Halosarpheia kandeliae sp. nov. on intertidal bark of the mangrove tree *Kandelia candel* in Hong Kong

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Halosarpheia kandeliae sp. nov. collected on intertidal bark of *Kandelia candel* is described and its unique ascus morphology illustrated. It is compared with other *Halosarpheia* spp. and *Aniptodera salsuginosa*.

During a study of fungi growing on *Kandelia candel* in Hong Kong, a *Halosarpheia* species was found on 5% of the 861 wood samples examined from four mangrove stands. It could not be assigned to any of the described species, and is described herein as new. *Halosarpheia* species are common in aquatic habitats with 15 and 5 found in marine and freshwater habitats respectively (Kohlmeyer & Volkmann-Kohlmeyer, 1991; Leong *et al*., 1991; Hsieh *et al*., 1995; Poon & Hyde, 1998; Hyde, Ho & Tsui, 1998). In marine habitats *Halosarpheia* is the genus with the most species after *Corollospora* (Kohlmeyer & Volkmann-Kohlmeyer, 1997). *Halosarpheia* species are well adapted for the aquatic environment with polar appendages that uncoil in water and aid in entrapment and adhesion to suitable substrata (Shearer & Crane 1980; Hyde, Greenwood & Jones, 1993).

MATERIAL AND METHODS

Dead attached intertidal branches of *Kandelia candel* were collected from four mangrove stands in Hong Kong (Three Fathoms Cove, Ting Kok, Ho Chung and Discovery Bay). The samples were returned to the laboratory in plastic bags, examined, incubated in plastic boxes, and re-examined after 4–6 wk. Ascospore appendage morphology was investigated at different salinity levels (0, 3, 5, 10, 20, 33‰) (Nakagiri & Ito, 1994). Photographs of sections and squash mounts were taken with a BH-2 Olympus differential interference contrast microscope. Materials for SEM were prepared as described by Moss & Jones (1977). Single-spore isolates were established on cornmeal seawater agar (CMSA) with added antibiotics: streptomycin and penicillin at 0·5 g l^{-1} each.

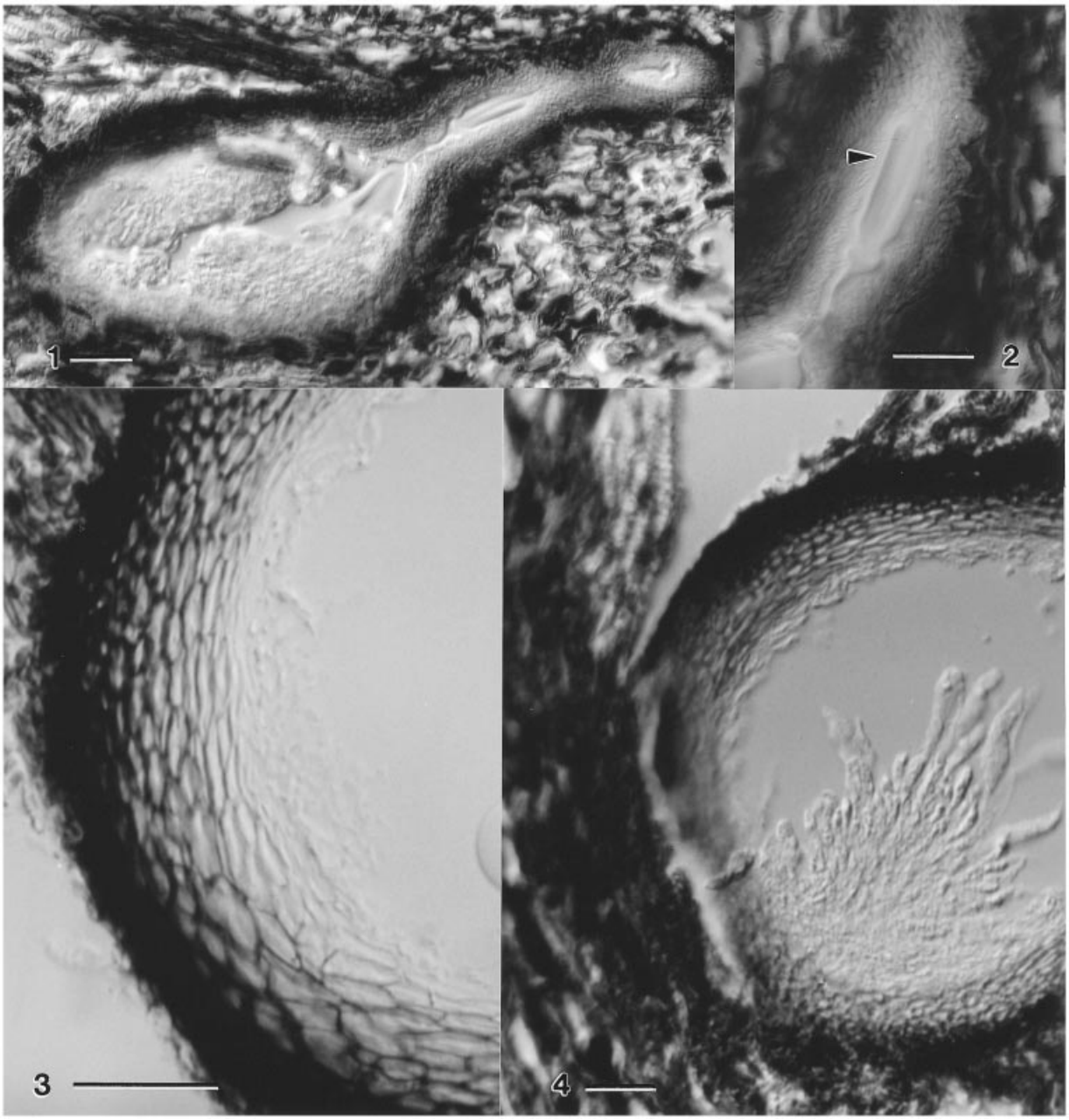
Halosarpheia kandeliae Abdel-Wahab & E. B. G. Jones, sp. nov.

Etymology: From *Kandelia candel*, the host.

Ascomata 200–310 μm alta, 120–180 μm lata, ellipsoidea, axe principali horizontali, nigra in partem superam, dilute brunnea in partem inferam, immersa, membranacea, ostiolata, papillata. *Peridiis* 25–42 μm crassis, bistratis, texturam angularem formantibus; stratum externo 10–15 μm crasso, 3–5 stratis cellularum poligonalis, atrobrunneis, luminibus grandibus, strato interno 15–28 μm crasso, 5–7 stratis cellularum elongatis, poligonalis, dilute brunneis, luminibus angustis, in pseudoparenchyma venteris transientis. *Collis* 110–137 μm longis, 40–77 μm latis, cylindricis, dilute brunneis, periphysatis; periphysibus 7·5–12·5 × 0·5^{-1} μm. *Catenophyses* praesentes. *Ascis* 50–110 × 12–22 μm, octosporis, clavatis, longistipitatis, 10–44 × 1–3 μm, leptodermis, persistentibus, in parte inferiore loculorum insertorum. *Ascosporis* 12–21 × 4–7·5 μm (appendicibus exclusis), ellipsoideis, uniseptatis, non constrictis, hyalinis, leptodermis, appendicibus apicalibus, bipolaribus, initio galericulatis et rigidis, 5–10 × 2–3·5 μm, postea in filamenta longa tenuia in aqua resolventes.

Substratum: in cortice e *Kandelia candel* (L.) Druce.

Ascomata 200–310 (av. = 234·6, S.D. = 55·4, n = 12) μm high, 120–180 (av. = 156·7, S.D. = 22·5, n = 12) μm wide, ellipsoidal, horizontal, black upper side, light brown bottom, immersed under blackened bark, membranous, ostiolate and papillate (Fig. 1). *Peridium* 25–42 (av. = 32·7, S.D. = 4·7, n = 9) μm thick, two layered, forming textura angularis, outer stratum 10–15 (av. = 11·7, S.D. = 1·8, n = 9) μm thick, 3–5 layers of polygonal dark brown melanized cells; inner stratum 15–28 (av. = 20·8, S.D. = 4·8, n = 9) μm thick, 5–7 layers of elongated polygonal, light brown cells, merging with the pseudoparenchyma of the venter (Fig. 3). *Necks* lateral bending upward, 110–137 (av. = 129·5, S.D. = 47·5, n = 7) μm long, 40–77 (av. = 63·4, S.D. = 12·9, n = 7) μm wide, cylindrical, light brown, ostiolar canal periphysate, periphyses 7·5–12·5 (av. = 11·5, S.D. = 1·7, n = 10) μm long, 0·5–1 (av. = 0·9, S.D. = 0·19, n = 10) μm wide (Fig. 2). *Catenophyses* present. *Asci* 50–110 (av. = 79·0, S.D. = 12·4, n = 30) μm long, 12–22 (av. = 19·1, S.D. = 2·1, n = 30) μm wide, eight-spored, clavate, with narrow long stalks 10–44 (av. = 27·5, S.D. = 9·4, n = 28) μm long, 1–3 (av. = 2·3, S.D. = 0·5, n = 28) μm wide, thin-walled, persistent, without an apical apparatus, developing at the base of the ascoma venter on small-celled ascogenous tissue which is separated from the peridium by thin-walled



Figs 1–4. *Halosarpheia kandeliae*, light micrographs. **Fig. 1.** V.S. of ascoma immersed in bark. Bar = 50 μm. **Fig. 2.** L.S. through neck of ascoma; ostiolar canal with periphyses (arrowheaded). Bar = 50 μm. **Fig. 3.** Section through peridium with an outer stratum of thick-walled melanized cells, inner stratum of light brown to hyaline, elongate cells. Bar = 20 μm. **Fig. 4.** V.S. of ascoma with immature asci. Bar = 20 μm.

polygonal cells (Figs 4–8). *Ascospores* 12–21 (av. = 14·8, S.D. = 2·2, $n = 54$) μm long (excluding appendages), 4–7·5 (av. = 5·8, S.D. = 1·4, $n = 54$) μm wide (excluding appendages), ellipsoidal, one-septate, not constricted at the septum, hyaline, thin-walled, with bipolar apical appendages, at first caplike and stiff, 5–12·5 (av. = 9·7, S.D. = 3·4, $n = 5$) μm long, 2–3·5 (av. = 2·6, S.D. = 0·71, $n = 5$) μm wide, rapidly unfurling in water into long, thin filaments (Figs 9–13).

Habitat: Bark of intertidal wood of *K. candel*.

Distribution: Hong Kong.

Holotype: CY 1492, in bark of intertidal wood of *K. candel* from Three Fathoms Cove mangrove, 5 Jan. 1998. IMI No. 379307.

Paratypes: CY 1493, CY 1494, CY 1495 in bark of *K. candel* from Ting Kok mangrove, 28 Jan. 1998; Ho Chung mangrove, 18 Mar. 1998 and Discovery Bay mangrove, 24 Apr. 1998, Hong Kong.

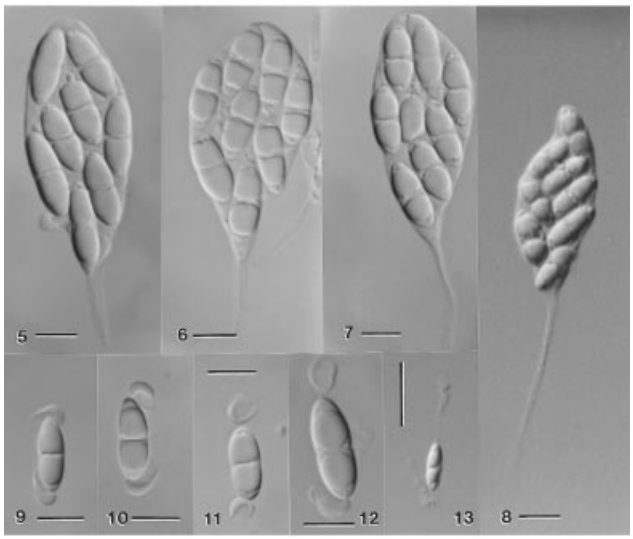
Single spore isolates of *H. kandeliae* growing on CMSA are hyaline to light brown with tufts of aerial mycelium, *ca* 10 mm diam. after one month. No sporulation structures were observed after 6 mo incubation.

In distilled water most ascospore appendages began unfurling into long, thin filaments immediately after submergence, but after 10 min no appendages were visible; either they deliquesced or were detached. At 3 and 5‰ salinity, unfurling began after 10 min, and was mostly completed after

Table 1. Comparison of ascomata, asci and ascospores of *Halosarpheia* species and *Aniptodera salsuginosa*

Arranged according to the dimensions of ascospores	*H. minuta**	*H. kandeliae*	*A. salsuginosa*†	*H. viscosa*‡	*H. rivulicola*§	*H. retorquens*‡	*H. phragmiticola*¶
Ascomata							
Shape	Flattened	Globose to subglobose	Flattened	Globose to subglobose	Globose to subglobose	Globose to subglobose	Globose to subglobose
Colour	Black	Black upper side, light brown bottom	Black upper side, hyaline bottom	Hyaline, becoming black	Black	Hyaline, becoming black	Black
Size (μm)	214–290–437 × 175–230–318	200–235–310 × 120–157–180	240–280 × 92–112	68–248–455 × 68–250–385	140–230	150–230–327 × 150–222–366	350–400
Main axis	Horizontal	Horizontal	Horizontal	Vertical	Vertical	Vertical	Vertical
Habit	Immersed to slightly superficial	Immersed	Immersed	Immersed to superficial	Immersed to superficial	Superficial to immersed	Immersed to superficial
Asci							
Stalk (% of the total ascus length)	Short (9)	Long (50)	Short (13)	Short (13)	Short (10)	Short (19)	Short (16)
Deliquescent	−	−	−	+	+	+	−
Thickened apical ring	+	−	+	−	−	−	−
Apical pore	+	−	+	−	−	−	+
Size (μm)	40–68 × 10–14	50–110 × 12–22	96–90 × 12–14	36–65–114 × 8·9–13–23	62–75 × 17–20	53–80–144 × 14·4–18–24	105–167·5 × 32·5–47·5
Ascospores							
Size (μm)	10–14 × 4–6	12–14·8–21 × 4–5·8–7·5	(12–) 14–20 (–23) × 4–7	13–20–26 × 4·4–6·6–8·4	19–27 × 7·5–9·5	20·4–27·3–33·6 × 7–8–10·8	25–35 × 7·7–10·5
Length to width ratio	2·3–2·5	2·8–2·6–3	3–3·2	2·8–3	2·5–2·8	2·9–3·4	3·2–3·3
Appendage to ascospore length ratio	0·4	0·5	0·4	0·5	0·4	0·7	0·4

* From Leong *et al.* (1991).
† From Nakagiri & Ito (1994).
‡ From Shearer & Crane (1980).
§ From Hyde *et al.* (1998)
¶ From Poon & Hyde (1998).



Figs 5–13. *Halosarpheia kandeliae*, light micrographs. **Figs 5–8.** Clavate asci with long stalks. **Figs 9–13.** Bicelled, hyaline ascospores with appendages at various stages of unfurling. Bars: Figs: 5–12 = 10 μm; Fig. 13 = 20 μm.

1 h. At 10–33‰, appendages unfurled after 15 min, and after 5 h in sea water.

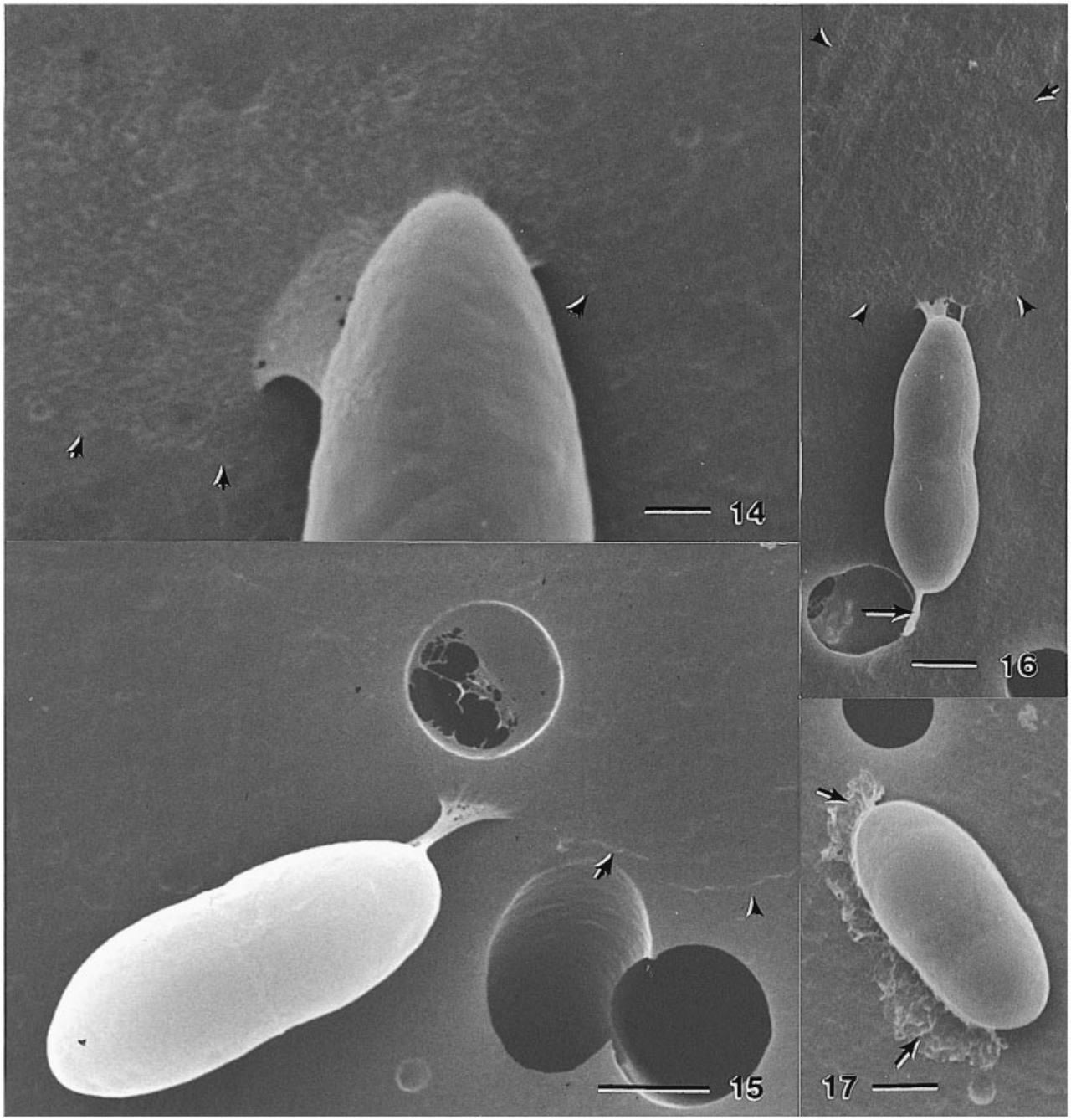
At the SEM level, ascospore appendages were of variable morphology ranging from sheet-like material (Figs 14–16) to amorphous material (Fig. 17), where the coiled nature of the appendage was not discernible, or uncoiled, narrow filaments (Figs 17–20). Future TEM studies are required to resolve the process of ascospore appendage ontogeny in *H. kandeliae*.

DISCUSSION

Halosarpheia was described by Kohlmeyer & Kohlmeyer (1977) from mangrove wood in the intertidal zone, Bermuda, with *H. fibrosa* Kohlm. & E. Kohlm. as the type species. It was established to accommodate species with persistent asci and hyaline ascospores with membranous apical appendages that become filamentous at maturity. Among the 19 species of *Halosarpheia* described, *H. kandeliae* most closely resembles *H. minuta* W. F. Leong, *H. rivulicola* K. D. Hyde, *H. viscosa* (I. Schmidt) Shearer & J. L. Crane ex Kohlm. & Volkm. – Kohlm. and *H. retorquens* Shearer & J. L. Crane, in that all have bicelled ascospores with stiff apical appendages at both ends that are of similar dimensions. *H. kandeliae* differs from all these species, however, in having asci which are clearly divided into a clavate sporal part and a long, thin stalk (Figs 4–8).

In ascoma habit, *H. kandeliae* resembles *H. minuta* in that both are formed horizontally beneath the bark of mangrove wood, with recurved long necks. However, *H. minuta* differs from *H. kandeliae* in having flattened ascomata, pedunculate asci with flattened, thickened apices with indistinct pores and smaller asci and ascospore dimensions (Leong *et al.*, 1991). *Halosarpheia rivulicola* K. D. Hyde, differs from *H. kandeliae* in having immersed to superficial vertical ascomata, pedunculate and shorter asci, and larger ascospore dimensions.

Halosarpheia kandeliae has smaller ascospores than *H. viscosa* and *H. retorquens* (Table 1) (Shearer & Crane, 1980; Kohlmeyer & Volkmann-Kohlmeyer, 1991). The latter two species also differ from *H. kandeliae* in that the ascomata are immersed to superficial, hyaline to black and the asci are deliquescent. *Halosarpheia kandeliae* and *Aniptodera salsuginosa* Nakagiri &



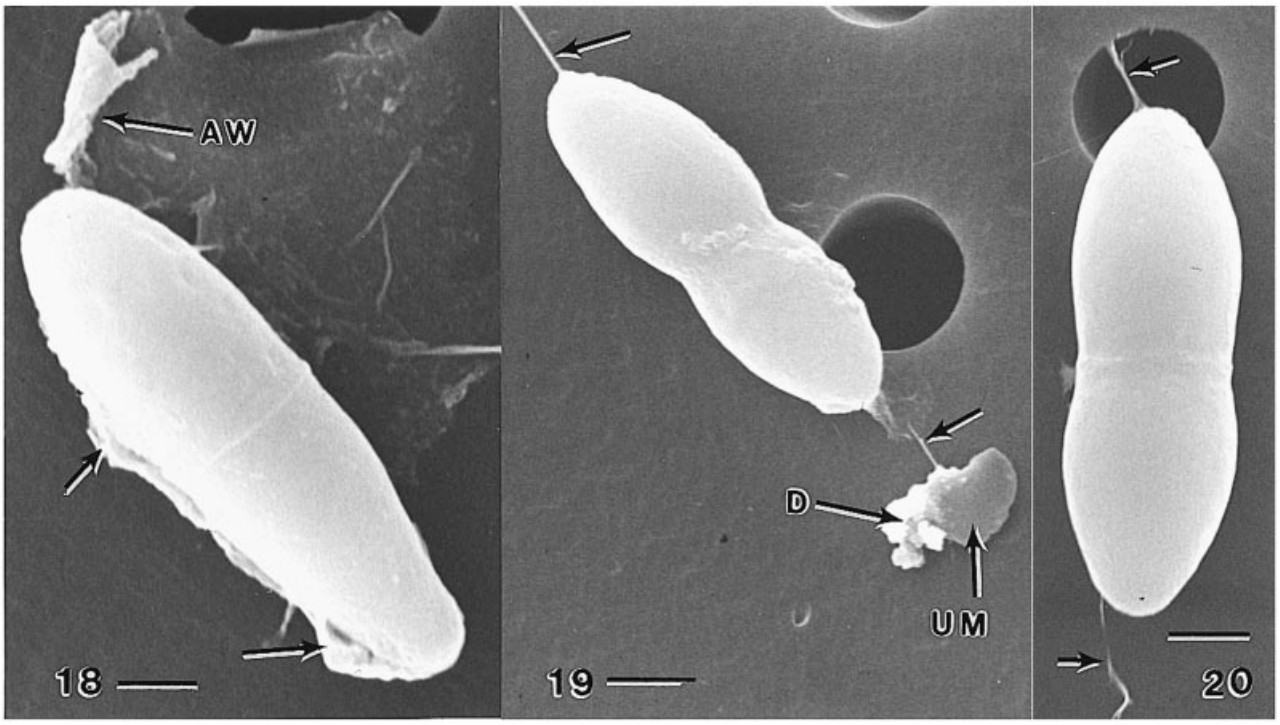
Figs 14–17. *Halosarpheia kandeliae*, scanning electron micrographs of ascospores and appendages. **Figs 14–16.** Amorphous polar appendages, spreading on the polycarbonate membrane. Arrow heads delimit the appendages. **Fig. 17.** Thread-like polar appendages (arrowed). Bars: 14 = 1 μm; 15–17 = 3 μm.

Tad. Ito have similar ascospore dimensions, but the latter has short pedunculate, cylindrical to clavate asci with an apical ring, and the cytoplasm is retracted in the subapical region (Nakagiri & Ito, 1994).

Haslosarpheia phragmiticola O. K. Poon & K. D. Hyde has recently been described from *Phragmites communis* L. at Mai Po marshes in Hong Kong (Poon & Hyde, 1998). *H. kandeliae* differs from it in having immersed, horizontal ascomata and smaller asci and ascospore dimensions.

Of the 19 *Halosarpheia* species described, nine are commonly collected in mangroves, habitats frequently subject to wide fluctuations in salinity. *Halosarpheia kandeliae* and *A. salsuginosa*, both mangrove species, exhibit similar patterns in the unfurling of the ascospore appendages. At high salinities appendages are slow to uncoil, but unfurling is rapid at low salinities. This attribute may confer advantages in the attachment and subsequent colonization of new substrata.

Morphological characters not usually considered when describing *Halosarpheia* species are the length of the ascus stalk and the length and degree of uncoiling of the polar appendages. In *H. kandeliae* the ascus stalk is extremely long and tapering in relation to the upper part of the ascus.



Figs 18–20. *Halosarpheia kandeliae*, scanning electron micrographs of ascospore appendages. **Fig. 18.** Hamate arrangement of polar appendages (arrowed). Remains of the deliquescing ascus wall (arrowed AW). **Fig. 19.** Thread-like polar appendage (arrowed), but terminal portion has not uncoiled (arrowed UM). D = debris attached to the polar appendage. **Fig. 20.** Uncoiled, thread-like polar appendages (arrowed). Bars = 2 μm.

Generally in *Halosarpheia* species the stalks are short; however, exceptions are *H. ratnagiriensis* S. D. Patil & B. D. Borse and *H. abonnis* Kohlm. (up to 30% of the total length of the ascus). In some *Halosarpheia* species, polar appendages (prior to unfurling) appear as small apical caps (e.g. *H. marina* (Cribb & J. W. Cribb) Kohlm. and *H. trullifera* (Kohlm.) E. B. G. Jones, S. T. Moss & Cuomo), while in others the hamate appendages extend to the central septum (e.g. *H. retorquens* and *H. ratnagiriensis*). Intermediate types include *H. minuta* and *H. viscosa*. Another character that might be useful in segregating *Halosarpheia* species is the rapidity of the unfurling of the polar appendages. In *H. fibrosa* and *H. retorquens* the uncoil relatively rapidly, while in *H. trullifera* and *H. abonnis* they take longer. In *H. kandeliae* and *A. salsuginosa* the rate of uncoiling is related to the salinity of the water in which the asci are mounted. As more *Halosarpheia* species are encountered (often differing in minor details) there may be a requirement to consider the above, and other, characters, before describing them as new.

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