Cylindrocladium parasiticum sp. nov., a new name for *C. crotalariae*

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In an examination of the type specimens of *Cylindrocladium ilicicola* and its teleomorph *Calonectria ilicicola*, two distinct *Cylindrocladium* spp. were found. Furthermore, the *Cylindrocladium* anamorph produced by the type culture from which *Calonectria ilicicola* was originally described, was identical to the *Cylindrocladium* anamorph present on the type of *Calonectria crotalariae*. This suggests that *Calonectria crotalariae* is conspecific with the earlier described *Calonectria ilicicola*. *Cylindrocladium crotalariae* can be distinguished from *Cylindrocladium ilicicola* by its larger conidia and sphaeropedunculate vesicles. However, the name *Cylindrocladium crotalariae* was never validly published due to the omission of a Latin description. *Cylindrocladium parasiticum* is therefore proposed as a new name for *Cylindrocladium crotalariae*, and *Calonectria ilicicola* shown as its teleomorph with *Calonectria crotalariae* as a synonym. The teleomorph of *Cylindrocladium ilicicola* is shown to be *Calonectria pyrochroa*, with synonyms *Calonectria daldiniana*, *Ophionectria puiggarii* and *Nectria abnormis*.

The genus *Cylindrocladium* Morgan, which has *Calonectria* de Not. teleomorphs, includes more than twenty species (Crous, Phillips & Wingfield, 1991; Peerally, 1991). Of these, *Cylindrocladium crotalariae* (Loos) Bell & Sobers is especially well-known for causing peg, pod and root necrosis of peanuts (Filer, 1970; Aragaki, Laemmlen & Nishijima, 1972; Nishijima & Aragaki, 1973; Sobers & Alfieri, 1972; Milholland, 1974; Peerally, 1974*b*; Sobers & Littrell, 1974; Alfenas *et al.*, 1979; Kuhlman, Cordell & Filer, 1980). This fungus also infects many other crops, and is widely distributed throughout the world (Bell & Sobers, 1966; Sobers & Alfieri, 1972; Misonou, 1973; Peerally, 1974*b*; Hwang & Ko, 1975; Hanounik, Pire & Osborne, 1977; Griffin, Roth & Powell, 1978; Phipps, 1990; Porter *et al.*, 1991).

In a recent review of *Cylindrocladium*, Crous *et al.* (1991) indicated that the name *Cylindrocladium crotalariae* is not validly published. Furthermore, the *Cylindrocladium* anamorph associated with the type of *Calonectria ilicicola* Boedijn & Reitsma has been illustrated with a vesicle morphology (Boedijn & Reitsma, 1950) similar to that of *Cylindrocladium crotalariae*. The aim of this study was to compare dried type and other herbarium specimens as well as cultures of *Cylindrocladium ilicicola* (Hawley) Boedijn & Reitsma and *Cylindrocladium crotalariae*, and to describe the variation present in these taxa. Comparisons were based on morphological criteria of both the anamorph and the teleomorph states, as well as their total protein banding patterns.

MATERIALS AND METHODS

Specimens and cultures of *Cylindrocladium crotalariae* and *Cylindrocladium ilicicola* compared in this study are shown in Table 1.

Single-conidial isolates of *Cylindrocladium crotalariae* and *Cylindrocladium ilicicola* were sub-cultured on to carnation-leaf agar (CLA) (Fisher *et al.*, 1982; Crous, Phillips & Wingfield, 1992), incubated at 25 °C under nuv light, and examined after 7 d. Differences have previously been observed in conidium and vesicle morphology of conidiophores occurring on water agar and carnation leaves (Crous *et al.*, 1992) and, therefore, only material occurring on the leaves was examined. Perithecia were examined before they became papillate and exuded ascospores, as ascospores were found to become elongated, multi-septate and atypical once discharged. Perithecia were placed for 2–12 h in 5% KOH and fixed for 12–24 h in 5% glutaraldehyde. They were subsequently washed in H_2O and placed in a gelatin solution (12·5 g gelatin, 37·5 ml H_2O, 0·5 g phenol). Longitudinal sections (10–15 µm) were made through perithecia using a Leitz Kryomat 1703 freezing microtome. Squash mounts were prepared in lactophenol cotton blue as well as in 3% aqueous KOH. All cultures and specimens examined were lodged at the National Collection of Fungi, Pretoria (PREM).

Single-conidial isolates of *Cylindrocladium crotalariae* and *Cylindrocladium ilicicola* were compared electrophoretically using total soluble protein banding patterns. Isolates were grown on 2% malt-extract agar (MEA), and four plugs of 7-d-old cultures transferred to 500 ml Erlenmeyer flasks (6 per

Table 1. Cultures and dry specimens of *Cylindrocladium crotalariae* and *C. ilicicola* examined

	Origin	Source	Collector	Accession no.	Date isolated	References
C. crotalariae	Brazil	*Euterpe edulis* Mart.	K. Rodriques	PPRI 4527	1991	Present study
	Ceylon	*Crotalaria anagyroides* H.B. & K.	C. A. Loos	IMI 35028	1949	Loos (1950)
	Honduras	*Ceratonia siliqua* L.	R. M. Stover	IMI 122262	1966	Present study
	Indonesia, Bogor	*Solanum tuberosum* L.	K. B. Boedijn & J. Reitsma	CBS 190·50	1948	Boedijn & Reitsma (1950)
	Taiwan	*Cinnamomum kanahirai* Hayata	M. J. Wingfield	PPRI 4213	1991	Present study
	U.S.A., Carolina	*Cissus rhombifolia* Vahl.	C. S. Semer	PPRI 4526, 4541	1991	Present study
	U.S.A., Florida	*Indigofera hirsuta* L.	N. El-Gholl	PPRI 4529	1991	Present study
	U.S.A., Georgia	*Arachis hypogaea* L.	D. K. Bell	IMI 264540	1982	Present study
	U.S.A., Hawaii	*Acacia koa* A. Grey	M. Aragaki	ATCC 24023	1970	Aragaki *et al.* (1972)
	U.S.A., Hawaii	*Carica papaya* L.	M. Aragaki	ATCC 24024	1970	Aragaki *et al.* (1972)
	U.S.A., Hawaii	*Carpetia* sp.	M. Aragaki	PPRI 4525	1991	Present study
	U.S.A., Hawaii	*Hotoria forsterana* Becc.	M. Aragaki	PPRI 4528	1988	Present study
	U.S.A., Hawaii	*Leea coccinea* Planch	M. Aragaki	PPRI 4530	1979	Present study
	U.S.A., Hawaii	*Mandevilla* sp.	M. Aragaki	PPRI 4531	1987	Present study
	U.S.A., Hawaii	*Medicago sativa* L.	M. Aragaki	PPRI 4532	1981	Present study
	U.S.A.	*A. hypogaea*	M. K. Beute	PPRI 4521–4524	1991	Present study
	U.S.A.	Soil	D. T. Krigsvold	ATCC 32832	1975	Present study
	U.S.A.	*C. rhombifolia*	S. A. Alfieri	ATCC 46133	1981	Alfieri *et al.* (1982)
	U.S.A.	—	M. K. Beute	PPRI 4533	1991	Present study
	U.S.A.	*A. hypogaea*	G. J. Griffin	PPRI 4540	1991	Present study
C. ilicicola	Brazil, Bahia	*Eucalyptus* sp.	A. C. Alfenas	PPRI 4151	1990	Crous *et al.* (1993)
	Ireland	*Ilex aquifolium* L.	H. C. Hawley	IMI 76542	1912	Peerally (1974*a*)

isolate), containing 100 ml glucose-yeast extract broth (Zumpetta, 1976), and incubated stationary for 7 d in the dark at 25°. Mycelium was harvested by suction onto Whatman No. 1 filter paper and rinsed under vacuum with distilled water to remove any residual medium. The mycelium was placed in a 100 ml beaker in an ice bath, and macerated with a Virtis 60K homogenizer (Virtis Company Inc., Gardiner NY, U.S.A.) in 0·1 M-Na_2HPO_4–KH_2PO_4 lysing buffer (pH 7), and maintained overnight at 10°. The extract was centrifuged at 10000 *g* for 7 min at 4°, the supernatant decanted, and dialysed against de-ionized H_2O at 10°. The dialysed supernatant was freeze-dried, and stored under vacuum.

Freeze-dried proteins were resuspended in 0·5 ml lysing buffer, and the concentrations determined using the Bio-Rad protein assay kit (Bio-Rad, California, U.S.A.). For electrophoresis, protein extracts were mixed in equal amounts with sample buffer (2 ml 75% glycerol, 4 ml 10% SDS, 2·5 ml Tris-HCl pH 6·8, 1 ml 2-mercapto-ethanol, 0·2 ml 0·25% w/v bromophenol blue, made up to 10 ml with distilled H_2O), and placed at 100° for 3 min. Sixty μg ml^{-1} of each sample was layered onto a 12% PAGE gel. A rainbow protein molecular weight kit (Amersham International, Buckinghamshire, England) was used to provide standard reference proteins. Buffer-soluble proteins were separated (Laemmli, 1970) on a discontinuous sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) system buffered with 0·1 M Tris-HCl (pH 6·8–8·8). Gels were run on a Hoefer SE 6000 (Hoefer Scientific Instruments, Tokai, South Africa) vertical slab apparatus at a constant current of 10 mA gel^{-1} at 10° and stained with 10 g l^{-1} Coomassie brilliant blue.

RESULTS AND DISCUSSION

Two holomorphs are considered in this study. These are *Calonectria crotalariae* Bell & Sobers with its anamorph *Cylindrocladium crotalariae*, as well as *Calonectria ilicicola*, the reported teleomorph of *Cylindrocladium ilicicola*.

Total protein electrophoresis

In this study, eleven isolates of *Cylindrocladium crotalariae* were compared by means of their total protein electrophoresis banding patterns. Although these isolates were collected from diverse hosts and geographic locations, they were found to have similar banding patterns. These similarities included three distinct bands in the vicinity of 69 kDa, one at 46 kDa, and one between 30 and 21·5 kDa (Fig. 1).

Results also showed that total protein electrophoresis banding patterns of isolates of *Cylindrocladium crotalariae* were consistent with similarities in vesicle, conidium and conidiophore morphology in these isolates. Morphologically, these isolates were all characterized by having 3-septate conidia (45)–62–(90) × (4·5)–6–(7) μm, and sphaeropedunculate vesicles (Snell & Dick, 1957). Correlation of protein electrophoresis banding patterns with cultural and morphological observations is well-documented for *Cylindrocladium* (Alfenas *et al.*, 1991; El-Gholl *et al.*, 1992; Crous, Alfenas & Wingfield, 1993; Crous *et al.*, 1993; El-Gholl *et al.*, 1993) as well as for many other fungal genera such as *Candida* Berkhout (Shechter, Landau & Dabrowa, 1972), *Armillaria* (Fr.) Staude (Lin, Dumas & Hubbes, 1989) *Gremmeniella* Morelet (Petrini *et al.*, 1989),

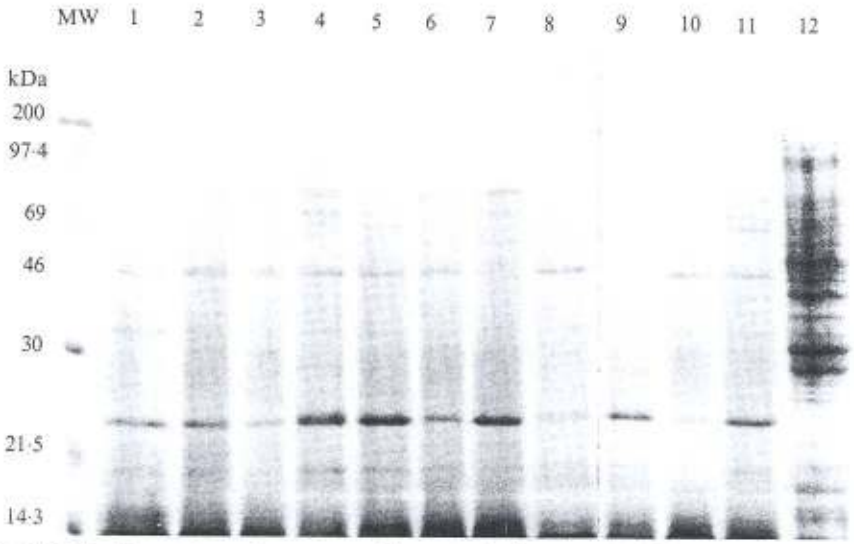

Fig. 1. Protein SDS–PAGE banding patterns. Lanes 1–11: *Calonectria ilicicola* strains ATCC 46133, CBS 190·50, PPRI 4522, PPRI 4529, PPRI 4524, ATCC 32832, PPRI 4213, PPRI 4540, PPRI 4527, PPRI 4533, PPRI 4541. Lane 12: *Calonectria pyrochroa* PPRI 4151. Molecular weights (MW) are given in kilodaltons, while data pertaining to the isolates are given in Table 1.

Table 2. Morphological characteristics and host range of *Calonectria ilicicola*

Conidia		Ascospores		Vesicles				
Dimensions (µm)	Septation	Dimensions (µm)	Septation	Width (µm)	Shape	Host	Accession no.	Reference
65–98 × 6	(1)–3	22–62 × 6–9	1–3	7–9	Globose	*Crotalariae anagyroides*	IMI 35028 (type)	Loos (1950)
49–69 × 4·5–6	(1)–3	38–57 × 5–7	1–(3)	5–8	Globose	*Solanum tuberosum*	CBS 190·50	Boedijn & Reitsma, (1950)
45–75 × 5–6	(1)–3	—	—	7–11	Sphaeropedunculate	*Solanum tuberosum*	CBS 190·50	Present study[a]
58–107 × 5–7	(1)–3	34–58 × 6–8	1–(3)	6–13	Globose to subglobose	*Arachis hypogaea*	IMI 264540	Bell & Sobers (1966)
59–107 × 5–7	(1)–3	29–59 × 6–8	1–3	6–13	Globose	—	—	Alfieri *et al.* (1982)
74 × 6	(1)–3	45 × 5	1–(3)	11	Globose	*Carica papaya*	ATCC 24024	Nishijima & Aragaki, (1973)
50–95 × 4–8	(1)–3	22–52 × 5–8	1–3	6–18	Globose	*Vaccinium corymbosum*	—	Milholland (1974)
60–103 × 5 × 7	(1)–3	31–62 × 6–8	1–3	6–13	Globose to subglobose	—	—	Peerally (1974*b*)
60–77 × 5·5–6·5	(1)–3	30–42 × 4·5–5	1–(3)	8–11	Sphaeropedunculate	*A. hypogaea*	ATCC 32832	Present study[a]
53–78 × 5–6	(1)–3	28–45 × 5–6	1–(3)	7–10	Sphaeropedunculate	*A. hypogaea*	PPRI 4523	Present study
56–85 × 5–6·5	(1)–3	—	1–(3)	7–9	Sphaeropedunculate	*A. hypogaea*	PPRI 4524	Present study
45–78 × 5–7	(1)–3	35–55 × 5–6	1–(3)	7–10	Sphaeropedunculate	*Cinnamomum kanahirai*	PPRI 4213	Present study
55–82 × 5–6	(1)–3	30–45 × 4·5–6	1–(3)	7–10	Sphaeropedunculate	*Cissus rhombifolia*	PPRI 4160	Present study
54–85 × 6–7	(1)–3	29–47 × 5–6	1–(3)	6–10	Sphaeropedunculate	*Euterpe edulis*	PPRI 4527	Present study
55–75 × 5–6	(1)–3	30–47 × 5–6	1–(3)	7–10	Sphaeropedunculate	*Indigofera hirsuta*	PPRI 4529	Present study
50–80 × 5–6	(1)–3	—	—	7–10	Sphaeropedunculate	*Rivinia humilis*	PPRI 4521	Present study
60–80 × 5–7	(1)–3	30–50 × 5–6	1–(3)	7–10	Sphaeropedunculate	*Acacia koa*	ATCC 24023	Present study
59–80 × 5–7	(1)–3	31–52 × 4·5–6	1–(3)	7–10	Sphaeropedunculate	*Medicago sativa*	PPRI 4532	Present study
61–83 × 5–7	(1)–3	30–55 × 5–6	1–(3)	7–10	Sphaeropedunculate	*Lens coccinea*	PPRI 4530	Present study
57–76 × 4·5–6	(1)–3	—	—	7–10	Sphaeropedunculate	—	PPRI 4533	Present study

[a] All determinations in this study made on carnation-leaf agar. Conidia and vesicles examined after 7 d at 25° under near-ultraviolet light. Perithecia examined before ascal dehiscence and ascospore discharge.

Leptostroma Fr. (Sieber-Canavesi, Petrini & Sieber, 1991) and *Pichia* Hansen (Kurtzman, 1992).

The type culture of *Calonectria ilicicola* (CBS 190·50) was morphologically indistinguishable from isolates of *Calonectria crotalariae*. Furthermore, it had a similar protein banding pattern to that of other isolates of *Calonectria crotalariae* (Fig. 1). This suggests that *Calonectria crotalariae* and *Calonectria ilicicola* are conspecific, and that *Calonectria ilicicola* is not the teleomorph of *Cylindrocladium ilicicola*, but rather of *Cylindrocladium crotalariae*. Banding patterns of isolates of *Cylindrocladium crotalariae* were, however, distinct from that of an isolate (PPRI 4151) morphologically similar to the dried type specimen of *Cylindrocladium ilicicola* (Fig. 1). Protein banding patterns as well as morphological characteristics (Tables 2, 3), therefore, support the contention that *Cylindrocladium crotalariae* is distinct from *Cylindrocladium ilicicola*.

Table 3. Morphological characteristics and host range of *Calonectria pyrochroa*

Conidia		Ascospores		Vesicles				
Dimensions (µm)	Septation	Dimensions (µm)	Septation	width (µm)	Shape	Host	Accession no.	Reference
37·5–68 × 4–5	(1)–3	—	—	5–11	Clavate	*Ilex aquifolium*	IMI 76542 (type)	Peerally (1974*a*)
49–60 × 3·5–4	3	55–70 × 5–6·5	3	6–10	Clavate	*Laurus nobilis*	IMI 299915	Brayford & Chapman (1987)
45–66 × 4–5	(1)–3	30–45 × 4·5–5	1–(3)	5–10	Clavate to spathulate	*Eucalyptus* sp.	PPRI 4151	Present study*

* All determinations in this study made on carnation-leaf agar. Conidia and vesciles examined after 7 d at 25° under near-ultraviolet light. Perithecia examined before ascal dehiscence and ascospore discharge.

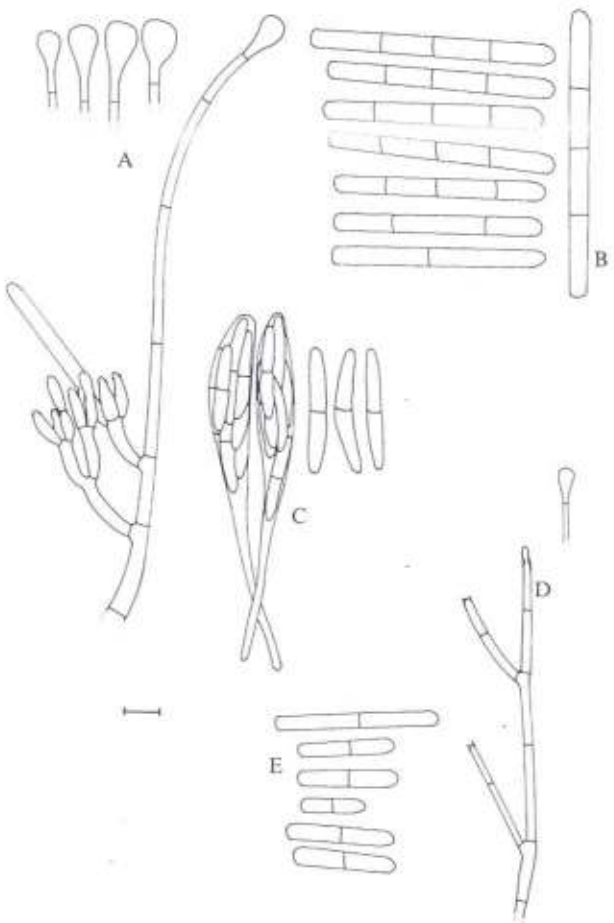

Fig. 2. *Calonectria ilicicola* and its anamorph *Cylindrocladium parasiticum*. Bar, 10 µm. A, Vesicles and macroconidiophore; B, macroconidia; C, asci and ascospores (redrawn from Boedijn & Reitsma, 1950); D, microconidiophore and microvesicle; E, microconidia (CBS 190·5 on CLA).

Cylindrocladium crotalariae

Petch (1917) described *Cercosporella theae* Petch as a new species from spots occurring on leaves of *Camellia sinensis* Kuntze. Loos (1950) collected and described the teleomorph of this fungus as *Calonectria theae* Loos. This author also collected a second *Calonectria* sp. from *Crotalaria anagyroides* H.B. & K., which could be distinguished from *Cercosporella theae* by perithecial colour, ascospore dimension, host range, and the presence of a terminal globose vesicle on the stipe of the *Cylindrocladium* anamorph. As Loos (1950) failed to provide a Latin diagnosis when he described the fungus from *Crotalaria anagyroides* as *Cercosporella theae* var. *crotalariae* Loos, the name is invalid. In this paper, Loos (1950) also commented on the similarity between this species and others placed in *Candelospora* Rea & Hawley.

Subsequently Bell & Sobers (1966) provided a new combination *Cylindrocladium crotalariae* (Loos) Bell & Sobers, citing *Candelospora theae* (Petch) Wakefield ex Gadd var. *crotalariae* Loos as the basionym. In the CMI description of *Cylindrocladium crotalariae* (Loos) Bell & Sobers, Peerally (1974*b*) regarded *Cercosporella theae* Petch to be the basionym of *Cylindrocladium crotalariae*. As there is no valid basionym for *Cylindrocladium crotalariae*, Rossman (1983) did not acknowledge the combination *Cylindrocladium crotalariae* (Loos) Bell & Sobers. This view is accepted in the present study, and a new name should therefore be provided for *Cylindrocladium crotalariae* (Loos) Bell & Sobers.

Cylindrocladium parasiticum Crous, Wingfield & Alfenas sp. nov. *Cercosporella theae* Petch var. *crotalariae* Loos, *Trans. Br. mycol. Soc.* **33**: 17 (1950)., *nom. inval.*

Candelospora theae (Petch) Wakefield ex Gadd var. *crotalariae* Loos, *Monographs of the Tea Production in Ceylon* No. 2, Tea Res. Inst. Ceylon, pp. 59–60 (1950), *nom. inval.*

Cylindrocladium crotalariae (Loos) Bell & Sobers, *Phytopathology* **56**: 1364 (1966)., *nom. illegit.*

Macroconidiophora septatum, hyalinum, terminans in vesicula sphaeropedunculata, (6)–8–(12) µm diam.; rami primarii non septati aut raro 1-septati, (16)–19–(25) × (4)–4·5–(5) µm; rami secundarii non septati aut raro 1-septati, (11)–14·5–(20) × (4)–4·5–(5) µm; rami tertiarii non septati, (9)–12–(14) × (4)–4·5–(5) µm; phialides doliiformes usque ad reniformes, hyalinae, non-septatae, (9)–12·5–(17) × 4–(4·5) µm. *Conidia* cylindrica, hyalina, (1)–3–septata, utrinque rotundata, (45)–62–(90) × (4·5)–6–(7) µm. Temperies ad crescendum necessaria: Minima temperies super 8°; maxima temperies infra 35°; optima temperies 25°. Chlamydospora complexa numerosa.

Microconidiophora septatum, hyalinum, terminans in vesicula sphaeropedunculata, (4)–5–(6) µm; rami primarii non septati (19)–21–(26) × 3·5–(4) µm; rami secundarii non septati (10)–12–(15) × (3)–3·5–(4) µm. Phialides, binae quaternaeque aggregatae, e terminali extremitate ullorum ramorum exoriri possunt; phialides cylindricae, hyalinae, non septatae, (10)–15–(19) × (3)–3·5–(4) µm. *Conidia* cylindrica, hyalina, 1-septata utrinque obtusa, (16)–30–(47) × 5–(6·5) µm.

U.S.A.: Georgia, *Arachidis hypogaeae*, D. K. Bell, 1982, IMI 264540, holotypus.

Macroconidiophores septate, hyaline, terminating in a sphaeropedunculate vesicle, (6)–8–(12) µm diam.; stipes (120)–175 –(240) µm long; primary branches non-septate or rarely 1-

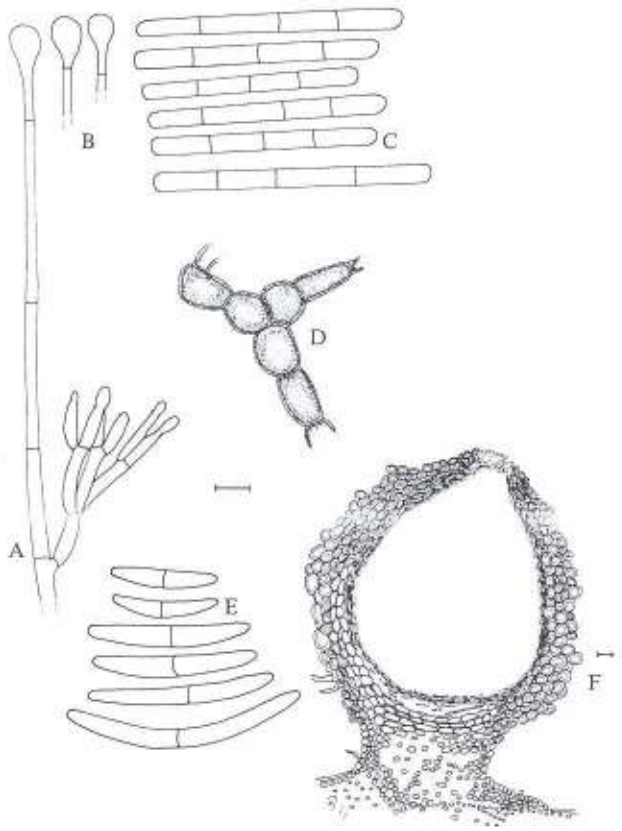

Fig. 3. *Calonectria ilicicola* and its anamorph *Cylindrocladium parasiticum*. A, Conidiophore; B, vesicles; C, conidia; D, chlamydospores on CLA (ATCC 32832); E, ascospores (bar, 10 μm); F, vertical section of a perithecium (ATCC 46133, bar, 20 μm).

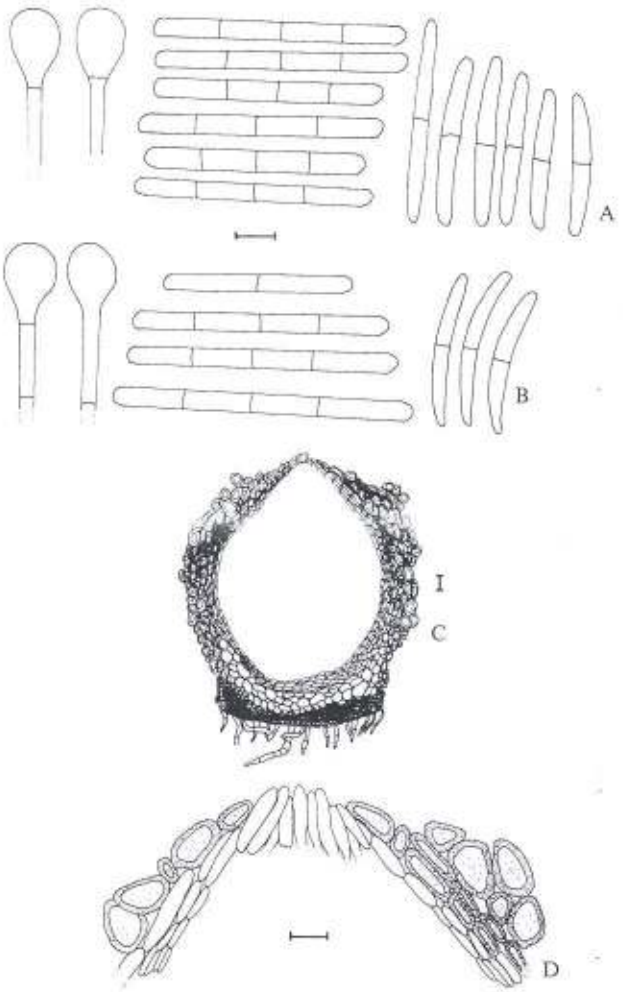

Fig. 4. *Calonectria ilicicola* and its anamorph *Cylindrocladium parasiticum*. A, Vesicles, conidia and ascospores (IMI 264540 on PSA); B, vesicles, conidia and ascospores (IMI 122262 on PDA) (bar, 10 μm); C, vertical section through a perithecium (IMI 122262) (bar, 20 μm); D, ostiolar region of a perithecium (IMI 122262) (bar, 10 μm).

septate, (16)–19–(25) × (4)–4·5–(5) μm; secondary branches non-septate or rarely 1-septate, (11)–14·5–(20) × (4)–4·5–(5) μm; tertiary branches non-septate, (9)–12–(14) × (4)–4·5–(5) μm. *Phialides* elongate, doliiform to reniform, hyaline, non-septate, (9)–12·5–(17) × (4)–4·5 μm. *Conidia* cylindrical, hyaline, (1)–3–septate, rounded at both ends, (45)–62–(90) × (4·5)–6–(7) μm. *Temperature requirements for growth*. Minimum temperature above 8°; maximum temperature below 35°; optimum temperature 25°. Colony colour on MEA after 7 d at 25° salmon-buff. *Chlamydospore* complexity extensive. *Microconidiophores* septate, hyaline, terminating in a sphaeropedunculate vesicle, (4)–5–(6) μm diam.; primary branches non-septate, (19)–21–(26) × 3·5–(4) μm; secondary branches non-septate, (10)–12–(15) × (3)–3·5–(4) μm. *Phialides* arise from the ends of branches, in groups of 2–4; phialides cylindrical, hyaline, non-septate, (10)–15–(19) × (3)–3·5–(4) μm. Collarettes present. *Conidia* cylindrical, hyaline, 1-septate with obtuse ends, (16)–30–(47) × 5–(6·5) μm.

Calonectria ilicicola *and* Calonectria crotalariae

The name *Calonectria ilicicola* Boedijn & Reitsma (1950) was established for an isolate collected from potatoes in Java. Asci are described as club-shaped and 100–140 × 16–18 μm, with ascospores 1–(3)-septate 38–57 × 5–7 μm (Boedijn & Reitsma, 1950). These measurements are similar to those given by Bell & Sobers (1966) for *Calonectria crotalariae*, being 95–138 × 13–19 μm with 1–(3)-septate ascospores 34–58 × 6·3–7·8 μm (Bell & Sobers, 1966).

In this study, we induced the type culture of *Calonectria ilicicola* (CBS 190·50) to sporulate on CLA (Fig. 2). The resulting anamorph formed sphaeropedunculate vesicles identical to those accepted for *Cylindrocladium parasiticum* (Bell & Sobers, 1966) (Figs 2, 4). Conidial and vesicle morphology was also similar to that of other isolates of *Cylindrocladium parasiticum* (Table 2), and distinct from that of the type of *Cylindrocladium ilicicola* (Table 3). Thus *Calonectria ilicicola* is not the teleomorph of *Cylindrocladium ilicicola*.

Furthermore, morphological similarities between the type of *Calonectria ilicicola* Boedijn & Reitsma (1950) (Fig. 2) and isolates of *Calonectria crotalariae* Bell & Sobers (1966) (Fig. 3), as well as their protein banding patterns (Fig. 1), indicate that these two names have been given to the same fungus. According to the International Code of Botanical Nomenclature (Art. 11·3), the name *Calonectria ilicicola* Boedijn & Reitsma (1950), which was described 16 yr before *Calonectria crotalariae* Bell & Sobers (1966) (Fig. 4A), has priority. These similarities therefore justify the following synonymy.

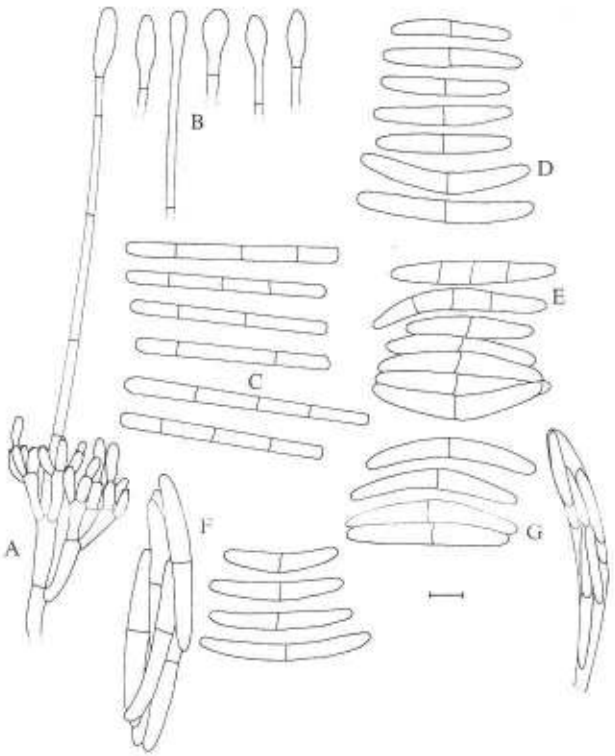

Fig. 5. *Calonectria pyrochroa* and its anamorph *Cylindrocladium ilicicola*. A, Conidiophore; B, vesicles; C, conidia; D, ascospores (PPRI 4151); E, ascospores (PC, *Calonectria pyrochroa*, type); F, ascospores (FH, *Nectria abnormis*, isotype); G, ascospores and broken ascus (FH, *Ophionectria puiggarii*, type) (bar, 10 µm).

Calonectria ilicicola Boedijn & Reitsma, *Reinwardtia* **1**: 58 (1950).

Calonectria theae Loos var. *crotalariae* Loos, *Trans. Br. mycol. Soc.* **33**: 18 (1950).

Calonectria crotalariae (Loos) Bell & Sobers, *Phytopathology* **56**: 1364 (1966).

Anamorph: *Cylindrocladium parasiticum* Crous, Wingfield & Alfenas sp. nov.

Isolates of this fungus are homothallic, producing orange to red perithecia, 300–500 µm high, 280–400 µm wide, which have a fairly warty outer layer, and a periphysate ostiolar region. Perithecia turn a blood colour in 3% KOH. Asci are clavate, long-stalked, eight-spored, 90–140 × 12–19 µm. Ascospores are fusoid to falcate, 1-septate, slightly or not constricted at the septum, (30)–45–(65) × (4·5)–5·5–(6·5) µm. Ascospores are primarily 1-septate before ascal dehiscence, but can eventually develop up to three septa.

Cylindrocladium ilicicola

In the first comprehensive treatment of *Cylindrocladium*, Boedijn & Reitsma (1950) included seven species. Amongst these was *Cylindrocladium ilicicola* (Hawley) Boedijn & Reitsma which was proposed as a new combination for *Candelospora ilicicola* Hawley apud Rea & Hawley. An examination of the type specimen of *Cylindrocladium ilicicola* (IMI 76542) showed condidia to be (1)–3-septate, 37·5–68 × 4–5 µm, with clavate to spathulate (widest below the middle) vesicles (Table 3). These are distinct from those of *Cylindrocladium parasiticum* (Table 2).

The teleomorph of Cylindrocladium ilicicola

In showing that the name *Calonectria ilicicola* refers to the teleomorph of *Cylindrocladium parasiticum*, a problem arises regarding the correct name for the teleomorph of *Cylindrocladium ilicicola*. Rossman (1979) re-examined the type specimens of *Calonectria pyrochroa* (Desm.) Sacc. and *Calonectria daldiniana* de Not., and subsequently synonymized the latter species with *Calonectria pyrochroa*. Furthermore, *Calonectria pyrochroa* was proposed as teleomorph of *Cylindrocladium ilicicola*, with several other species names being listed as synonyms (Rossman, 1983).

The teleomorph associated with *Cylindrocladium ilicicola* (PPRI 4151) (Fig. 5), was found to be similar to that on the type of *Calonectria pyrochroa* (Fig. 5) (lodged in PC, France). Ascospores were 1–(3)-septate in young perithecia, becoming primarily 3-septate in older perithecia, being (28)–45–(66) × (4·5)–5·5–(6·5) µm. The name *Calonectria pyrochroa* therefore represents the teleomorph of *Cylindrocladium ilicicola* (Rossman, 1979, 1983). However, all the synonyms of *Calonectria pyrochroa* as listed by Rossman (1983) are not recognized. *Calonectria* spp. that have been described with *Cylindrocladium* anamorphs will be treated elsewhere. Those described in the absence of anamorphs, are treated below. These include *Calonectria daldiniana*, *Ophionectria puiggarii* Speg., *Nectria abnormis* P. Henn., *Nectria leguminum* Rehm. and *Calonectria indusiata* Seaver.

Rossman (1979) stated that *Calonectria daldiniana* (type in RO, Italy) has ascospores that are 46–61 × 5–6 µm, 3-septate, 'hyaline to slightly yellow with age, generally loose in the ascocarp'. The fact that only older ascocarps were present, explains why only 3-septate ascospores were observed. *Calonectria daldiniana* is, therefore, a synonym of *Calonectria pyrochroa*.

The type material of *Nectria abnormis* (lodged at Farlow, FH) (Fig. 5) was examined and found to have ascospores which were 28–53 × 3·5–5 µm in size, larger than the published description 30–40 × 7–8·5 µm (Hennings, 1897). Part of the type lodged at the Botanischer Garten und Botanisches Museum, Berlin (B) was destroyed during the war, and the material lodged at Farlow (presumably isotype) is, therefore, the only material left representing this collection. Ascospore dimensions and septation support the conclusion that this collection is a synonym of *Calonectria pyrochroa*.

A collection of *Ophionectria puiggarii* lodged at FH (ex type) was examined, and found to have 1-septate ascospores, 38–53 × 4–6 µm in size (Fig. 5). This confirms that it is a synonym of *Calonectria pyrochroa* (Fig. 5).

The type of *Nectria leguminum* (Fig. 6) has been studied by numerous workers. Although the teleomorph is similar to *Calonectria pyrochroa* as suggested by Rossman (1983), an examination of the anamorph material found conidia to be 3–6-septate, 45–61 × 5–6 µm. The anamorph, therefore, is distinct from *Cylindrocladium ilicicola*, which suggests that the synonymy proposed by Rossman (1983) is not correct. It is

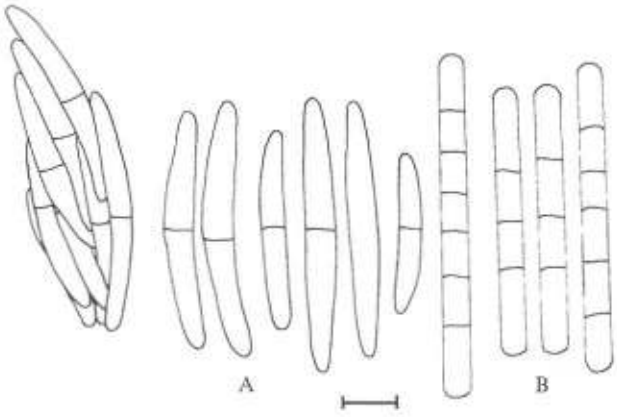

Fig. 6. *Nectria leguminum* (S, type) (bar, 10 μm). A. Ascospores; B. conidia.

possible that the name *N. leguminum* is applied to the teleomorph of an as yet undescribed *Cylindrocladium* sp.

Calonectria indusiata has been listed as a synonym of *Calonectria pyrochroa* (Rossman, 1979, 1983). Based on the large asci and ascospores, it is unlikely that this collection represents a synonym of *Calonectria pyrochroa*. *Calonectria indusiata* will therefore be discussed in a separate paper dealing with *Cylindrocladium* spp. and their *Calonectria* teleomorphs. The following names are recognized as synonyms of *Calonectria pyrochroa*:

Calonectria pyrochroa (Desm.) Sacc., *Michelia* **1**: 308 (1878).

Nectria pyrochroa Desm., *Pl. Crypt. France* ed. **2**: 372 (1856); *Bull. Soc. Bot. Fr.* **4**: 998 (1857).

Calonectria daldiniana De Not., *Comment. Soc. Crittogam. Ital.* **2**: 477 (1867).

Ophionectria puiggarii Speg., *Bol. Acad. Nac. Ci.* **11**: 532 (1889).

Nectria abnormis P. Hennings, *Hedwigia* **36**: 219 (1897).

Anamorph: *Cylindrocladium ilicicola* (Hawley) Boedijn & Reitsma, *Reinwardtia* **1**: 57 (1950).

Based on morphological and electrophoretic comparisons of type specimens and cultures, this study has clearly shown that *Cylindrocladium ilicicola* is a species distinct from *Cylindrocladium parasiticum*. These results also indicated that *Calonectria pyrochroa* is the correct teleomorph of *Cylindrocladium ilicicola*. Furthermore, *Calonectria ilicicola* was shown to be the teleomorph of *Cylindrocladium parasiticum*.

Results obtained in the present study support the observations of El-Gholl *et al.* (1992), that differences in soluble protein electrophoresis profiles of *Cylindrocladium* isolates compare favourably with differences in vesicle, conidium and conidiophore morphology. It can be concluded, therefore, that as found for other genera (Lin *et al.*, 1989; Petrini *et al.*, 1989; Sieber-Canavesi *et al.*, 1991; Kurtzman, 1992), protein banding patterns also support current species concepts in *Cylindrocladium*.

Subsequent to the incorrect assignment of *Calonectria ilicicola* to *Cylindrocladium ilicicola* by Boedijn & Reitsma (1950), there has been considerable confusion regarding the species concepts for *Cylindrocladium ilicicola* and *Cylindrocladium crotalariae*. We have found in this study that *Calonectria crotalariae* is a synonym of *Calonectria ilicicola*. The synonymy of *Calonectria crotalariae* as well as the invalid description of *Cylindrocladium crotalariae*, has left us with no option but to provide the new epithet, *Cylindrocladium parasiticum* for the important pathogen previously known as *Cylindrocladium crotalariae*. Although this might be considered unfortunate by some plant pathologists, it will avoid confusion in the future. Moreover, the recent description of a *Cylindrocladium* species closely resembling *Cylindrocladium parasiticum* (Crous *et al.*, 1993) has also demanded a more clearly defined species concept for the latter fungus.

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