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A MONOGRAPH OF *CYLINDROCLADIUM*, INCLUDING ANAMORPHS OF *CALONECTRIA*

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ABSTRACT

The genus *Cylindrocladium* and respective teleomorphs in the holomorph genus *Calonectria* are treated. The genera are circumscribed, while morphological characters of conidia, vesicles, phialides, stipes, conidiophore branches and cultural characteristics are compared under standardized conditions. Species are described and illustrated with reference to their pathogenicity and distribution. Comparisons are also made of perithecia, asci and ascospores of the *Calonectria* spp. Comparisons with type specimens, cultures and additional collections of various species worldwide are included. In *Cylindrocladium*, 22 species and one variety, *C. colhounii* var. *macroconidialis*, are recognized. Microconidial states are newly described for *C. ilicicola*, *C. reteaudii* and *C. quinqueseptatum*. Fifteen *Calonectria* species and one variety, *Calonectria colhounii* var. *macroconidialis*, are recognized. Dichotomous and synoptic keys are also provided.

SPECIES TREATED

1. *C. avesiculatum* Gill, Alfieri & Sobers
2. *C. candelabrum* Viegas
3. *C. citri* (Fawcett & Klotz) Boedijn & Reitsma
4. *C. clavatum* Hodges & May
5. *C. colhounii* Peerally var. *colhounii*
6. *C. colhounii* var. *macroconidialis* Crous, Wingfield & Alfenas

7. *C. curvatum* Boedijn & Reitsma
8. *C. floridanum* Sobers & Seymour
9. *C. gracile* (Bugnicourt) Boesewinkel
10. *C. hawksworthii* Peerally
11. *C. heptaseptatum* Sobers, Alfieri & Knauss
12. *C. ilicicola* (Hawley) Boedijn & Reitsma
13. *C. naviculatum* Crous & Wingfield
14. *C. ovatum* El-Gholl, Alfenas, Crous & Schubert
15. *C. parasiticum* Crous, Wingfield & Alfenas
16. *C. pteridis* Wolf
17. *C. quinqueseptatum* Boedijn & Reitsma
18. *C. reteaudii* (Bugnicourt) Boesewinkel
19. *C. scoparium* Morgan
20. *C. spathiphylli* Schoulties, El-Gholl & Alfieri
21. *C. spathulatum* El-Gholl, Kimbrough, Barnard, Alfieri & Schoulties
22. *C. theae* (Petch) Subramanian
23. *C. variabile* Crous, Janse, Victor, Marais & Alfenas

INTRODUCTION

The genus *Cylindrocladium* Morgan was first described in 1892 for *C. scoparium* Morgan (Morgan, 1892). Boedijn & Reitsma (1950) recognized seven species, and in a recent review (Peerally 1991a), 26 species were accepted. *Cylindrocladium* spp. have been associated worldwide with numerous plant hosts, causing a wide range of disease symptoms. Disease development is generally more prevalent under humid conditions, and this explains why *Cylindrocladium* spp. are regarded as more important in tropical and subtropical areas (Crous *et al.*, 1991).

In the original description of *Cylindrocladium* (Morgan 1892), no mention was made of the stipe and characteristic terminal vesicle, now considered typical of this genus. In the first review of *Cylindrocladium*, Boedijn & Reitsma (1950) mentioned that all the species had a vesicle, and they also illustrated these structures as differing in shape. Although Peerally (1991a) distinguished one *Cylindrocladium* sp. by its apparent inability to form a stipe and vesicle, the concept of *Cylindrocladium sensu lato* is one of a fungus having a septate stipe and an apical vesicle (Crous *et al.*, 1993b).

Many species of *Cylindrocladium* are known to have teleomorphs in the holomorph genus *Calonectria* De Not. Rossman (1983) studied the various *Calonectria* spp. with *Cylindrocladium* anamorphs. She was not able to distinguish all *Cylindrocladium* spp. by their teleomorphs, and therefore also rejected the vesicle as a criterion for distinguishing the various anamorph species. In some studies of *Cylindrocladium* (Sobers & Alfieri, 1972; Peerally, 1991a), vesicle morphology was found to be a reliable characteristic for species separation, while others (Zumpetta, 1976; Hunter & Barnett, 1978; Rossman, 1983) found it variable and unreliable, preferring to give preference to conidial characteristics. Recent studies (Crous *et al.*, 1992) have, however, shown that vesicle shape is influenced by the osmotic potential of the growing medium, and that it is a reliable criterion when examined on standardized media under prescribed growth conditions.

Boesewinkel (1982a) found that several of the small-spored *Cylindrocladium* species could be distinguished from the rest on the basis of cultural criteria, chlamydospore arrangement, two types of conidiophores, a *Nectria* (Fr.) Fr. holomorph, and a non-septate stipe. He accordingly transferred five species to a new genus, *Cylindrocladiella* Boesewinkel.

In his most recent review of *Cylindrocladium*, Peerally (1991a), treated the genus *Cylindrocladiella* as a synonym, and recognized 26 species. However, in our opinion, five species would be better accommodated in *Cylindrocladiella*, a genus we consider to be distinct from *Cylindrocladium* (Samuels *et al.*, 1991; Crous & Wingfield, 1993). Crous *et al.* (1991) found reference in the literature to six species of *Cylindrocladiella* and 34 of *Cylindrocladium*. In this study only the species of *Cylindrocladium* are considered in detail. *Cylindrocladiella* spp. have been treated elsewhere (Crous & Wingfield, 1993). Both teleomorph and anamorph characteristics are considered and illustrated. Keys to accepted species are also provided.

MATERIALS AND METHODS

Material examined

Standardization, both of media and conditions of incubation, are critical when studying and describing *Cylindrocladium* spp. (Crous *et al.*, 1992). Various media and different periods of incubation have previously been used by authors describing *Cylindrocladium* spp. Because of this variation, dried type specimens were inadequate for this study. Therefore, in addition to type specimens, type cultures (where available) and additional cultures were examined under standardized conditions.

Abbreviations used for herbaria are those cited by Holmgren & Kreuken (1974). Where type specimens were devoid of material, or depauperate, the original line drawings were redrawn, and the respective authors credited. All cultures and dried, colonized, pieces of carnation-leaf agar (CLA) (Fisher *et al.*, 1982; Crous *et al.*, 1992) were lodged at the National Collection of Fungi, Pretoria (**PREM**). A separate culture collection has been maintained at the Department of Plant Pathology, University of Stellenbosch (**CPC**), at 25 C on malt-extract agar (MEA) slants (20 g Oxoid malt extract, 15 g Difco agar, 1000 ml H_2O), on sterile soil, and under H_2O.

Evaluation of anamorphs

Single-conidial isolates were transferred to CLA, incubated at 25 C under near-ultraviolet lighting, and examined after 7 d. Only material occurring on the surface of the carnation leaves was examined. Material for microscopic examination was mounted in lactophenol containing cotton blue. Wherever possible, fifty examples of each structure were measured, means given, and extreme values placed in parentheses.

Evaluation of teleomorphs

Perithecia were derived from herbarium material as well as from fresh cultures. After material of anamorphs had been prepared for microscopic examination, plates were sealed and incubated until perithecia formed. Perithecia were examined before ascospores were released. This was because changes in spore length, septation and the degree of septal constriction were found to occur in older ascospores. Perithecia were placed in 5% KOH for 2-12 h, and fixed in 5% glutaraldehyde for 12-24 h. They were subsequently washed in H_2O, and placed in a gelatine solution (12.5 g gelatine, 37.5 ml H_2O, 0.5 g phenol). Longitudinal sections 10-15 μm thick were made using a Leitz Kryomat 1703 freezing microtome. Squash mounts were prepared in lactophenol cotton blue and 3% aqueous KOH.

Cultural criteria

Growth studies. To determine the maximum radial growth, agar plugs from 7-day-old colonies (3 mm diam.) of each fungus were placed at the centre of MEA plates and incubated at 25 C for 1 d to ensure active growth. After 1 d, the amount of growth was marked, and all isolates were incubated concurrently at 5, 8, 10, 15, 20, 25, 30, 33, and 35 C with three replicate plates of each culture at every temperature. It was therefore necessary to include these temperatures to establish minimum and maximium temperature requirements for growth. Because most *Cylindrocladium* spp. have an optimum growth between 25 and 30 C, growth was also assessed after 3 d at these temperatures to determine the value of this rapid method in species differentiation. Radial growth was assessed after incubation for 6 d in the dark. Average growth was calculated as an average of four colony radii on each of the three plates. The experiment was repeated at least once for all isolates. Species that grew below 10 C were considered low temperature species, while those that grew above 30 C were regarded as high temperature species.

Chlamydospores and colony colour. This characteristic is of limited taxonomic value in *Cylindrocladium*, as most species form numerous, dense chlamydospore masses. There are, however, exceptions, which will be discussed for individual species. Chlamydospore measurements were found to be unreliable in this study, and were therefore excluded. Colony colours and chlamydospore formation were determined after 6 d. Colour designations used were those of Rayner (1970) and Methuen (Kornerup & Wanscher, 1967).

EVALUATION OF MORPHOLOGICAL CHARACTERS

Anamorph

Vesicle shape and stipe length. Vesicles in *Cylindrocladium* were measured and illustrated (Appendix 1) after cultures were incubated on CLA for 7 d at 25 C under near-ultraviolet light. Because stipes frequently branch in this genus, only terminal vesicles on primary stipes were considered. Only vesicles that were on stipes which had at least one primary and secondary branch with phialides, were included in measurements. Vesicles that showed signs of further proliferation were ignored. Vesicle length was found to be highly variable, but widths were reliable for taxonomic evaluation. Vesicles were often absent in microconidial states.

Although Boesewinkel (1982b) erected new species on the basis of stipe septation, there does not necessarily appear to be a relationship between length and septation of stipes. Species with longer conidia do, however, tend to have longer stipes. Vesicle shape and stipe length (from highest primary branch to the vesicle tip) should thus be seen as additional criteria of taxonomic value in *Cylindrocladium*.

Microconidia. At the outset of this study, five *Cylindrocladium* species, *C. pteridis*, *C. heptaseptatum*, *C. ovatum*, *C. parasiticum*, and *C. variabile* were known to have microconidial states (Sobers, 1968; El-Gholl *et al.*,