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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 acuminate in the upper part of the ascus.

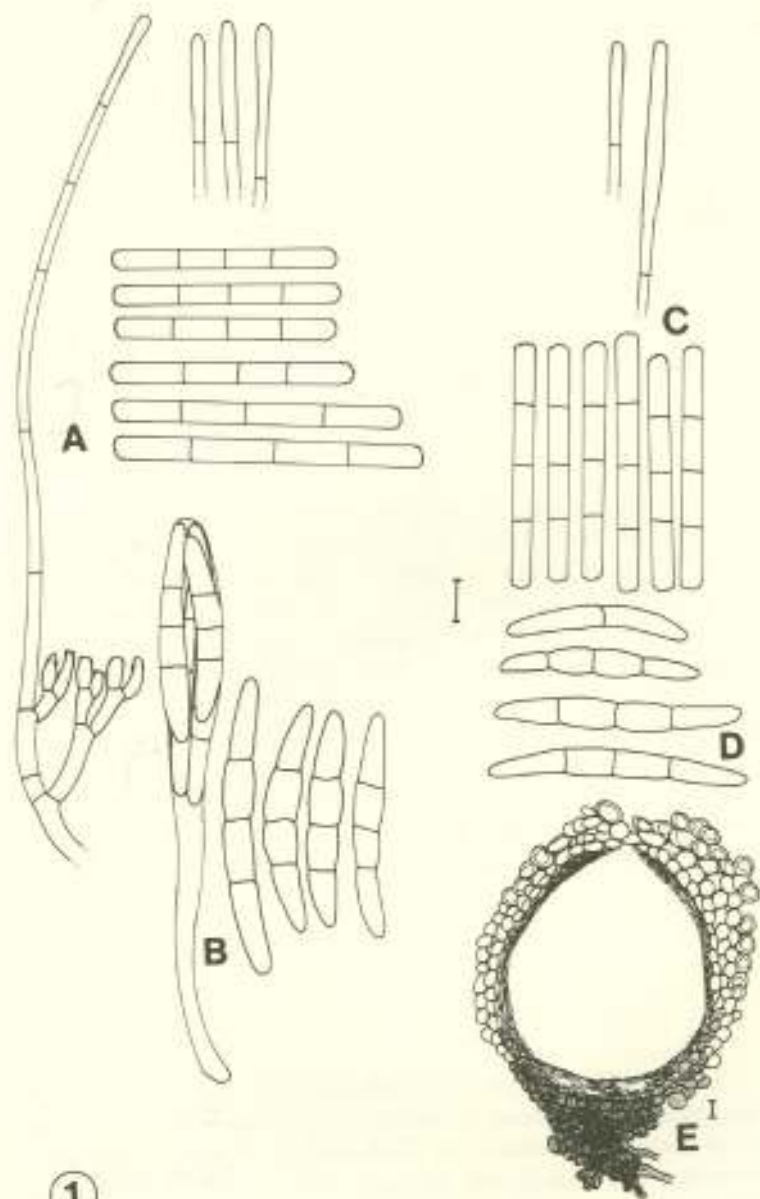
Although there have been several studies reporting the homothallic nature of *Cylindrocladium* spp. (Sobers, 1972; Peerally, 1973; Alfieri, El-Gholl & Schoulties, 1982; El-Gholl, Schoulties & Alfieri, 1983), few reports have referred to heterothallicism in *Cylindrocladium*, the first made only in 1974 (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 & Schoulties, 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 teleomorphs of *Cylindrocladium pteridis* Wolf, and a new variety of *Cylindrocladium colhounii* Peeraly, respectively. The third species is homothallic, and is the teleomorph of *Cylindrocladium gracile* (Bugn.) Boesewinkel.

Calonectria colhounii Peeraly was described from leaf spots on *Camellia sinensis* (L.) Kuntze in Mauritius in 1973 (Peeraly, 1973). This species is homothallic, and produces bright yellow perithecia with four 3-septate ascospores per ascus (Peeraly, 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 $38.0\text{--}84.0 \times 3.4\text{--}5.7 \mu\text{m}$, and stipes terminating in clavate vesicles (Peeraly, 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 $(86.0)\text{--}97.0\text{--}(112.0) \times (5.5)\text{--}6.5\text{--}(8.0) \mu\text{m}$ in size. Moreover, phialides were allantoid to cylindrical, and not doliiform as found on the type of *C. colhounii*. Stipes were also longer, being up to $320.0 \mu\text{m}$ in length, whereas those of *C. colhounii* were found to be less than $280.0 \mu\text{m}$ 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 = $10 \mu\text{m}$); E, vertical section through a perithecium on agar (IMI 167581, type) (bar = $20 \mu\text{m}$).



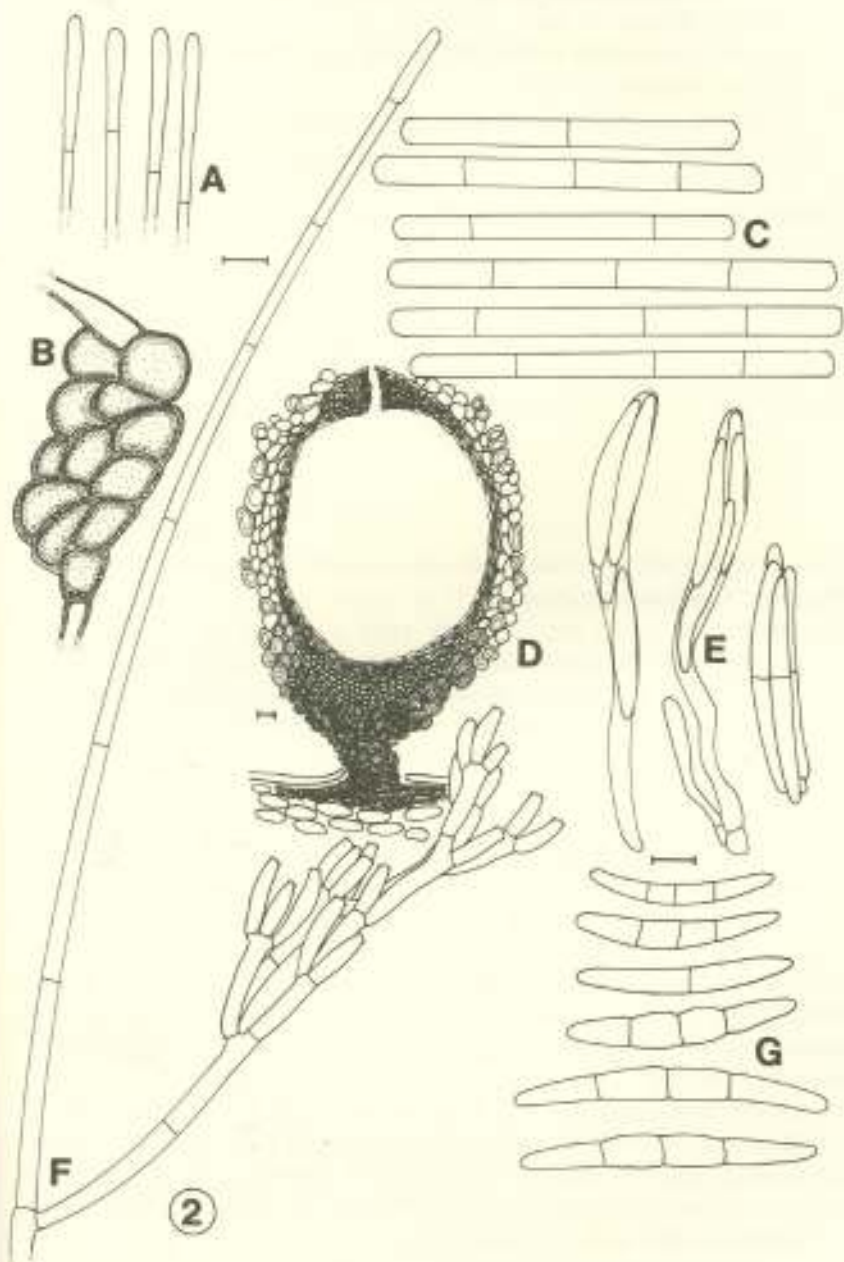
Based on the morphological differences mentioned above, as well as the identical and unique teleomorphs, the South African collection is described as a new variety of *Cylindrocladium colhounii* below:

Cylindrocladium colhounii Peeraly var. *colhounii* Fig. 1.
C. colhounii Peeraly var. *colhounii* *Trans. Br. mycol. Soc.* 61, 92 (1973).
 Holomorph: *Calonectria colhounii* Peeraly var. *colhounii*
Calonectria colhounii Peeraly *Trans. Br. mycol. Soc.* 61, 92 (1973).

Specimens: India: *Canavalia ensiformis* (L.) DC., 1986, IMI 308494. Mauritius: *Camellia sinensis*, M.A. Peeraly, 1972, IMI 167581 (holotype).

Cultures: Australia: *Arachis pintoi*, D. Hutton, N8335, N8710, N9060, N9190, PPRI 4735-4738. Colombia: *Centrosema* sp., J.M. Lenné, 1990, PPRI 4748. USA: unknown hosts, A. Alfenas, (Viçosa, Brazil, UFV 4A, UFV 52A, PPRI 4182, 4185; Hawaii, unknown host, Goos, UFV 22A, 51A, CTR 69-19, PPRI 4183-4184; North Carolina, Macon Co., Nantahala National Forest, Ellicott Rock Trail from Bull Pen Road along Chattooga River, on fallen cones of white pine, *Pinus strobus*, C.T. Rogerson & G.J. Samuels, 4 Oct. 1972, C.T. Rogerson 72-346, ATCC 44542, UFV 53A, 65A, PPRI 4186-4187; Louisiana, *Ficus carica* L., G.E. Holcomb, summer 1991, AR 2684, PPRI 4739; Virginia, Giles Co., Cascades Recreation site, 4 miles north of Pembroke, 37° 23' N, 80° 35' W, pericarp of *Carya* nut, G.J. Samuels, 18 Sept. 1991, GJS 91-117, PPRI 4740.

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 perithecium on *Eucalyptus* cuttings (bar = 20 µm); E, asci and ascospores; F, conidiophore; G, ascospores (PREM 51036) (bar = 10 µm).



Cylindrocladium colhounii Peerally var. *macroconidialis* Crous,
Wingfield & Alfenas var. nov. Fig. 2.
Holomorph: *Calonectria colhounii* Peerally var. *macroconidialis* Crous,
Wingfield & Alfenas var. nov.

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

Cylindrocladit colhounii var. *colhounii* similis, sed filum (240.0)-
280.0-(320.0) μm longum, *phialides* cylindrici ad allantoidei, (12.0)-20.0-
(25.0) \times (3.5)-4.0-(5.0) μm , *conidia* 3-septata, (86.0)-97.0-(112.0) \times (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) \times (3.5)-4.0-(5.0) μm , *conidia* 3-septata, (86.0)-97.0-(112.0) \times (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) \times
(4.0)-4.5-(5.0) μm ; secondary branches non-septate to rarely 1-septate,
(20.0)-25.0-(30.0) \times (4.0)-4.5-(5.0) μm ; tertiary and quaternary branches
non-septate, (18.0)-20.0-(30.0) \times (4.0)-4.5-(5.0) μm . *Phialides* allantoid to
cylindrical, hyaline, non-septate, (12.0)-20.0-(25.0) \times (3.5)-4.0-(5.0) μm .
Conidia cylindrical, hyaline, (1)-3-septate, rounded at both ends, (86.0)-
97.0-(112.0) \times (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 chlamydospores on
MEA after 6 d at 25 C in the dark.

Teleomorph. Perithecia morphologically and anatomically identical to those of var. *colhounii*.

Type: South Africa: E. Tvl., Frankfort, *Eucalyptus* cuttings, P.W. Crous, 16 Apr. 1991, PREM 51035, holotype of anamorph; E. Tvl., Frankfort, *E. grandis* cuttings, P.W. Crous, March 1990, PPRI 51036, holotype of teleomorph.

Symptoms: leaf spot, root rot, wilt.

Hosts: *Eucalyptus grandis* W. Hill : Maiden.

Distribution: South Africa.

Specimens: Africa: RSA, E. Tvl., Sabie, Frankfort, *Eucalyptus* cuttings, P.W. Crous, 16 Apr. 1991, PREM 51035, holotype of anamorph; RSA, E. Tvl., Sabie, Frankfort, *E. grandis* cuttings, P.W. Crous, March 1990, PREM 51036, holotype of teleomorph.

Cultures: Africa: RSA, E. Tvl., Sabie, Frankfort, *E. grandis* cuttings, P.W. Crous, March 1990, PPRI 4000; RSA, E. Tvl., Sabie, Frankfort, D.R. de Wet clone bank, *E. grandis* ramets (roots), P.W. Crous, 11 May 1990, PPRI 4001.

Cylindrocladium gracile was initially described as a *Cylindrocarpon* sp. from *Argyrea 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:

Cylindrocladium gracile (Bugn.) Boesewinkel *Trans. Br. mycol. Soc.* 78, 554 (1982). Fig. 3

Cylindrocarpon gracile Bugnicourt *Encycl. Mycol.* 11, 162 (1939).

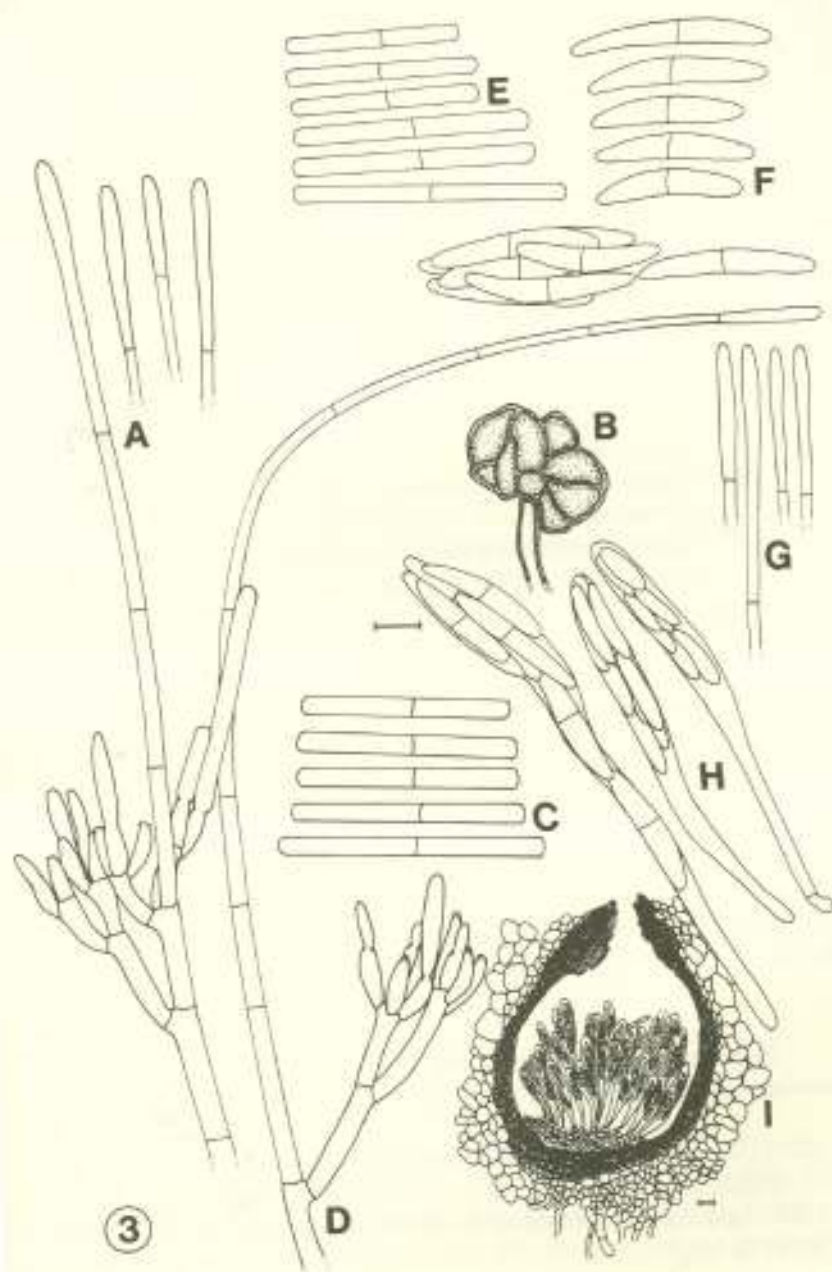
Holomorph: *Calonectria gracilis* Crous, Wingfield & Alfenas sp. nov.

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) \times (3.5)-4.0-(4.5) μm ; secondary branches non-septate, (12.0)-14.2-(16.0) \times (3.5)-4.0-(4.5) μm . *Phialides* doliform to reniform, hyaline, non-septate, (10.0)-12.5-(15.0) \times (3.5)-4.0-(4.5) μm . *Conidia* cylindrical, hyaline, 1-septate, rounded at both ends, (40.0)-56.0-(65.0) \times (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 \times 330.0-380.0 μm , rubri-brunnea usque ad rubra, pariete exteriori 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. *Ascospores* 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 .

Type: South East Asia (Indo China): *Argyreia splendens*, 1937, Bugnicourt, derived from Paris, PC 551197, PREM 51032, holotype of anamorph. Brazil: Para, near Belem, *Manilkara zapota* (L.) Van Royen, 1990, F. Carneiro de Albuquerque, PREM 51031, holotype of teleomorph.

Symptoms: root rot.

Hosts: *Manilkara zapota*, *Argyreia splendens*, *Pahudia cochinchinensis* Pierre, (Bugnicourt, 1939).

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

Specimens: South East Asia (Indo China): *C. gracile*, *Argyreia splendens*, 1937, Bugnicourt, derived from Paris No. 551197, IMI 117577.

Cultures: South East Asia (Indo China): Herb. Paris (PC) No. 551197, type culture. Brazil: PA, Belem, *M. zapota*, F. Albuquerque, PPRI 4176.

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. macrosporum* is a synonym of *C. pteridis* (Sobers, 1968), many researchers continue to use the name *C. macrosporum* (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. macrosporum* 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. macrosporum* 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:

Cylindrocladium pteridis Wolf J. Elisha Mitchell Sci. Soc. 42, 59 (1926).

Figs 4, 5

Cylindrocladium macrosporum Sherb. *Phytopathology* 18, 222 (1928).

Holomorph: *Calonectria pteridis* Crous, Wingfield & Alfenas sp. nov.

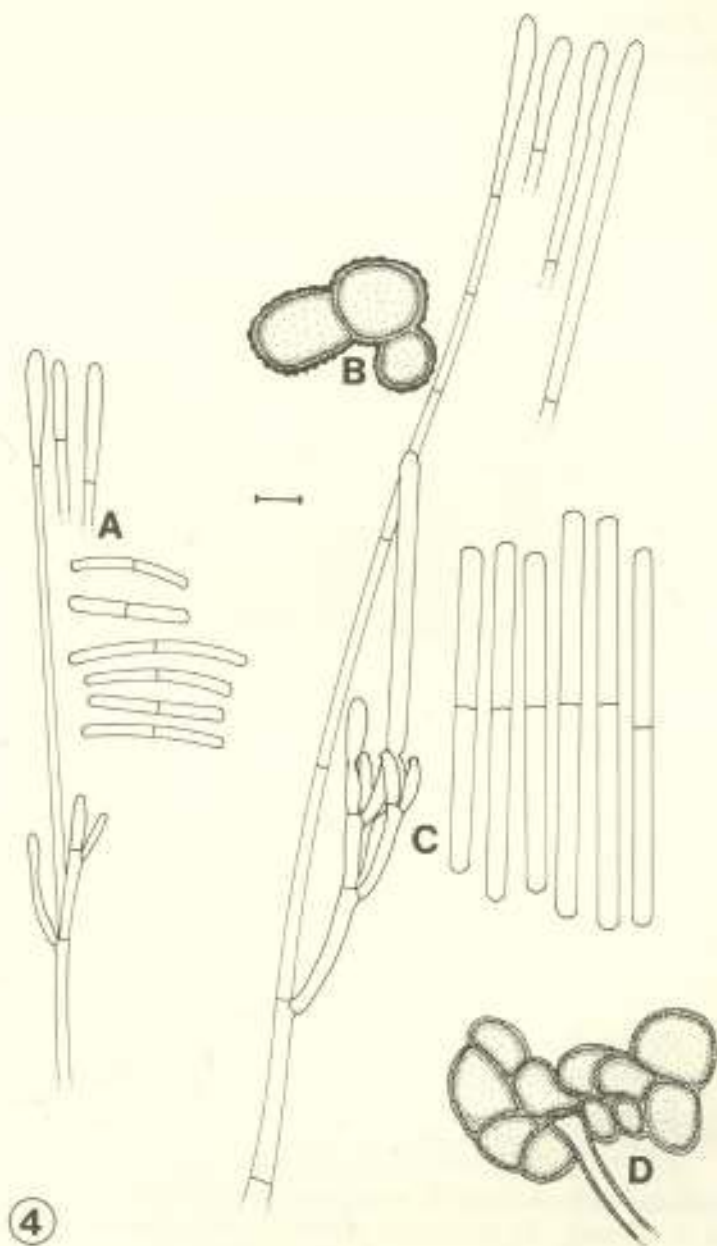
Macroconidiophores. Filament septate, hyaline, terminating in a clavate vesicle, (4.0)-4.5-(5.5) μm diam.; stipes (150.0)-240.0-(300.0) μm long. *Conidiophore branches*, primary branches non-septate or rarely 1-septate, (18.0)-28.0-(32.0) \times (4.0)-4.8-(6.0) μm ; secondary branches non-septate, (16.0)-20.0-(30.0) \times (3.5)-4.0-(5.5) μm ; tertiary branches non-septate, (14.0)-18.0-(22.0) \times (3.5)-4.0-(4.5) μm . *Phialides* elongate, dolliiform to reniform, hyaline, non-septate, (12.0)-15.5-(22.0) \times (3.5)-4.0-(5.0) μm . *Conidia* cylindrical, hyaline, 1-(3)-septate, rounded at both ends, (62.5)-82.0-(121.0) \times (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) \times (3.0)-4.5-(5.0) μm ; secondary branches non-septate to rarely 1-septate, (18.0)-20.0-(30.0) \times (2.5)-3.0-(3.5) μm ; tertiary branches non-septate, (16.0)-18.0-(23.0) \times (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) \times (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) \times (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 \times 300.0-350.0 μm , rubri-brunnea ad rubra, pariete exteriori aspero verrucosoque et ostiolo papillato. *Asci* hyalini, clavati ad caulem longum et tenuum contracti, (100.0)-120.0-(180.0) \times (9.0)-15.0-(27.0) μm , 1-8 ascospori. *Ascospores* hyalinae, rectae vel falcatae, 1-(3)-septatae, plerumque ad septum non constrictae (30.0)-51.5-(75.0) \times (4.5)-5.5-(7.0) μm . Ascospores 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.

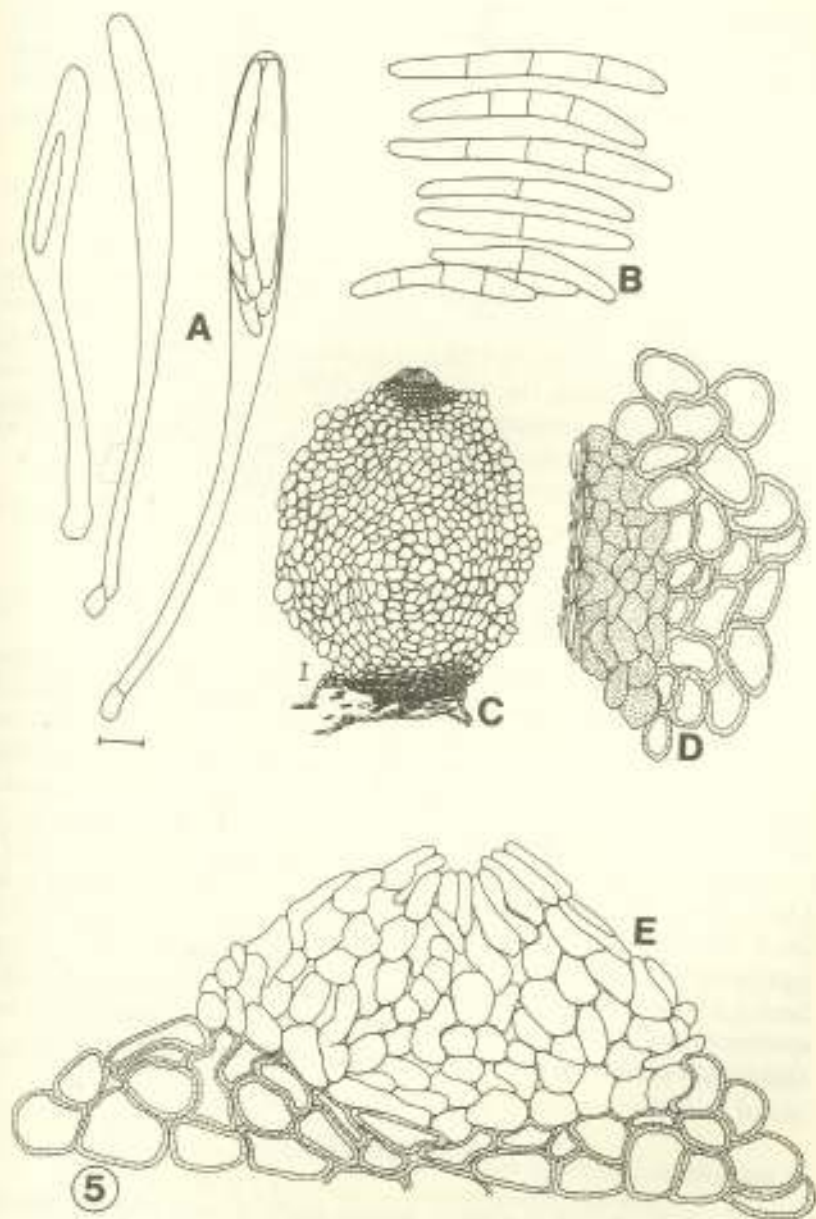
Type: USA: Florida, Orlando, *Rumohra adiantiformis* (G. Forst.) Ching (= *Polystichum adiantiforme* (Forst.) J.E. Sm.), F.A. Wolf, Oct. 1926, BPI 414564, holotype of anamorph. Brazil: *Pinus* sp., T.L. Krüchner, PPRI 4177 x *Eucalyptus grandis*, A.C. Alfenas, PPRI 4180, PREM 51033, holotype of teleomorph;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

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

Hosts: *Arachis hypogaea* L., *Arachnoides adiantiformis*, *Arecastrum romanzoffianum* (Cham.) Becc., *Asparagus plumosus* Baker, *Callistemon* spp., *Chamaedorea elegans* Schrad., *Cocos nucifera* L., *Collinia elegans* Liebm., *Dryopteris* sp., *Eucalyptus* spp., *Howeia* spp., *Lupinus* spp., *Melaleuca leucadendra* L., *Pinus caribaea* var. *hondurensis*, *Rhododendron obtusum* Planch., *Strelitzia reginae* Banks and *Washingtonia robusta* Wendl. (Wolf, 1926; Sherbakoff, 1928; Sobers, 1967; Sobers, 1968; Sobers & Alfieri, 1972).

Distribution: Africa, Brazil, India, Florida, U.S.A. (Wolf, 1926; Sobers, 1968; Sobers & Alfieri, 1972; Bedendo & Krüchner, 1987).

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).



Specimens: Africa: *Pinus caribaea* var. *hondurensis*, 1972, Ivory, IMI 164169; *P. oocarpa*, Ivory, IMI 164168. India: *Cocos nucifera*, ?, 1973, IMI 174348. USA: *Washingtonia robusta*, C.D. Sherbakoff, Jan. 1928, BPI 414558; Florida, near Sebring, *Washingtonia robusta*, C.D. Sherbakoff, Dec. 1927, BPI 414559; Florida, Orlando, *Rumohra adiantiformis*, F.A. Wolf, Oct. 1926, BPI 414564.

Cultures: Brazil: unknown host, J.C. Dianese, (Viçosa, Brazil, No. UFV 43), PPRI 4157; needles of a *Pinus* sp., T.L. Krüger, (Viçosa, Brazil, No. UFV 10) PPRI 4177; needles of *Pinus caribaea*, T.L. Krüger, (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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