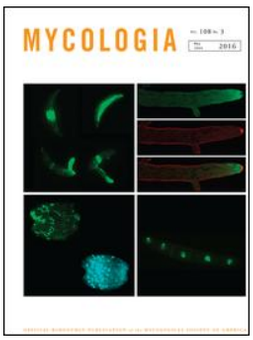

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Ten new species of *Calonectria* from Indonesia and Vietnam

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ABSTRACT

Vietnam and Indonesia have rapidly growing and extensive plantation forestry programs, especially of *Acacia* spp. and *Eucalyptus* spp. As these plantations expand, the threat from pests and diseases also increases. *Calonectria* species are among those pathogens causing diseases of trees in plantations and nurseries in these countries. Extensive surveys were conducted across plantations and nurseries of Vietnam and parts of Indonesia, where a large number of *Calonectria* isolates were retrieved from diseased leaves and soils associated with symptomatic trees. The aim of this study was to identify and resolve the phylogenetic relationships among these isolates using DNA sequence comparisons of four gene regions as well as morphological characters. From a collection of 165 isolates, the study revealed five known and 10 undescribed species. The relatively high diversity of *Calonectria* species found in this study supports the view that many more species in this genus remain to be discovered in other areas of Southeast Asia.

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KEYWORDS
Acacia; *Eucalyptus*; fungal diversity; phylogeny; taxonomy; 10 new taxa

INTRODUCTION

Species of *Calonectria* (*Hypocreales, Nectriaceae*) are globally distributed, particularly in tropical and subtropical regions where they occur on at least 335 plant species classified in approximately 100 plant families (Lombard et al. 2010a). *Calonectria* is best known as a genus of root, shoot, and foliar pathogens (Crous 2002) and is commonly associated with disease symptoms such as seedling damping-off, shoot blight, crown cankers, collar and root rots, leaf spots, leaf blight, stem lesions, tuber rot, and cutting rot (Sharma et al. 1984; Crous 2002; Lombard et al. 2010a). This makes disease management for these fungi complex because a wide range of strategies are needed to control the diseases (Crous 2002; Vitale et al. 2013).

Southeast Asia has fast-growing and extensive plantation forestry programs. These plantations include about 2.6 million ha of *Acacia* spp. and 4.3 million ha of *Eucalyptus* spp. (Harwood and Nambiar 2014). Vietnam and Indonesia have the most extensive plantations in Southeast Asia, and as these plantations expand, the threat from pests and diseases also grows (Wingfield et al. 2008, 2015; Paine et al. 2011). Leaf and shoot blight associated with *Calonectria* spp. is one of the most serious problems in high-rainfall regions in Southeast Asia and is especially serious in *Eucalyptus* plantations and nurseries (Booth et al. 2000; Kang et al. 2001; Crous 2002). Infections by *Calonectria* spp. have significant negative effects on the growth of *Eucalyptus* trees and have resulted in massive defoliation and deformation of main stems and crowns in Southeast Asia (Old et al. 2003). The observed mortality can reach 60–100% in some areas of Vietnam (Old et al. 1999, 2003; Booth et al. 2000). For tropical *Acacia* spp., calonectria leaf blight (CLB) has not had a major impact, but in some plantation areas where conducive microclimatic conditions frequently occur, these fungi can cause damage (Old et al. 2000).

Species of *Calonectria* are divided into two main phylogenetic groups: the Prolate group and Sphaero-Naviculate group, which are defined based on their morphological features (Lombard et al. 2010c). Of the more than 150 described species of *Calonectria*, only four have been reported in Vietnam. *Calonectria pentaseptata* and *Ca. reteaudii*, in the *Ca. reteaudii* complex, are among the most damaging, particularly causing CLB on *Eucalyptus* trees (Booth et al. 2000; Chen et al. 2011; Lombard et al. 2015a). *Calonectria insularis* and *Ca. pauciramosa* are

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associated with leaf spot symptoms on *Eucalyptus* spp. (Crous et al. 2002). Consistent with the known ecology of *Calonectria* spp., it is assumed that these species would occur in the soil. Previous studies suggested a hyperdiversity of *Calonectria* species isolated from soils in the tropics of South America and particularly South China and the neighboring regions of Southeast Asia (Alfenas et al. 2015; Lombard et al. 2015a; Li et al. 2017). There is, however, little knowledge regarding the diversity of *Calonectria* species in soil in forest plantations in Vietnam or Southeast Asia in general.

In this study, surveys for *Calonectria* spp. were conducted across plantations and nurseries of Vietnam and parts of Indonesia. The primary aim was to obtain a large number of isolates from diseased leaves and especially soils associated with symptomatic trees, to identify any potential new species and to resolve the phylogenetic relationships among isolates using DNA sequence comparisons. Overall, the goal was to provide some insight into the diversity and distribution of these fungi, particularly as putative tree pathogens in the Southeast Asia region.

MATERIALS AND METHODS

Isolates.—Surveys were conducted in Vietnam, including eight provinces (Hanoi, Hoa Binh, Lao Cai, Ninh Binh, Phu Tho, Tuyen Quang, Vinh Phuc, Yen Bai) in northern Vietnam, three provinces (Nghe An, Thanh Hoa, Quang Tri) in central Vietnam, and four provinces (Binh Duong, Binh Phuoc, Dong Nai, Tay Ninh) in southern Vietnam as well as in North Sumatra (Indonesia) (FIG. 1). Plant and soil samples were collected from plantations and nurseries as well as natural forests.

Symptomatic plant tissues were collected, incubated in moist chambers at room temperature, and examined daily for fungal sporulation. Soil samples associated with diseased trees were collected and baited with germinating alfalfa seeds (*Medicago sativa*) as described by Crous (2002). Direct isolations from fungal structures were made onto 2% (*w*/*v*) malt extract agar (MEA; Biolab, Midrand, South Africa) and incubated for 7 d at 25 C under continuous near-ultraviolet (UV) light. Single hyphal tip cultures from primary isolations were prepared on MEA and incubated at 25 C for 7 d to

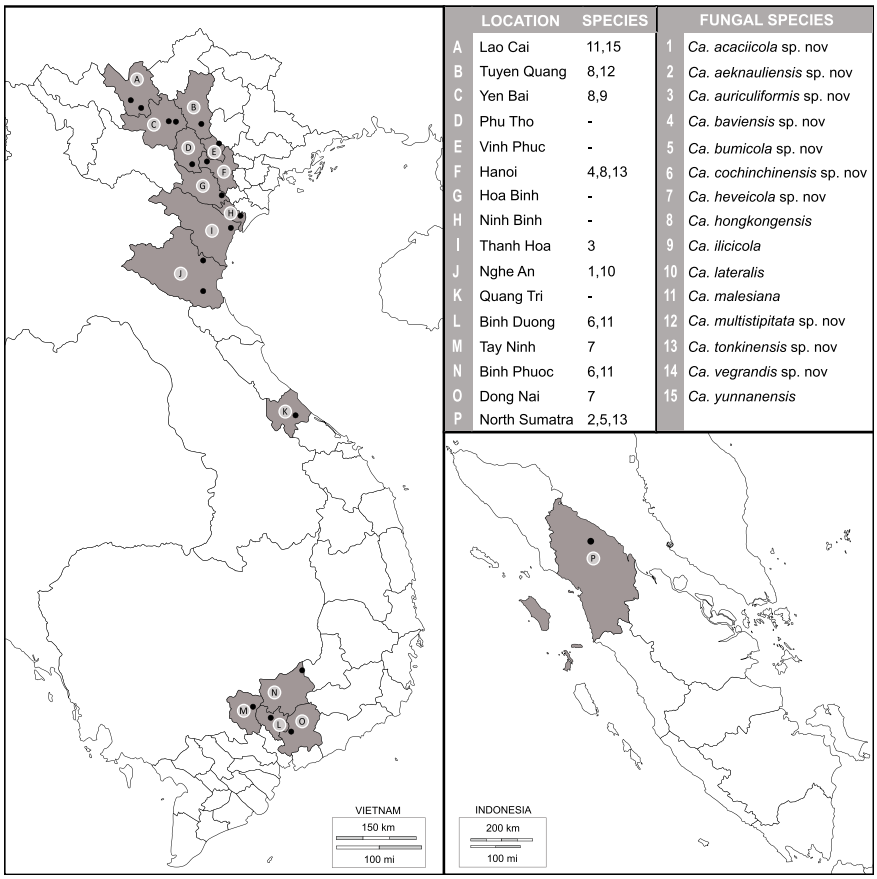

Figure 1. Map of Vietnam and Indonesia representing provinces (indicated as letters A–P) where surveys for *Calonectria* spp. were conducted (sampling localities indicated as black dots) and the fungal species (indicated as numbers 1–15) obtained from each location.

obtain pure cultures. These were deposited in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa, and the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, the Netherlands. Dried specimens of novel taxa were deposited in the National Collection of Fungi (PREM), Pretoria, South Africa.

DNA sequencing and phylogenetic analyses.—Genomic DNA was extracted from 7-d-old fungal cultures, grown on MEA at 25 C, using Prepman Ultra Sample Preparation Reagent (Thermo Fisher Scientific, Waltham, Massachusetts) following the manufacturer's instructions. Four loci were amplified and sequenced: (i) a fragment of the translation elongation factor 1-α (*TEF1*) gene region using primers EF1-728F (Carbone and Kohn 1999) and EF-2 (O'Donnell et al. 1998); (ii) a fragment of the β-tubulin (*TUB2*) gene with primers T1 (O'Donnell and Cigelnik 1997) and CYLTUB1R (Crous et al. 2004); (iii) part of the histone H3 (*HIS3*) gene region with primers CYLH3F and CYLH3R (Crous et al. 2004); and (iv) a fragment of the calmodulin (*CMDA*) gene with primers CAL-228F (Carbone and Kohn 1999) and CAL-2Rd (Groenewald et al. 2013).

Polymerase chain reaction (PCR) amplifications were performed in 25-μL reactions containing 5 μL 5× MyTaq buffer (Bioline, London, UK), 0.5 μL MyTaq DNA polymerases (Bioline), 1 μL DNA, 1 μL of each primer (10 mM), and sterile deionized water. Amplified fragments were purified using ExoSAP-IT PCR Product Cleanup Reagent (Thermo Fisher Scientific) and were sequenced in both directions using the same primers used for PCR amplification and using the BigDye terminator sequencing kit 3.1 (Applied Biosystems, Forster City, California). Sequences were obtained by running samples on an ABI PRISM 3100 DNA sequencer (Applied Biosystems).

Raw sequences were assembled and edited (edges trimmed) using Geneious 7.0 (Kearse et al. 2012). All sequence data of closely related *Calonectria* spp. used in this study were obtained from GenBank (http://www.ncbi.nlm.nih.gov). Those sequences were aligned using MAFFT 7 (http://mafft.cbrc.jp/alignment/server) (Katoh and Standley 2013) and then confirmed in MEGA7 (Kumar et al. 2016). The aligned sequence data sets were deposited in TreeBASE (No. 22930).

Phylogenetic analyses were based on both maximum likelihood (ML) and maximum parsimony (MP). The partition homogeneity test, using PAUP 4.0b10 (Swofford 2003), was applied to determine whether there was any conflict in single gene trees and whether the data sets could be combined (Cunningham 1997). For MP, analyses were conducted using PAUP 4.0b10 (Swofford 2003) with phylogenetic relationships estimated by heuristic searches with 1000 random stepwise addition sequences and tree bisection and reconnection (TBR) branch-swapping. Gaps were treated as a fifth character, and all characters were weighted equally. Measures calculated for parsimony included tree length (TL), retention index (RI), consistency index (CI), rescaled consistency indexes (RC), and homoplasy index (HI). Statistical support for branch nodes in the most parsimonious trees was assessed with 1000 bootstrap replicates.

For ML, the most appropriate model for each data set was obtained using jModelTest 2.1.5 (Posada 2008). Analyses was performed with PhyML 3.0 (Guindon and Gascuel 2003). Confidence levels for the nodes were determined with 1000 bootstrap replicates. All resulting trees were visualized using MEGA7 (Kumar et al. 2016).

Taxonomy.—For morphological identification, single hyphal tip cultures were transferred to synthetic low-nutrient agar (SNA; Nirenberg 1981) and incubated at 25 C for 7 d. In some cases, the presence of surface-sterilized *M. sativa* seedlings on SNA was required to induce production of conidial structures. Structures of the asexual morphs were studied by mounting them in 80% lactic acid and examining them using a Nikon H550L microscope (Nikon, Tokyo, Japan).

To induce formation of perithecia (sexual morph), crosses of single hyphal tip isolates of each putative new species (after preliminary identification based on DNA sequence analyses) were made in all possible combinations. Crosses were made on minimal salt agar (MSA) with sterile bamboo toothpicks placed on the agar surface as described by Lombard et al. (2010b, 2010c). To distinguish between homothallic and heterothallic mating systems, isolates were crossed with themselves and these served as controls. Plates bearing the crossed isolates were incubated at 25 C for 6–8 wk. Crosses were considered successful when they produced perithecia extruding ascospores. The sexual structures were then studied by mounting them in tissue-freezing medium (Leica Biosystems, Nussloch, Germany) and cutting 10-μm sections with a Microtome Cryostat Leica CM1100 (Leica Biosystems) at −20 C. The sections were mounted in 85% lactic acid and 3% KOH and examined in the same way as the asexual structures.

Thirty to fifty measurements were made for all taxonomically informative characters, depending on their availability; 95% confidence levels were determined, and extremes of conidial and ascospore measurements were calculated and presented as (min–)

(mean – standard deviation)–(mean + standard deviation)(–max). For all other fungal structures, only extremes were calculated.

Colony color and morphology were assessed using 7-d-old cultures on MEA growing at 25 C using the charts of Rayner (1970). To determine the optimal temperature for growth, cultures were transferred to MEA and incubated at temperatures ranging from 10 to 35 C with 5 C intervals. All description data were deposited in MycoBank (www.mycobank.org).

RESULTS

Isolates.—A total of 165 isolates were obtained, of which 105 were from northern Vietnam (four provinces: Hanoi, Lao Cai, Tuyen Quang, Yen Bai), 30 isolates were from central Vietnam (two provinces: Nghe An, Thanh Hoa), 25 were from northern Vietnam (four provinces: Binh Duong, Binh Phuoc, Dong Nai, Tay Ninh), and 5 were from Indonesia (FIG. 1). The majority of isolates were obtained by soil baiting (160 isolates), and five isolates were from diseased plant material. The *TEF1* gene region was initially sequenced for all 165 isolates. Based on the preliminary phylogenetic analysis of these *TEF1* sequences, isolates representing different substrates and localities with the same sequence were chosen for further sequencing of the *CMDA, HIS3*, and *TUB2* gene regions. This amounted to a total of 32 isolates (TABLE 1)

Phylogenetic analyses.—Amplicons with approximate sizes of 680 (*CMDA*), 430 (*HIS3*), 500 (*TEF1*), and 560 (*TUB2*) bp were generated. The sequence data for the 32 isolates amplified in this study were divided into two data sets representing the Prolate group (Lombard et al. 2010c), including isolates of *Ca. cylindrospora* and *Ca. reteaudii* species complexes, and the Sphaero-Naviculate group (Lombard et al. 2010c), which accommodates isolates in the *Ca. kyotensis* species complex. In both cases, sequences of other closely related species were included in the data sets (TABLE 1). The partition homogeneity test generated a *P*-value of 0.001 for both Prolate and Sphaero-Naviculate data sets. However, we chose to combine the different gene regions because it has been shown that combining incongruent data sets will improve phylogenetic accuracy (Cunningham 1997). Individual gene trees were shown in SUPPLEMENTARY FIGS. 1– 8. For both ML and MP analyses, two isolates of *Curvicladiella cignea* (CBS 101411 and CBS 109167; Lombard et al. 2015b) were used as the outgroup taxa for the Prolate group and the Sphaero-Naviculate group. A summary of important parameters applied in the phylogenetic analyses are presented in TABLE 2.

Sequence analyses for the Prolate group. The combined sequence data set for the Prolate group included 39 taxa, including the outgroup. The sequence data set consisted of 1966 bp, including alignment gaps. Of these, 676 were parsimony-informative and 1290 were excluded. The MP analysis yielded 1000 trees, and the statistical values generated for the MP analyses are presented in TABLE 2. The ML tree with bootstrap support values for both the ML and MP analyses is presented in FIG. 2. The 37 ingroup taxa were separated into two major phylogenetic groups representing the *Ca. reteaudii* species complex (ML/MP = 90/100) and *Ca. cylindrospora* species complex (ML/MP = 100/100).

In the *Ca. reteaudii* species complex, two clades were observed, with CBS 143563 and CBS 143564 forming one clade (ML/MP = 100/99) and CBS 143557 and CBS 143558 forming a second clade (ML/MP = 100/99) most closely related to *Ca. microconidialis*. In the *Ca. cylindrospora* species complex, a single isolate lineage (CBS 143576) clustered with a well-supported unique clade (ML/MP = 76/89) accommodating CBS 143561 and CBS 143562 (FIG. 2).

Sequence analyses for the Sphaero-Naviculate group. The combined sequence data set representing the Sphaero-Naviculate group of isolates contained 65 taxa, including the outgroup. This data set consisted of 1951 characters, including the alignment of gaps. Of these, 649 characters were parsimony-informative and 1302 were excluded. The MP analysis yielded 1000 trees, and the statistical values generated for the MP analyses are presented in TABLE 2. The ML tree with bootstrap support values for ML and MP analyses is presented in FIG. 3. In this tree, five isolates from Indonesia were separated in two closely related but unique clades and one lineage. One clade (ML/MP = 98/97) included CBS 143559 and CBS 143560, and the second clade (ML/MP = 95/92), most closely related to *Ca. curvispora*, included CBS 143565 and CMW 143567. The lineage was represented by CBS 143575 (ML/MP = 100/100) and was most closely related to *Ca. lantauensis*.

Three new distinct clades could be distinguished close to isolates representing *Ca. chinensis*. Two isolates (CBS 143573, CBS 143574) from Tuyen Quang formed a well-supported clade (ML/MP = 96/99) close to *Ca. chinensis* (CBS 112744, CBS 1142827). An additional two well-supported clades (ML/MP = 100/100 for both) occurred, each consisting of three isolates: CBS 143570, CBS 143571, CBS 143572 representing the one clade and CBS 143567, CBS 143568, CBS 143569 representing the second clade.

Table 1. Collection details and GenBank accessions of isolates included in the phylogenetic analyses.

Species	Isolate number[a,c]	Substrate	Locality	GenBank accessions[b]				Reference
				TUB2	*CMDA*	*HIS3*	*TEF1*	
Calonectria acacicola	**CBS 143557^{T}; CMW 47173**	Soil in *Acacia auriculiformis* plantation	Do Luong, Nghe An, Vietnam	MH119285	MH119252	MH119186	MH119219	This study
	CBS 143558; CMW 47174	Soil in *A. auriculiformis* plantation	Do Luong, Nghe An, Vietnam	MH119286	MH119253	MH119187	MH119220	This study
Ca. acicola	CBS 114812	*Phoenix canariensis*	New Zealand	DQ190590	GQ267359	DQ190692	GQ267291	Gadgil and Dick (2004)
	CBS 114813^{T}	*P. canariensis*	New Zealand	DQ190591	GQ267360	DQ190693	GQ267292	Gadgil and Dick (2004)
Ca. aeknauliensis	**CBS 143559^{T}; CMW 48253**	Soil in *Eucalyptus* plantation	Aek Nauli, North Sumatra, Indonesia	—[d]	MH119259	MH119193	MH119226	This study
	CBS 143560; CMW 48254	Soil in *Eucalyptus* plantation	Aek Nauli, North Sumatra, Indonesia	—	MH119260	MH119194	MH119227	This study
Ca. arbusta	CBS 136079^{T}; CMW 31370; CERC 1705	Soil in *Eucalyptus* plantation	Guangxi, China	KJ462904	KJ463018	KJ463135	KJ462787	Lombard et al. (2015a)
	CBS 136098; CPC 23519; CMW 37981; CERC 1944	Soil in *Eucalyptus* plantation	Guangxi, China	—	KJ463019	KJ463136	KJ462788	Lombard et al. (2015a)
Ca. auriculiformis	**CBS 143561^{T}; CMW 47178**	Soil in *A. auriculiformis* plantation	Hau Loc, Thanh Hoa, Vietnam	MH119287	MH119254	MH119188	MH119221	This study
	CBS 143562; CMW 47179	Soil in *A. auriculiformis* plantation	Hau Loc, Thanh Hoa, Vietnam	MH119288	MH119255	MH119189	MH119222	This study
Ca. australiensis	CBS 112954^{T}	*Ficus pleurocarpa*	Australia	DQ190596	GQ267363	DQ190699	GQ267293	Crous et al. (2006)
Ca. baviensis	**CBS 143563^{T}; CMW 47410**	*E. urophylla* leaf	Ba Vi, Hanoi, Vietnam	MH119289	MH119256	MH119190	MH119223	This study
	CBS 143564; CMW 47433	*E. pellita* leaf	Ba Vi, Hanoi, Vietnam	MH119290	MH119257	MH119191	MH119224	This study
Ca. bumicola	**CBS 143575^{T}; CMW 48257**	Soil in *Eucalyptus* plantation	Aek Nauli, North Sumatra, Indonesia	—	MH119271	MH119205	MH119238	This study
Ca. cerciana	CBS 123693^{T}; CMW 25309	*Eucalyptus* hybrid	Zhanjiang Prov., CERC nursery, China	FJ918510	GQ267369	FJ918528	FJ918559	Lombard et al. (2010d)
	CBS 123695; CMW 25290	*Eucalyptus* hybrid	Zhanjiang Prov., CERC nursery, China	FJ918511	GQ267370	FJ918529	FJ918560	Lombard et al. (2010d)
Ca. chinensis	CBS 112744; CMW 30986; CPC 4104	Soil	Hong Kong, China	AY725618	AY725746	AY725660	AY725709	Crous et al. (2004)
	CBS 114827^{T}; CMW 23674; CPC 4101	Soil	Hong Kong, China	AY725619	AY725747	AY725661	AY725710	Crous et al. (2004)
Ca. cochinchinensis	**CBS 143567^{T}; CMW 49915**	Soil in *Hevea brasiliensis* plantation	Duong Minh Chau, Tay Ninh, Vietnam	MH119292	MH119263	MH119197	MH119230	This study
	CBS 143568; CMW 47186	Soil in *A. auriculiformis* plantation	Song May, Dong Nai, Vietnam	MH119293	MH119264	MH119198	MH119231	This study
	CBS 143569; CMW 47187	Soil in *A. auriculiformis* plantation	Song May, Dong Nai, Vietnam	MH119294	MH119265	MH119199	MH119232	This study
Ca. colombiensis	CBS 112220^{T}; CPC 723	Soil	La Selva, Brazil	GQ267207	AY725748	AY725662	AY725711	Crous et al. (2004)
	CBS 112221; CPC 724	*E. grandis*	La Selva, Brazil	AY725620	AY725749	AY725663	AY725712	Crous (2002)
Ca. curvispora	CBS 116159^{T}; CPC 765	Soil	Tamatave, Madagascar	AF333394	GQ267374	AY725664	GQ267302	Crous (2002)
Ca. expansa	CBS 136078; CMW 31441; CERC 1776	Soil in *Eucalyptus* plantation	Guangdong, China	KJ462913	KJ463028	KJ463145	KJ462797	Lombard et al. (2015a)
	CBS 136247^{T}; CMW 31392; CERC 1727	Soil in *Eucalyptus* plantation	Guangdong, China	KJ462914	KJ463029	KJ463146	KJ462798	Lombard et al. (2015a)
Ca. guangxiensis	CBS 136092^{T}; CMW 35409; CERC 1900	Soil in *Eucalyptus* plantation	Guangxi, China	KJ462919	KJ463034	KJ463151	KJ462803	Lombard et al. (2015a)
	CBS 136094; CMW 35411; CERC 1902	Soil in *Eucalyptus* plantation	Guangxi, China	KJ462920	KJ463035	—	KJ462804	Lombard et al. (2015a)
Ca. hevicola	**CBS 143570^{T}; CMW 49913**	Soil in *H. brasiliensis* plantation	Bau Bang, Binh Duong, Vietnam	MH119295	MH119266	MH119200	MH119233	This study
	CBS 143571; CMW 49928	Soil in natural forest	Bu Gia Map National Park, Binh Phuoc, Vietnam	MH119296	MH119267	MH119201	MH119234	This study
	CBS 143572; CMW 49935	Soil in natural forest	Bu Gia Map National Park, Binh Phuoc, Vietnam	MH119297	MH119268	MH119202	MH119235	This study
Ca. hongkongensis	CBS 114711; CPC 686	Soil	Hong Kong, China	AY725621	AY725754	AY725666	AY725716	Crous et al. (2004)
	CBS 114828^{T}; CPC 4670	Soil	Hong Kong, China	AY725622	AY725755	AY725667	AY725717	Crous et al. (2004)
	CMW 47200	Soil in *Acacia* hybrid plantation	Tuyen Quang, Vietnam	MH119300	MH119272	MH119206	MH119239	This study
	CMW 47312	Soil in *A. mangium* plantation	Son Duong, Tuyen Quang, Vietnam	MH119301	MH119273	MH119207	MH119240	This study
	CMW 47329	Soil in *A. mangium* plantation	Tan Huong, Yen Bai, Vietnam	MH119302	MH119274	MH119208	MH119241	This study
	CMW 47429	Soil in *Eucalyptus* hybrid plantation	Bavi, Hanoi, Vietnam	MH119303	MH119275	MH119209	MH119242	This study
Ca. ilicicola	CBS 190.50^{T}; CMW 30998; IMI 299389	*Solanum tuberosum*	Bogor, Indonesia	AY725631	AY725764	AY725676	AY725726	Crous (2002)
	CBS 115897; CPC 493; UFV 108	*Anacardium* sp.	Brazil	AY725647	GQ267403	GQ267256	AY725729	Crous (2002)
	CMW 47411	Soil in *E. urophylla* plantation	Dai Dong, Yen Bai, Vietnam	—	MH119276	MH119210	MH119243	This study

(*Continued*)

Table 1. (Continued).

Species	Isolate number[a,c]	Substrate	Locality	GenBank accessions[b]				Reference
				TUB2	*CMDA*	*HIS3*	*TEF1*	
	CMW 47413	Soil in *E. urophylla* plantation	Dai Dong, Yen Bai, Vietnam	—	MH119277	MH119211	MH119244	This study
Ca. indonesiae	CBS 112823^{T}; CPC 4508	Soil	Warambunga, Indonesia	AY725623	AY725756	AY725668	AY725718	Crous et al. (2004)
	CBS 112840; CPC 4554	*Syzygium aromaticum*	Indonesia	AY725625	AY725758	AY725670	AY725720	Crous et al. (2004)
Ca. indonesiana	CBS 112826; CPC 4519	—	Indonesia	KX784630	KX784572	—	KX784700	Lombard et al. (2016)
	CBS 112936^{T}; CPC 4504	—	Indonesia	KX784631	KX784573	—	KX784701	Lombard et al. (2016)
Ca. insularis	CBS 114558^{T}; CPC 768	Soil	Tamatave, Madagascar	AF210861	GQ267389	FJ918526	FJ918556	Crous (2002)
	CBS 114559; CPC 954	Soil	Tamatave, Madagascar	AF210862	GQ267390	FJ918525	FJ918555	Crous (2002)
Ca. lantauensis	CBS 142887; CMW 47251; CERC 3301	Soil	Lantau, Lidao, Hong Kong, China	—	MF442906	MF442791	MF442676	Li et al. (2017)
	CBS 142888^{T}; CMW 47252; CERC 3302	Soil	Lantau, Lidao, Hong Kong, China	—	MF442907	MF442792	MF442677	Li et al. (2017)
Ca. lateralis	CBS 136629^{T}; CMW 31412; CERC 1747	Soil in *Eucalyptus* plantation	Fangchenggang, Guangxi, China	KJ462955	KJ463070	KJ463186	KJ462840	Lombard et al. (2015a)
	CMW 47414	Soil in *E. urophylla* plantation	Nghia Dan, Nghe An, Vietnam	MH119304	MH119278	MH119212	MH119245	This study
Ca. malesiana	CBS 112710; CPC 3899	Leaf litter	Thailand	AY725626	AY725759	AY725671	AY725721	Crous et al. (2004)
	CBS 112752^{T}; CPC 4223	Soil	Sumatra, Indonesia	AY725627	AY725760	AY725672	AY725722	Crous et al. (2004)
	CMW 49911	Soil in *H. brasiliensis* plantation	Bau Bang, Binh Duong, Vietnam	MH119305	MH119279	MH119213	MH119246	This study
	CMW 49933	Soil in natural forest	Bu Gia Map National Park, Binh Phuoc, Vietnam	MH119306	MH119280	MH119214	MH119247	This study
	CMW 49943	Soil in natural forest	Hoang Lien National Park, Lao Cai, Vietnam	MH119307	MH119281	MH119215	MH119248	This study
Ca. microconidialis	CBS 136633; CMW 31471; CERC 1806	*E. urophylla* × *E. grandis clone* seedling leaf	CERC nursery, Zhanjiang, Guangdong, China	KJ462957	KJ463072	KJ463188	KJ462842	Lombard et al. (2015a)
	CBS 136638^{T}; CMW 31487; CERC 1822	*E. urophylla* × *E. grandis* clone seedling leaf	CERC nursery, Zhanjiang, Guangdong, China	KJ462960	KJ463075	KJ463191	KJ462845	Lombard et al. (2015a)
Ca. multiseptata	CBS 112682^{T}; CMW 23692; CPC 1589	*Eucalyptus* sp.	Indonesia	DQ190573	GQ267397	DQ190659	FJ918535	Crous (2002)
Ca. multistipitata	**CBS 143573^{T}; CMW 47192**	Soil in *Acacia* hybrid plantation	Tuyen Quang, Vietnam	MH119298	MH119269	MH119203	MH119236	This study
	CBS 143574; CMW 47211	Soil in *Acacia* hybrid plantation	Tuyen Quang, Vietnam	MH119299	MH119270	MH119204	MH119237	This study
Ca. pacifica	CBS 109063^{T}; CPC 2534; IMI 354528	*Araucaria heterophylla*	Hawaii, USA	GQ267213	AY725762	GQ267255	AY725724	Crous (2002)
	CBS 114038; CPC 10717	*Ipomoea aquatica*	Auckland, New Zealand	AY725630	GQ267402	AY725675	GQ267320	Crous (2002)
Ca. papillata	CBS 136096; CMW 37972; CERC 1935	Soil in *Eucalyptus* plantation	Guangdong, China	KJ462963	KJ463078	KJ463194	KJ462848	Lombard et al. (2015a)
	CBS 136097^{T}; CMW 37976; CERC 1939	Soil in *Eucalyptus* plantation	Guangdong, China	KJ462964	KJ463079	KJ463195	KJ462849	Lombard et al. (2015a)
Ca. pauciramosa	CMW 5683^{T}; CPC 971	*E. grandis*	South Africa	FJ918514	GQ267405	FJ918531	FJ918565	Crous (2002)
	CMW 30823; CPC 416	*E. grandis*	South Africa	FJ918515	GQ280404	FJ918532	FJ918566	Crous (2002)
Ca. pentaseptata	CBS 136087; CMW 35177; CERC 1853	*Eucalyptus* leaf	Hainan, China	KJ462966	KJ463083	KJ463199	KJ462853	Lombard et al. (2015a)
	CBS 136089; CMW 35377; CERC 1879	*Eucalyptus* leaf	Hainan, China	KJ462967	KJ463084	KJ463200	KJ462854	Lombard et al. (2015a)
Ca. pseudocerciana	CBS 134823; LPF 366	*Eucalyptus* sp. (seeding)	Santana, Pará, Brazil	KM395961	KM396048	KM396131	KM395874	Alfenas et al. (2015)
	CBS 134824^{T}; LPF 367	*Eucalyptus* sp. (seeding)	Santana, Pará, Brazil	KM395962	KM396049	KM396132	KM395875	Alfenas et al. (2015)
Ca. pseudokyotensis	CBS 137332^{T}; CMW 31439; CERC 1774	Soil in *Eucalyptus* plantation	Fangchenggang, Guangxi, China	KJ462994	KJ463111	KJ463227	KJ462881	Lombard et al. (2015a)
Ca. pseudoreteaudii	CBS 123694^{T}; CMW 25310	*Eucalyptus* hybrid cutting	Guangdong, China	FJ918504	GQ267411	FJ918519	FJ918541	Lombard et al. (2010d)
	CBS 123696; CMW 25292	*Eucalyptus* hybrid cutting	Guangdong, China	FJ918505	GQ267410	FJ918520	FJ918542	Lombard et al. (2010d)
Ca. queenslandica	CBS 112146^{T}; CPC 3213	*E. urophylla*	Australia	AF389835	GQ267415	FJ918521	FJ918543	Lombard et al. (2010d)

(*Continued*)

Table 1. (Continued).

Species	Isolate number[a,c]	Substrate	Locality	GenBank accessions[b]				Reference
				TUB2	*CMDA*	*HIS3*	*TEF1*	
	CBS 112155; CPC 3210	*E. pellita*	Australia	AF389834	GQ267416	DQ190667	FJ918544	Lombard et al. (2010d)
Ca. reteaudii	CBS 112143; CPC 3200	*E. camaldulensis*	Vietnam	GQ240642	GQ267418	DQ190660	FJ918536	Crous (2002)
	CBS 112144; CPC 3201	*E. camaldulensis*	Vietnam	AF389833	GQ267417	DQ190661	FJ918537	Crous (2002)
Ca. sulawesiensis	CBS 125253; CMW 14879	*Eucalyptus* sp.	Sulawesi, Indonesia	GQ267220	GQ267432	GQ267269	GQ267340	Lombard et al. (2010c)
	CBS 125277^{T}; CMW 14878	*Eucalyptus* sp.	Sulawesi, Indonesia	GQ267222	GQ267434	GQ267271	GQ267342	Lombard et al. (2010c)
Ca. sumatraensis	CBS 112829^{T}; CPC 4518	Soil	Sumatra, Indonesia	AY725649	AY725771	AY725696	AY725733	Crous et al. (2004)
	CBS 112934; CPC 4516	Soil	Indonesia	AY725651	AY725773	AY725798	AY725735	Crous et al. (2004)
Ca. syzygiicola	CBS 112827; CPC 4512	*S. aromaticum*	Indonesia	KX784662	KX784597	—	KX784735	Lombard et al. (2016)
	CBS 112831^{T}; CPC 4511	*S. aromaticum*	Indonesia	KX784663	—	—	KX784736	Lombard et al. (2016)
Ca. terrae-reginae	CBS 112151^{T}; CPC 3202	*E. urophylla*	Queensland, Australia	FJ918506	GQ267451	FJ918522	FJ918545	Lombard et al. (2010d)
	CBS 112634; CPC 4233	*Xanthorrhoea australis*	Victoria, Australia	FJ918507	GQ267452	DQ190668	FJ918546	Lombard et al. (2010d)
Ca. terrestris	CBS 136642^{T}; CMW 35180; CERC 1856	Soil in *Eucalyptus* plantation	Guangdong, China	KJ463004	KJ463121	KJ463237	KJ462891	Lombard et al. (2015a)
	CBS 136645; CMW 35178; CERC 1854	Soil in *Eucalyptus* plantation	Guangdong, China	KJ463007	KJ463124	KJ463240	KJ462894	Lombard et al. (2015a)
Ca. tonkinensis	**CBS 143576^{T}; CMW 47430**	Soil in *Eucalyptus* hybrid plantation	Ba Vi, Hanoi, Vietnam	MH119291	MH119258	MH119192	MH119225	This study
Ca. turangicola	CBS 136077^{T}; CMW 31411; CERC 1746	Soil in *Eucalyptus* plantation	Fangchenggang, Guangxi, China	KJ463013	—	KJ463246	KJ462900	Lombard et al. (2015a)
	CBS 136093; CMW 35410; CERC 1901	Soil in *Eucalyptus* plantation	Guangxi, China	KJ463014	KJ463130	KJ463247	KJ462901	Lombard et al. (2015a)
Ca. uniseptata	CBS 413.67; CPC 2391; IMI 299577	*Paphiopedilum callosum*	Celle, Germany	GQ267208	GQ267379	GQ267248	GQ267307	Crous (2002)
	CBS 170.77; IMI 299388	*Idesia polycarpa*	Auckland, New Zealand	GQ267209	GQ267380	GQ267249	GQ267308	Crous (2002)
Ca. variabilis	CBS 112691; CPC 2506	*Theobroma grandiflorum*	Brazil	GQ267240	GQ267458	GQ267264	GQ267335	Crous (2002)
	CBS 114677; CPC 2436	*Schefflera morotoni*	Brazil	AF333424	GQ267457	GQ267263	GQ267334	Crous (2002)
Ca. vegrandis	**CBS 143565^{T}; CMW 48245**	Soil in *Eucalyptus* plantation	Aek Nauli, North Sumatra, Indonesia	—	MH119261	MH119195	MH119228	This study
	CBS 143566; CMW 48246	Soil in *Eucalyptus* plantation	Aek Nauli, North Sumatra, Indonesia	—	MH119262	MH119196	MH119229	This study
Ca. yunnanensis	CBS 142895; CMW 47642; CERC 5337	Soil in *Eucalyptus* plantation	ZhengXing, JingGu, PuEr, Yunnan, China	MF443086	MF442986	MF442871	MF442756	Li et al. (2017)
	CBS 142897^{T}; CMW 47644; CERC 5339	Soil in *Eucalyptus* plantation	ZhengXing, JingGu, PuEr, Yunnan, China	MF443088	MF442988	MF442873	MF442758	Li et al. (2017)
	CMW 47543	Soil in natural forest	Van Ban, Lao Cai, Vietnam	MH119308	MH119282	MH119216	MH119249	This study
	CMW 47544	Soil in natural forest	Van Ban, Lao Cai, Vietnam	MH119309	MH119283	MH119217	MH119250	This study
	CMW 47546	Soil in natural forest	Van Ban, Lao Cai, Vietnam	MH119310	MH119284	MH119218	MH119251	This study
Curvicladiella cignea	CBS 101411	Decaying seed	French Guiana	KM232001	KM231285	KM231459	KM231866	Lombard et al. (2015b)
	CBS 109167^{T}	Leaf litter	French Guiana	KM232002	KM231287	KM231461	KM231867	Lombard et al. (2015b)

[a]CBS = The culture collection of Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; CERC = China Eucalypt Research Centre, Zhanjiang, Guangdong Province, China; CMW = culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; CPC = Pedro Crous working collection housed at Westerdijk Fungal Biodiversity Institute; IMI = International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, UK; LPF = Laboratório de Patologia Florestal, Universidade Federal de Viçosa, Viçosa, Brazil; UFV = Universidade Federal de Viçosa, Viçosa, Brazil.
[b]*TUB2* = β-tubulin; *CMDA* = calmodulin; *HIS3* = histone H3; *TEF1* = translation elongation factor 1-α.
[c]Isolates obtained during the survey in this study are indicated in bold.
[d]"—" represents sequences that are not available.
[T] denotes ex-type cultures.

Table 2. Statistics resulting from MP and ML analyses.

Maximum parsimony			Maximum likelihood		
Parameter	Prolate group	Sphaero-Naviculate group	Parameter	Prolate group	Sphaero-Naviculate group
No. of taxa	39	65	Best substitution model	TIM2+I+G	TIM2+I+G
No. of base pairs	1966	1951	NST	6	6
PIC	676	649	Rate matrix	1.613	1.286
No. of trees	3	1302		4.405	3.399
Tree length	1254	1206		1.613	1.286
CI	0.726	0.716		1.000	1.000
RI	0.925	0.942		5.548	5.310
RC	0.672	0.675	Gamma shape	0.510	0.579
HI	0.273	0.284	P-inv	0.279	0.328

Note. PIC = number of parsimony informative characters; CI = consistency index; RI = retention index; RC = rescaled consistency index; HI = homoplasy index; NST = number of substitution rate categories.

TAXONOMY

DNA sequence and morphological comparisons for *Calonectria* isolates collected in our surveys showed that they represent five previously described and 10 undescribed species. The five known species are in the *Ca. kyotensis* species complex: *Calonectria hongkongensis* was represented by four isolates (CMW 47200, CMW 47312, CMW 47329, CMW 47429) from Hanoi, Tuyen Quang, and Yen Bai; *Ca. ilicicola* by two isolates (CMW 47411, CMW 47413) from Yen Bai; *Ca. lateralis* by one isolate (CMW 47414) from Nghe An; *Ca. malesiana* by three isolates (CMW 49911, CMW 49933, CMW 49943) from Binh Duong, Binh Phuoc, and Lao Cai; and *Ca. yunnanensis* by three isolates (CMW 47543, CMW 47544, CMW 47546) from Lao Cai.

The undescribed taxa included two species in the *Ca. cylindrospora* complex and two species in the *Ca. reteaudii* complex, accommodated in the Prolate group. The results also revealed six novel species in the *Ca. kyotensis* complex, placed in the Sphaero-Naviculate group. The isolates representing novel species are described below.

Prolate group

Calonectria cylindrospora species complex (Crous 2002; Lombard et al. 2010b, 2010c, 2010d, 2015a; Alfenas et al. 2013b, 2015)

Macroconidiophores consisting of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth; stipe extension septate, straight to flexuous, terminating in a pyriform to obpyriform or ovoid to ellipsoidal vesicle. Conidiogenous apparatus consisting of 3–7 branches, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, apex with minute periclinal thickening and inconspicuous collarette. Macroconidia cylindrical, rounded at both ends, straight, 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by hyaline slime.

Calonectria auriculiformis N.Q. Pham, T.Q. Pham & M.J. Wingf., sp. nov. FIGS. 4A, 5
MycoBank MB825527

Typification: VIETNAM. THANH HOA: Hau Loc, from soil in *Acacia auriculiformis* plantation, Nov 2013, *N.Q. Pham & T.Q. Pham* (**holotype** PREM 62109). Ex-type CBS 143561 = CMW 47178.

Etymology: The name refers to *Acacia auriculiformis*, the plantation tree species associated with the soil from which this fungus was isolated.

Description: Sexual morph not observed. Macroconidiophores consisting of a stipe bearing a penicillate arrangement of fertile branches, stipe extension, and terminal vesicle; stipe extensions septate, 164–268 µm long, 2–5 µm wide at apical septum, terminating in ellipsoidal to fusiform to obpyriform vesicle, 6–12 µm diam. Conidiogenous apparatus consisting of up to 5 branches. Macroconidia cylindrical, rounded at both ends, straight, (40–)41–45(–47) × (3–)4–5 µm (av. = 43 × 4 µm), 1-septate. Mega- and microconidia not observed.

Culture characters: Colonies buff to peach on the surface and sienna to umber in reverse; moderate to extensive aerial mycelium in the middle, mycelium immersed in the medium in the outer regions, irregular margins; moderate sporulation; chlamydospores abundant, occurring throughout the medium, forming microsclerotia. Optimal growth temperature at 25 C, no growth at 10 and 35 C; after 7 d, colonies at 15 C reach 23.4 mm, at 20 C 41.0 mm, at 25 C 57.8 mm, and at 30 C 46.7 mm.

Other specimen examined: VIETNAM. THANH HOA: Hau Loc, from soil in *A. auriculiformis* plantation, Nov 2013, *N.Q. Pham & T.Q. Pham*, PREM 62110, CBS 143562 = CMW 47179.

Notes: Calonectria auriculiformis is a member of the *Ca. cylindrospora* species complex. This species is phylogenetically closely related to *Ca. cerciana, Ca. papillata, Ca. terrestris*, and *Ca. tonkinensis. Calonectria auriculiformis* can be distinguished from the other species based on the number of branches of the conidiogenous apparatus,

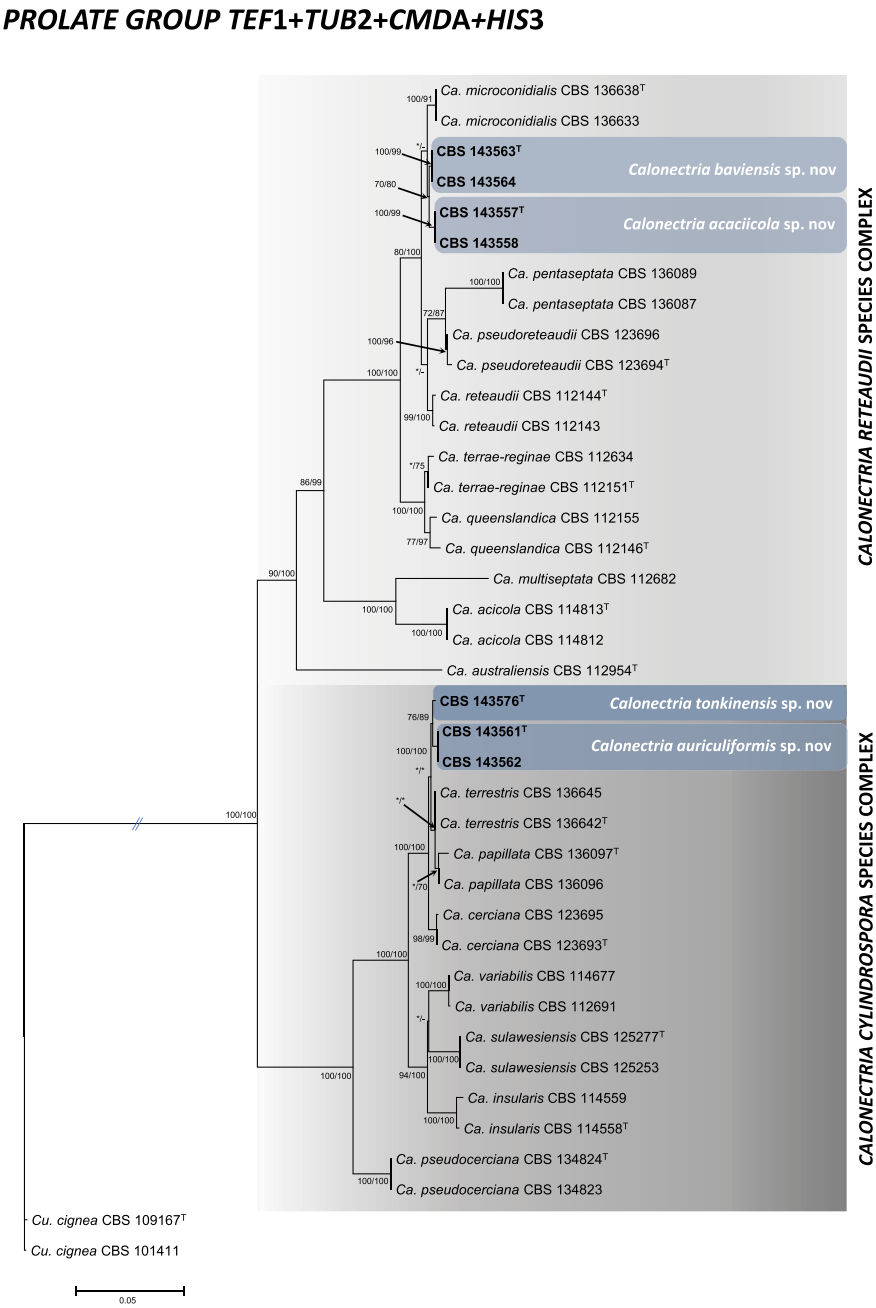

Figure 2. Phylogenetic tree based on maximum likelihood (ML) analysis of a combined DNA data set of *TEF1, HIS3, CMDA*, and *TUB2* sequences for *Calonectria* spp. in the *Ca. reteaudii* and *Ca. cylindrospora* species complex representing the Prolate group. Bootstrap values ≥70% for maximum parsimony (MP) and ML analyses are indicated at the nodes. Bootstrap values lower than 70% are marked with "*", and absent are marked with "–". Isolates representing ex-type material are marked with "T"; isolates collected in this study are highlighted in bold. *Curvicladiella cignea* (CBS 101411 and CBS 109167) represents the outgroup.

where *Ca. auriculiformis* has up to 5 branches, and *Ca. cerciana, Ca. papillate*, and *Ca. terrestris* have up to 4 each. Macroconidia of *Ca. auriculiformis* (av. = 43 × 4) are larger than those of *Ca. terrestris* (av. = 38.5 × 4.5) and *Ca. tonkinensis* (av. = 41.5 × 4), but smaller than those of *Ca. cerciana* (av. = 44 × 5) and *Ca. papillata* (av. = 45 × 4) (Lombard et al. 2010d, 2015a).

Calonectria tonkinensis N.Q. Pham, T.Q. Pham & M.J. Wingf., sp. nov. FIGS. 4B, 6
MycoBank MB825528

Typification: VIETNAM. HANOI: Ba Vi, from soil in *Eucalyptus* hybrid plantation, Nov 2013, *N.Q. Pham & T.Q. Pham* (**holotype** PREM 62124). Ex-type CBS 143576 = CMW 47430.

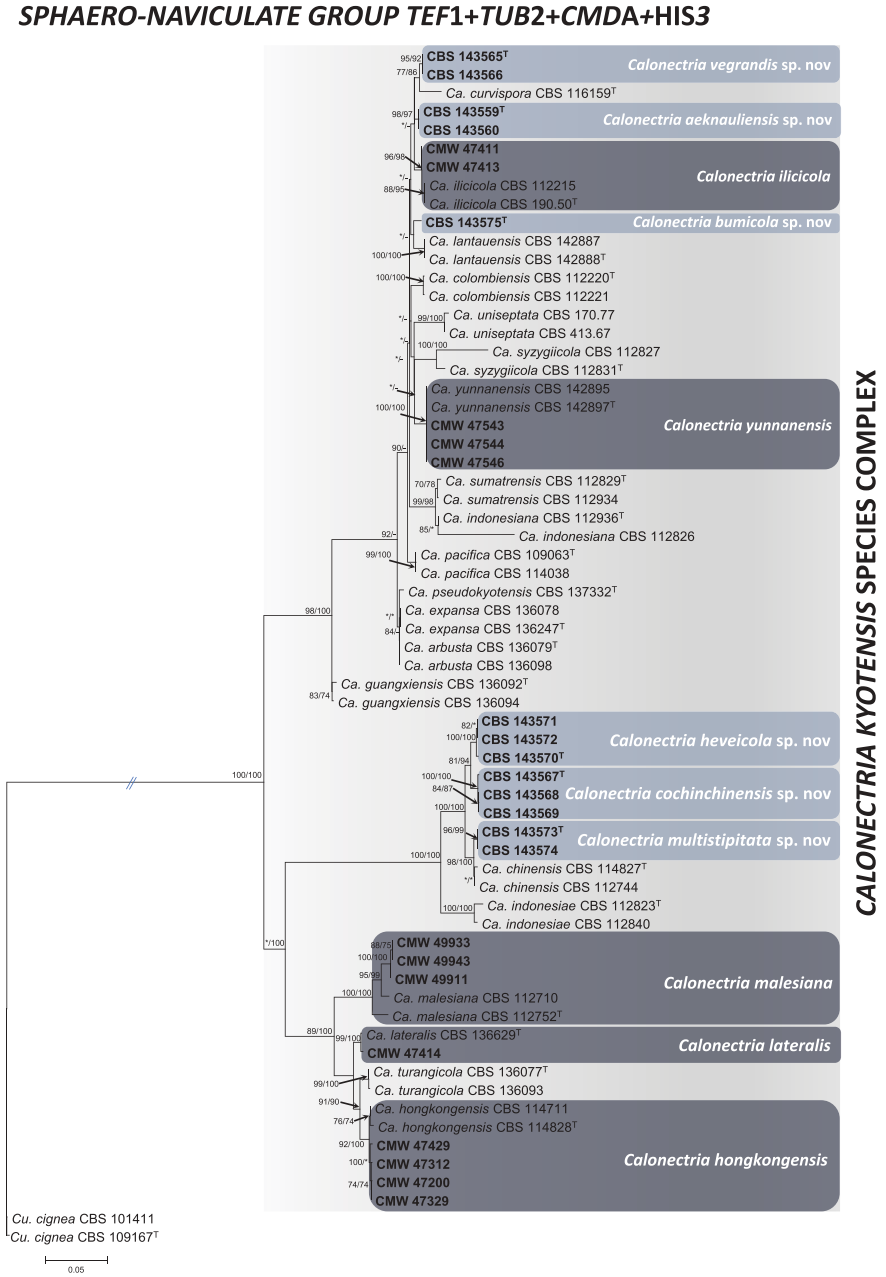

Figure 3. Phylogenetic tree based on maximum likelihood (ML) analysis of a combined DNA data set of *TEF1, HIS3, CMDA*, and *TUB2* gene sequences for the species of *Calonectria* in the *Ca. kyotensis* species complex representing the Sphaero-Naviculate group. Bootstrap values ≥70% for maximum parsimony (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 collected in this study are highlighted in bold. *Curvicladiella cignea* (CBS 101411 and CBS 109167) represents the outgroup.

Etymology: The name refers to the former name for North Vietnam, Tonkin, where this fungus was first isolated.

Description: Sexual morph not observed. Macroconidiophores consisting of a stipe bearing a penicillate arrangement of fertile branches, stipe extension, and terminal vesicle; stipe extensions septate, 107–164 µm long, 2–3 µm wide at apical septum, terminating in ellipsoidal to obpyriform vesicle, 3–7 µm diam. Conidiogenous apparatus consisting of up to 5 branches. Macroconidia cylindrical, rounded at both ends, straight, (36–)39–44(–50) × 3.5–4(–5) µm (av. = 41.5 × 4 µm), 1–septate. Megaconidia and microconidia not observed.

Culture characters: Colonies white to buff on the surface and sienna to brick to umber in reverse on MEA after 7 d; abundant aerial mycelium with profuse sporulation on the medium surface; chlamydospores

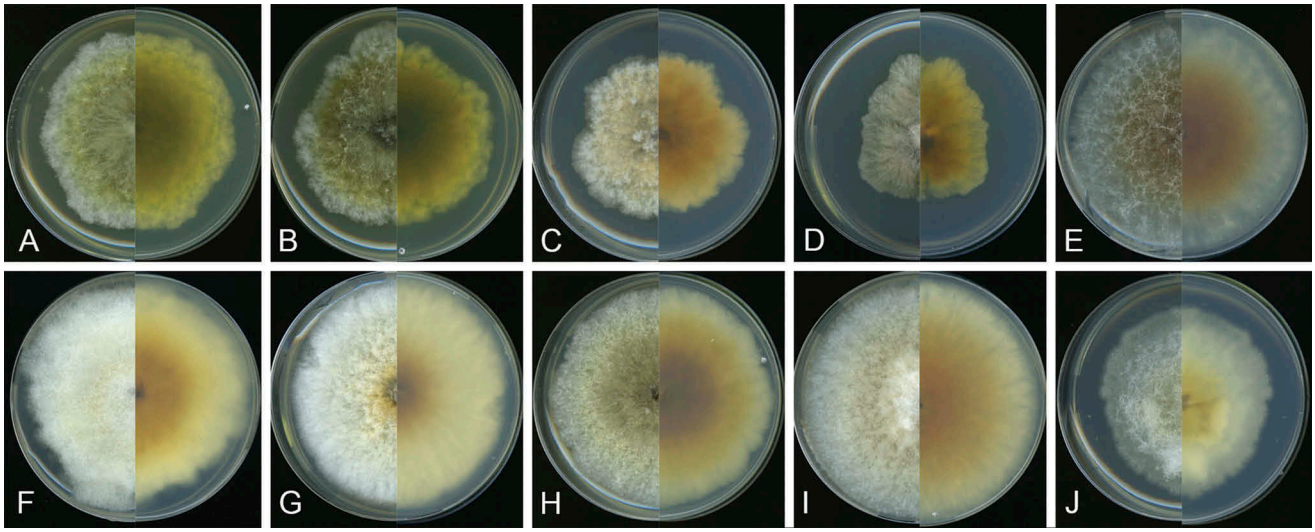

Figure 4. Colony morphology of *Calonectria* species grown at 25 C in the dark on MEA for 7 d. A. *Ca. auriculiformis*. B. *Ca. tonkinensis*. C. *Ca. acaciicola*. D. *Ca. baviensis*. E. *Ca. aeknauliensis*. F. *Ca. bumicola*. G. *Ca. cochinchinensis*. H. *Ca. heveicola*. I. *Ca. multistipitata*. J. *Ca. vegrandis*.

abundant, occurring throughout the medium, forming microsclerotia. Optimal growth temperature at 30 C, no growth at 10 and 35 C; after 7 d, colonies at 15 C reach 21.0 mm, at 20 C 35.3 mm, at 25 C 50.4 mm, and at 30 C 58.9 mm.

Notes: Calonectria tonkinensis is a member of the *Ca. cylindrospora* species complex. This species is phylogenetically closely related to *Ca. auriculiformis, Ca. cerciana, Ca. papillate*, and *Ca. terrestris. Calonectria tonkinensis* can be distinguished from the other species based on the number of branches of the conidiogenous apparatus where *Ca. tonkinensis* has up to 5 branches and *Ca. cerciana, Ca. papillate*, and *Ca. terrestris* have up to 4 each. Macroconidia of *Ca. tonkinensis* (av. = 41.5 × 4 µm) are larger than those of *Ca. terrestris* (av. = 38.5 × 4.5 µm) and smaller than those of *Ca. auriculiformis* (av. = 43 × 4 µm), *Ca. cerciana* (av. = 44 × 5 µm), and *Ca. papillata* (av. = 45 × 4 µm) (Lombard et al. 2010d, 2015a).

***Calonectria reteaudii* species complex** (Crous 2002; Lombard et al. 2010d, 2015a; Crous et al. 2012)

Macroconidiophores consisting of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth; stipe extension septate, straight to flexuous, terminating in a narrowly clavate to clavate vesicle. Conidiogenous apparatus consisting of 3–6 branches, each terminal branch producing 1–3 phialides; phialides cylindrical to allantoid, hyaline, aseptate, apex with minute periclinal thickening and inconspicuous collarette. Macroconidia cylindrical, rounded at both ends, straight, 1–8-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by hyaline slime. Microconidiophores present or absent, simple with some lateral branching, comprising a stipe and a penicillate or subverticillate arrangement of fertile branches. Microconidia cylindrical, straight, rounded at the apex, flattened at the base, 1–3-septate, held in fascicles by hyaline slime.

Calonectria acaciicola N.Q. Pham, T.Q. Pham & M.J. Wingf., sp. nov. FIGS. 4C, 7
MycoBank MB825529

Typification: VIETNAM. NGHE AN: Do Luong, from soil in *A. auriculiformis* plantation, Nov 2013, *N. Q. Pham & T.Q. Pham* (**holotype** PREM 62105). Ex-type CBS 143557 = CMW 47173.

Etymology: The name refers to *Acacia*, which comprised the plantations from which this fungus was isolated.

Description: Sexual morph not observed. Macroconidiophores consisting of a stipe bearing a penicillate arrangement of fertile branches, stipe extension, and terminal vesicle; stipe extensions septate, straight to flexuous, 199–527 µm long, 3–6 µm wide at apical septum, terminating in narrowly clavate vesicle, 4–7 µm diam. Conidiogenous apparatus consisting of up to 3 branches. Macroconidia cylindrical, rounded at both ends, straight, (85–)90–98(–105) × (6–)6.5–7.5 µm (av. = 94 × 7 µm), 5-septate. Mega- and microconidia not observed.

Culture characters: Colonies white to buff on the surface and sienna to umber in reverse on MEA after 7 d, undulate margins; sparse to moderate aerial mycelium,

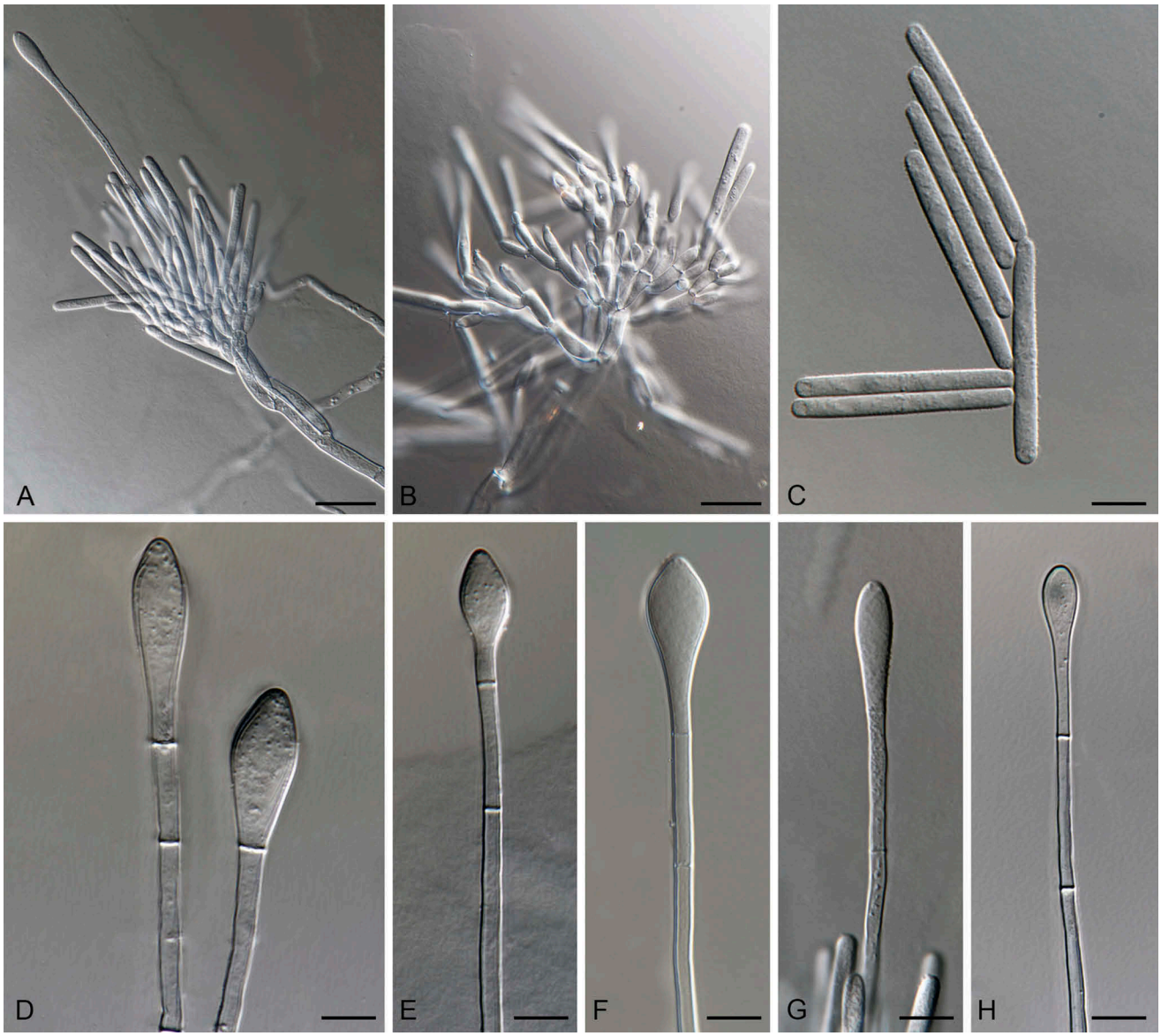

Figure 5. *Calonectria auriculiformis* (ex-type CBS 143561). A. Macroconidiophore. B. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. C. Macroconidia. D–H. Ellipsoidal to fusiform to obpyriform vesicles. Bars: A–B = 20 µm; C–H = 10 µm.

mycelium immersed in the medium with extensive sporulation on the medium surface; chlamydospores not observed. Optimal growth temperature at 25 C, no growth at 10 and 35 C; after 7 d, colonies at 15 C reach 18.2 mm, at 20 C 36.0 mm, at 25 C 51.7 mm, and at 30 C 46.8 mm.

Other specimen examined: VIETNAM. NGHE AN: Do Luong, from soil in *A. auriculiformis* plantation, Nov 2013, *N.Q. Pham & T.Q. Pham*, PREM 62106, culture CBS 143558 = CMW 47174.

Notes: Calonectria acaciicola is a member of the *Ca. reteaudii* species complex. This species is phylogenetically closely related to *Ca. baviensis, Ca. microconidialis*, and *Ca. pentaseptata*. The macroconidia of *Ca. acaciicola* (av. = 94 × 7 µm) are longer than those of *Ca. microconidialis* (av. = 88 × 8 µm) and smaller than those of *Ca. baviensis* (av. = 96 × 6.5 µm) and *Ca. pentaseptata* (av. = 98 × 7 µm). As with *Ca. pentaseptata, Ca. acaciicola* failed to produce microconidiophores and microconidia, distinguishing this species from *Ca. baviensis* and *Ca. microconidialis*, which form these structures in culture (Crous et al. 2012; Lombard et al. 2010d, 2015a).

Calonectria baviensis N.Q. Pham, T.Q. Pham & M. J. Wingf., sp. nov. FIGS. 4D, 8

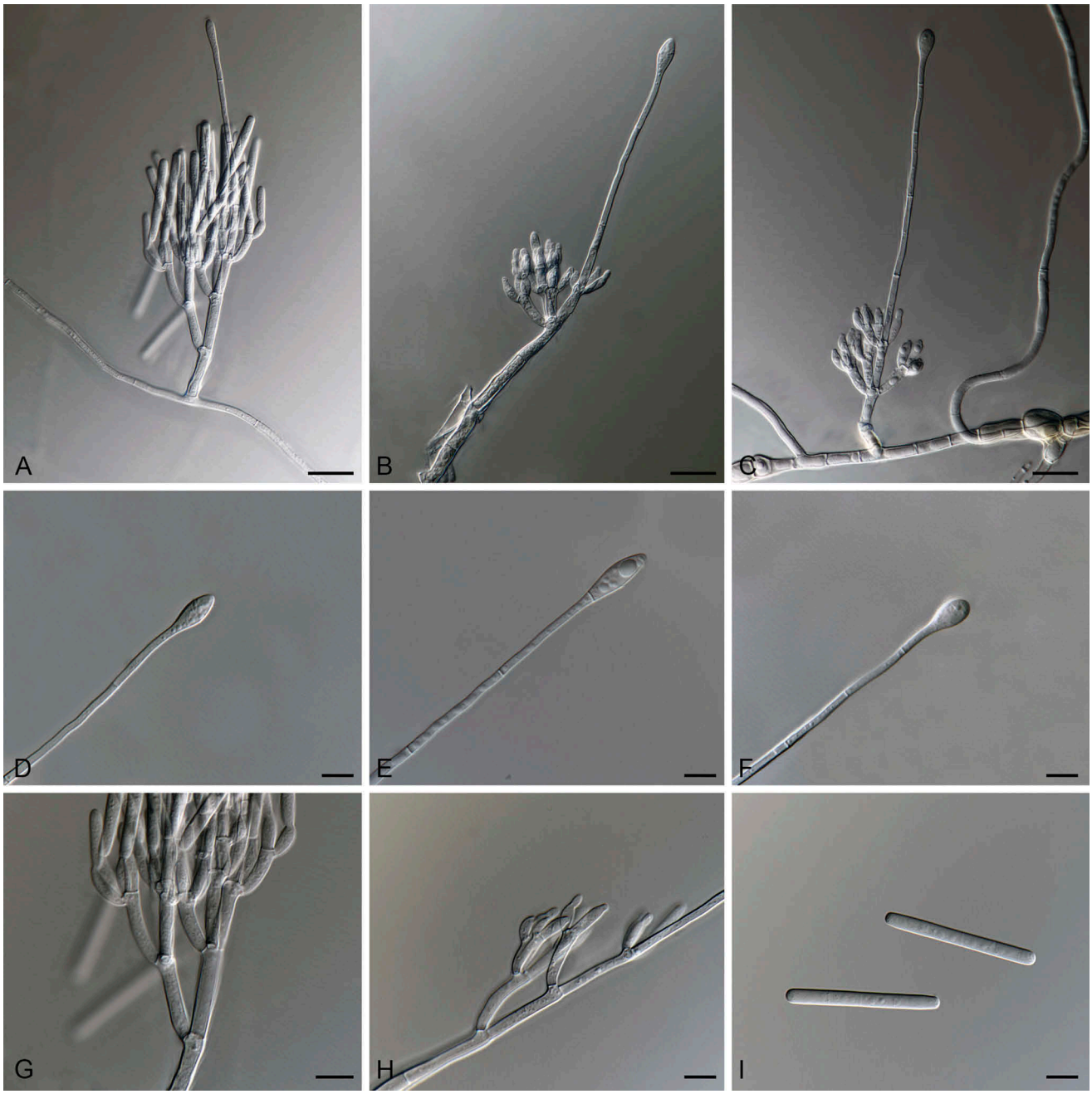

Figure 6. *Calonectria tonkinensis* (ex-type CBS 143576). A–C. Macroconidiophores. D–F. Ellipsoidal to obpyriform vesicles. G–H. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. I. Macroconidia. Bars: A–C = 20 µm; D–I = 10 µm.

MycoBank MB825530

Typification: VIETNAM. HANOI: Ba Vi, from leaf of *Eucalyptus urophylla*, Nov 2013, *N.Q. Pham & T.Q. Pham* (**holotype** PREM 62111). Ex-type CBS 143563 = CMW 47410.

Etymology: The name refers to the Ba Vi National Park, Hanoi, Vietnam, where the fungus was first collected.

Description: Sexual morph not observed. Macroconidiophores consisting of a stipe bearing a penicillate arrangement of fertile branches, stipe extension, and terminal vesicle stipe extensions septate, straight to flexuous, 263–398 µm long, 2–4 µm wide at apical septum, terminating in narrowly clavate vesicle, 3–6 µm diam. Conidiogenous apparatus consisting of up to 3 branches. Macroconidia cylindrical, rounded at both ends, straight, (82–)87.5–104.5(–120) × (5–)6–7(–8) µm (av. = 96 × 6.5 µm), 5-septate. Microconidiophores simple with some lateral branching, consisting of a stipe and a penicillate or subverticillate arrangement of fertile branches. Microconidia cylindrical, straight,

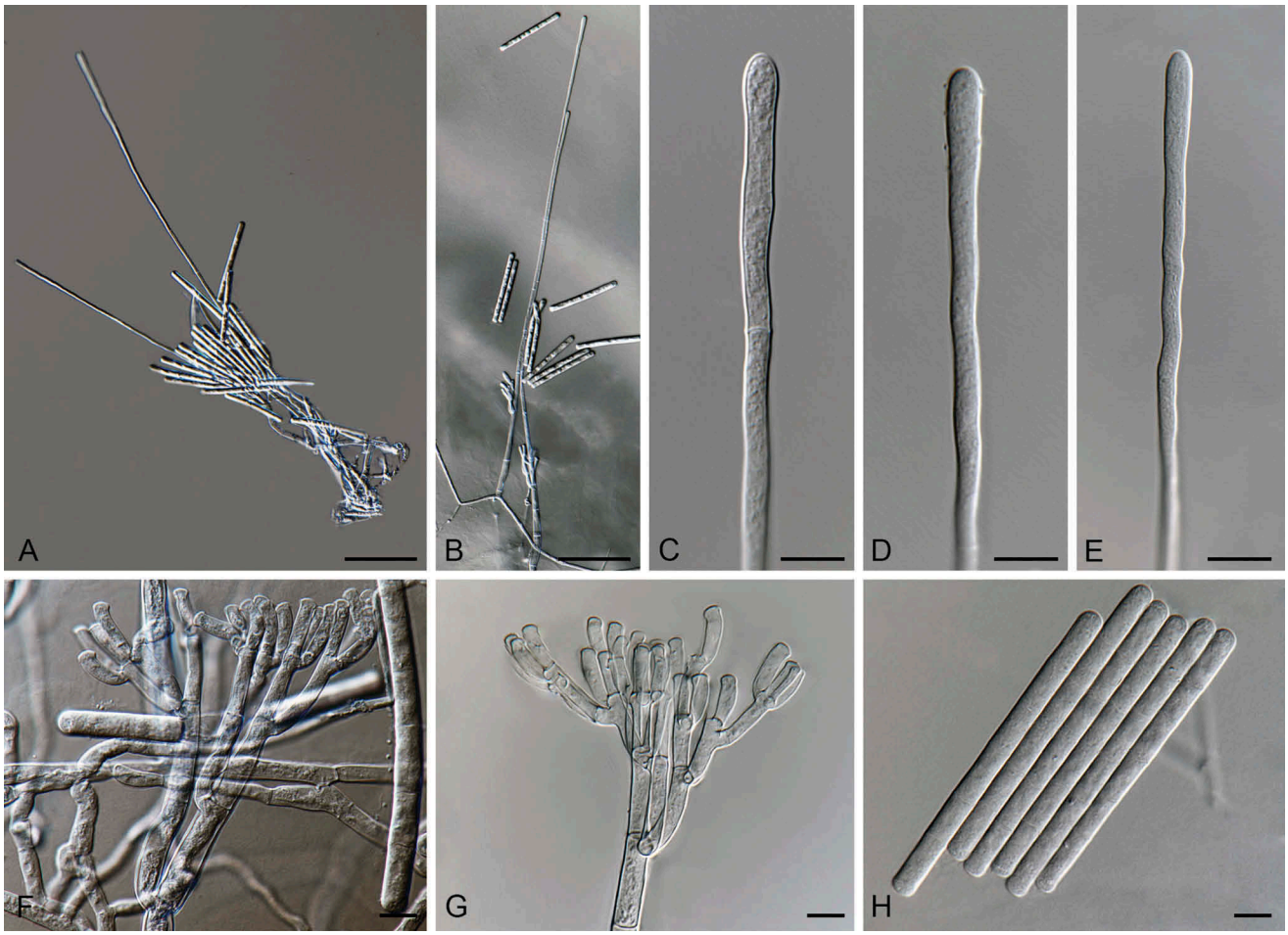

Figure 7. *Calonectria acaciicola* (ex-type CBS 143557). A–B. Macroconidiophores. C–E. Narrowly clavate vesicle. F–G. Conidiogenous apparatus with conidiophore branches and cylindrical to allantoid phialides. H. Macroconidia. Bars: A–B = 100 µm; C–H = 10 µm.

round at both ends, (22.5–)26–35(–38) × (3–)3.5–4.5 (–5) µm (av. = 30.5 × 4 µm), 1–3-septate. Megaconidia not observed.

Culture characters: Colonies white to amber on the surface and sienna to umber to sepia in reverse on MEA after 7 d, irregular margins; mycelium immersed in the medium with extensive sporulation on the medium surface, especially in the center of the colony; chlamydospores not observed. Optimal growth temperature at 30 C, no growth at 10 and 35 C; after 7 d, colonies at 15 C reach 10.4 mm, at 20 C 21.6 mm, at 25 C 36.3 mm, and at 30 C 38.4 mm.

Other specimen examined: VIETNAM. HANOI: Ba Vi, from leaf of *Eucalyptus pellita*, Nov 2013, *N.Q. Pham & T.Q. Pham*, PREM 62112, culture CBS 143564 = CMW 47433.

Notes: Calonectria baviensis is a member of the *Ca. reteaudii* species complex. This species is phylogenetically closely related to *Ca. acaciicola, Ca. microconidialis*, and *Ca. pentaseptata*. The macroconidia of *Ca. baviensis* (av. = 96 × 6.5 µm) are longer than those of *Ca. microconidialis* (av. = 88 × 8 µm) and *Ca. acaciicola* (av. = 94 × 7 µm) and smaller than those of *Ca. pentaseptata* (av. = 98 × 7 µm). The ability of *Ca. baviensis* and *Ca. microconidialis* to produce microconidiophores and microconidia distinguishes them from *Ca. acaciicola* and *Ca. pentaseptata*. The microconidia of *Ca. baviensis* (av. = 30.5 × 4 µm) are smaller than those of *Ca. microconidialis* (av. = 39 × 5 µm) (Crous et al. 2012; Lombard et al. 2010d, 2015a).

Sphaero-Naviculate group

***Calonectria kyotensis* species complex** (Crous 2002; Crous et al. 2004; Lombard et al. 2015a; Li et al. 2017)

Macroconidiophores consisting of a stipe bearing a penicillate arrangement of fertile branches, stipe extension, and terminal vesicle; stipe septate, hyaline, smooth; stipe extensions septate, straight to flexuous, terminating in sphaeropedunculate vesicle; lateral stipe extension (90° to main axis) present. Conidiogenous apparatus consisting of 3–8 branches, each terminal branch producing 2–4

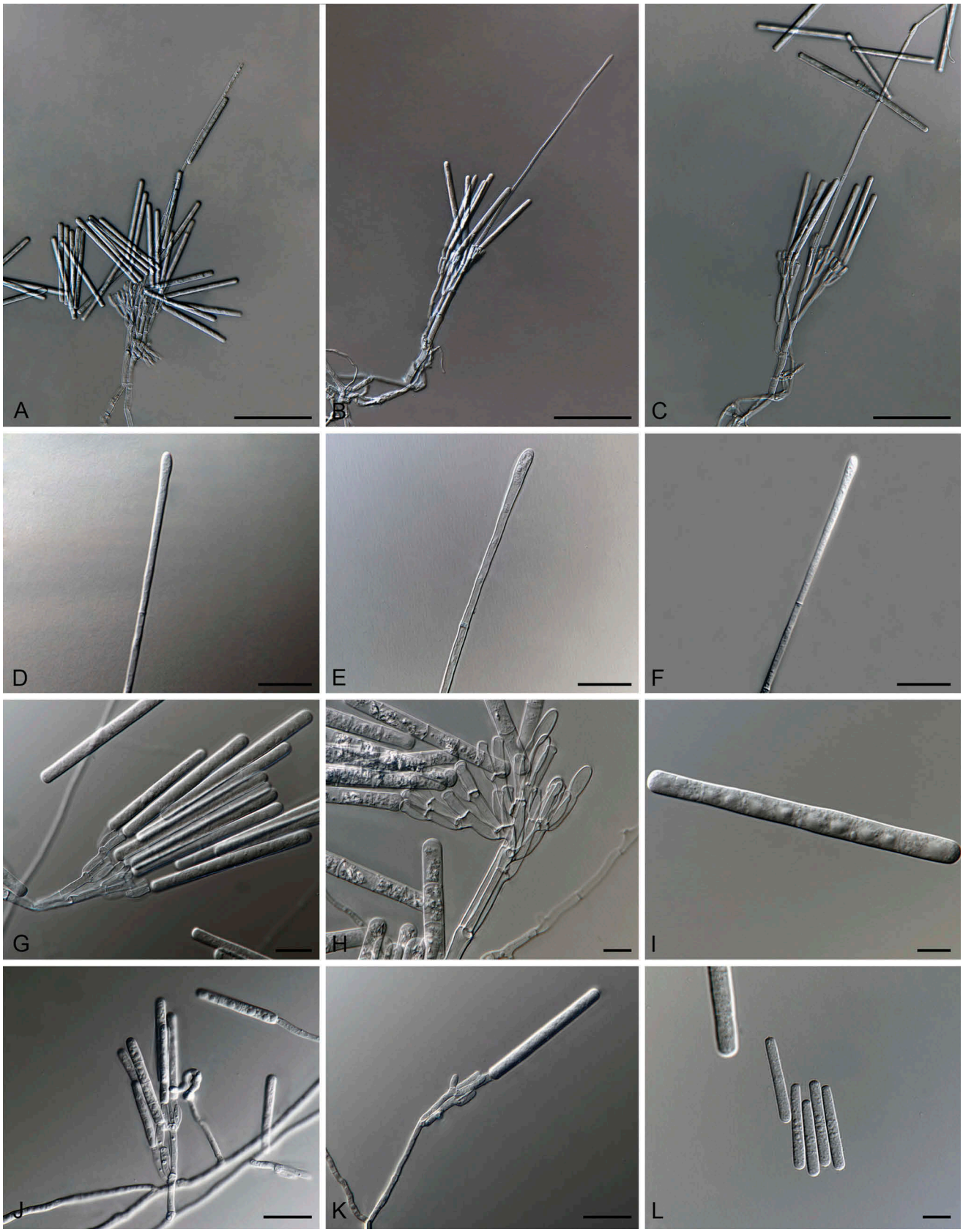

Figure 8. *Calonectria baviensis* (ex-type CBS 143563). A–C. Macroconidiophores. D–F. Narrowly clavate vesicles. G–H. Conidiogenous apparatus with conidiophore branches and cylindrical to allantoid phialides. I. Macroconidia. J–K. Microconidiophores. L. Microconidia. Bars: A–C = 100 µm; D–G = 20 µm; H–I = 10 µm; J–K = 20 µm; L = 10 µm.

phialides; phialides doliiform to reniform, hyaline, aseptate; apex with minute periclinal thickening and inconspicuous collarette. Macroconidia cylindrical, rounded at both ends, straight, 1-septate, lacking visible abscission scar, held in parallel cylindrical clusters by hyaline slime.

Calonectria aeknauliensis N.Q. Pham & M.J. Wingf., sp. nov. FIGS. 4E, 9
MycoBank MB825531

Typification: INDONESIA. NORTH SUMATRA: Aek Nauli, from soil in a *Eucalyptus* plantation, Feb 2013, *M.J. Wingfield* (**holotype** PREM 62107). Ex-type CBS 143559 = CMW 48253.

Etymology: The name refers to *Aek Nauli*, North Sumatra, Indonesia, where this fungus was first isolated.

Description: Sexual morph not observed. Macroconidiophores consisting of a stipe bearing a penicillate arrangement of fertile branches, stipe extension, and terminal vesicle; stipe extensions septate, straight to flexuous, 161–223 µm long, 3–6 µm wide at apical septum, terminating in sphaeropedunculate vesicle, 6–13 µm diam; lateral stipe extension (90° to main axis) rare. Conidiogenous apparatus consisting of up to 3 branches. Macroconidia cylindrical, rounded at both ends, straight, (38–)43–51(–56) × (4–)4.5–5.5 µm (av. = 47 × 5 µm), 1-septate. Mega- and microconidia not observed.

Culture characters: Colonies white to buff on the surface and sienna to umber in reverse on MEA after 7 d; moderate to abundant aerial mycelium with no sporulation on MEA and SNA; chlamydospores abundant, occurring throughout the medium, forming microsclerotia. Optimal growth temperature at 25 C, no growth at 10 and 35 C; after 7 d, colonies at 15 C reach 21.5 mm, at 20 C 37.9 mm, at 25 C 54.1 mm, and at 30 C 25.0 mm.

Other specimen examined: INDONESIA. NORTH SUMATRA: Aek Nauli, from soil in *Eucalyptus* plantation, Feb 2013, *M.J. Wingfield*, PREM 62108, culture CBS 143560 = CMW 48254.

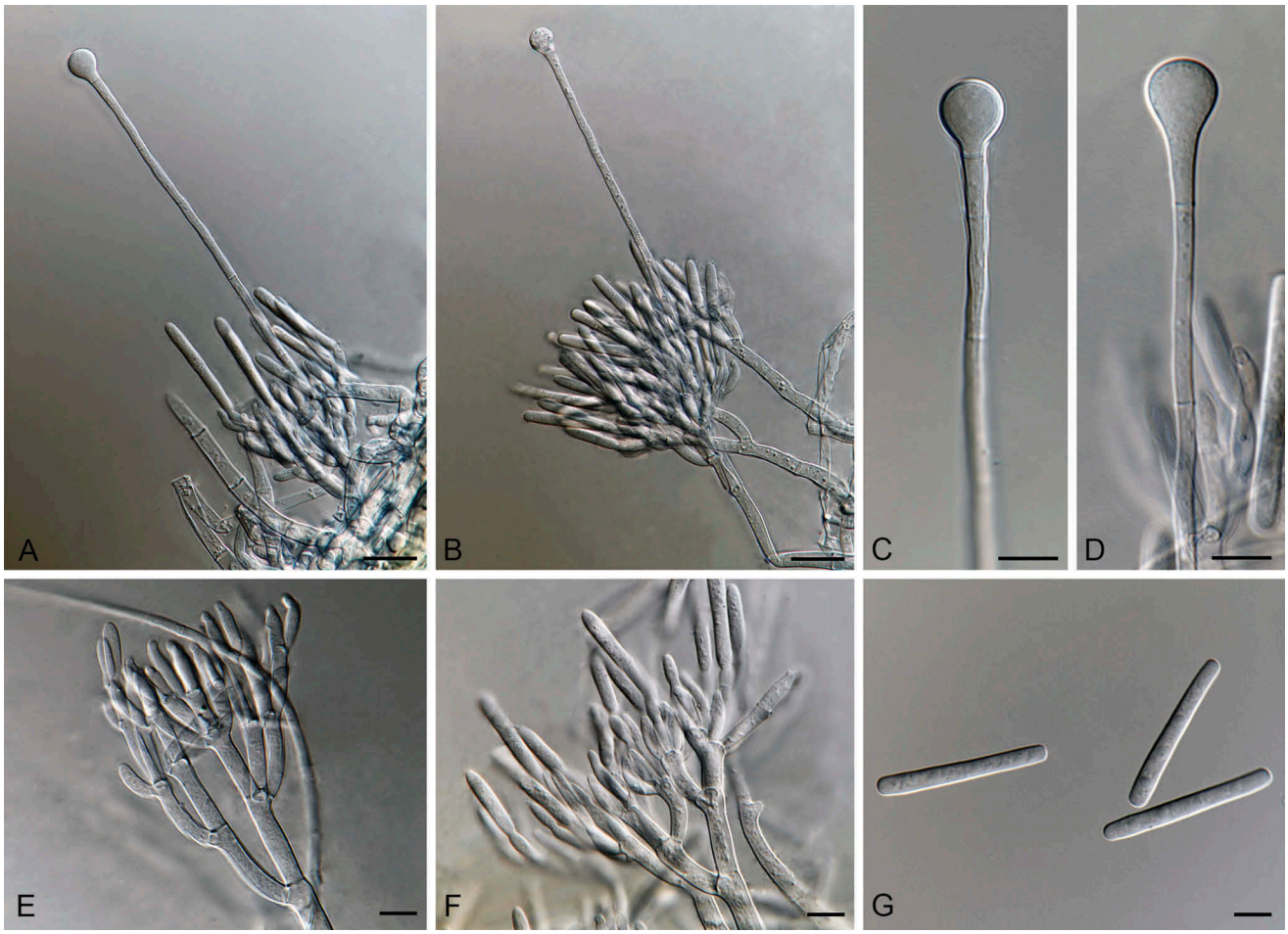

Figure 9. *Calonectria aeknauliensis* (ex-type CBS 143559). A–B. Macroconidiophores. C–D. Sphaeropedunculate vesicles. E–F. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. G. Macroconidia. Bars: A–B = 20 µm; C–G = 10 µm.

Notes: Calonectria aeknauliensis is a member of the *Ca. kyotensis* species complex. This species is closely related to *Ca. bumicola, Ca. curvispora, Ca. ilicicola, Ca. lantauensis*, and *Ca. vegrandis*. On average, macroconidia of *Ca. aeknauliensis* (av. = 47 × 5 µm) are larger than those of *Ca. vegrandis* (av. = 41 × 4.5 µm) and smaller than those of *Ca. curvispora* (av. = 60 × 5 µm), *Ca. ilicicola* (av. = 62 × 6 µm), and *Ca. lantauensis* (av. = 55 × 5 µm) (Crous et al. 2004; Li et al. 2017).

Calonectria bumicola N.Q. Pham & M.J. Wingf., sp. nov. FIG. 4F

MycoBank MB825532

Typification: INDONESIA. NORTH SUMATRA: Aek Nauli, from soil in *Eucalyptus* plantation, Feb 2013, *M.J. Wingfield* (**holotype** PREM 62123). Ex-type CBS 143575 = CMW 48257.

Etymology: Name refers to the Bahasa Indonesia word for soil ("bumi"), the substrate from which this fungus was first isolated.

Description: Asexual morph sporulation and sexual morph not observed. *Calonectria bumicola* can be distinguished from other closely related species by fixed nucleotides based on the alignments: *TEF1* positions 428 (C) and 462 (T); *HIS3* positions 267 (T) and 291 (G); *CMDA* positions 55 (–) and 423 (T).

Culture characters: Colonies white to buff to pale luteous on the surface and orange to sienna to umber in reverse on MEA after 7 d; extensive wooly aerial mycelium with no sporulation on MEA or SNA; chlamydospores abundant, occurring throughout the medium, forming microsclerotia. Optimal growth temperature at 25 C, no growth at 10 and 35 C; after 7 d, colonies at 15 C reach 21.3 mm, at 20 C 41.1 mm, at 25 C 61.9 mm, and at 30 C 39.6 mm.

Notes: All attempts to induce the asexual morph in *Ca. bumicola* failed. This included growing the fungus in the presence of *Medicago sativa* on SNA or on germinating *M. sativa* seeds on sterilized soil inoculated with *Ca. bumicola*.

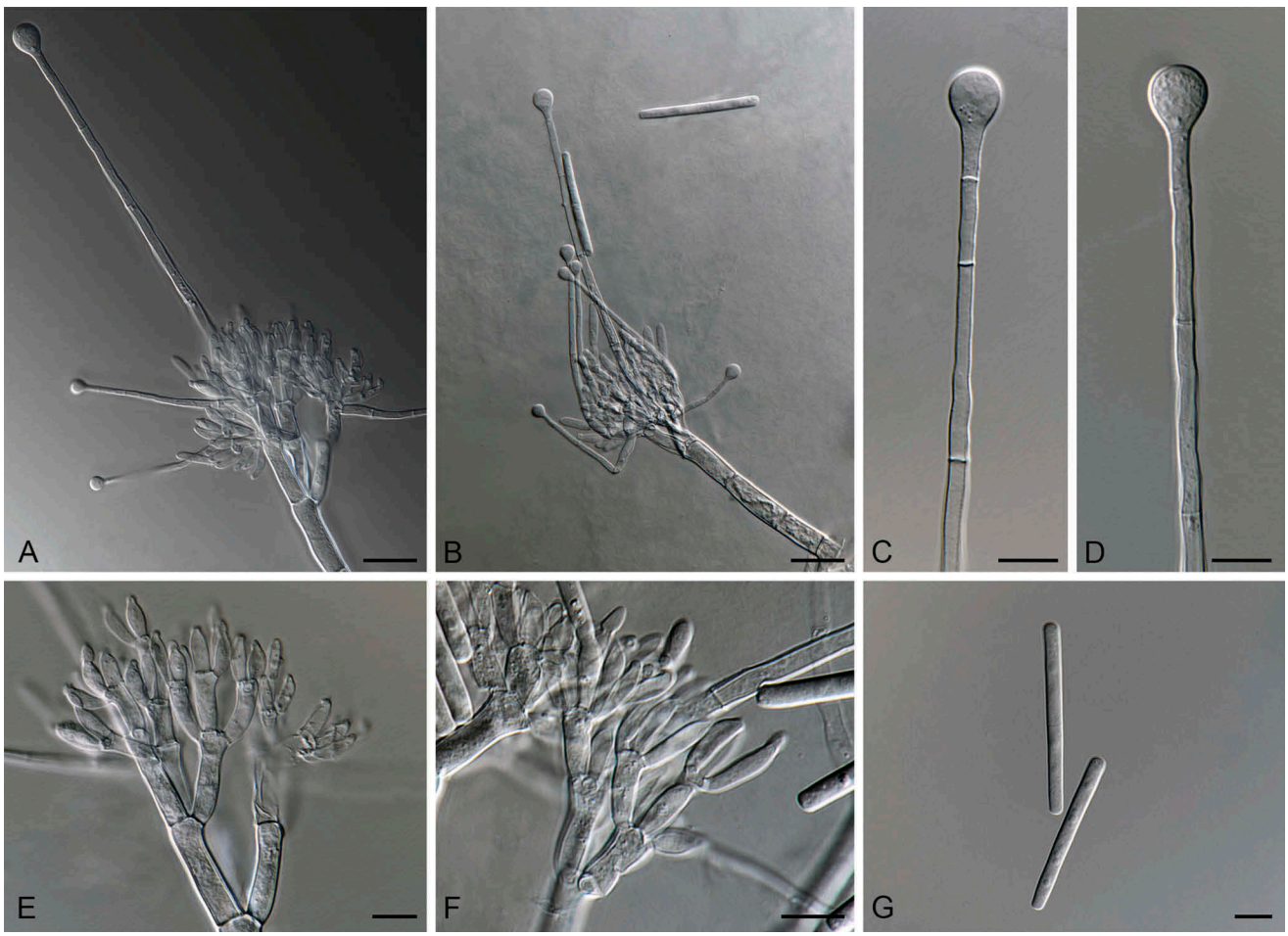

Figure 10. *Calonectria cochinchinensis* (ex-type CBS 143567). A–B. Macroconidiophores. C–D. Sphaeropedunculate vesicles. E–F. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. G. Macroconidia. Bars: A–B = 20 µm; C–G = 10 µm.

Calonectria cochinchinensis N.Q. Pham, T.Q. Pham & M.J. Wingf., sp. nov. FIGS. 4G, 10
MycoBank MB825533

Typification: VIETNAM. TAY NINH: Duong Minh Chau, from soil in *Hevea brasiliensis* plantation, Sep 2013, *N.Q. Pham, Q.N. Dang & T.Q. Pham* (**holotype** PREM 62115). Ex-type CBS 143567 = CMW 49915.

Etymology: The name refers to the former name of South Vietnam, "Cochinchina," where this fungus was first isolated.

Description: Sexual morph not observed. Macroconidiophores consisting of a stipe bearing a penicillate arrangement of fertile branches, stipe extension, and terminal vesicle; stipe extensions septate, straight to flexuous, 147–208 µm long, 2–3.5 µm wide at apical septum, terminating in sphaeropedunculate vesicle, 7–11 µm diam; lateral stipe extension (90° to main axis) abundant. Conidiogenous apparatus consisting of up to 3 branches. Macroconidia cylindrical, rounded at both ends, straight, (41.5–)44–48(–52) × 3–4(–5) µm (av. = 46 × 4 µm), 1-septate. Mega- and microconidia not observed.

Culture characters: Colonies white to salmon on the surface and flesh to sienna in reverse on MEA after 7 d; moderate aerial mycelium with sparse sporulation on the medium surface; chlamydospore moderate, occurring throughout the medium, forming microsclerotia. Optimal growth temperature at 25 C, no growth at 10 and 35 C; after 7 d, colonies at 15 C reach 18.7 mm, at 20 C 48.1 mm, at 25 C 67.7 mm, and at 30 C 61.1 mm.

Other specimens examined: VIETNAM. DONG NAI: Song May, from soil in *Acacia* hybrid plantation, Nov 2013, *N.Q. Pham & T.Q. Pham*, PREM 62116, culture CBS 143568 = CMW 47186; ibid., PREM 62117, culture CBS 143569 = CMW 47187.

Notes: Calonectria cochinchinensis is a member of the *Ca. kyotensis* species complex. This species is closely related to *Ca. chinensis, Ca. heveicola, Ca. indonesiae*, and *Ca. multistipitata*. Macroconidia of *Ca. cochinchinensis* (av. = 46 × 4 µm) are larger than those of *Ca. multistipitata* (av. = 32 × 3.5 µm) and smaller than those of *Ca. indonesiae* (av. = 50.5 × 4 µm), having a relatively similar size to *Ca. chinensis* (av. = 45 × 4 µm) and *Ca. heveicola* (av. = 44.5 × 4 µm). *Calonectria cochinchinensis* produces longer stipe extensions (147–208 µm) than those of *Ca. chinensis* (120–150 µm), *Ca. heveicola* (138–189 µm), *Ca. indonesiae* (110–130 µm), and *Ca. multistipitata* (100–171 µm) (Crous et al. 2004).

Calonectria heveicola N.Q. Pham, T.Q. Pham & M.J. Wingf., sp. nov. FIGS. 4H, 11
MycoBank MB825534

Typification: VIETNAM. BINH DUONG: Bau Bang, from soil in *Hevea brasiliensis* plantation, Sep 2013, *N. Q. Pham, Q.N. Dang & T.Q. Pham* (**holotype** PREM 62118). Ex-type CBS 143570 = CMW 49913.

Etymology: The name refers to the *Hevea* (rubber) plantations where this fungus was isolated.

Description: Ascomata perithecial, solitary or in groups, orange, becoming orange-brown with age; in section, apex and body orange, base red orange, subglobose to ovoid, 320–575 µm high, 270–470 µm diam, body turning red, and base dark red-brown (KOH+). Perithecial walls rough, consisting of two thick-walled layers; outside layer of textura globulosa, 38–85 µm wide, cells becoming more compressed towards inner layer of textura angularis, 11.5–28 µm wide, cells becoming thin walled and hyaline towards center; outer layer cells 14–52 × 11–37 µm, inner layer cells 7.5–23 × 3–6 µm; perithecial base up to 236.5 µm wide, consisting of dark red, angular cells, merging with an erumpent stroma; cells of outer wall layer continuing into pseudoparenchymatous cells of erumpent stroma. Asci 8-spored, clavate, 105–133.5 × 11–19.5 µm, tapering to a long thin stalk. Ascospores aggregate in upper third of ascus, hyaline, guttulate, fusoid with rounded ends, straight to curved, 1-septate, sometimes constricted at septum, (25–)30–39(–45) × (4–)4.5–5(–7) µm (av. = 34 × 5 µm). Heterothallic. Macroconidiophores consisting of a stipe bearing a penicillate arrangement of fertile branches, stipe extension, and terminal vesicle; stipe extensions septate, straight to flexuous, 138–189 µm long, 2–4 µm wide at apical septum, terminating in sphaeropedunculate vesicle, 7–10 µm diam; lateral stipe extension (90° to main axis) abundant. Conidiogenous apparatus consisting of up to 3 branches. Macroconidia cylindrical, rounded at both ends, straight, (37–)41–48(–50) × 3–4(–5) µm (av. = 44.5 × 4 µm), 1-septate. Mega- and microconidia not observed.

Culture characters: Colonies white to saffron on the surface and orange to sienna to umber in reverse on MEA after 7 d; moderate to extensive aerial mycelium with no sporulation on the medium surface, sparse sporulation on SNA; chlamydospore moderate, occurring throughout the medium, forming microsclerotia. Optimal growth temperature at 25 C, no growth at 10 and 35 C; after 7 d, colonies at 15 C reach 22.0 mm, at 20 C 45.0 mm, at 25 C 60.9 mm, and at 30 C 55.2 mm.

Other specimens examined: VIETNAM. BINH PHUOC: Bu Gia Map National Park, from soil in natural forest, Sep 2013, *N.Q. Pham, Q.N. Dang & T. Q. Pham*, PREM 62119, culture CBS 143571 = CMW

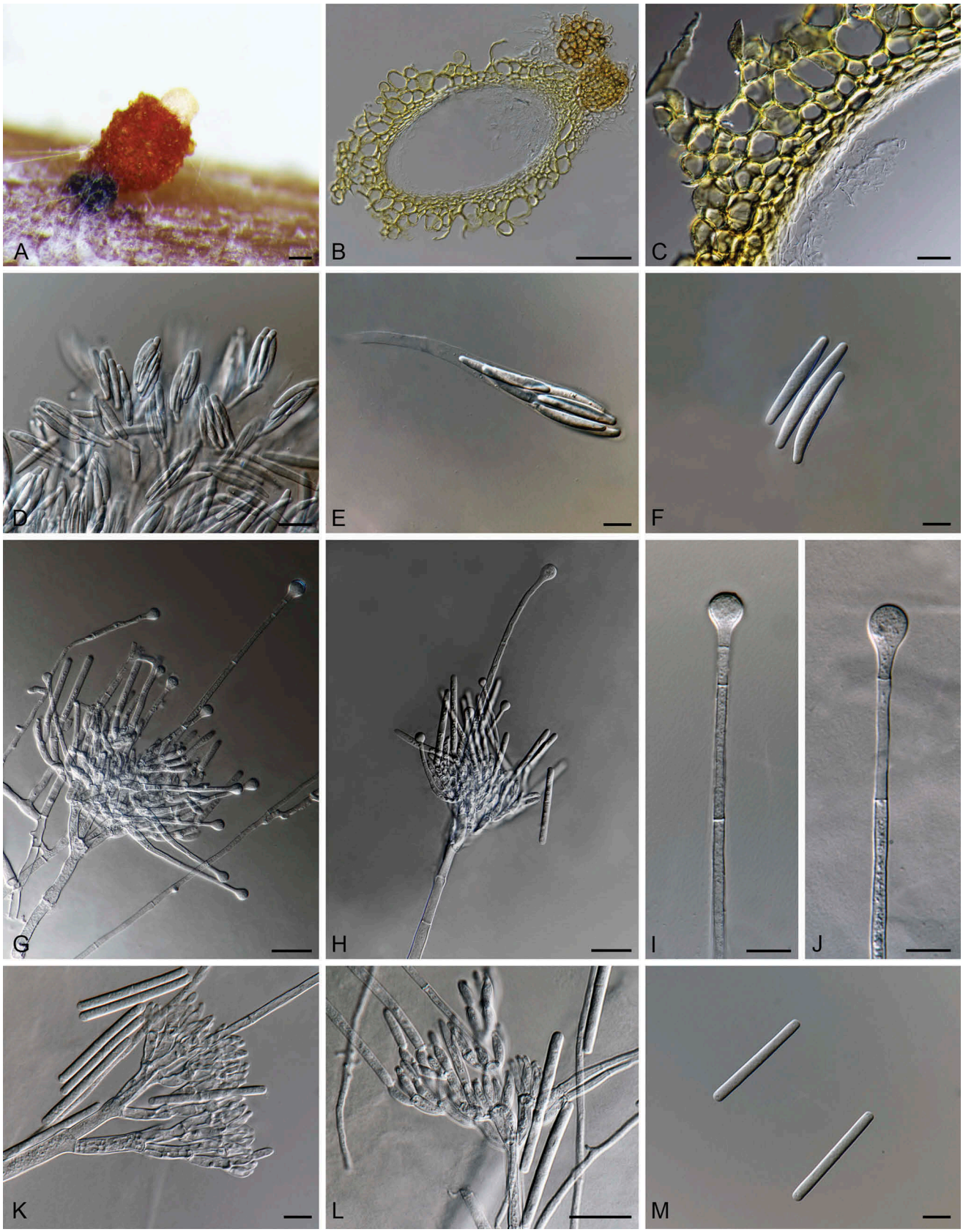

Figure 11. *Calonectria heveicola* (ex-type CBS 143570). A. Ascomata. B–C. Vertical section through ascomata, showing wall structure. D–E. Asci. F. Ascospores. G–H. Macroconidiophores. I–J. Sphaeropedunculate vesicle. K–L. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. M. Macrocondia. Bars: A–B = 100 µm; C–D = 20 µm; E–F = 10 µm; G–H = 20 µm; I–K = 10 µm; L = 20 µm; M = 10 µm.

49928; ibid., PREM 62120, culture CBS 143572 = CMW 49935.

Notes: Calonectria heveicola is a member of the *Ca. kyotensis* species complex. This species is closely related to *Ca. chinensis, Ca. cochinchinensis, Ca. indonesiae*, and *Ca. multistipitata*. Macroconidia of *Ca. heveicola* (av. = 44.5 × 4 µm) are larger than those of *Ca. multistipitata* (av. = 32 × 3.5 µm) and smaller than those of *Ca. indonesiae* (av. = 50.5 × 4 µm), having a relatively similar size to *Ca. chinensis* (av. = 45 × 4 µm) and *Ca. cochinchinensis* (av. = 46 × 4 µm). The stipe extensions in *Ca. heveicola* (138–189 µm) are shorter than those of *Ca. cochinchinensis* (147–208 µm) and longer than those of *Ca. chinensis* (120–150 µm), *Ca. indonesiae* (110–160 µm), and *Ca. multistipitata* (100–171 µm) (Crous et al. 2004).

Calonectria multistipitata N.Q. Pham, T.Q. Pham & M.J. Wingf., sp. nov. FIGS. 4I, 12
MycoBank MB825535

Typification: VIETNAM. TUYEN QUANG: From soil in *Acacia* hybrid plantation, Nov 2013, *N.Q. Pham & T.Q. Pham* (**holotype** PREM 62121). Ex-type CBS 143573 = CMW 47192.

Etymology: "multis" (Latin) = many + "stipitate" (Latin) = stipes, referring to the multiple lateral stipe extensions on the macroconidiophores of this fungus.

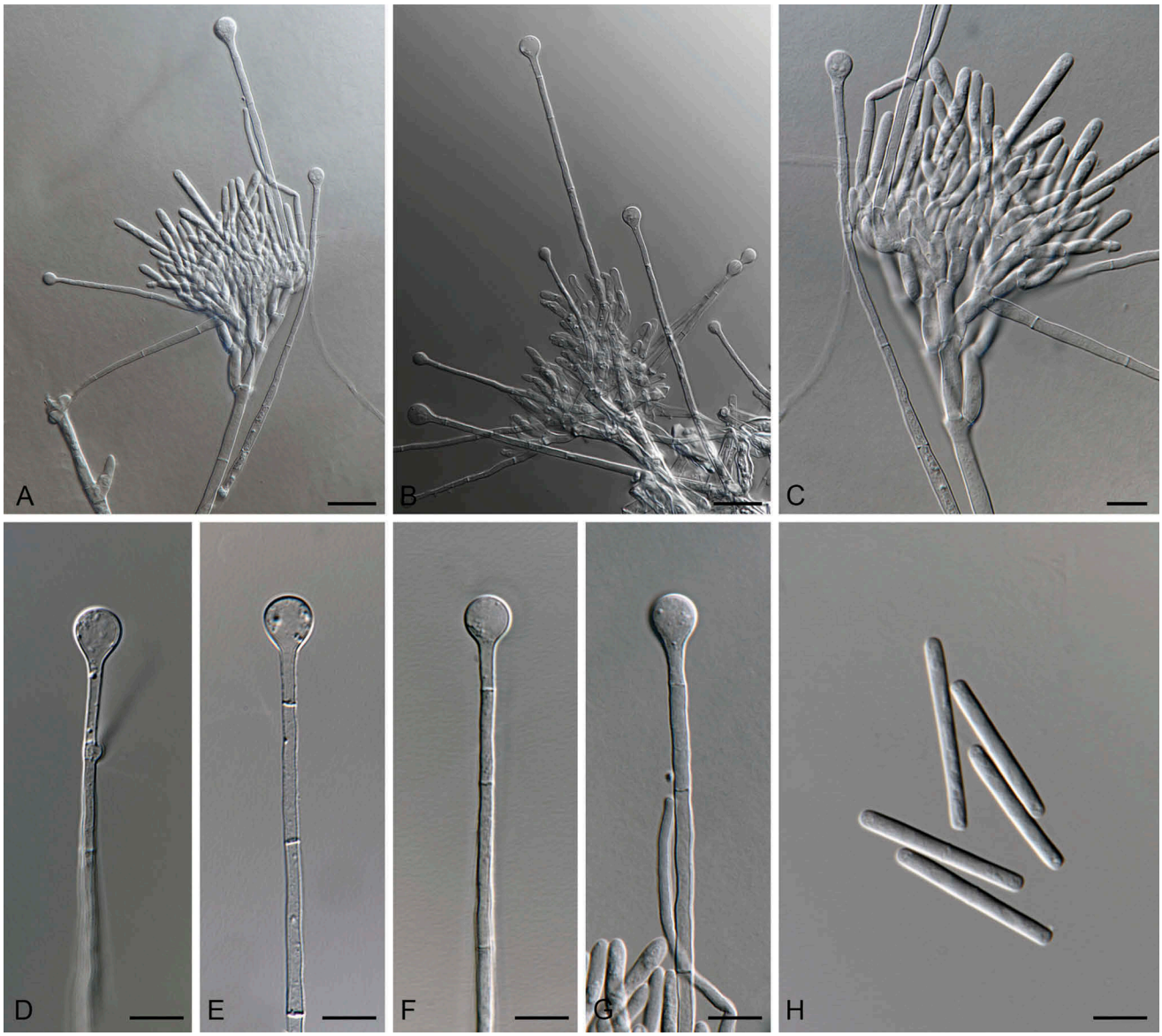

Figure 12. *Calonectria multistipitata* (ex-type CBS 143573). A–B. Macroconidiophores. C. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. D–G. Sphaeropedunculate vesicles. H. Macroconidia. Bars: A–B = 20 µm; C–H = 10 µm.

Description: Sexual morph not observed. Macroconidiophores consisting of a stipe bearing a penicillate arrangement of fertile branches, stipe extension, and terminal vesicle; stipe extensions septate, straight to flexuous, 100–171 µm long, 2–3 µm wide at apical septum, terminating in sphaeropedunculate vesicle, 5–10 µm diam; lateral stipe extension (90° to main axis) abundant. Conidiogenous apparatus consisting of up to 7 branches. Macroconidia cylindrical, rounded at both ends, straight, (28–)30–34(–36) × 3–4 µm (av. = 32 × 3.5 µm), 1-septate. Mega- and microconidia not observed.

Culture characters: Colonies white to sienna on the surface and saffron to sienna to umber in reverse on MEA after 7 d; moderate to extensive aerial mycelium with moderate sporulation on the medium surface and aerial mycelium in the outer regions; chlamydospore abundant, occurring throughout the medium, forming microsclerotia. Optimal growth temperature at 30 C, no growth at 10 and 35 C; after 7 d, colonies at 15 C reach 17.9 mm, at 20 C 38.7 mm, at 25 C 54.3 mm, and at 30 C 56.8 mm.

Other specimen examined: VIETNAM. TUYEN QUANG: From soil in *Acacia* hybrid plantation, Nov 2013, *N.Q. Pham & T.Q. Pham*, PREM 62122, culture CBS 143674 = CMW 47211.

Notes: Calonectria multistipitata is a member of the *Ca. kyotensis* species complex. This species is closely related to *Ca. chinensis, Ca. cochinchinensis, Ca. heveicola*, and *Ca. indonesiae. Calonectria multistipitata* can be distinguished from the other species based on the number of branches of the conidiogenous apparatus where *Ca. multistipitata* has up to 7 branches, *Ca. indonesiae* has up to 5, and *Ca. chinensis, Ca. cochinchinensis*, and *Ca. heveicola* have up to 3 each. Macroconidia of *Ca. multistipitata* (av. = 32 × 3.5 µm) are shorter than those of *Ca. chinensis* (av. = 45 × 4 µm), *Ca. cochinchinensis* (av. = 46 × 4 µm), *Ca. indonesiae* (av. = 50.5 × 4 µm), and *Ca. heveicola* (av. = 44.5 × 4 µm) (Crous et al. 2004).

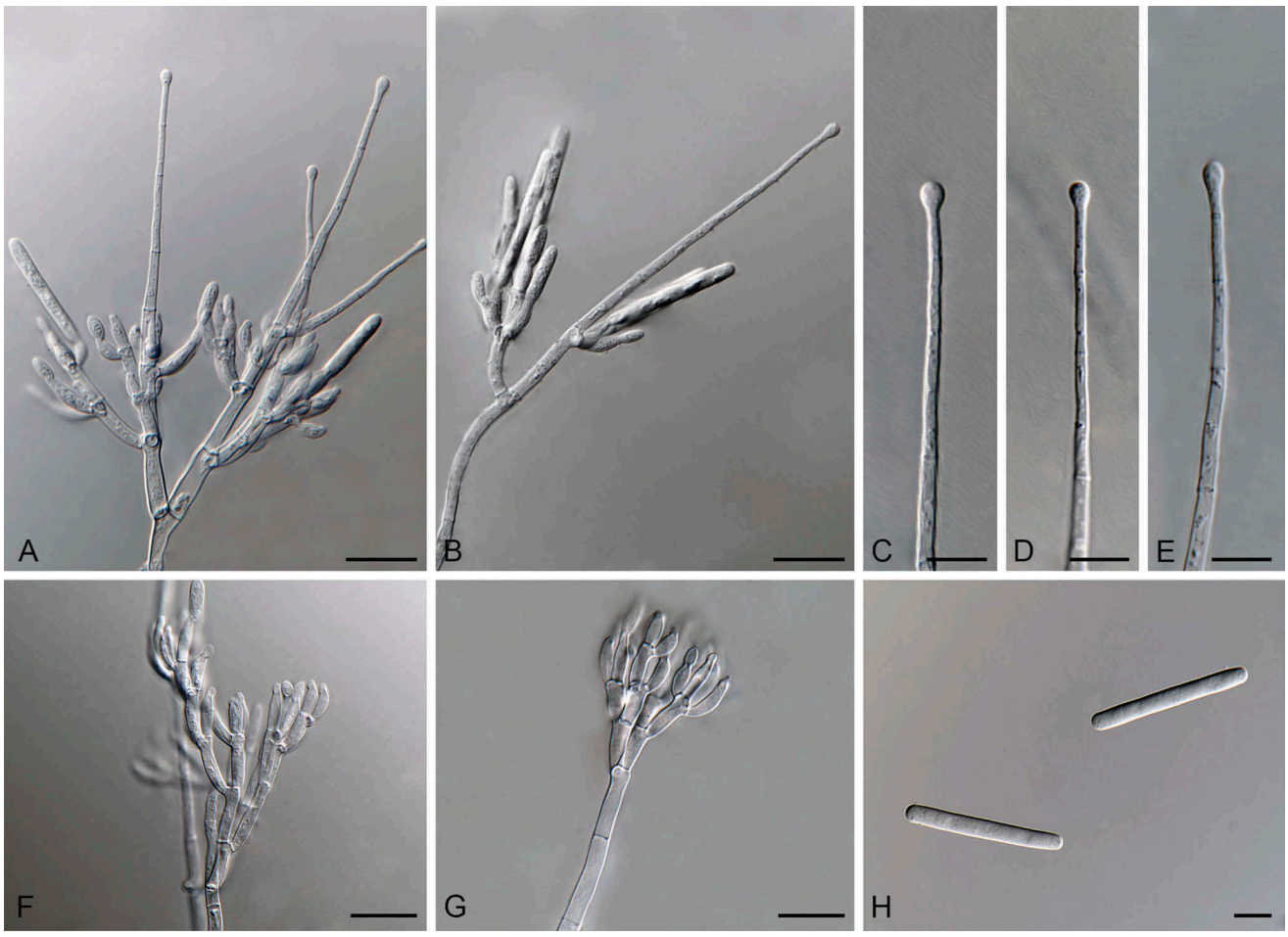

Figure 13. *Calonectria vegrandis* (ex-type CBS 143565). A–B. Macroconidiophores. C–E. Sphaeropedunculate vesicles. F–G. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. H. Macroconidia. Bars: A–B = 20 µm; C–E = 10 µm; F–G = 20 µm; H = 10 µm.

Calonectria vegrandis N.Q. Pham & M.J. Wingf., sp. nov. FIGS. 4J, 13

MycoBank MB825536

Typification: INDONESIA. NORTH SUMATRA: Aek Nauli, from soil in *Eucalyptus* plantation, Feb 2013, *M.J. Wingfield* (**holotype** PREM 62113). Ex-type CBS 143565 = CMW 48245.

Etymology: "ve-" (Latin) = not, less + "grandis" (Latin) = large, referring to the small stipe extension and terminating vesicles in this fungus.

Description: Sexual morph not observed. Macroconidiophores consisting of a stipe bearing a penicillate arrangement of fertile branches, stipe extension, and terminal vesicle; stipe extensions septate, straight to flexuous, 73–95 µm long, 1–2 µm wide at apical septum, terminating in sphaeropedunculate vesicle, 2–4 µm diam; lateral stipe extension (90° to main axis) moderate. Conidiogenous apparatus consisting of up to 6 branches. Macroconidia cylindrical, rounded at both ends, straight, (35–)38–44(–48) × 4–5 µm (av. = 41 × 4.5 µm), 1-septate. Mega- and microconidia not observed.

Culture characters: Colonies white to buff on the surface and ochreous to umber in reverse on MEA after 7 d; abundant aerial mycelium with no sporulation on MEA and SNA; chlamydospores abundant, occurring throughout the medium, forming microsclerotia. Optimal growth temperature at 25 C, no growth at 10 and 35 C; after 7 d, colonies at 15 C reach 19.0 mm, at 20 C 37.2 mm, at 25 C 50.8 mm, and at 30 C 32.1 mm.

Other specimen examined: INDONESIA. NORTH SUMATRA: Aek Nauli, from soil in *Eucalyptus* plantation, Feb 2013, *M.J. Wingfield*, PREM 62114, culture CBS 143566 = CMW 48246.

Notes: Calonectria vegrandis is a member of the *Ca. kyotensis* species complex. This species is closely related to *Ca. aeknauliensis, Ca. bumicola, Ca. curvispora, Ca. ilicicola*, and *Ca. lantauensis*. Macroconidia of *Ca. vegrandis* (av. = 41 × 4.5 µm) are smaller than those of *Ca. aeknauliensis* (av. = 47 × 5 µm), *Ca. curvispora* (av. = 60 × 5 µm), *Ca. ilicicola* (av. = 62 × 6 µm), and *Ca. lantauensis* (av. = 55 × 5 µm). Stipe extensions in *Ca. vegrandis* (73–95 µm) are shorter than those of *Ca. aeknauliensis* (161–223 µm), *Ca. curvispora* (110–150 µm), *Ca. ilicicola* (120–140 µm), and *Ca. lantauensis* (51–271 µm) (Crous 2002; Li et al. 2017). Macroconidiophores and macroconidia are formed only in SNA in the presence of *M. sativa* seedlings in the medium after 5–7 d.

DISCUSSION

This study represents the first exploration of *Calonectria* spp. from Vietnam including sequence data and phylogenetic inference. DNA sequence analyses have revolutionized the taxonomy of *Calonectria*, including revisions of the genus and substantially increasing the number of described species (Lombard et al. 2010b, 2010c, 2015a, 2016; Alfenas et al. 2015; Li et al. 2017). The present study revealed 15 *Calonectria* spp. collected from soils and plant material from numerous plantations, nurseries, and natural forests across Vietnam and a part of Indonesia. It includes the description of three novel species (*Ca. aeknauliensis, Ca. bumicola*, and *Ca. vegrandis*) from Indonesia and seven from Vietnam (*Ca. acaciicola, Ca. auriculiformis, Ca. baviensis, Ca. cochinchinensis, Ca. heveicola, Ca. multistipitata*, and *Ca. tonkinensis*) and the first report of five known species (*Ca. ilicicola, Ca. hongkongensis, Ca. lateralis, Ca. malesiana*, and *Ca. yunnanensis*) from Vietnam. Of these, only *Ca. baviensis* was collected from symptomatic plant tissue; the remaining species were baited from soil samples.

Calonectria is represented by 13 species complexes in two major phylogenetic groups (Lombard et al. 2010c). These are the Prolate group comprising *Calonectria* spp. that have clavate to pyriform to ellipsoidal terminal vesicles and the Sphaero-Naviculate group characterized by sphaeropedunculate and naviculate terminal vesicles (Lombard et al. 2010c). Four new species in the Prolate group and six new species in the Sphaero-Naviculate group were described. The Prolate group includes the majority of plant pathogenic *Calonectria* spp. and is reported in many parts of the world. In contrast, species of the Sphaero-Naviculate group show the highest species diversity in Asia (Crous et al. 2004; Lombard et al. 2010a, 2015a; Li et al. 2017), as also seen in the present study.

No clear patterns of host specificity or particular ecological adaptation emerged from the species complexes in this study. However, most of the newly described species expressed the morphological characteristics that define the two major groups. Although species can be assigned relatively easily to the complexes in which they occur based on morphological characters, phylogenetic inference based on multigene DNA sequence comparisons is required to provide accurate phylogenetic relationships of species within these complexes. This is a consequence of infraspecific variation in conidial dimensions and vesicle shapes in some species, reducing their reliability as diagnostic characters, as highlighted by Lombard et al. (2010a, 2010b, 2010c).

The descriptions of *Ca. acaciicola* and *Ca. baviensis* contribute two novel species to the *Ca. reteaudii* complex, where narrowly clavate to clavate terminal vesicles is a common morphological character (Crous et al.

2012; Lombard et al. 2010d, 2015a). *Calonectria acaciicola* was obtained from soil collected in *A. auriculiformis* plantations in Do Luong, Nghe An Province, whereas *Ca. baviensis* was the only new species in this study obtained from the symptomatic leaves of *Eucalyptus* spp. These two new species bring the number of species in the *Ca. reteaudii* complex discovered in Vietnam to a total of four. Species in the *Ca. reteaudii* complex are important causal agents of CLB on *Eucalyptus* spp. in tropical regions of Australia, India, China, Southeast Asia, and South America (Pitkethley 1976; Sharma and Mohanan 1982; Old et al. 2003; Rodas et al. 2005; Lombard et al. 2015a). The fact that *Ca. baviensis* was isolated from *E. urophylla* and *E. pellita* leaves displaying CLB symptoms suggests that this species could present a risk to *Eucalyptus* plantations in Vietnam.

Two new species, *Ca. auriculiformis* and *Ca. tonkinensis*, belong to the *Ca. cylindrospora* complex. This complex is thus expanded to 19 species (Alfenas et al. 2015; Lombard et al. 2015a, 2016), all of which have 1-septate macroconidia with stipe extensions terminating in ellipsoidal to ovoid or obpyriform to pyriform or clavate vesicles (Lombard et al. 2010c). Both *Ca. auriculiformis* and *Ca. tonkinensis* were baited from soil samples. Although some species in this complex cause leaf spot symptoms on species of *Acacia* and *Eucalyptus* in Indonesia and Vietnam (Schoch et al. 1999; Crous et al. 2002), nothing is known about the pathogenicity of the two newly described species.

Species in the *Ca. kyotensis* complex are characterized by sphaeropedunculate terminal vesicles with lateral stipe extensions on the conidiogenous apparatus (Crous et al. 2004; Lombard et al. 2015a). Members of this complex have commonly been isolated from soil and debris (Crous et al. 2004; Lombard et al. 2015a; Li et al. 2017). This study included the description of six new species, *Ca. aeknauliensis, Ca. bumicola, Ca. cochinchinensis, Ca. heveicola, Ca. multistipitata*, and *Ca. vegrandis*, and the isolation of five previously described species, *Ca. ilicicola, Ca. hongkongensis, Ca. lateralis, Ca. malesiana*, and *Ca. yunnanensis* in the *Ca. kyotensis* complex. These results support the fact that the highest species diversity of this species complex appears to be in Southeast Asia (Crous et al. 2004; Lombard et al. 2015a; Li et al. 2017). Among the species mentioned above, *Ca. ilicicola* is one of the most important *Calonectria* pathogens, causing black rot and red crown rot of peanut and soybean in many parts of the world (Bell and Sobers 1966; Crous 2002; Pan et al. 2012). In Brazil, it was also the causal agent of leaf blight and damping-off of *E. grandis*, resulting in severe defoliation (Alfenas and Ferreura 1979; Alfenas et al. 1979). The fact that *Ca. ilicicola* was first isolated from soil from *Eucalyptus* plantations in Yen Bai Province could highlight a potential danger to *Eucalyptus* plantation forestry.

Most of the novel taxa described here were from soils, with only one species originating from infected plant tissues. This is consistent with the known ecology of *Calonectria* spp. and the results of previous studies (Alfenas et al. 2015; Lombard et al. 2015a; Li et al. 2017). Although many different species can be found in soils in one location, relatively few occur on plant leaves causing disease (Crous 2002; Alfenas et al. 2013a, 2015; Lombard et al. 2015a). It remains unknown why some species in soil have the capacity to infect leaves, and this is a topic that deserves further study.

The majority of *Calonectria* species encountered in this, and previous, studies are known only from baited soils and thus cannot be associated with disease symptoms. The fact that they are isolated from freshly germinating alfalfa seedlings used for baiting implies some level of pathogenicity. Yet, little is known regarding host specificity in *Calonectria* spp., and it is possible that in the presence of susceptible hosts, most would cause disease. This is a situation similar to many canker pathogens of trees such as those classified in the Cryphonectriaceae and Botryosphaeriaceae (Wingfield 2003; Slippers and Wingfield 2007; Burgess and Wingfield 2016). Species in these families occur naturally as endophytes in natural forest ecosystems in the absence of disease symptoms, but when exposed to susceptible hosts in areas having environmental conditions conducive to infection, serious disease problems can emerge. Clearly a much deeper understanding of the biology and particularly host range is needed for species of *Calonectria*.

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