# *Hirsutella* species associated with hoppers (Homoptera) in Thailand

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*Hirsutella versicolor*, *H. citriformis* and *H. nivea* sp. nov. are described from Homoptera in Thailand. *H. versicolor* was usually associated with the ascomycete *Torrubiella pruinosa* and was isolated from ascospores. *H. citriformis* could not be linked with a teleomorph but was successfully isolated from conidia. *H. nivea* was not associated with a teleomorph and could not be isolated in pure culture.

*Hirsutella* Pat. was erected for *Hirsutella entomorrhiza* Pat. attacking an adult beetle (Coleoptera) (Patouillard, 1892). Patouillard considered this a clavariaceous hymenomycete but Speare (1920) questioned its placement in the basidiomycetes, presenting ample evidence that *Hirsutella* was 'a rather definite form genus of the Fungi Imperfecti'. This conclusion has, rightly, never been questioned.

Speare's redescription of *Hirsutella* noted it to be synnematous, 'composed of numerous somewhat interwoven but nearly parallel septate hyphae that adhere to one another tenaciously'. He noticed that the spores have 'a gelatinous substance which surrounds and renders them citriform in appearance'. Speare (1920) observed that 'the greater number of hosts are found among the Hemiptera, the family Fulgoridae being particularly conspicuous'. The 'Hemiptera' of Speare's paper are plant-, leaf-, tree- or froghoppers now placed in the insect order Homoptera.

Mains (1951) was the next to review the genus which, by this time, contained species that were not synnematous, species that did not have a mucilaginous coat around the conidia and species that were pathogenic to invertebrates other than insects. Mains (1951) particularly questioned the inclusion of mononematous species in a genus traditionally regarded within the Stilbaceae. He wrote for mononematous *Hirsutella* spp. that in 'the most generally accepted classification of the Moniliales such species belong in the Moniliaceae and those with synnemata, typified by *Hirsutella entomophila*, in the Stilbaceae'. Mains reviewed nine synnematous species in detail, accepting nine others and dismissing a further nine which were 'without synnemata and probably should be placed in the Moniliaceae'.

It was left to Minter & Brady (1980) to review the mononematous *Hirsutella* species noting that the monumental work of Hughes (1953) allowed the inclusion of mononematous and synnematous species within the same genus. These authors also mentioned the tendency to include mononematous and synnematous species within different sections of the same genus as Samson (1974) did for *Paecilomyces* Bainier. Consequently, Minter & Brady (1980) erected the section *Mononematosa* within *Hirsutella* and included ten species.

Speare (1920) predicted that teleomorphs, when found, would probably be 'species of *Cordyceps* or related genera'. Petch (1924) was the first to make this association noting that the ant pathogen *Cordyceps unilateralis* (Tul.) Sacc. had a *Hirsutella* anamorph which he later named *Hirsutella formicarum* Petch (Petch, 1935). There are now many records of *Hirsutella* associated with *Cordyceps* and the related *Torrubiella* Boudier bearing out Speare's prediction.

A 7-yr survey of invertebrate-associated fungi in Thailand has recognized several species of *Hirsutella* associated with clavicipitaceous teleomorphs. Two new *Cordyceps* were described from Lepidoptera larvae which produced mononematous *Hirsutella* species in pure culture (Hywel-Jones, 1994). *Torrubiella iriomoteana* Kobayasi and *Torrubiella siamensis* Hywel-Jones were described from scale insects (Homoptera) in Thailand and were associated in the field with mononematous *Hirsutella* species whose spores lacked a mucilaginous coat (Hywel-Jones, 1995*a*). *Cordyceps brunneapunctata* Hywel-Jones infecting elaterid larvae (Coleoptera) is also associated with a *Hirsutella* sp. both in the field and in pure culture (Hywel-Jones, 1995*b*). This paper describes three *Hirsutella* spp. recorded from hopper hosts in Thailand.

## MATERIALS AND METHODS

Surveys were made at Khao Yai National Park over a 7-yr period and, sporadically, at other National Parks and Wildlife Reserves in Thailand over a 3-yr period. Collections of invertebrate-associated fungi were made from the underside

of living leaves of herbs and saplings in natural forest. Material was returned to the laboratory in plastic boxes and stored in a refrigerator before being processed. Isolations were made using methods described elsewhere (Hywel-Jones, 1995 *b*).

## TAXONOMY

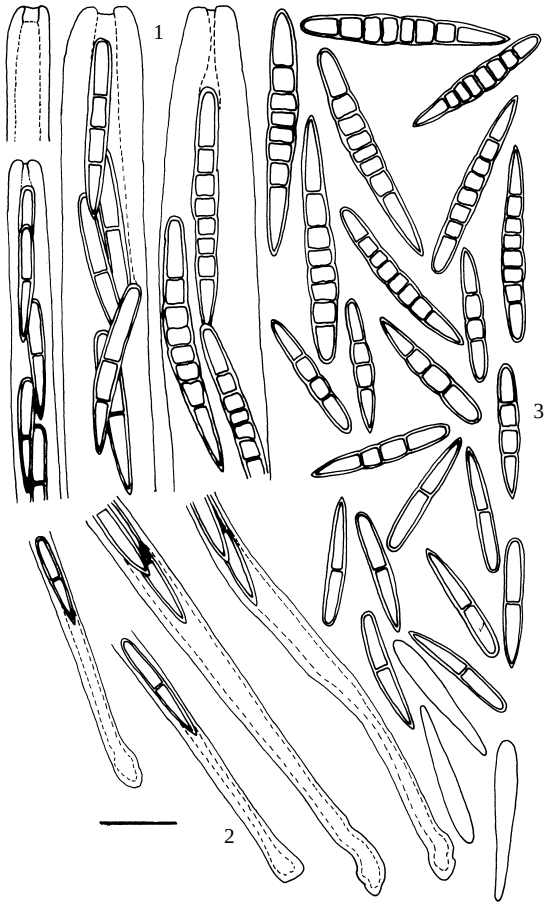
**Torrubiella pruinosa** (Petch) Minter & B. L. Brady, *Trans. Br. Mycol. Soc.* **74**: 278 (1980) (Figs 1–3)

*Calonectria pruinosa* Petch, *Trans. Br. Mycol. Soc.* **16**: 226 (1932)

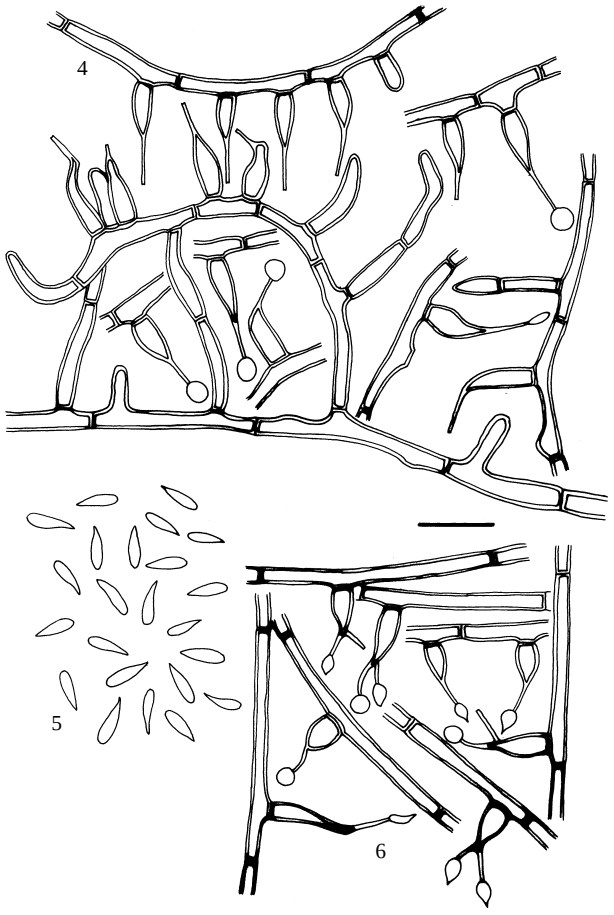
Anamorph: *Hirsutella versicolor* Petch, *Trans. Br. Mycol. Soc.* **16**: 227 (1932) (Figs 4–6)

*Stroma* sulphur yellow to pale brown or tawny brown. *Ascomata* crowded, immersed in stroma, ovoid, walls dark golden brown, 360–460 × 280–350 µm. *Asci* clavate, hyaline, 140–150 × 10 µm at maturity, 8-spored. *Ascospores* disto-septate with eight cells at maturity, 22–32 × 3·3–5·3 µm. *Anamorph Hirsutella versicolor* mononematous. *Conidiogenous cells* hyaline, smooth-walled. Basal part 8–12 × 2·5–3 µm with attenuated phialide 6–8 µm long – combined length of up to 22 µm. *Conidia* smooth-walled, hyaline, elongate oval 3·5–4·5 × 1–1·5 µm, surrounded by prominent mucous coat.

*Specimens examined in Thailand*: All collections in this study are stored in the BIOTEC invertebrate-fungus collection. All specimens were on Homoptera (Cicadellidae) attached to the underside of living leaves of dicotyledonous plants except where otherwise stated.




**Figs 1–3.** *Torrubiella pruinosa*. (Bar = 10 µm). **Fig. 1.** Tips of four asci showing development of ascospores. **Fig. 2.** The form of the ascus foot. **Fig. 3.** Examples of ascospores from immature (bottom) to mature (top).




**Figs 4–6. Fig. 4.** The *Hirsutella* state of *T. pruinosa* on branched and anastomosing hyphae (bar = 10 µm). **Fig. 5.** The shape of the conidia when not surrounded by a mucilaginous coat (bar = 7·5 µm). **Fig. 6.** Examples of phialides and developing conidia from pure culture (bar = 10 µm).

*Teleomorph and anamorph together*: NHJ616.01, 8 Oct. 1991, Khao Yai National Park – Wang Cham Pi, attached to the underside of a leaf of Zingiberaceae, N. L. Hywel-Jones; NHJ803.01, 25 Jun. 1992, Khao Yai National Park – road marker km 44·8, NLH-J, L. Manoch, A. Rongchitprapas & S. Sivichai; NHJ1009, 13 Jan. 1993, Khao Yai National Park – Heo Sawat, start of trail to Khao Laem, NLH-J; NHJ2664, 7 Dec. 1993, Khao Yai National Park – Gong Giao nature trail, NLH-J, R. Nasit, R. Plomhan & SS; NHJ3088, 15 Feb. 1994, Khao Yai National Park – Wang Cham Pi, NLH-J, RN, RP & SS. *Anamorph only*: NHJ373.04, 9 Jan. 1991, Khao Yai National Park – trail along tributary above Heo Narok waterfall, NLH-J.

*Isolates examined*: All isolates in this study are stored in the BIOTEC fungus collection with the author's codes.

NHJ616.01 from ascospores.

The host contains thick-walled hyphal bodies up to 35 µm long and 2–6 µm diam. A thick weft of tortuous but slightly branched sulphur yellow mycelium completely covers the insect. This extends over the surface of the leaf as a thin hyaline film of anastomosing hyphae. Perithecioid ascomata are immersed in the byssoid stroma with about 40–50 µm of the conic ostiole projecting. The projecting ascomata are dark golden brown.


**Fig. 7.** *Hirsutella citriformis*. Phialides with conidia (bar = 10 μm).

Inside the ascoma asci are at all stages of development (Figs 1–3). When immature, asci are 5·5–6·5 μm diam. across the apex while at maturity the asci are 10–12 μm across (Fig. 1). There is a prominent canal up to 2–2·5 μm across (Fig. 1). Asci are cylindrical tapering only at the foot to about 4 μm wide (Fig. 2). Immature ascospores are aseptate but become 7-septate at maturity (Fig. 3).

The stroma of *T. pruinosa* contains conidiogenous cells of a mononematous *Hirsutella* (Fig. 4). This is mainly monophialidic (rarely polyphialidic). The conidiogenous cells usually arise directly from the underlying hyphae but occasionally there is an intermediate cylindrical cell 7–12 μm long. On the host the developing conidia are usually surrounded by an opaque mucilaginous coat 2·5–3 μm diam. There is no evidence that spores develop in balls and they are not seen adhering to each other. Discharged conidia are sometimes still surrounded by the mucilaginous coat. More often this coat disappears and the shape of the conidia is seen (Fig. 5).

Isolations were not successful from conidia, but ascospores on PDA germinated to produce a single stout lateral germ-tube. Isolations were also secured from ascospores within whole asci. Growth was very slow for 3–4 months after which time the culture established itself on PDA. Isolations remained slow growing, stromatic, with a purple diffusible pigment. In the agar the cells were swollen. On the stromatic hyphae there was a *Hirsutella* anamorph which matched that found on the host (Fig. 6). More of the conidiogenous cells were polyphialidic in culture and the tip of the conidium usually protruded through the mucilaginous coat (Fig. 6).

**Hirsutella citriformis** Speare, *Mycologia* **12**: 70 (1920) (Fig. 7)

*Trichosterigma attenuatum* Petch, *Trans. Br. Mycol. Soc.* **8**: 215 (1923)

Teleomorph: Not known.

*Stroma* lacking external mycelium apart from brown to purple-brown sparse mycelium attaching host to substrate. *Synnemata* many, from all over the host, up to 15 mm long, 30–50 μm across. Composed of a tightly packed core of parallel strands of mycelium, simple or with short lateral branches, 100–150 μm long 30 μm diam. *Conidiogenous cells* monophialidic, with ellipsoid base tapering abruptly to long, phialidic neck, 18·5–52 × 3–3·5 μm wide. *Conidia* hyaline, aseptate, smooth-walled, fusiform or elliptical, 3·5–5 μm long, 1–1·5 μm wide, surrounded by mucilaginous coat.

*Specimens examined in Thailand*: All were on Homoptera and (except where stated otherwise) were attached to the underside of a dicotyledonous leaf.

NHJ298.01-04 6 Sep. 1990, Chachongsao – mature rice paddy, Delphacidae – *Nilaparvata lugens* Stähl (Brown Plant Hopper – BPH – of rice) attached to stems, leaves and ears of rice, NLH-J & P. Sommartya; NH662.01, 12 Dec. 1991, Khao Yai National Park – trail from Gong Giao to Heo Sawat, Cicadellidae – leafhopper, NLH-J; NHJ924.01, 25 Oct. 1990, Khao Yai National Park – trail to Tad Tha Phu waterfall, Cicadellidae – leafhopper, NLH-J; NHJ1034, 9 Feb. 1993, Khao Yai National Park – trail along tributary above Heo Narok waterfall, Fulgoridae – froghopper attached to the branch of a dicotyledonous shrub, NLH-J & B. Papierok.

*Isolates examined*: NHJ298.01, NH662·01, NHJ924.01.

The host is filled with a tightly packed mass of thin-walled hyphal bodies which are variable in size and shape. The synnemata emerge from all over the host and usually have small side branches. Conidiogenous cells are produced over the whole length of the synnema and are intercalary along the length of the mycelial strands as well as terminal towards the apex of the synnema. Conidiogenous cells have a prominent, swollen basal part with a long phialidic neck (Fig. 7). Conidia are normally in a prominent mucilaginous coat (Fig. 7) and are either solitary or occasionally paired.

Conidia on PDA germinated in 12–16 h. Colonies were hyaline to pale grey with a grey-brown reverse. They grew slowly (40 mm in 30 d at 22 °C). Lilac grey synnemata up to 10–15 mm long were produced after 4–6 wk when the fungus had grown to the edge of the 5 cm Petri plate. The synnemata had many conidiogenous cells with conidia.

**Hirsutella nivea** Hywel-Jones sp. nov. (Figs 8–9)

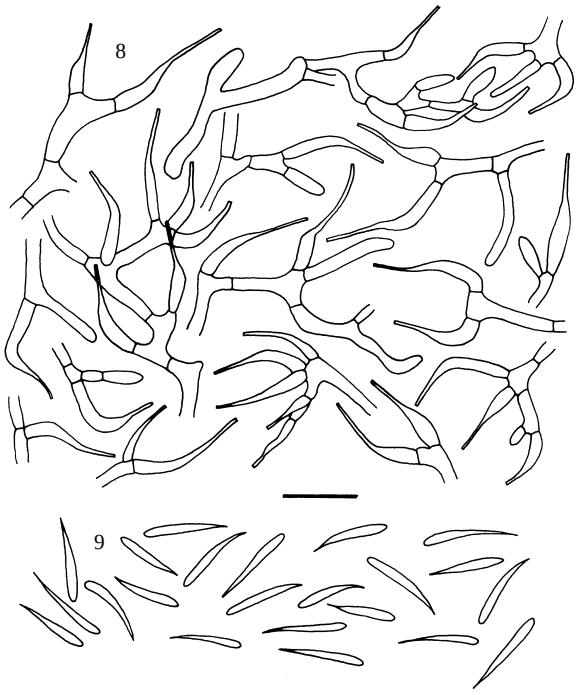
Teleomorph: Not known

Mycelium contegens hospitem membrana densa et nivea. *Synnemata* acerosa, singularia, 8–13 mm longa. *Cellulae conidiogenae* subulatae, 12·5–15·5 μm longae, basi 1–2 μm crassae, hyalinae. *Conidia* acerosa, 5·5–9·5 μm longa, 0·5 μm lata, tunica mucilagina non visa.

NHJ665.01 in homoptero (Cicadellidae), in arbore forestali, Gong Giao, Khao Yai National Park, Nakorn Ratchassima Province, Thailand, N. L. Hywel-Jones, 12 Dec. 1991, in herb. BIOTEC holotypus.

*Stroma* covering the insect in a compact white film, spreading over the leaf. *Synnemata* single (rarely double), from between the head and thorax of the host (rarely from the abdomen), needle-like, 8–13 mm long, 200–300 μm at the base, tapering to the tip. Composed of a loose association of parallel strands of mycelium. *Conidiogenous cells* monophialidic, subulate, 12·5–15·5 μm long, base 1–2 μm across, hyaline. *Conidia* acerose, hyaline, aseptate, smooth-walled, 5·5–9·5 μm long, 0·5 μm wide, lacking a mucilaginous coat.

Holotype NHJ665.01 on a leafhopper (Homoptera: Cicadellidae) attached to the underside of a dicotyledonous leaf in forest, trail from Gong Giao to Heo Sawat, Khao Yai National Park, Nakorn Ratchassima Province, Thailand, N. L. Hywel-Jones, 12 Dec. 1991.




**Figs 8–9.** *Hirsutella nivea*. **Fig. 8.** Phialides (bar = 10 μm). **Fig. 9.** Conidia (bar = 7·5 μm).

*Specimens examined in Thailand*: These were all on Homoptera – Cicadellidae attached to the underside of dicotyledonous leaves.

NHJ707.02, 15 Jan. 1992, Khao Yai National Park – road marker km 42·0, NLH-J; NHJ745.01, 12 Feb. 1992, Khao Yai National Park – Heo Narok forest trail to waterfall, NLH-J; NHJ3808–3813, 18 May 1994, Khao Luang National Park – Krung Ching forest, NLH-J, RN, RP, SS & S. Thienhirun.

The host is filled with a tightly packed mass of thin-walled hyphal bodies which are often in lines. There is a compact white byssoid stroma completely covering the host. A single synnema usually arises from between the head and thorax but occasionally two are formed. The synnema is broad at the base (300–600 μm), gradually tapering to a rounded tip 100–150 μm across. It is composed of loose, parallel strands of hyphae which trap air between them. The phialidic conidiogenous cells are scattered over the outer strands of the mycelium making up the synnema (Fig. 8). One or more phialides arise from each basal cell. Conidiogenous cells are also present on the repent mycelium covering the body of host. Acerose conidia are attached to the phialides at their rounded bases but are easily dislodged (Fig. 9). Although conidia were put on agar media none germinated.

## DISCUSSION

Petch (1932) described *T. pruinosa* (as *Calonectria*) from a leafhopper on bamboo in Sri Lanka. He also named the anamorph *H. versicolor* having previously identified this as *H. floccosa* Speare (Petch, 1924). In their review of mononematous *Hirsutella* species Minter & Brady (1980) discussed Petch's fungus. They noted that it was inappropriate to place the teleomorph in *Calonectria* 'which has been used in the past as a general repository for phragmosporous species with bright-coloured perithecia'. They re-assigned it to *Torrubiella* as *T. pruinosa* (Petch) Minter & B. L. Brady.

Kobayasi (1982) and Kobayasi & Shimizu (1982) did not record *T. pruinosa*, discussing only *T. hemipterigena* Petch from leafhopper hosts. Samson, Evans & Latgé (1988) illustrated *T. pruinosa* on a leafhopper but chose to retain it in *Calonectria* while regarding this genus as 'poorly known or taxonomically unclear'. Although *Calonectria* is not an appropriate genus for *T. pruinosa* as Minter & Brady (1980) discussed I do not believe that *Torrubiella* is wholly appropriate either.

All *Torrubiella* spp. recorded to date have hyaline, thin-walled, filiform ascospores (Kobayasi & Shimizu, 1982) which usually separate into part-spores. The ascospores of *T. pruinosa* are fusiform, distoseptate with a faint hint of pigmentation. Distoseptate, pigmented ascospores are characteristic of *Cordycepioideus* Stifler (Hypocreaceae) and *T. pruinosa* might be regarded as a non-clavate form of this genus. For now, I will accept with reservations the transfer of *T. pruinosa* from *Calonectria* to *Torrubiella* by Minter & Brady (1980).

The material gathered in Thailand compares with Petch's description although he did not illustrate the fungus. Comparison with the drawing of Minter & Brady suggested at first that there might be a different species involved. They reported the ascospores were 6–9 septate when mature whereas Petch (1932) noted that the ascospores were 7-septate. In Thai specimens ascospores were never seen with more than eight cells. Ascus and ascospore development was not synchronous and it did not appear to be synchronized within individual asci either. Individual asci can contain developing ascospores, some with a single septum, some with two and some with three.

Petch (1932) noted that the two ends of the ascospores were different. He described the apex as 'obtuse, lower end attenuated and aseptate for 6–10 μm'. In Thai material this arrangement is very clear (Fig. 3) with the 'obtuse' end of the ascospore toward the tip of the ascus while the 'attenuated' end is toward the foot of the ascus (Figs 1, 2). Petch (1932) described paraphyses as 'linear, shorter than the asci'. Minter & Brady (1980) made no mention of paraphyses. None were seen in the Thai material and I assume that what Petch considered paraphyses were immature asci.

Petch observed that the ascospores appeared to be surrounded 'sometimes with a mucilaginous coat, 1 μm thick'. This was undoubtedly the outer wall of the distoseptate spore. There was no evidence in Thai specimens that this was mucilaginous and spores were never seen adhering to each other. Although Petch (1932) did not culture either of the two states he linked them on the basis of their occurrence together on the same stroma. In Thai specimens the teleomorph was always associated with the *Hirsutella* state. There was a single collection where the teleomorph was absent and only the anamorph was present. The anamorph in the field matched with that from ascospores grown in pure culture. This helps to confirm the link between *T. pruinosa* and *H. versicolor*.

To date, *T. pruinosa* and *H. versicolor* have only been reported, in Thailand, from Khao Yai National Park. Apart from a single collection in June (wet season) this fungus appears to be more common in the cool and hot season (October to April) when rainfall is low. However, until more

extensive collections are made little more can be said about the temporal distribution.

*H. citriformis* is one of the most commonly reported *Hirsutella* species on insects. It appears to have migrated from natural forest and is often recorded as a mortality factor in agricultural hopper pests in the tropics (Roberts & Wraight, 1986; Rombach *et al*., 1986; Aguda *et al*., 1987). It does not seem to have been recorded from insects in natural habitats before. *H. citriformis* has (in Thailand) transferred from natural habitats to become an obvious mortality factor of the rice brown planthopper (BPH). Although it kills large numbers of BPH this is on rice that is close to harvesting. The overall effect of this pathogen on the BPH populations is unknown.

Hywel-Jones (unpubl. obs.) collected *H. citriformis* in large numbers from BPH in the Solomon Islands in April 1986. Also, in the Solomon Islands this species had transferred quickly to the Leucaena psyllid – *Heteropsylla cubana* Crawford (Homoptera; Psyllidae) which had arrived in the islands during the previous 12 months (MacFarlane, pers. comm.). *H. cubana* became a serious pest of *Leucaena* in Thailand after it was first reported in September 1986 (Napompeth, 1990). Although *H. citriformis* has transferred from the forest to BPH populations on rice it has not been recorded from the Leucaena psyllid yet (N. L. Hywel-Jones, unpubl. obs.).

The collection of *H. citriformis* from BPH was part of a large epizootic where there were 5–10 infected adults per 'hill' of rice. The epizootic was spectacular and only the occasional living adult was found. Some BPH must have been ready to migrate as they were attached to the rice seed whereas their normal feeding and resting sites are at the base of the rice. The other collections of *H. citriformis* from insects in natural forest yielded only three specimens in 1200 man hours of survey spread over 7 years. *H. citriformis* was particularly prevalent on BPH on rice toward the end of the monsoon wet season (September). However, collections from the natural forest were only from Khao Yai and were in the cool dry season (October to February).

The new species, *H. nivea*, was found on large cicadellids (body length up to 12 mm). Unlike the previous two species which seemed restricted to central Thailand this was found in tropical evergreen forest at Khao Yai and in the rain forest of southern Thailand at Khao Luang. At Khao Yai it was only reported in the cool dry season when rainfall and temperature were lower (January and February). At Khao Luang it was recorded in May during the hot dry season.

With its needle-shaped conidia *H. nivea* comes closest to the ant-pathogenic *Hirsutella acerosa* H. C. Evans & Samson (Evans & Samson, 1984). It differs significantly from this species in having smaller, more slender conidia (length/width ratio of 11–19 for *H. nivea* compared with 7·2–12 for *H. acerosa*) which are not aggregated in a mucous coat. The smaller subulate phialides of *H. nivea* are also different to those of *H. acerosa*. When Evans & Samson (1984) described *H. acerosa* they reported that the 'conidia of *H. acerosa* can be readily distinguished from those of any previously described species of *Hirsutella* and, although somewhat larger, strongly resemble the conidia of *Hymenostilbe formicarum*'. Both *H. acerosa* and the new species *H. nivea* are therefore quite distinct from other members of the genus.

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