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***Cordyceps owariensis* f. *viridescens* and its new *Nomuraea* anamorph**

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Abstract *Cordyceps owariensis* f. *viridescens* forma nov. (Nom. Jap., Amami-yarinohosemitake) is described from an underground nymph of *Platypleura kuroiwae* collected in Amami-oshima Island, southwestern Japan. Cultural isolations were made from ascospores of the fresh material of this fungus, and consequently a new hyphomycete was developed as its anamorph. *Nomuraea owariensis* is described to accommodate this anamorphic state. This is the first report on the association of a *Nomuraea* anamorph with cicadicolous *Cordyceps* species.

Key words *Cordyceps* · Entomogenous fungus · Hyphomycetes · *Nomuraea* · Systematics

Introduction

Cordyceps owariensis Kobayasi (Nom. Jap., Hayakawasemitake) was first described in 1939 as a cicadicolous fungus that was collected by A. Hayakawa at Shinkawamachi, Nishikasugai-gun, Aichi Prefecture, Japan (Kobayasi 1939, 1941; Kobayasi and Shimizu 1963, 1983; Shimizu 1994). This fungus appears to be very rare in nature and its distribution is restricted in western Japan, because further collections were made from Amami-oshima Island, Kagoshima Prefecture, in 1990 by K. Fujimoto, and from

Miki-cho, Kida-gun, Kagawa Prefecture, in 1991 by S. Mitani (Shimizu 1994). As well as the type material, these collections were always found on the nymph of cicadas (*Platypleura kaempferi* and *P. kuroiwae*) in the moist fallen leaf litter of laurel or deciduous broadleaf forests. The summarized characters of *C. owariensis* are (1) solitary, pale yellow, cylindrical stroma arising from the head of the host, (2) bifurcate (at lower part), glabrous stalk, (3) subulate fertile part that is not clearly defined from the stalk, (4) obliquely inserted, ampullaceous perithecia, (5) cylindrical, capitate asci, and (6) filiform, multiseptate ascospores. In the specimens of *C. owariensis*, there are no previous records of its anamorphic state. Comparison of *C. owariensis* with other species of *Cordyceps* from Cicadidae shows that, because of the formation of its obliquely inserted perithecia, this species is a very distinctive fungus and unlikely to be confused with any other so far known (Kobayasi and Shimizu 1963; Kobayasi 1982). *Cordyceps cicadicola* Teng has obliquely inserted perithecia, but this species differed significantly in its habitat because the small, gregarious to subcaespitose stromata developed on all parts of the ventral side of the adult cicada cadavers (Teng 1996).

Meantime, in 1990 and thereafter, several collections of *Cordyceps* sp. have been found on underground nymphs of cicada (*Platypleura kuroiwae*). Shimizu (1994) called that fungus “Amami-yarinohosemitake” in his Japanese description. In 1991, the same fungus was collected again from another locality, Kagawa Prefecture, Japan, on nymphs of *P. kaempferi*, by Mitani (Shimizu 1994). The fungus is characterized by its solitary, greenish, cylindrical stroma arising from the head of the host, obliquely inserted perithecia, cylindrical and capitate asci, and filiform, multiseptate ascospores (Shimizu 1994). It is very similar to Kobayasi’s original *C. owariensis* in general aspect, including size and morphology of stromata, asci, and ascospores, but only differs in the coloration of stroma (greenish in the latter fungus). In comparison with the specimens of *C. owariensis*, our results tempt us to consider these additional collections as variations within the species. Moreover, the host and locality of *C. owariensis* and the *Cordyceps* sp. almost overlapped. Thus, we consider them to represent

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one species and the latter could be identified as a greenish form of *C. owariensis*.

In June 2000, Uchiyama examined fresh material of the *Cordyceps* sp. from Amami-oshima Island, and successful isolations of its anamorph were made in a culture. Consequently, the isolate was determined to be an unknown species of the genus *Nomuraea* Maublanc (Kish et al. 1974; Hocking 1977; Samson et al. 1988; Tzean et al. 1992, 1993). Thus, this *Nomuraea* is taken to be the anamorph of *C. owariensis* (as the greenish form) and is described herein as a new anamorphic state. The link between these taxa is also reported.

Materials and methods

A fresh specimen of the *Cordyceps* sp. collected from Amami-oshima Island on June 16, 2000, was used as a source of isolation. The isolation was made on a Sabouraud glucose agar (SGA) from discharged ascospores as follows. After the specimen was rinsed in tap water and then sterile, distilled water for cleanup, the stromatal head of the specimen was dissected and was fixed downward on the inside of the lid of a sterile petri dish; the agar plate was turned over to let the spores shoot up evenly. Primary isolation was examined with the microscope through agar to look for evidence of germination. When this was noted, single-spore and multiple spore isolates were transferred to fresh SGA slants. The isolation procedure is detailed elsewhere (Uchiyama 1999).

For the identification of the isolate, cultures growing on SGA, malt extract agar (MEA), potato dextrose agar (PDA), potato-carrot agar (PCA), and oatmeal agar (OA) were used. The cultures were incubated at 10°, 15°, 20°, 25°, and 37°C under artificial daylight. For descriptions of colony appearance and microscopic features, the isolate was grown on MEA and SGA at 25°C. Colors designated are from the Kornerup and Wanscher (1978) color standard and the Rayner (1970) color chart and are referred to with the letters M and R, respectively.

The type specimens and specimens from which cultures were obtained were deposited at the Natural History Museum and Institute, Chiba, Japan (CBM).

Taxonomy

Cordyceps owariensis Kobayasi (Bull. Biogeogr. Soc. Japan 9:166, 1939) f. *viridescens* Uchiyama et Udagawa, forma nov. Fig. 1

A typo differt stromate griseo-viridi pro parte maxima.

Holotypus. CBM-FA-9168: On nymph of cicada (*Platypleura kuroiwae*), buried in moist fallen litter of laurel

forest, altitude 230–240m, Amami-oshima Island, Oshima-gun, Kagoshima Prefecture, Japan, June 16, 2000, collected by K. Fujimoto. The holotype is deposited in CBM.

Stroma arising from the head of the host, solitary, simple or 3–4 branched, greyish-green (M. 28D5) or dark herbage green (R), straight, somewhat swordshaped or cylindrical, 30–45 mm long; fertile part middle to terminal but not clearly defined from the stalk, 16–25 mm long, 2–4 mm thick, subulate apically with acute, white, and glabrous sterile tip; surface scabrous and inner layer of textura porrecta; stalk in the underground clavate, dark brown (M. 7F4) or sepia (R), 1.5–2.5 mm in diameter, slightly laterally compressed above, composed of hyphae that are septate, longitudinally and densely arranged, at lower part cylindrical or rhizomorphous. Perithecia obliquely inserted, ampullaceous, 440–640 × 180–320 μm, with a conical neck that is slightly protruded from the surface of stroma; peridium thin, hyaline to yellowish-white (M. 4A2), multilayered, 18–22 μm thick. Asci hyaline, 8-spored, cylindrical, 180–300 × 5–6.5 μm, with a 2–2.5 μm in thickness and 4–5 μm in diameter, perforated, depressed globose cap. Ascospores hyaline, filiform, 150–280 × 1–2 μm when discharged, flexuous, smooth, multiseptate, nonfragmenting. Anamorph: *Nomuraea owariensis*.

Nom. Jap., Amami-yarinohosemitake (Shimizu 1994, as *Cordyceps* sp. with an illustration).

Distribution: Amami-oshima, Oshima-gun, Kagoshima Prefecture, Japan, and Miki-cho, Kida-gun, Kagawa Prefecture, Japan.

Specimens examined: CBM-FA-9168 (holotype), on nymph of cicada (*Platypleura kuroiwae*), buried in moist fallen litter of laurel forest, Amami-oshima Island, Oshima-gun, Kagoshima Prefecture, Japan, collected June 16, 2000, by K. Fujimoto. Herb. CBM as CBM-FA-9168.

Additional specimens examined: SC042-01 (D. Shimizu's collection), on nymph of cicada (*P. kuroiwae*), from Amami-oshima Island, Oshima-gun, Kagoshima Prefecture, Japan, collected June 18, 1990, by D. Shimizu; and SC042-03, on nymph of cicada (*Platypleura kaempferi*), from Miki-cho, Kida-gun, Kagawa Prefecture, Japan, collected August 18, 1991, by S. Mitani.

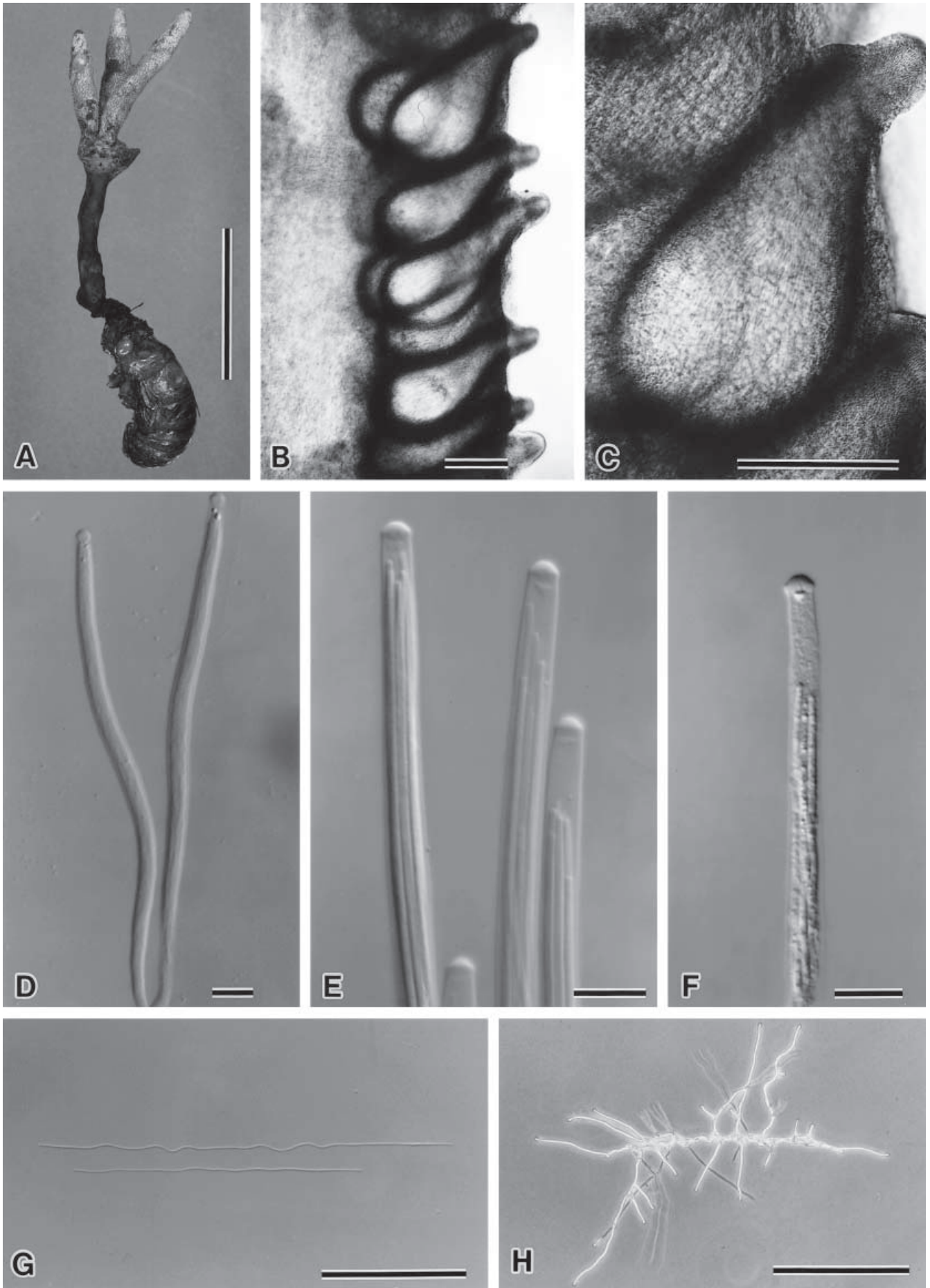
Differences from *C. owariensis* f. *owariensis* are only seen in the stroma coloration.

Specimens of *C. owariensis* f. *owariensis* for a comparison: SC041-01, on nymph of cicada (*P. kuroiwae*), from Amami-oshima Island, Oshima-gun, Kagoshima Prefecture, Japan, collected August 24, 1997, by K. Fujimoto; and SC042-02, on nymph of cicada (*P. kaempferi*), from Miki-cho, Kida-gun, Kagawa Prefecture, Japan, collected August 3, 1997, by H. Manabe.

Anamorph: *Nomuraea owariensis* Uchiyama et Udagawa, anam. sp. nov. Figs. 2, 3

Coloniae in agar maltoso effusae, planae, velutinae vel floccosae, ex mycelio basali coacto tenuiter constantes,

Fig. 1. *Cordyceps owariensis* f. *viridescens*. **A** Perithecial stroma arising from the host. **B** A part of a section through head of stroma with perithecia. **C** Close-up of perithecium. **D** Asci. **E,F** Tips of asci, showing the apical cap and ascospores. **G** Ascospores. **H** Germinating ascospore [on Sabouraud glucose agar (SGA) after 48h incubation at 25°C]. Bars **A** 20mm; **B, C** 200 μm; **D–F** 10 μm; **G, H** 100 μm



valde sporiferae, griseo-virides vel atrovirides; reversum incoloratum, deinde in centro rubro-brunneum.

Mycelium ex hyphis hyalinis ramosis saepe anastomosantibus septatis levibus 1–5 µm diam compositum. Apparatus conidici variabilis, vulgo penicilliformis et saepe valde divaricatus, interdum simplex.

Conidiophora mononematosa, erecta, ex mycelio basali vel hyphis aeriis oriunda, hyalina, recta vel sinuosa, 30–160 × 2–3.5 µm, septata, levia. Rami 12–30(–50) × 2–4 µm. Metulae hyalinae, late clavatae vel cylindraceae, vulgo 2–6-verticillatae, 5–12 × 4–5 µm, leves. Phialides hyalinae, 3–8-verticillatae, ovoideae vel ellipsoideae, adpressae, 6.5–12 × 3.3–5 µm, leves; collum destitutum. Conidia primo hyalina et guttulata, deinde dilute viridia vel viridia, unicellularia,

catenata, ellipsoidea vel cylindracea, interdum plus minusve curvata, (8–)9.5–11(–13) × 4–5.5 µm, incrassata et levia, saepe cum connectivo distincto instructa. Status teleomorphus: *Cordyceps owariensis* Kobayasi f. *viridescens* Uchiyama et Udagawa.

Holotypus. CBM-FD-9169: Colonia exsiccata in cultura ex ascosporis *Cordycipitis owariensis* f. *viridescens* in larva cicada, Amami-oshima, Kagoshima, in Japonia, November 2000, a S. Uchiyama isolata. The holotype is deposited in CBM.

Colonies on MEA growing rapidly, attaining a diameter of 55–59 mm in 14 days at 25°C, plane, velvety but covered with a floccose overgrowth of aerial hyphae in central areas, consisting of a thin basal mycelium, heavily sporulating

Fig. 2. *Nomuraea owariensis*. **A** Conidiogenous cells. **B** Conidia

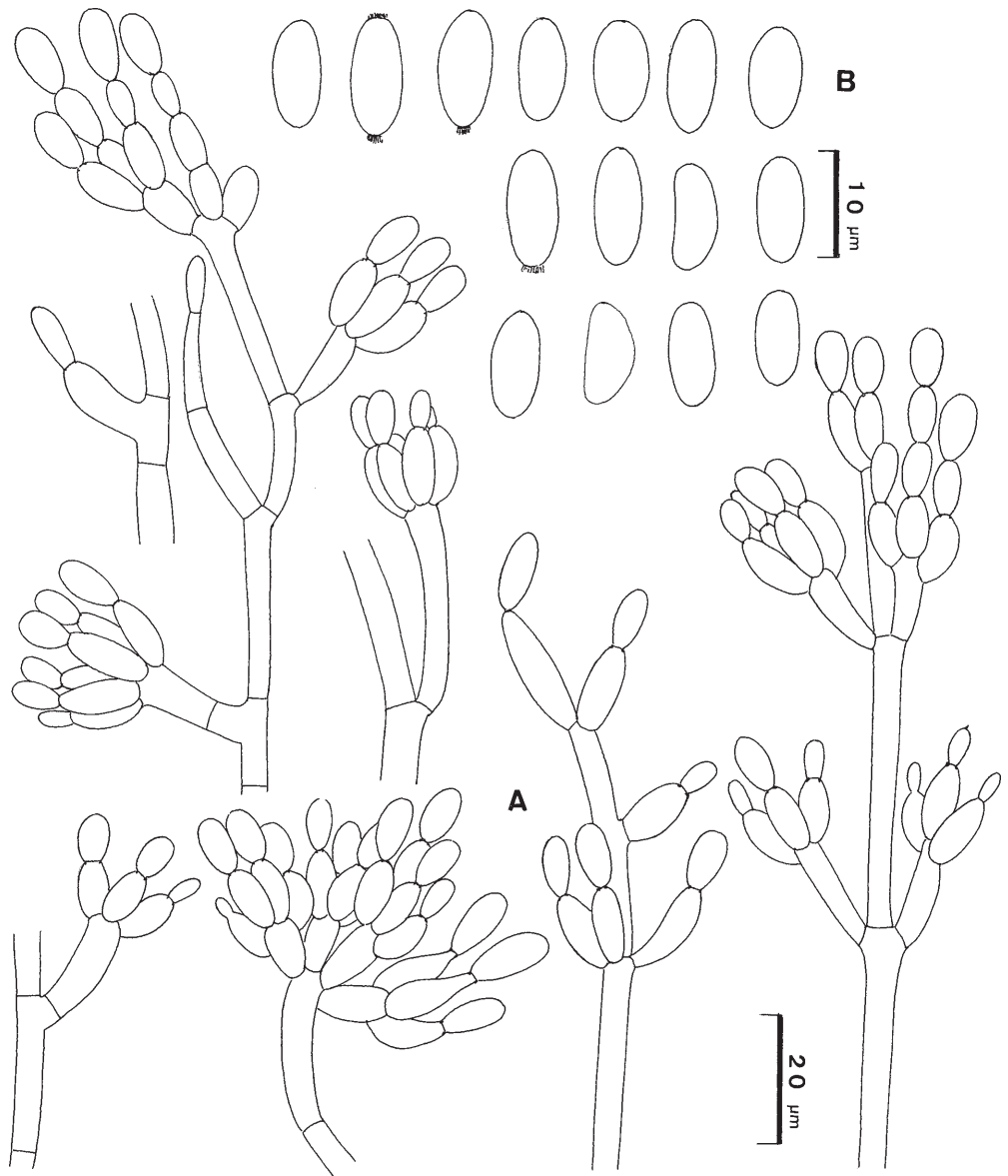


Fig. 3. *Nomuraea owariensis*. **A–F** Conidiogenous cells and conidia. Note distinct connections between conidia (arrows in **F**). **G, H** Conidia. Note the remnant of conidial connective (arrow in **H**). **I** Solitary phialide. Bars **A–D, I** 20 µm; **E, F** 20 µm; **G, H** 10 µm

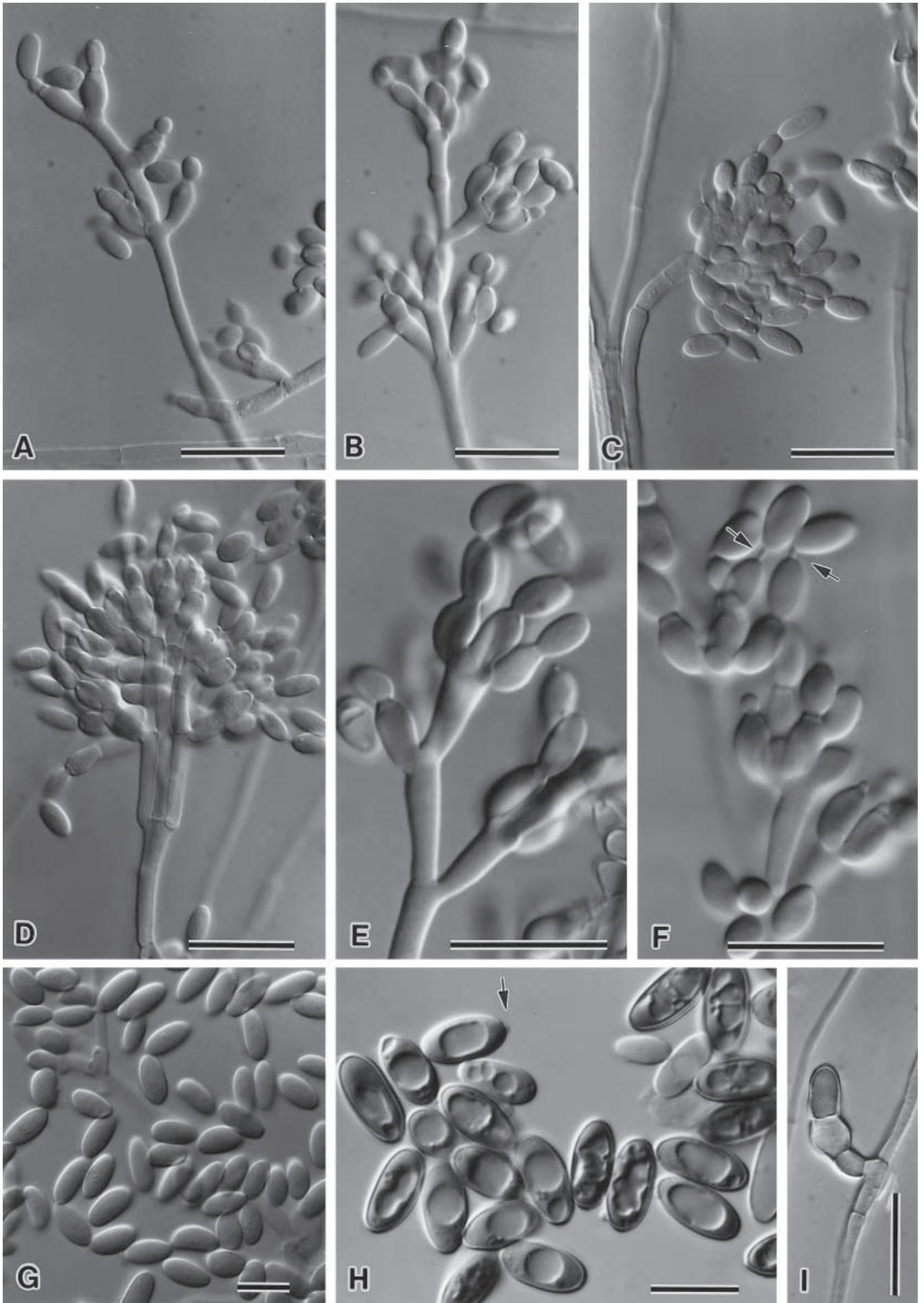


Table 1. Differences between *Nomuraea owariensis* and related *Nomuraea* species

	<i>N. owariensis</i>	<i>N. viridulus</i>	<i>N. cylindrospora</i>	<i>N. atypicola</i>
Source	This paper	Tzean et al. (1992)	Tzean et al. (1993)	Hywel-Jones and Sivichai (1995)
Host	Underground nymphs of cicadas (as teleomorph)	Cadavers of adult cicadas	Cadavers of adult cicadas	Ground-dwelling spiders
Teleomorph	<i>Cordyceps owariensis</i> f. <i>viridescens</i>	Unknown	Unknown	<i>Cordyceps cylindrica</i>
Growth on MEA (14 days, 25°C, mm)	55–59	15–23	29–31	5 ^a
Colony color	Grayish-green–dark green	Grayish-green–dull green	Grayish-green	Purple-gray
Colony reverse color (center)	Reddish-brown	Yellow–yellowish-brown	Brownish-yellow–yellowish-brown	Cream-white
Conidiophores (µm)	Mononematous, 30–160 × 2–3.5	Mononematous, up to 210 × 3.1–5	Mononematous, 46–270 × 2.5–3	Mononematous or synnematos, 100–150 × 2–2.5 ^b
Conidial apparatus	Variable, often divergent, verticillate	Variable, often divergent, verticillate	Variable, often divergent, verticillate	Typically verticillate, not divergent
Metulae (µm)	5–12 × 4–5	11.7–31.6 × 2.6–3.8	20–60 × 1.8–4.2	6.8–25 × 3 or 4–11 × 3–4 (side)
Phialides (µm)	6.5–12 × 3.5–5	5.3–9.2 × 3.3–4.6	5–8 × 3–4.2	4–8 × 2.5–3
Conidia (µm)	Monomorphic, (8–)9.5–11(–13) × 4–5.5	Monomorphic, 14.4–19.4 × 3.8–4.4	Dimorphic, 16.2–20.3 × 3.5–5, 3.3–6.7 × 3.3–4.2	Monomorphic, 3.8–6.4 × 1.1–1.9

MEA, malt extract agar

^aPDA, in 10 days at 22°C^bOn the host

throughout, greyish-green to dark green (M. 26E-F5) or malachite green (R); margins entire, thin; exudate lacking; odor none; reverse at first uncolored throughout, becoming reddish-brown (M. 9E5) or bay (R) at the colony center.

Colonies on SGA growing somewhat less rapidly, 47–52 mm in 14 days at 25°C, velvety to floccose, plane except for floccose overgrowth in the colony center, consisting of a white to brownish compact basal felt, heavily sporulating as on MEA, at first white to yellowish-white (M. 4A2), then becoming olive-brown (M. 4D4) to greyish-green (M. 29D5); margins entire, thin; exudate lacking; reverse reddish-brown (M. 8E4) or bay (R) to dull green (M. 29D4) or dark herbage green (R).

Mycelium composed of hyaline, branched, often anastomosed, septate, smooth-walled, 1–5 µm diameter hyphae. Conidial apparatus consisting of divergent, terminal, often verticillate penicilli. Conidiophores macronematous or semimacronematous, mononematous, erect, arising from the basal mycelium or aerial hyphae, hyaline, straight or sinuous, 30–160 × 2–3.5 µm, septate, smooth-walled. Penicilli often variable in pattern such as subterminal, lateral, or lower-level metulae or only solitary phialides; branches 12–30(–50) × 2–4 µm; metulae hyaline, broadly clavate or cylindrical, usually borne in verticils of 2–6, measuring 5–12 × 4–5 µm, smooth-walled; phialides hyaline, 3–8 in the verticil, ovoid or ellipsoidal, appressed, 6.5–12 × 3.5–5 µm, smooth-walled, without distinct neck, occasionally solitary and borne directly on the conidiophore. Solitary phialides sometimes present as a simple, thick-walled, proliferating branch on the hypha, cylindrical, with a thin, funnel-shaped and flaring collarette. Conidia at first hyaline and guttulate, becoming pale green to pastel green, one-celled, catenulate, ellipsoidal or cylindrical, sometimes slightly curved, (8–)9.5–11(–13) × 4–5.5 µm, thick and smooth-walled, often with a distinct connective.

At 10° or 37°C, growth is nil. At 15° or 20°C, growth is slower than that at 25°C.

Specimen examined: CBM-FD-9169 (holotype), a dried culture developed in the laboratory by S. Uchiyama in June 2000, isolated from ascospores of *Cordyceps owariensis* f. *viridescens* from Amami-oshima, Oshima-gun, Kagoshima Prefecture, Japan, collected June 16, 2000 (CBM-FA-9168). Herb. CBM as CBM-FD-9169; ex-type culture deposited as IFO-33258.

The salient differences between *N. owariensis* and related *Nomuraea* species including species recorded from cicada are summarized in Table 1.

Discussion

The genus *Nomuraea* was established by Maublanc (1903) on the basis of *N. pracina* (Delacr.) Maubl. (= *Botrytis pracina* Delacr.), which was found by Nomura (a Japanese entomopathologist) on the larvae of *Mamestra brassicae* in 1901 and sent to Delacroix for an identification. Kish et al. (1974) considered *Botrytis rileyi* Farl. (Farlow 1883) identical with *N. pracina*, and simultaneously they proposed, for reasons of priority, the new combination *N. rileyi* (Farl.) Samson as the type species of *Nomuraea*.

Nomuraea rileyi is an entomogenous hyphomycete capable of infecting several economically important lepidopterous insects. In Japan, Hirose (cf. Mitani 1929; Ishikawa 1936) first described an isolation of this fungus from silkworms (*Bombyx mori*) as a serious parasite causing the “green muscardine” disease in sericultural industry, although he erroneously assigned it to be *Oospora destructor* (Metschnikoff) Delacr. (= *Metarrhizium anisopliae* (Metschn.) Sorokin. The penicillate heads pro-

ducing cylindrical phialides and greenish, one-celled conidia in short, dry chains are characteristic of *Nomuraea*. Most of these features indicate a close relationship with the genus *Penicillium* Link. The key difference is seen at the apex of the phialide where is characterized by the absence of a distinct neck. In addition, most species of *Nomuraea* are entomogenous (Tzean et al. 1993), whereas *Penicillium* species are best known as widespread and omnivorous.

Nomuraea atypicola (Yasuda) Samson (= *Isaria atypicola* Yasuda), a second species in Samson's monograph (1974), appears to be a species common on a trapdoor spider (*Latouchia* spp.) in the temperate-subtropical latitudes of Japan (from central Honshu to Nansei-shoto), where it occurred along roadsides and in gardens, in precincts of shrines and temples, in a crack of a stone wall, and in laurel forests (Kobayasi and Shimizu 1983; Shimizu 1994; Hatamori and Kaneko 1998). As its teleomorph, *Cordyceps cylindrica* Petch (Petch 1937, 1939; Kobayasi and Shimizu 1977) has been linked to *N. atypicola* on the basis of association on the same host material or, in a recent verified case, through cultural studies (Hywel-Jones and Sivichai 1995; Hatamori and Kaneko 1998).

As shown in the taxonomic section, cultures of *C. owariensis* f. *viridescens* readily produced an anamorph of *Nomuraea* in culture, although it has not yet been found from nature. To date, no evidence was found to suggest that the remaining three species of *Nomuraea* have been proven to be a teleomorph (Hocking 1977; Tzean et al. 1992, 1993). Thus, *N. owariensis* becomes the second species of the genus known to produce an teleomorph. Also, this is the first report on the association of a *Nomuraea* anamorph with cicadicolous *Cordyceps* species. Further cultural studies are needed to determine the association of a *Nomuraea* anamorph for f. *owariensis*.

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References

- Farlow WG (1883) *Botrytis rileyi*. Rep US Comm Agric 1883:121
 Hatamori Y, Kaneko S (1998) Notes on teleomorph of *Nomuraea atypicola* (in Japanese). J Jpn Assoc *Cordyceps* (Tochukaso) 18:25–32
 Hocking AD (1977) *Nomuraea anemonoides* sp. nov. from Australian soil. Trans Br Mycol Soc 69:511–513
 Hywel-Jones NL, Sivichai S (1995) *Cordyceps cylindrica* and its association with *Nomuraea atypicola* in Thailand. Mycol Res 99:809–812
 Ishikawa K (1936) Silkworm pathology (Santaiyorigaku) (in Japanese). Meibundo, Tokyo
 Kish LP, Samson RA, Allen GE (1974) The genus *Nomuraea* Maublanc. J Invertebr Pathol 24:154–158
 Kobayasi Y (1939) On the genus *Cordyceps* and its allies on Cicadae from Japan. Bull Biogeogr Soc Jpn 9:145–176
 Kobayasi Y (1941) The genus *Cordyceps* and its allies. Sci Rep Tokyo Bunrika Daigaku Sect B 5:53–260
 Kobayasi Y (1982) Keys to the taxa of the genera *Cordyceps* and *Torrubiella*. Trans Mycol Soc Jpn 23:329–364
 Kobayasi Y, Shimizu D (1963) Monographic studies of *Cordyceps*. 2. Group parasitic on Cicadidae. Bull Natl Sci Mus 6(3):286–314
 Kobayasi Y, Shimizu D (1977) Some species of *Cordyceps* and its allies on spiders. Kew Bull 31:557–566
 Kobayasi Y, Shimizu D (1983) Iconography of vegetable wasps and plant worms (in Japanese). Hoikusha, Osaka
 Kornerup A, Wanscher JH (1978) Methuen handbook of colour, 3rd edn. Eyre Methuen, London
 Maublanc A (1903) Sur quelques espèces nouvelles de champignons inférieurs. Bull Soc Mycol Fr 19:291–296
 Mitani K (1929) Recent advances on diseases of silkworm, vol 2 (Saikinsanbyogaku, Chu-kan) (in Japanese). Meibundo, Tokyo
 Petch T (1937) Notes on entomogenous fungi 101–134. Trans Br Mycol Soc 21:34–67
 Petch T (1939) Notes on entomogenous fungi 135–160. Trans Br Mycol Soc 23:127–148
 Rayner RW (1970) A mycological colour chart. Commonwealth Mycological Institute, Kew, and British Mycological Society, London
 Samson RA (1974) *Paecilomyces* and some allied hyphomycetes. Stud Mycol 6:1–119
 Samson RA, Evans HC, Latgé J-P (1988) Atlas of entomopathogenic fungi. Springer, Berlin
 Shimizu D (1994) Color iconography of vegetable wasps and plant worms (in Japanese). Seibundo Shinkosha, Tokyo
 Teng SC (1996) In: Korf RP (ed) Fungi of China. Mycotaxon, Ithaca
 Tzean SS, Hsieh LS, Chen JL, Wu WJ (1992) *Nomuraea viridulus* a new entomogenous fungus from Taiwan. Mycologia 84:781–786
 Tzean SS, Hsieh LS, Chen JL, Wu WJ (1993) *Nomuraea cylindrospora* comb. nov. Mycologia 85:514–519
 Uchiyama S (1999) Methods for collection, detection and isolation: *Cordyceps* (in Japanese). Trans Mycol Soc Jpn 40:173–179