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A re-evaluation of *Cylindrocladiella*, and a comparison with morphologically similar genera

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Cylindrocladiella is confirmed as distinct from *Cylindrocladium*. Species of *Cylindrocladium* are primarily distinguished from *Cylindrocladiella* by septate stipes, more numerously branched conidiophores, only penicillate conidiophores and *Calonectria* teleomorphs. *Cylindrocladiella*, however, has non-septate stipes, small 1-septate conidia, penicillate and subverticillate conidiophores, chlamydospores that are arranged in chains, phialides with extending, prominent collarettes, conidia in slimy masses (as opposed to clusters), strong cultural odours and *Nectria* teleomorphs. Six *Cylindrocladiella* spp. are recognized. *C. peruviana* is placed in synonymy with *C. camelliae*, while *C. elegans* and *C. lageniformis* are described as new. *Gliocladiopsis* is distinguished from *Cylindrocladiella* by the absence of a stipe, the presence of more numerous conidiophore branches and the formation of conidia in slimy yellow masses. *Gliocladiopsis* is distinguished from *Cylindrocarpon* by having uniformly cylindrical conidia, more numerous conidiophore branches, and more numerous phialides per branch. *Cylindrocarpon tenue* is shown to be better accommodated in *Gliocladiopsis*, and the name *G. tenuis* proposed. The taxonomic position of *Acontiopsis* is uncertain, due to its vague generic description and the absence of type material.

The genus *Cylindrocladium* was established for the single species *C. scoparium* Morgan (1892). The primary distinguishing characteristic was the presence of cylindrical septate conidia. The stipe extension above the conidiogenous apparatus (referred to here as the stipe) was not mentioned in the diagnosis, but its existence became known later (Anderson, 1918; Boedijn & Reitsma, 1950).

In the first review of *Cylindrocladium*, *Candelospora* Hawley apud Rea & Hawley (1912) was treated as a synonym, and all relevant new combinations made (Boedijn & Rietsma, 1950; Tubaki, 1958). Subsequently, *Tetracytum* Vanderwalle (1945) was also recognized as a synonym of *Cylindrocladium* (Subramanian, 1971; Domsch, Gams & Anderson, 1980; Brayford & Chapman, 1987).

Boesewinkel (1982) believed that several species of *Cylindrocladium* with small conidia were distinct from those with larger conidia and septate stipes. On this basis he transferred the names of these fungi to a new genus, *Cylindrocladiella* Boesewinkel. Species of *Cylindrocladiella* were also distinguished from *Cylindrocladium* by cultural criteria, chlamydospore arrangement, the presence of penicillate as well as subverticillate conidiophores, *Nectria* (Fr.) Fr. as opposed to *Calonectria* De Not. teleomorphs, non-septate, unbranched, central stipes and obvious collarettes (*sensu* Sutton, 1980) on the phialides, conidial arrangement on conidiophores, as well as the small, 1-septate conidia (Boesewinkel, 1982).

Species of *Cylindrocladiella* are frequently associated with leaf spot and root rot symptoms of various hosts (Peerally, 1974; Boesewinkel, 1982; Sharma & Mohanan, 1982). Although there are conflicting reports regarding the pathogenicity of *C. parva* (Anderson) Boesewinkel (Sharma & Mohanan, 1982; Sobers & Alfieri, 1982; Crous, Phillips & Wingfield, 1992*b*), other species in the genus have been shown to be pathogenic to various hosts (Crous *et al.*, 1992*b*), and some have been reported to cause significant damage to economic plants (Mohanan & Sharma, 1985; Peerally, 1991).

The status of *Cylindrocladiella* as a genus has recently been contested (Peerally, 1991; Sharma & Mohanan, 1991). This study was undertaken to consider the distinction between *Cylindrocladiella* and other morphologically similar genera such as *Cylindrocladium* (Peerally, 1991), *Acontiopsis* Negru (Kendrick & Carmichael, 1973; Peerally, 1991), *Cylindrocarpon* Wollenw. (syn. *Moeszia* Bubak) (Tubaki, 1958; von Arx, 1970) and *Gliocladiopsis* Saksena (Agnihothrudu, 1959; Barron, 1968).

MATERIALS AND METHODS

Evaluation of anamorphs

Cultures derived from single conidia were plated on to carnation-leaf agar (CLA) (Fisher *et al.*, 1982; Crous, Phillips & Wingfield, 1992*a*), incubated at 25 °C under nuv, and examined after 7 d. Only material occurring on the leaves was examined. Mounts were prepared in lactophenol cotton blue. All measurements were made under the (100 ×) oil-immersion objective. Wherever possible, fifty examples of each structure were measured. The data were analysed (Table 1) by Least Significant Difference (L.S.D.) at a probability level of $P = 0{\cdot}05$. Where less material was available, or when it was of insufficient quality, averages are not included. Cultures and types are lodged at the National Collection of Fungi, Pretoria (PREM).

Vesicle shape and stipe length. Vesicle shape was determined on CLA after 7 d incubation. Vesicles examined were all on stipes of conidiophores, with at least one primary and one secondary branch bearing phialides. Vesicles that showed signs of proliferation were ignored. Vesicle width was measured at the widest point, and stipe length measured from the basal septum to the vesicle tip.

Collarettes and conidiophores. Conidiophores were examined after 7 d on CLA to determine if they were either penicillate or subverticillate, or if both conidiophore types were present. Phialides were also examined for the presence or absence of collarettes.

Cultural characteristics

Growth studies. To determine the maximum radial growth of species in culture, agar plugs from the periphery of 7-d-old colonies (3 mm diam.) of each fungus were plated at the centre of malt-extract agar (MEA) (20 g Oxoid malt extract, 15 g Difco agar, 1000 ml H_2O) plates, and incubated at 25° for 1 d to ensure active growth. Growth after 1 d was marked, and thereafter plates were placed at the respective temperatures under consideration. Growth was assessed for all isolates after 6 d of incubation in the dark at 5, 8, 10, 15, 20, 25, 33 and 35°, with three replicate plates of each isolate at each temperature. Radial growth was also determined after 3 d at 25 and 30°. Average growth was calculated from four radial measurements from each of the three plates. The experiment was repeated at least once for all isolates. The rationale for including 8 and 33° with the other 5° intervals was that the growth of many species began to slow or stop between 5 and 10 and between 30 and 35°. These temperatures were therefore necessary to determine a finer distinction between minimum and maximum temperature requirements for growth.

Chlamydospores and colony colour. Chlamydospore measurements were found to be variable, and were therefore ignored. Production of chlamydospores and microsclerotia was, however, rated for the extent of thickened, pigmented hyphae present after 6 d (viewed from the underside of plates), at the completion of the growth studies. Dark brown, medium brown and light brown colonies were used as categories to define extensive, medium and slight chlamydospore production respectively. Colony colours were rated simultaneously with chlamydospore production, as the amount of chlamydospores formed directly influenced the colony colours. To ensure homogeneity of results, ratings were made independently by two observers and results compared. Colours were taken from Methuen (Kornerup & Wanscher, 1967) and Rayner (1970).

RESULTS

Evaluation of anamorphs

Six vesicle classes were recognized for *Cylindrocladiella* spp.: irregularly lanceolate, ellipsoid to lanceolate, ellipsoid to clavate, cylindrical, clavate to pyriform and lageniform to ovoid (Figs 1–6). Although *C. parva* and *C. novae-zelandiae* (Boesew.) Boesew. were both characterized by penicillate conidiophores without obvious collarettes on doliiform to cymbiform phialides, stipe lengths of *C. parva* were significantly shorter ($P = 0{\cdot}05$) than those of *C. novae-zelandiae* (Table 1). *C. elegans* sp. nov., *C. infestans* Boesew. and *C. novae-zelandiae* had very similar conidial dimensions when examined on CLA, making it difficult to distinguish these species solely on this basis. Similar conidial dimensions were also observed for *C. camelliae* (Venkataramani & Venkata Ram) Boesew. and *C. lageniformis* sp. nov. (Table 1). In contrast to *Cylindrocladium*, stipes were nearly always formed at the centre of the conidiophore, and were never branched.

Cultural characteristics

Cylindrocladiella spp. could easily be distinguished from each other by their minimum and maximum temperature requirements for growth. The ability to grow at either very high or low temperatures enabled species to be grouped into either high (growing above 30°) or low (growing below 10°) temperature classes (Figs 7–12). *Cylindrocladiella* spp. could also easily be distinguished in the same way from *Gliocladiopsis sagariensis* Saksena, which was capable of growing at much higher temperatures than any species of *Cylindrocladiella*.

As reported by Boesewinkel (1981, 1982), chlamydospore formation can be used to further distinguish *Cylindrocladiella* spp. from each other (Table 1). Some degree of variation was, however, found in different isolates of certain species, thus reducing the value of this criterion. After 6 d on MEA, colony pigmentation in all spp. except *C. lageniformis* was chiefly the result of chlamydospore production. In *C. lageniformis*, however, a reddish pigment diffused readily into the medium, resulting in a darker colony.

TAXONOMY

Cylindrocladiella Boesewinkel, *Can. J. Bot.* **60**: 2289 (1982).

Conidiophores hyaline, single, subverticillate, as well as penicillate, with primary and secondary branches. *Phialides* terminal, hyaline, in whorls of 2–4, with or without obvious collarettes. *Stipe* mostly centrally arranged on conidiophore, with a single basal septum, terminating in a thin-walled, hyaline vesicle of characteristic shape. *Conidia* hyaline, (0)–1-septate, sometimes becoming swollen at one end with age. *Chlamydospores* more frequently arranged in chains than clusters.

Teleomorph: *Nectria*.