Chapter 5

Phylogeny and systematics of the genus *Calonectria*

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 this 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. orientalis*, 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 morphological features. Dichotomous and synoptic keys to all *Calonectria* spp. currently recognised are also provided.

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 done 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.) anamorph states (Rossman 1993, Crous 2002). 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 state 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).

Species of *Calonectria* are primarily distinguished based anamorph characters, such as 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). Although the reliability of vesicle shape as a distinguishing morphological character has been questioned (Sober & Alfieri 1972, Hunter & Barnett 1978, Rossman 1983), 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 state, 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 microcondia 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).

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.* 2009a).

Several molecular approaches have been 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, 1997, Crous *et al.* 1998a), random amplification of polymorphic DNA (RAPD) (Overmeyer *et al.* 1996, Victor *et al.* 1997, Schoch *et al.* 2000a, Riséde & 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; Riséde & Simoneau 2001) and DNA hybridization (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, Riséde & 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). 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 *Medicago sativa* seed, 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), 10× 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 a 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. 2009a). Controls were of isolates crossed with themselves, 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 CBS 125523 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. 2009a) 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. 2009b). 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) 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 test (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, CBS 125274, CBS 125275 and CBS 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, CBS 125259 and CBS 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, CBS 125259 and CBS 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, isolates CBS 125254, CBS 125255, CBS 125256 and CBS 125257 from Ecuador, clustered closely but separately from *Ca. scoparia* (CMW 31000 and CMW 31001) and other species in the *Ca. pauciramosa* complex.
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 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, CBS 125250, CBS 125251, CBS 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 because they add little taxonomic value. However, all ITS and LSU sequences generated in this study have been deposited in GenBank and TreeBase as SN4777 (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 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, CBS 125274, CBS 125275 and CBS 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 unknown *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 (Hawksworth 2005), the teleomorph name takes precedence over the anamorph name when both types belong to the same holomorph. Subsequently, the species below are described as new species in *Calonectria*, which represents the older generic name for these holomorphs. All *Cylindrocladium* species without a *Calonectria* state, are subsequently also transferred to *Calonectria*. 
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 and that are absent from the most closely related 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 a globose to ovoid to sphaeropedunculate vesicle, 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.


Culture characteristics: Colonies fast growing with optimal growth temperature at 25 ºC (growth at 15–35 ºC) on MEA, reverse umber (15m) to verona-brown (13”k) after 7 d; moderate white aerial mycelium with moderate sporulation; chlamydomspores 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 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 penicillate suites of 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 a broadly clavate vesicle, 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 doliform 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. *Mega- and microconidia* not seen.


*Culture characteristics:* Colonies fast growing with optimal growth temperature at 25 ºC (growth at 15–30 ºC) on MEA, reverse colour tawny brown (13’i) 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. 5) 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 a globose to ovoid to sphaeropedunculate vesicle, 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 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.


Culture characteristics: Colonies fast growing with optimal growth temperature at 25 ºC (growth at 15–35 ºC) on MEA, reverse umber (15m) to verona-brown (13”k) after 7 d; moderate white aerial mycelium with moderate sporulation; chlamydoospores 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 Cy. candelabro similis sed 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 a fusiform to pyriform or broadly clavate vesicle, 8–12 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.

*Specimen 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 (13i) 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. However, morphological comparisons showed that it is most similar to species in the Ca. scoparia and Ca. morganii complexes with stipe extensions terminating in fusiform to pyriform or broadly clavate vesicles. 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.

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. brachiatiae 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 difftert

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 a clavate vesicle, 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.

**Culture characteristics:** Colonies fast growing with optimal growth temperature at 25 ºC (growth at 15–30 ºC) on MEA, reverse amber (13k) to sepia-brown (13i) 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.* 2009b). Macroconidia of *Ca. pini* (av. 44 × 5 µm) are shorter than those of *Ca. brassicae* (av. 53 × 4.5 µm) and *Ca. gracilis* (56 × 4.5 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.* (2009b), 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 periclinal incrassatis colloículo 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 differt

**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 a obpyriform to ellipsoidal vesicle, 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.


*Culture characteristics:* Colonies fast growing with optimal growth temperature at 25 ºC (growth at 10–30 ºC) on MEA, reverse amber (13k) to sepia-brown (13i) after 7 d; colony margins irregular with sparse to moderate white aerial mycelium with moderate sporulation; chlamydomspores 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 (Fig. 6) 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.
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 a broadly clavate to ellipsoidal vesicle, 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.

*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 °C (growth at 15–30 °C) on MEA, reverse amber (13k) to sepia-brown (13i) after 7 d; abundant white aerial mycelium with moderate to extensive sporulation; chlamydospores extensive throughout the medium, forming microsclerotia.

*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).
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.


**Calonectria australiensis** (Crous & K.D. Hyde) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515537.


**Calonectria canadensis** (J.C. Kang, Crous & C.L. Schoch) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515538.


**Calonectria chinensis** (Crous) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515539.


**Calonectria citri** (Boedjin & Reitsma) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515540.

*Basionym:* Cylindrocladium citri (H.S. Fawc. & Klotz) Boedjin & Reitsma, Reinwardtia 1: 57. 1950.


**Calonectria curvata** (Boedjin & Reitsma) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515541

*Basionym:* Cylindrocladium curvatum Boedjin & Reitsma, Reinwardtia 1: 54. 1950.

**Calonectria curvispora** (Crous & D. Victor) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515542

**Calonectria ecuadoriae** (Crous & M.J. Wingf.) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515543.


**Calonectria gordoniae** (Leahy, T.S. Schub. & El-Gholl) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515544.


**Calonectria hawksworthii** (Peerally) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515545.


**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, Taxonomy and pathology of *Cylindrocladium* (Calonectria) and allied genera: 185. 2002.


**Calonectria indonesiae** (Crous) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515547.


**Calonectria leucothoës** (El-Gholl, Leahy & T.S. Schub.) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515548.


**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, P. Simoneau & J.-M. Risède) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515550.


**Calonectria pacifica** (J.C. Kang, Crous & C.L. Schoch) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515551.


**Calonectria penicilloides** (Tubaki) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515552


**Calonectria pseudonaviculata** (Crous, J.Z. Groenew. & C.F. Hill) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515554


**Calonectria sumatrensis** (Crous) L. Lombard, M.J. Wingf. & Crous, **comb. nov.** MycoBank MB515555.


**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 E. grandis, adds a new species to 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. 2009b). Calonectria pini was isolated from P. 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. 2009b). In contrast, Ca. orientalis was isolated from soils collected in Indonesia and nothing is known regarding its pathogenicity. Phylogenetic inference and SNP 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. (2009b), 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 macroconidia 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 world-wide (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 and displaying 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. 2009c). 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. 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) but 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 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 (C. scoparium), respectively. 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, Gadgill & Dick 2004, Crous et al. 2006, Lombard et al. 2009c). 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. 2009b). 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 only 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 Cylindrocladium spp., irrespective of whether their perithecial states are known or not, have been provided names in Calonectria. This is consistent with the view that 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, Mcniell 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 takes 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:

2. *Ca. angustata* (Crous & El-Gholl) L. Lombard, M.J. Wingf. & Crous
3. *Ca. asiatica* Crous & N.L.Hywel-Jones
10. *Ca. cerciana* L. Lombard, M.J. Wingf. & Crous
11. *Ca. chinensis* (Crous) L. Lombard, M.J. Wingf. & Crous
14. *Ca. colhounii* Peerally
15. *Ca. colombiana* L. Lombard, M.J. Wingf., Crous
16. *Ca. colombiensis* Crous
17. *Ca. curvata* (Boedjin & Reitsma) L. Lombard, M.J. Wingf. & Crous
18. *Ca. curvispora* (Crous & D. Victor) L. Lombard, M.J. Wingf. & Crous
20. *Ca. ecuadoriae* (Crous & M.J. Wingf.) L. Lombard, M.J. Wingf. & Crous
22. *Ca. gracilipes* Crous & G.R.A. Mchau
23. *Ca. gracilis* Crous, M.J. Wingf. & Alfenas
26. *Ca. hederae* C. Booth & J.S. Murray
27. *Ca. hongkongensis* 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
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, P. Simoneau & J.-M. Risède) L. Lombard, M.J. Wingf. & Crous
43. *Ca. multiisptata* 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. polizzi* 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. pseudopathiphylli* 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


64. *Ca. sulawesiensis* L. Lombard, M.J. Wingf. & Crous

65. *Ca. sumatrensis* (Crous) L. Lombard, M.J. Wingf. & Crous


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, 50, 53, 56, 58, 59, 60, 64, 66
   c. Ellipsoidal to pyriform to obovoid
      8, 12, 25, 26, 41, 45, 55, 61, 63
   d. Ellipsoidal to ovoid
      46
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, 28, 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, 30, 31, 39, 42, 47, 55, 56, 62, 65, 67
   c. Cylindrical to allantoids
      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. Microconidia 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. Microconidia 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. Microconidia 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
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. Macroconidia 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. Macroconidia 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
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
2. Stipe extensions thick-walled, terminating in an acicular to clavate vesicle; 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*
3. Teleomorph state unknown ................................................................. 4
4. 56–66
   4, 5, 12, 13, 18, 23, 24, 25, 26, 35, 57, 61, 65
5. 67–75
   1, 21, 36, 46, 58, 62, 67
6. 76–95
   32, 37, 56, 59, 66
7. above 95
   29, 53, 60
4. Macroconidia 1-septate ............................................................................. 5
4. Macroconidia more than 1-septate ................................................................. 6

5. Stipe extensions terminating in a clavate vesicle; fertile branches –5; phialides
doliiform to reniform; macroconidia 1-septate, 53 × 4.5 µm .................. Ca. brassicae
5. Stipe extensions terminating in clavate vesicle; fertile branches –3; phialides
doliiform to reniform; macroconidia 1-septate, 44 × 5 µm ................. Ca. pini

6. Macroconidia 1(–2)-septate, 44 × 5 µm; stipe extensions terminating in a clavate
vesicle; lateral stipe extensions present; fertile branches –5; phialides doliiform to
reniform ........................................................................................................... Ca. brachiatica
6. Macroconidia 1(–3)-septate to multi-septate ................................................ 7

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

8. Macroconidia 1(–3)-septate, 104 × 8 µm; stipe extension terminate in clavate vesicle;
fertile branches –3; phialides cylindrical to allantoid; microconidiophores lacking
stipe extension; microconidia 1–3-septate, 44 × 4 µm ............... Ca. pseudoreteaudii
8. Macroconidia more than 1-septate ............................................................... 10

9. Macroconidia (1–)3-septate, 63 × 6.5 µm; stipe extensions terminating in clavate
vesicle; fertile branches –6; phialides cylindrical to allantoid ........ Ca. australiensis
9. Macroconidia 1(–3)-septate, 51 × 4.5 µm; stipe extensions terminating in clavate
vesicles; fertile branches –7; phialides doliiform to reniform ........ Ca. ecuadoriae

10. Macroconidia longer than 100 µm with more than 6 septa ....................... 11
10. Macroconidia shorter than 100 µm with 6 or less septa ........................... 12
11. 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

11. 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

12. 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

12. 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

13. 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

13. Macroconidial state known …………………………………………………………… 14

14. Teleomorph state known and macroconidia 1-septate to 1(–3)septate .......... 15

14. Teleomorph state known and macroconidia multi-septate ……………………… 18

15. Teleomorph homothallic …………………………………………………………….. 16

15. Teleomorph heterothallic …………………………………………………………….. 17

16. 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
16. 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

17. 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

17. 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

18. Macroconidia 3-septate ............................................................... 19

18. Macroconidia 3- to multi-septate ................................................ 23

19. Perithecia yellow to orange ............................................................ 20

19. Perithecia yellow ................................................................. 21

20. 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

20. Teleomorph state homothallic; perithecia orange to red; ascospores (1–)3-septate, 53 × 7 μm; stipe extensions terminating in narrowly clavate vesicle; 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

21. 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
21. Macroconidia and ascospores longer than 65 µm ................................. 22

22. 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

22. 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

23. Macroconidiophore branches –2 or less .................................................. 24

23. Macroconidiophore with more than 2 series of branches ...................... 25

24. 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

24. 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

25. 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

26. 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
27. Vesicles sphaeropedunculate, globose or ovoid ................................. 28
27. Vesicles not as above ........................................................................ 48

28. Vesicles consistently ovate; teleomorph state heterothallic; perithecia orange;
ascospores 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
28. Vesicles not consistently ovate .............................................................. 29

29. Macroconidia 1(–3)-septate ................................................................. 30
29. Macroconidia only 1-septate ............................................................... 35

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

31. 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
31. Perithecia orange to red ................................................................. 32

32. 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
32. Teleomorph state homothallic ......................................................... 33
33. 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*

33. Lateral stipe extensions absent ................................................................. 34

34. 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*

34. Ascospores 1(–3)-septate, 45 × 6 µm; stipe extensions terminating in sphaeropedunculate vesicle; fertile branches –3; phialides elongate-doliiform to reniform; macroconidia (1–)3-septate, 62 × 6 µm; microconidia 1-septate, 30 × 4.5 µm ........................................................................................................... *Ca. ilicicola*

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

35. Stipe thin-walled .......................................................................................... 36

36. Teleomorph state known ............................................................................... 37

36. Teleomorph state unknown .......................................................................... 39

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

37. Macroconidiophore branches –5 ................................................................. 38

38. 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

38. 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

39. Lateral stipe extensions absent ........................................  40
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40. Macroconidia curved, 1-septate, 40–46 × 3–4 µm; stipe extensions terminating in sphaeropedunculate vesicle; fertile branches –2  ..................................  Ca. curvata

40. Macroconidia straight .......................................................  41

41. Stipe extensions terminating in globose to ovoid to sphaeropedunculate vesicle; fertile branches –3; phialides doliiform to reniform; macroconidia 1-septate, 51 × 5 µm  

.................................................................  Ca. humicola

41. Stipe extensions terminating in sphaeropedunculate vesicles; fertile branches –5; phialides elongate-doliiform to reniform; macroconidia 1-septate, 50.5 × 4 µm  

.................................................................  Ca. indonesiae

42. 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

42. Lateral stipe extensions abundant ........................................  43

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

44. Macroconidiophore branches –4; stipe extension terminating in globose to ovoid to sphaeropedunculate vesicles; phialides doliiform to reniform; macroconidia 1-septate, 54 × 6 µm  

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

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

45. Macroconidia longer than 45 µm ................................................................. 46

46. Stipe extensions terminating in sphaeropedunculate vesicle; phialides elongate-doliiform to reniform; macroconidia 1-septate, 55 × 4.5 µm .............. *Ca. pacifica*

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

47. Vesicles pyriform to ellipsoidal or clavate, rarely ovoid, never obpyriform ........ 48

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48. Macroconidia more than 1-septate ........................................................................ 49

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49. 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*

50. 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*
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51. Stipe extensions up to 200 µm long ........................................ 52
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52. **Teleomorph state unknown; stipe extensions terminating in fusiform vesicle; fertile branches –3; phialides doliiform to reniform; macroconidia 1-septate, 38 × 3.5 µm ................................................................. *Ca. brasiliensis*

53. **Teleomorph state unknown; stipe extensions terminating in fusiform to pyriform or clavate vesicle; fertile branches –5; phialides doliiform to reniform; macroconidia 1-septate, 48 × 4 µm ................................................................. *Ca. orientalis*

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

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55. **Macroconidia 1-septate** ..................................................... 56
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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 vesicle; 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

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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; 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_

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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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<sup>1</sup> 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 Vicsa, Brazil.  
<sup>2</sup> 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.  
<sup>3</sup> References used for species descriptions.  
<sup>3</sup> Ex-type cultures.
**Table 2.** Single nucleotide polymorphisms comparisons between *Ca. eucalypti* and *Ca. colhounii.*

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

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### Table 4. Single nucleotide polymorphisms comparisons between *Ca. scoparia* and *Ca. pseudoscoparia*.

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

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

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

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**Fig. 1.** The most parsimonious trees obtained from a heuristic search with 1000 random additions 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 1000 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.
**Fig. 2.** The most parsimonious trees obtained from a heuristic search with 1000 random additions of the combined BT, HIS3 and TEF-1α sequence alignments of the *Calonectria brassicae* complex. Scale bar shows 10 changes and bootstrap support values (bold) from 1000 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.
Fig. 3. One of two most parsimonious trees obtained from a heuristic search with 1000 random additions of the combined BT, HIS3 and TEF-1α sequence alignments of the *Calonectria scoparia* complex. Scale bar shows 10 changes and bootstrap support values (bold) from 1000 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.
Fig. 4. One of three most parsimonious trees obtained from a heuristic search with 1000 random additions of the combined BT, HIS3 and TEF-1α sequence alignments of the *Calonectria morganii* complex. Scale bar shows 10 changes and bootstrap support values (bold) from 1000 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.
Fig. 5. One of 24 most parsimonious trees obtained from a heuristic search with 1000 random additions of the combined actin, β-tubulin, calmodulin, histone H3 and translation elongation factor 1-alpha sequence alignments of the Calonectria spp. Scale bar shows 10 changes and bootstrap support values (bold) from 1000 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 C. lageniformis (CBS 112898) and C. peruviana (CPC 5614). Phylogenetic groups are indicated on the right.
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. Green 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*.
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**Fig. 7.** *Calonectria densa*. A–B. Macroconidiophore of *Ca. densa*. B. Macroconidiophore with lateral stipe extensions. C–D. Fertile branches of macroconidiophore with doliiform to reniform phialides. E–F. Vesicles. G–H. Macroconidia. Scale bars in A–B = 20 µm, C–H = 10 µm.