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BRIEF ARTICLE

NOMURAEA CYLINDROSPORA COMB. NOV.

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Guo et al. (1986) described three species of *Metarhizium*. Among them, *M. cylindrospora* Chen & Guo was recovered from the larvae of an unidentified insect pest inhabiting the rhizosphere of tea trees in Guizhou Province, southern Mainland China. Colonies of *M. cylindrospora* on potato dextrose agar are velutinous with dense mycelium, initially white turning to bright green when sporulating; hyphae are hyaline, septate, branched, 2.5–3 μm wide. The apex of the mycelium bears four to six short flask-shaped phialides from which catenulate conidia are formed. Conidia are light green when single and olive green in mass, long, cylindrical, with tapering ends, 17.2–19.7 \times 2.5–3.7 μm (Guo et al., 1986). Shimazu (1989) reported a fungal epizootic in a population of the large brown cicada *Graptopsaltria nigrofuscata* Motschulski in forests of Japanese alder. The macroconidia of the entomopathogen are cylindrical and 16.8 \times 3.8 μm , and the microconidia are ellipsoidal and 5.4 \times 2.7 μm (Shimazu, 1989). In a personal communication, Guo et al. conceded that the microconidia reported by Shimazu were neglected and not illustrated when they first described the fungus (Shimazu, 1989).

During the course of a study of the entomogenous fungi from Taiwan, a greenish fungus was isolated from a small brown cicada *Pomponia linearis* Walker (Homoptera). Morphological characteristics of this fungus were almost identical to *M. cylindrospora*. A critical examination of the diagnostic characters of the Taiwanese isolate indicated that the placement of this fungus in the genus *Metarhizium* (Tulloch, 1976) was incorrect and that it is better placed in *Nomuraea*, because both on the insect host and on culture media, it produced apical verticillate or subterminal, lateral metulae, which were solitary or two to five in groups randomly growing along

the length of the conidiophores. The phialides arising from the metulae or from the fertile hyphae were oval-cylindrical with a barely perceptible neck. All these characteristics are referable to *Nomuraea* Maublanc (Kish et al., 1974). In contrast *Metarhizium* produces conidiophores of variable length, usually shorter than *Nomuraea*, with apical loosely penicillate branches, each branch bearing two to five metulae and phialides. The metulae are clavate; the phialides are elongate to cylindrical with a central constriction; conidiophores are usually aggregated into sporodochia with a stromatic base; and conidia usually laterally adhere to form a prismatic column (Samson, 1974; Rombach et al., 1986, 1987).

Metarhizium cylindrospora is therefore transferred to another genus as *Nomuraea cylindrospora*. Illustrations and diagnosis of the Taiwanese isolate of *N. cylindrospora* were based on cultures of malt-extract agar (MEA) (Samson, 1974) and on cicada. Color nomenclature for colonies, mycelia and conidia was adapted from the color standard of Korerup and Wanscher (1978). Preparation of materials for scanning electron microscopy followed previously described methods (Tzean and Estey, 1978).

Nomuraea cylindrospora Tzean, Hsieh, Chen et Wu, *comb. nov.* FIGS. 1–8

BASIONYM: *Metarhizium cylindrospora* Chen & Guo, *Acta Mycologica Sinica* 5: 180. 1986.

Colonies on MEA growing slowly, 2.9–3.1 cm diam under diffuse light at 25 C after 14 days; plane, velutinous to slightly floccose, inconspicuous, farinose when sporulating; greyish green near ocean green, pistache or Nile (27–28 C4-6); outermost margin floccose, fasciculate, white. Reverse center brownish yellow to yellowish brown (5C-D6-8), subcenter to margin deep green (29E6-8, 28D-E7-8) to colorless; odor and soluble pigment lacking. Exudate colorless. Syn-

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nemata absent. Mycelium white composed of branched, septate, smooth-walled, hyaline hyphae, 2.5–4.2 μm wide. *Conidiophores* ascending mostly from aerial, occasionally from surface or submerged, hyphae; septate, smooth-walled, hyaline, 46–270 \times 2.5–3.3 μm ; bearing divergent, terminal, verticillate, or irregularly subterminal lateral or lower level metulae, single or two to five in whorls arising most often below septa; *metulae* broadly clavate, or cylindrical, 20–60 \times 1.8–4.2 μm , occasionally proliferating; *phialides* in a group of two to eight, closely appressed, rarely solitary and borne directly on the conidiophores, oval-cylindrical, ellipsoidal, neck absent or barely perceptible, 5–8 \times 3–4.2 μm . *Conidia* catenulate, basipetal, one-celled, smooth-walled, dimorphic; *microconidia* formed first, ovoid, ellipsoidal, or subglobose, 3.3–6.7 \times 3.3–4.2 μm ; *macroconidia* formed later, mostly cylindrical, ellipsoidal, usually slightly curved, 16.2–20.3 \times 3.5–5.0 μm , single, hyaline to greenish white (30A2), in mass greyish green (29B-C5-6) to dark green or deep green (28E-F7-8). With the scanning electron microscope (FIGS. 7, 8), the seceded micro- or macroconidia apiculate or somewhat truncate. Chlamydo-spores absent. Colonies on *Sabouraud's dextrose agar* growing slowly, 2.2–2.8 μm at 25 C under diffuse light after 14 days; center to subcenter distinctly floccose, raised, composed of white mycelium, margin velutinous, deeply or slightly sulcate, dull green to dark green (26E-F4, 29F4-6), with heavy sporulation, outermost margin floccose, mycelium white. Microscopic characteristics comparable to those on MEA. *On insect host*, mycelium covering the sutures, initially white, then turning pale green, greyish green (27D-E5-7), velutinous in age with heavy sporulation; conidiophores 25–147 \times 1.6–3.6 μm , phialides 5.6–7.1 \times 2.8–3.6 μm , microconidia 3.6–8.7 \times 2.5–3.3 μm , macroconidia 14.2–22.5 \times 3.7–4.6 μm ; microscopic features including conidiogenous structures, micro- and macroconidia comparable to that in culture media except with somewhat shorter phialides and larger macroconidia (compare FIG. 1a, b). Synnemata and chlamydo-spores absent.

SPECIMEN EXAMINED: REPUBLIC OF CHINA. TAIWAN: Taipei, Wulai, from cicada, *Pomponia linearis*, Homoptera, Ho.137 and PPH 13 (dried cultures) and PPH 13E (living culture), 1 Jun. 1990 (deposited in Department of Plant Pathology and Entomology, National Taiwan University, Taipei); PPH 13E was also deposited in the Culture Collection and Research Center (CCRC 32599), Hsinchu, Taiwan, in herb. NY and

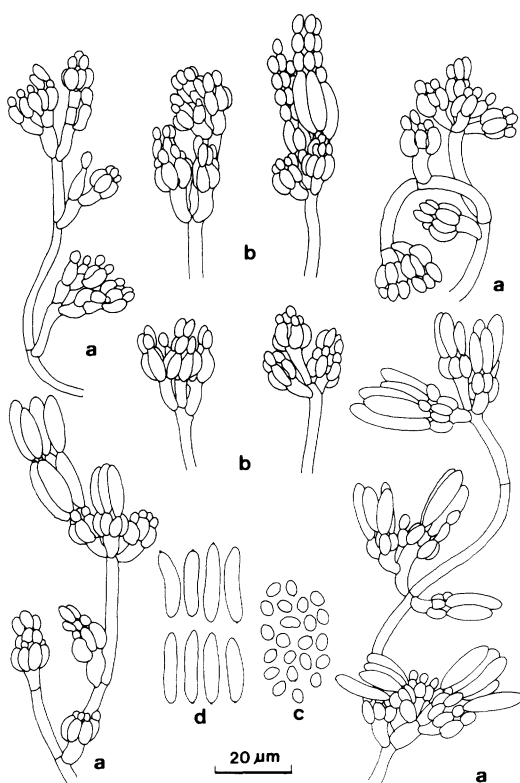
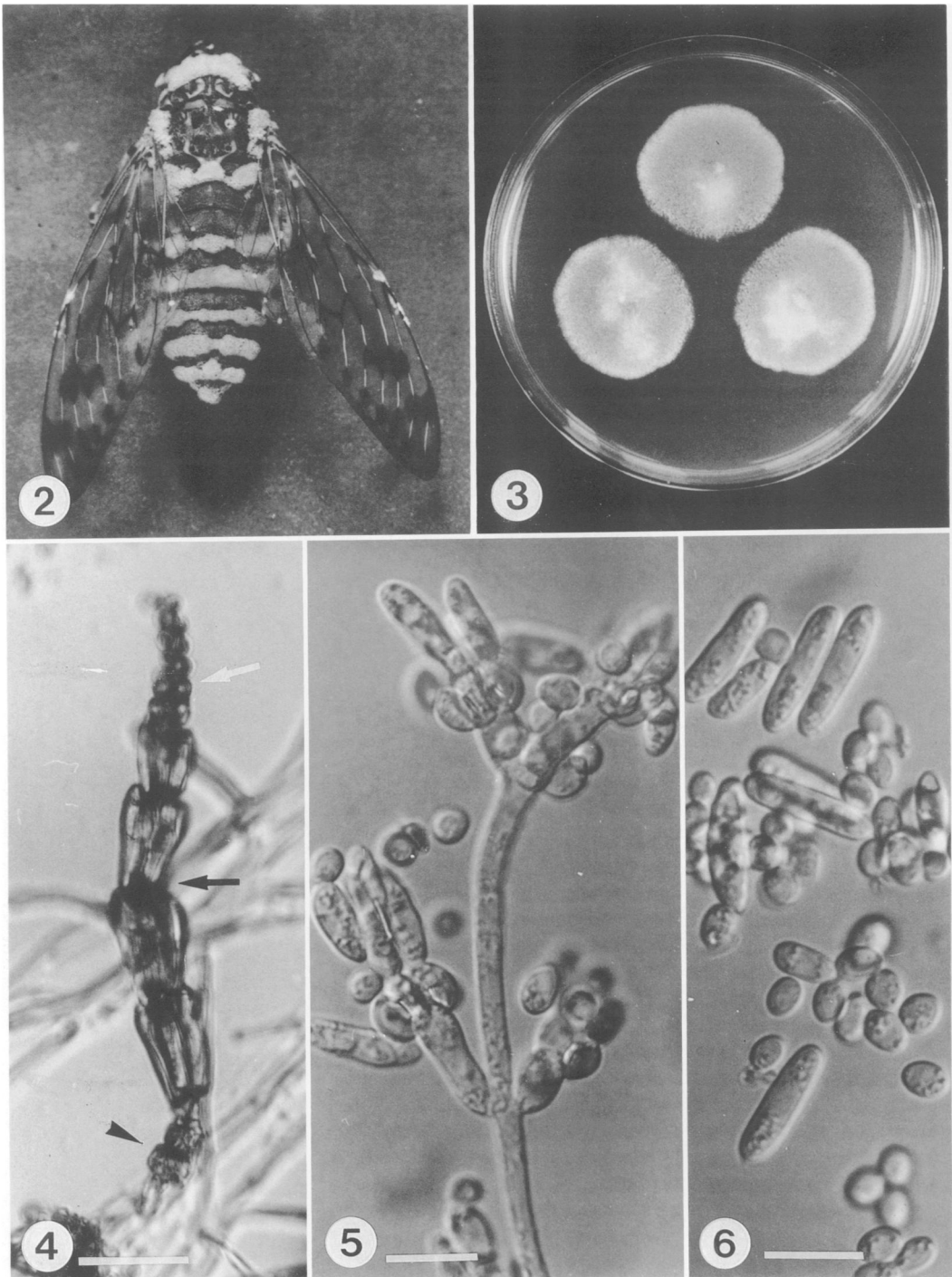


FIG. 1. a–d. *Nomuraea cylindrospora*. Characteristics of conidiophores, metulae, phialides and conidia on malt-extract agar (a), and on cicada *Pomponia linearis* (b). Conidia of two types: microconidia ovoid, subglobose, formed in early stage, ca 3 days (c); macroconidia cylindrical, slightly attenuate toward both ends, dominant in late stage, after 7 days, on MEA (d).

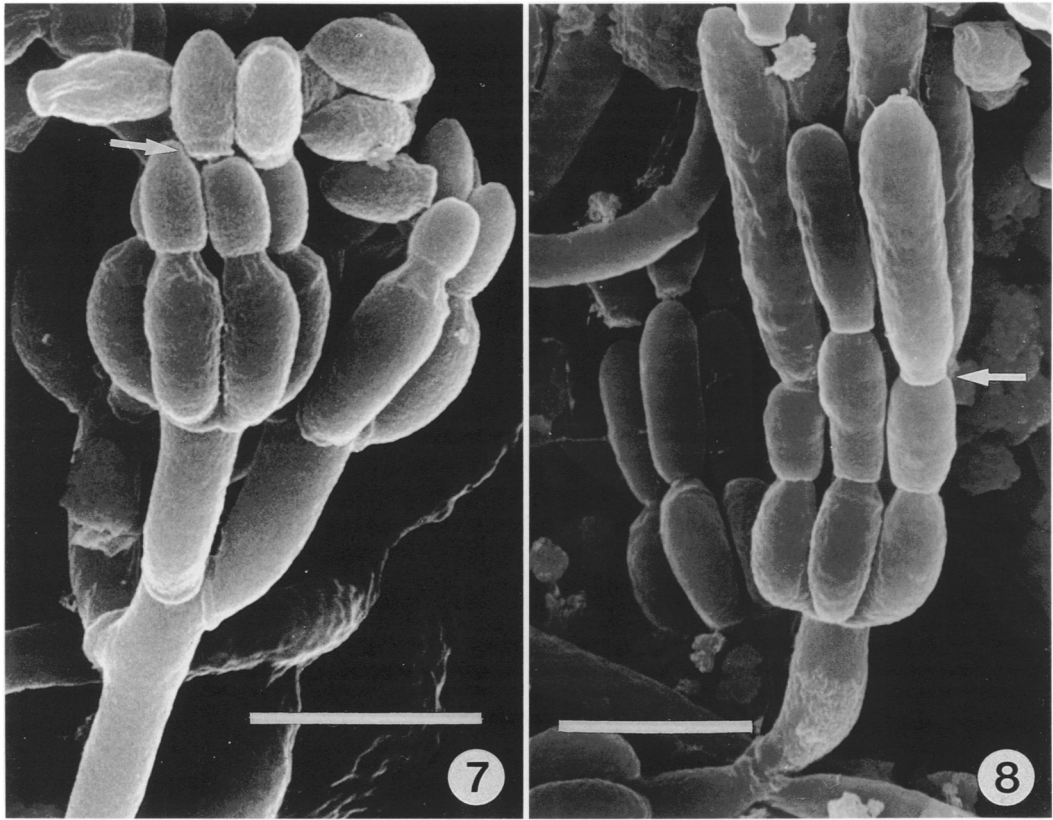
IMI. CHINA. GUIZHOU: unknown tea tree insect pest, *Metarhizium cylindrospora* ex-type (living culture from holotype ACCC 30114). JAPAN. Tsukuba, from brown cicada *Graptopsaltria nigrofuscata*, living culture of *Metarhizium cylindrospora*.

Close examination and comparing of the ex-type (living culture) obtained from holotype material of *Metarhizium cylindrospora* (ACCC 30114) (FIG. 9) and a Japanese collection of *M. cylindrospora* by Shimazu (1989) with the specimens and cultures of Taiwan collections of *N. cylindrospora* disclosed the common morphological and structural characteristics. Therefore, all these strains of different geographic origins are unequivocally conspecific.

Nomuraea cylindrospora showed noticeable resemblance both in macroscopic and microscopic characteristics to a newly described taxon *Nomuraea viridulus* Tzean, Hsieh, Chen & Wu



FIGS. 2-6. *Nomuraea cylindrospora*. 2. Naturally infected cicada *Pomponia linearis*; head and body junctures overlaid with velutinous mycelia and pale- to light green conidia, $\times 1.2$. 3. Colony on MEA at 25 C after 14 days without illumination, ca $\times 0.75$. 4. Habit of catenulate, basipetally formed conidia of two types. Terminal conidia small, ovoid, subglobose (white arrow); basal conidia large, cylindrical (black arrow) ascending from



FIGS. 7, 8. Scanning electron micrographs of *Nomuraea cylindrospora*. 7. Phialides borne on cylindrical or clavate metulae, oval-cylindrical, apex attenuated to form extremely short collula; microconidia formed in chains, ovoid, base somewhat truncate (arrow). 8. Macroconidia basipetal, formed on oval-cylindrical phialides, broadly cylindrical, base somewhat truncate (arrow). Bars = 10 μm .

(Tzean et al., 1992). However, both species differed sharply in respect to host specificity, occurrence and the kinds of conidia produced. During a 3-yr survey, without exception *N. viridulus* was encountered exclusively on black cicada, *Cryptotympana facialis* Walker, with a high occurrence rate, and produced only one type of conidia: macroconidia on insect hosts or culture media. Conversely *N. cylindrospora* with a lower occurrence rate, was encountered only twice on brown cicadas *Pomponia linearis* and produced two types of conidia: micro- and macroconidia both on the insect host and on culture media.

Cultures of *N. cylindrospora* from single micro- or macroconidia when young, i.e., 3–6 days old, first formed microconidia, but after 7–14 days a transition from microconidia to macroconidia occurred. At this stage macroconidia predominate; microconidia are sporadically formed. The viewpoint that the discrepancy in producing only one type of conidia by *N. viridulus* and two types of conidia by *N. cylindrospora* is due to stable genetic traits rather than physiological conditions is further strengthened by electrophoretic profiles of general proteins and esterase isozymes (Hsieh and Tzean, unpubl. results). In the his-

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conidial initials (arrow head). 5. Conidiophore bearing metulae, phialides and conidia; phialides oval-cylindrical, neck absent or barely perceptible, verticillate. 6. Microconidia ovoid, subglobose; macroconidia cylindrical, straight or slightly curved. Bar = 20 μm in FIG. 4, 10 μm in FIGS. 5, 6.

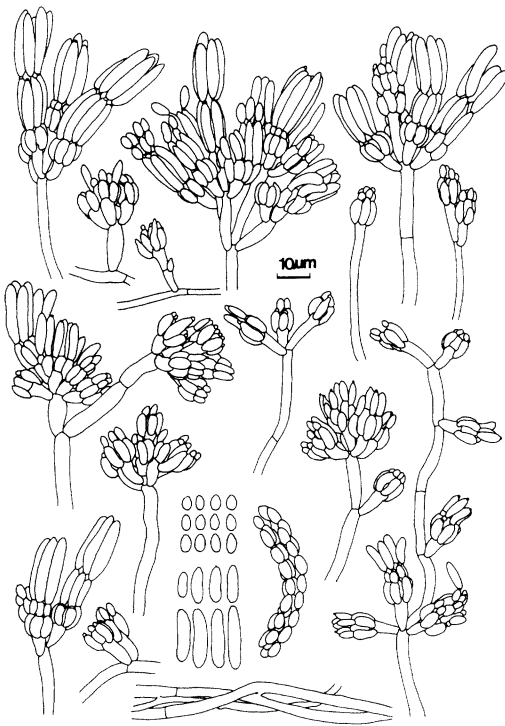
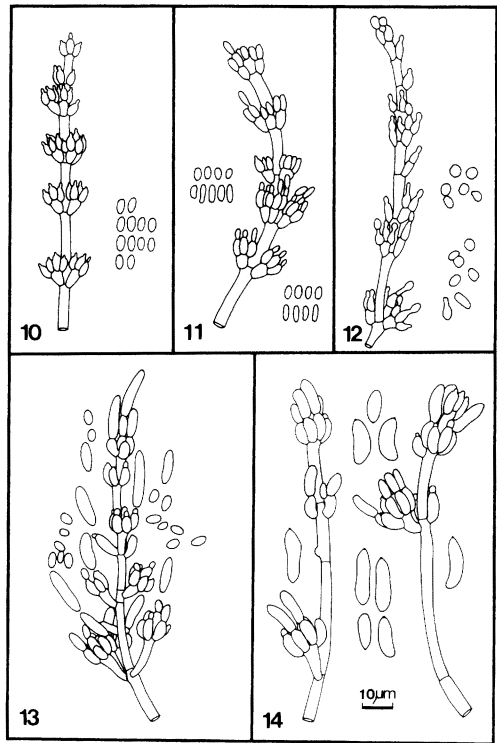


FIG. 9. Morphological characteristics of exotype of *Metarhizium cylindrospora* (ACCC 30114).

togram *N. cylindrospora* showed a pronounced difference to *N. viridulus* but a small difference when compared to an additional conspecific isolate (Ho.178) that showed a tendency to form metulae and phialides in compact clusters encircling the stalk mostly below the septa along the whole length of the conidiophores, a characteristic closer to *N. rileyi* (Farlow) Samson and *N. atypicola* (Yasuda) Samson (Samson, 1974). The results led to the conclusion that *N. cylindrospora* and *N. viridulus* are distinct species and not intraspecific strains, though showing close affinity.

Of the five species of *Nomuraea*, *N. atypicola* is a typical pathogen of spiders but it also different from other *Nomuraea* species by its 1) purple color, 2) often synnematos conidiophores, and 3) *Cordyceps* teleomorph (Samson, 1974). Although the type culture of *N. anemonioides* Hocking was originally isolated from soil, its entomogenous nature (occurrence on a soil-borne insect) is quite feasible (Hocking, 1977). *Nomuraea rileyi* mostly parasitizes lepidoterans and shows a high potential for biocontrol of insect pests, while *N. cylindrospora* and *N. viridulus*



FIGS. 10-14. Synoptic illustrations of conidiophores, metulae, phialides, and conidia in five species of *Nomuraea*: *N. rileyi* (FIG. 10), *N. atypicola* (FIG. 11), *N. anemonioides* (FIG. 12), *N. cylindrospora* (FIG. 13), and *N. viridulus* (FIG. 14). *Nomuraea rileyi*, *N. atypicola* and *N. anemonioides* redrawn with permission from Samson (1974) and Hocking (1977). All figures are to the same scale.

both are parasites of cicada and their biological activity deserves to be explored further.

Primarily based on the colony textures, habitats, host preference and specificity, structure and morphology of the metulae, phialides and conidia, a key and synoptic illustrations (FIGS. 10-14) to the taxon of the currently described *Nomuraea* species are provided.

KEY TO *NOMURAEA* SPECIES

1. Colony purple; parasite of spider . . . *N. atypicola*
1. Colony greenish; saprophyte or entomopathogen 2
 2. Saprophyte or facultative entomopathogen; conidia globose, ellipsoid to ellipsoid-pedunculate *N. anemonioides*
 2. Entomopathogen; conidia ellipsoid, broadly ellipsoidal, cylindrical or allantoid 3
3. Conidia broadly ellipsoidal or cylindrical, small 3.5-4.5 × 2.0-3.1 µm *N. rileyi*

3. Conidia ellipsoidal, cylindrical, broadly cylindrical or allantoid, macroconidia larger than $14.4\text{--}19.4 \times 3.8\text{--}4.4 \mu\text{m}$ 4
 4. Conidia dimorphic; microconidia subglobose, ovoid, ellipsoidal; macroconidia cylindrical with tapering ends *N. cylindrospora*
 4. Conidia monomorphic, broadly cylindrical with somewhat rounded ends or allantoid *N. viridulus*

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Key Words: Hyphomycetes, *Metarhizium*, *Nomuraea*, taxonomy

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