Phylogeny and systematics of the genus Calonectria

L. Lombard1*, P.W. Crous2, B.D. Wingfield3 and M.J. Wingfield1

1Department of Microbiology and Plant Pathology, Tree Protection Co-operative Programme, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa; *CBS-KNAW Fungal Biodiversity Centre, Uppsalaalan 5, 3584 CT Utrecht, The Netherlands; 2Department of Genetics, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa

Correspondence: Lorenzo Lombard, lorenzo.lombard@fabi.up.ac.za

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. pseudosporangia and Ca. sulawesiensis, bringing the total number of currently accepted Calonectria species to 68. A multigene phylogeny was subsequently constructed for all available Calonectria spp., employing seven gene regions, namely actin, β-tubulin, calmodulin, histone H3, the internal transcribed spacer regions 1 and 2 and the 5.8S gene of the ribosomal RNA, 28S large subunit RNA gene and translation elongation 1-alpha. Based on these data 13 phylogenetic groups could be distinguished within the genus Calonectria that correlated with morphological features. Dichotomous and synoptic keys to all Calonectria spp. currently recognised are also provided.

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

Taxonomic novelties: New combinations - Calonectria angustata (Crous & El-Gholl) L. Lombard, M.J. Wingf. & Crous, Ca. australiensis (Crous & H.D. Hyde) L. Lombard, M.J. Wingf. & Crous, Ca. canadensis (J.C. Kang, Crous & C.L. Schoch) L. Lombard, M.J. Wingf. & Crous, Ca. chinensis (Lombard, M.J. Wingf. & Crous, Ca. citri (H.S. Fawc. & Klotz) L. Lombard, M.J. Wingf. & Crous, Ca. curvata (Boedijn & Rettem) L. Lombard, M.J. Wingf. & Crous, Ca. curvispora (Crous & D. Victor) L. Lombard, M.J. Wingf. & Crous, Ca. eucalypti (Crous & M.J. Wingf.) L. Lombard, M.J. Wingf. & Crous, Ca. gordoniae (Leahy, T.S. Schub. & El-Gholl) L. Lombard, M.J. Wingf. & Crous, Calonectria hawksworthii (Peerauly) L. Lombard, M.J. Wingf. & Crous, Calonectria hureae (Crous) L. Lombard, M.J. Wingf. & Crous, Calonectria indonesiae (Crous) L. Lombard, M.J. Wingf. & Crous, Ca. leucothoei (El-Gholl, Leahy & T.S. Schub.) L. Lombard, M.J. Wingf. & Crous, Ca. malesiana (Crous) L. Lombard, M.J. Wingf. & Crous, Ca. multiphialidica (Crous, Simoneau & Risède) L. Lombard, M.J. Wingf. & Crous, Ca. pacifica (J.C. Kang, Crous & C.L. Schoch) L. Lombard, M.J. Wingf. & Crous, Ca. peniciloides (Tubaali) L. Lombard, M.J. Wingf. & Crous, Ca. pseudosporangia (Crous, J.Z. Groenew. & C.F. Hill) L. Lombard, M.J. Wingf. & Crous, Ca. sumatrensis (Crous) L. Lombard, M.J. Wingf. & Crous. New species - Ca. densa L. Lombard, M.J. Wingf. & Crous, Ca. eucalypti L. Lombard, M.J. Wingf. & Crous, Ca. humicola L. Lombard, M.J. Wingf. & Crous, Ca. orientalis L. Lombard, M.J. Wingf. & Crous, Ca. pinii L. Lombard, M.J. Wingf. & Crous, Ca. pseudosporangia L. Lombard, M.J. Wingf. & Crous, Ca. sulawesiensis L. Lombard, M.J. Wingf. & Crous.

INTRODUCTION

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

The reliability of vesicle shape as a distinguishing morphological character has been questioned (Sober & Afieri 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. multi septata (Peerally 1991, Crous & Wingfield 1994, Crous et al. 1998b, Crous 2002). Mega- and microconidia are less frequently encountered and these are not regarded as important characters to distinguish between species (Sober 1971, Crous & Wingfield 1994, Crous & Seifert 1998, Crous 2002). Similar to vesicle shape,
significant variability can occur in the production of all conidial types, so that this feature alone is not always a reliable taxonomic character to define species.

Both homothallic and heterothallic mating systems are found amongst species of Calonectria (Afferi 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 (protopodidya) 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) (Ovreymeyer et al. 1996, Victor et al. 1997, Schoch et al. 2000a, Risède & Simioneau 2004), restriction fragment length polymorphisms (RFLP) (Crous et al. 1993b, Crous et al. 1995, Crous et al. 1997, Bengt et al. 1997, Victor et al. 1997, Risède & Simioneau 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 Bengt et al. (1997) to distinguish between Cy. scoparium and Cy. floridanum isolates.

Subsequently, it was found that this gene region contains few informative characters for members of the genus (Crous et al. 1999, Schoch et al. 1999, Risède & Simioneau 2001, Schoch et al. 2001b). As a consequence, this resulted in the β-tubulin (BT) (Schoch et al. 2001b) and histone H3 (HIS3) (Kang et al. 2001b) gene regions being widely employed to improve the resolution of phylogenetic trees for species of Calonectria.

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

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

MATERIALS AND METHODS

Isolates

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

DNA extraction and amplification

Identification of unknown Calonectria isolates

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

Phylogenetic relationships amongst Calonectria spp.

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

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

DNA sequencing and analysis

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

Maximum-parsimony genealogies, for single genes and the combined genes were estimated in PAUP, by heuristic searches based on 1 000 random addition sequences and tree bisection-reconnection, with the branch swapping option set on “best trees” only. All characters were weighted equally and alignment gaps were treated as missing data. Statistics calculated for parsimony included tree length (TL), consistency index (CI), retention index (RI) and rescaled 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 et al. 1990).

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

Sexual compatibility

Based on the results of the DNA sequence analyses, single conidial isolates of Calonectria spp. of unknown identity were crossed with closely related species in all possible combinations. Where available, mating tester strains defined in previous studies were 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; Gueber & Correll 2001, Halleen et al. 2006) with sterile toothpicks placed on the surface of the agar (Lombard et al. 2010a). Controls consisted of isolates self-crossed, making it possible to distinguish between those having heterothallic or homothallic mating systems. Isolates CBS 125273–125276 from Indonesia were mated with Ca. macroconidialis (CBS 114880). Colombian isolates CBS 123698 and CMW 31210 and Indonesian isolates CBS 125258–125260 were crossed with Ca. brachiatica (CBS 123700 and CMW 25302) and Ca. brassicaceae (CBS 114178 and CBS 111869) in all possible combinations. Isolates CBS 125248, CBS 125253, CBS 125277 and CMW 14883 were crossed with Ca. cerciana (CBS 123693 and CBS 123695), Ca. brasiliensis (CBS 230.51 and CBS 114257) and mating tester strains of Ca. insularis (CBS 114558 and CBS 114559; Schoch et al. 1999). Similarly, isolates CBS 125249–125252, CBS 125261 and CBS 125269 were crossed with mating tester strains of Ca. spatiphyllyi (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).
Table 1. Isolates of *Calonectria* spp. studied.

| Species          | Isolate number¹ | Other collections¹ | ACT   | BT    | CAL   | HIS3  | ITS   | LSU   | TEF-1α | Reference²     |
|------------------|-----------------|--------------------|-------|-------|-------|-------|-------|-------|--------|----------------|
| *Ca. acicola*    | CBS 114812      |                    | GQ280424 | DQ190590 | GQ267539 | DQ190692 | GQ280546 | GQ280668 | GQ267291 | Gadgil & Dick (2004) |
| *Ca. angustata*  | CBS 114813      | CMW 30996          | GQ280425 | DQ190591 | GQ267360 | DQ190693 | GQ280547 | GQ280669 | GQ267292 | Crous (2002)    |
| *Ca. asiatica*   | CBS 110731      | CPC 3898           | GQ280429 | AY725613 | AY725738 | AY725655 | GQ280551 | GQ280673 | AY725702 | Crous et al. (2004b) |
| *Ca. australiensis* | CBS 112711     | CMW 25298          | GQ280433 | FJ696388 | GQ267366 | FJ696396 | GQ280555 | GQ280677 | GQ267296 | Lombard et al. (2009) |
| *Ca. australiensis* | CBS 112954     | CMW 23782          | CMW 30982 | CPC 3800 | GQ267538 | DQ190593 | GQ280549 | GQ280670 | FJ918552 | Lombard et al. (2009) |
| *Ca. brassicae*  | CBS 114778      | CMW 30981          | GQ280435 | AF348212 | AY725743 | AF348228 | GQ280556 | GQ280678 | GQ267294 | Crous (2002)    |
| *Ca. brasiliensis* | CBS 113571     | CMW 32949          | CMW 23690 | CPC 2390 | GQ267241 | GQ267259 | GQ280624 | GQ280746 | GQ267328 | Lombard et al. (2009c) |
| *Ca. canadensis* | CBS 110817      | CMW 25293          | GQ280502 | GQ267242 | GQ267259 | GQ267296 | GQ280505 | GQ280677 | GQ267296 | Lombard et al. (2009) |
| *Ca. cerciana*   | CBS 123693      | CMW 25200          | GQ280437 | FJ918510 | GQ267369 | FJ918528 | GQ280559 | GQ280681 | FJ918559 | Lombard et al. (2010c) |
| *Ca. chinensis*  | CBS 112744      | CMW 30986          | GQ280440 | AY725618 | AY725746 | AY725660 | GQ280562 | GQ280684 | AY725709 | Crous et al. (2004b) |
| *Ca. colhounii*  | CBS 114527      | CMW 23674          | GQ280390 | AY725619 | AY725747 | AY725661 | GQ280561 | GQ280683 | AY725710 | Lombard et al. (2010a) |
| *Ca. colombiana* | CBS 115127      | CMW 30871          | GQ280441 | AF333333 | GQ267371 | GQ267247 | GQ280563 | GQ280685 | GQ267299 | Crous (2002)    |
| *Ca. colombiensis* | CBS 115457     | CMW 30994          | GQ280449 | AF333336 | GQ267377 | DQ190623 | GQ280571 | GQ280693 | GQ267305 | Crous (2002)    |
| *Ca. colombiensis* | CBS 114666     | CMW 30994          | GQ280450 | DQ190594 | GQ267378 | DQ190624 | GQ280572 | GQ280694 | GQ267306 | Lombard et al. (2009) |
| *Ca. colomnii*   | CBS 293.79      | CMW 30999          | GQ280443 | DQ190564 | GQ267373 | DQ190639 | GQ280565 | GQ280687 | GQ267301 | Crous (2002)    |
| *Ca. colombiana* | CBS 114704      | CMW 30964          | GQ280442 | DQ190563 | GQ267372 | DQ190638 | GQ280564 | GQ280686 | GQ267300 | Lombard et al. (2010a) |
| *Ca. colombiana* | CBS 115127      | CMW 30871          | GQ280538 | FJ972423 | GQ267455 | FJ972442 | GQ280660 | GQ280782 | FJ972492 | Lombard et al. (2010a) |
| *Ca. colombiana* | CBS 115639      | CMW 30763          | GQ280539 | FJ972422 | GQ267456 | FJ972441 | GQ280661 | GQ280783 | FJ972491 | Lombard et al. (2010a) |
| *Ca. colombiana* | CBS 112220      | CMW 23676          | GQ280444 | GQ267207 | AY725748 | AY725662 | GQ280566 | GQ280688 | AY725711 | Crous et al. (2004b) |
| *Ca. colombiana* | CBS 112221      | CMW 30986          | GQ280445 | AY725620 | AY725749 | AY725663 | GQ280567 | GQ280689 | AY725712 | Crous (2002)    |

¹ Isolate number and other collections refer to the species name followed by the number assigned to the isolate and the code for the collection. ² GenBank accession number for each isolate.
## Table 1. (Continued).

| Species         | Isolate number | Other collections | GenBank accession nr. |
|-----------------|----------------|-------------------|-----------------------|
| **Ca. curvispora** | CBS 116159    | CMW 23693         | GQ280446 AF33394 GQ267374 AT275664 GQ280568 GQ280690 GQ267302 Crous (2002) |
| **Ca. densa**    | CBS 125249    | CMW 31184         | GQ280523 GQ267230 GQ26742 GQ267729 GQ280845 GQ280767 GQ267350 This study |
| **Ca. eucalypti** | CBS 125273    | CMW 14890         | GQ280510 GQ267217 GQ267429 GQ267266 GQ280832 GQ280754 GQ267337 This study |
| **Ca. gordoniae** | CBS 111406    | CMW 23677         | GQ280447 DQ190600 GQ267375 DQ190705 GQ280569 GQ280691 GQ267303 |
| **Ca. gracilipes** | CBS 111141    | CMW 31390         | GQ280457 DQ190566 GQ267385 DQ190644 GQ280579 GQ280701 GQ267311 Crous (2002) |
| **Ca. gracilis** | CBS 111284    | CMW 23677         | GQ280458 AF333406 GQ267233 GQ267445 GQ267282 GQ280848 GQ280770 GQ267353 This study |
| **Ca. hawksworthii** | CBS 111870   | CPC 2405          | GQ280458 AF333407 GQ267386 GQ267431 GQ267288 GQ280834 GQ267566 GQ267339 |
| **Ca. hongkongensis** | CBS 114711   | CMW 30995         | GQ280460 AY1725621 AY1725754 AY1725666 GQ280852 GQ280704 AY1725716 Crous et al. (2004b) |
| **Ca. humicola** | CBS 125251    | CMW 31183         | GQ280526 GQ267233 GQ267445 GQ267282 GQ280848 GQ280770 GQ267354 This study |
| **Ca. hurae**   | CBS 114581    | CPC 2405          | GQ280461 AF333408 GQ267387 GQ280649 GQ280580 GQ280702 FJ918558 Crous (2002) |
| **Ca. indici**  | CBS 190.50    | CMW 30995         | GQ280483 AY1725631 AY1725764 AY1725666 GQ280852 GQ280704 AY1725716 Crous et al. (2004b) |
| **Ca. indonesiae** | CBS 112623   | CMW 23663         | GQ280472 AY1725631 AY1725764 AY1725666 GQ280852 GQ280704 AY1725716 Crous et al. (2004b) |
| **Ca. indusiata** | CBS 112640    | CPC 4547          | GQ280464 AY1725625 AY1725758 AY1725670 GQ280586 GQ280708 AY1725720 Crous et al. (2004b) |
| **Ca. insularis** | CBS 114558    | CMW 30991         | GQ280459 AF210861 GQ267389 FJ918526 GQ280857 GQ280709 FJ918556 Crous (2002) |
| **Ca. kyotensis** | CBS 170.77    | CMW 23679         | GQ280536 GQ267239 GQ267453 GQ280658 GQ280780 GQ267332 Crous (2002) |
| **Ca. leguminum** | CBS 115897    | CMW 23684         | GQ280472 AY1725625 AY1725758 AY1725670 GQ280586 GQ280708 AY1725720 Crous et al. (2004b) |
| **Ca. leucothoës** | CBS 114599    | CMW 30992         | GQ280464 AF210862 GQ267390 FJ918525 GQ280588 GQ280710 FJ918555 Crous (2002) |
| **Ca. macroconidialis** | CBS 114880 | CPC 307           | GQ280469 AF232865 GQ267393 LQ190655 GQ280591 GQ280713 GQ267313 Crous (2002) |
| Species              | Isolate number | Other collections | GenBank accession nr. | Reference       |
|---------------------|----------------|-------------------|-----------------------|-----------------|
| Ca. madagascariensis | CBS 114571     | CMW 30983         | GQ280471 DQ190571 GQ267395 DQ190657 GQ280593 GQ280715 GQ267315 | Crous (2002)    |
|                     | CBS 114572     | CMW 23686         | GQ280470 DQ190572 GQ267394 DQ190658 GQ280592 GQ280714 GQ267314 |                 |
| Ca. malesiana       | CBS 112710     | CPC 3899          | GQ280473 AY725626 AY725759 AY725671 GQ280595 GQ280717 AY725721 | Crous et al. (2004b) |
| Ca. mexicana        | CBS 112752     | CMW 23687         | GQ280472 AY725627 AY725760 AY725672 GQ280594 GQ280716 AY725722 |                 |
| Ca. morganii        | CBS 110666     | CMW 30978         | GQ280504 FJ918509 GQ267423 FJ918527 GQ280626 GQ280748 FJ918857 | Crous (2002)    |
| Ca. multiphialidica | CBS 112678     | CMW 23689         | GQ280475 AY725628 AY725761 AY725673 GQ280597 GQ280719 AY725723 | Crous et al. (2004b) |
| Ca. multiseptata    | CBS 112682     | CMW 23692         | GQ280476 DQ190573 GQ267397 DQ190659 GQ280598 GQ280720 FJ918535 | Crous (2002)    |
| Ca. naviculata      | CBS 101121     | CMW 30974         | GQ280478 GQ267211 GQ267399 GQ267252 GQ280600 GQ280722 GQ267317 | Crous (2002)    |
| Ca. orientalis      | CBS 116080     | CMW 16723         | GQ280477 AF333409 GQ267398 GQ267251 GQ280599 GQ280721 GQ267316 |                 |
| Ca. ovata           | CBS 125259     | CMW 20272         | GQ280531 GQ267238 GQ267450 GQ267287 GQ280653 GQ280775 GQ267358 |                 |
| Ca. ovata           | CBS 125260     | CMW 20291         | GQ280532 GQ267236 GQ267448 GQ267285 GQ267651 GQ280773 GQ267356 |                 |
| Ca. pacifica        | CBS 111307     | CMW 30979         | GQ280480 AF210868 GQ267401 GQ267254 GQ280602 GQ280724 GQ267319 |                 |
| Ca. paucirotans     | CBS 114038     | CMW 30988         | GQ280482 AY725630 GQ267402 AY725675 GQ280604 GQ280726 GQ267320 |                 |
| Ca. paucinovacula   | CMW 5683       | CPC 971           | GQ280486 FJ918514 GQ267405 FJ918531 GQ280608 GQ280730 FJ918565 | Crous (2002)    |
| Ca. penicilloides   | CBS 174.55     | CMW 23696         | GQ280487 AF333414 GQ267406 GQ267257 GQ280609 GQ280731 GQ267322 | Crous (2002)    |
| Ca. pini            | CBS 123698     | CMW 31209         | GQ280517 GQ267224 GQ267436 GQ267273 GQ280639 GQ280761 GQ267344 |                 |
| Ca. polizzi         | CBS 125270     | CMW 7804          | GQ280544 FJ972417 GQ267461 FJ972436 GQ268066 GQ280788 FJ972486 | Lombard et al. (2010a) |
| Ca. pseudonaviculata| CBS 114417     | CMW 23672         | GQ280490 GQ267214 GQ267409 GQ267258 GQ280612 GQ280734 GQ267325 | Crous et al. (2002) |
| Ca. pseudoreteaudii | CBS 123694     | CMW 25310         | GQ280492 FJ918504 GQ267411 FJ918519 GQ280614 GQ280736 FJ918541 | Lombard et al. (2010c) |
| Ca. pseudovulcanoides| CBS 123699    | CMW 25292         | GQ280491 FJ918505 GQ267410 FJ918520 GQ280613 GQ280735 FJ918542 |                 |
| Ca. pseudoscoparia  | CBS 125254     | CMW 15214         | GQ280519 GQ267226 GQ267438 GQ267275 GQ280641 GQ280763 GQ267346 | This study      |
| Ca. pseudospathiphylli| CBS 109162   | CMW 30976         | GQ280493 FJ918513 GQ267412 AF348241 GQ280615 GQ280737 FJ918562 | Crous (2002)    |
| Ca. pteridis        | CBS 111793     | CMW 16736         | GQ280494 DQ190578 GQ267413 DQ190679 GQ280616 GQ280738 FJ918563 | Crous (2002)    |
| Ca. pyrochae        | CBS 749.70     | CMW 23682         | GQ280482 GQ267210 GQ267388 GQ267250 GQ280594 GQ280766 GQ267312 | Crous et al. (2006) |
| Species          | Isolate number | Other collections | GenBank accession nr. | Reference |
|------------------|----------------|-------------------|-----------------------|-----------|
| Ca. queenslandica | CBS 112146     | CMW 30604 = CPC 3213 | GQ280496 AF389835 GQ267415 FJ918521 Q280618 GQ280740 FJ918543 Lombard et al. (2010c) |
|                  | CBS 112155     | CMW 30603 = CPC 3210 | GQ280497 AF389834 GQ267416 Q2190667 Q280619 GQ280741 FJ918544 |
| Ca. reteaudii     | CBS 112143     | CMW 16738 = CPC 3200 | GQ280499 GQ240642 GQ267418 Q2190660 Q280621 GQ280743 FJ918536 Crous (2002) |
|                  | CBS 112144     | CMW 30984 = CPC 3201 | GQ280498 AF389833 GQ267417 Q2190661 Q280620 GQ280742 FJ918537 |
| Ca. rumohrae      | CBS 109062     | CMW 30989 = CPC 1603 | GQ280501 AF322873 GQ267420 Q2190676 Q280623 GQ280745 FJ918550 Crous (2002) |
|                  | CBS 111431     | CMW 23697 = CPC 1716 | GQ280500 AF322871 GQ267419 Q2190675 Q280622 GQ280744 FJ918549 |
| Ca. scoparia      | CMW 31000      | CPC 1675 = UFV 117  | GQ280435 FJ972426 GQ267367 FJ972476 Q280557 GQ280679 FJ972525 Crous (2002) |
|                  | CMW 31001      | UFV 126            | GQ280436 FJ972427 GQ267366 GQ267246 Q280558 GQ280680 GQ267246 |
| Ca. spathiphylli  | CBS 114540     | CMW 16742          | GQ280505 AF348214 GQ267424 AF348230 Q280627 GQ280749 GQ267330 Crous (2002) |
|                  | CBS 116168     | CMW 30997          | GQ280506 FJ918512 GQ267425 FJ918530 Q280628 GQ280750 FJ918561 |
| Ca. spathulata    | CBS 555.92     | CMW 16744          | GQ280508 GQ267215 GQ267427 GQ267261 Q280630 GQ280752 GQ267331 Crous (2002) |
|                  | CBS 112689     | CMW 16745          | GQ280507 AF308463 GQ267426 FJ918524 Q280629 GQ280751 FJ918554 |
| Ca. sulawesiensis| CBS 125248     | CMW 14857          | GQ280516 GQ267223 GQ267435 GQ267272 Q280638 GQ280760 GQ267343 This study |
|                  | CBS 125253     | CMW 14879          | GQ280513 GQ267220 GQ267432 GQ267269 Q280635 GQ280757 GQ267340 |
|                  | CBS 125277     | CMW 14878          | GQ280515 GQ267222 GQ267434 GQ267271 Q280637 GQ280759 GQ267342 |
|                  | CMW 14883      | GQ280514 GQ267221 GQ267433 GQ267270 Q280636 GQ280758 GQ267341 |
| Ca. sumatrensis   | CBS 112829     | CMW 23696 = CPC4518| GQ280532 AY725649 AY725771 AY725696 Q280654 GQ280776 AY725733 Crous et al. (2004b) |
|                  | CBS 112934     | CMW 30987 = CPC 4516| GQ280533 AY725651 AY725773 AY725798 Q280655 GQ280777 AY725735 |
| Ca. terrae-reginae| CBS 112151     | CMW 30601 = CPC 3202| GQ280534 FJ918506 GQ267451 FJ918522 Q280656 GQ280778 FJ918545 Lombard et al. (2010c) |
|                  | CBS 112634     | CMW 30602 = CPC 4233| GQ280535 FJ918507 GQ267452 Q2190668 Q280657 GQ280779 FJ918546 |
| Ca. variabilis    | CBS 112691     | CMW 2914           | GQ280541 GQ267240 GQ267458 GQ267264 Q280663 GQ280785 GQ267335 Crous (2002) |
|                  | CBS 114677     | CMW 3187           | GQ280540 AF333424 GQ267457 GQ267263 Q280662 GQ280764 GQ267334 |
| Ca. zuluensis     | CBS 125268     | CMW 9188          | GQ280542 FJ972414 GQ267459 FJ972433 Q280664 GQ280786 FJ972483 Lombard et al. (2010a) |
|                  | CBS 125272     | CMW 9896           | GQ280543 FJ972415 GQ267460 FJ972434 Q280665 GQ280787 FJ972484 |

1 CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Pedro Crous working collection housed at CBS; IMI: International Mycological Institute, CABIL-Bioscience, Egham, Bakeham Lane, U.K.; ATCC: American Type Culture Collection, Virginia, U.S.A.; UFV: Universidade 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.
Fig. 1. The most parsimonious trees obtained from a heuristic search with 1 000 random additions sequences of the combined BT, HIS3 and TEF-1α sequence alignments of the Ca. colhounii complex. Scale bar shows 10 changes and bootstrap support values (bold) from 1 000 replicates and Bayesian posterior probability values are indicated at the nodes. Red lines indicate bootstrap values of 100 and posterior probabilities of 1.00. Thickened lines indicate branches in the strict consensus tree and the consensus tree of the Bayesian analyses.

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

| Species                | Table no. | β-tubulin |
|------------------------|-----------|-----------|
|                        | 167 207 398 507 | 58 290 362 454 456 43 105 106 107 108 109 264 457 472 |
| Ca. colhounii           | CBS 293.79 | AGACCA    |
|                        | CBS 114704 | AGACCA    |
| Ca. eucalypti          | CBS 125273 | GTGT-     |
|                        | CBS 125274 | GTGT-     |
|                        | CBS 125275 | GTGT-     |
|                        | CBS 125276 | GTGT-     |
| Ca. macroconidialis    | CBS 114880 | CGAC      |
| Ca. madagascariensis   | CBS 114571 | CGAT      |
|                        | CBS 114572 | CGAT      |
as outgroup taxa. For Bayesian analyses, a HKY+I+G model was selected for BT and TEF-1α, and GTR+I+G for HIS3 for all four data sets, which was incorporated in the analyses. The consensus trees obtained from the Bayesian analyses confirmed the tree topologies obtained with maximum-parsimony as well as bootstrap support. Therefore, only maximum-parsimony trees are presented with bootstrap values and posterior probabilities shown for well-supported branches.

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

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

The data set representing the Ca. brassicaceae 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 313 characters parsimony-informative. Parsimony analysis yielded one most parsimonious tree (Fig. 2; TL = 569 steps; CI = 0.931; RI = 0.918; RC = 0.791). In the tree, isolates CBS 123698 and CBS 125523 clustered close to but separate from Ca. brassicaceae (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. brassicaceae 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 18 unique alleles and isolates CBS 125258–125260, from Indonesia, clustered together closely related to Ca. brassicaceae and Ca. brachiatica. These Indonesian isolates also share 14 unique alleles, distinguishing them from Ca. brassicaceae and Ca. brachiatica (Table 3).

The third data set, represented by 16 ingroup taxa residing in the Ca. scoparia complex and closely related species, consisted of 1 530 characters including gaps for the three gene regions analysed. Of these characters, 1 114 were constant, 136 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.

| Species       | Isolate no. | β-tubulin | TEF-1α |
|---------------|-------------|-----------|--------|
| Ca. scoparia  | CMW 31000   | T         | -      |
|               | CMW 31001   | T         | -      |
| Ca. pauciramosa| CMW 5683    | T         | -      |
|               | CMW 30823   | T         | -      |
| Ca. pseudoscoparia | CBS 125254 | C         | C      |
|               | CBS 125255 | C         | C      |
|               | CBS 125256 | C         | C      |
|               | CBS 125257 | C         | C      |
of the aligned sequences yielded three most parsimonious trees (TL = 977 steps; CI = 0.784; RI = 0.825; RC = 0.647), one of which is presented in Fig. 4. In the tree, isolates CBS 125249–125252, CBS 125261 and CBS 125269 from Ecuador clustered in a clade (BP = 99 and PP = 1.00) with Ca. spathiphylli (CBS 114540 and CBS 116168) and Ca. pseudospathiphylli (CBS 109165), whereas isolates CBS 125248, CBS 125253, CBS 125277 and CMW 14883 from Indonesia clustered close to Ca. brasiliensis (CBS 230.51 and CBS 114257) but with low support (BP = 52; PP = 0.90) in a separate, well-supported clade (BP = 100; PP = 1.00). Isolates CBS 125248, CBS 125253, CBS 125277 and CMW 14883, that also clustered together in a well-supported clade (BP = 81; PP = 1.00). Both clades were separate from Ca. spathiphylli and Ca. pseudospathiphylli but closely related to these species.

The SNP analyses showed that isolates CBS 125249, CBS 125250 and CBS 125261 shared four unique alleles and CBS 125251, CBS 125252 and CBS 125269 shared seven unique alleles for the three gene regions. These isolates also shared an additional 33 alleles, distinguishing them from Ca. spathiphylli (Table 5). Isolates CBS 125248, CBS 125253, CBS 125277 and CMW 14883 shared eight unique alleles, distinguishing them from Ca. brasiliensis (CBS 230.51 and CBS 114257), Ca. cerciana (CBS 123693 and CBS 123695) and Ca. insularis (CBS 114558 and CBS 114559) (Table 6).

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

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

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

| Species | Isolate no. | β-tubulin | Histone H3 | TEF-1α |
|---------|-------------|-----------|------------|--------|
| Ca. spathiphylli | CBS 114540 | A T G C C G C T T C T G C T A C C A C T T T T A C T T - G A G - - - - T A C T | A T G C C G C T T C T G C T A C C A C T T T T A C T T - G A G - - - - T A C T |
| Ca. densa | CBS 125249 | A - A G A A T C C T T C T C T G G T C C C C G T C C C A C C C - A G A C A - - C T T G | A - A G A A T C C T T C T C T G G T C C C C G T C C C A C C C - A G A C A - - C T T G |
| Ca. humicola | CBS 125251 | T - A G A A T T T T C C T C T T C G T C C C C G T C C C C A C C C A G A C A C A T A T G | T - A G A A T T T T C C T C T T C G T C C C C G T C C C C A C C C A G A C A C A T A T G |

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

The data for the ITS and LSU datasets were treated separately, but these are not presented.
because they add little taxonomic value. However, all ITS and LSU sequences generated in this study have been deposited in GenBank and TreeBase (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. 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 Cylindrocladella lageniformis (CBS 112898) and C. peruviana (CPC 5614). Phylogenetic groups are indicated on the right.
Table 6. Single nucleotide polymorphisms comparisons between Ca. brasiliensis, Ca. insularis and Ca. sulawesiensis compared to Ca. cerciana.

| Species          | Isolate no. | β-tubulin | Histone H3 | TEF-1α |
|------------------|-------------|-----------|------------|--------|
|                  |             | 95 100    | 253 259    | 300 417| 452 98 103 104 105 109 143 263 439 |
| Ca. brasiliensis | CBS 230.51  | C A T C   | G C G A C T TA G | - - - - | G T C G |
|                  | CBS 114257  | C A T C   | G C G A C T TA G | - - - - | G T C G |
| Ca. cerciana     | CBS 123693  | T A T A C C C T C G | - - C G A - C G |
|                  | CBS 123695  | T A T A C C C T C G | - - C G A - C G |
| Ca. insularis    | CBS 114558  | T G A C C A C C A C A | G C A C A A A C A |
|                  | CBS 114559  | T G A C C A C C A C A | G C A C A A A C A |
| Ca. sulawesiensis| CBS 125248  | T A G T T T T G T T C C G A C G A - T A |
|                  | CBS 125253  | T A G T T T T G T T C C G A C G A - T A |
|                  | CBS 125277  | T A G T T T T G T T C C G A C G A - T A |
|                  | CMW 14883   | T A G T T T T G T T C C G A C G A - T A |

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

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

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

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

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

Sexual compatibility

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

Taxonomy

Based on morphological observations, phylogenetic inference and mating, numerous isolates of Calonectria spp. included in this study represent undescribed species. Species of Cylindrocladium (1892) represent anamorph states of Calonectria (1867) (Rossman et al. 1999). In an attempt to move to a single nomenclature for pleomorphic fungi, the teleomorph name takes precedence over the anamorph name when both types belong to the same holomorph. The species below are described as new species in Calonectria, which represents the older generic name for these holomorphs and follows Lombard et al. (2009, 2010a, c). All Cylindrocladium species without a Calonectria state, are also transferred to Calonectria.
**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 ellipsoid to sphaeropedunculate vesicles, 10–12 µm diam; lateral stipe extensions (90° to the axis) also present. **Conidiogenous apparatus** with a stipe bearing sphaeropedunculate vesicles, 10–12 µm diam; lateral stipe extensions septate, straight to flexuous, 149–192 µm long, 5–6 µm wide at the apical septum, terminating in ovoid to ellipsoid to sphaeropedunculate vesicles. **Macroconidia** cylindrical, rounded at both ends, (47–)50–58(–62) × (5–)6 µm (av. = 54 × 6 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. **Microconidia** not seen.

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

**Culture characteristics:** Colonies fast growing with optimal growth temperature at 25 ºC (growth at 15–35 ºC) on MEA, reverse umber 49–78 µm long, and 63–123 µm wide; primary branches asceptate, 20–29 × 5–6 µm; secondary branches asceptate, 16–20 × 4–6 µm; tertiary and additional branches (–4) asceptate, 9–16 × 3–5 µm, each terminal branch producing 2–4 phialides; phialides doliform to reniform, hyaline, asceptate, 11–16 × 2–4 µm; apex with minute periclinal thickening and inconspicuous collarette. **Macroconidia** cylindrical, rounded at both ends, straight, (47–)50–58(–62) × (5–)6 µm (av. = 54 × 6 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. **Mega- and microconidia** not seen.
to verona-brown after 7 d; moderate white aerial mycelium with moderate sporulation; chlamydospores extensive throughout the medium forming microsclerotia.

**Substrate:** Soil.

**Distribution:** Ecuador.

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

---

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

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

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

Perithecia solitary or in groups, yellow to orange, becoming brown with age; in section apex and body yellow to orange, base red-brown, sub-globose to ovoid, 325–510 µm high, 285–360 µm diam, body turning dark red, and base dark red-brown (KOH+). Perithecial walls rough consisting of 2 thick-walled layers: outside
Fig. 8. Calonectria eucalypti. A. Perithecium. B. Section through ostiolar region of a perithecium. C. A vertical section through a perithecium, showing wall layers. D. Ascus. E–G. Ascospores. H–L. Macroconidiophores. M–P. Conidiogenous apparatus with conidiophore branches and doliform to reniform or allantoid phialides. Q–U. Clavate to broadly clavate vesicles. V–W. Three-septate macroconidia. Scale bars: A = 90 µm, H–I = 70 µm, Other bars = 10 µm.
layer of *textura globulosa*, 45–90 µm wide; becoming more compressed towards inner layer of *textura angularis*, 12–18 µm wide; becoming thin-walled and hyaline towards the centre, outer cells 24–50 × 10–40 µm; inner cells 6–19 × 3–6 µm: perithelial 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 perithecium, 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 × 10–25 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 asceptate or 1-septate, 21–29 × 5–6 µm; secondary branches asceptate, 14–21 × 3–5 µm; tertiary branches and additional branches (–5), aseptate, 11–16 × 3–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, asceptate, 10–14 × 3–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (66–)69–75(–80) × (5–)6 µm (av. = 72 × 6 µm), 3-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Megaconidia* and *microconidia* not seen.

Specimens examined: **Indonesia**, Sumatra Utara, Aek Nauli, on leaf of Eucalyptus grandis, May 2005, M.J. Wingfield, Herb. PREM 60298, holotype of *Ca. eucalypti*, culture ex-type CMW 18444 = CBS 125275; Aek Nauli, on leaf of Eucalyptus grandis, May 2005, M.J. Wingfield, PREM 60299, culture CMW 149890 = CBS 125273; Aek Nauli, on leaf of Eucalyptus grandis, May 2005, M.J. Wingfield, culture CMW 18445 = CBS 125274.

**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**: Eucalyptus grandis.

**Distribution**: Indonesia.

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

*Calonectria humidula* 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 macroconidios cylindricus utrinque rotundatis reductis (45–)48–54(–56) × 4–5 µm mediocriter 51 × 5 µm, semel septatis, sine cicatrice abscissionis manifesta, cum muro 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 asceptate, 20–29 × 4–6 µm; secondary branches asceptate, 12–19 × 3–5 µm; tertiary branches asceptate, 9–16 × 3–5 µm, each terminal branch producing 2–4 phialides; phialides elongated doliiform to reniform, hyaline, asceptate, 10–15 × 3–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (45–)48–54(–56) × (4–)5 × 5 µm (av. = 51 × 5 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega- and microconidia* not seen.

Specimens examined: **Ecuador**, Pichincha Province, Las Golondrinas, from soil, Dec. 2004, M.J. Wingfield, Herb. PREM 60369 holotype of *Ca. humidula*, culture ex-type CMW 31183 = CBS 125251; Las Golondrinas, from soil, Jan. 2006, L. Lombard, culture CMW 31186 = CBS 125262; Las Golondrinas, from soil, Jan. 2006, L. Lombard, (Herb. PREM 60368) culture CMW 31187 = CBS 125269.

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

**Substrate**: Soil.

**Distribution**: Ecuador.

**Notes**: *Ca. humidula* is morphologically very similar to *Ca. densa*, *Ca. pseudopathiothecia* and *Ca. spathiphylli*. However, no lateral stipe extensions occur in this species, whereas these are common in *Ca. densa*. *Macroconidia* of *Ca. humidula* (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. pseudopathiothecia* (av. 52 × 4 µm).

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

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

Teleomorpha ignota. *Anamorpha* Ca. brachiaticae similis sed conidioporum tres vel minus sine extensionibus lateralis stipae, macroconidios cylindricis utrinque rotundatis reductis (43–)46–50(–53) × 4–5 µm mediocriter 46 × 4 µm, semel septatis, sine cicatrice abscissionis manifesta, cum muro hyalino in fasciculis parallelis cylindricis differt.
Telemorph unknown. Conidiophores with a stipe bearing penicillate suites of fertile branches, stipe extensions, and terminal vesicles; stipe seporate, hyaline, smooth, 60–169 × 6–12 µm; stipe extensions seporate, straight to flexuous, 90–218 µm long, 5–10 µm wide at the apical septum, terminating in clavate to broadly clavate vesicles, 5–10 µm diam. Conidiogenous apparatus 54–174 µm long, and 67–92 µm wide; primary branches aseptate, 19–30 × 4–7 µm; secondary branches aseptate, 16–29 × 4–6 µm; tertiary and additional branches (–5) aseptate, 10–20 × 5–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 10–19 × 2–5 µm; apex with minute periclinal thickening and inconspicuous collarette. Macroconidia cylindrical, rounded at both ends, straight, (43–)46–50(–53) × 4(–5) µm (av. = 48 × 4 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. Mega- and microconidia not seen.

Culture characteristics: Colonies fast growing with optimal growth temperature at 25 °C (growth at 15–35 °C) on MEA, reverse sepiabrown 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 telemorph structures for this group of fungi.

Specimens examined: Indonesia, Langam, from soil, June 2005, M.J. Wingfield, Herb. PREM 60003, holotype of Ca. orientalis, culture ex-type CMW 20291 = CBS 125260; Teso East, from soil, June 2005, M.J. Wingfield culture CMW 20273 = CBS 125259; Teso East, from soil, June 2005, M.J. Wingfield, culture CMW 20272 = CBS 125258.
Fig. 9. *Calonectria humicola*. A–F. Macroconidiophores. G–I. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. J–N. Globose to ovoid to sphaeropedunculate vesicles. O–P. One-septate macroconidia. Scale bars = 10 µm.

Fig. 10. *Calonectria orientalis*. A–C. Macroconidiophores. D–K. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. L–Q. Clavate vesicles. R–S. One-septate macroconidia. Scale bars = 10 µm.
Calonectria pini 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 ignota. Anamorpha Ca. brachiaticae similis sed ramis conidiophorae tres vel minus sine extensionibus lateribus stipae, macroconidios cylindricis utrinque rotundatis rectis (37–)40–48(–50) × 4–6 µm mediocriter 44 × 5 µm, semel septatis, sine cicatrice abscissionis manifesta, cum muco hyalino in fasciculis parallelis cylindricis differ.

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

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

Specimens examined: **Colombia**, Valle del Cauca, Buga, from *Pinus patula*, Sept. 2007, C.A. Rodas, Herb. PREM 60304, holotype of *C. pini*, culture ex-type CMW 31209 = CBS 123898; Buga, from *Pinus patula*, Sept. 2007, C.A. Rodas; Buga, from *Pinus patula*, Sept. 2007, C.A. Rodas, culture CMW 31210 = CBS 125253.

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

**Substrate:** *Eucalyptus grandis*.

**Distribution:** Ecuador.

**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 *C. pini* (av. 44 × 5 µm) are shorter than those of *Ca. brassicae* (av. 53 × 4.5 µm), *Ca. gracilis* (56 × 4.5 µm) and *Ca. orientalis* (av. 48 × 4 µm). This species also has fewer conidiophore branches than those mentioned above. *Calonectria pini* failed to produce perithecia when crossed with *Ca. brachiatica* and *Ca. brassicae*. This supports the findings of Crous et al. (2004b) and Lombard et al. (2009), that teleomorph structures are rarely observed in members of the *Ca. brassicae* complex.

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

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

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

**Teleomorph unknown.** *Conidiophores* with a stipe bearing penicillate suites of fertile branches, stipe extensions, and terminal vesicles; stipe septate, hyaline, smooth, 37–139 × 5–11 µm; stipe extensions septate, straight to flexuous, 113–262 µm long, 5–7 µm wide at the apical septum, terminating in 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 colarette. Macroconidia cylindrical, rounded at both ends, straight, (41–)45–51(–52) × 3–5 µm (av. = 48 × 4 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. Mega- and microconidia not seen.

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

**Etymology:** Name refers to the Indonesian island of Sulawesi, where the fungus was collected.
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.
Calonectria angustata (Crous & El-Gholl) L. Lombard, M.J. Wingf. & Crous, comb. nov. MycoBank MB515536. 
Basionym: Cylindrocladium angustumatum Crous & El-Gholl, Mycosen 41: 522. 2000.

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

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

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

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

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

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

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

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

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

Calonectria hurae (Linder & Whetzel) L. Lombard, M.J. Wingf. & Crous, comb. nov. MycoBank MB515546. 
Basionym: Cercospora hurae Linder & Whetzel, Mycosen 29: 656. 1937.
≡ Cylindrocladiopsis hurae (Linder & Whetzel) U. Braun, Mycotaxon 51: 40. 1994.

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

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

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

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

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

Calonectria penicilloides (Tubaki) L. Lombard, M.J. Wingf. & Crous, comb. nov. MycoBank MB515552. 
Basionym: Candelospora penicilloides Tubaki, Nogaoa 2: 58. 1952.
≡ Cylindrocladium penicilloides (Tubaki) Tubaki, J. Hattori Bot. Lab. 20: 154. 1955.

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

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

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

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

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

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

The two newly described species, *Ca. densa* and *Ca. humicola*, isolated from Ecuadorian soils reside in the *Ca. spatiphylli* complex as defined by Kang et al. (2001b). *Calonectria pseudospatiphylli* and *Ca. spatiphylli*, 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. pseudospatiphylli* being homothallic and *Ca. spatiphylli* being heterothallic (Kang et al. 2001b, Crous 2002). The mating strategies of *Ca. densa* and *Ca. humicola* could not be determined in this study. This complex of species appears to originate from Central and South America (Chase & Poole 1987, Kang et al. 2001b, Crous 2002).

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

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

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

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

The geographic distribution of the *Calonectria* spp. representing the various sub-clades of the unifying Prolate Group of isolates...
shows some correlation in their distribution. Calonectria spp. in the sub-clade representing the Ca. refeaudii 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 heterothalic and homothallic species (Crous 2002, Crous et al. 2004b).

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

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

The teleomorph state has not been seen for several species of Calonectria. Nonetheless Cylindrocladium spp., irrespective of whether their perithecial states are known or not, have been provided names in Calonectria. This is consistent with the view that 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 1992 (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
4. Ca. australiensis (Crous & K.D. Hyde) L. Lombard, M.J. Wingf. & Crous
5. Ca. avesiculata T.S. Schuh., El-Gholl, Alfieri & Schoult.
6. Ca. brachichila L. Lombard, M.J. Wingf. & Crous
7. Ca. brassicae (Panwar & Borha) L. Lombard, M.J. Wingf. & Crous
8. Ca. brasiliensis (Peerally) L. Lombard, M.J. Wingf. & Crous
9. Ca. canadensis (J.C. Kang, Crous & C.L. Schoch) L. Lombard, M.J. Wingf. & Crous
10. Ca. cerciana L. Lombard, M.J. Wingf. & Crous
11. Ca. chinensis (Crous) L. Lombard, M.J. Wingf. & Crous
12. Ca. citri (H.S. Fawc. & Klotz) L. Lombard, M.J. Wingf. & Crous
13. Ca. clavata Alfieri, El-Gholl & E.L. Barnard
14. Ca. colhounii Peerally
15. Ca. colombiana L. Lombard, M.J. Wingf. & Crous
16. Ca. colombiensis Crous
17. Ca. curvata (Boedijn & Reitsma) L. Lombard, M.J. Wingf. & Crous
18. Ca. curvispora (Crous & D. Victor) L. Lombard, M.J. Wingf. & Crous
19. Ca. densa L. Lombard, M.J. Wingf. & Crous
20. Ca. ecuadoriae (Crous & M.J. Wingf.) L. Lombard, M.J. Wingf. & Crous
21. Ca. eucalypti L. Lombard, M.J. Wingf. & Crous
22. Ca. gracilipes Crous & G.R.A. Mchau
23. Ca. gracilis Crous, M.J. Wingf. & Alfenas
24. Ca. gordoniae (Leahy, T.S. Schub. & El-Gholl) L. Lombard, M.J. Wingf. & Crous
25. Ca. hederae (Peerally) L. Lombard, M.J. Wingf. & Crous
26. Ca. hederae C. Booth & J.S. Murray
27. Ca. hongkongensis Crous
28. *Ca. humicola* L. Lombard, M.J. Wingf. & Crous
29. *Ca. hurae* (Linder & Whetzel) L. Lombard, M.J. Wingf. & Crous
30. *Ca. illicicola* Boedijn & Reitsma
31. *Ca. indonesiae* (Crous) L. Lombard, M.J. Wingf. & Crous
32. *Ca. industrata* (Seaver) Crous
33. *Ca. insularis* C.L. Schoch & Crous
34. *Ca. kyotensis* Tersh.
35. *Ca. leguminum* (Rehm) Crous
36. *Ca. leucothoës* (El-Gholl, Leahy & T.S. Schub.) L. Lombard, M.J. Wingf. & Crous
37. *Ca. macroconidialis* (Crous, M.J. Wingf. & Alfenas) Crous
38. *Ca. madagascariensis* Crous
39. *Ca. malesiana* (Crous) L. Lombard, M.J. Wingf. & Crous
40. *Ca. mexicana* C.L. Schoch & Crous
41. *Ca. morganii* Crous, Alfenas & M.J. Wingf.
42. *Ca. multiphialidica* (Crous, Simoneau & Risède) L. Lombard, M.J. Wingf. & Crous
43. *Ca. multisepata* 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. pteridis* Crous, M.J. Wingf. & Alfenas
50. *Ca. pteridis* (Desm.) Sacc.
51. *Ca. pseudonaviculata* (Crous, J.Z. Groenew. & C.F. Hill) L. Lombard, M.J. Wingf. & Crous
52. *Ca. pseudoreteaudii* L. Lombard, M.J. Wingf. & Crous
53. *Ca. pseudospathiphylli* J.C. Kang, Crous & C.L. Schoch
54. *Ca. pyrochoa* (Bun.) Crous & M.J. Wingf.
55. *Ca. queenslandica* L. Lombard, M.J. Wingf. & Crous
56. *Ca. reteaudii* (Bun.) C. Booth
57. *Ca. rumohrae* El-Gholl & Alfenas
58. *Ca. scoparia* Peerally
59. *Ca. spathiphylli* El-Gholl, J.Y. Uchida, Alfenas, T.S. Schub., Alfieri & A.R. Chase
60. *Ca. spathulata* El-Gholl, Kimbr., E.L. Barnard, Alfieri & Schoult.
61. *Ca. sulawesiensis* L. Lombard, M.J. Wingf. & Crous
62. *Ca. sumatrensis* (Crous) L. Lombard, M.J. Wingf. & Crous
63. *Ca. terrae-reginae* L. Lombard, M.J. Wingf. & Crous
64. *Ca. variabilis* Crous, B.J.H. Janse, D. Victor, G.F. Marias & Alfenas
65. *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, 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, 46, 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
      14, 26, 37, 38, 42, 53, 59, 60
   b. 100–200
      9, 11, 12, 15, 16, 18, 19, 25, 27, 28, 31, 34, 39, 41, 44, 51, 52, 57, 58, 68
   c. 201–300
      2, 3, 10, 13, 14, 21, 22, 24, 26, 30, 33, 35, 36, 40, 45, 46, 47, 48, 50, 54, 55, 56, 61, 62, 63, 64, 65, 66, 67
   d. Above 300
      4, 5, 6, 7, 20, 23, 29, 32, 37, 38, 42, 53, 59, 60

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

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

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

g. Globose  
   19, 28, 62
h. Naviculate  
   44, 52

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

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

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

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

Dichotomous key to Calonectria species

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

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

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

3. Teleomorph state unknown ............................................................................................................... 4
3. Teleomorph state known .................................................................................................................. 15
4. Macroconidia 1-septate only ................................................................. 5
4. Macroconidia more than 1-septate ........................................................... 8
5. Fertile branches –3; phialides doliform to reniform; macroconidia 1-septate, 44 × 5 µm; stipe extensions terminating in clavate vesicles .................................................. Ca. pini
5. Fertile branches –5 ................................................................. Ca. brachiatica
6. Lateral stipe extensions present; macroconidia 1–(2)-septate, 44 × 5 µm; stipe extensions terminating in clavate vesicles; fertile branches –5; phialides doliform to reniform .............................................................. 6
6. Lateral stipe extensions absent .......................................................... 7
7. Stipe extensions terminating in clavate vesicles; fertile branches –5; phialides doliform to reniform; macroconidia 1-septate, 53 × 4.5 µm ............................................................... Ca. brassicae
7. Stipe extensions terminating in clavate vesicles; fertile branches –5; phialides doliform 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; microconidia 1–3-septate, 44 × 4 µm ................................................................. Ca. pseudoretaudii
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 doliiform to reniform ................................................................. Ca. australiensis
10. Macroconidia 1(–)3-septate ................................................................. 11
11. Fertile branches –7; phialides doliform to reniform; macroconidia 51 × 4.5 µm; stipe extensions terminating in clavate vesicles ................................................................. Ca. ecuadoriae
11. Fertile branches –4; phialides doliform 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. multisepata
15. Macroconidial state known ................................................................. 16
16. Teleomorph state known and macroconidia 1-septate to 1(–3)-septate ................................................................. 17
16. Teleomorph state known and macroconidia multi-septate ................................................................. 20
17. Teleomorph homothallic ................................................................. 18
17. Teleomorph heterothallic ................................................................. 19
18. Perithecia orange with a red apex; ascospores 1-septate, 35 × 6.5 µm; stipe extensions terminating in clavate vesicles; fertile branches –4; phialides doliform to reniform; macroconidia 1-septate, 45 × 4.5 µm ................................................................. Ca. gracilipes
18. Perithecia red; ascospores 1-septate, 37 × 5 µm; stipe extensions terminating in narrowly clavate vesicles; fertile branches –4; phialides doliiform to reniform; macroconidia 1–(3)-septate, 56 × 4.5 µm .......................... **Ca. gracilis**

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

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

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

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

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

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

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

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

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

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

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

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

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

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

26. Teleomorph state homothallic; perithecia orange-brown; ascospores 3–6–(9)-septate, 90 × 6.5 µm; stipe extensions terminating in narrowly clavate vesicles; fertile branches –2; phialides cylindrical; 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 red-brown; ascospores 3–4-septate, 70 × 6 µm; stipe extensions, when present, terminating in narrowly clavate vesicles; fertile branches –1; macroconidia 5–7-septate, 75 × 7 µm; microconidia 1–3-septate, 10–30 × 3–5 µm .......................... **Ca. acicola**

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

42. Stipe extensions terminating in globose to ovoid to sphaeropedunculate vesicles; fertile branches –3; phialides doliiform to reniform; macroconidia 1-septate, 51 × 5 µm .............................................................. Ca. humicola
42. Stipe extensions terminating in sphaeropedunculate vesicles; fertile branches –5; phialides elongate-doliiform to reniform; macroconidia 1-septate, 50.5 × 4 µm .................................................................................. Ca. indonesiae
43. Lateral stipe extensions rare; stipe extensions terminating in pyriform to sphaeropedunculate vesicles; fertile branches –3; phialides doliiform to reniform; macroconidia 1-septate, 50 × 4 μm ........................................... Ca. canadensis
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 doliiform to reniform; macroconidia 1-septate, 47.5 × 4 μm ................................................................. Ca. malesiana
47. Stipe extensions terminating in sphaeropedunculate vesicles; phialides elongate-doliiform to reniform; macroconidia 1-septate, 55 × 4.5 μm ................................................................. Ca. pacifica
48. Vesicles pyriform to ellipsoidal or clavate, rarely ovoid, never obpyriform ........................................................................... 49
49. Macroconidia more than 1-septate .................................................................................................................................................... 50
50. Teleomorph state unknown; stipe extensions terminating in narrowly ellipsoidal to pyriform or ovoid to sphaeropedunculate vesicles; fertile branches –3; phialides doliiform to reniform; macroconidia (1–)3-septate, 58 × 4 μm ................................................ Ca. citri
51. Teleomorph state homothallic; perithecia orange-red; ascospores 1(–3)-septate, 33.5–69 × 4.5–7 μm; stipe extensions terminating in clavate to ovoid or ellipsoidal vesicles; fertile branches –4; phialides doliiform to reniform; macroconidia (1–)3(–5)-septate, (44–)50–70(–102) × 5–7(–8) μm ................................ Ca. hederae
52. Teleomorph state heterothallic; perithecia yellow to orange; ascospores 1-septate, 37 × 6 μm; stipe extensions terminating in ellipsoidal to pyriform or clavate vesicles; fertile branches –6; phialides doliiform to reniform; macroconidia 1-septate, 45 × 4 μm ................................................................. Ca. morganii
53. Macroconidia curved, 1-septate, 56 × 4 μm, stipe extensions terminating in ellipsoidal to clavate vesicles; fertile branches –4; phialides doliiform to reniform; teleomorph state unknown ........................................ Ca. hawksworthii
54. Macroconidia straight, 1-septate, 48 × 4 μm Teleomorph state unknown; stipe extensions terminating in broadly clavate to ellipsoidal vesicles; fertile branches –5; phialides doliiform to reniform; ............................................ Ca. sulawesiensis
55. Vesicles obpyriform to ellipsoidal ................................................................................................................................................... 55
56. Macroconidia 1-septate ................................................................................................................................................................. 56
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. 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. paucidicola

59. Macroconidia longer than 45 µm ..................................................................... 60

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

61. Vescules broadly ellipsoid 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

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

63. Macroconidiophore branches –6; 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

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. leucotricha

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

66. 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

67. 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

68. Teleomorph state unknown; fertile branches –3; phialides doliiform to reniform; macroconidia 1-septate, 42–68 × 4–6 µm ........................................... Ca. pseudonaviculata

ACKNOWLEDGEMENTS

We thank members of the Tree Protection Co-operative Programme (TPCP), the Centraalbureau voor Schimmelcultures (CBS), and the University of Pretoria for financial and technical support to undertake this study. We also thank Dr H. Glen, South African National Botanical Institute (SANBI), for the Latin descriptions and for valuable suggestions in naming the new species. The first author further acknowledges Drs. J.Z. Groenewald, G.C. Hunter and C. Guadan for advice regarding DNA sequence analyses.

REFERENCES

Alfieri SA, El-Gholl NE, Schouties CL (1982). Homothallism in Calonectria ilicicola. Mycologia 74: 513–514.
Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990). Basic Local Alignment Search Tool. Journal of Molecular Biology 215: 403–410.
Boedijn KB, Reitsma J (1950). Notes on the genus Cylindrocladium. Reinwardtia 1: 51–60.
Boesewinkel HJ (1982). Heterogeneity within Cylindrocladium and its teleomorphs. Transactions of the British Mycological Society 78: 553–556.
Carbone I, Kohn LM (1999). A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91: 553–556.
Chase AR, Poole RT (1987). Effects of potting medium pH and air temperature on severity of Cylindrocladium root and petiole rot of Spathiphyllum sp. Plant Disease 71: 509–511.
Crous PW (2002). Taxonomy and pathogenicity of Cylindrocladium (Calonectria) and allied genera. APS Press, St. Paul, Minnesota, U.S.A.
Crous PW, Alfenas AC, Junghans TG (1998a). Variability within Calonectria ovata and its anamorph Cylindrocladium ovatum from Brazil. Sydowia 50: 1–13.
Crous PW, Alfenas AC, Wingfield MJ (1993a). Calonectria scoparia and Calonectria morganii sp. nov., and variation among isolates of their Cylindrocladium anamorphs. Mycological Research 97: 701–708.
Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G (2004a). MycoBank: an online initiative to launch mycology in the 21st century. Studies in Mycology 50: 19–22.
Crous PW, Groenewald JZ, Hill CF (2002). Cylindrocladium pseudonaviculatum sp. nov. from New Zealand, and new Cylindrocladium records from Vietnam. Sydowia 54: 23–33.
Crous PW, Groenewald JZ, Riséide J-M, Simonpeau P, Hyde KD (2006). Calonectria species and their Cylindrocladium anamorphs: species with clavate vesicles. Studies in Mycology 55: 213–226.
Crous PW, Groenewald JZ, Riséide J-M, Simonpeau P, Hywel-Jones NL (2004b). Calonectria species and their Cylindrocladium anamorphs: species with sphaeropedunculate vesicles. Studies in Mycology 50: 415–430.
Rossman AY (1983). The phragmosporous species of Nectria and related genera. Mycological Papers 150: 1–164.

Rossman AY (1993). Holomorph hypocrealean fungi: Nectria sensu stricto and telemorphs of Fusarium. In: The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics. (Reynolds DR, Taylor JW, eds). CAB International, Wallingford, U.K.: 149–160.

Rossman AY, Samuels GJ, Rogerson CT, Lowen R (1999). Genera of Bionectriaceae, Hypocreaceae and Nectriaceae (Hypocreales, Ascomycetes). Studies in Mycology 42: 1–248.

Schoch CL, Crous PW, Polizzi G, Koike ST (2001a). Female fertility and single nucleotide polymorphism comparisons in Cylindrocladium pauciramosum. Plant Disease 85: 941–946.

Schoch CL, Crous PW, Wingfield BD, Wingfield MJ (1999). The Cylindrocladium candelandrum species complex includes four distinct mating populations. Mycologia 91: 286–298.

Schoch CL, Crous PW, Wingfield BD, Wingfield MJ (2001b). Phylogeny of Calonectria based on comparisons of β-tubulin DNA sequences. Mycological Research 105: 1045–1052.

Schoch CL, Crous PW, Cronright G, Withuhn RC, El-Gholl NE, Wingfield BD (2000a). Recombination in Calonectria morganii and phylogeny with other heterothallic small-spored Calonectria species. Mycologia 92: 665–673.

Schoch CL, Crous PW, Wingfield MJ, Wingfield BD (2000b). Phylogeny of Calonectria and selected hypocrealean genera with cylindrical macroconidia. Studies in Mycology 45: 45–62.

Schoch CL, Sung G-H, López-Giráldez F, Townsend JP, Miadlikowska J, et al. (2000). The Ascomycota Tree of Life: A phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. Systematic Biology 58: 224–239.

Schubert TS, El-Gholl NE, Alfieri SA, Schouttens CL (1989). Calonectria avesiculata sp. nov. Canadian Journal of Botany 67: 2414–2419.

SoberEK (1971). A macro-conidial form of Cylindrocladium theae occurring on glycerol-water agar. Georgia Academy of Science Bulletin 29: 98.

Sobers EK, Alfieri SA (1972). Species of Cylindrocladium and their hosts in Florida and Georgia. Proceedings of the Florida State Horticultural Society 85: 366–369.

Swoford DL (2002). PAUP*. Phylogenetic analysis using parsimony (* and other methods), v. 4.0b10. Computer programme. Sunderland, Massachusetts, U.S.A.: Sinauer Associates.

Taylor JW, Jacobson DJ, Kroken SM, Kasuga T, Geiser DM, et al. (2000). Phylogenetic species recognition and species concepts in fungi. Fungal Genetics and Biology 31: 21–32.

Victor D, Crous PW, Janse BJH, Wingfield MJ (1997). Genetic variation in Cylindrocladium floridanum and other morphologically similar Cylindrocladium species. Systemic and Applied Microbiology 20: 268–285.

Vitagly R, Hester M (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. Journal of Bacteriology 172: 4238–4246.

Vitale A, Polizzi G (2008). First record of leaf spots and stem lesions on Pistacia lentiscus caused by Cylindrocladium pauciramosum and C. scoparium in Italy. Plant Pathology 57: 384.

White TJ, Burns T, Lee S, Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols: a guide to methods and applications. (Innis MA, Gelfand DH, Snisky JJ, White TJ, eds) Academic Press, U.S.A.: 282–287.