

## AUTECOLOGY OF ANTENNOSPORA (FUNGI: ASCOMYCOTA: SORDARIOMYCETIDAE: HALOSPHERIALES) AND ITS PHYLOGENY

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**ABSTRACT.** – Maximum parsimony and likelihood analyses were performed on the partial LSU rRNA gene sequences of *Antennospora quadricornuta* and *A. salina* to determine if *Antennospora* and *Halosphaeria* (Halosphaeriales) are congeneric. The molecular data corroborate the ultrastructural evidence that *Antennospora* and *Halosphaeria* are valid genera and they should be kept separate. *Antennospora* (*A. quadricornuta*) is situated in a clade with weak inter-generic support but this group is distantly related to *Halosphaeria* (*Halosphaeria appendiculata*). Two isolates of *Antennospora salina* form a well-supported clade with *Arenariomyces trifurcatus*, sharing similar development of subpolar ascospore appendages at both ends, which are outgrowths of the mesosporium. They differ significantly, however, in ascospore and ascospore appendage morphology and ontogeny. Based on these results, *Antennospora* and *Halosphaeria* are regarded as separate genera and *Haiyanga*, K. L. Pang & E. B. G. Jones, gen. nov. is established to accommodate *A. salina*. An autecological review of the geographical distribution, temperature and salinity requirements, and occurrence on different substrata of *Antennospora quadricornuta* and *Haiyanga salina*, K. L. Pang & E. B. G. Jones, comb. nov., is provided.

**KEY WORDS.** – *Arenariomyces*, *Haiyanga*, *Halosphaeria*, Halosphaeriaceae, marine fungi, *Okeanomyces*, *Periconia*.

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### INTRODUCTION

Kohlmeyer (1972) assigned 12 marine species to *Halosphaeria* Linder but this was not generally accepted (Hughes, 1975; Jones & Moss, 1980). Taxonomic revision, including transmission and scanning electron microscopy, resulted in the splitting up of *Halosphaeria* into a number of genera (Jones et al., 1984). Accepted genera were: *Antennospora* Meyers, *Halosarpheia* Kohlm. & E. Kohlm., *Halosphaeriopsis* T. W. Johnson, *Ondiniella* E. B. G. Jones, R. G. Johnson & S. T. Moss and *Remispora* Linder, leaving only two species in *Halosphaeria* (*Halosphaeria appendiculata*, *Halosphaeria cucullata*). Recently, *Halosphaeria cucullata* (Kohlm.) Kohlm. was transferred to a new genus, *Okeanomyces* K. L. Pang & E. B. G. Jones, based on morphology and sequence analysis (Pang et al., 2004). Thus, *Halosphaeria* became a monotypic genus.

Revision of *Halosphaeria* relied mainly on the morphology and ontogeny of the ascospore appendages. For example, *Remispora* species (*R. quadriremis* (Höhnk) Kohlm., *R. stellata* Kohlm.) differ from *Halosphaeria appendiculata* Linder in having ascospores surrounded by a sheath that fragments at maturity to form polar appendages, comprising ground amorphous material and fibres, the latter arising from the episporium (Manimohan et al., 1993a, b; Jones, 1995). *Halosarpheia trullifera* (Kohlm.) E. B. G. Jones, S. T. Moss & Cuomo can be differentiated from *Halosphaeria appendiculata* by its bipolar cap-like appendages that unfurl to form long threads (Jones et al., 1983). Ascospore appendages of *Ondiniella torquata* (Kohlm.) E. B. G. Jones, R. G. Johnson & S. T. Moss are extensions of the episporium and differ significantly in their morphology from those of *Halosphaeria appendiculata* (Jones et al., 1984). The characteristic crescent-like equatorial and cup-like polar ascospore appendages of

*Halosphaeriopsis mediosetigera* (Cribb & J. W. Cribb) T. W. Johnson are the result of exospore fragmentation and partial dissolution of the sheath and differ significantly from those of *H. appendiculata* (Jones et al., 1984). *Okeanomyces cucullatus* (Kohlm.) K. L. Pang & E. B. G. Jones is distinguishable from *Halosphaeria appendiculata* by having cylindrical ascospores with a cap-like, deciduous appendage at one end (Pang et al., 2004). However, this appendage is ephemeral and only readily observed in very young material. While *O. cucullatus* has an anamorphic stage (*Periconia prolifica* Anastasiou), none has been reported for *Halosphaeria appendiculata* (Shearer, 1986; Chatmala et al., 2002). *Antennospora quadricornuta* (Cribb & J. W. Cribb) T. W. Johnson and *A. salina* (Meyers) Yusoff, E. B. G. Jones & S. T. Moss, which lack equatorial appendages, differ from *Halosphaeria appendiculata* by the shape and position of the appendages (Jones et al., 1984; Yusoff et al., 1994). In addition, the mature appendages of *Halosphaeria appendiculata* are reticulate in appearance while those of *A. quadricornuta* and *A. salina* have parallel striations running the length of the appendage (Yusoff et al., 1994).

Not all species that were transferred from *Halosphaeria* have been widely accepted, for example, *Antennospora* is still regarded by Kohlmeyer (1984) and Kohlmeyer & Volkmann-Kohlmeyer (1991a) as a synonym of *Halosphaeria*. Kohlmeyer & Volkmann-Kohlmeyer (1987) argued that the difference in ascospore appendage ontogeny between the two, viz. reticulate electron-dense strands in *Halosphaeria appendiculata* and peripheral parallel electron-dense fibrils in *A. quadricornuta*, do not warrant generic separation.

In the present study, we establish a new genus: *Haiyanga*, K. L. Pang & E. B. G. Jones to accommodate *A. salina*. We report on the autecology (geographical distribution, temperature, salinity and occurrence on different substrata) of *A. quadricornuta* and *Haiyanga salina*, K. L. Pang & E. B. G. Jones, comb. nov., and analyse their phylogenetic affinity in the Halosphaeriales, and with the species *Halosphaeria appendiculata*, *Okeanomyces cucullatus* and *Periconia prolifica*, by sequence analysis of the partial nuclear LSU rRNA gene.

## MATERIALS AND METHODS

**Cultures.** – An isolate of *Antennospora quadricornuta* (GR89 from Florida Keys, USA) and two isolates of *A. salina* (CY5062 from Lantau Island, Hong Kong and GR85 from Koh Chang, Thailand), were cultured. Cultures were grown on seawater potato dextrose agar (PDA).

**DNA extraction.** – Aerial mycelium was scraped off directly from the PDA plates and transferred to 400 µl DNA extraction buffer (1% hexadecyltrimethylammonium bromide (CTAB), 1 M NaCl, 100 mM Tris-HCl, 20 mM EDTA) in an 1.5 ml Eppendorf tube, and vigorously vortexed for 1 minute. The tube was microwaved for 30 seconds (10 s-10 s-10 s) at maximum power, and subsequently incubated at 70°C in a water bath for 15 min (Lee & Taylor, 1990). Instead of

closing the tube, it was wrapped with cling film as pressure would build up inside the tube during microwaving with the cap closed. One volume of chloroform:isoamyl alcohol (24:1, by vol.) was added to the tube and inverted several times. The tube was centrifuged (14,000 rpm) for 10 min at room temperature, and the upper aqueous layer transferred to a new 1.5 ml Eppendorf tube. Half volume of ammonium acetate (7.5 M) and 2.5 volume of absolute ethanol were added to the tube and left on ice for 10 min. The tube was centrifuged (14,000 rpm) for 10 min at 4°C. The DNA pellet in the tube was washed once with 100 µl 70% ethanol and was centrifuged for another 10 min. The DNA was air-dried and resuspended in Tris-EDTA buffer (pH 8.5).

**PCR amplification and DNA sequencing.** – The partial nuclear large subunit rRNA gene was amplified from genomic DNA using primers JS1 (Landvik, 1996) and LR7 (Bunyard et al., 1994). PCR reactions were performed in 50 µl volume containing 1 µl genomic DNA, 0.2 µM of each primer, 0.2 mM of each dNTP, 2.5 mM MgCl<sub>2</sub>, 1X PCR buffer and 1U FINNZYMES (MACHEREY-NAGEL). The amplification cycle consisted of an initial denaturation step of 95°C for 2 min followed by 35 cycles of (i) denaturation (95°C for 1 min), (ii) annealing (55°C for 1 min) and (iii) elongation (72°C for 2 min) and a final 2 min elongation step at 72°C. The PCR products were analysed by agarose gel electrophoresis and purified using NucleoSpin<sup>R</sup> Plant DNA purification kit (MACHEREY-NAGEL) according to the manufacturer's instructions. Purified PCR products were sent to Macrogen Inc., Korea for sequencing.

**Sequence alignment and phylogenetic analysis.** – Returned sequences were checked and assembled. Multiple alignment of 51 fungal LSU rDNA sequences (obtained from the GenBank database; accession numbers are given in the tree) was performed using Clustal W (Thompson et al., 1994). Adjustment to the multiple alignment was made manually in Se-Al v1.0a1 (Rambaut, 1999). Two insertions in some of the taxa at positions 854–932 and 1036–1130 and two ambiguous regions at positions 127–142 and 613–620 in the alignment were excluded from analysis. Species of the Xylariales were designated as the outgroup taxa in all analyses.

Parsimony analysis was run initially in PAUP\* 4.0b10 (Swofford, 2002) with the following options: heuristic search with random sequence addition of 100 replicas and a tree-bisection-reconnection (TBR) branch-swapping algorithm. The resulting trees were then subjected to TBR branch-swapping using maximum likelihood. Support for the inferred clades in the trees was obtained by 1000 parsimony bootstrap analysis (random sequence addition of 10 replicas).

## RESULTS

### Autecology of *Antennospora* species

**Geographical distribution.** – Both *Antennospora quadricornuta* and *A. salina* are widely distributed in tropical and subtropical areas (Kohlmeyer, 1983, 1984; Zainal &

Jones, 1984; Hyde & Jones, 1989b), generally occurring on lignocellulosic substrata (Vrijmoed et al., 1982a, b, 1986a, b; Kohlmeyer, 1984). Fig. 1 summarises the distribution of both species in tropical and subtropical zones, with most records from tropical waters, and to a lesser extent from subtropical locations (e.g. Hong Kong, Kuwait, Taiwan). *Antennospora quadricornuta* is more common in its distribution than *A. salina*, although both can occur on the same wood samples or locations (Kohlmeyer, 1981, 1983, 1984; Kohlmeyer & Volkmann-Kohlmeyer, 1987, 1988, 1991b; Hyde & Jones, 1989b; Tan & Leong, 1990; Alias et al., 1999; Alias & Jones, 2000a, b; Jones & Vrijmoed, 2003; Prasannarai & Sridhar, 2001, 2003; Jones et al., 2006; Vittal & Sarma, 2006). Both tend to occur in the lower intertidal zone (Alias & Jones, 2000b), and are late colonizers of submerged mangrove test blocks, occurring after 72–90 weeks' exposure.

Abdel-Wahab (2000) exposed test blocks of *Bruguiera parviflora*, *Kandelia candel* and *Sonneratia alba* submerged in the sea in Hong Kong waters at three tidal levels, upper, middle and low tide. All three test blocks were colonized by *A. quadricornuta* at low tide at Clear Water Bay with a percentage occurrences of 88.9%, 88.9% and 80.6%, respectively and was the dominant species at this site. The fungus occurred throughout the exposure period at this site (8–48 weeks), with the highest percentage occurrence during the latter exposure period (16–48 weeks). However, it did not occur on any of the test blocks/zones at Mai Po mangrove where the salinity was much lower. In a similar experiment at Safaga mangrove, Egypt, the same trend was observed, with *A. quadricornuta* occurring only at the low water site with an occurrence of 8.3% and 2.8% on *B. parviflora* and *S. alba*, respectively. *Antennospora salina* did not occur on any of the test blocks or tidal zone in either study.

*Antennospora quadricornuta* can be the dominant species in some locations, for example at three sites in the Seychelles on submerged driftwood, it was present on 145 out of 327

samples (Hyde & Jones, 1989a). On submerged teak wood in Mangalore Harbour, Southern India, *A. quadricornuta* was the most frequent species (89.6%) followed by *Torpedospora radiata* Meyers (23.5%) (Prasannarai et al., 1999). It was also a common species on pine and teak test blocks submerged at different locations in Hong Kong (Vrijmoed et al., 1982a). Although absent on exposed test blocks at Mandai mangrove, Singapore (Tan et al., 1989; Leong et al., 1991), it was a common species on driftwood from five sites in Singapore and neighbouring areas of Malaysia (Tan, 2002). However, on driftwood collected in Kuwait coastal waters, *A. salina* was the dominant species with 19 collections, while only 8 collections were made for *A. quadricornuta* (Zainal & Jones, 1984). In other studies, they were infrequently collected: Brunei mangroves (Hyde, 1988), Mauritian mangroves (Poonyth et al., 1999), Ranong mangrove, Thailand (Hyde et al., 1990), and mangroves of the Andaman and Nicobar Islands (Chinnaraj, 1993). Neither species were reported on mangrove wood collected at Pagbilao, Philippines (Jones et al., 1988), driftwood collected in New Zealand (Lintott & Lintott, 2002), marine and mangrove wood at St. Lucia to Nahoon, South Africa (Steinke & Jones, 1993), driftwood at Hainan Island (Vrijmoed et al., 1996), mangrove wood at Udyavara, southwest India, and Godavari and Krishna deltas, east coast India (Sarma & Vittal, 2000; Maria & Sridhar, 2003, 2004). Such a variation in species occurrence can be attributed to different sampling procedure, temperature and salinity regimes, and substrata (Jones, 2000). In some mangroves, salinity varies greatly from 1.1‰ in the monsoon season to 34.1‰ (Udyavara, India) or from 3.9 to 37‰ (Krishna delta, India) (Sarma & Vittal, 2000; Maria & Sridhar, 2003, 2004). Patil & Borse (2001) noted that *A. quadricornuta* and *A. salina* were the most common species on driftwood from beach sites (percentage occurrence of 15.4% and 20.7%, respectively).

There is evidence to suggest that *A. quadricornuta* and *A. salina* may be host specific as neither have been reported

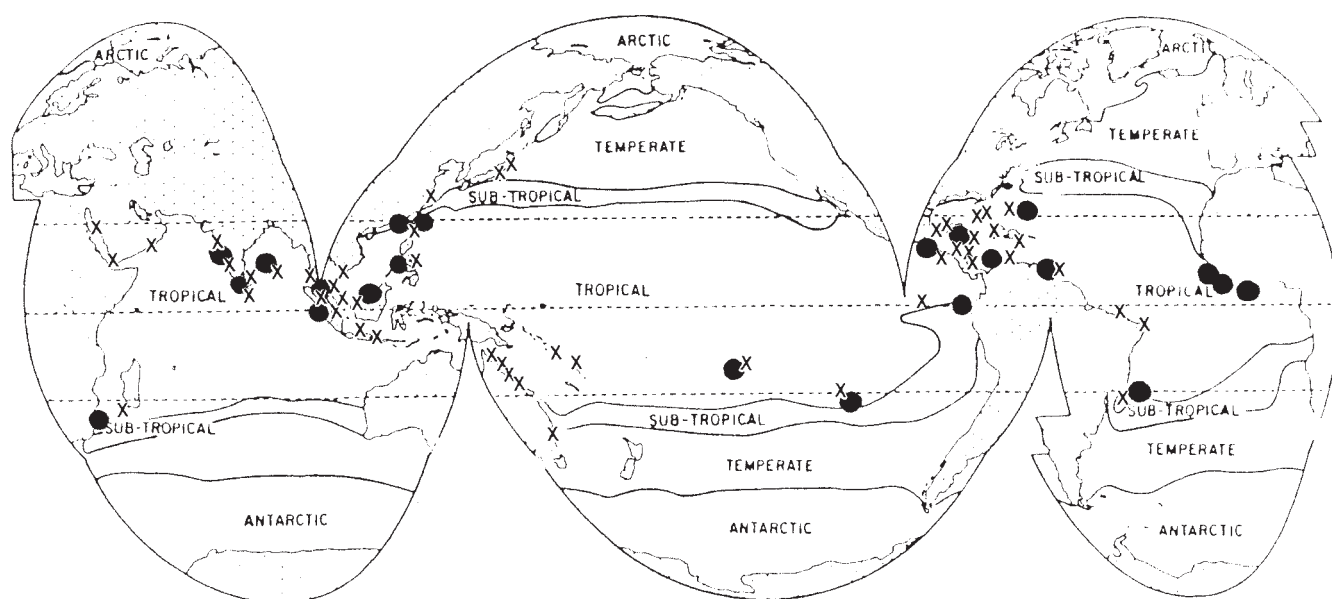


Fig. 1. Geographical distributions of *Antennospora quadricornuta* (x) and *Haiyanga salina* (•).

on the brackish water palm *Nypa fruticans* Wurm (Hyde & Alias, 2000; Pilantanapak et al., 2005; Hyde & Sarma, 2006). Their absence in the study of Hyde & Sarma (2006) may be attributable to the salinity of the water, i.e. “0% salinity at times of high river flow”. *Antennospora quadricornuta* and *A. salina* were not found on angiosperm leaves, brown algae or sea-grasses in the Seychelles, although common on mangrove wood (Hyde & Jones, 1989a). Test panels of mangrove trees submerged for 90 weeks at Kuala Selangor mangrove were preferentially colonized by *A. quadricornuta*, with 73 collections on *Avicennia marina* (Forsk.) Vierh. but only 4 on *Bruguiera parviflora* (Roxb.) Wight & Arn. ex Griff. (Alias & Jones, 2000a). Although 4 collections of *A. salina* were made on *A. marina*, none were made on *B. parviflora* (Alias & Jones, 2000a).

**Temperature.** – Few studies have examined the optimum temperature for the growth of *Antennospora* species. Kohlmeyer (1968) observed that ascospores of *A. quadricornuta* germinated at 28°C but not at 20°C, and this fungus grew well at temperatures between 28°C and 30°C (Tubaki, 1968; Kohlmeyer, 1968). Vrijmoed et al. (1982a) reported a significant positive correlation with temperature in the occurrence of *A. quadricornuta* colonizing pine test blocks in Hong Kong coastal waters. Colony numbers were low during August through to April, but peaked from May to July when sea surface temperature was 26.4–30.2°C. El Sharouney et al. (1998) noted that this fungus was more common in the summer season than in the winter, with a percentage occurrence of 20.8 and 15.4%, respectively.

**Salinity.** – Vrijmoed et al. (1982a), in a study of seasonal colonization of submerged test blocks in coastal Hong Kong waters, reported a significant negative correlation between colonization by *A. quadricornuta* and salinity. Vrijmoed et al. (1982b) reported that *A. quadricornuta* occurred with increasing frequency from Site 1 (mean salinity 12.51%) to Site 5 (mean salinity 27.56%).

**Occurrence on different substrata.** – Most collections of *Antennospora quadricornuta* have been from mangrove wood (*Avicennia marina*, *Aegiceras corniculatum* (L.) Blanco, *Bruguiera gymnorrhiza* (L.) Lam., *Cocos nucifera* L., *Hibiscus tiliaceus* L., *Prosopis* sp., *Pithecellobium* sp., *Rhizophora apiculata* Blume, *Rh. mangle* L., *Rh. mucronata* Lam., *Sonneratia alba* Griff., *Sonneratia griffithii* Kurz, test blocks of *Albizia Durazz.* (Kohlmeyer, 1969, 1984; Vrijmoed et al., 1982a, b, 1986a, b; Suhrman & Jones, 1983; Kohlmeyer & Volkmann-Kohlmeyer, 1987; Hyde, 1989; Hyde & Jones, 1989a; Hyde et al., 1990; Chinnaraj, 1993; El-Sharouney et al., 1998; Prasannarai et al., 1999; Poonyth et al., 2001), while fewer reports and substrata have been reported for *A. salina* (*Avicennia germinans* L.; bamboo; *Canavalina rosea* (Sw.) DC., *Hibiscus tiliaceus*, *Sonneratia alba*, and foraminifera attached to wood, as well as teredinid tunnels of various molluscs) (Kohlmeyer, 1968, 1984; Kohlmeyer & Volkmann-Kohlmeyer, 1991b; Chinnaraj, 1993).

Kohlmeyer (1956) reported strong cellulolytic activity on

cellulose foil with hyphae growing “parallel to the micellar structure”. Kohlmeyer (1984), in noting the fungus growing under the empty shipworm tunnels, suggested it was decomposing the organic matrix of the calcium carbonate, making it more brittle. However, no enzymatic studies were undertaken. Other marine fungi have been reported as growing on the chonchyolin of oyster shells (Alderman, 1976).

In conclusion, both *A. quadricornuta* and *A. salina* are predominantly tropical species, occurring less frequently in subtropical locations (Fig. 1); both occur on a wide spectrum of substrata; and are more common in oceanic waters than in estuarine mangroves. *Antennospora quadricornuta* occurs more frequently than *A. salina*, and is more-frequently collected.

### Phylogenetic Analysis

With a total of 1,426 characters, 336 parsimony-informative characters were used in searching for the best trees. Eight shortest trees with a tree length of 1,394 steps, a C.I. of 0.4878 and a R.I. of 0.6711 were found. Subsequently, these trees were subjected to TBR branch-swapping using maximum likelihood criterion. Seven trees were discarded at the start of the branch-swapping and 34,084 rearrangements were tried on the remaining tree. The resulting tree has an -Ln likelihood score of 9922.58282, which is shown in Fig. 2.

The topology of the tree is similar to those of the previous phylogenetic studies on the Halosphaeriales (Campbell et al., 2003; Pang et al., 2003a, b; Pang et al., 2004). Terminal groups are generally well-supported while inter-generic relationships, except for the group with *Halosphaeria appendiculata*, are poorly resolved. *Halosphaeria appendiculata* groups with *Neptunella longirostris* (Cribb & J. W. Cribb) K. L. Pang & E. B. G. Jones with weak support (50%). Two isolates of *Antennospora quadricornuta* are situated in a group, where internal nodes are not supported by parsimony bootstrapping, and are distantly placed from *Halosphaeria appendiculata*. This weakly-supported group also includes *Arenariomyces* Höhnk., *Corollospora* Werderm., *Cucullosporella* (K. D. Hyde & E. B. G. Jones) K. D. Hyde & E. B. G. Jones, *Halosarphaea*, *Halosphaeriopsis*, *Nereiospora* E. B. G. Jones, R. G. Johnson & S. T. Moss, *Pseudolignincola* Chatmala & E. B. G. Jones and *Saagaromyces* K. L. Pang & E. B. G. Jones. Two isolates of *Antennospora salina* are also in the same clade but instead of clustering with *A. quadricornuta*, form a moderately-supported group which includes the two isolates of *Arenariomyces trifurcatus* Höhnk. (85%).

### DISCUSSION

**Relationship of *Antennospora* and *Halosphaeria*.** – *Antennospora* was established by Meyers (1957) to accommodate *A. caribbea* Meyers, a species at the time already described as *Halosphaeria quadricornuta* Cribb & J. W. Cribb (Cribb & Cribb, 1956). Johnson (1958) transferred *Halosphaeria quadricornuta* to *Antennospora*

Table 1. Morphological differences between *Arenariomyces trifurcatus* and *Haiyanga salina*. (Jones et al. 1983, Yusoff et al. 1994)

	<i>Arenariomyces trifurcatus</i> (Figs. 3–4)	<i>Haiyanga salina</i> (Figs. 5–7)
<b>Ascomata</b>		
Substrata	On sand grains	On wood
Texture	Carbonaceous	Membraneous, coriaceous to subcarbonaceous
<b>Ascospores</b>		
Shape	Fusiform	Ellipsoidal
Constriction	Constricted at septum	Slightly or not constricted at septum
<b>Appendages</b>		
Position	Subpolar	Subpolar
Number	Consistently three	Three to four
Shape	Slender, rigid, round shaft, terminating in an apical thickening, pad or bifurcated structure	Obclavate, acuminate, attenuate, claw-like/curved
Ultrastructure	Episporium lacinated, outer surface of the basal third of the appendage composed of 2–4 laminae, the shaft containing a granular core bounded by an electron-opaque homogeneous layer	Attached to mesosporium via an isthmus of electron-dense material, outer electron-dense boundary enclosing peripheral longitudinal electron-dense striations, with amorphous core, striations arise from the electron-dense layer of mesosporium, with a drop of mucilage exuded from the appendage tips

and reduced *A. caribbea* to synonymy. A revision of the Halosphaeriaceae resulted in the transfer of *A. quadricornuta* back to *Halosphaeria* (Kohlmeyer, 1972). Jones & Moss (1980) and Jones et al. (1984) then, based on ultrastructural data, reassigned it to *Antennospora*. Kohlmeyer (1984) had difficulty in accepting the taxonomic status of *Antennospora* as he opined that the similarity in ascospore appendage ontogeny of both *Halosphaeria appendiculata* (type species of *Halosphaeria*) and *Halosphaeria quadricornuta*, i.e. arising as outgroups from the mesosporium, did not warrant generic separation.

*Antennospora* (*A. quadricornuta*) and *Halosphaeria* (*Halosphaeria appendiculata*) should be kept as separate genera as inferred from the analyses in the present study, although the relationships of the two species with other taxa in the Halosphaeriales remain unresolved. *Halosphaeria appendiculata* is always located within a well-supported branch (89%, Fig. 2) and this result has also been reported in other studies (Pang et al., 2003a, b, 2004). However, due to the great morphological variations exhibited by these taxa, the apomorphic character(s) supporting this group is(are) unknown. Both *A. quadricornuta* and *Halosphaeria appendiculata* possess ascospore appendages that are outgrowths of the mesosporium and connected by an isthmus of electron-dense material (Yusoff et al., 1994). Morphological differences between the two taxa exist and include: (a) position of the appendages (polar and equatorial in *Halosphaeria appendiculata* but subpolar in *A. quadricornuta*, (b) shape of the appendage (obclavate with a deep spoon-shaped structure at the point of attachment in *Halosphaeria appendiculata* but cylindrical in *A. quadricornuta*), and (c) ascospore appendage ultrastructure (reticulate arrangement

of fibres in *Halosphaeria appendiculata* but parallel in *A. quadricornuta*). These observations were also found in *Halosphaeria salina* (Meyers) Kohlm. and Yusoff et al. (1994) subsequently transferred *Halosphaeria salina* to *Antennospora*.

***Antennospora salina*.** – *Antennospora salina* was originally described as *Arenariomyces* but it was subsequently transferred to *Remispora* and *Halosphaeria* based on the superficial similarity in appendage morphology (Kohlmeyer, 1968; 1972). Most recently, the species was moved to *Antennospora* based on ultrastructural observations (Yusoff et al., 1994). *Antennospora salina* is not related phylogenetically to *Halosphaeria* (*Halosphaeria appendiculata*) or *A. quadricornuta* but forms a rather well-supported group with *Arenariomyces trifurcatus*, as shown in Fig. 2. *Antennospora salina* and *Halosphaeria appendiculata* differ mainly in the position and shape of ascospore appendages, i.e. three (-four) polar/subpolar, obclavate, acuminate, attenuate and curved appendages with a drop of mucilage exuded from the appendage tips in *A. salina* (Zainal & Jones, 1984; Yusoff et al., 1994) and polar and equatorial, obclavate with deep spoon-shaped structure at attachment region in *Halosphaeria appendiculata*. Also, *Halosphaeria appendiculata* has a temperate distribution while *A. salina* has only been reported from tropical/subtropical locations.

*Antennospora salina* consistently groups with *Arenariomyces trifurcatus* with a moderate bootstrap support and a comparatively long branch length. Both species have hyaline ascospores which are constricted at the septum and polar/subpolar ascospore appendages arising from the mesosporium (Jones, 1995). However, they differ in many morphological

features which are listed in Table 1. Consequently, we propose the erection of a new genus to accommodate *A. salina*, namely *Haiyanga* K. L. Pang & E. B. G. Jones. Four *Arenariomyces* species have been described: *A. majusculus* Kohlm. & Volkm.-Kohlm., *A. parvulus* J. Koch, *A. trifurcatus* and *A. triseptatus* Kohlm., but only cultures of *A. trifurcatus* have been available for this study. All *Arenariomyces* species have been reported from sand associated with buried wood,

while *Haiyanga salina* K. L. Pang & E. B. G. Jones is always found on submerged wood, or intertidal wood and has never been reported from sand (Sundari et al., 1996). *Haiyanga salina*, is a tropical to subtropical warm water species; *A. trifurcatus* is cosmopolitan, while *A. majusculus*, *A. parvulus* and *A. triseptatus* were described from tropical locations (Kohlmeyer, 1984; Koch, 1986; Kohlmeyer & Volkmann-Kohlmeyer, 1989).

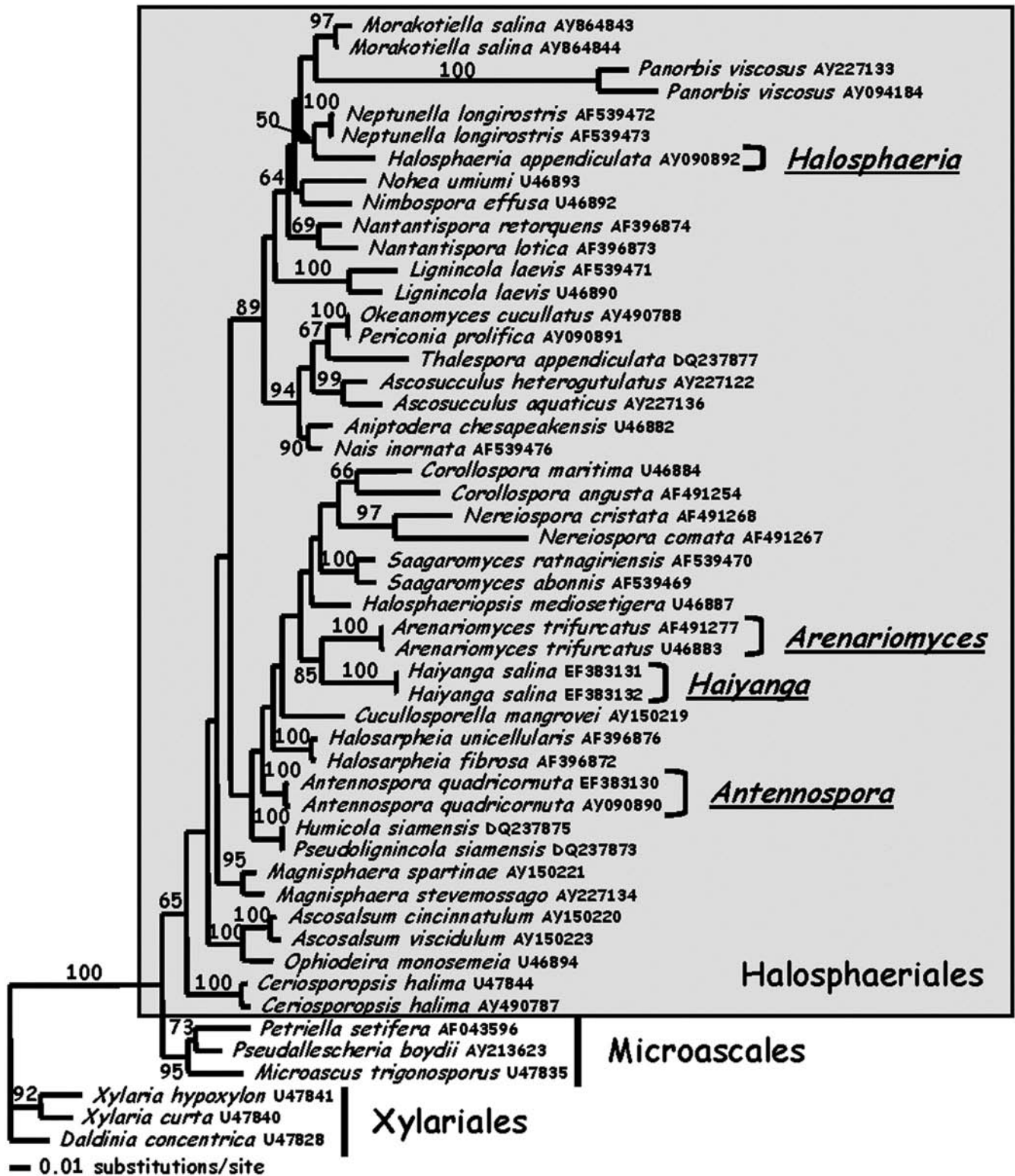
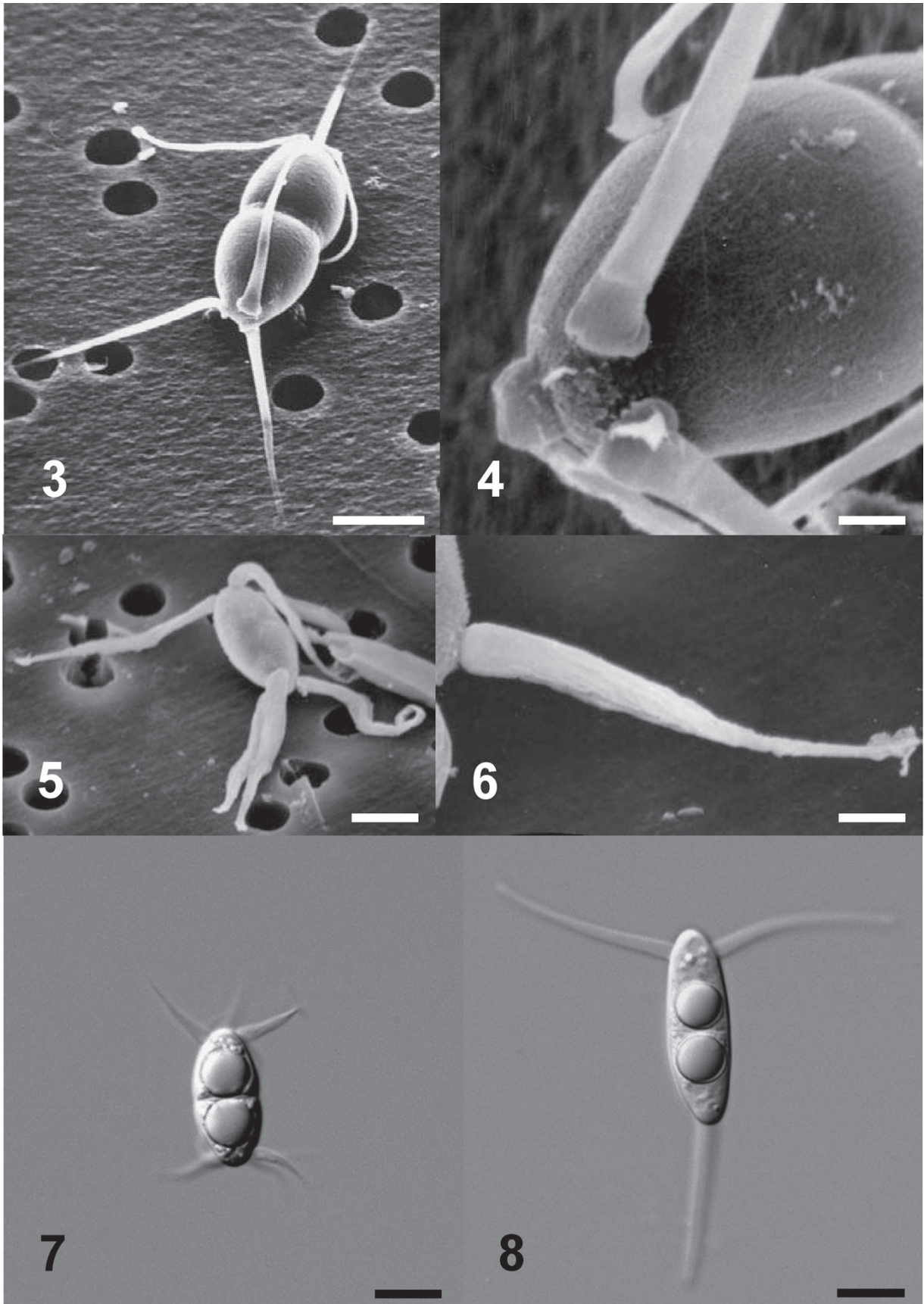


Fig. 2. Maximum likelihood tree from TBR branch-swapping of the trees resulted from maximum parsimony analysis. Bootstrap values (> 50%) from maximum parsimony are shown on the branches.



Figs. 3–8. *Arenariomyces trifurcatus*, scanning electron micrographs (Jones et al., 1983): 3, Fusiform and constricted ascospore with three subpolar appendages; 4, Rigid appendages with round shaft. *Haiyanga salina*, light and scanning electron micrographs: 5, Ellipsoidal ascospore slightly constricted at the septum with three subpolar appendages; 6, Appendage obclavate, acuminate, attenuate and curved; 7, Ascospore with three obclavate, curved and polar/bipolar appendages at both ends. *Antennospora quadricornuta*, light micrograph. Ascospores with two cylindrical appendages at both ends in juxtaposition. Scale bars: 3 = 10  $\mu$ m; 4 = 2  $\mu$ m. Scale bars: 5 = 5  $\mu$ m; 6 = 2  $\mu$ m; 7 = 10  $\mu$ m; 8 = 10  $\mu$ m.

## TAXONOMY

With the results discussed above, *Antennospora* and *Halosphaeria* are considered distinct genera and *Haiyanga*, new genus, is proposed to accommodate *A. salina*.

### *Haiyanga* K. L. Pang & E. B. G. Jones gen. nov.

**Type species.** – *Haiyanga salina* (Meyers) K. L. Pang & E. B. G. Jones.

**Diagnosis.** – Ascomata immersa vel exposita, fulva vel nigra, membranacea vel subcarbonacea, ostiolata, papillata. Asci unitunicati, octospori, clavati, pristina deliquescentes. Ascospores ellipsoideae, hyalinae, cum appendicibus.

**Description.** – Ascomata immersed or superficial, brown or black, membranous to subcarbonaceous, ostiolate, papillate. Asci unitunicate, 8-spored, clavate, early deliquescing. Ascospores ellipsoidal with round apices, hyaline, slightly constricted at the septum. Appendages polar to subpolar, obclavate, acuminate, attenuate, curved.

**Etymology.** – ‘hai yang’ meaning ‘ocean’ in Mandarin.

### *Haiyanga salina* (Meyers) K. L. Pang & E. B. G. Jones, comb. nov. (Figs. 5–7)

Basionym: *Arenariomyces salina* Meyers Mycologia 49: 505. 1957.

Synonyms: *Remispora salina* (Meyers) Kohlm. Mycologia 60: 262. 1968.

*Halosphaeria salina* (Meyers) Kohlm. Can. J. Bot. 50: 1957. 1972.

*Antennospora salina* (Meyers) Yusoff, E. B. G. Jones & S. T. Moss Can. J. Bot. 72: 1003. 1994.

### *Antennospora quadricornuta* (Cribb & J. W. Cribb) T. W. Johnson J. Elisha Mitchell Sci. Soc. 74: 46. 1958. (Fig. 8)

Basionym: *Halosphaeria quadricornuta* Cribb & J. W. Cribb Univ. Queensl. Pap. Dep. Bot. 3: 99. 1956.

Synonym: *Antennospora caribbea* Meyers Mycologia 49: 503. 1957.

### *Halosphaeria appendiculata* Linder Farlowia 1: 412. 1944.

Synonym: *Remispora ornata* T. W. Johnson & Cavaliere Nova Hedwigia 6: 188. 1963.

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