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Cryptometrion aestuescens gen. sp. nov. (Cryphonectriaceae) pathogenic to *Eucalyptus* in Indonesia

M. Gryzenhout[A,C], *M. Tarigan*[A], *P. A. Clegg*[B] *and M. J. Wingfield*[A]

[A]Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 2002, South Africa.
[B]Toba Pulp Lestari, Porsea, Sumatra, Indonesia.
[C]Corresponding author. Email: Marieka.Gryzenhout@fabi.up.ac.za

Abstract. The recently described Cryphonectriaceae (Diaporthales) includes numerous important tree pathogens such as the chestnut blight pathogen *Cryphonectria parasitica*, and various species of *Chrysoporthe* that cause serious stem canker diseases on *Eucalyptus*. A recent investigation of dying *Eucalyptus grandis* clonal hedges in northern Sumatra, Indonesia, revealed the presence of an unknown member of the Cryphonectriaceae. DNA sequence comparisons with other members of the Cryphonectriaceae showed that the Indonesian fungus represents a new genus and species in the family, for which we provide the name *Cryptometrion austuescens* gen. sp. nov. It can be distinguished from other Cryphonectriaceae on *Eucalyptus*, such as *Chrysoporthe*, *Microthia*, *Holocryphia* and *Cryphonectria*, based on its orange, limited stromatic tissue, single septate, fusoid to ellipsoid ascospores and the absence of paraphyses among the conidiogenous cells in the anamorph. Inoculations with this fungus on two clones of *E. grandis* showed that it is highly pathogenic and has the capacity to cause serious losses to *Eucalyptus* plantations in the region.

Introduction

The Cryphonectriaceae (Diaporthales) is a recently described family including numerous important tree pathogens (Gryzenhout *et al*. 2006*a*, 2009). These include the chestnut blight pathogen *Cryphonectria parasitica* (Heiniger and Rigling 1994), and *Chrysoporthe* species that cause serious stem canker diseases on *Eucalyptus* (Gryzenhout *et al*. 2006*b*). The Cryphonectriaceae includes 10 genera and 22 species, the majority of which have been described relatively recently (Gryzenhout *et al*. 2006*c*, 2009). These new genera and species have been defined based on DNA sequence comparisons of the internal transcribed spacer (ITS) region and β-tubulin genes, and morphological characteristics of anamorph structures, ascostromata and ascospores (Gryzenhout *et al*. 2009).

Species of *Chrysoporthe* cause serious stem cankers and tree mortality (Wingfield 2003) and were previously collectively treated as *Cryphonectria cubensis* (Gryzenhout *et al*. 2004). Of these, *Chr. cubensis*, *Chr. austroafricana* and *Chr. doradensis* are known from *Eucalyptus* species, with *Chr. cubensis* occurring in tropical and subtropical areas of the world (Gryzenhout *et al*. 2004), *Chr. austroafricana* in southern Africa (Gryzenhout *et al*. 2004; Nakabonge *et al*. 2006*a*) and *Chr. doradensis* in Ecuador (Gryzenhout *et al*. 2005).

Several Cryphonectriaceae infect *Eucalyptus* trees but are not considered important pathogens, for example, *Holocryphia eucalypti*, which was previously known as *Cryphonectria eucalypti* (Gryzenhout *et al*. 2006*d*), is an opportunistic pathogen on both native and commercially propagated *Eucalyptus* species in Australia and South Africa (Van der Westhuizen *et al*. 1993; Yuan and Mohammed 2000; Nakabonge *et al*. 2008). A fungus occurring on *Eucalyptus* but apparently not causing serious disease, is *Microthia havanensis*, previously known as *Cryphonectria* (=*Endothia*) *havanensis* (Barnard *et al*. 1987; Gryzenhout *et al*. 2006*d*). Another pathogen morphologically similar to *Chr. cubensis* occurs in Vietnamese and Indian plantations (Sharma *et al*. 1985; Old *et al*. 2003). This fungus has been treated under the name *Cryphonectria gyrosa* in the past, but is not related to that fungus and has not been formally named (Gryzenhout *et al*. 2009). *Cryphonectria parasitica* and other unknown *Cryphonectria* spp. have also been found to infect *Eucalyptus* spp. in Japan (Old and Kobayashi 1988) but there is no information regarding the accuracy of their identification in relation to the recent taxonomic changes for this group of fungi, or of their relative importance.

In South-East Asia, *Chr. cubensis* is one of the most important pathogens of plantation-grown *Eucalyptus* (Old *et al*. 2003). Recently, a fungus with orange fruiting structures characteristic of the Cryphonectriaceae, was found on girdling cankers on *E. grandis* clonal hedge plants in Northern Sumatra, Indonesia (Fig. 1), as well as on a small number of trees in the plantations. The aim of this study was to characterise this fungus within the Cryphonectriaceae and to assess its pathogenicity to *E. grandis*.

Materials and methods

Disease symptoms and samples

Clonal *E. grandis* hedge plants near Tele, Northern Sumatra, were severely affected by girdling cankers (Fig. 1). Infections mostly

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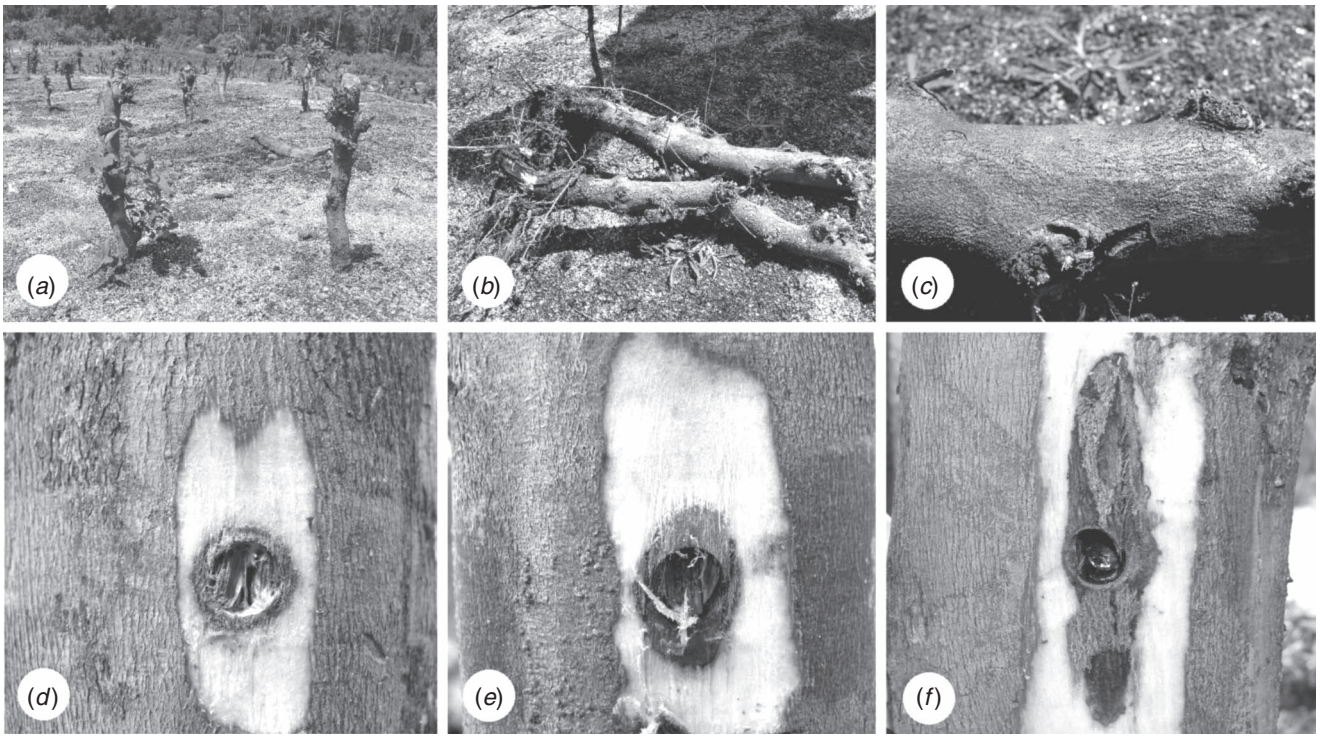


Fig. 1. (*a–c*) Disease symptoms and fungal sporulation caused by *Cryptometrion aestuescens* (*c*) on *Eucalyptus grandis* clonal hedges in Sumatra. (*d–f*) Artificial inoculations 6 weeks after inoculation with a clean agar block (*d*), CMW 18970 (*e*) and CMW 18793 (*f*) on a *Eucalyptus* clone in Sumatra.

originated from pruning wounds or from cut stem surfaces. Infections subsequently moved down the stems, in many cases reaching ground level, at which stage the plants were usually dead. Dead bark covering cankers was covered in orange fruiting structures typical of those of the Cryphonectriaceae. In the field, cankers were found on the stems of ~10 trees in a single compartment and were similar to those caused by *Chr. cubensis*. These cankers sometimes girdled the stems resulting in a dead top or death of the entire tree.

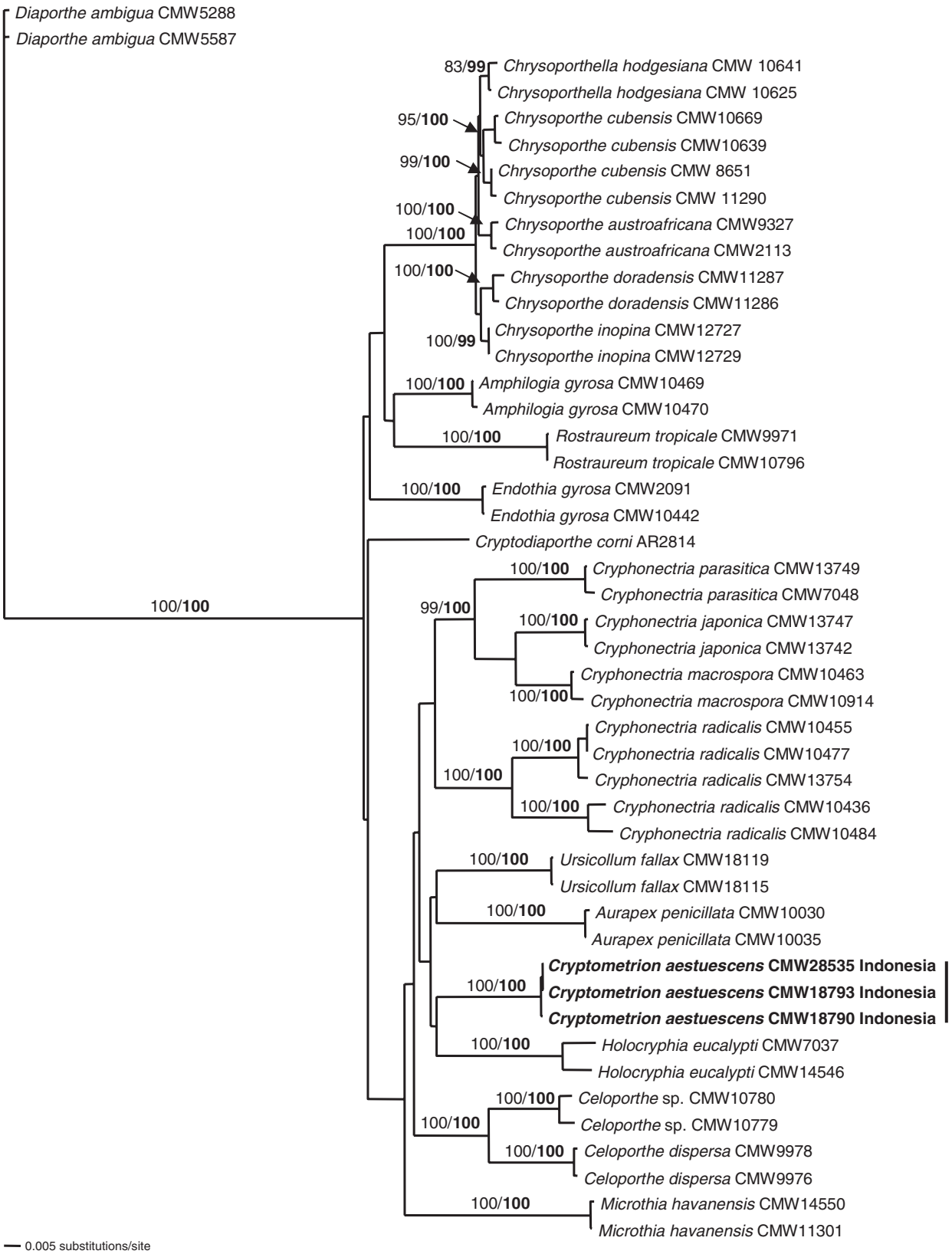
Bark material was removed from the dying plants and transported to the laboratory for detailed study. Fruiting bodies were excised from these samples and single-spore isolations were obtained, or isolations were made directly from the fruiting bodies by removing the centrum masses and plating them directly onto malt extract agar (MEA 20 g/L; Biolab, Merck, Midrand, South Africa). Cultures are maintained in the culture collection (CMW) of the Forestry and Agricultural and Biotechnology Institute, University of Pretoria, South Africa, and the Centraalbureau voor Schimmelcultures (CBS), Utrecht, Netherlands. Original bark specimens connected to isolates were deposited in the herbarium of the National Collection of Fungi, Pretoria, South Africa (PREM).

DNA sequence comparisons

DNA was extracted from representative isolates (CMW22535 – ex-holotype, CMW18790, CMW18793) and the parts of the internal transcribed spacer (ITS) region and β-tubulin gene regions were amplified and sequenced using the method previously presented by Gryzenhout *et al*. (2004). DNA sequences were deposited in GenBank (numbers cited in Fig. 2) and included in the datamatrix (TreeBASE S2003, M3737) from Gryzenhout *et al*. (2009). The datasets were aligned using the online sequence alignment tool MAFFT ver. 6 (http://align.bmr.kyushu-u.ac.jp/mafft/online/server/, last accessed March 2009; Katoh *et al*. 2002) and checked manually.

Phylogenetic analyses were done based on parsimony, and confirmed using neighbour-joining (NJ) analyses. Maximum parsimony analyses (MP) were made using PAUP (phylogenetic analysis using parsimony) ver. 4.0b10 (Swofford 2002) with the heuristic search algorithm (100 random sequence additions, tree-bisection-reconnection branch swapping, MULTREES off, uninformative characters excluded, remaining characters reweighted according to consistency index or CI). The rRNA and β-tubulin gene regions were analysed separately but were combined after tree congruence was tested with a 500-replicate partition homogeneity test (Farris *et al*.

Fig. 2. Neighbour-joining tree resulting from a combined dataset of ribosomal and β-tubulin gene sequences. Confidence levels (1000 replicate bootstrap analysis) more than 70% are indicated on the tree branch nodes, and Bayesian posterior probabilities are indicated after the bootstrap values. Isolates in bold type face (indicated with vertical line) were sequenced in this study (GenBank numbers for β-tubulin sequences for CMW 22535 is GQ 369454, CMW 18790 are GQ 369455, CMW 18793 is GQ 369456, and internal transcribed spacer sequences for CMW 22535 is GQ 369457, CMW 18790 is GQ 369458, CMW 18793 is GQ 369459). *Diaporthe ambigua* was used as an outgroup.


Diaporthe ambigua CMW5288
Diaporthe ambigua CMW5587
83/99
Chrysoporthella hodgesiana CMW 10641
Chrysoporthella hodgesiana CMW 10625
95/100
Chrysoporthe cubensis CMW10669
Chrysoporthe cubensis CMW10639
99/100
Chrysoporthe cubensis CMW 8651
Chrysoporthe cubensis CMW 11290
100/100
Chrysoporthe austroafricana CMW9327
100/100
Chrysoporthe austroafricana CMW2113
100/100
Chrysoporthe doradensis CMW11287
Chrysoporthe doradensis CMW11286
Chrysoporthe inopina CMW12727
100/99
Chrysoporthe inopina CMW12729
100/100
Amphilogia gyrosa CMW10469
Amphilogia gyrosa CMW10470
100/100
Rostraureum tropicale CMW9971
Rostraureum tropicale CMW10796
100/100
Endothia gyrosa CMW2091
Endothia gyrosa CMW10442
Cryptodiaporthe corni AR2814
100/100
Cryphonectria parasitica CMW13749
Cryphonectria parasitica CMW7048
100/100
99/100
Cryphonectria japonica CMW13747
Cryphonectria japonica CMW13742
Cryphonectria macrospora CMW10463
100/100
Cryphonectria macrospora CMW10914
Cryphonectria radicalis CMW10455
100/100
Cryphonectria radicalis CMW10477
100/100
Cryphonectria radicalis CMW13754
100/100
Cryphonectria radicalis CMW10436
Cryphonectria radicalis CMW10484
100/100
Ursicollum fallax CMW18119
Ursicollum fallax CMW18115
100/100
Aurapex penicillata CMW10030
Aurapex penicillata CMW10035
Cryptometrion aestuescens CMW28535 Indonesia
100/100
Cryptometrion aestuescens CMW18793 Indonesia
Cryptometrion aestuescens CMW18790 Indonesia
100/100
Holocryphia eucalypti CMW7037
Holocryphia eucalypti CMW14546
100/100
Celoporthe sp. CMW10780
100/100
Celoporthe sp. CMW10779
100/100
Celoporthe dispersa CMW9978
Celoporthe dispersa CMW9976
100/100
Microthia havanensis CMW14550
Microthia havanensis CMW11301
0.005 substitutions/site

1994). The correct model for the datasets was found using MODELTEST ver. 3.5 (Posada and Crandall 1998). The GTR + I + G model (Base = 0.1874, 0.3350, 0.2288; Nst = 6; Rmat = 0.9330, 3.1432, 1.1267, 0.9440, 3.9389; Rates = gamma, Shape = 0.8837, Pinvar = 0.4512) was shown to be the appropriate model (Tavaré 1986). Bootstrap analyses (1000 replicates) were executed to assess the confidence levels of the branch nodes of the phylogenetic trees obtained with MP and NJ. Bayesian inference with the Markov chain Monte Carlo algorithm (Larget and Simon 1999) in the program Mr. Bayes ver. 3.1.1 (Huelsenbeck and Ronquist 2001) tested the probabilities of branches. For the analyses, the number of generations was 5 000 000, sample frequency was 100, number of chains was four (one cold, three hot) and a burn in of 5000 was set. Four of these analyses were run.

Morphology

Fruiting bodies on bark specimens connected to isolates used in this study (PREM 60247, PREM 60248, PREM 60249), were sectioned and examined microscopically following methods previously described (Gryzenhout *et al*. 2006*d*). Colony growth was evaluated on two representative isolates (CMW 18790, CMW 18793) to obtain the maximum and minimum temperatures for growth and to describe culture morphology. The isolates were grown in the dark at temperatures ranging from 10 to 35°C at intervals of 5° on MEA, and growth was assessed as described previously (Gryzenhout *et al*. 2004). Growth comparisons were repeated once.

Pathogenicity tests

Field pathogenicity tests were conducted on 1.5-year-old *E. grandis* trees representing two different clones and ranging in diameter from 90 to 110 mm. Inoculations were made on the stems ~1.3 m above the ground. Two isolates (CMW 18790, CMW 18793) of the unknown fungus were used. Twenty trees of each of the two *Eucalyptus* clones were used for each isolate and an equal number of trees served as controls. Before inoculation, a wound was made on the tree stems using a sterilised cork borer (10 mm diameter). An agar disc, taken from the edges of actively growing colonies on 2% MEA, was inserted into the wound with the mycelium facing inwards. Controls were made using sterile 2% MEA discs. After inoculation, the wounds were sealed with masking tape to reduce contamination and desiccation of the inoculum and wounds.

The masking tape was removed 6 weeks after inoculation. The diameter at the inoculation point and the lengths of lesions produced on the stems were measured. Isolations were made from pieces of symptomatic tissue from a representative set of trees and isolates were taken from the area associated with the inoculation points to confirm that the lesions were associated with the inoculated fungus. Data obtained were analysed with ANOVA using SAS ver. 8.2 statistical analyses (SAS Institute 2001).

Results

DNA sequence comparisons

The DNA dataset consisted of 47 taxa with *Diaporthe ambigua* defined as outgroup taxon. The rRNA dataset consisted of 596 characters (200 informative, 385 constant, 11 uninformative) and yielded three trees differing only in branch length (tree length or TL 294, CI 0.753, retention index or RI 0.893, g1 value 0.452). The β-tubulin dataset consisted of 1006 characters (373 informative, 600 constant, 33 uninformative) and yielded one tree (TL 614, CI 0.702, RI 0.875, g1 value 0.405). Combining the datasets was shown to have no conflict (*P*-value = 0.226) and trees based on the ITS and β-tubulin gene regions showed the same distinct groupings of isolates, including those of the isolates of the unknown fungus. Therefore, the datasets were combined (TL 884.80, CI 0.693, RI 0.877, g1 value 0.48). These analyses yielded two nearly identical trees that differed only in branch lengths. The trees based on distance and Bayesian analyses (Fig. 2) reflected the same topology, in that the isolates from Indonesia grouped distinct (bootstrap support 100%, posterior probability 100%) from any of the known genera in the Cryphonectriaceae (Fig. 2).

Morphology

The morphology of the fungus from *Eucalyptus* in Indonesia was similar to that of species of Cryphonectriaceae with orange, globose to pulvinate conidiomata, and where the anamorph is the same colour and shape as the teleomorph. Such genera include *Cryphonectria*, *Microthia* and *Holocryphia* (Gryzenhout *et al*. 2009). These characteristics clearly distinguished the unknown fungus from *Chr. cubensis* that is common in Indonesia and that has black, pyriform conidiomata and black perithecial necks (Gryzenhout *et al*. 2009). Furthermore, stromata of the unknown fungus are semi-immersed with convoluted conidial linings. Perithecial necks above the stromatal surface are covered with orange tissue.

Morphological differences between the unknown fungus and species in *Cryphonectria*, *Microthia* and *Holocryphia* (Gryzenhout *et al*. 2009) included the following. Fruiting structures of the unknown fungus (Fig. 3*a*, *b*, *d*) were not as strongly developed nor as large as species of *Cryphonectria*, and thus are more similar to those of *Microthia* and *Holocryphia*. Species accommodated in *Microthia* and *Holocryphia* have long, prominent paraphyses among the conidiogenous cells, which are absent in the species from Indonesia (Fig. 3*i*). This characteristic can, however, also differ at the species level, as *C. macrospora* has been shown to have paraphyses-like cells, which are absent in other *Cryphonectria* species (Gryzenhout *et al*. 2009). Ascospores of the unknown fungus from Indonesia were single septate (Fig. 3*g*) and thus similar to those of *Cryphonectria* and *Microthia*, but different from the aseptate ascospores of *Holocryphia*.

Culture morphology of the fungus from Indonesia was also different from that in other genera and species in the Cryphonectriaceae (Gryzenhout *et al*. 2009). Colonies were initially white but with age turned sienna to umber, with sienna to luteous patches. The colony reverse colour was sienna to umber. This is most similar to *Celoporthe dispersa*, which has umber to hazel to chestnut patches in its colonies (Nakabonge *et al*. 2006*b*), but different from species in *Chrysoporthe* that have white to cinnamon cultures (Gryzenhout *et al*. 2004). This also differs from cultures of species in *Cryphonectria*, *Holocryphia* and *Microthia*, which

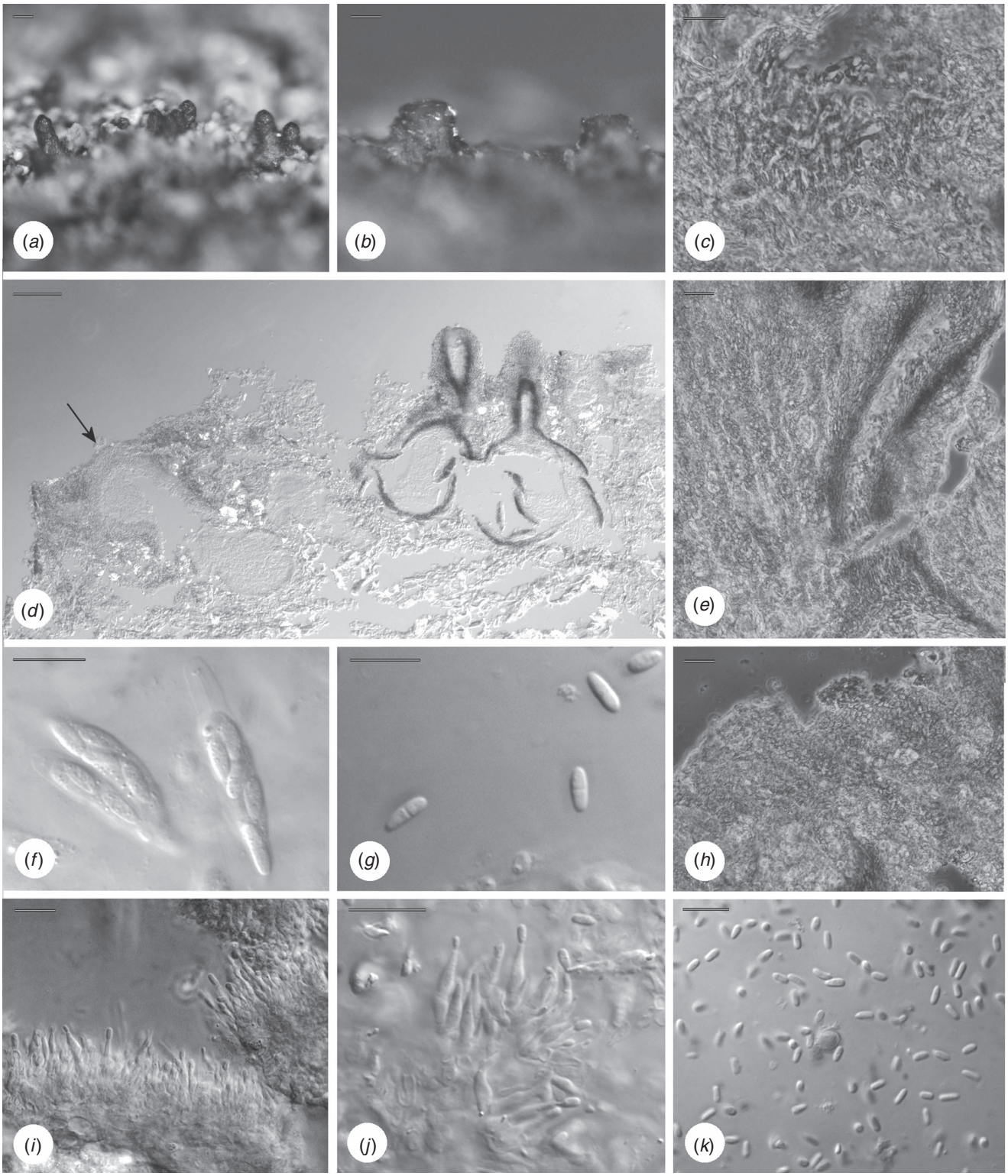


Fig. 3. Fruiting structures of *Cryptometrion aestuescens*. (*a*) Ascostromata on bark. (*b*) Conidiomata on bark. (*c*, *e*) Prosenchymatous tissue in centre of stromata. (*d*) Longitudinal sections through a conidioma (indicated with arrow) and ascostroma. (*f*) Asci. (*g*) Ascospores. (*h*) Pseudoparenchymatous tissue (*i*, *j*) Conidiophores. (*k*) Conidia. Scale bars *a*, *b*, *d* = 100 µm; *c*, *e*, *h* = 20 µm; *f*, *g*, *i*–*k* = 10 µm.

are generally white and fluffy, with orange to straw yellow patches (Gryzenhout *et al*. 2009). An unusual feature of the isolates from Indonesia was that they had an optimal growth temperature of 20°C, and showed no growth at 30–35°C. This is different from *Chr. cubensis* that has an optimal growth temperature of 30°C (Gryzenhout *et al*. 2004).

Pathogenicity tests

Both isolates (CMW 18790, CMW 18793) of the unknown fungus from *Eucalyptus* produced lesions on the inoculated *E. grandis* clones (Fig. 1). The lesion lengths on the treated trees ranged from 60 to 69 mm on clone 1 and 94 to 133 mm on clone 2 (Fig. 4). Isolate CMW 18793 consistently produced longer lesions than isolate CMW 18790 on both inoculated clones (Fig. 4). Both isolates produced significantly longer lesions on clone 1 than on clone 2. Lesions were not produced on the control trees and the points of inoculation had been closed by callus growth. Isolates that were morphologically similar to those of the test fungus were reisolated from lesions on inoculated trees but not from the control trees.

Taxonomy

Although the fungus from Indonesia resembles *Cryphonectria*, *Microthia* and *Holocryphia* very closely and could be difficult to differentiate from species in these genera, it can be distinguished based on a combination of characteristics of the stromata, the presence or absence of paraphyses and ascospore septation. The isolates from Indonesia also clearly grouped separately from these genera based on DNA sequence comparisons. Following the taxonomic scheme that has been developed for the Cryphonectriaceae (Gryzenhout *et al*. 2009), the new fungus represents a new genus and species, for which we provide the following description.

Cryptometrion Gryzenh. & M.J. Wingf., gen. nov. Mycobank MB 514188

Etymology: Greek, crypto = hidden, metrion = middle, alluding to the fact that this genus shares a combination of characteristics with other genera in the Cryphonectriaceae.

Ascostromata pulvinata vel globosa, in cortice subimmersa, aurantiaca, collis perithecii in superficie stromatis in forma ostiolorum nigrorum textura aurantiaca stromatis tectorum erumpentibus papillas formantibus. *Ascosporae* fusoideae vel ellipsoideae, semel septatae. *Conidiomata* globosa subimmersa aurantiaca uniloculares, sine *paraphysibus*, *conidiis* cylindricis non-septatis.

Ascostromata on host gregarious or single, pulvinate to globose, semi-immersed in bark, orange, pseudoparenchymatous at edge of stroma, prosenchymatous at centre. *Perithecia* diatrypoid, sometimes forced by host tissue into a valsoid position, embedded in host tissue at base of stroma, fuscous black, necks emerge at stromatal surface as black ostioles covered with orange stromatal tissue to form papillae. *Asci* fusiform, unitunicate, 8-spored. *Ascospores* hyaline, fusoid to ellipsoid, one medial to submedial septum.

Conidiomata formed as part of ascostromata as conidial locules, or as separate structures, globose, semi-immersed, orange, uniloculate, with the same tissue morphology and stromatic structure as the ascostromata, spores expelled through opening. *Conidiophores* hyaline, occasionally with separating septa and branched, conidiogenous cells cylindrical or flask-shaped with apices attenuated or not, paraphyses absent. *Conidia* hyaline, cylindrical, aseptate, exuded as orange droplets.

Cryptometrion aestuescens Gryzenh. & M.J. Wingf., sp. nov. Mycobank MB 514189, Fig. 3

Etymology: Greek, 'feeling hot' referring to the fact that this species has an optimal growth temperature more suitable to cooler climates but occurs in a tropical area, albeit at a high altitude with cool temperatures.

Ascostromata pulvinata vel globosa, in cortice subimmersa, aurantiaca, collis perithecii in superficie stromatis in forma ostiolorum nigrorum textura aurantiaca stromatis tectorum

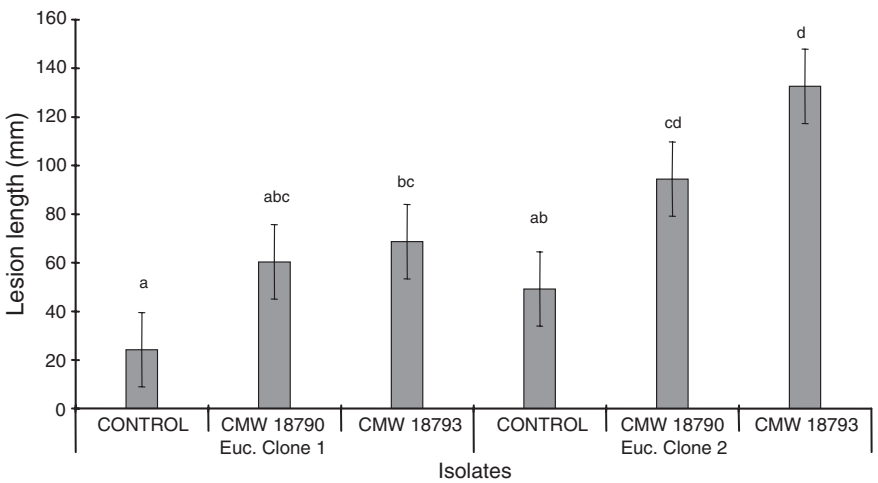


Fig. 4. Lesion lengths associated with inoculations using two isolates of *Cryptometrion aestuescens* (CMW 18790, CMW 18793) on 1.5-year-old *Eucalyptus* hybrid clones 1 and 2 in an Indonesian plantation, 6 weeks after inoculation. Bars on the graph indicated with the same letter are not significantly different from each other (*P*-value = 0.05).

erumpentibus papillas formantibus. *Ascosporae* fusoideae vel ellipsoideae, semel septatae, (5.5–)6.5–7.5(–8) × 2–2.5(–3) µm. *Conidiomata* globosa subimmersa aurantiaca uniloculares, sine *paraphysibus*, *conidiis* cylindricis non-septatis, (3–)3.5–4.5(–5) × 1.5–2 µm. *Coloniae* albae vel sieninae vel umbrinae maculis sieninis vel luteis, infra sieninae vel umbrinae; crescunt optime ad 20°C, non-crescunt ad 30–35°C.

Ascostromata on host gregarious or single, pulvinate to globose, typically 60–130 µm high, 160–350 µm diam., semi-immersed in bark, orange, pseudoparenchymatous on edge of stroma, prosenchymatous in centre. *Perithecia* diatrypoid but can be forced by host tissue into a valsoid position, embedded in host tissue at base of stroma, fuscous black, necks emerge at stromatal surface as black ostioles covered with orange stromatal tissue to form papillae extending up to 230 µm above stromatal surface. *Asci* (24–)27.5–32(–34.5) × (5–)5.5–6.5(–7) µm, unitunicate, fusiform, 8-spored. *Ascospores* (5.5–)6.5–7.5(–8) × 2–2.5(–3) µm, hyaline, fusoid to ellipsoid, one medial to submedial septum.

Conidiomata part of ascostromata as conidial locules, or as separate structures, globose, 80–180 µm high, 90–230 µm diam., semi-immersed, orange, uniloculate, with the same tissue morphology and stromatic structure as the ascostromata, spores expelled through opening. *Conidiophores* (6.5–)8–13.5 (–18) µm long, occasionally with separating septa and branched, hyaline, conidiogenous cells 1.5–2(–2.5) µm wide, cylindrical or flask-shaped with apices attenuated or not, no paraphyses present. *Conidia* (3–)3.5–4.5(–5) × 1.5–2 µm, hyaline, cylindrical, aseptate, exuded as orange droplets.

Cultural characteristics: white and fluffy when young, turning sienna to umber with sienna to luteous patches after 7–8 days, colony reverse sienna to umber; optimal growth temperature 20°C covering the plates after 7 days, no growth at 30–35°C and colonies at 10°C reaching 23 mm in 7 days. Asexual fruiting structures occasionally form in primary isolations of the fungus, similar to those of other Cryphonectriaceae (Gryzenhout *et al*. 2009).

Substrate*:* Eucalyptus grandis

Distribution: North Sumatra, Indonesia.

Specimens examined: INDONESIA, Sumatra, Tele, *Eucalyptus grandis*, 30 March 2006, *M.J. Wingfield*, PREM 60247, holotype, culture ex-type CMW 22535/CBS 124009, PREM 60248, living culture CMW 22537, PREM 60249, living culture CMW 18790/CBS 124008 and living culture CMW 18793/CBS 124007.

Discussion

Results of this study revealed a new species of Cryphonectriaceae infecting *E. grandis* trees in northern Sumatra. The fungus peripherally resembles other members of the family but phylogenetic inference showed that it resides in a distinct group equivalent to the other genera in the family. The new genus *Cryptometrion* was described to accommodate the new species *C. aestuescens.* The fungus was shown to be highly pathogenic on two clones of *E. grandis* in inoculation tests.

The most notable characteristics distinguishing *C. aestuescens* from other genera in the Cryphonectriaceae with globose to pulvinate conidiomata, are the absence of paraphyses among conidiogenous cells, the overall simple structure of the stromata and single septate ascospores. However, based on morphology this genus could easily be confused with other genera and species in the Cryphonectriaceae, such as those in *Microthia* and *Cryphonectria*, which have a similar overall appearance. Likewise, in the absence of the teleomorph *Cryptometrion* could be confused with species of *Holocryphia* (Gryzenhout *et al*. 2009). This is especially true because both genera occur on *Eucalyptus* spp. and careful microscopic examination is needed to provide robust identifications.

DNA sequence comparisons for the ITS and β-tubulin gene regions can clearly distinguish among genera in the Cryphonectriaceae. These generic boundaries are supported by distinct morphological differences that are too inordinately diverse to merely represent species differences (Gryzenhout *et al*. 2009). Although *C. aestuescens* has been described based on relatively subtle morphological differences, it is clearly distinct from other genera based on DNA sequence comparisons.

Growth studies showed that *C. aestuescens* has an optimum temperature of 20°C, showing no growth at 30–35°C. This is unlike most species of Cryphonectriaceae that cause disease on *Eucalyptus* in the tropics and subtropics. Yet it is consistent with the area in which *C. aestuescens* was discovered. Although in the tropics, this area is at high altitude (1800 m.a.s.l.) above Lake Toba in northern Sumatra and has typically low temperatures. Interestingly, the Asian form of *Chr. cubensis* is a common cause of stem cankers on *Eucalyptus* in plantations relatively close to those in which *C. aestuescens* was discovered. *Cryptometrion cubensis* has a typical growth temperature of 30°C (Gryzenhout *et al*. 2004). The plantations where *Chr. cubensis* occurs are, however, at low altitudes and subjected to the high temperature and humidity more typical of the tropics.

The discovery of a new *Eucalyptus* pathogen in Indonesia raises interesting questions regarding its origin. Previous studies have shown that species in *Chrysoporthe* that infect *Eucalyptus* could have originated on native hosts in the Myrtales (Gryzenhout *et al*. 2004, Gryzenhout *et al*. 2006*b*; Rodas *et al*. 2005). Thus, *Chr. austroafricana*, which is an important pathogen of *Eucalyptus* in southern Africa, appears to have undergone a host shift (Slippers *et al*. 2005) from native *Syzygium* spp. (Heath *et al*. 2006; Nakabonge *et al*. 2006*a*). Likewise, *Chr. cubensis* has been found on many native species of the Melastomataceae in South and Central America and these appear to be its native hosts (Rodas *et al*. 2005; Gryzenhout *et al*. 2006*b*). Previously, we have discovered the Asian form of *Chr. cubensis* on native *Melastoma malabathricum* (Melastomataceae) in Sumatra (Gryzenhout *et al*. 2006*b*), where it appears to have undergone a host jump to *Eucalyptus*. It thus seems likely that *C. aestuescens* has a native host in northern Sumatra and this is likely to be a species of the Melastomataceae or Myrtaceae that is well suited to the cool climates found at high altitude sites.

The appearance of new *Eucalyptus* pathogens in virtually all areas of the world where these trees are grown as non-natives in plantations (Wingfield 2003) is cause for considerable concern (Wingfield *et al*. 2008). Most of the pathogens residing in the Cryphonectriaceae cause serious diseases (Gryzenhout *et al*. 2009). Virtually all of them have been found on native trees and not always necessarily very closely related hosts, and they

have evidently undergone host shifts to be able to infect *Eucalyptus*. It thus seems likely that additional new *Eucalyptus* pathogens such as *C. aestuescens* will arise in the future and these need to be carefully considered in terms of disease management. The fact that they also represent pathogens new to *Eucalyptus* means that they are potentially threatening to *Eucalyptus* and other Myrtaceae where these trees are native. This is a similar situation to that found for the intriguing and serious *Eucalyptus* rust, *Puccinia psidii*, which was initially known from *Psidium guajava* (guava, Myrtaceae) but subsequently infected related *Eucalyptus* trees (Coutinho *et al*. 1998; Glen *et al*. 2007).

C. aestuescens is clearly a potentially important pathogen of *Eucalyptus* in northern Sumatra and it will require attention in terms of disease management in the future. Where it was found in the clonal propagation mother plants, more than 50% of the plants had been killed. In contrast, very few infected trees were found in plantations and it is unknown how important it might become in the future. Its biology appears to be similar to that of *Chr. cubensis* that requires wounds for infection although this does not detract from the damage that it can cause when susceptible trees are used to establish plantations. If *C. aestuescens* becomes more common in plantations, it should be possible to reduce its impact. This would be consistent with the fact that substantial progress has been made in reducing the impact of canker diseases on *Eucalyptus* caused by Cryphonectriaceae in various parts of the world (Wingfield 2003). This has mainly been achieved by selecting clones that are resistant to infection. The fact that the two clones tested in this study differed in susceptibility to *C. aestuescens* suggests that a breeding and selection approach will make it possible to avoid serious disease problems in the future. Furthermore, *C. aestuescens* is clearly a pathogen suited to cool environments and it is not likely to cause serious disease in the large plantation areas that are in close proximity, yet at low altitude, and thus growing in a more typical tropical environment.

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