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Torrubiella iriomoteana from scale insects in Thailand and a new related species *Torrubiella siamensis* with notes on their respective anamorphs

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Torrubiella iriomoteana is recorded for the first time since the type collection and is connected with an anamorph best assigned to *Hirsutella*. A second species, *Torrubiella siamensis*, and its *Hirsutella* anamorph, is described as new. These species are compared with other *Torrubiella* from scale insects.

Kobayasi & Shimizu (1982) recognized 56 species of *Torrubiella* Boud. (of these, eleven were from scale insects (Homoptera)). In a study of insect-pathogenic fungi on living leaves of forest herbs and saplings, in Thailand, two species of *Torrubiella* were found infrequently. One had previously been described as *Torrubiella iriomoteana* Kobayasi & Shimizu from Japan. The other does not agree with any species known from scale insects and is described as new. Details are presented on the anamorphs of both species which are best assigned to *Hirsutella*.

MATERIALS AND METHODS

Surveys of the underside of living leaves of herbs and saplings were made throughout the year within Khao Yai National Park over a 5-yr period. Sporadic surveys of other National Parks in Thailand were also made. Collected material was returned to the laboratory in plastic sandwich bags and stored in a refrigerator before processing.

Isolations were made on Potato Dextrose Agar from asci, ascus part-spores, conidia and from stromatal material. These were incubated at 22 °C in the dark and examined each day for signs of growth. Slides were prepared for examination using an Olympus BH microscope with a drawing tube.

RESULTS

Torrubiella iriomoteana Kobayasi & Shimizu *Bull. Nat. Sci. Mus.*, Tokyo, Ser. B 8, 73 (1982)

Kobayasi & Shimizu (1982) gave a brief description in Latin with figures (fig. 46A–G). The following description is based on the specimen from Thailand.

Stromata covering the host body, flattened discoid, tomentose, with smooth margin; white; hyphae interwoven, 2–4 μm diam., septate, branched. *Perithecia* scattered or partly aggregated, completely immersed, yellow-brown, globose or ovoid, 260–280 × 200–230 μm, densely hairy, hamathecium and paraphyses absent. *Asci* immature, 3 μm diam., cap 2 μm diam. *Ascospores* not seen. *Anamorph* within the same stroma. *Conidiophores* reduced to sessile conidiogenous cells, terminal or sub-terminal, polyphialidic, 10–15 × 3–3·5 μm. *Phialides* terete, ends truncate, 4–6 μm. *Conidia* hyaline, aseptate, smooth, reniform, 9·3–12·7 × 4–6 μm.

Specimens examined: NHJ314.04, Khao Yai road marker km 29·2, 11 Sept. 1990, N. L. Hywel-Jones.

A single specimen was attached to the underside of a monocotyledonous leaf (Zingiberaceae). No host was found under the mycelium but the superficial habit of the fungus and the absence of damage to the leaf suggest a scale insect was involved. Perithecia were scattered over the stroma with occasional but random groupings. There was a slight thickening of the stroma where the perithecia were located and the darkened tips and ostioles were exposed above the stromatal material (Fig. 1). The asci were all immature and the ascus cytoplasm had not differentiated into ascospores (Fig. 2). Part-spores were not seen. The asci had a prominent ascus foot (Fig. 3).

Examination of the stromatal material from Thailand confirmed the presence of an anamorph. Large conidia were associated with conidiogenous cells best described as a mononematous, polyphialidic, *Hirsutella* species (Fig. 4). These were mature, loose within the stroma and conidia were easily detached from the phialides. The conidia did not seem to have a mucous coat.

A second specimen, found at the same time of the year at another site within Khao Yai National Park, was sufficiently different from *T. iriomoteana* and other known *Torrubiella* spp. to justify describing a new taxon.

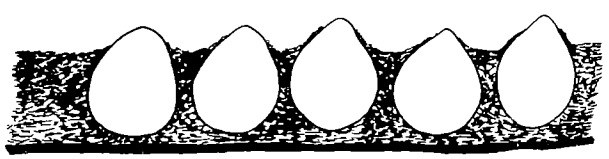

Fig. 1. Perithecia of *T. iriomoteana* within the stroma. The host has been completely digested and the fungus is superficial on the leaf (thick black line). Bar, 400 μm.

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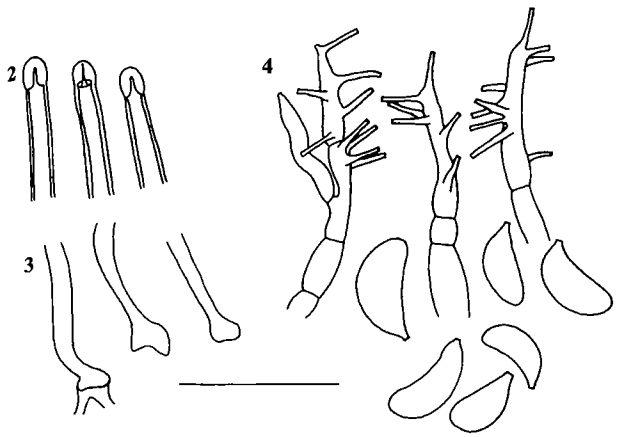


Figs 2–4. Fig. 2. Three immature asci of *T. iriomoteana* showing undifferentiated cytoplasm and the form of the developing ascus tip. Bar, 20 μm. **Fig. 3.** Three examples of the immature ascus foot of *T. iriomoteana*. Bar, 20 μm. **Fig. 4.** Conidiogenous cells of the *Hirsutella* anamorph of *T. iriomoteana* from the stroma, with examples of detached conidia. Bar, 20 μm.

Torrubiella siamensis Hywel-Jones, sp. nov. (Figs 5–8)

Stromata corpus hospitis contegentia, complanata discoidea, alba. *Mycelio* copioso, superficiali, hyalino vel albo, septato, ramoso, cum hyphis latitudinem usque ad 4 μm attingentibus. *Perithecia* aggregata, superficialia, elongata ovoidea, ochracea, 580–660 × 300–330 μm. *Asci* filiformes, capitati, octospori, 570 μm longi, 5 μm lati. Articulae ascosporae, 11·3–15·3 × 1·3–1·5 μm, utrinque truncatae. *Conidiophoris* ad cellulas conidiogenas sessiles reductis, terminales et sub-terminales. *Cellulis conidiogenis* polyphialidicis, hyalinis, 7–11 × 3–4·5 μm, levibus. *Phialidis* teretibus, 4·5–7·3 μm. *Conidiis* hyalinis, aseptatis, levibus, reniformibus, 14·6–19·3 μm longis, 5·7–8·7 μm latis, tunica mucilagina non visa.

Holotypus NHJ297.01 in Homoptera, in arbore forestali, Gong Giao, Khao Yai, Thailand 2 Sept. 1990.

Stromata flattened discoid, white; 4 mm diameter. *Mycelium* superficial, hyaline to white, septate and branched. *Perithecia* clustered, superficial, elongated ovoid, ochraceous, 580–660 × 300–330 μm. *Asci* filiform, capitate, 8-spored, up to 570 μm long, 5 μm diam. *Ascospores* filiform, breaking into elongated cylindric part-spores, ends truncate, 11·3–15·3 × 1·3–1·5 μm. *Conidiophores* reduced to sessile conidiogenous cells, terminal or sub-terminal, polyphialidic, 7–11 × 3–4·5 μm. *Phialides* terete, ends truncate, 4·5–7·3 μm. *Conidia* hyaline, aseptate, smooth, reniform, 14·6–19·3 × 5·7–8·7 μm.

Holotype NHJ297.01 (stored in the NBCRC insect-fungus collection) from Homoptera (scale insect), Gong Giao, Khao Yai, Thailand, 2 Sept. 1990.

The perithecia were not surrounded by mycelium and were superficial on the stroma (Fig. 5). There was no evidence of a hamathecium or of paraphyses within the perithecia. Asci were at different stages of development (Fig. 6). There was no obvious septation of the mature ascospores (Fig. 6) and there was no obvious ascus cap. However, ascospores readily broke into part-spores (Fig. 7). Natural release of part-spores was not observed.

Conidiogenous cells (Fig. 8) were sessile within the stroma. The conidia were easily detached and rarely seen on the phialides (Fig. 8). No evidence was seen of conidial development and there was no sign of a mucous coat. All conidia looked mature with many appearing vacuolate (Fig. 8).

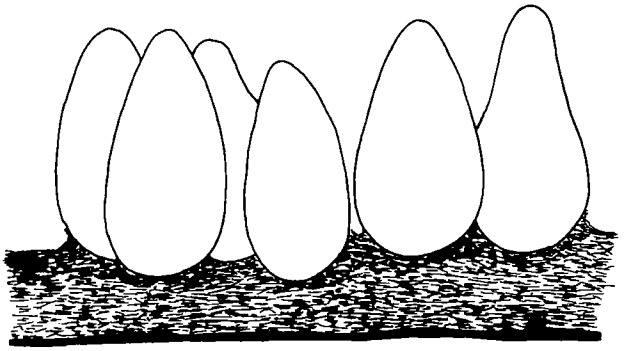
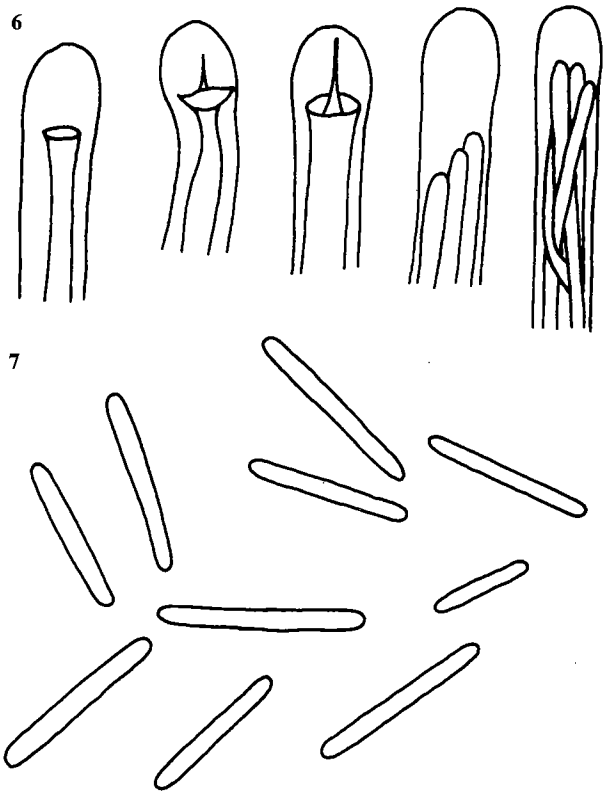


Fig. 5. Superficial perithecia of *T. siamensis*. The host was entirely digested and the stroma was superficial on the leaf (thick black line). Bar, 400 μm.



Figs 6–7. Fig. 6. Five ascus tips of *T. siamensis* at different stages of development. Three are immature and two are mature. Bar, 20 μm. **Fig. 7.** Examples of typical ascus part-spores of *T. siamensis*. Bar, 20 μm.

Isolation of the fungi

All attempts at isolating *T. iriomoteana* and *T. siamensis* from asci, part-spores, conidia and stromatal material on PDA failed. There was no germination after 10 d of observation.

DISCUSSION

Although the material was immature the shape and size of the perithecia of the Thai *T. iriomoteana* match those of Kobayasi & Shimizu (fig. 46B) who recorded perithecia 270–300 × 200–250 μm. Kobayasi & Shimizu (1982) noted the occurrence of conidia amongst the stromatal hyphae but did not describe the origin of these conidia. The conidia of the Thai material (Fig. 4) agree in shape with fig. 46E (mislabelled as 'part of the

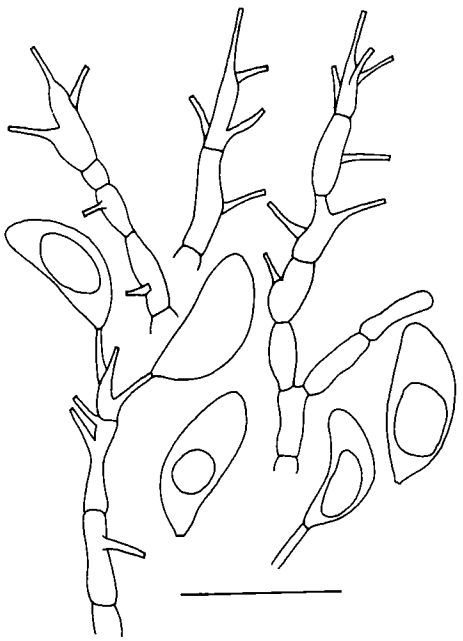


Fig. 8. Conidiogenous cells of the *Hirsutella* anamorph of *T. siamensis* with examples of attached and detached conidia. Bar, 20 μm.

ascus') of Kobayasi & Shimizu (1982). Kobayasi & Shimizu (1982) described the conidia as ellipsoid, 7·5–12 × 4–4·5 μm, hyaline, unequal and apiculate. These dimensions are slightly smaller than for the Thai material. In the Thai material it was clear the conidia arose from polyphialidic conidiogenous cells immersed in the stroma (Fig. 4). The specimen from Thailand differs from the Japanese one only in colour (white instead of the pale ochre yellow of Kobayasi) and in having a smooth rather than fimbriate margin. These character differences may be due to the immaturity of the Thai specimen.

T. iriomoteana has previously only been recorded from Japan, apparently also from a single specimen. The finding of a single record in Thailand suggests *T. iriomoteana* and its anamorph may have a range that at least covers the Asia–Pacific region. The area of forest where the specimen was originally found was visited in the following 2 yr at the same time but no further specimens were collected. With so few records it would seem this is a very rare fungus. This rarity may result from a very narrow host range.

T. siamensis had characteristics between *T. iriomoteana* and *T. superficialis*. The glabrous, ovoid superficial perithecia of *T. siamensis* were similar to those of *T. superficialis* which Kobayasi & Shimizu (1982) also reported from scale insects attached to a decorticated branch of a *Hydrangea*. Microscopically, the Thai specimen differs from *T. superficialis* in the longer ascus part-spores.

T. siamensis differs from *T. iriomoteana* in having larger, superficial perithecia which were ovoid instead of globose and immersed and in having part-spores up to 10 times longer than those of the Japanese species (Fig. 7). The ascus tips of *T. siamensis* (Fig. 6) were stouter than those recorded for *T. iriomoteana*. The *Hirsutella* state of *T. siamensis* (Fig. 8) was similar to that described for *T. iriomoteana* (Fig. 4) but was larger in all respects. Kobayasi & Shimizu (1982) did not describe an anamorph for *T. superficialis*.

The three *Torrubiella* species *T. iriomoteana*, *T. superficialis* and *T. siamensis* are closely related but appear different from other species of *Torrubiella* so far recorded from scale insects (Petch, 1923; Kobayasi & Shimizu, 1982; Hywel-Jones, 1993). It is significant that these three species have ascospores which break easily into part-spores. In contrast, those of *T. luteorostrata* and *T. tenuis* remain whole at maturity (Hywel-Jones, 1993). While attempts at culturing *T. iriomoteana* and *T. siamensis* from part-spores and conidia failed (this study) it was relatively easy to culture *T. luteorostrata* and *T. tenuis* from ascospores or conidia (Hywel-Jones, 1993).

T. luteorostrata and *T. tenuis* were commonly found at many sites within Khao Yai throughout much of the year (Hywel-Jones, 1993). Also, collections of *T. luteorostrata* or *T. tenuis* often had several stromata on a single leaf. *T. iriomoteana* and *T. siamensis*, in contrast, were found only in September and on one occasion each. As no other scale insects were found on the leaf the host must be a solitary species of scale insect.

Evans & Prior (1990) reviewed the fungi pathogenic to Diaspid scales and noted the presence of *Torrubiella* spp. Evans & Hywel-Jones (1990), in discussing *Hypocrella* on scale insects, warned against assuming accurate host identity beyond the level of Order when these were obliterated by the fungus. For now I assume that, on the basis of habit, a scale insect (*sensu lato*) was the host but cannot place this to family.

The anamorphs are distinctive and different from those usually described as *Hirsutella*. The very large conidia appeared to be formed singly on each phialide and did not appear to be surrounded by a mucous sheath. However, examination of the genus indicates that it is in need of revision as there are many different types of *Hirsutella* which bear only a superficial relationship with each other as a whole.

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