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Acanthostigma and Tubeufia species, including T. claspisphaeria sp. nov., from submerged wood in Hong Kong

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Abstract: Acanthostigma scopulum, Tubeufia claspisphaeria sp. nov. and T. paludosa were identified from submerged wood collected in a small forest stream on Lantau Island, Hong Kong. The collections of Acanthostigma scopulum and Tubeufia paludosa differed slightly from the original descriptions. Tubeufia claspisphaeria differs from previously described species in that it has hook-shaped setae that form radially around the ostiole. This new species is described and illustrated and compared with the most similar species. A dichotomous key to the 16 accepted species of Tubeufia is provided.

Key words: new species, saprobic fungi, systematics

INTRODUCTION
There are 22 genera in the Tubeufiaceae (Barr 1980, Rossman 1987, Kirk et al 2001). Some genera, such as Melioliphila and Uredinophila, are hyperparasites on sooty molds and on rust fungi, while Podonectria is parasitic fungi on scale insects. Acanthostigma and Tubeufia are saprobes, usually found on old, rotten wood. Previous examinations of freshwater fungi occurring on submerged wood in streams in the tropics and subtropics have yielded numerous new fungi, including hyphomycetes (Hyde et al 2002, McKenzie et al 2002), coelomycetes (Hyde 1993) and ascomycetes (Wong and Hyde 1999, Cai et al 2002). During our survey of fungi occurring on naturally submerged wood, we collected an Acanthostigma and two Tubeufia species, one being a species new to science, from a small forest stream in Hong Kong. The three species are described, illustrated and compared with known taxa. A key to the 16 accepted species of Tubeufia is provided.

MATERIALS AND METHODS
Submerged wood was retrieved from a stream near the Trappist Monastery, Lantau Island, Hong Kong, in Apr 2002 and returned to the laboratory, where it was incubated in zip-lock plastic bags at room temperature. Humidity was maintained by adding moistened paper towels. Samples were examined within 3 d and periodically over 1 mo for the presence of sporulating structures. Cultures of fungi were obtained where possible from single spores (Choi et al 1999). To stimulate sporulation, 1 cm² blocks of colonized agar were placed in water in a bubble chamber overnight.

TAXONOMY
Acanthostigma De Not. and Tubeufia Penz. & Sacc. Acanthostigma currently includes six species and recently has been reviewed by Réblová and Barr (2000). The genus is characterized by vinaceous, reddish-brown or dark brown ascomata that are covered with dark brownish-black, often opaque, obtuse or acute setae. The ascospores are hyaline, multisepitate and cylindrical-fusiform to elongate fusiform. The anamorphs are in Helicosporium and Helicomyces. In species of Tubeufia the ascomata are hyaline, whitish or yellowish to pinkish, but may become dark at maturity. They are smooth, or are covered with protruding cells, thick-walled hyphal appendages, or short dark setae (Réblová and Barr 2000).

Acanthostigma scopulum (Cooke & Peck) Peck, Bull. New York State Mus. 1:22 (1887) (Figs. 1–6)
Ascomata 145–250 μm diam, superficial, globose, solitary or gregarious, dark brown to black, membranous, ostiolate, with setae; setae (56–)80–90(–99) μm wide, 4–6 μm wide at base, 1.5–2 μm wide at apex, dark brown, 1–2-septate, thick-walled, straight. Asci (64–)90–120(–130) × 8–10 μm

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Fig. 1–6. *Acanthostigma scopulum* (from HKU[M] 17121). 1. Ascomata on natural substratum. 2. Dark brown, septate setae with acute apex. 3–4. A cluster of mature and immature asci. 5. Ascus. 6. Ascospore. Pseudoparaphyses in Fig. 3–4 (arrowed). Bars: 1 = 200 μm; 2–5 = 30 μm; 6 = 25 μm. Figs. 2–3 mounted in water, other figures mounted in lactophenol.

(\(x = 103 \times 9.2 \ \mu m, \ n = 20\)), 8-spored, cylindric-clavate, bitunicate, short stalked, pseudoparaphyses. Ascospores (66–70–85(–90) × 3–3.5(–4) μm (\(x = 78 \times 3.4 \ \mu m, \ n = 30\)), fasciculate, long-fusiform to cylindrical-fusiform, hyaline, straight or slightly curved, (5–)9–12-septate, smooth-walled, guttulate, lacking appendages and sheaths.


Colonies on potato-dextrose agar olive-colored, reaching 1 cm diam in 10 d at room temperature (~28 C), no pigment diffusing into agar, not sporulating even after submergence overnight in a bubble chamber.

Substratum. Wood submerged in streams, decaying wood (*Fagus sylvatica*, *Pinus* sp. and *Tsuga* sp.)

Known distribution. Australia, Europe, Hong Kong and U.S.A.

Specimen examined. HONG KONG. Lantau Island, Trappist Monastery, small stream in forest, on wood partially submerged, 21 Apr 2002, K.D. Hyde (HKU[M] 17121; living culture HKUCC 9117).

Notes. A key to species of *Acanthostigma* was provided by Réblová and Barr (2000). This collection differs from the description for *A. scopulum* provided by Réblová and Barr (2000) in that it has septate setae and wider ascospores 3–3.5(–4) μm versus (2–)2.5–3(–3.5) μm but in other aspects it is similar.

*Tubefia claspisphaeria* Kodsube, sp. nov.

(Figs. 7–16)

Ascomata 185–330(–350) μm diametro, superficialia, globosa, solitaria vel aggregata, hyalina vel pallide brunnea ubi immatura, atrobunnea vel atris ubi maturus, membranea, ostiolata, setosa; setae usque 47 μm longa, 10 μm lata, (0–)11(–2)–septata, atrobunnea, hamatus. Peries ascomati usque 60 μm lata crassus, vinacea-brunnea, e cellulis pseudoparenchymatis compositum, 4–5 stratosus, textura globulosa. Asci (93–)105–120(–127) × (12–)14–16(–18) μm, octospori, cylindrico-clavati, bitunicati, pedicellati. Ascosporae (34–)45–60(–64) × 5–7(–8) μm, fasciculatae, elongatae-fusiformis, (3–)5–6(–8)–septatae, hyalinae, curvatae, guttulatae.

Etymology. In reference to the clasp-like setae on the ascomata. Ascomata 185–330(–350) μm diam, superficial, globose, solitary or grouped, hyaline to pale brown when immature, dark brown becoming black when mature, membranous, ostiolate, with setae that form around ostiole. Setae from surface of ascomata, up to 47 μm long and 10 μm wide, (0–)1(–2)–septate, dark brown, thick-walled, hook-shaped. Peridium up to 60 μm wide, vinaceous brown, comprising 4–5 lay-
**Figs. 7–16. Tubeufia claspisphaeria (from holotype).** 7. Ascomata on natural substratum. 8. Longitudinal section through an ascoma. 9. Setae. 10. A cluster of mature and immature asci. 11. Asci. 12–16. Ascospores. Pseudoparaphyses in Fig. 10 (arrowed). Bars: 7 = 250 μm; 8 = 50 μm; 9 = 25 μm; 10–11 = 30 μm; 12 = 15 μm; 13–16 = 10 μm. Figs. 8–10 and 12 mounted in water, other figures mounted in lactophenol.

Table I. *Tubefisia* species and their present synonyms (accepted name in bold)

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<tr>
<th>Taxa</th>
<th>Synonym</th>
<th>Notes</th>
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<tr>
<td><em>T. acaciae</em> Tilak &amp; S. B. Kale</td>
<td>None</td>
<td>Although not examined this taxon may not be a <em>Tubefisia</em> with its violet to red ascomata and multisepate ascospores</td>
</tr>
<tr>
<td><em>T. aciculospora</em> Katum. &amp; Y. Harada</td>
<td>None</td>
<td>This species is unusual as it has ascospores with pointed ends Katumoto and Harada (1979)</td>
</tr>
<tr>
<td><em>T. alpina</em> L. Holm &amp; Nogr Osek</td>
<td><em>Acanthostigmina longisporum</em></td>
<td>This was considered to be synonymous with <em>A. longisporum</em> by Réblova and Barr (2000)</td>
</tr>
<tr>
<td><em>T. asclepiadis</em> Bat. &amp; Garnier</td>
<td><em>Saccardomyces socius</em> Henn.</td>
<td>Rossman (1979)</td>
</tr>
<tr>
<td><em>T. aurantiella</em> (Penz. &amp; Sacc.) Rossman</td>
<td>(=<em>Calonectria aurantiella</em>)</td>
<td>Mentioned in Crane et al (1998), transferred from <em>Calonectria</em> (Rossman 1979)</td>
</tr>
<tr>
<td><em>T. clintonii</em> (Peck) M. E. Barr</td>
<td><em>Acanthostigma perpusillum</em></td>
<td>This was considered to be synonymous with <em>A. perpusillum</em> by Réblova and Barr (2000)</td>
</tr>
<tr>
<td><em>T. coccicola</em> (Ellis &amp; Everh.)</td>
<td><em>Podonectria coccicola</em> Petch</td>
<td>Rossman (1987)</td>
</tr>
<tr>
<td><em>T. cylindrothecia</em> (Seaver) Höhn.</td>
<td><em>Tubefisia paludosa</em></td>
<td>Synonymized by Rossman (1977)</td>
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<tr>
<td><em>T. dactylariae</em> Chang</td>
<td>None</td>
<td>Chang (2003)</td>
</tr>
<tr>
<td><em>T. eriodermae</em> Etayo</td>
<td>None</td>
<td>Etayo (2002)—possibly <em>Chaetosphaerulina</em></td>
</tr>
<tr>
<td><em>T. minutia</em> Munk</td>
<td><em>Herpotrichiella</em></td>
<td>Barr (1980)</td>
</tr>
<tr>
<td><em>T. nigrotuberculata</em> T. Hino &amp; Katun.</td>
<td><em>Herpotrichia nigrotuberculata</em></td>
<td>Pirozynski (1972)</td>
</tr>
<tr>
<td><em>T. pachythrix</em> (Rehm) Rossman</td>
<td>None</td>
<td>For discussion see Rossman (1979)</td>
</tr>
<tr>
<td><em>T. panamia</em> Etayo</td>
<td>None</td>
<td>Etayo (2002)—possibly <em>Chaetosphaerulina</em></td>
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<tr>
<td><em>T. parvula</em> Dennis</td>
<td>None</td>
<td>Drawn in Ellis and Ellis (1985)</td>
</tr>
<tr>
<td><em>T. pezizula</em> (Berk. &amp; M. A. Curtis) M. E. Barr</td>
<td><em>Thaxteriella pezizula</em></td>
<td>Type species of <em>Thaxteriella</em> (Sivanesan 1984, Barr 1980)</td>
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ers of thick-walled textura globulosa, outer cells brown-walled, inner cells with hyaline walls. Pseudo-paraphyses 1.5–2.5 μm wide, hypha-like, numerous, cellular, unbranched. Ascii (93–)105–120(–127) × (12–)14–16(–18) μm (x = 109 × 15.1 μm, n = 15), 8-spored, cylindric-clavate, bitunicate, with a small ocular chamber, persistently pedicellate. Ascospores (34–)45–60(–64) × 5–7(–8) μm (x = 49 × 5.9 μm, n = 35), fasciculate, elongate-fusiform, (3–)5–6(–8)-septate, hyaline, sometimes slightly curved, smooth-walled, guttulate, lacking appendages or sheaths.

Anamorph. Unknown.

Colonies on potato-dextrose agar dark green to black, 1 cm diam in 1 wk at room temperature (~28 C). Mycelium mostly immersed, aerial mycelium velvety to fluffy, no pigment diffusing into agar, not sporulating even after submergence overnight in a bubble chamber. Mycelium less dense at the outer edge than in center, with branching mycelial strands extending from edge of colony.

Substratum. Wood submerged in streams.

Known distribution. Bermuda, Brazil, Columbia, Europe, Hong Kong, India, Indonesia, Panama, Trinidad, U.S.A. and Venezuela.

Specimen examined. HONG KONG. Lantau Island, Trappist Monastery, small stream in forest, on wood partially submerged, 21 Apr 2002, K.D. Hyde (HKU[M] 17122; living culture HKUCC 9118).

The genus Tubeufia has included at least 40 epi-theats as listed in IndexFungorum (http://www.indexfungorum.org/Names/Names.asp). Many of these taxa have been transferred to other genera (Barr 1980, Crane et al 1998) as summarized in Table I. Our two species are typical of Tubeufia because the ascomata are initially pale and have either
a glabrous surface or only short setae (Fig. 24). In ascospore size and morphology, Tubeufia claspisphaeria is most similar to T. acaciae, T. pachythrix and T. stromaticola. It differs from these species in that it has a row of hook-like setae that form radially around the ostiole. The ascospores of T. claspisphaeria, T. pachythrix and T. stromaticola are elongate-fusiform, while those of T. acaciae are cylindrical or vermiform. Ascospores of T. claspisphaeria are wider than those of the other three species. Our collection of Tubeufia paludosa is similar to the description given by Barr (1980) but differs in that it has globose ascomata and mucilaginous pads at both ends of the ascospore.

Tubeufia species have rarely been reported from freshwater habitats (Shearer 1993) and are more commonly found on rotting vegetation (Barr 1980). Tubeufia paludosa previously has been reported from submerged grasses (Shearer 1993), T. cylindrothecia and T. palmarum from submerged wood (Ho et al 2001, Sivichai et al 2002). The anamorphs, however, commonly are reported from submerged freshwater litter (Ho et al 2002, Sivichai et al 2002).

ACKNOWLEDGMENTS

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DICHOTOMOUS KEY TO THE SPECIES OF TUBEUFIA

1. Lichenicolous fungi .......................................................... 2
   1. Not lichenicolous fungi .................................................. 3
   3. Ascomata orange-yellow to grayish-yellow, ascospores 40–50 × (3.5–4.5 μm, fusiform, 5–8-septate .... T. parvula
   4. Asospores with <10 septa ............................................. 4
   5. Ascomata shorter than 21 μm .......................................... 5
   6. Ascomata longer than 21 μm ......................................... 6
   7. Ascomata reddish-brown, ascospores 14–15 × 3.5–4 μm, oblong-elliptical, 3-septate ........ T. parvula
   8. Ascomata hyaline to pale yellow, ascospores (13–)14–18 × (3.5–)4–5(–7) μm, fusoid, 3-septate ... T. brevispina
   9. Ascomata up to 7 septa ............................................... 7
   10. Ascomata >7 septa ................................................... 11
   11. Ascomata narrower than 6 μm ..................................... 8
   12. Ascomata less than 3.5 μm wide .................................... 9
   13. Ascomata more than 3.5 μm wide ................................ 10
   14. Ascomata 65–75 × 2.5–3.5 μm, acicular, 4–7-septate ............... T. aciculospora
   15. Ascomata 66–150 × 2–3 μm, filiform, 5–7-septate ................... T. helicomyces
   16. Ascomata 40–57 × 4–5 μm, long-fusiform, multisepitate (4-celled according to Rehm 1907) .... T. pachythrix
   17. Ascomata 50–70 × 4–5 μm, long-fusoid, 5–7-septate ............... T. stromaticola
   18. Ascomata with a row of hook-like setae, form radially around the ostiole, ascospores (34–)45–60(–64) × 5–7(–8) μm, elongate-fusiform, (3–)5–6(–7)-septate ........ T. claspisphaeria
   19. Ascomata not as above ............................................ 12
   20. Ascospores 12–36 × 6–7 μm, fusiform, 7–8-septate ................ T. dactylariae
   21. Ascomata narrower than 6 μm .................................... 13
   22. Ascospores 30–40 × 5–6 μm, fusiform with subacute to round ends, 5–7(–9)-septate ........ T. palmarum
   23. Ascospores 45–66 × 3.5–5 μm, narrowly fusiform to cylindric, 7–9-septate ............... T. aurantiella
   24. Ascospores shorter than 70 μm .................................. 15
   25. Ascospores (70–)100–200(–230) × (2–)3.5–7(–8) μm, fusiform with acute ends, up to 35 septa .... T. paludosa
   26. Ascospores 40–55(–65) × (2.5–)3–5 μm, elongate clavate or fusoid, (5–)7–9(–13)-septate ....... T. cylindrothecia
   27. Ascospores (27–)30–52 × 2.5–3.5(–4.5) μm, elongate fusoid, often curved, (5–)7–10(–13)-septate ...... T. cerea


