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RESEARCH ARTICLE

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Novel species of *Calonectria* associated with *Eucalyptus* leaf blight in Southeast China

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Key words

Cylindrocladium
Eucalyptus plantations
FuJian
pathogenicity

Abstract Leaf blight caused by *Calonectria* spp. is an important disease occurring on *Eucalyptus* trees grown in plantations of Southeast Asia. Symptoms of leaf blight caused by *Calonectria* spp. have recently been observed in commercial *Eucalyptus* plantations in FuJian Province in Southeast China. The aim of this study was to identify these *Calonectria* spp. employing morphological characteristics, DNA sequence comparisons for the β-tubulin, histone H3 and translation elongation factor-1α gene regions and sexual compatibility. Four *Calonectria* spp. were identified, including *Ca. pauciramosa* and three novel taxa described here as *Ca. crousiana*, *Ca. fujianensis* and *Ca. pseudocolhounii*. Inoculation tests showed that all four *Calonectria* spp. found in this study were pathogenic on two different *E. urophylla* × *E. grandis* hybrid clones, commercially utilised in eucalypt plantations in China.

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INTRODUCTION

Species of *Calonectria* (*Ca.*) (anamorph state: *Cylindrocladium* (*Cy.*)) are pathogenic to a wide range of plant hosts in tropical and subtropical areas of the world (Crous & Wingfield 1994, Crous 2002). Symptoms associated with infection by these fungi include stem cankers, leaf and shoot blight as well as root rot on many agronomic and forestry crop plants (Crous 2002, Old et al. 2003, Crous et al. 2004b, Lechat et al. 2010). *Calonectria* spp., particularly in their *Cylindrocladium* anamorph form, are especially well-known as pathogens of *Eucalyptus* trees in plantations where they cause the disease known as Cylindrocladium leaf blight (CLB) (Sharma & Mohanan 1991, 1992, Booth et al. 2000, Crous 2002, Old et al. 2003, Rodas et al. 2005). These fungi are also important causal agents of cutting rot and seedling blight in *Eucalyptus* nurseries (Sharma et al. 1984, Crous et al. 1991, Crous 2002, Old et al. 2003, Lombard et al. 2010c, d).

Symptoms of CLB on *Eucalyptus* include both leaf blotch and shoot blight, which develops upwards from the base of the trees and can result in tree mortality due to defoliation (Crous 2002, Old et al. 2003, Rodas et al. 2005). Symptoms begin as water-soaked lesions on young and mature leaves on the lower branches. These lesions coalesce and develop into extensive necrotic areas very rapidly. Under conditions of high humidity and frequent rainfall, the lesions can cover the entire leaf surface and infection of young shoot tips can result in dramatic blight. Defoliation typically moves upwards from the base and centres of affected trees and this can result in total defoliation of trees (Crous 2002, Old et al. 2003, Rodas et al. 2005). Severely affected trees can suffer reduction in growth vigour, with crowns and main stems becoming deformed (Booth et al. 2000, Old et al. 2003).

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In South and Southeast Asia, CLB is one of the most prominent diseases associated with *Eucalyptus* trees grown in commercial plantations (Old et al. 2003). In these regions, CLB is caused by several *Calonectria* spp., including *Ca. asiatica*, *Ca. brassicae*, *Ca. hurae*, *Ca. ilicicola*, *Ca. indusiata*, *Ca. kyotensis*, *Ca. multiseptata*, *Ca. pauciramosa*, *Ca. pteridis*, *Ca. reteaudii* and *Ca. sumatrensis* (Sharma et al. 1984, Booth et al. 2000, Kang et al. 2001, Crous 2002, Old et al. 2003, Crous et al. 2004b). Of these *Calonectria* spp., *Ca. reteaudii* is regarded as the most important pathogen and it occurs primarily on *Eucalyptus* trees in tropical regions of Southeast Asia and India (Booth et al. 2000, Kang et al. 2001, Crous 2002, Old et al. 2003).

Commercial plantations of *Eucalyptus* are distributed over 19 provinces in Central and South China (Qi 2006). Approximately 2.6 M ha of *Eucalyptus* plantations have recently been established in GuangXi, GuangDong, HaiNan, FuJian and YunNan Provinces (Xie 2006, Iglesias-Trabad & Wilstermann 2008), to meet the high demand in pulp products in China. Similar to the situation in other countries (Wingfield et al. 2008), these trees are affected by pests and diseases, for which limited information is available in China (Zhou et al. 2008). Leaf and shoot blight caused by *Calonectria* spp. is regarded as one of the most serious threats to commercial *Eucalyptus* plantations and nurseries in this country (Wang 1992, Sun & Liu 2004, Zhou et al. 2008, Lombard et al. 2010d). Recent surveys of tree diseases in the FuJian Province in Southeast China revealed numerous examples of CLB on *Eucalyptus* spp. The aim of this study was to determine the identity of the *Calonectria* spp. collected from these trees. In addition, the pathogenicity of selected isolates was tested on various *Eucalyptus* clones commercially grown in China.

MATERIAL AND METHODS

Isolates

Eucalyptus leaves showing symptoms of CLB were collected from commercially propagated *Eucalyptus* trees in plantations in FuJian Province in 2007 (Table 1). Conidial masses were transferred directly from infected leaves to malt extract agar

Table 1 Isolates used in the phylogenetic analyses and pathogenicity trials.

Species	Isolate number[1]	β-tubulin[2]	Histone H3[2]	TEF-1α[2]	Host	Origin	Collector
Calonectria acicola	CBS 114812	DQ190590	DQ190692	GQ267291	*Phoenix canariensis*	New Zealand	H. Pearson
	CBS 114813^{T}	DQ190591	DQ190693	GQ267292	*P. canariensis*	New Zealand	H. Pearson
Ca. brachiatica	CMW 25302	FJ716708	FJ716712	GQ267295	*Pinus tecunumanii*	Colombia	M.J. Wingfield
	CBS 123700 (= CMW25298)^{T}	FJ696388	FJ696396	GQ267296	*P. maximinoi*	Colombia	M.J. Wingfield
Ca. brassicae	CBS 111869^{T}	AF232857	DQ190720	FJ918568	*Argyreia* sp.	Southeast Asia	
	CBS 111478	DQ190611	DQ190719	FJ918567	Soil	Brazil	A.C. Alfenas
Ca. cerciana	CBS 123639 (= CMW 25309)^{T}	FJ918510	FJ918528	FJ918559	*Eucalyptus urophylla* × *E. grandis* cutting	GuangDong, China	M.J. Wingfield & X.D. Zhou
	CBS 123695 (= CMW 25290)	FJ918511	FJ918529	FJ918560	*E. urophylla* × *E. grandis* cutting	GuangDong, China	M.J. Wingfield & X.D. Zhou
Ca. chinensis	CBS 112744	AY725618	AY725660	AY725709	Soil	China	E.C.Y. Liew
Ca. colhounii	CBS 293.79 (= CMW 30999)^{T}	DQ190564	DQ190639	GQ267301	–	Indonesia	—
	CBS 114704	DQ190563	DQ190638	GQ267300	*Arachis pintoi*	Australia	D. Hutton
Ca. colombiana	CBS 115638	FJ972422	FJ972441	FJ972491	Soil	Colombia	M.J. Wingfield
	CBS 115127	FJ972423	FJ972442	FJ972492	Soil	Colombia	M.J. Wingfield
Ca. colombiensis	CBS 112221	AY725620	AY725663	AY725712	Soil	Colombia	M.J. Wingfield
Ca. crousiana	**CMW 27249**^{AT} (= CBS 127198)	HQ285794	HQ285808	HQ285822	*E. grandis*	FuJian, China	M.J. Wingfield
	CMW 27253 (= CBS 127199)	HQ285795	HQ285809	HQ285823	*E. grandis*	FuJian, China	M.J. Wingfield
	CMW 27258	HQ285796	HQ285810	HQ285824	*E. grandis*	FuJian, China	M.J. Wingfield
	CMW 27267^{A} (= CBS 127203)	HQ285797	HQ285811	HQ285825	*E. grandis*	FuJian, China	M.J. Wingfield
Ca. eucadoriae	CBS 111394	DQ190599	DQ190704	GQ267304	Soil	Ecuador	M.J. Wingfield
	CBS 111406	DQ190600	DQ190705	GQ267303	Soil	Ecuador	M.J. Wingfield
Ca. eucalypti	CBS 125273 (= CMW 14890)	GQ267217	GQ267266	GQ267337	*E. grandis*	Indonesia	M.J. Wingfield
	CBS 125275 (= CMW 18444)^{T}	GQ267218	GQ267267	GQ267338	*E. grandis*	Indonesia	M.J. Wingfield
Ca. fujianensis	**CMW 27254**^{A} (= CBS 127200)	HQ285791	HQ285805	HQ285819	*E. grandis*	FuJian, China	M.J. Wingfield
	CMW 27257^{AT} (= CBS 127201)	HQ285792	HQ285806	HQ285820	*E. grandis*	FuJian, China	M.J. Wingfield
	CMW 27263^{A} (= CBS 127202)	HQ285793	HQ285807	HQ285821	*E. grandis*	FuJian, China	M.J. Wingfield
Ca. insulare	CBS 114558	AF210861	FJ918526	FJ918556	Soil	Madagascar	P.W. Crous
	CBS 114559	AF210862	FJ918525	FJ918555	Soil	Madagascar	C. L. Schoch
Ca. macroconidialis	CBS 114880^{T}	AF232855	DQ190655	GQ267313	*E. grandis*	South Africa	P.W. Crous
Ca. madagascariensis	CBS 114572 (= CMW23686)^{T}	DQ190572	DQ190658	GQ267314	–	Madagascar	P.W. Crous
	CBS 114571 (= CMW 30993)	DQ190571	DQ190657	GQ267315	–	Madagascar	P.W. Crous
Ca. morganii	CBS 110666	FJ918509	FJ918527	FJ918557	*Rosa* sp.	USA	N.E. Ell-Gholl
Ca. multiseptata	CBS 112682^{T}	DQ190573	DQ190659	FJ918535	*Eucalyptus* sp.	Indonesia	M.J. Wingfield
Ca. pauciramosa	CMW 30823	FJ918515	FJ918532	FJ918566	*E. grandis*	South Africa	P.W. Crous
	CMW 5683	FJ918514	FJ918531	FJ918565	*Eucalyptus* sp.	Brazil	A.C. Alfenas
	CMW 27199^{A}	HQ285784	HQ285798	HQ285812	*E. dunnii*	FuJian, China	M.J. Wingfield
	CMW 27203	HQ285785	HQ285799	HQ285813	*E. dunnii*	FuJian, China	M.J. Wingfield
	CMW 27283	HQ285786	HQ285800	HQ285814	*E. dunnii*	FuJian, China	M.J. Wingfield
	CMW 27292^{A}	HQ285787	HQ285801	HQ285815	*E. dunnii*	FuJian, China	M.J. Wingfield
Ca. polizzii	CMW 7804	FJ972417	FJ972436	FJ972486	*Callistemon citrinus*	Italy	G. Polizzi
	CMW 10151	FJ972418	FJ972437	FJ972487	*Arbustus unedo*	Italy	G. Polizzi

Ca. pseudocolhounii	**CMW 27209**[AT] (= CBS 127195)	HQ285788	HQ285802	HQ285816	*E. dunnii*	FuJian, China	M.J. Wingfield
	CMW 27213[A] (= CBS 127196)	HQ285789	HQ285803	HQ285817	*E. dunnii*	FuJian, China	M.J. Wingfield
	CMW 27214[A] (= CBS 127197)	HQ285790	HQ285804	HQ285818	*E. dunnii*	FuJian, China	M.J. Wingfield
Ca. pseudoreteaudii	CBS 123694 (= CMW 25310)[T]	FJ918504	FJ918519	FJ918541	*E. urophylla* × *E. grandis* cutting	GuangDong, China	M.J. Wingfield & X.D. Zhou
	CBS 123696 (= CMW 25296)	FJ918505	FJ918520	FJ918542	*E. urophylla* × *E. grandis* cutting	GuangDong, China	M.J. Wingfield & X.D. Zhou
Ca. pteridis	CBS 111793	DQ190578	DQ190679	FJ918563	*Arachnoides adiantiformis*	USA	–
	CBS 111871	DQ190579	DQ190680	FJ918564	*Pinus* sp.	Spain	T.L. Krugner
Ca. queenslandica	CBS 112146 (= CMW30604)[T]	AF389835	FJ918521	FJ918543	*E. urophylla*	Australia	B. Brown
	CBS 112155 (= CMW30603)	AF389834	DQ190667	FJ918544	*E. pellita*	Australia	K.M. Old
Ca. reteaudii	CBS 112144[T]	AF389833	DQ190661	FJ918537	*E. camaldulensis*	Vietnam	M.J. Dudzinski
	CBS 112143	GQ240642	DQ190660	FJ918536	*E. camaldulensis*	Vietnam	M.J. Dudzinski
Ca. spathulata	CBS 112689	AF308463	FJ918524	FJ918554	*E. viminalis*	Brazil	N.E. Ell-Gholl
	CBS 555.92	GQ267215	GQ267261	GQ267331	*Araucaria angustifolia*	Brazil	C. Hodges
Ca. terrae-reginae	CBS 112151 (= CMW 30601)[T]	FJ918506	FJ918522	FJ918545	*E. urophylla*	Australia	C. Hanwood
	CBS 112634 (= CMW 30602)	FJ918507	DQ190668	FJ918546	*Xanthorrhoea australis*	Australia	T. Baigent
Ca. zuluensis	CMW 9188[T]	FJ972414	FJ972433	FJ972483	*E. grandis* × *E. urophylla* cutting	South Africa	L. Lombard
	CMW 9896	FJ972415	FJ972434	FJ972484	*E. grandis* × *E. urophylla* cutting	South Africa	L. Lombard

[1] CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CMW: culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; isolate number in **bold** were sequenced in this study.
[2] GenBank accession numbers.
[A] Isolates used for pathogenicity tests on *Eucalyptus* seedlings in China.
[T] Ex-type cultures.

(2 % w/v; MEA: Biolab Diagnostic Ltd., Midrand, South Africa) and incubated at 25 °C under continuous near-ultraviolet light for 7 d. Isolates were transferred to MEA and further incubated at 25 °C for 7 d. Single conidial isolates were prepared and lodged in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa (Table 1), and a duplicate set of isolates is maintained in a culture collection housed at the China Eucalypt Research Centre (CERC), Chinese Academy of Forestry (CAF), China. Representative isolates were also deposited with the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands (Table 1), and herbarium specimens in the National Collection of Fungi (PREM), Pretoria, South Africa.

DNA sequence comparisons

Single conidial cultures (Table 1) were grown on MEA for 7 d at 25 °C. Total genomic DNA was extracted using the method described by Smith et al. (2001). Three loci were amplified, using the primers T1 (O'Donnell & Cigelnik 1997) and Bt-2b (Glass & Donaldson 1995) to amplify a fragment of the β-tubulin (BT) gene region, part of the histone H3 (HIS3) gene region with primers H3-1a and H3-1b (Glass & Donaldson 1995), and primers EF1-728F and EF1-986R (Carbone & Kohn 1999) to amplify a fragment of the translation elongation factor-1 alpha (TEF-1α) gene region.

The PCR mixtures used to amplify the different loci consisted of 2.5 units Fast Start *Taq* polymerase (Roche Applied Science, USA), 1 × PCR buffer, 1–1.5 mM $MgCl_2$, 0.25 mM of each dNTP, 0.5 µm of each primer and approximately 30 ng of fungal genomic DNA, made up to a total reaction volume of 25 µL with sterile de-ionised water. Amplified fragments were purified using High Pure PCR Product Purification Kit (Roche, USA) and sequenced in both directions with the same primers used for the DNA amplifications. For this purpose, the BigDye terminator sequencing kit v3.1 (Applied Biosystems, USA) and an ABI PRISM™ 3100 DNA sequencer (Applied Biosystems, USA) were used. All PCRs and sequencing reactions were performed on an Eppendorf Mastercycler Personal PCR (Eppendorf AG, Germany) with cycling conditions as described in Crous et al. (2004b, 2006) for all loci amplified.

Sequences generated were added to other sequences for *Calonectria* obtained from GenBank (http://www.ncbi.nlm.nih.gov) and were assembled and aligned using Sequence Navigator v1.0.1 (Applied Biosystems, USA) and MAFFT v5.11 (Katoh et al. 2002), respectively. The aligned sequences were then manually corrected where needed. Single nucleotide polymorphisms (SNPs) were determined for each gene region analysed using DnaSP v5.00.07 (Librado & Rozas 2009).

PAUP (Phylogenetic Analysis Using Parsimony, v4.0b10; Swofford 2002) was used to analyse the DNA sequence datasets. A partition homogeneity test (Farris et al. 1994) and a 70 % reciprocal bootstrap method (Mason-Gamer & Kellog 1996, Gueidan et al. 2007) were applied to evaluate the feasibility of combining the datasets. Phylogenetic relationships were estimated by heuristic searches based on 1 000 random addition sequences and tree bisection-reconnection, with the branch swapping option set on 'best trees' only.

All characters were weighed equally and alignment gaps were treated as missing data. Measures calculated for parsimony included tree length (TL), consistency index (CI), retention index (RI) and rescaled consistence index (RC). Bootstrap analyses (Hillis & Bull 1993) were based on 1 000 replications. The phylogenetic analyses included 57 partial gene sequences per gene, representing 27 *Calonectria* species (Table 1) closely related to the isolates studied. *Calonectria colombiensis* (CBS 112221) and *Ca. chinensis* (CBS 112744) were used as the

outgroup taxa (Lombard et al. 2009, 2010d). All sequences were deposited in GenBank and the alignments in TreeBASE (http://www.treebase.org).

A Markov Chain Monte Carlo (MCMC) algorithm was used to generate phylogenetic trees with Bayesian probabilities using MrBayes v3.1.1 (Ronquist & Huelsenbeck 2003) for the combined sequence datasets. Models of nucleotide substitution for each of the three genes were determined using MrModeltest (Nylander 2004) and included for each gene partition, which were used for the combined sequence analyses. Two independent runs of four MCMC chains were run simultaneously from random trees for 1 000 000 generations and sampled every 100 generations for the combined analysis of the gene partitions. Both runs converged on the same likelihood score and tree topology, and the first 1 000 trees were discarded as the burn-in phase of each analysis. Posterior probabilities were determined from the remaining 9 000 trees.

Sexual compatibility

Single conidial *Calonectria* isolates of unknown identity from China were crossed among themselves in all possible combinations. Crosses were made as described in Schoch et al. (1999) on minimal salt agar (MN) to which sterile tooth picks had been placed on the agar surface (Guerber & Correll 2001, Lombard et al. 2010a, b, d). Controls were of isolates crossed with themselves and it was thus also possible to distinguish between those species with heterothallic or homothallic mating systems. The plates were stacked in plastic containers and incubated at 20 °C for 6–8 wk. Crosses were regarded as successful when isolate combinations produced perithecia extruding viable ascospores.

Taxonomy

For morphological identification of *Calonectria* isolates, single conidial isolates were prepared on MEA and synthetic nutrient-poor agar (SNA) (Nirenburg 1981, Lombard et al. 2009, 2010a, b, d). Inoculated plates were incubated at room temperature and examined after 7 d. Gross morphological characteristics of the anamorph state were determined by mounting fungal structures in lactic acid and 30 measurements at ×1 000 magnification were made for each isolate. Teleomorph morphology was determined by mounting perithecia obtained from the sexual compatibility tests in Leica mountant (Setpoint Premier, Johannesburg, South Africa) and hand-sectioned with a Leica CM1100 cryostat (Setpoint Technologies) at –20 °C. The 12 µm sections were mounted in lactophenol and 3 % KOH. Gross morphological characteristics were determined as mentioned for the anamorph state. The 95 % confidence levels were determined and extremes of measurements are given in parentheses.

Optimal growth conditions for cultures were determined in the dark on MEA for each isolate, at temperatures ranging from 5–35 °C at 5 °C intervals. This was repeated three times for each isolate examined. Colony colours were determined after 7 d on MEA at 25 °C in the dark, using the colour charts of Rayner (1970). All descriptions, illustrations and nomenclatural data were deposited in MycoBank (www.mycobank.org; Crous et al. 2004a).

Pathogenicity tests

In order to test the pathogenicity of the *Calonectria* spp. collected in this study, 10 profusely sporulating isolates, representing different *Calonectria* species identified based on morphology and DNA sequence comparisons were selected for inoculation trails (Table 1). The isolates were transferred to MEA, and incubated for 10 d at 25 °C. A spore suspension was prepared for each isolate, by adding 2 mL of sterile water to the plates and dislodging conidia with a sterile glass rod. The spore suspension was strained through a layer of cheesecloth and the concentration adjusted to 3.3×10^5 conidia/mL. To ensure that conidia would adhere to the surface of the inoculated leaves, 2 mL Tween 80 (ChangJiang JingXi HuaGongChang, GuangZhou, China) was added to the suspension.

Two *E. urophylla* × *E. grandis* hybrid clones, CEPT-9 and CEPT-10 (height 30–40 cm), selected for inoculation, were acclimatised for 2 wk in a shade house subjected to natural climatic conditions (temperature 26–32 °C and humidity 60–90 %). For each of the 10 selected *Calonectria* isolates, nine plants of each clone were inoculated with the spore suspensions by spraying the leaves until run-off. The plants were covered with plastic bags for 48 h allowing sufficient humidity for infection. Control inoculations were done in a similar fashion with sterile water amended with 2 mL of Tween 80.

Pathogenicity tests were evaluated 14 d after inoculation. For every inoculated seedling, the percentage of the infected/diseased leaves was calculated. Results were analysed in SAS v8 using the PROC GLM (general linear model) (SAS Institute 1999). Analysis of variance (ANOVA) was used to determine the effects of fungal strain on lesion length. Prior to ANOVA, homogeneity of variance across treatments was verified. For significance tests amongst means, Fisher's protected test was used. F values with $P < 0.05$ were considered significant. Isolations were made from lesions on the leaves of the test plants in each plot to ensure the presence of the inoculated fungi.

RESULTS

Isolates

A total of 97 isolates were collected from leaves in *Eucalyptus* plantations in the FuJian Province during the survey in 2007 (Table 1). Of these, 77 isolates were from diseased leaves on five *E. dunnii* trees, and an additional 20 isolates were obtained from diseased leaves on two *E. grandis* trees.

DNA sequence comparisons

Amplicons of approximately 500 bp were generated for the BT and TEF-1α gene regions and those for the HIS3 region were approximately 450 bp. Partition homogeneity tests for all possible combinations of the three gene regions used, consistently yielded a P-value of 0.001. The 70 % reciprocal bootstrap trees showed no conflict in tree topologies for the three gene regions. Based on the tree topologies and a P-value of 0.001 (Cunningham 1997, Dettman et al. 2003), the gene regions were combined. This resulted in a dataset consisting of 1 522 characters including gaps. Of these characters, 1 046 were constant and parsimony uninformative. The 476 parsimony informative characters included in the parsimony analyses yielded 54 equally most parsimonious trees (TL = 1110, CI = 0.722, RI = 0.879, RC = 0.634), of which the first tree is presented (Fig. 1). For Bayesian analyses, a HKY+I model was selected for BT, GTR+I+G model for HIS3 and a GTR+G model for TEF-1α and incorporated into the analyses. The consensus tree obtained for the Bayesian analyses confirmed the topology of the consensus tree obtained with the parsimony analysis (Fig. 1).

The phylogenetic tree showed a number of well-supported clades. Some isolates grouped in a clade representing *Ca. pauciramosa* with a bootstrap value (BP) of 55 and a Bayesian posterior probability (PP) value of 0.95. Other isolates grouped close to *Ca. reteaudii*, but in a distinct clade (BP = 100, PP = 1.00). Several isolates also clustered with *Ca. colhounii* and *Ca. eucalypti*, but separated from them to form a monophyletic group (BP = 84, PP = 0.84). These isolates also clustered into two well-supported clades (BP = 81, PP = 1.00 and

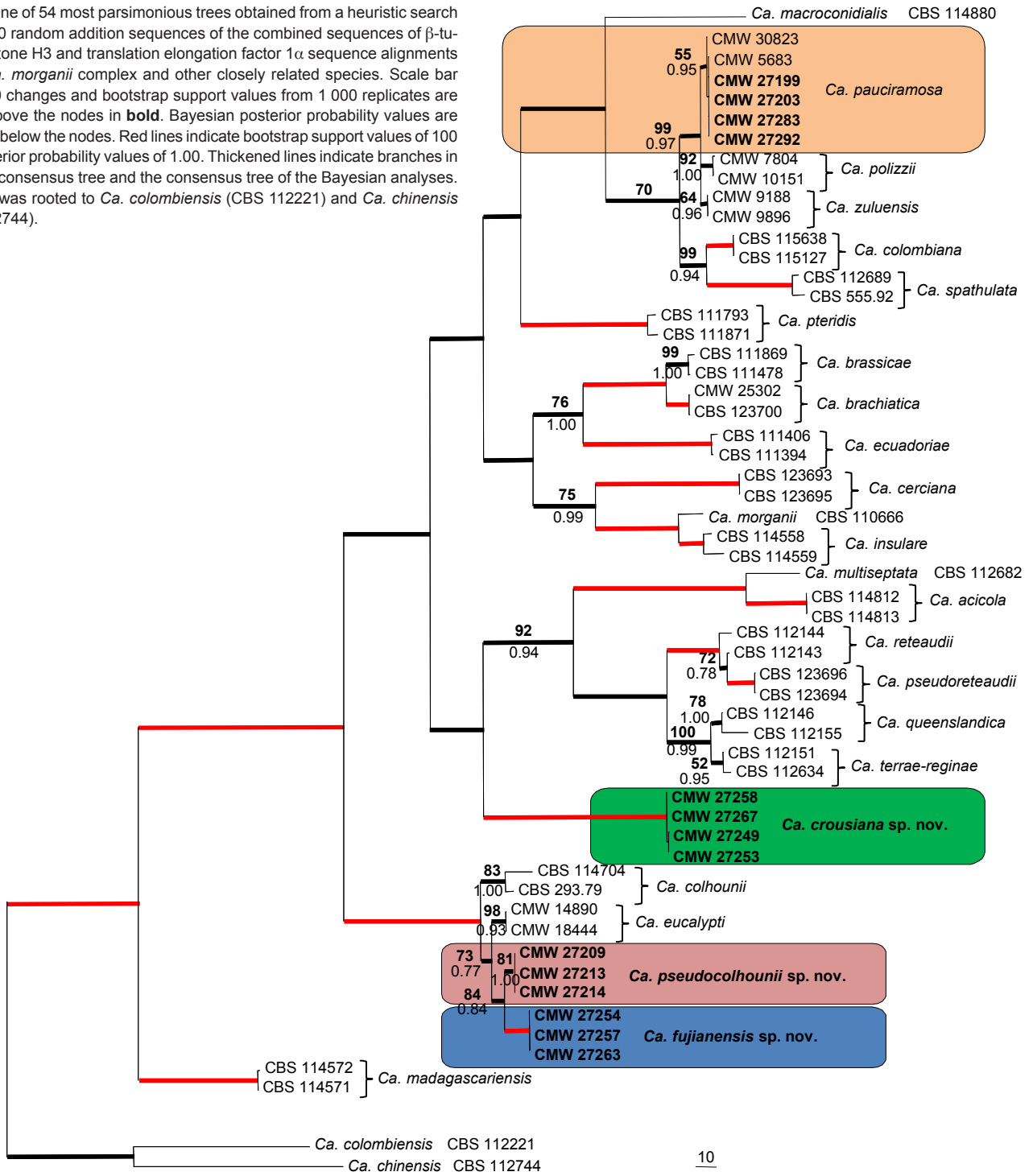

Fig. 1 One of 54 most parsimonious trees obtained from a heuristic search with 1 000 random addition sequences of the combined sequences of β-tubulin, histone H3 and translation elongation factor 1α sequence alignments of the *Ca. morganii* complex and other closely related species. Scale bar shows 10 changes and bootstrap support values from 1 000 replicates are shown above the nodes in **bold**. Bayesian posterior probability values are indicated below the nodes. Red lines indicate bootstrap support values of 100 and posterior probability values of 1.00. Thickened lines indicate branches in the strict consensus tree and the consensus tree of the Bayesian analyses. The tree was rooted to *Ca. colombiensis* (CBS 112221) and *Ca. chinensis* (CBS 112744).

BP = 100, PP = 1.00, respectively). SNP analyses for isolates CMW 27209, CMW 27213 and CMW 27214 showed that they shared two unique alleles, while isolates CMW 27254, CMW 27257 and CMW 27263 shared ten unique alleles for the three gene regions analysed, clearly distinguishing them from each other. Furthermore, these six Chinese isolates also shared three unique alleles, distinguishing them from *Ca. colhounii* and *Ca. eucalypti* (Table 2).

Sexual compatibility

Protoperithecia formed within 3 wk and mating tests produced perithecia within 6 wk on sterilised toothpicks on MN medium. Except for isolates of *Ca. pauciramosa* (CMW 27199, CMW 27203, CMW 27283, CMW 27292), all the control crosses of *Calonectria* isolates, included in this study, produced perithecia with viable ascospores. These results show that all the *Calonectria* isolates, except those of *Ca. pauciramosa* are self-fertile (homothallic).

Taxonomy

Based on morphology and DNA sequence comparisons (Fig. 1), *Calonectria* isolates from *Eucalyptus* trees in FuJian Province reside in four taxa that include *Ca. pauciramosa* and three previously undescribed species. Isolates CMW 27199, CMW 27203, CMW 27283 and CMW 27292 clearly represent *Ca. pauciramosa*, with obpyriform to ellipsoidal vesicles, and macroconidia being 40–65 × 3–5 µm (av. = 50 × 5 µm). The remaining isolates are described in the genus *Calonectria* as follows:

Table 2 Single nucleotide polymorphism comparisons between *Calonectria colhounii*, *Ca. eucalypti*, *Ca. pseudocolhounii* and *Ca. fujianensis*.

		β-tubulin							Histone H3								
Species	Isolate no.	53	378	397	398	407	420	516	57	248	290	311	362	371	386	454	455
Ca. colhounii	CBS 293.79	C	C	C	T	A	C	C	A	T	A	T	C	T	C	A	C
	CBS 114704	C	C	G	T	A	T	T	A	T	A	T	C	T	C	A	C
Ca. eucalypti	CBS 125237	C	C	G	**C**	**G**	T	T	**–**	T	**T**	T	**T**	T	C	**C**	**A**
	CBS 125275	C	C	G	**C**	**G**	T	T	**–**	T	**T**	T	**T**	T	C	**C**	**A**
Ca. pseudocolhounii	CMW 27209	C	**T**	G	T	A	C	C	A	**C**	A	T	C	T	C	A	C
	CMW 27213	C	**T**	G	T	A	C	C	A	**C**	A	T	C	T	C	A	C
	CMW 27214	C	**T**	G	T	A	C	C	A	**C**	A	T	C	T	C	A	C
Ca. fujianensis	CMW 27254	**T**	C	**C**	T	A	C	C	A	T	A	**C**	C	**C**	**A**	A	C
	CMW 27257	**T**	C	**C**	T	A	C	C	A	T	A	**C**	C	**C**	**A**	A	C
	CMW 27263	**T**	C	**C**	T	A	C	C	A	T	A	**C**	C	**C**	**A**	A	C

		TEF-1α																		
Species	Isolate no.	1	31	92	93	94	95	96	123	127	181	182	183	184	261	454	458	469	474	500
Ca. colhounii	CBS 293.79	**G**	**C**	**C**	**A**	**C**	**A**	**A**	**–**	A	–	–	–	–	**G**	**C**	**C**	**C**	**C**	C
	CBS 114704	**G**	**C**	**C**	**A**	**C**	**A**	**A**	**–**	A	–	–	–	–	**G**	**C**	**C**	**C**	**C**	C
Ca. eucalypti	CBS 125237	C	A	–	–	–	–	–	T	A	–	–	–	–	A	T	T	T	T	C
	CBS 125275	C	A	–	–	–	–	–	T	A	–	–	–	–	A	T	T	T	T	C
Ca. pseudocolhounii	CMW 27209	C	A	–	–	–	–	–	T	A	–	–	–	–	A	T	T	T	T	–
	CMW 27213	C	A	–	–	–	–	–	T	A	–	–	–	–	A	T	T	T	T	–
	CMW 27214	C	A	–	–	–	–	–	T	A	–	–	–	–	A	T	T	T	T	–
Ca. fujianensis	CMW 27254	C	A	–	–	–	–	–	T	**G**	**A**	**A**	**A**	**A**	A	T	T	T	T	–
	CMW 27257	C	A	–	–	–	–	–	T	**G**	**A**	**A**	**A**	**A**	A	T	T	T	T	–
	CMW 27263	C	A	–	–	–	–	–	T	**G**	**A**	**A**	**A**	**A**	A	T	T	T	T	–

[1] Highlight and **bold** = unique polymorphisms; highlight = shared polymorphisms.

Calonectria crousiana S.F. Chen, L. Lombard, M.J. Wingf. & X.D. Zhou, *sp. nov*. — MycoBank MB518855; Fig. 2

Teleomorpha *Calonectriae indusiatae* similis sed ascosporibus maioribus (56–)58–69(–76) × (5–)6.5–7.5(–8) µm, mediocriter 64 × 7 µm, differt. Anamorpha *Cy. theae* similis sed macroconidiis cylindricis utrinque rotundatis rectis (59–)61–67(–75) × (4–)4.5–5.5(–6) µm, mediocriter 64 × 5 µm, (semel vel) ter septatis, sine cicatrice abscissionis visibile, in fasciculis parallelis cylindricis muco contenitis, differt.

Etymology. This species is named for Prof. P.W. Crous recognising his monumental contributions to the taxonomy of *Calonectria* spanning more than two decades.

Perithecia solitary or in groups of up to five, orange, becoming red-brown with age; in section apex and body orange, base red-brown, subglobose to ovoid, (321–)352–499(–550) µm high, (260–)262–403(–465) µm diam, body turning dark orange to slightly red, and base dark red-brown in 3 % KOH; perithecial walls rough, consisting of two thick-walled layers: outside layer of *textura globulosa*, (32–)33–76(–90) µm wide, becoming more compressed towards inner layer of *textura angularis*, (10–)12–23(–30) µm wide, becoming thin-walled and hyaline towards the centre; outer cells (22–)26–38(–40) × (9–)16–29(–36) µm, inner cells (8–)9–15(–18) × (2.5–)3.5–6(–7) µm; perithecial base up to 241 µm wide, consisting of dark red, angular cells, merging with an erumpent stroma; cells of the outer wall layer continuing into the pseudoparenchymatous cells of the erumpent stroma. *Asci* 8-spored, clavate, (109–)120–175(–186) × (23–)24–25 µm, tapering to a long thin stalk. *Ascospores* aggregate in the upper third of the asci, hyaline, guttulate, fusoid with rounded ends, straight to slightly curved, (1–)3-septate, not or slightly constricted at the septum, (56–)58–69(–76) × (5–)6.5–7.5(–8) µm (av. = 64 × 7 µm). *Macroconidiophores* consisting of a stipe, a suite of penicillate arranged fertile branches, a stipe extension, and a terminal vesicle; stipe septate, hyaline, smooth (61–)63–160(–220) × (4.5–)6–8(–9.5) µm; stipe extensions septate, straight to flexuous (195–)225–404(–475) µm long, 3–6 µm wide at the apical septum, terminating in a clavate vesicle, (4–)4.5–5(–6) µm diam. *Conidiogenous apparatus* (63–)76–117(–138) µm long, (40–)53–98(–116) µm wide; primary branches aseptate to 1-septate, (19–)21–42(–70) × (4–)4.5–5.5(–6) µm; secondary branches aseptate, (13–)17–25(–28) × (3.5–)4–5 µm; tertiary branches aseptate, (11–)11.5–15(–18) × (3–)3.5–4(–4.5) µm; additional branches (–5), aseptate, (10–)10.5–14(–16) × (2.5–)3–4 µm; each terminal branch producing 1–4 phialides; phialides doliiform to allantoid, hyaline, aseptate, (9.5–)10.5–13.5(–15) × 34.5 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (59–)61–67(–75) × (4–)4.5–5.5(–6) µm (av. = 64 × 5 µm), (1–)3-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime.

Culture characteristics — Colonies reaching 64–80 mm diam after 7 d on MEA in the dark with optimal growth temperature at 25 °C. Colonies fast growing forming white to sienna aerial mycelium, with feathery, irregular margins. Surface and reverse with mikado-orange to sienna outer margin, and russet inner region, becoming argus-brown towards the centre. Chlamydospores arrange in chains, abundant throughout the medium, forming microsclerotia.

Substratum — *Eucalyptus grandis*.

Distribution — FuJian Province, China.

Specimens examined. China, FuJian Province, on leaves of *Eucalyptus grandis*, Aug. 2007, *M.J. Wingfield*, Herb. PREM 60453, holotype of *Ca. crousiana*, culture ex-type CMW 27249 = CBS 127198; FuJian Province, on leaves of *E. grandis*, Aug. 2007, *M.J. Wingfield*, Herb. PREM 60454, culture CMW 27253 = CBS 127199; FuJian Province, on leaves of *E. grandis*, Aug. 2007, *M.J. Wingfield*, Herb. PREM 60455, culture CMW 27267 = CBS 127203; FuJian Province, on leaves of *E. grandis*, Aug. 2007, *M.J. Wingfield*, culture CMW 27258.

Notes — *Calonectria crousiana* is morphologically similar to *Ca. indusiata*, *Ca. australiensis* and species in the *Ca. colhounii* complex, that includes *Ca. colhounii*, *Ca. eucalypti*, *Ca. macroconidialis* and *Ca. madagascariensis* (Crous et al. 2006, Lombard et al. 2010b). With the exception of *Ca. macroconidialis* (macroconidia (1–)3(–6)-septate), all of these species produce clavate vesicles and (1–)3-septate macroconidia. *Calonectria crousiana* can be distinguished from species in the *Ca. colhounii* complex by its distinctly orange to red perithecia. This fungus can also be distinguished from *Ca. indusiata* and *Ca. aus-*

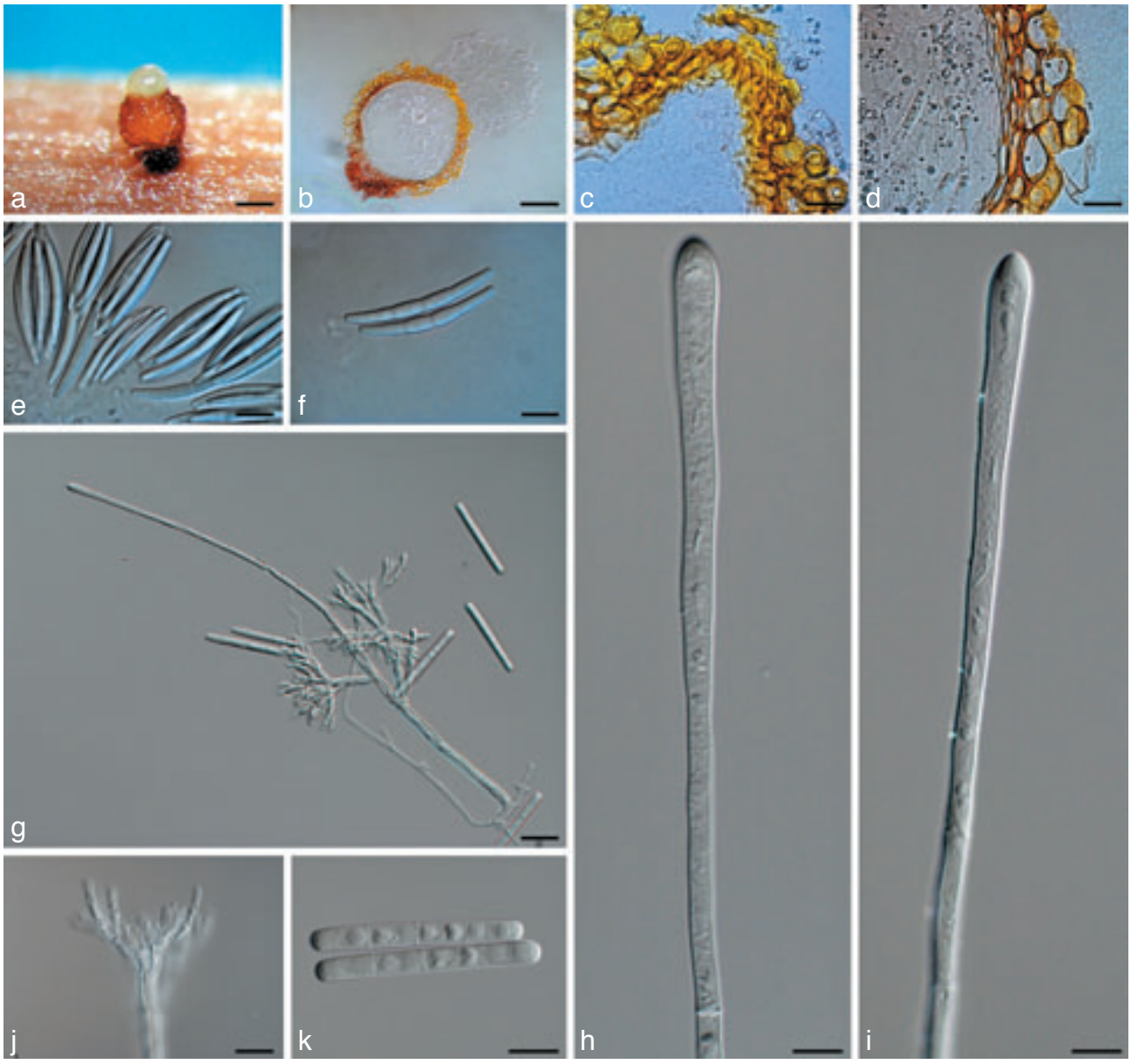

Fig. 2 *Calonectria crousiana*. a–f. Teleomorph state; g–k. anamorph state. a. Perithecium; b. vertical section through a perithecium; c. cells around ostiolar region of perithecium; d. section through lateral perithecial wall; e. asci; f. ascospores; g. macroconidiophore; h, i. clavate vesicles; j. fertile branches; k. macroconidia. — Scale bars: a = 200 µm; b = 100 µm; c–e, g, j = 20 µm; f, k = 10 µm; h, i = 5 µm.

traliensis based on the dimensions of the macroconidia, with *Ca. crousiana* (av. = 64 × 5 µm) having shorter macroconidia than those of *Ca. indusiata* (av. = 81 × 6.0 µm) and narrower than those of *Ca. australiensis* (av. = 63 × 6.5 µm).

Calonectria pseudocolhounii S.F. Chen, L. Lombard, M.J. Wingf. & X.D. Zhou, *sp. nov*. — MycoBank MB518856; Fig. 3

Teleomorpha *Calonectria colhounii* similis sed ascosporis hyalinis guttulatis fusoidibus extremis rotundatis, rectis vel subcurvatis, (semel vel) ter septatis, in septo non vel leviter constrictis, (44–)50–62(–74) × (5–)6–7(–8) µm, mediocriter 56 × 6.5 µm, differt. Anamorpha *Cy. colhounii* similis sed macroconidiis cylindricis utrinque rotundatis rectis (49–)55–65(–74) × (3.5–)4–5(–5.5) µm, mediocriter 60 × 4.5 µm, (semel vel) ter septatis, sine cicatrice abscissionis visibile, in fasciculis parallelis cylindricis muco contenitis, differt.

Etymology. The name reflects the fact that this fungus is morphologically similar to *Calonectria colhounii*.

Perithecia solitary or in groups of up to four, bright yellow, becoming orange with age; in section apex and body yellow, base red-brown, subglobose to ovoid, (330–)350–453(–495) µm high, (227–)258–330(–390) µm diam, body turning dark yellow, and base dark red-brown in KOH+; perithecial walls rough consisting of two thick-walled layers: outside layer of *textura globulosa*, (26–)33–59(–65) µm wide, becoming more compressed towards inner layer of *textura angularis*, (10–)12–18(–22) µm wide, becoming thin-walled and hyaline towards the centre; outer cells (17–)21–34(–42) × (11–)12–21(–27) µm, inner cells (10–)11–14(–20) × (3–)5–6.5(–7) µm, perithecial base up to 180 µm wide, consisting of dark red, angular cells merging with an erumpent stroma, cells of the outer wall layer continuing into the pseudoparenchymatous cells of the erumpent stroma. *Asci* 4-spored, clavate, (130–)135–162(–167) × (16–)18–24(–30) µm, tapering to a long thin stalk. *Ascospores* aggregate in the upper third of the asci, hyaline, guttulate, fusoid with rounded ends, straight to slightly curved, (1–)3-septate, not or slightly constricted at the septum, (44–)50–62(–74) × (5–)6–7(–8) µm (av. = 56 × 6.5 µm). *Macroconidiophores* consisting of a stipe, a suite of penicillate arranged fertile branches, a stipe extension, and a terminal vesicle; stipe septate, hyaline, smooth (45–)53–192(–217) × (5.5–)6–7(–8) µm; stipe extensions septate, straight to flexuous (133–)168–252(–300) µm long, 3–6

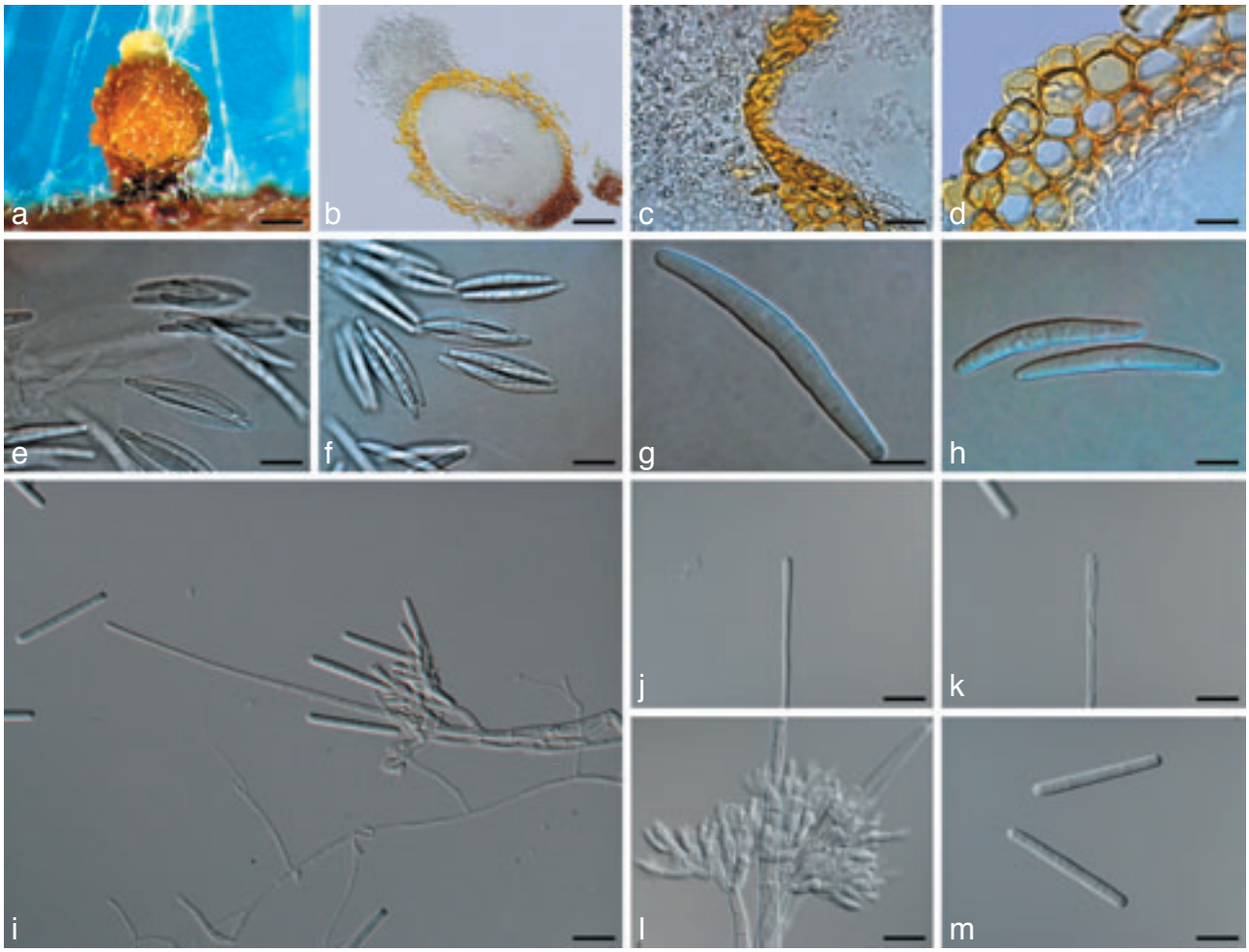

Fig. 3 *Calonectria pseudocolhunii*. a–h. Teleomorph state; i–m. anamorph state. a. Perithecium; b. vertical section through a perithecium; c. cells around ostiolar region of perithecium; d. section through lateral perithecial wall; e, f. asci; g, h. ascospores; i. macroconidiophore; j, k. clavate vesicles; l. fertile branches; m. macroconidia. — Scale bars: a = 200 µm; b = 100 µm; c = 40 µm; d–f, i–k = 20 µm; g, h, l, m = 10 µm.

µm wide at the apical septum, terminating in a clavate vesicle, (3.5–)4–5(–6) µm diam. *Conidiogenous apparatus* (41–)44–74(–91) µm long, (35–)38–65(–84) µm wide; primary branches aseptate to 1-septate, (13–)15–26(–33) × 3.5–4.5(–5) µm; secondary branches aseptate, (9–)11.5–20(–23) × 3–4(–4.5) µm; tertiary branches aseptate, 8.5–14(–17) × 3–4 µm; additional branches (–5), aseptate, (8–)8.5–13(–15) × 2.5–3(–3.5) µm; each terminal branch producing 2–4 phialides; phialides doliiform to reniform, hyaline, aseptate, (8–)9–12.5(–14) × 2.5–3(–3.5) µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (49–)55–65(–74) × (3.5–)4–5(–5.5) µm (av. = 60 × 4.5 µm), (1–)3-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime.

Culture characteristics — Colonies reaching 38–44 mm diam after 7 d on MEA in the dark with optimal growth temperature at 25 °C. Colonies with white aerial mycelium in the centre, with feathery, irregular margins at the edges. Surface and reverse with white to buff-yellow outer margins, and russet inner region, becoming liver-brown towards the centre. Chlamydospores arrange in chains, abundant throughout the medium, forming microsclerotia.

Substratum — *Eucalyptus dunnii*.

Distribution — FuJian Province, China.

Specimens examined. China, FuJian Province, on leaves of *Eucalyptus dunnii*, Aug. 2007, *M.J. Wingfield*, Herb. PREM 60456, holotype of *Ca. pseudocolhounii*, culture ex-type CMW 27209 = CBS 127195; FuJian Province, on leaves of *E. dunnii*, Aug. 2007, *M.J. Wingfield*, Herb. PREM 60457, culture CMW 27213 = CBS 127196; FuJian Province, on leaves of *E. dunnii*, Aug. 2007, *M.J. Wingfield*, Herb. PREM 60458, culture CMW 27214 = CBS 127197.

Notes — *Calonectria pseudocolhounii* is similar to species in the *Ca. colhounii* complex that all have yellow perithecia, (1–)3-septate ascospores and clavate vesicles in the anamorph state. *Calonectria pseudocolhounii* is morphologically most similar to *Ca. colhounii* (macroconidia av. = 65 × 5 µm), but can be distinguished from this species by having smaller and narrower macroconidia (av. = 60 × 4.5 µm). The ascospores of *Ca. pseudocolhounii* (av. = 56 × 6.5 µm) are larger, while the macroconidia (av. = 60 × 4.5 µm) are smaller than those of *Ca. eucalypti* (ascospores av. = 33 × 6 µm; macroconidia av. = 72 × 6 µm).

Calonectria fujianensis S.F. Chen, L. Lombard, M.J. Wingf. & X.D. Zhou, *sp. nov.* — MycoBank MB518857; Fig. 4

Teleomorpha *Calonectria colhounii* similis sed ascosporis hyalinis guttulatis fusoidibus extremis rotundatis, rectis vel subcurvatis, (semel vel) ter septatis, in septo non vel leviter constrictis, (38–)49–62(–72) × (5–)6–7.5(–8) µm, mediocriter 55.5 × 6.8 µm, differt. Anamorpha *Cy. colhounii* similis sed macroconidiis cylindricis utrinque rotundatis rectis (48–)50–55(–60) × (2.5–)3.5–4.5(–5) µm, mediocriter 52.5 × 4 µm, (semel vel) ter septatis, sine cicatrice abscissionis visibile, in fasciculis parallelis cylindricis muco contenitis, differt.

Etymology. Named after the FuJian Province of China where the fungus was first collected.

Perithecia solitary or in groups of up to four, bright yellow, becoming orange with age; in section apex and body yellow, base red-brown, subglobose to ovoid, (310–)351–465(–492) µm high, (206–)226–329(–382) µm diam, body turning dark yellow, and base dark red-brown in KOH+; perithecial walls

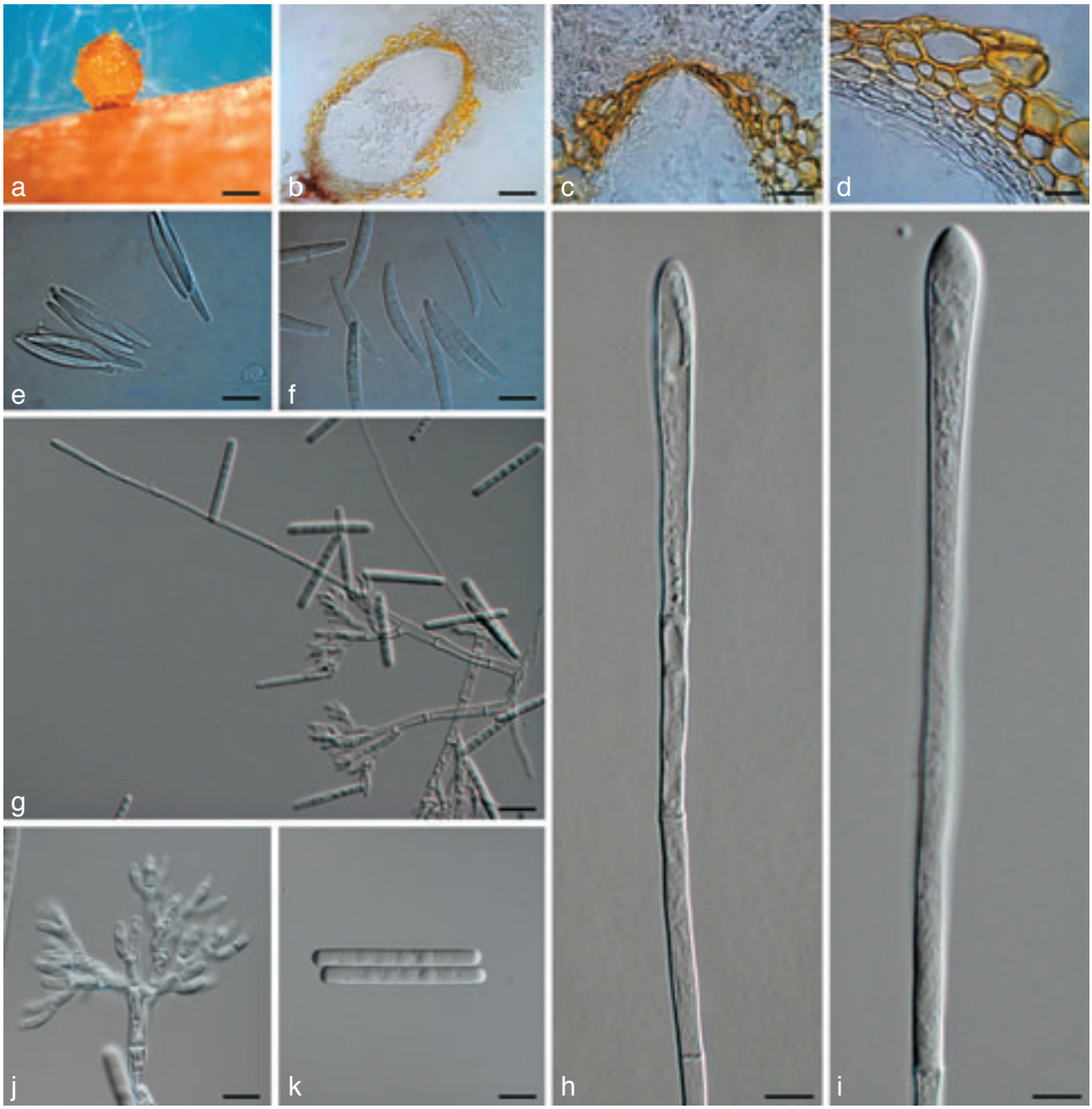

Fig. 4 *Calonectria fujianensis*. a–f. Teleomorph state; g–k. anamorph state. a. Perithecium; b. vertical section through a perithecium; c. cells around ostiolar region of perithecium; d. section through lateral perithecial wall; e. asci; f. ascospores; g. macroconidiophore; h, i. clavate vesicles; j. fertile branches; k. macroconidia. — Scale bars: a = 200 µm; b = 100 µm; c = 40 µm; d–g = 20 µm; h, i = 5 µm; j, k = 10 µm.

rough consisting of two thick-walled layers: outside layer of *textura globulosa*, (26–)35–58(–61) µm wide, becoming more compressed towards inner layer of *textura angularis*, (10–)12–21(–24) µm wide, becoming thin-walled and hyaline towards the centre; outer cells (15–)17–35(–41) × (8–)11–20(–24) µm, inner cells (9–)10–20(–26) × (2.5–)3.5–6.0(–6.5) µm; perithecial base up to 180 µm wide, consisting of dark red, angular cells, merging with an erumpent stroma, cells of the outer wall layer continuing into the pseudoparenchymatous cells of the erumpent stroma. *Asci* 4-spored, clavate, (118–)132–152(–155) × (14–)16–23(–29) µm, tapering to a long thin stalk. *Ascospores* aggregate in the upper third of the ascus, hyaline, guttulate, fusoid with rounded ends, straight to slightly curved, (1–)3-septate, not or slightly constricted at the septum, (38–)49–62(–72) × (5–)6–7.5(–8) µm (av = 55.5 × 6.8 µm). *Macroconidiophores* consisting of a stipe, a suite of penicillate arranged fertile branches, a stipe extension, and a terminal vesicle; stipe septate, hyaline, smooth, (33–)36–152(–210) × (3.5–)4.5–8(–8.5) µm; stipe extensions septate, straight to flexuous (147–)167–248(–261) µm long, 3–5 µm wide at the apical septum, terminating in a clavate vesicle, (3–)3.5–4.5(–5) µm diam. *Conidiogenous apparatus* (36–)43–72(–89) µm long, (21–)31–61(–65) µm wide; primary branches aseptate to 1-septate, (11–)12–28(–32) × (3–)3.5–4.5 µm; secondary branches aseptate, 8–20(–26) × 3–4(–4.5) µm; tertiary branches aseptate, (8–)10–12(–12.5) × 2.5–3(–4) µm; additional branches (–5), aseptate, (8–)9–10 × 2.5–3(–3.5) µm; each terminal branch producing 2–4 phialides; phialides doliiform to reniform, hyaline, aseptate, (6.5–)8–11 × (2–)2.5–3 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (48–)50–55(–60) × (2.5–)3.5–4.5(–5) µm (av. = 52.5 × 4 µm), (1–)3-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime.

Culture characteristics — Colonies reaching 44–52 mm diam after 7 d on MEA in the dark with optimal growth temperature at

25 °C. Colonies with white to cream-coloured aerial mycelium in the centre, with feathery, irregular margins at the edges. Surface and reverse with cream coloured to white outer margins, and russet inner region, becoming argus-brown towards the centre. Chlamydospores arranged in chains, abundant throughout the medium, forming microsclerotia.

Substratum — *Eucalyptus grandis*.

Distribution — FuJian Province, China.

Specimens examined. China, FuJian Province, on leaves of *Eucalyptus grandis*, Aug. 2007, *M.J. Wingfield*, Herb. PREM 60460, holotype of *Ca. fujianensis*, culture ex-type CMW 27257 = CBS 127201; FuJian Province, on leaves of *E. grandis*, Aug. 2007, *M.J. Wingfield*, Herb. PREM 60461, culture CMW 27263 = CBS 127202; FuJian Province, on leaves of *E. grandis*, Aug. 2007, *M.J. Wingfield*, Herb. PREM 60459, culture CMW 27254 = CBS 127200.

Notes — *Calonectria fujianensis* is morphologically distinguishable from *Ca. colhounii* and *Ca. pseudocolhounii* having smaller macroconidia (av. = 52.5 × 4 µm) than *Ca. colhounii* (av. = 65 × 5 µm) and *Ca. pseudocolhounii* (av. = 60 × 4.5 µm). The ascospores of *Ca. fujianensis* (av. = 55.5 × 6.8 µm), *Ca. pseudocolhounii* (av. = 56 × 6.5 µm) and *Ca. colhounii* (av. = 55 × 6 µm), are larger than those of *Ca. eucalypti* (av. = 33 × 6 µm), while the macroconidia of the former three species are smaller than those of *Ca. eucalypti* (av. = 72 × 6 µm).

Pathogenicity tests

All plants representing the two *Eucalyptus* clones inoculated with *Calonectria* spp. (*Ca. crousiana*, *Ca. fujianensis*, *Ca. pauciramosa*, *Ca. pseudocolhounii*) in this study, developed leaf spot symptoms whereas no disease was observed on the leaves of the control plants (Fig. 5). The inoculated fungi were successfully re-isolated from the leaf spots and no *Calonectria* spp. was isolated from the control plants. The average percentage of leaf surface affected by the test isolates showed no significant differences between the two experimental plots (P = 0.0578), and the interactions between the two experiments and two clones were not significantly different (P = 0.0535). Subsequently, the data for the two plots were combined and analysed collectively. The combined results showed significant isolate × clone interaction (P < 0.05), indicating that not all *Calonectria* isolates reacted similarly to the two *Eucalyptus* clones tested. The percentage of infected leaves arising from inoculation with *Ca. pauciramosa* (CMW 27199, CMW 27192), *Ca. pseudocolhounii* (CMW 27209, CMW 27213, CMW 27214), *Ca. crousiana* (CMW 27249, CMW 27267) and *Ca. fujianensis* (CMW 27263) were significantly different (P < 0.05) on the two clones tested. In contrast, there was no significant difference (P > 0.05) in the percentage of infected leaves for the two clones inoculated with isolates (CMW 27254, CMW 27257) of *Ca. fujianensis* (Fig. 5).

The *Eucalyptus* clone CEPT-10 displayed a significantly (P < 0.05) higher percentage of infected leaves when inoculated with *Ca. pauciramosa* (CMW 27199, CMW 27292), *Ca. pseudocolhounii* (CMW 27209, CMW 27213, CMW 27214) and *Ca. fujianensis* (CMW 27254, CMW 27257, CMW 27263) than with *Ca. crousiana* (CMW 27249, CMW 27267) (Fig. 5). Isolate CMW 27254 (*Ca. fujianensis*) displayed the highest average percentage of leaf surface infected on clone CEPT-10 (Fig. 5).

The *Eucalyptus* clone CEPT-9 showed a significantly (P < 0.05) higher percentage of infected leaves caused by isolates of *Ca. pseudocolhounii* (CMW 27209, CMW 27213, CMW 27214) and *Ca. fujianensis* (CMW 27254, CMW 27257, CMW 27263) than with those of *Ca. pauciramosa* (CMW 27199, CMW 27292) and *Ca. crousiana* (CMW 27249, CMW 27267) (Fig. 5). Isolate CMW 27214 (*Ca. pseudocolhounii*) resulted in the highest average percentage of leaves infected for CEPT-9 (Fig. 5).

DISCUSSION

In this study, four *Calonectria* spp. were identified from leaves collected on diseased *Eucalyptus* trees grown in commercial plantations of FuJian Province in Southeast China. These included *Ca. pauciramosa* and three previously undescribed species for which the names *Ca. crousiana*, *Ca. fujianensis* and *Ca. pseudocolhounii* are provided. The identification of these fungi was supported by DNA sequence comparisons as well as by morphological characteristics. Based on phylogenetic inference, *Ca. crousiana* is closely related to taxa in the *Ca. reteaudii* species complex, whereas *Ca. pseudocolhounii* and *Ca. fujianensis* reside in the *Ca. colhounii* complex. Pathogenicity tests showed that all four species are capable of causing leaf infections on two of the most widely planted *E. urophylla* × *E. grandis* clones in South China.

Calonectria pauciramosa resides in the *Ca. scoparia* species complex (Crous et al. 1993, Schoch et al. 1999, Lombard et al. 2010b, d) and was recently found killing plants in a commercial *Eucalyptus* nursery in the GuangDong Province of China (Lombard et al. 2010d). This study represents the first report of this pathogen infecting leaves of *Eucalyptus* trees growing in plantations. In the past, *Ca. pauciramosa* has been associated with nursery diseases in Australia, Italy, South Africa, Spain and USA (Koike et al. 1999, Polizzi & Crous 1999, Schoch et

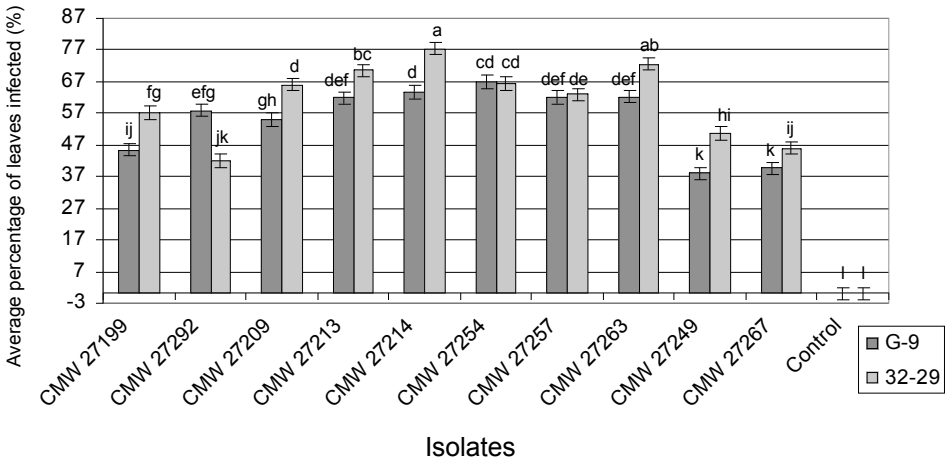

Fig. 5 Histogramme indicating the average percent leaves infected (%) resulting from inoculation trials of two *Eucalyptus urophylla* × *E. grandis* clones inoculated with isolates of *Calonectria pauciramosa* (CMW 27199, CMW 27192), *Ca. pseudocolhounii* (CMW 27209, CMW 27213, CMW 27214), *Ca. fujianensis* (CMW 27254, CMW 27257, CMW 27263), *Ca. crousiana* (CMW 27249, CMW 27267) and the controls. Bars represent 95 % confidence limits for each treatment. Different letters above the bars indicate treatments that were statistically significantly different (P = 0.05).

al. 1999, 2001, Koike & Crous 2001, Polizzi et al. 2006, 2009, Perez-Sierra et al. 2007). This fungus has also been isolated from tropical areas of GuangDong Province (Lombard et al. 2010d) and in this study was found in an area that has a subtropical climate. The climatic conditions of these regions differ significantly, supporting the view that *Ca. pauciramosa* can tolerate a wide range of temperature conditions.

Based on phylogenetic inference, *Ca. crousiana* is closely related to *Calonectria* spp. in the *Ca. reteaudii* complex. Similar to *Ca. reteaudii*, *Ca. crousiana* also produces orange to red perithecia and has a *Cylindrocladium* state with clavate vesicles. However, septation of the macroconidia is distinct in these species with *Ca. crousiana* having (1–)3-septate macroconidia that distinguish it from the other species in the *Ca. reteaudii* complex, including *Ca. reteaudii* ((1–)5(–6)-septate), *Ca. pseudoreteaudii* (1(–3)-septate), *Ca. queenslandica* ((1–)3(–6)-septate) and *Ca. terrae-reginae* ((1–)3(–6)-septate) (Crous 2002, Lombard et al. 2010b). Morphological comparisons showed that *Ca. crousiana* is very similar to *Ca. indusiata* and *Ca. australiensis*, which have clavate vesicles and (1–)3-septate macroconidia (Crous 2002, Crous et al. 2006, Lombard et al. 2010b).

Previous studies have shown that *Ca. indusiata* and species in the *Ca. reteaudii* species complex are pathogens causing leaf blight and cutting rot on *Eucalyptus* trees and seedlings in Australia, South America and Southeast Asia (Pikethley 1976, Bolland et al. 1985, Sharma & Mohanan 1991, 1992, Booth et al. 2000, Crous & Kang 2001, Crous 2002, Rodas et al. 2005, Lombard et al. 2010d). In this study, *Ca. crousiana* was isolated from diseased leaves on *E. grandis* trees in FuJian Province. Based on the results of pathogenicity tests on two *Eucalyptus* hybrid clones, *Ca. crousiana* should be regarded as an important pathogen of *Eucalyptus* in China.

Past studies have shown that *Ca. colhounii* is closely related to *Ca. madagascariensis* and *Ca. macroconidialis* (Crous et al. 1999). Recently, a newly described species, *Ca. eucalypti*, was also identified in this complex (Lombard et al. 2010b). This *Calonectria* complex is characterised by having unique yellow perithecia, (1–)3-septate ascospores and clavate vesicles (Crous et al. 1999, Crous 2002, Lombard et al. 2010b). In the present study, *Ca. pseudocolhounii* and *Ca. fujianensis* were described as new species with both species sharing unique morphological characteristics with the other species in the complex. There are, however, a number of morphological differences distinguishing *Ca. pseudocolhounii* and *Ca. fujianensis* from the other species in this complex. All species other than *Ca. madagascariensis* (8-spore asci) produce asci with four ascospores. Macroconidia of *Ca. fujianensis* (av. = 52.5 × 4 µm) are smaller than those of *Ca. pseudocolhounii* (av. = 60 × 4.5 µm), while these structures in both species are smaller than those of *Ca. colhounii* (av. = 65 × 5 µm) and *Ca. eucalypti* (av. = 72 × 6 µm). Species in the *Ca. colhounii* complex have been isolated from the *Eucalyptus* trees or soil under these trees in Africa, America and Southeast Asia (Crous 2002, Lombard et al. 2010d). Pathogenicity tests in this study showed that *Ca. pseudocolhounii* and *Ca. fujianensis* are both aggressive pathogens on the *Eucalyptus* clones tested.

Pathogenicity tests in this study showed that all four species of *Calonectria* found in FuJian Province are important pathogens of *Eucalyptus*. *Calonectria pseudocolhounii* and *Ca. fujianensis* were more pathogenic than *Ca. pauciramosa* and *Ca. crousiana*, while *Ca. pauciramosa* was more pathogenic than *Ca. crousiana*. These results also showed that the tolerance of the two tested *Eucalyptus* hybrid clones are significantly different for some of the isolates tested. This implies that it might be possible to select disease tolerant planting stock based on nursery screening.

Leaf and shoot blight associated with *Calonectria* spp. is one of the most serious threats to commercial *Eucalyptus* plantations and nurseries in China (Wang 1992, Sun & Liu 2004, Zhou et al. 2008, Lombard et al. 2010d). Although *Ca. reteaudii* has been regarded as the dominant pathogen responsible for CLB in South America and Southeast Asia (Pikethley 1976, Bolland et al. 1985, Sharma & Mohanan 1991, 1992, Booth et al. 2000, Crous & Kang 2001, Crous 2002, Rodas et al. 2005), no isolates of this fungus were obtained during this study. This could be due to the cooler climatic conditions of the region surveyed, as *Ca. reteaudii* has been only reported from tropical regions (Booth et al. 2000, Crous 2002).

This study has added considerably to the base of knowledge of the species of *Calonectria* and their *Cylindrocladium* anamorphs in China. The discovery of three new species was surprising and this suggests that additional species await discovery in that country. *Calonectria* spp. are well-known to have wide host ranges. Results of this study add substance to the view that those species occurring in the soil below *Eucalyptus* spp., are likely to infect the leaves of these trees, assuming that climatic conditions are favourable for infection. Very little is known regarding the host specificity of these important pathogens but inoculation tests in this study show clearly that different clones respond differently to inoculation by different species of *Calonectria*. This could provide opportunities to tailor planting to avoid damage due to CLB. However, given the large number of *Calonectria* spp. that are now known to occur in China, such complex deployment of clones may not be financially feasible.

Calonectria spp. are important *Eucalyptus* pathogens (Crous 2002, Old et al. 2003, Rodas et al. 2005, Lombard 2010d). The fact that they are soil-borne also contributes to the ease with which they might be moved globally. In this regard, very little is known regarding their origins. Some species with wide global distributions in agricultural and forestry environments, such as *Ca. pauciramosa*, seem very likely to have been moved to new environments. It is difficult to predict how these fungi might respond to new host encounters. However, they add to the growing threats that pathogens pose to *Eucalyptus* plantation forestry (Wingfield et al. 2008) and every effort should be made to avoid their movement.

This study represents an important contribution to the taxonomy of species of *Calonectria*, and highlights the distribution of these pathogens in *Eucalyptus* plantations in China. The first pathogenicity tests using these fungi on *Eucalyptus* clones in this country were also conducted. These results will offer valuable information on the management of *Calonectria* pathogens in *Eucalyptus* plantations, and will advance breeding strategies aimed at developing resistant *Eucalyptus* clones in China.

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