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# Systematics of *Halosarpheia* based on morphological and molecular data

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***Abstract:*** The genus *Halosarpheia* (Halosphaeriales) was established for marine ascomycetes with obpyriform to sub-globose, coriaceous, brown to black ostiolate ascomata with long necks; hamathecia of catenophyses; thin-walled, unitunicate, persistent asci with thick-walled apices; and ellipsoid, one septate, hyaline ascospores equipped with coiled, threadlike apical appendages that unfurl in water. Emphasis on ascospore appendage morphology has led to the inclusion in the genus of morphologically disparate fungi from a variety of marine and freshwater habitats. To better understand the evolutionary relationships of *Halosarpheia* species, phylogenetic analyses were conducted on 16 *Halosarpheia* species, 13 other species of Halosphaeriales and representatives of the Microascales, Hypocreales, Sordariales and Xylariales using 18S and 28S rDNA sequence data. All of the *Halosarpheia* species occurred on the Halosphaeriales clade. The type species of the genus, *H. fibrosa*, occurred on a well-supported clade with two morphologically similar species, *H. trullifera* and *H. unicellularis*. This clade, which phylogenetically was distant from the clades of other *Halosarpheia* species, represents the genus *Halosarpheia* sensu stricto. The other *Halosarpheia* species were distributed among eight other well-supported clades clearly separated from one another based on molecular data. New generic names are established for six of these clades, one new species is described, and one species is transferred to *Aniptodera*. A table (TABLE I) comparing the morphology, habitat, substrate and distribution of the genera of aquatic ascomycetes with coiled, threadlike apical appendages treated in this study is provided, along with a key for their identification.

***Key words:*** aquatic, Ascomycetes, Halosphaeriales, phylogenetics, rDNA

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## INTRODUCTION

Jan and Erika Kohlmeyer established the genus *Halosarpheia* (Halosphaeriaceae, Halosphaeriales), typified by *Halosarpheia fibrosa*, in 1977 (Kohlmeyer and Kohlmeyer 1977). Anderson et al (2001) reviewed the history of *Halosarpheia* and the phylogenetic and systematic problems associated with the genus, which now contains 22 species. In essence, taxonomic emphasis on morphologically similar unfurling, threadlike ascospore appendages has led to the inclusion in *Halosarpheia* of species morphologically distinct enough from one another to question their placement in the genus.

Recently, molecular sequence data have been used to shed light on the phylogeny of *Halosarpheia* and the putatively related genera, *Aniptodera*, *Lignincola* and *Nais*. Chen et al (1999) found that, based on 18S rDNA sequence data, *H. retorquens* occurred on the same clade with *Lignincola laevis*, *Nais inornata* and *Aniptodera chesapeakensis*. Bootstrap support for this clade was 97%. In 2000, Kong et al, using 18S rDNA sequence data, found that four *Halosarpheia* species, two that occur in marine habitats (*H. fibrosa*, *H. trullifera*) and two that occur in freshwater, brackish and marine habitats (*H. retorquens*, *H. viscosa*), occurred on two separate clades. *Halosarpheia fibrosa* and *H. trullifera* occurred on the same clade with 76% bootstrap support and were separate from the other *Halosarpheia* species. *Halosarpheia retorquens* occurred on a clade with *A. chesapeakensis* with 100% bootstrap support, while *H. viscosa* was a sister taxon to a clade containing *Halosphaeria appendiculata*, *L. laevis* and *N. inornata*, but with a bootstrap support below 50%. Abdel-Wahab et al (2001), using 28S rDNA sequence data and the same taxa, but different isolates in three cases, and a new species, *H. unicellularis*, found that *H. fibrosa*, *H. trullifera* and *H. unicellularis* were well supported on a single clade and were distant to a clade composed of *H. lotica* and *H. retorquens*.

In another recent molecular study of nine species of *Halosarpheia* based on 18S rDNA (Anderson et al 2001), species were resolved into eight well-supported clades, thereby providing further support for the polyphyly of the genus. Anderson et al (2001) declined to make nomenclatural changes in their publication on the basis that additional studies, both mo-

lecular and morphological, were needed to further resolve relationships among *Halosarpheia* species and related taxa. They also thought that more *Halosarpheia* species should be included in the analyses before nomenclatural changes were made.

This study was undertaken, therefore, to obtain and analyze additional sequence data (18S and 28S rDNA) for more *Halosarpheia* species than included in previous studies (Chen et al 1999, Kong et al 2000, Abdel-Wahab et al 2001, Anderson et al 2001) and other morphologically similar species to further delineate monophyletic clades within the *Halosarpheia* complex. In addition, morphology of the *Halosarpheia* species in these analyses was compared using protologues, recent descriptions, new collections and fruiting cultures where available. Based on the results of this study, nomenclatural changes are proposed.

## MATERIALS AND METHODS

*Rationale for selection of genera.*—In addition to species of *Halosarpheia*, we sequenced other morphologically similar species with unfurling apical appendages (*Aniptodera* and *Haligena*). A further reason for the inclusion of *Haligena* was because some species of *Halosarpheia* (*H. spartinae* and *H. unicaudata*) originally had been placed in *Haligena*. Thus the validity of these transfers could be tested. Representatives from all genera in the monotypic family Halosphaeriaceae that were currently available in GenBank also were included in our study, as well as species from other orders of pyrenomycetous fungi (Microascales, Hypocreales, Sordariales). Species of Xylariales were used as outgroup taxa (see below).

*Fungal isolates.*—Methods for collection, isolation and characterization of fungal species are described by Fallah and Shearer (2001). Ascomata were removed and processed for sectioning, according to the procedures of Fallah and Shearer (2001). Squash mounts were made for examination of the hamathecia, asci and ascospores. Staining reactions of the ascus apex were tested with Melzer's reagent (MLZ, 0.5 g iodine, 1.5 g KI, 20 g chloral hydrate, 20 mL distilled water) and aqueous cotton blue. All measurements were made of material fixed in glycerin or lactic acid. Photographs were taken with Nomarski optics on an Olympus BHII microscope and a Spot RT Digital Camera.

Cultures were obtained from single ascospores or asci, according to the procedures of Shearer (1993) (prefix A, TABLE II). Additional cultures were obtained from Drs. Brigitte Volkmann-Kohlmeyer and Jan Kohlmeyer (prefix K, TABLE II), the culture collection at Portsmouth University, U.K. (prefix PP, TABLE II) and the American Type Culture Collection (prefix ATCC, TABLE II). To stimulate isolates to reproduce to confirm culture identities, cultures were grown on cornmeal agar (CMA) with strips of balsa wood or alfalfa stems, which had been submerged in distilled water (with 15 ppt sea salts added for marine isolates) and then autoclaved 1 h. Colonized substrates were transferred to moist chambers (sterile glass Petri dishes containing three pieces of Whatman No. 1 filter paper moistened with sterile distilled water), sealed with parafilm and incubated until reproduction occurred. All prefix A cultures were confirmed in this way. None of the cultures obtained from other sources fruited in culture.

*DNA extraction, amplification and sequencing.*—Isolates were transferred to peptone-yeast-glucose (PYG) agar plates (0.125% peptone, 0.125% yeast extract, 0.6% glucose, 1.8% agar) and grown 6–8 weeks in the dark at 24 C. DNA was extracted and amplified, according to the procedures of Campbell et al (2003). Universal fungal primers NS1 and NS4 (White et al 1990) were used to amplify 1100 bp of the 18S rDNA, and LROR (Bunyard et al 1994) and either LR7 (Bunyard et al 1994) or LR6 (Vilgalys and Hester 1990) were used to amplify 1400 bp and 1100 bp regions of the 28S rDNA. PCR reactions were carried out in 0.5 mL microcentrifuge tubes in 50 μL volume containing 50 ng template DNA, 1× PCR buffer, 1.5 mM $MgCl_2$, 2 mM dNTP, 0.5 μM each primer and 1.25 U Taq polymerase. Cycling parameters were, NS1–NS4: following the procedure of Anderson et al (2001); LROR–LR6: following the protocol of Campbell et al (2003); LROR–LR7: following the procedure of Campbell et al (2002). The PCR products were purified to remove excess primers, dNTPs and nonspecific amplification products with a Qiaquick PCR Purification Kit (Qiagen 1999). PCR products were sequenced directly with primers NS1 and NS4 (White et al 1990) for 18S, LROR (Bunyard et al 1994) and either LR7 (Bunyard et al 1994) or LR6 (Vilgalys and Hester 1990) for 28S rDNA at the University of Illinois Biotechnology Center using BigDye® Terminators v3.1 (Applied Biosystems, U.S.A.) on ABI 373A automated sequencers. The sequences were aligned with published sequence data (TABLE III) in Clustal X (Thompson et al 1997), then refined manually in Se-Al (Rambaut 2001), with ambiguous regions of alignment excluded from the analyses.

*Phylogenetic analyses.*—The congruence of the two datasets was tested with the incongruence length difference (ILD) test (Farris et al 1994, 1995), using the partition homogeneity test in PAUP* 4.0b 10 (Swofford 2002). Cladistic analyses were performed in PAUP* using maximum-parsimony and weighted-parsimony criteria on the 18S rDNA data (35 taxa), the 28S rDNA data (52 taxa), and the combined 18S and 28S rDNA data (52 taxa, of which 35 had 18S data available). The 28S rDNA dataset and the combined 18S and 28S dataset were imported into MacClade (Maddison and Maddison 1992) and manipulated to constrain all *Halosarpheia* species into one clade to make two constrained trees, one for each dataset. Outgroup comparison was used to polarize the character states and hence root the trees (Stevens 1980, Watrous and Wheeler 1981, Farris 1982, Darlu and Tassey 1987), employing the strategies of Maddison et al (1984) and Campbell (1999). These strategies demonstrate that global parsimony is best achieved when the pleisomorphic states are indicated in a more distant ancestor than the most recent common ancestor of the ingroup taxa. Several basal ascomycetes and basidiomycetes were explored as outgroup taxa (data not shown) and species of

TABLE I. Morphological characteristics of the genera with threadlike, coiled ascospore appendages included in this study. Refs: 1. Hyde (1992). 2. Shearer and Miller (1977). 3. Shearer and Crane (1980). 4. Kohlmeyer (1984). 5. Kohlmeyer and Kohlmeyer (1977). 6. Jones (1962). 7. Kohlmeyer and Kohlmeyer (1965). 8. Kohlmeyer and Volkmann-Kohlmeyer (1988). 9. Patil and Borse (1982). 10. Baker (1991). 11. Hyde et al (1999). 12. Anderson and Shearer (2002) Further information was obtained from examination of specimens cited herein. Line drawings adapted from Kohlmeyer and Volkmann-Kohlmeyer (1991), except *Aquicalidophilus* and *Panorbium*, which are original (all scale bars = 10 μm)

| | *Natantispora*<br>Ref. 3, 11<br>Clade A | *H. marina*<br>Ref. 4<br>Clade B | *Panorbis*<br>Ref. 3, 10, 11<br>Clade C | *Ascosacculus*<br>Ref. 1, 11, 12<br>Clade D | *Aniptodera*<br>Ref. 2, 11 |
|---|---|---|---|---|---|
| Species | *N. retorquens*<br>*N. lotica* | *H. marinus* | *P. viscosa* | *A. aquaticus*<br>*A. heteroguttulatus* | *A. chesapeakensis*[a] |
| Ascomal shape | globose to subglobose | bottle shaped | globose to subglobose | globose to subglobose | globose to subglobose |
| Ascomal size | ~200–400 μm diam | 140–300 μm diam | 70–450 μm diam | less than 200 μm diam | 130–325 μm diam |
| Ascomal pigmentation | hyaline, becoming gray brown | subhyaline to light brown | hyaline, becoming gray to black | pale brown, brown, transparent | hyaline, transparent |
| Peridium | membranous, one layered, of textura angularis in face view | not reported | membranous, of textura angularis in face view | membranous, thin-walled, one layered, of textura angularis in face view | membranous, thin-walled, one layered, of textura angularis in face view |
| Neck | long, cylindrical, periphysate, hyaline at apex, pigmented toward base | long, cylindrical, light colored, periphysate | long, cylindrical, periphysate, hyaline at apex, pigmented toward base | long, cylindrical, narrow, thin-walled, hyaline at apex, pale brown at base, periphysate | long, cylindrical, hyaline with darkened areas, periphysate |
| Hamathecium at maturity | catenophyses | absent | absent? | catenophyses | catenophyses |
| Ascus shape | clavate, thin-walled throughout, pedunculate | clavate to subcylindrical, pedunculate | ellipsoid to clavate, thin-walled throughout | saccate, ellipsoid, thin-walled throughout, pedunculate | clavate, thin-walled except at apex, pedunculate |
| Ascus apex | ascus wall not thickened at apex, lacking an apical pore and apical apparatus | wall thickened, apical pore present, cytoplasm retracted below apex | ascus wall not thickened at apex, lacking an apical pore and apical apparatus | ascus wall not thickened at apex, lacking an apical pore and apical apparatus | ascus wall thickened at apex, apical pore present, cytoplasm retracted below apex |
| Ascus persistence | deliquescent | persistent | persistent to deliquescent | early deliquescent | persistent, forcible ascospore discharge observed |
| Ascospore shape | tapered fusoid-ellipsoid | cylindrical, ellipsoidal | ellipsoidal, tapered or rounded at apices, flattened on one side | fusiform, cylindrical, tapering toward one end, cells unequal in size | ellipsoidal to fusiform, thick-walled |
| Ascospore septation | one septate, septum median | one septate, septum median | one septate, septum median | one septate, septum supra-median or median | one septate, septum median |

TABLE I. Continued

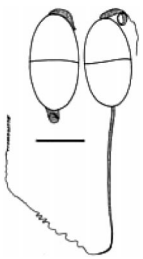
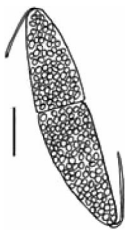
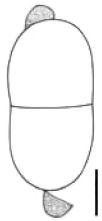
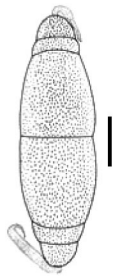
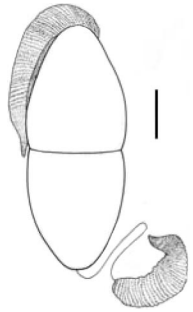
| | *Natantispora*<br>Ref. 3, 11<br>Clade A | *H. marina*<br>Ref. 4<br>Clade B | *Panorbis*<br>Ref. 3, 10, 11<br>Clade C | *Ascosacculus*<br>Ref. 1, 11, 12<br>Clade D | *Aniptodera*<br>Ref. 2, 11 |
|---|---|---|---|---|---|
| Ascospore guttulation | single large guttule in each cell | single large guttule in each cell | single large guttule in each cell | with many small guttules or with 1 or 2 large guttules in one cell, polyguttulate in the other cell | single large guttule in each cell |
| Ascospore surface[b] | smooth | smooth | smooth | smooth | smooth |
| Ascospore pigmentation | hyaline | hyaline | hyaline | hyaline | hyaline |
| Appendages | one at each apex, threadlike, coiled, unfurling in water | one at each apex, threadlike, coiled, unfurling in water | one at each apex, threadlike, coiled, unfurling in water | one at each apex, threadlike, coiled, unfurling in water | when present, one at each apex, threadlike, coiled, unfurling in water |
| Appendage size when coiled | large, equal to or longer than 1/2 ascospore length | small, cap-like, less than 1/2 ascospore length | small, less than or equal to 1/2 ascospore length | equal to or slightly longer than 1/2 ascospore length | equal to or longer than 1/2 ascospore length |
| Colony pigmentation | hyaline at first, becoming gray to black | not reported | hyaline, becoming gray with age | gray brown, forming chains of brown chlamydospores, *Trichocladium* anamorph for *A. heteroguttulata* | hyaline, becoming gray brown with age |
| Habitat | marine, brackish, freshwater | marine, mangroves | marine, brackish, freshwater | freshwater | freshwater, brackish, marine |
| Distribution | temperate, tropical, widespread | tropical | temperate, widespread | tropical, widespread | temperate, tropical, widespread |
| Substrate | woody and herbaceous debris | mangrove wood | woody and herbaceous debris | woody debris | woody and herbaceous debris |
| Ascospore of type species |  |  |  |  |  |

TABLE I. Continued

| | *Ascosalsum* Ref. 3, 7 Clade F | *Ophiodeira* Ref. 8 | *Halosarpheia* sensu stricto Ref. 5, 10 Clade G | *Magnisphaera* Ref. 3, 6 Clade H | *Littispora* Ref. 4, 9, 10 Clade I |
|---|---|---|---|---|---|
| Species | *A. viscidulum*<br>*A. cincinnatulum*<br>*A. unicaudatum* | *O. monosemeia* | *H. fibrosa*<br>*H. trullifera*<br>*H. unicellularis* | *M. spartinae*<br>*M. stevemossago* | *L. ratnagiriensis*<br>*L. abonnis* |
| Ascomal shape | globose to subglobose | ellipsoidal, under a thin stroma | obpyriform, broadly ellipsoidal, subglobose | globose, flattened globose | ellipsoidal |
| Ascomal size | 100–250 μm diam | 210–320 μm diam | 200–750 μm diam | 200–500 μm diam | 300–650 μm diam |
| Ascomal pigmentation | hyaline | light brown | cream, brown to black | dark brown to black | hyaline to light brown |
| Peridium | one layered, 13–25 μm thick, of thick-walled round to elongate cells, of textura angularis in face view | 15–20 μm thick, of 5–7 layers of cells, cell walls thickened toward the outside | two layered, 40–60 μm thick, coriaceous, of textura angularis in face view | two-layered, 32–40 μm thick, coriaceous, of textura angularis in face view | two layered, 20–45 μm thick, of textura angularis |
| Neck | long, cylindrical, hyaline, periphysate | long, curved, dark brown, periphysate | long (up to 500–1,000 μm), cylindrical, subconical, thick-walled, dark pigmented, periphysate | short or absent, black, periphysate | long, up to 1000 μm, cylindrical, periphysate |
| Hamathecium at maturity | absent | catenophyses | catenophyses, sterile pseudoparenchyma subtending asci | catenophyses | catenophysess |
| Ascus shape | clavate or ellipsoidal, thin-walled | clavate, thin-walled, pedunculate | clavate, thin-walled, thickened at apex, pedunculate | clavate, thin-walled, pedunculate | clavate, thin-walled, pedunculate |
| Ascus apex | thin-walled, without an apical pore or apparatus | thin-walled, rounded, without an apical pore or apparatus | thin-walled, rounded or beaked, without an apical pore or apparatus | thin-walled, rounded, without an apical pore or apparatus | apical plate, apical pore, no apical apparatus |
| Ascus persistence | deliquescent | deliquescent | persistent, (deliquescent in *H. unicellularis*), asci separating from ascogenous hyphae | deliquescent | persistent |
| Ascospore shape | long cylindrical or fusiform | ellipsoidal | ellipsoidal, broadly rounded at apices | ellipsoidal to fusiform | ellipsoidal |
| Ascospore septation | phragmoseptate | one septate, septum median | non- to one-septate, septum median | 2–9 septate | one septate, septum median |
| Ascospore surface[b] | smooth | smooth | smooth | rough walled | smooth |

TABLE I. Continued

| | *Ascosalsum* Ref. 3, 7 Clade F | *Ophiodeira* Ref. 8 | *Halosarpheia* sensu stricto Ref. 5, 10 Clade G | *Magnisphaera* Ref. 3, 6 Clade H | *Littispora* Ref. 4, 9, 10 Clade I |
|---|---|---|---|---|---|
| Ascospore pigmentation | hyaline | hyaline | hyaline, pale grayish brown | hyaline to pale gold, becoming subhyaline with age | hyaline |
| Appendages | one at one apex, (except *A. viscidula* which has an appendage at both apices) threadlike, coiled, unfurling in water | single threadlike appendage at one apex, coiled, unfurling in water | one at each apex, threadlike, coiled, unfurling in water | one at each apex, threadlike, coiled, unfurling in water | one at each apex, threadlike, coiled, unfurling in water |
| Appendage length when coiled | less than 1/2 ascospore length | equal to or less than 1/2 ascospore length | less than 1/2 ascospore length | less than 1/2 ascospore length | equal to or longer than 1/2 ascospore length, very large |
| Colony pigmentation | hyaline to cream colored | not reported | not reported | dark brown | not reported |
| Habitat | marine, mangroves, brackish | marine, mangroves | marine | marine, brackish, fresh water | marine, mangroves |
| Distribution | temperate, tropical | tropical | tropical, subtropical | temperate | tropical |
| Substrate | woody debris | sub-tidal wood | mangrove wood, palm leaf | woody debris | immersed wood, bark of mangroves |
| Ascospore of type species |  |  |  |  |  |

[a] Characters given only for *A. chesapeakensis*, type of the genus, because the genus is unlikely to be monophyletic as currently circumscribed (Campbell and Shearer unpubl).

[b] As viewed with light microscope.

TABLE II. Fungal isolates sequenced for this study

| Species | Isolate No.[a] | GenBank accession No. 18S | GenBank accession No. 28S |
|---|---|---|---|
| *Aniptodera lignatilis* K.D. Hyde | A460-1 | — | AY227115 |
| *Haligena salina* C.A. Farrant et E.B.G. Jones | A437-1D | — | AY227116 |
| *Halosarpheia abonnis* Kohlm. | K5163B | — | AY227117 |
| *Halosarpheia abonnis* Kohlm. | K5304A | AY227137 | AY227118 |
| *Halosarpheia aquatica* K.D. Hyde | A444-1D | — | AY227136 |
| *Halosarpheia cincinnatula* Shearer et J.L. Crane | A318-1C | — | AY227120 |
| *Halosarpheia heteroguttulata* S.W. Wong, K.D. Hyde et E.B.G. Jones | A108-7D | AY227138 | AY227121 |
| *Halosarpheia heteroguttulata* S.W. Wong, K.D. Hyde et E.B.G. Jones | A108-11B | — | AT227122 |
| *Halosarpheia lotica* Shearer | A214-3A | — | AY227123 |
| *Halosarpheia lotica* Shearer | A333-1A | — | AY227124 |
| *Halosarpheia marina* (Cribb et J.W. Cribb) Kohlm. | K5103B | — | AY227125 |
| *Halosarpheia ratnagiriensis* Patil et Borse | PP1910 | — | AY227126 |
| *Halosarpheia retorquens* Shearer et J.L. Crane | ATCC 38867 | — | AY227127 |
| *Halosarpheia retorquens* Shearer et J.L. Crane | A231-1D | — | AY227128 |
| *Halosarpheia sp.* | A481-1 | — | AY227119 |
| *Halosarpheia spartinae* (E.B.G. Jones) Shearer et J.L. Crane | A221-1C | — | AY227129 |
| *Halosarpheia spartinae* (Shearer et J.L. Crane) | A330-1A | — | AY227130 |
| *Halosarpheia viscidula* (Kohlm. et E. Kohlm.) Shearer et J.L. Crane | PP0218 | — | AY227131 |
| *Halosarpheia viscosa* (I. Schmidt) Shearer et J.L. Crane ex Kohlm. et Volkm.-Kohlm. | A231-2B | AY227139 | AY227132 |
| *Halosarpheia viscosa* (I. Schmidt) Shearer et J.L. Crane ex Kohlm. et Volkm.-Kohlm. | K5380A | — | AY227133 |
| *Magnisphaera stevemossago* J. Campb., J. L. Anderson et Shearer | A409-1B | AY227140 | AY227134 |
| *Magnisphaera stevemossago* J. Campb., J.L. Anderson et Shearer | A409-4D | — | AY227135 |

[a] Isolates with the prefix A are from the culture collection of C.A. Shearer; K are from B. and J. Kohlmeyer; PP are from the University of Portsmouth, UK; ATCC are from the American Type Culture Collection.

Xylariales were chosen on the basis of reduced homoplasy and increased congruence in the phylogenetic reconstructions. Maximum-parsimony analyses were performed with heuristic searches employing random starting trees, random stepwise addition on 100 replicates, gaps treated as missing data and a tree-bisection-reconnection branch-swapping algorithm. Parsimony tree scores for the consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) were calculated in PAUP* for each tree generated. Weighted parsimony analyses were performed with a step matrix to weight nucleotide transformations based on the reciprocal of the observed transition: transversion (ti/tv) ratio (Spatafora et al 1998), which was calculated using MacClade. The alternative tree topologies for each dataset were tested with the Kishino-Hasegawa (K-H) maximum-likelihood test (Kishino and Hasegawa 1989) to find the best or most likely tree for each dataset. These trees then were compared with the two constrained trees (TABLE IV). Bootstrap analyses (Felsenstein 1985) were performed on the ''best'' tree using a full heuristic search on 1000 replicates and the appropriate evolutionary model criteria, as above, and inferred in the K-H test. Decay indices (Bremer 1988, 1994) were calculated in AutoDecay (Eriksson 1998). Bayesian inference of phylogeny (Rannala and Yang 1996, Zhaxybayeva and Gogarten 2002) was calculated using MrBayes (Huelsenbeck and Ronquist 2001), which determines the posterior probability that a tree topology is correct for a given dataset using Markov Chain Monte Carlo methods. The dataset was analyzed using MrBayes with the general time reversible model of substitution (Rodriguez et al 1990) assumed with among-site rate variation described by gamma distribution. Four simultaneous Markov chains were run from random starting trees for 1 000 000 generations and sampled every 100 generations (generating 10 001 trees). The first 27 000 generations (270 trees) of the chain were discarded as burn-in (the time for the chain to reach stability), hence inferences of posterior probability were made on 9731 trees.

## RESULTS

*28S rDNA data.*—Of 879 unambiguously aligned characters, 388 were parsimony informative (~44%).

TABLE III. Sequences obtained from databases

| Species | GenBank accession No. 18S | 28S |
|---|---|---|
| Ascomycota, Halosphaeriales: | | |
| *Aniptodera chesapeakenis* Shearer et M.A. Mill | AF050483 | U46882 |
| | | AF279374 |
| *Corollospora maritima* Werderm. | U46871 | AF491260 |
| *Corollospora quinqueseptata* A. Nakagiri et Tokura | — | AF491262 |
| *Halosarpheia cincinnatula* Shearer et J.L. Crane | AF352077 | — |
| *Halosarpheia fibrosa* Kohlm. et E. Kohlm. | AF352078 | U46886 |
| *Halosarpheia lotica* Shearer | AF352079 | AF396873 |
| | AF352080 | |
| *Halosarpheia marina* (Cribb et J.W. Cribb) Kohlm. | AF352082 | — |
| *Halosarpheia retorquens* Shearer et J.L. Crane | AF352086 | — |
| | AF352087 | |
| *Halosarpheia spartinae* (Jones) Shearer et J.L. Crane | AF352076 | — |
| *Halosarpheia trullifera* (Kohlm.) E.B.G. Jones, S.T. Moss et Cuomo | — | AF396875 |
| *Halosarpheia unicellularis* Abdel-Wahab et E.B.G. Jones | — | AF396876 |
| *Halosarpheia viscosa* (I. Schmidt) Shearer et J.L. Crane ex Kohlm. et Volkm.-Kohlm. | AF352084 | — |
| *Halosphaeria appendiculata* Linder | U46872 | U46885 |
| *Halosphaeriopsis mediosetigera* (Cribb et J.W. Cribb) T.W. Johnson | U32420 | U46888 |
| *Lignincola laevis* Höhnk | U46873 | U46890 |
| *Nereiospora comata* (Kohlm.) E.B.G. Jones, R.G. Johnson et S.T. Moss | AF050485 | AF491267 |
| *Nereisopora cristata* (Kohlm.) E.B.G. Jones, R.G. Johnson et S.T. Moss | — | AF491268 |
| *Nimbospora effusa* Koch | U46877 | U46892 |
| *Nohea umiumi* Kohlm. et Volkm.-Kohlm. | U46878 | U46893 |
| *Ophiodeira monosemeia* Kohlm. et Volkm.-Kohlm. | U46879 | U46894 |
| Microascales: | | |
| *Microascus trigonosporus* C.W. Emmons et B.O. Dodge | L36987 | U47835 |
| *Petriella setifera* (J.C. Schmidt) Curzi | U43908 | U48421 |
| Neolectales: | | |
| *Bionectria aureofulva* (Cooke & Ellis) Schroers et Samuels | AB013010 | U88123 |
| *Bionectria ochroleuca* (Schwein.) Schroers et Samuels | AB012952 | U00750 |
| *Melanospora fallax* Zukal | U47842 | U17404 |
| *Malanospora zamiae* Corda | U78356 | U17405 |
| *Neocosmospora endophytica* Polishook, Bills et Rossman | — | U17411 |
| *Viridispora diparietospora* (J.H. Mill., Giddens et A.A. Foster) Samuels et Rossman | — | U17413 |
| Sordariales: | | |
| *Cercophora septentrionalis* N. Lundq. | U32400 | U47823 |
| *Chaetomium globosum* Kuntze | AB048285 | U47825 |
| *Sordaria fimicola* (Roberge ex Desm.) Ces. et De Not. | X69851 | AF132330 |
| Xylariales: | | |
| *Daldinia concentrica* (Bolton: Fr.) Ces. et De Not. | U32402 | U47828 |
| *Xylaria curta* Fr. | U32417 | U47840 |
| *Xylaria hypoxylon* (L.: Fr.) Grev. | U20378 | U47841 |

Maximum-parsimony analysis resulted in eight most-parsimonious trees and weighted-parsimony analysis (ti/tv = 1.26) generated one most-parsimonious tree. A K-H test of all nine trees indicated that the tree from the parsimony-weighted analysis was the best phylogenetic hypothesis for this dataset (FIG. 1).

*18S rDNA data.*—Of 1080 unambiguously aligned characters, 171 were parsimony informative (~16%). Maximum-parsimony analysis resulted in 12 most-parsimonious trees and weighted-parsimony analysis (ti/tv = 1.27) generated six most-parsimonious trees. A K-H test of all 18 trees indicated that one of the trees

TABLE IV. Results of Kishino-Hasegawa tests on alternative tree topologies

| Analysis | Tree length | −ln likelihood | Difference −ln L | *P* value |
|---|---|---|---|---|
| Weighted parsimony 18S + 28S data | 2603 | 16446.64255 | (best) | |
| Weighted parsimony 28S data | 2614 | 16493.15004 | 46.50748 | 0.000[a] |
| Constrained tree 18S + 28S data | 2874 | 17214.68701 | 768.04445 | 0.000[a] |
| Constrained tree 28S data | 2878 | 17239.84938 | 793.20683 | 0.000[a] |

[a] Significant difference at $P < 0.05$.

from the parsimony-weighted analysis was the best phylogenetic hypothesis for this dataset. A strict consensus tree of the six parsimony-weighted trees is shown in FIG. 2.

Homogeneity partition tests indicated that the two datasets were incongruent ($P = 0.01$). However, Cunningham (1997), Soltis et al (1998), and Hibbett and Donoghue (2001) found that combined analyses of incongruent datasets produced trees with greater resolution and support than the independent analyses, suggesting that phylogenetic accuracy might be improved in combined analyses of datasets even when ILD tests suggest incongruency. The 18S and 28S datasets thus were combined and the tree topologies evaluated with the K-H test and bootstrapping support.

*18S + 28S rDNA data.*—Of 1959 total characters, 578 were parsimony informative (~30%). Maximum-parsimony analysis resulted in one most-parsimonious tree and weighted-parsimony analysis (ti/tv = 1.19) generated one most-parsimonious tree. A K-H test of the two trees indicated that the tree from the parsimony-weighted analysis was the best phylogenetic hypothesis for this dataset (FIG. 3).

*Constrained analyses.*—Both the 28S data and the combined dataset were constrained to force the monophyly of *Halosarpheia*. A K-H test (TABLE IV) determined that the trees recovered in each of the constrained analyses were significantly less likely than the unconstrained trees.

*Phylogenetic signal.*—The unweighted combined analysis recovered one most-parsimonious tree with all nodes resolved, which provided more phylogenetic resolution than the trees generated in the independent analyses. Twelve trees were recovered in the unweighted analysis of the 18S dataset with ~18% unresolved nodes in the strict consensus tree. Eight trees were recovered in the unweighted analysis of the 28S dataset with ~12% unresolved nodes in the strict consensus tree. K-H tests determined that parsimony-weighted trees were the best fit for each dataset used. In the parsimony-weighted analyses, the combined analysis and the analysis of the 28S dataset each recovered one most-parsimonious tree with complete resolution of all nodes, compared to the 18S dataset, which recovered six most-parsimonious trees. A majority rule consensus of these six trees (FIG. 2) indicated that ~12% of the nodes were unresolved. In addition, the combined analysis had more strongly supported nodes, as measured by bootstrapping, than the independent analyses. In the combined analysis, the 50% majority rule bootstrap consensus tree had 70% of resolved nodes supported by at least 75% and the 28S analysis had 68% of resolved nodes supported by at least 75%. In contrast, the 18S analysis had only 2% of resolved nodes supported by at least 75%.

Because the parsimony-weighted analysis of the 28S dataset and the combined dataset both recovered trees with fully supported nodes and similar bootstrap support, a K-H test was performed (TABLE IV) to compare the trees using maximum likelihood. This determined that the tree inferred in the combined dataset (FIG. 3) was the best phylogenetic hypothesis for the data and the other tree topologies were significantly less likely.

All these results suggest that the combined dataset has a stronger phylogenetic signal than independent datasets. Therefore the tree generated in the weighted-parsimony analyses of the combined dataset (FIG. 3) was used for purposes of discussion concerning generic changes. This tree demonstrates that *Halosarpheia* is polyphyletic and separated into nine clades within the Halosphaeriaceae. The type of the genus, *H. fibrosa*, is placed on a clade with *H. unicellularis* and *H. trullifera*. The high statistical support for the terminal clades, as measured by bootstrapping and Bayesian posterior probability, and the rejection of *Halosarpheia* being monophyletic in the K-H test (TABLE IV), confirm that the polyphyly of *Halosarpheia* is not an analytical artifact of long branch attraction (Felsenstein 1978) or long branch repulsion (Siddall 1998).

## DISCUSSION

*Implications of molecular data.*—Although species of *Halosarpheia* all were included in the Halosphaeriales

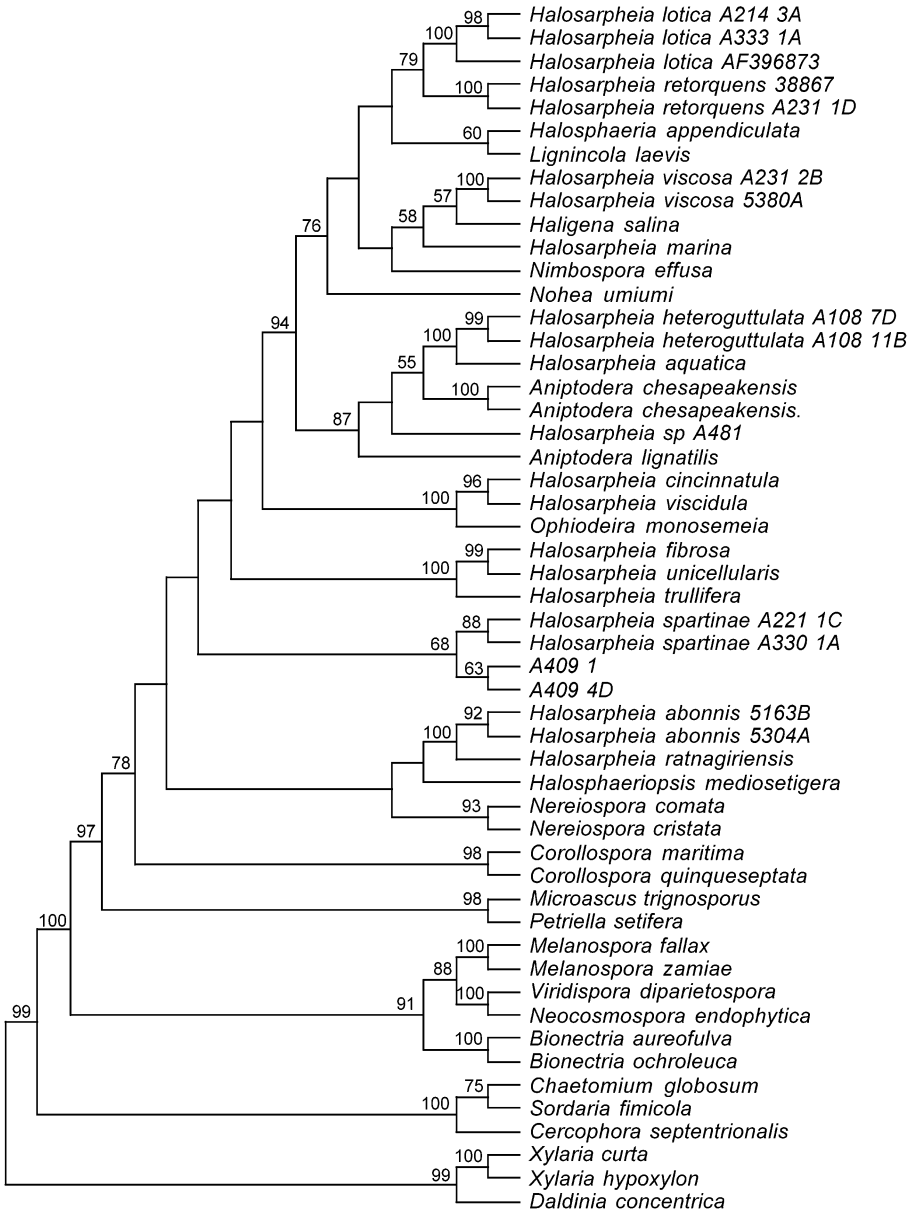



FIG. 1. Cladogram generated with weighted-parsimony analyses (length 2614, CI = 0.52, RI = 0.64, RC = 0.33 and HI = 0.48), inferred from 28S rDNA sequence data with Xylariales as outgroup taxa. Bootstrap values are shown above the branches for values greater than 50%.

clade, according to molecular sequence data (FIG. 3), the monophyly of the genus was not supported (FIG. 4, and TABLE IV). Species of *Halosarpheia* were distributed among numerous well-supported clades that phylogenetically are distant from one another (FIG. 3). These results are in agreement with previous molecular studies (Kong et al 2000, Abdel-Wahab et al 2001, Anderson et al 2001). One possible explanation is that the unfurling appendages used to define the genus *Halosarpheia* are not homologous in development and structure at the ultrastructural level, even though they appear similar at the light microscope level. However, scanning electron microscope (SEM) and transmission electron microscope (TEM) studies on a limited number of species to date do not indicate any heterogeneity in structure or ontogeny. *Halosarpheia fibrosa* (TEM only), *H. viscosa* (TEM only), *H. minuta*, *H. hamata*, *H. abonnis*, *H. ratnagiriensis* (Baker 1991) contain a pore in the apical region of the ascospore epispore wall, and the apical appendages extrude through the pores from material stored in the mesospore wall. *Halosarpheia marina* (Farrant 1986, 1988) has a field of pores in the episporial wall located beneath a collar, and *H. hetero-*

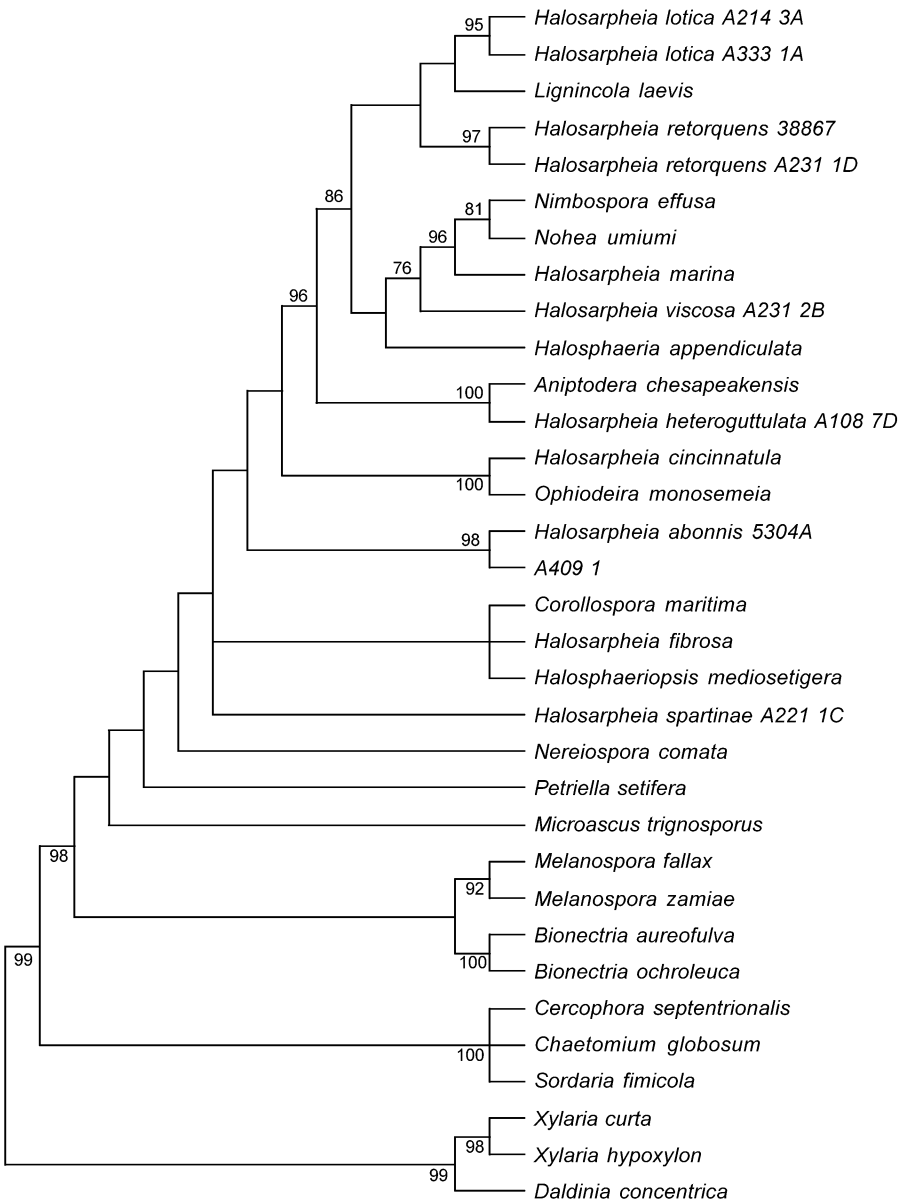



FIG. 2. Cladogram generated with weighted-parsimony analyses (length 1212, CI = 0.38, RI = 0.13, RC = 0.05 and HI = 0.62), inferred from 18S rDNA sequence data with Xylariales as outgroup taxa. Bootstrap values are shown above the branches for values greater than 50%.

*guttulata* (Wong et al 1998) also is reported to have an episporial pore field. Although the size and shape of the appendages of the foregoing species differ (TABLE I), all the appendages are reported to develop the same way, by extrusion through pores in the epispore wall. Additional ultrastructural studies are needed, however, on other species with threadlike unfurling appendages to learn more about their method of appendage development.

*Taxonomic revisions.*—All *Halosarpheia* species in this study share a suite of morphological characters including: (1) thin, threadlike apical ascospore appendages, which are coiled into a hamate structure that is adpressed to the ascospore from when the ascospores are in the ascus to when they are released. When ascospores are released into water, the appendages unfurl to form long threadlike structures that are very sticky; (2) unitunicate asci; (3) ascomata composed of textura angularis; and (4) presence of a neck with periphyses. Molecular sequence data do not reveal that these features are phylogenetically informative for the group of *Halosarpheia* species studied. Each of the phylogenetically supported clades of *Halosarpheia* species (FIG. 3), however, can be de-

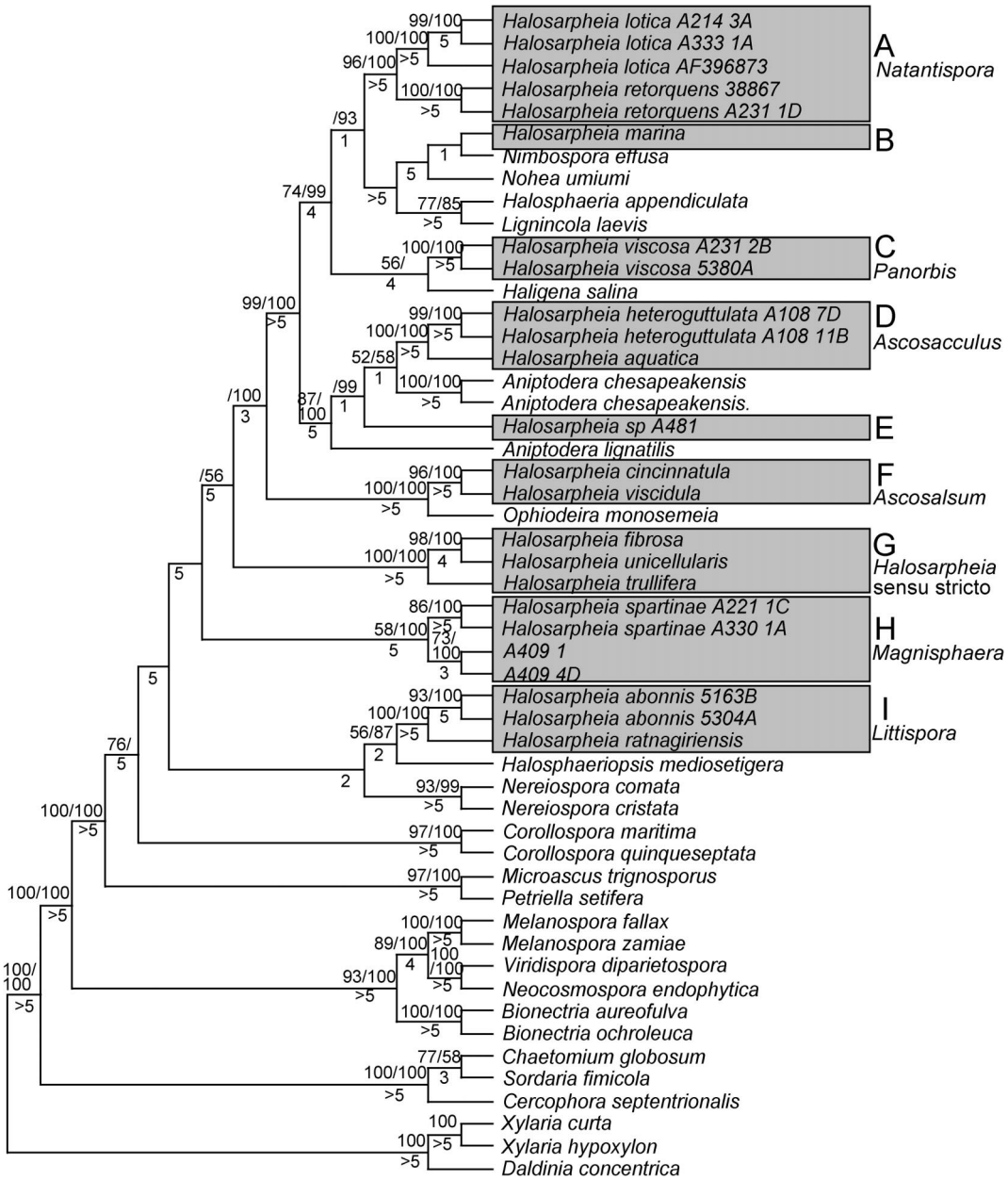



FIG. 3. Cladogram generated with weighted-parsimony analyses (length 2603, CI = 0.52, RI = 0.64, RC = 0.34 and HI = 0.48), inferred from the combined 18S and 28S sequence dataset with Xylariales as outgroup taxa. Maximum-parsimony bootstrap values and Bayesian posterior probabilities, respectively, are shown for values greater than 50% above the branches, and decay indices are shown below the branches.

fined by a distinctive set of morphological characteristics (TABLE I).

*Clade G, Halosarpheia sensu stricto.* The type species of *Halosarpheia*, *H. fibrosa* and its sister taxa, *H. trullifera* and *H. unicellularis* (Clade G, FIG. 3), all share these characteristics: large, ellipsoid or sub-globose, coriaceous, ostiolate ascomata; long, cylindrical to subconical, periphysate necks; relatively wide, two-layered peridia with the outer layer usually darker than the inner layer, and the inner layer hyaline and intergrading with the centrum pseudoparenchyma; a hamathecium of pseudoparenchyma that separates into catenophyses; asci that are relatively thin-walled throughout but slightly thickened at the apex, clavate, pedunculate and lacking an apical pore and apical apparatus; broadly ellipsoid ascospores that are broadly rounded at the apices, hyaline and non-septate or one septate; coiled, hamate appendages at both apices that are small, cap-like and shorter than the length of a single ascospore cell when coiled and

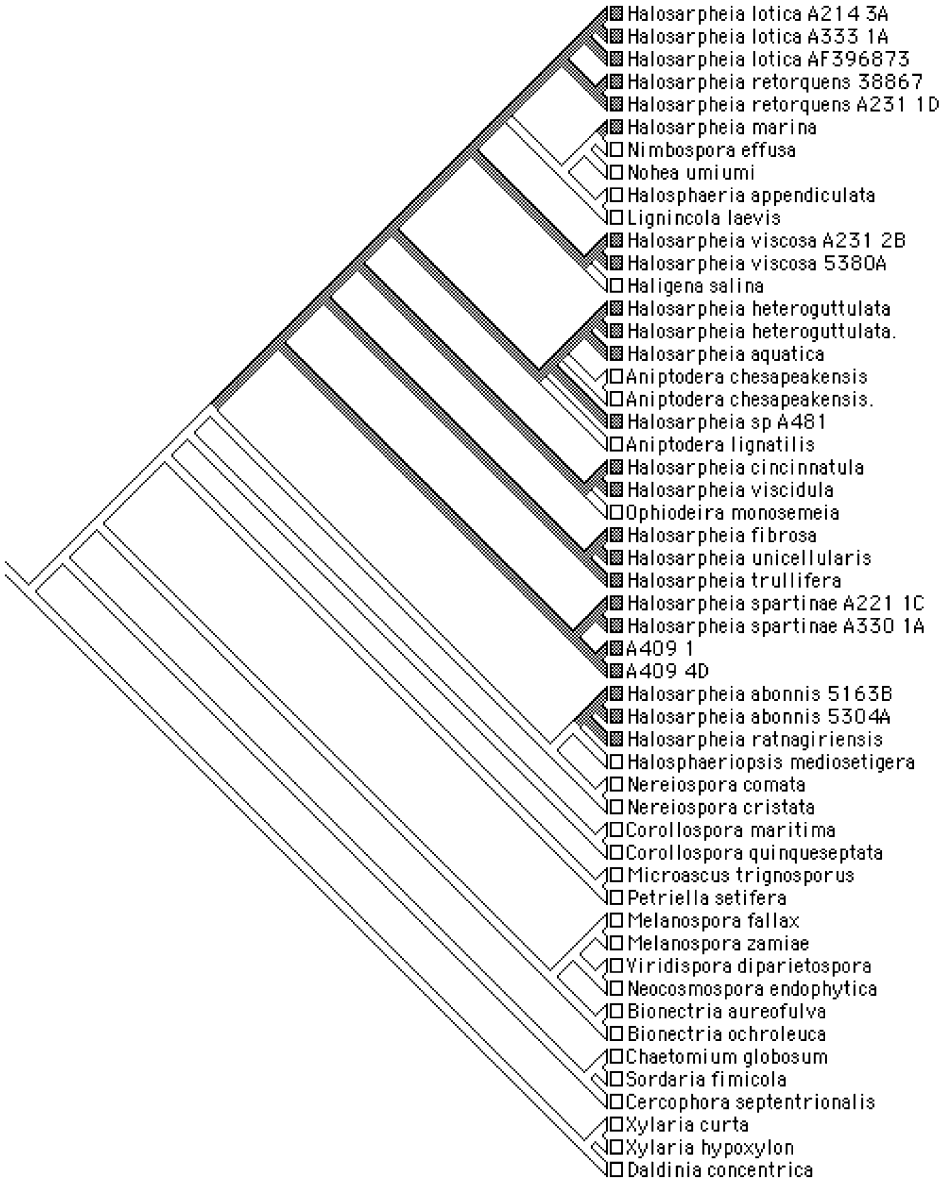



FIG. 4. Slanted cladogram of the weighted-parsimony analysis of the combined 18S + 28S dataset, indicating the (■) presence or (□) absence of unfurling, threadlike appendages, as reported in *Halosarpheia* species.

which unfurl in water to form long, sticky, threadlike structures; occurrence on wood submerged in seawater in the tropics or subtropics (TABLE I). These species comprise *Halosarpheia* sensu stricto (Clade G, FIG. 3) and differ from one another in ascomal shape and pigmentation, peridial cell wall thickening, presence or absence of a beak in immature asci, degree of ascus deliquescence, and ascospore size and septation.

*Clade A*. The *H. retorquens*/*H. lotica* clade (Clade A, FIG. 3) consists of two species that originally were described from freshwater habitats (Shearer and Crane 1980, Shearer 1984). Both species since have been reported from brackish and marine habitats (Schmit and Shearer 2003, http://fm5web.life.uiuc.edu:23523/mangrove/) and are among the very few species of Halosphaeriales that occur in both freshwater and marine habitats. These two species differ from *Halosarpheia* sensu stricto (Clade G, FIG. 3) in ascomal shape, peridium anatomy, ascus deliquescence and ascospore morphology (TABLE I). The peridium in *Halosarpheia* sensu stricto is distinctly two-layered and composed of up to 14–15 layers of cells, while that of the Clade A species is composed of a single layer of up to seven thin-walled cells, with the outer three or four cell layers pigmented brown. The asci of *H. fibrosa* are described as persistent and thin-walled, except below the ascus apex (Kohlmeyer and

Kohlmeyer 1977), while those of *H. retorquens* and *H. lotica* deliquesce very early, often before ascospore maturity and are thin-walled throughout. In fact, the asci of *H. lotica* deliquesce so early that putative delimiting membranes can be seen around the ascospores (Anderson et al 2001). Ascospores of the three species of *Halosarpheia* sensu stricto are broadly ellipsoid with the apices broadly rounded to flattened, while those of *H. retorquens* and *H. lotica* have a fusoid to ellipsoid shape and are more narrowly tapered at the apex. In addition, the ascospore appendages in the hamate stage are larger and adpressed along the sides of the ascospore to the midseptum in *H. retorquens* and *H. lotica*, while those of *H. fibrosa*, *H. trullifera* and *H. unicellularis* are smaller and more cap-like. Furthermore, the species in *Halosarpheia* sensu stricto occur on wood in marine habitats, while *H. retorquens* and *H. lotica* occur in freshwater, brackish and marine habitats and on woody and herbaceous debris (Shearer 2001, Schmit and Shearer 2003, http://fm5web.life.uiuc.edu:23523/mangrove/). Based on the foregoing phenotypic differences and analyses of molecular sequence data that indicate that *H. lotica* and *H. retorquens* phylogenetically are distinct from the type species of *Halosarpheia* and from other clades of *Halosarpheia* species, a new genus, *Natantispora*, is proposed to accommodate *H. retorquens* and *H. lotica*.

The characteristics that define *Natantispora* are: immersed to superficial, ostiolate, membranous, black ascomata; a thin-walled peridium, composed of pseudoparenchyma in longitudinal section, of textura angularis in surface view; long, cylindrical, periphysate necks, dark-pigmented, gradually lightening toward the apex; hamathecia of catenophyses; clavate, thin-walled asci lacking any thickening of the apical wall, an apical pore or an apical apparatus; asci deliquescing before or at ascospore maturity; hyaline, fusiform to ellipsoidal, one septate ascospores with a single appendage at each apex; appendages threadlike, coiled into a hamate structure equal to or longer than a single ascospore cell, unfurling in water to form a long, fine, sticky, threadlike structure.

**Natantispora** J. Campb., J.L. Anderson et Shearer, gen. nov.

Ascomata immersa vel superficiala, ostiolata, membranacea, nigra, longicolla. Peridium e textura angulari constitutum. Collum longum, cylindracum, fuscatum, gradatim ilustrans versus cacumen, periphysatum. Hamathecium catenophysibus. Asci clavati, tenuitunicati, sine apparatu apicali et poro, deliquescentes. Ascosporae hyalinae, fusiformes vel ellipsoideae, uniseptatae, appendiculatae. Ascosporarum appendices bipolares, e capillamento uno conspirato vel complicato, primo hamato, denique in aqua retorquente in filum longum compositae.

*Species typica. Natantispora retorquens* (Shearer & J.L. Crane) J. Campb., J.L. Anderson et Shearer

*Etymology.* From the Latin *spora* and *natans* = floating, in relation to the spores floating in water.

**Natantispora retorquens** (Shearer & J.L. Crane) J. Campb., J.L. Anderson et Shearer comb. nov.

Basionym: *Halosarpheia retorquens* Shearer & J.L. Crane. Bot. Mar. 23: 608. 1980.

*Specimens examined.* USA. MASSACHUSETTS: Barnstable County, Head of Meadow Beach (Atlantic Ocean), 42° 3′ 14″ N, 82° 4′ 49″ W, 30 June 1994, on herbaceous debris, *J.L. Crane, A4-11* (ILL); MINNESOTA: West arm of Lake Itasca, Lake Itasca State Park, 47° 11′ 51″ N, 95° 13′ 25″ W, water temp 2.5 C, pH 5.5, on submerged *Typha* sp., 22 Oct 1993, *J.L. Crane & C.A. Shearer, A4-10* (ILL).

**Natantispora lotica** (Shearer) J. Campb., J.L. Anderson et Shearer comb. nov.

Basionym: *Halosarpheia lotica* Shearer. Mycotaxon 20: 505. 1984.

*Specimens examined.* USA. WISCONSIN: Oneida County, Tomahawk River, 45° 50′ 06″ N, 89° 48′ 23″ W, on submerged, decorticated wood, 27 Jul 1995, *P. Fallah. A214-3* (ILL); Iron county, Manitowish River at jct. with Rt. 51, 46° 8′ 14″ N, 89° 54′ 42″ W, on submerged wood, 19 June 1996, *P. Fallah & J.L. Crane, A333-1* (ILL).

*Clade B. Halosarpheia marina* is placed on a clade that includes species of *Nimbospora*, *Nohea*, *Halosphaeria* and *Lignincola* (FIG. 3) but with no significant posterior probability or bootstrap support, indicating that its position here is tenuous. This is further demonstrated in analyses on the 28S dataset, in which *H. marina* is placed on a clade that consists of species of *Nimbospora*, *Haligena* and *Halosarpheia viscosa* (FIG. 1). This placement has no significant bootstrap support: <50% for the clade, 57% for the placement of *H. salina*. Morphologically, *H. marina* has characteristics of both *Halosarpheia* sensu stricto and *Aniptodera* but, based on molecular data, is not closely related to species in either genus. This taxon requires further molecular and morphological study, and thus no taxonomic changes are proposed at this time for *H. marina*.

*Clade C.* Clade C (FIG. 3) consists of two different isolates of *H. viscosa* (TABLE II). *Halosarpheia viscosa* differs from *Halosarpheia* sensu stricto in having globose as opposed to obpyriform to ellipsoidal ascomata, in lacking catenophyses although present in some collections (Hyde et al 1999, B. and J. Kohlmeyer pers comm), and in the shape and size of the ascospores and ascospore appendages (TABLE I). In addition, species of *Halosarpheia* sensu stricto have been reported only from marine habitats in the tropics while *H. viscosa* has been reported from both temperate and tropical areas and freshwater, brackish and marine habitats. *Halosarpheia viscosa* also is phy-

logenetically distant from *Halosarpheia* sensu stricto and all of the other *Halosarpheia* species (FIG. 3). Based on morphological and molecular data, this species cannot be included in *Halosarpheia* sensu stricto (Clade G, FIG. 3) or in any of the new genera based on *Halosarpheia* species established herein, thus a new genus, *Panorbis*, is erected for *H. viscosa*.

The genus *Panorbis* is characterized by: globose to subglobose ostiolate ascomata that are hyaline at first but become black with age; a membranous, thin-walled peridium 10–13 cells wide, of textura angularis in surface view; long cylindrical periphysate necks that are hyaline at the apex and pigmented at the base; hamathecia absent or present as catenophyses; ellipsoid to clavate, thin-walled, persistent to deliquescent asci separating from the ascogenous hyphae and lacking an apical pore and apical apparatus; hyaline, one septate, ellipsoidal ascospores tapered or rounded at the apices and often flattened on one side, with an apical appendage at each end; appendages small, hamate at first, less than or equal to one-half the ascospore length, unfurling in water to form long, sticky, threadlike structures; colonies on CMA agar composed of dark gray aerial hyphae, dark brown immersed hyphae, staining agar brown; occurrence on woody and herbaceous plant debris in freshwater, brackish and marine habitats.

**Panorbis** J. Campb., J.L. Anderson et Shearer, gen. nov.

Ascomata immersa ad superficiala, globosa, ostiolata, fuscogrisea vel nigra, saepe pellucida, membranacea, longicolla. Collum longum, cylindracum, periphysatum. Peridium tenuitunicatum, e textura angulari constitutum. Hamathecium absens vel catenophysibus. Asci clavati, tenuitunicati, sine apparatu apicali et poro, deliquescentes. Ascosporae cylindracae vel fusiformes, complanatae aut concavae in uno latere, hyalinae, uniseptatae, appendiculatae. Ascosporarum appendices bipolares, e capillamento uno conspirato vel complicato, primo hamato, denique in aqua retorquente in filum longum compositae.

*Species typica. Panorbis viscosus* (I. Schmidt) J. Campb., J.L. Anderson et Shearer

*Etymology.* From the Latin *pan* = throughout, and *Orbis* = the world, in relation to its worldwide distribution.

**Panorbis viscosus** (I. Schmidt) J. Campb., J.L. Anderson et Shearer comb. nov.

Basionym: *Halosphaeria viscosa* I. Schmidt. Mycotaxon 24: 420, 1985.

*Specimens examined.* USA. MASSACHUSETTS: Barnstable County, Head of Meadow Beach (Atlantic Ocean), 42° 3′ 14″ N, 82° 4′ 49″ W, 30 May 1994, on herbaceous debris, *J.L. Crane, A231-1* (ILL), Salt Pond, 41° 50′ 10″ N, 69° 58′ 15″ W, on herbaceous debris and small corticated woody branches, 07 May 1996, *J.L. Crane, A231-2* (ILL).

*Panorbis viscosus* is most similar in morphology to *Natantispora retorquens*. The two species often co-occur on the same substrate and are difficult to identify from field samples. Molecular data, however, indicate that the two species are not closely related phylogenetically (Clades C and A, FIG. 3). A careful comparison of the morphology of *N. retorquens* and *P. viscosus* indicates that the morphology of the ascospores might be the most reliable feature for identification. The ascospores of *P. viscosus* are shorter and often slightly flattened or inwardly curved on one side. When stained in lactic acid with trypan blue or cotton blue, the ascospores of *P. viscosus* stain intensely around the midseptum and at both apices while those of *N. retorquens* do not. In addition, whether *P. viscosus* has catenophyses is questionable but they are prominent in *N. retorquens*. Catenophyses were not reported for *P. viscosus* by Schmidt (1974) or Shearer and Crane (1980) but were reported by Hyde et al (1999). We did not observe catenophyses in our recent collections of *P. viscosus*, including the isolate that was sequenced. The asci of *P. viscosus* are more persistent than those of *N. retorquens*, and they separate from the ascogenous hyphae and lie free in the venter cavity. However, observation of these characters depends upon the age of the material being examined. Fully mature ascomata of both species may contain few or no asci and many liberated ascospores.

*Clade D.* Clade D (FIG. 3) consists of two isolates of *H. heteroguttulata* and one isolate of *H. aquatica*. These species are well supported as sister taxa and are morphologically similar to one another. Both of these taxa differ from *H. fibrosa* in having: globose, membranous ascomata; early deliquescent asci; tapering, fusiform to cylindrical ascospores; distinctive guttulation patterns; and occurrence in freshwater habitats. In addition, based on molecular data, they are phylogenetically distant from *Halosarpheia* sensu stricto (Clade G, FIG. 3) and therefore cannot be accommodated in *Halosarpheia*. These two taxa also cannot be accommodated in the genus of their closest sister species, *Aniptodera chesapeakensis* (type species of the genus *Aniptodera*), due to morphological differences in ascomata, asci and ascospores. The ascomata of Clade D species are dark pigmented, while those of *Aniptodera* are hyaline; the asci of Clade D species are saccate, deliquescent, thin-walled throughout and lack an apical pore and retraction of cytoplasm below the ascus apex, while those of *Aniptodera* are clavate, persistent, have a thickened apical wall and pore and retraction of cytoplasm below the apex; and cylindrical thin-walled ascospores that are polyguttulate compared to those of *Aniptodera*, which are ellipsoid, thick-walled and contain a single large guttule in each cell (TABLE I). A new genus,

*Ascosacculus*, therefore is established for *H. aquatica* and *H. heteroguttulata*.

The suite of phenotypic characters that both species of *Ascosacculus* share include: small, globose to subglobose, membranous, brown, ostiolate ascomata with long, narrow, thin-walled, hyaline to pale brown periphysate necks; thin-walled peridium of textura angularis in surface view; thin-walled, early deliquescent asci lacking an apical pore and apical apparatus; fusiform to cylindrical, hyaline, one septate ascospores filled with many small guttules and having a hamate appendage at each apex that unfurls to form long, threadlike, sticky appendages; and occurrence on submerged woody debris in tropical freshwater habitats.

**Ascosacculus** J. Campb., J.L. Anderson et Shearer gen. nov.

Ascomata globosa vel subglobosa, immersa ad superficialia, membranacea, brunnea, ostiolata, longicolla. Peridium e textura angulari constitutum. Hamathecium catenophysibus. Asci saccati tenuitunicati, sine apparatu apicali et poro, deliquescentes. Ascosporae fusiformes vel cylindraceae, hyalinae, guttulatae, appendiculatae, uniseptatae; septa media vel supra media. Ascosporarum appendices bipolares, e capillamento uno conspirato vel complicato, primo hamato, denique in aqua retorquente in filum longum compositae.

*Species typica. Ascosacculus aquaticus* (K.D. Hyde) J. Campb., J.L. Anderson et Shearer

*Etymology.* From the Latin *asci* and *sacculus* = a little sac, in relation to the saccate asci.

**Ascosacculus aquaticus** (K.D. Hyde) J. Campb., J.L. Anderson et Shearer, comb. nov.

Basionym: *Halosarpheia aquatica* K.D. Hyde. Aust. Syst. Bot. 5: 407, 1992.

*Specimens examined.* COSTA RICA. HEREDIA: La Selva Biological Station, stream, 10° 25′ 08″ N, 84° 0′ 22″ W, altitude 35.56 m, water temp 25 C, pH 5.5, on submerged decorticated wood, 19 May 2000, *J. Anderson* and *R. Wulffen, A444-1* (ILL).

*Known distribution.* Australia, Costa Rica

**Ascosacculus heteroguttulatus** (S.W. Wong, K.D. Hyde & E.B.G. Jones) J. Campb., J.L. Anderson et Shearer, comb. nov.

Basionym: *Halosarpheia heteroguttulata* S.W. Wong, K.D. Hyde & E.B.G. Jones. Can. J. Bot. 76: 1858, 1998.

*Specimens examined.* COSTA RICA. HEREDIA: La Selva Biological Station, stream at Sura 30, 10° 25′ 47″ N, 84° 0′ 27″ W, water temp 26 C, pH 7, on submerged, decorticated woody debris, 17 May 2000, *J. Anderson & R. Wulffen, A108-11* (ILL); USA. FLORIDA: Fakahatchee Strand Preserve State Park, Collier County, 26° 1′ 28″ N, 81° 24′ 60″ W, on submerged, decorticated wood, 13 May 1993, *J.L. Crane, A108-7* (ILL).

*Known distribution.* Australia, Brunei, Costa Rica, Hong Kong, Mauritius, Philippines, South Africa, USA (Florida).

*Ascosacculus heteroguttulatus* differs from *A. aquaticus* in having shorter and wider ascospores and one to two large guttules in one cell, rather than being uniformly polyguttulate in both cells.

*Clade E.* Unidentified *Halosarpheia* species A481-1 on Clade E (FIG. 3) differs from its closest relatives in *Aniptodera* and *Halosarpheia* based on both molecular and morphological data. The ascospores of A481-1 are ellipsoid, one to three septate and have coiled apical appendages at both apices. The appendages are extremely long and strap-like and differ from all the appendages previously described for *Halosarpheia* and *Aniptodera* species. This taxon requires further molecular and morphological study and will not be treated further in this paper.

*Clade F.* The two species on Clade F (FIG. 3), *H. cincinnatula* and *H. viscidula*, share several morphological features that differ distinctly from those of *Halosarpheia* sensu stricto (Clade G, FIG. 3). The ascomata are relatively small (~100–300 μm diam) and hyaline to lightly pigmented. The peridium is narrow, one layered and consists of only 3–7 layers of cells. Centrum pseudoparenchyma apparently disintegrates so that no catenophyses are present in mature ascomata. The asci deliquesce before or at ascospore maturity. Both *H. cincinnatula* and *H. viscidula* form pale, white to cream-colored colonies in culture, while all *Halosarpheia* sensu stricto species form dark-pigmented colonies. Again, based on morphological differences and molecular based phylogenetic distance, the taxa on Clade F (FIG. 3) do not belong in *Halosarpheia* sensu stricto (Clade G, FIG. 3). There is significant support (100% bootstrap, 100% posterior probability, decay index of more than 5) for *O. monosemeia* as a sister taxon to *H. cincinnatula* and *H. viscidula*. Even though *O. monosemeia* and *H. cincinnatula* both have only a single apical appendage, morphological differences and a distinct dichotomy in ascospore morphology do not support transfer of the two *Halosarpheia* species into *Ophiodeira*. The ascospores of *O. monosemeia* are ellipsoidal, between 16–21 μm long and 6–8 μm wide and one septate. The ascospores of *H. cincinnatula* and *H. viscidula* are narrowly cylindrical, multiseptate and 34–60 × 3.5–5, 45–80 × 4–6.5 μm, respectively. In addition, ascomata of *O. monosemeia* are formed under a thin stroma while those of *H. cincinnatula* and *H. viscidula* are not. Based on both morphology and molecular data, a new genus, *Ascosalsum*, is proposed for *H. cincinnatula* and *H. viscidula*.

The morphological features defining *Ascosalsum* include: hyaline, globose to subglobose ascomata

with long, cylindrical, hyaline, periphysate necks; thin-walled, hyaline, membranous peridium of textura angularis; hamathecia absent; asci clavate or ellipsoid, thin-walled throughout, lacking an apical thickening, apical pore and apical apparatus, deliquescing to release ascospores; long, cylindrical or narrowly fusiform, hyaline, phragmoseptate ascospores with threadlike, coiled apical appendages at one or both apices, appendages unfurling in water; colonies on peptone, glucose, yeast extract agar made with 15 ppt seawater white- to cream-colored, mostly immersed, consisting of hyaline septate hyphae.

**Ascosalsum** J. Campb., J.L. Anderson et Shearer, gen. nov.

Ascomata globosa vel subglobosa, alba, cremea vel furca, perlucidula, ostiolata, longicolla. Collum cylindraceum, irregulare, hyalinum, periphysatum. Hamathecium absens ad maturitatem. Asci ellipsoidei vel clavati, tenuitunicati, deliquescentes, sine apparatu apicali et poro. Ascosporae cylindracae vel fusiformes, multiseptatae, hyalinae, appendiculatae. Ascosporarum appendices bipolares, e capillamento uno conspirato vel complicato, primo hamato, denique in aqua retorquente in filum longum compositae.

*Species typica. Ascosalsum viscidulum* (Kohlm.) J. Campb., J.L. Anderson et Shearer

*Etymology.* From the Latin *asci* and *salsum* = brackish, in relation to the brackish habitat of these species

**Ascosalsum viscidulum** (J. & E. Kohlm.) J. Campb., J.L. Anderson et Shearer comb. nov.

Basionym: *Haligena viscidula* J. & E. Kohlm. Nova Hedwigia 9: 92. 1965.

=*Halosarpheia viscidula* (J. & E. Kohlm.) Shearer & J.L. Crane Bot. Mar. 23: 608. 1980.

**Ascosalsum cincinnatulum** (Shearer & J.L. Crane) J. Campb., J.L. Anderson et Shearer, comb. nov.

Basionym: *Halosarpheia cincinnatula* Shearer & J.L. Crane. Bot. Mar. 23: 613. 1980.

*Specimens examined.* USA. FLORIDA: Everglades National Park, Mangrove Swamp, Snake Bite Trail, 25° 12′ 6″ N, 80° 55′ 19″ W, on submerged, corticated wood, 23 Jan 1997, *J.L. Crane & J.D. Schoknecht, A318-1* (ILL).

*Clade H. Halosarpheia spartinae* and undescribed isolates A409-1 and A409-4D (Clade H, FIG. 3) differ morphologically in ascomal morphology and pigmentation and ascospore shape, pigmentation and wall roughness from *Halosarpheia* sensu stricto (Clade G, FIG. 3) and from all other new genera established herein (TABLE I). The ascomata of *H. spartinae* are globose and black and have short necks, in contrast to the obpyriform, broadly ellipsoidal and cream-colored to brown or black ascomata with very long necks in *Halosarpheia* sensu stricto. The ascospores of *H. spartinae* differ from those of *Halosarpheia* sensu stricto in being tapered at the apices rather than broadly rounded, phragmoseptate as opposed to one septate, and pigmented and rough walled as opposed to hyaline and smooth walled (TABLE I). Based on molecular and morphological data, therefore, a new genus, *Magnisphaera*, is proposed for *H. spartinae* and undescribed isolates A409-1 and A409-4D.

The genus *Magnisphaera* is characterized by large, black, globose to flattened globose ascomata with a central neck that is short in proportion to the length of the ascoma. The peridium is two-layered and composed of about 8–10 large cells forming a textura angularis with the cell walls darkened and occluded with brown amorphous material towards the outside. The asci are ellipsoid, thin-walled throughout, early deliquescent and lack an apical pore and apical apparatus. The ascospores are broadly acerose, phragmoseptate, constricted at the septa, with a large guttule in each of the central cells and a pattern of lipid droplets about the mid-septum and at the ascospore apices. The ascospores are hyaline to pale reddish gold at first and then become subhyaline to gray brown and the ascospore wall is warted. The bipolar ascospore appendages are coiled into short, flat, hamate structures that unfurl in water to form a long threadlike structure.

Undescribed isolates A409-1 and A409-4D, sister taxa basal to *H. spartinae* (Clade H, FIG. 3), morphologically are similar in all aspects to *H. spartinae*, except that the ascospores of A409 have three rather than three to nine septa and are shorter and broader than those of *H. spartinae*. In addition, the A409 isolates are from freshwater habitats while *H. spartinae* is known only from marine and brackish water habitats. Undescribed isolates A409-1 and A409-4D are morphologically indistinguishable from one another and are described herein as a new species.

**Magnisphaera** J. Campb., J.L. Anderson et Shearer gen. nov

Ascomata magna, globosa vel subglobosa, immersa ad superficialia, nigra, coriacea, rugulosa, ostiolata. Rostrum breve, conicum vel cylindraceum, nigrum, periphysatum. Hamathecium catenophysibus. Peridium bistratosum. Asci unitunicati, tenuitunicati, clavati vel elliptici, deliquescentes, sine apparatu apicali et poro. Ascosporae ellipsoideae vel fusiformes, septatae, hyalinae, fulvescentes, verruculosae, uniguttulatae, appendiculatae. Ascosporarum appendices bipolares, e capillamento uno conspirato vel complicato, primo hamato, denique in aqua retorquente in filum longum compositae.

*Species typica. Magnisphaera spartinae* (E.B.G. Jones) J. Campb., J.L. Anderson et Shearer

*Etymology.* From the Latin *magnus* = large, and

*sphaera* = globose, in relation to the large, globose ascomata.

**Magnisphaera spartinae** (E.B.G. Jones) J. Campb., J.L. Anderson et Shearer, comb. nov.

Basionym: *Haligena spartinae* E.B.G. Jones. Trans. Br. Mycol. Soc. 45: 245. 1962.

=*Halosarpheia spartinae* (E.B.G. Jones) Shearer & J.L. Crane. Bot. Mar. 23: 608. 1980.

*Specimens examined.* USA. MASSACHUSETTS: Barnstable County, Palmet River salt marsh (Atlantic Ocean), 42° 0′ 52″ N, 82° 3′ 47″ W, 30 June 1994, on herbaceous debris, *J.L. Crane, A221-1* (ILL); Salt Pond, 41° 50′ 10″ N, 69° 58′ 15″ W, on herbaceous debris, 05 Jul 1996, *J.L. Crane, A330-1* (ILL).

**Magnisphaera stevemossago** J. Campb., J.L. Anderson et Shearer, sp. nov. (FIGS. 5–15)

Ascomata magna, globosa vel subglobosa, immersa ad superficialia, nigra, coriacea, 340–520 μm in diametro, rugulosa, cum aut sine ostiolata. Rostrum breve, conicum vel cylindraceum, nigrum, periphysatum, 71 × 60 μm. Hamathecium catenophysibus. Peridium 32–40 μm latum, 7–10 cellulae crassum, bistratosum. Asci unitunicati, tenuitunicati, clavati vel elliptici, 124–246 × 28–49 μm, sine apparatu apicali et poro, deliquescentes. Ascosporae ellipsoideae vel fusiformes, 45–64 × 16–31 μm, triseptatae, hyalinae, fulvescentes, verruculosae, uniguttulatae, appendiculatae. Ascosporarum appendices bipolares, e capillamento uno conspirato vel complicato, primo hamato, denique in aqua retorquente in filum longum compositae. Coloniae in frumento agaro distendentes, aetheriae hyphae canae, immersae hyphae atrobrunneae. Anamorph non observatum.

Ascomata large, globose to subglobose (FIG. 5), partially immersed, black, coriaceous, 340–520 μm diam, somewhat rough-walled, with or without an ostiole, with a short neck when ostiolate. Peridium 32–40 μm wide, 7–10 cells thick, two-layered, outer layer of dark walled isodiametric cells with thin or slightly thickened walls, inner layer of hyaline, thin-walled, elongated cells (FIG. 6). Neck short, conical to cylindrical, black, periphysate, 71 × 60 μm (FIG. 7). Hamathecium of catenophyses (FIG. 8). Asci unitunicate, thin-walled throughout, clavate to ellipsoid, 124–246 × 28–49 μm, lacking an apical pore or apparatus, deliquescent (FIGS. 9–10). Ascospores ellipsoidal to fusiform, 45–64 × 16–31 μm, 3 septate (FIGS. 11–15), hyaline to pale golden brown, wall finely warted (FIG. 15), with a large guttule in each cell (FIGS. 11, 13) and lipid droplets about the mid-septum and at the apices (FIG. 15), with threadlike appendages at both apices (FIG. 14). Appendages of long, coiled threads that form a hamate structure and unwind in water to form a long sticky thread. Colonies on CMA spreading, aerial hyphae gray, immersed hyphae dark brown. No anamorph observed.

*Etymology.* Named after Steve Moss, in his memory and in recognition of all that he achieved (Latin *agere* = to achieve).

*Holotype.* USA. COLORADO: Deckers at swampy, marshy fork in Platt River, 39° 15′ 06″ N, 105° 14′ 05″ W, elevation ~2100 m, water temp 19 C, pH 5.5–6.0, on submerged, decorticated wood, 26 Jul 1998, *C.A. Shearer, A409-1* (ILL);

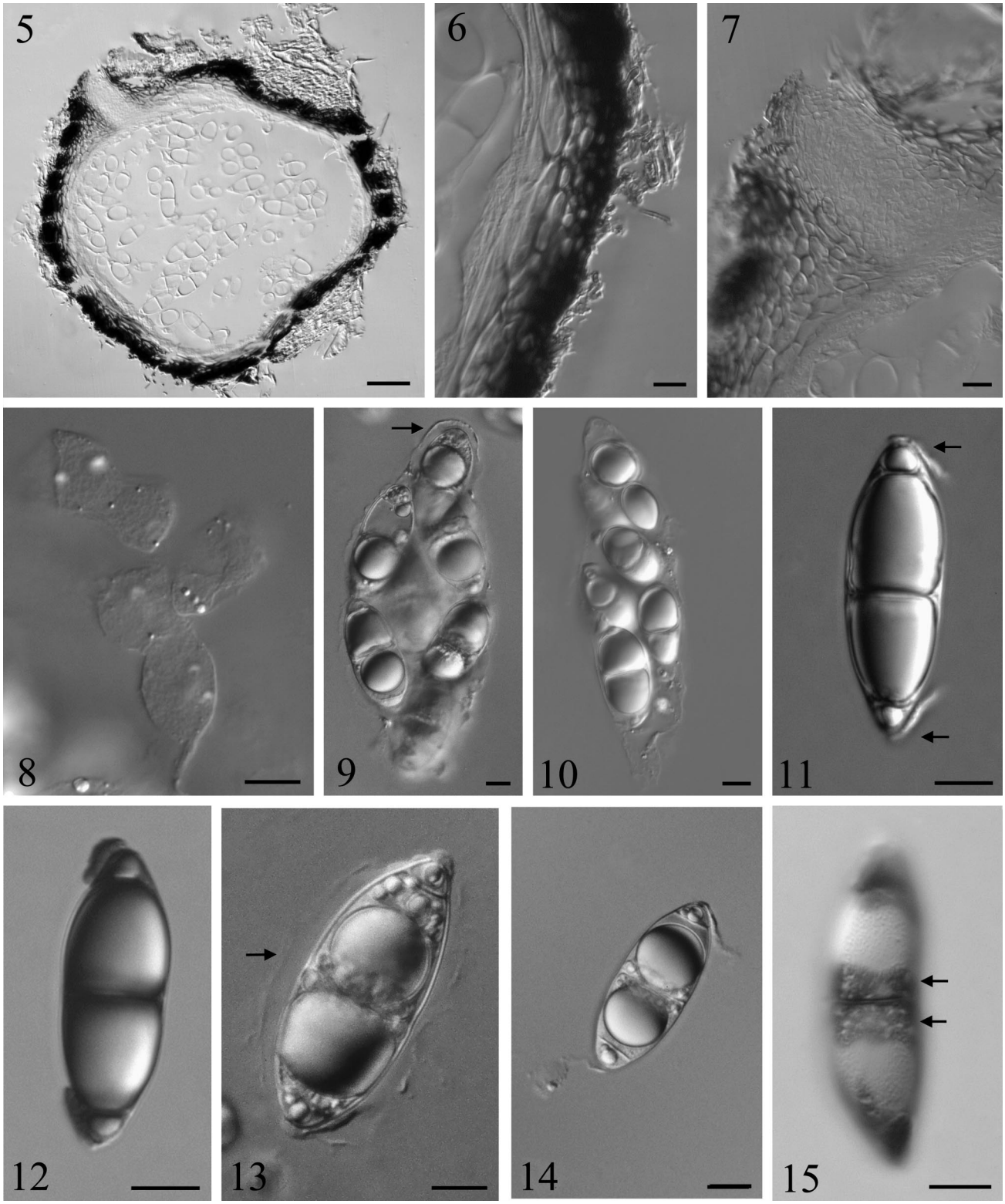
*Additional specimens examined.* USA. ALASKA: Chatanika River at Cripple Creek Campground Access, 65° 16′ 25″ N, 146° 38′ 54″ W, water temp 6.7 C, pH 5.5, 03 Jul 2000, *C.A. Shearer & W.L. Hurley, A409-3* (ILL), Chatanika River, Cripple Creek Campground Access, 65° 16′ 25″ N, 146° 38′ 54″ W, water temp 6.7 C, pH 5.5, 03 Jul 2000, *C.A. Shearer & W.L. Hurley, A409-3* (ILL), pond near Chena River (access mile 28.6), 65° 53′ 01″ N, 146° 41′ 51″ W, water temp 18 C, pH 6, 04 Jul 2000, *C.A. Shearer & W.L. Hurley, A409-4* (ILL), Chatanika River at Chatanika River Campground, Steese Highway (Mile 41), 65° 11′ 27″ N, 147° 15′ 55″ W, water temp 10.3 C, pH 5.5, 29 Jun 2000, *C.A. Shearer, W.L. Hurley, G. Laursen, A409-5* (ILL).

*Clade I.* Clade I (FIG. 3) consists of two species, *H. abonnis* and *H. ratnagiriensis*, with a very distinctive morphology that differs from both *Halosarpheia* sensu stricto (Clade G, FIG. 3) and other taxa previously placed in *Halosarpheia* (TABLE I). The sister taxa to *H. abonnis* and *H. ratnagiriensis* are species of *Nereiospora* and *Halosphaeriopsis* (FIG. 3), although there is no significant bootstrap or Bayesian support for their inclusion in this clade. Furthermore, *Nereiospora* and *Halosphaeriopsis* differ distinctively in the morphology of their ascomata, asci and ascospores from *H. abonnis* and *H. ratnagiriensis*. A new genus, *Littispora*, therefore is established for these two species.

The unifying morphological features of *Littispora* are: hyaline to light brown, coriaceous, ellipsoidal ascomata with long, cylindrical, periphysate necks; thick-walled, two-layered peridium of textura angularis; persistent, clavate asci with an apical plate and apical pore; one-septate, hyaline, ellipsoid ascospores with an appendage at each apex; appendages large hamate structures that unfurl in water to form long threadlike structures; on mangrove wood in the tropics.

**Littispora** J. Campb., J.L. Anderson et Shearer, gen. nov.

Ascomata immersa, ellipsoidea, ostiolata, longicolla, coriacea, hyalina vel subfusca. Peridium bistratosum, e textura angulari constitutum. Collum longum, cylindracum, hyalinum, periphysatum, insertum centraliter aut lateraliter. Hamathecium catenophysibus. Asci unitunicati, clavati, pedunculati, tenuitunicati, inspissatus apicem versus, persistentes, sine apparatu apicali et poro. Ascosporae ellipsoideae, uniseptatae, subconstrictae ad septum, hyalinae, appendiculatae. Ascosporarum appendices magnae bipolares, e cap-




FIGS. 5–15. *Magnisphaera stevemossago*. Bar in FIG. 5 = 50 μm, Bar in FIGS. 6–15 = 10 μm. 5. Longitudinal median section through an ascoma. 6. Longitudinal median section through peridium. 7. Longitudinal median section through beak illustrating periphyses. 8. Catenophyses. 9 & 10. Asci. Arrow indicates thin-walled ascus apex lacking a pore and apical apparatus. 11. Unstained ascospore illustrating lipid guttules and apical appendages in the hamate stage. 12. Ascospore stained with aqueous nigrosin illustrating apical appendages in hamate stage. 13. Unstained ascospore with possible delimiting membrane surrounding ascospore and lipid guttules. 14. Ascospore with unfurling appendages. FIG. 15. Ascospores stained in aqueous nigrosin showing the band around the midseptum and fine warting on the ascospore wall.

illamento uno conspirato vel complicato, primo hamato, denique in aqua retorquente in filum longum compositae.

*Species typica*. *Littispora ratnagiriensis* (Patil & Borse) J. Campb., J.L. Anderson et Shearer

*Etymology*. From the Latin *spora* and *littus* = seashore, in relation to the habitat of these species.

**Littispora ratnagiriensis** (Patil & Borse) J. Campb., J.L. Anderson et Shearer, comb. nov.

Basionym: *Halosarpheia ratnagiriensis* Patil & Borse. Indian Bot. Rep. 1: 102, 1982.

**Littispora abonnis** (J. Kohlm.) J. Campb., J.L. Anderson et Shearer, comb. nov.

Basionym: *Halosarpheia abonnis* J. Kohlm. Mar. Ecol. (P.S.Z.N.I) 5: 339, 1984

*Taxa not sequenced*. Seven of the 22 species of *Halosarpheia* were not included in our study due to lack of material, sequence data and/or cultures for sequencing. These species are: *H. aquadulcis* S.Y. Hsieh, H.S. Chang & E.B.G. Jones (Hsieh et al 1995), *H. bentonensis* Koch (Koch 1982), *H. culmiperda* Kohlm, Volkm.-Kohlm. & O.E. Erikss. (Kohlmeyer et al 1995), *H. kandeliae* Abdel-Wahab & E.B.G. Jones (Abdel-Wahab et al 1999), *H. minuta* W.F. Leong (Leong et al 1991), *H. phragmiticola* O.K. Poon & K.D. Hyde (Poon and Hyde 1998), and *H. unicaudata* (E.B.G. Jones & Le Camp.-Als.) R.G. Johnson, E.B.G. Jones & S.T. Moss ex Kohlm. & Volkm.-Kohlm. (Kohlmeyer and Volkmann-Kohlmeyer 1991). Of these seven species, *H. aquadulcis* clearly does not belong in *Halosarpheia* sensu stricto based on morphology. This species also does not fit in any of the other genera established herein for species of *Halosarpheia*, rather it has a combination of morphological characteristics similar to those of *Aniptodera*. These characteristics include: sub-globose to ellipsoidal, membranous, hyaline to light brown ascomata; cylindrical to conical, hyaline to pale brown, periphysate neck; hamathecium of catenophyses; clavate, pedicellate, persistent to semi-persistent asci with a somewhat thickened region in the ascus apex, as well as an apical pore, and retraction of the cytoplasm below the apex; ellipsoidal, thick-walled, uniseptate ascospores, not constricted at the septum; ascospore appendages at both apices; appendages adpressed to the spore, hamate, composed of a single filament, which initially is coiled but uncoils in water to form a long thin thread (Hsieh et al 1995). In addition, based on ultrastructural features, the ascus of *H. aquadulcis* conforms to that reported for species of *Aniptodera* (Hsieh et al 1995). *Halosarpheia aquadulcis* most closely resembles *A. chesapeakensis*, the type species of the genus, but differs slightly in ascomal size and shape. *Halosarpheia aquadulcis* has been reported only from freshwater habitats and although *A. chesapeakensis* initially was described from brackish water, it since has been reported from fresh water and seawater as well (Shearer 1993, 2001, Hyde et al 1999, Schmit and Shearer 2003). Because *H. aquadulcis* agrees in every respect with the unifying characters for *Aniptodera* and does not fit well in *Halosarpheia* sensu stricto and any of the new genera erected herein for other species of *Halosarpheia*, it is transferred to *Aniptodera*.

**Aniptodera aquadulcis** (S.Y. Hsieh, H.S. Chang, & E.B.G. Jones) J. Campb., J. Anderson & Shearer comb. nov.

Basionym: *Halosarpheia aquadulcis* S.Y. Hsieh, H.S. Chang, & E.B.G. Jones. Mycol. Res. 99: 49. 1995.

*Halosarpheia bentonensis* does not belong in *Halosarpheia* sensu stricto due to its hyaline, pyriform to globose ascomata, deliquescent asci and fusiform three septate ascospores. This species also does not belong in any other genera established herein for other *Halosarpheia* species. Molecular data therefore is needed to establish the appropriate placement of this species, and it is retained in *Halosarpheia* until material becomes available for sequencing.

*Halosarpheia culmiperda* agrees in all respects with the delimiting characteristics of *Halosarpheia*, based on morphology of the ascomata, hamathecia, asci and ascospores. It therefore is retained in this genus.

*Halosarpheia kandeliae* has ascomata that resemble those of *Halosarpheia* sensu stricto, while it has asci that resemble those of *L. ratnagiriensis* and *L. abonnis* and ascospores that resemble those of *P. viscosus* and *N. retorquens*. Molecular sequence data are necessary to resolve the systematics of this taxon, thus no nomenclatural changes are proposed at this time.

*Halosarpheia minuta* also agrees in most respects with the defining characteristics of *Halosarpheia* sensu stricto, except that this species apparently lacks catenophyses since they were not mentioned or illustrated in the species description (Leong et al 1991). Until material becomes available for sequencing this species is retained in *Halosarpheia*.

*Halosarpheia phragmiticola* differs from *Halosarpheia* sensu stricto in morphology and anatomy of the ascomata, in the asci, which have a pore and retraction of cytoplasm below the apex and in the ascospores that are ellipsoid fusiform. It is not clear at this point in which genus this species belongs and, lacking molecular data, no nomenclatural changes are proposed at this time.

*Halosarpheia unicaudata* is very similar in morphology to *A. cincinnatulum* and *A. viscidulum*. An unpublished sequence was obtained from Pang and Jones (pers comm), which places *H. unicaudata* as a sister taxon to *A. cincinnatulum* and *A. viscidulum*

with 100% bootstrap support (Campbell and Shearer unpubl). On the basis of morphology and molecular data, *H. unicaudata* is transferred to *Ascosalsum*.

**Ascosalsum unicaudatum** (E.B.G. Jones & Le Camp.-Als.) J. Campb., J.L. Anderson et Shearer, comb. nov.

Basionym: *Haligena unicaudata* E.B.G. Jones & Le Camp.-Als. Nova Hedwigia 19: 574. 1970.

=*Halosarpheia unicaudata* (E.B.G. Jones & Le Camp.-Als.) R.G. Johnson, E.B.G. Jones & S.T. Moss ex Kohlm. & Volkm.-Kohlm. Bot. Mar. 34:22. 1991.

*Conclusions.* Ascospore appendage morphology and development have been used extensively over the past 20 years to define genera of marine ascomycetes (Jones and Moss 1978, 1980, 1987, Shearer and Crane 1980, Johnson et al 1984, 1987, Jones et al 1986, Jones 1995). Ascospore appendages play an important role in attachment of ascospores to substrates (Rees and Jones 1984, Hyde and Jones 1989, Hyde et al 1989) and help to ensure that a fungus is able to colonize fresh substrates in moving water. Characters that have important ecological functions and are adaptive to a particular habitat likely result from strong selection for particular attributes. Such characters might be misleading with respect to taxon genealogy due to convergence or parallel evolution (Mayr and Ashlock 1991). Coiled, threadlike ascospore appendages that unfurl in water to produce long, sticky threadlike structures may be effective in entangling ascospores with herbaceous debris and enhance the ability of an ascospore to attach to a substrate and hence can be considered an adaptive character for the aquatic environment. Molecular data (FIG. 4) show clearly that this appendage type is not homologous and therefore not a good indicator of phylogeny. Further molecular studies of other taxa in Halosphaeriales with different types of ascospore appendages are warranted to evaluate the usefulness of appendage morphology in reconstructing phylogenies and defining genera.

KEY TO THE GENERA OF HALOSPHAERIALES WITH THREADLIKE, UNFURLING ASCOSPORE APPENDAGES.

1a. Asci with retraction of cytoplasm below the ascus apex . . 2
1b. Asci without retraction of cytoplasm below the ascus apex . . . . . 3
  2a. Ascospores hyaline . . . . . *Aniptodera*
  2b. Ascospores pale brown . . . . . *Phaeonectriella*
3a. Ascospores long, cylindrical, narrow, phragmoseptate . . . . . *Ascosalsum*
3b. Ascospores ellipsoidal, fusiform or short cylindrical . . . . . 4
  4a. Ascospores 3 or more septate, rough walled, hyaline to subhyaline . . . . . *Magnisphaera*
  4b. Ascospores 0–1 septate, smooth walled . . . . . 5
5a. Ascospores broadly ellipsoidal and rounded at apices . . . 6
5b. Ascospores fusiform and tapering at apex . . . . . 7
  6a. Ascospores with small, cap-like appendages at both apices . . . . . *Halosarpheia*
  6b. Ascospores with an apical appendage at only one apex . . . . . *Ophiodeira*
7a. Ascospores cylindrical to fusiform, densely polyguttulate . . . . . *Ascosacculus*
7b. Ascospores ellipsoidal or fusiform with one large guttule in each cell . . . . . 8
  8a. Ascospores ellipsoidal, with large apical appendages in the coiled stage (4–7 μm thick, 10–18 μm wide), asci persistent and thick-walled at the apex . . . . . *Littispora*
  8b. Ascospores ellipsoidal to fusiform, with apical appendages in the coiled stage not as thick and wide as in 8a; asci thin-walled throughout, early deliquescent . . . . . 9
9a. Ascospores fusoid to ellipsoid, tapered at the apex, mostly over 25 μm long, catenophyses present . . . . . *Natantispora*
9b. Ascospores ellipsoid, often flattened on one side, mostly under 25 μm long, catenophyses present or absent . . . . . *Panorbis*

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