Leaf blight in a Eucalyptus plantation caused by Calonectria spp. originating from both leaves and soils

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Research

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Abstract

Calonectria leaf blight (CLB) is one of the best-known diseases of *Eucalyptus* spp., particularly in Asia and South America. Recently, typical symptoms of leaf and shoot blight caused by *Calonectria* spp. were observed in a *Eucalyptus* plantation in the YunNan Province of southwestern China. Isolations were made from diseased leaves and soil samples collected from below the infected trees to determine the causal agent of the disease and to consider the distribution characteristics of the *Calonectria* species. This resulted in 417 isolates, of which 228 were from leaves and 189 were from soils. Based on comparisons of DNA sequences for the *act* (actin), *cmdA* (calmodulin), *his3* (histone H3), *rpb2* (the second largest subunit of RNA polymerase), *tef1* (translation elongation factor 1-alpha) and *tub2* (β-tubulin) gene regions, as well as morphological characteristics, 11 *Calonectria* species were identified. These included *Calonectria aciculata* (0.7%), *Ca. colhounii* (1.2%), *Ca. eucalypti* (10.6%) and *Ca. honghensis* (43.2%) in the *Ca. colhounii* species complex, and *Ca. aconidialis* (15.3%), *Ca. asiatica* (9.8%), *Ca. hongkongensis* (1.0%), *Ca. ilicicola* (6.0%), *Ca. kyotensis* (0.5%), and *Ca. yunnanensis* (11.3%) in the *Ca. kyotensis* species complex. In addition, a novel species, accounting for 0.5% of the isolates, was discovered and described here as *Ca. dianii* sp. nov. in the *Ca colhounii* species complex. Most (99.1%) of the isolates collected from the leaves resided in the *Ca. colhounii* species complex and a majority (95.8%) of those from the soils were in *Ca. kyotensis* species complex. These results suggest that *Calonectria* spp. in the *Ca. colhounii* species complex infecting leaves are specifically adapted to that niche and likewise those in the *Ca. kyotensis* species complex are better adapted to a soil habitat.

Introduction

As areas of China planted to *Eucalyptus* have expanded during the past four decades and growing numbers of diseases have emerged as threats to these trees (Zhou and Wingfield 2011; Liu and Xie 2020). Prominent disease problems include stem canker caused by the *Botryosphaeriaceae* (Li et al. 2018, 2020) and the *Cryphonectriaceae* (Wang et al. 2018, 2020), Coniothyrium canker caused by *Teratosphaeria zuluensis* (Chen et al. 2011a), wilt associated with infections of the bacterium *Ralstonia solanacearum* (Old et al. 2003; Carstensen et al. 2017; Jiang et al. 2017), leaf spot caused by species of *Mycosphaerella* (Burgess et al. 2007) and *Teratosphaeria* (Burgess et al. 2006; Havenga et al. 2020). Amongst the most common and often serious disease problems is leaf blight caused by *Calonectria* spp. (Li et al. 2017; Wang and Chen 2020; Wu and Chen 2021), particularly in southern China plantations.

Species of *Calonectria* are widely distributed in tropical and subtropical regions of the world, resulting in a wide variety of symptoms on a large number of agronomic, horticultural and forestry crops (Crous 2002; Lombard et al. 2010a; Alfenas et al. 2013, 2015). There are currently 133 accepted names in *Calonectria* (Crous et al. 2018, 2019, 2021a, b; Wang et al. 2019; Liu et al. 2020, 2022; Mohali and Stewart 2021; Pham et al. 2022; Sanchez-Gonzalez et al. 2022) distributed across 11 species complexes residing in either one of two morphological assemblages known as the Prolate or Sphaero-Naviculate Groups (Lombard et al. 2010a; Liu et al. 2020). The Prolate Group includes species with clavate to pyriform to ellipsoidal vesicles and those in the Sphaero-Naviculate Group have sphaeropedunculate and naviculate vesicles (Liu et al. 2020).

Calonectria species are well-known to infect the leaves of *Eucalyptus* spp. but also to occur in the soils associated with these trees in China (Li et al. 2017; Wu and Chen 2021; Liu et al. 2021). There are currently 28 species of *Calonectria* known from China, and of these seven (*Ca. aciculata*, *Ca. crousiana*, *Ca. eucalypti*, *Ca. fujianensis*, *Ca. hawksworthii*, *Ca. pauciramosa* and *Ca. queenslandica*) have been isolated from diseased *Eucalyptus* tissues (Chen et al. 2011b; Lombard et al. 2010b, 2015; Li et al. 2017, 2022; Wang and Chen 2020). Eleven other species (*Ca. asiatica*, *Ca. auriculiformis*, *Ca. chinensis*, *Ca. honghensis*, *Ca. hongkongensis*, *Ca. ilicicola*, *Ca. kyotensis*, *Ca. lateralis*, *Ca. yunnanensis*, *Ca. minensis* and *Ca. orientalis*) have been isolated from soils in *Eucalyptus* plantations (Crous et al. 2004; Lombard et al. 2015; Li et al. 2017; Liu et al. 2020, 2022; Wu and Chen 2021; Liu and Chen 2022) and four (*Ca. aconidialis*, *Ca. cerciana*, *Ca. pseudoreteaudii* and *Ca. reteaudii*) are known to occur both in soils and on infected *Eucalyptus* tissues (Lombard et al. 2010b, 2015; Wu and Chen 2021; Li et al. 2022).

Broadly, *Calonectria* spp. are known as soil-associated fungi with the ability to infect young plant tissues under favourable environmental conditions. Anecdotal observations, based on extensive field collections suggest that some species occurring in the soil are more likely to infect plants than others. However, very little is known regarding the species diversity and distribution characteristics of these fungi isolated from the diseased *Eucalyptus* tissues and soils. To the best of our knowledge, only one study has considered *Calonectria* species from diseased leaves of *Eucalyptus* and the soils associated with those trees (Wu and Chen 2021). That study showed that *Ca. pseudoreteaudii* occurred both on diseased leaves and in the associated soils in one *Eucalyptus* plantation, but the other four species (*Ca. aconidialis*, *Ca. auriculiformis*, *Ca. hongkongensis* and *Ca. reteaudii*) occurred only in the soil samples. The study of Wu and Chen (2021) was of a preliminary nature and included relatively few isolates. The present study was undertaken when a single *Eucalyptus* planation in the YunNan Province was seriously damaged by Calonectria Leaf Blight (CLB), providing an opportunity to intensively sample both infected leaves and the soils associated with the affected trees. The aim of this study was thus to determine the species diversity and the distribution characteristics of *Calonectria* spp. in diseased *Eucalyptus* leaves and soil samples associated with those trees.

Materials And Methods

Sample collection and fungal isolation

Extensive disease surveys of *Eucalyptus* plantations were conducted in JingGu County, PuEr Region, YunNan Province, southwestern China (23°23'58"N, 100°50'37"E) in December 2016. Typical symptoms of CLB were detected in an approximately 10 ha plantation of one-year-old *Eucalyptus urophylla* × *E. grandis*. Two hundred and fifty-one diseased trees in the plantation were randomly selected for leaf sampling and one symptomatic leaf was taken from each tree. The same number of soil samples were collected randomly in the plantation and the leaf litter was removed before collecting soil samples from the upper 0–20 cm of the soil profile. The leaf and soil samples were then transported to a laboratory for further study.

Diseased *Eucalyptus* leaves were incubated in moist Petri dishes for one to two days at 25°C and checked regularly throughout the incubation period for fungal sporulation. Soil samples were baited with germinating *Medicago sativa* seeds (alfalfa, surface-disinfested in 75% ethanol) as described by Crous (2002) and after seven to ten days incubation at 25°C, conidiophores emerged on the tissues of the germinating plants. Conidial masses were transferred from the *Eucalyptus* leaves and the infected alfalfa tissues onto 2% (v/v) Malt Extract Agar (MEA) using a sterile needle under a dissection microscope (Stemi 2000C, Carl Zeiss, Germany). After two days incubation at 25°C, a single hyphae tip from each culture was transferred to fresh 2% MEA plates and these were incubated at 25°C for seven days to obtain axenic cultures.

All cultures were deposited in the Culture Collection (CSF) at the Research Institute of Fast-growing Trees (RIFT) (previous institution: China Eucalypt Research Centre, CERC), Chinese Academy of Forestry (CAF), ZhanJiang, GuangDong Province, China. Representative isolates were stored in the China General Microbiological Culture Collection Centre (CGMCC), Beijing, China. The dried specimens were deposited in the mycological fungarium of the Institute of Microbiology, Chinese Academy of Sciences (HMAS), Beijing, China.

DNA extraction, PCR amplifications and sequencing

Genomic DNA was extracted from cultures grown on MEA for seven days, following the CTAB method as described by Van Burik et al. (1998). Six gene regions including the *act* (actin), *cmdA* (calmodulin), *his3* (histone H3), *rpb2* (the second largest subunit of RNA polymerase), *tef1* (translation elongation factor 1-alpha) and *tub2* (β-tubulin) were amplified using the primer pairs and protocols described by Liu et al. (2020). All the PCR products were sequenced in both directions using the same primers used for amplification. All sequences obtained in this study were deposited in GenBank (http://www.ncbi.nlm.nih.gov) (Table 1, Additional file 1: Table S1).

Table 1
Isolates sequenced and used for phylogenetic analyses and morphological studies in this study

Species[a]	Isolate No.[b]	Genotype[c]	Substrate	Sampling site	Collector	GenBank accession No.[d]				
						act	*cmdA*	*his3*	*rpb2*	*tef1*
Calonectria colhounii species complex										
Ca. aciculata	CSF6495	AAAAAA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321585	OP321689	OP321839	OP321990	OP3220
	CSF6515	AAAABA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321586	OP321690	OP321840	OP321991	OP3220
	CSF6528	ABBBCB	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321587	OP321691	OP321841	OP321992	OP3220
Ca. colhounii	CSF6433	AAAAAA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321588	OP321692	OP321842	OP321993	OP3220
	CSF6471	AAAABB	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321589	OP321693	OP321843	OP321994	OP3220
	CSF6589	ABABCA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321590	OP321694	OP321844	OP321995	OP3220
	CSF6592	ACBADA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321591	OP321695	OP321845	OP321996	OP3221
	CSF6642	ADABCA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321592	OP321696	OP321846	OP321997	OP3221
Ca. dianii **sp. nov.**	**CSF6520**[e – g]; **CGMCC3.20446**	AAAAAA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321593	OP321697	OP321847	OP321998	OP3221
	CSF6439[e,f] **CGMCC3.20445**	AAAAAA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321594	OP321698	OP321848	OP321999	OP3221

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

[b] CSF: Culture Collection at the Research Institute of Fast-growing Trees (RIFT)/China Eucalypt Research Centre (CERC), ZhanJiang, GuangDong Province, Ch China General Microbiological Culture Collection Center, Beijing, China.

[c] Genotype within each identified species, determined by sequences of *act*, *cmdA*, *his3*, *rpb2*, *tef1* and *tub2* regions.

[d] *act*, actin; *cmdA*, calmodulin; *his3*, histone H3; *rpb2*, the second largest subunit of RNA polymerase; *tef1*, translation elongation factor 1-alpha; *tub2*, β-tubuli

[e] Isolates used in morphological and culture growth studies.

[f] Isolates used for mating studies.

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

Species[a]	Isolate No.[b]	Genotype[c]	Substrate	Sampling site	Collector	GenBank accession No.[d]				
						act	*cmdA*	*his3*	*rpb2*	*tef1*
Ca. eucalypti	CSF5407	AAAAAA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321595	OP321699	OP321849	OP322000	OP3221
	CSF6466	AAAAAA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321596	OP321700	OP321850	OP322001	OP3221
	CSF6483	AAAAAB	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321597	OP321701	OP321851	OP322002	OP3221
	CSF6645	AAAAAB	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321598	OP321702	OP321852	OP322003	OP3221
	CSF6536	AAAAAC	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321599	OP321703	OP321853	OP322004	OP3221
	CSF6610	AAAAAD	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321600	OP321704	OP321854	OP322005	OP3221
	CSF6650	AAAAAE	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321601	OP321705	OP321855	OP322006	OP3221
	CSF6587	AAAABA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321602	OP321706	OP321856	OP322007	OP3221
	CSF6564	AAAABD	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321603	OP321707	OP321857	OP322008	OP3221
	CSF6504	AAAACA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321604	OP321708	OP321858	OP322009	OP3221
	CSF6646	AAAACA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321605	OP321709	OP321859	OP322010	OP3221

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[c] Genotype within each identified species, determined by sequences of *act*, *cmdA*, *his3*, *rpb2*, *tef1* and *tub2* regions.

[d] *act*, actin; *cmdA*, calmodulin; *his3*, histone H3; *rpb2*, the second largest subunit of RNA polymerase; *tef1*, translation elongation factor 1-alpha; *tub2*, β-tubuli

[e] Isolates used in morphological and culture growth studies.

[f] Isolates used for mating studies.

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

Species[a]	Isolate No.[b]	Genotype[c]	Substrate	Sampling site	Collector	GenBank accession No.[d]				
						act	*cmdA*	*his3*	*rpb2*	*tef1*
	CSF6571	AAAACF	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321606	OP321710	OP321860	OP322011	OP3221
	CSF6572	AAAACF	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321607	OP321711	OP321861	OP322012	OP3221
	CSF6461	AAAADA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321608	OP321712	OP321862	OP322013	OP3221
	CSF6473	AAAAEA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321609	OP321713	OP321863	OP322014	OP3221
	CSF6667	AAAAEA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321610	OP321714	OP321864	OP322015	OP3221
	CSF6533	AAAAGF	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321611	OP321715	OP321865	OP322016	OP3221
	CSF6607	AAAAHA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321612	OP321716	OP321866	OP322017	OP3221
	CSF6608	AAAAHA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321613	OP321717	OP321867	OP322018	OP3221
	CSF13848	ABAAAA	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321614	OP321718	OP321868	OP322019	OP3221
	CSF13849	ABAAAA	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321615	OP321719	OP321869	OP322020	OP3221
	CSF6531	ABAAFA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321616	OP321720	OP321870	OP322021	OP3221

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[c] Genotype within each identified species, determined by sequences of *act*, *cmdA*, *his3*, *rpb2*, *tef1* and *tub2* regions.

[d] *act*, actin; *cmdA*, calmodulin; *his3*, histone H3; *rpb2*, the second largest subunit of RNA polymerase; *tef1*, translation elongation factor 1-alpha; *tub2*, β-tubuli

[e] Isolates used in morphological and culture growth studies.

[f] Isolates used for mating studies.

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

Species[a]	Isolate No.[b]	Genotype[c]	Substrate	Sampling site	Collector	GenBank accession No.[d]				
						act	*cmdA*	*his3*	*rpb2*	*tef1*
	CSF6453	ACAACF	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321617	OP321721	OP321871	OP322022	OP3221
Ca. honghensis	CSF5381	AAAAAA	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321618	OP321725	OP321876	OP322023	OP3221
	CSF6459	AAAAAA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321619	OP321726	OP321877	OP322024	OP3221
	CSF6474	AAAAAB	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321620	OP321727	OP321878	OP322025	OP3221
	CSF6517	AAAAAG	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321621	OP321728	OP321879	OP322026	OP3221
	CSF5399	AAAABA	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321622	OP321729	OP321880	OP322027	OP3221
	CSF6462	AAAABA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321623	OP321730	OP321881	OP322028	OP3221
	CSF13721	AAAABA	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321624	OP321731	OP321882	OP322029	OP3221
	CSF13722	AAAABA	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321625	OP321732	OP321883	OP322030	OP3221
	CSF5415	AAAABB	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321626	OP321733	OP321884	OP322031	OP3221
	CSF6477	AAAABB	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321627	OP321734	OP321885	OP322032	OP3221

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[d] *act*, actin; *cmdA*, calmodulin; *his3*, histone H3; *rpb2*, the second largest subunit of RNA polymerase; *tef1*, translation elongation factor 1-alpha; *tub2*, β-tubuli

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Species[a]	Isolate No.[b]	Genotype[c]	Substrate	Sampling site	Collector	GenBank accession No.[d]				
						act	*cmdA*	*his3*	*rpb2*	*tef1*
	CSF6436	AAAABC	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321628	OP321735	OP321886	OP322033	OP3221
	CSF6525	AAAABD	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321629	OP321736	OP321887	OP322034	OP3221
	CSF6498	AAAABF	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321630	OP321737	OP321888	OP322035	OP3221
	CSF6500	AAAABG	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321631	OP321738	OP321889	OP322036	OP3221
	CSF6573	AAAABG	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321632	OP321739	OP321890	OP322037	OP3221
	CSF6561	AAAABI	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321633	OP321740	OP321891	OP322038	OP3221
	CSF6634	AAAABJ	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321634	OP321741	OP321892	OP322039	OP3221
	CSF5417	AAAACA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321635	OP321742	OP321893	OP322040	OP3221
	CSF6669	AAAACA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321636	OP321743	OP321894	OP322041	OP3221
	CSF6532	AAAACG	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321637	OP321744	OP321895	OP322042	OP3221
	CSF6428	AAAADA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321638	OP321745	OP321896	OP322043	OP3221

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[c] Genotype within each identified species, determined by sequences of *act*, *cmdA*, *his3*, *rpb2*, *tef1* and *tub2* regions.

[d] *act*, actin; *cmdA*, calmodulin; *his3*, histone H3; *rpb2*, the second largest subunit of RNA polymerase; *tef1*, translation elongation factor 1-alpha; *tub2*, β-tubuli

[e] Isolates used in morphological and culture growth studies.

[f] Isolates used for mating studies.

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

Species[a]	Isolate No.[b]	Genotype[c]	Substrate	Sampling site	Collector	GenBank accession No.[d]				
						act	*cmdA*	*his3*	*rpb2*	*tef1*
	CSF13771	AAAADA	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321639	OP321746	OP321897	OP322044	OP3221
	CSF13772	AAAADA	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321640	OP321747	OP321898	OP322045	OP3221
	CSF6508	AAAADH	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321641	OP321748	OP321899	OP322046	OP3221
	CSF6625	AAAADH	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321642	OP321749	OP321900	OP322047	OP3221
	CSF6440	AAAAED	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321643	OP321750	OP321901	OP322048	OP3221
	CSF6605	AAAAFA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321644	OP321751	OP321902	OP322049	OP3221
	CSF6613	AAAAFB	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321645	OP321752	OP321903	OP322050	OP3221
	CSF6512	AAAAFD	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321646	OP321753	OP321904	OP322051	OP3221
	CSF6442	AAAAFE	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321647	OP321754	OP321905	OP322052	OP3221
	CSF6456	AAAAGA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321648	OP321755	OP321906	OP322053	OP3221
	CSF6619	AAAAGA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321649	OP321756	OP321907	OP322054	OP3221

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

[b] CSF: Culture Collection at the Research Institute of Fast-growing Trees (RIFT)/China Eucalypt Research Centre (CERC), ZhanJiang, GuangDong Province, Ch China General Microbiological Culture Collection Center, Beijing, China.

[c] Genotype within each identified species, determined by sequences of *act*, *cmdA*, *his3*, *rpb2*, *tef1* and *tub2* regions.

[d] *act*, actin; *cmdA*, calmodulin; *his3*, histone H3; *rpb2*, the second largest subunit of RNA polymerase; *tef1*, translation elongation factor 1-alpha; *tub2*, β-tubuli

[e] Isolates used in morphological and culture growth studies.

[f] Isolates used for mating studies.

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

Species[a]	Isolate No.[b]	Genotype[c]	Substrate	Sampling site	Collector	GenBank accession No.[d]				
						act	*cmdA*	*his3*	*rpb2*	*tef1*
	CSF6481	AAAAHA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321650	OP321757	OP321908	OP322055	OP3221
	CSF6568	AAAAIA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321651	OP321758	OP321909	OP322056	OP3221
	CSF6437	AABACA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321652	OP321759	OP321910	OP322057	OP3221
	CSF6522	AACABD	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321653	OP321760	OP321911	OP322058	OP3221
	CSF6612	BBBAHA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321654	OP321761	OP321912	OP322059	OP3221
Calonectria kyotensis species complex										
Ca. aconidialis	CSF13739	AAAAAA	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321655	OP321785	OP321936	OP322060	OP3223
	CSF13743	AAAAAA	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321656	OP321786	OP321937	OP322061	OP3223
	CSF13753	AAAAAB	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321657	OP321787	OP321938	OP322062	OP3223
	CSF13774	AAAAAB	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321658	OP321788	OP321939	OP322063	OP3223
	CSF13780	AAAAAC	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321659	OP321789	OP321940	OP322064	OP3223

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[b] CSF: Culture Collection at the Research Institute of Fast-growing Trees (RIFT)/China Eucalypt Research Centre (CERC), ZhanJiang, GuangDong Province, Ch China General Microbiological Culture Collection Center, Beijing, China.

[c] Genotype within each identified species, determined by sequences of *act*, *cmdA*, *his3*, *rpb2*, *tef1* and *tub2* regions.

[d] *act*, actin; *cmdA*, calmodulin; *his3*, histone H3; *rpb2*, the second largest subunit of RNA polymerase; *tef1*, translation elongation factor 1-alpha; *tub2*, β-tubuli

[e] Isolates used in morphological and culture growth studies.

[f] Isolates used for mating studies.

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

Species[a]	Isolate No.[b]	Genotype[c]	Substrate	Sampling site	Collector	GenBank accession No.[d]				
						act	*cmdA*	*his3*	*rpb2*	*tef1*
	CSF13850	AAAAAD	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321660	OP321790	OP321941	OP322065	OP3223
	CSF13851	AAAAAD	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321661	OP321791	OP321942	OP322066	OP3223
	CSF13862	ABAAAB	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321662	OP321792	OP321943	OP322067	OP3223
Ca. asiatica	CSF6468	AAAAAA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321663	OP321799	OP321950	OP322068	OP3223
	CSF13795	AAAAAA	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321664	OP321800	OP321951	OP322069	OP3223
	CSF13741	AAAAAB	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321665	OP321801	OP321952	OP322070	OP3223
	CSF13734	AACAAB	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321666	OP321802	OP321953	OP322071	OP3223

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[b] CSF: Culture Collection at the Research Institute of Fast-growing Trees (RIFT)/China Eucalypt Research Centre (CERC), ZhanJiang, GuangDong Province, Ch China General Microbiological Culture Collection Center, Beijing, China.

[c] Genotype within each identified species, determined by sequences of *act*, *cmdA*, *his3*, *rpb2*, *tef1* and *tub2* regions.

[d] *act*, actin; *cmdA*, calmodulin; *his3*, histone H3; *rpb2*, the second largest subunit of RNA polymerase; *tef1*, translation elongation factor 1-alpha; *tub2*, β-tubuli

[e] Isolates used in morphological and culture growth studies.

[f] Isolates used for mating studies.

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

Species[a]	Isolate No.[b]	Genotype[c]	Substrate	Sampling site	Collector	GenBank accession No.[d]				
						act	*cmdA*	*his3*	*rpb2*	*tef1*
	CSF13751	AACBAB	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321667	OP321803	OP321954	OP322072	OP3223
	CSF13713	ABAAAB	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321668	OP321804	OP321955	OP322073	OP3223
	CSF13760	ABABAB	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321669	OP321805	OP321956	OP322074	OP3223
	CSF13704	ABCAAB	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321670	OP321806	OP321957	OP322075	OP3223
	CSF13701	BAAAAB	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321671	OP321807	OP321958	OP322076	OP3224
	CSF13731	BABAAB	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321672	OP321808	OP321959	OP322077	OP3224
Ca. hongkongensis	CSF13786	AAAAAA	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321673	OP321810	OP321961	OP322078	OP3224
	CSF13790	AAAAAA	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321674	OP321811	OP321962	OP322079	OP3224
	CSF13812	ABAAAB	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321675	OP321812	OP321963	OP322080	OP3224
	CSF13813	ABAAAB	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321676	OP321813	OP321964	OP322081	OP3224
Ca. ilicicola	CSF13767	AAAAAA	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321677	OP321814	OP321965	OP322082	OP3224

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[b] CSF: Culture Collection at the Research Institute of Fast-growing Trees (RIFT)/China Eucalypt Research Centre (CERC), ZhanJiang, GuangDong Province, Ch China General Microbiological Culture Collection Center, Beijing, China.

[c] Genotype within each identified species, determined by sequences of *act*, *cmdA*, *his3*, *rpb2*, *tef1* and *tub2* regions.

[d] *act*, actin; *cmdA*, calmodulin; *his3*, histone H3; *rpb2*, the second largest subunit of RNA polymerase; *tef1*, translation elongation factor 1-alpha; *tub2*, β-tubuli

[e] Isolates used in morphological and culture growth studies.

[f] Isolates used for mating studies.

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

Species[a]	Isolate No.[b]	Genotype[c]	Substrate	Sampling site	Collector	GenBank accession No.[d]				
						act	*cmdA*	*his3*	*rpb2*	*tef1*
	CSF13819	AAAAAA	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321678	OP321815	OP321966	OP322083	OP3224
Ca. kyotensis	CSF13723	AAAAAA	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321679	OP321824	OP321975	OP322084	OP3224
	CSF13724	AAAAAA	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321680	OP321825	OP321976	OP322085	OP3224
Ca. yunnanensis	CSF6506	AAAAAA	*E. urophylla* × *E. grandis* leaf	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321681	OP321826	OP321977	OP322086	OP3224
	CSF13694	AAAAAA	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321682	OP321827	OP321978	OP322087	OP3224
	CSF13783	AAAAAB	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321683	OP321828	OP321979	OP322088	OP3224
	CSF13832	AAAAAB	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321684	OP321829	OP321980	OP322089	OP3224
	CSF13852	AAABAC	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321685	OP321830	OP321981	OP322090	OP3224
	CSF13853	AAABAC	Soil (*Eucalyptus* plantation)	JingGu, PuEr, YunNan, China	G.Q. Li & C.F. Liu	OP321686	OP321831	OP321982	OP322091	OP3224

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

[b] CSF: Culture Collection at the Research Institute of Fast-growing Trees (RIFT)/China Eucalypt Research Centre (CERC), ZhanJiang, GuangDong Province, Ch China General Microbiological Culture Collection Center, Beijing, China.

[c] Genotype within each identified species, determined by sequences of *act*, *cmdA*, *his3*, *rpb2*, *tef1* and *tub2* regions.

[d] *act*, actin; *cmdA*, calmodulin; *his3*, histone H3; *rpb2*, the second largest subunit of RNA polymerase; *tef1*, translation elongation factor 1-alpha; *tub2*, β-tubuli

[e] Isolates used in morphological and culture growth studies.

[f] Isolates used for mating studies.

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

Phylogenetic analyses

The *tef1* and *tub2* gene regions were amplified and sequenced for all isolates in this study. These sequences were then used in a standard nucleotide BLAST search in the NCBI data base (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to allow for a preliminary identification of these species and to determine the complexes in which they reside. Based on a preliminary identification, representative isolates were selected for PCR amplification and sequencing of four additional gene regions (*act*, *cmdA*, *his3* and *rpb2*). Sequences of the representative isolates and all the published species in the relevant complexes were used for phylogenetic analyses. Sequence datasets were aligned in MAFFT v. 7 (Katoh and Standley 2013) via the online version using an FFT-NS-i strategy and were curated in MEGA v. 6.0.5 software (Tamura et al. 2013).

Both maximum parsimony (MP) and maximum likelihood (ML) analyses were conducted using the method described by Liu et al. (2020). Sequence datasets for each of the six gene regions and a concatenated dataset for those regions were used for phylogenetic inference. A partition homogeneity test (PHT, Farris et al. 1994) was conducted to determine whether there was conflict between datasets. Sequence data from two isolates of *Curvicladiella cignea* (CBS 109167 and CBS 109168) were used as outgroups (Table 2).

Table 2
Isolates from other studies and used in the phylogenetic analyses for this study

Code B[a]	**Species**	**Isolates No.**[b,c]	**Other collection number**[b]	**Substrate**	**Area of occurrence**	**Collector**	**GenBank accession No.**[d]	**References**
							act*; *cmdA*; *his3*;** ***rpb2*; *tef1*; *tub2	
Species in *Calonectria colhounii* species complex								
B3	*Ca. aciculata*	CERC 5342^{T}	CBS 142883; CMW 47645	*Eucalyptus urophylla* × *E. grandis*	YunNan, China	S.F. Chen & J.Q. Li	MT334937; MT335164; MT335403; MT412478; MT412694; MT412934	Li et al. 2017; Liu et al. 2020
B27	*Ca. colhounii*	CBS 293.79^{T}	CMW 30999	*Camellia sinensis*	Mauritius	A. Peerally	GQ280443; GQ267373; DQ190639; KY653376; GQ267301; DQ190564	Peerally 1973; Crous 2002; Crous et al. 2006; Lombard et al. 2010a
B36	*Ca. eucalypti*	CMW 18444^{T}	CBS 125275	*E. grandis*	Aek Nauli, Sumatra Utara, Indonesia	M.J. Wingfield	MT335013; MT335243; MT335483; MT412545; MT412774; MT412992	Lombard et al. 2010a; Liu et al. 2020
		CMW 18445	CBS 125276	*E. grandis*	Aek Nauli, Sumatra Utara, Indonesia	M.J. Wingfield	MT335014; MT335244; MT335484; MT412546; MT412775; MT412993	Lombard et al. 2010a; Liu et al. 2020
B39	*Ca. fujianensis*	CMW 27257^{T}	CBS 127201	*E. grandis*	FuJian, China	M.J. Wingfield	MT335019; MT335249; MT335489; MT412551; MT412780; MT412998	Chen et al. 2011b; Liu et al. 2020

[a] Codes (B1 to B120) of the 120 accepted *Calonectria* species resulting from Liu et al. (2020).

[b] ATCC: American Type Culture Collection, Virginia, USA; CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CERC: China Eucalypt Research Centre, ZhanJiang, GuangDong Province, China; CGMCC: China General Microbiological Culture Collection Center, Beijing, 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 Westerdijk Fungal Biodiversity Institute; CSF: Culture Collection at the Research Institute of Fast-growing Trees (RIFT)/China Eucalypt Research Centre (CERC), ZhanJiang, GuangDong Province, China; IMI: International Mycological Institute, CABI Bioscience, Egham, Bakeham Lane, UK; MUCL: Mycotheque, Laboratoire de Mycologie Systematique st Appliqee, l'Universite, Louvian-la-Neuve, Belgium; PPRI: Plant Protection Research Institute, Pretoria, South Africa; STE-U: Department of Plant Pathology, University of Stellenbosch, South Africa; "−" represent no other collection number.

[c] "T" represents ex-type isolates of the species.

[d] *act*, actin; *cmdA*, calmodulin; *his3*, histone H3; *rpb2*, the second largest subunit of RNA polymerase; *tef1*, translation elongation factor 1-alpha; *tub2*, β-tubulin. GenBank accession number obtained in this study are indicated in bold.

[e] "N/A" represents information not available.

Code B[a]	Species	Isolates No.[b,c]	Other collection number[b]	Substrate	Area of occurrence	Collector	GenBank accession No.[d]	References
							act; *cmdA*; *his3*; *rpb2*; *tef1*; *tub2*	
		CMW 27254	CBS 127200	*E. grandis*	FuJian, China	M.J. Wingfield	MT335020; MT335250; MT335490; MT412552; MT412781; MT412999	Chen et al. 2011b; Liu et al. 2020

[a] Codes (B1 to B120) of the 120 accepted *Calonectria* species resulting from Liu et al. (2020).

[b] ATCC: American Type Culture Collection, Virginia, USA; CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CERC: China Eucalypt Research Centre, ZhanJiang, GuangDong Province, China; CGMCC: China General Microbiological Culture Collection Center, Beijing, 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 Westerdijk Fungal Biodiversity Institute; CSF: Culture Collection at the Research Institute of Fast-growing Trees (RIFT)/China Eucalypt Research Centre (CERC), ZhanJiang, GuangDong Province, China; IMI: International Mycological Institute, CABI Bioscience, Egham, Bakeham Lane, UK; MUCL: Mycotheque, Laboratoire de Mycologie Systematique st Appliqee, l'Universite, Louvian-la-Neuve, Belgium; PPRI: Plant Protection Research Institute, Pretoria, South Africa; STE-U: Department of Plant Pathology, University of Stellenbosch, South Africa; "–" represent no other collection number.

[c] "T" represents ex-type isolates of the species.

[d] *act*, actin; *cmdA*, calmodulin; *his3*, histone H3; *rpb2*, the second largest subunit of RNA polymerase; *tef1*, translation elongation factor 1-alpha; *tub2*, β-tubulin. GenBank accession number obtained in this study are indicated in bold.

[e] "N/A" represents information not available.

Code B[a]	Species	Isolates No.[b,c]	Other collection number[b]	Substrate	Area of occurrence	Collector	GenBank accession No.[d]	References
							act*; *cmdA*; *his3*; *rpb2*; *tef1*; *tub2	
B47	*Ca. honghensis*	CERC 5572^{T}	CBS 142885; CMW 47669	Soil (*Eucalyptus* plantation)	HongHe, YunNan, China	S.F. Chen & J.Q. Li	MT335026; MT335256; MT335496; MT412557; MT412787; MT413005	Li et al. 2017; Liu et al. 2020
		CERC 5571	CBS 142884; CMW 47668	Soil (*Eucalyptus* plantation)	HongHe, YunNan, China	S.F. Chen & J.Q. Li	MT335027; MT335257; MT335497; MT412558; MT412788; MT413006	Li et al. 2017; Liu et al. 2020
B53	*Ca. indusiata*	CBS 144.36^{T}	CMW 23699	*Camellia sinensis*	Sri lanka	N/A	GQ280536; GQ267453; GQ267262; KY653396; GQ267332; GQ267239	Crous 2002; Lombard et al. 2010a, 2016; Marin-Felix et al. 2017
		CBS 114684	CMW 51213; CPC 2446; UFV16	*Rhododendron* sp.	Florida, USA	N.E. El-Gholl	GQ280537; GQ267454; DQ190653; N/A; GQ267333; AF232862	Crous et al. 1999, 2006; Crous 2002
B62	*Ca. lichi*	CERC 8866^{T}	–	Soil	HeNan, China	S.F. Chen	MT335046; MT335278; MT335518; MT412575; MT412809; MT413023	Liu and Chen 2017; Liu et al. 2020
		CERC 8850	–	Soil	HeNan, China	S.F. Chen	MT335047; MT335279; MT335519; MT412576; MT412810; MT413024	Liu and Chen 2017; Liu et al. 2020

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[a] Codes (B1 to B120) of the 120 accepted *Calonectria* species resulting from Liu et al. (2020).

[b] ATCC: American Type Culture Collection, Virginia, USA; CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CERC: China Eucalypt Research Centre, ZhanJiang, GuangDong Province, China; CGMCC: China General Microbiological Culture Collection Center, Beijing, 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 Westerdijk Fungal Biodiversity Institute; CSF: Culture Collection at the Research Institute of Fast-growing Trees (RIFT)/China Eucalypt Research Centre (CERC), ZhanJiang, GuangDong Province, China; IMI: International Mycological Institute, CABI Bioscience, Egham, Bakeham Lane, UK; MUCL: Mycotheque, Laboratoire de Mycologie Systematique st Appliqee, l'Universite, Louvian-la-Neuve, Belgium; PPRI: Plant Protection Research Institute, Pretoria, South Africa; STE-U: Department of Plant Pathology, University of Stellenbosch, South Africa; "–" represent no other collection number.

[c] "T" represents ex-type isolates of the species.

[d] *act*, actin; *cmdA*, calmodulin; *his3*, histone H3; *rpb2*, the second largest subunit of RNA polymerase; *tef1*, translation elongation factor 1-alpha; *tub2*, β-tubulin. GenBank accession number obtained in this study are indicated in bold.

[e] "N/A" represents information not available.

Code B[a]	**Species**	**Isolates No.**[b,c]	**Other collection number**[b]	**Substrate**	**Area of occurrence**	**Collector**	**GenBank accession No.**[d]	**References**
							act*; *cmdA*; *his3*; *rpb2*; *tef1*; *tub2	
B64	*Ca. macroconidialis*	CBS 114880$^{\mathrm{T}}$	CMW 51219; CPC 307; PPRI 4000	*E. grandis*	Sabie, Mpumalanga, South Africa	P.W. Crous	MT335050; MT335282; MT335522; MT412579; MT412813; MT413027	Crous et al. 1993; Crous 2002; Lombard et al. 2010a; Liu et al. 2020
B65	*Ca. madagascariensis*	CMW 23686$^{\mathrm{T}}$	CBS 114572; CPC 2252	Soil	Rona, Madagascar	J.E. Taylor	MT335052; MT335284; MT335524; MT412581; MT412815; MT413029	Crous 2002; Crous et al. 2006; Lombard et al. 2010a; Liu et al. 2020
		CMW 30993	CBS 114571; CPC 2253	Soil	Rona, Madagascar	J.E. Taylor	MT335053; MT335285; MT335525; MT412582; MT412816; MT413030	Crous 2002; Crous et al. 2006; Lombard et al. 2010a; Liu et al. 2020
	Ca. minensis	CSF9941$^{\mathrm{T}}$	CGMCC3.18877	Soil (*Eucalyptus* plantation)	XinLuo, LongYan, FuJian, China	S.F. Chen, Q.L. Liu & F.F. Liu	OK253121; OK253259; OK253403; OK253477; OK253814; OK253967	Liu et al. 2022
		CSF9975	CGMCC3.18881	Soil (natural forest area)	LianCheng, LongYan, FuJian, China	S.F. Chen, Q.L. Liu & F.F. Liu	OK253123; OK253261; OK253405; OK253479; OK253816; OK253969	Liu et al. 2022

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[c] "T" represents ex-type isolates of the species.

[d] *act*, actin; *cmdA*, calmodulin; *his3*, histone H3; *rpb2*, the second largest subunit of RNA polymerase; *tef1*, translation elongation factor 1-alpha; *tub2*, β-tubulin. GenBank accession number obtained in this study are indicated in bold.

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Code B[a]	Species	Isolates No.[b,c]	Other collection number[b]	Substrate	Area of occurrence	Collector	GenBank accession No.[d]	References
							act; *cmdA*; *his3*; *rpb2*; *tef1*; *tub2*	
B70	*Ca. monticola*	CBS 140645^{T}	CPC 28835	Soil	Chiang Mai, Thailand	P.W. Crous	N/A; KT964771; N/A; N/A; KT964773; KT964769	Crous et al. 2015

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		CPC 28836	–	Soil	Chiang Mai, Thailand	P.W. Crous	N/A; KT964772; N/A; N/A; KT964774; KT964770	Crous et al. 2015
B81	*Ca. paracolhounii*	CBS 114679^{T}	CMW 51212; CPC 2445	N/A	USA	A.Y. Rossman	N/A; KX784582; N/A; KY653423; KX784714; KX784644	Lombard et al. 2016; Marin-Felix et al. 2017
		CBS 114705	CMW 51215; CPC 2423	Fruit of *Annona reticulata*	Australia	D. Hutton	N/A; N/A; N/A; KY653424; KX784715; KX784645	Lombard et al. 2016; Marin-Felix et al. 2017
B123	*Ca. xianrensis*	CSF12909^{T}	CGMCC3.19584	Soil (near *Eucalyptus* plantation)	Dacheng Town, Gaozhou County, Maoming Region, GuangDong, China	S.F. Chen, Q.C. Wang & W. Wang	**OP321687**; MK962845; MK962857; **OP322092**; MK962869; MK962833	Wang et al. 2019; This study
		CSF12908	CGMCC3.19518	Soil (near *Eucalyptus* plantation)	Dacheng Town, Gaozhou County, Maoming Region, GuangDong, China	S.F. Chen, Q.C. Wang & W. Wang	**OP321688**; MK962844; MK962856; **OP322093**; MK962868; MK962832	Wang et al. 2019; This study
Species in *Calonectria kyotensis* species complex								

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Code B[a]	**Species**	**Isolates No.**[b,c]	**Other collection number**[b]	**Substrate**	**Area of occurrence**	**Collector**	**GenBank accession No.**[d]	**References**
							act*; *cmdA*; *his3*; *rpb2*; *tef1*; *tub2	
B4	*Ca. aconidialis*	CMW 35174[T]	CBS 136086; CERC 1850	Soil (*Eucalyptus* plantation)	HaiNan, China	X. Mou & S.F. Chen	MT334938; MT335165; MT335404; MT412479; MT412695; N/A[e]	Lombard et al. 2015; Liu et al. 2020
		CMW 35384	CBS 136091; CERC 1886	Soil (*Eucalyptus* plantation)	HaiNan, China	X. Mou & S.F. Chen	MT334939; MT335166; MT335405; N/A; MT412696; N/A	Lombard et al. 2015; Liu et al. 2020
B5	*Ca. aeknauliensis*	CMW 48253[T]	CBS 143559	Soil (*Eucalyptus* plantation)	Aek Nauli, North Sumatra, Indonesia	M.J. Wingfield	MT334953; MT335180; MT335419; MT412486; MT412710; N/A	Pham et al. 2019; Liu et al. 2020
		CMW 48254	CBS 143560	Soil (*Eucalyptus* plantation)	Aek Nauli, North Sumatra, Indonesia	M.J. Wingfield	MT334954; MT335181; MT335420; MT412487; MT412711; N/A	Pham et al. 2019; Liu et al. 2020
B8	*Ca. asiatica*	CBS 114073[T]	CMW 23782; CPC 3900	Debris (leaf litter)	Prathet Thai, Thailand	N.L. Hywel-Jones	GQ280428; AY725741; AY725658; N/A; AY725705; AY725616	Crous et al. 2004; Lombard et al. 2010a
B17	*Ca. brassicicola*	CBS 112841[T]	CMW 51206; CPC 4552	Soil at *Brassica* sp.	Indonesia	M.J. Wingfield	N/A; KX784561; N/A; N/A; KX784689; KX784619	Lombard et al. 2016

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							act; *cmdA*; *his3*; *rpb2*; *tef1*; *tub2*	
B19	*Ca. bumicola*	CMW 48257^{T}	CBS 143575	Soil (*Eucalyptus* plantation)	Aek Nauli, North Sumatra, Indonesia	M.J. Wingfield	MT334975; MT335205; MT335445; MT412509; MT412736; N/A	Pham et al. 2019; Liu et al. 2020
B20	*Ca. canadiana*	CMW 23673^{T}	CBS 110817; STE-U 499	*Picea* sp.	Canada	S. Greifenhagen	MT334976; MT335206; MT335446; MT412510; MT412737; MT412958	Kang et al. 2001; Crous 2002; Lechat et al. 2010; Liu et al. 2020
		CERC 8952	–	Soil	HeNan, China	S.F. Chen	MT335058; MT335290; MT335530; MT412587; MT412821; MT413035	Liu and Chen 2017; Liu et al. 2020
B23	*Ca. chinensis*	CMW 23674^{T}	CBS 114827; CPC 4101	Soil	Hong Kong, China	E.C.Y. Liew	MT334990; MT335220; MT335460; MT412524; MT412751; MT412972	Crous et al. 2004; Lombard et al. 2010a; Liu et al. 2020
		CMW 30986	CBS 112744; CPC 4104	Soil	Hong Kong, China	E.C.Y. Liew	MT334991; MT335221; MT335461; MT412525; MT412752; MT412973	Crous et al. 2004; Lombard et al. 2010a; Liu et al. 2020

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B26	*Ca. cochinchinensis*	CMW 49915^{T}	CBS 143567	Soil (*Hevea brasiliensis* plantation)	Duong Minh Chau, Tay Ninh, Vietnam	N.Q. Pham, Q.N. Dang & T.Q. Pham	MT334995; MT335225; MT335465; MT412529; MT412756; MT412977	Pham et al. 2019; Liu et al. 2020
		CMW 47186	CBS 143568	Soil (*A. auriculiformis* plantation)	Song May, Dong Nai, Vietnam	N.Q. Pham & T.Q. Pham	MT334996; MT335226; MT335466; MT412530; MT412757; MT412978	Pham et al. 2019; Liu et al. 2020
B29	*Ca. colombiensis*	CMW 23676^{T}	CBS 112220; CPC 723	Soil (*E. grandis* trees)	La Selva, Colombia	M.J. Wingfield	MT334998; MT335228; MT335468; MT412532; MT412759; MT412980	Crous et al. 2004; Liu et al. 2020
		CMW 30985	CBS 112221; CPC 724	Soil (*E. grandis* trees)	La Selva, Colombia	M.J. Wingfield	MT334999; MT335229; MT335469; MT412533; MT412760; MT412981	Crous et al. 2004; Liu et al. 2020
B31	*Ca. curvispora*	CMW 23693^{T}	CBS 116159; CPC 765	Soil	Tamatave, Madagascar	P.W. Crous	MT335002; MT335232; MT335472; MT412536; MT412763; N/A	Victor et al. 1997; Crous 2002; Lombard et al. 2010a, 2015; Liu et al. 2020
		CMW 48245	CBS 143565	Soil (*Eucalyptus* plantation)	Aek Nauli, North Sumatra, Indonesia	M.J. Wingfield	MT335003; MT335233; MT335473; MT412537; MT412764; N/A	Pham et al. 2019; Liu et al. 2020

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B46	*Ca. heveicola*	CMW 49913$^{\mathrm{T}}$	CBS 143570	Soil (*H. brasiliensis* plantation)	Bau Bang, Binh Duong, Vietnam	N.Q. Pham, Q.N. Dang & T.Q. Pham	MT335025; MT335255; MT335495; N/A; MT412786; MT413004	Pham et al. 2019; Liu et al. 2020
		CMW 49928	CBS 143571	Soil	Bu Gia Map National Park, Binh Phuoc, Vietnam	N.Q. Pham, Q.N. Dang & T.Q. Pham	MT335048; MT335280; MT335520; MT412577; MT412811; MT413025	Pham et al. 2019; Liu et al. 2020
B48	*Ca. hongkongensis*	CBS 114828$^{\mathrm{T}}$	CMW 51217; CPC 4670	Soil	Hong Kong, China	M.J. Wingfield	MT335028; MT335258; MT335498; MT412559; MT412789; MT413007	Crous et al. 2004; Liu et al. 2020
		CERC 3570	CMW 47271	Soil (*Eucalyptus* plantation)	BeiHai, Guangxi, China	S.F. Chen, J.Q. Li & G.Q. Li	MT335030; MT335260; MT335500; MT412561; MT412791; MT413009	Li et al. 2017; Liu et al. 2020
B51	*Ca. ilicicola*	CMW 30998$^{\mathrm{T}}$	CBS 190.50; IMI 299389; STE-U 2482	*Solanum tuberosum*	Bogor, Java, Indonesia	K.B. Boedijn & J. Reitsma	MT335036; MT335266; MT335506; MT412564; MT412797; N/A	Boedijn and Reitsma 1950; Crous 2002; Lombard et al. 2010a; Liu et al. 2020
B52	*Ca. indonesiae*	CMW 23683$^{\mathrm{T}}$	CBS 112823; CPC 4508	*Syzygium* *aromaticum*	Warambunga, Indonesia	M.J. Wingfield	MT335037; MT335267; MT335507; MT412565; MT412798; MT413015	Crous et al. 2004; Liu et al. 2020

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		CBS 112840	CMW 51205; CPC 4554	*S. aromaticum*	Warambunga, Indonesia	M.J. Wingfield	MT335038; MT335268; MT335508; MT412566; MT412799; MT413016	Crous et al. 2004; Liu et al. 2020
B55	*Ca. kyotensis*	CBS 114525^{T}	ATCC 18834; CMW 51824; CPC 2367	*Robinia pseudoacacia*	Japan	T. Terashita	MT335039; MT335271; MT335511; MT412569; MT412802; MT413019	Terashita 1968; Crous 2002; Lombard et al. 2016; Liu et al. 2020
		CBS 114550	CMW 51825; CPC 2351	Soil	China	M.J. Wingfield	MT335016; MT335246; MT335486; MT412548; MT412777; MT412995	Lombard et al. 2016; Liu et al. 2020
B57	*Ca. lantauensis*	CERC 3302^{T}	CBS 142888; CMW 47252	Soil	LiDao, Hong Kong, China	M.J. Wingfield & S.F. Chen	MT335040; MT335272; MT335512; MT412570; MT412803; N/A	Li et al. 2017; Liu et al. 2020
		CERC 3301	CBS 142887; CMW 47251	Soil	LiDao, Hong Kong, China	M.J. Wingfield & S.F. Chen	MT335041; MT335273; MT335513; N/A; MT412804; N/A	Li et al. 2017; Liu et al. 2020
B58	*Ca. lateralis*	CMW 31412^{T}	CBS 136629	Soil (*Eucalyptus* plantation)	GuangXi, China	X. Zhou, G. Zhao & F. Han	MT335042; MT335274; MT335514; MT412571; MT412805; MT413020	Lombard et al. 2015; Liu et al. 2020

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							act*; *cmdA*; *his3*; *rpb2*; *tef1*; *tub2	
B66	*Ca. malesiana*	CMW 23687^{T}	CBS 112752; CPC 4223	Soil	Northern Sumatra, Indonesia	M.J. Wingfield	MT335054; MT335286; MT335526; MT412583; MT412817; MT413031	Crous et al. 2004; Liu et al. 2020
		CBS 112710	CMW 51199; CPC 3899	Leaf litter	Prathet, Thailand	N.L. Hywel-Jones	MT335055; MT335287; MT335527; MT412584; MT412818; MT413032	Crous et al. 2004; Liu et al. 2020
B80	*Ca. pacifica*	CMW 16726^{T}	A1568; CBS 109063; IMI 354528; STE-U 2534	*Araucaria heterophylla*	Hawaii, USA	M. Aragaki	MT335079; MT335311; MT335551; MT412604; MT412842; N/A	Kang et al. 2001; Crous 2002; Crous et al. 2004; Liu et al. 2020
		CMW 30988	CBS 114038	*Ipomoea aquatica*	Auckland, New Zealand	C.F. Hill	MT335080; MT335312; MT335552; MT412605; MT412843; N/A	Crous 2002; Crous et al. 2004; Lombard et al. 2010a; Liu et al. 2020
B86	*Ca. penicilloides*	CMW 23696^{T}	CBS 174.55; STE-U 2388	*Prunus* sp.	Hatizyo Island, Japan	M. Ookubu	MT335106; MT335338; MT335578; MT412631; MT412869; MT413081	Tubaki 1958; Crous 2002; Liu et al. 2020

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[b] ATCC: American Type Culture Collection, Virginia, USA; CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CERC: China Eucalypt Research Centre, ZhanJiang, GuangDong Province, China; CGMCC: China General Microbiological Culture Collection Center, Beijing, 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 Westerdijk Fungal Biodiversity Institute; CSF: Culture Collection at the Research Institute of Fast-growing Trees (RIFT)/China Eucalypt Research Centre (CERC), ZhanJiang, GuangDong Province, China; IMI: International Mycological Institute, CABI Bioscience, Egham, Bakeham Lane, UK; MUCL: Mycotheque, Laboratoire de Mycologie Systematique st Appliqee, l'Universite, Louvian-la-Neuve, Belgium; PPRI: Plant Protection Research Institute, Pretoria, South Africa; STE-U: Department of Plant Pathology, University of Stellenbosch, South Africa; "−" represent no other collection number.

[c] "T" represents ex-type isolates of the species.

[d] *act*, actin; *cmdA*, calmodulin; *his3*, histone H3; *rpb2*, the second largest subunit of RNA polymerase; *tef1*, translation elongation factor 1-alpha; *tub2*, β-tubulin. GenBank accession number obtained in this study are indicated in bold.

[e] "N/A" represents information not available.

Code B[a]	Species	Isolates No.[b,c]	Other collection number[b]	Substrate	Area of occurrence	Collector	GenBank accession No.[d]	References
							act*; *cmdA*; *his3*; *rpb2*; *tef1*; *tub2	
	Ca. singaporensis	CBS 146715[T]	MUCL 048320	leaf litter (submerged in a small stream)	South East Asian rainforest, Mac Ritchie Reservoir, Singapore	C. Decock	MW890022; MW890042; MW890055; MW883409; MW883804; N/A; MW890086; MW890124	Crous et al. 2021a

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[e] “N/A” represents information not available.

Code B[a]	Species	Isolates No.[b,c]	Other collection number[b]	Substrate	Area of occurrence	Collector	GenBank accession No.[d]	References
							act*; *cmdA*; *his3*; *rpb2*; *tef1*; *tub2	
		CBS 146713	MUCL 048171	leaf litter (submerged in a small stream)	South East Asian rainforest, Mac Ritchie Reservoir, Singapore	C. Decock	MW890020; MW890040; MW890053; MW883407; MW883802; N/A; MW890084; MW890123	Crous et al. 2021a
B112	*Ca. sumatrensis*	CMW 23698^{T}	CBS 112829; CPC 4518	Soil	Northern Sumatra, Indonesia	M.J. Wingfield	MT335145; MT335382; MT335622; MT412674; MT412913; N/A	Crous et al. 2004; Liu et al. 2020
		CMW 30987	CBS 112934; CPC 4516	Soil	Northern Sumatra, Indonesia	M.J. Wingfield	MT335146; MT335383; MT335623; MT412675; MT412914; N/A	Crous et al. 2004; Liu et al. 2020
B113	*Ca. syzygiicola*	CBS 112831^{T}	CMW 51204; CPC 4511	*S. aromaticum*	Sumatra, Indonesia	M.J. Wingfield	N/A; N/A; N/A; N/A; KX784736; KX784663	Lombard et al. 2016
B116	*Ca. uniseptata*	CBS 413.67^{T}	CMW 23678; CPC 2391; IMI 299577	*Paphiopedilum callosum*	Celle, Germany	W. Gerlach	GQ280451; GQ267379; GQ267248; N/A; GQ267307; GQ267208	Lombard et al. 2016
B120	*Ca. yunnanensis*	CERC 5339^{T}	CBS 142897; CMW 47644	Soil (*Eucalyptus* plantation)	YunNan, China	S.F. Chen & J.Q. Li	MT335157; MT335396; MT335636; MT412687; MT412927; MT413134	Li et al. 2017; Liu et al. 2020

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[c] "T" represents ex-type isolates of the species.

[d] *act*, actin; *cmdA*, calmodulin; *his3*, histone H3; *rpb2*, the second largest subunit of RNA polymerase; *tef1*, translation elongation factor 1-alpha; *tub2*, β-tubulin. GenBank accession number obtained in this study are indicated in bold.

[e] "N/A" represents information not available.

Code B[a]	Species	Isolates No.[b,c]	Other collection number[b]	Substrate	Area of occurrence	Collector	GenBank accession No.[d]	References
							act; *cmdA*; *his3*; *rpb2*; *tef1*; *tub2*	
		CERC 5337	CBS 142895; CMW 47642	Soil (*Eucalyptus* plantation)	YunNan, China	S.F. Chen & J.Q. Li	MT335158; MT335397; MT335637; MT412688; MT412928; MT413135	Li et al. 2017; Liu et al. 2020
Outgroups								

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[a] Codes (B1 to B120) of the 120 accepted *Calonectria* species resulting from Liu et al. (2020).

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[c] "T" represents ex-type isolates of the species.

[d] *act*, actin; *cmdA*, calmodulin; *his3*, histone H3; *rpb2*, the second largest subunit of RNA polymerase; *tef1*, translation elongation factor 1-alpha; *tub2*, β-tubulin. GenBank accession number obtained in this study are indicated in bold.

[e] "N/A" represents information not available.

Code B[a]	Species	Isolates No.[b,c]	Other collection number[b]	Substrate	Area of occurrence	Collector	GenBank accession No.[d]	References
							act*; *cmdA*; *his3*; *rpb2*; *tef1*; *tub2	
	Curvicladiella cignea	CBS 109167^{T}	CPC 1595; MUCL 40269	Decaying leaf	French Guiana	C. Decock	KM231122; KM231287; KM231461; KM232311; KM231867; KM232002	Decock and Crous 1998; Crous et al. 2006; Lombard et al. 2015

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[c] “T” represents ex-type isolates of the species.

[d] *act*, actin; *cmdA*, calmodulin; *his3*, histone H3; *rpb2*, the second largest subunit of RNA polymerase; *tef1*, translation elongation factor 1-alpha; *tub2*, β-tubulin. GenBank accession number obtained in this study are indicated in bold.

[e] “N/A” represents information not available.

Code B[a]	Species	Isolates No.[b,c]	Other collection number[b]	Substrate	Area of occurrence	Collector	GenBank accession No.[d]	References
							act; *cmdA*; *his3*; *rpb2*; *tef1*; *tub2*	
		CBS 109168	CPC 1594; MUCL 40268	Decaying seed	French Guiana	C. Decock	KM231121; KM231286; KM231460; KM232312; KM231868; KM232003	Decock and Crous 1998; Crous et al. 2006; Lombard et al. 2015

[a] Codes (B1 to B120) of the 120 accepted *Calonectria* species resulting from Liu et al. (2020).

[b] ATCC: American Type Culture Collection, Virginia, USA; CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CERC: China Eucalypt Research Centre, ZhanJiang, GuangDong Province, China; CGMCC: China General Microbiological Culture Collection Center, Beijing, 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 Westerdijk Fungal Biodiversity Institute; CSF: Culture Collection at the Research Institute of Fast-growing Trees (RIFT)/China Eucalypt Research Centre (CERC), ZhanJiang, GuangDong Province, China; IMI: International Mycological Institute, CABI Bioscience, Egham, Bakeham Lane, UK; MUCL: Mycotheque, Laboratoire de Mycologie Systematique st Appliqee, l’Universite, Louvian-la-Neuve, Belgium; PPRI: Plant Protection Research Institute, Pretoria, South Africa; STE-U: Department of Plant Pathology, University of Stellenbosch, South Africa; “–” represent no other collection number.

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[d] *act*, actin; *cmdA*, calmodulin; *his3*, histone H3; *rpb2*, the second largest subunit of RNA polymerase; *tef1*, translation elongation factor 1-alpha; *tub2*, β-tubulin. GenBank accession number obtained in this study are indicated in bold.

[e] “N/A” represents information not available.

Morphology and taxonomy

Based on DNA sequence comparisons, representative isolates were selected for morphological identification. To induce asexual structures, mycelium plugs from pure cultures were transferred onto synthetic nutrient-poor agar (SNA, Nirenburg 1981) and incubated at 25°C for four to seven days. The asexual structures were mounted in 85% lactic acid and examined with a Zeiss Axio Imager A1 microscope (Carl Zeiss Ltd., Germany).

An attempt to induce sexual structures was made by crossing isolates of the novel species in all possible combinations on Minimum Salt Agar (MSA, Guerber and Correll 2001) amended with sterile bamboo toothpicks and incubated at 25°C for 2–8 wk.

For the novel species, 50 measurements were made for each taxonomically informative structure in the isolate selected to represent the holotype specimen, and 30 measurements were made for the paratypes. Minimum, maximum and average (mean) values are presented as (minimum–) (average – standard deviation) – (average + standard deviation) (–maximum).

The optimal growth temperature for the novel species was determined by transferring mycelial plugs taken from the actively growing margins of cultures to fresh MEA and incubating these at temperatures ranging from 5°C to 35°C at 5°C intervals, with five replicate plates per temperature per isolate. Colony diameters were measured after seven days. Colony colour and morphology was described based on the colour charts of Rayner (1970) using 7-d-old cultures on MEA incubated at 25°C. All descriptions were deposited in MycoBank (www.mycobank.org).

Results

Sample collection and fungal isolation

A total of 251 symptomatic *Eucalyptus* leaves and associated soil samples were collected from an equal number of diseased *E. urophylla* × *E. grandis* trees and sampling points. Based on the morphological characteristics of the cultures, between one and three isolates were retained for each of the leaf samples and between one and four isolates were retained for each of the soil samples. In total, 228 *Calonectria* isolates were obtained from 198 diseased trees, and 189 isolates were obtained from 88 soil samples. This amounted to a collection of 417 isolates with typical morphological characteristics of *Calonectria* species (Additional file 1: Table S1).

DNA extraction, PCR amplifications and sequencing

DNA was extracted and the *tef1* and *tub2* gene regions were sequenced for all isolates obtained (Additional file 1: Table S1). Based on the combined genotype of *tef1* and *tub2* as well as the sampling source, 151 isolates were selected for further analyses by sequencing the *cmdA* and *his3* gene regions (Additional file 1: Table S1). Based on the combined genotype of *tef1, tub2*, *cmdA* and *his3*, 102 representative isolates were further chosen to amplify *act* and *rpb2* gene regions. Analysis of the sequence data from all six gene regions for these 102 isolates resulted in a total of 72 genotypes (Table 1). The approximate size of the amplicons generated for the six loci were *act*: 300bp, *cmdA*: 700bp, *his3*: 500 bp, *rpb2*: 860 bp, *tef1*: 500 bp, and *tub2*: 600 bp. Overall, 102 isolates were identified based on sequence comparisons for six gene regions and the remaining 315 *Calonectria* isolates were identified based on two to four gene regions.

Phylogenetic analyses

A total of 65 sequences (including all ex-type isolates of the respective complexes) were downloaded from NCBI and used in phylogenetic analyses (Table 2). Both MP and ML methods based on the six individual gene regions and the combined sequence datasets were used to infer phylogenetic relationships. Only ML trees were utilised and bootstrap values for the MP and ML analyses were annotated on the tree branches (Fig. 1, Additional files 3–8: Figs S1–S6). Statistical values for MP and ML analyses and parameters are provided in Additional file 2: Table S2. The PHT test (P = 0.001) showed that the different datasets were congruent and could be combined for analysis.

Based on the six gene combined phylogeny (Fig. 1), the 102 isolates clustered in eleven groups (Groups 1 to 11). Of these, 70 isolates belonged to the *Ca. colhounii* species complex in the Prolate group of *Calonectria* and were identified as five species (Groups 1 to 5, Fig. 1). Three isolates (CSF6495, CSF6515 and CSF6528) grouped with *Ca. aciculata* (Group 1), five isolates were most closely related to *Ca. colhounii* (Group 2), two isolates (CSF6439 and CSF6520) formed an independent clade that was distinct from all known species (Group 3), 23 isolates clustered with *Ca. eucalypti* (Group 4) and 37 isolates in Group 5 were identified as *Ca. honghensis*.

Thirty-two isolates resided in the *Ca. kyotensis* species complex of the Sphaero-Naviculate group were identified as six species (Groups 6 to 11). Of these, eight isolates grouped with *Ca. aconidialis* (Group 6), ten isolates with *Ca. asiatica* (Group 7), four isolates with *Ca. hongkongensis* (Group 8), two isolates (CSF13767 and CSF13819) with *Ca. ilicicola* (Group 9), two isolates (CSF13723 and CSF13724) with *Ca. kyotensis* (Group 10) and six isolates with *Ca. yunnanensis* (Group 11).

Based on multi-gene phylogenetic inference (Fig. 1, Additional files 3–8: Figs. S1–S6) and morphological comparisons, eleven *Calonectria* species were identified, including ten described species and one novel species. Five of these (*Ca. aciculata*, *Ca. colhounii*, *Ca. eucalypti*, *Ca. honghensis* and the novel species) resided in the *Ca. colhounii* species complex, and six (*Ca. aconidialis*, *Ca. asiatica*, *Ca. hongkongensis*, *Ca. ilicicola*, *Ca. kyotensis* and *Ca. yunnanensis*) were in the *Ca. kyotensis* species complex.

All *Calonectria* isolates obtained in this study were identified based on the two to six gene regions sequenced. Consequently, the 417 isolates obtained were identified as *Ca. aciculata* (three isolates), *Ca. colhounii* (five isolates), *Ca. eucalypti* (44 isolates), *Ca. honghensis* (180 isolates), *Ca. aconidialis* (64 isolates), *Ca. asiatica* (41 isolates), *Ca. hongkongensis* (four isolates), *Ca. ilicicola* (25 isolates), *Ca. kyotensis* (two isolates), *Ca. yunnanensis* (47 isolates) and two isolates of a novel species.

Morphology and taxonomy

The mating tests with the two isolates (CSF6439 and CSF6520) representing a novel species did not result in sexual structures. Asexual structures were however common in these isolates on the SNA medium. The novel species is described as follows:

Calonectria dianii Q.L. Liu & S.F. Chen, **sp. nov.**

MycoBank MB845488. (Fig. 2).

Etymology

Name refers to the Chinese short name Dian of YunNan Province, where this fungus was isolated.

Diagnosis: *Calonectria dianii* can be distinguished phylogenetically from the most closely related species *Ca. aciculata*, *Ca. eucalypti*, *Ca. honghensis* and *Ca. minensis* and morphologically by its macroconidial dimensions (see notes below).

Type: **China**: YunNan Province, PuEr Region, JingGu County (23°23'58"N, 100°50'37"E), from leaves collected in a *E. urophylla × E. grandis* plantation, 27 December 2014, *G.Q. Li* & *C.F. Liu* (HMAS350282 – holotype, CSF6520 = CGMCC3.20446 – ex-type culture).

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, 52–206 × 5–9 µm, stipe extension septate, straight to flexuous 129–248 µm long, 2–4 µm wide at the apical septum, terminating in a broadly clavate to clavate vesicle, 3–5 µm diam; lateral stipe extensions (90° to main axis) absent. *Conidiogenous apparatus* 23–99 µm wide, and 34–96 µm long; primary branches aseptate, 14–30 × 3–6 µm; secondary branches aseptate, 9–23 × 3–6 µm; tertiary branches aseptate, 8–14 × 2–5 µm, each terminal branch producing 2–4 phialides; phialides elongate doliiform to reniform, hyaline, aseptate, 6–13 × 3–5 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (43.5–)45.5–54(–62.5) × (4.5–)5–5.5(–6) µm (av. = 49.5 × 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 woolly white aerial mycelium at 25°C on MEA, moderate sporulation; surface white to buff (45); reverse ochreous (44) to sienna (8) after 7 d. Chlamydospores not observed. 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 14.5 mm, 34.2 mm, 50.5 mm, 61.3 mm and 31.8 mm, respectively.

Host: *E. urophylla* × *E. grandis*

Distribution

Currently only known from PuEr Region inYunNan Province, China.

Notes: Calonectria dianii resides in the *Ca. colhounii* species complex, and is most closely related to *Ca. aciculata*, *Ca. eucalypti*, *Ca. honghensis* and *Ca. minensis*. It can easily be distinguished from those species by the dimensions of its macroconidia (Table 3) as follows: The macroconidia of *Ca. dianii* (av. = 49.5 × 5.5 µm) are shorter than those of *Ca. aciculata* (av. = 69 × 5.5 µm; Li et al. 2017), *Ca. eucalypti* (av. = 72 × 6 µm; Lombard et al. 2010a), *Ca. honghensis* (av. = 54 × 5.5 µm; Li et al. 2017) and *Ca. minensis* (av. = 60.5 × 5.5 µm; Liu et al. 2022). The total number of SNP differences between the ex-type isolate of *Ca. dianii* (CSF6520), and the ex-type isolates of *Ca. aciculata* (CERC 5342), *Ca. eucalypti* (CMW 18444), *Ca. honghensis* (CERC 5572) and *Ca. minensis* (CSF9941) for six gene regions combined, varied between 20–25.

Table 3
Morphological comparisons of *Calonectria dianii* and its phylogenetically closely related species

Species[a]	Macroconidia (L × W)[abc]	Macroconidia average (L × W)[ab]	Macroconidia septation	Vesicle (Min.–Max.)[a]	References or source of data
Calonectria dianii	(43.5–)45.5–54(–62.5) × (4.5–)5–5.5(–6)	49.5 × 5.5	3	3–5	this study
Ca. aciculata	(53–)62–76(–86) × (4.5–)5–6(–7)6	69 × 5.5	3	(2–)2.5–3.5(–5)	Li et al. 2017
Ca. eucalypti	(66–)69–75(–80) × (5–)6	72 × 6	3	4–6	Lombard et al. 2010a
Ca. honghensis	(43–)49–59(–66) × (4.5–)5–5.5(–6)	54 × 5.5	3	(2.5–)3–4.5(–5.5)	Li et al. 2017
Ca. minensis	(51–)55–66(–79) × (4.5–)5–6(–7.5)	60.5 × 5.5	(1–)3	3–5	Liu et al. 2022

[a] All measurements are in µm.

[b] L × W = length × width.

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

Additional specimens examined: **China**: YunNan Province, PuEr Region, JingGu County (23°23'58"N, 100°50'37"E), from leaves collected in a *E. urophylla × E. grandis* plantation, 27 December 2014, *G.Q. Li* & *C.F. Liu* (HMAS350281, culture CSF6439 = CGMCC3.20445).

Calonectria **species diversity in leaves and soil**

The 417 isolates collected in this study represented 11 *Calonectria* species residing in the *Ca. colhounii* and *Ca. kyotensis* species complexes. Of these, 234 (56.1%) isolates were in the *Ca. colhounii* species complex and 183 (43.9%) resided in the *Ca. kyotensis* species complex (Fig. 3a, 3b). There were 228 (54.7%) and 189 (45.3%) isolates from diseased leaves and soil samples, respectively (Fig. 3c, 3d). Of these, most isolates from leaves (99.1%) resided in the *Ca. colhounii* species complex, and 95.8% from soils were in the *Ca. kyotensis* species complex (Fig. 3c, 3d). *Calonectria eucalypti*, *Ca. honghensis*, *Ca. asiatica* and *Ca. yunnanensis* were found in both leaves and soils, *Ca. aciculata*, *Ca. colhounii* and *Ca. dianii* were isolated only from diseased leaves, and *Ca. aconidialis*, *Ca. hongkongensis*, *Ca. ilicicola* and *Ca. kyotensis* were isolated only from soils (Fig. 3c, 3d).

The 234 isolates in the *Ca. colhounii* complex included *Ca. aciculata*, *Ca. colhounii*, *Ca. dianii*, *Ca. eucalypti* and *Ca. honghensis* (Fig. 4). *Calonectria honghensis* and *Ca. eucalypti* were the dominant species in this complex accounting for 76.9% and 18.8% of isolates, respectively. The remaining isolates included *Ca. colhounii* (2.1%), *Ca. aciculata* (1.3%) and *Ca. dianii* (0.8%) (Fig. 4). Of the total collection of 234 isolates, 226 (96.6%) were obtained from diseased *Eucalyptus* leaves, and the remaining eight isolates (3.4%) were collected from soil samples (Fig. 4). The majority of the *Ca. honghensis* (96.7%) and *Ca. eucalypti* (95.5%), were isolated from leaves. The remaining three species were only collected from leaves (Fig. 4).

The 183 isolates in the *Ca. kyotensis* species complex were identified as *Ca. aconidialis*, *Ca. asiatica*, *Ca. hongkongensis*, *Ca. ilicicola*, *Ca. kyotensis* and *Ca. yunnanensis* (Fig. 4). They accounted for 35% (64), 22.4% (41), 2.2% (4), 13.7% (25), 1.1% (2) and 25.7% (47) of the isolates respectively. Most (98.9%) were collected from soil samples, and the remaining 1.1% were from leaves (Fig. 4).

Four species were collected from both *Eucalyptus* leaves and soils. In the case of *Ca. honghensis*, the three genotypes from the soils were also found in isolates from diseased leaves (26 genotypes) (Table 4). *Ca. asiatica* and *Ca. yunnanensis* isolates from leaves each represented a single genotype and these were also found in isolates from soil samples (nine and three genotypes, respectively) (Table 4).

Table 4
Species and genotypic diversity of *Calonectria* obtained from diseased *Eucalyptus* leaves and soils in this study

Species ID and species names	**Isolate number from leaf**	**Isolate number from soil**	**Isolate number of each genotype[a] of each species obtained from leaf**	**Isolate number of each genotype[a] of each species obtained from soil**
Calonectria colhounii species complex				
1. *Ca. aciculata*	3	0	GT1 (1), GT2 (1), GT3 (1)	No[b]
2. *Ca. colhounii*	5	0	GT1 (1), GT2 (1), GT3 (1), GT4 (1), GT5 (1)	No
3. *Ca. dianii*	2	0	GT1 (2)	No
4. *Ca. eucalypti*	42	2	GT1 (2), GT2 (2), GT3 (1), GT4 (1), GT5 (1), GT6 (1), GT7 (1), GT8 (2), GT9 (2), GT10 (1), GT11 (2), GT12 (1), GT13 (2), GT14 (1), GT15 (1)	GT16[c] (2)
5. *Ca. honghensis*	174	6	GT1[d] (1), GT2 (1), GT3 (1), GT4 (1), GT5 (2), GT6 (1), GT7 (1), GT8 (1), GT9 (2), GT10 (1), GT11 (1), GT12 (2), GT13 (1), GT14 (1), GT15 (2), GT16 (1), GT17 (1), GT18 (1), GT19 (1), GT20 (1), GT21 (2), GT22 (1), GT23 (1), GT24 (1), GT25 (1), GT26 (1)	GT1 (1), GT4 (3), GT14 (2)
Calonectria kyotensis species complex				
6. *Ca. aconidialis*	0	64	No	GT1 (2), GT2 (2), GT3 (1), GT4 (2), GT5 (1)
7. *Ca. asiatica*	1	40	GT1 (1)	GT1 (1), GT2 (1), GT3 (1), GT4 (1), GT5 (1) GT6 (1), GT7 (1), GT8 (1), GT9 (1)
8. *Ca. hongkongensis*	0	4	No	GT1 (2), GT2 (2)
9. *Ca. ilicicola*	0	25	No	GT1 (2)
10. *Ca. kyotensis*	0	2	No	GT1 (2)
11. *Ca. yunnanensis*	1	46	GT1 (1)	GT1 (1), GT2 (2), GT3 (2)

[a] "GT1–GT26" represent Genotype1 to Genotype26, determined by *act*, *cmdA*, *his3*, *rpb2*, *tef1* and *tub2* gene regions, the isolate number of each genotype was in bracket.

[b] "No" means no *Calonectria* isolate was obtained.

[c] representing the unique genotype only observed from soil.

[d] the bolded and underlined genotype was observed from both leaf and soil

Discussion

A total of 417 *Calonectria* isolates were collected from diseased *Eucalyptus* leaves or soil samples collected beneath these trees in YunNan Province. Based on multi-gene phylogenetic analyses and morphological characteristics, 11 species were identified, including *Ca. aciculata*, *Ca. colhounii*, *Ca. dianii sp. nov*, *Ca. eucalypti* and *Ca. honghensis* in the *Ca. colhounii* species complex, and *Ca. aconidialis*, *Ca. asiatica*, *Ca. hongkongensis*, *Ca. ilicicola*, *Ca. kyotensis* and *Ca. yunnanensis* in the *Ca. kyotensis* species complex. *Calonectria eucalypti*, *Ca. honghensis* and all five species in the *Ca. colhounii* species complex were isolated from diseased leaves; *Ca. asiatica*, *Ca. yunnanensis* and all six species in the *Ca. kyotensis* species complex were isolated from soils. *Calonectria eucalypti*, *Ca. honghensis*, *Ca. asiatica* and *Ca. yunnanensis* were found on both the diseased leaves and in soils; all remaining species were found exclusively in either on leaves or in soils. *Calonectria honghensis* and *Ca. eucalypti* were the predominant species in the diseased *Eucalyptus* leaves while *Ca. aconidialis*, *Ca. yunnanensis*, *Ca. asiatica* and *Ca. ilicicola* were prevalent in the soil samples.

The majority of the isolates obtained from diseased leaves resided in the *Ca. colhounii* species complex. *Calonectria aciculata* and *Ca. honghensis* were originally found and described by Li et al. (2017) in YunNan Province, and have never been identified outside that area. *Calonectria colhounii*, originally described from *Camellia sinensis* in Mauritius (Peerally, 1973) was reported from *Vaccinium* spp. (blueberry) in the LiaoNing Province of North-eastern China (Feng et al. 2007) and is now also known to occur on *Eucalyptus* in China. *Calonectria eucalypti*, commonly found in this study was originally described from diseased *Eucalyptus* in Indonesia (Lombard et al. 2010a), and has previously also been found on these trees in FuJian (Chen et al. 2011b; Liu et al. 2020) and YunNan Provinces (Li et al. 2017).

Calonectria dianii described as new in this study represents a new member to the *Ca. colhounii* species complex. This species is closely related to *Ca. aciculata*, *Ca. eucalypti*, *Ca. honghensis* and *Ca. minensis*, but all those species can be distinguished using DNA sequences in the *act*, *cmdA* and *tub2* gene regions, and macroconidial dimensions (Lombard et al. 2010a; Li et al. 2017; Liu et al. 2022). The *Ca. colhounii* species complex now includes 14 species (Liu et al. 2020, 2022), of which 11 have been found in Asia. Of these, nine have been reported from China (Chen et al. 2011b; Li et al. 2017; Liu and Chen 2017; Wang et al. 2019; Liu et al. 2022), *Ca. indusiata* was reported in Sri Lanka (Crous 2002) and *Ca. monticola* was described in Thailand (Crous et al. 2015). This species complex includes some *Calonectria* species associated with the CLB on *Eucalyptus* spp., such as *Ca. aciculata*, *Ca. colhounii*, *Ca*. *eucalypti*, *Ca*. *fujianensis* and *Ca*. *macroconidialis* (Crous et al. 1993, 2006; Chen et al. 2011b; Li et al. 2017).

Calonectria honghensis and *Ca. eucalypti* were dominant species from diseased leaves in the *Ca. colhounii* species complex, which is different to that observed in other provinces (Li et al. 2017; Wang and Chen 2020; Wu and Chen 2021). Previous studies have shown that *Ca. pseudoreteaudii*, in the *Ca. reteaudii* species complex, was most frequently collected in diseased *Eucalyptus* plantations or nurseries in four provinces (Lombard et al. 2015; Li et al. 2017; Ye et al. 2018; Liu et al. 2020; Wang and Chen 2020; Wu and Chen 2021) and was regarded as the most important species causing CLB in southern China. It is intriguing that *Ca. pseudoreteaudii* was not observed in the present study. In contrast, *Ca. eucalypti* has been found in various of regions of China and seems to be emerging as a threat to *Eucalyptus* plantations.

The majority of the isolates obtained from soils resided in the *Ca. kyotensis* species complex. Previous studies have found that *Ca. aconidialis*, *Ca. hongkongensis* and *Ca. kyotensis* were the most widely distributed species in the soils of southern China, and they have been frequently been collected from soils in the FuJian, GuangDong, GuangXi and HaiNan Provinces (Lombard et al. 2015; Li et al. 2017). Our results provide added support for the fact that these species are predominantly soil-inhabiting fungi and they add a new geographical distribution record for them.

Calonectria ilicicola was first described from *Solanum tuberosum* (Boedijn and Reitsma 1950) and is a well-known soil-borne pathogen, causing severe black rot on peanut and red crown rot on soybean (Gai et al. 2017; Akamatsu et al. 2020). This species has been reported in many countries of the world including Australia (Johnson 1985), China (Gai et al. 2017), Japan (Akamatsu et al. 2020), United States (Kleczewski et al. 2019) and South Korea (Sung 1980) where it occurs on soybean, and Brazil, India and Kenya (Alfenas et al. 1979; Sharma and Mohanan 1982; Crous et al. 1989) associated with Eucalytus leaf disease. It has previously been reported in Chinese *Eucalyptus* plantations (Liu et al. 2022; Liu and Chen 2022) and was not surprising that it emerged in the present study.

The majority of the *Ca. colhounii* species complex isolates were from diseased leaves in contrast to most species in the *Ca. kyotensis* species complex being from soil samples. This suggests that *Ca. colhounii* complex species are mainly leaf-infecting fungi and isolates recovered from the soil samples likely originated from diseased leaves. Species in the *Ca. kyotensis* species complex appear to be more specifically soil inhabiting fungi but having the ability to cause leaf disease under suitable conditions, as has recently been shown in inoculation studies (Wu and Chen 2021).

Conclusions

Results of this study revealed a remarkably rich *Calonectria* diversity in the sampled *Eucalyptus* plantations. There were also clear differences in species diversity and distribution relating to either the leaf or soil environments where they were found. These results provide a foundation on which to pursue an understanding of their pathogenicity to *Eucalyptus*. Furthermore, it is becoming increasingly apparent that some species are predominantly soil inhabitants and different to those that cause plant diseases. The basis of these differences deserves further study.

Declarations

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Author’s contributions

Q.L. Liu performed isolations, sequencing, data analysis and wrote the first drat manuscript, M.J. Wingfield, T.A. Duong, B.D. Wingfield and S.F. Chen advised the project and critically reviewed the draft manuscript, S.F. Chen conceived, supervised and administered the study and acquired funding. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Ethics approval and consent to participate

Not applicable.

Adherence to national and international regulations

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

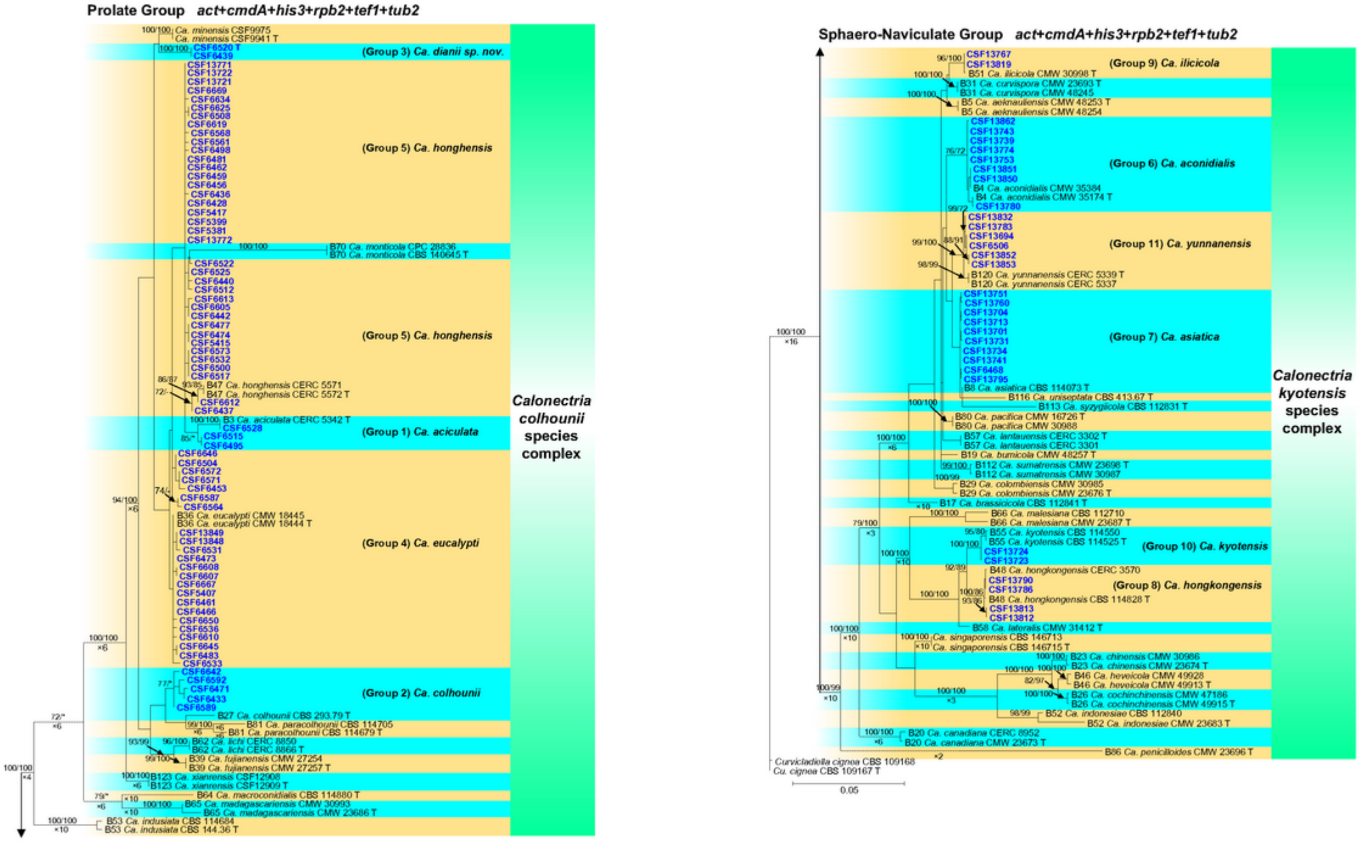

Figure 1

Maximum Likelihood (ML) tree inferred from the combined dataset of *act*, *cmdA*, *his3*, *rpb2*, *tef1*, and *tub2* sequences. Bootstrap value ≥ 70 % for ML and MP analyses are presented above the branches as ML/MP. Bootstrap values lower than 70 % are marked with “*”, whereas absent of clade from a given analysis is indicated with “-”. Ex-type isolates are marked with “T”. Isolates sequenced in this study are highlighted in blue and **bold**. The “B” species codes are consistent with the recently published results in Liu et al. (2020). The tree was rooted to *Curvicladiella cignea* (CBS 109167 and CBS 109168)

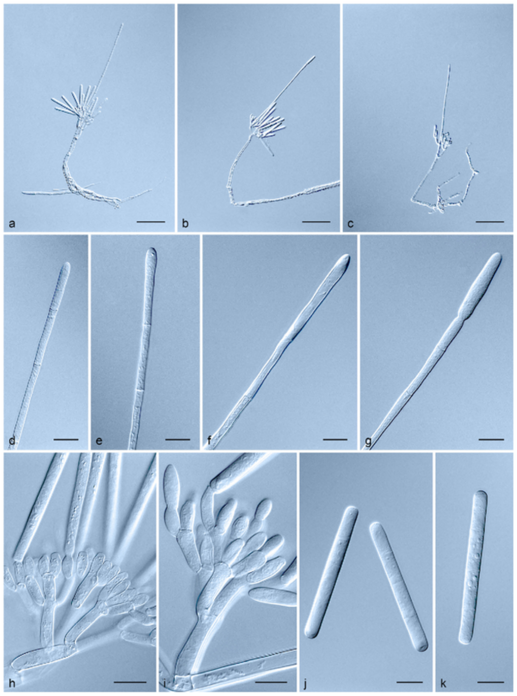

Figure 2

Calonectria dianii. **a–c.** macroconidiophore; **d–g**. broadly clavate to clavate vesicle; **h, i**. conidiogenous apparatus with conidiophore branches and elongate doliiform to reniform phialides; **j, k**. macroconidia. — Scale bars: a–c = 50 μm; h–k = 10 μm; d–g = 5 μm

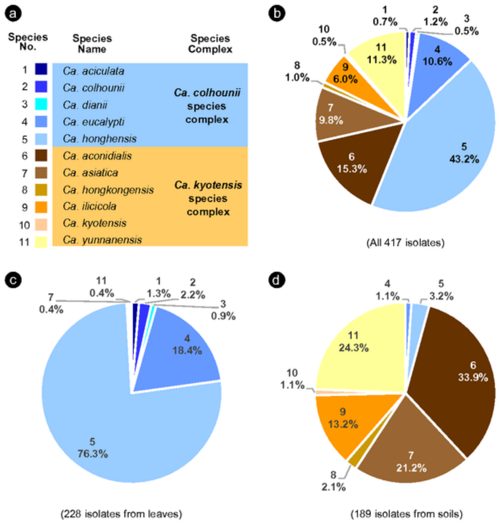

Figure 3

Calonectria species were observed from one *Eucalyptus* plantation in YunNan Province, southwestern China. **a.** Eleven *Calonectria*species were identified in this study and belong to two species complexes.Different species are indicated by numbers with different colours; **b.** The percentage of each *Calonectria* species accounted for all of the species obtained in this study; **c.** The percentage of each *Calonectria* species accounted for the species isolated from diseased leaves; **d.** The percentage of each *Calonectria* species accounted for the species isolated from soils.

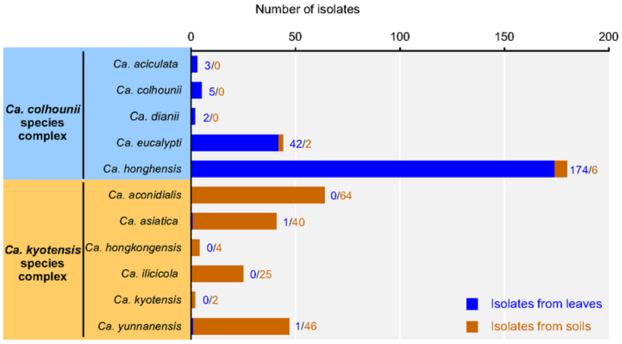

Figure 4

Isolation sources of 11 *Calonectria*species reside in the *Ca. colhounii* species complex and *Ca. kyotensis*species complex.

Supplementary Files

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