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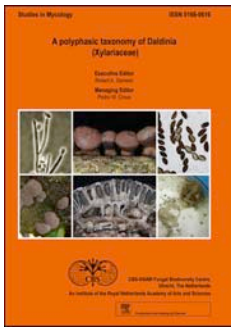

The forgotten *Calonectria* collection: pouring old wine into new bags

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The forgotten *Calonectria* collection: pouring old wine into new bags

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Abstract: The genus *Calonectria* with its *Cylindrocladium* asexual morphs has been subject to several taxonomic revisions in the past. These have resulted in the recognition of 116 species, of which all but two species (*C. hederae* and *C. pyrochroa*) are supported by ex-type cultures and supplemented with DNA barcodes. The present study is based on a large collection of unidentified *Calonectria* isolates that have been collected over a period of 20 years from various substrates worldwide, which has remained unstudied in the basement of the CBS-KNAW Fungal Biodiversity Centre. Employing a polyphasic approach, the identities of these isolates were resolved and shown to represent many new phylogenetic species. Of these, 24 are newly described, while *C. uniseptata* is reinstated at species level. We now recognise 140 species that include some of the most important plant pathogens globally.

Key words: *Cylindrocladium*, cryptic species, phylogeny, taxonomy.

Taxonomic novelties: New species: *Calonectria amazonica* L. Lombard & Crous, *C. amazoniensis* L. Lombard & Crous, *C. brasiliana* L. Lombard & Crous, *C. brassicicola* L. Lombard & Crous, *C. brevistipitata* L. Lombard & Crous, *C. cliffordiicola* L. Lombard & Crous, *C. ericae* L. Lombard & Crous, *C. indonesiana* L. Lombard & Crous, *C. lageniformis* L. Lombard & Crous, *C. machaerinae* L. Lombard & Crous, *C. multilateralis* L. Lombard & Crous, *C. paracolhounii* L. Lombard & Crous, *C. parva* L. Lombard & Crous, *C. plurilateralis* L. Lombard & Crous, *C. pseudoecuadoriae* L. Lombard & Crous, *C. pseudouxmalensis* L. Lombard & Crous, *C. putriramosa* L. Lombard & Crous, *C. stipitata* L. Lombard & Crous, *C. syzygiicola* L. Lombard & Crous, *C. tereticornis* L. Lombard & Crous, *C. terricola* L. Lombard & Crous, *C. tropicalis* L. Lombard & Crous, *C. uxmalensis* L. Lombard & Crous & *C. venezuelana* L. Lombard Crous.

INTRODUCTION

The genus *Calonectria*, first introduced in 1867 (Rossman 1979), has been the subject of numerous taxonomic studies since the 1990's (Crous & Wingfield 1993, Crous 2002, Lombard *et al*. 2010b, 2015a, Alfenas *et al*. 2015). These studies have resulted in the recognition of 116 species, of which all but two (*C. hederae* and *C. pyrochroa*) are supported by ex-type cultures and supplemented by DNA barcodes (Crous 2002, Lechat *et al*. 2010, Lombard *et al*. 2010b). This large number of species has arisen mainly due to the introduction of DNA sequence data and subsequent phylogenetic inference enabling delimitation of numerous previously unrecognised cryptic taxa. These species often share the same plant hosts, informing knowledge of the epidemiology and fungicide resistance (Graça *et al*. 2009, Vitale *et al*. 2013, Gehesquière *et al*. 2016).

Calonectria spp. are characterised by sexual morphs that have yellow to dark red perithecia, with scaly to warty ascocarp walls, and *Cylindrocladium* asexual morphs in which the cylindrical and septate conidia are produced from phialides clustered below and surrounding a stipe extention terminating in variously shaped vesicles (Rossman 1993, Crous 2002, Lombard *et al*. 2010b, c). For many years these fungi were best known by their *Cylindrocladium* names associated with important plant diseases (Crous & Wingfield 1994, Crous 2002, Lombard *et al*. 2010c). Following convention that only one scientific name should be used for a fungal species (Hawksworth *et al*. 2011, Hawksworth 2011, 2012, McNeill *et al*. 2012), *Calonectria* has been chosen (Rossman *et al*. 2013). This newly adopted convention should resolve confusion regarding their names (Wingfield *et al*. 2011). However, it is important to recognise that the asexual *Cylindrocladium* morph represents the life phase most commonly found in nature and many species are known only in this form, which also plays a major role in the dissemination of *Calonectria* spp.

Calonectria spp. cause important diseases in numerous plant hosts worldwide. This includes leaf blight, cutting rot, damping-off and root rot (Crous 2002, Vitale *et al*. 2013, Lombard *et al*. 2010c, 2015a, Alfenas *et al*. 2015). The majority of the diseases caused by *Calonectria* spp. are associated with forestry-related plants (see Lombard *et al*. 2010c), where Calonectria leaf blight (CLB) is an important constraint to plantation productivity in South America (Rodas *et al*. 2005, Alfenas *et al*. 2015) and Southeast Asia (Crous & Kang 2001, Old *et al*. 2003, Chen *et al*. 2011, Lombard *et al*. 2015a). In other regions, such as southern Africa and Australia, *Calonectria* spp. appear mostly to be limited to forestry nurseries (Crous 2002, Lombard *et al*. 2009, 2010a,b,c). In agricultural and horticultural crops, *Calonectria* spp. have chiefly been reported only from South America and the Northern Hemisphere, where they are mostly associated with nursery diseases (Lombard *et al*. 2010c,

Vitale *et al*. 2013), Cylindrocladium black rot of peanut (Bell & Sobers 1966, Beute & Rowe 1973, Hollowell *et al*. 1998) and box blight of *Buxus* spp. (Henricot *et al*. 2000, Crepel & Inghelbrecht 2003, Brand 2005, Saracchi *et al*. 2008, Saurat *et al*. 2012, Mirabolfathy *et al*. 2013, Gehesquière *et al*. 2016).

The present study is based on a large collection of unidentified *Calonectria* isolates that were collected over a period of 20 years from various substrates worldwide. This collection of isolates, deposited in the CBS-KNAW culture collection in 2002 has remained unstudied in the basement of the institute and hence, the title of this study "the forgotten basement collection". The large majority of these isolates were initially identified based solely on morphology and at a time when robust and multigene DNA sequence data were not commonly available. This implied that cryptic species could not be resolved (Lombard *et al*. 2010b, 2015a, Alfenas *et al*. 2015). The aim of the present study was to employ a polyphasic approach to identify these isolates.

MATERIALS AND METHODS

Isolates

Calonectria strains were obtained from the culture collection of the CBS-KNAW Fungal Biodiversity Centre (CBS), Utrecht, The Netherlands and the working collection of the senior author (CPC) housed at the CBS (Table 1).

Phylogeny

Total genomic DNA was extracted from 7-d-old axenic cultures, grown on MEA at room temperature, using the UltraClean™ Microbial DNA isolation kit (Mo Bio Laboratories, Inc., California, USA) following the protocols provided by the manufacturer. Based on previous studies (Lombard *et al*. 2010b, 2015b, Alfenas *et al*. 2015), partial gene sequences were determined for β-tubulin (*tub2*), calmodulin (*cmdA*), and the translation elongation factor 1-alpha (*tef1*) regions as these regions provided the best phylogenetic signal at species level for the genus *Calonectria*. Therefore, the primers and protocols described by Lombard *et al.* (2015b) were used to determine these regions.

To ensure the integrity of the sequences, the amplicons were sequenced in both directions using the same primers used for amplification. Consensus sequences for each locus were assembled in MEGA v. 7 (Kumar *et al*. 2016) and compared with representative sequences from Alfenas *et al.* (2013a,b, 2015), Chen *et al*. (2011) and Lombard *et al*. (2010a,b, 2011, 2015a). Subsequent alignments for each locus were generated in MAFFT v. 7.110 (Katoh & Standley 2013) and the ambiguously aligned regions of both ends were truncated. Congruency of the three loci were tested using the 70 % reciprocal bootstrap criterion (Mason-Gamer & Kellogg 1996) following the protocols of Lombard *et al*. (2015b).

Phylogenetic analyses of the individual gene regions and the combined dataset were based on Bayesian inference (BI), Maximum Likelihood (ML) and Maximum Parsimony (MP). For BI and ML, the best evolutionary models for each locus were determined using MrModeltest (Nylander 2004) and incorporated into the analyses. MrBayes v. 3.2.1 (Ronquist & Huelsenbeck 2003) was used for BI to generate phylogenetic trees under optimal criteria for each locus. A Markov Chain Monte Carlo (MCMC) algorithm of four chains was initiated in parallel from a random tree topology with the heating parameter set at 0.3. The MCMC analysis lasted until the average standard deviation of split frequencies was below 0.01 with trees saved every 1 000 generations. The first 25 % of saved trees were discarded as the "burn-in" phase and posterior probabilities (PP) were determined from the remaining trees.

The ML analyses were preformed using RAxML v. 8.0.9 (randomised accelerated (sic) maximum likelihood for high performance computing; Stamatakis 2014) through the CIPRES website (http://www.phylo.org) to obtain another measure of branch support. The robustness of the analysis was evaluated by bootstrap support (BS) with the number of bootstrap replicates automatically determined by the software.

For MP, analyses were done using PAUP (Phylogenetic Analysis Using Parsimony, v. 4.0b10; Swofford 2003) with phylogenetic relationships estimated by heuristic searches with 1 000 random addition sequences. Tree-bisection-reconnection was used, with branch swapping option set on "best trees" only. All characters were weighted equally and alignment gaps treated as fifth state. Measures calculated for parsimony included tree length (TL), consistency index (CI), retention index (RI) and rescaled consistence index (RC). Bootstrap analyses (Hillis & Bull 1993) were based on 1 000

replications. All new sequences generated in this study were deposited in GenBank (Table 1) and alignments and trees in TreeBASE.

Taxonomy

Axenic cultures were transferred to synthetic nutrient-poor agar (SNA; Nirenburg 1981) and incubated at room temperature for 7 d. Gross morphological characteristics were studied by mounting the fungal structures in 85 % lactic acid and 30 measurements were made at × 1 000 magnification for all taxonomically informative characters using a Zeiss Axioscope 2 microscope with differential interference contrast (DIC) illumination. The 95 % confidence levels were determined for the conidial measurements with extremes given in parentheses. For all other fungal structures measured, only the extremes are provided. Colony colour was assessed using 7-d-old cultures on MEA incubated at room temperature and the colour charts of Rayner (1970). All descriptions, illustrations and nomenclatural data were deposited in MycoBank (Crous *et al.* 2004a).

RESULTS

Phylogenetic analyses

Approximately 500−550 bases were determined for the three gene regions included in this study. The congruency analyses revealed no conflicts in tree topologies, with only minor differences in branch support. Therefore, the sequences of the three loci determined here were combined in a single dataset for analyses. For the BI and ML analyses, a HKY+I+G model was selected for all three gene regions and incorporated into the analyses. The ML tree topology confirmed the tree topologies obtained from the BI and MP analyses, and therefore, only the ML tree is presented.

The combined *cmdA*, *tef1* and *tub2* sequences dataset included 278 ingroup taxa and *Curvicladiella cignea* (CBS 109167) as outgroup taxon. This dataset consisted of 1 680 characters, of which 507 were constant, 198 parsimony-uninformative and 975 parsimony-informative. The MP analysis yielded 1 000 trees (TL = 6 998; CI = 0.344; RI = 0.867; RC = 0.298) and a single best ML tree with –InL = −32198.651254 which is presented in Fig. 1. The BI lasted for 10 M generations, and the consensus tree, with posterior probabilities, was calculated from 15 002 trees left after 5 000 trees were discarded as the 'burn-in' phase. In the phylogenetic tree (Fig. 1) the previously unnamed *Calonectria* species resolved in 21 distinct clades that were either well or strongly supported and 17 single lineages, each representing probable novel phylogenetic taxa.

Taxonomy

Based on phylogenetic inference supported by morphological observations, numerous *Calonectria* isolates included in this study represent novel species. No sexual morphs were observed for any of the novel taxa described below, even after 6 wk of incubation at room temperature. Fifteen of the lineages (CBS 111423, CBS 111468, CBS 111706, CBS 112152, CBS 112753, CBS 113496, CBS 113627, CBS 114164, CBS 114691, CBS 114755, CBS 116108, CBS 116249, CBS 116265, CBS 116305, CBS 116319) identified based on phylogenetic inference are not provided with names because they form part of a separate study (Crous *et al. in prep.*) or more taxa are required to resolve their phylogenetic position.

Calonectria amazonica L. Lombard & Crous, **sp. nov.** MycoBank MB818698. Fig. 2.
Etymology – Name refers to the Amazonian region of Brazil where this fungus was collected.

Macroconidiophores consist of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 75–190 × 6–8 µm; stipe extension septate, straight to flexuous, 180–270 µm long, 4–5 µm wide at the apical septum, terminating in a clavate vesicle, 5–6 µm diam. *Conidiogenous apparatus* 45–55 µm wide, and 60–80 µm long; primary branches aseptate, 22–32 × 4–6 µm; secondary branches aseptate, 14–24 × 3–5 µm; tertiary branches aseptate, 10–18 × 2–4 µm; quaternary branches aseptate, 10−15 × 3 µm, each terminal branch producing 2–4 phialides; phialides allantoid to elongate doliiform to reniform, hyaline, aseptate, 9–20 × 3–4 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight to slightly curved, (68–)74–84(–88) × (4−)4.5−5.5(−6) µm

(av. 79 × 5 µm), 1(−3)-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies moderately fast growing (40−65 mm diam) on MEA after 7 d at room temperature; surface sienna to sepia with moderate white, wooly aerial mycelium and moderate sporulation on the aerial mycelium and colony surface; reverse sienna to sepia with abundant chlamydospores throughout the medium, forming microsclerotia.

Specimens examined: **Brazil**, Amazon, from foliar lesion of *Eucalyptus tereticornis*, 1993, P.W. Crous & A.C. Alfenas (**holotype** CBS-H22750, culture ex-type CBS 116250 = CPC 3534); ibid., cultures CBS 115486 = CPC 3894.

Notes: *Calonectria amazonica* resides in the *C. pteridis* complex. The macroconidia of *C. amazonica* [(68–)74–84(–88) × (4−)4.5−5.5(−6) µm (av. 79 × 5 µm)] are slightly smaller than those of *C. pteridis* and *C. pseudopteridis* [(50–)70–100(–130) × (4−)5−6 µm (av. 82 × 5.5 µm); Crous 2002, Alfenas *et al*. 2015], but larger than those of *C. amazoniensis*, *C. lageniformis* and *C. tropicalis* (see below).

Calonectria amazoniensis L. Lombard & Crous, **sp. nov.** MycoBank MB818699. Fig. 3.
Etymology: Name refers to the Amazonian region of Brazil where this fungus was collected.

Macroconidiophores consist of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 45–240 × 6–9 µm; stipe extension septate, straight to flexuous, 140–280 µm long, 4–5 µm wide at the apical septum, terminating in a clavate vesicle, 5–7 µm diam; lateral stipe extensions (90° to main axis) few, 80–95 µm long, 2–4 µm wide at the apical septum, terminating in clavate vesicles, 2–3 µm diam. *Conidiogenous apparatus* 30–110 µm wide, and 30–100 µm long; primary branches aseptate, 15–31 × 4–6 µm; secondary branches aseptate, 10–26 × 3–5 µm; tertiary branches aseptate, 9–31 × 3–5 µm; quaternary branches and additional branches (−5) aseptate, 9−18 × 3–5 µm each terminal branch producing 2–4 phialides; phialides elongate doliiform to reniform, hyaline, aseptate, 7–17 × 3–5 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight to slightly curved, (56–)64–74(–75) × (4−)4.5−5.5(−6) µm (av. 69 × 4 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies moderately fast growing (40−65 mm diam) on MEA after 7 d at room temperature; surface sienna to amber with moderate white, wooly aerial mycelium and moderate sporulation on the aerial mycelium and colony surface; reverse sienna with abundant chlamydospores throughout the medium, forming microsclerotia.

Specimens examined: **Brazil**, Amazon, from foliar lesion of *Eucalyptus tereticornis*, 1993, P.W. Crous & A.C. Alfenas (**holotype** CBS-H22751 culture ex-type CBS 115440 = CPC 3885); ibid., cultures CBS 115438 = CPC 3890, CBS 115439 = CPC 3889.

Notes: *Calonectria amazoniensis* resides in the *C. pteridis* complex. This species can be distinguished from other species in the *C. pteridis* complex by its greater number (−5) of branches in the conidiogenous apparatus and the presence of lateral stipe extensions (Crous 2002, Alfenas *et al*. 2015).

Calonectria brasiliana L. Lombard & Crous, **sp. nov.** MycoBank MB818700. Fig. 4.
Etymology: Name refers to Brazil, the country where this fungus was collected.

Macroconidiophores consist of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 40–240 × 5–10 µm; stipe extension septate, straight to flexuous, 117–172 µm long, 4–6 µm wide at the apical septum, terminating in an ellipsoid to obpyriform vesicle, 6–9 µm diam. *Conidiogenous apparatus* 45–100 µm wide, and 40–70 µm long; primary branches aseptate, 16–23 × 4–6 µm; secondary branches aseptate, 10–17 × 3–6 µm; tertiary branches aseptate, 7–13 × 3–5 µm; quaternary branches and additional branches (–5) aseptate, 7–14 × 3–4 µm each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 7–12 × 3–4 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (36–)38–42(–46) × (3−)3.5−4.5(−5) µm (av. 40 × 4 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies moderately fast growing (30−60 mm diam) on MEA after 7 d at room temperature; surface cinnamon to brick with sparse, felty, white aerial mycelium and abundant sporulation on the aerial mycelium and colony surface; reverse cinnamon to sepia with abundant chlamydospores throughout the medium, forming microsclerotia.

Specimens examined: **Brazil**, from soil, Jun. 1998, A.C. Alfenas (**holotype** CBS-H22752, culture ex-type CBS 111484 = CPC 1924); ibid., culture CBS 111485 = CPC 1929.

Notes: *Calonectria brasiliana* is a new species in the *C. candelabrum* complex (Schoch *et al*. 1999, Lombard *et al*. 2010a, b, 2015a). The macroconidia of *C. brasiliana* [(36–)38–42(–46) × (3−)3.5−4.5(−5) µm (av. 40 × 4 µm)] are smaller than those of its closest phylogenetic neighbours (Fig. 1): *C. candelabrum* [(45–)58–68(–80) × 4−5(−6) µm (av. 60 × 4.5 µm); Crous 2002], *C. eucalypticola* [(43–)49–52(–55) × 3−5 µm (av. 50 × 4 µm); Alfenas *et al*. 2015], *C. glaebicola* [(45–)50–52(–55) × 3−5 µm (av. 50 × 4 µm); Alfenas *et al*. 2015], *C. metrosideri* [(40–)44–46(–51) × 3−5 µm (av. 45 × 4 µm); Alfenas *et al*. 2013a, 2015], *C. pseudometrosideri* [(40–)49–52(–60) × (3−)4.5(−5) µm (av. 51 × 4.5 µm); Alfenas *et al*. 2015] and *C. pseudoscoparia* [(41–)45–51(–52) × 3−5 µm (av. 48 × 4 µm); Lombard *et al*. 2010b].

Calonectria brassicicola L. Lombard & Crous, **sp. nov.** MycoBank MB818701. Fig. 5.
Etymology: Name refers to the host plant, *Brassica*, from which this fungus was isolated.

Macroconidiophores consist of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicles; stipe septate, hyaline, smooth, 30–90 × 6–9 µm; stipe extension septate, straight to flexuous, 90–140 µm long, 4–5 µm wide at the apical septum, terminating in a sphaeropedunculate vesicle, 6–10 µm diam; lateral stipe extensions (90° to main axis) sparse, 30–50 µm long, 2–4 µm wide at the apical septum, terminating in sphaeropedunculate vesicles, 3−5 µm. *Conidiogenous apparatus* 45–80 µm wide, and 35–50 µm long; primary branches aseptate, 12–20 × 4–6 µm; secondary branches aseptate, 8–13 × 3–5 µm; tertiary branches aseptate, 8–12 × 3–6 µm; quaternary branches aseptate, 8–11 × 2–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 7–15 × 3–4 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (36−)39–45(–48) × (4−)4.5–5.5(−6) µm (av. 42 × 5 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies moderately fast growing (50−65 mm diam) on MEA after 7 d at room temperature; surface buff with abundant white to buff, wooly aerial mycelium, and moderate sporulation on the colony surface; reverse sienna, chlamydospores not observed.

Specimens examined: **Indonesia**, from soil at *Brassica* sp., 1990's, M.J. Wingfield (**holotype** CBS-H22753, culture ex-type CBS 112841 = CPC 4552); ibid., culture CBS 112756 = CPC 4502. **New Zealand**, substrate unknown, 2001, C.F. Hill, Lynfield 484, culture CBS 112947 = CPC 4668.

Notes: *Calonectria brassicicola* is similar to *C. sumatrensis* in having few lateral stipe extensions (Crous *et al*. 2004b). The macroconidia of *C. brassicicola* [(36−)39–45(–48) × (4−)4.5–5.5(−6) µm (av. 42 × 5 µm)] are smaller than those of *C. sumatrensis* [(45−)55–65(–70) × (4.5–)5(−6) µm (av. 58 × 5 µm); Crous *et al*. 2004b].

Calonectria brevistipitata L. Lombard & Crous, **sp. nov.** MycoBank MB818702. Fig. 6.
Etymology: Name refers to the short stipe extensions of the macroconidiophores in this fungus.

Macroconidiophores consist of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 50–210 × 5–12 µm; stipe extension septate, straight to flexuous, 90–135 µm long, 2–5 µm wide at the apical septum, terminating in an fusiform to obpyriform vesicle, 5–8 µm diam; lateral stipe extensions (90° to main axis) abundant, 60–80 µm long, 2–3 µm wide at the apical septum, terminating in broadly clavate vesicles, 2–3 µm diam. *Conidiogenous apparatus* 45–75 µm wide, and 45–70 µm long; primary branches aseptate, 13–25 × 4–6 µm; secondary branches aseptate, 10–19 × 3–5 µm; tertiary branches aseptate, 8–16 × 3–5 µm; quaternary branches aseptate, 7–11 × 3–4 µm each terminal branch producing 2–6 phialides; phialides elongate doliiform to reniform, hyaline, aseptate, 6–11 × 2–4 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, 29–

33(–35) × 3−4 µm (av. 31 × 3.5 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies moderately fast growing (40−70 mm diam) on MEA after 7 d at room temperature; surface cinnamon to brick to sienna with abundant, wooly, white to buff aerial mycelium and abundant sporulation on the aerial mycelium and colony surface; reverse cinnamon to sepia with abundant chlamydospores throughout the medium, forming microsclerotia.

Specimens examined: **Mexico**, from soil, Apr. 1994, P.W. Crous (**holotype** CBS-H22754, culture ex-type CBS 115671 = CPC 949); ibid., cultures CBS 110837 = CPC 913, CBS 110928 = CPC 951.

Notes: *Calonectria brevistipitata* is a new species in the *C. candelabrum* complex. The lateral stipe extensions (up to 80 µm long) and macroconidia [29–33(–35) × 3−4 µm (av. 31 × 3.5 µm) of *C. brevistipitata* are shorter than the lateral stipe extensions (up to 125 µm long) and macroconidia [(35–)36–40(–43) × (3−)3.5−4.5(−5) µm (av. 38 × 4 µm)] of *C. machaerinae*, the only other species in the *C. candelabrum* complex to produce lateral stipe extensions.

Calonectria cliffordiicola L. Lombard & Crous, **sp. nov.** MycoBank MB818703. Fig. 7.
Etymology: Name refers to plant host plant genus, *Cliffordia*, from which this fungus was isolated.

Macroconidiophores consist of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 65–130 × 7–10 µm; stipe extension septate, straight to flexuous, 127–180 µm long, 4–6 µm wide at the apical septum, terminating in an ellipsoid to obpyriform vesicle, 7–9 µm diam. *Conidiogenous apparatus* 57–100 µm wide, and 40–85 µm long; primary branches aseptate, 15–32 × 4–6 µm; secondary branches aseptate, 11–23 × 3–6 µm; tertiary branches aseptate, 7–13 × 3–5 µm; quaternary branches aseptate, 8–13 × 3–4 µm each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 7–11 × 3–4 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (35–)38–42(–44) × (3−)3.5−4.5(−6) µm (av. 40 × 4 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies moderately fast growing (35−65 mm diam) on MEA after 7 d at room temperature; surface cinnamon to brick with sparse, felty, white to buff aerial mycelium and moderate sporulation on the aerial mycelium and colony surface; reverse cinnamon to sepia with abundant chlamydospores throughout the medium, forming microsclerotia.

Specimens examined: **South Africa**, Western Cape Province, George, from *Cliffordia feruginea*, 14 Apr. 1998, P.W. Crous (**holotype** CBS-H22755, culture ex-type CBS 111812 = CPC 2631); Stellenbosch, from *Prunus avium* saplings, 1 May 1999, C. Linde, cultures CBS 111814 = CPC 2617, CBS 111819 = CPC 2604.

Notes: *Calonectria cliffordiicola* is a new species in the *C. candelabrum* complex (Schoch *et al*. 1999, Lombard *et al*. 2010a, b, 2015a). Morphologically, this species shows some overlap with *C. brasiliana*, but can be distinguished by its shorter stipe extensions (up to 180 µm) compared to *C. brasiliana* (up to 240 µm).

Calonectria ericae L. Lombard & Crous, **sp. nov.** MycoBank MB818704. Fig. 8.
Etymology: Name refers to host plant genus, *Erica*, from which this species was isolated.

Macroconidiophores consist of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 40–100 × 6–9 µm; stipe extension septate, straight to flexuous, 105–160 µm long, 3–7 µm wide at the apical septum, terminating in an ellipsoid to obpyriform vesicle, 6–10 µm diam. *Conidiogenous apparatus* 40–75 µm wide, and 35–70 µm long; primary branches aseptate, 15–23 × 3–5 µm; secondary branches aseptate, 10–19 × 2–6 µm; tertiary branches aseptate, 6–16 × 2–5 µm; quaternary branches aseptate, 6–13 × 2–5 µm each terminal branch producing 2–6 phialides; phialides elongate doliiform to reniform, hyaline, aseptate, 6–11 × 2–4 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (29–)34–40(–42) × (3−)3.5−4.5(−5) µm (av. 37 × 4 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies moderately fast growing (40−65 mm diam) on MEA after 7 days at room temperature; surface cinnamon to brick with sparse, felty, white aerial mycelium and moderate sporulation on the aerial mycelium and colony surface; reverse cinnamon to umber with abundant chlamydospores throughout the medium, forming microsclerotia.

Specimens examined: **USA**, California, from *Erica capensis*, Sep. 1998, S.T. Koike (**holotype** CBS-H22756, culture ex-type CBS 114458 = CPC 2019); ibid., cultures CBS 114456 = CPC 1984, CBS 114457 = CPC 1985.

Notes: *Calonectria ericae* is a new species in the *C. candelabrum* complex. This species produces the smallest macroconidia in the *C. candelabrum* complex. Koike *et al*. (1999) initially identified these isolates as *C. pauciramosa* based on morphology and mating studies using the *C. pauciramosa* mating tester strains (Schoch *et al*. 1999, Lombard *et al*. 2010a).

Calonectria indonesiana L. Lombard & Crous, **sp. nov.** MycoBank MB818705. Fig. 9.
Etymology: Name refers to Indonesia, the country where this fungus was collected.

Macroconidiophores consist of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicles; stipe septate, hyaline, smooth, 35–115 × 6–9 µm; stipe extension septate, straight to flexuous, 110–130 µm long, 3–5 µm wide at the apical septum, terminating in a sphaeropedunculate vesicle, 8–10 µm diam; lateral stipe extensions (90° to main axis) sparse, 30–50 µm long, 3–4 µm wide at the apical septum, terminating in sphaeropedunculate vesicles, 4−5 µm. *Conidiogenous apparatus* 40–100 µm wide, and 40–70 µm long; primary branches aseptate, 11–20 × 4–6 µm; secondary branches aseptate, 8–17 × 4–7 µm; tertiary branches aseptate, 9–14 × 3–6 µm; quaternary branches and additional branches (−6) aseptate, 7–12 × 3–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 7–14 × 3–5 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (38−)40–46(–48) × (3−)4.5–5.5(−6) µm (av. 43 × 5 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies moderately fast growing (50−65 mm diam) on MEA after 7 d at room temperature; surface buff with abundant white to buff, wooly aerial mycelium, and moderate sporulation on the colony surface; reverse sienna, chlamydospores not observed.

Specimens examined: **Indonesia**, north Sumatera, from soil, 1998, M.J. Wingfield (**holotype** CBS-H22757, culture ex-type CBS 112936 = CPC 4504); ibid., culture CBS 112826 = CPC 4519.

Notes: *Calonectria indonesiana* is similar to *C. brassicicola* and *C. sumatrensis* in having few lateral stipe extensions (Crous *et al*. 2004b). *Calonectria indonesiana* (−6) can be distinguished from *C. brassicicola* (−4) and *C. sumatrensis* (−3) by the number of branches of the conidiogenous apparatus (Crous *et al*. 2004b).

Calonectria lageniformis L. Lombard & Crous, **sp. nov.** MycoBank MB818706. Fig. 10.
Etymology: Name refers to the characteristic lageniform vesicles in this fungus.

Macroconidiophores consist of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 65–220 × 4–9 µm; stipe extension septate, straight to flexuous, 135–185 µm long, 4–6 µm wide at the apical septum, terminating in a lageniform to ellipsoid vesicle, 6–10 µm diam. *Conidiogenous apparatus* 20–80 µm wide, and 35–60 µm long; primary branches aseptate, 16–28 × 4–6 µm; secondary branches aseptate, 10–18 × 3–6 µm; tertiary branches aseptate, 8–13 × 3–6 µm, each terminal branch producing 2–4 phialides; phialides doliiform to reniform, hyaline, aseptate, 7–11 × 3–4 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (35–)37–43(–45) × (3−)4.5−5.5(−6) µm (av. 40 × 5 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies fast growing (60−90 mm diam) on MEA after 7 d at room temperature; surface sepia with sparse buff, felty aerial mycelium and moderate sporulation on the aerial mycelium and colony surface; reverse sepia with abundant chlamydospores throughout the medium, forming microsclerotia.

Specimens examined: **Brazil**, from leaf lesion on *Eucalyptus* sp., 1993, P.W. Crous & A.C. Alfenas, culture CBS 112685 = CPC 3418. **Mauritius**, Rivière Noire, from foliar lesion on *Eucalyptus* sp., 10 Apr. 1996, H. Smith (**holotype** CBS-H22758 culture ex-type CBS 111324 = CPC 1473).

Note: *Calonectria lageniformis* is the only species that has lageniform vesicles (Crous 2002, Lombard *et al*. 2010b, 2015a, Alfenas *et al*. 2015).

Calonectria machaerinae L. Lombard & Crous, **sp. nov.** MycoBank MB818707. Fig. 11.
Etymology: Name refers to plant host genus, *Machaerina*, from which this species was isolated.

Macroconidiophores consist of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 40–115 × 5–10 µm; stipe extension septate, straight to flexuous, 105–170 µm long, 3–5 µm wide at the apical septum, terminating in an ellipsoid to obpyriform vesicle, 6–9 µm diam; lateral stipe extensions (90° to main axis) few, 80–125 µm long, 3–5 µm wide at the apical septum, terminating in broadly clavate vesicles, 5–6 µm diam. *Conidiogenous apparatus* 40–80 µm wide, and 55–90 µm long; primary branches aseptate, 18–28 × 4–6 µm; secondary branches aseptate, 13–23 × 3–6 µm; tertiary branches aseptate, 8–19 × 3–5 µm; quaternary branches and additional branches (–6) aseptate, 7–15 × 3–5 µm each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 6–11 × 2–4 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (35–)36–40(–43) × (3−)3.5−4.5(−5) µm (av. 38 × 4 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies fast growing (60−85 mm diam) on MEA after 7 d at room temperature; surface cinnamon to brick with sparse, wooly, white aerial mycelium and abundant sporulation on the aerial mycelium and colony surface; reverse cinnamon to umber with abundant chlamydospores throughout the medium, forming microsclerotia.

Specimen examined: **New Zealand**, Auckland, Auckland University Campus, from foliar lesion of *Machaerina sinclairii*, 27 Jan. 2008, C.F. Hill (**holotype** CBS-H22760, culture ex-type CBS 123183 = CPC 15378).

Notes: *Calonectria machaerinae* is a new species in the *C. candelabrum* complex. This species, along with *C. brevistipitata*, are the only two species to produce lateral stipe extensions in the *C. candelabrum* complex (Schoch *et al*. 1999, Lombard *et al*. 2010a, b, 2015a). See note under *C. brevistipitata* for additional distinguishing characters.

Calonectria multilateralis L. Lombard & Crous, **sp. nov.** MycoBank MB818708. Fig. 12.
Etymology: Name refers to the multiple lateral stipe extensions on the macroconidiophores of this species.

Macroconidiophores consist of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicles; stipe septate, hyaline, smooth, 25–130 × 4–8 µm; stipe extension septate, straight to flexuous, 135–375 µm long, 5–6 µm wide at the apical septum, terminating in a naviculate vesicle, 6–11 µm diam; lateral stipe extensions (90° to main axis) numerous, 55–100 µm long, 3–5 µm wide at the apical septum, terminating in naviculate vesicles, 4−8 µm. *Conidiogenous apparatus* 45–95 µm wide, and 30–70 µm long; primary branches aseptate, 10–25 × 3–6 µm; secondary branches aseptate, 6–20 × 3–5 µm; tertiary branches aseptate, 7–15 × 3–5 µm; quaternary branches and additional branches (–7) aseptate, 6–13 × 2–4 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform to elongate reniform, hyaline, aseptate, 6–12 × 2–4 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (27−)31–35(–38) × 3–4 µm (av. 33 × 3 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies fast growing (55−85 mm diam) on MEA after 7 d at room temperature; surface buff with abundant white, wooly aerial mycelium and abundant sporulation on the colony surface; reverse buff to sienna, chlamydospores not observed.

Specimens examined: **Mexico**, Uxmal, from soil, Apr. 1994, P.W. Crous (**holotype** CBS-H22762, culture ex-type CBS 110932 = CPC 957); ibid., cultures CBS 110926 = CPC 947, CBS 110927 = CPC 948, CBS 110931 = CPC 956, CBS 115615 = CPC 915.

Notes: *Calonectria multilateralis* is a new species in the *C. naviculata* complex (Alfenas *et al*. 2015). The macroconidia of *C. multilateralis* [31–35(–38) × 3–4 µm (av. 33 × 3 µm)] are smaller than those of *C. naviculata* [(40−)42–50 × 3(–4) µm (av. 45 × 3 µm); Crous 2002] and *C. multinaviculata* [(40−)44–49(–52) × (2.5−)3.5(–4) µm (av. 46 × 3.5 µm); Alfenas *et al*. 2015].

Calonectria paracolhounii L. Lombard & Crous, **sp. nov.** MycoBank MB818709. Fig. 13.
Etymology: Name refers to the fact that this species has an asexual morph that is very similar to that of *C. colhounii*.

Macroconidiophores consist of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 21–75 × 5–9 µm; stipe extension septate, straight to flexuous, 82–178 µm long, 3–5 µm wide at the apical septum, terminating in a narrowly clavate vesicle, 3–5 µm diam. *Conidiogenous apparatus* 31–77 µm wide, and 25–54 µm long; primary branches aseptate, 11–23 × 3–6 µm; secondary branches aseptate, 7–13 × 3–6 µm; tertiary branches aseptate, 7–12 × 2–4 µm, each terminal branch producing 2–6 phialides; phialides elongate doliiform to doliiform to reniform, hyaline, aseptate, 6–12 × 2–5 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (37–)39–43(–45) × 4−5 µm (av. 41 × 5 µm), 3-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies moderately fast growing (25−55 mm diam) on MEA after 7 d at room temperature; surface buff to sienna with abundant buff to white, felty to wooly aerial mycelium and moderate sporulation on the aerial mycelium and colony surface; reverse buff to sienna to umber with abundant chlamydospores throughout the medium, forming microsclerotia.

Specimens examined: **USA**, substrate unknown, 1990's, A.Y. Rossman (**holotype** CBS-H22763 culture ex-type CBS 114679 = CPC 2445). **Australia**, fruit of *Annona reticulata*, 1988, D. Hutton, culture CBS 114705 = CPC 2423.

Notes: *Calonectria paracolhounii* is a new species in the *C. colhounii* complex (Lombard *et al*. 2010b, Chen *et al*. 2011). The macroconidia of *C. paracolhounii* [(37–)39–43(–45) × 4−5 µm (av. 41 × 5 µm)] are smaller than those of *C. colhounii* [(45–)60–70(–80) × (4−)5(−6) µm (av. 65 × 5 µm); Crous 2002], *C. eucalypti* [(66–)69–75(–80) × (5−)−6 µm (av. 72 × 6 µm); Lombard *et al*. 2010b], *C. fujianensis* [(48–)50–55(–60) × (2.5−)3.5−4.5(−5) µm (av. 52.5 × 4 µm); Chen *et al*. 2011], *C. monticola* 46–51(–56) × 4−5 µm (av. 49 × 5 µm); Crous *et al*. 2015b] and *C. pseudocolhounii* [(49–)55–65(–74) × (3.5−)4−5(−5.5) µm (av. 60 × 4.5 µm); Chen *et al*. 2011]. Hutton & Sanewski (1989) initially identified isolate CBS 114705 as *C. colhounii*, associated with leaf and fruit spots of custard apple (*Annona reticulata*). Their identification was based on morphological comparisons, as no DNA sequence data was available for the genus *Calonectria* at that time.

Calonectria parva L. Lombard & Crous, **sp. nov.** MycoBank MB818710. Fig. 14.
Etymology: Name refers to the small macroconidiophores in this fungus.

Macroconidiophores consist of a stipe bearing a penicillate arrangement of fertile branches, and rarely a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 43–149 × 5–7 µm; stipe extension septate, straight to flexuous, 65–95 µm long, 2–4 µm wide at the apical septum, terminating in a narrowly clavate vesicle, 3–5 µm diam. *Conidiogenous apparatus* 18–33 µm wide, and 24–43 µm long; primary branches aseptate, 11–21 × 3–5 µm; secondary branches aseptate, 11–15 × 3–4 µm, each terminal branch producing 2–4 phialides; phialides cylindrical to allantoid, hyaline, aseptate, 9–19 × 3–5 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (60–)66–78(–83) × 5−7 µm (av. 72 × 6 µm), (1−)3-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies fast growing (55−85 mm diam) on MEA after 7 d at room temperature; surface buff with abundant buff to white, felty aerial mycelium and sparse to moderate sporulation on the aerial mycelium and colony surface; reverse buff; chlamydospores not observed.

Specimen examined: **South Africa**, Mpumalanga, Sabie, D.R. de Wet nursery, from *Eucalyptus grandis* ramets (roots), 11 May 1990, P.W. Crous (**holotype** CBS-H22764, culture ex-type CBS 110798 = CPC 410 = PPRI 4001).

Note: *Calonectria parva* can be distinguished from other species in the genus by its relatively small macroconidiophores, which rarely bear a stipe extension.

Calonectria plurilateralis L. Lombard & Crous, **sp. nov.** MycoBank MB818711. Fig. 15.
Etymology: Name refers to the multiple lateral stipe extensions on the macroconidiophores of this fungus.

Macroconidiophores consist of a stipe bearing a penicillate arrangement of fertile branches, and numerous lateral stipe extensions terminating in vesicles, lacking a central stipe extension; stipe septate, hyaline, smooth, 50–130 × 4–7 µm; stipe extension septate, straight to flexuous, 110–180 µm long, 4–7 µm wide at the apical septum, terminating in obpyriform to ellipsoid vesicles, 7–11 µm diam; lateral stipe extensions (90° to main axis) abundant, 75–105 µm long, 3–6 µm wide at the apical septum, terminating in obpyriform to ellipsoid vesicles, 5−7 µm diam. *Conidiogenous apparatus* 25–80 µm wide, and 25–85 µm long; primary branches aseptate, 11–39 × 2–9 µm; secondary branches aseptate, 7–17 × 3–5 µm; tertiary branches aseptate, 6–12 × 3–5 µm; quaternary branches aseptate, 8 × 4 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 4–11 × 3–4 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (27−)30–38(–41) × (3−)3.5−4.5(−5) µm (av. 34 × 4 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies fast growing (60−85 mm diam) on MEA after 7 d at room temperature; surface sienna to sepia with moderate white, wooly aerial mycelium and abundant sporulation on the colony surface; reverse sienna to sepia, chlamydospores throughout the medium, forming microsclerotia.

Specimen examined: **Ecuador**, from soil, 20 Jun. 1997, M.J. Wingfield (**holotype** CBS-H22766, culture ex-type CBS 111401 = CPC 1637).

Note: *Calonectria plurilateralis* can be distinguished from other members of the *C. cylindrospora* complex by its numerous lateral stipe extensions.

Calonectria pseudoecuadoriae L. Lombard & Crous, **sp. nov.** MycoBank MB818712. Fig. 16.
Etymology: Name refers to the fact that this species has an asexual morph that is very similar to that of *C. ecuadoriae*.

Macroconidiophores consist of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 40–210 × 7–10 µm; stipe extension septate, straight to flexuous, 160–250 µm long, 4–5 µm wide at the apical septum, terminating in a clavate vesicle, 4–7 µm diam. *Conidiogenous apparatus* 70–105 µm wide, and 50–90 µm long; primary branches aseptate, 18–30 × 5–7 µm; secondary branches aseptate, 9–22 × 3–7 µm; tertiary branches aseptate, 7–17 × 3–5 µm; quaternary branches and additional branches (–6) aseptate, 7–12 × 3–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 8–12 × 3–4 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (34–)36–40(–43) × 3−4 (−5) µm (av. 38 × 3.5 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies moderately fast growing (30−60 mm diam) on MEA after 7 d at room temperature; surface cinnamon to brick with sparse white, wooly aerial mycelium and abundant sporulation on the aerial mycelium and colony surface; reverse buff to cinnamon with abundant chlamydospores throughout the medium, forming microsclerotia.

Specimens examined: **Ecuador**, soil, 20 Jun. 1997, M.J. Wingfield (**holotype** CBS-H22768, culture ex-type CBS 111402 = CPC 1639); ibid., culture CBS 111412 = CPC 1648.

Notes: *Calonectria pseudoecuadoriae* is morphologically similar to *C. ecuadoriae*. The macroconidia of *C. pseudoecuadoriae* [(34–)36–40(–43) × 3−4 (−5) µm (av. 38 × 3.5 µm)] are smaller than those of *C. ecuadoriae* [(45–)48–55(–65) × (4−)4.5(−5) µm (av. 51 × 4.5 µm); Crous *et al*. 2006]. Furthermore,

C. pseudoecuadoriae has six tiers of branches in its conidiogenous apparatus in comparison to the seven in *C. ecuadoriae* (Crous *et al*. 2006), although these differences are relatively minor.

Calonectria pseudouxmalensis L. Lombard & Crous, **sp. nov.** MycoBank MB818713. Fig. 17.

Etymology: Name refers to the fact that this species has an asexual morph that is very similar to that of *C. uxmalensis*.

Macroconidiophores consist of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 30–60 × 6–8 µm; stipe extension septate, straight to flexuous, 100–140 µm long, 4–6 µm wide at the apical septum, terminating in a obpyriform to ellipsoidal vesicle sometimes with a papillate apex, 5–9 µm diam. *Conidiogenous apparatus* 25–65 µm wide, and 30–60 µm long; primary branches aseptate, 14–21 × 4–6 µm; secondary branches aseptate, 8–16 × 2–5 µm; tertiary branches aseptate, 5–13 × 2–5 µm; quaternary branches and additional branches (–6) aseptate, 5–9 × 2–4 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 6–9 × 3–4 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (26–)28–30(–32) × 3−4 µm (av. 29 × 3 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies fast growing (75−90 mm diam) on MEA after 7 d at room temperature; surface sienna with abundant white, felty to wooly aerial mycelium and abundant sporulation on the aerial mycelium and colony surface; reverse sienna with abundant chlamydospores throughout the medium, forming microsclerotia.

Specimens examined: **Mexico**, from soil, Apr. 1994, P.W. Crous (**holotype** CBS-H22769, culture ex-type CBS 110924 = CPC 942); ibid., cultures CBS 110923 = CPC 941, CBS 115677 = CPC 943.

Notes: *Calonectria pseudouxmalensis* can be distinguished from *C. uxmalensis* by its lack of lateral stipe extensions. The macroconidia of *C. pseudouxmalensis* [(26–)28–30(–32) × 3−4 µm (av. 29 × 3 µm)] are smaller than those of *C. mexicana* [(35–)40–48(–52) × 3−4(−4.5) µm (av. 45 × 3 µm); Crous 2002, Schoch *et al*. 1999], *C. pseudomexicana* [(40–)43–48(–49) × (4−)5−6 µm (av. 45 × 5 µm); Lombard *et al*. 2011] and *C. tunisiana* [(43–)47–51(–53) × 4−6 µm (av. 49 × 5 µm); Lombard *et al*. 2011]. Schoch *et al*. (1999) was able to induce the sexual morph of *C. mexicana* through the heterothallic mating of CBS 110918 (= CPC 927) with CBS 110923 (= CPC 941), which was deposited as the holotype (PREM 55763) of *C. mexicana*. However, phylogenetic inference in this study showed that the one mating tester strain CBS 110923 (Schoch *et al*. 1999) is distinct from the other mating tester strain (CBS 110918; ex-type of *Cylindrocladium mexicanum*). This phenomenon is not new to the genus *Calonectria*, as Neubauer & Zinkernagel (1995) and Overmeyer *et al*. (1996) have shown that fertile perithecia can be induced in some *Calonectria* species when they are cultured in the presence of other species, but where sexual outcrossing has not occurred.

Calonectria putriramosa L. Lombard & Crous, **sp. nov.** MycoBank MB818714. Fig. 18.

Etymology: Name refers to cutting rot, the disease symptoms that are associated with infection by this fungus.

Macroconidiophores consist of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 40–170 × 5–10 µm; stipe extension septate, straight to flexuous, 145–185 µm long, 4–7 µm wide at the apical septum, terminating in an ellipsoid to obpyriform vesicle, 7–9 µm diam. *Conidiogenous apparatus* 45–60 µm wide, and 30–90 µm long; primary branches aseptate, 12–34 × 3–6 µm; secondary branches aseptate, 9–21 × 3–6 µm; tertiary branches aseptate, 9–17 × 3–5 µm; quaternary branches aseptate, 4–13 × 3–5 µm each terminal branch producing 2–6 phialides; phialides elongate reniform to allantoid to cylindrical, hyaline, aseptate, 6–15 × 3–5 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (35–)40–46(–49) × (4−)4.5−5.5(−6) µm (av. 43 × 5 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies moderately fast growing (35−75 mm diam) on MEA after 7 d at room temperature; surface cinnamon to brick with sparse, wooly, white to buff aerial mycelium and

moderate sporulation on the aerial mycelium and colony surface; reverse cinnamon to sepia with abundant chlamydospores throughout the medium, forming microsclerotia.

Specimens examined: **Brazil**, from *Eucalyptus* cuttings, Jun. 1998, A.C. Alfenas (**holotype** CBS-H22770, culture ex-type CBS 111449 = CPC 1951); Bahia do Sol, from *Eucalyptus* cuttings, Apr. 1993, P.W. Crous, culture CBS 116076 = CPC 604; from soil, Jun. 1998, A.C. Alfenas, cultures CBS 111470 = CPC 1940, CBS 111477 = CPC 1928.

Notes: *Calonectria putriramosa* is a new species in the *C. candelabrum* complex (Schoch *et al*. 1999, Lombard *et al*. 2010a, b, 2015a). The macroconidia of *C. putriramosa* [(35–)40–46(–49) × (4−)4.5−5.5(−6) µm (av. 43 × 5 µm)] are smaller than those of its closest phylogenetic neighbours (see notes under *C. brasiliana*), but slightly larger than those of *C. brasiliana* [(36–)38–42(–46) × (3−)3.5−4.5(−5) µm (av. 40 × 4 µm)].

Calonectria stipitata L. Lombard & Crous, **sp. nov.** MycoBank MB818715. Fig. 19.
Etymology: Name refers to the lateral stipe extensions produced by this fungus.

Macroconidiophores consist of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 35–85 × 6–9 µm; stipe extension septate, straight to flexuous, 105–195 µm long, 4–6 µm wide at the apical septum, terminating in an ellipsoid to obpyriform vesicle, 7–11 µm diam; lateral stipe extensions (90° to main axis) abundant, 70–135 µm long, 3–6 µm wide at the apical septum, terminating in broadly clavate vesicles, 3–6 µm diam. *Conidiogenous apparatus* 50–120 µm wide, and 40–75 µm long; primary branches aseptate, 15–29 × 4–5 µm; secondary branches aseptate, 9–18 × 3–6 µm; tertiary branches aseptate, 8–19 × 2–5 µm; quaternary branches and additional branches (–6) aseptate, 6–14 × 2–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 7–13 × 2–5 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (27–)29–35(–37) × (3−)3.5−4.5(−6) µm (av. 32 × 4 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies fast growing (60−85 mm diam) on MEA after 7 d at room temperature; surface sienna to sepia with abundant wooly, white aerial mycelium and abundant sporulation on the aerial mycelium and colony surface; reverse sienna to sepia with abundant chlamydospores throughout the medium, forming microsclerotia.

Specimen examined: **Colombia**, from *Eucalyptus* sp., 1990's, M.J. Wingfield (**holotype** CBS-H22771, culture ex-type CBS 112513 = CPC 3851).

Notes – *Calonectria stipitata*, like *C. brevistipitata* and *C. machaerinae*, produce lateral stipe extensions, a characteristic not usually associated with members of the *C. candelabrum* complex (Schoch *et al*. 1999, Lombard *et al*. 2010a, b, 2015a). The lateral stipe extensions of *C. stipitata* (up to 135 µm) are longer than those of *C. brevistipitata* (up to 80 µm) and *C. machaerinae* (up to 125 µm).

Calonectria syzygiicola L. Lombard & Crous, **sp. nov.** MycoBank MB818716. Fig. 20.
Etymology: Name refers to the host plant, *Syzygium aromaticum* from which this fungus was isolated.

Macroconidiophores consist of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 30–170 × 4–8 µm; stipe extension septate, straight to flexuous, 65–105 µm long, 3–4 µm wide at the apical septum, terminating in a sphaeropedunculate vesicle, 4–7 µm diam; lateral stipe extensions (90° to main axis) sparse, 40–50 µm long, 2–3 µm wide at the apical septum, terminating in sphaeropedunculate vesicles, 3–6 µm diam. *Conidiogenous apparatus* 30–70 µm wide, and 30–45 µm long; primary branches aseptate, 12–21 × 4–6 µm; secondary branches aseptate, 8–14 × 3–5 µm; tertiary branches aseptate, 9–12 × 3–5 µm; quaternary branches aseptate, 8–10 × 2–3 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 7–11 × 2–4 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (39–)41–49(–56) × (4−)4.5−5.5(−7) µm (av. 45 × 5 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies moderately fast growing (45−65 mm diam) on MEA after 7 d at room temperature; surface amber to sienna with abundant wooly, white to buff aerial mycelium, and

abundant sporulation on the aerial mycelium and colony surface; reverse sienna with abundant chlamydospores throughout the medium, forming microsclerotia.

Specimens examined: **Indonesia**, Sumatra, from soil under *Syzygium aromaticum*, 1998, M.J. Wingfield (**holotype** CBS-H22772, culture ex-type CBS 112831 = CPC 4511), culture CBS 112827 = CPC 4512.

Notes: *Calonectria syzygiicola* is closely related to *C. asiatica* (Fig. 1). However, the macroconidia of *C. syzygiicola* [(39–)41–49(–56) × (4−)4.5−5.5(−7) µm (av. 45 × 5 µm)] are smaller than those of *C. asiatica* [(42–)48–55(–65) × (4−)5(−5.5) µm (av. 53 × 5 µm); Crous *et al*. 2004b].

Calonectria tereticornis L. Lombard & Crous, **sp. nov.** MycoBank MB818717. Fig. 21.
Etymology: Name refers to the host plant, *Eucalyptus tereticornis*, from which this fungus was isolated.

Macroconidiophores consist of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 70–270 × 6–11 µm; stipe extension septate, straight to flexuous, 140–245 µm long, 3–7 µm wide at the apical septum, terminating in a fusiform to ovoid vesicle, 8–14 µm diam. *Conidiogenous apparatus* 35–65 µm wide, and 45–75 µm long; primary branches aseptate, 18–34 × 4–10 µm; secondary branches aseptate, 11–26 × 3–7 µm, each terminal branch producing 2–4 phialides; phialides elongate doliiform to allantoid, hyaline, aseptate, 9–15 × 3–5 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (51–)55–63(–71) × (3−)4.5−5.5(−6) µm (av. 59 × 5 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies fast growing (55−75 mm diam) on MEA after 7 d at room temperature; surface cinnamon to sienna with sparse buff to white, wooly aerial mycelium and abundant sporulation on the aerial mycelium and colony surface; reverse sienna to umber with abundant chlamydospores throughout the medium, forming microsclerotia.

Specimens examined: **Brazil**, Tucurui, from leaves of *Eucalyptus tereticornis*, 20 Sep. 1996, P.W. Crous (**holotype** CBS-H22773 culture ex-type CBS 111301 = CPC 1429).

Notes: *Calonectria tereticornis* is closely related to *C. gordoniae* and *C. ovata* (Fig. 1). The macroconidia of *C. tereticornis* [(51–)55–63(–71) × (3−)4.5−5.5(−6) µm (av. 59 × 5 µm)] are smaller than those of *C. gordoniae* [(44–)50–70(–80) × (4−)5−6 µm (av. 65 × 5 µm); Crous 2002] and *C. ovata* [(50–)65–80(–110) × 4−5 (−6) µm (av. 70 × 5 µm); Crous 2002].

Calonectria terricola L. Lombard & Crous, **sp. nov.** – MycoBank MB818718. Fig. 22.
Etymology: Name refers to soil, the substrate from which this fungus was isolated.

Macroconidiophores consist of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 30–100 × 5–9 µm; stipe extension septate, straight to flexuous, 135–175 µm long, 4–5 µm wide at the apical septum, terminating in a fusiform to ovoid vesicle, 8–12 µm diam. *Conidiogenous apparatus* 30–100 µm wide, and 45–65 µm long; primary branches aseptate, 14–26 × 3–6 µm; secondary branches aseptate, 13–22 × 2–5 µm; tertiary branches aseptate, 15–18 × 4–5 µm, each terminal branch producing 2–4 phialides; phialides elongate doliiform to reniform, hyaline, aseptate, 9–17 × 3–5 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (40–)43–49(–53) × (3−)4−5(−6) µm (av. 46 × 4.5 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies moderately fast growing (45−65 mm diam) on MEA after 7 d at room temperature; surface brick to sienna with sparse, buff to white, wooly aerial mycelium and moderate sporulation on the aerial mycelium and colony surface; reverse sienna with abundant chlamydospores throughout the medium, forming microsclerotia.

Specimens examined: **Brazil**, from soil in *Eucalyptus* plantation, 1996, P.W. Crous (**holotype** CBS-H22774; culture ex-type CBS 116247 = CPC 3583); ibid., culture CBS 116248 = CPC 3536.

Notes: *Calonectria terricola* is a new species in the *C. pteridis* complex. The macroconidia of *C. terricola* [(40–)43–49(–53) × (3−)4−5(−6) µm (av. 46 × 4.5 µm)] are smaller than those of *C. ovata*

[(50–)65–80(–110) × 4−5 (−6) µm (av. 70 × 5 µm); Crous 2002], *C. pseudovata* [(55–)67–70(–80) × (4−)5 (−7) µm (av. 69 × 5 µm); Alfenas *et al*. 2015] and *C. tereticornis* [(51–)55–63(–71) × (3−)4.5−5.5(−6) µm (av. 59 × 5 µm)].

Calonectria tropicalis L. Lombard & Crous, **sp. nov.** MycoBank MB818719. Fig. 23.
Etymology: Name refers to the tropical region in Brazil where this fungus was collected.

Macroconidiophores consist of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 120–210 × 7–8 µm; stipe extension septate, straight to flexuous, 190–270 µm long, 4–6 µm wide at the apical septum, terminating in a clavate vesicle, 5–6 µm diam. *Conidiogenous apparatus* 50–70 µm wide, and 60–90 µm long; primary branches aseptate, 20–32 × 4–6 µm; secondary branches aseptate, 12–29 × 3–6 µm; tertiary branches aseptate, 12–20 × 2–4 µm, each terminal branch producing 2–4 phialides; phialides elongate doliiform to reniform, hyaline, aseptate, 10–16 × 2–5 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight to slightly curved, (69–)74–86(–89) × (4−)4.5−5.5(−6) µm (av. 80 × 5 µm), 1(−3)-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies moderately fast growing (45−65 mm diam) on MEA after 7 days at room temperature; surface sienna to sepia with moderate white, wooly aerial mycelium and moderate sporulation on the aerial mycelium and colony surface; reverse sienna to sepia with abundant chlamydospores throughout the medium, forming microsclerotia.

Specimens examined: **Brazil**, Amazon, from foliar lesion of *Eucalyptus* sp., 1993, P.W. Crous & A.C. Alfenas (**holotype** CBS-H22776 culture ex-type CBS 116271 = CPC 3559); ibid., cultures CBS 116242 = CPC 3543.

Notes: *Calonectria tropicalis* resides in the *C. pteridis* complex. This species can be distinguished from other species in the complex by the smaller numbers of fertile branches in its conidiogenous apparatus.

Calonectria uniseptata Gerlach, Phytopathol. Z. 61: 379. 1968. MycoBank MB327268.

Specimen examined: **Germany**, Celle, from root of *Paphiopedilum callosum*, May 1967, W. Gerlach, culture ex-type CBS 413.67 = IMI 299577.

Notes: Sobers (1972) reduced *C. floridana* and *C. uniseptata* to synonymy with *C. kyotensis* based on their similarities in morphology and pathogenicity. Phylogenetic inference in this study showed that the ex-type of *C. uniseptata* (CBS 413.67; Gerlach 1968) is distinct from *C. kyotensis*. Therefore, *C. uniseptata* is reinstated here as a distinct species of *Calonectria*.

Calonectria uxmalensis L. Lombard & Crous, **sp. nov.** MycoBank MB818720. Fig. 24.
Etymology: Name refers to the ancient Maya city Uxmal, Mexico, the locality where this fungus was collected.

Macroconidiophores consist of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 35–155 × 6–8 µm; stipe extension septate, straight to flexuous, 60–140 µm long, 3–6 µm wide at the apical septum, terminating in a obpyriform to ellipsoidal vesicle sometimes with a papillate apex, 5–8 µm diam; lateral stipe extensions (90° to main axis) few, 88–100 µm long, 3–4 µm wide at the apical septum, terminating in broadly clavate to obpyriform to ellipsoid vesicles, 5–6 µm diam. *Conidiogenous apparatus* 30–90 µm wide, and 35–60 µm long; primary branches aseptate, 14–19 × 3–6 µm; secondary branches aseptate, 10–16 × 3–6 µm; tertiary branches aseptate, 7–11 × 3–5 µm; quaternary branches and additional branches (–6) aseptate, 7–11 × 3–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 8–11 × 2–4 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (26–)27–33(–35) × 3−4 µm (av. 30 × 3 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* not observed.

Culture characteristics: Colonies fast growing (65−85 mm diam) on MEA after 7 d at room temperature; surface buff to sienna with abundant buff to white, felty to wooly aerial mycelium and moderate sporulation on the aerial mycelium and colony surface; reverse sienna to umber with abundant chlamydospores throughout the medium, forming microsclerotia.

Specimens examined: **Mexico**, Uxmal, from soil, Apr. 1994, P.W. Crous (**holotype** CBS-H22761, culture ex-type CBS 110925 = CPC 945); ibid., culture CBS 110919 = CPC 928.

Notes: *Calonectria uxmalensis* can be distinguished from *C. mexicana*, *C. pseudomexicana* and *C. tunisiana* by its lateral stipe extensions, a characteristic not known for the latter three species (Schoch *et al*. 1999, Crous 2002, Lombard *et al*. 2011).

Calonectria venezuelana L. Lombard & Crous, **sp. nov.** MycoBank MB818721. Fig. 25.
Etymology: Name refers to Venezuela, the country from which this fungus was collected.

Macroconidiophores consist of a stipe bearing a penicillate arrangement of fertile branches, and a stipe extension terminating in a vesicle; stipe septate, hyaline, smooth, 35–100 × 4–8 µm; stipe extension septate, straight to flexuous, 85–190 µm long, 3–6 µm wide at the apical septum, terminating in a fusiform to ovoid to ellipsoid vesicle, 5–9 µm diam. *Conidiogenous apparatus* 25–60 µm wide, and 25–65 µm long; primary branches aseptate, 15–30 × 4–8 µm; secondary branches aseptate, 11–24 × 3–5 µm; tertiary branches aseptate, 8–14 × 3–6 µm, each terminal branch producing 2–4 phialides; phialides elongate doliiform to reniform, hyaline, aseptate, 8–17 × 2–5 µm, apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (48–)54–62(–65) × (4−)4.5−5.5(−7) µm (av. 58 × 5 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Microconidiophores* consists of a stipe and a penicillate or subverticillate arrangement of fertile branches; stipe septate, hyaline, smooth, 25–40 × 3–4 µm; primary branches aseptate, 8–12 × 2–4 µm, terminating in 1–4 phialides that are cylindrical, straight to slightly curved, 7–15 × 2–4 µm, apex with minute periclinal thickening and inconspicuous collarette. *Microconidia* cylindrical, straight to slightly curved, rounded at the apex and flattened at the base, 16–20(–22) × (2−)2.5−3.5(−4) µm (av. 18 × 3 µm), (0−)1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Megaconidia* not observed.

Culture characteristics: Colonies fast growing (50−75 mm diam) on MEA after 7 d at room temperature; surface cinnamon to amber with sparse, buff to white, wooly aerial mycelium and abundant sporulation on the aerial mycelium and colony surface; reverse sienna to amber with abundant chlamydospores throughout the medium, forming microsclerotia.

Specimen examined: **Venezuela**, Acarigua, from soil, 27 Jun. 1995, M.J. Wingfield (**holotype** CBS-H22778 culture ex-type CBS 111052 = CPC 1183).

Notes: *Calonectria venezuelana* forms a single lineage closely related to *C. eucalypticola* (Fig. 1). The macroconidia of *C. venezuelana* [(48–)54–62(–65) × (4−)4.5−5.5(−7) µm (av. 58 × 5 µm)] are larger than those of *C. eucalypticola* [(43–)49–52(–55) × 3−5 µm (av. 50 × 4 µm); Alfenas *et al*. 2015].

DISCUSSION

A collection of isolates stored for many years and tentatively identified as species of *Calonectria* based on morphology, were shown to represent 24 new species. At the time that they were collected, it would not have been possible to recognise them as novel taxa and this vividly illustrates the power of the DNA-based sequencing tools that are now available to facilitate accurate species recognition. These species emerging from this study were isolated from various substrates collected globally over a 20 year period, and this study therefore highlights the value of the careful storage and maintenance of cultures for further study when appropriate opportunities arise to do so. This paper also highlights the fact that many undescribed species most likely remain hidden in culture collections, requiring a re-evaluation based on DNA sequence comparisons.

Most of the isolates collected in Brazil formed part of the *C. pteridis* species complex. This is regarded as one of the most prominent species complexes associated with CLB on *Eucalyptus* in that country (Alfenas *et al.* 2004, 2013c, 2015, Graça et al. 2009). *Calonectria amazonica*, *C. amazoniensis*, *C. lageniformis*, *C. tereticornis* and *C. tropicalis* were all isolated from CLB leaf lesions on *Eucalyptus* spp. propagated commercially as non-natives in plantations. Results of this study have raised the number of species known from Brazil to 55 (Alfenas *et al*. 2013a,b, 2015). *Calonectria terricola*, isolated from soil collected in a *Eucalyptus* plantation in Brazil, also formed part of the *C. pteridis* complex in this study.

Calonectria parva, isolated from soil collected in South Africa, formed a basal lineage to the *C. colhounii* species complex. This species can be readily distinguished from other species in the *C. colhounii* species complex by its relatively small macroconidiophores, which rarely bear stipe extensions.

Both *C. uxmalensis* and *C. pseudouxmalensis*, isolated from soil collected in Mexico, are new additions to the *C. mexicana* species complex, which now include five species (Lombard *et al*. 2011). This complex is characterised by the papillate apices of the vesicles terminating the stipe extensions (Lombard *et al*. 2011). *Calonectria uxmalensis* can be distinguished from the other species in this complex by the formation of lateral stipe extensions, whereas macroconidial dimensions can distinguish *C. pseudouxmalensis* from the species in this complex.

Calonectria paracolhounii, collected in the USA and Australia, is a new addition to the *C. colhounii* complex (Crous 2002, Crous *et al*. 2006, Chen *et al*. 2011). This species complex now includes seven species (Crous 2002, Crous *et al*. 2006, 2015b, Chen *et al*. 2011, Xu *et al*. 2012), and is characterised by the formation of unique bright yellow perithecia. Although no perithecia were observed for *C. paracolhounii* in this study, the macroconidia of *C. paracolhounii* were smaller than those of the other species known in this complex.

The *C. candelabrum* species complex (Schoch *et al.* 1999) accommodates the greatest number of species in the genus and includes 27 species (Schoch *et al*. 1999, Crous 2002, Lombard *et al*. 2010a, 2011, 2015a, Crous *et al*. 2013, Alfenas et al. 2015) after the addition of *C. brasiliana* (Brazil), *C. brevistipitata* (Mexico), *C. cliffordiicola* (South Africa), *C. ericae* (USA), *C. machaerinae* (New Zealand), *C. putriramosa* (Brazil), *C. stipitata* (Colombia) and *C. venezuelana* (Venezuela) recognised in this study. Although some unique morphological characters could be identified to distinguish these eight new species, DNA sequence comparisons are required to provide accurate species identification.

Calonectria pseudoecuadoriae and *C. plurilateralis* (Ecuador) are both new additions to the *C. brassicae* and *C. cylindrospora* species complexes, respectively (Crous 2002, Lombard *et al*. 2009, Alfenas *et al*. 2015. *Calonectria pseudoecuadoriae* is morphologically similar to *C. ecuadoriae* (Crous *et al*. 2006) except for the additional branches in the conidiogenous apparatus and smaller macroconidia. *Calonectria plurilateralis* is the only species in the *C. cylindrospora* complex known to produce lateral stipe extensions, distinguishing it from other species in this complex.

Calonectria brassicicola (Indonesia and New Zealand), *C. indonesiana* (Indonesia), and *C. syzygiicola* (Indonesia) are new additions to the *C. kyotensis* species complex (Crous 2002, Crous *et al*. 2004, Lombard *et al*. 2010b, 2015a). Species in this complex are characterised by their sphaeropedunculate vesicles and the formation of lateral stipe extensions on the conidiogenous apparatus (Crous *et al*. 2004, Lombard *et al*. 2010b, 2015a). The three new species introduced in this study can be distinguished from their closest phylogenetic neighbours as well as from each other by the number of branches in the conidiogenous apparatus and their macroconidial dimensions.

Calonectria spp. are soil-borne fungi that are able to exist in this substrate for long periods of time due to their abundant production of sclerotia (Crous 2002). This also implies that they can be and most likely have been extensively moved between countries and continents. Given their importance as plant pathogens, it is ironical that very little is known regarding their genetic diversity or pathways of movement globally. This study has shown that there are many more species of *Calonectria* than has been recognised and it likely that many more species have yet to be discovered. Genomes have yet to be sequenced for *Calonectria* spp. and as these emerge, tools will become available to answer questions regarding the global movement of these fungi (Crous *et al*. 2016). They will also contribute to reducing the impact of, for example, tree pathogens that are resulting in serious losses to planted forests (Wingfield *et al*. 2015).

When the 24 species newly described in this study were collected, the genus *Calonectria* had only been peripherally studied. At that time, most species had been described based on their morphological characteristics, which included vesicle shape and macroconidial dimensions and septation (Crous & Wingfield 1994, Crous 2002). However, with a large number of DNA sequences now available from recent taxonomic studies of the genus *Calonectria* (Lombard *et al*. 2010b, 2015a, Alfenas *et al*. 2015), the initial identifications could be either confirmed or corrected. This study, vividly highlights the impact that DNA sequence data have had in providing more accurate identifications of filamentous fungi (Crous *et al*. 2015a, 2016). Identifications at this level are already impacting substantially on agricultural and forestry practices as well as in the trade in food and fibre products (Crous *et al*. 2016).

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Fig. 1. The ML consensus tree inferred from the combined *cmdA*, *tef1* and *tub2* sequence alignments. Thickened lines indicate branches present in the ML, MP and Bayesian consensus trees. Branches with ML-BS & MP-BS = 100 % and PP = 1.00 are in blue. Branches with ML-BS & MP-BS ≥ 75 % and PP ≥ 0.95 are in red. Dashed lines indicate branches shortened ×10. The scale bar indicates 0.09 expected changes per site. The tree is rooted to *Curvicladiella cignea* (CBS 109167). Epi- and ex-type strains are indicated in **bold**.

Fig. 2. *Calonectria amazonica* (ex-type CBS 116250). A. Macroconidiophore. B−C. Conidiogenous apparatus with conidiophore branches and allantoid to elongate doliiform to reniform phialides. D−E. Clavate vesicles. F−G. Macroconidia. Scale bars: A = 50 µm; B−G = 10 µm.

Fig. 3. *Calonectria amazoniensis* (ex-type CBS 115440). A−C. Macroconidiophores. D−E. Conidiogenous apparatus with conidiophore branches and elongate doliiform to reniform phialides. F. Conidiogenous apparatus with lateral stipe extension. G−J. Clavate vesicles. K. Macroconidia. Scale bars: A−C = 50 µm; D−K = 10 µm.

Fig. 4. *Calonectria brasiliana* (ex-type CBS 111484). A−C. Macroconidiophores. D−F. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. G−J. Ellipsoid to obpyrifom vesicles. K. Macroconidia. Scale bars: A−C = 50 µm; D−K = 10 µm.

Fig. 5. *Calonectria brassicicola* (ex-type CBS 112841). A–C. Macroconidiophores. D–F. Conidiogenous apparatus with lateral stipe extensions and doliiform to reniform phialides. G–J. Sphaeropedunculate vesicles. K. Macroconidia. Scale bars: A–C = 50 µm; D–K = 10 µm.

Fig. 6. *Calonectria brevistipitata* (ex-type CBS 115671). A–C. Macroconidiophores. D–E. Conidiogenous apparatus with conidiophore branches and elongate doliiform to reniform phialides. F. Conidiogenous apparatus with lateral stipe extension. G–J. Fusiform to ellipsoid vesicles. K. Macroconidia. Scale bars: A–C = 50 µm; D–K = 10 µm.

Fig. 7. *Calonectria cliffordiicola* (ex-type CBS 111812). A–C. Macroconidiophores. D–F. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. G–J. Ellipsoid to obpyrifom vesicles. K. Macroconidia. Scale bars: A–C = 50 µm; D–K = 10 µm.

Fig. 8. *Calonectria ericae* (ex-type CBS 114458). A–C. Macroconidiophores. D–F. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. G–J. Ellipsoid to obpyriform vesicles. K. Macroconidia. Scale bars: A–C = 50 µm; D–K = 10 µm.

Fig. 9. *Calonectria indonesiana* (ex-type CBS 112936). A–C. Macroconidiophores. D–E. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. F. Conidiogenous apparatus with lateral stipe extension. G–J. Sphaeropedunculate vesicles. K. Macroconidia. Scale bars: A–C = 50 µm; D–K = 10 µm.

Fig. 10. *Calonectria lageniformis* (ex-type CBS 111324). A–B. Macroconidiophores. C–E. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. F–I. Lageniformis to ellipsoid vesicles. J. Macroconidia. Scale bars: A–B = 50 µm; C–J = 10 µm.

Fig. 11. *Calonectria machaerinae* (ex-type CBS 123183). A–C. Macroconidiophores. D–E. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. F. Conidiogenous apparatus with lateral stipe extension. G–J. Ellipsoid to obpyriform vesicles. K. Macroconidia. Scale bars: A–C = 50 µm; D–K = 10 µm.

Fig. 12. *Calonectria multilateralis* (ex-type CBS 110932). A–C. Macroconidiophores. D–E. Conidiogenous apparatus with conidiophore branches and doliiform to reniform to elongate reniform phialides. F. Conidiogenous apparatus with lateral stipe extension. G–J. Naviculate vesicles. K. Macroconidia. Scale bars: A–C = 50 µm; D–K = 10 µm.

Fig. 13. *Calonectria paracolhounii* (ex-type CBS 114679). A–B. Macroconidiophores. C–D. Clavate vesicles. E–F. Conidiogenous apparatus with conidiophore branches and elongate doliiform to doliiform to reniform phialides. G. Macroconidia. Scale bars: A–B = 50 µm; C–G = 10 µm.

Fig. 14. *Calonectria parva* (ex-type CBS 110798). A. Macroconidiophore. B–C. Conidiogenous apparatus with conidiophore branches and cylindrical to allantoid phialides. D–E. Narrowly clavate vesicles. F. Macroconidia. Scale bars = 10 µm.

Fig. 15. *Calonectria plurilateralis* (ex-type CBS 111401). A–C. Macroconidiophores with lateral stipe extensions. D. Conidiogenous apparatus with lateral stipe extensions. E–F. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. G–H. Obpyriform to ellipsoidal vesicles. I. Macroconidia. Scale bars: A–C = 50 µm; D–K = 10 µm.

Fig. 16. *Calonectria pseudoecuadoriae* (ex-type CBS 111402). A–B. Macroconidiophores. C–E. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. F–I. Clavate vesicles. J. Macroconidia. Scale bars: A–B = 50 µm; C–J = 10 µm.

Fig. 17. *Calonectria pseudouxmalensis* (ex-type CBS 110924). A–C. Macroconidiophores. D–F. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. G–J. Obpyriform to ellipsoidal vesicles. K. Macroconidia. Scale bars: A–C = 50 µm; D–K = 10 µm.

Fig. 18. *Calonectria putriramosa* (ex-type CBS 111449). A–B. Macroconidiophores. C–E. Conidiogenous apparatus with conidiophore branches and elongate reniform to allantoid to cylindrical phialides. F–I. Ellipsoid to obpyrifom vesicles. J. Macroconidia. Scale bars: A–B = 50 µm; C–J = 10 µm.

Fig. 19. *Calonectria stipitata* (ex-type CBS 112513). A–C. Macroconidiophores. D–E. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. F. Conidiogenous apparatus with lateral stipe extension. G–J. Ellipsoid to obpyriform vesicles. K. Macroconidia. Scale bars: A–C = 50 µm; D–K = 10 µm.

Fig. 20. *Calonectria syzygiicola* (ex-type CBS 112831). A–B. Macroconidiophores. C–E. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. F–H. Sphaeropedunculate vesicles. I. Macroconidia. Scale bars: A–B = 50 µm; C–I = 10 µm.

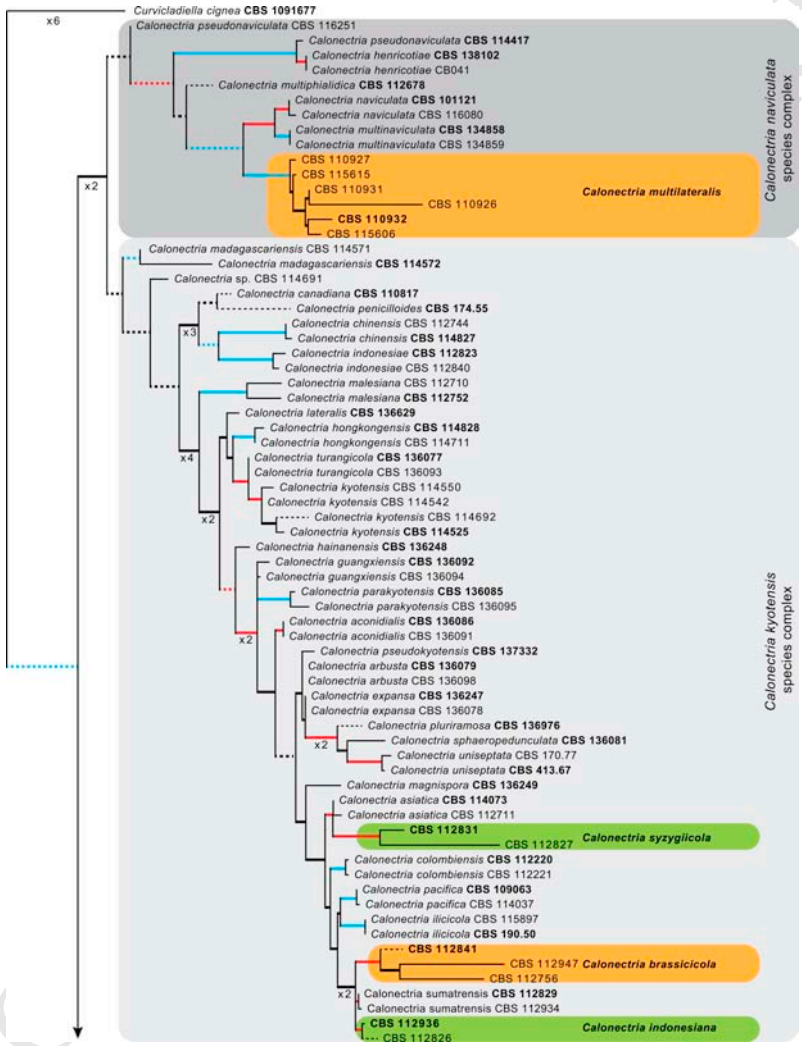
Fig. 21. *Calonectria tereticornis* (ex-type CBS 111301). A–C. Macroconidiophores. D–F. Conidiogenous apparatus with conidiophore branches and elongate doliiform to reniform phialides. G–J. Fusiform to ovoid vesicles. K. Macroconidia. Scale bars: A–C = 50 µm; D–K = 10 µm.

Fig. 22. *Calonectria terricola* (ex-type CBS 116247). A–C. Macroconidiophores. D–F. Conidiogenous apparatus with conidiophore branches and elongate doliiform to reniform phialides. G–J. Fusiform to ovoid vesicles. K. Macroconidia. Scale bars: A–C = 50 µm; D–K = 10 µm.

Fig. 23. *Calonectria tropicalis* (ex-type CBS 116271). A–C. Macroconidiophores. D–E. Conidiogenous apparatus with conidiophore branches and elongate doliiform to reniform phialides. F–I. Clavate vesicles. J. Macroconidia. Scale bars: A–C = 50 µm; D–J = 10 µm.

Fig. 24. *Calonectria uxmalensis* (ex-type CBS 110925). A−C. Macroconidiophores with lateral stipe extensions. D−F. Conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides. G−J. Obpyriform to ellipsoidal vesicles. K. Macroconidia. Scale bars: A−C = 50 µm; D−K = 10 µm.

Fig. 25. *Calonectria venezuelana* (ex-type CBS 111052). A−B. Macroconidiophores. C−D. Conidiogenous apparatus with conidiophore branches and elongate doliiform to reniform phialides. E−F. Fusiform to ovoid to ellipsoid vesicles. G. Macroconidia. H. Microconidia. I. Micro− and macroconidia. Scale bars: A−B = 50 µm; C−I = 10 µm.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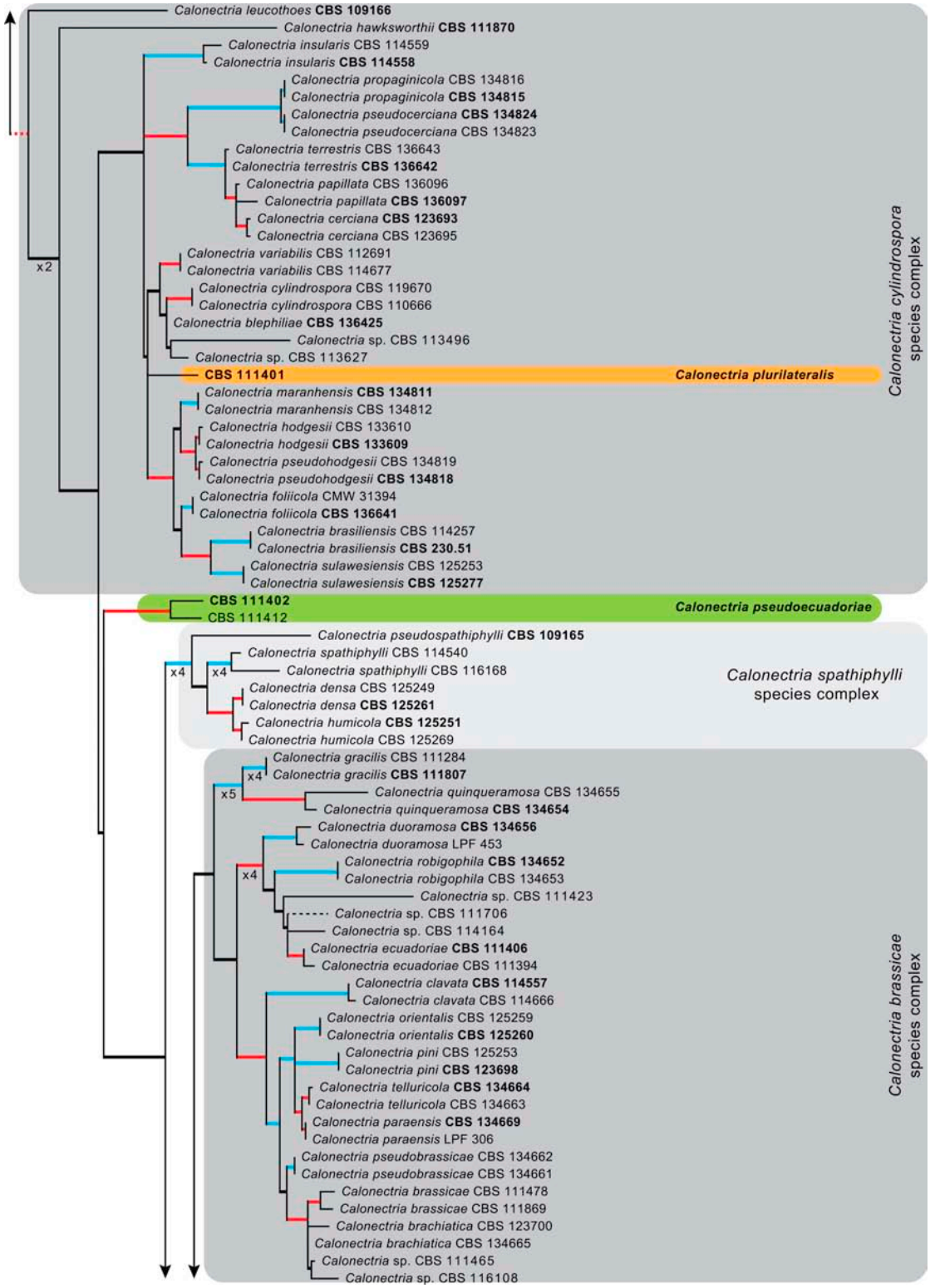
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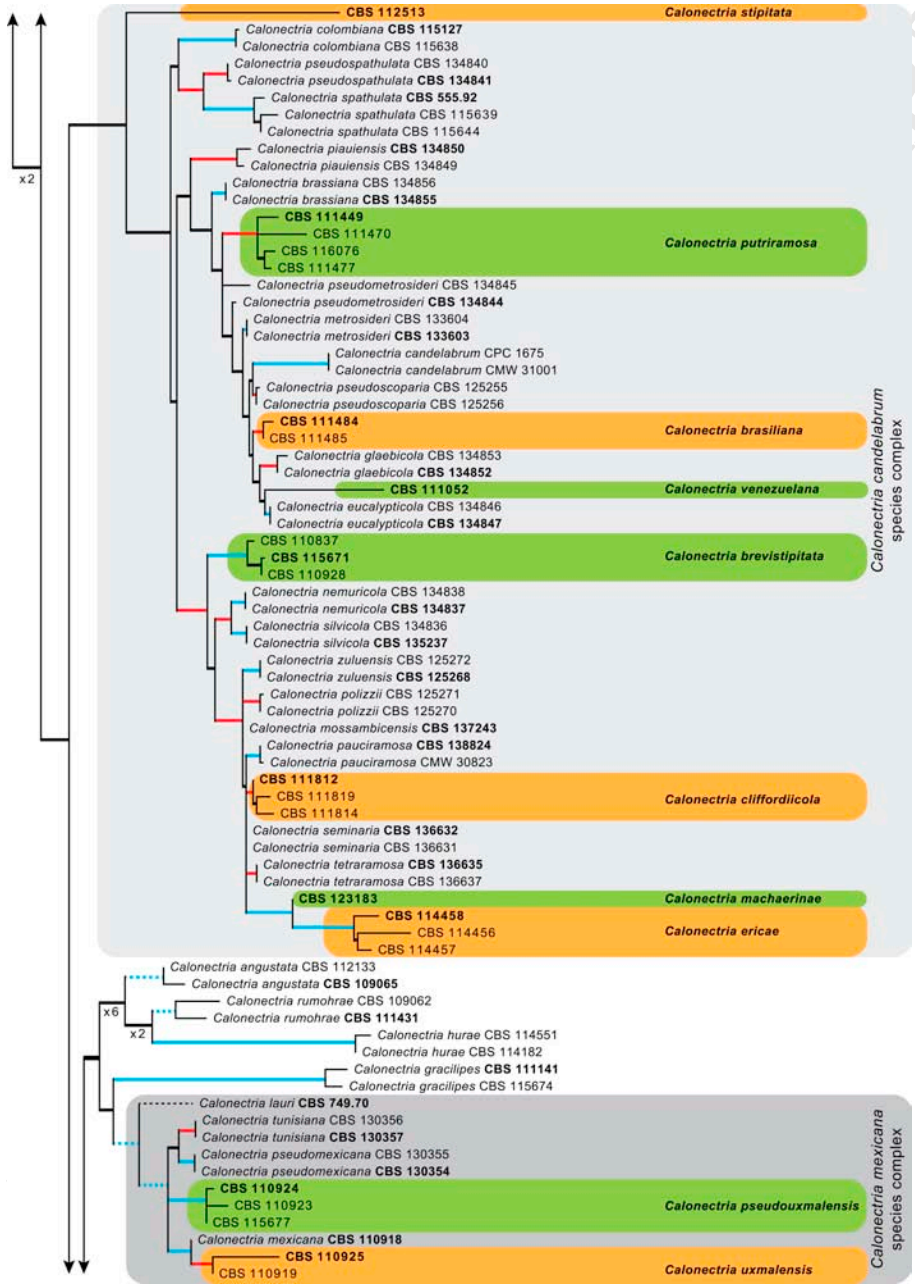
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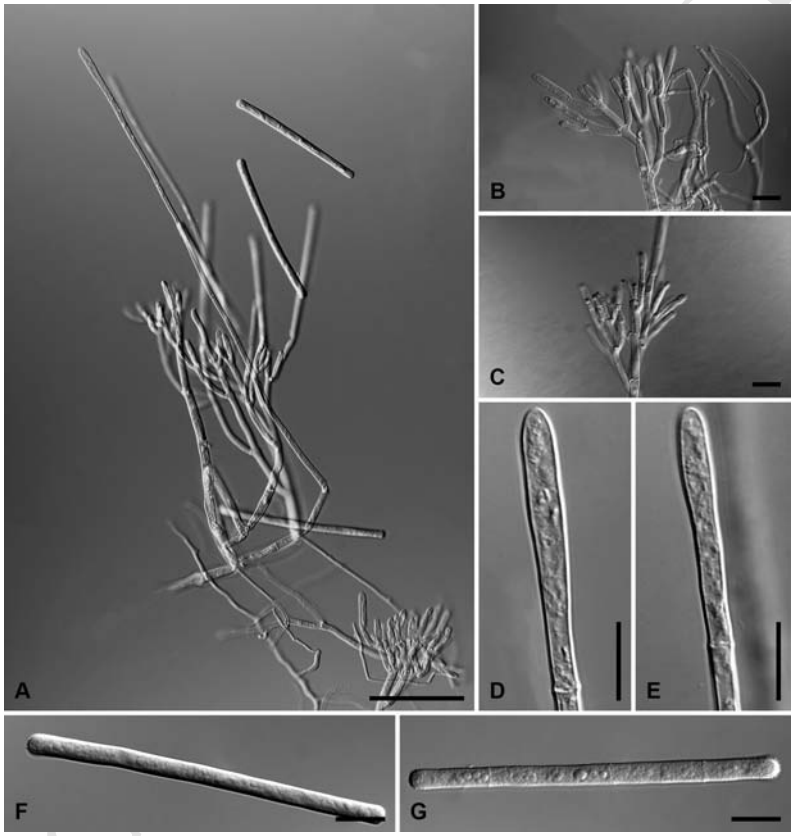

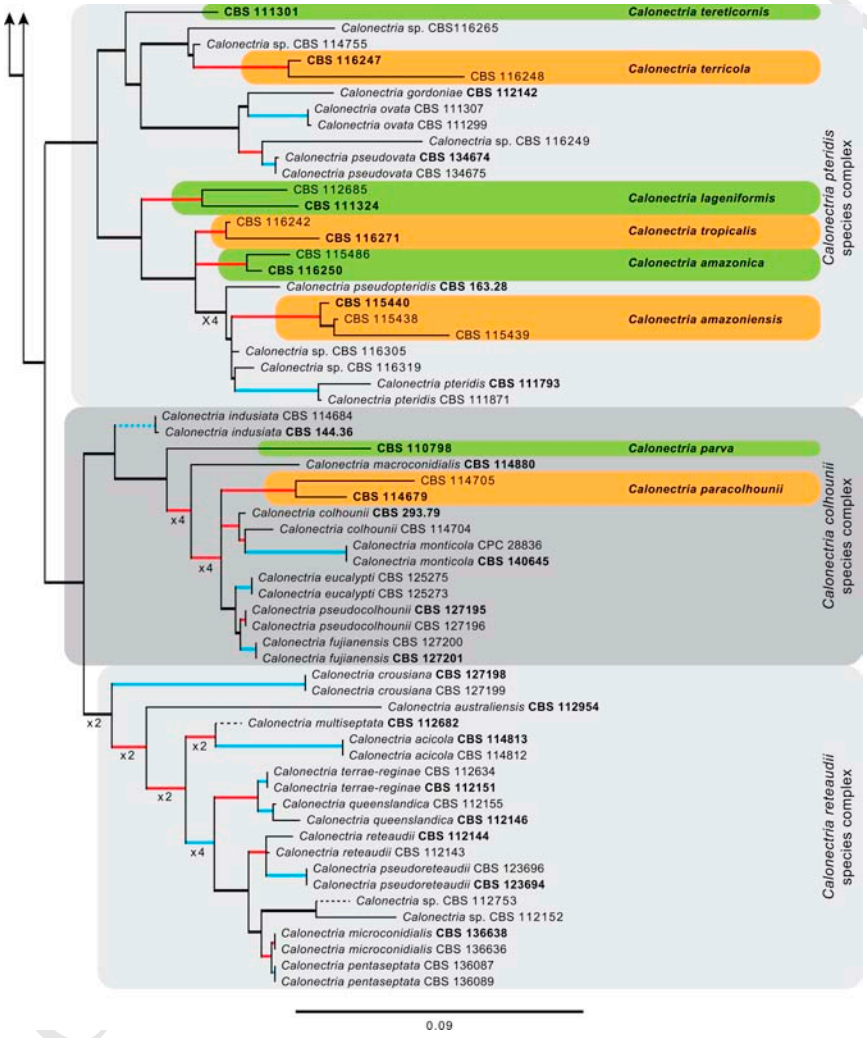
CBS 111301
Calonectria tereticornis
Calonectria sp. CBS116265
Calonectria sp. CBS 114755
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Calonectria gordoniae CBS 112142
Calonectria ovata CBS 111307
Calonectria ovata CBS 111299
Calonectria sp. CBS 116249
Calonectria pseudovata CBS 134674
Calonectria pseudovata CBS 134675
CBS 112685
CBS 111324
Calonectria lageniformis
CBS 116242
CBS 116271
Calonectria tropicalis
CBS 115486
CBS 116250
Calonectria amazonica
Calonectria pseudopteridis CBS 163.28
CBS 115440
CBS 115438
CBS 115439
Calonectria amazoniensis
X4
Calonectria sp. CBS 116305
Calonectria sp. CBS 116319
Calonectria pteridis CBS 111793
Calonectria pteridis CBS 111871
Calonectria pteridis species complex
Calonectria indusiata CBS 114684
Calonectria indusiata CBS 144.36
CBS 110798
Calonectria parva
Calonectria macroconidialis CBS 114880
CBS 114705
CBS 114679
Calonectria paracolhounii
x4
Calonectria colhounii CBS 293.79
Calonectria colhounii CBS 114704
Calonectria monticola CPC 28836
Calonectria monticola CBS 140645
x4
Calonectria eucalypti CBS 125275
Calonectria eucalypti CBS 125273
Calonectria pseudocolhounii CBS 127195
Calonectria pseudocolhounii CBS 127196
Calonectria fujianensis CBS 127200
Calonectria fujianensis CBS 127201
Calonectria colhounii species complex
Calonectria crousiana CBS 127198
Calonectria crousiana CBS 127199
Calonectria australiensis CBS 112954
x2
Calonectria multiseptata CBS 112682
x2
Calonectria acicola CBS 114813
Calonectria acicola CBS 114812
x2
Calonectria terrae-reginae CBS 112634
Calonectria terrae-reginae CBS 112151
Calonectria queenslandica CBS 112155
Calonectria queenslandica CBS 112146
x4
Calonectria reteaudii CBS 112144
Calonectria reteaudii CBS 112143
Calonectria pseudoreteaudii CBS 123696
Calonectria pseudoreteaudii CBS 123694
Calonectria sp. CBS 112753
Calonectria sp. CBS 112152
Calonectria microconidialis CBS 136638
Calonectria microconidialis CBS 136636
Calonectria pentaseptata CBS 136087
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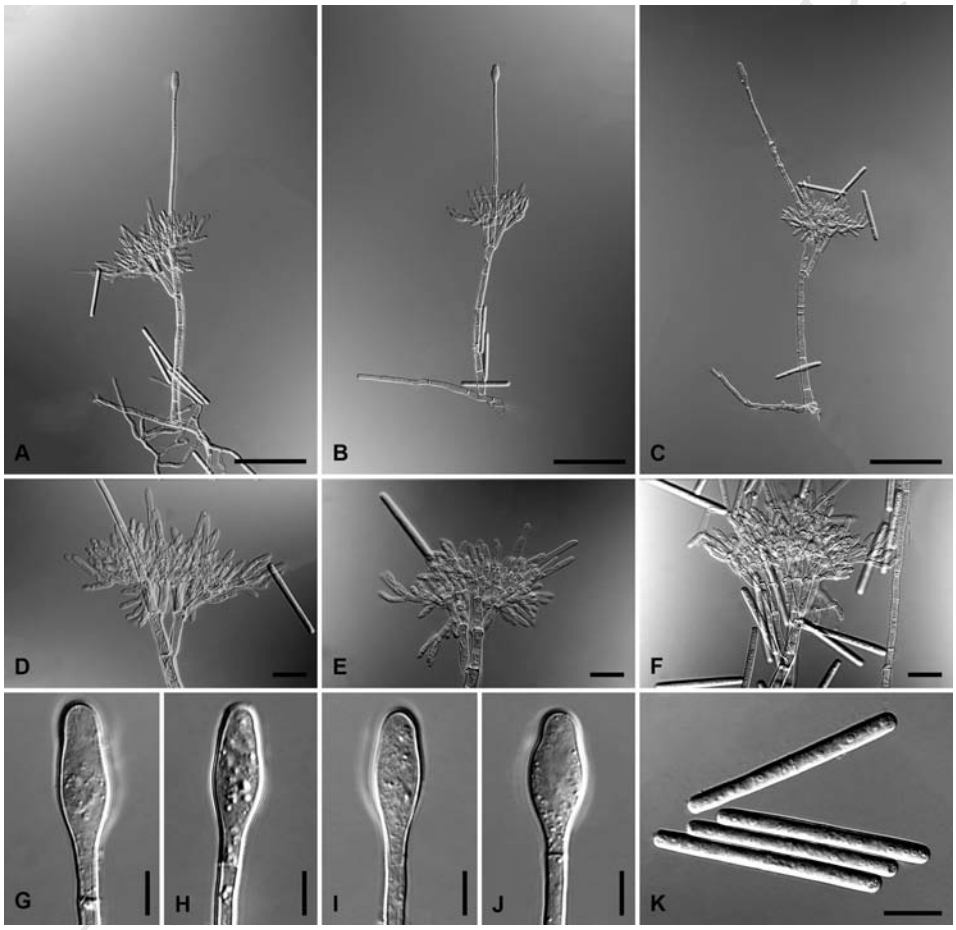
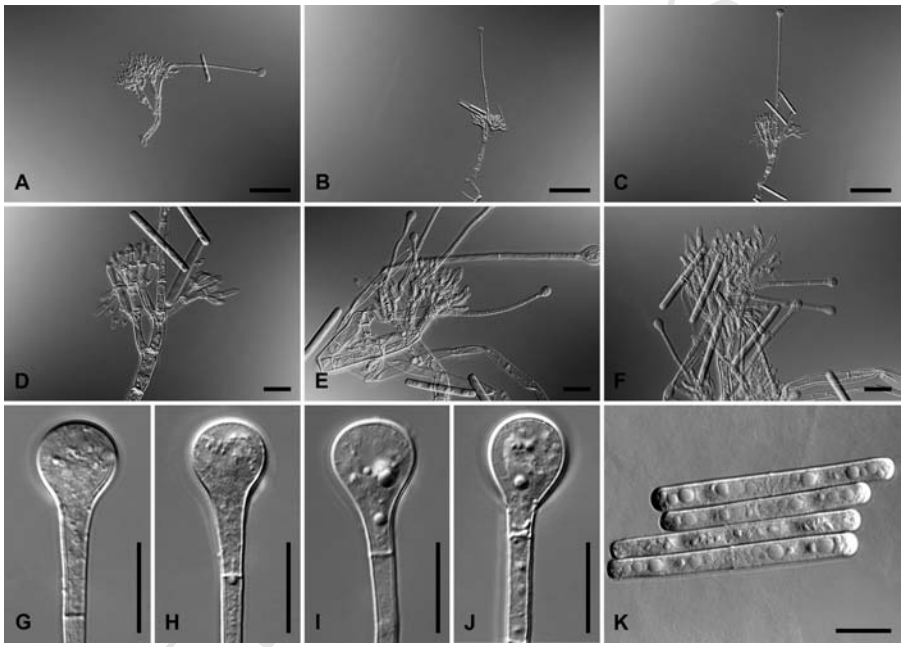
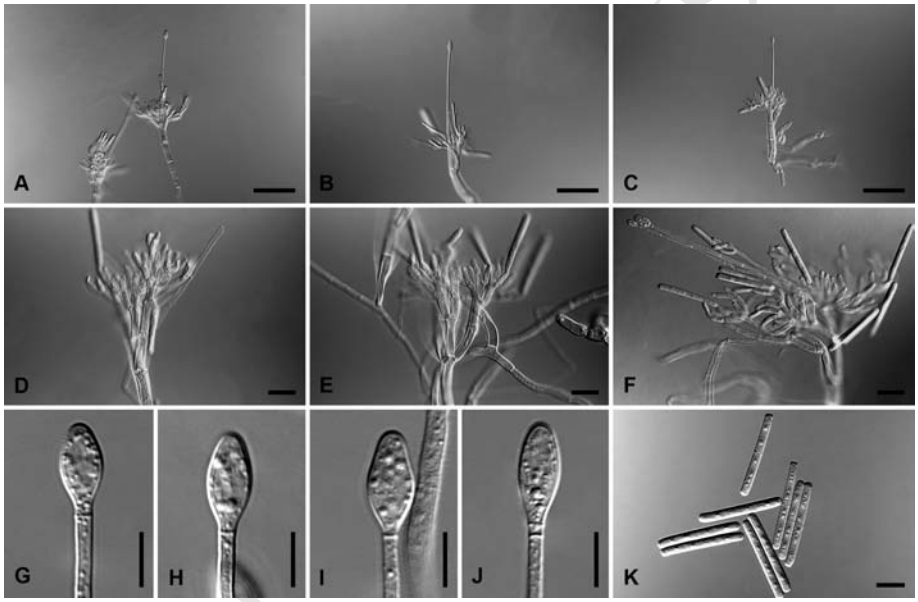
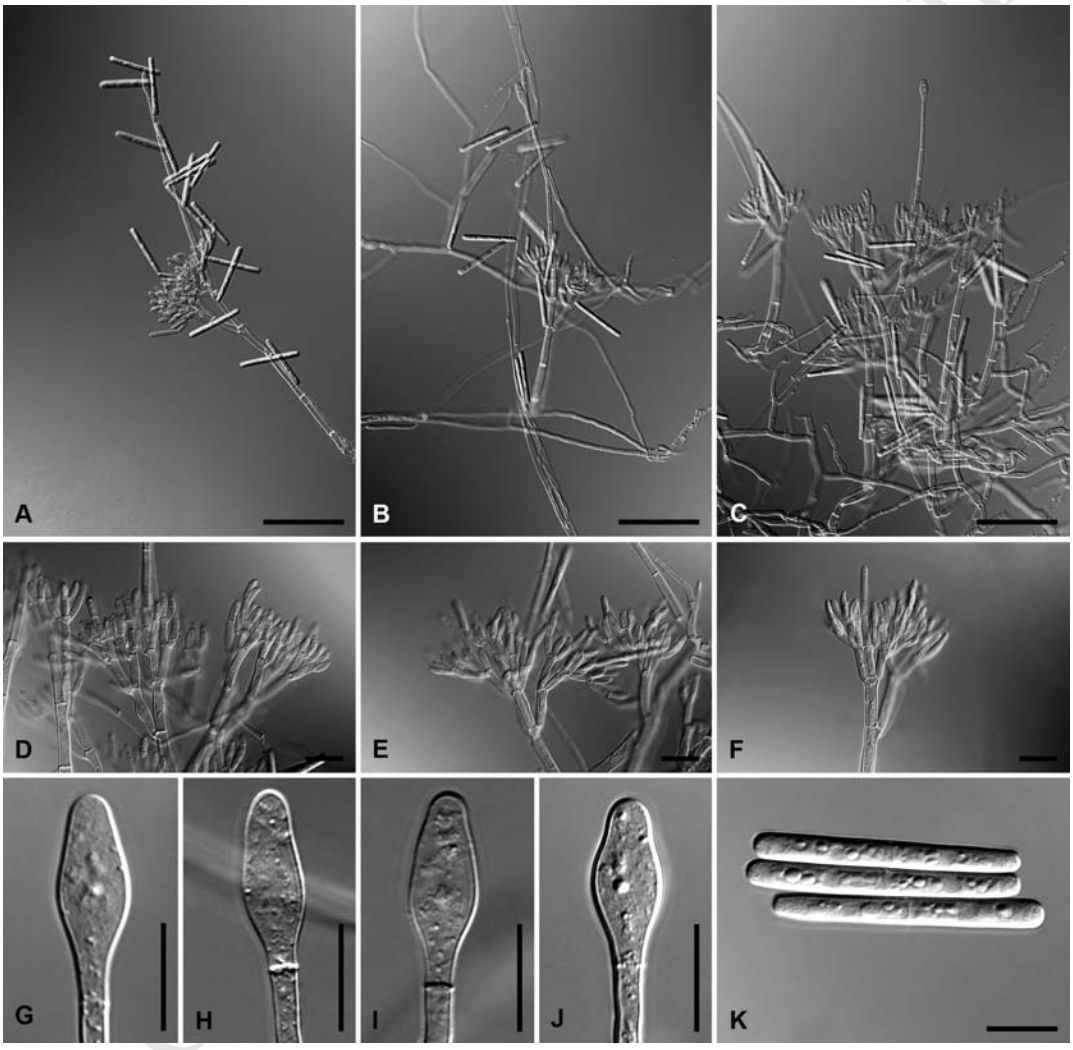
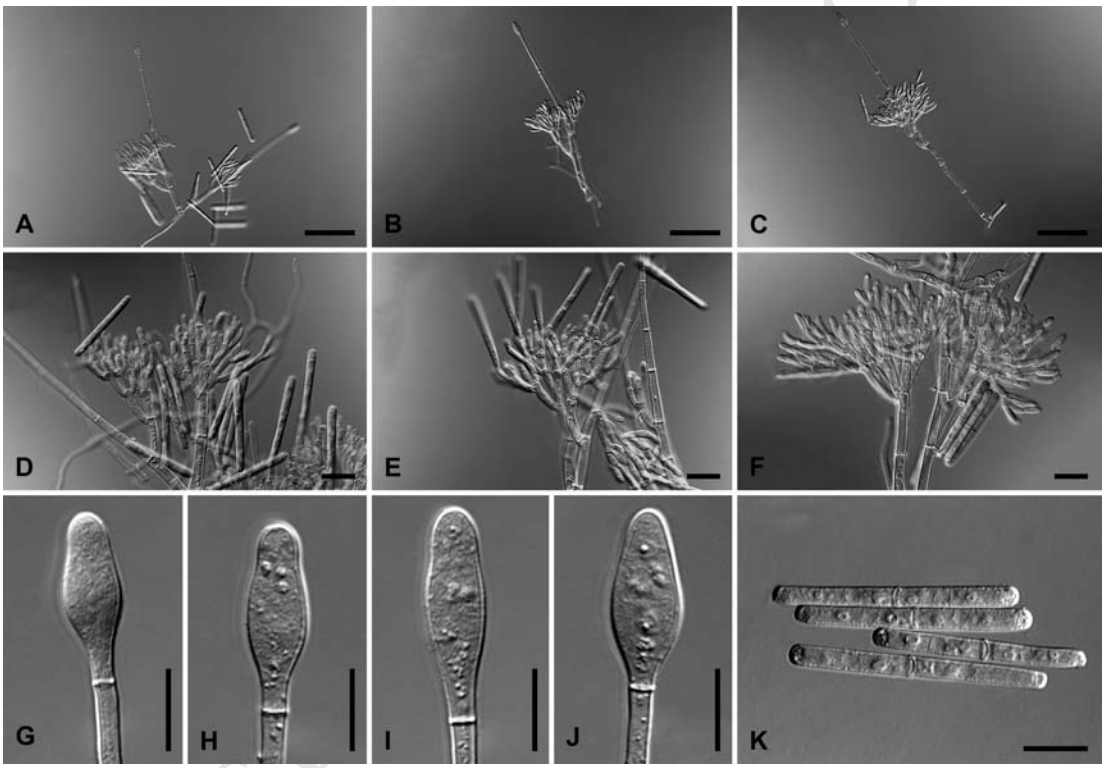
Calonectria reteaudii species complex
0.09

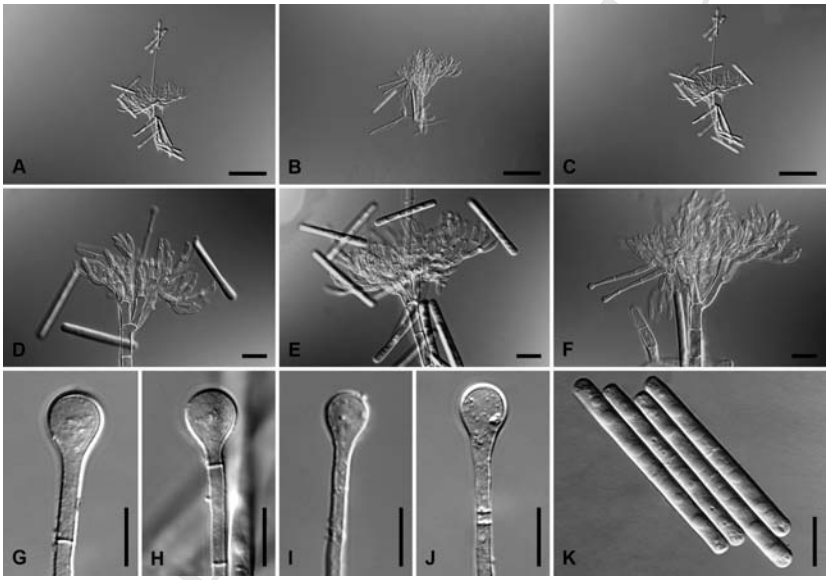


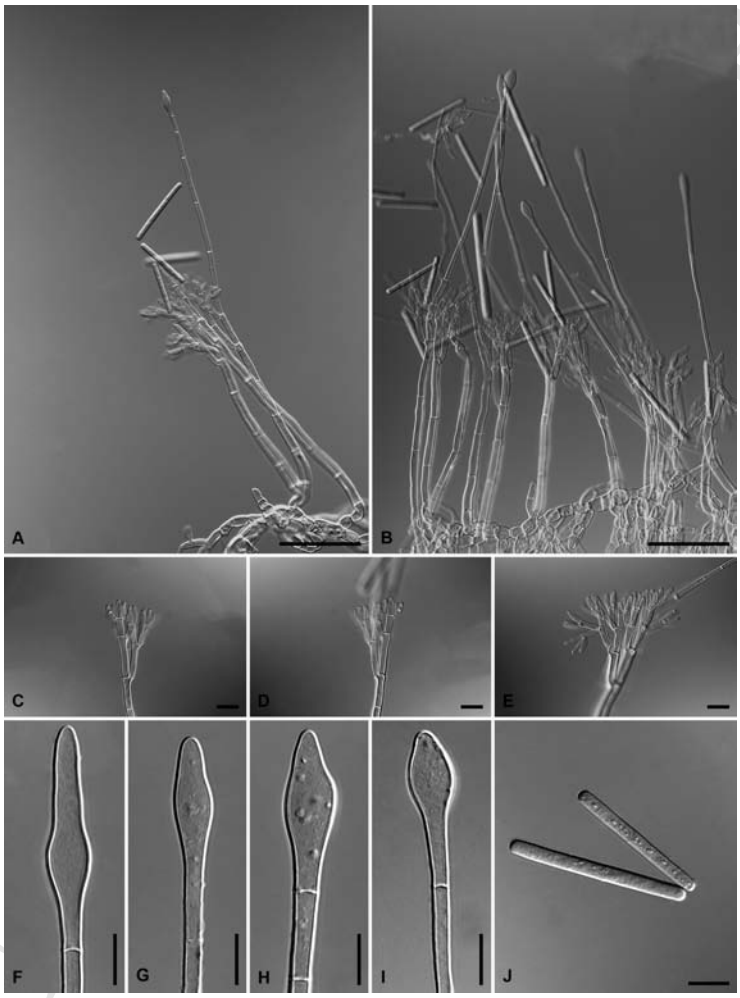


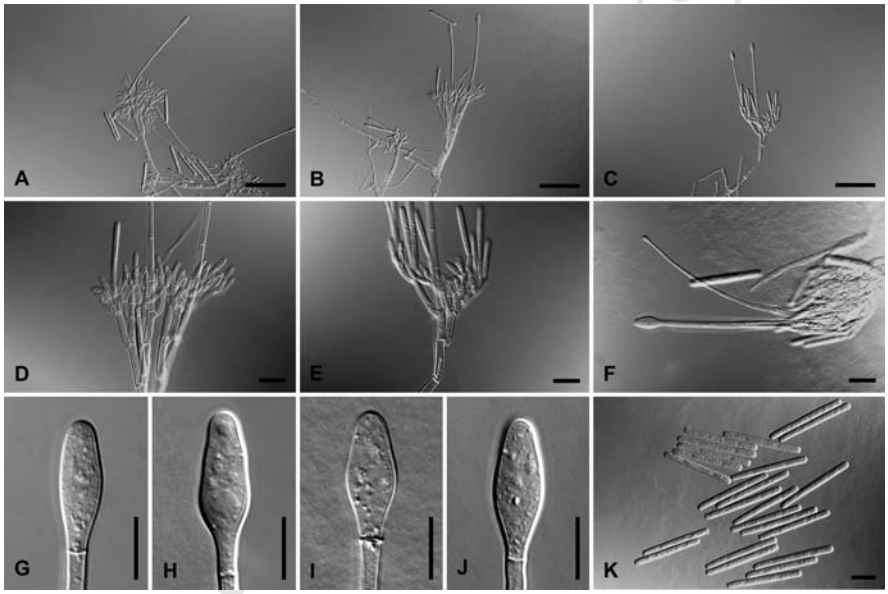


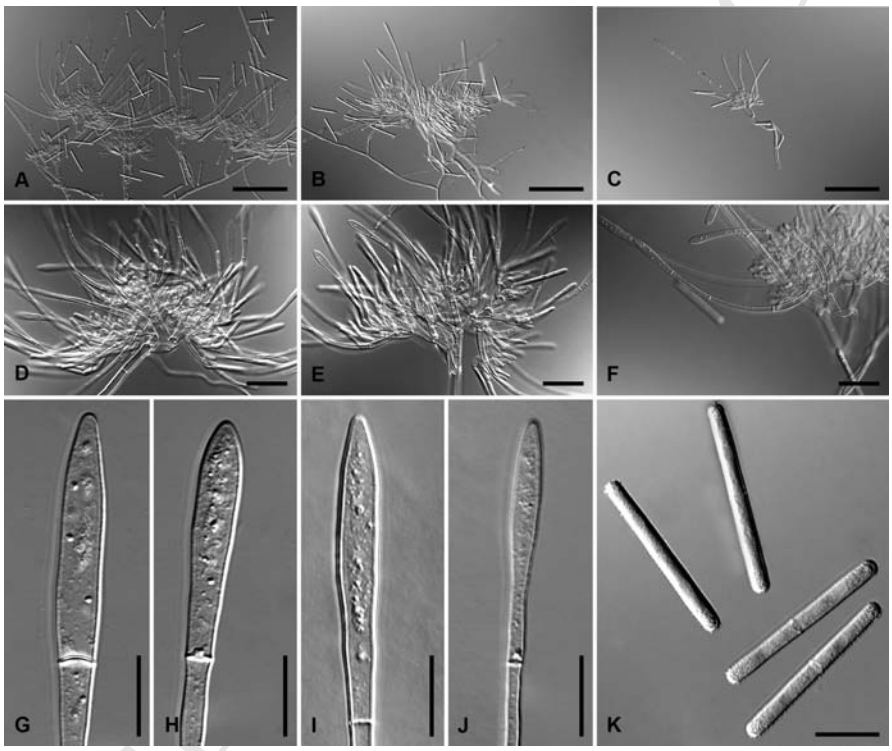


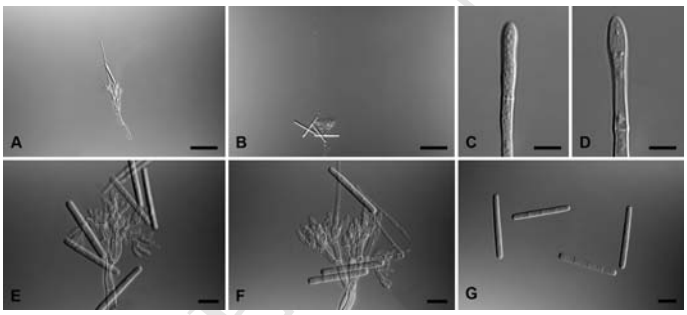


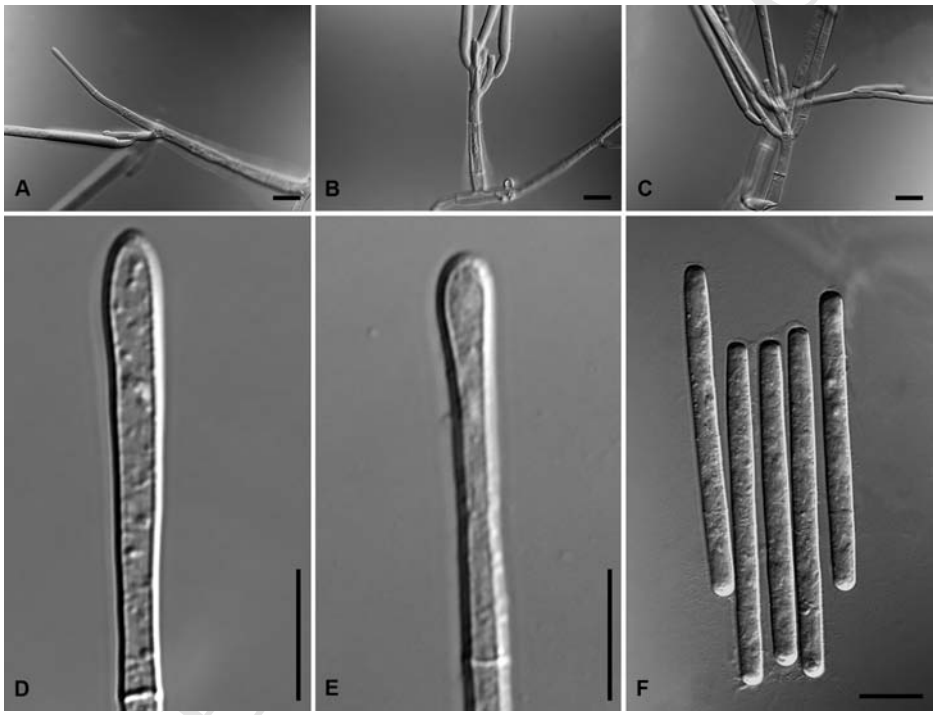


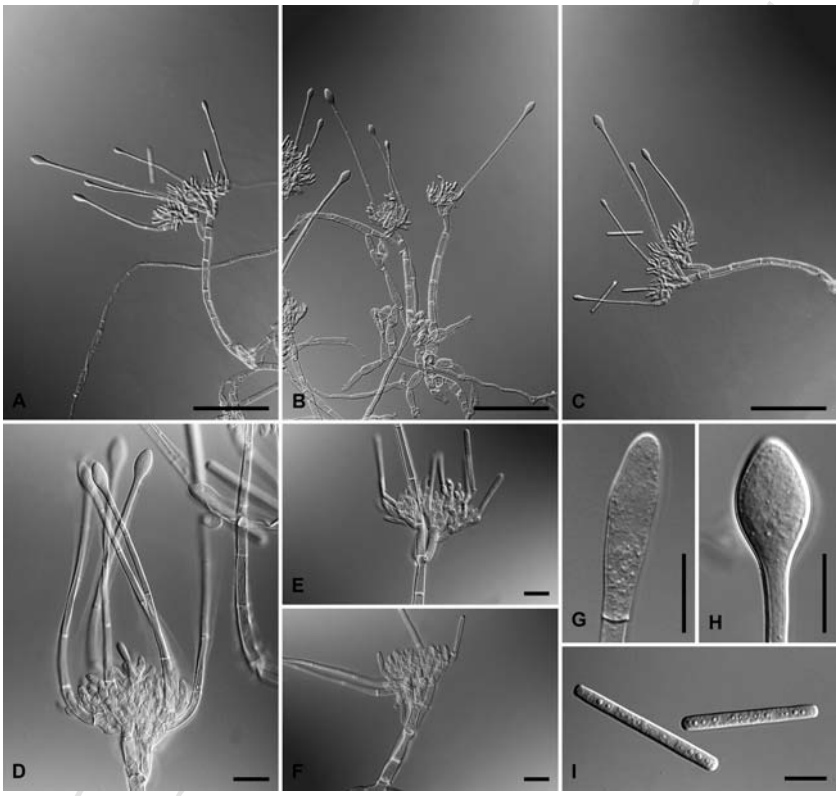


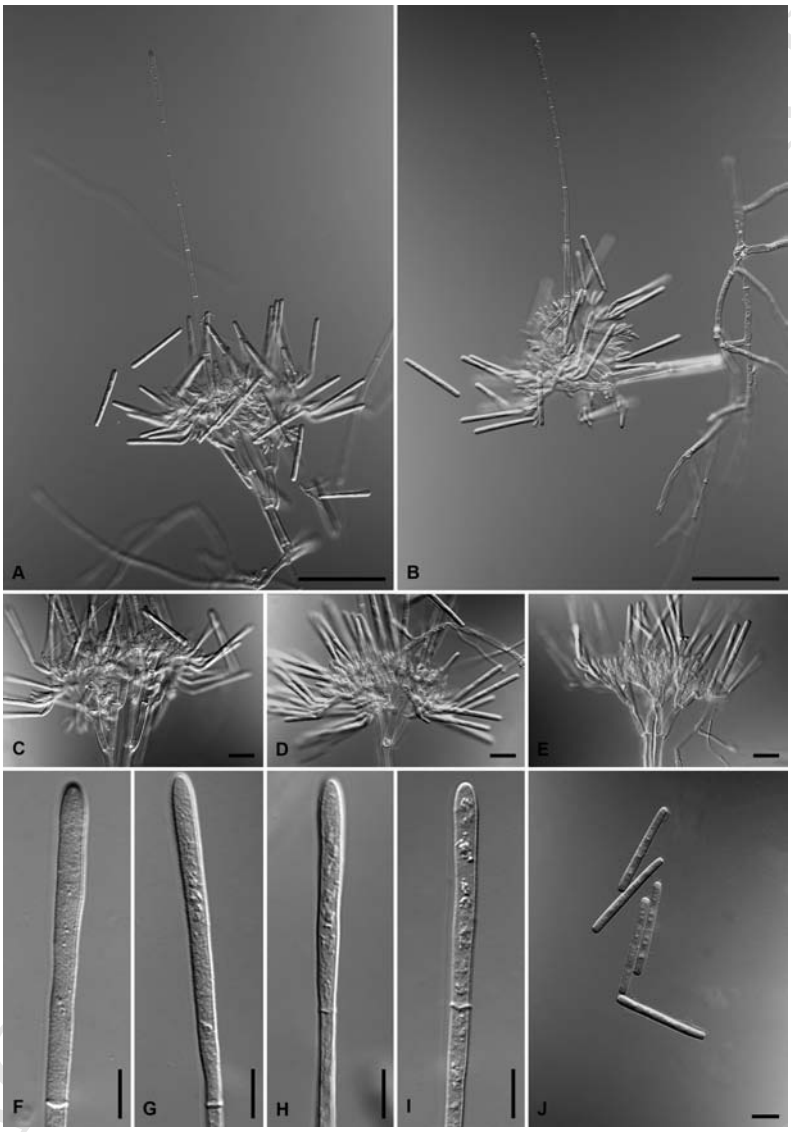


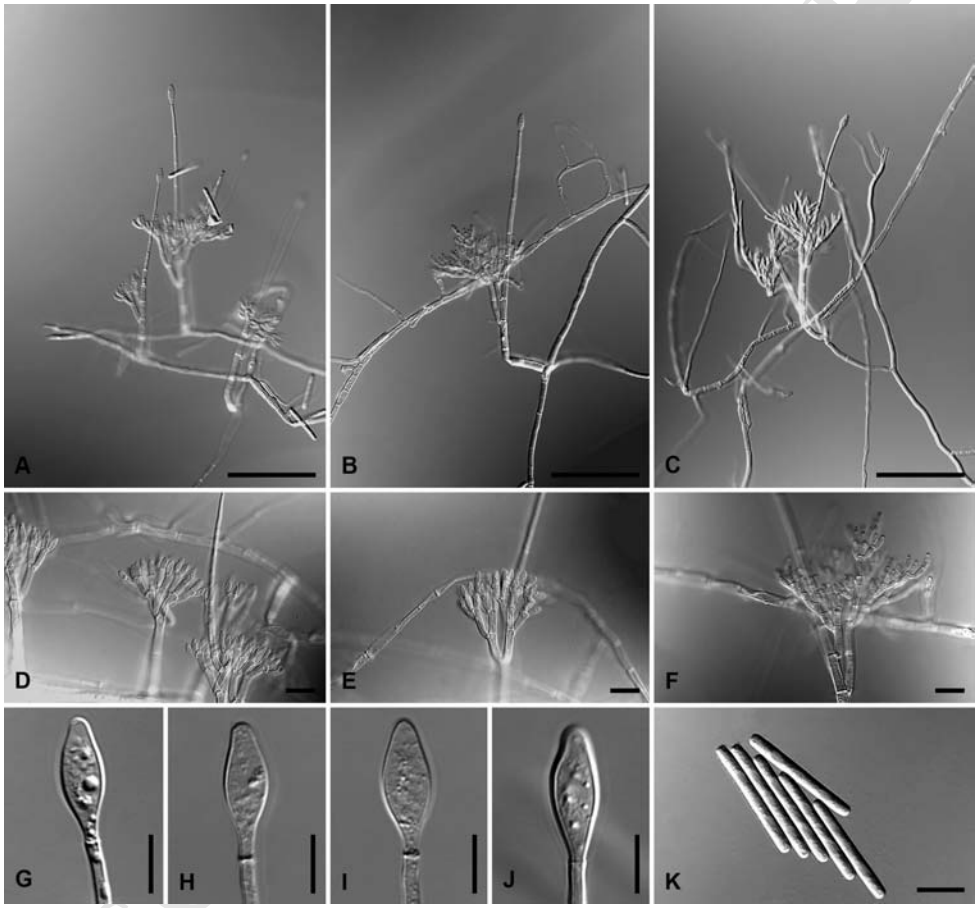


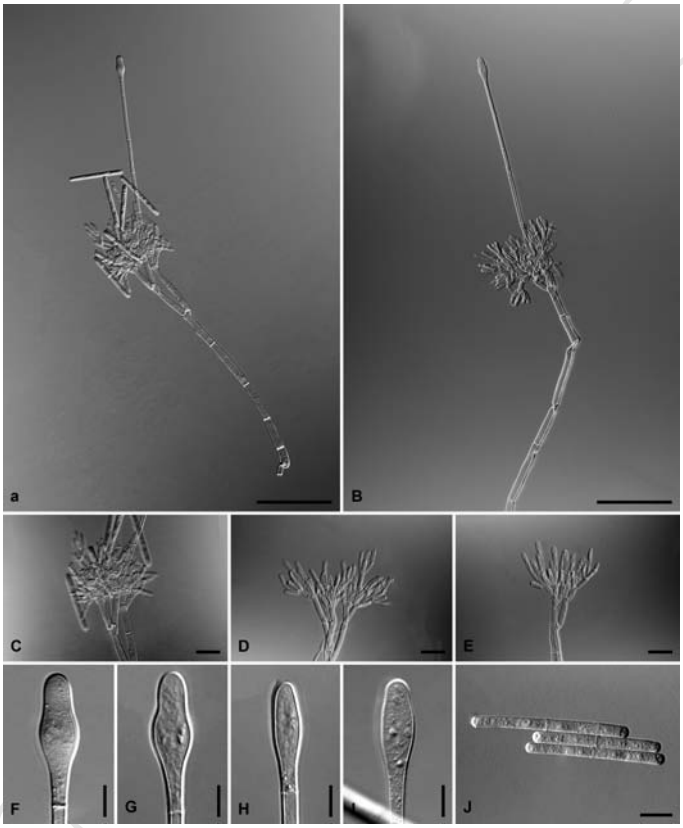


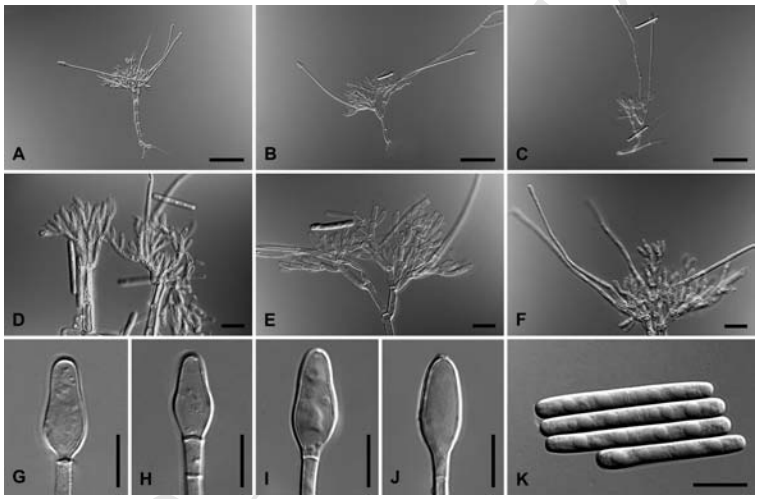


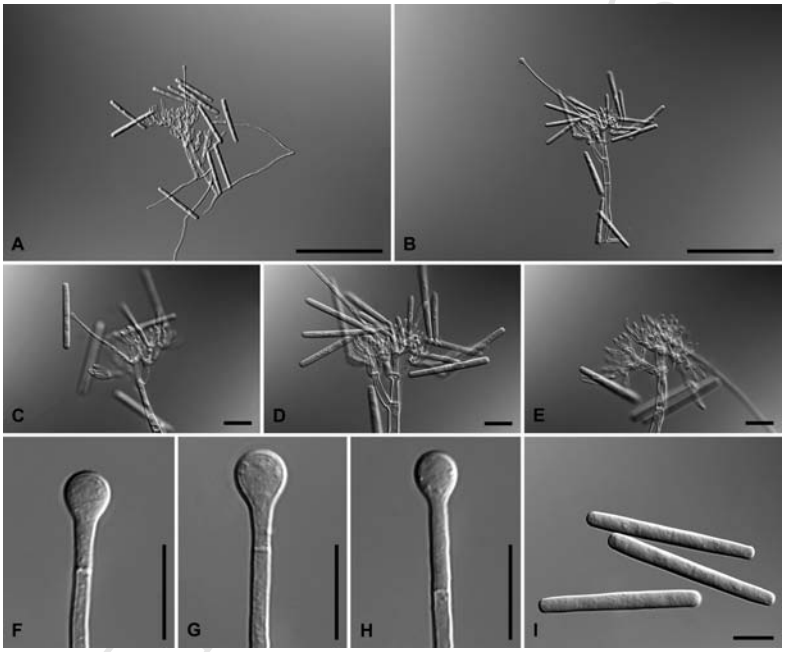


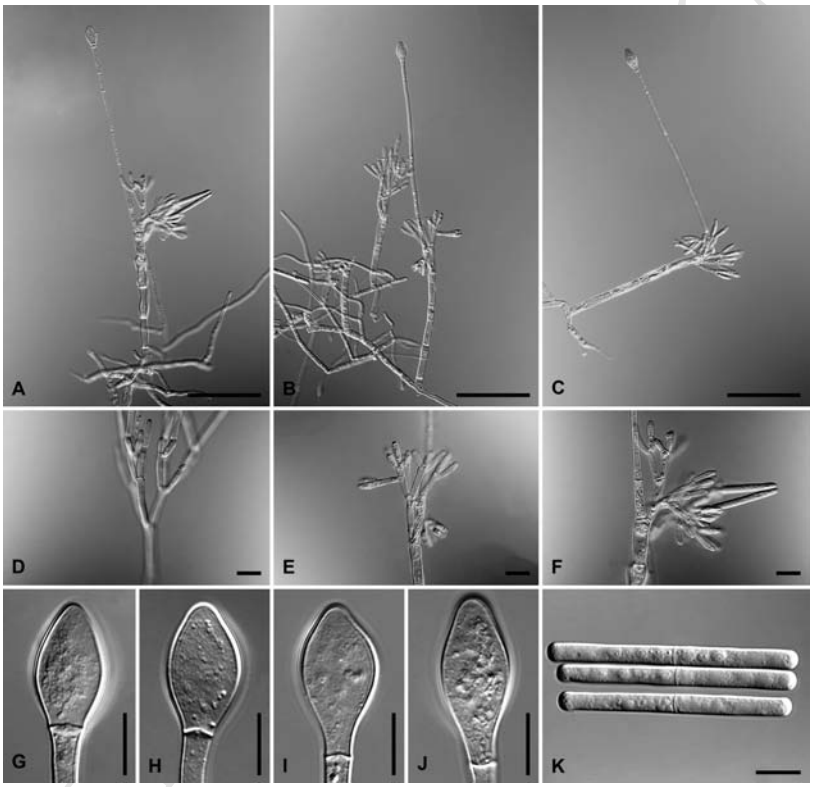


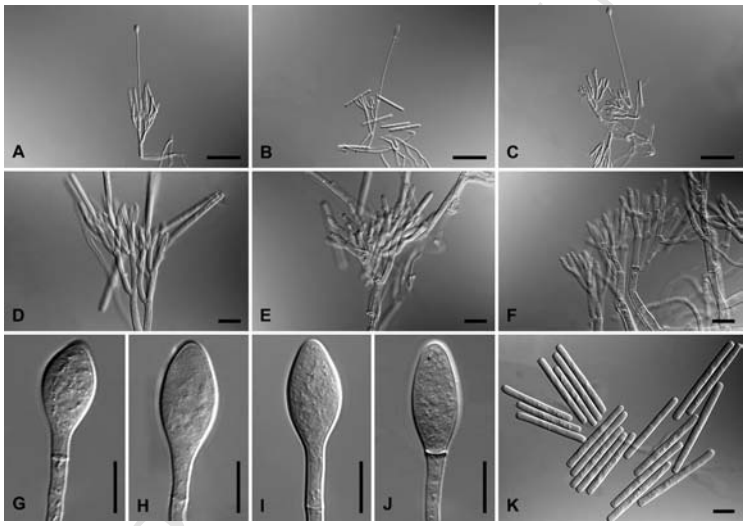


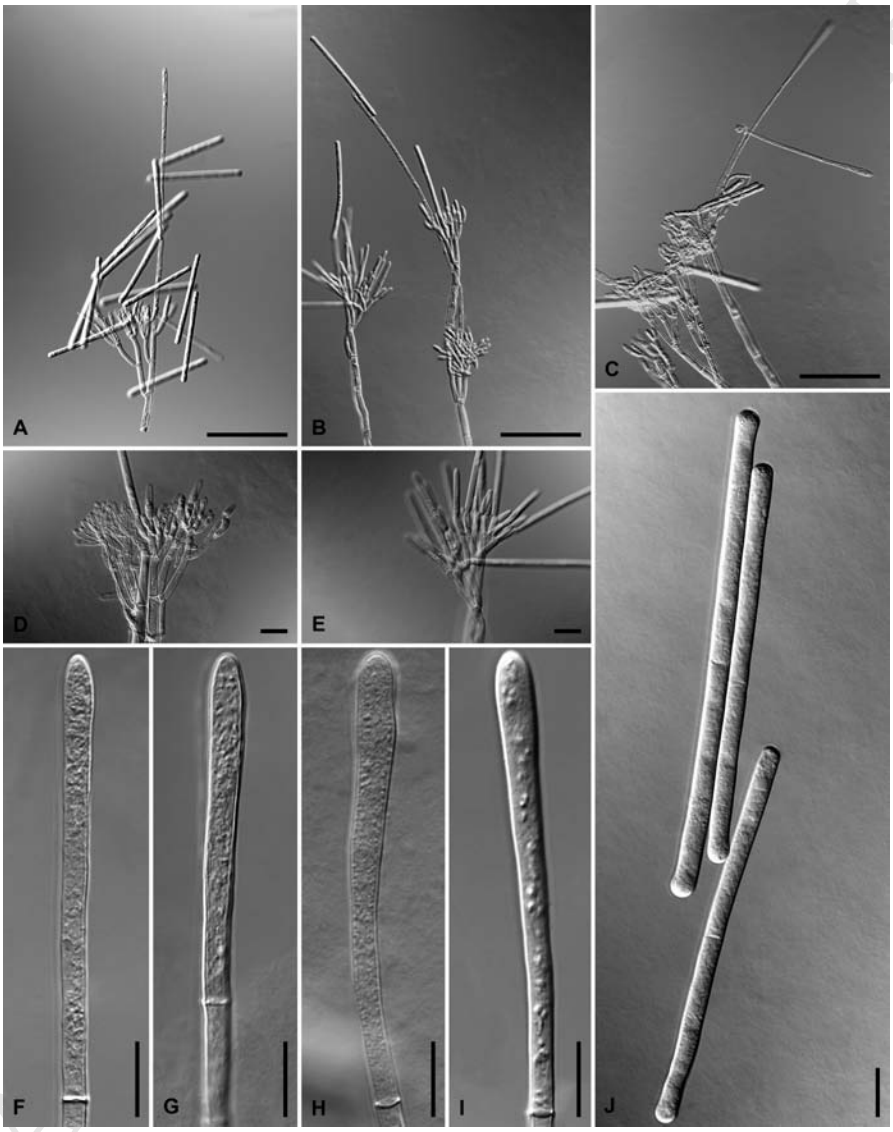








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