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Torrubiella luteorostrata: a pathogen of scale insects and its association with *Paecilomyces cinnamomeus* with a note on *Torrubiella tenuis*

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Torrubiella luteorostrata and *Paecilomyces cinnamomeus* are reported for the first time from Thailand. Field observation and cultural study has linked *P. cinnamomeus* with *T. luteorostrata*. The *Torrubiella* state appears during the rainy season while the *Paecilomyces* anamorph occurs at the beginning and end of the wet season. The fungus does not appear during the hot, dry season. *Torrubiella tenuis* is also reported for the first time from Thailand but it was not possible to link this to an anamorph.

Petch (1923) provides the only detailed account of *Torrubiella* spp. on scale insects (Homoptera) although more recently Kobayasi & Shimizu (1982) and Kobayasi (1982) reviewed 56 known species of *Torrubiella*. Of these, 12 were from scale insects and 4 are known from Asia. Very little is known of these insect pathogens. Two species were commonly found on scale insects in tropical monsoon (seasonal) forest in Khao Yai National Park, Thailand. Through field observation and cultural study it was possible to identify an anamorph for one species and to collect information on temporal distribution.

TAXONOMY

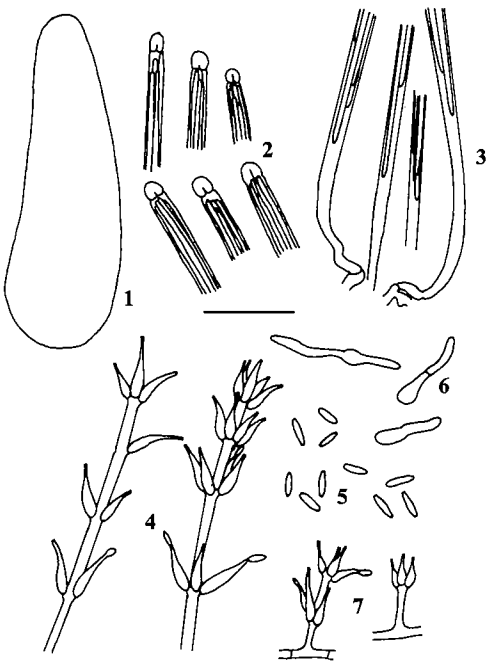
Torrubiella luteorostrata Zimm., *Centralbl. f. Bakt.* **7**, 872 (1909).

?*Torrubiella brunnea* Keissl., *Annls Mycol.* **7**, 292 (1909).

The following description is modified from Petch (1923) and allows for variation in the Thai material.

Stromata flattened pulvinate to discoid, tomentose, 1·5–5 mm diam., covering the host with thick-walled hyaline, yellow to yellow-brown or vinaceous-brown or purple hyphae, smooth, 3–7 µm diam. *Hypothallus* of thin, smooth-walled, hyaline hyphae radiating over substratum. *Perithecia* produced on the stromata or (more commonly) from the hypothallus, elongated flask-shaped to elongated conic, purple-red or purple-brown, or yellow-brown or Sanford brown, 600–900 µm long, 250–350 (–500) µm diam. below neck; neck at first dark purple-red becoming yellow and horny when mature, broken in old specimens. Asci cylindrical, capitate, 8-spored, at least 500 µm long, 4–5(–10) µm diam. *Ascospores* filiform, 460–590 × 1·5–2·0 µm, septate, dividing into part-spores, 3–6 × 1–2 µm (part-spores not seen in Thai material).

Specimens examined from Thailand: All on scale insects (Homoptera) on the underside of living dicotyledonous leaves within Khao Yai National Park. Specimens have been deposited in the NBCRC insect-fungus collection. Mor Singh To tributary of Lumtakhlong River, 13 June 1989, N. L. Hywel-Jones; Heo Narok, tributary 500 m up from main river, 6 July 1989, N. L. Hywel-Jones; Phakrajai, 23 Aug. 1989, N. L. Hywel-Jones; Heo Sawat, Lumtakhlong River 500 m up from waterfall, 17 Aug. 1989, N. L. Hywel-Jones; Phakrajai, 18 Sept. 1989, N. L. Hywel-Jones; Heo Narok Tributary at road marker 18·7 km, 18 Sept. 1989, N. L. Hywel-Jones; Phakrajai, 10 Oct. 1989,



Figs 1–7. Microscopic detail of *T. luteorostrata* and its anamorph, *P. cinnamomeus*. **Fig. 1.** Typical perithecium, **Fig. 2.** Tips of six mature asci. **Fig. 3.** Ascus base showing arrangement of the ascospores and the form of the ascus foot. **Fig. 4.** Typical conidiophores of the anamorph. **Fig. 5.** Conidia. **Fig. 6.** Three conidia germinating on PDA after 24 h. **Fig. 7.** Simple conidiophores on repent hyphae which produce conidia in slime balls. Bar: Fig. 1, 200 µm; Figs 2–7, 20 µm.

N. L. Hywel-Jones; Heo Narok tributary 500 m up from main river, 19 Dec. 1989, N. L. Hywel-Jones; Mor Singh To tributary of Lumtakhlong River, 29 Sept. 1990, N. L. Hywel-Jones; Heo Sai, Lumtakhlong River, 8 May 1990, N. L. Hywel-Jones; Darn Charng, 19 May 1990, N. L. Hywel-Jones; Heo Narok tributary 500 m up from main river, 1 Aug. 1990, N. L. Hywel-Jones; road marker 17·8 km, 11 Sept. 1990, N. L. Hywel-Jones; Than Rattana, 25 Oct. 1990, N. L. Hywel-Jones; road marker 44·8 km, 13 Nov. 1990, N. L. Hywel-Jones; road marker 31·6 km, 13 Nov. 1990, N. L. Hywel-Jones; Heo Narok tributary 500 m up from main river, 25 Oct. 1990, N. L. Hywel-Jones; road marker 44·8 km, 13 Nov. 1990, N. L. Hywel-Jones; tributary of the Lumtakhlong river 500 m up from Heo Sawat waterfall, 27 Nov. 1990, N. L. Hywel-Jones; Phakrajai, 8 Jan. 1991, N. L. Hywel-Jones & J. Drummond; Heo Narok 500 m up from main river, 9 Jan. 1991, N. L. Hywel-Jones; road marker 43·3 km, 29 Aug. 1991, N. L. Hywel-Jones.

The hot dry season in central Thailand occurs from February to April. All collections were made from May to January corresponding to the wet season or the cool dry season. The large collection of material from Thailand generally fits within the descriptions given by Petch (1923) and Dingley (1953). The perithecial neck was sometimes as obvious as that depicted by Petch (1923, Fig. 1.1) but most often it appeared as a gradual extension of the base (Fig. 1). A significant difference is that ascospores were never seen breaking within the ascus into part-spores in Thai material. Whole, mature ascospores fractured irregularly but these could not be considered part-spores. Between 4 and 6 filiform ascospores could always be seen below the ascus cap (Fig. 2). A thin canal in the ascus cap could sometimes be seen. Ascospores at the foot of the ascus were more spaced out (Fig. 3). The ascus foot was not clearly visible but when it could be discerned this was usually a further 20–30 μm behind the last ascospore. There was no apparent spiralling of the ascospores as is often found with other insect–pathogenic ascomycetes (Hywel-Jones, unpubl. obs.) and the ascospores were more or less parallel with one another.

Petch (1923) noted the stroma was 'purple-red, becoming purple-brown, or Sanford brown, rarely white'. He gave no indication of the freshness of the material but it is likely that some collections were up to twenty years old. Thai specimens had stromata ranging from partly white with purple perithecia through pale-brown, yellow-brown, rust-brown, cinnamon to purple-red when fresh. The colour range could be found within a single collection. The stromata of Thai specimens were generally 1·5–2·5 mm diam. with a hypothallus extending to 7–8 mm. This is larger than measurements given by Petch (1923) who reported stromata only up to 1·5 mm. Dingley (1953), however, recorded the stromata as up to 5 mm. Stromatal size is probably related to host size and maturity.

In Thailand, the host was obliterated by the fungus and no attempt was made to determine host identity beyond the assumption that a scale insect was involved. Dingley (1953) also noted the stromata 'smothered' the host. However, Petch (1923) recorded both Aleyrodidae and Coccididae as hosts. To what extent Petch based these identities on healthy scales remaining on the leaves is now impossible to establish. It is probable that *T. luteorostrata* is confined to one or other or both of these superfamilies within the order Homoptera.

Previous records (Petch, 1923; Dingley, 1953) list *T. luteorostrata* from the Seychelles, Sri Lanka, Java, Samoa and New Zealand. Recently, Kobayasi & Shimizu (1982) gave a record for the far east U.S.S.R. but no other details. Apart from the U.S.S.R. record all others are from islands and those for Thailand are the first from tropical mainland Asia. Given this distribution it is presumed that *T. luteorostrata* may be present throughout tropical and sub-tropical Asia.

Petch (1923) and Dingley (1953) did not report an anamorph of *T. luteorostrata*. In Thailand, many stromata identical to *T. luteorostrata* lacked perithecia. Careful examination of these revealed a hyphomycete growing from the stromata or from the hypothallus. This is a previously unrecognised anamorph which corresponds with the rarely recorded *Paecilomyces cinnamomeus* (Petch) Samson & Gams.

Anamorph: *Paecilomyces cinnamomeus* (Petch) Samson & Gams, in Samson, *Stud. Mycol.* **6**, 62 (1974).

Verticillium cinnamomeun Petch, *Trans. Br. mycol. Soc.* **16**, 233 (1932).

Verticillium heterocladum Penzig *sensu* Fawcett, Fungi Parasitic on *Aleyrodes citri*. 22 (1908).

The stromata and hypothallus of the anamorph were identical to those of the teleomorph. Instead of producing perithecia erect conidiophores formed. The following description is modified for Thai material from Samson (1974).

Conidiophores erect, mononematous, 150–240 × 2·0–3·0 μm, hyaline at top but pigmented at base, smooth-walled with verticillate branches of 2–8 phialides or whorls of phialides directly from the conidiophore. *Phialides* 9·0–14·0 × 2·5–3·0 μm, flask-shaped. *Conidia* catenate on host or catenate or in slime balls on agar, fusiform or cylindric, ends truncate, smooth-walled, hyaline, 5·0–7·5 × 1·5–2·5 μm.

The *Paecilomyces* state occurred as erect, mononematous conidiophores loosely scattered on the hypothallus or slightly more crowded on the stromata.

Specimens examined from Thailand: All on scale insects on underside of living dicotyledonous leaves within Khao Yai National Park. Specimens have been deposited in the NBCRC insect-fungus collection. Gong Giao, Lumtakhlong River, 14 Jan. 1990, N. L. Hywel-Jones & H. C. Evans; Mor Singh To tributary of Lumtakhlong River, 29 Sept. 1990, N. L. Hywel-Jones; Heo Sai trail along true left bank of Lumtakhlong River, 8 May 1990, N. L. Hywel-Jones; Darn Charng, 19 May 1990, N. L. Hywel-Jones; road marker 17·8 km, 11 Sept. 1990, N. L. Hywel-Jones; road marker 17·8 km, 2 Oct. 1990, N. L. Hywel-Jones.

Verticillium heterocladum Penzig was a name applied to a fungus infecting scale insects in Florida (Fawcett, 1908). Petch (1926) doubted whether this could be the same species as that described by Penzig from Europe. Later, Petch (1932) described the Florida fungus as a new species, *Verticillium cinnamomeum*. Samson (1974) transferred this to *Paecilomyces* because it had 'rather stout conidiophores with flask-shaped phialides with a distinct neck and (its) catenate conidia'. The stout conidiophores and phialides (Fig. 4) match those of Samson (1974, Fig. 25). Conidial shape and size was variable (Fig. 5). Specimens of *P. cinnamomeus* examined by Samson (1974) were from North and Central America and from Ghana. Material collected in Thailand agrees well with these

descriptions of *P. cinnamomeus* and suggests a pan-tropical distribution.

Isolation and culture of *T. luteorostrata* and *P. cinnamomeus* from Thailand linked these two states. Isolates were made from whole asci, ascus fragments, whole ascospores, ascospore fragments and conidia on Sabouraud dextrose agar, potato dextrose agar and malt extract agar. Conidia swelled notably before germinating from one or both of the conidial ends (Fig. 6). Colonies on PDA grew slowly (15 mm in 20 d and 40 mm after 50 d at 22 °C). The colony margin was hyaline with a basal felt of ochraceous red to purple mycelium similar to that found on the host insect. Oxygen-limited cultures readily produced a deep purple-red diffusible pigment in the agar. Isolates from ascospores or conidia sporulated readily but sparsely producing the *Paecilomyces* form as on the insect. Simple conidiophores and single phialides also arose from repent hyphae (Fig. 7). These appeared to be an *Acremonium* state with conidia in balls rather than chains. Both anamorphs occurred together in culture. This *Acremonium* form of *P. cinnamomeus* was not observed on the host insect and it is not known if this was a cultural artifact. A strain isolated from ascospores produced perithecia with asci after 6 months (at 20° in darkness) while a second strain from multiple conidia also produced perithecia after 6 months.

It was not practical to demonstrate the *in vitro* production of the *Paecilomyces* state from single ascospores but it was produced readily from solitary asci and from ascospore fragments. Weresub & Pirozynski (in Kendrick & DiCosmo, 1979) developed a scheme for affiliation between the anamorph and teleomorph. Under their scheme the relationship between *T. luteorostrata* and *P. cinnamomeus* may be coded as 2.3.1. In summary, the teleomorph and anamorph were found frequently in the field on morphologically similar stromata, the anamorph was grown in culture from single asci or multiple conidia and the teleomorph was grown from single asci and multiple-conidia.

Petch (1923) considered *T. luteorostrata* to be an Asian equivalent of the South American *Torrubiella rubra* Patouillard & Lagherheim. Given the variation in Thai material it is possible that *T. rubra* and *T. luteorostrata* may prove to be the same species. The fact that *P. cinnamomeus* was first recorded from the Americas suggests this might be linked with *T. rubra*. Petch (1923, Fig. 2) described, briefly, a conidial state present on a single stroma of *T. rubra*. From his figure it can not be determined whether this is a correlated anamorph or not. However, a similar condition was found in old Thai material where a chemical reaction between the pigmented hyphae and the stain produces rounded bodies along the hyphae which might be confused with conidia.

There was a seasonality for the two states, *T. luteorostrata* and *P. cinnamomeus*, which were found through the year apart from the hot dry season. The teleomorph was found between May and November corresponding exactly with the wet season. The anamorph was found at the start and the end of the wet season. Sterile forms were recorded from August to January. The lack of records in the hot dry season may be accounted for as the fungus survives this unfavourable period incubating slowly in host scales where it is protected from desiccation.

A second, related, species *Torrubiella tenuis* was also recorded from scale insects in Thailand.

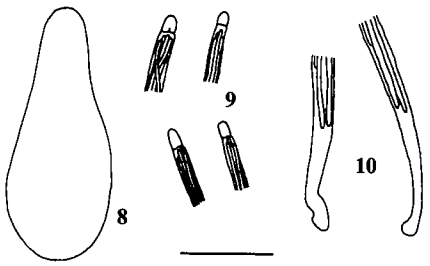
Torrubiella tenuis Petch, *Ann. Perad.* 7, 323 (1923).

Stromata pulvinate, flattened pulvinate or almost plane, up to 1·5 mm diam., white, tomentose, rather loose internally, surrounded by a broad, hyaline, fibrillose margin or hypothallus. *Perithecia* usually on the thicker part of the stroma, sometimes on the margin or hypothallus, sometimes occurring singly on scales which do not bear any stroma except a slight weft of hyphae at the base of the perithecium, scattered or clustered, usually 3–6 (extremely, more than 50), elongated flask-shaped or elongated conic, 500–900 µm long, 200–250 (–320) µm diam. below, pale amber to pale yellow-brown by transmitted light, subtranslucent, covered with hyphae up to two-thirds their height, or almost glabrous. Asci long, up to 400–500 (–570) µm, cylindrical, capitate, eight-spored, 7 µm diam. *Ascospores* filiform, 380–500 × 1·5–2·0 µm septate, dividing into cylindrical part-spores, 3–6 × 1 µm (part-spores not seen in Thai material).

Specimens examined from Thailand: All collections were from the underside of living leaves of dicotyledonous plants in Khao Yai National Park except for one from Sam Lan National Park. Specimens have been deposited in the NBCRC insect-fungus collection. Gong Giao, Lumtakhlong River, 20 June 1989, N. L. Hywel-Jones; Heo Sawat 500 m up from waterfall on true left bank, 17 Aug. 1989, N. L. Hywel-Jones; Phakrajai, 18 Sept. 1989, N. L. Hywel-Jones; Mor Singh To tributary of Lumtakhlong River, 29 Apr. 1990, N. L. Hywel-Jones; Mor Singh To tributary of Lumtakhlong River, 1 Aug. 1990, N. L. Hywel-Jones; Tad Tha Phu trail, 28 Aug. 1990, N. L. Hywel-Jones; Tad Tha Phu, 2 Oct. 1990, N. L. Hywel-Jones; Sam Lan waterfall, Sam Lan National Park, 30 Oct. 1990, N. L. Hywel-Jones; road marker 44·8 km, 13 Nov. 1990, N. L. Hywel-Jones; Orchid Falls on Lumtakhlong River, 18 Jan. 1991, N. L. Hywel-Jones; Heo Narok waterfall 500 m up from main river, 15 Mar. 1991, N. L. Hywel-Jones; Tad Tha Phu trail to Mor Singh To, 16 Mar. 1991, N. L. Hywel-Jones, G. Batten, S. Batten & S. Spriggs; road marker 42·0 km, 18 Sept. 1991, N. L. Hywel-Jones.

This species is known only from the Sri Lankan type locality (Petch, 1923) and was mentioned only briefly by Kobayasi & Shimizu (1982) in their monograph of the genus. Collections in Thailand over three years had one to several specimens in each sample. Stromata of Thai specimens were white and (when fresh) shiny, glabrous becoming dry and papery when old up to 3 mm diam. with a hyaline hypothallus to 10 mm diam. Stromata were often immature or had old perithecia hollowed out and lacking asci. No anamorph was found with these stromata in nature and Petch (1923) did not describe an anamorph.

Stromata of *T. tenuis* were generally larger and more pulvinate than the flattened stromata of *T. luteorostrata*. Perithecia (Fig. 8) swelled and were translucent when in a water-saturated atmosphere or when detached from the stromata and immersed in water. On rupturing the perithecium, asci could be removed which broke into fragments. The ascus tip of *T. tenuis* (Fig. 9) was not as bulbous as that of *T. luteorostrata* (Fig. 2). However, the ascus foot of *T. tenuis* (Fig. 10) was stouter than that of *T. luteorostrata*. As with *T. luteorostrata* there was no evidence to support Petch's



Figs 8–10. Microscopic detail of *T. tenuis*. **Fig. 8.** Typical perithecium. **Fig. 9.** Tips of four mature asci. **Fig. 10.** Ascus base showing arrangement of the ascopores and the form of the ascus foot. Bar: Fig. 8, 200 μm; Figs 9, 10, 20 μm.

observation that the ascospores of *T. tenuis* divided naturally into part-spores.

As with *T. luteorostrata* the hosts were assumed to be scale insects. Although Petch (1923) recorded the hosts to species, *T. tenuis* in Thailand completely obliterated the host making certain identification questionable. The known distribution of Sri Lanka and Thailand suggests the true distribution could at least encompass tropical southern Asia. The fact that Kobayasi failed to record *T. tenuis* in his many writings and collections over 50 years suggests its distribution might not include sub-tropical or temperate Asia (i.e. China, Taiwan and Japan). Collections of *T. tenuis* were not as common as for *T. luteorostrata* and *P. cinnamomeus* and it was not possible to define clearly its temporal distribution. However, collections in March and April would indicate this species is more tolerant of dry conditions than *T. luteorostrata*.

When whole asci, ascus fragments, whole ascospores or ascospore fragments were put on SDA, PDA and MEA they germinated within 24 h. Sometimes a whole ascus would produce germ tubes along its length although germ tubes were more common at the apical end of the ascus. This suggests ascospore maturation is from the apex down. It was not possible to start cultures from immature ascospores where cytoplasm had not differentiated into ascospores. Natural release of ascospores was not observed. Isolates grew slowly reaching 18 mm in 20 d at 22° with a sterile grey-white to cream-white mycelium and a cream-brown reverse. Petch (1923) did not report any attempt at culturing this fungus. While it was possible to isolate *T. tenuis* from ascospores there was no evidence of an anamorph growing in culture and none was found on insects from the forest.

DISCUSSION

It is difficult to explain the release of long, filiform ascospores unless they operate in surface water films (Bandoni & Koske, 1974). Cox (1983) noted that in aquatic plants and fungi there is a tendency for aquatic spores to have extremely long axes compared with non-aquatic spores. He regards this adaptation as one that will increase search and anchorage efficiency of the spores. The fact that the *Torrubiella* state of *T. luteorostrata* appears to be confined to the wet season suggests that the leaf-surface water-films may be important for the survival of these ascospores. Following the theory of Cox (1983), it may be concluded that the whole ascospores of *T. luteorostrata* and *T. tenuis* are suited to dispersal in an aqueous medium and for attachment to an immobile, flattened host. Susceptibility to desiccation make such spores unsuitable for dispersal in an aerial environment. The *Paecilomyces* state of *T. luteorostrata* may be active against the crawler stage of the host life-cycle which may be expected to brush passed the erect conidiophores as they search for feeding sites. Alternatively, the production of dry *Paecilomyces* conidia at the beginning and end of the rainy season might allow for aerial dispersal and potential infection of scale insects on other plants.

Early workers assumed the long filiform ascospores of insect–pathogenic genera such as *Cordyceps*, *Torrubiella* and *Hypocrella* naturally broke into part-spores. While this is the case for many species within these genera there is increasing evidence that certain species have ascospores which remain whole at maturity. Petch (1931) recognized this condition in *Cordyceps* and separated species producing whole, mature ascospores into a new genus *Ophiocordyceps*.

Earlier, Petch (1921) studied the genus *Hypocrella* in detail but failed to recognize that it too contains species with whole ascospores (Hywel-Jones & Evans, 1993). The type species of *Hypocrella*, *Hypocrella discoidea* (Berk. & Br.) Sacc. has whole, mature ascospores (Hywel-Jones & Evans, 1993). The recognition that two *Torrubiella* species produce whole, mature ascospores suggest this condition is more common than previously realised. Further study of these ascomycetous insect-pathogenic genera will likely reveal more species producing whole ascospores. It is not proposed to revise the taxonomy of *Torrubiella* on the basis of these few records.

These species were found only in undisturbed tropical forest. Although scale insects are serious pests of many crops there are no records of these fungi occurring in artificial ecosystems in Thailand. The slow growth of these fungi in culture and the sparse sporulation would not make these suitable candidates for biological control.

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