A re-evaluation of the leafhopper pathogen *Torrubiella hemipterigena*, its anamorph *Verticillium hemipterigenum* and *V. pseudohemipterigenum* sp. nov.

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Torrubiella hemipterigena and its anamorph, *Verticillium hemipterigenum*, are re-described from collections made on leafhoppers in Thailand. A new species, *Verticillium pseudohemipterigenum*, collected on scale insects on bamboo in Trinidad and Surinam, is proposed. Based on these collections, the taxonomic status of the genera *Verticillium* and *Hirsutella* is discussed.

Torrubiella Boud. (Clavicipitaceae, Hypocreales) is most frequently reported as a pathogen of spiders (Araneida), with 34 confirmed species, and insects of the order Homoptera, with 12 species (Kobayasi & Shimizu, 1982). Within the Homoptera, *Torrubiella* is especially common on 'scale insects' (Coccidae or Lecaniidae) (Petch, 1923; Kobayasi & Shimizu, 1982; Hywel-Jones, 1993, 1995), but host identification has generally been rudimentary (Evans & Hywel-Jones, 1996).

During the course of a survey for entomopathogenic fungi in Thailand, specimens of a *Torrubiella* species were regularly encountered on leafhoppers belonging to the family Cicadellidae. This was confirmed as *Torrubiella hemipterigena* Petch, with its associated anamorph *Verticillium hemipterigenum* Petch (Petch, 1932). Since this is a rarely reported species, a re-evaluation of its taxonomy and biology is considered appropriate. As a result of this study, a scale insect fungus from Trinidad, originally assigned to *V. hemipterigenum*, is described as a new species.

MATERIALS AND METHODS

Regular surveys for entomopathogenic fungi were undertaken at Khao Yai National Park (north-east Thailand) over a 6-yr period, and more sporadically at other national parks. Collections of diseased leafhoppers were made from the undersides of leaves of herbs and shrubs in natural forest stands. Details of their processing are as reported elsewhere (Hywel-Jones, 1995). A scanning electron microscope (Hitachi S570, fitted with an Emscope SP 2000 cryopreparation unit), was employed to show details of conidiogenesis. All Thailand collections are deposited at the National Science and Technology Development Agency (Herb. BIOTEC).

Asci, ascospores and conidia were streaked onto either full-strength or diluted (10%) potato dextrose agar (PDA), incubated at 20–25 °C and observed daily. Germinating spores were transferred to full-strength PDA plates containing streptomycin sulphate.

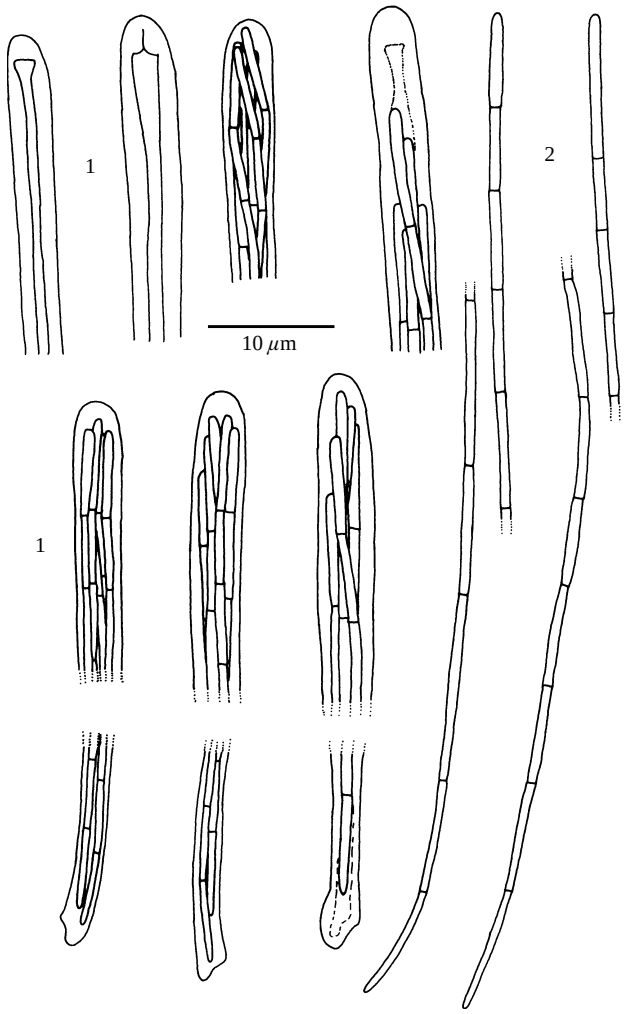
TAXONOMY

Torrubiella hemipterigena Petch, *Trans. Br. Mycol. Soc.* **16**: 236 (1932) (Figs 1–4)

Anamorph: *Verticillium hemipterigenum* Petch, *loc. cit.*: 237

Stroma dense, yellow, completely covering host; hyphae sulphur yellow, smooth-walled, septate, tortuous and anastomosing, 1·5–2·0 µm diam., spreading over leaf surface as a thin, hyaline film. *Ascomata* perithecioid, superficial, scattered to linearly arranged on stroma, dark yellow, elongate ovoid, 700–800 × 330–450 µm; wall bi-layered, inner hyaline, outer dark yellow. *Asci* hyaline, cylindrical, capitate, eight-spored, up to 540 µm when mature, 3·5–4·5 µm wide, tapering to a narrow foot region, 1·5–2·0 µm diam. *Ascospores* smooth, filiform, hyaline, flexuous, multiseptate, with cells 6·5–15 µm long, not breaking into part-spores, up to 440 µm long, < 1·0 µm diam. *Conidiophores* scattered, usually erect, commonly around periphery of stroma or on leaf surface, up to 60 µm in length, 1·5–2·0 µm diam., septate, smooth, hyaline, typically with a terminal and subterminal verticil. *Phialides* in whorls of 6–9 (–12), with a swollen elongate flask-shaped base, 4·5–6·5 × 1·5–2·0 µm diam., narrowing to a long, thread-like tip, 5·5–14 × 0·4–0·5 µm. *Conidia* hyaline, smooth, aseptate, fusoid to sickle-shaped or subfalcate, apices acute or rounded at base, 4·5–8·0 × 0·9–1·5 µm, produced singly, sometimes adhering in pairs, mucus not evident.

The above description is based on the following material collected on adult leafhoppers (Cicadellidae) attached to the underside of leaves of dicotyledonous understorey plants in Thailand.



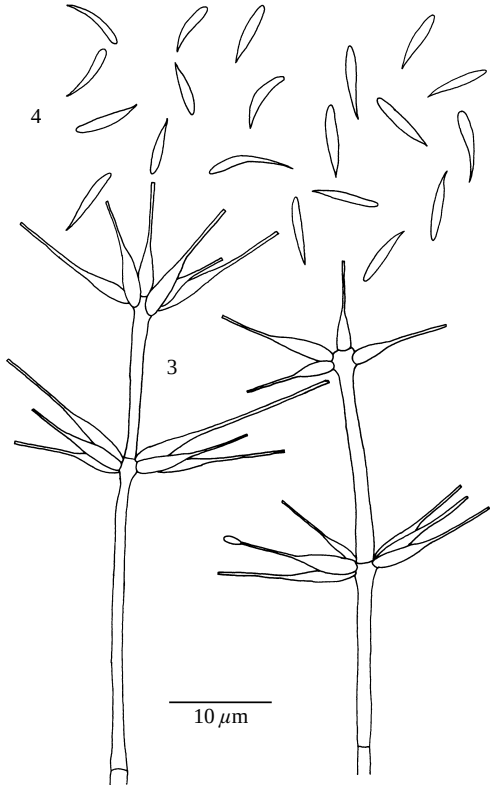
Figs 1–2. *Torrubiella hemipterigena* (NHJ 173.01). **Fig. 1.** Developing asci with maturing ascospores. **Fig. 2.** Mature ascospores.

Teleomorph and anamorph: NHJ173.01 19 Dec. 1989, Khao Yai National Park – trail along tributary above Heo Narok waterfall, N. L. Hywel-Jones, M. C. Hywel-Jones, R. E. Hywel-Jones, L. Manoch & C. V. Subramanian; NHJ614.01, 8 Oct. 1991, Khao Yai National Park – Heo Narok forest trail to waterfall, N. L. Hywel-Jones.

Anamorph only: NHJ337.01 & NHJ338.03, 30 Oct. 1990, Sam Lan National Park, N. L. Hywel-Jones; NHJ416.02, 25 Feb. 1991, Khao Yai National Park – Mor Singh To, N. L. Hywel-Jones; NHJ614.03, 8 Oct. 1991, Khao Yai National Park – Heo Narok forest trail to waterfall, N. L. Hywel-Jones: NHJ2460, 25 Oct. 1993, Khao Yai National Park – Heo Narok forest trail to waterfall, S. Sivichai & A. Rongchitprapas; NHJ2847, 11 Jan. 1994, Khao Yai National Park – Mor Singh To, N. L. Hywel-Jones, R. Nasit, R. Plomhan & S. Sivichai; NHJ3172, 26 Feb. 1994, Khao Sok National Park – Ban Por Tha, A. J. S. Whalley, S. Sivichai & S. Thienhirun; NHJ06076, 2 Feb. 1996, Khlong Naka Wildlife Reserve, N. L. Hywel-Jones, S. Sivichai & S. Thienhirun.

An ascospore isolate was obtained from NHJ614.01, and a conidial isolate from NHJ06076. The following description is based on these isolates.

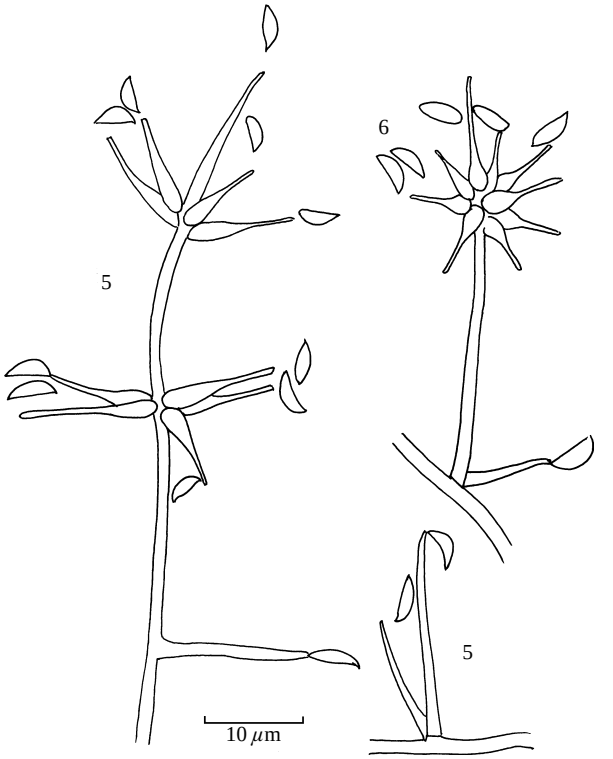
Colonies on 10% PDA slow growing 2·3–2·5 cm diam. after 14 d at 25°; white, cotton-like aerial mycelium and dense white basal skin or pseudostroma, creamish yellow reverse; sporulation absent to poor; ascomatal initials occasionally formed, in NJH06076; however, perithecia may develop, often in clumps, which remain significantly smaller than those on the host (maximum size 300 × 120 μm) and effete.



Figs 3–4. *Verticillium hemipterigenum* (NHJ 173.01), conidiophores and conidia from host.

The original description (Petch, 1932) was based on more than 20 collections (Herb. K) made over the period 1926–8 on leafhoppers attached to forest understorey plants (principally bamboos and *Psychotria* shrubs) in Nuwara Eliya, Sri Lanka. Gams (1971) proposed a lectotype (R21) based on one of the oldest and richest collections with numerous mature perithecia collected on a leafhopper on *Arundinaria debilis* on 17 Oct. 1926. Several of the Thai specimens also have a single, yellow, cylindrical synnema arising from the upper thorax, as reported by Petch (1932). Grams (1971) also noted the presence of chlamydospore-like structures in the stroma covering the host, and the complex terminal verticils consisting of up to 10 phialides, although Petch had reported only a maximum of six phialides in a whorl.

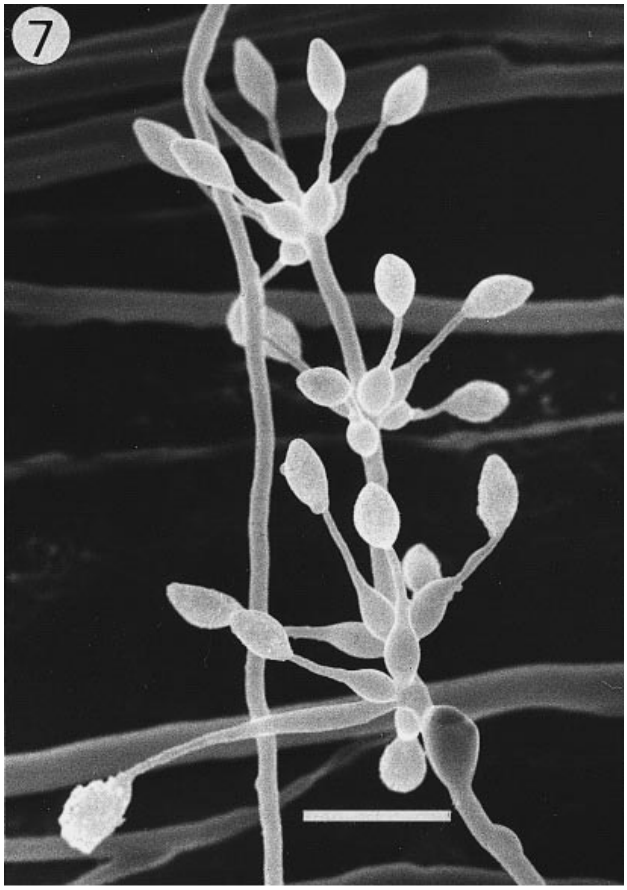
In his re-assessment of *Verticillium*, Gams (1971) proposed two subdivisions: *Verticillium* section *Verticillium*, producing phialides on upright, clearly differentiated conidiophores which emerge directly from the substrate, and *Verticillium* section *Prostrata* W. Gams, with phialides formed on repent hyphae, not clearly differentiated from the aerial mycelium. Gams observed that *V. hemipterigenum* is the only species described by Petch, of those belonging to the section *Prostrata*, which was not originally placed in *Cephalosporium* (with conidial slime balls), and he further characterized this species not only on the basis of the phialidic whorls but also on the basally swollen phialides tapering to a thread-like tip.

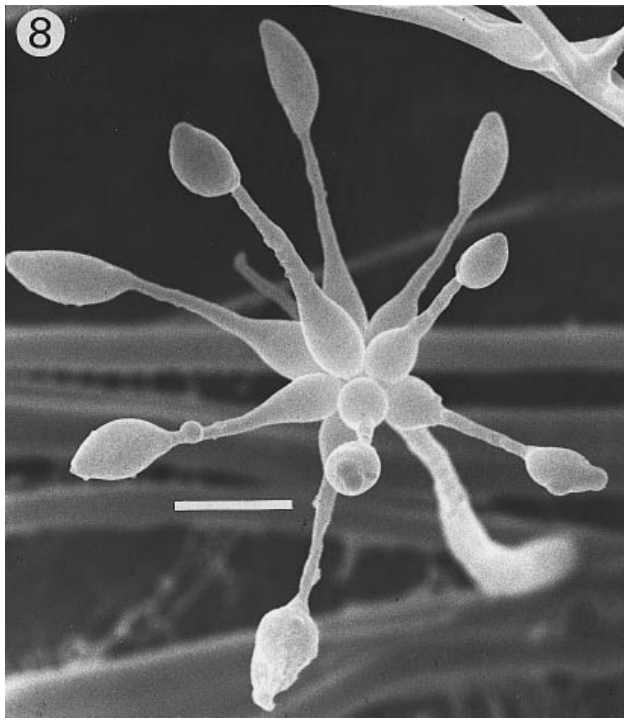


Figs 5–6. *Verticillium pseudohemipterigenum* sp. nov. (IMI 331563, ex type culture). **Fig. 5.** Verticillate and solitary conidiogenous cells. **Fig. 6.** Conidia.

Evans & Samson (1986) concluded that, due to the presence of subulate phialides with markedly attenuated tips and solitary conidia, *V. hemipterigenum* would be better accommodated in *Hirsutella* section *Mononematosa* Minter & Brady (1980).

Several species in this section, *H. guyana* Minter & B. L. Brady and *H. verticillioides* Charles, produce phialides in whorls but these are much more robust than in *V. hemipterigenum*, and often polyphialidic, with typically large conidia embedded in a pigmented mucous sheath. The delicate phialides of *V. hemipterigenum* most closely resemble those of *H. versicolor* Petch, which is also a pathogen of leafhoppers in Asia (Petch, 1932; Samson *et al*., 1988; Rombach & Roberts, 1989), except that these are markedly polyphialidic and the conidia are usually in mucoid heads. The occasional occurrence of well-organized synnemata in *V. hemipterigenum* would also suggest affinities with *Hirsutella*, where it appears to be closest to *H. darwinii* H. C. vans & Samson, in terms of conidial morphology. Indeed, Evans & Samson (1982) remarked on the similarity of the awl-shaped phialides of *H. darwinii* to those of *Verticillium* section *Prostrata* but concluded that '…the solitary not verticillate phialides scattered along distinct synnemata justify its placement in *Hirsutella*'. If this argument were followed here, then the characteristic verticils of *V. hemipterigenum* would justify the retention of this species in *Verticillium*, even though its phialide morphology resembles that of many *Hirsutella* species. Gams & Van Zaayen (1981), Evans & Samson (1986) and Jun *et al*. (1991) discussed the conceptual problems of *Verticillium*, which the latter authors regarded as a catch-all genus, '…a large heterogeneous assemblage of taxa grouped according to relatively simple and ill-defined





Figs 7–8. *Verticillium pseudohemipterigenum* sp. nov. (IMI 331563), SEM cryopreparation from 10 d culture. **Fig. 7.** Aerial mycelium bearing terminal and lateral verticils. **Fig. 8.** Terminal verticil with nine subulate phialides. Scale bars: **Fig. 7,** 9 μm; **Fig. 8,** 5 μm.

Table 1. Comparison of *Verticillium hemipterigenum* and *V. pseudohemipterigenum*

	V. hemipterigenum	*V. pseudohemipterigenum*
Distribution	Asia (Sri Lanka, Thailand)	Neotropics (Trinidad, Surinam)
Host	Cicadellidae	Coccidae (Asterolecaniidae)
In vivo	Mycelium yellow to ochraceous, dense stromatic, synnemata present, teleomorph present (*Torrubiella*)	Mycelium white, sparse, synnemata absent, teleomorph unknown
In vitro	Slow-growing, white, lanose and pseudostromatic; sporulation absent to poor	Slow-growing, white to pale brown, lanose; sporulation abundant on aerial mycelium
Phialides	In whorls, 6–9 (up to 12), 10–20·5 × 1·5–2·0 μm, tapering to a thread-like tip, 0·4–0·5 μm diam.	Solitary or in whorls 4–8 (up to 10), 8–25 μm long, either swollen at base (2·5 μm) and narrowing to needle-like neck (> 0·3 μm diam.), or awl-shaped, tapering gradually from 1·0–1·5 μm at base to 0·3–0·5 μm at apex
Conidia	Fusoid, sickle-shaped to subfalcate, 4·5–8·0 × 0·9–1·5 μm produced singly or in pairs	Normally fusiform to ellipsoidal, (3·5–) 4·5–6·5 × 1·5–2·0 μm, produced singly

characters'. Thus, until generic concepts are better defined, it is concluded that the anamorph of *T. hemipterigena* should be retained in *Verticillium*.

This conclusion was further endorsed following an examination of cultures derived from both conidial and ascospore isolations of Thai material. Colony morphology is more reminiscent of *Verticillium* species, such as *V. lecanii* (Zimm.) Viégas, which also has a *Torrubiella* teleomorph (Evans & Samson, 1982, 1986), rather than *Hirsutella* species (Rombach & Roberts, 1989). A fungus assigned to *V. hemipterigenum* in Herb. IMI, and included in a chemotaxonomic analysis of *Verticillium* (Jun *et al*., 1991), was also compared with the Thai isolates and this prompted a critical analysis of both herbarium and culture material. On the basis of this examination a new species is proposed.

Verticillium pseudohemipterigenum H. C. Evans & Y. Jun sp. nov. (Figs 5–8)

Mycelio ex insecto Coccidarum oriente, sparso, hyalino; hyphis levibus, septatis, usque ad 3 μm attingentibus. *Conidiophoris* ad cellulas conidiogenas sessiles reductis, verticillatim, usque 10 phialidibus compositim, vel singulatim, ex hyphis vegetabilibus exorientibus. *Cellulis conidiogenis* saepe, verticillatis, phialidicis, hyalinis, levibus, (8–) 10–16 (–20) μm longis, prope baso inflatis, usque 2·5 μm diam., ad collum rectum 4–10 μm, apice > 0·3 μm latitudinae attenuatis. *Phialides* solitaribus, anguste aculeatae, usque 25 μm longae, 1·0–1·5 μm ad 0·3–0·5 μm angustatae. *Conidiis* hyalinis, aseptatis, levibus, singulatim, anguste fusiformibus vel ellipsoideis, muco carentis, (3·5–) 4·5–6·5 × 1·5–2·0 μm. Teleomorphosis ignota.

Holotypus: IMI 300309, in larvae Coccidarum ad partem inferiorem foli bambusarum inventum, Northern Range, Trinidad, 13 Dec. 1985, lectus C. Prior; culto-typus IMI 331563.

Mycelium, white, sparsely produced on coccid host; hyphae smooth, septate, up to 3·0 μm wide. *Conidiophores* not distinct, reduced to solitary conidiogenous cells or complex verticils arising from the vegetative hyphae. *Conidiogenous cells* phialidic, predominantly verticillate, composed of 4–8 (–10) phialides, hyaline, smooth, (8–) 10–16 (–20) μm long, often inflated at the base, up to 2–5 μm diam., tapering to a needle-like neck, 4–10 μm long, and less than 0·3 μm at apex. *Solitary phialides*, awl-shaped, up to 25 μm in length, tapering gradually from 1·0–1·5 μm to 0·3–0·5 μm. *Conidia* produced singly, hyaline, aseptate, smooth, mucus or mucoid sheath not evident, narrowly fusiform to ellipsoidal, often in the shape of an orange segment (3·5–) 4·5–6·5 × 1·5–2·0 μm. Teleomorph unknown.

Holotype: IMI 300309 on coccid larvae (*Bambusaspis* sp., Asterolecaniidae, Coccoidea) on lower leaf surface of bamboo, Northern Range, Trinidad, 13 Dec. 1985, C. Prior; dried culture ex type deposited as IMI 331563.

Colonies on agar (10% PDA), attaining diam of 2·7–3·0 cm after 14 d at 25°; 3·8–4·5 cm after 28 d; white, initially low, appressed, but later with dense, cotton-like aerial mycelium becoming pale pinkish brown and stromatic centrally; phialides abundant, solitary or more usually in dense verticils; reverse white to cream to pale brown, odour slightly earthy, exudate absent.

Paratypes: IMI 374402, on *Bambusaspis* sp. (pit scale), on bamboo leaf, Parrylands, Siparia, Trinidad, 1 Nov. 1996, H. C. Evans; IMI 374403 on *Bambusaspis* sp., on bamboo leaf, Paramaribo, Surinam, H. C. Evans, 5 Nov. 1996. Dried cultures deposited under the same IMI number.

As observed on the holotype, sporulation on the host paratypes is difficult to detect but abundant *in vitro*. Colonies as above, but the Surinam isolate is faster growing, up to 4 cm after 21 d on 10% PDA at 25°.

The description is based mainly on the ex type culture since sporulation on the host is sparse. *V. pseudohemipterigenum* is distinguished from the anamorph of *T. hemipterigena* on the basis of the characteristics listed in Table 1.

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