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Ultrastructural observations of asci, ascospores and appendages of *Massarina armatispora* (Ascomycota)

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***Massarina armatispora* (Ascomycota, Dothideales, Lophiostomataceae) is described for the first time at the ultrastructural level. Two new structures were observed for this species. Firstly, the ascospores were shown to possess polar chambers formed from the episporium and which contained a fibrillar material and secondly, a lateral fibrillar appendage-like structure. The similarities between marine ascomycetes *Massarina armatispora*, *Massarina thalassiae* and *Paraliomyces lentiferus* are discussed.**

Key Words——bitunicate ascus; Loculoascomycetes; mangrove fungus; ultrastructure.

A recent study of the intertidal mangrove fungi of Macau, Hong Kong and India (Hyde et al., 1992) yielded a new marine loculoascomycete *Massarina armatispora* K. D. Hyde, Vrijmoed, Chinnaraj et E. B. G. Jones. This species differs from other marine species of *Massarina* in that the ascospore sheath is extended at the poles to form appendages. Most studies of marine ascomycetes have been confined to species with unitunicate deliquescent asci, largely within the Halosphaeriaceae (Moss, 1990; Jones, 1995). The ascospores of these species frequently possess elaborate appendages and only rarely form mucilaginous sheaths, e.g., *Nimbospora effusa* J. Koch and *N. bipolaris* K. D. Hyde et E. B. G. Jones (Read et al., 1993). Ultrastructural studies of genera with bitunicate asci have only recently been initiated and it has been suggested that elaborate appendages would impede ascospore release in species with active spore discharge (Read et al., 1992).

The aim of this investigation was to extend our knowledge of the marine, intertidal and mangrove Loculoascomycetes, in particular to examine the asci, ascospores and ascospore sheath of *M. armatispora*. These structures are discussed in relation to the information which already exists for unitunicate and other bitunicate marine ascomycetes.

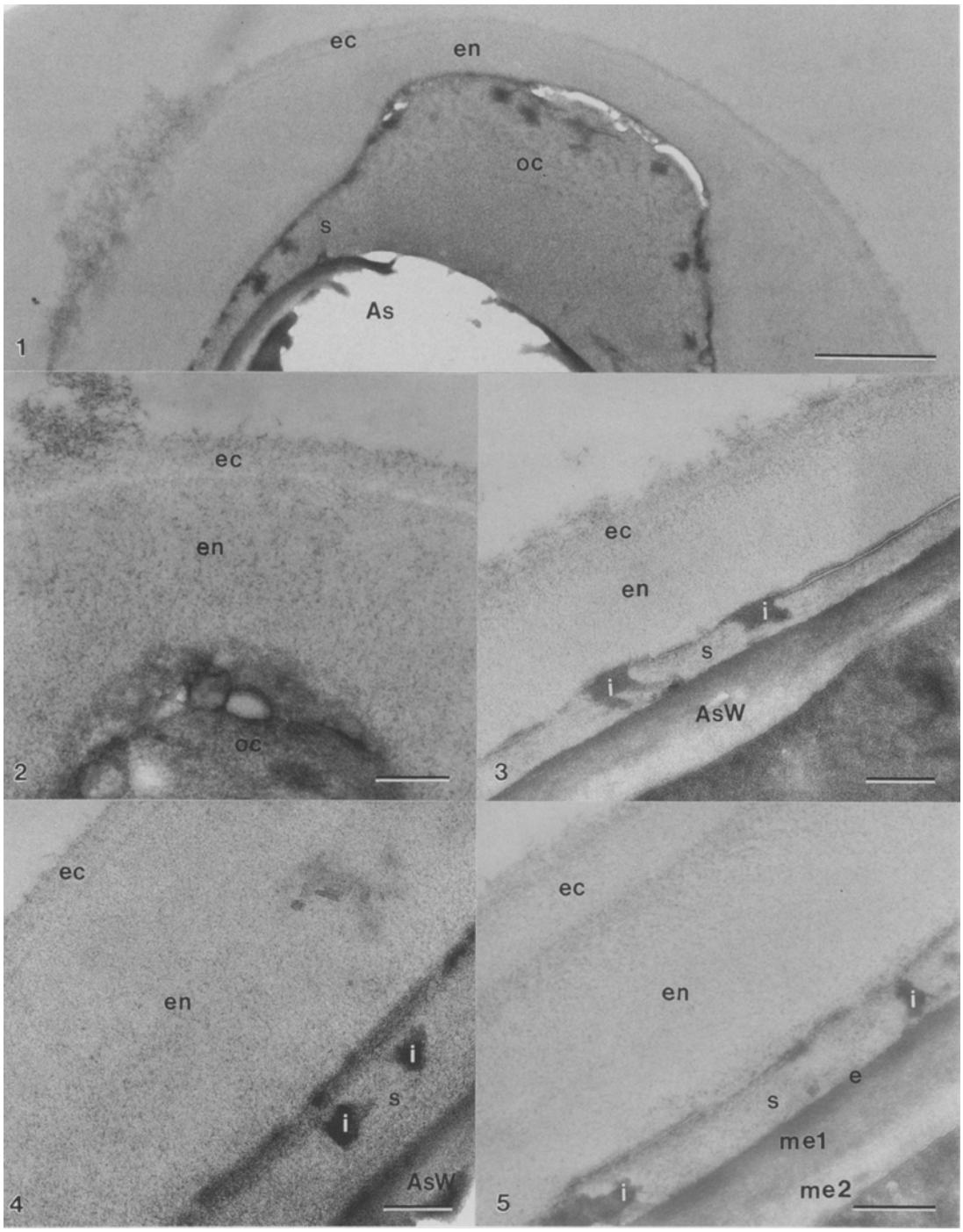
Materials and Methods

Intertidal mangrove wood was collected at Three Fathoms Cove, New Territories, Hong Kong, incubated in the laboratory and examined periodically for ascomata. Ascospores for scanning electron microscopy (SEM) were settled on to nucleopore membranes, fixed with 2% (w/v) aqueous osmium tetroxide, dehydrated through a graded ethanol series, transferred to acetone and critical point dried. Dried material was coated with gold and examined in a JEOL T20 SEM at 20 kV. Asci and ascospores for transmission electron microscopy (TEM) were embedded in 2% (w/v) ion agar, fixed in 2% (w/v) potassium permanganate, washed, dehydrated through a graded ethanol series, transferred to acetone and embedded in Mollenhauer's resin (Mollenhauer, 1964). Ultrathin sections were stained with lead citrate, post stained with uranyl acetate and examined at 80 kV in a JEOL 100S transmission electron microscope.

Results

Asci Asci were bitunicate (Figs. 1–5) although the ascus wall layers showed little contrast. At the apex of the intact ascus, the ectoascus appeared to be undifferentiated (Figs. 1, 2). Subapically the endoascus was thickened to ~1–1.1 μm whereas at the tip of the ascus, the endoascus was 350–400 nm thick, and formed an ocular chamber (Fig. 1). The endoascus was amorphous and thicker than the ectoascus and both layers were thinner at the apex (Figs. 3, 4) (endoascus = 460–470 nm; ectoascus = 85–100 nm) than at the base (Fig. 5) (endoascus = 650–680 nm; ectoascus = 260–300 nm). External to the ectoascus was a disorganized layer of fibrillar material (Figs. 3–5).

Ascospores Whilst within the ascus mature ascospores had a bipartite mesosporium (me1 = 250–270 nm; me2 = 235–250 nm) and a 35–40 nm thick, electron-dense episporium (Fig. 5). The episporium was surrounded by a 170–300 nm thick, fibrillar sheath which contained numerous inclusions of different electron-density (Figs. 3–5). The sheath extended polarly to form up to 7 μm long, tapering, curved, polar appendages (Figs. 6–8). Electron-dense inclusions were also present wi-



Figs. 1–5. *Massarina armatispora*. Transmission electron micrographs. Ascus apex and ascus wall structure.
1. Ascus apex is subapically thickened and contains an ocular chamber (oc). The ascus wall at the apex comprises the ectoascus (ec) and an endoascus (en). The polar extension of the mucilaginous sheath (s) of the terminal ascospore (As) is compressed within the ocular chamber. 2. Wall layers at the ascus apex: ectoascus (ec) is narrower than the endoascus (en); oc=ocular chamber. 3, 4. Subapically, the ectoascus (ec) and the endoascus (en) are much thinner than at the apex. Note that the ascospore wall (AsW) is surrounded by a sheath (s) which contains electron-dense inclusions (i). 5. Ectoascus (ec) and endoascus (en) at the base of the ascus. The ascospore wall comprises a mesosporium (me1, me2) and an episporium (e) and is surrounded by a sheath (s) which contains electron-dense inclusions (i).
Scale bars: Fig. 1=1 μm; Figs. 2–5=250 nm.

thin the polar appendages (Figs. 7, 8). At each pole of the ascospore, within the mucilaginous polar appendage, the episporium was irregularly evaginated to delimit a polar end chamber (Figs. 7–11). This ranged from a simple structure (Fig. 9) to a complex and convoluted chamber up to 1 μm across and 1.5 μm in length (Figs. 7, 8, 10, 11). This difference in its complexity may be accounted for by the plane of the sections. The contents were similar in morphology to that of the polar appendage.

In mature ascospores within asci the mucilaginous sheath possessed an electron-dense outer margin which was associated with the electron-dense inclusions 130–175 nm across and which in places were contiguous with the episporium (Figs. 12, 16, 17). Where the inclusions were most numerous, particularly adjacent to the septum on one side of the ascospore and external to the sheath, was a layer comprising electron-dense fibrils (Figs. 14, 15, 17). This layer was 200–420 nm thick and extended, in some cases, as far as the polar appendages where it was much narrower, only ~75 nm thick (Fig. 16). In oblique sections the majority of the fibrils was orientated along the longitudinal axis of the ascospore. There was some evidence for continuity between the fibrils and the episporium via the electron-dense inclusions within the mucilaginous sheath (Figs. 16, 17).

Discussion

Massarina armatispora is a species only recently described at the light microscope level (Hyde et al., 1992). Ultrastructural examination has revealed structures that could not be resolved at the light microscope level. The polar chambers and the layer of fibrils external to the mucilaginous sheath were not reported in the original description. Recently Kohlmeyer et al. (1995) have described a new marine *Massarina* (*M. ricifera* Kohlm., Volkm.-Kohlm. et O. E. Eriksson: 1995) which has a two-layered gelatinous sheath. This was observed only at the light microscope level.

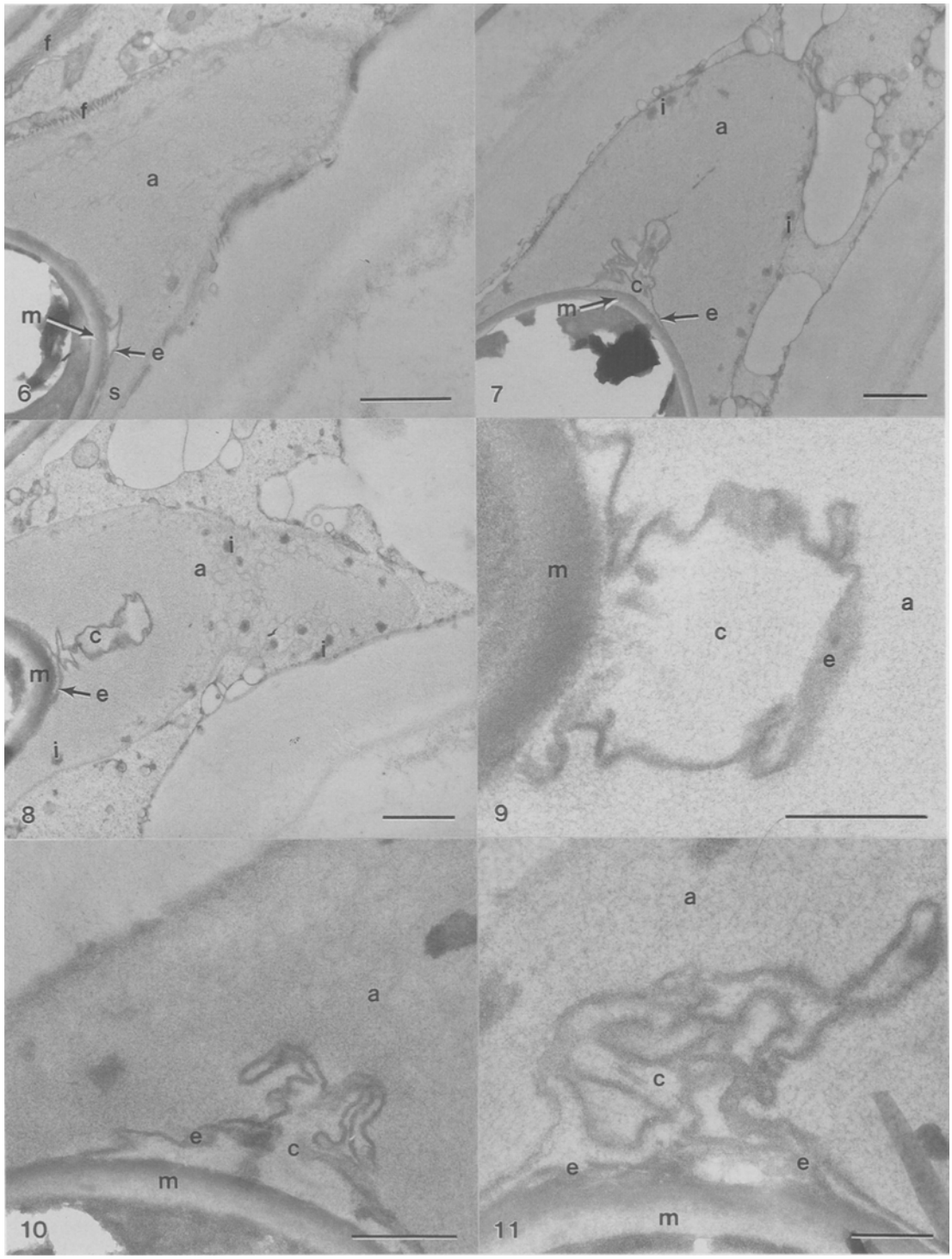
Polar chambers formed by extension of the episporium in *M. armatispora* have also been observed in *M. thalassiae* Kohlm. et Volkm.-Kohlm. (Read et al., 1994). However, the polar chambers were more highly developed in *M. armatispora* than in *M. thalassiae*. Similar polar chambers are to be found within the Halosphaeriales, e.g., *Ondiniella torquata* (Kohlm.) E. B. G. Jones, R. G. Johnson et S. T. Moss, *Kohlmeyeriella tubulata* (Kohlm.) E. B. G. Jones, R. G. Johnson et S. T. Moss and in *Ceriosporopsis tubulifera* (Kohlm.) Kirk (Jones, 1995). However, in *O. torquata* the appendages are formed as direct outgrowths of the episporium (Jones et al., 1984), whereas in *K. tubulata* a polar chamber containing mucilage is formed from both the episporium and the outer region of the mesosporium (Jones et al., 1983) while in *C. tubulifera* a polar chamber is formed from the exosporium (Johnson et al., 1987). Therefore, the formation of polar chambers solely by extension of the episporium observed in *M. armatispora* and *M. thalassiae* is unique among the marine bitunicate ascomycetes examined to date (Table 1).

The layer of fibrils external to the mucilaginous sheath in *M. armatispora* was remarkably similar, in both position and structure, to the lateral appendage of *Paraliomyces lentiferus* Kohlm. another marine bitunicate species (Read et al., 1992). Species of *Massarina* and *Paraliomyces* have many other features in common. All possess bitunicate asci with similar wall layers, ascus apical structures and ascospore walls comprising a mesosporium, episporium and an extensive extracellular mucilaginous sheath. In both *P. lentiferus* and *M. armatispora* a layer of fibrils is present in a localized area on one side of the ascospore and, it is hypothesized, formed by secretion of an electron-dense material through the mucilaginous sheath.

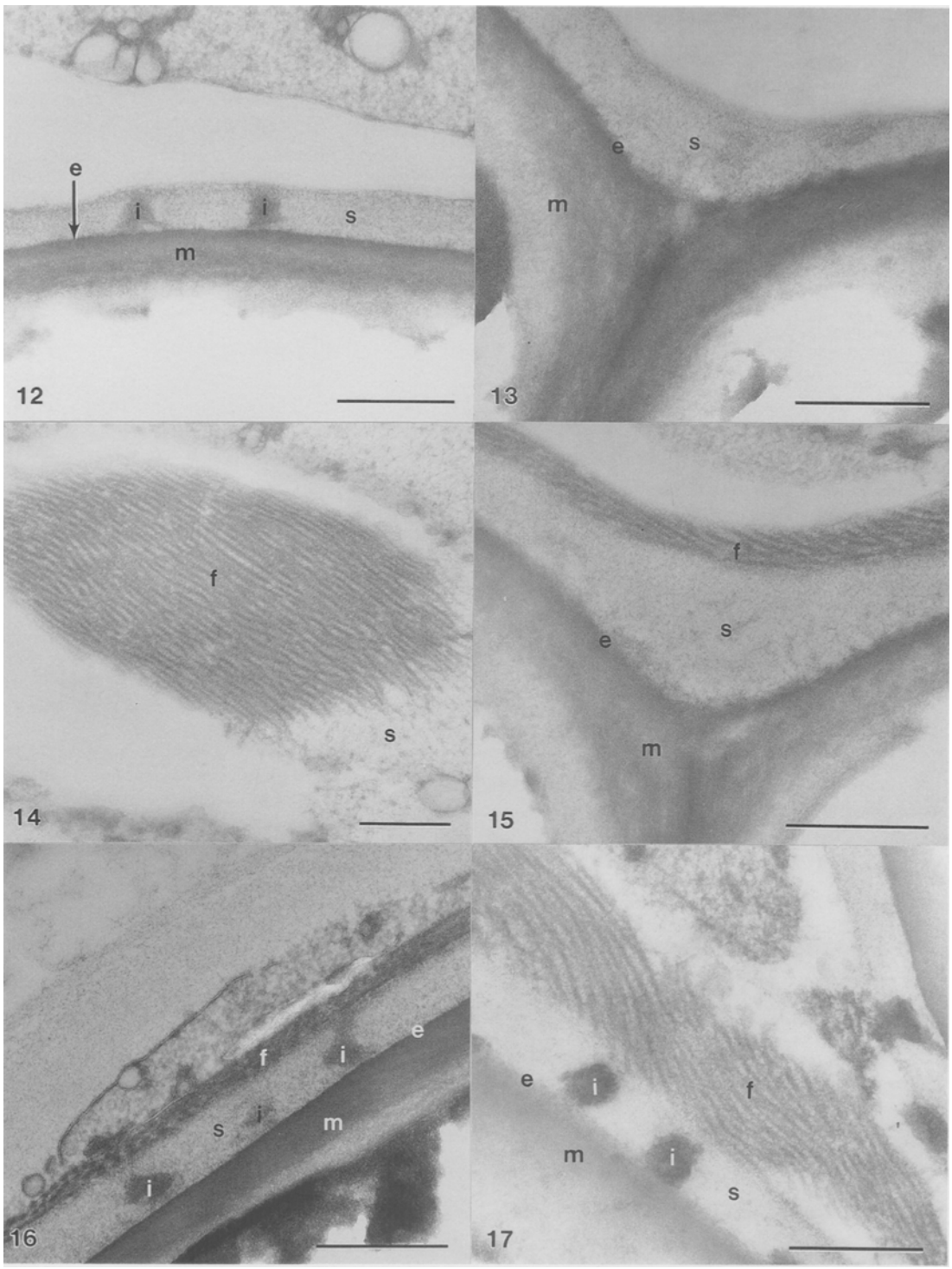
It is of interest to compare and contrast the ultrastructure of the asci and ascospores of *M. armatispora* with those of the Halosphaeriaceae. The asci of *M. armatispora* are functionally bitunicate and persistent, in contrast to those of the Halosphaeriaceae which are unitunicate and frequently deliquesce to release their ascospores passively. Very few species of the Halosphaeriaceae possess ascospores with extensive, persistent mucilaginous sheaths, namely three *Nimbospora* species. In *N. bipolaris* the sheath is formed prior to the mesosporium and is present in a highly folded state. Subsequent to ascospore release the sheath swells and unfolds while the equatorial appendages penetrate through the sheath (Read et al., 1993). Conversely, in *M. armatispora*, although the sheath is formed prior to the mesosporium, it is not folded, rather it is present in a condensed state.

The presence of both polar chambers and a lateral fibrillar layer in *M. armatispora* raises an interesting possibility. It could be argued that these characteristics of *M. armatispora* create a link between *M. thalassiae* which possesses a polar chamber and *P. lentiferus* with an extensive lateral fibrillar appendage. However, since *Paraliomyces* is a monospecific genus and only two *Massarina* species have been examined ultrastructurally it is too early to contemplate reassigning any of the three species involved. This study, and those on *M. thalassiae* (Read et al., 1994) and *Paraliomyces lentiferus* (Read et al., 1992) have illustrated that the initial criteria used for the delimitation of ascomycete genera may need revision based on ultrastructural details.

Based on the results presented in this paper *M. armatispora* is distinct from *M. thalassiae* in possession of a layer of fibrils which may be homologous to the lateral fibrillar appendage in *P. lentiferus*. The possession of polar chambers would seem to have developed independently in several evolutionary lines within the marine fungi, although they are generally associated with secretory activity. Taxonomic changes must avoid the use of convergent characters and thus the presence of polar end chambers must not be considered of primary taxonomic importance. The ultrastructure of *M. thalassiae* and *M. armatispora* indicates many similarities but more detailed studies, perhaps at the molecular level, may be needed to resolve the affinity of these species to each other and to



Figs. 6–11. *Massarina armatispora*. Transmission electron micrographs. Wall structure and appendages of unreleased ascospores. 6. Polar extension of the sheath (s) which constitutes the primary appendage (a). Note the presence of electron-dense fibrils (f) external to the primary appendage and a more extensive region of fibrils on an adjacent ascospore. The ascospore wall comprises a mesosporium (arrowed m) and an extended episporium (arrowed e). 7, 8. Median sections through the mucilaginous primary appendages (a) of two ascospores. There is a polar chamber (c), external to the mesosporium (arrowed m), formed from the episporium (arrowed e) within the primary appendage. Note the electron-dense inclusions (i) within the sheath. 9–11. Higher magnification of the mucilaginous appendages (a) and polar chambers (c) of different ascospores. The polar chamber may be simple (Fig. 9) or very elaborate (Figs. 10, 11) but always formed by extension of the episporium (e). Mesosporium = m.
Scale bars: Figs. 6–8 = 1 μm; Figs. 9, 10 = 500 nm; Fig. 11 = 250 nm.



Figs. 12–17. *Massarina armatispora*. Transmission electron micrographs. Wall layers and appendages of unreleased ascospores. 12. The electron-dense ascospore wall comprises an episporium (arrowed e) and mesosporium (m) and is surrounded by a less electron-dense mucilaginous sheath (s) which contains electron-dense inclusions (i). 13. Adjacent to one side of the ascospore septum the outer region of the mesosporium (m) is electron-dense and surrounded by the episporium (e) and a less electron-dense sheath (s). 14. An oblique section through an ascospore showing the presence of fibrils (f) external to the mucilaginous sheath (s). 15. Micrograph of the opposite side of the ascospore shown in Fig. 13 showing the ascospore wall; mesosporium (m), episporium (e) and mucilaginous sheath (s). Note the presence of electron-dense fibrils (f) external to the sheath. 16, 17. The ascospore wall: *mesosporium* (m), *episporium* (e) and fibrils (f) external to the mucilaginous sheath (s). The fibrils are connected to the episporium via electron-dense inclusions (i) within the sheath.
Scale bars: Figs. 12–17 = 500 nm.

Table 1. Comparison of the ascospores and sheaths of three marine *Massarina* species and *Paraliomyces lentiferus*.

Species	*M. thalassiae*	*M. armatispora*	*M. ramunculicola* Hyde	*P. lentiferus*
Spore size	28–44(–47) × 10–15 μm	28.0–38.2 × 7.0–9.8 μm	35.0–42.5 × 12.5–18.0 μm	17.7–28.6 × 8.6–13.6 μm
Septation	1–(1–3)	1	1	1
Sheath and appendage	1 expanding to ~3 μm after release with an outer electron-dense fibrillar layer that is discontinuous at the poles. No appendages are present.	~1 μm thick with an outer electron-dense fibrillar sheath extended to surround the end chambers. An electron-dense fibrillar layer is located at one side of the ascospore external to the sheath.	1.5 μm expanding to 5 μm after release with an outer fibrillar electron-dense layer that is discontinuous at the poles. Electron-dense fibrils extend through the discontinuities in the outer layer of the sheath to form a cap.	~0.8 μm thick, not discontinuous at the poles, no electron-dense outer layer. An electron-dense, fibrillar appendage is located at one side of the septum external to the sheath.
Polar caps	Simple polar caps formed from the episporium	Convoluted polar caps formed from the episporium	None	None

P. lentiferus.

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