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FULL PAPER

Yuuri Hirooka · Takao Kobayashi

Taxonomic studies of nectrioid fungi in Japan. I: The genus *Neonectria*

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Abstract Five species of the genus *Neonectria* (Nectriaceae, Hypocreales) collected from Japan are described with discussion and illustrated. Among them, one found on dead twigs of a broad-leaved tree is described as a new species, *Neonectria tokyoensis* (anamorph: *Cylindrocarpon tokyoense*). *Neonectria cinnamomea* and *Neo*. *discophora* are newly added to the Japanese mycobiota. *Nectria azureo-ostiolata*, recorded in 1977 from Japan, is reexamined as a synonym of *Neo*. *jungneri*. *Neonectria radicicola*, teleomorph of *Cy*. *destructans*, a well-known soil-borne plant pathogen in Japan, is newly recorded from Japan. Additional distribution records are provided for the *Neonectria* species hitherto recorded in Japan.

Key words *Cylindrocarpon* · Hypocreales · Nectriaceae · New species · Taxonomy

Introduction

Fries (1849) proposed the genus *Nectria* in the Hypocreales (Ascomycetes). Thereafter, the number of species belonging to the genus *Nectria* and related genera, so-called nectrioid fungi, increased year by year. Nectrioid fungi were mainly characterized by slightly bright colored and generally superficial perithecia containing ascospores with articulated cells, and their various phialidic anamorphs. The reexamination of nectrioid fungi has been carried out continuously since 1950 by several taxonomists (Booth 1959; Rogerson 1970; Samuels 1976; Seifert 1985; Samuels et al. 1990; Rossman et al. 1999; Lieckfeldt and Seifert 2000; Rossman 2000; Schroers 2001).

Y. Hirooka (✉)
Department of International Agricultural Development, Graduate School of Agriculture, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya-ku, Tokyo 156-8502, Japan
Tel. +81-3-5477-2412; Fax +81-3-5477-4032
e-mail: 70040001@nodai.ac.jp

T. Kobayashi
Department of International Agricultural Development, Tokyo University of Agriculture, Tokyo, Japan

In a recent taxonomic concept (Kirk et al. 2001), nectrioid fungi are distributed among six families with bright colored perithecia on/in the fleshy stroma in the Hypocreales: these are Bionectriaceae, Ceratostomataceae, Clavicipitaceae, Hypocreaceae, Nectriaceae, and Niessliaceae. Among them, Nectriaceae, Bionectriaceae, and Hypocreaceae are relatively close to each other.

Recently, nectrioid fungi have been noted for their biological activities, for parasitism on plants, fungi, and insects, and as producers of antibiotics and/or mycotoxins (Rossman 1996). About 600 species have been recognized throughout the world, in 23 genera of Nectriaceae and 27 genera of Bionectriaceae, after detailed reexamination (Rossman et al. 1999; Schroers et al. 1999; Schoch et al. 2000). However, only about 80 species of 9 genera belonging to these two families have been recorded in Japan.

The purpose of the present series is to reexamine the previously recorded Japanese nectrioid fungi under the recent taxonomic concepts (Rossman et al. 1999; Rossman 2000) and to describe the species newly found in Japan. In this first article, members of the genus *Neonectria* Wollenweber (Nectriaceae, Hypocreales) are presented.

Since the establishment of the genus *Neonectria* (Wollenweber 1917), its generic concept has been variously interpreted, and until recently it had long been synonymized under *Nectria* (for historical background, see Rossman et al. 1999). When Rossman et al. (1999) established a new family, Bionectriaceae, in the Hypocreales, *Neonectria* was revived with some other old genera, all of them long treated as synonyms under *Nectria*. According to Rossman et al. (1999), the genus *Neonectria* has *Cylindrocarpon* anamorph in common and is divided into five groups, the *Nectria coccinea*/*galligena* group, *N. mammoidea* group, *N. rugulosa* group, *N. radicicola* group, and *N. veuillotiana* group, based on the difference of surface structure of ascospores, the structure of perithecial walls, and the productivity of macro- and/or microconidia.

Since 2001, phylogenetic analysis has been used to support the taxonomy of *Neonectria* (Mantiri et al. 2001; Brayford et al. 2004; Halleen et al. 2004; Hirooka et al. 2005, 2006). Mantiri et al. (2001) and Brayford et al. (2004) analyzed mitochondrial small subunit ribosomal DNA (mSSU rDNA) sequence data of some *Neonectria* and *Cylindrocarpon* Wollenw. species, and their data supported the intrageneric grouping. Based on mitochondrial large subunit ribosomal DNA (mLSU rDNA), β-tubulin and nuclear ribosomal internal transcribed spacer (nrDNA ITS) region sequence data, Halleen et al. (2004) separated some species of the genus *Cylindrocarpon* included in the *N. mammoidea* group but without a known teleomorph to establish a new genus: *Campylocarpon* Halleen, Schroers & Crous.

At present, *Neonectria* includes more than 40 species (Booth 1959, 1966; Samuels 1988; Samuels and Brayford 1990, 1993, 1994; Brayford and Samuels 1993; Rossman et al. 1999; Mantiri et al. 2001; Brayford et al. 2004; Halleen et al. 2004; Hirooka et al. 2005, 2006; Kobayashi et al. 2005) in the world.

In Japan, eight *Neonectria* species with *Cylindrocarpon* anamorph have been recorded. In this article, five additional species, including one new species, of the genus *Neonectria* newly collected from Japan are described and illustrated. In addition, some additional records of distribution of the hitherto known *Neonectria* species in Japan are given.

Materials and methods

Specimens examined

Fresh specimens of the teleomorph and anamorph of the *Neonectria* and the single ascospore isolates were collected for the present study. All specimens were deposited in the Herbaria of Forest Mycology and Pathology (TFM), Forestry and Forest Products Research Institute (FFPRI), Tsukuba, Ibaraki, Japan.

Isolation

To obtain cultures from the fresh material, ascospore suspension in water taken from crushed perithecia was streaked on 2% (W/V) water agar (WA) and incubated at 25ºC. After 24 h, a single germinating ascospore was transferred directly to potato dextrose agar (PDA; Difco, Detroit, MI, USA) slants. Monoascospore isolates were preserved at MAFF Genebank, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan.

Morphological observation

Perithecia were picked up from the substrate with a fine needle. A drop of Shear's mounting fluid (mounting media; Kirk et al. 2001) was applied directly to the perithecia for rehydration. Slides were prepared by hand-sectioning from some dried materials. The morphological characteristics were examined by light microscopy (Olympus BX50; Olympus, Tokyo, Japan). The color reaction test for the perithecia was carried out using 3% KOH and 100% lactic acid (LA) (Rossman et al. 1999). The isolates were grown on PDA in 9-cm plastic Petri dishes at 25ºC for 7 days in the dark to evaluate the growth rates, colony color, and odor. For observation of the *Cylindrocarpon* anamorph, cultures were grown on synthetic low-nutrient agar (SNA; Nirenberg and O'Donnell 1998). SNA plates were incubated for 2 weeks at 25ºC in complete darkness, then for 2 weeks at 20ºC in either complete darkness or under continuous black light (BLB; Nirenberg 1990). In each case, 50 conidia randomly selected from individual isolate were measured. Color of the colony on PDA from the top and reverse view was described according to Kornerup and Wanscher (1978). For the scanning electron microscope (SEM) observation of the ascospore ornamentation, a small portion of dried specimen was crushed on aluminum foil to release ascospores. The aluminum foil was affixed on the stub, and the ascospores were platinum-palladium coated in an ion sputter (Hitachi E-102; Hitachi, Tokyo Japan), and observed under a SEM (Hitachi S-4000) operating at 5.0 kV.

Descriptions

Neonectria Wollenw., Ann. Mycol. 15: 52, 1917.

Type species: *Neonectria ramulariae* Wollenw. [≡*Nectria ramulariae* (Wollenw.) E. Müll.].

Synonym: *Chitinonectria* M. Morelet, Bull. Soc. Sci. Nat. Archéol. Toulon Var 178: 6, 1968 [Type species: *C. coccinea* (Pers.: Fr.) M. Morelet (≡*Sphaeria coccinea* Pers.: Fr.)].

1. ***Neonectria tokyoensis*** Hirooka & Tak. Kobay., sp. nov. Figs. 1–7, 11a–c

Perithecia in cortice emortuo, gregaria vel sparsa, ovalia vel pyriformia, 390–490 µm alta, 380–500 µm diametro, luteola, apice discoidea. Asci 105–130 × 10–22 µm, unitunicati, cylindrici vel leviter clavati, octospori. Ascosporae ellipsoideae vel fusiformes, 15–25 × 6–10 µm, 0–1-septatae, laeves vel verruculosae, hyalinae vel brunneae.

Holotypus: On dead twigs of broad-leaved tree, Okutama-cho, Tokyo, November 20, 2003, by Y. Hirooka (Y.H.) (TFM FPH-7809).

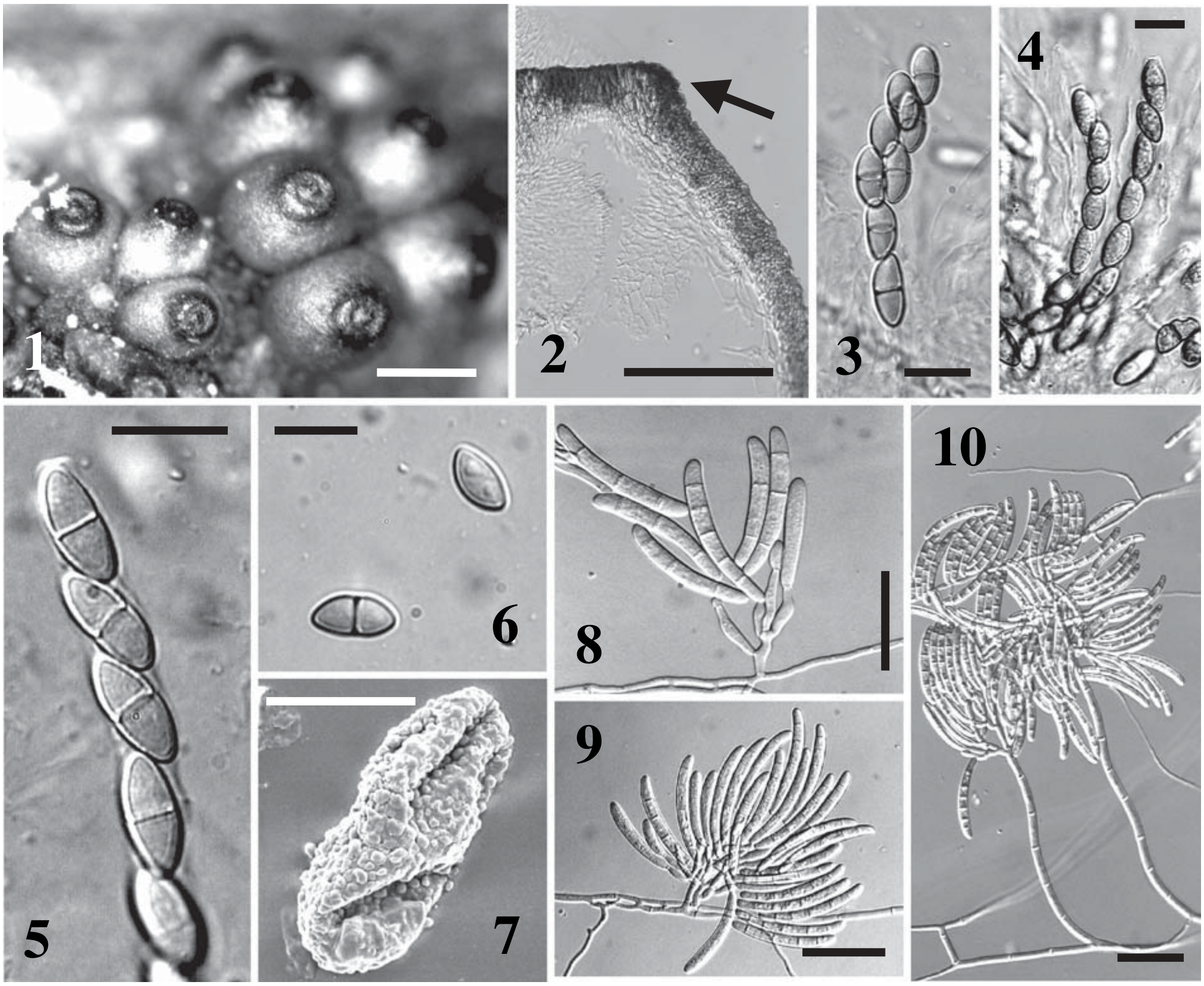
Etymology: Tokyo + -ensis; indicates the collected place of the type material.

Anamorph: *Cylindrocarpon tokyoense* Hirooka & Tak. Kobay., anam. nov. Figs. 8–10, 11d–f

Coloniae in PDA post 7 die ad 25ºC 15–25 mm diametro attingentes, spadiceae vel nigrae; reversum nigrum. Macroconidia in SNA cylindrica vel leviter fusiformia, ad apicaem leviter hamata, basi truncata, parum curvata, hyalina, 2–6-septata; illa 2-septata 25–41 × 6–6.5 µm; illa 3-septata 45–57 × 6–7 µm; illa 4-septata 52.5–62.5 × 6–7.5 µm; illa 5-septata 55–75 × 6–7.5 µm; illa 6-septata 57.5–78 × 6–7.5 µm. Microconidia et clamydosporae nulla.

Holotypus: On dead twigs of broad-leaved tree, Okutama-cho, Okutama-gun, Tokyo, November 20, 2003, by Y.H. (TFM FPH-7810).

Etymology: Tokyo + -ense; indicates the collected place of the type material.



Figs. 1–10. 1–7. *Neonectria tokyoensis* (TFM FPH-7809). **1** Perithecia on bark of a broad-leaved tree. **2** Median section of perithecial apex (*arrow*). **3** Ascus with eight ascospores arranged in biseriate. **4** Asci with eight ascospores arranged in uniseriate. **5** Close-up of ascal apex. **6** Ascospores showing 1 or 2 cells. **7** Surface ornamentation of ascospore by scanning electron microscope. **8–10** *Cylindrocarpon tokyoense* (TFM FPH-7810, MAFF 239825). **8** Aerial conidiophores and macroconidia. **9** Short sporodochial conidiophores and macroconidia. **10** Long sporodochial conidiophores and macroconidia. *Bars* **1**, **2** 200 µm; **3–6** 20 µm; **7** 10 µm; **8–10** 30 µm

Mycelium visible around perithecia or host. Stromata slightly immersed in the epidermal layer of outer bark, then erumpent, "textura intricata," "textura epidermoidea" or "textura angularis," sometimes producing conidia. Perithecia solitary or gregarious, 5–10 in group, subglobose to pyriform, narrowly constricted just below the papillate protrusion, 390–490 µm in height and 380–500 µm in diameter, not collapsed when dry, orange to pale red with black apex, not changing color in KOH and LA, smooth, with a papillary pore 135–260 µm wide. Cells at surface of perithecial wall composed of intertwined hyphae and becoming circular to angular near the perithecial apex. Perithecial wall 30–40 µm in thickness, composed of two layers. Asci cylindrical or clavate, 105–130 × 10–22 µm, without any structure at the apex, containing 8 spores in one to two rows. Ascospores elliptic to fusiform, 15–19 × 6–10 µm (1-celled), 17.5–25 × 7.5–10 µm (2-celled), smooth, but with minute spinulose under SEM, hyaline to brown.

Colony on PDA attaining a diameter of 15–25 mm in 7 days at 25°C, covered with concentric rings of initially buff-colored, becoming black-colored, sporodochia; reverse dark blood to black in the center and pale black at the margin; odor strongly acidic. Macroconidia normally produced on SNA and PDA. Sporulation on SNA in the dark starting within 3 weeks on aerial mycelium or conidiophores directly from the hyphae on agar surface, not produced richly. On SNA in the dark, conidia formed on the tips of conidiophores. Aerial conidiophores loosely to densely branched, sometimes verticillate, up to 17–52 µm long, 4–5 µm wide. Sporodochial conidiophores dimorphic, short or long. Short sporodochial conidiophores densely branched, up to 43–55 µm long, 4–5.5 µm wide. Long sporodochial conidiophores densely branched, up to 93–242 µm long, 5–6 µm wide. Aerial and sporodochial conidiogenous cells cylindrical, monophialidic, 10–20 × 3–5 µm, with apical thickenings and collarettes. Macroconidia cylindrical, rounded at the apex, rounded to truncate at the base, slightly curved, usually broadest in their upper third, hyaline, 2–6-septate, mostly 5-septae; 2-septate: 25–41 × 6–6.5 µm; 3-septate: 45–57 × 6–7 µm; 4-septate: 52.5–62.5 × 6–7.5 µm; 5-septate:

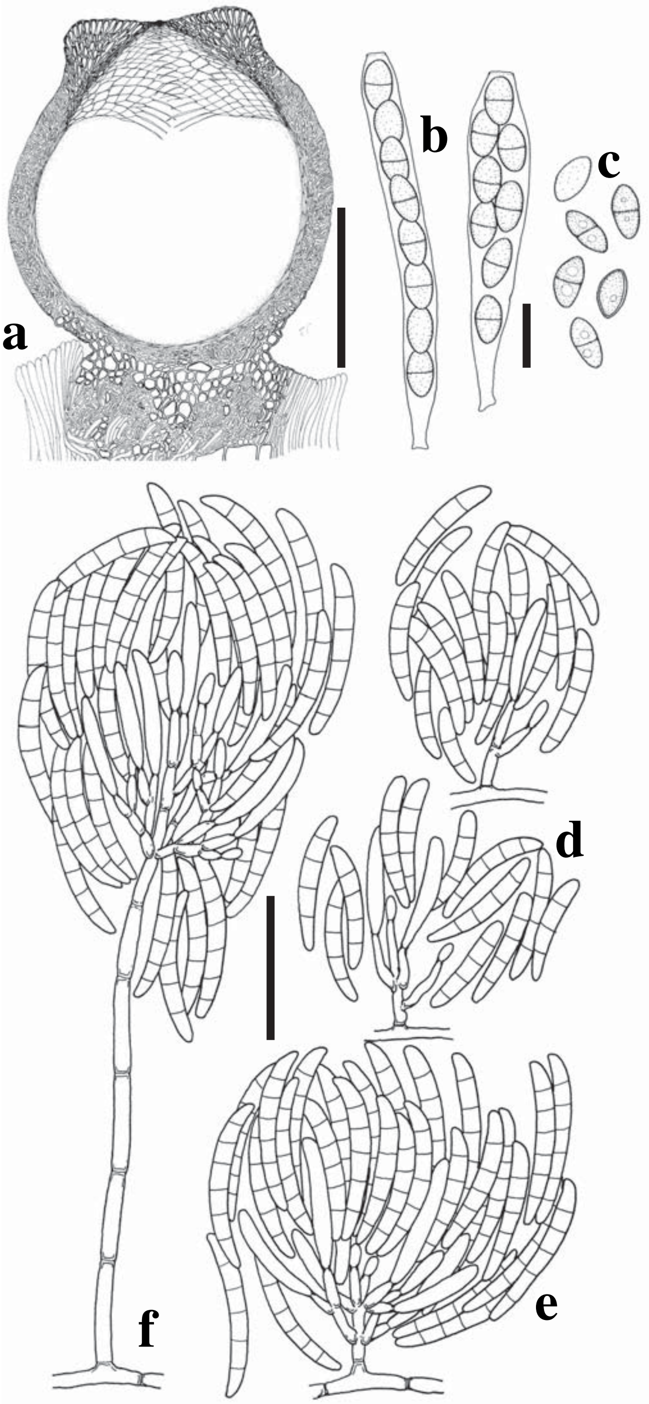


Fig. 11. Schematic figure of *Neonectria tokyoensis* on natural substratum (TFM FPH-7809) and *Cylindrocarpon tokyoense* on synthetic low-nutrient agar (SNA) in the dark (TFM FPH-7809, MAFF239825). **a** Median section of perithecium. **b** Asci with eight ascospores arranged in uniseriate or biseriate. **c** Ascospores. **d** Aerial conidiophores and macroconidia. **e** Short sporodochial conidiophores and macroconidia. **f** Long sporodochial conidiophores. *Bars* **a** 200 μm; **b**, **c** 20 μm; **d–f** 50 μm

55–75 × 6–7.5 μm; 6-septate: 57.5–78 × 6–7.5 μm. Microconidia and chlamydospores absent, but cells of old macroconidia may become swollen and thickened.

Isolate examined: On dead twigs of broad-leaved tree, Okutama-cho, Tokyo, November 20, 2003, by Y.H. (culture: MAFF 239825 ex holotype, TFM FPH-7810).

Note: *Neonectria tokyoensis* (anamorph: *Cylindrocarpon tokyoense*) is similar to *Neo. discophora* (Mont.) Mantiri & Samuels (*Cy. ianthothele* Wollenw. var. *majus* Wollenw.) in its hyphal perithecial wall cells, shape of ascospores, colony color, and absence of microconidia and chlamydospores (Figs. 1–11). However, it differs from the latter in orange to pale red color of perithecia (red to dark red in *Neo. discophora*) presence of papillate protrusion of perithecia (absence in *Neo. discophora*), negative KOH and LA reaction of perithecia (positive in *Neo. discophora*), larger ascospores (10–20 × 4–6 μm in *Neo. discophora*), and thicker macroconidia (43–73 × 4–6 μm in *Neo. discophora*) (Brayford et al. 2004). *Neonectria tokyoensis* is clearly distinguished from *Neo. discophora* in papillate protrusion in the perithecia are composed of circular or angular cells (Figs. 2, 11a), and presence of some unicellular ascospores (Fig. 6). Moreover, *Neo. tokyoensis* (*Cy. tokyoense*) on SNA is characterized by long sporodochial conidiophores (Figs. 10, 11f). This character is not found in any known species of *Cylindrocarpon* (Brayford et al. 2004). The present fungus belongings to the *Nectria mammoidea* (*Neo. discophora*) group.

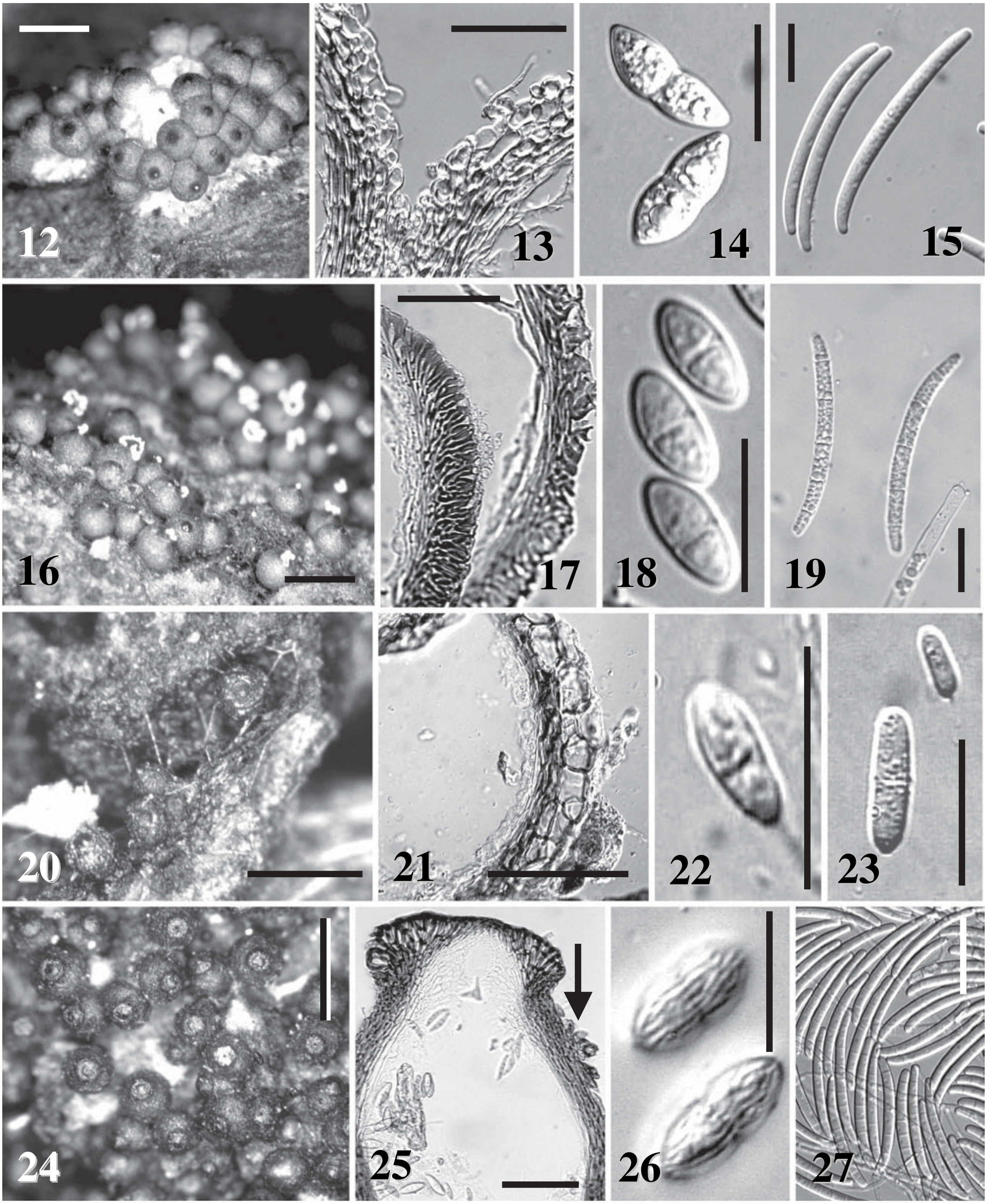
2. ***Neonectria cinnamomea*** (Brayford & Samuels) amuels & Brayford, Mycologia 96: 572, 2004. Figs. 12–14, 28a–c

≡*Nectria cinnamomea* Brayford & Samuels, Mycologia 85: 617, 1993.

Anamorph: *Cylindrocarpon cinnamomeum* Brayford & Samuels, Mycologia 85: 619, 1993. Fig. 15

Mycelium sometimes visible around perithecia. Perithecia solitary to gregarious in groups of 5–50, seated on scanty stroma, globose to pyriform, 250–550 μm in height and 200–420 μm in diameter, not collapsing when dry, light brown to dark brown, with dark-colored papilla, not changing color in KOH and LA, scaly to slightly warted. Cells at surface of perithecial wall composed of variable shape or circular to slightly angular, 4–15 μm in size, 1–3 μm in thickness. Perithecial wall 40–100 μm in thickness, composed of three layers. Asci clavate, 100–117 × 15–22.5 μm, without any structure at the apex, 8 spores in two rows. Ascospores ellipsoidal to fusiform, 22.5–32.5 × 7.5–14 μm, equally 2-celled, not constricted at the septum, smooth, hyaline, with slightly wrinkled sheath, completely filling each ascus.

Colony on PDA attaining a diameter of 20–25 mm in 7 days at 25°C, cottony with mycelium or pionnotes, whitish-brown; reverse brick-red to black; odor absent. Macroconidia abundant on PDA and SNA. Aerial and sporodochial macroconidia on SNA cylindrical, uniformly curved at the tip, slightly curved, with a rounded apex and tapering evenly toward a flat and truncate basal cell, 3-septate, 67–95 × 5 7.5 μm. Perithecia produced on PDA. Chlamydospores formed poorly in conidia, globose, terminal, single, hyaline, smooth, 10–12 μm in diameter. Microconidia and sclerotia absent.



Figs. 12–27. 12–15. *Neonectria cinnamomea* (anamorph: *Cylindrocarpon cinnamomeum*) (TFM FPH-7789, 7790, MAFF 239835). **12** Perithecia on natural substratum. **13** Section through lateral perithecial wall. **14** Ascospores in surface view. **15** Macroconidia. **16–19** *Neo. discophora* (*Cy. ianthothele* var. *majus*) (TFM FPH-7801, 7802, MAFF 239840). **16** Perithecia on natural substratum. **17** Section through lateral perithecial wall. **18** Ascospores in surface view. **19** Macroconidia. **20–23** *Neo. radicicola* (*Cy. destructans*) (TFM FPH-7807, 7808, MAFF 239831). **20** Perithecia on natural substratum. **21** Section through lateral perithecial wall. **22** Ascospore in surface view. **23** Microconidia. **24–27** *Neo. jungneri* (*Cy. victoriae*) (TFM FPH-7775, 7776, MAFF 239839). **24** Flask-shaped perithecia on natural substratum. **25** Median section of perithecial apex (*arrow*). **26** Ascospores showing coarsely striate surface. **27** Macroconidia on SNA. *Bars* **12**, **16**, **20**, **24** 1000 µm; **13**, **17**, **21**, **25** 100 µm; **14**, **15**, **18**, **19**, **22**, **23**, **26** 20 µm; **27** 50 µm

Specimens and isolate examined: On *Cinnamomum camphora* (L.) J. Presl, Forest Research Station of Kagoshima University, Tarumizu-shi, Kagoshima Pref., August 26, 2002, by Y.H. (teleomorph: TFM FPH-7789; anamorph: TFM FPH-7790; culture: MAFF 239835).

Note: *Neonectria cinnamomea* (*Cylindrocarpon cinnamomeum*) was newly added to the Japanese mycobiota. The present fungus has brown perithecia with negative KOH reaction, surface of wrinkled sheath ascospores, only 3-septate conidia on culture, and brick-red colony color (Figs. 14, 15, 28b,c). Macroscopic and microscopic characteristics of Japanese specimens almost matched to those of *Neo*. *cinnamomea* (*Cy*. *cinnamomeum*) provided by Brayford and Samuels (1993), except for its ascospores without conspicuous sheath.

3. ***Neonectria discophora*** (Mont.) Mantiri & Samuels, Can. J. Bot. 79: 339, 2001. var. ***discophora*** Figs. 16–18, 29a–c

≡*Sphaeria discophora* Mont., Ann. Sci. Nat. Bot. II 3: 353, 1835.

=*Nectria mammoidea* W. Phillips & Plower., Grevillea 3: 126, 1875.

≡*Creonectria mammoidea* (W. Phillip. & Plower.) Seaver, Mycologia 1: 188, 1909.

=*Nectria umbilicata* Henn., Hedwigia 41: 3, 1902.

=*Nectria nelumbicola* Henn., Verh. Bot. Vereins Prov. Brandenburg 40: 151, 1898. Fide Höhnel & Weese, Ann. Mycol. 8: 467, 1910.

=*Nectria pinea* Dingley, Trans. Roy. Soc. New Zealand 79: 198, 1951.

Anamorph: *Cylindrocarpon ianthothele* Wollenw. var. *majus* Wollenw., Z. Parasitenkd. (Berlin), 1: 161, 1928. Fig. 19

=*Cylindrocarpon ianthiothele* var. *rugulosum* C. Booth, Mycol. Pap. 104: 25, 1966.

=*Cylindrocarpon pineum* C. Booth, Mycol. Pap. 104: 26, 1966.

Mycelium not visible around perithecia or on host. Perithecia solitary or gregarious in groups of 5–20, with minute erumpent hyphal stroma, nonpapillate, globose, 280–550 μm in height and 220–460 μm in diameter, not collapsing when dry, red to dark red with dark red papilla, uniformly purple in KOH, yellow in LA, smooth to slightly warted. Cells at surface of perithecial wall composed of intertwined hyphae. Perithecial wall 30–50 μm in thickness, composed of two layers. Asci cylindrical, 70–103 × 7.5–10 μm, with a ring at the apex, with 8 spores in one row. Ascospores ellipsoidal, 11–17 × 4–8 μm, equally 2-celled, spinulose, colorless or light brown, completely filling each ascus.

Colony on PDA attaining a diameter of 20–25 mm in 7 days at 25ºC, violet to dark violet, restricted to center or margin; reverse dark violet; odor absent. Macroconidia abundant on PDA and SNA. Aerial and sporodochial macroconidia on SNA cylindrical, uniformly curved at the tip, slightly curved, with a round apex and tapering evenly towards a flat and truncate basal cell, mainly 3–5-septate; 3-septate: 33–60 × 4–6 μm; 4-septate: 60–70 × 5–6 μm; 5-septate: 65–80 × 5–6 μm. Perithecia produced on PDA. Chlamydospores, microconidia and sclerotia absent.

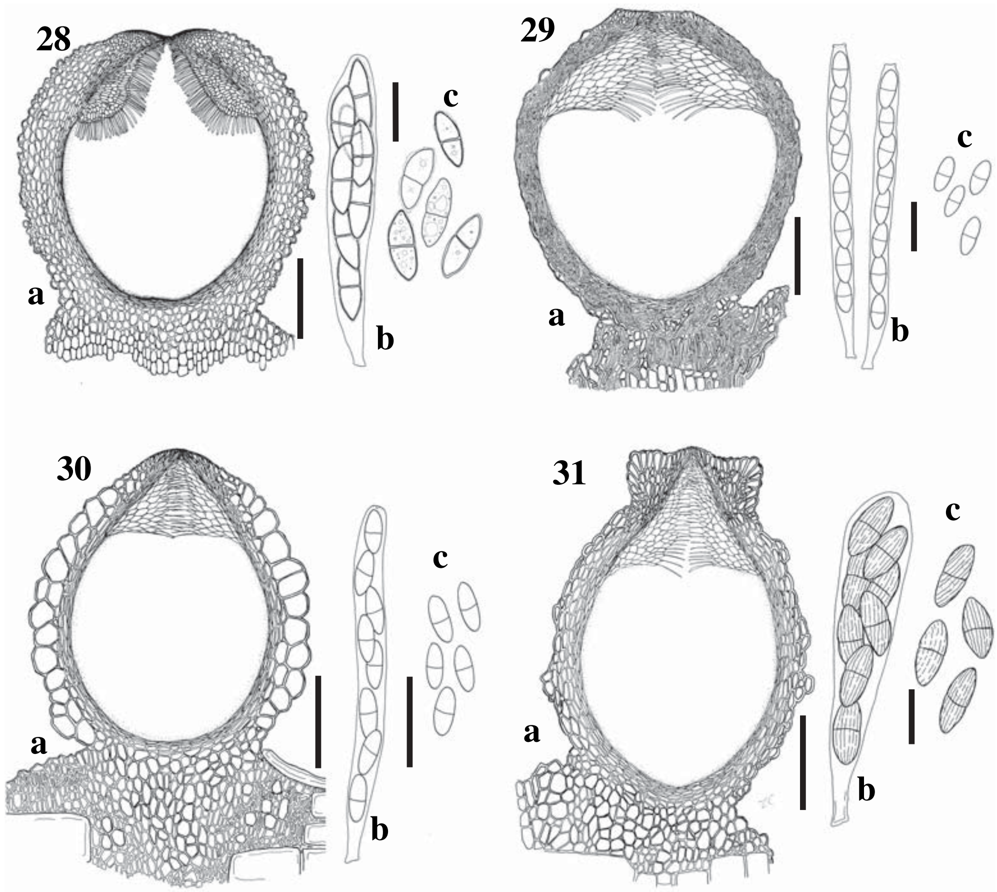
Specimens and isolates examined: On bark, Tosa-cho, Kochi Pref., August 4, 2003, by Y.H. (teleomorph: TFM FPH-7791; anamorph: TFM FPH-7792; culture: MAFF 239837); on twigs of *Cryptomeria japonica* (L. f.) D. Don, Tosa-cho, Kochi Pref., August 4, 2003, by Y.H. (teleomorph: TFM FPH-7793; anamorph: TFM FPH-7794; culture: MAFF 239822); on bark of decayed tree, Okutama-cho, Tokyo, November 20, 2003, by Y.H. (teleomorph: TFM FPH-7795; anamorph: TFM FPH-7796; culture: MAFF 239843); on bark of fallen twigs, Akiyu-cho, Taihaku-ku, Miyagi-shi, Miyagi Pref., August 4, 2004, by Y.H. (teleomorph: TFM FPH-7797; anamorph: TFM FPH-7798; culture: MAFF 239836); on bark of dead twigs, Rifu-cho, Miyagi-gun, Miyagi Pref., August 5, 2004, by Y.H. (teleomorph: TFM FPH-7799; anamorph: TFM FPH-7800; culture: MAFF 239821); on bark of dead twigs, Yamakita-cho, Asigarakami-gun, Kanagawa Pref., October 30, 2004, by Y.H. (teleomorph: TFM FPH-7801; anamorph: TFM FPH-7802; culture: MAFF 239840); on *Fagus crenata* Blume, Idenzawa, Nakagawa, Yamakita-cho, Asigarakami-gun, Kanagawa Pref., April 17, 2005, by Y.H. (teleomorph: TFM FPH-7803; anamorph: TFM FPH-7804; culture: MAFF 239844); on bark of fallen tree, Sakaigatake, Hahajima, Ogasawara-mura, Tokyo (Bonin Islands), June 22, 2005, by Y.H. (teleomorph: TFM FPH-7805; anamorph: TFM FPH-7806; culture: MAFF 239838).

Note: Teleomorph state of *Neonectria discophora* was newly added to the Japanese mycobiota. It was collected in various parts of Japan. Its anamorph state, *Cylindrocarpon ianthothele* var. *majus*, was known as a pathogenic fungus of *Camellia* spp. in Japan (Hirokawa and Takaya 1970). Ono and Kobayashi (2001) recorded a variety of this species, *Neo*. *discophora* var. *rubi* (Osterw.) Brayford & Samuels (*Cylindrocarpon ianthothele* var. *ianthothele* Wollenw.), on *Cercidiphyllum japonicum* Siebold & Zucc. in Aomori Prefecture [as "*Nectria mammoidea* W. Phillips & Plowr. var. *rubi* (Osterw.) Weese"]. Matsushima (1975) also recorded anamorph states of *Neo*. *discophora* var. *rubi* in soil collected in Hokkaido.

Booth (1959, 1966) asserted that *Nectria mammoidea* (=*Neo*. *discophora*) is a heterogeneous species based on the morphology of the teleomorph and anamorph. More recently, molecular data support the presence of paraphyletic groups of this species (Brayford et al. 2004; Halleen et al. 2004). Halleen et al. (2004) established the new genus *Campylocarpon* for some anamorph groups of *Neo*. *discophora* on the basis of the morphological and molecular characteristics. At present, the teleomorph of *Campylocarpon* is unknown.

4. ***Neonectria radicicola*** (Gerlach & L. Nilsson) Mantiri & Samuels, Can. J. Bot. 79: 339, 2001. Figs. 20–22, 30a–c

≡*Nectria radicicola* Gerlach & L. Nilsson, Phytopath. Z. 48: 255, 1963.



Figs. 28–31. 28 Schematic figures of *Neonectria cinnamomea* (TFM FPH-7789). **a** Median section of perithecium. **b** Ascus with biseriate ascospores. **c** Ascospores. **29** Schematic figures of *Neo. discophora* (TFM FPH-7801). **a** Median section of perithecium. **b** Asci with uniseriate ascospores. **c** Ascospores. **30** Schematic figures of *Neo. radicicola* (TFM FPH-7807). **a** Median section of perithecium. **b** Ascus with irregularly uniseriate ascospores. **c** Ascospores. **31** Schematic figures of *Neo. jungneri* (TFM FPH-7775). **a** Median section of perithecium. **b** Ascus with biseriate ascospores. **c** Ascospores. *Bars* (all) **a** 100 µm; **b**, **c** 20 µm

Anamorph: *Cylindrocarpon destructans* (Zinssm.) Scholten, Netherl. J. Plant Pathol. 70 (suppl.) (2): 9, 1964. Fig. 23

≡*Ramularia destructans* Zinssm., Phytopathology 8: 570, 1918.

≡*Cylindrocarpon radicicola* Wollenw., Fus. autogr. delin. 2: 651, 1924.

Mycelium not visible around perithecia or on host. Perithecia solitary to gregarious in small groups, erumpent, with scanty stroma, subglobose to pyriform, 200–340 µm in height and 180–250 µm in diameter, collapsing when dry, red with darker red papilla, not changing color in KOH and LA. Cells at surface of perithecial wall circular to angular, 15–35 µm in thickness. Perithecial wall 20–55 µm wide, composed of two layers. Asci clavate, 30–60 × 5–7.5 µm, with a ring at the apex, with 8 spores in one to two rows. Ascospores ellipsoidal, 7.5–12.5 × 2.5–5 µm, equally 2-celled, coarsely smooth, sometimes appearing spinulose, colorless, completely filling each ascus.

Colony on PDA attaining a diameter of 40–50 mm in 7 days at 25ºC; aerial mycelium white to buff, eventually covered with concentric rings of chestnut brown colored sporodochia; reverse dark brown at the center and chestnut-brown at the margin; odor absent. Microconidia and macroconidia abundant on PDA and SNA. Aerial and sporodochial microconidia produced on SNA, cylindrical, ellipsoid, or globose with rounded ends, 0–1-septate; 0-septate: 5–10 × 2.5–5 µm; 1-septate: 10–12.5 × 2.5–5 µm. Sporodochial macroconidia cylindrical, seldom curved, with a round apex and protruding basal abscission scar, 0-8-septate; but mostly 3-septate and 25–38 × 5–7.5 µm. Perithecia not produced in culture. Chlamydospores and sclerotia absent.

Specimens and isolate examined: On bark of fallen twigs, Takao, Hachioji-shi, Tokyo, July 21, 2003, by S. Inaba (teleomorph: TFM FPH-7807; anamorph: TFM FPH-7808; culture: MAFF 239831).

Note: The morphological characteristics of our specimens and isolate agreed well with the previous description given for *Nectria radicicola* by Samuels and Brayford (1990). Phylogenetically, *Neo. radicicola* (*Cy. destructans*) has been known to have a wide variation (Seifert et al. 2003; Halleen et al. 2004). Seifert et al. (2003) analyzed β-tubulin genes and nrDNA ITS sequence data of some *Cy. destructans* and concluded that there are two anamorphic varieties of *Neo. radicicola*. The teleomorph of this fungus was newly recorded from Japan. *Cylindrocarpon destructans* is well known as a widely distributed soil-borne fungus in Japan. It often causes seedling blight and root rot of various plants (Phytopathological Society of Japan 2000).

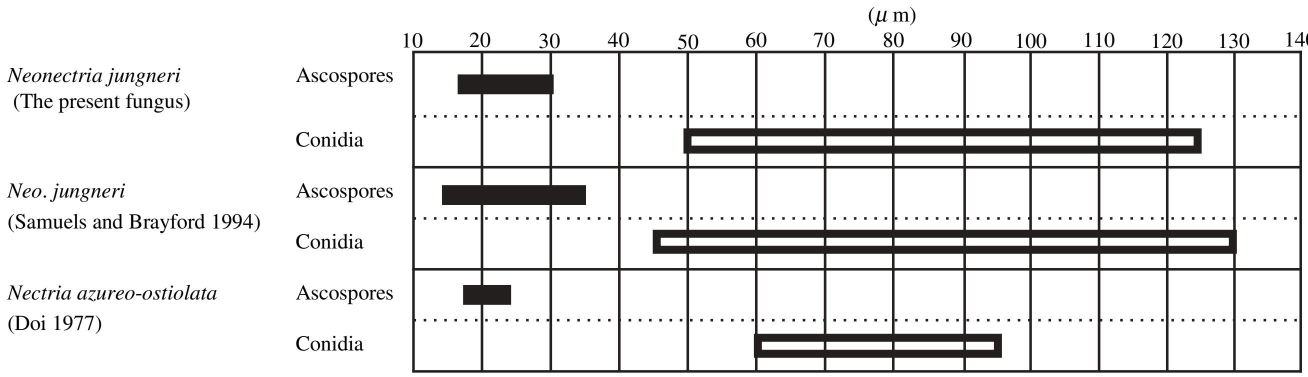


Fig. 32. Comparison of length of ascospores and conidia among *Neonectria jungneri* (the present fungi), *Neo. jungneri*, and *Nectria azureo-ostiolata*. Size ranges of ascospores and conidia are shown as *black and white lines*, respectively

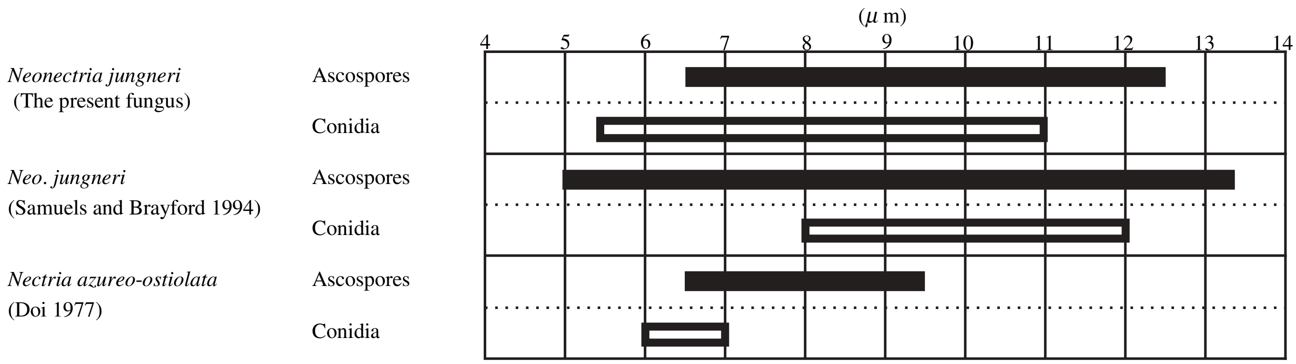


Fig. 33. Comparison of width of ascospores and conidia among *Neonectria jungneri* (the present fungi), *Neo. jungneri*, and *Nectria azureo-ostiolata*. Size ranges of ascospores and conidia are shown as *black and white lines*, respectively

5. ***Neonectria jungneri*** (Henn.) Samuels & Brayford, Mycologia 96: 580, 2004. Figs. 24–26, 31a–c

≡*Nectria jungneri* Henn., Engler's Bot. Jahrb. Syst. 22: 75, 1895.

=*Nectria striatospora* Zimm., Centralbl. Bakteriol. II, 7: 105, 1901.

=*Sphaerostilbe marmellosensis* Henn., Hedwigia 43: 245, 1904.

=*Nectria theobromae* Massee, Kew Bull. 1908: 218, 1908.

=*Nectria azureoostiolata* Yoshim. Doi, Mem. Natn. Sci. Mus., Tokyo 10: 23, 1977.

Anamorph: *Cylindrocarpon victoriae* Wollenw., Z. Parasitenk. (Berlin) 1: 161, 1928. Fig. 27

Mycelium not visible around perithecia or host. Stromata formed in epidermal layer of outer bark, "textura angularis." Perithecia gregarious, 10–100 in group, subglobose to pyriform, or elongated to flask shaped, narrowly constricted just below the papillate protrusion, 270–470 μm in height and 210–380 μm in diameter, not collapsing when dry, red with pale purplish black to black papilla, uniformly dark red in KOH and yellow in LA, slightly warted; with papillary pores 135–260 μm wide. Cells at surface of perithecial wall composed of variable shapes or circular to angular in outline, 12–20 μm in size, wall 2–3.5 μm. Perithecial wall 22–40 μm in thickness, consisting of two layers. Asci clavate, 70–100 × 10–25 μm, having simple structure at the apex, with 8 spores in two rows. Ascospores fusiform, 17–30 × 6.5–12.5 μm, equally 2-celled, ornamented coarsely warts in striate, hyaline, completely filling each ascus.

Colony on PDA attaining a diameter of 35–50 mm in 7 days at 25ºC, cottony with aerial mycelia with concentric rings of yellow to buff; reverse buff at the center and yellow at the margin; odor absent. Macroconidia abundant on PDA and SNA. Sporulation on SNA in the dark starts within 2 weeks in aerial mycelium or conidiophores arising directly from the agar surface richly. Aerial conidiophores unbranched, becoming loosely to densely branched, sometimes verticillate, up to 13–28 μm long, 3.5–5 μm wide. Sporodochial conidiophores densely branched, up to 115–212 μm long, 4–6 μm wide. Aerial and sporodochial conidiogenous cells cylindrical, monophialidic, 13–27 × 3–4 μm, with apical thickenings and collarettes. Macroconidia long cylindrical, usually broadest in their upper third, slightly curved, with a rounded apex and tapering evenly toward a flat and truncate basal cell, 4–9-septate; 4-septate: 50–90 × 5.5–7.5 μm; 5-septate: 69–100 × 5.5–7.5 μm; 6-septate: 75–95 × 5.5–7.5 μm; 7-septate: 80–102 × 6.5–8.5 μm; 8-septate: 83–121 × 6.5–10.5 μm; 9-septate: 85–125 × 6.5–11 μm. Perithecia not produced in culture. Chlamydospores and microconidia absent.

Table 1. Distribution of *Neonectria* species hitherto known in Japan

Species of *Neonectria*	Locality[a]	References of the Japanese species
Neonectria amamiensis (*Cylindrocarpon amamiense*)[b]	**Kagoshima**, **Okinawa (3)**[c]	Hirooka et al. 2006
Neo. castaneicola (*Cy. castaneicola*)	Miyagi, **Gunma**, **Tokyo (11)**, **Kanagawa**, **Yamanashi**, **Shizuoka**, Hyogo, **Kochi**, **Kumamoto (3)**	Kobayashi et al. 2005; Hirooka et al. 2005; Yamamoto and Oyasu 1958
Neo. coccinea (*Cy. candidum*)	**Miyagi**, **Ibaraki**, **Tokyo (2)**, **Kanagawa (4)**, **Niigata**, **Yamanashi**, **Nagano (2)**, **Shizuoka**, **Kochi (2)**, **Fukuoka**, Oita	Yasuda 1922
Neo. galligena (*Cy. heteronema*)	Hokkaido, Aomori, **Miyagi**, Fukushima	Komiya 1982; Sasaki 1985; Terui 1959
Neo. discophora var. *rubi* (*Cy. ianthothele* var. *ianthothele*)	Aomori	Ono and Kobayashi 2001
Neo. lucida (*Cy. lucidum*)	No locality data	Brayford et al. 2004
Neo. rugulosa (*Cy.rugulosum*)	Gunma, Tokyo (7), Kanagawa (3), Shizuoka, Kagoshima, Okinawa (6)	Hirooka et al. 2005
Neo. veuillotiana (*Cy. candidulum*)	**Miyagi**, **Tokyo**, **Yamanashi**, **Kagawa**, Tottori	Brayford and Samuels 1993

[a] The names of localities described by bold font indicate newly recorded localities of hitherto known Japanese species
[b] Anamorphic name in parentheses
[c] Number of specimens

Specimens and isolates examined: On bark of fallen twigs, Ishikawa-shi, Okinawa Pref. (Okinawa Island), January 20, 2003, by Y.H. (teleomorph: TFM FPH-7775; anamorph: TFM FPH-7776; culture: MAFF 239839); on bark of old tree, Onna-son Kunigami-gun, Okinawa Pref. (Okinawa Island), June 22, 2003, by Y.H. (teleomorph: TFM FPH-7777; anamorph: TFM FPH-7778; culture: MAFF 239845); on bark of fallen twigs, Nakamura-shi, Kochi Pref., August 6, 2003, by Y.H. (teleomorph: TFM FPH-7779; anamorph: TFM FPH-7780; culture: MAFF 239834); on bark of tree stump, Kurokami, Kumamoto-shi, Kumamoto Pref., June 6, 2004, by Y.H. (teleomorph: TFM FPH-7781; anamorph: TFM FPH-7782; culture: MAFF 239830); on bark of fallen twigs, Hakusan-cho, Ichisi-gun, Mie Pref., August 25, 2004, by Y.H. (teleomorph: TFM FPH-7783; anamorph: TFM FPH-7784; culture: MAFF 239833); on bark of fallen twigs, Mt. Shigure, Chichijima, Ogasawara-mura, Tokyo (Bonin Islands), June 17, 2005, by Y.H. (teleomorph: TFM FPH-7785; anamorph: TFM FPH-7786; culture: MAFF 239846); on bark of fallen twigs, Sakaigatake, Hahajima, Ogasawara-mura, Tokyo (Bonin Islands), June 22, 2005, by Y.H. (teleomorph: TFM FPH-7787; anamorph: TFM FPH-7788; culture: MAFF 239832).

Note: All our specimens showed the same characteristics of *Neo. jungneri* (anamorph: *Cy. victoriae*) in the size of ascospores (17–30 × 6.5–12.5 µm vs. 14.7–36 × 5–13.3 µm) and macroconidia (50–125 × 5.5–11 µm vs. 46–130 × 8–12 µm) (Figs. 26, 27, 31c, 32, 33). Morphologically, *Nectria azureo-ostiolata* (*Fusarium*-type) recorded in Chichijima, Bonin Islands, Japan by Doi (1977) is very similar to *Neo. jungneri*.

Nectria azureo-ostiolata (*Fusarium*-type) and *Neo. jungneri* (*Cy. victoriae*) have similar morphological characteristics except for the surface of perithecial walls (slightly warted vs. smooth), length of ascospores (17–23 × 6.5–9.5 µm vs. 14.7–36 × 5–13.3 µm), and width of macroconidia (60–95 × 6–7 µm vs. 46–130 × 8–12 µm) (Figs. 32, 33). Samuels and Brayford (1994) were uncertain whether to include *N. azureo-ostiolata* as a synonym of *Neo. jungneri* because they could not find the type material of *N. azureo-ostiolata*. However, the sizes of ascospores and macroconidia of our data were completely included in the range of those of *N. azureo-ostiolata* (Figs. 32, 33). In addition, we observed the authentic material collected by Doi, and on the basis of these examinations, we treated *N. azureo-ostiolata* as a synonym of *Neo. jungneri*.

Distribution of *Neonectria* species hitherto known in Japan

Based on the published reports and on the collected specimens by the authors, five *Neonectria* species were found to be newly distributed in various parts of Japan (Table 1).

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