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 46 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 obovoid 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, 1976; 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 Alfers, 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 ellipsoid (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; EI-Gholl et al., 1986; EI-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 x 3–4 µm, and apical vesicles were oval to ellipsoid to unlobate (Peerally, 1991). In contrast, the apical vesicles of C. candelabrum, however, were illustrated as ellipsoid, while conidia were described as 1-septate, 40–88 x 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 et 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

<table>
<thead>
<tr>
<th>Vesicle morphology</th>
<th>Source</th>
<th>Origin</th>
<th>Collector</th>
<th>Accession no.</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elliptical to</td>
<td>Eucalyptus sp.</td>
<td>Natal, R.S.A.</td>
<td>M. J. Wingfield</td>
<td>PPRI 4202</td>
<td>1</td>
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<tr>
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<td>Eucalyptus sp.</td>
<td>Eastern Transvaal, R.S.A.</td>
<td>P. W. Crous</td>
<td>PPRI 4199</td>
<td>2</td>
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</tr>
<tr>
<td>Elliptical to</td>
<td>Eucalyptus sp.</td>
<td>Virgianopolis, MG, Brazil</td>
<td>A. C. Afiea</td>
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<td>3</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elliptical to</td>
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<td>Sio Mateus, ES, Brazil</td>
<td>A. C. Afiea</td>
<td>PPRI 4161</td>
<td>5</td>
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</tr>
<tr>
<td>Elliptical to</td>
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<td>U.S.A.</td>
<td>A. Rossman</td>
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<tr>
<td>Elliptical to</td>
<td>Mahonia sp.</td>
<td>Florida, U.S.A.</td>
<td>S. A. Alberi</td>
<td>ATCC 38227</td>
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<td>Elliptical to</td>
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<td>North Carolina, U.S.A.</td>
<td>D. M. Benson</td>
<td>ATCC 46300</td>
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<tr>
<td>Elliptical to</td>
<td>Anacardium sp.</td>
<td>Fortaleza, CE, Brazil</td>
<td>D. O. Freiie</td>
<td>PPRI 4731</td>
<td>9</td>
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<td>Gainesville, U.S.A.</td>
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</tr>
<tr>
<td>Elliptical to</td>
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<td>Brazil</td>
<td>T. R. Ciferri</td>
<td>CBS 230.51</td>
<td>11</td>
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</table>

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 H2O and placed in a gelatin solution (12.5 g gelatin, 27.5 g H2O, 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 43868, 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-Na2HPO4-KH2PO4 buffer (pH 7.0) (lyzing buffer), and filtered. The extract was centrifuged at 10000 g for 7 min, the supernatant extracted, and dialysed in de-ionized H2O. The dialysed supernatant was freeze-dried, and stored under vacuum. Proteins were resuspended in lyzing buffer and the concentrations determined (BioRad protein assay kit). A volume equivalent to 60 μg 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).
Cylindrocladium candelabrum was described from Brazil (Viegas, 1946), with 1-septate conidia, 40-68 x 5-6 µm in size, and ellipsoidal vesicles. The illustration based on the type (Viegas 1940) showed ellipsoidal vesicles and doliiform phialides. The type collection (JACM 440) was examined and found to be almost completely devoid of material, but those conidia found were 46.0-70.0 x 3.5-5.0 µm in size. Vesicles were ellipsoidal to obpyriform. Other than the type, Viegas also lodged another collection of C. candelabrum (JACM 2682). The conidia of JACM 2682 were determined to be 5-septate and the apical vesicles narrowly clavate, which agrees with the description of C. quinqueseptatum included in Peerally (1991). In contrast, the conidia of C. candelabrum (JACM 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 (Peerally, 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 x 3.5-4.0-5.0 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 Battista & Ciferri) with a noticeably higher virulence (Battista, 1951). Conidia on the type specimen (IMI 43688) were 25.0-31.5-34.0 x 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 x 3.0-3.5-4.0 µm in size, being much larger than those on the type specimen. The opinion of Allenas 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 progeny were obtained amongst as well as between Brazilian and South African isolates of C. candelabrum (Figs 4, 5). Compatible isolates that produced proterothecia 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

<table>
<thead>
<tr>
<th>Accession no.</th>
<th>C. candelabrum</th>
<th>C. scoparium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Brazil</td>
<td>South Africa</td>
</tr>
<tr>
<td>PPR1 4153</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>PPR1 4161</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>PPR1 4163</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>PPR1 4146</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>PPR1 4202</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>ATCC 46300</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>ATCC 38227</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>PPR1 4731</td>
<td>(-)</td>
<td>(+)</td>
</tr>
</tbody>
</table>

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

Fig. 4. C. scoparium (paired Brazilian and South African isolates PPR1 4153 x PPR1 4198). A. Vertical section through a perithecium (bar = 20 μm); B. ascospores (bar = 10 μm).

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.

Isolates could be placed in either C. scoparium or C. candelabrum based on their vesicle morphologies and could also be distinguished by their protein banding patterns (Fig. 6). North American and Brazilian isolates typical
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 apical vesicles with rough warted outer wall and papillate ostiole (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, Allenas & Wingfield sp. nov.

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


Conidia bi- to tri-septate, hyaline, 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.


Diplocadium cylindrosporum Ell. & Everh., Bull. Torrey Bot. Cl. 27: 58 (1900).


Diplocadium cylindrosporum Ell. & Everh., Bull. Torrey Bot. Cl. 27: 58 (1900).


Leucothia candelabrum, A. C. Batista, 11 Sept. 1950, 1MI 43688 (ex holotype, C. p., W, Crous, A. C Alfenas and M. J. Wingfield PPRI (Viçosa, Brazil, No. UFV 39), PPRI 4154; North Carolina, (Viçosa, Brazil, No. UFV 11). PPRI 4144; unknown host A. Rossman ellipficr;m, ex 38227 (c. D. M. Benson, 1981, A TCC 4733, P90. 1445, PPRI 4732; Florida, PREM 1992, P. W. Crous, be representative of Brazilian and South African isolates with description is thus emended as follows.

- **Branches non-septate, 10-(15)-22 x 3'-Q--{3'S}-4'0 ~m;**
- **Ter- rarely 1-septate, (180}-290 ~m long;**
- **Primary branches non-septate or**
- **Obpyrifonn vesicle 6'0-(8'0)-10'0 ~m diam;**
- **Stipes 105-**
- **Tertiary branches non-septate, 10---(14}-16 x 3'Q--{3'5}-4'0 ~m.**

**Phialides**

- **Conidia**

**Ascospores**

- **72-{90}-120 x 6'O-{8'O)-15 ~m,**
- **1-septate rounded at both ends, 33-{45)-66 x 3'5-{4'O)-

**Temperature requirements for growth and**

**Critical characters as for C. scoparium.**

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

**Perichaeta of Calonectria scoparia have frequently been**

**Calonectria scoparia Ribeiro & Matsuoka ex Peerally, Mycota 40: 341 (1991).**


**Perithecia superficial, borne singly or in small groups, globose or subglobose, 280-520 x 280-400 μm, red-brown to red, with rough warty outer wall and papillate ostiole lined with periphyses. Ascii unitunicate, hyaline clavate, 72-(90)-120 x 60-(80)-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 x 4.(5-0)-6.5 μm. Ascospores developing up to three septa after discharge from ascus.**

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

**Specimen of teleomorph examined, Brazil: BA Sul, Piccadia, Conceição da Barra, A.C. Alfenas & F. A. Ferreira, Eucalyptus grandis clone 172 (branch), 27 Apr. 1992, PREM 51045 (neotype).**

**Specimens examined:** Brazil: Copener-Bahia, Eucalyptus sp., PPRI 4146 (Eucalyptus sp.) x PREM 4153 (Eucalyptus sp.), July 1992, P. W. Crous, PREM 51047; PPRI 4161 (E. grandis) x PPRI 5133 (Eucalyptus sp.), July 1992, P. W. Crous, PREM 51048; PPRI 4146 (Eucalyptus sp.) x PPRI 4153 (Eucalyptus sp.), July 1992, P. W. Crous, PREM 51046; Florida, PREM 51040 (teleomorph occurring naturally on cuttings), PPRI 4202 (E. macrocarpa) x PPRI 4199 (Eucalyptus sp.), July 1992, P. W. Crous, PREM 51050.

Eucalyptus has become a repository for fungi representing at least two species distinguishable on their vesicle morphology and species, parts of the world, currently accommodated within the North America, Brazil and South Africa has been considered. We thank the curators of the American Type Culture Collection (ATCC, Parklawn Drive, Rockville, U.S.A.), International Mycological Institute (IMI, Bakeham Lane, Egham, Surrey, U.K.), Beltsville, Agricultural Research Centre (BPI, Maryland, U.S.A.) and Herbario da Secção de Botânico/Instituto Agronômico, Campinas, Brazil) for placing their material and cultures at our disposal. We are also grateful to Dr Amy Rossman (BPI) for useful comments on an earlier version of this paper.

REFERENCES


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