

New species, hyper-diversity and potential importance of *Calonectria* spp. from *Eucalyptus* in South China

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Abstract: Plantation forestry is expanding rapidly in China to meet an increasing demand for wood and pulp products globally. Fungal pathogens including species of *Calonectria* represent a serious threat to the growth and sustainability of this industry. Surveys were conducted in the Guangdong, Guangxi and Hainan Provinces of South China, where *Eucalyptus* trees in plantations or cuttings in nurseries displayed symptoms of leaf blight. Isolations from symptomatic leaves and soils collected close to infected trees resulted in a large collection of *Calonectria* isolates. These isolates were identified using the Consolidated Species Concept, employing morphological characters and DNA sequence comparisons for the β -tubulin, calmodulin, histone H3 and translation elongation factor 1-alpha gene regions. Twenty-one *Calonectria* species were identified of which 18 represented novel taxa. Of these, 12 novel taxa belonged to Sphaero-Naviculate Group and the remaining six to the Prolate Group. Southeast Asia appears to represent a centre of biodiversity for the Sphaero-Naviculate Group and this fact could be one of the important constraints to *Eucalyptus* forestry in China. The remarkable diversity of *Calonectria* species in a relatively small area of China and associated with a single tree species is surprising.

Key words: *Calonectria*, *Cylindrocladium* leaf blight, *Eucalyptus*, Soil, Taxonomy.

Taxonomic novelties: New species: *Calonectria aconidialis* L. Lombard, Crous & S.F. Chen, *C. arbusta* L. Lombard, Crous & S.F. Chen, *C. expansa* L. Lombard, Crous & S.F. Chen, *C. foliicola* L. Lombard, Crous & S.F. Chen, *C. guangxiensis* L. Lombard, Crous & S.F. Chen, *C. hainanensis* L. Lombard, Crous & S.F. Chen, *C. lateralis* L. Lombard, Crous & S.F. Chen, *C. magnispora* L. Lombard, Crous & S.F. Chen, *C. microconidialis* L. Lombard, Crous & S.F. Chen, *C. papillata* L. Lombard, Crous & S.F. Chen, *C. parakyotensis* L. Lombard, Crous & S.F. Chen, *C. pluriramosa* L. Lombard, Crous & S.F. Chen, *C. pseudokyotensis* L. Lombard, Crous & S.F. Chen, *C. seminaria* L. Lombard, Crous & S.F. Chen, *C. sphaeropedunculata* L. Lombard, Crous & S.F. Chen, *C. terrestris* L. Lombard, Crous & S.F. Chen, *C. tetraramosa* L. Lombard, Crous & S.F. Chen, *C. turangicola* L. Lombard, Crous & S.F. Chen.

Published online 23 January 2015; <http://dx.doi.org/10.1016/j.simyco.2014.11.003>. Hard copy: March 2015.

INTRODUCTION

Eucalyptus plantation forestry has grown rapidly during the course of the past two decades in China. This is due to the country being the world's leading consumer of wood products (Turnbull 2007) and a growing global forest products market. In order to service this market, large-scale plantations of fast-growing trees and especially *Eucalyptus* spp. have been established in South and Central China. The area spans 19 provinces (Chen *et al.* 2011a, b, c, d, Zhou & Wingfield 2011) and the aim is to establish 13.3 M ha by 2015 (Turnbull 2007). As is true in other parts of the world, pests and diseases represent a significant challenge to reaching this goal (Zhou *et al.* 2008, Wingfield *et al.* 2010, Wingfield *et al.* 2013).

A recent survey of commercial *Eucalyptus* plantations and nurseries in the Guangdong, Guangxi, Yunnan and Hainan Provinces resulted in the identification of several important *Eucalyptus* pathogens. These included leaf pathogens belonging to the genera *Mycosphaerella* (Burgess *et al.* 2007), *Quambalaria* (Zhou *et al.* 2007) and *Teratosphaeria* (Burgess *et al.* 2006). Stem pathogens found included species of *Botryosphaeriaceae* (Chen *et al.* 2011a), *Celoporthe* (Chen *et al.* 2011b), *Ceratocystis* (Chen *et al.* 2013), *Chrysoporthe* (Chen *et al.* 2010) and *Teratosphaeria* (Chen *et al.* 2011c). In eucalypt nurseries, only isolates belonging to the genus *Calonectria* (as *Cylindrocladium*)

were found and these were shown (Lombard *et al.* 2010d) to represent two novel taxa, *C. cerciana* and *C. pseudoreteauidii*, and the well-known *Eucalyptus* nursery pathogen, *C. pauciramosa* (Koike *et al.* 1999, Polizzi & Crous 1999, Schoch *et al.* 1999, Crous 2002, Lombard *et al.* 2010a, d). A more recent survey of *Eucalyptus* leaves showing symptoms of *Calonectria* Leaf Blight (CLB) in the Fujian Province resulted in the identification of three novel taxa, *C. crousiana*, *C. fujianensis* and *C. pseudocolhounii*, and the first record of *C. pauciramosa* as plantation pathogen (Chen *et al.* 2011d). Pathogenicity test showed that all four *Calonectria* species are aggressive pathogens of two important *Eucalyptus* hybrid clones extensively deployed in plantations (Chen *et al.* 2011d).

The genus *Calonectria* accommodates well-known pathogens of various agricultural, horticultural and forestry crops, worldwide (Crous 2002, Lechat *et al.* 2010, Lombard *et al.* 2010a, b, c, 2011). Diseases associated with these fungi include cutting and root rot, stem cankers as well as leaf and shoot blight (Crous 2002, Lombard *et al.* 2010a, b, 2011). In Asia, several *Calonectria* species have been reported on *Eucalyptus* trees grown in plantations with most species associated with CLB (Sharma *et al.* 1984, Booth *et al.* 2000, Kang *et al.* 2001a, Crous 2002, Old *et al.* 2003, Crous *et al.* 2004b, Chen *et al.* 2011d). Of these species, members of the *C. reteaudii* complex (Lombard *et al.* 2010d) have most frequently been found on *Eucalyptus* trees, especially in

tropical regions of Asia (Booth *et al.* 2000, Kang *et al.* 2001a, b, Crous 2002, Old *et al.* 2003, Lombard *et al.* 2010d).

Studies by Lombard *et al.* (2010d) and Chen *et al.* (2011d) suggested a high level of diversity of *Calonectria* species associated with *Eucalyptus* in plantations and nurseries in Southeast China. The aim of this study was to undertake surveys to further assess the limits of diversity of *Calonectria* in a relatively small area of China associated with *Eucalyptus* plantations.

MATERIALS AND METHODS

Isolates

An extensive survey for *Calonectria* species was conducted in *Eucalyptus* plantations in the Guangdong, Guangxi and Hainan Provinces, China in 2008 and 2009. Where present, leaves of *Eucalyptus* trees showing symptoms were collected in these plantations. In addition, soil samples were collected associated with the symptomatic trees and these baited with germinating *Medicago sativa* (alfalfa) seeds using the technique described by Crous (2002). *Eucalyptus* cuttings showing CLB symptoms were also collected in the nursery of the China Eucalypt Research Centre (CERC) in Guangdong Province.

Plant samples were incubated in moist chambers at room temperature for up to 14 d and inspected daily for fungal structures. Isolations were made directly from these structures onto malt extract agar (2 % w/v; MEA; Biolab, Midrand, South Africa) and incubated for 7 d at 24 °C under continuous near-ultraviolet light. From these primary isolations, single conidial cultures were prepared on MEA and these are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa, the CBS-KNAW Fungal Biodiversity Centre (CBS), Utrecht, The Netherlands, the research collection of P.W. Crous (CPC) maintained at CBS, and the culture collection of CERC, Zhanjiang, Guangdong Province, China.

DNA sequence comparisons

Total genomic DNA was extracted from 7-d-old cultures established from single-conidial propagules, grown on MEA at room temperature, using the UltraClean™ Microbial DNA isolation kit (Mo Bio Laboratories, Inc., California, USA) following the protocols provided by the manufacturer. Partial gene sequences were determined for β -tubulin (*tub2*), calmodulin (*cmdA*), histone H3 (*his3*), and the translation elongation factor 1-alpha (*tef1*) regions using the primers and protocols described by Lombard *et al.* (2010b). To ensure the integrity of the sequences, the amplicons were sequenced in both directions using the same primers used for amplification. Consensus sequences for each locus were assembled in MEGA v. 5.1 (Tamura *et al.* 2011) and compared with representative sequences from Lombard *et al.* (2010b) and Alfenas *et al.* (2015). Subsequent alignments for each locus were generated in MAFFT v. 7.110 (Katoh & Standley 2013) and the ambiguously aligned regions of both ends were truncated.

Phylogenetic analyses were based on both Bayesian inference (BI) and Maximum Parsimony (MP). For BI, the best evolutionary model for each locus was determined using

MrModeltest (Nylander 2004) and incorporated into the analyses. MrBayes v. 3.2.1 (Ronquist & Huelsenbeck 2003) was used to generate phylogenetic trees under optimal criteria for each locus. A Markov Chain Monte Carlo (MCMC) algorithm of four chains was initiated in parallel from a random tree topology with the heating parameter set at 0.3. The MCMC analysis lasted until the average standard deviation of split frequencies was below 0.01 with trees saved every 1 000 generations. The first 25 % of saved trees were discarded as the “burn-in” phase and posterior probabilities (PP) were determined from the remaining trees.

For MP, analyses were done using PAUP (Phylogenetic Analysis Using Parsimony, v. 4.0b10; Swofford 2003) with phylogenetic relationships estimated by heuristic searches with 1 000 random addition sequences. Tree-bisection-reconnection was used, with branch swapping option set on “best trees” only. All characters were weighted equally and alignment gaps treated as fifth state. Measures calculated for parsimony included tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency index (RC). Bootstrap analyses (Hillis & Bull 1993) were based on 1 000 replications.

Phylogenetic analyses were conducted on two separate sequence datasets. Datasets were separated based on morphological characteristics into the Prolate Group and Sphaero-Naviculate Group as defined by Lombard *et al.* (2010b), making it possible to reduce the number of ambiguously aligned regions for the loci analysed. The dataset representing the Prolate Group of species was rooted to *C. hongkongensis* (CBS 114711 & CBS 114828) and the dataset representing the Sphaero-Naviculate Group was rooted to *C. pauciramosa* (CMW 5683 & CMW 30823).

Taxonomy

Axenic cultures were sub-cultured onto synthetic nutrient-poor agar (SNA; Nirenburg 1981) and incubated at room temperature for 7 d. Gross morphological characteristics of the asexual morphs were studied by mounting the structures in 85 % lactic acid and 30 measurements were made at $\times 1\,000$ magnification for all taxonomically informative characters.

Axenic cultures of *Calonectria* species of unknown identity and identified based on DNA sequence analyses were crossed among themselves in all possible combinations. Crosses were made on minimal salt agar (MN) with sterile toothpicks placed on the agar surface as described by Lombard *et al.* (2010b, d). Isolates were crossed with themselves as controls, thus making it possible to distinguish between heterothallic and homothallic mating systems of the isolates. 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 ascospores extruding viable ascospores.

Morphological characteristics of the sexual morphs were studied by mounting ascospores in tissue freezing medium (Leica Biosystems, Nussloch, Germany) and cutting sections with a Leica CM1100 cryostat (Leica Biosystems, Nussloch, Germany). The 10 μm sections were mounted in 85 % lactic acid and 3 % KOH. The 95 % confidence levels were calculated for the conidia and ascospores with extremes provided in parentheses. For all other fungal structures measured, only the extremes are provided. Colony colour was assessed using 7-d-old cultures on MEA incubated at 25 °C and the colour charts of Rayner (1970).

Table 1. *Calonectria* spp. used in phylogenetic analyses.

Species	Isolate nr. ¹	Substrate	Locality	GenBank Accession no. ²			
				<i>tub2</i>	<i>cmdA</i>	<i>his3</i>	<i>tef1</i>
<i>Calonectria aconidialis</i>	CBS 136086; CMW 35174; CERC 1850	Soil in <i>Eucalyptus</i> plantation	Hainan, China	–	KJ463017	KJ463133	KJ462785
	CBS 136091; CMW 35384; CERC1886	Soil in <i>Eucalyptus</i> plantation	Hainan, China	–	–	KJ463134	KJ462786
<i>C. arbusta</i>	CBS 136079; CMW 31370; CERC1705	Soil in <i>Eucalyptus</i> plantation	Guangxi, China	KJ462904	KJ463018	KJ463135	KJ462787
	CBS 136098; CPC 23519; CMW37981; CERC 1944	Soil in <i>Eucalyptus</i> plantation	Guangxi, China	–	KJ463019	KJ463136	KJ462788
	CPC 23481; CMW 31369; CERC1704	Soil in <i>Eucalyptus</i> plantation	Guangxi, China	KJ462905	KJ463020	KJ463137	KJ462789
	CPC 23483; CMW 31371; CERC 1706	Soil in <i>Eucalyptus</i> plantation	Guangxi, China	KJ462906	KJ463021	KJ463138	KJ462790
	CMW 31367; CERC 1702	Soil in <i>Eucalyptus</i> plantation	Fangchenggang, Guangxi, China	KJ462907	KJ463022	KJ463139	KJ462791
	CMW 31368; CERC 1703	Soil in <i>Eucalyptus</i> plantation	Guangxi, China	KJ462908	KJ463023	KJ463140	KJ462792
	<i>C. asiatica</i>	CBS 112711; CPC 3898	Leaf litter	Thailand	AY725613	AY725738	AY725655
CBS 114073; CPC 3900		Leaf litter	Thailand	AY725616	AY725741	AY725658	AY725705
<i>C. brasiliensis</i>	CBS 230.51; CPC 2390	<i>Anacardium</i> sp.	Brazil	GQ267241	GQ267421	GQ267259	GQ267328
	CBS 114257; CPC 1944	<i>Eucalyptus</i> leaf	Brazil	GQ267242	GQ267422	GQ267260	GQ267329
<i>C. brassiana</i>	CBS 134855	Soil	Teresina, Piauí, Brazil	KM395969	KM396056	KM396139	KM395882
	CBS 134856	Soil	Teresina, Piauí, Brazil	KM395970	KM396057	KM396140	KM395883
<i>C. canadania</i>	CBS 110817; CPC 499		Canada	AF348212	AY725743	AF348228	GQ267297
<i>C. candellabra</i>	CPC 1675; CMW 31000	<i>Eucalyptus</i> sp.	Brazil	FJ972426	GQ267367	FJ972476	FJ972525
	CMW 31001	<i>Eucalyptus</i> sp.	Brazil	FJ972427	GQ267368	GQ267246	GQ267246
<i>C. cerciana</i>	CBS 123693; CMW 25309	<i>Eucalyptus</i> cutting	Zhanjiang, China	FJ918510	GQ267369	FJ918528	FJ918559
	CBS 123695; CMW 25290	<i>Eucalyptus</i> cutting	Zhanjiang, China	FJ918511	GQ267370	FJ918529	FJ918560
<i>C. chinensis</i>	CBS 112744; CPC 4104	Soil	Hong Kong, China	AY725618	AY725746	AY725660	AY725709
	CBS 114827; CPC 4101	Soil	Hong Kong, China	AY725619	AY725747	AY725661	AY725710
	CBS 136082; CMW 35367; CERC 1871	Soil in <i>Eucalyptus</i> plantation	Guangdong, China	KJ462909	KJ463024	KJ463141	KJ462793
	CBS 136083; CMW 35179; CERC 1855	Soil in <i>Eucalyptus</i> plantation	Guangdong	KJ462910	KJ463025	KJ463142	KJ462794
	CBS 136088; CMW 35376; CERC 1878	Soil in <i>Eucalyptus</i> plantation	Hainan, China	KJ462911	KJ463026	KJ463143	KJ462795
<i>C. colhounii</i>	CBS 136090; CMW 35379; CERC 1881	Soil in <i>Eucalyptus</i> plantation	Hainan, China	KJ462912	KJ463027	KJ463144	KJ462796
	CBS 293.79	<i>Camellia sinensis</i>	Bandung, Indonesia	DQ190564	GQ267373	DQ190639	GQ267301
<i>C. colombiensis</i>	CBS 114704	<i>Arachis pintoi</i>	Australia	DQ190563	GQ267372	DQ190638	GQ267300
	CBS 112220; CPC 723	Soil	La Selva, Brazil	GQ267207	AY725748	AY725662	AY725711
<i>C. crousiana</i>	CBS 112221; CPC 724	<i>Eucalyptus grandis</i>	La Selva, Brazil	AY725620	AY725749	AY725663	AY725712
	CBS 127198; CMW 27249	<i>E. grandis</i>	Fujian, China	HQ285794	–	HQ285808	HQ285822
<i>C. curvispora</i>	CBS 127199; CMW 27253	<i>E. grandis</i>	Fujian, China	HQ285795	–	HQ285809	HQ285823
	CBS 116159; CPC 765	Soil	Tamatave, Madagascar	AF333394	GQ267374	AY725664	GQ267302
<i>C. cylindrospora</i>	CBS 110666; CPC 496		USA	FJ918509	GQ267423	FJ918527	FJ918557
	CBS 119670; CPC 12766	<i>Pistacia lentiscus</i>	Italy	DQ521600	–	DQ521602	GQ421797
<i>C. eucalypticola</i>	CBS 134846	<i>Eucalyptus</i> leaf	Eunápolis, Bahia, Brazil	KM395963	KM396050	KM396133	KM395876
	CBS 134847	<i>Eucalyptus</i> seedling	Santa Bárbara, Minas Gerais, Brazil	KM395964	KM396051	KM396134	KM395877
<i>C. expansa</i>	CBS 136078; CMW 31441; CERC 1776	Soil in <i>Eucalyptus</i> plantation	Guangdong, China	KJ462913	KJ463028	KJ463145	KJ462797

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Table 1. (Continued).

Species	Isolate nr. ¹	Substrate	Locality	GenBank Accession no. ²			
				<i>tub2</i>	<i>cmdA</i>	<i>his3</i>	<i>tef1</i>
<i>C. foliicola</i>	CBS 136247; CMW 31392; CERC 1727	Soil in <i>Eucalyptus</i> plantation	Guangxi, China	KJ462914	KJ463029	KJ463146	KJ462798
	CMW 31413; CERC 1748	Soil in <i>Eucalyptus</i> plantation	Guangxi, China	KJ462915	KJ463030	KJ463147	KJ462799
	CBS 136641; CMW 31393; CERC 1728	<i>E. urophylla</i> × <i>E. grandis</i> clone leaf	Guangxi, China	KJ462916	KJ463031	KJ463148	KJ462800
	CMW 31394; CERC 1729	<i>E. urophylla</i> × <i>E. grandis</i> clone leaf	Guangxi, China	KJ462917	KJ463032	KJ463149	KJ462801
	CMW 31395; CERC 1730	<i>E. urophylla</i> × <i>E. grandis</i> clone leaf	Guangxi, China	KJ462918	KJ463033	KJ463150	KJ462802
<i>C. fujianensis</i>	CBS 127200; CMW 27254	<i>E. grandis</i>	Fujian, China	HQ285791	–	HQ285805	HQ285819
	CBS 127201; CMW 27257	<i>E. grandis</i>	Fujian, China	HQ285792	–	HQ285806	HQ285820
<i>C. glaeboicola</i>	CBS 134852	Soil	Martinho Campos, Minas Gerais, Brazil	KM395966	KM396053	KM396136	KM395879
	CBS 134853	Soil	Bico do Papagaio, Tocantins, Brazil	KM395967	KM396054	KM396137	KM395880
<i>C. guangxiensis</i>	CBS 136092; CMW 35409; CERC 1900	Soil in <i>Eucalyptus</i> plantation	Guangxi, China	KJ462919	KJ463034	KJ463151	KJ462803
	CBS 136094; CMW 35411; CERC 1902	Soil in <i>Eucalyptus</i> plantation	Guangxi, China	KJ462920	KJ463035	–	KJ462804
<i>C. hainanensis</i>	CBS 136248; CMW 35187; CERC 1863	Soil in <i>Eucalyptus</i> plantation	Hainan, China	–	KJ463036	KJ463152	KJ462805
<i>C. hawksworthii</i>	CBS 111870; CPC 2405; MUCL 30866	<i>Nelumbo nucifera</i>	Mauritius	AF333407	GQ267386	DQ190649	FJ918558
<i>C. hodgesii</i>	CBS 133609; LPF 245	<i>Anadenanthera peregrina</i>	Viçosa, Brazil	KC491228	KC491222	–	KC491225
	CBS 133610; LPF 261	<i>Azadirachta indica</i>	Viçosa, Brazil	KC491229	KC491223	–	KC491226
<i>C. hongkongensis</i>	CBS 114711; CPC 686	Soil	Hong Kong, China	AY725621	AY725754	AY725666	AY725716
	CBS 114828; CPC 4670	Soil	Hong Kong, China	AY725622	AY725755	AY725667	AY725717
	CBS 136080; CMW 31443; CERC 1778	Soil in <i>Eucalyptus</i> plantation	Fangchenggang, Guangxi, China	KJ462921	KJ463037	KJ463153	KJ462806
	CBS 136246; CMW 31374; CERC 1709	Soil in <i>Eucalyptus</i> plantation	Fangchenggang, Guangxi, China	KJ462922	KJ463038	KJ463154	KJ462807
	CPC 23478; CMW 31438; CERC 1773	Soil in <i>Eucalyptus</i> plantation	Shiling, Zhanjiang, Guangdong, China	KJ462923	KJ463039	KJ463155	KJ462808
	CPC 23480; CMW 31414; CERC 1749	Soil in <i>Eucalyptus</i> plantation	Fangchenggang, Guangxi, China	KJ462924	KJ463040	KJ463156	KJ462809
	CPC 23499; CMW 35175; CERC 1851	Soil in <i>Eucalyptus</i> plantation	Hainan, China	KJ462925	KJ463041	KJ463157	KJ462810
	CPC 23877; CERC 1932	Soil in <i>Eucalyptus</i> plantation	Hainan, China	KJ462926	KJ463042	KJ463158	KJ462811
	CPC 23878; CMW 37973; CERC 1936	Soil in <i>Eucalyptus</i> plantation	Guangdong, China	KJ462927	KJ463043	KJ463159	KJ462812
	CMW 31375; CERC 1710	Soil in <i>Eucalyptus</i> plantation	Fangchenggang, Guangxi, China	KJ462928	KJ463044	KJ463160	KJ462813
	CMW 31377; CERC 1712	Soil in <i>Eucalyptus</i> plantation	Fangchenggang, Guangxi, China	KJ462929	KJ463045	KJ463161	KJ462814
	CMW 31382; CERC 1717	Soil in <i>Eucalyptus</i> plantation	Fangchenggang, Guangxi, China	KJ462930	KJ463046	KJ463162	KJ462815
	CMW 31383; CERC 1718	Soil in <i>Eucalyptus</i> plantation	Fangchenggang, Guangxi, China	KJ462931	KJ463047	KJ463163	KJ462816
	CMW 31384; CERC 1719	Soil in <i>Eucalyptus</i> plantation	Fangchenggang, Guangxi, China	KJ462932	KJ463048	KJ463164	KJ462817
	CMW 31385; CERC 1720	Soil in <i>Eucalyptus</i> plantation	Fangchenggang, Guangxi, China	KJ462933	KJ463049	KJ463165	KJ462818
CMW 31387; CERC 1722	Soil in <i>Eucalyptus</i> plantation	Fangchenggang, Guangxi, China	KJ462934	KJ463050	KJ463166	KJ462819	

Table 1. (Continued).

Species	Isolate nr. ¹	Substrate	Locality	GenBank Accession no. ²			
				<i>tub2</i>	<i>cmdA</i>	<i>his3</i>	<i>tef1</i>
	CMW 31388; CERC 1723	Soil in <i>Eucalyptus</i> plantation	Fangchenggang, Guangxi, China	KJ462935	KJ463051	KJ463167	KJ462820
	CMW 31399; CERC 1734	Soil in <i>Eucalyptus</i> plantation	Shiling, Zhanjiang, Guangdong, China	KJ462936	–	KJ463168	KJ462821
	CMW 31400; CERC 1735	Soil in <i>Eucalyptus</i> plantation	Shiling, Zhanjiang, Guangdong, China	KJ462937	KJ463052	KJ463169	KJ462822
	CMW 31401; CERC 1736	Soil in <i>Eucalyptus</i> plantation	Shiling, Zhanjiang, Guangdong, China	KJ462938	KJ463053	KJ463170	KJ462823
	CMW 31404; CERC1739	Soil in <i>Eucalyptus</i> plantation	Shiling, Zhanjiang, Guangdong, China	KJ462939	KJ463054	KJ463171	KJ462824
	CMW 31432; CERC1767	Soil in <i>Eucalyptus</i> plantation	Shiling, Zhanjiang, Guangdong, China	KJ462940	KJ463055	KJ463172	KJ462825
	CMW 31433; CERC1768	Soil in <i>Eucalyptus</i> plantation	Shiling, Zhanjiang, Guangdong, China	KJ462941	KJ463056	KJ463173	KJ462826
	CMW 31434; CERC1769	Soil in <i>Eucalyptus</i> plantation	Shiling, Zhanjiang, Guangdong, China	KJ462942	KJ463057	KJ463174	KJ462827
	CMW 31442; CERC1777	Soil in <i>Eucalyptus</i> plantation	Fangchenggang, Guangxi, China	KJ462943	KJ463058	KJ463175	KJ462828
	CMW 35186; CERC1862	Soil in <i>Eucalyptus</i> plantation	Hainan, China	KJ462944	KJ463059	KJ463176	KJ462829
	CMW 35188; CERC1864	Soil in <i>Eucalyptus</i> plantation	Hainan, China	KJ462945	KJ463060	KJ463177	KJ462830
	CMW 35190; CERC1865	Soil in <i>Eucalyptus</i> plantation	Guangxi, China	KJ462946	KJ463061	KJ463178	KJ462831
	CMW 35192; CERC1867	Soil in <i>Eucalyptus</i> plantation	Guangxi, China	KJ462947	KJ463062	–	KJ462832
	CMW 35371; CERC1874	Soil in <i>Eucalyptus</i> plantation	Guangdong, China	KJ462948	KJ463063	KJ463179	KJ462833
	CMW 35378; CERC1880	Soil in <i>Eucalyptus</i> plantation	Hainan, China	KJ462949	KJ463064	KJ463180	KJ462834
	CMW 35381; CERC1883	Soil in <i>Eucalyptus</i> plantation	Hainan, China	KJ462950	KJ463065	KJ463181	KJ462835
	CMW 35401; CERC1892	Soil in <i>Eucalyptus</i> plantation	Guangxi, China	KJ462951	KJ463066	KJ463182	KJ462836
	CMW 35404; CERC1895	Soil in <i>Eucalyptus</i> plantation	Guangxi, China	KJ462952	KJ463067	KJ463183	KJ462837
	CMW 35414; CERC1905	Soil in <i>Eucalyptus</i> plantation	Guangxi, China	KJ462953	KJ463068	KJ463184	KJ462838
	CMW 36270; CERC1928	Soil in <i>Eucalyptus</i> plantation	Hainan, China	KJ462954	KJ463069	KJ463185	KJ462839
<i>C. illicicola</i>	CBS 190.50 ; CMW 30998; IMI 299389	<i>Solanum tuberosum</i>	Bogor, Indonesia	AY725631	AY725764	AY725676	AY725726
	CBS 115897; CPC 493; UFV 108	<i>Anacardium</i> sp.	Brazil	AY725647	GQ267403	GQ267256	AY725729
<i>C. indonesiae</i>	CBS 112823 ; CPC 4508	Soil	Warambunga, Indonesia	AY725623	AY725756	AY725668	AY725718
	CBS 112840; CPC 4554	<i>Syzygium aromaticum</i>	Indonesia	AY725625	AY725758	AY725670	AY725720
<i>C. insularis</i>	CBS 114558 ; CPC 768	Soil	Tamatave, Madagascar	AF210861	GQ267389	FJ918526	FJ918556
	CBS 114559; CPC 954	Soil	Tamatave, Madagascar	AF210862	GQ267390	FJ918525	FJ918555
<i>C. kyotensis</i>	CBS 413.67; CPC 2391; IMI 299577	<i>Paphiopedilum callosum</i>	Celle, Germany	GQ267208	GQ267379	GQ267248	GQ267307
	CBS 170.77; IMI 299388	<i>Idesia polycarpa</i>	Auckland, New Zealand	GQ267209	GQ267380	GQ267249	GQ267308
<i>C. lateralis</i>	CBS 136629 ; CMW 31412; CERC 1747	Soil in <i>Eucalyptus</i> plantation	Fangchenggang, Guangxi, China	KJ462955	KJ463070	KJ463186	KJ462840
<i>C. leucothoes</i>	CBS 109166 ; CPC 2385; ATCC 64824	<i>Leucothoe axillaris</i>	Gainesville, Florida, USA	FJ918508	GQ267392	FJ918523	FJ918553
<i>C. magnispora</i>	CBS 136249 ; CMW 35184; CERC 1860	Soil in <i>Eucalyptus</i> plantation	Guangxi, China	KJ462956	KJ463071	KJ463187	KJ462841
<i>C. malesiana</i>	CBS 112710; CPC 3899	Leaf litter	Thailand	AY725626	AY725759	AY725671	AY725721
	CBS 112752 ; CPC 4223	Soil	Sumatra, Indonesia	AY725627	AY725760	AY725672	AY725722
<i>C. maranhensis</i>	CBS 134811	<i>Eucalyptus</i> sp.	Açailândia, Maranhão, Brazil	KM395948	KM396035	KM396118	KM395861
	CBS 134812	<i>Eucalyptus</i> sp.	Açailândia, Maranhão, Brazil	KM395949	KM396036	KM396119	KM395862

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Table 1. (Continued).

Species	Isolate nr. ¹	Substrate	Locality	GenBank Accession no. ²			
				<i>tub2</i>	<i>cmdA</i>	<i>his3</i>	<i>tef1</i>
	CBS 134858	Soil	Urbano Santos, Maranhão, Brazil	KM395951	KM396038	KM396121	KM395864
	CBS 134829	Soil	Urbano Santos, Maranhão, Brazil	KM395952	KM396039	KM396122	KM395865
<i>C. metrosideri</i>	CBS 133604 ; LPF 103	<i>Metrosideros polymorpha</i>	Viçosa, Brazil	KC294314	KC294305	KC294308	KC294311
	CBS 133605; LPF 104	<i>M. polymorpha</i>	Viçosa, Brazil	KC294315	KC294306	KC294309	KC294312
<i>C. microconidialis</i>	CBS 136633; CMW 31471; CERC 1806	<i>E. urophylla</i> × <i>E. grandis</i> clone seedling leaf	CERC Nursery, Zhanjiang, Guangdong, China	KJ462957	KJ463072	KJ463188	KJ462842
	CBS 136634; CMW 31473; CERC 1808	<i>E. urophylla</i> × <i>E. grandis</i> clone seedling leaf	CERC Nursery, Zhanjiang, Guangdong, China	KJ462958	KJ463073	KJ463189	KJ462843
	CBS 136636; CMW 31475; CERC 1810	<i>E. urophylla</i> × <i>E. grandis</i> clone seedling leaf	CERC Nursery, Zhanjiang, Guangdong, China	KJ462959	KJ463074	KJ463190	KJ462844
	CBS 136638 ; CMW 31487; CERC 1822	<i>E. urophylla</i> × <i>E. grandis</i> clone seedling leaf	CERC Nursery, Zhanjiang, Guangdong, China	KJ462960	KJ463075	KJ463191	KJ462845
	CBS 136640; CMW 31492; CERC 1827	<i>E. urophylla</i> × <i>E. grandis</i> clone seedling leaf	CERC Nursery, Zhanjiang, Guangdong, China	KJ462961	KJ463076	KJ463192	KJ462846
<i>C. nemuricola</i>	CBS 134837	Soil	Araponga, Minas Gerais, Brazil	KM395979	KM396066	KM396149	KM395892
	CBS 134838	Soil	Araponga, Minas Gerais, Brazil	KM395980	KM396067	KM396150	KM395893
<i>C. nymphaeae</i>	CBS 131802 ; HGUP 100003	<i>Nymphaea tetragona</i>	Guizhou, China	JN984864	–	–	KC555273
<i>C. pacifica</i>	CBS 109063 ; CPC 2534; IMI 354528	<i>Araucaria heterophylla</i>	Hawaii, USA	GQ267213	AY725762	GQ267255	AY725724
	CBS 114038; CPC 10717	<i>Ipomoea aquatica</i>	Auckland, New Zealand	AY725630	GQ267402	AY725675	GQ267320
<i>C. papillata</i>	CBS 136084; CMW 35165; CERC 1841	Soil in <i>Eucalyptus</i> plantation	Guangdong, China	KJ462962	KJ463077	KJ463193	KJ462847
	CBS 136096; CMW 37972; CERC 1935	Soil in <i>Eucalyptus</i> plantation	Guangdong, China	KJ462963	KJ463078	KJ463194	KJ462848
	CBS 136097 ; CMW 37976; CERC 1939	Soil in <i>Eucalyptus</i> plantation	Guangdong, China	KJ462964	KJ463079	KJ463195	KJ462849
	CBS 136251; CMW 37971; CERC 1934	Soil in <i>Eucalyptus</i> plantation	Guangxi, China	KJ462965	KJ463080	KJ463196	KJ462850
<i>C. parakyotensis</i>	CBS 136085 ; CMW 35169; CERC 1845	Soil in <i>Eucalyptus</i> plantation	Guangdong, China	–	KJ463081	KJ463197	KJ462851
	CBS 136095; CMW 35413; CERC 1904	Soil in <i>Eucalyptus</i> plantation	Guangxi, China	–	KJ463082	KJ463198	KJ462852
<i>C. pauciramosa</i>	CMW 5683	<i>E. grandis</i>	South Africa	FJ918514	GQ267405	FJ918531	FJ918565
	CMW 30823	<i>E. grandis</i>	South Africa	FJ918515	GQ280404	FJ918532	FJ918566
<i>C. pentaseptata</i>	CBS 133349	<i>Eucalyptus</i> hybrid	Bavi, Hanoi, Vietnam	JX855942	–	JX855946	JX855958
	CBS 133351	<i>Macadamia</i> sp.	Bavi, Hanoi, Vietnam	JX855944	–	JX855948	JX855960
	CBS 136087; CMW 35177; CERC 1853	<i>Eucalyptus</i> leaf	Hainan, China	KJ462966	KJ463083	KJ463199	KJ462853
	CBS 136089; CMW 35377; CERC 1879	<i>Eucalyptus</i> leaf	Hainan, China	KJ462967	KJ463084	KJ463200	KJ462854
	CBS 136250; CMW 35451; CERC 1923	<i>Eucalyptus</i> leaf	Guangdong, China	KJ462968	KJ463085	KJ463201	KJ462855
	CBS 136646; CMW 35436; CERC 1908	<i>Eucalyptus</i> leaf	Guangdong, China	KJ462969	KJ463086	KJ463202	KJ462856
	CMW 31332; CERC 1667	<i>Eucalyptus</i> clone U6 leaf	Shiling, Zhanjiang, Guangdong, China	KJ462970	KJ463087	KJ463203	KJ462857
	CMW 31333; CERC 1668	<i>Eucalyptus</i> clone U6 leaf	Shiling, Zhanjiang, Guangdong, China	KJ462971	KJ463088	KJ463204	KJ462858
	CMW 31336; CERC 1671	<i>Eucalyptus</i> clone U6 leaf	Shiling, Zhanjiang, Guangdong, China	KJ462972	KJ463089	KJ463205	KJ462859

Table 1. (Continued).

Species	Isolate nr. ¹	Substrate	Locality	GenBank Accession no. ²			
				tub2	cmdA	his3	tef1
	CMW 31340; CERC 1675	<i>E. urophylla</i> × <i>E. grandis</i> clone seedling leaf	CERC Nursery, Zhanjiang, Guangdong, China	KJ462973	KJ463090	KJ463206	KJ462860
	CMW 31343; CERC 1678	<i>E. urophylla</i> × <i>E. grandis</i> clone seedling leaf	CERC Nursery, Zhanjiang, Guangdong, China	KJ462974	KJ463091	KJ463207	KJ462861
	CMW 31344; CERC 1679	<i>E. urophylla</i> × <i>E. grandis</i> clone seedling leaf	CERC Nursery, Zhanjiang, Guangdong, China	KJ462975	KJ463092	KJ463208	KJ462862
	CMW 31345; CERC 1680	<i>E. urophylla</i> × <i>E. grandis</i> clone seedling leaf	CERC Nursery, Zhanjiang, Guangdong, China	KJ462976	KJ463093	KJ463209	KJ462863
	CMW 31346; CERC 1681	<i>E. urophylla</i> × <i>E. grandis</i> clone seedling leaf	CERC Nursery, Zhanjiang, Guangdong, China	KJ462977	KJ463094	KJ463210	KJ462864
	CMW 31347; CERC 1682	<i>E. urophylla</i> × <i>E. grandis</i> clone seedling leaf	CERC Nursery, Zhanjiang, Guangdong, China	KJ462978	KJ463095	KJ463211	KJ462865
	CMW 31348; CERC 1683	<i>E. urophylla</i> × <i>E. grandis</i> clone seedling leaf	CERC Nursery, Zhanjiang, Guangdong, China	KJ462979	KJ463096	KJ463212	KJ462866
	CMW 31355; CERC 1690	<i>E. urophylla</i> × <i>E. grandis</i> leaf	Hepu, Guangxi, China	KJ462980	KJ463097	KJ463213	KJ462867
	CMW 31356; CERC 1691	<i>E. urophylla</i> × <i>E. grandis</i> leaf	Hepu, Guangxi, China	KJ462981	KJ463098	KJ463214	KJ462868
	CMW 31357; CERC 1692	<i>E. urophylla</i> × <i>E. grandis</i> leaf	Hepu, Guangxi, China	KJ462982	KJ463099	KJ463215	KJ462869
	CMW 31358; CERC 1693	<i>E. urophylla</i> × <i>E. grandis</i> leaf	Hepu, Guangxi, China	KJ462983	KJ463100	KJ463216	KJ462870
	CMW 31359; CERC 1694	<i>E. urophylla</i> × <i>E. grandis</i> leaf	Hepu, Guangxi, China	KJ462984	KJ463101	KJ463217	KJ462871
	CMW 31363; CERC 1698	<i>E. urophylla</i> × <i>E. grandis</i> leaf	Hepu, Guangxi, China	KJ462985	KJ463102	KJ463218	KJ462872
	CMW 31422; CERC 1757	<i>Eucalyptus</i> clone U6 leaf	Shiling, Zhanjiang, Guangdong, China	KJ462986	KJ463103	KJ463219	KJ462873
	CMW 31497; CERC 1832	<i>E. urophylla</i> × <i>E. grandis</i> clone seedling leaf	CERC Nursery, Zhanjiang, Guangdong, China	KJ462987	KJ463104	KJ463220	KJ462874
	CMW 35385; CERC 1887	Soil in <i>Eucalyptus</i> plantation	Hainan, China	KJ462988	KJ463105	KJ463221	KJ462875
	CMW 35437; CERC 1909	<i>Eucalyptus</i> leaf	Guangdong, China	KJ462989	KJ463106	KJ463222	KJ462876
	CMW 35442; CERC 1914	<i>Eucalyptus</i> leaf	Guangdong, China	KJ462990	KJ463107	KJ463223	KJ462877
	CMW 35452; CERC 1924	<i>Eucalyptus</i> leaf	Guangdong, China	KJ462991	KJ463108	KJ463224	KJ462878
	CMW 35453; CERC 1925	<i>Eucalyptus</i> leaf	Guangdong, China	KJ462992	KJ463109	KJ463225	KJ462879
	CMW 35454; CERC 1926	<i>Eucalyptus</i> leaf	Guangdong, China	KJ462993	KJ463110	KJ463226	KJ462880
<i>C. piauiensis</i>	CBS 134850	Soil	Teresina, Piauí, Brazil	KM395973	KM396060	KM396143	KM395886
	CBS 134851	Soil	Teresina, Piauí, Brazil	KM395974	KM396061	KM396144	KM395887
<i>C. pluriramosa</i>	CBS 136976 ; CMW 31440; CERC 1775	Soil in <i>Eucalyptus</i> plantation	Fangchenggang, Guangxi, China	KJ462995	KJ463112	KJ463228	KJ462882
<i>C. polizzi</i>	CBS 125270; CMW 7804	<i>Callistemon citrinus</i>	Messina, Sicily, Italy	FJ972417	GQ267461	FJ972436	FJ972486
	CBS 125271; CMW 10151	<i>Arbustus unedo</i>	Catania, Sicily, Italy	FJ972418	GQ267462	FJ972437	FJ972487
<i>C. propaginicola</i>	CBS 134815	<i>Eucalyptus</i> cutting	Santana, Pará, Brazil	KM395953	KM396040	KM396123	KM395866
	CBS 134820	Used planting substrate	Santana, Pará, Brazil	KM395956	KM396043	KM396126	KM395869
	CBS 134821	Used planting substrate	Santana, Pará, Brazil	KM395957	KM396044	KM396127	KM395870
<i>C. pseudocerciana</i>	CBS 134824	<i>Eucalyptus</i> seedling	Santana, Pará, Brazil	KM395962	KM396049	KM396132	KM395875
<i>C. pseudocolhounii</i>	CBS 127195 ; CMW 27209	<i>E. dunnii</i>	Fujian, China	HQ285788	–	HQ285802	HQ285816
	CBS 127196; CMW 27213	<i>E. dunnii</i>	Fujian, China	HQ285789	–	HQ285803	HQ285817
<i>C. pseudohodgesii</i>	CBS 134818	<i>Azadirachta indica</i>	Viçosa, Minas Gerais, Brazil	KM395905	KM395991	KM396079	KM395817
	CBS 134819	<i>A. indica</i>	Viçosa, Minas Gerais, Brazil	KM395906	KM395992	KM396080	KM395818
<i>C. pseudokytensis</i>	CBS 137332 ; CMW 31439; CERC 1774	Soil in <i>Eucalyptus</i> plantation	Fangchenggang, Guangxi, China	KJ462994	KJ463111	KJ463227	KJ462881
<i>C. pseudometrosideri</i>	CBS 134843	Soil	Viçosa, Minas Gerais, Brazil	KM395907	KM395993	KM396081	KM395819
	CBS 134845	Soil	Maceió, Alagoas, Brazil	KM395909	KM395995	KM396083	KM395821

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Table 1. (Continued).

Species	Isolate nr. ¹	Substrate	Locality	GenBank Accession no. ²			
				<i>tub2</i>	<i>cmdA</i>	<i>his3</i>	<i>tef1</i>
<i>C. pseudoreteaudii</i>	CBS 123694; CMW 25310	<i>Eucalyptus</i> hybrid cutting	Guangdong, China	FJ918504	GQ267411	FJ918519	FJ918541
	CBS 123696; CMW 25292	<i>Eucalyptus</i> hybrid cutting	Guangdong, China	FJ918505	GQ267410	FJ918520	FJ918542
<i>C. pseudoscoparia</i>	CBS 125256; CMW 15216	<i>E. grandis</i>	Pichincha, Ecuador	GQ267228	GQ267440	GQ267277	GQ267348
	CBS 125257; CMW 15218	<i>E. grandis</i>	Pichincha, Ecuador	GQ267229	GQ267441	GQ267278	GQ267349
<i>C. pseudospathulata</i>	CBS 134840	Soil	Araponga, Minas Gerais, Brazil	KM395982	KM396069	KM396152	KM395895
	CBS 134841	Soil	Araponga, Minas Gerais, Brazil	KM395983	KM396070	KM396153	KM395896
<i>C. queenslandica</i>	CBS 112146 ; CPC 3213	<i>E. urophylla</i>	Australia	AF389835	GQ267415	FJ918521	FJ918543
	CBS 112155; CPC 3210	<i>E. pellita</i>	Australia	AF389834	GQ267416	DQ190667	FJ918544
<i>C. reteaudii</i>	CBS 112143 ; CPC 3200	<i>E. camaldulensis</i>	Vietnam	GQ240642	GQ267418	DQ190660	FJ918536
	CBS 112144; CPC 3201	<i>E. camaldulensis</i>	Vietnam	AF389833	GQ267417	DQ190661	FJ918537
<i>C. seminaria</i>	CBS 136630; CMW 31446; CERC 1781	<i>E. urophylla</i> × <i>E. grandis</i> clone seedling leaf	CERC Nursery, Zhanjiang, Guangdong, China	KJ462996	KJ463113	KJ463229	KJ462883
	CBS 136631; CMW 31449; CERC 1784	<i>E. urophylla</i> × <i>E. grandis</i> clone seedling leaf	CERC Nursery, Zhanjiang, Guangdong, China	KJ462997	KJ463114	KJ463230	KJ462884
	CBS 136632 ; CMW 31450; CERC 1785	<i>E. urophylla</i> × <i>E. grandis</i> clone seedling leaf	CERC Nursery, Zhanjiang, Guangdong, China	KJ462998	KJ463115	KJ463231	KJ462885
	CBS 136639; CMW 31489; CERC 1824	<i>E. urophylla</i> × <i>E. grandis</i> clone seedling leaf	CERC Nursery, Zhanjiang, Guangdong, China	KJ462999	KJ463116	KJ463232	KJ462886
	CBS 136648; CMW 37970; CERC 1933	<i>Eucalyptus</i> leaf	Guangxi, China	KJ463000	KJ463117	KJ463233	KJ462887
	CPC 23486; CMW 31447; CERC 1782	<i>E. urophylla</i> × <i>E. grandis</i> clone seedling leaf	CERC Nursery, Zhanjiang, Guangdong, China	KJ463001	KJ463118	KJ463234	KJ462888
	CPC 23487; CMW 31448; CERC 1783	<i>E. urophylla</i> × <i>E. grandis</i> clone seedling leaf	CERC Nursery, Zhanjiang, Guangdong, China	KJ463002	KJ463119	KJ463235	KJ462889
<i>C. silvicola</i>	CBS 134836	Soil	Araponga, Minas Gerais, Brazil	KM395975	KM396062	KM396145	KM395888
	CBS 135237	Soil	Araponga, Minas Gerais, Brazil	KM395978	KM396065	KM396148	KM395891
<i>C. sphaeropedunculata</i>	CBS 136081 ; CMW 31390; CERC 1725	Soil in <i>Eucalyptus</i> plantation	Guangxi, China	KJ463003	KJ463120	KJ463236	KJ462890
<i>C. sulawesiensis</i>	CBS 125248; CMW 14857	<i>Eucalyptus</i> sp.	Sulawesi, Indonesia	GQ267223	GQ267435	GQ267272	GQ267343
	CBS 125253; CMW 14879	<i>Eucalyptus</i> sp.	Sulawesi, Indonesia	GQ267220	GQ267432	GQ267269	GQ267340
<i>C. sumatrensis</i>	CBS 112829 ; CPC 4518	Soil	Sumatra, Indonesia	AY725649	AY725771	AY725696	AY725733
	CBS 112934; CPC 4516	Soil	Indonesia	AY725651	AY725773	AY725798	AY725735
<i>C. terrae-reginae</i>	CBS 112151 ; CPC 3202	<i>E. urophylla</i>	Queensland, Australia	FJ918506	GQ267451	FJ918522	FJ918545
	CBS 112634; CPC 4233	<i>Xanthorrhoea australis</i>	Victoria, Australia	FJ918507	GQ267452	DQ190668	FJ918546
<i>C. terrestris</i>	CBS 136642 ; CMW 35180; CERC 1856	Soil in <i>Eucalyptus</i> plantation	Guangdong, China	KJ463004	KJ463121	KJ463237	KJ462891
	CBS 136643; CMW 35364; CERC 1868	Soil in <i>Eucalyptus</i> plantation	Guangdong, China	KJ463005	KJ463122	KJ463238	KJ462892
	CBS 136644; CMW 35366; CERC 1870	Soil in <i>Eucalyptus</i> plantation	Guangdong, China	KJ463006	KJ463123	KJ463239	KJ462893
	CBS 136645; CMW 35178; CERC 1854	Soil in <i>Eucalyptus</i> plantation	Guangdong, China	KJ463007	KJ463124	KJ463240	KJ462894
	CBS 136647; CMW 35447; CERC 1919	<i>Eucalyptus</i> leaf	Guangdong, China	KJ463008	KJ463125	KJ463241	KJ462895
	CBS 136651; CMW 37974; CERC 1937	Soil	Guangdong, China	KJ463009	KJ463126	KJ463242	KJ462896
	CBS 136653; CMW 37980; CERC 1943	Soil	Guangxi, China	KJ463010	KJ463127	KJ463243	KJ462897

Table 1. (Continued).

Species	Isolate nr. ¹	Substrate	Locality	GenBank Accession no. ²			
				<i>tub2</i>	<i>cmdA</i>	<i>his3</i>	<i>tef1</i>
<i>C. tetraramosa</i>	CBS 136635; CMW 31474; CERC 1809	<i>E. urophylla</i> × <i>E. grandis</i> clone seedling leaf	CERC Nursery, Zhanjiang, Guangdong, China	KJ463011	KJ463128	KJ463244	KJ462898
	CBS 136637; CMW 31476; CERC 1811	<i>E. urophylla</i> × <i>E. grandis</i> clone seedling leaf	CERC Nursery, Zhanjiang, Guangdong, China	KJ463012	KJ463129	KJ463245	KJ462899
<i>C. turangicola</i>	CBS 136077; CMW 31411; CERC 1746	Soil in <i>Eucalyptus</i> plantation	Fangchenggang, Guangxi, China	KJ463013	–	KJ463246	KJ462900
	CBS 136093; CMW 35410; CERC 1901	Soil in <i>Eucalyptus</i> plantation	Guangxi, China	KJ463014	KJ463130	KJ463247	KJ462901
	CBS 136652; CMW 37977; CERC 1940 CMW 35383; CERC 1885	Soil Soil in <i>Eucalyptus</i> plantation	Guangxi, China Hainan, China	KJ463015 KJ463016	KJ463131 KJ463132	KJ463248 KJ463249	KJ462902 KJ462903

¹ ATCC: American Type Culture Collection, Virginia, USA; CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CERC: China Eucalypt Research Centre, Zhanjiang, Guangdong Province, China; CMW: culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; CPC: Pedro Crous working collection housed at CBS; HGUP: Plant Pathology Herbarium of Guizhou University, Guiyang 550025, China; IMI: International Mycological Institute, CABI-Bioscience, Egham, Boreham Lane, UK; LPF: Laboratório de Patologia Florestal, Universidade Federal de Viçosa, Viçosa, Brazil; MUC: Mycothèque, Laboratoire de Mycologie Systématique et Appliquée, l'Université, Louvain-la-Neuve, Belgium; UVF: Universidade Federal de Viçosa, Viçosa, Brazil. Isolates obtained during the survey indicated in grey blocks.

² *tub2* = β -tubulin, *cmdA* = calmodulin, *his3* = histone H3, *tef1* = translation elongation factor 1-alpha. Ex-type isolates indicated in **bold**. Sequences generated in this study indicated in *italics*.

All descriptions, illustrations and nomenclatural data were deposited in MycoBank (Crous *et al.* 2004a).

RESULTS

Isolates

A total of 278 isolates were collected of which 162 were from the Guangdong Province (44 isolates from soil; 45 isolates from *Eucalyptus* leaves on trees; 73 from cuttings in a single nursery), 87 isolates from Guangxi Province (63 from soil; 24 from *Eucalyptus* leaves in plantations), and 29 isolates from the Hainan Province (27 from soil; two from *Eucalyptus* leaves in plantations). One hundred and twenty of these isolates were selected for further study (Table 1) based on preliminary phylogenetic analysis of the *cmdA* and *tub2* gene region sequences (results not shown).

DNA sequence comparisons

Approximately 500–550 bases were determined for the four gene regions used in this study. For the Bayesian analyses, a HKY+I+G model was selected for *cmdA*, *tef1* and *tub2* and the GTR+I+G model for *his3*. These models were incorporated for each of the datasets analysed. The Bayesian consensus trees for both datasets confirmed the tree topologies obtained from the MP analyses, and therefore, only the MP trees are presented with bootstrap support values (BS) and posterior probabilities (PP) shown for well-supported nodes.

The dataset for the Prolate Group isolates included 127 ingroup taxa, with *C. hongkongensis* (CBS 114711 & CBS 114828) as the outgroup taxon. The sequence dataset consisted of 2018 characters, including alignment gaps. Of these, 1308 were constant, 73 were parsimony-uninformative and 637

parsimony-informative. The MP analysis yielded 1000 trees (TL = 1512; CI = 0.612; RC = 0.538; RI = 0.952) of which the first is presented (Fig. 1). The majority of the isolates included in this dataset clustered in the clade (BS < 50; PP = 1.00) representing *C. pentaseptata* (ex-type CBS 133349) with five isolates (CBS 136633, CBS 136634, CBS 136636, CBS 136638 & CBS 136640) forming a sister clade (BS < 50; PP = 0.96) to the *C. pentaseptata* clade. A clade (BS = 75; PP = 0.99) incorporating seven isolates (CBS 136630, CBS 136631, CBS 136632, CBS 133639, CBS 1336640, CPC 23486 & CPC 23487), with an additional two isolates (CBS 136635 & CBS 136637) forming a sister clade (BS = 81; PP = 1.00), clustered close but separate from *C. pauciramosa* (ex-type CMW 5683) and *C. polizzii* (CBS 125270 & CBS 125271). A further seven isolates (CBS 136642, CBS 136643, CBS 136644, CBS 136645, CBS 136647, CBS 136651 & CBS 136653) formed a clade (BS = 60; PP = 1.00) close but separate to *C. cerciana* (ex-type CBS 123693) with four isolates (CBS 136084, CBS 136096, CBS 136097 & CBS 136251) forming a sister clade (BS = 72; PP = 1.00) to these seven isolates. Three isolates (CBS 136641, CMW 31394 & CMW 31395) formed a clade (BS = 100; PP = 1.00) close but separate from *C. brasiliensis* (ex-type CBS 230.51) and *C. sulawesensis* (CBS 125248 & CBS 125253).

The dataset representing the Sphaero-Naviculate Group of isolates included 85 ingroup taxa, with *C. pauciramosa* (CMW 5683 & CMW 30823) as the outgroup taxon. This dataset consisted of 2016 characters, of which 1369 were constant, 127 were parsimony-uninformative and 520 were parsimony-informative. The MP analysis yielded 100 trees (TL = 1264; CI = 0.672; RC = 0.633; RI = 0.942) of which the first is presented (Fig. 2). In this tree, 35 isolates clustered within the clade (BS = 97; PP = 1.00) representing *C. hongkongensis* (ex-type CBS 114828) with four isolates (CBS 136077, CBS 136093, CBS 136652 & CMW 35383) forming a sister clade (BS = 78; PP = 1.00) to the *C. hongkongensis* clade. A single isolate (CBS 136629) formed a basal sister lineage to both these clades. Four isolates (CBS

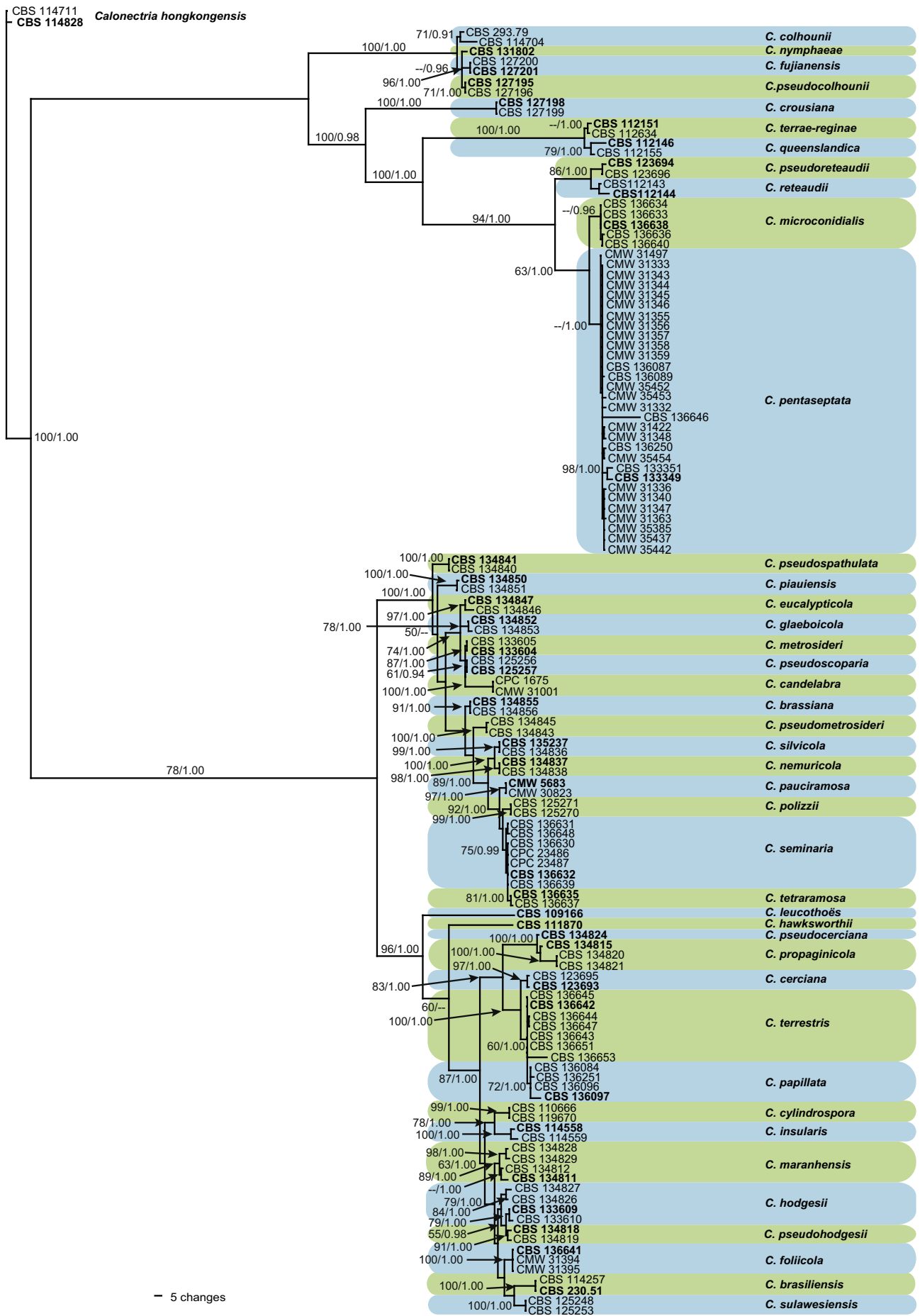


Fig. 1. One of 1 000 equally most parsimonious trees obtained from a heuristic search with 1 000 random taxon additions of the combined *cmdA*, *his3*, *tef1* and *tub2* sequence alignments of the Prolate group. Scale bar shows 5 changes. Bootstrap support values and Bayesian posterior probability values are shown at the nodes. The tree was rooted to *C. hongkongensis* (CBS 114711 & CBS 114828). Ex-type strains are indicated in bold.

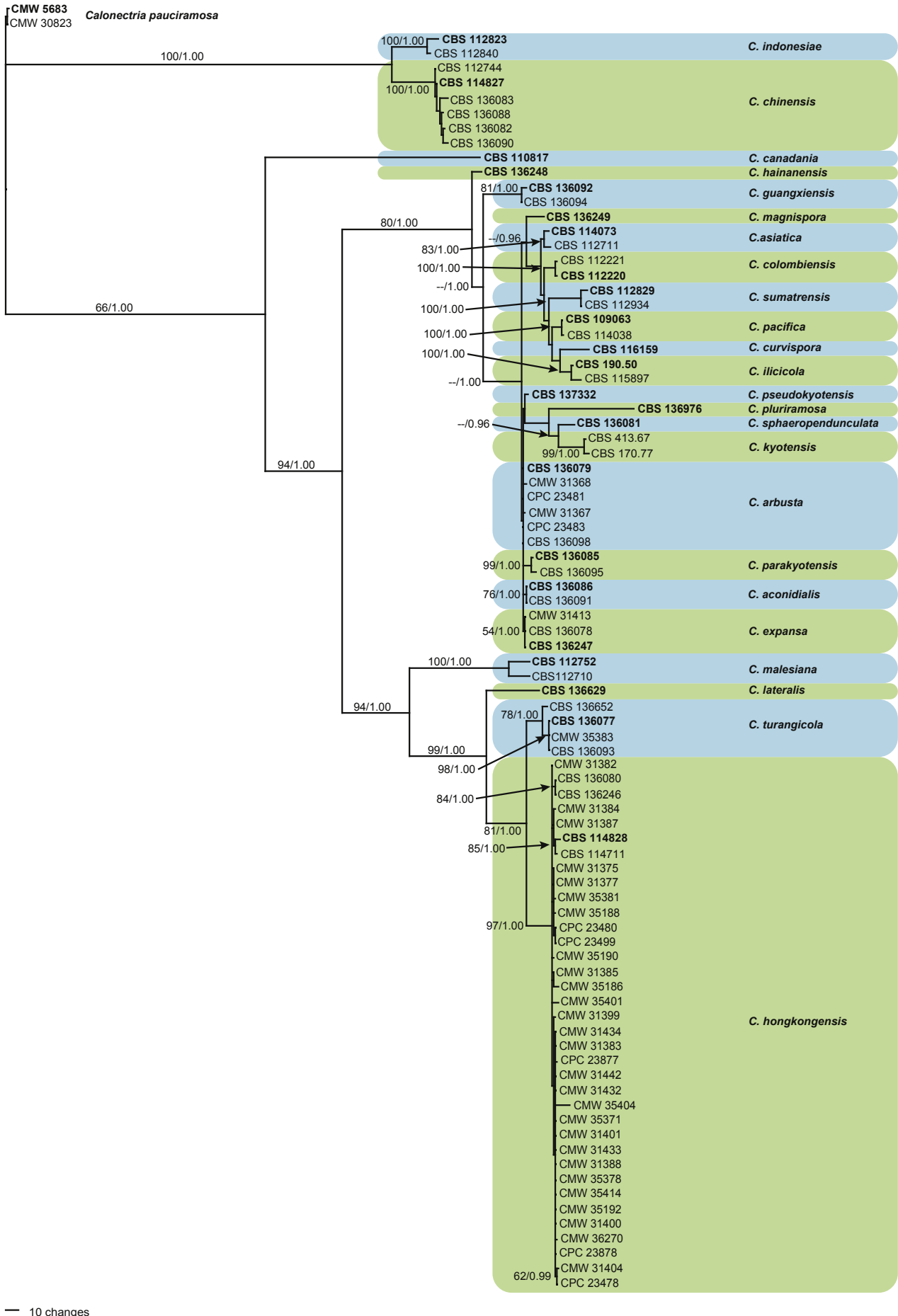


Fig. 2. One of 1 000 equally most parsimonious trees obtained from a heuristic search with 1 000 random taxon additions of the combined *cmdA*, *his3*, *tef1* and *tub2* sequence alignments of the Sphaero-Naviculate Group. Scale bar shows 10 changes. Bootstrap support values and Bayesian posterior probability values are shown at the nodes. The tree was rooted to *C. pauciramosa* (CMW 5683 & CMW 30823). Ex-type strains are indicated in **bold**.

136082, CBS 136083, CBS 136088 & CBS 136090) clustered in a clade (BS = 100; PP = 1.00) with *C. chinensis* (ex-type CBS 114827). Sixteen isolates clustered near the *C. kyotensis* (ex-type CBS 413.67) clade (BS = 99; PP = 1.00) of which three isolates (CBS 136081, CBS 136976 & CMW 31439) formed single lineages. The remaining isolates clustered in four separate clades, three of which were well supported (BS = 99; PP = 1.00, BS = 76; PP = 1.00 & BS = 54; PP = 1.00, respectively). Of the remaining four isolates, two (CBS 136248 & CBS 136249) formed single lineages and two (CBS 136092 & CBS 136094) formed a unique clade (BS = 81; PP = 1.00).

Taxonomy

Morphological observation supported by phylogenetic inference showed that the majority of strains included in this study belonged to *C. chinensis*, *C. hongkongensis* and *C. pentaseptata* (Figs 1 and 2; Table 1). The remaining strains are shown to represent several distinct taxa that are provided names in *Calonectria*. Important morphological characters are summarised in Table 2.

***Calonectria aconidialis* L. Lombard, Crous & S.F. Chen, sp. nov.** MycoBank MB809043. Fig. 3.

Etymology: Name refers to an absence of macroconidia in the fungus.

Ascomata perithecial, solitary or in groups of two, orange, becoming orange-brown with age; in section, apex and body orange, base red-brown, subglobose to ovoid, 297–366 µm high, 232–304 µm diam, body turning dark orange to red, and base dark red-brown in 3% KOH+; ascomatal wall rough, consisting of two thick-walled layers; outer layer of *textura globulosa*, 37–75 µm thick, cells becoming more compressed towards the inner layer of *textura angularis*, 16–23 µm thick, cells becoming thin-walled and hyaline towards the centre; outermost cells 14–45 × 12–35 µm, cells of inner layer 10–25 × 3–7 µm; ascomatal base up to 150 µm wide, consisting of dark red, angular cells, merging with an erumpent stroma; cells of the outer wall layer continuous with the pseudoparenchymatous cells of the erumpent stroma. *Asci* 8-spored, clavate, 111–113 × 15–18 µm, tapering into a long thin stalk. *Ascospores* aggregated in the upper third of the ascus, hyaline, guttulate, fusoid with rounded ends, straight to slightly curved, 1-septate, sometimes constricted at the septum, (28–)32–40(–44) × 5–7 µm (av. 36 × 6 µm). Homothallic. *Mega-*, *macro-* and *microconidia* not observed.

Culture characteristics: Colonies moderately fast growing at 24 °C on MEA with mycelium immersed in medium with no sporulation on the medium surface; surface and reverse white to pale luteous after 7 d.

Specimens examined: **China**, Hainan Province, from soil collected in a *Eucalyptus* plantation, Aug. 2009, X. Mou & S.F. Chen (**holotype** CBS H-21481, culture ex-type CBS 136086 = CMW 35174 = CERC 1850), CBS 136091 = CPC 23504 = CMW 35384 = CERC 1886.

Notes: All attempts to induce the asexual morph of *C. aconidialis* failed. *Ascomata* formed readily within 16 d on MEA, SNA and MSA, either on the surface or immersed in the medium, exuding viable ascospores after 20 d.

***Calonectria arbusta* L. Lombard, Crous & S.F. Chen, sp. nov.** MycoBank MB809045. Fig. 4.

Etymology: Name refers to a plantation and the environment from which this fungus was collected.

Ascomata perithecial, solitary, orange, becoming orange-brown with age; in section, apex and body orange, base red-brown, subglobose to ovoid, 357–444 µm high, 276–391 µm diam, body turning dark orange to red, and base dark red-brown in 3% KOH+; ascomatal wall rough, consisting of two thick-walled layers; outer layer of *textura globulosa*, 37–66 µm thick, cells becoming more compressed towards the inner layer of *textura angularis*, 18–20 µm thick, cells becoming thin-walled and hyaline towards the centre; outermost cells 34–71 × 34–55 µm, cells of inner layer 23–32 × 6–9 µm; ascomatal base up to 137 µm wide, consisting of dark red, angular cells, merging with an erumpent stroma; cells of the outer wall layer continuous with the pseudoparenchymatous cells of the erumpent stroma. *Asci* 8-spored, clavate, 97–119 × 16–19 µm, tapering into a long thin stalk. *Ascospores* aggregated in the upper third of the ascus, hyaline, guttulate, fusoid with rounded ends, straight to slightly curved, 1-septate, constricted at the septum, (30–)35–41(–43) × 5–7(–8) µm (av. 38 × 7 µm). Homothallic. *Macroconidiophores* consist of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 40–133 × 6–10 µm; stipe extension septate, straight to flexuous, 134–196 µm long, 3–6 µm wide at the apical septum, terminating in a sphaeropedunculate vesicle, 7–13 µm diam; lateral stipe extensions (90° to main axis) abundant. *Conidiogenous apparatus* 58–151 µm wide, and 54–108 µm long; primary branches aseptate, 18–42 × 5–8 µm; secondary branches aseptate, 10–27 × 4–7 µm; tertiary branches aseptate, 9–18 × 3–6 µm; quaternary branches and additional branches (–5) aseptate, 10–20 × 3–6 µm, each terminal branch producing 2–6 phialides; phialides doliform to reniform, hyaline, aseptate, 9–15 × 2–4 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (41–)42–48(–52) × 4–6 µm (av. 45 × 5 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies fast growing at 24 °C on MEA, producing abundant white to pale luteous aerial mycelium with profuse sporulation on the medium surface; reverse sienna to umber after 7 d; chlamydoconidia extensive throughout the medium forming microsclerotia.

Specimens examined: **China**, Guangxi Province, from soil collected in a *Eucalyptus* plantation, Mar. 2009, X. Zhou, G. Zhao & F. Han (**holotype** CBS H-21482, living ex-type culture CBS 136079 = CPC 23482 = CMW 31370 = CERC 1705), CPC 23481 = CMW 31369 = CERC 1704, CPC 23483 = CMW 31371 = CERC 1706, CMW 31368 = CERC 1703; Guangxi Province, Fangchenggang, from soil collected in a *Eucalyptus* plantation, Mar. 2009, X. Zhou, G. Zhao & F. Han, CMW 31367 = CERC 1702.

Note: *Calonectria arbusta* produces a larger conidiogenous apparatus than *C. kyotensis* and the ascospores and macroconidia of *C. arbusta* are also larger than those of *C. kyotensis* (Table 2).

Table 2. Morphological characteristics of *Calonectria* spp. included in this study.

Species	Perithecia		Asci	Ascospores		Conidiogenous apparatus		Stipe extension	Vesicle		Macroconidia		Reference
	Size (µm)	Shape	Size (µm)	Size (µm)	Septation	Size (µm)	Branches	(µm)	Diam (µm)	Shape	Size (µm)	Septation	
<i>Calonectria reteaudii</i> species complex													
<i>C. microconidialis</i>						26–92 × 35–95	3	175–441 × 4–7	3–7	Narrowly clavate	(69–)78–98(–113) × 7–9(10)	4–6(7)	This study
<i>C. pentatseptata</i>						23–90 × 70–99	3	168–350 × 3–6	2–6	Narrowly clavate	(75–)87–109(–115) × (5–)6–8(–10)	5(–8)	Crous <i>et al.</i> (2012)
<i>C. pseudoreteaudii</i>						26–82 × 45–103	3	193–313 × 5–6	3–5	Narrowly clavate	(61–)65–73(–78) × (4–)5–6(–7)	4–6	Lombard <i>et al.</i> (2010d)
<i>C. queenslandica</i>						27–68 × 39–64	3	105–156 × 4–5	3–4	Narrowly clavate	(61–)65–73(–78) × (4–)5–6(–7)	4–6	Lombard <i>et al.</i> (2010d)
<i>C. reteaudii</i>	350–450 × 250–350	Subglobose to ovoid	70–150 × 7–20	(50–)65–85(–100) × (4–)5–6(–7)	(1–)3(–5)	20–70 × 80–100	6	150–380 × 2.5–3.5	3–6	Clavate	(50–)75–95(–120) × (5–)6–7	(1–)5(–6)	Crous (2002)
<i>C. terrae-reginae</i>						33–48 × 35–54	4	127–235 × 4–6	3–5	Narrowly clavate	60–83(–87) × (4–)5–7(–8)	4–6	Lombard <i>et al.</i> (2010d)
<i>Calonectria candelabra</i> species complex													
<i>C. brassiana</i>						50–135 × 50–80	3	90–172 × 2–3	3–7	Ellipsoid to narrowly obpyriform	(35–)50–56(–65) × 3–5	1	Alfenas <i>et al.</i> (2015)
<i>C. candelabra</i>	350–450 × 300–350	Subglobose to ovoid	70–130 × 7–15	(40–)45–50(–60) × 5–6	1	30–70 × 50–80	5	100–220 × 3–3.5	5–8	Ellipsoid to narrowly obpyriform	(45–)58–68(–80) × 4–5(–6)	1	Crous (2002)
<i>C. eucalypticola</i>						45–75 × 35–62	3	145–170 × 2–4	5–7	Ellipsoid to obpyriform	(43–)49–52(–55) × 3–5	1	Alfenas <i>et al.</i> (2015)
<i>C. glaeboicola</i>						25–40 × 27–45	2	100–165 × 2–4	3–5	Ellipsoid to narrowly obpyriform	(45–)50–52(–55) × 3–5	1	Alfenas <i>et al.</i> (2015)
<i>C. metrosideri</i>						60–75 × 40–65	4	90–170 × 2–4	5–9	Spathulate to obpyriform	(40–)44–46(–51) × 3–5	1	Alfenas <i>et al.</i> (2013a)
<i>C. mossambicensis</i>						37–87 × 19–59	3	91–203 × 2–6	2–8	Obpyriform to ellipsoidal	(35–)38–46(–50) × 3–6	1	Crous <i>et al.</i> (2013)
<i>C. nemuricola</i>						50–80 × 40–60	4	150–205 × 6–12	7–13	Obpyriform	(40–)44–46(–50) × 3–5	1	Alfenas <i>et al.</i> (2015)
<i>C. pauciramosa</i>	250–400 × 170–300	Subglobose to ovoid	70–140 × 8–25	(30–)33–38(–40) × 6–7(–8)	1	20–50 × 35–85	3	120–230 × 2–3	5–11	Obpyriform to ellipsoidal	(30–)45–55(–60) × (3.5–)4–5	1	Schoch <i>et al.</i> (1999)
<i>C. piauiensis</i>						35–80 × 20–60	2	95–130 × 2–3	3–7	Ellipsoid to narrowly obpyriform	(38–)47–52(–60) × 3–5	1	Alfenas <i>et al.</i> (2015)
<i>C. polizzii</i>						28–51 × 27–57	3	111–167 × 5–6	6–9	Obpyriform to ellipsoidal	(31–)32–42(–49) × 3–5	1	Lombard <i>et al.</i> (2010a)
<i>C. pseudoscoparia</i>						52–74 × 34–87	4	124–201 × 4–6	6–10	Obpyriform to ellipsoidal	(41–)45–51(–52) × 3–3	1	Lombard <i>et al.</i> (2010b)

(continued on next page)

Table 2. (Continued)

Species	Perithecia		Asci	Ascospores		Conidiogenous apparatus		Stipe extension	Vesicle		Macroconidia		Reference
	Size (µm)	Shape	Size (µm)	Size (µm)	Septation	Size (µm)	Branches	(µm)	Diam (µm)	Shape	Size (µm)	Septation	
<i>C. pseudospathulata</i>						60–100 × 30–70	3	145–190 × 2–4	7–10	Obpyriform	(35–)41–44(–50) × 3–5	1	Alfenas et al. (2015)
<i>C. seminaria</i>						31–155 × 36–72	3	105–185 × 4–7	6–11	Obpyriform to ellipsoidal	(42–)45–49(–52) × 3.5–4.5(–7)	1	This study
<i>C. silvicola</i>						45–105 × 35–90	3	130–195 × 3–4	7–10	Obpyriform	(30–)40–42(–50) × 3–5	1	Alfenas et al. (2015)
<i>C. tetraramosa</i>						54–95 × 36–75	4	102–253 × 3–6	4–10	Obpyriform	(45–)46.5–49.5(–51) × (4–)4.5–5.5(–6)	1	This study
<i>C. zuluensis</i>	292–394 × 170–285	Subglobose to ovoid	92–140 × 10–16	(26–)29–34(–38) × 4–5	1	37–70 × 35–67	3	110–171 × 5–8	6–10	Ellipsoid to obpyriform	(31–)34–38(–40) × 3–5	1	Lombard et al. (2010a)
<i>Calonectria cylindrospora</i> species complex													
<i>C. brasiliensis</i>						81–103 × 58–90	3	204–266 × 6–7	7–11	Ellipsoid to obpyriform	(35–)36–40(–41) × 3–5	1	Lombard et al. (2010a)
<i>C. cerciana</i>						62–113 × 70–98	4	148–222 × 5–6	8–13	Fusiform to obpyriform	(37–)41–46(–49) × 5–6	1	Lombard et al. (2010d)
<i>C. cylindrospora</i>	280–520 × 280–400	Globose to subglobose	75–100 × 8–15	(24–)30–40(–49) × (4–)5–6(–8)	1	60–100 × 60–110	6	150–200 × 3–4	6–8	Ellipsoid to pyriform or clavate	(40–)42–50(–66) × 3–4(–5)	1	Crous (2002)
<i>C. foliicola</i>						76–180 × 59–130	7	140–215 × 4–6	6–13	Obpyriform to ellipsoidal	(41–)44–50(–52) × (3–)4–5(–6)	1	This study
<i>C. hawksworthii</i>						40–90 × 65–100	4	150–250 × 2–3	6–9	Ellipsoid to clavate	(38–)50–60(–76) × 4(–5)	1	Crous (2002)
<i>C. hodgesii</i>						61–72 × 45–65	3	136–196 × 2–4	6–11	Pyriform to ellipsoidal or ovoid to sphaeropedunculate	(44–)49–51(–55) × 3–5	1	Alfenas et al. (2013b)
<i>C. insularis</i>	350–450 × 300–350	Subglobose to ovoid	70–125 × 7–18	(27–)30–36(–42) × 5–6(–7)	1	45–90 × 45–80	6	110–250 × 4–5	4–13	Obpyriform to broadly ellipsoidal	(33–)40–50(–60) × 3.5–4	1	Crous (2002)
<i>C. leucothoëns</i>						25–50 × 50–80	6	160–250 × 3–6	6–11.5	Ellipsoid to obpyriform	(45–)68–78(–97) × (4–)5–5.5(–6.5)	(1–)3(–6)	Crous (2002)
<i>C. maranhensis</i>						45–65 × 45–71	3	125–190 × 3–5	7–11	Ellipsoid, obpyriform to sphaeropedunculate	(50–)56–58(–65) × (3–)5(–6)	1	Alfenas et al. (2015)
<i>C. mexicana</i>	400–450 × 350–450	Subglobose to ovoid	70–120 × 10–20	(35–)40–55(–65) × 5–6(–7)		25–60 × 40–70	3	160–250 × 2–3	7–12	Broadly ellipsoid with papillate apex	(35–)40–48(–52) × 3–4(–4.5)	1	Crous (2002)
<i>C. papillata</i>	425–455 × 345–395	Subglobose to ovoid	106–112 × 16–20	(27–)32–40(–46) × 5–6(–7)	1	45–114 × 33–82	4	163–218 × 4–7	8–14	Obpyriform to ellipsoidal with papillate apex	(40–)43–47(–50) × (3–)4–5	1	This study
<i>C. propaginicola</i>						40–75 × 31–85	4	130–250 × 2–5	5–12	Ellipsoid, obpyriform to sphaeropedunculate	(40–)48–51(–55) × 3–5	1	Alfenas et al. (2015)

Table 2. (Continued)

Species	Perithecia		Asci	Ascospores		Conidiogenous apparatus		Stipe extension	Vesicle		Macroconidia		Reference
	Size (µm)	Shape	Size (µm)	Size (µm)	Septation	Size (µm)	Branches	(µm)	Diam (µm)	Shape	Size (µm)	Septation	
<i>C. pseudocerciana</i>						50–90 × 40–95	3	130–190 × 2–5	7–12	Obpyriform to sphaeropedunculate	(35–)43–46(–55) × 3–5	1	Alfenas et al. (2015)
<i>C. pseudohodgesii</i>						50–90 × 40–95	3	130–190 × 2–5	7–12	Obpyriform to sphaeropedunculate	(35–)43–46(–55) × 3–5	1	Alfenas et al. (2015)
<i>C. sulawesiensis</i>						43–81 × 41–79	5	113–262 × 5–7	5–7	Broadly clavate to ellipsoid	(41–)45–51(–54) × (3–)4(–6)	1	Lombard et al. (2010b)
<i>C. terrestris</i>						35–89 × 35–102	4	147–228 × 4–7	5–12	Obpyriform to pyriform to broadly clavate	(33–)36–40(–41) × (3–)4–5	1	This study
<i>Calonectria kytensis</i> species complex													
<i>C. aconidialis</i>	297–366 × 232–304	Subglobose to ovoid	111–113 × 15–18	(28–)32–40(–44) × 5–7	1								This study
<i>C. arbusta</i>	357–444 × 276–391	Subglobose to ovoid	97–119 × 16–19	(30–)35–41(–43) × 5–7(–8)	1	58–151 × 54–108	5	134–196 × 3–6	7–13	Sphaeropedunculate	(41–)42–48(–52) × 4–6	1	This study
<i>C. asiatica</i>	280–400 × 200–350	Subglobose to ovoid	70–120 × 12–20	(28–)30–38(–40) × (5–)6(–7)	1	40–80 × 40–90	5	200–280 × 3–7	12–17	Sphaeropedunculate	(42–)48–55(–65) × (4–)5(–5.5)	1	Crous et al. (2004b)
<i>C. canadana</i>							3	100–180 × 3–4	6–10	Pyriform to sphaeropedunculate	(38–)48–55(–65) × 4(–5)	1	Kang et al. (2001b)
<i>C. chinensis</i>						40–60 × 40–60	3	120–150 × 2.5–3.5	6–9	Sphaeropedunculate	(38–)41–48(–56) × (3.5–)4(–4.5)	1	Crous et al. (2004b)
<i>C. colombiensis</i>	200–350 × 200–300	Subglobose to ovoid	90–150 × 11–23	(28–)30–35(–40) × (4–)5(–6)	1	25–60 × 40–60	5	130–200 × 3–4	7–12	Sphaeropedunculate	(33–)48–58(–60) × (4–)4.5(–5)	1(–3)	Crous et al. (2004b)
<i>C. curvispora</i>						15–30 × 35–50	3	110–150 × 2–3	5–10	Sphaeropedunculate	(45–)55–65(–70) × (4–)5–6	1(–3)	Crous (2002)
<i>C. expansa</i>	310–520 × 270–435	Subglobose to ovoid	107–146 × 16–21	(33–)36–41(–44) × (4–)5–7	1	26–116 × 45–82	5	124–216 × 3–7	8–16	Sphaeropedunculate	(44–)48–52(–57) × 4–6	1	This study
<i>C. guangxiensis</i>	295–435 × 265–355	Subglobose to ovoid	83–146 × 15–23	(23–)32–40(–42) × 5–7(–8)	1	31–95 × 55–85	4	175–193 × 5–7	11–14	Sphaeropedunculate	(42–)45–49(–52) × 4–6	1	This study
<i>C. hainanensis</i>	300–455 × 230–385	Subglobose to ovoid	91–110 × 15–22	(24–)30–38(–42) × (4–)5–7	1	54–119 × 41–80	5	112–186 × 5–9	7–14	Sphaeropedunculate	(41–)43–49(–52) × 4–6	1	This study
<i>C. hongkongensis</i>	350–550 × 300–450	Subglobose to ovoid	80–140 × 14–20	(25–)28–35(–40) × (4–)5–6(–7)	1	70–120 × 70–100	8	100–200 × 3–4	8–14	Sphaeropedunculate	(38–)45–48(–53) × 4(–4.5)	1	Crous et al. (2004b)
<i>C. ilicicola</i>	300–550 × 280–400	Subglobose to ovoid	90–140 × 12–19	(30–)37–50(–65) × (4–)5–6.5(–7)	1(–3)	25–100 × 55–100	3	120–140 × 3–4	6–12	Sphaeropedunculate	(45–)70–82(–90) × (4–)5–6.5(–7)	(1–)3	Crous (2002)
<i>C. indonesiae</i>						60–80 × 40–60	5	110–160 × 2.5–3	7–9	Sphaeropedunculate	(40–)45–55(–60) × (3–)4	1	Crous et al. (2004b)
<i>C. kytensis</i>	280–550 × 210–425	Subglobose to ovoid	70–140 × 13–22	(18–)28–40(–48) × (4–)5–6(–7)	1	40–100 × 40–90	5	100–200 × 3–4	6–12	Sphaeropedunculate	(35–)45–50(–55) × 3–4(–5)	1	Crous (2002)

(continued on next page)

Table 2. (Continued)

Species	Perithecia		Asci	Ascospores		Conidiogenous apparatus		Stipe extension	Vesicle		Macroconidia		Reference
	Size (µm)	Shape	Size (µm)	Size (µm)	Septation	Size (µm)	Branches	(µm)	Diam (µm)	Shape	Size (µm)	Septation	
<i>C. lateralis</i>						43–138 × 41–104	6	150–225 × 4–6	9–13	Sphaeropedunculate	(35–)37–41(–44) × 4–5	1	This study
<i>C. magnispora</i>	280–550 × 210–425	Subglobose to ovoid	91–125 × 14–17	(33–)36–44(–49) × 5–7(–8)	1	47–95 × 47–80	4	161–278 × 4–7	9–18	Sphaeropedunculate	(46–)49–55(–60) × 4–6(–7)	1	This study
<i>C. malesiana</i>						30–80 × 50–60	6	120–200 × 3–4	8–15	Sphaeropedunculate to globose	(34–)45–52(–55) × (3–)4	1	Crous <i>et al.</i> (2004b)
<i>C. pacifica</i>						20–60 × 30–80	3	150–250 × 3–4	7–15	Sphaeropedunculate	(38–)45–65(–75) × 4–5	1	Crous (2002)
<i>C. parakyotensis</i>						49–98 × 41–84	4	135–210 × 4–6	10–14	Sphaeropedunculate	(39–)42–46(–49) × 4–5(–6)	1	This study
<i>C. pluriramosa</i>						76–177 × 59–127	7	140–215 × 4–6	6–13	Sphaeropedunculate	(41–)44–50(–52) × (3–)4–5(–6)	1	This study
<i>C. pseudokyotensis</i>						43–103 × 76–109	4	145–320 × 5–7	10–13	Pyriiform to sphaeropedunculate	(43–)45–51(–53) × 5–7	1	This study
<i>C. sphaeropedunculata</i>	470–575 × 345–465	Subglobose to ovoid	82–144 × 11–23	(31–)33–40(–42) × 5–7(–8)	1	63–144 × 40–111	6	152–253 × 4–8	10–14	Sphaeropedunculate	(40–)43–47(–49) × 4–6	1	This study
<i>C. sumatrensis</i>						40–60 × 50–60	3	180–260 × 3–4	8–13	Sphaeropedunculate	(45–)55–65(–70) × (4.5–)5(–6)	1	Crous <i>et al.</i> (2004b)
<i>C. turangicola</i>						48–110 × 35–86	5	133–195 × 4–6	8–12	Sphaeropedunculate	(40–)42–46(–47) × 3–5	1	This study

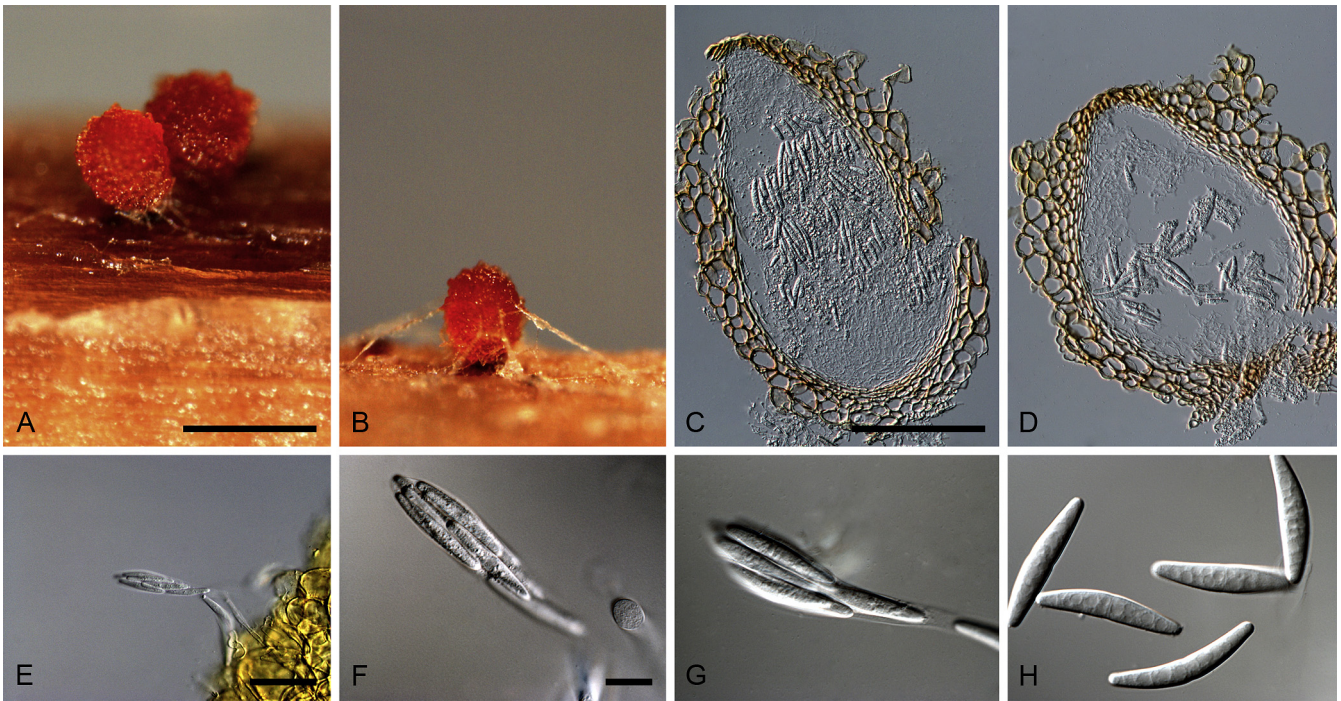


Fig. 3. *Calonectria aconidialis* (ex-type CBS 136086). A–B. Ascomata. C–D. Vertical section through ascomata, showing wall structure. E–G. Asci. H. Ascospores. Scale bars: A = 500 μ m; C = 100 μ m (apply to D); E = 50 μ m; F = 10 μ m (apply to G–H).

Calonectria expansa L. Lombard, Crous & S.F. Chen, **sp. nov.** MycoBank MB809046. **Fig. 5.**

Etymology: Name refers to Guangxi Province, the “Western Expanse”, where this fungus was first collected.

Ascomata perithecial, solitary, orange, becoming orange-brown with age; in section, apex and body orange, base red-brown, subglobose to ovoid, 310–520 μ m high, 270–435 μ m diam, body turning dark orange to red, and base dark red-brown in 3% KOH+; ascomatal wall rough, consisting of two thick-walled layers; outer layer of *textura globulosa*, 37–64 μ m thick, cells becoming more compressed towards the inner layer of *textura angularis*, 13–25 μ m thick, cells becoming thin-walled and hyaline towards the centre; outermost cells 13–31 \times 9–20 μ m, cells of inner layer 9–18 \times 3–5 μ m; ascomatal base up to 150 μ m wide, consisting of dark red, angular cells, merging with an erumpent stroma; cells of the outer wall layer continuous with the pseudoparenchymatous cells of the erumpent stroma. **Asci** 8-spored, clavate, 107–146 \times 16–21 μ m, tapering into a long thin stalk. **Ascospores** aggregated in the upper third of the ascus, hyaline, guttulate, fusoid with rounded ends, straight to slightly curved, 1-septate, sometimes constricted at the septum, (33–)36–41(–44) \times (4–)5–7 μ m (av. 39 \times 6 μ m). Homothallic. **Macroconidiophores** consist of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 61–169 \times 5–10 μ m; stipe extension septate, straight to flexuous, 124–216 μ m long, 3–7 μ m wide at the apical septum, terminating in a sphaeropedunculate vesicle, 8–16 μ m diam; lateral stipe extensions (90° to main axis) abundant. **Conidiogenous apparatus** 26–116 μ m wide, and 45–82 μ m long; primary branches aseptate, 18–29 \times 5–7 μ m; secondary branches aseptate, 12–22 \times 4–7 μ m; tertiary branches aseptate, 9–16 \times 3–6 μ m; quaternary branches and additional branches (–5) aseptate, 12–18 \times 3–5 μ m, each terminal branch producing

2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 10–18 \times 3–5 μ m, apex with minute periclinal thickening and inconspicuous collarete. **Macroconidia** cylindrical, rounded at both ends, straight, (44–)48–52(–57) \times 4–6 μ m (av. 52 \times 5 μ m), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. **Mega-** and **microconidia** not observed.

Culture characteristics: Colonies fast growing at 24 °C on MEA, producing abundant white aerial mycelium with profuse sporulation on the medium surface; reverse sienna to umber after 7 d; chlamydospores extensive throughout the medium forming microsclerotia.

Specimens examined: China, Guangxi Province, from soil collected in a *Eucalyptus* plantation, Mar. 2009, X. Zhou, G. Zhao & F. Han (**holotype** CBS H-21483, living ex-type culture CBS 136247 = CPC 23485 = CMW 31392 = CERC 1727); Guangxi Province, Fangchenggang, from soil collected in a *Eucalyptus* plantation, Mar. 2009, X. Zhou, G. Zhao & F. Han, CBS 136078 = CMW 31441 = CERC 1776, CMW 31413 = CERC 1748.

Note: *Calonectria expansa* can be distinguished from *C. arbusta* and *C. kyotensis* by its larger macroconidia and longer stipe extension (Table 2).

Calonectria foliicola L. Lombard, Crous & S.F. Chen, **sp. nov.** MycoBank MB809047. **Fig. 6.**

Etymology: Name refers to the natural habitat of this species, being a foliar pathogen.

Ascomata not observed. **Macroconidiophores** consist of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 47–190 \times 6–12 μ m; stipe extension septate, straight to flexuous, 140–215 μ m long, 4–6 μ m wide at the apical septum,

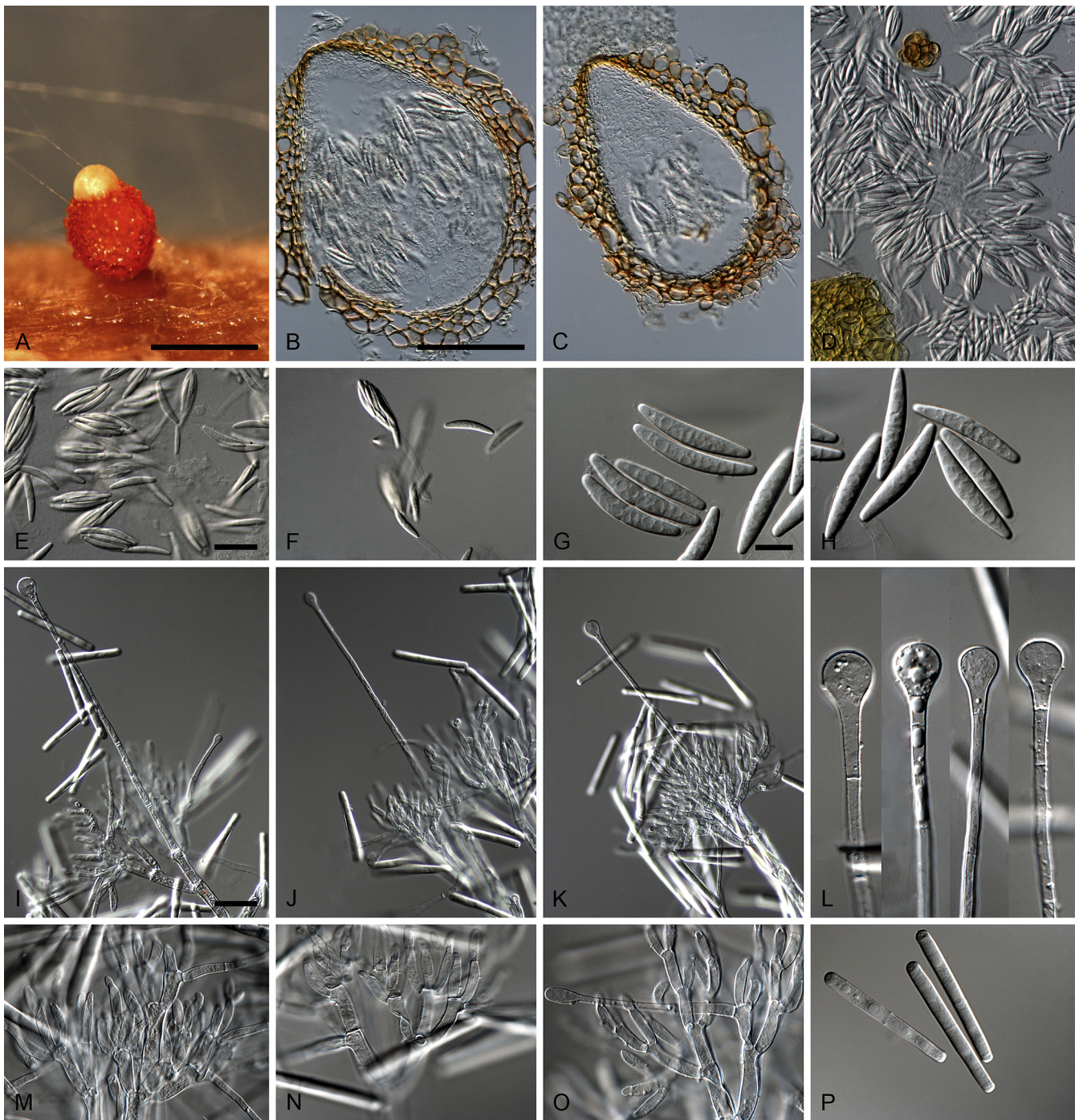


Fig. 4. *Calonectria arbusta* (ex-type CBS 136079). A. Ascoma. B–C. Vertical section through ascomata, showing wall structure. D–F. Asci. G–H. Ascospores. I–K. Macroconidiophores. L. Sphaeropedunculate vesicles. M–N. Conidiogenous apparatus with conidiophore branches and doliform to reniform phialides. O. Conidiogenous apparatus with lateral stipe extension. P. Macroconidia. Scale bars: A = 500 μm ; B = 100 μm (apply to C–D); E = 50 μm (apply to F, I–K), G = 10 μm (apply to H, L–P).

terminating in a obpyriform to ellipsoidal vesicle, 6–13 μm diam. *Conidiogenous apparatus* 76–180 μm wide, and 59–130 μm long; primary branches aseptate, 17–37 \times 5–8 μm ; secondary branches aseptate, 16–30 \times 4–7 μm ; tertiary branches aseptate, 11–23 \times 4–6 μm ; quaternary and additional branches (–7) aseptate, 9–20 \times 3–6 μm , each terminal branch producing 2–6 phialides; phialides doliform to reniform, hyaline, aseptate, 8–13 \times 3–5 μm , apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (41–)44–50(–52) \times (3–)4–5(–6) μm (av. 47 \times 5 μm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies fast growing at 24 °C on MEA, producing abundant white aerial mycelium and sporulating moderately on the medium surface; reverse sienna to umber after 7 d; chlamydospores formed abundantly throughout the medium, forming microsclerotia.

Specimen examined: China, Guangxi Province, from *E. urophylla* \times *E. grandis* clone leaf, Mar. 2009, X. Zhou & G. Zhao (**holotype** CBS H-21472, living ex-type culture CBS 136641 = CPC 23491 = CMW 31393 = CERC 1728), CPC 23492 = CMW 31394 = CERC 1729, CMW 31395 = CERC 1730.

Notes: *Calonectria foliicola* is closely related to *C. brasiliensis* and *C. sulawesiensis* and can be distinguished from these species by the formation of up to seven levels of conidiophore

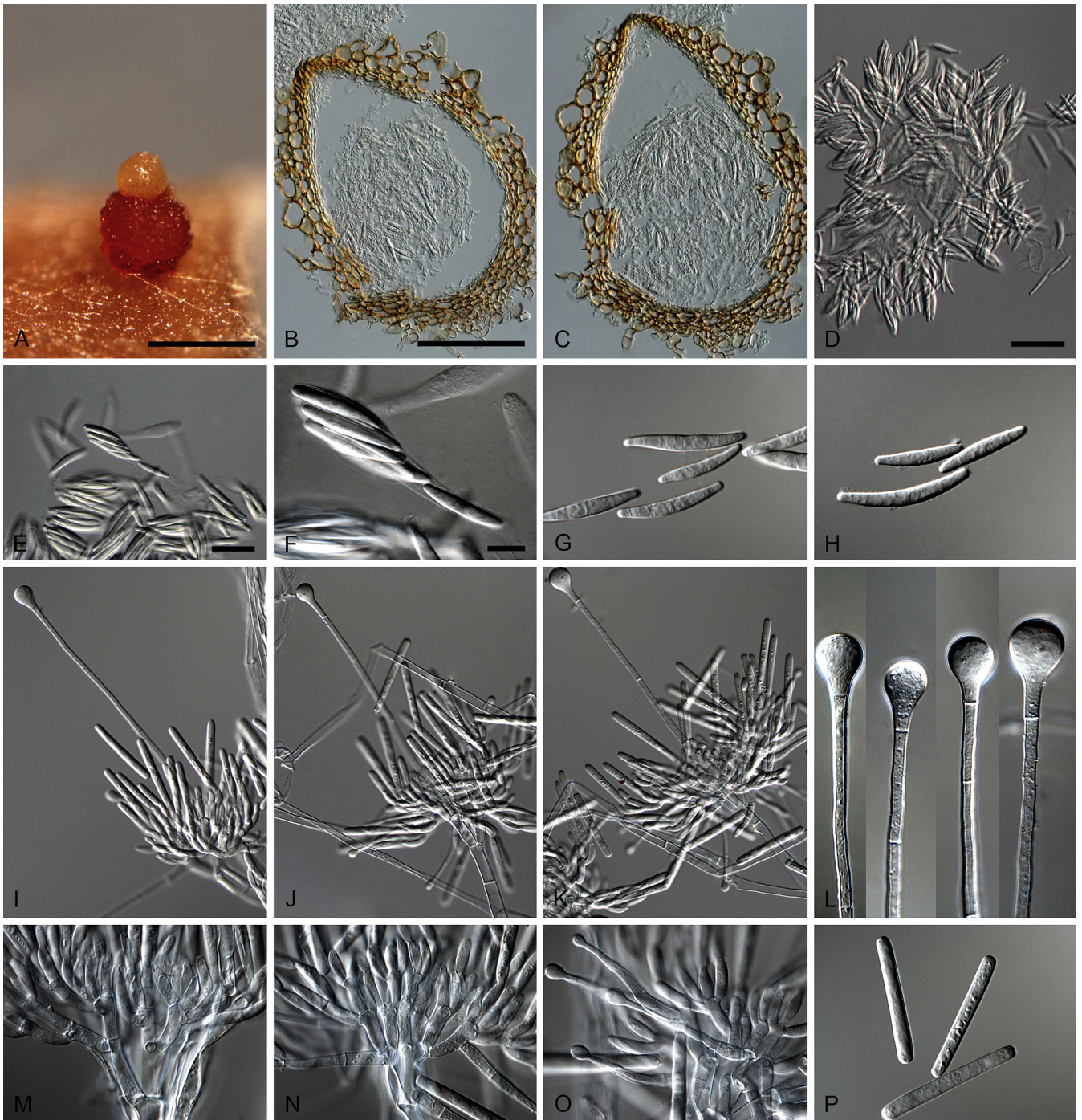


Fig. 5. *Calonectria expansa* (ex-type CBS 136247). A. Ascoma. B–C. Vertical section through ascomata, showing wall structure. D–F. Asci. G–H. Ascospores. I–K. Macroconidiophores. L. Sphaeropedunculate vesicles. M–N. Conidiogenous apparatus with conidiophore branches and doliform to reniform phialides. O. Conidiogenous apparatus with lateral stipe extension. P. Macroconidia. Scale bars: A = 500 μm ; B, D = 100 μm (apply to C); E = 50 μm (apply to I–K), F = 10 μm (apply to G–H, L–P).

branches. The macroconidia of *C. foliicola* are larger than those of *C. brasiliensis* but slightly smaller than those of *C. sulawesiensis* (Table 2).

Calonectria guangxiensis L. Lombard, Crous & S.F. Chen, *sp. nov.* MycoBank MB809049. Fig. 7.

Etymology: Name refers to the Guangxi Province of China where the fungus was first collected.

Ascomata perithecial, solitary or in groups of two, orange, becoming orange-brown with age; in section, apex and body orange, base red-brown, subglobose to ovoid, 295–435 μm high,

265–355 μm diam, body turning dark orange to red, and base dark red-brown in 3% KOH+; ascomatal wall rough, consisting of two thick-walled layers; outer layer of *textura globulosa*, 32–80 μm thick, cells becoming more compressed towards the inner layer of *textura angularis*, 14–22 μm thick, cells becoming thin-walled and hyaline towards the centre; outermost cells 13–26 \times 10–15 μm , cells of inner layer 11–15 \times 4–5 μm ; ascomatal base up to 175 μm wide, consisting of dark red, angular cells, merging with an erumpent stroma; cells of the outer wall layer continuous with the pseudoparenchymatous cells of the erumpent stroma. **Asci** 8-spored, clavate, 83–146 \times 15–23 μm , tapering into a long thin stalk. **Ascospores** aggregated in the upper third of the ascus, hyaline, guttulate, fusoid with rounded ends, straight to slightly

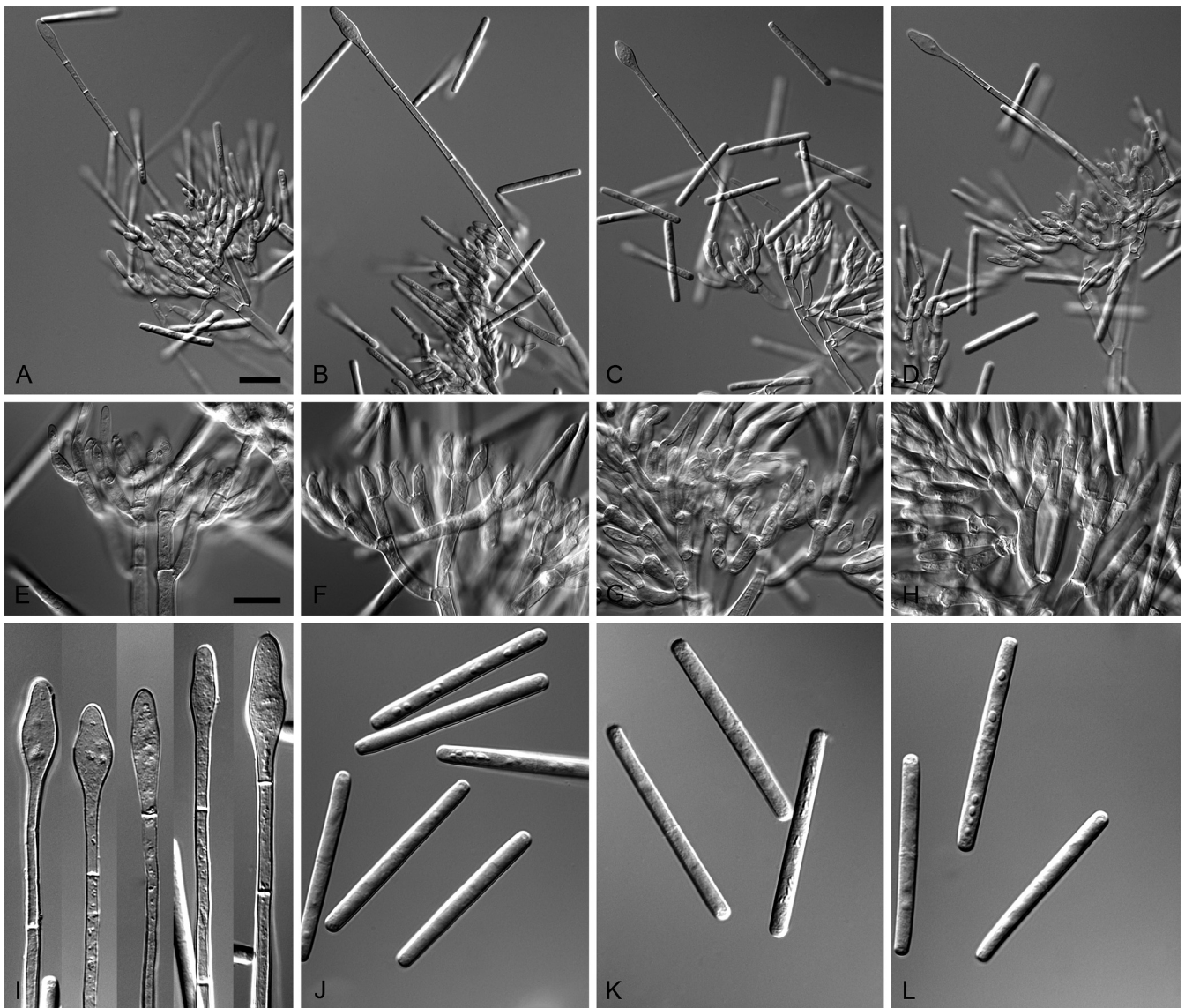


Fig. 6. *Calonectria foliicola* (ex-type 136641). A–D. Macroconidiophores. E–H. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. I. Obpyriform to ellipsoidal vesicles. J–L. Macroconidia. Scale bars: A = 50 μ m (apply to B–D); E = 10 μ m (apply to F–L).

curved, 1-septate, constricted at the septum, (23–) 32–40(–42) \times 5–7(–8) μ m (av. 36 \times 6 μ m). Homothallic. *Macroconidiophores* consist of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 91–182 \times 7–9 μ m; stipe extension septate, straight to flexuous, 175–193 μ m long, 5–7 μ m wide at the apical septum, terminating in a sphaeropedunculate vesicle, 11–14 μ m diam; lateral stipe extensions (90° to main axis) rare. *Conidiogenous apparatus* 31–95 μ m wide, and 55–85 μ m long; primary branches aseptate, 17–26 \times 4–7 μ m; secondary branches aseptate, 10–19 \times 3–6 μ m; tertiary branches aseptate, 9–17 \times 2–5 μ m; quaternary branches aseptate, 12–16 \times 3–5 μ m, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 8–19 \times 3–7 μ m, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (42–) 45–49(–52) \times 4–6 μ m (av. 47 \times 5 μ m), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies fast growing at 24 °C on MEA, producing abundant white to cream-coloured aerial mycelium and sporulating profusely on the medium surface at the edge of the

colony; reverse sienna to umber after 7 d; chlamydoconidia formed abundantly throughout the medium, forming microsclerotia.

Specimen examined: China, Guangxi Province, from soil collected in a *Eucalyptus* plantation, Aug. 2009, X. Mou & R. Chang (**holotype** CBS H-21484, culture ex-type CBS 136092 = CPC 23506 = CMW 35409 = CERC 1900), CBS 136094 = CPC 23507 = CMW 35411 = CERC 1902.

Notes: *Calonectria guangxiensis* can be distinguished from other species in the *C. kyotensis* complex by having fewer conidiophore branches and rarely forming lateral stipe extensions. The macroconidia of *C. guangxiensis* are slightly smaller than those of *C. expansa* and *C. kyotensis* and slightly larger than those of *C. arbusta* (Table 2).

***Calonectria hainanensis* L. Lombard, Crous & S.F. Chen, sp. nov.** MycoBank MB809050. Fig. 8.

Etymology: Name refers to the Hainan Province of China where the fungus was first collected.

Ascomata perithecial, solitary, orange, becoming orange-brown with age; in section, apex and body orange, base red-brown,

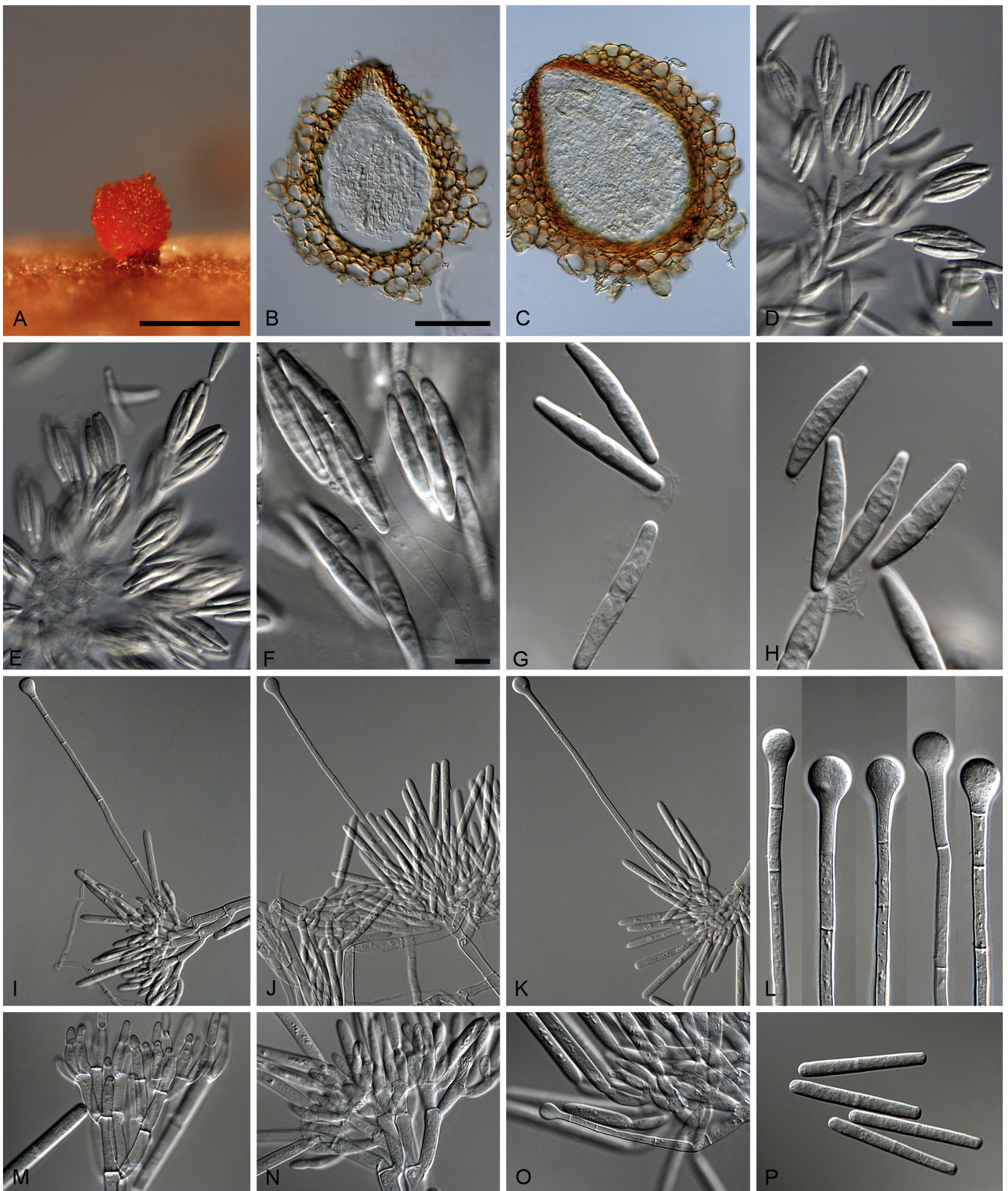


Fig. 7. *Calonectria guangxiensis* (ex-type CBS 136092). A. Ascoma. B–C. Vertical section through ascomata, showing wall structure. D–F. Asci. G–H. Ascospores. I–K. Macroconidiophores. L. Sphaeropedunculate vesicles. M–N. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. O. Conidiogenous apparatus with lateral stipe extension. P. Macroconidia. Scale bars: A = 500 μ m; B = 100 μ m (apply to C); D = 50 μ m (apply to E, I–K), F = 10 μ m (apply to G–H, L–P).

subglobose to ovoid, 300–455 μ m high, 230–385 μ m diam, body turning dark orange to red, and base dark red-brown in 3% KOH+; ascomatal wall rough, consisting of two thick-walled layers; outer layer of *textura globulosa*, 30–64 μ m thick, cells becoming more compressed towards the inner layer of *textura angularis*, 10–16 μ m thick, cells becoming thin-walled and hyaline towards the centre; outermost cells 16–42 \times 13–42 μ m, cells of inner layer 23–39 \times 8–10 μ m; ascomatal base up to 262 μ m wide, consisting

of dark red, angular cells, merging with an erumpent stroma; cells of the outer wall layer continuous with the pseudoparenchymatous cells of the erumpent stroma. Asci 8-spored, clavate, 91–110 \times 15–22 μ m, tapering into a long thin stalk. Ascospores aggregated in the upper third of the ascus, hyaline, guttulate, fusoid with rounded ends, straight to slightly curved, 1-septate, sometimes constricted at the septum, (24–)30–38(–42) \times (4–)5–7 μ m (av. 34 \times 6 μ m). Homothallic. Macroconidiophores



Fig. 8. *Calonectria hainanensis* (ex-type CBS 136248). A. Ascoma. B–C. Vertical section through ascomata, showing wall structure. D–F. Asci. G–H. Ascospores. I–K. Macroconidiophores. L. Sphaeropedunculate vesicles. M–N. Conidiogenous apparatus with conidiophore branches and dolliiform to reniform phialides. O. Conidiogenous apparatus with lateral stipe extension. P. Macroconidia. Scale bars: A = 500 μ m; B = 100 μ m (apply to C); D = 50 μ m (apply to I–K), E = 10 μ m (apply to F), G = 10 μ m (apply to H, L–P).

consisting of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 66–106 \times 8–14 μ m; stipe extension septate, straight to flexuous, 112–186 μ m long, 4–11 μ m wide at the apical septum, terminating in a sphaeropedunculate vesicle, 7–14 μ m diam; lateral stipe extensions (90° to main axis) abundant. *Conidiogenous apparatus* 54–119 μ m wide, and 41–80 μ m long; primary branches aseptate, 18–28 \times 5–9 μ m; secondary branches aseptate, 12–21 \times 5–8 μ m; tertiary

branches aseptate, 10–19 \times 3–6 μ m; quaternary branches and additional branches (–5) aseptate, 9–15 \times 3–5 μ m, each terminal branch producing 2–6 phialides; phialides dolliiform to reniform, hyaline, aseptate, 10–17 \times 3–5 μ m, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (41–)43–49(–52) \times 4–6 μ m (av. 46 \times 5 μ m), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

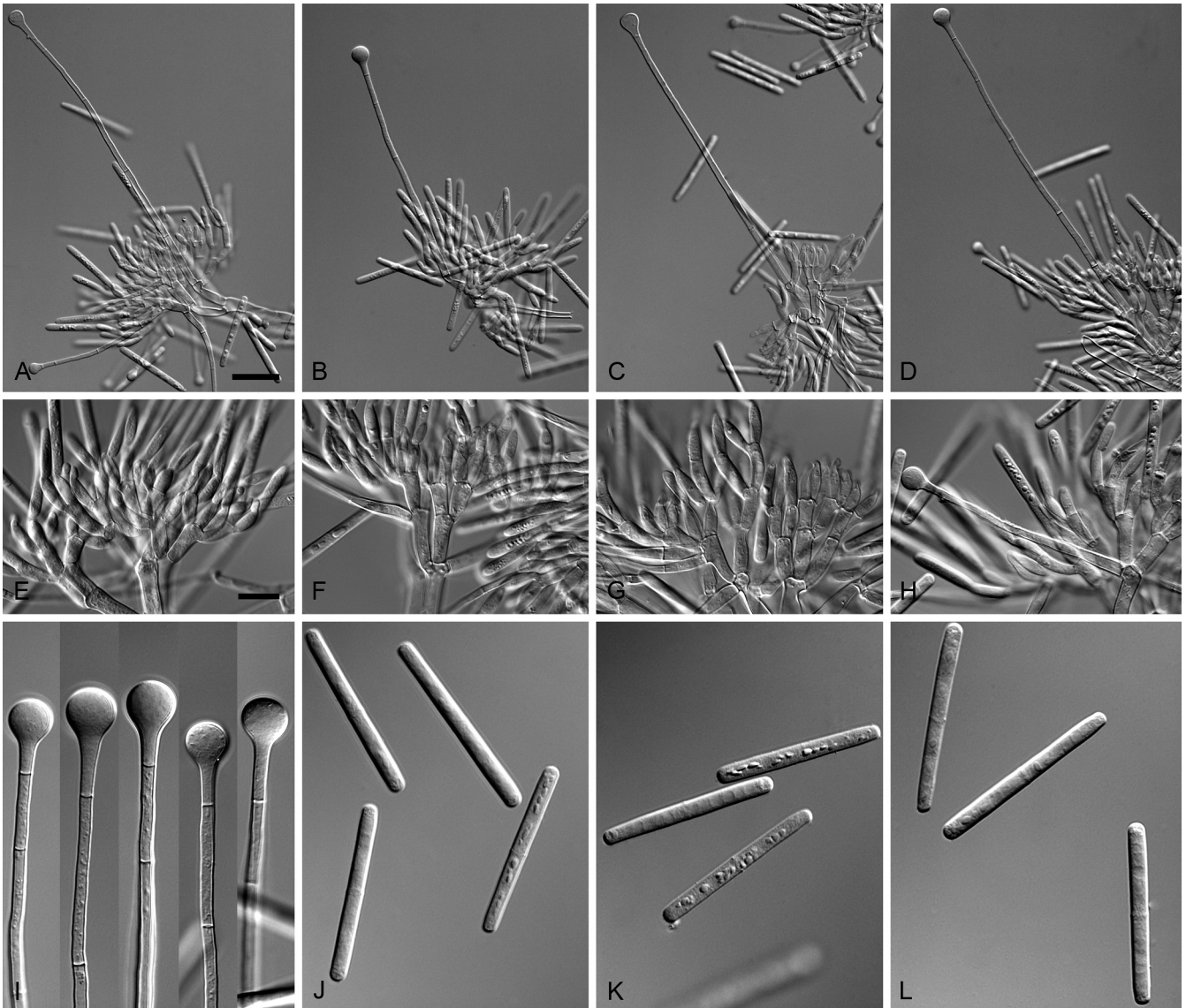


Fig. 9. *Calonectria lateralis* (ex-type CBS 136629). A–D. Macroconidiophores. E–H. Conidiogenous apparatus with conidiophore branches, doliiform to reniform phialides and lateral stipe extensions. I. Sphaeropedunculate vesicles. J–L. Macroconidia. Scale bars: A = 50 μm (apply to B–D); E = 10 μm (apply to F–L).

Culture characteristics: Colonies fast growing at 24 °C on MEA, producing abundant white to pale luteous aerial mycelium and sporulating profusely on the medium surface; reverse sienna to umber after 7 d; chlamydospores formed abundantly throughout the medium, forming microsclerotia.

Specimen examined: China, Hainan Province, from soil collected in a *Eucalyptus* plantation, Aug. 2009, X. Mou & S.F. Chen (**holotype** CBS H-21480, culture ex-type CBS 136248 = CPC 23505 = CMW 35187 = CERC 1863).

Notes: Based on morphological characteristics, *C. hainanensis* closely resembles *C. malesiana*. However, *C. hainanensis* readily produces fertile ascomata in culture, a feature not observed for *C. malesiana* (Crous *et al.* 2004b). Furthermore, *C. hainanensis* has fewer conidiophore branches than reported for *C. malesiana*, and the macroconidia of *C. hainanensis* are slightly smaller than those of *C. malesiana* (Table 2).

Calonectria lateralis L. Lombard, Crous & S.F. Chen, **sp. nov.** MycoBank MB809051. Fig. 9.

Etymology: Name refers to the lateral stipe extensions on its macroconidiophores.

Ascomata not observed. **Macroconidiophores** consisting of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 55–185 \times 4–8 μm ; stipe extension septate, straight to flexuous, 150–225 μm long, 4–6 μm wide at the apical septum, terminating in a sphaeropedunculate vesicle, 9–13 μm diam; lateral stipe extensions (90° to main axis) abundant. **Conidiogenous apparatus** 43–138 μm wide, and 41–104 μm long; primary branches aseptate, 17–28 \times 4–7 μm ; secondary branches aseptate, 11–26 \times 3–7 μm ; tertiary branches aseptate, 8–20 \times 3–6 μm ; quaternary and additional branches (–6) aseptate, 8–17 \times 3–5 μm , each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 7–13 \times 2–4 μm , apex with minute periclinal thickening and inconspicuous collarette. **Macroconidia** cylindrical, rounded at both ends, straight, (35–)37–41(–44) \times 4–5 μm (av. 39 \times 4 μm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. **Mega-** and **microconidia** not observed.

Culture characteristics: Colonies fast growing at 24 °C on MEA, producing abundant white to sienna aerial mycelium with profuse

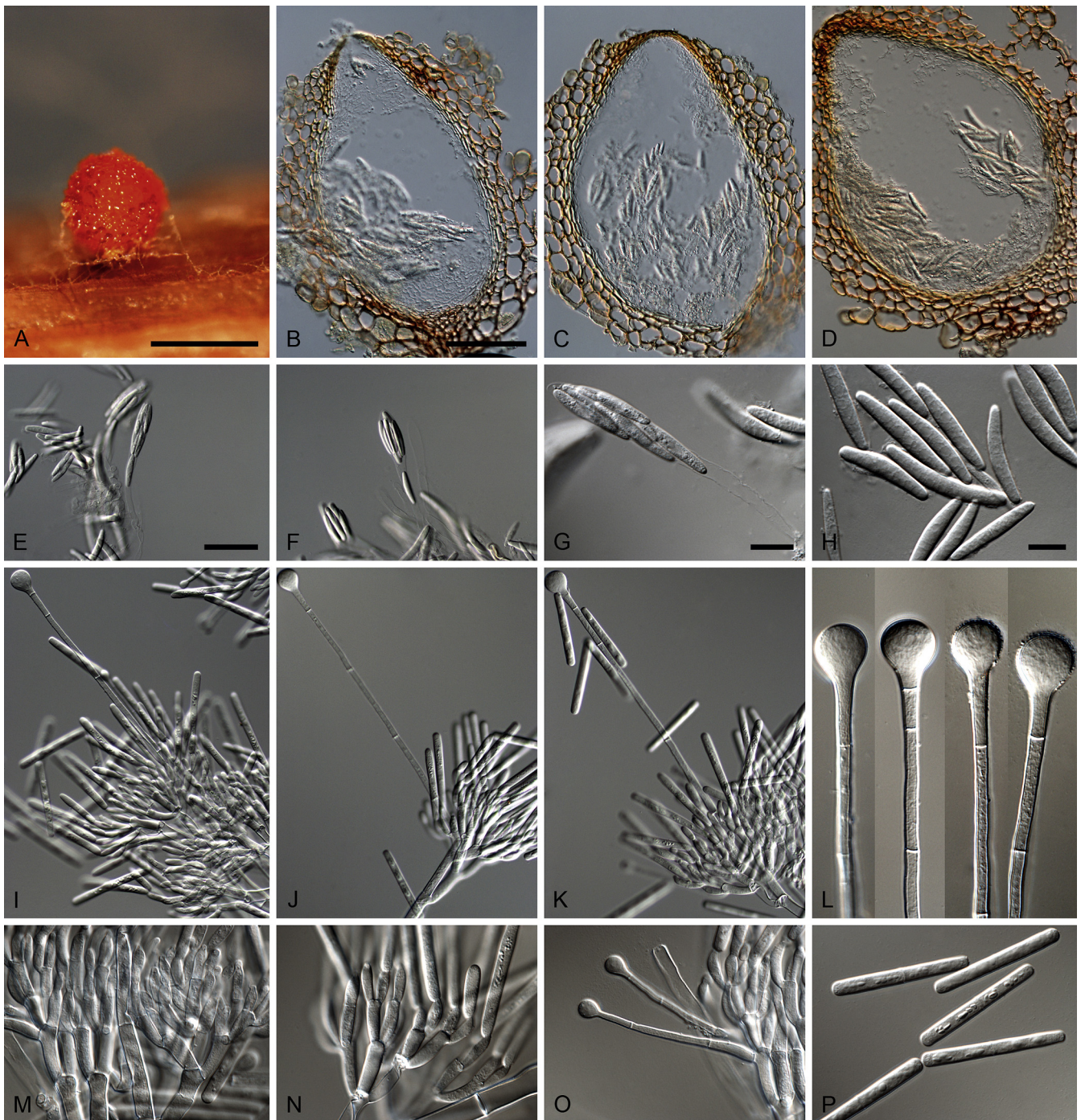


Fig. 10. *Calonectria magnispora* (ex-type CBS 136249). A. Ascoma. B–D. Vertical section through ascomata, showing wall structure. E–G. Asci. H. Ascospores. I–K. Macroconidiophores. L. Sphaeropedunculate vesicles. M–N. Conidiogenous apparatus with conidiophore branches and doliform to reniform phialides. O. Conidiogenous apparatus with lateral stipe extension. P. Macroconidia. Scale bars: A = 500 μ m; B = 100 μ m (apply to C–D); E = 50 μ m (apply to F, I–K), G = 20 μ m, H = 10 μ m (apply to L–P).

sporulation on the medium surface; reverse sienna to umber after 7 d; chlamydospores extensive throughout the medium forming microsclerotia.

Specimen examined: **China**, Guangxi Province, Fangchenggang, from soil collected in a *Eucalyptus* plantation, Aug. 2009, X. Zhou, G. Zhao & F. Han (**holotype** CBS H-21469, living ex-type CBS 136629 = CMW 31412 = CERC 1747).

Notes: *Calonectria lateralis* is closely related to *C. hongkongensis* and can be distinguished by having smaller macroconidia as compared to *C. hongkongensis*, and the stipe extensions of *C. lateralis* being longer than those of *C. hongkongensis* (Table 2).

***Calonectria magnispora* L. Lombard, Crous & S.F. Chen, sp. nov.** MycoBank MB809052. Fig. 10.

Etymology: Name reflects the characteristically large ascospores produced by this fungus.

Ascomata perithecial, solitary, orange, becoming orange-brown with age; in section, apex and body orange, base red-brown, subglobose to ovoid, 390–495 μ m high, 315–410 μ m diam, body turning dark orange to red, and base dark red-brown in 3% KOH+; ascomatal wall rough, consisting of two thick-walled layers; outer layer of *textura globulosa*, 53–91 μ m thick, cells becoming more compressed towards the inner layer of *textura*

angularis, 16–20 µm thick, cells becoming thin-walled and hyaline towards the centre; outermost cells 16–28 × 10–18 µm, cells of inner layer 9–20 × 3–6 µm; ascomatal base up to 166 µm wide, consisting of dark red, angular cells, merging with an erumpent stroma; cells of the outer wall layer continuous with the pseudoparenchymatous cells of the erumpent stroma. *Asci* 8-spored, clavate, 91–125 × 14–17 µm, tapering into a long thin stalk. *Ascospores* aggregated in the upper third of the ascus, hyaline, guttulate, fusoid with rounded ends, straight to slightly curved, 1-septate, not constricted at the septum, (33–) 36–44(–49) × 5–7(–8) µm (av. 40 × 6 µm). Homothallic. *Macroconidiophores* consisting of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 57–139 × 7–11 µm; stipe extension septate, straight to flexuous, 161–278 µm long, 4–7 µm wide at the apical septum, terminating in a sphaeropedunculate vesicle, 9–18 µm diam; lateral stipe extensions (90° to main axis) moderately formed. *Conidiogenous apparatus* 47–95 µm wide, and 47–80 µm long; primary branches aseptate, 18–35 × 5–9 µm; secondary branches aseptate, 13–23 × 3–7 µm; tertiary branches aseptate, 10–19 × 3–5 µm; quaternary branches aseptate, 12–16 × 3–5 µm, each terminal branch producing 2–6 phialides; phialides doliform to reniform, hyaline, aseptate, 8–16 × 3–5 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (46–)49–55(–60) × 4–6(–7) µm (av. 52 × 5 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies fast growing at 24 °C on MEA, producing abundant white aerial mycelium and sporulating profusely on the medium surface; reverse sienna to umber after 7 d; chlamydo-spores formed abundant throughout the medium, forming microsclerotia.

Specimen examined: China, Guangxi Province, from soil collected in a *Eucalyptus* plantation, Aug. 2009, X. Mou & R. Chang (holotype CBS H-21471, living ex-type culture CBS 136249 = CPC 23509 = CMW 35184 = CERC 1860).

Notes: *Calonectria magnispora* can be distinguished from *C. arbusta*, *C. expansa*, *C. guangxiensis*, *C. hainanensis* and *C. kyotensis* by having larger ascospores and macroconidia. The stipe extensions of *C. magnispora* are also longer than observed in these species (Table 2).

Calonectria microconidialis L. Lombard, Crous & S.F. Chen, **sp. nov.** MycoBank MB809053. Fig. 11.

Etymology: Name refers to the microconidial state that is readily produced by this species.

Ascomata not observed. *Macroconidiophores* consisting of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 59–195 × 7–11 µm; stipe extension septate, straight to flexuous, 175–441 µm long, 4–7 µm wide at the apical septum, terminating in a narrowly clavate vesicle, 3–7 µm diam. *Conidiogenous apparatus* 26–92 µm wide, and 35–95 µm long; primary branches aseptate or 1-septate, 23–34 × 5–7 µm; secondary branches aseptate, 16–28 × 3–6 µm; tertiary

branches aseptate, 14–24 × 3–6 µm, each terminal branch producing 1–3 phialides; phialides cylindrical to allantoid, hyaline, aseptate, 12–25 × 3–5 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (69–) 78–98(–113) × 7–9(–10) µm (av. 88 × 8 µm), 4–6(7)-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Microconidiophores* simple with some lateral branching, comprising a stipe and a penicillate or subverticillate arrangement of fertile branches. *Stipe* septate, hyaline, smooth, 53–86 × 7–8 µm; primary branches aseptate, straight, 19–26 × 4–5 µm, terminating in 1–3 phialides that are cylindrical to allantoid, 12–27 × 4–5 µm; apex with minute periclinal thickening and collarette. *Microconidia* cylindrical, straight, rounded at the apex, flattened at the base, (23–) 31–47(–58) × 4–6(–7) µm (av. 39 × 5 µm), 1–3-septate, held in fascicles by colourless slime. *Megaconidia* not observed.

Culture characteristics: Colonies slow growing at 24 °C on MEA with mycelia immersed in the media, sporulating profusely on the medium surface, forming white to amber colonies with irregular margins; reverse sienna to umber after 7 d. Chlamydo-spores not observed.

Specimens examined: China, Guangdong Province, Zhanjiang, CERC nursery, on *E. urophylla* × *E. grandis* clone seedling leaf, Mar. 2009, G. Zhao (holotype CBS H-21473, culture ex-type CBS 136638 = CMW 31487 = CERC 1822), CBS 136640 = CMW 31492 = CERC 1827 (Herb. CBS H-21474); Guangdong Province, Zhanjiang, CERC nursery, on *E. urophylla* × *E. grandis* clone seedling leaf, Mar. 2009, G. Zhao, CBS 136633 = CMW 31471 = CERC 1806, CBS 136634 = CMW 31473 = CERC 1808, CBS 136636 = CMW 31475 = CERC 1810.

Notes: *Calonectria microconidialis* resides in the *C. reteaudii* complex (Lombard *et al.* 2010d, Crous *et al.* 2012). The ability of *C. microconidialis* to produce microconidiophores and microconidia in culture distinguishes it from *C. pentaseptata*, *C. queenslandica* and *C. terrae-reginae* (Lombard *et al.* 2010d, Crous *et al.* 2012). The micro- and macroconidia of *C. microconidialis* are slightly larger than those of *C. reteaudii* but slightly smaller than those of *C. pseudoreteaudii* (Table 2).

Calonectria papillata L. Lombard, Crous & S.F. Chen, **sp. nov.** MycoBank MB809054. Fig. 12.

Etymology: Name refers to the papillate apices of the stipe vesicles.

Ascomata perithecial, solitary, orange, becoming orange-brown with age; in section, apex and body orange, base red-brown, subglobose to ovoid, 425–455 µm high, 345–395 µm diam, body turning dark orange to red, and base dark red-brown in 3 % KOH+; ascomatal wall rough, consisting of two thick-walled layers; outer layer of *textura globulosa*, 49–57 µm thick, cells becoming more compressed towards the inner layer of *textura angularis*, 21–22 µm thick, cells becoming thin-walled and hyaline towards the centre; outermost cells 25–52 × 21–38 µm, cells of inner layer 11–21 × 5–9 µm; ascomatal base up to 200 µm wide, consisting of dark red, angular cells, merging with an erumpent stroma; cells of the outer wall layer continuous with the pseudoparenchymatous cells of the erumpent stroma. *Asci* 8-spored, clavate, 106–112 × 16–20 µm, tapering into a long thin stalk. *Ascospores* aggregated in the upper third of the ascus, hyaline, guttulate, fusoid with rounded ends, straight to slightly

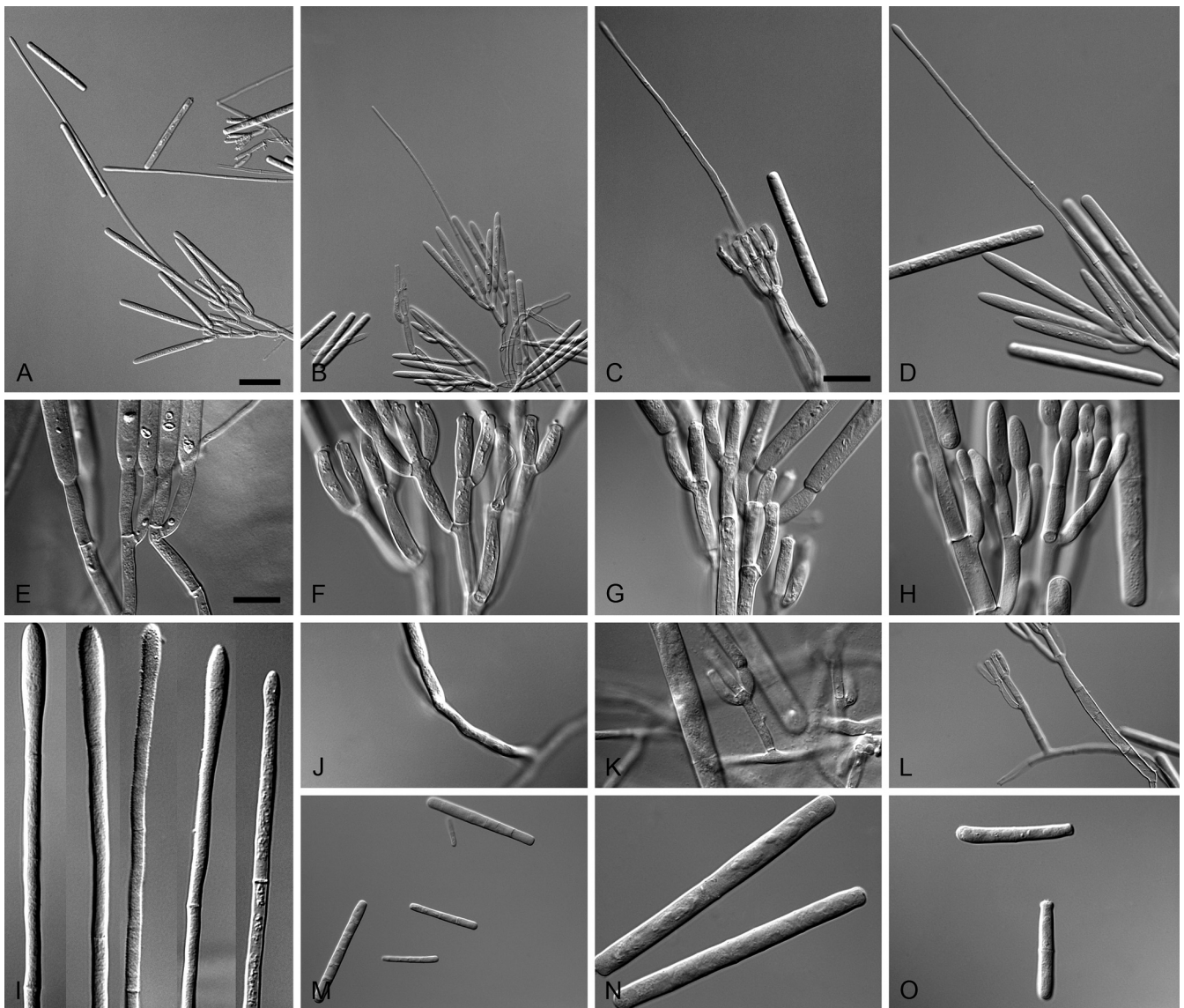


Fig. 11. *Calonectria microconidialis* (ex-type CBS 136638). A–D. Macroconidiophores. E–H. Conidiogenous apparatus with conidiophore branches and cylindrical to allantoid phialides. I. Narrowly clavate vesicles. J–L. Microconidiophores. M–O. Macro- and microconidia. Scale bars: A = 50 μm (apply to B, M); C = 20 μm (apply to D, L); E = 10 μm (apply to F–K, N–O).

curved, 1-septate, constricted at the septum, (27–) 32–40(–46) \times 5–6(–7) μm (av. 36 \times 6 μm). Homothallic. *Macroconidiophores* consisting of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 54–245 \times 6–11 μm ; stipe extension septate, straight to flexuous, 163–218 μm long, 4–7 μm wide at the apical septum, terminating in a obpyriform to ellipsoidal vesicle with a papillate apex, 8–14 μm diam. *Conidiogenous apparatus* 45–114 μm wide, and 33–82 μm long; primary branches aseptate, 18–32 \times 5–9 μm ; secondary branches aseptate, 11–25 \times 4–7 μm ; tertiary branches aseptate, 8–19 \times 2–5 μm ; quaternary branches aseptate, 9–12 \times 3–4 μm , each terminal branch producing 2–4 phialides; phialides doliform to reniform, hyaline, aseptate, 7–16 \times 3–4 μm , apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (40–)43–47(–50) \times (3–)4–5 μm (av. 45 \times 4 μm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies fast growing at 24 °C on MEA, producing abundant white aerial mycelium and sporulating profusely on the medium surface; reverse sienna to umber after 7 d; chlamydospores abundant throughout the medium, forming microsclerotia.

Specimens examined: **China**, Guangdong Province, from soil collected in a *Eucalyptus* plantation, Aug. 2009, X. Mou & R. Chang (**holotype** CBS H-21487, living ex-type culture CBS 136097 = CPC 23517 = CMW 37976 = CERC 1939), Guangdong Province, from soil collected in a *Eucalyptus* plantation, Aug. 2009, X. Mou & R. Chang, CBS 136084 = CPC 23497 = CMW 35165 = CERC 1841, CBS 136096 = CPC 23515 = CMW 37972 = CERC 1935, Guangxi Province, from soil collected in a *Eucalyptus* plantation, Aug. 2009, X. Mou & R. Chang, CBS 136251 = CPC 23514 = CMW 37971 = CERC 1934.

Notes: *Calonectria papillata* can be distinguished from both *C. cerciana* and *C. terrestris* by the papillate apices of the terminal vesicles on the stipe extension. This species is also homothallic, which is not the case for *C. cerciana* (Lombard et al. 2010d) or *C. terrestris*.

Calonectria parakytensis L. Lombard, Crous & S.F. Chen, **sp. nov.** MycoBank MB809055. Fig. 13.

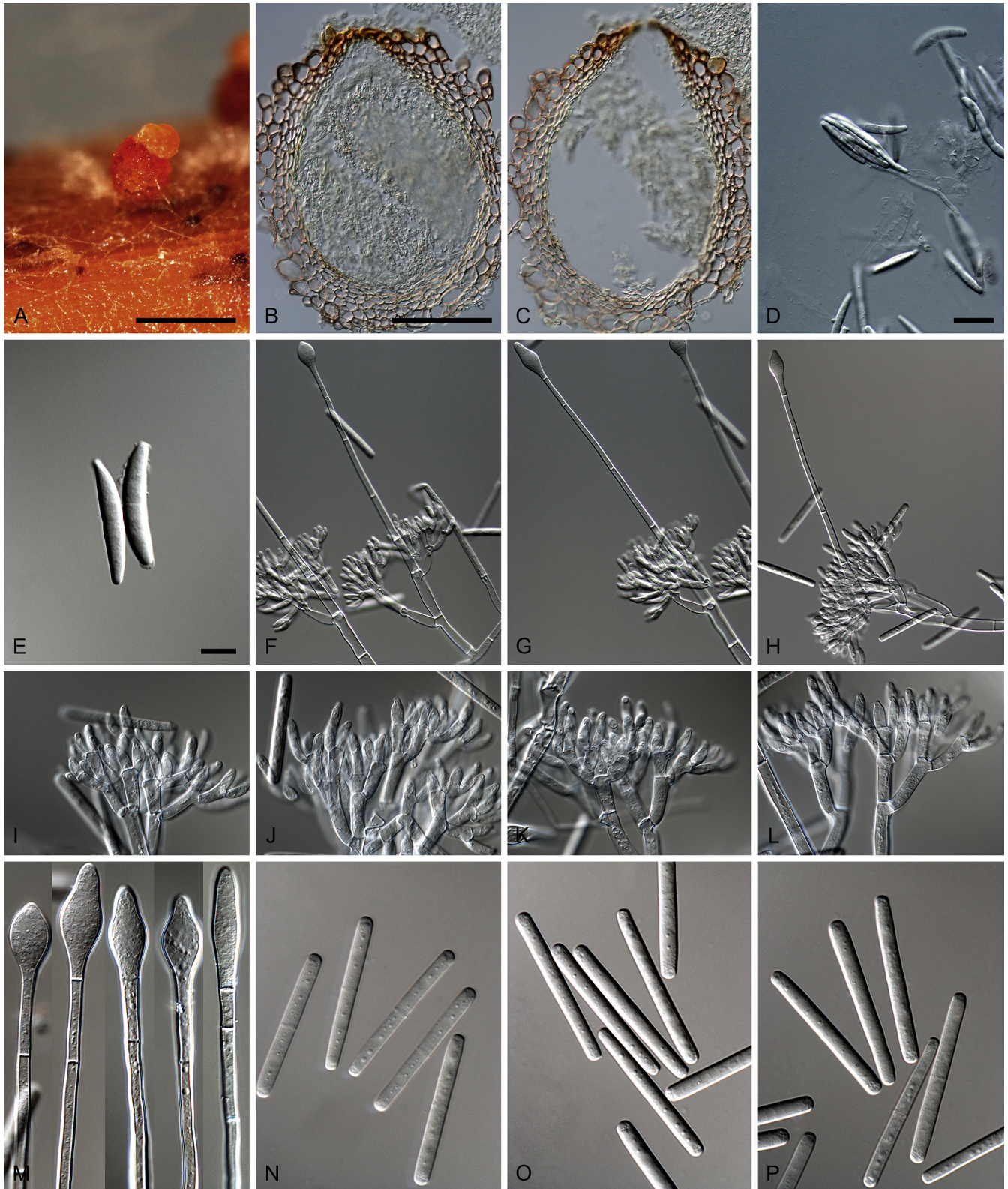


Fig. 12. *Calonectria papillata* (ex-type CBS 136097). A. Ascoma. B–C. Vertical section through ascomata, showing wall structure. D. Asci. E. Ascospores. F–H. Macroconidiophores. I–L. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. M. Obpyriform to ellipsoid vesicles with papillate apex. N–P. Macroconidia. Scale bars: A = 500 μm ; B = 100 μm (apply to C); D = 50 μm (apply to F–H); E = 10 μm (apply to I–P).

Etymology: Name refers to fact that this species has an asexual morph that is very similar to that of *C. kyotensis*.

Ascomata not observed. **Macroconidiophores** consisting of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 42–125 \times 5–9 μm ; stipe extension septate, straight to

flexuous, 135–210 μm long, 4–6 μm wide at the apical septum, terminating in a sphaeropedunculate vesicle, 10–14 μm diam; lateral stipe extensions (90° to main axis) rare. **Conidiogenous apparatus** 49–98 μm wide, and 41–84 μm long; primary branches aseptate, 15–34 \times 5–8 μm ; secondary branches aseptate, 10–17 \times 4–7 μm ; tertiary branches aseptate, 9–17 \times 3–6 μm ; quaternary branches aseptate,

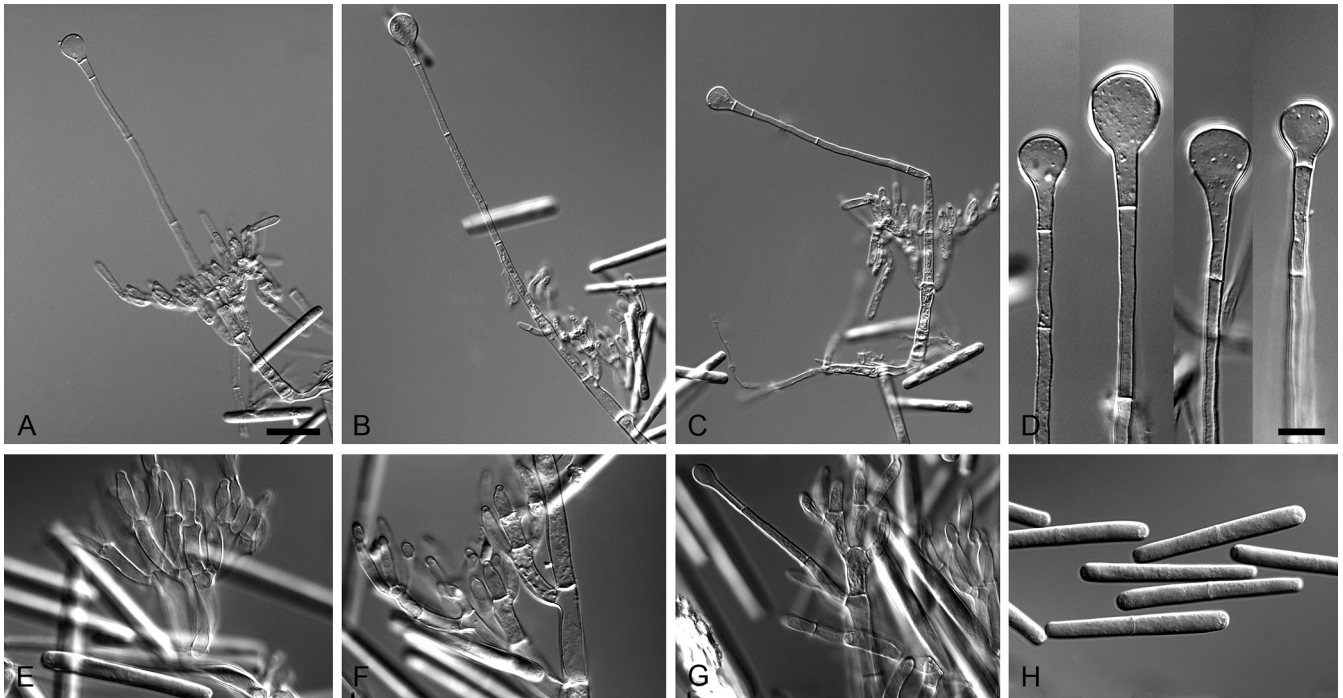


Fig. 13. *Calonectria parakyotensis* (ex-type CBS 136085). A–C. Macroconidiophores. D. Sphaeropedunculate vesicles. E–G. Conidiogenous apparatus with conidiophore branches, doliform to reniform phialides and lateral stipe extensions. H. Macroconidia. Scale bars: A = 50 μ m (apply to B–C); D = 10 μ m (apply to E–H).

11–18 \times 4–6 μ m, each terminal branch producing 2–6 phialides; phialides doliform to reniform, hyaline, aseptate, 10–18 \times 2–6 μ m, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (39–)42–46(–49) \times 4–5(–6) μ m (av. 44 \times 5 μ m), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies fast growing at 24 °C on MEA, producing abundant white aerial mycelium with profuse sporulation on the medium surface; reverse sienna to cinnamon after 7 d; chlamyospores extensive throughout the medium forming microsclerotia.

Specimens examined: **China**, Guangdong Province, from soil collected in a *Eucalyptus* plantation, Aug. 2009, X. Mou & R. Chang (**holotype** CBS H-21470, living ex-type CBS 136085 = CPC 23498 = CMW 35169 = CERC 1845); Guangxi Province, from soil collected in a *Eucalyptus* plantation, Aug. 2009, X. Mou & R. Chang, CBS 136095 = CPC 23508 = CMW 35413 = CERC 1904.

Note: *Calonectria parakyotensis* can be distinguished from other closely related species in the *C. kyotensis* complex by having fewer conidiophore branches and the fact that it rarely forms lateral stipe extensions.

***Calonectria pluriramosa* L. Lombard, Crous & S.F. Chen, sp. nov.** MycoBank MB809056. **Fig. 14.**

Etymology: Name refers to the numerous conidiophore branches formed by this species.

Ascomata not observed. *Macroconidiophores* consisting of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 47–185 \times 6–12 μ m; stipe extension septate, straight to flexuous, 140–215 μ m long, 4–6 μ m wide at the apical septum, terminating

in a sphaeropedunculate vesicle, 6–13 μ m diam; lateral stipe extensions (90° to main axis) rare. *Conidiogenous apparatus* 76–177 μ m wide, and 59–127 μ m long; primary branches aseptate or 1-septate, 17–37 \times 5–8 μ m; secondary branches aseptate, 16–30 \times 4–7 μ m; tertiary branches aseptate, 11–23 \times 4–6 μ m; quaternary branches and additional branches (–7) aseptate, 9–20 \times 3–6 μ m, each terminal branch producing 2–6 phialides; phialides doliform to reniform, hyaline, aseptate, 8–13 \times 3–5 μ m, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (41–)44–50(–52) \times (3–)4–5(–6) μ m (av. 47 \times 5 μ m), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies fast growing at 24 °C on MEA, producing abundant white to sienna aerial mycelium with profuse sporulation on the medium surface; reverse sienna to umber after 7 d; chlamyospores extensive throughout the medium forming microsclerotia.

Specimen examined: **China**, Guangxi Province, Fangchenggang, from soil collected in a *Eucalyptus* plantation, Mar. 2009, X. Zhou, G. Zhao & F. Han (**holotype** CBS H-21485, living ex-type culture CBS 136976 = CMW 31440 = CERC 1775).

Notes: *Calonectria pluriramosa* is closely related to *C. kyotensis* and *C. pseudokyotensis* but can be distinguished by having a greater number of conidiophore branches. The macroconidia of *C. pluriramosa* are larger than those of *C. kyotensis* (Table 2). Unlike the latter two species, *C. pluriramosa* also failed to produce viable ascomata in culture.

***Calonectria pseudokyotensis* L. Lombard, Crous & S.F. Chen, sp. nov.** MycoBank MB809057. **Fig. 15.**

Etymology: Name refers to the morphological similarity to the asexual morph of *C. kyotensis*.

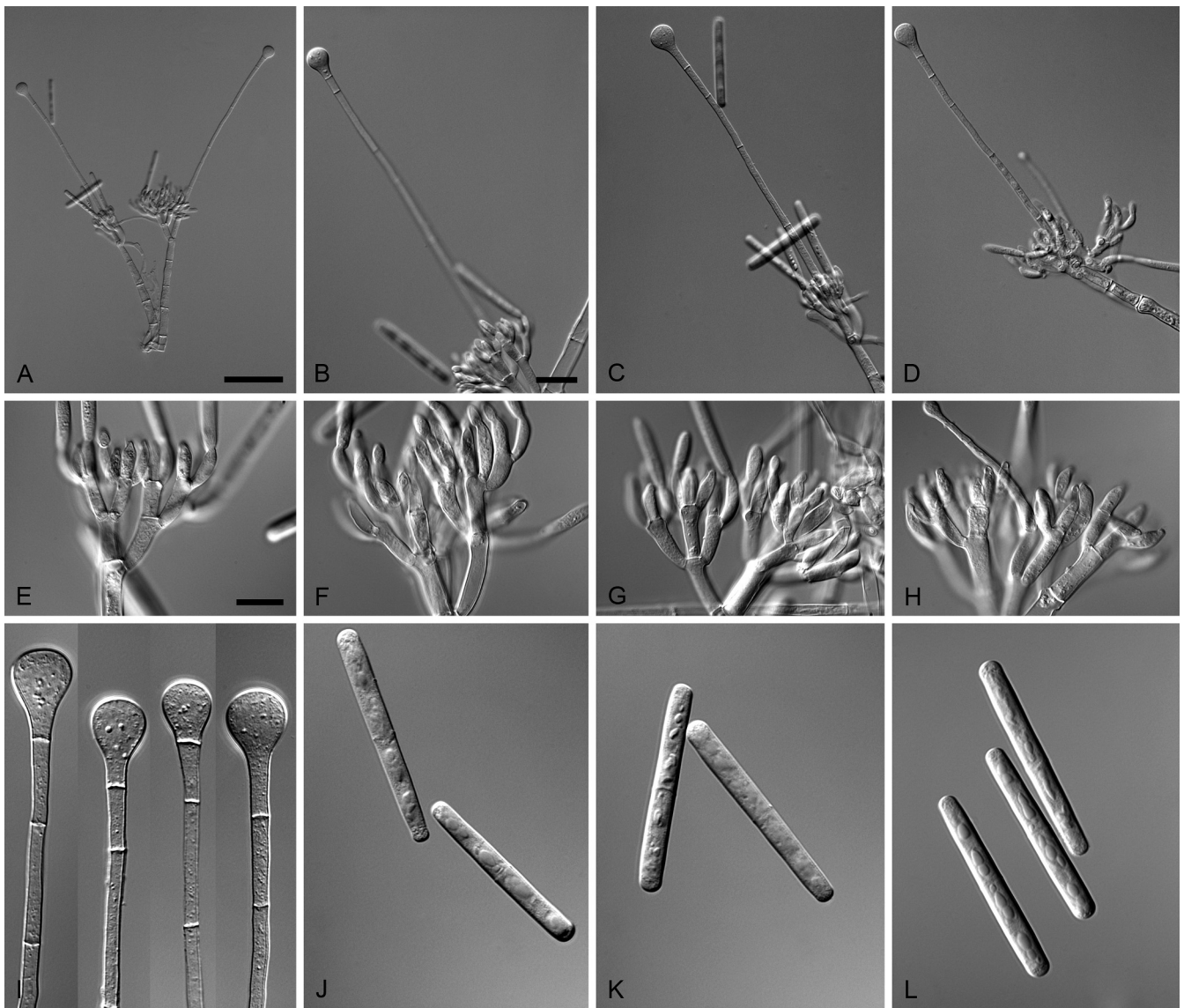


Fig. 14. *Calonectria pluriramosa* (ex-type CBS 136976). A–D. Macroconidiophores. E–H. Conidiogenous apparatus with conidiophore branches, dolliiform to reniform phialides and lateral stipe extensions. I. Sphaeropedunculate vesicles. J–L. Macroconidia. Scale bars: A = 100 μ m; B = 20 μ m (apply to C–D); E = 10 μ m (apply to F–L).

Ascomata not observed. *Macroconidiophores* consisting of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 85–205 \times 6–10 μ m; stipe extension septate, straight to flexuous, 145–320 μ m long, 5–7 μ m wide at the apical septum, terminating in a pyriform to sphaeropedunculate vesicle, 10–13 μ m diam; lateral stipe extensions (90° to main axis) moderate. *Conidiogenous apparatus* 42–103 μ m wide, and 76–109 μ m long; primary branches aseptate or 1-septate, 24–40 \times 5–8 μ m; secondary branches aseptate, 14–32 \times 5–7 μ m; tertiary branches aseptate, 13–25 \times 4–6 μ m; quaternary branches aseptate, 14–24 \times 4–6 μ m, each terminal branch producing 2–6 phialides; phialides elongate dolliiform to reniform, hyaline, aseptate, 10–20 \times 3–5 μ m, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (43–) 45–51(–53) \times 5–7 μ m (av. 48 \times 6 μ m), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies fast growing at 24 °C on MEA, producing abundant white aerial mycelium with profuse

sporulation on the medium surface; reverse sienna to umber after 7 d; chlamydospores extensive throughout the medium forming microsclerotia.

Specimen examined: **China**, Guangxi Province, Fangchenggang, from soil collected in a *Eucalyptus* plantation, Mar. 2009, X. Zhou, G. Zhao & F. Han (**holotype** CBS H-21774, living ex-type culture CBS 137332 = CMW 31439 = CERC 1774).

Notes: *Calonectria pseudokyotensis* has fewer fertile branches than *C. kyotensis*. Furthermore, the stipe extensions of *C. pseudokyotensis* are longer than those of *C. kyotensis*, terminating in pyriform to sphaeropedunculate vesicles, not observed in *C. kyotensis* (Table 2).

Calonectria seminaria L. Lombard, Crous & S.F. Chen, **sp. nov.** MycoBank MB809058. Fig. 16.

Etymology: Name refers the fact that this species was collected in a nursery.

Ascomata not observed. *Macroconidiophores* consisting of a stipe bearing a penicillate arrangement of fertile branches, and a stipe

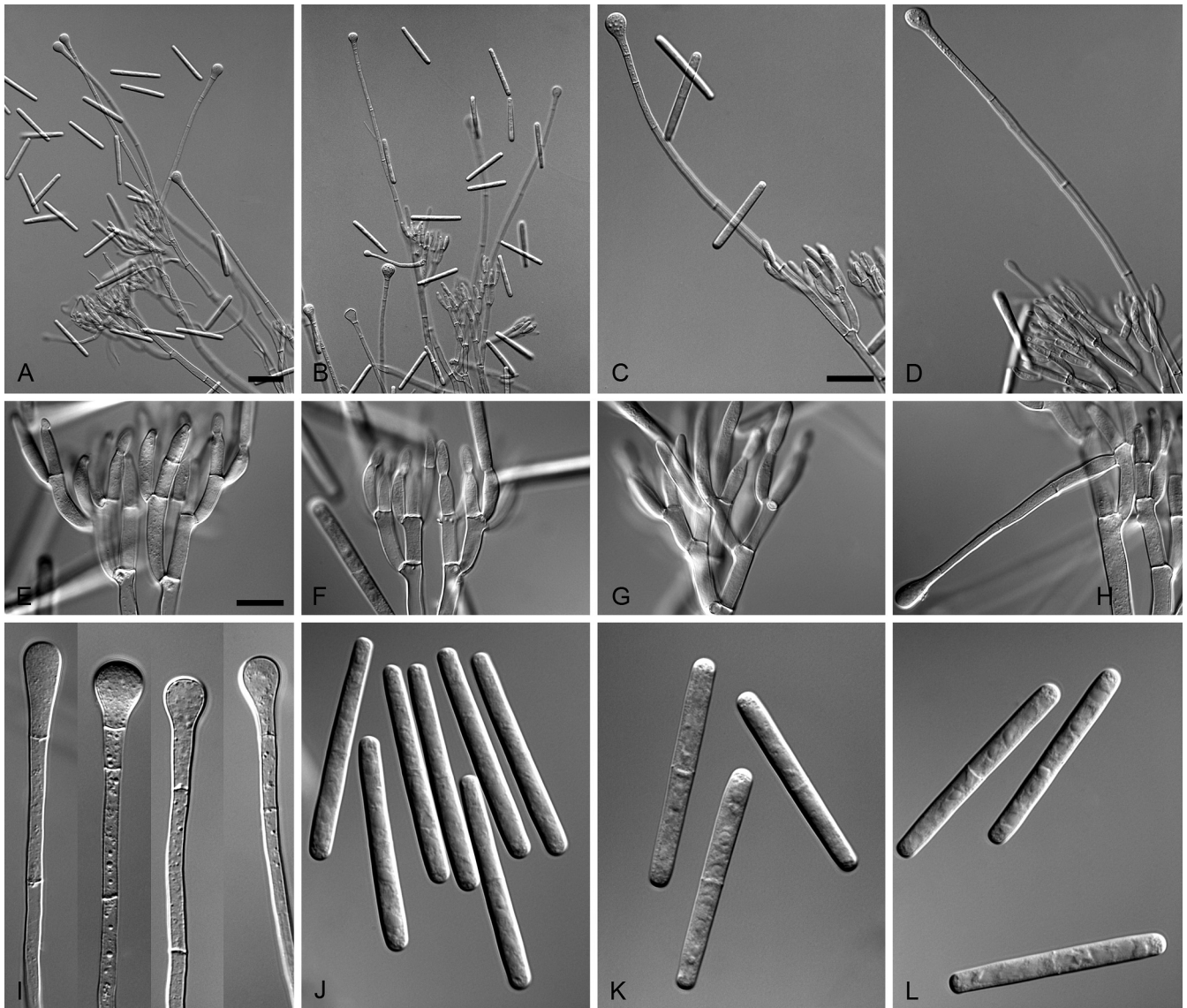


Fig. 15. *Calonectria pseudokyotensis* (ex-type CBS 137332). A–D. Macroconidiophores. E–H. Conidiogenous apparatus with conidiophore branches, doliiform to reniform phialides and lateral stipe extensions. I. Pyriform to sphaeropedunculate vesicles. J–L. Macroconidia. Scale bars: A = 50 μm (apply to B); C = 20 μm (apply to D); E = 10 μm (apply to F–L).

extension terminating in a vesicle; stipe septate, hyaline, smooth, 39–101 \times 6–10 μm ; stipe extension septate, straight to flexuous, 105–185 μm long, 4–7 μm wide at the apical septum, terminating in an obpyriform to ellipsoid vesicle, 6–11 μm diam. *Conidiogenous apparatus* 31–155 μm wide, and 36–72 μm long; primary branches aseptate or 1-septate, 13–27 \times 3–6 μm ; secondary branches aseptate, 8–19 \times 2–5 μm ; tertiary branches aseptate, 10–20 \times 2–6 μm , each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 7–14 \times 2–4 μm , apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (42–) 45–49(–52) \times 3.5–4.5(–7) μm (av. 47 \times 5 μm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies fast growing at 24 $^{\circ}\text{C}$ on MEA, producing abundant white aerial mycelium and sporulating profusely on the medium surface; reverse amber to sepia-brown after 7 d; chlamydospores formed extensively in the media, forming microsclerotia.

Specimens examined: **China**, Guangdong Province, Zhanjiang, CERC nursery, on *E. urophylla* \times *E. grandis* clone seedling leaf, Mar. 2009, G. Zhao (**holotype** CBS

H-21475, living ex-type culture CBS 136632 = CPC 23488 = CMW 31450 = CERC 1785); Guangdong Province, Zhanjiang, CERC nursery, on *E. urophylla* \times *E. grandis* clone seedling leaf, Mar. 2009, G. Zhao, CBS 136630 = CMW 31446 = CERC 1781, CBS 136631 = CMW 31449 = CERC 1784, CPC 23486 = CMW 31447 = CERC 1782, CPC 23487 = CMW 31448 = CERC 1783, CBS 136639 = CMW 31489 = CERC 1824; Guangxi Province, on leaf of *Eucalyptus* in plantation, Aug. 2009, X. Mou & R. Chang, CBS 136648 = CMW 37970 = CERC 1933.

Notes: *Calonectria seminaria* belongs to the *C. candelabra* species complex (Schoch et al. 1999, Lombard et al. 2010a; see Lombard et al. 2015), closely related to *C. pauciramosa* and *C. polizzii*. The macroconidia of *C. seminaria* are slightly smaller than those of *C. pauciramosa*, and larger than those of *C. polizzii* (Table 2).

Calonectria sphaeropedunculata L. Lombard, Crous & S.F. Chen, **sp. nov.** MycoBank MB809059. Fig. 17.

Etymology: Name refers to the sphaeropedunculate vesicles produced by this species.

Ascomata perithecial, solitary or in groups of two, orange, becoming orange-brown with age; in section, apex and body

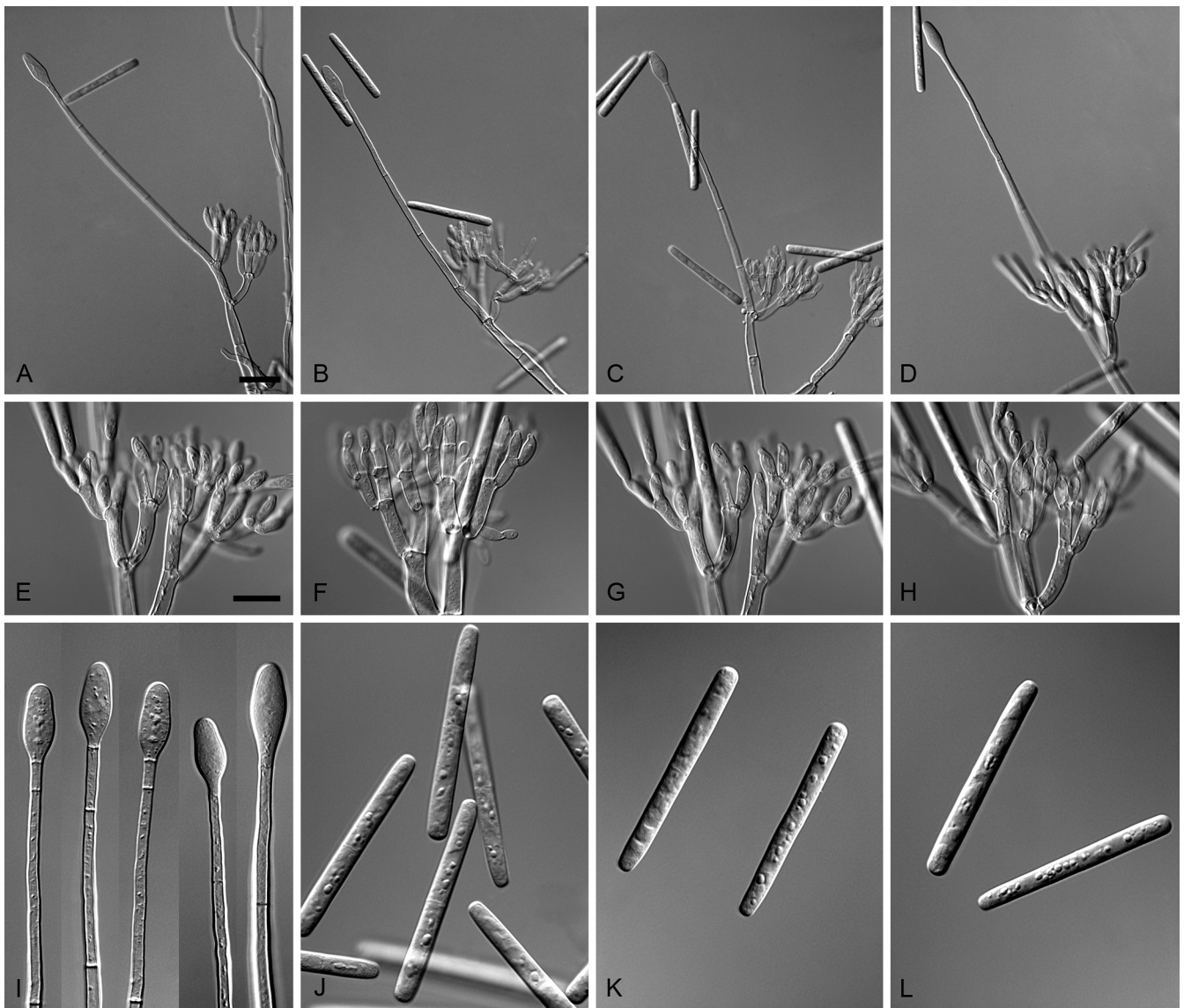


Fig. 16. *Calonectria seminaria* (ex-type CBS 136632). A–D. Macroconidiophores. E–H. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. I. Obpyriform to ellipsoid vesicles. J–L. Macroconidia. Scale bars: A = 100 μm (apply to B–D); E = 10 μm (apply to F–L).

orange, base red-brown, subglobose to ovoid, 470–575 μm high, 345–465 μm diam, body turning dark orange to red, and base dark red-brown in 3% KOH+; ascomatal wall rough, consisting of two thick-walled layers; outer layer of *textura globulosa*, 40–80 μm thick, cells becoming more compressed towards the inner layer of *textura angularis*, 14–21 μm thick, cells becoming thin-walled and hyaline towards the centre; outermost cells 22–36 \times 14–22 μm , cells of inner layer 13–32 \times 6–8 μm ; ascomatal base up to 216 μm wide, consisting of dark red, angular cells, merging with an erumpent stroma; cells of the outer wall layer continuous with the pseudoparenchymatous cells of the erumpent stroma. Asci 8-spored, clavate, 82–144 \times 11–23 μm , tapering into a long thin stalk. Ascospores aggregated in the upper third of the ascus, hyaline, guttulate, fusoid with rounded ends, straight to slightly curved, 1-septate, not constricted at the septum, (31–) 33–40(–42) \times 5–7(–8) μm (av. 37 \times 6 μm). Homothallic. Macroconidiophores consisting of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 62–183 \times 7–12 μm ; stipe extension septate, straight to flexuous, 152–253 μm long, 4–8 μm wide at the apical septum, terminating in a sphaeropedunculate vesicle, 10–14 μm diam; lateral stipe extensions

(90° to main axis) formed moderately. Conidiogenous apparatus 63–144 μm wide, and 40–111 μm long; primary branches aseptate or 1-septate, 18–36 \times 4–10 μm ; secondary branches aseptate, 11–29 \times 5–9 μm ; tertiary branches aseptate, 14–23 \times 5–8 μm ; quaternary and additional branches (–6) aseptate, 9–19 \times 4–7 μm , each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 9–17 \times 3–5 μm , apex with minute periclinal thickening and inconspicuous collarete. Macroconidia cylindrical, rounded at both ends, straight, (40–) 43–47(–49) \times 4–6 μm (av. 46 \times 5 μm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. Mega- and microconidia not observed.

Culture characteristics: Colonies fast growing at 24 °C on MEA, producing abundant white to cinnamon aerial mycelium with profuse sporulation on the medium surface; reverse sienna to umber after 7 d; chlamydospores extensive throughout the medium forming microsclerotia.

Specimen examined: China, Guangxi Province, from soil collected in a *Eucalyptus* plantation, Mar. 2009, X. Zhou, G. Zhao & F. Han (holotype CBS H-21486, culture ex-type CBS 136081 = CPC 23484 = CMW 31390 = CERC 1725).

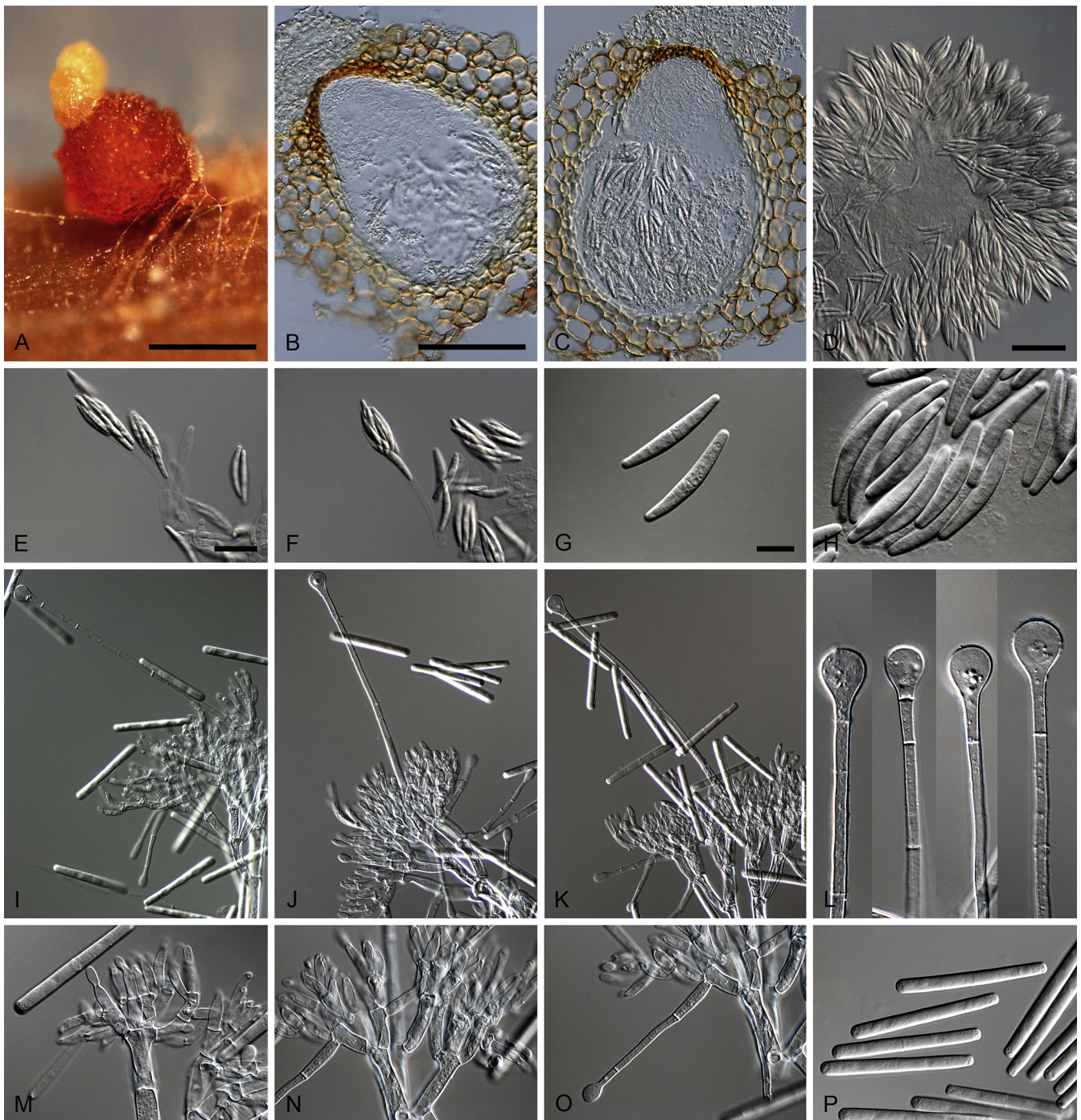


Fig. 17. *Calonectria sphaeropedunculata* (ex-type CBS 136081). A. Ascoma. B–C. Vertical section through ascomata, showing wall structure. D–F. Asci. G–H. Ascospores. I–K. Macroconidiophores. L. Sphaeropedunculate vesicles. M–O. Conidiogenous apparatus with conidiophore branches, dolliiform to reniform phialides and lateral stipe extension. P. Macroconidia. Scale bars: A = 500 μ m; B = 100 μ m (apply to C); D = 50 μ m (apply to I–K), E = 20 μ m (apply to F), G = 10 μ m (apply to H, L–P).

Notes: *Calonectria sphaeropedunculata* produces longer stipe extensions than those of *C. kyotensis* and *C. pluriramosa*, but shorter extensions than those of *C. pseudokyotensis*. The macroconidia of *C. sphaeropedunculata* are also smaller than those of *C. kyotensis*, *C. pluriramosa* and *C. pseudokyotensis* (Table 2).

Calonectria terrestris L. Lombard, Crous & S.F. Chen, **sp. nov.** MycoBank MB809060. Fig. 18.

Etymology: Name refers to the fact that this fungus was isolated from soil.

Ascomata not observed. **Macroconidiophores** consisting of a stipe bearing a penicillate arrangement of fertile branches, and a

stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 35–185 \times 6–10 μ m; stipe extension septate, straight to flexuous, 147–228 μ m long, 4–7 μ m wide at the apical septum, terminating in an obpyriform to pyriform to broadly clavate vesicle, 5–12 μ m diam. **Conidiogenous apparatus** 35–89 μ m wide, and 35–102 μ m long; primary branches aseptate, 21–35 \times 5–8 μ m; secondary branches aseptate, 15–27 \times 4–7 μ m; tertiary branches aseptate, 10–18 \times 4–6 μ m; quaternary branches aseptate, 9–14 \times 3–6 μ m, each terminal branch producing 2–4 phialides; phialides dolliiform to reniform, hyaline, aseptate, 8–12 \times 3–5 μ m, apex with minute periclinal thickening and inconspicuous collarette. **Macroconidia** cylindrical, rounded at both ends, straight, (33–)36–40(–41) \times (3–) 4–5 μ m (av. 38.5 \times 4.5 μ m), 1-septate, lacking a visible

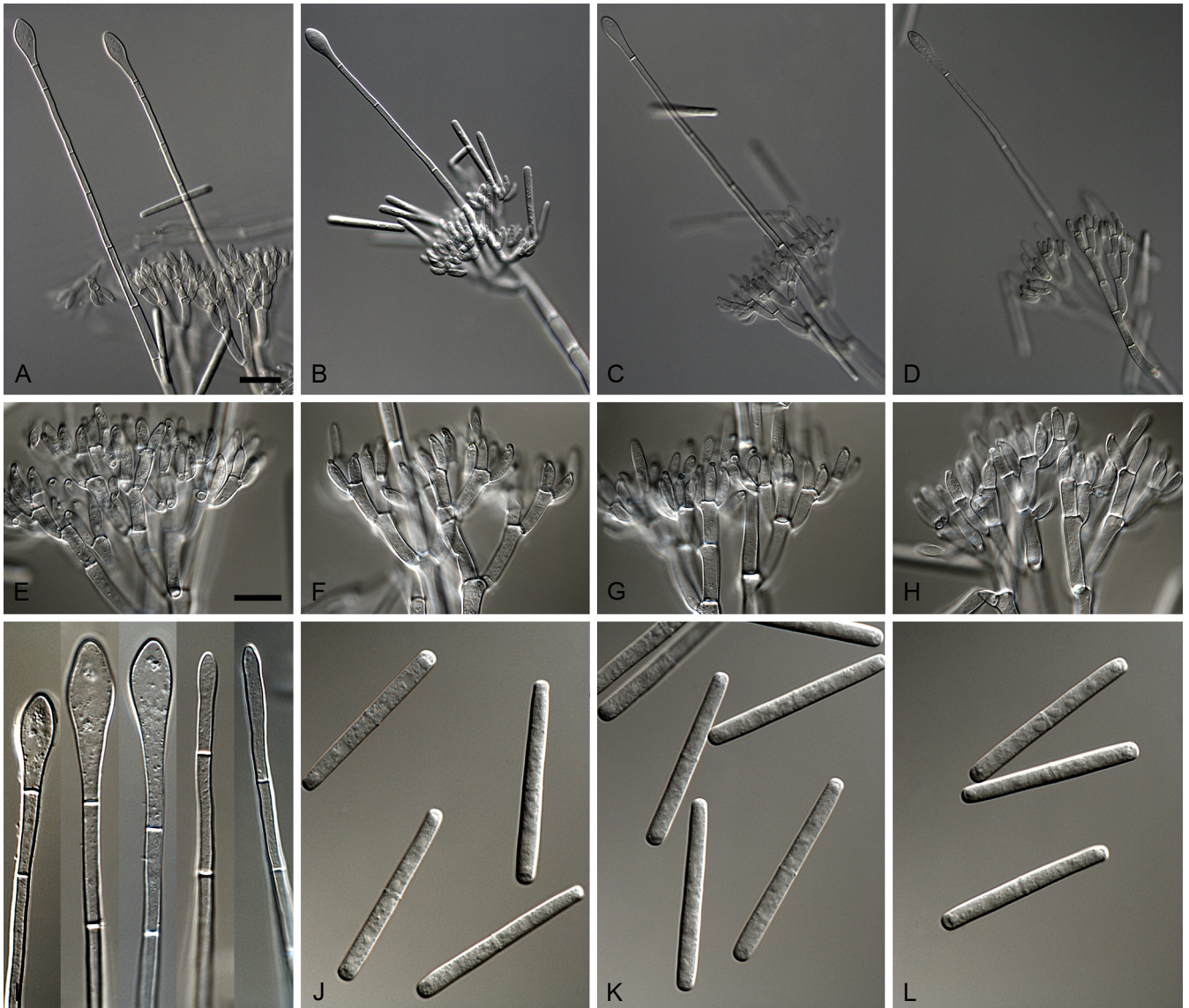


Fig. 18. *Calonectria terrestris* (ex-type CBS 136642). A–D. Macroconidiophores. E–H. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. I. Obpyriform to pyriform to broadly clavate vesicles. J–L. Macroconidia. Scale bars: A = 50 μ m (apply to B–D); E = 10 μ m (apply to F–L).

abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies fast growing at 24 °C on MEA, producing abundant white to pale luteous aerial mycelium and sporulating profusely on the medium surface; reverse sienna to umber after 7 d; chlamydospores formed abundant throughout the medium, forming microsclerotia.

Specimens examined: **China**, Guangdong Province, from soil collected in a *Eucalyptus* plantation, Aug. 2009, X. Mou & R. Chang (**holotype** CBS H-21478, culture ex-type CBS 136642 = CMW 35180 = CERC 1856), CBS 136643 = CPC 23493 = CMW 35364 = CERC 1868, CBS 136644 = CPC 23494 = CMW 35366 = CERC 1870, CBS 136645 = CPC 23496 = CMW 35178 = CERC 1854, CBS 136651 = CPC 23516 = CMW 37974 = CERC 1937 (CBS H-21479); Guangdong province, from leaf of *Eucalyptus*, Aug. 2009, X. Mou & R. Chang, CBS 136647 = CPC 23510 = CMW 35447 = CERC 1919; Guangxi Province, from soil collected in a *Eucalyptus* plantation, Aug. 2009, X. Mou & R. Chang, CBS 136653 = CPC 23518 = CMW 37980 = CERC 1943.

Notes: *Calonectria terrestris* can be distinguished from *C. cerciana* and *C. papillata* by its obpyriform to pyriform to broadly clavate vesicles rather than the fusiform to obpyriform vesicles of *C. cerciana*, and obpyriform to ellipsoidal vesicles

with papillate apex of *C. papillata*. The macroconidia of *C. terrestris* are also slightly smaller than those of *C. cerciana* and *C. papillata* (Table 2).

Calonectria tetraramosa L. Lombard, Crous & S.F. Chen, **sp. nov.** MycoBank MB809061. **Fig. 19.**

Etymology: Name refers to the four levels of fertile branches produced by this species.

Ascomata not observed. **Macroconidiophores** consisting of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 47–109 \times 6–9 μ m; stipe extension septate, straight to flexuous, 102–253 μ m long, 3–6 μ m wide at the apical septum, terminating in a obpyriform vesicle, 4–10 μ m diam. **Conidiogenous apparatus** 54–95 μ m wide, and 36–75 μ m long; primary branches aseptate, 15–29 \times 4–7 μ m; secondary branches aseptate, 10–20 \times 3–6 μ m; tertiary branches aseptate, 9–15 \times 3–6 μ m; quaternary branches aseptate, 10–13 \times 3–4 μ m, each terminal branch producing 2–6 phialides; phialides elongate doliiform to reniform, hyaline, aseptate,

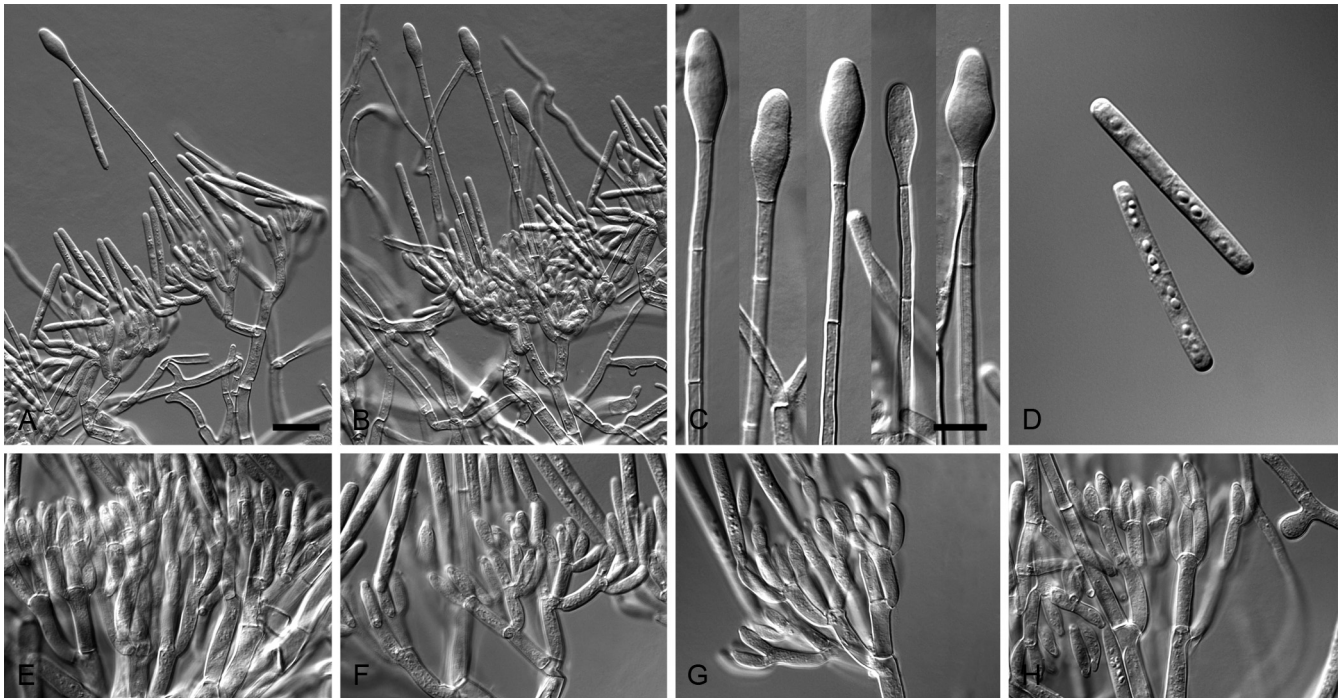


Fig. 19. *Calonectria tetraramosa* (ex-type CBS 136635). A–B. Macroconidiophores. C. Obpyriform vesicles. D. Macroconidia. E–H. Conidiogenous apparatus with conidiophore branches and doliform to reniform phialides. Scale bars: A = 50 μ m (apply to B); C = 10 μ m (apply to D–H).

8–14 \times 3–5 μ m, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (45–)46.5–49.5(–51) \times (4–)4.5–5.5(–6) μ m (av. 48 \times 5 μ m), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies fast growing at 24 $^{\circ}$ C on MEA, producing abundant white aerial mycelium and sporulating profusely on the medium surface; reverse amber to sepia-brown after 7 d; chlamydospores formed extensively in the media, forming microsclerotia.

Specimen examined: China, Guangdong Province, Zhanjiang, CERC nursery, on *E. urophylla* \times *E. grandis* clone seedling leaf, Mar. 2009, G. Zhao (**holotype** CBS H-21477, living ex-type culture CBS 136635 = CPC 23489 = CMW 31474 = CERC 1809), CBS 136637 = CMW 31476 = CERC 1811.

Notes: *Calonectria tetraramosa* is closely related to *C. pauciramosa*, *C. polizzii* and *C. seminaria* in the *C. candelabra* complex (Schoch et al. 1999, Lombard et al. 2010a). It can be distinguished from these three species by quaternary branches in the conidiogenous apparatus, which are not found in the other species. Furthermore, the macroconidia of *C. tetraramosa* are slightly smaller than those of *C. pauciramosa*, larger than those of *C. polizzii*, but similar to those of *C. seminaria*. The stipe extensions of *C. tetraramosa* are also longer than those of *C. pauciramosa*, *C. polizzii* and *C. seminaria* (Table 2).

Calonectria turangicola L. Lombard, Crous & S.F. Chen, sp. nov. MycoBank MB809062. Fig. 20.

Etymology: Name refers to the Chinese word for soil (Tǔrǎng), the substrate from which this fungus was first isolated.

Ascomata not observed. *Macroconidiophores* consisting of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 45–122 \times 6–9 μ m; stipe extension septate, straight to flexuous, 133–195 μ m long, 4–6 μ m wide at the apical septum, terminating in a sphaeropedunculate vesicle, 8–12 μ m diam; lateral stipe extensions (90 $^{\circ}$ to main axis) abundant. *Conidiogenous apparatus* 48–110 μ m wide, and 35–86 μ m long; primary branches aseptate, 16–30 \times 4–7 μ m; secondary branches aseptate, 10–18 \times 3–6 μ m; tertiary branches aseptate, 9–17 \times 3–5 μ m; quaternary and additional branches (–5) aseptate, 10–16 \times 3–5 μ m, each terminal branch producing 2–6 phialides; phialides doliform to reniform, hyaline, aseptate, 8–16 \times 3–7 μ m, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (40–)42–46(–47) \times 3–5 μ m (av. 44 \times 4 μ m), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies fast growing at 24 $^{\circ}$ C on MEA, producing abundant white to sienna aerial mycelium with profuse sporulation on the medium surface; reverse sienna to umber after 7 d; chlamydospores extensive throughout the medium forming microsclerotia.

Specimens examined: China, Guangxi Province, Fangchenggang, from soil collected in a *Eucalyptus* plantation, Mar. 2009, X. Zhou, G. Zhao & F. Han (**holotype** CBS H-21488, culture ex-type CBS 136077 = CPC 23479 = CMW 31411 = CERC 1746); Guangxi Province, from soil collected in a *Eucalyptus* plantation, Aug. 2009, X. Mou & R. Chang, CBS 136093 = CMW 35410 = CERC 1901, CBS 136652 = CMW 37977 = CERC 1940; Hainan Province, from soil collected in a *Eucalyptus* plantation, Aug. 2009, X. Mou & S.F. Chen, CMW 35383 = CERC 1885.

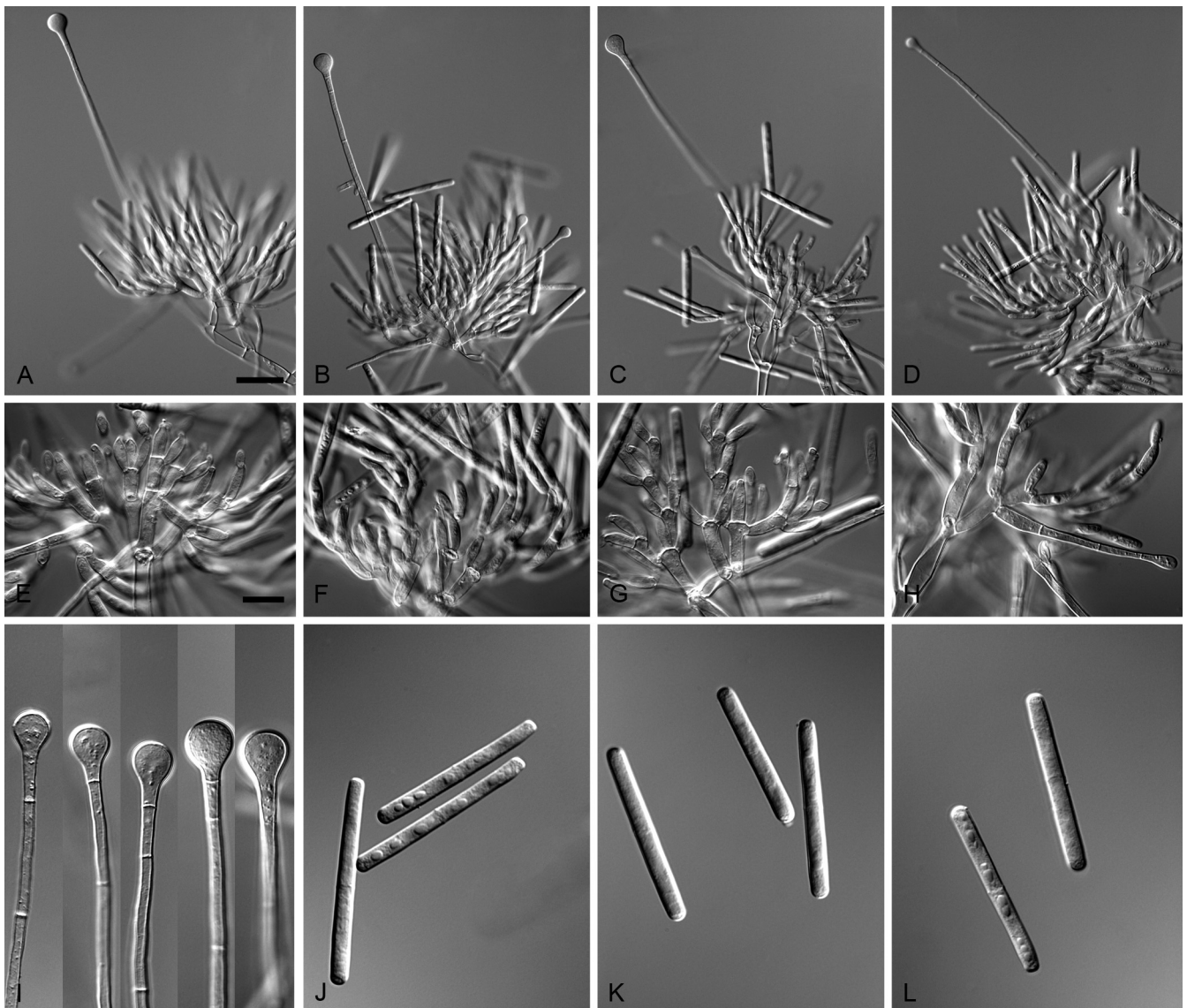


Fig. 20. *Calonectria turangicola* (ex-type CBS 136077). A–D. Macroconidiophores. E–H. Conidiogenous apparatus with conidiophore branches, doliiform to reniform phialides and lateral stipe extension. I. Sphaeropedunculate vesicles. J–L. Macroconidia. Scale bars: A = 50 μ m (apply to B–D); E = 10 μ m (apply to F–L).

Note: The macroconidia of *C. turangicola* are slightly smaller than those of *C. hongkongensis* but larger than those of *C. lateralis* (Table 2).

DISCUSSION

A surprisingly large number of *Calonectria* species were collected from soils and *Eucalyptus* tissue in a relatively small area of southern China. Phylogenetic inference was used to define the species boundaries but these were in most cases also well-supported by morphological features. The 18 new species described in this study add to the eleven species previously recognised in the Southern provinces of China (Crous *et al.* 2004b, Lombard *et al.* 2010d, Chen *et al.* 2011d, Xu *et al.* 2012).

Most of the isolates obtained from *Eucalyptus* leaves displaying symptoms of CLB were identified as *C. pentaseptata*, which was recently described in the *C. reteaudii* complex from Vietnam (Crous *et al.* 2012), making this the first report of the fungus from China. *Calonectria pentaseptata* was collected in all three provinces sampled, including the sampled nursery surveyed, with a single isolate obtained from soil collected in Hainan

Province. The collection data suggest that this fungus could be amongst the more important *Eucalyptus* leaf and shoot pathogens but this hypothesis will need testing experimentally.

Calonectria microconidialis, which was collected from *Eucalyptus* leaves in the nursery, resides in the *C. reteaudii* species complex, which now includes six species (Lombard *et al.* 2010d). The only other species in this complex known from China is *C. pseudoreteaudii* (Lombard *et al.* 2010d, Chen *et al.* 2011d). *Calonectria microconidialis* produces microconidiophores in culture, a characteristic shared with *C. reteaudii* and *C. pseudoreteaudii*, but distinguishing it from *C. pentaseptata*, *C. queenslandica* and *C. terrae-reginae* (Lombard *et al.* 2010d, Crous *et al.* 2012). Species of the *C. reteaudii* complex are well-known causal agents of CLB in Australia, South America and Southeast Asia (Pitkethley 1976, Bolland *et al.* 1985, Sharma & Mohanan 1991, 1992, Booth *et al.* 2000, Kang *et al.* 2001a, Crous 2002, Rodas *et al.* 2005, Lombard *et al.* 2010d), but the pathogenicity of *C. microconidialis* and *C. pentaseptata* will need to be tested experimentally.

Calonectria seminaria and *C. tetraramosa* were found together with *C. microconidialis* in the nursery sampled in this study. These species represent new members of the

C. candelabra complex (Schoch et al. 1999, Lombard et al. 2015), which includes several well-known nursery pathogens (Schoch et al. 1999, Koike et al. 1999, Polizzi & Crous 1999, Polizzi 2000, Koike & Crous 2001, Polizzi & Catara 2001, Polizzi & Vitale 2001, Crous 2002, Polizzi et al. 2006, 2007, 2009, Vitale et al. 2009, Lombard et al. 2010a, d, Vitale et al. 2013, Guarnaccia et al. 2014, Alfenas et al. 2015). The *C. candelabra* complex now includes 16 species (Schoch et al. 1999, Crous 2002, Lombard et al. 2010a, Alfenas et al. 2013a, 2015) and has the highest diversity of species found in South America (Schoch et al. 1999, 2001, Alfenas et al. 2015, see this volume). Although *C. pauciramosa* has been regarded as the dominant *Eucalyptus* nursery pathogen in previous studies (Schoch et al. 1999, Crous 2002, Lombard et al. 2010a), it was not isolated here. Although it has previously also been found in China (Lombard et al. 2010d) it is clearly not as common as it is elsewhere in the world such as in South America and Southern Africa.

This study included the description of three new species, *C. follicola*, *C. papillata* and *C. terrestris* in the *C. cylindrospora* complex (Crous et al. 1993, Schoch et al. 1999, 2001, Lombard et al. 2010d, Alfenas et al. 2013b, 2015; see Lombard et al. 2015), which displays a similarly high level of species diversity in South America (Alfenas et al. 2013b, 2015). *Calonectria papillata* and *C. terrestris* are sibling species of *C. cerciana*, but can be distinguished by their characteristic terminal vesicles and the morphology of their macroconidia. *Calonectria papillata* is also homothallic, a feature not known in *C. cerciana* (Lombard et al. 2010d) nor in *C. terrestris* described in this study. Both *C. papillata* and *C. terrestris* were isolated from soils collected in Guangdong Province, but only a single isolate of *C. terrestris* was obtained from a *Eucalyptus* leaf collected from the same province. *Calonectria follicola*, isolated from *Eucalyptus* leaves collected in Guangxi Province, is closely related to *C. brasiliensis* and *C. sulawesiensis*, but can be distinguished from those species based on its macroconidiophore morphology. Although some members of the *C. cylindrospora* complex are well-known pathogens (Crous 2002, Lombard et al. 2010c), nothing is known regarding the pathogenicity of *C. follicola*, *C. papillata* and *C. terrestris*.

Most isolates of *Calonectria* spp. baited from soils in this study belonged to *C. hongkongensis*, a member of the *C. kyotensis* complex (Crous et al. 2004b) and the Sphaero-Naviculate Group (Lombard et al. 2010b). This fungus is characterised by its sphaeropedunculate terminal vesicles, a common feature for all members of the *C. kyotensis* complex (Crous et al. 2004b), and they also all have up to eight conidiophore branches (Crous et al. 2004b). Like *C. hongkongensis*, its sibling species *C. turangicola* and *C. lateralis* described here, were also isolated exclusively from soil. These species can be distinguished from *C. hongkongensis* by having fewer conidiophore branches and from each other based on the morphology of their macroconidia.

Results of this study add 10 species to the *C. kyotensis* complex, which includes *C. aconidialis*, *C. arbusta*, *C. expansa*, *C. guangxiensis*, *C. hainanensis*, *C. magnispora*, *C. parakyotensis*, *C. pseudokyotensis*, *C. pluriramosa* and *C. sphaeropedunculata*. All 10 species were isolated from soils collected in all three provinces, although nothing is thus far known regarding their pathogenicity.

Calonectria aconidialis produced only its sexual morph in this study, despite many attempts to stimulate the production of

conidiophores and conidia. However, sibling species such as *C. arbusta* and *C. expansa* formed both morphs in cultures derived from single conidia, and were thus homothallic. *Calonectria parakyotensis*, also a sibling species of *C. aconidialis*, failed to produce a sexual morph during this study. Different mating systems in species within related groups of *Calonectria* spp. are well-known and have been reported for members of the *C. candelabra* (Lombard et al. 2010a) and *C. kyotensis* complexes (Crous et al. 2004b). *Calonectria aconidialis* was found only in samples from the Hainan Province, and *C. arbusta* only in Guangxi, whereas both *C. expansa* and *C. parakyotensis* were found in soils collected in Guangdong and Guangxi provinces. Whether these species are restricted geographically would be interesting but more intensive and structured sampling would be needed to resolve this question.

Calonectria pseudokyotensis, *C. pluriramosa* and *C. sphaeropedunculata* are closely related to *C. kyotensis* and are easily distinguished from each other and *C. kyotensis* based on morphological features and phylogenetic inference. All of these novel species were isolated from soils collected in the Guangxi Province. Only *C. sphaeropedunculata* displayed a homothallic mating system, a feature shared with *C. kyotensis* (Crous 2002, Crous et al. 2004b), whereas *C. pseudokyotensis* and *C. pluriramosa* did not produce any sexual morphs in culture during this study.

The large ascospores and macroconidia of *C. magnispora* distinguish this novel species from the other members of the *C. kyotensis* complex. This species, along with *C. guangxiensis*, was isolated from soils collected in the Guangxi Province. Together with *C. hainanensis*, isolated from soil collected in the Hainan Province, these novel species readily formed their sexual morphs in culture, and are homothallic. Several isolates were also identified as *C. chinensis* based on phylogenetic inference and morphological features. This species, known only from China (Crous et al. 2004b), belongs to the *C. kyotensis* complex, and nothing is known regarding its ability to infect plants.

The greatest diversity of species found in this study came from baiting of soils collected in the Guangxi Province, followed by the Guangdong and Hainan Provinces. Of the 29 *Calonectria* species now known from China, 16 belong to the Sphaero-Naviculate Group, and 13 to the Prolate Group as defined by Lombard et al. (2010b). Ten species in the former group have a homothallic mating system and the remaining six species are more likely to be heterothallic because single conidial isolates mated in culture did not produce ascospores. Interestingly, all homothallic species originated exclusively from a soil habitat, while those thought to be heterothallic were from both soil and plant material. It is possible that homothallism in these *Calonectria* species represents an adaptation to the soil environment where only short-distance spread is required, as ascospores are extremely susceptible to desiccation (Rowe & Beute 1975). The majority of the putative heterothallic *Calonectria* species in this study were isolated from leaves of different *Eucalyptus* clones displaying CLB symptoms. Since heterothallism results in sexual outcrossing and the generation of genetic diversity (Billiard et al. 2012, Heitman et al. 2013), such a mating system would be beneficial to fungi that infect plants where sexual outcrossing would facilitate the process of overcoming host resistance.

To better understand the genetic variation within these homothallic and putative heterothallic *Calonectria* species more knowledge of the population structure of these species is

required. Relatively few studies have focused on the population dynamics of *Calonectria* species (Wright *et al.* 2006, 2007, 2010), and therefore limited knowledge is available on the population structure, distribution of genetic diversity, gene flow, centres of origin and the role of mating strategies for these fungi. Population studies on these fungi, especially those associated with CLB in China would better facilitate our understanding of the epidemiology, and in turn, the management of CLB in *Eucalyptus* plantations in China. These studies would also allow the prediction of efficacy of host plant resistance to these fungi, necessary for the establishment of future commercial plantations in China.

Although several *Calonectria* species were isolated from *Eucalyptus* leaves displaying symptoms of CLB in this study, relatively little is known about their pathogenicity, and their roles as potential pathogens can only be assumed based on the symptoms they are associated with. Therefore, pathogenicity tests need to be done experimentally to determine whether these species are pathogenic to *Eucalyptus* and if they are host specific. These studies would help identify which *Calonectria* species are important to commercial *Eucalyptus* forestry in China. The high diversity of *Calonectria* species in a relatively small area of southern China, and especially in virgin soils, implies that more *Calonectria* species remain to be discovered as sampling is extended to more provinces in China, which would also have to be tested as possible threats to *Eucalyptus* production in China.

ACKNOWLEDGEMENTS

This study was initiated through the bilateral agreement between the Governments South Africa and China, and we are grateful for the funding via projects 2010KJJC015-03 (Forestry Science and Technology Innovation Project of Guangdong Province of China), 2012DFG31830 (International Science & Technology Cooperation Program of China), 31400546 (National Natural Science Foundation of China) and the NWO Joint Scientific Thematic Research Programme – Joint Research Projects 2012 ALW file number 833.13.005 “Building the fungal quarantine & quality barcode of life database to ensure plant health”. We also appreciate the financial support of members of the Tree Protection Cooperative Programme (TPCP). We thank Arien van Iperen, Trix Merck and Dr Seonju Marincowitz for their invaluable assistance with cultures.

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