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Cordyceps myrmecophila-like fungi infecting ants in the leaf litter of tropical forest in Thailand

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Two *Cordyceps* spp. were found infecting ants in leaf litter of tropical forest in Thailand. One species is compared with the poorly understood taxon *Cordyceps myrmecophila*. The second species is assigned to *Cordyceps irangiensis* which was previously known only from the type locality in Central Africa. A specimen of *Hymenostilbe*, not assignable to any recognized species, is described and named. The isolation of these three species in culture is noted.

Evans & Samson (1982, 1984) studied species of *Cordyceps* (Fr.) Link (Ascomycotina; Clavicipitales) infecting ants of the *Cephalotes* (Myrmicinae) and *Camponotus* (Formicinae) complexes. They noted the host specificity of certain *Cordyceps* species within a given ant complex. While studying the insect-pathogenic fungi of the forest floor in tropical monsoon forest of Khao Yai National Park formicine ants were found infected with two macroscopically identical *Cordyceps* species. Sporadic surveys in other National Parks in Thailand also revealed records of these ant-associated *Cordyceps* from the forest floor.

Both specimens of *Cordyceps* were macroscopically similar and could not be reliably separated by eye. However, microscopic detail revealed one species was near *Cordyceps myrmecophila* Ces. which has been frequently reported around the world (Saccardo, 1883; Kobayasi, 1941; Kobayasi & Shimizu, 1978). The other species was identified as *Cordyceps irangiensis* Moureau, known only from the type locality in Central Africa (Moureau, 1961). This paper reports on these two species and describes a new *Hymenostilbe* sp. which was also found on the same hosts and in the same micro-habitats.

MATERIALS AND METHODS

Surveys were made of the leaf litter of the forest floor at Khao Yai National Park over a 4-yr period throughout the year and sporadically at other National Parks in Thailand. Full details of collection and processing are described by Hywel-Jones (1995). All collections are deposited in the N.B.C.R.C insect fungus collection.

RESULTS

Distribution and description of C. cf. myrmecophila *in Thailand*

Specimens were found in the leaf litter of humid forest. At Khao Yai all specimens were found only during the wet season (May to October) or after sporadic heavy rainfall in the area of collection. Specimens from other National Parks were also more common in the wet season or at other times of the year in parks which receive significant rainfall in all months of the year (mainly in the south of Thailand).

The terminal 2–3 cm of the stroma was usually seen emerging from the leaf litter. Careful excavation of the surrounding leaves and twigs revealed the host lying beneath. Sometimes the stroma of the fungus was affixed by a pad of rhizoidal hyphae to the leaves or twigs it was growing against.

Clavus arising from between the head and thorax of adult worker ants (Hymenoptera; Formicinae), usually single, sometimes multiple-headed, 30–80 mm long, 200–300 (–500) µm diam., cream yellow to yellow orange. *Fertile head* terminal, ovoid, citriform to sub-cylindric, (1·5–)2·0–3·2(–4·2) × 1–2 mm. *Ascomata* immersed obliquely, ostioles slightly projecting, elongate flask-shaped, 600–650 × 180–200 µm, ascomatal walls hyaline. *Asci* cylindric, hyaline, capitate, 8-spored, 460–500 µm long by 5·5–6·5 µm wide. *Ascus cap* flattened, 6·7–8 × 4·7–6 µm. *Ascospores* filiform, almost as long as the asci, multiseptate and breaking into part-spores. *Part-spores* hyaline, barrel-shaped, ends truncate, 6–7·5 × 1·3–1·7 µm.

A single, simple clavus usually emerged from the host and terminated in a solitary, fertile head. Abnormal forms were sometimes found, usually associated with damage to the original stroma. In these, abnormally shaped heads developed as side branches where the main clavus was damaged. In a few specimens more than one (up to four) clavae could emerge from the host without any obvious reason for this.

The fertile heads were variable in shape and in intensity of the colour. The heads were firm with close-packed ascomata and if pressure was applied a longitudinal rupture resulted. It was not easy to separate the ascomata without breaking them

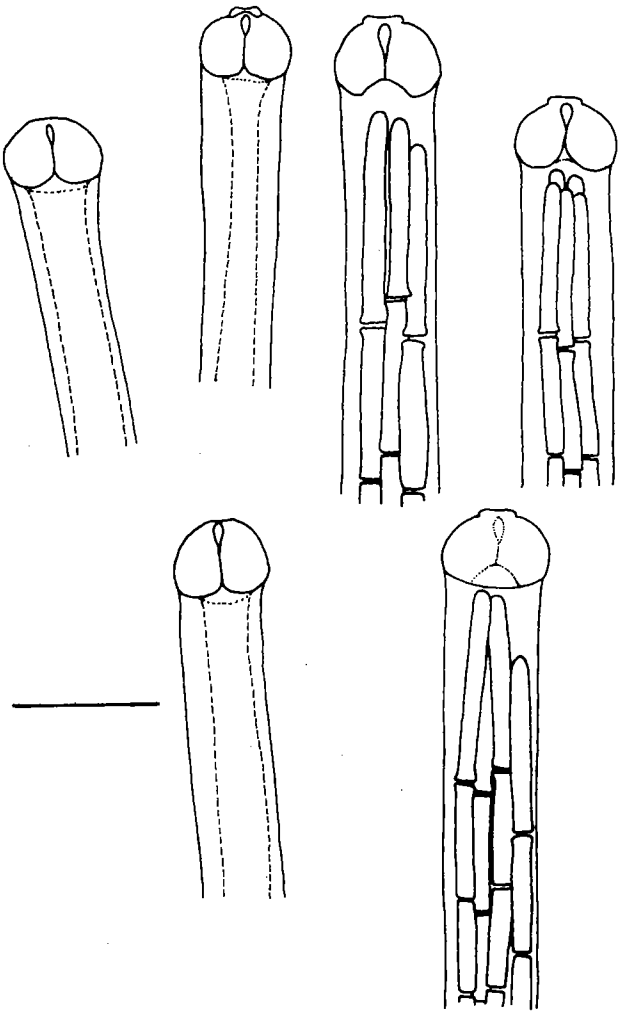


Fig. 1. The ascus tips of three immature (left) and three mature (right) asci of *C. cf. myrmecophila*. Bar, 10 μm.

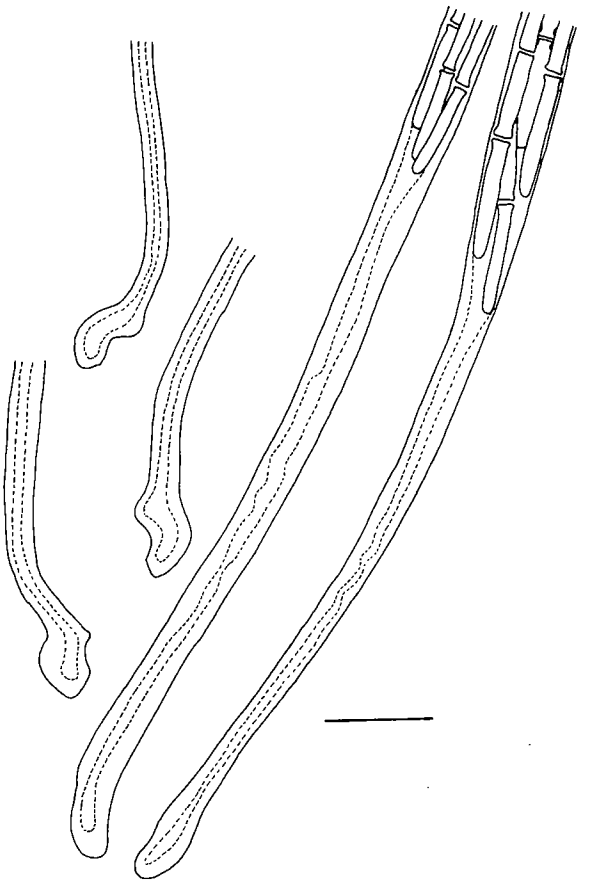


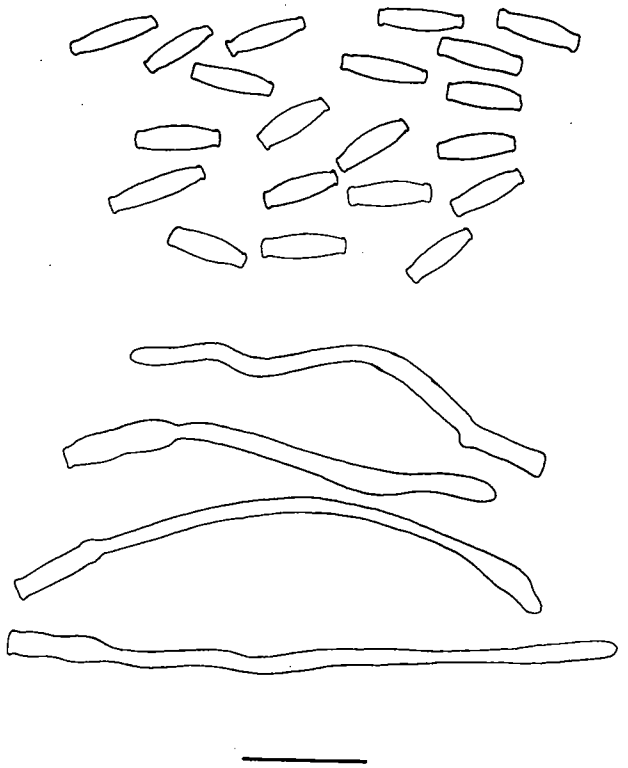
Fig. 2. The ascus foot of *C. cf. myrmecophila*. Bar, 10 μm.

as they were connected to the pigmented outer wall and to the hyaline cortex cells. Although the outer layer of the head was pigmented, all interior cells were hyaline.

Ascomata were packed with asci and there was no sign of a hamathecium. Asci did not mature simultaneously and could be found at all stages of development in a single ascoma. The apical cap was flattened slightly with a prominent doughnut structure (Fig. 1). The ascospores were crowded at the apex with three to five behind the ascus cap (Fig. 1). The ascus was a uniform 5·5–6·5 μm diam. but the base narrowed to 3 μm before widening to a pedicel (Fig. 2).

The ascospores readily separated into part-spores within the ascus. With some specimens it was possible to observe whole ascospores before they had broken. The central part-spores were a uniform length (6·7–7·5 × 1·3–1·7 μm). However, the cells at either end were longer (12·7–15·3 μm) and the penultimate cells of each ascospore wee 8·7–10·7 μm (Figs 1, 2). The released part-spores were barrel-shaped with a slight widening at either end (Fig. 3).

Specimens examined in Thailand: Most collections contain more than one specimen. NHJ535.01, Khao Yai–Heo Narok forest trail to waterfall, 16 Jul. 1991, N. L. Hywel-Jones; NHJ552.03, Khao Yai – road marker km 43·3, 29 Aug. 1991, N. L. Hywel-Jones, M. C. Hywel-Jones & R. E. Hywel-Jones; NHJ1567, Khao Yai – road marker km 42·0, 18 Sept. 1991, N. L. Hywel-Jones; NHJ614.05 and NHJ614.06, Heo Narok forest trail to waterfall, 8 Oct. 1991, N. L. Hywel-Jones; NHJ623.03, Khao Yai – trail along True Left Bank of Lumtakhlong to Heo Sai waterfall, 15 Oct. 1991, N. L. Hywel-Jones & K. Jones; NHJ807.05, Khao Yai–Phakrajai, 25 Jun. 1992, N. L. Hywel-Jones, L. Manoch, A. Rongchitprapas & S. Sivichai; NHJ833.01 & NHJ833.02, Khao Yai – road marker km 34·4, 7 Jul. 1992, N. L. Hywel-Jones; NHJ839.01, road marker km 34·4, 13 Jul. 1992, N. L. Hywel-Jones, A. Rongchitprapas & S. Sivichai; NHJ853.16, NHJ853.18, NHJ853.20, Khao Yai–Gong Giao nature trail, 26 Jul. 1992, N. L. Hywel-Jones & R. A. Samson; NHJ858.02, 858.08, Gong Giao nature trail, 28 Aug. 1992, N. L. Hywel-Jones, A. Rongchitprapas & S. Sivichai; NHJ926.02, NHJ926.03, Khao Yai – road marker km 38·8, 7 Oct. 1992, N. L. Hywel-Jones; NHJ956.06, Khao Yai–Wang Cham Pi, 19 Oct. 1992, N. L. Hywel-Jones, K. Ando, L. Manoch, A. Rongchitprapas & S. Sivichai; NHJ1117, Gong Giao nature trial, 8 Jun. 1993, N. L. Hywel-Jones & R. Nasit; NHJ1189, Heo Narok forest trail to waterfall, 22 Jun. 1993, N. L. Hywel-Jones & R. Nasit; NHJ1212, Heo Narok forest trail to waterfall, 29 Jun. 1993, N. L. Hywel-Jones, R. Nasit, R. Plomhan & S. Thienhirun; NHJ1777, Phakrajai, 6 Aug. 1993, N. L. Hywel-Jones, R. Nasit, R. Plomhan & S. Sivichai; NHJ1798, Heo Narok forest trail to waterfall, 10 Aug. 1993, N. L. Hywel-Jones, R. Nasit, R. Plomhan & S. Sivichai; NHJ2011, Heo Narok forest trail to waterfall, 2 Sep. 1993, N. L. Hywel-Jones, R. Nasit & S. Sivichai; NHJ2152, Gong Giao nature trail, 9 Sep. 1993, N. L. Hywel-Jones, R. Nasit, S. Sivichai & S. Thienhirun; NHJ2286, Doi Inthanon National Park – road marker km 27·0, 26 Sep. 1993, N. L. Hywel-Jones, K. Auncam, R. Nasit, S. Thienhirun & A. J. S. Whalley; NHJ2405, Heo Narok forest trail to waterfall, 19 Oct. 1993; NHJ2420, Khao Yai – trail



Figs 3–4. Fig. 3. Discharged part-spores of *C. cf. myrmecophila*. Bar, 10 μm. **Fig. 4.** Part-spores of *C. cf. myrmecophila* 36 h after being put on PDA. Bar 10 μm.

along True Left Bank of Lumtakhlong river to Pha Khloi Mai waterfall, 19 Oct. 1993, N. L. Hywel-Jones, R. Nasit, R. Plomhan & S. Sivichai; NHJ2810, Khao Yai – road marker km 29·2, 6 Jan. 1994, N. L. Hywel-Jones, R. Nasit & S. Sivichai; NHJ3583, Khlong Nakha wildlife reserve, 22 Apr. 1994, N. L. Hywel-Jones, R. Nasit, R. Plomhan, S. Sivichai & S. Thienhirun; NHJ3933 & NHJ3934, Phakrajai, 24 May 1994, N. L. Hywel-Jones & R. Nasit; NHJ4038, Khao Yai – road marker km 29·2, 15 Jun. 1994, N. L. Hywel-Jones, R. Nasit & S. Sivichai; NHJ4170 & NHJ4171, Kaeng Krachan National Park–Pala U waterfall, 22 Jun. 1994, N. L. Hywel-Jones, R. Nasit, R. Plomhan, S. Sivichai & S. Thienhirun.

Isolation of Cordyceps cf. myrmecophila

When mature specimens were kept in a moist chamber, part-spores were released onto the surface of the head. There was no evidence of violent discharge away from the fertile head. Instead the hyaline part-spores imparted a pruinose appearance to the head when dry but were glistening when wet. These part-spores were viable when transferred to nutrient agar. Whole asci and part-spores could also be released from mature ascomata by applying pressure to the fertile head. Where part-spores had differentiated in the ascus these germinated on nutrient agar, and it was possible to secure isolates from entire asci. Germination of part-spores began after 24–36 h. Polar swelling sometimes altered the shape from barrel to dumb-bell but more usually germination occurred without noticeable swelling of the spore (Fig. 4).

On PDA at 22 °C and in the dark, colonies were largely immersed, slow growing, reaching 12–15 mm after 10–12 wk and did not increase much in size beyond this. Colour was at first buff with a buff-cream reverse, darkening to a cream brown front and reverse. From 4–6 wk onward colonies produced pale ochraceous yellow synnemata, 400–500 μm diam. and up to 20–30 mm long. There was no sign of conidia being produced.

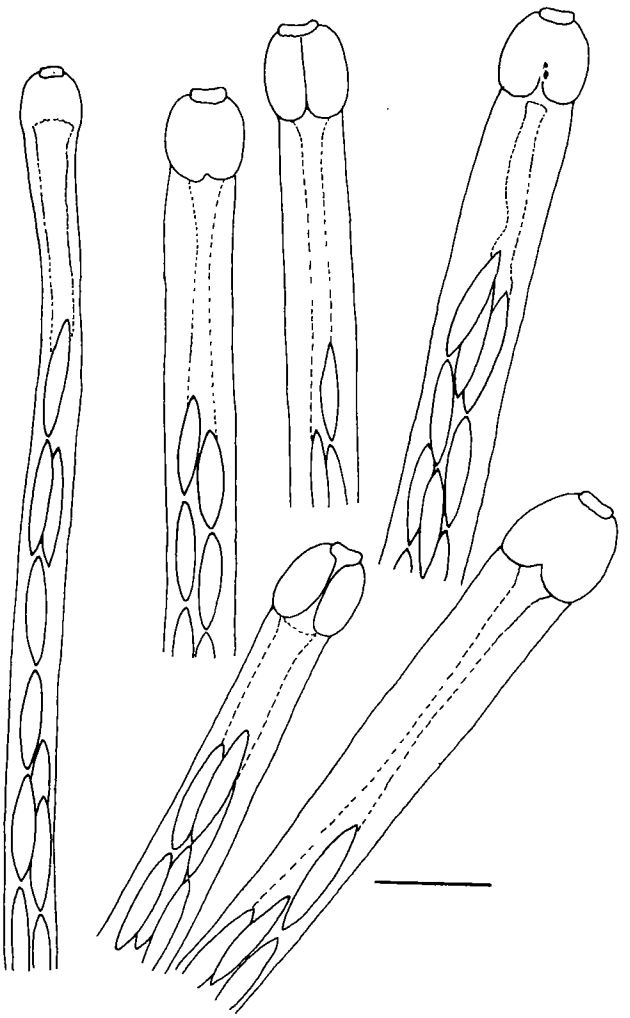


Fig. 5. The tips of six mature asci of *C. irangiensis*. Bar, 10 μm.

Cultures examined: Cultures are maintained in the N.C.G.E.B invertebrate–fungus collection: NHJ614.05, NHJ807.05, NHJ623.03, NHJ833.01, NHJ833.02, NHJ858.08, NHJ926.02, NHJ956.06.

Distribution and description of C. irangiensis *in Thailand*

These specimens were found in the same microhabitat as *C. cf. myrmecophila*. Collections included both species and in the field it was impossible to separate the two except by microscopic examination.

Clavus arising from between the head and thorax of adult worker ants (Hymenoptera; Formicinae), usually single, sometimes multiple, 25–60 mm, 200–700 μm diam., pale cream yellow to ochraceous yellow or orange. *Fertile head* terminal, ovoid to sub-cylindric, often citriform with a prominent, eccentric beak, 2·8–5·7 × 1·2–1·8 mm. *Ascomata* immersed obliquely, ostioles projecting, elongated flask-

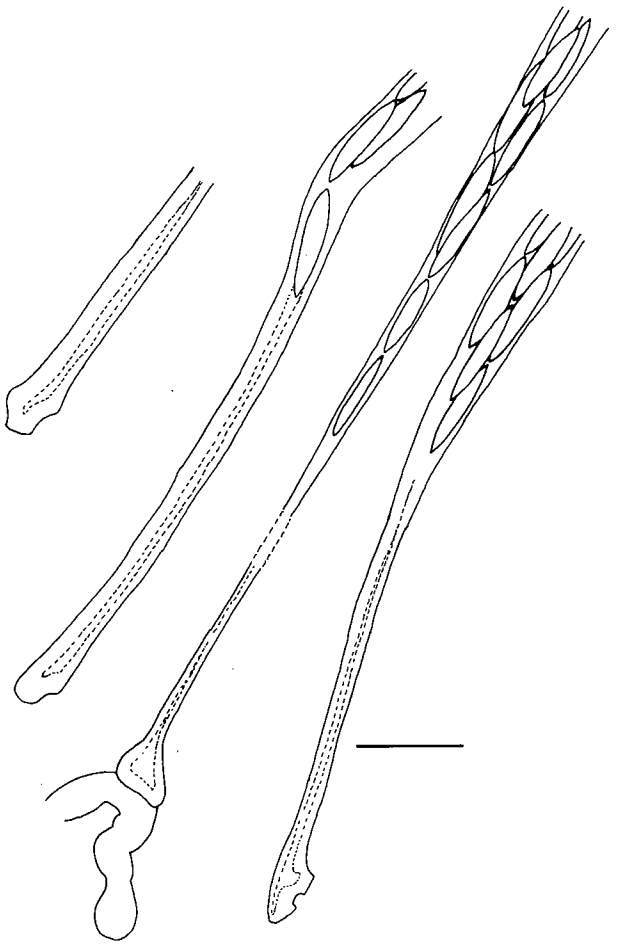


Fig. 6. The ascus foot of *C. irangiensis*. Bar, 10 μm.

shaped, up to 1000 μm long by 150–200 μm wide, ascomatal wall hyaline. *Asci* cylindric, hyaline, capitate, 8-spored, up to 900 long by 6–8 μm wide. *Ascus cap* rounded, 8–9 μm long by 6·7–9·3 μm wide. *Ascospores* filiform, up to 700 μm long, multiseptate, breaking into (about 64) part-spores. *Part-spores* hyaline, fusoid, 8·5–12·5 × 1·3–2·3 μm.

The ascomatal heads of *C. irangiensis* were variable in shape and colour. Ascomata were tightly packed and it was difficult to separate them. Ascomata contained no hamathecium and asci did not mature simultaneously. The ascus cap had a prominent canal running through the middle and was tipped with a doughnut structure (Fig. 5).

The ascospores readily separated into part-spores in the ascus. It was not possible to determine how many part-spores were formed from a single ascospore. There were usually one to three ascospores some 10–27 μm behind the cap of the ascus (Fig. 5).

The ascus was a uniform 6–8 μm in diameter but the foot of *C. irangiensis* narrowed to as little as 2 μm. Below the lowest ascospore there was an empty region of 20–50 μm (Fig. 6). In an extreme case this was almost 200 μm long. After discharge from the ascus part-spores were the same fusoid shape as those still in the ascus (Figs 5–7).

Specimens examined in Thailand: NHJ566.01, NHJ566.02, Khao Yai National Park – trail along True Left Bank of Lumtakhlong to Heo Sai waterfall, 30 Aug. 1991, N. L. Hywel-Jones, M. C. Hywel-Jones & R. E. Hywel-Jones; NHJ578.04, Khao Yai – road marker 43·3 km,

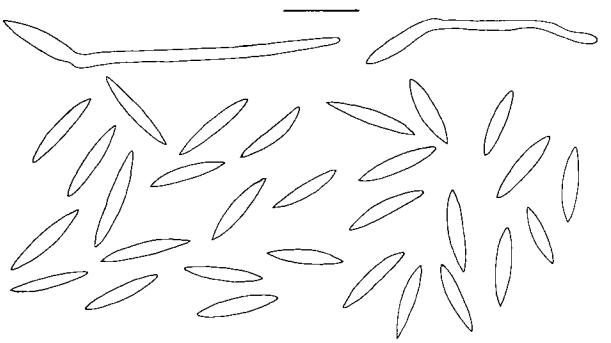


Fig. 7. Discharged part-spores of *C. irangiensis* including two examples of germinating part-spores. Bar, 10 μm.

31 Aug. 1991, N. L. Hywel-Jones, M. C. Hywel-Jones & R. E. Hywel-Jones; NHJ614.04, Heo Narok forest trail to waterfall, 8 Oct. 1991, N. L. Hywel-Jones; NHJ769.01, Phakrajai, 26 May 1992, N. L. Hywel-Jones; NHJ804.03, Khao Yai – road marker 44·8 km, 25 Jun. 1992, N. L. Hywel-Jones, L. Manoch, A. Rongchitprapas & S. Sivichai; NHJ807.04, Phakrajai, 25 Jun. 1992, N. L. Hywel-Jones, L. Manoch, A. Rongchitprapas & S. Sivichai; NHJ836.03 & 836.04 Khao Yai – road marker 34·4 km, 13 Jul. 1992, N. L. Hywel-Jones; NHJ840.02, Phakrajai, 13 Jul. 1992, N. L. Hywel-Jones; NHJ849.02, Gong Giao nature trail, 16 Jul. 1992, N. L. Hywel-Jones; NHJ853.24, NHJ853.26, Gong Giao nature trail, 26 Jul. 1992, N. L. Hywel-Jones & R. A. Samson; NHJ952.01 & NHJ953.05, Sam Lan National Park, 15 Oct. 1992, N. L. Hywel-Jones, A. Rongchitprapas & S. Sivichai; NHJ2012, Heo Narok forest trail to waterfall, 2 Sep. 1993, N. L. Hywel-Jones & R. Nasit; NHJ2287, Doi Inthanon National Park – road marker km 25·5, 26 Sep. 1993, N. L. Hywel-Jones, K. Auncam, R. Nasit, S. Thienhirun & A. J. S. Whalley; NHJ2425, NHJ2426, trail along True Left Bank of Lumtakhlong river to Pha Khloi Mai waterfall, 19 Oct. 1993, N. L. Hywel-Jones, R. Nasit, R. Plomhan & S. Sivichai; NHJ2446, Heo Narok forest trail to waterfall, 25 Oct. 1993, S. Sivichai & A. Rongchitprapas; NHJ3661, 3 May 1994, Wang Cham Pi, N. L. Hywel-Jones, R. Nasit, R. Plomhan & S. Sivichai; NHJ3899, 19 May 1994, Khao Luang National Park–Phrom Lok waterfall, N. L. Hywel-Jones, R. Nasit, R. Plomhan, S. Sivichai & S. Thienhirun; NHJ3938, 24 May 1994, Phakrajai, N. L. Hywel-Jones & R. Nasit; NHJ4001, 7 Jun. 1994, trail from Heo Sawat waterfall to Pha Khloi Mai waterfall, N. L. Hywel-Jones, R. Nasit, R. Reeder, S. Sivichai, M. Thomas & C. Watson; NHJ4072, 21 Jun. 1994, Kaeng Krachan National Park – road marker km 17·0, N. L. Hywel-Jones, R. Nasit, R. Plomhan, S. Sivichai & S. Thienhirun; NHJ4096, 21 Jun. 1994, Kaeng Krachan – road marker km 17·0, N. L. Hywel-Jones, R. Nasit, R. Plomhan, S. Sivichai & S. Thienhirun.

Isolation of Cordyceps irangiensis

Isolations were made from part-spores or part-spores within whole asci. Part-spores germinated in the dark at 22° on PDA after 24–36 h to produce single, polar germ-tubes (Fig. 7). Cultures grew slowly and were mainly immersed with a thin felty aerial mycelium. They reached 15–25 mm after 10–12 wk. The front was pale grey brown to dark brown with a dark brown reverse. The immersed margin of the colony was usually irregular. Some strains produced very thin pale ochre yellow sterile synnemata in culture after 6–12 wk. There was no evidence of an anamorph being produced in culture.

Cultures examined: NHJ578.04, NHJ614.04, NHJ804.03, NHJ807.04, NHJ836.03, NHJ849.02, NHJ952.01, NHJ953.05 and NHJ3938.

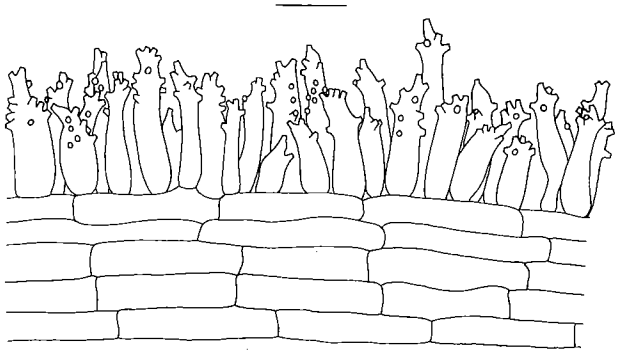


Fig. 8. The hymenium-like layer of conidiogenous cells of *H. aurantiaca*. Bar, 10 μm.

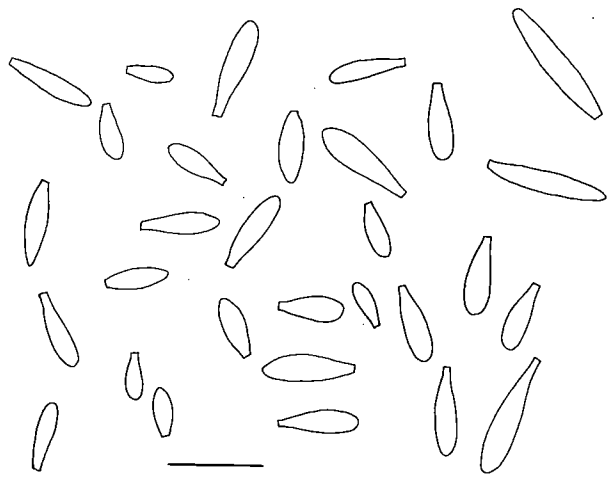


Fig. 9. Dislodged conidia of *H. aurantiaca*. Bar, 10 μm.

Distribution and description of Hymenostilbe aurantiaca *sp. nov. in Thailand*

A third species of entomogenous fungus was also found on formicine ants in the same localities and micro-habitat as infections of *C. cf. myrmecophila* and *C. irangiensis*. This was a hyphomycete best assigned to *Hymenostilbe*. Because it does not match any known species of this genus it is here described as new.

Hymenostilbe aurantiaca Hywel-Jones sp. nov. (Figs 8, 9)

Synnemata simplicia, cylindrica, solitaria raro plura, usque ad 150 mm longa, 150–200 μm crassa; armeniaca vel aurantiaca. *Cellulae conidiogenae* cylindricae vel clavatae, polyblasticae, aurantiacae, sursum denticulis, 9·3–25·0 μm longae, 3·0–6·0 μm crassae. *Conidia* solitaria, cymbiformia, obclavata, laeva, aurantiaca, 5·3–17·0 μm longa, 1·3–3·0 μm crassa.

Holotypus e hymenopteris adultus inter foliis sepultis, Herbarium N.B.C.R.C, NHJ592.01, Phakrajai, Khao Yai National Park, Thailand, 24 Sept. 1991.

Synnema emerging from between thorax and head of adult worker ants (Hymenoptera; Formicinae); usually single, rarely multiple, slender, cylindrical, up to 150 mm long, 150–200 μm diam., apricot orange to orange, composed of hyaline, parallel strands of septate hyphae 4·0–6·0 μm diam., fertile in upper 1/3 to 1/2. *Conidiogenous cells* pigmented, orange, clavate, 9·3–25·0 × 3·0–6 μm, indeterminate polyblastic with sympodial succession, denticles stout, protuberant, unthickened, crowded. *Conidia* solitary, cymbiform to obclavate to strongly obclavate, single-celled, smooth-walled, orange, 5·3–17·0 × 1·3–3·0 μm.

Holotype: NHJ592.01, 24 Sep. 1991, in leaf litter of Monsoon evergreen forest in Khao Yai National Park, Phakrajai deposited in the N.B.C.R.C invertebrate–fungus collection.

Very thin synnemata grew above the leaf litter. The fertile tips were usually a deeper colour than the sterile base. The tips were densely crowded with long conidiogenous cells which had many denticles (Fig. 8). When undisturbed specimens were examined, conidia were seen attached to the denticles. However, any processing for microscopic examination resulted in the dislodging of these conidia. Conidia could be easily dislodged at any stage in their development. This resulted in the wide range of size and shape of dislodged conidia (Fig. 9).

Specimens examined in Thailand: NHJ592.01, Khao Yai National Park–Phakrajai, 24 Sep. 1991, N. L. Hywel-Jones; NHJ617.02, Wang Cham Pi, 8 Oct. 1991, N. L. Hywel-Jones; NHJ1574, Heo Narok forest trail to waterfall, 8 Oct. 1991, N. L. Hywel-Jones; NHJ804.01, road marker km 44·8, 25 Jun. 1992, N. L. Hywel-Jones; NHJ807.01 & NHJ807.02, Phakrajai, 25 Jun. 1992, N. L. Hywel-Jones; NHJ836.02, Khao Yai – road marker km 34·4, 13 Jul. 1992, N. L. Hywel-Jones; NHJ842.01, 13 Jul. 1992, Phakrajai, N. L. Hywel-Jones; NHJ849.01, Gong Giao nature trail, 16 Jul. 1992, N. L. Hywel-Jones; NHJ853.01, NHJ853.09, NHJ853.14, NHJ853.25 & NHJ854.02, Gong Giao nature trail, 26 Jul. 1992, N. L. Hywel-Jones & R. A. Samson; NHJ858.01, NHJ858.02, NHJ858.03 & NHJ858.04, Gong Giao nature trail, 28 Aug. 1992, N. L. Hywel-Jones; NHJ876.06, Phaskrajai, 15 Sep. 1992, N. L. Hywel-Jones; NHJ926.01, Khao Yai – road marker km 38·8, 7 Oct. 1992, N. L. Hywel-Jones; NHJ934.03, Khao Yai – road marker km 29·2, 13 Oct. 1992, N. L. Hywel-Jones; NHJ1116, Gong Giao nature trail, 8 Jun. 1993, N. L. Hywel-Jones & R. Nasit; NHJ1225 & NHJ1227, Heo Narok forest trail to waterfall, 29 Jun. 1993, N. L. Hywel-Jones, R. Nasit, R. Plomhan & S. Thienhirun; NHJ1317, NHJ1319 & NHJ1337, Gong Giao nature trail, 6 Jul. 1993, N. L. Hywel-Jones & R. Nasit; NHJ1480, trail to Tad Tha Phu waterfall, 27 Jul. 1993, N. L. Hywel-Jones, R. Nasit, R. Plomhan & S. Sivichai; NHJ1754, NHJ1778–1784 & NHJ1787, Phakrajai, 6 Aug. 1993, N. L. Hywel-Jones, R. Nasit, R. Plomhan & S. Sivichai; NHJ1825, 10 Aug. 1993, Heo Narok forest trail to waterfall, N. L. Hywel-Jones, R. Nasit, R. Plomhan & S. Sivichai; NHJ1865–1867, Khao Yai – road marker km 44·8, 17 Aug. 1993, N. L. Hywel-Jones, R. Nasit & S. Sivichai; NHJ1880, trail along True Left Bank of Lumtakhlong river to Heo Sai waterfall, 17 Aug. 1993, N. L. Hywel-Jones, R. Nasit & S. Sivichai; NHJ1947–1949, NHJ1955, NHJ1958, NHJ1960–1962, NHJ1964–1969 & NHJ1971, Phakrajai, 24 Aug. 1993, N. L. Hywel-Jones, R. Nasit, S. Sivichai & C. Tangchit; NHJ2013, Heo Narok forest trail to waterfall, 2 Sep. 1993, N. L. Hywel-Jones & R. Nasit; NHJ2078 & NHJ2081, Mor Singh To, 8 Sep. 1993, N. L. Hywel-Jones, R. Nasit, R. Plomhan, S. Sivichai & S. Thienhirun; NHJ2143, Khao Yai – trail along True Left Bank of Lumtakhlong river downstream of Gong Giao waterfall, 9 Sep. 1993, N. L. Hywel-Jones, R. Nasit, R. Plomhan, S. Sivichai & S. Thienhirun; NHJ2154 & NHJ2155, Gong Giao nature trail, 9 Sep. 1993, N. L. Hywel-Jones, R. Nasit, R. Plomhan, S. Sivichai & S. Thienhirun; NHJ2285, Doi Inthanon National Park – road marker km 27·0, 26 Sep. 1993, N. L. Hywel-Jones, K. Auncam, R. Nasit, S. Thienhirun & A. J. S. Whalley; NHJ2341, Sam Lan National Park, 12 Oct. 1993, N. L. Hywel-Jones, R. Nasit & S. Sivichai; NHJ2359, NHJ2373 &

NHJ2375, Phakrajai, 13 Oct. 1993, N. L. Hywel-Jones, R. E. Hywel-Jones, R. Nasit, S. Sivichai, M. Vanabhuti & S. Vanabhuti; NHJ2398 & NHJ2399, Heo Narok forest trail to waterfall, 19 Oct. 1993, N. L. Hywel-Jones, R. Nasit, R. Plomhan & S. Sivichai; NHJ2443, Heo Narok forest trail to waterfall, 25 Oct. 1993, S. Sivichai & A. Rongchitprapas; NHJ2613, Heo Sawat, 19 Nov. 1993, N. L. Hywel-Jones; NHJ3446, NHJ3448 & NHJ3450, 29 Mar. 1994, Phakrajai, N. L. Hywel-Jones, R. Plomhan & S. Sivichai; NHJ3578, NHJ3584, 22 Apr. 1994, Khlong Nakha wildlife reserve, N. L. Hywel-Jones, R. Nasit, R. Plomhan, S. Sivichai & S. Thienhirun; NHJ3755 & NHJ3756, 17 May 1994, Khao Luang–Galom waterfall True Left Bank between steps 5 and 7, N. L. Hywel-Jones, R. Nasit, R. Plomhan, S. Sivichai & S. Thienhirun; NHJ3807, 18 May 1994, Khao Luang–Krung Ching forest, N. L. Hywel-Jones, R. Nasit, R. Plomhan, S. Sivichai & S. Thienhirun; NHJ3937, 24 May 1994, Phakrajai, N. L. Hywel-Jones & R. Nasit; NHJ3954 & NHJ3999, 7 Jun. 1994, trail from Heo Sawat waterfall to Pha Khloi Mai waterfall, N. L. Hywel-Jones, R. Nasit, R. Reeder, S. Sivichai, M. Thomas & C. Watson; NHJ4039, 15 Jun. 1994, Khao Yai – road marker km 29·2, N. L. Hywel-Jones, R. Nasit & S. Sivichai; NHJ4073 & NHJ4097, 21 Jun. 1994, Kaeng Krachan National Park – road marker km 17·0, N. L. Hywel-Jones, R. Nasit, R. Plomhan, S. Sivichai & S. Thienhirun; NHJ4172 & NHJ4173, 22 Jun. 1994, Kaeng Krachan–Pala U waterfall, N. L. Hywel-Jones, R. Nasit, R. Plomhan, S. Sivichai & S. Thienhirun; NHJ4227, 23 Jun. 1994, Kaeng Krachan – road marker km 27·0, N. L. Hywel-Jones, R. Nasit, R. Plomhan, S. Sivichai & S. Thienhirun.

Isolation of Hymenostilbe aurantiaca

Isolations were made by wiping the fertile terminal region of a synnema across the agar surface. Conidia germinated in 24–36 h on PDA, in the dark at 22°. On PDA colonies were largely immersed, slow growing, reaching 15 mm after *ca* 10 wk and not increasing much in size beyond this. Colour was at first buff to buff brown with a buff cream to buff brown reverse, darkening to a brownish front and reverse. From 4–6 wk onward colonies produced ochraceous synnemata, 200–500 µm and up to 30 mm long. There was no evidence of sporulation in culture.

Cultures examined: NHJ3450 and NHJ3755.

DISCUSSION

Saccardo (1883) cited records of *C. myrmecophila* from Italy, Finland, Britain, North America, Sri Lanka and Borneo. The record for Britain (Saccardo, 1883) was ignored by Petch (1948) in his revised list of British insect-pathogenic fungi. This British record was traced to Berkeley & Broome (1881) who erroneously described *C. myrmecophila* from an ichneumonid wasp. It is likely that Berkeley & Broome's record was *Cordyceps sphecocephala* (Klotzsch) Sacc. which is macroscopically similar to *C. myrmecophila* and, although not explicitly stated, Petch (1924, 1948) clearly assumed Berkeley & Broome's record was *C. sphecocephala*.

Mains (1940) noted that *C. myrmecophila* 'has been rarely collected and information concerning it is scanty'. When discussing Saccardo's (1883) records, Mains (1940) mentioned that hosts other than ants were recorded and concluded that 'probably other species of *Cordyceps* are included'. Besides these records, Kobayasi (1941) noted *C. myrmecophila* from Switzerland, Brazil, Vietnam (Annam) and China and later from Taiwan (Kobayasi & Shimizu, 1978). These reports would indicate a pan-global distribution for *C. myrmecophila*.

While many authors have described *C. myrmecophila* macroscopically, microscopic descriptions often lack detail. Few authors have described the shape of the part-spores, still fewer have figured them. With the description of the macroscopically similar *C. irangiensis* by Moureau clearly examination of the part-spore shape is important. Until careful microscopic examination is made of herbaria specimens the pan-global nature of *C. myrmecophila* should be questioned. Some specimens might be better placed in *C. irangiensis*.

In contrast to *C. myrmecophila*, *C. irangiensis* is known only from the type locality in Africa (Moureau, 1961) and now from Thailand (this study). Moureau (1961) noted that *C. irangiensis* was related to *C. myrmecophila*, *C. sphecocephala* and *C. formicarum* – all on Hymenoptera. Specimens of *C. irangiensis* from Thailand match Moureau's description. Although macroscopically similar to *C. cf. myrmecophila*, it differs microscopically. The shape of the ascus part-spore was an especially significant difference. These were fusoid for *C. irangiensis* but truncate barrel-shaped for *C. cf. myrmecophila*. The form of the ascus cap was also important. In *C. cf. myrmecophila* the cap was flattened while it was elongate for *C. irangiensis*. To a lesser degree, the form of the ascus foot and the packing of the ascus part-spores was also different between the two species.

In his key Kobayasi (1982) recognized three sub-genera – *Ophiocordyceps* (Petch) Kobayasi, *Eucordyceps* Kobayasi and *Neocordyceps* Kobayasi. Kobayasi (1982) correctly placed *C. myrmecophila* in the subgenus *Neocordyceps* but wrongly included *C. irangiensis* in the subgenus *Eucordyceps*, section *Cystocarpon* Kobayasi series *Mycogenae* Tul. He also keyed *C. irangiensis* to a group where the fertile part of the stroma has an apical appendage. While there was sometimes a slight beak to the citriform head this cannot be regarded as an apical appendage in the form that this is understood – i.e one that normally produces the anamorph. Considering the currently used criteria, *C. irangiensis* belongs in the subgenus *Neocordyceps* along with the related *C. myrmecophila* and *C. sphecocephala*.

A full review of the differences between the sub-genera will require a more extensive examination of the taxonomy, ecology and biology of many species. However, it may be noted here that the species placed in *Neocordyceps* appear to ooze their part-spores onto the surface of the fertile head. This contrasts with members of the sub-genera *Ophiocordyceps* (Hywel-Jones, 1994) and *Eucordyceps* (Hywel-Jones, unpubl. obs.) which actively discharge their ascospores.

In the field, the thin synnema of *H. aurantiaca* were often confused with roots of plants and easily overlooked. *Hymenostilbe aurantiaca* is morphologically similar to *H. longispora* Samson & H. C. Evans, and both occur on ants. However, *H. aurantiaca* differs significantly in not having a black base to the synnema, having larger conidiogenous cells (those of *H. longispora* are 3–13 × 4·5–6 µm) and more numerous and crowded denticles. Conidia of *H. aurantiaca* were also smaller than *H. longispora*.

Most conidia of *H. aurantiaca* were strongly obclavate. However, it was possible to find cymbiform conidia

5·3–9·0 μm long. At first it was thought these represented a separate species of *Hymenostilbe*. However, multiple collections showed that both spore shapes and size ranges occur on the same synnema. Smaller, stouter conidia were probably immature. Examination of squash preparations revealed it was very rare to find spores still attached to the conidiogenous cell. From this it was assumed the spore septum was laid down before elongation occurred.

Pigmentation was due to cytoplasmic oil globules. The stronger colour in the upper part of the synnema coincided with the fertile area of conidiogenous cells and conidia and with the part of the synnema which extended above the leaf litter. The infertile base of the synnema was pale cream orange with the parallel strands of hyphae containing orange globules. Pigmentation could be a protection from uv light. One specimen of *C. irangiensis* (NHJ4001) was collected in a well-lit clearing and was deep orange in colour.

Sutton & Hennebert (1994) have attempted to rationalize the interconnexions amongst anamorphs and their possible contribution to ascomycete systematics. They have noted that there are at least 43 combinations of conidiogenous events which can be organized into 12 broad groups. Examination of many conidiogenous cells suggested conidial development was by sympodial conidiogenous cell proliferation as in *Tritirachium* (Hawksworth, Sutton & Ainsworth, 1983, fig. 6*c*). *Hymenostilbe* has characteristics which put it in one of four main core groups. Typical characteristics of this group include multilocular, blastic conidiogenous cells with conidia produced successively and solitarily at each point.

Specimens of *H. aurantiaca* were never found on the same host as either of the two species of *Cordyceps*, but collecting has not been extensive enough to rule out this possibility. Specimens ascribable to *H. aurantiaca* were found in the same locale as *C. cf. myrmecophila* collections, and when they were isolated and grown in pure culture the cultural characteristics of the two were similar enough to suggest a teleomorph/anamorph connexion. However, until more conclusive evidence is available it is proposed that *H. aurantiaca* is kept separate.

Although all three species have been isolated it is not considered likely that these will have potential for biological control. The slow, sterile growth makes these fungi poor candidates for consideration when there are other more easily manipulated species available for study.

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