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Calonectria scoparia and *Calonectria morganii* sp. nov., and variation among isolates of their *Cylindrocladium* anamorphs

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Cylindrocladium scoparium and *C. candelabrum* are important pathogens of numerous hosts with wide geographical distributions. Single-conidial pairings of 48 isolates tentatively identified as either *C. scoparium* or *C. candelabrum* from Brazil, North America and South Africa have confirmed that at least two distinct species exist within this complex. These results were also confirmed with total protein discontinuous SDS–PAGE electrophoresis gels. The two species could also be distinguished morphologically on their vesicle morphology. *C. candelabrum* is accepted as the correct name for Brazilian and South African isolates with ellipsoidal to obpyriform apical vesicles (widest below the middle). North American and some Brazilian isolates with ellipsoidal to pyriform apical vesicles (widest above the middle) represent *C. scoparium*, with *C. ellipticum* and *C. brasiliensis* as synonyms. *Calonectria morganii* is newly described as the teleomorph of *Cylindrocladium scoparium*. *Calonectria scoparia* is shown to be the teleomorph of *Cylindrocladium candelabrum*.

Cylindrocladium scoparium Morgan is the type of *Cylindrocladium* Morgan (Morgan, 1892). This fungus is known from numerous collections worldwide (Booth & Gibson, 1973; French & Menge, 1978; Peerally, 1991). It is reported to cause a wide range of disease symptoms including damping off, root rot, cutting rot, stem cankers, and leaf-spot, as well as seedling and shoot blight (Cordell & Rowan, 1975; Cordell & Skilling, 1975; Ferreira, 1989). *C. scoparium* has also been reported to occur on an extensive range of hosts (Bertus, 1976; French & Menge, 1978; Peerally, 1991). *C. candelabrum* was originally described from the leaves of a *Luma* sp. in Brazil (Viegas, 1946). In contrast to the frequently cited *C. scoparium*, the only other reference found in the literature referring to *C. candelabrum* was that of Peerally (1991), who considered *C. candelabrum* to be the earlier name for *C. ellipticum* Alfieri, Seymour & Sobers.

When *C. scoparium* was first described from a dead pod of honey locust (*Gleditsia triacanthos* L.) in Ohio, the exact nature of the stipe and the vesicle was omitted from the description (Morgan, 1892). Subsequent studies on the *C. scoparium* group have reported the apical vesicle to be ovoid to ellipsoidal (Sobers & Seymour, 1967), and inequilaterally obovoid (Booth & Gibson, 1973). Currently, some workers accept vesicle morphology as one of the most important taxonomic criteria to separate species of *Cylindrocladium* (Sobers, 1968; Peerally, 1973; El-Gholl *et al.*, 1986; El-Gholl, Leahy & Schubert, 1989; Peerally, 1991), while others (Hunter & Barnett, 1978; Rossman, 1983) report it to be highly variable. Recent studies (Crous, Phillips & Wingfield, 1992) have found, however, that this criterion is reliable when examined on carnation-leaf agar (CLA) (Fisher *et al.*, 1982; Crous *et al.*, 1992) under predetermined conditions of incubation.

The concept of *C. scoparium* as a species has developed to such an extent that isolates were placed in this taxon if conidia were 1-septate, 33–59 × 3–4 μm, and apical vesicles were oval to ellipsoid to umbonate (Peerally, 1991). In contrast, the apical vesicles of *C. candelabrum*, however, were illustrated as ellipsoidal, while conidia were described as 1-septate, 40–88 × 5–6 μm (Viegas, 1946).

Sobers (1974) reported a *Calonectria* teleomorph for *C. scoparium* after ascocarps with fertile ascospores developed from pairings of single-conidial isolates from different locations. The *Calonectria* teleomorph was not named. Using different isolates, the experiment was repeated by Ribeiro (1978), who found that a teleomorph resulted from pairing different Brazilian isolates. The name *Calonectria scoparia* Ribeiro & Matsuoka was proposed for the teleomorph of *Cylindrocladium scoparium* (Ribeiro, 1978). Although reference is made to a holotype specimen lodged at Viçosa, Brazil (VIC), no such specimen could be located in the present study. Ribeiro (1978) did not publish the work from his thesis, and Peerally (1991) provided a Latin description to validate the name *Calonectria scoparia* Ribeiro & Matsuoka *ex* Peerally.

The aim of the present study was to reconsider the species

Table 1. Isolates of *Cylindrocladium candelabrum* and *C. scoparium* used in total protein electrophoretic studies

	Vesicle morphology	Source	Origin	Collector	Accession no.	Position
C. candelabrum	Elliptical to pyriform	*Eucalyptus* sp.	Natal, R.S.A.	M. J. Wingfield	PPRI 4202	1
		Eucalyptus sp.	Eastern Transvaal, R.S.A.	P. W. Crous	PPRI 4199	2
		Eucalyptus sp.	Virginopolis, MG, Brazil	A. C. Alfenas	PPRI 4146	3
		Eucalyptus sp.	Caronel Fabricano, MG, Brazil	A. C. Alfenas	PPRI 4163	4
		Eucalyptus sp.	São Mateus, ES, Brazil	A. C. Alfenas	PPRI 4161	5
C. scoparium	Elliptical to obpyriform	*Eucalyptus* sp.	U.S.A.	A. Rossman	PPRI 4154	6
		Mahonia sp.	Florida, U.S.A.	S. A. Alfieri	ATCC 38227	7
		Leucothoe sp.	North Carolina, U.S.A.	D. M. Benson	ATCC 46300	8
		Anacardium sp.	Fortaleza, CE, Brazil	D. O. Freire	PPRI 4731	9
		Rhododendron sp.	Gainsville, U.S.A.	C. R. Semer	PPRI 4144	10
		Eucalyptus sp.	Brazil	T. R. Ciferri	CBS 230.51	11

concept in *C. scoparium* and *C. candelabrum*, by studying isolates collected from Brazil, North America and South Africa. Sexual compatibility between isolates identified as either *C. scoparium* or *C. candelabrum* was also tested. Furthermore, variation in morphology of isolates from these countries was considered. A comparison was also made with isolates of the suspected synonyms, *C. brasiliensis* (Batista & Ciferri) Peerally and *C. ellipticum*, using morphological as well as total protein banding patterns.

MATERIALS AND METHODS

Morphology

Single-conidial isolates were subcultured on to CLA, incubated at 25 °C under nuv light and examined after 7 d (Crous *et al.*, 1992). Only material occurring on the carnation tissue was examined. Averages of all measurements are given in parentheses. Perithecia were examined before they became papillate and exuded spore masses. They were placed in 5% KOH for 2–12 h, fixed in 5% glutaraldehyde for 12–24 h and subsequently washed in H_2O and placed in a gelatin solution (12·5 g gelatin, 27·5 g 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 and 3% aqueous KOH. Abbreviations used for herbaria are those cited by Holmgren & Keuken (1974). Cultures and specimens examined were lodged with the National Collection of Fungi, Pretoria (PREM), and are maintained in the culture collection of the Department of Plant Pathology, University of Stellenbosch, Stellenbosch.

Validly described species in *Cylindrocladium* that are reported to have similar vesicle morphologies and 1-septate conidia were compared with isolates of *C. scoparium* and *C. candelabrum*. Where possible, they were also paired with all isolates used in this study. The species compared were *C. brasiliensis* (CBS 230.51, type culture; IMI 43688, type specimen) and *C. ellipticum* (ATCC 38227, type culture).

Sexual compatibility

Forty-eight single-conidial isolates (listed under cultures examined) including 19 from Brazil, 7 from North America and 22 from South Africa, were paired with each other in all possible combinations. Pairings were made on plates of CLA. Single-conidial isolates were grown on 2% malt-extract agar for 7 d at 25° under nuv light. Agar discs (3 mm diam.) from the periphery of the actively growing colonies were used to inoculate plates. In all pairings, two isolates were placed on opposite sides of a piece of carnation leaf. There were four pieces of carnation leaf per plate, three replicate plates per pairing, and the experiment was repeated once. Plates were sealed with a double layer of Parafilm, placed under nuv light at 25° and examined weekly until perithecia developed.

Total protein electrophoresis

Single-conidial isolates (Table 1) were compared electrophoretically on the basis of their total soluble protein banding patterns. Isolates were grown on 2% malt-extract agar (MEA), and plugs from seven-d-old cultures transferred to 500 ml Erlenmeyer flasks (six/isolate), containing 100 ml glucose–yeast extract broth (Zumpetta, 1976), and incubated for 7 d in the dark at 25°. Mycelium was extracted, and macerated in 0·1 $\text{M-Na}_2\text{HPO}_4\text{–KH}_2\text{PO}_4$ buffer (pH 7·0) (lysing buffer), and filtered. The extract was centrifuged at 10000 ***g*** for 7 min, the supernatant extracted, and dialysed in de-ionized H_2O. The dialysed supernatant was freezer-dried, and stored under vacuum. Proteins were resuspended in lysing buffer and the concentrations determined (Biorad protein assay kit). A volume equivalent to 60 $\mu g\ ml^{-1}$ was layered on to the gel. A rainbow protein molecular weight kit (Amersham Inter-

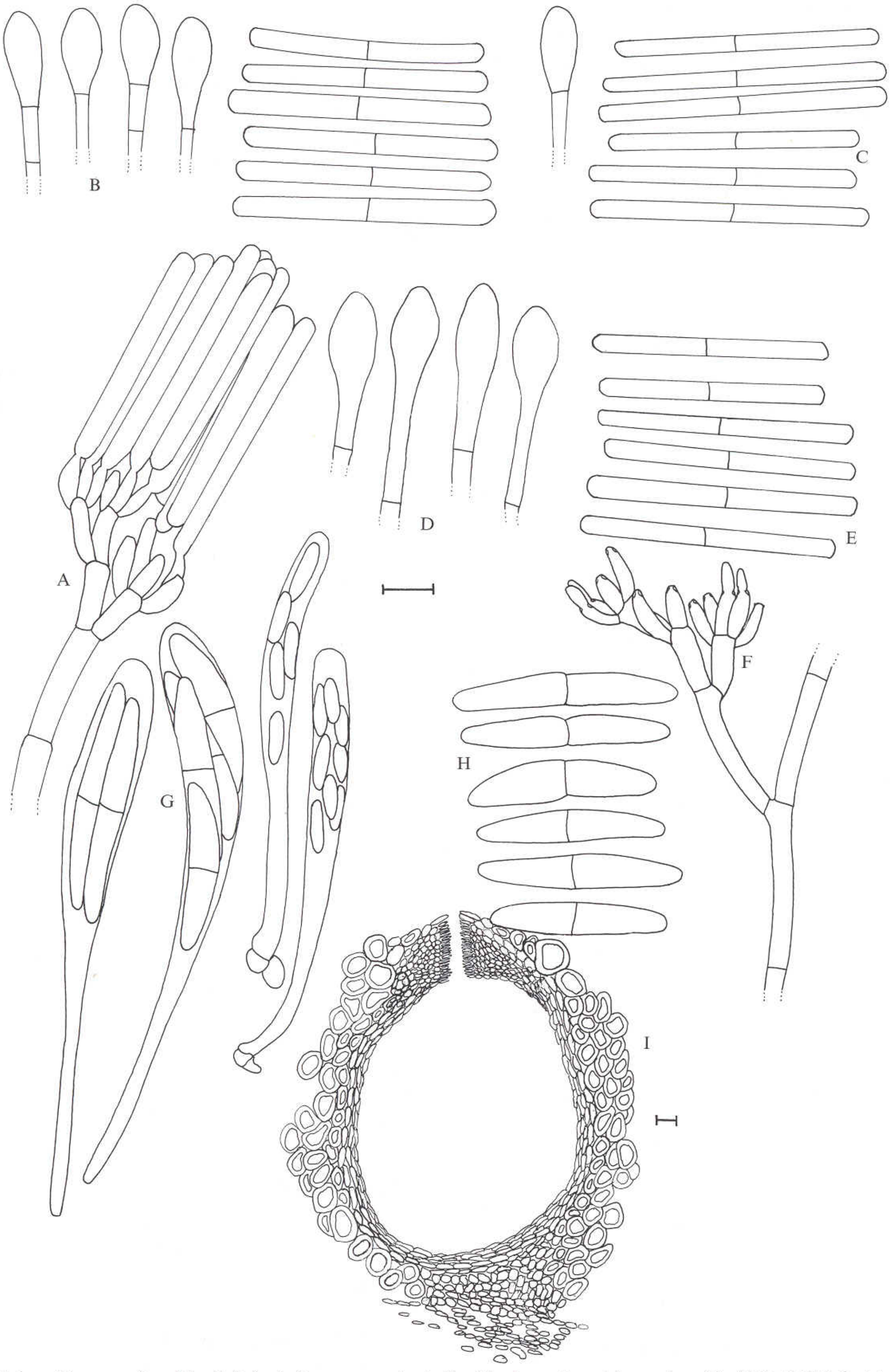

Fig. 1. *Calonectria morganii* and its *Cylindrocladium* anamorph. A, Conidiophore; B, vesicles and conidia (BPI 414576, lectotype); C, vesicle and conidia on CLA (ATCC 38227, type culture of *C. ellipticum*); D, vesicles; E, conidia; F, conidiophore on CLA (ATCC 46300); G, immature and mature asci of *Calonectria morganii* (PREM 51042); H, ascospores (bar = 10 μm); I, vertical section through a perithecium (bar = 20 μm).