***CALONECTRIA* SPECIES DIVERSITY ON EUCALYPTS IN INDONESIA**

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Marthin Tarigan[1,2], Nam Q. Pham[1,*], Fahimeh Jami[3,4], Leonardo S.S. Oliveira[5], Muhammad Agni Saha[2], Alvaro Durán[2], Michael J. Wingfield[1]

[1]Department of Plant and Soil Sciences, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa.

[2]Plant Health Program, Research and Development, Asia Pacific Resources International Holdings Ltd. (APRIL), Pangkalan Kerinci 28300, Riau, Indonesia.

[3]Department of Biochemistry, Genetic & Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa.

[4]Agricultural Research Council, Pretoria 0002, South Africa.

[5]Forest Management, Research & Development, Bracell, Alagoinhas 48030-300, Bahia, Brazil.

*Corresponding author: Nam.Pham@fabi.up.ac.za

ABSTRACT

Diseases increasingly threaten the rapidly expanding *Eucalyptus* plantation industry of Indonesia. Of these, leaf blight caused by *Calonectria* spp. is considered amongst the more important problems, imparting 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 *Eucalyptus* 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 *Eucalyptus* in artificial inoculation studies. *Calonectria lombardiana* was generally the most pathogenic species and *Eucalyptus* 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

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; Pham et al. 2019; Li et al. 2017; Lopes et al. 2018; Jiang 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).

Eucalyptus is the most widely planted tree 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 *Eucalyptus* 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 *Eucalyptus* worldwide (Crous et al. 2019; Liu et al. 2020). Several of these species were reported to cause serious leaf and shoot blight disease in *Eucalyptus* 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 programs reliant on *Eucalyptus* have expanded rapidly in Indonesia and especially in the islands of Sumatra and Kalimantan since the early 1990's (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). Particularly in the nursery situation, these pathogens are able to spread rapidly, and losses can seriously hamper nursery production or plantation establishment. The aims of this study were consequently to identify *Calonectria* species causing diseases in *Eucalyptus* nurseries and plantations in Indonesia and to assess their relative importance by 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 *Eucalyptus* nurseries and 26 plantation sites; and two *Acacia crassicarpa* plantation sites in proximity to *Eucalyptus* 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.

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 disinfested in 0.5 % sodium hypochlorite for 30 seconds and rinsed three times in sterile distilled water. Surface-disinfested plant segments were placed onto the surface of potato dextrose agar (PDA Acumedia®: 40 g/L) 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 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 mM), 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 CDMA; 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 CDMA; 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 GTR substitution matrix and 1,000 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 trees 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 the method described by 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 was 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 (approx. 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 the inoculum suspension was then diluted to 1×10^6 spores/mL.

Inoculations were conducted on a 14-week-old *E. grandis* x *E. pellita* clone (ECL05). Two mL of a 1 × 10^6 spore suspension of each isolate was sprayed onto the surface of 30 plants until run-off. After inoculation, a piece of wet cotton was placed at 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 randomized 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 in R statistical software, version 3.2.0 (R Core Team 2020).

Relative tolerance of *Eucalyptus* clones*.* Five *Eucalyptus* genotypes that included three *E. pellita* clones (ECL01, ECL02, and ECL03) and two *E. grandis* x *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. The trial was arranged in a completely randomized 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 (Figure 2; Figure 4). The distribution and relative occurrence of *Calonectria* spp. isolated in each region is presented in Figure 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 dataset used in the phylogenetic analyses included 73 ingroup taxa and 2214 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).

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, the majority 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 six 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 (p-value) < 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 plant and identified as representing the inoculated species. No symptoms appeared on the control plants.

Relative tolerance of *Eucalyptus* clones to *C. lombardiana*. Four days after inoculation, all five *Eucalyptus* 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* x *E. pellita*) were the most susceptible clones to *C. lombardiana*, where they showed significant differences from the other clones and the controls (P < 0.05) (Figure 10). ECL01, ECL02 and ECL03 (*E. pellita*) appeared to be more tolerant to infection by *C. lombardiana* than the hybrid clones (Figure 8). *Calonectria 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 characterized from diseased *Eucalyptus* 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 included *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 *Eucalyptus* 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 *Eucalyptus* 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; Liu 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 the predominance of this fungus, it is interesting that it has not previously been reported from Indonesia. This species was first isolated from *Xanthorrhoea australis* in Australia (Crous 2002). *Calonectria lombardiana* was collected from both nursery and plantation 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 *Eucalyptus* in Indonesia and China (Lombard et al. 2010b, 2015). In pathogenicity trials, it can cause leaf blight symptoms, however, was less aggressive than the other species tested in the present 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 *Eucalyptus*. 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 *Eucalyptus*.

When an isolate of the most commonly occurring species (*C. lombardiana*) was inoculated on different genotypes of *Eucalyptus*, 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 *Eucalyptus* genotypes to avoid CLB in the future, similar to the situation with various other *Eucalyptus* disease problems that have been resolved through active breeding and selection of disease tolerant planting stock (van Heerden et al. 2005; Wingfield 2003).

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ORCID IDS

Marthin Tarigan – https://orcid.org/0000-0002-9128-2650

Nam Q Pham – https://orcid.org/0000-0002-4938-9067

Fahimeh Jami – https://orcid.org/0000-0002-0550-3550

Leonardo Oliveira – https://orcid.org/0000-0002-4056-6987

Muhammad Agni Saha – https://orcid.org/0000-0002-9695-9309

Alvaro Duran – https://orcid.org/0000-0002-3035-9087

Michael J Wingfield – https://orcid.org/0000-0001-9346-2009

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Figures

Figure 1. Symptoms of *Calonectria* infection: (a) on leave of *Eucalyptus* seedlings; (b) on stems of *Eucalyptus* seedlings; (c) on leave of *Eucalyptus* tree in the field

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

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 colours.

Figure 5. Phylogenetic tree 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 boldface. Bootstrap values of ≥70% for ML analyses are indicated at the nodes. Bootstrap values <70% are marked with "*". Isolates representing ex-type material are marked with "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-d after inoculation (dai) with moderate leaf blight; (c) Infected plants at 4-dai with severely 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 $p \leq 0.05$.

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

Table

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

Supplementary files

Figure S1. Stack bar graphs representing the aggressiveness of different *Calonectria* spp. on *Eucalyptus* clone ECL05 assessed using 0–4 scale.

Figure S2. Stack bar graphs representing the aggressiveness of *C. lombardiana* (CMW 54860) on five *Eucalyptus* clones assessed using 0–4 scale.

Table S1. Collection details and GenBank accession numbers of isolates included in the phylogenetic analyses.

Figures

Figure 1.

Figure 2.

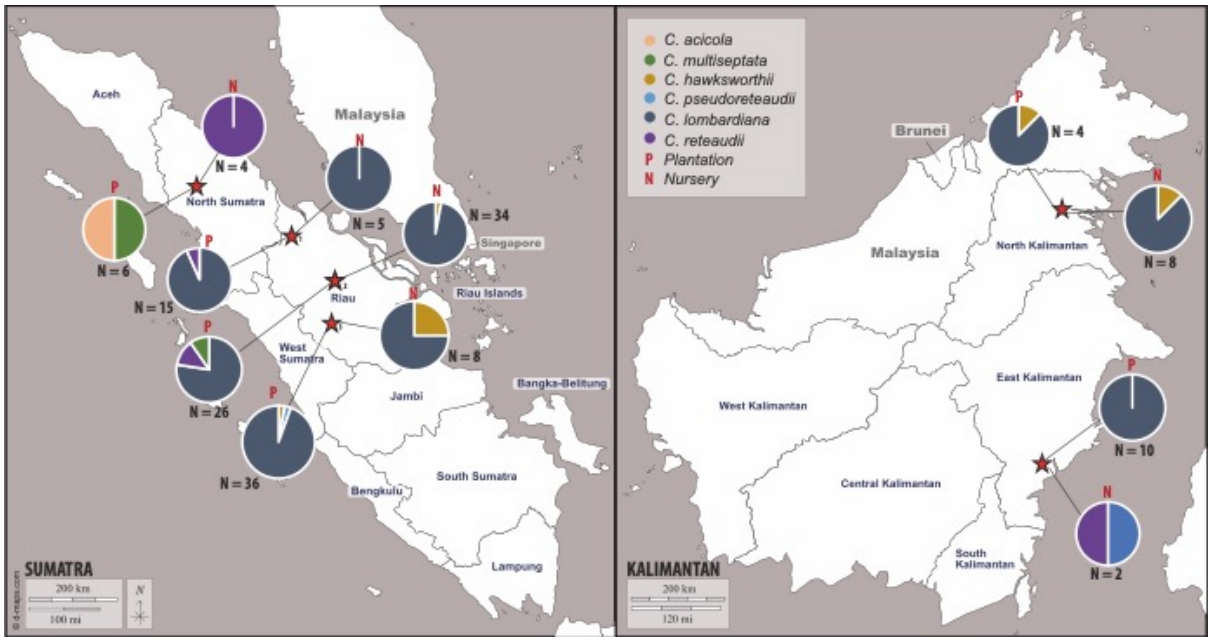
C. acicola
C. multiseptata
C. hawksworthii
C. pseudoreteaudii
C. lombardiana
C. reteaudii
P Plantation
N Nursery
Aceh
North Sumatra
Malaysia
Singapore
Riau
Riau Islands
West Sumatra
Jambi
Bangka-Belitung
South Sumatra
Bengkulu
Lampung
SUMATRA
N = 4
N = 6
N = 15
N = 26
N = 36
N = 5
N = 34
N = 8
Brunei
Malaysia
North Kalimantan
West Kalimantan
East Kalimantan
Central Kalimantan
South Kalimantan
KALIMANTAN
N = 4
N = 8
N = 10
N = 2

Figure 3.

SCORE 0
Healthy seedling, no leaves blight symptom

SCORE 1
1–25 % of inoculated leaves showing leaves blight symptom

SCORE 2
26–50 % of inoculated leaves showing leaves blight symptom

SCORE 3
51–75 % of inoculated leaves showing leaves blight symptom, up to 25 % leaves defoliated

SCORE 4
76–100 % of inoculated leaves showing leaves blight symptom, more than 25 % leaves defoliated

Figure 4.

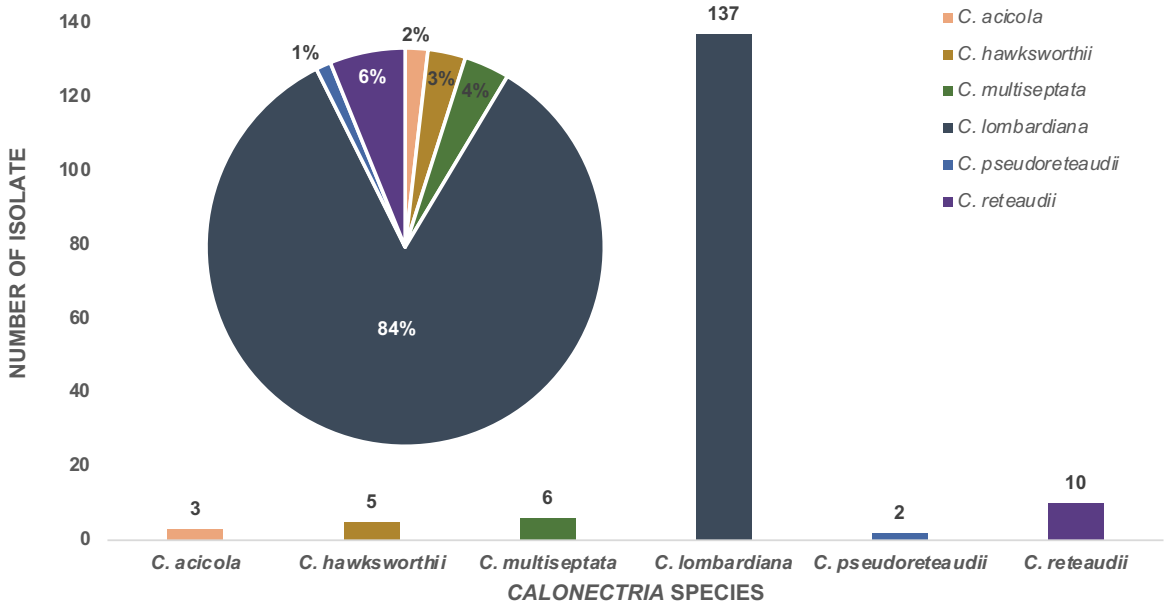

Figure 5.

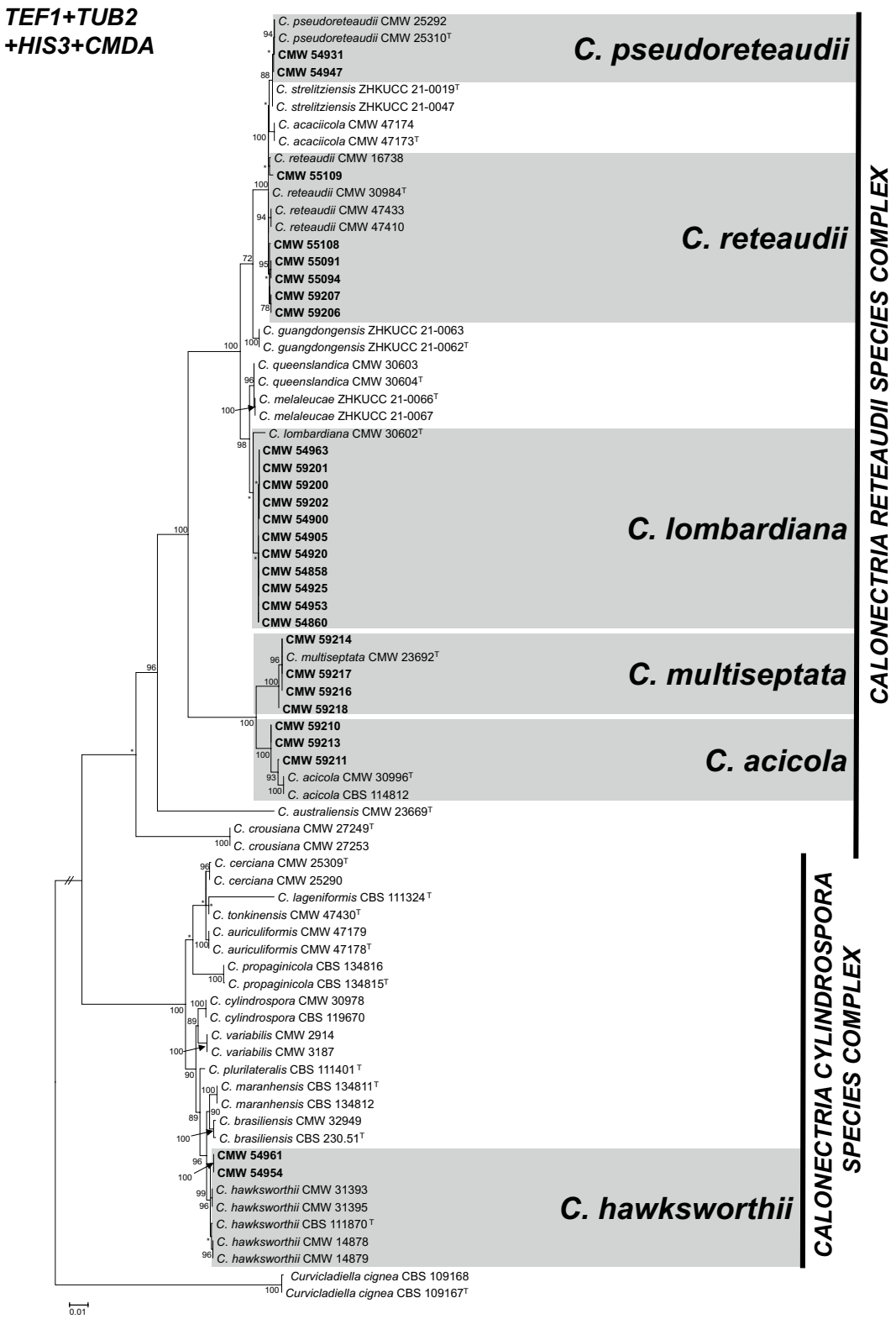
TEF1+TUB2
+HIS3+CMDA
C. pseudoreteaudii CMW 25292
C. pseudoreteaudii CMW 25310T
CMW 54931
CMW 54947
C. pseudoreteaudii
C. strelitziensis ZHKUCC 21-0019T
C. strelitziensis ZHKUCC 21-0047
C. acaciicola CMW 47174
C. acaciicola CMW 47173T
C. reteaudii CMW 16738
CMW 55109
C. reteaudii CMW 30984T
C. reteaudii CMW 47433
C. reteaudii CMW 47410
CMW 55108
CMW 55091
CMW 55094
CMW 59207
CMW 59206
C. reteaudii
C. guangdongensis ZHKUCC 21-0063
C. guangdongensis ZHKUCC 21-0062T
C. queenslandica CMW 30603
C. queenslandica CMW 30604T
C. melaleucae ZHKUCC 21-0066T
C. melaleucae ZHKUCC 21-0067
C. lombardiana CMW 30602T
CMW 54963
CMW 59201
CMW 59200
CMW 59202
CMW 54900
CMW 54905
CMW 54920
CMW 54858
CMW 54925
CMW 54953
CMW 54860
C. lombardiana
CMW 59214
C. multiseptata CMW 23692T
CMW 59217
CMW 59216
CMW 59218
C. multiseptata
CMW 59210
CMW 59213
CMW 59211
C. acicola CMW 30996T
C. acicola CBS 114812
C. acicola
C. australiensis CMW 23669T
C. crousiana CMW 27249T
C. crousiana CMW 27253
C. cerciana CMW 25309T
C. cerciana CMW 25290
C. lageniformis CBS 111324T
C. tonkinensis CMW 47430T
C. auriculiformis CMW 47179
C. auriculiformis CMW 47178T
C. propaginicola CBS 134816
C. propaginicola CBS 134815T
C. cylindrospora CMW 30978
C. cylindrospora CBS 119670
C. variabilis CMW 2914
C. variabilis CMW 3187
C. plurilateralis CBS 111401T
C. maranhensis CBS 134811T
C. maranhensis CBS 134812
C. brasiliensis CMW 32949
C. brasiliensis CBS 230.51T
CMW 54961
CMW 54954
C. hawksworthii CMW 31393
C. hawksworthii CMW 31395
C. hawksworthii CBS 111870T
C. hawksworthii CMW 14878
C. hawksworthii CMW 14879
C. hawksworthii
Curvicladiella cignea CBS 109168
Curvicladiella cignea CBS 109167T
0.01
CALONECTRIA RETEAUDII SPECIES COMPLEX
CALONECTRIA CYLINDROSPORA SPECIES COMPLEX

Figure 6.

Figure 7.

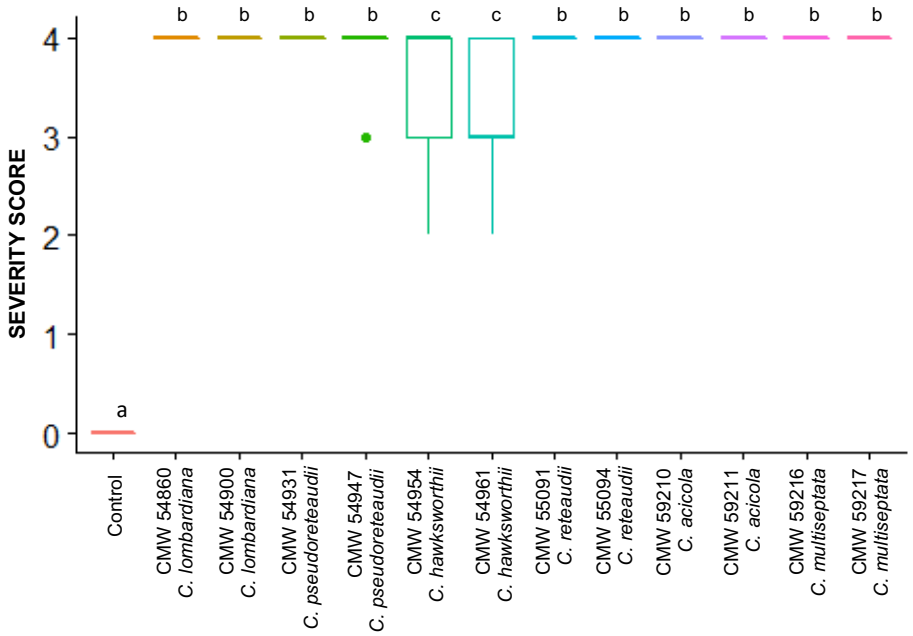

Figure 8.

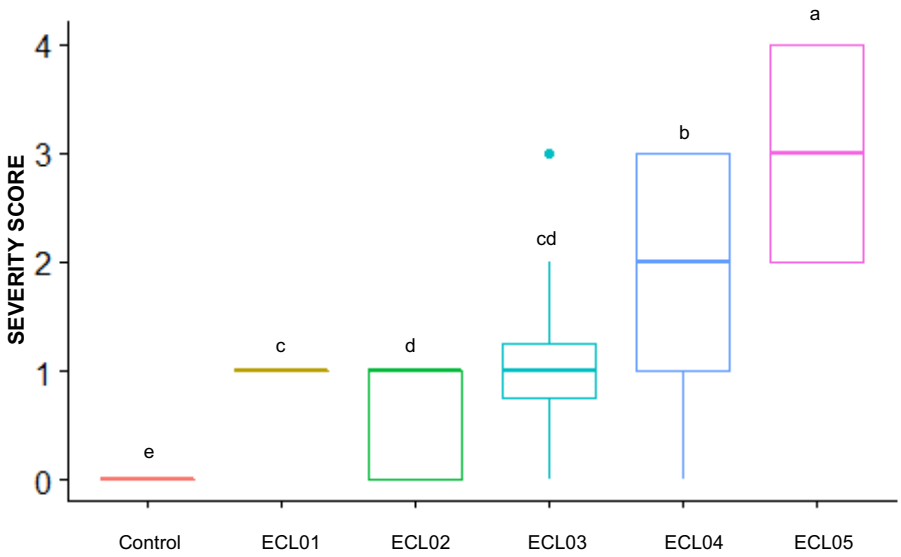
SEVERITY SCORE
0
1
2
3
4
a
b
cd
c
d
e
Control
ECL01
ECL02
ECL03
ECL04
ECL05

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

Region	Altitude (masl)	Nursery	Plantation	Total
North Sumatera (Porsea)	1200	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

Supplementary materials

Figure S1. Stack bar graphs representing the aggressiveness of different *Calonectria* spp. on *Eucalyptus* clone ECL05 assessed using 0–4 scale.

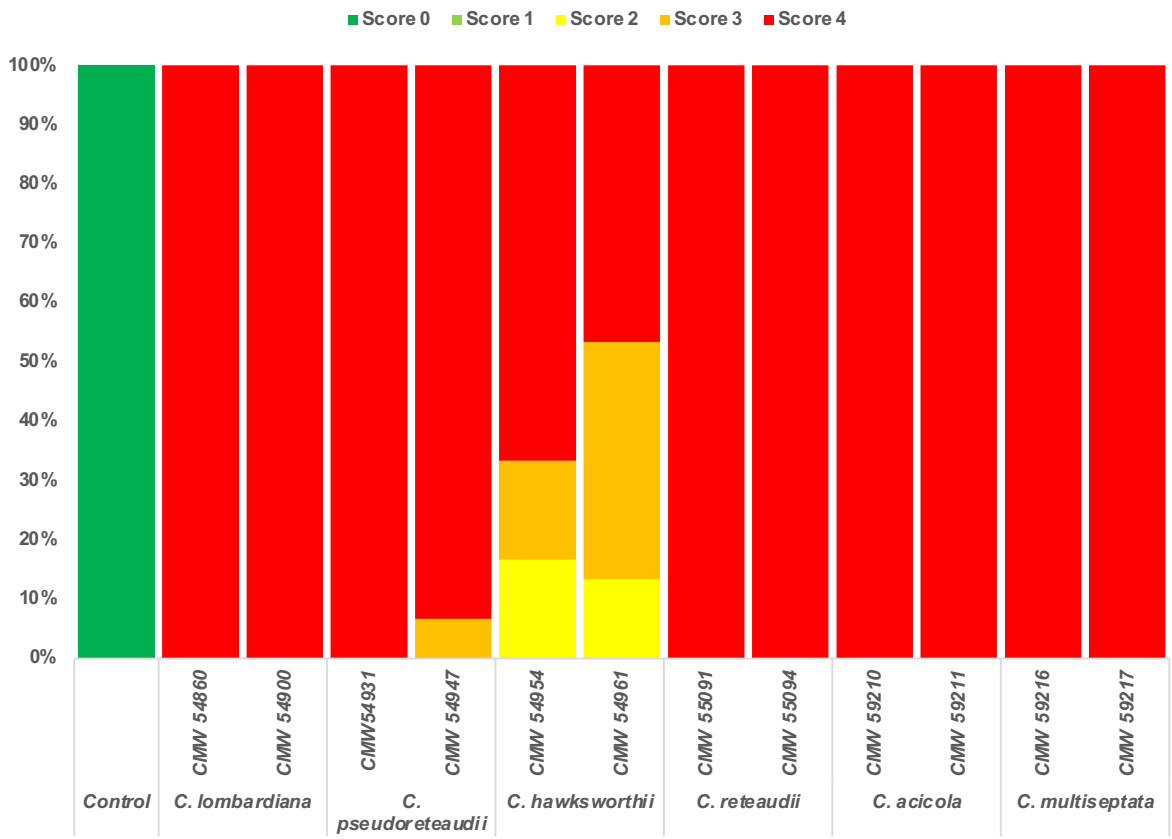

Figure S2. Stack bar graphs representing the aggressiveness of *C. lombardiana* (CMW 54860) on five *Eucalyptus* clones assessed using 0–4 scale.

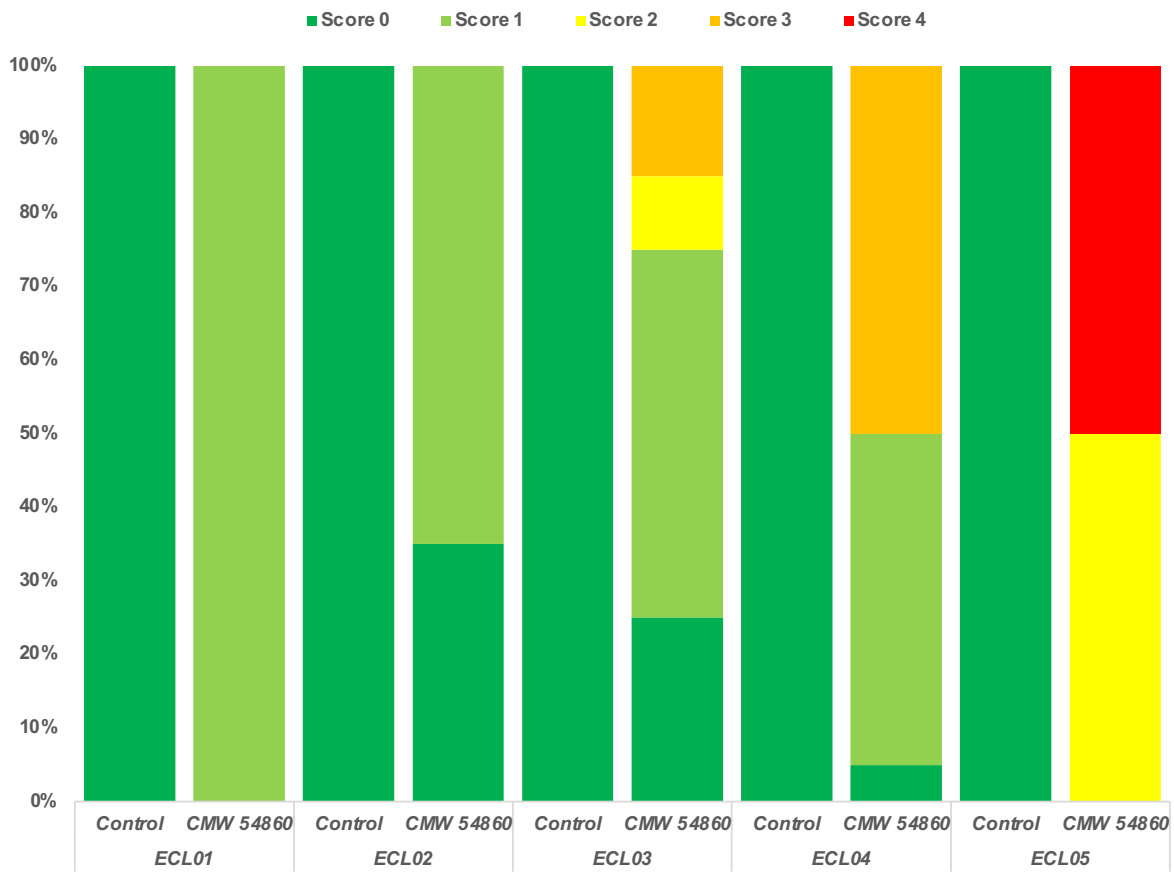

Table S1. Collection details and GenBank accessions of isolates included in the phylogenetic analyses.

Species	Isolate number	Host/substrate	Locality	GenBank accessions				References
				CMDA	*HIS3*	*TEF1*	*TUB2*	
Calonectria acaciicola	CMW 47173^{T} = CBS 143557	Soil	Vietnam	MT335160	MT335399	MT412690	MT412930	Liu et al. (2020)
Calonectria acaciicola	CMW 47174 = CBS 143558	Soil	Vietnam	MT335161	MT335400	MT412691	MT412931	Liu et al. (2020)
Calonectria acicola	**CMW 59210**	*Eucalyptus grandis x Eucalyptus urophylla*	Porsea, North Sumatra, Indonesia	OQ296456	OQ296482	OQ296505	OQ296532	This study
Calonectria acicola	**CMW 59211**	*Eucalyptus grandis x Eucalyptus urophylla*	Porsea, North Sumatra, Indonesia	OQ296457	OQ296483	OQ296506	OQ296533	This study
Calonectria acicola	**CMW 59213**	*Eucalyptus grandis x Eucalyptus urophylla*	Porsea, North Sumatra, Indonesia	OQ296458	OQ296484	OQ296507	OQ296534	This study
Calonectria acicola	CMW 30996^{T}	*Phoenix canariensis*	New Zealand	MT335162	MT335401	MT412692	MT412932	Liu et al. (2020)
Calonectria acicola	CBS 114812 = CMW 51216	*Phoenix canariensis*	New Zealand	MT335163	MT335402	MT412693	MT412933	Liu et al. (2020)
Calonectria auriculiformis	CMW 47178^{T} = CBS 143561	Soil	Vietnam	MT335190	MT335430	MT412721	MT412944	Liu et al. (2020)
Calonectria auriculiformis	CMW 47179 = CBS 143562	Soil	Vietnam	MT335191	MT335431	MT412722	MT412945	Liu et al. (2020)
Calonectria australiensis	CMW 23669^{T} = CBS 112954 = CPC 4714	*Ficus pleurocarpa*	Australia	MT335192	MT335432	MT412723	MT412946	Liu et al. (2020)
Calonectria brasiliensis	CBS 230.51^{T} = IMI 299576	*Eucalyptus* sp.	Brazil	MT335200	MT335440	MT412731	MT412953	Liu et al. (2020)
Calonectria brasiliensis	CMW 32949 = CBS 114257 = CPC 1944	*Eucalyptus* sp.	Brazil	MT335201	MT335441	MT412732	MT412954	Liu et al. (2020)
Calonectria cerciana	CMW 25309^{T} = CBS 123693	*Eucalyptus urophylla* × *Eucalyptus grandis*	China	MT335211	MT335451	MT412742	MT412963	Liu et al. (2020)
Calonectria cerciana	CMW 25290 = CBS 123695	*Eucalyptus urophylla* × *Eucalyptus grandis*	China	MT335212	MT335452	MT412743	MT412964	Liu et al. (2020)
Calonectria crousiana	CMW 27249^{T} = CBS 127198	*Eucalyptus grandis*	China	MT335230	MT335470	MT412761	MT412982	Liu et al. (2020)
Calonectria crousiana	CMW 27253 = CBS 127199	*Eucalyptus grandis*	China	MT335231	MT335471	MT412762	MT412983	Liu et al. (2020)
Calonectria cylindrospora	CMW 30978 = CBS 110666 = STE-U 497	*Ilex vomitoria*	USA	MT335237	MT335477	MT412768	MT412986	Liu et al. (2020)
Calonectria cylindrospora	CBS 119670 = CMW 51310 = CPC 12766	*Pistacia lentiscus*	Italy	MT335236	MT335476	MT412767	MT412985	Liu et al. (2020)
Calonectria guangdongensis	ZHKUCC 21-0062^{T}	*Heliconia metallica*	China	MZ491127	N/A	MZ491149	MZ491171	Zhang et al. (2022)
Calonectria guangdongensis	ZHKUCC 21-0063	*Heliconia metallica*	China	MZ491128	N/A	MZ491150	MZ491172	Zhang et al. (2022)
Calonectria hawksworthii	**CMW 54954**	*Eucalyptus grandis x Eucalyptus pellita*	Kalimantan, Indonesia	OQ296459	OQ296485	OQ296508	OQ296535	This study
Calonectria hawksworthii	**CMW 54961**	*Eucalyptus pellita*	Kalimantan, Indonesia	OQ296460	OQ296486	OQ296509	OQ296536	This study
Calonectria hawksworthii	CMW 14878^{T} = CBS 125277	*Eucalyptus* sp.	Indonesia	MT335378	MT335618	MT412909	MT413119	Liu et al. (2020)
Calonectria hawksworthii	CMW 14879 = CBS 125253	*Eucalyptus* sp.	Indonesia	MT335379	MT335619	MT412910	MT413120	Liu et al. (2020)
Calonectria hawksworthii	CMW 31395	*Eucalyptus urophylla* × *Eucalyptus grandis*	China	MT335248	MT335488	MT412779	MT412997	Liu et al. (2020)

Calonectria hawksworthii	CMW 31393 = CBS 136641	*Eucalyptus urophylla* × *Eucalyptus grandis*	China	MT335247	MT335487	MT412778	MT412996	Liu et al. (2020)
Calonectria hawksworthii	CBS 111870^{T} = CMW 51194 = CPC 2405	*Nelumbo nucifera*	Mauritius	MT335254	MT335494	MT412785	MT413003	Liu et al. (2020)
Calonectria lageniformis	CBS 111324^{T} = CMW 51177 = CPC 1473	*Eucalyptus* sp.	Mauritius	KX784574	N/A	KX784702	KX784632	Marin-Felix et al. (2017)
Calonectria lombardiana	CMW 30602^{T} = CBS 112634	*Xanthorrhoea australis*	Australia	MT335395	MT335635	MT412926	MT413133	Liu et al. (2020)
Calonectria lombardiana	**CMW 54858**	*Eucalyptus pellita*	Teso, Riau, Indonesia	OQ296461	OQ296487	OQ296510	OQ296537	This study
Calonectria lombardiana	**CMW 54860**	*Eucalyptus grandis x Eucalyptus pellita*	Pelalawan, Riau, Indonesia	OQ296462	OQ296488	OQ296511	OQ296538	This study
Calonectria lombardiana	**CMW 54900**	*Eucalyptus pellita*	Teso, Riau, Indonesia	OQ296463	N/A	N/A	OQ296539	This study
Calonectria lombardiana	**CMW 54905**	*Eucalyptus pellita*	Pelalawan, Riau, Indonesia	N/A	OQ296489	OQ296512	OQ296540	This study
Calonectria lombardiana	**CMW 54920**	*Eucalyptus pellita*	Pelalawan, Riau, Indonesia	OQ296464	OQ296490	OQ296513	OQ296541	This study
Calonectria lombardiana	**CMW 54925**	*Eucalyptus grandis x Eucalyptus pellita*	Teso, Riau, Indonesia	OQ296465	OQ296491	OQ296514	OQ296542	This study
Calonectria lombardiana	**CMW 54953**	*Eucalyptus grandis x Eucalyptus pellita*	Kalimantan, Indonesia	OQ296466	OQ296492	OQ296515	OQ296543	This study
Calonectria lombardiana	**CMW 54963**	*Eucalyptus grandis x Eucalyptus pellita*	Pelalawan, Riau, Indonesia	OQ296467	OQ296493	OQ296516	OQ296544	This study
Calonectria lombardiana	**CMW 59200**	*Eucalyptus grandis x Eucalyptus pellita*	Seikabaro, Riau, Indonesia	OQ296468	OQ296494	OQ296517	OQ296545	This study
Calonectria lombardiana	**CMW 59201**	*Eucalyptus grandis x Eucalyptus pellita*	Seikabaro, Riau, Indonesia	OQ296469	OQ296495	OQ296518	OQ296546	This study
Calonectria lombardiana	**CMW 59202**	*Eucalyptus grandis x Eucalyptus pellita*	Seikabaro, Riau, Indonesia	OQ296470	OQ296496	OQ296519	OQ296547	This study
Calonectria maranhensis	CBS 134811^{T} = LPF142	*Eucalyptus* sp.	Brazil	KM396035	KM396118	KM395861	KM395948	Alfenas et al. (2015)
Calonectria maranhensis	CBS 134812 = LPF143	*Eucalyptus* sp.	Brazil	KM396036	KM396119	KM395862	KM395949	Alfenas et al. (2015)
Calonectria melaleucae	ZHKUCC 21-0066^{T}	*Melaleuca bracteata*	China	MZ491110	N/A	MZ491132	MZ491154	Zhang et al. (2022)
Calonectria melaleucae	ZHKUCC 21-0067	*Melaleuca bracteata*	China	MZ491111	N/A	MZ491133	MZ491155	Zhang et al. (2022)
Calonectria multiseptata	**CMW 59214**	*Eucalyptus grandis x Eucalyptus urophylla Acacia crassicarpa*	Porsea, North Sumatra, Indonesia	OQ296471	OQ296497	OQ296520	OQ296548	This study
Calonectria multiseptata	**CMW 59216**	*Acacia crassicarpa*	Pelalawan, Riau, Indonesia	OQ296472	OQ296498	OQ296521	OQ296549	This study
Calonectria multiseptata	**CMW 59217**	*Acacia crassicarpa*	Pelalawan, Riau, Indonesia	OQ296473	N/A	OQ296522	OQ296550	This study
Calonectria multiseptata	**CMW 59218**	*Acacia crassicarpa*	Pelalawan, Riau, Indonesia	OQ296474	OQ296499	OQ296523	OQ296551	This study
Calonectria multiseptata	CMW 23692^{T} = CBS 112682 = CPC 1589	*Eucalyptus grandis*	Indonesia	MT335299	MT335539	MT412830	MT413044	Liu et al. (2020)
Calonectria plurilateralis	CBS 111401^{T} = CMW 51178 = CPC 1637	Soil	Ecuador	MT335340	MT335580	MT412870	MT413082	Liu et al. (2020)
Calonectria propaginicola	CBS 134815^{T} = LPF220	*Eucalyptus* sp.	Brazil	KM396040	KM396123	KM395866	KM395953	Alfenas et al. (2015)
Calonectria propaginicola	CBS 134816 = LPF222	*Eucalyptus* sp.	Brazil	KM396041	KM396124	KM395867	KM395954	Alfenas et al. (2015)
Calonectria pseudoreteaudii	**CMW 54931**	*Eucalyptus grandis x Eucalyptus pellita*	Teso, Riau, Indonesia	OQ296475	OQ296500	OQ296524	OQ296552	This study
Calonectria pseudoreteaudii	**CMW 54947**	*Eucalyptus grandis x Eucalyptus pellita*	Kalimantan, Indonesia	N/A	N/A	OQ296525	OQ296553	This study

Calonectria pseudoreteaudii	CMW 25310^{T} = CBS 123694	*Eucalyptus urophylla* × *Eucalyptus grandis*	China	MT335354	MT335594	MT412885	MT413096	Liu et al. (2020)
Calonectria pseudoreteaudii	CMW 25292 = CBS 123696	*Eucalyptus urophylla* × *Eucalyptus grandis*	China	MT335355	MT335595	MT412886	MT413097	Liu et al. (2020)
Calonectria queenslandica	CMW 30604^{T} = CBS 112146 = CPC 3213	*Eucalyptus urophylla*	Australia	MT335367	MT335607	MT412898	MT413108	Liu et al. (2020)
Calonectria queenslandica	CMW 30603 = CBS 112155 = CPC 3210	*Eucalyptus pellita*	Australia	MT335368	MT335608	MT412899	MT413109	Liu et al. (2020)
Calonectria reteaudii	**CMW 55091**	*Eucalyptus pellita*	Pelalawan, Riau, Indonesia	OQ296476	OQ296501	OQ296526	OQ296554	This study
Calonectria reteaudii	**CMW 55094**	*Eucalyptus pellita*	Pelalawan, Riau, Indonesia	OQ296477	OQ296502	OQ296527	OQ296555	This study
Calonectria reteaudii	**CMW 59206**	*Eucalyptus grandis x Eucalyptus urophylla*	Porsea, North Sumatra, Indonesia	OQ296478	N/A	OQ296528	OQ296556	This study
Calonectria reteaudii	**CMW 59207**	*Eucalyptus grandis x Eucalyptus urophylla*	Porsea, North Sumatra, Indonesia	OQ296479	OQ296503	OQ296529	OQ296557	This study
Calonectria reteaudii	**CMW 55108**	*Acacia crassicarpa*	Pelalawan, Riau, Indonesia	OQ296480	OQ296504	OQ296530	OQ296558	This study
Calonectria reteaudii	**CMW 55109**	*Acacia crassicarpa*	Pelalawan, Riau, Indonesia	OQ296481	N/A	OQ296531	OQ296559	This study
Calonectria reteaudii	CMW 30984^{T} = CBS 112144 = CPC 3201	*Eucalyptus camaldulensis*	Vietnam	MT335370	MT335610	MT412901	MT413111	Liu et al. (2020)
Calonectria reteaudii	CMW 16738 = CBS 112143 = CPC 3200	*Eucalyptus* sp.	Vietnam	MT335371	MT335611	MT412902	MT413112	Liu et al. (2020)
Calonectria reteaudii	CMW 47410 = CBS 143563	*Eucalyptus urophylla*	Vietnam	MT335193	MT335433	MT412724	N/A	Liu et al. (2020)
Calonectria reteaudii	CMW 47433 = CBS 143564	*Eucalyptus pellita*	Vietnam	MT335194	MT335434	MT412725	MT412947	Liu et al. (2020)
Calonectria strelitziae	ZHKUCC 210019^{T}	*Strelitzia reginae*	China	MZ491105	N/A	MZ491129	MZ491151	Zhang et al. (2022)
Calonectria strelitziae	ZHKUCC 210047	*Strelitzia reginae*	China	MZ491106	N/A	MZ491130	MZ491152	Zhang et al. (2022)
Calonectria tonkinensis	CMW 47430^{T} = CBS 143576	Soil	Vietnam	MT335384	MT335624	MT412915	MT413122	Liu et al. (2020)
Calonectria variabilis	CMW 2914 = CBS 112691 = CPC 2506	*Theobroma grandiflorum*	Brazil	MT335393	MT335633	MT412924	MT413131	Liu et al. (2020)
Calonectria variabilis	CMW 3187^{T} = AR2675 = CBS 114677 = CPC 2436	*Schefflera morototoni*	Brazil	MT335392	MT335632	MT412923	MT413130	Liu et al. (2020)

Note: N/A represents information that is not available. Isolates obtained in this study are indicated in **bold**. T denotes ex-type strain.

AR = Amy Y. Rossman working collection; CBS = The culture collection of Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; CMW = culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; CPC = Pedro Crous working collection housed at Westerdijk Fungal Biodiversity Institute; IMI = International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, UK; LPF = Laboratório de Patologia Florestal, Universidade Federal de Viçosa, Viçosa, Brazil; STE-U = Department of Plant Pathology, University of Stellenbosch, South Africa; ZHKUCC = Zhongkai University of Agriculture and Engineering Culture Collection.

CMDA = calmodulin; HIS3 = histone H3; TEF1 = translation elongation factor 1-alpha; TUB2 = β-tubulin.

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