

New species of *Calonectria* and *Cylindrocladium* isolated from soil in the tropics

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Abstract: Several *Calonectria* (*Ca.*) and *Cylindrocladium* (*Cy.*) species were recovered from alfalfa-baited soil samples gathered in Colombia and Venezuela. Perithecia of *Calonectria naviculata* sp. nov. formed in culture when Venezuelan strains of *Cy. naviculatum* were crossed with ex-type Brazilian strains. *Calonectria gracilipes* sp. nov. (anam. *Cy. gracilioideum* sp. nov.), a homothallic species, was isolated from Colombian soils. *Cylindrocladium gracilioideum*, *Cy. gracile*, *Cy. pteridis* and *Cy. pseudogracile* form a complex of morphologically similar species characterized by clavate vesicles and primarily 1-septate conidia. Each species could, however, be distinguished using RFLP banding patterns. A key to *Calonectria* spp. having *Cylindrocladium* anamorphs with 1-septate conidia is also presented.

Key Words: Hypocreales, mating studies, soil, systematics

INTRODUCTION

The hyphomycete genus *Cylindrocladium* Morgan represents species with hyaline, smooth, cylindrical conidia, and penicillate conidiophores with septate stipe extensions terminating in vesicles of characteristic shape. Where known, teleomorphs of *Cylindrocladium* spp. (*Cy.*) are best accommodated in *Calonectria* De Not. (*Ca.*).

More than 20 species of *Cylindrocladium* are recognized (Crous and Wingfield, 1994; Crous et al.,

1995, 1997; Victor et al., 1997). Of these, *Cy. gracile* (Bugnic.) Boesew. (= *Cy. clavatum* Hodges & May; Crous et al., 1995) has been described under various epithets, and has also been incorrectly linked to different *Calonectria* teleomorphs. In contrast to this species, *Cy. naviculatum* Crous & M.J. Wingf. is presently known only from its type collection, isolated from soil in the Amazonas Province, Brazil (Crous et al., 1994). In the present study, several soil samples were collected from *Eucalyptus* plantations in South America (Colombia and Venezuela). Some of the samples rendered isolates of *Cylindrocladium* spp., including *Cy. naviculatum* and a species morphologically similar to *Cy. gracile*. When cultured, two previously undescribed *Calonectria* states were induced for these species. This report describes the hitherto unknown teleomorph of *Cy. naviculatum*, and characterizes the *Calonectria* species isolated from Colombian soil.

MATERIALS AND METHODS

Isolation and identification.—Fifteen soil samples were randomly collected from different eucalypt plantations in Colombia and Venezuela. Each sample consisted of two petri dishes filled with moist soil collected in the upper 15 cm soil layer in a 1 m radius. Dishes were sealed, and transported to the laboratory, where soil samples were moistened, after which surface-disinfested (1 min in 1% NaOCl) alfalfa seeds were scattered onto the soil surface in each dish. After 14 d, the germinating seedlings were removed from the dishes, submerged in 1% NaOCl for 30 s, rinsed in sterile H₂O, and plated onto 2% malt extract agar (Biolab), amended with streptomycin sulphate (0.05 g/L) (MEA). Petri dishes were incubated for 7 d at 25 C under continuous near-ultraviolet light, after which single conidia from sporulating *Cylindrocladium* isolates were plated onto carnation leaf agar (CLA) (Crous et al., 1992). Cardinal temperature requirements for growth and cultural characteristics were determined after 6 d on MEA using the procedures of Crous et al. (1994). Cultures of *Cylindrocladium* were identified using the keys of Crous and Wingfield (1994). Wherever possible, 30 measurements were made of structures mounted in lactophenol, and extremes given in parentheses. Type

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specimens were lodged at the National Collection of Fungi in Pretoria (PREM), and ex-type cultures maintained in the culture collection of the Department of Plant Pathology at the University of Stellenbosch, South Africa (STE-U).

Sexual compatibility.—Isolates of *Cy. naviculatum* collected and identified in this study were mated on CLA with their ex-type strains (STE-U 627–629) in all possible combinations using the technique previously described by Crous et al. (1993a). Petri dishes were inspected weekly for perithecial development. Single conidial isolates of the Colombian species (STE-U 1153, 1213) readily formed perithecia in culture, and were, therefore, accepted as being homothallic.

Molecular comparisons.—Morphologically the *Cylindrocladium* sp. isolated from Colombian soil is characterized by having 1-septate conidia and clavate vesicles. Based on these observations, it was further compared with *Cylindrocladium* spp. known to have clavate vesicles, and primarily 1-septate conidia. The nuclear ribosomal DNA (nrDNA) of the Colombian *Cylindrocladium* sp. (STE-U 1153) was compared with ex-type strains of *Cy. gracile* (PC 551197, = *Cy. clavatum*, ATCC 22833), *Ca. pteridis* Crous et al. (anam. *Cy. pteridis* Wolf, PPRI 4157) and *Ca. gracilis* Crous et al. (anam. *Cy. pseudogracile* Crous, PPRI 4176). The nrDNA of these isolates was digested with the restriction enzymes *EcoRI*, *HindIII* and *XhoI*, and a Southern blot analysis was performed using the 6.3-kb ribosomal DNA repeat unit of *Neurospora crassa* as probe (Crous et al., 1995).

TAXONOMY

Calonectria gracilipes Crous et G.R.A. Mchau, sp. nov. FIGS. 1–4, 9–11

Anamorph. *Cylindrocladium graciloideum* Crous et G.R.A. Mchau, sp. nov.

Perithecia globosa ad ovoidea, 350–400 µm alta, 300–380 µm lata, crocea ad rubra, pariete exteriore verrucosa, ostiolo papillato. Asci clavati, in stipitem longum tenuem gradatim angustatae, 80–120 × 12–18 µm, 8-spori. Ascosporae hyalinae, fusiformes, 1-septatae, ad septum constrictae, (28–)33–40(–45) × (5–)6–7(–7.5) µm. Filum septatum, hyalinum (150–)200(–260) µm, in vesiculam clavatam 3(–4) µm diam terminans. Rami primarii non septati vel raro 1-septati, 15–25 × 4–5 µm; rami secundarii non septati, 10–15 × 4–6 µm. Phialides doliiformes ad reniformes, hyali-

nae, non septatae, 10–15 × 2.5–4 µm. Conidia cylindrica, hyalina, 1-septata, apicibus obtusis, (35–)40–48(–60) × 4–5(–6) µm. Microconidiophora ignota.

HOLOTYPE. COLOMBIA. La Selva, soil, Jun. 1995, *M.J. Wingfield* (PREM 54417 teleomorph, PREM 55299 anamorph, ex-type culture STE-U 1153).

Perithecia orange, globose to ovoid, 350–400 µm high, 300–380 µm wide, turning dark red in 3% KOH; ostiolo papillate, orange to red, generally darker than the perithecialium body. Perithecial wall consisting of two layers: outside layer of *textura globulosa*, 40–50 µm wide; inner layer of *textura angularis*, 10–30 µm wide; hymenial layer of *textura prismatica*, hyaline, 5–10 µm wide; perithecial base up to 100 µm wide, consisting of dark red, angular cells. *Asci* 8-spored, clavate, 80–120 × 12–18 µm, tapering to a long thin stalk. *Ascospores* hyaline, fusoid with rounded ends, straight to slightly curved, 1-septate, constricted at the septum, (28–)33–40(–45) × (5–)6–7(–7.5) µm. *Macroconidiophores* comprising a stipe, a sterile elongation and a penicillate arrangement of fertile branches. Stipe septate, (150–)200(–260) µm long, terminating in a narrowly clavate vesicle, 3(–4) µm diam; primary branches aseptate or rarely 1-septate, 15–25 × 4–5 µm; secondary and tertiary branches aseptate, 10–15 × 4–6 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, non-septate, 10–15 × 2.5–4 µm, apex with minute periclinal thickening. *Conidia* cylindrical, rounded at both ends, straight, (35–)40–48(–60) × 4–5(–6) µm, 1-septate, lacking a visible abscission scar, held in cylindrical packets by colorless slime. *Microconidiophores* not observed. *Chlamydo-spores* dark brown, walls thickened, formed in moderate numbers throughout the medium, and aggregated to form microsclerotia.

Cultures. Colony color (reverse) 13i sienna (Rayner, 1970). Colonies attaining a radius of 15–18 mm diam on MEA after 6 d in the dark at 25 C.

Cardinal temperature requirements for growth. Min. above 10 C, max. below 35 C, opt. 25 C. This is a high temperature species, with medium sporulation on aerial mycelium.

Substrate. Soil.

Distribution. Colombia.

Calonectria naviculata Crous et M.J. Wingf., sp. nov. FIGS. 5–8, 12–14

Anamorph. *Cylindrocladium naviculatum* Crous & M.J. Wingf., Mycotaxon 50: 443. 1994.

Perithecia rubri-brunnea, globosa ad ovoidea, 350–450 µm alta, 350–400 µm lata, pariete exteriore verrucosa, ostiolo papillato. Asci clavati, in stipitem longum tenuem gradatim angustatae, 70–100 × 8–12 µm, 2–8-spori. Ascosporae hyalinae, fusiformes, falcatae, 1(–3)-septatae, ad septa constrictae, guttulate, (20–)40–48(–52) × (3–)5–6(–6.5) µm.

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FIGS. 1–4. *Calonectria gracilipes* and its anamorph *Cylindrocladium graciloideum*. 1. Asci and ascospores. 2. Conidiophore, clavate vesicles and 1-septate conidia. 3. Section through a perithecialium wall showing the various wall layers. Bar = 10 µm. 4. Vertical section through a perithecialium. Bar = 20 µm.



