Diversity and potential impact of *Calonectria* species in *Eucalyptus* plantations in Brazil

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Abstract: Species in the genus *Calonectria* (*Hypocreales*) represent an important group of plant pathogenic fungi that cause serious losses to plant crops in tropical and subtropical climates. Calonectria leaf blight is currently one of the main impediments to *Eucalyptus* cultivation in Brazil, and various species of *Calonectria* have been associated with this disease. Since most previous identifications were solely based on morphological characters, much of the published literature needs to be re-evaluated. The aim of this study was thus to identify and determine the phylogenetic relationships among species that occur in the *Eucalyptus* growing regions of Brazil by using partial sequences of the β-tubulin, calmodulin, translation elongation factor 1-α and histone H3 gene regions. Based on extensive collections from soil and infected eucalypt leaf samples from plantations, phylogenetic inference revealed the *Ca. pteridis* complex to be the most common species complex present in *Eucalyptus* plantations in Brazil. By elucidating taxa in the *Ca. pteridis*, *Ca. cylindrospora* and *Ca. candelabra* species complexes, 20 novel *Calonectria* species were identified, and a new name in *Calonectria* provided for *Cylindrocladium macrosporum* as *Ca. pseudopteridis*.

Key words: Cylindrocladium, Calonectria leaf blight, Damping-off, Diversity, Taxonomy.

Taxonomic novelties: New species: *Calonectria brassiana* R.F. Alfenas, L. Lombard & Crous, *Ca. duoramosa* R.F. Alfenas, L. Lombard & Crous, *Ca. eucalypticola* R.F. Alfenas, L. Lombard & Crous, *Ca. glaebicola* R.F. Alfenas, L. Lombard & Crous, *Ca. maranhensis* R.F. Alfenas, L. Lombard & Crous, *Ca. metrosideri* R.F. Alfenas, O.L. Pereira, Crous & A.C. Alfenas, *Ca. multinaviculata* R.F. Alfenas, L. Lombard & Crous, *Ca. nemuricola* R.F. Alfenas, L. Lombard & Crous, *Ca. paraensis* R.F. Alfenas, L. Lombard & Crous, *Ca. piauiensis* R.F. Alfenas, L. Lombard & Crous, *Ca. propaginicola* R.F. Alfenas, L. Lombard & Crous, *Ca. pseudobrassicae* R.F. Alfenas, L. Lombard & Crous, *Ca. pseudocerciana* R.F. Alfenas, L. Lombard & Crous, *Ca. pseudohodgesii* R.F. Alfenas, L. Lombard & Crous, *Ca. pseudometrosideri* R.F. Alfenas, L. Lombard & Crous, *Ca. pseudospathulata* R.F. Alfenas, L. Lombard & Crous, *Ca. pseudovata* R.F. Alfenas, L. Lombard & Crous, *Ca. quinqueramosa* R.F. Alfenas, L. Lombard & Crous, *Ca. robigophila* R.F. Alfenas, L. Lombard & Crous, *Ca. silvicola* R.F. Alfenas, L. Lombard & Crous, *Ca. telluricola* R.F. Alfenas, L. Lombard & Crous; **New name:** *Ca. pseudopteridis* (Sherb.) R.F. Alfenas, L. Lombard & Crous.

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INTRODUCTION

Calonectria species (asexual morph previously known as *Cylindrocladium* (*Cy.*)) are widely distributed around the world and cause diseases on a broad range of host plants in tropical and subtropical climates (Crous 2002, Lombard *et al.* 2010a, Vitale *et al.* 2013). In Brazil, *Calonectria* species have been reported as pathogens of numerous important agronomic crops, such as potatoes (*Solanum tuberosum*; Dianese *et al.* 1986), soybeans (*Glycine max*; Bolkan *et al.* 1980), acerola (*Malpighia glabra*; Silva *et al.* 2001), mango (*Mangifera indica*; Tozetto & Ribeiro 1996), *Eugenia* spp. (Poltronieri *et al.* 2011), and several ornamentals (Reis *et al.* 2004). Thus far however, the majority of the reports from Brazil focused on forestry crops, such as *Pinus* and *Acacia* (Hodges & May 1972, Hodges *et al.* 1973, Alfenas 1986, Dianese *et al.* 1986, Novaes *et al.* 2012, Alfenas *et al.* 2013a,b) and in particular on the epidemiology and disease control of *Calonectria* spp. associated with diseases of *Eucalyptus* in commercial plantations and nurseries (Blum *et al.* 1992, Mafia *et al.* 2008, 2009, Graça *et al.* 2009, Ferreira *et al.* 2012, Alfenas *et al.* 2013c).

Based on the increasing global market for paper and wood pulp, and renewable energy, commercial *Eucalyptus* plantations in Brazil have expanded towards the warm and humid regions of northern and north-eastern Brazil where Calonectria leaf blight (CLB) has become the primary fungal leaf disease of this crop (Alfenas *et al.* 2009, 2013c). Other prominent diseases associated with *Calonectria* species on *Eucalyptus* in Brazil include damping-off, cutting rot and root rot (Alfenas & Ferreira 1979, Alfenas *et al.* 1979, Alfenas 1986, Alfenas *et al.* 2009). Calonectria leaf blight was first observed in commercial plantation trees of *E. grandis* in 1970, with more than 80 % of the trees showing severe defoliation (Alfenas & Ferreira 1979). Three *Calonectria* species were identified as the causal agents, which included *Calonectria cylindrospora* (= *Cylindrocladium scoparium;* see Lombard *et al.* 2015a, 2015b), *Ca. ilicicola* (= *Cy. parasiticum*) and *Ca. pyrochroa* (= *Cy. ilicicola*). Additional species also reported to cause CLB and damping-off of *Eucalyptus* in Brazil include *Ca. ovata* (= *Cy. ovatum*), *Ca. candelabra* (= *Cy. candelabrum*; see Lombard *et al.* 2015a, 2015b), and *Ca. brassicae* (= *Cy. gracile*) (Alfenas *et al.* 1979, Almeida & Bolkan 1981, Alfenas, 1986, El-Gholl *et al.* 1993, Crous *et al.* 1998). However, these *Calonectria* species have been identified based solely on morphological characters of the asexual morphs (conidial dimensions and vesicle shape; Alfenas 1986, Ferreira 1989, Alfenas *et al.* 2009), which could have resulted in incorrect identifications.

In the 1990's, *Eucalyptus* leaf blight and defoliation caused by *Ca. pteridis* (= *Cy. pteridis*), in south-eastern Bahia and Pará

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provinces resulted in severe defoliation of *E. grandis* trees in these regions (Ferreira *et al.* 1995). Since then, *Ca. pteridis* has become the most common species reported from commercial plantations, primarily on *E. camaldulensis*, *E. cloeziana*, *E. grandis*, *E. saligna*, *E. tereticornis*, *E. urophylla* and hybrid *E. grandis* × *E. urophylla* (Alfenas *et al.* 2009). For most *Eucalyptus* species, the disease is characterised by spots that are initially small, circular or elongated and pale grey to pale brown, progressing and extending throughout the leaf blade, resulting in leaf drop and in some cases severe defoliation (Alfenas & Ferreira 1979, Alfenas *et al.* 1979). It is believed that defoliation caused by CLB decreases timber volume due to the reduced photosynthetic area (Ferreira *et al.* 1995, Berger *et al.* 2007, Alfenas *et al.* 2009) and that weed growth is promoted due to light in the understory, which further subjects the trees to competition from weeds (Alfenas *et al.* 2009).

Planting of resistant genotypes is the most effective and economical method to control this disease in the field (Alfenas *et al.* 2009, Fonseca *et al.* 2010). However, selecting resistant genotypes has proven difficult since several *Calonectria* species appear to be associated with CLB. Pathogenicity trials done by Rehn *et al.* (2004) showed that several *Calonectria* species isolated from soil can be highly aggressive to *Eucalyptus*, but hardly any information is presently available on the diversity of *Calonectria* species occurring in soil in eucalypt plantations in Brazil.

Although morphological characters provide valuable information for species discrimination in *Calonectria*, incorporation of a polyphasic identification process with multi-gene DNA sequence data has elucidated various previously unknown species complexes (Crous *et al.* 2004b, 2006, Lombard *et al.* 2010b,c, Chen *et al.* 2011, Lombard *et al.* 2015a, 2015b). For some of these species complexes, cryptic members can only be accurately identified on the basis of DNA sequence data. Except for a few recent studies (Alfenas *et al.* 2013a,b), most previous reports of *Calonectria* species in Brazil need to be re-evaluated. Therefore, the aims of the present study were to conduct extensive surveys of soils and trees in various commercial *Eucalyptus* plantations in Brazil, cultivate as many isolates as possible, and subject them to DNA sequence analyses, to determine which morphological groups are dominant, and establish their distribution in Brazil.

MATERIAL AND METHODS

Sampling and isolation

Samples of *Eucalyptus* leaves showing characteristic symptoms of CLB were collected in the main eucalypt growing regions of Brazil. Since the clonal plantations are established in different Management Operational Units (MOU), according to the characteristics of the soil and climatic conditions, a sample of 30 leaves per infected clone/species was collected. A random soil sample (400 g in the 0–20 cm layer) was also collected for each MOU and another from the surrounding native vegetation (Table 1). Additionally, diseased *Azadirachta indica* and *Eucalyptus* cuttings were collected from nurseries in the states of Minas Gerais (Viçosa) and Pará (Santana). The symptomatic plant material were kept in paper bags and the soil samples in plastic bags and transported to the Forest Pathology Laboratory/Bioagro of the Universidade Federal de Viçosa. All the collected plant materials were incubated in moist chambers at room temperature (25 °C ± 3 °C) for up to 14 d and inspected daily for fungal sporulation. The collected soil samples were baited with mature leaf discs of castor bean (*Ricinus communis*) and eucalypt twig segments as described by Gonçalves *et al.* (2001). Direct isolations were made onto malt extract agar (2 % w/v; MEA; Vetec, Brazil) and incubated for 7 d at 25 °C under continuous near-ultraviolet light. From these primary isolations, single conidial cultures were prepared on MEA and deposited in the culture collection of the CBS-KNAW Fungal Biodiversity Centre (CBS), Utrecht, The Netherlands, the working collections of Pedro W. Crous (CPC) maintained at CBS, and Acelino C. Alfenas (LPF) maintained at the Forest Pathology Laboratory/Bioagro, Universidade Federal de Viçosa, Brazil.

DNA sequencing and phylogenetic analyses

Genomic DNA was isolated from 7-d-old fungal mycelium grown on MEA following the protocol of the Wizard® Genomic DNA Purification (Promega Corporation, WI, USA) kit. For amplification of gene regions, the DreamTaq™ Master Mix (MBI Fermentas, Vilnius, Lithuania) was used, following the manufacturer's protocol. Initially, partial gene sequences of the translation elongation factor 1-α (*tef1*) were determined for all isolates collected using the primers EF1-728F (O'Donnell *et al.* 1998) and EF-2 (Carbone & Kohn 1999) following the protocol and conditions outlined by Crous *et al.* (2004b). Subsequently, partial fragments of β-tubulin (*tub2*), calmodulin (*cmdA*) and histone 3 (*his3*), were determined following the protocols and primers outlined by Crous *et al.* (2004b) and Groenewald *et al.* (2013). DNA sequencing reactions were performed using the BigDye® Terminator Cycle Sequencing Kit v. 3.1 (Applied Biosystems Life Technologies, Carlsbad, CA, USA) following the protocol provided by the manufacturer. To ensure the integrity of the sequences, amplicons were sequenced in both directions using the same primers used for amplification. Purified sequence reactions were run on an ABI Prism 3730xl DNA Sequencer (Life Technologies, Carlsbad, CA, USA). The quality of the electropherograms generated were evaluated using Sequence Scanner Software v. 1.0 (Applied Biosystems) and PHPH (http://www.biomol.unb.br/phph/). Consensus sequences were determined using Seqman (DNAStar Inc., Madison, Wisconsin, USA). All sequences were manually corrected and the arrangement of nucleotides in ambiguous positions was corrected using comparisons of the sequences generated from both the forward and reverse primers. In addition to the sequences generated in this study, other sequences were obtained from NCBI's GenBank nucleotide database (www.ncbi.nlm.nih.gov) and added to the DNA sequence datasets generated in this study (Table 1).

Sequence datasets for the four loci were aligned in MAFFT v. 7.0 (Katoh & Standley 2013), and manually corrected where necessary using MEGA v. 5 (Tamura *et al.* 2011). Single nucleotide polymorphisms (SNP's) were determined for each gene region with the aid of DnaSP v. 5.00.06 (Librado & Rozas 2009). The best evolutionary model of nucleotide substitution for each gene region was selected according to Akaike Information Criterion (AIC) using MrModeltest v. 2.3 (Nylander 2004) and incorporated into the analyses.

An initial phylogenetic analysis was done for the aligned *tef1* data set which included 1019 taxa, including outgroup, using

Table 1. Collection details and GenBank assessions of *Calonectria* isolates included in this study.

Species	Isolate nr.[1]	Substrate	Locality	Collector	GenBank assession[2]			
					tub2	*cmdA*	*his3*	*tef1*
Calonectria brachiatica	CBS 111478; CMW 30981; CPC 1921	Soil	Brazil	A.C. Alfenas	DQ190611	GQ267383	DQ190719	FJ918568
	CBS 123699; CMW 25303	*Pinus tecunumanii*	Buga, Colombia	M.J. Wingfield	FJ716708	GQ267365	FJ716712	GQ267295
	CBS 123700; CMW 25298	*Pinus maximinoi*	Buga, Colombia	M.J. Wingfield	FJ696388	GQ267366	FJ696396	GQ267296
	CBS134665; LPF305	Soil (*Eucalyptus* plantation)	Monte Dourado, Pará, Brazil	R.F. Alfenas	*KM395933*	*KM396020*	*KM396103*	*KM395846*
	CBS134666; LPF298	Soil (*Eucalyptus* plantation)	Monte Dourado, Pará, Brazil	R.F. Alfenas	*KM395934*	*KM396021*	*KM396104*	*KM395847*
Ca. brasiliensis	**CBS 230.51**; IMI 299576; CPC 2390	*Eucalyptus* sp.	Brazil	R. Ciferri	GQ267241	GQ267421	GQ267259	GQ267328
	CBS 114257; CMW 32949; CPC 1944	*Eucalyptus* sp.	Brazil	A.C. Alfenas	GQ267242	GQ267422	GQ267260	GQ267329
Ca. brassiana	**CBS 134855**; LPF378	Soil (*E. brassiana* plantation)	Teresina, Piauí, Brazil	R.F. Alfenas	*KM395969*	*KM396056*	*KM396139*	*KM395882*
	CBS 134856; LPF379	Soil (*E. brassiana* plantation)	Teresina, Piauí, Brazil	R.F. Alfenas	*KM395970*	*KM396057*	*KM396140*	*KM395883*
	CBS 134857; LPF380	Soil (*E. brassiana* plantation)	Teresina, Piauí, Brazil	R.F. Alfenas	*KM395971*	*KM396058*	*KM396141*	*KM395884*
Ca. brassicae (= *Cy. clavatum*)	**CBS 111869**; CPC 2409; PC 551197	*Argyreia splendens*	Indonesia	F. Bugnicourt	AF232857	GQ267382	DQ190720	FJ918567
	CBS 143.72; ATCC 22833; IMI 164057	*Pinus caribaea*	Itabira, Minas Gerais, Brazil	C.S. Hodges	*KM395988*	*KM396075*	–	*KM395901*
	CBS 134657; LPF236	Soil (*Eucalyptus* plantation)	Mucuri, Bahia, Brazil	E. Zauza	*KM395918*	*KM396005*	*KM396088*	*KM395831*
	CBS 134658; LPF234	Soil (*Eucalyptus* plantation)	Mucuri, Bahia, Brazil	E. Zauza	*KM395919*	*KM396006*	*KM396089*	*KM395832*
	CBS 134659; LPF216	Soil	Salinas, Minas Gerais, Brazil	D.B. Pinho	*KM395920*	*KM396007*	*KM396090*	*KM395833*
	CBS 134660; LPF493	Soil	Salinas, Minas Gerais, Brazil	D.B. Pinho	*KM395921*	*KM396008*	*KM396091*	*KM395834*
	LPF235	Soil (*Eucalyptus* plantation)	Mucuri, Bahia, Brazil	E. Zauza	*KM395922*	*KM396009*	*KM396092*	*KM395835*
	LPF237	Soil (*Eucalyptus* plantation)	Mucuri, Bahia, Brazil	E. Zauza	*KM395923*	*KM396010*	*KM396093*	*KM395836*
Ca. candelabra	CMW 31000; CPC 1675	*Eucalyptus* sp.	Amazonas, Brazil	A.C. Alfenas	FJ972426	GQ267367	FJ972476	FJ972525
	CMW 31001; CPC 1679	*Eucalyptus* sp.	Amazonas, Brazil	A.C. Alfenas	GQ421779	GQ267368	GQ267246	GQ267298
Ca. cerciana	**CBS 123693**; CMW 25309	*Eucalyptus* hybrid	Zhanjiang Prov., CERC nursery, China	M.J. Wingfield & X.D. Zhou	FJ918510	GQ267369	FJ918528	FJ918559
	CBS 123695; CMW 25290	*Eucalyptus* hybrid	Zhanjiang Prov., CERC nursery, China	M.J. Wingfield & X.D. Zhou	FJ918511	GQ267370	FJ918529	FJ918560
Ca. clavata	**CBS 114557**; ATCC 66389; CPC 2536	*Callistemon viminalis*	USA	C.P. Seymour & E.L. Barnard	AF333396	GQ267377	DQ190623	GQ267305
	CBS 114666; CMW 30994; CPC 2537	Root debris in peat	USA	D. Ferrin & N.E. El-Gholl	DQ190549	GQ267378	DQ190624	GQ267306
Ca. colombiana	**CBS 115127**; CPC 1160	Soil	La Selva, Colombia	M.J. Wingfield	FJ972423	GQ267455	FJ972442	FJ972492
	CBS 115638; CPC 1161	Soil	La Selva, Colombia	M.J. Wingfield	FJ972422	GQ267456	FJ972441	FJ972491
Ca. colombiensis	**CBS 112220**; CPC 723	Soil	La Selva, Colombia	M.J. Wingfield	GQ267207	AY725748	AY725662	AY725711
	CBS 112221; CPC 724	Soil	La Selva, Colombia	M.J. Wingfield	AY725620	AY725749	AY725663	AY725712
Ca. cylindrospora	CBS 110666	*Rosa* sp.	USA	N.E. El-Gholl	FJ918509	GQ267423	FJ918527	FJ918557
Ca. densa	CBS 125249; CMW 31184	Soil	Las Golondrinas, Pichincha, Ecuador	M.J. Wingfield	GQ267230	GQ267442	GQ267279	GQ267350
	CBS 125261; CMW 31182	Soil	Las Golondrinas, Pichincha, Ecuador	M.J. Wingfield	GQ267232	GQ267444	GQ267281	GQ267352

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Table 1. (Continued).

Species	Isolate nr.[1]	Substrate	Locality	Collector	GenBank assession[2]			
					tub2	*cmdA*	*his3*	*tef1*
Ca. duoramosa	**CBS 134656**; LPF434	Soil (tropical rainforest)	Monte Dourado, Pará, Brazil	R.F. Alfenas	*KM395940*	*KM396027*	*KM396110*	*KM395853*
	LPF453	Soil (*Eucalyptus* plantation)	Monte Dourado, Pará, Brazil	R.F. Alfenas	*KM395941*	*KM396028*	*KM396111*	*KM395854*
Ca. ecuadoriae	CBS 111394; CPC 1628	Soil	Ecuador	M.J. Wingfield	DQ190599	GQ267376	DQ190704	GQ267304
	CBS 111406; CPC 1635	Soil	Ecuador	M.J. Wingfield	DQ190600	GQ267375	DQ190705	GQ267303
Ca. eucalypticola	CBS 134846; LPF121	*Eucalyptus* sp. (leaf)	Eunápolis, Bahia, Brazil	A.C. Alfenas	*KM395963*	*KM396050*	*KM396133*	*KM395876*
	CBS 134847; LPF124	*Eucalyptus* sp. (seeding)	Santa Bárbara, Minas Gerais, Brazil	A.C. Alfenas	*KM395964*	*KM396051*	*KM396134*	*KM395877*
	CBS 134848; LPF451	Soil (*Eucalyptus* plantation)	Monte Dourado, Pará, Brazil	R.F. Alfenas	*KM395965*	*KM396052*	*KM396135*	*KM395878*
Ca. glaebicola	**CBS 134852**; LPF406	Soil (*Eucalyptus* plantation)	Martinho Campos, Minas Gerais, Brazil	A.C. Alfenas	*KM395966*	*KM396053*	*KM396136*	*KM395879*
	CBS 134853; LPF407	*Eucalyptus* sp. (leaf)	Bico do Papagaio, Tocantins, Brazil	R.F. Alfenas	*KM395967*	*KM396054*	*KM396137*	*KM395880*
	CBS 134854; LPF408	*Eucalyptus* sp. (leaf)	Bico do Papagaio, Tocantins, Brazil	R.F. Alfenas	*KM395968*	*KM396055*	*KM396138*	*KM395881*
Ca. gordoniae	CBS 112142; CPC 3136; ATCC 201837	*Gordonia liasanthus*	USA	D. Chiappini	AF449449	GQ267381	DQ190708	GQ267309
Ca. gracilipes	**CBS 111141**	Soil	La Selva, Colombia	M.J. Wingfield	DQ190566	GQ267385	DQ190644	GQ267311
	CBS 115674	Soil	La Selva, Colombia	M.J. Wingfield	AF333406	GQ267384	DQ190645	GQ267310
Ca. gracilis	CBS 111284	Soil	Brazil	P.W. Crous	DQ190567	GQ267408	DQ190647	GQ267324
	CBS 111807	*Manilkara zapota*	Belém, Pará, Brazil	M. Aragaki	AF232858	GQ267407	DQ190646	GQ267323
Ca. hodgesii	CBS 133608; LPF244	*Piptadenia gonoacantha*	Viçosa, Minas Gerais, Brazil	R.F. Alfenas	KC491227	KC491221	–	KC491224
	CBS 133609; LPF245	*Anadenanthera peregrina*	Viçosa, Minas Gerais, Brazil	R.F. Alfenas	KC491228	KC491222	–	KC491225
	CBS 133610; LPF261	*Azadirachta indica*	Viçosa, Minas Gerais, Brazil	R.F. Alfenas	KC491229	KC491223	–	KC491226
Ca. humicola	**CBS 125251**	Soil	Las Golondrinas, Pichincha, Ecuador	M.J. Wingfield	GQ267233	GQ267445	GQ267282	GQ267353
	CBS 125269	Soil	Las Golondrinas, Pichincha, Ecuador	L. Lombard	GQ267235	GQ267447	GQ267284	GQ267355
Ca. insularis	**CBS 114558**; CPC 768	Soil	Tamatave, Madagascar	P.W. Crous	AF210861	GQ267389	FJ918526	FJ918556
	CBS 114559; CPC 954	Soil	Tamatave, Madagascar	P.W. Crous	AF210862	GQ267390	FJ918525	FJ918555
Ca. leucothoës	CBS 109166; ATCC 64824; CPC 2385	*Leucothoë axillaris*	USA	N.E. El-Gholl	FJ918508	GQ267392	FJ918523	FJ918553
Ca. pseudopteridis	**CBS 163.28**	*Washingtonia robusta*	USA	C.D. Sherbakoff	–	*KM396076*	–	*KM395902*
Ca. maranhensis	**CBS 134811**; LPF142	*Eucalyptus* sp. (leaf)	Açailândia, Maranhão, Brazil	A.C. Alfenas	*KM395948*	*KM396035*	*KM396118*	*KM395861*
	CBS 134812; LPF143	*Eucalyptus* sp. (leaf)	Açailândia, Maranhão, Brazil	A.C. Alfenas	*KM395949*	*KM396036*	*KM396119*	*KM395862*
	CBS 134825; LPF370	Soil (*Eucalyptus* plantation)	Imperatriz, Maranhão, Brazil	R.F. Alfenas	*KM395950*	*KM396037*	*KM396120*	*KM395863*
	CBS 134828; LPF441	Soil (*Eucalyptus* plantation)	Urbano Santos, Maranhão, Brazil	E. Zauza	*KM395951*	*KM396038*	*KM396121*	*KM395864*
	CBS 134829; LPF443	Soil (*Eucalyptus* plantation)	Urbano Santos, Maranhão, Brazil	E. Zauza	*KM395952*	*KM396039*	*KM396122*	*KM395865*
Ca. metrosideri	**CBS 133603**; LPF101	*Metrosideros polymorpha*	Viçosa, Minas Gerais, Brazil	R.F. Alfenas	KC294313	KC294304	KC294307	KC294310
	CBS 133604; LPF103	*Metrosideros polymorpha*	Viçosa, Minas Gerais, Brazil	R.F. Alfenas	KC294314	KC294305	KC294308	KC294311
	CBS 133605; LPF104	*Metrosideros polymorpha*	Viçosa, Minas Gerais, Brazil	R.F. Alfenas	KC294315	KC294306	KC294309	KC294312

Table 1. (Continued).

Species	Isolate nr.[1]	Substrate	Locality	Collector	GenBank assession[2]			
					tub2	*cmdA*	*his3*	*tef1*
Ca. multinaviculata	**CBS 134858**; LPF233	Soil (*Eucalyptus* plantation)	Mucuri, Bahia, Brazil	E. Zauza	*KM395985*	*KM396072*	*KM396155*	*KM395898*
	CBS 134859; LPF418	Soil (*Eucalyptus* plantation)	Monte Dourado, Pará, Brazil	R.F. Alfenas	*KM395986*	*KM396073*	*KM396156*	*KM395899*
	CBS 134862; LPF472	Soil (*Eucalyptus* plantation)	Mucuri, Bahia, Brazil	E. Zauza	*KM395987*	*KM396074*	*KM396157*	*KM395900*
Ca. multiphialidica	CBS 112678	Soil	Cameroon	Abadie	AY725628	AY725761	AY725673	AY725723
Ca. naviculata	CBS 101121	Leaf litter	João Pessoa, Brazil	R.F. Castañeda	GQ267211	GQ267399	GQ267252	GQ267317
	CBS 116080	Soil	Amazonas, Brazil	M.J. Wingfield	AF333409	GQ267398	GQ267251	GQ267316
Ca. nemuricola	**CBS 134837**; LPF085	Soil (tropical rainforest)	Araponga, Minas Gerais, Brazil	A.C. Alfenas & P.W. Crous	*KM395979*	*KM396066*	*KM396149*	*KM395892*
	CBS 134838; LPF090	Soil (tropical rainforest)	Araponga, Minas Gerais, Brazil	A.C. Alfenas & P.W. Crous	*KM395980*	*KM396067*	*KM396150*	*KM395893*
	CBS 134839; LPF094	Soil (tropical rainforest)	Araponga, Minas Gerais, Brazil	A.C. Alfenas & P.W. Crous	*KM395981*	*KM396068*	*KM396151*	*KM395894*
Ca. orientalis	CBS 125259	Soil	Teso East, Indonesia	M.J. Wingfield	GQ267237	GQ267449	GQ267286	GQ267357
	CBS 125260	Soil	Lagan, Indonesia	M.J. Wingfield	GQ267236	GQ267448	GQ267285	GQ267356
	LPF032	Soil (*Eucalyptus* plantation)	Monte Dourado, Pará, Brazil	R.F. Alfenas	*KM395910*	*KM395996*	–	*KM395822*
	LPF300	Soil (*Eucalyptus* plantation)	Monte Dourado, Pará, Brazil	R.F. Alfenas	*KM395911*	*KM395997*	–	*KM395823*
	LPF301	Soil (*Eucalyptus* plantation)	Monte Dourado, Pará, Brazil	R.F. Alfenas	*KM395912*	*KM395998*	–	*KM395824*
	LPF435	Soil (*Eucalyptus* plantation)	Monte Dourado, Pará, Brazil	R.F. Alfenas	*KM395913*	*KM395999*	–	*KM395825*
Ca. ovata	CBS 111299	*E. tereticornis*	Tucuruí, Pará, Brazil	P.W. Crous	GQ267212	GQ267400	GQ267253	GQ267318
	CBS 111307	*E. tereticornis*	Tucuruí, Pará, Brazil	P.W. Crous	AF210868	GQ267401	GQ267254	GQ267319
Ca. paraensis	**CBS 134669**; LPF430	Soil (*Eucalyptus* plantation)	Monte Dourado, Pará, Brazil	R.F. Alfenas	*KM395924*	*KM396011*	*KM396094*	*KM395837*
	LPF306	Soil (*Eucalyptus* plantation)	Monte Dourado, Pará, Brazil	R.F. Alfenas	*KM395925*	*KM396012*	*KM396095*	*KM395838*
	LPF308	Soil (*Eucalyptus* plantation)	Monte Dourado, Pará, Brazil	R.F. Alfenas	*KM395926*	*KM396013*	*KM396096*	*KM395839*
	LPF309	Soil (*Eucalyptus* plantation)	Monte Dourado, Pará, Brazil	R.F. Alfenas	*KM395927*	*KM396014*	*KM396097*	*KM395840*
	LPF429	Soil (tropical rainforest)	Monte Dourado, Pará, Brazil	R.F. Alfenas	*KM395928*	*KM396015*	*KM396098*	*KM395841*
Ca. pauciramosa	**CMW 5683**	*E. grandis*	Knysna, South Africa	P.W. Crous	FJ918514	GQ267405	FJ918531	FJ918565
	CMW 30823	Soil	Tzaneen, South Africa	S. de Buisson	FJ918515	GQ267404	FJ918532	FJ918566
Ca. piauiensis	CBS 134849; LPF291	Soil (tropical rainforest)	Serra das Confusões, Piauí	O.L. Pereira	*KM395972*	*KM396059*	*KM396142*	*KM395885*
	CBS 134850; LPF377	Soil (*Eucalyptus* plantation)	Teresina, Piauí, Brazil	R.F. Alfenas	*KM395973*	*KM396060*	*KM396143*	*KM395886*
	CBS 134851; LPF381	Soil (tropical rainforest)	Teresina, Piauí, Brazil	R.F. Alfenas	*KM395974*	*KM396061*	*KM396144*	*KM395887*
Ca. pini	**CBS 123698**	*Pinus patula*	Buga, Colombia	C.A. Rodas	GQ267224	GQ267436	GQ267273	GQ267344
	CBS 125253	*Pinus patula*	Buga, Colombia	C.A. Rodas	GQ267225	GQ267437	GQ267274	GQ267345
Ca. polizzii	CBS 125270	*Callistemon citrinus*	Sicily, Messina, Italy	G. Polizzi	FJ972417	GQ267461	FJ972436	FJ972486
	CBS 125271	*Arbustus unedo*	Sicily, Messina, Italy	G. Polizzi	FJ972418	GQ267462	FJ972437	FJ972487

(*continued on next page*)

Table 1. (Continued).

Species	Isolate nr.[1]	Substrate	Locality	Collector	GenBank assession[2]			
					tub2	*cmdA*	*his3*	*tef1*
Ca. propaginicola	**CBS 134815**; LPF220	*Eucalyptus* sp. (seeding)	Santana, Pará, Brazil	A.C. Alfenas	*KM395953*	*KM396040*	*KM396123*	*KM395866*
	CBS 134816; LPF222	*Eucalyptus* sp. (seeding)	Santana, Pará, Brazil	A.C. Alfenas	*KM395954*	*KM396041*	*KM396124*	*KM395867*
	CBS 134817; LPF223	*Eucalyptus* sp. (seeding)	Santana, Pará, Brazil	A.C. Alfenas	*KM395955*	*KM396042*	*KM396125*	*KM395868*
	CBS 134820; LPF287	Used planting substrate	Santana, Pará, Brazil	A.C. Alfenas	*KM395956*	*KM396043*	*KM396126*	*KM395869*
	CBS 134821; LPF289	Used planting substrate	Santana, Pará, Brazil	A.C. Alfenas	*KM395957*	*KM396044*	*KM396127*	*KM395870*
	LPF218	*Eucalyptus* sp. (seeding)	Santana, Pará, Brazil	A.C. Alfenas	*KM395958*	*KM396045*	*KM396128*	*KM395871*
	LPF221	*Eucalyptus* sp. (seeding)	Santana, Pará, Brazil	A.C. Alfenas	*KM395959*	*KM396046*	*KM396129*	*KM395872*
Ca. pseudobrassicae	CBS 134661; LPF260	Soil (*Eucalyptus* plantation)	Santana, Pará, Brazil	A.C. Alfenas	*KM395935*	*KM396022*	*KM396105*	*KM395848*
	CBS 134662; LPF280	Soil (*Eucalyptus* plantation)	Santana, Pará, Brazil	A.C. Alfenas	*KM395936*	*KM396023*	*KM396106*	*KM3* 95849
Ca. pseudocerciana	CBS 134822; LPF365	*Eucalyptus* sp. (seeding)	Santana, Pará, Brazil	A.C. Alfenas	*KM395960*	*KM396047*	*KM396130*	*KM395873*
	CBS 134823; LPF366	*Eucalyptus* sp. (seeding)	Santana, Pará, Brazil	A.C. Alfenas	*KM395961*	*KM396048*	*KM396131*	*KM395874*
	CBS 134824; LPF367	*Eucalyptus* sp. (seeding)	Santana, Pará, Brazil	A.C. Alfenas	*KM395962*	*KM396049*	*KM396132*	*KM395875*
Ca. pseudohodgesii	CBS 134813; LPF205	*Eucalyptus* sp. (seeding)	Viçosa, Minas Gerais, Brazil	R.F. Alfenas	*KM395903*	*KM395989*	*KM396077*	*KM395815*
	CBS 134814; LPF206	*Eucalyptus* sp. (seeding)	Viçosa, Minas Gerais, Brazil	R.F. Alfenas	*KM395904*	*KM395990*	*KM396078*	*KM395816*
	CBS 134818; LPF262	*Azadirachta indica* (leaf)	Viçosa, Minas Gerais, Brazil	R.F. Alfenas	*KM395905*	*KM395991*	*KM396079*	*KM395817*
	CBS 134819; LPF265	*Azadirachta indica* (leaf)	Viçosa, Minas Gerais, Brazil	R.F. Alfenas	*KM395906*	*KM395992*	*KM396080*	*KM395818*
Ca. pseudometrosideri	CBS 134843; LPF100	*Metrosideros polymorpha*	Viçosa, Minas Gerais, Brazil	A.C. Alfenas	*KM395907*	*KM395993*	*KM396081*	*KM395819*
	CBS 134844; LPF147	*Eucalyptus* sp. (leaf)	Açailândia, Maranhão, Brazil	R.F. Alfenas	*KM395908*	*KM395994*	*KM396082*	*KM395820*
	CBS 134845; LPF210	Soil (*Eucalyptus* plantation)	Maceió, Alagoas, Brazil	M.M. Coutinho	*KM395909*	*KM395995*	*KM396083*	*KM395821*
Ca. pseudonaviculata	CBS 114417; CPC 10926	*Buxus sempervirens*	West Auckland, New Zealand	C. Crepel	GQ267214	GQ267409	GQ267258	GQ267325
	CBS 116251; CPC 3399	*Buxus sempervirens*	West Auckland, New Zealand	C.R. MacDiarmid	AF449455	*KM396000*	–	*KM395826*
Ca. pseudoscoparia	**CBS 125255**	*E. grandis*	Pichincha, Ecuador	M.J. Wingfield	GQ267227	GQ267439	GQ267276	GQ267347
	CBS 125257	*E. grandis*	Pichincha, Ecuador	M.J. Wingfield	GQ267229	GQ267441	GQ267278	GQ267349
Ca. pseudospathulata	CBS 134840; LPF066	Soil (tropical rainforest)	Araponga, Minas Gerais, Brazil	A.C. Alfenas & P.W. Crous	*KM395982*	*KM396069*	*KM396152*	*KM395895*
	CBS 134841; LPF072	Soil (tropical rainforest)	Araponga, Minas Gerais, Brazil	A.C. Alfenas & P.W. Crous	*KM395983*	*KM396070*	*KM396153*	*KM395896*
	CBS 134842; LPF087	Soil (tropical rainforest)	Araponga, Minas Gerais, Brazil	A.C. Alfenas & P.W. Crous	*KM395984*	*KM396071*	*KM396154*	*KM395897*
Ca. pseudovata	CBS 134674; LPF267	Soil (*Eucalyptus* plantation)	Santana, Pará, Brazil	A.C. Alfenas	*KM395945*	*KM396032*	*KM396115*	*KM395858*
	CBS 134675; LPF285	Soil (*Eucalyptus* plantation)	Santana, Pará, Brazil	A.C. Alfenas	*KM395946*	*KM396033*	*KM396116*	*KM395859*
	LPF286	Soil (*Eucalyptus* plantation)	Santana, Pará, Brazil	A.C. Alfenas	*KM395947*	*KM396034*	*KM396117*	*KM395860*
Ca. pseudospathiphylli	**CBS 109165**; CPC 1623	Soil	Ecuador	M.J. Wingfield	FJ918513	GQ267412	AF348241	FJ918562

Table 1. (Continued).

Species	Isolate nr.[1]	Substrate	Locality	Collector	GenBank assession[2]			
					tub2	*cmdA*	*his3*	*tef1*
Ca. pteridis	**CBS 111793**; ATCC 34395; CPC 2372	*Arachnoides adiantiformis*	USA	P.W. Crous	DQ190578	GQ267413	DQ190679	FJ918563
	CBS 111871; CPC 2443	*Pinus* sp.	Spain	T.L. Krugner	DQ190579	GQ267414	DQ190681	FJ918564
	CBS 134670; LPF410	*Eucalyptus* sp. (leaf)	Imperatriz, Maranhão, Brazil	R.F. Alfenas	*KM395914*	*KM396001*	*KM396084*	*KM395827*
	CBS 134671; LPF422	*Eucalyptus* sp. (leaf)	Monte Dourado, Pará, Brazil	R.F. Alfenas	*KM395915*	*KM396002*	*KM396085*	*KM395828*
	CBS 134672; LPF201	*Eucalyptus* sp. (leaf)	Imperatriz, Maranhão, Brazil	R.F. Alfenas	*KM395916*	*KM396003*	*KM396086*	*KM395829*
	CBS 134673; LPF202	*Eucalyptus* sp. (leaf)	Imperatriz, Maranhão, Brazil	R.F. Alfenas	*KM395917*	*KM396004*	*KM396087*	*KM395830*
Ca. quinqueramosa	**CBS 134654**; LPF065	Soil (*Eucalyptus* plantation)	Monte Dourado, Pará, Brazil	R.F. Alfenas	*KM395942*	*KM396029*	*KM396112*	*KM395855*
	CBS 134655; LPF281	Soil (*Eucalyptus* plantation)	Santana, Pará, Brazil	A.C. Alfenas	*KM395943*	*KM396030*	*KM396113*	*KM395856*
	CBS 134863; LPF302	Soil (*Eucalyptus* plantation)	Monte Dourado, Pará, Brazil	R.F. Alfenas	*KM395944*	*KM396031*	*KM396114*	*KM395857*
Ca. robigophila	**CBS 134652**; LPF192	*Eucalyptus* sp. (leaf)	Açailândia, Maranhão, Brazil	R.F. Alfenas	*KM395937*	*KM396024*	*KM396107*	*KM395850*
	CBS 134653; LPF193	*Eucalyptus* sp. (leaf)	Açailândia, Maranhão, Brazil	R.F. Alfenas	*KM395938*	*KM396025*	*KM396108*	*KM395851*
	LPF190	*Eucalyptus* sp. (leaf)	Açailândia, Maranhão, Brazil	R.F. Alfenas	*KM395939*	*KM396026*	*KM396109*	*KM395852*
Ca. silvicola	CBS 134836; LPF079	Soil (tropical rainforest)	Araponga, Minas Gerais, Brazil	A.C. Alfenas & P.W. Crous	*KM395975*	*KM396062*	*KM396145*	*KM395888*
	CBS 135237; LPF081	Soil (tropical rainforest)	Araponga, Minas Gerais, Brazil	A.C. Alfenas & P.W. Crous	*KM395978*	*KM396065*	*KM396148*	*KM395891*
	CPC 18741; LPF071	Soil (tropical rainforest)	Araponga, Minas Gerais, Brazil	A.C. Alfenas & P.W. Crous	*KM395976*	*KM396063*	*KM396146*	*KM395889*
	CPC 18766; LPF096	Soil (tropical rainforest)	Mucuri, Bahia, Brazil	E. Zauza	*KM395977*	*KM396064*	*KM396147*	*KM395890*
Ca. spathiphylli	CBS 114540; ATCC 44730; CPC 2378	*Spathiphyllum* sp.	USA	S. A. Alfieri	AF348214	GQ267424	AF348230	GQ267330
	CBS 116168; CPC 789	*Spathiphyllum* sp.	Switzerland	L. Petrini	FJ918512	GQ267425	FJ918530	FJ918561
Ca. spathulata	CBS 555.92	*Eucalyptus viminalis*	Brazil	N.E. El-Gholl	AF308463	GQ267426	FJ918524	FJ918554
	CBS 112689	*Araucaria angustifolia*	São Paulo, Brazil	C.S. Hodges	GQ267215	GQ267427	GQ267261	GQ267331
Ca. sulawesiensis	CBS 125248	*Eucalyptus* sp.	Sulawesi, Indonesia	M.J. Wingfield	GQ267223	GQ267435	GQ267272	GQ267343
	CBS 125277	*Eucalyptus* sp.	Sulawesi, Indonesia	M.J. Wingfield	GQ267222	GQ267434	GQ267271	GQ267342
Ca. telluricola	CBS 134663; LPF214	Soil (tropical rainforest)	Salinas, Minas Gerais, Brazil	D.B. Pinho	*KM395929*	*KM396016*	*KM396099*	*KM395842*
	CBS 134664; LPF217	Soil (tropical rainforest)	Mucuri, Bahia, Brazil	E. Zauza	*KM395930*	*KM396017*	*KM396100*	*KM395843*
	CBS 134667; LPF263	Soil (*Eucalyptus* plantation)	Mucuri, Bahia, Brazil	E. Zauza	*KM395931*	*KM396018*	*KM396101*	*KM395844*
	CBS 134668; LPF254	Soil (*Eucalyptus* plantation)	Mucuri, Bahia, Brazil	E. Zauza	*KM395932*	*KM396019*	*KM396102*	*KM395845*
Ca. variabilis	CBS 112691; CPC 2506	*Theobroma grandiflorum*	Brazil	F. Carneiro	GQ267240	GQ267458	GQ267264	GQ267335
	CBS 114677; CPC 2436	*Schefflera morototoni*	Brazil	F. C. de Albuquerque	AF333424	GQ267457	GQ267263	GQ267334
Ca. zuluensis	**CBS 125268**	*E. grandis*	Kwa-Zulu Natal, Kwambonambi, South Africa	L. Lombard	FJ972414	GQ267459	FJ972433	FJ972483
	CBS 125272	*E. grandis*	Kwa-Zulu Natal, Kwambonambi, South Africa	L. Lombard	FJ972415	GQ267460	FJ972434	FJ972484

[1] ATCC: American Type Culture Collection, Virginia, U.S.A., CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, CMW: collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa, CPC: Pedro W. Crous working collection housed at CBS, IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham lane, U.K., LPF: Laboratório de Patologia Florestal, Universidade Federal de Viçosa, Viçosa, Brazil. Ex-type strains indicated in **bold**.

[2] *tub2* = β-tubulin, *cmdA* = calmodulin, *his3* = histone H3, *tef1* = translation elongation factor-1α; sequences generated in this study indicated in *italics*.

MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003) to identify the possible groups present in our samples. Based on this, 85 *Calonectria* isolates were selected for further study and divided into four separate datasets representing the (1) *Ca. brassicae* and *Ca. pteridis* complex, (2) *Ca. cylindrospora* complex, (3) *Ca. candelabra* complex and (4) *Ca. naviculata* complex, to reduce the number of gaps in the alignments and consequently improve the resolution of the analyses. To determine whether the four gene regions determined were congruent, congruence index trees (De Vienne *et al.* 2007) and a 70 % reciprocal bootstrap method (Gueidan *et al.* 2007) were applied to each gene region used.

Phylogenetic analyses were based on both Bayesian Inference (BI) and Maximum Parsimony (MP). For BI, the best evolutionary model of nucleotide substitution for each gene region was incorporated into the analyses. Analyses in MrBayes v. 3.2.1 (Ronquist & Huelsenbeck 2003) used the Markov Chain Monte Carlo (MCMC) algorithm and employed two sets of four chains started in parallel from a random tree topology with the heating parameter set at 0.3. The MCMC analysis ran until the average standard deviation of split frequencies came below 0.01, with trees saved every 1 000 generations. The first 25 % of saved trees were discarded as the "burn-in" phase and posterior probabilities (PP) determined from the remaining trees.

MP analyses were performed in PAUP (Phylogenetic Analysis Using Parsimony, v. 4.0b10; Swofford 2003) with phylogenetic relationships estimated by heuristic searches with 1 000 random addition sequences. The tree-bisection-reconnection option was used, with the branch swapping option set to "best trees" only. All characters were weighted equally and alignment gaps treated as fifth state. Measures calculated for parsimony included tree length (TL), consistency index (CI), retention index (RI) and rescaled consistence index (RC). Bootstrap analyses (Hillis & Bull 1993) were based on 1 000 replications. All resulting trees were illustrated using Geneious v. 5.5.4 (Drummond *et al.* 2011). Sequences derived in this study were deposited in GenBank (Table 1) and the alignments in TreeBASE (www.treebase.org/treebase/index.html).

Taxonomy

Single conidial isolates were grown on synthetic nutrient-poor agar (SNA; Nirenburg 1981) at 24 °C, following the protocol of Lombard *et al.* (2009). After 7 d of incubation, the morphological characteristics of the asexual morphs were determined by mounting fungal structures in clear lactic acid and 30 measurements at ×1 000 magnification were determined for each isolate using a Zeiss Axioscope 2 microscope with interference contrast (DIC) optics. Additionally, crosses were made as described by Lombard *et al.* (2010b, c) in all possible combinations based on the identities determined by DNA sequence analysis of the *tef1* gene region. Isolates were crossed with themselves as controls, thus making it possible to distinguish between heterothallic and homothallic mating systems of the isolates. The plates were stacked in plastic containers and incubated at room temperature (25°C ± 3 °C) for 6–8 wk. Crosses were regarded as successful when isolate combinations produced ascomata extruding viable ascospores. Morphological characteristics of the sexual morphs were determined by mounting ascomata in tissue freezing medium (Leica Biosystems, Nussloch, Germany) and cutting sections with a Leica CM1100 cryostate (Leica Biosystems, Nussloch, Germany). The 10 µm sections were mounted in 85 % lactic acid and 3 % KOH. The 95 % confidence levels were determined and extremes of conidial and ascospore measurements are given in parentheses. For other structures only extremes are presented. Colony morphology was assessed using 7-d-old cultures on MEA and oatmeal agar (OA) incubated at 24 °C and the colour charts of Rayner (1970). All descriptions, illustrations and nomenclatural data were deposited in MycoBank (Crous *et al.* 2004a).

RESULTS

Sampling and isolation

A total of 1 017 isolates were obtained of which 646 were from *Eucalyptus* leaves displaying symptoms of CLB in plantations, 320 isolates were baited from soils collected within the commercial *Eucalyptus* plantations, 13 isolates were obtained from *Eucalyptus* seedlings and two from *A. indica* seedlings in the nursery, and 36 isolates were obtained from the surrounding native vegetation. Eighty-five of these isolates were selected for further study (Table 1) based on preliminary phylogenetic analysis of the *tef1* gene region (results not shown).

Phylogenetic analyses

Approximately 500–550 bases were determined for the *his3*, *tef1* and *tub2* gene regions and 650 bases for the *cmdA* gene region. The preliminary *tef1* sequence analysis, which included 1 019 taxa as well as outgroup taxa (*Ca. colombiensis* CBS 112220 & CBS 112221), showed that the majority of the collected isolates from *Eucalyptus* with CLB symptoms belonged to the *Ca. pteridis* species complex (484 of 545 isolates; results not shown). The remaining isolates were divided among the *Ca. brassicae*, *Ca. cylindrospora*, *Ca. naviculata* and *Ca. candelabra* species complexes.

For the Bayesian analyses, the evolutionary model selected for each gene region for each dataset is presented in Table 2. The Bayesian consensus trees for each of the datasets

Table 2. Evolutionary substitution models determined for each gene region used in the Baysian phylogenetic inference.

***Calonectria* complex**	**Evolution model**			
	tef1	***tub2***	***cmdA***	***his3***
Ca. brassicae and *Ca. pteridis* complexes	HKY + G	HKY + G	GTR + G	GTR + I + G
Ca. cylindrospora complex	HKY + G	HKY + I + G	HKY + I + G	GTR + I + G
Ca. naviculata complex	HKY + I	HKY + I	HKY + I	GTR + I
Ca. candelabra complex	GTR + G	GTR + G	GTR + I + G	HKY + G

confirmed the tree topologies obtained from the MP analyses, and therefore, only the Bayesian consensus trees are presented with bootstrap support values (BS) and posterior probabilities (PP) shown for well-supported nodes. Congruency tests revealed no conflicts in tree topologies for the four gene regions used in each of the four separate datasets and were therefore combined.

The combined dataset for the *Ca. brassicae* and *Ca. pteridis* complexes included 61 ingroup taxa, with *Ca. colombiensis* (CBS 112220 & CBS 112221) as the outgroup taxon. The sequence dataset consisted of 1 958 characters, including alignment gaps. Of these, 1 289 were constant, 35 were parsimony-uninformative and 634 parsimony-informative. The MP analysis yielded 1 000 trees (TL = 1 304, CI = 0.685, RI = 0.902, RC = 0.618). The BI analysis lasted 1 625 000 generations and the consensus tree (Fig. 1) and posterior probabilities (PP) were calculated from 2 439 trees. In the tree, five main clades could be resolved. Clade 1 included four smaller clades, of which two of the clades represented *Ca. orientalis* (ex-type CBS 125260; BS = 77, PP = 0.97) and *Ca. pini* (ex-type CBS 123698; BS = 100, PP = 1.0), respectively. The remaining two clades (BS = 53, PP = 0.98 and BS = 81, PP = 0.98, respectively), which include CBS 134669, LPF 306, LPF308, LPF309, LPF429 in one clade, and CBS 134663, CBS 134664, CBS 134667, CBS 134668 in the other, appear to represent two distinct lineages. Clade 2 consisted of four smaller clades, of which three represented known *Calonectria* species. The clade representing *Ca. brassicae*, which included the ex-types of *Cy. gracile* (CBS 111869) and *Cy. clavatum* (CBS 134.71), could not be resolved in this study. Similarly, the clade representing *Ca. brachiatica* (ex-type CBS 123700) could not be resolved. Two isolates (CBS 134661, CBS 1346620) formed a basal sister clade (BS = 100, PP = 1.0) to the clades representing *Ca. brassicae* and *Ca. brachiatica*, possibly indicating a previously unrecognised lineage. Clade 3 included three well-supported sister clades, one of which (BS = 100, PP = 1.0) represents *Ca. ecuadoriae* (ex-type CBS 111406). The other two clades, one incorporating CBS 134652, CBS 134653 and LPF190 (BS = 100, PP = 1.0) and the other CBS 134656 and LPF453 (BS = 98, PP = 1.0), each represent novel lineages. Isolates CBS 134654, CBS 134655 and CBS 134863, in Clade 4, clustered together in a clade (BS = 91, PP < 0.95) closely related to but separate from the clade (BS = 100, PP = 1.0) representing *Ca. gracilis* (ex-type CBS 111807), representing a novel lineage. In Clade 5, three isolates obtained from *Eucalyptus* leaves (CBS 134674, CBS 134675, LPF286) formed a sister clade (BS = 93, PP = 1.0) closely related to but separate from the clade (BS = 100, PP = 1.0) representing *Ca. ovata* (CBS 111299 and CBS 111307). Four representative isolates (CBS 134670 – CBS 134673) collected during this study, clustered within the clade (BS = 100, PP = 1.0) representing *Ca. pteridis* (ex-type CBS 111793). The ex-type of *Cy. macrosporum* (CBS 163.28) formed a distinct basal lineage to the *Ca. pteridis* clade, indicating that this species was incorrectly synonymised under *Ca. pteridis* (Crous 2002).

The combined dataset for the *Ca. cylindrospora* species complex included 41 ingroup taxa, with *Ca. colombiensis* (CBS 112220 & CBS 112221) as the outgroup taxon. The sequence dataset consisted of 1 975 characters, including alignment gaps. Of these, 1 355 were constant, 85 were parsimony-uninformative and 535 parsimony-informative. The MP analysis yielded 1 000 trees (TL = 1 002, CI = 0.767, RI = 0.891, RC = 0.684). The BI analysis lasted 985 000 generations and the consensus tree (Fig. 2) and posterior probabilities (PP) were calculated from 1 480 trees. In the tree, four main clades could be resolved with the isolates collected in this study clustering in Clades 1 & 3. In Clade 1, isolates obtained during this study clustered in two smaller clades, one of which formed a distinct basal clade (BS = 98, PP = 1.0) to the clades representing *Ca. hodgesii* (ex-type CBS 133609), *Ca. brasiliensis* (ex-type CBS 230.51) and *Ca. sulawesiensis* (ex-type CBS 125277), representing a distinct lineage. The remaining isolates (CBS 134813, CBS 134814, CBS 134818, CBS 134819) formed a clade (BS = 79, PP = 0.99) closely related to, but distinct from, the *Ca. hodgesii* clade, also representing a previously unrecognised lineage. In Clade 3, the newly collected isolates also clustered in two well-supported but distinct clades (containing CBS 134815; BS = 69, PP = 0.95 & containing CBS 134824; BS = 73, PP < 0.95, respectively) with *Ca. cerciana* (ex-type CBS 123693) forming a basal clade to both these lineages.

The combined dataset for the *Ca. naviculata* species complex included eight ingroup taxa, with *Ca. colombiensis* (CBS 112220 & CBS 112221) as the outgroup taxon. The sequence dataset consisted of 1 994 characters, including alignment gaps. Of these, 1 431 were constant, 86 were parsimony-uninformative and 477 parsimony-informative. The MP analysis yielded 1 000 trees (TL = 764, CI = 0.919, RI = 0.931, RC = 0.855). The BI analysis lasted 275 000 generations and the consensus tree (Fig. 3) and posterior probabilities (PP) were calculated from 15 trees. Only three isolates (CBS 134858, CBS 134859, CBS 134862), collected in this study, grouped in this dataset. They formed a well-supported clade (BS = 91, PP < 0.95) closely related to, but distinct from, the clade representing *Ca. naviculata* (ex-type CBS 101121).

The combined dataset for the *Ca. candelabra* species complex included 42 ingroup taxa, with *Ca. colombiensis* (CBS 112220 & CBS 112221) as the outgroup taxon. The sequence dataset consisted of 1 930 characters, including alignment gaps. Of these, 1 448 were constant, six were parsimony-uninformative and 476 parsimony-informative. The MP analysis yielded 1 000 trees (TL = 690, CI = 0.813, RI = 0.922, RC = 0.750). The BI analysis lasted 1 565 000 generations and the consensus tree (Fig. 4) and posterior probabilities (PP) were calculated from 2 348 trees. In the tree, three main clades are resolved, with each clade incorporating isolates collected in this study. In Clade 1, the newly collected isolates clustered into five well-supported clades. The first of these clades (containing CBS 134845; BS = 61, PP = 0.99) is closely related but separate from the *Ca. metrosideri* clade (ex-type CBS 133603). The remaining four clades of newly collected isolates formed basal sister clades to *Ca. candelabra* (CMW31000 & CMW31001), *Ca. pseudoscoparia* (ex-type CBS 125257) and *Ca. metrosideri*. In Clade 2, the isolates from the current study clustered into two separate well-supported clades (containing CBS 135237; BS = 99, PP = 1.0, and containing CBS 134837; BS = 100, PP = 1.0) sister to the clades of *Ca. pauciramosa* (ex-type CMW5683), *Ca. polizzii* (CBS 125270 & CBS 125271) and *Ca. zuluensis* (ex-type CBS 125268). The newly collected isolates in Clade 3 clustered together in a well-supported clade (containing CBS 134841; BS = 100, PP = 0.99) closely related to, but distinct from, the *Ca. spathulata* clade (CBS 555.92 & CBS 112689).

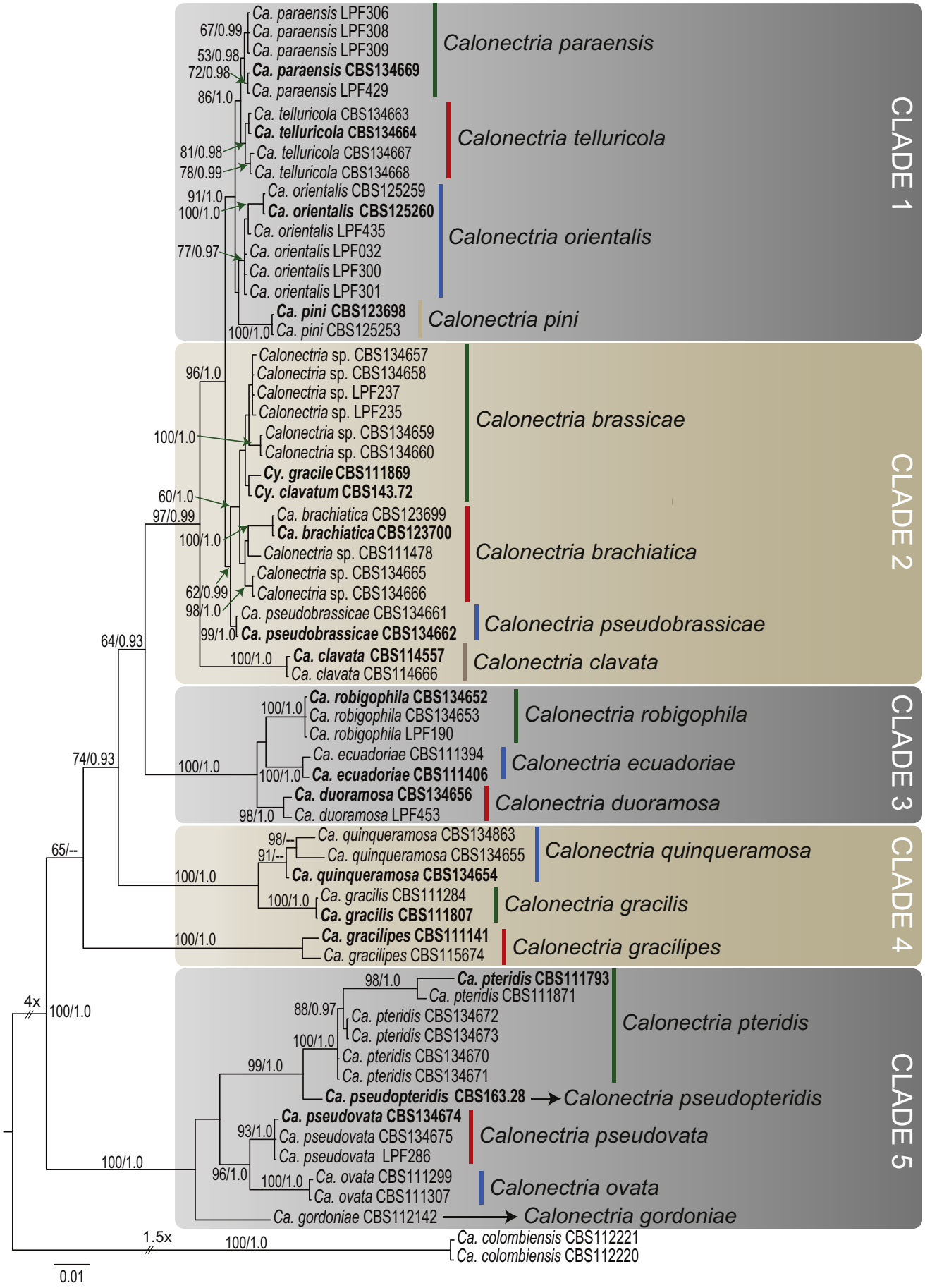

Fig. 1. Consensus phylogram of 2 439 trees resulting from a Bayesian analysis of the combined four gene sequence alignment of the *Calonectria brassicae* and *Ca. pteridis* complexes. Accession numbers in **bold** represent ex-type strains. Bayesian posterior probabilities and Maximum Parsimony bootstrap support values are indicated at the nodes and the scale bar represents the number of expected changes per site. The tree was rooted to *Ca. colombiensis* (CBS 112220, CBS 112221).

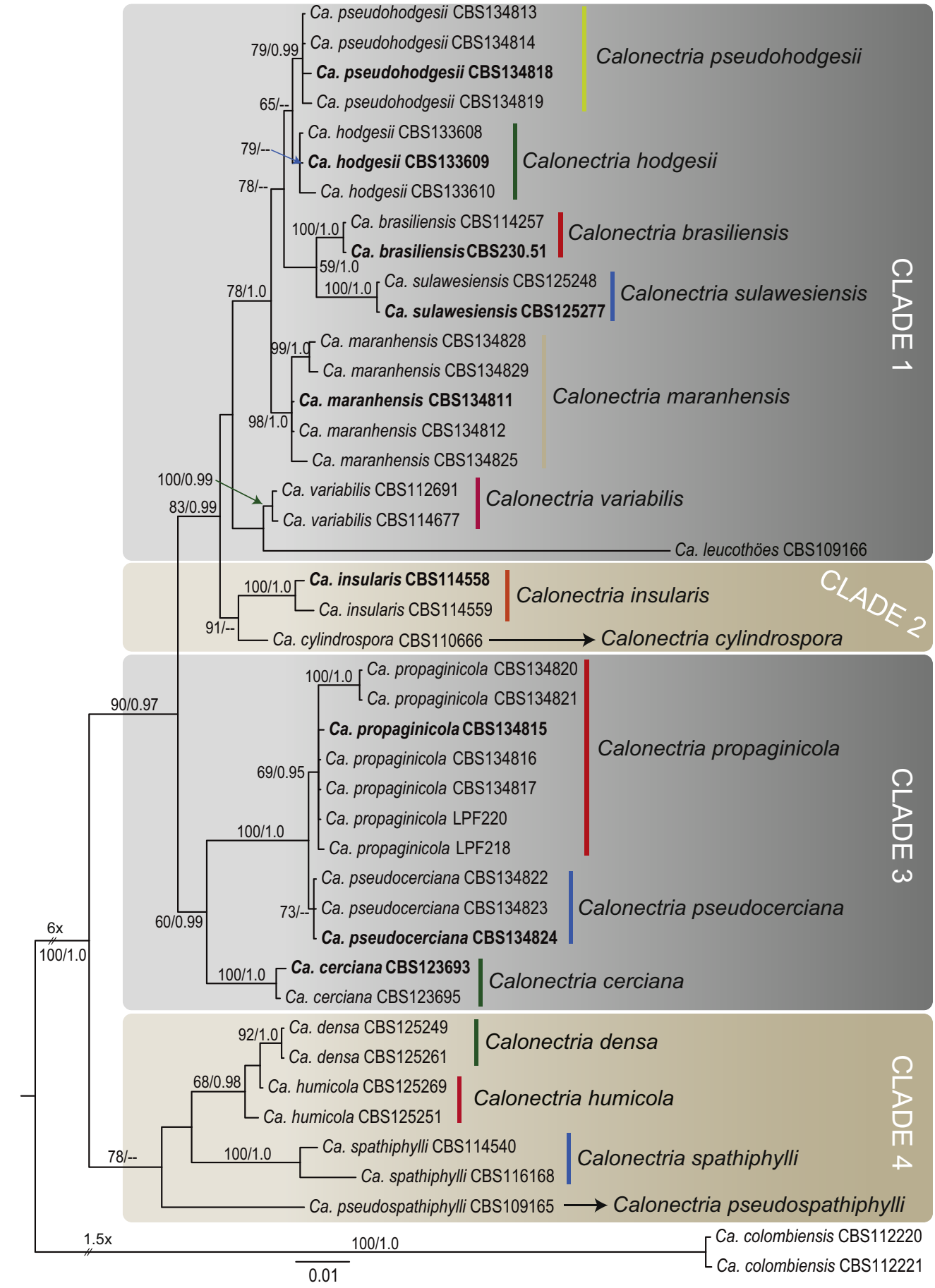

Fig. 2. Consensus phylogram of 1 480 trees resulting from a Bayesian analysis of the combined four gene sequence alignment of the *Calonectria cylindrospora* complex. Accession numbers in **bold** represent ex-type strains. Bayesian posterior probabilities and Maximum Parsimony bootstrap support values are indicated at the nodes and the scale bar represents the number of expected changes per site. The tree was rooted to *Ca. colombiensis* (CBS 112220, CBS 112221).

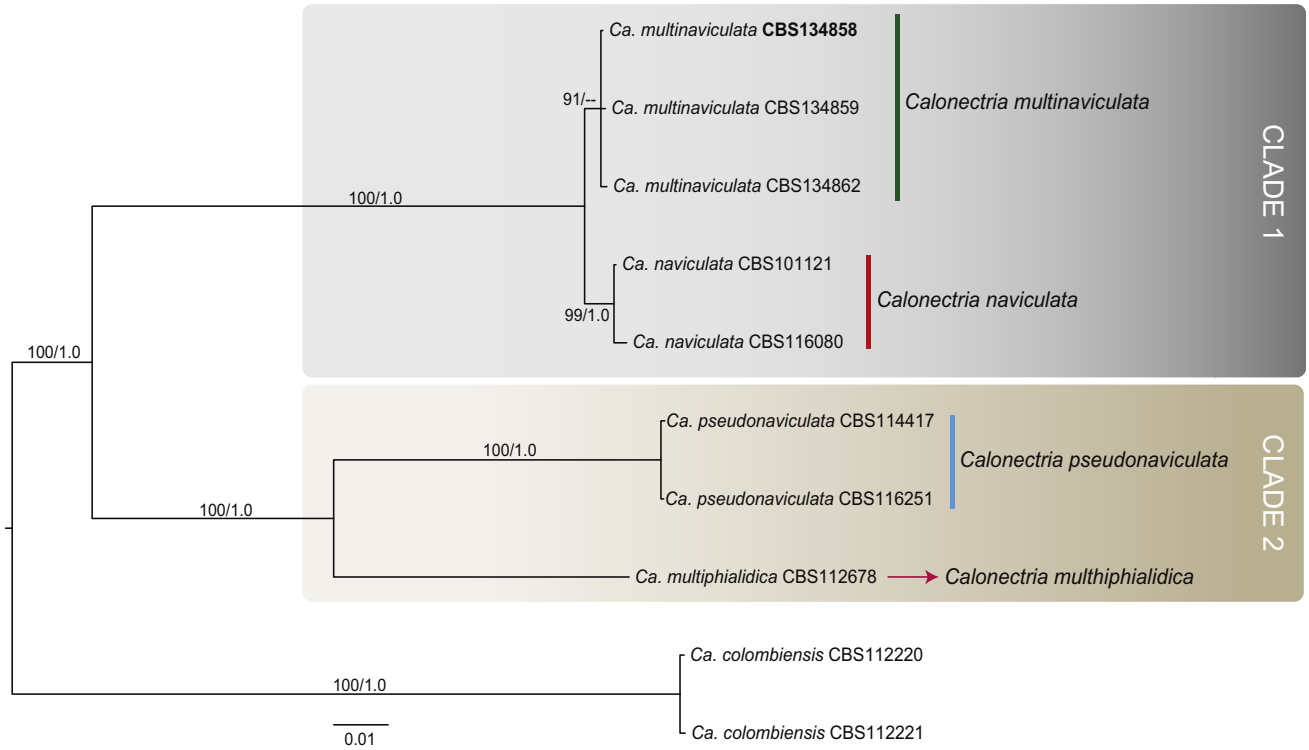

Fig. 3. Consensus phylogram of 15 trees resulting from a Bayesian analysis of the combined four gene sequence alignment of the *Calonectria naviculata* complex. Accession numbers in **bold** represent ex-type strains. Bayesian posterior probabilities and Maximum Parsimony bootstrap support values are indicated at the nodes and the scale bar represents the number of expected changes per site. The tree was rooted to *Ca. colombiensis* (CBS 112220, CBS 112221).

Taxonomy

Morphological observations (Table 3) supported by phylogenetic inference showed that the majority of the strains collected in this study belonged to *Ca. pteridis*. The remaining isolates are shown to represent several distinct taxa that are provided with names in *Calonectria*. Furthermore, *Ca. metrosideri* is invalid, as Alfenas *et al.* (2013a) did not include collection and specimen details and it is, therefore, validated here. *Calonectria pseudopteridis* (= *Cylindrocladium macrosporum*) is resurrected to species rank based on phylogenetic inference.

Calonectria brassiana R.F. Alfenas, L. Lombard & Crous, **sp. nov.** MycoBank MB810001. Fig. 5.

Etymology: Name refers to *Eucalyptus brassiana*, the plantation tree species associated with the soil from which this fungus was isolated.

Sexual morph not observed. *Conidiophores* consist of a stipe bearing a penicillate arrangement of fertile branches, stipe extension, and terminal vesicle; stipe septate, hyaline, smooth, 55–155 × 5–8 µm; stipe extensions septate, straight to flexuous, 90–172 µm long, 2–3 µm wide at the apical septum, terminating in ellipsoidal to narrowly obpyriform vesicles, 3–7 µm diam. *Conidiogenous apparatus* 50–80 µm long, 50–135 µm wide; primary branches aseptate, 20–30 × 4–6 µm, secondary branches aseptate, 15–25 × 3–6 µm, and tertiary branches aseptate, 10–17 × 3–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 9–15 × 3–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight to slightly curved, (35–)50–56(–65) × 3–5 µm (av. = 53 × 4 µm), L/W ratio = 12.91, 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies buff on the surface and sepia in reverse; extensive white aerial mycelium with moderate sporulation on the aerial mycelium; chlamydospores sparse, occurring throughout the medium and forming microsclerotia. Colonies moderately fast growing (40–60 mm diam) on MEA and OA, after 7 d at 25 °C.

Material examined: **Brazil**, Piauí state, Teresina, from soil collected in *Eucalyptus brassiana* plantation, Jul. 2011, R.F. Alfenas (**holotype** CBS H-21376, culture **ex-type** CBS 134855 = LPF378), CBS 134856 = LPF379, CBS 134857 = LPF380.

Note: The macroconidia of *Ca. brassiana* are larger than those of *Ca. eucalypticola*, *Ca. glaebicola*, *Ca. metrosideri*, *Ca. piauiensis*, *Ca. pseudoscoparia* and *Ca. pseudometrosideri,* but smaller than those of *Ca. candelabra* (Table 3).

Calonectria duoramosa R.F. Alfenas, L. Lombard & Crous, **sp. nov.** MycoBank MB810002. Fig. 6.

Etymology: Name refers to the two levels of fertile branches formed in the conidiogenous apparatus of this fungus.

Sexual morph not observed. *Conidiophores* consist of a stipe bearing a penicillate arrangement of fertile branches, stipe extension, and terminal vesicle; stipe septate, hyaline, smooth, 45–95 × 4–7 µm; stipe extensions septate, straight to flexuous, 175–310 µm long, 3–5 µm wide at the apical septum, terminating in acicular to clavate vesicles, 4–6 µm diam. *Conidiogenous apparatus* 20–60 µm long, 30–50 µm wide; primary branches aseptate, 20–30 × 4–6 µm and secondary branches aseptate, 10–20 × 3–5 µm, each terminal branch producing 2–6

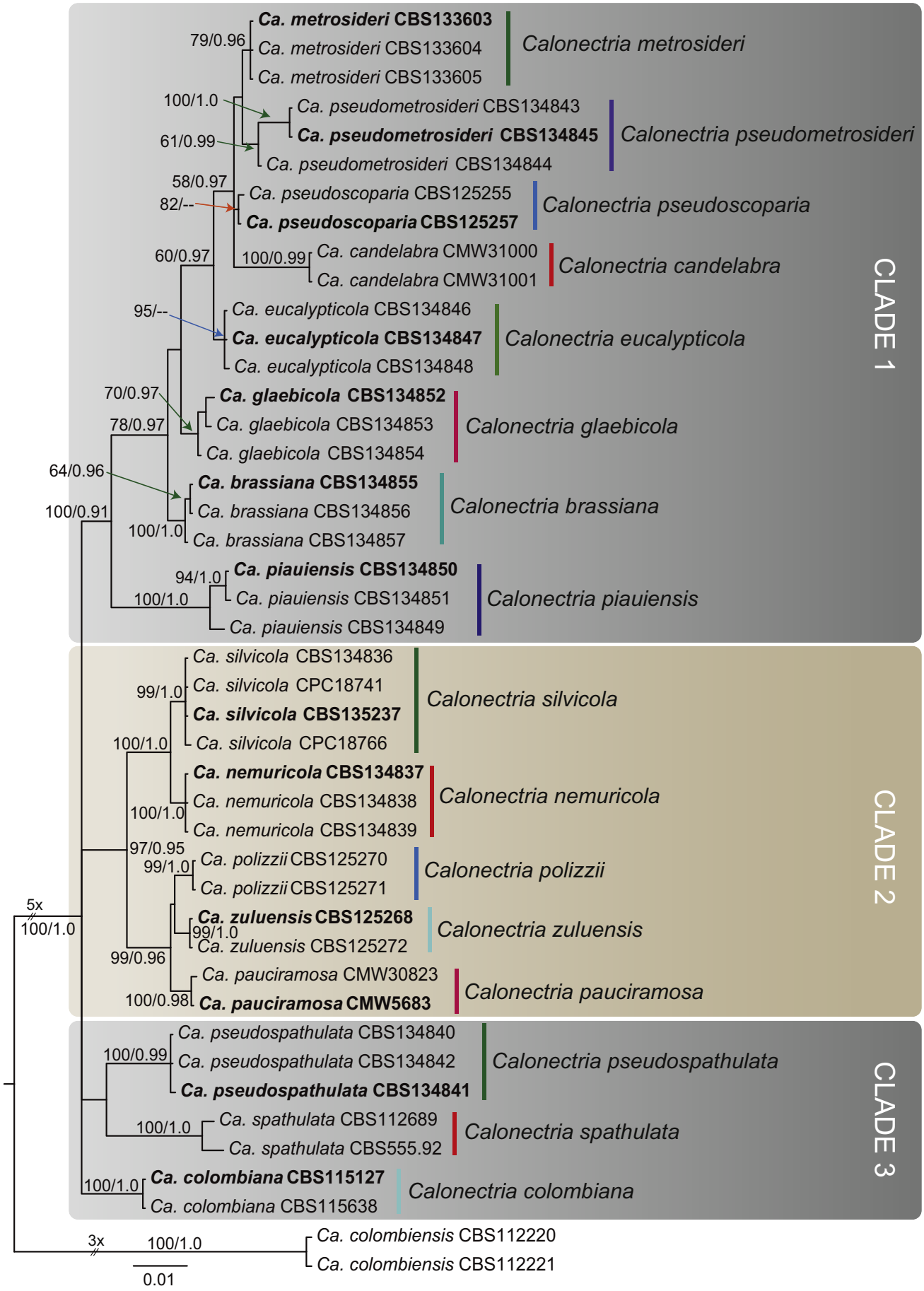

Fig. 4. Consensus phylogram of 2 348 trees resulting from a Bayesian analysis of the combined four gene sequence alignment of the *Calonectria candelabra* complex. Accession numbers in **bold** represent ex-type strains. Bayesian posterior probabilities and Maximum Parsimony bootstrap support values are indicated at the nodes and the scale bar represents the number of expected changes per site. The tree was rooted to *Ca. colombiensis* (CBS 112220, CBS 112221).

Table 3. Morphological characterisitics of *Calonectria* spp. included in this study.

Species	Perithecia		Asci	Ascospores		Conidiogenous apparatus		Stipe extention	Vesicle		Macroconidia			Reference
	Size (µm)	Shape	Size (µm)	Size (µm)	Septation	Size (µm)	Branches	Size (µm)	Diam (µm)	Shape	Size (µm)	Septation	Length/ Diam ratio	
***Calonectria brassicae* species complex**														
Ca. brachiatica						40–81 × 35–84	5	134–318 × 4–5	5–7	clavate	(37–)40–48(–50) × 4–6	1(–2)	8.8	Lombard *et al.* (2009)
Ca. brassicae						35–75 × 15–60	5	140–350 × 2.5–3	2–6	clavate	(38–)40–55(–65) × 3.5–6	1	11.78	Crous (2002)
Ca. clavata	360–630 × 290–500	subglobose to ovoid	53–155 × 10–22.5	(30–)40–50(–54) × (4–)5–6(–6.5)	1(–3)	40–70 × 25–50	4	60–110 × 5–6	3–4	narrowly clavate	(44–)50–70(–80) × 4–6	1(–3)	13	Crous (2002)
Ca. duoramosa						20–60 × 30–50	2	175–310 × 3–5	4–6	acicular to clavate	(35–)44–48(–55) × 3–5	1	11.38	This study
Ca. ecuadoriae						30–100 × 30–100	7	200–300 × 2–3	3–5	clavate	(45–)48–55(–65) × 4–5	1(–3)	11.33	Crous *et al.* (2006)
Ca. gracilipes	350–400 × 300–380	subglobose to ovoid	80–120 × 12–18	(28–)33–40(–45) × (5–)6–7(–7.5)	1	30–70 × 25–35	3	150–260 × 2.5–3	3–4	clavate	(35–)40–48(–60) × 4–6	1	10	Crous (2002)
Ca. gracilis	350–400 × 330–380	subglobose to ovoid	75–100 × 8–15	(27–)33–45(–50) × (4–)4.5–5(–6)	1	60–100 × 30–70	4	160–350 × 2–3	2–11	clavate	(40–)53–58(–65) × 3.5–**5**	1(–3)	12.44	Crous (2002)
Ca. orientalis						54–174 × 67–92	5	90–218 × 5–10	5–10	clavate to broadly clavate	(43–)46–50(–53) × 4–5	1	12	Lombard *et al.* (2010c)
Ca. paraensis						45–55 × 60–75	2	120–195 × 3–5	4–6	clavate	(35–)40–43(–50) × 3–6	1	8.85	This study
Ca. pini						49–81 × 35–84	3	121–266 × 5–7	4–6	clavate	(37–)40–48(–50) × 4–6	1	8.8	Lombard *et al.* (2010c)
Ca. pseudobrassicae						50–115 × 60–100	3	190–300 × 3–5	3–5	clavate	(30–)39–42(–48) × 4–6	1	8.04	This study
Ca. quinqueramosa	160–400 × 115–250	pyriform to subglobose	50–105 × 10–25	(25–)39–42(–50) × 5–7	1	30–60 × 35–65	5	170–340 × 2–4	3–5	narrowly clavate to clavate	(45–)57–61(–70) × 4–6	1	11.57	This study
Ca. robigophila						15–60 × 30–70	6	125–225 × 3–4	4–5	acicular to clavate	(45–)49–52(–60) × 3–5	1	12.6	This study
Ca. telluricola						45–95 × 40–80	4	100–225 × 2–4	3–6	clavate	(35–)40–42(–50) × 3–6	1	9.13	This study
***Calonectria candelabra* species complex**														
Ca. brassiana						50–135 × 50–80	3	90–172 × 2–3	3–7	ellipsoid to narrowly obpyriform	(35–)50–56(–65) × 3–5	1	12.91	This study
Ca. candelabra	350–450 × 300–350	subglobose to ovoid	70–130 × 7–15	(40–)45–50(–60) × 5–6	1	30–70 × 50–80	5	100–220 × 3–3.5	5–8	ellipsoid to narrowly obpyriform	(45–)58–68(–80) × 4–5(–6)	1	13.33	Crous (2002)
Ca. colombiana	270–410 × 175–285	subglobose to ovoid	87–162 × 12–18	(28–)31–36(–40) × 3–5	1	38–115 × 35–91	4	143–173 × 5–7	8–12	obpyriform to ellipsoidal	(33–)35–39(–40) × 3–4	1	12.33	Lombard *et al.* (2010b)
Ca. eucalypticola						45–75 × 35–62	3	145–170 × 2–4	5–7	ellipsoid to obpyriform	(43–)49–52(–55) × 3–5	1	12.2	This study

Table 3. (Continued).

Species	Perithecia		Asci	Ascospores		Conidiogenous apparatus		Stipe extention	Vesicle		Macroconidia			Reference
	Size (µm)	Shape	Size (µm)	Size (µm)	Septation	Size (µm)	Branches	Size (µm)	Diam (µm)	Shape	Size (µm)	Septation	Length/ Diam ratio	
Ca. glaebicola						25–40 × 27–45	2	100–165 × 2–4	3–5	ellipsoid to narrowly obpyriform	(45–)50–52(–55) × 3–5	1	12.06	This study
Ca. metrosideri						60–75 × 40–65	4	90–170 × 2–4	5–9	spathulate to obpyriform	(40–)44–46(–51) × 3–5	1	11.25	Alfenas *et al.* (2013a)
Ca. mossambicensis						37–87 × 19–59	3	91–203 × 2–6	2–8	obpyriform to ellipsoidal	(35–)38–46(–50) × 3–6	1	10.5	Crous *et al.* (2013)
Ca. nemuricola						50–80 × 40–60	4	150–205 × 6–12	7–13	obpyriform	(40–)44–46(–50) × 3–5	1	11.06	This study
Ca. pauciramosa	250–400 × 170–300	subglobose to ovoid	70–140 × 8–25	(30–)33–38(–40) × 6–7(–8)	1	20–50 × 35–85	3	120–230 × 2–3	5–11	obpyriform to ellipsoidal	(30–)45–55(–60) × (3.5–)4–5	1	12.5	Schoch *et al.* (1999)
Ca. piauiensis						35–80 × 20–60	2	95–130 × 2–3	3–7	ellipsoid to narrowly obpyriform	(38–)47–52(–60) × 3–5	1	11.27	This study
Ca. polizzii						28–51 × 27–57	3	111–167 × 5–6	6–9	obpyriform to ellipsoidal	(31–)32–42(–49) × 3–5	1	9.25	Lombard *et al.* (2010a)
Ca. pseudometrosideri						30–76 × 45–65	3	160–210 × 2–4	5–7	ellipsoid to obpyriform	(40–)49–52(–60) × (3–)4.5(–5)	1	11.34	This study
Ca. pseudoscoparia						52–74 × 34–87	4	124–201 × 4–6	6–10	obpyriform to ellipsoidal	(41–)45–51(–52) × 3–3	1	12	Lombard *et al.* (2010b)
Ca. pseudospathulata						60–100 × 30–70	3	145–190 × 2–4	7–10	obpyriform	(35–)41–44(–50) × 3–5	1	10.46	This study
Ca. silvicola						45–105 × 35–90	3	130–195 × 3–4	7–10	obpyriform	(30–)40–42(–50) × 3–5	1	9.17	This study
Ca. spathulata	300–500 × 200–350	subglobose to ovoid	90–150 × 13–17	(38–)45–55(–60) × (4.5–)5–6(–7)	(1)–3	60–100 × 30–70	3	150–300 × 3–4	6–10	ellipsoid to obpyriform to clavate	(48–)75–90(–100) × (4–)5–6	(1–)3(–6)	13.33	Crous 2002
Ca. zuluensis	292–394 × 170–285	subglobose to ovoid	92–140 × 10–16	(26–)29–34(–38) × 4–5	1	37–70 × 35–67	3	110–171 × 5–8	6–10	ellipsoid to obpyriform	(31–)34–38(–40) × 3–5	1	8	Lombard *et al.* (2010a)
***Calonectria cylindrospora* species complex**														
Ca. brasiliensis						81–103 × 58–90	3	204–266 × 6–7	7–11	ellipsoid to obpyriform	(35–)36–40(–41) × 3–5	1	10.86	Lombard *et al.* (2010a)
Ca. cerciana						62–113 × 70–98	4	148–222 × 5–6	8–13	fusiform to obpyriform	(37–)41–46(–49) × 5–6	1	8.8	Lombard *et al.* (2010d)
Ca. cylindrospora	280–520 × 280–400	globose to subglobose	75–100 × 8–15	(24–)30–40(–49) × (4–)5–6(–8)	1	60–100 × 60–110	6	150–200 × 3–4	6–8	ellipsoid to pyriform or clavate	(40–)42–50(–66) × 3–4(–5)	1	11.25	Crous (2002)
Ca. densa						49–78 × 63–123	4	149–192 × 5–6	10–12	ovoid to ellipsoid to sphaeropedunculate	(47–)50–58(–62) × (5–)6	1	9	Lombard *et al.* (2010d)
Ca. hodgesii						61–72 × 45–65	3	136–196 × 2–4	6–11		(44–)49–51(–55) × 3–5	1	12.5	Alfenas *et al.* (2013b)

(*continued on next page*)

Table 3. (Continued).

Species	Perithecia		Asci	Ascospores		Conidiogenous apparatus		Stipe extention	Vesicle		Macroconidia			Reference
	Size (µm)	Shape	Size (µm)	Size (µm)	Septation	Size (µm)	Branches	Size (µm)	Diam (µm)	Shape	Size (µm)	Septation	Length/ Diam ratio	
										pyriform to ellipsoidal or ovoid to sphaeropedunculate				
Ca. humicola						43–71 × 42–49	3	126–157 × 4–5	10–12	globose to ovoid to sphaeropedunculate	(45–)48–54(–56) × 4–5	1	10.2	Alfenas *et al.* (2013b)
Ca. insularis	350–450 × 300–350	subglobose to ovoid	70–125 × 7–18	(27–)30–36(–42) × 5–6(–7)	1	45–90 × 45–80	6	110–250 × 4–5	4–13	obpyriform to broadly ellipsoidal	(33–)40–50(–60) × 3.5–4	1	11.25	Crous (2002)
Ca. leucothoës						25–50 × 50–80	6	160–250 × 3–6	6–11.5	ellipsoid to obpyriform	(45–)68–78(–97) × (4–)5–5.5(–6.5)	(1–)3(–6)	14.6	Crous (2002)
Ca. maranhensis						45–65 × 45–71	3	125–190 × 3–5	7–11	ellipsoid, obpyriform to sphaeropedunculate	(50–)56–58(–65) × (3–)5(–6)	1	11.85	This study
Ca. propaginicola						40–75 × 31–85	4	130–250 × 2–5	5–12	ellipsoid, obpyriform to sphaeropedunculate	(40–)48–51(–55) × 3–5	1	12.67	This study
Ca. pseudocerciana						50–90 × 50–95	3	160–250 × 2–5	4–10	clavate or ellipsoidal to obpyriform	(45–)53–55(–65) × (3–)4.5(–5)	1	11.95	This study
Ca. pseudohodgesii						50–90 × 40–95	3	130–190 × 2–5	7–12	obpyriform to sphaeropendunculate	(35–)43–46(–55) × 3–5	1	11.95	This study
Ca. pseudospatiphylli	350–550 × 300–500	globose to subglobose	90–150 × 7–25	(30–)38–45(–55) × 5–6	1(–3)	70–100 × 25–70	4	100–250 × 2.5–3.5	8–12	sphaeropendunculate to ellipsoidal	(40–)47–55(–60) × 4–5	1(–3)	13	Crous (2002)
Ca. spathiphylli	380–655 × 340–650	subglobose to ovoid	120–230 × 7–25	(22–)40–52(–65) × (3–)4.5–5.5(–7)	1(–3)	60–150 × 40–90	4	170–260 × 3–4	8–15	globoid or ellipsoid to obpyriform	(45–)46–80(–120) × (5–)6(–7)	1(–3)	11.67	Crous (2002)
Ca. sulawesiensis						43–81 × 41–79	5	113–262 × 5–7	5–7	broadly clavate to ellipsoid	(41–)45–51(–54) × (3–)4(–6)	1	12	Lombard *et al.* (2010b)
Ca. variabilis	260–450 × 220–350	globose to ovoid	90–120 × 10–20	(34–)38–50(–60) × 4–5(–6)	1(–3)	40–70 × 20–100	3	130–250 × 2–3	6–11	sphaeropendunculate to ovoid or ellipsoid to clavate	(48–)68–77(–85) × 4–5(–7)	(1–)3(–4)	14.6	Crous (2002)
***Calonectria pteridis* species complex**														
Ca. gordoniae							4		3–6	narrowly clavate	(45–)62(–81) × 4–6	1	12.6	Leahy *et al.* (2000)
Ca. ovata	350–550 × 350–450	globose to ovoid	70–120 × 10–25	(35–)55–70(–90) × (4–)5–6	1	30–55 × 20–45	3	185–230 × 2.5–4	8–14	ovate	(50–)65–80(–110) × 4–6	1(–3)	10.9	Crous 2002
Ca. pseudovata						55–121 × 75–105	3	140–280 × 3–6	8–12	ovate to ellipsoidal	(55–)67–70(–80) × (4–)5(–7)	1	13.73	This study
Ca. pteridis	300–500 × 280–350	subglobose to ovoid	70–120 × 10–25	(30–)45–60(–75) × (4–)5–6(–7)	1(–3)	75–150 × 45–170	5	150–300 × 2.5–4	4–6	clavate to narrowly ellipsoidal	(50–)70–100(–130) × (4–)5–6	1(–3)	14.91	Crous 2002
***Calonectria naviculata* species complex**														
Ca. multinaviculata						30–65 × 40–70	3	75–140 × 2–5	4–7	naviculate	(40–)44–49(–52) × (2–)3.5(–4)	1	13.72	This study

Table 3. (Continued).

Species	Perithecia		Asci	Ascospores		Conidiogenous apparatus		Stipe extention	Vesicle		Macroconidia			Reference
	Size (µm)	Shape	Size (µm)	Size (µm)	Septation	Size (µm)	Branches	Size (µm)	Diam (µm)	Shape	Size (µm)	Septation	Length/ Diam ratio	
Ca. multiphialidica						70–150 × 70–150	8	170–300 × 4–5	8–16	sphaeropedunculate to clavate	(45–)48–55(–65) × (4–)4.5(–5)	1	11.78	Crous *et al.* (2004a,b)
Ca. naviculata	350–450 × 350–400	globose to ovoid	70–100 × 8–12	(20–)40–48(–52) × (3–)5–6(–6.5)	3	45–90 × 25–100	4	150–200 × 3–4.5	5–11	naviculate to ellipsoidal	(40–)42–50 × 3(–4)	1	15	Crous 2002
Ca. pseudonaviculata						30–60 × 30–45	4	120–180 × 3–4	4–8	naviculate	(50–)55–65(–80) × 4–5(–6)	1(–3)	18	Crous 2002

phialides; phialides doliiform to reniform, hyaline, aseptate, 7–15 × 3–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (35–)44–48(–55) × 3–5 µm (av. = 46 × 4 µm), L/W ratio = 11.38, 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies umber to fawn on the surface and dark brick in reverse; sparse aerial mycelium; chlamydospores sparse, occurring throughout the medium, with moderate to extensive sporulation on the aerial mycelium. Colonies slow growing (33–43 mm diam) on MEA, and fast growing (79–83 mm diam) on OA, after 7 d at 25 °C.

Materials examined: **Brazil**, Pará state, Monte Dourado, from soil collected in tropical rainforest, Aug. 2010, R.F. Alfenas (**holotype** CBS H-21380, culture **ex-type** CBS 134656 **=** LPF434); from soil collected in *Eucalyptus* plantation, Aug. 2010, R.F. Alfenas, culture LPF453.

Notes: Calonectria duoramosa characteristically forms only two levels of branching in its conidiogenous apparatus distinguishing it from *Ca. ecuadoriae* and *Ca. robigophila*. The macroconidia of *Ca. duoramosa* are also slightly smaller than those of *Ca. ecuadoriae* and *Ca. robigophila* (Table 3).

Calonectria eucalypticola R.F. Alfenas, L. Lombard & Crous, **sp. nov.** MycoBank MB810003. Fig. 7.

Etymology: Name refers to the host genus, *Eucalyptus*, from which this fungus was first isolated.

Sexual morph not observed. *Conidiophores* consist of a stipe bearing a penicillate arrangement of fertile branches, stipe extension, and terminal vesicle; stipe septate, hyaline, smooth, 50–242 × 5–10 µm; stipe extensions septate, straight to flexuous, 145–170 µm long, 2–4 µm wide at the apical septum, terminating in ellipsoidal to obpyriform vesicles, 5–7 µm diam. *Conidiogenous apparatus* 35–62 µm long, 45–75 µm wide; primary branches aseptate, 20–25 × 4–6 µm; secondary branches aseptate, 16–19 × 3–5 µm, tertiary branches aseptate, 9–16 × 2–4 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 6–12 × 2–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight to slightly curved, (43–)49–52(–55) × 3–5 µm (av. = 50 × 4 µm), L/W ratio = 12.20, 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies cinnamon to dark brick on the surface and sepia in reverse; moderate to extensive sporulation on the aerial mycelium, especially at the margins; chlamydospores moderate occurring throughout the medium forming microsclerotia. Colonies slow growing (40–45 mm diam) on MEA, and moderate growing (50–55 mm diam) on OA, after 7 d at 25 °C.

Materials examined: **Brazil**, Minas Gerais state, Santa Bárbara, from stem of *Eucalyptus* seedling, Dec. 2010, A.C. Alfenas (**holotype** CBS H-21359, culture **ex-type** CBS 134847 **=** LPF124); Bahia state, Eunápolis, from *Eucalyptus* leaf, Mar. 2012, A.C. Alfenas, CBS 134846 = LPF121; Pará state, Monte Dourado,

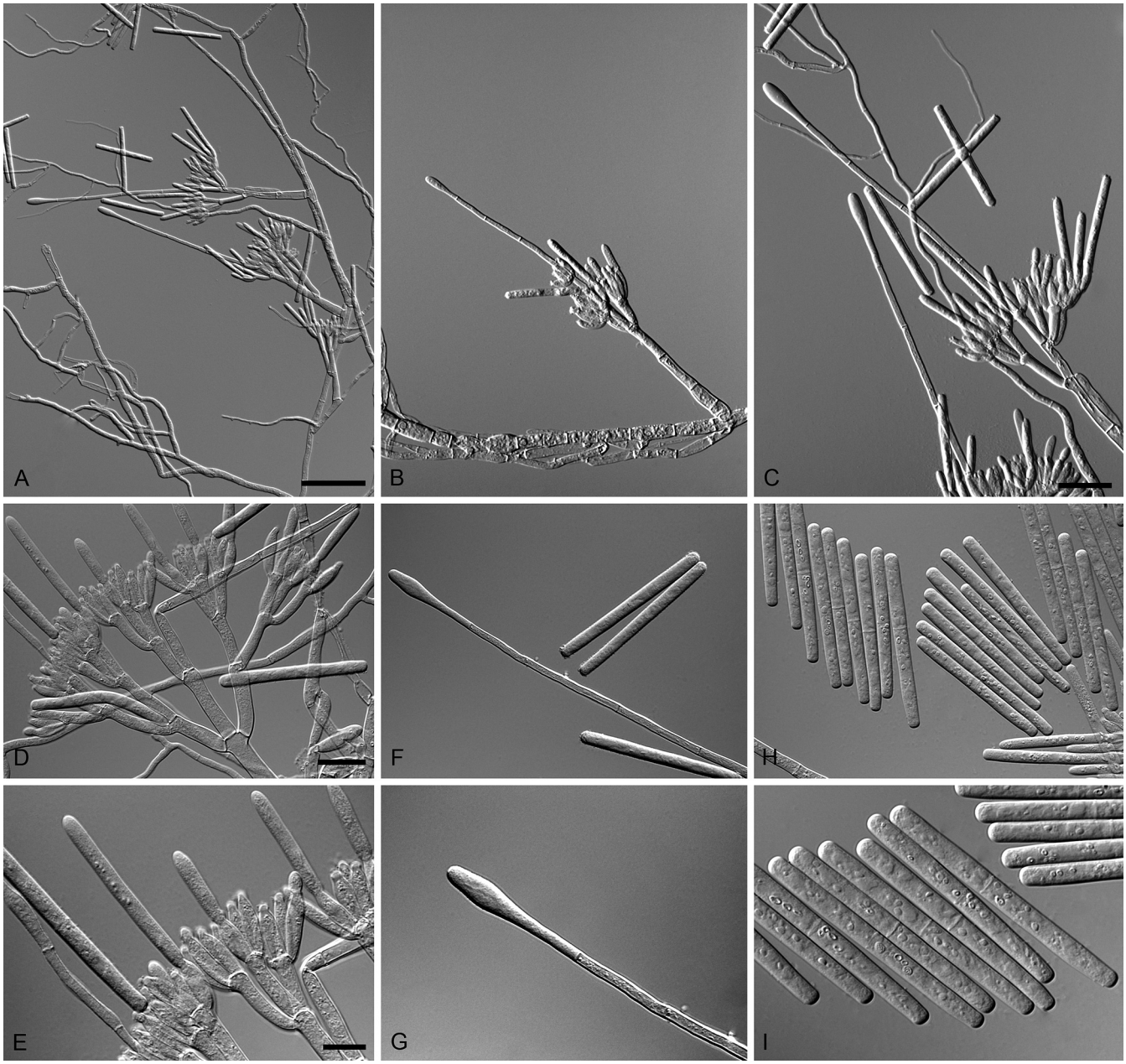

Fig. 5. *Calonectria brassiana* (ex-type CBS 134855). A–C. Macroconidiophores. D–E. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. F–G. Ellipsoidal to narrowly obpyriform vesicles. H–I. Macroconidia. Scale bars: A = 50 µm (apply to B); C = 20 µm; D = 10 µm (apply to F, H); E = 10 µm (apply to G, I).

from soil collected in *Eucalyptus* plantation, July 2012, R.F. Alfenas, CBS 134848 = LPF451.

Note: *Calonectria eucalypticola* can be distinguished from its closest relatives (Fig. 4) based on macroconidial dimensions and the number of fertile branches produced in the conidiogenous apparatus (Table 3).

Calonectria glaebicola R.F. Alfenas, L. Lombard & Crous, **sp. nov.** MycoBank MB810004. Fig. 8.

Etymology: Name refers to soil, the substrate from which this fungus was first isolated.

Sexual morph not observed. *Conidiophores* consist of a stipe bearing a penicillate arrangement of fertile branches, stipe extension, and terminal vesicle; stipe septate, hyaline, smooth, 50–130 × 5–7 µm; stipe extensions septate, straight to flexuous, 100–165 µm long, 2–4 µm wide at the apical septum, terminating in ellipsoidal to narrowly obpyriform vesicles, 3–5 µm diam. *Conidiogenous apparatus* 27–45 µm long, 25–40 µm wide; primary branches aseptate, 14–22 × 3–5 µm, secondary branches aseptate, 11–15 × 3–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 5–13 × 3–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight to slightly curved, (45–) 50–52(–55) × 3–5 µm (av. = 50 × 4 µm), L/W ratio = 12.06, 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies buff on the surface and sepia to umber in reverse; extensive aerial mycelium with moderate sporulation on the aerial mycelium; chlamydospores sparse, occurring throughout the medium forming microsclerotia.

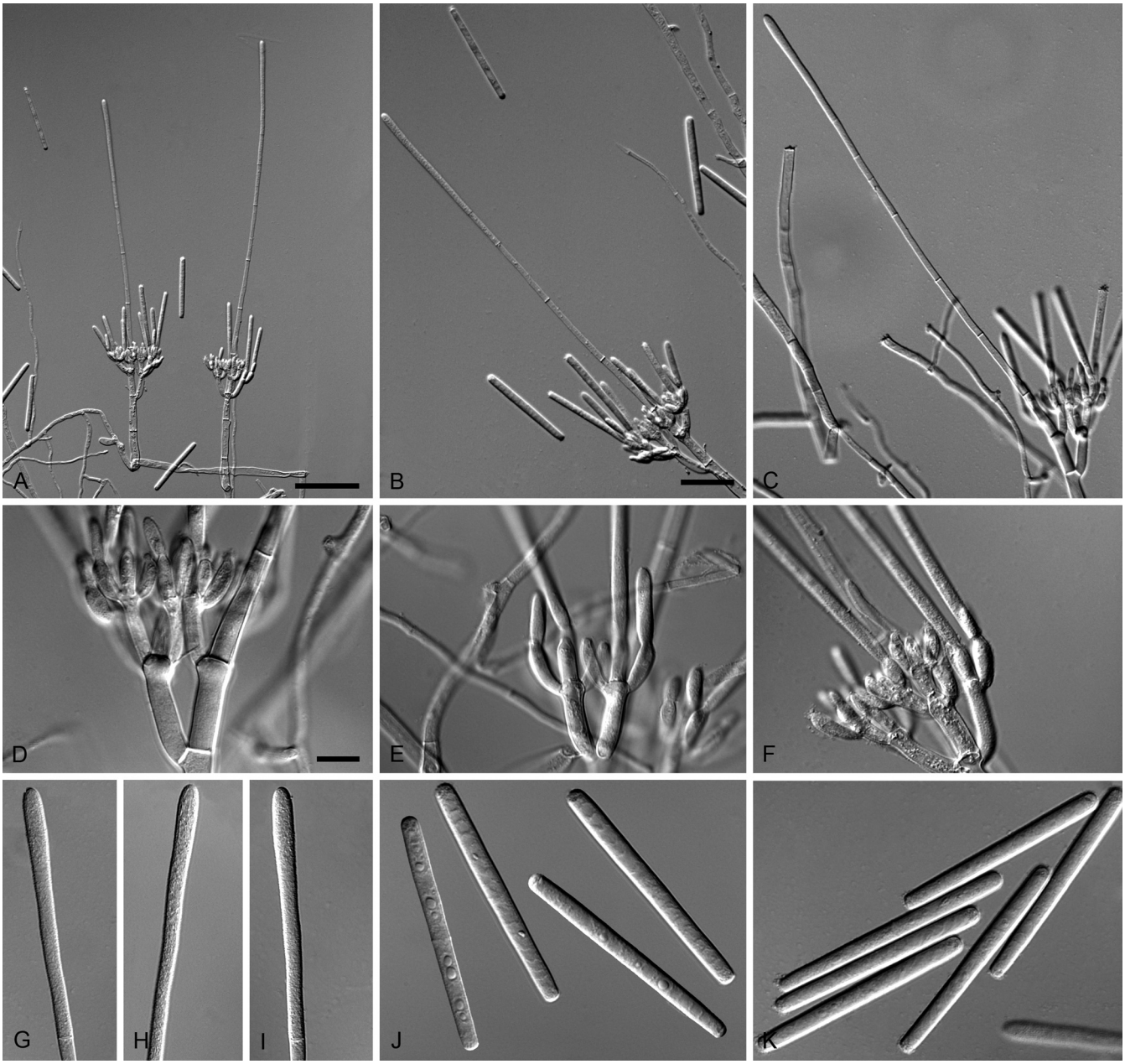

Fig. 6. *Calonectria duoramosa* (ex-type CBS 134656). A–C. Macroconidiophores. D–F. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. G–I. Clavate vesicles. J–K. Macroconidia. Scale bars: A = 50 µm; B = 50 µm (apply to C); D = 10 µm (apply to E–K).

Colonies moderate growing (45–60 mm diam) on MEA and OA, after 7 d at 25 °C.

Materials examined: **Brazil**, Minas Gerais state, Martinho Campos, from soil collected in *Eucalyptus* plantation, Jul. 2010; A.C. Alfenas (**holotype** CBS H-21378, culture **ex-type** CBS 134852 **=** LPF406); Tocantins, Bico do Papagaio, from *Eucalyptus* leaf, Aug. 2012, R.F. Alfenas, CBS 134853 = LPF407, CBS 134854 = LPF408.

Note: *Calonectria glaebicola* is morphologically similar to its closest relatives (Fig. 4), from which it can be distinguished only by phylogenetic inference.

Calonectria maranhensis R.F. Alfenas, L. Lombard & Crous, **sp. nov.** MycoBank MB810005. Fig. 9.

Etymology: Name refers to Maranhão state, Brazil, the region where this fungus was first collected.

Sexual morph not observed. *Conidiophores* consist of a stipe bearing a penicillate arrangement of fertile branches, stipe extension, and terminal vesicle; stipe septate, hyaline, smooth, 55–105 × 6–9 µm; stipe extensions septate, straight to flexuous, 125–190 µm long, 3–5 µm wide at the apical septum, terminating in ellipsoidal, obpyriform to sphaeropedunculate vesicles, 7–11 µm diam. *Conidiogenous apparatus* 45–71 µm long, 45–65 µm wide; primary branches aseptate, 20–45 × 3–6 µm; secondary branches aseptate, 15–20 × 3–5 µm, tertiary branches aseptate, 11–16 × 3–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 8–15 × 3–5 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight to slightly curved, (50–)56–58(–65) × (3–)5(–6) µm (av. = 57 × 5 µm), L/W ratio = 11.85, 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

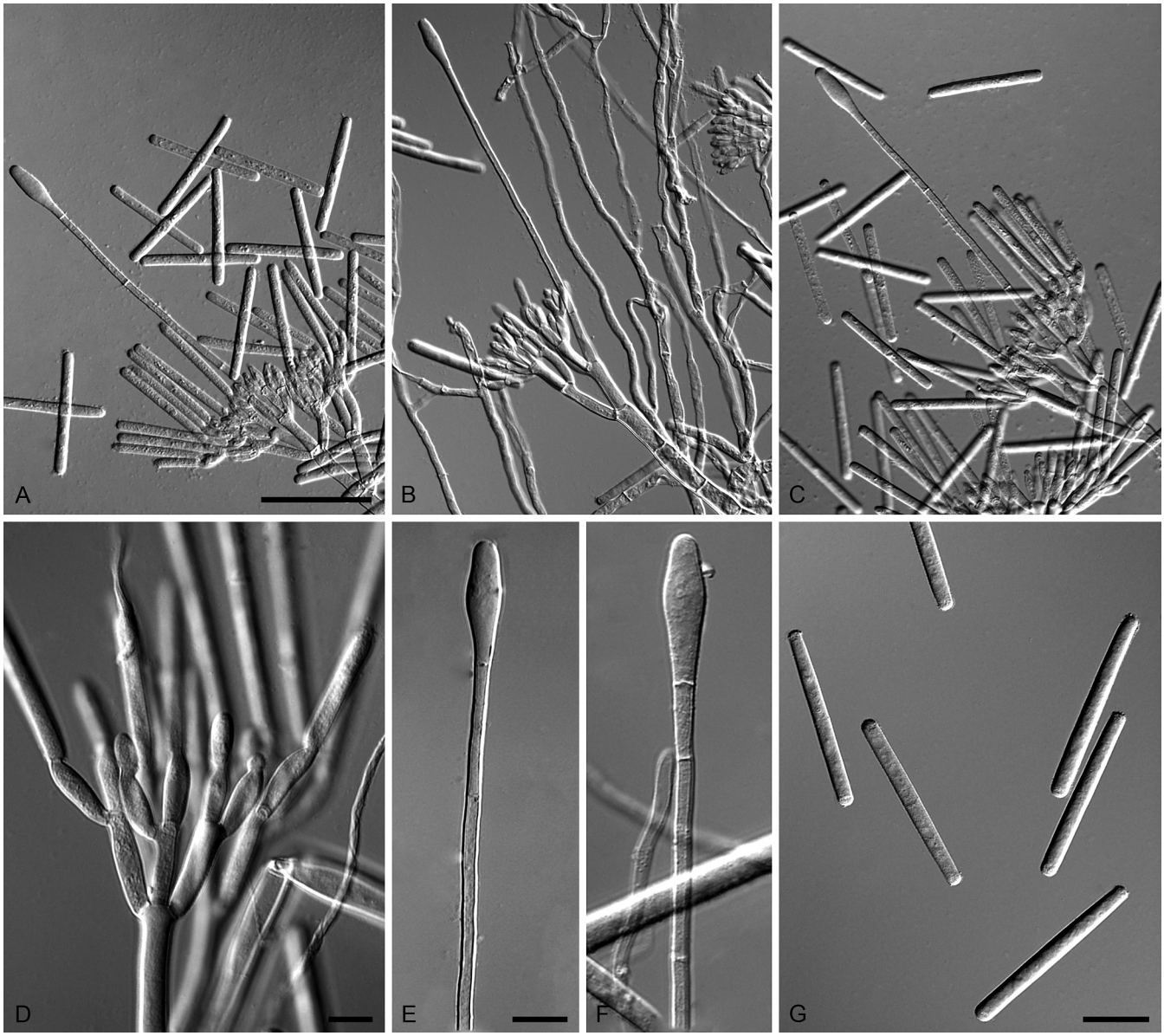

Fig. 7. *Calonectria eucalypticola* (ex-type CBS 134847). A–C. Macroconidiophores. D. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. E–F. Ellipsoidal to obpyriform vesicles. G. Macroconidia. Scale bars: A = 50 µm (apply to B–C); D = 10 µm; E = 10 µm (apply to F); G = 10 µm.

Culture characteristics: Colonies greyish sepia to dark brick on the surface and sepia to umber in reverse; extensive white aerial mycelium with moderate sporulation on the aerial mycelium; chlamydospores moderate to extensive occurring throughout the medium. Colonies moderately slow growing (50–55 mm diam) on MEA, and fast growing (80–85 mm diam) on OA, after 7 d at 25 °C.

Materials examined: **Brazil**, Maranhão state, Açailândia, from *Eucalyptus* leaf, May 2011, A.C. Alfenas (**holotype** CBS H-21360, culture **ex-type** CBS 134811 **=** LPF142), CBS 134812 = LPF143; Imperatriz, from soil in *Eucalyptus* plantation, May 2011, R.F. Alfenas, CBS 134825 = LPF370.

Note: Calonectria maranhensis can be distinguished from *Ca. brasiliensis, Ca. hodgesii, Ca. sulawesiensis* and *Ca. variabilis* by the dimensions of their macroconidia (Table 3).

Calonectria metrosideri R.F. Alfenas, O.L. Pereira, Crous & A.C. Alfenas, **sp. nov.** MycoBank MB810023.

≡ *Calonectria metrosideri* R.F. Alfenas, O.L. Pereira, Crous & A.C. Alfenas, Forest Pathology 43: 262. 2013. Nom. inval., Art 37.7.

See Alfenas *et al.* (2013a) for description and illustrations.

Material examined: **Brazil**, Minas Gerais state, Viçosa, Universidade Federal de Viçosa, forest nursery, isolated from leaf of *Metrosideros polymorpha*, Apr 2010, R.F. Alfenas (**holotype** CBS H-21146, culture **ex-type** CBS 133603).

Note: The original description of *Ca. metrosideri* is invalid, as no type specimen was designated. This issue is now addressed, and the name validly published.

Calonectria multinaviculata R.F. Alfenas, L. Lombard & Crous, **sp. nov.** MycoBank MB810006. Fig. 10.

Entymology: Name refers to the multiple naviculate terminal vesicles formed by this fungus.

Sexual morph not observed. *Conidiophores* consist of a stipe bearing a penicillate arrangement of fertile branches, stipe extension, and terminal vesicle; stipe septate, hyaline, smooth, 45–90 × 5–7 µm; stipe extensions septate, straight to flexuous, 75–140 µm long, 2–5 µm wide at the apical septum, terminating in naviculate vesicles, 4–7 µm diam, abundant lateral stipe extension also present. *Conidiogenous apparatus* 30–65 µm long,

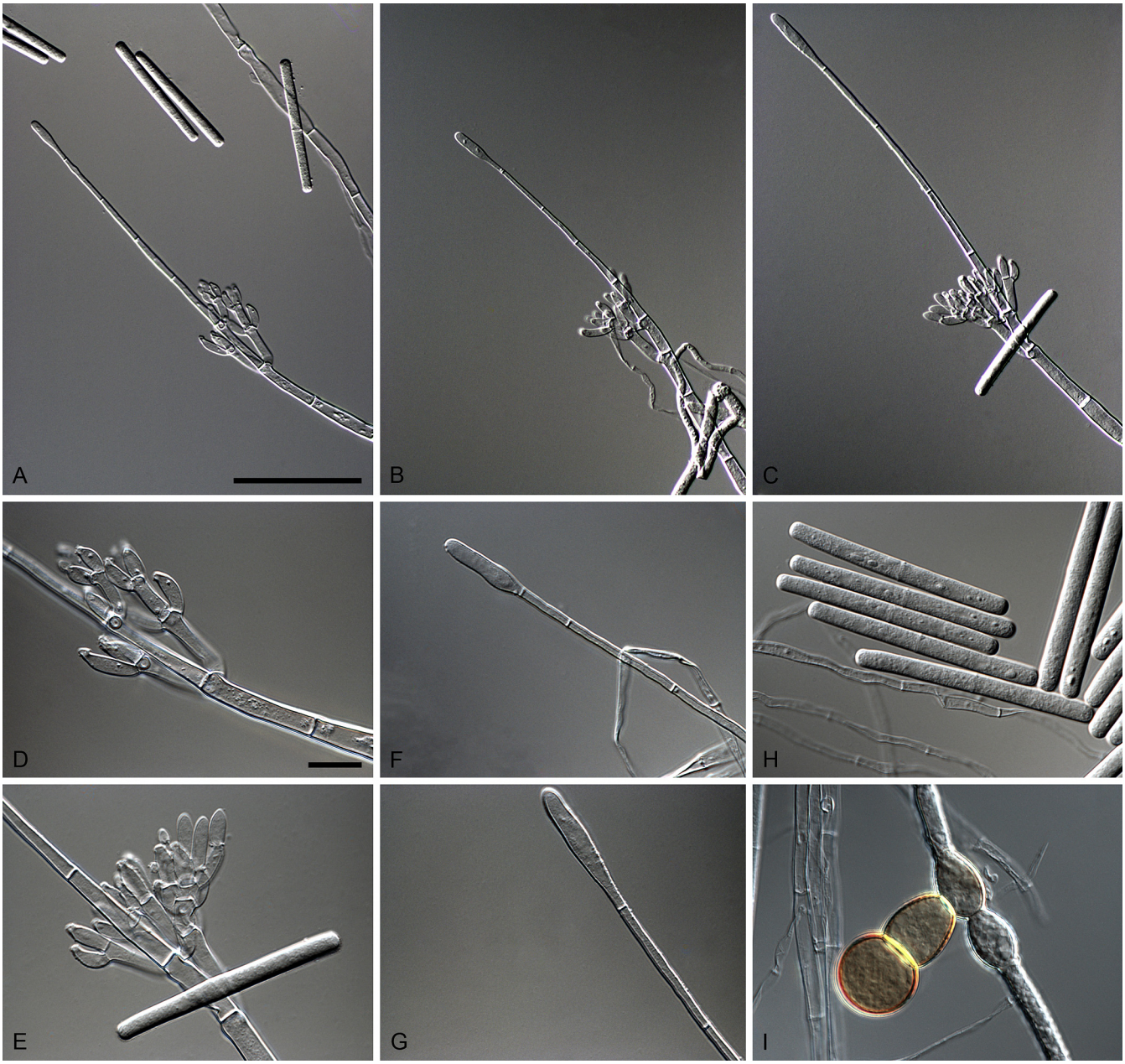

Fig. 8. *Calonectria glaebicola* (ex-type CBS 134852). A–C. Macroconidiophores. D–E. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. F–G. Ellipsoidal to narrowly obpyriform vesicles. H. Macroconidia. I. Chlamydospores. Scale bars: A = 50 µm (apply to B–C); D = 10 µm (apply to E–I).

40–70 µm wide; primary branches aseptate, 19–22 × 3–6 µm, secondary branches aseptate, 9–18 × 3–6 µm, tertiary branches aseptate, 9–12 × 2–4 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 6–12 × 2–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight to slightly curved, (40–)44–49(–52) × (2–)3.5(–4) µm (av. = 46 × 3.5 µm), L/W ratio = 13.72, 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies buff on the surface and sepia to umber in reverse; extensive white aerial mycelium with sparse to moderate sporulation on the aerial mycelium; chlamydospores not seen. Colonies moderately fast to fast growing (50–70 mm diam) on MEA and OA, after 7 d at 25 °C.

Materials examined: **Brazil**, Bahia state, Mucuri, from soil collected in *Eucalyptus* plantation, Aug. 2010; E. Zauza (**holotype** CBS 134858, preserved as metabolically inactive culture; culture **ex-type** CBS 134858 **=** LPF233), CBS 134862 = LPF472; Pará state, Monte Dourado, from soil collected in *Eucalyptus* plantation, July 2012, R.F. Alfenas, CBS 134859 = LPF418.

Note: *Calonectria multinaviculata* can be distinguished from *Ca. naviculata* by having fewer fertile branches in the conidiogenous apparatus, and having abundant lateral stipe extensions, which are absent in *Ca. naviculata* (Table 3).

Calonectria nemuricola R.F. Alfenas, L. Lombard & Crous, **sp. nov.** MycoBank MB810007. Fig. 11.

Etymology: Name refers to a forest, the habitat this fungus was collected from.

Sexual morph not observed. *Conidiophores* consist of a stipe bearing a penicillate arrangement of fertile branches, stipe extension, and terminal vesicle; stipe septate, hyaline, smooth, 50–105 × 6–12 µm; stipe extensions septate, straight to flexuous,

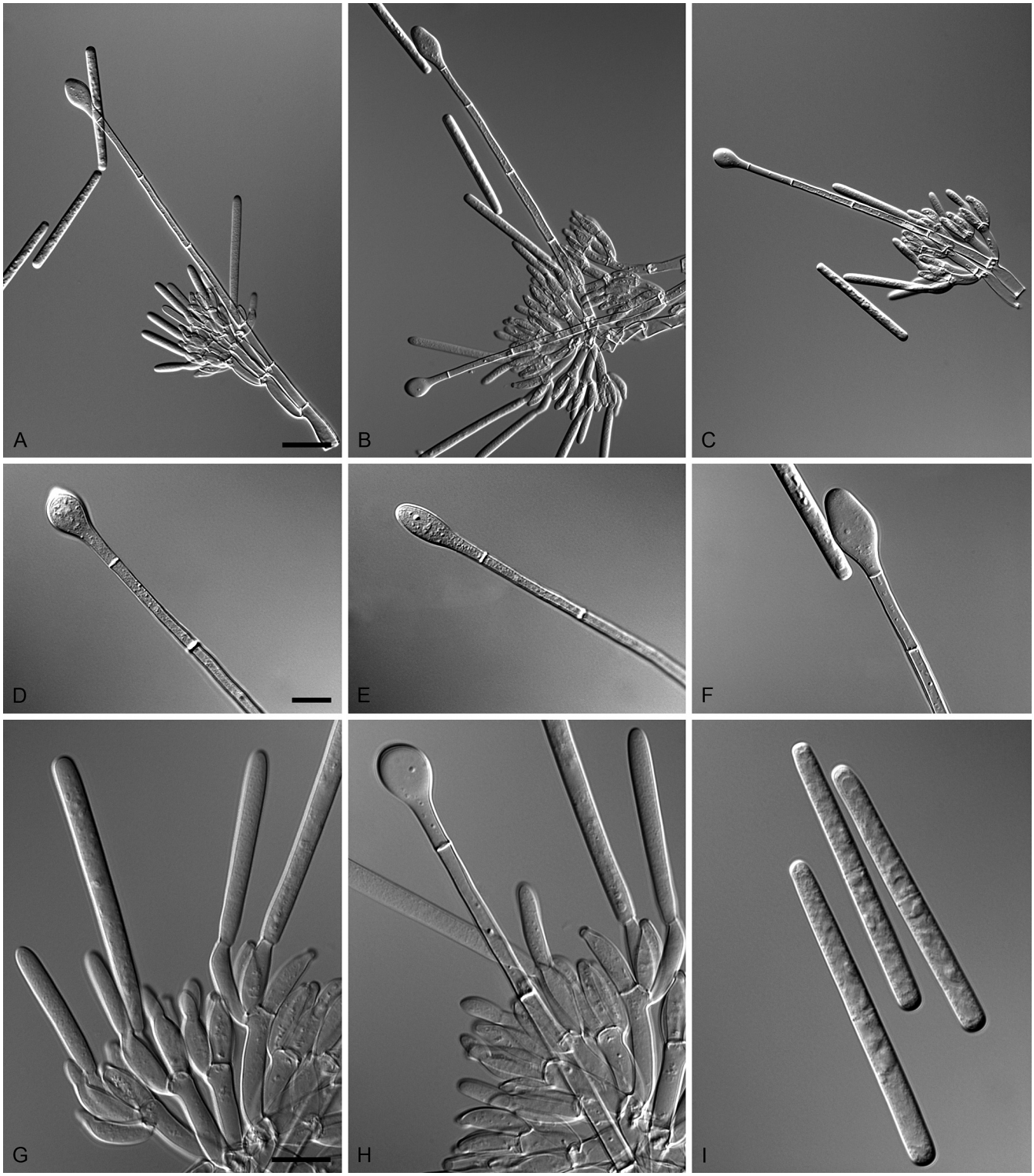

Fig. 9. *Calonectria maranhensis* (ex-type CBS 134811). A–C. Macroconidiophores. D–F. Ellipsoidal, obpyriform to sphaeropedunculate vesicles. G–H. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. I. Macroconidia. Scale bars: A = 20 µm (apply to B–C); D = 10 µm (apply to E–F); G = 10 µm (apply to H–I).

150–205 µm long, 2–4 µm wide at the apical septum, terminating in obpyriform vesicles, 7–13 µm diam. *Conidiogenous apparatus* 40–60 µm long, 50–80 µm wide; primary branches aseptate, 19–25 × 3–7 µm, secondary branches aseptate, 11–18 × 3–5 µm, tertiary branches aseptate, 9–12 × 3–5 µm, additional branches rare, (–4), aseptate, 7–10 × 3–4 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 5–11 × 2–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight to slightly curved, (40–) 44–46(–50) × 3–5 µm (av. = 45 × 4 µm), L/W ratio = 11.06, 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies buff on the surface and sepia to umber in reverse; extensive white aerial mycelium with sparse to moderate sporulation on the aerial mycelium; chlamydospores sparse, occurring throughout the medium, forming microsclerotia; Colonies fast growing (55–80 mm diam) on MEA and OA, after 7 d at 25 °C.

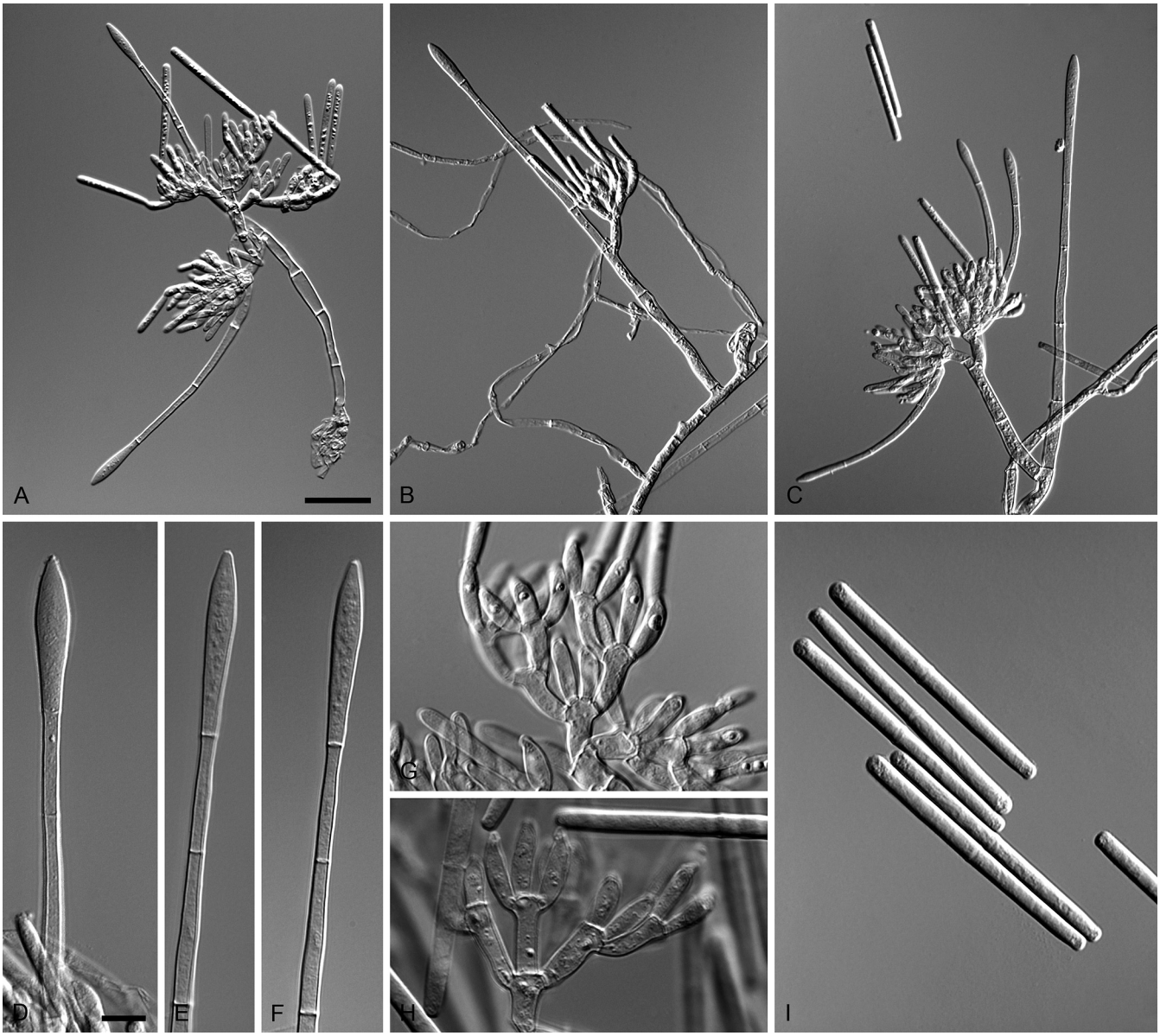

Fig. 10. *Calonectria multinaviculata* (ex-type CBS 134858). A–C. Macroconidiophores. D–F. Naviculate vesicles. G–H. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. I. Macroconidia. Scale bars: A = 50 µm (apply to B–C); D = 10 µm (apply to E–I).

Material examined: **Brazil**, Minas Gerais state, Araponga (Serra do Brigadeiro), from soil collected in tropical rainforest, Aug. 2010, A.C. Alfenas & P.W. Crous (**holotype** CBS H-21358, culture **ex-type** CBS 134837 **=** LPF085), CBS 134838 = LPF090, CBS 134839 = LPF094.

Note: The macroconidia of *Ca. nemuricola* are larger than those of *Ca. mossambicensis*, *Ca. polizzii*, *Ca. silvicola* and *Ca. zuluensis*, but smaller than those of *Ca. pauciramosa* (Table 3).

Calonectria paraensis R.F. Alfenas, L. Lombard & Crous, **sp. nov.** MycoBank MB810008. Fig. 12.

Etymology: Name refers to the Pará state in Brazil where the fungus was collected.

Sexual morph not observed. *Conidiophores* consist of a stipe bearing a penicillate suites of fertile branches, stipe extension, and terminal vesicle; stipe septate, hyaline, smooth, 52–110 × 5–7 µm; stipe extensions septate, straight to flexuous, 120–195 µm long, 3–5 µm wide at the apical septum, terminating in a clavate vesicle, 4–6 µm diam. *Conidiogenous apparatus* 45–55 µm long, 60–75 µm wide; primary branches aseptate, 18–24 × 4–6 µm, secondary branches aseptate, 14–23 × 3–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 7–11 × 2–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (35–)40–43(–50) × 3–6 µm (av. = 42 × 5 µm), L/W ratio = 8.85, 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies buff on the surface and ochraceous to umber in reverse; extensive aerial mycelium; chlamydospores not seen; sparse sporulation on aerial mycelium. Colonies moderate growing (40–60 mm diam) on MEA, and fast growing (75–85 mm diam) on OA, after 7 d at 25 °C.

Material examined: **Brazil**, Pará state, Monte Dourado, from soil in *Eucalyptus* plantation, Aug 2011, R.F. Alfenas (**holotype** CBS H–21379, culture **ex-type** CBS 134669 **=** LPF430), LPF429.

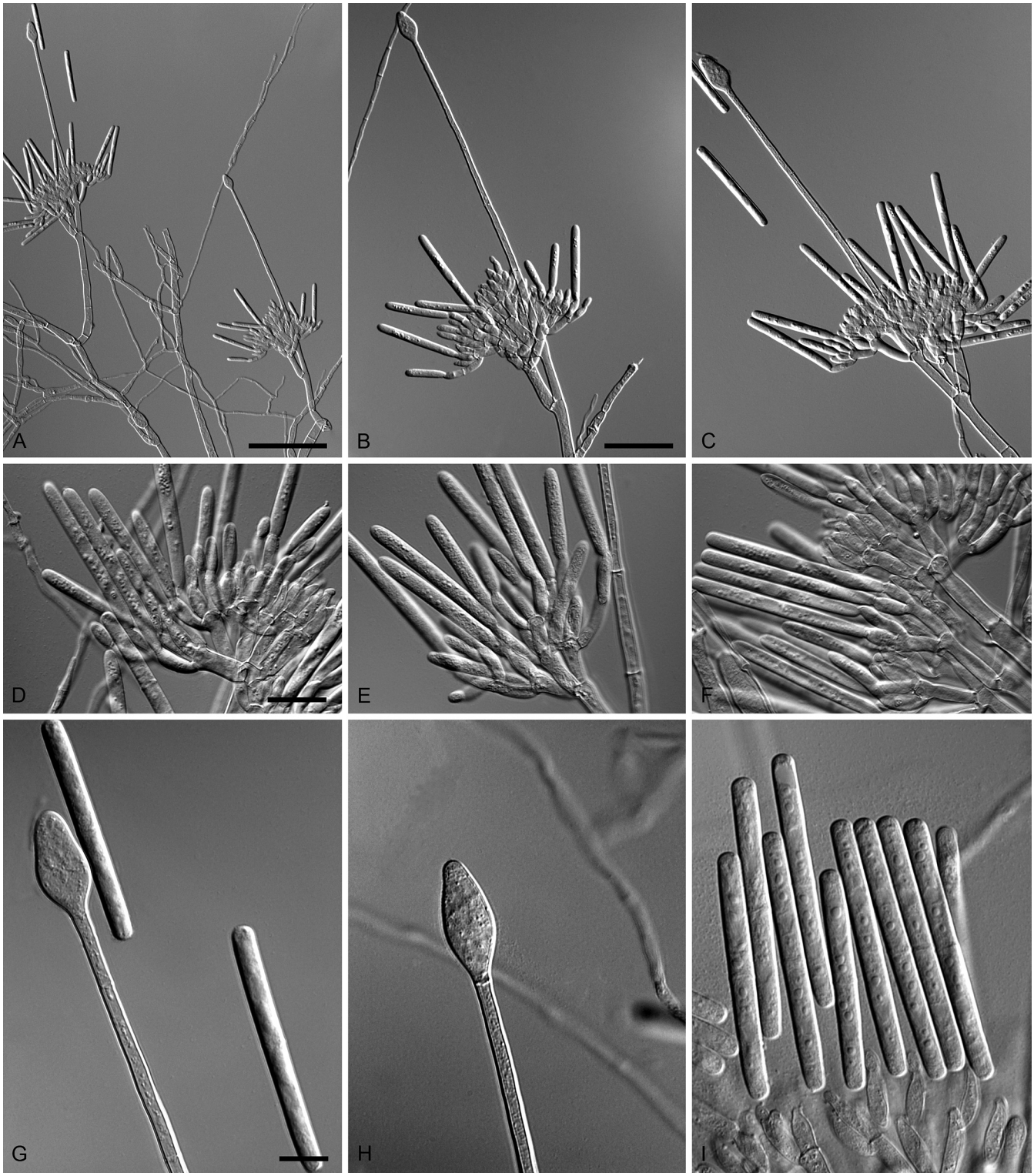

Fig. 11. *Calonectria nemuricola* (ex-type CBS 134837). A–C. Macroconidiophores. D–F. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. G–H. Obpyriform vesicles. I. Macroconidia. Scale bars: A = 100 µm; B = 50 µm (apply to C); D = 10 µm (apply to E–F); G = 10 µm (apply to H–I).

Note: *Calonectria paraensis* is a new member of the *Ca. brassicae* complex, distinguished from *Ca. pini* and *Ca. orientalis* by the size and length/diam ratio of its macroconidia, numbers of branches per conidiophores, and vesicle diameter (Table 3).

Calonectria piauiensis R.F. Alfenas, L. Lombard & Crous, **sp. nov.** MycoBank MB810009. Fig. 13.

Etymology: Name refers to Piauí state, Brazil, the region this fungus was isolated from.

Sexual morph not observed. *Conidiophores* consist of a stipe bearing a penicillate arrangement of fertile branches, stipe extension, and terminal vesicle; stipe septate, hyaline, smooth, 50–110 × 4–6 µm; stipe extensions septate, straight to flexuous, 95–130 µm long, 2–3 µm wide at the apical septum, terminating in ellipsoidal to narrowly obpyriform vesicles, 3–7 µm diam. Abundant lateral stipe extensions also present. *Conidiogenous apparatus* 20–60 µm long, 35–80 µm wide; primary branches aseptate, 12–20 × 3–5 µm, secondary branches aseptate, 8–10 × 3–4 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 6–12 ×

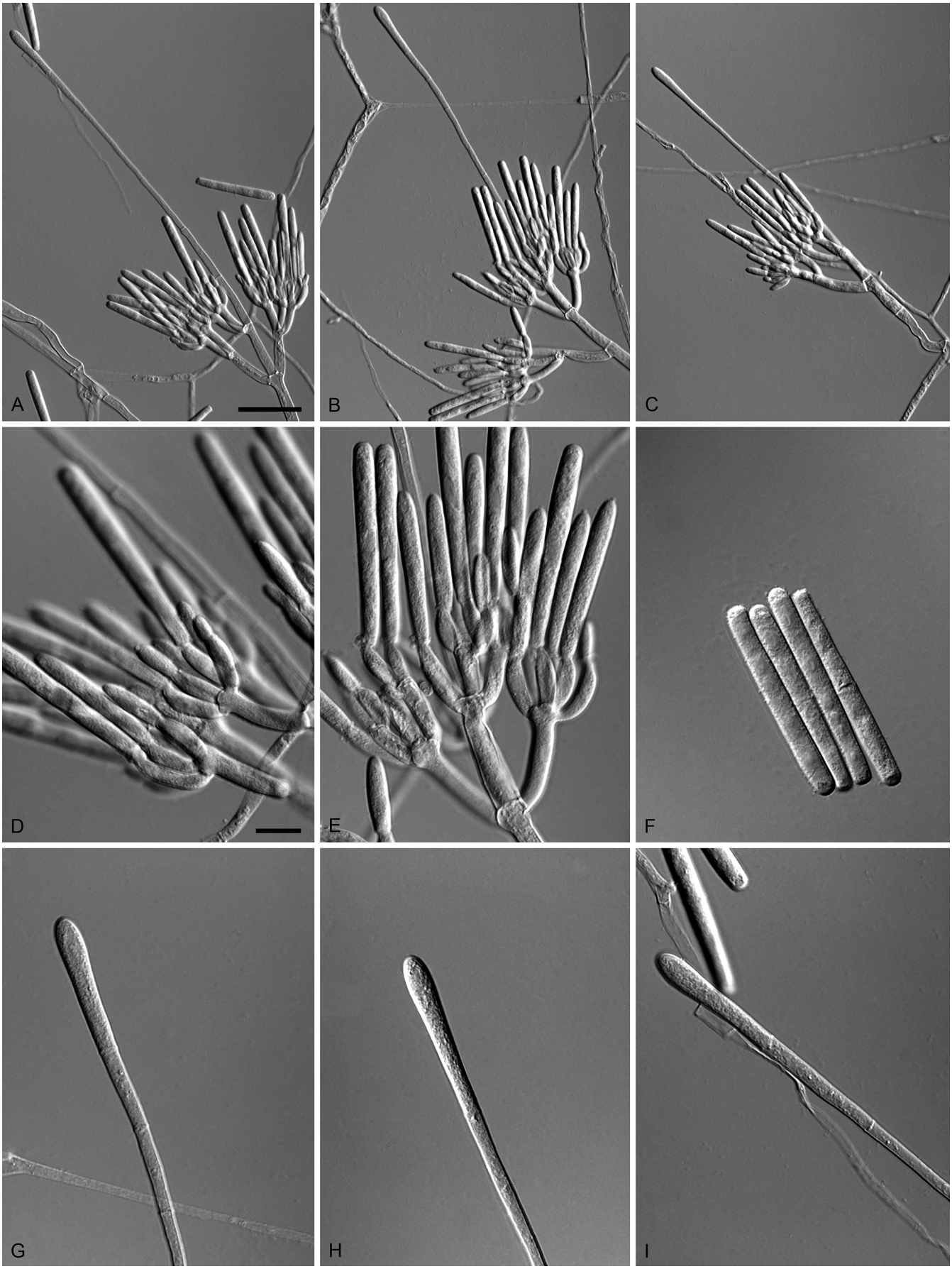

Fig. 12. *Calonectria paraensis* (ex-type CBS 134669). A–C. Macroconidiophores. D–E. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. F. Macroconidia. G–I. Clavate vesicles. Scale bars: A = 50 µm (apply to B–C); D = 10 µm (apply to E–I).

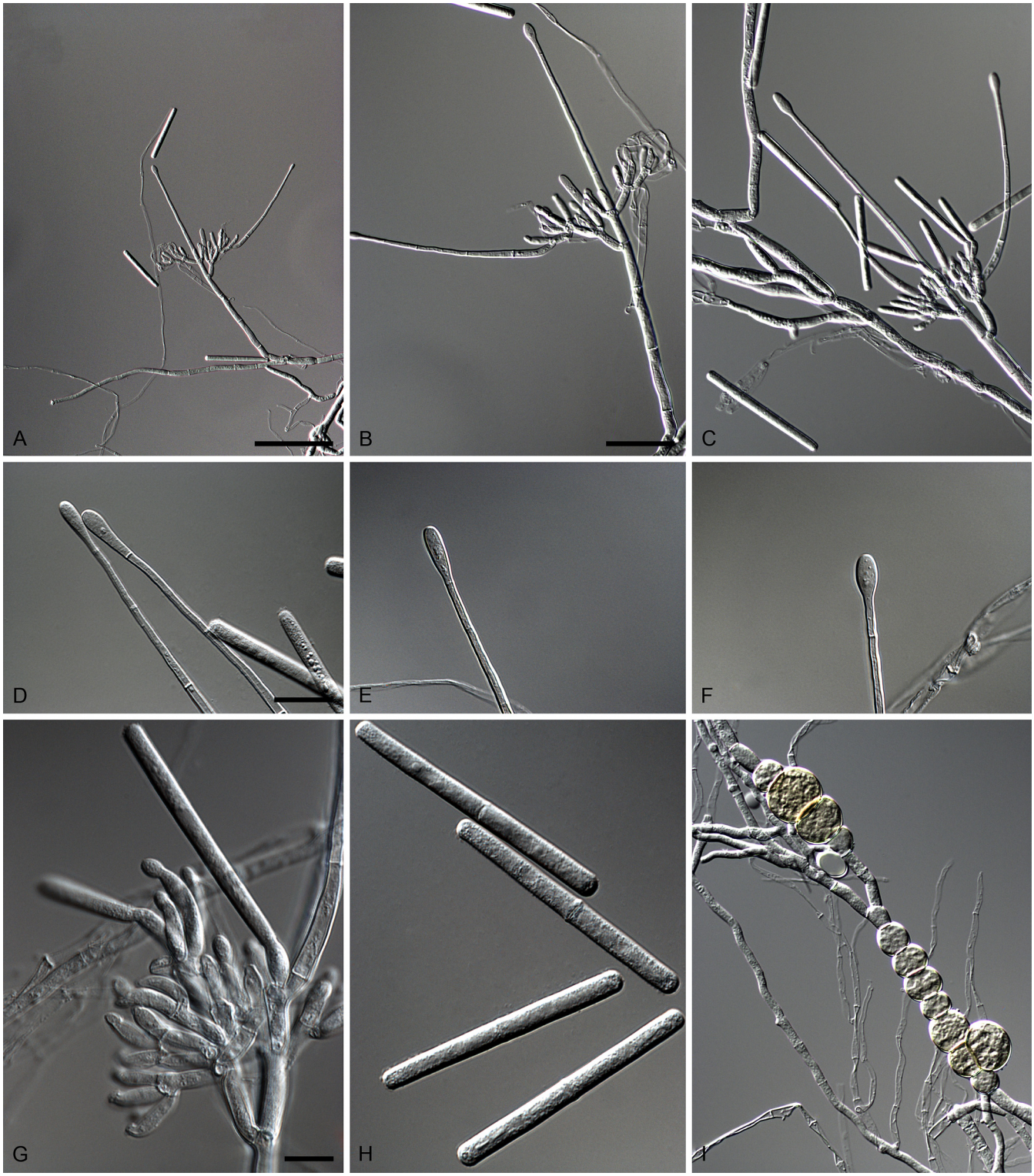

Fig. 13. *Calonectria piauiensis* (ex-type CBS 134850). A–C. Macroconidiophores. D–F. Ellipsoidal to narrowly obpyriform vesicles. G. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. H. Macroconidia. I. Chlamydospores. Scale bars: A = 100 µm; B = 50 µm (apply to C, I); D = 10 µm (apply to E–F); G = 10 µm (apply to H).

3–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight to slightly curved, (38–)47–52(–60) × 3–5 µm (av. = 49 × 4.5 µm), L/W ratio = 11.27, 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies buff on the surface and sepia in reverse; extensive white aerial mycelium with moderate sporulation on the aerial mycelium; chlamydospores sparse, occurring throughout the medium, forming microsclerotia. Colonies moderately fast growing (50–75 mm diam) after 7 d at 25 °C on MEA and OA.

Materials examined: **Brazil**, Piauí state, Teresina, from soil collected in *Eucalyptus brassiana* plantation, Jul. 2011, R.F. Alfenas (**holotype** CBS H-21375, culture **ex-type** CBS 134850 **=** LPF377), CBS 134851 = LPF381; Serra das Confusões, from soil and leaf litter collected in semi-arid vegetation, Jul. 2012, D.B. Pinho & O.L. Pereira, CBS 134849 = LPF291.

Note: *Calonectria piauiensis* can be distinguished from other closely related species in the *Ca. candelabra* complex by the abundant lateral stipe extensions produced on the

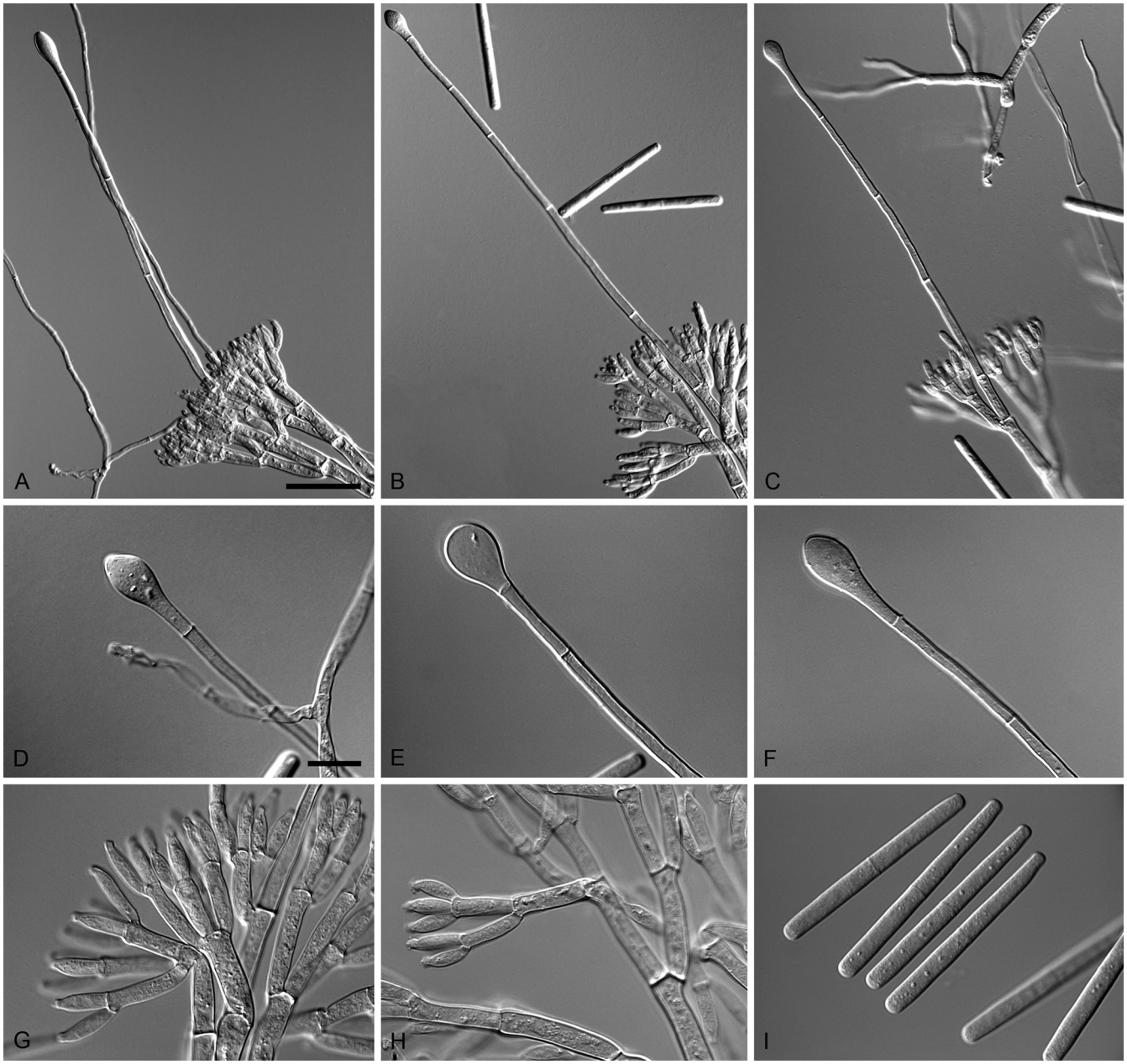

Fig. 14. *Calonectria propaginicola* (ex-type CBS 134815). A–C. Macroconidiophores. D–F. Ellipsoidal, obpyriform to sphaeropedunculate vesicles. G–H. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. I. Macroconidia. Scale bars: A = 50 µm (apply to B–C); D = 10 µm (apply to E–I).

conidiogenous apparatus not reported for other member species in the *Ca. candelabra* complex (Table 3).

Calonectria propaginicola R.F. Alfenas, L. Lombard & Crous, **sp. nov.** MycoBank MB810018. Fig. 14.

Etymology: Name refers to cuttings from which this fungus was first isolated.

Sexual morph not observed. *Conidiophores* containing a stipe bearing penicillate suites of fertile branches, stipe extension, and terminal vesicle; stipe septate, hyaline, smooth, 52–180 × 6–8 µm; stipe extensions septate, straight to flexuous, 130–250 µm long, 2–5 µm wide at the apical septum, terminating in ellipsoidal, obpyriform to sphaeropedunculate vesicles, 5–12 µm diam. *Conidiogenous apparatus* 31–85 µm long, 40–75 µm wide; primary branches 0–1-septate, 18–30 × 3–7 µm; secondary branches aseptate, 10–22 × 3–6 µm, tertiary branches aseptate, 11–20 × 3–5 µm, and additional branches (–4), aseptate, 9–15 × 3–4 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 5–12 × 3–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight to slightly curved, (40–) 48–51(–55) × 3–5 µm (av. = 49 × 4 µm), L/W ratio = 12.67, 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies buff to pale umber on the surface and sepia in reverse; moderate to extensive aerial mycelium with extensive sporulation on the aerial mycelium, especially in the centre of the colony; chlamydospores moderate, occurring throughout the medium, forming microsclerotia. Colonies fast growing (65–70 mm diam) on MEA, and (80–85 mm diam) on OA, after 7 d at 25 °C.

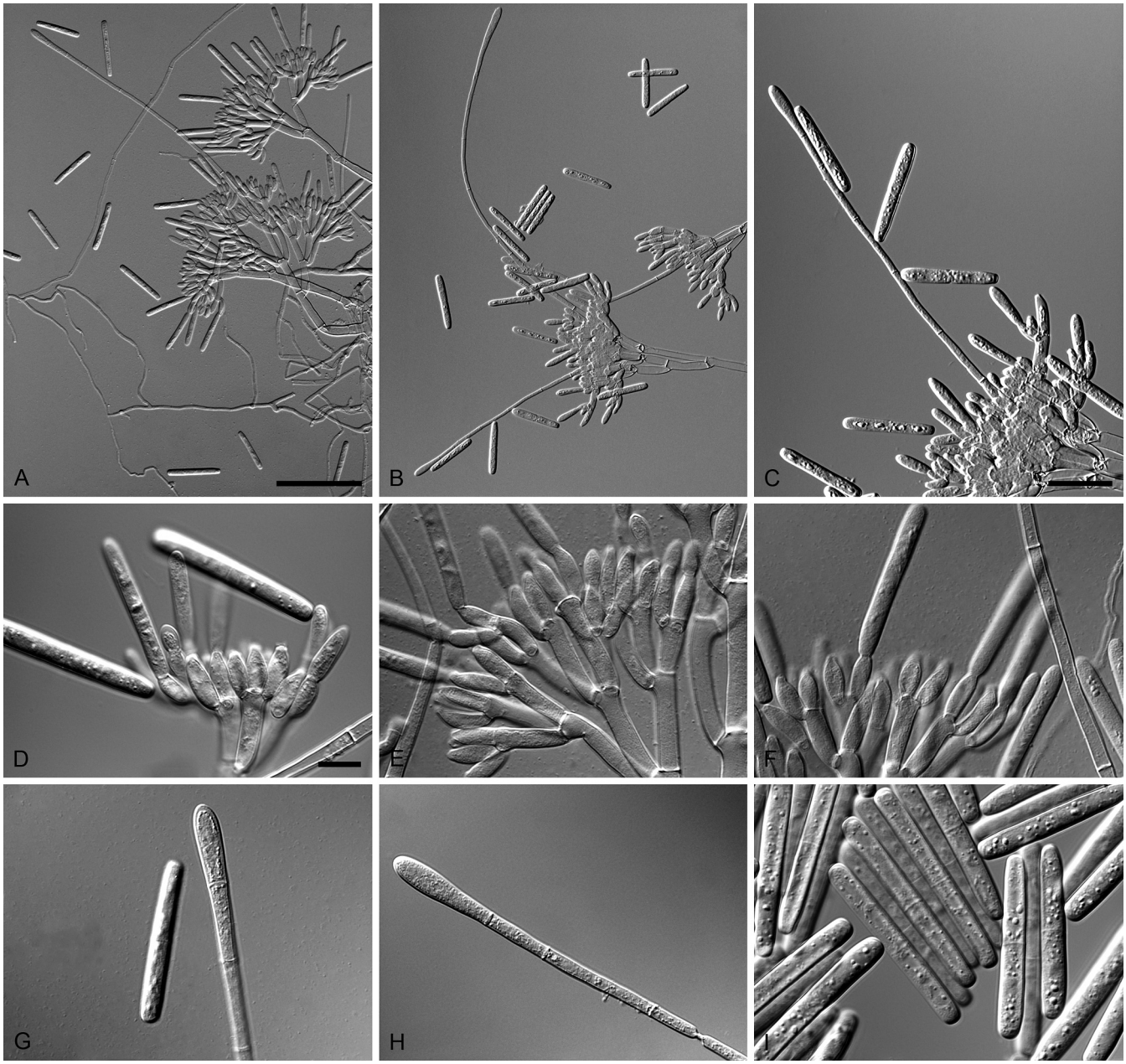

Fig. 15. *Calonectria pseudobrassicae* (ex-type CBS 134662). A–C. Macroconidiophores. D–F. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. G–H. Clavate vesicles. I. Macroconidia. Scale bars: A = 100 µm (apply to B); C = 50 µm; D = 10 µm (apply to E–I).

Material examined: **Brazil**, Pará state, Santana, from *Eucalyptus* seedling, Apr. 2011, A.C. Alfenas (**holotype** CBS H-21366, culture **ex-type** CBS 134815 **=** LPF220), CBS 134816 = LPF222.

Note: *Calonectria propaginicola* can be distinguished from *Ca. cerciana* and *Ca. pseudocerciana* based on its terminal vesicle morphology, and length/diam ratio of the macroconidia.

Calonectria pseudobrassicae R.F. Alfenas, L. Lombard & Crous, **sp. nov.** MycoBank MB810010. Fig. 15.

Etymology: Name refers to the fact that this species closely resembles *Calonectria brassicae.*

Sexual morph not observed. *Conidiophores* consist of a stipe bearing a penicillate arrangement of fertile branches, stipe extension, and terminal vesicle; stipe septate, hyaline, smooth, 50–125 × 5–8 µm; stipe extensions septate, straight to flexuous, 190–300 µm long, 3–5 µm wide at the apical septum, terminating in clavate vesicles, 5–6 µm diam. *Conidiogenous apparatus* 50–115 µm long, 60–100 µm wide; primary branches aseptate, 15–30 × 5–7 µm; secondary branches aseptate, 15–25 × 4–6 µm, tertiary branches aseptate, 10–20 × 3–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 7–15 × 3–5 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight to slightly curved, (30–)39–42(–48) × 4–6 µm (av. = 41 × 5 µm), L/W ratio = 8.04, 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies light amber, forming a rosy buff concentric ring on the surface, and ochraceous to umber in reverse; extensive aerial mycelium; sparse sporulation on the aerial mycelium; chlamydospores not seen. Colonies moderately fast growing (58–61 mm diam) on MEA, and fast growing (80–84 mm diam) on OA, after 7 d at 25 °C.

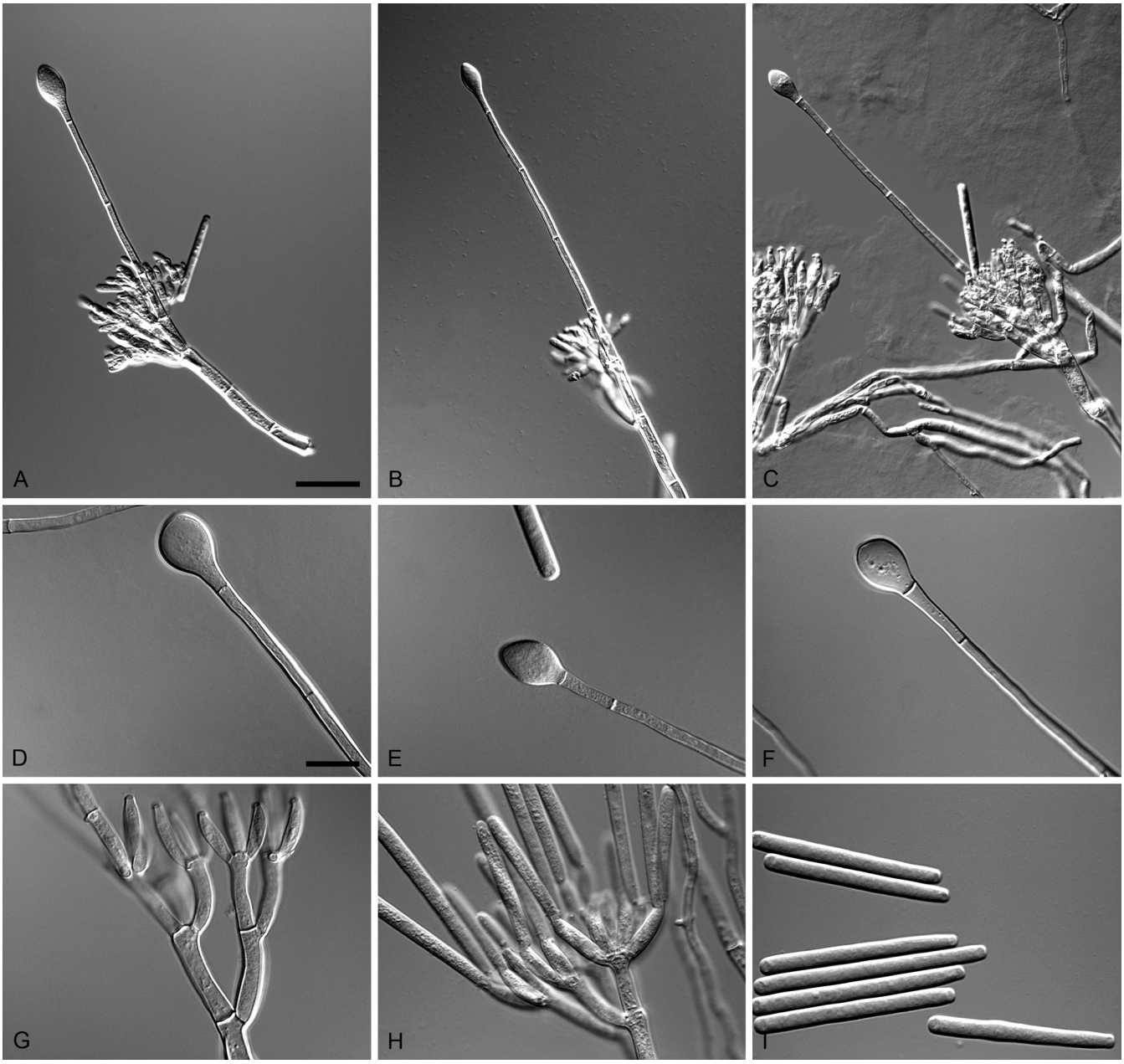

Fig. 16. *Calonectria pseudocerciana* (ex-type CBS 134824). A–C. Macroconidiophores. D–F. Obpyriform to sphaeropedunculate vesicles. G–H. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. I. Macroconidia. Scale bars: A = 50 µm (apply to B–C); D = 10 µm (apply to E–I).

Material examined: **Brazil**, Pará state, Santana, Apr. 2011, A.C. Alfenas (**holotype** CBS H-21371, culture **ex-type** CBS 134662 **=** LPF280), CBS 134661 = LPF260.

Note: *Calonectria pseudobrassicae* is morphologically distinguished from *Ca. brassicae* and *Ca. brachiatica* by the number of conidiophore branches and slightly smaller macroconidia (Table 3).

Calonectria pseudocerciana R.F. Alfenas, L. Lombard & Crous, **sp. nov.** MycoBank MB810011. Fig. 16.

Etymology: Name refers to the fact that this fungus closely resembles *Calonectria cerciana.*

Sexual morph not observed. *Conidiophores* consists of a stipe bearing a penicillate arrangement of fertile branches, stipe extension, and terminal vesicle; stipe septate, hyaline, smooth, 40–110 × 5–7 µm; stipe extensions septate, straight to flexuous, 130–190 µm long, 2–5 µm wide at the apical septum, terminating in obpyriform to sphaeropedunculate vesicles, 7–12 µm diam. *Conidiogenous apparatus* 40–95 µm long, 50–90 µm wide; primary branches 0–1-septate, 20–45 × 4–7 µm; secondary branches aseptate, 13–30 × 3–6 µm, tertiary branches aseptate, 8–18 × 3–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 5–15 × 3–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight to slightly curved, (35–)43–46(–55) × 3–5 µm (av. = 45 × 4 µm), L/W ratio = 10.6, 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies pale buff on the surface and cinnamon to sepia in reverse; extensive white aerial mycelium; sparse sporulation on the aerial mycelium; chlamydospores sparse, occurring throughout the medium, forming

microsclerotia. Colonies fast growing (65–70 mm diam) on MEA, and (70–80 mm diam) on OA, after 7 d at 25 °C.

Material examined: **Brazil**, Pará state, Santana, from stem of *Eucalyptus* seedling, Apr. 2011, A.C. Alfenas (**holotype** CBS H-21366, culture **ex-type** CBS 134824 **=** LPF367), CBS 134822 = LPF365.

Note: *Calonectria pseudocerciana* can be distinguished from *Ca. cerciana* and *Ca. propaginicola* based on the morphology of their terminal vesicle and length/diam ratio of the macroconidia.

Calonectria pseudohodgesii R.F. Alfenas, L. Lombard & Crous, **sp. nov.** MycoBank MB810012. Fig. 17.

Etymology: Name refers to the fact that this fungus closely resembles *Calonectria hodgesii.*

Sexual morph not observed. *Conidiophores* containing a stipe bearing penicillate suites of fertile branches, stipe extension, and terminal vesicle; stipe septate, hyaline, smooth, 35–160 × 5–8 µm; stipe extensions septate, straight to flexuous, 160–250 µm long, 2–5 µm wide at the apical septum, terminating in clavate (rarely), ellipsoidal to obpyriform vesicles, 4–10 µm diam (av. = 8 µm). *Conidiogenous apparatus* 50–90 µm long, 50–95 µm wide; primary branches aseptate, 20–35 × 4–7 µm; secondary branches aseptate, 15–30 × 4.5–6 µm, tertiary branches aseptate, 10–20 × 3–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 7–15 × 3–5 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight to slightly curved, (45–)53–55(–65) × (3–)4.5(–5) µm (av. = 54 × 4.5 µm), L/W ratio = 11.95, 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* were not observed.

Culture characteristics: Colonies fawn to cinnamon, rosy buff at the margin on the surface, and sepia in reverse; extensive white aerial mycelium with extensive sporulation on the aerial mycelium; chlamydospores moderate to extensive occurring throughout the medium. Colonies moderately fast growing (60–65 mm diam) on MEA, and fast growing (80–85 mm diam) on OA, after 7 d at 25 °C.

Materials examined: **Brazil**, Minas Gerais state, Viçosa, on leaf of rooted *Azadirachta indica* cutting, Mar. 2011, R.F. Alfenas (**holotype** CBS H-21368, culture **ex-type** CBS 134818 = LPF262), CBS 134819 = LPF265; from stem of *Eucalyptus* seedling, Mar. 2011, R.F. Alfenas, CBS 134813 = LPF205, CBS 134814 = LPF206.

Note: The macroconidia of *Ca. pseudohodgesii* are larger than those of *Ca. hodgesii* and the stipe extensions of *Ca. pseudohodgesii* are also longer than those of *Ca. hodgesii* (Table 3).

Calonectria pseudometrosideri R.F. Alfenas, L. Lombard & Crous, **sp. nov.** MycoBank MB810013. Fig. 18.

Etymology: Name refers to the fact that this fungus closely resembles *Calonectria metrosideri.*

Sexual morph not observed. *Conidiophores* containing a stipe bearing penicillate suites of fertile branches, stipe extension, and terminal vesicle; stipe septate, hyaline, smooth, 62–220 × 6–8 µm; stipe extensions septate, straight to flexuous, 160–210 µm long, 2–4 µm wide at the apical septum, terminating in ellipsoidal to obpyriform vesicles, 5–7 µm diam. *Conidiogenous apparatus* 30–76 µm long, 45–65 µm wide; primary branches 0(–1)-septate, 21–30 × 5–7 µm; secondary branches aseptate, 16–22 × 4–7 µm, tertiary branches aseptate, 10–17 × 3–5 µm, each terminal branch producing 2–6 phialides; phialides elongated doliiform to reniform, hyaline, aseptate, 9–17 × 3–5 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight to slightly curved, (40–)49–52(–60) × (3–)4.5(–5) µm (av. = 51 × 4.5 µm), L/W ratio = 11.34, 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies cinnamon to dark brick on the surface, and sepia in reverse; moderate aerial white mycelium with moderate to extensive sporulation on the aerial mycelium, especially at the margins; chlamydospores moderate to extensive, occurring throughout the medium forming microsclerotia. Colonies slow growing (35–40 mm diam) on MEA, and moderately slow growing (45–50 mm diam) on OA, after 7 d at 25 °C.

Materials examined: **Brazil**, Alagoas state, Maceió, from soil collected in *Eucalyptus* plantation, Apr. 2011, M.M. Coutinho (**holotype** CBS 134845, preserved as metabolically inactive culture, culture **ex-type** CBS 134845 **=** LPF210); Maranhão, Açailândia, from leaf of *Eucalyptus* sp., Aug 2012, A.C. Alfenas CBS 134844 = LPF147; Minas Gerais, Viçosa, from leaf of *Metrosideros polymorpha*, Mar. 2012, R.F. Alfenas, CBS 134843 = LPF100.

Note: *Calonectria pseudometrosideri* can be distinguished from *Ca. metrosideri* by their larger macroconidia and longer stipe extensions (Table 3).

Calonectria pseudopteridis (Sherb.) R.F. Alfenas, L. Lombard & Crous, **nom. nov.** MycoBank MB810024.

Basionym: *Cylindrocladium macrosporum* Sheb., Phytopathology 18: 219. 1928.

Notes: Sobers (1968) synonymised *Cy. macrosporum* under *Ca. pteridis* based on morphological similarities of the asexual morphs. This was further validated in the monographic studies of Crous & Wingfeld (1994) and Crous (2002). However, to our knowledge, the ex-type strain (CBS 163.28) of *Cy. macrosporum* has never been subjected to DNA sequence analysis. Phylogenetic inference in this study showed that the ex-type strain of *Cy. macrosporum* is closely related to, but distinct from, the ex-type strain (CBS 111793) of *Ca. pteridis*, and is therefore reinstated here as a separate species of *Calonectria*. As the name *Ca. macrospora* is already occupied, we provide a new name for this species.

Calonectria pseudospathulata R.F. Alfenas, L. Lombard & Crous, **sp. nov.** MycoBank MB810014. Fig. 19.

Etymology: Name refers to the fact that this species closely resembles *Calonectria spathulata.*

Sexual morph not observed. *Conidiophores* consist of a stipe bearing a penicillate arrangement of fertile branches, stipe extension, and terminal vesicle; stipe septate, hyaline, smooth,

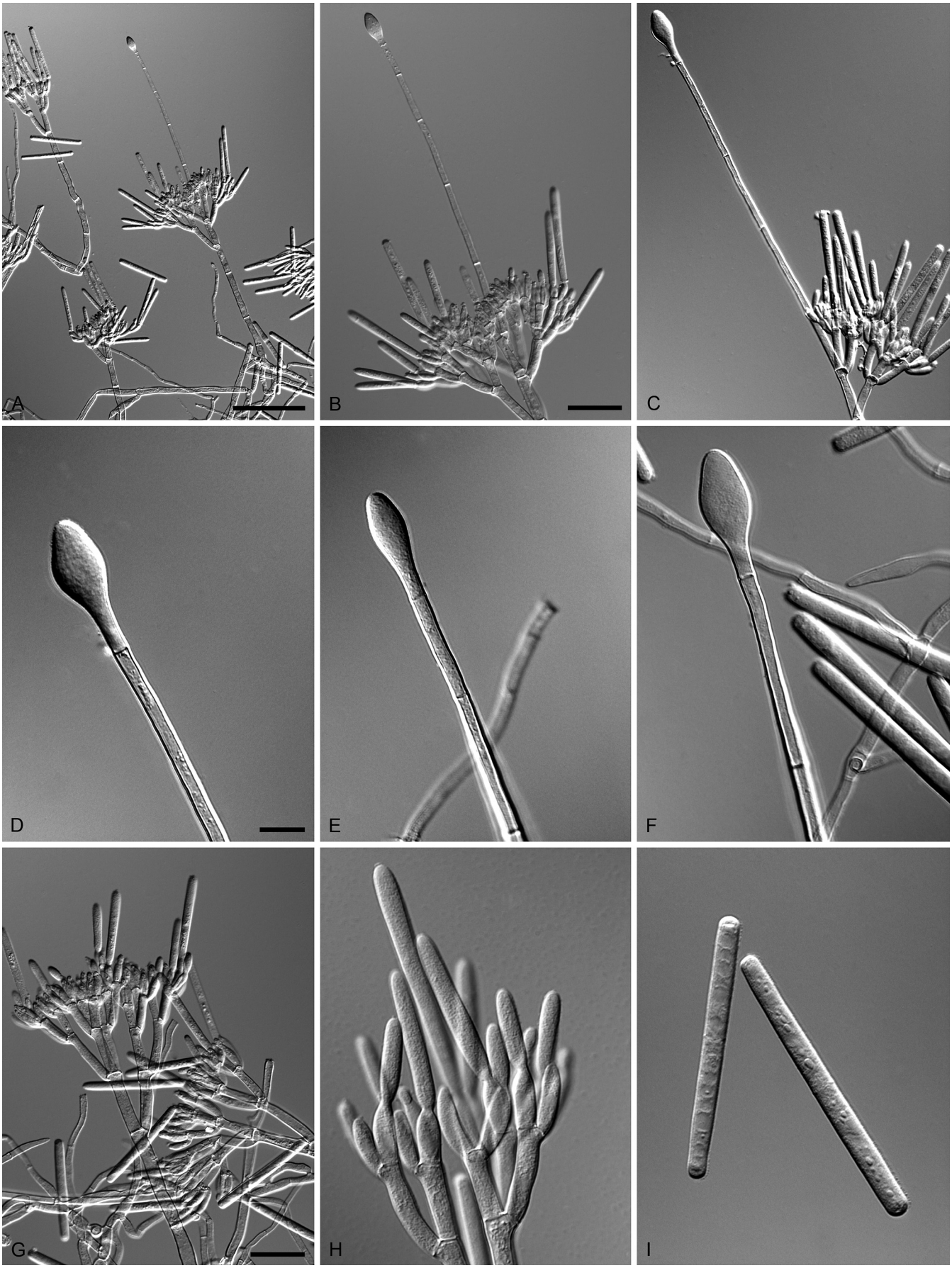

Fig. 17. *Calonectria pseudohodgesii* (ex-type CBS 134818). A–C. Macroconidiophores. D–F. Ellipsoidal to obpyriform vesicles. G–H. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. I. Macroconidia. Scale bars: A = 100 µm; B = 50 µm (apply to C); D = 10 µm (apply to E–F, H–I); G = 20 µm.

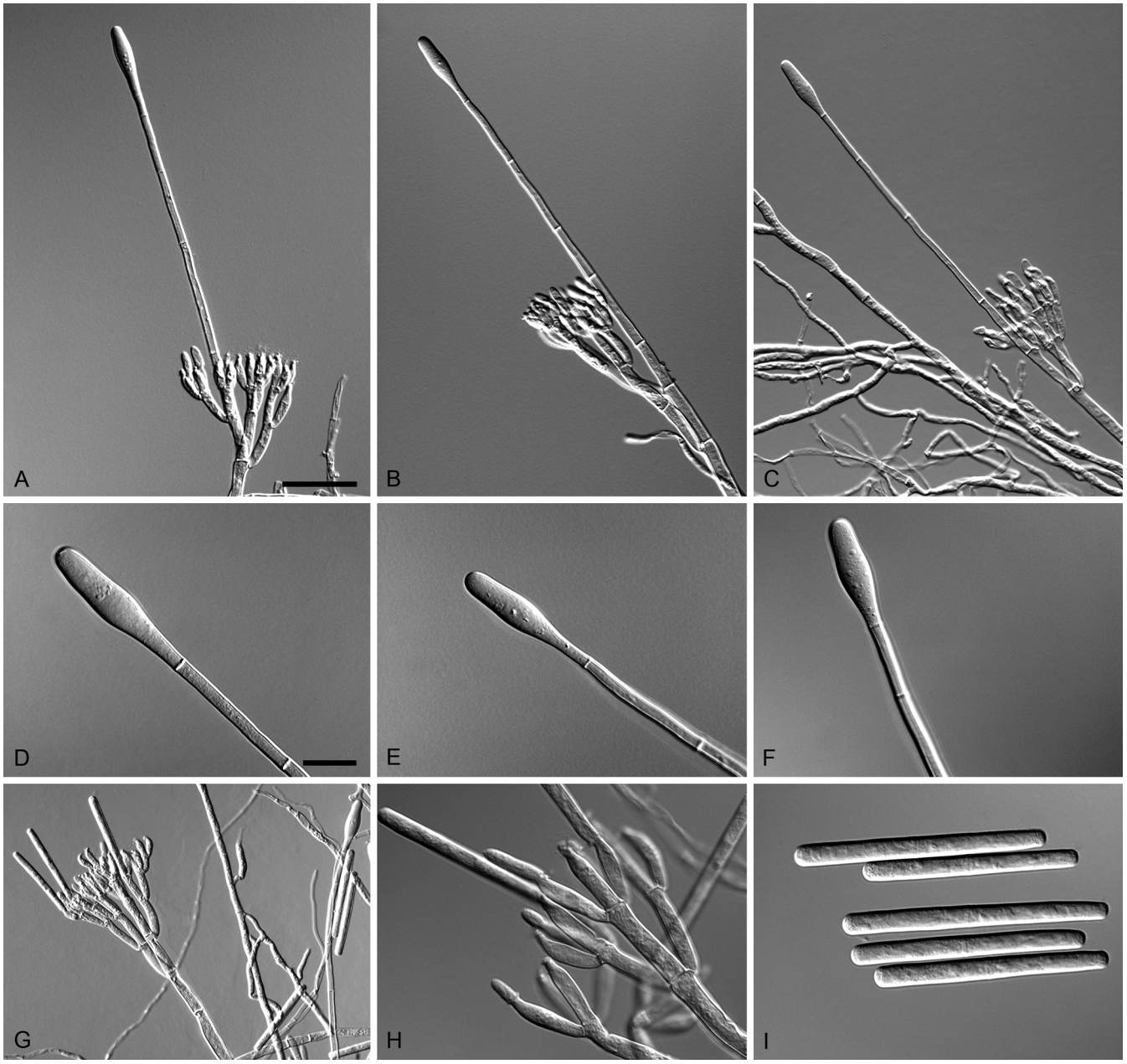

Fig. 18. *Calonectria pseudometrosideri* (ex-type CBS 134845). A–C. Macroconidiophores. D–F. Ellipsoidal to obpyriform vesicles. G–H. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. I. Macroconidia. Scale bars: A = 50 µm (apply to B, G); D = 10 µm (apply to E–F, H–I).

45–95 × 5–8 µm; stipe extensions septate, straight to flexuous, 145–190 µm long, 2–4 µm wide at the apical septum, terminating in obpyriform vesicles, 7–10 µm diam. *Conidiogenous apparatus* 30–70 µm long, 65–100 µm wide; primary branches aseptate, 15–25 × 4–7 µm, secondary branches aseptate, 12–20 × 4–5 µm, tertiary branches aseptate, 10–12 × 3–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 5–10 × 3–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight to slightly curved, (35–)41–44(–50) × 3–5 µm (av. = 43 × 4 µm), L/W ratio = 10.46, 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies cinnamon to dark brick on the surface and sepia to umber in reverse; extensive aerial white mycelium with moderate sporulation on the aerial mycelium; chlamydospores moderately abundant, occurring throughout the medium, forming microsclerotia. Colonies slow to moderately slow growing (40–60 mm diam) on MEA and OA, after 7 d at 25 °C.

Material examined: **Brazil**, Minas Gerais state, Araponga (Serra do Brigadeiro), from soil collected in tropical rainforest, Aug. 2010, A.C. Alfenas & P.W. Crous (**holotype** CBS H-21356, living **ex-type** CBS 134841 **=** LPF072), CBS 134840 = LPF066, CBS 134842 = LPF087.

Note: The macroconidia of *Ca. pseudospathulata* are smaller than those of *Ca. spathulata* (Table 3).

Calonectria pseudovata R.F. Alfenas, L. Lombard & Crous, **sp. nov.** MycoBank MB810015. Fig. 20.

Etymology: Name refers to the fact that the species closely resembles *Calonectria ovata.*

Sexual morph not observed. *Conidiophores* consist of a stipe bearing a penicillate arrangement of fertile branches, stipe

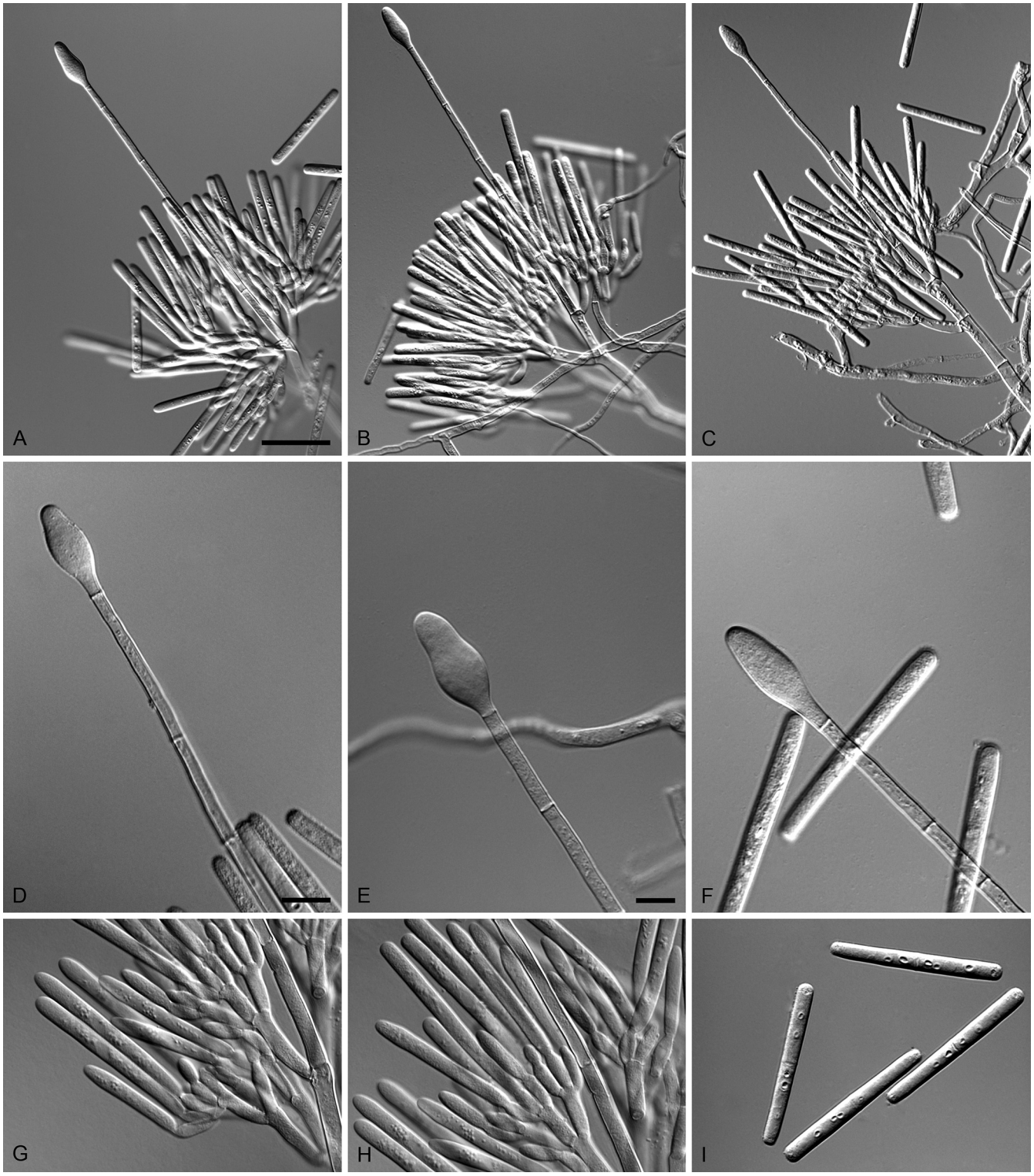

Fig. 19. *Calonectria pseudospathulata* (ex-type CBS 134841). A–C. Macroconidiophores. D–F. Obpyriform vesicles. G–H. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. I. Macroconidia. Scale bars: A = 50 µm (apply to B–C); D = 10 µm (apply to G–I); E = 10 µm (apply to F).

extension, and terminal vesicle; stipe septate, hyaline, smooth, 35–105 × 5–7 µm; stipe extensions septate, straight to flexuous, 140–280 µm long, 3–6 µm wide at the apical septum, terminating in fusiform, ovate to ellipsoidal vesicles, 8–12 µm diam. *Conidiogenous apparatus* 55–121 µm long, 75–105 µm wide; primary branches 0–1-septate, 25–75 × 5–8 µm; secondary branches aseptate, 15–35 × 4–7 µm, tertiary branches aseptate, 15–30 × 4–6 µm, each terminal branch producing 2–6 phialides; phialides elongate doliiform to reniform, hyaline, aseptate, 10–25 × 3–5 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (55–)67–70(–80) × (4–)5(–7) µm (av. = 69 × 5 µm), L/W ratio = 13.73, 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Microconidiophores* comprise a stipe, a stipe extension and a penicillate or subverticillate arrangement of fertile branches. Stipe extension septate, thin-walled, terminating in an ellipsoidal to ovoid vesicle, 3–5 µm diam. Primary branches aseptate, 8–15 × 2–4 µm, secondary branches aseptate, 5–10 × 2–4 µm, terminating in 1–3 phialides; phialides elongate doliiform to reniform, straight to slightly curved, hyaline, aseptate, 7–15 × 2–4 µm; apex with minute periclinal thickening and

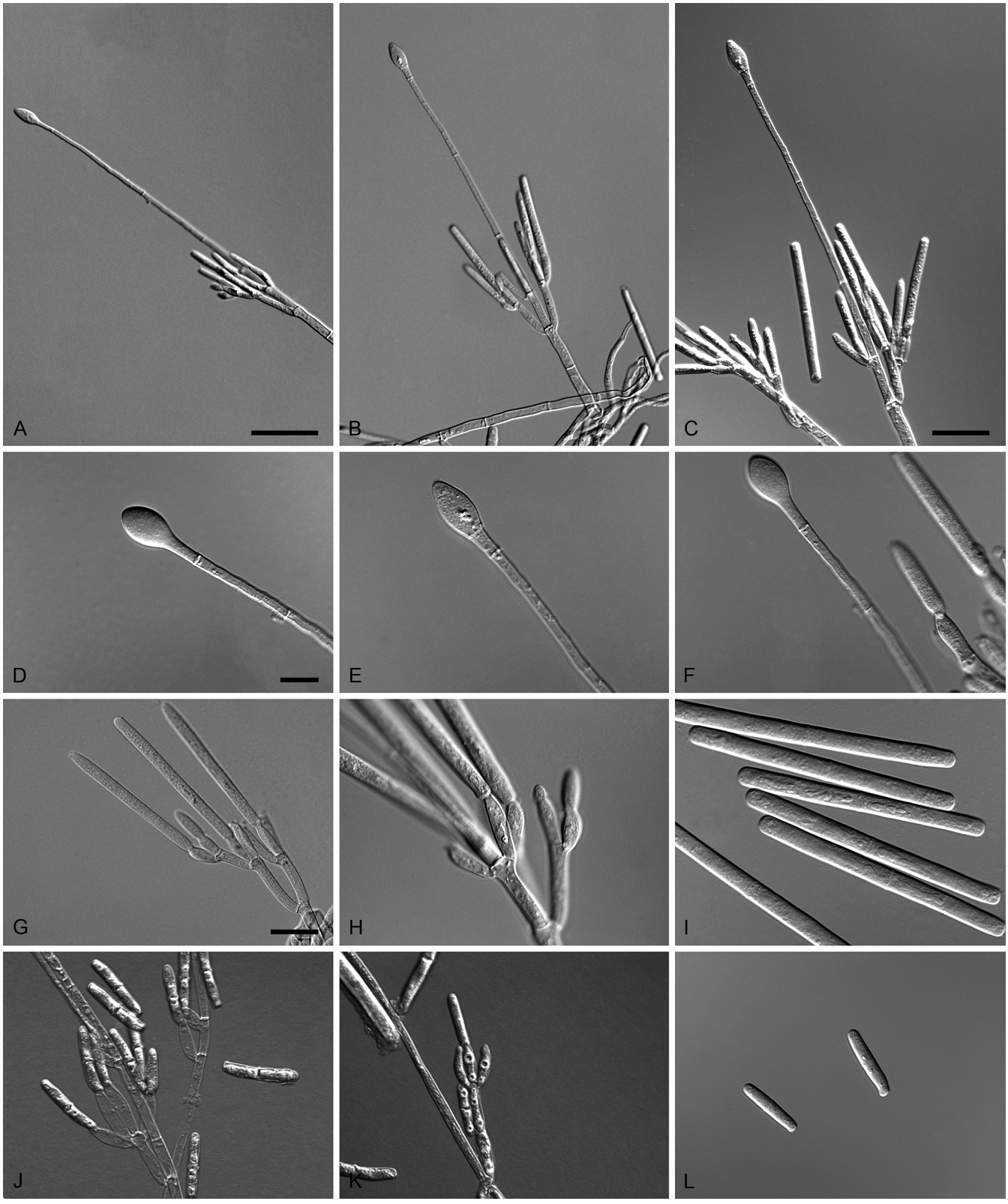

Fig. 20. *Calonectria pseudovata* (ex-type CBS 134674). A–C. Macroconidiophores. D–F. Ovate to ellipsoidal vesicles. G–H. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. I. Macroconidia. J–K. Microconidiophores. L. Microconidia. Scale bars: A = 50 µm (apply to B); C = 50 µm; D = 10 µm (apply to E–F, H–I); G = 10 µm (apply to J–L).

inconspicuous collarette. *Microconidia* cylindrical, straight to curved, rounded at apex, (10–)20–23(–30) × (3–)4(–6) µm (av. = 22 × 4 µm), L/W ratio = 5.38, 1-septate, held in fascicles by colourless slime. *Megaconidia* not observed.

Culture characteristics: Colonies ochraceous to rosy buff on the surface and umber in reverse; moderate to extensive aerial mycelium; sparse sporulation on the aerial mycelium; chlamydospores not seen. Colonies moderately fast growing (55–64 mm diam) on MEA and on OA, after 7 d at 25 °C.

Material examined: **Brazil**, Pará state, Santana, from soil in *Eucalyptus* plantation, Apr. 2011, A.C. Alfenas (**holotype** CBS H-21370, culture **ex-type** CBS 134674 **=** LPF267), CBS 134675 = LPF285, LPF286.

Notes: *Calonectria pseudovata* can be distinguished from *Ca. ovata* by the shape of the terminal vesicle and smaller macroconidia produced by *Ca. pseudovata*. The microconidia of *Ca. pseudovata* are also slightly smaller than those of *Ca. ovata*.

Calonectria quinqueramosa R.F. Alfenas, L. Lombard & Crous, **sp. nov.** MycoBank MB810016. Fig. 21.

Etymology: Name refers to the characteristic five branches formed in the conidiogenous apparatus of this fungus.

Ascomata perithecial, solitary or in groups, orange to red, becoming brown with age; in section apex and body orange to red, base red-brown, pyriform to sub-globose, 160–400 µm high, 115–250 µm diam, body turning dark red, and base dark red-brown (KOH+). Perithecial walls rough, consisting of two thick-walled layers: outside layer of *textura globulosa*, 25–85 µm wide; becoming more compressed towards inner layer of *textura angularis*, 10–30 µm wide; becoming thin-walled and hyaline towards the centre, outer layer cells 10–20 × 10–30 µm; inner cells 4–6 × 8–15 µm: perithecial base up to 135 µm wide; consisting of dark red, angular cells; merging with an erumpent stroma, cells of the outer wall layer continuing into the pseudoparenchymatous cells of the erumpent stroma. *Asci* 8-spored, clavate, 50–105 × 10–25 µm, tapering to a long thin stalk. *Ascospores* aggregated in the upper third of the ascus, hyaline, guttulate, fusoid with rounded ends, straight to curved, (1–)3-septate, slightly constricted at the septum, (25–)39–42(–50) × 5–7 µm (av. = 40 × 6 µm). Cultures were homothallic. *Conidiophores* consists of a stipe bearing a penicillate arrangement of fertile branches, stipe extension, and terminal vesicle; stipe septate, hyaline, smooth, 50–145 × 5–7 µm; stipe extensions septate, straight to flexuous, 170–340 µm long, 2–4 µm wide at the apical septum, terminating in narrowly clavate to clavate vesicles, 3–5 µm diam. *Conidiogenous apparatus* 30–60 µm long, 35–65 µm wide; primary branches aseptate, 10–35 × 3–6 µm; secondary branches aseptate, 10–30 × 3–5 µm; tertiary branches aseptate, 10–20 × 2–4 µm and additional branches (–5), aseptate, 10–15 × 3–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 6–18 × 2–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (45–)57–61(–70) × 4–6 µm (av. = 59 × 5 µm), L/W ratio = 11.57, 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* were not observed.

Culture characteristics: Colonies umber to fawn on the surface and dark brick in reverse; sparse aerial mycelium; chlamydospores sparse, occurring throughout the medium, with moderate to extensive sporulation on the aerial mycelium. Colonies moderately fast growing (57–70 mm diam) on MEA, and fast growing (78–81 mm diam) on OA, after 7 d at 25 °C.

Materials examined: **Brazil**, Pará state, Monte Dourado, from soil in *Eucalyptus* plantation, May 2011, R.F. Alfenas (**holotype** CBS H-21355, culture **ex-type** CBS 134654 **=** LPF065), LPF302; Santana, from soil in *Eucalyptus* plantation, Apr. 2011, A.C. Alfenas, CBS 134655 = LPF281.

Note: *Calonectria quinqueramosa* can be distinguished from *Ca. gracilis* and *Ca. gracilipes* by the size of its ascospores and macroconidia, and by the number of fertile branches formed in the conidiogenous apparatus (Table 3).

Calonectria robigophila R.F. Alfenas, L. Lombard & Crous, **sp. nov.** MycoBank MB810017. Fig. 22.

Etymology: Name refers to Calonectria leaf blight, the disease this fungus is associated with.

Sexual morph not observed. *Conidiophores* consist of a stipe bearing a penicillate arrangement of fertile branches, stipe extension, and terminal vesicle; stipe septate, hyaline, smooth, 65–120 × 5–8 µm; stipe extensions septate, straight to flexuous, 125–225 µm long, 3–4 µm wide at the apical septum, terminating in acicular to clavate vesicles, 4–5 µm diam. *Conidiogenous apparatus* 15–60 µm long, 30–70 µm wide; primary branches aseptate, 18–35 × 4–7 µm; secondary branches aseptate, 10–20 × 3–5 µm; tertiary branches aseptate, 10–20 × 3–5 µm and additional branches (–6), aseptate, 10–15 × 3–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 5–10 × 3–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (45–)49–52(–60) × 3–5 µm (av. = 50 × 4 µm), L/W ratio = 12.6, 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies umber to sienna on the surface, and umber to sepia in reverse; sparse to moderate aerial mycelium with moderate to extensive sporulation on the aerial mycelium; forming sparse chlamydospores occurring throughout the medium. Colonies slow growing (34–40 mm diam) on MEA, and fast growing (70–80 mm diam) OA, after 7 d at 25 °C.

Material examined: **Brazil**, Maranhão state, Açailândia, on leaves of *Eucalyptus* sp., May 2011, R.F. Alfenas (**holotype** CBS H-21361, living **ex-type** CBS 134652 **=** LPF192), LPF190, CBS 134653 = LPF193.

Notes: *Calonectria robigophila* can be distinguished from *Ca. ecuadoriae* by the dimensions and septation of its macroconidia. Furthermore, *Ca. robigophila* formed fewer fertile branches than reported for *Ca. ecuadoriae* (Crous *et al.* 2006, Table 3).

Calonectria silvicola R.F. Alfenas, L. Lombard & Crous, **sp. nov.** MycoBank MB810019. Fig. 23.

Etymology: Name refers to a forest, the habitat this fungus was isolated from.

Sexual morph not observed. *Conidiophores* consist of a stipe bearing a penicillate arrangement of fertile branches, stipe extension, and terminal vesicle; stipe septate, hyaline, smooth, 50–220 × 7–9 µm; stipe extensions septate, straight to flexuous, 130–195 µm long, 3–4 µm wide at the apical septum, terminating in obpyriform vesicles, 7–10 µm diam. *Conidiogenous apparatus* 35–90 µm long, 45–105 µm wide; primary branches aseptate, 20–30 × 3–6 µm, secondary branches aseptate, 13–26 × 3–6 µm, tertiary branches aseptate, 8–15 × 3–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 6–10 × 3–4 µm; apex with minute periclinal thickening and inconspicuous collarette.

Fig. 21. *Calonectria quinqueramosa* (ex-type CBS 134654). A. Ascoma. B–C. Vertical section through ascomata, showing wall structure. D–E. Asci. F. Ascospores. G–I. Macroconidiophores. J–L. Narrowly clavate to clavate vesicles. M–N. Conidiogenous apparatus with conidiophore branches, doliiform to reniform phialides. O. Macroconidia. Scale bars: A = 500 µm; B = 100 µm (apply to C); D = 50 µm (apply to I); E = 20 µm (apply to F, J–O); G = 50 µm (apply to H).

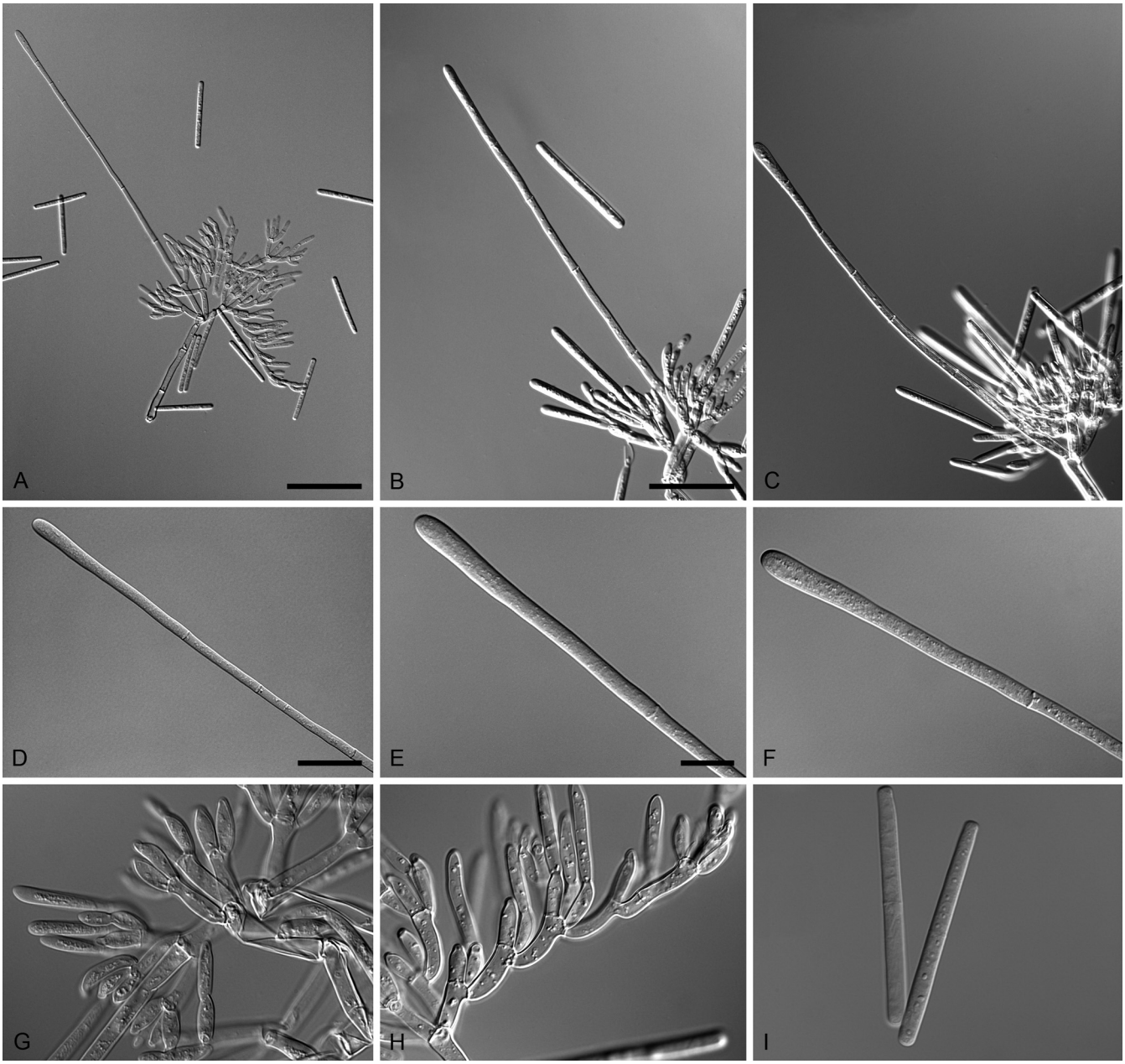

Fig. 22. *Calonectria robigophila* (ex-type CBS 134652). A–C. Macroconidiophores. D–F. Clavate vesicles. G–H. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. I. Macroconidia. Scale bars: A = 50 µm; B = 50 µm (apply to C); D = 10 µm (apply to I); E = 10 µm (apply to F–H).

Macroconidia cylindrical, rounded at both ends, straight to slightly curved, (30–)40–42(–50) × 3–5 µm (av. = 41 × 4.5 µm), L/W ratio = 9.17, 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies cinnamon to dark brick on the surface and sepia in reverse; moderate aerial mycelium with extensive sporulation on the aerial mycelium, especially at the centre; chlamydospores moderate to extensive, occurring throughout the medium, forming microsclerotia. Colonies slow growing (30–40 mm diam) on MEA, and moderately slow growing (45–50 mm diam) on OA, after 7 d at 25 °C.

Materials examined: **Brazil**, Bahia state, Mucuri, form soil collected in tropical rainforest, Aug. 2011, E. Zauza (**holotype** CBS H-21357, living **ex-type** CBS 135237 **=** LPF081); Minas Gerais state, Araponga, from soil collected in tropical rainforest, Aug. 2010, A.C. Alfenas & P.W. Crous, CBS 134836 = LPF079, CPC 18741 = LPF071, CPC 18766 = LPF096.

Note: The macroconidia of *Ca. silvicola* are larger than those of *Ca. polizzii* and *Ca. zuluensis*, but smaller than those of *Ca. mossambicensis*, *Ca. nemuricola* and *Ca. pauciramosa* (Table 3).

Calonectria telluricola R.F. Alfenas, L. Lombard & Crous, **sp. nov.** MycoBank MB810020. Fig. 24.

Etymology: Name refers to soil, the substrate this fungus was isolated from.

Sexual morph not observed. *Conidiophores* consist of a stipe bearing penicillate suites of fertile branches, stipe extension, and terminal vesicle; stipe septate, hyaline, smooth, 55–125 × 5–7 µm; stipe extensions septate, straight to flexuous, 100–225 µm long, 2–4 µm wide at the apical septum, terminating in clavate vesicles, 3–6 µm diam. *Conidiogenous apparatus* 45–95 µm long, 40–80 µm wide; primary branches aseptate, 20–30 × 5–8 µm; secondary branches aseptate, 15–30 ×

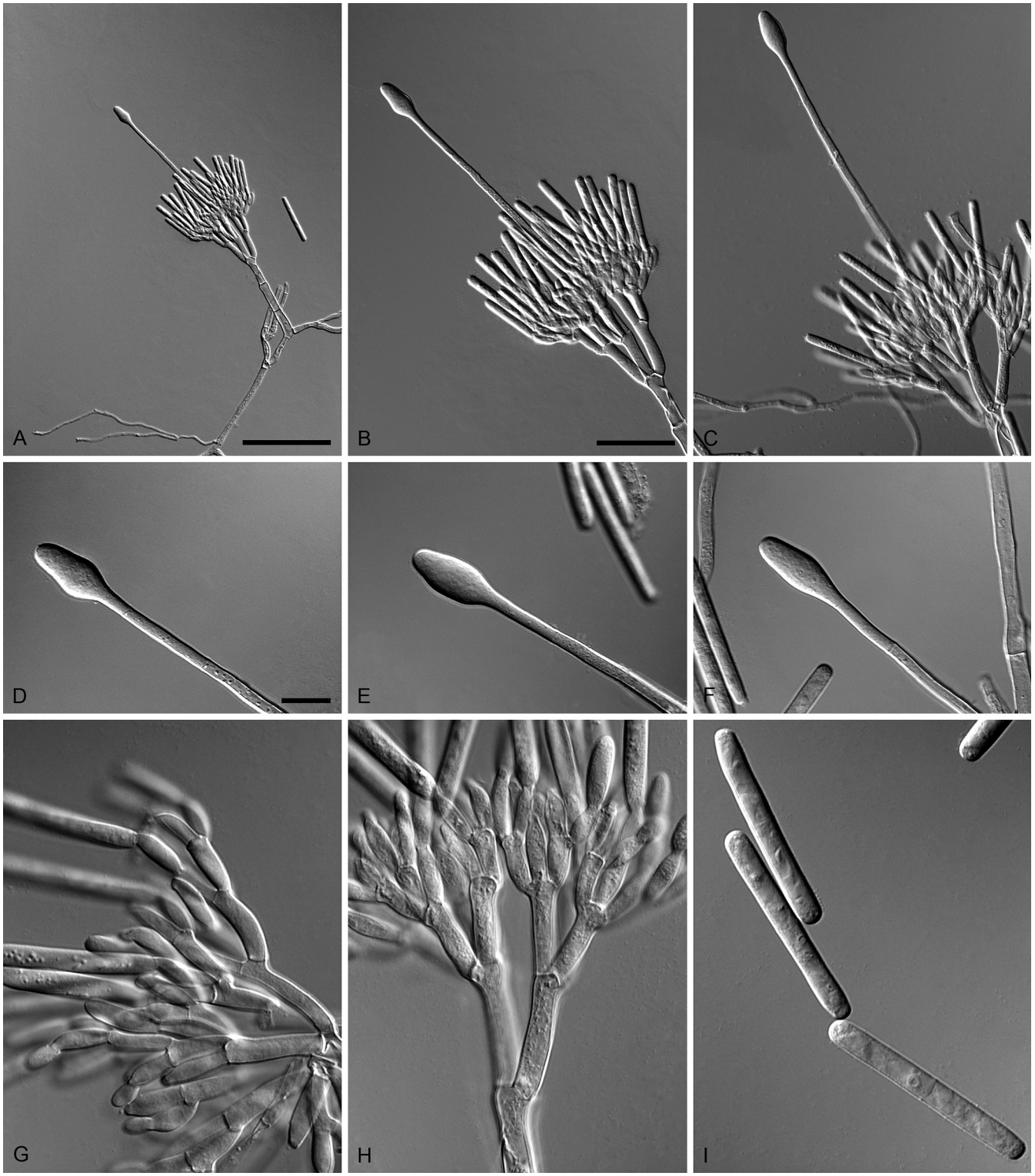

Fig. 23. *Calonectria silvicola* (ex-type CBS 135237). A–C. Macroconidiophores. D–F. Obpyriform vesicles. G–H. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. I. Macroconidia. Scale bars A = 100 µm; B = 50 µm (apply to C); D = 10 µm (apply to E–I).

4–5 µm; tertiary branches aseptate, 10–20 × 5–6 µm, and additional branches (–4), aseptate, 10–15 × 3–4 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 7–11 × 3–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (35–) 40–42(–50) × 3–6 µm (av. = 41 × 5 µm), L/W ratio = 9.13, 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies buff on the surface and ochraceous to umber in reverse; extensive aerial mycelium; chlamydospores not seen; sparse sporulation on the aerial mycelium. Colonies moderately fast growing (45–60 mm diam) at 25 °C on MEA, and fast growing (76–83 mm diam) on OA, after 7 d at 25 °C.

Materials examined: **Brazil**, Bahia state, Mucuri, from soil collected in tropical rainforest, Oct. 2011, E. Zauza (**holotype** CBS H–21365, culture **ex-type** CBS 134664 **=** LPF217); from soil collected in *Eucalyptus* plantations, Apr. 2011, E. Zauza, CBS 134667 = LPF263.

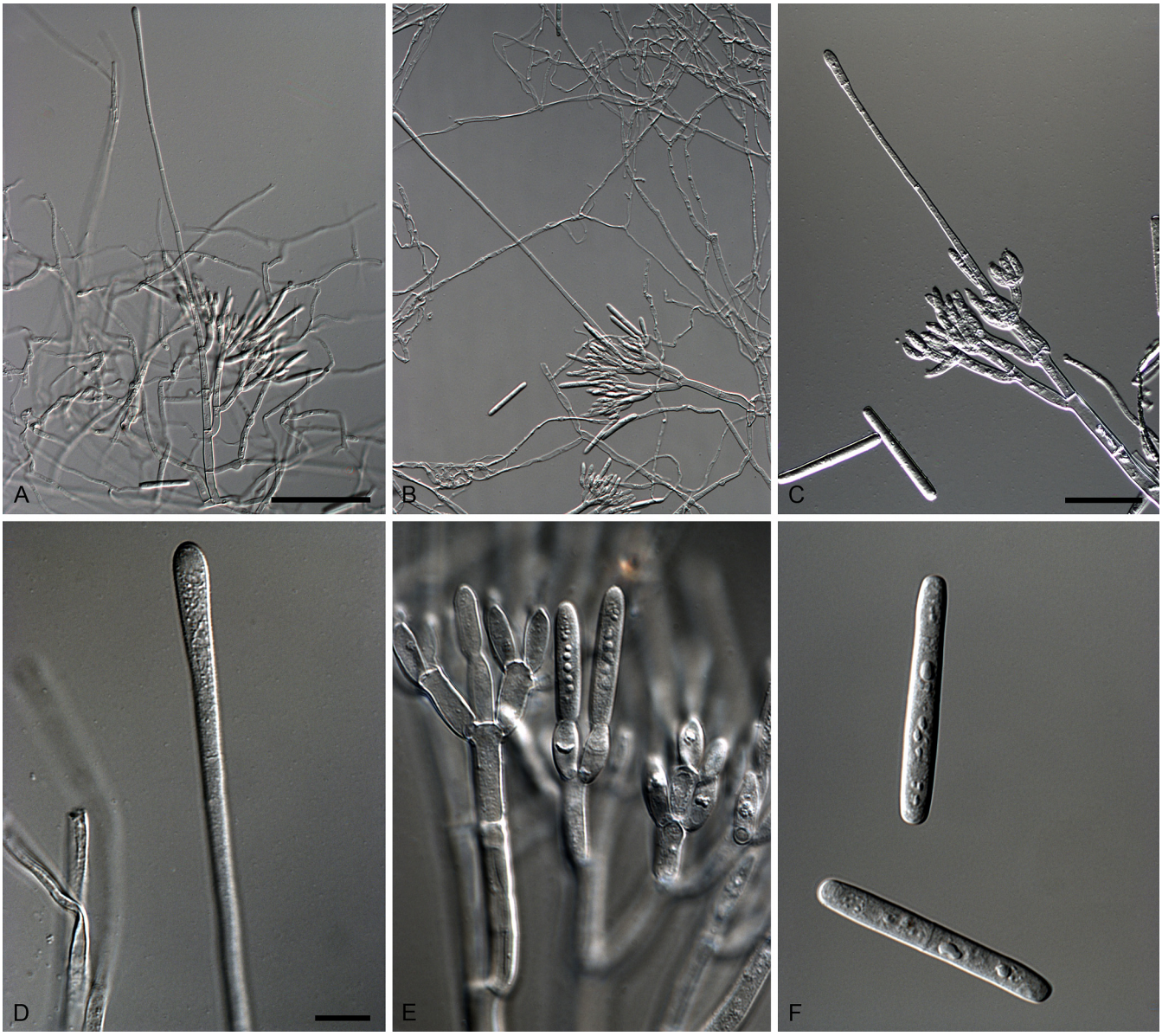

Fig. 24. *Calonectria telluricola* (ex-type CBS 134664). A–C. Macroconidiophores. D. Clavate vesicle. E. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. F. Macroconidia. Scale bars: A = 100 µm (apply to B); C = 20 µm; D = 10 µm (apply to E–F).

Note: *Calonectria telluricola* can be morphologically distinguished from other members of the *Ca. brassicae* complex by the length/diam ratio of its macroconidia, and number of conidiophore branches (Table 3).

DISCUSSION

The present study represents the largest number of *Calonectria* isolates and species from Brazil ever subjected to DNA sequence analyses. Phylogenetic studies published on the genus *Calonectria* in recent years have substantially influenced its taxonomy (Lombard *et al.* 2010c). In the past five years alone, the phylogenetic species recognition concept (Taylor *et al.* 2000) has led to the description of at least 20 additional new species of *Calonectria* (Lombard *et al.* 2010b,c, Chen *et al.* 2011, Xu *et al.* 2012, Alfenas *et al.* 2013a,b). In this study, a further 20 new *Calonectria* species are introduced from Brazil, with the name *Ca. pseudopteridis* being introduced for *Cy. macrosporum*, which is restored to species rank based on morphological characteristics and phylogenetic inference. The introduction of these novel *Calonectria* species supports the view that there are many more species in this genus to be discovered, particularly from the tropics and Southern Hemisphere (Crous *et al.* 2006, Lombard *et al.* 2010c).

Mendes & Urben (2014) list 23 *Calonectria* and 24 *Cylindrocladium* species in their list of fungi known from Brazil, although this is based on the old nomenclature and associated publications. However, recent literature (Crous 2002, Lombard *et al.* 2009, 2010c, Alfenas *et al.* 2013a, b) provide DNA proof for at least 25 *Calonectria* species from Brazil, which include *Ca. avesiculata*, *Ca, brasiliensis*, *Ca. brassicae*, *Ca. canadensis*, *Ca. cylindrospora, Ca. gracilis*, *Ca. hederae*, *Ca. hodgesii*, *Ca. hurae*, *Ca. ilicicola*, *Ca. indusiata*, *Ca. insularis*, *Ca. kyotensis*, *Ca. lauri*, *Ca. leguminum*, *Ca. metrosideri*, *Ca. naviculata*, *Ca. ovata*, *Ca. pauciramosa*, *Ca. penicilloides*, *Ca. pteridis*, *Ca. pyrochroa*, *Ca. spathiphylli*, *Ca. spathulata* and *Ca. variabilis*. Additionally, *Ca. rubropunctata* (Silva & Minter 1995) and *Ca. meliolae* (Herbário Virtual da Flora e dos Fungos – http://inct.splink.org.br) have also been reported from Brazil, but we regard these taxa as dubious, pending their re-examination.

Most of the isolates obtained from *Eucalyptus* leaves displaying symptoms of CLB were identified as *Ca. pteridis* in this study. *Calonectria pteridis* is regarded as the most important causal agent of CLB of *Eucalyptus* throughout Brazil (Alfenas *et al.* 2004, Graça *et al.* 2009, Alfenas *et al.* 2013c) and was first reported in Brazil by Hodges & May (1972), causing needle blight of *Pinus caribaea* var. *hondurensis*. Thereafter, this fungal pathogen has been reported on various plant hosts in Brazil, mostly associated with leaf spot diseases of these respective hosts (Silva & Souza 1981, Dianese *et al.* 1986, Silva 1996, Trindade *et al.* 1998, Crous 2002). Past taxonomic studies that included *Ca. pteridis* (Crous & Wingfeld 1994, Crous *et al.* 1997, Crous 2002, Crous *et al.* 2006) concluded that this species should be regarded as a species complex based on the morphological variation observed during these studies. However, phylogenetic inference in this study failed to identify any cryptic species among the hundreds of *Ca. pteridis* isolates obtained during this study. Only one new taxon, *Ca. pseudovata*, could be identified in the *Ca. pteridis* species complex, which in the past also included *Ca. gordoniae* and *Ca. ovata* (Crous *et al.* 1997, Crous *et al.* 2006, Lombard *et al.* 2010c). *Calonectria pseudovata* appears to have a limited distribution, as all isolates were obtained from soil collected in a commercial *Eucalyptus* plantation in the state of Pará, and its pathogenicity needs to be tested experimentally. Additionally, *Ca. pseudopteridis* is introduced for *Cy. macrosporum*, based on the phylogenetic inference in this study. This species was considered synonymous with *Ca. pteridis* (Sobers 1968, Crous & Wingfeld 1994, Crous 2002) based on morphology only, but DNA sequence analysis revealed it to be distinct from that species. However, more isolates need to be (re)collected and strains from previous studies (Renard & Viennot-Bourgin 1973, Renard & Quillec 1979, Ahmad & Ahmad 1982) need to be re-evaluated to determine the distribution and host range of *Ca. pseudopteridis*.

Species in the *Ca. brassicae* complex are characterised by having clavate vesicles and small (<60 µm), 1-septate macroconidia. Species known to belong to this complex include: *Ca. brachiatica, Ca. brassicae*, *Ca. clavata*, *Ca. ecuadoriae, Ca. gracilipes, Ca. gracilis, Ca. orientalis* and *Ca. pini* (Crous *et al.* 2006, Lombard *et al.* 2009, 2010c). In the study by Crous *et al.* (2006) re-evaluating *Calonectria* species with clavate vesicles, only two new species could be resolved at that time. However, in this study, six new *Calonectria* species were delineated within this complex, with only *Calonectria robigophila* associated with CLB. The remaining species (*Ca. duoramosa*, *Ca. paraensis*, *Ca. pseudobrassicae*, *Ca. quinqueramosa* and *Ca. telluricola*) were all isolated from soil, and the extent of their pathogenicity to *Eucalyptus* still needs to be assessed. Only *Ca. paraensis* and *Ca. telluricola* were isolated from soils collected in tropical rainforests surrounding established *Eucalyptus* plantations. However, whether these species originated from these natural environments and were introduced into plantations through the movement of soil, still needs to be determined.

The *Ca. cylindrospora* complex is characterised by having 1-septate macroconidia and vesicles varying from pyriform to obpyriform or ovoid to ellipsoidal, and includes *Ca. brasiliensis, C. cerciana*, *Ca. cylindrospora*, *Ca. hawksworthii*, *Ca. hodgesii, Ca. insularis*, *Ca. leucothöes*, *Ca. sulawesiensis* and *Ca. variabilis* (Crous 2002, Lombard *et al.* 2010c, Alfenas *et al.* 2013b). This complex has been extended in this study by the introduction of four new species (*Ca. maranhensis*, *Ca. pseudocerciana*, *Ca. pseudohodgesii* and *Ca. propaginicola*), based on phylogenetic inference and morphological features. Previous studies (Crous *et al.* 1993, Overmeyer *et al.* 1996, Schoch *et al.* 1999, 2000) focussing on the taxonomy of the *Ca. cylindrospora* complex initially regarded these species as either *Ca. cylindrospora* (= *Cy. scoparium*) or *Ca. candelabra* (= *Cy. candelabrum*) based on their morphological similarities. However, *Ca. cylindrospora* has been circumscribed as having ellipsoidal to pyriform vesicles and *Ca. candelabra* having ellipsoidal to obpyriform vesicles (Crous *et al.* 1993). Of the four species described in this complex, only *Ca. maranhensis* was isolated from soil and *Eucalyptus* leaves displaying CLB collected in commercial plantations. The remaining three species were all isolated from *Eucalyptus* seedlings displaying symptoms of damping-off, with *Ca. pseudohodgesii* also isolated from *Azadirachta indica* leaves showing CLB symptoms, collected at the same nursery in Viçosa.

The *Ca. candelabra* complex is characterised by having ellipsoidal to obpyriform vesicles and 1-septate macroconidia (Schoch *et al.* 1999, Crous 2002, Lombard *et al.* 2010b). This complex includes *Ca. candelabra*, *Ca. colombiana*, *Ca. metrosideri, Ca. mexicana*, *Ca. mossambicensis*, *Ca. pauciramosa*, *Ca. pseudoscoparia*, *Ca. polizzii*, *Ca. spathulata* and *Ca. zuluensis* (Schoch *et al.* 1999, Lombard *et al.* 2010b 2011, Crous *et al.* 2013, Alfenas *et al.* 2013a). Eight new species (*Ca. brassiana*, *Ca. eucalypticola*, *Ca. glaebicola*, *Ca. nemuricola*, *Ca. paiuiensis*, *Ca. pseudospathulata* and *Ca. silvicola*) are introduced in this complex from this study. Of these, *Ca. eucalypticola* was the only species isolated from *Eucalyptus* seedlings displaying symptoms of damping-off, and from soil and *Eucalyptus* leaves with CLB symptoms in commercial plantations. This suggests that this species could have been introduced into the plantation environment from infected seedlings supplied by the nursery. *Calonectria nemuricola*, *Ca. pseudospathulata* and *Ca. silvicola* were only isolated from soils collected in tropical rainforests neighbouring commercial *Eucalyptus* plantations, and therefore their pathogenicity on *Eucalyptus* still needs to be determined. *Calonectria piauiensis* was isolated from soils collected in both commercial plantations and neighbouring tropical rainforests, whereas *Ca. brassiana* was only isolated from soils collected in a commercial plantation of *E. brassiana*. Both *Ca. glaebicola* and *Ca. pseudometrosideri* were found in soils and on *Eucalyptus* leaves, with the latter also isolated from *Metrosideros polymorpha*. The *Ca. candelabra* complex represents an important pathogen complex, having been reported worldwide on numerous plant hosts, and being regarded as dominant in commercial forest nurseries (Schoch *et al.* 1999, Crous 2002, Lombard *et al.* 2010b, Vitale *et al.* 2013). Members of this complex have been reported in regions where the climatic conditions differ significantly, supporting the view that these species can tolerate a wide range of environmental conditions (Crous 2002, Lombard *et al.* 2010b, c). *Calonectria multinaviculata* is introduced here as a new species in the *Ca. naviculata* complex. This species was isolated from soil collected in commercial *Eucalyptus* plantations, and therefore nothing is known about its pathogenicity.

Considering the distribution and substrates from which the new species described here were collected, it is apparent that the majority of species were isolated from soils collected in commercial *Eucalyptus* plantations. It is still uncertain whether these soil-borne species were originally present in the soil or

were introduced during the establishment of these plantations. Extensive pathogenicity trials are, however, needed to determine if these species present a potential risk to *Eucalyptus* plantations in Brazil. The highest species diversity found in *Eucalyptus* plantations appears to be in the Brazilian states of Pará, Minas Gerais and Bahia, with fewer species recorded from the states of Maranhão and Piauí, and only single species from the states of Alagoas and Tocantins. More extensive surveys are required for the remaining areas in Brazil to obtain a clearer view on the species diversity of *Calonectria* in Brazil.

Recently, Lombard *et al.* (2010c) divided the genus *Calonectria* into two groups, the Prolate- and Sphaero-Naviculate groups, which corresponded well with terminal vesicle morphology of the respective species. The Prolate Group includes the majority of plant pathogenic *Calonectria* species, which appear to have distinct biogeographic distributions. For example, the *C. reteaudii* complex has only been reported from Australia, China, Indonesia and New Zealand, while the *C. brassicae* complex has only been reported from South and Central America, now including *C. orientalis* which is newly reported from Brazil here. The remaining members of the Prolate Group appear to have broad geographic distributions (Schoch *et al.* 2001, Lombard *et al.* 2010c). All novel taxa treated in this study, belonged to the Prolate Group. In the Sphaero-Naviculate Group there were no obvious patterns of distribution and pathogenicity, and only vesicle morphology appeared consistent. However, the highest species diversity in this group appears to be in the Northern Hemisphere and Asia (Crous *et al.* 2004b, Lombard *et al.* 2015a,b).

Other than clear global distribution patterns in the Prolate- and Sphaero-Naviculate groups of *Calonectria* species, there also appears to be a movement of taxa from natural forests to commercial forest nurseries, and again from nurseries to commercial plantations. The next logical question would be to establish if these species are also exported along with plant material in international trade. While this could be feasible in South America, no evidence yet has been found supporting such movement of any of the new species described here to other continents. There are however ample examples of the global movement of other pathogen groups along with this host, such as *Ceratocystis fimbriata* (Ferreira *et al.* 2011), *Teratosphaeria nubilosa* (Teratosphaeria leaf Blight; Hunter *et al.* 2009, Quaedvlieg *et al.* 2014), and *Chrysoporthe cubensis* (Van der Merwe *et al.* 2013). Global trade in forest products and plant material remains a serious concern for biosecurity, as *Calonectria* species could represent a more serious threat when introduced into favourable climate zones, and hence could pose a serious problem for the establisment of *Eucalyptus* plantations elsewhere in the world.

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