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Delineation of *Cylindrocladium* species with 1-3-septate conidia and clavate vesicles based on morphology and rDNA RFLPs

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Unidentified isolates of *Cylindrocladium* with 1–3-septate conidia and clavate vesicles were compared with *C. theae*, *C. colhounii* var. *colhounii*, *C. colhounii* var. *macroconidiale*, *C. gracile*, *C. pteridis* and *Calonectria gracilis*. Ribosomal DNA (rDNA) of these isolates were digested with the restriction enzymes *Eco*R I, *Hin*d III and *Xho* I, and Southern analysis performed with the 6·3-kb rDNA repeat unit of *Neurospora crassa* as DNA probe. Based on differences in general morphology, supported by their rDNA restriction fragment length polymorphisms, isolates of all species and varieties could be distinguished. Furthermore, the *Cylindrocladium* anamorph of *Calonectria gracilis* was shown to be distinct from *Cylindrocladium pteridis* and *C. gracile*. The name *Cylindrocladium pseudogracile* sp. nov. is, therefore, proposed for the undescribed anamorph of *Calonectria gracilis*.

Several species with 1–3-septate conidia and clavate vesicles were recently treated in a monograph of *Cylindrocladium* Morgan by Crous & Wingfield (1994). Within this complex, however, several distinct morphological groups can be recognized. *Cylindrocladium avesiculatum* D. L. Gill, Alfieri & Sobers is easily separated by its clavate to avesiculate vesicles and thick-walled stipe extensions, *C. colhounii* Peerally var. *colhounii* and var. *macroconidiale* Crous, M. J. Wingf. & Alfenas are distinguished by producing 3-septate conidia, being homothallic, and forming bright yellow perithecia with asci containing four 3-septate ascospores. *C. theae* (Petch) Subram. is also homothallic with 3-septate conidia, but forms red-brown perithecia with asci containing eight ascospores (Crous & Wingfield, 1994).

The remaining and more difficult group consists of a complex of three species. Crous & Wingfield (1994) recognized *C. clavatum* Hodges & L. C. May, *C. gracile* (Bugnic.) Boesew. and *C. pteridis* F. A. Wolf. In a subsequent study comparing the nuclear DNA polymorphisms of these species (Crous, Korf & Van Zyl, 1995), *C. clavatum* was reduced to synonymy under *C. gracile*, while their respective teleomorphs, *Calonectria clavata* Alfieri, El-Gholl & E. L. Barnard and *Calonectria gracilis* Crous, M. J. Wingf. & Alfenas were shown to be distinct biological species. Uncertainty remained, however, concerning the status of *C. gracilis*, as its banding pattern appeared similar to the only isolate of *Cylindrocladium pteridis* included in that study. Recently, another isolate with smaller conidia and slightly different vesicles than that ascribed to *C. pteridis* by Crous & Wingfield (1994) was isolated from soil. The aim of the present study, therefore, was to compare this isolate with isolates of *Calonectria gracilis* and *Cylindrocladium pteridis*, and to delineate the biological species present in this complex.

MATERIALS AND METHODS

***Isolates of* Cylindrocladium *and* Calonectria *spp. examined*:** *Cylindrocladium gracile* (= *C. clavatum*), Brazil, Minas Gerais, Horto Conceicao, near Itabira, *Pinus caribaea* roots, Hodges & May, 16 Mar. 1971, ATCC 22833; *Calonectria gracilis*, Brazil, Para, Belém, *Manilkara zapota*, F. Albuquerque, PPRI 4176, IMI 354519, AR 2677; *Cylindrocladium pteridis*, Brazil, unknown host, J. C. Dianese, (Viçosa, Brazil, No. UFV 43), PPRI 4157; *C. pteridis*, needles of *Pinus* sp., T. L. Krügner, (Viçosa, Brazil, No. 10), PPRI 4177, IMI 354524; *C. pteridis* of *Pinus caribaea*, T. L. Krügner, (Viçosa, Brazil, No. UFV 37), PPRI 4178; *C. pteridis*, *Eucalyptus grandis* leaves, A. C. Alfenas, (Brazil, Viçosa, No. UFV 105), PPRI 4180, IMI 354530; *C. pteridis*, U.S.A., *Arachnoides adiantiformis*, F. Schjickedanz, 1974, ATCC 34395; *C. pteridis*, Florida, *Rumohra adiantiformis* N. E. El-Gholl, (Brazil, Viçosa, No. UFV 50), PPRI 4179; *Cylindrocladium* sp., South Africa, Natal Province, soil, P. W. Crous, 18 Nov. 1993, STE-U 675; *C. theae*, U.S.A., Florida, *Rhododendron* sp., N. E. El-Gholl, (Brazil, Viçosa, No. UFV 16), PPRI 4188; *C. colhounii* var. *colhounii*, U.S.A., unknown host, A. Alfenas, (Viçosa, Brazil, UFV 22A), PPRI 4183; *C. colhounii* var. *macroconidiale*, E. Transvaal, Sabie, Frankfort, *E. grandis* cuttings, P. W. Crous, Mar. 1990, PPRI 4000.

Morphology

Single-conidial isolates were cultured on 2% malt extract agar (MEA) (Oxoid), plated onto carnation-leaf agar (CLA) (Crous, Phillips & Wingfield, 1992), incubated at 25 °C under nuv light, and examined after 7 d. Only material occurring on carnation leaves was examined. Mounts were prepared in lactophenol, and measurements made at × 1000 magnification.

Total DNA isolation

Single-conidial isolates were grown on MEA, and plugs of 7-d-old cultures transferred into 500 ml Erlenmeyer flasks containing 100 ml glucose-yeast extract broth (Biolab)

(Zumpetta, 1976). Cultures were incubated for 7–14 d in the dark at 30° until sufficient growth occurred. Mycelia were harvested by filtration (Whatman No. 1 filter paper), and freeze dried for 2 d. Total DNA was isolated according to Crous *et al.* (1993*b*), and subsequently redissolved in 200 µl TE buffer (pH 8·0) (Sambrook, Fritsch & Maniatis, 1989).

Restriction enzyme analysis and Southern hybridization

Total DNA (*ca* 5 µg) of each isolate was subjected to restriction digestion with *Eco*R I, *Hind* III and *Xho* I for 3 h respectively, according to the recommendation of the suppliers (Boehringer Mannheim). The DNA was separated on horizontal 1% agarose gels and transferred to Hybond-N nylon membranes (Amersham) according to standard procedures (Sambrook, *et al.*, 1989). The *Neurospora crassa* ribosomal DNA (rDNA) was purified from plasmid pMF2 (Russell *et al.*, 1984) as a 6·3-kb *Pst* I fragment and labelled with [α-^{32}P]dATP (Amersham) as described by Feinberg & Vogelstein (1983). The Southern hybridizations and stringency washes were performed according to the method of Sambrook *et al.* (1989).

RESULTS

Morphology

Calonectria gracilis (PPRI 4176) formed a *Cylindrocladium* anamorph with 1-septate, 40–65 × 4–5 µm conidia, which were longer than those of *C. gracile* (ATCC 22833) at 38–52 × 4–6 µm. Among the species with 1–3-septate conidia, *C. colhounii* var. *colhounii* (PPRI 4183) had the shortest conidia at 45–65 × 4·5–5 µm, followed by *C. theae* (PPRI 4188) at 65–90 × 5–6 µm and *C. colhounii* var. *macroconidiale* (*PPRI* 4000) *at* 86–112 × 5–8 µm. Brazilian isolates of *C. pteridis* (PPRI 4157, 4177–4180) had conidia within the range of 60–120 × 5–6 µm, while the Florida isolate (ATCC 34395) had slightly larger conidia at 65–130 × 4–7 µm. The *Cylindrocladium* species from South Africa (STE-U 675) had conidia similar in size to *C. theae*, *C. pteridis* and *Calonectria gracilis*, being 50–75(–100) × 4–5 µm, but were 1(–3)-septate, as found in *Cylindrocladium pteridis*. However, vesicles of STE-U 675 varied from narrowly ellipsoidal to clavate, and were not therefore strictly typical of *C. pteridis*.

Table 1. Nuclear DNA polymorphisms observed for species of *Cylindrocladium*

Species	Accession no.	DNA fragment sizes* *Eco*R I/ *Xho* I	*Hind* III	*Eco*R I/ *Hind* III
C. pteridis	STE-U 675	3000	6000	4900
		2600		2450
				800
C. pteridis	PPRI 4157	3000	6000	4900
		2600		2450
				800
C. gracile	ATCC 22833	3000	13000	3500
		2600		1600
C. theae	PPRI 4188	3000	13000	4900
		2600		3400
C. colhounii var. *macroconidiale*	PPRI 4000	4300	13000	4900
		3000		3400
C. colhounii var. *colhounii*	PPRI 4183	4300	6000	4900
		3000		3400

* Nuclear rDNA fragments highlighted by the 6·3-kb ribosomal repeat unit of *Neurospora crassa* ^{32}P-labelled probe.

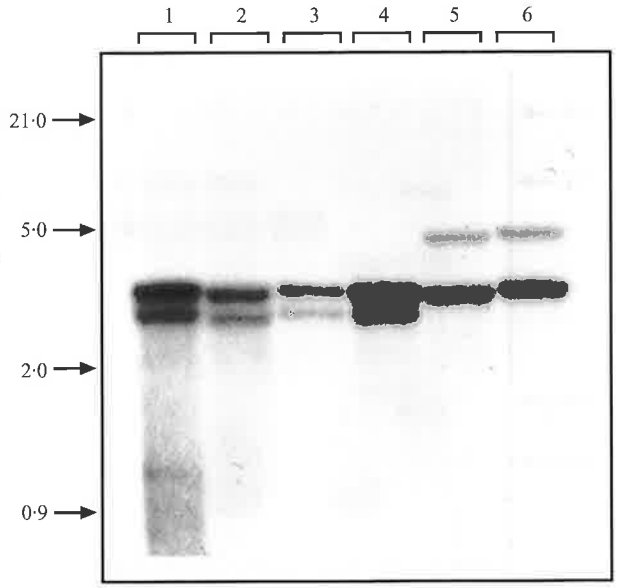

Fig. 1. Ribosomal DNA hybridization patterns for *Eco*R I and *Xho* I digested nDNA of strains of *Cylindrocladium* and *Calonectria* species. Lanes 1, 2. *Cylindrocladium pteridis* STE-U 675, PPRI 4157. Lane 3. *C. gracile* ATCC 22833. Lane 4. *C. theae* PPRI 4188. Lane 5. *C. colhounii* var. *macroconidiale*. Lane 6. *C. colhounii* var. *colhounii* PPRI 4183. Size markers are lambda DNA digested with *Eco*R I and *Hind* III.

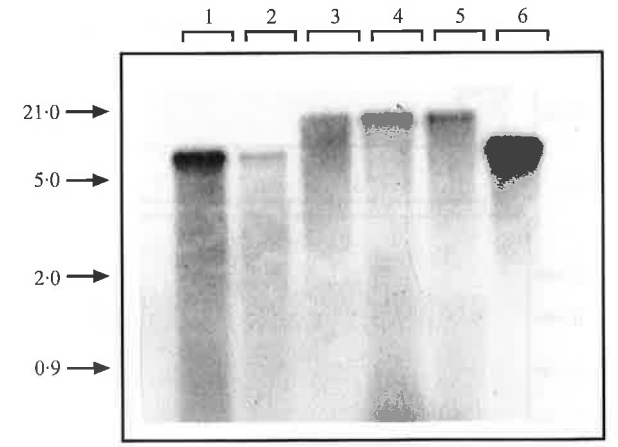

Fig. 2. Ribosomal DNA hybridization patterns for *Hind* III digested nDNA of strains of *Cylindrocladium* and *Calonectria* species. Details as in Fig. 1.

Restriction enzyme analysis and Southern hybridization

Based on the rDNA restriction fragment length polymorphism (RFLP) profiles obtained with the restriction enzyme *Eco*R I, *Hind* III and *Xho* I, the South African isolate STE-U 675 fell into the variation accepted for *C. pteridis* (Table 1). Using the restriction enzymes *Eco*R I and *Xho* I (Fig. 1), isolate STE-U

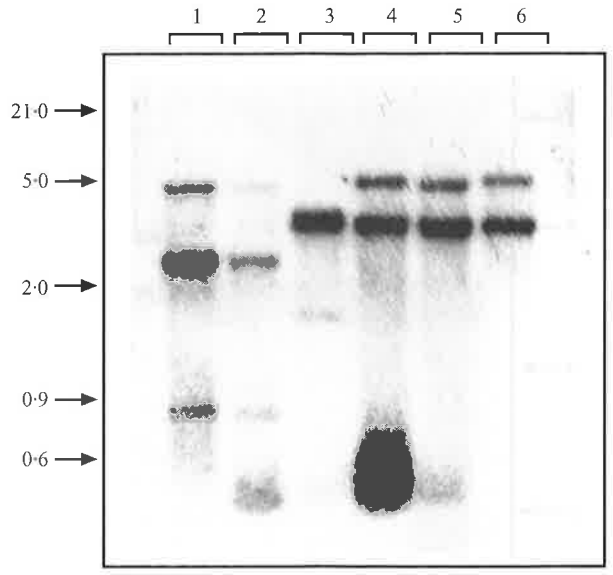

Fig. 3. Ribosomal DNA hybridization patterns for *Eco*R I and *Hind* III digested nDNA of strains of *Cylindrocladium* and *Calonectria* species. Details as in Fig. 1.

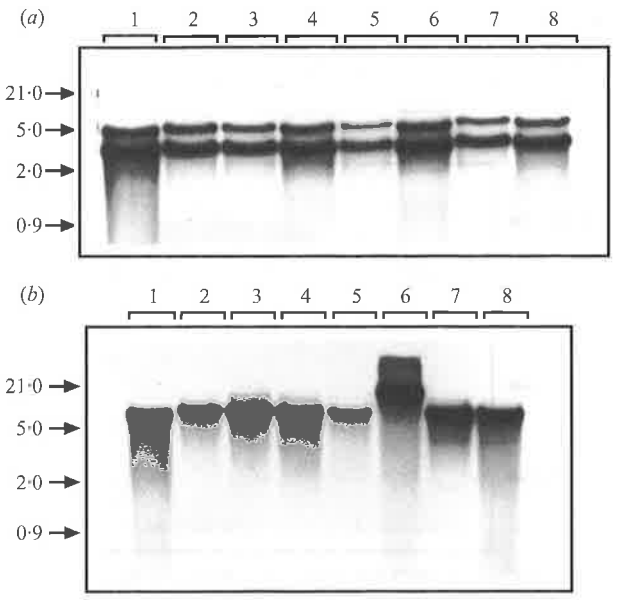

Fig. 4. Ribosomal DNA hybridization patterns for (a) *Eco*R I and (b) *Xho* I digested nDNA of strains of *Cylindrocladium pteridis* and *Calonectria gracilis*. Lanes 1–5, 7, 8. *C. pteridis*, STE-U 675, PPRI 4177, ATCC 34395, PPRI 4178, PPRI 4180, PPRI 4179 and PPRI 4157. Lane 6. *C. gracilis*, PPRI 4176. Size markers are lambda DNA digested with *Eco*R I and *Hind* III.

675 could not be distinguished from *C. pteridis* (PPRI 4157), *C. gracile* (ATCC 22833) or *C. theae* (PPRI 4188), but proved distinct from the two varieties of *C. colhounii*. When digested with the restriction enzyme *Hind* III, STE-U 675 and PPRI 4157 again proved similar (Fig. 2, Table 1). Although this enzyme distinguished the two varieties of *C. colhounii*, it could not distinguish *C. gracile* from *C. theae*. With the restriction enzymes *Eco*R I & *Hind* III (Fig. 3), however, *C. gracile* could be distinguished from *C. theae*. The three restriction enzymes used in the present study proved sufficient to distinguish the species treated, and to allocate STE-U 675 to *C. pteridis*. When DNA was digested with additional restriction enzymes such as *Sal* I, *Xba* I, *Stu* I, *Man*H I, *Bgl* II and *Pvu* II, only the latter restriction enzyme showed a difference in the position of the two bands occurring in STE-U 675 and PPRI 4157. Banding patterns generated by the restriction enzyme *Xho* I (Fig. 4) showed the type strain of *Calonectria gracilis* (PPRI 4176) to be distinct from strains of *Cylindrocladium pteridis* collected from Brazil, U.S.A. and South Africa in having a band larger than 20000 bp.

DISCUSSION

In a previous study of *Cylindrocladium* species with 1-septate conidia and clavate vesicles four species, namely *C. clavatum*, *C. pteridis*, *C. gracile* and *C. hawksworthii* Peerally, were treated (Crous *et al.*, 1995). Based on general morphology, *C. hawksworthii* could easily be distinguished from this group in having curved, 1-septate conidia and ellipsoidal to clavate vesicles. Results obtained using rDNA RFLPs firstly confirmed that the teleomorph *Calonectria clavata* was not conspecific with its purported anamorph, *Cylindrocladium clavatum*, and a new epithet *C. flexuosum* Crous was subsequently proposed. Furthermore, *C. clavatum* was shown to be synonymous with *C. gracile*, while once again, conspecifity could not be shown between *C. gracile* and its purported teleomorph *Calonectria gracilis*. The rDNA restriction patterns of *Eco*R I and *Hind* III showed only minor differences between *C. gracilis* and the strain of *Cylindrocladium pteridis* studied. It was only with a third enzyme, *Xho* I, that differences were detected between *Calonectria gracilis* and *Cylindrocladium pteridis*.

Conidia of *Calonectria gracilis* are 1-septate and 40–65 × 4–5 μm, while those of *C. pteridis* are 1(–3)-septate and 60–130 × 4–7 μm. In a previous study characterizing *Cylindrocladium gracile*, Crous *et al.* (1995) remained uncertain as to what variation was acceptable within *C. pteridis*, and accepted conidia to be 62–121 × 5–6 μm. Sobers (1968) stated that conidia of the latter are primarily 1-septate and 61–118 × 5–7 μm. Several isolates of *C. pteridis* have been studied that fail to develop 3-septate conidia. Although Sobers (1968) stated that microconidia of *C. pteridis* were curved, Crous & Wingfield (1994) reported that they could also be straight in some strains. Several strains seemed unable to produce a microconidial state, thereby lessening its value as a taxonomic feature in *C. pteridis*.

In *Cylindrocladium*, species identification relies on several features, of which conidial septation, dimensions, and vesicle shape are regarded to be of primary importance. Of those species with 1-septate conidia and more or less ovoid vesicles, small differences in vesicle morphology separate species such as *C. candelabrum* Viégas (obpyriform vesicles), *C. scoparium* Morgan (pyriform vesicles), *C. ovatum* El-Gholl, Alfenas, Crous & T. S. Schub. (ovoid vesicles) and *C. floridanum* Sobers & C. P. Seym. (sphaeropeduculate vesicles). However, as more data have become available, the separation of these species is further supported by differences in their protein (Crous, Alfenas & Wingfield, 1993*a*; El-Gholl *et al.*, 1993) and DNA banding patterns, as well as distinct teleomorphs (Victor *et al.*, 1996). Crous *et al.* (1993*b*) stated

that DNA RFLPs could distinguish variation between and among species, therefore enhancing attempts to identify isolates of *Cylindrocladium*. Using rDNA RFLPs, Lodolo, Van Zyl & Rabie (1992) and Crous *et al.* (1995) showed that this technique supported morphological differences in *Fusarium* and *Cylindrocladium*, respectively. Magee, D'Souza & Magee (1987) used rDNA RFLPs to distinguish several species of *Candida*, while Vilgalys & Hester (1990) successfully employed this technique to distinguish species of *Cryptococcus*. Furthermore, intra- and interspecific variation could also be indicated in genera such a *Verticillium*, *Tapesia* and *Fusarium* (Cader & Barbara, 1991; Nicholson Razanoor & Hollins, 1993; Appel & Gordon, 1995). Using the same probe as in the present study, Kohn *et al.* (1988) combined this technique with morphological and epidemiological criteria to distinguish species of *Sclerotinia*. Hausner *et al.* (1993) reported RFLPs to be ideal for studying relatedness among both distantly and closely related species. The findings of the present study thus concur with that of others, namely that RFLP patterns support the recognition of individual species, as well as strains within a species.

The results we obtained in the present study showed the RFLP profiles of STE-U 675 with the restriction enzymes *Eco*R I, *Hin*d III and *Xho* I, to be similar to that of *C. pteridis* (Table 1), but distinct from all other species studied. Isolate STE-U 675 was initially accepted as distinct from *C. pteridis* based on its smaller conidia, at 50–75 × 4–5 µm, as well as narrowly ellipsoidal vesicles with acutely rounded apices. In older cultures, STE-U 675 produced a few conidia up to 100 µm long, thus suggesting that although conidia were usually 50–75 µm long, conidia more comparable with typical isolates of *C. pteridis* could be formed. These findings suggest that in contrast to the earlier concept accepted for *C. pteridis* as having clavate vesicles and conidia above 60 µm in length (Wolf, 1926; Sherbakof, 1928; Boedijn & Reitsma, 1950; Sobers, 1968; Sobers & Alfieri, 1972; Peerally, 1991; Crous & Wingfield, 1994), vesicles can be narrowly ellipsoidal (widest in the middle or upper third of the vesicle with acutely rounded apices) or clavate, and conidia can be 1(–3)-septate and 50–130 × 4–7 µm.

The different banding pattern generated by the restriction enzyme *Xho* I (Fig. 4) for *Calonectria gracilis* (PPRI 4176) (presence of a band larger than 20000 bp), and strains of *Cylindrocladium pteridis* collected from Brazil, U.S.A. and South Africa, suggested the former species to be distinct. Conidia of *Calonectria gracilis* are 1-septate and 40–65 × 4–5 µm, overlapping to some degree with those of *C. pteridis*, which are 1(–3)-septate and 50–130 × 4–7 µm. However, identification based solely on the anamorph states can be problematic, especially as this study has shown that isolates at the lower end of the range of *C. pteridis*, such as STE-U 675, have conidia falling between what is typically associated with both *C. pteridis* and *Calonectria gracilis*. In the latter case, the teleomorph (when produced) will greatly assist in identification, as ascospores of the homothallic *C. gracilis* are 1-septate, (27–)30(–50) × (4–)5(–6) µm, while those of the heterothallic *Calonectria pteridis* Crous *et al.* are 1(–3)-septate, (30–)52(–75) × (4–)6(–7) µm (Crous, Wingfield & Alfenas, 1993*c*). Based on these differences between *C. pteridis* and *C. gracilis*, an epithet is provided for the *Cylindrocladium* anamorph of *Calonectria gracilis*, which is considered to be a species intermediate between *Cylindrocladium gracile* and *C. pteridis*.

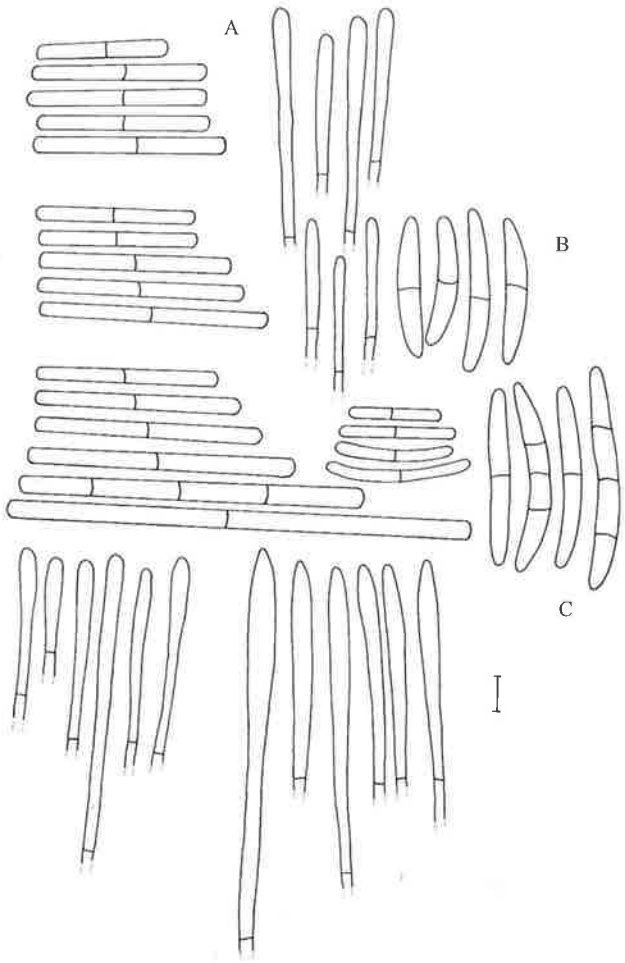

Fig. 5. Conidia, vesicles and ascospores of *Cylindrocladium* and *Calonectria* spp. A. One-septate conidia and clavate vesicles of *C. gracile* (ATCC 22833). B. One-septate conidia and clavate vesicles of *C. pseudogracile*, and ascospores of its teleomorph *Calonectria gracilis*. C. Range of 1–3-septate macroconidia (STE-U 675, PPRI 4157), microconidia, 1–3-septate ascospores (PREM 51033), and clavate to narrowly ellipsoidal vesicles (PPRI 4157, STE-U 675) of *C. pteridis* (bar, 10 µm).

Cylindrocladium pseudogracile Crous, sp. nov. (Fig. 5B)

Teleomorph: *Calonectria gracilis* Crous, M. J. Wingf. & Alfenas, *Mycotaxon* **46**: 224 (1993).

Etym.: morphologically intermediate between *C. gracile* and *C. pteridis*.

Macroconidiophora. *Filum* septatum, hyalinum, (160–)220(–350) µm, in vesiculam clavatam (2–)4(–5) µm diam. terminans. Rami primarii non septati vel raro 1-septati, (14–)18(–25) × (3–)4(–5) µm; rami secundarii non-septati, (12–)15(–16) × (3–)4(–5) µm. *Phialides* elongatae, doliiformes ad reniformes, hyalinae, non-septatae, (10–)13(–15) × (3–)4(–5) µm. *Conidia* cylindrical, hyalina, 1-septata, apicibus obtusis, (40–)56(–65) × (4–)5 µm. *Microconidiophora* ignota.

Macroconidiophores. *Stipe* septate, hyaline, terminating in a narrowly clavate vesicle, (2–)4(–5) µm diam.; stipes (160–)220(–350) µm long. *Conidiophore branches*: primary

branches non-septate or rarely 1-septate, (14–)18(–25) × (3–)4(–5) µm; secondary branches non-septate, (12–)15(–16) × (3–)4(–5) µm. *Phialides* doliiform to reniform, hyaline, non-septate, (10–)13(–15) × (3–)4(–5) µm. *Conidia* cylindrical, hyaline, 1-septate, rounded at both ends, (40–)56(–65) × (4–)5 µm. *Microconidiophores* unknown.

Cultural characteristics. *Colony colour* (reverse) 13K amber brown (Rayner, 1970). *Chlamydospores* extensive, dense, throughout medium, forming microsclerotia.

Cardinal temperature requirements for growth. Min. above 10°, max. above 35°, opt. 30°. This is a high temperature species, with medium sporulation on aerial mycelium.

Holotype: Brazil, Para, near Belém, *Manilkara zapota*, 1990, F. Carneiro de Albuquerque, PREM 51031.

This study has demonstrated once again the value of integrating molecular techniques with traditional morphology to assess morphological variation accurately, and also to confirm anamorph/teleomorph relationships. Furthermore, results have suggested that in *Cylindrocladium*, where species are identified primarily by minor differences in conidium size, septation and vesicle shape, isolates falling within the range of accepted species complexes can only safely be identified if those complexes have been adequately characterized. In contrast to the acceptable variation found for *C. pteridis* in this study, other studies (Crous *et al.*, 1993*b*; Victor *et al.*, 1996) have shown that morphologically similar isolates could easily belong to distinct mating populations, and possible distinct biological species within a *Cylindrocladium* complex. Research involving the integration of studies on morphology, mating behaviour, vegetative compatibility groups and molecular techniques are therefore required to further attempts to elucidate other species complexes within *Cylindrocladium* and *Calonectria*.

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REFERENCES

Appel, D. J. & Gordon, T. R. (1995). Intraspecific variation within populations of *Fusarium oxysporum* based on RFLP analysis of the intergenic spacer region of the rDNA. *Experimental Mycology* **19**, 120–128.

Boedijn, K. B. & Reitsma, J. (1950). Notes on the genus *Cylindrocladium*. *Reinwardtia* **1**, 51–60.

Carder, J. H. & Barbara, D. J. (1991). Molecular variation and restriction fragment length polymorphisms (RFLPs) within and between six species of *Verticillium*. *Mycological Research* **95**, 935–942.

Crous, P. W., Alfenas, A. C. & Wingfield, M. J. (1993*a*). *Calonectria scoparia* and *Calonectria morganii* sp. nov., and variation among isolates of their *Cylindrocladium* anamorphs. *Mycological Research* **97**, 701–708.

Crous, P. W., Janse, B. J. H., Victor, D., Marais, G. F. & Alfenas, A. C. (1993*b*). Molecular characterization of *Cylindrocladium* spp. with three-septate conidia and ovoid-like vesicles. *Systematic and Applied Microbiology* **16**, 266–273.

Crous, P. W., Korf, A. & Van Zyl, W. H. (1995). Nuclear DNA polymorphisms of *Cylindrocladium* species with 1-septate conidia and vesicles. *Systematic and Applied Microbiology* **18**, 224–250.

Crous, P. W., Phillips, A. J. L. & Wingfield, M. J. (1992). Effects of cultural conditions on vesicle and conidium morphology in species of *Cylindrocladium* and *Cylindrocladiella*. *Mycologia* **84**, 497–504.

Crous, P. W. & Wingfield, M. J. (1994). A monograph of *Cylindrocladium*, including anamorphs of *Calonectria*. *Mycotaxon* **51**, 341–435.

Crous, P. W., Wingfield, M. J. & Alfenas, A. C. (1993*c*). Additions to *Calonectria*. *Mycotaxon* **46**, 217–234.

El-Gholl, N. E., Alfenas, A. C., Crous, P. W. & Schubert, T. S. (1993). Description and pathogenicity of *Cylindrocladium ovatum* sp. nov. *Canadian Journal of Botany* **71**, 466–470.

Feinberg, A. P. & Vogelstein, B. A. (1983). A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Analytical Biochemistry* **132**, 6–13.

Hausner, G., Reid, J. & Klassen, G. R. (1993). Grouping of isolates and species of *Ceratocystis sensu lato* on the basis of molecular and morphological characters. In *Ceratocystis and Ophiostoma. Taxonomy, Ecology and Pathogenicity* (ed. M. J. Wingfield, K. A. Seifert & J. F. Webber), pp. 93–104. The American Phytopathological Society: St Paul, MN, U.S.A.

Kohn, L. M., Petsche, D. M., Bailey, S. R., Novak, L. A. & Anderson, J. B. (1988). Restriction fragment length polymorphisms in nuclear and mitochondrial DNA of *Sclerotinia* species. *Phytopathology* **78**, 1047–1051.

Lodolo, E. J., Van Zyl, W. H. & Rabie, C. J. (1992). A rapid molecular technique to distinguish *Fusarium* species. *Mycological Research* **97**, 345–346.

Magee, B. B., D'Souza, T. M. & Magee, P. T. (1987). Strain and species identification by restriction fragment length polymorphisms in the ribosomal DNA repeat of *Candida* species. *Journal of Bacteriology* **169**, 1639–1643.

Nicholson, P., Rezanoor, H. N. & Hollins, T. W. (1993). Classification of a world-wide collection of isolates of *Pseudocercosporella herpotrichoides* by RFLP analysis of mitochondrial and ribosomal DNA and host range. *Plant Pathology* **42**, 58–66.

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

Rayner, R. W. (1970). A mycological colour chart. CMI and British Mycological Society: Kew, Surrey, U.K.

Russell, P. J., Wagner, S., Rodland, K. D., Feinbaum, R. L., Russel, J. P., Bret-Harte, M. S., Free, S. J. & Metzenberg, R. L. (1984). Organization of the ribosomal ribonucleic acid genes in various wild-type strains and wild-collected strains of *Neurospora*. *Molecular & General Genetics* **196**, 275–282.

Sambrook, J., Fritsch, E. F., Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*. 2nd edn. Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY.

Sherbakoff, C. D. (1928). Washington palm leaf spot due to *Cylindrocladium macrosporum* n. sp. *Phytopathology* **18**, 219–225.

Sobers, E. K. (1968). Morphology and host range of *Cylindrocladium pteridis*. *Phytopathology* **58**, 1265–1270.

Sobers, E. K. & Alfieri, S. A. (1972). Species of *Cylindrocladium* and their hosts in Florida and Georgia. *Proceedings of the Florida State Horticultural Society* **85**, 366–369.

Victor, D., Crous, P. W., Janse, B. J. H. & Wingfield, M. J. (1996). Genetic variation in *Cylindrocladium floridanum* and other morphologically similar *Cylindrocladium* species. *Systematic and Applied Microbiology* (In press).

Vilgalys, R. & Hester, M. (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**, 4238–4246.

Wolf, F. A. (1926). Brown leaf spot of leather leaf fern. *Journal of the Elisha Mitchell Scientific Society* **42**, 55–62.

Zumpetta, G. M. (1976). An electrophoretic study of the genus *Cylindrocladium* as a possible taxonomic tool. M.Sc. Thesis, California State College, California, Pennsylvania.

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