Cordyceps sphecocephala and a *Hymenostilbe* sp. infecting wasps and bees in Thailand

NIGEL HYWEL-JONES*

National 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 sphecocephala and a *Hymenostilbe* sp. were recorded from Hymenoptera (wasps and bees) in natural forest in Thailand. These were isolated from hyphal bodies, ascus part-spores and from conidia. The possible relationship between the two fungi is discussed. These records are compared with other collections from around the world.

Sphaeria sphecocephala was described by Klotzsch and published by Berkeley (1843) from wasps in Central America. *S. sphecocephala* Klotzsch ex Berk. was later transferred to *Cordyceps* as *Cordyceps sphecophila* Berk. & M. A. Curtis. Mains (1958) noted that *C. sphecocephala* 'has frequently been given the name *C. sphecophila* apparently due to an error in publication by Berkeley and Curtis, who cited Tulasne but changed the name' of the epithet. Cooke (1892) noted problems concerning the spelling of the epithet and to prevent an 'unwarrantable multiplication of synonymy' chose to accept *Cordyceps sphecocephala* Klotzsch as the correct citation. This was accepted by Petch (1933; 1937). However, based upon the epithet provided by Klotzsch and based upon the currently accepted genus, the earliest correct citation is *C. sphecocephala* (Klotzsch) Sacc.

Petch (1937) considered *C. sphecocephala* to be the teleomorph of *Isaria sphecophila* Ditmar which he transferred to *Hymenostilbe*. Consequently, in a review of teleomorph–anamorph connections, Kendrick & DiCosmo (1979) listed *H. sphecophila* as the anamorph of *C. sphecocephala* citing Petch (1937; 1948). However, previously, Mains (1950) and Samson & Evans (1975) had considered Petch's *Hymenostilbe* as doubtful.

Petch (1932), Kobayasi (1941) and Mains (1958) discussed *C. sphecocephala* and several related species but came to separate conclusions on synonymy. In the last three years a large collection of *Cordyceps* and *Hymenostilbe* on wasps and bees in tropical forest in Thailand has been made. These specimens, and cultures derived from them, provide new information on *Cordyceps sphecocephala* and its possible relationship with a previously unaccepted *Hymenostilbe*.

* Address for correspondence: National Biological Control Research Centre, P.O. Box 9-52, Kasetsart University, Bangkok 10900, Thailand.

MATERIALS AND METHODS

Surveys were made of the leaf litter of the forest floor at Khao Yai National Park over a three year period throughout the year and sporadically at other National Parks in Thailand. Collections of infected insects were returned to the laboratory for processing. Isolations were made on Potato Dextrose Agar from abdominal hyphal bodies, ascus part-spores and from conidia. Slides were prepared for microscopic examination using an Olympus BH microscope with a drawing tube.

RESULTS

Distribution of the fungi in Thailand

All specimens were lying loose in, or on, moist leaf litter in the rainy season between May and October. All were found in forest between 100 and 1600 m above sea level. Forest types included tropical monsoon evergreen forest (at Khao Yai), tropical monsoon deciduous forest (at Sam Lan) and submontane evergreen forest (at Doi Inthanon). Many were found on their backs giving the impression they had fallen from the surrounding vegetation at death.

Description of the Cordyceps *and* Hymenostilbe *specimens from Thailand*

The inside of the host was packed with white, oval, hyphal bodies, 20–30 µm by 10–20 µm. A single stroma arose from any point on the insect but usually from between the head and thorax. This was up to 45 mm long including the head. Where these contacted a substrate such as leaves or twigs, rhizoidal growth fixed the stroma to this substrate.

The diameter of the stalk was 150–800 µm consisting of parallel strands of septate, tightly packed hyphae, 4–6 µm

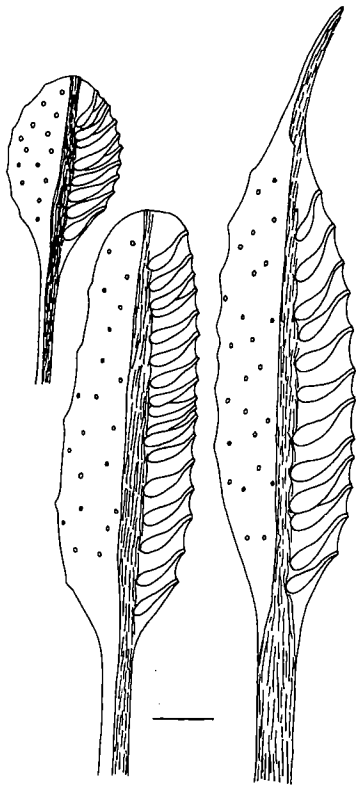

Fig. 1. Three fertile heads of *C. sphecocephala* showing the external appearance (left side) and internal appearance (right side). Scale bar, 1 mm.

across. There was no evidence of branching. Fertile heads were terminal, variable in size and shape, the smallest being 2·2 × 1·2 mm and the largest 11 × 1·9 mm (Fig. 1) but mostly in the range 4–8 × 1·4–1·8 mm. Heads were citriform to cylindric in shape, attenuating to a bent tip. Both stalk and head were ochraceous to cream yellow.

Dissection of the head showed the colour was confined to the outer walls. The interior contained perithecia with walls of tightly interwoven, hyaline hyphae in a loose context of hyaline cells around a central core which was an extension of the stalk (Fig. 1). Perithecia were oblique to the walls of the fertile head but with a distinctive curve on the neck ending almost at right-angles to the outer wall of the head. Perithecia were large 880–1000 × 200–260 µm with no evidence of a hamathecium. All seemed to mature at the same time. However, within each perithecium asci were found at different stages of development.

Asci were hyaline, filiform, at least 700 µm long and up to 7 µm diameter with a prominent apical cap (Fig. 2). A thickened plug was sometimes seen at the tip of the cap above the central canal (Fig. 2). This feature was not constant within or between specimens and did not appear to be related to the state of ascus development. At maturity, ascospores broke readily into hyaline, fusoid part-spores, 10–14 × 1·5–2·5 µm (Fig. 2).

Most specimens examined, and especially larger forms, were very mature and devoid of part-spores. For others, pressure applied to the fertile head was often enough to release part-spores into water. Natural release of part-spores was not observed.

On some specimens there were eggs on the stroma and dipteran larvae within the *Cordyceps* head. Larvae fed upon perithecia and stromatal contents within the fertile head. When this happened it was not possible to find any healthy perithecia or any spores.

When the *Cordyceps* head was damaged, it was possible for new growth to occur. This was either from the point of

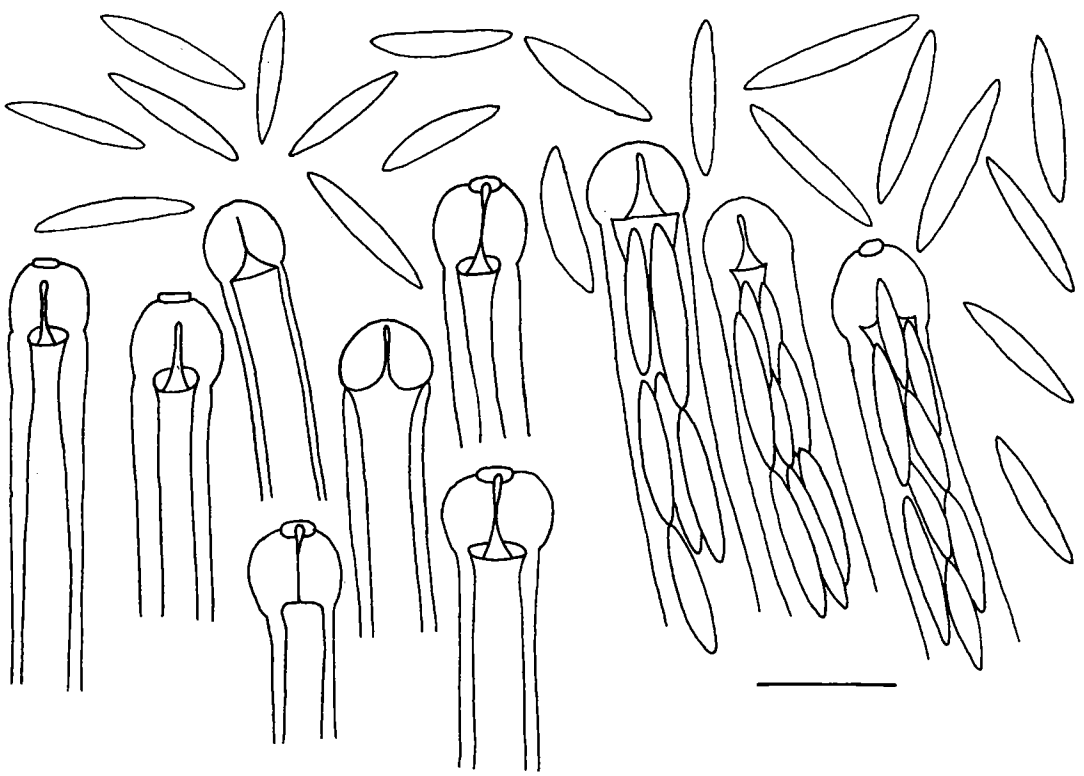

Fig. 2. Tips of seven immature asci and three mature asci with examples of part spores. Scale bar, 10 µm.

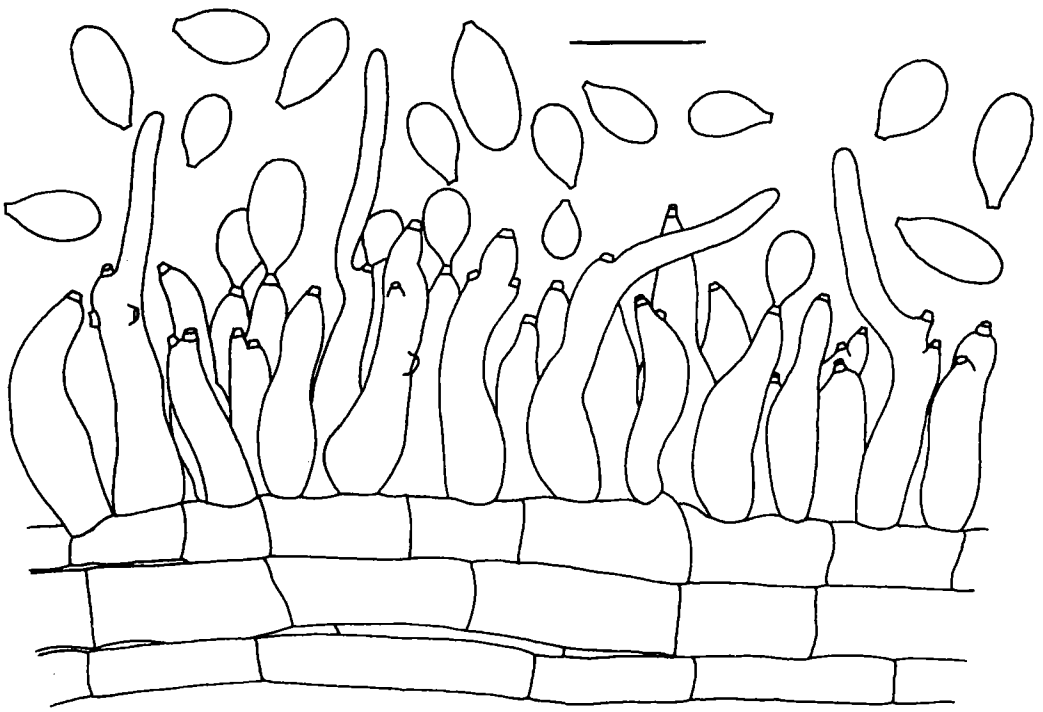

Fig. 3. Part of the hymenial layer showing the conidiogenous cells and conidia of *H. sphecocephala*. Scale bar, 10 μm.

damage or from the base of the fertile head. The new growth, however, appeared to be sterile and there was no evidence of a regrowth of the teleomorph or the development of an anamorph.

No evidence was found of an anamorph on the same stroma as the teleomorph. Occasionally, specimens were found which in all respects of habit matched the *Cordyceps* form. These differed in not producing a fertile teleomorph but in producing a stroma which remained the same diameter throughout rounding only at the tip. Examination revealed the presence of a *Hymenostilbe* state on the terminal part of the stroma.

The conidiogenous cells were very variable in size and shape as is typical of the genus. They were cylindrical, 10–20 × 3–6 μm with one to three stout denticles (Fig. 3). A notable feature of the conidiogenous cells were long sterile extensions up to 25 μm which gave the stroma a markedly setulose appearance at low power. Conidia were broadly clavate, strongly apiculate and 3–10 × 3–4·5 μm.

Specimens examined: All specimens were on adult wasps (Hymenoptera) except for one record on a bee. These were deposited in the insect–fungus collection at NBCRC with the author's codes.

Teleomorph: NHJ595.01, Khao Yai – Phakrajai, 24 Sept. 1991, N. L. Hywel-Jones; NHJ623.04, Khao Yai – Heo Sawat, 15 Oct. 1991, N. L. Hywel-Jones & K. Jones; NHJ804.02, Khao Yai – Heo Sawat road marker km 44·8, 25 June 1992, N. L. Hywel-Jones, L. Manoch, A. Rongchitprapas & S. Sivichai; NHJ858.04, Khao Yai – Gong Giao, 28 Aug. 1992, N. L. Hywel-Jones, A. Rongchitprapas & S. Sivichai; NHJ933.02, Khao Yai road marker km 29·2, 13 Oct. 1992, N. L. Hywel-Jones; NHJ948, NHJ953.03 & NHJ953.04, Sam Lan – waterfall, 15 Oct. 1992, N. L. Hywel-Jones, A. Rongchitprapas & S. Sivichai; NHJ1070, NHJ1075, Khao Yai – Phakrajai, 27 May 1993, N. L. Hywel-Jones & R. Nasit; NHJ1206, Khao Yai – Heo Narok, 22 June 1993, N. L. Hywel-Jones & R. Nasit; NHJ1224, Khao Yai – Heo Narok, 29 June 1993, N. L. Hywel-Jones, R. Nasit, R. Plomhan & S. Thienhirun; NHJ1316 & NHJ1743 & NHJ1752, Khao Yai – Gong Giao, 6 Aug. 1993, N. L. Hywel-Jones, R. Nasit, S. Sivichai & R. Plomhan; NHJ1800, NHJ1801, NHJ1807 & NHJ1822, Khao Yai – Heo Narok, 10 Aug. 1993, N. L. Hywel-Jones, R. Nasit & S. Sivichai; NHJ2133, Khao Yai – Gong Giao, 9 Sept. 1993, N. L. Hywel-Jones, R. Nasit, S. Sivichai & S. Thienhirun; NHJ2199, Doi Inthanon road marker km 25·5, 26 Sept. 1993, N. L. Hywel-Jones, K. Auncam, R. Nasit, S. Thienhirun & A. J. S. Whalley.

Anamorph: NHJ1332, Khao Yai – Phakrajai on an adult bee, 6 July 1993, S. Sivichai, L. Tangchit & C. Yomsopit; NHJ1741, Khao Yai – Phakrajai, 6 Aug. 1993, N. L. Hywel-Jones, R. Nasit & S. Sivichai; NHJ1858, Khao Yai road marker km 44·8, 17 Aug. 1993, N. L. Hywel-Jones, R. Nasit & S. Sivichai.

Immature: NHJ787.01, Khao Yai road marker km 29·2, 6 June 1992, N. L. Hywel-Jones.

Culture of the fungi

Cultures were started from hyphal bodies, part-spores, and from conidia. Initial isolations were on PDA. Part-spores and conidia germinated in the dark at 22 °C within 20 h. Hyphal bodies germinated after 5 d under the same conditions apart from brief periods of light each day for examination.

Isolates are stored in the NCGEB insect–fungus collection. Isolate numbers coincide with the herbarium numbers given to specimens. Isolates derived from part-spores were NHJ595.01, 623.04, 804.02, 948, 953.02, 953.03, 1743 and 1800. Isolates derived from conidia were NHJ1332, 1741, 1752 and 1858. Isolate NHJ787.01 was derived from abdominal hyphal bodies.

Isolations from all three sources were sterile but morphologically indistinguishable. Growth was very slow and

stromatic on PDA reaching 33 mm in 14 wk at 22°. Cultures were hyaline to pale grey brown with only sparse aerial mycelium. Some isolates developed an irregular, immersed, hyaline margin in contrast to the regular, immersed, hyaline margin that was also noted. The reverse was grey-white to pale grey brown. Several stout synnemata developed after 3 wk. They were silky pale cream and up to 400 μm diam. and appeared similar to the stromata on the hosts. There was no sign of either a teleomorph or an anamorph developing on these stromata.

DISCUSSION

Since Torrubia first noted the appearance of infected wasps in Cuba in 1749 (see Samson, Evans & Latgé, 1988) there have been many reports of *Cordyceps* infecting wasps around the world. Petch (1932) noted *Cordyceps gentilis* (Ces.) Sacc. on a hornet sent to him from Thailand. He considered *C. gentilis* distinct from *C. sphecocephala* (= *C. sphecophila* in Petch's early writings) noting the 'longer, more acute head' of *C. gentilis* which contrasted with the 'ovoid or narrow-oval, and acute' head of *C. sphecocephala*.

Petch (1933) later described the shape of the head of *C. sphecocephala* as variable and used the description and measurements of the Thai specimen of *C. gentilis* in support. In this paper, he considered *C. gentilis* synonymous with *C. sphecocephala* along with *C. lachnopoda* Penz. & Sacc., *C. oxycephala* Penz. & Sacc., *C. puiggarii* Speg. and *C. thyrsoides* Möller. Kobayasi (1941) described *C. sphecocephala* from Japan. He regarded *C. oxycephala* to be distinct from *C. sphecocephala* (based on the shape of the head) and considered *C. gentilis* a synonym of the former species along with *C. puiggarii*. Later, Mains (1948) showed that *C. puiggarii* was not the same as these wasp *Cordyceps*.

Mains (1958) re-described *Cordyceps sphecocephala* from a substantial collection of specimens from North America and concluded that there was much variation in the overall morphology which manifested itself in the many different names given to what, essentially was a single species. All early observations put weight upon gross morphology as a separator of species with little attention being paid to microscopic features.

The work of Mains (1958) and the present observations from Thailand, based upon substantial collections within well-defined geographical regions, suggest there is a single species, *C. sphecocephala* which shows gross morphological variation but is constant in microscopic detail. While the microscopic characteristics of *C. sphecocephala* in Thailand showed little variation there were large differences in the shape and size of the fertile head (Fig. 1). The Thai records suggest that maturity of the specimen, size of the host, micro-habitat conditions such as humidity and damage re-growth may all affect the form of the head.

Ditmar (1817) described and figured *Isaria sphecophila* from a hornet in Germany noting that the upper part of the simple, brown synnema was pilose and darkened. His figures indicate a minutely setulose condition with globose, hyaline conidia. Speare (1920) discussed Ditmar's fungus and, on the basis of the illustrations, suggested that this should be placed in *Hirsutella*. Petch (1932) agreed noting that it (Ditmar's fungus) 'does not agree with a *Hymenostilbe*'.

Later, Petch (1937) found a conidial state on an ichneumon wasp in England and considered this identical to Ditmar's fungus and concluded 'that *Isaria sphecophila* Ditm. is a *Hymenostilbe* and that it is the conidial stage of *Cordyceps sphecocephala* (Kl.) Cooke'. The description given by Petch (1937) was brief with no illustrations. However, he noted the synnemata were cream-coloured and 1·75 mm long. Ditmar's fungus had brown synnemata which were 90 mm long. Specimens examined by Petch (1937) had clavate or narrow-oval conidia in contrast to the globose conidia of Ditmar's fungus.

Mains (1950) noted for *H. sphecophila* that it 'is doubtful whether it is Ditmar's fungus'. Samson & Evans (1975) accepted nine species of *Hymenostilbe* and, like Mains (1950), they also regarded *H. sphecophila* as doubtful. No type specimen remains of Ditmar's fungus but it is possible this is *Hirsutella saussurei* Speare which also has long, brown synnemata and conidia that appear globose in a mucous coat.

In spite of these doubtful records, Kendrick & DiCosmo (1979) listed *H. sphecophila* as the anamorph of *C. sphecocephala* citing Petch (1937; 1948). Furthermore, in their scheme the *C. sphecocephala-H. sphecophila* association was coded 2.3.1 suggesting the link was documented with experimental evidence of one morph developing from the other in culture. There is no evidence in either of the cited works by Petch that he ever attempted to culture either fungus. Indeed, Petch (1948) was merely a listing of British entomogenous fungi in which he noted the association based upon his 1937 study of herbarium material. Furthermore, it seems as though there have been no attempts to isolate either fungus until now.

As Petch (1937) cites only a single record associating the two species, under the Kendrick & DiCosmo scheme it would have been better to code this association at a lower level of 2.2.1 – documented but circumstantial evidence of association based on a single observation of co-habitation. But as earlier workers (Mains, 1958; Samson & Evans, 1975) had already raised doubts as to whether Ditmar's fungus could be considered the same as Petch's *Hymenostilbe* records associated with *C. sphecocephala*, it would be prudent to say that a *Hymenostilbe* may be associated with *C. sphecocephala* based upon Petch's 1937 writings.

The *Hymenostilbe* sp. on wasps from Thailand had a setulose appearance due to sterile extensions of the conidiogenous cells. This feature has not been described before for *Hymenostilbe* (Samson & Evans, 1975) but the conidial state is clearly assignable to *Hymenostilbe* and not *Hirsutella*. The current work showed that *C. sphecocephala* is present on wasps in Thailand and that a *Hymenostilbe* sp. not considered by Samson & Evans (1975) is also present on wasps and bees in the same micro-habitats. Furthermore, it showed that isolations from the *Cordyceps* and *Hymenostilbe* are morphologically similar, suggesting they may be linked.

Until more collections can be made it is proposed that the *Hymenostilbe* sp. found in Thailand is possibly an anamorph of *C. sphecocephala* based upon its co-habitation and morphological similarity in culture and may be the same as the *Hymenostilbe* specimens examined by Petch (1937) and Mains

(1950). It is unlikely that the *Hymenostilbe* from Thailand is the same as Ditmar's fungus from Germany.

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