
A new family of freshwater ascomycetes

W.H. Ho* and **Kevin D. Hyde**

Centre for Research in Fungal Diversity, Department of Ecology and Biodiversity, The University of Hong Kong, Pokfulam Road, Hong Kong; * e-mail: wellcomeho@hotmail.com

Ho, W.H. and Hyde, K.D. (2000). A new family of freshwater ascomycetes. *Fungal Diversity* 4: 21-36.

Studies of freshwater fungi over the last ten years have resulted in the discovery of numerous new ascomycetes with various sheath and appendage types. Many of the unitunicate representatives have asci with a relatively massive apical ring. Several species have been reexamined at the ultrastructural level and a new family, the *Annulatascaceae*, was introduced to accommodate these fungi. The ontogeny of ascospore and sheath/appendage development has also been found to differ between taxa and has shown the family to comprise several genera. The family placement near to the Sordariales has been confirmed by analysis of molecular data.

Key words: *Annulatascaceae*, freshwater fungi, molecular study, Sordariales, systematics, ultrastructure.

Introduction

Marine fungi are a fascinating group comprising mostly ascomycetes in the Halosphaeriales. Asci in these taxa generally deliquesce early, while the ascospores may have elaborate appendages thought to be functional in dispersal and subsequent attachment to new substrates in the sea (Hyde and Jones, 1988). Jones and coworkers have examined the ontogeny of the ascospores in many of these marine taxa and as a result have described 42 genera in the family *Halosphaeriaceae* (Jones, 1995). The genera are characterised based mainly on the way in which the appendages are formed (Jones, 1995).

Freshwater ecosystems offer a similar habitat to the sea and therefore one might expect that there could be similar fungi adapted for life in freshwater streams or lakes. The immersed aquatic habitat, flowing water, foam in waves and submerged woody debris would indicate such a possibility. Ingoldian fungi are a unique group adapted for life in streams and are abundant in freshwater, but they are mostly found on submerged leaves (Ingold, 1942; Goh and Hyde, 1996). The ascomycetes previously known from freshwater habitats did not appear to be specifically adapted for their aquatic lifestyle, in such a way as marine or Ingoldian fungi.

Examination of the literature in 1990 revealed the fact that few tropical

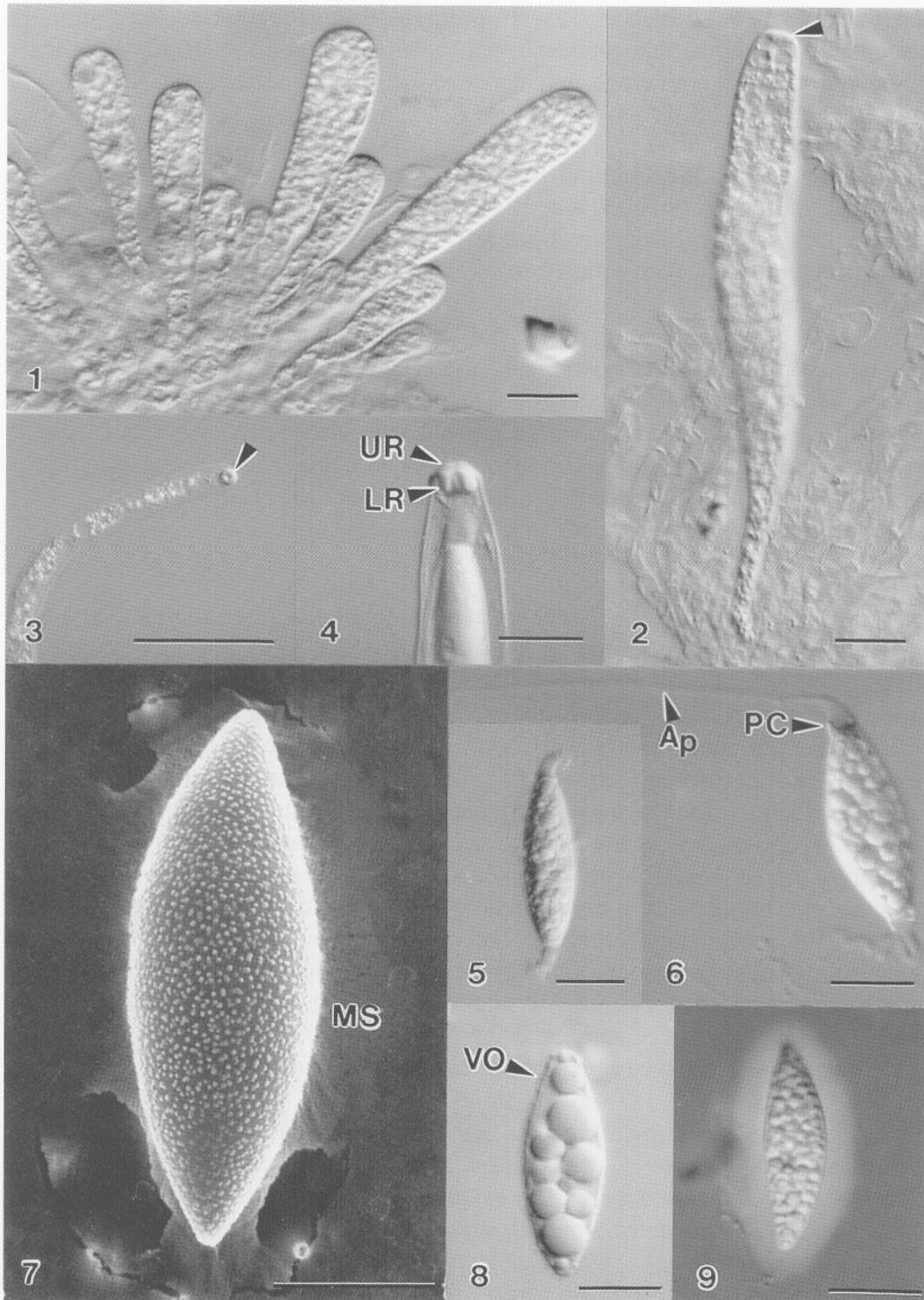
freshwater ascomycetes were known, as most studies had previously been carried out in temperate regions (Goh and Hyde, 1996). Most ascomycetes known from freshwater at that time, either had ascospores with simple sheaths or ascospores lacking external structures. Two of the temperate aquatic ascomycetes however, had ascospores with interesting sheaths or appendages. Ingold (1955) had described *Macrospora scirpicola* (D.C.: Fr.) Shoemaker and C.E. Babcock [as *Pleospora scirpicola* (D.C.) Karst.] and this beautiful taxon had ascospores with a massive spreading sheath. *Ceriospora caudae-suis* was also intriguing as it has unique appendages which had been beautifully illustrated by Ingold (1951). The appendages were somewhat similar to those found in *Aniptodera/Halosarpheia* species (Hyde *et al.*, 1999) but appeared to be more complex.

This small insight into the possible ascomycete spore type that may be present in streams lead to K.D. Hyde visiting Millaa Millaa falls in tropical north Queensland on the first day on 1990. While swimming he collected some submerged wood samples from the pool at the base of the waterfall. On examining these samples in the laboratory two days later, he found an unique and interesting taxon that he later described as *Annulatascus velatisporus* K.D. Hyde (Figs. 8, 9; Hyde, 1992). This led to intensive collections of freshwater ascomycetes over the following 10 years and the discovery of a previously unknown, yet unique group of freshwater fungi.

Annulatascus velatisporus was somewhat reminiscent of *Phomatospora* species in having unicellular ascospores and ascii with an apical ring. In *Phomatospora* species, however, the ascal ring is small and cylindrical, while in *Annulatascus* the ring was relatively massive (Hyde, 1992). The differences were too great to accommodate this taxon in *Phomatospora* and therefore Hyde (1992) described a new genus to accommodate it.

Subsequent collections of *Annulatascus*-like species revealed a wide assortment of similar fungi. These varied from those with brown spores and germ pores, to those with hyaline spores with sheaths, and various appendage types. These taxa were described in genera such as *Clohesia*, *Diluvicola*, *Frondicola* and *Submersisphaeria* (Hyde and Jones, 1988; Hyde, 1994, 1995,

Figs. 1-6. Light micrographs of *Cataractispora appendiculata*. **1.** Immature ascus without apical differentiation. **2.** Ascus with initial development of an apical ring (arrowed). **3.** Distorted apical ring (arrowed), illustrating the top view of the ring and the hole. **4.** Mature ascus with bipartite apical ring [comprising an upper region (UR) and a lower region (LR)]. **5.** Ascospore with intact bipolar appendages. **6.** Ascospore with appendages (Ap), extending from polar chambers (PC). **Fig. 7.** Scanning electron micrograph of an ascospore of *Annulatascus hongkongensis*, with verruculose surface and a mucilaginous sheath (MS). **Figs. 8, 9.** Light micrographs of *Annulatascus velatisporus*. **8.** Ascospore with verruculose ornamentations (VO). **9.** Ascospore in Indian ink. Note the mucilaginous sheath. Bars: 1, 2, 4-9 = 10 µm; 3 = 50 µm.



1996; Hyde *et al.*, 1997a, 1998). They were all aquatic species with similar ascomata, cylindrical-clavate ascospores with relatively massive refractive apical rings and various spore types, and appeared to form a related group.

Ultrastructural studies

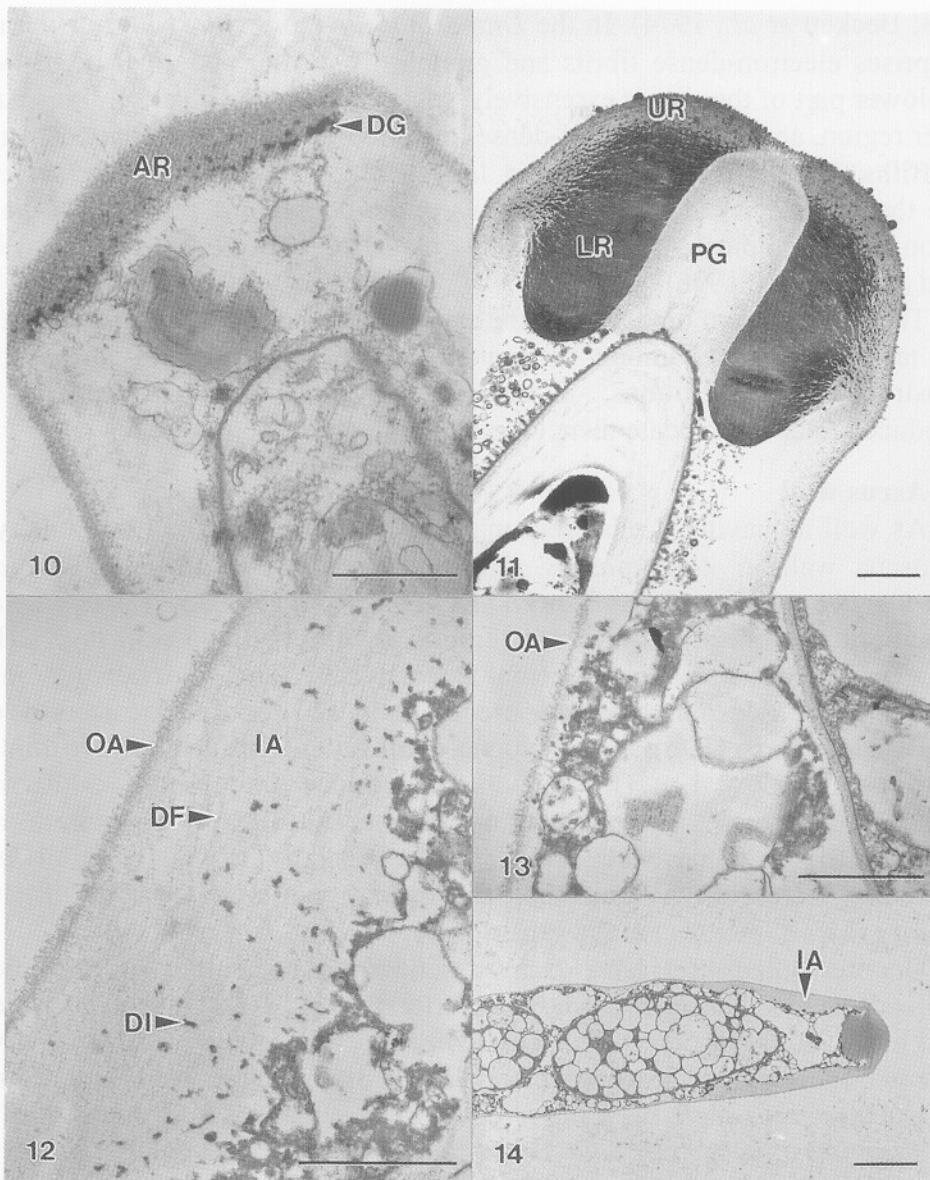
At this stage ultrastructural work was carried out on these *Annulatascus*-like taxa in order to establish whether they belong to a single genus or separate genera, and to establish which order would best accommodate the taxa. Ultrastructural work was initiated in 1993 in order to examine the morphology and ontogeny of ascospores. Fifteen species have been examined at this stage. This has resulted in the introduction of a new family, the *Annulatascaceae* based on amongst other characters, the bipartite ascospore ring (Fig. 4). The new genera *Cataractispora*, *Diluvicola*, *Fluminicola*, *Pseudoproboscispora* and *Torrentispora* have also been described based mainly upon the morphology and ontogeny of ascospores and their appendages or sheaths (Wong, 1997; Ho, 1998; Wong and Hyde, 1999).

Ascus structure and the Annulatascaceae

Ascus apical rings

One of the most distinctive characters of *Annulatascus* and related taxa are the relatively massive, refractive apical rings, which have a unique ontogeny (Figs. 1-4, 10, 11). In immature ascospores, the ascus walls are bilamellate at the apex, and similar in thickness to the periclinal wall. During early stages of ring formation, the inner ascus wall layer thickens at the apex and becomes electron-dense forming an apical plate. Some electron-dense granules accumulate at the base of the apical plate, except at central region (Fig. 10). As further electron-dense granules accumulate at the base of the apical plate, it becomes thicker, again with the exception of the central region. The plate extends proximally during ascospore maturation. At maturity, the inner ascus wall layer at the apex has differentiated into a massive bipartite ring with a thick, electron-dense upper region, and an extensively elongated lower region that is filled with compact electron-dense granules (Fig. 11). The cavity of the ring is filled with amorphous material and this is the plug (Fig. 11). The outer ascus wall layer is not involved in ring formation, and disintegrates at the apex (Fig. 11).

A bipartite ascus apical ring has been described in a number of unitunicate families, such as the *Clypeosphaeriaceae*, *Diaporthaceae*, *Diatrypaceae*, *Sordariaceae* and *Xylariaceae*. The apical rings in the *Clypeosphaeriaceae* and *Xylariaceae*, are however amyloid, and in the *Clypeosphaeriaceae* the ring comprises an upper region which is electron-dense, and a lower region which is amorphous and electron-translucent (Greenhalgh and Evans, 1967; Griffiths,



Figs. 10-14. Transmission electron micrographs of *Annulatascus* species. **10.** Median section of ascus of *A. incrassatus* with an apical ring (AR) at an early developmental stage. The layer of electron-dense granules (DG) at the base of the ring are less dense towards the centre. **11.** Mature ascus of *A. biatriisporus*. The apical ring is bipartite with upper (UR) and lower (LR) regions, and the ring is plugged (PG) with amorphous material. **12.** Immature ascus of *A. hongkongensis*. The wall is bilamellate, comprising thin outer (OA) and thick inner (IA) layers. Note the electron-dense fibrils (DF) and inclusions (DI) in the inner ascus wall layer. **13.** Mature ascus of *A. hongkongensis*. The inner ascus wall layer is absent and the outer layer (OA) is deliquescent. **14.** Ascus of *A. hongkongensis*. The inner wall layer (IA) disintegrated, except at the subapical region. Bars: 10-13 = 1 μm ; 14 = 5 μm .

1973; Beckett *et al.*, 1974). In the *Diaporthaceae* the upper region of the ring comprises electron-dense fibrils and granules as in the pericinal ascus wall. The lower part of the ring is extensively thickened, with an electron-transparent upper region, and small, electron-dense lower region, and lacks plug occlusions (Griffiths, 1973; Parguey-Leduc and Janex-Favre, 1980). In the *Sordariaceae* both the inner and outer ascus wall layers are continuous through the apical region, and the apical ring is relatively small (Reeves, 1971; Griffiths, 1973; Read and Beckett, 1996).

The anatomy and ontogeny of the ascus apical ring of these *Annulatascus*-like taxa are unique among the unitunicate ascomycetes. Based on these ultrastructural observations, a new family, the *Annulatascaceae*, was introduced to accommodate them (Wong *et al.*, 1998).

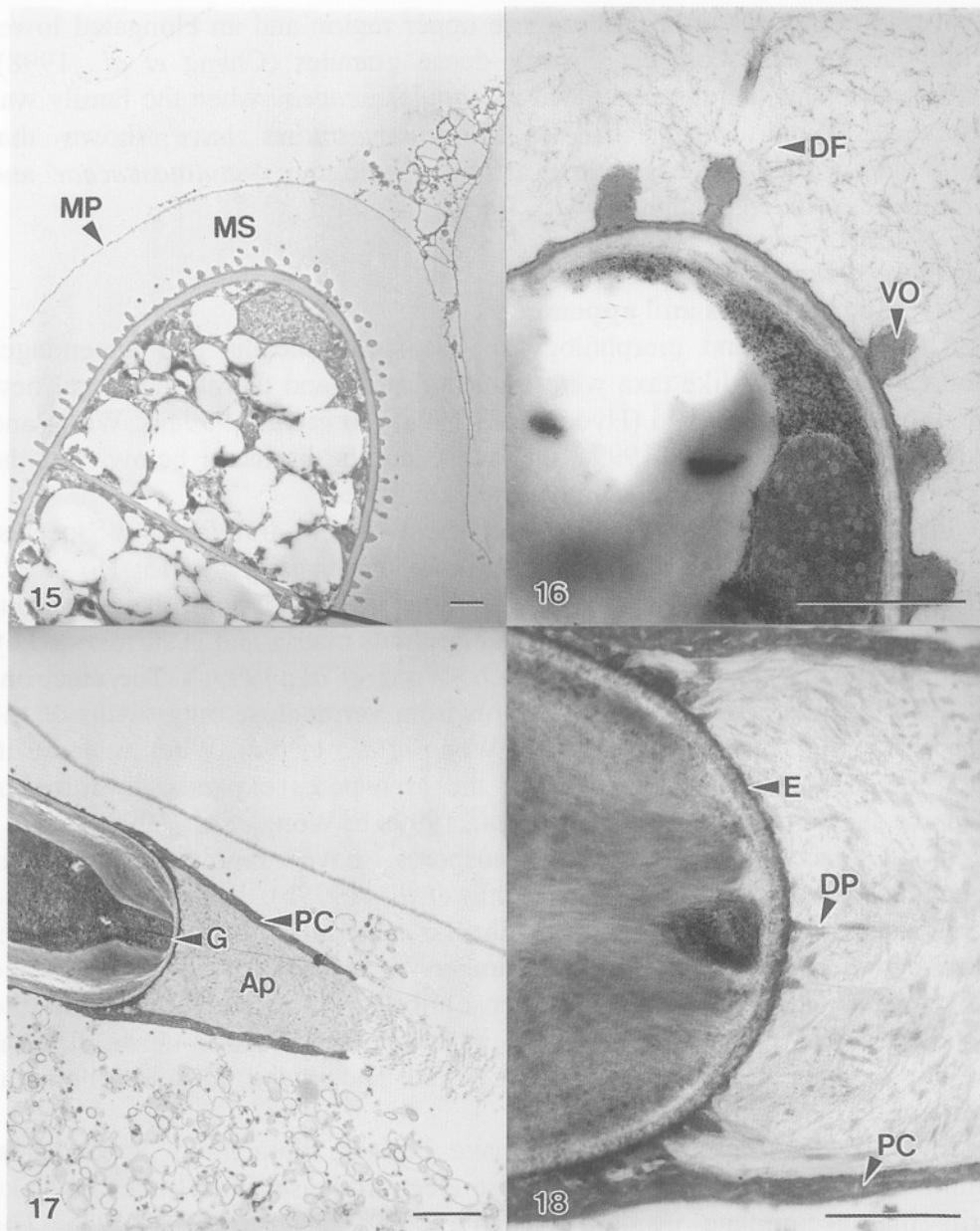
Ascus wall

As well as having a relatively massive bipartite ring, the morphology of the ascus wall in *Annulatascus*, *Cataractispora*, *Diluvicola*, *Fluminicola*, *Pseudoproboscispora*, *Torrentispora* and *Verticicola* are also similar. The asci are unitunicate, but at the ultrastructural level comprise two wall layers (Fig. 12). The ascus wall layers remain structurally and functionally intact throughout the ascospore discharge process as in other unitunicate ascomycetes (Read and Beckett, 1996). In immature asci the outer layer is relatively thin and electron-dense (Fig. 12). The inner layer is thicker and comprises electron-dense fibrillar material and/or electron-dense inclusions. During maturation the thickness of the inner wall layer and the quantity of the electron-dense inclusions decreases. In some taxa the inner ascus wall layer is absent at maturity (Fig. 13), except at the subapical region (Fig. 14).

Double-walled asci have been described in a number of unitunicate families, such as the *Amphisphaeriaceae* (Read *et al.*, 1993), *Ombrophiloideae* (Verkley, 1992) and *Sclerotiniaceae* (Verkley, 1993). The ascus wall of the latter two families, are however, bilamellate only in mature asci. Similar ascus wall substructures have been observed in other aquatic ascomycetes, such as *Marinosphaera mangrovei* K.D. Hyde, *Swampomyces armeniacus* Kohlm. and Volk. Kohlm. (Read *et al.*, 1995) and *Rivulicola appendispora* K.D. Hyde (Hyde *et al.*, 1997b). No fibrils or inclusions have been observed within the inner ascus wall of these aquatic ascomycetes, although this may result from different preparation methods used in transmission electron microscopy preparation and examination.

Other *Annulatascus*-like taxa

The morphology of the ascus in *Ascotaiwania* species is similar to that of species in the *Annulatascaceae*. Ultrastructural studies have shown that the



Figs. 15-18. Transmission electron micrographs. **15.** Mature ascospore of *Annulatascus hongkongensis*. The thick mucilaginous sheath (MS) is covered with a layer of membrane (MP). **16.** Mature ascospore of *Annulatascus velatisporus*. Note the electron-dense fibrils (DF) projected from the verruculose ornamentations (VO). **17.** Mature ascospore of *Cataractispora aquatica* with germ pore (G) and polar chamber (PC). The polar appendages (Ap) has been extruded through the opening of the polar chamber. **18.** Higher magnification of Fig. 17 at the region of polar chamber (PC). Some electron-dense projections (DP) extend from the episporium (E). Bars: 15, 17 = 1 μm ; 16, 18 = 0.5 μm .

apical ring comprises an electron-dense upper region and an elongated lower region that contains compact electron-dense granules (Chang *et al.*, 1998). *Ascotaiwania* was therefore included in *Annulatasceae* when the family was introduced (Wong *et al.*, 1998). Molecular studies have shown that *Ascotaiwania* does not have close affinities with the *Annulatasceae* and therefore is excluded (Ranghoo *et al.*, 1999).

Ascospore structure and new genera

Ascospore sheaths and appendages

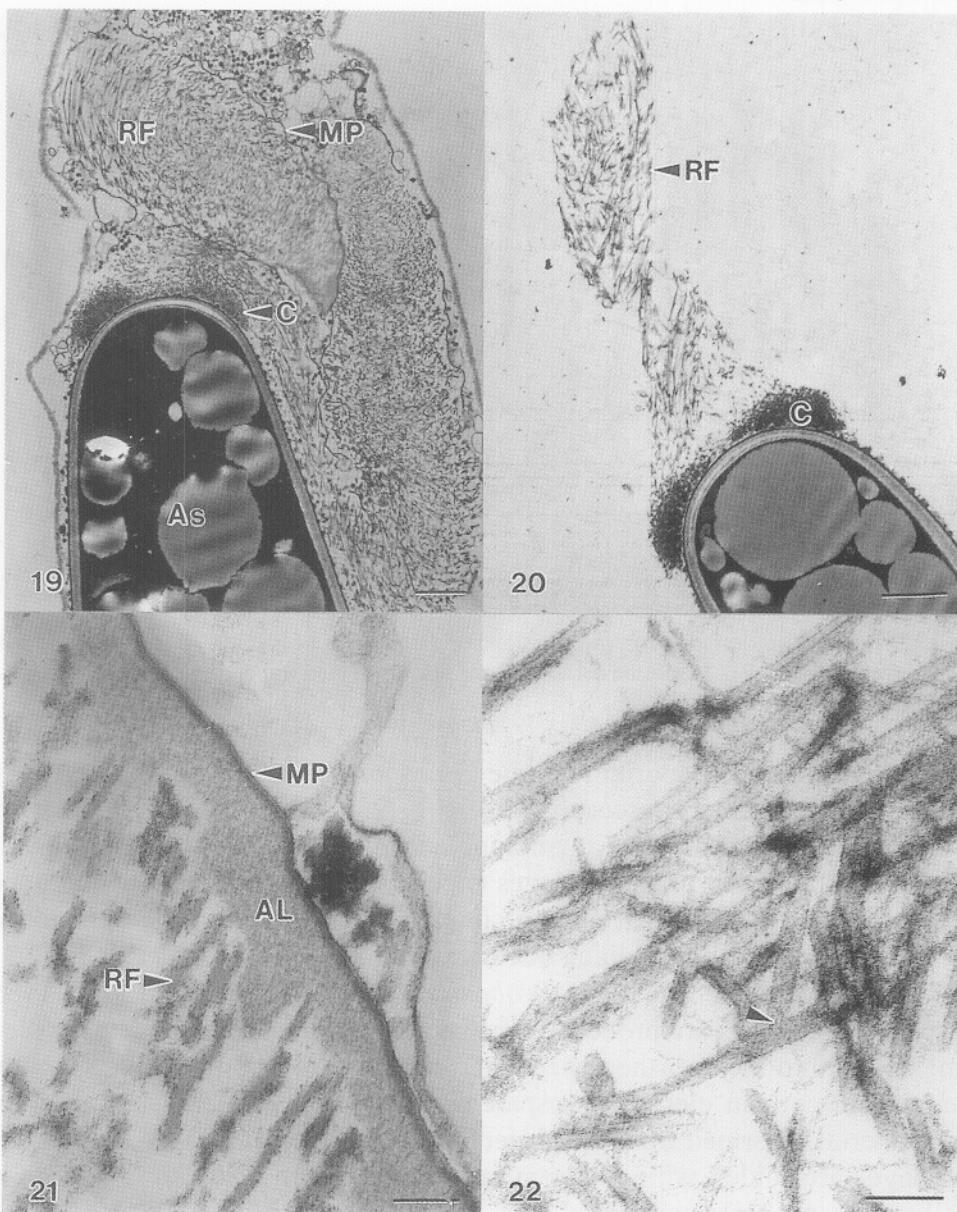
The ontogeny and morphology of ascospore sheaths and appendages among *Annulatascus*-like taxa were found to differ and therefore several new genera have been described (Hyde *et al.*, 1998; Ho *et al.*, 1999a,b; Wong and Hyde, 1999; Wong *et al.*, 1999a,b). Each genus is discussed below, and the ascospore sheath or appendage ontogeny is compared.

In *Annulatascus velatisporus* and most other *Annulatascus* species, ascospores are surrounded by a mucilaginous sheath (Figs. 9, 15; Ho *et al.*, 1999a,b; Wong *et al.*, 1999a). Within the ascus the sheath comprises electron-dense fibrillar material embedded in an amorphous matrix and is surrounded by a membrane (Fig. 15; Ho *et al.*, 1999a,b; Wong *et al.*, 1999a). The electron-dense fibrillar material is thought to form from verruculose outgrowths of the episporium (Fig. 16; Ho *et al.*, 1999b; Wong *et al.*, 1999a). When released in water, precursor material surrounding the ascospores expands, to form an extensive sheath (Figs. 9, 15, 16; Ho *et al.*, 1999a,b; Wong *et al.*, 1999a).

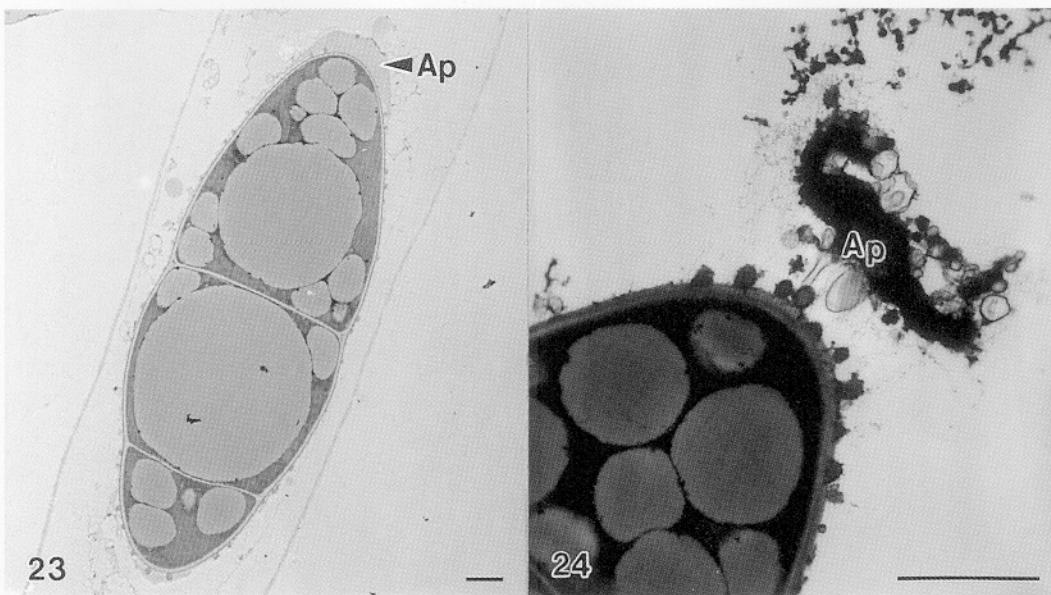
In *Cataractispora* species, ascospores have bipolar filamentous appendages (Figs. 5, 6; Ho, 1998; Wong *et al.*, 1999b). Within the asci the appendages comprise electron-dense fibrillar material embedded in a matrix that accumulates inside polar end chambers or within the end cells of the ascospores (Fig. 17). The electron-dense fibrillar material forms from the episporium (Fig. 18). When released in water, the electron-dense fibrillar material extends, and the appendages swell and extend into long bipolar threads (Fig. 6; Wong *et al.*, 1999b).

In *Diluvicola* ascospores also have bipolar filamentous appendages. Within the asci the appendages comprise electron-dense, inter-linked rod-like fibrils that accumulate inside detachable polar caps (Figs. 19, 22). The electron-dense rod-like fibrils are derived from exosporial material and accumulate within the polar caps and collars at the ascospore ends (Fig. 19). When released in water the appendages unfurl, partly from the collar and partly from the exosporial material within the caps to form long bipolar threads (Figs. 20, 21; Hyde *et al.*, 1998).

In *Fluminicola* ascospore appendages are bipolar and crown-like. Within the asci the appendages comprise electron-dense amorphous material that is



Figs. 19-22. Transmission electron micrographs of *Diluvicola capensis*. **19.** Ascospore (As) with a collar (C) of electron-dense material and a polar appendage filled with densely packed rod-like electron-dense fibrils (RF). The electron-dense collar is almost absent at the ascospore apex. The rod-like fibrils are surrounded by membrane (MP). **20.** Released ascospore with expanded rod-like fibrils (RF) that derived from the electron-dense collar (C). **21.** Higher magnification of polar appendage illustrating the rod-like fibrils (RF) being closely associated with the electron-dense amorphous material (AL) on the inner surface of the trilamellate membrane (MP) that enclose the appendage. **22.** Longitudinal section of the rod-like electron-dense fibrils of the ascospore appendage. The fibrils are inter-linked (arrowed). Bars: 19, 20 = 1 μm ; 21, 22 = 0.1 μm .



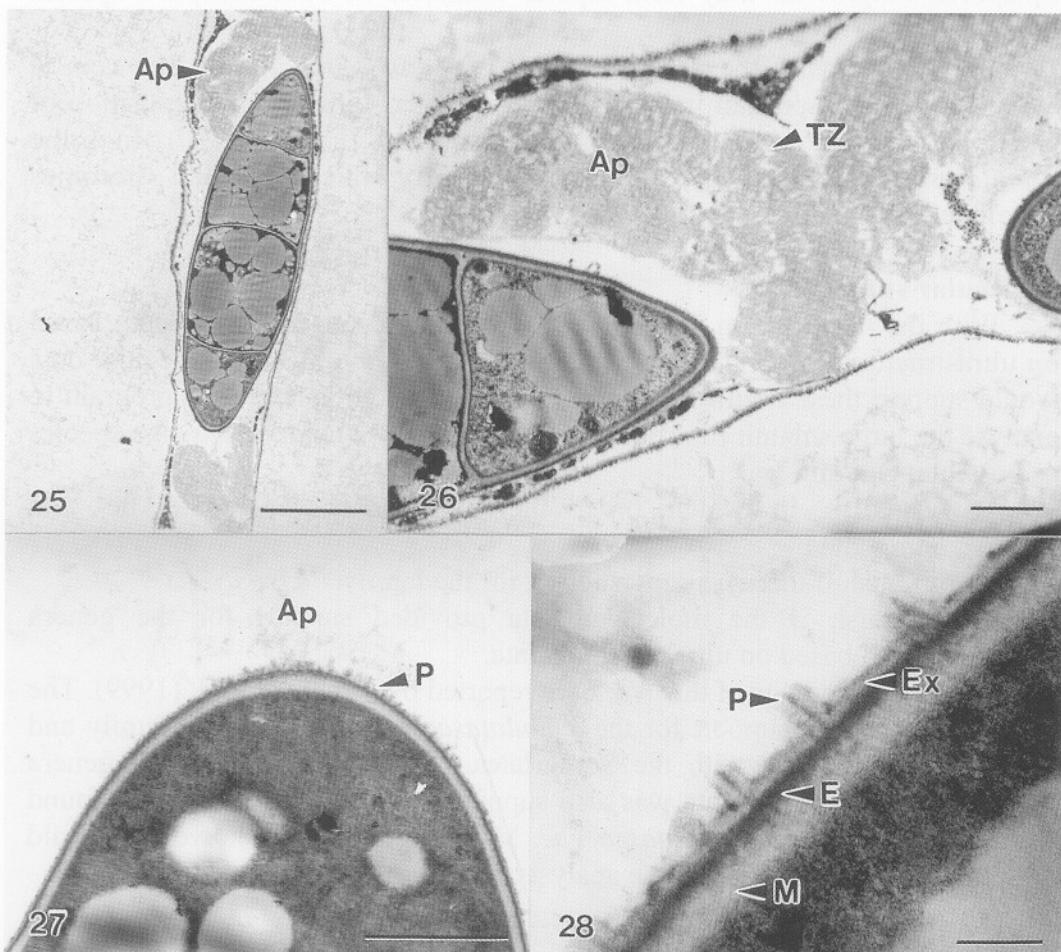
Figs. 23, 24. Transmission electron micrographs of *Fluminicola bipolaris*. 23. Ascospore with bipolar appendages (AP). 24. Released ascospore with unfurled appendage. Bars: 23, 24 = 1 μm .

formed from the mesosporium at the ascospore tips. The appendages are initially appressed to the ascospore tip (Fig. 23). The upper surface of the appendages is covered with a membrane complex, which was absent on the lower surface. In water the appendages expand, but the expansion of the upper part was restricted by the membrane complex. Subsequently, the appendages curl outwards to form crown-like structures (Fig. 24; Wong *et al.*, 1999c).

In *Pseudoproboscispora* (as *Proboscispora* in Wong and Hyde, 1999) ascospore appendages are proboscis-like and unfurl in water to become bipolar filaments. Within the asci the appendages comprise electron-dense amorphous material, and contain electron-transparent zones (Figs. 25, 26). The electron-dense material is formed from the projections of episporium (Figs. 27, 28). The appendages are folded within a membrane complex. In water the appendages uncoil and become less condensed (Fig. 27; Wong and Hyde, 1999).

Ascospore wall

The ascospore wall of *Annulatascus*-like taxa are mostly similar, but differ in some aspects (Ho, 1998; Hyde *et al.*, 1998; Ho *et al.*, 1999a,b; Wong and Hyde, 1999; Wong *et al.*, 1999a,c,d). In *Annulatascus* (Figs. 30, 31), *Cataractispora* (Fig. 32), *Diluvicola*, and *Fluminicola* species the wall comprises a mesosporium of 1-3 layers, and a single layered episporium. With



Figs. 25-28. Transmission electron micrographs of *Pseudoproboscispora aquatica*. **25.** Mature ascospore with bipolar appendages (Ap). **26.** Ascospore appendage (Ap) with electron-transparent zones (TZ). **27.** Ascospore with electron-dense projections (P) at the polar region. The appendage (Ap) is amorphous. **28.** Ascospore wall at the polar region. The ascospore is trilamellate with an outermost exosporium (Ex), an episporium (E), and an innermost mesosporium (M). The projections (P) extend from the exosporium (Ex) and episporium (E). Bars: 25 = 5 µm; 26, 27 = 1 µm; 28 = 0.1 µm.

the exception of *Pseudoproboscispora* that has smooth walls (Fig. 27), ascospores walls are covered with verrucose wall ornamentations (Figs. 15, 16), that are elaborations of the episporium. An exosporium is generally lacking, but this is found in ascospores of *Pseudoproboscispora* (Fig. 28).

Episporial outgrowths may form bipolar chambers in *Cataractispora* species (Fig. 17).

The ontogeny of the ascospore wall of these *Annulatascus*-like taxa is similar. The mesosporium is the first formed layer, followed by deposition of electron-dense materials that form the episporium (Figs. 29, 30). In some *Annulatascus*-like taxa the mesosporium may thicken and becomes lamellated (Fig. 32).

Molecular studies

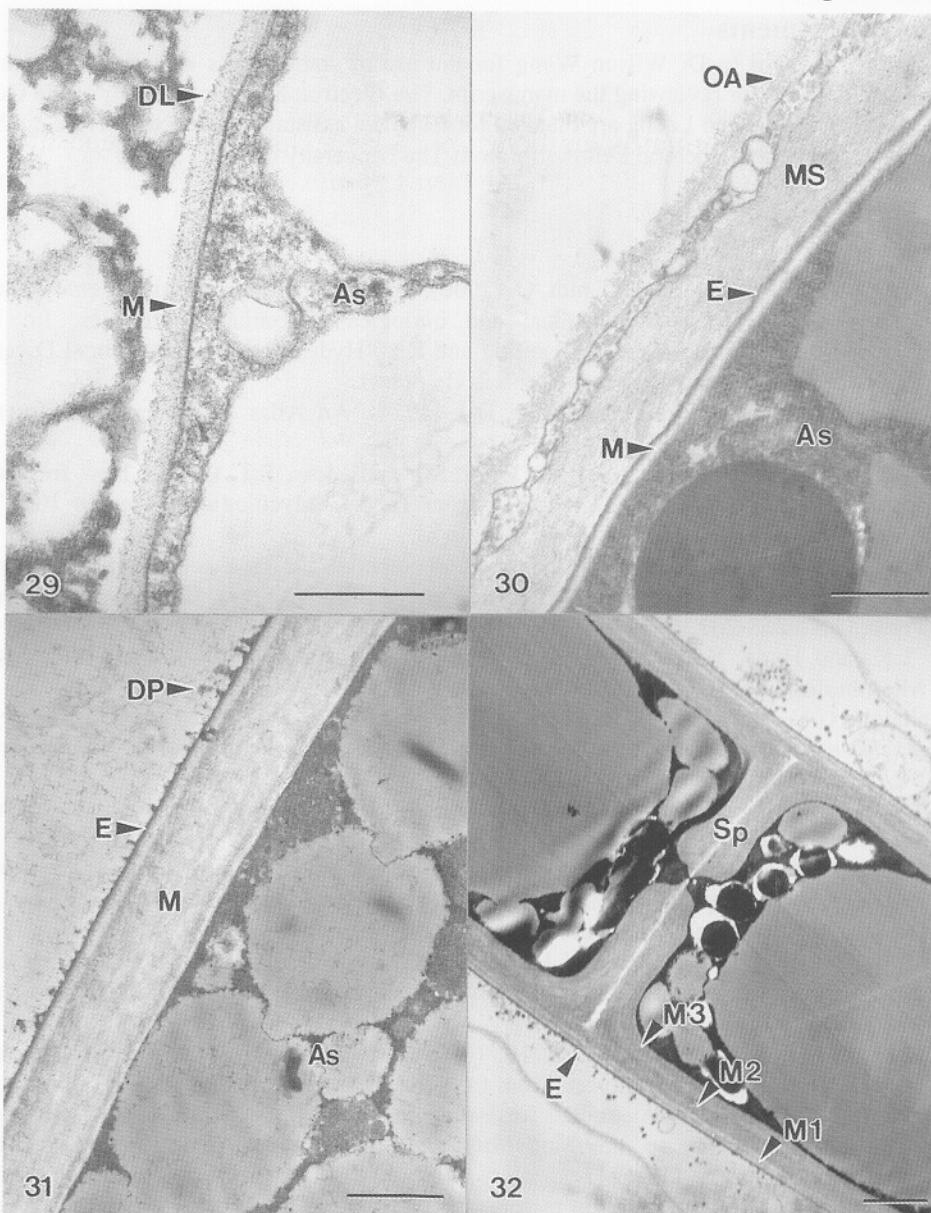
With the introduction the *Annulascaceae* and several new genera based on ultrastructural work, it was deemed necessary to establish if molecular data would support these findings. Therefore in 1997 we embarked on a program to analyse the large subunit ribosomal DNA of as many *Annulatascus*-like species as possible. Our aim was:

1. To establish in which order the *Annulascaceae* could be best accommodated.
2. To establish if there was any support for the family.
3. To establish if the molecular data provided support for the genera introduced based on ultrastructural data.

The initial findings of this work are reported by Ranghoo *et al.* (1999). The data provides some support for the *Annulascaceae* as a separate family and indicates relationships with the Sordariales. The description of new genera based on ultrastructural data was also supported, but *Ascotaiwania* was found to be unrelated to the *Annulascaceae*. Many more species, however, should be sequenced and included in the analysis, in order to strengthen the data.

Future work

Over the last decade, Hyde and coworkers have described 13 new genera of unitunicate freshwater ascomycetes (Hyde, 1992, 1994, 1995, 1996; Hyde *et al.*, 1997b, 1998; Ranghoo and Hyde, 1998; Wong *et al.*, 1998, 1999b,d; Wong and Hyde, 1999) and examined hundreds of fungi from the tropical streams. Over 1000 cultures have been obtained from freshwater habitats and are cryopreserved in The University of Hong Kong Culture collection. Fungal cultures have been shown to be an excellent source for bioactive chemical discovery (Fox, 1993; Ainsworth *et al.*, 2000; Hyde, 2000). Currently we are screening these fungi for novel antibiotics and enzymes, and for strains that are able to produce metabolites in higher doses *ex situ*. We are also examining species to species, and intra-specific competition among these fungi in order to establish if their dominance is controlled by their competitiveness among the fungal communities.



Figs. 29-32. Transmission electron micrographs of *Annulatascus* and *Cataractispora* species. **29.** Immature ascospore (As) of *A. hongkongensis*. The ascospore wall comprises mesosporium (M) lined with a thin layer of electron-dense deposits (DL). **30.** Ascospore (As) of *A. velatisporus* with thick mucilaginous sheath (MS). The ascospore wall is bilamellate comprising episporium (E) and mesosporium (M). The outer ascus wall (OA) is intact but the inner wall is absent. **31.** Ascospore (As) of *A. biatriisporus* with a thin episporium (E) and a much thickened mesosporium (M). Some electron-dense materials (DP) project from the episporium. **32.** Ascospore of *C. aquatica* with a thin episporium (E) and thick trilamellate mesosporium (M1-M3). The septum (Sp) is continuous with the M3 layer. Bars: 29, 30 = 0.5 μ m; 31, 32 = 1 μ m.

Acknowledgements

We are grateful to Dr Wilson Wong for the use of some of his electron micrographs. Doris Au is thanked for reviewing the manuscript. The Electron Microscopy Unit of the Queen Mary Hospital, and Helen Leung are thanked for technical assistance. Wellcome Ho is grateful for the award of a Post-Doctoral Fellowship from The University of Hong Kong.

References

- Ainsworth, A.M., Wrigley, S. and Fauth, U. (2000). Molecular and taxonomic diversity in drug discovery: experience of chemical and biological screening approaches. In: Bio-exploitation of Fungi (eds. S.B. Pointing and K.D. Hyde). Hong Kong, Fungal Diversity Press, Fungal Diversity Research Series 2: (In press).
- Beckett, A., Heath, I.B. and McLaughlin, D.J. (1974). An Atlas of Fungal Ultrastructure. Longman Group Ltd., U.K.
- Chang, H.S., Hsieh, S.Y., Jones, E.B.G., Read, S.J. and Moss, S.T. (1998). New freshwater species of *Ascotaiwania* and *Savoryella* from Taiwan. Mycological Research 102: 709-718.
- Fox, F.M. (1993). Tropical fungi: their commercial potential. In: *Aspects of Tropical Mycology* (eds. S. Isaac, J.C. Frankland, R. Watling and A.J.S. Whalley). Cambridge University Press, U.K.: 253-263.
- Goh, T.K. and Hyde, K.D. (1996). Biodiversity of freshwater fungi. Journal of Industrial Microbiology 17: 328-345.
- Greenhalgh, G.N. and Evans, L.V. (1967). The structure of the ascus apex in *Hypoxyylon fragiforme* with reference to ascospore release in this and related species. Transactions of the British Mycological Society 50: 183-188.
- Griffiths, H.B. (1973). Fine structure of seven unitunicate pyrenomycete asci. Transactions of the British Mycological Society 60: 261-271.
- Ho, W.H. (1998). Biodiversity, ecological and ultrastructural observations of fungi on wood submerged in tropical streams. Ph.D. Dissertation, Department of Ecology and Biodiversity, The University of Hong Kong, Hong Kong.
- Ho, W.H., Hyde, K.D. and Hodgkiss, I.J. (1999a). Ultrastructure of *Annulatasca aquaticus* sp. nov., a freshwater ascomycete on submerged wood from Hong Kong. Fungal Diversity 2: 121-130.
- Ho, W.H., Hyde, K.D. and Hodgkiss, I.J. (1999b). Ultrastructural study of *Annulatasca hongkongensis*, a new ascomycete from freshwater habitats. Mycologia 91: (In press).
- Hyde, K.D. (1992). Tropical Australian freshwater fungi. II. *Annulatasca velatispora* gen. et sp. nov., *A. bipolaris* sp. nov. and *Nais aquatica* sp. nov. (Ascomycetes). Australian Systematic Botany 5: 117-124.
- Hyde, K.D. (1994). Aquatic fungi on rachides of *Livistona* in the Western Province of Papua New Guinea. Mycological Research 98: 719-725.
- Hyde, K.D. (1995). Tropical Australian freshwater fungi. VII. New genera and species of Ascomycetes. Nova Hedwigia 61: 119-140.
- Hyde, K.D. (1996). Tropical Australian freshwater fungi. X. *Submersisphaeria aquatica* gen. et sp. nov. Nova Hedwigia 62: 171-175.
- Hyde, K.D. (2000). Cutting corners, the mycologists role in the bio-exploitation of fungi. In: Bio-exploitation of Fungi (eds. S.B. Pointing and K.D. Hyde). Hong Kong, Fungal Diversity Press, Fungal Diversity Research Series 1: (In press).
- Hyde, K.D. and Jones, E.B.G. (1988). Marine mangrove fungi. Marine Ecology 9: 15-33.

Fungal Diversity

- Hyde, K.D., Ho, W.H. and Tsui, C.K.M. (1999). The genera *Aniptodera*, *Halosarpheia*, *Nais* and *Phaenectriella* from freshwater. *Mycoscience* 40: 165-183.
- Hyde, K.D., Wong, S.W. and Jones, E.B.G. (1997a). Freshwater Ascomycetes. In: *Biodiversity of tropical microfungi* (ed. K.D. Hyde). Hong Kong University Press, Hong Kong: 179-188.
- Hyde, K.D., Wong, S.W. and Jones, E.B.G. (1998). *Diluvicola capensis* gen. et sp. nov., a freshwater ascomycete with unique polar caps on the ascospores. *Fungal Diversity* 1: 133-146.
- Hyde, K.D., Read, S.J., Jones, E.B.G. and Moss, S.T. (1997b). Tropical Australian freshwater fungi. XII. *Rivulicola incrustatus* gen. et sp. nov. and notes on *Ceratosphaeria lampadophora*. *Nova Hedwigia* 64: 185-196.
- Ingold, C.T. (1942). Aquatic hyphomycetes of decaying alder leaves. *Transactions of the British Mycological Society* 25: 339-417.
- Ingold, C.T. (1951). Aquatic ascomycetes: *Ceriospora caudaesuis* n. sp. and *Ophiobolus typhae*. *Transactions of the British Mycological Research* 34: 210-212.
- Ingold, C.T. (1955). Aquatic ascomycetes: Futher species from the English lake district. *Transactions of the British Mycological Research* 38: 157-168.
- Jones, E.B.G. (1995). Ultrastructure and taxonomy of the aquatic ascomycetes order Halosphaerales. *Canadian Journal of Botany* 73 (Supplement 1): S790-S801.
- Parguey-Leduc, A. and Janex-Favre, M.C. (1980). L'appareil apical de deux Diatrypales: Etude ultrastructurale. *Cryptogamie Mycologici* 1: 155-163.
- Ranghoo, V.M. and Hyde, K.D. (1998). *Ascolacicola aquatica* gen. et sp. nov., and a new species of *Ascotaiwania* from wood submerged in a reservoir in Hong Kong. *Mycologia* 90: 1055-1062.
- Ranghoo, V.M., Hyde, K.D., Liew, E.C.Y. and Spatafora, J.W. (1999). Family placement of *Ascotaiwania* and *Ascolacicola* based on DNA sequences from the large subunit rRNA gene. *Fungal Diversity* 2: 161-174.
- Read, N.D. and Beckett, A. (1996). Centenary review: Ascus and ascospores morphogenesis. *Mycological Research* 100: 1281-1314.
- Read, S.J., Jones, E.B.G. and Moss, S.T. (1993). Taxonomic studies of marine Ascomycotina: ultrastructure of the asci, ascospores, and appendages of *Savoryella* species. *Canadian Journal of Botany* 71: 273-283.
- Read, S.J., Jones, E.B.G., Moss, S.T. and Hyde, K.D. (1995). Ultrastructure of asci and ascospores of two mangrove fungi: *Swampomyces armeniacus* and *Marinosphaera mangrovei*. *Mycological Research* 99: 1465-1471.
- Reeves, F.B. (1971). The structure of the ascus apex in *Sordaria fimicola*. *Mycologia* 63: 204-212.
- Wong, S.W. (1996). Ultrastructural and taxonomic studies of freshwater Ascomycetes. Ph.D. Dissertation, Department of Ecology and Biodiversity, The University of Hong Kong, Hong Kong.
- Wong, S.W. and Hyde, K.D. (1999). *Proboscispora aquatica* gen. et sp. nov. from wood submerged in freshwater. *Mycological Research* 103: 81-87.
- Wong, S.W., Hyde, K.D. and Jones, E.B.G. (1998). *Annulatasccaceae*, a new ascomycete family from the tropics. *Systema Ascomycetum* 16: 17-25.
- Wong, S.W., Hyde, K.D., Jones, E.B.G. and Moss, S.T. (1999a). Ultrastructural studies on *Annulatascus velatisporus* and *A. triseptatus* sp. nov. *Mycological Research* 103: 561-571.
- Wong, S.W., Hyde, K.D. and Jones, E.B.G. (1999b). Ultrastructural studies on *Cataractispora aquatica* gen. et sp. nov., *C. appendiculata* sp. nov. and *C. viscosa* sp. nov. *Mycological*

- Research 103: 1019-1031.
- Wong, S.W., Hyde, K.D. and Jones, E.B.G. (1999c). Ultrastructural studies on freshwater ascomycetes, *Fluminicola bipolaris* gen. et sp. nov. Fungal Diversity 2: 195-203.
- Verkley, G.J.M. (1992). Ultrastructure of the apical apparatus of ascii in *Ombrophila violacea*, *Neobulgaria pura* and *Bulgaria inquinans* (Leotiales). Persoonia 15: 3-22.
- Verkley, G.J.M. (1993). Ultrastructure of the ascus apical apparatus in ten species of Sclerotiniaceae. Mycological Research 97: 179-194.

(Received 28 Aug. 1999, accepted 12 Jan. 2000)