

Cordyceps cylindrica and its association with *Nomuraea atypicola* in Thailand

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Nomuraea atypicola and *Cordyceps cylindrica* infecting spiders are reported from Thailand for the first time. Cultural studies with strains from both species confirm a previously suspected teleomorph–anamorph link between the two species.

Nomuraea atypicola (Yasuda) Samson was first described from Japan on a spider, *Kishinoyeus typicus* (Samson, 1974). Later, Petch (1937) described *Cordyceps cylindrica* from a single specimen collected by D. H. Linder on a trapdoor spider in Trinidad and noted the apparent similarity between the stroma of this and that of *N. atypicola*. Petch (1937, 1939) concluded that *N. atypicola* was probably related to *C. cylindrica*. Kobayasi (1941) reported *N. atypicola* to be endemic in Japan where it was common along roadsides and in gardens and endorsed Petch's association despite not having recorded *C. cylindrica* in Japan at that time. Later, Kobayasi & Shimizu (1977) noted a single record of *C. cylindrica* from Japan and concluded that in spite of the temporal separation there was the likelihood this species was linked with *N. atypicola*. With so few records of either fungus the association could only be accepted as of dubious character. According to the scheme of Kendrick & DiCosmo (1979) this would only rank as 2:1 – documented but casual affiliation without circumstantial or experimental evidence.

Invertebrate-associated fungi are being surveyed in Thailand. Over 5 yr a few collections were made of *N. atypicola* and, in 1993, a single specimen of *C. cylindrica*. Successful isolations were made from the *Cordyceps* and from the *Nomuraea*. Consequently, the link between these two species is reported on.

MATERIALS AND METHODS

Surveys were made at Khao Yai National Park over a 5 yr period throughout the year and sporadically at other National Parks in Thailand. Several micro-habitats were explored for invertebrate-associated fungi including the stems and underside of living leaves of trees, saplings and herbs of the forest

floor, the leaf litter and the shaded underside of water-splashed boulders in rivers and streams. Samples were usually returned to the laboratory on the day of collection. When this was not possible they were gently air-dried before storing in plastic pots. When not being used, fresh specimens were kept in a refrigerator in individual plastic pots.

Isolations were made on Potato Dextrose Agar from ascus part-spores and from conidia. Perithecia were dissected from the stroma using sterile fine insect pins. These were mounted in 0.05% Triton X-100 solution and dissected to release asci and ascus part-spores. A sterile loop was used to spread these to PDA. Conidia were plated directly to PDA. Primary isolations were examined with $\times 10$ objective with an Olympus BH compound microscope through agar to look for evidence of swelling or germination. When this was noted single-spore and multiple spore isolates were transferred to fresh PDA plates.

Slides were prepared for microscopic examination using an Olympus BH microscope with a drawing tube. Material was examined with oil immersion objective.

RESULTS

Distribution and description of Cordyceps cylindrica in Thailand

A single specimen was found on a well-drained slope in deciduous forest. It was on a large (body length 25 mm) ground-dwelling spider and was found under leaf litter at the start of the cool dry season.

Stroma single, cream-white, 60 mm long \times 1–1.5 mm diam. near the base. Upper 35 mm cylindrical and 5–6 mm diam. Perithecial ostioles yellow, projecting from cylindrical, terminal portion. *Perithecia* elongated flask-shaped, 1100–1267 μ m long by 200–300 μ m wide, mature. *Asci* filiform, at least 375 μ m long and 5 μ m diam. *Ascospores* broken within the asci to produce ascus part-spores. *Part spores* 3.0–3.8 \times 1.0–1.5 μ m, cylindrical with truncate ends.

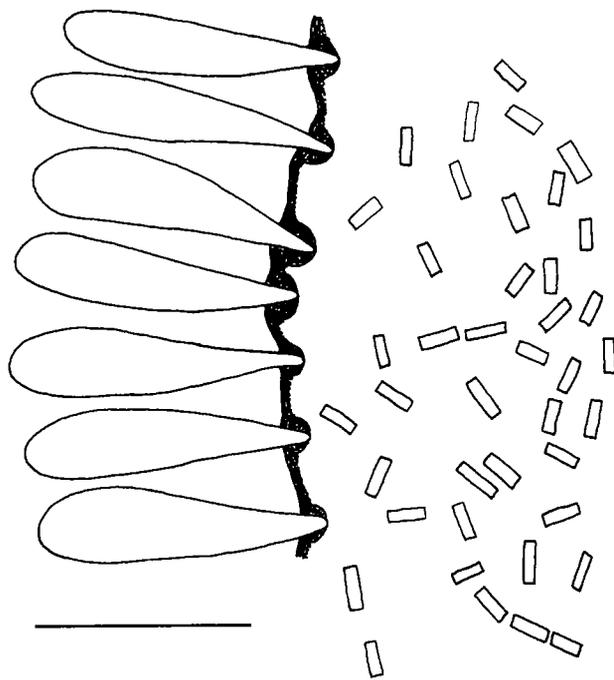


Fig. 1. Perithecia immersed within the fertile head of *Cordyceps cylindrica* with examples of part-spores. Scale bar, 1000 μm for the perithecia and 20 μm for the part-spores.

Specimen examined: This is held in the herbarium of the NBCRC. NHJ2347, Sam Lan National Park, 12 Oct. 1993, N. L. Hywel-Jones, R. Nasit & S. Sivichai.

The spider was covered in a web of white mycelium and had disintegrated within this so that only fragmented remains of the legs and cephalothorax were left. Within, there was no evidence of mycelium or hyphal bodies. After 3 months the herbarium specimen had turned blackish brown.

The fertile head was soft and the perithecia were arranged perpendicular to the wall (Fig. 1). There was no evidence of a hamathecium or of paraphyses within the perithecia. All asci had disintegrated so that the contents of the perithecia were a mass of part-spores (Fig. 1) and part-spores contained within ascus fragments of various lengths. From the ascus fragments it appeared that they were initially eight-spored before the disarticulation into part-spores.

Isolation of *Cordyceps cylindrica*

Ascus fragments and ascus part-spores swelled and germinated within 36–48 h. No isolation could be made from single part-spores but it was possible to get viable isolates from ascus fragments containing part-spores and from multiple part-spore isolations. Multiple spore cultures grew as a restricted, white, floccose growth reaching 5–6 mm after 14 d at 22 °C in the dark with a creamish reverse. After 2 wk the mycelium developed a greyish-purple colour due to conidial production on phialides (Fig. 2).

Conidiophores were 2.3 μm diam. and individual cells of the conidiophores were 6.0–12.0 μm long. Phialides were ovoid to cylindrical with short necks, 4.5–6.8 μm by 2.3–3.0 μm . These either arose directly from the conidiophores or were from small branches 4.5–7.5 \times 1.5–3.0 μm . Conidia were in dry chains, variable in size and shape, 2.6–6.4 \times 1.5–2.6 μm .

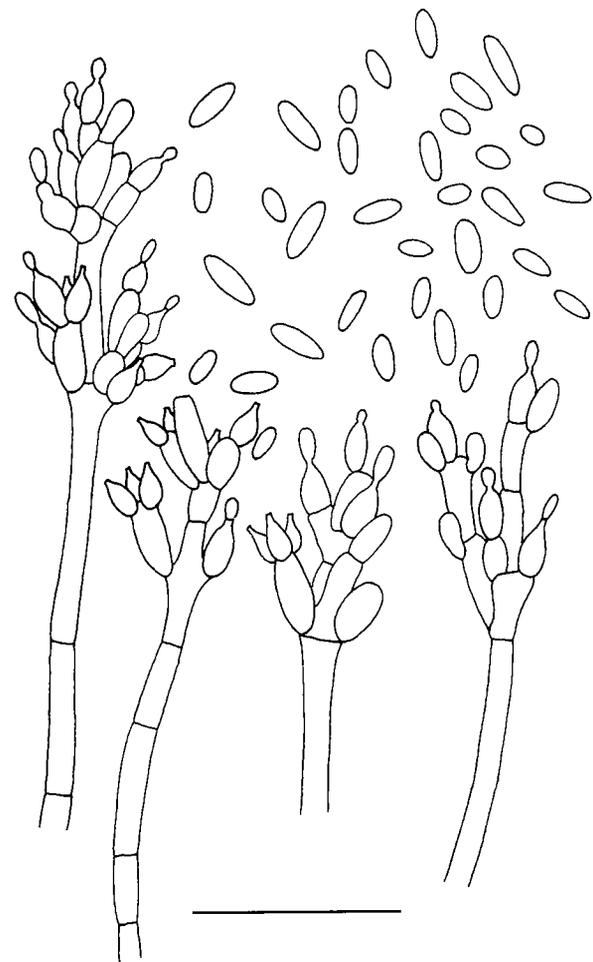


Fig. 2. The *Nomuraea* state produced in culture from *C. cylindrica* part-spores. Scale bar, 20 μm .

Isolates examined: NHJ2347 from multiple part-spores.

Distribution and description of *Nomuraea atypicola* in Thailand

N. atypicola was found on spiders attached to the underside leaves of forest herbs, saplings and trees and on spiders inhabiting the underside of boulders in rivers, especially in cool season between October and February. The hosts were small to medium-sized spiders (body length 5–15 mm).

Conidiophores mononematous or (rarely) synnematous 100–150 μm long by 2–2.5 μm wide. Phialides borne on conidiophore or on small side branches 5.0–7.0 \times 2.0–3.0 μm . Conidia purple-grey, variable in size and shape with individual specimens 3.5–6.0 \times 1.0–1.5 μm .

Specimens examined: These are held in the herbarium of the NBC. NHJ155.07, Khao Yai–Tad Tha Phu trail, 10 Oct. 1989, N. L. Hywel-Jones; NHJ198.02, Khao Yai–Mor Singh To tributary of Lumtakhlong river, 29 Apr. 1990, N. L. Hywel-Jones; NHJ2856, Khao Yai–Mor Singh To tributary of Lumtakhlong river, 11 Jan. 1994, N. L. Hywel-Jones, R. Nasit, S. Sivichai & R. Plomhan. NHJ3076, Khao Yai–Wong Cham Pi, 2 Feb. 1994, N. L. Hywel-Jones, R. Nasit, S. Sivichai & R. Plomhan.

In most specimens the conidiophores formed a dense covering over the host which appeared uniformly purple.

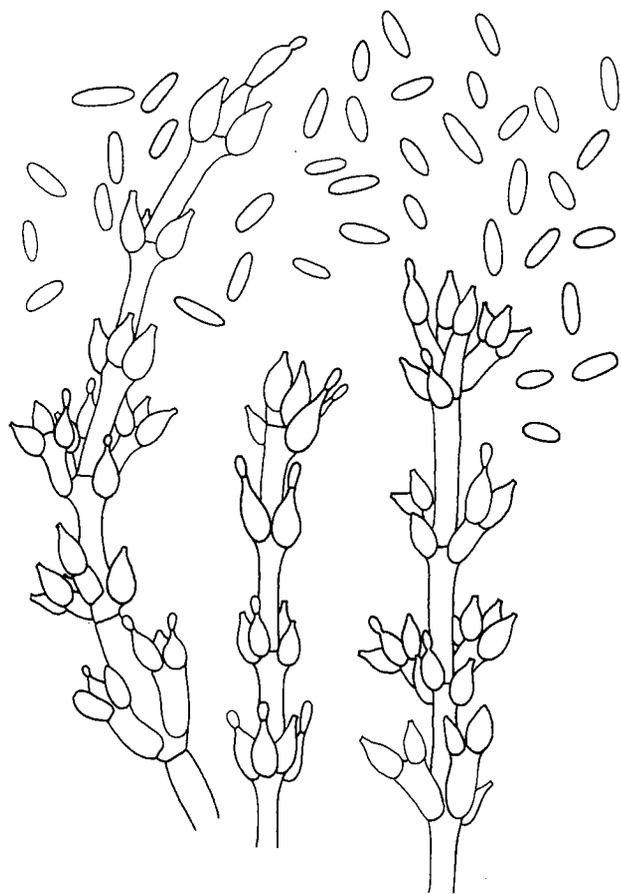


Fig. 3. The *Nomuraea* state produced in culture from conidia on the surface of the spider. Scale bar, 20 μ m.

grey. In specimen NJH155.07 there was a synnematal habit with short synnemata up to 1 mm long and 100–150 μ m in diameter. Specimen NHJ3076 was very young, conidia were sparse and in short chains and had not yet coloured the underlying white mycelium.

Isolation of *Nomuraea atypicola*

Conidia germinated within 24–36 h. Cultures were secured from multi-spore isolations but not from single spore isolations. The mycelium was white at first, floccose and restricted reaching 5 mm in 10 d at 22° in the dark. The reverse was cream-white. Within 1 wk patches of purple-grey areas of sporulation were seen on the white mycelium. Phialides were ovoid to cylindrical with short or absent necks, 4.0–8.0 \times 2.5–3.0 μ m and generally borne on the conidiophore with cells 6.8–25.0 \times 3.0 μ m, or sometimes on small side branches 4.0–11.0 \times 3.0–4.0 μ m (Fig. 3). Conidia were 3.8–6.4 \times 1.1–1.9 μ m.

Isolates examined: NHJ198.02, NHJ2856 and NHJ3076 all from conidia.

DISCUSSION

The *C. cylindrica* specimen was found in the immediate area of an epizootic of *Cordyceps pseudomilitaris* Hywel-Jones & Sivichai. The size of the perithecia of *C. cylindrica* in Thailand

was in very good agreement with Petch's record of 1.2 mm high by 250 μ m diam. Mains (1954) re-examined the type and noted the perithecia were 850–1200 \times 220–270 μ m. Kobayasi & Shimizu (1977) noted that specimens from Japan had slightly smaller perithecia (850–1000 \times 200–225 μ m) than the type material. With the published records and those reported in this study it seems the perithecia of *C. cylindrica* are from 850–1267 \times 200–300 μ m.

Petch (1937) failed to see asci or ascospores in the type material noting that there were 'a few spores, cylindrical or narrow-oval with rounded ends, 2.5–3.5 \times 1.0 μ m'. He doubted whether these were part-spores. His description does not match the usual definition of part-spores (Kobayasi & Shimizu, 1977; Hywel-Jones & Sivichai, this study). Mains (1954) in a re-examination of the type material also noted that there were no asci or ascospores visible.

Kobayasi & Shimizu (1977) were the first to report in detail on *C. cylindrica*. They could not measure the length of the asci but noted these were 4.5–5.5 μ m diam. It was not possible to determine the full length of the asci in the Thai material as these had already broken within the perithecia. There was no evidence of the ascus cap and the ascus foot was not seen either. While Kobayasi & Shimizu (1977) did not see whole ascospores they noted there were part-spores 3–4 \times 1.2 μ m with truncate ends. The measurements of part-spores from the Thai material and their shape agree well with published records. From these few reports of *C. cylindrica* it would seem that most specimens have been very mature.

N. atypicola was first recorded from Thailand in 1986 where it was found infecting lycosid and tetragnathid spiders in rice paddies in Suphan Buri and Pathum Thani Provinces (N. L. Hywel-Jones, unpubl. obs.). Because of the colouring it was thought to be *Paecilomyces fumosoroseus* (Wize) A. H. S. Br. & Sm. but this has finer conidiophores (1.5–2 μ m) and phialides (5.7–8.0 \times 1.0–2.0 μ m) with a tapering long and distinct neck about 0.5 μ m wide (Samson, 1974). The conidia of *P. fumosoroseus*, also, are smaller than those of *N. atypicola* being 3.0–4.0 \times 1.0–2.0 μ m (Samson, 1974).

Kobayasi (1941), in describing *N. atypicola* from Japan, noted that synnemata were club-shaped, usually solitary but occasionally 2 or 3. He made no mention of a mononematous form. Most specimens of the anamorph from Thailand were mononematous – one was multisynnematal. This generally mononematous form may be a feature of the small size of the host and was similar to the form that *Nomuraea rileyi* (Farl.) Samson takes on lepidoptera larvae. Samson (1974) and Samson & Evans (1977) also noted that some specimens of *N. atypicola* were synnematos, others mononematous.

Petch (1937) initially linked *C. cylindrica* and *N. atypicola* because they had similar shaped stromas. In his original description of *C. cylindrica* he noted the fertile head was pale yellow and the stalk white – both turning black with age and drying. This colour change was noted for the Thai specimen. The *Nomuraea* state was described by Petch (1939) as club-shaped and purple drying to the same colour and consistency as *C. cylindrica*. On the basis of similarities Petch (1937, 1939) concluded that *N. atypicola* was the anamorph of *C. cylindrica*.

This link was confirmed in our study as cultural work demonstrated that *N. atypicola* is an anamorph of *C. cylindrica*.

Nomuraea atypicola

Cultures from multiple part-spore isolations of *C. cylindrica* matched those from multiple conidia isolations of *N. atypicola*. Microscopic examination showed that the colouring was due to pigmented conidia on phialides which exactly match *N. atypicola* (Figs 2, 3). The survey conducted in Thailand and records of other workers suggest that the *Nomuraea* state is more commonly reported than the *Cordyceps*.

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