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Calonectria species and their *Cylindrocladium* anamorphs: species with sphaeropedunculate vesicles

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Abstract: Species of *Cylindrocladium* have wide host ranges, and are commonly distributed in soils of tropical and subtropical regions of the world. In the present study several isolates, which have been baited from soils from various parts of the world, are compared based on morphology, as well as DNA sequence data from their β-tubulin, histone, elongation factor 1-α and calmodulin gene regions. As a result of these studies, eight new species with sphaeropedunculate vesicles and 1-septate conidia are described. An emended key is provided to distinguish these species from others in the *Cy. floridanum* species complex.

Taxonomic novelties: *Calonectria asiatica* Crous & N.L. Hywel-Jones sp. nov. (anamorph *Cylindrocladium asiaticum* Crous & N.L. Hywel-Jones sp. nov.), *Calonectria colombiensis* Crous sp. nov. (anamorph *Cylindrocladium colombiense* Crous sp. nov.), *Calonectria hongkongensis* Crous sp. nov. (anamorph *Cylindrocladium hongkongense* Crous sp. nov.), *Cylindrocladium chinense* Crous sp. nov., *Cylindrocladium indonesiae* Crous sp. nov., *Cylindrocladium malesianum* Crous sp. nov., *Cylindrocladium multiphialidicum* Crous, P. Simoneau & J.-M. Risède sp. nov., *Cylindrocladium sumatrense* Crous sp. nov.

Key words: *Ascomycetes, Calonectria, Cylindrocladium, Hypocreales*, leaf spots, soil fungi, systematics.

INTRODUCTION

Species of *Cylindrocladium* Morgan (*Cy.*) are commonly associated with a wide range of disease symptoms, including leaf spot, stem rot, canker, blight, root and pod rot, to name but a few (Crous 2002). Wherever sexual reproduction is known to occur, species of *Cylindrocladium* have *Calonectria* De Not. (*Ca.*) (*Nectriaceae*, *Hypocreales*, *Ascomycetes*) teleomorphs (Rossman 1979). These have been reported for 28 of the 41 species currently recognized (Crous 2002, Crous *et al*. 2002). In the past, species were chiefly identified based on the morphology of their anamorph (Peerally 1991). In recent years a more integrated approach has been advocated, integrating morphology with DNA sequence data and sexual compatibility studies. New molecular data sets, however, have indicated considerable variation that was easily overlooked when morphology and sexual compatibility were employed as sole characters (Schoch *et al*. 1999, 2001, Kang *et al*. 2001). As shown for *Cy. gordoniae* Leahy, T.S. Shub. & El-Gholl (Crous *et al*. 2002), as well as several strains of *Cy. insulare* C.L. Schoch & Crous (Schoch *et al*. 2001), minute morphological differences that could be perceived as variation within a morphological or biological species could, in fact, be indicative of distinct but closely related species. Such species frequently remain sexually compatible with the other biological species, but are clearly separable based on molecular data.

Cylindrocladium species with sphaeropedunculate to globose vesicles have been reported as pathogens from a wide range of hosts in most tropical to subtropical countries (Victor *et al*. 1997, Crous 2002). To address speciation within this complex, Kang *et al*. (2001) used multi-allelic sequence data to delineate species within the *Cy. floridanum* Sobers & C.P. Seym. and *Cy. spathiphylli* Schoult., El-Gholl & Alfieri species complexes, describing *Cy. canadense* J.C. Kang, Crous & C.L. Schoch, *Cy. pacificum* J.C. Kang, Crous & C.L. Schoch and *Cy. pseudospathiphylli* J.C. Kang, Crous & C.L. Schoch as new species. As noted by Kang *et al.* (2001), however, some isolates did not fit in either of these taxa. This was also the case for some recent collections of *Cylindrocladium* isolates with sphaeropedunculate vesicles that were obtained from various localities. The aim of the present study, therefore, was to analyze these strains by means of morphology, and DNA sequence

analysis of their β-tubulin, calmodulin, elongation factor 1-α and histone gene regions.

MATERIALS AND METHODS

Isolates

Isolates were obtained from debris, or baited from soil as explained in Crous (2002). They were studied on divided plates containing 2 % malt extract agar (MEA) (2 g/L) (Biolab, Midrand, South Africa) in one half, and carnation leaf agar (CLA) [1 % water agar (1 g/L) (Biolab) with autoclaved carnation leaves placed onto the medium] in the other. These plates were incubated for 7 d at 25 °C under continuous near-UV light, to promote sporulation.

DNA phylogeny

The protocol of Lee & Taylor (1990) was used to isolate genomic DNA from fungal mycelium grown on MEA plates. Four loci were amplified, namely, part of the β-tubulin gene, amplified with primers T1 (O'Donnell & Cigelnik 1997) and Bt-2b (Glass & Donaldson 1995); part of the histone 3 (H3) gene with primers H3-1a and H3-1b (Glass & Donaldson 1995); part of the elongation factor 1-α gene with primers EF1-728F and EF1-986R (Carbone & Kohn 1999), and part of the calmodulin gene with primers CAL-228F and CAL-737R (Carbone & Kohn 1999). However, some of these primer pairs failed to amplify with all of the isolates included in this study, and therefore new primers were designed. For β-tubulin, we designed a primer (CYLTUB1R: 5'-AGT TGT CGG GAC GGA AGA G-3') annealing at a position 58 nt internal to the first nucleotide position of primer Bt-2b (nucleotide positions 371–389 of GenBank sequence AY305703). For histone two primers were designed, a forward primer (CYLH3F: 5'-AGG TCC ACT GGT GGC AAG-3'; nucleotide positions 1675–1692 of GenBank sequence AY062173) annealing at a point 18 nt internal to the first nucleotide position of primer H3-1a, and a reverse primer (CYLH3R: 5'-AGC TGG ATG TCC TTG GAC TG-3'; nucleotide positions 1273–1292 of GenBank sequence AY062173) annealing at seven nt internal to the first nucleotide position of primer H3-1b. A new reverse primer (CylEF-R2: 5'-CAT GTT CTT GAT GAA (A/G)TC ACG-3'; nucleotide positions 783–803 of GenBank sequence X96615) that allows amplification of an extended region of the 3' end of the elongation factor gene was designed from sequences available on GenBank. The PCR reaction mixture used to amplify the different loci consisted of 0.5 units Biotaq polymerase (Bioline, London, U.K.), 1× PCR buffer, 0.5–1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 5 pmol of each primer, approximately 10 to 30 ng of fungal genomic DNA and was made up to a total volume of 25 µL with sterile water. Reactions were performed on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA) with cycling conditions consisting of denaturation for 5 min at 96 °C, followed by 30 cycles at 96 °C (30 s), 52 °C (30 s), and 72 °C (60 s), with a final 5 min extension step at 72 °C to complete the reaction. PCR conditions were the same for all loci, except that the $MgCl_2$ concentration varied. Histone and elongation factor amplifications used 1.0 mM $MgCl_2$; β-tubulin used 0.5 mM and calmodulin used 1.5 mM. PCR products were separated by electrophoresis at 80 V for 1 h in a 0.8 % (w/v) agarose gel in 0.5 × TAE running buffer (0.4 M Tris, 0.05 M NaAc, and 0.01 M EDTA, pH 7.85) and visualised under UV light using a GeneGenius Gel Documentation and Analysis System (Syngene, Cambridge, U.K.) following ethidium bromide staining. The amplification products were purified according to the manufacturer's instructions using a commercial kit (GFX PCR DNA and Gel Band Purification Kit, Amersham Pharmacia Biotech Europe GmbH, Freiburg, Germany). Sequencing reactions were carried out using the PCR primers in ABI PRISM Big Dye Terminator Cycle v. 3.0 Sequencing Ready Reaction Kit (Applied Biosystems) according to the manufacturer's recommendations. The reaction was analysed on an ABI Prism 3100 Genetic Analyser (Applied Biosystems).

The sequences generated in this study were added to other sequences obtained from GenBank (http://www.ncbi.nlm.nih.gov) and TreeBASE (http://www.treebase.org) and the alignment was assembled using Sequence Alignment Editor v. 2.0a11 (Rambaut 2002) with manual adjustments for improvement made visually where necessary. Sequences for *Cylindrocladiella peruviana* (Bat., J.L. Bezerra & M.M.P. Herrera) Boesew. and *Cylindrocladiella lageniformis* Crous, M.J. Wingf. & Alfenas were added to the alignments as outgroups. The phylogenetic analyses of sequence data were done using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2000). Phylogenetic analysis of both datasets in PAUP consisted of distance and parsimony analysis. For distance analysis, neighbour-joining with the uncorrected ("p"), the Jukes-Cantor and the Kimura 2-parameter substitution model were performed. Alignment gaps were treated as missing data and all characters were unordered and of equal weight. Any ties were broken randomly when encountered. For parsimony analysis, alignment gaps were treated as a fifth character state and all characters were unordered and of equal weight. A heuristic search was performed for each dataset with 100 random taxon additions and tree bisection and reconstruction (TBR) as the branch swapping algorithm. Branches of zero-length were collapsed and all multiple, equally parsimonious trees were saved. Measures calculated for parsimony included tree length, consistency index, retention index and rescaled consistency index (TL, CI, RI and

RC, respectively). The robustness of the resulting phylogenetic trees was evaluated by 1000 bootstrap replications (Hillis & Bull 1993) and the trees were printed with TreeView v. 1.6.6 (Page 1996). A partition homogeneity test (Farris *et al.* 1994) was conducted in PAUP to evaluate the feasibility of combining the sequence data sets. Sequences were deposited in GenBank (Accession numbers AY725612–AY725775) and the alignments in TreeBASE (accession number S1147).

Taxonomy

All morphological examinations were made from cultures sporulating on CLA. Structures were mounted in lactic acid, and 30 measurements at × 1000 magnification were made of each structure. The 95 % confidence levels were determined, and the extremes of spore measurements given in parentheses. Colony reverse colours were noted after 6 d on MEA at 25 °C in the dark, using the colour charts of Rayner (1970) for comparison. All cultures studied are maintained in the culture collection of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands (Table 1).

RESULTS

DNA phylogeny

The partition homogeneity test showed that the β-tubulin and histone datasets could not be combined ($P > 0.05$); therefore these datasets were analysed separately. It was possible to combine the smaller subset of taxa ($P = 0.064$) sequenced for all four loci into a single analysis.

For the β-tubulin gene, approximately 480–550 bases were determined for the isolates indicated in Table 1. The manually adjusted alignment contained 54 taxa (including the two outgroups) and 523 characters including alignment gaps. Of the 523 characters used in the analysis, 216 were parsimony-informative, 81 were variable and parsimony-uninformative, and 226 were constant. Neighbour-joining analysis using the three substitution models, as well as parsimony analysis, yielded trees with similar topology and bootstrap values. Parsimony analysis of the alignment yielded 64 most parsimonious trees (TL = 673 steps; CI = 0.738; RI = 0.867; RC = 0.640), one of which is shown in Fig. 1. The phylogenetic tree obtained shows a number of well-supported clades. Although the first cluster does not have a bootstrap support value, it does contain several well-supported clades, namely *Cy. floridanum* (100 % bootstrap support), *Cy. hongkongense* (98 %), *Cy. malesianum* (83 %), *Cy. indonesiae* (92 %), *Cy. chinense* (100 %) and *Cy. canadense* (100 %). The *Cy. floridanum*, *Cy. hongkongense* and *Cy. malesianum* clades cluster together with a bootstrap support value of 100 %, as do the *Cy. indonesiae* and *Cy. chinense* clades. The second main clustering (bootstrap support value of 100 %) contains several taxa and clades, namely *Cy. curvisporum* CPC 765 and *Cy. parasiticum* CBS 112217; a *Cy. parasiticum* clade containing isolates mainly from Hawaii (Clade 1, 95 % bootstrap support), a second *Cy. parasiticum* clade containing isolates from Indonesia and U.S.A. (Clade 2, 51 % bootstrap support), *Cy. pacificum* (87 %), *Cy. asiaticum* (95 %), *Cy. colombiense* (67 %) and *Cy. sumatrense* (100 %). Basal to the other clades is a clade (100 %) containing *Cy. multiphialidicum* and *Cy. pseudonaviculatum* as sister taxa.

For the histone gene, approximately 430 bases were determined for the isolates in Table 1. The manually adjusted alignment contained 55 taxa (including the two outgroups) and, for each taxon, 489 characters including alignment gaps were analysed. Among these characters was an insertion of 55 nucleotides in the outgroup taxa, and this was coded as a single event for analysis purposes, leaving a total of 434 characters. Of these 175 were parsimony-informative, 65 were variable and parsimony-uninformative, and 194 were constant. Neighbour-joining analysis as described previously yielded trees with similar topology and bootstrap values, except in regard to the placement of *Cy. multiphialidicum* and *Cy. pseudonaviculatum*. Using the Kimura 2-parameter model, these two isolates formed a basal polytomy, whereas the other two models placed *Cy. pseudonaviculatum* as a sister clade (low bootstrap support value) to the clade containing *Cy. floridanum*, *Cy. hongkongense* and *Cy. malesianum*, leaving *Cy. multiphialidicum* sitting basal to all the other isolates (data not shown). Parsimony analysis yielded 24 most parsimonious trees (TL = 631 steps; CI = 0.648; RI = 0.838; RC = 0.543), one of which is shown in Fig. 2. The main difference between the neighbour-joining and parsimony analyses is in the placement of *Cy. multiphialidicum* and *Cy. pseudonaviculatum*; parsimony supported the placement given by the Kimura 2-parameter model (data not shown). Figure 2 shows a number of well-supported clades. As in the β-tubulin tree, the first clustering of clades lacks bootstrap support, but it does show excellent support for the same clades seen in the β-tubulin analysis. The *Cy. floridanum*, *Cy. hongkongense* and *Cy. malesianum* clades cluster together with 98 % bootstrap support, and the *Cy. indonesiae* and *Cy. chinense* clades together have a 100 % support. The second main cluster (98 % support) contains several entities, firstly a clade (71 %) containing *Cy. curvisporum* CPC 765 and *Cy. parasiticum* CBS 112217, as well as a *Cy. parasiticum* clade containing isolates mainly from Hawaii (Clade 1, 88 %) and a second *Cy. parasiticum* clade containing isolates from Indonesia and the U.S.A. (Clade 2, 62 %). The second main cluster also contains a *Cy. pacificum*

clade (92 %), a *Cy. colombiense* clade (99 %), and a *Cy. sumatrense* clade (98 %).

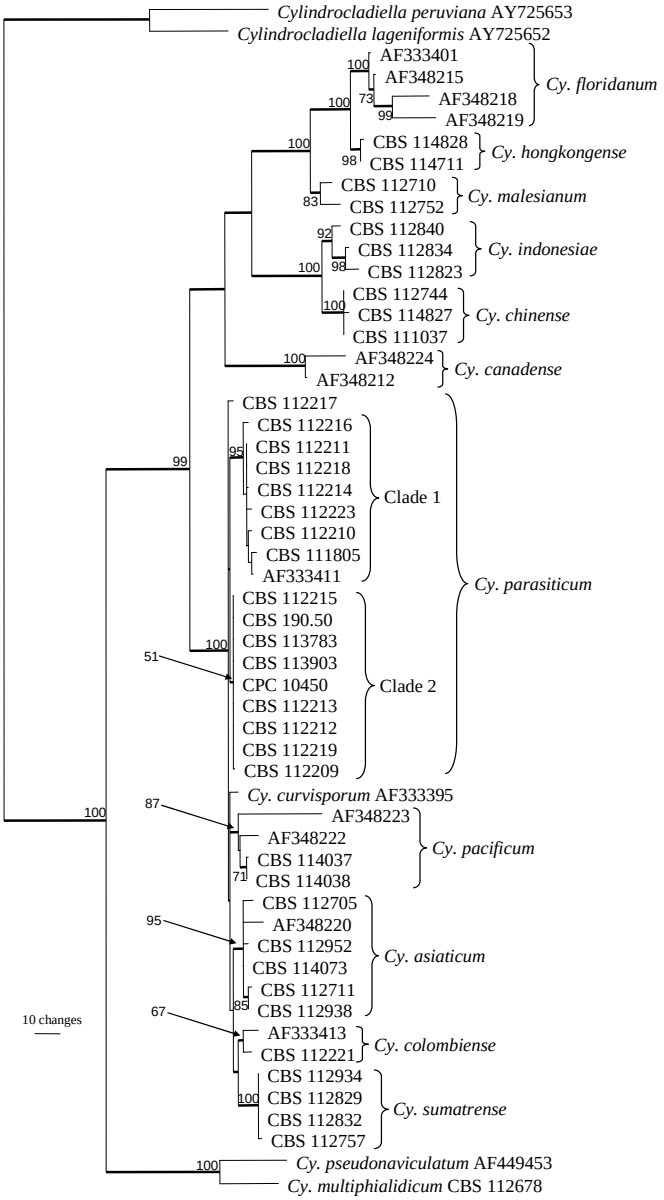


Fig. 1. One of 64 most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the β-tubulin sequence alignment. The scale bar shows 10 changes; bootstrap support values from 1000 replicates are shown at the nodes. Thickened lines indicate the strict consensus branches. The tree was rooted to two *Cylindrocladiella* species.

The *Cy. asiaticum* isolates did not form a clade but are a basal polytomy in this second main cluster. The two *Cy. canadense* isolates form a sister clade (100 %) to the first and second main clusters. Basal to the other clades is a clade (100 % support) containing *Cy. multiphialidicum* and *Cy. pseudonaviculatum* as sister taxa.

For the combined analysis of the four loci, 523, 434, 538 and 493 characters (including alignment gaps and respectively for β-tubulin, histone, elongation factor 1-α and calmodulin) were included (Table 1). The manually adjusted alignment contained 36 taxa, including the outgroup, and 1988 characters including alignment gaps were used in the analysis. Of these, 541 were parsimony-informative, 433 were parsimony-uninformative, and 1014 were constant.

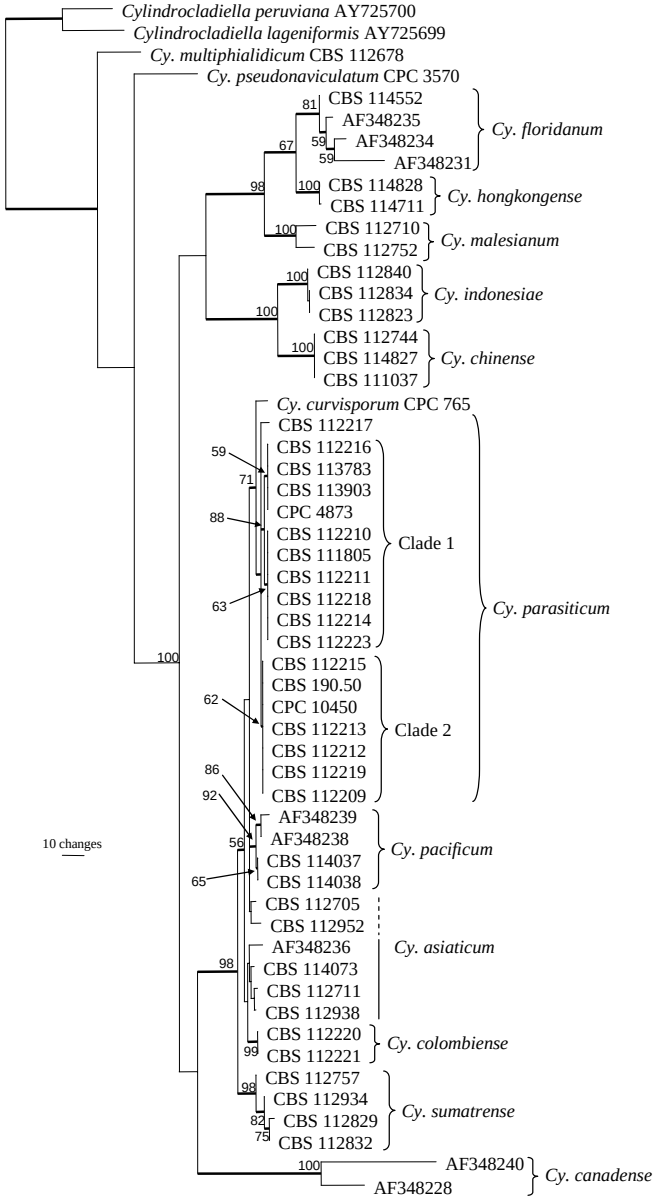


Fig. 2. One of 24 most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the histone sequence alignment. The scale bar shows 10 changes; bootstrap support values from 1000 replicates are shown at the nodes. The tree was rooted to two *Cylindrocladiella* species.

Neighbour-joining analysis using the three substitution models yielded trees with similar topology and bootstrap values, except that the placement of the clade containing *Cy. canadense*, *Cy. indonesiae* and *Cy. chinense* differed in the Jukes-Cantor substitution model. Parsimony analysis yielded twelve most parsimonious trees (TL = 1942 steps; CI = 0.754; RI = 0.849; RC = 0.640), one of which is shown in Fig. 3.

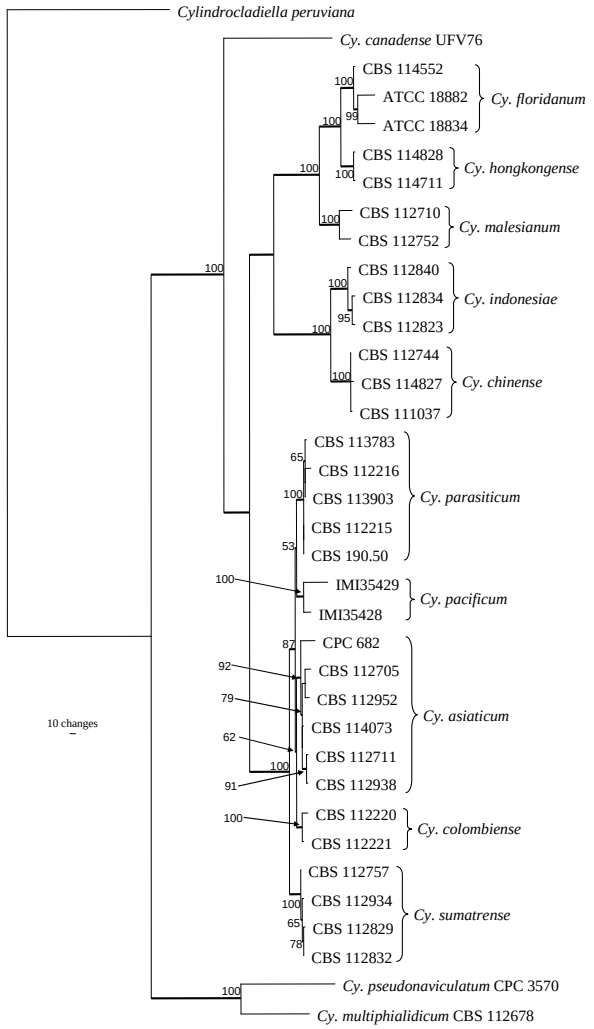


Fig. 3. One of 12 most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the combined β-tubulin, histone, elongation factor 1-α and calmodulin sequence alignment. The scale bar shows 10 changes and bootstrap support values from 1000 replicates are shown at the nodes. Thickened lines indicate the strict consensus branches. The tree was rooted to *Cylindrocladiella peruviana* (AY725653, AY725700, AY725736 and AY725775).

The main difference between the neighbour-joining and parsimony analyses was in bootstrap support values, which were higher in the parsimony analysis (data not shown). The parsimony tree shows a number of well-supported clades. As in the β-tubulin and histone phylograms, the first cluster of clades lacks bootstrap support, but it does contain the identical well-supported clades for *Cy. floridanum* and other species. *Cylindrocladium floridanum*, *Cy. hongkongense* and *Cy. malesianum* cluster together with 100 % support, as do *Cy. indonesiae* and *Cy. chinense*. The next cluster with 100 % support contains strongly supported clades for *Cy. parasiticum*, *Cy. pacificum*, *Cy. asiaticum*, *Cy. colombiense* and *Cy. sumatrense*. Basal to the other clades is a strongly supported clade containing *Cy. multiphialidicum* and *Cy. pseudonaviculatum*.

Taxonomy

Calonectria asiatica Crous & Hywel-Jones, **sp. nov.** MycoBank MB500102. Figs 4–10.
Anamorph: *Cylindrocladium asiaticum* Crous & Hywel-Jones, sp. nov.

Etymology: Named after Asia, from where it was collected.

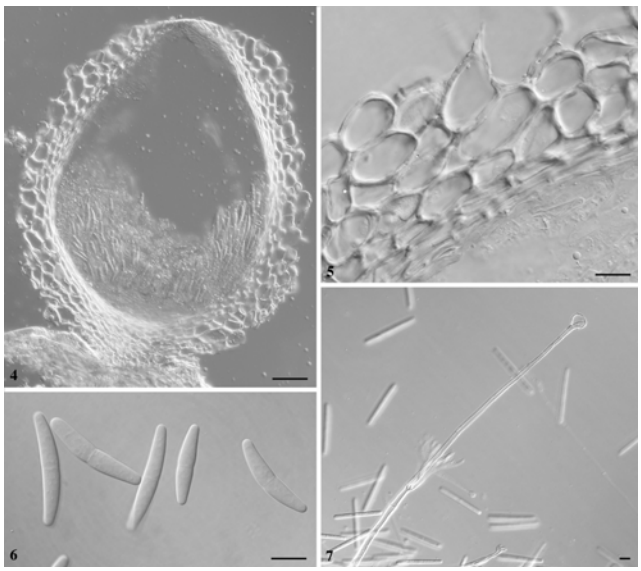
Calonectriae kyotensi similis, sed ascosporis brevioribus, (28–)30–38(–40) × (5–)6(–7) µm (in medio 33 × 6 µm).

Perithecia solitary or in groups of up to 6, orange, becoming orange-brown with age; in section, apex and body orange, base red-brown, subglobose to ovoid, 280–400 µm high, 200–350 µm diam, body turning red, and base dark red-brown (KOH+); perithecial walls rough, consisting of two thick-walled layers: outer layer of *textura globulosa*, 20–70 µm thick, cells becoming more compressed towards the inner layer of *textura angularis*, 10–15 µm thick, cells becoming thin-walled and hyaline towards the center; outermost cells 15–35 × 10–25 µm, cells of inner layer 8–15 × 3–6 µm; perithecial base up to 100 µm thick, consisting of dark red, angular cells, merging with an erumpent stroma; cells of the outer wall layer continuous with the pseudoparenchymatous cells of the erumpent stroma. *Asci* 8-spored, clavate, 70–120 × 12–20 µm, tapering into a long thin stalk. *Ascospores* aggregated in the upper third of the ascus, hyaline, guttulate, fusoid with rounded ends, straight to slightly curved, 1-septate, constricted at the septum, (28–)30–38(–40) × (5–)6(–7) µm (mean = 33 × 6 µm). Homothallic.

Cylindrocladium asiaticum Crous & Hywel-Jones, **sp. nov.** MycoBank MB500103.

Cylindrocladio floridano simile, sed vesiculis latioribus (12–17 µm diam) et conidiis maioribus (42–)48–55(–65) × (4–)5(–5.5) µm, in medio 53 × 5 µm.

Conidiophores consisting of a stipe bearing a penicillate arrangement of fertile branches, a stipe extension, and a terminal vesicle; stipe septate, hyaline, smooth, 60–170 × 7–8 µm; stipe extensions septate, straight to flexuous, 200–280 µm long, 4–5 µm wide at apical septum, terminating in a sphaeropedunculate vesicle, 12–17 µm diam; lateral stipe extensions (90° to main axis) also abundant.

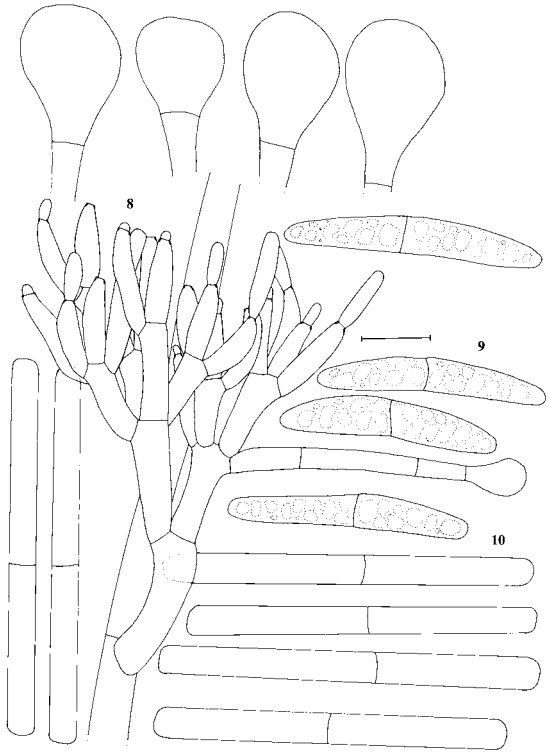


Figs 4–7. *Calonectria asiatica* and its anamorph *Cylindrocladium asiaticum* (CBS 114073). 4. Vertical section through a perithecium. 5. Section through lateral perithecial wall. 6. Ascospores. 7. Conidiophore. Scale bars: 4 = 45 µm, 5, 6 = 10 µm, 7 = 15 µm.

Conidiogenous apparatus 40–90 µm long, 40–80 µm wide; primary branches aseptate or 1-septate, 20–30 × 4–7 µm; secondary branches aseptate, 15–20 × 4–5 µm, tertiary branches aseptate, 10–15 × 3–5 µm, additional branches –5, aseptate, 10–15 × 3–4 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 10–13 × 3–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Conidia* cylindrical, rounded at both ends, straight, (42–)48–55(–65) × (4–)5(–5.5) µm (mean = 53 × 5 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colorless slime. *Megaconidia* and *microconidia* unknown.

Holotype: **Thailand**, Prathet Thai, on leaf litter, 2001, N.L. Hywel-Jones (herb. CBS 9889, **holotype** of *Calonectria asiatica* and *Cylindrocladium asiaticum*, cultures ex-type CBS 114073 = CPC 3900 = SFE 726).

Cultural characteristics: Colonies with feathery, irregular margins, abundant white to cream-coloured aerial mycelium, surface rust-coloured (7'i); reverse with cream-coloured to white outer margin, and rust (7'i) inner region, becoming chestnut (7'm) towards the centre. Colonies reaching 42–64 mm diam after 7 d on MEA in the dark at 25 °C.



Figs 8–10. *Calonectria asiatica* and its anamorph *Cylindrocladium asiaticum* (CBS 114073). 8. Conidiophore and sphaeropedunculate vesicles. 9. Ascospores. 10. Conidia. Scale bar = 10 µm.

Substrate: Debris, soil.

Distribution: Indonesia, Thailand.

Notes: *Cylindrocladium asiaticum* is morphologically similar to *Cy. floridanum* [vesicles 6–12 µm diam, conidia (35–)45–50(–55) × 3–4(–5) µm, mean = 40 × 3.5 µm], but can be distinguished by having wider vesicles (12–17 µm diam) and larger conidia (42–)48–55(–65) × (4–)5(–5.5) µm, mean = 53 × 5 µm.

Additional cultures examined: **Thailand**, Prathet Thai, on leaf litter, 2001, N.L. Hywel-Jones, CBS 112711 = CPC 3898 = SFE 744). **Indonesia**, Northern Sumatra, soil collected under canopies of *Eucalyptus* trees, 2001, M.J. Wingfield, CBS 112938 = CPC 4513.

Cylindrocladium chinense Crous, **sp. nov.**
MycoBank MB500104. Figs 11, 12.

Etymology: Named after the country from which it was collected, China.

Cylindrocladio floridano simile, sed conidiis longioribus (in medio 45 × 4 µm) et paucis ramis (–3) conidiophororum differens.

Teleomorph unknown. *Conidiophores* consisting of a stipe bearing a penicillate arrangement of fertile branches, a stipe extension, and a terminal vesicle;

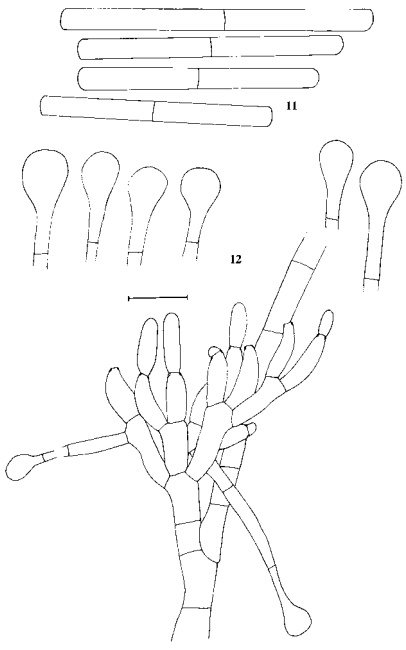
stipe septate, hyaline, smooth, 40–150 × 6–7 µm; stipe extensions septate, straight to flexuous, 120–150 µm long, 2.5–3.5 µm wide at the apical septum, terminating in a sphaeropedunculate vesicle, 6–9 µm diam; lateral stipe extensions (90° to main axis) common. *Conidiogenous apparatus* 40–60 µm long and wide; primary branches aseptate or 1-septate, 20–30 × 5–6 µm; secondary branches aseptate, 15–30 × 4–6 µm, tertiary branches aseptate, 10–20 × 4–5 µm, each terminal branch producing 2–4 phialides; phialides elongate doliiform to reniform, hyaline, aseptate, 10–20 × 3–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Conidia* cylindrical, rounded at both ends, straight, (38–)41–48(–56) × (3.5–)4(–4.5) µm (mean = 45 × 4 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colorless slime. *Megaconidia* and *microconidia* unknown.

Holotype: **China**, soil, 2000, E.C.Y. Liew (**holotype** herb. CBS 9887; culture ex-type CBS 114827 = CPC 4101).

Cultural characteristics: Colonies fast growing with abundant aerial mycelium, consisting of strands and tufts of white to cream-coloured hyphae; surface sienna (13i); reverse with cream-coloured to white outer region, sienna (13i) inner region, and rust-coloured (7'i) area near the centre. Colonies reaching 51–72 mm diam after 7 d on MEA in the dark at 25 °C.

Substrate: Soil.

Distribution: China.



Figs 11, 12. *Cylindrocladium chinense* (CBS 114827). 11. Conidia. 12. Conidiophore and vesicles. Scale bar = 10 µm.

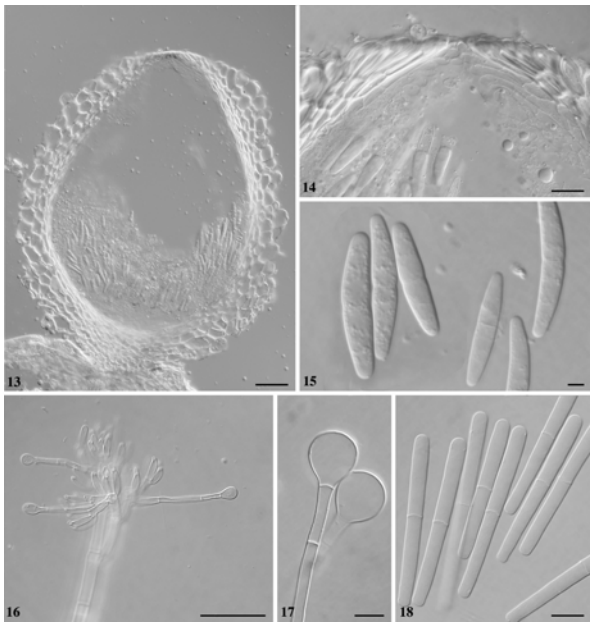
Notes: This species, which is part of the *Cylindrocladium floridanum* complex, is only known from China, inclusive of Hong Kong. Morphologically, *Cy. chinense* can be distinguished based on a combination of characters, namely having conidia of intermediate length (mean = 45 × 4 µm), up to three conidiophore branches, and, commonly, lateral stipe extensions.

Additional cultures examined: **Hong Kong**, soil, Jun. 1995, M.J. Wingfield, CBS 111037 = CPC 1154; **China**, soil, 2000, E.C.Y. Liew, CBS 112744 = CPC 4104.

Calonectria colombiensis Crous, **sp. nov.**
MycoBank MB500105. Figs 13–22.
Anamorph: *Cylindrocladium colombiense* Crous, sp. nov.

Etymology: Named after Colombia, from where it was collected.

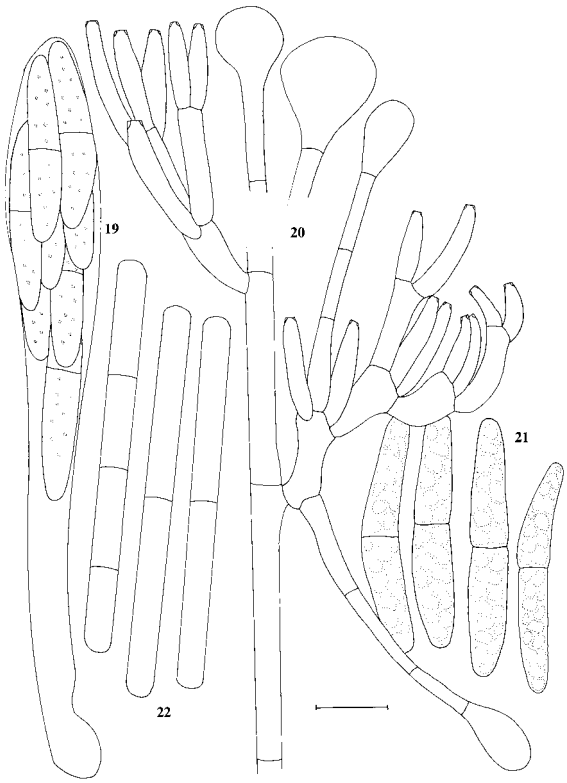
Calonectriae kyotensi simile, sed ascosporis brevioribus, (28–)30–35(–40) × (4–)5(–6) µm (in medio 33 × 5 µm) differens.



Figs 13–18. *Calonectria colombiensis* and its anamorph *Cylindrocladium colombiense* (CBS 112220). 13. Vertical section through a perithecium. 14. Cells around ostiolar region of perithecium. 15. Ascospores. 16. Conidiophore. 17. Vesicles. 18. Conidia. Scale bars: 13 = 45 µm, 14–18 = 10 µm.

Perithecia solitary or in groups of up to 6, orange, becoming orange-brown with age; in section, apex and body orange, base red-brown, subglobose to ovoid, 200–350 µm high, 200–300 µm diam, body turning red, and base dark red-brown (KOH+); perithecial walls rough, consisting of two thick-walled layers: outside layer of *textura globulosa*, 20–60 µm thick,

cells becoming more compressed towards inner layer of *textura angularis*, 10–15 µm thick, cells becoming thin-walled and hyaline towards the center, outermost cells 15–35 × 10–25 µm, cells of inner layer 8–17 × 3–6 µm; perithecial base up to 160 µm thick, consisting of dark red, angular cells, merging with an erumpent stroma; cells of the outer wall layer continuous with the pseudoparenchymatous cells of the erumpent stroma. *Asci* 8-spored, clavate, 90–150 × 11–23 µm, tapering into a long thin stalk. *Ascospores* aggregated in the upper third of the ascus, hyaline, guttulate, fusoid with rounded ends, straight to slightly curved, 1-septate, becoming constricted at the septum, (28–)30–35(–40) × (4–)5(–6) µm (mean = 33 × 5 µm). Cultures were homothallic.



Figs 19–22. *Calonectria colombiensis* and its anamorph *Cylindrocladium colombiense* (CBS 112220). 19. Ascus. 20. Conidiophore and vesicles. 21. Ascospores. 22. Conidia. Scale bar = 10 µm.

Cylindrocladium colombiense Crous, **sp. nov.** MycoBank MB500106.

Cylindrocladio parasitico simile, sed conidiis minoribus, (33–)48–58(–60) × (4–)4.5(–5) µm, in medio 53 × 4.5 µm differens.

Conidiophores consisting of a stipe bearing a penicillate arrangement of fertile branches, a stipe extension, and a terminal vesicle; stipe septate, hyaline, smooth, 50–70 × 6–7 µm; stipe extensions septate, straight to flexuous, 130–200 µm long, 3–4 µm wide at the apical septum, terminating in a sphaeropedunculate vesicle, 7–12 µm diam; lateral stipe extensions (90° to main axis) also abundant. *Conidiogenous apparatus* 40–60 µm long, 25–60 µm wide; primary branches aseptate or 1-septate, 16–20 × 4–6 µm; secondary branches aseptate, 10–20 × 3–5 µm, tertiary branches aseptate, 10–17 × 3–5 µm, additional branches –5, aseptate, 8–15 × 3–4 µm, each terminal branch producing 2–6 phialides; phialides elongate doliiform to reniform, hyaline, aseptate, 10–18 × 3–5 µm; apex with minute periclinal thickening and inconspicuous collarette. *Conidia* cylindrical, rounded at both ends, straight, (33–)48–58(–60) × (4–)4.5(–5) µm (mean = 53 × 4.5 µm), 1(–3)-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colorless slime. *Megaconidia* and *microconidia* unknown.

Holotype: **Colombia**, La Selva, soil under *Eucalyptus grandis* trees, M.J. Wingfield (herb. CBS 9890, **holotype** of *Calonectria colombiensis* and *Cylindrocladium colombiense*, cultures ex-type CBS 112220 = CPC 723, CBS 112221 = CPC 724, 725).

Cultural characteristics: Colonies with feathery, irregular margins, sparse white to sienna (13i) aerial mycelium, surface rust-coloured (7'i); reverse rust (7'i). Colonies reaching 26–39 mm diam after 7 d on MEA in the dark at 25 °C.

Substrate: Soil.

Distribution: Colombia.

Notes: Isolates of *Cy. colombiense* were initially treated under *Cy. parasiticum* by Crous (2002). This was based on observations that *Cy. colombiense* produces 3-septate conidia, and that the conidia are generally larger, (33–)48–58(–60) × (4–)4.5(–5) µm, mean = 53 × 4.5 µm, than those of *Cy. floridanum*, (35–)45–50(–55) × 3–4(–5) µm, mean = 40 × 3.5 µm, showing considerable overlap with those of *Cy. parasiticum*, (45–)70–82(–90) × (4–)5–6.5(–7) µm, mean = 62 × 6 µm. Although *Cy. parasiticum* has been reported in the literature to occur on eucalypts from various tropical countries (Crous 2002), cultures from this host are presently available to confirm this.

Calonectria hongkongensis Crous, **sp. nov**. MycoBank MB500107. Figs 23–29.

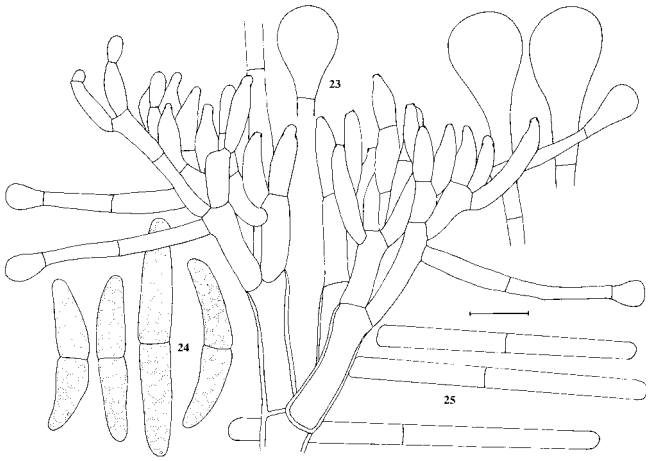
Anamorph: *Cylindrocladium hongkongense* Crous, sp. nov.

Etymology: Named after Hong Kong, from where it was collected.

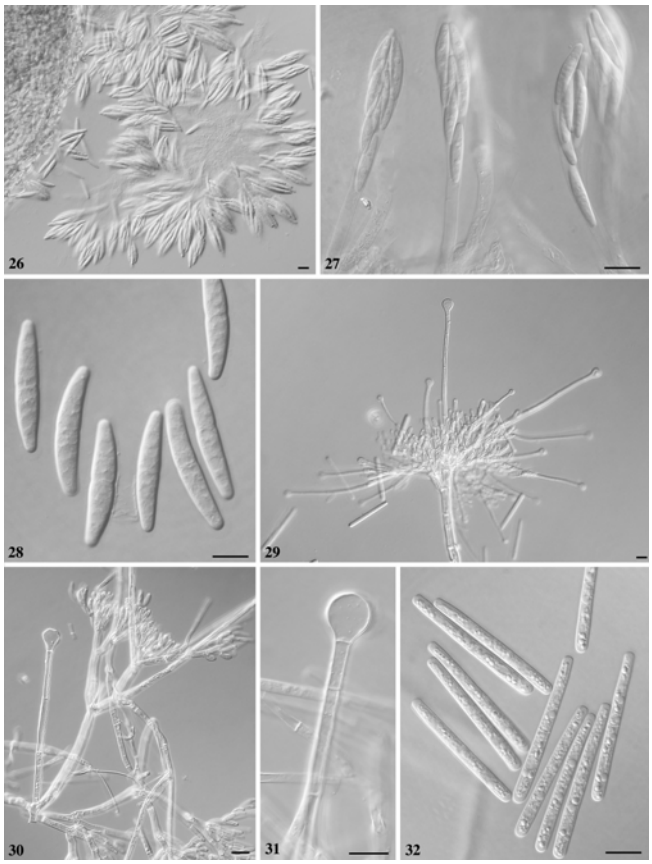
Calonectriae kyotensi simile, sed ascosporis brevioribus, latioribus, (25–)28–35(–40) × (4–)5–6(–7) µm (in medio 31 × 6 µm) differens.

Perithecia solitary or in groups of up to 3, orange, becoming red-brown with age; in section, apex and

body orange, base red-brown, subglobose to ovoid, 350–550 µm high, 300–450 µm diam, body turning red, and base dark red-brown (KOH+); perithecial



Figs 23–25. *Calonectria hongkongensis* and its anamorph *Cylindrocladium hongkongense* (CBS 114828). 23. Conidiophore and vesicles. 24. Ascospores. 25. Conidia. Scale bar = 10 µm.



Figs 26–32. *Calonectria hongkongensis* (CBS 114828) and *Cylindrocladium malesianum* (CBS 112752). 26–29. *Ca. hongkongensis.* 26, 27. Asci. 28. Ascospores. 29. Conidiophore. 30–32. *Cy. malesianum*. 30. Conidiophore. 31. Vesicle. 32. Conidia. Scale bars: 26, 27, 29–31 = 15 µm, 28, 32 = 10 µm

walls rough, consisting of two thick-walled layers: outside layer of *textura globulosa*, 20–40 µm thick, cells becoming more compressed towards inner layer of *textura angularis*, 10–15 µm thick, cells becoming thin-walled and hyaline towards the center, outermost cells 15–30 × 10–20 µm, cells of inner layer 8–15 × 3–6 µm; perithecial base up to 150 µm thick, consisting of dark red, angular cells, merging with an erumpent stroma, cells of the outer wall layer continuous with the pseudoparenchymatous cells of the erumpent stroma. *Asci* 8-spored, clavate, 80–140 × 14–20 µm, tapering into a long thin stalk. *Ascospores* aggregated in the upper third of the ascus, hyaline, guttulate, fusoid with rounded ends, straight to curved, 1-septate, becoming constricted at the septum, (25–)28–35(–40) × (4–)5–6(–7) µm (mean = 31 × 6 µm). Homothallic.

Cylindrocladium hongkongense Crous, **sp. nov.** MycoBank MB500108.

Cylindrocladio floridano simile, sed ramis conidiophororum numerosis (–8) et penicillo ad 100 µm alto et lato differens.

Conidiophores consisting of a stipe bearing a penicillate arrangement of fertile branches, a stipe extension, and a terminal vesicle; stipe septate, hyaline, smooth, 40–60 × 5–6 µm; stipe extensions septate, straight to flexuous, 100–200 µm long, 3–4 µm wide at apical septum, terminating in a sphaeropedunculate vesicle, 8–14 µm diam; lateral stipe extensions (90° to main axis) also abundant. *Conidiogenous apparatus* 70–100 µm long, 70–120 µm wide; primary branches aseptate or 1-septate, 17–25 × 4–7 µm; secondary branches aseptate, 15–20 × 3–5 µm, tertiary branches aseptate, 10–15 × 3–5 µm, additional branches –8, aseptate, 8–15 × 3–4 µm, each terminal branch producing 2–6 phialides; phialides elongate doliiform to reniform, hyaline, aseptate, 9–15 × 3–5 µm; apex with minute periclinal thickening and inconspicuous collarette. *Conidia* cylindrical, rounded at both ends, straight, (38–)45–48(–53) × 4(–4.5) µm (mean = 46.5 × 4 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colorless slime. *Megaconidia* and *microconidia* unknown.

Holotype: **Hong Kong**, soil, M.J. Wingfield (herb. CBS 9886, **holotype** of *Calonectria hongkongensis* and *Cylindrocladium hongkongense*, culture ex-type CBS 114828 = CPC 4670).

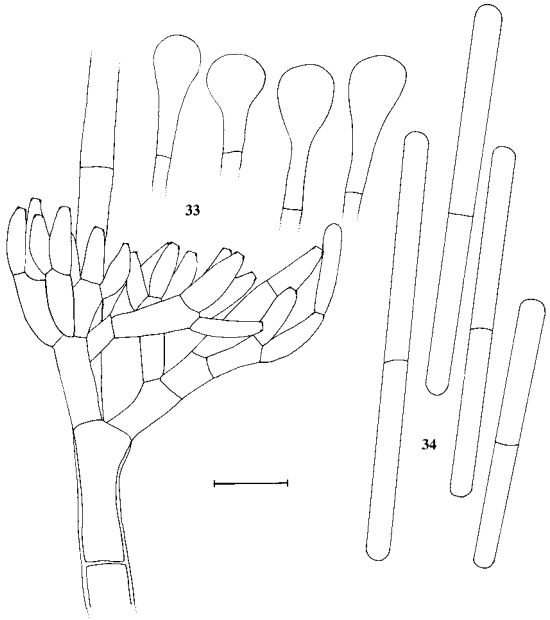
Cultural characteristics: Colonies irregular with feathery margins and abundant white to sienna (13i) aerial mycelium, surface rust-coloured (7'i) to pale white; reverse sienna (13i) to rust-coloured (7'i). Colonies reaching 15–30 mm diam after 7 d on MEA in the dark at 25 °C.
Substrate: Soil.

Distribution: China.

Notes: *Cylindrocladium honkongense* is distinguished from *Cy. floridanum* by having numerous conidiophore branches (–8), and a conidiogenous apparatus that is up to 100 µm wide and long, as well as conidia that have a longer average length (46.5 × 4 µm).

Additional culture examined: China, soil, 8 Nov. 1993, M.J. Wingfield, CPC 686 = CBS 114711.

Cylindrocladium indonesiae Crous, **sp. nov.** MycoBank MB500109. Figs 33, 34.



Figs 33, 34. *Cylindrocladium indonesiae* (CBS 112823). 33. Conidiophore and vesicles. 34. Conidia. Scale bar = 10 µm.

Etymology: Only known from Indonesia.

Cylindrocladio floridano simile, sed ramis conidiophororum numerosis (–5) et conidiis longioribus (in medio 50.5 × 4 µm) differens.

Teleomorph unknown. *Conidiophores* consisting of a stipe bearing a penicillate arrangement of fertile branches, a stipe extension, and a terminal vesicle; stipe septate, hyaline, but pale brown below, smooth, 40–80 × 5–6 µm; stipe extensions septate, straight to flexuous, 110–160 µm long, 2.5–3 µm wide at the apical septum, terminating in a sphaeropedunculate vesicle, 7–9 µm diam; lateral stipe extensions absent. *Conidiogenous apparatus* 40–60 µm long, 60–80 µm wide; primary branches aseptate or 1-septate, 18–25 × 4–5 µm; secondary branches aseptate, 10–20 × 4–5 µm, tertiary and additional branches, –5, aseptate, 10–15 × 4–5 µm, each terminal branch producing 2–6 phialides; phialides elongate doliiform to reniform, hyaline, aseptate, 8–15 × 3–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Conidia* cylindrical, rounded at both ends, straight, (40–)45–55(–60) × (3–)4 µm (mean = 50.5 × 4 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colorless slime. *Megaconidia* and *microconidia* unknown.

Holotype: **Indonesia**, Warambunga, soil, 9 Mar. 1996, M.J. Wingfield (**holotype** herb. CBS 9891, culture ex-type CBS 112823 = CPC 4508).

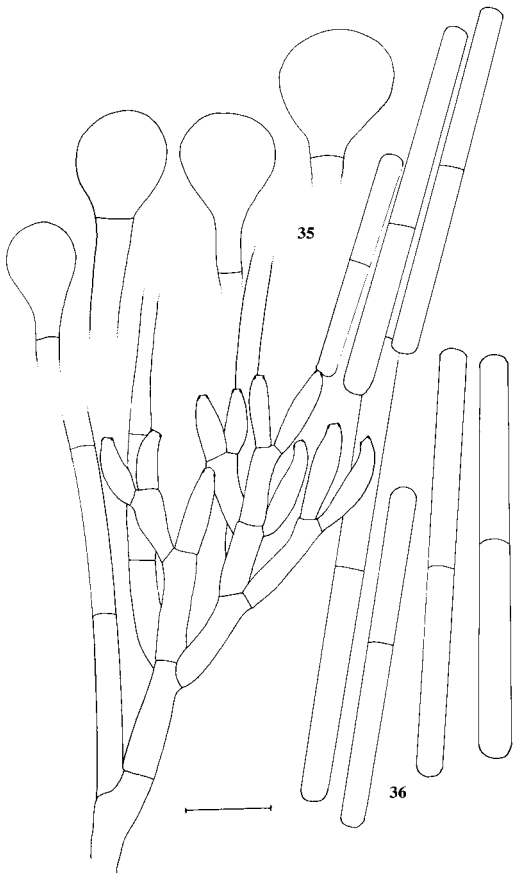
Cultural characteristics: Colonies fast-growing with feathery margins and moderate to abundant white aerial mycelium; surface umber (13'i); reverse with white outer margin, and umber (13'i) inner region, becoming rust-coloured (7'i) towards the centre. Colonies reaching 56–80 mm diam after 7 d on MEA in the dark at 25 °C.

Substrate: Soil.

Distribution: Indonesia.

Notes: *Cylindrocladium indonesiae* can be distinguished from other species in the *Cy. floridanum* complex by having numerous conidiophore branches, by having conidia of medium length (mean = 50.5 × 4 µm), and by lacking lateral stipe extensions.

Additional culture examined: **Indonesia**, Warambunga, soil, 9 Mar. 1996, M.J. Wingfield, CBS 112840 = CPC 4554, CBS 112834 = CPC 4547.



Figs 35, 36. *Cylindrocladium malesianum* (CBS 112752). 35. Conidiophore and vesicles. 36. Conidia. Scale bar = 10 µm.

Cylindrocladium malesianum Crous, **sp. nov.** MycoBank MB500110. Figs 30–32, 35, 36.

Etymology: Named after Malaysia, the region from which it was collected.

Cylindrocladio floridano simile, sed ramis conidiophororum numerosis (–6) et conidiis longioribus (in medio 47.5 × 4 µm) differens.

Teleomorph unknown. *Conidiophores* consisting of a stipe bearing a penicillate arrangement of fertile branches, a stipe extension, and a terminal vesicle; stipe septate, hyaline, smooth, 70–200 × 5–7 µm; stipe extensions septate, straight to flexuous, 120–200 µm long, 3–4 µm wide at the apical septum, terminating in a sphaeropedunculate to globose vesicle, 8–15 µm diam; lateral stipe extensions (90° to main axis) also present. *Conidiogenous apparatus* 50–60 µm long, 30–80 µm wide; primary branches aseptate or 1-septate, 20–30 × 5–6 µm; secondary branches aseptate, 10–30 × 5–6 µm, tertiary and additional branches, –6, aseptate, 10–15 × 4–6 µm, each terminal branch producing 2–6 phialides; phialides elongate doliiform to reniform, hyaline, aseptate, 7–15 × 2.5–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Conidia* cylindrical, rounded at both ends, straight, (34–)45–52(–55) × (3–)4 µm (mean = 47.5 × 4 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colorless slime. *Megaconidia* and *microconidia* unknown.

Holotype: **Indonesia**, Northern Sumatra, soil, 9 Mar. 1996, M.J. Wingfield (**holotype** herb. CBS 9885, culture ex-type CBS 112752 = CPC 4223).

Cultural characteristics: Colonies with feathery, irregular white margins, surface with moderate sienna (13i) aerial mycelium and inner region; reverse with thin sienna (13i) outer region, and chestnut (7'm) inner region. Colonies reaching 36–47 mm diam after 7 d on MEA in the dark at 25 °C.

Substrate: Soil.

Distribution: Indonesia, Thailand.

Notes: *Cylindrocladium malesianum* can be distinguished from other species in the *Cy. floridanum* complex by having numerous conidiophore branches, conidia of medium length (mean = 47.5 × 4 µm), and numerous lateral stipe extensions.

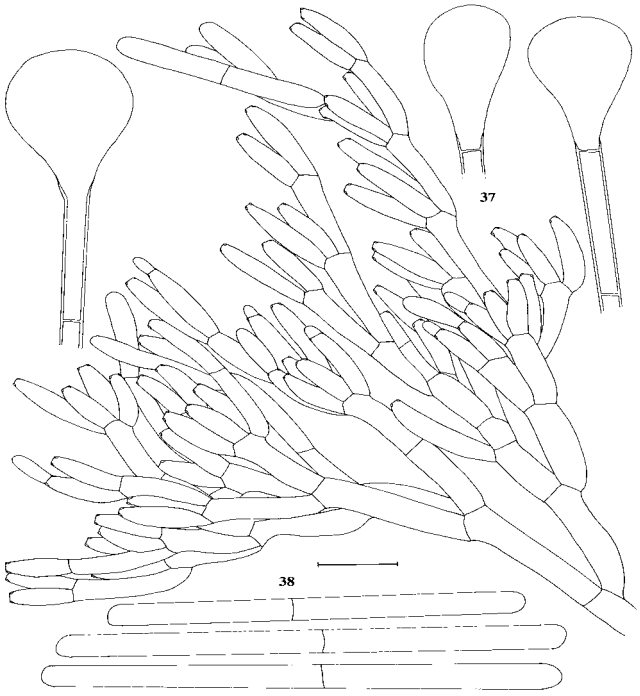
Additional culture examined: **Thailand**, Prathet, on leaf litter, 2001, N.L. Hywel-Jones, CBS 112710 = CPC 3899.

Cylindrocladium multiphialidicum Crous, P. Simoneau & J.-M. Risède, **sp. nov**. MycoBank MB500111. Figs 37–41.

Etymology: Named after its characteristic conidiophores that form numerous branches and phialides.

Cylindrocladio floridano simile, sed ramis conidiophororum numerosis (–8) et conidiis longioribus (in medio 53 × 4.5 µm) et appendicibus crassitunicatis differens.

Teleomorph unknown. *Conidiophores* consisting of a stipe bearing a penicillate arrangement of fertile branches, a stipe extension, and a terminal vesicle; stipe septate, hyaline to medium brown, smooth, 80–150 × 7–10 µm; stipe extensions septate, straight to flexuous, hyaline to pale brown, thick-walled, 170–300 µm long, 4–5 µm wide at apical septum, terminating in a clavate to sphaeropedunculate vesicle, 8–16 µm diam. *Conidiogenous apparatus* 70–150 µm long and wide; primary branches aseptate or 1-septate, 20–40 × 5–6 µm; secondary branches aseptate or 1-septate, 15–25 × 5–6 µm, tertiary branches aseptate, 10–20 × 5–6 µm, additional branches –8, aseptate, 10–15 × 4–5 µm, each terminal branch producing 2–6 phialides; phialides elongate doliiform to reniform, hyaline, aseptate, 8–15 × 3–4 µm; apex with minute periclinal thickening and inconspicuous collarette. *Conidia* cylindrical, rounded at both ends, guttulate, straight, (45–)48–55(–65) × (4–)4.5(–5) µm (mean = 53 × 4.5 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colorless slime. *Megaconidia* and *microconidia* unknown.



Figs 37, 38. *Cylindrocladium multiphialidicum* (CBS 112678). 37. Conidiophore and vesicles. 38. Conidia. Scale bar = 10 µm.

Holotype: **Cameroon**, on soil surrounding roots of *Musa* sp., Mar. 1998, Abadie, (**holotype** herb. CBS 9887, culture ex-type CBS 112678 = Cam 13).

Cultural characteristics: Colonies fast growing with irregular margins, moderate to abundant white aerial mycelium; surface sienna (13i); reverse with sienna (13i) outer margin, and chestnut (9'm) inner region. Colonies reaching 70–80 mm diam after 7 d on MEA in the dark at 25 °C. Microsclerotia (perithecial initials?) aggregating in clusters on agar surface, bright red, turning red-brown to brown with age, eventually becoming covered in mycelium.

Substrate: Soil.

Distribution: Cameroon.

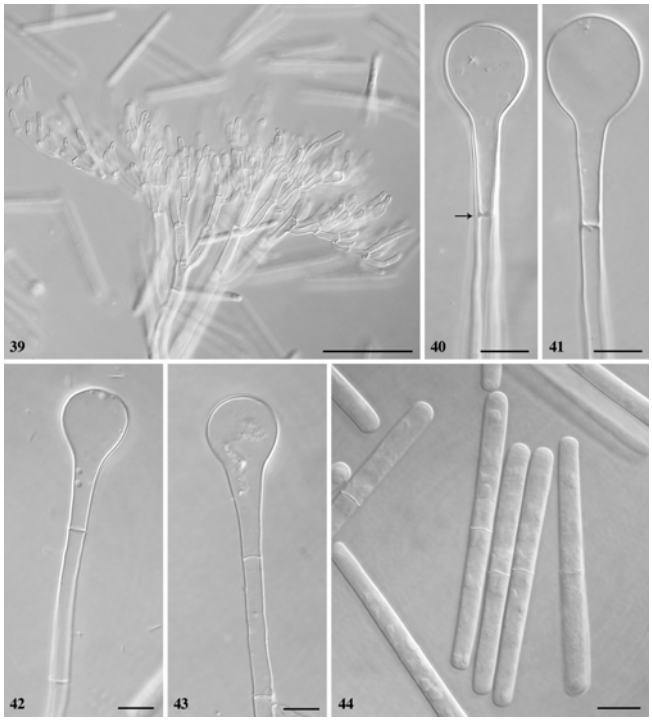
Notes: *Cylindrocladium multiphialidicum* resembles other taxa in the *Cy. floridanum* complex. Its conidial dimensions (mean 53 × 4.5 µm) are larger than those of *Cy. floridanum* (mean 40 × 3.5 µm), and more closely match those of *Cy. pacificum* (mean 55 × 4.5 µm). Conidial lengths of *Cy. pacificum* tend to have a broader range (38–)45–65(–75) µm than those of *Cy. multiphialidicum* (45–)48–55(–65) µm. Furthermore, stipes of *Cy. multiphialidicum* are thick-walled, which is not the case in *Cy. pacificum*. The most characteristic feature distinguishing *Cy. multiphialidicum* from other taxa in the *Cy. floridanum* complex is the numerous branches (–8) and phialides, that are formed on the conidiophores. *Cylindrocladium multiphialidicum* has thus far only been isolated once from *Musa* roots, which may be due to the fact that it has been shown to not be highly virulent to this host (Risède & Simoneau 2004).

Cylindrocladium sumatrense Crous, **sp. nov.** MycoBank MB500112. Figs 42–46.

Etymology: Named after the location from which it was collected, Sumatra, Indonesia.

Cylindricladio pacifico simile sed ramis conidiophororum paucis (–3) et nonnullis appendicibus in quoque conidiophoro.

Teleomorph unknown. *Conidiophores* consisting of a stipe bearing a penicillate arrangement of fertile branches, a stipe extension, and a terminal vesicle; stipe septate, hyaline, smooth, 40–70 × 6–7 µm; stipe extensions septate, straight to flexuous, 180–260 µm long, 3–4 µm wide at the apical septum, terminating in a sphaeropedunculate vesicle, 8–13 µm diam; lateral stipe extensions (90° to main axis) also present. *Conidiogenous apparatus* 50–80 µm long, 40–60 µm wide; primary branches aseptate or 1-septate, 20–30 × 4–5 µm; secondary branches aseptate, 10–20 × 4–5 µm, tertiary branches aseptate, 10–20 × 4–5 µm, each terminal branch producing 2–6 phialides; phialides elongate doliiform to reniform, hyaline, aseptate, 10–20 × 3–5 µm; apex with minute periclinal thickening and inconspicuous collarette. *Conidia* cylindrical, rounded at both ends, straight, (45–)55–65(–70) × (4.5–)5(–6) µm (mean = 58 × 5 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colorless slime. *Megaconidia* and *microconidia* unknown.



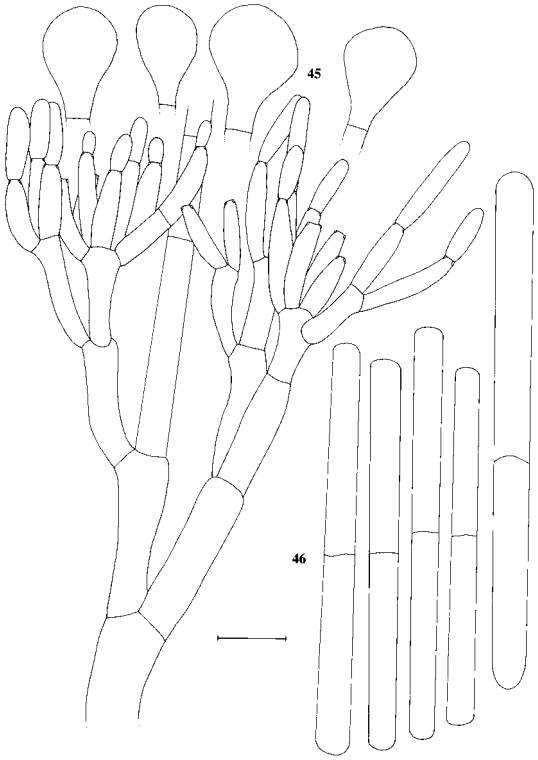
Figs 39–44. *Cylindrocladium multiphialidicum* (CBS 112678) and *Cy. sumatrense* (CBS 112829). 39–41. *Cy. multiphialidicum*. 39. Conidiophore. 40, 41. Thick-walled stipe extension (arrow). 42–44. *Cy. sumatrense*. 42, 43. Vesicles. 44. Conidia. Scale bars: 39 = 50 µm, 40, 41 = 8 µm, 42, 43 = 6 µm, 44 = 10 µm.

Holotype: **Indonesia**, Northern Sumatra, from soil collected under canopies of *Eucalyptus* trees, 2001, M.J. Wingfield (**holotype** herb. CBS 9888, culture ex-type CBS 112829 = CPC 4518).

Cultural characteristics: Colonies fast growing with irregular margins and abundant white to cream-coloured aerial mycelium, which makes the upper surface white to cream-coloured; reverse with broad cream-coloured to white outer margin, sienna (13i) inner region, and rust (7'i) central region. Colonies reaching 51–66 mm diam after 7 d on MEA in the dark at 25 °C.

Substrate: Soil.

Distribution: Indonesia.



Figs 45, 46. *Cylindrocladium sumatrense* (CBS 112829). 45. Conidiophore and vesicles. 46. Conidia. Scale bar = 10 µm.

Notes: *Cylindrocladium sumatrense* is similar to *Cy. pacificum* in having few conidiophore branches (–3), and similar conidial dimensions (38–75 × 4–5 µm for *Cy. pacificum*, and 45–70 × 4.5–6 µm for *Cy. sumatrense*). The two species can be distinguished, however, in that *Cy. pacificum* commonly forms lateral stipe extensions, while this is rarely observed in *Cy. sumatrense*.

Additional cultures examined: **Indonesia**, Northern Sumatra, soil collected under *Eucalyptus* forest, 2001, M.J. Wingfield, CBS 112934 = CPC 4516, CBS 112832 = CPC 4520.

DISCUSSION

The *Cy. floridanum* species complex has been the topic of several recent studies (Jeng *et al*. 1997, Victor *et al*. 1997, Kang *et al*. 2001, Crous 2002), which have integrated morphological, phylogenetic and biological species concepts to try and resolve the various species involved in this complex. Isolates in this complex have a six nucleotide insertion in their ITS2 region (Jeng *et al*. 1997, Risède & Simoneau 2001), which may be indicative of a single insertion event that occurred in a common ancestor to all these species. It is surprising, therefore, that in the present study we have been able to delineate yet another eight species within this complex. The concordant genealogy derived from the β-tubulin, calmodulin, elongation factor 1-α and histone sequence data corroborated the taxonomic relevance of minor morphological differences observed among these species. All species in this complex share similar vesicle morphology, and are distinguished primarily based on vesicle dimensions, conidiogenous apparatus (size and branching), conidial morphology, and the ability to produce a teleomorph in culture. The *Calonectria* teleomorphs, however, proved to be relatively similar to one another, and they added little information useful in species recognition.

The uncertainty surrounding the status of the Hawaiian isolates of *Cy. pacificum*, which lacked a teleomorph, and the homothallic strains from Thailand, which also have shorter conidia than those from Hawaii (Kang *et al*. 2001), is somewhat clarified with the description of *Cy. asiaticum*. In the four gene regions compared, however (Fig. 3), some isolates clustered within the *asiaticum* clade only with low bootstrap support, suggesting that this clade still contains cryptic elements that will eventually be resolved following more collections. *Cylindrocladium asiaticum* is similar to *Cy. sumatrense*, but can be distinguished by having abundant lateral stipe extensions, and up to 5 branches in its conidiogenous apparatus. *Cylindrocladium sumatrense* rarely produces lateral stipe extensions, and only has up to 3 branches in its conidiogenous apparatus.

Cylindrocladium parasiticum has sphaeropedunculate vesicles similar to species in the *Cy. floridanum* species complex, but can be distinguished by its wider, 3-septate macroconidia (45–90 × 4–7 µm). This species is a major pathogen of peanuts and soybean, causing Cylindrocladium black rot (CBR) on the former, and red crown rot (RCR) on the latter (Crous 2002). It has been speculated that this pathogen was introduced into the U.S.A. on *Indigofera tinctoria* L., from where it spread to other crops (Berner *et al*. 1988). Since it was first described, this pathogen has been recorded on numerous hosts in many tropical and subtropical regions of the world. Live cultural proofs to substantiate these records are, however, only available for some of these records. A question thus arose about whether all these reports did in fact represent the same fungus. As can be seen in Figs 1, 2, isolates of *Cy. parasiticum* clustered in two clades. One clade corresponded chiefly to isolates obtained from Hawaii, while the other represented strains from the U.S. mainland and Indonesia. These clades were not supported by sufficient bootstrap support or morphology, however, to argue that they could represent two species. The isolates that did cluster apart were those from soils under *Eucalyptus* canopies in Colombia, here described as *Cy. colombiense*. A reason why they could have been mistaken for *Cy. parasiticum* is that these isolates frequently produce up to 3-septate macroconidia that are slightly larger than those of *Cy. floridanum* and resemble those of *Cy. parasiticum*.

Cylindrocladium multiphialidicum is quite distinct from the other species described here because of its thick-walled stipe extensions and the numerous branches in its large conidiogenous apparatus. It is interesting, however, that this species clusters close to *Cy. pseudonaviculatum* Crous, J.Z. Groenewald & C.F. Hill (Jun. 2002) (= *Cy. buxicola* B. Henricot; Nov. 2002), which is a pathogen of *Buxus sempervirens* L. in New Zealand (Crous *et al*. 2002), as well as the U.K. (Henricot & Culham 2002), and Belgium (Crepel & Inghelbrecht 2003). *Cylindrocladium pseudonaviculatum* is distinct from *Cy. multiphialidicum* in having naviculate vesicles, and in having conidia larger (50–80 × 4–6 µm) than those of *Cy. multiphialidicum*.

Table 1. Isolates of *Cylindrocladium* (*Calonectria*) species studied.

	Accession number[1]	GenBank accession numbers (Tub, His, EF, Cal)[3]	Host	Country	Collector
Cy. asiaticum (*Ca. asiatica*)	CBS 112705 / CPC 3897	AY725612, AY725654, AY725701, AY725737	Debris	Thailand	N.L. Hywel-Jones
	CBS 112711 / CPC 3898	AY725613, AY725655, AY725702, AY725738	Debris	Thailand	N.L. Hywel-Jones
	CBS 112938 / CPC 4513	AY725614, AY725656, AY725703, AY725739	Soil	Indonesia	M.J. Wingfield
	CBS 112952 / CPC 4718	AY725615, AY725657, AY725704, AY725740	Soil	Australia	S. Abell
	CBS 114073 / CPC 3900[2]	AY725616, AY725658, AY725705, AY725741	Debris	Thailand	N.L. Hywel-Jones
	CPC 682	AF348220, AF348236, AY725706, AY725742	Soil	Thailand	M.J. Wingfield
Cy. canadense	CBS 110817 / CPC 499[2]	AF348212, AF348228, –, AY725743	*Picea* sp.	Canada	S. Greifenhagen
	UFV76	AF348224, AF348240, AY725707, AY725744	*Pinus* sp.	Canada	A.C. Alfenas
Cy. chinense	CBS 111037 / CPC 1154	AY725617, AY725659, AY725708, AY725745	Soil	Hong Kong	M.J. Wingfield
	CBS 112744 / CPC 4104	AY725618, AY725660, AY725709, AY725746	Soil	Hong Kong	E.C.Y. Liew
	CBS 114827 / STEU 4101[2]	AY725619, AY725661, AY725710, AY725747	Soil	Hong Kong	E.C.Y. Liew
Cy. colombiense (*Ca. colombiensis*)	CBS 112220 / CPC 723[2]	AF333413, AY725662, AY725711, AY725748	*Eucalyptus grandis*	Colombia	M.J. Wingfield
	CBS 112221 / CPC 724	AY725620, AY725663, AY725712, AY725749	*Eucalyptus grandis*	Colombia	M.J. Wingfield
Cy. curvisporum	CPC 765[2]	AF333395, AY725664, –, –	Soil	Madagascar	P.W. Crous
Cy. floridanum (*Ca. kyotensis*)	ATCC 18834[2]	AF348215, AF348231, AY725713, AY725750	*Robinia pseudoacacia*	Japan	T. Terashita
	ATCC 18882[2]	AF348218, AF348234, AY725714, AY725751	*Prunus persica*	U.S.A.	R.H. Morrison
	CBS 413.67 / IMI 299577[2]	AF348219, AF348235, –, AY725752	*Paphiopedilum callosum*	Germany	W. Gerlach
	CBS 114552 / CPC 2350	AF333401, AY725665, AY725715, AY725753	Soil	Hong Kong	M.J. Wingfield
Cy. hongkongense (*Ca. hongkongensis*)	CBS 114711 / CPC 686	AY725621, AY725666, AY725716, AY725754	Soil	Hong Kong	M.J. Wingfield
	CBS 114828 / STEU 4670[2]	AY725622, AY725667, AY725717, AY725755	Soil	Hong Kong	E.C.Y. Liew
Cy. indonesiae	CBS 112823 / CPC 4508[2]	AY725623, AY725668, AY725718, AY725756	*Syzygium aromaticum*	Indonesia	M.J. Wingfield
	CBS 112834 / CPC 4547	AY725624, AY725669, AY725719, AY725757	*Vanilla* sp.	Indonesia	M.J. Wingfield
	CBS 112840 / CPC 4554	AY725625, AY725670, AY725720, AY725758	*Syzygium aromaticum*	Indonesia	M.J. Wingfield
Cy. malesianum	CBS 112710 / CPC 3899	AY725626, AY725671, AY725721, AY725759	Debris	Thailand	N.L. Hywel-Jones
	CBS 112752 / CPC 4223[2]	AY725627, AY725672, AY725722, AY725760	Soil	Indonesia	M.J. Wingfield
Cy. multiphialidicum	CBS 112678[2]	AY725628, AY725673, AY725723, AY725761	*Musa* sp.	Cameroon	Abadie
Cy. pacificum	A1568 / IMI35428 / CPC 2534[2]	AF348222, AF348238, AY725724, AY725762	*Araucaria heterophylla*	Hawaii	M. Aragaki
	A1569 / IMI35429 / CPC 2535	AF348223, AF348239, AY725725, AY725763	*Araucaria heterophylla*	Hawaii	M. Aragaki
	CBS 114037 / CPC 10716	AY725629, AY725674, –, –	*Quercus palustris*	New Zealand	R. Peers
	CBS 114038 / CPC 10717	AY725630, AY725675, –, –	*Ipomoea aquatica*	New Zealand	C.F. Hill
Cy. parasiticum (= *Ca. ilicicola*)	ATCC 46133 / CPC 2381	AF333411, –, –, –	*Cissus rhombifolia*	U.S.A.	C.L. Schoulties
	CBS 190.50[2]	AY725631, AY725676, AY725726, AY725764	*Solanum tuberosum*	Indonesia	K.B. Boedijn & J. Reitsma
	CBS 111805 / CPC 2548	AY725632, AY725677, –, –	*Acacia koa*	Hawaii	M. Aragaki

	CBS 112209 / CPC 491	AY725633, AY725678, –, –	*Medicago sativa*	Hawaii	M. Aragaki
	CBS 112210 / CPC 490	AY725634, AY725679, –, –	*Mandevilla* sp.	Hawaii	M. Aragaki
	CBS 112211 / CPC 489	AY725635, AY725680, –, –	*Leea guineensis*	Hawaii	M. Aragaki
	CBS 112212 / UFV49 / CPC 495	AY725636, AY725681, –, –	*Cissus rhombifolia*	U.S.A.	C.R. Semer
	CBS 112213 / CPC 488	AY725637, AY725682, –, –	*Indigofera hirsuta*	U.S.A.	N.E. El-Gholl
	CBS 112214 / CPC 467	AY725638, AY725683, –, –	*Cinnamomum kanahirai*	Taiwan	M.J. Wingfield
	CBS 112215 / CPC 492	AY725639, AY725684, AY725727, AY725765	*Arachis hypogaea*	U.S.A.	Beute
	CBS 112216 / CPC 487	AY725640, AY725685, AY725728, AY725766	*Howea forsteriana*	Hawaii	M. Aragaki
	CBS 112217 / CPC 486	AY725641, AY725686, –, –	*Karya* sp.		K. Rodriques
	CBS 112218 / CPC 484	AY725642, AY725687, –, –	*Caryota* sp.	Hawaii	M. Aragaki
	CBS 112219 / CPC 482	AY725643, AY725688, –, –	*Arachis hypogaea*	U.S.A.	Beute
	CBS 112223 / CPC 2549	AY725644, AY725689, –, –	*Carica papaya*	Hawaii	M. Aragaki
	CBS 113783 / CPC 4872	AY725645, AY725690, AY725729, AY725767	*Arachis hypogaea*	U.S.A.	C. Mobley
	CBS 113903 / CPC 4871	AY725646, AY725691, AY725730, AY725768	*Arachis hypogaea*	U.S.A.	C. Mobley
	CPC 4873	–, AY725692, –, –	*Arachis hypogaea*	U.S.A.	C. Mobley
	CPC 10450	AY725647, AY725693, –, –	*Echeveria elegans*	Indonesia	C.F. Hill
Cy. pseudonaviculatum	CPC 3570	AF449453, AY725694, AY725731, AY725769	*Buxus sempervirens*	New Zealand	–
Cy. sumatrense	CBS 112757 / CPC 4501	AY725648, AY725695, AY725732, AY725770	Soil	Indonesia	M.J. Wingfield
	CBS 112829 / CPC 4518[2]	AY725649, AY725696, AY725733, AY725771	Soil	Indonesia	M.J. Wingfield
	CBS 112832 / CPC 4520	AY725650, AY725697, AY725734, AY725772	Soil	Indonesia	M.J. Wingfield
	CBS 112934 / CPC 4516	AY725651, AY725798, AY725735, AY725773	Soil	Indonesia	M.J. Wingfield

[1] CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Pedro Crous working collection housed at CBS; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.; ATCC: American Type Culture Collection, Virginia, U.S.A.; UFV: Univeridade Federal de Viçosa, Brazil.
[2] Ex-type cultures.
[3] Tub = β-tubulin, His = Histone H3, EF = Elongation factor 1-α, Cal = Calmodulin, - = not sequenced.

Key to *Cylindrocladium* species with sphaeropedunculate vesicles and 1-septate conidia (To be inserted in Crous 2002, p. 60, couplet no. 21)

21. Macroconidiophore branches –8; conidiogenous apparatus up to 100 µm long and wide ..... A
21. Macroconidiophore branches –6; conidiogenous apparatus up to 90 µm long and wide 22

A. Stipe thick-walled; conidia (45–)48–55(–65) × (4–)4.5(–5) µm, mean = 53 × 4.5 µm ..... ***Cy. multiphialidicum***
A. Stipe thin-walled; conidia (38–)45–48(–55) × 4(–4.5) µm, mean = 46.5 × 4 µm ..... ***Cy. hongkongense*** **(teleo. *Ca. hongkongensis*)**

22. Macroconidiophore branches 4–6 ..... a
22. Macroconidiophore branches –3 ..... e

a. Lateral stipe extensions absent; macroconidia (40–)45–55(–60) × 3(–4) µm, mean = 50.5 × 4 µm ***Cy. indonesiae***
a. Lateral stipe extensions present ..... b

b. Macroconidia up to 55 µm, mean length less than 50 µm c
b. Macroconidia longer than 55 µm, mean length exceeding 50 µm ..... d

c. Macroconidia (35–)45–50(–55) × 3–4(–5) µm, mean = 40 × 3.5 µm; teleomorph readily formed ..... ***Cy. floridanum*** **(teleo. *Ca. kyotensis*)**
c. Macroconidia (34–)45–52(–55) × (3–)4 µm, mean = 47.5 × 4 µm; teleomorph not observed .... ***Cy. malesianum***

d. Primary vesicles 7–12 µm diam; macroconidia 1(–3)-septate ..... ***Cy. colombiense*** **(teleo. *Ca. colombiensis*)**
d. Primary vesicles 12–17 µm diam; macroconidia 1-septate ..... ***Cy. asiaticum*** **(teleo. *Ca. asiatica*)**

e. Conidial mean length 50 µm or shorter ..... f
e. Conidial mean length above 50 µm ..... g

f. Vesicles pyriform to sphaeropedunculate; (38–)48–55(–65) × 4(–5) µm, mean = 50 × 4 µm ..... ***Cy. canadense***
f. Vesicles sphaeropedunculate; (38–)41–48(–56) × (3.5–)4(–4.5) µm (mean = 45 × 4 µm) .....***Cy. chinense***

g. Lateral stipe extensions common .....***Cy. pacificum***
g. Lateral stipe extensions rare .....***Cy. sumatrense***

REFERENCES

Berner DK, Berggren GT, Snow JP, White EP (1988). Distribution and management of red crown rot of soybean in Louisiana USA. *Applied Agricultural Research* **3**: 160–166.

Carbone I, Kohn LM (1999). A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* **91**: 553–556.

Crepel C, Inghelbrecht S (2003). First report of blight on *Buxus* spp. caused by *Cylindrocladium buxicola* in Belgium. *Plant Disease* **87**: 1539.

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

Crous PW, Groenewald JZ, Hill CF (2002). *Cylindrocladium pseudonaviculatum* sp. nov. from New Zealand, and new *Cylindrocladium* records from Vietnam. *Sydowia* **54**: 23–33.

Farris JS, Källersjö M, Kluge AG, Bult C (1994). Testing significance of incongruence. *Cladistics* **10**: 315–320.

Glass NL, Donaldson G (1995). Development of primer sets designed for use with PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* **61**: 1323–1330.

Henricot B, Culham A (2002). *Cylindrocladium buxicola*, a new species affecting *Buxus* spp., and its phylogenetic status. *Mycologia* **94**: 980–997.

Hillis DM, Bull JJ (1993). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* **42**: 182–192.

Jeng RS, Dumas M, Liu FH, Wang CL, Hubbes M (1997). DNA analysis of *Cylindrocladium floridanum* isolates from selected forest nurseries. *Mycological Research* **101**: 285–291.

Kang JC, Crous PW, Schoch CL (2001). Species concepts in the *Cylindrocladium floridanum* and *Cy. spathiphylli* complexes (*Hypocreaceae*) based on multi-allelic sequence data, sexual compatibility and morphology. *Systematic and Applied Microbiology* **24**: 206–217.

Lee SB, Taylor JW (1990). Isolation of DNA from fungal mycelia and single spores. In: *PCR protocols: a guide to methods and applications.* (Innis MA, Gelfand DA, Sninsky JJ, White TJ, eds). Academic Press, San Diego, CA, U.S.A.: 282–287.

O'Donnell K, Cigelnik E (1997). Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics and Evolution* **7**: 103–116.

Page RDM (1996). TREEVIEW: An application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* **12**: 357–358.

Peerally A (1991). The classification and phytopathology of *Cylindrocladium* species. *Mycotaxon* **40**: 323–366.

Rambaut A (2002). *Sequence alignment editor*. Version 2.0. Department of Zoology, University of Oxford, Oxford.

Rayner RW (1970). *A mycological colour chart.* CMI and British Mycological Society. Kew, Surrey, England.

Risède J-M, Simoneau P (2003). Typing *Cylindrocladium* species by analysis of ribosomal DNA spacers polymorphism: Application to field isolates from the banana rhizosphere. *Mycologia* **93**: 494–504.

Risède J-M, Simoneau P (2004). Pathogenic and genetic diversity of soilborne isolates of *Cylindrocladium* from banana cropping systems. *European Journal of Plant Pathology* **110**: 139–154.

Rossman AY (1979). *Calonectria* and its type species, *C. daldiniana*, a later synonym of *C. pyrochroa*. *Mycotaxon* **8**: 321–328.

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

Swofford DL (2000). *PAUP* 4.0: phylogenetic analysis using parsimony*. Sinauer Associates, Sunderland, MA, U.S.A.

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