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## Ultrastructure of the asci and ascospores of *Torrentispora fibrosa*

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The ascus apex and ascospores of *Torrentispora fibrosa* are illustrated at the ultrastructure level. *Torrentispora fibrosa* is typical of the *Annulatasceae* in having asci with a bilamellate wall and a bipartite apical ring with a plug. The ascospores differ from species of other genera in the family in being thick-walled and lacking verrucose wall ornamentations.

**Key words:** *Annulatasceae*, aquatic fungi, fine structure, ultrastructure.

### Introduction

Electron microscopy is widely used to investigate ascus and ascospore anatomy (Ho *et al.*, 1999a; Ho and Hyde, 2000, 2004) of freshwater fungi. New fungal taxa have been introduced based on characters seen only at the electron microscope level. Examples are members of the *Annulatasceae* (Hyde *et al.*, 1998, 1999; Wong and Hyde, 1998; Wong *et al.*, 1998, 1999).

*Torrentispora fibrosa* K.D. Hyde, W.H. Ho, E.B.G. Jones, K.M. Tsui & S.W. Wong was described and assigned to the *Annulatasceae* (Hyde *et al.*, 2000). This species occur on submerged wood in subtropical and tropical streams (Ho *et al.*, 1999c, 2002). The purpose of the present study was to investigate the ultrastructure of mature ascus apex and ascospores in order to determine if this taxon possesses distinctive characters that can only be resolved at the electron microscope level.

### Materials and methods

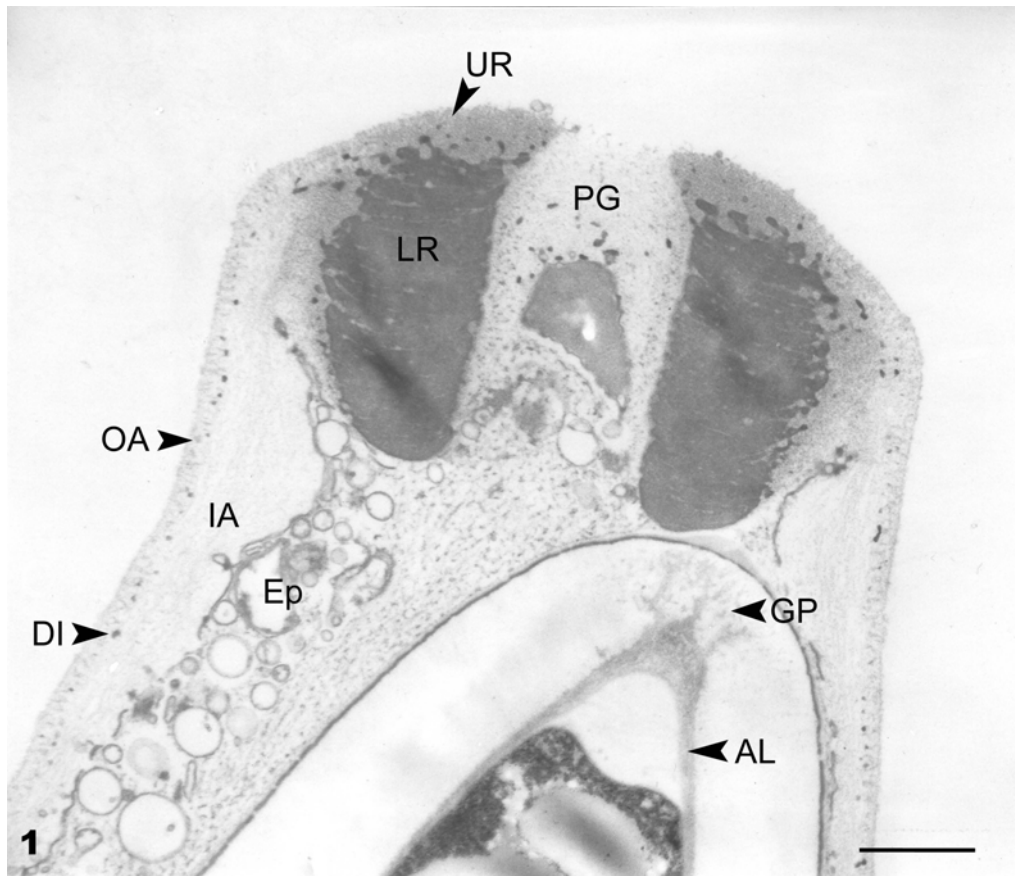
Material was obtained from wood submerged in Tai Po Kau Stream, Tai Po Kau Country Park, New Territories, Hong Kong. The methods used follow Ho *et al.* (1999b).

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## Results

The wall of a mature ascus was bilamellate comprising: (i) an outer, electron-dense layer (75-100 nm thick), containing tightly packed electron-dense fibrils orientated perpendicular to the ascus wall; and (ii) an inner, electron-translucent layer (100-200 nm thick), containing less electron-dense fibrillar material orientated parallel to the ascus wall (Fig. 1). Electron-dense inclusions (20-36 nm diam) occurred at the interface between the outer and the inner wall layers of the ascus (Fig. 1). The inner layer of the ascus wall was thickened (500-700 nm) near the base of the apical ring (Fig. 1). The outer layer of the ascus wall was absent at the opening of the apical ring (Fig. 1).



**Fig. 1.** Longitudinal median section of a mature ascus of *Torrentispora fibrosa*. Abbreviations: AL = additional layer of ascospore wall, DI = electron-dense inclusions, Ep = epiplasm, GP = germ pore, IA = inner ascus wall layer, LR = lower region of ascus apical ring, m = mesosporium, OA = outer ascus wall layer, PG = plug of ascus apical ring, UR = upper region of ascus apical ring. Bar = 1  $\mu$ m.

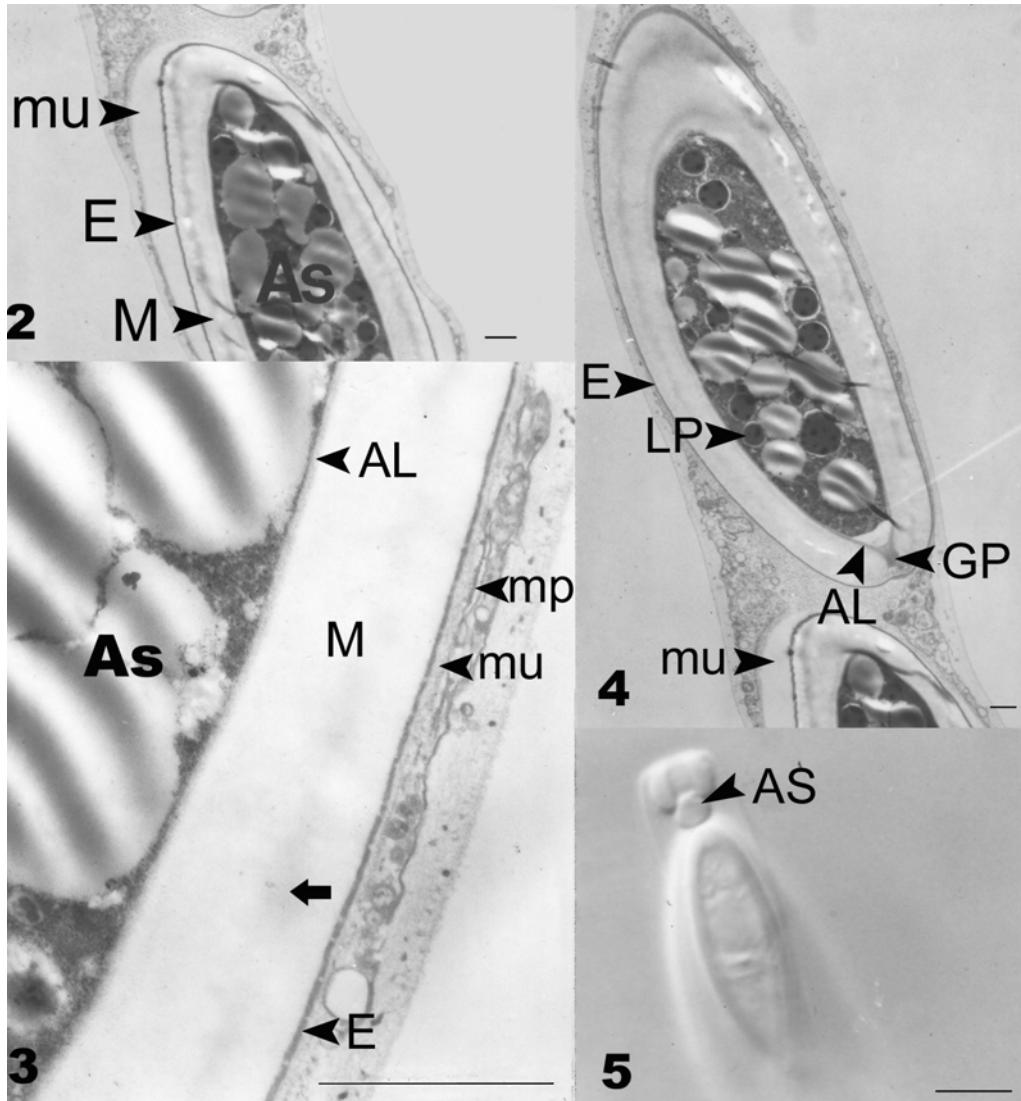
The ascus apex contained a bipartite ring (3  $\mu\text{m}$  long, 5  $\mu\text{m}$  diam.) comprising: (i) an upper region which was less electron-dense, fibrillar and appeared differentiated from the inner layer of the ascus wall; (ii) a lower region which was more electron-dense, with highly compact electron-dense granules (Fig. 1). Dispersed electron-dense granules were observed at the interface between the upper and the lower regions (Fig. 1). The granules became highly compacted to form the lower region of the apical ring (Fig. 1). Both the upper and the lower regions of the ascus ring were continuous with the inner ascus wall layer (Fig. 1). An electron-translucent apical plug (ca. 1  $\mu\text{m}$  diam.) was found at the top of the channel of the apical ring (Fig. 1). Some electron-dense deposits were present within the plug.

Mature ascospores were thick-walled, ellipsoidal to fusiform, and contained numerous lipid globules (Figs. 2-4). The ascospore wall comprised two distinct parts: (i) an outer, 20 nm thick, electron-dense episporium; and (ii) an inner, 650-850 nm thick electron-translucent mesosporium (Fig. 3). The mesosporium appeared to comprise two layers (Fig. 3). In addition, a 50-100 nm electron-dense layer appeared to line the inner surface of the mesosporium and this additional layer, possibly the endosporium, also lined the germ pores (Figs. 3, 4). One germ pore was observed at the polar end of some ascospores (Fig. 3). Some ascospores were surrounded by electron-transparent mucilage-like matrix (100-700 nm) which appeared to be bounded by a slightly folded membrane profile when the ascospores were still inside the ascus (Fig. 3). No episporial verrucose ornamentation was observed. Organelles were not shown clearly which may have been due to prolonged oxidation using osmium tetroxide.

## Discussion

Asci of *Torrentispora* were found to have a bilamellate wall layer at TEM level. This is similar to that found in *Pseudoproboscispora* (Wong and Hyde, 1998) and other taxa in the *Annulatasceae* (Ho and Hyde, 2000).

New observations on the ascospores at the TEM level include the polar germ pores, an additional wall layer inside the mesosporium and the electron-transparent mucilage-like matrix surrounding the ascospores. Germ pores were not observed at the light microscopic level and were possibly occluded by the mucilage layer surrounding the ascospores or were too small for observation. Germ pores are important features for germination in ascospores with thick walls and are found in other thick-walled taxa (e.g. *Caryospora* sp.). The additional wall layer has been observed in ascospores *Annulatascus biatriisporus* K.D. Hyde, which also has thick walls, but is not known in other



**Figs. 2-5.** Oblique longitudinal sections of asci of *Torrentispora fibrosa*. **2.** Thick-walled ascospores. **3.** Larger magnification of ascospore wall. The mesosporium appears to be bilamellate (arrowed). **4.** Ascospore with a polar germ pore. **5.** Light micrographs of ascus of *Torrentispora fibrosa*. The ascus has a large apical ring. Abbreviations: AL = additional layer of ascospore wall, As = Ascospore, E = episporium, GP = germ pore, LG = lipid globules, m = mesosporium, mp = membrane profile, mu = mucilage. Bars: 2-4 = 1  $\mu$ m; 5 = 10  $\mu$ m.

members of the *Annulatasceae*. The electron-transparent mucilage-like matrix surrounding the ascospores is clearly visible towards the end of the ascospores (Fig. 1, labelled Ep). A thinner layer surrounds the ascospores (Fig. 1, labelled Mu) and is bounded by a membrane. The mucilage layer most likely

corresponds to the fibrillar mucilage illustrated at the SEM level by Wong *et al.* (1998).

The mesosporium in *Torrentispora* is typical of other taxa in the *Annulatascaceae* comprising a thin, electron-dense episporium and a thicker, less electron-dense or electron-transparent mesosporium. The thickness and partition of the mesosporium however, varies among genera. *Cataractispora* (*ca.* 505 nm), *Torrentispora* (*ca.* 650-850 nm) and *Vertxicola* (*ca.* 404-660 nm) possess a relatively thicker mesosporium (Hyde *et al.*, 1999; Figs. 2-4; Ranghoo *et al.*, 2000, respectively). It has been shown in *Cataractispora* that the primary mesosporium is first formed, followed by a thick bilamellate secondary mesosporium that gives rise to a further tertiary layer (Hyde *et al.*, 1999).

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