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Cucullosporella mangrovei, ultrastructure of ascospores and their appendages

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The ultrastructure of *Cucullosporella mangrovei* ascospores is described. Mature ascospores possess two wall layers, an outer electron-dense episporium and an innermost tripartite mesosporium. Episporial elaborations form electron-dense spore wall ornamentations from which extend fibrils that may constitute a highly hydrated exosporium which was not visualised at either the scanning electron microscope or light microscope level. Ascospores possess a hamate appendage at each pole which unfolds in seawater to form a long thread. Ultrastructurally the polar appendage comprises folded fibro-granular electron-dense material and fine fibrils. The fibrils form a matrix around and within the fibro-granular appendage and around the entire unreleased ascospore. These fibrils have not been observed associated with the ascospore appendages in other species of the Halosphaeriales and are a discrete and new appendage component. The fibro-granular appendage and fibrils are bounded by the outer delimiting membrane which is absent around released ascospores. The nature of the spore appendage is compared with that of other marine and freshwater ascomycetes and the taxonomic assignment of the species is discussed.

Key Words——Halosphaeriales; marine fungi; ultrastructure.

Cucullosporella K. D. Hyde & E. B. G. Jones is monotypic, the type being *C. mangrovei* K. D. Hyde & E. B. G. Jones, which is characterized by hyaline one-septate ascospores with uncoiling polar appendages arising from a hood or cap-like structure. Owing to these characters the genus was assigned to the Halosphaeriaceae, Halosphaeriales (Jones, 1995).

In recent years a number of freshwater and marine ascomycetes have been described with polar, hamate appendages that uncoil in water. Many of these have been studied at the ultrastructural level and the results are summarised in Table 1, while others await publication (Wong et al., pers. commun.). *Cucullosporella mangrovei* differs from other species with polar, hamate appendages in that the polar appendages arise from a hood or cap-like structure (Hyde and Jones, 1986). This paper presents information on the ontogeny of this hood and the nature of the ascospore appendages and discusses the validity of the genus.

Materials and Methods

Material of *Cucullosporella mangrovei* was collected on *Bruguiera parviflora* Wight & Arnold ex Griffith test blocks exposed at Kuala Selangor, Malaysia mangrove stand after 32 wk exposure. Ascomata were crushed in filtered sterile seawater to form a suspension of asci and released ascospores. For scanning electron microscopy the ascus and ascospore suspension was filtered on to 5 μm pore size nucleopore membranes and then the membranes with attached material were fixed for 12 h at 4°C in 2% (w/v) aqueous osmium tetroxide (Moss and Jones, 1977). Fixed material was dehydrated in a graded ethanol series, critical point dried, sputter coated with gold and examined with a JEOL T20 SEM.

For transmission electron microscopy a spore suspension of asci and ascospores was embedded in 2% (w/v) ion agar and fixed in freshly prepared 2% (w/v) aqueous potassium permanganate for 15 min at room temperature. Fixed material was dehydrated in a graded ethanol series, transferred to acetone and embedded in Mollenhauer's resin (Mollenhauer, 1964). Ultrathin sections were stained sequentially in lead citrate and uranyl acetate and then examined with a JEOL 100S TEM.

Results

Light microscopy Ascospores of *C. mangrovei* were fusoid to ellipsoid, two-celled, hyaline and possessed polar appendages (Figs. 1, 2). The appendages were initially hamate but later unfolded to form single long thread-like appendages at each pole. At the region of appendage attachment was a polar cap or hood that formed a collar-

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Table 1. Genera of aquatic ascomycetes producing ascospores with unfurling polar appendages.

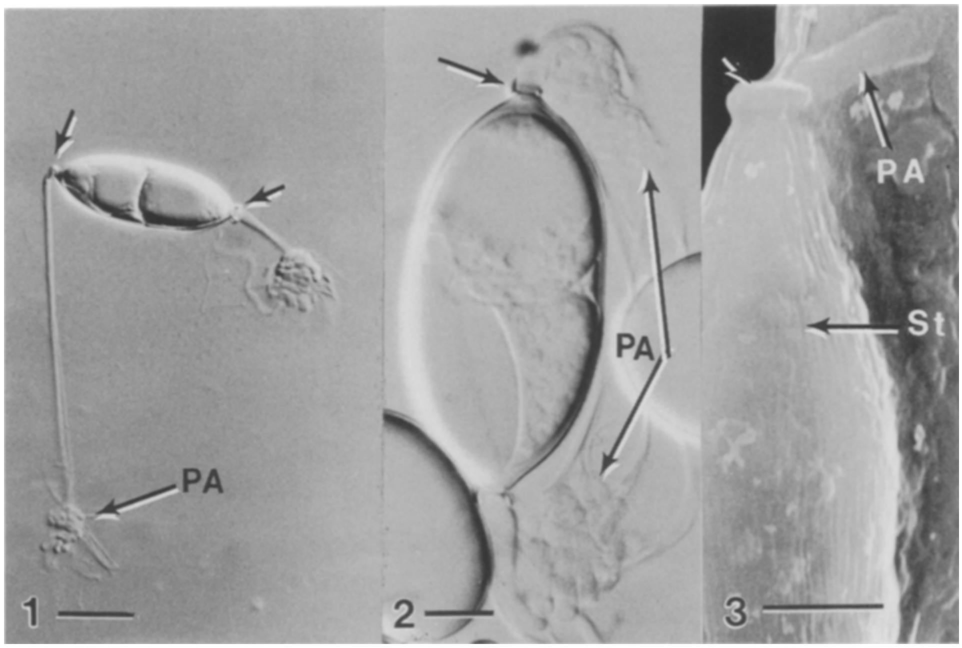
Fungus	Ascospore wall[a)] Exo-	Epi-	Meso-	Structure and origin of appendage
MARINE				
Aniptodera chesapeakensis Shearer & M. Miller (Jones, 1995)	−	+	+ Bipartite	Through episporial discontinuities
Cucullosporella mangrovei (K. D. Hyde & E. B. G. Jones) K. D. Hyde & E. B. G. Jones (Present study)	−	+	+ Tripartite	Polar appendages arise from a hood-like structure at the ascospore poles, which is an outgrowth of the mesosporium and bounded by the episporium Appendages may arise through pores in the episporium within the hood
Halosarpheia abonnis Kohlm., *H. viscosa* (I. Schmidt) Shearer & J. L. Crane (Baker, 1991)	−	+	+ Bipartite	Through episporial discontinuities
Halosarpheia minuta Leong (Baker, 1991)	−	+	+ Unipartite	Through episporial discontinuities
Halosarpheia trullifera (Kohlm.) E. B. G. Jones, S. T. Moss & Cuomo (Farrant, 1986)	+	−	+ Unipartite	Through episporial discontinuities
Ophiodeira monosemeia Kohlm. & Volkm.-Kohlm. (Kohlm. and Volkm.-Kohlm., 1988)	?	?	?	No details available
Tirispora unicaudata E. B. G. Jones & Vrijmoed (Jones et al., 1994)	−	+	+ Unipartite	May arise through episporial discontinuities
Trichomaris invadens Hibbits, G. C. Hughes & Sparks (Porter, 1982)	−	+	+ Unipartite	Outgrowth of the episporium
Tunicatispora australiensis K. D. Hyde (McKeown et al., 1996)	+	+	+ Unipartite	Through episporial discontinuities
FRESHWATER				
Cataractispora aquatica K. D. Hyde, S. W. Wong & E. B. G. Jones *C. appendiculata* K. D. Hyde, S. W. Wong & E. B. G. Jones *C. viscosa* K. D. Hyde, S. W. Wong & E. B. G. Jones (Wong et al., 1999a)	− − −	+ + +	+ + +	This genus has a polar chamber from which thread-like polar appendages are released. Polar chamber arises as an extension of the episporium
Diluvicola capensis S. W. Wong, K. D. Hyde & E. B. G. Jones (Hyde et al., 1998)	−	+	+	Derived from the polar electron-dense collar around the spore tip. Appendages rod-like with another electron-dense layer and a core of less electron-dense material
Fluminicola coronata S. W. Wong, K. D. Hyde & E. B. G. Jones (Wong et al., 1999b)	−	+	+	Appendage not strictly hamate; initially adpressed to spore wall but retracts to form a polar crown-like appendage Arises through discontinuities in the episporium
Halosarpheia aquadulcis S.-Y. Hsieh, H. S. Chang & E. B. G. Jones (Hsieh et al., 1995)	−	+	+	Through episporial discontinuities
Halosphaeria heteroguttulata S. W. Wong, K. D. Hyde & E. B. G. Jones (Wong et al., 1998)	−	+	+	Arises from a pore field
Phaeonectriella lignicola R. A. Eaton & E. B. G. Jones (Hyde et al., 1999)	?	?	?	No details available
Proboscispora aquatica S. W. Wong & K. D. Hyde (Wong and Hyde, 1999)	+	+	+	Not resolved May be associated with the exosporial projections at the ascospore tip

a) +, present; −, absent; ?, unknown; exo-, exosporium; epi-, episporium; meso-, mesosporium.

like structure around the base of the appendage (Figs. 1, 2). The asci were unitunicate, clavate and thin-walled.

Scanning electron microscopy Ascospores possessed longitudinal surface striations that extended between the poles. Polar appendages in released ascospores were unfolded, and originated from a polar collar-like structure (Fig. 3).

Transmission electron microscopy The wall of the ascospores of *C. mangrovei* comprised an outer episporium and an innermost mesosporium (Figs. 7, 9). The episporium was electron-dense and irregular in thickness (160–300 nm) with the thickened regions forming discrete surface ornamentation (Figs. 9–11) which constitute the wall striations observed at the scanning electron microscope level (Fig. 3). Extending from the episporium were fibrils (Fig. 11) that may form a sheath around the ascospore. Whether this sheath is an exosporium or an elaboration of the episporium requires clarification. The mesosporium was tripartite. The outermost region was 300–400 nm thick and consisted of fibres orientated radially and which terminated in a less organised region adjacent to the episporium (Figs. 8–10).



Figs. 1–3. *Cucullosporella mangrovei*. 1, 2. Light (DIC) micrographs. Ascospores fusoid to ellipsoid, 1-septate, hyaline with polar appendages (PA) initially hamate (Fig. 2) but later unfurl (Fig. 1) in water to form long thread-like appendages. Polar appendages originate from within a polar collar (arrowed). 3. Scanning electron micrograph. Ascospore within a collapsed ascus. The ascospore wall is striated (St) and the polar collar (arrowed) surrounds the region of appendage (PA) attachment. Scales: 1=20 μm; 2=10 μm; 3=5 μm.

The middle region of the mesosporium was 100–200 nm thick and fibro-granular, and the innermost region finely fibrillar (Fig. 10). Variation in the degree of differentiation between the three mesosporial regions (Figs. 5, 7–10) could not be attributed to maturity of the ascospore and may reflect only differences in the thickness of sections, staining procedures or other preparation parameters.

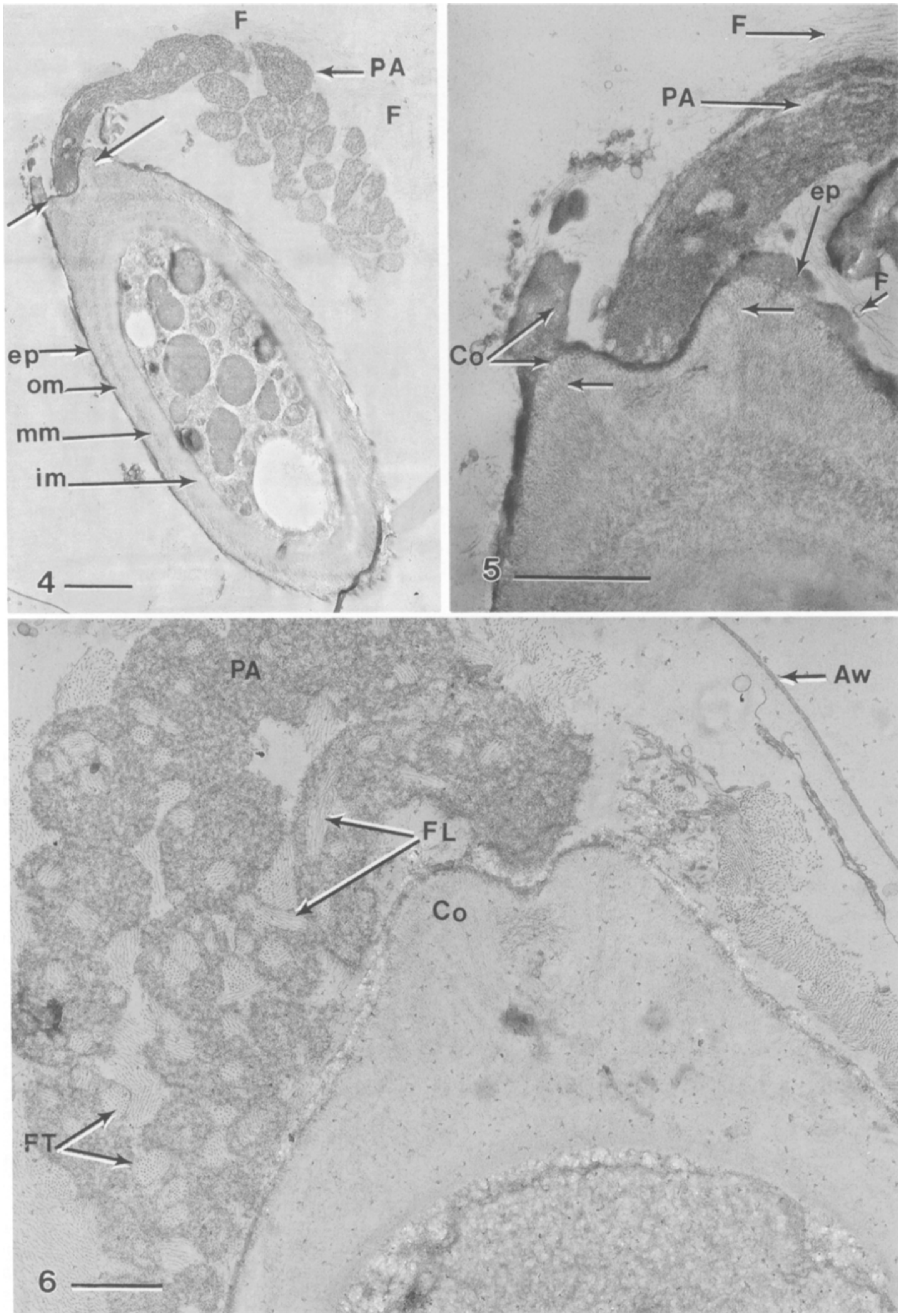
At the ascospore poles the outer region of the mesosporium, bounded by the episporium, was thickened to form a collar-like structure within which the base of the appendage was contiguous with the episporium (Figs. 4, 5). This thickening was also fibrillar, with the fibres extending from the middle layer of the mesosporium to the episporium (Figs. 5, 7, 10). In some sections (Figs. 4, 5) the collar was extended to surround the base of the appendage which may indicate that the collar is larger in immature ascospores.

The base of the appendage was contiguous with the episporium within the collar, although no differentiation of the episporium in this region was detected (Fig. 5). The appendages of unreleased ascospores comprised two discrete components: a coiled electron-dense fibro-granular material; fine fibrils that occurred within and around the coiled fibro-granular component of the appendage (Figs. 6–9). These fibrils also extended laterally adjacent to the ascospore wall (Figs. 6, 7). The fibro-granular region of the appendage was of similar electron density to the episporium and the fibrils appeared to be closely associated, or even continuous with the surface of the appendage (Fig. 5) as well as with the episporium. In sectioned material the fibrils were within discrete, 0.5–1.0 μm diam., "locules" (Figs. 6, 8), with most of the fibrils in a locule orientated in the same direction; an orientation that may result from the spatial constraints imposed by the fibro-granular region of the appendage. The fibrils also formed a matrix around the granular component of the appendage (Fig. 8). The outer delimiting membrane (Fig. 7) surrounded the fibro-granular material and fibrils of the appendages of unreleased ascospores, whereas in released ascospores the delimiting membrane was absent (Fig. 4).

Discussion

Spore appendages are thought to have an ecological role as a mechanism for spore dispersal and attachment/entrapment (Hyde et al., 1989). Attachment studies of marine ascomycetes have shown several types of appendages: release of a drop of mucilage from polar appendages; hair-like sticky appendages; pads composed of fibrillar material (Jones, 1994). Jones and Moss (1980, 1987), Jones et al. (1983, 1984, 1986), Jones (1995) and Johnson et al. (1987) concluded, after intensive ultrastructural studies of the ascospores of marine fungi, that ascospore appendages and their ontogeny are taxonomically important characters at the generic level in the Halosphaeriales.

A number of marine and freshwater ascomycetous genera have species with hamate polar appendages that uncoil at maturity when placed in water: *Aniptodera* Shearer & M. A. Mill.; *Buxetroldia* K. R. L. Petersen & J. Koch; *Halosarpheia* Kohlm. & E. Kohlm.; *Ophiodeira*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Kohlm. & Volkm.-Kohlm.; *Phaeonectriella* R. A. Eaton & E. B. G. Jones; *Tirispora* E. B. G. Jones & Vrijmoed; *Trichomaris* Hibbits, G. C. Hughes & Sparks; *Tunicatispora* K. D. Hyde. These genera can be separated on the basis of light microscope observations, e.g. in species of *Tirispora* and *Ophiodeira* the ascospores possess only one polar appendage, whereas in *C. mangrovei* the appendage arises from a hood-like structure at each pole. However, ontogeny of the appendages can only be determined by electron microscope studies.

In most species of the Halosphaeriales with unfurling appendages the ascospore wall comprises an episporium and mesosporium (e.g. *Tirispora unicaudata* E. B. G. Jones & Vrijmoed; Jones et al. 1994). However, in other species an exosporial layer is also present, e.g. *Halosarpheia trullifera* (Kohlm.) E. B. G. Jones, S. T. Moss & Cuomo (Farrant, 1986), *Tunicatispora australiensis* K. D. Hyde (McKeown et al., 1996). In species of *Aniptodera* and *Halosarpheia* the ascospore wall comprises an electron-dense unipartite episporium and an electron-transparent mesosporium (Farrant, 1986), although the mesosporium may vary in thickness and number of layers between species (Table 1). The significance of the complexity of the mesosporium requires further study in order to identify the effects of different fixation and staining procedures and changes that occur as a spore matures.

In species of *Aniptodera* and *Halosarpheia* polar appendages arise as outgrowths of the spore wall through discontinuities in the episporium (Hsieh et al., 1995). In *Trichomaris invadens* Hibbits, G. C. Hughes & Sparks the appendages are deposited within an enlarged spore sac and are described as outgrowths of the episporium although there is no evidence to suggest that the appendages are a product of extrusion from within the developing spore or fragmentation of pre-formed material (Porter, 1982).

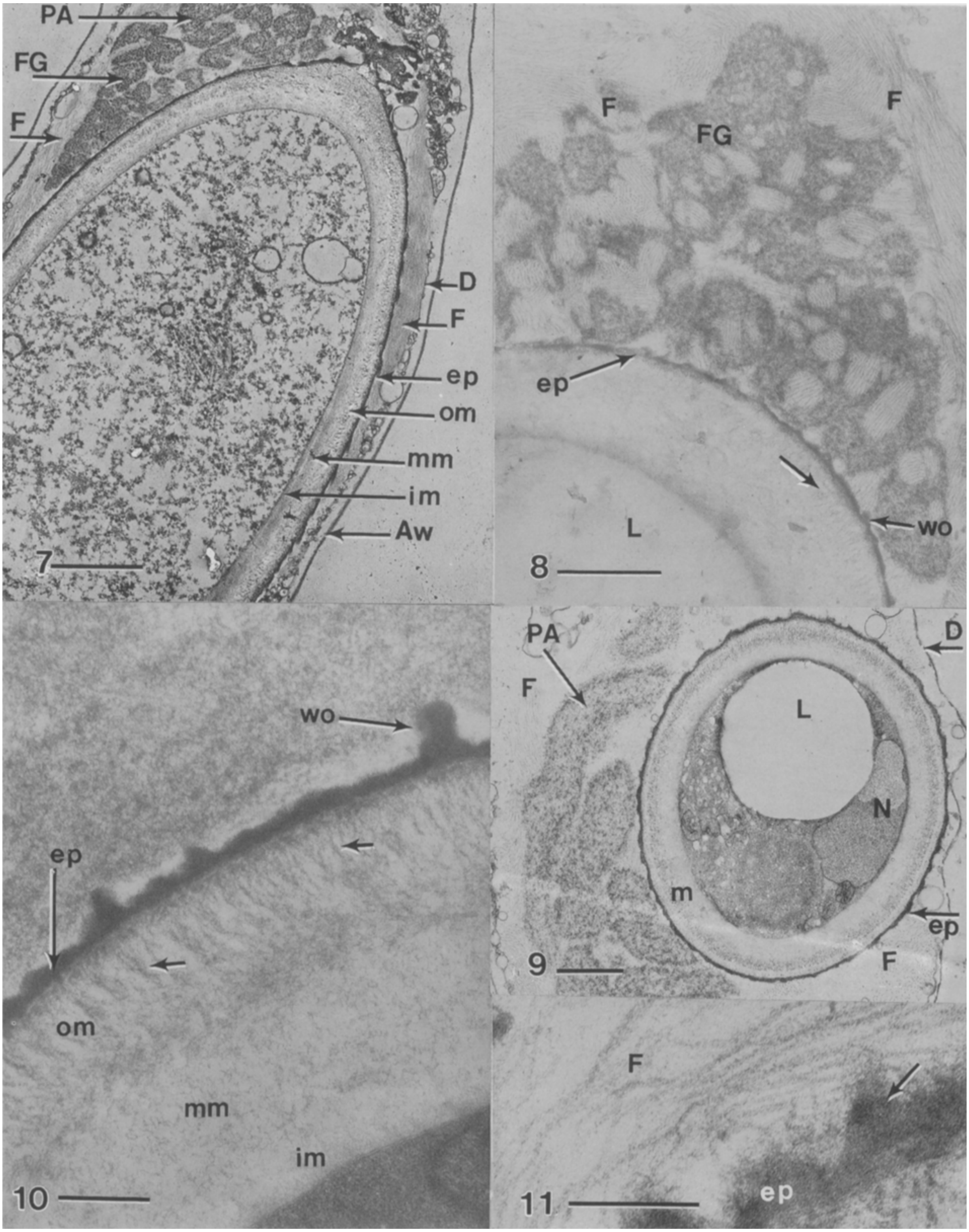
In *Cucullosporella mangrovei*, at the light microscope level, the polar appendage arises from a hood. Hyde and Jones (1986) examined this species at the scanning microscope level and suggested that the hood is an extension of the ascospore wall. Our studies show that a thickening of the outer, fibrillar region of the mesosporium forms a collar, extensions of this become the hood. The hood may enclose the developing appendage and ruptures to release the appendage. The appendage of *C. mangrovei* is distinct from those of all other species of the Halosphaeriales in that it has associated fibrils. These fibrils form a matrix between folds (coils) of the appendage and also extend laterally towards the midseptum. The origin of the fibrils is not known but they are continuous with the fibro-granular component of the appendage and also with the episporium at the poles. It is speculated that upon ascospore release the fibrils may absorb water and contribute to the unfolding of the appendage. In other species of the Halosphaeriales with polar unfolding appendages, the matrix around the appendage is fibro-granular or granular but never consists of regularly orientated fibrils as found in *C. mangrovei*. The fibrils that extend from the episporium in the non-polar regions of the ascospore may be structurally similar to those associated with the appendage but less organised in their orientation. Whether these fibrils represent an elaboration of the episporium or an exosporial sheath that hydrates in water requires clarification. There is no evidence of this layer (sheath) at either the light microscope or scanning electron microscope levels.

Ornamentations of the ascospore wall, excluding the appendages, in the Halosphaeriaceae can be observed in *Halosarpheia spartinae* (E. B. G. Jones) Shearer & J. L. Crane (Jones and Moss, 1987) and *C. mangrovei* (Hyde and Jones, 1986). In *H. spartinae* small papillae occur on the surface of the ascospore wall, while in *C. mangrovei* the surface of the ascospore wall is striated.

Ultrastructurally, the ascospore, and ascospore appendage morphology and ontogeny confirm that *Cucullosporella* is a valid genus in the Halosphaeriaceae but differs from other genera with unfurling polar append-

Figs. 4–6. *Cucullosporella mangrovei*. Transmission electron micrographs. 4. Oblique longitudinal section of a released ascospore with polar appendage (PA), and the polar collar (arrowed) formed from the episporium (ep) and outer region of the mesosporium (om). The polar appendage (PA) comprises folded electron-dense fibro-granular material with associated fibrils (F). mm, middle region of mesosporium; im, inner region of mesosporium. 5. Longitudinal section of a collar (Co), polar appendage (PA), fibrils (F). Fibrillar material (arrowed) within the mesosporium extends towards the episporium (ep). 6. Longitudinal section of a polar appendage (PA) arising from the depression within the collar (Co) showing the locules containing sectioned longitudinally (FL) and transversely (FT). Aw, ascus wall. Scales: 4=2 μm; 5=1 μm; 6=1 μm.

Figs. 7–11. *Cucullosporella mangrovei*. Transmission electron micrographs. 7. Oblique section of mature ascospore within an ascus (Aw). The ascospore wall comprises two layers: a mesosporium comprising an outer region (om), an inner region (im) and a middle region (mm); an electron-dense episporium (ep). The polar appendage (PA) is coiled into a hamate arrangement and consists of a fibro-granular strand (FG), 1.0–1.6 μm diam that is attached to the episporium at the pole where the mesosporium is thickened. Surrounding the appendage and extending laterally around the ascospore are regularly arranged fibrils (F). D, outer delimiting membrane. 8. Section through the hamate polar appendage. The fibro-granular component (FG) of the appendage is surrounded by fibrils (F). The spore wall ornamentation (wo) is a thickening of the episporium (ep). Fibres in the outer region of the mesosporium are arranged radially (arrowed), L, lipid body. 9. Transverse section through the sub-polar region of an immature ascospore showing the polar appendage (PA) and associated fibrils (F). The episporium (ep) is thickened to form surface ornamentations and the mesosporium (m) is tripartite. D, outer delimiting membrane; L, lipid body; N, nucleus. 10. Section through an ascospore wall showing the tripartite mesosporium: outer region (om) comprises fibres (arrowed) that extend to and are contiguous with the episporium; the middle region (mm) comprises less organised fibres; the inner region (im) possesses fibres orientated at right angles to the plasma-membrane. ep, episporium; wo, wall ornamentations. 11. Section through the ascospore wall showing striations (arrowed) of the episporium (ep) in transverse section and from which the fibrils arise (F). Scales: 7=5 μm; 8, 9=1 μm; 10, 11=250 nm.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ages in having: a polar hood formed by the thickening of the mesosporium bounded by the episporium; a polar appendage attached to the spore wall at the base of the hood; an appendage comprising folded fibro-granular electron-dense material with associated fine fibrils; and surface ornamentations (striae) formed from the episporium of the ascospore and from which extend fibrils.

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