Halosarpheia heteroguttulata sp.nov. from submerged wood in streams

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

Abstract: A new species of *Halosarpheia*, *H. heteroguttulata*, is described from wood submerged in streams and lakes in Australia, Brunei, Hong Kong, Mauritius, the Philippines, and South Africa. It differs from other species in the genus in ascospore dimensions, and consistently large guttule(s) in the apical cell, but many smaller guttules in the basal cell. The species is illustrated with light and scanning and transmission electron micrographs and compared with other *Halosarpheia* species.

Key words: appendage ontogeny, freshwater Ascomycete, *Halosarpheia*, taxonomy, ascospore ultrastructure.

Résumé : Les auteurs décrivent une nouvelle espèce d'*Halosarpheia*, l'*H. heteroguttulata*, récoltée sur du bois submergé dans des ruisseaux et des lacs de l'Australie, du Brunei, de Hong Kong, de l'île Maurice, des Philippines et de l'Afrique du sud. Il se distingue des autres espèces du genre par les dimensions des ascospores et la présence constante de grande(s) guttule(s) dans la cellule apicale, mais de plusieurs guttules plus petites dans la cellule basale. On illustre l'espèce à l'aide de micrographies photoniques et électroniques par balayage et transmission, et on la compare avec les autres espèces d'*Halosarpheia*.

Mots clés : ontogénie des appendices, ascomycètes d'eau douce, *Halosarpheia*, taxonomie, ultrastructure des ascospores.

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Introduction

Halosarpheia Kohlm. & E. Kohlm. (1977), typified by *Halosarpheia fibrosa* Kohlm. & E. Kohlm., was originally described from wood submerged in the sea. The genus is characterized by mostly immersed, soft-walled ascomata; clavate, unitunicate, persistent, or deliquescent asci; and hyaline, one-septate, or multiseptate ascospores with filamentous polar appendages. The ascospore appendages are initially hamate but unfurl in water and form long, filamentous threads (Kohlmeyer and Kohlmeyer 1977; Shearer and Crane 1980). Although *H. fibrosa* is an obligate marine species, Jones (1995) has suggested that more marine genera may be found to have freshwater counterparts as knowledge of freshwater ascomycetes increases. A number of marine genera, including *Halosarpheia*, *Aniptodera*, and *Nais*, have recently been reported from freshwater habitats (Hyde 1992*a*, 1992*b*; Shearer 1993; Hsieh et al. 1995). Three species of *Halosarpheia*: *H. aquatica* Hyde (Hyde 1992*b*), *H. aquadulcis* Hsieh et al. (Hsieh et al. 1995), and *H. lotica* Shearer (Shearer 1984) have been described solely from freshwater habitats or estuarine regions. In this article, *Halosarpheia heteroguttulata* is described as a new species of freshwater ascomycete. The ascospores in this species possess appendages similar to those found in other species of *Halosarpheia*, but the mature ascospores differ in having an apical cell with one or two large guttules and a basal cell with numerous small guttules. The ultrastructure of the ascospores and their appendages is examined using scanning and transmission electron microscopy.

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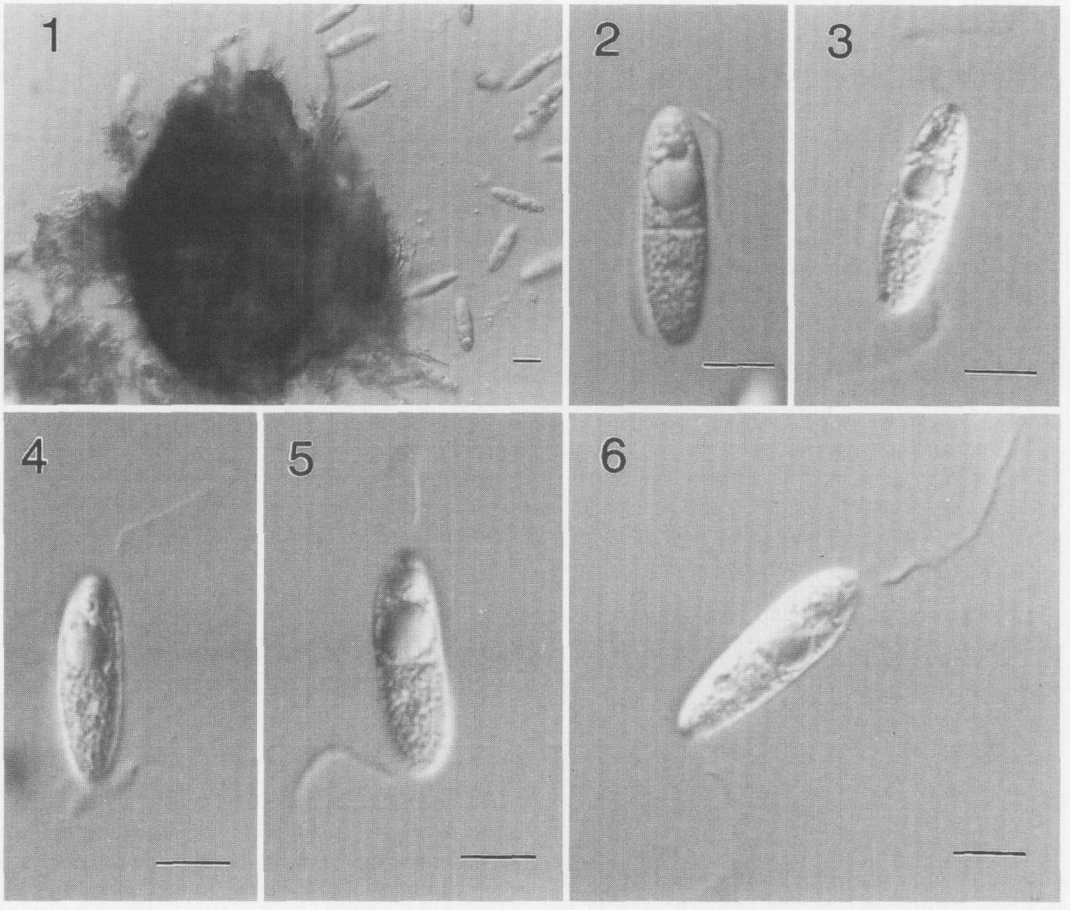
Materials and methods

Natural, decayed, and submerged wood from dicotyledonous plants was collected from Crystal Cascades in North Queensland, Australia; a river near the Kuala Belalong Field Studies Centre in Temburong, Brunei; Plover Cove Reservoir in New Territories, Hong Kong; the Black River in Mauritius; Natigbasan Creek in Impalutao, Mindanao, the Philippines; and the Palmiet River in Durban, South Africa. The wood was taken to the laboratory and incubated in plastic boxes containing moist tissue paper and periodically examined for fungal fruiting structures. Squash mounts of ascomata in water were prepared on glass slide and the face view of the ascomata, paraphyses, asci, and ascospores were examined and photographed using a Leitz Dialux 22EB interference microscope.

For scanning electron microscopy, a few drops of ascospore suspension were pipetted onto a polycarbonate membrane (Nucleopore) with pore size of 5 μm. The ascospores were allowed to settle for 5–10 min and then fixed in 2% (w/v) aqueous osmium tetroxide overnight at 4°C. Fixed material was dehydrated through a graded ethanol series from 10 to 90% (in 10% steps), then 95% and followed by three changes of absolute ethanol. Each of the above changes were for 15 min. Dehydrated material was critical point dried using carbon dioxide and coated with gold–palladium. Material was examined in a Leica Cambridge Stereoscan 440 scanning electron microscope (SEM) operated at 20 kV.

Figs. 1–6. *Halosarpheia heteroguttulata*. Interference contrast micrographs. Fig. 1. Ascoma with released ascospores. Scale bar = 10 μm. Figs. 2–6. Ascospores with a large guttule in the apical cell and numerous small guttules in the basal cell; and polar appendages. Scale bars = 10 μm.



For transmission electron microscopy, an ascospore suspension was embedded in 2% (w/v) ion agar and subsequently fixed in 4% (v/v) glutaraldehyde with added ruthenium red in 0.1 M sodium cacodylate buffer at pH 7.2 for 4 h at room temperature, and post-fixed in 2% (w/v) osmium tetroxide with added ruthenium red in 0.1 M sodium cacodylate buffer at pH 7.2 for overnight at 4°C. The dehydration process was the same as described for scanning electron microscopy but the material was finally transferred to absolute acetone (three times, 15 min each). Fixed and dehydrated material was infiltrated by 25 and 50% Möllenhauer's resin (Möllenhauer 1964) in acetone for 36 h at each concentration, then 75 and 90% resin in acetone for 24 h at each concentration. The almost-infiltrated material was then transferred to a flat plastic mould with 100% resin and placed in a vacuum chamber at approximately 20 cmHg (1 cmHg = 1333.22 Pa) at room temperature for 4 h. Finally, the embedded material was polymerized in an oven at 60°C for 3–5 days. Ultrathin sections (60–80 nm) were obtained using a Diatome diamond knife. The sections were stained with lead citrate (Reynolds 1963) for 15 min and uranyl acetate solution for 30 min. Finally, the specimens were examined using a JEOL 100SX transmission electron microscope (TEM) operated at 80 kV.

Taxonomy

Halosarpheia heteroguttulata S.W. Wong, K.D. Hyde & E.B.G. Jones, sp.nov. (Figs. 1–16)

Ascomata 112–168 μm diametro, globosa vel subglobosa, immersa vel superficiales, nigra, membranecea, ostiolata, papillata, periphysata. Asci 8-spori, deliquescentes. Catenophyses praedita. Ascosporae 27–37 × 12.5–17.5 μm, ellipsoideae, hyalinae, 1-septatae, cellula apicali, 1–2 guttulatae, cellula basim multiguttulata praeditae.

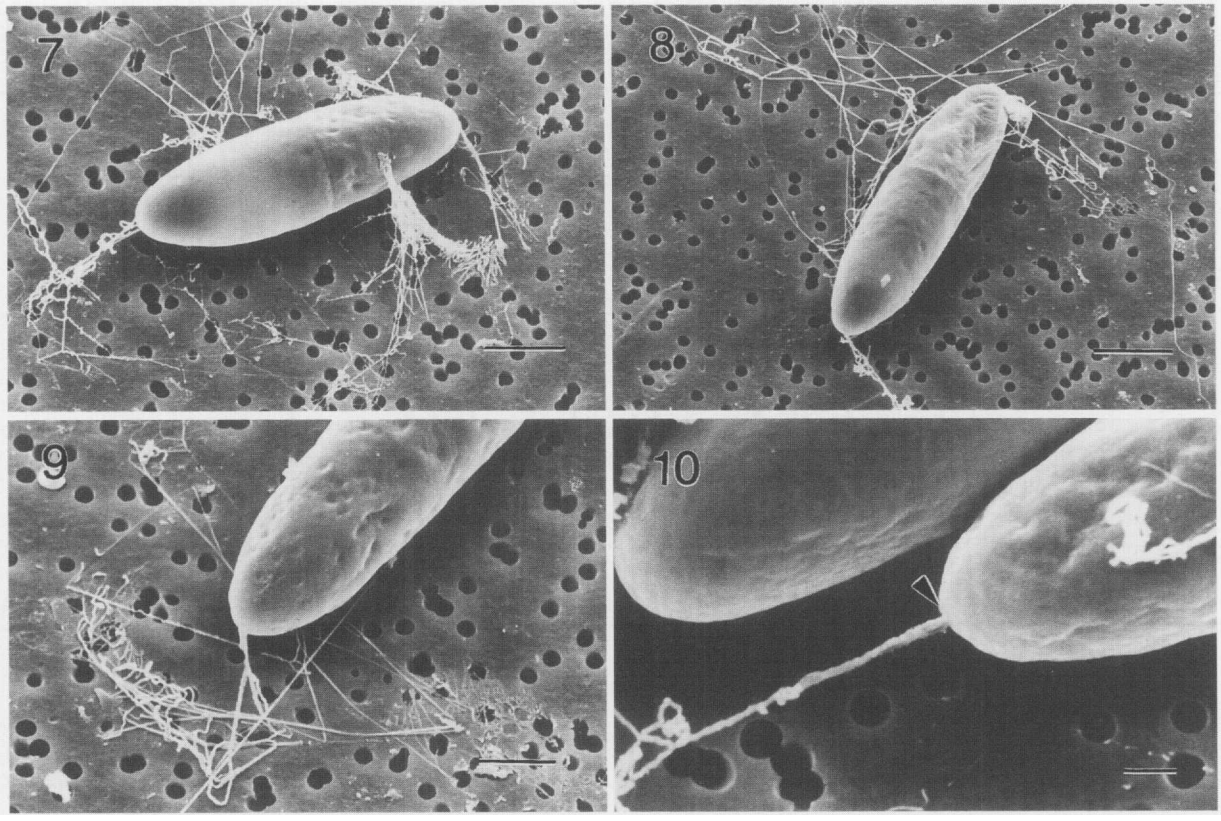
Ascomata 112–168 μm diam., globose to subglobose, immersed or superficial, black, membraneous, ostiolate, papillate, periphysate. Neck long. Asci eight-spored, deliquescing early. Peridium 20–28 μm thick, composed of several layers of brown elongate cells. Catenophyses present. Ascospores 27–37 × 9–17.5 μm, ellipsoidal, hyaline, one-septate, equally two-celled, apical cell with one or two large lipid guttule(s), basal cell with numerous small guttules, with bipolar, hamate, and highly coiled filamentous appendages that unfurl in water to form long strands, exosporium absent, appendage derived from the mesosporium through the discontinuities of episporium.

MODE OF LIFE: Saprobic.

HABITAT: On dicotyledonous wood submerged in freshwater.

KNOWN DISTRIBUTION: Australia, Brunei, Hong Kong, Mauritius, Philippines, South Africa.

Figs. 7–10. *Halosarpheia heteroguttulata*. Ascospores. Scanning electron micrographs. Figs. 7–9. Ascospores showing the polar appendages, which are thread-like and sticky. Scale bars = 5 μm. Fig. 10. Higher magnifications showing the polar appendages emerged from the ascospore tip (arrowed). Scale bar = 1 μm.



ETYMOLOGY: from the Latin *hetero* meaning half and *guttulata* meaning droplets, in reference to one cell having 1 or 2 large guttule(s) and the other having numerous smaller guttules.

HOLOTYPE: AUSTRALIA: North Queensland, Crystal Cascades, on submerged wood in small river, 28 Apr. 1996, T.M. & K.D. Hyde (HKU(M)2806)

MATERIAL EXAMINED: AUSTRALIA: North Queensland, Crystal Cascades, on submerged wood in small river, 28 Apr. 1996, T.M. & K.D. Hyde CC64 (HKU(M)2792); HONG KONG: Plover Cove Reservoir, on submerged wood, 15 Nov. 1996, M. Wong & K.D. Hyde PC24 (HKU(M)3305), ibid. (HKU(M)3306); MAURITIUS: Black River, on submerged wood, August 1995, A. Poonyth & K.D. Hyde (HKU(M)2383), ibid. (HKU(M)2385), ibid. (HKU(M)2393), ibid. (HKU(M)2394), ibid. (HKU(M)2422); SOUTH AFRICA: Durban, Palmiet River, on submerged wood, 15 Nov. 1994, T.S. Steinke & K.D. Hyde (HKU(M)2175).

Ultrastructure

Mature ascospores of *Halosarpheia heteroguttulata* are ellipsoidal with bipolar, threadlike appendages that are highly coiled in a hamate complex adpressed to the ascospore wall (Figs. 11 and 12). Subsequently, the distal region of the coiled appendage separates from the ascospore wall and uncoils and forms threadlike appendages (Figs. 3–10). The uncoiled appendages are thin, sticky in nature, closely adpressed to the polycarbonate membrane, and probably aid in the attachment of the ascospores to the substrate (Figs. 7–10). Threadlike appendages are connected at each pole of the ascospore where they are thicker in diameter (Figs. 9 and 10). The appendage comprises an electron-dense core surrounded by thick electron-transparent material (Figs. 15 and 16). Transverse sections of the coiled appendage indicates that it is a single filamentous appendage, which is folded and compact and closely adpressed to the electron-dense episporium.

Mature ascospores are one-septate with a different guttule deposition in each cell (Figs. 2–6 and 11). In the apical cell, one or two large guttule(s) are present, while in the basal cell, numerous small guttules occur.

The ascospore wall comprises two layers (Figs. 13 and 14): (*i*) a thin electron-dense episporium (25–30 nm) and (*ii*) a thick electron-transparent mesosporium (ca. 50 nm). The mesosporium is more electron-dense towards the ascospore tip, although an electron-transparent zone is located at the ascospore tip (Figs. 13 and 14). The episporium is discontinuous in the area where the appendage emerges (Fig. 14). The appendage presumably derived from the mesosporium through this pore field of the episporium (Fig. 14).

Discussion

In *H. heteroguttulata*, ascospores have a characteristic guttule deposition. This arrangement of guttules is consistent in all our collections. The presence of guttules in the asco-

Figs. 11–16. *Halosarpheia heteroguttulata*. Mature ascospores. Transmission electron micrographs. Fig. 11. Longitudinal section. The apical cell (AC) contains two large guttules and some smaller guttules. The basal cell (BC) contains numerous small guttules. Note the appendage (AP) adpressed to the ascospore wall near the ascospore tip. Scale bar = 1 μm. Fig. 12. Oblique longitudinal section of ascospore illustrating the appendage extending to the septum. The distal part of the coiled appendage (arrowed) is separated from the ascospore wall and uncoils into a filament. Scale bar = 1 μm. Fig. 13. Longitudinal section illustrating a coiled appendage (AP) adpressed to the ascospore wall. The mesosporium (M) is more electron-dense towards the ascospore tip with an electron-transparent zone (TZ) at the apex. Scale bar = 1 μm. Fig. 14. Ascospore tip showing the episporium (E) discontinuous or absent at the ascospore tip where the appendage emerges. Note the electron-transparent zone (TZ) in the mesosporium (M). Scale bar = 0.1 μm. Figs. 15 and 16. Transversely sectioned appendage composed of an electron-dense core (DC) surrounded by a thick electron-transparent matrix (TM). The ascospore wall comprises an electron-dense episporium (E) and an electron-transparent mesosporium (M). Scale bars = 0.1 μm.

Table 1. Comparisons of all freshwater *Halosarpheia* species.

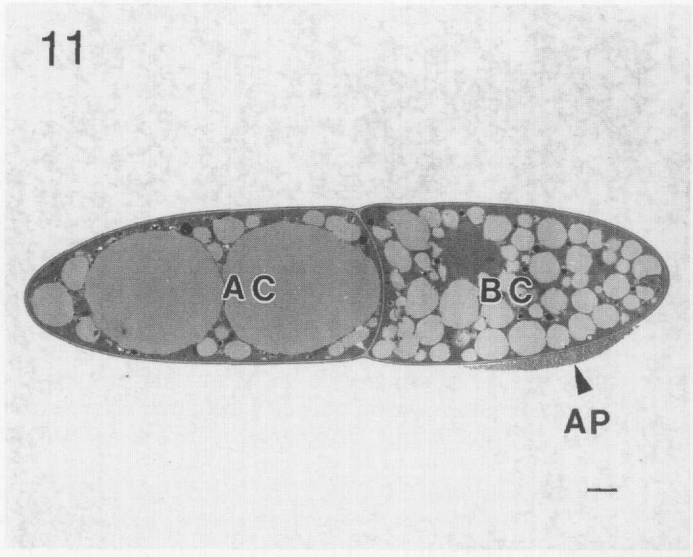
	H. aquatica Freshwater	*H. aquadulcis* Freshwater	*H. lotica* Freshwater	*H. retorquens* Freshwater and marine	*H. heteroguttulata* Freshwater
Ascus size (μm)	56 × 30	80–130 × 20–26	86–137 × 34–43	53–144 × 14.4–24	Unknown
Ascospore size (μm)	33.5–64 × 7–10	26.1–35.5 × 9.5–12	26.4–38.4 × 9.6–14.4	20.4–33.6 × 7–10.8	27–37 × 9–17.5
Ascospore guttules deposition	Numerous small guttules in each cell	One large guttule in each cell with small guttules at the apices and near the septum	One large guttule in each cell with small guttules at the apices and near the septum	One large guttule in each cell with small guttules at the apices and near the septum	One or two large guttule(s) in the polar cell and numerous small guttules in the basal cell

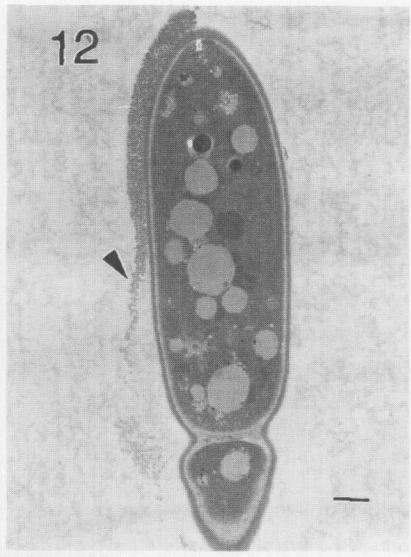
spores of *Halosarpheia* differs among species. Most species possess one large guttule in each cell with small guttules at the apices and near the midseptum, e.g., *H. lotica* and *Halosarpheia retorquens* Shearer & Crane (Shearer 1984; Shearer and Crane 1980). However, smaller guttules are present throughout the whole ascospore in *H. aquatica* (Hyde 1992*b*). Little is known of the function and significance of the guttules.

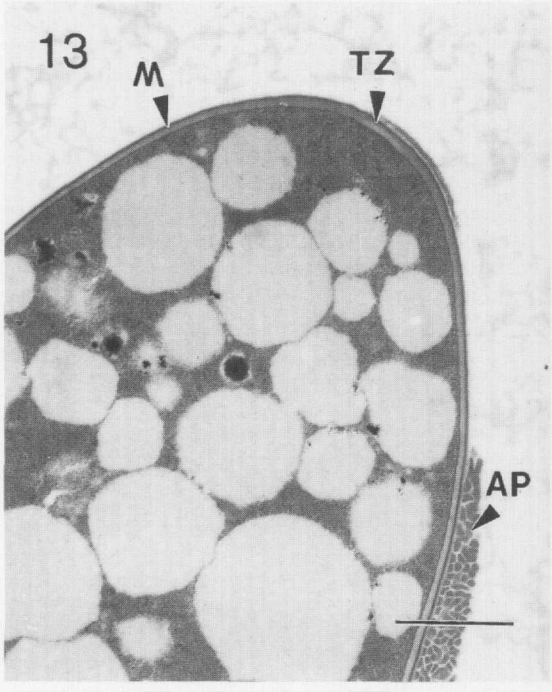
A synopsis of the characteristics of all freshwater *Halosarpheia* species is given in Table 1. Ascospores of *H. heteroguttulata* are similar to those of *H. aquadulcis* at the TEM level (Hsieh et al. 1995). Their common characters including a thin electron-dense episporium and a thick electron-transparent mesosporium. The electron density of the mesosporium increases at the ascospore tip where the appendage emerges, and the appendage is derived from the mesosporium through a discontinuity (pore) of the episporium. This appendage ontogeny type is similar to those described in marine *Halosarpheia* species (Jones 1995). Unlike *H. aquadulcis* and other marine *Halosarpheia* species, *H. heteroguttulata* has asymmetrically organized guttules in the ascospores.

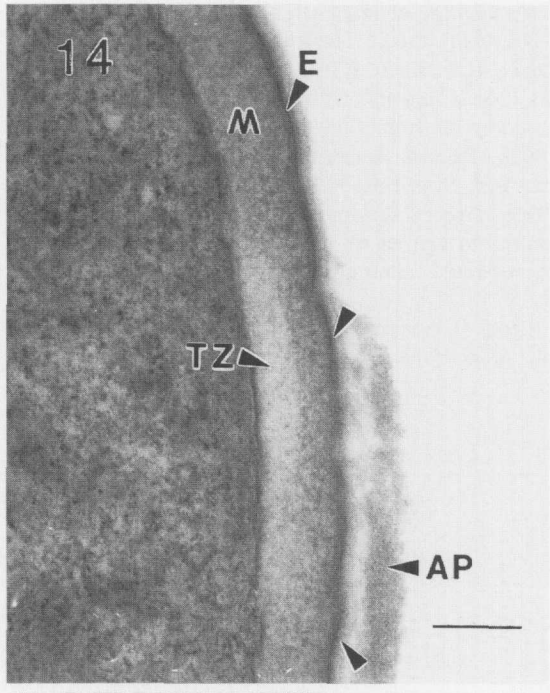
The coiled, polar appendages of the ascospore and their unfurling process in species of *Halosarpheia* have been studied extensively and described with SEM and (or) TEM micrographs (Baker 1991; Farrant 1986; Jones and Moss 1978, 1980; Moss 1990; Shearer and Crane 1980; Yusoff 1991). Uncoiling of this hamate appendage in water results in a long and sticky filamentous thread. The unfurled filament extends and becomes thinner. In addition to *Halosarpheia* species, ascospores that possess long, unfurling, polar appendages are common in other freshwater ascomycetes, e.g., *Aniptodera chesapeakensis* Shearer & Miller (Shearer and Crane 1980; Shearer and Miller 1977), *Annulatascus bipolaris* Hyde (Hyde 1992*a*), and the marine ascomycetes, e.g., *Tirispora unicaudata* E.B.G. Jones & Vrijmoed (Jones et al. 1994). This appendage type may provide the ascospores with a flexible and effective means for entrapment and attachment to substrata and may be an important morphological adaptation for life in fast-flowing rivers or in the sea.

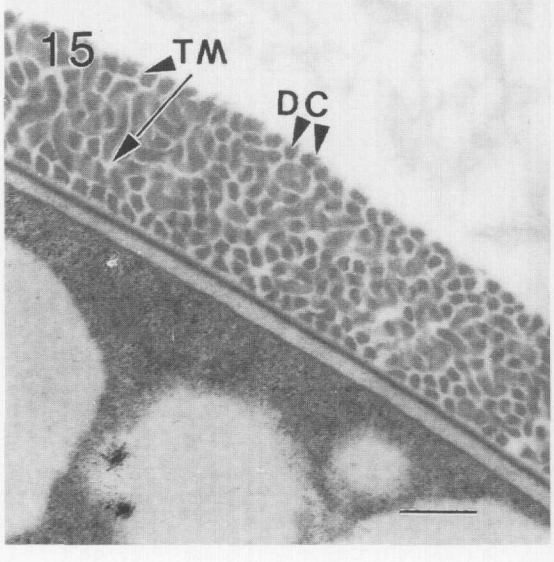
Although the unfurling process in *Halosarpheia* species have been extensively studied, the question of how the coiled appendage is prevented from sticking together has not been considered and is still unresolved. In the TEM, the mature ascospores of *Halosarpheia heteroguttulata* and a marine *Halosarpheia* species (Yusoff 1991) fixed with glutaraldehyde–osmium with added ruthenium red, comprise a highly coiled and folded filamentous appendage (electron-dense) surrounded by thick electron-transparent zones. Is this electron-transparent zone a "space" or an electron-transparent coating of the filament? The answer awaits further ultrastructural studies. Hsieh et al. (1995) fixed the ascospores of *H. aquadulcis* using potassium permanganate and showed that the polar appendages were amorphous and electron dense throughout without any electron-transparent zones. This implies the electron-transparent zones observed in the appendages of *H. heteroguttulata* are compact and there may be some kind of electon-transparent gel matrix. From SEM micrographs, the part of the appendage that is adpressed to the polycarbonate membrane is much thinner than the part close to the ascospore tip. In addition, these thicker strands are never attached to the membrane. Therefore, the electron-transparent gel matrix may be nonsticky and soluble in nature, "insulate" the sticky filaments (electron-dense core), and prevent clumping before unravelling. Once in water, this nonsticky coating may dissolve and allow the sticky filaments to unravel.

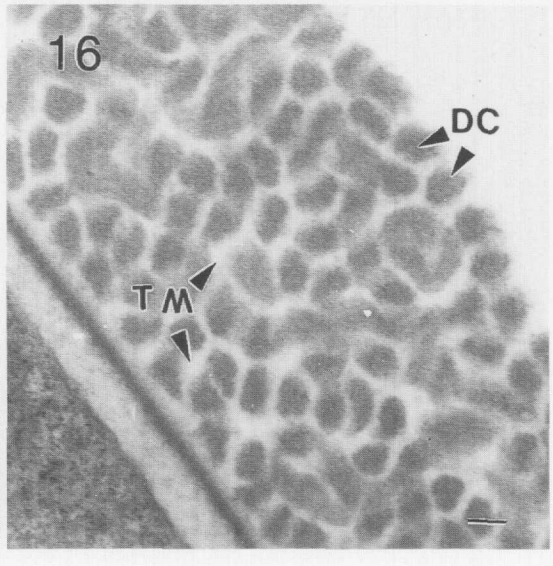












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