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Phylogeny and systematics of the genus *Calonectria*

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Abstract: Species of *Calonectria* are important plant pathogens, several of which have a worldwide distribution. Contemporary taxonomic studies on these fungi have chiefly relied on DNA sequence comparisons of the β-tubulin gene region. Despite many new species being described, there has been no phylogenetic synthesis for the group since the last monographic study almost a decade ago. In the present study, the identity of a large collection of *Calonectria* isolates from various geographic regions was determined using morphological and DNA sequence comparisons. This resulted in the discovery of seven new species; *Ca. densa*, *Ca. eucalypti*, *Ca. humicola*, *Ca. orientalis*, *Ca. pini*, *Ca. pseudoscoparia* and *Ca. sulawesiensis*, bringing the total number of currently accepted *Calonectria* species to 68. A multigene phylogeny was subsequently constructed for all available *Calonectria* spp., employing seven gene regions, namely actin, β-tubulin, calmodulin, histone H3, the internal transcribed spacer regions 1 and 2 and the 5.8S gene of the ribosomal RNA, 28S large subunit RNA gene and translation elongation 1-alpha. Based on these data 13 phylogenetic groups could be distinguished within the genus *Calonectria* that correlated with morphological features. Dichotomous and synoptic keys to all *Calonectria* spp. currently recognised are also provided.

Key words: *Cylindrocladium*, DNA phylogeny, sexual compatibility, taxonomy.
Taxonomic novelties: New combinations - *Calonectria angustata* (Crous & El-Gholl) L. Lombard, M.J. Wingf. & Crous, *Ca. australiensis* (Crous & H.D. Hyde) L. Lombard, M.J. Wingf. & Crous, *Ca. canadensis* (J.C. Kang, Crous & C.L. Schoch) L. Lombard, M.J. Wingf. & Crous, *Ca. chinensis* (Crous) L. Lombard, M.J. Wingf. & Crous, *Ca. citri* (H.S. Fawc. & Klotz) L. Lombard, M.J. Wingf. & Crous, *Ca. curvata* (Boedijn & Reitsma) L. Lombard, M.J. Wingf. & Crous, *Ca. curvispora* (Crous & D. Victor) L. Lombard, M.J. Wingf. & Crous, *Ca. ecuadoriae* (Crous & M.J. Wingf.) L. Lombard, M.J. Wingf. & Crous, *Ca. gordoniae* (Leahy, T.S. Schub. & El-Gholl) L. Lombard, M.J. Wingf. & Crous, *Calonectria hawksworthii* (Peerally) L. Lombard, M.J. Wingf. & Crous, *Calonectria hurae* (Crous) L. Lombard, M.J. Wingf. & Crous, *Calonectria indonesiae* (Crous) L. Lombard, M.J. Wingf. & Crous, *Ca. leucothoës* (El-Gholl, Leahy & T.S. Schub.) L. Lombard, M.J. Wingf. & Crous, *Ca. malesiana* (Crous) L. Lombard, M.J. Wingf. & Crous, *Ca. multiphialidica* (Crous, Simoneau & Risède) L. Lombard, M.J. Wingf. & Crous, *Ca. pacifica* (J.C. Kang, Crous & C.L. Schoch) L. Lombard, M.J. Wingf. & Crous, *Ca. penicilloides* (Tubaki) L. Lombard, M.J. Wingf. & Crous, *Ca. pseudonaviculata* (Crous, J.Z. Groenew. & C.F. Hill) L. Lombard, M.J. Wingf. & Crous, *Ca. sumatrensis* (Crous) L. Lombard, M.J. Wingf. & Crous. **New species -** *Ca. densa* L. Lombard, M.J. Wingf. & Crous, *Ca. eucalypti* L. Lombard, M.J. Wingf. & Crous, *Ca. humicola* L. Lombard, M.J. Wingf. & Crous, *Ca. orientalis* L. Lombard, M.J. Wingf. & Crous, *Ca. pini* L. Lombard, M.J. Wingf. & Crous, *Ca. pseudoscoparia* L. Lombard, M.J. Wingf. & Crous, *Ca. sulawesiensis* L. Lombard, M.J. Wingf. & Crous.

INTRODUCTION

The genus *Calonectria* (*Ca.*) was first described in 1867, with *Ca. daldiniana* as the type. This species was later reduced to synonymy with *Ca. pyrochroa* based on morphological comparisons by Rossman (1979). *Calonectria* spp. are *Euascomycetes* in the order *Hypocreales* (Hibbett *et al.* 2007, Schoch *et al.* 2009) and are characterised by their yellow to dark red perithecia, with scaly to warty ascocarp walls giving rise to long-stalked, clavate asci with 1–multi-septate ascospores and *Cylindrocladium* (*Cy.*) anamorphs (Rossman 1993, Crous 2002, Lombard *et al*. 2010b). The genus *Cylindrocladium* was described by Morgan (1892), and is characterised by branched conidiophores with stipe extensions terminating in characteristic vesicles and producing cylindrical, 1–multi-septate conidia (Crous & Wingfield 1994, Crous 2002). Morphologically, the anamorph provides the greatest number of distinguishing characters for *Calonectria* and it is also the state most frequently encountered in nature (Peerally 1991, Crous & Wingfield 1994, Schoch *et al.* 2001b, Crous 2002). Consequently, species of *Calonectria* are primarily distinguished by their anamorph characters, especially vesicle shape, stipe extension length, conidial septation, and dimensions on a standardised medium under defined growth conditions (Boesewinkel 1982, Peerally 1991, Crous & Wingfield 1994, Crous 2002). Despite, the use of standardised conditions, taxonomic confusion can result because some intraspecific variation in vesicle shape and conidial dimension is common (Crous & Peerally 1996, Crous *et al.* 1998a).

The reliability of vesicle shape as a distinguishing morphological character has been questioned (Sober & Alfieri 1972, Hunter & Barnett 1978, Rossman 1983), although Crous *et al.* (1992) demonstrated experimentally that the shape of this structure can be influenced by the osmotic potential of the medium and the age of the culture, but that it remains a reliable morphological feature if these aspects are standardised. In the original description of *Ca. morganii* (= *Cy. scoparium*), the type of the anamorph, Morgan (1892) failed to include details of the stipe extension and terminal vesicle, which is a defining characteristic in distinguishing anamorphs of *Calonectria* (Boesewinkel 1982, Peerally 1991, Crous & Wingfield 1994, Crous 2002).

Calonectria spp. produce three different morphological forms of conidia, of which the macroconidia are present in all but *Ca. multiseptata* (Peerally 1991, Crous & Wingfield 1994, Crous *et al.* 1998b, Crous 2002). Mega- and microconidia are less frequently encountered and these are not regarded as important characters to distinguish between species (Sober 1971, Crous & Wingfield 1994, Crous & Seifert 1998, Crous 2002). Similar to vesicle shape,

significant variability can occur in the production of all conidial types, so that this feature alone is not always a reliable taxonomic character to define species.

Both homothallic and heterothallic mating systems are found amongst species of *Calonectria* (Alfieri *et al.* 1982, Schubert *et al.* 1989, Crous & Wingfield 1994, Crous 2002). Heterothallic *Calonectria* spp. have a biallelic heterothallic mating system with the female structures (protoperithecia) spermatised by conidia or hyphae of an opposite mating type strain (Schoch *et al.* 1999, 2000a, 2001a). Some *Calonectria* spp. have retained the ability to recombine with other closely related *Calonectria* spp., although the progeny from these crosses have low levels of fertility (Crous 2002). This has complicated the application of the biological species concept for *Calonectria,* although it has been useful for some species (Schoch *et al.* 1999, Lombard *et al.* 2010a).

Difficulties experienced in morphological identification, have led to several molecular approaches being employed to identify *Calonectria* spp. These include total protein electrophoresis (Crous *et al.* 1993a, El-Gholl *et al.* 1993), isozyme electrophoresis (El-Gholl *et al.* 1992, El-Gholl *et al.* 1997, Crous *et al.* 1998a), random amplification of polymorphic DNA (RAPD) (Overmeyer *et al.* 1996, Victor *et al.* 1997, Schoch *et al.* 2000a, Risède & Simoneau 2004), restriction fragment length polymorphisms (RFLP) (Crous *et al.* 1993b, Crous *et al.* 1995, Crous *et al.* 1997, Jeng *et al.* 1997, Victor *et al.* 1997, Risède & Simoneau 2001) and DNA hybridisation (Crous *et al.* 1993a, 1995, 1997, Victor *et al.* 1997). However, DNA sequence comparisons and associated phylogenetic inference has had the most significant impact on the taxonomy of the group. It is also most widely applied in contemporary species descriptions. The 5.8S ribosomal RNA gene and flanking internally transcribed spacer (ITS) sequences made it possible for Jeng *et al.* (1997) to distinguish between *Cy. scoparium* and *Cy. floridanum* isolates. Subsequently, it was found that this gene region contains few informative characters for members of the genus (Crous *et al.* 1999, Schoch *et al.* 1999, Risède & Simoneau 2001, Schoch *et al.* 2001b). As a consequence, this resulted in the β-tubulin (BT) (Schoch *et al.* 2001b) and histone H3 (HIS3) (Kang *et al.* 2001b) gene regions being widely employed to improve the resolution of phylogenetic trees for species of *Calonectria*.

The first complete DNA sequence-based phylogenetic study using partial BT gene sequences (Schoch *et al.* 2001b) compared phenotypic, biological and phylogenetic species concepts used in the taxonomy of *Calonectria.* Results showed that the genus represents a well resolved monophyletic lineage. Subsequently, combined DNA sequence data for the ITS, BT and HIS3 gene regions have been used to resolve taxonomic questions for *Calonectria* (Schoch *et al.* 2000a, Henricot & Culham 2002, Crous *et al.* 2004b, 2006). Other DNA sequences recently used to distinguish between species include the translation elongation factor 1–alpha (TEF-1α) and calmodulin (CAL) gene regions (Crous *et al.* 2004b, Lombard *et al.* 2009, 2010a, b). However, sequence data for these regions on GenBank (www.ncbi.nlm.nih.gov) are incomplete for the group, substantially reducing their value.

The aim of this study was to consider the identity of a large collection of previously unidentified *Calonectria* isolates collected over a five year period from various parts of the world. Morphological characteristics, phylogenetic inference and mating compatibility were employed for this purpose. Subsequently, the phylogenetic relationships between *Calonectria* spp. were re-evaluated by constructing a multigene phylogeny for seven gene regions and considering these results together with morphological features for all species in the genus.

MATERIALS AND METHODS

Isolates

Plant material showing symptoms of *Calonectria* infections as well as soil samples were collected from various geographical regions over a period of five years. Diseased plant material was placed in moist chambers and incubated for 48 h at room temperature to induce sporulation. Direct isolations were made onto malt extract agar (2 % w/v; MEA; Biolab, Midrand, South Africa) and cultures were incubated for 7 d at 25 °C under continuous near-ultraviolet light. Baiting, using seeds of *Medicago sativa*, was applied for the soil samples following the technique of Crous (2002). For each isolate, single conidial cultures were prepared on MEA. Representative strains are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa and the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands (Table 1).

DNA extraction and amplification

Identification of unknown *Calonectria* isolates

Total genomic DNA was extracted from 7 d old *Calonectria* cultures using the methods presented in Lombard *et al.* (2008). Three loci were amplified and sequenced. These included a fragment of the BT gene region using primers T1 (O'Donnell & Cigelnik 1997) and CYLTUB1R (Crous *et al.* 2004b), a fragment of the HIS3 gene region using primers CYLH3F and CYLH3R (Crous *et al.* 2004b) and a fragment of the TEF-1α gene region using primers EF1-728F (Carbone & Kohn 1999) and EF2 (O'Donnell *et al.* 1998).

Phylogenetic relationships amongst *Calonectria* spp.

Total genomic DNA was extracted as above. Seven loci were amplified including the ITS gene region using primers V9G (De Hoog & van den Ende 1998) and ITS4 (White *et al.* 1990); the 28S large subunit RNA gene (LSU) using primers LR0R (Moncalvo *et al.* 1995) and LR5 (Vilgalys & Hester 1990); and parts of the TEF-1α gene region; the BT gene region; the HIS3 gene region with the same primer sets mentioned previously, the actin (ACT) gene region using primers ACT-512F and ACT-783R (Carbone & Kohn 1999) and CAL gene region using primers CAL-228F and CAL-737R (Carbone & Kohn 1999).

The PCR reaction mixture used to amplify the different loci consisted of 2.5 units FastStart *Taq* polymerase (Roche Applied Science, USA), 1× PCR buffer, 1–1.5 mM MgCl2, 0.25 mM of each dNTP, 0.5 µm of each primer and approximately 30 ng of fungal genomic DNA, made up to a total reaction volume of 25 µL with sterile deionised water. Amplified fragments were purified using High Pure PCR Product Purification Kit (Roche, U.S.A.).

DNA sequencing and analysis

Amplified fragments were sequenced in both directions using the same primer pairs used for amplification. For this purpose, the BigDye terminator sequencing kit v. 3.1 (Applied Biosystems, U.S.A.) and an ABI PRISM™ 3100 DNA sequencer (Applied Biosystems) were used. All PCRs and sequencing reactions were performed on an Eppendorf Mastercycler Personal PCR (Eppendorf AG, Germany) with cycling conditions as described in Crous *et al.* (2006) for all loci amplified.

In addition to the sequences generated in this study, *Calonectria* spp. sequences were obtained from GenBank. All sequences were assembled and aligned using Sequence Navigator v. 1.0.1 (Applied Biosystems) and MAFFT v. 5.11 (Katoh *et al.* 2005), respectively. The aligned sequences were then manually corrected where necessary. Single nucleotide polymorphisms (SNP's) were determined for the aligned DNA sequences of each gene region using DnaSP v. 5.00.06 (Librado & Rozas 2009)

To determine whether the DNA sequence data sets were congruent, a partition homogeneity test (PHT; Farris *et al.* 1994) of all possible combinations, with 1 000 replications on all informative characters was conducted in PAUP (Phylogenetic Analysis Using Parsimony, v. 4.0b10; Swofford 2002). A 70 % reciprocal bootstrap method using Neighbour-Joining with Maximum Likelihood distance (Mason-Gamer & Kellogg 1996; Gueidan *et al.* 2007) was also employed. Models of evolution were estimated in Modeltest v. 3.7 (Posada & Crandall 1998) using the Akaike Information Criterion (AIC) for each gene region. The bootstrap analyses were run in PAUP for 10 000 replicates. Resulting tree topologies were compared visually for conflict between the separate gene regions.

Maximum-parsimony genealogies, for single genes and the combined genes were estimated in PAUP, by heuristic searches based on 1 000 random addition sequences and tree bisection-reconnection, with the branch swapping option set on "best trees" only. All characters were weighted equally and alignment gaps were treated as missing data. Statistics calculated for parsimony included tree length (TL), consistency index (CI), retention index (RI) and rescaled consistence index (RC). Bootstrap analysis (Hillis & Bull 1993) was based on 1 000 replications. All sequences for the isolates studied were analysed using the Basic Local Alignment Search Tool for Nucleotide sequences (BLASTN, Altschul *et al.* 1990).

A Markov Chain Monte Carlo (MCMC) algorithm was used to generate phylogenetic trees for each gene region and combined sequence data subsets with Bayesian probabilities using MrBayes v. 3.1.1 (Ronquist & Huelsenbeck 2003). Models of nucleotide substitution for each gene were determined using MrModeltest (Nylander 2004) and included for each gene partition. Four MCMC chains were run simultaneously from random trees for one million generations, sampled every 100 generations and repeated twice. Both runs converged on the same likelihood score and tree topology for each gene. The first 1 000 trees were, therefore, discarded as the burn-in phase of each analysis and posterior probabilities were determined from the remaining trees.

Sexual compatibility

Based on the results of the DNA sequence analyses, single conidial isolates of *Calonectria* spp. of unknown identity were crossed with closely related species in all possible combinations. Where available, mating tester strains defined in previous studies were also used. Crosses were made as described in Schoch *et al.* (1999) on carnation leaf agar (CLA; Fisher *et al.* 1982, Crous *et al.* 1993a) and minimal salt agar (MSA; Guerber & Correll 2001, Halleen *et al.* 2006) with sterile toothpicks placed on the surface of the agar (Lombard *et al.* 2010a). Controls consisted of isolates self-crossed, making it possible to distinguish between those having heterothallic or homothallic mating systems. Isolates CBS 125273–125276 from Indonesia were mated with *Ca. macroconidialis* (CBS 114880). Colombian isolates CBS 123698 and CMW 31210 and Indonesian isolates CBS 125258–125260 were crossed with *Ca. brachiatica* (CBS 123700 and CMW 25302) and *Ca. brassicae* (CBS 111478 and CBS 111869) in all possible combinations. Isolates CBS 125248, CBS 125253, CBS 125277 and CMW 14883 were crossed with *Ca. cerciana* (CBS 123693 and CBS 123695), *Ca. brasiliensis* (CBS 230.51 and CBS 114257) and mating tester strains of *Ca. insularis* (CBS 114558 and CBS 114559; Schoch *et al.* 1999). Similarly, isolates CBS 125249–125252, CBS 125261 and CBS 125269 were crossed with mating tester strains of *Ca. spathiphylli* (CBS 114540 and CBS 116168; Crous 2002). Isolates CBS 125254–125257 were crossed with mating tester strains of *Ca. scoparia* (CMW 31000 and CMW 31001; Lombard *et al.* 2010a) and *Ca. pauciramosa* (CMW 5683 and CMW 30823; Schoch *et al.* 2001a). The plates were stacked in plastic containers and incubated at 22 °C for 6–8 wk. Crosses were regarded as successful when isolate combinations produced numerous perithecia extruding viable ascospores.

Taxonomy

For identification of *Calonectria* isolates based on morphology, single conidial cultures were prepared on MEA and synthetic nutrient-poor agar (SNA; Nirenburg 1981, Lombard *et al.* 2009, 2010a, c). Inoculated plates were incubated at room temperature and examined after 7 d. Gross morphological characteristics of the anamorph structures were determined by mounting fungal structures in lactic acid and 30 measurements at ×1 000 magnification were made for all taxonomically informative characters for each isolate. Teleomorph morphology was determined by mounting perithecia resulting from the sexual compatibility tests in Leica mountant (Setpoint Premier, Johannesburg, South Africa) and making sections using a Leica CM1100 cryostat (Setpoint Technologies) at -20 °C. The 10 µm sections were mounted in lactophenol or 3 % KOH. Gross morphological characteristics were determined in the same manner as for the anamorph states. The 95 % confidence levels were determined and extremes of conidial measurements are given in parentheses. For other structures, only extremes are presented in the descriptions. Optimal growth conditions for cultures were determined in the dark on MEA for each isolate, at temperatures ranging from 5–35 °C at 5 °C intervals with three replicate plates for each temperature tested. Two measurements of culture diameter perpendicular to each other were made daily for 7 d. Colony colours were determined after 7 d on MEA at 25 °C in the dark, using the colour charts of Rayner (1970). Descriptions, nomenclature and illustrations were deposited in MycoBank (Crous *et al.* 2004a).

RESULTS

DNA sequencing and analysis

Identification of unknown *Calonectria* isolates

Amplicons of approx. 500 bp were generated for the BT and TEF-1α gene regions and those for the HIS3 region were approx. 450 bp in length. Based on preliminary BT sequence comparisons and morphological characteristics, the sequence data sets for the unknown *Calonectria* spp. were divided into four separate data sets representing the *Ca. colhounii*, *Ca. brassicae*, *Ca. scoparia* and *Ca. morganii* complexes and other closely related species in each data set. These data sets were analysed separately with *Ca. colombiensis* (CBS 112221) and *Ca. chinensis* (CBS 112744)

Table 1. Isolates of *Calonectria* spp. studied.

Species	Isolate number[1]	Other collections[1]	GenBank accession nr.[2]							Reference[3]
			ACT	BT	CAL	HIS3	ITS	LSU	TEF-1α	
Ca. acicola	CBS 114812		GQ280424	DQ190590	GQ267359	DQ190692	GQ280546	GQ280668	GQ267291	Gadgil & Dick (2004)
	CBS 114813^T	CMW 30996	GQ280425	DQ190591	GQ267360	DQ190693	GQ280547	GQ280669	GQ267292	
Ca. angustata	CBS 109065^T	CMW 30990 = CPC 2347 = P99-0454	GQ280426	AF207543	GQ267361	DQ190696	GQ280548	GQ280671	FJ918551	Crous (2002)
	CBS 109169	CMW 30983 = CPC 3152 = P99-1321	GQ280427	DQ190593	GQ267362	DQ190695	GQ280549	GQ280670	FJ918552	
Ca. asiatica	CBS 112711	CPC 3898 = SFE 744	GQ280429	AY725613	AY725738	AY725655	GQ280551	GQ280673	AY725702	Crous *et al.* (2004b)
	CBS 114073^T	CMW 23782 = CPC 3900 = SFE 726	GQ280428	AY725616	AY725741	AY725658	GQ280550	GQ280672	AY725705	
Ca. australiensis	CBS 112954^T	CMW 23669 = CPC 4714	GQ280430	DQ190596	GQ267363	DQ190699	GQ280552	GQ280674	GQ267293	Crous *et al.* (2006)
Ca. avesiculata	CBS 313.92^T	CMW 23670 = CPC 2373 = ATCC 38226	GQ280431	AF333392	GQ267364	DQ190620	GQ280553	GQ280675	GQ267294	Crous (2002)
Ca. brachiatica	CBS 123700^T	CMW 25298	GQ280433	FJ696388	GQ267366	FJ696396	GQ280555	GQ280677	GQ267296	Lombard *et al.* (2009)
	CMW 25302		GQ280432	FJ716708	GQ267365	FJ716712	GQ280554	GQ280676	GQ267295	
Ca. brassicae	CBS 111478	CMW 30981	GQ280455	DQ190611	GQ267383	DQ190719	GQ280577	GQ280699	FJ918567	Crous (2002)
	CBS 111869^T	CMW 30982 = CPC 2409 = PC 551197	GQ280454	AF232857	GQ267382	DQ190720	GQ280576	GQ280698	FJ918566	
Ca. brasiliensis	CBS 230.51^T	CMW 23670 = CPC 2390	GQ280502	GQ267241	GQ267421	GQ267259	GQ280624	GQ280746	GQ267328	Lombard *et al.* (2009c)
	CBS 114257	CMW 32949 = CPC 1944	GQ280503	GQ267242	GQ267422	GQ267260	GQ280625	GQ280747	GQ267329	
Ca. canadensis	CBS 110817^T	CMW 23673 = CPC 499	GQ280434	AF348212	AY725743	AF348228	GQ280556	GQ280678	GQ267297	Crous (2002)
Ca. cerciana	CBS 123693^T	CMW 25309	GQ280437	FJ918510	GQ267369	FJ918528	GQ280559	GQ280681	FJ918559	Lombard *et al.* (2010c)
	CBS 123695	CMW 25290	GQ280438	FJ918511	GQ267370	FJ918529	GQ280560	GQ280682	FJ918560	
Ca. chinensis	CBS 112744	CMW 30986 = CPC 4104	GQ280440	AY725618	AY725746	AY725660	GQ280562	GQ280684	AY725709	Crous *et al.* (2004b)
	CBS 114827^T	CMW 23674 = CPC 4101	GQ280390	AY725619	AY725747	AY725661	GQ280561	GQ280683	AY725710	
Ca. citri	CBS 186.36^T	CMW 23675	GQ280441	AF333393	GQ267371	GQ267247	GQ280563	GQ280685	GQ267299	Crous (2002)
Ca. clavata	CBS 114557^T	CMW 23690 = CPC 2536 = ATCC 66389	GQ280449	AF333396	GQ267377	DQ190623	GQ280571	GQ280693	GQ267305	Crous (2002)
	CBS 114666^T	CMW 30994 = CPC 2537	GQ280450	DQ190549	GQ267378	DQ190624	GQ280572	GQ280694	GQ267306	
Ca. colhounii	CBS 293.79^T	CMW 30999	GQ280443	DQ190564	GQ267373	DQ190639	GQ280565	GQ280687	GQ267301	Crous (2002)
	CBS 114704		GQ280442	DQ190563	GQ267372	DQ190638	GQ280564	GQ280686	GQ267300	
Ca. colombiana	CBS 115127^T	CMW 30871 = CPC 1160	GQ280538	FJ972423	GQ267455	FJ972442	GQ280660	GQ280782	FJ972492	Lombard *et al.* (2010a)
	CBS 115638	CMW 30766 = CPC 1161	GQ280539	FJ972422	GQ267456	FJ972441	GQ280661	GQ280783	FJ972491	
Ca. colombiensis	CBS 112220^T	CMW 23676 = CPC 723	GQ280444	GQ267207	AY725748	AY725662	GQ280566	GQ280688	AY725711	Crous *et al.* (2004b)
	CBS 112221	CMW 30985 = CPC 724	GQ280445	AY725620	AY725749	AY725663	GQ280567	GQ280689	AY725712	Crous (2002)

Table 1. (Continued).

Species	Isolate number[1]	Other collections[1]	GenBank accession nr.[2]							Reference[3]
			ACT	BT	CAL	HIS3	ITS	LSU	TEF-1α	
Ca. curvispora	CBS 116159^T	CMW 23693	GQ280446	AF333394	GQ267374	AY725664	GQ280568	GQ280690	GQ267302	Crous (2002)
Ca. densa	CBS 125249	CMW 31184	GQ280523	GQ267230	GQ267442	GQ267279	GQ280645	GQ280767	GQ267350	This study
	CBS 125250	CMW 31185	GQ280524	GQ267231	GQ267443	GQ267280	GQ280646	GQ280768	GQ267351	
	CBS 125261^T	CMW 31182	GQ280525	GQ267232	GQ267444	GQ267281	GQ280647	GQ280769	GQ267352	
Ca. ecuadoriae	CBS 111394	CMW 30980 = CPC 1628	GQ280448	DQ190599	GQ267376	DQ190704	GQ280570	GQ280692	GQ267304	Crous *et al.* (2006)
	CBS 111406^T	CMW 23677 = CPC 1635	GQ280447	DQ190600	GQ267375	DQ190705	GQ280569	GQ280691	GQ267303	
Ca. eucalypti	CBS 125273	CMW 14890	GQ280510	GQ267217	GQ267429	GQ267266	GQ280632	GQ280754	GQ267337	This study
	CBS 125274	CMW 18443	GQ280509	GQ267216	GQ267428	GQ267265	GQ280631	GQ280753	GQ267336	
	CBS 125275^T	CMW18444	GQ280511	GQ267218	GQ267430	GQ267267	GQ280633	GQ280755	GQ267338	
	CBS 125276	CMW 18445	GQ280512	GQ267219	GQ267431	GQ267268	GQ280634	GQ280756	GQ267339	
Ca. gordoniae	CBS 112142	CMW 23694 = CPC 3136 = ATCC 201837	GQ280453	AF449449	GQ267381	DQ190708	GQ280575	GQ280697	GQ267309	Leahy *et al.* (2000)
Ca. gracilipes	CBS 111141^T		GQ280457	DQ190566	GQ267385	DQ190644	GQ280579	GQ280701	GQ267311	Crous (2002)
	CBS 115674		GQ280456	AF333406	GQ267384	DQ190645	GQ280578	GQ280700	GQ267310	
Ca. gracilis	CBS 111284		GQ280489	DQ190567	GQ267408	DQ190647	GQ280611	GQ280733	GQ267324	Crous (2002)
	CBS 111807		GQ280488	AF232858	GQ267407	DQ190646	GQ280610	GQ280734	GQ267323	
Ca. hawksworthii	CBS 111870^T	CPC 2405 = MUCL 30866	GQ280458	AF333407	GQ267386	DQ190649	GQ280580	GQ280702	FJ918558	Crous (2002)
Ca. hongkongensis	CBS 114711	CMW 30995	GQ280460	AY725621	AY725754	AY725666	GQ280582	GQ280704	AY725716	Crous *et al.* (2004b)
	CBS 114828^T		GQ280459	AY725622	AY725755	AY725667	GQ280581	GQ280703	AY725717	
Ca. humicola	CBS 125251^T	CMW 31183	GQ280526	GQ267233	GQ267445	GQ267282	GQ280648	GQ280770	GQ267353	This study
	CBS 125252	CMW 31186	GQ280527	GQ267234	GQ267446	GQ267283	GQ280649	GQ280771	GQ267354	
	CBS 125269	CMW31187	GQ280528	GQ267235	GQ267447	GQ267284	GQ280650	GQ280772	GQ267355	
Ca. hurae	CBS 114551	CMW 16720 = CPC 2344	GQ280461	AF333408	GQ267387	DQ190728	GQ280583	GQ280705	FJ918548	Crous (2002)
Ca. ilicicola	CBS 190.50^T	CMW 30998 = CPC 2482 = IMI 299389	GQ280483	AY725631	AY725764	AY725676	GQ280605	GQ280727	AY725726	Crous (2002)
	CBS 115897		GQ280484	AY725647	GQ267403	GQ267256	GQ280606	GQ280728	AY725729	
Ca. indonesiae	CBS 112823^T	CMW 23683 = CPC 4508	GQ280463	AY725623	AY725756	AY725668	GQ280585	GQ280707	AY725718	Crous *et al.* (2004b)
	CBS 112840	CPC 4547	GQ280464	AY725625	AY725758	AY725670	GQ280586	GQ280708	AY725720	
Ca. indusiata	CBS 144.36	CMW 23699	GQ280536	GQ267239	GQ267453	GQ267262	GQ280658	GQ280780	GQ267332	Crous (2002)
	CBS 114684	CPC 2446 = UFV 16A	GQ280537	AF232862	GQ267454	DQ190652	GQ280659	GQ280781	GQ267333	
Ca. insularis	CBS 114558^T	CMW 30991	GQ280465	AF210861	GQ267389	FJ918526	GQ280587	GQ280709	FJ918556	Crous (2002)
	CBS 114559	CMW 30992	GQ280466	AF210862	GQ267390	FJ918525	GQ280588	GQ280710	FJ918555	
Ca. kyotensis	CBS 170.77	CMW 23679 = IMI 299388	GQ280452	GQ267209	GQ267380	GQ267249	GQ280574	GQ280696	GQ267308	Crous (2002)
	CBS 413.67	CMW 23678 = CPC 2391	GQ280451	GQ267208	GQ267379	GQ267248	GQ280573	GQ280695	GQ267307	
Ca. leguminum	CBS 728.68^T	CMW 23684 = IMI 299578	GQ280467	AF389837	GQ267391	DQ190654	GQ280589	GQ280711	FJ918547	Crous (2002)
Ca. leucothoës	CBS 109166	CMW 30977 = CPC 3612 = P97-2605	GQ280468	FJ918508	GQ267392	FJ918523	GQ280590	GQ280712	FJ918553	Crous (2002)
Ca. macroconidialis	CBS 114880^T	CPC 307	GQ280469	AF232855	GQ267393	DQ190655	GQ280591	GQ280713	GQ267313	Crous (2002)

Table 1. (Continued).

Species	Isolate number[1]	Other collections[1]	GenBank accession nr.[2]							Reference[3]
			ACT	BT	CAL	HIS3	ITS	LSU	TEF-1α	
Ca. madagascariensis	CBS 114571	CMW 30993 = CPC 2253	GQ280471	DQ190571	GQ267395	DQ190657	GQ280593	GQ280715	GQ267315	Crous (2002)
	CBS 114572^{T}	CMW 23686 = CPC 2252	GQ280470	DQ190572	GQ267394	DQ190658	GQ280592	GQ280714	GQ267314	
Ca. malesiana	CBS 112710	CPC 3899	GQ280473	AY725626	AY725759	AY725671	GQ280595	GQ280717	AY725721	Crous *et al.* (2004b)
	CBS 112752^{T}	CMW 23687 = CPC 4223	GQ280472	AY725627	AY725760	AY725672	GQ280594	GQ280716	AY725722	
Ca. mexicana	CBS 110918^{T}	CMW 9055	GQ280474	AF210863	GQ267396	FJ972460	GQ280596	GQ280718	FJ972526	Crous (2002)
Ca. morganii	CBS 110666	CMW 30978 = P90.1479	GQ280504	FJ918509	GQ267423	FJ918527	GQ280626	GQ280748	FJ9188557	Crous (2002)
Ca. multiphialidica	CBS 112678	CMW 23688	GQ280475	AY725628	AY725761	AY725673	GQ280597	GQ280719	AY725723	Crous *et al.* (2004b)
Ca. multiseptata	CBS 112682	CMW 23692 = CPC 1589	GQ280476	DQ190573	GQ267397	DQ190659	GQ280598	GQ280720	FJ918535	Crous (2002)
Ca. naviculata	CBS 101121^{T}	CMW 30974	GQ280478	GQ267211	GQ267399	GQ267252	GQ280600	GQ280722	GQ267317	Crous (2002)
	CBS 116080	CMW 16723	GQ280477	AF333409	GQ267398	GQ267251	GQ280599	GQ280721	GQ267316	
Ca. orientalis	CBS 125258	CMW 20272	GQ280531	GQ267238	GQ267450	GQ267287	GQ280653	GQ280775	GQ267358	This study
	CBS 125259	CMW 20273	GQ280530	GQ267237	GQ267449	GQ267286	GQ280652	GQ280774	GQ267357	
	CBS 125260^{T}	CMW 20291	GQ280529	GQ267236	GQ267448	GQ267285	GQ267651	GQ280773	GQ267356	
Ca. ovata	CBS 111299	CMW 16724	GQ280479	GQ267212	GQ267400	GQ267253	GQ280601	GQ280723	GQ267318	Crous (2002)
	CBS111307	CMW 30979	GQ280480	AF210868	GQ267401	GQ267254	GQ280602	GQ280724	GQ267319	
Ca. pacifica	CBS 109063	CMW 16726 = IMI 35428	GQ280481	GQ267213	AY725762	GQ267255	GQ280603	GQ280725	AY725724	Crous (2002)
	CBS 114038	CMW 30988	GQ280482	AY725630	GQ267402	AY725675	GQ280604	GQ280726	GQ267320	
Ca. pauciramosa	CMW 5683^{T}	CPC 971	GQ280486	FJ918514	GQ267405	FJ918531	GQ280608	GQ280730	FJ918565	Crous (2002)
	CMW30823	CPC 416	GQ280485	FJ918515	GQ280404	FJ918532	GQ280607	GQ280729	FJ918566	
Ca. penicilloides	CBS 174.55^{T}	CMW 23696	GQ280487	AF333414	GQ267406	GQ267257	GQ280609	GQ280731	GQ267322	Crous (2002)
Ca. pini	CBS 123698^{T}	CMW 31209	GQ280517	GQ267224	GQ267436	GQ267273	GQ280639	GQ280761	GQ267344	This study
	CBS 125523	CMW 31210	GQ280518	GQ267225	GQ267437	GQ267274	GQ280640	GQ280762	GQ267345	
Ca. polizzii	CBS 125270	CMW 7804	GQ280544	FJ972417	GQ267461	FJ972436	GQ280666	GQ280788	FJ972486	Lombard *et al.* (2010a)
	CBS 125271	CMW 10151	GQ280545	FJ972418	GQ267462	FJ972437	GQ280667	GQ280789	FJ972487	
Ca. pseudonaviculata	CBS 114417^{T}	CMW 23672	GQ280490	GQ267214	GQ267409	GQ267258	GQ280612	GQ280734	GQ267325	Crous *et al.* (2002)
Ca. pseudoreteaudii	CBS 123694^{T}	CMW 25310	GQ280492	FJ918504	GQ267411	FJ918519	GQ280614	GQ280736	FJ918541	Lombard *et al.* (2010c)
	CBS 123696	CMW 25292	GQ280491	FJ918505	GQ267410	FJ918520	GQ280613	GQ280735	FJ918542	
Ca. pseudoscoparia	CBS 125254	CMW 15214	GQ280519	GQ267226	GQ267438	GQ267275	GQ280641	GQ280763	GQ267346	This study
	CBS 125255	CMW 15215	GQ280520	GQ267227	GQ267439	GQ267276	GQ280642	GQ280764	GQ267347	
	CBS 125256	CMW 15216	GQ280521	GQ267228	GQ267440	GQ267277	GQ280643	GQ280765	GQ267348	
	CBS 125257^{T}	CMW 15218	GQ280522	GQ267229	GQ267441	GQ267278	GQ280644	GQ280766	GQ267349	
Ca. pseudospathiphylli	CBS 109162^{T}	CMW 30976 = CPC 1623	GQ280493	FJ918513	GQ267412	AF348241	GQ280615	GQ280737	FJ918562	Crous (2002)
Ca. pteridis	CBS 111793^{T}	CMW 16736 = CPC 2372 = ATCC 34395	GQ280494	DQ190578	GQ267413	DQ190679	GQ280616	GQ280738	FJ918563	Crous (2002)
	CBS 111871	CMW 30982 = CPC 2443	GQ280495	DQ190579	GQ267414	DQ190680	GQ280617	GQ280739	FJ918564	
Ca. pyrochoa	CBS 749.70^{T}	CMW 23682	GQ280462	GQ267210	GQ267388	GQ267250	GQ280584	GQ280706	GQ267312	Crous *et al.* (2006)

Table 1. (Continued).

Species	Isolate number[1]	Other collections[1]	GenBank accession nr.[2]							Reference[3]
			ACT	BT	CAL	HIS3	ITS	LSU	TEF-1α	
Ca. queenslandica	CBS 112146^{T}	CMW 30604 = CPC 3213	GQ280496	AF389835	GQ267415	FJ918521	GQ280618	GQ280740	FJ918543	Lombard *et al.* (2010c)
	CBS 112155	CMW 30603 = CPC 3210	GQ280497	AF389834	GQ267416	DQ190667	GQ280619	GQ280741	FJ918544	
Ca. reteaudii	CBS 112143	CMW 16738 = CPC 3200	GQ280499	GQ240642	GQ267418	DQ190660	GQ280621	GQ280743	FJ918536	Crous (2002)
	CBS 112144^{T}	CMW 30984 = CPC 3201	GQ280498	AF389833	GQ267417	DQ190661	GQ280620	GQ280742	FJ918537	
Ca. rumohrae	CBS 109062	CMW 30989 = CPC 1603	GQ280501	AF232873	GQ267420	DQ190676	GQ280623	GQ280745	FJ918550	Crous (2002)
	CBS 111431^{T}	CMW 23697 = CPC 1716	GQ280500	AF232871	GQ267419	DQ190675	GQ280622	GQ280744	FJ918549	
Ca. scoparia	CMW 31000	CPC 1675 = UFV 117	GQ280435	FJ972426	GQ267367	FJ972476	GQ280557	GQ280679	FJ972525	Crous (2002)
	CMW 31001	UFV 126	GQ280436	FJ972427	GQ267368	GQ267246	GQ280558	GQ280680	GQ267246	
Ca. spathiphylli	CBS 114540	CMW 16742	GQ280505	AF348214	GQ267424	AF348230	GQ280627	GQ280749	GQ267330	Crous (2002)
	CBS 116168	CMW 30997	GQ280506	FJ918512	GQ267425	FJ918530	GQ280628	GQ280750	FJ918561	
Ca. spathulata	CBS 555.92	CMW 16744	GQ280508	GQ267215	GQ267427	GQ267261	GQ280630	GQ280752	GQ267331	Crous (2002)
	CBS 112689	CMW 16745	GQ280507	AF308463	GQ267426	FJ918524	GQ280629	GQ280751	FJ918554	
Ca. sulawesiensis	CBS 125248	CMW 14857	GQ280516	GQ267223	GQ267435	GQ267272	GQ280638	GQ280760	GQ267343	This study
	CBS 125253	CMW 14879	GQ280513	GQ267220	GQ267432	GQ267269	GQ280635	GQ280757	GQ267340	
	CBS 125277^{T}	CMW 14878	GQ280515	GQ267222	GQ267434	GQ267271	GQ280637	GQ280759	GQ267342	
	CMW 14883		GQ280514	GQ267221	GQ267433	GQ267270	GQ280636	GQ280758	GQ267341	
Ca. sumatrensis	CBS 112829^{T}	CMW 23698 = CPC4518	GQ280532	AY725649	AY725771	AY725696	GQ280654	GQ280776	AY725733	Crous *et al.* (2004b)
	CBS 112934	CMW 30987 = CPC 4516	GQ280533	AY725651	AY725773	AY725798	GQ280655	GQ280777	AY725735	
Ca. terrae-reginae	CBS 112151^{T}	CMW 30601 = CPC 3202	GQ280534	FJ918506	GQ267451	FJ918522	GQ280656	GQ280778	FJ918545	Lombard *et al.* (2010c)
	CBS 112634	CMW 30602 = CPC 4233	GQ280535	FJ918507	GQ267452	DQ190668	GQ280657	GQ280779	FJ918546	
Ca. variabilis	CBS 112691	CMW 2914	GQ280541	GQ267240	GQ267458	GQ267264	GQ280663	GQ280785	GQ267335	Crous (2002)
	CBS 114677	CMW 3187	GQ280540	AF333424	GQ267457	GQ267263	GQ280662	GQ280764	GQ267334	
Ca. zuluensis	CBS 125268	CMW 9188^{T}	GQ280542	FJ972414	GQ267459	FJ972433	GQ280664	GQ280786	FJ972483	Lombard *et al.* (2010a)
	CBS 125272	CMW 9896	GQ280543	FJ972415	GQ267460	FJ972434	GQ280665	GQ280787	FJ972484	

[1] CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Pedro Crous working collection housed at CBS; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.; ATCC: American Type Culture Collection, Virginia, U.S.A.; UFV: Univeridade Federal de Viçosa, Brazil. [2] ACT = Actin, BT = β-tubulin, CAL = Calmodulin, HIS3 = Histone H3, ITS = Internal transcribed spacer regions 1 and 2 and the 5.8S gene of the ribosomal RNA, LSU = 28S large subunit RNA, TEF-1α = Translation elongation factor 1-alpha. [3] References used for species descriptions. ^{T} Ex-type cultures.

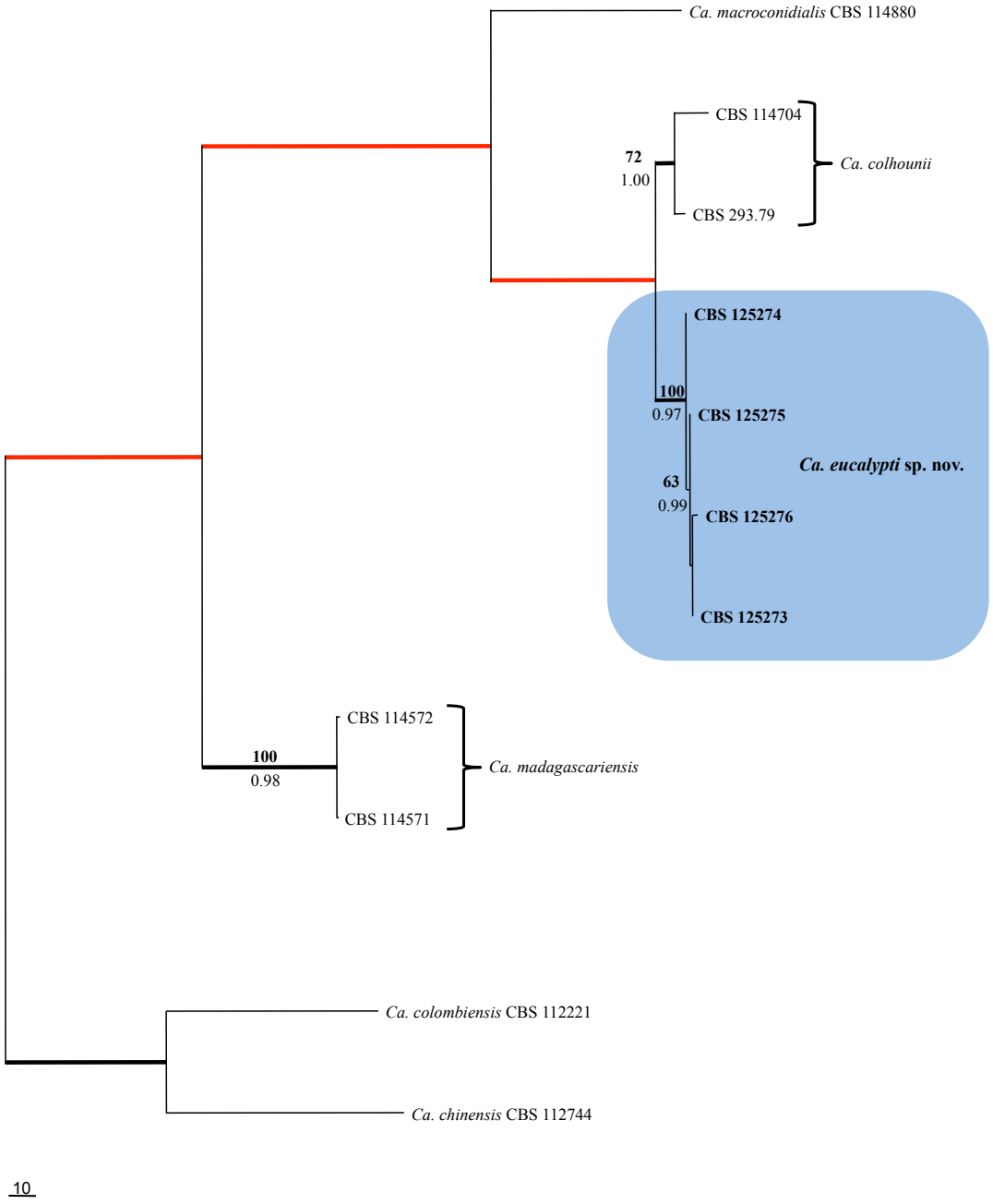

Fig. 1. The most parsimonious trees obtained from a heuristic search with 1 000 random additions sequences of the combined BT, HIS3 and TEF-1α sequence alignments of the *Ca. colhounii* complex. Scale bar shows 10 changes and bootstrap support values (bold) from 1 000 replicates and Bayesian posterior probability values are indicated at the nodes. Red lines indicate bootstrap values of 100 and posterior probabilities of 1.00. Thickened lines indicate branches in the strict consensus tree and the consensus tree of the Bayesian analyses.

Table 2. Single nucleotide polymorphisms comparisons between *Ca. eucalypti* and *Ca. colhounii*, compared to *Ca. macroconidialis* and *Ca. madagascariensis*.

Species	Isolate no.	β-tubulin				Histone H3					TEF-1α								
		167	207	398	507	58	290	362	454	455	43	105	106	107	108	109	264	457	472
Ca. colhounii	CBS 293.79	A	G	A	C	A	A	C	A	C	C	A	C	A	A	C	G	C	C
	CBS 114704	A	G	A	C	A	A	C	A	C	C	A	C	A	A	C	G	C	C
Ca. eucalypti	CBS 125273	G	T	G	T	-	T	T	C	A	A	-	-	-	-	-	A	T	T
	CBS 125274	G	T	G	T	-	T	T	C	A	A	-	-	-	-	-	A	T	T
	CBS 125275	G	T	G	T	-	T	T	C	A	A	-	-	-	-	-	A	T	T
	CBS 125276	G	T	G	T	-	T	T	C	A	A	-	-	-	-	-	A	T	T
Ca. macroconidialis	CBS 114880	C	G	A	C	A	A	T	A	C	C	C	A	A	C	C	C	T	C
Ca. madagascariensis	CBS 114571	C	G	A	T	T	A	G	A	C	C	C	C	A	C	C	C	C	A
	CBS 114572	C	G	A	T	T	A	G	A	C	C	C	C	A	C	C	C	C	A

Table 3. Single nucleotide polymorphisms from the sequence datasets for *Ca. pini* and *Ca. orientalis* compared to *Ca. brachiatica* and *Ca. brassicae.*

Species	Isolate no.	β-tubulin								Histone H3										
		84	91	121	202	380	382	395	518	12	58	59	61	62	65	71	105	255	268	270
Ca. brachiatica	CBS 123700	A	G	A	A	T	C	A	-	T	-	T	C	A	T	C	C	T	A	A
	CMW 25302	A	G	A	A	T	C	A	-	T	-	T	C	A	T	C	C	T	A	A
Ca. brassicae	CBS 111478	A	G	C	G	G	T	G	-	T	A	T	C	C	C	C	C	C	C	A
	CBS 111869	A	G	C	G	G	T	G	-	T	A	T	C	C	C	C	C	C	C	A
Ca. pini	CBS 123698	A	C	C	G	G	T	G	C	G	-	-	T	C	C	-	A	C	C	A
	CMW 31210	A	C	C	G	G	T	G	C	G	-	-	T	C	C	-	A	C	C	A
Ca. orientalis	CBS 125258	G	G	C	G	G	T	G	-	T	A	T	C	C	C	C	C	C	C	G
	CBS 125259	G	G	C	G	G	T	G	-	T	A	T	C	C	C	C	C	C	C	G
	CBS 125260	G	G	C	G	G	T	G	-	T	A	T	C	C	C	C	C	C	C	G

Species	Isolate no.	TEF-1α																															
		4	12	49	61	62	65	79	93	124	141	142	186	194	195	196	197	198	199	200	201	236	240	246	259	273	428	447	448	449	465	473	493
Ca. brachiatica	CBS 123700	C	T	C	G	C	C	C	A	T	T	T	G	T	-	-	-	-	C	A	T	C	T	G	T	C	G	C	C	A	G	G	-
	CMW 25302	C	T	C	G	C	C	C	A	T	T	T	G	T	-	-	-	-	C	A	T	C	T	G	T	C	G	C	C	A	G	G	-
Ca. brassicae	CBS 111478	T	C	-	G	C	C	C	A	T	-	-	G	T	-	-	-	-	C	A	T	C	T	G	T	C	G	C	C	A	C	G	C
	CBS 111869	T	C	-	G	C	C	C	A	T	-	-	G	T	-	-	-	-	C	A	T	C	T	G	T	C	G	C	C	A	C	G	C
Ca. pini	CBS 123698	G	T	C	C	A	G	C	G	A	T	T	A	A	A	A	A	A	G	C	C	T	A	C	C	C	A	-	-	-	G	A	C
	CMW 31210	G	T	C	C	A	G	C	G	A	T	T	A	A	A	A	A	A	G	C	C	T	A	C	C	C	A	-	-	-	G	A	C
Ca. orientalis	CBS 125258	T	T	C	C	C	C	T	G	T	T	T	G	A	-	-	-	-	G	C	C	T	A	C	C	T	A	-	-	-	G	G	C
	CBS 125259	T	T	C	C	C	C	T	G	T	T	T	G	A	-	-	-	-	G	C	C	T	A	C	C	T	A	-	-	-	G	G	C
	CBS 125260	T	T	C	C	C	C	T	G	T	T	T	G	A	-	-	-	-	G	C	C	T	A	C	C	T	A	-	-	-	G	G	C

as outgroup taxa. For Bayesian analyses, a HKY+I+G model was selected for BT and TEF-1α, and GTR+I+G for HIS3 for all four data sets, which was incorporated in the analyses. The consensus trees obtained from the Bayesian analyses confirmed the tree topologies obtained with maximum-parsimony as well as bootstrap support. Therefore, only maximum-parsimony trees are presented with bootstrap values and posterior probabilities shown for well-supported branches.

The partition homogeneity tests for all possible combinations of the three gene regions used, consistently yielded a P-value of 0.001 for the four separate data sets. The 70 % reciprocal bootstrap trees showed no conflict in tree topologies for the three gene regions in each of the four separate data sets. Based on the tree topologies of the 70 % reciprocal bootstrap trees and a P-value of 0.001 in the PHT (Cunningham 1997, Dettman *et al.* 2003) the DNA sequences for the three gene regions were combined for each of the four separate data sets.

The combined sequence data set representing the *Ca. colhounii* complex, with 10 taxa including outgroups, consisted of 1 497 characters, including gaps. Of these characters, 1 051 were constant, 133 were parsimony-uninformative and 313 characters were parsimony informative. Parsimony analysis of the aligned sequences yielded one most parsimonious tree (Fig. 1; TL = 649 steps; CI = 0.888; RI = 0.891; RC = 0.791). In the tree, isolates CBS 125273–125276, from Indonesia, grouped close to but separate from *Ca. colhounii* (CBS 293.79 and CBS 114704) with 100 % bootstrap support (BP) and a posterior probability (PP) of 0.97. The SNP analyses showed 16 unique alleles for the Indonesian isolates with one shared unique allele with *Ca. madagascariensis* (CBS 114571 and CBS 114572) and two shared alleles with *Ca. macroconidialis* (CBS 114880) for the three gene regions analysed (Table 2). These unique alleles, however, distinguish the Indonesian isolates from *Ca. colhounii*, *Ca. macroconidialis* and *Ca. madagascariensis.*

The data set representing the *Ca. brassicae* complex consisted of 15 taxa including the outgroups, while the combined sequence alignment was made up of 1 509 characters, including gaps. These characters represented 1 092 constant, 127 parsimony-uninformative and 290 parsimony-informative characters. Parsimony analysis yielded one most parsimonious tree (Fig. 2; TL = 569 steps; CI = 0.931; RI = 0.918; RC = 0.855). In the tree, Colombian isolates CBS 123698 and CBS 125523 clustered close to *Ca. brassicae* (CBS 111869 and CBS 111478) and *Ca. brachiatica* (CBS 123700 and CMW 25302) but separately from both these species with high support (BP = 100 and PP = 1.00). Similarly, isolates CBS 125258–125260, from Indonesia, clustered together closely related to *Ca. brassicae* and *Ca. brachiatica*. These Indonesian isolates were also closely related to the Colombian isolates but grouped separately from them in a clade with high support (BP = 97 and PP = 1.00). The SNP analyses showed that isolates CBS 123698 and CBS 125523 have 18 unique alleles and isolates CBS 125258–125260 have four unique alleles distinguishing them from each other for the three gene regions analysed. These isolates also share 14 unique alleles, distinguishing them from *Ca. brassicae* and *Ca. brachiatica* (Table 3).

The third data set, represented by 16 ingroup taxa residing in the *Ca. scoparia* complex and closely related species, consisted of 1 530 characters including gaps for the three gene regions analysed. Of these characters, 1 114 were constant, 138 were parsimony-uninformative and 278 characters were parsimony informative. Parsimony analysis of the aligned sequences yielded two most parsimonious trees (TL = 551 steps; CI = 0.902; RI = 0.925; RC = 0.834), one of which is presented in Fig. 3. In the tree,

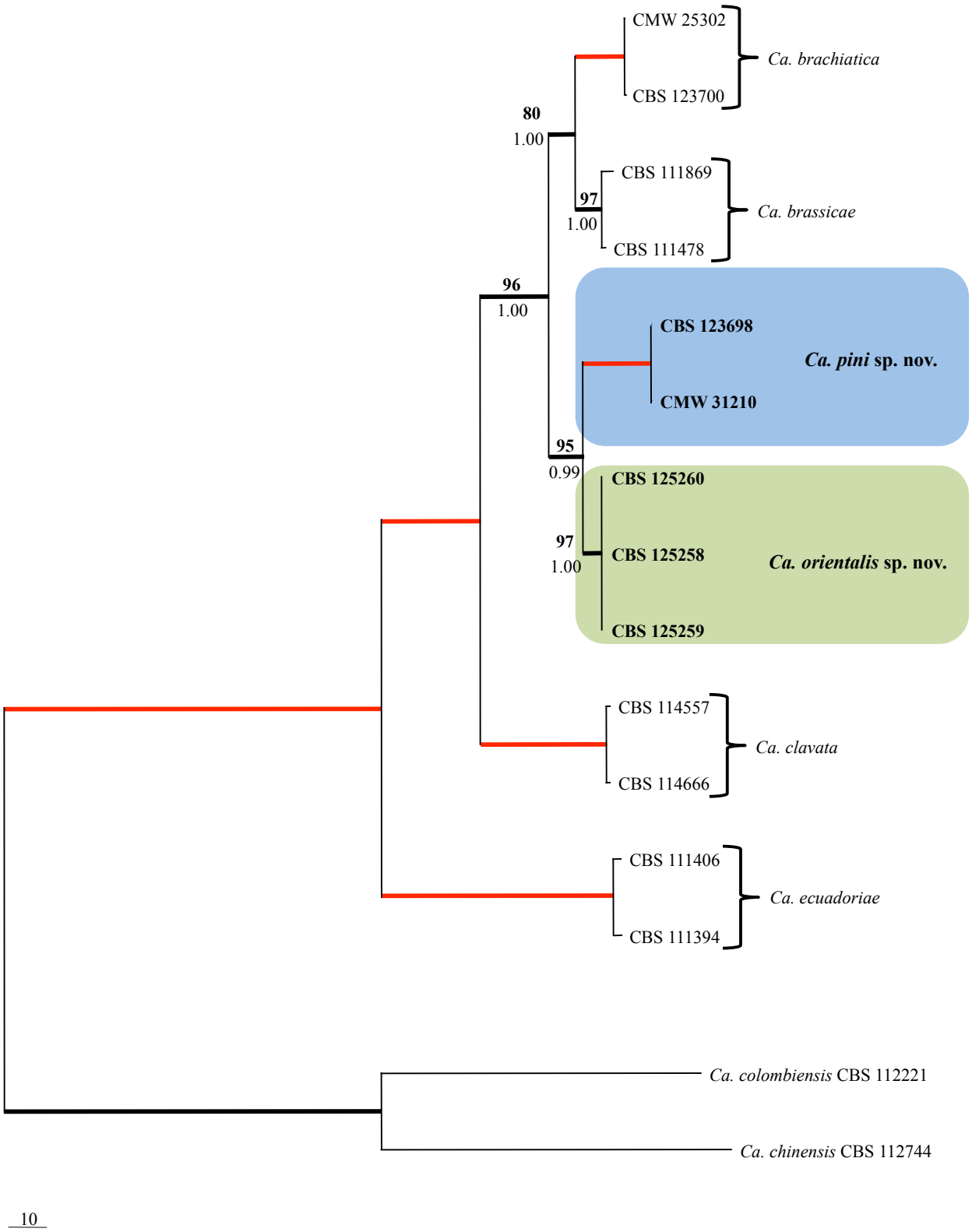

Fig. 2. The most parsimonious trees obtained from a heuristic search with 1 000 random additions sequences of the combined BT, HIS3 and TEF-1α sequence alignments of the *Ca. brassicae* complex. Scale bar shows 10 changes and bootstrap support values (bold) from 1 000 replicates and Bayesian posterior probability values are indicated at the nodes. Red lines indicate bootstrap values of 100 and posterior probabilities of 1.00. Thickened lines indicate branches in the strict consensus tree and the consensus tree of the Bayesian analyses.

isolates CBS 125254–125257 from Ecuador, clustered closely but separately from *Ca. scoparia* (CMW 31000 and CMW 31001) and other species in the *Ca. pauciramosa* complex with low support (BP = 63 and PP = 1.00). The Ecuadorian isolates also had three unique alleles separating them from *Ca. scoparia* and *Ca. pauciramosa* (CMW 5683 and CMW 30823) for the BT and TEF-1α regions, but there were no unique alleles for these isolates in the HIS3 region (Table 4).

The aligned sequence data set for the *Ca. morganii* complex included 25 ingroup taxa consisting of 1 535 characters. Of these characters, 975 were constant, 211 were parsimony-uninformative and 349 characters were parsimony-informative. Parsimony analysis

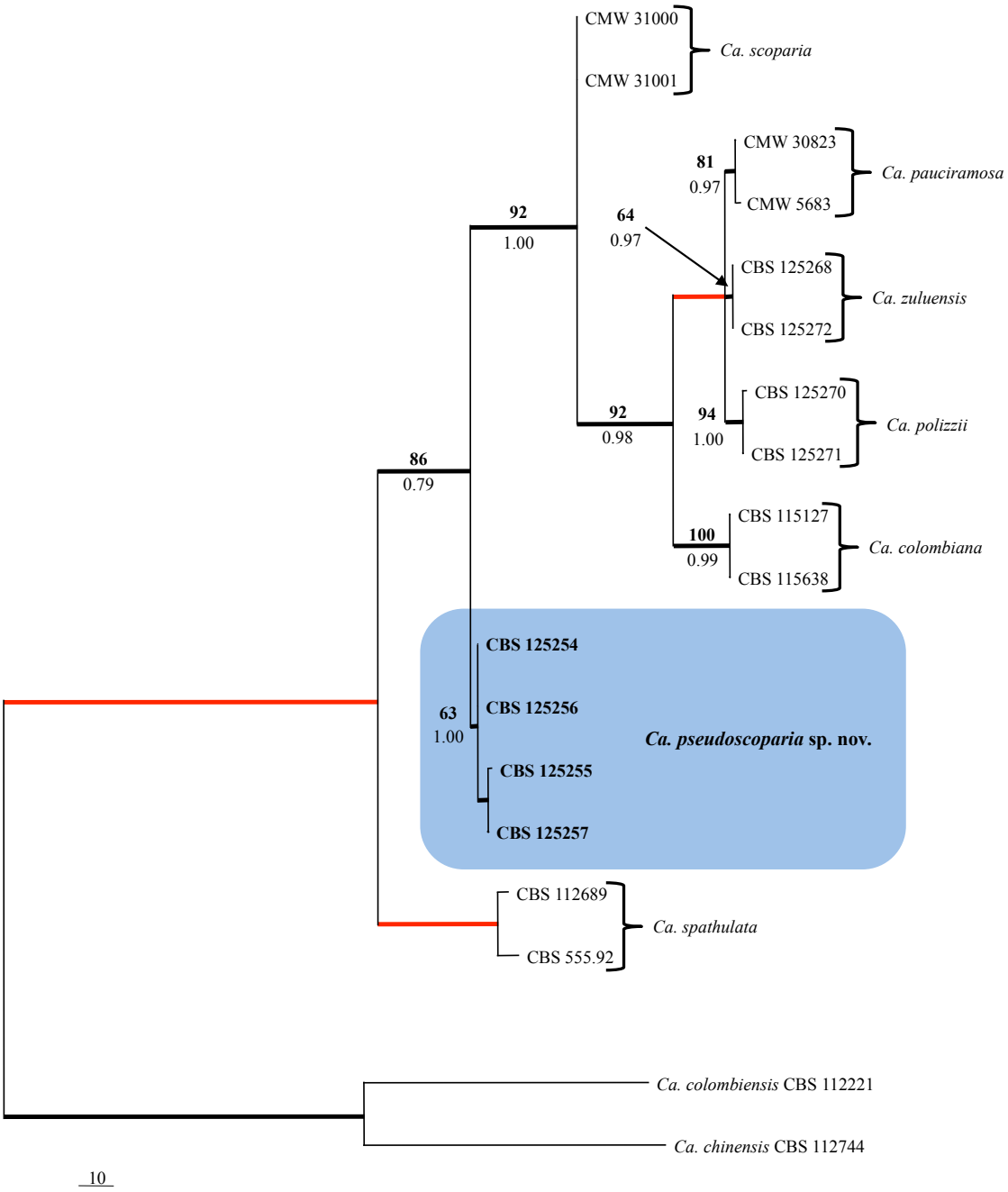

Fig. 3. One of two most parsimonious trees obtained from a heuristic search with 1 000 random additions sequences of the combined BT, HIS3 and TEF-1α sequence alignments of the *Ca. scoparia* complex. Scale bar shows 10 changes and bootstrap support values (bold) from 1 000 replicates and Bayesian posterior probability values are indicated at the nodes. Red lines indicate bootstrap values of 100 and posterior probabilities of 1.00. Thickened lines indicate branches in the strict consensus tree and the consensus tree of the Bayesian analyses.

Table 4. Single nucleotide polymorphisms comparisons between *Ca. scoparia* and *Ca. pseudoscoparia*, compared to *Ca. pauciramosa*.

Species	Isolate no.	β-tubulin	TEF-1α	
		193	288	490
Ca. scoparia	CMW 31000	T	-	-
	CMW 31001	T	-	-
Ca. pauciramosa	CMW 5683	T	-	-
	CMW 30823	T	-	-
Ca. pseudoscoparia	CBS 125254	C	C	C
	CBS 125255	C	C	C
	CBS 125256	C	C	C
	CBS 125257	C	C	C

of the aligned sequences yielded three most parsimonious trees (TL = 977 steps; CI = 0.784; RI = 0.825; RC = 0.647), one of which is presented in Fig. 4. In the tree, isolates CBS 125249–125252, CBS 125261 and CBS 125269 from Ecuador clustered in a clade (BP = 99 and PP = 1.00) with *Ca. spathiphylli* (CBS 114540 and CBS 116168) and *Ca. pseudospathiphylli* (CBS 109165), whereas isolates CBS 125248, CBS 125253, CBS 125277 and CMW 14883 from Indonesia clustered close to *Ca. brasiliensis* (CBS 230.51 and CBS 114257) but with low support (BP = 52; PP = 0.90) in a separate, well-supported clade (BP = 100; PP = 1.00). Isolates CBS 125249, CBS 125250 and CBS 125261 clustered together in a well-supported clade (BP = 93; PP = 1.00) separate from CBS 125251, CBS 125252 and CBS 125269, that also clustered together in a well-supported clade (BP = 81; PP = 1.00). Both clades were separate from *Ca. spathiphylli* and *Ca. pseudospathiphylli* but closely related to these species. The SNP analyses showed that isolates CBS 125249, CBS 125250 and CBS 125261 shared four unique alleles and CBS 125251, CBS 125252 and CBS 125269 shared seven unique alleles for the three gene regions. These isolates also shared an additional 33 alleles, distinguishing them from *Ca. spathiphylli* (Table 5). Isolates CBS 125248, CBS 125253, CBS 125277 and CMW 14883 shared eight unique alleles, distinguishing them from *Ca. brasiliensis* (CBS 230.51 and CBS 114257), *Ca. cerciana* (CBS 123693 and CBS 123695) and *Ca. insularis* (CBS 114558 and CBS 114559) (Table 6).

Phylogenetic relationships amongst *Calonectria* spp.

Approximately 250 bases were determined for ACT, 450 bases for HIS3, 500 for BT, CAL and TEF-1α, 700 for ITS and 880 for LSU. The adjusted sequence alignments for each gene region consisted of 122 ingroup taxa with *Cylindrocladiella lageniformis* (CBS 112898) and *C. peruviana* (CPC 5614) as outgroup taxa for each gene region. For Bayesian analyses, a K80+G model was selected for ACT, HKY+I+G for BT, CAL and TEF-1α, GTR+I+G for HIS3 and LSU, and SYM+I+G for ITS and incorporated in the analyses. The consensus trees obtained from the Bayesian analyses confirmed the tree topologies obtained with maximum-parsimony as well as bootstrap support.

Individual analyses of the gene regions showed similar tree topologies for the protein coding regions (ACT, BT, CAL, HIS3 and TEF-1α) with well-supported clades for *Calonectria* spp. with similar morphological characteristics. In contrast, the non-coding gene regions (ITS and LSU) provided little or no support for the clades that emerged from the protein coding regions, with several *Calonectria* spp. clustering together with no significant similarities. The trees for the ITS and LSU regions showed a single monophyletic clade for all *Calonectria* spp. and did not reveal the two clades observed for the coding gene regions. The phylogeny constructed based on CAL sequences showed the best resolution of the species and it had the highest support for the individual clades, followed by TEF-1α gene region. Statistical data for the individual trees (not shown) are presented in Table 7.

The partition homogeneity tests for all possible combinations of the seven gene regions used, consistently yielded a P-value of 0.001. The 70 % reciprocal bootstrap trees showed no conflict in tree topologies for the five coding gene regions (ACT, BT, CAL, HIS3 and TEF-1α), however conflicts were observed between the non-coding gene regions (ITS and LSU) and the coding gene regions. Based on the tree topologies and a P-value of 0.001 (Cunningham 1997, Dettman *et al.* 2003) the sequence data for coding gene regions were combined. The data for the ITS and LSU datasets were treated separately, but these are not presented

Table 5. Single nucleotide polymorphisms from the sequence datasets for *Ca. densa* and *Ca. humicola* compared to *Ca. spathiphylli*.

Species	Isolate no.	β-tubulin															Histone H3															TEF-1α													
		8	74	103	151	193	220	225	234	235	241	388	393	515	524	527	71	83	101	103	105	127	209	253	256	261	262	266	279	459	460	49	72	84	100	102	104	113	114	115	116	207	262	454	469
Ca. spathiphylli	CBS 114540	A	T	G	C	C	G	C	T	T	C	T	G	C	T	A	C	C	A	C	T	T	T	T	A	C	T	T	T	A	C	T	T	-	G	A	G	-	-	-	-	T	A	C	T
	CBS 116168	A	T	G	C	C	G	C	T	T	C	T	G	C	T	A	C	C	A	C	T	T	T	T	A	C	T	T	T	A	C	T	T	-	G	A	G	-	-	-	-	T	A	C	T
Ca. densa	CBS 125249	A	-	A	G	A	A	T	C	C	T	T	C	T	C	T	T	G	G	T	C	C	C	C	G	T	C	C	C	A	C	C	C	-	A	G	A	C	A	-	-	C	T	T	G
	CBS 125250	A	-	A	G	A	A	T	C	C	T	T	C	T	C	T	T	G	G	T	C	C	C	C	G	T	C	C	C	A	C	C	C	-	A	G	A	C	A	-	-	C	T	T	G
	CBS 125261	A	-	A	G	A	A	T	C	C	T	T	C	T	C	T	T	G	G	T	C	C	C	C	G	T	C	C	C	A	C	C	C	-	A	G	A	C	A	-	-	C	T	T	G
Ca. humicola	CBS 125251	T	-	A	G	A	A	T	T	T	T	C	C	T	C	T	T	C	G	T	C	C	C	C	G	T	C	C	C	C	A	C	C	C	A	G	A	C	A	C	A	T	A	T	G
	CBS 125252	T	-	A	G	A	A	T	T	T	T	C	C	T	C	T	T	C	G	T	C	C	C	C	G	T	C	C	C	C	A	C	C	C	A	G	A	C	A	C	A	T	A	T	G
	CBS 125269	T	-	A	G	A	A	T	T	T	T	C	C	T	C	T	T	C	G	T	C	C	C	C	G	T	C	C	C	C	A	C	C	C	A	G	A	C	A	C	A	T	A	T	G

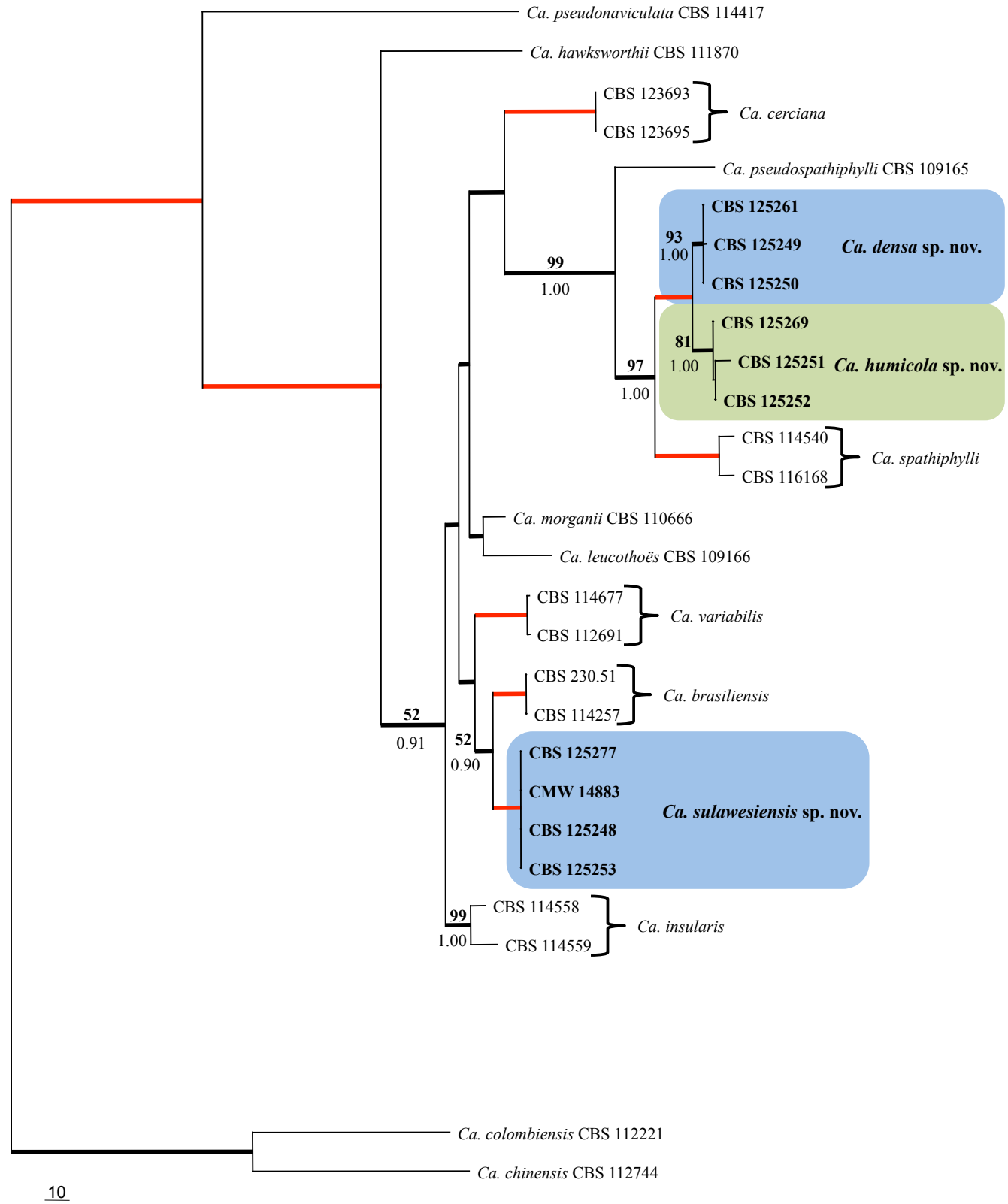

Fig. 4. One of three most parsimonious trees obtained from a heuristic search with 1 000 random additions sequences of the combined BT, HIS3 and TEF-1α sequence alignments of the *Ca. morganii* complex. Scale bar shows 10 changes and bootstrap support values (bold) from 1 000 replicates and Bayesian posterior probability values are indicated at the nodes. Red lines indicate bootstrap values of 100 and posterior probabilities of 1.00. Thickened lines indicate branches in the strict consensus tree and the consensus tree of the Bayesian analyses.

because they add little taxonomic value. However, all ITS and LSU sequences generated in this study have been deposited in GenBank and TreeBase (Table 1).

The combined sequence alignment of the five coding gene regions consisted of 2 472 characters, including gaps. Of these characters, 925 were constant, 267 were parsimony-uninformative and 1 280 characters were parsimony-informative. Parsimony analysis of the aligned sequences yielded 24 most parsimonious trees (TL = 7319 steps; CI = 0.397; RI = 0.820; RC = 0.326), one of which is presented in Fig. 5. The tree topology obtained with

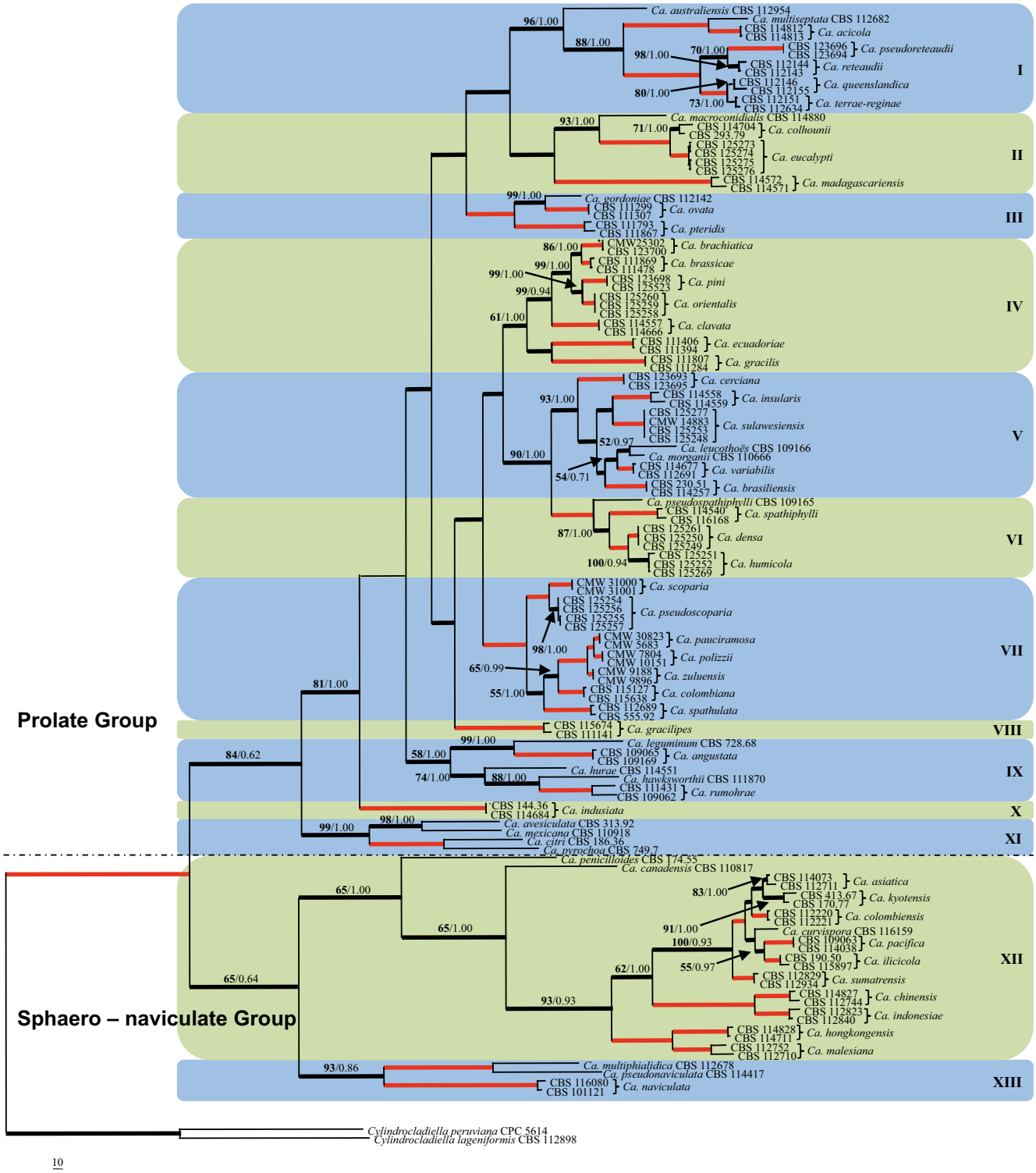

Fig. 5. One of 24 most parsimonious trees obtained from a heuristic search with 1 000 random additions sequences of the combined actin, β-tubulin, calmodulin, histone H3 and translation elongation factor 1-alpha sequence alignments of the *Calonectria*. Scale bar shows 10 changes and bootstrap support values (bold) from 1 000 replicates and Bayesian posterior probability values are indicated at the nodes. Thickened lines indicate branches in the strict consensus tree and the consensus tree of the Bayesian analyses. Red lines indicate bootstrap values of 100 and posterior probabilities of 1.00. The tree was rooted to *Cylindrocladiella lageniformis* (CBS 112898) and *C. peruviana* (CPC 5614). Phylogenetic groups are indicated on the right.

Table 6. Single nucleotide polymorphisms comparisons between *Ca. brasiliensis*, *Ca. insularis* and *Ca. sulawesiensis* compared to *Ca. cerciana*.

Species	Isolate no.	β-tubulin					Histone H3								TEF-1α									
		117	360	395	472	509	95	100	253	259	260	390	417	452	98	100	103	104	105	109	143	263	439
Ca. brasiliensis	CBS 230.51	C	A	A	T	C	G	C	G	A	C	T	T	A	G	-	-	-	-	G	T	C	G
	CBS 114257	C	A	A	T	C	G	C	G	A	C	T	T	A	G	-	-	-	-	G	T	C	G
Ca. cerciana	CBS 123693	T	A	A	T	T	A	C	C	A	C	C	T	C	G	-	-	C	G	A	-	C	G
	CBS 123695	T	A	A	T	T	A	C	C	A	C	C	T	C	G	-	-	C	G	A	-	C	G
Ca. insularis	CBS 114558	T	G	A	C	C	A	C	G	A	C	C	C	A	G	C	A	C	A	A	-	C	A
	CBS 114559	T	G	A	C	C	A	C	G	A	C	C	C	A	G	C	A	C	A	A	-	C	A
Ca. sulawesiensis	CBS 125248	T	A	G	T	T	A	T	T	G	T	T	T	C	C	G	A	C	G	A	-	T	A
	CBS 125253	T	A	G	T	T	A	T	T	G	T	T	T	C	C	G	A	C	G	A	-	T	A
	CBS 125277	T	A	G	T	T	A	T	T	G	T	T	T	C	C	G	A	C	G	A	-	T	A
	CMW 14883	T	A	G	T	T	A	T	T	G	T	T	T	C	C	G	A	C	G	A	-	T	A

Table 7. Statistical information on the sequence dataset and maximum parsimony trees for each locus.

	Actin	β-tubulin	Calmodulin	Histone H3	ITS	LSU	TEF-1α
Aligned characters	290	532	531	499	706	887	596
Variable characters	15	42	39	62	32	10	57
Informative characters	151	268	323	223	112	37	337
Most parsimonious trees	2622	91	1000	372	1000	100	9970
Tree length	573	1454	1282	1843	296	91	1641
CI	0.490	0.431	0.467	0.352	0.618	0.538	0.477
RI	0.867	0.840	0.849	0.793	0.882	0.913	0.871
RC	0.425	0.569	0.397	0.648	0.545	0.492	0.416

the combined sequence dataset was similar to that obtained for the individual gene regions analysed and therefore the only tree presented is that of the combined dataset.

In the tree (Fig. 5), the *Calonectria* spp. were found to clearly reside in two main clades which was consistent for the analyses for these gene regions separately. One of these clades (BP = 82, PP = 0.62) which we refer to as representing the Prolate Group, includes *Calonectria* spp. with clavate to pyriform to ellipsoidal vesicles. This clade (Fig. 5) is made up of two sub-clades, one (BP = 81, PP = 1.00) of which includes 10 minor clades representing *Calonectria* spp. that have vesicles and conidia that have similar morphology. The second sub-clade (BP = 99, PP = 1.00) representing the Prolate Group includes taxa represented by single isolates and for which there were no obvious unifying morphological characters.

The second main clade (BP = 65, PP = 0.64) which is referred to as the Sphaero-Naviculate Group of species included *Calonectria* spp. characterised by sphaeropedunculate and naviculate vesicles and these were also seen in the analyses based on the individual gene regions. This clade is further sub-divided into two clades. The first of these sub-clades (BP = 65, PP = 1.00) includes *Calonectria* spp. characterised by sphaeropedunculate vesicles. The second sub-clade (BP = 93, PP = 0.86) accommodates *Calonectria* spp. with naviculate vesicles.

Sexual compatibility

The only isolates in the mating tests that yielded perithecia were CBS 125273–125276 (Fig. 6). These isolates all produced perithecia containing viable ascospores within 6 wk when mated with themselves, indicating that they are self-fertile (homothallic). All other control inoculations with the selected isolates failed to yield perithecia, indicating that they were either self-sterile (heterothallic) and non-compatible, or that they had lost the ability to undergo sexual recombination.

Taxonomy

Based on morphological observations, phylogenetic inference and mating, numerous isolates of *Calonectria* spp. included in this study represent undescribed species. Species of *Cylindrocladium* (1892) represent anamorph states of *Calonectria* (1867) (Rossman *et al.* 1999). In an attempt to move to a single nomenclature for pleomorphic fungi, the teleomorph name takes precedence over the anamorph name when both types belong to the same holomorph. The species below are described as new species in *Calonectria,* which represents the older generic name for these holomorphs and follows Lombard *et al.* (2009, 2010a, c). All *Cylindrocladium* species without a *Calonectria* state, are also transferred to *Calonectria*.

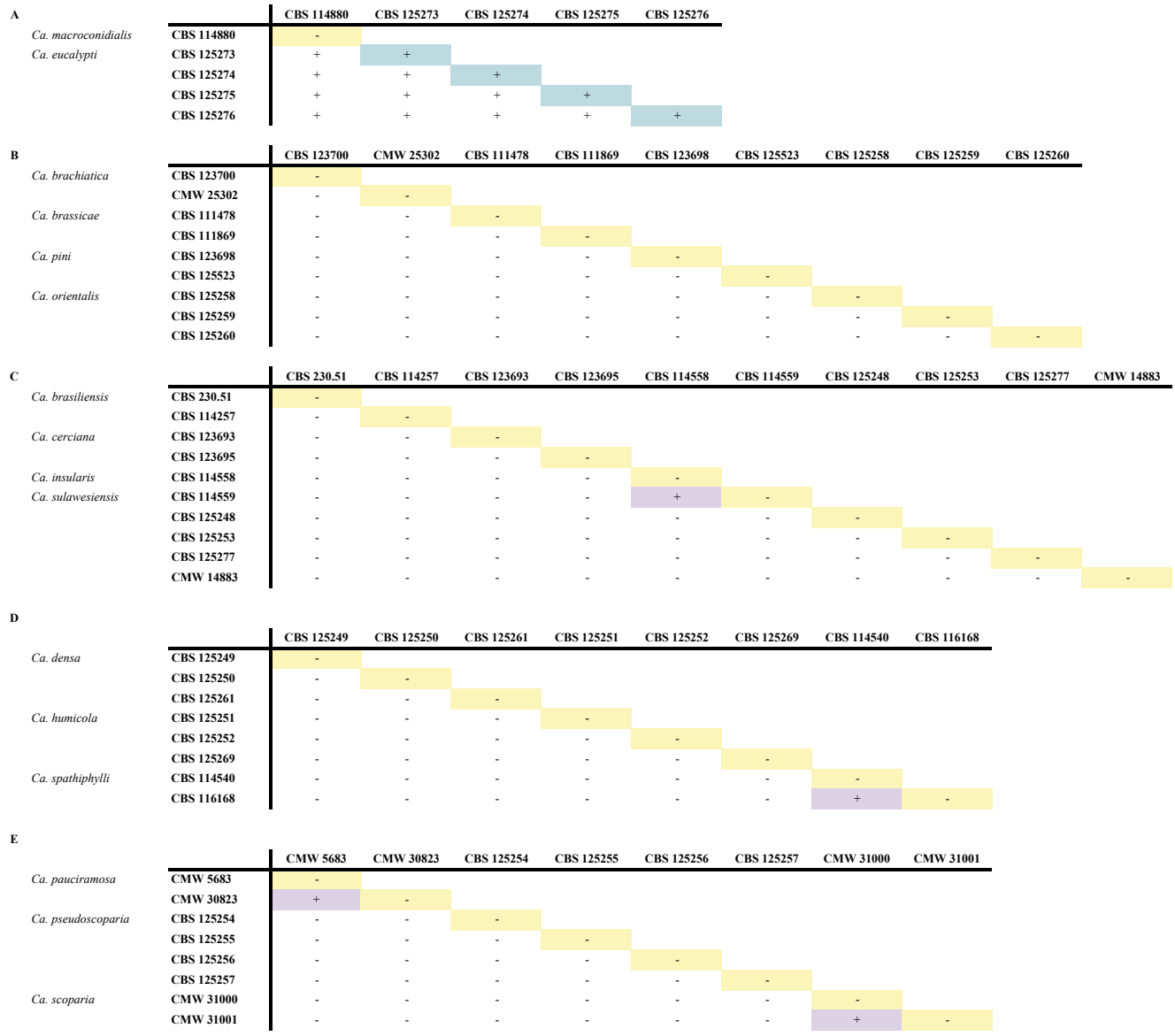

Fig 6. Results of sexual compatibility tests. Successful matings are indicated by (+) and unsuccessful matings is indicated with (-). Blue highlighted blocks indicate homothallic matings. Yellow blocks highlight unsuccessful self-self matings. Purple blocks indicate mating tester strain matings. A. Matings between isolates of *Ca. macroconidialis* and *Ca. eucalypti*. B. Matings between isolates of *Ca. brachiatica*, *Ca. brassicae*, *Ca. pini* and *Ca. orientalis*. C. Matings between isolates of *Ca. brasiliensis*, *Ca. cerciana*, *Ca. insularis* and *Ca. sulawesiensis*. D. Matings between isolates of *Ca. densa*, *Ca. humicola* and *Ca. spathiphylli*. E. Matings between isolates of *Ca. pauciramosa*, *Ca. pseudoscoparia* and *Ca. scoparia*.

Calonectria densa L. Lombard, M.J. Wingf. & Crous, **sp. nov.** MycoBank MB515529, Fig. 7.

Etymology: Name refers to the fact that lateral stipe extensions are readily formed in this species, giving it a bushy appearance.

Teleomorpha ignota. Anamorpha *Cy. spathiphylli* similis sed extensiones laterales stiparum facit, macroconidiis cylindricis utrinque rotundatis rectis (47–)50–58(–62) × 5–6 µm mediocriter 54 × 6 µm, semel septatis, sine cicatrice abscissionis manifesta, cum muco hyalino in fasciculis parallelis cylindricis differ.

Teleomorph unknown. *Conidiophores* with a stipe bearing penicillate suites of fertile branches, stipe extensions, and terminal vesicles; stipe septate, hyaline, smooth, 54–90 × 6–10 µm; stipe extensions septate, straight to flexuous, 149–192 µm long, 5–6 µm wide at the apical septum, terminating in ovoid to ellipsoid to sphaeropedunculate vesicles, 10–12 µm diam; lateral stipe extensions (90˚ to the axis) also present. *Conidiogenous apparatus* 49–78 µm long, and 63–123 µm wide; primary branches aseptate, 20–29 × 5–6 µm; secondary branches aseptate, 16–20 × 4–6 µm; tertiary and additional branches (–4) aseptate, 9–16 × 3–5 µm, each terminal branch producing 2–4 phialides; phialides doliiform to reniform, hyaline, aseptate, 11–16 × 2–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (47–)50–58(–62) × (5–)6 µm (av. = 54 × 6 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not seen.

Specimens examined: **Ecuador**, Pichincha Province, Las Golondrinas, from soil, Dec. 2004, M.J. Wingfield, Herb. PREM 60302, **holotype** of *Ca. densa*, culture ex-type CMW 31182 = CBS 125261; Pichincha Province, Las Golondrinas, from soil, Dec. 2004, M.J. Wingfield, cultures CMW 31184 = CBS 125249; Las Golondrinas, from soil, Dec. 2004, M.J. Wingfield, culture CMW 31185 = CBS 125250.

Culture characteristics: Colonies fast growing with optimal growth temperature at 25 ºC (growth at 15–35 ºC) on MEA, reverse umber

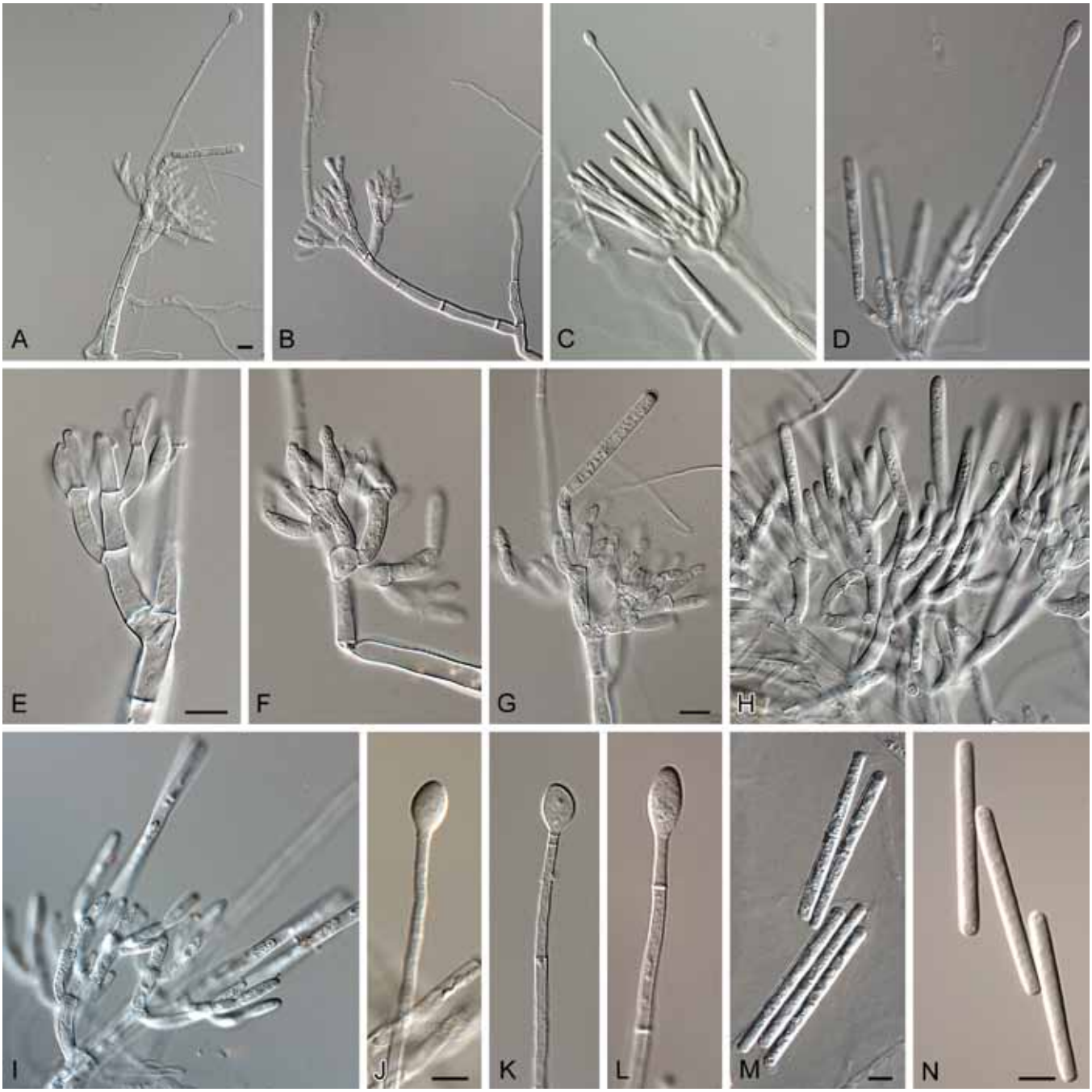

Fig. 7. *Calonectria densa*. A–D. Macroconidiophores. E–I. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. J–L. Ovoid to ellipsoid vesicles. M–N. One-septate macroconidia. Scale bars = 10 µm.

to verona-brown after 7 d; moderate white aerial mycelium with moderate sporulation; chlamydospores extensive throughout the medium forming microsclerotia.

Substrate: Soil.

Distribution: Ecuador.

Notes: Morphologically, *Ca. densa* is very similar to *Ca. spathiphylli* and *Ca. pseudospathiphylli*. However, macroconidia of *Ca. densa* (av. 54 × 6 µm) are smaller than those of *Ca. spathiphylli* (av. 70 × 6 µm), but slightly larger and broader than those of *Ca. pseudospathiphylli* (av. 52 × 4 µm). *Calonectria densa* also readily forms lateral stipe extensions, not reported for the other two species.

Calonectria eucalypti L. Lombard, M.J. Wingf. & Crous, **sp. nov.** MycoBank MB515530, Fig. 8.

Etymology: Name refers to *Eucalyptus* from which the fungus was isolated.

Teleomorpha *Ca. colhounii* similis sed ascocarpo flavo vel aurantiaco differt. Anamorpha *Cy. colhounii* similis sed macroconidiis cylindricis utrinque rotundatis rectis (66–)69–75(–80) × 5–6 µm mediocriter 72 × 6 µm, ter septatis, sine cicatrice abscissionis manifesta, cum muco hyalino in fasciculis parallelis cylindricis, differt.

Perithecia solitary or in groups, yellow to orange, becoming brown with age; in section apex and body yellow to orange, base red-brown, sub-globose to ovoid, 325–510 µm high, 285–360 µm diam, body turning dark red, and base dark red-brown (KOH+). Perithecial walls rough consisting of 2 thick-walled layers: outside

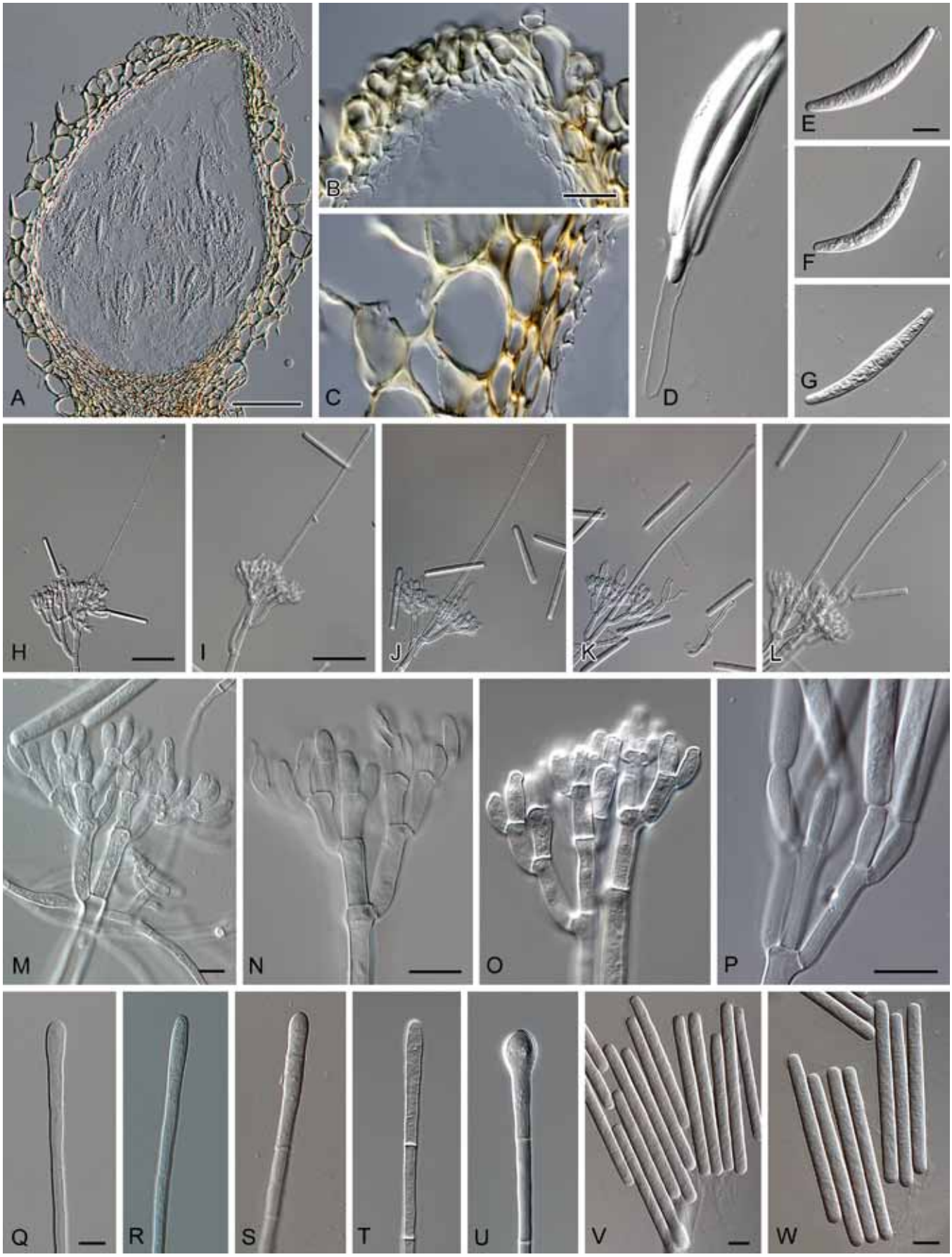

Fig. 8. *Calonectria eucalypti*. A. Perithecium. B. Section through ostiolar region of a perithecium. C. A vertical section through a perithecium, showing wall layers. D. Ascus. E–G. Ascospores. H–L. Macroconidiophores. M–P. Conidiogenous apparatus with conidiophore branches and doliiform to reniform or allantoid phialides. Q–U. Clavate to broadly clavate vesicles. V–W. Three-septate macroconidia. Scale bars: A = 90 µm, H–I = 70 µm, Other bars = 10 µm.

layer of *textura globulosa*, 45–90 µm wide; becoming more compressed towards inner layer of *textura angularis,* 12–18 µm wide; becoming thin-walled and hyaline towards the centre, outer cells 24–50 × 10–40 µm; inner cells 6–19 × 3–6 µm: perithecial base up to 125 µm wide; consisting of dark red, angular cells; merging with an erumpent stroma, cells of the outer wall layer continuing into the pseudoparenchymatous cells of the erumpent stroma. *Asci* 4-spored, clavate, 92–188 × 10–27 µm, tapering to a long thin stalk. *Ascospores* aggregated in the upper third of the ascus, hyaline, guttulate, fusoid with rounded ends, straight to slightly curved, (1–)3-septate, not or slightly constricted at the septum, (25–)30–36(–56) × (3–)5–6(–8) µm (av. = 33 × 6 µm). Cultures were homothallic. *Conidiophores* with a stipe bearing a suit of penicillate, fertile branches, a stipe extension, and a terminal vesicle; stipe septate, hyaline, smooth, 45–91 × 7–10 µm; stipe extensions septate, straight to flexuous, 110–235 µm long, 5–6 µm wide at the apical septum, terminating in broadly clavate vesicles, 4–6 µm diam. *Conidiogenous apparatus* 52–82 µm long, and 40–95 µm wide; primary branches aseptate or 1-septate, 21–29 × 5–6 µm; secondary branches aseptate, 14–21 × 3–5 µm; tertiary branches and additional branches (–5), aseptate, 11–16 × 3–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 10–14 × 3–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (66–)69–75(–80) × (5–)6 µm (av. = 72 × 6 µm), 3-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Megaconidia* and *microconidia* not seen.

Specimens examined: **Indonesia**, Sumatra Utara, Aek Nauli, on leaf of *Eucalyptus grandis*, May 2005, M.J. Wingfield, Herb. PREM 60298, **holotype** of *Ca. eucalypti*, culture ex-type CMW 18444 = CBS 125275; Aek Nauli, on leaf of *Eucalyptus grandis*, May 2005, M.J. Wingfield, PREM 60299, culture CMW 14890 = CBS 125273; Aek Nauli, on leaf of *Eucalyptus grandis*, May 2005, M.J. Wingfield, culture CMW 18443 = CBS 125274, Aek Nauli, on leaf of *Eucalyptus grandis*, May 2005, M.J. Wingfield, culture CMW 18445 = CBS 125276.

Culture characteristics: Colonies fast growing with optimal growth temperature at 25 ºC (growth at 15–30 ºC) on MEA, reverse colour tawny-brown after 7 d; abundant white aerial mycelium and sporulation; chlamydospores abundant throughout the medium, forming microsclerotia.

Substrate: *Eucalyptus grandis.*

Distribution: Indonesia.

Notes: The perithecia of *Ca. eucalypti* can be distinguished from *Ca. colhounii* and *Ca. macroconidialis* based on their yellow to orange colour in KOH. Macroconidia of *Ca. eucalypti* (av. 72 × 6 µm) are also larger than those of *Ca. colhounii* (av. 55 × 6 µm) and *Ca. madagascariensis* (av. 55 × 4.5 µm), but smaller than those of *Ca. macroconidialis* (av. 90 × 6.5 µm). Mating tests (Fig. 6) also showed that *Ca. eucalypti* is homothallic, a characteristic shared by *Ca. colhounii* and *Ca. madagascariensis* but not with *Ca. macroconidialis*, which is heterothallic (Crous 2002).

Calonectria humicola L. Lombard, M.J. Wingf. & Crous, **sp. nov.** MycoBank MB515531, Fig. 9.

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

Teleomorpha ignota. Anamorpha Cy. spathiphylli similis sed macroconidiis cylindricis utrinque rotundatis rectis (45–)48–54(–56) × 4–5 µm mediocriter 51 × 5 µm, semel septatis, sine cicatrice abscissionis manifesta, cum muco hyalino in fasciculis parallelis cylindricis differt.

Teleomorph unknown. *Conidiophores* with a stipe bearing penicillate suites of fertile branches, stipe extensions, and terminal vesicles; stipe septate, hyaline, smooth, 44–90 × 6–8 µm; stipe extensions septate, straight to flexuous, 126–157 µm long, 4–5 µm wide at the apical septum, terminating in globose to ovoid to sphaeropedunculate vesicles, 10–12 µm diam. *Conidiogenous apparatus* 43–71 µm long, and 42–49 µm wide; primary branches aseptate, 20–29 × 4–6 µm; secondary branches aseptate, 12–19 × 3–5 µm; tertiary branches aseptate, 9–16 × 3–5 µm, each terminal branch producing 2–4 phialides; phialides elongated doliiform to reniform, hyaline, aseptate, 10–15 × 3–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (45–)48–54(–56) × (4–)5 µm (av. = 51 × 5 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not seen.

Specimens examined: **Ecuador**, Pichincha Province, Las Golondrinas, from soil, Dec. 2004, M.J. Wingfield, Herb. PREM 60369 **holotype** of *Ca. humicola*, culture ex-type CMW 31183 = CBS 125251; Las Golondrinas, from soil, Jan. 2006, L. Lombard, culture CMW 31186 = CBS 125252; Las Golondrinas, from soil, Jan. 2006, L. Lombard, (Herb. PREM 60368) culture CMW 31187 = CBS 125269.

Culture characteristics: Colonies fast growing with optimal growth temperature at 25 ºC (growth at 15–35 ºC) on MEA, reverse umber to verona-brown after 7 d; moderate white aerial mycelium with moderate sporulation; chlamydospores extensive throughout the medium, forming microsclerotia.

Substrate: Soil.

Distribution: Ecuador.

Notes: *Calonectria humicola* is morphologically very similar to *Ca. densa*, *Ca. pseudospathiphylli* and *Ca. spathiphylli*. However, no lateral stipe extensions occur in this species, whereas these are common in *Ca. densa*. Macroconidia of *Ca. humicola* (av. 51 × 5 µm) are slightly smaller than those of *Ca. densa* (av. 54 × 6 µm) and *Ca. spathiphylli* (av. 70 × 6 µm), but slightly broader than those of *Ca. pseudospathiphylli* (av. 52 × 4 µm).

Calonectria orientalis L. Lombard, M.J. Wingf. & Crous, **sp. nov.** MycoBank MB515532, Fig. 10.

Etymology: Name refers to the East Asian region, where the fungus was isolated.

Teleomorpha ignota. Anamorpha *Ca. brachiaticae* similis sed ramis conidiophorae tres vel minus sine extensionibus lateralibus stipae, macroconidiis cylindricis utrinque rotundatis rectis (43–)46–50(–53) × 4–5 µm mediocriter 48 × 4 µm, semel septatis, sine cicatrice abscissionis manifesta, cum muco hyalino in fasciculis parallelis cylindricis differt.

Teleomorph unknown. *Conidiophores* with a stipe bearing penicillate suites of fertile branches, stipe extensions, and terminal vesicles; stipe septate, hyaline, smooth, 60–169 × 6–12 µm; stipe extensions septate, straight to flexuous, 90–218 µm long, 5–10 µm wide at the apical septum, terminating in clavate to broadly clavate vesicles, 5–10 µm diam. *Conidiogenous apparatus* 54–174 µm long, and 67–92 µm wide; primary branches aseptate, 19–30 × 4–7 µm; secondary branches aseptate, 16–29 × 4–6 µm; tertiary and additional branches (–5) aseptate, 10–20 × 5–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 10–19 × 2–5 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (43–)46–50(–53) × 4(–5) µm (av. = 48 × 4 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not seen.

Specimens examined: **Indonesia**, Langam, from soil, June 2005, M.J. Wingfield, Herb. PREM 60303, **holotype** of *Ca. orientalis*, culture ex-type CMW 20291 = CBS 125260; Teso East, from soil, June 2005, M.J. Wingfield culture CMW 20273 = CBS 125259; Teso East, from soil, June 2005, M.J. Wingfield, culture CMW 20272 = CBS 125258.

Culture characteristics: Colonies fast growing with optimal growth temperature at 25 ºC (growth at 15–35 ºC) on MEA, reverse sepia-brown after 7 d; abundant white aerial mycelium with moderate to extensive sporulation; chlamydospores extensive throughout the medium, forming microsclerotia.

Substrate: Soil.

Distribution: Indonesia.

Notes: *Calonectria orientalis* is closely related to *Calonectria* spp. in the *Ca. brassicae* complex, based on phylogenetic inference and SNP analyses. Morphological comparisons showed that the macroconidia of *Ca. orientalis* (av. 48 × 4 µm) are shorter than those of *Ca. brassicae* (av. 53 × 4.5 µm), *Ca. clavata* (av. 65 × 5 µm) and *Ca. gracilis* (av. 56 × 4.5 µm) but larger than those of *Ca. brachiatica* (av. 44 × 5 µm) and *Ca. gracilipes* (av. 45 × 4.5 µm). As with *Ca. pini*, perithecia could not be induced when this species was mated with *Ca. brachiatica* and *Ca. brassicae*, highlighting the rarity of teleomorph structures for this group of fungi.

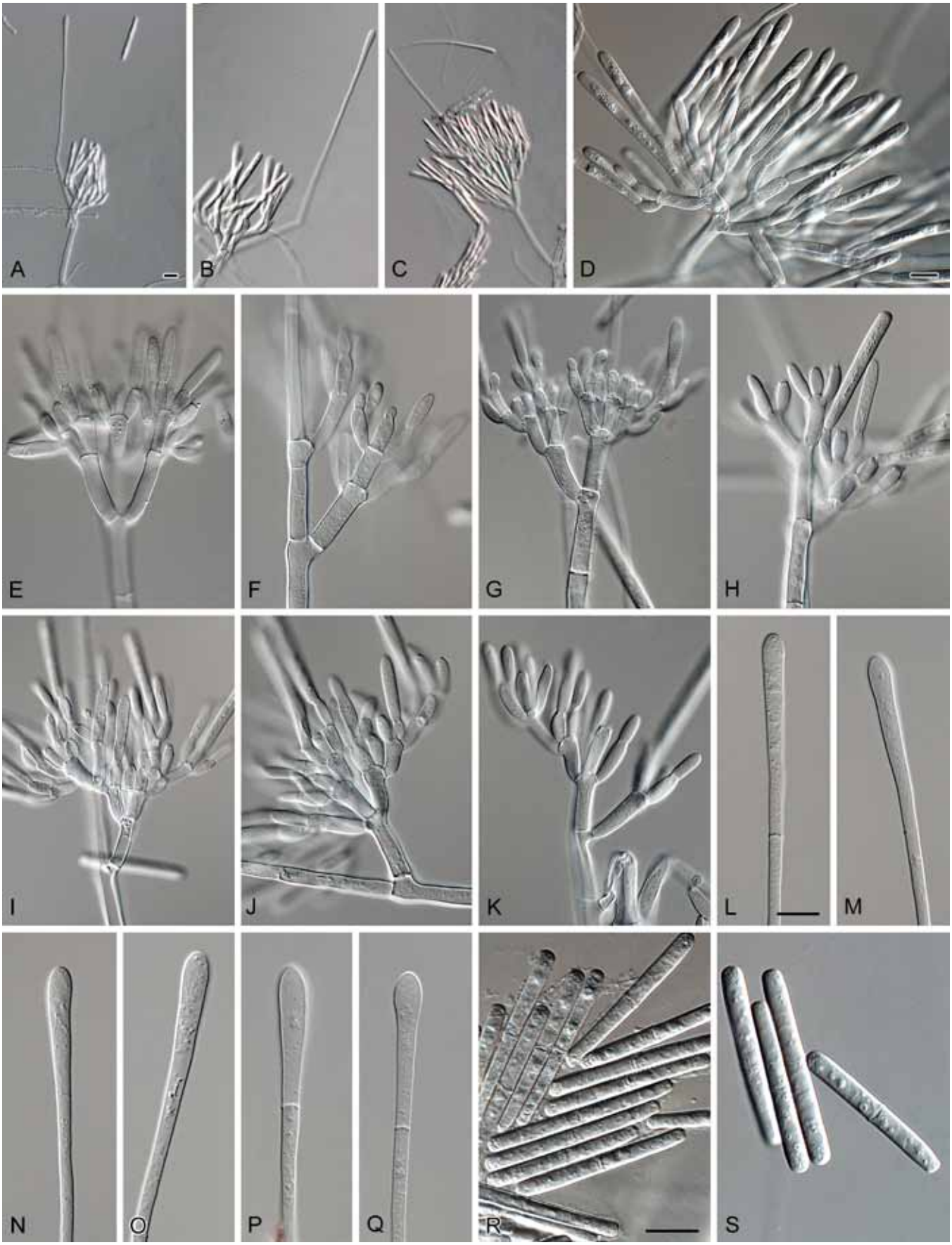

Fig. 10. *Calonectria orientalis*. A–C. Macroconidiophores. D–K. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. L–Q. Clavate vesicles. R–S. One-septate macroconidia. Scale bars = 10 µm.

Fig. 9. (p. 50) *Calonectria humicola*. A–F. Macroconidiophores. G–I. Conidiogenous apparatus with conidiophore branches and somewhat elongated, doliiform to reniform phialides. J–N. Globose to ovoid to sphaeropedunculate vesicles. O–P. One-septate macroconidia. Scale bars = 10 µm.

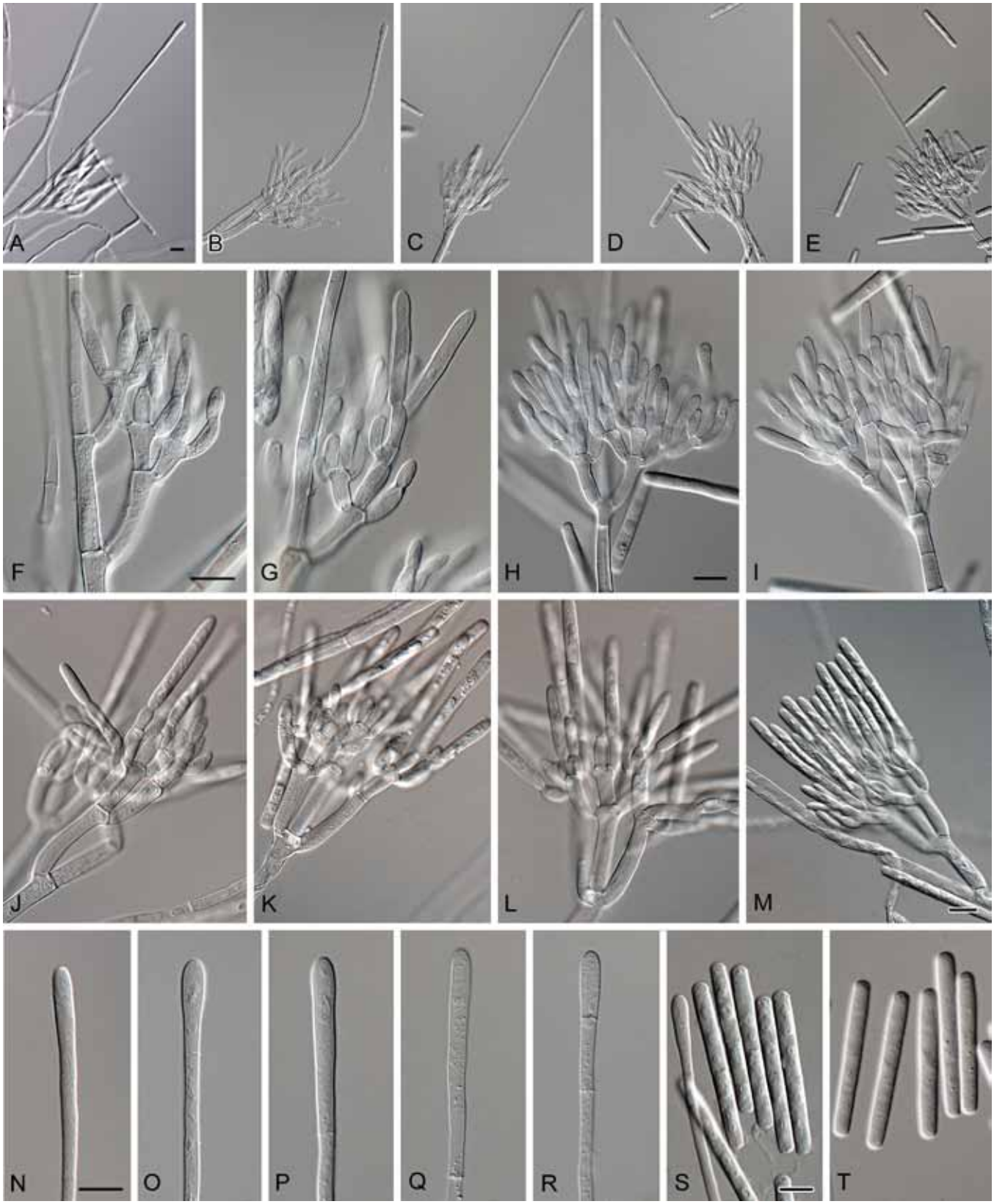

Fig. 11. *Calonectria pini*. A–E. Macroconidiophores. F–M. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. N–R. Clavate vesicles. S–T. One-septate macroconidia. Scale bars = 10 µm.

Calonectria pini L. Lombard, M.J. Wingf. & Crous, **sp. nov.** MycoBank MB515533, Fig. 11.

Etymology: Name refers to *Pinus,* the host from which the fungus was isolated.

Teleomorpha ignota. Anamorpha *Ca. brachiaticae* similis sed ramis conidiophorae tres vel minus sine extensionibus lateralibus stipae, macroconidiis cylindricis utrinque rotundatis rectis (37–)40–48(–50) × 4–6 µm mediocriter 44 × 5 µm, semel septatis, sine cicatrice abscissionis manifesta, cum muco hyalino in fasciculis parallelis cylindricis differ.

Teleomorph unknown. *Conidiophores* with a stipe bearing penicillate suites of fertile branches, stipe extensions, and terminal vesicles; stipe septate, hyaline, smooth, 40–99 × 6–7 µm; stipe extensions septate, straight to flexuous, 121–266 µm long, 5–7

µm wide at the apical septum, terminating in clavate vesicles, 4–6 µm diam. *Conidiogenous apparatus* 49–81 µm long, and 35–84 µm wide; primary branches aseptate, 20–30 × 4–6 µm; secondary branches aseptate, 13–22 × 3–5 µm; tertiary branches aseptate, 11–15 × 3–4 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 10–15 × 3–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (37–)40–48(–50) × 4–6 µm (av. = 44 × 5 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not seen.

Specimens examined: **Colombia**, Valle del Cauca, Buga, from *Pinus patula*, Sept. 2007, C.A. Rodas, Herb. PREM 60304, **holotype** of *Ca. pini*, culture ex-type CMW 31209 = CBS 123698; Buga, from *Pinus patula*, Sept. 2007, *C.A. Rodas*; Buga, from *Pinus patula*, Sept. 2007, C.A. Rodas, culture CMW 31210 = CBS 125523.

Culture characteristics: Colonies fast growing with optimal growth temperature at 25 ºC (growth at 15–30 ºC) on MEA, reverse amber to sepia-brown after 7 d; abundant white aerial mycelium with moderate to extensive sporulation; chlamydospores extensive throughout the medium forming microsclerotia.

Substrate: *Pinus patula.*

Distribution: Colombia.

Notes: *Calonectria pini* is very similar to *Ca. brachiatica*, but can be distinguished morphologically by the fact that it has three or fewer conidiophore branches and no lateral stipe extensions (Lombard *et al.* 2009). Macroconidia of *Ca. pini* (av. 44 × 5 µm) are shorter than those of *Ca. brassicae* (av. 53 × 4.5 µm), *Ca. gracilis* (56 × 4.5 µm) and *Ca. orientalis* (av. 48 × 4 µm). This species also has fewer conidiophore branches than those mentioned above. *Calonectria pini* failed to produce perithecia when crossed with *Ca. brachiatica* and *Ca. brassicae*. This supports the findings of Crous *et al.* (2004b) and Lombard *et al.* (2009), that teleomorph structures are rarely observed in members of the *Ca. brassicae* complex.

Calonectria pseudoscoparia L. Lombard, M.J. Wingf. & Crous, **sp. nov.** MycoBank MB515534, Fig. 12.

Etymology: Name reflects the fact that the species resembles the anamorph state of *Ca. scoparia*.

Teleomorpha ignota. Anamorpha *Ca. scopario* similis sed phialidibus elongato-doliiformibus vel reniformibus hyalinis non septatis 7–11 × 2–4 µm apice minute periclinale incrassatis colliculo inconspicuo, macroconidiis cylindricis utrinque rotundatis rectis (41–)45–51(–52) × 3–5 µm mediocriter 48 × 4 µm, semel septatis, sine cicatrice abscissionis manifesta, cum muco hyalino in fasciculis parallelis cylindricis differ.

Teleomorph unknown. *Conidiophores* with a stipe bearing penicillate suites of fertile branches, stipe extensions, and terminal vesicles; stipe septate, hyaline, smooth, 56–107 × 6–10 µm; stipe extensions septate, straight to flexuous, 124–201 µm long, 4–6 µm wide at the apical septum, terminating in obpyriform to ellipsoidal vesicles, 6–10 µm diam. *Conidiogenous apparatus* 34–87 µm long, and 52–74 µm wide; primary branches aseptate, 26–38 × 4–7 µm; secondary branches aseptate, 17–28 × 4–6 µm; tertiary branches and additional branches (–4) aseptate, 14–19 × 3–4 µm, each terminal branch producing 2–6 phialides; phialides elongate-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, (41–)45–51(–52) × 3–5 µm (av. = 48 × 4 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not seen.

Specimens examined: **Ecuador**, Pichincha Province, Las Golondrinas, Buenos Aires Nursery, from *Eucalyptus grandis* cutting, Dec. 2004, M.J. Wingfield, Herb. PREM 60305, **holotype** of *Ca. pseudoscoparia*, culture ex-type CMW 15218 = CBS 125257; Buenos Aires Nursery, from *Eucalyptus grandis* cutting, Dec. 2004, M.J. Wingfield, Herb. PREM 60306, cultures from different cuttings, CMW 15214 = CBS 125254, CMW 15215 = CBS 125255, CMW 15216 = CBS 125256.

Culture characteristics: Colonies fast growing with optimal growth temperature at 25 ºC (growth at 10–30 ºC) on MEA, reverse amber to sepia-brown after 7 d; colony margins irregular with sparse to moderate white aerial mycelium with moderate sporulation; chlamydospores extensive throughout the medium forming microsclerotia.

Substrate: *Eucalyptus grandis.*

Distribution: Ecuador.

Notes: *Calonectria pseudoscoparia* (conidia av. 48 × 4 µm) can be distinguished from *Ca. scoparia* (conidia av. 60 × 4.5 µm) based on smaller macroconidia and the fact that it has elongated-doliiform to reniform phialides unlike those of *Ca. pauciramosa* and *Ca. scoparia*. Mating tests between this fungus and *Ca. scoparia* and *Ca. pauciramosa* failed to produce perithecia. Control crosses with both *Ca. pauciramosa* (CMW 5683 and CMW 30823) and *Ca. scoparia* tester isolates (CMW 31000 and CMW 31001) produced perithecia with viable ascospores showing that culture conditions were appropriate for mating.

Calonectria sulawesiensis L. Lombard, M.J. Wingf. & Crous, **sp. nov.** MycoBank MB515535, Fig. 13.

Etymology: Name refers to the Indonesian island of Sulawesi, where the fungus was collected.

Teleomorpha ignota. Anamorpha *Ca. morganii* similis sed vesiculo terminali late clavato vel ellipsoideo 5–7 µm diametro, macroconidiis cylindricis utrinque rotundatis rectis (41–)45–51(–54) × (3–)4–6 µm mediocriter 48 × 4 µm, semel septatis, sine cicatrice abscissionis manifesta, cum muco hyalino in fasciculis parallelis cylindricis differt.

Teleomorph unknown. *Conidiophores* with a stipe bearing penicillate suites of fertile branches, stipe extensions, and terminal vesicles; stipe septate, hyaline, smooth, 37–139 × 5–11 µm; stipe extensions septate, straight to flexuous, 113–262 µm long, 5–7 µm wide at the apical septum, terminating in broadly clavate to ellipsoidal vesicles, 5–7 µm diam. *Conidiogenous apparatus* 41–79 µm long, and 43–81 µm wide; primary branches aseptate, 17–41 × 3–6 µm; secondary branches aseptate, 10–27 × 3–6 µm; tertiary branches and additional branches (–5), aseptate, 9–15 × 3–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 9–15 × 2–5 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (41–)45–51(–54) × (3–)4(–6) µm (av. = 48 × 4 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not seen.

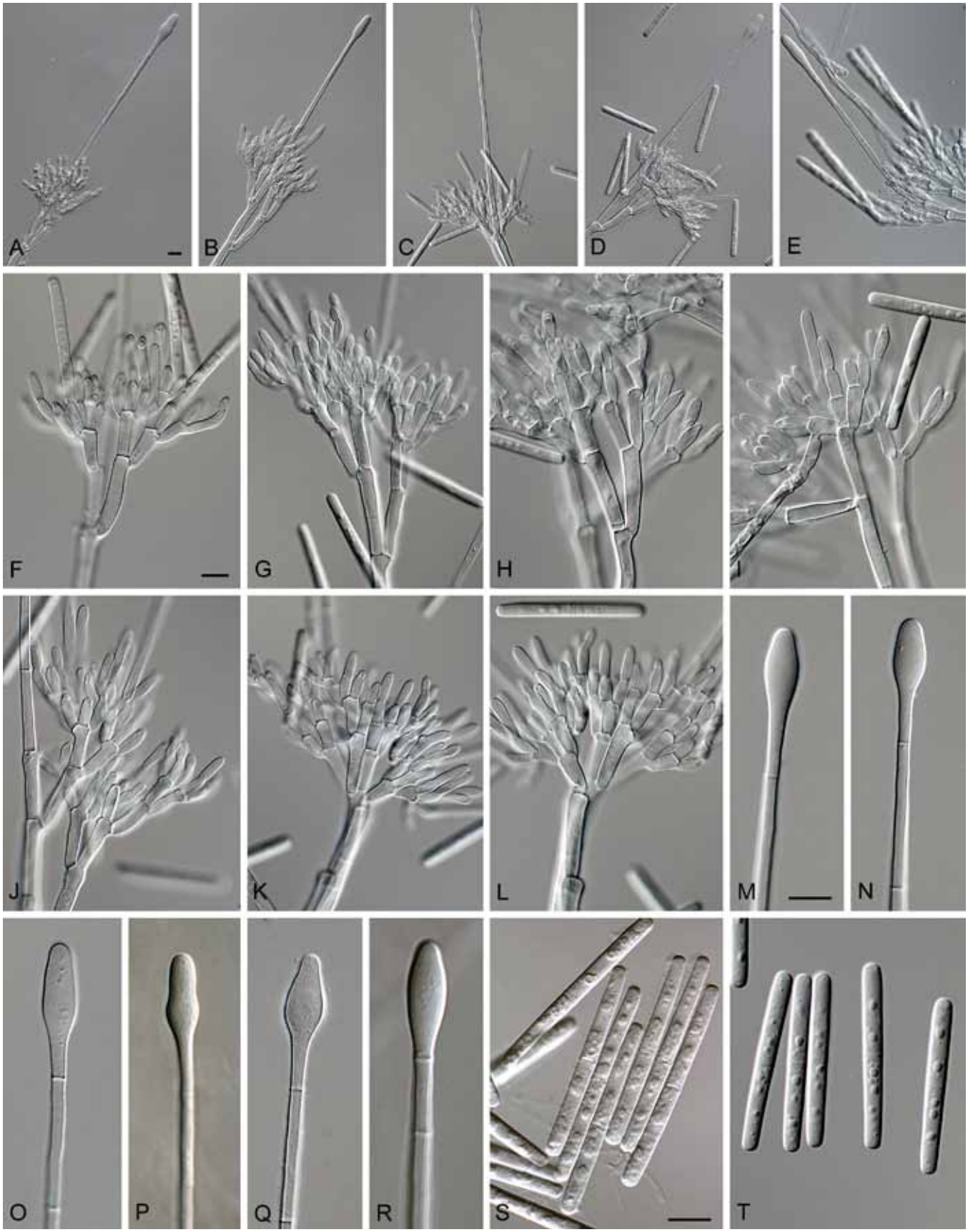

Fig. 12. *Calonectria pseudoscoparia*. A–E. Macroconidiophores. F–L. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. M–R. Obpyriform to ellipsoidal vesicles. S–T. One-septate macroconidia. Scale bars = 10 µm.

Specimens examined: **Indonesia**, Sulawesi, from leaf of *Eucalyptus* sp., July 2003, M.J. Wingfield, Herb. PREM 60300, **holotype** of *Ca. sulawesiensis*, culture ex-type CMW 14878 = CBS 125277; Sulawesi, from leaf of *Eucalyptus* sp., July 2003, M.J. Wingfield, PREM 60301 culture CMW 14883; from different leaves, culture CMW 14859 = CBS 125248, CMW 14879 = CBS 125253.

Culture characteristics: Colonies fast growing with optimal growth temperature at 25 ºC (growth at 15–30 ºC) on MEA, reverse amber to sepia-brown after 7 d; abundant white aerial mycelium with moderate to extensive sporulation; chlamydospores extensive throughout the medium, forming microsclerotia.

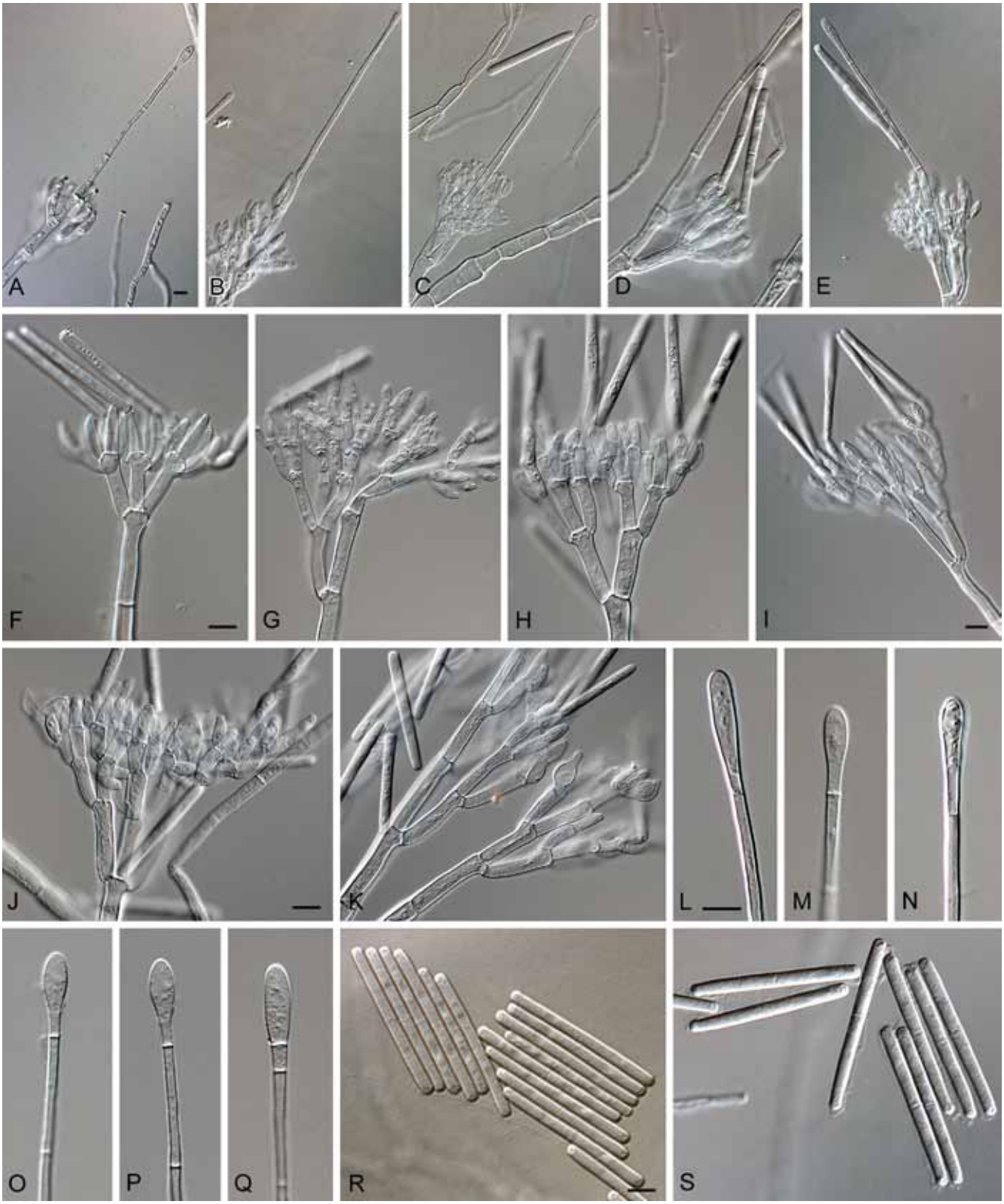

Fig. 13. *Calonectria sulawesiensis*. A–E. Macroconidiophores. F–K. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. L–Q. Clavate to ellipsoidal vesicles. R–S. One-septate macroconidia. Scale bars = 10 µm.

Substrate: *Eucalyptus* sp.

Distribution: Indonesia.

Notes: There are a few morphological differences distinguishing *Ca. sulawesiensis* from other species in the *Ca. morganii* complex. Macroconidia of *Ca. sulawesiensis* (av. 48 × 4 µm) are slightly larger than those of *Ca. brasiliensis* (av. 30 × 4 µm), *Ca. cerciana* (av. 44 × 5 µm), *Ca. insularis* (av. 45 × 4 µm) and *Ca. morganii* (av. 45 × 4 µm), but smaller than those of *Ca. hawksworthii* (av. 56 × 4 µm), *Ca. leucothoës* (av. 73 × 5 µm) and *Ca. variabilis* (av. 73 × 5 µm). Mating tests where *Ca. sulawesiensis* was crossed with *Ca. brasiliensis*, *Ca. cerciana* and *Ca. insularis* failed to produce perithecia, or produced perithecia without viable ascospores.

Calonectria angustata (Crous & El-Gholl) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515536.
Basionym: *Cylindrocladium angustatum* Crous & El-Gholl, Mycoscience 41: 522. 2000.

Calonectria australiensis (Crous & K.D. Hyde) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515537.
Basionym: *Cylindrocladium australiense* Crous & K.D. Hyde, Stud. Mycol. 55: 221. 2006.

Calonectria canadensis (J.C. Kang, Crous & C.L. Schoch) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515538.
Basionym: *Cylindrocladium canadense* J.C. Kang, Crous & C.L. Schoch, Syst. Appl. Microbiol. 24: 210. 2001.

Calonectria chinensis (Crous) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515539.
Basionym: *Cylindrocladium chinense* Crous, Stud. Mycol. 50: 420. 2004.

Calonectria citri (H.S. Fawc. & Klotz) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515540.
Basionym: *Candelospora citri* H.S. Fawc. & Klotz, Mycologia 29: 213. 1937.
≡ *Cylindrocladium citri* (H.S. Fawc. & Klotz) Boedijn & Reitsma, Reinwardtia 1: 57. 1950.

Calonectria curvata (Boedijn & Reitsma) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515541.
Basionym: *Cylindrocladium curvatum* Boedijn & Reitsma, Reinwardtia 1: 54. 1950.

Calonectria curvispora (Crous & D. Victor) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515542.
Basionym: *Cylindrocladium curvisporum* Crous & D. Victor, Syst. Appl. Microbiol. 20: 283. 1997.

Calonectria ecuadoriae (Crous & M.J. Wingf.) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515543.
Basionym: *Cylindrocladium ecuadoriae* Crous & M.J. Wingf., Stud. Mycol. 55: 222. 2006.

Calonectria gordoniae (Leahy, T.S. Schub. & El-Gholl) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515544.
Basionym: *Cylindrocladium gordoniae* Leahy, T.S. Schub. & El-Gholl, Mycotaxon 76: 80. 2000.

Calonectria hawksworthii (Peerally) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515545.
Basionym: *Cylindrocladium hawksworthii* Peerally, Mycotaxon 40: 375. 1991.

Calonectria hurae (Linder & Whetzel) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515546.
Basionym: *Cercosporella hurae* Linder & Whetzel, Mycologia 29: 656. 1937.
≡ *Cylindrocladiopsis hurae* (Linder & Whetzel) U. Braun, Mycotaxon 51: 40. 1994.
≡ *Cylindrocladium hurae* (Linder & Whetzel) Crous, In: *Taxonomy and pathology of Cylindrocladium (Calonectria) and allied genera:* 185. 2002.
= *Cylindrocladium heptaseptatum* Sober, Alfieri & Knauss, Phytopathology 65: 333. 1975.
= *Cylindrocladiopsis lagerstroemiae* J.M. Yen, Mycotaxon 8: 236. 1979.

Calonectria indonesiae (Crous) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515547.
Basionym: *Cylindrocladium indonesiae* Crous, Stud. Mycol. 50: 424. 2004.

Calonectria leucothoës (El-Gholl, Leahy & T.S. Schub.) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515548.
Basionym: *Cylindrocladium leucothoës* El-Gholl, Leahy & T.S. Schub., Canad. J. Bot. 67: 2530. 1989.
= *Cylindrocladium perseae* T.S. Schub., Leahy & El-Gholl, Mycotaxon 73: 474. 1999.

Calonectria malesiana (Crous) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515549.
Basionym: *Cylindrocladium malesianum* Crous, Stud. Mycol. 50: 425. 2004.

Calonectria multiphialidica (Crous, Simoneau & Risède) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515550.
Basionym: *Cylindrocladium multiphialidicum* Crous, Simoneau & Risède, Stud. Mycol. 50: 425. 2004.

Calonectria pacifica (J.C. Kang, Crous & C.L. Schoch) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515551.
Basionym: *Cylindrocladium pacificum* J.C. Kang, Crous & C.L. Schoch, Syst. Appl. Microbiol. 24: 213. 2001.

Calonectria penicilloides (Tubaki) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515552.
Basionym: *Candelospora penicilloides* Tubaki, Nogaoa 2: 58. 1952.
≡ *Cylindrocladium penicilloides* (Tubaki) Tubaki, J. Hattori Bot. Lab. 20: 154. 1958.

Calonectria pseudonaviculata (Crous, J.Z. Groenew. & C.F. Hill) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515554.
Basionym: *Cylindrocladium pseudonaviculatum* Crous, J.Z. Groenew. & C.F. Hill, Sydowia 54: 26. 2002.
= *Cylindrocladium buxicola* Henricot, Mycologia 94: 993. 2002.

Calonectria sumatrensis (Crous) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515555.
Basionym: *Cylindrocladium sumatrense* Crous, Stud. Mycol. 50: 426. 2004.

DISCUSSION

In this study, a collection of isolates of unknown identity were shown to represent seven new species of *Calonectria*. These species, provided with the names *Ca. eucalypti*, *Ca. orientalis* and *Ca. sulawesiensis* from Indonesia, *Ca. densa*, *Ca. humicola* and *Ca. pseudoscoparia* from Ecuador and *Ca. pini* from Colombia were recognised based on morphological characteristics and phylogenetic inference. Recognition of a relatively large number of new species, mainly from soil samples collected in areas not previously intensively sampled, suggests that many more species of *Calonectria* remain to be discovered, particularly from the tropics and Southern Hemisphere.

Calonectria eucalypti, isolated from the leaves of *Eucalyptus grandis*, adds a new species to the *Ca. colhounii* complex (Crous 2002, Crous *et al.* 2006), which includes *Ca. colhounii*, *Ca. macroconidialis* and *Ca. madagascariensis*. Members of this complex are characterised by their unique yellow perithecia (Crous 2002). Although *Ca. eucalypti* was isolated from lesions typical of Cylindrocladium leaf blight, its importance as a pathogen is unknown. *Calonectria eucalypti* was shown to be homothallic, which is a characteristic that this species shares with *Ca. colhounii* and *Ca. madagascariensis.*

The descriptions of *Ca. pini* and *Ca. orientalis* add two species to the *Ca. brassicae* complex (Crous *et al.* 2006, Lombard *et al.* 2009). *Calonectria pini* was isolated from *Pinus patula* rooted cuttings with symptoms similar to those associated with root and collar infections caused by *Ca. brassicae* and *Ca. brachiatica* on other *Pinus* spp. (Lombard *et al.* 2009). In contrast, *Ca. orientalis* was isolated from soils collected in Indonesia and nothing is known regarding its pathogenicity. Phylogenetic inference and SNP allele analyses showed that these are closely related sibling species (Taylor *et al.* 2000) with genetic isolation having apparently occurred recently. Crosses between isolates of *Ca. pini* and *Ca. orientalis* as well as those with themselves and other *Calonectria* spp. in the group failed to produce perithecia. This is consistent with the observations of Crous *et al.* (2006) and Lombard *et al.* (2009), that *Calonectria* spp. in this complex rarely produce teleomorph structures in culture. *Calonectria sulawesiensis* resides in the *Ca. morganii* complex, closely related to *Ca. brasiliensis* and *Ca. insularis*. Morphologically, *Ca. sulawesiensis* can be distinguished from other species in the complex based only on macroconidial dimensions. Therefore phylogenetic inference based on DNA sequence data is necessary to distinguish it from other members of the *Ca. morganii* complex. Members of this complex are well-known pathogens of various hosts worldwide (Crous 2002), but nothing is known regarding the pathogenicity of *Ca. sulawesiensis.*

Calonectria pseudoscoparia is a new species in the *Ca. scoparia* complex (Schoch *et al.* 1999), isolated from *E. grandis* cuttings collected in Ecuador that displayed basal rot symptoms. *Calonectria* spp. in this group are well known causal agents of cutting rot in commercial forestry nurseries worldwide (Crous *et al.* 1991, Crous 2002, Lombard *et al.* 2010a). However, the pathogenicity of *Ca. pseudoscoparia* is only assumed based on the symptoms with which the fungus was associated.

The two newly described species, *Ca. densa* and *Ca. humicola*, isolated from Ecuadorian soils reside in the *Ca. spathiphylli* complex as defined by Kang *et al.* (2001b). *Calonectria pseudospathiphylli* and *Ca. spathiphylli*, that define this complex, are not easily distinguished based on morphology and DNA sequence comparisons are required for their identification. They can, however, be distinguished based on their mating strategies, with *Ca. pseudospathiphylli* being homothallic and *Ca. spathiphylli* being heterothallic (Kang *et al.* 2001b, Crous 2002). The mating strategies of *Ca. densa* and *Ca. humicola* could not be determined in this study. This complex of species appears to originate from Central and South America (Chase & Poole 1987, Kang *et al.* 2001b, Crous 2002).

DNA sequence data for the ITS, BT and HIS3 have been used more extensively to explore phylogenetic relationships amongst *Calonectria* spp. (Schoch *et al*. 1999, Kang *et al*. 2001a, 2001b, Henricot & Culham 2002, Crous *et al*. 2004b, 2006). In this regard, BT is the gene region that provides the most valuable insights into relationships between all species of *Calonectria* (Schoch *et al.* 2000b, 2001b, Crous 2002, Henricot & Culham 2002). Application of the CAL and TEF-1α partial gene sequences has only recently been introduced for *Calonectria* spp. (Crous *et al.* 2004b, 2006, Lombard *et al.* 2009, 2010a, c) and data for these gene regions have been available for only a small sub-set of species. The present study has attempted to address this problem and also introduce the ACT and LSU gene sequences that have not been employed previously for *Calonectria* spp. It has also provided sequence data for all seven gene regions for all accepted species in the genus.

The ITS and LSU sequences provided little valuable information to separate *Calonectria* spp. In contrast, sequence data for the protein-coding gene regions ACT, BT, CAL, HIS3 and TEF-1α provided good resolution of *Calonectria* spp., confirming the results of previous studies (Schoch *et al.* 1999, 2001a, Crous 2002, Henricot & Culham 2002, Crous *et al.* 2004b, 2006). This study also introduced sequence data for the ACT gene region, although it had few informative sites, consistent with the results of previous studies on other groups of fungi (Helgason *et al.* 2003, Hunter *et al.* 2006). Phylogenetic analyses of the individual coding gene regions and single nucleotide polymorphisms showed that CAL sequence data provide the best resolution distinguishing *Calonectria* spp. from each other followed by sequence data for the TEF-1α, HIS3, BT and ACT gene regions.

In addition to identifying the most useful gene regions to accurately identify species of *Calonectria*, an important goal of this study was to re-consider the phylogenetic relationships between all the species in this genus. Having determined that the ACT, BT, CAL, HIS3 and TEF-1α gene regions give the best resolution when identifying species of *Calonectria*, a phylogenetic tree for the genus was generated. This showed that the group includes two major clades and that these define morphologically similar groups of *Calonectria* spp. These two major clades have substantial sub-structure with all of the 66 species of *Calonectria* residing in one of 13 sub-clades. Eleven of these sub-clades, that include 50 species, represent the Prolate Group of isolates and two sub-clades that include 16 species representing the Sphaero-Naviculate Group of isolates.

The Prolate group of isolates incorporates the majority of the plant pathogenic *Calonectria* spp. and includes the type species for *Calonectria* (*Ca. pyrochoa*) and *Cylindrocladium* (*Cy. scoparium*). Most of these pathogenic species have been reported from forestry crops (Peerally 1991, Crous & Wingfield 1994, Crous 2002, Crous *et al.* 2006) but a few have also been found to infect horticultural and agronomic crops (Boedijn & Reitsma 1950, Kim *et al.* 1998, Crous 2002, Polizzi *et al.* 2007, Vitale *et al.* 2008). None of the sub-clades in this group could, however, be correlated with any specific host type.

The geographic distribution of the *Calonectria* spp. representing the various sub-clades of the unifying Prolate Group of isolates

shows some correlation in their distribution. *Calonectria* spp. in the sub-clade representing the *Ca. reteaudii* complex (Sub-clade I) have been reported only from Australia, China, Indonesia and New Zealand (Crous 2002, Gadgil & Dick 2004, Crous *et al.* 2006, Lombard *et al.* 2010c). Another sub-clade of isolates that appears to have geographical structure resides in the *Ca. brassicae* complex (Sub-clade IV). Species in this sub-clade, with the exception of *Ca. orientalis*, have all been reported from South and Central America (Crous 2002, Crous *et al.* 2004b, Lombard *et al.* 2009). Isolates in other sub-clades appeared to have broad geographic distribution and not to occur in any defined part of the world.

Species residing in the Sphaero-Naviculate Group had no obvious patterns of pathogenicity, or distribution. This group consisted of two sub-clades in which only vesicle morphology was a consistent character. The majority of the species in the *Ca. kyotensis* complex (sub-clade XII) have been isolated from debris and soil (Crous *et al.* 2004b) but a few such as *Ca. kyotensis*, *Ca. ilicicola* and *Ca. pacifica* are important pathogens of agronomic and forestry crops (Crous 2002, Crous *et al.* 2004b). Members of this sub-clade also had a broad distribution with the majority reported from Asia (Crous *et al.* 2004b) and they included both heterothallic and homothallic species (Crous 2002, Crous *et al.* 2004b).

The second sub-clade in the Sphaero-Naviculate Group of isolates (sub-clade XIII) included three *Calonectria* spp., only two of which have morphological similarities. *Calonectria multiphialidica* is morphologically similar to the *Calonectria* spp. in sub-clade XII but there were no obvious patterns of distribution and pathogenicity for this group.

The intention of this phylogenetic study was to include all *Calonectria* spp. recognised to date. *Calonectria curvata* and *Ca. hederae* were, however, not included because there are no cultures for them as has previously been mentioned by Crous (2002). Furthermore, *Ca. rajasthanensis*, *Cy. avesiculatum* var. *microsporum*, *Cy. bambusae*, *Cy. couratarii*, *Cy. crataegi*, *Cy. intermedium* and *Cy. musae* were not included due either to the fact that they have not been validly described or not recognised as true species of *Calonectria* (Crous 2002). Based on the results of this study, 68 *Calonectria* spp. are recognised as valid and cultures are available for 66 of them.

The teleomorph state has not been seen for several species of *Calonectria*. Nonetheless C*ylindrocladium* spp., irrespective of whether their perithecial states are known or not, have been provided names in *Calonectria.* This is consistent with the view that for all newly described pleomorphic fungal species, the teleomorph name or the oldest typified name takes precedence over the anamorph or more recent name when both types belong to the same holomorph taxon (Hawksworth 2005, McNeill *et al.* 2005). It has already been established that *Calonectria* spp. have only *Cylindrocladium* anamorphs (Rossman *et al.* 1999, Schoch *et al.* 2001b), with micro- and megaconidial states that have thus far not been named. The name *Calonectria* was typified in 1867 (Rossman 1979) whereas that of *Cylindrocladium* was typified in 1892 (Morgan 1892). Therefore *Calonectria* has preference above *Cylindrocladium* and should henceforth be used for all species irrespective of whether the perithecial state has been found.

KEYS

Both synoptic and dichotomous keys to species of *Calonectria* are presented. In the synoptic key, numbers grouped with each character refer to the species that are alphabetically arranged below:

1. *Ca. acicola* P.D. Gadgill & M.A. Dick
2. *Ca. angustata* (Crous & El-Gholl) L. Lombard, M.J. Wingf. & Crous
3. *Ca. asiatica* Crous & N.L. Hywel-Jones
4. *Ca. australiensis* (Crous & K.D. Hyde) L. Lombard, M.J. Wingf. & Crous
5. *Ca. avesiculata* T.S. Schub., El-Gholl, Alfieri & Schoult.
6. *Ca. brachiatica* L. Lombard, M.J. Wingf. & Crous
7. *Ca. brassicae* (Panwar & Borha) L. Lombard, M.J. Wingf. & Crous
8. *Ca. brasiliensis* (Peerally) L. Lombard, M.J. Wingf. & Crous
9. *Ca. canadensis* (J.C. Kang, Crous & C.L. Schoch) L. Lombard, M.J. Wingf. & Crous
10. *Ca. cerciana* L. Lombard, M.J. Wingf. & Crous
11. *Ca. chinensis* (Crous) L. Lombard, M.J. Wingf. & Crous
12. *Ca. citri* (H.S. Fawc. & Klotz) L. Lombard, M.J. Wingf. & Crous
13. *Ca. clavata* Alfieri, El-Gholl & E.L. Barnard
14. *Ca. colhounii* Peerally
15. *Ca. colombiana* L. Lombard, M.J. Wingf. & Crous
16. *Ca. colombiensis* Crous
17. *Ca. curvata* (Boedijn & Reitsma) L. Lombard, M.J. Wingf. & Crous
18. *Ca. curvispora* (Crous & D. Victor) L. Lombard, M.J. Wingf. & Crous
19. *Ca. densa* L. Lombard, M.J. Wingf. & Crous
20. *Ca. ecuadoriae* (Crous & M.J. Wingf.) L. Lombard, M.J. Wingf. & Crous
21. *Ca. eucalypti* L. Lombard, M.J. Wingf. & Crous
22. *Ca. gracilipes* Crous & G.R.A. Mchau
23. *Ca. gracilis* Crous, M.J. Wingf. & Alfenas
24. *Ca. gordoniae* (Leahy, T.S. Schub. & El-Gholl) L. Lombard, M.J. Wingf. & Crous
25. *Ca. hawksworthii* (Peerally) L. Lombard, M.J. Wingf. & Crous
26. *Ca. hederae* C. Booth & J.S. Murray
27. *Ca. hongkongensis* Crous

28. *Ca. humicola* L. Lombard, M.J. Wingf. & Crous
29. *Ca. hurae* (Linder & Whetzel) L. Lombard, M.J. Wingf. & Crous
30. *Ca. ilicicola* Boedijn & Reitsma
31. *Ca. indonesiae* (Crous) L. Lombard, M.J. Wingf. & Crous
32. *Ca. indusiata* (Seaver) Crous
33. *Ca. insularis* C.L. Schoch & Crous
34. *Ca. kyotensis* Tersh.
35. *Ca. leguminum* (Rehm) Crous
36. *Ca. leucothoës* (El-Gholl, Leahy & T.S. Schub.) L. Lombard, M.J. Wingf. & Crous
37. *Ca. macroconidialis* (Crous, M.J. Wingf. & Alfenas) Crous
38. *Ca. madagascariensis* Crous
39. *Ca. malesiana* (Crous) L. Lombard, M.J. Wingf. & Crous
40. *Ca. mexicana* C.L. Schoch & Crous
41. *Ca. morganii* Crous, Alfenas & M.J. Wingf.
42. *Ca. multiphialidica* (Crous, Simoneau & Risède) L. Lombard, M.J. Wingf. & Crous
43. *Ca. multiseptata* Crous & M.J. Wingf.
44. *Ca. naviculata* Crous & M.J. Wingf.
45. *Ca. orientalis* L. Lombard, M.J. Wingf. & Crous
46. *Ca. ovata* D. Victor & Crous
47. *Ca. pacifica* (J.C. Kang, Crous & C.L. Schoch) L. Lombard, M.J. Wingf. & Crous
48. *Ca. pauciramosa* C.L. Schoch & Crous
49. *Ca. penicilliodes* (Tubaki) L. Lombard, M.J. Wingf. & Crous
50. *Ca. pini* L. Lombard, M.J. Wingf. & Crous
51. *Ca. polizzii* L. Lombard, M.J. Wingf. & Crous
52. *Ca. pseudonaviculata* (Crous, J.Z. Groenew. & C.F. Hill) L. Lombard, M.J. Wingf. & Crous
53. *Ca. pseudoreteaudii* L. Lombard, M.J. Wingf. & Crous
54. *Ca pseudoscoparia* L. Lombard, M.J. Wingf. & Crous
55. *Ca. pseudospathiphylli* J.C. Kang, Crous & C.L. Schoch
56. *Ca. pteridis* Crous, M.J. Wingf. & Alfenas
57. *Ca. pyrochoa* (Desm.) Sacc.
58. *Ca. queenslandica* L. Lombard, M.J. Wingf. & Crous
59. *Ca. reteaudii* (Bugn.) C. Booth
60. *Ca. rumohrae* El-Gholl & Alfenas
61. *Ca. scoparia* Peerally
62. *Ca. spathiphylli* El-Gholl, J.Y. Uchida, Alfenas, T.S. Schub., Alfieri & A.R. Chase
63. *Ca. spathulata* El-Gholl, Kimbr., E.L. Barnard, Alfieri & Schoult.
64. *Ca. sulawesiensis* L. Lombard, M.J. Wingf. & Crous
65. *Ca. sumatrensis* (Crous) L. Lombard, M.J. Wingf. & Crous
66. *Ca. terrae-reginae* L. Lombard, M.J. Wingf. & Crous
67. *Ca. variabilis* Crous, B.J.H. Janse, D. Victor, G.F. Marias & Alfenas
68. *Ca. zuluensis* L. Lombard, M.J. Wingf. & Crous

Synoptic key to *Calonectria* species

1. Teleomorph:
    a. Teleomorph state known
        1, 3, 5, 13, 14, 15, 16, 21, 22, 23, 26, 27, 29, 30, 31, 32, 33, 34, 35, 37, 38, 40, 41, 43, 44, 46, 48, 55, 56, 57, 59, 60, 61, 62, 63, 67, 68
    b. Teleomorph state unknown
        2, 4, 6, 7, 8, 9, 10, 11, 12, 17, 18, 19, 20, 24, 25, 28, 36, 39, 42, 45, 47, 49, 50, 51, 52, 53, 54, 58, 64, 65, 66

2. Ascocarps:
    a. Red-brown to red in colour, changing to dark-red in 3 % KOH
        1, 23, 44, 56, 61, 67
    b. Orange to red in colour, changing to dark-red in 3 % KOH
        3, 5, 15, 16, 22, 26, 30, 32, 33, 34, 40, 43, 55, 62, 68
    c. Orange to red-brown in colour, changing to dark-red in 3 % KOH
        13, 27, 35, 46, 48, 57, 59, 60, 63
    d. Yellow to orange in colour, only base and stroma changing to dark-red in 3 % KOH
        14, 21, 37, 38, 41

3. Asci:
    a. 8-spored and clavate
        1, 3, 5, 13, 15, 16, 22, 23, 26, 27, 30, 32, 33, 34, 35, 38, 40, 41, 43, 44, 46, 48, 55, 56, 57, 59, 60, 61, 62, 63, 67, 68
    b. 4-spored and clavate
        14, 21, 37

4. Ascospore septation:
    a. 1-septate
        3, 15, 16, 22, 23, 27, 33, 34, 40, 41, 48, 61, 68
    b. (1–)3-septate
        5, 13, 14, 21, 26, 30, 32, 35, 37, 38, 44, 46, 55, 56, 57, 59, 62, 63, 67
    c. (3–)4-septate
        1
    d. (1–)3–6(–9) septate
        43, 60

5. Ascospore width (av. in µm)
    a. 4–5
        15, 16, 22, 34, 44, 62, 67, 68
    b. 5.5–6
        1, 3, 5, 13, 14, 21, 26, 27, 30, 33, 37, 38, 40, 41, 46, 55, 56, 57, 59, 61, 63
    c. 6.5–7
        22, 32, 35, 43, 48, 60

6. Ascospore length (av. in µm)
    a. 30–39
        3, 15, 16, 21, 22, 23, 27, 33, 34, 41, 48, 68
    b. 40–49
        5, 13, 30, 44, 55, 57, 61, 62, 67
    c. 50–59
        14, 26, 32, 37, 38, 40, 56, 63
    d. 60–69
        46
    e. 70 and above
        1, 35, 43, 59, 60

7. Stipe length (av. in µm)
    a. 40–100
        1, 5, 6, 9, 10, 16, 18, 20, 21, 27, 30, 31, 33, 34, 36, 38, 40, 44, 47, 48, 49, 50, 57, 58, 61, 63, 65, 66, 68
    b. 101–150
        4, 7, 11, 13, 15, 24, 32, 41, 42, 51, 53, 54, 60, 62, 64,
    c. 151–200
        2, 3, 12, 14, 19, 22, 23, 28, 29, 35, 39, 45, 46, 52, 56, 67
    d. above 200
        25, 26, 37, 55, 59

8 Stipe extension length (av. in µm)
    a. Less than 100
        1
    b. 100–200
        9, 11, 12, 15, 16, 18, 19, 25, 27, 28, 31, 34, 39, 41, 44, 51, 52, 57, 58, 68
    c. 201–300
        2, 3, 10, 13, 14, 21, 22, 24, 26, 30, 33, 35, 36, 40, 45, 46, 47, 48, 50, 54, 55, 56, 61, 62, 63, 64, 65, 66, 67
    d. Above 300
        4, 5, 6, 7, 20, 23, 29, 32, 37, 38, 42, 53, 59, 60

9. Vesicle shape
    a. Avesiculate to clavate
        5
    b. Clavate
        1, 2, 4, 6, 7, 13, 14, 20, 21, 22, 23, 24, 29, 32, 35, 37, 38, 43, 45, 50, 53, 56, 58, 59, 60, 64, 66
    c. Ellipsoidal to pyriform to obovoid
        8, 12, 25, 26, 41, 55, 61, 63

d. Ellipsoidal to ovoid
   19, 46
e. Ellipsoidal to obpyriform
   10, 15, 33, 36, 40, 48, 51, 54, 57, 68
f. Sphaeropedunculate
   3, 9, 11, 16, 17, 18, 19, 27, 30, 31, 34, 39, 42, 47, (49), 64, 67
g. Globose
   19, 28, 62
h. Naviculate
   44, 52

10. Shape of phialides on macroconidiophore
    a. Reniform to doliiform
       3, 6, 7, 8, 9, 10, 12, 15, 17, 19, 20, 21, 22, 23, 24, 25, 26, 33, 34, 36, 40, 41, 44, 45, 46, 48, 49, 50, 51, 52, 54, 57, 61, 63, 64, 68
    b. Elongate reniform to doliiform
       5, 11, 13, 14, 16, 18, 27, 28, 30, 31, 39, 42, 47, 55, 56, 62, 65, 67
    c. Cylindrical to allantoid
       1, 2, 4, 29, 32, 35, 37, 38, 53, 58, 59, 60, 66

11. Number of fertile branches on macroconidiophore
    a. 1–3
       1, 5, 8, 9, 11, 12, 17, 18, 28, 30, 46, 48, 49, 50, 51, 52, 53, 57, 58, 60, 63, 66, 67, 68
    b. 4–6
       2, 3, 4, 6, 7, 14, 16, 19, 21, 24, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 44, 45, 46, 47, 54, 55, 56, 59, 61, 62, 64, 65
    c. More than 6
       20, 27, 42

12. Microconidia
    a. Microconidia absent
       2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25, 26, 27, 28, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 44, 45, 47, 48, 49, 50, 51, 52, 54, 55, 57, 58, 61, 63, 64, 65, 66, 68
    b. Microconidia present
       1, 13, 24, 29, 30, 43, 46, 53, 56, 59, 60, 62, 67

13. Microconidial septation
    a. 1-septate
       13, 29, 30, 46, 56, 62, 67
    b. 1(–3)-septate
       24, 59, 60
    c. 1–3-septate
       1, 43, 53

14. Microconidial width (mean in µm)
    a. Up to 3
       13, 29, 43, 46, 56, 59
    b. Up to 4
       24, 53, 62, 67
    c. Up to 5
       1, 30, 60

15. Microconidial length (mean in µm)
    a. Below 20
       29
    b. 20–30
       1, 30, 46, 56, 59, 60, 67
    c. 31–40
       13, 24, 62
    d. above 40
       43, 53

16. Macroconidial septation
    a. 1-septate
       3, 6, 7, 8, 9, 10, 11, 12, 15, 17, 19, 22, 25, 27, 28, 31, 33, 34, 39, 40, 41, 42, 44, 45, 47, 48, 50, 51, 52, 54, 61, 64, 65, 68
    b. 1(–3)-septate
       5, 13, 16, 18, 20, 23, 24, 36, 46, 53, 55, 56, 62
    c. (1–)3-septate
       4, 14, 21, 30, 32, 38, 49, 57,
    d. (1–)3(–6)-septate
       26, 37, 58, 66
    e. (1–)5(–6)-septate
       1, 26, 35, 59, 60
    f. (1–)7(–8)-septate
       29
    g. More than 8-septate
       2
17. Macroconidial width (av. in µm)
    a. 3–4
       8, 9, 11, 12, 15, 17, 25, 27, 31, 33, 34, 39, 40, 41, 44, 45, 51, 54, 55, 63, 64, 68
    b. 4.5–5
       3, 5, 6, 7, 10, 13, 14, 16, 18, 20, 22, 23, 24, 28, 35, 36, 38, 42, 46, 47, 48, 49, 50, 52, 61, 65, 67
    c. 5.5–6
       19, 21, 26, 30, 32, 56, 57, 58, 62, 66
    d. 6.5–7
       1, 4, 37, 59
    e. above 7
       2, 29, 53, 60

18. Macroconidial length (av. in µm)
    a. Less than 40
       8, 15, 51, 68
    b. 40–46
       6, 10, 11, 17, 22, 30, 33, 34, 40, 41, 44, 50
    c. 47–55
       3, 7, 9, 14, 16, 19, 20, 27, 28, 31, 38, 39, 42, 45, 47, 48, 49, 52, 54, 55, 63, 64
    d. 56–66
       4, 5, 12, 13, 18, 23, 24, 25, 26, 35, 57, 61, 65
    e. 67–75
       1, 21, 36, 46, 58, 62, 67
    f. 76–95
       32, 37, 56, 59, 66
    g. above 95
       29, 53, 60

Dichotomous key to *Calonectria* species

The following key is an adaptation of the key provided by Crous (2002) to include all *Calonectria* spp. described subsequent to 2002. Measurements and observations are those of Crous (2002) and other authors who have described species subsequent to 2002 (Table 1). Only average conidial dimensions, where available, and a few distinguishing characters are presented in the key. Complete descriptions should be consulted to determine species variations. *Calonectria penicilloides* has been omitted from the keys, due to the fact that there is little morphological information available for this species.

1. Stipe extensions thick-walled; acicular to clavate vesicles 2
1. Stipe extensions and vesicles not as above 28

2. Stipe extensions thick-walled, terminating in acicular to clavate vesicles; fertile branches –3; phialides elongate-doliiform to reniform; macroconidia 1(–3)-septate, 64 × 5 µm; perithecia orange to red; ascospores 1(–3)-septate, 40 × 6 µm ..... ***Ca. avesiculata***
2. Stipe extensions not thick-walled and vesicles clavate 3

3. Teleomorph state unknown 4
3. Teleomorph state known 15

4. Macroconidia 1-septate only 5
4. Macroconidia more than 1-septate 8

5. Fertile branches –3; phialides doliiform to reniform; macroconidia 1-septate, 44 × 5 µm; stipe extensions terminating in clavate vesicles ..... ***Ca. pini***
5. Fertile branches –5 6

6. Lateral stipe extensions present; macroconidia 1(–2)-septate, 44 × 5 µm; stipe extensions terminating in clavate vesicles; fertile branches –5; phialides doliiform to reniform ..... ***Ca. brachiatica***
6. Lateral stipe extensions absent 7

7. Stipe extensions terminating in clavate vesicles; fertile branches –5; phialides doliiform to reniform; macroconidia 1-septate, 53 × 4.5 µm ..... ***Ca. brassicae***
7. Stipe extensions terminating in clavate vesicles; fertile branches –5; phialides doliiform to reniform; macroconidia 1-septate, 48 × 4 µm ..... ***Ca. orientalis***

8. Macroconidia longer than 100 µm 9
8. Macroconidia shorter than 100 µm 10

9. Macroconidia 5–8-septate, 104 × 8 µm; stipe extension terminate in clavate vesicles; fertile branches –3; phialides cylindrical to allantoid; microconidiophores lacking stipe extension; microconidia 1–3-septate, 44 × 4 µm ..... ***Ca. pseudoreteaudii***
9. Macroconidia 1–3-septate 12

10. Macroconidia (1–)3-septate, 63 × 6.5 µm; stipe extensions terminating in clavate vesicles; fertile branches –6; phialides cylindrical to allantoid ..... ***Ca. australiensis***
10. Macroconidia 1(–3)-septate 11

11. Fertile branches –7; phialides doliiform to reniform; macroconidia 51 × 4.5 µm; stipe extensions terminating in clavate vesicles ..... ***Ca. ecuadoriae***
11. Fertile branches –4; phialides doliiform to reniform; macroconidia 62 × 5 µm; stipe extensions terminating in clavate vesicles ..... ***Ca. gordoniae***

12. Macroconidia longer than 100 µm with more than 6 septa 13
12. Macroconidia shorter than 100 µm with 6 or less septa 14

13. Stipe extensions terminating in narrowly clavate vesicles; fertile branches –4; phialides cylindrical; macroconidia (1–)7–10(–12)-septate with slight swelling in the middle, 110 × 10 µm; Mega- and microconidia absent ..... ***Ca. angustata***
13. Stipe extensions terminating in narrowly clavate vesicles; fertile branches –3; phialides cylindrical; microconidia present, 1-septate, 18 × 3 µm; macroconidia (1–)7(–8)-septate, 120 × 7.5 µm; megaconidia present, 9–16-septate, bent or curved, (150–)200–250(–270) × 6–7(–8) µm ..... ***Ca. hurae***

14. Stipe extensions terminating in narrowly clavate vesicles; fertile branches –3; phialides cylindrical to allantoid, obpyriform when carried singly; macroconidia 4–6-septate, 69 × 6 µm ..... ***Ca. queenslandica***
14. Stipe extensions terminating in a narrowly clavate vesicles; fertile branches –3; phialides cylindrical to allantoid, obpyriform when carried singly; macroconidia 4–6-septate, 76 × 6 µm ..... ***Ca. terrae-reginae***

15. Macroconidial state unknown; megaconidiophores with stipe extensions terminating in clavate vesicles when present; megaconidia 6–10-septate, boomerang-shaped or curved, (120–)150–170(–220) × 8–9 µm; microconidia 1–3-septate, straight or curved, 20–65 × 2.5–3.5 µm ..... ***Ca. multiseptata***
15. Macroconidial state known 16

16. Teleomorph state known and macroconidia 1-septate to 1(–3)-septate 17
16. Teleomorph state known and macroconidia multi-septate 20

17. Teleomorph homothallic 18
17. Teleomorph heterothallic 19

18. Perithecia orange with a red apex; ascospores 1-septate, 35 × 6.5 µm; stipe extensions terminating in clavate vesicles; fertile branches –4; phialides doliiform to reniform; macroconidia 1-septate, 45 × 4.5 µm ..... ***Ca. gracilipes***

18. Perithecia red; ascospores 1-septate, 37 × 5 µm; stipe extensions terminating in narrowly clavate vesicles; fertile branches –4; phialides doliiform to reniform; macroconidia 1(–3)-septate, 56 × 4.5 µm ..... ***Ca. gracilis***

19. Perithecia orange; ascospores 1(–3)-septate, 44 × 5.5 µm; stipe extensions terminating in narrowly clavate vesicles; fertile branches –4; phialides elongate-doliiform to reniform; macroconidia 1(–3)-septate, 65 × 5 µm; microconidia 1-septate, 32 × 3 µm ..... ***Ca. clavata***

19. Perithecia red-brown; ascospores 1(–3)-septate, 52 × 6 µm; stipe extensions terminating in clavate to narrowly ellipsoidal vesicles; fertile branches –5; phialides elongate-doliiform to reniform; macroconidia 1(–3)-septate, 82 × 5.5 µm; microconidia 1-septate, 30 × 3.5 µm ..... ***Ca. pteridis***

20. Macroconidia 3-septate 21

20. Macroconidia 3- to multi-septate 25

21. Perithecia yellow to orange 22

21. Perithecia yellow 23

22. Teleomorph state homothallic; perithecia yellow to orange; ascospores (1–)3-septate, 33 × 6 µm; stipe extensions terminating in broadly clavate vesicles; fertile branches –5; phialides doliiform to reniform; macroconidia 3-septate, 72 × 6 µm ..... ***Ca. eucalypti***

22. Teleomorph state homothallic; perithecia orange to red; ascospores (1–)3-septate, 53 × 7 µm; stipe extensions terminating in narrowly clavate vesicles; fertile branches –5; phialides allantoid to reniform; macroconidia (1–)3-septate, 81 × 6 µm; megaconidia 7–9(–14)-septate, boomerang-shaped to curved, 130–200 × 5–6 µm ..... ***Ca. indusiata***

23. Macroconidia and ascospores shorter than 65 µm; teleomorph state homothallic; perithecia bright yellow; ascospores (1–)3-septate, 50 × 5.5 µm; stipe extensions terminating in clavate vesicles; fertile branches –5; phialides allantoid to cylindrical; macroconidia (1–)3-septate, 55 × 4.5 µm ..... ***Ca. madagascariensis***

23. Macroconidia and ascospores longer than 65 µm 24

24. Teleomorph state homothallic; perithecia bright yellow; ascospores (1–)3-septate, 55 × 6 µm; stipe extensions terminating in clavate vesicles; fertile branches –5; phialides elongate-doliiform to reniform; macroconidia (1–)3-septate, 65 × 5 µm ..... ***Ca. colhounii***

24. Teleomorph state heterothallic; perithecia dirty yellow, ascospores (1–)3-septate, 55 × 6 µm; stipe extensions terminating in clavate vesicles; fertile branches –5; phialides allantoid to cylindrical; macroconidia (1–)3(–4)-septate, 90 × 6.5 µm ..... ***Ca. macroconidialis***

25. Macroconidiophore branches –2 or less 26

25. Macroconidiophore with more than 2 series of branches 27

26. Teleomorph state homothallic; perithecia orange-brown; ascospores 3–6(–9)-septate, 90 × 6.5 µm; stipe extensions terminating in narrowly clavate vesicles; fertile branches –2; phialides cylindrical; microconidia 1(–3)-septate, (8–)15–30(–50) × 3–5 µm; macroconidia 5(–7)-septate, 110 × 9 µm; megaconidia 7–13-septate, bent or curved, (120–)180–230 × (8–)10–11(–13) µm ..... ***Ca. rumohrae***

26. Teleomorph state homothallic; perithecia red to red-brown; ascospores 3–4-septate, 70 × 6 µm; stipe extensions, when present, terminating in narrowly clavate vesicles; fertile branches –1; macroconidia 5–7-septate, 75 × 7 µm; microconidia 1–3-septate, 10–30 × 3–5 µm ..... ***Ca. acicola***

27. Teleomorph state homothallic; perithecia orange to red-brown; ascospores (1–)3-septate, 70 × 6.5 µm; stipe extensions terminating in narrowly clavate vesicles; fertile branches –6; phialides cylindrical to allantoid; macroconidia (1–)3–5(–6)-septate, 60 × 5 µm ..... ***Ca. leguminum***

27. Teleomorph state heterothallic; perithecia orange to red-brown; ascospores (1–)5(–6)-septate, 70 × 5.5 µm; stipe extensions terminating in clavate vesicles; fertile branches –6; phialides cylindrical to allantoid; macroconidia (1–)5(–6)-septate, 84 × 6.5 µm; microconidia 1(–3)-septate, 30 × 3 µm ..... ***Ca. reteaudii***

28. Vesicles sphaeropedunculate, globose or ovoid 29

28. Vesicles not as above 48

29. Vesicles consistently ovate; teleomorph state heterothallic; perithecia orange; ascocpores 1–3(–7)-septate, 60 × 5.5 µm; fertile branches –3; phialides doliiform to reniform; macroconidia straight or curved, 1(–3)-septate, 70 × 5 µm; microconidia 1-septate, 21 × 3 µm ..... ***Ca. ovata***

29. Vesicles not consistently ovate 30

30. Macroconidia 1(–3)-septate 31
30. Macroconidia only 1-septate 36

31. Teleomorph state unknown; stipe extensions terminating in sphaeropedunculate vesicles; fertile branches –3; phialides elongate-doliiform to reniform; macroconidia 1(–3)-septate, 60 × 5 µm ..... ***Ca. curvispora***
31. Teleomorph state known 32

32. Perithecia red-brown; teleomorph state homothallic; ascospores 1(–3)-septate, 42 × 5 µm; stipe extensions terminating in sphaeropedunculate to ovoid or ellipsoidal to clavate vesicles; fertile branches –3; phialides elongate-doliiform to reniform; macroconidia (1–)3(–4)-septate, 73 × 5 µm; microconidia 1-septate, 27 × 4 µm ..... ***Ca. variabilis***
32. Perithecia orange to red 33

33. Teleomorph state heterothallic; perithecia orange to red; ascospores 1(–3)-septate, 45 × 5 µm; stipe extensions terminating in globoid or ellipsoid to obpyriform vesicles; fertile branches –5; phialides elongate-doliiform to reniform; macroconidia 1(–3)-septate, 70 × 6 µm; microconidia 1-septate, 39 × 4 µm ..... ***Ca. spathiphylli***
33. Teleomorph state homothallic 34

34. Lateral stipe extensions abundant; perithecia orange; ascospores 1-septate, 33 × 5 µm; stipe extensions terminating in sphaeropedunculate vesicles; fertile branches –5; phialides elongate-doliiform to reniform; macroconidia 1(–3)-septate, 53 × 4.5 µm ..... ***Ca. colombiensis***
34. Lateral stipe extensions absent 35

35. Ascospores 1(–3)-septate, 42 × 5.5 µm; stipe extensions terminating in sphaeropedunculate to ellipsoidal vesicles; fertile branches –4; phialides elongate-doliiform to reniform; macroconidia 1(–3)-septate, 52 × 4 µm ..... ***Ca. pseudospathiphylli***
35. Ascospores 1(–3)-septate, 45 × 6 µm; stipe extensions terminating in sphaeropedunculate vesicles; fertile branches –3; phialides elongate-doliiform to reniform; macroconidia (1–)3-septate, 62 × 6 µm; microconidia 1-septate, 30 × 4.5 µm ..... ***Ca. ilicicola***

36. Stipe thick-walled; teleomorph state unknown; stipe extensions terminating in clavate to sphaeropedunculate vesicles; fertile branches –8; phialides elongate-doliiform to reniform; macroconidia 1-septate, 53 × 4.5 µm ..... ***Ca. multiphialidica***
36. Stipe thin-walled 37

37. Teleomorph state known 38
37. Teleomorph state unknown 40

38. Macroconidiophore branches –8; perithecia orange; teleomorph state homothallic; perithecia orange; ascospores 1-septate, 31 × 6 µm; stipe extensions terminating in sphaeropedunculate vesicles; phialides elongate-doliiform to reniform; macroconidia 1-septate, 46.5 × 4 µm ..... ***Ca. hongkongensis***
38. Macroconidiophore branches –5 39

39. Teleomorph state homothallic; perithecia orange; ascospores 1-septate, 33 × 6 µm; stipe extensions terminating in sphaeropedunculate vesicles, lateral stipe extensions abundant; phialides doliiform to reniform; macroconidia 1-septate, 53 × 5 µm ..... ***Ca. asiatica***
39. Teleomorph state homothallic; perithecia orange to red; ascospores 1-septate, 35 × 5 µm; stipe extensions terminating in sphaeropedunculate vesicles, lateral stipe extensions abundant; phialides doliiform to reniform; macroconidia 1-septate, 40 × 3.5 µm ..... ***Ca. kyotensis***

40. Lateral stipe extensions absent 41
40. Lateral stipe extensions present 43

41. Macroconidia curved, 1-septate, 40–46 × 3–4 µm; stipe extensions terminating in sphaeropedunculate vesicles; fertile branches –2 ..... ***Ca. curvata***
41. Macroconidia straight 42

42. Stipe extensions terminating in globose to ovoid to sphaeropedunculate vesicles; fertile branches –3; phialides doliiform to reniform; macroconidia 1-septate, 51 × 5 µm ..... ***Ca. humicola***
42. Stipe extensions terminating in sphaeropedunculate vesicles; fertile branches –5; phialides elongate-doliiform to reniform; macroconidia 1-septate, 50.5 × 4 µm ..... ***Ca. indonesiae***

43. Lateral stipe extensions rare; stipe extensions terminating in pyriform to sphaeropedunculate vesicles; fertile branches – 3; phialides doliiform to reniform; macroconidia 1-septate, 50 × 4 µm ..... ***Ca. canadensis***
43. Lateral stipe extensions abundant 44

44. Macroconidiophore branches 4–6 45
44. Macroconidiophore branches –3 46

45. Macroconidiophore branches –4; stipe extension terminating in globose to ovoid to sphaeropendunculate vesicles; phialides doliiform to reniform; macroconidia 1-septate, 54 × 6 µm ..... ***Ca. densa***
45. Macroconidiophore branches –6; stipe extensions terminating in sphaerpendunculate vesicles; phialides elongate-doliiform to reniform; macroconidia 1-septate, 47.5 × 4 µm ..... ***Ca. malesiana***

46. Macroconidia 45 × 4 µm, 1-septate; stipe extensions terminating in sphaeropedunculate vesicles; phialides elongate-doliiform to reniform ..... ***Ca. chinensis***
46. Macroconidia longer than 45 µm 47

47. Stipe extensions terminating in sphaeropedunculate vesicles; phialides elongate-doliiform to reniform; macroconidia 1-septate, 55 × 4.5 µm ..... ***Ca. pacifica***
47. Stipe extensions terminating in sphaeropedunculate vesicles; phialides elongate-doliiform to reniform; macroconidia 1-septate, 58 × 5 µm ..... ***Ca. sumatrensis***

48. Vesicles pyriform to ellipsoidal or clavate, rarely ovoid, never obpyriform 49
48. Vesicles not as above 54

49. Macroconidia more than 1-septate 50
49. Macroconidia 1-septate 51

50. Teleomorph state unknown; stipe extensions terminating in narrowly ellipsoidal to pyriform or ovoid to sphaeropedunculate vesicles; fertile branches –3; phialides doliiform to reniform; macroconidia (1–)3-septate, 58 × 4 µm ..... ***Ca. citri***
50. Teleomorph state homothallic; perithecia orange-red; ascospores 1(–3)-septate, 33.5–69 × 4.5–7 µm; stipe extensions terminating in clavate to ovoid or ellipsoidal vesicles; fertile branches –4; phialides doliiform to reniform; macroconidia (1–)3(–5)-septate, (44–)50–70(–102) × 5–7(–8) µm ..... ***Ca. hederae***

51. Stipe extensions up to 200 µm long 52
51. Stipe extensions longer than 200 µm 53

52. Teleomorph state heterothallic; perithecia yellow to orange; ascospores 1-septate, 37 × 6 µm; stipe extensions terminating in ellipsoidal to pyriform or clavate vesicles; fertile branches –6; phialides doliiform to reniform; macroconidia 1-septate, 45 × 4 µm ..... ***Ca. morganii***
52. Teleomorph state unknown; stipe extensions terminating in oval to ellipsoidal to fusiform vesicles; fertile branches –3; phialides doliiform to reniform; macroconidia 1-septate, 38 × 3.5 µm ..... ***Ca. brasiliensis***

53. Macroconidia curved, 1-septate, 56 × 4 µm, stipe extensions terminating in ellipsoidal to clavate vesicles; fertile branches –4; phialides doliiform to reniform; teleomorph state unknown ..... ***Ca. hawksworthii***
53. Macroconidia straight, 1-septate, 48 × 4 µm Teleomorph state unknown; stipe extensions terminating in broadly clavate to ellipsoidal vesicles; fertile branches –5; phialides doliiform to reniform; ..... ***Ca. sulawesiensis***

54. Vesicles obpyriform to ellipsoidal 55
54. Vesicles naviculate 66

55. Macroconidia 1-septate 56
55. Macroconidia more than 1-septate 64

56. Macroconidiophore branches –3 57
56. Macroconidiophore branches 4–6 59

57. Teleomorph state homothallic; perithecia orange to red; ascospores 1-septate, 32 × 4 µm; stipe extensions terminating in broadly clavate to obpyriform vesicles; phialides doliiform to reniform; macroconidia 1-septate, 36 × 4 µm ..... ***Ca. zuluensis***
57. Teleomorph state heterothallic 58

58. Perithecia orange to red-brown; ascospores 1-septate, 35 × 6.5 µm; stipe extensions terminating in obpyriform to ellipsoidal vesicles; phialides doliiform to reniform; macroconidia 1-septate, 50 × 4.5 µm ..... ***Ca. pauciramosa***
58. Teleomorph state unknown; stipe extensions terminating in broadly clavate to obpyriform vesicles; phialides doliiform to reniform; macroconidia 1-septate, 37 × 4 µm ..... ***Ca. polizzii***

59. Macroconidia up to 45 µm long 60
59. Macroconidia longer than 45 µm 63

60. Macroconidiophore branches –6; teleomorph state heterothallic; perithecia orange to red; ascospores 1-septate, 33 × 6 µm; stipe extensions terminating in obpyriform to broadly ellipsoidal vesicles; phialides doliiform to reniform; macroconidia 1-septate, 45 × 4 µm ..... ***Ca. insularis***
60. Macroconidiophore branches –4 61

61. Vesicles broadly ellipsoidal with a papillate apex; phialides doliiform to reniform; macroconidia 1-septate, 45 × 4 µm; teleomorph state heterothallic; perithecia orange to red; ascospores 1-septate, 50 × 5.5 µm ..... ***Ca. mexicana***
61. Vesicles fusiform to obpyriform 62

62. Teleomorph state homothallic; perithecia yellow to orange; ascospores 1-septate, 34 × 4 µm; phialides doliiform to reniform; macroconidia 1-septate, 37 × 3 µm ..... ***Ca. colombiana***
62. Teleomorph state unknown; phialides doliiform to reniform; macroconidia 1-septate, 44 × 5 µm ..... ***Ca. cerciana***

63. Teleomorph state heterothallic; perithecia red-brown; ascospores 1-septate, 48 × 5.5 µm; stipe extensions terminating in ellipsoidal to narrowly obpyriform vesicles; fertile branches –5; phialides doliiform to reniform; macroconidia 1-septate, 60 × 4.5 µm ..... ***Ca. scoparia***
63. Teleomorph state unknown; stipe extensions terminating in obpyriform to ellipsoidal vesicles; fertile branches –4; phialides doliiform to reniform; macroconidia 1-septate, 48 × 4 µm ..... ***Ca. pseudoscoparia***

64. Macroconidiophore branches –6; stipe extensions terminating in ellipsoidal to obpyriform vesicles; phialides cylindrical, straight or doliiform to reniform; macroconidia 1(–3)-septate, 73 × 5 µm ..... ***Ca. leucothoës***
64. Macroconidiophore branches –3 65

65. Teleomorph state homothallic; perithecia orange to red-brown; ascospores 1(–3)-septate, 50 × 5.5 µm; stipe extensions terminating in obpyriform to broadly ellipsoidal vesicles; phialides doliiform to reniform; macroconidia (1–)3-septate, 50–70 × 5–6 µm ..... ***Ca. pyrochoa***
65. Teleomorph state homothallic; perithecia orange; ascospores (1–)3-septate, 50 × 5.5 µm; stipe extensions terminating in ellipsoidal to obpyriform or clavate vesicles; phialides cylindrical, straight or doliiform to reniform; macroconidia (1–)3(–6)-septate, 55 × 4 µm ..... ***Ca. spathulata***

66. Teleomorph state heterothallic; perithecia red-brown; ascospores 1(–3)-septate, 40 × 5 µm; fertile branches –5; phialides doliiform to reniform; macroconidia 1-septate, 45 × 3 µm ..... ***Ca. naviculata***
66. Teleomorph state unknown; fertile branches –3; phialides doliiform to reniform; macroconidia 1-septate, 42–68 × 4–6 µm ..... ***Ca. pseudonaviculata***

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