Cordyceps brunneapunctata sp. nov. infecting beetle larvae in Thailand

NIGELL HYWEL-JONES

Director Centre for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Ministry of Science, Technology and Environment Building, Rama VI Road, Bangkok 10400, Thailand

Cordyceps brunneapunctata is described from elaterid beetle larvae buried in forest soils in Thailand where it is found associated with a Coleoptera. Isolations were made from ascus part-spores, conidia and from hyphal material within the host and all grew in an anamorphic manner.

MATERIALS AND METHODS

Survey were made of the leaf litter of the forest floor at Khao Yai National Park during a 4 yr period, throughout the year and especially at other National Parks in Thailand. Stromata emerging from the soil were excavated carefully to ensure collection of the buried host. The surface layer of leaves and litter was removed and a stout knife was used to excavate soil at least 5 cm from the stroma. After an excavation 9 cm deep was made it was possible to work back toward the stroma carefully cutting through roots. If this was not possible to return immediately to the laboratory, specimens were taken to the laboratory in plastic containers and stored in a refrigerator when not being examined. If it was not possible to return immediately to the laboratory, specimens were dried for 1-2 d before being stored in plastic pots.

Hyphal material was excavated from the host abdomen by sterilizing part of the abdomen with 100 % alcohol, breaking the exoskeleton and extracting the contents with a flame-sterilized watchmaker's forceps. These hyphal bodies were dispersed in 0-05 % Triton X-100 solution using flame-sterilized insect pins. Ascomata were re-hydrated in 0-05 % Triton X-100 solution and dissected with fine forceps and a scalpel. Asci were further dissected using sterilized insect pins.

Isolations were made on potato dextrose agar (PDA) from abdominal hyphal bodies, ascus part-spores and conidia. A sterilized inoculation loop was used to spread ascomatal and hyphal bodies on PDA plates. Conidia were spread directly on PDA plates. Herbarium specimens were sealed in plastic pots after being rendered inert in an alcohol atmosphere. All herbarium specimens are stored with the author's codes in the NBCRC insect-fungus collection.

TAXONOMY

Cordyceps brunneapunctata Hywel-Jones, sp. nov. (Figs 1-12)

Etym.: named for the brown-dotted appearance of the fertile head

Stromata solitaria raro usque ad tria, simplicia, (25-)40-90 mm alta. Stipites simplices, cylindracei, squamosi, basi bruneo, capitulo distincto, subterminalis, cinnamonei et brunneapunctati, 5-15 mm alta, 1-1.8 mm crassi. Ascomata immersa, apice vix prominua, atrobreunnea, ovatia vel piriformia, parietibus atrobreunnea, 270-335 μm alta, 110-160 μm crassa. Asci hyalini, cylindracei, capitati, octospori, 280-295 μm longi, 6-7 μm crassi. Ascosporae hyalinae, filiformes, flexuosae, multisetae, in articula cylindraceae, trunci, truncatae 4-6 μm longae et 1-1.5 μm crasseae. Status anamorphicus synematosus, terminalis, pallidus griseus vel albusculus, teres, 8-12 mm longus. Cellulae conidiogenae monophialidicae, nonnumquam polyphialidicae hyalinae, laevae 15-25 μm crasseae. Conidia hyalinae, aseptatae, laevae, sphaericae, 1.5-2.5 μm, in muco involuta.

In larva coleopteron, Thailand, Nakorn Nayok Province, Khao Yai National Park, road marker km 29-2, 16 July 1991. N. L. Hywel-Jones, NHJ539.02, in herb. NBCRC holotypus.

Stroma solitaria rarely up to 3, simple, (25-)40-90 mm high. Stipe simple, cylindric, base reddish-brown, squamous, head distinct, subterminal, cinnamon-coloured with brown dots,
Cordyceps brunneapunctata sp. nov.

Figs 1-3. Cordyceps brunneapunctata from the abdomen of an infected elaterid larva. (Scale bar, 10 μm.) Fig. 1. Hyphal strands. Fig. 2. Hyphal strands with terminal bulbous growths. Fig. 3. Hyphal bodies.

5–15 mm high, 1.0–1.8 mm across. Ascomata immersed, perithecioid, brown, ovate to pyriform, brown-walled, 270–335 μm high, 110–160 μm across. Ascii hyaline, cylindrical, capitate, 8-spored, 280–295 μm long, 6–7 μm wide. Ascospores hyaline, filiform, flexuous, multi-septate, breaking; spores, cylindrical, curved, truncate, 4–6 μm long and across. Anamorph synnematous, terminal, pale grey, whitish, terete, 8–12 mm long. Conidiogenous cells phialidic, rarely polyphialidic, hyaline, 5.5–7.5 × 2.5–3 μm at the base, up to 15 × 0.5 μm enveloped by a mucous sheath.


The fungus was not common but appeared consistently in Khao Yai National Park over 4 yr of searching soil and decaying insect carcasses.
Cordyceps brunneapunctata. Fig. 4. Tip of stroma showing luminous nature of the surface below the anamorph-bearing region (scale bar, 5 mm). Fig. 5. Two stromata illustrating the form of the teleomorph-bearing region and its relationship to the terminal anamorph-bearing region (scale bar, 5 mm). Fig. 6. A section through the part of the teleomorph showing the shape and arrangement of the perithecoid ascomata (scale bar, 300 μm).

Habitats. When one specimen was found at a site it was usually possible to find 2–5 other specimens during 6–10 man hours of searching. It also appeared in collections from other National Parks and wildlife reserves in the south of Thailand. Specimens were never found loose in the leaf litter. The host was always buried in the soil to a depth of some 2–5 cm. A single stroma usually grew from the host but sometimes two or three were formed.

The host abdomen was packed with parallel strands of hyphae running the length of the body (Figs 1, 2) intermixed with some hyphal bodies (Fig. 3). The stroma arose from any point on the host and grew straight up through the soil. The stroma was thicker where it emerged from the host. Above ground it was brown with sometimes a reddish-purple or cinnamon hue and a few mm below the fertile part it was luminous (Figs 4, 5). In some specimens, the stroma tapered gradually to form a greyish spike bearing the anamorph (Fig. 1).

Perithecoid ascomata appeared as distinct brown spots on a paler cinnamon or pale brown background (Figs 5, 6). No evidence was seen of a hamathecium or of paraphyses within the ascomata. The cap of the undifferentiated ascus was distinct with cytoplasm forming a conical canal within (Fig. 7) and the cylindrical ascus had a simple foot (Fig. 8). Whole, septate ascospores were not observed in the ascus.

After differentiation of the ascospores within the ascus the part-spores were up to 10 μm behind the cap (Fig. 9). Two or three ascospores occupied the ascus just behind the cap (Fig. 9). A single ascospore could be found usually near the base of the ascus foot (Fig. 10). Before release part-spores were cylindrical with truncate ends (Figs 9, 10) but, after release, part-spores were sometimes curved with slightly rounded ends (Fig. 11).

The anamorph was always terminal and either solitary (Fig. 4) or connected with the teleomorph (Fig. 5). It consisted of a layer of crowded monophialidic, occasionally polyphialidic, conidiogenous cells (Fig. 12). The spherical conidia were enveloped in a barely visible mucous coat (Fig. 12).

Mycelium/hyphal bodies, ascus part-spores and conidia readily germinated on PDA to produce slow-growing, flattened, cinnamon and white stromatic cutures. These readily produced the Hirsutella state either mononematously on hyaline hyphae or, after 10–12 wk, on thin, cinnamon synnemata with a pruinose white fertile zone bearing conidiogenous cells and conidia and a cream tip.
**DISCUSSION**

Seven species of *Cordyceps* are known from elaterid beetles: *C. elateridicola* Kobayasi & Shimizu, *C. gracilis* Durieu & Mont., *C. luntii* Giard, *C. rubra* A. Møller, *C. salebrosa* Mains, *C. stylophora* Berk. & Broome and *C. viperina* Mains. Of these, *C. stylophora* appears to be the more common although Mains (1941) still regarded it as ‘rarely collected’.

Specimens of *C. brunneapunctata* from Thailand did not match any of these species although there was a superficial resemblance to *C. stylophora*, which also had a terminal fertile zone with a *Hirsutella* state. This species has an ochraceous tawny to dark cinnamon-brown stroma. The perithecioid ascomata of *C. stylophora* are larger, at up to 420 µm, than those of *C. brunneapunctata* while the asci are generally smaller at up to 220 µm long. The most significant difference is, however, the ascospores which remain whole in *C. stylophora*, not regularly breaking into part-spores.

Comparison of *C. brunneapunctata* with other species of *Cordyceps* from Coleoptera larvae shows that it comes close to *C. rubripunctata* Moureau reported from lamellicorn larvae in Zaire (Moureau, 1949). Moureau reported that species was frequently found on larvae buried in the humid forest. A distinctive feature that *C. brunneapunctata* shares with *C. rubripunctata* is the shape of the part-spores which Moureau considered was characteristic. The presence of a squamous layer below the fertile part of the stroma is another feature the two species have in common.

Moureau (1949) noted the stalk of *C. rubripunctata* olivaceous-brown, becoming tawny, with the apex yellow becoming whitish. The fertile region was white with vivid red perithecia. These colours contrast with the generally brown or reddish-brown colour of *C. brunneapunctata*. The ascomata of the two species are quite different in size being 350–550 × 200–350 µm in *C. brunneapunctata* compared with 270–335 × 110–160 µm in *C. rubripunctata*.

The stromata of *C. brunneapunctata* were of three types; teleomorph only, anamorph only and teleomorph and anamorph together. Specimens where only the teleomorph was present all seemed old. In some the ascomata locally were devoid of contents having been excavated by mycophagous insects. It appeared that in these specimens the anamorph-bearing region had been lost through aging although the anamorph was found alone these specimens always fresh. However, no evidence was found to suggest the teleomorph later grew from the anamorph as there were examples of immature perithecia developing on or from the anamorph.

It is a pleasure to thank the National Research Council of Thailand for providing logistical support for this project. Banpot Napompeth and his staff at the NBCRC continue to provide a pleasant environment in which to work. Mr Phinyo Thienhiru and the Royal Forest Department are thanked for their support. Miss Rungrat Nasit and Mr Somsak Plomhan located many of these specimens and helped with processing and isolation of the specimens while Mr. Plomhan provided technical assistance.

**REFERENCES**

