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 multiplicidalicum 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. (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 Lealhy, 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 temperature of 60 °C. For histone two β-tubulin, calmodulin, elongation factor 1-α and histone gene regions.

For histone two β-tubulin, calmodulin, elongation factor 1-α and histone gene regions.
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 = 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. 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 value, 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. indonesiae and Cy. pseudonaviculatum as sister taxa.

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 %).

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. 1](image1.png)

**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.

![Fig. 2](image2.png)

**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.
supported clades for The next cluster with 100 % support contains strongly well-supported clades. As in the
histone phylograms, the first cluster of clades lacks bootstrap support, but it does contain the identical
changes and bootstrap support values from 1000 replicates in the scale bar shows 10 changes and bootstrap support values from 1000 replicates.

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


Anamorph: Cylindrocladium asiaticum Crous & Hywel-Jones, *sp. nov.*

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

Calonectriae kyotensi similis, sed ascosporis brevieribus, (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. 

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 similis, sed vesiculis latoribus (12–17 µm diam) et conidis maioribus (42–)48–55(–65) × (4–)5(–5.5) µm, in medio 53 × 5 µm.

Conidiophores consisting of a stipe bearing a penicilate 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.

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 doliform 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. Microconidia and megaconidia unknown.


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.


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.


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

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

Cylindrocladio floridano similis, sed conidiis longioribus (in medio 45 × 4 µm) et paucis ramos (–3) conidiophorum 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 sphäropedunculate 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, (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.


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.

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.


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) differs.

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.


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

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

Conidiophores consisting of a stipe bearing a penicil late 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 doliform 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 color less slime. *Megaconidium* and *microconidium* 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 *Cyl. colombiense* were initially treated under *Cyl. parasiticum* by Crous (2002). This was based on observations that *Cyl. 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 *Cyl. floridanum*, (35–)45–50(–55) × 3–4(–5) µm, mean = 40 × 3.5 µm, showing considerable overlap with those of *Cyl. parasiticum*, (45–)70–82(–90) × (4–)5–6.5(–7) µm, mean = 62 × 6 µm. Although *Cyl. 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 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.

Cylindrocladium floridanum similis, sed ramis conidiophororum numerosis (–8) et penicillo ad 100 µm alto et latu differens.

Conidiophores consisting of a stipe bearing a penicilate 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 colarette. 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.

*Cylindrocladion floridanum* 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 septe, hyaline, but pale brown below, smooth, 40–80 × 5–6 µm; stipe extensions septe, 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 asperate or 1-septe, 18–25 × 4–5 µm; secondary branches asperate, 10–20 × 4–5 µm, tertiary and additional branches, –5, asperate, 10–15 × 4–5 µm, each terminal branch producing 2–6 phialides; phialides elongate doliform to reniform, hyaline, asperate, 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-septe, lacking a visible abscission scar, held in parallel cylindrical clusters by colorless slime. *Megaconidia* and *microconidia* unknown.


**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.


**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.

*Cylindrocladium flavidano* 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. *Megaconidium* and *microconidium* 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.


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**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.

*Cylindrocladium floridanum* simile, sed ramis conidiophororum numerosis (–8) et conidiis longioribus (in medio 53 × 4.5 µm) et appendicibus crassitunicatibus 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. *Megaconidium* and *microconidium* unknown.
Crous et al.


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'i) 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 conidiophorum 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 dolliform 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.


Holotype: Indonesia, Northern Sumatra, from soil collected under canopies of Eucalyptus trees, 2001, M.J. Wingfield (holotype herb. CBS 9887, 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.

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. buxicolor 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.

<table>
<thead>
<tr>
<th>Accession number</th>
<th>GenBank accession numbers (Tub, His, EF, Cal)</th>
<th>Host</th>
<th>Country</th>
<th>Collector</th>
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<tr>
<td>Cy. asiaticum (Ca. asiatica)</td>
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<td>CBS 112711 / CPC 3898</td>
<td>AY725613, AY725655, AY725702, AY725738</td>
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<td>Cy. colombiensis (Ca. colombiensis)</td>
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<td>AY725620, AY725662, AY725711, AY725748</td>
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<td>Cy. curvisporum (Ca. kytosensis)</td>
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<td>Solanum tuberosum</td>
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<td>CBS 111805 / CPC 2548</td>
<td>AY725632, AY725677, ~, ~</td>
<td>Acacia koa</td>
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</table>
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 µm, mean = 46.5 × 4 µm.................................

........... Cy. hongkongense (teleo. Ca. hongkongensis)

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

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..............

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

1 CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Pedro Crous working collection housed at CBS; IMI: International Mycological Institute, CABI-Bioscience, Egham, Berkshire, U.K.; ATCC: American Type Culture Collection, Virginia, U.S.A.; UFV: Universidade Federal de Viçosa, Brazil.

2 Ex-type cultures.

3 Tub = β-tubulin, His = Histone H3, EF = Elongation factor 1-α, Cal = Calmodulin, - = not sequenced.

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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**


