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ARTICLE

Calonectria species isolated from *Eucalyptus* plantations and nurseries in South China

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Abstract: Diseases caused by species of *Calonectria* (*Ca.*) represent a serious threat to the growth and sustainability of *Eucalyptus* plantations in China. Symptoms caused by these fungi mainly include leaf blight on trees in plantations and rotting of stems and leaves in nurseries. Extensive surveys have recently been conducted where *Calonectria* species were collected in *Eucalyptus* plantations and nurseries in the FuJian, GuangDong, GuangXi, and YunNan Provinces of South China. Additional isolates were baited from soil samples in the Hong Kong Region. The aim of this study was to identify the 115 *Calonectria* isolates obtained using comparisons of DNA sequence data for the β-tubulin (*tub2*), calmodulin (*cmdA*), histone H3 (*his3*) and partial translation elongation factor-1α (*tef1*) gene regions as well as their morphological features. Seven known species were identified, including *Calonectria arbusta*, *Ca. asiatica*, *Ca. chinensis*, *Ca. eucalypti*, *Ca. hongkongensis*, *Ca. mossambicensis* and *Ca. pentaseptata*. In addition, six novel taxa were collected and are described here as *Ca. aciculata*, *Ca. honghensis*, *Ca. lantauensis*, *Ca. pseudoturangicola*, *Ca. pseudoyunnanensis,* and *Ca. yunnanensis* spp. nov. Overall, the results reflect a high diversity of *Calonectria* species in China.

Key words:
Cylindrocladium
forest pathogens
Nectriaceae
phylogeny
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INTRODUCTION

The genus *Calonectria* (*Hypocreales*, *Nectriaceae*) includes numerous important pathogens that cause significant damage to a large number of herbaceous and woody plants worldwide (Crous 2002, Lombard *et al*. 2010a). Approximately 335 plant species residing in about 100 plant families are hosts to *Calonectria* species, including important plantation tree crops such as species of *Eucalyptus*, *Pinus,* and *Acacia* (Crous 2002, Lombard *et al*. 2010a). To date, at least 149 species of *Calonectria* have been described and verified based on comparisons of DNA sequence data (Lombard *et al*. 2016, Marin-Felix *et al*. 2017). *Calonectria* species are soil-borne fungi (Thies & Patton 1970, Hwang & Ko 1976, Gilligan 1983, Crous 2002) and disease symptoms resulting from infection include cutting rot, damping-off, leaf blight, red crown rot, root rot, seedling rot, shoot blight and stem canker (Crous *et al*. 1991, Brown & Ferreira 2000, Crous 2002, Lombard *et al*. 2010a, 2015).

In China, plantation forestry utilizing rapidly-growing *Eucalyptus* species has expanded during the course of the past two decades, to meet an increasing need for wood products. Approximately 4.5 M ha of *Eucalyptus* plantations have been established in South China (Chen & Chen 2013) and these are threatened by disease and insect pest problems (Zhou *et al*. 2008). Recent surveys of *Eucalyptus* plantations in South China have recorded several important emerging diseases, which include stem diseases caused by *Teratosphaeria zuluensis* (Cortinas *et al*. 2006, Chen *et al*. 2011a), species of *Botryosphaeriaceae* (Chen *et al*. 2011d) and *Cryphonectriaceae* (Chen *et al*. 2010, 2011b), and also *Ceratocystis* species (Chen *et al*. 2013). Leaf and shoot diseases caused by species of *Mycosphaerellaceae* and *Teratosphaeriaceae* (Burgess *et al*. 2006, 2007), *Quambalaria* species (Zhou *et al*. 2007), and *Calonectria* species (Crous *et al*. 2004, Lombard *et al*. 2010d, 2015, Chen *et al*. 2011c) have become widespread. Of these, *Calonectria* associated diseases are considered amongst the most threatening.

Pathogenic *Calonectria* species can cause significant losses to the *Eucalyptus* industry in China. The most important factor contributing to *Calonectria* infection and disease development is high humidity and free moisture (Crous 2002,

Rodas *et al*. 2005). Common conditions in many parts of China where *Eucalyptus* species are propagated, serious disease problems emerge.

Twenty-eight species of *Calonectria* have been identified in China (Crous *et al*. 2004, Lombard *et al*. 2010d, 2015, Chen *et al*. 2011c, Xu *et al*. 2012). With the exception of *Ca. nymphaeae* (Xu *et al*. 2012), all species have been isolated from leaves, seedlings, and soil collected in *Eucalyptus* plantations and nurseries in South China (Supplementary Table 1). Twelve of these species were isolated from symptomatic *Eucalyptus* tissues, 17 were reported from soil associated with *Eucalyptus* trees or in *Eucalyptus* nurseries, and *Ca. pentaseptata* and *Ca. terrestris* were isolated from symptomatic *Eucalyptus* tissues as well as soil (Crous *et al*. 2004, Lombard *et al*. 2010d, 2015, Chen *et al*. 2011c). Pathogenicity tests have shown that 15 *Calonectria* species, including four known only from soil, are pathogenic to two tested *E. urophylla* × *E. grandis* hybrid clones commonly planted in South China (Chen *et al*. 2011c, Li *et al*. 2014a, b). Nothing is known regarding the pathogenicity of the remaining 11 *Calonectria* species known only from soil (Supplementary Table 1).

Species of *Calonectria* are characterised by a sexual morph having yellow to dark red perithecia, scaly to warty ascomatal walls and 4–8-spored clavate asci. The asexual morphs produce branched conidiophores, cylindrical, septate conidia and stipe extensions with terminal vesicles of characteristic shape (Crous 2002, Lombard *et al*. 2010c, 2016). These asexual morphs provide the best diagnostic characters for identification, especially in conidial and vesicle morphology (Schoch *et al*. 2000, Crous 2002). Based on phylogenetic inference that matches with the distribution of vesicle shapes, species of *Calonectria* are divided into two main groups. These include the Prolate Group including species with clavate to pyriform to ellipsoidal vesicles and the Sphaero-Naviculate Group that accommodates species with sphaeropedunculate and naviculate vesicles (Lombard *et al*. 2010c). At present, 14 species of *Calonectria* found in China reside in the Prolate Group and these include four species complexes: the *Ca. candelabrum* complex (*Ca. pauciramosa*, *Ca. seminaria,* and *Ca. tetraramosa*), *Ca. colhounii* complex (*Ca. fujianensis*, *Ca. nymphaeae,* and *Ca. pseudocolhounii*), *Ca. cylindrospora* complex (*Ca. cerciana*, *Ca. foliicola*, *Ca. papillata,* and *Ca. terrestris*), and *Ca. reteaudii* complex (*Ca. crousiana*, *Ca. pentaseptata*, *Ca. microconidialis,* and *Ca. pseudoreteaudii*) (Supplementary Table 1). The remaining 14 known species in China reside in the Sphaero-Naviculate Group and they all cluster in the *Ca. kyotensis* complex (Crous *et al*. 2004, Lombard *et al*. 2010d, 2015, Chen *et al*. 2011c, Xu *et al*. 2012) (Supplementary Table 1).

Previous research has suggested a relatively high *Calonectria* species diversity in South China (Chen *et al*. 2011c, Lombard *et al*. 2015). This study was undertaken in order to provide a more comprehensive overview of *Calonectria* species associated with planted *Eucalyptus* in the provinces of South China.

MATERIALS AND METHODS

Isolates

Surveys for *Calonectria* species were conducted in *Eucalyptus* plantations and nurseries of the FuJian, GuangDong, GuangXi, and YunNan Provinces in South China (Table 1). Leaves on trees showing blight symptoms were collected in *Eucalyptus* plantations. In *Eucalyptus* nurseries, seedlings showing stem and leaf rot symptoms were selected. Soil in the *Eucalyptus* plantations, and soil samples or planting substrate in *Eucalyptus* nurseries, were also sampled. In addition, soil samples were collected in a naturally forested area on Lantau Island in Hong Kong (Table 1). At each sampling site, between five and 25 *Eucalyptus* trees or seedlings were sampled, and between 10 and 25 soil samples were collected between March 2014 and May 2015. The symptomatic tissues were incubated in moist chambers at room temperature for 1–7 d to induce *Calonectria* sporulation. Soil samples were baited with germinating *Medicago sativa* (alfalfa) seeds using the method described by Crous (2002).

Conidial masses were transferred directly from *Eucalyptus* or *M. sativa* infected tissues to 2 % (v/v) malt extract agar (MEA) under a AxioCam Stemi 2000C dissecting microscope (Carl Zeiss, Germany). After incubation at room temperature for 2–5 d, a single hyphal tip from each culture was transferred to MEA plates and incubated at room temperature for 1 wk to obtain pure cultures.

Cultures were deposited in the Culture Collection of the China Eucalypt Research Centre (CERC), Chinese Academy of Forestry (CAF), ZhanJiang, GuangDong Province, China, and in the culture collection (CMW) of the Forestry Agricultural and Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa. Representative isolates including the ex-type cultures were deposited in the culture collection (CBS) of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands. Dried specimens of sporulating cultures were deposited in the National Collection of Fungi (PREM), Pretoria, South Africa.

DNA extraction, PCR and sequence reactions

Isolates from sampled trees, seedlings and soil representing all sampling sites were used for total genomic DNA extraction and sequence comparisons. The DNA was extracted from 5–7-d-old cultures, using the CTAB method "5" as described by Van Burik *et al*. (1998). DNA concentrations were determined using a NanoDrop ND-2000 Spectrometer (Thermo Fisher Scientific, Waltham, MA). Four gene regions including the partial β-tubulin (*tub2*), calmodulin (*cmdA*), histone H3 (*his3*) and translation elongation factor 1-alpha (*tef1*) were amplified using the primers and protocols described by Lombard *et al*. (2010c). The TopTaqTM Master Mix Kit (Qiagen, Hilden) was used to amplify these gene regions. All PCR products were sequenced in both directions, using the same primers used for PCR amplification by the Beijing Genomics Institution, Guangzhou, China. All sequences obtained in this study were edited using Geneious v. 7.0 (Kearse *et al*. 2012) and were deposited in GenBank (Table 1).

Phylogenetic analyses

All sequences representing the different *Calonectria* species in this study were used together with published

Table 1. Species of *Calonectria* collected in this study.

Species[1]	Isolate No.[2]	Haplotype[3]	Substrate	Sampling site	Collector	GenBank accession No.[4]			
						tef1	*his3*	*cmdA*	*tub2*
Ca. aciculata	**CMW 47645**[5–8]; CERC 5342; CBS 142883	AAAA	*Eucalyptus urophylla* × *E. grandis* leaf in plantation	WeiYuan, JingGu, PuEr, YunNan, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442644	MF442759	MF442874	MF442989
Ca. arbusta	CMW 47502; CERC 9516	AAA–[9]	Soil in *Eucalyptus* plantation	XiaoPingYang, XingBin, LaiBin, GuangXi, China	S.B. Liang	MF442645	MF442760	MF442875	–
	CMW 47503; CERC 9520	AAA–	Soil in *Eucalyptus* plantation	XiaoPingYang, XingBin, LaiBin, GuangXi, China	S.B. Liang	MF442646	MF442761	MF442876	–
	CMW 47504; CERC 9522	AAA–	Soil in *Eucalyptus* plantation	XiaoPingYang, XingBin, LaiBin, GuangXi, China	S.B. Liang	MF442647	MF442762	MF442877	–
	CMW 47505; CERC 9523	AAA–	Soil in *Eucalyptus* plantation	XiaoPingYang, XingBin, LaiBin, GuangXi, China	S.B. Liang	MF442648	MF442763	MF442878	–
	CMW 47506; CERC 9525	AAA–	Soil in *Eucalyptus* plantation	XiaoPingYang, XingBin, LaiBin, GuangXi, China	S.B. Liang	MF442649	MF442764	MF442879	–
	CMW 47507; CERC 9526	AAA–	Soil in *Eucalyptus* plantation	XiaoPingYang, XingBin, LaiBin, GuangXi, China	S.B. Liang	MF442650	MF442765	MF442880	–
	CMW 47508[5]; CERC 9527	AAA–	Soil in *Eucalyptus* plantation	XiaoPingYang, XingBin, LaiBin, GuangXi, China	S.B. Liang	MF442651	MF442766	MF442881	–
	CMW 47509; CERC 9528	AAA–	Soil in *Eucalyptus* plantation	XiaoPingYang, XingBin, LaiBin, GuangXi, China	S.B. Liang	MF442652	MF442767	MF442882	–
	CMW 47637[5]; CERC 5320	AAA–	Soil in *Eucalyptus* plantation	XiDi, LongXu, WuZhou, GuangXi, China	S.F. Chen, J.Q. Li & W. Lu	MF442653	MF442768	MF442883	–
	CMW 47638; CERC 5322	AAA–	Soil in *Eucalyptus* plantation	XiDi, LongXu, WuZhou, GuangXi, China	S.F. Chen, J.Q. Li & W. Lu	MF442654	MF442769	MF442884	–
	CMW 47639; CERC 5324	AAA–	Soil in *Eucalyptus* plantation	XiDi, LongXu, WuZhou, GuangXi, China	S.F. Chen, J.Q. Li & W. Lu	MF442655	MF442770	MF442885	–
Ca. asiatica	CMW 47641[5]; CERC 5333	AAA–	Soil in *Eucalyptus* plantation	ZhengXing, JingGu, PuEr, YunNan, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442656	MF442771	MF442886	–
	CMW 47654[5]; CERC 5373	ABB–	Soil in *Eucalyptus* plantation	WeiYuan, JingGu, PuEr, YunNan, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442657	MF442772	MF442887	–
Ca. chinensis	CMW 47256[5]; CERC 3339	AAAA	Soil	Lantau, Lidao, Hong Kong, China	M.J. Wingfield & S.F. Chen	MF442658	MF442773	MF442888	MF442990
	CMW 47258[5]; CERC 3349	ABAA	Soil	Lantau, Lidao, Hong Kong, China	M.J. Wingfield & S.F. Chen	MF442659	MF442774	MF442889	MF442991
	CMW 47259[5]; CERC 3350	ABAA	Soil	Lantau, Lidao, Hong Kong, China	M.J. Wingfield & S.F. Chen	MF442660	MF442775	MF442890	MF442992
	CMW 47260; CERC 3351	ABAA	Soil	Lantau, Lidao, Hong Kong, China	M.J. Wingfield & S.F. Chen	MF442661	MF442776	MF442891	MF442993

Table 1. (Continued).

Species[1]	Isolate No.[2]	Haplotype[3]	Substrate	Sampling site	Collector	GenBank accession No.[4]			
						tef1	*his3*	*cmdA*	*tub2*
Ca. eucalypti	CMW 47660[5]; CERC 5401	AAAA	*E. urophylla* × *E. grandis* leaf in plantation	WeiYuan, JingGu, PuEr, YunNan, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442662	MF442777	MF442892	MF442994
Ca. honghensis	CMW 47667[5]; CERC 5568	AAAA	Soil in *Eucalyptus* plantation	XinXian, PingBian, HongHe, YunNan, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442663	MF442778	MF442893	MF442995
	CMW 47668[5–7]; CERC 5571; CBS 142884	AAAA	Soil in *Eucalyptus* plantation	XinXian, PingBian, HongHe, YunNan, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442664	MF442779	MF442894	MF442996
	CMW 47669[5–8]; CERC 5572; CBS 142885	AAAA	Soil in *Eucalyptus* plantation	XinXian, PingBian, HongHe, YunNan, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442665	MF442780	MF442895	MF442997
	CMW 47670[5–7]; CERC 5573; CBS 142886	AAAA	Soil in *Eucalyptus* plantation	XinXian, PingBian, HongHe, YunNan, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442666	MF442781	MF442896	MF442998
	CMW 47671[5]; CERC 5574	AAAA	Soil in *Eucalyptus* plantation	XinXian, PingBian, HongHe, YunNan, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442667	MF442782	MF442897	MF442999
Ca. hongkongensis	CMW 47257[5]; CERC 3341	AAAA	Soil	Lantau, Lidao, Hong Kong, China	M.J. Wingfield & S.F. Chen	MF442668	MF442783	MF442898	MF443000
	CMW 47271; CERC 3570	AAAA	Soil in *Eucalyptus* plantation	ChangLe, HePu, BeiHai, GuangXi, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442669	MF442784	MF442899	MF443001
	CMW 47274[5]; CERC 3573	AAAA	Soil in *Eucalyptus* plantation	ChangLe, HePu, BeiHai, GuangXi, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442670	MF442785	MF442900	MF443002
	CMW 47495; CERC 7125	AAAA	Soil	FAFU, CangShan, FuZhou, FuJian, China	S.F. Chen	MF442671	MF442786	MF442901	MF443003
	CMW 47499; CERC 7132	AAAA	Soil	FAFU, CangShan, FuZhou, FuJian, China	S.F. Chen	MF442672	MF442787	MF442902	MF443004
	CMW 47500; CERC 7133	AAAA	Soil	FAFU, CangShan, FuZhou, FuJian, China	S.F. Chen	MF442673	MF442788	MF442903	MF443005
	CMW 47501; CERC 7137	AAAA	Soil	FAFU, CangShan, FuZhou, FuJian, China	S.F. Chen	MF442674	MF442789	MF442904	MF443006
	CMW 47619; CERC 3288	AAAA	Soil	Lantau, Lidao, Hong Kong, China	M.J. Wingfield & S.F. Chen	MF442675	MF442790	MF442905	MF443007
Ca. lantauensis	CMW 47251[5–7]; CERC 3301; CBS 142887	AAA–	Soil	Lantau, Lidao, Hong Kong, China	M.J. Wingfield & S.F. Chen	MF442676	MF442791	MF442906	–
	CMW 47252[5–8]; CERC 3302; CBS 142888	AAA–	Soil	Lantau, Lidao, Hong Kong, China	M.J. Wingfield & S.F. Chen	MF442677	MF442792	MF442907	–
Ca. mossambicensis	CMW 47465[5]; CERC 6979	AAAA	Medium of *E. urophylla* × *E. grandis* seedling in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442678	MF442793	MF442908	MF443008
	CMW 47466; CERC 6990	AAAA	Medium of *E. urophylla* × *E. grandis* seedling in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442679	MF442794	MF442909	MF443009

Table 1. (Continued).

Species[1]	Isolate No.[2]	Haplotype[3]	Substrate	Sampling site	Collector	GenBank accession No.[4]			
						tef1	*his3*	*cmdA*	*tub2*
	CMW 47467; CERC 6996	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442680	MF442795	MF442910	MF443010
	CMW 47469; CERC 7004	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442681	MF442796	MF442911	MF443011
	CMW 47472; CERC 7022	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442682	MF442797	MF442912	MF443012
	CMW 47476; CERC 7038	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442683	MF442798	MF442913	MF443013
	CMW 47478; CERC 7048	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442684	MF442799	MF442914	MF443014
	CMW 47479; CERC 7056	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442685	MF442800	MF442915	MF443015
	CMW 47481; CERC 7072	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442686	MF442801	MF442916	MF443016
	CMW 47484[5]; CERC 7085	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442687	MF442802	MF442917	MF443017
Ca. pentaseptata	CMW 47261; CERC 3529	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen & J.Q. Li	MF442688	MF442803	MF442918	MF443018
	CMW 47262; CERC 3533	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen & J.Q. Li	MF442689	MF442804	MF442919	MF443019
	CMW 47263; CERC 3535	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen & J.Q. Li	MF442690	MF442805	MF442920	MF443020
	CMW 47264; CERC 3536	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen & J.Q. Li	MF442691	MF442806	MF442921	MF443021
	CMW 47265; CERC 3537	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen & J.Q. Li	MF442692	MF442807	MF442922	MF443022

Table 1. (Continued).

Species[1]	Isolate No.[2]	Haplotype[3]	Substrate	Sampling site	Collector	GenBank accession No.[4]			
						tef1	*his3*	*cmdA*	*tub2*
	CMW 47266; CERC 3542	AAAA	Soil in *Eucalyptus* nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen & J.Q. Li	MF442693	MF442808	MF442923	MF443023
	CMW 47267; CERC 3552	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen & J.Q. Li	MF442694	MF442809	MF442924	MF443024
	CMW 47268; CERC 3559	AAAA	*E. urophylla* × *E. grandis* seedling medium in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen & J.Q. Li	MF442695	MF442810	MF442925	MF443025
	CMW 47269; CERC 3560	AAAA	*E. urophylla* × *E. grandis* seedling medium in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen & J.Q. Li	MF442696	MF442811	MF442926	MF443026
	CMW 47270[5]; CERC 3565	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen & J.Q. Li	MF442697	MF442812	MF442927	MF443027
	CMW 47277[5]; CERC 3652	AAAA	*E. urophylla* × *E. grandis* leaf in plantation	HengShan, LianJiang, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442698	MF442813	MF442928	MF443028
	CMW 47278; CERC 3655	AAAA	*E. urophylla* × *E. grandis* leaf in plantation	HengShan, LianJiang, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442699	MF442814	MF442929	MF443029
	CMW 47279; CERC 3658	AAAA	*E. urophylla* × *E. grandis* leaf in plantation	HengShan, LianJiang, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442700	MF442815	MF442930	MF443030
	CMW 47280; CERC 3660	AAAA	*E. urophylla* × *E. grandis* leaf in plantation	HengShan, LianJiang, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442701	MF442816	MF442931	MF443031
	CMW 47281; CERC 3664	AAAA	*E. urophylla* × *E. grandis* leaf in plantation	HengShan, LianJiang, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442702	MF442817	MF442932	MF443032
	CMW 47282; CERC 3672	AAAA	*E. urophylla* × *E. grandis* leaf in plantation	HengShan, LianJiang, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442703	MF442818	MF442933	MF443033
	CMW 47283; CERC 3680	AAAA	*E. urophylla* × *E. grandis* leaf in plantation	HengShan, LianJiang, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442704	MF442819	MF442934	MF443034
	CMW 47284; CERC 3708	AAAA	*E. urophylla* × *E. grandis* leaf in plantation	HengShan, LianJiang, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442705	MF442820	MF442935	MF443035

Table 1. (Continued).

Species[1]	Isolate No.[2]	Haplotype[3]	Substrate	Sampling site	Collector	GenBank accession No.[4]			
						tef1	*his3*	*cmdA*	*tub2*
	CMW 47285; CERC 3720	AAAA	*E. urophylla* × *E. grandis* leaf in plantation	HengShan, LianJiang, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442706	MF442821	MF442936	MF443036
	CMW 47463; CERC 6963	AAAA	*E. urophylla* × *E. grandis* seedling leaf in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen & G.Q. Li	MF442707	MF442822	MF442937	MF443037
	CMW 47464; CERC 6973	AAAA	*E. urophylla* × *E. grandis* seedling leaf in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen & G.Q. Li	MF442708	MF442823	MF442938	MF443038
	CMW 47468; CERC 6999	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442709	MF442824	MF442939	MF443039
	CMW 47470; CERC 7012	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442710	MF442825	MF442940	MF443040
	CMW 47471; CERC 7018	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442711	MF442826	MF442941	MF443041
	CMW 47473; CERC 7024	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442712	MF442827	MF442942	MF443042
	CMW 47474; CERC 7030	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442713	MF442828	MF442943	MF443043
	CMW 47475; CERC 7036	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442714	MF442829	MF442944	MF443044
	CMW 47477; CERC 7047	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442715	MF442830	MF442945	MF443045
	CMW 47480; CERC 7060	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442716	MF442831	MF442946	MF443046
	CMW 47482; CERC 7074	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442717	MF442832	MF442947	MF443047
	CMW 47483; CERC 7081	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442718	MF442833	MF442948	MF443048

Table 1. (Continued).

Species[1]	Isolate No.[2]	Haplotype[3]	Substrate	Sampling site	Collector	GenBank accession No.[4]			
						tef1	*his3*	*cmdA*	*tub2*
	CMW 47485; CERC 7087	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442719	MF442834	MF442949	MF443049
	CMW 47486; CERC 7095	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442720	MF442835	MF442950	MF443050
	CMW 47487; CERC 7104	AAAA	*E. urophylla* × *E. grandis* seedling stem in nursery	LingBei, SuiXi, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442721	MF442836	MF442951	MF443051
	CMW 47510[5]; CERC 9529	AAAA	*Eucalyptus* clone seedling stem in nursery	CERC, XiaShan, ZhanJiang, GuangDong, China	J.Q. Li & S.F. Chen	MF442722	MF442837	MF442952	MF443052
	CMW 47511; CERC 9533	AAAA	*Eucalyptus* clone seedling stem in nursery	CERC, XiaShan, ZhanJiang, GuangDong, China	J.Q. Li & S.F. Chen	MF442723	MF442838	MF442953	MF443053
	CMW 47512; CERC 9541	AAAA	*Eucalyptus* clone seedling stem in nursery	CERC, XiaShan, ZhanJiang, GuangDong, China	J.Q. Li & S.F. Chen	MF442724	MF442839	MF442954	MF443054
	CMW 47513; CERC 9556	AAAA	*Eucalyptus* clone seedling leaf in nursery	CERC, XiaShan, ZhanJiang, GuangDong, China	J.Q. Li & S.F. Chen	MF442725	MF442840	MF442955	MF443055
	CMW 47514; CERC 9565	AAAA	*Eucalyptus* clone seedling stem in nursery	CERC, XiaShan, ZhanJiang, GuangDong, China	J.Q. Li & S.F. Chen	MF442726	MF442841	MF442956	MF443056
	CMW 47515; CERC 9572	AAAA	*Eucalyptus* clone seedling stem in nursery	CERC, XiaShan, ZhanJiang, GuangDong, China	J.Q. Li & S.F. Chen	MF442727	MF442842	MF442957	MF443057
	CMW 47620; CERC 3722	AAAA	*E. urophylla* × *E. grandis* leaf in plantation	HengShan, LianJiang, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442728	MF442843	MF442958	MF443058
	CMW 47621; CERC 3730	AAAA	*E. urophylla* × *E. grandis* stem in plantation	HengShan, LianJiang, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442729	MF442844	MF442959	MF443059
	CMW 47622; CERC 3736	AAAA	*E. urophylla* × *E. grandis* leaf in plantation	HengShan, LianJiang, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442730	MF442845	MF442960	MF443060
	CMW 47623; CERC 3742	AAAA	*E. urophylla* × *E. grandis* leaf in plantation	HengShan, LianJiang, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442731	MF442846	MF442961	MF443061

Table 1. (Continued).

Species[1]	Isolate No.[2]	Haplotype[3]	Substrate	Sampling site	Collector	GenBank accession No.[4]			
						tef1	*his3*	*cmdA*	*tub2*
	CMW 47624; CERC 3752	AAAA	*E. urophylla* × *E. grandis* leaf in plantation	HengShan, LianJiang, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442732	MF442847	MF442962	MF443062
	CMW 47625; CERC 3758	AAAA	*E. urophylla* × *E. grandis* leaf in plantation	HengShan, LianJiang, ZhanJiang, GuangDong, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442733	MF442848	MF442963	MF443063
	CMW 47626; CERC 4987	AAAA	*E. urophylla* × *E. grandis* leaf in plantation	JiuHe, ZiJin, HeYuan, GuangDong, China	S.F. Chen & J.Q. Li	MF442734	MF442849	MF442964	MF443064
	CMW 47627; CERC 4989	AAAA	*E. urophylla* × *E. grandis* leaf in plantation	JiuHe, ZiJin, HeYuan, GuangDong, China	S.F. Chen & J.Q. Li	MF442735	MF442850	MF442965	MF443065
	CMW 47628[5]; CERC 4992	AAAA	*E. urophylla* × *E. grandis* leaf in plantation	JiuHe, ZiJin, HeYuan, GuangDong, China	S.F. Chen & J.Q. Li	MF442736	MF442851	MF442966	MF443066
	CMW 47629; CERC 4994	AAAA	*E. urophylla* × *E. grandis* leaf in plantation	JiuHe, ZiJin, HeYuan, GuangDong, China	S.F. Chen & J.Q. Li	MF442737	MF442852	MF442967	MF443067
	CMW 47630; CERC 5005	AAAA	*E. urophylla* × *E. grandis* leaf in plantation	TongHe, PingNan, GuiGang, GuangXi, China	S.F. Chen & J.Q. Li	MF442738	MF442853	MF442968	MF443068
	CMW 47631[5]; CERC 5009	AAAA	*E. urophylla* × *E. grandis* leaf in plantation	TongHe, PingNan, GuiGang, GuangXi, China	S.F. Chen & J.Q. Li	MF442739	MF442854	MF442969	MF443069
	CMW 47632; CERC 5022	AAAA	*E. urophylla* × *E. grandis* leaf in plantation	TongHe, PingNan, GuiGang, GuangXi, China	S.F. Chen & J.Q. Li	MF442740	MF442855	MF442970	MF443070
	CMW 47633; CERC 5307	AAAA	*E. urophylla* × *E. grandis* leaf in plantation	ChengYue, SuiXi, ZhanJiang GuangDong, China	S.F. Chen & J.Q. Li	MF442741	MF442856	MF442971	MF443071
	CMW 47634; CERC 5310	AAAA	*E. urophylla* × *E. grandis* leaf in plantation	ChengYue, SuiXi, ZhanJiang GuangDong, China	S.F. Chen & J.Q. Li	MF442742	MF442857	MF442972	MF443072
	CMW 47635[5]; CERC 5313	AAAA	*E. urophylla* × *E. grandis* leaf in plantation	ChengYue, SuiXi, ZhanJiang GuangDong, China	S.F. Chen & J.Q. Li	MF442743	MF442858	MF442973	MF443073
	CMW 47636; CERC 5317	AAAA	*E. urophylla* × *E. grandis* leaf in plantation	ChengYue, SuiXi, ZhanJiang GuangDong, China	S.F. Chen & J.Q. Li	MF442744	MF442859	MF442974	MF443074
Ca. pseudoturangicola	CMW 47247[5]; CERC 3250	AAAA	Soil	Lantau, Lidao, Hong Kong, China	M.J. Wingfield & S.F. Chen	MF442745	MF442860	MF442975	MF443075
	CMW 47248[5]; CERC 3251	AAAA	Soil	Lantau, Lidao, Hong Kong, China	M.J. Wingfield & S.F. Chen	MF442746	MF442861	MF442976	MF443076

Table 1. (Continued).

Species[1]	Isolate No.[2]	Haplotype[3]	Substrate	Sampling site	Collector	GenBank accession No.[4]			
						tef1	*his3*	*cmdA*	*tub2*
	CMW 47488[5]; CERC 7111	AAAA	Soil in *Eucalyptus* plantation	BaiSha, MinHou, FuZhou, FuJian, China	S.F. Chen	MF442747	MF442862	MF442977	MF443077
	CMW 47489[5–7]; CERC 7115; CBS 142889	AAAA	Soil in *Eucalyptus* plantation	BaiSha, MinHou, FuZhou, FuJian, China	S.F. Chen	MF442748	MF442863	MF442978	MF443078
	CMW 47490[5]; CERC 7116	AAAA	Soil in *Eucalyptus* plantation	BaiSha, MinHou, FuZhou, FuJian, China	S.F. Chen	MF442749	MF442864	MF442979	MF443079
	CMW 47496[5–8]; CERC 7126; CBS 142890	AAAA	Soil	FAFU, CangShan, FuZhou, FuJian, China	S.F. Chen	MF442750	MF442865	MF442980	MF443080
	CMW 47497[5–7]; CERC 7127; CBS 142891	AAAB	Soil	FAFU, CangShan, FuZhou, FuJian, China	S.F. Chen	MF442751	MF442866	MF442981	MF443081
	CMW 47498[5]; CERC 7131	AAAB	Soil	FAFU, CangShan, FuZhou, FuJian, China	S.F. Chen	MF442752	MF442867	MF442982	MF443082
Ca. pseudoyunnanensis	**CMW 47655**[5–8]; CERC 5376; CBS 142892	AAAA	Soil in *Eucalyptus* plantation	WeiYuan, JingGu, PuEr, YunNan, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442753	MF442868	MF442983	MF443083
	CMW 47656[5–7]; CERC 5377; CBS 142893	AAAA	Soil in *Eucalyptus* plantation	WeiYuan, JingGu, PuEr, YunNan, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442754	MF442869	MF442984	MF443084
	CMW 47657[5–7]; CERC 5378; CBS 142894	AAAA	Soil in *Eucalyptus* plantation	WeiYuan, JingGu, PuEr, YunNan, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442755	MF442870	MF442985	MF443085
Ca. yunnanensis	CMW 47642[5–7]; CERC 5337; CBS 142895	AAAA	Soil in *Eucalyptus* plantation	ZhengXing, JingGu, PuEr, YunNan, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442756	MF442871	MF442986	MF443086
	CMW 47643[5–7]; CERC 5338; CBS 142896	AAAA	Soil in *Eucalyptus* plantation	ZhengXing, JingGu, PuEr, YunNan, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442757	MF442872	MF442987	MF443087
	CMW 47644[5–8]; CERC 5339; CBS 142897	AAAA	Soil in *Eucalyptus* plantation	ZhengXing, JingGu, PuEr, YunNan, China	S.F. Chen, J.Q. Li & G.Q. Li	MF442758	MF442873	MF442988	MF443088

[1] New species described in this study are indicated in bold.

[2] 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. CBS: culture collection of Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands.

[3] Haplotype within each identified species, determined by sequences of *tef1*, *his3*, *cmdA* and *tub2* regions.

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

[5] Isolates used in phylogenetic analyses.

[6] Isolates used in morphological studies.

[7] Isolates used in growth studies.

[8] Isolates that represent ex-type cultures are indicated in **bold**.

[9] "**–**" represents sequences that are not available.

sequences from ex-type strains of *Calonectria* downloaded from NCBI (http://www.ncbi.nlm.nih.gov) and subjected to phylogenetic analyses. Sequences generated in this study and those from NCBI were aligned using the online version of MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server; Katho & Standley 2013) with the interactive refinement method (FFT-NS-i) setting. The aligned sequences were edited manually in MEGA v. 6 (Tamura *et al*. 2013) where necessary and deposited in TreeBASE (http://treebase.org). Single nucleotide polymorphisms (SNPs) were determined for each gene region between novel species identified in this study and their phylogenetically closest related species.

Based on sequences for *cmdA, his3*, *tef1* and *tub2* gene regions, the haplotypes of obtained *Calonectria* isolates were determined. Isolates representing different haplotypes and representing all the sampling sites were selected for the phylogenetic analyses. For the new species identified here, all the isolates were included in the analyses. The datasets were separated into two groups based on morphological characteristics representing the Prolate Group and the Sphaero-Naviculate Group, as defined by Lombard *et al*. (2010c). Phylogenetic analyses were conducted separately on the datasets for each of the four gene regions and combined data for three or four gene regions for the two groups, depending on the availability of *tub2* sequences for the *Calonectria* species selected for the phylogenetic analyses. A partition homogeneity test (PHT) was used to test whether conflict existed between the different datasets, the sequence data for coding gene regions were combined if no significant conflict (Cunningham 1997, Dettman *et al*. 2003). Data were analysed using Maximum Parsimony (MP) with PAUP* v. 4.0b10 (Swofford 2003) and Maximum likelihood (ML) with PhyML v. 3.0 (Guindon & Gascuel 2003).

For MP analyses, gaps were treated as a fifth character (Ogden & Rosenberg 2007) and the characters were unordered and of equal weight with 1000 random addition replicates. The most parsimonious trees were generated using the heuristic search option with random stepwise addition of 1000 replicates and tree bisection and reconstruction (TBR) branch swapping. Zero-length branches were collapsed. Statistical support for internal nodes in trees was set with 1000 bootstrap replicates. Statistics estimated for parsimony included tree length (TL), retention index (RI), consistency index (CI), rescaled consistency indexes (RC) and homoplasy index (HI) (Hillis & Bull 1993).

For ML analyses, the appropriate models were obtained with jModeltest v. 2.1.5 (Posada 2008). The maximum number of retained trees was set to 1000 and the confidence levels for node support were determined using non-parametric bootstrapping with 1000 replicates. *Calonectria hongkongensis* (CBS 114828 and CBS 114711) and *Ca. pauciramosa* (CMW 5683 and CMW 30823) were used as the outgroup taxa for the Prolate Group and Sphaero-Naviculate Group, respectively. For all the analyses, the phylogenetic trees were viewed using MEGA v. 6 (Tamura *et al*. 2013).

Morphology

Isolates were examined to define the characteristics of the asexual sporing structures. Single hyphal tip isolates were transferred to synthetic nutrient-poor agar (SNA; Nirenberg 1981, Lombard *et al*. 2010b, c) and incubated at room temperature for 7–15 d. The structures were examined and recorded using a Zeiss Axio Imager A1 microscope and a Zeiss AxioCam MRc digital camera with Zeiss Axio Vision Rel. 4.8 software (Carl Zeiss, Munchen). Morphological characteristics were studied by mounting the structures in a drop of 85 % lactic acid on glass slides. For the known *Calonectria* species, the structures were compared with those published of the type specimens. For those species shown to represent novel phylogenetic species, for ascospores, asci, conidia, and vesicles, 50 measurements were made for the isolates selected to represent the holotype specimen. In addition, 30 measurements were made for paratype specimens. Minimum, maximum and average (mean) values were calculated and are presented as (minimum–) (average – standard deviation)–(average + standard deviation) (– maximum). For all other taxonomic informative structures, only the extremes are given.

The optimal growth conditions for cultures representing novel species were determined on MEA in the dark at temperatures ranging from 5–35 °C with 5 °C intervals. Four replicates were used for each isolate at each temperature. Two diameter measurements, perpendicular to each other, were measured daily for 7 d. Colony morphology and colour were determined on MEA after growth at 25 °C in the dark for 7 d using the colour charts of Rayner (1970). All descriptions were deposited in MycoBank (www.mycobank.org; Crous *et al*. 2004).

Sexual compatibility

Isolates of each novel *Calonectria* species identified based on multi-gene phylogenetic analyses were crossed with each other in all possible combinations. Crosses were made on minimal salt agar (MSA) on which sterile toothpicks had been placed on the surface of the media (Geurber & Correll 2001, Lombard *et al*. 2010b, c) and incubated at room temperature. Isolates crossed with themselves served as controls, and it was thus possible to distinguish between those species with heterothallic or homothallic mating systems. Crosses were regarded as successful when isolate combinations produced perithecia extruding viable ascospores.

After 4–6 wk of incubation, the perithecia obtained from the sexual compatibility tests were mounted in Leica Biosystems Tissue Freezing Medium (Leica Biosystems Nussloch, Nussloch, Germany) and sectioned using a Microtome Cryostat Microm HM550 (Microm International, Thermo Fisher Scientific, Walldorf, Germany) at –20 °C to observe characteristics of the ascomata and ascostromatic tissues. The 12 µm sections were mounted in 85 % lactic acid and 3 % KOH, and all taxonomically informative structures were measured in the same manner as that for the asexual structures.

RESULTS

Isolates

A total of 115 isolates having morphological characteristics typical of *Calonectria* spp. were obtained. Of these, 64 isolates were from GuangDong Province, 16 from GuangXi

Province, 15 from YunNan Province, 10 from FuJian Province, and 10 isolates were from soil in a natural forested area in the Hong Kong Region. All of these isolates were either from soil samples (mostly from beneath *Eucalyptus* trees), from infected leaves on *Eucalyptus* trees or from *Eucalyptus* plants in nurseries (Table 1).

Phylogenetic analyses

All 115 isolates obtained in this study were sequenced (Table 1). Thus, approximately 475 bp were generated for the *cmdA* gene region, 435 bp for the *his3* gene region, 500 bp for the *tef1* gene region and 565 bp for the *tub2* gene region. The 115 isolates represent 16 haplotypes determined by sequences for the four gene regions (Table 1). In total, 40 isolates collected in this study which represent all the 16 haplotypes were selected for phylogenetic analyses. Based on the comparsions for four gene region sequences generated in this study and published sequences from ex-type strains of *Calonectria* downloaded from NCBI, sequences for 65 ex-type and other strains representing 34 species closely related to specie emerging from this study were used for analyses (Supplementary Table 2). For the 40 isolates selected for phylogenetic analyses, 15 resided in the Prolate Group and 25 isolates formed part of the Sphaero-Naviculate Group. For the MP and ML trees based on the single and combined sequence datasets (TreeBASE no 21167) in Prolate Group or Sphaero-Naviculate Group, although the relative positions of individual *Calonectria* species differed slightly, while the overall topologies were similar.

Species residing in the Prolate Group: The partition homogeneity tests (PHT) for combinations of the *tef1*, *his3*, *cmdA* and *tub2* gene regions yielded a P-value of 0.001, and consequently, the sequence data for coding gene regions were combined (Cunningham 1997, Dettman *et al*. 2003). The combined dataset included 51 taxa and consisted of 1993 characters, including alignment gaps, of which 1414 were parsimony-uninformative and 579 were parsimony-informative. Statistical values for the trees for the MP analyses and parameters for the best-fit substitution models of ML are provided in Supplementary Table 3. The ML tree of combined sequence dataset is presented in Fig. 1.

In total, the 15 isolates collected in this study residing in the Prolate Group clustered in three phylogenetic groups (Group A, Group B and Group C), which belong to the *Ca. colhounii*, *Ca. reteaudii* and *Ca. candelabrum* complexes, respectively (Fig. 1). In Group A, five isolates (CMW 47667, CMW 47668, CMW 47669, CMW 47670 and CMW 47671) grouped in a novel monophyletic cluster (ML/MP: 89 % / 92 %) with a single isolate, CMW 47645, forming a novel distinct basal lineage, both of two novel lineages were closely related to, but separate from *Ca. monticola* and *Ca. colhounii* (Fig. 1). The total number of the fixed unique differences (SNPs) between the four clades for all four gene regions combined varied between 12–26 (Supplementary Table 4). One isolate (CMW 47660) was identified as *Ca. eucalypti* (Fig. 1).

In Group B, six isolates (CMW 47270, CMW 47277, CMW 47510, CMW 47628, CMW 47631 and CMW 47635) resided in the same clade as *Ca. pentaseptata*. Two isolates (CMW 47465 and CMW 47484) clustered within the clade representing *Ca. mossambicensis* in Group C (Fig. 1).

Species in the Sphaero-Naviculate Group: For this Group, sequences for the *tub2* gene region were not available for some taxa due to multiple sequence copies occur in single *Calonectira* isolates. The PHT comparing the *tef1*, *his3* and *cmdA* gene regions gave a P = 0.077 value. This showed that there was no significant conflict between the three gene regions and the sequence data for three gene regions were combined (Cunningham 1997, Dettman *et al*. 2003). The combined sequence dataset included 58 taxa and consisted of 1 415 characters, including alignment gaps. Of these, 1015 were parsimony-uninformative and 400 were parsimony-informative. Statistical values for the MP trees and parameters for the best-fit substitution models of ML are provided in Supplementary Table 3. The ML tree is presented in Fig. 2.

The 25 isolates placed in the Sphaero-Naviculate Group of *Calonectria* collected in this study clustered into three phylogenetic groups (Groups D–F), which all belong to the *Ca. kyotensis* complex (Fig. 2). Group D included six isolates residing in two distinct sister clades; CMW 47642, CMW 47643 and CMW 47644 in one clade, and CMW 47655, CMW 47656 and CMW 47657 in another clade (ML/MP: 85 % / 80 %, ML/MP: 71 % / 73 %, respectively). Three and four SNPs could be identified in each of the two clades for *his3* and *tub2* gene sequences (Supplementary Table 5). These two clades were phylogenetically most closely related to *Ca. asiatica* and *Ca. colombiensis* (Fig. 2). The total number of SNP differences between isolates in these two clades, *Ca. asiatica* and *Ca. colombiensis,* for all four gene regions combined, varied between 7–28 (Supplementary Table 5). Two isolates (CMW 47251 and CMW 47252) formed a single independent clade that was distinct from any known *Calonectria* species and this was supported by high bootstrap values (ML/MP: 100 % / 100 %) (Fig. 2). The total number of SNP differences between this clade accommodating isolates CMW 47251 and CMW 47252, and other phylogenetically closely related *Calonectria* species (*Ca. curvispora, Ca. ilicicola, Ca. pacifica and Ca. sumatrensis*) for three gene regions combined varied between 15–44 (Supplementary Table 6). Isolates CMW 47641 and CMW 47654 resided in the clade representing *Ca. asiatica*, however, with low bootstrap support (Fig. 2). In addition, isolates CMW 47508 and CMW 47637 did not resided in a distinct clade but were closely related to *Ca. arbusta* (Fig. 2).

In Group E, eight isolates (CMW 47247, CMW 47248, CMW 47488, CMW 47489, CMW 47490, CMW 47496, CMW 47497 and CMW 47498) formed a well-resolved clade (ML/MP: 81 %/98 %), close to, but distinct from *Ca. turangicola* (Fig. 2). Several SNPs could be identified for this clade and *Ca. turangicola,* for three of the four gene regions analysed (Supplementary Table 7). The total number of SNP differences between this clade and the species most closely related to it for all four gene regions combined, varied between 6–34 (Supplementary Table 7). Two isolates, CMW 47257 and CMW 47274 clustered with *Ca. hongkongensis* (Fig. 2).

In Group F, three isolates (CMW 47256, CMW 47258 and CMW 47259), representing two haplotypes, grouped in a clade, although, lacking bootstrap support. These isolates were most closely related to *Ca. chinensis* (Fig. 2).

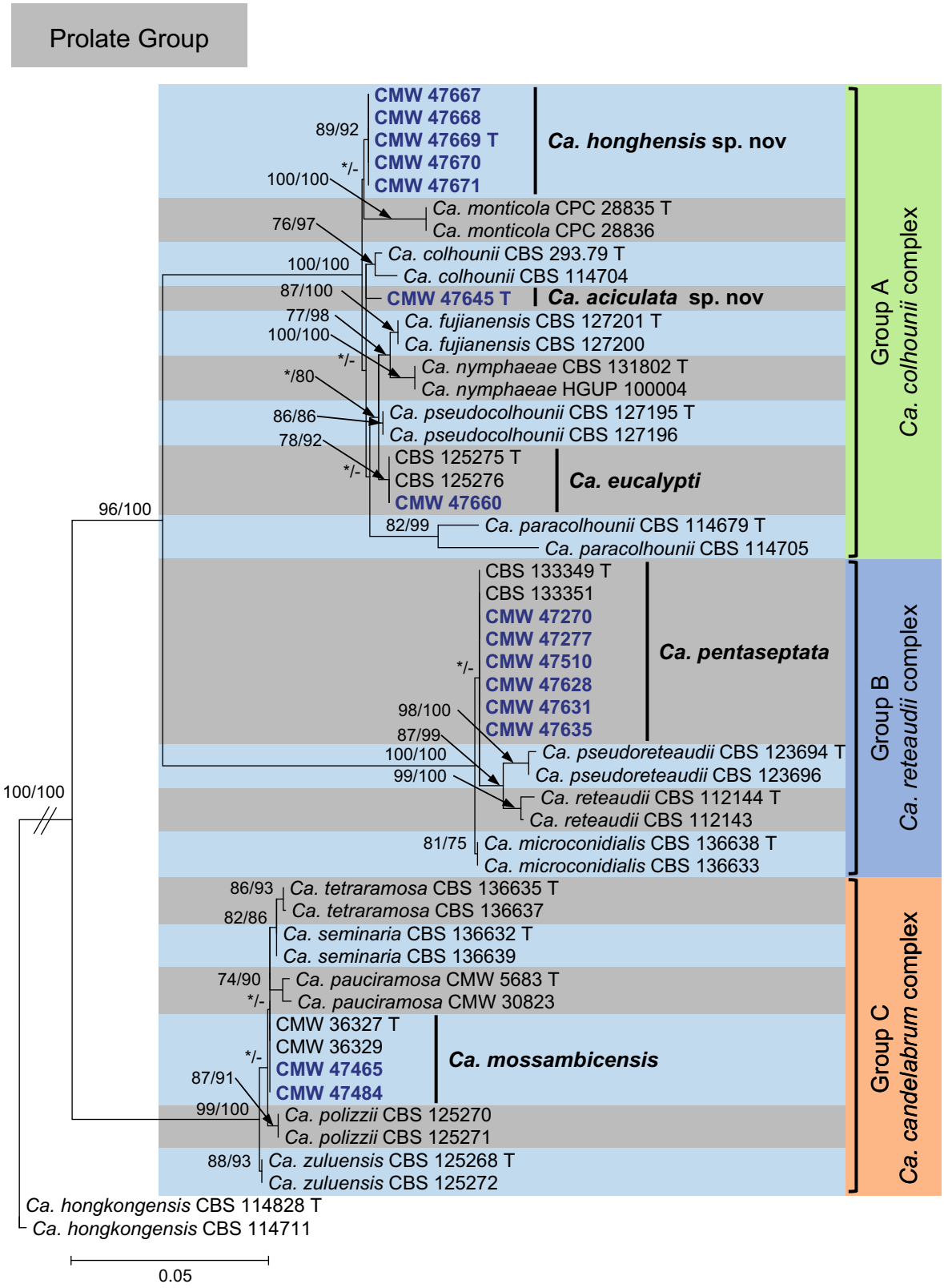

Fig. 1. Phylogenetic tree based on ML analysis of a combined DNA dataset of *tef1*, *his3*, *cmdA* and *tub2* gene sequences for the species of *Calonectria* in the Prolate Group. Bootstrap value ≥ 70 % for MP and ML analyses are presented at the branches. Bootstrap values lower than 70 % are marked with "*", and absent are marked with "-". Isolates, representing ex-type material, are marked with "T", isolates sequenced in this study are highlighted in blue and bold. The tree was rooted to *Ca. hongkongensis* (CBS 114828 and CBS 114711).

Sexual compatibility

Sixteen isolates belonging to three of the novel taxa (*Ca. honghensis*, *Ca. pseudoturangicola,* and *Ca. yunnanensis*) were able to produce sexual structures when crossed with themselves. These included isolates CMW 47247, CMW 47248, CMW 47488–47490, CMW 47496–47498,

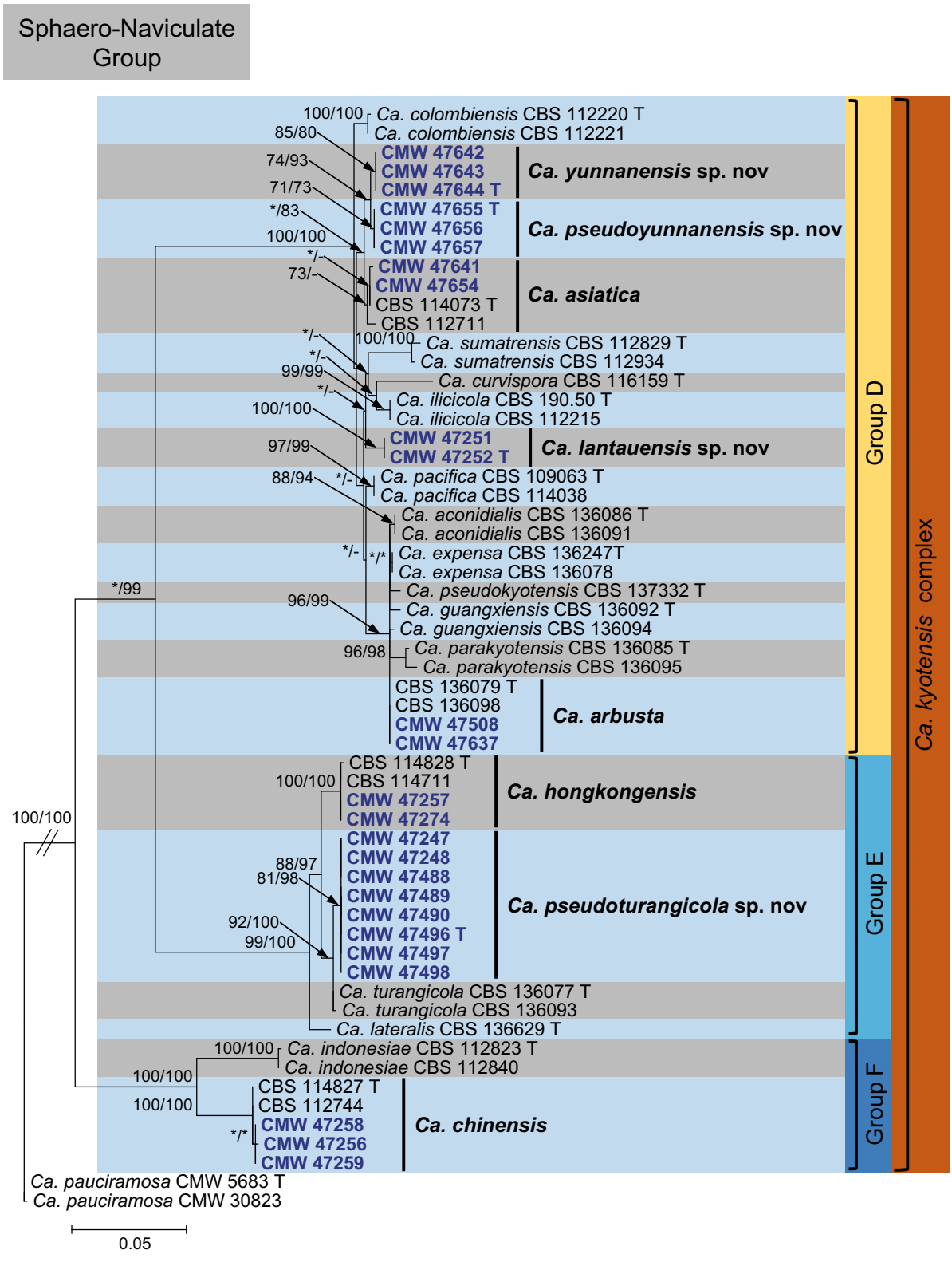

Fig. 2. Phylogenetic tree based on ML analysis of a combined DNA dataset of *tef1*, *his3* and *cmdA* gene sequences for the species of *Calonectria* in the Sphaero-Naviculate Group. Bootstrap value ≥ 70 % for MP and ML analyses are presented at the branches. Bootstrap values lower than 70 % are marked with "*", and absent are marked with "-". Isolates, representing ex-type material, are marked with "T", isolates sequenced in this study are highlighted in blue and bold. The tree was rooted to *Ca. pauciramosa* (CMW 5683 and CMW 30823).

CMW 47642–47644 and CMW 47667–47671 that formed protoperithecia within 2–3 wk and perithecia within 4–6 wk. They were consequently recognised as homothallic. The remaining isolates identified as novel *Calonectria* species failed to yield any perithecia in crosses, indicating that they were either self-sterile (heterothallic) or they lacked the ability to recombine to produce fertile progeny in culture.

Morphology and taxonomy

Based on DNA sequence comparisons (Figs 1–2) and morphology, isolates collected in this study resided in either the Prolate or Sphaero-Naviculate Group of *Calonectria* species as defined by Lombard *et al*. (2010c). For the 40 isolates selected for phylogenetic analyses, 18 resolved as known species in six groups and respectively represented *Ca. eucalypti* (Group A; *Ca. colhounii* species complex), *Ca. pentaseptata* (Group B; *Ca. reteaudii* species complex), *Ca. mossambicensis* (Group C; *Ca. candelabrum* species complex), *Ca. asiatica* and *Ca. arbusta* (Group D), *Ca. hongkongensis* (Group E) and *Ca. chinensis* (Group F), the latter three groups all clustered in the *Ca. kyotensis* species complex (Figs 1–2). The former three species resided in the Prolate Group and the latter four known species all clustered in the Sphaero-Naviculate Group (Figs 1–2).

The remaining isolates grouped in six distinct clades (Figs 1–2) that represent novel taxa: *Calonectria aciculata* and *Ca. honghensis* spp. nov. in the Prolate Group; and *Ca. lantauensis*, *Ca. pseudoturangicola*, *Ca. pseudoyunnanensis*, and *Ca. yunnanensis* spp. nov. in Sphaero-Naviculate Group. The morphological characters of isolates identified as new species were compared with the species phylogenetically most closely related to them, and these characteristics are summarized in Table 2. Based on phylogenetic inference and morphological features, these isolates represent six previously undescribed species of *Calonectria* described below:

TAXONOMY

Species in the Prolate Group

Calonectria aciculata J.Q. Li, Q.L. Liu & S.F. Chen, **sp. nov.**
MycoBank MB821632
(Fig. 3)

Etymology: After the acicular vesicles in this species.

Diagnosis: *Calonectria aciculata* can be distinguished from the phylogenetically closely related *Ca. colhounii*, *Ca. honghensis,* and *Ca. monticola* in the longer macroconidia.

Type: **China**: *YunNan Province*: PuEr Region, JingGu County, WeiYuan Town, on leaves of an *E. urophylla* × *E. grandis* hybrid clone, 16 Nov. 2014, *S.F. Chen & J.Q. Li* (PREM 61941 – holotype; CMW 47645 = CERC 5342 = CBS 142883 – ex-type cultures).

Description: *Sexual morph* unknown. *Macroconidiophores* consisting of a stipe, a suite of penicillate arranged fertile branches, a stipe extension, and a terminal vesicle; stipe septate, hyaline, smooth, 48–176 × 3–7 µm; stipe extensions septate, straight to flexuous 90–193 × 2.5–4 µm long, 2–4 µm wide at the apical septum, terminating in acicular to clavate vesicles, (2.0–)2.5–3.5(–5) µm diam. *Conidiogenous apparatus* 19–110 µm long, 27–145 µm wide; primary branches aseptate to 1-septate, 13–38 × 3.5–6 µm; secondary branches aseptate, 11–24 × 3.5–5.5 µm; tertiary branches aseptate, 9–14 × 3.5–4.5 µm, each terminal branch producing 2–4 phialides; phialides doliiform to reniform, hyaline, aseptate, 6–14 × 2.5–5 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (53–)62–76(–86) × (4.5–)5–6 (–7) µm (av. = 69 × 5.5 µm), 3-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: *Colonies* forming abundant white aerial mycelium on MEA at 25 °C after 7 d; moderate sporulation, feathery, irregular margins, reverse pale ochraceous-salmon (13'f) to sanford's brown (11k). *Chlamydospores* common throughout the medium forming microsclerotia. *Growth characteristics,* optimal growth temperature 25 °C, no growth at 5 °C and 35 °C, after 7 d, colonies at 10 °C, 15 °C, 20 °C, 25 °C and 30 °C reached 11.3 mm, 32.1 mm, 48.3 mm, 60.3 mm and 30.8 mm, respectively.

Notes: *Calonectria aciculata* differs from the phylogenetically closely related species *Ca. colhounii*, *Ca. honghensis,* and *Ca. monticola* with respect to the size of its macroconidia. The average sizes of the macroconidia of *Ca. aciculata* (av. = 69 × 5.5 µm) are longer than the average sizes of *Ca. colhounii* (av. = 65 × 5 µm), *Ca. honghensis* (av. = 54 × 5.5 µm) and *Ca. monticola* (av. = 49 × 5 µm) (Crous 2002, Crous *et al*. 2015b).

Calonectria honghensis J.Q. Li, Q.L. Liu & S.F. Chen, **sp. nov.**
MycoBank MB821633
(Fig. 4)

Etymology: From the HongHe Region of China where the fungus was first collected.

Diagnosis: *Calonectria honghensis* differs from the phylogenetically closely related *Ca. aciculata*, *Ca. colhounii* and *Ca. monticola* in the dimensions of the macroconidia and ascospores.

Type: **China**: *YunNan Province:* HongHe Region, PingBian County, XinXian Town, from soil collected in a *Eucalyptus* plantation, 14 Nov. 2014, *S.F. Chen & J.Q. Li* (PREM 61943 – holotype; CMW 47669 = CERC 5572 = CBS 142885 – ex-type cultures).

Description: *Perithecia* solitary or in groups of up to four, yellow, becoming orange with age; in section apex and body yellow, base red-brown, subglobose to ovoid, 208–423 µm high, 233–406 µm diam, body turning dark yellow, and base dark red in 3 % KOH; perithecial walls rough consisting of two thick-walled layers: outside layer of *textura globulosa*, 10–57 µm wide, becoming more compressed towards inner layer of *textura angularis*, 10–23 µm wide, becoming thin-walled and hyaline towards the centre; outer cells 9–41 × 7–24 µm, inner cells 10–19

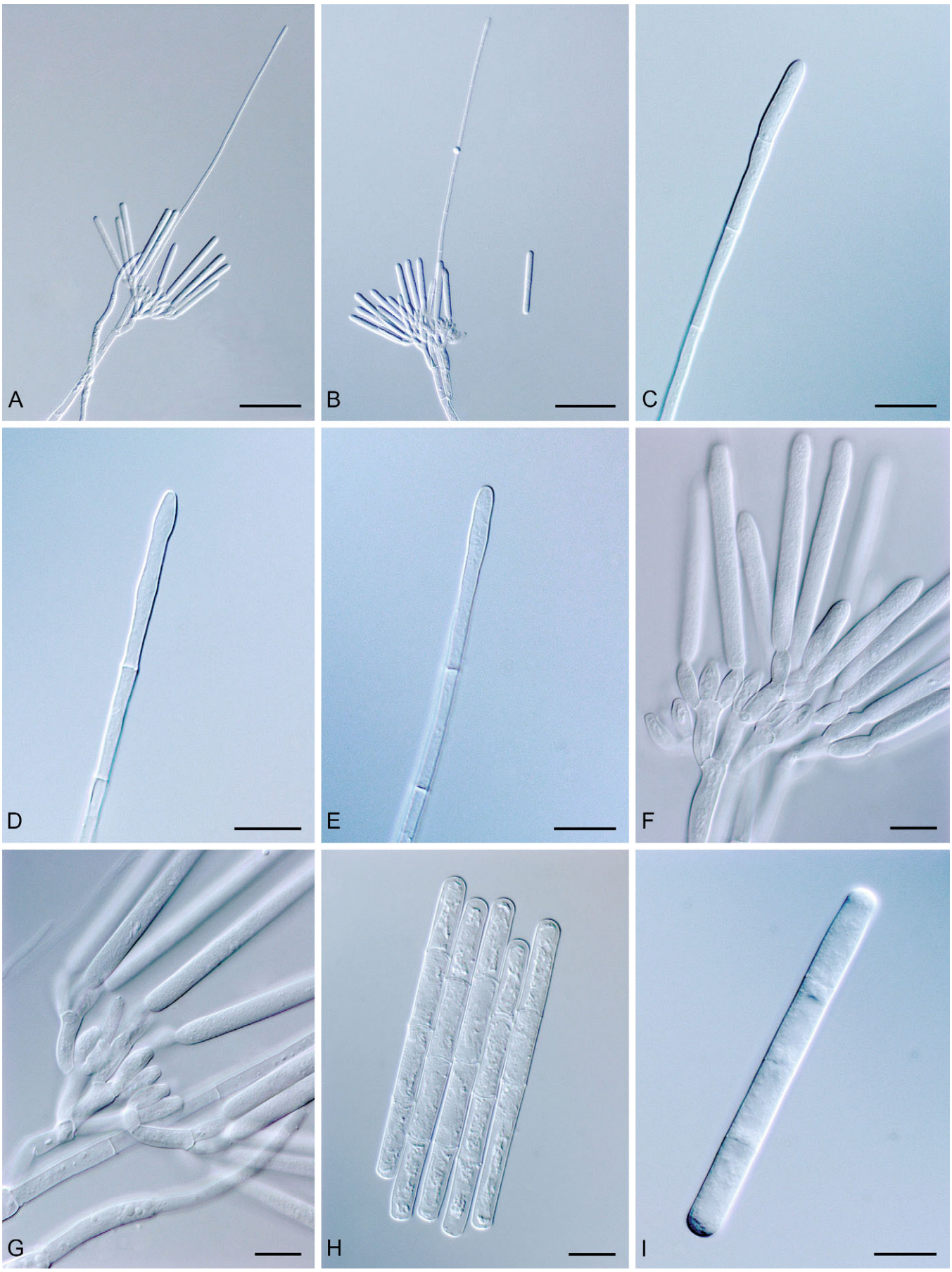

Fig. 3. *Calonectria aciculata*. **A–B.** Macroconidiophore. **C–E.** Acicular to clavate vesicles. **F–G.** Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. **H–I.** Macroconidia. Bars: A–B = 50 µm; C–I = 10 µm.

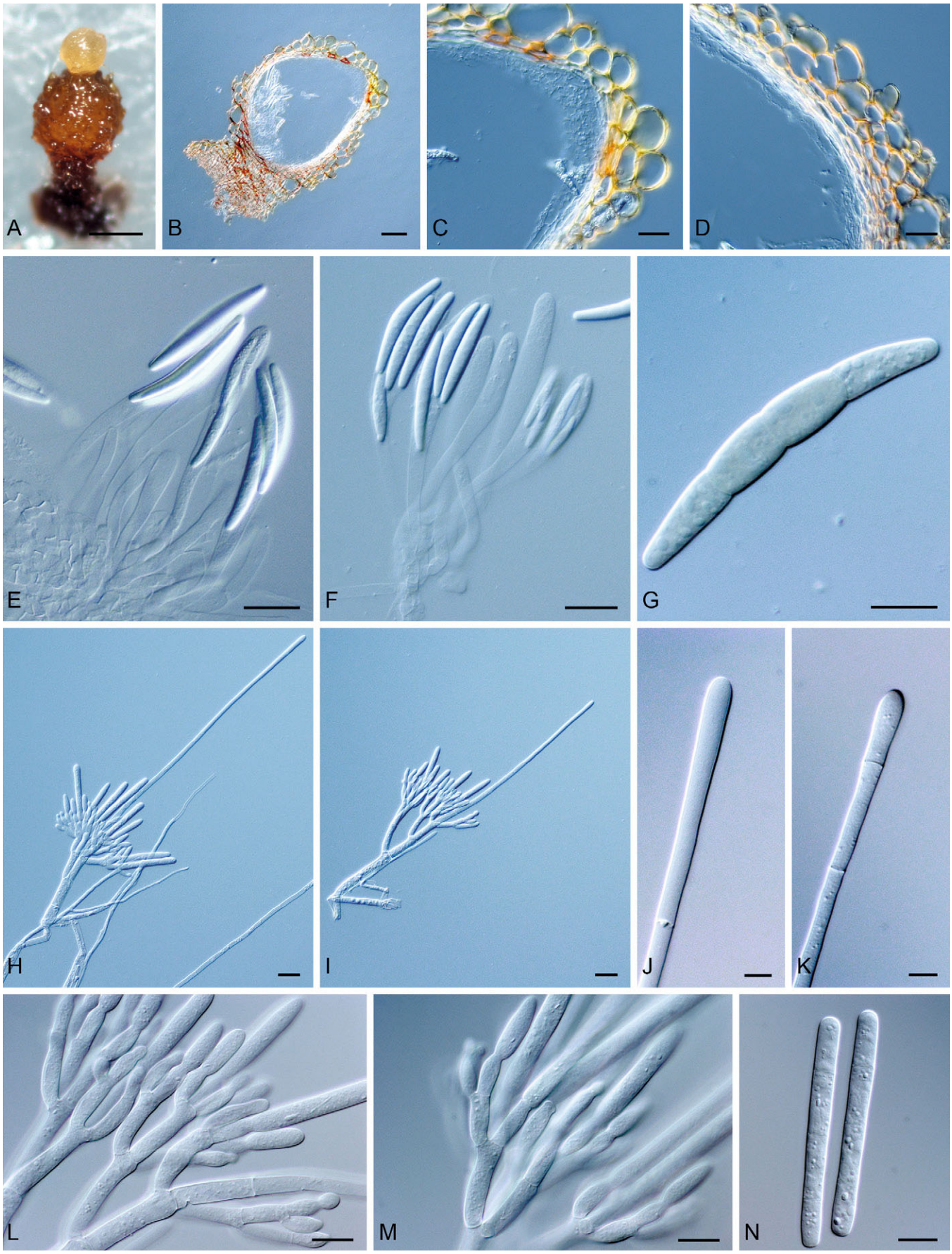

Fig. 4. *Calonectria honghensis*. **A.** Perithecium. **B.** Vertical section through a perithecium. **C.** Cells around ostiolar region of perithecium. **D.** Section through lateral perithecial wall. **E–F.** Asci. **G.** Ascospores. **H–I.** Macroconidiophore. **J–K.** Clavate vesicles. **L–M.** Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. **N.** Macroconidia. Bars: A = 200 µm; B = 50 µm; C–F and H–I = 20 µm; G and L–N = 10 µm; J and K = 5 µm.

Table 2. Morphological comparisons of *Calonectria* species examined in this study and other phylogenetically closely related species.

Species	Ascospores (L × W)[1,2]	Ascospores average (L × W)[1,2]	Ascospores septation	Macroconidia (L × W)[1,2]	Macroconidia average (L × W)[1,2]	Macroconidia septation	Vesicle (Min.–Max.)[3]	Vesicle shape	Reference
Ca. aciculata[4]	N/A[5]	N/A	N/A	(53–)62–76(–86) × (4.5–)5–6(–7)[6]	69 × 5.5	3	(2–)2.5–3.5(–5)	acicular to clavate	This study
Ca. honghensis	(35–)43–55(–65) × (4.5–)5.5–6.5(–7.5)	49 × 6	3	(43–)49–59(–66) × (4.5–)5–5.5(–6)	54 × 5.5	3	(2.5–)3–4.5(–5.5)	clavate	This study
Ca. colhounii	(30–)50–65(–75) × (4–)5–6(–8)	55 × 6	(1–)3	(45–)60–70(–80) × (4–)5–(–6)	65 × 5	(1–)3	3–4	clavate	Crous (2002)
Ca. monticola	N/A	N/A	N/A	46–51(–56) × 4–6(–7)	49 × 5	3	4–6	broadly clavate	Crous *et al*. (2015b)
Ca. lantauensis	N/A	N/A	N/A	(49–)52–58(–62) × (4.5–)5–5.5(–6)	55 × 5	1	(7.5–)8.5–12.5(–17.5)	sphaeropedunculate	This study
Ca. curvispora	N/A	N/A	N/A	(45–)55–65(–70) × (4–)5–6	60 × 5	1(–3)	(5–)8(–10)	sphaeropedunculate	Crous (2002)
Ca. ilicicola	(30–)37–50(–65) × (4–)5–6.5(–7)	45 × 6	1(–3)	(45–)70–82(–90) × (4–)5–6.5(–7)	62 × 6	(1–)3	(6–)7–10(–12)	sphaeropedunculate	Crous (2002)
Ca. pacifica	N/A	N/A	N/A	(38–)45–65(–75) × 4–5	55 × 4.5	1	7–15	sphaeropedunculate	Crous (2002)
Ca. sumatrensis	N/A	N/A	N/A	(45–)55–65(–70) × (4.5)5(–6)	58 × 5	1	8–13	sphaeropedunculate	Crous *et al*. (2004)
Ca. pseudoturangicola	(24–)27–35(–43) × (4.5–)5.5–7.5(–9.5)	31 × 6.5	1(–3)	(33–)36–44(–50) × (2.5–)3.5–4	40 × 3.5	1	(4.5–)5–8.5(–12)	sphaeropedunculate	This study
Ca. hongkongensis	(25–)28–35(–40) × (4–)5–6(–7)	31 × 6	1	(38–)45–48(–53) × 4(–4.5)	46.5 × 4	1	8–14	sphaeropedunculate	Crous *et al*. (2004)
Ca. turangicola	N/A	N/A	N/A	(40–)42–46(–47) × 3–5	44 × 4	1	8–12	sphaeropedunculate	Lombard *et al*. (2015)
Ca. pseudoyunnanensis	N/A	N/A	N/A	(40–)44–50(–55) × (4–)4.5–5.5(–6)	47.5 × 5	1	(2.5–)3.5–5	ellipsoidal, obpyriform to sphaeropedunculate	This study
Ca. yunnanensis	(28–)31–41(–55) × (5–)5.5–6.5(–8)	36 × 6	1(–3)	(36–)39–47(–52) × (4–)4.5–5(–5.5)	43 × 4.5	1	(2–)2.5–3.5(–4.5)	sphaeropedunculate	This study
Ca. asiatica	(28–)30–38(–40) × (5–)6(–7)	33 × 6	1	(42–)48–55(–65) × (4–)5(–5.5)	53 × 5	1	12–17	sphaeropedunculate	Crous *et al*. (2004)
Ca. colombiensis	(28–)30–35(–40) × (4–)5(–6)	33 × 5	1	(33–)48–58(–60) × (4–)4.5(–5)	53 × 4.5	1(–3)	7–12	sphaeropedunculate	Crous *et al*. (2004)

[1] All measurements are in µm.

[2] L × W = length × width.

[3] Min.–Max. = minimum–maximum.

[4] Species indicated in bold are described in this study.

[5] N/A = not available.

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

× 3–13 µm, perithecial base to 190 µm wide, consisting of dark red, angular cells merging with an erumpent stroma, cells of the outer wall layer continuing into the pseudoparenchymatous cells of the erumpent stroma. *Asci* 4-spored, clavate, (75–)91–115(–153) × (13–)14–24(–37) µm (av. = 103 × 19 µm), tapering to a long thin stalk. *Ascospores* aggregate in the upper third of the asci, hyaline, guttulate, fusoid with rounded ends, straight to slightly curved, 3-septate, not or slightly constricted at the septum, (35–)43–55(–65) × (4.5–)5.5–6.5(–7.5) µm (av. = 49 × 6 µm). *Macroconidiophores* consisting of a stipe, a suite of penicillate arranged fertile branches, a stipe extension, and a terminal vesicle; stipe septate, hyaline, smooth, 42–192 × 4–10 µm; stipe extensions septate, straight to flexuous, 70–215 µm long, 3–5 µm wide at the apical septum, terminating in a clavate vesicle, (2.5–)3.0–4.5(–5.5) µm diam. *Conidiogenous apparatus* 33–114 µm long, 21–75 µm wide; primary branches aseptate to 1-septate, 14–57 × 4–7.5 µm; secondary branches aseptate, 10–26 × 4–5.5 µm; tertiary branches aseptate, 9–19 × 3.5–6 µm; additional branches (–4), aseptate, 9.5–14.5 × 3.5–5 µm; each terminal branch producing 2–4 phialides; phialides doliiform to reniform, hyaline, aseptate, 7–12 × 3–5 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (43–)49–59(–66) × (4.5–)5–5.5(–6) µm (av. = 54 × 5.5 µm), 3-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: *Colonies* forming white to sienna aerial mycelium on MEA at 25 °C after 7 d, profuse sporulation, feathery, irregular margins, reverse capucine buff (13f) to umber (9). *Chlamydospores* common throughout the medium forming microsclerotia. *Growth characteristics*, optimal growth temperature 25 °C, no growth at 5 °C and 35 °C, after 7 d, colonies at 10 °C, 15 °C, 20 °C, 25 °C and 30 °C reached 14.3 mm, 31.6 mm, 43.5 mm, 51.9 mm and 17.3 mm, respectively.

Additional material examined: **China**: *YunNan Province*: HongHe Region, PingBian County, XinXian Town: from soil collected in a *Eucalyptus* plantation, 14 Nov. 2014, *S.F. Chen & J.Q. Li* (PREM 61942, culture CMW 47668 = CERC 5571 = CBS 142884; PREM 61944, culture CMW 47670 = CERC 5573 = CBS 142886).

Notes: *Calonectria honghensis* is phylogenetically most closely related to *Ca. aciculata*, *Ca. colhounii,* and *Ca. monticola*. However, *Ca. honghensis* can be distinguished from these species by the dimensions of the macroconidia and ascospores. The average size of the macroconidia of *Ca. honghensis* (av. = 54 × 5.5 µm) is shorter than that of *Ca. aciculata* (av. = 69 × 5.5 µm) and *Ca. colhounii* (av. = 65 × 5 µm), but longer than that of *Ca. monticola* (av. = 49 × 5 µm) (Crous 2002, Crous *et al*. 2015b). The average size of the ascospores of *Ca. honghensis* (av. = 49 × 6 µm) is shorter than for *Ca. colhounii* (av. = 55 × 6 µm) (Crous 2002); sexual structures are not known for *Ca. aciculata* and *Ca. monticola* (Crous *et al*. 2015b).

Species in the Sphaero-Naviculate Group

Calonectria lantauensis J.Q. Li, Q.L. Liu & S.F. Chen, **sp. nov.**
MycoBank MB821634
(Fig. 5)

Etymology: After Lantau Island in Hong Kong, China, where the fungus was first collected.

Diagnosis: *Calonectria lantauensis* can be distinguished from the phylogenetically closely related species *Ca. curvispora*, *Ca. ilicicola*, *Ca. pacifica* and *Ca. sumatrensis* by the size of the macroconidia.

Type: **China**: *Hong Kong Region*: LiDao Distict, Lantau Island, from soil collected in roadside near Hong Kong airport, 12 Mar. 2014, *M.J. Wingfield & S.F. Chen* (PREM 61946 – holotype; CMW 47252 = CERC 3302 = CBS 142888 – ex-type cultures).

Description: *Sexual morph* unknown. *Macroconidiophores* consisting of a stipe, a suite of penicillate arranged fertile branches, a stipe extension, and a terminal vesicle; stipe septate, hyaline, smooth, 44–216 × 4.5–12.5 µm; stipe extension septate, straight to flexuous 51–271 µm long, 2–5.5 µm wide at the apical septum, terminating in a sphaeropedunculate vesicle, (7.5–)8.5–12.5(–17.5) µm diam; lateral stipe extensions absent. *Conidiogenous apparatus* 45–173 µm long, 34–114 µm wide; primary branches aseptate to 1-septate, 16–83 × 4.5–12.5 µm; secondary branches aseptate, 10–19 ×4.5–7.5 µm; tertiary branches aseptate, 7.5–13 × 3.5–6 µm; each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 5.5–13 × 3–8 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (49–)52–58(–62) × (4.5–)5–5.5(–6) µm, (av. = 55 × 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* forming abundant white to buff aerial mycelium on MEA at 25 °C after 7 d, moderate sporulation, feathery, irregular margins, reverse sienna (8) to umber (9). *Growth characteristic,* optimal growth temperature 25 °C, no growth at 5 °C and 35 °C, after 7 d, colonies at 10 °C, 15 °C, 20 °C, 25 °C and 30 °C reached 7.2 mm, 28.8 mm, 54.2 mm, 78.0 mm and 71.6 mm, respectively.

Additional material examined: **China**: *Hong Kong Region*: LiDao Distict, Lantau Island, from soil collected in roadside near Hong Kong airport, 12 Mar. 2014, *M.J. Wingfield & S.F. Chen* (PREM 61945, culture CMW 47251 = CERC 3301 = CBS 142887).

Notes: *Calonectria lantauensis* is closely related to *Ca. curvispora*, *Ca. ilicicola*, *Ca. pacifica,* and *Ca. sumatrensis*. *Calonectria lantauensis* can be distinguished from *Ca. curvispora*, *Ca. ilicicola* and *Ca. sumatrensis* by the average size of the macroconidia. The macroconidia of *Ca. lantauensis* (av. = 55 × 5 µm) are shorter than those of

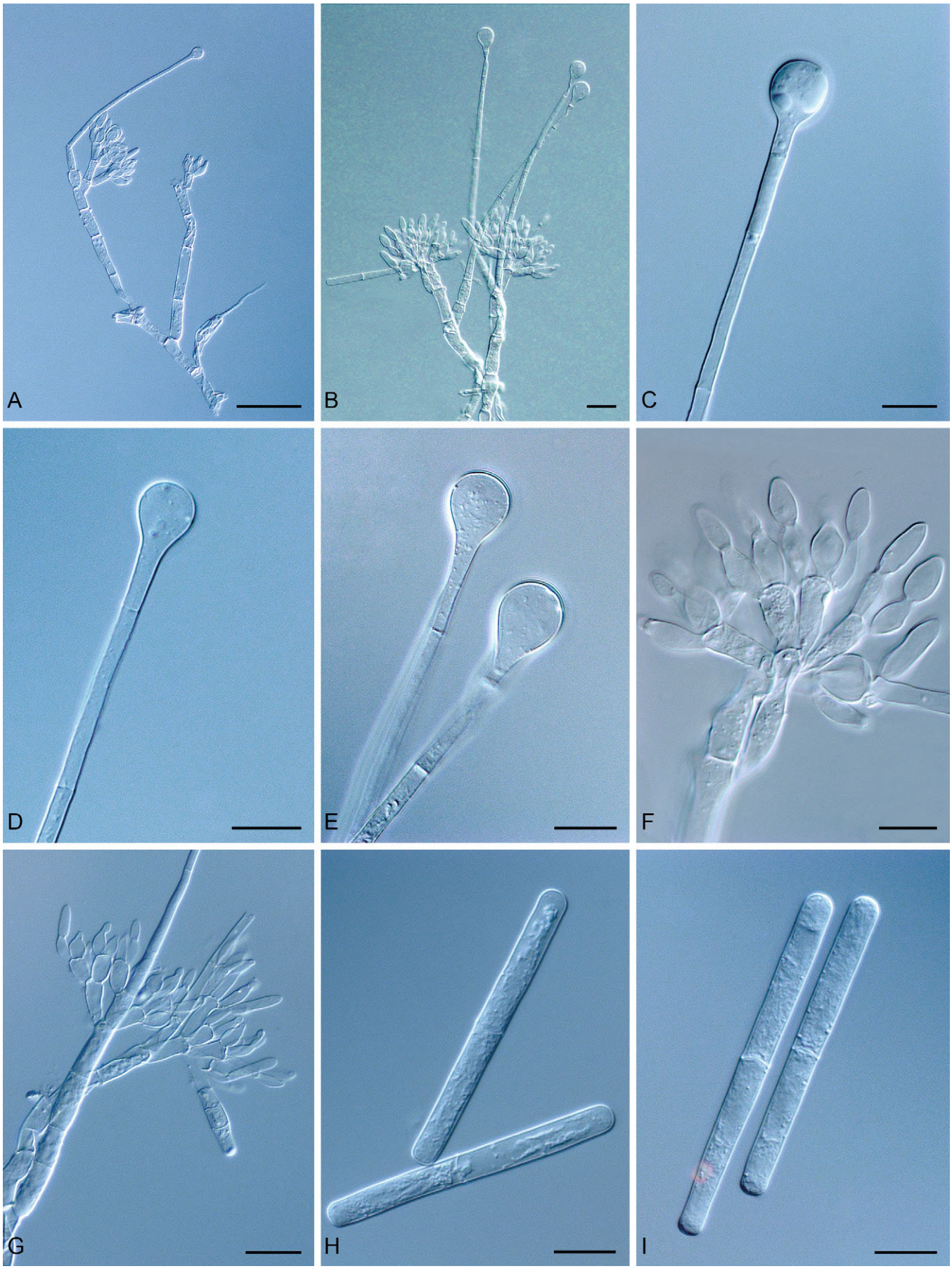

Fig. 5. *Calonectria lantauensis*. **A–B.** Macroconidiophore. **C–E.** Sphaeropedunculate vesicles. **F–G.** Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. **H–I.** Macroconidia. Bars: A = 50 µm; B and G = 20 µm; C–F and H–I = 10 µm.

Ca. curvispora (av. = 60 × 5 µm), *Ca. ilicicola* (av. = 62 × 6 µm) and *Ca. sumatrensis* (av. = 58 × 5 µm) (Crous 2002, Crous *et al*. 2004). No lateral stipe extensions were found in *Ca. lantauensis*, *Ca. curvispora* or *Ca. ilicicola,* while these structures are commonly observed in *Ca. pacifica* but rarely observed in *Ca. sumatrensis* (Crous 2002, Crous *et al*. 2004).

Calonectria pseudoturangicola J.Q. Li, Q.L. Liu & S.F. Chen, **sp. nov.**
MycoBank MB821635
(Fig. 6)

Etymology: From the close resemblance to *Calonectria turangicola.*

Diagnosis: *Calonectria pseudoturangicola* can be distinguished from the phylogenetically closely related species *Ca. hongkongensis* and *Ca. turangicola* in the shorter and narrower macroconidia.

Type: **China**: *FuJian Province:* FuZhou City, CangShan District, from soil collected in the campus of Fujian Agriculture and Forestry University (FAFU), 14 Dec. 2014, *S.F. Chen* (PREM 61948 – holotype; CMW 47496 = CERC 7126 = CBS 142890 – ex-type cultures).

Description: *Perithecia* solitary or in groups of up to five, orange, becoming orange-brown with age; in section, apex and body orange, base red-brown, subglobose to ovoid, 241–511 µm high, 242–456 µm diam, body turning red, and base dark red-brown in 3 % KOH; perithecial walls rough consisting of two thick-walled layers: outside layer of *textura globulosa*, 21–49 µm wide, becoming more compressed towards inner layer of *textura angularis*, 8–16 µm wide, becoming thin-walled and hyaline towards the centre; outer cells 17–60 × 10–33 µm, inner cells 7–44 × 2–15 µm; perithecial base to 191 µm wide, consisting of dark red, angular cells merging with an erumpent stroma, cells of the outer wall layer continuing into the pseudoparenchymatous cells of the erumpent stroma. *Asci* 8-spored, clavate, (71–) 84–114(–142) × (8–)11–17(–22) µm (av. = 99 × 14 µm), tapering to a long thin stalk. *Ascospores* aggregate in the upper third of the asci, hyaline, guttulate, fusoid with rounded ends, straight to slightly curved, 1(–3)-septate, not or slightly constricted at the septum, (24–)27–35(–43) × (4.5–)5.5–7.5(–9.5) µm (av. = 31 × 6.5 µm). *Macroconidiophores* consisting of a stipe, a suite of penicillate arranged fertile branches, a stipe extension, and a terminal vesicle; stipe septate, hyaline, smooth, 32–146 × 3.5–7.5 µm; stipe extension septate, straight to flexuous 35–217 µm long, 1.5–3.5 µm wide at the apical septum, terminating in a sphaeropedunculate vesicle, (4.5–)5–8.5(–12) µm diam; lateral stipe extensions (90° to main axis) abundant, septate, straight to flexuous 21–143 µm long, terminating in a sphaeropedunculate vesicle, 1–4 µm diam. *Conidiogenous apparatus* 32–187 µm long, 23–126 µm wide; primary branches aseptate to 1-septate, 13–53 × 3.5–6 µm; secondary branches aseptate, 11–28 × 3–5.5 µm; tertiary branches aseptate, 8.5–21 × 3–5 µm; additional branches (–5), aseptate, 6–11.5 × 2–4.5 µm; each terminal branch producing 2–4 phialides; phialides doliiform to reniform, hyaline, aseptate, 7–13.5 × 2–4.5 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (33–) 36–44(–50) × (2.5–)3.5–4 µm, (av. = 40 × 3.5 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: *Colonies* forming abundant white to saffron aerial mycelium on MEA at 25 °C after 7 d, profuse sporulation, feathery, irregular margins, reverse capucine buff (13f) to russet (13'k). *Chlamydospores* common throughout the medium forming microsclerotia. *Growth characteristics,* optimal growth temperature 25 °C, no growth at 5 °C and 35 °C, after 7 d, colonies at 10 °C, 15 °C, 20 °C, 25 °C and 30 °C reached 9.3 mm, 28.9 mm, 43.3 mm, 68.7 mm and 57.2 mm, respectively.

Additional material examined: **China**: *FuJian Province*: Fuzhou City, MinHou County, BaiSha Town: from soil collected in a *Eucalyptus* plantation, 12 Dec. 2014, *S.F. Chen* (PREM 61947, culture CMW 47489 = CERC 7115 = CBS 142889); FuZhou City, CangShan District: from soil collected in the campus of Fujian Agriculture and Forestry University (FAFU), 14 Dec. 2014, *S.F. Chen* (PREM 61949, culture CMW 47497 = CERC 7127 = CBS 142891).

Notes: *Calonectria pseudoturangicola* is phylogenetically closely related to *Ca. hongkongensis* and *Ca. turangicola*, but the macroconidia of *Ca. pseudoturangicola* (av. = 40 × 3.5 µm) are shorter and narrower than those of *Ca. hongkongensis* (av. = 46.5 × 4 µm) and *Ca. turangicola* (av. = 44 × 4 µm) (Crous *et al*. 2004, Lombard *et al*. 2015).

Calonectria pseudoyunnanensis J.Q. Li, Q.L. Liu & S.F. Chen, **sp. nov.**
MycoBank MB821636
(Fig. 7)

Etymology: From the close resemblance to *Calonectria yunnanensis*.

Diagnosis: *Calonectria pseudoyunnanensis* can be distinguished from the phylogenetically closely related *Ca. asiatica*, *Ca. colombiensis,* and *Ca. yunnanensis* by the size of macroconidia and the shape of vesicles.

Type: **China**: *YunNan Province*: PuEr Region, JingGu County, WeiYuan Town, from soil collected in a *Eucalyptus* plantation, 16 Nov. 2014, *S.F. Chen & J.Q. Li* (PREM 61950 – holotype; CMW 47655 = CERC 5376 = CBS 142892 – ex-type cultures).

Description: *Sexual morph* unknown. *Macroconidiophores* consisting of a stipe, a suite of penicillate arranged fertile branches, a stipe extension, and a terminal vesicle; stipe septate, hyaline, smooth, 38–89 × 5–8 µm; stipe extension septate, straight to flexuous 22–94 µm long, 1.5–2.5 µm wide at the apical septum, terminating in ellipsoidal, obpyriform to sphaeropedunculate vesicles, (2.5–)3.5–5.0 µm diam; lateral stipe extensions (90° to main axis) abundant, septate, straight to flexuous 18–64 µm long, terminating in a obpyriform to

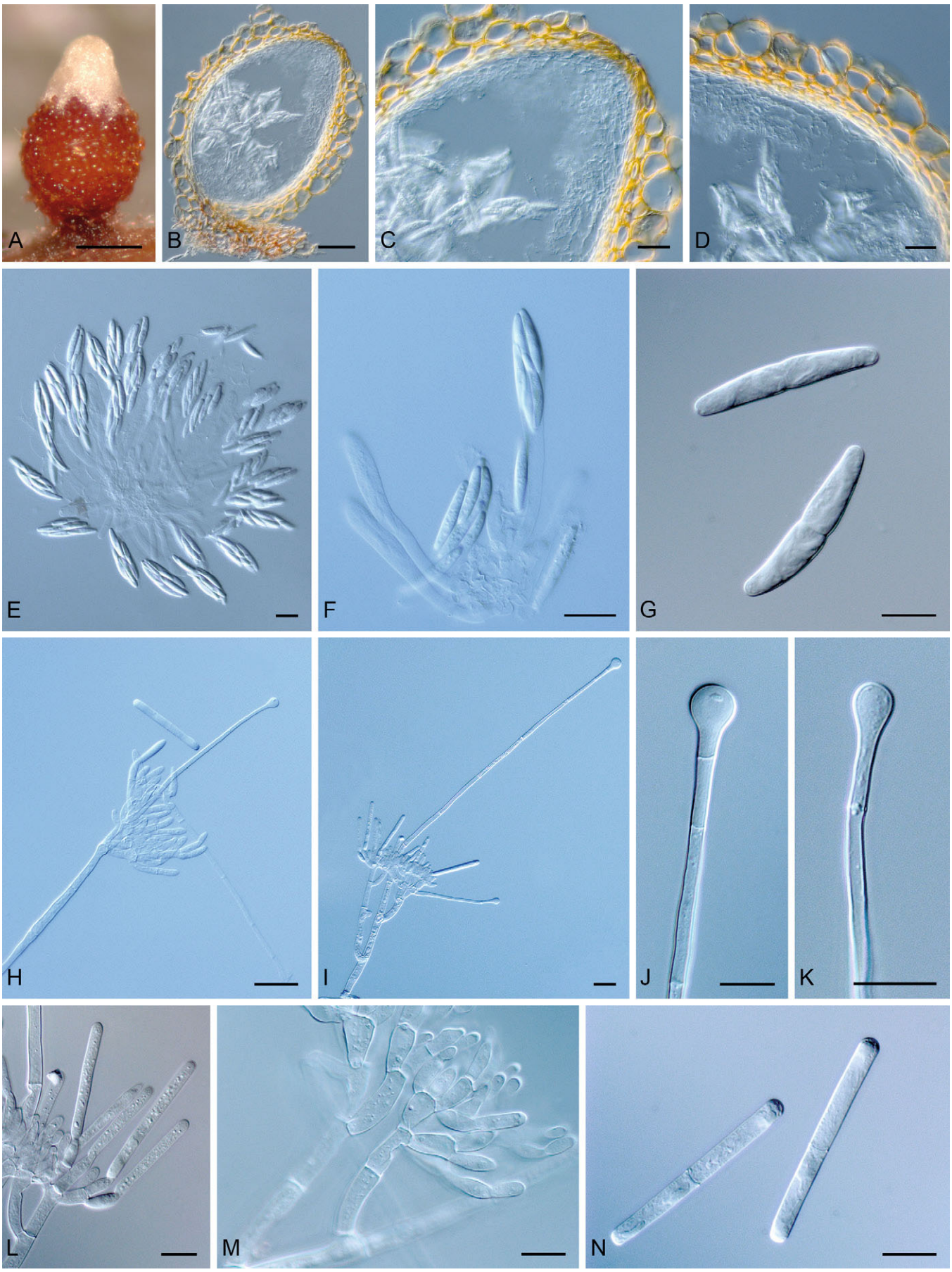

Fig. 6. *Calonectria pseudoturangicola*. **A.** Perithecium. **B.** Vertical section through a perithecium. **C.** Cells around ostiolar region of perithecium. **D.** Section through lateral perithecial wall. **E–F.** Asci. **G.** Ascospores. **H–I.** Macroconidiophore. **J–K.** Sphaeropedunculate vesicles. **L–M.** Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. **N.** Macroconidia. Bars: A = 200 µm; B = 50 µm; C–F and H–I = 20 µm; G and J–N = 10 µm.

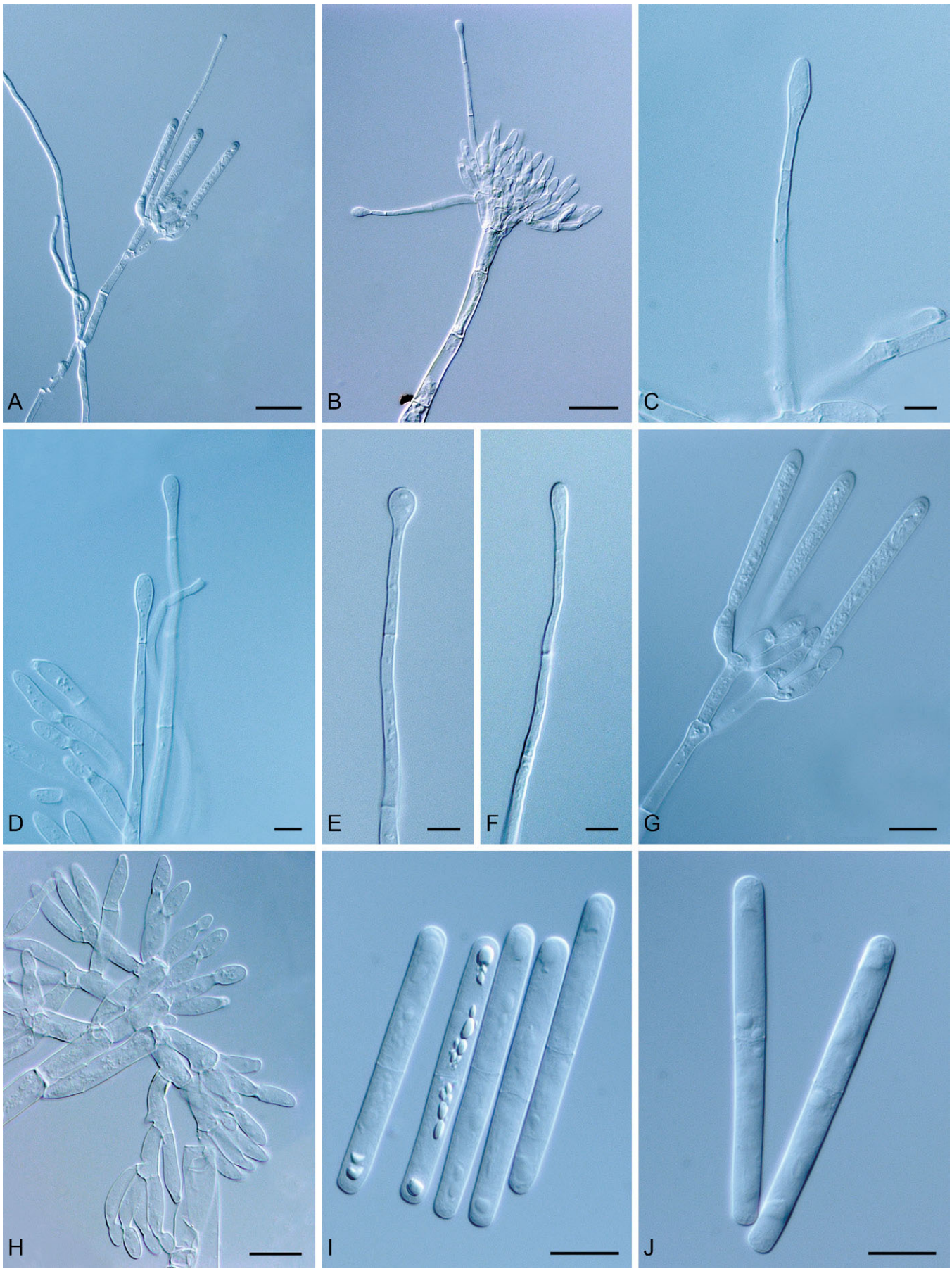

Fig. 7. *Calonectria pseudoyunnanensis*. **A–B.** Macroconidiophore. **C–F.** Ellipsoidal, obpyriform to sphaeropedunculate vesicles. **G–H.** Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. **I–J.** Macroconidia. Bars: A–B = 20 µm; G–J = 10 µm; C–F = 5 µm.

sphaeropedunculate vesicle, 1–3 µm diam. *Conidiogenous apparatus* 28–87 µm long, 32–83 µm wide; primary branches aseptate to 1-septate, 16–42 × 3.5–6.5 µm; secondary branches aseptate, 11–19 × 3.5–5.5 µm; tertiary branches aseptate, 7–13 × 3–5 µm; each terminal branch producing 2–4 phialides; phialides doliiform to reniform, hyaline, aseptate, 6–15 × 3–5 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (40–)44–50(–55) × (4–)4.5–5.5(–6) µm (av. = 47.5 × 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* forming abundant white aerial mycelium on MEA at 25 °C after 7 d, moderate sporulation, feathery, irregular margins, reverse pale yellow-orange (15f) to sienna (8). *Chlamydospores* common throughout the medium forming microsclerotia. *Growth characteristics,* optimal growth temperature 25 °C, no growth at 5 °C and 35 °C, after 7 d, colonies at 10 °C, 15 °C, 20 °C, 25 °C and 30 °C reached 6.4 mm, 30.4 mm, 53.7 mm, 78.4 mm and 55.3 mm, respectively.

Additional material examined: **China**: *YunNan Province*: PuEr Region, JingGu County, WeiYuan Town, from soil collected in a *Eucalyptus* plantation, 16 Nov. 2014, *S.F. Chen & J.Q. Li* (PREM 61951, CMW 47656 = CERC 5377 = CBS 142893; PREM 61952, culture CMW 47657 = CERC 5378 = CBS 142894).

Notes: *Calonectria pseudoyunnanensis* is most closely related to *Ca. asiatica*, *Ca. colombiensis,* and *Ca. yunnanensis*. It can be distinguished from these three species by the average size of the macroconidia. Those of *Ca. pseudoyunnanensis* (av. = 47.5 × 5 µm) are longer and broader than those of *Ca. yunnanensis* (av. = 43 × 4.5 µm), but shorter than those of *Ca. asiatica* (av. = 53 × 5 µm) and *Ca. colombiensis* (av. = 53 × 4.5 µm) (Crous *et al*. 2004). Furthermore, the vesicle shape of *Ca. pseudoyunnanensis* (ellipsoidal, obpyriform to sphaeropedunculate) is different to those of *Ca. asiatica* (sphaeropedunculate) and *Ca. colombiensis* (sphaeropedunculate) (Crous *et al*. 2004, Lombard *et al*. 2010c).

Calonectria yunnanensis J.Q. Li, Q.L. Liu & S.F. Chen, **sp. nov.**
MycoBank MB821637
(Fig. 8)

Etymology: From YunNan Province, China, where this fungus was first collected.

Diagnosis: *Calonectria yunnanensis* can be distinguished from the phylogenetically closely related *Ca. asiatica*, *Ca. colombiensis,* and *Ca. pseudoyunnanensis* by the size of macroconidia and ascospores.

Type: **China**: *YunNan Province*: PuEr Region, JingGu County, ZhengXing Town, from soil collected in a *Eucalyptus* plantation, 16 Nov. 2014, *S.F. Chen & J.Q. Li* (PREM 61955 – holotype; CMW 47644 = CERC 5339 = CBS 142897 – ex-type cultures).

Description: *Perithecia* solitary or in groups of up to five, orange, becoming orange-brown with age; in section, apex and body orange, base red-brown, subglobose to ovoid, 303–511 µm high, 322–567 µm diam, body turning red, and base dark red-brown in 3 % KOH; perithecial walls rough consisting of two thick-walled layers: outside layer of *textura globulosa*, 24–72 µm wide, becoming more compressed towards inner layer of *textura angularis*, 10–22 µm wide, becoming thin-walled and hyaline towards the centre; outer cells 19–37 × 12–21 µm, inner cells 14–39 × 3–11 µm; perithecial base up to 260 µm wide, consisting of dark red, angular cells merging with an erumpent stroma, cells of the outer wall layer continuing into the pseudoparenchymatous cells of the erumpent stroma. *Asci* 8-spored, clavate, (84–)97–133(–163) × (10–)15–21(–27) µm (av. = 115 × 18 µm), tapering to a long thin stalk. *Ascospores* aggregate in the upper third of the asci, hyaline, guttulate, fusoid with rounded ends, straight to slightly curved, 1(–3)-septate, not or slightly constricted at the septum, (28–)31–41(–55) × (5–)5.5–6.5(–8) µm (av. = 36 × 6 µm). *Macroconidiophores* consisting of a stipe, a suite of penicillate arranged fertile branches, a stipe extension, and a terminal vesicle; stipe septate, hyaline, smooth, 43–230 × 2.5–7 µm; stipe extension septate, straight to flexuous 25–102 µm long, 1.5–3.5 µm wide at the apical septum, terminating in a sphaeropedunculate vesicle, (2–)2.5–3.5(–4.5) µm diam; lateral stipe extensions (90° to main axis) abundant, septate, straight to flexuous 25–69 µm long, terminating in a sphaeropedunculate vesicle, 1–4 µm diam. *Conidiogenous apparatus* 20–130 µm long, 23–135 µm wide; primary branches aseptate to 1-septate, 13–49 × 3–6.5 µm; secondary branches aseptate, 12–17 × 3–5 µm; tertiary branches aseptate, 4–13 × 1.5–4 µm; each terminal branch producing 2–4 phialides; phialides doliiform to reniform, hyaline, aseptate, 6–16 × 2.5–4.5 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (36–)39–47(–52) × (4–)4.5–5(–5.5) µm, (av. = 43 × 4.5 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: *Colonies* forming abundant white to white-buff aerial mycelium on MEA at 25 °C after 7 d, profuse sporulation, feathery, irregular margins, reverse salmon (13'd) to sienna (8). *Chlamydospores* common throughout the medium forming microsclerotia. *Growth characteristics,* optimal growth temperature 25 °C, no growth at 5 °C and 35 °C, after 7 d, colonies at 10 °C, 15 °C, 20 °C, 25 °C and 30 °C reached 7.3 mm, 33.0 mm, 53.9 mm, 76.4 mm and 53.9 mm, respectively.

Additional material examined: **China**: *YunNan Province*: PuEr Region, JingGu County, ZhengXing Town, from soil collected in a *Eucalyptus* plantation, 16 Nov. 2014, *S.F. Chen & J.Q. Li* (PREM 61953, culture CMW 47642 = CERC 5337 = CBS 142895; PREM 61954, culture CMW 47643 = CERC 5338 = CBS 142896).

Notes: *Calonectria yunnanensis* is closely related to *Ca. asiatica*, *Ca. colombiensis* and *Ca. pseudoyunnanensis*. It can be distinguished from these three species by the

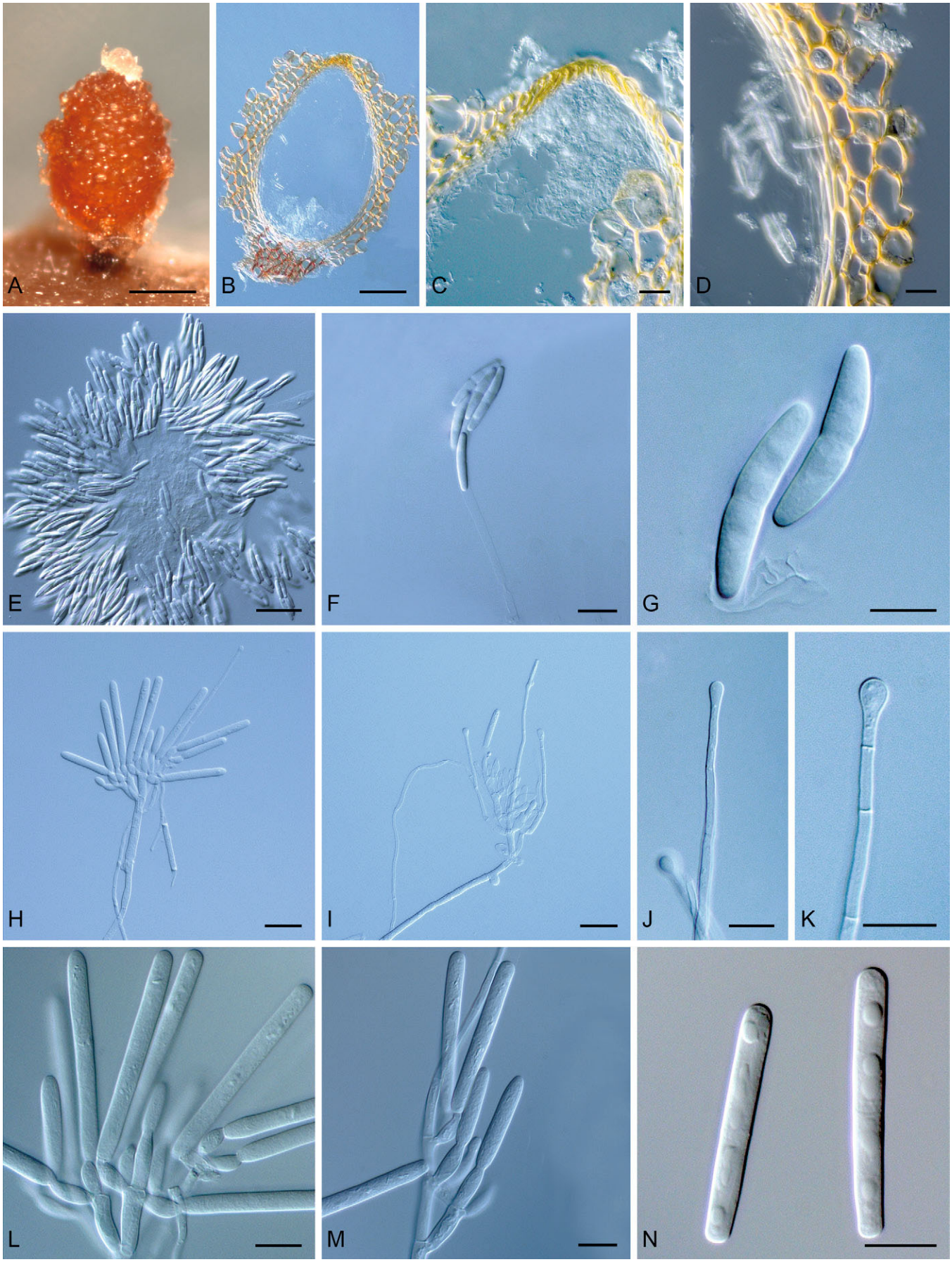

Fig. 8. *Calonectria yunnanensis*. **A.** Perithecium. **B.** Vertical section through a perithecium. **C.** Cells around ostiolar region of perithecium. **D.** Section through lateral perithecial wall. **E–F.** Asci. **G.** Ascospores. **H–I.** Macroconidiophore. **J–K.** Sphaeropedunculate vesicles. **L–M.** Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. **N.** Macroconidia. Bars: A = 200 µm; B = 100 µm; E = 50 µm; C–D, F and H–I = 20 µm; G and J–N = 10 µm.

average size of the macroconidia. The macroconidia of *Ca. yunnanensis* (av. = 43 × 4.5 µm) are shorter than those of *Ca. asiatica* (av. = 53 × 5 µm), *Ca. colombiensis* (av. = 53 × 4.5 µm) and *Ca. pseudoyunnanensis* (av. = 47.5 × 5 µm) (Crous *et al*. 2004). The ascospores of *Ca. yunnanensis* (av. = 36 × 6 µm) are slightly longer than those of *Ca. asiatica* (av. = 33 × 6 µm) and *Ca. colombiensis* (av. = 33 × 5 µm) (Crous *et al*. 2004).

DISCUSSION

Results of this study revealed 13 species of *Calonectria* from infected *Eucalyptus* tissues collected in plantations and nurseries, or baited soil samples from *Eucalyptus* plantations, and a naturally forested area in South China. These species include *Ca. arbusta*, *Ca. asiatica*, *Ca. chinensis*, *Ca. eucalypti*, *Ca. hongkongensis*, *Ca. mossambicensis*, *Ca. pentaseptata,* and six previously undescribed taxa (*Ca. aciculata*, *Ca. honghensis*, *Ca. lantauensis*, *Ca. pseudoturangicola*, *Ca. pseudoyunnanensis,* and *Ca. yunnanensis*). The six novel species were strongly supported by DNA sequence data and morphological observations. Five of the 13 species, including *Ca. aciculata*, *Ca. eucalypti*, *Ca. honghensis*, *Ca. mossambicensis,* and *Ca. pentaseptata,* resided in the Prolate Group and eight in the Sphaero-Naviculate Group. With the exception of the newly described species, this is the first report of *Ca. asiatica*, *Ca. eucalypti,* and *Ca. mossambicensis* from China. *Calonectria chinensis* and *Ca. lantauensis* were isolated only from soil in natural forested areas, while 11 species were all collected from *Eucalyptus* plantations or nurseries.

Calonectria pentaseptata, identified in this study, resides in the *Ca. reteaudii* species complex and was widely distributed in different regions causing disease on *Eucalyptus* in plantations and nurseries in South China. Amongst the 115 *Calonectria* isolates collected in this study, approximately half (57) were identified as *Ca. pentaseptata*, and this fungus occurred at six different sites in the GuangDong and GuangXi Provinces. This is consistent with previous studies showing that *Ca. pentaseptata* is widely distributed in *Eucalyptus* plantations and nurseries in South China (Lombard *et al*. 2015). The *Ca. reteaudii* complex, which includes species that are well-known causal agents of Calonectria Leaf Blight (CLB) of *Eucalyptus* (Crous 2002, Rodas *et al*. 2005, Lombard *et al*. 2010b). *Calonectria pentaseptata* is the fourth species in the *Ca. reteaudii* species complex to have been found in China; the other three include *Ca. crousiana*, *Ca. microconidialis* and *Ca. pseudoreteaudii*. Pathogenicity tests have shown that all four of these species cause rot on inoculated *Eucalyptus* leaves (Chen *et al*. 2011c, Li *et al*. 2014a, b). Overall, the results of this study support the view (Lombard *et al*. 2015) that *Ca. pentaseptata* is an important *Eucalyptus* pathogen both in plantations and nurseries in China.

Calonectria mossambicensis is the fourth species in the *Ca. candelabrum* complex to have been reported from China together with *Ca. pauciramosa*, *Ca. seminaria* and *Ca. tetraramosa*. All four species were isolated from diseased seedlings in *Eucalyptus* nurseries (Lombard *et al*. 2010d, 2015). Species in the *Ca. candelabrum* complex include some important nursery pathogens (Crous 2002, Lombard *et al*. 2010b, d, Guarnaccia *et al*. 2014, Alfenas *et al*. 2015). Inoculation studies have also shown that *Ca. pauciramosa*, *Ca. seminaria* and *Ca. tetraramosa* are differentially pathogenic to *Eucalyptus* clones (Chen *et al*. 2011c, Li *et al*. 2014a, b). *Calonectria mossambicensis* was originally described from diseased cuttings of *E. grandis* × *E. camaldulensis* clones in Mozambique (Crous *et al*. 2013) and it is likely to be a *Eucalyptus* nursery pathogen in China, since this fungus causes rot on *Eucalyptus* cutting rot in Mozambique.

Three species residing in the *Ca. colhounii* complex were identified in this study. They include *Ca. eucalypti* and the newly described *Ca. aciculata* and *Ca. honghensis.* Species in the *Ca. colhounii* complex are characterized by bright yellow perithecia (Crous 2002, Lombard *et al*. 2010c, Chen *et al*. 2011c, Xu *et al*. 2012). *Calonectria aciculata* and *Ca. honghensis* are closely related to *Ca. colhounii* and these three species can easily be distinguished from each other based on phylogenetic inference, as well as by their macroconidial dimensions. Other *Calonectria* species known in China and that reside in the *Ca. colhounii* complex include *Ca. fujianensis*, *Ca. nymphaeae,* and *Ca. pseudocolhounii* (Chen *et al*. 2011c, Xu *et al*. 2012). Other than *Ca. honghensis* isolated from soil collected in a *Eucalyptus* plantation, and *Ca. nymphaeae* from diseased leaves of *Nymphaea tetragona* (Xu *et al*. 2012), the remaining four species in the *Ca. colhounii* complex were all isolated from diseased *Eucalyptus* leaves in commercial plantations (Chen *et al*. 2011c). Inoculation studies have shown that *Ca. crousiana*, *Ca. fujianensis* and *Ca. pseudocolhounii* are all pathogenic to inoculated *Eucalyptus* clones (Chen *et al*. 2011c, Li *et al*. 2014a, b).

Four new species in the Sphaero-Naviculate Group reside in the *Ca. kyotensis* complex, *Ca. lantauensis*, *Ca. pseudoturangicola*, *Ca. pseudoyunnanensis,* and *Ca. yunnanensis*. *Calonectria pseudoyunnanensis* and *Ca. yunnanensis* are sister species based on phylogenetic inference but they can easily be distinguished by DNA sequence comparisons of the *his3* and *tub2* gene regions, and vesicle shape differences. *Calonectria pseudoturangicola* appears as a sister species to *Ca. turangicola* but can be distinguished based on DNA sequence differences in the *tef1*, *cmdA* and *tub2* gene regions, and macroconidial dimensions (Lombard *et al*. 2015). *Calonectria lantauensis* formed a basal clade in the *Ca. kyotensis* species complex, and lateral stipe extensions were absent in this species making it readily distinguishable from other species in the *Ca. kyotensis* species complex (Crous *et al*. 2004, Lombard *et al*. 2010c, 2015).

The remaining four known species (*Ca. arbusta*, *Ca. asiatica*, *Ca. chinensis,* and *Ca. hongkongensis*) found in this study reside in the *Ca. kyotensis* complex. To date, 19 species in the *Ca. kyotensis* complex have been found in China and the only other species in the complex, *Ca. asiatica,* was first described from Thailand (Crous *et al*. 2004, Lombard *et al*. 2015). These 19 species were all isolated exclusively from soil (Lombard *et al*. 2015) and the results of this study suggest that many more species in this complex have yet to be discovered from soil in China.

Overall, the results of this study revealed 37 species

of *Calonectria* from China. Other than *Ca. asiatica*, *Ca. eucalypti*, *Ca. mossambicensis, Ca. pauciramosa,* and *Ca. pentaseptata*, all of these species were first discovered in this country (Crous *et al*. 2004, Lombard *et al*. 2010d, 2015, Chen *et al*. 2011c, Xu *et al*. 2012). The results highlight the significant impact that DNA sequence comparisons have had in revealing new species of filamentous fungi, including species of *Calonectria* (Lombard *et al*. 2010c, 2015, 2016, Crous *et al*. 2015a).

With the exception of *Ca. nymphaeae* isolated from diseased leaves of *N. tetragona* (Xu *et al*. 2012), and *Ca. lantauensis* from a naturally forested area in Hong Kong, all of the other 35 species found in China were from *Eucalyptus* plantations or nurseries. This appears to be an environment surprisingly rich in species of *Calonectria*, although future sampling in China should be expanded to include other environments. Inoculation tests conducted in previous studies have shown that 15 species of *Calonectria* found in China are pathogenic to several *Eucalyptus* clones (Chen *et al.* 2011c, Li *et al*. 2014a, b). Future work should include a more comprehensive understanding of the species diversity, distribution, pathogenicity and population biology of *Calonectria* in China. This will contribute to the development of integrated management strategies for the diseases caused by these fungi in *Eucalyptus* plantations and nurseries.

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