Calonectria pentaseptata causes severe leaf disease on cultivated *Eucalyptus* in Leizhou Peninsula of southern China

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Abstract

Eucalyptus (Myrtaceae, Myrtales) trees are widely cultivated for commercial purposes worldwide. Calonectria leaf blight is one of the most prominent diseases associated with *Eucalyptus* trees grown in plantations in Asia and South America. Recently, symptoms of leaf blight, shoot blight, tree death and seedling rot caused by *Calonectria* species have been observed in commercial *Eucalyptus* plantations and nurseries in Leizhou Peninsula, which presents one of the most densely *Eucalyptus*-planted areas in southern China. Disease samples were collected from 10 *Eucalyptus* species and a number of *Eucalyptus grandis*, *E. tereticornis* and *E. urophylla* hybrid genotypes, which were planted in plantations at 13 sites and one experimental nursery. A total of 773 isolates of *Calonectria* were obtained from 683 plantation trees and nursery seedlings. Fifty-five representative isolates from all the surveyed sites and *Eucalyptus* species/genotypes were selected for molecular identification. These 55 isolates were identified by DNA sequence analyses based on the calmodulin (*cmdA*), histone H3 (*his3*), translation elongation factor 1-alpha (*tef1*), and β-tubulin (*tub2*) gene regions, as well as a combination of morphological characteristics. The results indicated that these 55 isolates

present one single species, *Calonectria pentaseptata*. Determined by sequences of *cmdA*, *his3*, *tef1* and *tub2* gene regions, only two genotypes were identified among the 55 representative isolates; 54 of these isolates share the same genotype, which suggests that the genetic diversity of *Ca. pentaseptata* collected in this study is relatively low. A growth study indicated that *Ca. pentaseptata* is a high-temperature species. The mating test results suggest that *Ca. pentaseptata* is heterothallic or lacks the ability to recombine to produce fertile progeny. Inoculation results showed that *Ca. pentaseptata* causes leaf blight and stem rot, resulting in tree death of the two widely planted *Eucalyptus* genotypes in southern China, and the two genotypes differ significantly in their susceptibilities to infection by *Ca. pentaseptata*. It is urgent to initiate a selection program to develop *Eucalyptus* planting stocks with high levels of resistance to Calonectria leaf blight in China in the long term.

Introduction

Eucalyptus (Myrtaceae, Myrtales) species were first introduced to China in 1890 (Qi 2002). Currently, *Eucalyptus* trees are planted in large areas in southern China because of their fast growth, strong adaptability and broad applications (Xie et al. 2017), and more than 4.5 million hm^2 of *Eucalyptus* plantations have been established (Xie et al. 2017). In 2015, China's *Eucalyptus* timber production reached 30 million m^3, accounting for about 27% of the country's total annual domestic timber production. *Eucalyptus* plantations make a substantial contribution to safeguarding the security of China's wood supply (Chinese Society of Forestry 2016). In commercial plantations, the hybrids of *Eucalyptus camaldulensis*, *E. grandis*, *E. pellita*, *E. tereticornis* and *E. urophylla* are the most widely planted (Xie et al. 2017).

The *Eucalyptus* plantations in China typically have limited genotypes of hybrids of few species; thus, diseases spread rapidly after they break out in small regions (Chen 2014; Zhou and Wingfield 2011). Currently, the important diseases that threaten *Eucalyptus* plantations include leaf spot/blight caused by species of Mycosphaerellaceae and Teratosphaeriaceae (Burgess et al. 2006, 2007), *Calonectria* (Chen et al. 2011c; Li et al. 2017) and *Quambalaria* (Zhou et al. 2007; Chen et al. 2017); stem canker/wilt caused by species of Botryosphaeriaceae (Chen et al.

2011d; Li et al. 2018) and Cryphonectriaceae (Chen et al. 2010, 2011b; Wang et al. 2018), as well as *Ceratocystis* (Chen et al. 2013b; Liu et al. 2015) and *Teratosphaeria zuluensis* (Chen et al. 2011a; Cortinas et al. 2006), and bacterial wilt caused by *Ralstonia pseudosolanacearum* (Carstensen et al. 2017). Leaf blight caused by species of *Calonectria* is considered one of the most serious threats to *Eucalyptus* plantations in China (Xie et al. 2017; Zhou and Wingfield 2011).

Species of *Calonectria* are widely distributed in sub-tropical and tropical regions around the world (Crous 2002; Lombard et al. 2010a). With respect to *Eucalyptus*, *Calonectria* species mainly infect the leaves of plantation trees and leaves and stems of nursery seedlings, causing leaf blight and seedling rot, respectively (Alfenas et al. 2015, 2016; Chen et al. 2011c; Crous 2002; Fernandes et al. 2016; Rodas et al. 2005). On *Eucalyptus* in China, the disease caused by species of *Calonectria* was first reported on nursery seedlings in HaiNan Province in 1985 (Feng and Zheng 1986). On *Eucalyptus* plantations, leaf blight was first observed on one–two-year old trees in GuangXi Province in 1991 (Meng 1993). Subsequently, disease caused by *Calonectria* was reported in *Eucalyptus* plantations and nurseries in GuangDong and FuJian Provinces (Chen 2004; Deng et al. 1997; Zhu et al. 2002). Since then, outbreaks have frequently been reported in other *Eucalyptus* plantations in southern China (Chen et al. 2013a; Chen et al. 2011c; Li et al. 2017; Lombard et al. 2010d).

The use of resistant genotypes is a valuable method for controlling disease caused by *Calonectria* on species of *Eucalyptus* and *Corymbia* (Alfenas et al. 2016; Chen et al. 2011c; Old et al. 2003; Rodas et al. 2005). Evaluation of susceptibility of *Eucalyptus* and *Corymbia* species to *Calonectria* revealed broad inter and intraspecific variability of the species among *Eucalyptus* or *Corymbia* in Brazil (Alfenas et al. 2016). Chen et al (2011c) showed that the tolerance of *Eucalyptus* hybrid clones significantly differs among isolates of *Calonectria* in China. In Colombia, evaluation of 42 different *E. grandis* clones indicated that clones differ markedly in term of susceptibility to *Calonectria* species (Rodas et al. 2005). The results of previous studies imply that it might be possible to select disease-tolerant planting stocks based on nursery and field screening.

In 2017 and 2018, during the *Eucalyptus* disease surveys on plantation trees and nursery seedlings in Leizhou Peninsula in GuangDong Province in southern China, leaf blight of trees and leaf and stem rot of seedlings were frequently observed in different regions/sites. White masses of conidiophores and conidia with the typical morphological characteristics of *Calonectria* were consistently observed on the diseased trees and seedlings. Disease samples were collected, and *Calonectria* fungi were isolated. The aims of this study were thus to (1) identify the *Calonectria* fungi isolated from diseased trees and seedlings based on DNA sequence comparisons and morphological characteristics; and (2) test their pathogenicities by inoculating two *Eucalyptus urophylla* hybrid genotypes that are widely planted in the sampling regions and in other regions in southern China.

Materials and Methods

Disease symptoms, samples, and fungal isolations

From June to August 2017 and from April to November 2018, disease surveys in *Eucalyptus* plantations and nurseries were conducted in Leizhou Peninsula, GuangDong Province in southern China, which is one of the most densely *Eucalyptus*-planted areas in China. In plantations, the leaves and shoots of *Eucalyptus* infected by pathogens present blight and the leaves dry out; defoliation typically moves upwards from the bases and centers of the affected trees, resulting in total defoliation and ultimately tree death (Fig. 1A, B and C). On the infected trees, the disease is first observed as greyish water-soaked spots on young and mature leaves on the lower branches; these spots coalesce and develop into extensive necrotic areas (Fig. 1D). Under conditions of high humidity and frequent rainfall, necrotic lesions cover the entire area of the leaf, and young shoot tips are killed, resulting in leaf and shoot blight symptoms (Fig. 1E and F). In locations with high humidity, white masses of conidiophores with typical morphological characteristics of *Calonectria* species were frequently observed on main stems, branches and shoots of *Eucalyptus* trees (Fig. 1G, H and I). The disease symptoms were mainly observed on plantation *Eucalyptus* less than two years old. The main infected *Eucalyptus*

species include *Eucalyptus grandis* and *E. pellita*, and the hybrids among *E. grandis*, *E. tereticornis* and *E. urophylla* (Fig. 1J, K, L, M and N). In nurseries, the typical disease symptoms include seedling stem rot (Fig. 2A, B and C) and leaf rot (Fig. 2F); white masses of conidiophores with typical morphological characteristics of *Calonectria* fungi cover the infected stems, resulting in seedling death (Fig. 2D and E) and a rapid onset of leaf rot (Fig. 2F). Typical round lesions are produced on leaves of different species of *Eucalyptus* (Fig. 2G, H and I); the leaves drop after infection, which results in seedling death in most species (Fig. 2J). The diseases symptoms were observed in most *Eucalyptus* plantations in the Leizhou Peninsula; diseased samples included blighted leaves and twigs or young branches with blight that were collected from 13 sites distributed in different regions in the peninsula (Fig. 3). Samples from diseased seedlings, including rotten stems and leaves, were collected from the South China Experimental Nursery (SCEN), which is the largest forest tree seedling nursery in Leizhou Peninsula (Fig. 3). Diseased leaves, branches or stems were collected from each of the sampled trees/seedlings. In the plantations, samples were collected from twenty to 150 trees at each site, depending on the area of the sampled plantation. In one nursery, samples were collected from 230 seedlings from all four seedling-cultivated sites. Samples of diseased materials were transported to the laboratory for isolation, morphological examination and further assessment.

The symptomatic tissues were incubated in moist dishes at room temperature for one to three days to induce *Calonectria* sporulation. By using sterile needles, the conidial masses of *Calonectria* were transferred directly from diseased *Eucalyptus* materials to 2% malt extract agar (MEA) (20 g malt extract powder and 20 g agar powder per liter of water: malt extract powder was obtained from the Beijing Shuangxuan microbial culture medium products factory, Beijing, China; the agar powder was obtained from Beijing Solarbio Science & Technology Co., Ltd., Beijing, China). The conidial masses were incubated at room temperature for three to five days; a single hyphal tip from each culture was transferred to a 2% MEA plate and incubated at room temperature for seven to ten days to obtain pure cultures. The pure cultures were deposited in the culture collection (CSF) located at the China Eucalypt Research Centre (CERC), Chinese Academy of Forestry (CAF), ZhanJiang, GuangDong Province, China. The

specimens (pure dried fungal cultures) were deposited in the Collection of Central South Forestry Fungi of China (CSFF), GuangDong Province, China.

DNA extraction, PCR and sequencing

Representative isolates were selected for DNA sequence analyses; these isolates were obtained from leaves, branches and stems of different *Eucalyptus* species/genotypes collected from *Eucalyptus* plantations and nursery at different sites. The actively growing mycelium was scraped from 10-day-old cultures using a sterilized scalpel and transferred into 2.0-mL Eppendorf tubes. The total genomic DNA was extracted following the protocols "Extraction method 5: grinding and CTAB" described by Van Burik et al. (1998). The extracted DNA was dissolved in 30 μL TE buffer (1 M Tris-HCl and 0.5 M EDTA, pH 8.0), and a Nano-Drop 2000 spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to measure the concentration.

Based on previous research results, partial gene regions including calmodulin (*cmdA*), histone H3 (*his3*), translation elongation factor 1-alpha (*tef1*) and β-tubulin (*tub2*) were used as successful DNA barcodes at the species level, being able to clearly distinguish between intra- and inter-specific divergence of the *Calonectria* genus (Alfenas et al. 2015; Li et al. 2017; Liu and Chen 2017; Lombard et al. 2010b, c, 2015a, 2016). Fragments of the *cmdA*, *his3*, *tef1* and *tub2* genes were amplified by the respective primer pairs CAL-228F/CAL-2Rd, CYLH3F/CYLH3R, EF1-728F/EF2 and T1/CYLTUB1R (Lombard et al. 2010d). Polymerase chain reaction (PCR) was conducted as described by Liu and Chen (2017).

All PCR products were sequenced in both directions using the same primers that were used for the PCR amplification. Sequence reactions were run by the Beijing Genomics Institute, Guangzhou, China. The nucleotide sequences were read and edited using MEGA v. 6.0.5 software (Tamura et al. 2013). All sequences obtained in this study were submitted to GenBank (http://www.ncbi.nlm.nih.gov).

Phylogenetic analyses

Sequences generated in this study were compared to sequences of type specimen strains of closely related *Calonectria* species downloaded from GenBank for phylogenetic analyses. Sequences of each of the *cmdA*, *his3*, *tef1* and *tub2* gene regions as well as the sequence combination of the four gene regions were aligned using MAFFT online v. 7 (http://mafft.cbrc.jp/alignment/server/), with the alignment strategy FFT-NS-i (slow; interactive refinement method). The alignments were edited manually using MEGA v. 6.0.5 software (Tamura et al. 2013).

Phylogenetic analyses were conducted for each of the four gene sequence datasets, as well as for the combination of all gene regions. Two methods, Maximum Parsimony (MP) and Maximum Likelihood (ML), were used for phylogenetic analyses. The MP and ML analyses were conducted using the methods described in Liu and Chen (2017). The phylogenetic trees were viewed using MEGA v. 6.0.5 (Tamura et al. 2013) for both MP and ML analyses.

Sexual compatibility

To determine whether the *Calonectria* species identified in the current study had a heterothallic or a homothallic mating system, representative isolates of each of the identified *Calonectria* species were crossed with each other in all possible combinations. Isolates crossed with themselves served as controls. Crosses were performed on minimal salt agar (MSA; Guerber and Correll 2001) on which sterile toothpicks had been placed on the surface of the medium (Lombard et al. 2010b, c). The cultures were incubated at 25°C for six weeks. The isolate combinations were considered successful when the isolate combinations produced perithecia and viable ascospores.

Morphology

The representative isolates of *Calonectria* species identified by DNA sequence comparisons

were selected for morphological studies. The sexual and asexual structures were produced by using MSA and synthetic nutrient-poor agar (SNA; Nirenburg 1981), respectively, by the method described in Liu and Chen (2017).

The sexual structures were studied by transferring perithecia to a tissue-freezing medium (Leica Biosystems, Nussloch, Germany) and were hand-sectioned using an HM550 Cryostat Microtome (Microm International GmbH, Thermo Fisher Scientific, Walldorf, Germany) at –20°C. The 10-µm sections were mounted in 80% lactic acid and 3% KOH. The asci and ascospores and the asexual structures produced on the surface of the SNA medium were mounted in one drop of sterile water on glass slides and examined under an Axio Imager A1 microscope (Carl Zeiss Ltd., Munchen, Germany) and with an AxioCam ERc 5S digital camera controlled by Zeiss Axio Vision Rel. 4.8 software (Carl Zeiss Ltd., Munchen, Germany).

Thirty measurements were made for each morphological structure of the isolates selected as the specimen. Since the size of macroconidia and width of vesicles are the most typical characteristics using for morphological comparisons in *Calonectria* (Alfenas et al. 2015; Li et al. 2017; Lombard et al. 2010c, 2015a, 2016), 100 and 50 measurements of macroconidia and vesicles, respectively, were made for the specimens. To understand the variations of macroconidia and vesicles among isolates identified as the same *Calonectria* species, 50 measurements of these structures were made for representative isolates. Minimum, maximum and average (mean) values were determined and they are presented as follows: (minimum–) (average – standard deviation) – (average + standard deviation) (–maximum).

To determine the effect of temperature on the mycelial growth rate of the representative isolates of identified *Calonectria* species, mycelial agar plugs (5 mm diam.) were transferred from these cultures to new 2% MEA Petri dishes and incubated in darkness under different temperatures ranging from 5°C to 35°C at 5°C intervals. Five replicates of each selected isolate were incubated at each temperature in each experiment. The experiment was repeated once. Colony diameters were measured orthogonally after seven days incubation, and the data were used to calculate growth rates. The average growth rates at each of the seven temperatures were

240 determined by calculating the values of ten replicate plates in the two experiments. The colony
241 characteristic was determined after the isolates were inoculated on fresh MEA at 25°C for seven
242 days.
243
244 **Pathogenicity tests**
245
246 To test the pathogenicity of the *Calonectria* species identified in this study, representative
247 isolates obtained from different geographic sites and identified based on DNA sequence
248 comparisons were selected for inoculation trials. Mycelial plugs with abundant conidia were
249 transferred to fresh 2% MEA 9-cm-Petri dishes, and the conidia were spread on the surface of
250 MEA Petri dishes by moving the mycelium plugs. The culture was incubated at 25°C for three
251 days until sporulation occurred on the culture medium surface. A conidial suspension was
252 prepared for each selected isolate, by adding 75 ml of sterile water to the culture surface
253 followed by scraping with a sterilized, soft-bristled paint brush to release conidia using the
254 method described in Graça et al. (2009). The conidial suspension was measured using a
255 hemocytometer and the concentration was adjusted to 1×10^5 conidia/mL.
256
257 Two *Eucalyptus* genotypes, *E. urophylla* × *E. tereticornis* hybrid genotype CEPT1845 and *E.*
258 *urophylla* × *E. grandis* hybrid genotype CEPT1846, which are widely planted in Leizhou
259 Peninsula, were selected for inoculation. The inoculated seedlings were three months old and
260 approximately 40 cm tall. For each of the selected *Calonectria* isolates, eight seedlings of each
261 genotype were inoculated with the conidial suspension by spraying the leaves until run-off.
262 Sterile water was sprayed onto the seedlings and subjected to the same treatment as the control.
263 The seedlings were covered with plastic chambers and were subjected to stable climatic
264 conditions (temperature 24°C–26°C and humidity 95%–100%) for three days, allowing
265 sufficient humidity for infection. The negative control inoculations were conducted in a similar
266 fashion with sterile water. The experiment was repeated once using the same methodology.
267
268 The plastic chambers were removed three days after inoculation. The percentage of
269 rotten/blighted leaves was calculated for every inoculated seedling. For re-isolations, small

pieces of discolored leaf from the edges of the resultant lesions were cut and placed on 2% MEA at room temperature. Re-isolations of randomly selected leaves from all seedlings were inoculated as negative controls, and from four randomly selected seedlings for each inoculated isolate. The identities of the re-isolated fungi were confirmed by culture morphological comparisons, and the fruiting structures (macroconidiophore and macroconidia) and the disease symptoms produced on the leaves and stems of the inoculated seedlings with the original fungi were used for the inoculations. The inoculation results were analyzed using SPSS Statistics 20 software (BM Corp., Armonk, NY, USA) by one-way analysis of variance (ANOVA). The inoculations were performed in April 2019 at the China Eucalypt Research Centre, located in Leizhou Peninsula in southern China.

Results

Fungal isolations

Fungal isolates with typical morphological characteristics of *Calonectria* were isolated from sampled materials; one to two *Calonectria* isolates from each tree or seedling were deposited in the culture collection (CSF) at CERC, depending on the varieties of originally diseased materials (tree leaf, tree branch, seedling leaf or seedling stem), and the culture morphology among the isolates was obtained from the same tree (Table 1). In total, 773 isolates were obtained from 14 sites in Leizhou Peninsula; these include 513 isolates from 455 *Eucalyptus* trees in plantations at 13 sites and 260 isolates from 228 *Eucalyptus* seedlings in one nursery (Table 1, Fig. 3). The isolates obtained from plantations were isolated mainly from *E. urophylla* × *E. tereticornis* hybrids, followed by *E. urophylla* × *E. grandis* hybrids, and a relatively small number of isolates were from *E. grandis* × *E. urophylla* hybrids, *E. urophylla* hybrids, *E. grandis*, *E. pellita* and *E. saligna*. The 260 isolates obtained from one nursery were isolated from *E. urophylla* hybrids, *E. urophylla* × *E. grandis* hybrids, *E. urophylla* × *E. tereticornis* hybrids and 10 *Eucalyptus* species. Based on the morphological characteristics, all the isolates collected in this study reside in the Prolate Group of *Calonectria* (Lombard et al. 2010b).

Phylogenetic analyses

One to two isolates from each geographic site × *Eucalyptus* genotype were selected for molecular identification by sequences, depending on the varieties of originally diseased materials (tree leaf, tree branch, seedling leaf and seedling stem). Fifty-five isolates were ultimately selected for further analyses (Table 2). All of the 55 selected isolates reside in the Prolate Group of *Calonectria*. Determined by sequences of the *cmdA*, *his3*, *tef1* and *tub2* gene regions, two genotypes were generated for the 55 sequenced isolates; with the exception of isolate CSF13337, all of the remaining 54 isolates presented the same genotype (Table 2). Sixteen isolates obtained from different geographic sites that represent the two genotypes were used in phylogenetic analyses (Table 2, Fig. 3). Sequences for 12 ex-type specimen strains and other strains of 12 *Calonectria* species closely related to isolates obtained in this study were downloaded from GenBank (Table 3). *Curvicladiella cignea* (CBS 109167 and CBS 109168) was used as the outgroup taxon. The partition homogeneity test (PHT) comparing the combined *cmdA, his3, tef1,* and *tub2* gene datasets generated a *P* value of 0.01, indicating some incongruence in the datasets for the four loci, and the accuracy of the combined data suffered relative to the individual partitions (Cunningham 1997). Although the *P* value was low, sequences of the four regions were combined for presentation purposes. These four datasets were combined and were subjected to phylogenetic analyses. For the phylogenetic trees based on *cmdA, his3, tef1,* and *tub2* individually and the combined sequence datasets, the overall topologies were similar, although the relative position of some *Calonectria* species was slightly different between the MP and ML trees, the ML trees are shown (Figs, 4 and 5). The number of parsimony informative characters, the statistical values for the phylogenetic trees of the MP analyses, and the parameters for the best-fit substitution models of the ML analyses are presented in Table 4.

The phylogenetic analyses of each of the four individual and combined sequence datasets showed that all 16 isolates obtained in the current study reside in the *Ca. reteaudii* species complex. The analyses of four individual sequence datasets showed these 16 isolates grouped in the same clade with *Ca. pentatseptata* based on each of the *his3, tef1* and *tub2* trees (the

cmdA sequence was not available for ex-type of *Ca. pentatseptata*) (Fig. 4A, B, C and D), and grouped in the same clade with *Ca. microconidialis* in each of the *cmdA* and *tub2* trees (Fig. 4A and D), but separate from *Ca. microconidialis* in the *his3* and *tef1* trees (Fig. 4B and C). The analyses of the combined sequence dataset showed that the isolates obtained in the current study were grouped in the same clade with *Ca. pentaseptata*, while *Ca. microconidialis* formed one independent clade supported by relatively high bootstrap values (ML and MP: 87% and 85%) (Fig. 5). The phylogenetic analysis results indicated that the 55 selected isolates obtained in this study were all identified as *Ca. pentaseptata* (Table 2, Fig. 5).

Sexual compatibility

Six isolates (CSF12825, CSF13036, CSF13040, CSF13317, CSF13452 and CSF13636) of *Ca. pentaseptata* obtained from five geographic sites were selected for mating tests on MSA (Table 2). After six weeks, all six isolates and the crosses failed to yield any perithecia. These results indicate that they were either self-sterile (heterothallic) or they lacked the ability to recombine to produce fertile progeny.

Morphology

Since all six isolates and the crosses failed to yield any perithecia, the sexual state is unavailable for *Ca. pentaseptata* obtained in this study. Based on the two isolates (CSF13036 and CSF13636) selected as the specimens, the fungi are described as follows: the macroconidiophores consist of a stipe, a suite of penicillate arranged fertile branches, a stipe extension, and a terminal vesicle (Fig. 6A, B and C); stipe septate, hyaline, smooth, (39.5–)92.5–215.5(–330) × (4–)5.5–8.5(–13.5) μm, stipe extension septate, straight to flexuous (67–)116–224.5(–371.5) μm long, (2–)3–4(–5.5) μm wide at the apical septum (Fig. 6A, B and C), terminating in clavate vesicle, (2–)2.5–4(–5) μm diam (average of 100 vesicles: 3.5 μm) (Fig. 6G and H); lateral stipe extensions (90° to main axis) absent (Fig. 6A, B and C). Conidiogenous apparatus (20.5–)34.5–58(–75.5) μm wide, and (32–)42.5–74(–104) μm long (Fig. 6D, E and F); primary branches aseptate to 1 septate, (10.5–)14–22.5(–28.5) × (3–)4–5(–

6.5) μm; secondary branches aseptate, (9.5–)12.5–18.5(–22) × (3–)3.5–4(–5.5) μm; tertiary branches aseptate, (9–)9–14.5(–15.5) × (3–)3–4(–4) μm, each terminal branch producing 2–4 phialides; phialides cylindrical to allantoid, hyaline, aseptate, (10.5–)12–17(–23.5) × (3–)3.5–4.5(–6) μm, apex with minute periclinal thickening and inconspicuous collarette (Fig. 6D, E and F). Macroconidia cylindrical, rounded at both ends, straight, (69–)80.5–100(–113.5) × (5.5–)6.5–7.5(–9) μm (average of 200 macroconidia: 90 × 7 μm), 5 septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colorless slime (Fig. 6I and J). Megaconidia and microconidia not observed.

Specimens examined were from China, GuangDong Province, ZhanJiang Region, SuiXi County, LingBei Town, 21º15'31.74"N, 110º06'35.17"E, from leaves of the *E. urophylla* × *E. tereticornis* hybrid genotype G1 seedling, 04 August 2018, GuoQing Li, QianLi Liu and Wen Wang, CSFF 2047, living culture CSF13036; and GuangDong Province, ZhanJiang Region, SuiXi County, LingBei Town, 21º15'51.80"N, 110º07'27.93"E, from the branch of a 2-year-old *E. urophylla* × *E. tereticornis* hybrid tree, 13 September 2018, ShuaiFei Chen, CSFF 2048, living culture CSF13636.

Five isolates (CSF13036, CSF13040, CSF13317, CSF13452 and CSF13636) obtained from five geographic sites (Table 2) were selected to evaluate the size variations of macroconidia and vesicles among isolates identified as *Ca. pentaseptata*. The measurements of macroconidia showed that significant length variations exist among some of the five isolates. Based on the measurement average, the conidia of isolate CSF13317 (average length 100.5 μm) are 16.5 um longer than those of isolate CSF13636 (average length 84 μm) (Table 5). Isolates CSF13636 and CSF13452 (average length 90 μm) are much shorter than the originally described strains of *Ca. pentaseptata* (Crous et al. 2012) (Table 5). The measurements further showed no differences in the conidia length among five isolates obtained in the current study and the originally described strains of *Ca. pentaseptata* (Crous et al. 2012). All five isolates produced 5-septate macroconidia; the vesicle widths were 2–6 μm, similar to the originally described strains of *Ca. pentaseptata* (Crous et al. 2012).

Six isolates (CSF12825, CSF13036, CSF13040, CSF13317, CSF13452 and CSF13636) of *Ca. pentaseptata* obtained from five geographic sites were selected for a growth study (Table 2, Fig. 7). The results of the average growth rates showed no growth at 5°C and 35°C for all six tested isolates. The optimal growth temperatures among the six isolates are different; 25°C is the optimal growth temperature for isolates CSF12825, CSF13036 and CSF13452, compared with 30°C for isolates CSF13040, CSF13317 and CSF13636 (Fig. 7). The growth results indicated that *Ca. pentaseptata* obtained in this study is a high-temperature species. Colonies formed abundant buff and wooly aerial mycelia on MEA at 25°C after seven days, with feathery, irregular margins at the edges, sporulation abundant and more concentrated in the colony center.

Pathogenicity tests

Five isolates (CSF13036, CSF13040, CSF13317, CSF13452 and CSF13636) of *Ca. pentaseptata* obtained from five geographic sites (Table 2) were inoculated on seedlings of *Eucalyptus* genotypes CEPT1845 and CEPT1846. In both experiments, all seedlings representing the two *Eucalyptus* genotypes inoculated with *Ca. pentaseptata* developed leaf spot, shoot blight and stem rot symptoms, whereas no disease symptoms were observed on the tissues of the negative control seedlings. The fungi have the same morphological characteristics as the originally inoculated *Calonectria* that were successfully re-isolated from the diseased *Eucalyptus* tissues, while no *Calonectria* were isolated from the negative control seedlings.

The inoculated *Calonectria* produced water-soaked spots on both young and old leaves of two *Eucalyptus* genotypes after inoculation for 24 hours; the spots coalesced and developed into extensive necrotic areas (Fig. 8A–H). The tips of seedlings rotted after infection within 48 hours (Fig. 8J and N); abundant white masses of conidiophores of *Ca. pentaseptata* were produced on infected tips and leaves, resulting in seedling stem rot (Fig. 8J, K and N). The tips and leaves of seedlings rotted and blighted after inoculation within 72 hours (Fig. 8L and P).

The average percentage of blighted leaves affected by the test isolates showed some differences existed between the two experiments, especially for the inoculation results for *Eucalyptus*

genotype CEPT1846 (Fig. 9A and B); this may be due to the inconsistency of climatic conditions during the two experiments. Subsequently, the data for the two experiments were analyzed separately. For the tested *Eucalyptus* genotype CEPT1845, in the two experiments, 82% to 100% of the seedling leaves were rotted and blighted after infection by inoculated *Ca. pentaseptata* isolates (Fig. 9A and B). The average percentages of blighted leaves caused by the five *Ca. pentaseptata* isolates were not significantly different within each of the two experiments, with the exception of CSF13636 in Experiment Two ($P < 0.05$). Isolate CSF13036 displayed the highest average percentage of leaf blight infection in *Eucalyptus* genotype CEPT1845 in both experiments. For the tested *Eucalyptus* genotype CEPT1846, though the average percentages of blighted leaves caused by the five *Ca. pentaseptata* isolates were not consistent between the two experiments, the two experiments consistently showed that more than 20% of the seedlings leaves rotted and were blighted after infection by inoculated *Ca. pentaseptata* isolates, with the exception of isolate CSF13040 in Experiment One (Fig. 9A and B).

Analyses of variance indicated that there were significant differences in the susceptibility of the two *Eucalyptus* genotypes to the isolates we tested. In the two experiments, the average percentages of blighted leaves caused by all *Calonectria* isolates on *Eucalyptus* genotype CEPT1845 were significantly higher than those on genotype CEPT1846 ($P < 0.05$). The results suggested that CEPT1846 is much more tolerant than CEPT1845 to *Ca. pentaseptata* tested in the current study.

Discussion

In this study, a severe disease was observed in *Eucalyptus* plantations and nursery in Leizhou Peninsula in southern China. The disease mainly caused leaf blight and leaf defoliation of plantation *Eucalyptus* and leaf spot and stem rot of nursery seedlings. Fruiting structures with typical morphological characteristics of *Calonectria* species were observed on the diseased leaves, branches and seedlings. Disease samples were collected from 10 *Eucalyptus* species and a number of hybrid genotypes, which were planted in plantations at 13 sites, and an

experimental nursery at one site. Representative isolates were identified based on DNA sequence comparisons, which were combined with morphological characteristics. These fungi were consistently identified as *Calonectria pentaseptata*. The genetic diversity of *Ca. pentaseptata*, which is widely distributed in different geographic sites and *Eucalyptus* genotypes is relatively low. Pathogenicity tests showed that the inoculated isolates of *Ca. pentaseptata* caused leaf blight and stem rot, resulting in tree death of the two tested *Eucalyptus* genotypes, which are widely planted throughout Leizhou Peninsula and other regions in southern China.

In China, the first *Calonectria* fungus that caused eucalypt disease was identified as *Cylindrocladium quinqueseptatum* based on morphological characteristics (Boedijn and Reitsma 1950; Feng and Zheng 1986). The fungus was reported on seedlings of *Eucalyptus parvula* and *Corymbia citriodora* (Myrtaceae, Myrtales) in one nursery in HaiNan Province in 1985 (Feng and Zheng 1986). Based on the morphological characteristics described in previous studies, the *Calonectria* species isolated from Leizhou Peninsula was identified as *Cy. quinqueseptatum* (referred to as *Ca. reteaudii* in 2001) (Deng et al. 1997; Kang et al. 2001). The morphological characteristics of *Cy. quinqueseptatum* in Deng et al. (1997) are similar to those of *Ca. pentaseptata* reported in the current study, but it is difficult to prove whether these are the same species because the *Cy. quinqueseptatum* isolates reported in Deng et al. (1997) are not available.

Calonectria pentaseptata was first isolated from a *Eucalyptus* hybrid and *Macadamia* sp. in Vietnam, but whether *Ca. pentaseptata* caused disease of *Eucalyptus* hybrid and *Macadamia* sp. was not tested at that time (Crous et al. 2012). In China, *Ca. pentaseptata* has occasionally been isolated from leaves of *E. urophylla* × *E. grandis* clones in both nurseries and plantations in Leizhou Peninsula (Li et al. 2017; Lombard et al. 2015a), whereas no outbreaks of leaf blight caused by *Calonectria* species in this region have occurred during the last few years (Li et al. 2017; Lombard et al. 2010d, 2015a). It is known that *Calonectria* species responsible for leaf disease in *Eucalyptus* plantations can change over time (Rodas et al. 2005). It is possible that *Ca. pentaseptata* can reside in Leizhou Peninsula for a long time, because the morphology of

480 *Ca. pentaseptata* isolated in the current study is similar to that of *Cy. quinqueseptatum* reported
481 more than two decades ago (Deng et al. 1997), and the outbreaks of disease mentioned in the
482 current study only happened recently.
483
484 *Calonectria pentaseptata* resides in the *Ca. reteaudii* species complex based on phylogenetic
485 analyses (Lombard et al. 2015a). Species in this complex, such as *Ca. reteaudii*, have been
486 regarded as the predominant pathogen responsible for *Calonectria* leaf blight in South America
487 and Southeast Asia (Booth et al. 2000; Crous 2002; Crous and Kang 2001; Rodas et al. 2005;
488 Sharma and Mohanan 1991, 1992). Combined with the results in a previous study (Crous et al.
489 2012), *Ca. pentaseptata* may thus be a pathogen similar to *Ca. reteaudii*, because it has been
490 also reported in multiple regions in Vietnam and China, and the growth results indicated that
491 *Ca. pentaseptata* obtained in this study is a high-temperature species, which is similar to *Ca.*
492 *reteaudii*, because it has only been reported in tropical and sub-tropical regions (Booth et al.
493 2000; Crous 2002).
494
495 The identification of the *Calonectria* isolates obtained in the current study was mainly based
496 on DNA sequence comparisons of multiple gene regions. These sequences have been widely
497 used to clearly distinguish between intra- and inter-specific divergence of the *Calonectria* genus
498 (Alfenas et al. 2015; Li et al. 2017; Liu and Chen 2017; Lombard et al. 2010b, c, 2015a, 2016;
499 Marin-Felix et al. 2017; Pham et al. 2019). The morphological characteristics of macroconidia
500 are considered important features to distinguish *Calonectria* species (Crous 2002; Lombard et
501 al. 2010a). The morphological results in this study showed that significant variations in
502 macroconidia size exist among different isolates of *Ca. pentaseptata*, which suggests significant
503 morphological differences exist among different *Calonectria* isolates of the same species,
504 although these isolates share the same genotype based on sequences of multiple gene regions.
505
506 In China, 39 *Calonectria* species have been isolated and identified (Chen et al. 2011c; Crous et
507 al. 2004; Li et al. 2017; Liu and Chen 2017; Lombard et al. 2015a; Xu et al. 2012). Of these
508 species, 16 were isolated from *Eucalyptus* trees or seedlings, and the other 19 species were
509 isolated from soil collected from *Eucalyptus* plantations (Chen et al. 2011c; Crous et al. 2004;

Li et al. 2017; Liu and Chen 2017; Lombard et al. 2015a; Xu et al. 2012). The results of the current study indicated that *Ca. pentaseptata* is an important pathogen affecting cultivated *Eucalyptus* in China. The results of previous studies have indicated that species diversity of *Calonectria* in China is relatively higher than expected (Li et al. 2017; Liu and Chen 2017; Lombard et al. 2015a). Because species of *Calonectria* include many important pathogens that cause serious disease in economically important crops and forest trees (Crous 2002; Lombard et al. 2010a), more intensive studies need to be conducted to improve our understanding of its diversity and pathogenicity in China and other regions around the world.

The inoculation results of the current study showed that all tested isolates of *Ca. pentaseptata* found in Leizhou Peninsula are pathogenic to the two tested *Eucalyptus* genotypes. These results also showed that the two genotypes differ significantly in terms of susceptibility to infection by *Ca. pentaseptata*. This result is consistent with observations pertaining to disease caused by *Calonectria* elsewhere around the world (Alfenas et al. 2016; Crous 2002; Rodas et al. 2005). This implies that it might be possible to select disease-resistant *Eucalyptus* genotypes to reduce the impact of diseases caused by *Calonectria* species in China.

Results obtained in this study also indicated that a single genotype of *Ca. pentaseptata* was widely distributed across different geographic sites and different *Eucalyptus* genotypes in Leizhou Peninsula. Previous research has shown that one single genotype of a plant pathogen may spread and cause disease to its hosts in different geographic regions (Hurtado-Gonzales et al. 2008), which indicates that a single genotype of *Ca. pentaseptata* may cause disease to *Eucalyptus* plantations in other regions in southern China. Previous research results have also revealed that the species diversity of *Calonectria* in China is relatively high (Chen et al. 2011c; Li et al. 2017; Lombard et al. 2015a), which is similar to that of other counties such as Brazil, where a relative large number of *Calonectria* species were identified and described recently (Alfenas et al. 2015). In Brazil, several *Calonectria* species appear to be associated with Calonectria leaf blight on *Eucalyptus*, and some species were widely distributed across different *Eucalyptus* genotypes and geographic regions (Alfenas et al. 2013, 2015, 2016). The leaf blight caused by *Calonectria* is considered one of the most important diseases in both Brazil and China

(Alfenas et al. 2015, 2016; Chen et al. 2011c; Lombard et al. 2015a). The risk of spread of other *Calonectria* species also needs to be considered in China and other regions around the world.

This study reported and described an influential disease that occurs in *Eucalyptus* plantations and nurseries in southern China and expanded our understanding of the geographic distribution, host range, genetic diversity, morphological characteristics, growth feature and pathogenicity of *Ca. pentaseptata*. In recent years, the sustainable development of *Eucalyptus* plantations in China has been increasingly threatened by pathogens (Zhou and Wingfield 2011). The results of the current study offer valuable information on the management of *Calonectria* pathogens in *Eucalyptus* plantations, and will advance breeding strategies to develop disease resistant *Eucalyptus* genotypes in southern China.

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790 **Figure legends**

791

792 **Fig. 1.** Disease symptoms on plantation *Eucalyptus* hybrids and species caused by species of
793 *Calonectria*. A and B, Intense defoliation in *Eucalyptus urophylla* × *E. grandis* hybrid
794 plantations. C, The *E. urophylla* × *E. grandis* hybrid trees died after infection. D, The early
795 stage of infection of one *E. urophylla* × *E. grandis* hybrid genotype. E, The infected leaves
796 became blighted and dried. F, *Eucalyptus* trees in the whole plantation were infected and
797 defoliated. G, H and I, White mass of conidiophores of *Calonectria* species on the main stem
798 (G), branch (H) and shoot (I) of *Eucalyptus* trees. J and K, Two *E. urophylla* × *E. grandis* hybrid
799 genotypes showing leaf spot caused by species of *Calonectria*. L, *Eucalyptus pellita* with leaf
800 spot caused by *Calonectria* species. M and N, Two *E. urophylla* × *E. tereticornis* hybrid
801 genotypes showing leaf spot caused by species of *Calonectria*.

802

803 **Fig. 2.** Disease symptoms on nursery *Eucalyptus* hybrids and seedlings caused by species of
804 *Calonectria*. A, *Eucalyptus urophylla* × *E. grandis* hybrid seedlings infected by *Calonectria*
805 species. B, Dark spots on seedling stems showing the early stage of infection. C and D, White
806 mass of conidiophores of *Calonectria* species on stems of *E. urophylla* × *E. grandis* hybrid
807 seedlings. E, *Calonectria* species causing widespread death of seedlings. F, The early stage of
808 infection on young leaves of one *E. urophylla* × *E. grandis* hybrid genotype. G, H and I, Typical
809 small and rounded lesions caused by *Calonectria* species on seedlings of one *E. urophylla*
810 hybrid genotype (G), *E. pellita* (H) and *E. urophylla* (I). J, The *E. smithii* seedlings died after
811 infection by *Calonectria* species.

812

813 **Fig. 3.** Map of Leizhou Peninsula in southern China showing where *Eucalyptus* trees in
814 plantations and seedlings in a nursery were sampled, and the diversity of *Eucalyptus* species
815 and genotypes. The 14 sampled sites are indicated as number 1 to 14, followed by the species
816 or genotypes of *Eucalyptus*.

817

818 **Fig. 4.** Phylogenetic trees based on Maximum Likelihood (ML) analyses for species in the
819 *Calonectria reteaudii* species complex. A, Calmodulin (*cmdA*) region. B, Histone H3 (*his3*)

region. C, Translation elongation factor 1-alpha (*tef1*) region. D, β-tubulin (*tub2*) region. Bootstrap support values ⩾60 % for ML and MP are indicated above the branches as follows: ML/MP. Bootstrap support values < 60% are marked with *, and absence is marked with -. Isolates highlighted in bold and blue were isolated in this study; isolates representing ex-type material are marked with "T". *Curvicladiella cignea* (CBS 109167 and CBS 109168) was used as the outgroup taxon.

Fig. 5. Phylogenetic tree of *Calonectria* species based on Maximum Likelihood (ML) analyses of the dataset of the combined *cmdA*, *his3*, *tef1* and *tub2* gene sequences. Bootstrap support values ⩾60 % for ML and MP are indicated above the branches as follows: ML/MP. Bootstrap support values < 60% are marked with *, and absence is marked with -. Isolates highlighted in bold and in blue were isolated in this study; isolates representing ex-type material are marked with "T". *Curvicladiella cignea* (CBS 109167 and CBS 109168) was used as the outgroup taxon.

Fig. 6. Morphological features of asexual structures of *Calonectria pentaseptata* obtained in this study. A, B and C, Macroconidiophores with stipes bearing conidiogenous apparatus, conidia and terminating in a vesicle. D, E and F, Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. G and H, Clavate vesicles. I and J, Cylindrical, straight, 5-septate macroconidia. Scale bars: A to C = 50 μm, D to F, and I to J = 20 μm, G to H = 10 μm.

Fig. 7. Effect of temperature on mycelial radial growth of isolates of *Calonectria pentaseptata* obtained in the current study. Vertical bars represent the standard error of the means. Each value represents the average of 10 replicates.

Fig. 8. Symptoms on seedlings of *Eucalyptus urophylla* × *E. tereticornis* hybrid genotype CEPT1845 and *E. urophylla* × *E. grandis* hybrid genotype CEPT1846 inoculated by spray inoculation of conidial suspensions of *Calonectria pentaseptata* isolates. A, The non-infected leaves of *E. urophylla* × *E. tereticornis* hybrid genotype CEPT1845 seedlings. B, C and D, Leaves of *E. urophylla* × *E. tereticornis* hybrid seedlings sprayed by conidial suspension, after

24 hours (B), 48 hours (C) and 72 hours (D). E, The non-infected leaves of *E. urophylla* × *E. grandis* hybrid genotype CEPT1846 seedling. F, G and H, Leaves of *E. urophylla* × *E. grandis* hybrid seedlings sprayed by conidial suspension, after 24 hours (F), 48 hours (G) and 72 hours (H). I, The non-infected seedling of *Eucalyptus* genotype CEPT1845. J and K, White mass of conidiophores of *Ca. pentaseptata* on infected young shoots and leaves (J), and stem (K) of *Eucalyptus* genotype CEPT1845 seedlings. L, All leaves of *Eucalyptus* genotype CEPT1845 blighted and the seedling died after infection by *Ca. pentaseptata*. M, The non-infected seedling of *Eucalyptus* genotype CEPT1846. N, White mass of conidiophores of *Ca. pentaseptata* on infected young shoots and leaves of seedlings of *Eucalyptus* genotype CEPT1846. O, Dark spots on seedling stem showing the early stage of infection. P, Partial leaves of *Eucalyptus* genotype CEPT1846 seedling exhibited blighted after infection by *Ca. pentaseptata*. Q and R, Different levels of leaf blight on two *Eucalyptus* genotypes CEPT1845 (left) and CEPT1846 (right) infected by isolates CSF13452 in Experiment One (Q) and Two (R). S, No disease symptoms on two *Eucalyptus* genotypes CEPT1845 (left) and CEPT1846 (right) without infection by *Ca. pentaseptata*.

Fig. 9. Column chart indicating the average percentage of blighted leaves resulting from inoculation trials of two *Eucalyptus* hybrid genotypes inoculated with five isolates of *Calonectria pentaseptata* (CSF13036, CSF13040, CSF13317, CSF13452 and CSF13636) and the controls. Vertical bars represent the standard error of the means. Bars with different letters indicate treatment means that are significantly different (P = 0.05). Two experiments were conducted, A, Results of Experiment One. B, Results of Experiment Two.

Table 1. Sampling sites, *Eucalyptus* genotypes surveyed, and isolates obtained in this study.

Site No.	Habitat/Substratum	*Eucalyptus* genotype	Isolate No.	Isolate details
1	Plantation tree	1.5-year-old *Eucalyptus grandis*	2	two isolates from leaves of two trees
1	Plantation tree	1.5-year-old *E. saligna*	11	11 isolates from leaves of six trees
1	Plantation tree	1.5-year-old *E. urophylla* hybrid genotype CEPT28	1	one isolate from leaf of one tree
1	Plantation tree	0.5- to 1.5-year old *E. urophylla* × *E. tereticornis* hybrid genotype G1	108	108 isolates from leaves of 57 trees
2	Nursery seedling	*E. badjensis*	7	seven isolates from stems of six seedlings
2	Nursery seedling	*E. dorrigoensis*	12	12 isolates from stems of 10 seedlings
2	Nursery seedling	*E. dunnii*	8	eight isolates from leaves of six seedlings
2	Nursery seedling	*E. grandis*	51	51 isolates from leaves of 46 seedlings
2	Nursery seedling	*E. nitens*	4	four isolates from stems of four seedlings
2	Nursery seedling	*E. pellita*	10	10 isolates from leaves of 10 seedlings
2	Nursery seedling	*E. saligna*	9	nine isolates from leaves of seven seedlings
2	Nursery seedling	*E. smithii*	4	four isolates from stems of four seedlings
2	Nursery seedling	*E. urophylla*	31	31 isolates from leaves of 29 seedlings
2	Nursery seedling	*E. urophylla* hybrid genotype U6	13	13 isolates from leaves of nine seedlings
2	Nursery seedling	*E. urophylla* hybrid genotype W1	5	five isolates from leaves of three seedlings
2	Nursery seedling	*E. urophylla* × *E. grandis* hybrid	73	73 isolates from stems of 65 seedlings
2	Nursery seedling	*E. urophylla* × *E. tereticornis* hybrid genotype G1	14	14 isolates from leaves of 12 seedlings
2	Nursery seedling	*E. viminalis*	10	10 isolates from stems of 10 seedlings
2	Nursery seedling	Unknown *Eucalyptus* species	9	nine isolates from stems of seven seedlings
3	Plantation tree	2-year-old *E. urophylla* × *E. tereticornis* hybrid	11	10 isolates from leaves of 10 trees; one isolate from branches of one tree
4	Plantation tree	2-year-old *E. grandis* × *E. urophylla* hybrid genotype G9	5	five isolates from leaves of five trees
4	Plantation tree	2-year-old *E. pellita*	3	three isolates from leaves of two trees
4	Plantation tree	3-year-old *E. urophylla* × *E. tereticornis* hybrid	24	21 isolates from leaves of 21 trees; three isolates from branches of three trees
5	Plantation tree	2-year-old *E. urophylla* × *E. grandis* hybrid	25	23 isolates from leaves of 23 trees; two isolates from branches of two trees
5	Plantation tree	2-year-old *E. urophylla* × *E. tereticornis* hybrid	7	seven isolates from leaves of seven trees
6	Plantation tree	2-year-old *E. urophylla* × *E. tereticornis* hybrid	144	86 isolates from leaves of 86 trees; 58 isolates from branches of 58 trees
7	Plantation tree	2-year-old *E. urophylla* × *E. tereticornis* hybrid	17	13 isolates from leaves of 12 trees; four isolates from branches of four trees

8	Plantation tree	1-year-old *E. urophylla* × *E. tereticornis* hybrid	32	14 isolates from leaves of 14 trees; 18 isolates from branches of 18 trees
9	Plantation tree	2-year-old *E. urophylla* × *E. tereticornis* hybrid	37	21 isolates from leaves of 21 trees; 16 isolates from branches of 16 trees
10	Plantation tree	1-year-old *E. urophylla* × *E. tereticornis* hybrid	12	seven isolates from leaves of seven trees; five isolates from branches of five trees
11	Plantation tree	2-year-old *E. urophylla* × *E. grandis* hybrid	12	eight isolates from leaves of eight trees; four isolates from branches of four trees
11	Plantation tree	2-year-old *E. urophylla* × *E. tereticornis* hybrid	24	11 isolates from leaves of 11 trees; 13 isolates from branches of 13 trees
12	Plantation tree	2-year-old *E. urophylla* × *E. tereticornis* hybrid	3	three isolates from leaves of three trees
13	Plantation tree	1-year-old *E. urophylla* × *E. tereticornis* hybrid	14	14 isolates from leaves of 14 trees
14	Plantation tree	2-year-old *E. urophylla* × *E. grandis* hybrid	14	eight isolates from leaves of eight trees; six isolates from branches of six trees
14	Plantation tree	2-year-old *E. urophylla* × *E. tereticornis* hybrid	7	seven isolates from leaves of seven trees

Table 2. Isolates used for phylogenetic analyses, morphological comparisons and pathogenicity tests in this study.

Site No.	Host	Isolate No.[a]	Genotype[b]	Location	GPS information	Collector	GenBank accession No.[c]			
							cmdA	*his3*	*tef1*	*tub2*
1	1.5-year-old *Eucalyptus grandis* tree leaf	CSF13424	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°15'45.24"N, 110°05'55.87"E	S. F. Chen, Q. C. Wang and W. X. Wu	MN096268	MN115837	MN115892	MN115947
1	1.5-year-old *E. saligna* tree leaf	CSF13444	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°15'45.24"N, 110°05'55.87"E	S. F. Chen, Q. C. Wang and W. X. Wu	MN096269	MN115838	MN115893	MN115948
1	1.5-year-old *E. urophylla* hybrid genotype CEPT28 tree leaf	CSF13451	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°15'45.24"N, 110°05'55.87"E	Q. C. Wang	MN096270	MN115839	MN115894	MN115949
1	0.5-to 1.5-year old *E. urophylla* × *E. tereticornis* hybrid genotype G1 tree leaf	CSF13452[defgh]	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°16'01.72"N, 110°05'30.64"E	Q. C. Wang	MN096271	MN115840	MN115895	MN115950
1	0.5-to 1.5-year old *E. urophylla* × *E. tereticornis* hybrid genotype G1 tree leaf	CSF13477	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°16'01.72"N, 110°05'30.64"E	Q. C. Wang	MN096272	MN115841	MN115896	MN115951
2	*E. badjensis* seedling stem	CSF12761	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°15'31.74"N, 110°06'35.17"E	Q. L. Liu, G. Q. Li and S. F. Chen	MN096273	MN115842	MN115897	MN115952
2	*E. dorrigoensis* seedling stem	CSF12768	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°15'31.74"N, 110°06'35.17"E	Q. L. Liu, G. Q. Li and S. F. Chen	MN096274	MN115843	MN115898	MN115953
2	*E. dorrigoensis* seedling stem	CSF12825[dfg]	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°15'31.74"N, 110°06'35.17"E	Q. L. Liu, G. Q. Li and S. F. Chen	MN096275	MN115844	MN115899	MN115954
2	*E. dunnii* seedling leaf	CSF12776	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°15'31.74"N, 110°06'35.17"E	Q. L. Liu, G. Q. Li and S. F. Chen	MN096276	MN115845	MN115900	MN115955
2	*E. grandis* seedling leaf	CSF12654	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°15'31.74"N, 110°06'35.17"E	S. F. Chen, Q. L. Liu and Q. C. Wang	MN096277	MN115846	MN115901	MN115956
2	*E. grandis* seedling leaf	CSF12785	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°15'31.74"N, 110°06'35.17"E	Q. L. Liu, G. Q. Li and S. F. Chen	MN096278	MN115847	MN115902	MN115957
2	*E. nitens* seedling stem	CSF12829	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°15'31.74"N, 110°06'35.17"E	Q. L. Liu, G. Q. Li and S. F. Chen	MN096279	MN115848	MN115903	MN115958
2	*E. pellita* seedling leaf	CSF12877	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°15'31.74"N, 110°06'35.17"E	Q. L. Liu, G. Q. Li and S. F. Chen	MN096280	MN115849	MN115904	MN115959
2	*E. saligna* seedling leaf	CSF12833	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°15'31.74"N, 110°06'35.17"E	Q. L. Liu, G. Q. Li and S. F. Chen	MN096281	MN115850	MN115905	MN115960
2	*E. smithii* seedling stem	CSF12842	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°15'31.74"N, 110°06'35.17"E	Q. L. Liu, G. Q. Li and S. F. Chen	MN096282	MN115851	MN115906	MN115961
2	*E. urophylla* seedling leaf	CSF12849	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°15'31.74"N, 110°06'35.17"E	Q. L. Liu, G. Q. Li and S. F. Chen	MN096283	MN115852	MN115907	MN115962

2	*E. urophylla* hybrid genotype U6 seedling leaf	CSF12647	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°15'31.74"N, 110°06'35.17"E	S. F. Chen, Q. L. Liu and Q. C. Wang	MN096284	MN115853	MN115908	MN115963
2	*E. urophylla* hybrid genotype W1 seedling leaf	CSF13426	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°15'31.74"N, 110°06'35.17"E	S. F. Chen, G. Q. Li and Q. C. Wang	MN096285	MN115854	MN115909	MN115964
2	*E. urophylla* × *E. grandis* hybrid seedling stem	CSF12674	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°15'31.74"N, 110°06'35.17"E	S. F. Chen, Q. L. Liu and Q. C. Wang	MN096286	MN115855	MN115910	MN115965
2	*E. urophylla* × *E. grandis* hybrid seedling stem	CSF12690	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°15'31.74"N, 110°06'35.17"E	S. F. Chen, Q. L. Liu and Q. C. Wang	MN096287	MN115856	MN115911	MN115966
2	*E. urophylla* × *E. grandis* hybrid seedling stem	CSF12743	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°15'31.74"N, 110°06'35.17"E	S. F. Chen, Q. L. Liu and Q. C. Wang	MN096288	MN115857	MN115912	MN115967
2	*E. urophylla* × *E. tereticornis* hybrid genotype G1 seedling leaf	CSF12638	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°15'31.74"N, 110°06'35.17"E	S. F. Chen, Q. L. Liu and Q. C. Wang	MN096289	MN115858	MN115913	MN115968
2	*E. urophylla* × *E. tereticornis* hybrid genotype G1 seedling leaf	CSF12641	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°15'31.74"N, 110°06'35.17"E	S. F. Chen, Q. L. Liu and Q. C. Wang	MN096290	MN115859	MN115914	MN115969
2	*E. urophylla* × *E. tereticornis* hybrid genotype G1 seedling leaf	CSF13036[defgh]	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°15'31.74"N, 110°06'35.17"E	G. Q. Li, Q. L. Liu and W. Wang	MN096291	MN115860	MN115915	MN115970
2	*E. viminalis* seedling stem	CSF12887	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°15'31.74"N, 110°06'35.17"E	Q. L. Liu, G. Q. Li and S. F. Chen	MN096292	MN115861	MN115916	MN115971
2	*Eucalyptus* unknown species seedling stem	CSF12664	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°15'31.74"N, 110°06'35.17"E	S. F. Chen, Q. L. Liu and Q. C. Wang	MN096293	MN115862	MN115917	MN115972
3	2-year-old *E. urophylla* × *E. tereticornis* hybrid tree leaf	CSF13628	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°15'51.80"N, 110°07'27.93"E	S. F. Chen	MN096294	MN115863	MN115918	MN115973
3	2-year-old *E. urophylla* × *E. tereticornis* hybrid tree branch	CSF13636[defgh]	AAAA	LingBei, SuiXi, ZhanJiang, GuangDong	21°15'51.80"N, 110°07'27.93"E	S. F. Chen	MN096295	MN115864	MN115919	MN115974
4	2-year-old *E. pellita* tree leaf	CSF13277[d]	AAAA	ChengYue, SuiXi, ZhanJiang, GuangDong	21°08'0.75"N, 110°04'37.02"E	S. F. Chen, G. Q. Li and Q. L. Liu	MN096296	MN115865	MN115920	MN115975
4	3-year-old *E. urophylla* × *E. tereticornis* hybrid tree leaf	CSF13253	AAAA	ChengYue, SuiXi, ZhanJiang, GuangDong	21°08'0.75"N, 110°04'37.02"E	S. F. Chen, G. Q. Li and Q. L. Liu	MN096297	MN115866	MN115921	MN115976
4	3-year-old *E. urophylla* × *E. tereticornis* hybrid tree branch	CSF13256	AAAA	ChengYue, SuiXi, ZhanJiang, GuangDong	21°08'0.75"N, 110°04'37.02"E	S. F. Chen, G. Q. Li and Q. L. Liu	MN096298	MN115867	MN115922	MN115977
5	2-year-old *E. urophylla* × *E. grandis* hybrid tree leaf	CSF13285[d]	AAAA	KeLu, LeiZhou, ZhanJiang, GuangDong	21°06'53.74"N, 110°0'55.43"E	S. F. Chen, Q. C. Wang and W. X. Wu	MN096299	MN115868	MN115923	MN115978
5	2-year-old *E. urophylla* × *E. grandis* hybrid tree branch	CSF13301	AAAA	KeLu, LeiZhou, ZhanJiang, GuangDong	21°06'53.74"N, 110°0'55.43"E	S. F. Chen, Q. C. Wang and W. X. Wu	MN096300	MN115869	MN115924	MN115979
5	2-year-old *E. urophylla* × *E. tereticornis* hybrid tree leaf	CSF13310	AAAA	KeLu, LeiZhou, ZhanJiang, GuangDong	21°06'53.74"N, 110°0'55.43"E	S. F. Chen, Q. C. Wang and W. X. Wu	MN096301	MN115870	MN115925	MN115980

6	2-year-old *E. urophylla* × *E. tereticornis* hybrid tree leaf	CSF13040[defgh]	AAAA	TaiPing , MaZhang, ZhanJiang, GuangDong	21º03'12.40"N, 110º09'19.15"E	S. F. Chen, Q. C. Wang and W. X. Wu	MN096302	MN115871	MN115926	MN115981
6	2-year-old *E. urophylla* × *E. tereticornis* hybrid tree branch	CSF13045	AAAA	TaiPing , MaZhang, ZhanJiang, GuangDong	21º03'12.40"N, 110º09'19.15"E	S. F. Chen, Q. C. Wang and W. X. Wu	MN096303	MN115872	MN115927	MN115982
7	2-year-old *E. urophylla* × *E. tereticornis* hybrid tree leaf	CSF13317[defgh]	AAAA	YangJia, LeiZhou, ZhanJiang, GuangDong	20º53'14.74"N, 109º56'39.58"E	S. F. Chen, Q. C. Wang and W. X. Wu	MN096304	MN115873	MN115928	MN115983
7	2-year-old *E. urophylla* × *E. tereticornis* hybrid tree leaf	CSF13324	AAAA	YangJia, LeiZhou, ZhanJiang, GuangDong	20º53'14.74"N, 109º56'39.58"E	S. F. Chen, Q. C. Wang and W. X. Wu	MN096305	MN115874	MN115929	MN115984
7	2-year-old *E. urophylla* × *E. tereticornis* hybrid tree branch	CSF13327	AAAA	YangJia, LeiZhou, ZhanJiang, GuangDong	20º53'14.74"N, 109º56'39.58"E	S. F. Chen, Q. C. Wang and W. X. Wu	MN096306	MN115875	MN115930	MN115985
7	2-year-old *E. urophylla* × *E. tereticornis* hybrid tree branch	CSF13333	AAAA	YangJia, LeiZhou, ZhanJiang, GuangDong	20º53'14.74"N, 109º56'39.58"E	S. F. Chen, Q. C. Wang and W. X. Wu	MN096307	MN115876	MN115931	MN115986
8	1-year-old *E. urophylla* × *E. tereticornis* hybrid tree leaf	CSF13221[d]	AAAA	LeiGao, LeiZhou, ZhanJiang, GuangDong	20º48'32.52"N, 110º13'23.84"E	S. F. Chen, Q. C. Wang and W. X. Wu	MN096308	MN115877	MN115932	MN115987
8	1-year-old *E. urophylla* × *E. tereticornis* hybrid tree branch	CSF13223	AAAA	LeiGao, LeiZhou, ZhanJiang, GuangDong	20º48'32.52"N, 110º13'23.84"E	S. F. Chen, Q. C. Wang and W. X. Wu	MN096309	MN115878	MN115933	MN115988
9	2-year-old *E. urophylla* × *E. tereticornis* hybrid tree leaf	CSF13184[d]	AAAA	NanXing, LeiZhou, ZhanJiang, GuangDong	20º45'3.56"N, 110º04'3.39"E	S. F. Chen, Q. C. Wang and W. X. Wu	MN096310	MN115879	MN115934	MN115989
9	2-year-old *E. urophylla* × *E. tereticornis* hybrid tree branch	CSF13186	AAAA	NanXing, LeiZhou, ZhanJiang, GuangDong	20º45'3.56"N, 110º04'3.39"E	S. F. Chen, Q. C. Wang and W. X. Wu	MN096311	MN115880	MN115935	MN115990
10	1-year-old *E. urophylla* × *E. tereticornis* hybrid tree leaf	CSF13373[d]	AAAA	LongMen, LeiZhou, ZhanJiang, GuangDong	20º37'04.52"N, 110º01'38.65"E	S. F. Chen, Q. C. Wang and W. X. Wu	MN096312	MN115881	MN115936	MN115991
10	1-year-old *E. urophylla* × *E. tereticornis* hybrid tree branch	CSF13374	AAAA	LongMen, LeiZhou, ZhanJiang, GuangDong	20º37'04.52"N, 110º01'38.65"E	S. F. Chen, Q. C. Wang and W. X. Wu	MN096313	MN115882	MN115937	MN115992
11	2-year-old *E. urophylla* × *E. tereticornis* hybrid tree leaf	CSF13337[d]	AAAB	WuShi, LeiZhou, ZhanJiang, GuangDong	20º35'34.27"N, 109º53'31.38"E	S. F. Chen, Q. C. Wang and W. X. Wu	MN096314	MN115883	MN115938	MN115993
11	2-year-old *E. urophylla* × *E. tereticornis* hybrid tree branch	CSF13340	AAAA	WuShi, LeiZhou, ZhanJiang, GuangDong	20º35'34.27"N, 109º53'31.38"E	S. F. Chen, Q. C. Wang and W. X. Wu	MN096315	MN115884	MN115939	MN115994
11	2-year-old *E. urophylla* × *E. grandis* hybrid tree branch	CSF13361[d]	AAAA	WuShi, LeiZhou, ZhanJiang, GuangDong	20º35'34.27"N, 109º53'31.38"E	S. F. Chen, Q. C. Wang and W. X. Wu	MN096316	MN115885	MN115940	MN115995
11	2-year-old *E. urophylla* × *E. grandis* hybrid tree leaf	CSF13362	AAAA	WuShi, LeiZhou, ZhanJiang, GuangDong	20º35'34.27"N, 109º53'31.38"E	S. F. Chen, Q. C. Wang and W. X. Wu	MN096317	MN115886	MN115941	MN115996
12	2-year-old *E. urophylla* × *E. tereticornis* hybrid tree leaf	CSF13334[d]	AAAA	WuShi, LeiZhou, ZhanJiang, GuangDong	20º34'41.50"N, 109º51'59.44"E	S. F. Chen, Q. C. Wang and W. X. Wu	MN096318	MN115887	MN115942	MN115997
13	1-year-old *E. urophylla* × *E. tereticornis* hybrid tree leaf	CSF13406[d]	AAAA	ChengBei, XuWen, ZhanJiang, GuangDong	20º20'06.95"N, 110º03'04.38"E	S. F. Chen, Q. C. Wang and W. X. Wu	MN096319	MN115888	MN115943	MN115998

14	2-year-old *E. urophylla* × *E. grandis* hybrid tree branch	CSF13388[d]	AAAA	LongTang, XuWen, ZhanJiang, GuangDong	20º19'45.75"N, 110º15'34.54"E	S. F. Chen, Q. C. Wang and W. X. Wu	MN096320	MN115889	MN115944	MN115999
14	2-year-old *E. urophylla* × *E. grandis* hybrid tree leaf	CSF13389	AAAA	LongTang, XuWen, ZhanJiang, GuangDong	20º19'45.75"N, 110º15'34.54"E	S. F. Chen, Q. C. Wang and W. X. Wu	MN096321	MN115890	MN115945	MN116000
14	2-year-old *E. urophylla* × *E. tereticornis* hybrid tree leaf	CSF13402	AAAA	LongTang, XuWen, ZhanJiang, GuangDong	20º19'45.75"N, 110º15'34.54"E	S. F. Chen, Q. C. Wang and W. X. Wu	MN096322	MN115891	MN115946	MN116001

[a] CSF: Culture Collection located at China Eucalypt Research Centre (CERC), Chinese Academy of Forestry, ZhanJiang, GuangDong Province, China.

[b] Genotype within *Ca. pentaseptata*, determined by sequences of the *cmdA*, *his3*, *tef1* and *tub2* regions.

[c] *cmdA* = calmodulin, *his3* = histone H3, *tef1* = translation elongation factor 1-alpha, and *tub2* = β-tubulin.

[d] Isolates used for phylogenetic analyses.

[e] Isolates used for morphological study.

[f] Isolates used for culture growth.

[g] Isolates used to test sexual compatibility.

[h] Isolates used in pathogenicity tests.

Table 3. All the described *Calonectria* species with molecular data in the *Calonectria reteaudii* species complex used in the phylogenetic analyses in the current study.

Species	Isolate No.[a]	Substrate	Sampling site	Collector	GenBank accession No.[b]				Reference
					cmdA	*his3*	*tef1*	*tub2*	
Calonectria acacicola	**CMW 47173**[c]	Soil in *Acacia uriculiformis* plantation	Do Luong, Nghe An, Vietnam	N. Q. Pham and T. Q. Pham	MH119252	MH119186	MH119219	MH119285	Pham et al. 2019
	CMW 47174	Soil in *A. uriculiformis* plantation	Do Luong, Nghe An, Vietnam	N. Q. Pham and T. Q. Pham	MH119253	MH119187	MH119220	MH119286	Pham et al. 2019
Ca. acicola	**CBS 114813**	*Pinus radiata*	New Zealand	H. Pearson	GQ267360	DQ190693	GQ267292	DQ190591	Gadgil and Dick, 2004
	CBS 114812	*P. radiata*	New Zealand	H. Pearson	GQ267359	DQ190692	GQ267291	DQ190590	Gadgil and Dick, 2004
Ca. australiensis	**CBS 112954**	*Ficus pleurocarpa*	Australia	C. Pearce and B. Paulu	GQ267363	DQ190699	GQ267293	DQ190596	Crous et al. 2006
Ca. baviensis	**CMW 47410**	*Eucalyptus urophylla* leaf	Bavi, Hanoi, Vietnam	N. Q. Pham and T. Q. Pham	MH119256	MH119190	MH119223	MH119289	Pham et al. 2019
	CMW 47433	*Eucalyptus pellita* leaf	Bavi, Hanoi, Vietnam	N. Q. Pham and T. Q. Pham	MH119257	MH119191	MH119224	MH119290	Pham et al. 2019
Ca. crousiana	**CBS 127198**	*Eucalyptus grandis*	FuJian, China	M. J. Wingfield	MF527084	HQ285808	HQ285822	HQ285794	Chen et al. 2011c, Liu and Chen, 2017
	CBS 127199	*E. grandis*	FuJian, China	M. J. Wingfield	MF527085	HQ285809	HQ285823	HQ285795	Chen et al. 2011c, Liu and Chen, 2017
Ca. microconidialis	**CBS 136638**	*E. urophylla* × *E. grandis* clone seedling leaf	GuangDong, China	G. Zhao	KJ463075	KJ463191	KJ462845	KJ462960	Lombard et al. 2015a
	CBS 136633	*E. urophylla* × *E. grandi s*clone seedling leaf	GuangDong, China	G. Zhao	KJ463072	KJ463188	KJ462842	KJ462957	Lombard et al. 2015a
Ca. multiseptata	**CBS 112682**	*Eucalyptus* sp.	Indonesia	M. J. Wingfield	GQ267397	DQ190659	FJ918535	DQ190573	Lombard et al. 2010c
Ca. pentaseptata	**CBS 133349**	*Eucalyptus* hybrid	Vietmam	P. Q. Thu	N/A[d]	JX855946	JX855958	JX855942	Cous et al. 2012
	CBS 133351	*Macadamia* sp.	Vietmam	P. Q. Thu	N/A	JX855948	JX855960	JX855944	Cous et al. 2012
Ca. pseudoreteaudii	**CBS 123694**	*E. urophylla* × *E. grandis* cutting	GuangDong, China	M. J. Wingfield	GQ267411	FJ918519	FJ918541	FJ918504	Lombard et al. 2010c
	CBS 123696	*E. urophylla* × *E. grandis* cutting	GuangDong, China	M. J. Wingfield	GQ267410	FJ918520	FJ918542	FJ918505	Lombard et al. 2010c
Ca. queenslandica	**CBS 112146**	*E. urophylla*	Australia	B. Brown	GQ267415	FJ918521	FJ918543	N/A	Lombard et al. 2010c

Ca. reteaudii	**CBS 112144**	*E. camaldulensis*	Vietnam	M. J. Dudzinski	GQ267417	DQ190661	FJ918537	AF389833	Lombard et al. 2010c
	CBS 112143	*E. camaldulensis*	Vietnam	M. J. Dudzinski	GQ267418	DQ190660	FJ918536	GQ240642	Lombard et al. 2010c
Ca. terrae-reginae	**CBS 112151**	*E. urophylla*	Australia	C. Hanwood	GQ267451	FJ918522	FJ918545	FJ918506	Lombard et al. 2010c
Curvicladiella cignea	**CBS 109167**	Leaf litter	French Guiana	C. Decock	KM231287	KM231461	KM231867	KM232002	Lombard et al. 2015b
	CBS 109168	Decaying seed	French Guiana	C. Decock	KM231286	KM231460	KM231868	KM232003	Lombard et al. 2015b

[a] CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CMW: culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

[b] *cmdA* = calmodulin, *his3* = histone H3, *tef1* = translation elongation factor 1-alpha, and *tub2* = β-tubulin.

[c] Isolates represent ex-type and are indicated in bold.

[d] "N/A" represents sequences that are not available.

Table 4. Statistical results from the phylogenetic analyses conducted in this study.

Dataset	Phylogenetic group	No. of taxa	No. of bp[a]	Maximum Parsimony						
				PIC[b]	No. of trees	Tree length	CI[c]	RI[d]	RC[e]	HI[f]
cmdA	Prolate	36	473	332	12	189	0.8677	0.9129	0.7921	0.1323
his3	Prolate	38	464	314	6	255	0.8275	0.9018	0.7462	0.1725
tef1	Prolate	38	473	293	5	297	0.8215	0.8868	0.7285	0.1785
tub2	Prolate	37	519	374	6	205	0.8878	0.9238	0.8202	0.1122
cmdA/*his3*/*tef1*/*tub2*	Prolate	38	1929	1313	1	981	0.8165	0.8815	0.7198	0.1835
Dataset	Phylogenetic group	Maximum Likelihood								
		Subst. model[g]	NST[h]	Rate matrix					Rates	
cmdA	Prolate	TPM1uf+G	6	1.0000	3.8960	0.3603	0.3603	3.8960	Gamma	
his3	Prolate	TPM2uf+I	6	1.9238	7.1094	1.9238	1.0000	7.1094	Gamma	
tef1	Prolate	TIM3+G	6	0.5918	1.0501	1.0000	0.5918	2.4342	Gamma	
tub2	Prolate	TPM1uf+G	6	1.0000	5.2988	1.6531	1.6531	5.2988	Gamma	
cmdA/*his3*/*tef1*/*tub2*	Prolate	TIM2+G	6	1.6095	4.0105	1.6095	1.0000	5.5704	Gamma	

[a] bp = base pairs.

[b] PIC = number of parsimony informative characters.

[c] CI = consistency index.

[d] RI = retention index.

[e] RC = rescaled consistency index.

[f] HI = homoplasy index.

[g] Subst. model = best fit substitution model.

[h] NST = number of substitution rate categories.

Table 5. Morphological comparisons of *Calonectria pentaseptata* isolates obtained in the current study.

Isolate/species	Macroconidia (L × W)[a,b,c]	Macroconidia average (L × W)[a,b]	Macroconidia septation	Vesicle width[a,c]	Vesicle width average[a]	Reference
CSF13036	(77–)88.5–104.5(–113.5)×(6–)6.5–7.5(–8.5)	96.5 × 7	5	(2–)2.5–3.5(–4.5)	3	This study
CSF13040	(80–)90–102(–110)×(6–)6.5–7.5(–8)	96.5 × 7	5	(2–)2.5–4(–6)	3.5	This study
CSF13317	(83–)94–107(–116)×(6–)6.5–7.5(–8.5)	100.5 × 7	5	(2–)2.5–3(–3.5)	3	This study
CSF13452	(75.5–)84.5–97(–110.5)×(6–)6.5–7.5(–8.5)	90 × 7	5	(2.5–)2.5–3.5(–4.5)	3	This study
CSF13636	(69–)77.5–90.5(–101)×(5.5–)6.5–7.5(–9)	84 × 7	5	(2–)3–4–(–5)	3.5	This study
Ca. pentaseptata	(75–)87–109(–115)×(5–)6–8(–10)	98 × 7	5(–8)	2–6	N/A[d]	Crous et al. 2012

[a] All measurements are in µm.

[b] L × W = length × width.

[c] Measurements are presented in the format [(minimum–) (average – standard deviation) – (average + standard deviation) (–maximum)].

[d] N/A represents data that is not available.

Fig. 1. Disease symptoms on plantation Eucalyptus hybrids and species caused by species of Calonectria. A and B, Intense defoliation in Eucalyptus urophylla × E. grandis hybrid plantations. C, The E. urophylla × E. grandis hybrid trees died after infection. D, The early stage of infection of one E. urophylla × E. grandis hybrid genotype. E, The infected leaves became blighted and dried. F, Eucalyptus trees in the whole plantation were infected and defoliated. G, H and I, White mass of conidiophores of Calonectria species on the main stem (G), branch (H) and shoot (I) of Eucalyptus trees. J and K, Two E. urophylla × E. grandis hybrid genotypes showing leaf spot caused by species of Calonectria. L, Eucalyptus pellita with leaf spot caused by Calonectria species. M and N, Two E. urophylla × E. tereticornis hybrid genotypes showing leaf spot caused by species of Calonectria.

177x258mm (300 x 300 DPI)

Fig. 2. Disease symptoms on nursery Eucalyptus hybrids and seedlings caused by species of Calonectria. A, Eucalyptus urophylla × E. grandis hybrid seedlings infected by Calonectria species. B, Dark spots on seedling stems showing the early stage of infection. C and D, White mass of conidiophores of Calonectria species on stems of E. urophylla × E. grandis hybrid seedlings. E, Calonectria species causing widespread death of seedlings. F, The early stage of infection on young leaves of one E. urophylla × E. grandis hybrid genotype. G, H and I, Typical small and rounded lesions caused by Calonectria species on seedlings of one E. urophylla hybrid genotype (G), E. pellita (H) and E. urophylla (I). J, The E. smithii seedlings died after infection by Calonectria species.

177x225mm (300 x 300 DPI)

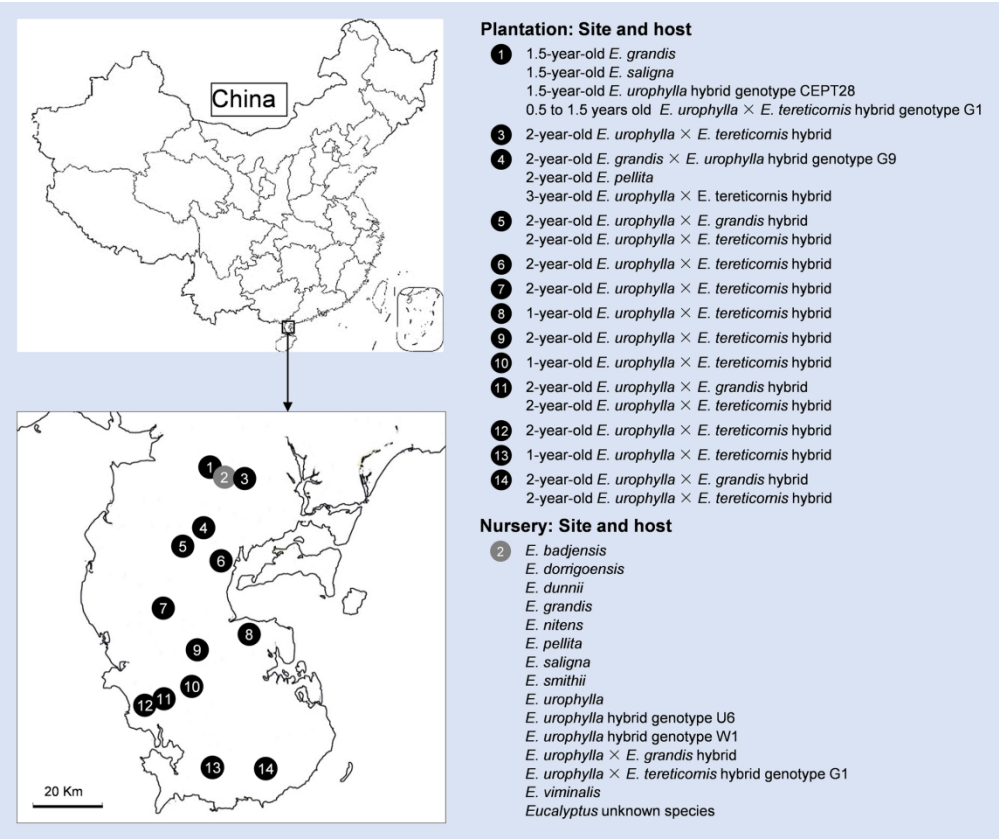

Fig. 3. Map of Leizhou Peninsula in southern China showing where Eucalyptus trees in plantations and seedlings in a nursery were sampled, and the diversity of Eucalyptus species and genotypes. The 14 sampled sites are indicated as number 1 to 14, followed by the species or genotypes of Eucalyptus.

177x149mm (300 x 300 DPI)

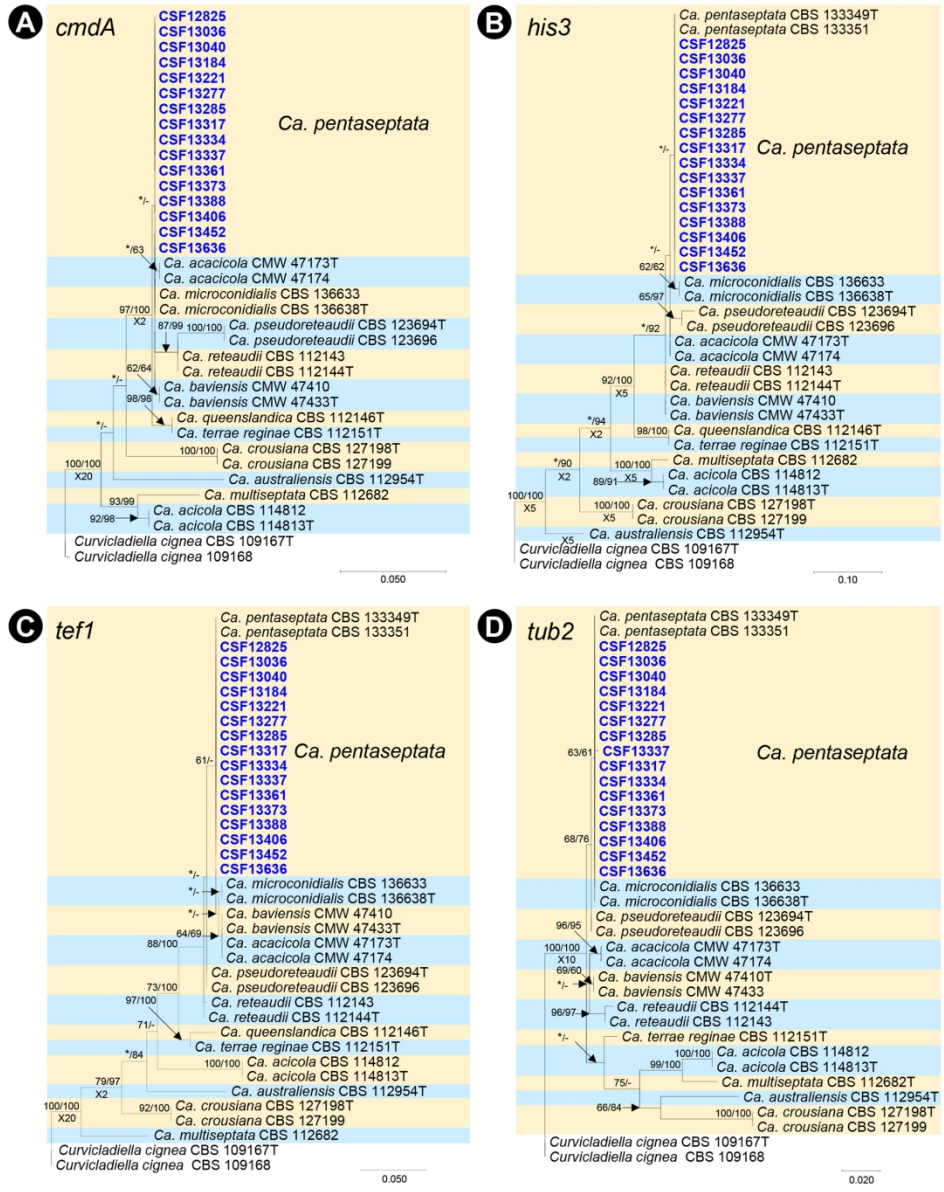

Fig. 4. Phylogenetic trees based on Maximum Likelihood (ML) analyses for species in the Calonectria reteaudii species complex. A, Calmodulin (cmdA) region. B, Histone H3 (his3) region. C, Translation elongation factor 1-alpha (tef1) region. D, β-tubulin (tub2) region. Bootstrap support values ≥60 % for ML and MP are indicated above the branches as follows: ML/MP. Bootstrap support values < 60% are marked with *, and absence is marked with -. Isolates highlighted in bold and blue were isolated in this study; isolates representing ex-type material are marked with "T". Curvicladiella cignea (CBS 109167 and CBS 109168) was used as the outgroup taxon.

177x224mm (300 x 300 DPI)

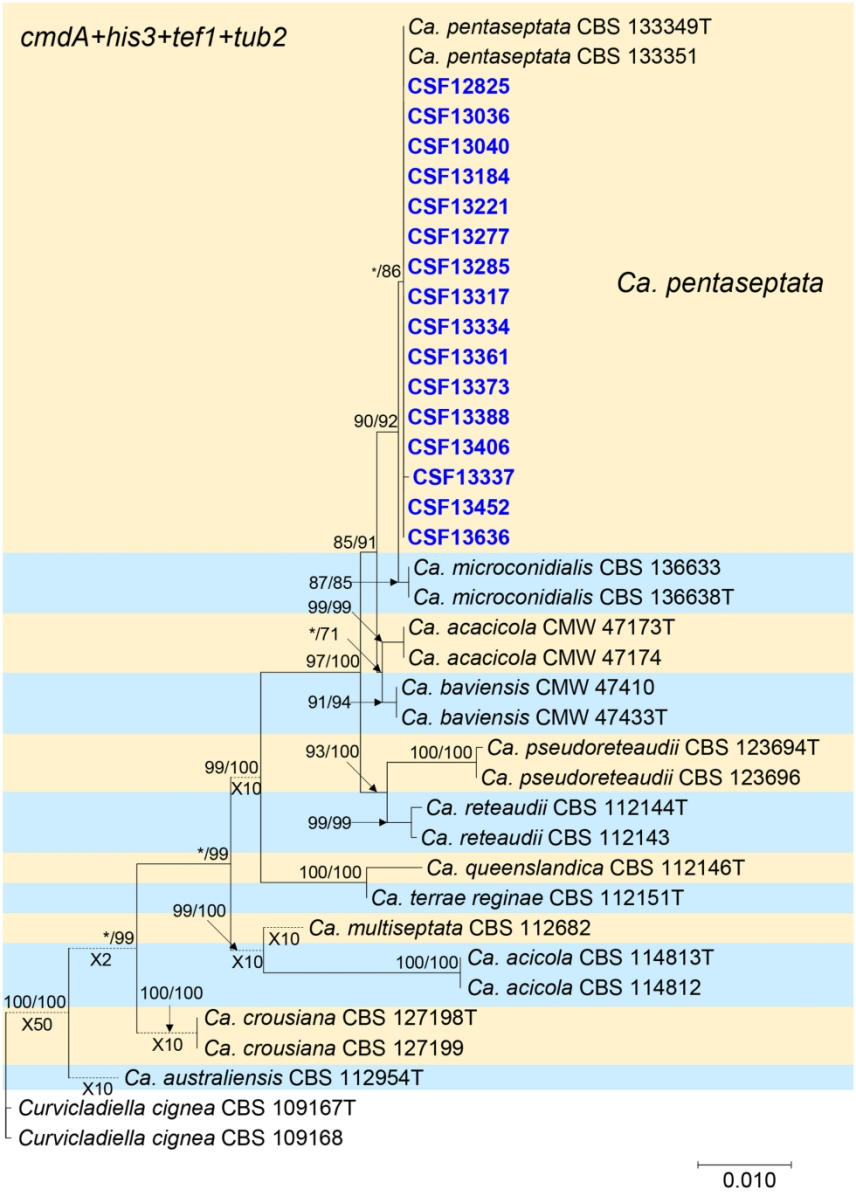

Fig. 5. Phylogenetic tree of Calonectria species based on Maximum Likelihood (ML) analyses of the dataset of the combined cmdA, his3, tef1 and tub2 gene sequences. Bootstrap support values ≥60 % for ML and MP are indicated above the branches as follows: ML/MP. Bootstrap support values < 60% are marked with *, and absence is marked with -. Isolates highlighted in bold and in blue were isolated in this study; isolates representing ex-type material are marked with "T". Curvicladiella cignea (CBS 109167 and CBS 109168) was used as the outgroup taxon.

177x245mm (300 x 300 DPI)

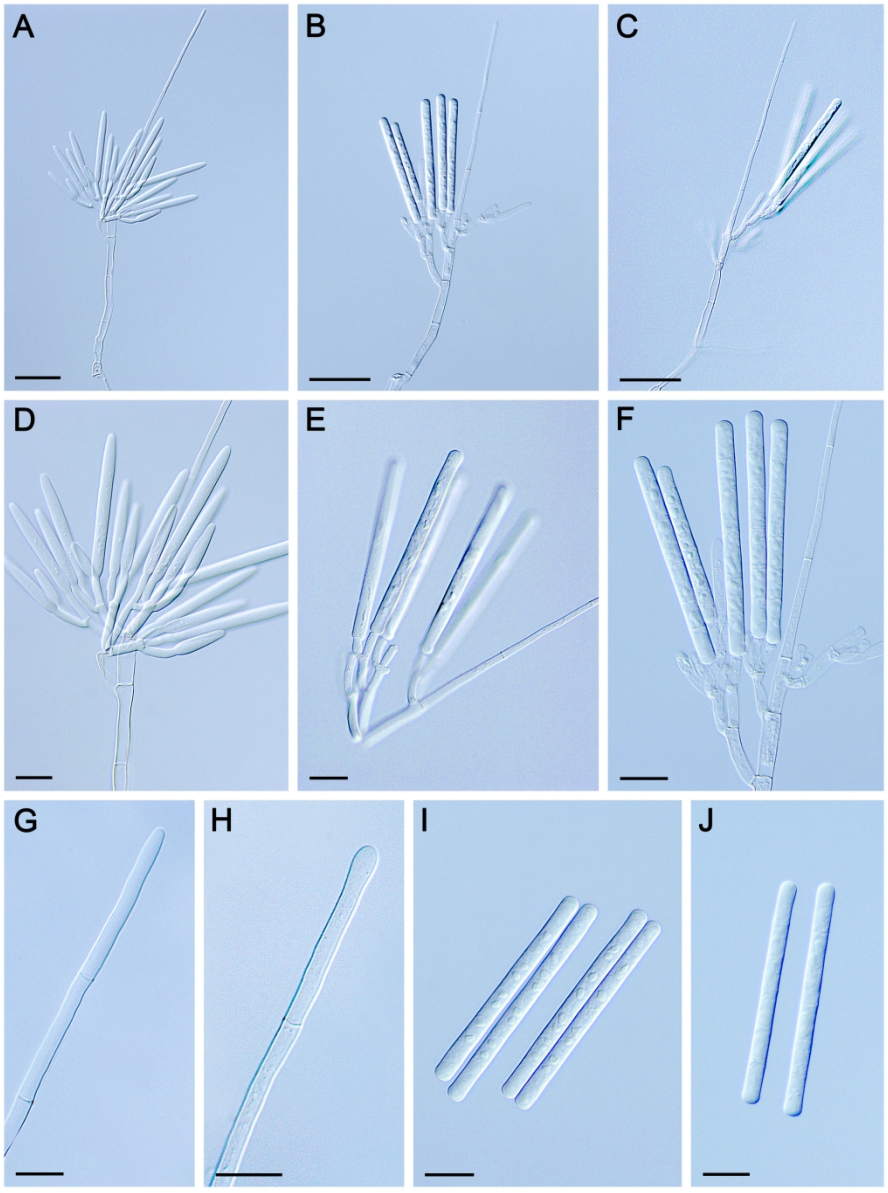

Fig. 6. Morphological features of asexual structures of Calonectria pentaseptata obtained in this study. A, B and C, Macroconidiophores with stipes bearing conidiogenous apparatus, conidia and terminating in a vesicle. D, E and F, Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. G and H, Clavate vesicles. I and J, Cylindrical, straight, 5-septate macroconidia. Scale bars: A to C = 50 µm, D to F, and I to J = 20 µm, G to H = 10 µm.

177x237mm (300 x 300 DPI)

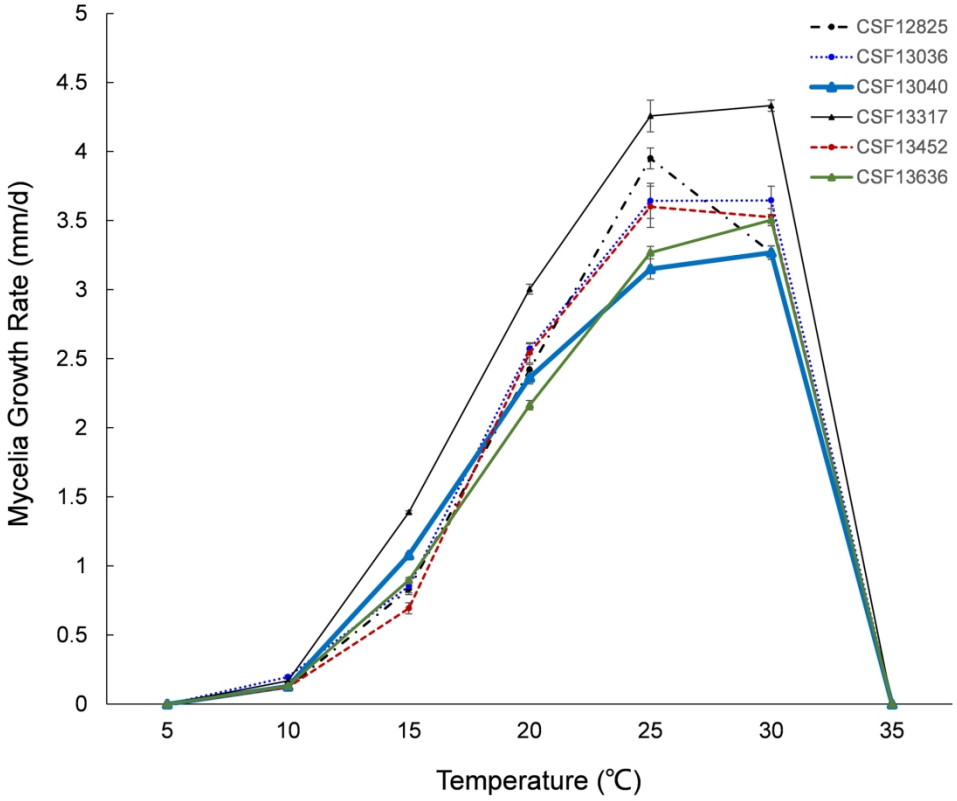

Fig. 7. Effect of temperature on mycelial radial growth of isolates of Calonectria pentaseptata obtained in the current study. Vertical bars represent the standard error of the means. Each value represents the average of 10 replicates.

177x145mm (300 x 300 DPI)

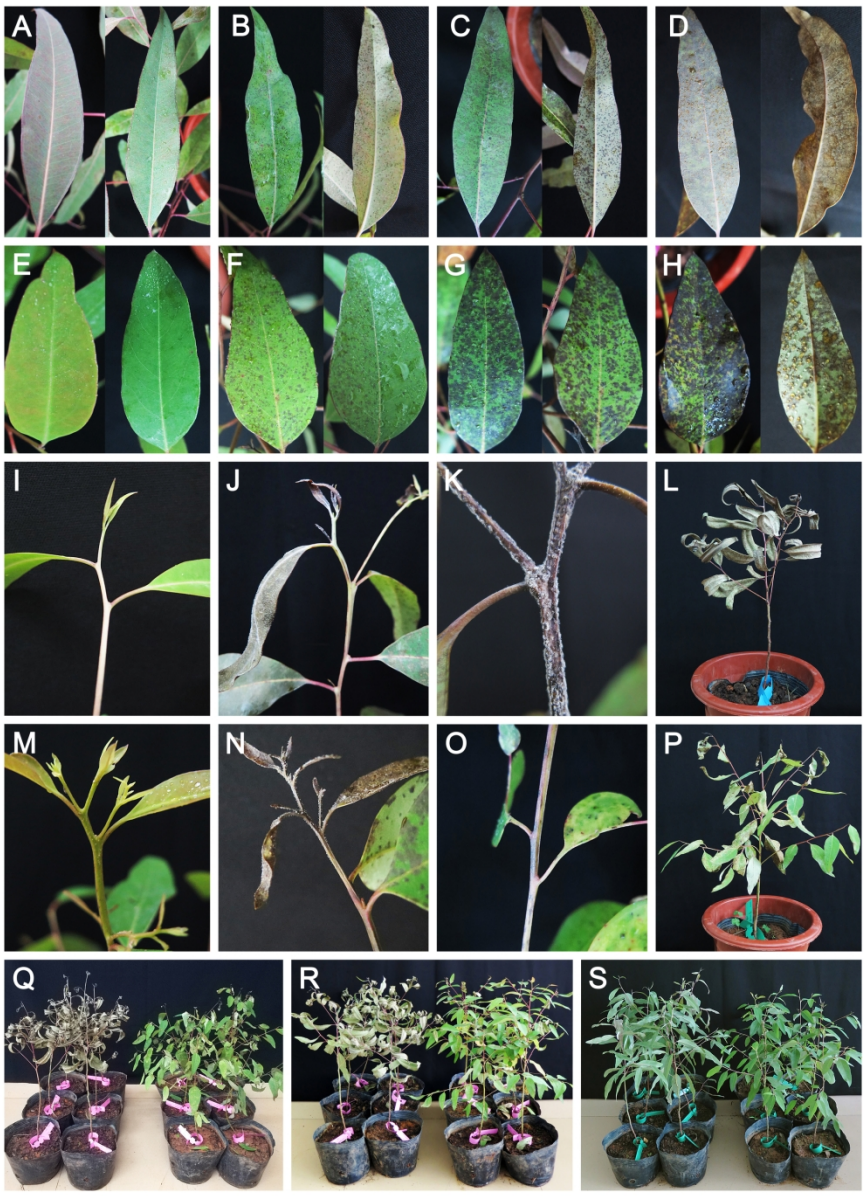

Fig. 8. Symptoms on seedlings of Eucalyptus urophylla × E. tereticornis hybrid genotype CEPT1845 and E. urophylla × E. grandis hybrid genotype CEPT1846 inoculated by spray inoculation of conidial suspensions of Calonectria pentaseptata isolates. A, The non-infected leaves of E. urophylla × E. tereticornis hybrid genotype CEPT1845 seedlings. B, C and D, Leaves of E. urophylla × E. tereticornis hybrid seedlings sprayed by conidial suspension, after 24 hours (B), 48 hours (C) and 72 hours (D). E, The non-infected leaves of E. urophylla × E. grandis hybrid genotype CEPT1846 seedling. F, G and H, Leaves of E. urophylla × E. grandis hybrid seedlings sprayed by conidial suspension, after 24 hours (F), 48 hours (G) and 72 hours (H). I, The non-infected seedling of Eucalyptus genotype CEPT1845. J and K, White mass of conidiophores of Ca. pentaseptata on infected young shoots and leaves (J), and stem (K) of Eucalyptus genotype CEPT1845 seedlings. L, All leaves of Eucalyptus genotype CEPT1845 blighted and the seedling died after infection by Ca. pentaseptata. M, The non-infected seedling of Eucalyptus genotype CEPT1846. N, White mass of conidiophores of Ca. pentaseptata on infected young shoots and leaves of seedlings of Eucalyptus genotype CEPT1846. O, Dark spots on seedling stem showing the early stage of infection. P, Partial leaves of Eucalyptus genotype CEPT1846 seedling exhibited blighted after infection by Ca. pentaseptata. Q and R,

Different levels of leaf blight on two Eucalyptus genotypes CEPT1845 (left) and CEPT1846 (right) infected by isolates CSF13452 in Experiment One (Q) and Two (R). S, No disease symptoms on two Eucalyptus genotypes CEPT1845 (left) and CEPT1846 (right) without infection by Ca. pentaseptata.

177x244mm (300 x 300 DPI)

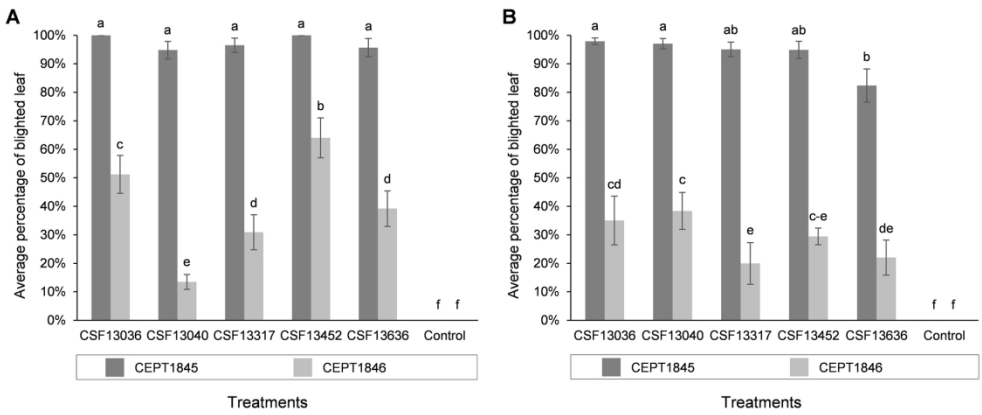

Fig. 9. Column chart indicating the average percentage of blighted leaves resulting from inoculation trials of two Eucalyptus hybrid genotypes inoculated with five isolates of Calonectria pentaseptata (CSF13036, CSF13040, CSF13317, CSF13452 and CSF13636) and the controls. Vertical bars represent the standard error of the means. Bars with different letters indicate treatment means that are significantly different (P = 0.05). Two experiments were conducted, A, Results of Experiment One. B, Results of Experiment Two.

177x76mm (300 x 300 DPI)