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Cylindrocladium and *Cylindrocladiella* species new to Taiwan

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Abstract. Two fungal species, *Cylindrocladium floridanum* Sob. & Seymour and *Cylindrocladiella infestans* Boesew., both new to Taiwan, are described and illustrated. Both fungi were isolated from soils and were able to produce their sexual stage on 10% V-8 juice agar and on malt-extract agar media. The teleomorphs of *Cylindrocladium floridanum* and *Cylindrocladiella infestans* were *Calonectria kyotensis* Terashita and *Nectria camelliae* (Shipton) Boesewinkel, respectively.

Key words: *Cylindrocladium floridanum*; *Cylindrocladiella infestans*; Taiwan.

Introduction

The genus *Cylindrocladium* was established by Morgan in 1892 for a mucedinaceous fungus with *Penicillium*-like branching and long, cylindrical, two-celled conidia (Morgan, 1892). After about sixty years, Boedijin and Reitsma (1950) reported their notes on the genus, which consists of seven species. Since then numerous species of *Cylindrocladium* have been described. Most of them are considered to be devastating pathogens, particularly on woody plants, including hardwoods and conifers, with a host range of approximately 100 ornamental plant species (McRitchie, 1982). More than 35 species have been erected in the genus, some of which have been incompletely described while some others are only synonyms (Peerally, 1991). Peerally (1991) presented a comprehensive key for identification of 26 species of *Cylindrocladium*, and the conidial and vesicle morphology have been emphasized as important criteria. Although Boesewinkel (1982) argued for the separation of the small-conidial *Cylindrocladium* species into a new genus, *Cylindrocladiella*, Peerally (1991) reduced *Cylindrocladiella* to synonymy with *Cylindrocladium* due to insufficient characters. All the sexual states of *Cylindrocladium* belong to *Calonectria* de Not. A teleomorph has not been observed for several species of *Cylindrocladium* and this is possibly due to the occurrence of heterothallism (Sobers, 1973; Shipton, 1979). In total, ten teleomorphs of *Cylindrocladium* have been recognized (Peerally, 1991).

Calonectia kyotensis Terashita (a teleomorph of *Cylindrocladium floridanum* Sob. & Seymour) caused a serious black root rot of *Cinnamomum kanehirai* Hay cuttings in Taiwan (Chang, 1991). However, this isolate did not produce typical conidia of *Cylindrocladium floridanum*. I therefore considered that it was a different taxon. Later, I sent the fungus to Dr. P. Crous, Department of Plant Pathology, University of Stellenbosch for confirmation of the identification. He believed that it was *Calonectria crotalariae* (Loos) Bell & Sob. (a teleomorph of *Cylindrocladium crotalariae* [Loos] Bell & Sob.), rather than *Cal. kyotensis* (personal communication). Based on the detailed observation, I agreed with his identification. It has been reported that *Cyl. clavatum* causes leaf blight of *Cin. kanehirai* cuttings (Chang, 1992a). It has been pointed out that *Cal. theae* Loos (anamorph: *Cyl. theae* [Petch] Alfieri & Sob.) causes a disease of *Cinnamomum osmophloeum* Kaneh. (Chang, 1992b). In addition, Matsushima (1980) listed three species of the genus which were obtained from Taiwan: *Cylindrocladiella camelliae* Venkataramani & Vendata Ram, *Cylindrocladium ilicicola* (Hawley) Boedijn & Reitsma, and *Cylindrocladium scoparium* Morgan. In this report, two species in the

genera *Cylindrocladium* and *Cylindrocladiella* new to Taiwan are described and illustrated.

Materials and Methods

Isolation from Soils

Soils used for isolation of *Cylindrocladium* and *Cylindrocladiella* species were collected mostly from second growth forests around the island of Taiwan. Soils were assayed for presence of the fungi by baiting with azalea leaves. Soil samples (approximately 100 ml) were placed in 500 ml beakers and distilled water was added to a total volume of 150-200 ml. Five to seven leaf pieces (ca. 3 × 3 cm) were floated in each beaker as bait, for 3-6 days at room temperature. Lesion bait was washed under running tap water for 1 h and blotted dry with paper towels. Pieces of lesion bait (ca. 0.5 × 0.5 cm) were placed on 10% V-8 juice agar (10% V-8 juice, 0.02% CaCO3 and 2% Bacto agar) supplemented with 2 mg/l each of streptomycin, tetracycline, and neomycin (Rossman, 1983). After incubation at 25 C for 5-7 days, fungal mycelia growing from leaf pieces were transferred to 10% V-8 juice agar.

Growth of Fungi

Cylindrocladium and *Cylindrocladiella* were isolated from soils and were grown on 10% V-8 juice agar for 5-7 days. Agar discs (5 mm diam.) cut from the periphery of the colonies with a sterile cork borer were used as inocula. One agar disc inoculum was transferred to the edge of a plate (9 cm diam.) of 10% V-8 juice agar or MEA (2% malt-extract and 2% Bacto agar) To determine the affect of temperature on the growth of each fungal species, inoculated agar plates were incubated at 10, 15, 20, 25, 30, and 35 C in darkness. Colonies were measured 1 week after inoculation. Four plates were used for each treatment and the experiment was repeated once.

Identification

The classification keys (Crous and Wingfield, 1993; Peerally, 1991) and original descriptions were used for identification of the isolates. Morphological and physiological characteristics of each culture were compared with those described in taxonomy papers. All cultures are maintained in sterile distilled water at 20 C (Boese-

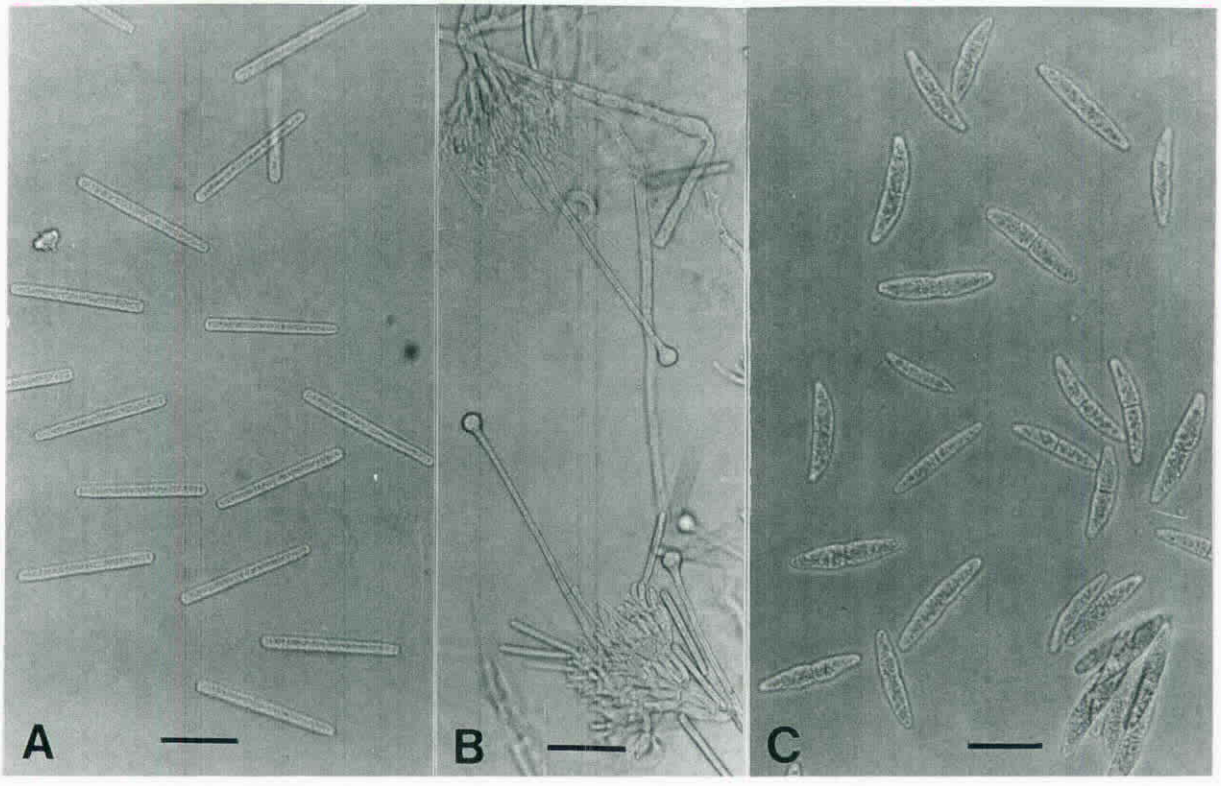

Fig. 1. *Cylindrocladium floridanum*. A, conidia; B, conidiophores and apical vesicles; C, ascospores. Bar = 28 μm.

winkel, 1976) and deposited at the Laboratory of Forest Pathology, Taiwan Forestry Research Institute.

Species Descriptions

Cylindrocladium floridanum Sob. & Seymour, Phytopathology 57: 389, 1967. Fig. 1

The fungus grows and sporulates well on MEA and 10% V-8 juice agar. The aerial mycelium is at first white but rapidly turns reddish brown. The reverse is reddish brown also. Chlamydospores are globose, formed in chains, brown, 5–15 μm diameter and formed in the substratum or the aerial mycelium. Conidiophores arise laterally on a filament stipe, dichotomously or trichotomously branched; primary branches nonseptate, 15–27 μm; secondary branches nonseptate, 11–24 μm; tertiary branches nonseptate, 8–16 μm; phialides mostly doliform, nonseptate, hyaline, 8.0–15.5 × 3.0–7.5 μm. The sterile appendages septate, olivaceous at the base, up to 10 μm wide, 230–440 μm long, hyaline and narrower toward the apex, terminating in a hyaline sphaeropedunculate (Snell and Dick, 1971) vesicle 6.8–17.5 μm in diam. Conidia are hyaline, granular, cylindric, straight, wider at the base than the apex, rounded at both ends, one septate, 38–54 × 3.0–6.0.

Teleomorph: *Calonectria kyotensis* Terashita, Trans. Mycol. Soc. Japan 8: 124, 1968.

Ascocarps solitary or in small groups, superficial on substrate, without stroma, firmly attached to substrate, base of ascocarp and substrate blackened around point of attachment. Ascocarps orange to scarlet, turning blood color in KOH, globose to ovoid with flattened apex, laterally pinched or not collapsing when dry, 280–530 × 230–420 μm, without papilla, ascocarp surface with concolorous warts up to 50 μm high, of loose, globose cells sloughing off from outer wall region. Asci unitunicate, 75–130 × 15–25 μm, narrowly clavate with truncate apex without specialized apical discharge mechanism, eight-spored. Ascospores 25–42 × 4.5–7.5 μm, fusiform with broadly rounded ends, often slightly curved, 1-septate, hyaline smooth, multiseriate.

Optimum temperature for growth of *C. floridanum* was 25–30 C (Fig. 3-A). Temperatures above 35 C and below 10 C partly and completely inhibited fungal growth, respectively.

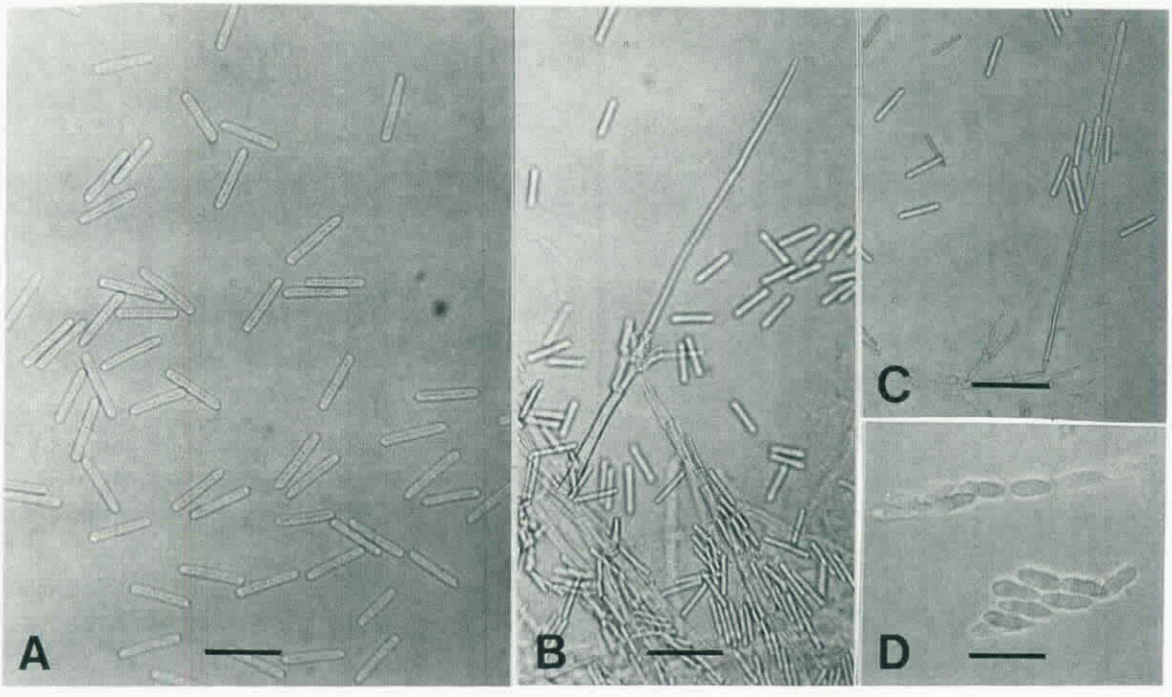

Fig. 2. *Cylindrocladiella infestans*. A, conidia, Bar = 28 μm; B & C, conidia, conidiophores and apical vesicles, Bar = 55 μm; D, ascospores. Bar = 28 μm.

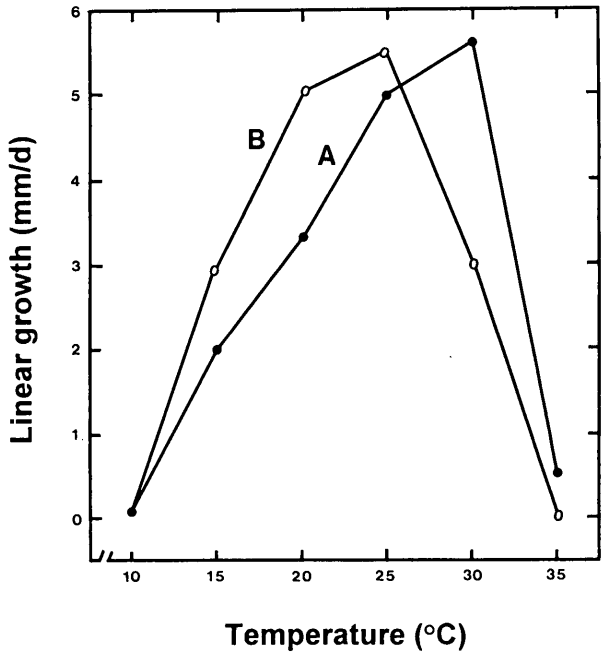

Fig. 3. Linear growth of *Cylindrocladium floridanum* (A) and *Cylindrocladiella infestans* (B) on V8JA.

Cylindrocladiella infestans Boesewinkel, Can. J. Bot. 60: 2290, 1982. Fig. 2

The fungus grows and sporulates well on MEA and 10% V-8 juice agar. The aerial mycelium is at first white but later turns ocher or brown. Chlamydospores are globose, produced in chains, brown, 7.5-15 μm diam and formed in the substratum or the aerial mycelium. Microsclerotia are seldom formed in the substratum. Simple, subverticillate or penicillately branched conidiophores arise from the substratum and the aerial mycelium. Conidiophores which are penicillately branched measure 180-260 × 3.0-7.5 μm; primary branches, 15-17 × 3 μm; two-five secondary branches occur and measure 8.0-13 × 2.5-3 μm; 2-8 phialides occur, which are navicular or cylindrical and measure 10-13 × 2-2.5 μm. Conidia are cylindric, hyaline, rounded on both ends, 0-1-septate, 14-23 × 2.5-3.7 μm. The sterile appendages are 80-140 μm long and 1.5-5 μm wide, and end in a narrowly clavate not septate vesicle of 20-70 × 4-5 μm.

Teleomorph: *Nectria camelliae* (Shipton) Boesewinkel. Trans. Brit. Mycol. Soc. 78: 555, 1982.

Ascocarps solitary or in small groups, superficial on substrate, without a stroma. Ascocarps scarlet, darker when dry, turning blood color in KOH, globose, oval, or pyriform, not collapsing when dry, described as 180-320 × 120-250 μm, found to be smaller after months in culture, 90-140 μm high × 80-110 μm diam., with small, broadly rounded papilla, ascocarp surface smooth or with a few, loose, globose cells. Asci unitunicate, 50-68 × 5-7 μm, clavate to cylindric, without specialized apical discharge mechanism, éight-spored. Ascospores 8.5-14 × 3-4 μm ellipsoid or slightly clavate, 1-septate, hyaline, smooth, uniseriate or biseriate toward apex of ascus.

Optimum temperature for fungal growth was 20-25 C (Fig. 3-B). Temperatures above 35 C and below 10 C completely inhibited the fungal growth.

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台灣新記錄之 *Cylindrocladium* 和 *Cylindrocladiella*

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本文報導兩種台灣新記錄之 *Cylindrocladium* 和 *Cylindrocladiella*: *Cylindrocladium floridanum* 和 *Cylindrocladiella infestans*。上述兩種眞菌均分離自森林土壤且它們都可以形成有性世代。*Cylindrocladium floridanum* 和 *Cylindrocladiella infestans* 的有性代分別是 *Calonectria kyotensis* 和 *Nectria camelliae*。