

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 in *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 novel 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

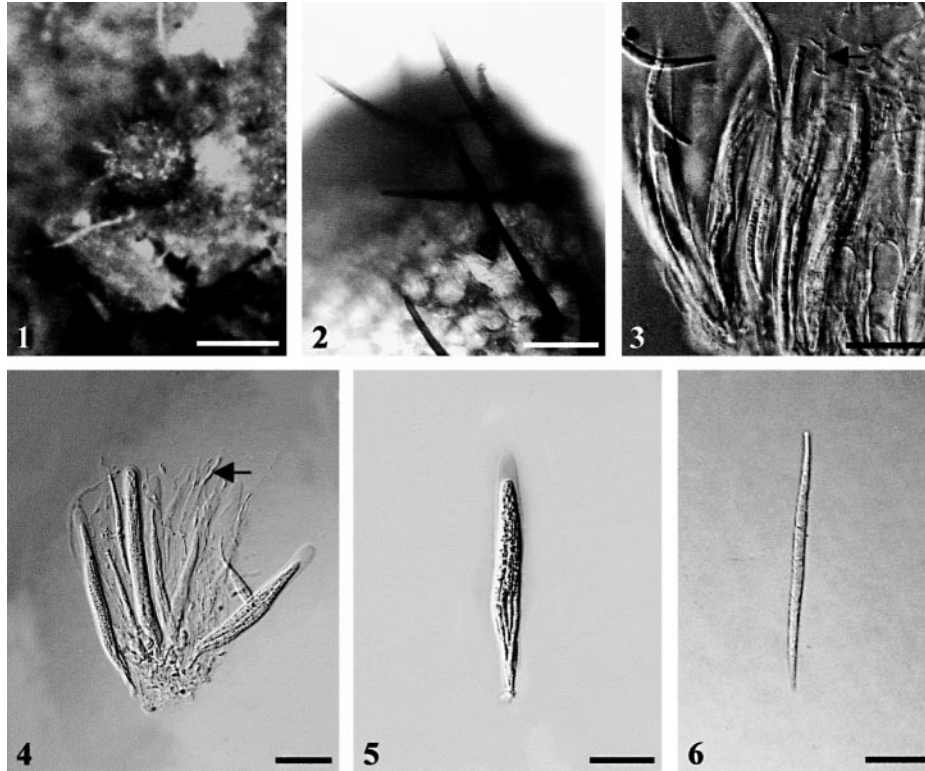
Submerged wood was retrieved from a stream near the Trappist Monastery, Lantau Island, Hong Kong, in Apr 2002 and returned to the laboratory, where it was incubated in zip-lock plastic bags at room temperature. Humidity was maintained by adding moistened paper towels. Samples were examined within 3 d 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, multiseptate and cylindrical-fusiform to elongate fusiform. The anamorphs are in *Helicosporium* and *Helicomycetes*. 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 (56–)80–90(–99) µm long, 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



FIGS. 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.

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

Anamorph. *Helicosporium aureum* (Corda) Linder, Ann. Missouri Bot. Gard. 16:279, 1929, from Réblová and Barr (2000).

Colonies on potato-dextrose agar olive-colored, reaching 1 cm diam in 10 d at room temperature (\sim 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

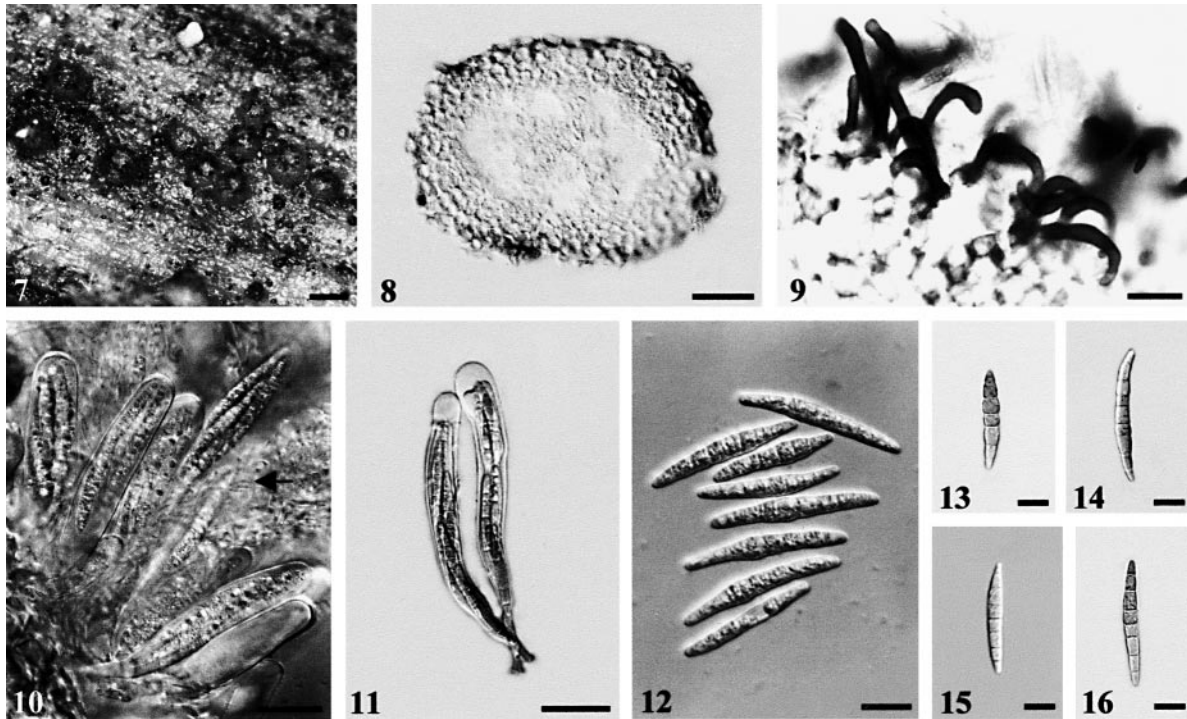
setae and wider ascospores 3–3.5(–4) μm versus (2–)2.5–3(–3.5) μm but in other aspects it is similar.

***Tubeufia claspisphaeria* Kodsueb, sp. nov.**

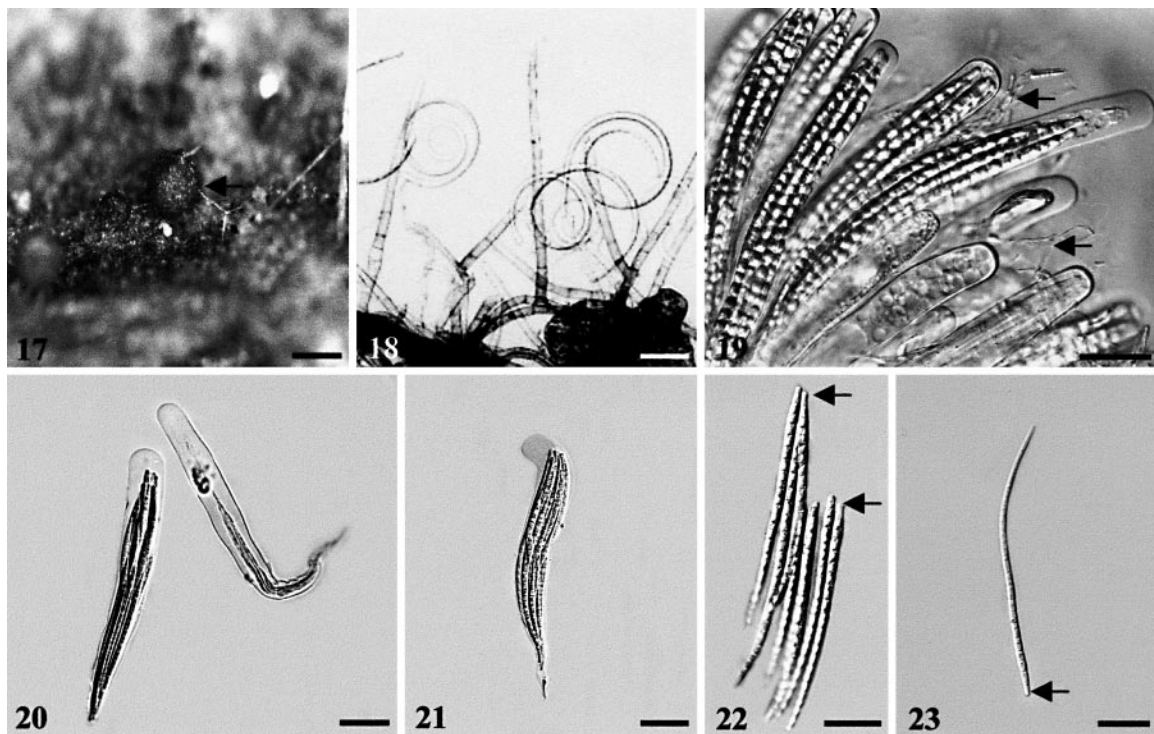
(FIGS. 7–16)

Ascomata 185–330(–350) μm diametro, superficialia, globosa, solitaria vel aggregata, hyalina vel pallide brunnea ubi immatura, atrobunnea vel atris ubi maturus, membranosa, ostiolata, setosa; setae usque 47 μm longa, 10 μm lata, (0–)1(–2)-septata, atrobunnea, hamatus. Periescomati usque 60 μm lata crassus, vinacea-brunnea, e cellulis pseudoparenchymatis compositum, 4–5 stratosus, textura globulosa. Asci (93–)105–120(–127) \times (12–)14–16(–18) μm , octospori, cylindrico-clavati, bitunicati, pedicellati. Ascospores (34–)45–60(–64) \times 5–7(–8) μm , fasciculatae, elongatae-fusiformis, (3–)5–6(–8)-septatae, hyalinae, curvatae, guttulate.

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.



FIGS. 17–23. *Tubeufia paludosa* (from HKU[M] 17122). 17. Ascomata on natural substratum (arrowed). 18. Anamorph (*Helicosporium* sp.). 19. A cluster of mature and immature asci. 20–21. Asci. 22–23. Ascospores. Pseudoparaphyses in FIG. 19 (arrowed), Mucilaginous pads in FIG. 22–23 (arrowed). Bars: 17 = 200 μm ; 18–21 = 30 μm ; 22–23 = 25 μm . FIGS. 18, 20–21 and 23 mounted in lactophenol, other figures mounted in water.

TABLE I. *Tubeufia* species and their present synonyms (accepted name in bold)

Taxa	Synonym	Notes
<i>T. acaciae</i> Tilak & S. B. Kale	None	Although not examined this taxon may not be a <i>Tubeufia</i> with its violet to red ascomata and multiseptate ascospores
<i>T. aciculospora</i> Katum. & Y. Harada	None	This species is unusual as it has ascospores with pointed ends Katumoto and Harada (1979)
<i>T. albo-ostiolata</i> Rossman	<i>Thaxteriella alboostiolata</i>	Transferred in Crane et al (1998)
<i>T. alpina</i> L. Holm & Nogrased	<i>Acanthostigmia longisporum</i>	This was considered to be synonymous with <i>A. longisporum</i> by Réblová and Barr (2000)
<i>T. amazonensis</i> Samuels, Rossman & E. Müll.	<i>Thaxteriella amazonensis</i>	Transferred in Crane et al (1998)
<i>T. anceps</i> Penz. & Sacc.	<i>Tubeufia paludosa</i>	Synonymized by Rossman (1977)
<i>T. asclepiadis</i> Bat. & Garnier	<i>Saccardomyces socius</i> Henn.	Rossman (1979)
<i>T. aurantiella</i> (Penz. & Sacc.) Rossman	Section Nectrioidea (= <i>Calonectria aurantiella</i>)	Mentioned in Crane et al (1998), transferred from <i>Calonectria</i> (Rossman 1979)
<i>T. brevispina</i> (M. E. Barr & Roger-son) J. L. Crane, Shearer & M. E. Barr	(= <i>Acanthostigmella brevispina</i>)	Crane et al (1998)
<i>T. cerea</i> (Berk. M. A. Curtis) Höhn.	Section Nectrioidea	Mentioned in Crane et al (1998), for description see Sivanesan (1984)
<i>T. clintonii</i> (Peck) M. E. Barr	<i>Acanthostigma perpusillum</i>	This was considered to be synonymous with <i>A. perpusillum</i> by Réblová and Barr (2000)
<i>T. coccicola</i> (Ellis & Everh.)	<i>Podonectria coccicola</i> Petch	Rossman (1987)
<i>T. coronata</i> Penz. & Sacc.	<i>Tubeufia paludosa</i>	Synonymized by Rossman (1977)
<i>T. corynespora</i> Munk	<i>Thaxteriella corynespora</i>	Transferred in Crane et al (1998)
<i>T. cylindrothecia</i> (Seaver) Höhn.	<i>Tubeufia paludosa</i>	Synonymized by Rossman (1977)
<i>T. dactylariae</i> Chang	None	Chang (2003)
<i>T. eriodermiae</i> Etayo	None	Etayo (2002)—possibly <i>Chaetosphaerulina</i>
<i>T. genuflexa</i> Höhn.	<i>Acanthostigmella genuflexa</i>	Barr (1977)
<i>T. hebridensis</i> Dennis	<i>Taphrophila hebridensis</i>	Réblová and Barr (2000)
<i>T. helicoma</i> (W. Phillips & Plowr.) Piroz.	<i>Thaxteriella helicoma</i>	Transferred in Crane et al (1998)
<i>T. helicomyces</i> Höhn.	<i>Tubeufia paludosa</i>	Synonymized by Rossman (1977)
<i>T. indica</i> (Dharne & E. Müll.) Deoray & Ujjainkar	<i>Thaxteriella indica</i>	Mentioned in Crane et al (1998)
<i>T. javanica</i> Penz. & Sacc.	<i>Tubeufia paludosa</i>	Synonymized by Rossman (1977)
<i>T. minuta</i> Munk	<i>Herpotrichiella</i>	Barr (1980)
<i>T. miscanthi</i> W. H. Hsieh, C. Y. Chen & Sivan.	<i>Taphrophila miscanthi</i>	Réblová and Barr (2000)
<i>T. nigrotuberculata</i> T. Hino & Katum.	<i>Herpotrichia nigrotuberculata</i>	Pirozynski (1972)
<i>T. ovatum</i> Rossman	<i>Thaxteriella ovata</i>	Transferred in Crane et al (1998)
<i>T. pachytrix</i> (Rehm) Rossman	None	For discussion see Rossman (1979)
<i>T. palmarum</i> (Torrend) Samuels, Rossman & E. Müll.	Section Nectrioidea	Samuels et al (1979); mentioned in Crane et al (1998)
<i>T. paludosa</i> (P. Crouan & H. Crouan) Rossman	Section Tubeufia	Samuels et al (1979); mentioned in Crane et al (1998)
<i>T. pannariae</i> Etayo	None	Etayo (2002)—possibly <i>Chaetosphaerulina</i>
<i>T. parvula</i> Dennis	None	Drawn in Ellis and Ellis (1985)
<i>T. pezizula</i> (Berk. & M. A. Curtis) M. E. Barr	<i>Thaxteriella pezizula</i>	Type species of <i>Thaxteriella</i> (Sivanesan 1984, Barr 1980)
<i>T. roraimensis</i> (Samuels & E. Müll.)	<i>Thaxteriella roraimensis</i>	Samuels and Müller (1978); also mentioned in Crane et al (1998)

TABLE I. Continued

Taxa	Synonym	Notes
<i>T. rugosa</i> C. Booth	<i>Tubeufia helicoma</i>	Barr (1980)
<i>T. scopula</i> (Cooke & Peck), M. E. Barr	<i>Acanthostigma scopulum</i>	Barr (1980), Réblová and Barr (2000)
<i>T. setosa</i> Sivan. & W. H. Hsieh	<i>Acanthostigma minutum</i>	Réblová and Barr (2000)
<i>T. stromaticola</i> (Henn.) Rossman	Transferred from <i>Calonectria</i>	Rossman (1979)
<i>T. trichella</i> (Sacc., E. Bommer & M. Rousseau) Scheuer	<i>Taphrophila trichela</i>	Réblová and Barr (2000)
<i>T. trichospora</i> (Berk. & Broome) Petch	<i>Ophionectria trichospora</i>	Rossman (1977)
<i>T. vermicularispora</i> (T. Hino & Katum.) Sivan.	<i>Chaetosphaerulina vermicularispora</i>	This has black carbonaceous ascomata and is probably not a <i>Tubeufia</i> . It was described as a <i>Chaetosphaerulina</i> (Hino and Katumoto 1954)
<i>T. yasudae</i> (T. Hino) Sivan.	<i>Chaetosphaerulina yasudae</i>	Mentioned in Crane et al (1998)

ers of thick-walled textura globulosa, outer cells brown-walled, inner cells with hyaline walls. Pseudoparaphyses 1.5–2.5 μm wide, hypha-like, numerous, cellular, unbranched. Asci (93–)105–120(–127) \times (12–)14–16(–18) μm (\bar{x} = 109 \times 15.1 μm , n = 15), 8-spored, cylindric-clavate, bitunicate, with a small ocular chamber, persistently pedicellate. Ascospores (34–)45–60(–64) \times 5–7(–8) μm (\bar{x} = 49 \times 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. Hong Kong.

Holotype. HONG KONG. Lantau Island, Trappist Monastery, small stream in forest, on wood partially submerged, 21 Apr 2002, *K.D. Hyde* (HKU[M] 17123). Living cultures ex holotype HKUCC 9116, HKUCC 9119.

Tubeufia paludosa (P. Crouan & H. Crouan) Rossman, *Mycologia* 69:383 (1977) (FIGS. 17–23)

Ascomata 185–350 μm diam, superficial, globose, brown to dark-brown, solitary, sparse, surface roughened, membranous. Asci (162–)170–180 \times (14–)15–

18(–19) μm (\bar{x} = 170 \times 16.1 μm ; n = 17), numerous, 8-spored, cylindric-oblong, bitunicate, hyaline, with a subapical ring, rounded at apex, persistently pedicellate, pseudoparaphyses. Ascospores (77–)120–135(–155) \times 4–5(–6) μm (\bar{x} = 127 \times 4.3 μm , n = 30), fasciculate, narrowly elongate, cylindric or filiform, hyaline, (18–)20–25-septate, straight or slightly curved, smooth-walled, guttulate, with small mucilaginous pads at each end.

Anamorph. *Helicosporium* sp.

Colonies on potato-dextrose agar 3.5 cm diam in 3 wk. Mycelium mostly immersed, no pigment diffusing into agar, hyphae less dense in outer zone, sporulating, forming a brownish-gray turf of upright setiform conidiophores, becoming ochraceous or brownish with age; conidiogenous cells produced laterally as thin-walled pegs. Conidia coiled two or three times, multiseptate, coils 37–86.4 μm diam, cells 4.6–5.4 μm wide.

Substratum. Wood submerged in streams, decaying woody fruit, palm fruit peduncle and bamboo.

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 epithets 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

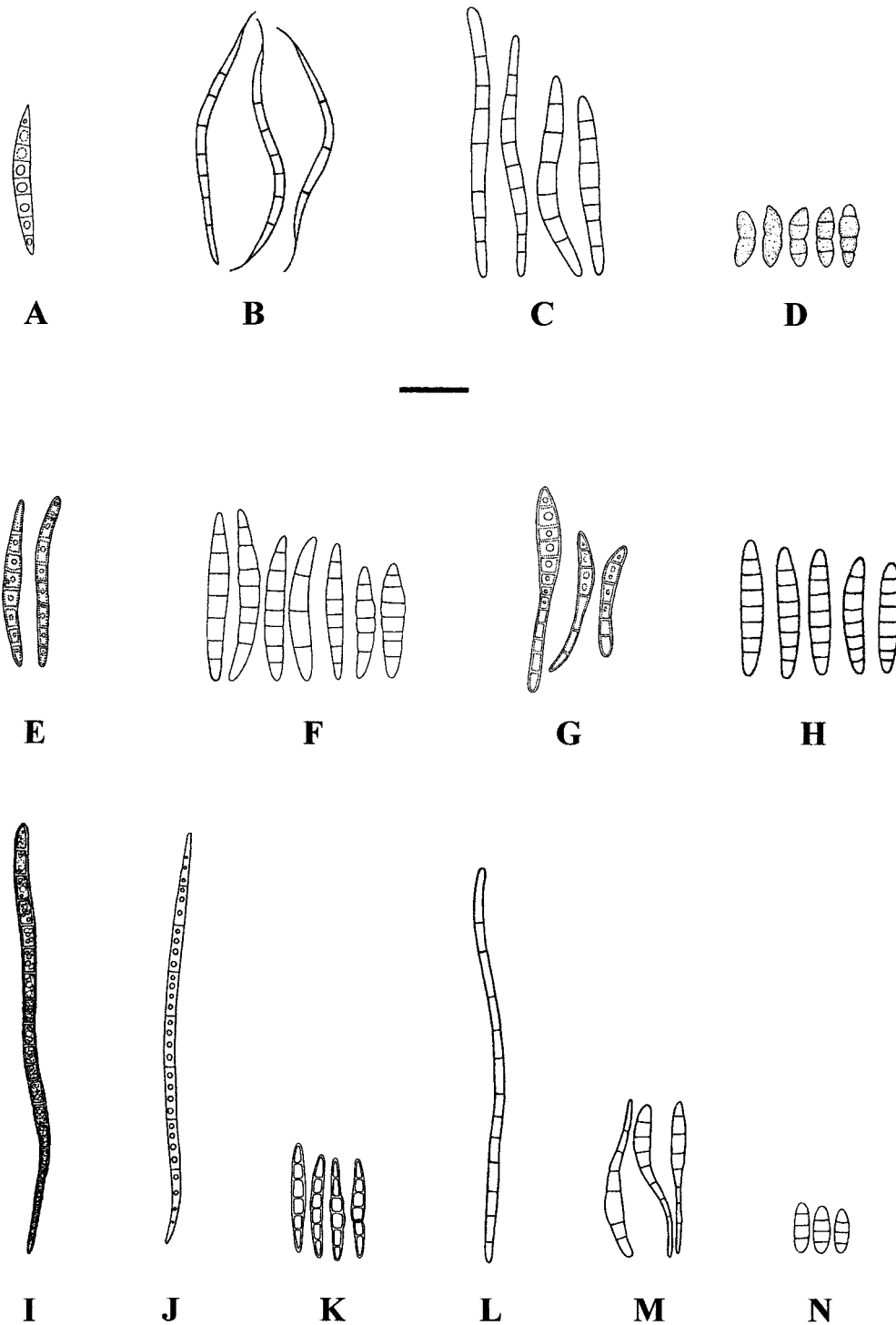


FIG. 24. Diagrammatic representation of ascospores of *Tubeufia* species drawn to the same scale for comparison. A. *T. acaciae* (from Tilak and Kale 1970). B. *T. aciculospora* (from Katumoto and Harada 1979). C. *T. aurantiella* (from Rossman 1987). D. *T. brevispina* (from Barr and Rogerson 1983). E. *T. cerea* (from Barr 1980). F. *T. claspisphaeria* (this paper). G. *T. cylindrothecia* (from Barr 1980). H. *T. dactylariae* (from Chang 2003). I. *T. eriodermiae* (from Etayo 2002). J. *T. helicomyces* (from Ellis and Ellis 1985). K. *T. palmarum* (from Samuels et al 1979). L. *T. paludosa* (from Sivanesan 1984). M. *T. pannariae* (from Etayo 2002). N. *T. parvula* (from Ellis and Ellis 1985). Bar = 20 μm .

a glabrous surface or only short setae (FIG. 24). In ascospore size and morphology *Tubeufia claspisphaeria* is most similar to *T. acaciae*, *T. pachythrinx* 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. pachythrinx* 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).

DICHOTOMOUS KEY TO THE SPECIES OF *TUBEUFIA*

1. Lichenicolous fungi 2
1. Not lichenicolous fungi 3
 2. Ascomata white, ascospores $105\text{--}145 \times 4\text{--}5 \mu\text{m}$, acicular, 17–19-septate *T. eriodermiae*
 2. Ascomata orange-yellow to grayish-yellow, ascospores $40\text{--}50 \times (3.5\text{--}4.5 \mu\text{m})$, fusiform, 5–8-septate *T. pannariae*
3. Ascospores with <10 septa 4
3. Ascospores with >10 septa 14
 4. Ascospores shorter than $21 \mu\text{m}$ 5
 4. Ascospores longer than $21 \mu\text{m}$ 6
5. Ascomata reddish-brown, ascospores $14\text{--}15 \times 3.5\text{--}4 \mu\text{m}$, oblong-elliptical, 3-septate *T. parvula*
5. Ascomata hyaline to pale yellow, ascospores $(13\text{--})14\text{--}18\text{--}(21) \times (3.5\text{--})4\text{--}5\text{--}(7) \mu\text{m}$, fusoid, 3-septate *T. brevispina*
 6. Ascospores up to 7 septa 7
 6. Ascospores >7 septa 11
7. Ascospore $48\text{--}56 \times 6\text{--}7 \mu\text{m}$, cylindrical or worm-like, often curved, pointed of both ends, 5–7-septate *T. acaciae*
7. Ascospores narrower than $6 \mu\text{m}$ 8
 8. Ascospores less than $3.5 \mu\text{m}$ wide 9
 8. Ascospores more than $3.5 \mu\text{m}$ wide 10
9. Ascospores $65\text{--}75 \times 2.5\text{--}3.5 \mu\text{m}$, acicular, 4–7-septate *T. aciculospora*
9. Ascospores $66\text{--}150 \times 2\text{--}3 \mu\text{m}$, filiform, 5–7-septate *T. helicomyces*
10. Ascospores $40\text{--}57 \times 4\text{--}5 \mu\text{m}$, long-fusiform, multiseptate (4-celled according to Rehm 1907) *T. pachythrinx*
10. Ascospores $50\text{--}70 \times 4\text{--}5 \mu\text{m}$, long-fusoid, 5–7-septate *T. stromaticola*
11. Ascomata with a row of hook-like setae, form radially around the ostiole, ascospores $(34\text{--})45\text{--}60\text{--}(64) \times 5\text{--}7\text{--}(8) \mu\text{m}$, elongate-fusiform, (3–)5–6(–7)-septate *T. claspisphaeria*
11. Ascomata not as above 12
 12. Ascospores $32\text{--}36 \times 6\text{--}7 \mu\text{m}$, fusiform, 7–8-septate *T. dactylariae*
 12. Ascospores narrower than $6 \mu\text{m}$ 13
13. Ascospores $30\text{--}40\text{--}(50) \times 3\text{--}4 \mu\text{m}$, fusiform with subacute to round ends, 5–7(–9)-septate *T. palmarum*
13. Ascospores $45\text{--}66 \times 3.5\text{--}5 \mu\text{m}$, narrowly fusiform to cylindric, 7–9-septate *T. aurantiella*
 14. Ascospores shorter than $70 \mu\text{m}$ 15
 14. Ascospores $(70\text{--})100\text{--}200\text{--}(230) \times (2\text{--})3.5\text{--}7\text{--}(8) \mu\text{m}$, fusiform with acute ends, up to 35 septa *T. paludosa*
15. Ascospores $40\text{--}55\text{--}(65) \times (2.5\text{--})3\text{--}5 \mu\text{m}$, elongate clavate or fusoid, (5–)7–9(–13)-septate *T. cylindrothecia*
15. Ascospores $(27\text{--})30\text{--}52 \times 2.5\text{--}3.5\text{--}(4.5) \mu\text{m}$, elongate fusoid, often curved, (5–)7–10(–13)-septate *T. cerea*

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