

# 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 *Lima* 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;

Rossmann, 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
<i>C. candelabrum</i>	Elliptical to pyriform	<i>Eucalyptus</i> sp.	Natal, R.S.A.	M. J. Wingfield	PPRI 4202	1
		<i>Eucalyptus</i> sp.	Eastern Transvaal, R.S.A.	P. W. Crous	PPRI 4199	2
		<i>Eucalyptus</i> sp.	Virginópolis, MG, Brazil	A. C. Alfenas	PPRI 4146	3
		<i>Eucalyptus</i> sp.	Caronel Fabriciano, MG, Brazil	A. C. Alfenas	PPRI 4163	4
		<i>Eucalyptus</i> sp.	São Mateus, ES, Brazil	A. C. Alfenas	PPRI 4161	5
<i>C. scoparium</i>	Elliptical to obpyriform	<i>Eucalyptus</i> sp.	U.S.A.	A. Rossman	PPRI 4154	6
		<i>Mahonia</i> sp.	Florida, U.S.A.	S. A. Alferi	ATCC 38227	7
		<i>Leucothoe</i> sp.	North Carolina, U.S.A.	D. M. Benson	ATCC 46300	8
		<i>Anacardium</i> sp.	Fortaleza, CE, Brazil	D. O. Freire	PPRI 4731	9
		<i>Rhododendron</i> sp.	Gainesville, U.S.A.	C. R. Semer	PPRI 4144	10
		<i>Eucalyptus</i> 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) Peeraly 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<sub>2</sub>O and placed in a gelatin solution (12.5 g gelatin, 27.5 g H<sub>2</sub>O, 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.*

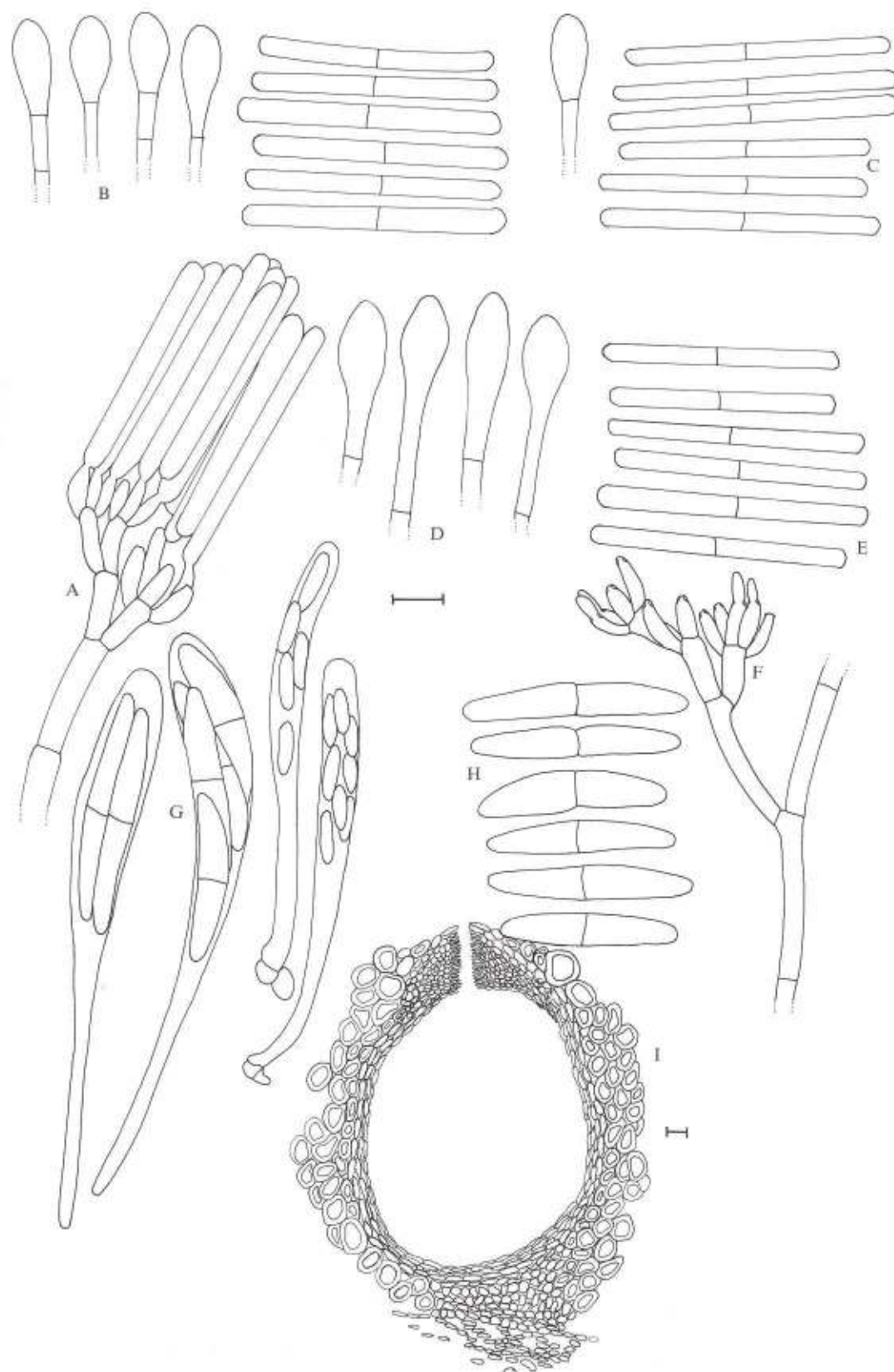
*brasiliensis* (CBS 230.51, type culture; IMI 43688, type specimen) and *C. ellipticum* (ATCC 38227, type culture).

### 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 °C 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 °C 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 °C. Mycelium was extracted, and macerated in 0.1 M-Na<sub>2</sub>HPO<sub>4</sub>-KH<sub>2</sub>PO<sub>4</sub> buffer (pH 7.0) (lysing buffer), and filtered. The extract was centrifuged at 10 000 g for 7 min, the supernatant extracted, and dialysed in de-ionized H<sub>2</sub>O. 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 µg ml<sup>-1</sup> was layered on to the gel. A rainbow protein molecular weight kit (Amersham Inter-



**Fig. 1.** *Calonectria morganii* and its *Cylindrocladion* 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  $\mu$ m); I, vertical section through a perithecium (bar = 20  $\mu$ m).



national) was used as standard reference proteins. Buffer-soluble proteins were separated electrophoretically on a discontinuous SDS-PAGE system (Laemmli, 1970), 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 at 10°. Gels were stained with 10 g l<sup>-1</sup> Coomassie brilliant blue. One isolate was grown up in three separate batches, and run on the same, as well as separate gels to confirm intra- and intergel repeatability of the system.

## RESULTS

### Morphology

Vesicles of North American isolates and one Brazilian isolate were ellipsoidal to pyriform with papillate apices, corresponding to the type of *C. scoparium* (Fig. 1), whereas those from South Africa and all other Brazilian isolates were ellipsoidal to obpyriform, corresponding to the type of *C. candelabrum* (Fig. 2). Vesicles that were ellipsoidal to pyriform are typical of those of *C. scoparium* present on material collected by Morgan (BPI 414576) (Fig. 1).

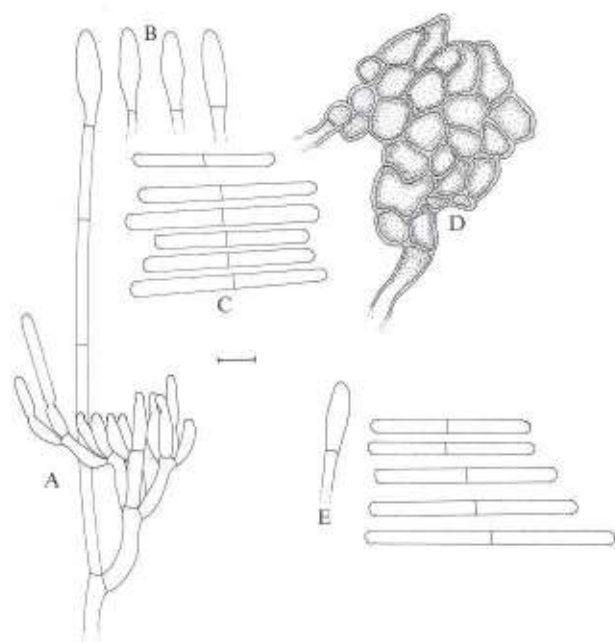


Fig. 2. *Cylindrocladium candelabrum*. A, Conidiophore; B, vesicles; C, conidia; D, chlamydospores (PPRI 3989); E, vesicle and conidia (IACM, 440, type) (bar = 10 µm).

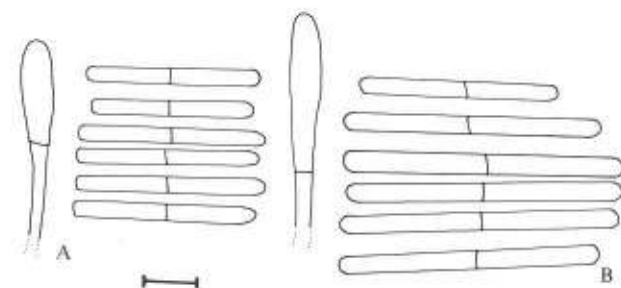


Fig. 3. *Cylindrocladium brasiliensis*. A, Obovoid to clavate vesicle and conidia (IMI 43688, type specimen); B, conidia on CLA (CBS 230.51, type culture).

*Cylindrocladium candelabrum* was described from Brazil (Viegas, 1946), with 1-septate conidia, 40–88 × 5–6 µm in size, and ellipsoidal vesicles. The illustration based on the type (Viegas, 1946) showed ellipsoidal vesicles and doliiform phialides. The type collection (IACM 440) was examined and found to be almost completely devoid of material, but those conidia found were 46.0–70.0 × 3.5–5.0 µm in size. Vesicles were ellipsoidal to obpyriform. Other than the type, Viegas also lodged another collection of *C. candelabrum* (IACM 2682). The conidia of IACM 2682 were determined to be 5-septate and the apical vesicles narrowly clavate, which agrees with the description of *C. quinqueseptatum* included in Peeraly (1991). In contrast, the conidia of *C. candelabrum* (IACM 440, holotype) are 1-septate and the apical vesicles wider below the middle. These results suggest that many isolates that have in recent years been treated as *C. scoparium* (Peeraly, 1991), are in fact representative of *C. candelabrum*. In order to ensure clarity, the name *C. candelabrum* is used for South African and Brazilian isolates with ellipsoidal to obpyriform vesicles in subsequent parts of this study.

*C. ellipticum* is known only from the type specimen collected in North America, having larger conidia, reniform phialides, and smaller vesicles than *C. scoparium* (Alfieri, Seymour & Sobers, 1970). An examination of the type culture (ATCC 38227) (Fig. 1) revealed conidia to be 47.0–(53.5)–66.5 × 3.5–(4.0)–4.5 µm. Vesicles and phialides were also similar to those of North American isolates of *C. scoparium*.

*C. brasiliensis* was originally described from *Eucalyptus* seedlings as a small-spored variety of *C. scoparium* (*C. scoparium* var. *brasiliensis* Batista & Ciferri) with a noticeably higher virulence (Batista, 1951). Conidia on the type specimen (IMI 43688) were 25.0–(31.5)–34.0 × 2.5–(3.0)–3.5 µm in size. The type culture (CBS 230.51) was reported as sterile (Hodges & May, 1972), but sporulated sparsely on CLA in this study (Fig. 3). The conidia formed on CLA were 39.0–(45.0)–51.0 × 3.0–(3.5)–4.0 µm in size, being much larger than those on the type specimen. The opinion of Alfenas *et al.* (1979) – that the smaller conidia present on the type specimen could have resulted from an inappropriate growth medium – might therefore be correct. This isolate has unfortunately lost its ability to form vesicles in culture, so comparisons of vesicle morphology under standardized conditions on CLA were not possible.

### Sexual compatibility

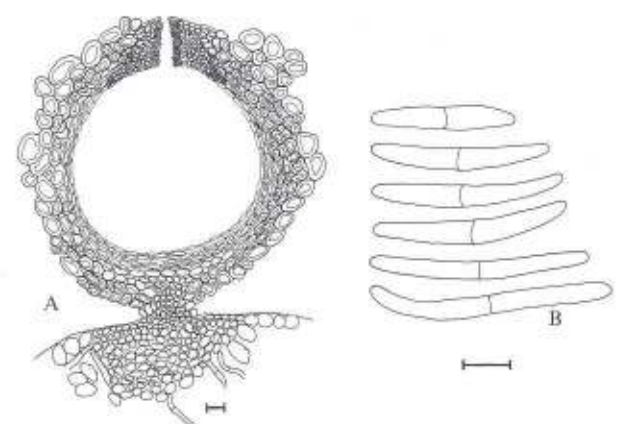
Mature perithecia containing ascospores developed after 8 wk, and were obtained from only 9 of the 1176 combinations of the 48 isolates paired. Paired isolates that resulted in the formation of perithecia are given in Table 2. No perithecia were obtained in pairings of *C. scoparium* with *C. candelabrum*. Fertile progenies were obtained among as well as between Brazilian and South African isolates of *C. candelabrum* (Figs 4, 5). Compatible isolates that produced protoperithecia when incubated alone were considered positive (+), and those that fertilized them as negative (–).

Of the seven North American isolates of *C. scoparium* tested, one pairing produced fertile perithecia, namely ATCC 46300 (+) × ATCC 38227 (–). The same North American

**Table 2.** Single conidial pairings within isolates of *Cylindrocladium candelabrum* and *C. scoparium* which resulted in the formation of fertile perithecia<sup>1</sup>

Accession no.	<i>C. candelabrum</i>							<i>C. scoparium</i>		
	Brazil				South Africa			North America		Brazil
	(+)	(-)	(-)	(-)	(+)	(-)	(-)	(+)	(-)	(-) <sup>2</sup>
	PPRI	PPRI	PPRI	PPRI	PPRI	PPRI	PPRI	ATCC	ATCC	PPRI
	4153	4161	4163	4146	4202	4199	4198	46300	38227	4731
PPRI 4153	+									
PPRI 4161	+	+								
PPRI 4163	+		+							
PPRI 4146	+			+						
PPRI 4202					+					
PPRI 4199	+				+					
PPRI 4198	+				+					
ATCC 46300								+		
ATCC 38227									+	
PPRI 4731										+

<sup>1</sup> Pairings were done on carnation-leaf agar at 25° under nuv light and included 48 isolates paired in all possible combinations.  
<sup>2</sup> Information pertaining to isolates screened is presented in the cultures examined section.  
<sup>3</sup> +, Fertile perithecia formed; -, no fertile perithecia formed.



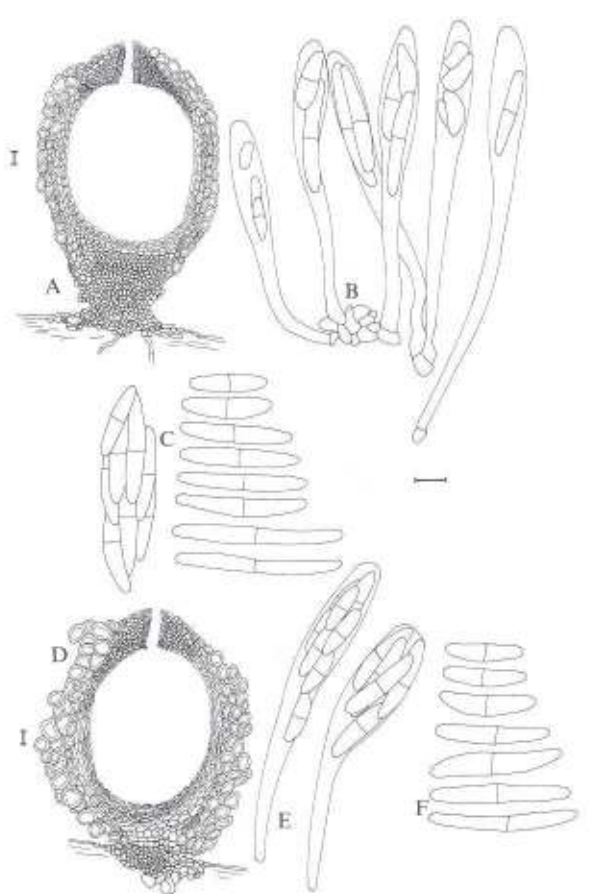
**Fig. 4.** *Calonectria scoparia* (paired Brazilian and South African isolates PPRI 4153 × PPRI 4198). A, Vertical section through a perithecium (bar = 20 µm); B, ascospores (bar = 10 µm).

isolate (ATCC 46300) (+) was interfertile with the Brazilian isolate PPRI 4731 (-) of *C. scoparium*.

The perithecia resulting from compatible pairings amongst Brazilian isolates of *C. candelabrum* were larger and more elongated than those from pairings between the South African isolates. The latter structures tended to be globose. Furthermore, cells on the outer layer of perithecia from pairings amongst Brazilian isolates were smaller and the ascospores longer and narrower than those from pairings amongst South African isolates. Pairings between Brazilian and South African isolates of *C. candelabrum* resulted in the formation of perithecia and ascospores of a size more typical of the larger Brazilian than the smaller South African progeny (Figs 4, 5).

**Total protein electrophoresis**

South African isolates assigned the name *C. candelabrum* had uniform protein banding patterns, which were distinct from those of North American isolates of *C. scoparium*. However, Brazilian isolates of *C. candelabrum* showed much more variation than those from South Africa.



**Fig. 5.** *Calonectria scoparia*. A, Vertical section through a perithecium (bar = 20 µm); B, immature and mature asci; C, ascospores (paired Brazilian isolates PPRI 4163 × PPRI 4153, bar = 10 µm); D, vertical section through a perithecium (bar = 20 µm); E, ascospores (paired South African isolates PPRI 4202 × PPRI 4199, bar = 10 µm).

Isolates could be placed in either *C. scoparium* or *C. candelabrum* based on their vesicle morphologies and could also be distinguished by means of their protein banding patterns (Fig. 6). North American and Brazilian isolates typical



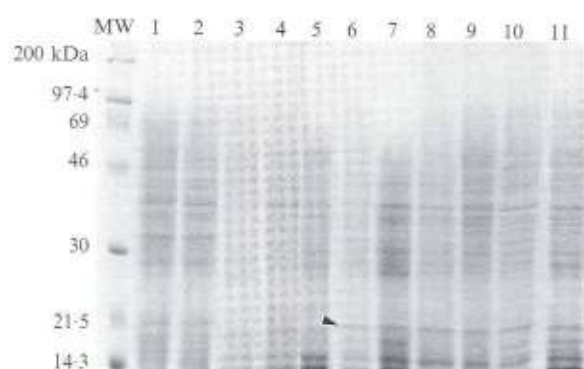


Fig. 6. SDS electrophoresis gel of *Cylindrocladium candelabrum* and *C. scoparium* isolates. Molecular weights (mw) are given in kilodaltons, while data pertaining to the isolates are given in Table 1. Arrow indicates a distinct band present in isolates of *C. scoparium*, but absent in those of *C. candelabrum*.

of *C. scoparium* had a distinct band in the vicinity of 21.5 kDa, which was missing in South African and Brazilian isolates typical of *C. candelabrum*. The banding patterns of the type cultures of *C. ellipticum* (ATCC 38227) and *C. brasiliensis* (CBS 230.51) were typical of isolates of *C. scoparium*.

## DISCUSSION

Results obtained in this study, including morphological characteristics, electrophoretic comparisons and sexual compatibility tests, indicate that there are at least two distinct species within the fungal population until now generally regarded as *C. scoparium*. Isolates characterized by ellipsoidal to pyriform vesicles (widest above the middle) represent *C. scoparium*, while those characterized by ellipsoidal to obpyriform vesicles (widest below the middle) represent *C. candelabrum*. The latter species would include all isolates from South Africa and the majority of those from Brazil.

Peerally (1991) treated *C. ellipticum* as a synonym of the previously described *C. candelabrum* after comparing their conidium dimensions. *C. ellipticum* should rather be a synonym of *C. scoparium*, as these fungi have vesicles widest above the middle, are sexually compatible and have similar protein banding patterns. Furthermore, the type culture of *C. brasiliensis* has also been shown to have conidia of a similar size, and a similar protein banding pattern to isolates of *C. scoparium*. It would therefore be appropriate to include them as synonyms of *C. scoparium* along with two previously recognized synonyms as follows.

*Cylindrocladium scoparium* Morgan, Bot. Gaz. 17: 191 (1892).

*Cylindrocladium pithecolobii* Petch, Ann. R. Bot. Gard. Peradeniya 6: 244 (1917).

*Diplocladium cylindrosporium* Ell. & Everh., Bull. Torrey Bot. Cl. 27: 58 (1900).

*Cylindrocladium scoparium* var. *brasiliensis* Batista & Ciferri, Boletim da S.A.L.C. Pernambuco 18: 190 (1951).

*Cylindrocladium brasiliensis* (Batista & Ciferri) Peerally, CMI Descriptions of Pathogenic Fungi and Bacteria No. 427 (1974).

*Cylindrocladium ellipticum* Alfieri, Seymour & Sobers, Phytopathology 60: 1213 (1970).

Conidiophores septate, hyaline, terminating in an ellipsoid to pyriform vesicle, 6.0–(6.5)–8.0 µm diam; stipes 150–(173)–195 µm long; primary branches non-septate or rarely 1-septate, 11–(24)–40 × 4.0–(4.5)–5.0 µm; secondary branches non-septate, 11–(16)–20 × (4.0)–4.5 µm; tertiary branches non-septate, 10–(13)–15 × (4.0)–4.5 µm. Phialides arise from the terminal ends of branches, in groups of 2, 3 or 4; doliform to reniform, hyaline, 9.0–(11)–15 × 3.0–(3.5)–4.0 µm. Conidia cylindrical, hyaline, 1-septate, rounded at both ends, 40–(44.5)–66 × 3.5–(4.0)–4.5 µm.

**Cultural characteristics.** Minimum temperature above 5°; maximum temperature above 35°; optimum temperature range, 25–30°. This is both a high- and a low-temperature species, growing below 10° and above 30°, with medium sporulation on aerial mycelium. Colony colour (bottom), on MEA after 7 d in the dark (13K–17°I–15°I), amber brown to buckthorn brown to sayal brown (Rayner, 1970), (5D6–6D8 or 5C6–6C7) pompeian yellow to light brown, or oak brown to reddish golden (Kornerup & Wanscher, 1967). Chlamydospores abundant, in chains or clusters, forming microsclerotia in moderate numbers (Fig. 1).

Single conidial isolates of *C. scoparium* produced a *Calonectria* teleomorph when paired in culture. The name *Calonectria scoparia* Ribeiro & Matsuoka ex Peerally represents the teleomorph of isolates characterized by apical vesicles widest below the middle (Ribeiro, 1978), thus typical of *Cylindrocladium candelabrum*. Another species epithet therefore must be chosen for the teleomorph of *C. scoparium*. This *Calonectria* state is described below.

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

Etym.: named after Dr A. P. Morgan, who described the genus *Cylindrocladium* and its type species *C. scoparium*.

*Perithecia* superficialia portata, singulatim aut in turmis parvis, globosa vel subglobosa, 280–520 × 280–400 µm, rubri-brunnea usque ad rubra, pariete exteriore aspero verrucosoque et ostiolo papillato. Asci hyalini, clavati ad longum et tenuum caulem 75–(80)–100 × 8.0–(10)–15 µm, capientes 1–8 ascosporas. Ascosporae hyalinae, rectae vel facatae, 1-septatae, nihil vel leviter medium ad septum constrictae 24–(37)–49 × 4.0–(6.3)–8.0 µm. Ascosporae evolentes usque ad tres septa dimissae ab asco.

Holotypus: ATCC 46300 (U.S.A., *Leucothoe catesbaei*) × PPRI 4731 (Brazil, *Anacardium* sp.), July 1992, P. W. Crous, PREM 51042 (type specimen).

*Perithecia* superficial, borne singly or in small groups, globose or subglobose, 280–520 × 280–400 µm, red-brown to red, with rough warted outer wall and papillate ostiole (Fig. 1I). Asci hyaline, unitunicate, clavate, 75–(80)–100 × 8.0–(10.0)–15.0 µm, tapering to a long thin stalk, containing 1–8 ascospores at maturity (Fig. 1G). Ascospores hyaline, straight to falcate, tapering to rounded ends, 1-septate, smooth, not or slightly constricted at central septum, 24–(37)–49 × 4.0–(6.3)–8.0 µm (Fig. 1H). Ascospores developing up to three septa after discharge from ascus.

**Specimens of teleomorph examined:** ATCC 46300 (U.S.A., *Leucothoe catesbaei*) × PPRI 4731 (Brazil, *Anacardium* sp.), July 1992, P. W. Crous, PREM 51042 (holotype specimen); ATCC 46300 (U.S.A.,



*Leucothoe catesbaei* × ATCC 38227 (U.S.A., *Mahonia bealei*), July 1992, P. W. Crous, PREM 51043 (paratype specimen).

*Specimens of anamorph examined*: Brazil: Recife, *Eucalyptus* seedlings, A. C. Batista, 11 Sept. 1950, IMI 43688 (ex holotype, *C. brasiliensis*); U.S.A.: Preston, Ohio, *Gleditsia triacanthos*, A. P. Morgan, 1893, BPI 414576 (neotype designated here), BPI 414577; Preston, Ohio, *Juglans nigra*, A. P. Morgan, 1893, BPI 414578.

*Cultures examined*: Brazil: CE, Fortaleza, *Anacardium* sp., D. O. Freire, PPRI 4731; T. R. Ciferri, CBS 230.51 ex type of *C. brasiliensis* (IMI 299576). U.S.A.: *Mahonia bealei*, S. A. Alfieri, 1970, ATCC 38227 (*C. ellipticum*, ex type); Florida, *Rhododendron* sp., C. R. Semer (Viçosa, Brazil, No. UFV 11), PPRI 4144; unknown host, A. Rossman (Viçosa, Brazil, No. UFV 39), PPRI 4154; North Carolina, *Leucothoe catesbaei*, D. M. Benson, 1981, ATCC 46300; *Rosa* sp., N. El-Gholl, P90.1445, PPRI 4732; Florida, *Ilex vomitoria*, N. El-Gholl, P90.1479, PPRI 4733.

An examination of the type of *C. candelabrum* found it to be representative of Brazilian and South African isolates with ellipsoidal to obpyriform vesicles. The original description of *C. candelabrum* is poor and incomplete. Moreover, numerous additional isolates have now been studied, and the species description is thus emended as follows.

***Cylindrocladium candelabrum* Viegas, *Bragantia* 6: 370 (1946).**

*Conidiophores* septate, hyaline, terminating in an ellipsoid to obpyriform vesicle 6.0–(8.0)–10.0 µm diam; stipes 105–(180)–290 µm long; primary branches non-septate or rarely 1-septate, 13–(18.5)–27 × 3.0–(3.5)–4.0 µm; secondary branches non-septate, 10–(15)–22 × 3.0–(3.5)–4.0 µm; tertiary branches non-septate, 10–(14)–16 × 3.0–(3.5)–4.0 µm. *Phialides* arise from the terminal ends of branches, in groups of 2, 3 or 4; doliiform to reniform, hyaline, 7.5–(13)–19 × 3.0–(3.5)–4.0 µm. *Conidia* cylindrical, hyaline, 1-septate rounded at both ends, 33–(45)–66 × 3.5–(4.0)–4.5 µm (Fig. 2). Temperature requirements for growth and cultural characteristics as for *C. scoparium*.

*Neotype* (designated here): Brazil, Copener-Bahia, *Eucalyptus* sp., A. C. Alfenas, UFV 63, PPRI 4153, PREM 51044 (neotype specimen).

*Perithecia* of *Calonectria scoparia* have frequently been found associated with collections of *Cylindrocladium candelabrum* on *Eucalyptus* cuttings in forest nurseries in Brazil and South Africa. Furthermore, single-conidial pairings of isolates of *C. candelabrum* also gave rise to perithecia with a viable progeny. As no holotype specimen could be located in VIC, specimens of the teleomorph collected in this study are briefly described below, and a neotype designated.

***Calonectria scoparia* Ribeiro & Matsuoka ex Peeraly, *Mycotaxon* 40: 341 (1991).**

*Calonectria scoparia* Ribeiro & Matsuoka, Ribeiro, M.Sc. thesis (1978) *Heterotalismo em C. scoparium* Morgan p. 28. (nom. illegit.).

*Perithecia* superficial, borne singly or in small groups, globose or subglobose, 280–520 × 280–400 µm, red-brown to red, with rough warted outer wall and papillate ostiole lined with periphyses. *Asci* unitunicate, hyaline, clavate, 72–(90)–120 × 6.0–(8.0)–15 µm, tapering to a long thin stalk,

containing 1–8 ascospores at maturity. *Ascospores* hyaline, straight to falcate, tapering to rounded ends, 1-septate, not or slightly constricted at central septum, 28–(41)–68 × 4.0–(5.0)–6.5 µm. *Ascospores* developing up to three septa after discharge from ascus.

*Neotype*: Brazil, BA Sul, Picadao, Conceição da Barra, A. C. Alfenas & F. A. Ferreira, *Eucalyptus grandis* clone 172 (branch), 27 Apr. 1992, PREM 51045.

*Specimens of teleomorph examined*: Brazil: BA Sul, Picadao, Conceição da Barra, A. C. Alfenas & F. A. Ferreira, *Eucalyptus grandis* clone 172 (branch), 27 Apr. 1992, PREM 51045 (neotype); PPRI 4163 (*Eucalyptus* sp.) × PPRI 4153 (*Eucalyptus* sp.), July 1992, P. W. Crous, PREM 51047; PPRI 4161 (*E. grandis*) × PPRI 4153 (*Eucalyptus* sp.), July 1992, P. W. Crous, PREM 51048; PPRI 4146 (*Eucalyptus* sp.) × PPRI 4153 (*Eucalyptus* sp.), July 1992, P. W. Crous, PREM 51049. Africa: R.S.A., Eastern Transvaal, Sabie, Frankfort, *E. grandis* cuttings, A. C. Alfenas, 24 Mar. 1992, PREM 51046 (teleomorph occurring naturally on cuttings); PPRI 4202 (*E. macarthuri*) × PPRI 4199 (*Eucalyptus* sp.), July 1992, P. W. Crous, PREM 51050.

*Specimens of anamorph examined*, Africa: Kenya, *E. saligna* Sm., I. Gibson, 1966, IMI 123734; R.S.A., Natal, Cedara, *Syncarpia gummiifera*, M. J. Wingfield, 17 July 1978, PPRI 45419. Brazil: Luma sp., Campinas, Herbario da Seccao de Botanico IACM 440 (specimen almost devoid of material). (*C. candelabrum*, holotype); *Ammonia* sp., Campinas, Herbario da Seccao de Botanico, IACM 8155. India: *Eucalyptus* sp., C. Booth, 1964, IMI 108770 (as *C. theobromae*). Mauritius: unknown host, M. A. Peerally, IMI 167982 (as *C. sclerotiorum*).

*Cultures examined*, Africa: R.S.A.: Swellendam, *Medicago truncatula*, S. Lamprecht, 16 March 1986, PPRI 3554; Natal, Seven Oaks, debris, 1 Feb. 1990, I. Rong, PPRI 3812; Natal, Kwambonambi, *E. grandis*, G. Kemp, May 1990, PPRI 3988; [Eastern] [Transvaal], Frankfort, *Eucalyptus* sp., S. H. Koch, 1988, PPRI 3989; E. Tvl, White River, *Rhododendron* hybrid, P. W. Crous, 11 May 1990, PPRI 4085; Natal, Pietermaritzburg, *E. nitens*, P. W. Crous, March 1991, PPRI 4189; Natal, Kwambonambi, *E. grandis*, P. W. Crous, Feb. 1990, PPRI 4191; Natal, Kwambonambi, *E. grandis*, P. W. Crous, Feb. 1990, PPRI 4192; E. Tvl, White River, *E. grandis*, P. W. Crous, Feb. 1990, PPRI 4193; Natal, Pietermaritzburg, *E. fastigiata*, P. W. Crous, Feb. 1990, PPRI 4194; Natal, Kwambonambi, *Eucalyptus* sp., P. W. Crous, Feb. 1990, PPRI 4195; Natal, Kwambonambi, *Eucalyptus* sp., P. W. Crous, May 1990, PPRI 4196; E. Tvl, White River, *Eucalyptus* sp., P. W. Crous, 29 Sept. 1989, PPRI 4197; E. Tvl, Klipkraal, *E. grandis*, P. W. Crous, Feb. 1990, PPRI 4198; E. Tvl, Sabie, D. R. De Wet, *Eucalyptus* roots, P. W. Crous, Feb. 1990, PPRI 4199; N. Tvl, Tzaneen, *Eucalyptus* sp., P. W. Crous, 28 June 1990, PPRI 4201; Natal, Commendale, M. J. Wingfield, *E. macarthuri*, 5 Jan. 1987, PPRI 4202; E. Tvl, *E. nitens* (Deane & Maid.) Maid., M. J. Wingfield, ?PPRI 4203; E. Tvl, Sabie, *E. grandis* roots, M. J. Wingfield, ?PPRI 4204; E. Tvl, White River, *Eucalyptus grandis* cuttings, P. W. Crous, 29 Sept. 1989, PPRI 4205; ?*Acacia cyclops*, M. Morris, 10 July 1990, PPRI 4206; Natal, Kwambonambi, *Eucalyptus* leaves, P. W. Crous, May 1990, PPRI 4208. Brazil: MG, João Pinheiro, *E. cloeziana*, A. C. Alfenas, PPRI 4151; MG, Virginópolis, *Eucalyptus* sp., PPRI 4146; ?UNB, J. C. Dianese, PPRI 4147; Copener-Bahia, *Eucalyptus* sp., PPRI 4148; UNB, J. C. Dianese, PPRI 4149; Viveiro, *Eucalyptus* sp., A. C. Alfenas, PPRI 4150; Copener-Bahia, *Eucalyptus* sp., A. C. Alfenas, PPRI 4152; Copener-Bahia, *Eucalyptus* sp., A. C. Alfenas, PPRI 4153; ?UNB, J. C. Dianese, PPRI 4155; CAF, *Eucalyptus* sp., A. C. Alfenas, PPRI 4156; MG, Ipatinga, *Eucalyptus* sp., A. C. Alfenas, PPRI 4158; MG, Ipatinga, *Eucalyptus* sp., A. C. Alfenas, PPRI 4159; Viveiro Florestas Rio Doce, *E. grandis*, A. C. Alfenas, PPRI 4161; ?UNB, C. J. Dianese, PPRI 4162; MG, Coronel Fabriciano, *Eucalyptus* sp., A. C.

Alfenas, PPRI 4163; PE, Recife, *Eucalyptus* sp., R. L. R. Mariano, PPRI 4165; ES, Aracruz, *Eucalyptus* sp., A. C. Alfenas, UFV 117, PPRI 4734.

The present study has shown that the name *C. scoparium* has become a repository for fungi representing at least two species distinguishable on their vesicle morphology and sexual compatibility. A substantial collection of isolates from North America, Brazil and South Africa has been considered. It seems likely that additional collections of isolates from other parts of the world, currently accommodated within the broader *C. scoparium* species concept, might include additional species.

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