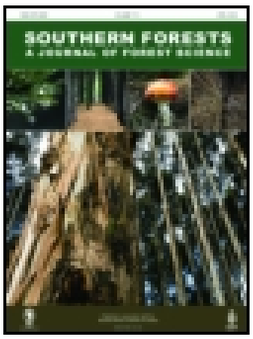

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Calonectria species diversity on eucalypts in Indonesia

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Diseases increasingly threaten the rapidly expanding eucalypt plantation industry of Indonesia. Of these, leaf blight caused by *Calonectria* spp. is considered amongst the more important problems, causing losses both in production nurseries and plantations. Using DNA sequence data based on the translation elongation factor 1-alpha, β-tubulin, calmodulin, and histone H3 gene regions, 163 isolates of *Calonectria* spp. obtained from diseased eucalypt seedlings in nurseries and infected leaves in plantations were identified as *Calonectria acicola, C. hawksworthii, C. lombardiana, C. multiseptata, C. pseudoreteaudii* and *C. reteaudii.* Of these, *C. lombardiana* was by far the most commonly isolated and accounted for approximately 84% of the isolates. Given the predominance of this fungus, it is interesting that it has not previously been reported from Indonesia. This is also the first report of *C. pseudoreteaudii* and *C. acicola* from the country. All six species of *Calonectria* were found to be pathogenic to eucalypts in artificial inoculation studies. *Calonectria lombardiana* was generally the most pathogenic species and eucalypt genotypes displayed different levels of susceptibility, providing confidence that disease caused by this fungus can be reduced by selecting disease-tolerant planting stock.

Keywords: *Cylindrocladium*, forestry, leaf and shoot blight, multi-gene phylogeny

Online supplementary material: Supplementary data for this article are available at https://doi.org/10.2989/20702620.2023.2179441

Introduction

Calonectria (Nectriaceae, Hypocreales) is a genus that accommodates numerous important pathogens that are widely distributed, especially in tropical and sub-tropical regions of the world (Crous 2002; Lombard et al. 2010a; Marin-Felix et al. 2017). These fungi are mainly soil-borne pathogens but infect most plant tissues on susceptible hosts (Crous 2002; Li et al. 2017; Lopes et al. 2018; Jiang et al. 2019; Pham et al. 2019). Liu et al. (2020) produced the most comprehensive recent taxonomic study on these fungi, defining 120 species based on sequence data for eight gene regions. These included many species known as causal agents of diseases on important forest plantation trees including *Pinus* (Hodges and May 1972; Lombard et al. 2009), *Acacia* (Lombard et al. 2010a) and *Eucalyptus* (Lombard et al. 2015; Li et al. 2017).

Eucalypts are the most widely planted trees used to establish short-rotation plantations globally (Couto et al. 2011; Harwood and Nambiar 2014). Many diseases have been reported on these trees, including those caused by a variety of *Calonectria* spp. (Booth et al. 2000; Rodas et al. 2005; Crous et al. 2019). These fungi are amongst the most common pathogens of eucalypts in plantations and nurseries causing *Calonectria* leaf blight (CLB) as well as root disease and cutting rot (Crous 2002; Lombard et al. 2010b). Twenty-seven species of *Calonectria* are currently known to occur on eucalypts worldwide (Crous et al. 2019; Liu et al. 2020). Several of these species were reported to cause serious leaf and shoot blight disease in eucalypt plantations in Southeast Asia (Crous et al. 1998; Old et al. 2003; Chen et al. 2011; Lombard et al. 2015; Li et al. 2017; Pham et al. 2019; Pham et al. 2022).

Industrial forest plantation programmes reliant on eucalypts have expanded rapidly in Indonesia and especially in the islands of Sumatra and Kalimantan since the early 1990s (Harwood and Nambiar 2014). Concomitant with this growing industry, there has been an increase in disease problems on these trees (Wingfield et al. 1996; Crous et al. 1998; Gryzenhout et al. 2010; Coetzee et al. 2011; McTaggart et al. 2016; Bophela et al. 2019; Siregar et al. 2020; Pham et al. 2021; Jami et al. 2022). Of these, leaf blight caused by species of *Calonectria* has become increasingly common (Pham et al. 2019; Pham et al. 2022). These pathogens are

able to spread rapidly in nurseries, and losses can seriously hamper nursery production or plantation establishment. The aims of this study were consequently to identify *Calonectria* species causing diseases in eucalypt nurseries and plantations in Indonesia and to assess their relative importance using pathogenicity tests.

Materials and methods

Sample collections and fungal isolations

Leaves and seedlings showing CLB symptoms (Figure 1) were collected in both nurseries and plantations in Kalimantan and Sumatra during regular disease surveys in 2018–2019. These included eight eucalypt nurseries and 26 plantation sites, and two *Acacia crassicarpa* plantation sites in proximity to eucalypt plantations. This resulted in a collection of 61 diseased seedlings and leaves from 102 diseased trees (Table 1). Samples were collected from Riau, Central Sumatra, including Sei Kebaro (15 leaves and 5 seedlings), Pelalawan (31 leaves and 34 seedlings) and Kuantan Singingi (36 leaves and 8 seedlings); from North Sumatra including Porsea (6 leaves and 4 seedlings); from Kalimantan, including East Kalimantan (10 leaves and 2 seedlings) and North Kalimantan (4 leaves and 8 seedlings) (Table 1, Figure 2). The number of samples collected depended on the disease incidence at the sampling sites.

Figure 1: Symptoms of *Calonectria* infection: (a) on leaves of *Eucalyptus* seedlings; (b) on stems of *Eucalyptus* seedlings; (c) on leaf of *Eucalyptus* trees in the field

All collected samples were placed in individual brown paper bags and transported to the laboratory for further study. Pieces (0.5 × 0.5 cm^2) of leaf or shoot tissue were cut from the border of the lesions, surface disinfected in 0.5% sodium hypochlorite for 30 seconds and rinsed three times in sterile distilled water. Surface-disinfected plant segments were placed onto the surface of potato dextrose agar (PDA Acumedia®: 40 g l^{-1}) and incubated for 3–4 days at 25 °C. Colonies showing typical morphology of *Calonectria* spp., especially orange-brownish aerial hyphae, were transferred to clean PDA in petri dishes and all isolates were purified by sequentially transferring hyphal tips to clean PDA. All isolates considered in this study have been stored in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from the fungi using Prepman® Ultra Sample Preparation Reagent (Thermo Fisher Scientific, Waltham, MA, USA) from 4-day-old fungal cultures. A fragment of the translation elongation factor 1-α (*TEF1*) gene was amplified using primers EF1-728F (Carbone and Kohn 1999) and EF-2 (O'Donnell et al. 1998); a fragment of the β-tubulin (*TUB2*) gene using primers T1 (O'Donnell and Cigelnik 1997) and CYLTUB1R (Crous et al. 2004); a fragment of the histone H3 (*HIS3*) gene region using primers CYLH3F and CYLH3R (Crous et al. 2004); and a fragment of the calmodulin (*CMDA*) gene using primers CAL-228F (Carbone and Kohn 1999) and CAL-2Rd (Groenewald et al. 2013). Initially, the *TEF1* and *TUB2* gene regions were amplified for all isolates. Based on the preliminary sequencing results, isolates representing the

Table 1: Number of samples collected from nurseries and plantations in Sumatra and Kalimantan regions

Region	Altitude (m.a.s.l.)	Nursery	Plantation	Total
North Sumatra (Porsea)	1 200	4	6	10
Central Sumatra/Riau 1 (Sei Kebaro)	56	5	15	20
Central Sumatra/Riau 2 (Pelalawan)	33	34	31	65
Central Sumatra/Riau 3 (Kuantan Singingi)	52	8	36	44
East Kalimantan (IHM complex)	70	2	10	12
North Kalimantan (AHL complex)	585	8	4	12
Total		61	102	163

range of genotypes revealed by these two loci were chosen for further study.

Polymerase chain reaction (PCR) amplifications were performed in 12 µl reactions containing 2 µl 5× MyTaq buffer (Bioline, London, UK), 0.1 µl MyTaq DNA polymerases (Bioline), 1 µl DNA, 0.5 µl of each primer (10 µm) and sterile SABAX water. The PCR protocol used included an initial denaturation (94 °C, 5 min), 10 amplification cycles (95 °C, 30 s; 55 °C for *HIS3* and *CMDA*; 52 °C for *TEF1* and *TUB2*), 45 s; 72 °C, 1 min), 30 amplification cycles with auto delta 5s (95 °C, 30 s; 55 °C for *HIS3* and *CMDA*; 52 °C for *TEF1* and *TUB2*, 45 s; 72 °C, 1 min) and a final extension (72 °C, 10 min) (Pham et al. 2019). All the amplicons were purified using ExoSAP-IT PCR Product Cleanup Reagent (Thermo Fisher Scientific, Waltham, MA, USA) and were sequenced in both directions using the BigDye terminator sequencing kit 3.1 (Applied Biosystems, Forster City, CA, USA). Sequences were obtained by running samples on an ABI PRISM 3100 DNA sequencer (Applied Biosystems, Forster City, CA, USA). CLC Main Workbench V20.1 (Qiagen, Hilden, Germany) was used to assemble and edit the raw sequences. All the sequences emerging from this study were deposited in GenBank (http://www.ncbi.nlm.nih.gov/) (Table S1).

Phylogenetic analyses

Sequences of previously published *Calonectria* spp. were obtained from GenBank database (http://www.ncbi.nlm.nih.gov/) for comparison with those generated in this study. Alignments of all sequences were assembled using the online version of MAFFT v.7 (http://mafft.cbrc.jp/alignment/server/) (Katoh and Standley 2013) and then confirmed manually in MEGA v.7 (Kumar et al. 2016). ML analyses were conducted using RaxML v.8.2.4 on the CIPRES Science Gateway v.3.3 (Stamatakis 2014) with default general time reversible (GTR) substitution matrix and 1000 rapid bootstraps. Sequences for two isolates of *Curvicladiella cignea* (CBS 109167 and CBS 109168) were used as the outgroup taxa in all phylogenetic analyses. Phylogenetic dendrograms were viewed using MEGA v.7 (Kumar et al. 2016).

Pathogenicity tests

Preliminary assessment of isolate pathogenicity

A total of 12 *Calonectria* isolates, including two of each species identified, were selected for pathogenicity tests. These selections were made specifically to include a diversity of areas of origin and/or host. The isolates were grown on 2% PDA for 10 days at 28 °C. Sporulation was induced using a method based on Alfenas et al. (2013) as follows: 10 ml of sterile distilled water was poured onto the surface of the cultures in petri dishes and the aerial mycelium was scraped from the cultures using a sterile spatula. The remaining colonies on the agar surface were rinsed with sterile distilled water to ensure that all aerial mycelium had been removed. Subsequently, 20 ml of distilled water were added to the petri dishes and the sub-surface mycelium was kept submerged for 48 hours. The excess water was then removed, and the colonies were dried using sterile tissue paper. Finally, the colonies were incubated for 48 hours in a laminar air flow cabinet at room temperature (approximately 25 °C) with the petri dish lids removed. After 48 hours, the conidia forming on the surfaces of the colonies were harvested by pouring 10 ml of sterile distilled water into the petri dishes and diluting the inoculum suspension to 1 × 10^6 spores/ml.

Inoculations were conducted on a 14-week-old *E. grandis* × *E. pellita* clone (ECL05): 2 ml of a 1 × 10^6 spore suspension of each isolate was sprayed onto the surface of 30 plants until runoff. After inoculation, a piece of wet cotton was placed at

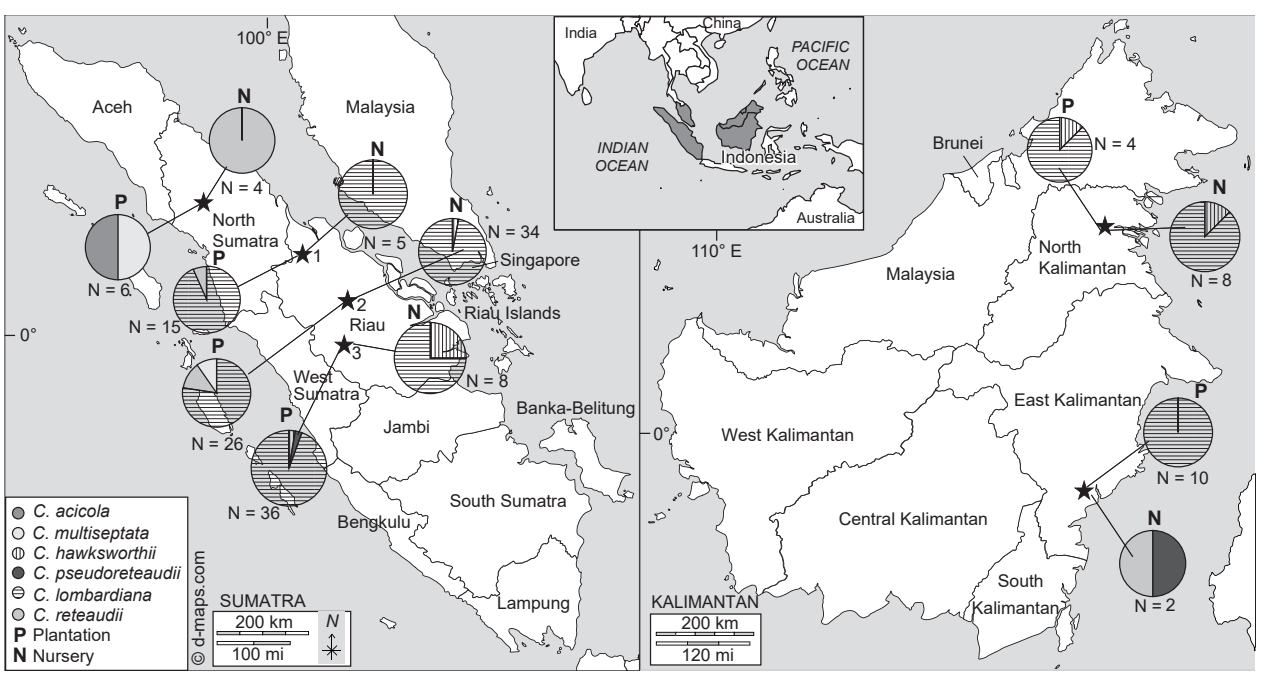

Figure 2: Geographic location of the sampling sites in Indonesia and the diversity of *Calonectria* spp. isolated in each region

the collar of the plant stem and each plant was covered with a transparent plastic bag to ensure leaf wetness and to maintain a high level of humidity. After 48 hours, the plastic bags were removed, and the plants maintained for 48 hours at room temperature. Control plants were treated in a similar manner, but the inoculum was replaced with sterile distilled water. The trial was arranged in a completely randomised design.

Disease severity was assessed four days after inoculation using a five-level rating scale where 0 = 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75% and 4 = 76–100% of the leaves infected on each plant (Figure 3). To fulfil Koch's postulates, isolations were made from inoculated tissue and the resulting isolates were identified based on morphology. Data were analysed using Kruskal–Wallis tests to determine whether there were statistically significant differences between the treatments. Pairwise comparisons were then conducted using Wilcoxon rank sum test with continuity correction. All statistical analyses were performed using R statistical software, version 3.2.0 (R Core Team 2020).

Relative tolerance of eucalypt clones

Five eucalypt genotypes that included three *E. pellita* clones (ECL01, ECL02 and ECL03) and two *E. grandis* × *E. pellita* hybrid clones (ECL04 and ECL05) commonly deployed in plantations were selected to screen against the most aggressive and predominant *Calonectria* species found in this study. Twenty 14-week-old plants of each clone were inoculated as described above with an equal number of plants used as controls, in a completely randomised design. Disease severity was assessed four days after inoculation using the same rating scale described for the preliminary inoculation trial. The inoculated fungus was re-isolated from symptomatic tissue and identified based on morphology. Data were analysed in the same manner as the initial inoculation trial.

Results

Isolates

In total, 163 isolates were obtained from diseased leaves and shoots. Most of the isolates (129) were obtained from symptomatic leaves on trees in plantations or seedlings in nurseries in Riau, Central Sumatra, as the disease was most common in this area (Table 1; Figure 2). Of these, five isolates were collected from *A. crassicarpa* plantations. In addition, 10 isolates were obtained from North Sumatra and 24 from Kalimantan (Table 1; Figure 2). The most commonly isolated species accounted for approximately 84% of the isolates (Figures 2 and 4). The distribution and relative occurrence of *Calonectria* spp. isolated in each region is presented in Figures 2 and 4.

Phylogenetic analyses

Based on the preliminary sequencing results of the *TEF1* and *TUB2* loci for all 163 isolates, 28 representative isolates were chosen for further sequencing of the *CMDA* and *HIS3* gene regions. Amplicons of approximately 660 bp were generated for the *CMDA* gene region, 430 bp for the *HIS3*, 500 bp for the *TEF1* and 560 bp for the *TUB2*. The combined sequence data set used in the phylogenetic analyses included 73 ingroup taxa and 2 214 characters. The ML tree with bootstrap support values is presented in Figure 5. Phylogenetic analyses resulted in the recognition of species residing in two species complexes, including the *Calonectria reteaudii* complex and *Calonectria cylindrospora* complex (Figure 5).

SCORE 0
Healthy seedling, no blight symptoms

SCORE 1
1–25% of innoculated leaves showing blight symptoms

SCORE 2
26–50% of innoculated leaves showing blight symptoms

SCORE 3
51–75% of innoculated leaves showing blight symptoms, up to 25% leaves defoliated

SCORE 4
76–100% of innoculated leaves showing blight symptoms, more than 25% leaves defoliated

Figure 3: Disease severity scoring chart

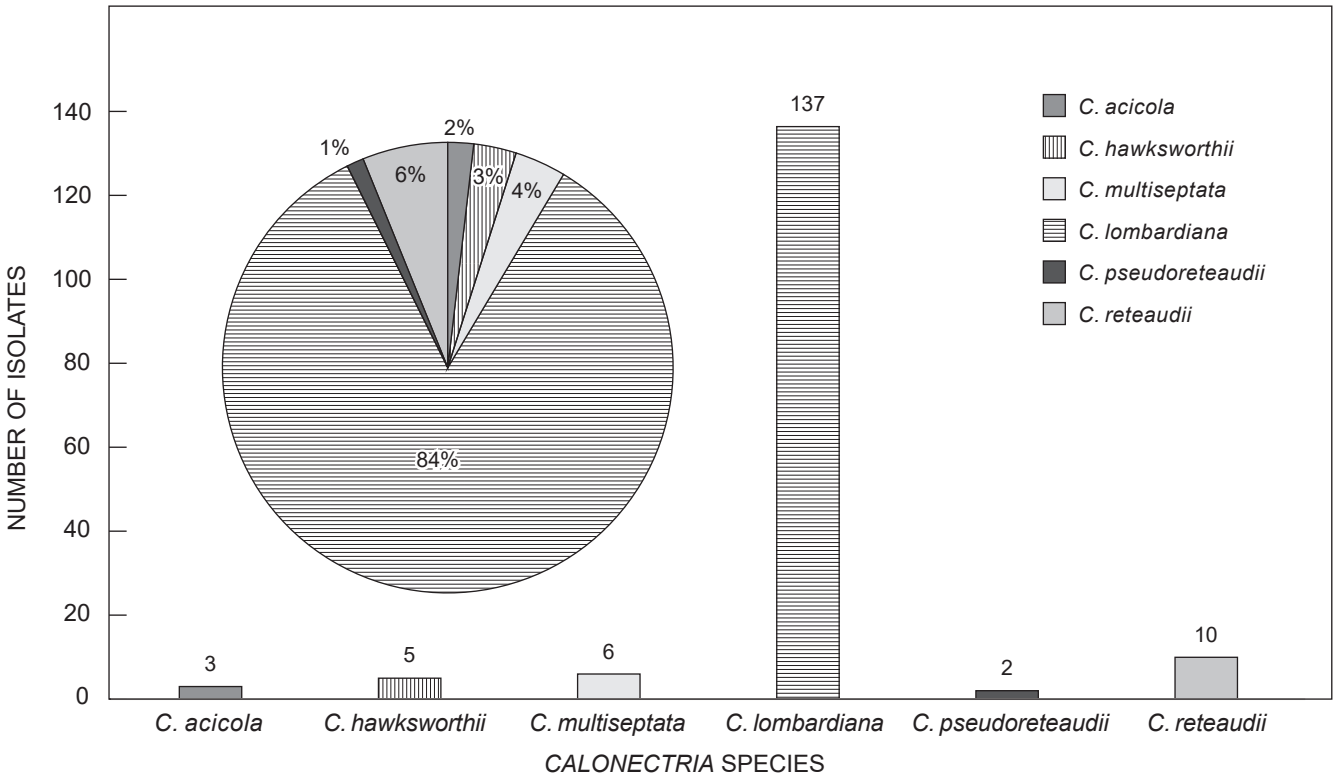

Figure 4: Relative occurrence of the *Calonectria* species from plantations and nurseries in Indonesia. Different species are represented by different patterns

Of the 28 isolates subjected to four gene region phylogenetic analyses, 26 were in the *C. reteaudii* complex and clustered in five clades. Of these, most of the isolates (11) grouped with the ex-type isolate of *C. lombardiana.* In addition, two isolates grouped with *C. pseudoreteaudii,* six with *C. reteaudii*, four with *C. multiseptata* and three with *C. acicola*. The remaining isolates resided in the *C. cylindrospora* complex, of which two isolates were identified as *C. hawksworthii* (Figure 5).

Pathogenicity tests

Preliminary screening

All 12 *Calonectria* isolates, representing 6 species: *C. lombardiana, C. pseudoreteaudii, C. reteaudii, C. acicola, C. multiseptata* and *C. hawksworthi,* were shown to be pathogenic to *Eucalyptus* clone ECL05. Four days after inoculation, all isolates produced severe leaf blight symptoms (Figure 6). The Kruskal–Wallis test (h = 282.05, df = 12 and p < 2.2e-16) confirmed that there were significant differences among the *Calonectria* isolates. No disease symptoms were observed on the plants inoculated as controls (Figure 7, Figure S1). Among all six species, *C. hawksworthii* yielded a lower disease severity score and was thus considered less aggressive (Figure S1). *Calonectria* spp. were re-isolated from lesions on all inoculated plants and identified as representing the inoculated species. No symptoms appeared on the control plants.

Relative tolerance of eucalypt clones to C. lombardiana

Four days after inoculation, all five eucalypt clones inoculated with an isolate of *C. lombardiana* (CMW 54860), shown to be the predominant species in this study, displayed extensive symptoms of leaf blight. In some cases, an infected clone (i.e. ECL03) showed variation in its level of susceptibility (Figure S2). Based on Kruskal–Wallis test results, there were significant differences in susceptibility among the tested clones (h = 80.574, df = 5 and p = 6.365e-16). ECL05 and ECL04 (*E. grandis* × *E. pellita*) were the most susceptible clones to *C. lombardiana*, and showed significant differences from the other clones and the controls (p < 0.05) (Figure S2). ECL01, ECL02 and ECL03 (*E. pellita*) appeared to be more tolerant of infection by *C. lombardiana* than the hybrid clones (Figure 8). *C. lombardiana* was re-isolated from lesions on all inoculated plants. No symptoms appeared on the control plants.

Discussion

A total of 163 isolates of *Calonectria* spp. were characterised from diseased eucalypt seedlings in nurseries or leaves in plantations of North and Central Sumatra as well as East and North Kalimantan, Indonesia. Based on multigene phylogenetic analyses, six species residing in two species complexes were identified. These were *Calonectria lombardiana*, *C. reteaudii, C. acicola, C. multiseptata, C. pseudoreteaudii* and *C. hawksworthii*. An inoculation trial showed that all six *Calonectria* species were pathogenic and that eucalypt

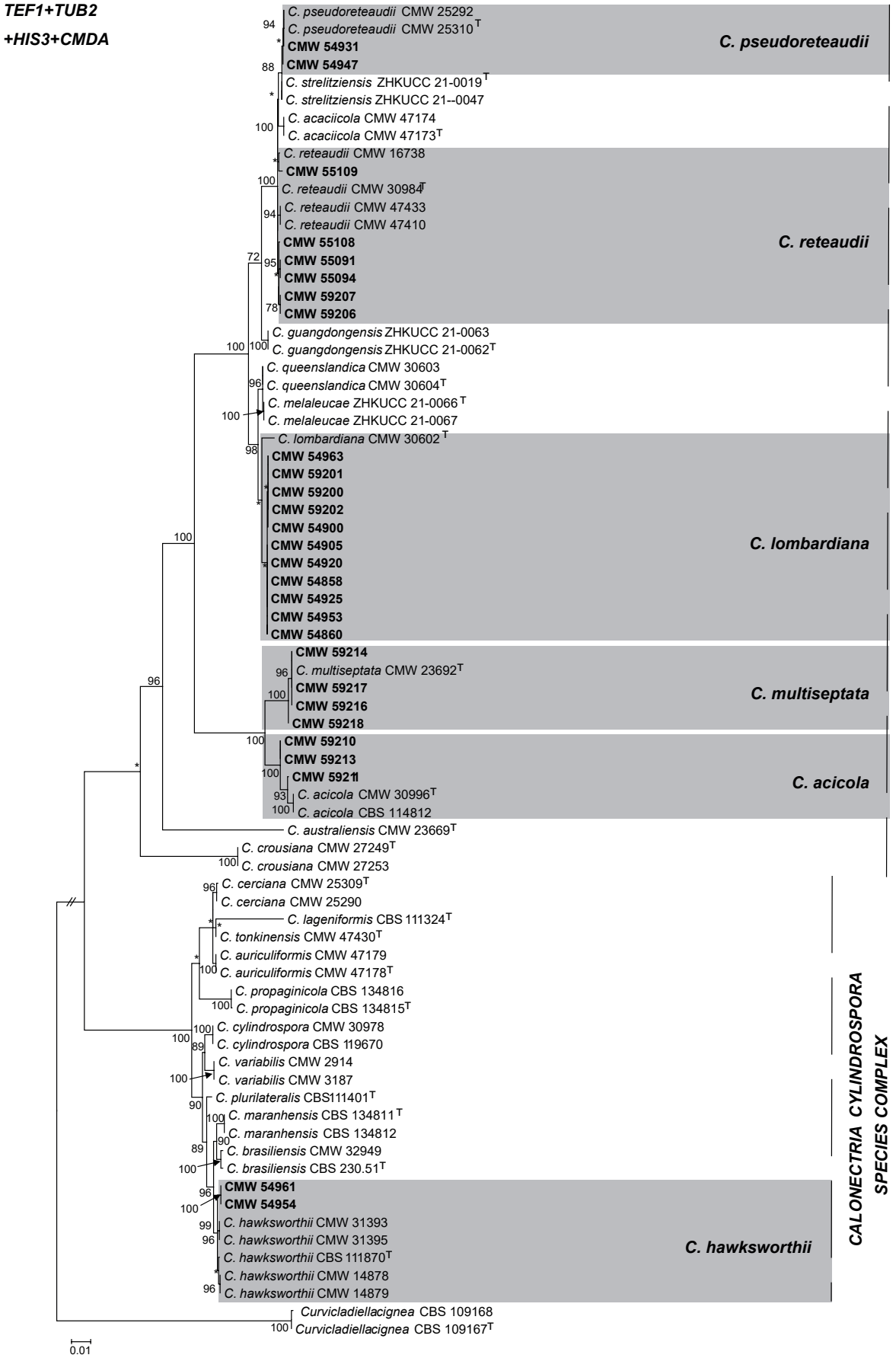

Figure 5: Phylogenetic dendrogram based on maximum likelihood (ML) analysis of a combined data set of *TEF1, TUB2, HIS3* and *CMDA* sequences for *Calonectria* spp. Isolates sequenced in this study are presented in bold face. Bootstrap values of ≥ 70% for ML analyses are indicated at the nodes. Bootstrap values < 70% are marked *. Isolates representing ex-type material are marked **T**. *Curvicladiella cignea* (isolate CBS 109167 and CBS 109168) represents the outgroup

Figure 6: Results of the pathogenicity test on *Eucalyptus* clone ECL05: (a) healthy plants; (b) infected plants 2 days after inoculation (dai) with moderate leaf blight; (c) infected plants at 4 dai with severe leaf blight resulting in plant die-off and defoliation

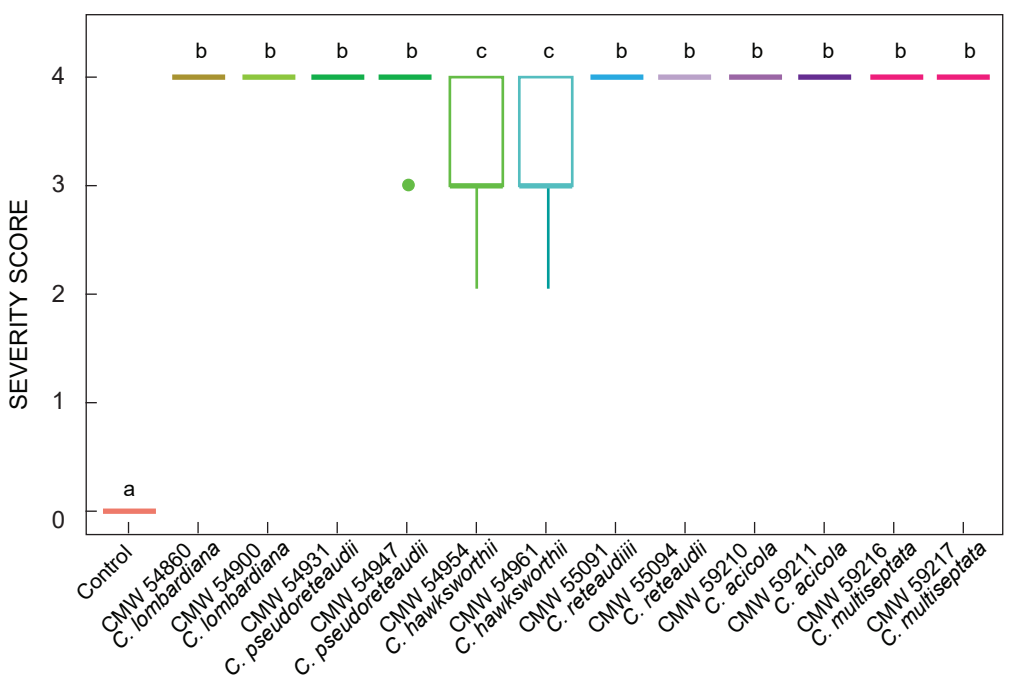

Figure 7: Graphical representations of *Eucalyptus* clone ECL05 pathogenicity trials using 12 different *Calonectria* isolates representing six different *Calonectria* spp. Vertical bars represent the standard error of the means. Different letters indicate statistically significance at $p \leq 0.05$

genotypes differed in their susceptibility to *C. lombardiana,* which was the most commonly isolated species.

Species in the *C. reteaudii* species complex emerged as the most diverse in this study. Most species in this complex are well-known pathogens associated with leaf and shoot blight on eucalypts and they have predominantly been found in tropical and subtropical regions of Southeast Asia, South China and Australasia (Crous 2002; Old et al. 2003; Crous et al. 2006; Lombard et al. 2010b; Li et al. 2017; Pham et al. 2019; Liu et al. 2020; Wang and Chen 2020; Li et al. 2022). This is the first report of *C. acicola*, *C. pseudoreteaudii* and *C. lombardiana* from Indonesia.

Calonectria lombardiana was the predominant species in all sampling areas and accounted for approximately 84% of the isolates. Given its predominance, it is interesting that this fungus has not previously been reported from Indonesia.

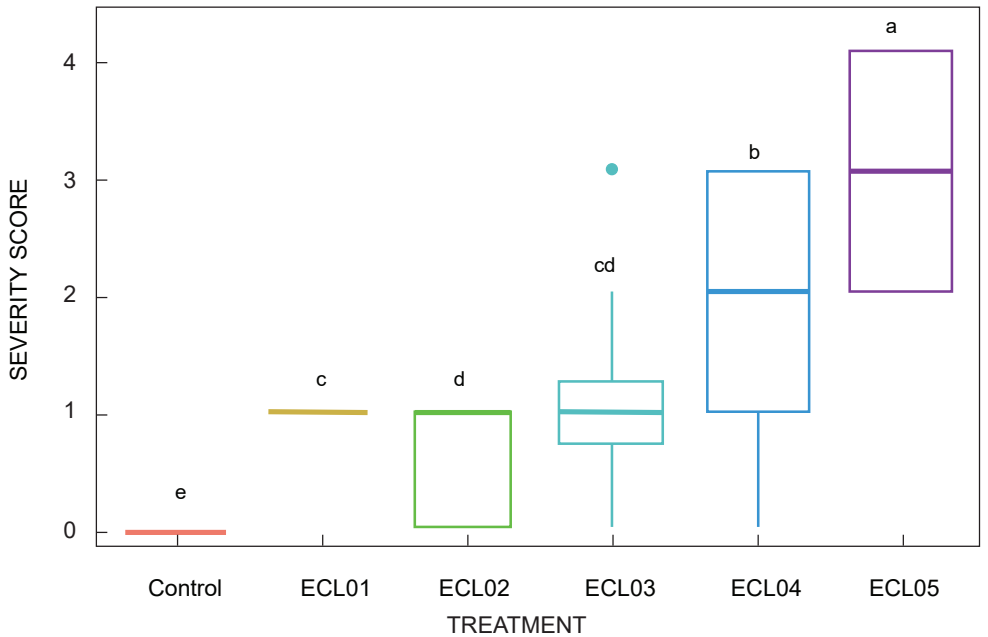

Figure 8: Box plot indicating the severity score resulting from inoculation trials of five eucalypt genotypes inoculated with *C. lombardiana* (CMW 54860) and the controls. Vertical bars represent the standard error of the means. Different letters indicate statistical significance at $p \leq 0.05$

This species was first isolated from *Xanthorrhoea australis* in Australia (Crous 2002). *Calonectria lombardiana* was collected from both nurseries and plantations in all sampling sites in Central Sumatra, East Kalimantan and North Kalimantan, but was not found in North Sumatra. Besides being the most commonly occurring species, *C. lombardiana* emerged as one of the most aggressive species in pathogenicity tests.

Calonectria hawksworthii was the only species in the *C. cylindrospora* complex found in this study. This species was previously found to cause leaf spots on *Nelumbo nucifera* in Mauritius (Crous 2002) and on eucalypts in Indonesia and China (Lombard et al. 2010b; Lombard et al. 2015). In pathogenicity trials, it can cause leaf blight symptoms, although less aggressively than the other species tested in this study.

Pathogenicity tests in this study showed that all six species of *Calonectria* were pathogenic to a single clone of *Eucalyptus*. However, *C. hawksworthii* was clearly less aggressive than the other five species. Of those five species, four species (*C. lombardiana*, *C. multiseptata*, *C. reteaudii* and *C. pseudoreteaudii*) have been previously reported on eucalypts. The remaining species (*C. acicola*) was previously known only from *Pinus radiata* in New Zealand (Gadgil and Dick 2004). This is the first report of *C. acicola* infecting eucalypts.

When an isolate of the most commonly occurring species (*C. lombardiana*) was inoculated on different genotypes of eucalypt, these plants were shown to differ in their susceptibility to infection. In this study, hybrids of *E. pellita* and *E. grandis* were more susceptible to leaf blight than pure *E. pellita* genotypes. This highlights the importance of selecting disease resistant eucalypt genotypes to avoid CLB in the future, similar to the situation with various other eucalypt disease problems that have been resolved through active breeding and selection of disease-tolerant planting stock (Wingfield 2003; van Heerden et al. 2005).

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References

Alfenas RF, Pereira OL, Freitas RG, Freitas CS, Dita MAD, Alfenas AC. 2013. Mass spore production and inoculation of *Calonectria pteridis* on *Eucalyptus* spp. under different environmental conditions. *Tropical Plant Pathology* 38: 406–413.

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

Bophela KN, Venter SN, Wingfield MJ, Duran A, Tarigan M, Coutinho TA. 2019. *Xanthomonas perforans*: a tomato and pepper pathogen associated with bacterial blight and dieback of *Eucalyptus pellita* seedlings in Indonesia. *Australasian Plant Pathology* 48: 543–551.

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

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.

Coetzee MPA, Wingfield BD, Golani GD, Tjahjono B, Gafur A,

Wingfield MJ. 2011. A single dominant *Ganoderma* species is responsible for root rot of *Acacia mangium* and *Eucalyptus* in Sumatra. *Southern Forests* 73: 175–180.

Couto L, Nicholas I, Wright L. 2011. Short rotation eucalypt plantations for energy in Brazil. *IEA Bioenergy Task* 43: 02.

Crous PW. 2002. *Taxonomy and pathology of Cylindrocladium (Calonectria) and allied genera*. Minnesota: American Phytopathological Society (APS Press).

Crous PW, Wingfield MJ, Mohammed C, Yuan ZQ. 1998. New foliar pathogens of *Eucalyptus* from Australia and Indonesia. *Mycological Research* 102: 527–532.

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

Crous PW, Wingfield MJ, Cheewangkoon R, Carnegie AJ, Burgess TI, et al. 2019. Foliar pathogens of eucalypts. *Studies in Mycology* 94: 125–298.

Gadgil PD, Dick MA. 2004. Fungi silvicolae novazelandiae: 5. *New Zealand Journal of Forestry Science* 34: 316–323.

Groenewald JZ, Nakashima C, Nishikawa J, Shin HD, Park JH, et al. 2013. Species concepts in *Cercospora*: spotting the weeds among the roses. *Studies in Mycology* 75: 115–170.

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.

Harwood CE, Nambiar EKS. 2014. *Sustainable plantation forestry in South-East Asia.* Report EP14685. Canberra: Australian Centre for International Agricultural Research Sustainable Agriculture Flagship and CSIRO Ecosystem Sciences.

Hodges CS, May LC. 1972. A root disease of pine, *Araucaria*, and *Eucalyptus* in Brazil caused by a new species of *Cylindrocladium*. *Phytopathology* 62: 898–901.

Jami F, Marincowitz S, Durán A, Slippers B, Abad JIM, et al. 2022. Botryosphaeriaceae diversity on *Eucalyptus* clones in different climate zones of Indonesia. *Forest Pathology* 52: e12737.

Jiang GZ, Gao F, Yue H, Tao L, He XY. 2019. First report of fruit spot of *Macadamia* sp. caused by *Calonectria pentaseptata* in China. *Plant Disease* 104: 575.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870–1874.

Li JQ, Wingfield MJ, Liu QL, Barnes I, Roux J, et al. 2017. *Calonectria* species isolated from *Eucalyptus* plantations and nurseries in South China. *IMA Fungus* 8: 259–286.

Li WW, Chen SF, Wingfield MJ, Duong TA. 2022. *Calonectria queenslandica*: Causal agent of eucalyptus leaf blight in southern China. *Plant Disease* 10.1094/PDIS-01-22-0196-RE.

Liu QL, Li JQ, Wingfield MJ, Duong TA, Wingfield BD, et al. 2020. Reconsideration of species boundaries and proposed DNA barcodes for *Calonectria*. *Studies in Mycology* 97: 100106.

Liu QL, Wingfield MJ, Doung TA, Wingfield BD, Chen SF. 2022. Diversity and distribution of *Calonectria* species from plantation and forest soils in Fujian Province, China. *Journal of Fungi* 8: 811.

Lombard L, Chen SF, Mou X, Zhou XD, Crous PW, Wingfield MJ. 2015. New species, hyper-diversity and potential importance of *Calonectria* spp. from *Eucalyptus* in south China. *Studies in Mycology* 80: 151–188.

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, 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. 2010b. *Calonectria* species associated with cutting rot of *Eucalyptus*. *Persoonia* 24: 1–11.

Lopes UP, Alfenas RF, Zambolim L, Crous PW, Costa H, Pereira OL. 2018. A new species of *Calonectria* causing rot on ripe strawberry fruit in Brazil. *Australasian Plant Pathology* 47: 1–11.

Marin-Felix Y, Groenewald JZ, Cai L, Chen Q, Marincowitz S, et al. 2017. Genera of phytopathogenic fungi: GOPHY 1. *Studies in Mycology* 86: 99–216.

McTaggart AR, Roux J, Granados GM, Gafur A, Tarigan M, et al. 2016. Rust (*Puccinia psidii*) recorded in Indonesia poses a threat to forests and forestry in South-East Asia. *Australasian Plant Pathology* 45: 83–89.

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.

O'Donnell K, Kistler HC, Cigelnik E, Ploetz RC. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. *Proceedings of the National Academy of Sciences of the USA* 95: 2044–2049.

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

Pham NQ, Barnes I, Chen SF, Liu FF, Dang QN, et al. 2019. Ten new species of *Calonectria* from Indonesia and Vietnam. *Mycologia* 111: 78–102.

Pham NQ, Marincowitz S, Chen SF, Yaparudin Y, Wingfield MJ. 2022. *Calonectria* species, including four novel taxa, associated with *Eucalyptus* in Malaysia. *Mycological Progress* 21: 181–197.

Pham NQ, Marincowitz S, Solís M, Duong TA, Wingfield BD, et al. 2021. Eucalyptus scab and shoot malformation: A new and serious foliar disease of *Eucalyptus* caused by *Elsinoe necatrix* sp. nov. *Plant Pathology* 70: 1230–1242.

R Core Team. 2020. *R: a language and environment for statistical computing*. Vienna, Austria: *R foundation for statistical computing*. Available at https://www.R-project .org

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

Siregar BA, Giyanto, Hidayat SH, Siregar IZ, Tjahjono B. 2020. Epidemiology of bacterial wilt disease on *Eucalyptus pellita* F. Muell. in Indonesia. *IOP Conference Series: Earth and Environmental Science* 468: 012033.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.

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

Wang QC, Chen SF. 2020. *Calonectria pentaseptata* causes severe leaf disease of cultivated *Eucalyptus* on the Leizhou peninsula of southern China. *Plant Disease* 104: 493–509.

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

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