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Cordyceps khaoyaiensis and *C. pseudomilitaris*, two new pathogens of lepidopteran larvae from Thailand

NIGEL L. HYWEL-JONES

National Biological Control Research Centre, PO Box 9-52, Kasetsart University, Bangkhen, Bangkok 10900, Thailand

Cordyceps khaoyaiensis and *C. pseudomilitaris* resemble *C. vinosa* and *C. militaris* respectively in general form, but differ significantly in producing ascospores which did not fragment while in the ascus or on release. While *C. khaoyaiensis* appears to be rare, *C. pseudomilitaris* is able to affect large numbers of a larval population. Both grew easily in culture, producing anamorphs best assigned to *Hirsutella*.

Cordyceps Fries is particularly well represented on the insect order Lepidoptera, where it usually infects the immature stages (Kobayasi, 1982). In a recent survey of the insect-pathogenic fungi of Thailand two *Cordyceps* species were found infecting lepidoptera larvae. They both have superficial similarities with recognized species but differ in microscopic detail and are described here as new.

Cordyceps khaoyaiensis Hywel-Jones, sp. nov.

Etym: named after the type locality

Stromata solitaria, simplicia, albo vel albo-purpurascentia, 55 mm alta. Stipites glabri, carnosi, flexuosi, cylindracei compressi, basi albo-purpurascente, capitulo concolore, 15–20 mm alti, 2–4 mm crassi. Capitula terminalia, plus minusve clavata sed apice obtuso-rotundata et fertilia. *Perithecia* immersa, apice vix prominula, purpurea, ovoidea, parietibus hyalinis, 335–410 × 200–270 µm. *Asci* hyalini, cylindrici, capitati, octospori, 215–340 × 5–6 µm. *Ascoporae* hyalinae, filiformes, flexuosae, multiseptatae, in ascis numquam individuales in partes secedentes, 165–240 × 1 µm.

Status anamorphicus in cultura PDA, celeriter crescens, mycelio aerio lanato indutus, hyphis cum vegetabilibus ramosis, septatis, albis, 1–2 µm latis, reverso cremoris colore, dein purpurascente, mononematosus. *Cellulae conidiogenae* monophialidicae, hyalinae, leves 15–22 × 1·5–2 µm. *Conidia* hyalina, aseptata, levia, lacrymiformia, 2–3·5 × 1·5–2 µm, in muco involuta.

Holotypus e lepidopterarum larva inter foliis sepultis, Herbarium N.B.C.R.C., NHJ 885 Heo Sai waterfall, Khao Yai National Park, Thailand, 15 Sep. 1992.

Stroma solitary, simple from dead lepidopteran larva, white to whitish-purple, becoming purple around ostioles, 55 mm high, up to 4 mm wide; stipe glabrous, fleshy, flexuous, flattened cylindric, base whitish-purple. Internal hyphae hyaline, smooth, septate, parallel with stroma. Fertile part terminal, concolorous, 15–20 mm high, 2–4 mm wide, more or less clavate with an obtusely rounded apex. *Perithecia* immersed, ovoid, single-layered hyaline wall, 335–410 µm high and 200–270 µm wide, maturing in unison. Hamathecium absent. *Asci* hyaline, cylindric, capitate, 8-spored, 215–340 µm long and 5–6 µm wide when mature. Immature and mature asci in same perithecium. *Ascospores* smooth, filiform, hyaline, flexuous, multiseptate, non-fragmenting, 165–240 µm long and 1 µm wide.

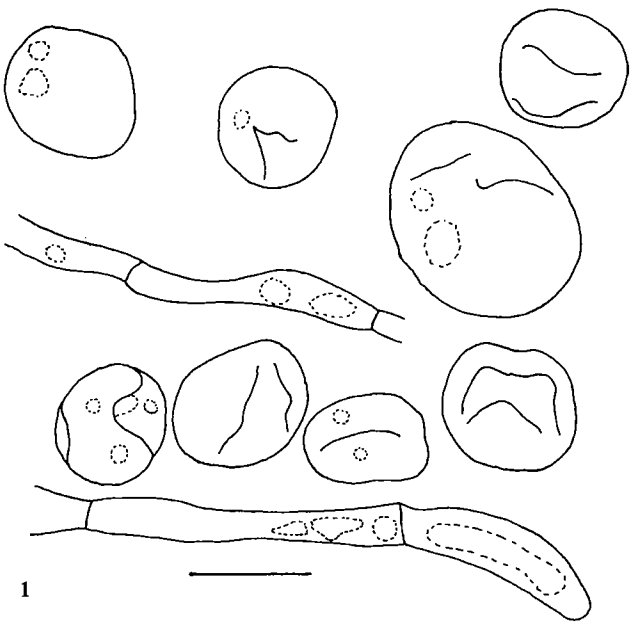
Anamorphic state in PDA culture, growing rapidly (30 mm in 3 days at 22 °C in the dark) with woolly aerial mycelium, branching vegetative hyphae, septate, white, 1–2 µm wide, reverse cream becoming purplish. *Conidiogenous cells* mononematous on undifferentiated vegetative hyphae, monophialidic, hyaline, smooth, 15–22 µm long, 1·5–2 µm wide. *Conidia* solitary or a few on each phialide, hyaline, aseptate, smooth, lacrymoid, 2–3·5 µm long, 1·5–2 µm wide, in a mucilaginous sheath.

Holotype from lepidopteran larva in moist fallen leaf litter of tropical monsoon evergreen forest. Herbarium N.B.C.R.C., NHJ 885 Heo Sai waterfall, Khao Yai National Park, 15 Sep. 1992.

The host abdomen was packed with cream-white spherical hyphal bodies intermixed with hyphae (Fig. 1), giving the appearance of a mycelium ramifying throughout the host before breaking into hyphal bodies. This condition is similar to that found in some Entomophthorales (Hywel-Jones, unpubl. obs.). Unlike the more typical endosclerotial forms of *Cordyceps*, the body of the larva did not become rigid but remained flexuous. The hyphal bodies and hyphae from the abdomen were mostly devoid of cytoplasm and the viable part of the fungus was restricted to the stroma and fertile region.

The ovoid perithecia of *C. khaoyaiensis* (Fig. 2) were within a thin wall of hyphae, which enclosed the whole fertile region giving the overall purple appearance. This wall was integrated with the perithecia around the ostiole and the colour was deeper here, making the presence of the perithecia beneath clear. There was little hyphal material between the perithecia, and these were easily separated from each other when dissected out.

The asci of *C. khaoyaiensis* were much longer than the ascospores and typically there was an empty region of



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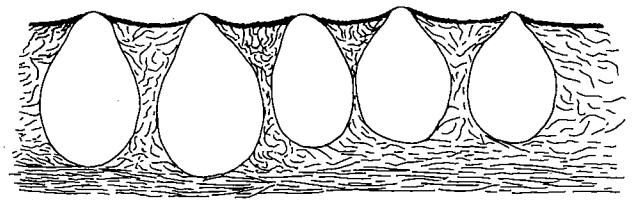

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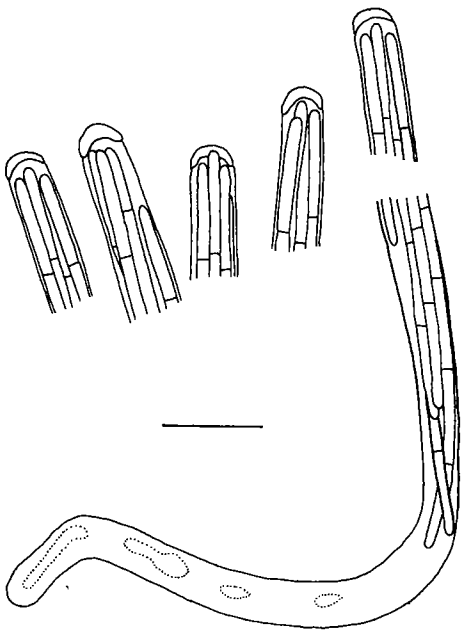

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20–80 μm following the last ascospore (Fig. 3). The ascospores of *C. khaoyaiensis* were aligned more or less parallel in the ascus, unlike most *Cordyceps* species, which have spirally arranged ascospores (Hywel-Jones, unpubl. obs.). Three to five were seen in the apex behind the cap (Fig. 3). Release of these was observed in sterile water. Pressure appeared to build in the ascus, forcing the spores one at a time through an indistinct pore in the ascus cap. The fresh specimen released whole ascospores on to the surface of the stroma, and these could be germinated within 5 h on PDA. *C. khaoyaiensis* grew rapidly on PDA producing the anamorph within 3 d (Fig. 4). The anamorph was not found on the host.

A second species found on lepidoptera larvae occurred in evergreen monsoon forest at Khao Yai. It was solitary, infecting just a few individuals here, but occurred as an epizootic in deciduous monsoon forest of Sam Lan National Park. This species bore a superficial resemblance to *Cordyceps militaris* (Vuill.) Fr.

Cordyceps pseudomilitaris Hywel-Jones & Sivichai, sp. nov.

Etym.: indicating its superficial similarity to *C. militaris*.

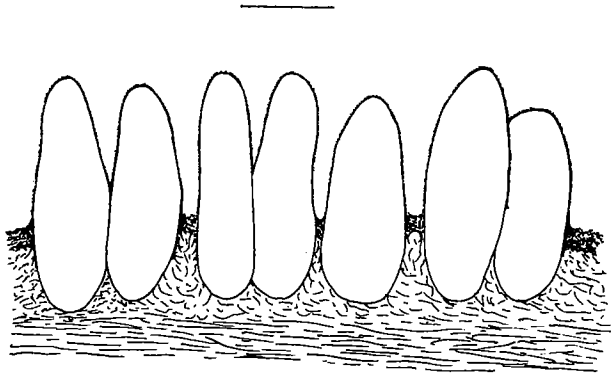
Stromata solitaria vel caespitosa, simplicia vel ramosa, 15–30 mm alta, 0·9–3 mm lata. Stipes glabri, carnosi, fragiles, flexuosi, cylindracei, basi albo vel albo-aurantiaco, sursum aurantiaco, capitulo concolore. Capitula terminalia, clavata vel obclavata, 2–8 mm longa, 1·2–4·0 mm lata. *Perithecia* plus minusve superficialia, basi immersa, apice prominentes, elongato-ellipsoidea vel elongato-ovoidea, 290–570 μm longa, 120–245 μm lata; parietibus et intus hyalinis. *Asci* hyalini, filiformes, capitati, octospori, 290–410 × 5–6 μm. *Ascosporae* hyalinae, filiformes, flexuosae, multiseptatis, in ascis numquam individuales in partes secedentes, 280–390 × 1 μm.

Status anamorphicus in cultura PDA celeriter crescens (40 mm tres dies 22°), mycelio aerio lanato indulus, hyphis cum vegetabilibus ramosis, septatis, albis, 1–2 μm latis, reverso cremoris colore. Cellulae conidiogenae monophialidicae, hyalinae, leves 8–21 × 1–1·5 μm. *Conidia* hyalina, aseptata, levia, elliptica, 4–6 × 1·5–2 μm, tunica mucilagina non visa.

Holotypus e lepidopterorum larva inter foliis sepultis, N.B.C.R.C. herbarium NHJ 945, Sam Lan National Park, Thailand, 12 Oct. 1992.

Stroma solitary or in tufts, simple or branched, from dead lepidoptera larvae, 15–30 mm high, 0·9–3 mm wide, fleshy, orange, cylindric to flattened cylindric fertile head terminal, variably clavate to flattened clavate or obclavate, 2–8 mm long by 1·2–4·0 mm wide. *Perithecia* almost superficial with one-quarter to one-third immersed, elongate ellipsoid to elongate ovoid, 290–570 μm long × 120–245 μm wide; perithecial wall two-layered, inner wall hyaline with a thin orange outer layer. *Asci* hyaline, cylindric, capitate, 8-spored, 290–410 μm × 5–6 μm. *Ascospores* smooth, filiform, hyaline, flexuous, multiseptate, not breaking into part-spores, 280–390 μm × 1 μm.

Figs 1–4. Microscopic detail of *C. khaoyaiensis* and its anamorph. **Fig. 1.** Hyphal bodies and hyphae from host abdomen. **Fig. 2.** Arrangement of the perithecia in the stroma. **Fig. 3.** Tips of five asci showing the cap and the arrangement of the ascophores. The foot of one ascus is shown with the arrangement of the ascophores. **Fig. 4.** Typical phialides and conidia of the anamorph from culture. Bar: Figs 1, 3 and 4, 10 μm; Fig. 2, 200 μm.



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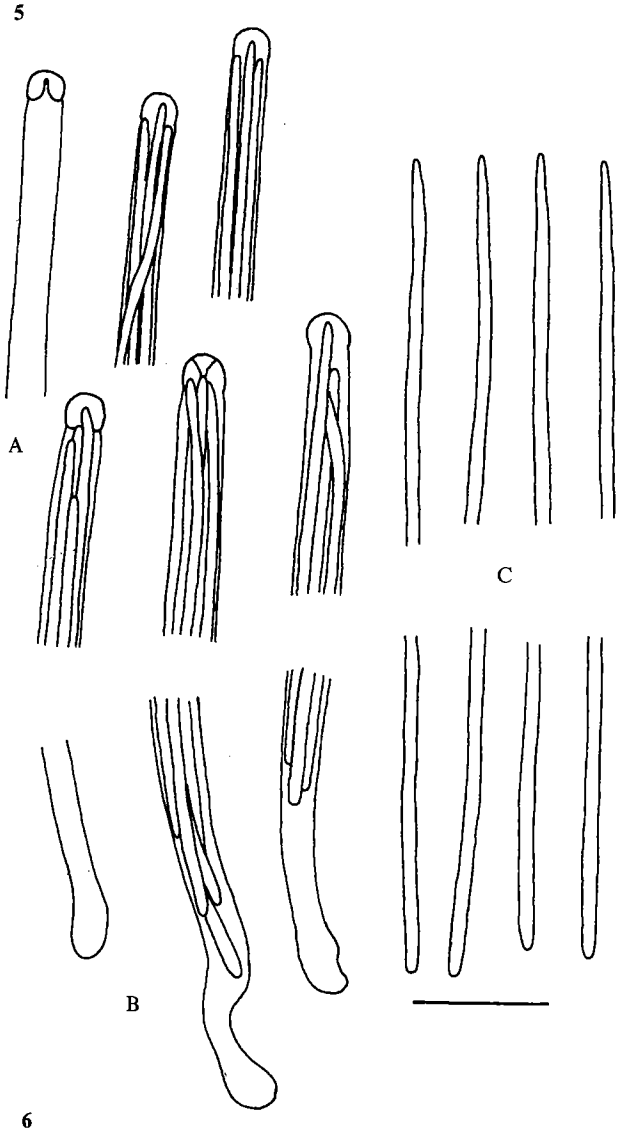

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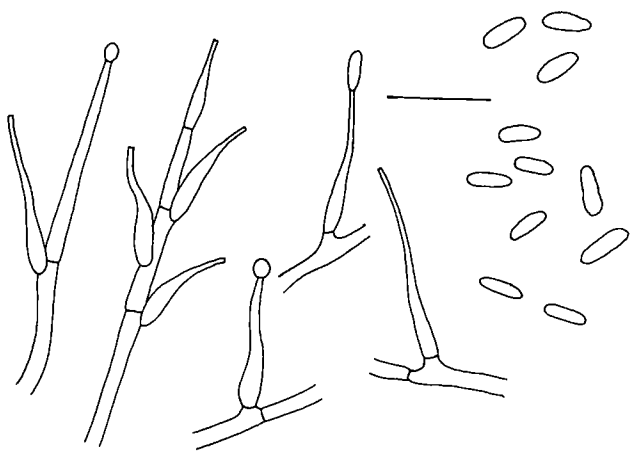

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Figs 5–7. Microscopic detail of *C. pseudomilitaris* and its anamorph. **Fig. 5.** Arrangement of the perithecia on the stroma. **Fig. 6A.** Tips of one immature and five mature asci showing arrangement of the ascospores. **Fig. 6B.** Base of 3 asci showing the form of the ascus foot and the arrangement of the ascospores. **Fig. 6C.** The tips and bases of four ascospores. **Fig. 7.** Phialides and conidia of the anamorph from culture. Bar: Fig. 5, 200 µm; Figs 6 and 7, 10 µm.

Anamorphic state in PDA culture, growing rapidly (40 mm, 3 d, 22°), aerial mycelium white, vegetative hyphae branched, septate, white, 1–2 µm wide, reverse cream. *Conidiogenous cells* mononematous on undifferentiated vegetative hyphae, monophialidic, hyaline, smooth, 8–21 µm long, 1–1·5 µm wide. *Conidia* hyaline, aseptate, smooth, elliptical, 4–6 µm, 1·5–2 µm wide, mucilaginous coat not seen.

Holotype from lepidoptera larva buried in moist fallen leaf litter of deciduous monsoon forest. N.B.C.R.C. herbarium NHJ 945, Sam Lan National Park, Thailand, 12 Oct. 1992.

The larvae were ground-dwelling lepidoptera, and specimens were usually 2–5 cm below the soil surface. The dead infected larva was surrounded by hyphae which had aggregated into a loose network of rhizomorph-like structures enveloping the larva. These structures grew separately through the soil, occasionally uniting and again separating before coalescing at the surface to form the stroma. When the host was horizontal in the soil several separate stromata could develop from the many rhizomorph-like structures which grew up from the insect. Occasionally the insect was buried vertically and then the rhizomorph-like structures grew in a confined region, all uniting to form a single stroma at the surface.

Stromatal colour ranged from deep orange-red to orange-yellow; its form was highly variable, with some specimens having a single stroma while others were multi-stromatal. The orange-red to orange-yellow colour occurred on material exposed to light. Below ground the stroma was rhizoidal, branched and cream-white. The degree of branching may be partly related to the size of the host.

The size and shape of the perithecia of this species was extremely variable (Fig. 5). Small specimens tended to have smaller, more ovoid perithecia, while the larger specimens were more elongated. This variability was observed within individual specimens.

In common with *C. khaoyaiensis* the filiform ascospores were arranged more or less parallel (Fig. 6A, B). Although multiseptate like *C. khaoyaiensis*, the septation was not so well defined. Unlike *C. khaoyaiensis* the ascospores were almost the same size as the asci (Fig. 6B). The ascospores of this fungus were very long, averaging over 300 µm, filiform in shape but with the apical end more acute than the pedicel end (Fig. 6C).

Specimens which were kept refrigerated readily released ascospores after being brought in to the laboratory environment. Whole, filiform ascospores were discharged from the perithecia. These fell slowly, landing up to 3–4 cm distant from the stroma. Those which landed on the dry Petri-plate

base rapidly dried out and were no longer viable. Those which landed on water or agar remained viable, germinating within 7 h and producing moderately fast-growing colonies on PDA.

Cultures of *C. pseudomilitaris* rapidly produced an anamorph in culture (Fig. 7). The anamorph was mononematous, with conidiogenous cells 8–21 μm × 1–1·5 μm. These sometimes had a swollen base, but there were also examples which were awl-shaped. Conidia are 4–6 μm × 1·5–2 μm and elliptical without a noticeable mucous sheath.

DISCUSSION

Cordyceps vinosa Moureau was described from lepidopteran larvae in tropical forest of the Belgian Congo (Moureau, 1961), but no further records are known. The single specimen of *C. khaoyaiensis* collected from leaf litter of tropical monsoon forest in Khao Yai National Park, Thailand appeared macroscopically similar to *C. vinosa*, but microscopic examination revealed significant differences.

In common with *C. vinosa* the Thai material consisted of a fleshy stroma more or less purple in colour. The new taxon differs significantly from the African material in having oval perithecia with hyaline walls as opposed to the purple walls of *C. vinosa*. Furthermore, the perithecia are significantly larger, being 335–410 × 200–270 μm compared with 260–360 × 100–180 μm for *C. vinosa*.

A significant difference between *C. vinosa* and the Thai material was that the ascospores of the latter remained whole at maturity, unlike *C. vinosa* in which the ascospores cleave to produce part-spores 7–12 μm long by 1–1·5 μm across. *Cordyceps khaoyaiensis* spores remained intact, and although the long, filiform ascospores were notably multiseptate there was no evidence of natural cleavage into part-spores either within or without the ascus.

Petch (1931) was the first to suggest the importance of the non-fragmenting ascospores within *Cordyceps* and he erected a new genus, *Ophiocordyceps*, to accommodate such species. This separation has not generally been accepted, and most authors accept that while *Cordyceps* is very heterogeneous our current state of knowledge is such that it is better to accommodate these many and varied species within the one genus (Kobayasi, 1941, 1982).

Cordyceps militaris is the best-known and most studied *Cordyceps* species. Mains (1947) described a similar species from lepidopteran pupae, which he named *Cordyceps washingtonensis*. This differed significantly from *C. militaris* in having a sulphur-yellow stroma and ascospores which remained whole, not dividing into part-spores. The Thai material resembled *C. militaris* in having vivid orange stromata but was also like *C. washingtonensis* in having ascospores which remained whole at maturity. Mains (1958) noted that *C. militaris* was more frequently found on pupae of lepidoptera than larvae. *Cordyceps pseudomilitaris* in contrast was found only on the larvae.

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The apparent random growth of the rhizomorph-like structures through the soil contrasted with the organized manner in which they united at the surface to form the perithecial stroma. It is possible the rhizomorph-like structures which first reach the surface emit chemical signals, possibly in response to light, which result in growth of others toward these stimuli. The stromatal colour certainly appears to be light-mediated, as the stroma is pale when covered with leaf litter, becoming strongly coloured when exposed to light.

The evergreen forest of Khao Yai has a closed canopy and the brightly coloured species of fungi appear to be less common than in the open-canopied deciduous forest of Sam Lan (Hywel-Jones, unpubl. obs.). It is possible the bright orange-red colour of *C. pseudomilitaris* may be an adaptation to protect the hyaline spores from uv light during development.

The many reports for *C. militaris* indicate that this is, macroscopically, a very variable species (Kobayasi, 1941). Material of *C. pseudomilitaris* was also highly variable, macroscopically. While the overall appearance was similar to *C. militaris* the microscopic details differed. Similarly, there were differences between the Thai material and *C. washingtonensis*. The main difference is that *C. pseudomilitaris*, like *C. khaoyaiensis*, produced and discharged whole ascospores rather than part-spores. These observations indicate that, apart from *C. militaris*, there is a complex of superficially similar *Cordyceps* species which are pathogenic to the larvae and pupae of Lepidoptera.

Both the new taxa from Thailand readily produced an anamorph in culture. The genus *Hirsutella* seems best suited to accommodate both anamorphs. However, as these were not found from nature it was decided not to recognize them as distinct from the teleomorph.

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