ADDITIONS TO CALONECTRIA

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The genus Calonectria De Not. was circumscribed by Rossman (1979),
who examined the type species, Calonectria pyrochroa (Desm.) Sacc. In
subsequent treatments (Boesewinkel, 1982, Rossman, 1983), the genus
was characterised as having Cylindrocladium Morgan anamorphs, scarlet
to dark umber (KOH +) ascocarps with scaly to warty walls, and
narrowly-stalked, broadly clavate asci, with curved to fusoid ascospores,
having one to several septa, and accumulate in the upper part of the ascus.

Although there have been several studies reporting the
homothallic nature of Cylindrocladium spp. (Sobers, 1972; Peerally, 1973;
Alfieri, El-Gholl & Schoultes, 1982; El-Gholl, Schoultes & Alfieri,
1983), few reports have referred to heterothallism in Cylindrocladium, the
first made only in 1974 (Sobers, 1974), referring to the teleomorph of
Cylindrocladium scoparium Morgan. Subsequent to this report, several
Calonectria spp. have been described from paired haploid conidial
isolates (Ribeiro, 1978; Schubert, El-Gholl, Alfieri & Schoultes, 1989;
Peerally, 1991). We therefore assume that many well-known
Cylindrocladium spp. that consistently occur in the absence of their
teleomorphs, are probably heterothallic.
The present study reports three new *Calonectria* spp. Two of these are the heterothallic telemorphs of *Cylindrocladium pteridis* Wolf, and a new variety of *Cylindrocladium colhounii* Peerally, respectively. The third species is homothallic, and is the teleomorph of *Cylindrocladium gracile* (Bugn.) Boesewinkel.

*Calonectria colhounii* Peerally was described from leaf spots on *Camellia sinensis* (L.) Kunze in Mauritius in 1973 (Peerally, 1973). This species is homothallic, and produces bright yellow perithecia with four 3-septate ascospores per ascus (Peerally, 1973). The presence of yellow perithecia with 4-spored asci is an unique feature in *Calonectria* (Rossman, 1983). The *Cylindrocladium* anamorph, however, is distinguished from other *Cylindrocladium* spp. by having 3-septate conidia, measuring 38.0-84.0 x 3.4-5.7 μm, and stipes terminating in clavate vesicles (Peerally, 1974).

During routine collections from a forest nursery in the Sabie area, Eastern Transvaal, South Africa, a severe disease of *Eucalyptus* cuttings was found. The causal organism was characterized by having large, warty, yellow perithecia, with four 3-septate ascospores per ascus, typical of *C. colhounii*. Single-ascospore isolations produced a *Cylindrocladium* anamorph with 3-septate conidia and stipes terminating in clavate vesicles. The teleomorph could, however, not be induced in culture on either 2 % malt-extract agar (MEA) or carnation-leaf agar (CLA) (Crous, Phillips & Wingfield, 1992). Although the *Calonectria* state was similar to that of the type of *Calonectria colhounii* (IMI 167581), the anamorph exhibited some very clear differences. Conidia were 3-septate, but much larger than those of *C. colhounii*, being (86.0)-97.0-(112.0) x (5.5)-6.5-(8.0) μm in size. Moreover, phialides were allantoid to cylindrical, and not doliform as found on the type of *C. colhounii*. Stipes were also longer, being up to 320.0 μm in length, whereas those of *C. colhounii* were found to be less than 280.0 μm in length.

Fig. 1A-E. *Calonectria colhounii* var. *colhounii* and its anamorph *Cylindrocladium colhounii* var. *colhounii* on CLA. A, conidiophore, vesicles and conidia; B, ascus and ascospores (PPRI 4183); C, vesicles and conidia; D, ascospores (bar = 10 μm); E, vertical section through a perithecium on agar (IMI 167581, type) (bar = 20 μm).
Based on the morphological differences mentioned above, as well as the identical and unique teleomorphs, the South African collection is described as a new variety of *Cylindrocladium colhounii* below:

**Cylindrocladium colhounii** Peerally var. *colhounii*  
Holomorph: *Calonectria colhounii* Peerally var. *colhounii*  


Fig. 2A-G. *Calonectria colhounii* var. *macroconidialis* and its anamorph *Cylindrocladium colhounii* var. *macroconidialis*. A, vesicles; B, chlamydospores; C, conidia on CLA (bar = 10 μm); D, v.s. through a peritheciun on *Eucalyptus* cuttings (bar = 20 μm); E, asci and ascospores; F, conidiophore; G, ascospores (PREM 51036) (bar = 10 μm).
Cylindrocladium colhounii var. macroconidialis Crous, Wingfield & Alfenas var. nov.

Cylindrocladium colhounii var. macroconidialis Crous, Wingfield & Alfenas var. nov.

Cylindrocladium colhounii var. colhounii similis, sed filum (240.0)-280.0-(320.0) μm longum, phialides cylindrici ad allantoidei, (12.0)-20.0-(25.0) x (3.5)-4.0-(5.0) μm, conidia 3-septata, (86.0)-97.0-(112.0) x (5.5)-6.5-(8.0) μm.

Status holomorphicus: Calonectria colhounii var. macroconidialis Crous, Wingfield & Alfenas var. nov.

Omnia var. colhounii sed statu anamorphico differt, filum (240.0)-280.0-(330.0) μm longum, phialides cylindrici ad allantoidei, (12.0)-20.0-(25.0) x (3.5)-4.0-(5.0) μm, conidia 3-septata, (86.0)-97.0-(112.0) x (5.5)-6.5-(8.0) μm.

Morphologically similar to var. colhounii. Macroconidiophores. Filament septate, hyaline, terminating in a narrowly clavate vesicle, (3.0)-4.0-(5.0) μm diam.; stipes (240.0)-280.0-(320.0) μm long. Conidiophore branches, primary branches non-septate to 1-septate, (20.0)-30.5-(48.0) x (4.0)-4.5-(5.0) μm; secondary branches non-septate to rarely 1-septate, (20.0)-25.0-(30.0) x (4.0)-4.5-(5.0) μm; tertiary and quaternary branches non-septate, (18.0)-20.0-(30.0) x (4.0)-4.5-(5.0) μm. Phialides allantoid to cylindrical, hyaline, non-septate, (12.0)-20.0-(25.0) x (3.5)-4.0-(5.0) μm. Conidia cylindrical, hyaline, (1)-3-septate, rounded at both ends, (86.0)-97.0-(112.0) x (5.5)-6.5-(8.0) μm. Cultural characteristics identical to var. colhounii. Isolates of var. macroconidialis do, however, grow faster than those of var. colhounii and have medium numbers of chlamydoospores on MEA after 6 d at 25 C in the dark.
Teleomorph. Perithecia morphologically and anatomically identical to those of var. colhounii.


Symptoms: leaf spot, root rot, wilt.

Hosts: Eucalyptus grandis W. Hill : Maiden.

Distribution: South Africa.


Cylindrocladium gracile was initially described as a Cylindrocarpon sp. from Argyreia splendens (Roxb.) Sweet in Indo China (Bugnicourt, 1939). In a review of Cylindrocarpon Wollenw., Booth (1966) discussed the morphology of this species, and noted that the conidiophore apparatus did not fit the general trend for the genus. The differences observed by Booth were recognized by Boesewinkel as representative of the genus Cylindrocladium, leading him to transfer this species to the latter genus as C. gracile (Bugn.) Boesew. (Boesewinkel, 1982). Boesewinkel justified the establishment of a new species of Cylindrocladium by stating that it had 3-6-septate stipes, 1-septate conidia
and narrowly clavate vesicles. A comparison of the type of *C. gracile* (PC 551197) with that of a similar species, *C. clavatum* Hodges & May (BPI 414550), showed that *C. gracile* could be distinguished by having longer stipes and larger conidia.

*Cylindrocladium gracile* has recently been reported from Canada (Chang & Blenis, 1987), but this is the first report of this species from Brazil. Single-conidial isolates also gave rise to a previously undescribed *Calonectria* species on CLA. The teleomorph is described for the first time as follows:

*Cylindrocarpon gracile* Bugnicourt Encycl. Mycol. 11, 162 (1939).
Holomorph: *Calonectria gracilis* Crous, Wingfield & Alfenas sp. nov.

Macroconidiophores. *Filament* septate, hyaline, terminating in a narrowly clavate vesicle, (2.5)-3.6-(5.0) μm diam.; stipes (160.0)-220.0-(350.0) μm long. *Conidiophore branches*, primary branches non-septate or rarely 1-septate, (14.0)-18.0-(25.0) x (3.5)-4.0-(4.5) μm; secondary branches non-septate, (12.0)-14.2-(16.0) x (3.5)-4.0-(4.5) μm. *Phialides* doliform to reniform, hyaline, non-septate, (10.0)-12.5-(15.0) x (3.5)-4.0-(4.5) μm. *Conidia* cylindrical, hyaline, 1-septate, rounded at both ends, (40.0)-56.0-(65.0) x (4.0)-4.5-(5.0) μm.

*Calonectria gracilis* Crous, Wingfield & Alfenas sp. nov.

*Perithecia* superficialia, singulatim aut in turmis parvis portata, globosa vel subglobosa, 350.0-400.0 x 330.0-380.0 μm, rubri-brunnea usque ad rubra, pariete exteriore aspero verrucosoque et ostiolo

Fig. 3A-I. *Calonectria gracilis* and its anamorph *Cylindrocladium gracile* on CLA. A, conidiophore and vesicles; B, chlamydospores; C, conidia (PC 551197, type); D, conidiophore (PREM 51031, type of teleomorph); E, conidia; F, ascospores; G, vesicles; H, asci (bar = 10 μm); I, v.s. through a perithecium (bar = 20 μm).
papillato. Asci hyalini, clavati ad longum caulem et tenuum contracti, (75.0)-90.0-(100.0) x (8.0)-10.0-(15.0) μm, 8 ascospori. Ascosporae hyalinae, rectae vel falcatae, guttulatae, 1-septatae, ad septam medianam non constrictae, (27.0)-36.5-(50.0) x (4.0)-5.0-(6.0) μm.

Perithecia superficial, borne singly or in small groups, globose to subglobose, 350.0-400.0 x 330.0-380.0 μm, with warty outer wall and papillate ostiole, red to red-brown, turning blood-red in 3 % KOH. Asci hyaline, clavate, (75.0)-90.0-(100.0) x (8.0)-10.0-(15.0) μm, tapering to a long thin stalk, containing 8 ascospores. Ascospores hyaline, straight or falcate, guttulate, 1-septate, not constricted at median septum, (27.0)-36.5-(50.0) x (4.0)-5.0-(6.0) μm.


Symptoms: root rot.


Distribution: Brazil, Canada, South East Asia (Indo China) (Bugnicourt, 1939; Chang & Blenis, 1987).


Cylindrocladium pteridis has the longest conidia of all 1-septate Cylindrocladium spp., frequently being longer than 100.0 μm. Furthermore, it is the only Cylindrocladium sp. that produces curved microconidia (Peerally, 1991). Although C. macrosorum is a synonym of C. pteridis (Sobers, 1968), many researchers continue to use the name C. macrosorum (Renard & Viennot-Bourgin, 1973; Renard & Quillec, 1979; Ahmad & Ahmad, 1982) rather than the correct name C. pteridis.

Sobers (1968) showed that the type culture of C. macrosorum could produce microconidia when cultured on water agar. This was the first time that a microconidial state had been described in a species of Cylindrocladium. In the original description (Sherbakoff, 1928), microconidia are illustrated, but were not mentioned prior to the study in which Sobers (1968) placed C. macrosorum in synonymy under C. pteridis. Sobers (1968) stated that microconidia did not give rise to both conidial types when single-spored. In the present study, single microconidia from C. pteridis commonly gave rise to both conidial types in culture.

One isolate (ATCC 34395) was observed to form protoperithecia when cultured on CLA. When this isolate was paired on CLA with other isolates of C. pteridis using the methods explained in Ribeiro (1978), perithecia developed. However, although perithecia developed easily on CLA, they were never fertile. Fertile perithecia could only be obtained by incubating plates at 15°C for 2 mo. Cultures used in the pairings were representative of two types, (-) and (+) respectively. Fertile perithecia were obtained by pairing one (-) isolate with a (+) isolate. Isolates used in pairings for fertile perithecia were the following: (+) PPRI 4180, 4181; (-) PPRI 4157, 4177, 4178, ATCC 34395. Calonectria pteridis, the teleomorph of Cylindrocladium pteridis, is described below:

Figs 4, 5
Cylindrocladium macrosorum Sherb. Phytopathology 18, 222 (1928).
Holomorph: Calonectria pteridis Crous, Wingfield & Alfenas sp. nov.
Macroconidiophores. Filament septate, hyaline, terminating in a clavate vesicle, (4.0)-4.5-(5.5) μm diam.; stipes (150.0)-240.0-(300.0) μm long. Conidiophore branches, primary branches non-septate or rarely 1-septate, (18.0)-28.0-(32.0) x (4.0)-4.8-(6.0) μm; secondary branches non-septate, (16.0)-20.0-(30.0) x (3.5)-4.0-(5.5) μm; tertiary branches non-septate, (14.0)-18.0-(22.0) x (3.5)-4.0-(4.5) μm. Phialides elongate, doliiform to reniform, hyaline, non-septate, (12.0)-15.5-(22.0) x (3.5)-4.0-(5.0) μm. Conidia cylindrical, hyaline, 1-(3)-septate, rounded at both ends, (62.5)-82.0-(121.0) x (4.5)-5.0-(6.0) μm.

Microconidiophores. Filament septate, hyaline, terminating in a clavate vesicle. Conidiophore branches, primary branches non-septate to rarely 1-septate, (20.0)-28.0-(50.0) x (3.0)-4.5-(5.0) μm; secondary branches non-septate to rarely 1-septate, (18.0)-20.0-(30.0) x (2.5)-3.0-(3.5) μm; tertiary branches non-septate, (16.0)-18.0-(23.0) x (2.5)-3.0-(3.5) μm. Phialides arise from the ends of branches, in groups of 2-4; phialides cylindrical, hyaline, non-septate, (10.0)-15.0-(21.0) x (2.5)-3.0-(3.5) μm, collarettes absent in some isolates, inconspicuous in others. Conidia cylindrical, curved or straight, hyaline, 1-septate with obtuse ends, (18.5)-29.5-(40.0) x (2.5)-3.5-(4.0) μm.

Calonectria pteridis Crous, Wingfield & Alfenas sp. nov. Perithecia superficialia, singulatim aut in turmis parvis portata, globosa vel subglobosa, 400.0-500.0 x 300.0-350.0 μm, rubri-brunnea ad rubra, pariete exteriore aspero verrucosoque et ostiolo papillato. Asci hyalini, clavati ad caulem longum et tenuem contracti, (100.0)-120.0-(180.0) x (9.0)-15.0-(27.0) μm, 1-8 ascospori. Ascosporeae hyalinae, rectae vel falcatae, 1-(3)-septatae, plerumque ad septum non constrictae (30.0)-51.5-(75.0) x (4.5)-5.5-(7.0) μm. Ascosporeae usque ad 6 septis ubi ab asco dimissae.

Fig. 4A-D. Cylindrocladium pteridis, anamorph of Calonectria pteridis on CLA (bar = 10 μm). A, microconidiophore, vesicles and conidia on CLA; B, chlamydospores on MEA after 7d; C, macroconidiophore, vesicles and conidia on CLA; D, chlamydospores on CLA after 7d (PPRI 4157).
Perithecia superficial, borne singly or in small groups, globose to subglobose, 400.0-500.0 x 300.0-350.0 μm, with warty outer wall and papillate ostiole, red to red-brown, turning blood-red in 3 % KOH. Asci hyaline, clavate, (100.0)-120.0-(180.0) x (9.0)-15.0-(27.0) μm, tapering to a long thin stalk, containing 1-8 ascospores. Ascospores hyaline, straight or falcate, 1-(3)-septate, generally not constricted at septa, (30.0)-51.5-(75.0) x (4.5)-5.7-(7.0) μm. Ascospores with up to 6 septa once discharged from the ascus.

Type: USA: Florida, Orlando, Rumohra adiantiformis (G. Forst.) Ching (=Polystichum adiantiforme (Forst.) J.E. Sm.), F.A. Wolf, Oct. 1926, BPI 414564, holotype of anamorph. Brazil: Pinus sp., T.L. Krügener, PPRI 4177 x Eucalyptus grandis, A.C. Alfenas, PPRI 4180, PREM 51033, holotype of teleomorph; Pinus caribaea, T.L. Krügener, PPRI 4178 x Eucalyptus grandis, A.C. Alfenas, PPRI 4180, PREM 51034, paratype of teleomorph.

Symptoms: causing leaf spots and root disease (Sobers, 1968).


Fig. 5A-E. Calonectria pteridis, teleomorph of Cylindrocladium pteridis on CLA. A, asci; B, ascospores (bar = 10 μm); C, perithecium (bar = 20 μm); D, transverse section through perithecium wall; E, ostiolar region of a perithecium (PREM 51033, type) (bar = 10 μm).

Cultures: Brazil: unknown host, J.C. Dianese, (Viçosa, Brazil, No. UFV 43), PPRI 4157; needles of a Pinus sp., T.L. Krügner, (Viçosa, Brazil, No. UFV 10) PPRI 4177; needles of Pinus caribaea, T.L. Krügner, (Viçosa, Brazil, No. UFV 37) PPRI 4178; Eucalyptus grandis leaves, A.C. Alfenas, (Brazil, Viçosa, No. UFV 105) PPRI 4180. USA: Arachnoides adiantiformis, F. Schickedanz, 1974, ATCC 34395; Florida, Rumohra adiantiformis, N.E. El-Gholl, (Brazil, Viçosa, No. UFV 50) PPRI 4179; Eucalyptus sp., C.S. Hodges, (Brazil, Viçosa, No. UFV 92) PPRI 4181.

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