

SHORT COMMUNICATION

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## *Cylindrocladium* species and the related fungi isolated in the Ogasawara (Bonin) Islands and the Ryukyu Islands, Japan

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**Abstract** A total of 28 deuteromycetous isolates obtained from forest environments in the Ogasawara (Bonin) Islands and the Ryukyu Islands, Japan, were identified to five *Cylindrocladium* and related fungal species (*Calonectria kyotoensis* (anamorph: *Cylindrocladium floridanum*), *Cylindrocladiella lageniformis*, *Cylindrocladium camelliae*, *Cylindrocladium citri*, and *Cylindrocladium tenue*), excluding two unknowns. *Cylindrocladiella lageniformis* is a new record, and the others are rarely reported in Japan.

**Key words** Identification · Mitosporic fungi · Morphology · Teleomorph

During studies on the diversity of fungi in various substrates and habitats and their biology, various noteworthy fungi were isolated in the Ogasawara (Bonin) Islands and the Ryukyu Islands, Japan, including *Cylindrocladium* species, and several new species (Watanabe et al. 2001a–c).

Some *Cylindrocladium* species are well known as plant pathogens (Peerally 1991). In addition, one of the authors (T.W.) noted the discoloration of wood that might be related to wood decay during etiological studies of the decline of *Phellodendron amurense* Ruprecht in Tokyo in 1995 (Watanabe et al. 1995).

The purpose of this study is to identify a total of 28 isolates of *Cylindrocladium* and the related fungi, in relation to the lignocellulose-decomposing abilities to be described later in a separate paper.

The soil samples from the Ogasawara (Bonin) Islands were previously reported elsewhere (Watanabe et al. 2001a). Those from the Ryukyu Islands were obtained from

mountainous forest soils of Komidake in Iriomote Island, Omotodake, in Ishigaki Island, and Nagodake in Okinawa Island, Japan, in 2002. Samples of the fruit bodies including basidiomata, or decayed wood, were collected from nearly the same locations with soil samples in 2000–2002. Fungi were isolated using direct inoculation or the cucumber seed or toothpick bait method from soil samples (Watanabe et al. 2001a–c) and tissue isolation from basidiocarp or ascocarp tissues (Watanabe et al. 2003). In addition, spores dropped off or released from their fruit bodies within 2 min after their exposure on water agar (WA) were collected in plates in situ and isolated later by single sporing.

Some living isolates are deposited at the MAFF GeneBank, National Institute of Agrobiological Sciences (with the MAFF number) in Tsukuba, Japan and the Institute of Biological Resources and Functions (with TW number), National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki, Japan.

A total of 28 *Cylindrocladium* and related fungal isolates consisted of 19 from the Ogasawara (Bonin) Islands and 9 from the Ryukyu Islands. The Ogasawara fungi include 15 soil isolates obtained with cucumber seed (1 isolate) and toothpick bait (5), and direct inoculation (9), and 3 spores, and 1 tissue isolate from unknown basidiocarps. Three of 9 Ryukyu isolates were obtained with direct inoculation and 6 with the toothpick bait method from soil samples.

In most of these isolates, conidiophores are penicillate and subverticillate, bearing spore masses composed of cylindrical conidia and stipes and apical vesicles elongated from conidiophores, although some of them did not form stipes and vesicles. All these fungi are classified into a small conidium group, with less than 20- $\mu$ m-long aseptate or 1-septate conidia, and a large conidium group with 3-septate conidia more than 36  $\mu$ m long. Vesicles are globose, ellipsoidal, hastate, or various in shape. Chlamydospores are mostly catenulate. Microsclerotia and fruit bodies are formed by a few isolates. Their fresh colonies are pale brown, brown to dark brown, or dark reddish-brown, but during transfers, some isolates faded their brown colors and also their abilities to form chlamydospores gradually, mutating from conidial type to whitish mycelial type.

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These isolates were identified to five species, i.e., *Calonectria kyotoensis* Terashita (anamorph *Cylindrocladium floridanum* Sobers & Seymour) (4 isolates), *Cylindrocladiella lageniformis* Crous, Wingfield & Alfenas (2), *Cylindrocladium camelliae* Venkataramani & Venkata Ram (8), *Cylindrocladium citri* (Fawcett & Klotz) Boedijn & Reitsma (1), *Cylindrocladium tenue* (Bugnicourt) T. Watanabe (11), and unknown species (2). These identified fungi have not been previously reported in the Japanese mountainous forest habitats including the Ogasawara (Bonin) Islands and the Ryukyu Islands. The morphology and dimensions of each organ in these species are based on potato-dextrose agar (PDA "Nissui"; Nissui Pharmaceutical, Tokyo, Japan) or Difco malt agar cultures.

The names of these fungi were adopted following the respective original usage of *Cylindrocladiella* and *Cylindrocladium* species, because the separation of *Cylindrocladiella* from *Cylindrocladium* species (Boesewinkel 1982; Crous and Wingfield 1994; Peerally 1991) is still controversial. However, we believe the fungi in both genera are more or less common in morphology, and they may be better differentiated to one another on the species level, but not on the genus level, and therefore, we concur with Peerally (1991).

## Taxonomy

***Calonectria kyotoensis*** Terashita (anamorph: *Cylindrocladium floridanum* Sobers & Seymour), Trans. Mycol. Soc. Japan 8: 124–129, 1968.

Figs. 1–10

Four isolates are nearly identical with the original or previous descriptions of *Calonectria kyotoensis* and *Cylindrocladium floridanum* (Matsushima 1971; Morrison and French 1969; Sobers and Seymour 1967; Terashita 1968; Peerally 1974a, 1991). Perithecia reddish-brown, ellipsoidal to globose, 270–350 µm tall; peridium brown, pseudoparenchymatous, the cells nearly 14 µm in diameter; asci 8-spored, unitunicate, clavate 78–110 × 6.2–19 µm; ascospores hyaline, two-celled, ellipsoidal, curved, constricted at septum 26–33 × 5–6 µm.

Anamorph: Conidiophores 270–350 µm tall, (4.8–) 6–8 µm wide basally, 3–4 µm wide apically; primary branches 10–18.5 × 2.8–5 µm, secondary branches 10–14 (–20) × 2.8–3.6 µm, tertiary branches 9–14 × 2.8–3.6 µm, phialides 7–19 × 2.4–4 µm. Spore masses 18–28 µm in diameter. Stipes septate, narrowing toward apices, mostly 14–56 µm tall from the attachment of conidiophores to the vesicle, 4–6 µm wide basally. Vesicles globose, 8–14 µm in diameter. Conidia hyaline, 1–3-septate, 36–52 × 3–4 µm. Chlamydospores catenulate, 12.5–27.5 µm in diameter. Microsclerotia 54–115 (–135) µm in diameter.

Habitat: Forest soil and as an associate of unknown basidiomycete.

Specimens examined: Japan. Cultures from unknown basidiocarp tissue from Kuwanoki-yama, Hahajima, the

Ogasawara (Bonin) Islands, Tokyo, Jan. 14, 2001, collected and isolated by T. Watanabe (TW 01-273), and from forest soil at Omotodake, Ishigaki Island, Okinawa, Jan. 9, 2002, T. Watanabe (TW 02-191), from forest soil at Komidake, Iriomote Island, Okinawa, Jan. 11, 2002, T. Watanabe (TW 02-224) and at Nagodake, Okinawa Island, Okinawa, Jan. 13, 2002, T. Watanabe (TW 02-252).

The isolate TW 01-273 was obtained by tissue isolation from the Ogasawara sample and three others (TW 02-191, -224, -252) by the toothpick bait method from Okinawa samples.

Perithecia were formed by two isolates (TW 02-191, -252) studied on PDA or water agar with inocula from PDA or malt agar (MA) cultures within 20 days after inoculation under laboratory conditions nearly at 25°C.

***Cylindrocladiella lageniformis*** Crous, Wingfield & Alfenas, Mycol. Res. 97: 441–442, 1992.

Figs. 11–14

Two isolates are nearly identical morphologically with the original description of *Cylindrocladiella lageniformis* (Crous and Wingfield 1993). Conidiophores 134–210 µm tall, 3–6 µm wide basally; primary branches 12–18 × 2.4–3.6 µm, secondary branches 14–18 × 2.5–4 µm, phialides 12.8–18(–26) × 2–3.6 µm. Spore masses 18–28 µm in diameter. Stipes 20–82 µm tall from the attachment of conidiophores to the vesicle. Vesicles, characteristically hastate, rarely 1-septate, 11–37 µm long, 4–5 µm wide. Conidia hyaline, aseptate or 1-septate, 10–14 × 1.8–2.4 µm. Chlamydospores catenulate, granulate, pale brown, 10–16 µm in diameter.

Habitat: Forest soil.

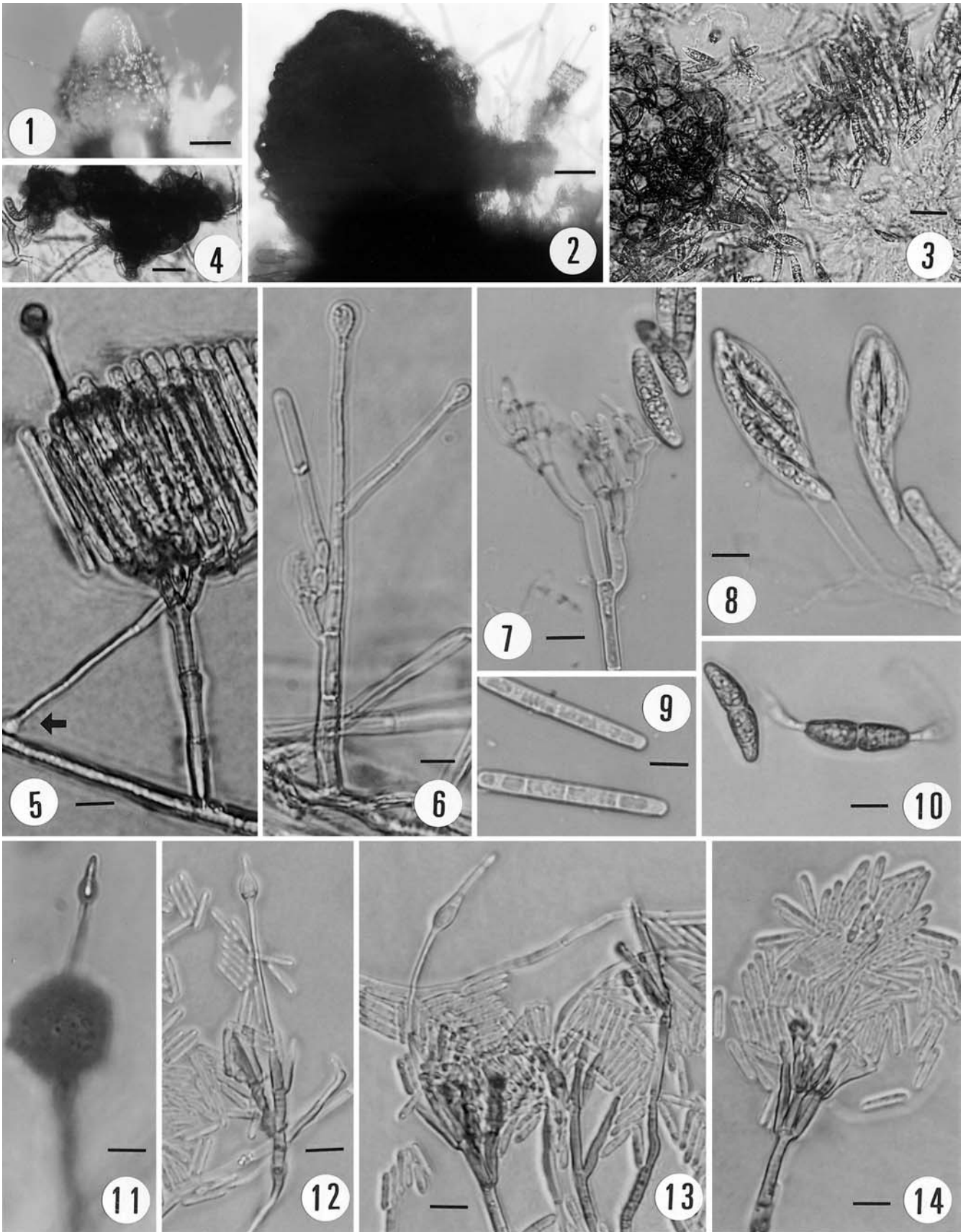
Specimens examined: The Ogasawara (Bonin) Islands, Tokyo, Japan, Cultures from forest soils of Kuwanokiyama, Hahajima, Jan. 13, 2001, collected and isolated by T. Watanabe (TW 01-127, -165).

The two isolates obtained by cucumber seed and toothpick bait method from Ogasawara samples were identified on the basis of vesicle morphology and named following the original name belonging to the genus *Cylindrocladiella*.

***Cylindrocladium camelliae*** Venkataramani & Venkata Ram, Curr. Sci. 30: 186, 1961.

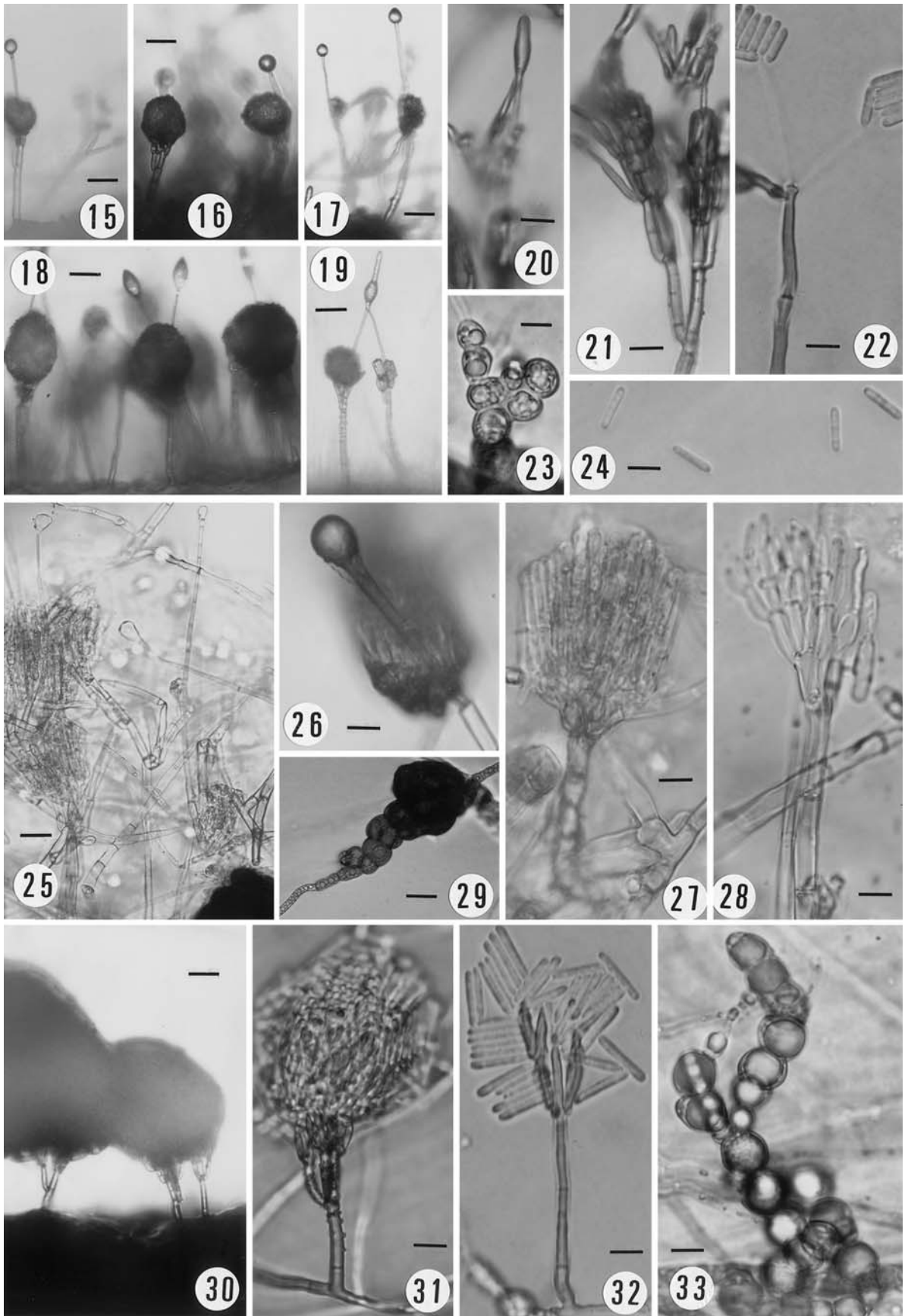
Figs. 15–24

Eight isolates are nearly identical morphologically with the previous descriptions of *Cylindrocladium camelliae* (Peerally 1974b; Terashita 1969; Watanabe 1994). Conidiophores (66–)80–300 µm tall, 3–6 µm wide basally; primary branches 20–26 × 2.8–3.6 µm, secondary branches 17–20 × 2.8–3.6 µm, tertiary branches 15.7–17 × 2.8–3.6 µm, phialides 10–24(–34) × 2.8–3.6 µm. Spore masses 18–28 µm in diameter. Stipes 20–80 µm tall from the attachment of conidiophores to the vesicle. Vesicles ellipsoidal, globose, cylindrical, or lanceolate 5–32 µm long, 7.5–18 µm wide. Conidia aseptate or 1-septate, 11–14 × 1.8–2.4 µm.



**Figs. 1–10.** *Calonectria kyotoensis* (anamorph: *Cyliandrocladium floridanum*). **1,2** Perithecia. **3** Peridium, asci, and ascospores. **4** Microsclerotia. **5,6** Habit showing conidiophores, stipes, vesicles, and mass of conidia (**5**). Note laterally elongated stipe and vesicle (*arrow*). **7** Part of conidiophore and ascospores. **8** Asci with ascospores. **9** Three-

septate conidia. **10** Ascospores germinated (*right*) and ungerminated. **Figs. 11–14.** *Cyliandrocladiella lageniformis*. **11** Habit showing conidiophore, stipe, hastate vesicle, and spore mass. **12–14** Penicillate (**14**) and subverticillate conidiophores, stipes, hastate vesicles (**12, 13**), and conidia. *Bars* **1** 100  $\mu$ m; **2** 50  $\mu$ m; **3, 4** 20  $\mu$ m; **5–14** 10  $\mu$ m



**Figs. 15–24.** *Cyindrocladium camelliae*. 15–20 Habit showing conidiophores, stipes, vesicles, and spore mass. 21, 22 Penicillate (21) and subverticillate conidiophores with conidia (22). 23 Catenulate chlamydospores. 24 Detached 1-septate conidia. **Figs. 25–29.** *Cyindrocladium citri*. 25 Habit showing conidiophores, stipes, vesicles, and part of microsclerotium. 26 Part of stipe and vesicle, and spore mass. 27, 28

Conidiophores with spore mass (27) and penicillate fertile portion (28). 29 Microsclerotium. **Figs. 30–33.** *Cyindrocladium tenue*. 30 Habit showing conidiophores and spore mass. 31, 32 Conidiophores with spore mass (31), penicillate fertile portion, and conidia (32). 33 Catenulate chlamydospores. Bars 15–19, 25, 29, 30 20  $\mu\text{m}$ ; 20–24, 26–28, 31–33 10  $\mu\text{m}$

Chlamydoconidia catenulate, pale yellowish-brown, 12–13 µm in diameter.

Habitat: Forest soil and as a spore associate of an unknown basidiomycete.

Specimens examined: Japan. Cultures from forest soil of Chibusayama, Hahajima, the Ogasawara (Bonin) Islands, Tokyo, Jan. 15, 2000, collected and isolated by T. Watanabe (TW 00-49), from forest soil of Kuwanokiyama, Hahajima, Jan. 14, 2001, T. Watanabe (TW 01-166, -167, -204, -205), from forest soil of Oogamiyama, Chichijima, Ogasawara, Tokyo, Jan. 15, 2001, T. Watanabe (TW 01-478), from spores associated with an unknown basidiocarp collected at Chichijima, Jan. 14, 2001, T. Watanabe (TW 01-485), and from forest soil at Komidake, Iriomote Island, Okinawa, Jan. 11, 2002, T. Watanabe (TW 02-110).

Among a total of eight isolates, six of seven isolates from soil samples (TW 00-49, 01-166, -167, -204, -205, 02-110) were obtained by the direct inoculation and another one (TW 01-478) by the toothpick bait method. One isolate (TW 01-485) was obtained as a spore associate of an unknown basidiomycete. Their vesicles are ellipsoidal (Fig. 18), globose (Figs. 15–17), or lanceolate (Fig. 20), often with apical cylindrical elongation (Fig. 19), and more than two shapes were often observed in single isolates. For example, both globose (Fig. 15) and lanceolate vesicles with apical cylindrical elongation (Fig. 19) were formed by isolate TW 02-110. In addition, occasionally vesicles were not readily observed.

*Cylindrocladium citri* (Fawcett & Klotz) Boedijn & Reitsma, Reinwardtia 1: 54–60, 1950.

Figs. 25–29

Isolate TW 00-52 was previously described as *Cylindrocladium* sp. (Watanabe 2002), but reidentified in this study. It is nearly identical to the original or previous descriptions of *Cylindrocladium citri* (Boedijn and Reitsma 1950; Crous and Wingfield 1994; Fawcett and Klotz 1937). Conidiophores mostly 140–330 µm tall, 6–8 µm wide basally, 3–4 µm wide apically; primary branches 11–23 × 3–5.8 µm, secondary branches 13.5–20 × 3.5–4.2 µm, tertiary branches 8.5–14 × 2.8–3.5 µm, phialides 7–19 × 2.4–4 µm. Spore masses 18–28 µm in diameter. Stipes septate, narrowing toward apices, 20–30 µm tall from the attachment of conidiophores to the vesicle. Vesicles globose or subglobose 14–20 × 6–14 µm. Conidia 3-septate, 32–48 × 3–4 µm. Chlamydoconidia catenulate, 12.5–27.5 µm in diameter. Microsclerotia 54–115 µm in diameter.

Habitat: Forest soil.

Specimens examined: The Ogasawara (Bonin) Islands, Tokyo, Japan, Culture from forest soils of Chibusayama, Hahajima, Jan. 14, 2000, collected and isolated by T. Watanabe (TW 00-52 = MAFF 238171).

The chlamydoconidia and sclerotia were not originally described by Boedijn and Reitsma (1950), but were later described by Crous and Wingfield (1994).

*Cylindrocladium tenue* (Bugnicourt) T. Watanabe, Mycologia 86: 151–156, 1994.

Figs. 30–33

A total of 11 isolates are nearly identical morphologically with the previous descriptions of *Cylindrocladium tenue* (Watanabe 1994). Conidiophores 60–300 µm tall, 3–6 µm wide basally; primary branches 10–32.8 × 2–4.2 µm, secondary branches 12.8–20 × 2–4.2 µm, phialides 10–34 × 2–4.2 µm. Spore masses 18–145 µm in diameter. Vesicles and stipes not formed. Conidia aseptate or 1-septate, (8.4–)11–16(–20) × 1.8–2.4 µm. Chlamydoconidia catenulate, 7–19 µm in diameter.

Habitat: Forest soil and as associates of unknown basidiomycete.

Specimens examined: Japan. Cultures from forest soils of Chibusayama, Hahajima, the Ogasawara (Bonin) Islands, Tokyo, Jan. 14, 2000, collected and isolated by T. Watanabe (TW 00-57), from spores associated with unknown basidiocarps collected at Ohgiura, Chichijima, the Ogasawara (Bonin) Islands, Tokyo, Jan. 13, 2001, collected and isolated by T. Watanabe (TW 01-79, -80), from forest soil, Kuwanokiyama, Hahajima, Jan. 12, 2001, T. Watanabe (TW 01-162, -424, -425, -426), from forest soil, Oogamiyama, Chichijima, Jan. 15, 2001, T. Watanabe (TW 01-479), from forest soil, Omotodake, Ishigaki Island, Okinawa, Jan. 9, 2002, T. Watanabe (TW 02-67), and from forest soil at Komidake, Iriomote Island, Okinawa, Jan. 11, 2002, T. Watanabe (TW 02-131, -215).

Nine of 11 isolates studied were obtained from soil samples by direct inoculation (TW 00-57, 01-162, 02-67, -131) or toothpick bait method (TW 01-424, -425, -426, -479, 02-215). Two others (TW 01-79, -80) were originated from spores associated with unknown basidiocarps. These fungi were identified on the basis of small aseptate or 1-septate cylindrical conidia, absence of stipe and vesicle, and catenulate chlamydoconidia.

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