Torrentispora fibrosa gen. sp. nov. (*Annulatascaceae*) from freshwater habitats

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Torrentispora fibrosa gen. sp. nov. (*Ascomycota, Annulatascaceae*) is described based on specimens from submerged wood collected from streams in Tai Po Kau Forest Reserve, Hong Kong. *T. fibrosa* is characterized by immersed to superficial ascomata with a peridium of black cells arranged in irregular rows, wide septate paraphyses, long cylindrical asci with a relatively massive refractive apical ring, and unicellular ascospores with a fibrillar sheath. Illustrations from light and scanning electron microscopy are provided. It is compared with species in the genus *Annulatascus*, from which it differs in ascoma peridium and ascospore sheath morphology, and with other aquatic ascomycetes possessing ascospores with a similar fibrillar sheath structure.

INTRODUCTION

In a continuing study of freshwater fungi in the tropics (Hyde 1992, Ho, Hyde & Hodgkiss 1997, Goh, Hyde & Ho 1998, Wong, Hyde & Jones 1999a), an *Annulatascus*-like taxon was commonly collected which could not be accommodated in any existing genus of the *Annulatascaceae* (Wong, Hyde & Jones 1998, 1999a, Wong & Hyde 1999). It was similar to *Annulatascus velatisporus*, in having immersed beaked ascomata with dark walls, wide tapering paraphyses, long cylindrical asci with relatively massive refractive apical rings, and hyaline unicellular ascospores arranged uniseriately in the asci. It differed, however, in having a peridium, in surface view, comprising black, thick-walled cylindrical cells, arranged in irregular rows, in having asci with a much greater length to width ratio ($> 25:1$), and smaller ascospores (< 20 µm long) which produced a distinct fibrillar sheath. Although there are similarities with species of *Annulatascus*, for example the relatively massive refractive ascus apical ring, this new taxon cannot be accommodated in *Annulatascus* because of the above differences. In particular, it is considered that the different sheath and peridium structure warrant the introduction of a new genus (Hyde, Wong & Jones 1998b). The new genus and species *Torrentispora fibrosa* is, therefore, introduced to accommodate this taxon. It is compared with species of *Annulatascus* and with other aquatic ascomycetes possessing ascospores with a similar type of fibrillar sheath.

MATERIALS AND METHODS

Submerged wood collected from various streams in Hong Kong was returned to the laboratory, incubated in plastic boxes lined with damp tissue paper and examined periodically for the presence of ascomata. Squash mounts of ascomata were prepared in water and all measurements are from specimens mounted thus. Scanning electron micrographs were made according to Ho, Hyde and Hodgkiss (1999a).

TAXONOMY

Torrentispora K. D. Hyde, W. H. Ho, E. B. G. Jones, K. M. Tsui & S. W. Wong, **gen. nov.**

Etym.: from the Latin *torrens* meaning torrent, in relation to the habitat.

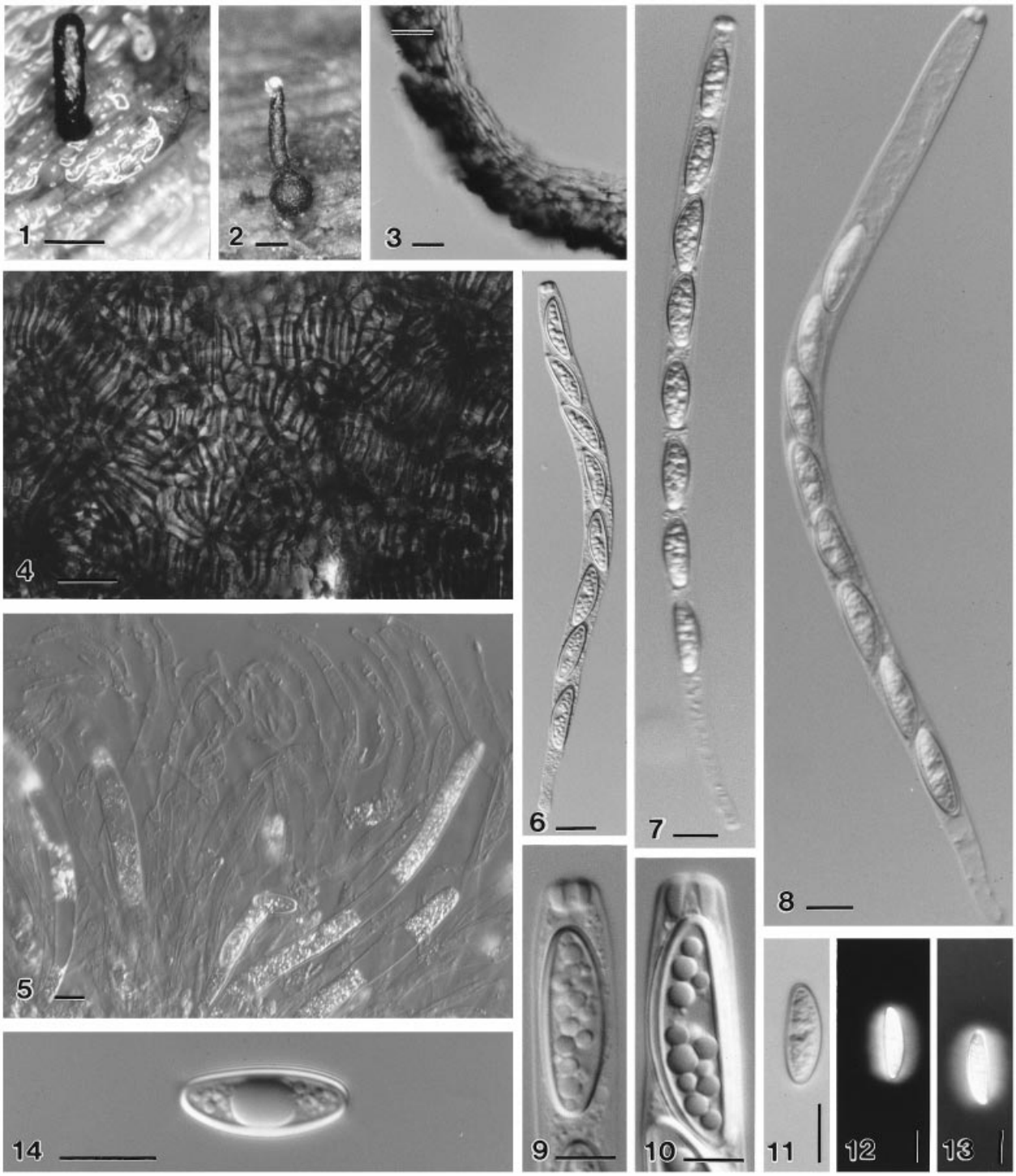
Ascomata globosa ad subglobosa, immersa ad superficialia, nigra, coriacea, papillata, ostiolata, paraphysata et solitaria. *Collum* longum et nigrum. *Asci* 8-spori, longe cylindrici, unitunicati, pedicellati, apparatu apicale praediti. *Ascosporae* uniseriatae, ovalis ad fusiformes, unicellulae, hyalinae, tunica fibrillae praeditae.

Typus: *Torrentispora fibrosa* K. D. Hyde, W. H. Ho, E. B. G. Jones, K. M. Tsui & S. W. Wong.

Ascomata globose or subglobose, immersed to superficial, black, coriaceous, papillate, ostiolate, paraphysate and solitary. *Neck* long and black. *Peridium* in section composed of an outer layer of dark-brown, angular cells, and an inner layer of hyaline, compressed cells; in surface view comprising black, thick-walled cylindrical cells, arranged in irregular rows. *Paraphyses* wide, septate and tapering distally. *Asci* 8-spored, long-cylindrical, unitunicate, pedicellate, with a relatively massive refractive apical ring. *Ascospores* uniseriate, oval to fusiform, occasionally flattened on one side, unicellular, hyaline, surrounded by a narrow fibrillar sheath (observed with SEM).

Torrentispora fibrosa K. D. Hyde, W. H. Ho, E. B. G. Jones, K. M. Tsui & S. W. Wong, **sp. nov.** (Figs 1–16).

Etym.: from the Latin *fibrosa* meaning fibrillar, in relation to the appearance of the ascospore sheath.

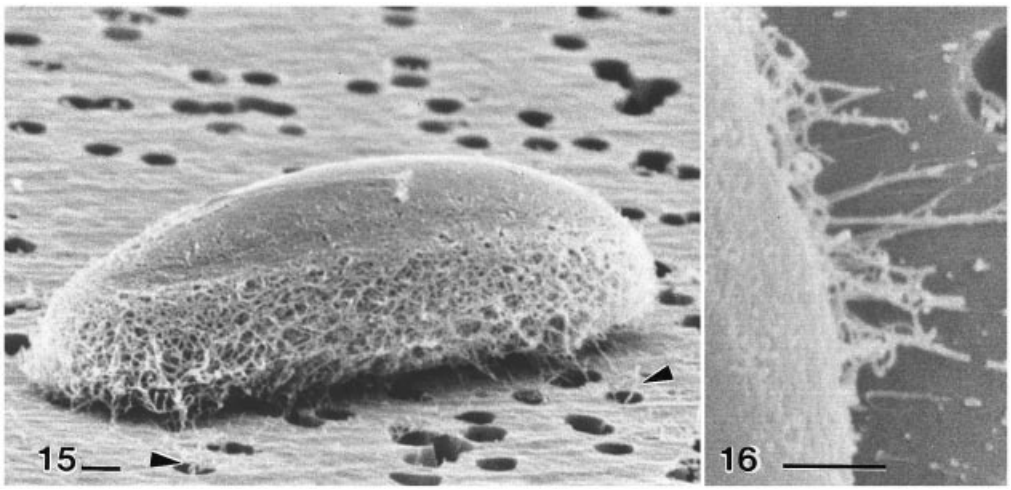


Figs 1–14. *Torrentispora fibrosa*. Interference contrast micrographs. **Fig. 1.** Immersed ascoma with a long, black neck. **Fig. 2.** Superficial ascoma. **Fig. 3.** Section of peridium comprising an outer layer of black, thick-walled, angular cells, and an inner hyaline compressed cells. **Fig. 4.** Surface view of peridium. Note the arrangement of the black, thick-walled, cylindrical cells. **Fig. 5.** Wide, hyaline and septate paraphyses and young asci. **Figs 6–8.** Long cylindrical asci with uniseriate ascospores. Note the relatively massive refractive apical rings. **Figs 9–10.** Higher magnification of apical rings. **Figs 11–14.** Hyaline and non-septate ascospores. Note that the sheath is indistinct, except when India Ink is added (Figs 12–13). Bars: 1–2 = 100 μm; 3–8, 11–14 = 10 μm; 9–10 = 5 μm.

Ascomata 135–255 μm diam, globosa ad subglobosa, immersa ad superficialia, nigra, coriacea, papillata, ostiolata, paraphysata et solitaria. *Collum* longum et nigrum. *Asci* 154–254 × 6–9 μm, 8-spori, longe cylindrici, pedicellati, apparatu apicale 2.5 μm longi, 4.5 μm diam praediti. *Ascosporae* 13.5–19.5 × 5–7 μm, uniseriatae, ovalis ad fusiformes, hyalinae, unicellulae, tunica fibrillae praeditae.

Typus: **Hong Kong**: New Territories, Tai Po Kau Forest Reserve, Tai Po Kau Forest Stream, in ligno putredinis submersis, 10 Dec. 1995, *W. H. Ho* (HKU(M) 4519–holotypus).

Ascomata 135–255 μm diam, globose to subglobose, immersed or superficial, black, coriaceous, papillate, ostiolate,



Figs 15–16. *Torrentispora fibrosa*. Scanning electron micrographs. **Fig. 15.** Ascospore with a thin fibrillar sheath. Note the fibrillar residues (arrowed) on the polycarbonate membrane near to the ascospores. **Fig. 16.** Fibrillar material radiating from the ascospore wall. Bars = 1 μm.

Table 1. Comparison of some important characteristics in *T. fibrosa* and *Annulatascus* species.

	T. fibrosa	*A. aquaticus*	*A. hongkongensis*	*A. biatriisporus*	*A. palminetensis*	*A. triseptatus*	*A. velatisporus*
Ascus range (μm)	154–254 × 6–9	150–175 × 10–12	250–275 × 13–16	210–260 × 12–17	98–142 × 7–10.5	140–218 × 9–14	220–290 × 12–18
Ascus length : width	25–28 : 1	14–15 : 1	17–19 : 1	15–17 : 1	13–14 : 1	15–16 : 1	16–18 : 1
Ascospore range (μm)	13.5–19.5 × 5–7	19–24 × 6–7	35–37.5 × 12.5–15	40–58 × 8–10	20–26 × 6–7	18–33 × 6–12	26–42 × 9–12
Verruculose wall ornamentation	Not seen	Sparse	Numerous	Numerous	Not resolved	Sparse	Numerous
Fine structure of sheath under SEM	Thick layer of exosporial fibrillar material without gel matrix	—	Thin network of exosporial fibrillar material, gel matrix dissolved	—	—	Gel matrix	Gel matrix
Fine structure of sheath under TEM	—	Exosporial fibrillar material embedded in a gel matrix	Exosporial fibrillar material embedded in a gel matrix	Sheath absent	—	Exosporial fibrillar material embedded in a gel matrix	Exosporial fibrillar material embedded in a gel matrix
Reference	This paper	Ho *et al.* (1999a)	Ho *et al.* (1999b)	Hyde (1995), Ho *et al.* (unpubl.)	Hyde, Goh & Steinke (1998a)	Wong *et al.* (1999b)	Hyde (1992), Wong *et al.* (1999b)

— = Data not available.

solitary (Fig. 2). *Neck* long, black, periphysate (Fig. 1). *Peridium* to 21.5 μm wide, in vertical section composed of an outer layer of dark-brown, angular cells, heavily pigmented and amorphous at the periphery, and an inner layer of hyaline, compressed cells (Fig. 3); in surface view comprising black, thick-walled cylindrical cells, arranged in irregular rows (Fig. 4). *Paraphyses* to 5.5 μm wide, filamentous, numerous, septate, slightly constricted at the septa, and tapering distally (Fig. 5). *Asci* 154–254 × 6–9 μm ($\bar{x}$ = 184 × 8 μm, n = 25), 8-spored, long cylindrical, pedicellate, thin-walled, with a relatively massive refractive apical ring, ca 2.5 μm long and 4.5 μm wide (Figs 6–10). *Ascospores* 13.5–19.5 × 5–7 μm ($\bar{x}$ = 17 × 6 μm, n = 50), uniseriate, oval to fusiform, occasionally flattened on one side, hyaline, unicellular, thick-walled, surrounded by a narrow fibrillar sheath (visible in India Ink and with the SEM) (Figs 11–16).

Ecology: Saprobic on submerged wood in streams.
Distribution: Hong Kong.

Other material examined: **Hong Kong**: New Territories, Tai Po, Lam Tsuen River, on submerged decaying wood, 1 Oct. 1997, *K. M Tsui* (HKU(M) 8093); *ibid.* (HKU(M) 8107); Tai Po Kau Forest Reserve, Tai Po Kau Forest Stream, on submerged decaying wood, 27 Jun. 1996, *K. D. Hyde* (HKU(M) 4534); *ibid.* (HKU(M) 4538); *ibid.* 21 Sep. 1996, *W. H. Ho* & *K. M. Tsui* (HKU(M) 4818); *ibid.*, 20 Dec. 1996, *W. H. Ho* & *S. Y. Ho* (HKU(M) 5919); *ibid.* (HKU(M) 5932); *ibid.* 28 Jun. 1997, *W. H. Ho* (HKU(M) 6076); *ibid.* (HKU(M) 6084); *ibid.* (HKU(M) 6086); *ibid.* (HKU(M) 6089); *ibid.* (HKU(M) 6099); *ibid.* (HKU(M) 6101); *ibid.* (HKU(M) 6115); *ibid.* (HKU(M) 6118); *ibid.* 28 Sep. 1997, *W. H. Ho* (HKU(M) 6167); *ibid.* (HKU(M) 6178); Shing Mun Reservoir, on submerged wood, 14 Jan. 1998, *K. M. Tsui* (HKU(M) 8129); *ibid.* (HKU(M) 8197).

SCANNING ELECTRON MICROSCOPY

Mature ascospores of *Torrentispora fibrosa* are ellipsoidal and surrounded by a thin layer of fibrillar material (Fig. 15). It appears as a network of condensed fibrillar material which radiates outwards from the ascospore wall, mostly occurring on the surface of the ascospore adjacent to the polycarbonate membrane (Figs 15, 16). The fibrillar material probably aids in the attachment of ascospores to the substratum surface, in this case the polycarbonate membrane.

DISCUSSION

The ascospores of *T. fibrosa* are similar to those found in species of *Annulatascus* (Hyde 1992, Wong *et al*. 1999b). The major difference between species of *Annulatascus* and *T. fibrosa* is that the peridium in *Torrentispora* comprises black, thick-walled cylindrical cells, arranged in irregular rows in surface view, while in species of *Annulatascus* it is *textura epidermoidea* (Hyde 1992). The ascospores in *T. fibrosa* are also relatively small (< 20 µm long) as compared to those in species of *Annulatascus* (> 20 µm long) (Table 1). The length to width ratio of asci in *T. fibrosa* is 25–28 : 1 while in *Annulatascus* species it is 13–19 : 1 (Table 1). The ascospores of *Annulatascus* species are surrounded by fibrillar exosporial sheath which is embedded in a gel matrix. This gel matrix may dissolve during the preparation procedures in SEM, as in *A. hongkongensis* where a thin mat of fibrillar network was observed under SEM (Ho, Hyde & Hodgkiss 1999b). In contrast, the ascospores of *T. fibrosa* are surrounded by a thick sheath which comprises dense exosporial fibrillar material (Table 1).

Ascospores with fibrillar sheaths are also found in several other genera of marine ascomycetes, for example *Appendichordella amicta* (Johnson, Jones & Moss 1987), *Carbosphaerella leptosphaerioides* (Johnson, Jones & Moss 1984, Hyde & Jones 1989, Moss 1990), and *Marinospora longissima* (Jones, Johnson & Moss 1983). As observed with the SEM, the ascospores of *Appendichordella amicta* differ from those in *T. fibrosa* in having thick coiled thread-like appendages which form loops around the ascospores (Johnson *et al*. 1987). *Carbosphaerella leptosphaerioides* has ascospores with a reticulate or net-like sheath which becomes fibrillar at maturation (Jones & Moss 1987). Sticky fibrillar material is only present on the immature ascospores of *Marinospora longissima*, but this disappears with maturity. *Nereiospora comata* and species of *Remispora* (e.g. *R. stellata*) have also been described as possessing ascospores with fibrillar appendages (Jones & Moss 1980, Jones *et al*. 1983). However, the ascospore appendages in these species are only restricted to the polar and/or equatorial regions. In addition, the appendages radiate from the ascospore wall.

The origin of the fibrillar material in these fungi varies. In *Appendichordella amicta* and species of *Nereiospora* the fibrillar material arises from the episporium and mesosporium respectively (Jones *et al*. 1983, Johnson *et al*. 1987, Jones 1995). In species of *Remispora*, fibrillar appendages are formed by fragmentation of the episporium, although the fibres are embedded in a matrix of unknown origin (Manimohan, Jones & Moss 1993a,b). In *Carbosphaerella leptosphaerioides*, the fibrillar sheath is derived by the fragmentation of an intricate exosporial sheath (Johnson *et al*. 1984). There does not appear to be any gel matrix associated with the fibrillar material of the ascospores of *T. fibrosa*.

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