Diluviocola capensis gen. and sp. nov., a freshwater ascomycete with unique polar caps on the ascospores

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Diluviocola capensis gen. and sp. nov. is described from wood submerged in a river in Brunei. The fungus is similar to species in *Annulatascus*, but it has unique ascospore appendages. In *D. capensis* ascospores have polar conical caps. After release from the ascus these polar caps detach from the tip, and a thread-like appendage unfurls from within the cap. EM micrographs indicate that the appendage comprises a network of inter-linked rod-like fibrils. This appendage structure is compared with the appendage structure in *Annulatascus bipolaris* and *Halosarpheia* species. *Diluviocola* is also compared to *Annulatascus*, *Rivulicola* and *Proboscispora*, genera which share some similar characteristics at the light and electron microscope levels.

Introduction

Ascospores with unfurling polar thread-like appendages are common amongst tropical freshwater ascomycetes e.g. *Aniptodera lignatilis* K.D. Hyde (Hyde, 1992a), *Annulatascus bipolaris* K.D. Hyde (Hyde, 1992b), *Halosarpheia aquadulcis* S.Y. Hsieh, H.S. Chang and E.B.G. Jones (Hsieh *et al.*, 1995), *H. heteroguttulata* S.W. Wong, K.D. Hyde and E.B.G. Jones (Wong, Hyde and Jones, 1998a) and *Proboscispora aquatica* S.W. Wong and K.D. Hyde (Wong and Hyde, 1998). However, the structure of these appendages differs between genera. In a collection of a freshwater *Annulatascus*-like species made in Brunei, ascospores had bipolar caps which unfurled internally producing thread-like appendages. The structure of these caps and appendages is unique when compared to other fungi with filamentous appendages. A new genus, *Diluviocola*, is therefore proposed to accommodate it. Electron micrographs of mature asci and ascospores are presented to illustrate the uniqueness of the appendages. *Diluviocola capensis* is compared with species in other genera of aquatic ascomycetes.

Materials and methods

Submerged wood was collected from Sungai Esu, near Kuala Belalong Field Studies Centre in Brunei and examined for the presence of fungi. Asci and ascospores were prepared for light microscopy, SEM and TEM following the methods used by Wong *et al.* (1998a).

Taxonomy

Diluviocola K.D. Hyde, S.W. Wong and E.B.G. Jones, gen. nov.

Etymology: from Latin *Diluvium* and *cola* meaning "a flood" and "loving" respectively.

Ascomata obpyriforma vel ellipsoidea, immersa vel semi-immersa, coriacea, paraphysata, nigra, solitaria et papillata. *Asci* 8-spori, unitunicati, cylindrici, pedicellati, apparato apicale refractibus praediti. *Ascosporae* uniseriatae, hyalinae, fusiformes, unicellulae vel septatae, verruculosae, appendiculatae.

Typus generis: *Diluviocola capensis* K.D. Hyde, S.W. Wong and E.B.G. Jones

Ascomata obpyriform or ellipsoidal, immersed or semi-immersed, coriaceous, beaked, black and solitary. *Peridium* comprising several layers of compressed brown walled cells. *Paraphyses* wide, septate and tapering distally. *Asci* 8-spored, unitunicate, thin-walled, cylindrical, pedicellate. *Ascus apical ring* relatively massive, bipartite, upper part differentiated from the inner ascus wall. *Ascospores* uniseriate or overlapping uniseriate, hyaline, fusiform with rounded ends, unicellular or septate, wall with episporial verruculose ornamentations, with polar conical caps attached at each end, appendage material filling the polar caps. Once released in water, the polar caps detach from the collar-like structure at the ascospore tip and are held by a sticky, thin and flexible thread, eventually unfurling from within the cap to form thread-like polar appendages. The appendage material comprises rod-like fibrils inter-linked to each other to form a complicated but systematic framework.

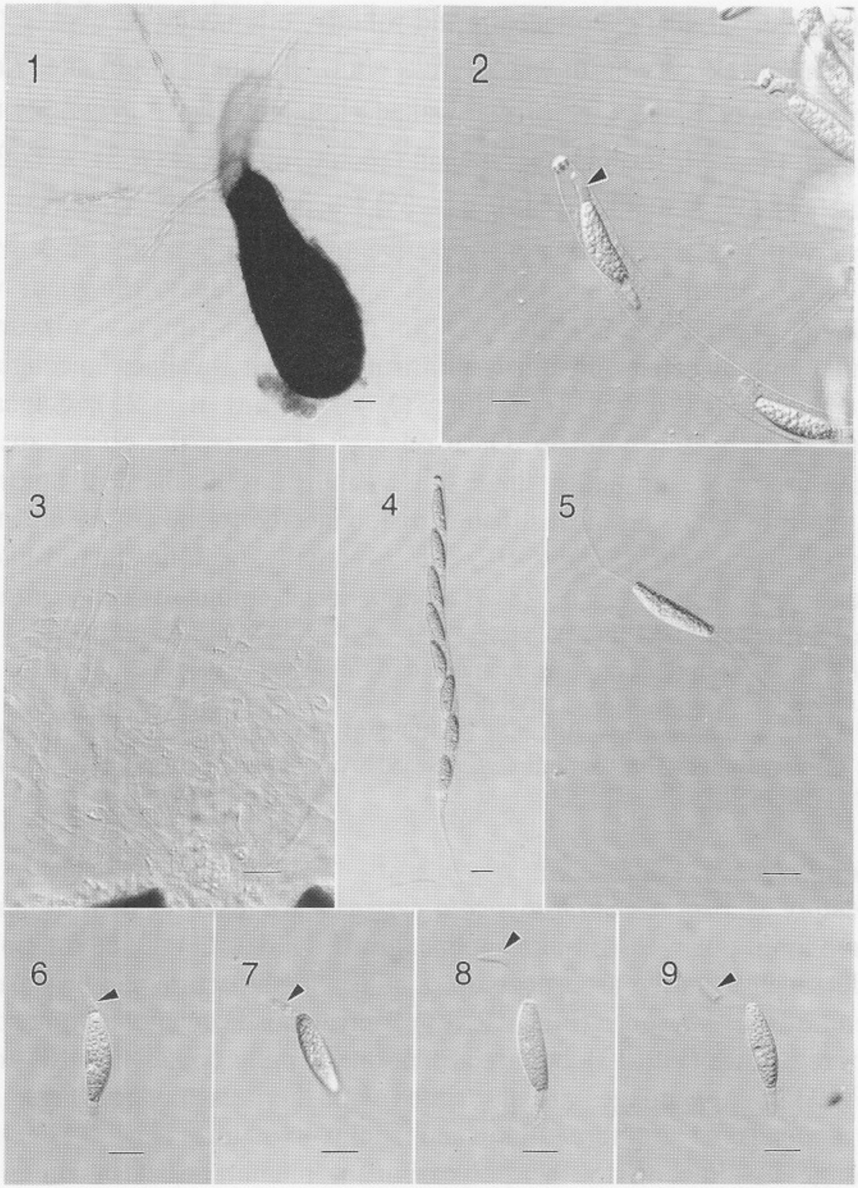
Diluviocola capensis K.D. Hyde, S.W. Wong and E.B.G. Jones, sp. nov.
(Figs. 1-28)

Etymology : from the Latin *capensis*, which refers to the ascospore polar caps.

Ascomata 95-130 µm longa, 32-80 µm in diametro, obpyriformes, immersa vel semi-immersa, nigra, coriacea, paraphysata, papillata, periphysata. *Asci* 265-325 × 11-14 µm ($\overline{x}$ = 305 × 13 µm, n = 25), 8-spori, longe cylindrici, pedicellati, apparato apicale refractibus, 3-4 µm × 2-3 µm praeditae. *Ascosporae* 26-34 × 6-11 µm ($\overline{x}$ = 29 × 8 µm, n = 50), uniseriatae, fusiformes, hyalinae, unicellulae, appendiculatae.

Holotypus: BRUNEI DARUSSALAM, Temburong, Kuala Belalong Field Studies Centre, Sungai Esu, ad lignum submersum, Feb. 1994, K.D. Hyde and S.W. Wong (HKU(M) 3125).

Ascomata 93-128 µm long, 32-80 µm diam., obpyriform (Fig. 1), immersed



Figs. 1-9. *Diluviocola capensis* (from holotype). Interference contrast micrographs. **1.** Obpyriform ascoma with discharged asci. **2, 4.** Asci, with relatively massive apical rings. Ascospores are uniseriate or overlapping uniseriate within the ascus. Ascospores with polar caps are clearly visible within the ascus (arrowed). **3.** Wide, septate paraphyses. **5.** Ascospore with long polar thread-like appendages. **6-9.** Ascospore appendage unfurling process: Note the detaching polar caps (arrowed). 6. Polar caps intact; 7. One of the caps begins to detach from the ascospore; 8, 9. A thread-like appendage forms between the detached cap and the ascospore tip. Bars = 10 μm.

or semi-immersed, black, coriaceous and solitary, often lying parallel to the host surface with neck curving upwards. *Neck* short, hyaline, periphysate. *Peridium* comprising several layers, or brown walled elongate cells. *Paraphyses* up to 4 µm wide at the base, hyaline, septate and tapering distally (Fig. 3). *Asci* 266-326 × 11-14 µm ($\bar{x}$ = 305 × 13 µm, n = 25), 8-spored, long cylindrical, pedicel tapering, with a relatively massive, refractive apical ring, 3-4 µm × 2-3 µm (Figs. 2, 4). *Ascus apical ring* bipartite, upper part connected to the ascus wall (at TEM level). *Ascospores* 26-34 × 6-11 µm ($\bar{x}$ = 29 × 8 µm, n = 50), uniseriate or overlapping uniseriate, fusiform with truncate ends, hyaline, unicellular, with polar caps. *Polar caps* 5-13 µm long ($\bar{x}$ = 8.3 µm, n = 25), conical, base attached at each ascospore tip. Once released in water, the polar caps detach from the ascospore tip and a single sticky, thin, flexible filament unfurls from within the caps. The end of this filament is connected to the collar-like structure at the ascospore tip (Figs. 6-9), and eventually, a long thread-like polar appendage is formed (Figs. 5, 28). The appendage comprises dense inter-linked rod-like fibrils (at TEM level).

Mode of life: Saprobic on wood submerged in freshwater.

Known distribution: Brunei Darussalam.

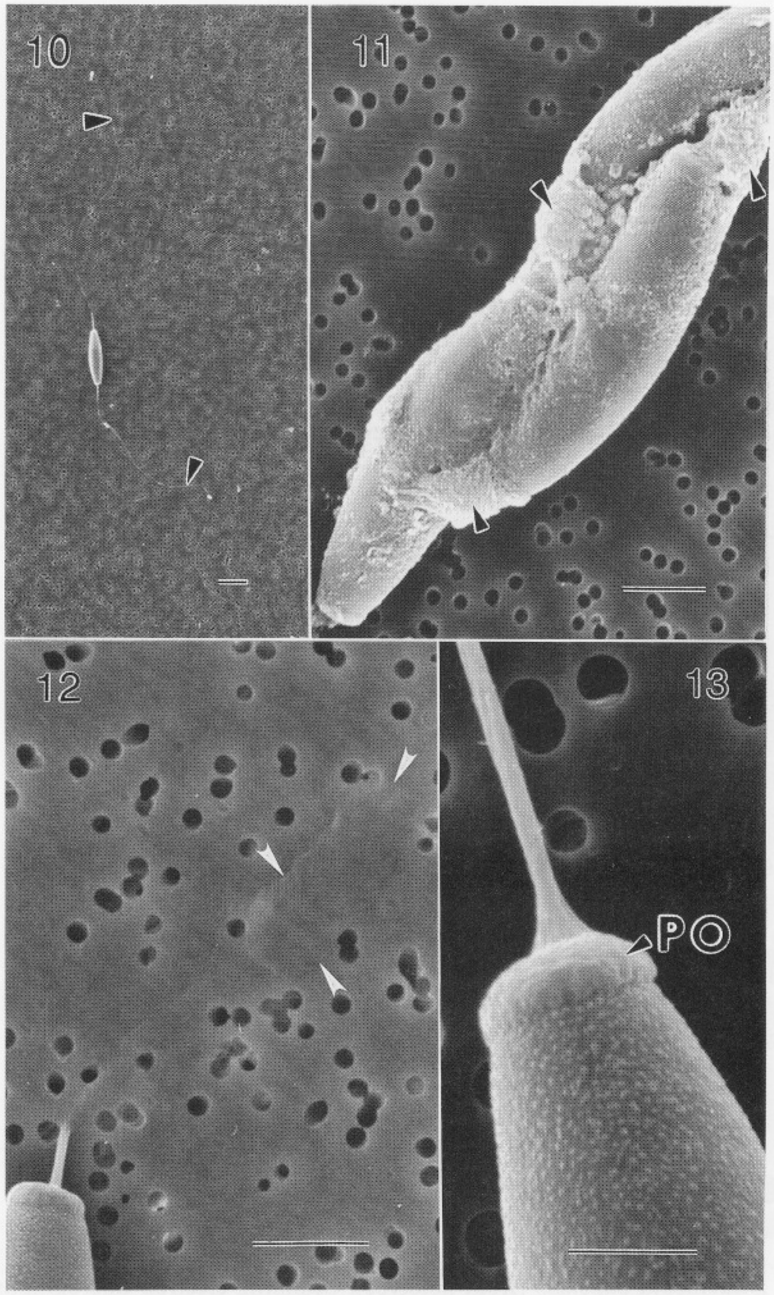
Other material examined: BRUNEI DARUSSALAM, Temburong, Kuala Belalong Field Studies Centre, Sungai Esu, on submerged wood, Aug. 1997, K.D. Hyde and S.W. Wong (HKU(M) BRUNEI 29).

Scanning electron microscopy

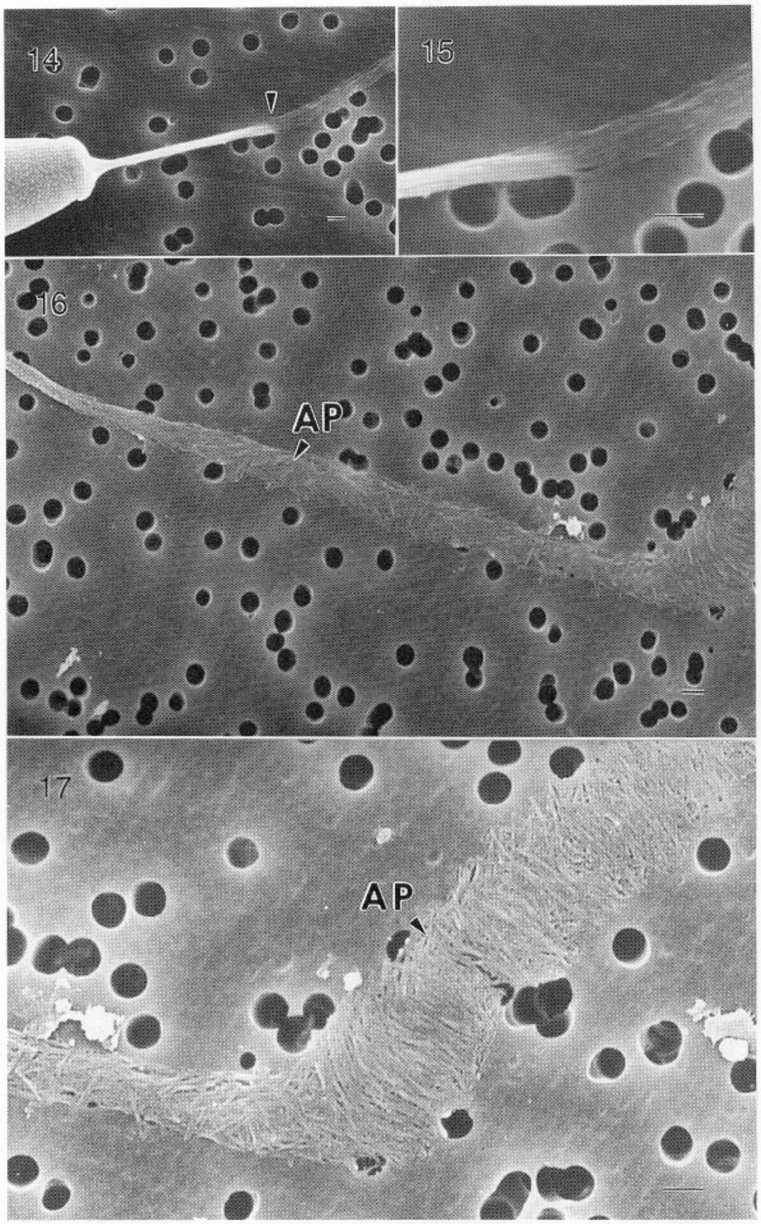
Mature ascospores of *Diluviocola capensis* are fusiform with truncate ends and have long, polar, thread-like appendages (Figs. 10, 11). Once the polar caps have detached from the ascospore tip, a collar-like structure can be observed at each end of the ascospore (Figs. 12, 13). Verruculose ornamentations cover the ascospore surface (Fig. 13), but are absent on the polar collar (Fig. 13) and polar cap (Fig. 12). Appendages are associated with the polar collars (Figs. 13, 14). These appendages are thread-like and sticky in nature (Fig. 14). Once the appendage is attached to the polycarbonate membrane, it becomes closely adpressed to the surface (Fig. 15). The appendage comprises dense inter-linked rod-like fibrils which form a complicated, but systematic framework (Figs. 16, 17).

Transmission electron microscopy

The wall of mature asci (*ca* 45-50 nm thick) comprise an outer thin electron-dense layer and an inner less electron-dense layer (Figs. 18, 23). The inner ascus wall layer increases in thickness towards the apical ring (up to *ca* 870 nm thick) and appears to differentiate to form the upper part of the apical



Figs. 10-13. *Diluviocola capensis* (from holotype). Scanning electron micrographs. **10.** Ascospore with extremely long polar thread-like appendages (arrowed). **11.** Ascospores with polar caps (arrowed) that have a warty surface. **12.** Ascospore with detached cap linked to the ascospore tip by an appendage (arrowed). **13.** Higher magnification of ascospore tip illustrating the polar collar-like structure (PO) from which the thread-like appendage emerges. Note the verruculose ornamentations on the ascospore wall. Bars: 10-12 = 5 µm, 13 = 1 µm.



Figs. 14-17. *Diluviocola capensis* (from holotype). Scanning electron micrographs. **14.** Polar appendage emerging from the collar and adhering to the polycarbonate membrane (arrowed). **15.** Higher magnification of the ascospore appendage which is close to the ascospore tip. Note the smooth and compact structure of the appendage. **16.** Distal portion of the ascospore appendage (AP). Note the inter-linked rod-like fibrils. **17.** Higher magnification of ascospore appendage (AP) illustrating the inter-linked rod-like fibrils which form a systematic framework. Bars = 1 µm.

ring (Figs. 22, 23). The mature apical ring consists of two parts with similar electron density. The upper part is connected to the inner ascus wall and the lower part, which is closely associated with the upper part, appears to elongate downwards into the epiplasm. Electron-dense granular deposits occur at the interface between the upper and lower parts of the apical ring (Figs. 22, 23). In addition, some of these granular deposits extend into the inner ascus wall layer. An opening channel is present within the apical ring (Fig. 23).

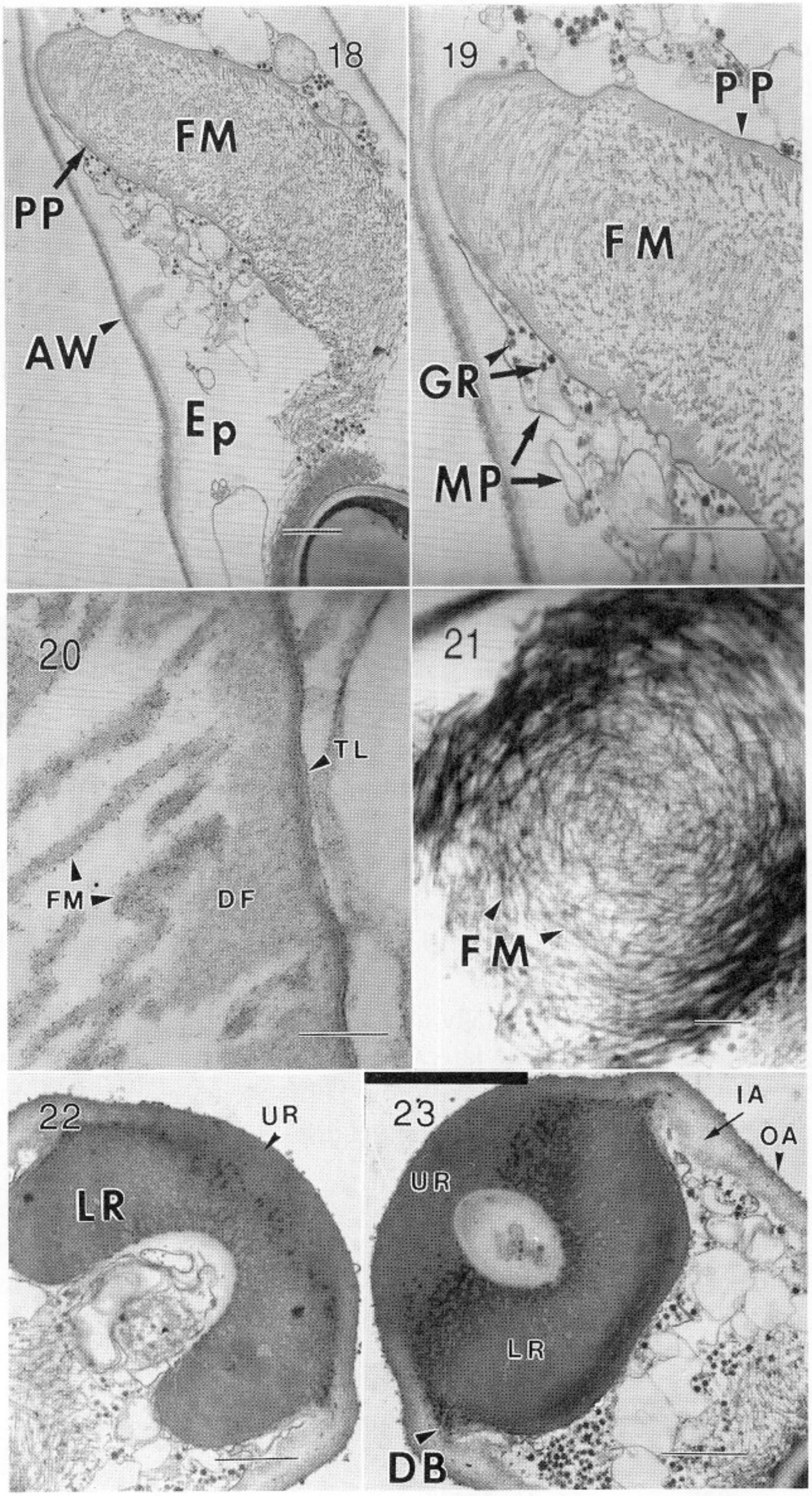
The polar region of mature ascospores has a collar of electron-dense material and a polar appendage which appears to have been derived from the collar (Fig. 18). The unfurled appendage is composed of densely packed, electron-dense, rod-like fibrils (Figs. 24-27) which are inter-linked to form long threads. Thick tangential sections (*ca* 0.2 μm) of the appendages indicate that the rod-like fibrils are arranged in a circular fashion (Fig. 21). In ultrathin longitudinal sections (*ca* 60-70 nm), the rod-like fibrils are sectioned transversely at the centre (elongated), and longitudinally at the periphery (dot-like) (Fig. 19). This indicates that the rod-like fibrils are arranged in a helical or cylindrical network.

The polar cap is conical and surrounded by a tripartite “layer” comprising an inner, thin, electron-dense layer; a median thick and less electron-dense layer; and an outer thin and more electron-dense layer (Fig. 20). The internal surface of the caps consists of an irregular layer of amorphous material (100-150 nm) to which the rod-like fibrils fuse (Fig. 20). Higher magnifications indicate that this electron-dense material is closely associated with the rod-like fibrils. Both are amorphous and electron-dense. Some electron-dense deposits occur on the rod-like appendage material (Fig. 20).

The collar is less dense or totally absent at the ascospore apex, where the appendages emerge (Fig. 24). The lateral part of the collar is compact, whereas the upper part comprises numerous rod-like fibrils which expand by absorbing water and separate from the upper part (Figs. 26, 27). Each fibril has an amorphous core surrounded by an electron-dense layer and is particularly well defined in the collar (Figs. 25-27). The wall of mature ascospores comprise an electron-dense episporium (*ca* 35 nm thick) and an less electron-dense mesosporium (180 nm thick) which comprises a central electron-transparent layer (Fig. 24).

Discussion

Diluviocola capensis possesses a relatively massive apical ring in mature asci, similar to that found in species of *Annulatascus* (Wong *et al*., 1998a), *Proboscispora* (Wong and Hyde, 1998) and *Rivulicola* (Hyde *et al*., 1997). At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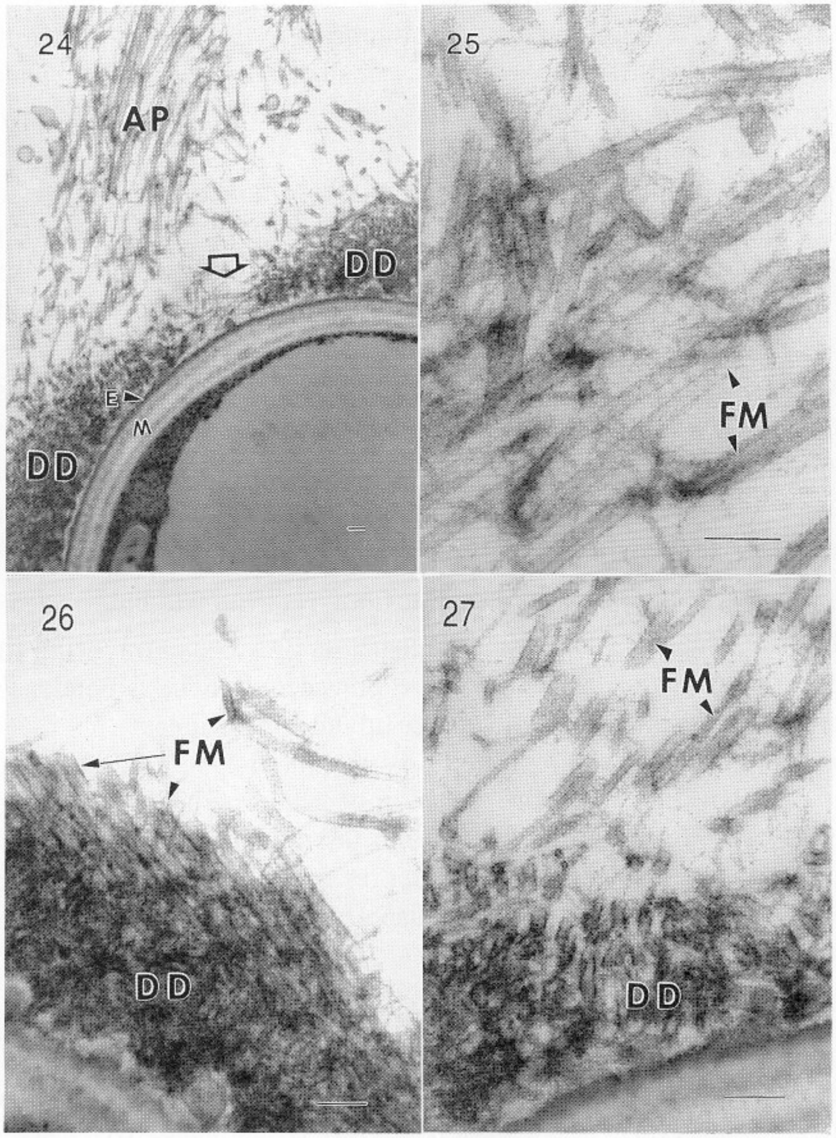
the TEM level, although the general structure of the apical ring differs, they are all bipartite. The upper part of the mature apical ring is differentiated from the outer ascus wall layer, and the lower part appears to elongate downwards and is closely associated with the upper part. Apical rings appear to be similar in all *Annulatascus*-like genera and these fungi have been assigned to a new family, the Annulatascaceae (Wong, Hyde and Jones, 1998b).

Ascospores of *Diluviocola capensis* possess a unique appendage form and unfurling mechanism. Mucilage-filled caps detach from the ascospore poles in water and a long thread-like appendage is formed between the ascospore tip and base of the released cap. The appendages mainly form from inside the caps, whereas, in other species with thread-like appendages, the appendages form from the ascospore (Farrant, 1986). The appendage is a network of dense inter-linked rod-like fibrils which are helically arranged. These rod-like fibrils may originate from (i) the electron-dense polar collar around the spore tip; and/or (ii) the amorphous, electron-dense material at the inner surface of the cap. Some reaction may be involved in converting this material into fibrils. Under the light microscope, it appears that the appendage unravels from within the released cap, so that the cap becomes progressively smaller as it extends away from the spore tip. The material in the cap is therefore considered to be a condensed form and/or precursor of the fibrils which make up the appendages.

The arrangement of the rod-like fibrils and the unique unfurling mechanism (Figs. 17, 28) of the ascospore polar appendages exhibited in *Diluviocola capensis* probably relates to its life style in fast-flowing rivers. The absence of long appendages in newly released ascospores may allow them to drift for short distances which may be important in dispersal. Subsequently, the appendages unfurl and aid ascospore attachment.

Figs. 18-23. *Diluviocola capensis* (from holotype). Transmission electron micrographs. **18, 19.** Longitudinal sections illustrating ascospores with detached caps (PP) filled with rod-like fibrils (FM). The rod-like fibrils are sectioned transversely at the centre (elongate), and longitudinally at the periphery (dot-like). The ascus is thin-walled (AW) and contains numerous glycogen rosettes (GR). **20.** Longitudinal section illustrating the rod-like fibrils (FM) which are closely associated with the electron-dense amorphous material (DF) on the internal surface of the polar caps. The membrane of the polar cap is surrounded by a tripartite layer (TL). Note the electron-dense deposits on the rod-like fibrils. **21.** Transverse section of ascospore appendage illustrating the rod-like fibrils (FM) arranged in a circular orientation. **22, 23.** Oblique longitudinal sections illustrating bipartite apical rings including an upper part (UR) connected to the thick inner ascus wall (IA) and the outer ascus wall (OA); and a lower part (LR) which elongates downwards into the epiplasm. Some electron-dense granular deposits (DB) occur in the inner ascus wall and the interface of the upper and lower apical ring. Note the opening channel within the apical ring. Bars: 18 = 5 µm, 19, 21-23 = 1 µm, 20 = 0.1 µm.



Figs. 24-27. *Diluviocola capensis* (from holotype). Transmission electron micrographs. **24.** Longitudinal section illustrating appendage (AP) derived from the electron-dense collar (DD). The electron-dense collar is almost absent at the ascospore apex (large arrow). Note the ascospore wall which comprises an electron-dense episporium (E) and an electron-transparent mesosporium (M). **25.** Higher magnification of the expanded appendage material illustrating its rod-like (FM) and inter-linked substructure. **26, 27.** Longitudinal (26) and transverse sections (27) of the polar appendage (AP) illustrating the rod-like fibrils (FM) derived from the polar electron-dense collar (DD). The rod-like fibrils expand and separate from the ascospore tip and are inter-linked (arrowed). Bars = 0.1 μm.

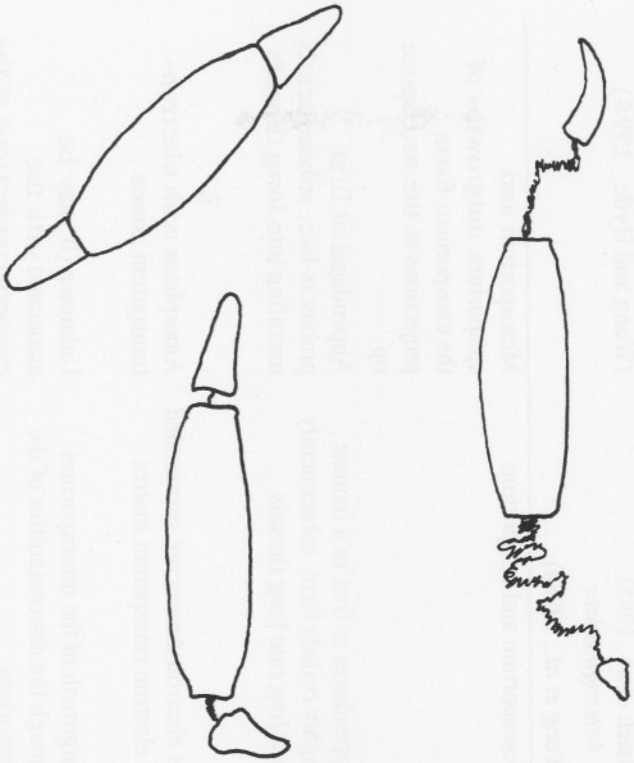


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Fig. 28. Unfurling of ascospore appendage in *Diluviocola capensis*. **a.** Initially, the base of each polar cap is attached at each ascospore tip. **b.** Once in water, the cap detaches from the ascospore tip and a filaments unfurls from inside the caps. **c.** Eventually, a long thread-like appendage is formed.

Several *Halosarpheia* and *Aniptodera* species which have been reported from freshwater (e.g. *H. aquatica* K.D. Hyde, *H. aquadulcis*, *H. lotica* Shearer, *H. retorquens* Shearer and Crane, *H. heteroguttulata*, *A. chesapeakensis* Shearer and Miller and *A. lignatilis*), possess ascospores with unfurling polar appendages (Shearer and Crane, 1980; Shearer, 1984; Farrant, 1986; Hyde, 1992a, b; Hsieh *et al*., 1995; Wong *et al*., 1998a). *Diluviocola capensis* differs from these species in the ontogeny and unfurling mechanism of these appendages (Table 1). In *Halosarpheia* and *Aniptodera* species, the appendages are pre-formed, highly coiled and adpressed to the ascospore wall prior to their release from the ascus. These coiled hamate appendages unfurl and, as a result, long polar thread-like appendages are formed. The appendage material is derived from the mesosporium through the discontinuities of the episporial at the

Table 1. Comparison of ascospore characters of selected freshwater ascomycetes, with unfurling polar appendages, at the light and ultrastructural microscope levels.

	Annulatascus bipolaris (Hyde, 1992b)	*Diluviocola capensis* (this paper)	*Halosarpheia aquadulcis* (Hsieh *et al.*, 1995) *H. heteroguttulata* (Wong *et al.*, 1998a)	*Proboscispora aquatica* (Wong and Hyde, 1998)
Ascospore wall	Unknown	Mesosporium and episporium which elaborates to form verruculose ornamentations	Mesosporium and episporium	Mesosporium and episporium, outgrowths of the exosporium form projections at the ascospore tip
Appendage unfurling mechanism	Appendages at first pad-like, subsequently extending into long threads	Appendages at first within a polar cap, subsequently, detaching and a long thread is formed from inside the cap	Appendages at first in a hamate (highly coiled) form, subsequently uncoiling into long threads	Appendages at first proboscis-like, subsequently uncoiling into long threads
Appendage composition	Unknown	Dense, inter-linked, rod-like fibrils which form a helical framework	An electron-dense core surrounded by electron transparent matrix	Amorphous with electron-transparent zones
Appendage ontogeny	Unknown	Derived from the polar, electron-dense collar around the spore tip and/or from the electron-dense material lying on the inner surface of the polar caps	Outgrowth of the mesosporium through the discontinuities of the episporium	Unknown (It may be associated with the exosporial projections at the ascospore tip)

ascospore tip (Jones, 1995). However, no pre-formed and coiled appendages have been observed in the ascospores of *Diluviocola capensis*. The ascospore appendages in *D. capensis* are formed by the expansion of appendage material in the collar or from the inner surface of the cap. No discontinuity of episporium has been found in *D. capensis*, and the origin of the appendage material is unknown. In addition, the network of rod-like fibrils which occur in *D. capensis* have not been described in *Halosarpheia* and *Aniptodera* species (Shearer and Miller, 1977; Shearer and Crane, 1980; Farrant, 1986; Wong *et al.*, 1998a). *Annulatascus bipolaris* and *Proboscispora aquatica* also differs from *Diluviocola capensis* in lacking polar caps bounded by a tripartite layer (Table 1).

Most thread-like polar appendages reported from ascospores of aquatic ascomycetes are fibrillar in nature, e.g. *Lautisporopsis cirvumvestita* (Yusoff, Jones and Moss, 1994), *Lulworthia* spp. (Yusoff, Jones and Moss, 1995). In some ascomycetes, the appendages are more compact and amorphous e.g. *Ceriosporopsis tubulifera* (Johnson, Jones and Moss, 1987), *Kohlmeyeriella tubulata* (Jones, Johnson and Moss, 1983), *Halosarpheia* spp. (Yusoff, 1991; Hsieh *et al.*, 1995). Therefore, the composition of the ascospore appendage in *Diluviocola* appears to be unique.

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