

# **A serious shoot and leaf disease caused by *Colletotrichum theobromicola* discovered on eucalypts in South Africa**

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## **Abstract**

Eucalypt plantations in South Africa make up an important part of the local forestry industry. Recently, one-year-old nursery plants of a *Eucalyptus grandis* × *Eucalyptus urophylla* variety displayed symptoms of leaf and shoot anthracnose disease. Samples were collected from these plants and isolations were made from the disease symptoms. Isolates were identified based on their morphological characteristics and DNA sequence data for eight gene regions. Phylogenetic analyses led to the isolates being identified as *Colletotrichum theobromicola* and the reduction of *Colletotrichum pseudotheobromicola* to synonymy with the former species. Pathogenicity trials with isolates of *C. theobromicola* were conducted on clones of *E. grandis* and hybrids of *E. grandis* × *E. urophylla* and *E. grandis* × *Eucalyptus camaldulensis*. Resulting symptoms were similar to those found on naturally infected plants and the fungus was re-isolated from the infections. *Colletotrichum theobromicola* is known to cause anthracnose on various plants including eucalypts in Brazil, but this is the first record of the pathogen in South Africa.

## **Keywords:**

- anthracnose disease
- *Eucalyptus* hybrids
- fungal pathogens
- nursery diseases

## Introduction

The genus *Eucalyptus* (Myrtaceae), includes approximately 700 species that are native to Australia, Indonesia, the Philippines and New Guinea (Potts and Pederick 2000). Plantations of these trees, mostly as non-natives, are amongst the most important sources of wood and pulp products in the southern hemisphere, covering an area of more than 20 million ha worldwide (Turnbull 2000, Booth 2013).

South Africa has approximately 1.2 million hectares of commercial forest plantation, with *Eucalyptus* making up a substantial component of this resource (<https://www.forestry.co.za/statistical-data/>). As is true, for many other regions where plantation forestry is based on non-native species, the accidental introduction of non-native pathogens has resulted in serious losses for the industry (Wingfield et al. 2015, Burgess and Wingfield 2017). Likewise, host shifts where native pathogens have adapted to infect non-native eucalypts have added to these disease problems (Slippers et al. 2005, Burgess and Wingfield 2017).

Apart from the many diseases that affect *Eucalyptus* spp. in plantations, diseases of seedlings and cutting plants in nurseries also result in considerable economic losses (Sharma et al. 1984, Old et al. 2003). In this regard, diseases in nurseries can result in failure to produce sufficient planting material, loss of important germplasm and down-stream losses in biomass from plantations (Keane 2000, Old et al. 2003).

Most *Eucalyptus* nursery pathogens are known to be either soil, seed or water-borne and they can arise from the time of sowing through to out-planting in the field (Sharma et al. 1984, Mohanan 2014). Among the more commonly encountered pathogens that can affect *Eucalyptus* in nurseries are species of *Ralstonia* (Alfenas et al. 2006), *Phytophthora* (Simamora et al. 2017), *Botrytis* (Viljoen et al. 1992), *Calonectria* (Lombard et al. 2010) and *Colletotrichum* (Rodrigues et al. 2014).

In 2021, during routine observations of *Eucalyptus* plants in a Pretoria nursery, symptoms of a serious leaf and shoot disease were observed on one-year-old *E. grandis* × *E. urophylla* hybrid plants. The aim of this study was to identify the causal agent of this disease. This was achieved using routine isolation procedures, identification of the associated fungus based on morphological characteristics as well as DNA sequence analyses, after which pathogenicity of the putative pathogen was tested on a number of different *Eucalyptus* varieties.

## Materials and methods

### Disease symptoms, sampling and fungal isolation

Symptoms of a leaf and shoot disease were observed on an *E. grandis* × *E. urophylla* variety in a nursery where the conditions included a range of temperatures from 20 °C to 32 °C and high levels of relative humidity (>80%). The disease was typified by anthracnose symptoms including circular to irregular, red-brown to black necrotic leaf spots on young and newly expanded leaves (Figure 1). In more advanced stages of the disease, necrotic spots were found on the stems, resulting in rotting of the young shoots (Figure 1).

Fresh *E. grandis* × *E. urophylla* leaves showing typical anthracnose symptoms were collected and placed in paper bags and transferred to the laboratory for isolations. Direct isolations of spores were made from fungal structures using a sterile needle and placed onto the surface of Malt Extract Agar (MEA; 20 g malt extract, 20 g Difco agar, 1 L ionized water) in Petri dishes and incubated for 3–5 days at 25 °C. Single hyphal tips from primary isolations were transferred to MEA plates and incubated at 25 °C for 7 days to obtain pure cultures. Resulting isolates were deposited in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

### DNA extraction, PCR sequencing and phylogenetic analyses

Single hyphal-tip isolates grown on MEA for seven days at 25 °C were used for DNA extraction. Mycelium was scraped from the surface of the cultures using a sterile needle and transferred to 1.5 ml Eppendorf tubes. DNA was extracted using Prepman® Ultra Sample Preparation Reagent (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's protocols. Eight gene regions, namely, *ACT*, *CAL*, *CHS1*, *GAPDH*, *GS*, *ITS*, *ApMat* and *TUB2*, were used for PCR amplification and subsequent sequencing (Table 1). Initially, the ITS region was amplified for all isolates. Based on these preliminary results, representative isolates were chosen for further sequencing of the remaining seven gene regions for phylogenetic analyses.

The PCR reactions were conducted using an Applied Biosystems ProFlex PCR System (Thermo Fisher Scientific, Waltham, MA, USA) following conditions suggested by Gan et al. (2013) and Khodadadi et al. (2020). Amplified fragments were purified using ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Fisher Scientific, Waltham, MA, USA). Amplicons

were sequenced in both directions using an ABI PRISM™ 3100 DNA sequencer (Applied Biosystems, USA) at the Sequencing Facility of the Faculty of Natural and Agricultural Sciences, University of Pretoria, South Africa. Raw sequences were assembled and edited using Geneious Prime 2021.1.1 (<https://www.geneious.com>). All sequences generated in this study were deposited in GenBank (<http://www.ncbi.nlm.nih.gov>) with details provided in (Table 2).

Sequences of species related to those emerging from this study were sourced from the GenBank database (<http://www.ncbi.nlm.nih.gov/>) (Table 2). Alignments of all sequences were assembled using MAFFT v. 7 (<http://mafft.cbrc.jp/alignment/server>) (Katoh and Standley 2013), then confirmed manually in MEGA v. 7 (Kumar et al. 2016). A concatenated data set was generated comprising of ACT, CAL, CHS1, GAPDH, GS, ITS and TUB2 sequences. Alignment of ApMat sequences was analysed individually due to the fact that sequence data were not equally available for the gene regions and species considered. Maximum likelihood (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. Final consensus trees were viewed using MEGA v. 7 (Kumar et al. 2016).

### **Morphological observations**

Morphological characteristics of the isolates obtained from diseased tissues were observed using three representative cultures (CMW 56826, CMW 56827 and CMW 56828). The cultures were grown on MEA for 7 days and incubated at 25°C in the dark to induce sporulation. Fungal structures that emerged were initially mounted in water and this was then replaced with 85% lactic acid. Morphological observations were made using an Eclipse Ni, SMZ 18 (Nikon, Tokyo, Japan) stereoscope and an Axioskop 2 plus (Zeiss, Oberkochen, Germany) microscope.

### **Pathogenicity tests**

To confirm pathogenicity of the putative causal agent of the disease, plants of *E. grandis* × *E. urophylla*, *E. grandis* and *E. grandis* × *E. camaldulensis* varieties were used. Plants were grown in pots in a greenhouse at 25 °C with 16h light/8h dark cycles. Two representative isolates (CMW 56826 and CMW 56827) were selected for the inoculations, and these were grown on MEA at 25 °C for seven days.

A conidial suspension was prepared by adding sterile distilled water to the cultures and gently scraping their surface with a sterilized scalpel. The suspension was filtered through a layer of cheesecloth to remove mycelial fragments and the concentration of the conidia was adjusted to  $10^6$  spores/ml using a haemocytometer. Six plants for each *Eucalyptus* variety were inoculated with conidia from the two isolates by spraying these onto the apical parts of the plants until run-off. Six additional plants for each variety were inoculated with sterile water and maintained as controls.

Foliage of the inoculated plants and the controls were enclosed in plastic bags to which balls of cotton wool, soaked in sterile distilled water, had been added to ensure the leaf wetness and to retain high levels of humidity. Plants were maintained in a growth chamber at 25 °C and subjected to a 16h light/8h dark cycle. Inoculated plants were monitored daily until symptoms appeared. To comply with Koch's postulates, re-isolations were made from symptomatic leaves on the inoculated plants. The resulting isolates were identified based on morphological characteristics and DNA sequence data for the ITS region.

## **Results**

### **Fungal isolations**

Twenty isolates, morphologically resembling a *Colletotrichum* species, were obtained from the disease symptoms. All of these isolates were either from leaf samples or from infected stems. All the colonies had a similar morphology on MEA and grew rapidly to cover the surface of the agar in the Petri dishes within four days.

### **Phylogenetic analyses**

Based on the preliminary sequencing results for the ITS region, all *Collectotrichum* isolates resided in the *Colletotrichum gloeosporioides* complex. Six representative isolates were chosen for further study. Amplicons of approximately 260 bp were generated for the *ACT* gene region, 730 bp for the *CAL*, 240 bp for the *CHS1*, 620 bp for the *GAPDH*, 910 bp for the *GS*, 590 bp for the *ITS*, 800 bp for the *ApMat* and 730 bp for the *TUB2*.

The 7-locus combined sequence dataset used in the phylogenetic analyses included 61 ingroup taxa, with *C. boninense* (CBS 123755) as the outgroup. The dataset contained 4750 characters including the alignment gaps. Phylogenetic analysis of the aligned sequences for the ApMat

locus included 30 taxa and 892 characters with alignment gaps. The tree was rooted with *C. alatae* (CBS 304.67). The phylogenetic trees for the 7-locus combined dataset and the ApMat locus with bootstrap support values are presented in Figures 2 and 3, respectively. The two trees were found to be congruent, with similar topologies overall.

The phylogenetic tree presented in Figure 2 showed that all South African isolates considered in this study were all identical and clustered in a clade (ML = 100), residing together with representative isolates of *C. theobromicola sensu stricto* (Weir et al. 2012). This was further supported by the ApMat tree where these six isolates from *Eucalyptus* also formed a monophyletic clade with sequences for isolate GJS B1160843 and the ex-type isolate CBS 124945, representing *C. theobromicola* (Figure 3).

Isolate MFLUCC 18–1602, previously recognized as *C. pseudotheobromicola* based on a multilocus analysis (Chethana et al. 2019), clustered with *C. theobromicola* in our seven-gene multilocus analysis (Figure 2). Sequences for this isolate formed a clade together with isolates ICMP 18565 and ICMP 18576, previously identified as *C. theobromicola* (Weir et al. 2012).

Sequences for three isolates, GM529 (from *Mangifera indica*), GA002 and GA006 (from *Persea americana*), were previously identified as *C. theobromicola* (Sharma et al. 2013; 2017). However, in our analyses, sequences for these isolates fell outside of the main *C. theobromicola* clade and grouped more closely with those representing *C. grossum* (Figure 2). The same pattern was observed in the ApMat analysis, where sequences for these three isolates clustered with isolate INIFAT-4144 of *C. grossum* and were clearly distinct from the clade accommodating the ex-type isolate of *C. theobromicola* (Figure 3). Hence, isolates GM529, GA002 and GA006 were considered as representing *C. grossum*.

## **Taxonomy**

*Collectotrichum pseudotheobromicola*, identified from diseased leaves of *Prunus avium* in China (Chethana et al. 2019), was shown not to be phylogenetically distant from *C. theobromicola* based on phylogenetic analyses of sequence data generated in this study. *Collectotrichum pseudotheobromicola* was primarily distinguished from the ex-type of *C. theobromicola* based on ITS, GAPDH, CHS1, ACT and TUB sequences data (Chethana et al. 2019). Sequences for this isolate failed to form a genetically distinct lineage when a greater number of *C. theobromicola* isolates were included in the analysis compared to one conducted

with limited number of reference sequences in the original description of the species. Based on this result, *C. pseudotheobromicola* is reduced to synonymy with *C. theobromicola*, as follows:

*Colletotrichum theobromicola* Delacr., Bulletin de la Société Mycologique de France 21: 191 (1905)

= *Colletotrichum pseudotheobromicola* Chethana, J.Y. Yan, X.H. Li & K.D. Hyde, Mycosphere 10: 518 (2019)

### **Morphology**

The isolates grown on MEA medium produced a white to gray mycelium and the underside of the colony was uniformly black (Figure 4). After five to seven days, the aerial mycelium became darker with orange spore masses obvious (Figure 4). Setae were produced on black acervuli on cultures after 10 days, and these were simple, light brown in colour, with 6–10 septa (Figure 4). Conidia were hyaline, straight to cylindrical, aseptate and  $7\text{--}8 \times 2\text{--}3 \mu\text{m}$  in size (Figure 4).

### **Pathogenicity**

The two isolates used in the pathogenicity tests produced circular anthracnose-like leaf spots two to three days after inoculation. These symptoms were similar to those found on naturally infected plants in the nursery (Figure 5). Symptoms included brown-red leaf spots on both leaf surfaces, and these became black and necrotic at ten days post inoculation, with the presence of fungal structures at the site of infection (Figure 5). These symptoms developed only on the *E. grandis*  $\times$  *E. urophylla* hybrid plants. No symptoms developed on the inoculated *E. grandis* or *E. grandis*  $\times$  *E. camaldulensis* plants (Figure 5) or those used as controls.

*Colletotrichum theobromicola* was successfully re-isolated on MEA from the lesions on all the *E. grandis*  $\times$  *E. urophylla* plants inoculated. The isolates were morphologically identical to those used for the pathogenicity test and this identification was confirmed based on sequence data for the ITS region.

### **Discussion**

The results of this study showed that a potentially serious leaf and shoot disease that has emerged in a South African *Eucalyptus* nursery was caused by *C. theobromicola*. This was

based on isolations from symptomatic tissues, identification of the resulting cultures using analyses of DNA sequences for eight gene regions and pathogenicity tests. This is the first record of *C. theobromicola* from South Africa.

The morphological characteristics of the isolates obtained in this study are typical of species in the *C. gloeosporioides* species complex (von Arx 1970, Sutton 1992, Weir et al. 2012). It is well-known that differentiating among species within this species complex is challenging due to a lack of distinctive morphological features and a plasticity of their phenotypic characters (Cai et al. 2009, Weir et al. 2012, Jayawardena et al. 2016). For this reason, identification of *Colletotrichum* spp. is carried out using a polyphasic approach that combines morphological and cultural characteristics with multilocus phylogenetic analyses of DNA sequences, which has transformed the taxonomy of the genus (Cai et al. 2009, Weir et al. 2012, Liu et al. 2016). We used the combination of eight gene regions to effectively resolve the phylogenetic placement of the isolates associated with a newly observed disease of *Eucalyptus* in South Africa. In addition, the resulting data provided justification to reduce *C. pseudotheobromicola* to synonymy with *C. theobromicola*.

*Colletotrichum theobromicola* occurs mostly in tropical, subtropical, and temperate regions and it has a relatively wide host range including economically important crops (Bragança et al. 2014, Araújo et al. 2018, Hawk et al. 2018, Lima et al. 2019). It is also known to cause anthracnose in Brazilian *Eucalyptus* nurseries causing symptoms similar to those observed in this study (Rodrigues et al. 2014). The disease described from Brazil also appeared to be specific to shoots and stems of a *E. grandis* × *E. urophylla* hybrid clone. Importantly, this pathogen is able to cause severe defoliation and death of plants, and this can occur rapidly when conditions are conducive to disease development.

Some differences in morphology and DNA sequence data were observed in this study for *C. theobromicola* isolates from South Africa, compared to those reported from Brazil. Conidia produced on culture in this study (avg.  $7\text{--}8 \times 2\text{--}3 \mu\text{m}$ ) were smaller than those reported on *Eucalyptus* in Brazil (avg.  $10\text{--}17 \times 4\text{--}6 \mu\text{m}$ ) (Rodrigues et al. 2014). While phylogenetic analyses showed that the South African isolates clustered together as a well-supported clade with representative isolates of *C. theobromicola*, they clustered separately from two Brazilian isolates. This might suggest that *C. theobromicola* in South Africa does not share a similar origin to those causing diseases on *Eucalyptus* in Brazil.

Inoculation tests in this study showed that *C. theobromicola* was specifically pathogenic on a single variety of an *E. grandis* × *E. urophylla* hybrid. This is an intriguing result given that this fungus has a wide host range including for example olive (*Olea europaea*), onion (*Allium fistulosum*) (Matos et al. 2017) and sapote (*Manilkara zapota*) (Martins et al. 2018). While fungal pathogens of *Eucalyptus* are well-known to display differential pathogenicity on different varieties of these (van Heerden et al. 2005, Chen et al. 2013), it seems likely that *C. theobromicola* has a broader host range than has emerged from this study. Consequently, *Eucalyptus* nursery surveys will need to be conducted to clarify this important question, which has a direct impact on the management program for this emerging disease.

The origin of *C. theobromicola* causing disease on a *Eucalyptus* variety in this study is unknown. However, it is well known that *Colletotrichum* spp. can be seed borne (Meon and Nik 1988, Begum et al. 2007, Pecchia et al. 2019). And consistent with this fact, two other *Colletotrichum* spp., *Colletotrichum ciggaro* and *Colletotrichum fructicola* were recently recorded for the first time in South Africa after being isolated from the seed of various *Eucalyptus* spp. (Mangwende et al. 2021). It is thus plausible to hypothesise that *C. theobromicola* was accidentally introduced into this country with seed imported from elsewhere. One possibility would be Brazil where the fungus is known to occur on *Eucalyptus* (Rodrigues et al. 2014)

Various important tree pathogens are thought to have been introduced into South Africa via seed used for plantation establishment. Notable examples are the pine pitch canker pathogen *Fusarium circinatum* (Coutinho et al. 2007) and the *Eucalyptus* canker pathogen *Teratosphaeria zuluense* (Jimu et al. 2016). The appearance of *C. theobromicola* in this study as well as two other *Colletotrichum* spp. in the recent study of Mangwende et al. (2021) suggests a need for increased vigilance to be paid to seed pathogens that might accidentally be introduced into South Africa in the future.

*Colletotrichum theobromicola* appears to be a pathogen of increasing prevalence in countries such as Argentina (Lima et al. 2019), Australia (Wang et al. 2021), Israel (Sharma et al. 2016), United States (Hawk et al. 2018) and Brazil (Rodrigues et al. 2014), where it is found in both agriculture and forestry settings. Generally, little is known regarding its impact and given its recent discovery, this is also true for South Africa. Surveys should now be undertaken in South African forestry nurseries to determine where the pathogen occurs and how it might be managed.

## Conclusions

Infections by *C. theobromicola* found in this study were associated with plants in a greenhouse and where plants were exposed to high levels of humidity and regular periods of leaf wetness. This might imply that anthracnose caused by the pathogen was facilitated by these conditions, which could be managed in commercial forestry nurseries. Furthermore, there was clear evidence of tolerance to infection in some *Eucalyptus* varieties, reducing the concern that the pathogen will emerge as an important constraint to large-scale propagation of these plants. It is also important to recognise that *Colletotrichum* spp. are well-known to be opportunistic pathogens, which would also allow for effective management of *C. theobromicola* in the future.

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## References

- Alfenas AC, Mafia RG, Sartório RC, Binoti DH, Silva RR, Lau D, Vanetti CA. 2006. *Ralstonia solanacearum* em viveiros clonais de eucalipto no Brasil. *Fitopatologia Brasileira* 31: 357–366.
- Araújo MBM, Lima CS, Rabelo Filho F de A, Ootani MA, Bezerra AME, Cardoso JE. 2018. First report of *Colletotrichum theobromicola* and *C. tropicale* causing anthracnose on fruits of carnauba palm in Brazil. *Plant Disease* 102: 244.
- Begum MM, Sariah M, Puteh AB, Abidin MZ. 2007. Detection of seed-borne fungi and site of infection by *Colletotrichum truncatum* in naturally-infected soybean seeds. *International Journal of Agricultural Research* 2: 812–819.
- Berbee M, Pirseyedi M, Hubbard S. 1999. *Cochliobolus* phylogenetics and the origin of known, highly virulent pathogens, inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. *Mycologia* 91: 964–977.
- Booth TH. 2013. Eucalypt plantations and climate change. *Forest ecology and management* 301: 28–34.

- Bragança CAD, Junior AN, Rogério F, Massola Jr NS. 2014. First report of anthracnose caused by *Colletotrichum theobromicola* on barbados cherry (*Malpighia emarginata*) in Brazil. *Plant Disease* 98: 1272.
- Burgess TI, Wingfield MJ. 2017. Pathogens on the Move: A 100-Year Global Experiment with Planted Eucalypts. *BioScience* 67: 14–25
- Cai L, Hyde KD, Taylor PWJ, Weir B, Waller J, Abang MM, Zhang JZ, Yang YL, Phoulivong S, Liu ZY. 2009. A polyphasic approach for studying *Colletotrichum*. *Fungal Diversity* 39: 183–204.
- Cannon PF, Damm U, Johnston PR, Weir BS. 2012. *Colletotrichum* – current status and future directions. *Studies in Mycology* 73: 181–213.
- Carbone I, Kohn L. 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* 91: 553–556.
- Chen SF, Van Wyk M, Roux J, Wingfield MJ, Xie Y, Zhou X. 2013. Taxonomy and pathogenicity of *Ceratocystis* species on *Eucalyptus* trees in South China, including *C. chinaeucensis* sp. nov. *Fungal Diversity* 58: 267–279.
- Chethana KWT, Jayawardene RS, Zhang W, Zhou YY, Liu M, Hyde KD, Li XH, Wang J, Zhang KC, Yan JY. 2019. Molecular characterization and pathogenicity of fungal taxa associated with cherry leaf spot disease. *Mycosphere* 10: 490–530.
- Coutinho TA, Steenkamp ET, Mongwaketsi K, Wilmot M, Wingfield MJ. 2007. First outbreak of pitch canker in a South African pine plantation. *Australasian Plant Pathology* 36: 256–261.
- de Silva D, Groenewald J, Crous P, Ades P, Nasruddin A, Mongkolporn O, Taylor P. 2019. Identification, prevalence and pathogenicity of *Colletotrichum* species causing anthracnose of *Capsicum annuum* in Asia. *IMA Fungus* 10: 1–32.
- Diao YZ, Zhang C, Liu F, Wang WZ, Liu L, Cai L, Liu XL. 2017. *Colletotrichum* species causing anthracnose disease of chili in China. *Persoonia: Molecular Phylogeny and Evolution of Fungi* 38: 20–37
- Gan P, Ikeda K, Irieda H, Narusaka M, O’Connell RJ, Narusaka Y, Takano Y, Kubo Y, Shirasu K. 2013. Comparative genomic and transcriptomic analyses reveal the hemibiotrophic stage shift of *Colletotrichum* fungi. *New Phytologist* 197: 236–249.

- Gardes M, Bruns T. 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113–118.
- Glass N, Donaldson G. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* 61: 1323-1330.
- Hamm PB, Hansen EM. 1982. Pathogenicity of *Phytophthora* species to Pacific Northwest conifers. *European Journal of Forest Pathology* 12: 167–174.
- Hawk T, Rhodes SC, McBride S, Ong KL. 2018. First report of boxwood dieback caused by *Colletotrichum theobromicola* in Texas. *Plant Disease* 102: 242.
- 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.
- Jayawardena RS, Hyde KD, Damm U, Cai L, Liu M, Li XH, Zhang W, Zhao WS, Yan JY. 2016. Notes on currently accepted species of *Colletotrichum*. *Mycosphere* 7: 1192–1260.
- Jimu L, Kemler M, Wingfield MJ, Mwenje E, Roux J. 2016. The *Eucalyptus* stem canker pathogen *Teratosphaeria zuluensis* detected in seed samples. *Forestry* 89: 316–324.
- Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.
- Keane PJ. 2000. Diseases and pathogens of eucalypts. Clayton: CSIRO Publishing.
- Khodadadi F, González JB, Martin PL, Giroux E, Bilodeau GJ, Peter KA, Doyle VP, Aćimović SG. 2020. Identification and characterization of *Colletotrichum* species causing apple bitter rot in New York and description of *C. noveboracense* sp. nov. *Scientific Reports* 10: 1–19
- Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 333: 1870–1874.
- Lima NB, Pastor SE, Maza CE, Conforto C, Vargas-Gil S, Roca M. 2019. First Report of Anthracnose of Olive Fruit Caused by *Colletotrichum theobromicola* in Argentina. *Plant Disease* 104: 589.

- Liu F, Wang M, Damm U, Crous PW, Cai L. 2016. Species boundaries in plant pathogenic fungi: a *Colletotrichum* case study. *BMC evolutionary biology* 16: 1–14.
- Liu F, Weir B, Damm U, Crous P, Wang Y, Liu B, Wang M, Zhang M, Cai L. 2015. Unravelling *Colletotrichum* species associated with *Camellia*: employing ApMat and GS loci to resolve species in the *C. gloeosporioides* complex. *Persoonia: Molecular Phylogeny and Evolution of Fungi* 35: 63–86.
- Lombard L, Crous PW, Wingfield BD, Wingfield MJ. 2010. Species concepts in *Calonectria* (*Cylindrocladium*). *Studies in Mycology* 66: 1–13.
- Mangwende E, Truter M, Aveling TAS, Chirwa PW. 2021. Anthracnose leaf spot pathogens, *Colletotrichum fruticola* and *Colletotrichum cigarro*, associated with *Eucalyptus* seed produced in South Africa. *Australasian Plant Pathology*. 50: 533–543.
- Manzano León AM, Serra Hernández W, García Pérez L, Crespo K, Guarnaccia V. 2018. First report of leaf anthracnose caused by *Colletotrichum grossum* on mango (*Mangifera indica*) in Cuba. *Journal of Plant Pathology* 100: 329.
- Martins MVV, Lima JS, Araújo FSA, Ootani MA, Viana FMP, Cardoso JE, Coutinho IBL, Gonçalves FJT, Fonseca WL. 2018. First report of *Colletotrichum theobromicola* causing leaf spot in sapote (*Manilkara zapota*) seedlings in Brazil. *Plant Disease* 102: 2641.
- Matos KS, Santana KFA, Catarino AM, Hanada RE, Silva GF. 2017. First report of anthracnose on welsh onion (*Allium fistulosum*) in Brazil caused by *Colletotrichum theobromicola* and *C. truncatum*. *Plant Disease* 101: 1055.
- Meon S, Nik WZW. 1988. Seed-borne infection and development of *Colletotrichum capsici* in naturally infected chili seed. *PERTANIKA* 11: 341–344.
- Mohanan C. 2014. Diseases in Eucalypts: status and management. In: *Eucalypts in India*. ENVIS Centre on Forestry, National Forest Library and Information Centre, Forest Research Institute. pp 281–314.
- O'Donnell K, Nirenberg HI, Aoki T, Cigelnik E. 2000. A multigene phylogeny of the *Gibberella fujikuroi* species complex: detection of additional phylogenetically distinct species. *Mycoscience* 41: 61–78.

Old KM, Wingfield MJ, Yuan ZQ. 2003. A manual of diseases of *Eucalyptus* in South-East Asia. Bogor: CIFOR.

Paine TD, Steinbauer MJ, Lawson SA. 2011. Native and exotic pests of *Eucalyptus*: A worldwide perspective. *Annual Review of Entomology* 56: 181–201.

Pecchia S, Caggiano B, Da Lio D, Cafà G, Le Floch G, Baroncelli R. 2019. Molecular detection of the seed-borne pathogen *Colletotrichum lupini* targeting the hyper-variable IGS region of the ribosomal cluster. *Plants* 8: 222.

Potts BM, Pederick LA. 2000. Morphology, phylogeny, origin, distribution and genetic diversity of eucalypts. Clayton: CSIRO Publishing.

Rodrigues AL, Pinho DB, Lisboa DO, Nascimento RJ, Pereira OL, Alfenas AC, Furtado GQ. 2014. *Colletotrichum theobromicola* causes defoliation, stem girdling and death of mini-cuttings of eucalyptus in Brazil. *Tropical Plant Pathology* 39: 326–330.

Rojas EI, Rehner SA, Samuels GJ, Van Bael SA, Herre EA, Cannon P, Chen R, Pang J, Wang R, Zhang Y. 2010. *Colletotrichum gloeosporioides* sl associated with *Theobroma cacao* and other plants in Panama: multilocus phylogenies distinguish host-associated pathogens from asymptomatic endophytes. *Mycologia* 102: 1318–1338.

Sharma G, Maymon M, Freeman S. 2016. First report of *Colletotrichum theobromicola* causing leaf spot of *Cyclamen persicum* in Israel. *Plant Disease* 100: 1790.

Sharma G, Maymon M, Freeman S. 2017. Epidemiology, pathology and identification of *Colletotrichum* including a novel species associated with avocado (*Persea americana*) anthracnose in Israel. *Scientific Reports* 7: 1–16.

Sharma G, Kumar N, Weir BS, Hyde KD, Shenoy BD. 2013. The ApMat marker can resolve *Colletotrichum* species: a case study with *Mangifera indica*. *Fungal Diversity* 61: 117–138.

Sharma JK, Mohanan C, Maria Florence EJ. 1984. Nursery diseases of *Eucalyptus* in Kerala 1. *European Journal of Forest Pathology* 14: 77–89.

Simamora AV, Paap T, Howard K, Stukely MJ, Hardy GESJ, Burgess TI. 2018. *Phytophthora* contamination in a nursery and its potential dispersal into the natural environment. *Plant Disease* 102: 132–139.

- Slippers B, Stenlid J, Wingfield MJ. 2005. Emerging pathogens: fungal host jumps following anthropogenic introduction. *Trends in Ecology & Evolution* 20: 420–421.
- Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.
- Stephenson S, Green J, Manners J, Maclean D. 1997. Cloning and characterisation of glutamine synthetase from *Colletotrichum gloeosporioides* and demonstration of elevated expression during pathogenesis on *Stylosanthes guianensis*. *Current Genetics* 31: 447–454.
- Sutton B. 1992. The genus *Glomerella* and its anamorph *Colletotrichum*. Wallingford: CAB International.
- Turnbull JW. 2000. Economic and social importance of eucalypts. Clayton: CSIRO Publishing.
- Vieira W, Lima W, Nascimento E, Michereff S, Câmara M, Doyle V. 2017. The impact of phenotypic and molecular data on the inference of *Colletotrichum* diversity associated with *Musa*. *Mycologia* 109: 912–934.
- Viljoen A, Wingfield MJ, Crous PW. 1992. Fungal pathogens in *Pinus* and *Eucalyptus* seedling nurseries in South Africa: a review. *South African Forestry Journal* 161: 45–51.
- Von Arx J. 1970. A revision of the fungi classified as *Gloeosporium*. New York: Lehre, J. Cramer.
- Wang W, de Silva DD, Moslemi A, Edwards J, Ades PK, Crous PW, Taylor PW. 2021. *Colletotrichum* species causing anthracnose of citrus in Australia. *Journal of Fungi* 7: 47.
- Weir BS, Johnston PR, Damm U. 2012. The *Colletotrichum gloeosporioides* species complex. *Studies in Mycology* 73: 115–180
- Wingfield MJ, Brouwerhoff EG, Wingfield BD, Slippers B. 2015. Planted forest health: the need for a global strategy. *Science* 349: 832–836.
- White T, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications* 18: 315–322.

## Figures legends

**Figure 1.** Symptoms of anthracnose on *E. grandis* × *E. urophylla* (a) Leaf spots in shoots and young leaves in the upper leaf surface. (b) Leaf spots present in the abaxial leaf surface. (c) Stem and shoot rot. (d) Leaf spots in new shoots and stem. (e) Necrotic spots on the adaxial leaf surface with sporulation of the pathogen.

**Figure 2.** Phylogenetic tree based on maximum likelihood (ML) analysis of a combined DNA data set of ACT, CAL, CHS1, GAPDH, GS, ITS and TUB2 sequences for *Colletotrichum* spp. Isolates sequenced in this study are presented in bold face. Host species are highlighted in blue and the countries of origin of the isolates are in red. Bootstrap values for ML analyses are indicated at the nodes. Isolates representing ex-type material are marked with a “T”. *Colletotrichum boninense* (isolate CBS 123755) represents the outgroup taxon.

**Figure 3.** Phylogenetic tree based on maximum likelihood (ML) analysis of ApMat locus sequences for *Colletotrichum* spp. Isolates used in this study are presented in bold face. Host species are highlighted in blue and the countries of origin of the isolates are given in red. Bootstrap values for ML analyses are indicated at the nodes. Sequences representing the ex-type isolates are marked with “T”. The tree was rooted to *C. alatae* (CBS 304.67).

**Figure 4.** Morphology of *C. theobromicola* (a) Seven-day-old colony from above and from below. (b) Spore masses exuding from conidiomata on MEA. (c) Setae on 10-day-old culture. (d) Conidiophores. (e) Conidia. Scale bars: b = 500 µm; c–e = 10 µm.

**Figure 5.** Results of the pathogenicity test with *C. theobromicola* on different *Eucalyptus* varieties. (a) *E. grandis* × *E. urophylla*, control (left) and leaf spots three days after inoculation (right). (b) *E. grandis*, control (left) and after three days (right). (c) *E. grandis* × *E. camaldulensis*, control (left) and after three days (right). (d) *E. grandis* × *E. urophylla*, 10 days after inoculation, black necrotic spots in the abaxial leaf surface, sporulation in stems and defoliation.

**Figure 1.**



Figure 2.

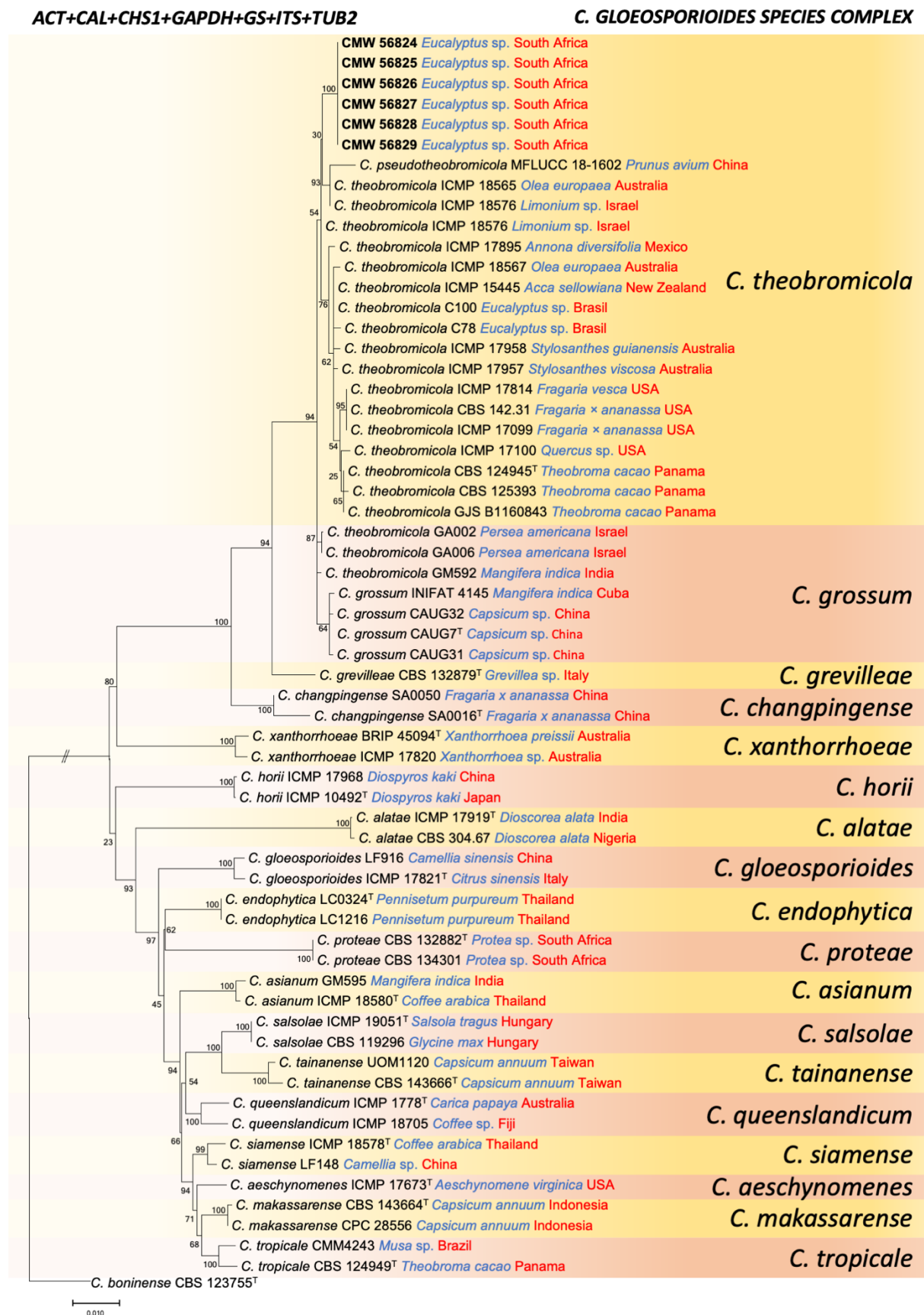
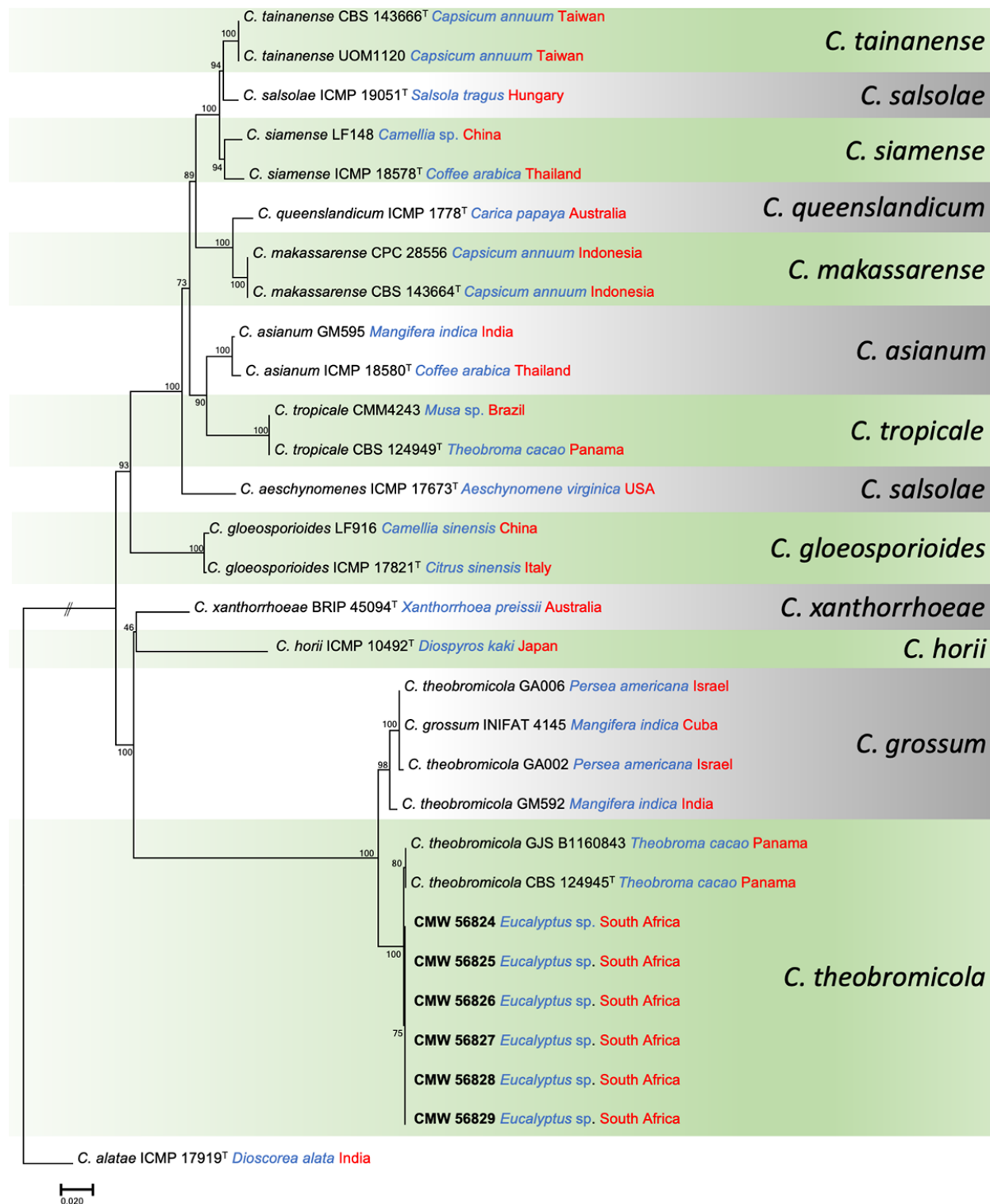


Figure 3.

ApMat

*C. GLOEOSPORIOIDES SPECIES COMPLEX*



**Figure 4.**

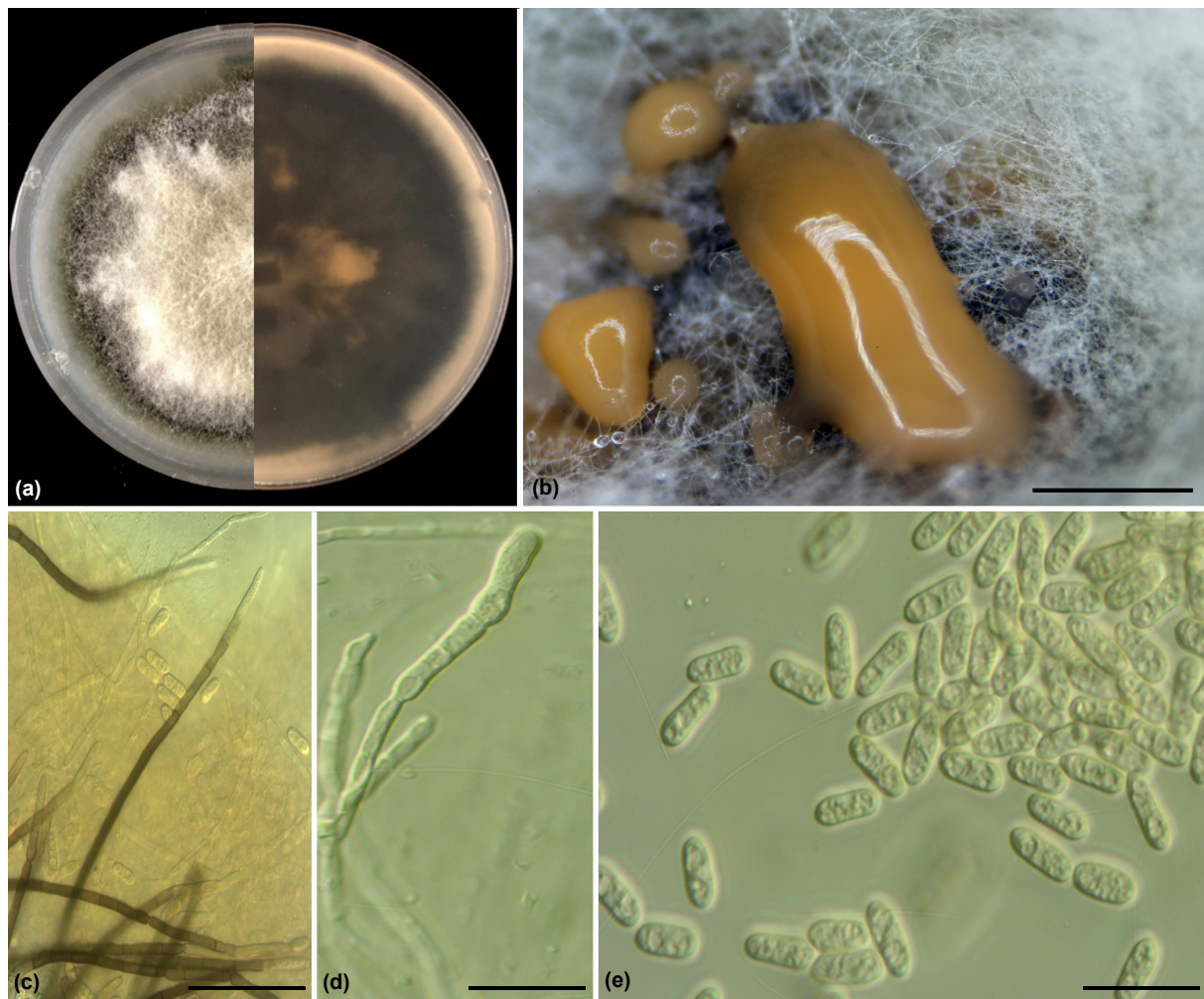
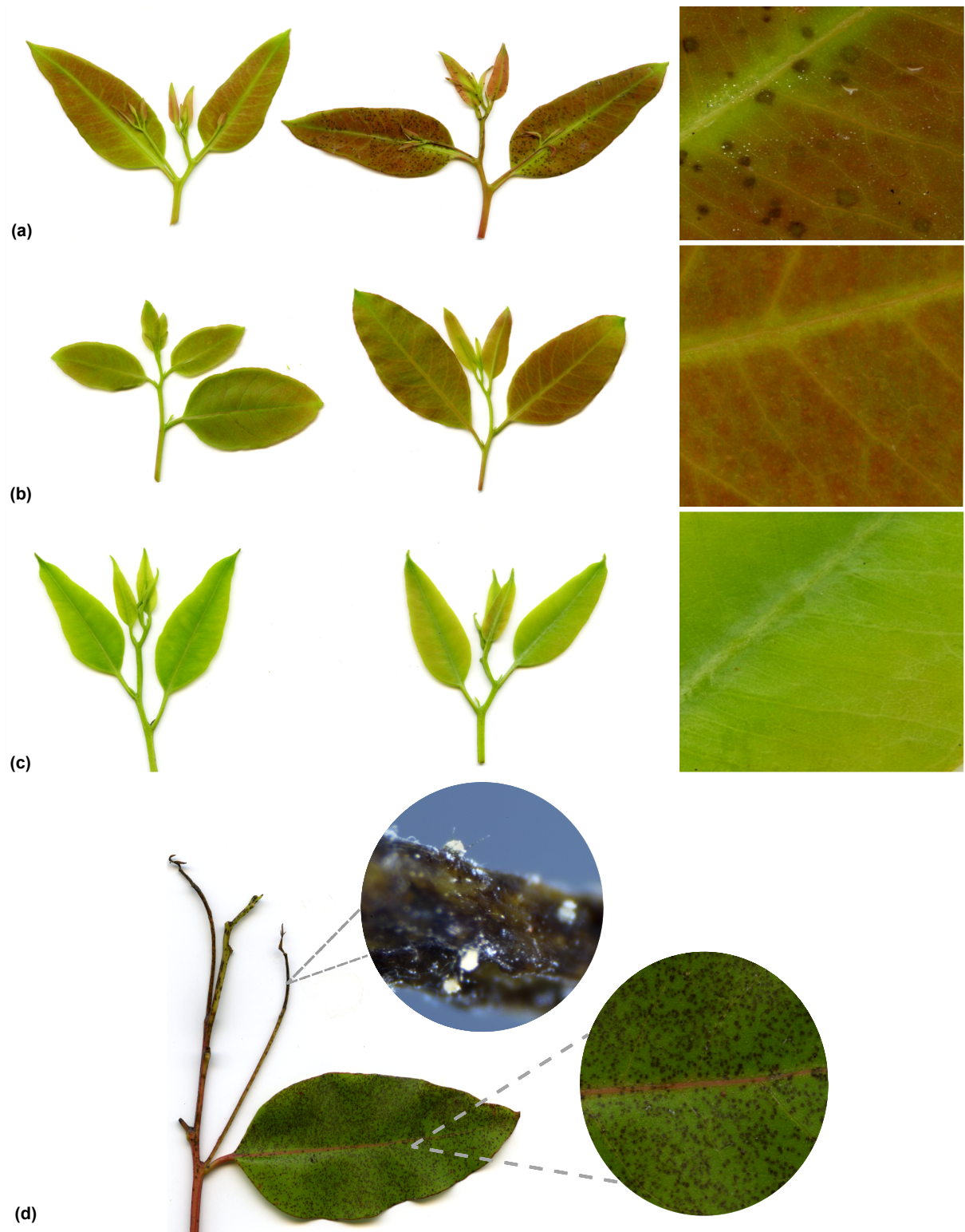


Figure 5.



**Table 1.** Primers used in this study, with sequences and sources

Region	Primer	Direction	Sequence	References
Actin (ACT)	ACT-512F	Forward	ATGTGCAAGGCCGGTTTCGC	Carbone and Kohn (1999)
	ACT-783R	Reverse	TACGAGTCCTTCTGGCCCAT	Carbone and Kohn (1999)
Calmodulin (CAL)	CL1	Forward	GARTWCAAGGAGGCCTTCTC	O'Donnell et al. (2000)
	CL2a	Reverse	TTTTTGCATCATGAGTTGGAC	O'Donnell et al. (2000)
Chitin synthase (CHS1)	CHS-79F	Forward	TGGGGCAAGGATGCTTGAAGAAG	Carbone and Kohn (1999)
	CHS-345R	Reverse	TGGAAGAACCATCTGTGAGAGTTG	Carbone and Kohn (1999)
Glutamine synthetase (GS)	GSF	Forward	ATGGCCGAGTACATCTGG	Stephenson et al. (1997)
	GSR	Reverse	GAACCGTCGAAGTTCCAC	Stephenson et al. (1997)
Glyceraldehyde-3-phosphate (GAPDH)	GDP1	Forward	CAACGGCTTCGGTCGCATTG	Berbee et al. (1999)
	GDP2	Reverse	GCCAAGCAGTTGGTTGTGC	Berbee et al. (1999)
Intergenic spacer and partial MAT1-2 gene (ApMat)	CgDL-F6	Forward	AGTGGAGGTGCCGGGACGTT	Rojas et al. (2010)
	CgMAT1F2	Reverse	TGATGTATCCCGACTACCG	Rojas et al. (2010)
Internal transcribed spacer (ITS)	ITS-1F	Forward	CTTGGTCATTTAGAGGAAGTAA	Gardes and Bruns (1993)
	ITS-4	Reverse	TCCTCCGCTTATTGATATGC	White et al. (1990)
$\beta$ -Tubulin 2 (TUB2)	T1	Forward	AACATGCGTGAGATTGTAAGT	O'Donnell and Cigel'nik (1997)
	Bt2b	Reverse	ACCCTCAGTGTAGTGACCCTTGGC	Glass and Donaldson (1995)

**Table 2.** Collection details and GenBank accessions of isolates included in the phylogenetic analyses

Species	Isolate number	Host/Substrate	Country	GenBank accession number							Reference	
				ACT	ApMat	CAL	GAPDH	GS	ITS	TUB2		CHS
<i>Colletotrichum aeshynomenes</i>	ICMP 17673, ATCC 201874T	<i>Aeschynomene virginica</i>	USA	JX009483	KM360145	JX009721	JX009930	JX010081	JX010176	JX010392	JX009799	Vieira et al. (2017)
<i>C. alatae</i>	CBS 304.67, ICMP 17919T	<i>Dioscorea alata</i>	India	JX009471	KC888932	JX009738	JX009990	JX010065	JX010190	JX010383	JX009837	Liu et al. (2015)
<i>C. alatae</i>	ICMP 18122	<i>Dioscorea alata</i>	Nigeria	JX009470	N/A	JX009739	JX010011	JX010136	JX010191	JX010449	JX009846	Liu et al. (2015)
<i>C. asianum</i>	GM595, MTCC 11680	<i>Mangifera indica</i>	India	JQ894545	JQ894554	KC790789	JQ894623	N/A	JQ894679	JQ894601	JQ894616	Liu et al. (2015)
<i>C. asianum</i>	ICMP 18580, CBS 130418T	<i>Coffea arabica</i>	Thailand	JX009584	FR718814	FJ917506	JX010053	JX010096	FJ972612	JX010406	JX009867	Liu et al. (2015)
<i>C. boniense</i>	MAFF 305972, CBS 123755T	<i>Crinum asiaticum</i> var. <i>sinicum</i>	Japan	JQ005501	N/A	JQ005674	JQ005240	N/A	JQ005153	JQ005588	JQ005327	Liu et al. (2015)
<i>C. changpingense</i>	MFLUCC 15-0022, SA0016	Rhizome of <i>Fragaria</i> × <i>ananassa</i>	China	KP683093	N/A	N/A	KP852469	N/A	KP683152	KP852490	KP852449	Jayawardena et al. (2016)
<i>C. changpingense</i>	SA0050	Rhizome of <i>Fragaria</i> × <i>ananassa</i>	China	KY214470	N/A	N/A	KY214472	N/A	KY214473	KY214474	KY214471	Jayawardena et al. (2016)
<i>C. endophytica</i>	MFLUCC 130417, LC1216	<i>Pennisetum purpureum</i>	Thailand	KC692467	N/A	KC810017	KC832853	N/A	KC633853	N/A	N/A	Vieira et al. (2017)
<i>C. endophytica</i>	MFLUCC 130418, LC0324T	<i>Pennisetum purpureum</i>	Thailand	KF306258	N/A	KC810018	KC832854	N/A	KC633854	N/A	N/A	Vieira et al. (2017)
<i>C. gloesporioides</i>	LC3686, LF916	<i>Camellia sinensis</i>	China	KJ954493	KJ954629	KJ954777	KJ954927	KJ955076	KJ955226	KJ955371	N/A	Liu et al. (2015)
<i>C. gloesporioides</i>	IMI 356878T, ICMP 17821, CBS 112999	<i>Citrus sinensis</i>	Italy	JX009531	JQ807843	JX009731	JX010056	JX010085	JX010152	JX010445	JX009818	Vieira et al. (2017)
<i>C. grevilleae</i>	CBS 132879T, CPC 15481	<i>Grevillea</i> sp.	Italy	KC296941	N/A	KC296963	KC297010	KC297033	KC297078	KC297102	KC296987	Vieira et al. (2017)
<i>C. grossum</i>	CAUG7T	<i>Capsicum</i> sp.	China	KP890141	N/A	KP890147	KP890159	N/A	KP890165	KP890171	KP890153	Diao et al. (2017)
<i>C. grossum</i>	CAU31	<i>Capsicum</i> sp.	China	KP890142	N/A	KP890148	KP890160	N/A	KP890166	KP890172	KP890154	Diao et al. (2017)
<i>C. grossum</i>	CAUG32	<i>Capsicum</i> sp.	China	KP890143	N/A	KP890149	KP890161	N/A	KP890167	KP890173	KP890155	Diao et al. (2017)
<i>C. grossum</i>	INIFAT-4144	<i>Magifera indica</i>	Cuba	MG826117	MG826119	MG826121	MG826116	MG826120	MG812307	MG826118	N/A	Manzano León et al. (2018)
<i>C. horii</i>	ICMP 17968	<i>Diospyros kaki</i>	China	JX009547	N/A	JX009605	GQ329682	JX010068	JX010212	JX010378	JX009811	Liu et al. (2015)
<i>C. horii</i>	ICMP 10492T	<i>Diospyros kaki</i>	Japan	JX009438	JQ807840	JX009604	GQ329681	JX010137	GQ329690	JX010450	JX009752	Vieira et al. (2017)
<i>C. makassarensae</i>	CPC 28556	<i>Capsicum annuum</i>	Indonesia	MH781478	MH728833	N/A	MH728821	MH748262	MH728815	MH846561	N/A	de Silva et al. (2019)
<i>C. makassarensae</i>	CBS 143664, CPC 28612T	<i>Capsicum annuum</i>	Indonesia	MH781480	MH728831	N/A	MH728820	MH748264	MH728812	MH846563	MH805850	de Silva et al. (2019)
<i>C. proteae</i>	CBS 132882, CPC 14859T	<i>Protea</i> sp.	South Africa	KC296940	N/A	KC296960	KC297009	KC297032	KC297079	KC297101	KC296986	Liu et al. (2015)
<i>C. proteae</i>	CBS 134301, CPC 14860	<i>Protea</i> sp.	South Africa	KC842373	N/A	KC842375	KC842379	KC842381	KC842385	KC842387	KC842377	Liu et al. (2015)
<i>C. pseudotheobromicola</i>	MFLUCC 18–1602T			MH853681	N/A	N/A	MH853675	N/A	MH817395	MH853684	MH853678	Chethana et al. (2019)
<i>C. queenslandicum</i>	ICMP 18705	<i>Coffea</i> sp.	Fiji	JX009490	N/A	JX009694	JX010036	JX010102	JX010185	JX010412	JX009890	Liu et al. (2015)

<i>C. queenslandicum</i>	ICMP 1778T	<i>Carica papaya</i>	Australia	JX009447	KC888928	JX009691	JX009934	JX010104	JX010276	JX010414	JX009899	Vieira et al. (2017)
<i>C. salsolae</i>	ICMP 19051T	<i>Salsola tragus</i>	Hungary	JX009562	KC888925	JX009696	JX009916	JX010093	JX010242	JX010403	JX009863	Vieira et al. (2017)
<i>C. salsolae</i>	CBS 119296, ICMP 18693	<i>Glycine max</i> (inoculated)	Hungary	JX009559	N/A	JX009695	JX009917	N/A	JX010241	N/A	JX009791	Weir et al. (2012)
<i>C. siamense</i>	LC2940, LF148	<i>Camellia</i> sp.	China	KJ954370	KJ954504	KJ954641	KJ954789	KJ954939	KJ955088	KJ955237	N/A	Liu et al. (2015)
<i>C. siamense</i>	ICMP 18578T, CBS 130417	<i>Coffea arabica</i>	Thailand	FJ907423	JQ899289	FJ917505	JX009924	JX010094	JX010171	JX010404	JX009865	Vieira et al. (2017)
<i>C. tainanense</i>	CBS 143666, CPC 30245T	<i>Capsicum annuum</i>	Taiwan	MH781475	MH728836	N/A	MH728823	MH748259	MH728818	MH846558	MH805845	de Silva et al. (2019)
<i>C. tainanense</i>	UOM1120, Coll1298	<i>Capsicum annuum</i>	Taiwan	MH781487	MH728824	N/A	MH728819	MH748271	MH728805	MH846570	MH805857	de Silva et al. (2019)
<i>C. theobromicola</i>	GM592 = MTCC 11673	<i>Mangifera indica</i>	India	JQ894544	JQ894578	KC790788	JQ894631	N/A	JQ894678	JQ894593	JQ894615	Sharma et al. (2013)
<i>C. theobromicola</i>	CBS 124945T, ICMP 18649	<i>Theobroma cacao</i>	Panama	JX009444	KC790726	JX009591	JX010006	JX010139	JX010294	JX010447	JX009869	Vieira et al. (2017)
<i>C. theobromicola</i>	GJS B1160843	<i>Theobroma cacao</i>	Panama	N/A	GU994447	N/A	N/A	N/A	GU994356	GU994476	N/A	Vieira et al. (2017)
<i>C. theobromicola</i>	GA002	<i>Persea americana</i>	Israel	KX620137	KX620170	KX620198	KX620234	KX620267	KX620300	KX620333	N/A	Sharma et al. (2017)
<i>C. theobromicola</i>	GA006	<i>Persea americana</i>	Israel	KX620138	KX620171	KX620199	KX620235	KX620268	KX620301	KX620334	N/A	Sharma et al. (2017)
<i>C. theobromicola</i>	ICMP 18566	<i>Olea europaea</i>	Australia	JX009496	N/A	JX009593	JX009953	JX010071	JX010282	JX010376	JX009801	Weir et al. (2012)
<i>C. theobromicola</i>	ICMP 18565	<i>Olea europaea</i>	Australia	JX009449	N/A	JX009594	JX010029	JX010070	JX010283	JX010374	JX009802	Weir et al. (2012)
<i>C. theobromicola</i>	ICMP 18567	<i>Olea europaea</i>	Australia	JX009457	N/A	JX009599	JX009985	JX010069	JX010287	JX010377	JX009803	Weir et al. (2012)
<i>C. theobromicola</i>	MUCL 42295, ICMP 17958, CBS 124250	<i>Stylosanthes guianensis</i>	Australia	JX009498	N/A	JX009598	JX009948	JX010067	JX010291	JX010381	JX009822	Weir et al. (2012)
<i>C. theobromicola</i>	ICMP 18576	<i>Limonium</i> sp.	Israel	JX009532	N/A	JX009595	JX010022	N/A	JX010279	N/A	JX009771	Weir et al. (2012)
<i>C. theobromicola</i>	ICMP 17895	<i>Annona diversifolia</i>	Mexico	JX009568	N/A	JX009600	JX010057	JX010066	JX010284	JX010382	JX009828	Weir et al. (2012)
<i>C. theobromicola</i>	ICMP 15445	<i>Acca sellowiana</i>	New Zealand	JX009509	N/A	JX009601	JX010027	N/A	JX010290	N/A	JX009893	Weir et al. (2012)
<i>C. theobromicola</i>	CBS 125393, ICMP 18650	<i>Theobroma cacao</i>	Panama	JX009503	N/A	JX009590	JX009982	N/A	JX010280	N/A	JX009872	Weir et al. (2012)
<i>C. theobromicola</i>	ICMP 17099	<i>Fragaria × ananassa</i>	USA	JX009493	N/A	JX009588	JX009957	N/A	JX010285	N/A	JX009778	Weir et al. (2012)
<i>C. theobromicola</i>	ICMP 17100	<i>Quercus</i> sp.	USA	JX009507	N/A	JX009596	JX009947	N/A	JX010281	N/A	JX009781	Weir et al. (2012)
<i>C. theobromicola</i>	IMI 348152, ICMP 17814	<i>Fragaria vesca</i>	USA	JX009448	N/A	JX009589	JX010003	JX010062	JX010288	JX010379	JX009819	Weir et al. (2012)
<i>C. theobromicola</i>	CBS 142.31, ICMP 17927	<i>Fragaria × ananassa</i>	USA	JX009516	N/A	JX009592	JX010024	JX010064	JX010286	JX010373	JX009830	Weir et al. (2012)
<i>C. theobromicola</i>	MUCL 42294, ICMP 17957, CBS 124251	<i>Stylosanthes viscosa</i>	Australia	JX009575	N/A	JX009597	JX009962	JX010063	JX010289	JX010380	JX009821	Weir et al. (2012)
<i>C. theobromicola</i>	CMM 3559	<i>Eucalyptus</i> sp.	Brazil	N/A	N/A	N/A	KF768562	N/A	KF768556	KF768559	N/A	Rodrigues et al. (2014)
<i>C. theobromicola</i>	CMM 3561	<i>Eucalyptus</i> sp.	Brazil	N/A	N/A	N/A	KF768561	N/A	KF768555	KF768558	N/A	Rodrigues et al. (2014)

<i>C. theobromicola</i>	<b>CMW 56824</b>	<i>Eucalyptus</i> sp.	South Africa	OK584040	OK584046	OK584034	OK584058	OK584052	OK485037	OK584022	OK584028	This study
<i>C. theobromicola</i>	<b>CMW 56825</b>	<i>Eucalyptus</i> sp.	South Africa	OK584041	OK584047	OK584035	OK584059	OK584053	OK485038	OK584023	OK584029	This study
<i>C. theobromicola</i>	<b>CMW 56826</b>	<i>Eucalyptus</i> sp.	South Africa	OK584042	OK584048	OK584036	OK584060	OK584054	OK485039	OK584024	OK584030	This study
<i>C. theobromicola</i>	<b>CMW 56827</b>	<i>Eucalyptus</i> sp.	South Africa	OK584043	OK584049	OK584037	OK584061	OK584055	OK485040	OK584025	OK584031	This study
<i>C. theobromicola</i>	<b>CMW 56828</b>	<i>Eucalyptus</i> sp.	South Africa	OK584044	OK584050	OK584038	OK584062	OK584056	OK485041	OK584026	OK584032	This study
<i>C. theobromicola</i>	<b>CMW 56829</b>	<i>Eucalyptus</i> sp.	South Africa	OK584045	OK584051	OK584039	OK584063	OK584057	OK485042	OK584027	OK584033	This study
<i>C. tropicale</i>	CBS 124949T, ICMP 18653	<i>Theobroma cacao</i>	Panama	JX009489	GU994425	JX009719	JX010007	JX010097	JX010264	GU994454	JX009870	Vieira et al. (2017)
<i>C. tropicale</i>	CMM 4243	<i>Musa</i> sp.	Brazil	KU213596	KU213597	KU213599	KU213601	KU213602	KU213603	KU213604	KU213600	Vieira et al. (2017)
<i>C. xanthorrhoeae</i>	IMI 350817a, ICMP 17820	<i>Xanthorrhoea</i> sp.	Australia	JX009479	N/A	JX009652	JX010008	N/A	JX010260	N/A	JX009814	Liu et al. (2015)
<i>C. xanthorrhoeae</i>	BRIP 45094, ICMP 17903, CBS 127831T	<i>Xanthorrhoea preissii</i>	Australia	JX009478	KC790689	JX009653	JX009927	JX010138	JX010261	JX010448	JX009823	Liu et al. (2015)

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

#### Abbreviations:

ATCC = American Type Culture Collection, Virginia, USA; BRIP = Queensland Plant Pathology Herbarium, Australia; CBS = The culture collection of Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; CMM = Culture Collection of Phyto-pathogenic Fungi Prof. Maria Menezes, Federal Rural University of Pernambuco, Brazil; CMW = culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), South Africa; CPC = Pedro Crous working collection housed at Westerdijk Fungal Biodiversity Institute; ICMP = International Collection of Microorganisms from Plants; MFLUCC = Mae Fah Luang University Culture Collection, Thailand; IMI = CABI Genetic Resource Collection, UK; LC = Working collection of Lei Cai, housed at CAS, China; LF = Working collection of Fang Liu, housed at CAS,

China; MAFF = Ministry of Agriculture, Forestry and Fisheries, Japan; MUCL = Belgian Co-ordinated Collections of Micro-organisms, (agro)industrial fungi and yeasts, Belgium; UOM = University of Melbourne culture collection, Victoria, Australia.

ACT = actin; CAL = calmodulin; CHS1 = Chitin synthase; GS = Glutamine synthetase; GAPDH = Glyceraldehyde-3-phosphate; ApMat = Intergenic spacer and partial MAT1-2 gene; ITS = Internal transcribed spacer; TUB2 =  $\beta$ -tubulin 2.