929, and CMW26932) from different locations and *Eucalyptus* genotypes in China (Table 1) were selected for inoculations. These isolates were inoculated into trees of a susceptible *Eucalyptus grandis* clone under glasshouse conditions. This was done to select the most virulent isolates for field inoculations. The trees were approximately 2 m tall and had diameters of approximately 10 mm. Before inoculation, the trees were allowed to acclimatize to the glasshouse conditions of 25°C and 14 h of daylight with 10 h of darkness. Fungal isolates were grown at 25°C under continuous fluorescent light for 6 days prior to inoculation.

In order to expose the cambium, wounds were made in the bark at a constant height (about 300 mm above the seedling medium) using a cork borer (7 mm in diameter). Discs of the same size from the actively growing colonies were inserted into the wounds with the mycelium facing the xylem. To prevent desiccation and contamination, wounds were covered with Parafilm (Pechiney Plastic Packing, Chicago). The six isolates were inoculated into the stems of 10 trees each. Ten trees were also inoculated with sterile MEA plugs to serve as controls. The 70 inoculated trees were arranged randomly in the glasshouse.

Trees were inoculated in April 2008 and results evaluated after 6 weeks by measuring the lengths of lesions in the cambium. Reisolations were made from the resultant lesions by plating small pieces of discoloured xylem onto 2% MEA at 25°C. Reisolations were made from four randomly selected trees per isolate and from all trees inoculated as controls. Results were analyzed in SAS (version 8) using the PROC general linear model (GLM) (SAS Institute Inc 1999). Analysis of variance (ANOVA) was used to determine the effects of fungal strain on lesion length. Prior to ANOVA, homogeneity of variance across treatments was verified. For significance tests among means, Fisher's protected test was used. F values with $P < 0.05$ considered significant.

2.4.2. Field trials

To evaluate the relative susceptibility of commercially planted *Eucalyptus* spp. and clones grown in South China, field inoculations were done using isolates (CMW26892 and CMW26929) identified in the glasshouse inoculations as most virulent. Three isolates (CMW24909, CMW24919, and CMW24921) originating from *S. cumini* (Table 1) were also used in the study. The field trial was situated in the ZhanJiang area of GuangDong Province and consisted of seven *Eucalyptus* genotypes, including pure species and hybrids. These included an *E. grandis* clone (CEPT-1), an *E. grandis* × *E. tereticornis* clone (CEPT-2), an *E. pellita* genotype (CEPT-3), two *E. urophylla* × *E. grandis* clones (CEPT-4 and CEPT-7), an *E. urophylla* clone (CEPT-5), and an *E. wetarensis* clone (CEPT-6). The isolates originating from *S. cumini* were inoculated onto two of the *Eucalyptus* genotypes (CEPT-6 and CEPT-7) due to limited availability of trees for inoculation. At the time of inoculation, trees were 1 year old and 6 to 10 trees of each genotype were inoculated per isolate. An equal number of trees were inoculated with sterile MEA discs to serve as negative controls. Wounds were made in the bark at a constant height (about 400 to 800 mm above the ground) using a cork borer (9 mm in diameter). The inoculation trail was conducted in October 2008.

To evaluate the susceptibility of *S. cumini* to the *Chrysoporthe cubensis* isolates collected from South China, the same isolates as those used in field inoculations of *Eucalyptus* genotypes were inoculated onto the branches of *S. cumini* trees in ZhanJiang. For the five selected isolates, 10 branches from each of five *S. cumini* trees were inoculated per isolate. An equal number of branches were inoculated with sterile MEA discs to serve as negative controls. Inoculations were conducted in a similar way to those for the field and glasshouse inoculations, using a 9-mmdiameter cork borer. Branches were inoculated in September 2008, and the inoculation trial was repeated once, 7 days after the first inoculations on five different *S. cumini* trees.

Lesion lengths were recorded after 5 weeks for the *Eucalyptus* trees and 6 weeks for the *S. cumini* trees. To evaluate the pathogenicity of the isolates of *Chr. cubensis* to *Eucalyptus* genotypes and *S. cumini*, the lengths of the lesions in the cambium below the bark were recorded. Results from the experiments were analyzed separately in SAS (version 8) using the

PROC GLM (SAS Institute Inc 1999) and in a similar manner as for the greenhouse inoculation tests.

3. RESULTS

3.1. Sampling

Symptoms typical of infection by *Chr. cubensis*. were commonly observed on *Eucalyptus* and *S. cumini* trees in South China. These included dying branches, cracked bark, and cankers girdling the stems (Figs 2A, 2B). Fruiting structures typical of *Chr. cubensis*, including ascostromata and conidiomata, were found on the surface of the cankers on *Eucalyptus* trees, whereas only ascostromata were observed on *S. cumini*. Isolates were obtained from a total of 25 *Eucalyptus* trees and 19 *S. cumini* trees. Of these, isolates were obtained from three *E. camaldulensis* and three *E. urophylla* × *E. grandis* trees in HaiNan Province, four *E. urophylla* × *E. grandis* trees in GuangXi Province, one each of three trees representing *Eucalyptus* clones W5, U6, and EC48, and one tree of an unknown *Eucalyptus* sp. in GuangDong Province (Table 1). Isolates were also collected from cankers on five *Eucalyptus* trees, including *E. grandis*, in GuangDong Province. All isolates of *Chr. cubensis* from *S. cumini* were collected from GuangDong Province (Table 1, Fig 1).

3.2 Morphology

The ascostromata on the bark taken from cankers on *Eucalyptus* trees were semi-immersed, 120 to 200 μm (average: 150 μm) high above the level of the bark, and 250 to 410 μm (average: 350 μm) wide above the surface of the bark (Fig 3A). The perithecial stromata were valsoid with perithecia partially embedded in the bark tissue. Perithecia were fuscous black in color and the tops of the perithecial bases were covered with cinnamon to orange fungal tissue, which was occasionally visible above the bark surface. The necks of the perithecia emerging from the stromata were covered with fuscous black tissue and were cylindrical, up to 520 μm (average: 410 μm) in length and 100 to 150 μm (average: 120 μm) wide (Fig 3A). Asci were 19.5–22 to 26–27 × 4.5–5 to 6–6.5 μm, fusoid to ellipsoidal, containing eight ascospores. Ascospores were 5–5.5 to 7–8 × 2–3 μm, hyaline, 1-septate, fusoid to oval, with the ends tapering (Fig 3F).

Stromatic conidiomata formed on the surfaces of the ascostromata or as separate structures, and were superficial to slightly immersed, pyriform to clavate (Fig 3C). Stromatic conidiomatal bases were 130 to 430 µm (average: 170 µm) high above the level of bark and 190 to 750 µm (average: 320 µm) wide. Stromatic conidiomatal necks were up to 320 µm long and 80 to 160 µm wide (Fig 3C). Stromatic conidiomatal locules with even to convoluted inner surface were occasionally multilocular, with single locules connected to one or several necks. Conidiophores were hyaline with globose to rectangular basal cells that were rounded off, 2.5–3 to 6.5–9 × 2–2.5 to 4.5–6 µm, and branched irregularly at the base or above into cylindrical cells that were delimited by septa or not; conidiophores were 13–14.5 to 21.5–26.5 µm in length and conidiogenous cells were cylindrical to flask-shaped with attenuated apices, 1.5–2 to 3–3.5 µm wide (Fig 3G). Conidia were 3–3.5 to 4.5–5 × 1.5–2 to 2.5–3 µm, hyaline, oblong, aseptate, and exuded as bright luteous tendrils or droplets (Fig 3H).

The morphology of ascostromata on *S. cumini* trees was similar to that on the *Eucalyptus* trees (Fig 3B), and conidiomata were not observed. The emerging necks of the perithecia from stromata were up to 900 µm (average: 610 µm) long, 80 to 140 µm (average: 110 µm) wide, and covered with fuscous black tissue (Fig 3B). Asci were 19.5–21.5 to 25.5–27.5 × 4.5–5 to 6–6.5 µm, fusoid to ellipsoidal in shape, and eight spored (Fig 3D). Ascospores were 5–5.5 to 6.5–7 × 2 to 3 µm, hyaline, 1-septate, fusoid to oval, with tapered ends and septa that could be in various positions in the spore but usually central (Fig 3E).

Specimens examined were China, GuangDong Province, bark of *Eucalyptus* clone, December 2006, M. J. Wingfield and X. D. Zhou, PREM60451, living culture CMW26891; and GuangDong Province, bark of *S. cumini*, December 2006, M. J. Wingfield and X. D. Zhou, PREM60452, living culture CMW24921.

3.3. DNA sequence comparisons

After alignment of sequence data, a partition homogeneity test on the three separate data sets gave a PHT value of $P = 0.014$, showing that the data sets (ITS and βt) were significantly incongruent. However, based on the results of previous studies (Gryzenhout *et al.* 2004, 2006), they were combined in the phylogenetic analysis. The combined ITS and β-tubulin data set had 1 327 characters of equal weight, with 1 019 constant characters, of which 31 were parsimony uninformative and 277 were parsimony informative. In all, 36 most parsimonious

trees were retained, and one of them (50% majority rule), with tree length = 312 steps, CI = 0.939, RI = 0.956, RC = 0.897, and HI = 0.061, was chosen to obtain a representative tree of the data (Fig 4).

Results of the DNA sequence analyses showed that the isolates collected from *Eucalyptus* spp. in South China represented *Chr. cubensis* (Fig 4). Isolates from the *S. cumini* trees were similar to those from *Eucalyptus* spp. Isolates from China grouped within the Asian clade of *Chr. cubensis* (Myburg *et al.* 2002b; Gryzenhout *et al.* 2006) together with isolates originating from Australia, Hawaii, Indonesia, and Tanzania, with 97% bootstrap support, and separate from the South American clade of *Chr. cubensis*, with a 90% bootstrap (Fig 4).

Differences were observed between isolates from China. Some of these isolates (CMW12746, CMW12748, CMW12749, CMW26891, and CMW26892) differed from the other isolates with two fixed nucleotide differences in the ITS gene region whereas, for the β-tubulin gene region, no differences were found between these isolates. The bootstrap values within the *Chr. cubensis* clade representing sequence differences are not significant (Fig 4), suggesting that these reflect intraspecific sequence differences rather than interspecies variation.

3.4. Pathogenicity tests

3.4.1 Glasshouse trials

All *Chr. cubensis* isolates from China tested for pathogenicity on the *Eucalyptus* clone produced lesions within 6 weeks, while small lesions were produced for the control inoculations (Fig 5). ANOVAs showed significant differences in susceptibility to the fungal isolates on the inoculated *Eucalyptus* clone ($P < 0.001$). The mean comparison tests showed that the lesion lengths produced by the *Chr. cubensis* isolates were significantly longer ($P < 0.0001$ to $P = 0.0036$) than those of the controls (Fig 5). Of the isolates tested, lesions produced by isolate CMW26929 were significantly longer ($P < 0.0001$ to $P = 0.0004$) than those of the other isolates (Fig 5). All the inoculated fungi were successfully reisolated from the lesions, whereas no *Chr. cubensis* was isolated from the controls. Based on the glasshouse trial, isolates CMW26929 and CMW26892 were most virulent and, therefore, used for field inoculations.

3.4.2. Field trials in China

The two *Chr. cubensis* isolates (CMW26892 and CMW26929) inoculated on seven *Eucalyptus* genotypes gave rise to distinct lesions. ANOVAs showed significant differences in susceptibility to the fungal isolates between the various *Eucalyptus* genotypes ($P < 0.001$). Statistical analyses of the data showed that not all the isolates of *Chr. cubensis* reacted in the same manner to the tested *Eucalyptus* genotypes. For example, lesions produced by CMW26929 on *Eucalyptus* genotype CEPT-2 were significantly longer ($P = 0.0437$) than those of CMW26892 (Fig 6) whereas, for the other *Eucalyptus* genotypes, the lesions produced by the two *Chr. cubensis* isolates were not significantly different ($P = 0.2477$ to $P = 0.6777$) (Fig 6). The mean comparison tests further showed that the lesions produced by the two *Chr. cubensis* isolates (CMW26892 and CMW26929) from *Eucalyptus* were significantly longer ($P < 0.0001$ to $P = 0.0477$) than those of the controls, except for isolate CMW26892 ($P = 0.053$) on *Eucalyptus* CEPT-5 (Fig 6). The results further showed that *Eucalyptus* genotype CEPT-2 is the most susceptible while genotype CEPT-5 is the most tolerant to infection by *Chr. cubensis* (Fig 6).

Under field conditions, five *Chr. cubensis* isolates (CMW26892, CMW26929, CMW24909, CMW24919, and CMW24921) inoculated on two *Eucalyptus* genotypes (CEPT-6 and CEPT-7) also resulted in lesions. ANOVAs showed significant differences in susceptibility to the fungal isolates between the two *Eucalyptus* genotypes ($P < 0.001$). The three *Chr. cubensis* isolates (CMW24909, CMW24919, and CMW24921) originating from *S. cumini* trees produced lesions on both *Eucalyptus* genotypes (CEPT-6 and CEPT-7). The mean comparison tests showed that lesions produced by two of the isolates (CMW24909 and CMW24919) were significantly longer ($P < 0.0001$ to $P = 0.0085$) than those produced by the controls (Fig 7). However, lesions produced by isolates (CMW26892 and CMW26929) originating from *Eucalyptus* trees were significantly longer ($P < 0.0001$) than those produced by the two isolates (CMW24919 and CMW24921) from *S. cumini* trees (Fig 7).

Where *S. cumini* branches were inoculated with *Chr. cubensis* isolates from *Eucalyptus* spp. (CMW26892 and CMW26929) and *S. cumini* (CMW24909, CMW24919, and CMW24921), distinct lesions developed after 6 weeks. For the first inoculation experiment, ANOVAs showed significant differences in susceptibility to the fungal isolates on the inoculated *S. cumini* branches ($P = 0.0516$). Statistical analyses of the data showed that not all the isolates

of *Chr. cubensis* reacted in the same manner to all the tested *S. cumini* branches. For example, lesions produced by CMW26929 were significantly longer than those of CMW26892 (P = 0.0269) and CMW24909 (P = 0.0058) (Fig 8). In the second test, ANOVAs showed no significant differences in susceptibility to the fungal isolates on the inoculated *S. cumini* branches (P = 0.9730). For each experiment, all five isolates produced significantly longer lesions (P < 0.0001) than those of the controls (Fig 8).

4. DISCUSSION

Results of the present study confirmed the presence of *C. cubensis* on *Eucalyptus* spp. in mainland China. The fungus had previously been reported only from Hong Kong, China (Sharma *et al.* 1985; Hodges *et al.* 1986; Myburg *et al.* 1999; Gryzenhout *et al.* 2009). This is especially important because recent studies (Gryzenhout *et al.* 2009) have shown that there are various closely related species of *Chrysoporthe* that can be confused with each other and that multigene DNA sequence analysis is needed to obtain unequivocal identifications. As part of this study, the new host *S. cumini* is recorded for *Chr. cubensis*. Inoculation trials confirmed the pathogenicity of *Chr. cubensis* on the *Eucalyptus* genotypes used for plantation forestry in China as well as on *S. cumini*. clones of *Eucalyptus* were shown to differ in their susceptibility to this pathogen.

Comparisons of sequence data for the ITS and β-tubulin gene regions provided definitive evidence that isolates of *Chrysoporthe* collected from China were those of *Chr. cubensis*. Previous research has shown that, based on ITS, β-tubulin, and Histone H3 gene sequence data, *Chr. cubensis* isolates reside in two very distinct phylogenetic clades. These clades represent isolates from Southeast Asia and South America (Myburg *et al.* 2002b; Gryzenhout *et al.* 2004, 2006). Based on sequence data for ITS and β-tubulin gene regions, isolates from South China group within the Southeast Asian clade of this species.

Field inoculation studies using different *Eucalyptus* genotypes grown in South China provided valuable data for the selection of future planting material in the country. The susceptibility of the genotypes tested differed significantly from each other. This is similar to results of screening trials in South Africa (Conradie *et al.* 1992; Van der Westhuizen *et al.* 1992; Van Heerden *et al.* 2005), which have resulted in the effective management of canker caused by *Chr. austroafricana* through the identification of disease-resistant planting stock

(Van Heerden & Wingfield 2002; Wingfield 2003; Van Heerden *et al.* 2005). Thus, in South Africa, it is currently difficult to find *Chr. austroafricana* infections on *Eucalyptus* trees in plantations due to the success of selection and breeding programs that have been supported by diseases screening via inoculation (Roux, unpublished). As a further aid to the selection of disease-tolerant planting material, genetic markers have been developed in South Africa to aid in effective screening of species, hybrids, and clones (Lezar *et al.* 2004). This is an approach that might be useful in China.

An interesting outcome of this study was the discovery of *Chr. cubensis* on *S. cumini*. This tree is planted as an ornamental in South China and is native to nearby countries of Bangladesh, India, Indonesia, Nepal, and Pakistan. In this regard, it seems likely that it is also a natural host of the pathogen in Southeast Asia. This would be consistent with the fact that *Chr. cubensis* has been found on other native members of the order Myrtales in Southeast Asia and in South and Central America and has apparently undergone a host shift (Slippers *et al.* 2005) to infect *Eucalyptus* spp. (Roux *et al.* 2003; Gryzenhout *et al.* 2006; Nakabonge *et al.* 2006). Similarly, the closely related pathogen *Chr. austroafricana* is found on native *Syzygium* spp. in South Africa and it has apparently undergone a host shift to infect *Eucalyptus* spp. (Heath *et al.* 2006; Nakabonge *et al.* 2006). Pathogenicity tests in this study also showed that *S. cumini* is susceptible to infection by *Chr. cubensis*. This could explain the extensive cankering and branch die-back observed regularly on *S. cumini* trees.

This study represents the first comprehensive work that investigates the pathogenicity of *Chr. cubensis* on *Eucalyptus* and *S. cumini* trees in China. Moreover, the identification of commercially available *Eucalyptus* genotypes tolerant to Chrysoporthe canker will promote the selection of tolerant stocks for wide-scale planting. This approach has been shown to effectively reduce the disease severity in other parts of the world (Hodges *et al.* 1976; Van Heerden & Wingfield 2002; Van Heerden *et al.* 2005) and it should also be useful in China.

5. REFERENCES

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.

Campinhos E, Ikemori YK, 1983. Mass production of *Eucalyptus* spp. by rooting cuttings. *Silviculture* **8**: 770–775.

Chungu D, Gryzenhout M, Muimba-Kankolongo A, Wingfield MJ, Roux J, 2010. Taxonomy and pathogenicity of two novel *Chrysoporthe* species from *Eucalyptus grandis* and *Syzygium guineense* in Zambia. *Mycolological Progress* **9**: 379–393.

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

Conradie E, Swart WJ, Wingfield MJ, 1992. Susceptibility of *Eucalyptus grandis* to *Cryphonectria cubensis*. *European Journal of Forest Pathology* **22**: 312–315.

Farris JS, Källersjö M, Kluge AG, Bult C, 1995. Testing significance of incongruence. *Cladistics* **10**: 315–319.

Felsenstein J, 1985. Confidence intervals on phylogenetics: an approach using bootstrap. *Evolution* **39**: 783–791.

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.

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, 2005. *Chrysoporthe doradensis* sp. nov. pathogenic to *Eucalyptus* in Ecuador. *Fungal Diversity* **20**: 39–57.

Gryzenhout M, Rodas CA, Portales JM, Clegg P, Wingfield BD, Wingfield MJ, 2006. Novel hosts of the *Eucalyptus* canker pathogen *Chrysoporthe cubensis* and a new *Chrysoporthe* species from Colombia. *Mycological Research* **110**: 833–845.

Gryzenhout M, Wingfield BD, Wingfield MJ, 2009. *Taxonomy, phylogeny, and ecology of bark-inhabiting and tree pathogenic fungi in the Cryphonectriaceae*. APS Press, St. Paul, Minnesota, USA.

Heath R, Gryzenhout M, Roux J, Wingfield MJ, 2006. Discovery of the canker pathogen *Chrysoporthe austroafricana* on native *Syzygium* spp. in South Africa. *Plant Disease* **90**: 433–438.

Hodges CS, Alfenas AC, Ferreira FA, 1986. The conspecificity of *Cryphonectria cubensis* and *Endothia eugeniae*. *Mycologia* **78**: 343–350.

Hodges CS, Reis MS, Ferreira FA, Henfling JDM, 1976. O cancro do eucalipto causado por *Diaporthe cubensis*. *Fitopatologia Brasileira* **1**: 129–167.

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.

Lezar S, Myburg AA, Berger DK, Wingfield MJ, Wingfield BD, 2004. Development and assessment of microarray-based DNA fingerprinting in *Eucalyptus grandis*. *Theoretical and Applied Genetics* **109**: 1329–1336.

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

Myburg H, Gryzenhout M, Wingfield BD, Wingfield MJ, 2002b. 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* **44**: 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, Roux J, Gryzenhout M, Wingfield MJ, 2006. Distribution of Chrysoporthe canker pathogens on *Eucalyptus* and *Syzygium* species in eastern and southern Africa. *Plant Disease* **90**: 734–740.

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

Old KM, Wingfield MJ, Yuan ZQ, 2003. *A Manual of Diseases of Eucalypts in South-East Asia*. Centre for International Forestry Research, Indonesia.

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

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.

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

SAS Institute Inc, 1999. *SAS/STAT Users Guide*, Version 8. SAS Institute Inc., Cary, NC.

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

Sharma JK, Mohanan C, Florence EJM, 1985. Occurrence of Cryphonectria canker disease of *Eucalyptus* in Kerala, India. *Annals of Applied Biology* **106**: 265–276.

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

Swofford DL, 2002. *PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). 4. 0b10*. Computer program. Sinauer Associates, Sunderland, Massachusetts, USA.

Tamura K, Dudley J, Nei M, Kumar S, 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**: 1596–1599.

Van der Westhuizen IP, Wingfield MJ, Kemp GHJ, Swart WJ, 1992. Comparative susceptibility of *Eucalyptus grandis* clones and hybrids to *Cryphonectria cubensis*. *Phytophylactica* **24**: 107.

Van Heerden SW, Amerson HV, Preisig O, Wingfield BD, Wingfield MJ, 2005. Relative pathogenicity of *Cryphonectria cubensis* on *Eucalyptus* clones differing in the tolerance to *C. cubensis*. *Plant Disease* **89**: 659–66.

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.

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

Wingfield MJ, 2003. Increasing threat of diseases to exotic plantation forests in the southern hemisphere: lessons from *Cryphonectria* canker. *Australasian Plant Pathology* **32**: 133–139.

Wingfield MJ, Rodas C, Wright J, Myburg H, Venter M, Wingfield BD, 2001 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.

Xie YJ, 2006. *Eucalypt research at the beginning of 21st century*. China Forestry House Press, Beijing, China. (In Chinese).

Zhou XD, Xie YJ, Chen SF, Wingfield MJ, 2008. Diseases of eucalypt plantations in China: challenges and opportunities. *Fungal Diversity* **32**: 1–7.

Table 1. Isolates used for phylogenetic analysis and pathogenicity trials in this study.

Isolate[a] CMW no.	Alternative isolate no.	Species identity	Host	Origin	Collector[b]	GenBank no. ITS[c]	GenBank no. β-Tubulin 1	GenBank no. β-Tubulin 2
CMW12746[d]	…	*Chrysoporthe cubensis*	*Eucalyptus* sp.	GuangDong, China	TIB	HM142105	HM142121	HM142137
CMW12748[d]	⋯	*Chr. cubensis*	*Eucalyptus* sp.	GuangDong, China	TIB	HM142106	HM142122	HM142138
CMW12749[d]	⋯	*Chr. cubensis*	*Eucalyptus* sp.	GuangDong, China	TIB	HM142100	HM142116	HM142132
CMW24693[d]	⋯	*Chr. cubensis*	*Eucalyptus* sp.	GuangDong, China	MJW	HM142101	HM142117	HM142133
CMW24695[d]	⋯	*Chr. cubensis*	*Eucalyptus grandis*	GuangDong, China	MJW	HM142093	HM142109	HM142125
CMW24697[d]	⋯	*Chr. cubensis*	*Eucalyptus* sp.	GuangDong, China	MJW	HM142102	HM142118	HM142134
CMW24909[d,f,g]	⋯	*Chr. cubensis*	*Syzygium cumini*	GuangDong, China	MJW & XDZ	HM142094	HM142110	HM142126
CMW24919 [d,f,g]	⋯	*Chr. cubensis*	*S. cumini*	GuangDong, China	MJW & XDZ	HM142104	HM142120	HM142136
CMW24921 [d,f,g]	⋯	*Chr. cubensis*	*S. cumini*	GuangDong, China	MJW & XDZ	HM142103	HM142119	HM142135
CMW26888[d,e]	⋯	*Chr. cubensis*	*E. urophylla* × *E. grandis*	GuangXi, China	MJW & XDZ	HM142095	HM142111	HM142127
CMW26890[d]	⋯	*Chr. cubensis*	*E. urophylla* × *E. grandis*	GuangXi, China	MJW & XDZ	HM142096	HM142112	HM142128
CMW26891[d,e]	⋯	*Chr. cubensis*	*Eucalyptus* U6 clone	GuangDong, China	MJW & XDZ	HM142107	HM142123	HM142139
CMW26892[d,e,f,g]	⋯	*Chr. cubensis*	*Eucalyptus* U6 clone	GuangDong, China	MJW & XDZ	HM142108	HM142124	HM142140
CMW26895[d,e]	⋯	*Chr. cubensis*	*Eucalyptus* W5 clone	GuangDong, China	MJW & XDZ	HM142097	HM142113	HM142129
CMW26929[d,e,f,g]	⋯	*Chr. cubensis*	*Eucalyptus camaldulensis*	HaiNan, China	MJW & XDZ	HM142098	HM142114	HM142130
CMW26932[d,e]	⋯	*Chr. cubensis*	*Eucalyptus* U6 clone	HaiNan, China	MJW & XDZ	HM142099	HM142115	HM142131
CMW1856	⋯	*Chr. cubensis*	*Eucalyptus* sp.	Kauai, Hawaii	NA	AY083999	AY084022	AY084010
CMW9903	⋯	*Chr. cubensis*	*Syzygium aromaticum*	Kalimantan, Indonesia	CSH	AF292044	AF273066	AF273461
CMW11288	CBS115736	*Chr. cubensis*	*Eucalyptus* sp.	Indonesia	MJW	AY214302	AY214230	AY214266
CWM11290	CBS115738	*Chr. cubensis*	*Eucalyptus* sp.	Indonesia	MJW	AY214304	AY214232	AY214268
CMW8650	CBS115719	*Chr. cubensis*	*S. aromaticum*	Sulawesi, Indonesia	MJW	AY084001	AY084024	AY084013
CMW8651	CBS115718	*Chr. cubensis*	*S. aromaticum*	Sulawesi, Indonesia	MJW	AY084002	AY084014	AY084026
CMW10774	⋯	*Chr. cubensis*	*S. aromaticum*	Zanzibar, Tanzania	NA	AF492130	AF492131	AF492132
CMW2631	⋯	*Chr. cubensis*	*E. marginata*	Australia	ED	AF543823	AF543824	AF543825
CMW2632	⋯	*Chr. cubensis*	*E. marginata*	Australia	ED	AF046893	AF273078	AF375607
CMW10453	CBS505.63	*Chr. cubensis*	*Eucalyptus saligna*	Republic of Congo	Unknown	AY063476	AY063478	AY063480
CMW10669	CBS115751	*Chr. cubensis*	*Eucalyptus* sp.	Republic of Congo	JR	AF535122	AF535124	AF535126
CMW10671	CBS115752	*Chr. cubensis*	*Eucalyptus* sp.	Republic of Congo	JR	AF254219	AF254221	AF254223
CMW10639	CBS115747	*Chr. cubensis*	*E. grandis*	Colombia	CAR	AY263419	AY263420	AY263421
CMW14394	CBS118654	*Chr. cubensis*	*E. grandis*	Cuba	MJW	DQ368773	DQ368798	DQ368799
CMW1853	⋯	*Chr. cubensis*	*S. aromaticum*	Brazil	NA	AF046891	AF273070	AF273465
CMW10777	⋯	*Chr. cubensis*	*S. aromaticum*	Brazil	CSH	AY084005	AY084029	AY084017
CMW10778	CBS115755	*Chr. cubensis*	*S. aromaticum*	Brazil	CSH	AY084006	AY084030	AY084018

CMW2113	CBS112916	*Chr. austroafricana*	*E. grandis*	South Africa	MJW	AF046892	AF273067	AF273462
CMW9327	CBS115843	*Chr. austroafricana*	*Tibouchina granulosa*	South Africa	MJW	AF273473	AF273060	AF273455
CMW14561	⋯	*Chr. austroafricana*	*Syzygium cordatum*	South Africa	GN	DQ246605	DQ246559	DQ246582
CMW13976	⋯	*Chr. austroafricana*	*S. cordatum*	Zambia	JR	DQ246614	DQ246568	DQ246591
CMW11286	CBS115734	*Chr. doradensis*	*E. grandis*	Ecuador	MJW	AY214289	AY214217	AY214253
CMW11287	CBS115735	*Chr. doradensis*	*E. grandis*	Ecuador	MJW	AY214290	AY214218	AY214254
CMW10625	CBS115744	*Chr. hodgesiana*	*Miconia theaezans*	Colombia	CAR	AY956970	AY956979	AY956980
CMW10641	CBS115854	*Chr. hodgesiana*	*Tibouchina semidecandra*	Colombia	RA	AY692322	AY692326	AY692325
CMW12727	CBS118659	*Chr. inopina*	*T. lepidota*	Colombia	RA	DQ368777	DQ368806	DQ368807
CMW12729	CBS118658	*Chr. inopina*	*T. lepidota*	Colombia	RA	DQ368778	DQ368808	DQ368809
CMW7048	⋯	*Cryphonectria parasitica*	*Quercus virginiana*	United States	FFL	AF368330	AF273076	AF273470
CMW13749	⋯	*C. parasitica*	*Castanea mollisima*	Japan	NA	AY697927	AY697943	AY697944

[a]CMW = culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; CBS = the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

[b]TIB = T. I. Burgess, MJW = M. J. Wingfield, XDZ = X. D. Zhou, NA = not applicable, CSH = C. S. Hodges, ED = E. Davison, JR = J. Roux, CAR = C. A. Rodas, GN = G. Nakabonge, RA = R. Arbaleaz, and FFL = F.F. Lombard.

[c]Internal transcribed spacer.

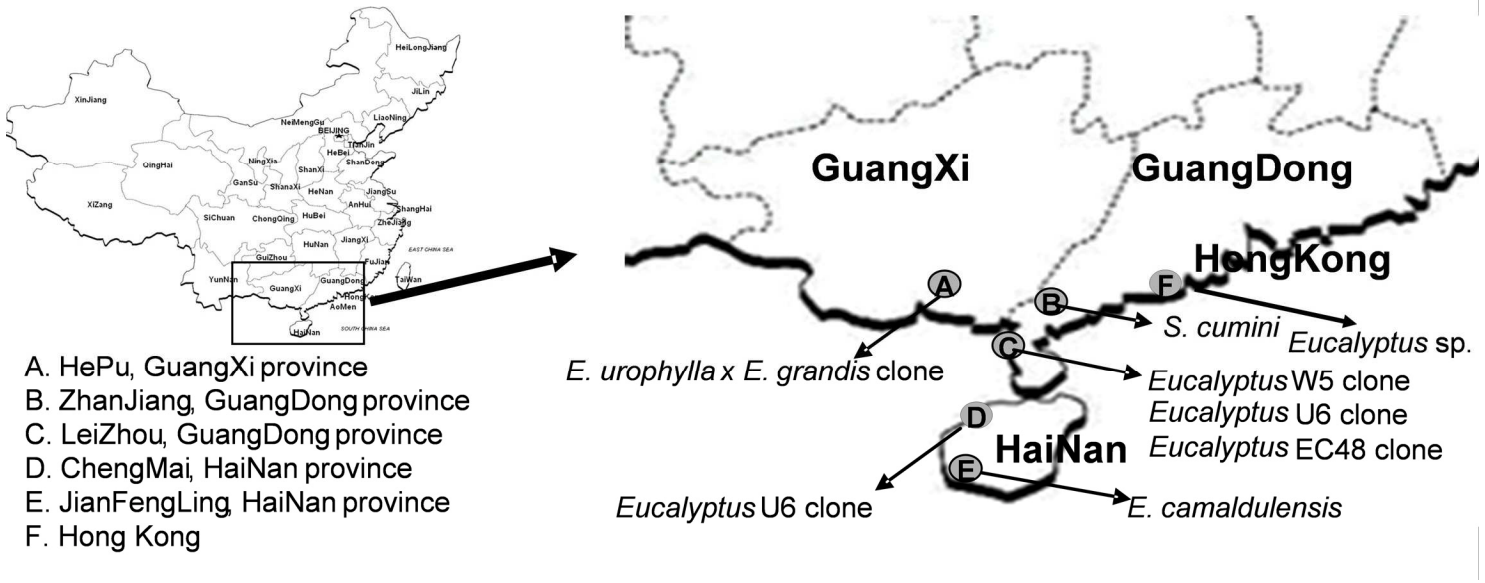
[d]Isolates obtained and used in phylogenetic analysis in this study.

[e]Isolates used for pathogenicity tests on *E. grandis* clone seedlings in glasshouse.

[f]Isolates used for pathogenicity tests on *Eucalyptus* plantations in field in China.

[g]Isolates used for pathogenicity tests on *S, cumini* branches in field in China.

Fig 1. Map of China indicating areas and species of *Eucalyptus* and *Syzygium* from which *Chrysoporthe cubensis* isolates were collected and identified. Records of *Chr. cubensis* on *Eucalyptus* spp. in Hong Kong are from previous studies (Hodges *et al*. 1976; Sharma *et al*. 1985; Myburg *et al*. 1999)



A. HePu, GuangXi province
B. ZhanJiang, GuangDong province
C. LeiZhou, GuangDong province
D. ChengMai, HaiNan province
E. JianFengLing, HaiNan province
F. Hong Kong

Fig 2. Symptoms of infection by *Chrysoporthe cubensis* on A. *Eucalyptus* spp. and B. *Syzygium cumini* in China.

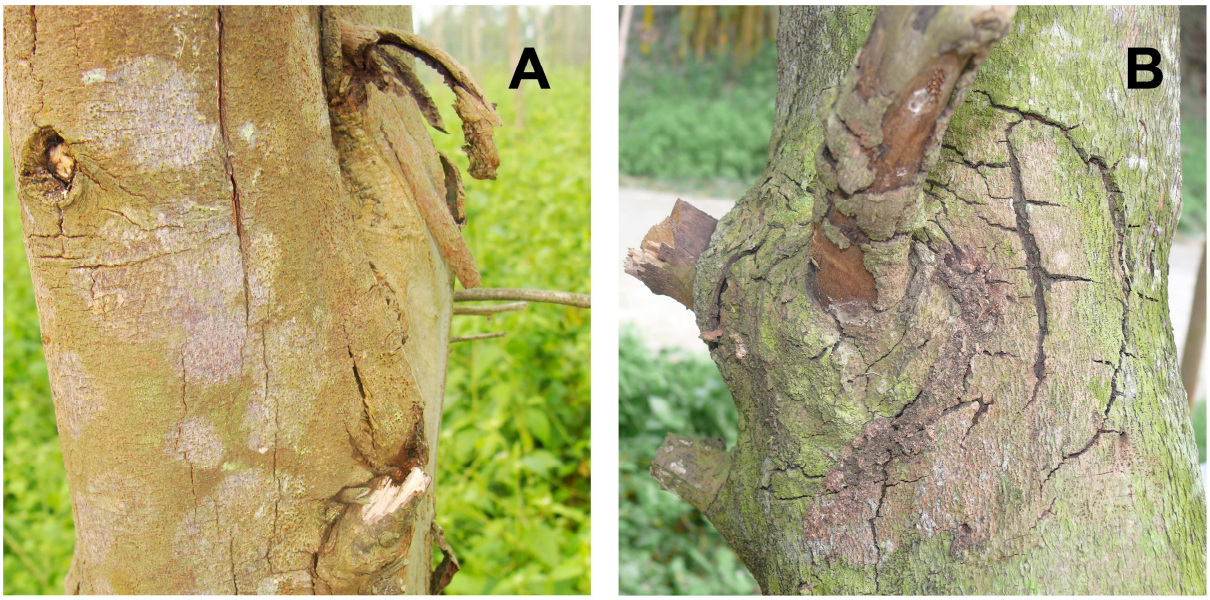


Fig 3. Fruiting structures of *Chrysoporthe cubensis* from a *Eucalyptus* sp. and *Syzygium cumini* in China. A. Ascoma on bark of *Eucalyptus* sp.; B. Ascoma on bark of *S. cumini*; C. Conidioma on bark of *Eucalyptus* sp.; D. Asci from *S. cumini* tree; E. Ascospores from *S. cumini* bark; F. Ascospores from *Eucalyptus* sp.; G. Conidiophores from *Eucalyptus* sp.; H. Conidia from *Eucalyptus* sp. Scale bars: A–C = 100; D–H = 10 µm.

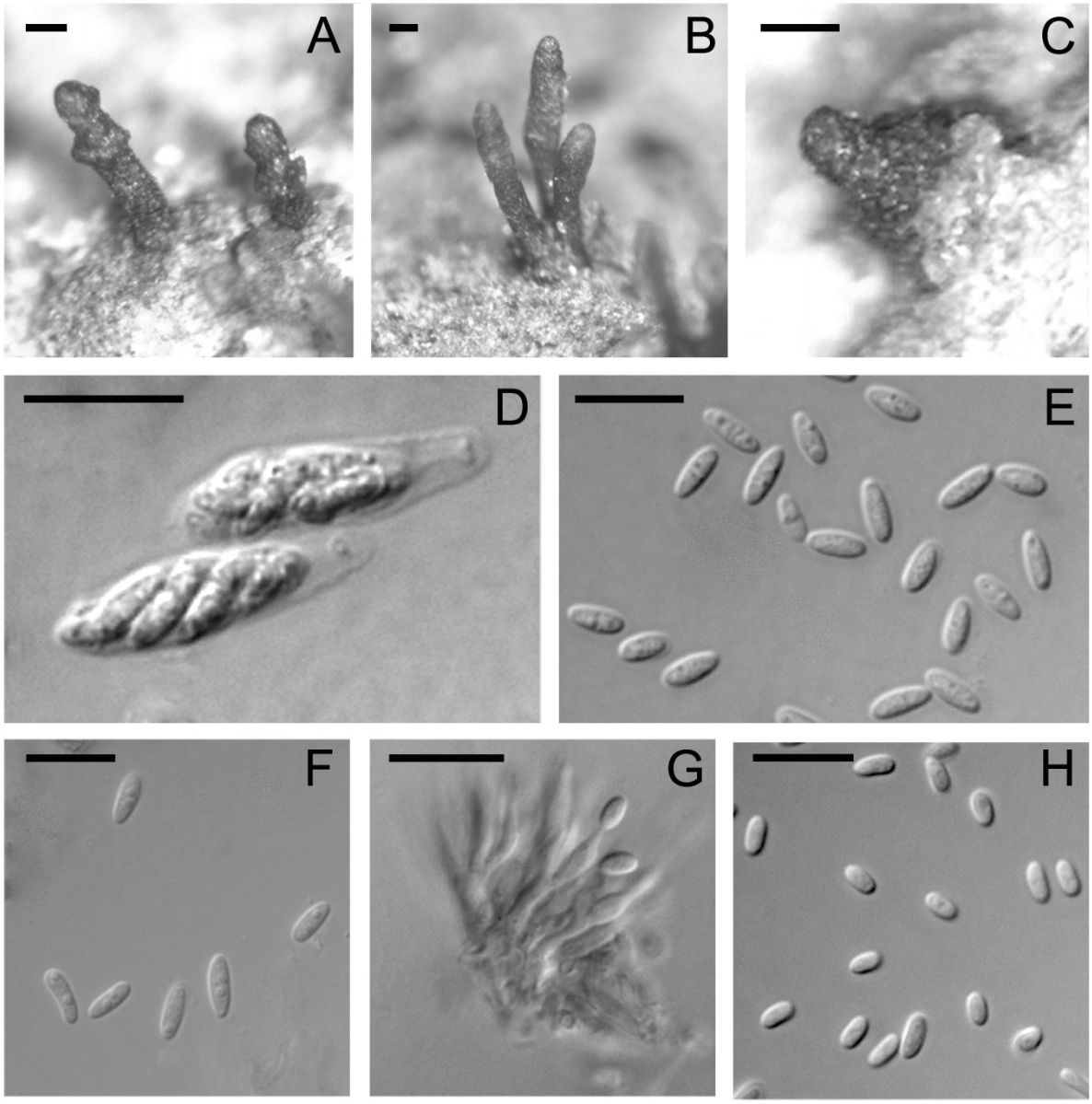


Fig 4. One of 36 most parsimonious trees generated from combined sequence data of the internal transcribed spacer ribosomal DNA and β-tubulin gene sequence data and generated from heuristic searches performed on the combined data set (tree length of 348, CI of 0.937, and RI of 0.954). Bootstrap values (1 000 replicates) above 75% are indicated on the branches. Isolates sequenced in this study are in bold. *Cryphonectria parasitica* was used to root the tree.

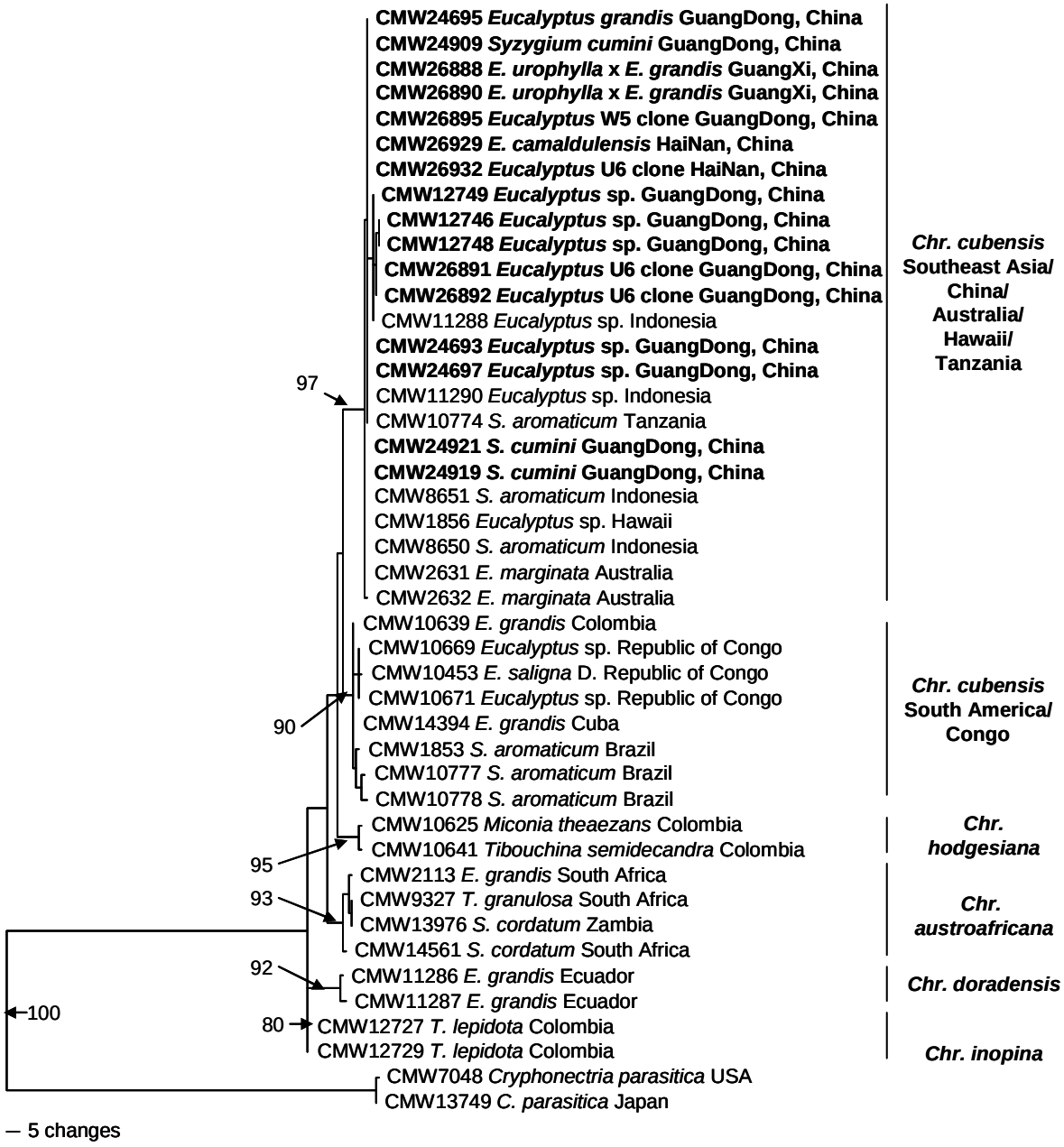


Fig 5. Column chart indicating the average lesion length (in millimeters) resulting from inoculation trials onto an *Eucalyptus grandis* clone (TAG-5) under glasshouse conditions. Six isolates of *Chrysoporthe cubensis* were used that was identified from *Eucalyptus* trees in China. Bars represent 95% confidence limits for each treatment. Different letters above the bars indicate treatments that were statistically significantly different (P = 0.05).

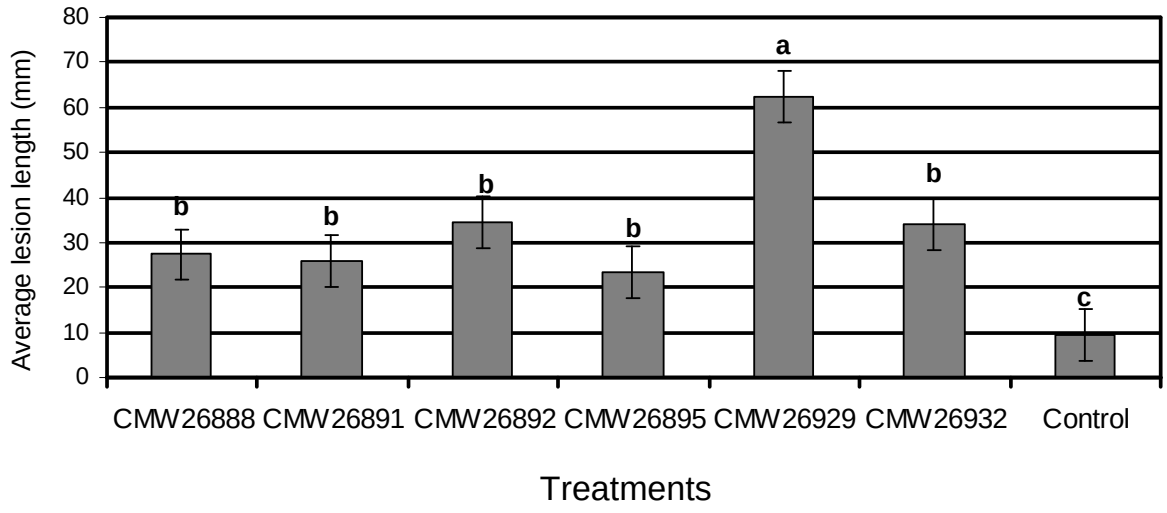


Fig 6. Column chart indicating the average lesion lengths (in millimeters) resulting from inoculation trials on seven *Eucalyptus* genotypes. Lesion lengths were produced by two *Chrysoporthe cubensis* isolates (CMW26892 and CMW26929). Bars represent 95% confidence limits for each treatment. Different letters above the bars indicate treatments that were statistically significantly different (P = 0.05).

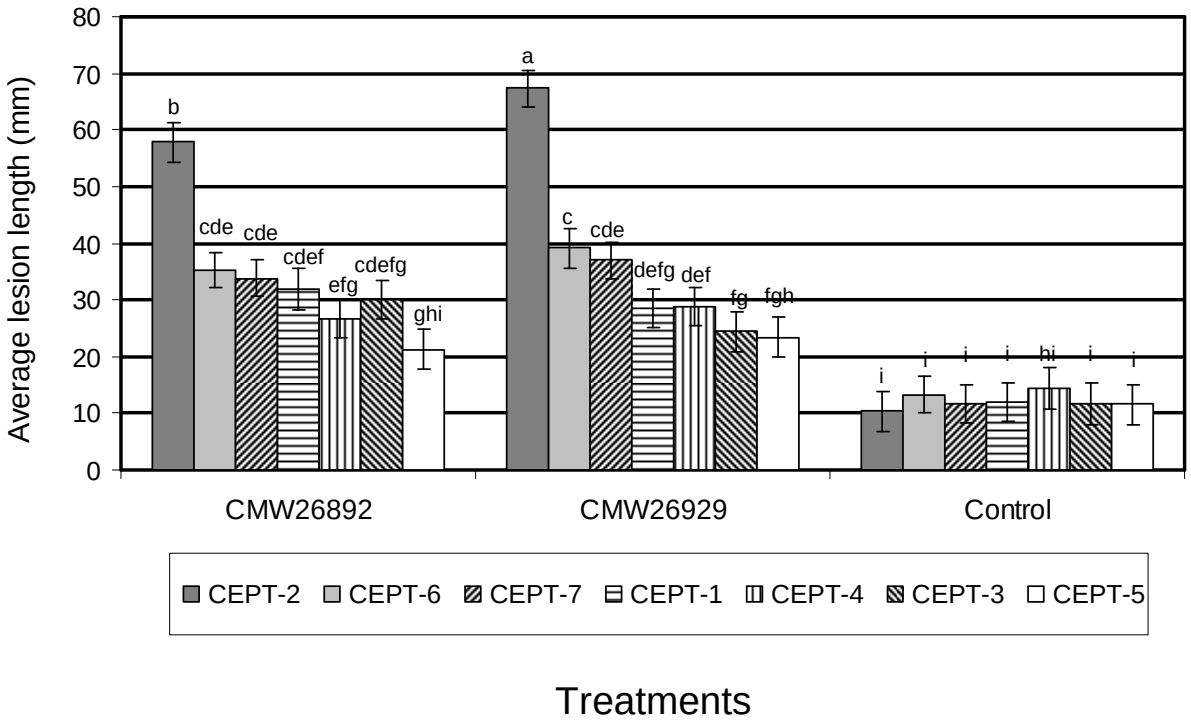


Fig 7. Column chart indicating the average lesion lengths (in millimeters) resulting from inoculation trials on two *Eucalyptus* genotypes (CEPT-6 and CEPT-7) in the field. Five *Chrysoporthe cubensis* isolates were used, with two isolates (CMW26892 and CMW26929) identified from *Eucalyptus* trees and three isolates (CMW24909, CMW24919, and CMW24921) identified from *Syzygium cumini* trees. Bars represent 95% confidence limits for each treatment. Different letters above the bars indicate treatments that were statistically significantly different (P = 0.05).

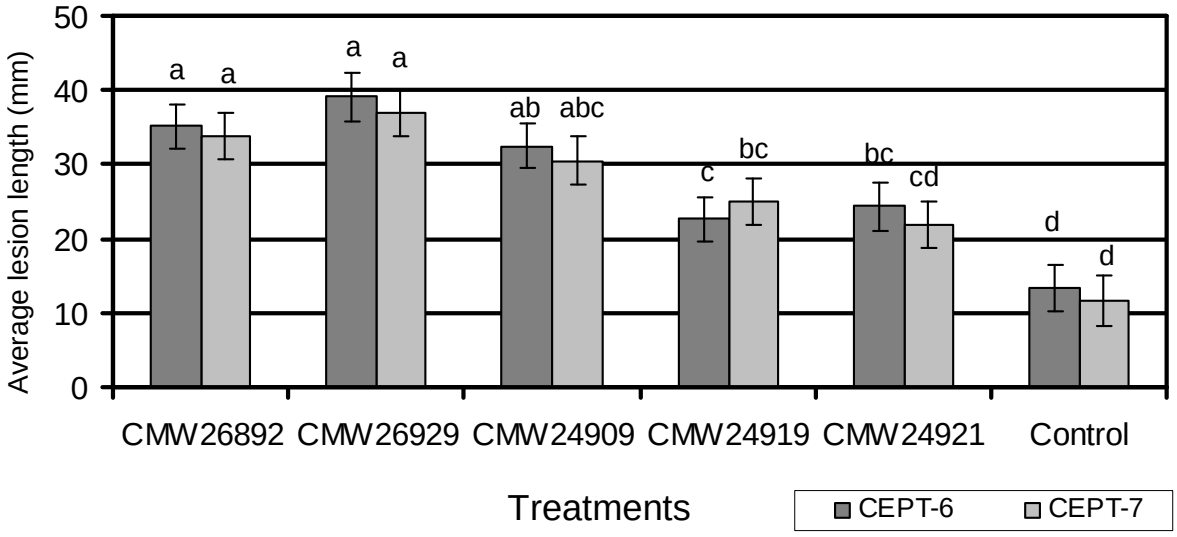
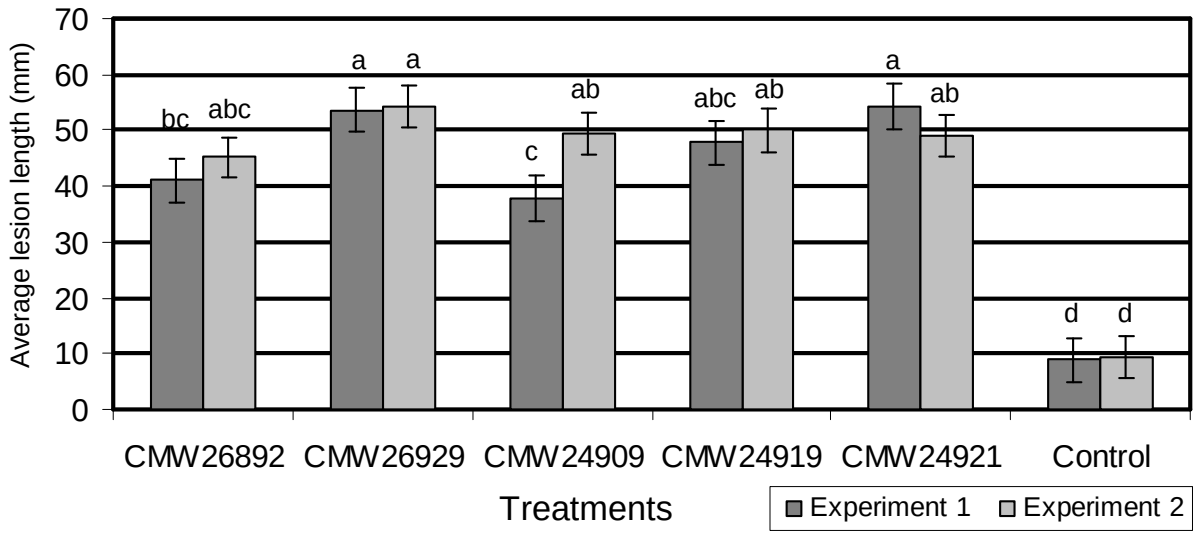


Fig 8. Column chart indicating the average lesion lengths (in millimeters) resulting from inoculation trials on branches of *Syzygium cumini* that were inoculated with five *Chrysoporthe cubensis* isolates. Two of these isolates (CMW26892 and CMW26929) were identified from *Eucalyptus* trees and three isolates (CMW24909, CMW24919, and CMW24921) were identified from *S. cumini* trees. Bars represent 95% confidence limits for each treatment. Different letters above the bars indicate treatments that were statistically significantly different (P = 0.05).



Chapter 3

Novel species of *Celoporthe* from *Eucalyptus* and *Syzygium* trees in China and Indonesia

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This chapter has been submitted for publication as: Chen SF, Gryzenhout M, Roux J, Xie YJ, Wingfield MJ, Zhou XD. Novel species of *Celoporthe* from *Eucalyptus* and *Syzygium* trees in China and Indonesia. (Submitted to *Mycologia*, on Jan. 5, 2011).

ABSTRACT

Many species in the Cryphonectriaceae cause diseases of trees, including those in the genera *Eucalyptus* and *Syzygium*. During disease surveys on these trees in South China, fruiting structures typical of fungi in the Cryphonectriaceae and associated with dying branches and stems were observed. Morphological comparisons indicated that these fungi were distinct from the well-known *Chrysoporthe deuterocubensis*, also found on these trees in China. The aim of this study was to identify these fungi and evaluate their pathogenicity to *Eucalyptus* clones/species as well as *Syzygium cumini*. Three morphologicaly similar fungal isolates collected previously from Indonesia were also included in the study. Isolates were characterized based on comparisons of their morphology and DNA sequence data for the partial LSU and ITS nuclear ribosomal DNA, β-tubulin, and TEF-1α gene regions. Following glasshouse trials to select virulent isolates, field inoculations were undertaken to screen different commercial *Eucalyptus* clones/species and *S. cumini* trees for susceptibility to infection. Phylogenetic analyses showed that the Chinese isolates and those from Indonesia reside in a clade close to previously identified South African *Celoporthe* isolates. Based on morphology and DNA sequence comparisons, four new *Celoporthe* spp. were identified and they are described as *C. syzygii*, *C. eucalypti*, *C. guangdongensis* and *C. indonesiensis*. Field inoculations indicated that the three tested Chinese *Celoporthe* spp., namely *C. syzygii*, *C. eucalypti* and *C. guangdongensis*, are pathogenic to all tested *Eucalyptus* and *S. cumini* trees. Significant differences in the susceptibility of the inoculated *Eucalyptus* clones/species suggest that it will be possible to select disease tolerant planting stock for forestry operations in the future.

Keywords: Cryphonectriaceae, stem canker pathogens, Myrtales, plantation forestry, Southeast Asia

1. INTRODUCTION

The Cryphonectriaceae Gryzenh. & M.J. Wingf. (Diaporthales) represents a group of bark and/or wood-infecting fungi of trees and shrubs in various parts of the world (Gryzenhout *et al.* 2009). Species of Cryphonectriaceae exist naturally as virulent pathogens, facultative parasites or saprophytes on woody hosts. Some species have been introduced into new environments causing diseases on important trees such as those grown commercially in plantations or for their ornamental value, and include some of the most important tree pathogens in the world (Gryzenhout *et al.* 2009). Except for *Cryphonectria* (Sacc.) Sacc. & D. Sacc., which is the type genus, thirteen other genera have been described in this family (Nakabonge *et al.* 2006a; Cheewangkoon *et al.* 2009; Gryzenhout *et al.* 2009, 2010; Begoude *et al.* 2010; Vermeulen *et al.* 2010).

Several species of Cryphonectriaceae have been collected from trees in China in the past. *Cryphonectria parasitica* (Murrill) M.E. Barr, best known for causing a devastating canker disease of chestnuts (*Castanea* spp.) in the USA and Europe (Anagnostakis 1987, 1992) also causes canker and die-back on Chinese chestnut (*Castanea mollissima* Blume) trees in their native range (Fairchild 1913; Shear & Stevens 1913, 1916). *Cryphonectria japonica* (Tak. Kobay. & Kaz. Itô) Gryzenh. & M.J. Wingf. [= *Cryphonectria nitschkei* (G.H. Otth) M.E. Barr], which was first collected in Japan, and *Endothia gyrosa* (Schwein.: Fr.) Fr., have been found on a *Quercus* sp. in China (Teng 1934; Kobayashi & Itô 1956; Myburg *et al.* 2004; Gryzenhout *et al.* 2009). *Chrysoporthe deuterocubensis* Gryzenh. & M.J. Wingf., previously treated as *Chr. cubensis* (Bruner) Gryzenh. & M.J. Wingf. (Van Der Merwe *et al.* 2010) has been reported from species of *Eucalyptus* and *Syzygium* in South China from a wide range of locations (Sharma *et al.* 1985; Hodges *et al.* 1986; Myburg *et al.* 2002; Zhou *et al.* 2008; Chen *et al.* 2010).

The genus *Celoporthe* Nakab., Gryzenh., Jol. Roux & M.J. Wingf., based on *C. dispersa* Nakab., Gryzenh., Jol. Roux & M.J. Wingf., is a recently described genus in the Cryphonectriaceae, described from both native and introduced Myrtales in South Africa (Nakabonge *et al.* 2006a). The fungus was associated with dying branches and stems on these trees. Currently, the genus is represented by a single species, despite DNA-based comparisons that showed the presence of three different but closely related phylogenetic sub-clades within the genus (Nakabonge *et al.* 2006a). *C. dispersa* is represented by isolates from native

Syzygium cordatum Hochst.: C.Kraus, native *Heteropyxis canescens* Oliv. and non-native *Tibouchina granulosa* Cogn. (Nakabonge *et al.* 2006a) in South Africa. Based on DNA sequences comparisons, isolates previously collected from *S. aromaticum* (L.) Merr. & L.M.Perry in Indonesia (Myburg *et al.* 2003) also grouped closely with *C. dispersa*, but morphological evaluation was impossible due to an absence of specimens (Myburg *et al*. 2003; Nakabonge *et al.* 2006a). Inoculation trials showed that *C. dispersa* is more pathogenic on *Eucalyptus grandis* W. Hill clone than *T. granulosa* (Nakabonge *et al.* 2006a).

During surveys in South China for pathogens of trees in the Myrtaceae especially species of *Eucalyptus* and *Syzygium*, several pathogens affecting these trees were identified (Zhou *et al.* 2008). Besides *Chr. deuterocubensis* (Chen *et al.* 2010; Van Der Merwe *et al*. 2010), these surveys yielded a fungus on *Eucalyptus* and *S. cumini* trees resembling species of *Celoporthe*. The aim of the present study was to characterize these isolates based on morphology and DNA sequence comparisons and to assess their pathogenicity to *Eucalyptus* and *S. cumini* using glasshouse and field inoculations.

2. MATERIALS AND METHODS

2.1 Sampling

Eucalyptus (Myrtales) plantations in the GuangDong Province of South China were investigated for the presence of fungal diseases during the periods November to December 2006, January 2007, and September to November 2008. Where present in these areas, *S. cumini* trees (Myrtales) were also examined for the presence of fungi in the Cryphonectriaceae, as these fungi are known to occur on *Syzygium* spp. (Heath *et al*. 2006; Gryzenhout *et al.* 2009). Sections of bark bearing fruiting structures (ascostromata and conidiomata) resembling the Cryphonectriaceae were collected from symptomatic trees and transported to the laboratory in order to make isolations.

Samples were incubated in moist chambers for 1–3 days to induce the production of spores from the fruiting bodies. Single tendrils of spores were transferred to 2% Malt Extract Agar (MEA) (Biolab, Merck, Midrand, South Africa) (20 g Biolab Malt Extract, 20 g Biolab Agar, 1 L water) and incubated at 25°C. From the resultant cultures, single hyphal tips were transferred to fresh 2% MEA to obtain pure cultures. Three unidentified isolates from

Indonesia, originating from *S. aromaticum* and resembling fungi in the Cryphonectriaceae, obtained from the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa, were also inclued in this study (Table 1). All cultures collected from China are maintained in the culture collection (CMW), and a duplicate set of isolates is maintained in a culture collection housed at the China Eucalypt Research Centre (CERC), Chinese Academy of Forestry (CAF), China. Representative isolates were also deposited with the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands (Table 1). The original bark material bearing fruiting structures was deposited in the National Collection of Fungi (PREM), Pretoria, South Africa.

2.2 DNA sequence comparisons

2.2.1. DNA extraction, PCR and sequencing reactions

Isolates originating from different *Eucalyptus* species/clones and *S. cumini* from the various areas sampled, as well as those showing differing culture and fruiting structure morphology (Table 1), were selected for DNA sequence comparisons. Prior to DNA extraction, isolates were grown in 2% MEA at 25°C for 5–7 days. For each isolate, actively growing mycelium from one MEA plate per isolate was scraped from the surface of the medium using a sterile scalpel and transferred to 1.5 mL Eppendorf tubes. DNA was extracted using the method described by Myburg *et al.* (1999). DNA was separated by electrophoresis on a 1% agarose gel, stained with ethidium bromide and visualised under ultraviolet (UV) light. Samples were treated with 3 µL RNase (1mg/mL) and left overnight at 37°C to degrade RNA.

Gene regions amplified using the Polymerase Chain Reaction (PCR) included the conserved nuclear Large Subunit (LSU), the β-tubulin gene region 1 (BT1) and 2 (BT2), the Internal Transcribed Spacer (ITS) regions including the 5.8S gene of the ribosomal DNA operon (Gryzenhout *et al.* 2009) as well as the translation elongation factor 1-alpha (TEF-1α) gene region. Part of the LSU rDNA gene region was amplified using the primers LR0R and LR7 (Vilgalys & Hester 1990; Rehner & Samuels 1994), two regions within the BT gene were amplified using the primer pairs βt1a/βt1b and βt2a/βt2b, respectively (Glass & Donaldson 1995), the ITS regions including the 5.8S rDNA operon were amplified using the primer pairs ITS1 and ITS4 (White *et al.* 1990), and a fragment of the TEF-1α gene region was amplified using the primer pairs EF1-728F and EF1-986R (Carbone & Kohn 1999). PCR conditions for

the LSU gene region were as outlined by Castlebury *et al.* (2002), those for the BT1/2 and ITS gene regions followed the protocols of Myburg *et al.* (2002), and the TEF-1α gene region was amplified using the method described by Slippers *et al.* (2004). PCR products were visualised with UV light on 1% agarose (ethidium bromide-stained) gels. Using 6% Sephadex G-50 columns (Steinheim, Germany), the amplified products were purified as suggested by the manufacturers.

Each PCR product was sequenced in both directions with the same primers used for PCR reactions. The ABI PRISM™ Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Applied Biosystems, Foster City, California) was used to perform the sequencing reactions. Sephadex G-50 columns (6%) were used to purify the sequence products, wherafter electropherograms were generated on an ABI PRISM 3100 autosequencer (Perkin-Elmer Applied Biosystems, Foster City, California). Nucleotide sequences were edited using MEGA4 (Tamura *et al.* 2007). All sequences obtained in this study have been deposited in GenBank (Table 1).

2.2.2. Generic placement and species identification

To determine the generic placement of the isolates collected from *Eucalyptus* and *S. cumini* in South China, as well as those previously collected in Indonesia, sequences of the LSU gene region were analysed. These analyses were supplemented by analyses of the conserved 5.8S operon of the ITS region and the exon regions of the BT (including partial exon 4, exon 5, partial exon 6, and partial exon 7) gene regions for previously described species in the Cryphonectriaceae (Gryzenhout *et al.* 2009; Begoude *et al.* 2010; Gryzenhout *et al.* 2010; Vermeulen *et al.* 2010). For analyses of the LSU, the datasets of Gryzenhout *et al.* (2006a, 2009) were used as templates. Sequences were also supplemented with those of other recently published isolates of new genera in the Cryphonectriaceae, including *Aurifilum* Begoude, Gryzenh. & Jol. Roux, *Cryptometrion* Gryzenh. & M.J. Wingf., and *Latruncella* M. Verm., Gryzenh. & Jol. Roux (Begoude *et al.* 2010; Gryzenhout *et al.* 2010; Vermeulen *et al.* 2010). *Togninia minima* (Tul. & C. Tul.) Berl., *T. fraxinopennsylvanica* (T.E. Hinds) Georg Hausner, Eyjólfsd. & J. Reid, and *Phaeoacremonium aleophilum* W. Gams, Crous, M.J. Wingf. & Mugnai were used as outgroups (Gryzenhout *et al.* 2009). Two isolates of *Diaporthe ambigua* Nitschke were used as outgroups for analyses of the 5.8S gene and BT exon regions (Gryzenhout *et al.* 2009). A partition homogeneity test (PHT) was used to determine the

congruence of the datasets for these two gene regions (Farris *et al.* 1995; Huelsenbeck *et al.* 1996). After an outcome indicating congruence between the datasets, phylogenetic analyses were done in PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford 2002) of the individual and combined datasets.

To determine the species identities and phylogenetic relationships between the isolates from China and previously described species of Cryphonectriaceae, sequences of the BT1, BT2, ITS and TEF-1α gene regions were analysed separately and in combination. A partition homogeneity test (PHT) was used to determine the congruence of the datasets (Farris *et al.* 1995; Huelsenbeck *et al.* 1996). After an outcome indicating congruence between the datasets, phylogenetic analyses were done in PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford 2002).

All sequences were aligned using the iterative refinement method (FFT-NS-i settings) of the online version of MAFFT v. 5.667 (Katoh *et al.* 2002), and adjusted and edited manually where necessary in MEGA 4 (Tamura *et al.* 2007). The sequence alignments for each of the datasets were deposited in TreeBASE (http://www.treebase.org). Three different phylogenetic analyses were conduced for each of the datasets. Maximum Parsimony (MP) analyses were done in PAUP 4.0b10 (Swofford 2002), Maximum Likelihood (ML) tests were conducted using PhyML 3.0 (Guindon & Gascuel 2003), and Bayesian inference was determined using the Markov Chain Monte Carlo (MCMC) algorithm in MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003).

For MP analyses, gaps were treated as a fifth character and the characters were all unordered and of equal weight with 1 000 random addition replicates. By using the heuristic search function and tree bisection and reconstruction (TBR) as branch swapping algorithms, the most parsimonious trees were obtained. Maxtrees were unlimited and branch lengths of zero were collapsed. A bootstrap analysis (50% majority rule, 1 000 replicates) was done to determine the confidence levels of the tree-branching points (Felsenstein 1985). Tree length (TL), consistency index (CI), retention index (RI) and the homoplasy index (HI) were used to assess the trees (Hillis & Huelsenbeck 1992). For ML and Bayesian analyses of each dataset, the best models of nucleotide substitution were established using Modeltest 3.7 (Posada & Crandall 1998) and MrModelTest version 2.3 (Nylander 2004), respectively. For the ML analyses, additional ML parameters in PhyML included the retention of the maximum number

of 1 000 trees, and the determination of nodal support by nonparametric bootstrapping using 1 000 replicates. For Bayesian analyses, four chains were run simultaneously for the MCMC analyses, over a 1 000 000 generations. After every 100th generation, trees were saved. The burn-in number was established graphically from likelihood scores, and the posterior probabilities (50% majority rule) were determined from the remaining trees. MEGA4 was used to construct consensus trees (Tamura *et al.* 2007).

2.3. Morphology

Only herbarium samples from *S. cumini* were available for detailed study, and collections from *Eucalyptus* species/clones were available only as cultures. Asexual structures of the Cryphonectriaceae are not always produced and sexual states are almost never induced in culture. For this reason, thin branches of *E. grandis* and *S. cordatum*, a native species of Myrtaceae in South Africa, were used to induce the production of fruiting structures. Freshly cut branch sections (1–2 cm in diameter and 15 cm in length) of *E. grandis* and *S. cordatum* were artificially inoculated under glasshouse conditions with nine single-spore isolates (CWM10781, CWM12750, CWM24912, CWM24914, CWM24917, CWM26900, CWM26908, CWM26911 and CWM26913) that originated from different hosts and locations in China and Indonesia (Table 1).

Prior to inoculation, branch sections were surface-disinfected by swabbing with 75% alcohol. Each section was inoculated by removing a 7 mm diameter cambial disc with a cork borer and placing a disc of equivalent size from a 7-day-old MEA culture of these fungi into the wound. Sterile MEA discs were placed in wounds and these served as controls. Each inoculated wound was covered with parafilm (Pechiney plastic packing, Chicago, USA). In one method, the top ends of the section were coated with melted paraffin-wax, and the other ends were placed in containers of water to ensure that the sections continued to absorb water. In a second method, both ends of the branch sections were coated with melted paraffin-wax, and the inoculated sections were placed in covered plastic boxes on damp paper towels. Three replicates of each treatment were made, including six branches each of an *E. grandis* clone and *S. cordatum* and inoculated with each isolate. All the inoculated branch sections were incubated at 25˚C. After six months of incubation, fruiting structures formed on the bark were examined.

To obtain an indication of the minimum and maximum size ranges of the stromata, measurements were made from both anamorph and telemorph structures on *Eucalyptus* and *S. cumini* bark. To study the internal morphology of the fungi collected, fruiting structures were cut from the original field collected, as well as glasshouse generated bark specimens under a dissection microscope, boiled for two minutes and sectioned (12 µm thick) using a Leica CM1100 cryostat (Setpoint Technologies, Johannesburg, South Africa) at –20ºC (Gryzenhout *et al.* 2004). In order to study the asci, ascospores, conidiophores, conidiogenous cells and conidia, fruiting structures were crushed on microscope slides in 85% lactic acid or 3% KOH. For the holotype specimen, 50 measurements were made for each of these characteristics, while 20 measurements per character were made for the remaining specimens. A HRc Axiocam digital camera with Axiovision 3.1 software (Carl Zeiss Ltd., Germany) was used to capture digital images and to obtain digital measurements. Characteristics of specimens were compared with those published for closely related species in the Cryphonectriaceae (Gryzenhout *et al.* 2009; Begoude *et al.* 2010; Gryzenhout *et al.* 2010; Vermeulen *et al.* 2010). The results were presented as (min–) (mean – st.dev.) – (mean + st.dev.) (–max).

For studies of culture characteristics, five representative isolates (CMW34023, CMW34024, CMW24912, CMW24914, CMW24917) from *S. cumini*, four isolates (CMW26900, CMW26908, CMW26911, CMW26913) from an *Eucalyptus* clone, one isolate (CMW12750) from an *Eucalyptus* sp., as well as an isolate (CMW10781) from *S. aromaticum* in Indonesia, were used. Isolates were incubated on 2% MEA at 25˚C for seven days. Mycelial plugs (6 mm) were taken from the actively growing margins of these cultures and transferred to the centers of 90 mm Petri dishes containing 2% MEA. These were incubated at seven different temperatures ranging from 5˚C to 35˚C at five degree intervals with four replicates of each isolate at every temperature. Two measurements perpendicular to each other were taken daily until the fastest growing culture had covered the plate. Averages were computed for each temperature using Microsoft Excel 2003. The entire experiment was repeated once. Colour designations were obtained for the descriptions of cultures and fruiting bodies using the colour charts of Rayner (1970).

2.4. Pathogenicity tests

2.4.1. Glasshouse trials

Three isolates (CMW26900, CMW26908, CMW26911) from an *Eucalyptus* clone in China (Table 1) were selected for inoculations. These isolates represented a subset of a larger collection of one of the sub-clades identified by DNA sequence comparisons. In order to select the most virulent isolates for field inoculations in China, the three isolates were inoculated on trees of a susceptible *E. grandis* clone (TAG-5) in the glasshouse. The trees were approximately 2 m tall and with diameters of ~10 mm. Before conducting inoculations, all the trees were allowed to adapt to the glasshouse environment of 25°C with 14 hrs daylight and 10 hrs darkness for approximately one month. Before inoculation, the three isolates were grown at 25°C under continuous fluorescent light for six days.

Wounds were made on the bark of trees at the same height (~ 300 mm above the medium) using a cork borer (7 mm in diameter) to expose the cambium. Agar discs 7 mm in diameter were taken from the margins of actively growing fungal cultures and placed into the wounds with the mycelium facing the exposed cambium. In order to prevent contamination and desiccation, wounds were covered with Parafilm (Pechiney Plastic Packing, Chicago). Each of the three isolates was inoculated into the stems of ten trees. For the negative controls, ten trees were inoculated with sterile MEA plugs. The 40 inoculated trees were arranged randomly in a single glasshouse.

After six weeks, the lesion lengths in the cambium were measured. Small pieces of discoloured xylem were taken from four randomly selected trees for each isolates as well as all the trees inoculated as controls, placed onto the surface of 2% MEA and incubated at 25˚C. In this way, it was possible to ascertain that the inoculated fungi were responsible for causing the lesions.

The results of the inoculations were analysed in SAS (Version 8) using PROC GLM (general linear model) (SAS Institute, 1999). In order to determine the effects of the fungal strains on lesion length, analysis of variance (ANOVA) was conducted. Before ANOVA, homogeneity of variance across treatments was confirmed. Fisher's protected test was used to test the significance amongst means and $P < 0.05$ for the F value taken as significant in difference.

2.4.2. Field trials

For the field inoculations, isolate CMW26908, selected based on the results of the greenhouse trial and isolate CMW12750 also from *Eucalyptus*, were used to test the susceptibility of commercially grown *Eucalyptus* species and clones in South China (Table 1). Additionally, to compare the aggressiveness of isolates collected from *S. cumini* with the isolates from *Eucalyptus* trees, three isolates (CMW24912, CMW24914, CMW24917) collected from *S. cumini* in China (Table 1) were used. The five isolates selected for field inoculations thus represented three different phylogenetic sub-clades emerging from the DNA sequence comparisons.

Seven *Eucalyptus* genotypes, including hybrids and pure species, were selected for field inoculation trials. The genotypes included an *E. grandis* clone (CEPT-1), an *E. grandis* × *E. tereticornis* Sm. clone (CEPT-2), an *E. pellita* F.Muell genotype (CEPT-3), two *E. urophylla* S.T.Blake × *E. grandis* clones (CEPT-4, CEPT-7), an *E. urophylla* clone (CEPT-5), and an *E. wetarensis* L.D.Pryor clone (CEPT-6). Isolates CMW12750 and CMW26908 were inoculated onto all seven the *Eucalyptus* genotypes. Because there were a limited number of *Eucalyptus* trees available for inoculation, the isolates (CMW24912, CMW24914, CMW24917) collected from *S. cumini* trees were inoculated only onto *Eucalyptus* genotypes CEPT-6 and CEPT-7.

Inoculated trees were approximately one-year-old at the time of the experiment. For each isolate, six to ten trees of each genotype were inoculated. For the negative controls, ten trees were inoculated with sterile MEA discs. Inoculations were made by using a 9-mm-in-diameter cork borer to remove the bark and expose the cambium at the same height (~400–800 mm) above the ground. Agar discs taken for the actively growing margins of the test isolates were placed in the wounds with the mycelium facing the cambium. In the case of the negative controls, sterile MEA was used. All inoculation wounds were covered with masking tape to reduce contamination and desiccation of the inoculum.

To test the susceptibility of *S. cumini* trees to the fungi isolated from South China, and also to compare the aggressiveness of isolates collected from *Syzygium* trees with those from the *Eucalyptus* on *S. cumini*, the same isolates (CMW12750, CMW26908, CMW24912, CMW24914, CMW24917) as those used for the *Eucalyptus* field tests were also inoculated onto branches of healthy *S. cumini* trees (Table 1). Ten branches were inoculated for each of the five selected isolates, and ten *S. cumini* branches were inoculated with sterile MEA as

negative controls. Inoculations were conducted using a method similar to that for the *Eucalyptus* genotype inoculation trials. The entire trial was repeated once.

The field trials were conducted during September to October of 2008 in ZhanJiang of GuangDong Province, China. After five and six weeks for the inoculations on *Eucalyptus* trees and the *S. cumini* branches, respectively, lesion lengths in the cambium of the trees were measured. Data for lesion length were analysed using PROC GLM (general linear model) in SAS (SAS Institute, 1999) in a manner similar to that for the greenhouse inoculations.

3. RESULTS

3.1. Sampling

During the disease surveys on *Eucalyptus* and *S. cumini* trees in GuangDong Province, typical symptoms of infection by fungi in the Cryphonectriaceae were observed. These included cracking of the bark and the formation of girdling stem cankers (Figs 1A, 1B). Apart from symptoms caused by *Chr. deuterocubensis* (Chen *et al*. 2010), anamorph and teleomorph fruiting structures of a fungus resembling species of *Celoporthe* were observed on diseased bark of *Eucalyptus* and *Syzygium* trees. These fruiting structures (anamorph and teleomorph) were often completely orange with orange to umber perithecial necks, and could be distinguished from fruiting structures of *Chr. deuterocubensis* that were always fuscous black to umber, with black and much longer perithecial necks (Gryzenhout *et al.* 2009; Chen *et al.* 2010). Ascostromata and conidiomata of the unknown fungus were often found on the cankers on the *S. cumini* trees, while only conidiomata were observed on *Eucalyptus* trees. Isolates were obtained from two *S. cumini* trees, one tree of an unknown *Eucalyptus* sp. and one tree representing *Eucalyptus* clone EC48 (Table 1).

3.2. DNA sequence comparisons

3.2.1. Generic placement

The aligned sequences of the LSU dataset consisted of 66 taxa and 639 characters (TreeBASE: http://purl.org/phylo/treebase/phylows/study/TB2:S11111). Statistical values for obtained trees for the parsimony analysis, number of informative characters and parameters for the best

fit substitution models are shown in Table 2. For the analyses of Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian analyses, the position of the genera in relation to each other was different, but genera could still be distinguished consistently from each other. A total of 100 most parsimonius trees were obtained after analyses of the LSU dataset, of which the first tree was saved for presentation (Fig 2). Isolates collected from *Eucalyptus* and *S. cumini* trees in China and *S. aromaticum* in Indonesia clearly resided in the genus *Celoporthe*, with *Celoporthe* representing a distinct lineage in the Cryphonectriaceae (Fig 2) (ML bootstrap = 63%).

The partition homogeneity test (PHT) comparing the exons in the the β-tubulin gene region and the 5.8S rRNA gene datasets gave a PHT value of P = 0.988, indicating that these two datasets were congruent and could be combined in the phylogenetic analyses. The aligned sequences of the combined datasets consisted of 61 taxa and 760 characters (TreeBASE: http://purl.org/phylo/treebase/phylows/study/TB2:S11111) (Table 2). Similar to results of the LSU dataset, the position of the genera in relation to each other differed slightly based on the three different analyses, but the topology was similar for the genera. Results showed that all the genera in Cryphonectriaceae resided in discrete clades with high support values in at least one of the three analyses (Fig 3). Only one most parsimonious tree was obtained (Fig 3). Based on the phylogenetic analyses of the combined BT exons and 5.8S gene sequences, the isolates from *Eucalyptus* and *S. cumini* trees in China and Indonesia grouped closely with those of *C. dispersa*, and separately from other genera in the Cryphonectriaceae (MP bootstrap = 87%, ML bootstrap = 89%, Bayesian posterior probabilities = 100%) (Fig 3). Within the *Celoporthe* clade, the isolates from Asia (China and Indonesia) formed several sub-clades separate from that representing *C. dispersa* from South Africa (MP bootstrap = 92%, ML bootstrap = 71%, Bayesian posterior probabilities = 99%) (Fig 3).

3.2.2. Species identification

The LSU phylogenetic analyses and the BLAST searches for the β-tubulin and ITS gene sequences showed that the isolates considered in this study were phylogenetically most closely related to *Celoporthe* spp. The type species of this genus, *C. dispersa* (Nakabonge *et al.* 2006a), and *Celoporthe* isolates (CMW10779, CMW10780, CMW10781) collected from *S. aromaticum* trees in Indonesia (Myburg *et al.* 2003; Nakabonge *et al.* 2006a), were included with the isolates collected from China for the species identification. The partition

homogeneity test (PHT) comparing the BT, ITS and TEF-1α datasets gave a PHT value of P = 0.998, indicating that these three datasets were congruent and could be combined for phylogenetic analyses. This was supported by the topologies of the separate trees of the three gene datasets that consistently showed the same phylogenetic groups (Figs 4A–4C).

The three datasets (TreeBASE: http://purl.org/phylo/treebase/phylows/study/TB2:S11111) were analysed separately. Four equally most parsimonious trees were obtained for the two BT gene regions (Fig 4A), while one most parsimonious tree was obtained for the ITS (Fig 4B) and TEF-1α (Fig 4C) datasets, respectively. Results for all three gene regions showed that the Asian isolates are distinct from *C. dispersa*, with long phylogenetic branch distances and high support values (Branch distances 17–42; MP bootstrap value 100% for each of the three genes) (Figs 4A–4C). The phylogenetic analyses for each of the BT, ITS and TEF-1α gene regions further showed that the Asian isolates formed four sub-clades supported by long phylogenetic branch distances and high support values (Branch distances up to 6; MP bootstrap value up to 100%; ML bootstrap value up to 99%; Bayesian posterior probabilities up to 100%). In these sub-clades, isolates (CMW24912, CMW24914, CMW24917, CMW34023, CMW34024) from *S. cumini* trees in China formed a single sub-clade, isolates CMW26900, CMW26908, CMW26911, CMW26913) rom a Chinese *Eucalyptus* clone formed a second clade, one isolate (CMW12750) from a Chinese *Eucalyptus* sp. resided in a third clade and isolates(CMW10779, CMW10780, CMW10781) from *S. aromaticum* trees in Indonesia formed the fourth clade (Fig 4A–4C).

Single nucleotide polymorphism (SNP) analyses supported the distinction of four sub-clades for the Asian (Chinese and Indonesian) *Celoporthe* isolates considered in this study. Unique SNPs could be identified for all four phylogenetic groups for each of the gene regions sequenced (Tables 3, 4). Comparisons of the three gene regions showed that each sub-clade was separated from the other three sub-clades by 4–11 unique SNPs for each gene region (Table 3). The total number of SNP differences between the four sub-clades, for all three gene regions combined varied between 21–31 unique SNPs (Table 4). No nucleotide differences could be detected in the BT, ITS and TEF-1α gene regions for the isolates within each of the three Chinese *Celoporthe* sub-clades. In the Indonesian *Celoporthe* sub-clade, some nucleotide differences in the BT (10 bp) and ITS (12 bp) regions were found between isolate CWM10780 and the other two (CMW10781, CMW10779) isolates. This could not be

confirmed for sequence data of the TEF-1α gene region for these isolates (CMW10779, CWM10780) since no living cultures exist for them.

3.3. Morphology

For the morphological examination of material for the various phylogenetic groups arising from DNA sequence comparisons, fruiting bodies of the teleomorph and anamorph states from natural infections were available only for the *S. cumini* isolates from South China. Naturally infected tissue from *Eucalyptus* in China, as well as those from Indonesian *S. aromaticum* (Myburg *et al*. 2003) was unavailable. The fungus on *S. cumini* trees in China was characterized by fruiting structures (Fig 5) that are morphologically similar to those of *C. dispersa* (Nakabonge *et al.* 2006a). Both of their teleomorph states were characterised by short, extending, orange to umber, cylindrical perithecical necks (Fig 5A), semi-immersed stromata (Fig 5B) and hyaline ascospores with one median septum (Fig 5G). For the anamorph, conidiomata were all pulvinate to conical without a prominent neck (Figs 5H–5J), orange when young (Fig 5H) and fuscous-black when mature. Their conidiomatal base tissue was prosenchymatous (Fig 5L), and paraphyses or cylindrical sterile cells were present (Fig 5M). Cultures were fluffy with uneven margins and white when young. A number of differences between *C. dispersa* and the specimens from *S. cumini* were visible. Asci of the fungus from *S. cumini* (av. 34.0 × 6.0 µm) were longer than those of *C. dispersa* (av. 26.5 × 6.3 µm). The conidiomatal locules in the *S. cumini* specimens were multilocular, seldom unilocular, while the structures of *C. dispersa* were unilocular, seldom multilocular. The conidia of the *S. cumini* specimens (av. 3.0 × 1.2 µm) were smaller than those of *C. dispersa* (av. 3.5 × 1.5 µm). Cultures of the fungus from *S. cumini* turned yellow white to sulphur yellow with sienna/umber patches, while those of *C. dispersa* turned umber to hazel to chestnut with grey patches (Nakabonge *et al.* 2006a).

Due to the unavailibility of naturally infected tissue representing all the four sub-clades from Asia, fruiting structures produced on the inoculated branch sections of *S. cordatum* and the *E. grandis* clone after six months, were used to determine morphological differences between the fungi in the sub-clades. For the branch sections where both ends were coated with melted paraffin-wax, abundant anamorph structures covered the surface of the bark (Figs 1C, 1D). For the branch sections where only the top ends were coated, substantially fewer anamorph

structures were produced. No teleomorph structures were produced on any of the inoculated branch sections.

Fruiting structures produced on the inoculated branch stubs were similar to those on naturally infected tissue. Except for the shape, size and position of conidiomata in the bark, the remaining morphology of structures resulting from the inoculations was similar to that on naturally infected *S. cumini* bark (Figs 5, 6). For isolates in the same phylogenetic groups, fruiting structures on the inoculated *S. cordatum* and *E. grandis* branch sections were similar in terms of conidiomatal position on the inoculated branch sections, shape, colour and tissue of conidiomata, structure of conidiomatal locules, as well as shape and size of conidiophores, conidiogenous cells, paraphyses and conidia. Only the sizes of conidiomata on *S. cordatum* were larger than those on *E. grandis* (Figs 6–9). Similar differences were observed by Hodges *et al*. (1986) who inoculated excised branch stubs to produce fruiting structures.

The four Asian phylogenetic sub-clades of *Celoporthe* could be distinguished from *C. dispersa* based on morphological characteristics observed on the limited naturally infected tissue as well as the artificially inoculated branch pieces. The conidiomata of *C. dispersa* are usually unilocular (Table 5) (Nakabonge *et al.* 2006a), while the conidiomata of isolates in all Asian sub-clades were predominantly multilocular (Table 5, Figs 5–9). The paraphyses of *C. dispersa* were shorter than those for all the isolates in the Asian sub-clades (Table 5). Lastly, the naturally infected bark from *S. cumini* trees showed that the asci of *C. dispersa* are much shorter than those of the Asian isolates (Table 5).

The four Asian phylogenetic sub-clades of *Celoporthe* could also be distinguished from *C. dispersa* by growth characteristics in culture. The optimal temperature for the growth of *C. dispersa* was 25ºC (Nakabonge *et al.* 2006a), while that for isolates in all of the Asian *Celoporthe* sub-clades was 30ºC.

Isolates representing the four Asian sub-clades of *Celoporthe* could be destinghuished from each other based on several morphological features, including length of conidiophores, and especially conidial shape and sizes as well as the length of paraphyses (Table 5, Figs 10A, 10B). Lengths of the paraphyses ($P < 0.01$ to $P = 0.018$) differed between isolates for each of the four Asian sub-clades. Except for conidial length between isolates of the two sub-clades from the Chinese *Eucalyptus* clone and an *Eucalyptus* species ($P = 0.74$), length of the conidia

($P < 0.01$) was significantly different for isolates representing the four Asian sub-clades (Figs 10A, 10B). For example, the conidia of isolates in the Indonesian *Celoporthe* sub-clade were the longest ($P < 0.01$) of all isolates in the four Asian sub-clades, while the paraphyses of these isolates were shorter ($P < 0.01$ to $P = 0.018$) than those of isolates in the two Chinese *Celoporthe* sub-clades from *Eucalyptus* trees (Table 5, Figs 10A, 10B).

3.4. Taxonomy

Based on phylogenetic analyses, the Asian isolates characterized in this study reside in the genus *Celoporthe*. These isolates could be divided into four phylogenetic sub-clades, separate from that of the type species, *C. dispersa.* These sub-clades and *C. dispersa* could also be distinguished from each other and *C. dispersa*, based on characteristics of the asci, conidiomata, conidiophores, conidia, paraphyses and growth in culture. The Asian isolates, therefore, represent four distinct species and are described as follows:

Celoporthe syzygii S.F. Chen, Gryzenh., M.J. Wingf. & X.D. Zhou, sp. nov.
(Figs 5, 6)
MycoBank no.: MB519066
Etymology: The name refers to the fact that the species was isolated from *Syzygium* trees.

Asci fusoidei (29.5–)31.5–36.5(–43) × (5–)5.5–6.5(–7) µm. Ascosporae oblongo-ellipsoideae (5–)6–7.5(–8.5) × 2.5–3(–3.5) µm. Loculi conidiomatum multiloculares raro uniloculares. Conidiophorae (4.5–)6.0–11.0(–16.0) µm longae. Paraphyses usque ad 52 µm. Conidia oblonga vel cylindrica raro allantoidea, (2.3–)2.8–3.4(–3.8) × (1.0–)1.3(–1.6) µm. A speciebis aliis cladi asiatici Celoporthis (*C. eucalypti*, *C. guangdongensis*, *C. indonesiensis*) nucleotidis unice fixis in tribus locis nuclearibus differt: β-tubulinum-1 sitis 141 (T), 202 (A); β-tubulinum-2 sitis 223 (A), 241 (G), 327 (A), loco vulgo “internal transcribed spacer rDNA” dicto (ITS1, 5.8S et ITS2) sitis 92 (T), 93 (C), 97 (T), 100 (C) et 132 (–) et loco vulgo “translation elongation factor 1-alpha” dicto sitis 47 (T) et 48 (T).

Ascostromata on the bark taken from cankers on *S. cumini* trees gregarious, seldom single, immersed or semi-immersed; recognizable by short, extending, umber, cylindrical perithecial necks, occasionally erumpent, limited; orange to umber ascostromatic tissue covering the tops of the perithecial bases; ascostromata 40–240 µm (av. 120 µm) high above the level of bark

and 280–580 µm (av. 300 µm) wide above the surface of the bark (Fig 5A). **Stromatic tissue** pseudoparenchymatous at the edges, prosenchymatous in the centre (Fig 5E). **Perithecia** valsoid, 1–15 per stroma; bases immersed in the bark, black, globose to subglobose, 60–380 µm (av. 170 µm) diam; perithecial wall 10–35 µm (av. 18 µm) thick (Figs 5B, 5C); perithecial necks black, emerging through the stromatal surface, covered in orange to umber stromatic tissue of *textura porrecta* (Fig 5D), necks emerge at stromatal surface as black ostioles covered with umber to brown stromatal tissue to form papillae extending up to 40 µm long, 40–90 µm (av. 55 µm) wide (Fig 5C). **Asci** 8-spored, biseriate, unitunicate, free when mature, non-stipitate with a non-amyloid refractive ring, fusoid, (29.5–)31.5–36.5(–43) × (5–)5.5–6.5(–7) µm (Fig 5F). **Ascospores** hyaline, with one median septum, oblong-ellipsoidal, with rounded ends, (5–)6–7.5(–8.5) × 2.5–3(–3.5) µm (Fig 5G).

Conidiomata part of ascomata as conidial locules or as solitary structures; immersed to semi-immersed to superficial; pulvinate without necks, occasionally with a neck that is attenuated, orange when young, umber to brown to fuscous-black when mature; conidiomatal base 90–300 µm (av. 170 µm) high above the level of bark and 180–900 µm (av. 420 µm) wide above the surface of the bark (Figs 5H, 5I). **Conidiomatal locules** multilocular, seldom unilocular, locules 50–650 µm (av. 260 µm) diam (Figs 5J, 5K). **Stromatic tissue** of base prosenchymatous (Fig 5L). **Conidiophores** hyaline, branched irregularly at the base or above into cylindrical cells, with or without separating septa, (4.5–) 6.0–9.0(–12.5) µm (av. 8.0 µm) long (Fig 5N, 5O). **Conidiogenous cells** phialidic, cylindrical with or without attenuated apices, 1.0–2.0(–2.5) µm (av. 2.0 µm) wide (Figs 5N, 5O). **Paraphyses** or cylindrical sterile cells occur among conidiophores, up to 44 µm long (av. 17 × 1.5 µm) (Fig 5M). **Conidia** hyaline, non-septate, oblong to cylindrical, occasionally allantoid, pushed through opening at stromatal surface as orange droplets (Fig 5I), (2.3–)2.8–3.3(–3.7) × (1.0–)1.1–1.3(–1.5) µm (av. 3.0 × 1.2 µm) (Fig 5P).

No ascostroma were observed on either inoculated *S. cordatum* or *E. grandis* branch tissue. The inoculated material on both the *S. cordatum* and *E. grandis* branch tissue was similar in morphology to the structures on field-collected *S. cumini* bark (Figs 5, 6). However, **conidiomata** on inoculated *S. cordatum* and *E. grandis* branches were eustromatic, superficial to slightly immersed (Figs 6B, 6H); conidiomatal base on *S. cordatum* 100–400 µm (av. 160 µm) high above the level of bark and 200–700 µm (av. 320 µm) wide above the surface of the bark (Fig 6A); on *E. grandis* 80–280 µm (av. 140 µm) high above the level of bark and 140–

620 µm (av. 290 µm) wide above the surface of the bark (Fig 6G). **Conidiomatal locules** on *S. cordatum* 70–540 µm (av. 250 µm) diam (Fig 6B), on *E. grandis* 40–490 µm (av. 240 µm) diam (Fig 6H). **Paraphyses** or cylindrical sterile cells on *S. cumini* branches up to 42 µm long (av. 18 × 1.5 µm) (Fig 6D), on *E. grandis* branches up to 52 µm long (av. 24 × 1.5 µm).

Celoporthe syzygii differs from other species in the Asian clade of *Celoporthe* (*C. eucalypti*, *C. guangdongensis*, *C. indonesiensis*) by uniquely fixed DNA nucleotides in three nuclear loci: β-tubulin-1 positions 141 (T) and 202 (A), β-tubulin-2 positions 223 (A), 241 (G) and 327 (A); internal transcribed spacer rDNA (ITS1, 5.8S, ITS2) positions 92 (T), 93 (C), 97 (T), 100 (C) and 132 (–); translation elongation factor 1-alpha positions 47 (T) and 48 (T).

Culture Characteristics: On MEA, *C. syzygii* fluffy with an uneven margin, white when young, turning yellow white to sulphur yellow with sienna/umber patches after 10 days. Colony reverse white to yellow white. Optimal growth temperature 30°C, covering the 90 mm plates after five days. No growth at 5°C and 35°C, and colonies at 10 °C reaching 31.5 mm in 30 days (reach 6 mm in seven days). Asexual fruiting structures occasionally form in primary isolations of the fungus.

Substrate: Bark of *Syzygium cumini*.

Distribution: China.

Specimens examined: China, GuangDong Province, *S. cumini*. September, 2008, S.F. Chen, holotype PREM 60462, ex-type culture CMW34023 = CBS127218; paratype PREM 60463, living culture CMW34024; December, 2006, M.J. Wingfield & X.D. Zhou, paratype PREM 60464 (isolate CMW24912= CBS127188, artificial inoculation on South African *S. cordatum* and *E. grandis* branch tissue in April, 2008, S.F. Chen); December, 2006, M.J. Wingfield & X.D. Zhou, paratype PREM 60465 (isolate CMW24914 = CBS127189, artificial inoculation on South African *S. cordatum* and *E. grandis* branch tissue in April, 2008, S.F. Chen).

Notes: *Celoporthe syzygii* is morphologically distinguishable from *C. dispersa* by having longer asci (av. 34 × 6 µm, L/W = 5.7) than *C. dispersa* (av. 26 × 6.3 µm, L/W = 4.1). The inoculated conidiomata of *C. syzygii* are more superficial than those of structures formed in nature. The conidia of *C. syzygii* (2.3–3.8 µm) are shorter than those of *C. guangdongensis* (2.4–4.3 µm), *C. eucalypti* (2.6–4.4 µm), *C. indonesiensis* (3.1–4.7 µm) and *C. dispersa* (2.5–5.5 µm).

Celoporthe eucalypti S.F. Chen, Gryzenh., M.J. Wingf. & X.D. Zhou, sp. nov.

(Fig 7)

MycoBank no.: MB519067

Etymology: Name refers to *Eucalyptus* and the first host from which this fungus was collected.

Loculi conidiomatum multiloculares raro uniloculares. Conidiophorae (4.5–)9.0–14.0(–25.0) µm longae. Paraphyses usque ad 68 µm. Conidia (2.6–)3.1–3.8(–4.4) × (1.1–)1.4–1.6(–1.8) µm. A speciebis aliis cladi asiatici Celoporthis (*C. syzygii*, *C. guangdongensis*, *C. indonesiensis*) nucleotidis unice fixis in duobus locis nuclearibus differt: β-tubulinum-1 sitis 119 (T) et 192 (T), β-tubulinum-2 sito 24 (T), et loco vulgo “translation elongation factor 1-alpha” dicto sito 35 (T).

No ascostromata were observed on either the field-collected *Eucalyptus* bark or on inoculated *S. cordatum* and *E. grandis* branch tissue. Both on *S. cordatum* and *E. grandis*, the conidiomata on inoculated branch tissue were eustromatic, superficial to slightly immersed, pulvinate without necks, sometimes conical, orange to umber when young, fuscous-black when mature (Figs 7A, 7H). **Stromatic tissue** of base prosenchymatous (Fig 7C). However, the conidiomatal base on *S. cordatum* 120–600 µm (av. 260 µm) high above the level of bark and 150–700 µm (av. 400 µm) wide above the surface of the bark (Fig 7H), on *E. grandis* 100–410 µm (av. 210 µm) high above the level of bark and 140–650 µm (av. 340 µm) wide above the surface of the bark (Fig 7A). **Conidiomatal locules** multilocular, seldom unilocular, locules on *S. cordatum* 50–510 µm (av. 240 µm) diam (Fig 7I), locules on *E. grandis* 35–580 µm (av. 260 µm) diam (Fig 7B). **Conidiophores** hyaline, branched irregularly at the base or above into cylindrical cells, with or without separating septa, on *S. cordatum* (5.5–)9.5–13.5(–22.5) µm (av. 12.0 µm) long (Fig 7F), on *E. grandis* (4.5–)9.0–14.0(–25.0) µm (av. 12 µm) long (Fig 7E). **Conidiogenous cells** phialidic (Fig 7F), cylindrical with or without attenuated apices, on *S. cordatum* 1.5–2.5(–3.0) µm (av. 2.0 µm) wide, on *E. grandis* 1.5–3.0(–3.5) µm (av. 2.5 µm) wide (Fig 7E). **Paraphyses** or cylindrical sterile cells occur among conidiophores, on *S. cordatum* up to 68 µm long (av. 44 × 1.7 µm) (Fig 7J), on *E. grandis* up to 62 µm long (av. 40 × 1.6 µm) (Fig 7D). **Conidia** hyaline, non-septate, oblong to cylindrical, occasionally allantoid, extend through opening at stromatal surface as orange droplets, on *S. cordatum* (2.6–)3.2–3.7(–4.1) × (1.2–)1.4–1.6(–1.8) µm, (av. 3.5 × 1.5 µm), on *E. grandis* (2.7–)3.1–3.8(–4.4) × (1.1–)1.5–1.7(–1.8) µm, (av. 3.5 × 1.6 µm) (Fig 7G).

Celoporthe eucalypti differs from other species in the Asian clade of *Celoporthe* (*C. syzygii*, *C. guangdongensis*, *C. indonesiensis*) by uniquely fixed DNA nucleotides in two nuclear loci: β-tubulin-1 positions 119 (A) and 192 (T), β-tubulin-2 position 24 (T); translation elongation factor 1-alpha position 35 (T).

Culture Characteristics: On MEA, *C. eucalypti* fluffy with an uneven margin, white when young, turning pale luteous to luteous after 10 days. Colony reverse sulphur yellow to pale luteous after 10 days. Optimal growth temperature 30°C covering the 90 mm plates after six days. No growth at 5°C and 35°C, colonies at 10°C reaching 16 mm in 30 days (reach 4 mm in seven days). Asexual fruiting structures occasionally form in primary isolations of the fungus.

Substrate: Bark of *Eucalyptus* clone.

Distribution: China.

Specimens examined: China, GuangDong Province, *Eucalyptus* clone. January, 2007, X.D. Zhou & S.F. Chen. Holotype PREM 60467 (isolate CMW26908, artificial inoculation on South African *S. cordatum* and *E. grandis* branch tissue in April, 2008, S.F. Chen), ex-type culture CMW26908 = CBS127190; paratype PREM 60466 (isolate CMW26900 = CBS127191, artificial inoculation on South African African *S. cordatum* and *E. grandis* branch tissue in April, 2008, S.F. Chen).

Notes: *Celoporthe eucalypti* is morphologically similar to *C. guangdongensis*, but can be distinguished by having shorter paraphyses (up to 68 µm) than *C. guangdongensis* (up to 91 µm). *C. eucalypti* can be distinguished from *C. dispersa* (paraphyses up to 39 µm), *C. indonesiensis* (paraphyses up to 50 µm) and *C. syzygii* (paraphyses up to 52 µm) by its longer paraphyses. At 10 C, on 2% MEA after 30 d, *C. eucalypti* grew faster (av. colony diam. = 16 mm) than *C. guangdongensis* (av. colony diam. = 7 mm) and *C. indonesiensis* (av. colony diam. = 1.5 mm), but slower than *C. syzygii* (av. colony diam. = 31.5 mm).

Celoporthe guangdongensis S.F. Chen, Gryzenh., M.J. Wingf. & X.D. Zhou, sp. nov.

(Fig 8)

MycoBank no.: MB519068

Etymology: Name reflects the Guangdong Province of China where this fungus was first found.

Loculi conidiomatum multiloculares raro uniloculares. Conidiophorae (4.5–)10.0–16.0(–27.5) µm longae. Paraphyses usque ad 91 µm. Conidia (2.4–)3.1–3.9(–4.3) × (1.1–)1.3–1.7(–1.9) µm. A speciebis aliis cladi asiatici Celoporthis (*C. syzygii*, *C. guangdongensis*, *C. indonesiensis*) nucleotidis unice fixis in tribus locis nuclearibus differt: β-tubulinum-1 sitis 71 (T), 188 (T), 189 (C), 194 (T); β-tubulinum-2 sitis 233 (–) et 234 (–); loco vulgo "internal transcribed spacer rDNA" dicto (ITS1, 5.8S et ITS2) sitis 181 (A), 489 (T) et loco vulgo "translation elongation factor 1-alpha" dicto sitis 230 (G), 240 (C) et 249 (C).

No ascostromata were observed on *Eucalyptus* bark collected in the field, nor on inoculated *S. cordatum* or *E. grandis* branch tissue. Both on *S. cordatum* and *E. grandis*, the conidiomata on inoculated branch tissue were eustromatic, superficial to slightly immersed, pulvinate without necks, sometimes conical, orange to umber when young, fuscous-black when mature (Figs 8A, 8H). **Stromatic tissue** of base prosenchymatous (Fig 8C). However, the conidiomatal base on *S. cordatum* 90–720 µm (av. 280 µm) high above the level of bark and 140–750 µm (av. 430 µm) wide above the surface of the bark (Fig 8H), on *E. grandis* 110–500 µm (av. 230 µm) high above the level of bark and 120–700 µm (av. 360 µm) wide above the surface of the bark (Fig 8A). **Conidiomatal locules** multilocular, seldom unilocular, locules on *S. cordatum* 40–590 µm (av. 270 µm) diam (Fig 8I), on *E. grandis* 35–640 µm (av. 290 µm) diam (Fig 8B). **Conidiophores** hyaline, branched irregularly at the base or above into cylindrical cells, with or without separating septa, on *S. cordatum* (4.5–)10.0–15.0(–24.5) µm (av. 13.0 µm) long, on *E. grandis* (5.0–)11.0–16.0(–27.5) µm (av. 14 µm) long (Figs 8E, 8F). **Conidiogenous cells** phialidic, cylindrical with or without attenuated apices, on *S. cordatum* 1.5–2.5(–3.0) µm (av. 2.0 µm) wide, on *E. grandis* 1.5–3.0(–3.5) µm (av. 2.0 µm) wide (Figs 8E, 8F). **Paraphyses** or cylindrical sterile cells occur among conidiophores, on *S. cordatum* up to 82 µm long (av. 58 × 1.9 µm) (Fig 8J), on *E. grandis* up to 91 µm long (av. 52 × 1.8 µm) (Fig 8D). **Conidia** hyaline, non-septate, oblong to cylindrical, occasionally allantoid, extending through opening at stromatal surface as orange droplets, on *S. cordatum* (2.4–)3.3–3.7(–4.0) × (1.1–)1.3–1.6(–1.8) µm, (av. 3.5 × 1.5 µm), on *E. grandis* (2.5–)3.1–3.9(–4.3) × (1.1–)1.4–1.7(–1.9) µm, (av. 3.5 × 1.5 µm) (Fig 8G).

Celoporthe guangdongensis differs from other species in the Asian clade of *Celoporthe* (*C. syzygii*, *C. eucalypti*, *C. indonesiensis*) by uniquely fixed DNA nucleotides in three nuclear loci: β-tubulin-1 positions 71 (T), 188 (T), 189 (C) and 194 (T); β-tubulin-2 positions 233 (–)

and 234 (–); internal transcribed spacer rDNA (ITS1, 5.8S, ITS2) positions 181 (A) and 489 (T); translation elongation factor 1-alpha positions 230 (G), 240 (C) and 249 (C).

***Culture Characteristics**:* On MEA, *C. guangdongensis* is fluffy with uneven margins, white when young, turning greenish grey to greenish black after 10 days. Colony reverse smoke grey to greenish grey after 10 days. Optimal growth temperature 30°C covering 90 mm plates after seven days. No growth at 5°C and 35°C, and colonies at 10°C reaching 7 mm in 30 days (nearly no growth in seven days). Asexual fruiting structures occasionally form in primary isolations of the fungus.

Substrate: Bark of *Eucalyptus* sp.

Distribution: China.

Specimens examined: China, GuangDong Province, *Eucalyptus* sp., T.I. Burgess, holotype PREM 60468 (isolate CMW12750, artificial inoculation on South African *S. cordatum* and *E. grandis* branch tissue in April, 2008, S.F. Chen), ex-type culture CMW12750 = CBSXXXXXX.

Notes: *Celoporthe guangdongensis* is morphologically different to *C. dispersa* (paraphyses up to 39 µm), *C. indonesiensis* (paraphyses up to 50 µm), *C. syzygii* (paraphyses up to 52 µm) and *C. eucalypti* (paraphyses up to 68 µm) because of its longer paraphyses (up to 91 µm). This species was described based on the single isolate CMW12750 but supported by strong phylogenetic data and morphological differences with other *Celoporthe* spp.

Celoporthe indonesiensis S.F. Chen, Gryzenh., M.J. Wingf. & X.D. Zhou, gen. nov.
(Fig 9)
MycoBank no.: MB519069
Etymology: Name refers to Indonesia, the country where the the fungus was first collected.

Loculi conidiomatum multiloculares raro uniloculares. Conidiophorae (5.5–)12.0–18.0(–32.0) µm longae. Paraphyses usque ad 50 µm. Conidia (3.1–)3.5–4.2(–4.7) × (1.1–)1.2–1.5(–1.6) µm. A speciebis aliis cladi asiatici Celoporthis (*C. syzygii*, *C. guangdongensis*, *C. eucalypti*) nucleotidis unice fixis in tribus locis nuclearibus differt: β-tubulinum-2 sitis 235 (T), 236 (T) 238 (T); loco vulgo “internal transcribed spacer rDNA” dicto (ITS1, 5.8S et ITS2) sitis 40 (T), 82 (A), 84 (T), 85 (T), 211 (A) et loco vulgo “translation elongation factor 1-alpha” dicto sitis 76 (G), 215 (C).

No ascostromata were observed on the bark of *S. aromaticum* collected in the field, nor on inoculated *S. cordatum* and *E. grandis* branch tissue. Both on *S. cordatum* and *E. grandis*, the conidiomata on inoculated branch tissue were eustromatic, superficial to slightly immersed, pulvinate without necks, orange when young, umber to fuscous-black when mature (Fig 9A, 9H). **Stromatic tissue** of base, prosenchymatous (Fig 9C). However, the conidiomatal base on *S. cordatum* 60–260 µm (av. 120 µm) high above the level of bark and 120–550 µm (av. 260 µm) wide above the surface of the bark (Fig 9A), on *E. grandis* 60–250 µm (av. 100 µm) high above the level of bark and 100–470 µm (av. 190 µm) wide above the surface of the bark (Fig 9H). **Conidiomatal locules** multilocular, seldom unilocular, locules on *S. cordatum* 50–610 µm (av. 310 µm) diam (Fig 9B), on *E. grandis* 35–530 µm (av. 280 µm) diam (Fig 9I). **Conidiophores** hyaline, branched irregularly at the base or above into cylindrical cells, with or without separating septa, on *S. cordatum* (6.0–)14.0–18.0(–29.0) µm (av. 16.0 µm) long (Fig 9E, 9F), on *E. grandis* (5.5–)12.0–17.5(–32.0) µm (av. 15.0 µm) long. **Conidiogenous cells** phialidic, cylindrical with or without attenuated apices, on *S. cordatum* 1.5–2.5(–3.5) µm (av. 2.0 µm) wide (Figs 9E, 9F), on *E. grandis* 1.0–3.0 µm (av. 2.5 µm) wide. **Paraphyses** or cylindrical sterile cells occur among conidiophores, on *S. cordatum* up to 48 µm long (av. 26 × 1.5 µm) (Fig 9D), on *E. grandis* up to 50 µm long (av. 28 × 1.5 µm). **Conidia** hyaline, non-septate, cylindrical, occasionally oblong or allantoid, extending through opening at stromatal surface as orange droplets, on *S. cordatum* (3.1–)3.5–4.0(–4.6) × (1.1–)1.2–1.4(–1.5) µm, (av. 3.8 × 1.3 µm) (Fig 9G), on *E. grandis* (3.5–)3.7–4.2(–4.7) × (1.1–)1.2–1.5(–1.6) µm, (av. 3.9 × 1.4 µm) (Fig 9J).

Celoporthe indonesiensis differs from other species in the Asian clade of *Celoporthe* (*C. syzygii*, *C. eucalypti*, *C. guangdongensis*) by uniquely fixed DNA nucleotides in three nuclear loci: β-tubulin-2 positions 235 (T), 236 (T), and 238 (T); internal transcribed spacer rDNA (ITS1, 5.8S, ITS2) positions 40 (T), 82 (A), 84 (T), 85 (A) and 211 (A); translation elongation factor 1-alpha positions 76 (G) and 215 (C).

Culture Characteristics: On MEA, *C. indonesiensis* fluffy with an uneven margin, white when young, turning sulphur yellow to pale luteous after 10 days. Colony reverse yellow white to sulphur yellow after 10 days. Optimal growth temperature 30°C, covering the 90 mm plates after six days. No growth at 5°C and 35°C, and colonies at 10°C reaching 1.5 mm in 30

days (nearly no growth in seven days). Asexual fruiting structures occasionally form in primary isolations of the fungus.

Substrate: Bark of *Syzygium aromaticum*.

Distribution: Indonesia.

Specimens examined: Indonesia, North Sumatra, *S. aromaticum*, September 1997, M.J. Wingfield, holotype PREM 60469 (isolate CMW10781, artificial inoculation on South African *S. cordatum* and *E. grandis* branch tissue in April, 2008, S.F. Chen), ex-type culture CMW10781 = CBS115844.

Notes: *Celoporthe indonesiensis* is morphologically different from *C. syzygii* (conidia 2.3–3.8 µm; paraphyses up to 52 µm), *C. eucalypti* (conidia 2.6–4.4 µm; paraphyses up to 68 µm) and *C. guangdongensis* (conidia 2.4–4.3 µm; paraphyses up to 91 µm) because of its longer conidia (3.1–4.7 µm) and shorter paraphyses (up to 50 µm). Furthermore, its conidia are shorter and paraphyses are longer than that of *C. dispersa* (conidia 2.5–5.5 µm; paraphyses up to 39 µm). At 10 C, on 2% MEA after 30 d, *C. indonesiensis* grew slower (av. colony diam. = 1.5 mm) than *C. guangdongensis* (av. colony diam. = 7 mm), *C. eucalypti* (av. colony diam. = 16 mm), and *C. syzygii* (av. colony diam. = 31.5 mm). This species is described based on the phylogenetic placement of three isolates (CMW10779, CMW10780, CMW10781), and the morphology of a single isolate (CMW10781), and is supported by strong phylogenetic and morphological differences from other *Celoporthe* spp.

Dichotomous Key to *Celoporthe* species

The following dichotomous key is based on characteristics of naturally occurring and artificially induced conidia and paraphyses, as well as growth in culture:

1a. Optimal growth at 30ºC; conidia shorter than 5 µm; paraphyses longer than 45 µm; conidiomata multilocular, occasionally unilocular; Asci* longer than 40 µm…….2

1b. Optimal growth at 25ºC; conidia longer than 5 µm; paraphyses shorter than 40 µm; conidiomata unilocular, occasionally multilocular; Asci* shorter than 35 µm……*C. dispersa*

2a. Conidia shorter than 4.5 µm, oblong, occasionally allantoid…….3

2b. Conidia longer than 4.5 µm, cylindrical, occasionally oblong……..*C. indonesiensis*

3a. Conidia longer than 4 µm……...4

3b. Conidia shorter than 4 µm……*C. syzygii*

4a. Paraphyses longer than 90 µm……...*C. guangdongensis*

4b. Paraphyses shorter than 70 µm…….*C. eucalypti*

* Teleomorph structures have not been seen for *C. eucalypti, C. guangdongensis* or. *indonesiensis*.

Synoptic Key to *Celoporthe* Species

Optimum growth:

a. 25ºC: *C. dispersa*

b. 30ºC: *C. eucalypti, C. guangdongensis, C. indonesiensis, C. syzygii*

Ascus size*:

a. Asci shorter than 35 µm: *C. dispersa*

b. Asci longer than 40 µm: *C. syzygii*

Conidiomatal locules:

a. Unilocular, occasionally multilocular: *C. dispersa*

b. Multilocular, occasionally unilocular: *C. eucalypti, C. guangdongensis, C. indonesiensis, C. syzygii*

Conidia morphology (length):

a. Up to 4 µm: *C. syzygii*

b. Up to 4.5 µm: *C. eucalypti, C. guangdongensis*

c. Up to 4.75 µm: *C. indonesiensis*

d. Up to 5.5 µm: *C. dispersa*

Paraphyses morphology (length):

a. Up to 40 µm: *C. dispersa*

b. Up to 50 µm: *C. indonesiensis*

c. Slightly longer than50 µm: *C. syzygii*

d. Up to 70 µm: *C. eucalypti*

e. Up to 90 µm: *C. guangdongensis*

Conidial morphology:

a. Conidia mostly cylindrical, occasionally oblong or allantoid: *C. indonesiensis*

b. Conidia mostly oblong, occasionally allantoid: *C. dispersa, C. eucalypti, C. guangdongensis, C. syzygii*

* Teleomorph structures have not been seen for *C. eucalypti, C. guangdongensis* or. *indonesiensis*.

3.5. Pathogenicity tests

3.5.1. Glasshouse trials

The three isolates of *C. eucalypti* (CMW26900, CMW26908, CMW26911) tested for pathogenicity on *Eucalyptus* clone TAG-5 in the glasshouse, produced lesions within six weeks, while wounds were covered with callus tissue in the case of the control inoculations (Fig 11). The mean comparison tests showed that the lesion lengths produced by the *C. eucalypti* isolates were significantly longer ($P < 0.0001$ to $P = 0.0047$) than those of the controls (Fig 11). Analyses of variance showed significant differences in susceptibility of the *Eucalyptus* clone to the fungal isolates ($P = 0.0433$). Of the isolates tested, lesions produced by isolate CMW26908 were the longest, differing significantly from isolate CMW26900 and the controls (Fig 11). All the inoculated fungi were successfully re-isolated from the lesions, while *Celoporthe* was not isolated from the control inoculations. Based on the glasshouse trial, the most virulent isolate CMW26908 was chosen for use in field inoculations.

3.5.2. Field trials

Statistically significant differences in lesion lengths on the seven *Eucalyptus* genotypes were found between the isolates identified as *C. eucalypti* (CMW26908) and *C. guangdongensis* (CMW12750), and the control inoculations ($P < 0.0001$ to $P = 0.0230$) after five weeks under field conditions (Fig 12). *Eucalyptus* genotypes CEPT-2 and CEPT-6 were the most susceptible to both *Celoporthe* spp., while genotypes CEPT-3, CEPT-4 and CEPT-5 were relatively tolerant to infection by *C. eucalypti* and *C. guangdongensis* (Fig 12).

The trial including three *C. syzygii* isolates (CMW24912, CMW24914, CMW24917), *C. eucalypti* (CMW26908) and *C. guangdongensis* (CMW12750) on two *Eucalyptus* genotypes (CEPT-6, CEPT-7), showed that the *Celoporthe* isolates produced significantly longer lesions ($P < 0.0001$ to $P = 0.0380$) on the two tested *Eucalyptus* genotypes than the control inoculations that had started to heal through the production of callus tissue (Fig 13A). Analyses of variance showed significant differences ($P < 0.001$) in susceptibility between the two *Eucalyptus* genotypes, with CEPT-6 being more susceptible to infection by *C. guangdongensis* (CMW12750) and *C. syzygii* (CMW24912, CMW24914, CMW24917) than

CEPT-7 (P < 0.0001 to P = 0.0302) (Fig 13A). No statistically significant differences were found between CEPT-6 and CEPT-7 for *C. eucalypti* (CMW26908) (P = 0.1621). No differences were found between the *C. syzygii* isolates and the two *Celoporthe* spp. that originated from *Eucalypts* on clone CEPT-6 (P = 0.0676 to P = 1.0000), however, on CEPT-7 the *Eucalyptus* isolates, CMW26908 (*C. eucalypti*) and CMW12750 (*C. guangdongensis* showed higher virulence on *Eucalypts* than the *C. syzygii* isolates.

The three *Celoporthe* spp. inoculated onto the branches of *S. cumini* produced significantly **(**P < 0.0001) longer lesions than the control inoculations after six weeks (Fig 13B). The analyses showed no statistical differences between the two sets of inoculations (P = 0.2421) and, therefore, the data were combined. Analyses of variance for the combined data showed significant differences in susceptibility to the fungal isolates (P = 0.0004). For example, the *C. eucalypti* isolate (CMW26908) produced significantly (P = 0.0001 to P = 0.0215) longer lesions than the *C. guangdongensis* isolate (CMW12750) and the *C. syzygii* isolates (CMW24912, CMW24914; Fig 13B).

4. DISCUSSION

In this study, four previously undescribed species of *Celoporthe* were found on bark collected from diseased *Syzygium* and *Eucalyptus* trees in Asia. *C. syzygii* was collected from *S. cumini* trees in China, both *C. eucalypti* and *C. guangdongensis* from non-native *Eucalyptus* trees in China, and *C. indonesiensis* isolates originated from *S. aromaticum* trees in Indonesia. The identification of these species was supported by DNA sequence comparisons as well as morphological characteristics. The species from *Eucalyptus* in China were also shown to be capable of causing lesions on commercially utilised *Eucalyptus* genotypes and on the branches of *S. cumini* trees.

In this study, anamorph fruiting structures were successfully induced on *Eucalyptus* and *Syzygium* branch sections for all of the *Celoporthe* isolates. Differences were observed between the naturally occuring anamorph structures for *C. syzygii* and those formed on the inoculated branch sections, especially relating to the shape and position of conidiomata. However, characteristics such as diameter of conidiomata, sizes of conidiophores, conidigenous cells and conidia in *C. syzygii* were similar in the artificially induced and

naturally occurring fruiting structures and they were, therefore, useful in distinguishing between the four Asian phylogenetic *Celoporthe* spp.

Phylogenetic comparisons for isolates of *Celoporthe* suggested clustering of species based on geographic origin and host. Thus, the three Chinese *Celoporthe* spp. were more closely related to each other than to Indonesian *C. indonesiensis*. In contrast the Chinese *C. eucalypti* and *C. guangdongensis* from *Eucalyptus* in China were more closely related to each other than to *C. syzygii* from *Syzygium*. Similarly, the *Celoporthe* species formed two major sub-clades, one including *C. dispersa* from Africa and the other including the Asian species described in the present study. Studies by Nakabonge *et al*. (2006a) also suggested clustering of *Celoporthe* isolates from South Africa, based on host and regions of collection, but they were not able to explain these results due to the limited number of isolates available (Nakabonge *et al*. 2006a).

The discovery of the *Celoporthe* spp. in Asia raises interesting questions regarding the origin of these fungi. Previous research results suggest that *C. dispersa* may have originated from native African Myrtales (Nakabonge *et al*. 2006a). Similarly, all four *Celoporthe* spp. that were identified from non-native trees in this study may have originated from native Myrtales in South or Southeast Asia. This is especially likely due to the species diversity of *Celoporthe* in the region. Additional surveys are, however, necessary to expand the host and geographic ranges of *Celoporthe* spp. in Southeast Asia and other regions to obtain a better indication of their possible origins.

Previous research has shown that some pathogens in the Cryphonectriaceae may undergo host shifts (Slippers *et al*. 2005) enabling them to infect tree species other than their native hosts (Wingfield 2003; Gryzenhout *et al*. 2004, 2006b, 2009, 2010; Heath *et al*. 2006; Nakabonge *et al*. 2006a, b; Begoude *et al*. 2010; Vermeulen *et al*. 2010). It has further been suggested that species of *Chrysoporthe* that infect non-native plantion-grown *Eucalyptus* have originated from native trees in the Myrtales (Gryzenhout *et al*. 2004; Rodas *et al*. 2005; Heath *et al*. 2006; Gryzenhout *et al*. 2006b, 2009, 2010). A similar host shift could have occurred with *C. dispersa* and the four *Celoporthe* spp. described in this study. In the present study, all four *Celoporthe* spp. were collected from non-native trees. This includes *C. indonesiensis*, which was found on planted *S. aromaticum* trees in North Sumatra, Indonesia, where these trees do not occur naturally. The ability of the four *Celoporthe* spp. to infect *Syzygium* trees

that are native to South and Southeast Asia, suggests that these fungi could have originated from these or related trees and that they have undergone host shifts to infect *Eucalyptus* species.

The pathogenicity tests in this study showed that Chinese *Celoporthe* spp. from *S. cumini* and *Eucalyptus* are pathogenic to various *Eucalyptus* genotypes and *S. cumini* trees. Similar lesion sizes were also produced on both *Eucalyptus* genotypes and *S. cumini* trees by *Chr. deuterocubensis* (Chen *et al*. 2010), which suggests that *Celoporthe* spp. may be equally important potential pathogens of *Eucalyptus* in China. Similar to the results of the screening trials with *Chr. deuterocubensis* in China (Chen *et al*. 2010), the inoculations in this study showed that *Eucalyptus* genotypes differ in susceptibility to infection by *Celoporthe* spp. These results further imply that it will be possible to select *Eucalyptus* planting stock with tolerance to infection by these fungi.

Pathogenicity tests showed that cross-infectivity between *Eucalyptus* and *Syzygium* is possible by the *Celoporthe* spp. described in thus study. For example, the isolates of *C. syzygii* collected from *Syzygium* trees were more pathogenetic on the tested *Eucalyptus* genotype CEPT-6 than isolates of *C. eucalypti* and *C. guangdongensis* from *Eucalyptus* trees. Likewise, the isolate of *C. eucalypti* collected from an *Eucalyptus* clone was more pathogenic on *Syzygium* trees than the isolates of *C. syzygii* collected from *Syzygium*. Previous pathogenicity studies with *C. dispersa* have shown that an isolate of this fungus collected from *Tibouchina granulosa* Cogn.: Britton was more pathogenic on an *Eucalyptus* clone than on *T. granulosa* plants (Nakabonge *et al*. 2006a). The cross-infectivity of *Celoporthe* spp. on different hosts suggests that quarantine regulations should consider these alternative hosts to prevent these pathogens from moving to new areas.

5. REFERENCES

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

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

Begoude BAD, Gryzenhout M, Wingfield MJ, Roux J, 2010. *Aurifilum*, a new fungal genus in the Cryphonectriaceae from *Terminalia* species in Cameroon. *Antonie Van Leeuwenhoek* **98**: 263–278.

Carbone I, Kohn LM, 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* **91**: 553–556.

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.

Cheewangkoon R, Groenewald JZ, Summerell BA, Hyde KD, To-anun C, Crous PW, 2009. Myrtaceae, a cache of fungal biodiversity. *Persoonia* **23**: 55–85.

Chen SF, Gryzenhout M, Roux J, Xie YJ, Wingfield MJ, Zhou XD, 2010. Identification and pathogenicity of *Chrysoporthe cubensis* on *Eucalyptus* and *Syzygium* species in South China. *Plant Disease* **94**: 1143–1150.

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

Farris JS, Kallersjo M, Kluge AG, Bult C, 1995. Testing significance of incongruence. *Cladistics* **10**: 315–319.

Felsenstein J, 1985. Confidence intervals on phylogenetics: an approach using bootstrap. *Evolution* **39**: 783–791.

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

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, Wingfield MJ, 2006a. *Cryphonectriaceae* (Diaporthales), a new family including *Cryphonectria*, *Chrysoporthe*, *Endothia* and allied genera. *Mycologia* **98**: 239–249.

Gryzenhout M, Rodas CA, Menas Portales J, Clegg P, Wingfield BD, Wingfield MJ, 2006b. Novel hosts of the *Eucalyptus* canker pathogen *Chrysoporthe cubensis* and a new *Chrysoporthe* species from Colombia. *Mycological Research* **110**: 833–845.

Gryzenhout M, Tarigan M, Clegg PA, Wingfield MJ, 2010. *Cryptometrion aestuescens* gen. sp. nov. (*Cryphonectriaceae*) pathogenic to *Eucalyptus* in Indonesia. *Australasian Plant Pathology* **39**: 161–169.

Gryzenhout M, Wingfield BD, Wingfield MJ, 2009. *Taxonomy, phylogeny, and ecology of bark-inhabiting and tree-pathogenic fungi in the Cryphonectriaceae*. APS Press, St. Paul, MN. USA.

Guindon S, Gascuel O, 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52**: 696–704.

Heath R, Gryzenhout M, Roux J, Wingfield MJ, 2006. Discovery of the canker pathogen *Chrysoporthe austroafricana* on native *Syzygium* spp. in South Africa. *Plant Disease* **90**: 433–438.

Hillis DM, Huelsenbeck JP, 1992. Signal, noise, and reliability in molecular phylogenetic analyses. *Journal of Heredity* **83**: 189–195.

Hodges CS, Alfenas AC, Ferreira FA, 1986. The conspecificity of *Cryphonectria cubensis* and *Endothia eugeniae*. *Mycologia* **78**: 343–350.

Huelsenbeck JP, Bull JJ, Cunningham CW, 1996. Combining data in phylogenetic analysis. *TREE* **11**: 152–158.

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, Itô K, 1956. Two new species of *Endothia*. *Annual Phytopathology Sociedad Japan* **21**: 151–152.

Myburg H, Gryzenhout M, Wingfield BD, Milgroom MG, Shigeru K, Wingfield MJ, 2004. 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, Wingfield MJ, 2002. 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* **44**: 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, 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**: 255–267.

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* **90**: 734–740.

Nylander JAA, 2004. *MrModeltest* 2.3. *Program distributed by the author*. Evolutionary Biology Centre, Uppsala University, Sweden.

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

Rayner RW, 1970. *A mycological colour chart*. Commonwealth Mycological Institute and British Mycological Society, Kew, Surrey.

Rehner SA, Samuels GJ, 1994. Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. *Mycological Research* **98**: 625–634.

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.

Ronquist F, Huelsenbeck JP, 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.

SAS Institute Inc, 1999. *SAS/STAT Users Guide*, Version 8. SAS Institute Inc., Cary, NC. ISBN 1-58025-494-2.

Sharma JK, Mohanan C, Florence EJM, 1985. Occurrence of *Cryphonectria* canker disease of *Eucalyptus* in Kerala, India. *Annals of Applied Biology* **106**: 265–276.

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.

Slippers B, Crous PW, Denman S, Coutinho TA, Wingfield BD, Wingfield MJ, 2004. Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. *Mycologia* **96**: 83–101.

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

Swofford DL, 2002. *PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods).* Version 4. Sunderland, Massachusetts: Sinauer Associates.

Tamura K, Dudley J, Nei M, Kumar S, 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**: 1596–1599.

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

Van Der Merwe NA, Gryzenhout M, Steenkamp ET, Wingfield BD, Wingfield MJ, 2010. Multigene phylogenetic and population differentiation data confirm the existence of a cryptic species within *Chrysoporthe cubensis*. *Fungal Biology* **114**: 966–979.

Vermeulen M, Gryzenhout M, Wingfield MJ, Roux J, 2010. New records of Cryphonectriaceae from southern Africa including *Latruncellus aurorae* gen. sp. nov. *Mycologia*. (Accepted).

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.

White TJ, Bruns T, Lee S, Taylor J, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis AM, Gelfand DH, Sninsky JJ, White TJ (eds), *PCR Protocols: a Guide to Methods and Applications*. Academic Press, San Diego, pp. 315–322.

Wingfield MJ, 2003. Increasing threat of diseases to exotic plantation forests in the Southern Hemisphere: lessons from Cryphonectria canker. *Australasian Plant Pathology* **32**: 133–139.

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

Zhou X, Xie Y, Chen SF, Wingfield MJ, 2008. Diseases of eucalypt plantations in China: challenges and Opportunities. *Fungal Diversity* **32**: 1–7.

Table 1. Isolates generated and used for phylogenetic analyses and pathogenicity trials in this study.

Species	Culture no.[a]	Other no.	Host	Location	Collector[b]	GenBank Accession no[c]				
						β-Tubulin 1	β-Tubulin 2	ITS	TEF-1α	LSU
Aurifilum marmelostoma	CMW28285	CBS124929	*Terminalia mantaly*	Yaounde, Cameroon	DB & JR	FJ900585	FJ900590	FJ882855	n/a[d]	**HQ730873**
	CMW28288	CBS124930	*T. ivorensis*	Mbalmayo,Cameroon	DB & JR	FJ900586	FJ900591	FJ882856	n/a	**HQ730874**
Celoporthe dispersa	CMW9976	CBS118782	*Syzygium cordatum*	Limpopo, South Africa	MG	DQ267136	DQ267142	DQ267130	HQ730840	**HQ730853**
	CMW9978	CBS118781	*S. cordatum*	Limpopo, South Africa	MG	DQ267135	DQ267141	AY214316	HQ730841	**HQ730854**
C. eucalypti	CMW26900[e]	CBS127191	*Eucalyptus* EC48 clone	GuangDong, China	XDZ & SFC	**HQ730816**	**HQ730826**	**HQ730836**	**HQ730849**	**HQ730862**
	CMW26908[efg]	CBS127190	*Eucalyptus* EC48 clone	GuangDong, China	XDZ & SFC	**HQ730817**	**HQ730827**	**HQ730837**	**HQ730850**	**HQ730863**
	CMW26911[e]	CBS127192	*Eucalyptus* EC48 clone	GuangDong, China	XDZ & SFC	**HQ730818**	**HQ730828**	**HQ730838**	**HQ730851**	**HQ730864**
	CMW26913		*Eucalyptus* EC48 clone	GuangDong, China	XDZ & SFC	**HQ730819**	**HQ730829**	**HQ730839**	**HQ730852**	**HQ730865**
C. guangdongensis	CMW12750[fg]	CBSXXXXX	*Eucalyptus* sp.	GuangDong, China	TIB	**HQ730810**	**HQ730820**	**HQ730830**	**HQ730843**	**HQ730856**
C. indonesiensis	CMW10781	CBS115844	*S. aromaticum*	North Sumatra, Indonesia	MJW	AY084021	AY084033	AY084009	**HQ730842**	**HQ730855**
	CMW10779		*S. aromaticum*	Somosir, Indonesia	MJW	AY084019	AY084031	AY084007	n/a	n/a
	CMW10780		*S. aromaticum*	Somosir, Indonesia	MJW	AY084020	AY084032	AY084008	n/a	n/a
Celoporthe sp.	CMW14853	CBS534.82	*S. aromaticum*	Indonesia	SM	n/a	n/a	n/a	n/a	AF277142
C. syzygii	CMW34023	CBS127218	*S. cumini*	GuangDong, China	SFC	**HQ730811**	**HQ730821**	**HQ730831**	**HQ730844**	**HQ730857**
	CMW34024		*S. cumini*	GuangDong, China	SFC	**HQ730812**	**HQ730822**	**HQ730832**	**HQ730845**	**HQ730858**
	CMW24912[fg]	CBS127188	*S. cumini*	GuangDong, China	MJW & XDZ	**HQ730813**	**HQ730823**	**HQ730833**	**HQ730846**	**HQ730859**
	CMW24914[fg]	CBS127189	*S. cumini*	GuangDong, China	MJW & XDZ	**HQ730814**	**HQ730824**	**HQ730834**	**HQ730847**	**HQ730860**
	CMW24917[fg]		*S. cumini*	GuangDong, China	MJW & XDZ	**HQ730815**	**HQ730825**	**HQ730835**	**HQ730848**	**HQ730861**
Cryptometrion aestuescens	CMW18790	CBS124008	*Eucalyptus grandis*	Sumatra, Indonesia	MJW	GQ369455	GQ369455	GQ369458	n/a	**HQ730869**
	CMW18793	CBS124007	*E. grandis*	Sumatra, Indonesia	MJW	GQ369456	GQ369456	GQ369459	n/a	**HQ730870**
Latruncella aurorae	CMW28274	CBS124904	*Galpinia transvaalica*	Mpumalanga, South Africa	JR	GU726958	GU726958	GU726946	n/a	**HQ730871**
	CMW28276	CBS125526	*G. transvaalica*	Mpumalanga, South Africa	JR	GU726959	GU726959	GU726947	n/a	**HQ730872**

[a]CMW = Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; CBS = the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

[b]DB = D. Begoude, JR = J. Roux, MG = M Gryzenhout, XDZ = X.D. Zhou, SFC = S.F. Chen, TIB = T.I. Burgess, MJW = M.J. Wingfield, SM = S. Mandang.

[c]GenBank no. in bold were sequenced in this study.

[d]n/a, not available.

[e]Isolates used for glasshouse pathogenicity tests on an *Eucalyptus grandis* clone.

[f]Isolates used for pathogenicity tests on *Eucalyptus* in field inoculations in China.

[g]Isolates used for pathogenicity tests on *S. cumini* branches in field inoculations in China.

Table 2. Statistics resulting from phylogenetic analyses.

Dateset	No. of taxa	No. of bp[a]	Maximum Parsimony					
			PIC[b]	No.of trees	Tree length	CI[c]	RI[d]	HI[e]
LSU	66	639	125	100	283	0.541	0.820	0.459
Conserved ITS/β-tubulin	61	760	114	1	246	0.581	0.870	0.419
β-tubulin	15	874	49	4	69	0.884	0.925	0.116
ITS	15	535	52	1	57	0.947	0.967	0.053
TEF-1α	13	297	27	1	32	0.906	0.933	0.094

Dateset	Maximum Likelihood						
	Subst[f] model	NST[g]	Rate matrix	Ti/tv[h] ratio	Rates	I[i]	G[j]
LSU	GTR+I+G	6	(1.4570, 6.3657, 3.7129, 1.3405, 20.3689, 1.0000)		gamma	0.5297	0.511
Conserved ITS/β-tubulin	GTR+I	6	(0.3846, 1.9433, 0.2760, 1.0209, 6.3795, 1.0000)		equal	0.7623	
β-tubulin	HKY+I	2		1.6439	equal	0.8686	
ITS	TrNef	6	(1.0000, 1.2798, 1.0000, 1.0000, 3.5337, 1.0000)		equal	0	
TEF-1α	HKY	2		2.8872	equal	0	

Dateset	MrBayes				
	Subst[f] model	Prset statefreqpr	NST[g]	Rates	Burn-in
LSU	GTR+I+G	dirichlet(1,1,1,1)	6	invgamma	100 000
Conserved ITS/β-tubulin	GTR+I	dirichlet(1,1,1,1)	6	propinv	100 000
β-tubulin	HKY+I	dirichlet(1,1,1,1)	2	propinv	50 000
ITS	K80	fixed(equal)	2	equal	100 000
TEF-1α	HKY	dirichlet(1,1,1,1)	2	equal	50 000

[a]bp = base pairs. [b]PIC = number of parsimony informative characters. [c]CI = consistency index. [d]RI = retention index. [e]HI = homoplasy index. [f]Subst. model = best fit substitution model. [g]NST = number of substitution rate categories. [h]Ti/Tv ratio = transition/transversion ratio. [i]I = proportion of invariable sites. [j]G = gamma distribution shape parameter.

Table 3. Summary of polymorphic nucleotides found within the β-tubulin (1 and 2), ribosomal ITS and the partial TEF-1α gene regions generated for the phylogenetic groups of *Celoporthe syzygii*, *C. eucalypti*, *C. guangdongensis* and *C. indonesiensis*. Only polymorphic nucleotides occurring in all of the isolates are shown, and not alleles that partially occur in individuals per phylogenetic group. Fixed polymorphisms for each group are highlighted and in bold, those fixed but shared between two groups are highlighted. Numerical positions of the nucleotides in the DNA sequence alignments are indicated.

Species	Isolate number	β-tubulin (Bt1a/1b)							
		71	119	141	188	189	192	194	202
C. syzygii	CMW34023	C	G	**T**	C	T	C	C	**A**
	CMW34024	C	G	**T**	C	T	C	C	**A**
	CMW24912	C	G	**T**	C	T	C	C	**A**
	CMW24914	C	G	**T**	C	T	C	C	**A**
	CMW24917	C	G	**T**	C	T	C	C	**A**
C. eucalypti	CMW26900	C	**A**	C	C	T	**T**	C	C
	CMW26908	C	**A**	C	C	T	**T**	C	C
	CMW26911	C	**A**	C	C	T	**T**	C	C
	CMW26913	C	**A**	C	C	T	**T**	C	C
C. guangdongensis	CMW12750	**T**	G	C	**T**	**C**	C	**T**	C
C. indonesiensis	CMW10779	C	G	C	C	T	C	C	C
	CMW10780	C	G	C	C	T	C	C	C
	CMW10781	C	G	C	C	T	C	C	C

Species	Isolate number	β-tubulin (Bt2a/2b)									
		24	223	233	234	235	236	237	238	241	327
C. syzygii	CMW34023	C	**A**	C	C	–	–	C	C	**G**	**A**
	CMW34024	C	**A**	C	C	–	–	C	C	**G**	**A**
	CMW24912	C	**A**	C	C	–	–	C	C	**G**	**A**
	CMW24914	C	**A**	C	C	–	–	C	C	**G**	**A**
	CMW24917	C	**A**	C	C	–	–	C	C	**G**	**A**
C. eucalypti	CMW26900	**T**	C	C	T	–	–	–	C	C	C
	CMW26908	**T**	C	C	T	–	–	–	C	C	C
	CMW26911	**T**	C	C	T	–		–	C	C	C
	CMW26913	**T**	C	C	T	–	–	–	C	C	C
C. guangdongensis	CMW12750	C	C	**–**	**–**	–	–	–	C	C	C
C. indonesiensis	CMW10779	C	C	C	C	**T**	**T**	C	**T**	C	C
	CMW10780	C	C	C	C	**T**	**T**	C	**T**	C	C
	CMW10781	C	C	C	C	**T**	**T**	C	**T**	C	C

Species	Isolate number	ITS1/5.8S/ITS2														
		40	82	84	85	92	93	97	100	118	132	180	181	211	212	489
C. syzygii	CMW34023	C	T	–	–	**T**	**C**	**T**	**C**	C	**–**	–	–	–	A	–
	CMW34024	C	T	–	–	**T**	**C**	**T**	**C**	C	**–**	–	–	–	A	–
	CMW24912	C	T	–	–	**T**	**C**	**T**	**C**	C	**–**	–	–	–	A	–
	CMW24914	C	T	–	–	**T**	**C**	**T**	**C**	C	**–**	–	–	–	A	–
	CMW24917	C	T	–	–	**T**	**C**	**T**	**C**	C	**–**	–	–	–	A	–
C. eucalypti	CMW26900	C	T	–	–	C	T	C	G	–	G	A	–	–	–	–
	CMW26908	C	T	–	–	C	T	C	G	–	G	A	–	–	–	–
	CMW26911	C	T	–	–	C	T	C	G	–	G	A	–	–	–	–
	CMW26913	C	T	–	–	C	T	C	G	–	G	A	–	–	–	–
C. guangdongensis	CMW12750	C	T	–	–	C	T	C	G	C	G	A	**A**	–	–	**T**
C. indonesiensis	CMW10779	**T**	**A**	**T**	**A**	C	T	C	G	–	G	–	–	**A**	A	–
	CMW10780	**T**	**A**	**T**	**A**	C	T	C	G	–	G	–	–	**A**	A	–
	CMW10781	**T**	**A**	**T**	**A**	C	T	C	G	–	G	–	–	**A**	A	–

Species	Isolate number	TEF-1α												
		35	47	48	76	128	129	138	214	215	230	234	240	249
C. syzygii	CMW34023	C	**T**	**T**	A	T	T	A	–	–	A	G	T	T
	CMW34024	C	**T**	**T**	A	T	T	A	–	–	A	G	T	T
	CMW24912	C	**T**	**T**	A	T	T	A	–	–	A	G	T	T
	CMW24914	C	**T**	**T**	A	T	T	A	–	–	A	G	T	T
	CMW24917	C	**T**	**T**	A	T	T	A	–	–	A	G	T	T
C. eucalypti	CMW26900	**T**	G	C	A	T	T	A	–	–	A	G	T	T
	CMW26908	**T**	G	C	A	T	T	A	–	–	A	G	T	T
	CMW26911	**T**	G	C	A	T	T	A	–	–	A	G	T	T
	CMW26913	**T**	G	C	A	T	T	A	–	–	A	G	T	T
C. guangdongensis	CMW12750	C	G	C	A	–	–	C	A	–	**G**	A	**C**	**C**
C. indonesiensis	CMW10781	C	G	C	**G**	–	–	C	A	**C**	A	A	T	T

Table 4. Number of unique alleles between *Celoporthe syzygii*, *C. eucalypti*, *C. guangdongensis* and *C. indonesiensis*.

β-tubulin/ITS/TEF-1α[a]	*C. eucalypti*	*C. guangdongensis*	*C. indonesiensis*
C. syzygii	21 (10/8/3)	31(12/9/10)	29 (9/11/9)
C. eucalypti		21 (9/3/9)	24 (9/7/8)
C. guangdongensis			26 (11/10/5)

[a]The order of the three genes: β-tubulin, ITS and TEF-1α.

Table 5. Comparison of morphological characteristics between *Celoporthe* species.

		C. dispersa	*C. syzygii*	*C. eucalypti*	*C. guangdongensis*	*C. indonesiensis*
Species collected from	country	South Africa	China	China	China	Indonesia
	host	*S. cordatum*	*S. cumini*	*Eucalyptus* clone	*Eucalyptus* sp.	*S. aromaticum*
Conidiomata	heigth	**300–500** [a]	**80–400 (av. 163)**	**100–600 (av. 235)**	**90–720 (av. 255)**	**60–260 (av. 110)**
(above the bark)	width	**200–1000**	**140–900 (av. 343)**	**140–700 (av. 370)**	**120–750 (av. 395)**	**100–550 (av. 225)**
Conidiomatal locules	uni-or	**unilocular,**	**multilocular,**	**multilocular,**	**multilocular,**	**multilocular,**
	multilocular	**occasionally multilocular**	**seldom unilocular**	**seldom unilocular**	**seldom unilocular**	**seldom unilocular**
	diameter	**100–550**	**40–650 (av. 250)**	**35–580 (av. 250)**	**35–640 (av. 280)**	**35–610 (av. 295)**
Conidiophores	length	**(9.5–)12.0–17.0(–19.5)**	**(4.5–)6.0–11.0(–16.0)**	**(4.5–)9.0–14.0(–25.0)**	**(4.5–)10.0–16.0(–27.5)**	**(5.5–)12.0–18.0(–32.0)**
		(av. 14.5)	**(av. 9.0)**	**(av. 12.0)**	**(av. 13.5)**	**(av. 15.5)**
Conidiogenous cells	width	(1.5–)2.0–3.0	1.0–2.0(–2.5) (av. 2.0)	1.5–2.5(–3.5) (av. 2.2)	1.5–2.5(–3.5) (av. 2.0)	(1.0–3.5) (av. 2.2)
Paraphyses or sterile cells	length	**up to 39 (av. 20)**	**up to 52 (av. 20)**	**up to 68 (av. 42)**	**up to 91 (av. 55)**	**up to 50 (av. 27)**
Conidia	shape	oblong (to cylindrical), occasionally allantoid	oblong (to cylindrical), occasionally allantoid	oblong (to cylindrical), occasionally allantoid	oblong (to cylindrical), occasionally allantoid	cylindrical, occasionally oblongor or allantoid
	length	(2.5–)3–4(–5.5) (av. 3.5)	(2.3–)2.8–3.4(–3.8) (av. 3.1)	(2.6–)3.1–3.8(–4.4) (av. 3.5)	(2.4–)3.1–3.9(–4.3) (av. 3.5)	(3.1–)3.5–4.2(–4.7) (av. 3.9)
	width	**(1.0–)1.5(–2.5) (av. 1.5)**	**(1.0–)1.3(–1.6) (av. 1.3)**	**(1.1–)1.4-1.6(–1.8) (av. 1.6)**	**(1.1–)1.3-1.7(–1.9) (av. 1.5)**	**(1.1–)1.2-1.5(–1.6) (av. 1.3)**
	length/width	2.3	2.5	2.2	2.3	3
Ascostromata	heigth	100–400	40–240 (av. 120)	n/a[b]	n/a	n/a
(above the bark)	width	320–505	280–580 (av. 300)	n/a	n/a	n/a
Perithecia	no. perithecia	**1–6**	**1–15**	n/a	n/a	n/a
	per ascostroma			n/a	n/a	n/a
	diameter	100–300	60–380 (av. 170)	n/a	n/a	n/a
Perithecial necks	length	50	40	n/a	n/a	n/a
	width	100–150	40–90	n/a	n/a	n/a
Asci	shape	fusoid to ellipsoidal	fusoid	n/a	n/a	n/a
	length	**(19.5–)23.5–29.5(–33.5)**	**(29.5–)31.5–36.5(–43)**	n/a	n/a	n/a
		(av. 26.5)	**(av. 34.0)**	n/a	n/a	n/a
	width	**(4.5–)5.5–7(–7.5) (av. 6.3)**	**(5–)5.5–6.5(–7) (av. 6.0)**	n/a	n/a	n/a
	length/width	**4.2**	**5.7**	n/a	n/a	n/a
Ascospores	shape	oblong ellipsoidal	oblong ellipsoidal	n/a	n/a	n/a
	length	**(4.5–)6–7(–8) (av. 6.5)**	**(5–)6–7.5(–8.5) (av. 6.8)**	n/a	n/a	n/a
	width	(2–)2.5–3(–3.5) (av. 2.8)	2.5–3(–3.5) (av. 2.8)	n/a	n/a	n/a
	length/width	2.3	2.4	n/a	n/a	n/a

[a]The measurements are in µm, and the distinctive characters are in bold.

[b]n/a, not available.

Fig 1. The symptoms infection on *S. cumini* trees caused by *C. syzygii*, as well as inoculated branches of *S. cordatum* and an *E. grandis* clone with isolates of a *C. sysygii*. A. Cracks and cankers on *S. cumini* trees caused by *C. syzygii* (arrow indicates fruiting structures); B. Fruiting structures of *C. syzygii* on bark of *S. cumini*; C. Conidiomata of *C. syzygii* on inoculated branches of *S. cordatum*; D. Conidiomata of *C. syzygii* on inoculated branches of an *E. grandis* clone.

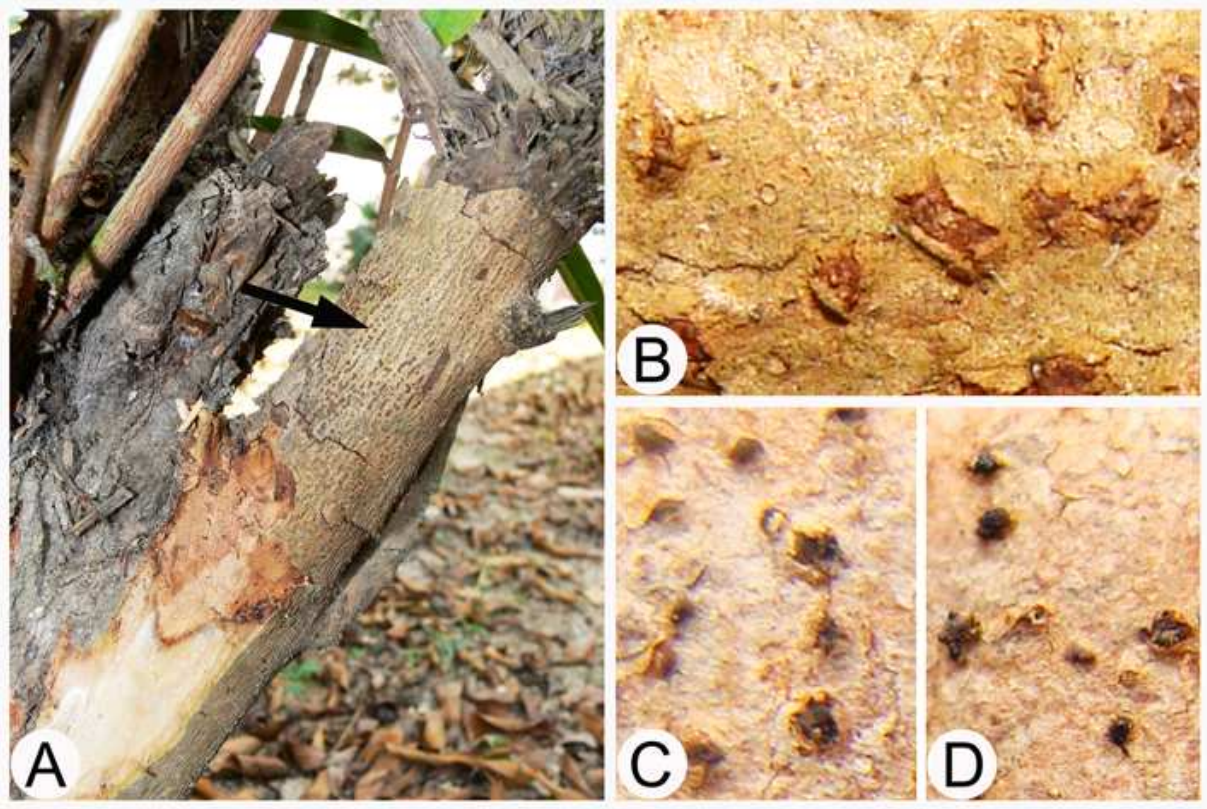


Fig 2. Phylogram based on maximum parsimony (MP) analysis of LSU DNA sequences for various genera in the Diaporthales. Isolates in bold were sequenced in this study. Sequences with AF and AY numbers originated from Zhang & Blackwell (2001) and Castlebury *et al.* (2002), while others were obtained from Gryzenhout *et al.* (2006a, 2009). Bootstrap values > 60 % for MP and Maximum likelihood (ML), and posterior probabilities > 80% obtained from Bayesian anlyses are presented above branches as follows: MP/ML/Bayesian. Bootstrap values lower than 60%, and posterior probabilities lower than 80% are marked with "*", and absent analysis values are marked with "-". *Togninia minima* (AY761082), *T. fraxinopennsylvanica* (AY761083) and *Phaeoacremonium aleophilum* (AY249088) in the family of Togniniaceae represent the outgroups.

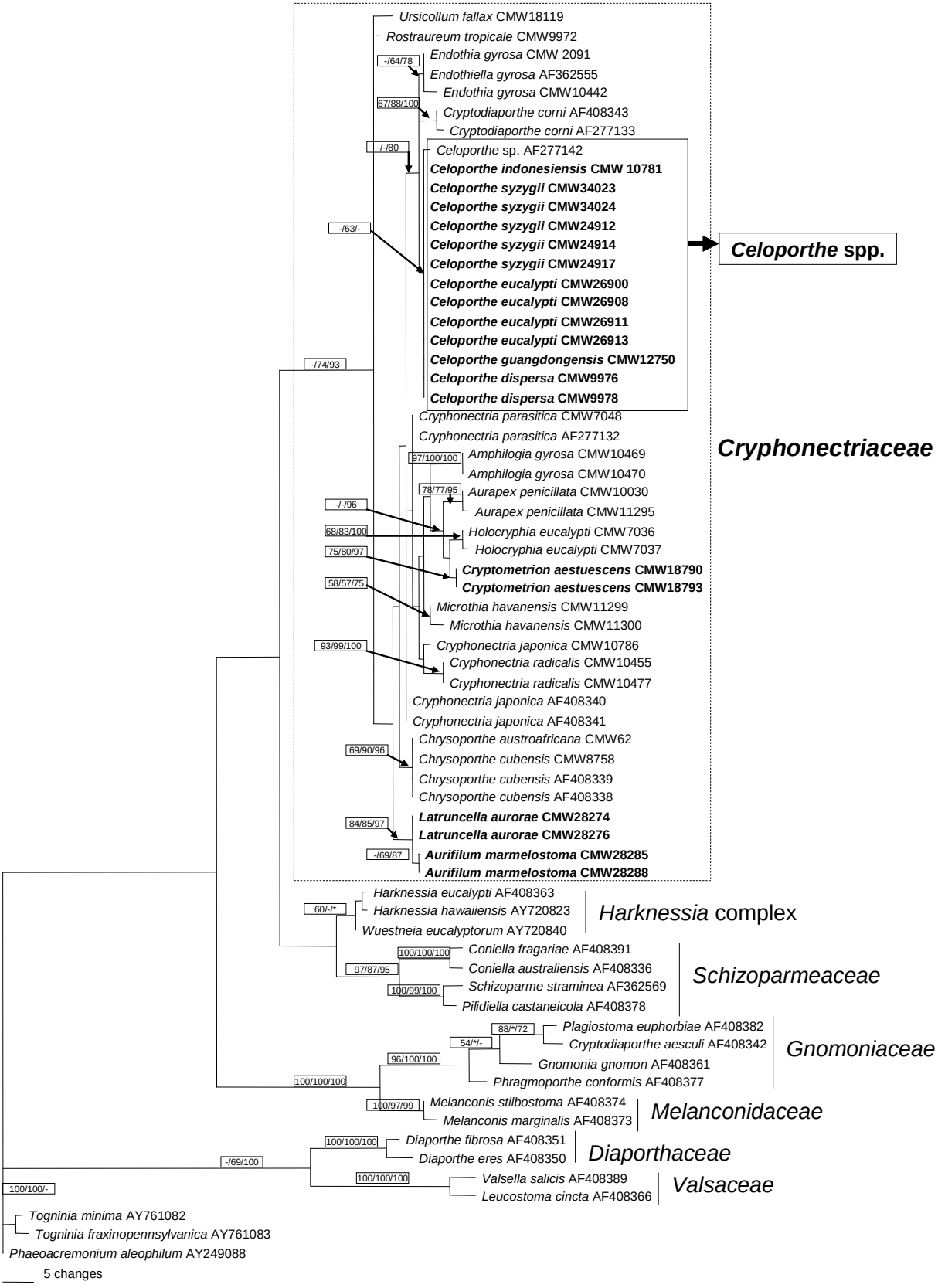


Fig 3. Phylogram based on maximum parsimony (MP) analysis of a combined DNA sequence dataset of gene regions of the the partial exon 4, exon 5, partial exon 6 and partial exon 7 of the β-tubulin genes, and the 5.8S rRNA gene regions of the ribosomal operon. Bootstrap values > 60 % for MP and Maximum likelihood (ML), and posterior probabilities > 80% obtained from Bayesian anlyses are presented above branches as follows: MP/ML/Bayesian. Bootstrap values lower than 60%, posterior probabilities lower than 80% are marked with "*", and analysis value absent is marked with "-". Two isolates of *Diaporthe ambigua* (CMW5587, CMW5288) represent the outgroup.

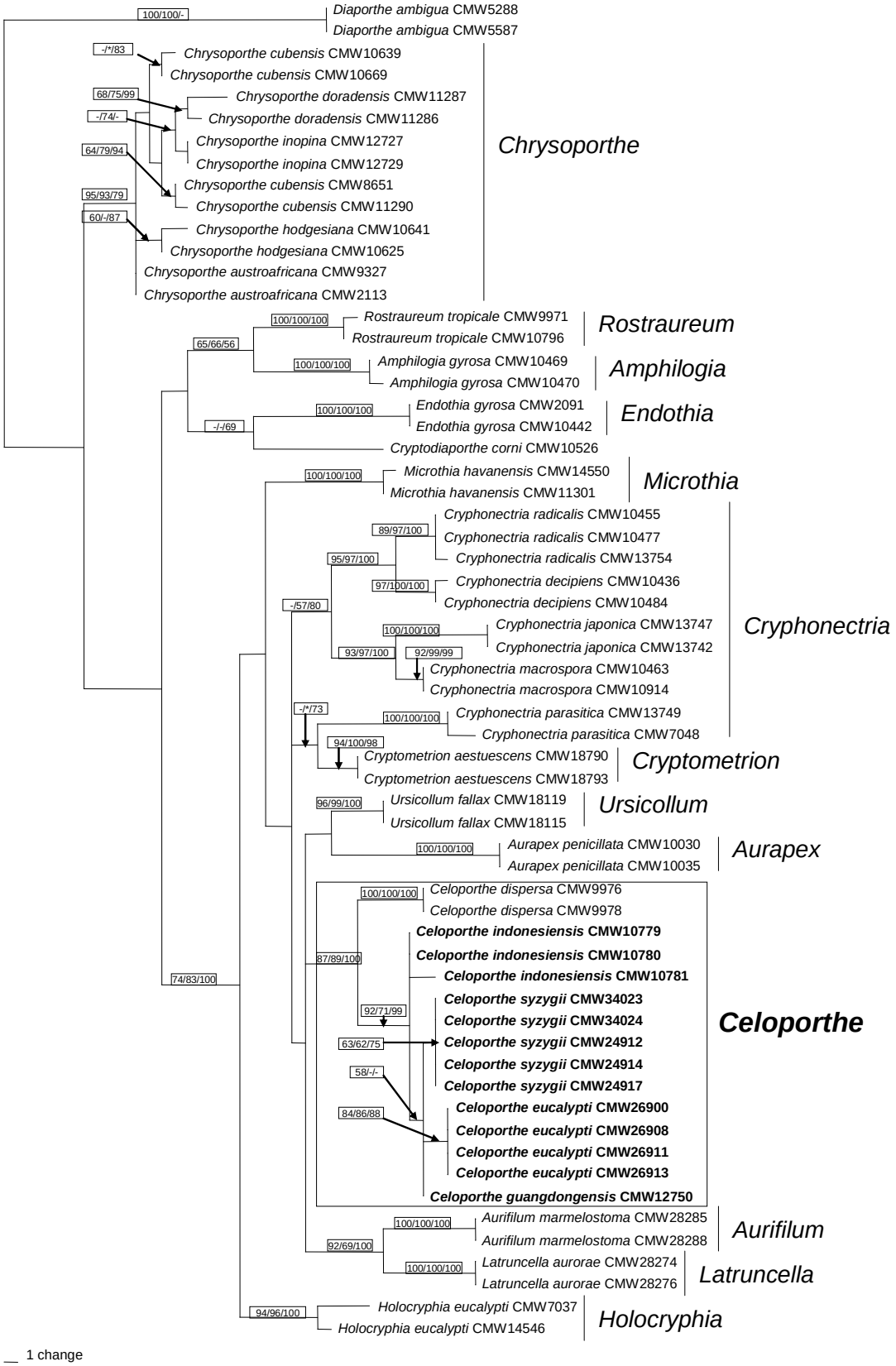


Fig 4. Unrooted phylogenetic tree obtained with parsimony from DNA sequence datasets of three gene regions. A. β-tubulin gene region (BT1 and BT2). B. ITS1, ITS2 and 5.8S rRNA regions of the ribosomal operon. C. A fragment of the translation elongation factor 1-alpha (TEF-1α) gene region. Branch length (BL), bootstrap values > 60 % for MP and Maximum likelihood (ML), and posterior probabilities > 80% obtained from Bayesian anlyses are presented above branches as follows: BL/MP/ML/Bayesian. Bootstrap values lower than 60%, posterior probabilities lower than 80% are marked with "*", and analysis value absent is marked with "-".

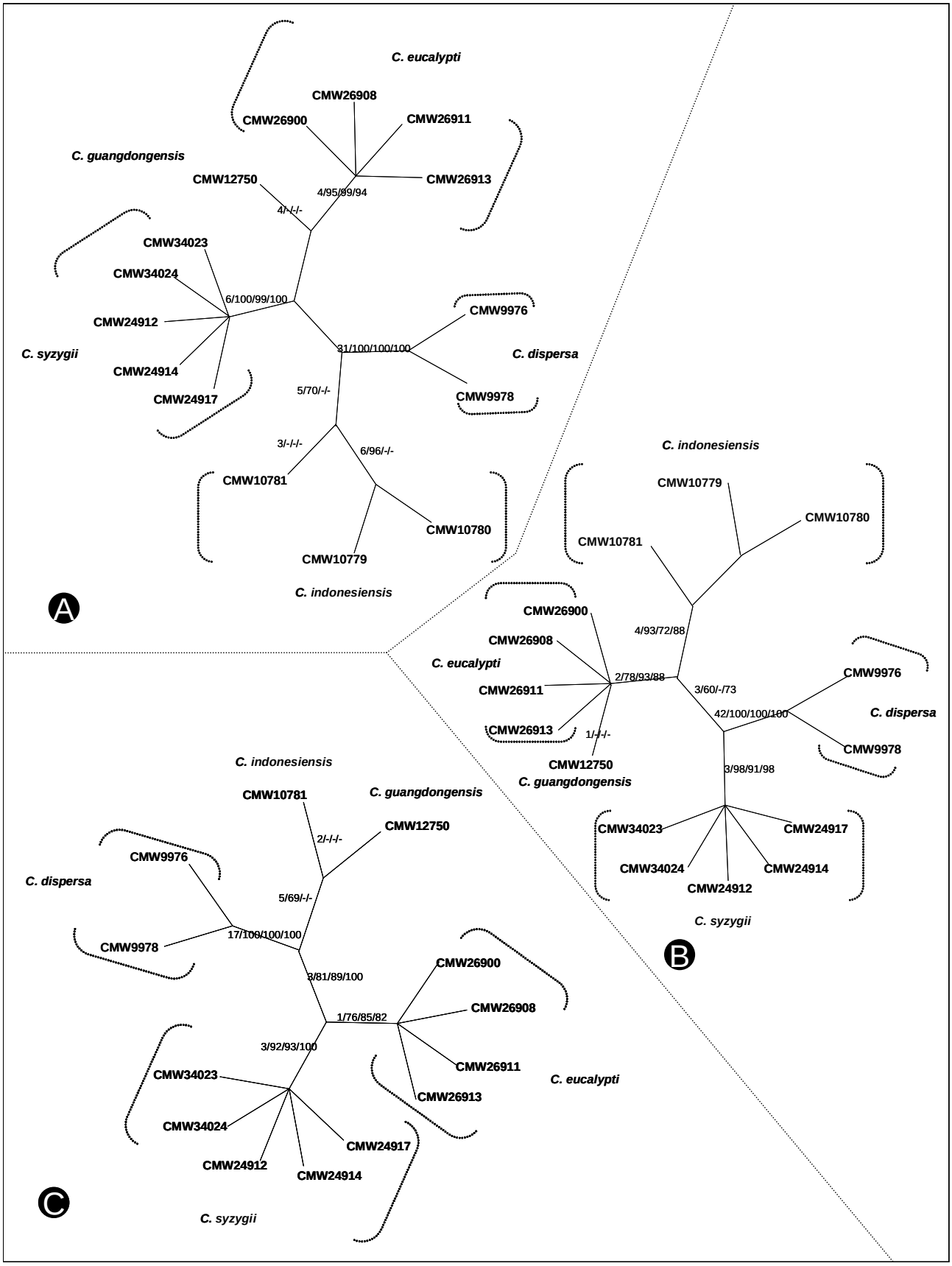


Fig 5. Fruiting structures of *C. syzygii* on natural *S. cumini* trees. A. Ascostroma on bark; B, C. Longitudinal section through ascostroma; D. Perithecial neck tissue; E. Stromatic tissue of ascostroma; F. Ascus with ascospores; G. Ascospores; H, I. Conidomata on the bark (arrow indicate conidial spore mass); J. Longitudinal section through conidioma; K. Latitudinal section through conidioma; L. Stromatic tissue of conidioma; M. Paraphyses; N, O. Conidiophores and conidigenous cells; P. Conidia. Scale bars: A–C, I–K = 100 μm; H = 200 μm; D = 20 μm; E–G, L–P = 10 μm.

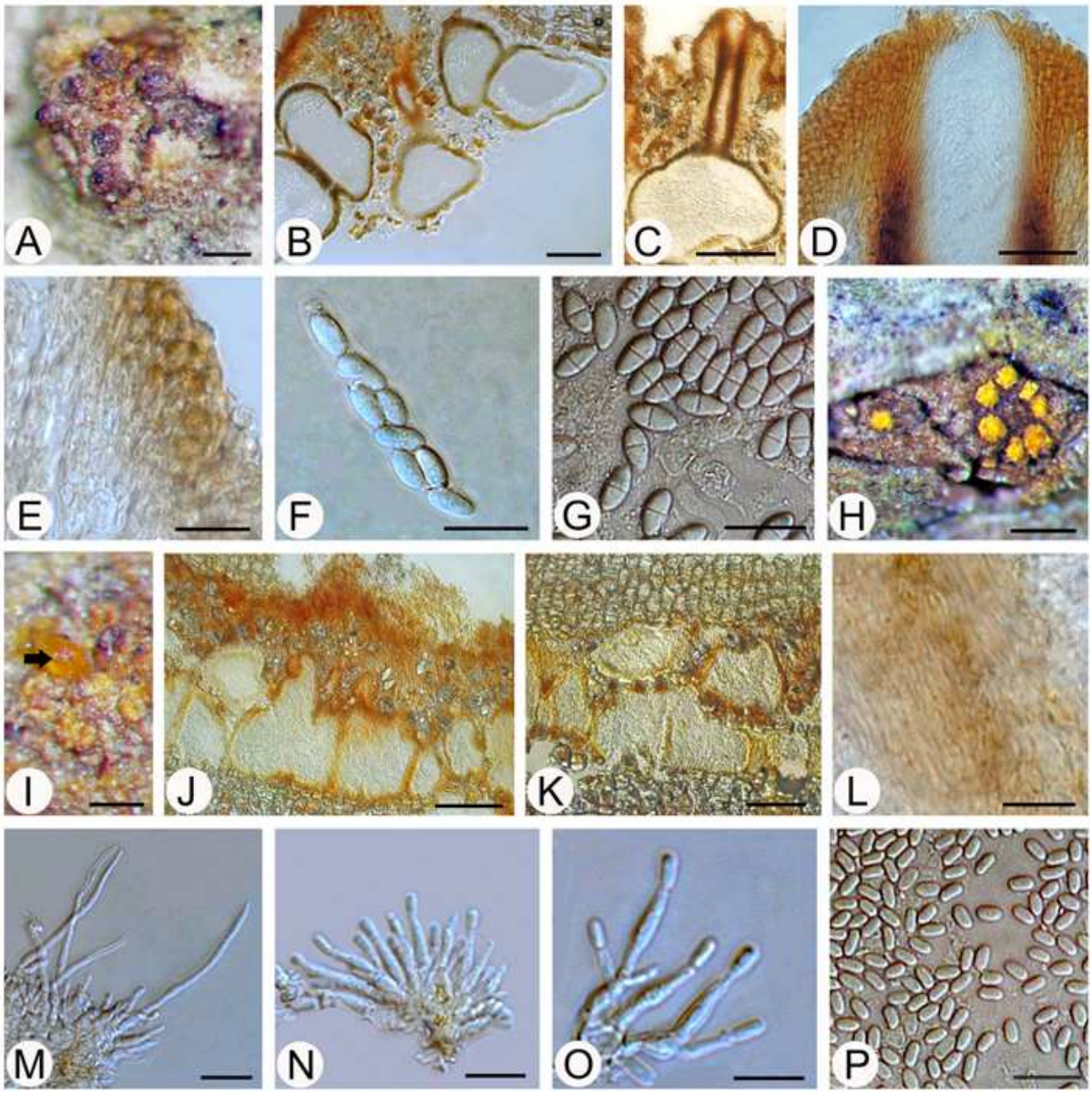


Fig 6. Fruiting structures of *C. syzygii* on inoculated branch tissue of *S. cordatum* (A–F) and an *E. grandis* clone (G–I). A, G. Conidiomata on the bark; B, H. Longitudinal section through conidioma; C. Stromatic tissue of conidioma; D. Paraphyses; E. Conidiophores and conidigenous cells; F, I. Conidia. Scale bars: A, B, G, H = 100 µm; C = 20 µm; D–F, I = 10 µm.

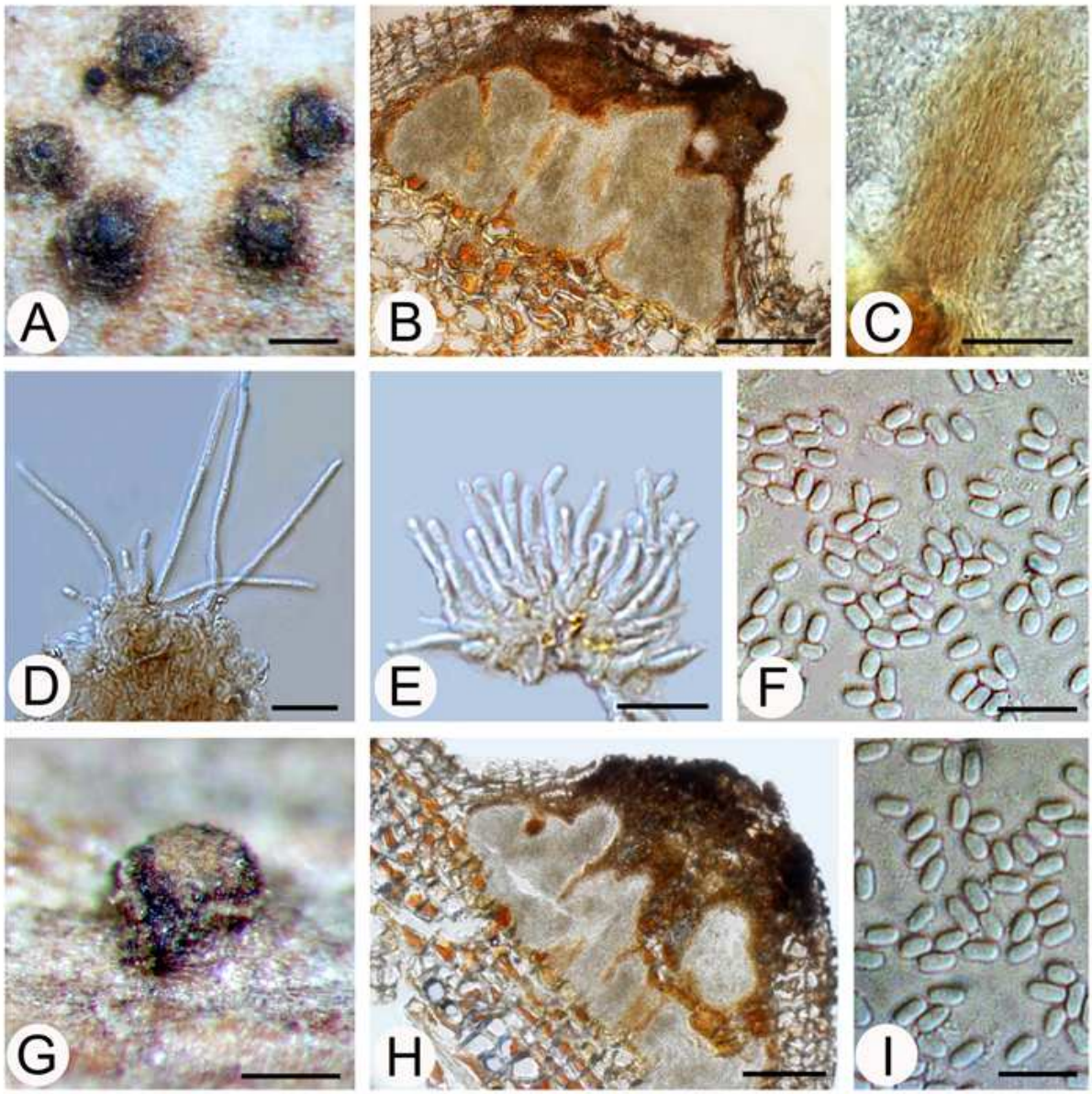


Fig 7. Fruiting structures of *C. eucalypti* on inoculated branch tissue of an *E. grandis* clone (A–G) and *S. cordatum* (H–J). A, H. Conidiomata on the bark; B, I. Longitudinal section through conidioma; C. Stromatic tissue of conidioma; D, J. Paraphyses; E, F. Conidiophores and conidigenous cells; G. Conidia. Scale bars: A, B, H, I = 100 μm; C = 20 μm; D–G, J = 10 μm.

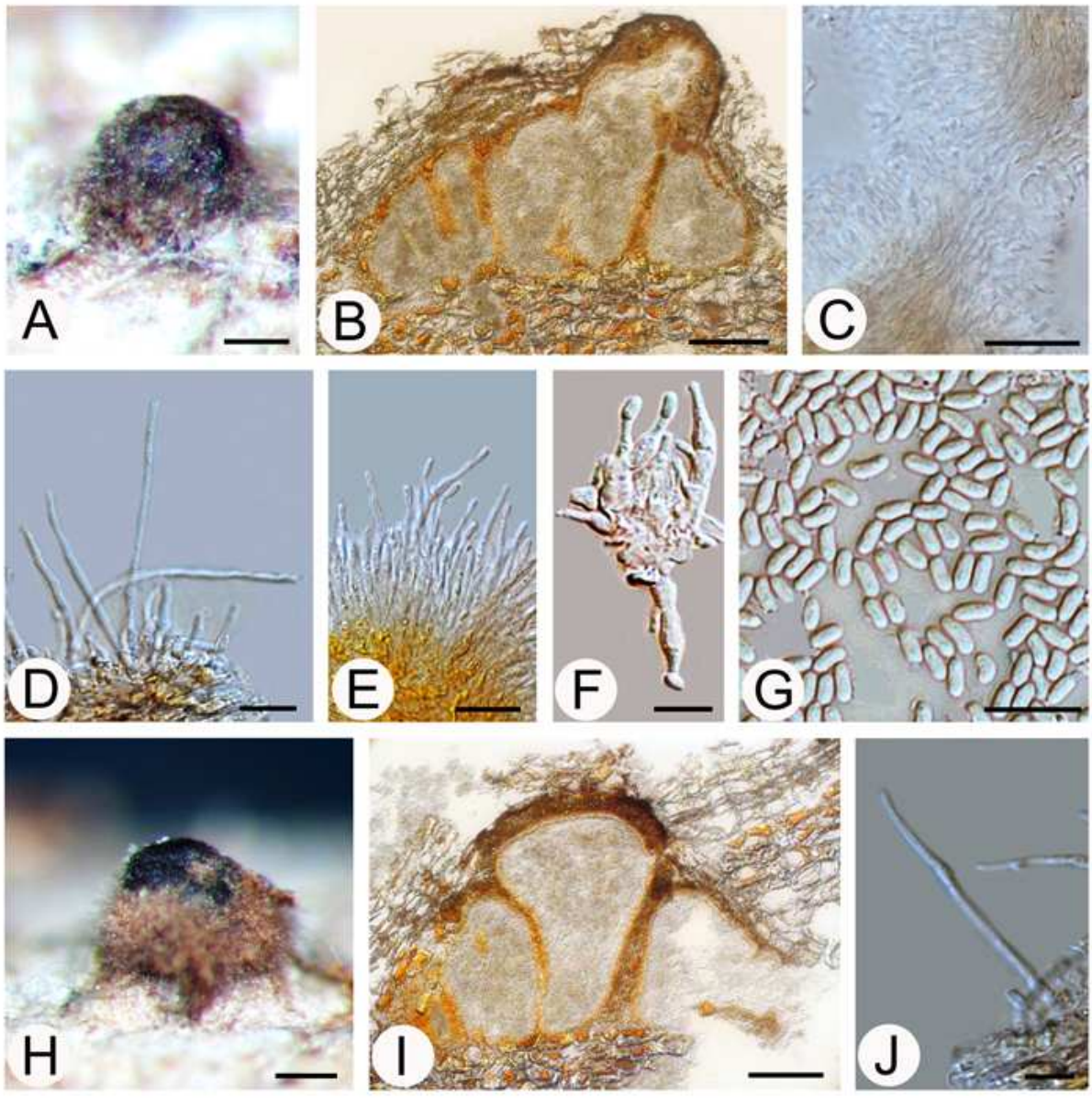


Fig 8. Fruiting structures of *C. guangdongensis* on inoculated branch tissue of an *E. grandis* clone (A–G) and *S. cordatum* (H–J). A, H. Conidiomata on the bark; B, I. Longitudinal section through conidioma; C. Stromatic tissue of conidioma; D, J. Paraphyses; E, F. Conidiophores and conidigenous cells; G. Conidia. Scale bars: A, B, H, I = 100 μm; C = 20 μm; D–G, J = 10 μm.

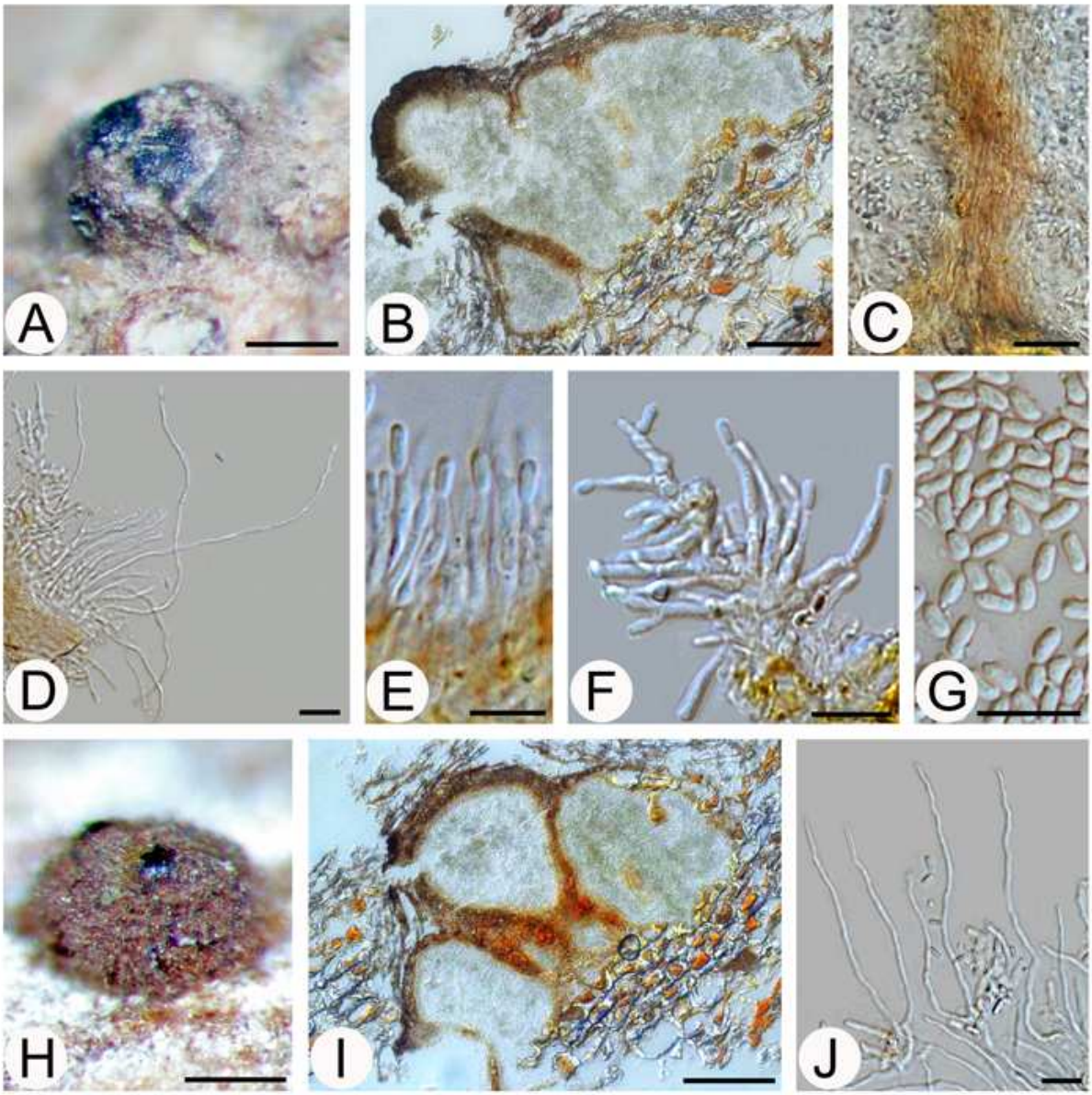


Fig 9. Fruiting structures of *C. indonesiensis* on inoculated branch tissue of *S. cordatum* (A–G) and A *E. grandis* clone (H–J). A, H. Conidiomata on the bark; B, I. Longitudinal section through conidioma; C. Stromatic tissue of conidioma; D. Paraphyses; E, F. Conidiophores and conidigenous cells; G, J. Conidia. Scale bars: A, B, H, I = 100 µm; C = 20 µm; D–G, J = 10 µm.

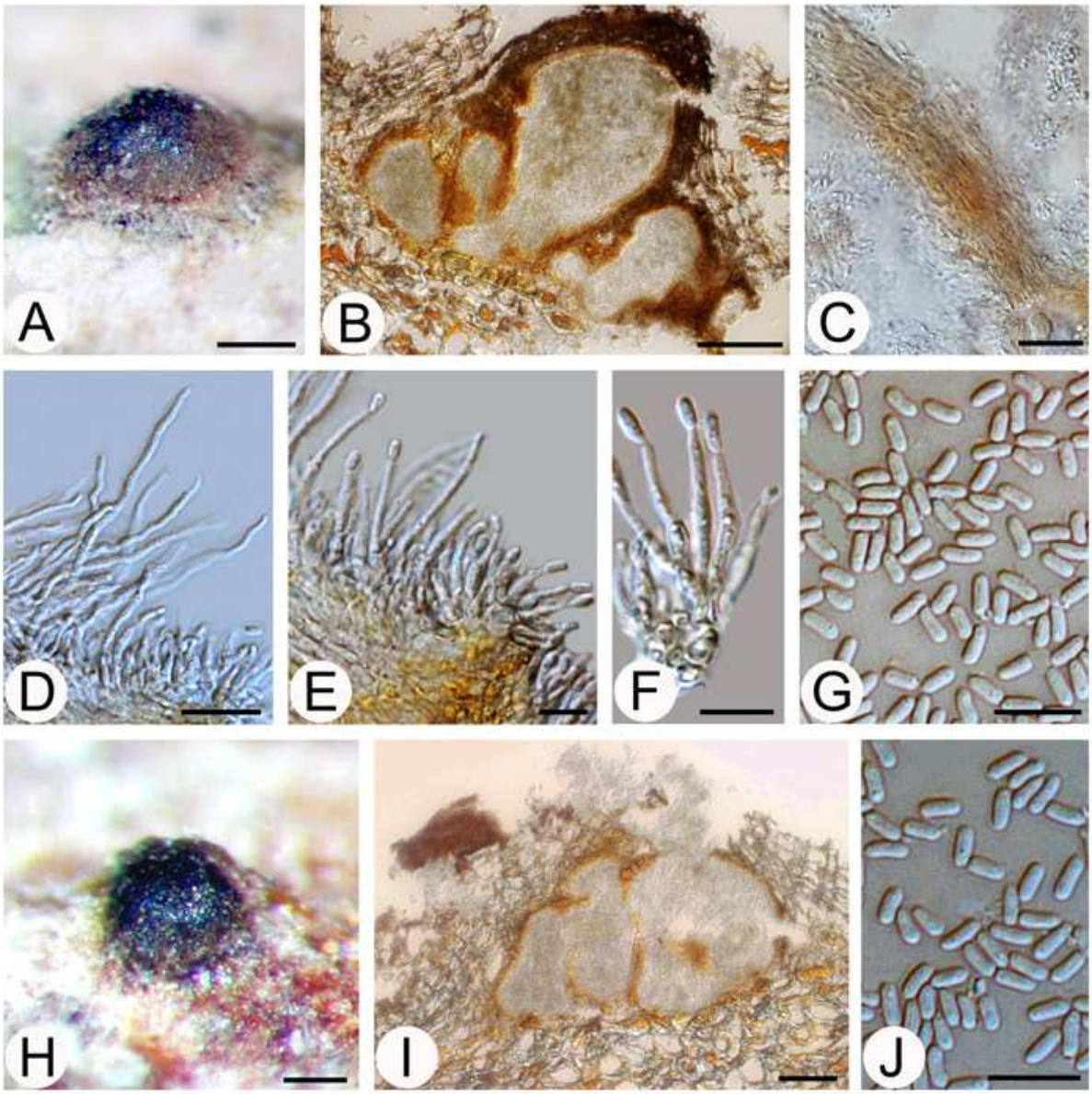
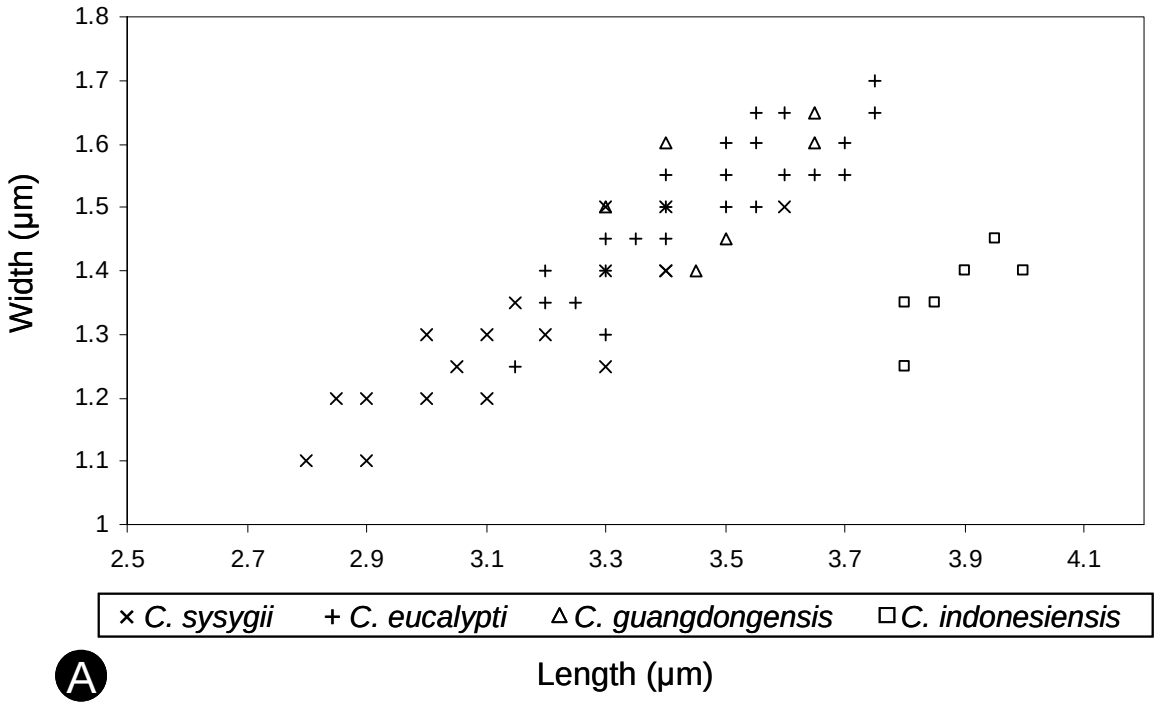
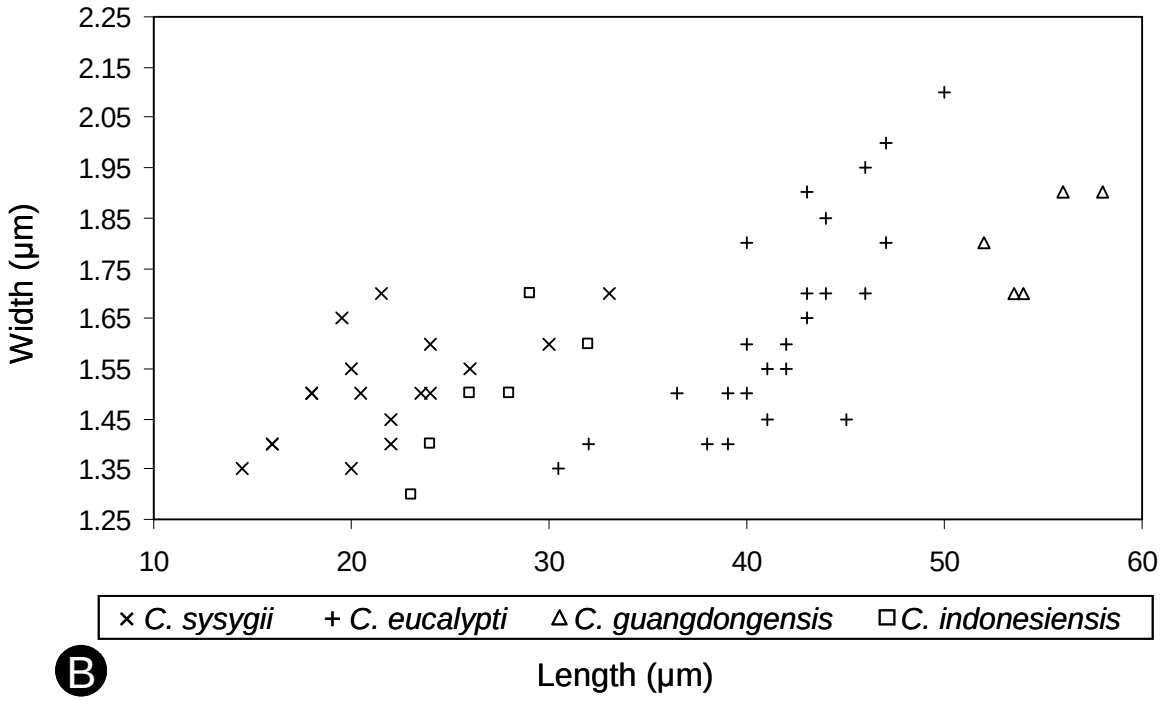


Fig 10. The average lengths and widths of 10 conidia and 10 paraphyses measured for each of the inoculated *S. cordatum* and *E. grandis* branche stubs by four species of *Celoporthe*. A. Conidia. B. Paraphyses.



A



B

Fig 11. Histogram showing the average lesion lengths (mm) resulting from inoculations onto *E. grandis* clone (TAG-5) under glasshouse conditions. Three isolates of *C. eucalypti* from *Eucalyptus* trees in China were used. Bars represent 95% confidence limits for each treatment. Different letters above the bars indicate treatments that were statistically significantly different (P = 0.05).

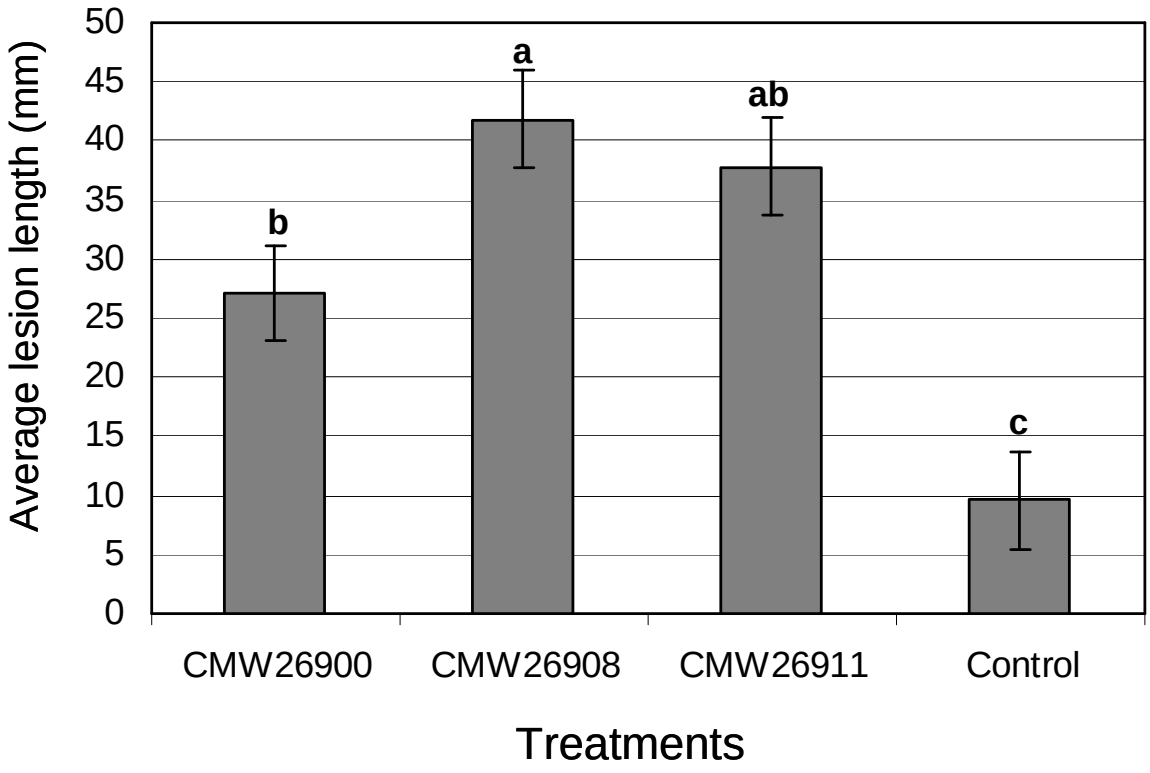


Fig 12. Histogram showing the average lesion lengths (mm) resulting from inoculations of seven *Eucalyptus* genotypes with isolate CMW12750 (*C. guangdongensis*) and isolate CMW26908 (*C. eucalypti*). Bars represent 95% confidence limits for each treatment. Different letters above the bars indicate treatments that were statistically significantly different (P = 0.05).

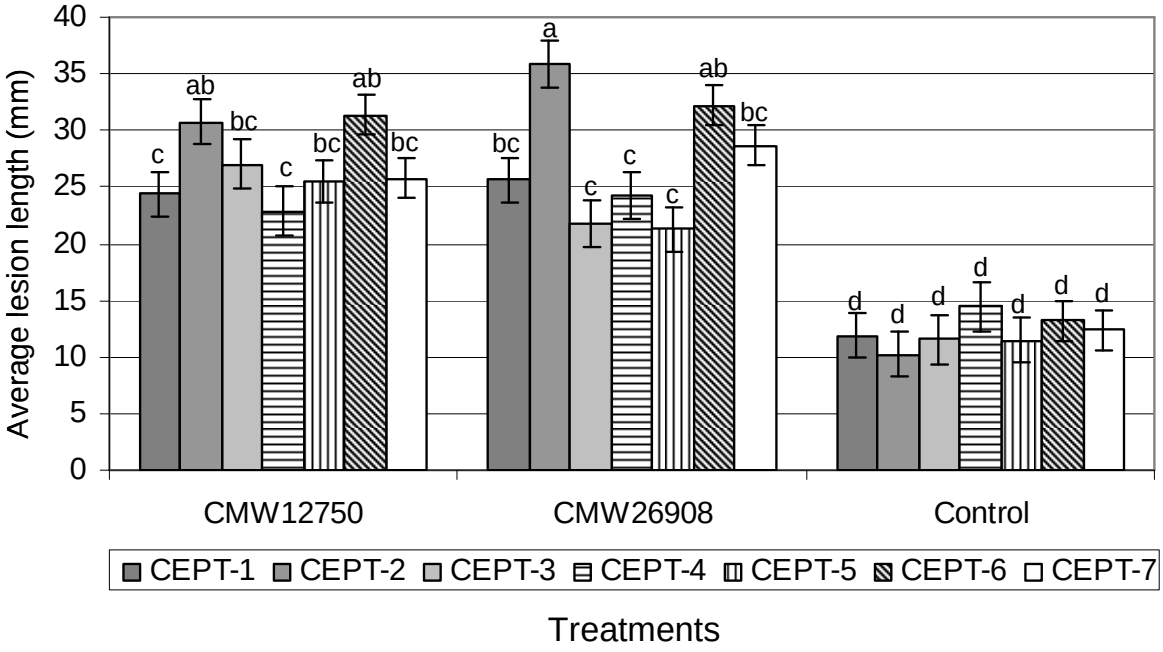
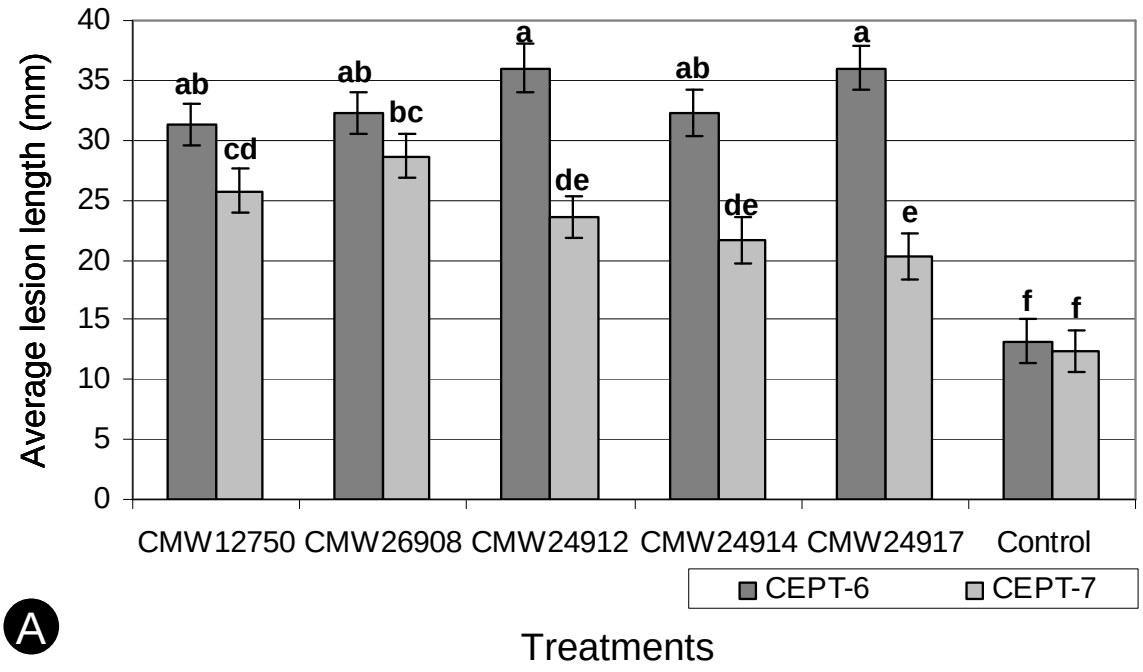
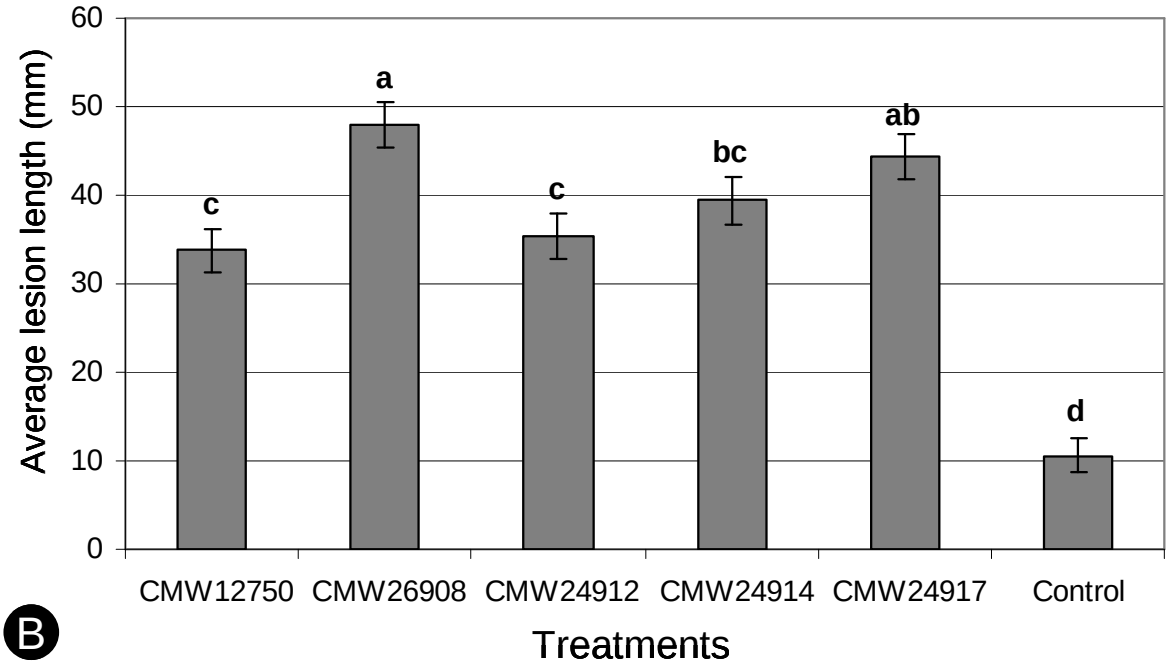


Fig 13. Histogram showing average lesion lengths (mm) resulting from inoculation of (A). Two *Eucalyptus* genotypes (CEPT-6, CEPT-7) and (B). *S. cumini* branches with three isolates (CMW24912, CMW24914, CMW24917) of *C. syzygii*, one isolate (CMW26908) of *C. eucalypti*, and one isolate (CMW12750) of *C. guangdongensis*. Bars represent 95% confidence limits for each treatment. Different letters above the bars indicate treatments that were statistically significantly different (P = 0.05).



A



B

Chapter 4

Characterization of Botryosphaeriaceae from plantation-grown *Eucalyptus* species in South China

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This chapter has been accepted for publication as: Chen SF, Pavlic D, Roux J, Xie YJ, Wingfield MJ, Zhou XD, 2011. Characterization of Botryosphaeriaceae from plantation-grown *Eucalyptus* species in South China. *Plant Pathology*.

ABSTRACT

The Botryosphaeriaceae is a species-rich family that includes pathogens of a wide variety of trees, including *Eucalyptus* species. Symptoms typical of infection by the Botryosphaeriaceae have recently been observed in *Eucalyptus* plantations in South China. The aim of this study was to identify the Botryosphaeriaceae associated with these symptoms. Isolates were collected from branch cankers and senescent twigs of different *Eucalyptus* spp. All isolates resembling Botryosphaeriaceae were separated into groups based on conidial morphology. Initial identifications were made using PCR-RFLP fingerprinting, by digesting the ITS region of the rRNA operon with the restriction enzymes (RE) *Cfo*I and *Ksp*I. Furthermore, to distinguish isolates in the *Neofusicoccum parvum/N. ribis* complex, a locus (BotF15) previously shown to define these species, was amplified and restricted with *Cfo*I. Selected isolates were then identified using comparisons of DNA sequence data for the ITS rDNA and translation elongation factor 1-alpha (TEF-1α) gene regions. Based on anamorph morphology and DNA sequence comparisons, five species were identified including *Lasiodiplodia pseudotheobromae, L. theobromae, Neofusicoccum parvum, N. ribis sensu lato* and one undescribed taxon, for which the name *Fusicoccum fabicercianum* sp. nov. is provided. Isolates of all species gave rise to lesions on the stems of an *E. grandis* clone in glasshouse inoculation trials and on the stems of five *Eucalyptus* genotypes inoculated in the field, where *L. pseudotheobromae* and *L. theobromae* were most pathogenic. The five *Eucalyptus* genotypes differed in their susceptibility to the Botryosphaeiaceae species suggesting that breeding and selection offers opportunity for disease avoidance in the future.

Keywords: *Botryosphaeria,* canker, endophyte, *Fusicoccum fabicercianum, Lasiodiplodia, Neofusicoccum*

1. INTRODUCTION

The Botryosphaeriaceae (Botryosphaeriales, Ascomycetes) incorporates a family of fungi that have a cosmopolitan distribution and occur on a wide range of monocotyledonous, dicotyledonous and gymnosperm hosts (Barr 1987; Crous *et al.* 2006; Slippers & Wingfield 2007). These fungi are generally regarded as weak, opportunistic pathogens that cause disease symptoms on plants exposed to stressful environmental conditions such as those associated with drought, frost, hail and damage caused by other pathogens and pests (Smith *et al.* 1994; Slippers & Wingfield 2007). It has also been shown that the Botryosphaeriaceae occur in asymptomatic tissues as endophytes and latent pathogens on a variety of trees, including *Eucalyptus* spp. (Smith *et al.* 1996; Mohali *et al.* 2007; Pavlic *et al.* 2007; Slippers & Wingfield 2007; Slippers *et al.* 2009). These fungi can cause different symptoms on *Eucalyptus*, but are typically associated with cankers and die-back followed by kino exudation and in severe cases tree death (Smith *et al.* 1996; Slippers & Wingfield 2007).

Species of the Botryosphaeriaceae are considered to be a significant threat to the production and sustainability of plantations of *Eucalyptus* spp., where they are grown as non-native crops (Smith *et al.* 1994, 2001; Slippers *et al.* 2004b, 2009; Mohali *et al.* 2009; Rodas *et al.* 2009). At least 23 species of Botryosphaeriaceae have been associated with *Eucalyptus* spp. in commercially grown plantations worldwide (Slippers *et al.* 2009). Some of these species, such as *Botryosphaeria dothidea* (Moug.) Ces. & De Not. and *Neofusicoccum ribis* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips were the most commonly reported species from *Eucalyptus* spp. in the past (Slippers *et al.* 2009). However, the application of DNA-based molecular tools for identification of species in the Botryosphaeriaceae has shown these two species are rare on *Eucalyptus* spp. Furthermore, a large number of new or cryptic sister species have been identified on *Eucalyptus* spp., mostly in the genera *Neofusicoccum, Pseudofusicoccum* and *Lasiodiplodia* (Slippers *et al.* 2009).

Eucalyptus plantations are of increasing importance in China where they provide the country with pulp products such as paper and structural timber. Approximately 2.6 million hectares of *Eucalyptus* plantations have been established in China to meet the needs of the country, especially for the rapidly growing pulp industry (Xie *et al.* 2006; Iglesias-Trabad & Wilstermann 2008). *Eucalyptus* trees are thus important in China, both for the development of

the economy and protection of native ecosystems that have been strictly protected from logging since the beginning of the 21st century.

Similar to the situation in other countries (Wingfield *et al.* 2008), *Eucalyptus* plantations in China are threatened by various pests and diseases (Zhou *et al.* 2008). Limited research has, however, been conducted on *Eucalyptus* diseases in this country. A recent survey of *Eucalyptus* plantations in South China reported a number of fungal pathogens, including species within the Botryosphaeriaceae (Zhou *et al.* 2008). The aims of the current study were to identify the Botryosphaeriaceae occurring on *Eucalyptus* spp. in the FuJian, GuangXi and HaiNan Provinces in South China, using PCR-RFLP fingerprinting analyses, DNA sequence comparisons and morphology of the anamorph stages. Furthermore, the pathogenicity of these fungi was tested on *Eucalyptus* clones in glasshouse trials and on different genotypes in field trials.

2. MATERIALS AND METHODS

2.1. Isolates

Isolates used in this study were collected from different *Eucalyptus* clones/species in plantations in FuJian, GuangXi and HaiNan during the period of 2006 and 2007. Isolations were made from diseased branches, and from pycnidia formed on senescing branches following the protocols described by Pavlic *et al.* (2007, 2008). Cultures were maintained on 2% malt extract agar (MEA) (20 g malt extract, 15 g agar, 1 L water; Biolab, Midrand, South Africa) at 25°C under near-fluorescent light and stored at 5°C. To induce sporulation of cultures, isolates were transferred to 2% water agar (WA) (20 g agar, 1 L water; Biolab, Midrand, South Africa) with sterilized pine needles placed on the agar surface and incubated at 25°C for 10–14 days until fruiting structures appeared on the surfaces of the pine needles.

Conidial masses from fruiting structures were spread on the surface of WA in sterile drops of water. Single germinating conidia were isolated after 4–12 hours, and transferred to clean 2% MEA plates. All the single-spore cultures are maintained in the Culture Collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa and the China Eucalypt Research Centre (CERC), Chinese Academy of Forestry (CAF), China.

2.2. DNA extraction and PCR amplification

For DNA extraction, single-conidial cultures were grown on 2% MEA for 7–10 days at 25ºC in the dark. Mycelium used for DNA extraction was scraped directly from the medium using a sterile scalpel, and transferred to 1.5 µL Eppendorf tubes. DNA was extracted following a modified DNA extraction method (Raeder & Broda 1985; Smith *et al.* 2001). The DNA pellets were re-suspended in 50 µL sterile SABAX water. RNAse (1 mg/mL) was added to DNA suspensions and incubated overnight at 37°C in a water bath for RNA degradation. DNA was separated by electrophoresis on a 1% agarose gel, stained with ethidium bromide and visualized under ultraviolet (UV) light.

The internal transcribed spacer (ITS) regions, ITS1 and ITS2, and the 5.8S gene of the ribosomal DNA (rDNA) operon, were amplified using the primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.* 1990). Part of the translation elongation factor 1-alpha (TEF-1α) gene was amplified using the primers EF1-728F (5'-CATCGAGAAGTTCGAGAAGG-3') and EF1-986R (5'-TACTTGAAGGAACCCTTACC-3') (Carbone *et al.* 1999). The PCR reactions were performed following the PCR protocol described in Slippers *et al.* (2004a). In the case of isolates that were difficult to amplify using primers EF1-728F and EF1-986R, the primers EF1F (5'-TGCGGTGGTATCGACAAGCGT-3') and EF2R (5'-AGCATGTTGTCGCCGTTGAAG-3') (Jacobs *et al.* 2004) were used. The PCR amplicons were viewed on a 1% agarose gel, stained with ethidium bromide and visualized under UV light. The fragment sizes were estimated against a standard 100 bp molecular weight marker (Roche Molecular Biochemicals, Mannheim, Germany). The PCR products were cleaned using 6% Sephadex G-50 columns (Steinheim, Germany).

2.3. PCR-RFLP analyses

A PCR-RFLP fingerprinting technique was applied to identify groups among the collected isolates and to select representative isolates for sequencing. The ITS rDNA locus was amplified from all the isolates using primers ITS1 and ITS4 and amplicons were digested with the restriction enzymes (RE) *Cfo*I and *Ksp*I (Roche Diagnostics, Indianapolis, U.S.A.). Isolates identified as representing the *Neofusicoccum parvum* (Pennycook & Samuels) Crous,

Slippers & A.J.L. Phillips /*N. ribis* complex were further separated by PCR-RFLP analyses of the amplicons obtained using primers BotF15 and BotF16 (Slippers *et al.* 2004b) and digested with *Cfo*I. The RFLP reaction mixtures consisted of 20 µL PCR products, 0.30 µL RE and 2.5 µL matching enzyme buffers. The reaction mixtures were incubated for 18 hours at 37ºC. The resulting restriction fragments were separated on 2% agarose gels, stained with ethidium bromide and visualized under UV light. A standard 100 bp molecular marker was used to estimate the fragment sizes. The banding patterns were compared with those previously published (Slippers *et al.* 2004b).

2.4. DNA sequencing and analyses

Representative isolates from all groups identified based on PCR-RFLP analyses were sequenced (Table 1). To determine their identities, ITS and TEF-1α sequences of known Botryosphaeriaceae species were obtained from GenBank and included in the analysis (Table 1). The PCR products were sequenced in both directions using the same primers that were used for PCR reactions, and were purified using 6% Sephadex G-50 columns. Sequence reactions were run on an ABI PRISM 3100^{TM} autosequencer (Perkin-Elmer Applied Biosystems, Foster City, California).

Nucleotide sequences were analyzed using MEGA4 software (Tamura *et al.* 2007). Sequence alignments were conducted online using MAFFT version 5.667 (Katoh *et al.* 2002) with the iterative refinement method (FFT-NS-i settings) and adjusted manually. Gaps were treated as a fifth character and all characters were unordered and of equal weight. A partition homogeneity test (PHT) was used to determine the congruence of the ITS and TEF-1α datasets (Farris *et al.* 1995; Huelsenbeck *et al.* 1996). After an outcome indicating congruence between the datasets, phylogenetic analyses of the combined datasets were done in PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford 2002). Most parsimonious trees were found using the heuristic search function with 1000 random addition replicates and tree bisection and reconstruction (TBR) as branch swapping algorithm. Maxtrees were unlimited, branches of zero length were collapsed, and all parsimonious trees were saved. A bootstrap analysis (1 000 replicates) was done to determine the confidence levels of the tree-branching points (Felsenstein 1985). The sequence of *Guignardia philoprina* (Berk. & M.A. Curtis) Aa from GenBank was used as the outgroup taxon (table 1).

By using the Markov Chain Monte Carlo (MCMC) method, Bayesian analyses were performed to ascertain the topology of the trees obtained with PAUP. These were conducted on the combined datasets of ITS and TEF-1α sequence as those used in the parsimony analysis. First MrModeltest v.2.3 (Nylander 2004) was used to determine the best nucleotide substitution model. Phylogenetic analyses were performed with MrBayes v. 3.1 (Ronquist & Huelsenbeck 2003). GTR+G was chosen as best-fitting model for the ITS and TEF-1α combined dataset. Two independent runs of MCMC with four chains were run for 1 000 000 generations**.** Trees were sampled every 100th generation for a total of 10 000 trees. The first 1 000 trees were discarded as the burn-in phase of each analysis, well after the likelihood values converted to stationary, leaving 9 000 trees from which the consensus trees and posterior probabilities were calculated. The sequence of *Guignardia philoprina* from GenBank was used as the outgroup taxon (table 1).

2.5. Morphology

Single-spore cultures of each species identified using DNA sequence data were transferred to WA media with sterilized pine needles placed on the agar surface and incubated at 25ºC to induce sporulation. Released conidia from the pycnidia formed on pine needles were mounted in 85% lactic acid on glass slides and examined microscopically. Digital images were taken using a light microscope, a HRc Axiocam digital camera and accompanying software (Carl Zeiss Ltd., Munich, Germany). Widths and lengths of twenty conidia were measured for each taxon and average (mean), standard deviation (std. dev), minimum (min) and maximum (max) measurements presented as [(min–)(average – std. dev.) – (average + std. dev.)(–max)], and their length: width ratios were calculated. The morphology of fungal colonies and the growth rates of cultures were conducted following the method described in Begoude *et al*. (2010b).

2.6. Pathogenicity tests

2.6.1. Glasshouse trials

Fourteen isolates, representing different species of the Botryosphaeriaceae identified based on PCR-RFLP fingerprinting, anamorph morphology and DNA sequence comparisons, were used in a glasshouse pathogenicity trial (Table 1). These isolates were randomly selected to

represent different locations and hosts. Six-day-old isolates grown on 2% MEA at 25ºC were used for inoculations.

Trees of a *Eucalyptus grandis* W.Hill clone (TAG-5) were selected for pathogenicity tests under glasshouse conditions. The trees were approximately 2 m tall and had diameters of ~10 mm. Before conducting the inoculations, the trees were allowed to acclimatize to the glasshouse conditions of 25°C and 14 hours daylight, with 10 hours darkness, for one month. Each of the 14 selected isolates was inoculated into the stems of 10 trees. Ten trees were also inoculated with sterile MEA plugs to serve as controls. The total of 150 inoculated trees was randomly arranged in a glasshouse.

For inoculations, wounds were made on the stems of the seedlings using a six-mm-diameter cork borer to remove the bark and expose the cambium. Wounds were made on the stems of the trees, approximately 300 mm above soil level. Plugs of mycelium were taken from 10-day-old cultures grown on MEA using the same size cork borer, and were placed into the wounds with the mycelium facing the cambium. Inoculated wounds were sealed with laboratory film (Parafilm M, Pechiney Plastic Packaging) to prevent desiccation and contamination.

After six weeks, the bark of the inoculated plants was removed and internal lesion lengths (mm) on the cambium were measured. The inoculated fungi were re-isolated by cutting small pieces of wood from the edges of lesions and plating them on 2% MEA at 25ºC. Re-isolations were made from four randomly selected trees per isolate and from all trees inoculated as controls. Results were analysed in SAS (Version 8) using PROC GLM (General Linear Model) (SAS Institute, 1999). Analysis of variance (ANOVA) was used to determine the effects of fungal strain on lesion length. Prior to ANOVA, homogeneity of variance across treatments was verified. For significance tests amongst means, Fisher's protected test was used. F values with $P < 0.05$ considered significant.

2.6.2. Field inoculation trials

The eight most aggressive isolates, belonging to five different species of Botryosphaeriaceae, as identified in glasshouse trails, were selected for field trails in China (Table 1). The field inoculations were conducted in an experimental *Eucalyptus* plantation in the ZhanJiang area

of South China. Five *Eucalyptus* genotypes, commonly grown in commercial plantations in South China were used in the field trial. These included an *E. grandis* clone (CEPT-1), an *E. grandis* × *E. tereticornis* Sm. clone (CEPT-2), an *E. pellita* F.Muell. genotype (CEPT-3), an *E. urophylla* S.T.Blake × *E. grandis* clone (CEPT-4) and an *E. urophylla* clone (CEPT-5). Each of the eight isolates were inoculated into eight trees for every *Eucalyptus* genotype. Eight trees of each *Eucalyptus* genotype were also inoculated with sterile MEA plugs to serve as controls.

Inoculations were made on the stems of trees 50–100 cm above the soil level. A nine-mm-diameter sterile metal cork borer was used to remove the bark and to expose the cambial layer. Discs of mycelium of the same size were taken from seven-day-old cultures and placed into the exposed wounds with the mycelium facing the cambium. The inoculated wounds were sealed with Parafilm to protect them from desiccation and contamination. The inoculations were conducted in October–November, 2008. Internal lesion lengths in the cambium were measured after five weeks. Results from the filed trials were analysed separately in SAS (Version 8) using PROC GLM (SAS Institute, 1999) and in a similar manner as for the glasshouse inoculation tests.

3. RESULTS

3.1 Isolates

Forty-eight isolates of Botryosphaeriaceae were collected from 14 trees in three provinces during collection trips in 2006 and 2007 (Table 1). Six of these isolates were collected from a single *E. grandis* hybrid tree and two trees of an unknown *Eucalyptus* clone at two sites in GuangXi Province in November of 2006. Three isolates were each collected from three different trees of an *E. urophylla* × *E. tereticornis* clone at a site in the HaiNan Province in June of 2007. Thirty-nine isolates were collected from four different sites of FuJian Province in September of 2007, nine isolates were collected from a single *E. urophylla* × *E. grandis* clone at one site, four isolates from two trees of an *E. dunnii* clone at another site, and 26 isolates were collected from five trees of an unknown *Eucalyptus* sp. at two different sites. Nine isolates from the GuangXi and HaiNan Provinces were collected from diseased twigs, while the 39 isolates from FuJian Province were collected from senescing twigs on different trees (Table 1).

3.2. PCR-RFLP analyses

The 48 isolates of Botryosphaeriaceae from South China were separated into four groups based on PCR-RFLP profiles. After digestion of ITS rDNA amplicons with *Cfo*I, the 48 isolates were separated into three groups (Figs 1a, 1b). These profiles were compared with those published by Slippers *et al.* (2004b). Four isolates, identified as *Lasiodiplodia* spp. based on morphology, produced the same profile (Group 1) (Fig 1a) and 17 isolates in Group 2 represented members of the *N. parvum/N. ribis* complex (Figs 1a, 1b). Profiles of 27 isolates in Group 3 matched those of the *B. dothidea* complex or *N. luteum/N. australe* (Figs 1a, 1b). The ITS amplicons of these isolates were, therefore, also digested with *Ksp*I and identified as *B. dothidea* complex (Fig 1c).

The 17 isolates residing in the *N. parvum/N. ribis* complex (Group 2) produced two profiles after digestion of BotF15 and BotF16 amplicons with RE *Cfo*I (Fig 1d). These profiles matched those of *N. parvum* (16 isolates) and *N. ribis s. l.* (one isolate) as described by Slippers *et al.* (2004b).

3.3. Phylogenetic analyses

The partition homogeneity test comparing the ITS and TEF-1α datasets gave a PHT value of P = 0·297, indicating that these two datasets were congruent and could be combined in the phylogenetic analyses. The aligned sequences of the combined datasets were submitted to TreeBASE (11035; www.TreeBASE.org), and consisted of 863 characters of which 481 were constant and 382 were parsimony informative. Heuristic searches resulted in 20 most parsimonious trees. A consensus tree (50% majority rule) (tree length = 784 steps; CI = 0·802; RI = 0·969; RC = 0·777) was computed (Fig. 2; TreeBASE 11035).

In the Bayesian analysis, the position of the genera in relation to each other was different, but within each genus the topology was similar to the parsimony tree (TreeBASE SNXXX). Three clades were identified, each corresponding to a separate genus and each supported with high Bayesian posterior probabilities (PP) and bootstrap support (BS) (Fig 2, PP = 0.99, BS = 100%; PP = 0.95, BS = 99%; PP = 0.99, BS = 100%; respectively). These were Clade 1 (*Neofusicoccum*), Clade 2 (*Botryosphaeria*) and Clade 3 (*Lasiodiplodia*). Within the

Neofusicoccum clade, a single Chinese isolate was phylogenetically distant from other species within this clade, while other Chinese isolates in this clade resided with *N. parvum*. Within the *Botryosphaeria* clade, the Chinese isolates were found to be distinct from the known species in the genus by congruent distinction in both the datasets, and high Bayesian posterior probability and high bootstrap value (PP = 0.98, BS = 99%), suggesting that they represent an undescribed species. Within the *Lasiodiplodia* clade, the isolates in this study resided in clades identified as *L. theobromae* (Pat.) Griffon & Maubl. and *L. pseudotheobromae* A.J.L. Phillips, A. Alves & Crous, respectively (Fig 2).

3.4. Morphology and taxonomy

All 48 isolates of the Botryosphaeriaceae from *Eucalyptus* plantations in South China produced anamorph fruiting structures on pine needles on WA media within 2–3 weeks. Teleomorph structures were not observed. All isolates were separated into two main groups based on conidial morphology. Forty-four isolates produced hyaline, *Fusicoccum*-like conidia, and four isolates produced dark, septate and striate conidia typical for *Lasiodiplodia* spp. Based on PCR-RFLP fingerprinting, anamorph morphology and DNA sequence comparisons, five species were identified. The taxonomy of the undescribed species and the conidial morphology of the other four species are described as follows:

Fusicoccum fabicercianum S.F. Chen, D. Pavlic, M.J. Wingf. & X.D. Zhou, sp. nov.
(Fig 3)
MycoBank no.: MB519065
Etymology: Name refers to the co-operation between FABI (Forestry and Agricultural Biotechnology Institute, South Africa) and CERC (China Eucalypt Research Centre, China), that led to the discovery of this new species.

Conidiomata superficialia solitaria vel aggregata atrobrunnea globosa, hyphis vel mycelio tecta. Conidiophorae absunt. Cellulae conidiogenae cylindricae vel lageniformes, hyalinae laeves parietibus tenuibus holoblasticae conidium unicum in apice formantes. Paraphyses absunt. Conidia hyalina parietibus tenuibus laevia contento granuloso, unicellularia non septata, fusiformia medio vel in tertia parte summa latissima, apice acuta, basi truncata fimbriis minutis marginalibus, mediocriter 22.0 × 5.8 µm, 3.8–plo longiora quam latiora; unum vel duo septa ante germinationem formantes.

Conidiomata (Fig 3a): Developing in culture on pine needles after 10 days and producing conidia after 14 days, superficial, solitary to aggregated, dark brown, globose, covered with hyphae/mycelium, diameter (245–)346–470(–525) µm (average of 50 conidiomata 408 µm); wall composed of three layers: an outer thick-walled dark to light brown *textura angulari*; a middle layer of thin-walled light brown cells; and an inner layer of thin-walled hyaline cells. **Conidiophores** absent. **Conidiogenous cells** (Figs 3b, 3c) cylindrical to lageniform, hyaline, smooth, thin-walled, holoblastic producing a single conidium at the tip, rarely proliferating at same level giving rise to periclinal thickenings, (6.5–)10.5–13.5(–16.0) × (2.0–)2.5–3.5(–4.5) µm (average of 50 conidiogenous cells 12.0 × 3.0 µm). **Paraphyses** absent. **Conidia** (Figs 3d–3g) hyaline, thin-walled, smooth (Fig 3d) with granular contents (Fig 3e); unicellular, aseptate, fusiform, widest in the middle to upper third, apex acute, base truncate with a minute marginal frill, (16.7–)19.6–24.4(–26.1) × (4.5–)5.2–6.4(–7.5) µm (average of 100 conidia 22.0 × 5.8 µm, l/w = 3.8); forming one or two septa before germination (Figs 3f, 3g).

Culture characteristics: Colony mycelium fluffy, initially white turning smoke gray (21""f) from the middle of colonies within 4–6 days, with an appressed mycelial mat, sparse to moderately dense. Cottony aerial mycelium toward the edge of colony, becoming pale olivaceous grey (21""d) to olivaceous grey (21""i), and greenish black (33""k) (reverse) within 12–16 days (Figs 3h, 3i). Optimal temperature for growth 25(–30) ºC, colony covering the 90 mm diam Petri dish after 5 days in the dark.

Teleomorph: Not observed, but expected to be *Botryosphaeria*-like based on phylogenetic inference.

Habitat: Branches and twigs of *Eucalyptus* species and hybrids.

Hosts and Distributions: *Eucalyptus urophylla* × *E. grandis* and a *Eucalyptus* sp. in FuJian Province; *E. urophylla* × *E. tereticornis* in HaiNan Province; *Eucalyptus grandis* hybrid in GuangXi Province; China.

Specimens examined: China. FuJian Province, from senescing twigs of an unknown *Eucalyptus* sp. Aug., 2007, M.J. Wingfield, Herb. PREM 60449, holotype of *F. fabicercianum* sp. nov., culture ex-type CMW 27094 = CBS 127193; FuJian Province, from

senescing twigs of unknown *Eucalyptus* sp. Aug., 2007, M.J. Wingfield, Herb. PREM 60450, culture CMW 27121 = CBS 127194; GuangXi Province, from senescing twigs of *Eucalyptus grandis* hybrid, Nov., 2006, M.J. Wingfield & X.D. Zhou, culture CMW 24703 = CBS 127187; HaiNan Province, from diseased living twigs of *E. urophylla* × *E. tereticorni*; June, 2007, M.J. Wingfield & X.D. Zhou, culture CMW 27091.

Notes: *Fusicoccum fabicercianum* sp. nov. is morphologically similar to *Botryosphaeria cortices* (Demaree & Wilcox) Arx & E. Müll., but can be distinguished from that species by having smaller conidiogenous cells and smaller conidia. *Botryosphaeria corticis*: conidiogenous cells 12.5–17.5 × 2.5–4.5 µm, conidia (20.5–)23.5–32.5(–34.5) × (5–)5.5–7(–7.5) µm, av. = 28.9 × 6.4 µm, //w = 4.5 (Phillips *et al.* 2006). Compared to the conidial morphology of other species of *Fusicoccum*, the conidia of *F. fabicercianum* sp. nov. are bigger than those of *F. ramosum* Pavlic, T.I. Burgess, M.J. Wingf. (13.4 × 5.7 µm in culture) (Pavlic *et al*, 2008), similar to *B. dothidea* (24.7 × 4.9 µm in culture, 19.6 × 4.8 µm on a natural *Prunus* sp.) (Slippers *et al.* 2004a), smaller than *F. atrovirens* J. W. M. Mehl & B. Slippers (33.5 × 8.5 µm in culture) (Mehl *et al.* 2010) and *B. mamane* D.E. Gardner (33.5 × 6.1 µm in culture) (Mohali *et al.* 2007). *Fusicoccum fabicercianum* sp. nov. is also characterized by developing one or two septa in the conidia before germination, which is not uncommon amongst species of *Botryosphaeria* (Slippers *et al.* 2004a).

Twenty-seven isolates were identified as *F. fabicercianum* sp. nov. in this study. One isolate was from a single unknown *E. grandis* hybrid tree in GuangXi Province, three isolates were from three different trees of an *E. urophylla* × *E. tereticornis* clone in the HaiNan Province, twenty-one isolates were from four trees of an unknown *Eucalyptus* sp. and an additional two isolates from a single *E. urophylla* × *E. grandis* tree were identified from the FuJian Province.

Two isolates from one tree of an unknown *Eucalyptus* sp. in GuangXi Province were identified as *L. pseudotheobromae*. Conidia of *L. pseudotheobromae* were ellipsoidal, apices and bases rounded, widest in their middle to upper two-thirds, thick-walled, initially hyaline and aseptate, becoming one-septate and dark walled, (26.5–)27–29(–30.5) × (13–)14.5–16(–17) µm (average of 40 conidia 28.1 × 15.2 µm, l/w 1.8) (Fig 4a).

Two isolates from a single unknown *Eucalyptus* tree in GuangXi Province were identified as *L. theobromae*. Conidia of *L. theobromae* were ellipsoid or oblong, broadly rounded at their

apices, tapering to truncate bases, widest in their middle to upper one-fourth sections, initially hyaline and aseptate, becoming one-septate and dark brown, (22.5–)23.5–26(–27) × (12–)13–14.5(–16) µm (average of 40 conidia 24.7 × 13.9 µm, l/w 1.8) (Fig 4b).

Conidia of isolates identified as *N. parvum* and *N. ribis s. l.* were fusiform to ellipsoidal, aseptate, hyaline, smooth with granular contents. Conidia of *N. parvum* (Fig 4c) were (16.5–)17–20(–23.0) × (5–)5.5–6.5(–7) µm (average of 100 conidia 18.7 × 6.0 µm, l/w 3.1) and *N. ribis s. l.* (Fig 4d) were (19–)19.5–21(–22.5) × (5.5–6.5) µm, (average of 20 conidia 20.6 × 6.0 µm, l/w 3.5). Sixteen isolates were identified as *N. parvum*. One isolate was from a single *E. grandis* hybrid tree in GuangXi Province, fifteen isolates from FuJian Province, including four isolates from two trees of an unknown *Eucalyptus* sp., seven isolates from a single *E. urophylla* × *E. grandis* tree and another four isolates from two *E. dunnii* trees. One isolate, from a single unknown *Eucalyptus* tree in FuJian Province was identified as *N. ribis s. l.*

3.5. Pathogenicity tests

3.5.1. Glasshouse trials

All isolates of the Botryosphaeriaceae inoculated on the *Eucalyptus* TAG-5 clone produced lesions within six weeks, while small lesions were produced for the control inoculations (Fig 5). Statistical analyses showed that the lesions produced by the isolates of Botryosphaeriaceae were significantly longer than those of the controls ($P < 0.0001$) (Fig 5). The inoculated fungi were successfully re-isolated from the lesions. Although small lesions were produced on the controls, no Botryosphaeriaceae were re-isolated from those trees.

Lesions produced by isolates of *L. pseudotheobromae* (CMW 24699, CMW 24700) and *L. theobromae* (CMW 24701, CMW 24702) were significantly longer than those induced by isolates of *F. fabicercianum* sp. nov. (CMW 24703, CMW 27091, CMW 27094, CMW 27106, CMW 27121), *N. parvum* (CMW 24704, CMW 27110, CMW 27125, CMW 27135) and *N. ribis s. l.* (CMW 27119) ($P < 0.05$) (Fig 5). Isolates of *L. pseudotheobromae* were more aggressive than those of *L. theobromae* (Fig 5). The two most aggressive isolates of each species of *L. pseudotheobromae* (CMW 24699, CMW 24700), *L. theobromae* (CMW 24701, CMW 24702) and *F. fabicercianum* sp. nov. (CMW 24703, CMW 27094), and one most

aggressive isolate of *N. parvum* (CMW 27125) and *N. ribis s. l.* (CMW 27119) were selected for field inoculations.

3.5.2. Field trials

All isolates used in the field trial produced lesions on the *Eucalyptus* genotypes. Small lesions were also produced for the control inoculations (Fig 6). Lesions produced by all isolates were significantly longer than those of the controls ($P < 0.05$) (Fig 6). Statistical analyses showed significant isolate × genotype interaction ($P < 0.0001$), indicating that not all the Botryosphaeriaceae isolates reacted in the same manner to all the tested *Eucalyptus* genotypes. For example, lesions produced by *L. pseudotheobromae* (CMW 24699, CMW 24700) on *Eucalyptus* genotypes CEPT-1, CEPT-2 and CEPT-4 were significantly longer than those on CEPT-3 and CEPT-5 ($P < 0.05$) (Fig 6), while no significant differences in lesion length were found for the five *Eucalyptus* genotypes inoculated with isolates of *F. fabicercianum* sp. nov. (CMW 24703, CMW 27094) ($P > 0.05$) (Fig 6). Similar to the glasshouse inoculations, *L. pseudotheobromae* was found to be the most aggressive. Results from field inoculations showed that the *Eucalyptus* genotypes CEPT-1, CEPT-2 and CEPT-4 are more susceptible to infection by the species of Botryosphaeriaceae tested in this study than are genotypes CEPT-3 and CEPT-5 (Fig 6).

4. DISCUSSION

This study represents the most comprehensive consideration of species of Botryosphaeriaceae from *Eucalyptus* spp. in China to date. Using comparisons of ITS rDNA and TEF-1α sequence data combined with PCR-RFLP analyses and morphology of the anamorph structures, it was possible to identify five species from a small collection of isolates. They included *L. pseudotheobromae*, *L. theobromae*, *N. parvum*, *N. ribis sensu lato* and *F. fabicercianum* sp. nov. Except for *N. parvum* (Slippers *et al.* 2009), all species are reported for the first time from *Eucalyptus* trees in China.

The distribution of the species collected in this study varied among the regions. *Fusicoccum fabicercianum* sp. nov. was found on diseased *Eucalyptus* trees in the GuangXi and HaiNan Provinces, and also on dying *Eucalyptus* twigs in FuJian Province. *Lasiodiplodia pseudotheobromae* and *L. theobromae* were identified on a diseased *Eucalyptus* sp. in

GuangXi Province. *Neofusicoccum parvum* was found on diseased *Eucalyptus* trees in GuangXi Province and dying twigs in FuJian Province. *Neofusicoccum ribis s. l.* was collected from senescing twigs on a *Eucalyptus* sp. in FuJian Province. *Fusicoccum fabicercianum* sp. nov., *N. parvum* and *N. ribis s. l.* were isolated from senescing twigs on the same tree in FuJian Province, indicating that different species of Botryosphaeriaceae share the same ecological environment in China, which is similar to that found in other studies (Slippers & Wingfield 2007).

The relatively limited distribution of *L. pseudotheobromae* and *L. theobromae* is most likely related to a collecting bias rather than to particular ecological boundaries for these fungi. This view emerges from the fact that these fungi are known from numerous countries, especially from tropical regions (Punithalingam 1980; Mohali *et al.* 2005, 2007; Pavlic *et al.* 2007; Alves *et al.* 2008; Begoude *et al.* 2010a). It would thus be unusual for them to occur only in a limited area of South China. These fungi are also known to have a wide host range on native and introduced trees (Punithalingam 1976; Roux *et al.* 2000, 2001; Burgess *et al.* 2006; Mohali *et al.* 2005, 2007; Pavlic *et al.* 2007; Alves *et al.* 2008; Begoude *et al.* 2010a), making it unlikely that the *Eucalyptus* spp. considered in this study were a determining factor in the distribution of the species.

Fusicoccum fabicercianum sp. nov. resides in the *Botryosphaeria* (anamorph *Fusicoccum*) clade of the Botryosphaeriaceae and pathogenicity tests indicate that it is a weak pathogen. Although the species was most commonly isolated in samples taken in three provinces of South China, it was less pathogenic than the other species collected. Phylogenetically, *F. fabicercianum* sp. nov. is closely related to *Botryosphaeria corticis*, *B. dothidea* and *F. ramosum*. Interestingly, our results further showed that *Botryosphaeria* spp. with *Fusicoccum* anamorphs clustered in two clades supported by high statistical values. One clade includes *B. corticis*, *B. dothidea*, *F. fabicercianum* sp. nov. and *F. ramosum*, while *B. mamane* and *F. atrovirens* reside in the other clade (Fig 2). Previous studies have shown that there are conidial differences amongst the species residing in the two clades, for example the conidia of species in the former clade are shorter (average: less than 30 µm) than those of *B. mamane* and *F. atrovirens* (average: more than 30 µm) (Slippers *et al.* 2004a; Phillips *et al.* 2006; Mohali *et al.* 2007; Pavlic *et al.* 2008; Mehl *et al.* 2010). More representative species and further work is needed to better understand the relevance of this divergence, and whether these groups validate distinct generic descriptions.

Neofusicoccum parvum is a well known pathogen of *Eucalyptus* spp. (Slippers *et al.* 2004b; Burgess *et al.* 2005; Pavlic *et al.* 2007; Slippers & Wingfield 2007; Mohali *et al.* 2009; Rodas *et al.* 2009). The pathogen has been identified from *Eucalyptus* trees in Australia (Slippers *et al.* 2004b), Africa (Gezahgne *et al.* 2004; Slippers *et al.* 2004b), South America (Mohali *et al.* 2007) and Southeast Asia (Slippers *et al.* 2009). Results of this study also showed that *N. parvum* was amongst the most pathogenic species, supporting the view that *N. parvum* is an important pathogen of *Eucalyptus* spp. (Slippers *et al.* 2004b; Slippers & Wingfield 2007).

A single isolate that could be identified only as *N. ribis s. l.* was isolated from a *Eucalyptus* sp. in FuJian Province. The taxonomy of *N. ribis* and closely related species has been confused for many years. Isolates of *N. ribis* were initially separated from its sister species *N. parvum,* based on concordance between sequence data of multiple gene regions and PCR-RFLP analyses and were treated as *N. ribis s. l.* (Slippers *et al.* 2004b). Subsequently, *N. ribis* has been reported from *Eucalyptus* in Australia (Barber *et al.* 2005), Colombia (Rodas *et al.* 2009) and Venezuela (Mohali *et al.* 2007). Recently, four species, *N. batangarum* Begoude, Jol. Roux, Slippers, *N. cordaticola* Pavlic, Slippers & M.J. Wingf., *N. kwambonambiense* Pavlic, Slippers & M.J. Wingf. and *N. umdonicola* Pavlic, Slippers & M.J. Wingf. were identified in the *N. ribis s. l.* complex based on multiple gene genealogies (Pavlic *et al.* 2009a, b; Begoude *et al.* 2010b). Thus, isolates previously identified as *N. ribis* based on PCR-RFLP analyses could represent any of a number of cryptic species. The isolate included in this study grouped closely, but separated from *N. batangarum*, *N. cordaticola* and *N. umdonicola* in the phylogenetic analysis of the ITS rDNA and TEF-1α sequences. Thus, it could represent another cryptic species in the *N. parvum/N. ribis* complex. Additional isolates and DNA sequence data for a greater number of gene regions will be required to resolve its identity. Pathogenicity trials in this study showed that this isolate has the ability to produce lesions on *Eucalyptus* trees, although there was no indication that it might be an important pathogen.

Two *Lasiodiplodia* species, *L. pseudotheobromae* and *L. theobromae,* were identified in this study. The latter species is a widely distributed plant pathogen that has most commonly been reported from the tropics and subtropics (Punithalingam 1980). It has been associated with more than 500 host plants including *Eucalyptus* (Punithalingam 1976; Roux *et al.* 2000, 2001; Burgess *et al.* 2006; Mohali *et al.* 2007). This pathogen has been reported from *Eucalyptus* trees in Africa (Roux *et al.* 2000, 2001), Australia (Burgess *et al.* 2006) and South America

(Mohali *et al.* 2007), and is considered the most aggressive species of Botryosphaeriaceae on these trees (Pavlic *et al.* 2007; Mohali *et al.* 2009). In the current study, *L. theobromae* was second only to *L. pseudotheobromae* in terms of its pathogenicity, which further supports the view that *L. theobromae* is one of the most damaging species of Botryosphaeriaceae on *Eucalyptus* (Slippers & Wingfield 2007).

Lasiodiplodia pseudotheobromae is a sister species to *L. theobromae* and was recently described from species of *Acacia*, *Citrus*, *Coffea*, *Gmelina* and *Rosa* (Alves *et al.* 2008). It has also been identified on *Eucalyptus* spp. in eastern Australia (Mohali *et al.* 2005; Alves *et al.* 2008; Slippers *et al.* 2009) and Venezuela (Mohali *et al.* 2005; Alves *et al.* 2008; Slippers *et al.* 2009). In this study, isolates of this species were the most aggressive of all the Botryosphaeriaceae tested. Previous inoculations on *Terminalia* trees also showed that *L. pseudotheobromae* is the most pathogenic among all the tested species of Botryosphaeriaceae on those trees (Begoude *et al.* 2010a).

Pathogenicity trials, both in the glasshouse and field showed that all the isolates of Botryosphaeriaceae considered in this study can cause lesions on inoculated *Eucalyptus* trees. Other than *N. ribis s. l.*, all the Botryosphaeriaceae species found in this study occurred on diseased *Eucalyptus* tissue in HaiNan and GuangXi Provinces. Symptoms from which these species were collected included branch die-back and distinct lesions on branches of *Eucalyptus* in South China. Results of pathogenicity test confirmed that all species of Botryosphaeriaceae collected have the potential to cause disease in *Eucalyptus* plantations of China.

Overall results of this study suggest that it is important to consider the impact of genotype by isolate and environment interaction when conducting artificial screening trials. For example, in the field pathogenicity trials on *Eucalyptus* genotypes CEPT-3, CEPT-4 and CEPT-5, lesions produced by isolate CMW 24702 of *L. theobromae* were significantly longer than those of *N. parvum* and the controls. In Venezuela, lesions produced by *L. theobromae* were significantly shorter than those of *N. parvum*, while the lesions produced by *L. theobromae* and the controls were not significantly different (Mohali *et al.* 2009). It is thus important to select the appropriate isolates for screening trials and to consider the involvement of isolate by tree genotype interaction, which has also been shown to be important for the important

Eucalyptus stem pathogen *Chrysoporthe austroafricana* Gryzenh. & M.J. Wingf. (Van Heerden *et al.* 2005; Gryzenhout *et al.* 2009).

Species of Botryosphaeriaceae are considered to be a significant threat to the production and sustainability of *Eucalyptus* plantations. These fungi can infect native and introduced or cultivated hosts and appear to have been moved easily between different countries and continents (Slippers & Wingfield 2007; Slippers *et al.* 2009). The results of this study provide a foundation for future work aimed at managing diseases caused by the Botryosphaeriaceae in *Eucalyptus* plantations in China.

5. REFERENCES

Alves A, Crous, PW, Correia A, Phillips AJL, 2008. Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. *Fungal Diversity* **28**: 1–13.

Barber P, Burgess T, Hardy GJ, Slippers B, Wingfield MJ, 2005. Morphology and DNA sequence comparisons show that well defined species of *Botryosphaeria* from *Eucalyptus* in Australia have *Dichomera*-like anamorphs. *Mycological Research* **109**: 1347–1363.

Barr ME, 1987. Prodromus to class Loculoascomycetes. Hamilton I. Newell, Inc. Amherst. MA, USA.

Begoude BAD, Slippers B, Wingfield MJ, Roux J, 2010a. The pathogenic potential of endophytic Botryosphaeriaceous fungi on *Terminalia* species in Cameroon. *Forest Pathology*. doi: 10.1111/j.1439-0329.2010.00671.x. (In Press).

Begoude BAD, Slippers B, Wingfield MJ, Roux J, 2010b. Botryosphaeriaceae associated with *Terminalia catappa* in Cameroon, South Africa and Madagascar. *Mycological Progress* **9**: 101–123

Burgess TI, Barber PA, Hardy GEStJ, 2005. *Botryosphaeria* spp. associated with eucalypts in Western Australia, including the description of *Fusicoccum macroclavatum* sp. nov. *Australasian Plant Pathology* **34**: 557–567.

Burgess TI, Barber PA, Mohali S, Pegg G, De Beer ZW, Wingfield MJ, 2006. Three new *Lasiodiplodia* spp. from the tropics, recognised based on DNA sequence comparisons and morphology. *Mycologia* **98**: 423–435.

Carbone I, Anderson JB, Kohn LM, 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* **3**: 553–556.

Crous PW, Slippers B, Wingfield MJ, Rheeder J, Marasas WFO, Philips AJL, Alves A, Burgess T, Barber P, Groenewald JZ, 2006. Phylogenetic lineages in the Botryosphaeriaceae. *Studies in Mycology* **55**: 235–253.

Farris JS, Kallersjo M, Kluge AG, Bult C, 1995. Testing significance of incongruence. *Cladistics* **10**: 315–319.

Felsenstein J, 1985. Confidence intervals on phylogenetics: an approach using bootstrap. *Evolution* **39**: 783–791.

Gezahgne A, Roux J, Slippers B, Wingfield MJ, 2004. Identification of the causal agent of *Botryosphaeria* stem canker in Ethiopian *Eucalyptus* Plantations. *South African Journal of Botany* **70**: 241–248.

Gryzenhout M, Wingfield BD, Wingfield MJ, 2009. *Taxonomy, phylogeny, and ecology of bark-inhabiting and tree pathogenic fungi in the Cryphonectriaceae*. APS Press, St. Paul, Minnesota, USA.

Huelsenbeck JP, Bull JJ, Cunningham CW, 1996. Combining data in phylogenetic analysis. *TREE* **11**: 152–158.

Iglesias-Trabad G, Wilstermann D, 2008. *Eucalyptus universalis*. Global cultivated eucalypt forest map 2008 Version 1.0.1 in GIT Forestry Consulting's EUCALYPTOLOGICS: Information resources on *Eucalyptus* cultivation worldwide. Retrieved from http://www.git-forestry.com (March 29th 2009).

Jacobs K, Bergdahl DR, Wingfield MJ, Halk S, Seifert KA, Bright DE, Wingfield BD, 2004. *Leptographium wingfieldii* introduced into North America and found associated with exotic *Tomicus piniperda* and native bark beetles. *Mycological Research* **108**: 411–418.

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.

Mehl JWM, Slippers B, Roux J, Wingfield MJ, 2010. Botryosphaeriaceae associated with *Pterocarpus angolensis* (kiaat) in South Africa. *Mycologia*. (in press).

Mohali S, Burgess TI, Wingfield MJ, 2005. Diversity and host association of the tropical tree endophyte *Lasiodiplodia theobromae* revealed using SSR markers. *Forest Pathology* **35**: 385–396.

Mohali S, Slippers B, Wingfield MJ, 2007. Identification of Botryosphaeriaceae species from *Eucalyptus*, *Acacia* and *Pinus* in Venezuela. *Fungal Diversity* **25**: 143–165.

Mohali S, Slippers B, Wingfield MJ, 2009. Pathogenicity of seven species of the Botryosphaeriaceae on *Eucalyptus* clones in Venezuela. *Australasian Plant Pathology* **38**: 135–140.

Nylander JAA, 2004. *MrModeltest 2.3. Program distributed by the author*. Uppsala University, Sweden: Evolutionary Biology Centre.

Pavlic D, Slippers B, Coutinho TA, Wingfield MJ, 2007. Botryosphaeriaceae occurring on native *Syzygium cordatum* in South Africa and their potential threat to *Eucalyptus*. *Plant Pathology* **56**: 624–636.

Pavlic D, Slippers B, Coutinho TA, Wingfield MJ, 2009a. Multiple gene genealogies and phenotypic data reveal cryptic species of the Botryosphaeriaceae: A case study on

the *Neofusicoccum parvum/N. ribis* complex. *Molecular Phylogenetics and Evolution* **51**: 259–268.

Pavlic D, Slippers B, Coutinho TA, Wingfield MJ, 2009b. Molecular and phenotypic characterization of three phylogenetic species discovered within the *Neofusicoccum parvum/N. ribis* complex. *Mycologia* **5**: 636–647.

Pavlic D, Wingfield MJ, Barber P, Slippers B, Hardy GEStJ, Burgess TI, 2008. Seven new species of the Botryosphaeriaceae from baobab and other native trees in Western Australia. *Mycologia* **100**: 851–866.

Phillips AJL, Oudemans PV, Correia A, Alves A, 2006. Characterisation and epitypification of *Botryosphaeria corticis*, the cause of blueberry cane canker. *Fungal Diversity* **21**: 141–155.

Punithalingam E, 1976. *Botryodiplodia theobromae*. Kew, UK: Commonwealth Mycological Institute: CMI descriptions of pathogenic fungi and bacteria no. 519.

Punithalingam E, 1980. Plant diseases attributed to *Botryodiplodia theobromae* Pat. Kew, UK: Commonwealth Mycological Institute.

Raeder U, Broda, P, 1985. Rapid preparation of DNA from filamentous fungi. *Letters in Applied Microbiology* **1**: 17–20.

Rodas CA, Slippers B, Gryzenhout M, Wingfield MJ, 2009. Botryosphaeriaceae associated with *Eucalyptus* canker diseases in Colombia. *Forest Pathology* **39**: 110–123.

Ronquist F, Huelsenbeck JP, 2003. MrBayes: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.

Roux J, Coutinho TA, Mujuni Byabashaija D, Wingfield MJ, 2001. Diseases of plantation *Eucalyptus* in Uganda. *South African Journal of Science* **97**: 16–18.

Roux J, Coutinho TA, Wingfield MJ, Bouillet J-P, 2000. Diseases of plantation *Eucalyptus* in the Republic of Congo. *South Africa Journal of Science* **96**: 454–456.

SAS Institute Inc, 1999. *SAS/STAT Users Guide*. Version 8. Cary NC: SAS Institute Inc. ISBN 1-58025-494-2.

Slippers B, Burgess T, Pavlic D, Ahumada R, Maleme H, Mohali S, Rodas C, Wingfield MJ, 2009. A diverse assemblage of Botryosphaeriaceae infect *Eucalyptus* in native and non-native environments. *Southern Forests* **71**: 101–110.

Slippers B, Crous PW, Denman S, Coutinho TA, Wingfield BD, Wingfield MJ, 2004a. Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. *Mycologia* **96**: 83–101.

Slippers B, Fourie G, Crous PW, Coutinho TA, Wingfield BD, Carnegie AJ, Wingfield MJ, 2004b. Species and distribution of *Botryosphaeria* spp. on native and introduced *Eucalyptus* trees in South Africa and Australia. *Studies in Mycology* **50**: 343–358.

Slippers B, Wingfield MJ, 2007. Botryosphaeriaceae as endophytes and latent pathogens of woody plants: diversity, ecology and impact. *Fungal Biology Reviews* **21**: 90–106.

Smith H, Crous PW, Wingfield MJ, Coutinho TA, Wingfield BD, 2001. *Botryosphaeria eucalyptorum* sp. nov., a new species in the *B. dothidea*-complex on *Eucalyptus* in South Africa. *Mycologia* **93**: 277–285.

Smith H, Kemp GHJ, Wingfield MJ, 1994. Canker and die-back of *Eucalyptus* in South Africa caused by *Botryosphaeria dothidea*. *Plant Pathology* **43**: 1031–1034.

Smith H, Wingfield MJ, Petrini O, 1996. *Botryosphaeria dothidea* endophytic in *Eucalyptus grandis* and *Eucalyptus nitens* in South Africa. *Forest Ecology and Management* **89**: 189–195.

Swofford DL, 2002. *PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). 4. 0b10*. Computer program. Sinauer Associates, Sunderland, Massachusetts, USA.

Tamura K, Dudley J, Nei M, Kumar S, 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**: 1596–1599.

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

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

Wingfield MJ, Slippers B, Hurley BP, Coutinho TA, Wingfield BD, Roux J, 2008. Eucalypt pests and diseases: growing threats to plantations productivity. *Southern Forests* **70:** 139–144.

Xie YJ, 2006. *Eucalypt research at the beginning of 21st century*. China Forestry House Press, Beijing, China. (In Chinese).

Zhou XD, Xie YJ, Chen SF, Wingfield MJ, 2008. Diseases of eucalypt plantations in China: challenges and Opportunities. *Fungal Diversity* **32**: 1–7.

Table 1. Isolates considered in the phylogenetic study and pathogenicity tests.

Isolate No.[ab]	Other No.[a]	Identify	Host	Location	Collector	GeneBank[c] ITS	GeneBank[c] TEF 1-a
CBS 119047		*Botryosphaeria corticis*	*Vaccinium corymbosum*	New Jersey, USA	P.V. Oudemans	DQ299245	EU017539
ATCC 22927		*B. corticis*	*Vaccinium* sp.	North Carolina, USA	R.D. Millholland	DQ299247	EF614931
CMW 7780		*Botryosphaeria dothidea*	*Fraxinus excelsior*	Molinizza, Switzerland	B. Slippers	AY236947	AY236896
CMW 8000		*B. dothidea*	*Prunus* sp.	Crocifisso, Switzerland	B. Slippers	AY236949	AY236898
CMW 13425	CBS 117445	*Botryosphaeria mamane*	*Acacia mangium*	Portuguesa state, Venezuela	S. Mohali	EF118046	GU134939
CMW 13429	CBS 117446	*B. mamane*	*Eucalyptus* hybrid	Cojedes state, Venezuela	S. Mohali	EF118048	GU134940
CMW 22674	CBS 124934	*Fusicoccum atrovirens*	*Pterocarpus angolensis*	South Africa	J. Mehl & J. Roux	FJ888473	FJ888456
CMW 22682	CBS 124935	*F. atrovirens*	*P. angolensis*	South Africa	J. Mehl & J. Roux	FJ888476	FJ888457
CMW 26167	CBS 122069	*Fusicoccum ramosum*	*E. camaldulensis*	Bell Gorge, Australia.	T. Burgess	EU144055	EU144070
CMW 24703[cd]	CBS 127187	*Fusicoccum fabicercianum*	*E. grandis* hybrid	GuangXi, China	M.J. Wingfield & X.D. Zhou	HQ332195	HQ332211
CMW 27091[c]		*F. fabicercianum*	*E. urophylla* × *E. tereticornis* clone	HaiNan, China	M.J. Wingfield & X.D. Zhou	HQ332196	HQ332212
CMW 27094[cd]	CBS 127193	*F. fabicercianum*	*Eucalyptus* sp.	FuJian, China	M.J. Wingfield	HQ332197	HQ332213
CMW 27106[c]		*F. fabicercianum*	*Eucalyptus* sp.	FuJian, China	M.J. Wingfield	HQ332199	HQ332215
CMW 27108		*F. fabicercianum*	*Eucalyptus* sp.	FuJian, China	M.J. Wingfield	HQ332200	HQ332216
CMW 27121[c]	CBS 127194	*F. fabicercianum*	*Eucalyptus* sp.	FuJian, China	M.J. Wingfield	HQ332198	HQ332214
CMW 7063		*Guignardia philoprina*	*Terminalia baccata*	Netherlands	H.A. van der Aa	AY236956	AY236905
CMW 27801		*Lasiodiplodia mahajangana*	*T. catappa*	Madagascar	J. Roux	FJ900595	FJ900641
CMW 27820		*L. mahajangana*	*T. catappa*	Madagascar	J. Roux	FJ900597	FJ900643
CBS 356.59		*Lasiodiplodia parva*	*Theobroma cacao*	Sri Lanka	A. Riggenbach	EF622082	EF622062
CBS 494.78		*L. parva*	*Cassava-field* soil	Colombia	O. Rangel	EF622084	EF622064
STEU 5803		*Lasiodiplodia plurivora*	*Prunus salic*	South Africa	U.Damm F	EF445362	EF445395
STEU 4583		*L. plurivora*	*Vitis vinifera*	South Africa	Halleen	AY343482	EF445396
CMW 24699[cd]		*Lasiodiplodia pseudotheobromae*	Eucalyptus sp.	GuangXi, China	M.J. Wingfield & X.D. Zhou	HQ332191	HQ332207
CMW 24700[cd]		*L. pseudotheobromae*	Eucalyptus sp.	GuangXi, China	M.J. Wingfield & X.D. Zhou	HQ332192	HQ332208
CBS 116459		*L. pseudotheobromae*	Gmelina arborea	Costa Rica	J. Carranza-Velásquez	EF622077	EF622057
CBS 304.79		*L. pseudotheobromae*	Rosa sp.	Netherlands	Na[e]	EF622079	EF622061
CMW 24701[cd]		*Lasiodiplodia theobromae*	*Eucalyptus* sp.	GuangXi, China	M.J. Wingfield & X.D. Zhou	HQ332193	HQ332209
CMW 24702[cd]		*L. theobromae*	*Eucalyptus* sp.	GuangXi, China	M.J. Wingfield & X.D. Zhou	HQ332194	HQ332210
CMW 18420	BOT 979	*L. theobromae*	*Casuarina cunninghamii*	Uganda	J. Roux	DQ103534	DQ103564
CMW 9074		*L. theobromae*	*Pinus* sp.	Mexico	T. Burgess	AY236952	AY236901
CBS 164.96		*L. theobromae*	Fruit along coral reef coast	New Guinea	Na[e]	AY640255	AY640258
CBS 111530		*L. theobromae*	Na[e]	Na[e]	Na[e]	EF622074	EF622054
CMW 13511	WAC 12539	*Lasiodiplodia venezuelensis*	*A. mangium*	Acarigua, Venezuela	S. Mohali	DQ103547	DQ103568

WAC 12540		*L. venezuelensis*	*A. mangium*	Venezuela	S. Mohali	DQ103547	DQ103568
CMW 28315		*Neofusicoccum batangarum*	*Terminalia catappa*	Cameroon	D. Begoude & J. Roux	FJ900606	FJ900652
CMW 28363		*N. batangarum*	*T. catappa*	Cameroon	D. Begoude & J. Roux	FJ900607	FJ900653
CMW 13992	CBS 123634	*Neofusicoccum cordaticola*	*Syzygium cordatum*	Sodwana Bay, South Africa	D. Pavlic	EU821898	EU821868
CMW 14151	CBS 123637	*N. cordaticola*	*S. cordatum*	Sabi, South Africa	D. Pavlic	EU821922	EU821982
CMW 10125	CBS 115791	*Neofusicoccum eucalyptorum*	*E. grandis*	Mpumalanga, South Africa	H. Smith	AF283686	AY236891
CMW 11705		*N. eucalyptorum*	*E. nitens*	South Africa	B. Slippers	AY339248	AY339264
CMW 14023	CBS 123639	*Neofusicoccum kwambonambiense*	*S. cordatum*	Kwambonambi, South Africa	D. Pavlic	EU821900	EU821870
CMW 14123	CBS 123643	*N. kwambonambiense*	*S. cordatum*	Ricards Bay, South Africa	D. Pavlic	EU821894	EU821954
CMW 9079	ICMP 7933	*Neofusicoccum parvum*	*Actinidia deliciosa*	New Zealand	S.R. Pennycook	AY236941	AY236886
CMW 9081	ICMP 8003	*N. parvum*	*Populus nigra*	New Zealand	G.J. Samuels	AY236943	AY236888
CMW 994	ATCC 58189	*N. parvum*	*Malus sylvestris*	New Zealand	G.J. Samuels	AF243395	AY236883
CMW 24704[c]		*N. parvium*	*E. grandis* hybrid	GuangXi, China	M.J. Wingfield & X.D. Zhou	HQ332201	HQ332217
CMW 27110[c]		*N. parvium*	*Eucalyptus* sp.	FuJian, China	M.J. Wingfield	HQ332202	HQ332218
CMW 27111		*N. parvium*	*Eucalyptus* sp.	FuJian, China	M.J. Wingfield	HQ332203	HQ332219
CMW 27125[cd]		*N. parvium*	*E. urophylla* × *E. grandis* clone	FuJian, China	M.J. Wingfield	HQ332204	HQ332220
CMW 27135[c]		*N. parvium*	*E. dunnii*	FuJian, China	M.J. Wingfield	HQ332205	HQ332221
CMW 7772		*Neofusicoccum ribis*	*Ribes* sp.	New York, USA	B. Slippers & G. Hudler	AY236935	AY236877
CMW 7773		*N. ribis*	*Ribes* sp.	New York, USA	B. Slippers & G. Hudler	AY236936	AY236878
CMW 27119[cd]		*N. ribis s. l.*	*Eucalyptus* sp.	FuJian, China	M.J. Wingfield	HQ332206	HQ332222
CMW 14058	CBS 123645	*N. umdonicola*	*S. cordatum*	Kosi Bay, South Africa	D. Pavlic	EU821904	EU821874
CMW 14127	CBS 123648	*N. umdonicola*	*S. cordatum*	Kwambonambi, South Africa	D. Pavlic	EU821926	EU821896

[a]Designation of isolates and culture collections: CMW = Tree Protection Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria; ATCC = American Type Culture Collection; CBS = Centraal Bureau voor Scimmelcultures, Utrecht, Netherlands; ICMP = International Collection of Microorganisms from Plants, Auckland, New Zealand; WAC = Department of Agriculture, Western Australia Plant Pathogen Collection, Perth, Australia. STEU = University of Stellenbosch, South Africa.

[b]Isolates sequenced in this study are given in bold.

[c]Isolates used in glasshouse pathogenicity trails.

[d]Isolates used in field pathogenicity trails in China.

[e]Na: Not available

Fig 1. Agarose gel showing restriction fragments for the restriction enzyme *Cfo*I of the ITS and DNA locus BotF15 PCR, *Ksp*I of the ITS PCR products of different clades of Botryosphaeriaceae from China. (a–b) All the isolates are distinguished into threes groups by using restriction enzyme *Cfo*I to digest ITS PCR products, (a) Group 1 (dash arrow): *Lasiodiplodia pseudotheobromae* and *L. theobromae*, (a–b) Group 2 (bold arrow): *Neofusicoccum parvum*/*N. ribis* species complex; Group 3 (thin arrow): species within *Botryosphaeria dothidea* complex; (c) ITS PCR products of isolates of *B. dothidea* complex digested by restriction enzyme *Ksp*I; (d) DNA locus BotF15 PCR products of isolates of *N. parvum*/*N. ribis* species complex digested by restriction enzyme *Cfo*I, one isolate is in group of *N. ribis s. l.*, and other isolates in *N. parvum*. The oblique arrow indicates the 500 bp marker, and lane "M" contains a 100 bp size marker.

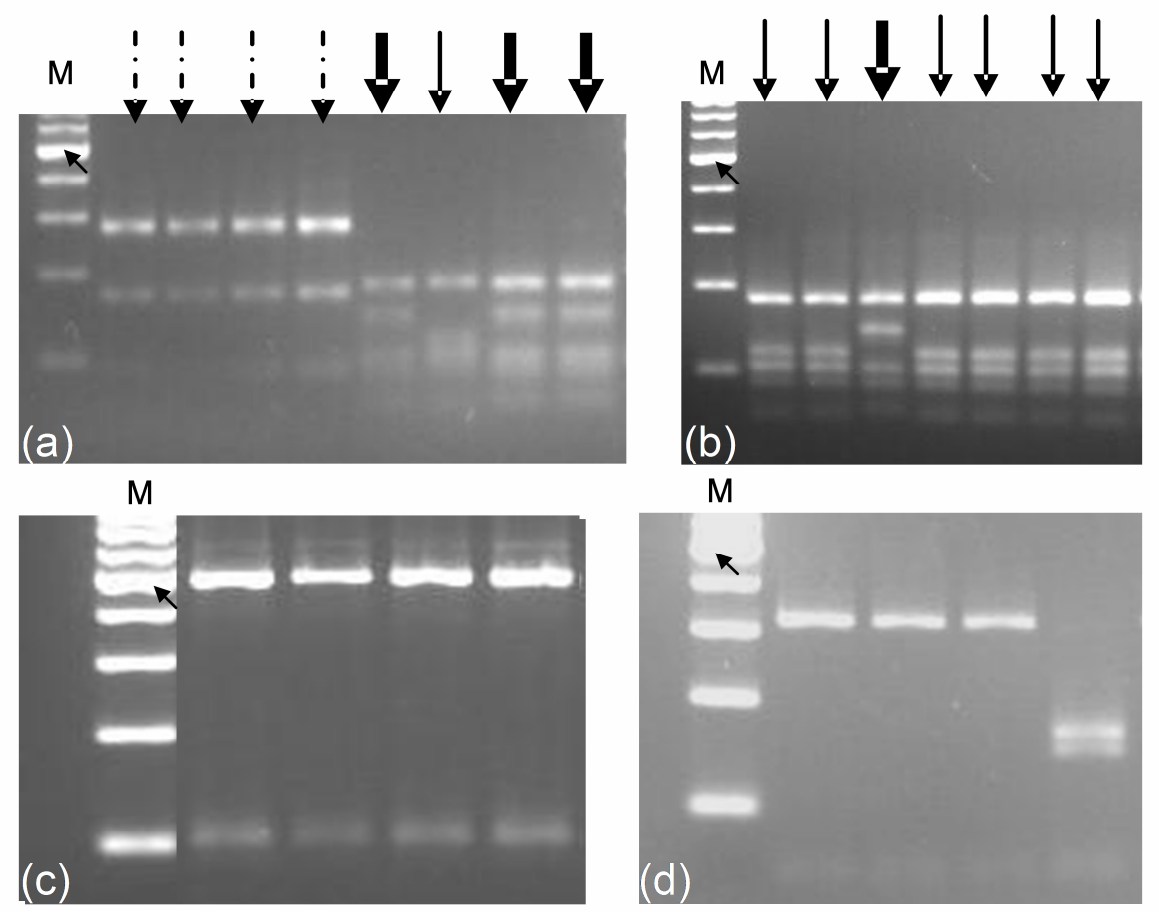


Fig 2. One of the twenty most-parsimonious trees obtained from Maximum Parsimony analyses of the combined ITS and TEF-1α sequence data of the the representative taxa of the Botryosphaeriaceae. Posterior probabilities followed by bootstrap support (%) from 1000 replications are given on the branches (PP/BS). Isolates marked in bold represent those obtained from *Eucalyptus* trees in China.

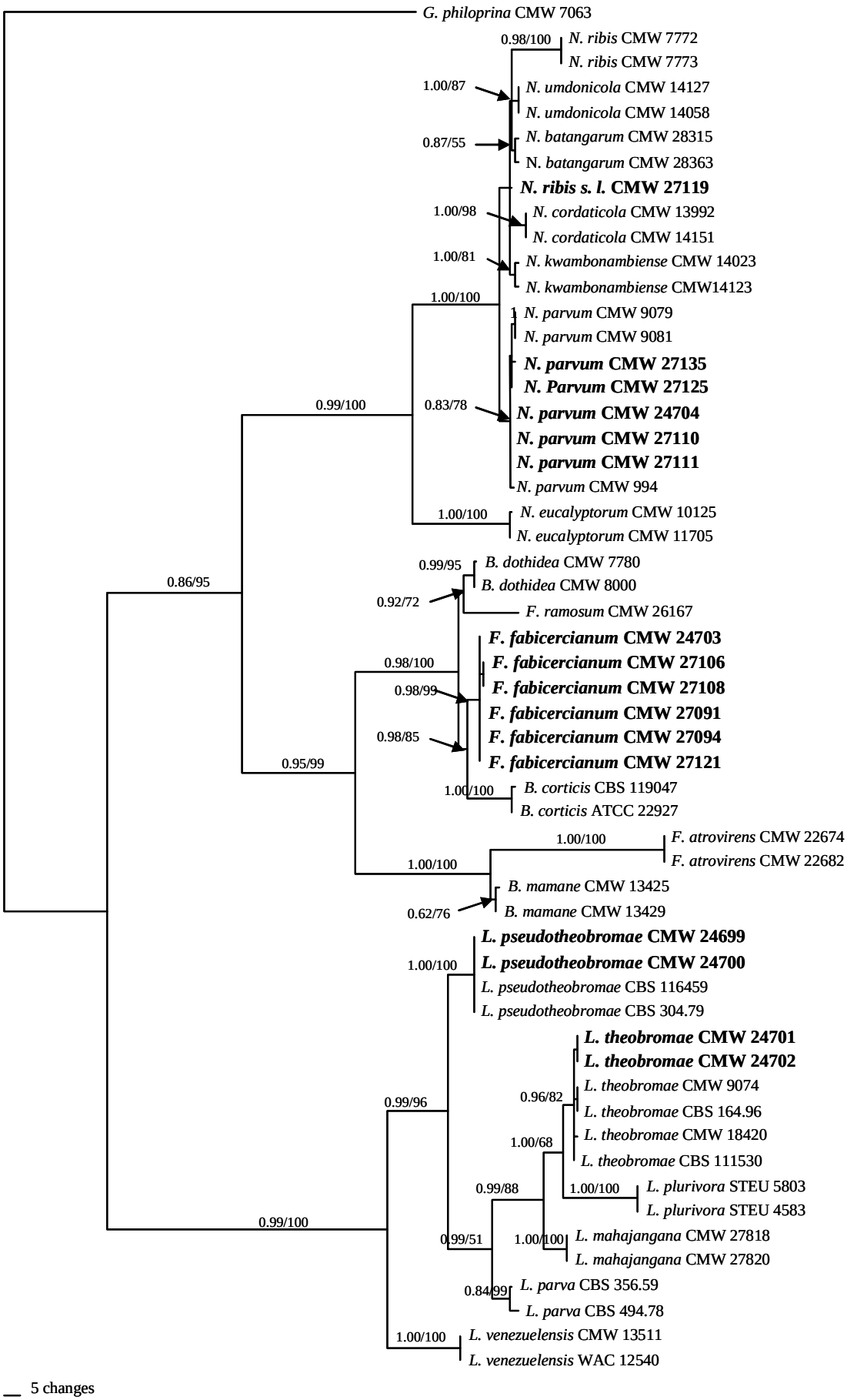


Fig 3. *Fusicoccum fabicercianum* sp. nov. CMW 27094 = CBS 127193 (culture ex-epitype). (a) Conidiomata formed in culture on pine needle; (b) Immature, developing conidia produced by conidiogenous cells; (c) Conidiogenous cells and developing conidia; (d) Smooth, aseptate, fusiform mature conidia; (e) Mature conidia with granular contents. (f) Germinating conidia with one or two septa; (g) A typical germinating conidium with two septums; (h) Living culture after growing 10 days on MEA (front); (i) Living culture after growing 10 days on MEA (reverse). Bars: a = 100 μm; b–g = 10 μm; h–i = 10 cm.

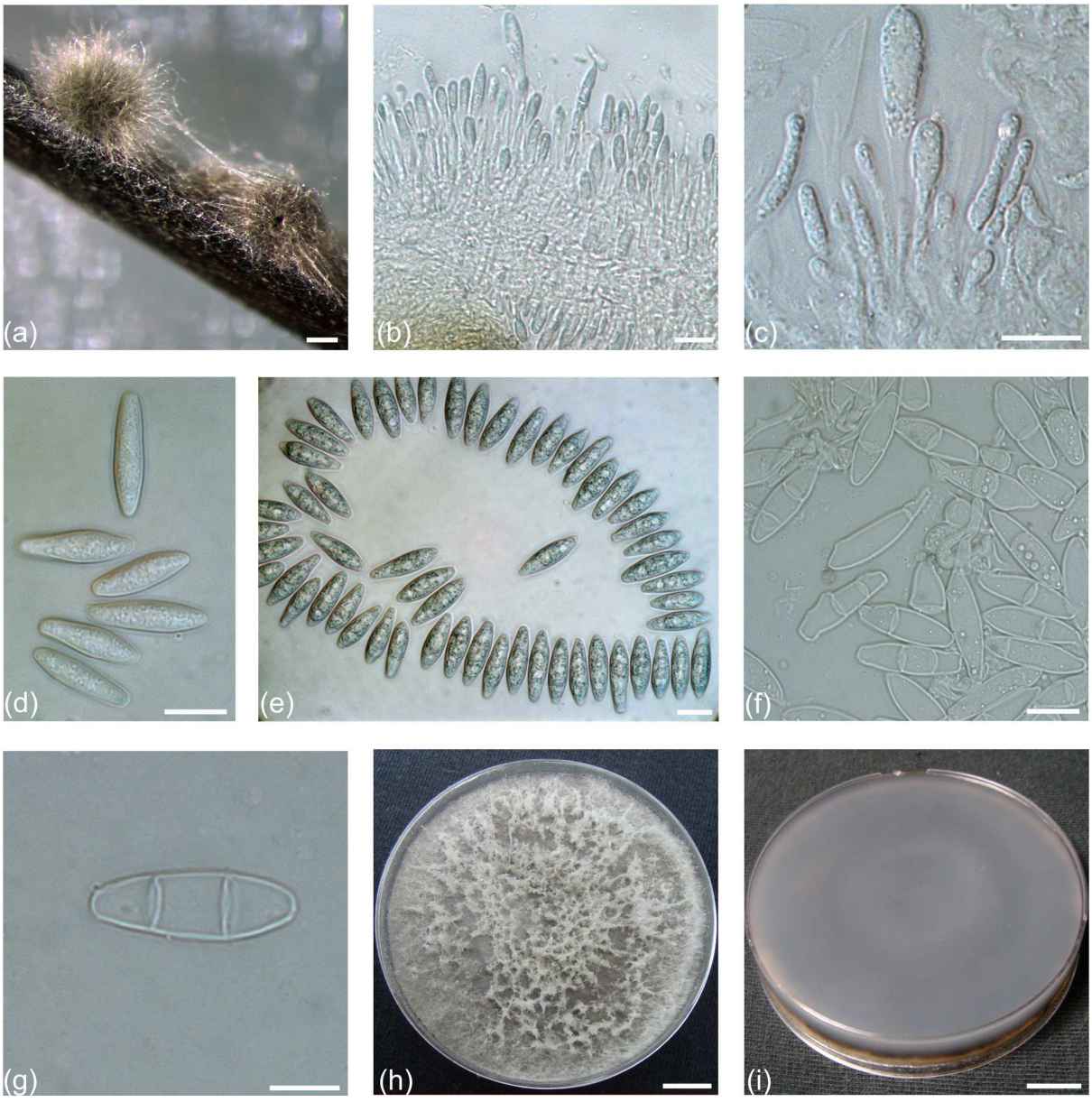


Fig 4. Conidia of *Lasiodiplodia*, *Fusicoccum* and *Neofusicoccum* species isolated from Chinese *Eucalyptus* plantations. (a) *Lasiodiplodia pseudotheobromae*. (b) *L. theobromae*. (c) *Neofusicoccum parvum*. (d) *N. ribis s. l.* Bars = 10 μm.

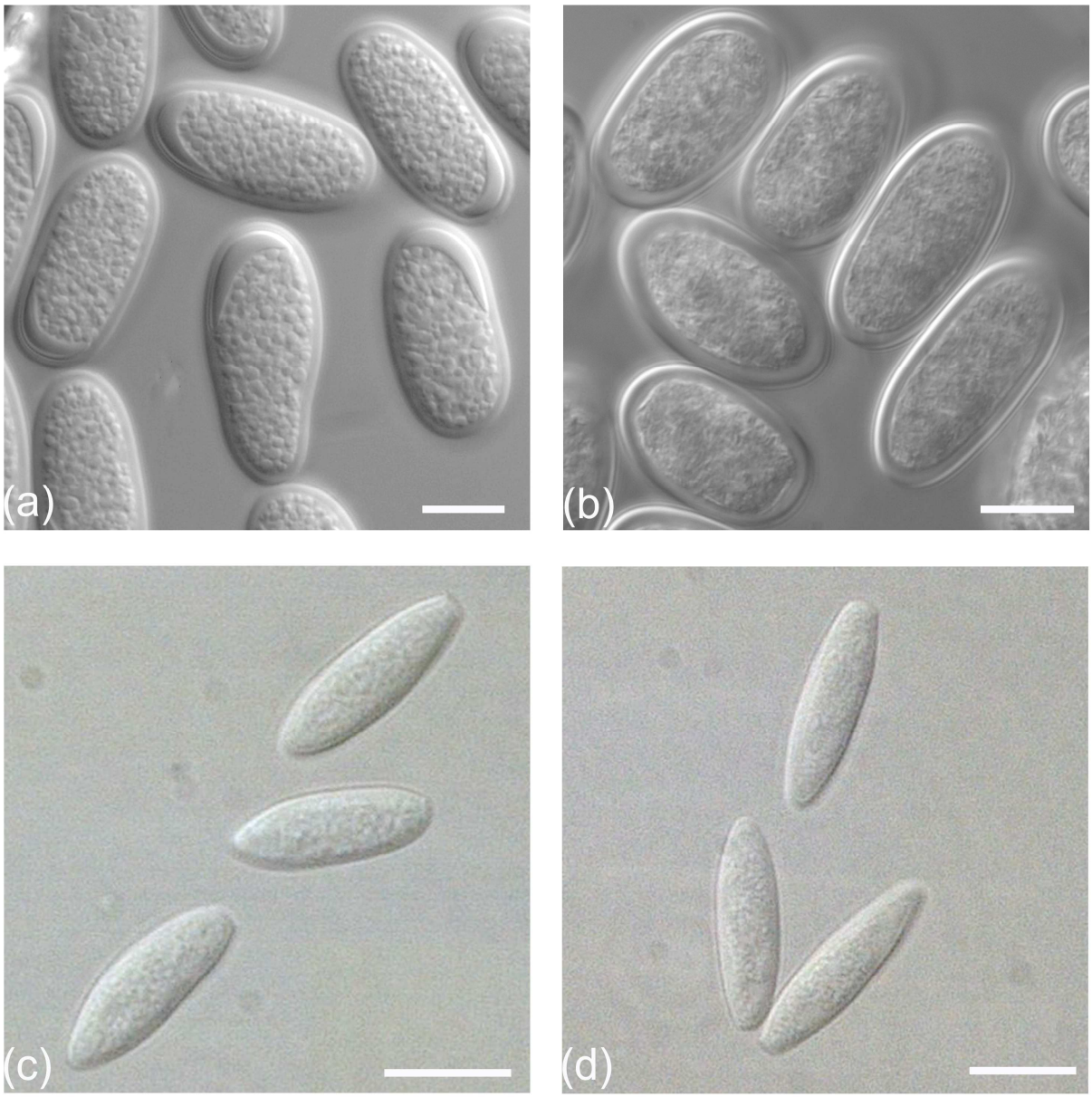


Fig 5. Column chart indicating the mean lesion lengths (mm) resulting from inoculation trials with *L. pseudotheobromae* (CMW 24699, CMW 24700), *L. theobromae* (CMW 24701, CMW 24702), *Fusicoccum fabicercianum* sp. nov. (CMW 24703, CMW 27091, CMW 27094, CMW 27106, CMW 27121), *Neofusicoccum parvum* (CMW 24704, CMW 27110, CMW 27125, CMW 27135), *N. ribis s. l.* (CMW 27119) onto *E. grandis* clone (TAG-5) under glasshouse conditions. Bars represent 95% confidence limits for each treatment. Different letters above the bars indicate treatments that were statistically significantly different (P = 0.05).

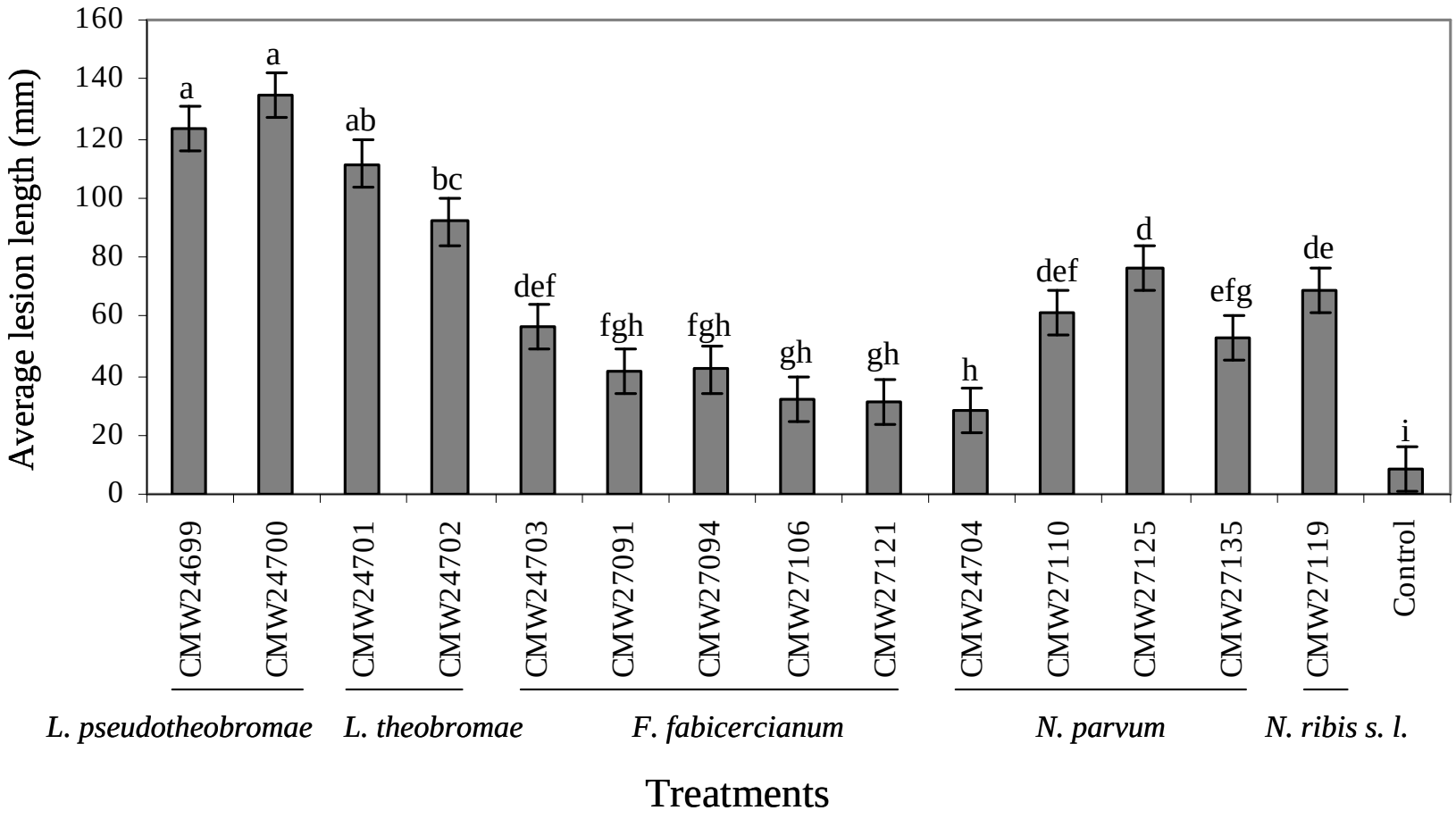
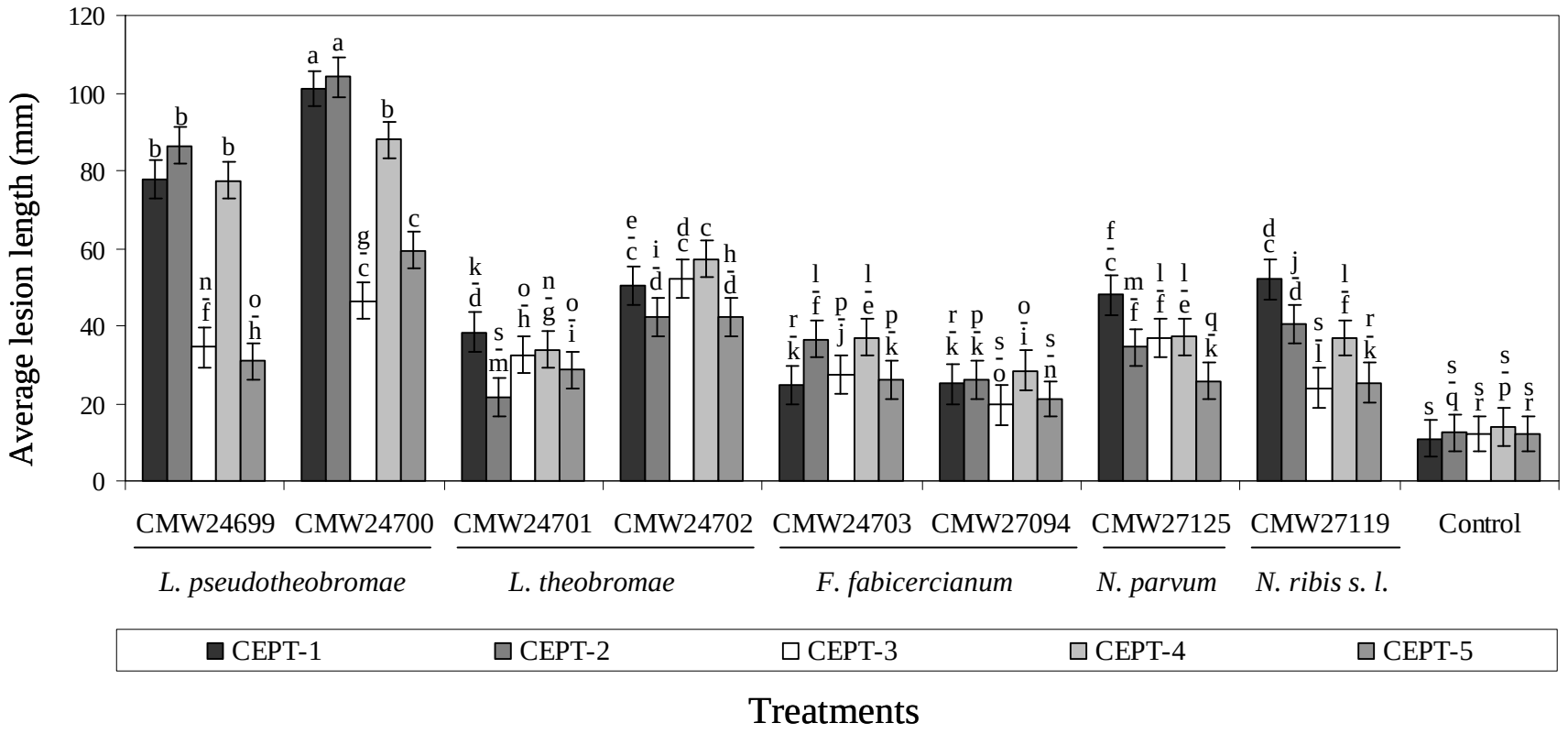


Fig 6. Column chart indicating the mean lesion lengths (mm) resulting from inoculation trials with *Lasiodiplodia pseudotheobromae* (CMW 24699, CMW 24700), *L. theobromae* (CMW 24701, CMW 24702), *Fusicoccum fabicercianum* sp. nov. (CMW 24703, CMW 27094), *Neofusicoccum parvum* (CMW 27125), *N. ribis s. l.* (CMW 27119) onto five *Eucalyptus* genotypes in Chinese plantations. Bars represent 95% confidence limits for each treatment. Different letters above the bars indicate treatments that were statistically significantly different (P = 0.05).



Chapter 5

Novel species of *Calonectria* associated with *Eucalyptus* leaf blight in Southeast China

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This chapter has been published as: Chen SF, Lombard L, Roux J, Xie YJ, Wingfield MJ, Zhou XD, 2011. Novel species of *Calonectria* associated with *Eucalyptus* leaf blight in Southeast China. *Persoonia* **26**: 1–12.

ABSTRACT

Leaf blight caused by *Calonectria* spp. is an important disease occurring on *Eucalyptus* trees grown in plantations in Southeast Asia. Symptoms of leaf blight caused by *Calonectria* spp. have recently been observed in commercial *Eucalyptus* plantations in FuJian Province in Southeast China. The aim of this study was to identify these *Calonectria* spp. employing morphological characteristics, DNA sequence comparisons for the β-tubulin, histone H3 and translation elongation factor 1-lpha gene regions and sexual compatibility. Four *Calonectria* spp. were identified, including *Ca. pauciramosa* and three novel taxa described here as *Ca. crousiana*, *Ca. fujianensis* and *Ca. pseudocolhounii*. Inoculation tests showed that all four *Calonectria* spp. found in this study were pathogenic to two different *E. urophylla* × *E. grandis* hybrid clones, commercially utilised in eucalypt plantations in China.

Keywords: *Cylindrocladium*, *Eucalyptus* plantations, FuJian, pathogenicity

1. INTRODUCTION

Species of *Calonectria* (*Ca*.) (anamorph state: *Cylindrocladium* (*Cy*.)) are pathogenic to a wide range of plant hosts in tropical and subtropical areas of the world (Crous & Wingfield 1994; Crous 2002). Symptoms associated with infection by these fungi include stem cankers, leaf and shoot blight as well as root rot on many agronomic and forestry crop plants (Crous 2002; Old *et al*. 2003; Crous *et al*. 2004b). *Calonectria* spp., particularly in their *Cylindrocladium* anamorph form, are especially well-known as pathogens of *Eucalyptus* trees in plantations where they cause the disease known as Cylindrocladium leaf blight (CLB) (Sharma & Mohanan 1991, 1992; Booth *et al*. 2000; Crous 2002; Old *et al*. 2003; Rodas *et al*. 2005). These fungi are also important causal agents of cutting rot and seedling blight in *Eucalyptus* nurseries (Sharma *et al*. 1984; Crous *et al*. 1991; Crous 2002; Old *et al*. 2003; Lombard *et al*. 2010c, d).

Symptoms of CLB on *Eucalyptus* include both leaf blotch and shoot blight, which develops upwards from the base of the trees and can result in tree mortality due to defoliation (Crous 2002; Old *et al*. 2003; Rodas *et al*. 2005). Symptoms begin as water-soaked lesions on young and mature leaves on the lower branches. These lesions coalesce and develop into extensive necrotic areas very rapidly. Under conditions of high humidity and frequent rainfall, the lesions can cover the entire leaf surface and infection of young shoot tips can result in dramatic blight. Defoliation typically moves upwards from the base and centres of affected trees and this can result in total defoliation of trees (Crous 2002; Old *et al*. 2003; Rodas *et al*. 2005). Severely affected trees can suffer reduction in growth vigour, with crowns and main stems becoming deformed (Booth *et al*. 2000; Old *et al*. 2003).

In South and Southeast Asia, CLB is one of the most prominent diseases associated with *Eucalyptus* trees grown in commercial plantations (Old *et al*. 2003). In these regions, CLB is caused by several *Calonectria* spp., including *Ca. asiatica* Crous & N.L. Hywel-Jones, *Ca. brassicae* (Panwar & Borha) L. Lombard, M.J. Wingf. & Crous, *Ca. hurae* (Linder & Whetzel) L. Lombard, M.J. Wingf. & Crous, *Ca. ilicicola* Boedijn & Reitsma, *Ca. indusiata* (Seaver) Crous, *Ca. kyotensis* Tersh., *Ca. multiseptata* Crous & M.J. Wingf., *Ca. pauciramosa* C.L. Schoch & Crous, *Ca. pteridis* Crous, M.J. Wingf. & Alfenas, *Ca. reteaudii* (Bugn.) C. Booth, and *Ca. sumatrensis* (Crous) L. Lombard, M.J. Wingf. & Crous (Sharma *et al*. 1984; Booth *et al*. 2000; Kang *et al*. 2001; Crous 2002; Old *et al*. 2003; Crous *et al*.

2004b). Of these *Calonectria* spp., *Ca. reteaudii* is regarded as the most important pathogen and it occurs primarily on *Eucalyptus* trees in tropical regions of Southeast Asia and India (Booth *et al*. 2000; Kang *et al*. 2001; Crous 2002; Old *et al*. 2003).

Commercial plantations of *Eucalyptus* are distributed over 19 Provinces in Central and South China (Qi 2006). Approximately 2.6 million hectares of *Eucalyptus* plantations have recently been established in FuJian, GuangDong, GuangXi, HaiNan and YunNan Provinces (Xie 2006; Iglesias-Trabad & Wilstermann 2008), to meet the high demand in pulp products in China. Similar to the situation in other countries (Wingfield *et al*. 2008), these trees are affected by pests and diseases, for which limited information is available in China (Zhou *et al*. 2008). Leaf and shoot blight caused by *Calonectria* spp. is regarded as one of the most serious threats to commercial *Eucalyptus* plantations and nurseries in this country (Wang 1992; Sun & Liu 2004; Zhou *et al*. 2008; Lombard *et al*. 2010d). Recent surveys of tree diseases in the FuJian Province in Southeast China revealed numerous examples of CLB on *Eucalyptus* spp. The aim of this study was to determine the identity of the *Calonectria* spp. collected from these trees. In addition, the pathogenicity of selected isolates was tested on various *Eucalyptus* clones commercially grown in China.

2. MATERIALS AND METHODS

2.1. Isolates

Eucalyptus leaves showing symptoms of CLB were collected from commercially propagated *Eucalyptus* trees in plantations in FuJian Province in 2007 (Table 1). Conidial masses were transferred directly from infected leaves to malt extract agar (2% w/v; MEA: Biolab Diagnostic Ltd., Midrand, South Africa) and incubated at 25°C under continuous near-ultraviolet light for 7 d. Isolates were transferred to MEA and further incubated at 25°C for 7 d. Single conidial isolates were prepared and lodged in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa (Table 1), and a duplicate set of isolates is maintained in a culture collection housed at the China Eucalypt Research Centre (CERC), Chinese Academy of Forestry (CAF), China. Representative isolates were also deposited with the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands (Table 1), and herbarium specimens in the National Collection of Fungi (PREM), Pretoria, South Africa.

2.2. DNA sequence comparisons

Single conidial cultures (Table 1) were grown on MEA for 7 d at 25ºC. Total genomic DNA was extracted using the method described by Smith *et al*. (2001). Three loci were amplified, using the primers T1 (O'Donnell & Cigelnik 1997) and Bt-2b (Glass & Donaldson 1995) to amplify a fragment of the β-tubulin (BT) gene region, part of the histone H3 (HIS3) gene region with primers H3-1a and H3-1b (Glass & Donaldson 1995), and primers EF1-728F and EF1-986R (Carbone & Kohn 1999) to amplify a fragment of the translation elongation factor 1-lpha (TEF-1α) gene region.

The PCR mixtures used to amplify the different loci consisted of 2.5 units Fast Start *Taq* polymerase (Roche Applied Science, USA), 1 × PCR buffer, 1–1.5 mM $MgCl_2$, 0.25 mM of each dNTP, 0.5 µm of each primer and approximately 30 ng of fungal genomic DNA, made up to a total reaction volume of 25 µL with sterile de-ionised water. Amplified fragments were purified using High Pure PCR Product Purification Kit (Roche, USA) and sequenced in both directions with the same primers used for the DNA amplifications. For this purpose, the BigDye terminator sequencing kit v. 3.1, (Applied Biosystems, USA) and an ABI PRISM™ 3100 DNA sequencer (Applied Biosystems, USA) were used. All PCRs and sequencing reactions were performed on an Eppendorf Mastercycler Personal PCR (Eppendorf AG, Germany) with cycling conditions as described in Crous *et al*. (2004b, 2006) for all loci amplified.

Sequences generated were added to other sequences for *Calonectria* obtained from GenBank (http://www.ncbi.nlm.nih.gov) and were assembled and aligned using Sequence Navigator v. 1.0.1 (Applied Biosystems, USA) and MAFFT v. 5.11 (Katoh *et al*. 2002), respectively. The aligned sequences were then manually corrected where needed. Single nucleotide polymorphisms (SNPs) were determined for each gene region analysed using DnaSP v. 5.00.07 (Librado & Rozas 2009).

PAUP (Phylogenetic Analysis Using Parsimony, v. 4.0b10; Swofford 2002) was used to analyse the DNA sequence datasets. A partition homogeneity test (Farris *et al*. 1994) and a 70% reciprocal bootstrap method (Mason-Gamer & Kellog 1996; Gueidan *et al*. 2007) were applied to evaluate the feasibility of combining the datasets. Phylogenetic relationships were

estimated by heuristic searches based on 1 000 random addition sequences and tree bisection-reconnection, with the branch swapping option set on 'best trees' only.

All characters were weighed equally and alignment gaps were treated as missing data. Measures calculated for parsimony included tree length (TL), consistency index (CI), retention index (RI) and rescaled consistence index (RC). Bootstrap analyses (Hillis & Bull 1993) were based on 1 000 replications. The phylogenetic analyses included 57 partial gene sequences per gene, representing 27 *Calonectria* species (Table 1) closely related to the isolates studied. *Ca. colombiensis* Crous (CBS112221) and *Ca. chinensis* (Crous) L. Lombard, M.J. Wingf. & Crous (CBS112744) were used as the outgroup taxa (Lombard *et al*. 2009, 2010d). All sequences were deposited in GenBank and the alignments in TreeBASE (http://www.treebase.org).

A Markov Chain Monte Carlo (MCMC) algorithm was used to generate phylogenetic trees with Bayesian probabilities using MrBayes v. 3.1.1 (Ronquist & Huelsenbeck 2003) for the combined sequence datasets. Models of nucleotide substitution for each of the three genes were determined using MrModeltest (Nylander 2004) and included for each gene partition, which used for the combined sequence analyses. Two independent runs of four MCMC chains were run simultaneously from random trees for 1 000 000 generations and sampled every 100 generations for the combined analysis of the gene partitions. Both runs converged on the same likelihood score and tree topology, and therefore, the first 1 000 trees were discarded as the burn-in phase of each analysis and posterior probabilities determined from the remaining 9 000 trees.

2.3. Sexual compatibility

Single conidial *Calonectria* isolates of unknown identity from China were crossed among themselves in all possible combinations. Crosses were made as described in Schoch *et al*. (1999) on minimal salt agar (MN) to which sterile tooth picks had been placed on the agar surface (Guerber & Correll 2001; Lombard *et al*. 2010a, b, d). Controls were of isolates crossed with themselves and it was thus also possible to distinguish between those species with heterothallic or homothallic mating systems. The plates were stacked in plastic containers and incubated at 20 ˚C for 6–8 wk. Crosses were regarded as successful when isolate combinations produced perithecia extruding viable ascospores.

2.4. Taxonomy

For morphological identification of *Calonectria* isolates, single conidial isolates were prepared on MEA and synthetic nutrient-poor agar (SNA) (Nirenburg 1981; Lombard *et al*. 2009, 2010a, b, d). Inoculated plates were incubated at room temperature and examined after 7 d. Gross morphological characteristics of the anamorph state were determined by mounting fungal structures in lactic acid and 30 measurements at × 1 000 magnification were made for each isolate. Teleomorph morphology was determined by mounting perithecia obtained from the sexual compatibility tests in Leica mountant (Setpoint Premier, Johannesburg, South Africa) and hand-sectioned with a Leica CM1100 cryostat (Setpoint Technologies) at –20°C. The 12 µm sections were mounted in lactophenol and 3% KOH. Gross morphological characteristics were determined as mentioned for the anamorph state. The 95% confidence levels were determined and extremes of measurements are given in parentheses.

Optimal growth conditions for cultures were determined in the dark on MEA for each isolate, at temperatures ranging from 5–35°C at 5°C intervals. This was repeated three times for each isolate examined. Colony colours were determined after 7 d on MEA at 25°C in the dark, using the colour charts of Rayner (1970). All descriptions, illustrations and nomenclature were deposited in MycoBank (www.mycobank.org; Crous *et al*. 2004a).

2.5. Pathogenicity tests

In order to test the pathogenicity of the *Calonectria* spp. collected in this study, ten profusely sporulating isolates, representing different *Calonectria* species identified based on morphology and DNA sequence comparisons were selected for inoculation trails (Table 1). The isolates were transferred to MEA, and incubated for 10 d at 25°C. A spore suspension was prepared for each isolate, by adding 2 mL of sterile water to the plates and dislodging conidia with a sterile glass rod. The spore suspension was strained through a layer of cheesecloth and the concentration adjusted to 3.3×10^5 conidia/mL. To ensure that conidia would adhere to the surface of the inoculated leaves, 2 mL Tween-80 (ChangJiang JingXi HuaGongChang, GuangZhou, China) was added to the suspension.

Two *E. urophylla* S.T.Blake × *E. grandis* W.Hill hybrid clones, CEPT-9 and CEPT-10 (height: 30–40 cm), selected for inoculation, were acclimatised for 2 wk in a shade house subjected to natural climatic conditions (temperature 26–32°C and humidity 60–90%). For each of the 10 selected *Calonectria* isolates, nine plants of each clone were inoculated with the spore suspensions by spraying the leaves until run-off. The plants were covered with plastic bags for 48 h allowing sufficient humidity for infection. Control inoculations were done in a similar fashion with sterile water amended with 2 mL of Tween-80.

Pathogenicity tests were evaluated 14 d after inoculation. For every inoculated seedling, the percentage of the infected/diseased leaves was calculated. Results were analysed in SAS v. 8 using the PROC GLM (general linear model) (SAS Institute, 1999). Analysis of variance (ANOVA) was used to determine the effects of fungal strain on lesion length. Prior to ANOVA, homogeneity of variance across treatments was verified. For significance tests amongst means, Fisher's protected test was used. F values with $P < 0.05$ were considered significant. Isolations were made from lesions on the leaves of the test plants in each plot to ensure the presence of the inoculated fungi.

3. RESULTS

3.1. Isolates

A total of 97 isolates were collected from leaves in *Eucalyptus* plantations in the FuJian Province during the survey in 2007 (Table 1). Of these, 77 isolates were isolated from diseased leaves on five *E. dunnii* Maiden trees, and an additional 20 isolates were obtained from diseased leaves on two *E. grandis* trees.

3.2. DNA sequence comparisons

Amplicons of approximately 500 bp were generated for the BT and TEF-1α gene regions and those for the HIS3 region were approximately 450 bp. Partition homogeneity tests for all possible combinations of the three gene regions used, consistently yielded a P-value of 0.001. The 70% reciprocal bootstrap trees showed no conflict in tree topologies for the three gene regions. Based on the tree topologies and a P-value of 0.001 (Cunningham 1997; Dettman *et al*. 2003), the gene regions were combined. This resulted in a dataset consisting of 1 522

characters including gaps. Of these characters, 1 046 were constant and parsimony uninformative. The 476 parsimony informative characters included in the parsimony analyses yielded 54 equally most parsimonious trees (TL = 1110, CI = 0.722, RI = 0.879, RC = 0.634), of which the first tree is presented (Fig 1). For Bayesian analyses, a HKY+I model was selected for BT, GTR+I+G model for HIS3 and a GTR+G model for TEF-1α and incorporated into the analyses. The consensus tree obtained for the Bayesian analyses confirmed the topology of the consensus tree obtained with the parsimony analysis (Fig 1).

The phylogenetic tree showed a number of well supported clades. Some isolates grouped in a clade representing *Ca. pauciramosa* with a bootstrap value (BP) of 55 and a Bayesian posterior probability (PP) value of 0.95. Other isolates grouped close to *Ca. reteaudii*, but in a distinct clade (BP = 100, PP = 1.00). Several isolates also clustered with *Ca. colhounii* Peerally and *Ca. eucalypti* L. Lombard, M.J. Wingf. & Crous, but separated from them to form a monophyletic group (BP = 84, PP = 0.84). These isolates also clustered into two well-supported clades (BP = 81, PP = 1.00 and BP = 100, PP = 1.00, respectively). SNP analyses for isolates CMW27209, CMW27213 and CMW27214 showed that they shared two unique alleles, while isolates CMW27254, CMW27257 and CMW27263 shared ten unique alleles for the three gene regions analysed, clearly distinguishing them from each other. Furthermore, these six Chinese isolates also shared three unique alleles, distinguishing them from *Ca. colhounii* and *Ca. eucalypti* (Table 2).

3.3. Sexual compatibility

Protoperithecia formed within 3 wk and mating tests produced viable perithecia within 6 wk on sterilised toothpicks on MN medium. Except for isolates of *Ca. pauciramosa* (CMW27199, CMW27203, CMW27283, CMW27292), all the control crosses of *Calonectria* isolates, included in this study, produced perithecia with viable ascospores. These results show that all the *Calonectria* isolates, except those of *Ca. pauciramosa* are self-fertile (homothallic).

3.4. Taxonomy

Based on morphology and DNA sequence comparisons (Fig 1), *Calonectria* isolates from *Eucalyptus* trees in FuJian Province reside in four taxa that include *Ca. pauciramosa* and three previously undescribed species. Isolates CMW27199, CMW27203, CMW27283 and

CMW27292 clearly represent *Ca. pauciramosa*, with obpyriform to ellipsoidal vesicles, and macroconidia being 40–65 × 3–5 µm (av. = 50 × 5 µm). The remaining isolates are described in the genus *Calonectria* as follows:

Calonectria crousiana S.F. Chen, L. Lombard, M.J. Wingf. & X.D. Zhou, sp. nov.
MycoBank no.: MB518855
(Fig 2)

Etymology: This species is named for Prof. P.W. Crous recognising monumental contributions to the taxonomy of *Calonectria* spanning more than two decades.

Teleomorpha *Calonectria indusiatae* similis sed ascosporibus maioribus (56–)58–69(–76) × (5–)6.5–7.5(–8) µm, mediocriter 64 × 7 µm, differt. Anamorpha *Cy. theae* similis sed macroconidiis cylindricis utrinque rotundatis rectis (59–)61–67(–75) × (4–)4.5–5.5(–6) µm, mediocriter 64 × 5 µm, (semel vel) ter septatis, sine cicatrice abscissionis visibile, in fasciculis parallelis cylindricis muco contenitis, differt.

Perithecia solitary or in groups of up to five, orange, becoming red-brown with age; in section apex and body orange, base red-brown, subglobose to ovoid, (321–)352–499(–550) µm high, (260–)262–403(–465) µm diam, body turning dark orange to slightly red, and base dark red-brown in 3% KOH; perithecial walls rough, consisting of two thick-walled layers: outside layer of *textura globulosa*, (32–)33–76(–90) µm wide, becoming more compressed towards inner layer of *textura angularis*, (10–)12–23(–30) µm wide, becoming thin-walled and hyaline towards the centre; outer cells (22–)26–38(–40) × (9–)16–29(–36) µm, inner cells (8–)9–15(–18) × (2.5–)3.5–6(–7) µm; perithecial base up to 241 µm wide, consisting of dark red, angular cells, merging with an erumpent stroma; cells of the outer wall layer continuing into the pseudoparenchymatous cells of the erumpent stroma. **Asci** 8-spored, clavate, (109–)120–175(–186) × (23–)24–25 µm, tapering to a long thin stalk. **Ascospores** aggregate in the upper third of the asci, hyaline, guttulate, fusoid with rounded ends, straight to slightly curved, (1–)3-septate, not or slightly constricted at the septum, (56–)58–69(–76) × (5–)6.5–7.5(–8) µm (av. = 64 × 7 µm). **Macroconidiophores** consisting of a stipe, a suite of penicillate arranged fertile branches, a stipe extension, and a terminal vesicle; stipe septate, hyaline, smooth (61–)63–160(–220) × (4.5–)6–8(–9.5) µm; stipe extensions septate, straight to flexuous (195–)225–404(–475) µm long, 3–6 µm wide at the apical septum, terminating in a

clavate vesicle, (4–)4.5–5(–6) µm diam. **Conidiogenous apparatus** (63–)76–117(–138) µm long, (40–)53–98(–116) µm wide; primary branches aseptate to 1-septate, (19–)21–42(–70) × (4–)4.5–5.5(–6) µm; secondary branches aseptate, (13–)17–25(–28) × (3.5–)4–5 µm; tertiary branches aseptate, (11–)11.5–15(–18) × (3–)3.5–4(–4.5) µm; additional branches (–5), aseptate, (10–)10.5–14(–16) × (2.5–)3–4µm; each terminal branch producing 1–4 phialides; phialides doliiform to allantoid, hyaline, aseptate, (9.5–)10.5–13.5(–15) × 34.5 µm, apex with minute periclinal thickening and inconspicuous collarette. **Macroconidia** cylindrical, rounded at both ends, straight, (59–)61–67(–75) × (4–)4.5–5.5(–6) µm (av. = 64 × 5 µm), (1–)3-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime.

Culture characteristics: Colonies reaching 64–80 mm diam after 7 d on MEA in the dark with optimal growth temperature at 25 °C; Colonies fast growing forming white to sienna (13b) aerial mycelium, with feathery, irregular margins. Surface and reverse with mikado-orange (13b) to sienna (13i) outer margin, and russet (13'k) inner region, becoming argus-brown (13m) towards the centre. Chlamydospores arrange in chains, abundant throughout the medium, forming microsclerotia.

Substrate: *Eucalyptus grandis*.

Distribution: FuJian Province, China.

Specimens examined: China, FuJian Province, on leaves of *E. grandis*, Aug. 2007, M.J. Wingfield, Herb. PREM60453, holotype of *Ca. crousiana*, culture ex-type CMW27249 = CBS127198; FuJian Province, on leaves of *E. grandis*, Aug. 2007, *M.J. Wingfield*, Herb. PREM60454, culture CMW27253 = CBS127199; FuJian Province, on leaves of *E. grandis*, Aug. 2007, M.J. Wingfield, Herb. PREM60455, culture CMW27267 = CBS127203; FuJian Province, on leaves of *E. grandis*, Aug. 2007, *M.J. Wingfield*, culture CMW27258.

Notes: *Calonectria crousiana* is morphologically similar to *Ca. indusiata*, *Ca. australiensis* (Crous & K.D. Hyde) L. Lombard, M.J. Wingf. & Crous and species in the *Ca. colhounii* complex, that includes *Ca. colhounii*, *Ca. eucalypti*, *Ca. macroconidialis* (Crous, M.J. Wingf. & Alfenas) Crous and *Ca. madagascariensis* Crous (Crous *et al*. 2006, Lombard *et al*. 2010b). With the exception of *Ca. macroconidialis* (macroconidia (1–)3(–6)-septate), all of these species produce clavate vesicles and (1–)3-septate macroconidia. *Ca. crousiana* can be

distinguished from species in the *Ca. colhounii* complex by its distinctly orange to red perithecia. This fungus can also be distinguished from *Ca. indusiata* and *Ca. australiensis* based on the dimensions of the macroconidia, with *Ca. crousiana* (av. = 64 × 5 µm) having shorter macroconidia than those of *Ca. indusiata* (macroconidia av. = 81 × 6.0 µm), and narrower than those of *Ca. australiensis* (macroconidia av. = 63 × 6.5 µm).

Calonectria pseudocolhounii S.F. Chen, L. Lombard, M.J. Wingf. & X.D. Zhou, sp. nov. MycoBank no.: MB518856

(Fig 3)

Etymology: The name reflects the fact that this fungus is morphologically similar to *Ca. colhounii*.

Teleomorpha *Calonectria colhounii* similis sed ascosporis hyalinis guttulatis fusoidibus extremis rotundatis, rectis vel subcurvatis, (semel vel) ter septatis, in septo non vel leviter constrictis, (44–)50–62(–74) × (5–)6–7(–8) µm, mediocriter 56 × 6.5 µm, differt. Anamorpha *Cy. colhounii* similis sed macroconidiis cylindricis utrinque rotundatis rectis (49–)55–65(–74) × (3.5–)4.0–5(–5.5) µm, mediocriter 60 × 4.5 µm, (semel vel) ter septatis, sine cicatrice abscissionis visibile, in fasciculis parallelis cylindricis muco contenitis, differt.

Perithecia solitary or in groups of up to four, bright yellow, becoming orange with age; in section apex and body yellow, base red-brown, subglobose to ovoid, (330–)350–453(–495) µm high, (227–)258–330(–390) µm diam, body turning dark yellow, and base dark red-brown in KOH+; perithecial walls rough consisting of two thick-walled layers: outside layer of *textura globulosa*, (26–)33–59(–65) µm wide, becoming more compressed towards inner layer of *textura angularis*, (10–)12–18(–22) µm wide, becoming thin-walled and hyaline towards the centre; outer cells (17–)21–34(–42) × (11–)12–21(–27) µm, inner cells (10–)11–14(–20) × (3–)5–6.5(–7) µm, perithecial base up to 180 µm wide, consisting of dark red, angular cells merging with an erumpent stroma, cells of the outer wall layer continuing into the pseudoparenchymatous cells of the erumpent stroma. **Asci** 4-spored, clavate, (130–)135–162(–167) × (16–)18–24(–30) µm, tapering to a long thin stalk. **Ascospores** aggregate in the upper third of the asci, hyaline, guttulate, fusoid with rounded ends, straight to slightly curved, (1–)3-septate, not or slightly constricted at the septum, (44–)50–62(–74) × (5–)6–7(–8) µm (av. = 56 × 6.5 µm). **Macroconidiophores** consisting of a stipe, a suite of penicillate arranged fertile branches, a stipe extension, and a terminal vesicle; stipe septate, hyaline, smooth (45–

)53–192(–217) × (5.5–)6–7(–8) µm; stipe extensions septate, straight to flexuous (133–)168–252(–300) µm long, 3–6 µm wide at the apical septum, terminating in a clavate vesicle, (3.5–)4–5(–6) µm diameter. **Conidiogenous apparatus** (41–)44–74(–91) µm long, (35–)38–65(–84) µm wide; primary branches aseptate to 1-septate, (13–)15–26(–33) × 3.5–4.5(–5) µm; secondary branches aseptate, (9–)11.5–20(–23) × 3–4(–4.5) µm; tertiary branches aseptate, 8.5–14(–17) × 3–4 µm; additional branches (–5), aseptate, (8–)8.5–13(–15) × 2.5–3(–3.5) µm; each terminal branch producing 2–4 phialides; phialides doliiform to reniform, hyaline, aseptate, (8–)9–12.5(–14) × 2.5–3(–3.5)µm, apex with minute periclinal thickening and inconspicuous collarette. **Macroconidia** cylindrical, rounded at both ends, straight, (49–)55–65(–74) × (3.5–)4–5(–5.5) µm (av. = 60 × 4.5 µm), (1–)3-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime.

Culture characteristics: Colonies reaching 38–44 mm diam after 7 d on MEA in the dark with optimal growth temperature at 25 °C. Colonies with white aerial mycelium in the centre, with feathery, irregular margins at the edges. Surface and reverse with white to buff-yellow (19d) outer margins, and russet (13'k) inner region, becoming liver-brown (7'm) towards the centre. Chlamydospores arrange in chains, abundant throughout the medium, forming microsclerotia.

Substrate: *Eucalyptus dunnii*.

Distribution: FuJian Province, China.

Specimens examined: China, FuJian Province, on leaves of *E. dunnii*, Aug. 2007, M.J. Wingfield, Herb. PREM60456, holotype of *Ca. pseudocolhounii*, culture ex-type CMW27209 = CBS127195; FuJian Province, on leaves of *E. dunnii*, Aug. 2007, M.J. Wingfield, Herb. PREM60457, culture CMW27213 = CBS127196; FuJian Province, on leaves of *E. dunnii*, Aug. 2007, M.J. Wingfield, Herb. PREM60458, culture CMW27214 = CBS127197.

Notes: *Calonectria pseudocolhounii* is similar to species in the *Ca. colhounii* complex that all have yellow perithecia, (1–)3-septate ascospores and clavate vesicles in the anamorph state. *Ca. pseudocolhounii* is morphologically most similar to *Ca. colhounii* (macroconidia av. = 65 × 5 µm), but can be distinguished from this species by having smaller and narrower macroconidia (av. = 60 × 4.5 µm). The ascospores of *Ca. pseudocolhounii* (av. = 56 × 6.5 µm)

are larger, while the macroconidia (av. = 60 × 4.5 µm) are smaller than those of *Ca. eucalypti* (ascospores av. = 33 × 6 µm; macroconidia av. = 72 × 6 µm).

Calonectria fujianensis S.F. Chen, L. Lombard, M.J. Wingf. & X.D. Zhou, sp. nov.
MycoBank no.: MB518857
(Fig 4)

Etymology: Named after the FuJian Province of China, from where the fungus was first collected.

Teleomorpha *Calonectria colhounii* similis sed ascosporis hyalinis guttulatis fusoidibus extremis rotundatis, rectis vel subcurvatis, (semel vel) ter septatis, in septo non vel leviter constrictis, (38–)49–62(–72) × (5–)6–7.5(–8) µm, mediocriter 55.5 × 6.8 µm, differt. Anamorpha *Cy. colhounii* similis sed macroconidiis cylindricis utrinque rotundatis rectis 48–)50–55(–60) × (2.5–)3.5–4.5(–5) µm, mediocriter 52.5 × 4 µm, (semel vel) ter septatis, sine cicatrice abscissionis visibile, in fasciculis parallelis cylindricis muco contenitis, differt.

Perithecia solitary or in groups of up to four, bright yellow, becoming orange with age; in section apex and body yellow, base red-brown, subglobose to ovoid, (310–)351–465(–492) µm high, (206–)226–329(–382) µm diam, body turning dark yellow, and base dark red-brown in KOH+; Perithecial walls rough consisting of two thick-walled layers: outside layer of *textura globulosa*, (26–)35–58(–61) µm wide, becoming more compressed towards inner layer of *textura angularis*, (10–)12–21(–24) µm wide, becoming thin-walled and hyaline towards the centre; outer cells (15–)17–35(–41) × (8–)11–20(–24) µm, inner cells (9–)10–20(–26) × (2.5–)3.5–6.0(–6.5) µm; perithecial base up to 180 µm wide, consisting of dark red, angular cells, merging with an erumpent stroma, cells of the outer wall layer continuing into the pseudoparenchymatous cells of the erumpent stroma. **Asci** 4-spored, clavate, (118–)132–152(–155) × (14–)16–23(–29) µm, tapering to a long thin stalk. **Ascospores** aggregate in the upper third of the ascus, hyaline, guttulate, fusoid with rounded ends, straight to slightly curved, (1–)3-septate, not or slightly constricted at the septum, (38–)49–62(–72) × (5–)6–7.5(–8) µm (av = 55.5 × 6.8 µm). ***Macroconidiophores*** consisting of a stipe, a suite of penicillate arranged fertile branches, a stipe extension, and a terminal vesicle; stipe septate, hyaline, smooth, (33–)36–152(–210) × (3.5–)4.5–8(–8.5) µm; stipe extensions septate, straight to flexuous (147–)167–248(–261) µm long, 3–5 µm wide at the apical septum,

terminating in a clavate vesicle, (3–)3.5–4.5(–5) µm diameter. **Conidiogenous apparatus** (36–)43–72(–89) µm long, (21–)31–61(–65) µm wide; primary branches aseptate to 1-septate, (11–)12–28(–32) × (3–)3.5–4.5 µm; secondary branches aseptate, 8–20(–26) × 3–4(–4.5) µm; tertiary branches aseptate, (8–)10–12(–12.5) × 2.5–3(–4) µm; additional branches (–5), aseptate, (8–)9–10× 2.5–3(–3.5) µm; each terminal branch producing 2–4 phialides; phialides doliiform to reniform, hyaline, aseptate, (6.5–)8–11 × (2–)2.5–3 µm, apex with minute periclinal thickening and inconspicuous collarette. **Macroconidia** cylindrical, rounded at both ends, straight, (48–)50–55(–60) × (2.5–)3.5–4.5(–5) µm (av. = 52.5 × 4 µm), (1–)3-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime.

Culture characteristics: Colonies reaching 44–52 mm diam after 7 d on MEA in the dark with optimal growth temperature at 25 °C. Colonies with white to cream-coloured aerial mycelium in the centre, with feathery, irregular margins at the edges. Surface and reverse with cream coloured to white outer margins, and russet (13'k) inner region, becoming argus-brown (13m) towards the centre. Chlamydospores arranged in chains, abundant throughout the medium, forming microsclerotia.

Substrate: *Eucalyptus grandis*.

Distribution: FuJian Province, China.

Specimens examined: China, FuJian Province, on leaves of *E. grandis*, Aug. 2007, M.J. Wingfield, Herb. PREM60460, holotype of *Ca. fujianensis*, culture ex-type CMW27257 = CBS127201; FuJian Province, on leaves of *E. grandis*, Aug. 2007, M.J. Wingfield, Herb. PREM60461, culture CMW27263 = CBS127202; FuJian Province, on leaves of *E. grandis*, Aug. 2007, M.J. Wingfield, Herb. PREM60459, culture CMW27254 = CBS127200.

Notes: *Calonectria fujianensis* is morphologically distinguishable from *Ca. colhounii* and *Ca. pseudocolhounii* having smaller macroconidia (av. = 52.5 × 4 µm) than *Ca. colhounii* (av. = 65 × 5 µm) and *Ca. pseudocolhounii* (av. = 60 × 4.5 µm). The ascospores of *Ca. fujianensis* (av. = 55.5 × 6.8 µm), *Ca. pseudocolhounii* (av. = 56 × 6.5 µm) and *Ca. colhounii* (av. = 55 × 6 µm), are larger than those of *Ca. eucalypti* (av. = 33 × 6 µm), while the macroconidia of the former three species are smaller than those of *Ca. eucalypti* (av. = 72 × 6 µm).

3.5. Pathogenicity tests

All tested plants representing the two *Eucalyptus* clones inoculated with *Calonectria* spp. (*Ca. crousiana*, *Ca. fujianensis*, *Ca. pauciramosa*, *Ca. pseudocolhounii*) in this study, developed leaf spot symptoms whereas no disease was observed on the leaves of the control plants (Fig 5). The inoculated fungi were successfully re-isolated from the leaf spots and no *Calonectria* spp. was isolated from the control plants. The average percentage of leaf surface affected by the test isolates showed no significant differences between the two experimental plots ($P = 0.0578$), and the interactions between the two experiments and two clones were not significantly different ($P = 0.0535$). Subsequently, the data for the two plots were combined and analysed collectively. The combined results showed significant isolate × clone interaction ($P < 0.05$), indicating that not all *Calonectria* isolates reacted similarly to the two *Eucalyptus* clones tested. The percentage of infected leaves arising from inoculation with *Ca. pauciramosa* (CMW27199, CMW27192), *Ca. pseudocolhounii* (CMW27209, CMW27213, CMW27214), *Ca. crousiana* (CMW27249, CMW27267) and *Ca. fujianensis* (CMW27263) were significantly different ($P < 0.05$) on the two clones tested. In contrast, there was no significant difference ($P > 0.05$) in the percentage of infected leaves for the two clones inoculated with isolates (CMW27254, CMW27257) of *Ca. fujianensis* (Fig 5).

The *Eucalyptus* clone CEPT-10 displayed a significantly ($P < 0.05$) higher percentage of infected leaves when inoculated with *Ca. pauciramosa* (CMW27199, CMW27292), *Ca. pseudocolhounii* (CMW27209, CMW27213, CMW27214) and *Ca. fujianensis* (CMW27254, CMW27257, CMW27263) than with *Ca. crousiana* (CMW27249, CMW27267) (Fig 5). Isolate CMW27254 (*Ca. fujianensis*) displayed the highest average percentage of leaf surface infected on clone CEPT-10 (Fig 5).

The *Eucalyptus* clone CEPT-9 showed a significantly ($P < 0.05$) higher percentage of infected leaves caused by isolates of *Ca. pseudocolhounii* (CMW27209, CMW27213, CMW27214) and *Ca. fujianensis* (CMW27254, CMW27257, CMW27263) than with those of *Ca. pauciramosa* (CMW27199, CMW27292) and *Ca. crousiana* (CMW27249, CMW27267) (Fig 5). Isolate CMW27214 (*Ca. pseudocolhounii*) resulted in the highest average percentage of leaves infected for CEPT-9 (Fig 5).

4. DISCUSSION

In this study, four different *Calonectria* spp. were identified from leaves collected on diseased *Eucalyptus* trees grown in commercial plantations of FuJian Province in Southeast China. These included *Ca. pauciramosa* and three previously undescribed species for which the names *Ca. crousiana*, *Ca. fujianensis* and *Ca. pseudocolhounii* are provided. The identification of these fungi was supported by DNA sequence comparisons as well as by morphological characteristics. Based on phylogenetic inference, *Ca. crousiana* is closely related to taxa in the *Ca. reteaudii* species complex, whereas *Ca. pseudocolhounii* and *Ca. fujianensis* reside in the *Ca. colhounii* complex. Pathogenicity tests showed that all four species are capable of causing leaf blight on two of the most widely planted *E. urophylla* × *E. grandis* clones in South China.

Calonectria pauciramosa resides in the *Ca. scoparia* Peerally species complex (Schoch *et al*. 1999; Crous *et al*. 1993; Lombard *et al*. 2010b, d) and was recently found killing plants in a commercial *Eucalyptus* nursery in the GuangDong Province of China (Lombard *et al*. 2010d). This study represents the first report of this pathogen infecting leaves of *Eucalyptus* trees growing in plantations. In the past, *Ca. pauciramosa* has been associated with nursery diseases in Australia, Italy, South Africa, Spain and USA (Koike *et al*. 1999; Polizzi & Crous 1999; Schoch *et al*. 1999, 2001; Koike & Crous 2001; Polizzi *et al*. 2006, 2009; Perez-Sierra *et al*. 2007). This fungus has also been isolated from tropical areas of GuangDong Province (Lombard *et al*. 2010d) and in this study was found in an area that has a sub-tropical climate. The climatic conditions of these regions differ significantly, supporting the view that *Ca. pauciramosa* can tolerate a wide range of temperature conditions.

Based on phylogenetic inference, *Ca. crousiana* is closely related to *Calonectria* spp. in the *Ca. reteaudii* complex. Similar to *Ca. reteaudii*, *Ca. crousiana* also produces orange to red perithecia and has a *Cylindrocladium* state with clavate vesicles. However, septation of the macrocondia is distinct in these species with *Ca. crousiana* having (1–)3-septate macroconia that distinguish it from the other species in the *Ca. reteaudii* complex, including *Ca. reteaudii* ((1–)5(–6)-septate), *Ca. pseudoreteaudii* L. Lombard, M.J. Wingf. & Crous (1(–3)-septate), *Ca. queenslandica* L. Lombard, M.J. Wingf. & Crous ((1–)3(–6)-septate) and *Ca. terrae-reginae* L. Lombard, M.J. Wingf. & Crous ((1–)3(–6)-septate) (Crous 2002; Lombard *et al*. 2010b). Morphological comparisons showed that *Ca. crousiana* is very similar to *Ca.*

indusiata and *Ca. australiensis*, which have clavate vesicles and (1–)3-septate macroconidia (Crous 2002; Crous *et al*. 2006; Lombard *et al*. 2010b).

Previous studies have shown that *Ca. indusiata* and species in the *Ca. reteaudii* species complex are pathogens causing leaf blight and cutting rot on *Eucalyptus* trees and seedlings in Australia, South America and Southeast Asia (Pikethley 1976; Bolland *et al*. 1985; Sharma & Mohanan 1991, 1992; Booth *et al*. 2000; Crous & Kang 2001; Crous 2002; Rodas *et al*. 2005; Lombard *et al*. 2010d). In this study, *Ca. crousiana* was isolated from diseased leaves on *E. grandis* trees in FuJian Province. Based on the results of pathogenicity tests on two *Eucalyptus* hybrid clones, *Ca. crousiana* should be regarded as an important pathogen of *Eucalyptus* in China.

Past studies have shown that *Ca. colhounii* is closely related to *Ca. madagascariensis* and *Ca. macroconidialis* (Crous *et al*. 1999). Recently, a newly described species, *Ca. eucalypti*, was also identified in this complex (Lombard *et al*. 2010b). This *Calonectria* complex is characterised by having unique yellow perithecia, (1–)3-septate ascospores and clavate vesicles (Crous *et al*. 1999, Crous 2002, Lombard *et al*. 2010b). In the present study, *Ca. pseudocolhounii* and *Ca. fujianensis* were described as new species with both species sharing unique morphological characteristics with the other species in the complex. There are, however, a number of morphological differences distinguishing *Ca. pseudocolhounii* and *Ca. fujianensis* from the other species in this complex. All species other than *Ca. madagascariensis* (8-spore asci) produce asci with four ascospores. Macroconidia of *Ca. fujianensis* (av. 52.5 × 4 µm) are smaller than that of *Ca. pseudocolhounii* (av. = 60 × 4.5 µm), while these structures in both species are smaller than those of *Ca. colhounii* (av. = 65 × 5 µm) and *Ca. eucalypti* (av. = 72 × 6 µm). Species in the *Ca. colhounii* complex have been isolated from the *Eucalyptus* trees or soil under these trees in Africa, America and Southeast Asia (Crous 2002; Lombard *et al*. 2010d). Pathogenicity tests in this study showed that *Ca. pseudocolhounii* and *Ca. fujianensis* are both aggressive pathogens on the *Eucalyptus* clones tested.

Pathogenicity tests in this study showed that all four species of *Calonectria* found in FuJian Province are important pathogens of *Eucalyptus*. *Ca. pseudocolhounii* and *Ca. fujianensis* were more virulent than *Ca. pauciramosa* and *Ca. crousiana*, while *Ca. pauciramosa* was more virulent than *Ca. crousiana*. These results also showed that the tolerance of the two

tested *Eucalyptus* hybrid clones are significantly different for some of the isolates tested. This implies that it might be possible to select disease tolerant planting stock based on nursery screening.

Leaf and shoot blight associated with *Calonectria* spp. is one of the most serious threats to commercial *Eucalyptus* plantations and nurseries in China (Wang 1992; Sun & Liu 2004; Zhou *et al*. 2008; Lombard *et al*. 2010d). Although *Ca. reteaudii* has been regarded as the dominant pathogen responsible for CLB in South America and Southeast Asia (Pikethley 1976; Bolland *et al*. 1985; Sharma & Mohanan 1991, 1992; Booth *et al*. 2000; Crous & Kang 2001; Crous 2002; Rodas *et al*. 2005), no isolates of this fungus was obtained during this study. This could be due to the cooler climatic conditions of the region surveyed, as *Ca. reteaudii* has only been reported from tropical regions (Booth *et al*. 2000; Crous 2002).

This study has added considerably to our knowledge of the species of *Calonectria* and their *Cylindrocladium* anamorphs in China. The discovery of three new species was surprising and this suggests that additional species await discovery in that country. *Calonectria* spp. are well-known to have wide host ranges and the results of this study add substance to the view that those species occurring in the soil below *Eucalyptus* spp., are likely to infect the leaves of these trees, assuming that climatic conditions are favourable for infection. Very little is known regarding the host specificity of these important pathogens but inoculation tests in this study show clearly that different clones respond differently to inoculation by different species of *Calonectria*. This could provide opportunities to tailor planting to avoid damage due to CLB. However, given the large number of *Calonectria* spp. that are now known to occur in China, such complex deployment of clones may not be financially feasible.

Calonectria spp. is important *Eucalyptus* pathogens (Crous 2002; Old *et al*. 2003; Rodas *et al*. 2005; Lombard 2010d). The fact that they are soil-borne also contributes to the ease with which they might be moved globally. In this regard, very little is known about their origins. Some species with wide global distributions in agricultural and forestry environments, such as *Ca. pauciramosa*, seem very likely to have been moved to new environments. It is difficult to predict how these fungi might respond to new host encounters. However, they add to the growing threats that pathogens pose to *Eucalyptus* plantation forestry (Wingfield *et al*. 2008) and every effort should be made to avoid their movement and introduction.

This study represents an important contribution to the taxonomy of species of *Calonectria*, and highlights the distribution of these pathogens in *Eucalyptus* plantations in China. We conducted the first pathogenicity tests of these species on *Eucalyptus* clones in this country. These results will offer valuable information on the management of *Calonectria* pathogens in *Eucalyptus* plantations, and will advance breeding strategies aimed at developing resistant *Eucalyptus* clones in China.

5. REFERENCES

Bolland L, Tierney JW, Tierney BJ, 1985. Studies on leaf spot and shoot blight of *Eucalyptus* caused by *Cylindrocladium quinqueseptatum*. *European Journal of Forest Pathology* **15**: 385–397.

Booth TH, Jovanovic T, Old KM, Dudzinski MJ, 2000. Climatic mapping to identify high-risk areas for *Cylindrocladium quinqueseptatum* leaf blight on eucalypts in main land South East Asia and around the world. *Environmental Pollution* **108**: 365–372.

Carbone I, Kohn LM, 1999. A method for designing primer sets for the speciation studies in filamentous ascomycetes. *Mycologia* **91**: 553–556.

Crous PW, 2002. *Taxonomy and pathology of Cylindrocladium (Calonectria) and allied genera*. APS Press, St. Paul, Minnesota, USA.

Crous PW, Alfenas AC, Wingfield MJ, 1993. *Calonectria scoparia* and *Calonectria morganii* sp. nov., and variations among isolates of their *Cylindrocladium* anamorphs. *Mycological Research* **97**: 701–708.

Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G, 2004a. MycoBank: an online initiative to launch mycology into the 21st century. *Studies in Mycology* **50**: 19–22.

Crous PW, Groenewald JZ, Risède J-M, Simoneau P, Hyde KD, 2006. *Calonectria* species and their *Cylindrocladium* anamorphs: species with clavate vesicles. *Studies in Mycology* **55**: 213–226.

Crous PW, Groenewald JZ, Risède J-M, Simoneau P, Hywel-Jones NL, 2004b. *Calonectria* species and their *Cylindrocladium* anamorphs: species with sphaeropedunculate vesicles. *Studies in Mycology* **50**: 415–430.

Crous PW, Kang J-C, 2001. Phylogenetic confirmation of *Calonectria spathulata* and *Cylindrocladium leucothoes* based on morphology, and sequence data of the β-tubulin and ITS rRNA genes. *Mycoscience* **42**: 51–57.

Crous PW, Kang J-C, Schoch CL, Machau GRA, 1999. Phylogenetic relationship of *Cylindrocladium pseudogracile* and *Cylindrocladium rumohrae* with morphologically similar taxa, based on morphology and DNA sequence of internal transcribed spacers and β-tubulin. *Canadian Journal of Botany* **77**: 1813–1820.

Crous PW, Phillips AJL, Wingfield MJ, 1991. The genera *Cylindrocladium* and *Cylindrocladiella* in South Africa, with special reference to forest nurseries. *South African Forestry Journal* **157**: 69–85.

Crous PW, Wingfield MJ, 1994. A monograph of *Cylindrocladium*, including anamorphs of *Calonectria*. *Mycotaxon* **51**: 341–435.

Cunningham CW, 1997. Can three incongruency tests predict when data should be combined? *Molecular Biology and Evolution* **14**: 733–740.

Dettman JR, Jacobson DJ, Taylor JW, 2003. A multilocus genealogical approach to phylogenetic species recognition in the model eukaryote Neurospora. *Evolution* **57**: 2703–2720.

Farris JS, Källersjö M, Kluge AG, Bult C, 1994. Testing significance of incongruence. *Cladistics* **10**: 315–320.

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

Gueidan C, Roux C, Lutzoni F, 2007. Using multigene phylogeny analysis to assess generic delineation and character evolution in Verrucariaceae (Verrucariales, Ascomycota). *Mycological Research* **111**: 1145–1168.

Guerber JC, Correll JC, 2001. Characterization of *Glomerella acutata*, the teleomorph of *Colletotrichum acutatum*. *Mycologia* **93**: 216–229.

Hillis DM, Bull JJ, 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* **42**: 182–192.

Iglesias-Trabad G, Wilstermann D, 2008. *Eucalyptus universalis*. Global cultivated eucalypt forest map 2008 Version 1.0.1 in GIT Forestry Consulting's EUCALYPTOLOGICS: Information resources on *Eucalyptus* cultivation worldwide. Retrieved from http://www.git-forestry.com (March 29th 2009).

Kang J-C, Crous PW, Old K, Dudzinski MJ, 2001. Non-conspecificity of *Cylindrocladium quinqueseptatum* and *Calonectria quinqueseptata* based on a β-tubulin gene phylogeny and morphology. *Canadian Journal of Botany* **79**: 1241–1247.

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.

Koike ST, Crous PW, 2001. First report of a root and crown rot disease of myrtle in California caused by *Cylindrocladium pauciramosum*. *Plant Disease* **85**: 44.

Koike ST, Henderson DM, Crous PW, Schoch CL, Tjosvold SA, 1999. A new root and crown rot disease of heath in California caused by *Cylindrocladium pauciramosum*. *Plant Disease* **83**: 589.

Librado P, Rozas J, 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**: 1451–1452.

Lombard L, Crous PW, Wingfield BD, Wingfield MJ, 2010a. Multigene phylogeny and mating tests reveal three cryptic species related to *Calonectria pauciramosa*. *Studies in Mycology* **66**: 15–30.

Lombard L, Crous PW, Wingfield BD, Wingfield MJ, 2010b. Phylogeny and systematics of the genus *Calonectria*. *Studies in Mycology* **66**: 31–69.

Lombard L, Crous PW, Wingfield BD, Wingfield MJ, 2010c. Species concepts in *Calonectria* (*Cylindrocladium*). *Studies in Mycology* **66**: 1–14.

Lombard L, Rodas CA, Crous PW, Wingfield BD, Wingfield MJ, 2009. *Calonectria* (*Cylindrocladium*) species associated with dying Pinus cuttings. *Persoonia* **23**: 41–47.

Lombard L, Zhou XD, Crous PW, Wingfield BD, Wingfield MJ, 2010d. *Calonectria* species associated with cutting rot of *Eucalyptus*. *Persoonia* **24**: 1–11.

Mason-Gamer R, Kellogg E, 1996. Testing for phylogenetic conflict among molecular datasets in the tribe Triticeae (Graminae). *Systematic Biology* **45**: 524–545.

Nirenburg HI, 1981. A simplified method for identifying *Fusarium* spp. occurring on wheat. *Canadian Journal of Botany* **59**: 1599–1609

Nylander JAA, 2004. *MrModeltest v2.3. Program distributed by the author*. Evolutionary Biology Centre, Uppsala University, Sweden.

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

O'Donnell K, Cigelnik E, 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics and Evolution* **7**: 103–116.

Perez-Sierra A, Alvarez LA, Leon M, Abad-Campos P, Armengol J, Garcia-Jimenez J, 2007. First Report of Leaf Spot, Blight, and Stem Lesions Caused by *Cylindrocladium pauciramosum* on Callistemon in Spain. *Plant Disease* **91**: 1057.

Pikethley RN, 1976. *Cylindrocladium quinqueseptatum* on myrtaceous tree seedlings. *Australian Plant Pathology Newsletter* **5**: 57.

Polizzi G, Crous PW, 1999. Root and collar rot of milkwort caused by *Cylindrocladium pauciramosum*, a new record for Europe. *European Journal of Plant Pathology* **105**: 407–411.

Polizzi G, Vitale A, Aiello D, Castello I, Guarnaccia V, Parlavecchio G, 2009. First Report of Crown Rot Caused by *Cylindrocladium pauciramosum* on Brush Cherry in Italy. *Plant Disease* **93**: 547.

Polizzi G, Vitale A, Aiello D, Parlavecchio G, 2006. First Record of Crown and Root Rot Caused by *Cylindrocladium pauciramosum* on California Lilac in Italy. *Plant Disease* **90**: 1459.

Qi SX, 2006. The introduction and development situation of *Eucalyptus* in China. *Guangxi Forestry Science* **35**: 250–252. (in Chinese).

Rayner RW, 1970. *A mycological colour chart*. British Mycological Society, Commonwealth Mycological Institute, Kew, Surry.

Rodas CA, Lombard L, Gryzenhout M, Slippers B, Wingfield MJ, 2005. Cylindrocladium blight of *Eucalyptus grandis* in Colombia. *Australasian Plant Pathology* **34**: 143–149.

Ronquist F, Huelsenbeck JP, 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.

SAS Institute, 1999. SAS/STAT Users Guide, Version 8, Cary NC: SAS Institute Inc. ISBN 1-58025-494-2.

Schoch CL, Crous PW, Polizzi G, Koike ST, 2001. Female fertility and single nucleotide polymorphism comparisons in *Cylindrocladium pauciramosum*. *Plant Disease* **85**: 941–946.

Schoch CL, Crous PW, Wingfield BD, Wingfield MJ, 1999. The *Cylindrocladium candelabrum* species complex includes four distinct mating populations. *Mycologia* **91**: 286–298.

Sharma JK, Mohanan C, 1991. Pathogenic variation in *Cylindrocladium quinqueseptatum* causing leaf blight of *Eucalyptus*. *European Journal of Forest Pathology* **21**: 210–217.

Sharma JK, Mohanan C, 1992. Relative susceptibility of *Eucalyptus* provenances to Cylindrocladium leaf blight in Kerala, India. *European Journal of Forest Pathology* **22**: 257–265.

Sharma JK, Mohanan C, Florence EJM, 1984. Nursery diseases of *Eucalyptus* in Kerala. *European Journal of Forest Pathology* **14**: 77–89.

Smith H, Crous PW, Wingfield MJ, Coutinho TA, Wingfield BD, 2001. *Botryosphaeria eucalyptorum* sp. nov., a new species in the *B. dothidea*-complex on *Eucalyptus* in South Africa. *Mycologia* **93**: 277–285.

Sun YX, Liu JF, 2004. Current situation and control of diseases and insect pests in eucalypts. *Forest Pest and Disease* **4**: 36–38. (In Chinese).

Swofford DL, 2002. *PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). 4. 0b10*. Computer program. Sinauer Associates, Sunderland, Massachusetts, USA.

Wang WY, 1992. Survey of *Eucalyptus* diseases in Taiwan. *Bulletin Taiwan Forest Research Institute* **7**: 179–194. (In Chinese).

Wingfield MJ, Slippers B, Hurley BP, Coutinho TA, Wingfield BD, Roux J, 2008. Eucalypt pests and diseases: growing threats to plantation productivity. *Southern Forests* **70**: 139–144.

Xie YJ, 2006. *Eucalypt research at the beginning of 21st century*. China Forestry House Press, Beijing, China. (In Chinese).

Zhou XD, Xie YJ, Chen SF, Wingfield MJ, 2008. Diseases of eucalypt plantations in China: challenges and opportunities. *Fungal Diversity* **32**: 1–7.

Table 1. Isolates used in the phylogenetic analyses and pathogenicity trials.

Species	Isolate number[a]	β-tubulin[b]	Histone H3[b]	TEF-1α[b]	Host	Origin	Collector
Calonectria acicola	CBS114812	DQ190590	DQ190692	GQ267291	*Phoenix canariensis*	New Zealand	H Pearson
	CBS114813[d]	DQ190591	DQ190693	GQ267292	*P. canariensis*	New Zealand	H Pearson
Ca. brachiatica	CMW25302	FJ716708	FJ716712	GQ267295	*Pinus tecunumanii*	Colombia	MJ Wingfield
	CBS123700 (= CMW25298)[d]	FJ696388	FJ696396	GQ267296	*P. maximinoi*	Colombia	MJ Wingfield
Ca. brassicae	CBS111869[d]	AF232857	DQ190720	FJ918568	*Argyreia* sp.	Southeast Asia	n/a
	CBS111478	DQ190611	DQ190719	FJ918567	Soil	Brazil	AC Alfenas
Ca. cerciana	CBS123639 (= CMW25309)[d]	FJ918510	FJ918528	FJ918559	*Eucalyptus urophylla* × *E. grandis* cutting	GuangDong, China	MJ Wingfield & XD Zhou
	CBS123695 (= CMW25290)	FJ918511	FJ918529	FJ918560	*E. urophylla* × *E. grandis* cutting	GuangDong, China	MJ Wingfield & XD Zhou
Ca. chinensis	CBS112744	AY725618	AY725660	AY725709	Soil	China	ECY Liew
Ca. colhounii	CBS293.79 (= CMW30999)[d]	DQ190564	DQ190639	GQ267301	n/a[e]	Indonesia	n/a
	CBS114704	DQ190563	DQ190638	GQ267300	*Arachis pintoi*	Australia	D Hutton
Ca. colombiana	CBS115638	FJ972422	FJ972441	FJ972491	Soil	Colombia	MJ Wingfield
	CBS115127	FJ972423	FJ972442	FJ972492	Soil	Colombia	MJ Wingfield
Ca. colombiensis	CBS112221	AY725620	AY725663	AY725712	Soil	Colombia	MJ Wingfield
Ca. crousiana	**CMW27249**[cd] (= CBS127198)	HQ285794	HQ285808	HQ285822	*E. grandis*	FuJian, China	MJ Wingfield
	CMW27253 (= CBS127199)	HQ285795	HQ285809	HQ285823	*E. grandis*	FuJian, China	MJ Wingfield
	CMW27258	HQ285796	HQ285810	HQ285824	*E. grandis*	FuJian, China	MJ Wingfield
	CMW27267[c] (= CBS127203)	HQ285797	HQ285811	HQ285825	*E. grandis*	FuJian, China	MJ Wingfield
Ca. eucadoriae	CBS111394	DQ190599	DQ190704	GQ267304	Soil	Ecuador	MJ Wingfield
	CBS111406	DQ190600	DQ190705	GQ267303	Soil	Ecuador	MJ Wingfield
Ca. eucalypti	CBS125273 (= CMW14890)	GQ267217	GQ267266	GQ267337	*E. grandis*	Indonesia	MJ Wingfield
	CBS125275 (= CMW18444)[d]	GQ267218	GQ267267	GQ267338	*E. grandis*	Indonesia	MJ Wingfield
Ca. fujianensis	**CMW27254**[c] (= CBS127200)	HQ285791	HQ285805	HQ285819	*E. grandis*	FuJian, China	MJ Wingfield
	CMW27257[cd] (= CBS127201)	HQ285792	HQ285806	HQ285820	*E. grandis*	FuJian, China	MJ Wingfield
	CMW27263[c] (= CBS127202)	HQ285793	HQ285807	HQ285821	*E. grandis*	FuJian, China	MJ Wingfield
Ca. insulare	CBS114558	AF210861	FJ918526	FJ918556	Soil	Madagascar	PW Crous
	CBS114559	AF210862	FJ918525	FJ918555	Soil	Madagascar	CL Schoch
Ca. madagascariensis	CBS114572 (= CMW23686)[d]	DQ190572	DQ190658	GQ267314	n/a	Madagascar	PW Crous
	CBS114571 (= CMW30993)	DQ190571	DQ190657	GQ267315	n/a	Madagascar	PW Crous
Ca. marcoconidialis	CBS114880[d]	AF232855	DQ190655	GQ267313	*E. grandis*	South Africa	PW Crous
Ca. morganii	CBS110666	FJ918509	FJ918527	FJ918557	*Rosa* sp.	USA	NE Ell-Gholl
Ca. multiseptata	CBS112682[d]	DQ190573	DQ190659	FJ918535	*Eucalyptus* sp.	Indonesia	MJ Wingfield
Ca. pauciramosa	CMW30823	FJ918515	FJ918532	FJ918566	*E. grandis*	South Africa	PW Crous
	CMW5683	FJ918514	FJ918531	FJ918565	*Eucalyptus* sp.	Brazil	AC Alfenas
	CMW27199[c]	HQ285784	HQ285798	HQ285812	*E. dunnii*	FuJian, China	MJ Wingfield
	CMW27203	HQ285785	HQ285799	HQ285813	*E. dunnii*	FuJian, China	MJ Wingfield
	CMW27283	HQ285786	HQ285800	HQ285814	*E. dunnii*	FuJian, China	MJ Wingfield

	CMW27292[c]	HQ285787	HQ285801	HQ285815	*E. dunnii*	FuJian, China	MJ Wingfield
Ca. polizzii	CMW7804	FJ972417	FJ972436	FJ972486	*Callistemon citrinus*	Italy	G Polizzi
	CMW10151	FJ972418	FJ972437	FJ972487	*Arbustus unedo*	Italy	G Polizzi
Ca. pseudocolhounii	**CMW27209**[cd] (= CBS127195)	HQ285788	HQ285802	HQ285816	*E. dunnii*	FuJian, China	MJ Wingfield
	CMW27213[c] (= CBS127196)	HQ285789	HQ285803	HQ285817	*E. dunnii*	FuJian, China	MJ Wingfield
	CMW27214[c] (= CBS127197)	HQ285790	HQ285804	HQ285818	*E. dunnii*	FuJian, China	MJ Wingfield
Ca. pseudoreteaudii	CBS123694 (= CMW25310)[d]	FJ918504	FJ918519	FJ918541	*E. urophylla* × *E. grandis* cutting	GuangDong, China	MJ Wingfield & XD Zhou
	CBS123696 (= CMW25296)	FJ918505	FJ918520	FJ918542	*E. urophylla* × *E. grandis* cutting	GuangDong, China	MJ Wingfield & XD Zhou
Ca. pteridis	CBS111793	DQ190578	DQ190679	FJ918563	*Arachnoides adiantiformis*	USA	n/a
	CBS111871	DQ190579	DQ190680	FJ918564	*Pinus* sp.	Spain	TL Krugner
Ca. queenslandica	CBS112146 (= CMW30604)[d]	AF389835	FJ918521	FJ918543	*E. urophylla*	Australia	B Brown
	CBS112155 (= CMW30603)	AF389834	DQ190667	FJ918544	*E. pellita*	Australia	KM Old
Ca. reteaudii	CBS112144[d]	AF389833	DQ190661	FJ918537	*E. camaldulensis*	Vietnam	MJ Dudzinski
	CBS112143	GQ240642	DQ190660	FJ918536	*E. camaldulensis*	Vietnam	MJ Dudzinski
Ca. spathulata	CBS112689	AF308463	FJ918524	FJ918554	*E. viminalis*	Brazil	NE Ell-Gholl
	CBS555.92	GQ267215	GQ267261	GQ267331	*Araucaria angustifolia*	Brazil	C Hodges
Ca. terrae-reginae	CBS112151 (= CMW30601)[d]	FJ918506	FJ918522	FJ918545	*E. urophylla*	Australia	C Hanwood
	CBS112634 (= CMW30602)	FJ918507	DQ190668	FJ918546	*Xanthorrhoea australis*	Australia	T Baigent
Ca. zuluensis	CMW9188[d]	FJ972414	FJ972433	FJ972483	*E. grandis* × *E. urophylla* cutting	South Africa	L Lombard
	CMW9896	FJ972415	FJ972434	FJ972484	*E. grandis* × *E. urophylla* cutting	South Africa	L Lombard

[a]CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CMW: culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; isolate number in bold were sequenced in this study.

[b]GenBank accession numbers.

[c]Isolates used for pathogenicity tests on *Eucalyptus* seedlings in China.

[d]Ex-type cultures.

[e]n/a, not available.

Table 2. Single nucleotide polymorphism comparisons between *Ca. colhounii*, *Ca. eucalypti*, *Ca. pseudocolhounii* and *Ca. fujianensis*.

Species	Isolate no.	β-tubulin							Histone H3								
		53	378	397	398	407	420	516	57	248	290	311	362	371	386	454	455
Ca. colhounii	CBS293.79	C	C	C	T	A	C	C	A	T	A	T	C	T	C	A	C
	CBS114704	C	C	G	T	A	T	T	A	T	A	T	C	T	C	A	C
Ca. eucalypti	CBS125237	C	C	G	**C**	**G**	T	T	**–**	T	**T**	T	**T**	T	C	**C**	**A**
	CBS125275	C	C	G	**C**	**G**	T	T	**–**	T	**T**	T	**T**	T	C	**C**	**A**
Ca. pseudocolhounii	CMW27209	C	**T**	G	T	A	C	C	A	**C**	A	T	C	T	C	A	C
	CMW27213	C	**T**	G	T	A	C	C	A	**C**	A	T	C	T	C	A	C
	CMW27214	C	**T**	G	T	A	C	C	A	**C**	A	T	C	T	C	A	C
Ca. fujianensis	CMW27254	**T**	C	**C**	T	A	C	C	A	T	A	**C**	C	**C**	**A**	A	C
	CMW27257	**T**	C	**C**	T	A	C	C	A	T	A	**C**	C	**C**	**A**	A	C
	CMW27263	**T**	C	**C**	T	A	C	C	A	T	A	**C**	C	**C**	**A**	A	C

Species	Isolate no.	TEF-1α																		
		1	31	92	93	94	95	96	123	127	181	182	183	184	261	454	458	469	474	500
Ca. colhounii	CBS293.79	**G**	**C**	**C**	**A**	**C**	**A**	**A**	**-**	A	–	–	–	–	**G**	**C**	**C**	**C**	**C**	C
	CBS114704	**G**	**C**	**C**	**A**	**C**	**A**	**A**	**-**	A	–	–	–	–	**G**	**C**	**C**	**C**	**C**	C
Ca. eucalypti	CBS125237	C	A	–	–	–	–	–	T	A	–	–	–	–	A	T	T	T	T	C
	CBS125275	C	A	–	–	–	–	–	T	A	–	–	–	–	A	T	T	T	T	C
Ca. pseudocolhounii	CMW27209	C	A	–	–	–	–	–	T	A	–	–	–	–	A	T	T	T	T	–
	CMW27213	C	A	–	–	–	–	–	T	A	–	–	–	–	A	T	T	T	T	–
	CMW27214	C	A	–	–	–	–	–	T	A	–	–	–	–	A	T	T	T	T	–
Ca. fujianensis	CMW27254	C	A	–	–	–	–	–	T	**G**	**A**	**A**	**A**	**A**	A	T	T	T	T	–
	CMW27257	C	A	–	–	–	–	–	T	**G**	**A**	**A**	**A**	**A**	A	T	T	T	T	–
	CMW27263	C	A	–	–	–	–	–	T	**G**	**A**	**A**	**A**	**A**	A	T	T	T	T	–

[a] Highlight and bold = unique polymorphisms; highlight = shared polymorphisms.

Fig 1. One of 54 most parsimonious trees obtained from a heuristic search with 1 000 random addition sequences of the combined sequences of β-tubulin, histone H3 and translation elongation factor 1-lpha sequence alignments of the *Ca. morganii* Crous, Alfenas & M.J. Wingf. complex and other closely related species. Scale bar shows 10 changes and bootstrap support values from 1 000 replicates are shown above the nodes in bold. Bayesian posterior probability values are indicated below the nodes. Red lines indicate bootstrap support values of 100 and posterior probability values of 1.00. Thickened lines indicate branches in the strict consensus tree and the consensus tree of the Bayesian analyses. The tree was rooted to *Ca. colombiensis* (CBS112221) and *Ca. chinensis* (CBS112744).

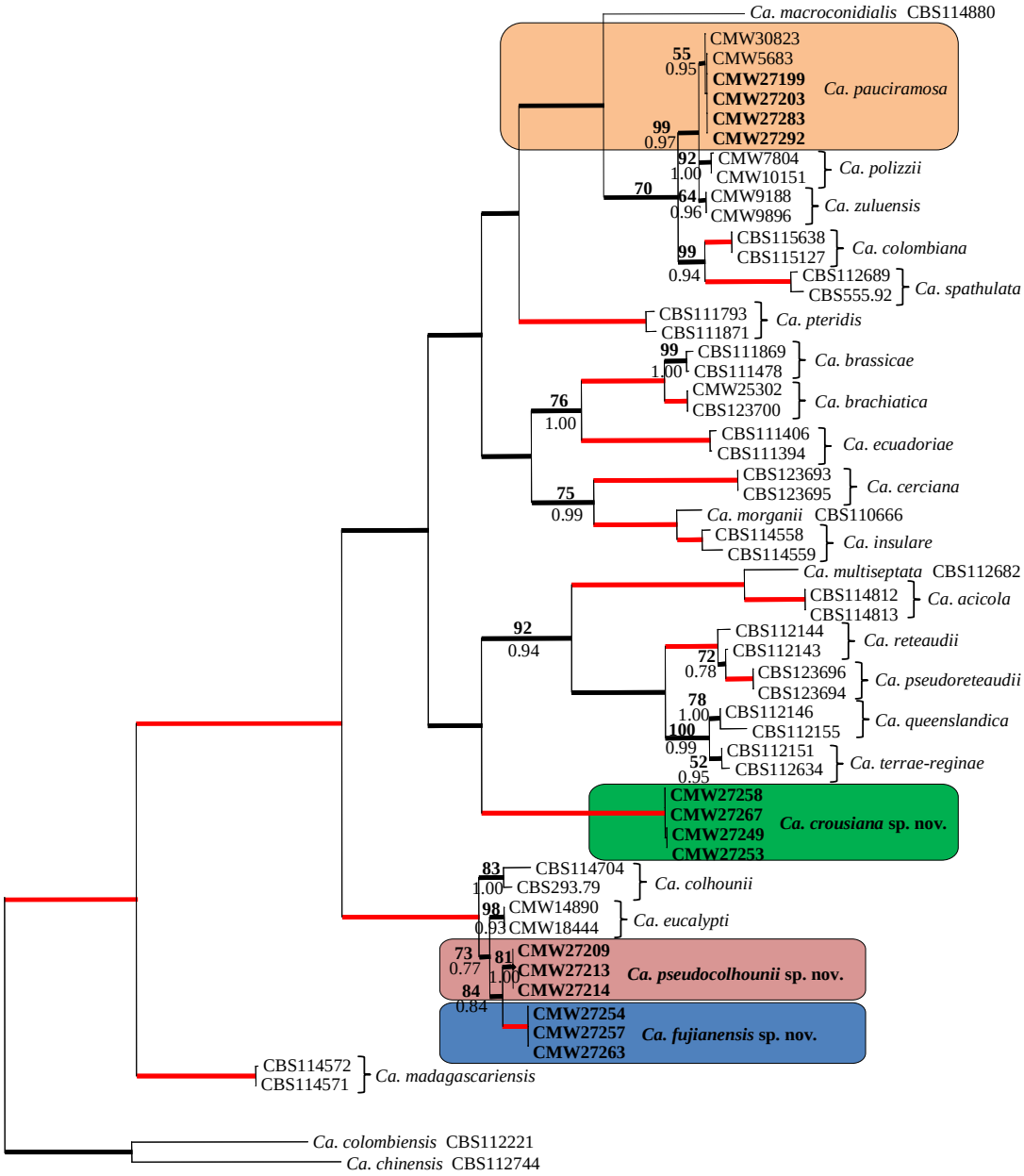


Fig 2. *Calonectria crousiana*. a–f. Teleomorph state of *Ca. crousiana*. g–k. Anamorph state of *Ca. crousiana*. a. Perithecium; b. Vertical section through a perithecium; c. Cells around ostiolar region of perithecium; d. Section through lateral perithecial wall; e. Asci; f. Ascospores; g. Macroconidiophore; h, i. Clavate vesicles; j. Fertile branches; k. Macroconidia. Scale bars: a = 200 µm; b = 100 µm; c–e, g, j = 20 µm; f, k = 10 µm; h, i = 5 µm.

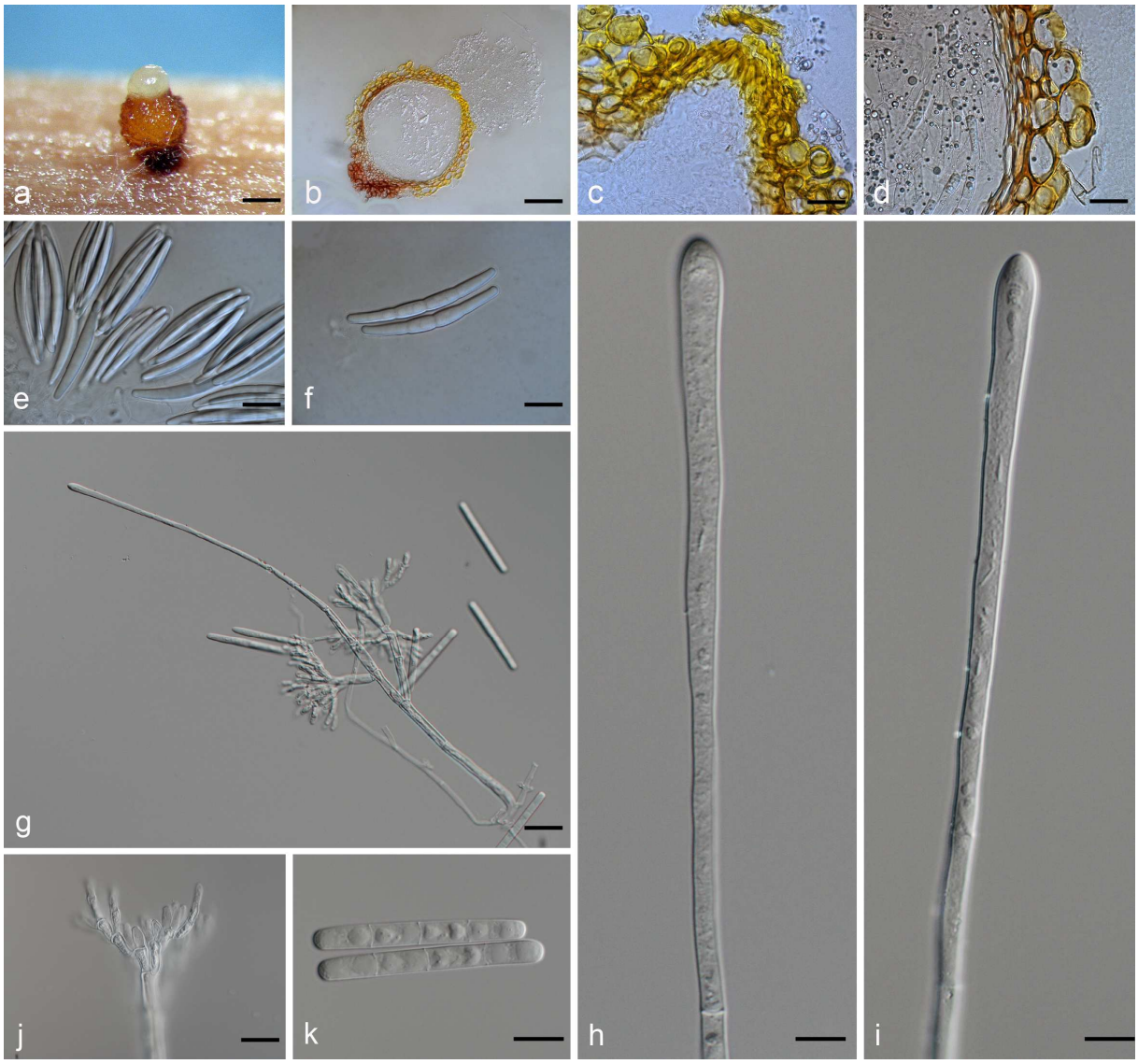


Fig 3. *Calonectria pseudocolhunii*. a–h. Teleomorph state of *Ca. pseudocolhounii*. i–m. Anamorph state of *Ca. pseudocolhounii*. a. Perithecium; b. Vertical section through a perithecium; c. Cells around ostiolar region of perithecium; d. Section through lateral perithecial wall; e, f. Asci; g, h. Ascospores; i. Macroconidiophore; j, k. Clavate vesicles; l. Fertile branches; m. Macroconidia. Scale bars: a = 200 μm; b = 100 μm; c = 40 μm; d–f, i–k = 20 μm; g, h, l, m = 10 μm.

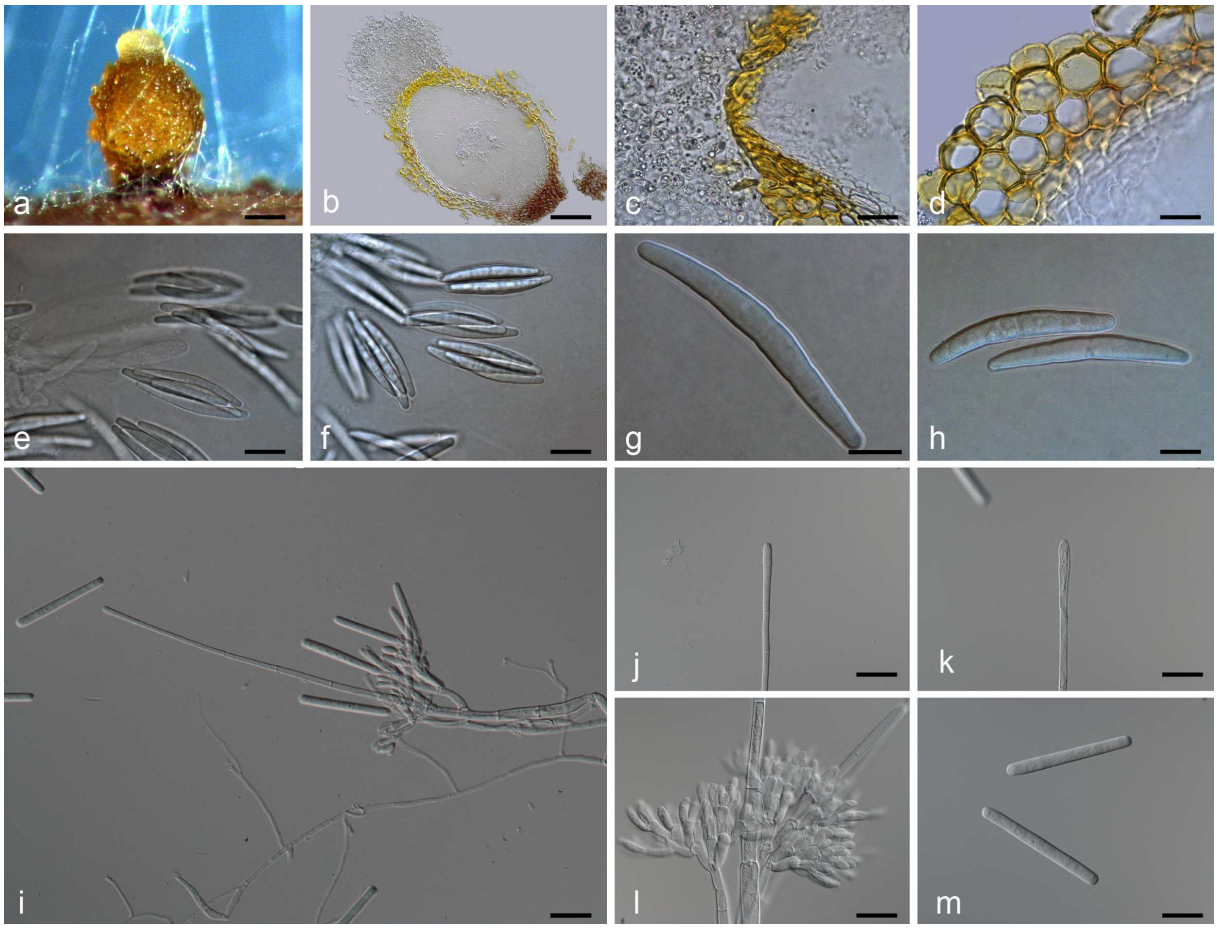


Fig 4. *Calonectria fujianensis*. a–f. Teleomorph state of *Ca. fujianensis*. g–k. Anamorph state of *Ca. fujianensis*. a. Perithecium; b. Vertical section through a perithecium; c. Cells around ostiolar region of perithecium; d. Section through lateral perithecial wall; e. Asci; f. Ascospores; g. Macroconidiophore; h, i. Clavate vesicles; j. Fertile branches; k. Macroconidia. Scale bars: a = 200 µm; b = 100 µm; c = 40 µm; d–g = 20 µm; j, k = 10 µm; h, i = 5 µm.

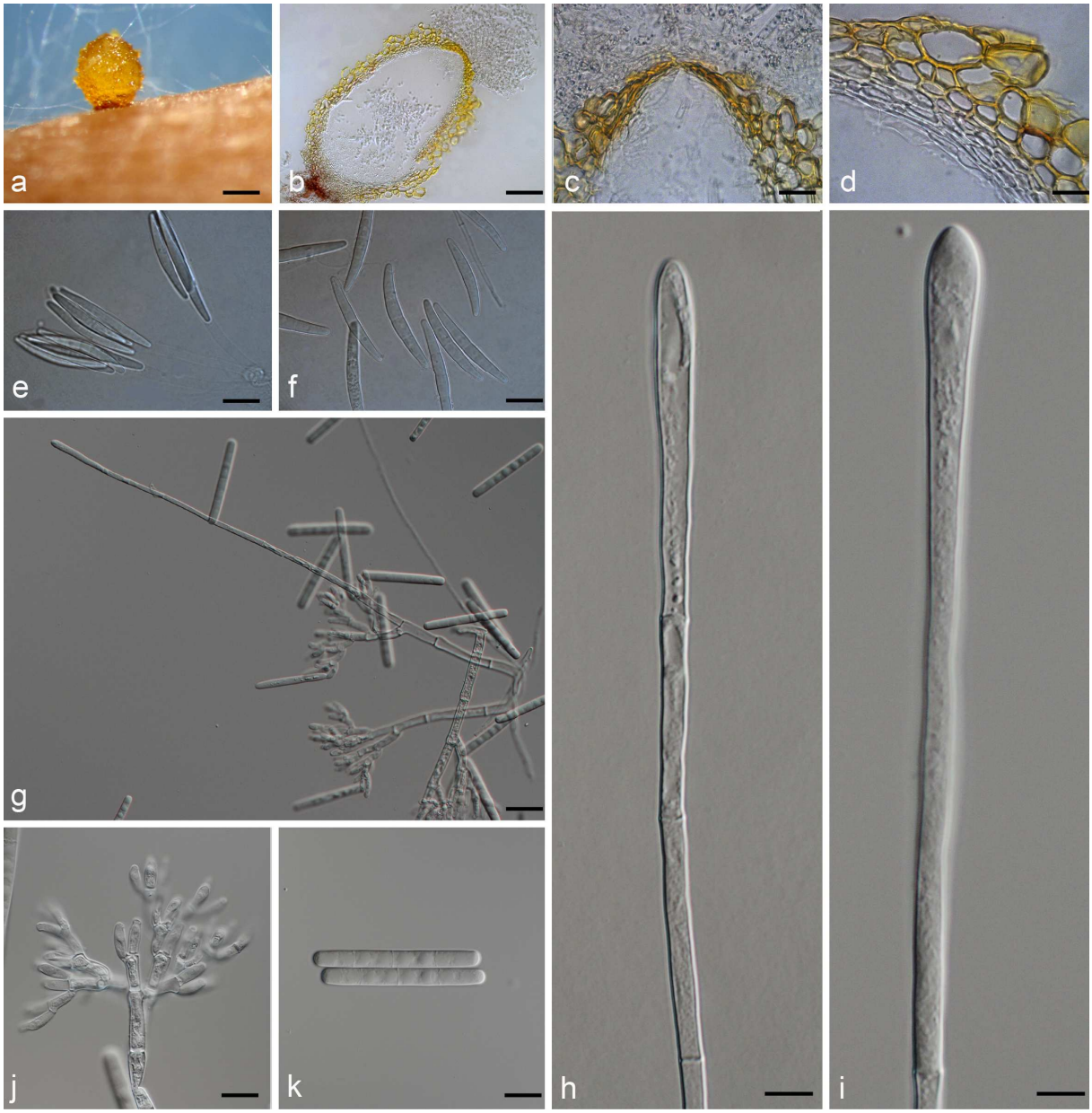
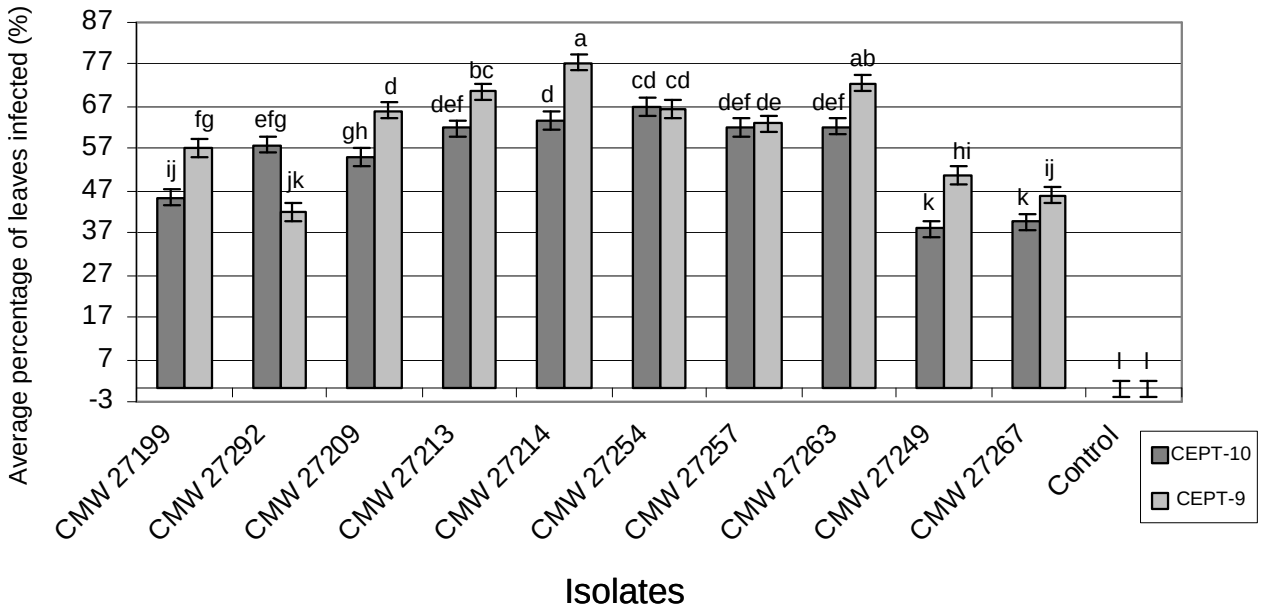


Fig 5. Histogramme indicating the average percent leaves infected (%) resulting from inoculation trials of two *E. urophylla* × *E. grandis* clones inoculated with isolates of *Ca. pauciramosa* (CMW27199, CMW27192), *Ca. pseudocolhounii* (CMW27209, CMW27213, CMW27214), *Ca. fujianensis* (CMW27254, CMW27257, CMW27263), *Ca. crousiana* (CMW27249, CMW27267) and the controls. Bars represent 95% confidence limits for each treatment. Different letters above the bars indicate treatments that were statistically significantly different (P = 0.05).



Chapter 6

High population diversity and increasing importance of the *Eucalyptus* stem canker pathogen, *Teratosphaeria zuluensis*, in South China

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This chapter has been submitted for publication as: Chen SF, Barnes I, Chungu D, Roux J, Wingfield MJ, Xie YJ, Zhou XD. High population diversity and increasing importance of the *Eucalyptus* stem canker pathogen, *Teratosphaeria zuluensis*, in South China. (Submitted to *Australasian Plant Pathology*, on Dec. 7, 2010)

ABSTRACT

Coniothyrium stem canker caused by *Teratosphaeria zuluensis*, is one of the most important diseases of plantation-grown *Eucalyptus* trees in tropical and sub-tropical areas of the world. Previous research on the population structure of *T. zuluensis* in China, Malawi and South Africa has suggested that *T. zuluensis* in these countries had independent origins with the highest genetic diversity found in a population from South China. In this study, the genetic diversity of three *T. zuluensis* populations from different regions in South China was determined using ten microsatellite markers. Results showed that more than one genotype of *T. zuluensis* can occur on a single tree in all three populations and that a moderate to high genetic diversity exists within the populations. Population differentiation was evident between populations, and in one population there was evidence for a low level of genetic recombination. Comparisons among the three populations of *T. zuluensis* from South China suggest that they originated independently of each other.

Keywords: Coniothyrium canker, genetic diversity, microsatellites, plantation health

1. INTRODUCTION

Coniothyrium canker is a serious stem and branch disease of *Eucalyptus* trees (Wingfield *et al*. 1996). Two different species, *Teratosphaeria zuluensis* (M.J. Wingf., Crous & T.A. Cout.) M.J. Wingf. & Crous [previously *Coniothyrium zuluense* M.J. Wingf., Crous & T.A. Cout.; *Colletogloeopsis zuluensis* (M.J. Wingf., Crous & T.A. Cout.) M.-N. Cortinas, M.J. Wingf. & Crous; *Readeriella zuluensis* (M.J. Wingf., Crous & T.A. Cout.) Crous & U. Braun; *Kirramyces zuluensis* (M.J. Wingf., Crous & T.A. Cout.) Andjic & M.J. Wingf.] and *T. gauchensis* (M.-N. Cortinas, Crous & M.J. Wingf.) M.J. Wingf. & Crous [previously *Colletogloeopsis gauchensis* M.-N. Cortinas, Crous & M.J. Wingf.; *Readeriella gauchensis* (M.-N. Cortinas, Crous & M.J. Wingf.) Crous & U. Braun; *Kirramyces gauchensis* (M.-N. Cortinas, Crous & M.J. Wingf.) Andjic, M.-N. Cortinas & M.J. Wingf.] are known to cause the disease (Cortinas *et al*. 2006c; Andjic *et al*. 2007; Crous *et al*. 2007, 2009). The disease is typically characterized by small necrotic lesions on the branches and stems, which coalesce to from large cankers resulting in deformed branches and stems (Wingfield *et al*. 1996). These large cankers reduce wood quality and can lead to growth loss, stunting of growth and tree death (Wingfield *et al*. 1996; Roux *et al*. 2002; Van Zyl *et al*. 2002a; Old *et al*. 2003; Cortinas *et al*. 2006b).

Coniothyrium canker caused by *T. zuluensis* was first discovered in the mid 1980's in South Africa and the causal agent was determined and described based on this discovery (Wingfield *et al*. 1996). Since then, the disease has been reported from China (Cortinas *et al*. 2006b), Malawi (Roux *et al*. 2005; Cortinas *et al*. 2006c), Mexico (Roux *et al*. 2002), Thailand (Van Zyl *et al*. 2002b), Vietnam (Gezahgne *et al*. 2003; Old *et al*. 2003) and Zambia (Chungu *et al*. 2010b).

Teratosphaeria zuluensis was first described as a species of *Coniothyrium* (Wingfield *et al*. 1996). DNA sequence comparison of isolates from Ethiopia, Mexico, South Africa, Thailand and Uganda, however, showed that the fungus is most closely related to species in what is now known as the Teratosphaeriaceae (Gezaghne *et al*. 2005; Cortinas *et al*. 2006*c*; Crous *et al*. 2009). Cortinas *et al*. (2006c) also discovered the existence of a second species, *T. gauchensis* causing Coniothyrium canker in some countries, it has been found only in Argentina (Gezahgne *et al.* 2003), Ethiopia (Gezahgne *et al.* 2005), Hawaii (Cortinas *et al*. 2004), Uganda (Gezahgne *et al.* 2005) and Uruguay (Cortinas *et al.* 2006c).

The origin of *T. zuluensis* is unknown. Because it had not been found elsewhere in the world and based on the fact that it appeared in countries that had imported *Eucalyptus* germplasm from South Africa, it was at one time suggested that this might be the origin of the pathogen (Wingfield *et al*. 1996). A recent study, however comparing populations of *T. zuluensis* from China, Malawi and South Africa, surprisingly, found the highest genetic diversity for isolates collected in China and Malawi (Cortinas *et al*. 2010), where plantation establishment using *Eucalyptus* spp. is a more recent practice than in South Africa (Nkaonja 1982; Geldenhuys 1997; Qi 2002). The study also found high numbers of private alleles in all three populations and significant population differentiation between them. Cortinas *et al*. (2010) thus concluded that *T. zuluensis* in China, Malawi and South Africa originated independently, through multiple introductions from a source that is yet to be discovered.

Coniothyrium canker caused by *T. zuluensis* on *Eucalyptus* in China appears to be spreading with the disease appearing in increasing numbers of plantations in South China. In China, the pathogen was first reported on *E. urophylla* S.T.Blake trees in GuangDong Province in 2006 (Cortinas *et al*. 2006b). At that stage, infection levels were low. Two years later, the disease was reported from additional regions in South China (Zhou *et al*. 2008).

The aim of this study was to extend surveys for Coniothyrium stem canker in South China and to obtain an improved understanding of its distribution and impact in the region. An additional aim was to collect populations of *T. zuluensis* in greater numbers than has previously been possible to further investigate the genetic diversity and population structure of this pathogen in China.

2. MATERIALS AND METHODS

2.1. Sampling and isolation

During the course of a survey of diseases of *Eucalyptus* trees in South China between August and November 2008, stems showing symptoms of Coniothyrium canker were observed. Trees included *E. grandis* W.Hill and its hybrid clones with *E. urophylla* (Table 1). A collection of samples from these infected trees was made from one region in the GuangXi Province (Region One) and two regions in the GuangDong Provinces (Region Two and Three) (Table 1,

Fig 1). In Region One, collections were made from two plantations that are ~90 Km apart. In Region Two, samples were collected from one plantation and two parks, which are approximately 30 Km apart from each other. In Region Three, collections were made from two plantations that are ~60 Km apart from each other. Regions One and Two are ~270 Km apart, Region Two and Three ~350 Km apart and Region Three, ~500 Km from Region One (Fig 1).

Samples for population diversity studies were collected from trees showing typical symptoms of Coniothyrium stem canker. Representative samples were collected from the main stems of trees (two to three-years-old) at approximately 2 m above ground level. The samples were collected from *Eucalyptus* plantations using the same method described by Cortinas *et al*. (2010) where five to ten diseased trees at the centre of a plantation were selected as the central group for the collection. Samples were then collected from trees randomly selected in the plantations around the central group of trees, extending outwards. For the two parks in Region Two, samples were collected from randomly chosen and infected *Eucalyptus* trees (Table 1).

Sampling consisted of collecting two to three bark pieces bearing single lesions from the stems of each tree. Bark pieces were incubated in moist chambers to induce the production of spores from the fruiting bodies. Spore drops taken from each bark sample were transferred to 2% malt extract agar (MEA) (20 g malt extract, 20 g agar, 1 L water: Biolab, Midrand, South Africa). Single hyphal tip isolations were made later following the method described by Chungu (2010a). An isolate of *T. zuluensis* (identity confirmed in this study) from *E. grandis* that was collected in GuangXi Province in 2006 was also included in this study (Table 1). Isolates were grown on 2% malt extract agar (MEA) at 25°C for 30 days prior to DNA extraction.

2.2. DNA extraction and isolate identification

Actively growing mycelium was scraped from the agar surface using a sterile scalpel and transferred to a 1.5 µL Eppendorf tubes. DNA was extracted from the mycelium using the phenol/chloroform method described by Myburg *et al*. (1999). Samples were treated with 3 µL RNase (1mg/mL) and left overnight at room temperature to degrade the RNA. DNA was separated by electrophoresis on a 1% agarose gel, stained with ethidium bromide and visualised under ultraviolet (UV) light.

To confirm the identity of the isolates collected, DNA sequence data were derived for all isolates. The internal transcribed spacer (ITS) regions, ITS1 and ITS2, and the 5.8S gene of the ribosomal DNA (rDNA) operon, were amplified using the primers ITS1 (5' TCCGTAGGTGAACCTGCGG) and ITS4 (5' TCCTCCGCTTATTGATATGC) (White *et al*. 1990). PCR and sequencing reactions were performed as described in Cortinas *et al*. (2006b, c). Sequence data for the isolates collected in this study were compared with those in GenBank (http://blast.ncbi.nlm.nih.gov/) using a Blast search, after which they were compared more closely with sequences of the authentic strains of *T. zuluensis* and *T. gauchensis* (Cortinas *et al*. 2006c).

2.3. Simple sequence repeat (SSR) - PCR and gene scan analyses

For all *T. zuluensis* isolates, polymorphic loci were amplified using ten pairs of fluorescently labelled species-specific primers (Cortinas *et al*. 2006a, 2010) (Table 2). PCR reactions and conditions were performed as described in Cortinas *et al*. (2006a, 2010). Amplified DNA was separated by electrophoresis on a 2% agarose gel, stained with ethidium bromide and visualised under ultraviolet (UV) light.

PCR products were size-separated on an ABI PRISM^{TM} 3100 Automated DNA Sequencer (Applied Biosystems, Foster City, USA) together with the internal size standard GENSCAN LIZ 500 (-250) (Applied Biosystems, Warringon, UK). Alleles were determined using the software GENEMAPPER version 3.0 (Applied Biosystems, Foster City, USA), and based on the size of each amplicon. Alleles for each locus were assigned an alphabetical character and together provided a multilocus genotype for each isolate. Isolates with the same multilocus genotype at each region were considered clones.

The number of alleles, private alleles at each locus as well as the allelic frequency of each population was calculated using the program POPGENE version 1.31 (Yeh *et al*. 1999). The allelic richness of each population was calculated using FSTAT version 2.9.3.2 (FSTAT for windows, version 2.9.3.2 (http://www2.unil.ch/popgen/softwares/fstat.htm) (Goudet 2001).

2.4. Genotypic diversity versus the number of loci

In order to ascertain whether the sample size for each of the three regions, and the number of loci used in this study was sufficiently large to support statistical significance of genotypic diversity for *T. zuluensis*, a plot for mean genotypic diversity against the number of loci was obtained. This was done using MULTILOCUS version 1.3 (Agapow & Burt 2001) and allowed us to statistically define the populations for further analyses.

2.5. Gene and genotypic diversity

The gene diversity for each clone-corrected population was determined using the program POPGENE version 1.31 (Yeh *et al*. 1999) based on the equation $H = 1–\Sigma x_k^2$, where x_k is the frequency of the k^{th} allele (Nei 1973),. Genotypic diversity (G) was estimated by using the equation $G = 1/\Sigma p_i^2$, where p_i is the observed frequency of the i^{th} genotype in each of the non clone-corrected populations (Stoddart & Taylor 1988). The maximum percentage of genotypic diversity ($\hat{G}$), obtained from the equation $\hat{G} = G/N \times 100\%$ (where N is the population size), was used to compare the genotypic diversities between populations (Chen *et al*. 1994). The significance of differences in genotypic diversity between populations was calculated using a *t*-test (Chen *et al*. 1994). For each of the three populations, the different observations regarding the number of genotypes obtained from the variable number of isolates per tree were documented.

2.6. Population differentiation and assignment

In order to detect the differences in allele frequencies at individual loci between clone-corrected populations of *T. zuluensis*, the program POPGENE version 1.31 (Yeh *et al*. 1999) was used. The significance of differences in allele frequencies was determined with Chi square tests (Workman & Niswander 1970).

Population differentiation was calculated using theta (θ) (Weir 1996), which is a modification of F_{ST} (Wright 1978). Theta (θ) values were measured using MULTILOCUS version 1.3 (Agapow & Burt 2001). The observed value was compared to that of 1 000 randomizations in which individuals were randomized across populations. The significance of θ was evaluated by the P value.

To test for structure in the isolates, the model-based Bayesian clustering method in STRUCTURE version 2.2 (Pritchard *et al*. 2000; Falush *et al*. 2003) was used. This method uses the allelic frequencies at each locus of each isolate and excludes any prior information about location. To test for the optimal number of groups 'K', the model of admixture ancestry and independent allele frequency was used. Twenty iterations with 'K' set from 1 to 10 were simulated with 300 000 runs each with a burn-in set at 30 000 runs. The likelihood values were plotted against the ln likelihood and delta likelihood values to determine the 'K' with higher likelihood and lower standard deviation (Evanno *et al*. 2005). For the assignment of individuals into the optimal 'K' obtained above, a second analysis was conducted by setting the runs at 1 000 000 with a burn-in of 100 000.

2.7. Mode of reproduction

In order to consider the reproduction mode for each of the clone-corrected populations, the Index of Association (I_A) using the program MULTILOCUS version 1.3, was calculated (Maynard Smith *et al*. 1993; Agapow & Burt 2001). The I_A tests the likelihood that two different individuals sharing the same allele at one locus will share the same allele at another locus. By comparing the observed data with the expected data of 1 000 randomly recombining datasets, recombination in each of the three populations could be determined (Taylor *et al*. 1999). If the observed data remain within the distribution range of the recombined data, the population is likely to be undergoing recombination. Otherwise, the population is most likely clonal and not undergoing recombination.

3. RESULTS

3.1. Sampling and isolate identification

Bark samples showing symptoms typical of Coniothyrium canker were collected from a total of 89 trees in the three regions sampled (Table 1, Fig 1). One to four isolates were obtained from each tree with a total of 238 isolates resembling species of *Teratosphaeria*. Blast results of the ITS gene sequences of the isolates from China showed that 97 of them represented *T. zuluensis* (Table 1). Most of the remaining isolates represented different species in the Mycosphaerellaceae and Teratosphaeriaceae. Of the isolates confirmed are representing *T.*

zuluensis, twenty-one originated from 12 trees in Region One, 38 isolates from 21 trees in Region Two and 38 isolates from 24 trees in Region Three (Table 1, Fig 1).

3.2. Polymorphic microsatellite loci

For the 97 isolates of *T. zuluensis*, a total of 50 different alleles were amplified by the ten species-specific polymorphic microsatellite markers (Table 2). Forty-two different alleles were observed in the population from Region One, thirty-nine from Region Two and 31 from Region Three. There were two to eight alleles per locus. Private alleles were observed in all three populations. In total, eleven private alleles were identified, four of which were detected in Regions One and Two, and three in Region Three. The allelic richness was highest in the population from Region One (4.17) followed by those from Regions Two (3.70) and Three (3.10). Locus Czulu3 was monomorphic in the population from Region Two and locus Czulu1 was monomorphic for the population from Region Three (Table 2). No monomorphic loci were detected in the population from Region One (Table 2).

3.3. Genotypic diversity against the number of loci

The plot for mean genotypic diversity against the number of loci for isolates from the three regions showed that a plateau of genotypic diversity had been reached using the set of ten microsatellite markers. This suggested that for each of the three regions, the genotypic diversity calculated for the isolates was sufficient to characterize the populations. When isolates from individual plantations or parks were considered, statistical support was not obtained in all cases. Populations for further analyses were, therefore, based on isolates from regions and not from individual plantations or parks.

3.4. Gene and genotypic diversity

The gene diversity levels in the three *T. zuluensis* populations were moderate to high. Values were $H = 0.57$ for the Population One, 0.55 for Population Two and 0.46 for Population Three (Table 2). The levels of clonality for each population were 4.8%, 5.3% and 55.3% respectively (Table 2). Seventy-two genotypes were identified across all the Chinese *T. zuluensis* isolates, with twenty different genotypes in the Population One, thirty-six in Population Two and 17 in Population Three. Only one genotype was shared between any of

the populations and this was between Population One and Two (Table 2). The maximum genotypic diversity was $\hat{G}$ = 91% for Population One, 90% for Population Two and 28% for Population Three (Table 2). There was no significant difference (P = 0.033) for the genotypic diversity between Populations One and Two, while the genotypic diversities between Populations One and Three, as well as Populations Two and Three were significant (P = 1.61, P = 1.67, respectively).

In all three populations, there were situations where more than one genotype of *T. zuluensis* was present on a single *Eucalyptus* tree. The greatest number of genotypes obtained from a single tree was in Population Two with four genotypes obtained from four isolates on a single tree. In Population Three, one genotype was identified among six isolates from four trees.

3.5. Population differentiation and assignment

Significant differences in allelic frequencies were found between loci for the populations in the pairwise comparisons. The majority of these differences were detected between Populations One and Three, and between Populations Two and Three (Table 3).

Results showed that all three populations were significantly different from each other (P < 0.001) based on calculations of theta (θ). The largest differentiation was found between Populations One and Three (θ = 0.207) (Table 4).

Structure analyses showed that the number of groups (K) obtained with the highest likelihood (ln and delta K), and lowest standard deviation was equal to four. Assignment of individuals into these groups resulted in the majority of isolates from Region One (Population One) being assigned to Group One (38%) and Two (36%). Most of the isolates of Region Two (Population Two) were assigned to Group One (44%) and Three (37%) and more than half (61%) of the isolates of Region Three (Population Three) were assigned to Group Four (Fig 1).

3.6. Mode of reproduction

The I_A values of the observed data for Populations One and Three fell outside the distribution range for a recombining population (P < 0.001) (Table 5), indicating that no recombination is

evident in these populations. There was marginal evidence for recombination in Population Two (P = 0.011) (Table 5).

4. DISCUSSION

In this study, the genetic diversity and structure of three geographically different populations of *T. zuluensis* from *Eucalyptus* plantations in South China were analyzed using ten microsatellite markers. The results showed that multiple genotypes of the pathogen exist on single infected tree. In all three populations, the genetic diversity of the pathogen was relatively high and private alleles were present. A significant amount of population differentiation was observed between the different populations, suggesting that the three *T. zuluensis* populations in China most likely originated independently of each other.

The genetic diversity of two populations considered in this study and that of a previous Chinese population analysed by Cortinas *et al*. (2010) were the highest for any population of *T. zuluensis* studied thus far. This includes that of a population from South Africa, which was previously hypothesised to represent a possible of origin of this pathogen (Wingfield *et al*. 1996). This, together with evidence for sexual recombination (out-crossing) in Chinese populations from *Eucalyptus* in the current and in a previous study (Cortinas *et al*. 2010), support the view that *T. zuluensis* is not native to South Africa. It is possible that this pathogen is native to Australia but the pathogen has never been found in that country. This might be due to the fact that the intensively managed and highly selected *Eucalyptus* clones planted in China enable the expression of a disease by a pathogen that is unable to produce symptoms on *Eucalyptus* in their native range in Australia. Alternatively, the pathogen is present in Australia but is rare and not seen in the native forest.

The collection of several different genotypes of *T. zuluensis* from the same *Eucalyptus* tree was unexpected. This has not been observed for populations of the pathogen studied from South Africa or Malawi (Cortinas *et al*. 2010). However, a similar observation has been made for the closely related pathogens *T. nubilosa* (Cooke) Crous & U. Braun in South Africa (Hunter *et al*. 2008; Pérez *et al*. 2010), and *Mycosphaerella populorum* G.E. Thomps. from North-eastern America, where more than 90% of the genetic diversity was distributed within a single tree (Feau *et al*. 2005). The high level of genotypic diversity for isolates collected on

the same tree reflects a diverse population in the area, with multiple and independent infections occurring on individual trees.

The genetic diversities obtained for isolates of *T. zuluensis* from the three different geographic regions correlate with the history of *Eucalyptus* plantation development in these regions of South China. *Eucalyptus* trees have a longer history of planting in Region One (GuangXi Province) and Region Two (GuangDong Province) than in Region Three also in GuangDong Province (Qi 2002). Similarly, the genetic diversity of the populations in Regions One and Two was higher than that in Region Three. In the early stage of *Eucalyptus* development in China, *Eucalyptus* trees were generally established from seeds, most of which were imported from Australia (Qi 2002). It has been suggested that species of Teratosphaeriaceae and Mycosphaerellaceae infecting *Eucalyptus* have been moved between countries on seeds (Keane *et al*. 2000; Zhan *et al*. 2003; Burgess *et al*. 2007; Hunter *et al*. 2008; Pérez *et al*. 2009). It is thus possible that *T. zuluensis* was introduced into China on infected *Eucalyptus* seeds. The pathogen would then have spread to different regions of China over time, leading to the genetic diversity for its populations in different regions of China being different, and correlating with the history of *Eucalyptus* plantation development.

In order to manage the disease caused by *T. zuluensis*, the introduction and exchange of different genotypes of this fungus to new regions needs to be prevented. This is especially important to reduce the development of new and possibly more virulent genotypes. Significant evidence for the movement of *T. zuluensis* between the regions sampled in this study was observed. This was reflected in the presence of a shared genotype in Regions One and Two, as well as by results of the allele frequencies and assignment analyses. Isolates from Regions One and Two were more similar to each other than those from Region Three. These results reflect the frequent trade and exchange of *Eucalyptus* breeding material that occur between regions that are in close proximity as suggested by Wingfield *et al.* (2008).

It is clear that *T. zuluensis* is becoming increasingly important in Chinese *Eucalyptus* plantations. Compared to previous studies, the distribution of Coniothyrium canker and associated *T. zuluensis* on *Eucalyptus* spp. and clones in China continue to increase. Although no girdling cankers were observed on trees in this study and tree death was not a concern, *T. zuluensis* results in lesions in the wood and this is especially important in *Eucalyptus* sawn

timber products. Selection and breeding programmes focusing on developing *Eucalyptus* genotypes tolerant to infection by *T. zuluensis* will be important in the future.

5. REFERENCES

Agapow PM, Burt A, 2001. Indices of multilocus linkage disequilibrium. *Molecular Ecology Notes* **1**: 101–102.

Andjic V, Barber PA, Carnegie AJ, Hardy GSJ, Wingfield MJ, Burgess TI, 2007. Phylogenetic reassessment supports accommodation of *Phaeophleospora* and *Colletogloeopsis* from eucalypts in *Kirramyces*. *Mycological Research* **111**: 1184–1198.

Burgess TI, Barber PA, Sufaati S, Xu DP, Hardy GES, Dell B, 2007. *Mycosphaerella* spp. on *Eucalyptus* in Asia: new species; new hosts and new records. *Fungal Diversity* **24**: 135–157.

Chen RS, Boeger JM, McDonald BA, 1994. Genetic stability in a population of a plant pathogenic fungus over time. *Molecular Ecology* **3**: 209–218.

Chungu D, 2010a. Pathogens associated with plantation tree diseases in Zambia. MSc thesis, University of Pretoria. Pretoria, South Africa.

Chungu D, Muimba-Kankolongo A, Wingfield MJ, Roux J, 2010b. Identification of fungal pathogens occurring in eucalypt and pine plantations in Zambia by comparing DNA sequences. *Forestry*. **83**: 507–515.

Crous PW, Braun U, Groenewald JZ, 2007. *Mycosphaerella* is polyphyletic. *Studies in Mycology* **58**: 1–32.

Crous PW, Groenewald JZ, Summerell BA, Wingfield BD, Wingfield MJ, 2009. Co-occurring species of *Teratosphaeria* on *Eucalyptus*. *Persoonia* **22**: 38–48.

Cortinas MN, Barnes I, Wingfield BD, Wingfield MJ, 2006a. Polymorphic microsatellite markers for the *Eucalyptus* fungal pathogen *Colletogloeopsis zuluensis*. *Molecular Ecology Notes* **6**: 780–783.

Cortinas MN, Barnes I, Wingfield MJ, Wingfield BD, 2010. Genetic diversity in the *Eucalyptus* stem pathogen *Kirramyces zuluensis*. *Australasian Plant Pathology* **39**: 383–393.

Cortinas MN, Burgess T, Dell B, Xu D, Crous PW, Wingfield BD, Wingfield MJ, 2006b. First record of *Colletogloeopsis zuluense* comb. nov., causing stem canker of *Eucalyptus* in China. *Mycological Research* **110**: 229–236.

Cortinas MN, Crous PW, Wingfield BD, Wingfield MJ, 2006c. Multilocus gene phylogenies and phenotypic characters distinguish two species within the *Colletogloeopsis*

zuluensis complex associated with *Eucalyptus* stem cankers. *Studies in Mycology* **55**: 135–148.

Cortinas MN, Koch N, Thain J, Wingfield BD, Wingfield MJ, 2004. First record of the *Eucalyptus* stem canker pathogen, *Coniothyrium zuluense* from Hawaii. *Australasian Plant Pathology* **33**: 309–312.

Evanno G, Regnaut S, Goudet J, 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**: 2611–2620.

Falush D, Stephens M, Pritchard JK, 2003. Inference of Population Structure Using Multilocus Genotype Data: Linked Loci and Correlated Allele Frequencies. *Genetics* 164: 1567–1587.

Feau N, Hamelin RC, Vandecasteele C, Stanosz GR, Bernier L, 2005. Genetic structure of *Mycosphaerella populorum* (anamorph *Septoria musiva*) populations in north-central and northeastern north America. *Phytopathology* **95**: 608–616.

Geldenhuys CJ, 1997. Native forest regeneration in pine and eucalypt plantations in Northern Province, South Africa. *Forest Ecology and Management* **99**: 101–115.

Gezahgne A, Cortinas MN, Wingfield MJ, Roux J, 2005. Characterisation of the Coniothyrium stem canker pathogen on *Eucalyptus camaldulensis* in Ethiopia. *Australasian Plant Pathology* **34**: 85–90.

Gezahgne A, Roux J, Thu PQ, Wingfield MJ, 2003. Coniothyrium stem canker of *Eucalyptus*, new to Argentina and Vietnam. *South African Journal of Science* **99**: 587–588.

Goudet J, 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3.2). Available from http://www2.unil.ch/popgen/softwares/fstat.htm.

Hunter GC, van der Merwe NA, Burgess TI, Carnegie AJ, Wingfield BD, Crous PW, Wingfield MJ, 2008. Global movement and population biology of *Mycosphaerella nubilosa* infecting leaves of cold-tolerant *Eucalyptus globulus* and *E. nitens*. *Plant Pathology* **57**: 235–242.

Keane PJ, Kile GA, Podger FD, Brown BN, 2000. *Diseases and Pathogens of Eucalypts*. CSIRO Publishing, Australia.

Maynard Smith J, Smith NH, O'Rourke M, Spratt BG, 1993. How clonal are bacteria? *Proceedings of the National Academy of Sciences of the United States of America* **90**: 4384–4388.

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

Nei M, 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences* **70**: 3321–3323.

Nkaonja RWS, 1982. The Silviculture of Indigenous and Exotic Forest Trees other than Pines and Eucalypts in Malawi. *South African Forestry Journal* **121**: 28–37.

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

Pérez CA, Wingfield MJ, Altier NA, Blanchette RA, 2009. Mycosphaerellaceae and Teratosphaeriaceae associated with *Eucalyptus* leaf diseases and stem cankers in Uruguay. *Forest Pathology* **39**: 349–360.

Pérez G, Slippers B, Wingfield BD, Hunter GC, Wingfield MJ, 2010. Micro- and macrospatial scale analyses illustrates mixed mating strategies and extensive geneflow in populations of an invasive haploid pathogen. *Molecular Ecology* **19**: 1801–1813.

Pritchard JK, Stephens M, Donnelly P, 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–959.

Qi SX, 2002. *Eucalyptus in China*. China Forestry House Press, Beijing, China. (In Chinese).

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

Roux J, Wingfield MJ, Cibrián D, 2002. First report of Coniothyrium canker of *Eucalyptus* in Mexico. *Plant Pathology* **51**: 382.

Stoddart JA, Taylor JF, 1988. Genotypic diversity: estimation and prediction in samples. *Genetics* **118**: 705–711.

Taylor JW, Jacobson DJ, Fisher MC, 1999. The evolution of asexual fungi: reproduction, speciation and classification. *Annual Review of Phytopathology* **37**: 197–246.

Van Zyl LM, Coutinho TA, Wingfield MJ, 2002a. Morphological, cultural and pathogenic characteristics of *Coniothyrium zuluense* isolates from different plantation regions in South Africa. *Mycopathologia* **155**: 149–153.

Van Zyl LM, Coutinho TA, Wingfield MJ, Pongpanich K, Wingfield BD, 2002b. Morphological and molecular relatedness of geographically diverse isolates of *Coniothyrium zuluense* from South Africa and Thailand. *Mycological Research* **106**: 51–59.

Weir BS, 1996. *Genetic Data Analysis II*. Sinauer Associates, Sunderland, MA.

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

Wingfield MJ, Crous PW, Couthinho TA, 1996. A serious new canker disease of *Eucalyptus* in South Africa caused by a new species of *Coniothyrium. Mycopathologia* 136: 139–145.

Wingfield MJ, Slippers B, Hurley BP, Coutinho TA, Wingfield BD, Roux J, 2008. Eucalypt pests and diseases: growing threats to plantation productivity. *Southern Forests* **70**: 139–144.

Workman PL, Niswander JD, 1970. Population studies in South-western Indian tribes. II. Local genetic differentiation in the Papago. *American Journal of Human Genetics* **22**: 24–49.

Wright S, 1978. *Evolution and the Genetics of Populations, vol.4: Variability Within and Among Natural Populations*. University of Chicago Press, Chicago, USA.

Yeh FC, Yang R-C, Boyle T, 1999. POPGENE. Version 1.31. Microsoft windows based freeware for population genetic analysis. University of Alberta, Alberta.

Zhan J, Pettway RE, McDonald BA, 2003. The global genetic structure of the wheat pathogen *Mycosphaerella graminicola* is characterized by high nuclear diversity, low mitochondrial diversity, regular recombination, and gene flow. *Fungal Genetics and Biology* **38**: 286–297.

Zhou XD, Xie YJ, Chen SF, Wingfield MJ, 2008. Diseases of eucalypt plantations in China: challenges and Opportunities. *Fungal Diversity* **32**: 1–7.

Table 1. Origin and hosts of *T. zuluensis* isolates used in this study.

Region	Origin	Host	No. Isolates	No. Trees	No. Genotypes	Collectors[a]	Collection Date	GenBank accession no[b]
Region One	ChongZuo & QinZhou, GuangXi Province	*E. grandis*, *Eucalyptus* clone/species	21	12	20	JR, XDZ, MJW & SFC	Nov. 2006, Aug. 2008	DQ239961, DQ239963 & DQ239965
Region Two	ZhanJiang, GuangDong Province	*E. urophylla* × *E. grandis* clone	38	21	36	SFC & GXZ	Sept. to Oct. 2008	DQ239961, DQ239963 & DQ239965
Region Three	ZhaoQing & YunFu, GuangDong Province	*E. urophylla* × *E. grandis* clone	38	24	17	JR, XDZ & SFC	Aug. 2008	DQ239961, DQ239963 & DQ239965

[a]JR = J. Roux, XDZ = X.D. Zhou, MJW = M.J. Wingfield, SFC = S.F. Chen, GXZ = G.X. Zhao.

[b]ITS sequences produced in this study for each region are identical to those with GenBank numbers presented.

Table 2. Diversity indices of three populations of *T. zuluensis* from China obtained using ten microsatellite loci.

Locus	Allele size	Population One	Population Two	Population Three
Czulu1	150	0.250	0.111	
	152	0.700	0.722	1.000
	156	0.050	0.167	
Czulu2	178	0.500	0.194	0.294
	186	0.500	0.472	0.294
	188		0.333	0.412
Czulu3	163	0.050		0.177
	172	0.950	1.000	0.824
Czulu6	322	0.250		
	325	0.400	0.194	0.118
	331	0.250	0.389	0.882
	334	0.100	0.417	
Czulu7	209	0.150		
	213	0.150	0.222	0.294
	221	0.550	0.583	0.412
	224	0.150	0.194	0.294
Kzulu5	234			0.059
	236		0.028	
	246	0.100	0.139	
	254	0.050		0.294
	256	0.500	0.667	0.471
	258	0.050		
	260	0.200	0.083	0.059
	270	0.100	0.083	0.118
Kzulu10	335	0.050	0.417	0.118
	365	0.250	0.139	
	385	0.050	0.083	0.882
	414	0.650	0.167	
	426		0.194	
Kzulu12	254			0.118
	260	0.050	0.028	
	268			0.471
	288	0.050	0.028	
	296	0.300		
	298	0.500	0.639	0.294
	304	0.100	0.306	0.118
Kzulu13	121	0.050		0.588
	123	0.350	0.139	
	127	0.350	0.389	0.118
	131		0.028	
	138	0.150	0.333	0.177
	152	0.050	0.083	0.118
	154	0.050	0.028	
Kzulu14	254	0.050		0.529
	256	0.350	0.139	0.059
	260	0.350	0.361	0.118
	264		0.028	
	270	0.150	0.333	0.177
	282	0.050	0.111	0.118

	284	0.050	0.028	
Ni[a]		21	38	38
Ng[b]		20	36	17
Na[c]		42	39	31
Npa[d]		4	4	3
AR[e]		4.17	3.70	3.10
H[f]		0.57	0.55	0.46
Npl[g]		10	9	9
G[h]		19.17	34.38	10.78
$\hat{G}$[i]		91%	90%	28%

[a]Ni = Number of isolates (non clone-corrected).

[b]Ng = Number of genotypes.

[c]Na = Number of alleles.

[d]Npa = Number of private alleles.

[e]AR = Allelic Richness.

[f]*H* = Gene Diversity (Nei 1973).

[g]Npl = Number of polymorphic loci.

[h]*G* = Genotypic Diversity (Stoddart & Taylor 1988).

[i]$\hat{G}$ = G/N% = Percentage maximum diversity of genotype.

Table 3. Pairwise Chi-square comparisons of allelic frequencies among three Chinese *T. zuluensis* populations.

Pairs of populations		Locus										Number of significantly different loci[a]
		Czulu1	Czulu2	Czulu3	Czulu6	Czulu7	Kzulu5	Kzulu10	Kzulu12	Kzulu13	Kzulu14	
Population One and Two	Chi^2	2.95	4.82	1.83	16.01*	5.91	6.34	19.88*	13.92*	7.07	7.36	3 out of 10
	df	2	2	1	3	3	6	4	4	6	6	
Population One and Three	Chi^2	6.09*	7.00*	1.52	15.46*	4.68	8.50	30.54*	19.55*	18.35*	14.87*	7 out of 10
	df	2	2	1	3	3	6	3	6	5	5	
Population Two and Three	Chi^2	5.82	1.59	6.73*	12.62*	1.39	16.51*	33.43*	26.38*	28.44*	24.08*	7 out of 10
	df	2	2	1	2	2	6	4	5	6	6	

[a]In the pairwise comparison, the total number of loci where the frequency differed significantly from each other (*$P < 0.05$), is shown in the last column.

Table 4. Pairwise comparisons of population differentiation (θ, above the diagonal) among three Chinese *T. zuluensis* populations.

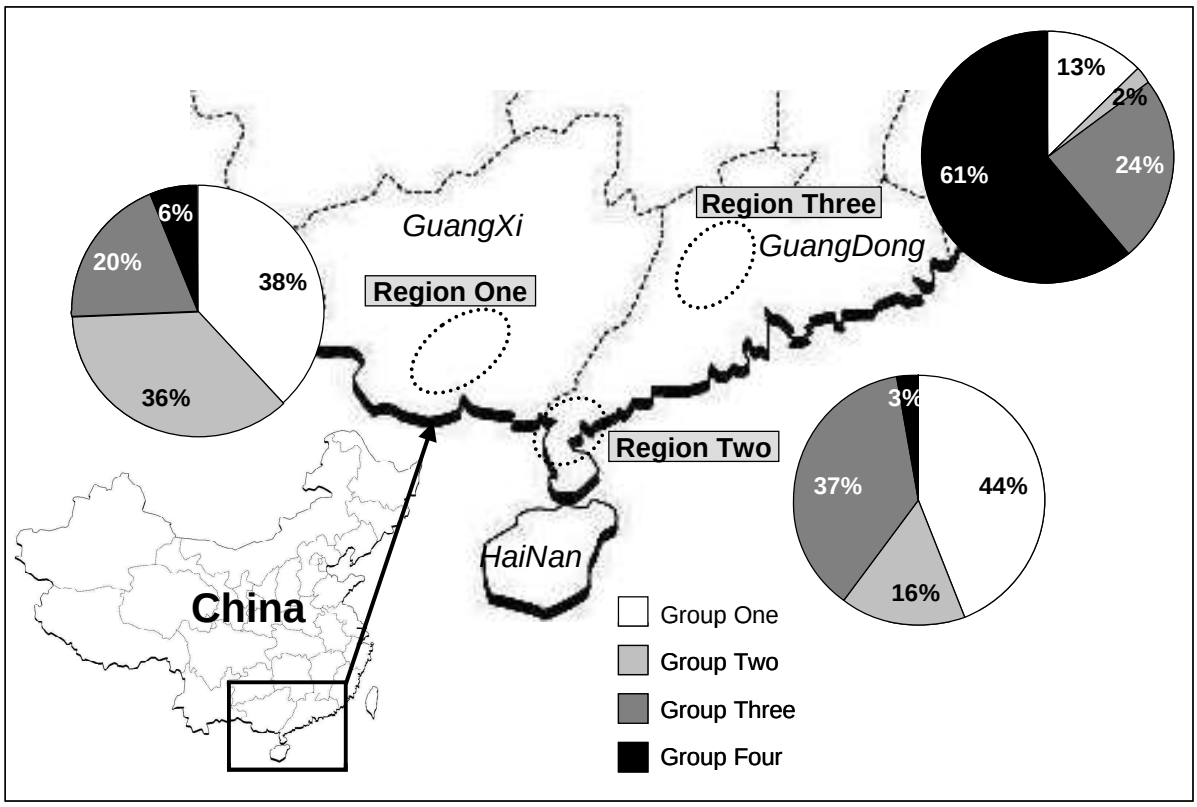
Teratosphaeria zuluensis	Population One	Population Two	Population Three
Population One	—	0.058***	0.207***
Population Two		—	0.183***
Population Three			—

For θ, asterisks represent the level of significance (***$P < 0.001$).

Table 5. Observed Index of Association (I_A) and range of I_A values obtained for the three Chinese *T. zuluensis* populations, after 1 000 randomizations.

Populations	Observed I_A	Range of obtained I_A values	Obs. I_A within randomized the data range. (i.e. evidence for recombination)	*P* value
Population One	0.867	–0.329 – 0.431	No	$P < 0.001$
Population Two	0.198	–0.224 – 0.260	Yes	$P = 0.011$
Population Three	0.831	–0.364 – 0.550	No	$P < 0.001$

Fig 1. Map showing the position of the sampling regions for this study in South China. Samples from Region One were collected in the province of GuangXi and those from Region Two and Three were collected in the province of GuangDong. The assignment of individuals (from the three regions) based on their allele frequencies into one of four groups, as determined with STRUCTURE, is represented as percentages in the pie charts.



Summary

The eucalypt industry in China is expanding substantially, while information on the species identity, origin and impact of fungal pathogens in the country remains very limited. Studies presented in this dissertation investigated some of the most common disease problems experienced in eucalypt plantations in South China during 2006–2008. The causal agents of five common eucalypt diseases were characterized using morphology and DNA sequence data. Furtheremore, the population diversity and structure of *Teratosphaeria zuluensis* was investigated using microsattelite markers. Species in two genera of Cryphonectriaceae, *Chrysoporthe* and *Celoporthe*, species in the Botryosphaeriaceae, *Calonectria* spp. and *T. zuluensis* were identified as pathogens to eucalypts in South China. Although the origin of most of these pathogens remains unclear, it is highly likely that some of them originated from native trees in Asia and have adapted to non-native plantation grown eucalypts. They are now causing diseases on these trees as a result of a lack of natural resistance through co-evolution. For example *T. zuluensis* was found to have a very high genetic diversity in China, and unlike other population studies, suggesting the presence of sexual recombination in the region. Additionally, pathogenicity trials were conducted to screen various commercially grown eucalypt genotypes for their susceptibility to the identified pathogens. These inoculation studies highlighted the importance of knowledge regarding the pathogen genotypes present in a region, and especially the importance of isolate selection when considering artificial screening for disease tolerance. Research presented in this dissertation represents the most detailed investigation of eucalypt fungal diseases in China to date. Clearly, the number of disease and pest problems on plantation eucalypts in China is, and will continue to grow. This is especially true because of the increased movement of forestry material in the form of seed, timber and other products between regions, countries and continents. A combination of management strategies as well as close interaction between foresters, tree breeders and pathologists/entomologists will be needed to ensure a sustainable *Eucalyptus* forestry industry in China.