Phylogeny and systematics of the genus *Calonectria*

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Abstract: Species of *Calonectria* are important plant pathogens, several of which have a worldwide distribution. Contemporary taxonomic studies on these fungi have chiefly relied on DNA sequence comparisons of the β-tubulin gene region. Despite many new species being described, there has been no phylogenetic synthesis for the group since the last monographic study almost a decade ago. In the present study, the identity of a large collection of *Calonectria* isolates from various geographic regions was determined using morphological and DNA sequence comparisons. This resulted in the discovery of seven new species; *Ca. densa*, *Ca. eucalypti*, *Ca. humicola*, *Ca. orientalis*, *Ca. pinii*, *Ca. pseudospongia* and *Ca. sulawesiensis*, bringing the total number of currently accepted *Calonectria* species to 68. A multigenic 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. 26S large subunit RNA gene and translation elongation 1-alpha. Based on these data, 13 phylogenetic groups could be distinguished within the genus *Calonectria* that correlate with morphological features. Dichotomous and synoptic keys to all *Calonectria* spp. currently recognised are also provided.

Key words: Cylindrocladium, DNA phylogeny, sexual compatibility, taxonomy.


INTRODUCTION

The genus *Calonectria* *(Ca.)* was first described in 1867, with *Ca. daldiniana* as the type. This species was later reduced to synonymy with *Ca. pyrochroa* based on morphological comparisons by Rossman (1979). *Calonectria* spp. are *Eusacomyctes* 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 ascosporic walls giving rise to long-stalked, clavate ascii with 1–multi-septate ascospores and *Cylindrocladium* (Cy.) anamorphs (Rossman 1993, Crous 2002, Lombard et al. 2010b).

The genus *Cylindrocladium* was described by Morgan (1892), and is characterised by branched conidiophores with stipe extensions terminating in conical to cylindrical, 1–multi-septate conidia (Crous & Wingfield 1994, Crous 2002). Morphologically, the anamorph provides the greatest number of distinguishing characters for *Calonectria* and it is also the state most frequently encountered in nature (Peerally 1991, Crous & Wingfield 1994, Schoch 2001b, Crous 2002). Consequently, species of *Calonectria* are primarily distinguished by their anamorph characters, especially vesicle shape, stipe extension length, conidial septation, and dimensions on a standardised medium under defined growth conditions (Boesewinkel 1982, Peerally 1991, Crous & Wingfield 1994, Crous 2002).

Besides 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. 1999).

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

*Calonectria* spp. produce three different morphological forms of conidia, of which the macroconidia are present in all but *Ca. multisepata* (Peerally 1991, Crous & Wingfield 1994, Crous et al. 1998b, Crous 2002). Mega- and microconidia are less frequently encountered and these are not regarded as important characters to distinguish between species (Schober 1971, Crous & Wingfield 1994, Crous & Seifert 1998, Crous 2002).
significant variability can occur in the production of all conidial types, so that this feature alone is not always a reliable taxonomic character to define species.

Both homothallic and heterothallic mating systems are found amongst species of Calonectria (Allerèi 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 (protophore/panicles) spermatised by conidia or hyphae of an opposite mating type strain (Schoch et al. 1999, 2000a, 2001a). Some Calonectria spp. have retained the ability to recombine with other closely related Calonectria spp., although the progeny from these crosses have low levels of fertility (Crous 2002). This has complicated the application of the biological species concept for Calonectria, although it has been useful for some species (Schoch et al. 1999, Lombard et al. 2010a).

Difficulties experienced in morphological identification, have led to several molecular approaches being employed to identify Calonectria spp. These include total protein electrophoresis (Crous et al. 1993a, El-Gholl et al. 1993), isozyme electrophoresis (El-Gholl et al. 1992, El-Gholl et al. 1997, Crous et al. 1998a), random amplification of polymorphic DNA (RAPD) (Overmeyer et al. 1996, Victor et al. 1997, Schoch et al. 2000a, Risède & Simouone 2004), restriction fragment length polymorphisms (RFLP) (Crous et al. 1993b, Crous et al. 1995, Crous et al. 1997, Jeng et al. 1997, Victor et al. 1997, Risède & Simouone 2001) and DNA hybridisation (Crous et al. 1993a, 1995, 1997, Victor et al. 1997). However, DNA sequence comparisons and associated phylogenetic inference has had the most significant impact on the taxonomy of the group. It is also most widely applied in contemporary species descriptions. The 5.8S ribosomal RNA gene and flanking internally transcribed spacer (ITS) sequences made it possible for Jeng et al. (1997) to distinguish between *C. scaparium* and *C. floridanum* isolates. Subsequently, it was found that this gene region contains few informative characters for members of the genus (Crous et al. 1999, Schoch et al. 1999, Risède & Simouone 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 & Cumalham 2002, Crous et al. 2004b, 2006). Other DNA sequences recently used to distinguish between species include the translation elongation factor 1–α (TEF-1α) and calmodulin (CAL) gene regions (Crous et al. 2004b, Lombard et al. 2009, 2010a, b). However, sequence data for these regions on GenBank (www.ncbi.nlm.nih.gov) are incomplete for the group, substantially reducing their value.

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

### MATERIALS AND METHODS

#### Isolates

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

#### DNA extraction and amplification

**Identification of unknown *Calonectria* isolates**

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

**Phylogenetic relationships amongst *Calonectria* spp.**

Total genomic DNA was extracted as above. Seven loci were amplified including the ITS gene region using primers V9G (De Hoog & van den Ende 1998) and ITS4 (White et al. 1990), the 28S large subunit RNA gene (LSU) using primers LR0R (Moncalvo et al. 1995) and LR5 (Vilgalys & Hester 1990); and parts of the TEF-1α gene region; the BT gene region; the HIS3 gene region with the same primer sets mentioned previously, the actin (ACT) gene region using primers ACT-512F and ACT-738R (Carbone & Kohn 1999) and CAL gene region using primers CAL-228F and CAL-737R (Carbone & Kohn 1999).

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

#### DNA sequencing and analysis

Amplified fragments were sequenced in both directions using the same primer pairs used for amplification. For this purpose, the BigDye terminator sequencing kit v. 3.1 (Applied Biosystems, U.S.A.) and an ABI PRISM™ 3100 DNA sequencer (Applied Biosystems) were used. All PCRs and sequencing reactions were performed on an Eppendorf Mastercycler Personal PCR (Eppendorf AG, Germany) with cycling conditions as described in Crous et al. (2006) for all loci amplified.
In addition to the sequences generated in this study, Calonectria spp. sequences were obtained from GenBank. All sequences were assembled and aligned using Sequence Navigator v. 1.0.1 (Applied Biosystems) and MAFFT v. 5.11 (Katoh et al. 2005), respectively. The aligned sequences were then manually corrected where necessary. Single nucleotide polymorphisms (SNPs) were determined for the aligned DNA sequences of each gene region using DnaSP v. 5.00.06 (Librado & Rozas 2009).

To determine whether the DNA sequence data sets were congruent, a partition homogeneity test (PHT; Farris et al. 1994) of all possible combinations, with 1,000 replications on all informative characters was conducted in PAUP (Phylogenetic Analysis Using Parsimony, v. 4.0b10; Swofford 2002). A 70% reciprocal bootstrap method using Neighbour-Joining with Maximum Likelihood distance (Mason-Gamer & Kellogg 1996; Gueidan et al. 2007) was also employed. Models of evolution were estimated in Modeltest v. 3.7 (Posada & Crandall 1998) using the Akaike Information Criterion (AIC) for each gene region. The bootstrap analyses were run in PAUP for 10,000 replications. 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 consistency 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 & Bull 1993) 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 & Bull 1993).

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

**Sexual compatibility**

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

**Taxonomy**

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

**RESULTS**

**DNA sequencing and analysis**

**Identification of unknown Calonectria isolates**

Amplicons of approx. 500 bp were generated for the BT and TEF-1α gene regions and those for the HIS3 region were approx. 450 bp in length. Based on preliminary BT sequence comparisons and morphological characteristics, the sequence data sets for the unknown Calonectria spp. were divided into four separate data sets representing the Ca. colhounii, Ca. brassicaceae, Ca. scoparia and Ca. morgani 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)
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Table 1. Isolates of *Calonectria* spp. studied.
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1 CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Pedro Crous working collection housed at CBS; IMI: International Mycological Institute, CABl-Bioscience, Egham, Bakeham Lane, U.K.; ATCC: American Type Culture Collection, Virginia, U.S.A.; UFV: Univeridade Federal de Viçosa, Brazil. 2 ACT = Actin, BT = β-tubulin, CAL = Calmodulin, HIS3 = Histone H3, ITS = Internal transcribed spacer regions 1 and 2 and the 5.8S gene of the ribosomal RNA, LSU = 28S large subunit RNA, TEF-1α = Translation elongation factor 1-alpha. 3 References used for species descriptions. 4 Ex-type cultures.
Table 2. Single nucleotide polymorphisms comparisons between *Ca. eucalypti* and *Ca. colhounii*, compared to *Ca. macroconidialis* and *Ca. madagascariensis*.

<table>
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<th>Species</th>
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<th>β-tubulin</th>
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<th>TEF-1α</th>
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Fig. 1. The most parsimonious trees obtained from a heuristic search with 1 000 random additions sequences of the combined BT, HIS3 and TEF-1α sequence alignments of the *Ca. colhounii* complex. Scale bar shows 10 changes and bootstrap support values (bold) from 1 000 replicates and Bayesian posterior probability values are indicated at the nodes. Red lines indicate bootstrap values of 100 and posterior probabilities of 1.00. Thickened lines indicate branches in the strict consensus tree and the consensus tree of the Bayesian analyses.
Table 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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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 with maximum-parsimony as well as bootstrap support. Therefore, only maximum-parsimony trees are presented with bootstrap values and posterior probabilities shown for well-supported branches.

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

The combined sequence data set representing the Ca. colhounii complex, with 10 taxa including outgroups, consisted of 1 497 characters, including gaps. Of these characters, 1 051 were constant, 133 were parsimony-uninformative and 313 characters were parsimony informative. Parsimony analysis of the aligned sequences yielded one most parsimonious tree (Fig. 1; TL = 649 steps; CI = 0.888; RI = 0.891; RC = 0.791). In the tree, isolates CBS 125273–125276, from Indonesia, grouped close to but separate from Ca. colhounii (CBS 293.79 and CBS 114704) with 100 % bootstrap support (BP = 100 and PP = 1.00). Similarly, isolates CBS 125258–125260, from Indonesia, clustered closely related to Ca. brassicae (CBS 114571 and CBS 114572) and two shared alleles with Ca. macroconidialis (CBS 114880) for the three gene regions analysed (Table 2). These unique alleles, however, distinguish the Indonesian isolates from Ca. colhounii, Ca. macroconidialis and Ca. madagascariensis.

The data set representing the Ca. brassicae complex consisted of 15 taxa including the outgroups, while the combined sequence alignment was made up of 1 509 characters, including gaps. These characters represented 1 092 constant, 127 parsimony-uninformative and 290 parsimony-informative characters. Parsimony analysis yielded one most parsimonious tree (Fig. 2; TL = 569 steps; CI = 0.931; RI = 0.918; RC = 0.855). In the tree, Colombian isolates CBS 123698 and CBS 125523 clustered close to Ca. brassicae (CBS 111869 and CBS 111478) and Ca. brachiatica (CBS 123700 and CMW 25302) but separately from both these species with high support (BP = 100 and PP = 1.00). Similarly, isolates CBS 125258–125260, from Indonesia, clustered together closely related to Ca. brassicae and Ca. brachiatica. These Indonesian isolates were also closely related to the Colombian isolates but grouped separately from them in a clade with high support (BP = 97 and PP = 1.00). The SNP analyses showed 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 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 125254–125257 from Ecuador, clustered closely but separately from Ca. scoparia (CMW 31000 and CMW 31001) and other species in the Ca. pauciramosa complex with low support (BP = 63 and PP = 1.00). The Ecuadorian isolates also had three unique alleles separating them from Ca. scoparia and Ca. pauciramosa (CMW 5683 and CMW 30823) for the BT and TEF-1α regions, but there were no unique alleles for these isolates in the HIS3 region (Table 4).

The aligned sequence data set for the Ca. morganii complex included 25 ingroup taxa consisting of 1 535 characters. Of these characters, 975 were constant, 211 were parsimony-uninformative and 349 characters were parsimony-informative. Parsimony analysis
Table 4. Single nucleotide polymorphisms comparisons between Ca. scoparia and Ca. pseudoscoparia, compared to Ca. pauciramosa.

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<td>Ca. pauciramosa</td>
<td>CMW 5683</td>
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<td>CMW 30823</td>
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</table>
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.

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. In contrast, the non-coding gene regions (ITS and LSU) provided little or no support for the clades that emerged from the coding gene regions. The phylogeny constructed based on CAL sequences showed the best resolution for the individual clades, followed by TEF-1α. The analyses were performed in MrBayes (Ronquist et al. 2005) with a Bayeroian model as outlined for each gene region. For Bayesian analyses, a K80+G model was selected for ACT, HKY+I+G for BT 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 from the maximum-parsimony as well as bootstrap support.

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

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

The combined sequence alignment of the five coding gene regions consisted of 2,472 characters, including gaps. Of these characters, 925 were constant, 267 were parsimony-uninformative and 1,280 characters were parsimony-informative. Parsimony analysis of the aligned sequences yielded 24 most parsimonious trees (TL = 7,319 steps; CI = 0.397; RI = 0.820; RC = 0.326), one of which is presented in Fig. 5. The tree topology obtained with
Red lines indicate bootstrap values of 100 and posterior probabilities of 1.00. The tree was rooted to 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. Fig. 5 and translation elongation factor 1-alpha sequence alignments of the Phylogenetic groups are indicated on the right.

Fig. 5. One of 24 most parsimonious trees obtained from a heuristic search with 1 000 random additions sequences of the combined actin, β-tubulin, calmodulin, histone H3 and translation elongation factor 1-alpha sequence alignments of the Calonectria. Scale bar shows 10 changes and bootstrap support values (bold) from 1 000 replicates and Bayesian posterior probability values are indicated at the nodes. Thickened lines indicate branches in the strict consensus tree and the consensus tree of the Bayesian analyses. Red lines indicate bootstrap values of 100 and posterior probabilities of 1.00. The tree was rooted to Cylindrocladiella lageniformis (CBS 112682) and C. peruviana (CPC 5614). Phylogenetic groups are indicated on the right.
Table 6. Single nucleotide polymorphisms comparisons between Ca. brasiliensis, Ca. insularis and Ca. sulawesiensis compared to Ca. cerciana.

<table>
<thead>
<tr>
<th>Species</th>
<th>Isolate no.</th>
<th>β-tubulin</th>
<th>Histone H3</th>
<th>TEF-1α</th>
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</thead>
<tbody>
<tr>
<td>Ca. brasiliensis</td>
<td>CBS 230.51</td>
<td>C A T C</td>
<td>G C G A</td>
<td>T T A</td>
</tr>
<tr>
<td></td>
<td>CBS 114257</td>
<td>C A T C</td>
<td>G C G A</td>
<td>T T A</td>
</tr>
<tr>
<td>Ca. cerciana</td>
<td>CBS 123693</td>
<td>T A T T</td>
<td>A C C A</td>
<td>C C T</td>
</tr>
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<td>CBS 123695</td>
<td>T A T T</td>
<td>A C C A</td>
<td>C C T</td>
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<tr>
<td>Ca. insularis</td>
<td>CBS 114558</td>
<td>T G A C C</td>
<td>A C G A</td>
<td>C C A</td>
</tr>
<tr>
<td></td>
<td>CBS 114559</td>
<td>T G A C C</td>
<td>A C G A</td>
<td>C C A</td>
</tr>
<tr>
<td>Ca. sulawesiensis</td>
<td>CBS 125248</td>
<td>T A G T T</td>
<td>A T T G</td>
<td>T T C</td>
</tr>
<tr>
<td></td>
<td>CBS 125253</td>
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<td>A T T G</td>
<td>T T C</td>
</tr>
<tr>
<td></td>
<td>CBS 125277</td>
<td>T A G T T</td>
<td>A T T G</td>
<td>T T C</td>
</tr>
<tr>
<td></td>
<td>CMW 14883</td>
<td>T A G T T</td>
<td>A T T G</td>
<td>T T C</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Actin</th>
<th>β-tubulin</th>
<th>Calmodulin</th>
<th>Histone H3</th>
<th>ITS</th>
<th>LSU</th>
<th>TEF-1α</th>
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<td>706</td>
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<td>39</td>
<td>62</td>
<td>32</td>
<td>10</td>
<td>57</td>
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<tr>
<td>Informative characters</td>
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<td>268</td>
<td>323</td>
<td>223</td>
<td>112</td>
<td>37</td>
<td>337</td>
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<tr>
<td>Most parsimonious trees</td>
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<td>91</td>
<td>1000</td>
<td>372</td>
<td>1000</td>
<td>100</td>
<td>9970</td>
</tr>
<tr>
<td>Tree length</td>
<td>573</td>
<td>1454</td>
<td>1282</td>
<td>1843</td>
<td>296</td>
<td>91</td>
<td>1641</td>
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<tr>
<td>CI</td>
<td>0.490</td>
<td>0.431</td>
<td>0.467</td>
<td>0.352</td>
<td>0.618</td>
<td>0.538</td>
<td>0.477</td>
</tr>
<tr>
<td>RI</td>
<td>0.867</td>
<td>0.840</td>
<td>0.849</td>
<td>0.793</td>
<td>0.882</td>
<td>0.913</td>
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<tr>
<td>RC</td>
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<td>0.569</td>
<td>0.397</td>
<td>0.648</td>
<td>0.545</td>
<td>0.492</td>
<td>0.416</td>
</tr>
</tbody>
</table>

Sexual compatibility

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

Taxonomy

Based on morphological observations, phylogenetic inference and mating, numerous isolates of Calonectria spp. included in this study represent undescribed species. Species of Cylindrocladium (1892) represent anamorph states of Calonectria (1867) (Rossman et al. 1999). In an attempt to move to a single nomenclature for pleomorphic fungi, the teleomorph name takes precedence over the anamorph name when both types belong to the same holomorph. The species below are described as new species in Calonectria, which represents the older generic name for these holomorphs and follows Lombard et al. (2009, 2010a, c). All Cylindrocladium species without a Calonectria state, are also transferred to Calonectria.
### Fig 6. Results of sexual compatibility tests. Successful matings are indicated by (+) and unsuccessful matings is indicated with (-). Blue highlighted blocks indicate homothallic matings. Yellow blocks highlight unsuccessful self-self matings. Purple blocks indicate mating tester strain matings. A. Matings between isolates of *Ca. macroconidialis*. B. Matings between isolates of *Ca. brasiliensis*, *Ca. brassicae*, *Ca. pini* and *Ca. orientalis*. C. Matings between isolates of *Ca. brasiliensis*, *Ca. cerciana*, *Ca. insularis* and *Ca. sulawesiensis*. D. Matings between isolates of *Ca. densa*, *Ca. humicola* and *Ca. spathiphylli*. E. Matings between isolates of *Ca. pauciramosa*, *Ca. pseudoscoparia* and *Ca. scoparia*.

### Calonectria densa

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

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

Teleomorph unknown. Conidiophores with a stipe bearing penicillate suites of fertile branches, stipe extensions, and terminal vesicles; stipe septate, hyaline, smooth, 54–90 × 6–10 µm; stipe extensions septate, straight to flexuous, 149–192 µm long, 5–6 µm wide at the apical septum, terminating in ovoid to ellipsoidal to sphaeropedunculate vesicles, 10–12 µm diam; lateral stipe extensions (90° to the axis) also present. Conidiogenous apparatus 49–78 µm long, and 63–123 µm wide; primary branches aseptate, 20–29 × 5–6 µm; secondary branches aseptate, 16–20 × 4–6 µm; tertiary and additional branches (–4) aseptate, 9–16 × 3–5 µm, each branch bearing 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–)6–10 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...
to verona-brown after 7 d; moderate white aerial mycelium with moderate sporulation; chlamydospores extensive throughout the medium forming microsclerotia.

**Substrate:** Soil.

**Distribution:** Ecuador.

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

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**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 macroconidios cylindricis utrinque rotundatis rectis (66–169–75–80) × 5–6 µm mediocre 72 × 6 µm, ter septatis, sine cicatrice abscissionis manifesta, cum moco hyalinum in fasciculis parallellis 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: peripherial base up to 125 µm wide; consisting of dark red, angular cells; merging with an erumpent stroma, cells of the outer wall layer continuing into the pseudoparenchymatous cells of the erumpent stroma. Asci 4-spored, clavate, 92–188 × 10–27 µm, tapering to a long thin stalk. Ascospores aggregated in the upper third of the ascus, hyaline, guttulate, fusoid with rounded ends, straight to slightly curved, (1–)3-septate, not or slightly constricted at the septum, (25–)30–36(–56) × (3–)5–(6–)8 µm (av. = 33 × 6 µm).

Cultures were homothallic. Conidiophores with a stipe bearing a suit of penicillate, fertile branches, a stipe extension, and a terminal vesicle; stipe septate, hyaline, smooth, 45–91 × 7–10 µm; stipe extensions septate, straight to flexuous, 110–235 µm long, 5–6 µm wide at the apical septum, terminating in broadly clavate vesicles, 4–6 µm diam. Conidiogenous apparatus 52–82 µm long, and 40–95 µm wide; primary branches asperate or 1-septate, 21–29 × 5–6 µm; secondary branches asperate, 14–21 × 3–5 µm; tertiary branches and additional branches (–5), asperate, 11–16 × 3–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform 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).

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

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

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

Teleomorpha ignota. Anamorpha Cy. spathiphylli similis sed macroconidis cylindricis utrinque rotundatis relictis (45–)48–54(–56) × 4–5 µm mediocter 51 × 5 µm, semel septata, sine cicatrice abscissionis manifesta, cum mucro hyalino in fasciculis parallelis cylindricis differt.

Teleomorph unknown. Conidiophores with a stipe bearing penicillate suites of fertile branches, stipe extensions, and terminal vesicles; stipe septate, hyaline, smooth, 44–90 × 6–8 µm; stipe extensions septate, straight to flexuous, 126–157 µm long, 4–5 µm wide at the apical septum, terminating in globose to ovoid to sphaeropedunculate vesicles, 10–12 µm diam. Conidiogenous apparatus 43–71 µm long, and 42–49 µm wide; primary branches asperate, 20–29 × 4–6 µm; secondary branches asperate, 12–19 × 3–5 µm; tertiary branches and additional branches (–5), asperate, 11–16 × 3–5 µm, each terminal branch producing 2–4 phialides; phialides elongated doliiform to reniform, hyaline, aseptate, 10–15 × 3–4 µm; apex with minute pericinal thickening and inconspicuous collarate. Macroconidia cylindrical, rounded at both ends, straight, (45–)48–54(–56) × (4–)5–6 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 °C (growth at 15–35 °C) on MEA, reverse colour to verona-brown after 7 d; moderate white aerial mycelium with moderate sporulation; chlamydospores extensive throughout the medium, forming microsclerotia.

Substrate: Soil.

Distribution: Ecuador.

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

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

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

Teleomorpha ignota. Anamorpha Ca. brachiatcaae similis sed ramis conidiophorae tres vel minus sine extensionibus lateralis et stipae, macroconidios cylindricis utrinque rotundatis redcis (45–)48–54(–56) × 4–5 µm mediocter 48 × 4 µm, semel septata, sine cicatrice abscissionis manifesta, cum mucro hyalino in fasciculis parallelis cylindricis differt.
Teleomorph unknown. Conidiophores with a stipe bearing penicillate suites of fertile branches, stipe extensions, and terminal vesicles; stipe separte, hyaline, smooth, 60–169 × 6–12 µm; stipe extensions separte, straight to flexuos, 90–219 µm long, 5–10 µm wide at the apical septum, terminating in clavate to broadly clavate vesicles, 5–10 µm diam. Conidiogenous apparatus 54–174 µm long, and 67–92 µm wide; primary branches aseptate, 19–30 × 4–7 µm; secondary branches aseptate, 16–29 × 4–6 µm; tertiary and additional branches (~5) aseptate, 10–20 × 5–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 10–19 × 2–5 µm; apex with minute periclinal thickening and inconspicuous collarette. Macroconidia cylindrical, rounded at both ends, straight, (43–)46–50(--53) × 4(–5) µm (av. = 48 × 4 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. Mega- and microconidia not seen.


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

Substrate: Soil.

Distribution: Indonesia.

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

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.

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

µm wide at the apical septum, terminating in clavate vesicles, 4–6 µm diam. Conidiogenous apparatus 49–81 µm long, and 35–84 µm wide; primary branches aseptate, 20–30 × 4–6 µm; secondary branches aseptate, 13–22 × 3–5 µm; tertiary branches aseptate, 11–15 × 3–4 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 10–15 × 3–4 µm; apex with minute periclinal thickening and inconspicuous collarette. Macroconidia cylindrical, rounded at both ends, straight, (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 to sepia-brown after 7 d; abundant white aerial mycelium with moderate to extensive sporulation; chlamydospores extensive throughout the medium forming microsclerotia.

Substrate: Pinus patula.

Distribution: Colombia.

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

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

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

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 flexuosus, 124–201 µm long, 4–6 µm wide at the apical septum, terminating in obpyriform to ellipsoidal vesicles, 6–10 µm diam. Conidiogenous apparatus 34–87 µm long, and 52–74 µm wide; primary branches aseptate, 26–38 × 4–7 µm; secondary branches aseptate, 17–28 × 4–6 µm; tertiary branches and additional branches (–4) aseptate, 14–19 × 3–4 µm, each terminal branch producing 2–6 phialides; phialides elongate-doliiform to reniform, hyaline, aseptate, 7–11 × 2–4 µm; apex with minute periclinal thickening and inconspicuous collarette. Macroconidia cylindrical, rounded at both ends, straight, (41–)45–51(–52) × 3–5 µm (av. = 48 × 4 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. Mega- and microconidia not seen.


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

Substrate: Eucalyptus grandis.

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

Calonectria sulwasiensis 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.

Teleomorph ignota. Anamorpha Ca. morgani similis sed phialidibus elongato-doliiformibus vel reiniformibus hyalinis non septatis 7–11 × 2–4 µm apice minute periclinae incrassatis colliculo incompisco, macroconidios cylindricis utrinque rotundatis rectis (41–)45–51(–52) × 3–5 µm mediciorton 48 × 4 µm, semel septatis, sine cicatrice abscissionis manifesta, cum mucro hyalino in fasciculis parallelis cylindricis differt.
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 to sepia-brown 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). 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.


≡ *Cylindrocladium citri* (H.S. Fawk. & Klotz) Boedijn & Reitsma, Reinwardtia 1: 57. 1950. 

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


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. pseudospathiphylli from Ecuador and Ca. pini from Colombia were recognised based on morphological characteristics and phylogenetic inference. Recognition of a relatively large number of new species, mainly from soil samples collected in areas not previously intensively sampled, suggests that many more species of Calonectria remain to be discovered, particularly from the tropics and Southern Hemisphere.

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

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

Calonectria pseudospathiphylli is a new species in the Ca. scoparia complex (Schoch et al. 1999), isolated from E. grandis cuttings collected in Ecuador that displayed basal rot symptoms. Calonectria spp. in this group are well known causal agents of cutting rot in commercial forestry nurseries worldwide (Crous et al. 1991, Crous 2002, Lombard et al. 2010a). However, the pathogenicity of Ca. pseudospathiphylli 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. 2004a, 2006). In this regard, BT is the gene region that provides the most valuable insights into relationships amongst all species of Calonectria (Schoch et al. 2000b, 2001b, Crous 2002, Henricot & Culham 2002). Application of the CAL and TEF-1α partial gene sequences has only recently been introduced for Calonectria spp. (Crous et al. 2004b, 2006, Lombard et al. 2009, 2010a, c) and data for these gene regions have been available for only a small sub-set of species. The present study has attempted to address this problem and also introduce the ACT and LSU gene sequences that have not been employed previously for Calonectria spp. It has also provided sequence data for all seven gene regions for all accepted species in the genus.

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

In addition to identifying the most useful gene regions to accurately identify species of Calonectria, an important goal of this study was to re-consider the phylogenetic relationships between all the species in this genus. Having determined that the ACT, BT, CAL, HIS3 and TEF-1α gene regions give the best resolution when identifying species of Calonectria, a phylogenetic tree for the genus was generated. This showed that the group includes two major clades and that these define morphologically similar groups of Calonectria spp. These two major clades have substantial sub-structure with all of the 66 species of Calonectria residing in one of 13 sub-clades. Eleven of these sub-clades, that include 50 species, represent the Prolate Group of isolates and two sub-clades that include 16 species representing the Sphaero-Navicate 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. pyrochloa) and Cylindrocladium (Cy. scoparium). Most of these pathogenic species have been reported from forestry crops (Peeraly 1991, Crous & Wingfield 1994, Crous 2002, Crous et al. 2006) but a few have also been found to infect horticultural and agronomic crops (Boedijn & Reitsma 1950, Kim et al. 1998, Crous 2002, Polizzi et al. 2007, Vitale et al. 2008). None of the sub-clades in this group could, however, be correlated with any specific host type.

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

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

The second sub-clade in the Sphaero-Naviculate Group of isolates (sub-clade XIII) included three *Calonectria* spp., only two of which have morphological similarities. *Calonectria multiphalidica* 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. *microsorum*, *Cy. bambusae*, *Cy. couraritii*, *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 for all newly described pleomorphic fungal species, the teleomorph name or the oldest typified name takes precedence over the anamorph or more recent name when both types belong to the same holomorph taxon (Hawksworth 2005, McNeill et al. 2005). It has already been established that *Calonectria* spp. have only *Cylindrocladium* anamorphs (Rossman et al. 1999, Schoch et al. 2001b), with micro- and megaconidial states that have thus far not been named. The name *Calonectria* was typified in 1867 (Rossman 1979) whereas that of *Cylindrocladium* was typified in 1892 (Morgan 1892). Therefore *Calonectria* has preference above *Cylindrocladium* and should henceforth be used for all species irrespective of whether the perithecial state has been found.

**KEYS**

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

1. *Ca. acicola* P.D. Gadgil & M.A. Dick
2. *Ca. angustata* (Crous & El-Gholl) L. Lombard, M.J. Wingf. & Crous
3. *Ca. asiatica* Crous & N.L. Hywel-Jones
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* (Boedijn & 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. Michau
23. *Ca. gracilis* Crous, M.J. Wingf. & Alfenas
26. *Ca. hederae* C. Booth & J.S. Murray
27. *Ca. hongkongensis* Crous
28. Ca. humicola L. Lombard, M.J. Wingf. & Crous
29. Ca. hurae (Linder & Whetzel) L. Lombard, M.J. Wingf. & Crous
30. Ca. ilicicola L. Lombard, M.J. Wingf. & Crous
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. multiformis (Crous, Simoneau & Risède) L. Lombard, M.J. Wingf. & Crous
43. Ca. multiseptata Crous & M.J. Wingf.
44. Ca. naviculata Crous & M.J. Wingf.
45. Ca. orientalis L. Lombard, M.J. Wingf. & Crous
46. Ca. ovata D. Victor & Crous
47. Ca. pacifica (J.C. Kang, Crous & C.L. Schoch) L. Lombard, M.J. Wingf. & Crous
48. Ca. pauciramosa C.L. Schoch & Crous
49. Ca. penicilliiodes (Tubaki) L. Lombard, M.J. Wingf. & Crous
51. Ca. pteridis Crous, M.J. Wingf. & Alfenas
52. Ca. pyrochoa (Desm.) Sacc.
53. Ca. queenslandica L. Lombard, M.J. Wingf. & Crous
54. Ca. reteaudii (Bgn.) C. Booth
55. Ca. rumohrae El-Gholl & Alfenas
56. Ca. scoparia Peerally
59. Ca. sulawesiensis L. Lombard, M.J. Wingf. & Crous
60. Ca. sumatrensis (Crous) L. Lombard, M.J. Wingf. & Crous
61. Ca. terrae-reginae L. Lombard, M.J. Wingf. & Crous
63. Ca. zuluensis L. Lombard, M.J. Wingf. & Crous

Synoptic key to *Calonectria* species

1. Teleomorph:
   a. Teleomorph state known
      1, 3, 5, 13, 14, 15, 16, 21, 22, 23, 26, 27, 29, 30, 31, 32, 33, 34, 35, 37, 38, 40, 41, 43, 44, 46, 48, 55, 56, 57, 59, 60, 61, 62, 63, 67, 68

   b. Teleomorph state unknown
      2, 4, 6, 7, 8, 9, 10, 11, 12, 17, 18, 19, 20, 24, 25, 28, 36, 39, 42, 45, 47, 49, 50, 51, 52, 53, 54, 58, 64, 65, 66

2. Ascocarps:
   a. Red-brown to red in colour, changing to dark-red in 3 % KOH
      1, 23, 44, 56, 61, 67

   b. Orange to red in colour, changing to dark-red in 3 % KOH
      3, 5, 15, 16, 22, 26, 30, 32, 33, 34, 40, 43, 55, 62, 68

   c. Orange to red-brown in colour, changing to dark-red in 3 % KOH
      13, 27, 35, 46, 48, 57, 59, 60, 63

   d. Yellow to orange in colour, only base and stroma changing to dark-red in 3 % KOH
      14, 21, 37, 38, 41
3. Asci:
   a. 8-spored and clavate
      1, 3, 5, 13, 15, 16, 22, 23, 26, 27, 30, 32, 33, 34, 35, 38, 40, 41, 43, 44, 46, 48, 55, 56, 57, 59, 60, 61, 62, 63, 67, 68
   b. 4-spored and clavate
      14, 21, 37

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

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

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

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

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

9. Vesicle shape
   a. Avesiculate to clavate
      5
   b. Clavate
      1, 2, 4, 6, 7, 13, 14, 20, 21, 22, 23, 24, 29, 32, 35, 37, 38, 43, 45, 50, 53, 56, 58, 59, 60, 64, 66
   c. Ellipsoidal to pyriform to obovoid
      8, 12, 25, 26, 41, 55, 61, 63
d. Ellipsoidal to ovoid  
   19, 46

e. Ellipsoidal to obpyriform  
   10, 15, 33, 36, 40, 48, 51, 54, 57, 68

f. Sphaeroapedunculate  
   3, 9, 11, 16, 17, 18, 19, 27, 30, 31, 34, 39, 42, 47, (49), 64, 67

g. Globose  
   19, 28, 62

h. Naviculate  
   44, 52

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

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

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

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

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

15. Microconidial length (mean in µm)  
   a. Below 20  
      29
   b. 20–30  
      1, 30, 46, 56, 59, 60, 67
   c. 31–40  
      13, 24, 62
   d. above 40  
      43, 53
16. Macroconidial septation
   a. 1-septate
      3, 6, 7, 8, 9, 10, 11, 12, 15, 17, 19, 22, 25, 27, 28, 31, 33, 34, 39, 40, 41, 42, 44, 45, 47, 48, 50, 51, 52, 54, 61, 64, 65, 68
   b. 1(–3)-septate
      5, 13, 16, 18, 20, 23, 24, 36, 46, 53, 55, 56, 62
   c. (1–)3-septate
      4, 14, 21, 30, 32, 38, 49, 57,
   d. (1–3(–6)-septate
      26, 37, 58, 66
   e. (1–)5(–6)-septate
      1, 26, 35, 59, 60
   f. (1–)7(–8)-septate
      29
   g. More than 8-septate
      2

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

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

Dichotomous key to Calonectria species

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

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

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

3. Teleomorph state unknown ............................................................................................................ 4
3. Teleomorph state known .................................................................................................................. 15
4. Macroconidia 1-septate only ................................................................. 5
4. Macroconidia more than 1-septate .......................................................... 8
5. Fertile branches –3; phialides doliiform to reniform; macroconidia 1-septate, 44 × 5 µm; stipe extensions terminating in clavate vesicles ............................................................... Ca. pini
5. Fertile branches –5 .................................................................................. 6
6. Lateral stipe extensions present; macroconidia 1–2-septate, 44 × 5 µm; stipe extensions terminating in clavate vesicles; fertile branches –5; phialides doliiform to reniform ................................. Ca. brachiatica
6. Lateral stipe extensions absent ............................................................... 7
7. Stipe extensions terminating in clavate vesicles; fertile branches –5; phialides doliiform to reniform; macroconidia 1-septate, 53 × 4.5 µm ...................................................... Ca. brassicae
7. Stipe extensions terminating in clavate vesicles; fertile branches –5; phialides doliiform to reniform; macroconidia 1-septate, 48 × 4 µm .................................................. Ca. orientalis
8. Macroconidia longer than 100 µm ......................................................... 9
8. Macroconidia shorter than 100 µm ........................................................ 10
9. Macroconidia 5–8-septate, 104 × 8 µm; stipe extension terminate in clavate vesicles; fertile branches –3; phialides cylindrical to allantoid; microconidiophores lacking stipe extension; microconidia 1–3-septate, 44 × 4 µm ...................................................... Ca. pseudoreteaudii
9. Macroconidia 1–3-septate ..................................................................... 12
10. Macroconidia (1–)3-septate, 63 × 6.5 µm; stipe extensions terminating in clavate vesicles; fertile branches –6; phialides cylindrical to allantoid .................................................. Ca. australiensis
10. Macroconidia 1(–)3-septate ................................................................. 11
11. Fertile branches –7; phialides doliiform to reniform; macroconidia 51 × 4.5 µm; stipe extensions terminating in clavate vesicles .............................................................. Ca. ecuadoriae
11. Fertile branches –4; phialides doliiform to reniform; macroconidia 62 × 5 µm; stipe extensions terminating in clavate vesicles .................................................. Ca. gordoniae
12. Macroconidia longer than 100 µm with more than 6 septa.................. 13
12. Macroconidia shorter than 100 µm with 6 or less septa ......................... 14
13. Stipe extensions terminating in narrowly clavate vesicles; fertile branches –4; phialides cylindrical; macroconidia (1–)7–10(–12)-septate with slight swelling in the middle, 110 × 10 µm; mega- and microconidia absent ........................................ Ca. angustata
13. Stipe extensions terminating in narrowly clavate vesicles; fertile branches –3; phialides cylindrical; microconidia present, 1-septate, 18 × 3 µm; macroconidia (1–)7(–8)-septate, 120 × 7.5 µm; megaconidia present, 9–16-septate, bent or curved, (150–)200–250(–270) × 6–7(–8) µm ..................... Ca. hurae
14. Stipe extensions terminating in narrowly clavate vesicles; fertile branches –3; phialides cylindrical to allantoid, obpyriform when carried singly; macroconidia 4–6-septate, 69 × 6 µm ....................................................... Ca. queenslandica
14. Stipe extensions terminating in a narrowly clavate vesicles; fertile branches –3; phialides cylindrical to allantoid, obpyriform when carried singly; macroconidia 4–6-septate, 76 × 6 µm ....................................................... Ca. terrae-reginae
15. Macroconidial state unknown; megaconidiophores with stipe extensions terminating in clavate vesicles when present; megaconidia 6–10-septate, boomerang-shaped or curved, (120–)150–170(–220) × 8–9 µm; microconidia 1–3-septate, straight or curved, 20–65 × 2.5–3.5 µm ................................................ Ca. multiseptata
15. Macroconidial state known .................................................................. 16
16. Teleomorph state known and macroconidia 1-septate to 7(–)3-septate ................................................................. 17
16. Teleomorph state known and macroconidia multi-septate ...................... 20
17. Teleomorph homothallic ..................................................................... 18
17. Teleomorph heterothallic ................................................................... 19
18. Perithecia orange with a red apex; ascospores 1-septate, 35 × 6.5 µm; stipe extensions terminating in clavate vesicles; fertile branches –4; phialides doliiform to reniform; macroconidia 1-septate, 45 × 4.5 µm ................................. Ca. gracilipes
18. Perithecia red; ascospores 1-septate, 37 × 5 µm; stipe extensions terminating in narrowly clavate vesicles; fertile branches –4; phialides doliform to reniform; macroconidia 1(–3)-septate, 56 × 4.5 µm .......................... Ca. gracilis

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

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

20. Macroconidia 3-septate ................................................................. 21

20. Macroconidia 3- to multi-septate .................................................. 25

21. Perithecia yellow to orange ............................................................. 22

21. Perithecia yellow ........................................................................... 23

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

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

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

23. Macroconidia and ascospores longer than 65 µm .......................... 24

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

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

25. Macroconidiophore branches –2 or less ........................................... 26

25. Macroconidiophore with more than 2 series of branches .................. 27

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

26. Teleomorph state homothallic; perithecia red to 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; macroconidia 1–3-septate, 10–30 × 3–5 µm .................................................. Ca. acicola

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

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

28. Vesicles sphaeropedunculate, globose or ovoid .................................. 29

28. Vesicles not as above ....................................................................... 48

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

29. Vesicles not consistently ovate ........................................................... 30
30. Macroconidia 1(–3)-septate ................................................................. 31
30. Macroconidia only 1-septate ................................................................ 36

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

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

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

34. Lateral stipe extensions abundant; perithecia orange; ascospores 1-septate, 33 × 5 µm; stipe extensions terminating in sphaeropedunculate vesicles; fertile branches –5; phialides elongate-doliiform to reniform; macroconidia 1(–3)-septate, 54 × 4.5 µm .......................... Ca. colombiensis
34. Lateral stipe extensions absent ................................................................ 35

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

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

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

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

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

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

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

42. Stipe extensions terminating in globose to ovoid to sphaeropedunculate vesicles; fertile branches –3; phialides doliiform to reniform; macroconidia 1-septate, 51 × 5 µm ............................................. Ca. humicola
42. Stipe extensions terminating in sphaeropedunculate vesicles; fertile branches –5; phialides elongate-doliiform to reniform; macroconidia 1-septate, 50.5 × 4 µm ............................... Ca. indonesiae
43. Lateral stipe extensions rare; stipe extensions terminating in pyriform to sphaeropedunculate vesicles; fertile branches – 3; phialides doliiform to reniform; macroconidia 1-septate, 50 × 4 µm ......................................................... Ca. canadensis

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

45. 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

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

47. Macroconidia more than 1-septate .................................................................................................................................................... 50

49. Macroconidia 1-septate, 58 × 5 µm ........................................................................................................................................... Ca. hederae

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

51. Macroconidia 1-septate, 55 × 4.5 µm .............................................................................................................................................. Ca. morganii

52. Teleomorph state unknown; stipe extensions terminating in narrowly ellipsoidal to pyriform or ovoid to sphaeropedunculate vesicles; fertile branches –3; phialides doliiform to reniform; macroconidia (1–)3-septate, 58 × 4 µm ............................................................. Ca. brasiliensis

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

54. Macroconidia 1-septate .............................................. 56

55. Macroconidia more than 1-septate .................................................. 57

56. Macroconidiophore branches –3 .................................................. 58

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

58. Teleomorph state heterothallic .................................................. 59
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. polizzi

59. Macroconidia up to 45 μm long ................................................................. 60

59. Macroconidia longer than 45 μm ................................................................. 63

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

60. Macroconidiophore branches –4 ................................................................. 61

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

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

62. Teleomorph state unknown; phialides doliiform to reniform; macroconidia 1-septate, 44 × 5 μm .......................... Ca. cerciana

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

63. Teleomorph state unknown; stipe extensions terminating in obpyriform to ellipsoidal vesicles; fertile branches –4; phialides doliiform to reniform; macroconidia 1-septate, 48 × 4 μm .......................... Ca. pseudoscoparia

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

64. Macroconidiophore branches –3 ................................................................. 65

65. Teleomorph state homothallic; perithecia orange to red-brown; ascospores 1(–3)-septate, 50 × 5.5 μm; stipe extensions terminating in broadly ellipsoidal vesicles; phialides doliiform to reniform; macroconidia (1–)3-septate, 50–70 × 5–6 μm .......................... Ca. pyrochona

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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REFERENCES

Crous PW (2002). Taxonomy and pathology of Cylindrocladium (Calonectria) and allied genera. APS Press, St. Paul, Minnesota, U.S.A.


