Notes on *Cordyceps nutans* and its anamorph, a pathogen of hemipteran bugs in Thailand

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Cordyceps nutans is described from collections made in Thailand and the first report of *Hymenostilbe nutans* is given for Thailand. The *Hymenostilbe* state is redescribed from fresh material and its association with the teleomorph is discussed. The fungus was successfully cultured from the anamorph but did not survive *in vitro* for long.

Cordyceps nutans Pat. was first described from Japan and there are now many records from that country (Kobayasi, 1941). Petch (1939) reviewed *C. nutans* and noted that it was also collected from New Guinea. Kobayasi (1941) later listed China and Moureau (1949) also described it from the Belgian Congo (now Zaire) as *C.* (*Eucordyceps*) *bicephala* Berk. subsp. *nutans* (Pat.) Moureau. Schumacher (1982) recorded 34 species of ascomycetes from northern Thailand with *C. nutans* the only insect ascomycete reported. In a revision of *Cordyceps* Kobayasi (1981) briefly recorded *C. nutans* from East Siberia, China and Colombia. All these records discuss only the teleomorph. Samson & Evans (1975) described a *Hymenostilbe* state associated with the *Cordyceps* from specimens collected in Ghana.

In a continuing survey of invertebrate-associated fungi from National Parks and Wildlife Sanctuaries in Thailand *C. nutans* and *H. nutans* were occasionally found in leaf litter or buried in soil. As it is such a rarely reported fungus and as it is a distinctive species the following records and notes are given.

MATERIALS AND METHODS

Surveys of invertebrate-associated fungi of tropical forest were made by examining micro-habitats including the underside of living leaves of forest herbs and saplings, the branches and stems of these and the leaf litter. Most work was done at several sites within Khao Yai National Park but sporadic surveys were also made at other National Parks in Thailand.

Specimens were taken to the laboratory in plastic containers and kept fresh in a refrigerator when not being examined. When it was not possible to return specimens immediately to the laboratory these were air-dried for 1–2 d before being stored in plastic pots. Herbarium specimens were prepared by air-drying and then sealing in plastic pots after being rendered inert in an alcohol atmosphere.

Perithecial heads were re-hydrated in 0·05% Triton X-100 solution and dissected with fine forceps and a scalpel while asci were disrupted using sterilized insect pins. Isolations were attempted on Potato Dextrose Agar from abdominal hyphal bodies, ascus part-spores and from conidia. A sterilized inoculation loop was used to spread perithecial material and hyphal bodies on PDA plates. Conidia were wiped directly on to PDA plates.

Isolations were examined at × 10 with an Olympus BH compound microscope to look for germination of the hyphal bodies or spores. Hyphal body, single ascus, single part-spore/conidia and multiple part-spore/conidia isolations were made. Permanent slides were prepared from material mounted in lactophenol cotton blue. These were examined with an immersion objective and drawn with the aid of a drawing tube.

RESULTS

Distribution and description of Cordyceps nutans *in Thailand*

Many records of this fungus were from monsoon evergreen forest in Khao Yai National Park. However, specimens at Khao Yai were always found in the early part of the rainy season. The largest single collection was from mixed deciduous (teak dominated) and *Pinus* forest at Doi Inthanon in northern Thailand. In contrast to collections from Khao Yai these were in the latter part of the rainy season. Collections of the anamorph were also made in April from primary rain forest at Khlong Naka Wildlife Reserve and in May from disturbed primary rain forest at Khao Luang National Park.

Clavus solitary on small hosts (body size 10–15 mm), multiple on larger specimens (body size 20–30 mm), 50–90 mm long, 400–800 μm across, marasmoid black or

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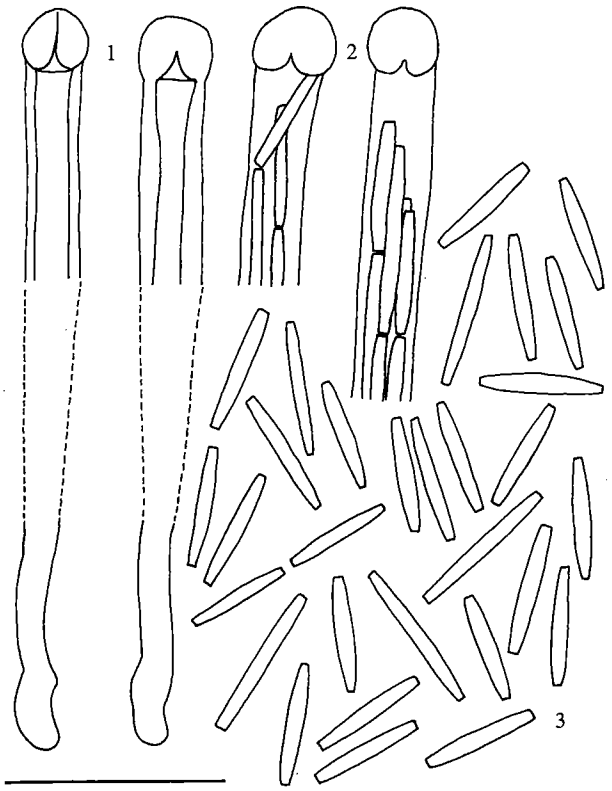

Figs. 1–3. Fig. 1. Two immature asci showing the form of the cap and of the ascus foot (Scale bar in Figs 1–5, 20 μm). **Fig. 2.** Two mature ascus tips showing the arrangement of the part-spores. **Fig. 3.** Examples of released part-spores showing the range of size and shape.

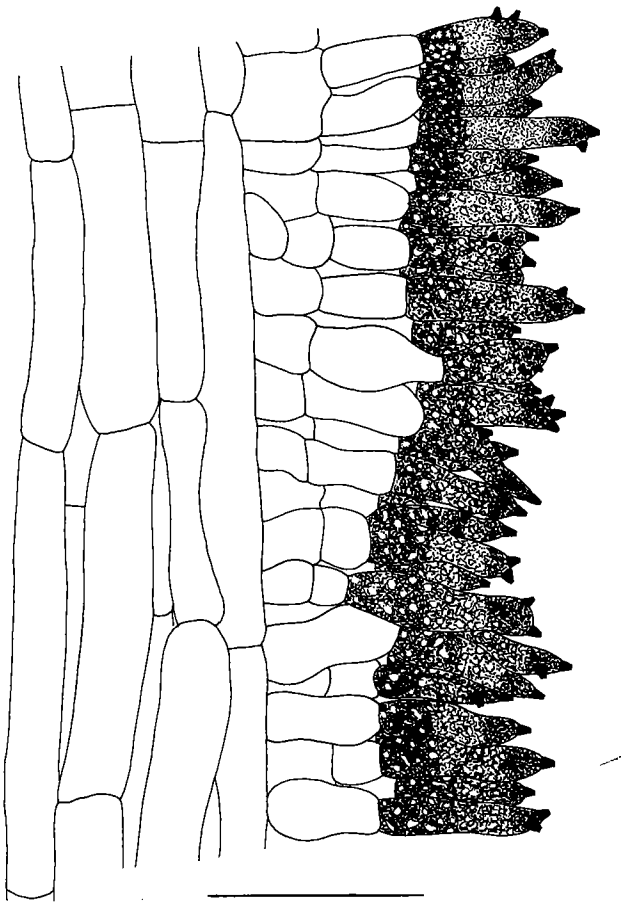

Fig. 4. The arrangement of the conidiogenous cells of *H. nutans*.

blackish brown, becoming red 8–12 mm below the fertile head which was red, salmon-pink, orange or orange-yellow, 6–17 × 3–5 mm. *Perithecia* immersed, hyaline-walled, oblique with a curved neck, 550–800 × 130–200 μm. *Ostioles* visible on the outer surface. *Asci* cylindric, up to 780 μm × 7–8 μm diam., 8-spored. *Ascospores* broke easily in to part-spores. *Part-spores* cylindric or slightly barrel-shaped, ends blunt, 9·3–15·0 × 1·5–2·0 μm. *Anamorph* subterminal and over the perithecial head or independent of the teleomorph and terminal. *Conidiogenous cells* crowded along the pigmented apical region, 10·6–14·3 × 3·3–4·6 μm, red to deep salmon pink. *Conidia* 4·3–7 × 2·7–3·3 μm, red to deep salmon pink.

The perithecial heads were usually cylindric but occasionally irregular in larger or damaged specimens. The perithecia appeared to develop beneath the pigmented anamorph and, as they did so, the terminal part of the stroma swelled from *ca* 500 μm to 3–5 mm. Beneath the pigmented outer wall of the fertile head the inner context was hyaline. When mature, the head was very tough and it was not possible to compress it without rupturing it. Perithecia were tightly held in a context of cells and not easily separated from these without breaking them.

There was no evidence of a hamathecium or of paraphyses within the perithecia. Asci had a distinct cap and appeared to be at different stages of development within the perithecia (Figs 1, 2). The ascus foot was not prominent and was almost vertical with the rest of the ascus (Fig. 1). When mature in the ascus, the ascospores broke easily in to part-spores (Fig. 2). Natural release of part-spores was noted from specimens kept in a water-saturated atmosphere (Fig. 3). Part-spores oozed from the ostioles on to the surface of the fertile head and imparted a pruinose appearance to the red head.

For the anamorph the conidiogenous cells were densely crowded each with one to several deeply pigmented denticles (Fig. 4). No conidia were seen developing from these denticles and it looked as if these were easily separated. The conidiogenous cells were heavily coloured with an oily pigment that gave the cytoplasm a granular appearance (Fig. 4). Conidia also contained this pigment and, when fresh, had the same granular appearance as the conidiogenous cells (Fig. 5).

Observation in Thailand indicated that fresh material was always red to some degree but that on drying, and with time, this colour faded from the conidiogenous cells and conidia to leave an ochre-yellow tip. The red colour was confined to the conidiogenous cells and to the spores.

Specimens examined in Thailand: These are all deposited in the insect–fungus collection at NBCRC with the author's codes.

Teleomorph and anamorph: NHJ806.02, Khao Yai National Park – Phakrajai, 25 Jun. 1992, N. L. Hywel-Jones, L. Manoch, S. Sivichai & A. Rongchitprapas; NHJ853.12, Khao Yai – Gong Çiao, 26 Jul. 1992, N. L. Hywel-Jones & R. A. Samson; NHJ1305, Khao Yai – Phakrajai, 6 Jul. 1993, S. Sivichai, L. Tangchit & C. Yamsopit; NHJ2177, 2178, 2308, 2309, 2310 & 2311, Doi Inthanon National Park – road marker km 25·5, 26 Sept. 1993, N. L. Hywel-Jones, A. J. S. Whalley, R. Nasit, S. Thienhirun & K. Auncam; NHJ4079, 4080 & 4087, Kaeng Krachan National Park – road marker km 17·0, 21 June

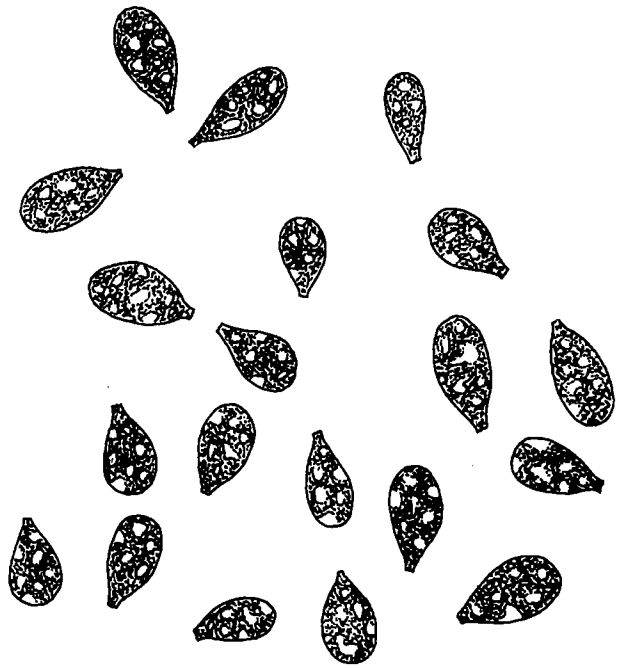

Fig. 5. Examples of mature conidia of *H. nutans*.

1994, N. L. Hywel-Jones, R. Nasit, R. Plomhan, S. Sivichai & S. Thienhirun.

Anamorph only: NHJ854.01, Khao Yai – Gong Giao, 26 Jul. 1992, N. L. Hywel-Jones, S. Sivichai & A. Rongchitprapas; NHJ1112 & NHJ1144, Khao Yai – Gong Giao, 8 Jun. 1993, N. L. Hywel-Jones & R. Nasit; NHJ1226, Khao Yai – Heo Narok, 29 Jun. 1993, N. L. Hywel-Jones, R. Nasit, R. Plomhan & S. Thienhirun; NHJ3543, 3544, 3545, 3546, Khlong Naka Wildlife Reserve, 21 Apr. 1994, N. L. Hywel-Jones, R. Nasit, R. Plomhan, S. Sivichai & S. Thienhirun; NHJ3587, Khlong Naka, 22 Apr. 1994, N. L. Hywel-Jones, R. Nasit, R. Plomhan, S. Sivichai & S. Thienhirun; NHJ3897, Khao Luang National Park – Phrom Lok Waterfall, 19 May 1994, N. L. Hywel-Jones, R. Nasit, R. Plomhan, S. Sivichai & S. Thienhirun.

Isolation of the fungus

Conidia and part-spores germinated within 24–48 h. Further development was very slow and usually resulted in the unsuccessful establishment of the culture. However, a viable isolate was established from conidia of one specimen. Hyphae were hyaline, sparse and totally immersed reaching 10 mm after 30 d at 22 °C in the dark on PDA. The isolate survived monthly subculturing for 6 months before expiring.

Culture examined: NHJ1226 isolated from conidia of specimen NHJ1226.

DISCUSSION

While the type of *C. nutans* was described as having a nutant head (hence the epithet) specimens from Thailand always had an erect head as generally reported by other workers (Kobayasi, 1941; Petch, 1939). Early workers did not report the presence of an anamorph and Samson & Evans (1975) were the first to describe this, noting that it was absent from the type material of *C. nutans*. In describing *H. nutans* Samson & Evans (1975) stated it was present with the teleomorph and included this detail in their key. Specimens from Thailand were sometimes separate from the teleomorph. However, in Thailand, when the teleomorph occurred the anamorph was also found.

It seems, from observation of material in Thailand, that the *Cordyceps* develops underneath the anamorph and that the head gradually swells as the perithecia develop within. In fresh specimens it also seems that the *Cordyceps* form is a paler red than the anamorph on its own. This could be due to the expanding head having fewer conidiogenous cells in a given area and the colour then becoming more diffused or it could be because the older conidiogenous cells have begun to lose the pigment.

The description given by Samson & Evans (1975) for the teleomorph is at odds with all previous descriptions as they report the teleomorph (and anamorph) to be yellowish on black stipes. The colour of the fresh living material of *Cordyceps* spp. is often very different to that of the dried (N. L. Hywel-Jones, unpubl. obs.) and there is a clear need to examine material as fresh as possible. In the description given for *H. nutans* (Samson & Evans, 1975) the conidia were described as hyaline. While this appears to be the case in the dried material fresh conidia from Thailand were red or pinkish red.

All previous reports of *C. nutans* were of very sporadic findings. This survey, in progress for over 5 yr at Khao Yai and other National Parks, suggests this fungus is present only in the rainy season. Specimens from Khao Yai, Doi Inthanon and Khaeng Krachan were from central or northern Thailand and were collected during the monsoon season (May to September). Specimens from Khlong Naka and from Khao Luang (collected in April and May respectively) were also from moist forest as they were from the south of Thailand where significant rain falls in all months of the year.

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