

# Taxonomy and ecology of *Hypocrella discoidea* and its anamorph, *Aschersonia samoensis*

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An examination of the holotype of *Hypocrella discoidea*, the type species of the genus, and fresh specimens collected in south-east Asia, shows that the ascospores remain whole at maturity. This is in contrast to previous descriptions and generic diagnoses which report the ascospores as fragmenting within the ascus. Ascospore isolations confirmed the association of the coelomycete, *Aschersonia samoensis*, with *H. discoidea*. A new method of ascospore germination is reported, in which secondary spores are produced on narrow sporogenous cells arising from the ascospore wall.

The genus *Hypocrella* (Clavicipitales) was erected by Saccardo (1878) to accommodate four species previously assigned to *Hypocrea* Fr. (Hypocreales). Only one of these is now in the entomopathogenic genus *Hypocrella* as recognized by Petch (1914, 1921) and Mains (1959*a*). This is *Hypocrella discoidea* (Berk. & Broome) Sacc., designated the lectotype (Seaver, 1910), and its associated anamorph *Aschersonia samoensis* P. Henn.

Since the monumental work of Petch (1921), there has been little systematic collection of *Hypocrella* spp. from natural habitats. Recent collections in Thailand and Malaysia have revealed new information on *H. discoidea* and its association with *A. samoensis*.

## Taxonomy

***Hypocrella discoidea*** (Berk. & Broome) Sacc., *Michelia* 1: 322 (1878). (Figs 1, 4-9)

*Hypocrea discoidea* Berk. & Broome, Jr. *Linn. Soc.* 14: 113 (1873).

*Hypocrella zingiberis* Masee, *Kew Bull.*: 174 (1899).

*H. zimmermanniana* P. Henn., *Hedwigia*: 142 (1902).

*H. grewiae* Koord., *Bot. Untersuch.*: 179 (1907).

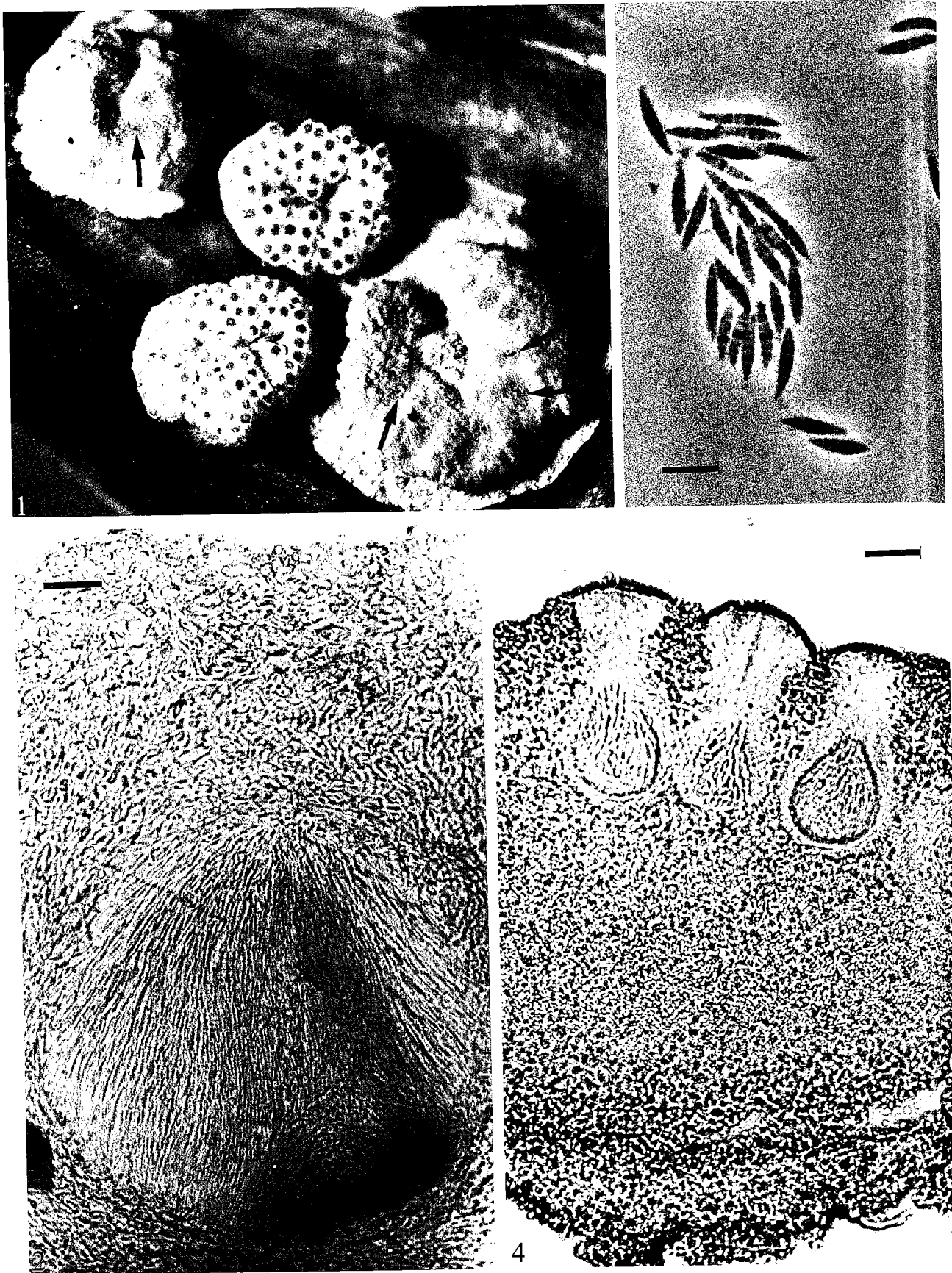
Anamorph: *Aschersonia samoensis* P. Henn., *Engler's Bot. Jahrbuch.* 23: 289 (1896). (Figs 1-3)

Petch (1921) comprehensively described the teleomorph and anamorph. His description is repeated here with variations from Thai and Malaysian material in parentheses.

**Stromata** usually discoid (to stud-shaped) up to 4 mm (0.5-3+ mm) diam. and 1.25 mm (up to 2.0 mm) high, upper surface almost plane, edge vertical or rounded, sometimes umbilicate in the centre (umbilicate when a solitary ostiole is present), with or without a thin, fibrillose hypothallus; or irregularly pulvinate 1-2 mm diam. (flattened pulvinate in very small specimens); minutely pulverulent (to verrucose in

large perithecial forms); orange-red, or more rarely yellow (pale orange to dark orange or orange red), internally yellowish white; substance rather soft (brittle when dry). **Perithecia** scattered (to crowded); ostioles red-brown, or dark brown, slightly projecting; perithecia flask-shaped, 0.35 mm deep, 0.15 mm diam. **Asci** 140-180 × 8 μm (up to 10 μm in middle when mature), 4- to 8-spored (always 8-spored); **part-spores** 8-12 × 2 μm, cylindric, ends rounded (*ascospores* remain whole, non-fragmenting, 60-90 × 4-5 μm, elongate clavate, blunt at one end, tapering to rounded tip). **Conidiomata** flattened globose or laterally oval, 0.3 mm diam., neck short. **Conidiomatal ostioles** sunken, each in the centre of a scarious area about 0.1 mm diam., red-brown, usually circularly arranged (sometimes solitary in the centre, especially when in association with the teleomorph). **Conidia** narrow-oval (in fresh material) or fusoid (irregular when dry), sometimes slightly inequilateral, ends pointed and slightly attenuated, 10-15 × 1.5-2.0 μm (8-15 × 1.5-2.0 μm). **Paraphyses** up to 180 μm long (up to 225 μm), rather stout, 1.0-1.5 μm diam.

Petch described the conidiophores of *Aschersonia* as simple, although he noted branched 'basidia' in four species. South-east Asian examples of *A. samoensis* have branched conidiophores which arise with the paraphyses. Spore age, degree of packing and exposure to desiccation affect the shape and size of conidia. Fresh spores from culture are narrow-oval. Packed, dry or old spores are thinner and often irregular in form. Stromatal size is dependent partly on the size of the host insect and partly on the maturity of the specimen. In Thai collections, *Hypocrella* stromata are generally larger than the *Aschersonia* stromata (average 2.2 mm and 1.5 mm respectively), although the Malaysian collections of *A. samoensis* tend to be larger than the teleomorph (Fig. 1). Petch (1921) observed that: 'The pycnidia and perithecia are not usually found in the same stroma'. Most Thai specimens were in the anamorph state (63%), 26% were in the teleomorph and 11%



Figs 1-4. *Hypocrella discoidea* (IMI 353553) ex Malaysia. Fig. 1. Habitat on insect host on Zingiberaceous leaf, Genting Ridge, Malaysia. Note the variation in size of the *Aschersonia* stromata and the sunken ostioles (arrowed) of the conidiomata. The two smaller central *Hypocrella* stromata have crowded perithecia with prominent necks (x ca 18). Fig. 2 L. S. of *Hypocrella* stroma showing buried conidioma packed with long paraphyses. Bar = 40  $\mu$ m. Fig. 3. Fusoid conidia with attenuated ends. Bar = 10  $\mu$ m. Fig. 4. L. S. of upper part of *Hypocrella* stroma showing flash-shaped perithecia. Bar = 80  $\mu$ m.

were a mixture. However, as Fig. 2 shows, seemingly exclusively *Hypocrella* stromata may contain buried conidiomata. The stromata consist of tightly coiled hyphal fragments, 5–8 µm diam., with thick walls and a prominent lumen (Figs 2, 4, 6). The hyphae of the hypothallus are more regular, less tortuous and thinner (3–4 µm diam.), but still thick-walled with a prominent lumen (Fig. 5).

Perithecia are recognizable as small, scattered to crowded cones giving the upper surface of the stroma a verrucose appearance (Fig. 1). Size and shape of the perithecia are variable, although they are generally flask-shaped (Figs 4–6). Asci have a blunt tip and there is no evidence of an ascus cap. Mature asci contain eight ascospores arranged with four in the upper two-thirds and four in the lower two-thirds of the ascus. The ascus is fattest where the two groups overlap. Ascospores are blunt at the end, which is always at the tip of the ascus. In all south-east Asian specimens, ascospores are triseptate and slightly constricted at the septa. Examination of the type material (Herb. K, 5061) shows indistinct septation in the whole ascospores making it difficult to determine the number of septa. In the fresh material, with ascospores oozing from the ostioles, there is no evidence of fragmentation into part-spores as assumed by Petch (1921). This led him to conclude that 'Nearly all the collections of the *Hypocrella* stage are immature', and that previous descriptions of part-spores 'were merely pycnosporous'.

### Ecology

Petch (1921) noted that *H. discoidea* was common in the East, citing records from Sri Lanka, India, Burma, Malaysia, Indonesia, the Philippines and Samoa. He also considered that *N. napoleonae* Pat. & Har. from Africa is synonymous with *A. samoensis*. *H. discoidea* is one of the most commonly encountered entomopathogenic fungi in Khao Yai national park, Thailand and is similarly ubiquitous on Zingiberaceae shrubs in submontane forest in Malaysia.

*Specimens examined from Thailand:* All on Homopteran insects within Khao Yai national park. *Teleomorph:* Heo Sawat, Lumtakhlong river, 500 m upriver of waterfall, 2 July 1989, N. L. Hywel-Jones, same locality, 25 July 1989, N. L. Hywel-Jones; Heo Narok, tributary 500 m up river of waterfall, 19 Dec. 1989, N. L. Hywel-Jones; Heo Sawat, tributary of Lumtakhlong river, 500 m up from waterfall, 15 Jan. 1990, N. L. Hywel-Jones & H. C. Evans; Heo Sawat, Lumtakhlong river, 500 m up-riber of waterfall, 18 July 1990, N. L. Hywel-Jones; Heo Narok, tributary 500 m up-riber of waterfall, 1 Aug. 1990, N. L. Hywel-Jones; Heo Sawat, road marker km 44·8, 13 Nov. 1990, N. L. Hywel-Jones; Heo Narok, tributary 500 m up-riber of waterfall, 4 June 1991, N. L. Hywel-Jones. *Anamorph:* Gong Giao, Lumtakhlong river, 20 June 1989, N. L. Hywel-Jones; Salika, river above waterfall, 22 June 1989, N. L. Hywel-Jones; Heo Sawat, Lumtakhlong river, 50 m up river of waterfall, 2 July 1989, N. L. Hywel-Jones; Heo Narok, tributary 500 m up-riber of waterfall, 6 July 1989, N. L. Hywel-Jones; Heo Sawat, Lumtakhlong river, 500 m up-riber of waterfall, 17 Aug. 1989, N. L. Hywel-Jones; Phakrajai, 23 Aug. 1989, N. L. Hywel-Jones; Heo Narok tributary at road marker km 18·7, 18 Sept. 1989, N. L. Hywel-Jones; Tad Tha Phu, 10 Oct. 1989, N. L. Hywel-Jones; Heo Narok, tributary 500 m up river of waterfall, 19 Dec. 1989, N. L. Hywel-Jones; Heo Narok, tributary up-riber of waterfall, 17 Jan. 1990, N. L. Hywel-Jones &

H. C. Evans; Heo Sawat, Lumtakhlong river tributary 500 m up-riber of waterfall, 15 Jan. 1990, N. L. Hywel-Jones & H. C. Evans; Heo Sai, Lumtakh long river, 8 May 1990, N. L. Hywel-Jones; Heo Narok tributary 500 m up-riber of waterfall, 19 May 1990, N. L. Hywel-Jones; Heo Sawat, tributary 500 m up-riber of waterfall, 1 Aug. 1990, N. L. Hywel-Jones; Mor Singh To, tributary of Lumtakhlong river, 1 Aug. 1990, N. L. Hywel-Jones; Heo Sawat, road marker km 44·8, 13 Nov. 1990, N. L. Hywel-Jones; Mor Singh To tributary of Lumtakhlong river, 8 Jan. 1991, N. L. Hywel-Jones & J. Drummond; Heo Sawat, Lumtakhlong river, 500 m up-riber of waterfall, 21 Nov. 1991, N. L. Hywel-Jones.

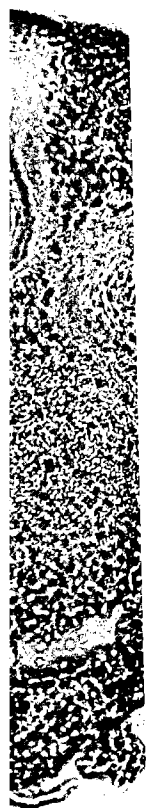
*Specimens examined from Malaysia:* teleomorph and anamorph; Homopteran colonies (?Aleyrodiidae), Genting Ridge, Genting Highlands, 31 Aug. 1991, H. C. Evans, IMI 353553.

Khao Yai national park is 150 km north-east of Bangkok and covers 2000 km<sup>2</sup>. Elevation is from sea level to 1300 m and is primary, disturbed and secondary tropical forest. Slash and burn agriculture, which stopped over thirty years ago, resulted in areas of savannah now managed for recreation or forest regeneration. Dry evergreen forest and deciduous woodland occur at lower elevations in and around the park.

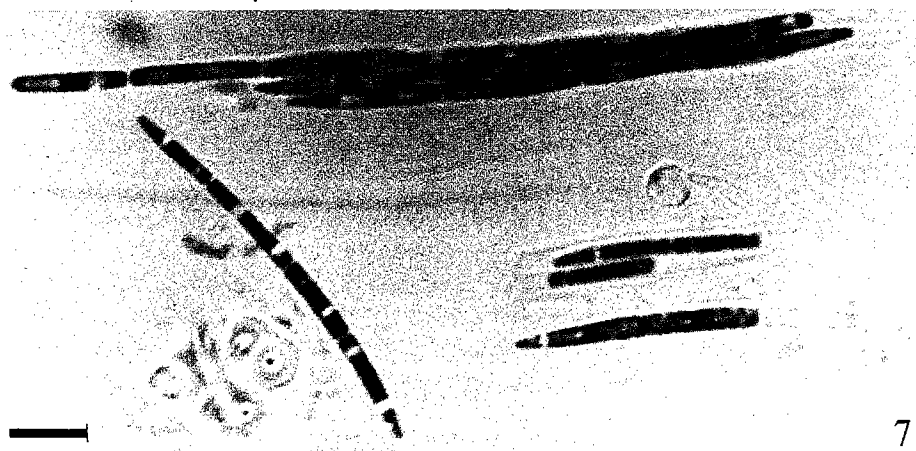
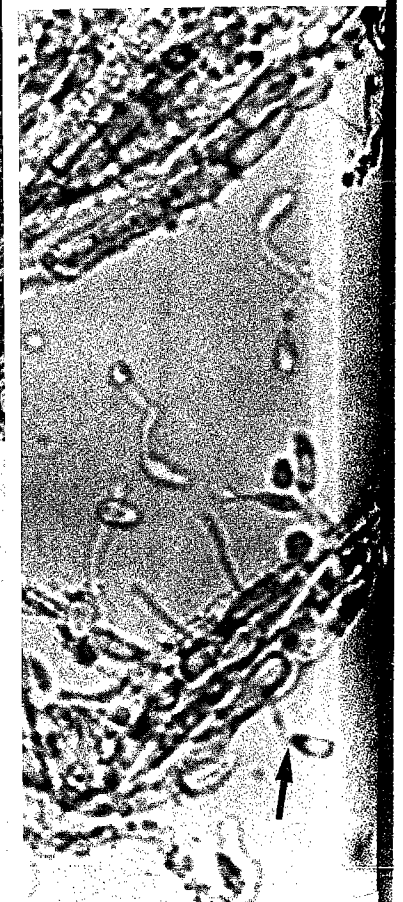
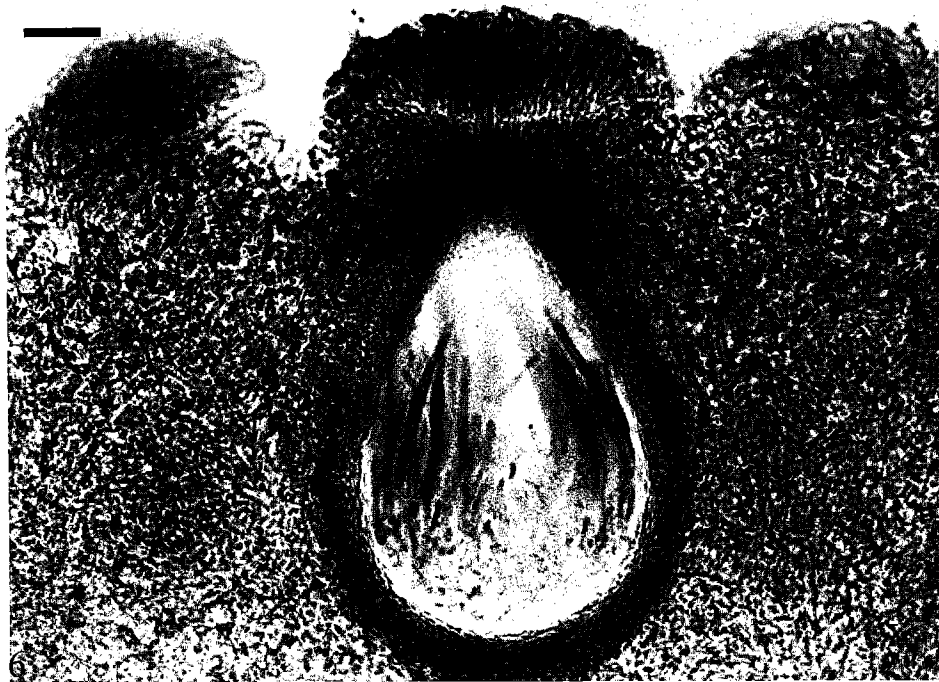
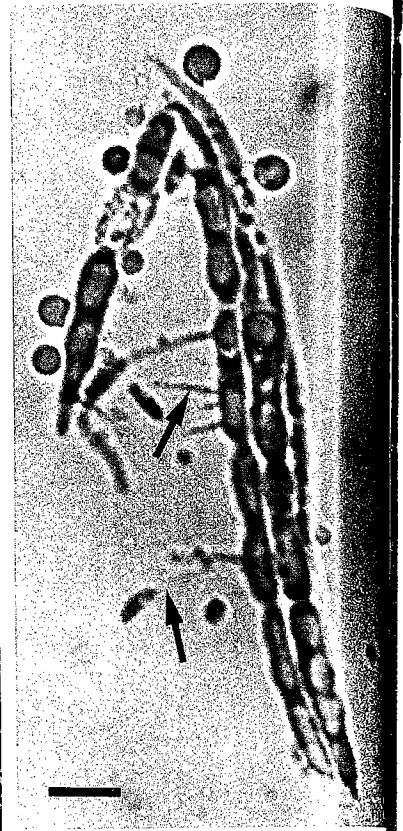
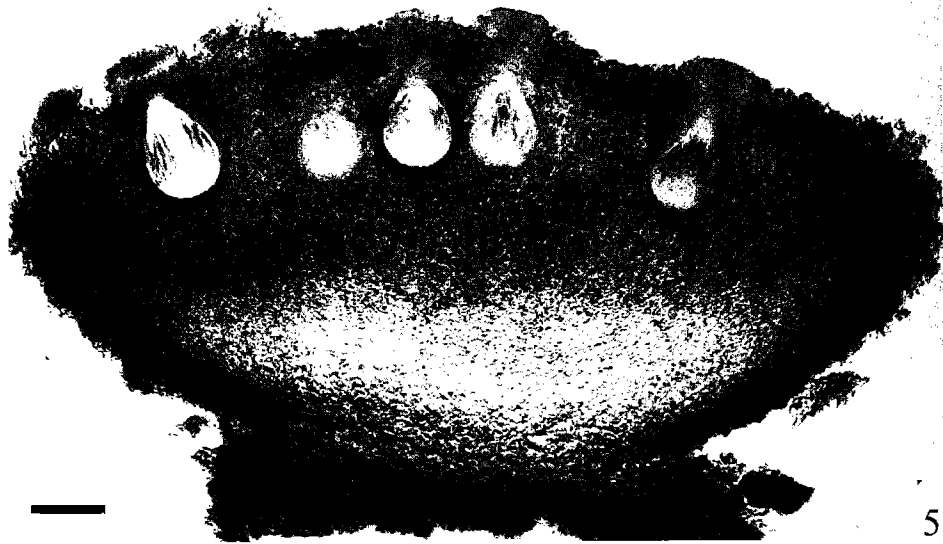
Each collection or record had from one to over a hundred individual stromata. The anamorph was found throughout the year except in February, March and April. These three months are the driest at Khao Yai (rain is from May to October/November followed by a cool dry season from November to February and a hot season from February to May). The teleomorph was found from June to January. Records were from 200 m above sea level (Heo Narok and Salika) to above 1000 m (Gong Giao). Although *H. discoidea* was found in primary forest away from rivers (eg. Phakrajai), it was most often found on Homopteran hosts on vegetation along rivers and in small stream valleys. It was not found in deciduous forest at lower elevations, where the hills are limestone blocks with poor water-retaining ability.

The teleomorph and anamorph were commonly found on the underside of leaves of *Ctenolophon* spp. (Zingiberaceae). This is a ground flora herb in dry evergreen and rain forest but absent from deciduous forest (unpubl. obs.). Examples were occasionally found on the upper leaf surface of these plants. Significantly, *H. discoidea* has been associated with Zingiberaceae by other workers (Petch, 1921). Collections at Khao Yai were not exclusively on the monocotyledonous *Ctenolophon* spp. There were also records from insects on dicotyledonous herbs and trees. Petch (1921) recorded *H. discoidea* attacking a black *Aleyrodes* sp. on dicotyledonous plants.

The host insect is usually completely destroyed by the fungus, but some immature stromata confirmed that one host was a black Aleyrodiidae commonly found infesting *Ctenolophon* spp. at Khao Yai. Petch (1921) also considered that the collections of *Aschersonia* recorded by Parkin (1906) on *Aleyrodes* sp. on *Zingiber*, *Diospyros* and *Memecylon* from Sri Lanka are conspecific with *A. samoensis*. The genus is divided into two sub-groups according to the presence or absence of paraphyses (Petch, 1921). The aparaphysate species are considered to be confined to soft-scale insect hosts (Lecaniidae), whilst those with paraphyses have whitefly (Aleyrodiidae) hosts. However, this requires verification, and Evans &



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Figs 5-9. For legend see opposite.

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