Torrubiella petchii, a new species of scale insect pathogen from Thailand

NIGEL L. HYWEL-JONES

National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Ministry of Science, Technology and Environment Building, Rama VI Road, Bangkok, 10400, Thailand

Torrubiella petchii is described from scale insects that infest bamboo leaves in tropical forest. It is compared with four other species of *Torrubiella* described from Thailand and with other species known to infect scale insects. *T. petchii* was successfully isolated from whole ascospores. No anamorph was found in the material collected from the field. One isolate produced a *Hirsutella* anamorph. This is compared with *Hirsutella*-like anamorphs, collected from the field, of two other scale-insect *Torrubiella* spp.

Petch (1923) was the first to discuss the taxonomy of the invertebrate pathogenic genus *Torrubiella* Boud. He recognized *Torrubiella* species from scale insects ('coccids') as forming a distinct group and gave a detailed review of seven recognized species. More recently, Kobayasi & Shimizu (1982) reviewed 11 species of *Torrubiella* from scale insects, four of which are found in Asia. Hywel-Jones (1993) reported on *T. luteorostrata* Zimm. and *T. tenuis* Petch which are commonly found in Thailand and later (Hywel-Jones, 1995) described *T. iriomoteana* Kobayasi & Shimizu and *T. siamensis* Hywel-Jones which are rare species found at Khao Yai National Park, Thailand. This paper describes a fifth species from Thailand and discusses its taxonomy with what is known already of *Torrubiella* spp. in Thailand and elsewhere.

MATERIALS AND METHODS

Surveys of the underside of living leaves of herbs and saplings were made throughout the year, and over a 7-yr period, at several sites within Khao Yai National Park. Sporadic surveys at other National Parks and Wildlife Reserves in Thailand were also made. Collected material was returned to the laboratory in plastic bags or plastic pots and stored in a refrigerator before being processed.

Isolations were made on Malt Extract Agar and Potato Dextrose Agar from asci and/or ascospores. Plates were incubated at 22 °C in the dark and examined each day for signs of growth. Permanent slides were made for examination with an Olympus BH microscope plus a drawing tube. Drawings were made using a × 100 objective with immersion oil.

RESULTS

Torrubiella petchii Hywel-Jones sp. nov.
(Figs 1–4, anamorph Fig. 5)

Etym.: In recognition of Tom Petch's work with scale-insect fungi

Stromata corpus hospitis obtegentia, ochracea, annularia, 4–5 µm diam. *Ascomata* gregaria, superficialia, ampulliformia, luteo brunnea, in mycelio luteo formata, 630–680 × 200–300 µm. *Asci* cylindrici, capitati, 480–600 × 5–6 µm, 8-sporis. *Ascosporae* ad maturitatem completae, filiformes, 450–500 × 1–1·2 µm.

Holotypus ex larvis homopterorum (*Coccus* sp.) in culmo delapso 'bamboo' (*Neohouzeaua* sp.), 500 m secundo flumine Heo Sawat Waterfall, true left bank fluminis Lumtakhlong, Khao Yai National Park, 29 May 1991, leg. N. Hywel-Jones, Herb. N.B.C.R.C., NHJ 459.01.

Stroma covering the host body, ochraceous, ring-like, 4–5 µm diameter. *Ascomata* crowded, superficial, yellow mycelium, 630–680 × 200–300 µm, flask-shaped. *Asci* cylindric, capitate, 480–600 × 5–6 µm, 8–spored. *Ascospores* remaining whole at maturity, filiform, 450–500 × 1–1·2 µm.

Holotype from homopteran scale insect (*Coccus* sp.) on fallen bamboo (*Neohouzeaua* sp.) leaves in tropical monsoon evergreen forest. Herbarium N.B.C.R.C., NHJ459·01 500 m down river of Heo Sawat Waterfall on the true left bank of the Lumtakhlong river, Khao Yai National Park, 29 May 1991, N. L. Hywel-Jones.

Specimens from the type locality were on a tall bamboo (*ca* 15 m) and the only leaves that could be easily examined had fallen to the forest floor. Specimens were in large numbers at all sites with several stromata usually on a single leaf.

The perithecioid ascomata were arranged mainly in an irregular toroid (Fig. 1) and in some specimens the host could be seen in the middle of the ring. The basal stroma over the host was red but the overall appearance was of an ochraceous

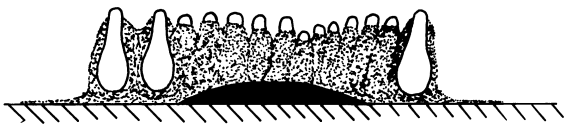

Fig. 1. Habit sketch of the form of the stroma cut open to show the shape of three ascomata. The diagonal lines represent the bamboo substrate of the host (solid black arc). (Scale bar, 500 µm.)

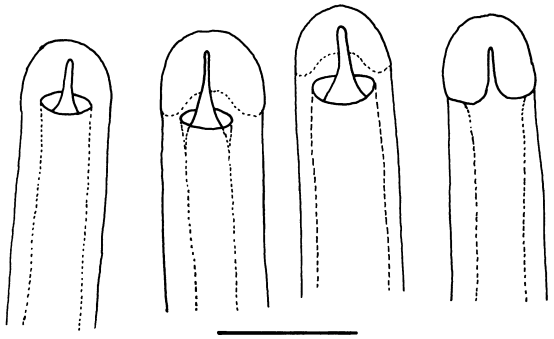

Fig. 2. The tips of four immature asci showing the cytoplasmic intrusion in to the developing cap. (Scale bar = 10 μm.)

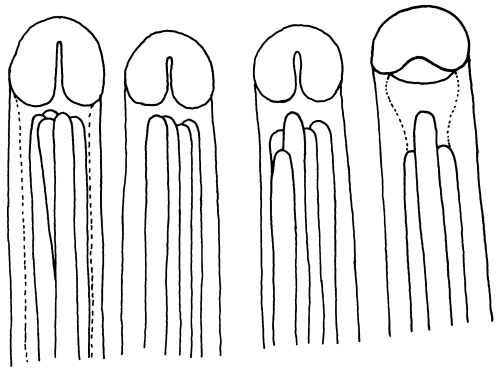

Fig. 3. Four mature ascus tips showing the form of the cap with canal and the arrangement of the ascospores. (Scale bar, 10 μm.)

yellow ring containing the ascomata. Each stroma had 50–100 ampulliform ascomata embedded in coarse hyphae (Fig. 1) with only the tip of the ascomatal neck protruding. The ascomata contained yellow oil globules between the asci. There was no sign of a hamathecium or of paraphyses. No anamorph was noted in field collected material.

Immature asci had a cone of cytoplasm that protruded in to the ascus cap (Fig. 2). As the ascospores matured the cytoplasm in the cone was lost or retreated but a prominent canal remained (Fig. 3). Ascospores were arranged parallel with no evidence of regular spiralling (Fig. 3). Four or five of the eight ascospores were arranged almost level with each other below the cap while the remaining ascospores were an indeterminate way back. The ascus foot was not easy to discern (Fig. 4) but it was clear that no ascospores originated in the foot.

Other specimens examined: These are all stored in the insect–fungus collection of the N.B.C.R.C., NHJ2746, Khao Sabap National Park – Trok Nong Waterfall, 15 Dec. 1993, NLH-J, R. Nasit, R. Plomhan, S. Sivichai & S. Thienhirun; NHJ3700, 500 m downstream of Heo Sawat Waterfall on the true left bank of the Lumtakhlong river, 10 May 1994, NLH-J, RN & SS; NHJ5137, Khao Yai National Park – Heo Sawat trail to Heo Sai, 23 Nov. 1994, NLH-J, RN & SS; NHJ5152, Phu Lua National Park, 27 Nov. 1994, NLH-J; NHJ5318 & 5319, Kaeng Krachan National Park – trail to Tor Tip Waterfall, 24 May 1995, RN, SS & ST; NHJ5369, Khao Laem National Park – trail behind headquarters, 20 Jun. 1995, NLH-J, RN, SS & ST; NHJ5670, Nam Nao National Park – trail behind headquarters, 5 Sep. 1995, NLH-J& RN; NHJ5738, Khlong Lan National Park – Khlong Lan Waterfall, 18 Oct. 1995, NLH-J, RN, SS & ST.

Isolation of the fungus. Isolations were made from asci containing whole, mature ascospores and from released ascospores on MEA and PDA. Germ tubes emerged from the anterior ends of ascospores still contained within asci. Colonies were at first white, becoming cream yellow and then ochraceous yellow as on the host. Colonies were slow growing, reaching 1·5 cm after 3 wk at 22° in the dark.

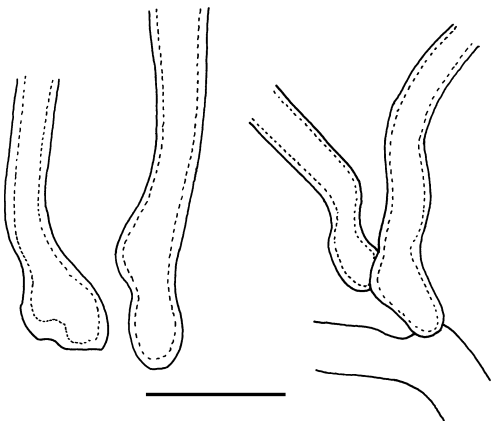

Fig. 4. The form of the ascus foot. (Scale bar, 10 μm.)

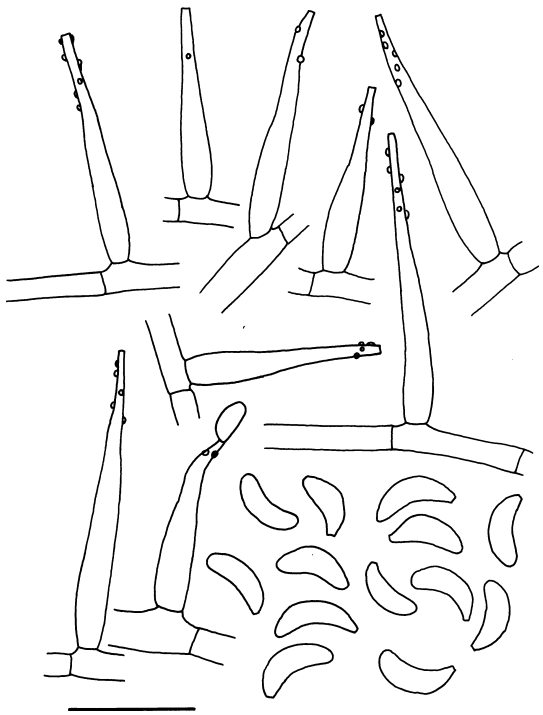

Fig. 5. The *Hirsutella* anamorph of *T. petchii* from culture. (Scale bar, 10 μm.)

A *Hirsutella* anamorph (Fig. 5) was noted in one of the cultures – NHJ5152. Conidiogenous cells arose from basal hyphae which were 1·6–2·4(–3·1) μm diam. These were produced at irregular intervals along the length of the aerial mycelium. All conidiogenous cells developed adjacent to hyphal septa. The hyaline conidiogenous cells were 14·9–23·8 μm long. They were 2·2–2·7 μm diam. near their base but tapered to a long thin neck 0·5 μm diam. The terminal third of the conidiogenous cell was verrucose. Conidia, formed singly, were reniform with a truncate attachment point and were 5·1–6·5 μm long by 1·9–2·2 μm across.

Table 1. Summary of important characteristics separating the five recognized species of scale insect *Torrubiella* in Thailand

	Stroma (form)	Ascoma (shape)	Ascospore (size µm)	Ascospore (type, size µm)	Anamorph
T. luteorostrata	Planar	Flask-shaped	600–900 × 250–500	Whole, 460–590 × 1·5–2	*Paecilomyces*
T. tenuis	Planar	Flask-shaped	500–900 × 200–320	Whole, 380–500 × 1·5–2	Unknown
T. petchii	Toroidal	Flask-shaped	630–680 × 200–300	Whole, 450–500 × 1–1·2	*Hirsutella*
T. iriomoteana	Flattened discoid	Globose/ovoid	270–330 × 200–250	Part-spores, 1 × 2	*Hirsutella*
T. siamensis	Flattened discoid	Pear-shaped	500–600 × 180–250	Part-spores, 11·3–15·3 × 1·3–1·5	*Hirsutella*

Cultures examined: Cultures are stored in the N.C.G.E.B. insect–fungus collection. NHJ459·01, NHJ5152 and NHJ5318 all from whole ascospores.

DISCUSSION

T. petchii was found only on bamboo and was assumed to be specific to a bamboo scale insect. This was the first record of a *Torrubiella* associated with scale insects of bamboo in Thailand. Surveys were made at several sites in Khao Yai National Park and, to date, the type locality is the only place where *T. petchii* was found in this National Park. Other sites in Thailand where bamboo occurred (naturally on the slopes of steep river valleys and as secondary growth in cleared forest) were surveyed for this fungus. All other collections were from tropical monsoon evergreen forest in Central and Northern Thailand. There were no records of this from the south of Thailand where rainfall is more evenly distributed through the year. Specimens were collected throughout the rainy and the cool season (May to December). However, until more extensive collections are made little can be said of the temporal distribution.

With the description of *T. petchii*, five species of scale insect *Torrubiella* have been recorded from Thailand. The important characteristics of these five are summarized in Table 1. Petch (1923) described seven species of *Torrubiella* from scale insects and commented particularly on the shape of their ascomata. The ascomatal shape of *T. petchii* came between that of *T. rubra* Pat. & Lagerh. and *T. luteorostrata*. No clear evidence was seen of septation of the ascospores and, in this respect, it may be compared also with *T. luteorostrata* and *T. tenuis* (Hywel-Jones, 1993). The ascus cap was bulbous, more like that of *T. luteorostrata* than the elongate cap of *T. tenuis*. Also, the ascus foot was more like that of *T. luteorostrata*, not stout as for *T. tenuis*, As with *T. luteorostrata* and *T. tenuis*, there was no apparent spiralling of the ascospores within the ascus. Another feature that *T. petchii* shared with *T. luteorostrata* and *T. tenuis* was the relative ease with which it could be isolated. The observation that germ tubes sometimes emerged just from the anterior end of the ascus suggests that ascospore differentiation was from the anterior downwards.

Apart from *T. luteorostrata* and *T. tenuis*, recognized from Petch's study, the other three species seem to be rare in SE Asia. *T. iriomoteana* and *T. siamensis* have proved to be rare in Thailand and only on solitary scale insects. *T. petchii* seems to be restricted in its host and the host's food plant. However, it does attack a gregarious host and, when found, is in large numbers.

T. iriomoteana and *T. siamensis* differed from *T. petchii* in having ascospores that separated into part-spores. The anamorph connection in four of the five species from Thailand has been made. It is noteworthy that while *T. petchii* has characters more in common with *T. luteorostrata* and *T. tenuis* it nevertheless produces a *Hirsutella* anamorph like *T. iriomoteana* and *T. siamensis*. The *Hirsutella* anamorphs of these three species all have stout, reniform conidia that lack a mucilaginous coat. *T. iriomoteana* and *T. siamensis* have not been isolated yet and their polyphialidic anamorphs are known only from the field. In contrast, the monophialidic anamorph of *T. petchii* is known only from culture.

When Petch reviewed the scale insect *Torrubiella* spp. in 1923 there were only seven species known. *T. petchii* was compared with *T. sublintea* Petch, *T. tomentosa* Pat. and *T. barda* Petch in having yellow as the predominant colour of the stroma. However, these three species are South American and from the descriptions given by Petch (1923) are different from *T. petchii* as all produce part-spores at maturity. The shape and size of the ascomata of *T. petchii* were also different from *T. tomentosa* and *T. barda*. The ascomata of *T. sublintea* had a similar shape to *T. petchii* but are larger and fewer as Petch (1923) reported only two or three ascomata on a stroma. Later, Kobayasi & Shimizu (1982) reviewed the genus and recognized eleven species from scale insects. Seven years of surveys in Thailand have confirmed the presence of three of the eleven species recognized by Kobayasi & Shimizu (1982) and have added two more (Hywel-Jones, 1995; and this study).

The National Research Council of Thailand is thanked for providing the opportunity to conduct this work in the early stages. The Royal Forest Department provided access to the sites. Most especially, Mrs Surang Thienhirun of the Royal Forest Department is thanked for her behind the scenes help. Dr Banpot Napompeth and his staff at the National Biological Control Research Center are especially thanked for providing me with a friendly atmosphere in which to work.

REFERENCES

Hywel-Jones, N. L. (1993). *Torrubiella luteorostrata*: a pathogen of scale insects and its association with *Paecilomyces cinnamomeus* with a note on *Torrubiella tenuis*. *Mycological Research* **97**, 1126–1130.

Hywel-Jones, N. L. (1995). *Torrubiella iriomoteana* from scale insects in Thailand and a new related species *Torrubiella siamensis* with notes on their respective anamorphs. *Mycological Research* **99**, 330–332.

Kobayasi, Y. & Shimizu, D. (1982). Monograph of the genus *Torrubiella*. *Bulletin of the National Science Museum, Tokyo*, Series B **8**, 43–78.

Petch, T. (1923). Studies in entomogenous fungi. III. *Torrubiella*. *Transactions of the British Mycological Society* **9**, 108–128.

(*Accepted 3 May 1996*)