

Chapter V

General Discussion and Conclusion

This thesis sets out to study the molecular phylogeny of two selected groups of marine Ascomycota:

1) genera assigned to the Halosphaeriales on morphological characteristics; but not verified at the molecular level (*Bathyascus*, *Haligena*, *Marinospora*, *Nautosphaeria*, *Ocostaspora* and *Remispora*), and,

2) genera not currently assigned to existing orders, or those only tentatively assigned to an order (*Marinosphaera*, *Pedumispora*, *Swampomyces* and *Torpedospora*).

The major aims of this thesis are:

- 1) to test the monophyly within these genera,
- 2) to examine their phylogenetic relationships,
- 3) to confirm assignment to the Halosphaeriales,
- 4) to evaluate whether ascospore appendages can be used in the delineation of selected genera of marine Ascomycota,
- 5) to explore their taxonomic position within the Sordariomycetes, and,
- 6) to outline future areas for further study.

A number of topics are worthy of further discussion and conclusions drawn.

1. Phyletic inference of marine Ascomycota

1.1 Monophyly of *Torpedospora*, *Swampomyces*, *Marinospora*

1.1.1 *Torpedospora*

Torpedospora species, *T. radiata* (the type species) and *T. ambispinosa*, are inferred to be monophyletic in origin. The generic morphological characters that support their monophyly are type of centrum development and the hamathecium arrangement, although the spore shape, and appendage morphology appear to be different. This may imply that convergence in ascospore and appendage morphology has occurred in this genus, and that care should be used in their selection for the delineation of marine ascomycetes.

1.1.2 *Swampomyces*

Three *Swampomyces* species are monophyletic and closely related (*S. armeniacus* (the type species), *S. aegyptiacus* and *S. clavatisporus*) but *S. triseptatus*, groups occasionally with weak support. These three species share some morphological characters in common, e.g. ascoma morphology, ascospores apricot-colored in mass, branched paraphyses in a gel and the asci with a thickened apex. *Swampomyces triseptatus* nestles within the *Torpedospora* clade but differs from the type species with its deeply immersed ascomata with a neck leading to the surface without a clypeus (Kohlmeyer and Volkmann-Kohlmeyer, 1987). The ascus of *S. triseptatus* is different in its dehiscence and in the structure of the ascus apex at the ultrastructural level (Hyde and Nakagiri, 1992). Whether this species is conspecific remains to be further studied.

1.1.3 *Marinospora*

Marinospora is shown to be monophyletic and well delineated in the Halosphaeriales, and *M. calyptrata* (the type species) is closely related to *M. longissima*. Distinct primary and secondary equatorial appendages with cup-like structures at their apices may be of taxonomic importance in generic delineation in the Halosphaeriales. Morphological features that have been used to distinguish these two species are ascospore size and the length of primary polar appendages (Johnson *et al.*, 1984). *Marinospora* forms a consistent adjoining subclade to *Ceriosporopsis halima*, although with weak support. This may reflect their ascospore appendage ontogeny formed by a combination of wall outgrowth and elaboration of the outer exosporial wall layer (Jones, 1995). Therefore, this appendage ontogeny type may be a stable character in the delineation of *Marinospora* and *Ceriosporopsis*.

1.2 Polyphyly of *Haligena*, *Remispora*

1.2.1 *Haligena*

The genus *Haligena* is inferred to be polyphyletic but well placed in the Halosphaeriales. *Haligena elaterophora* and *H. salina* differ significantly in the nature of ascospore appendages: wider, more sticky and strap-like in *H. elaterophora*, however they are spoon-shaped at the point of attachment in *H. salina*, longer and narrower, with finely drawn out filaments. The type species always forms a supportive basal clade to the order, while *H. salina* constitutes a sister clade with *Ocostaspora*. A new genus, *Morakotiella*, is introduced to accommodate *H. salina*.

1.2.2 *Remispora*

Six species have been referred to the genus *Remispora* (*R. maritima*, *R. stellata*, *R. quadriremis*, *R. pilleata*, *R. galerita* and *R. crispa*), and the genus was regarded as being well delineated in the Halosphaeriales (Hughes, 1974; Jones, 1980). However, molecular data indicates that *Remispora* species are polyphyletic in origin. The type species, *R. maritima*, and its sister taxa, *R. pilleata*, *R. stellata* and *R. quadriremis* can be regarded as *Remispora sensu stricto*. They share similar morphological characteristics at the light microscope level: globose or subglobose, cream-colored to yellowish ascomata (except for *R. pilleata*), well-developed periphysate necks, clavate asci, pedunculate and deliquescing early, catenophyses present, ellipsoidal, thin-walled ascospore (except thick-walled and rhomboid shape in *R. pilleata*), and the polar pleomorphic appendages that are initially wrapped around the ascospores. *Remispora crispa* and *R. galerita* are distantly placed in relation to the type species of *Remispora*. Appendages of *R. crispa* are initially subgelatinous and envelop the ascospore, later spread out from the wall but remain attached at the polar regions. The lower part of the appendages swell and parallel fibers become apparent that emerge fountain-like from the thickened tip of the spore wall. Ultimately, the whole appendage is transformed into fibers, except for the part initially attached to the side of the spore which becomes spoon-shaped (Kohlmeyer, 1981; Hyde and Jones, 1989). *Remispora galerita* differs from other *Remispora* species in the distinct subglobose cap-like appendages that appear to be more compact, contain a greater number of strands than the other *Remispora* species. Pleomorphic polar appendages may be acquired by convergent evolution, therefore, it

may not be of phyletic importance in the delineation of this genus. *Remispora crispa* and *R. galerita* are considered for transferring to new genera.

2. Confirmation of taxonomic assignment

2.1 Confirmation of genera assigned to the Halosphaeriales

2.1.1 *Naufragella spinibarbata*

Naufragella is correctly assigned to the genus and order. The unique ascospore appendage morphology of *N. spinibarbata* with two types of appendages: 1) long polar appendages stretching to form a band-like undulating appendage and 2) a mucilaginous sheath that fragments at the central septum into subpolar soft spines (Koch, 1986), can be used in the delineation of this genus. The transfer of *Remispora spinibarbata* to the new genus *Naufragella* (Kohlmeyer and Volkmann-Kohlmeyer, 1998) is supported by the molecular evidence.

2.1.2 *Nautosphaeria cristaminuta*

Nautosphaeria, a monotypic genus, is correctly assigned to the order and the molecular data supports the distant relationship to *Nereiospora*. Although *Nautosphaeria* groups consistently with *Remispora galerita*, they are not congeneric. Morphological and molecular evidence confirms its placement in a different genus.

2.1.3 *Ocostaspora apilongissima*

Ocostaspora, is correctly assigned to the order. It is distantly related to *Halosphaeriopsis* and *Halosphaeria* although they also possess polar and equatorial appendages. Phylogenetically it shares the clade with *Morakotiella salina*, but they are not congeneric, therefore, its placement as a monotypic genus is confirmed.

Concluding remarks

All the genera considered in section 2.1 above have morphological features that support their placement in the order, e.g. perithecial ascomata, necks usually with periphyses, central pseudoparenchymatous tissue, presence or absence of catenophyses, unitunicate, thin-walled asci that deliquescing early and appendaged ascospores (Jones, 1995).

2.2 Confirmation of Ascomycota *incertae sedis* genera in the Sordariomycetes

2.2.1 *Torpedospora* and *Swampomyces*

Our results clearly show that *Torpedospora* does not have an affinity with the Halosphaeriales morphologically and phylogenetically, and supports the views of Kohlmeyer (1972) and Kohlmeyer and Kohlmeyer (1979) that this genus should be included elsewhere. Although *Torpedospora* possesses appendaged ascospores, this does not guarantee placement in the Halosphaeriaceae. This character may be the result of convergent evolution with modification or adaptation to the marine environment (Shearer, 1993; Jones, 1995).

Molecular data conclusively indicates that *Swampomyces* does not belong in the Phyllachorales, as suspected by Kohlmeyer and Volkmann-Kohlmeyer (1987), although they share some morphological similarities in the possession of a clypeus, the presence of paraphyses and the apical apparatus to the ascus. These morphological characters may be the result of convergent evolution, and the loss and rapid modification of characters (Alexopoulos *et al.*, 1996; Samuels and Blackwell, 2001).

Torpedospora and *Swampomyces* share a monophyletic clade and group within the subclass Hypocreomycetidae, class Sordariomycetes with the Halosphaeriales, Hypocreales, Microascales and Phyllachorales as the sister orders. However, placement in an order, or family cannot be made at this time.

2.2.2 *Marinosphaera*

The molecular results confirm that *Marinosphaera* does not have affinities with the Phyllachorales, or the *Swampomyces/Torpedospora* clade. Phylogenetically *Marinosphaera* is located between the Halosphaeriales and Microascales but without any closely related taxa. However, *Marinosphaera* is clearly distinguished from the Halosphaeriales and Microascales by the presence of paraphyses, persistent cylindrical asci that possess a subapical plate (Hyde, 1989a).

More ascomycete taxa from other habitats, especially from mangrove habitats, should be collected, studied, described, sequenced and compared with *Marinosphaera*.

3. The evolution of the Halosphaeriales

3.1 Phylogeny of ascospore appendages

The Halosphaeriales is a large order of predominantly marine Ascomycota with genera and species exhibiting great morphological diversity, especially the appendaged ascospores. Although it has been shown that marine ascomycetes have evolved from a terrestrial ancestor (Spatafora *et al.*, 1998), no hypothesis has been proposed for the evolution of appendaged ascospores within the Halosphaeriales. Speculation as to which of these appendage types are primitive or derived characters

have been voiced. However, it has been demonstrated that they serve as an aide to floatation and in entrapment and attachment to substrata (Jones, 1994; 1995).

Within the Halosphaeriales how these appendaged ascospores have evolved is not clear. Kirk (1986) presented a scheme representing genera that might share a close relationship, based on ascospore appendage morphology and histochemistry of cell wall layers. He proposed that ascospores in the Halosphaeriaceae may have evolved along two complex lines, one group possessing an exosporium (cluster 1-6), but absent in the other (cluster 7-9) (Figure 31).

The ancestral species of *Lignincola* was proposed to give rise to *Nais* in cluster 9, while it was suggested that *Aniptodera* be independently evolved or shared an ancestral group (Figure 31). My results, based on rRNA sequences, demonstrate that *Lignincola* is placed adjacent to *Halosphaeria appendiculata* in clade A of the Halosphaeriales, and distantly related to *Nais inornata* (Figure 32). However, *Aniptodera* species are placed in the same clade as *N. inornata*, although with weak support (clade B, Figure 32). I can not determine whether clade A gave rise to clade B, or the other way round, as both clades have evolved at more or less the same rate with an equal rate of base substitutions.

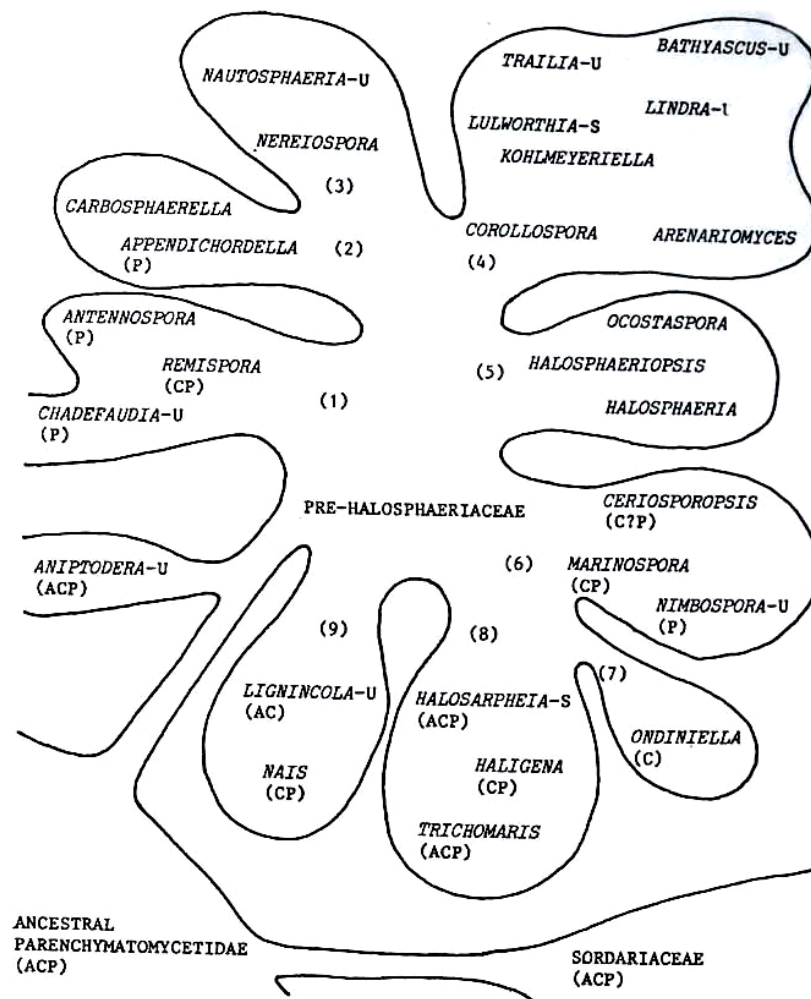


Figure 31. Scheme of proposed evolutionary trends within the Halosphaeriaceae. Numbers refer to clusters of genera thought to have a common ancestry (A = asci persistent, C = catenophyses, P = periphyses in some species, -U = ultrastructure unobserved, -S = SEM only observed) (followed Kirk, 1986)

The ancestral species of *Halosarpheia* were proposed to evolve into species similar to *Haligena* and *Trichomaris* (cluster 8, Figure 31). We do not have molecular evidence for *Trichomaris* to confirm this hypothesis. However, recent morphological and molecular evidences for *Halosarpheia* and *Haligena* species reveal that they are distantly related. At present, as the result of taxonomic changes, only three species remain in the genus *Halosarpheia* (*H. fibrosa*, *H. trullifera*, *H. unicellularis*), while only the type species of *Haligena* (*H. elaterophora*) remains in that genus. Molecular data confirm the polyphyletic origin of both genera.

Our molecular data suggests the recent common ancestor of *Halosarpheia sensu stricto* may not have given rise to *Haligena elaterophora* or to other genera, which were previously assigned to *Haligena* (*Magnisphaera*, *Ascosalsum*). *Halosarpheia sensu stricto* forms a distinct clade with *Antennospora quadricornuta* (Cribb and J. W. Cribb) T. W. Johnson and *Cucullosporella mangrovei* in clade C (Figure 32), although with weak support, while *H. elaterophora* nestles with other genera in clade F of the order (Figure 32).

In cluster 1, ancestral species of *Remispora* are thought to give rise to all other clusters (2-5), except cluster 6 (Figure 31). The molecular data proved that cluster 4, which comprises genera with scolecosporous ascospores (*Lulworthia*, *Lindra* and *Kohlmeriella*), does not have an affinity to any of the Halosphaeriales lineage (Spatafora *et al.*, 1998; Kohlmeyer *et al.*, 2000; Campbell *et al.*, 2002). *Remispora sensu stricto* is well placed in clade B, which has affinity with clade A, while other genera were thought to have relationships with *Remispora* (*Nautosphaeria*, *Nereiospora*, *Corollospora*, *Arenariomyces* and *Halosphaeriopsis*) are distantly related (Figure 32). Moreover, we cannot determine the phylogenetic position of

Chadefaudia, *Carbospharella*, *Appendichordella* and *Trailia*, due to lack of sequence data. However, morphological data does not support a relationship between these genera.

Antennospora quadricornuta has no affinity with *Remispora sensu stricto*, as it clusters with *Cucullosporella mangrovei* and *Halosarpheia sensu stricto* in clade C. *Nautosphaeria* and *Nereiospora* were proposed to share the same cluster (cluster 3), however, their appendage ontogeny and phylogenetic position confirm that they are not congeneric (Figure 32).

Kirk's scheme proposed that genera that are adapted to sand and shell (e.g. *Nereiospora*, *Arenariomyces*, *Kohlmeyeriella*, *Corollospora* and *Lulworthia lignoarenaria* Koch and E. B. G. Jones) might have radiated from the same ancestors, possibly *Halosphaeriopsis* (Kirk, 1986). However, molecular results show that *Kohlmeyeriella* and *L. lignoarenaria* have no affinity with the halosphaerialean members (Kohlmeyer, *et al.*, 2000; Campbell, *et al.*, 2002). *Corollospora* and *Nereiospora* group in clade E, while the placement of *Arenariomyces trifurcatus* is uncertain (clade F, Figure 32). It is possible that the ancestral species of *Halosphaeriopsis* may have radiated to these arenicolous species, as its phylogenetic position indicates a primitive origin (clade F, Figure 32).

Kirk grouped *Ocostaspora* and *Halosphaeria* in cluster 5 (Figure 31). Recent molecular results reveal that they are located in clade A (Figure 32) but distantly placed from each other including, *Halosphaeriopsis* which was thought to be the ancestral species, for this cluster.

Cluster 6 genera were thought to be closely related and this is congruent with our recent molecular data for *Ceriosporopsis* and *Marinospora* which are

monophyletic genera (clade D, Figure 32). This indicates the stability of the appendage ontogeny that could be used in delineation of these genera. However, although *Nimbospora* was thought to share relationships to *Marinospora* and *Ceriosporopsis*, it is distantly placed from them and well grouped in clade A (Figure 32).

In Figure 33 I have superimposed the various type of appendaged ascospore morphology onto a recent phylogenetic tree of halosphaerialean taxa. The clades comprise a number of genera with much variation in ascospore appendage morphology. Development of an exosporial wall layer (gain or loss) has been observed for a wide range of genera that are not phylogenetically related (Figure 33). Therefore Kirk's hypothesis on the evolution of the exosporium is not supported by sequence analysis.

Fragmentation pattern of the exosporium of *Remispora maritima* was proposed to be an ancestral state resembling the hypothetical prototype of the Halosphaeriaceae (Kirk, 1986). However, this is not supported by recent molecular data, as it has occurred several times, not only *Remispora* (which is polyphyletic) but also in *Corollospora*, *Marinospora*, *Ocostaspora* and *Halosphaeriopsis* (Figure 33).

Appendages that arise as an outgrowth of the exosporium (*Haligena*, *Morakotiella*); direct outgrowth from one or more spore wall layers (*Nereiospora*, *Arenariomyces*, *Nautosphaeria*); or exuded through pores (*Halosarpheia sensu stricto*, *Ascosacculus*, *Ophiodeira*, *Magnisphaera*) are present in number of genera throughout the order (Figure 33). The appendage types that seem to be stable for certain genera include appendages that arise as an outgrowth and fragmentation of the

spore wall (*Corollospora*), and as outgrowth of spore wall and with the elaboration of an exosporium (*Marinospora*, *Ceriosporopsis*) (Figure 33).

Kirk's hypothesis as to the evolution of appendages within the Halosphaeriales was an attempt to correlate different morphological features of the ascospore. However, molecular data, although incomplete, does not support his hypothesis. Therefore, the evolution of ascospore appendages remains unresolved. What emerges is that ascospore appendages, with different wall layers and pattern of development, arose many times within the Halosphaeriales. The only group that shows commonality in ascospore appendage development is those with end chambers from which a drop of mucilage is exuded, and these referred to the Lulworthiales (*Lulworthia*, *Lindra*), and, *Kohlmeyeriella* and *Spathulospora* (in which molecular data suggests their inclusion in this order). The morphological data is well supported by molecular evidence (Kohlmeyer *et al.*, 2000).

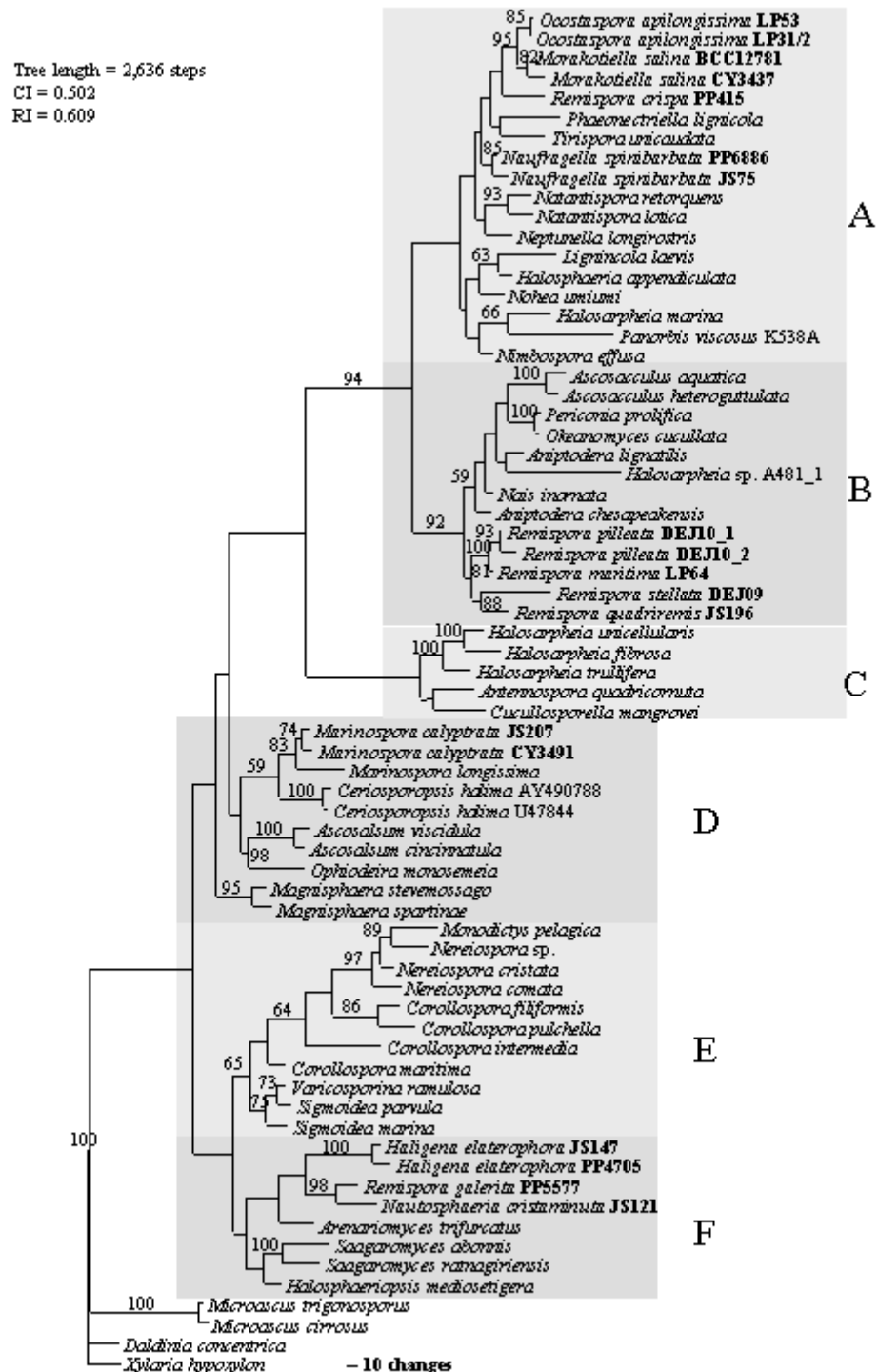


Figure 32. One of three MPTs inferred from LSU rRNA sequences of all halosphaeriales taxa, generated with maximum parsimony analysis. Bootstrap values higher than 50% are given above branches. Scale bar indicates 10 character state changes.

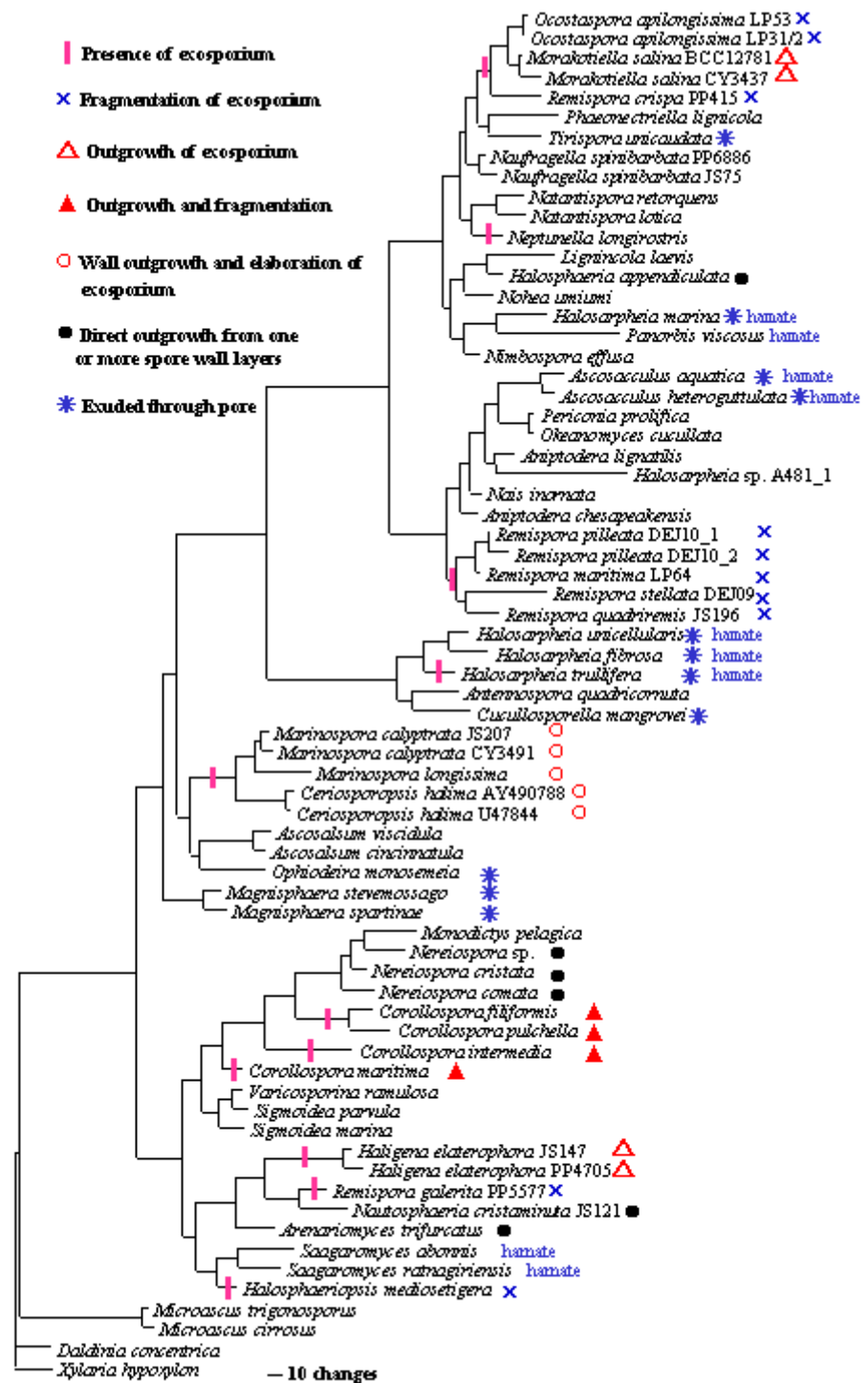


Figure 33. One of three MPIs inferred from LSU rRNA sequences of all halosphaeralean taxa, generated with maximum parsimony analysis, with the different type of appendage ontogenies indicated.

3.2 Why only one family assigned to the Halosphaeriales?

The Halosphaeriales is a large order and it is intriguing that currently only one family has been described for it. The Halosphaeriaceae was established by Müller and von Arx (1962) to accommodate marine fungi growing on a variety of substrata with ascomata that are subglobose, cylindrical to pyriform, light to dark color, and immersed or superficial on substratum. Perithecial necks variable in length, periphysate or with thin-walled pseudoparenchyma. Peridium variable, ranging from membranous to carbonaceous and composed of more than one layer. Paraphyses are absent, but the centrum pseudoparenchymatous tissue may break to form catenophyses. Asci clavate or fusiform, lacking an apical apparatus, unitunicate, thin-walled, sometimes with a thickened wall at the apex, deliquescing early before ascospores maturity, rarely persistent and form at the base of the ascocarp as a layer of cells. Ascospores mostly hyaline, unicellular to many septate, usually with appendages (Jones and Moss, 1987). The Halosphaeriaceae was initially referred to the Sphaeriales but subsequently was erected to accommodate a new order, Halosphaeriales (Kohlmeyer and Kohlmeyer, 1979).

Many marine fungi have been described and placed in this order, however, it has been questioned whether the genera in the Halosphaeriaceae constitute a natural classification. For example, Kohlmeyer (1972) synonymised a number of taxa in *Halosphaeria* (e.g. *Remispora*), while *Corollospora* was clearly not monophyletic (Schmidt, 1974). To resolve the taxonomic position, ultrastructural studies were undertaken (Moss and Jones, 1977; Jones, *et al.*, 1983a, b; Johnson *et al.*, 1984; 1987), mainly focusing on ascospore appendage development and ontogeny.

Jones, *et al.* (1986) reviewed the characters available for the delineation of genera within the Halosphaeriaceae and concluded that characters of little value in the delineation of genera include ascus structure and morphology, and ascocarp and ascospore phenology (color, size, texture). The important secondary taxonomic characters include presence or absence of catenophyses, periphyses and ascocarp wall structure. They have shown that ascospore appendage ontogeny is a primary character for characterization of genera within the Halosphaeriaceae (Jones, *et al.*, 1986; Jones, 1995).

Recently, Pang (2002) reviewed the variable morphological features of ascoma phenology e.g. color (hyaline to melanized), texture (carbonaceous to membranous), peridial wall layers (1-3 layers) in the Halosphaeriales. These characters have been mentioned repeatedly as they may be the result of environment conditions (Cavaliere and Johnson, 1966; Jones, *et al.*, 1986). Therefore, in this discussion I will focus on certain characters (e.g. presence or absence of periphyses and catenophyses, persistent or deliquescent ascus, ascus apical structures and appendage morphology) and explore their phylogenetic significance in the Halosphaeriales.

3.2.1 Presence or absence of periphyses and catenophyses

Presence of periphyses has been observed and well defined for a wide range of genera, in particular in clades A, B, C, D, F (Figure 34). However, periphyses of some genera in these clades are also absent even within the same genus (e.g. *Remispora sensu stricto*). Taxa in clade E, which are mostly associated with sand grains (*Corollospora*, *Nereiospora*, *Arenariomyces*), lack periphyses or are

degenerate at maturity. These arenicolous genera are morphologically adapted to this habitat. The wall is usually thick, hard, carbonaceous, with short necks or may be absent, the ostiole is inconspicuous and usually situated near the base, close to the place of attachment to the substratum (Kohlmeyer and Kohlmeyer, 1979). These morphological features may help to protect against desiccation or abrasion. Thus, the loss of periphyses may be an advantage for better survival.

Catenophyses are persistent chains of utricular, thin-walled cells formed by the vertical separation of the pseudoparenchyma in the centrum (Kirk *et al.*, 2001). Presence of catenophyses has been observed in a wide range of genera e.g. *Aniptodera*, *Marinospora*, *Haligena*, *Halosarpheia*, *Remispora*, *Lignincola*, *Nais*, *Phaeonectriella* R. A. Eaton and E. B. G. Jones, *Tirispota* E. B. G. Jones and Vrijmoed, *Naufragella* and *Morakotiella* (Figure 34). This character can be used to distinguish between certain genera, such as, *Remispora* from *Halosphaeria*, *Marinospora* from *Ceriosporopsis*, but may not be used to characterize such genera as *Halosarpheia* from *Saggaromyces* and *Ascosacculus*. Catenophyses have not been reported in arenicolous genera (*Corollospora*, *Nereiospora*, *Arenariomyces*).

Therefore, presence or absence of periphyses and catenophyses may not be indicators of phylogenetic relationships of taxa within the Halosphaerales. Gain or loss of these morphological features may be the result of adaptation to different habitats or substrata, however, observation of these features depends upon the age of materials being examined.

3.2.2 Asci structures

Persistent or deliquescing asci occur several times within different clades in the Halosphaeriales (Figure 35). Ascospores accumulate in the ascocarp venter and are liberated passively through the ostiole, before finally being dispersed by water currents. This is an adaptation for release and dispersal in aquatic environments. Persistent asci also occur in many genera e.g. *Phaeonectriella*, *Tirispora*, *Natantispora*, *Halosarpheia*, *Panorbis*, *Aniptodera*, *Cucullosporella* and *Saagaromyces* (Figure 35), although actual forcible release of spores has only rarely been observed. Therefore, persistent or deliquescing asci do not indicate any trend in the phylogenetic relationship of genera in the Halosphaeriales

Although the ascus shape in the Halosphaeriales is commonly clavate, it can vary from fusiform, ellipsoidal, saccate, clavate-broadly fusoid, elongate clavate, clavate to ellipsoidal and cylindrical. Pang (2002) suggested that the length to width ratio of the asci should be closely considered. Ascus stalk length should also be examined for a relationship between different taxa e.g. a long stalk is observed in *Halosarpheia kandeliae*, *Saagaromyces abonnis* and *S. ratnagiriensis*, while short stalks are present in *Halosarpheia sensu stricto*.

Presence of apical structures (apical pore, apical plate, apical thickening and retraction of the plasmalemma) has evolved independently from different common ancestors (Figure 35). Genera possessing these structures are located in clades A, B, C, D, F (e.g. *Phaeonectriella*, *Tirispora*, *Neptunella*, *Halosarpheia marina*, *Aniptodera*, *Cucullosporella* and *Saagaromyces*), but these characters do not give any clue of their phylogenetic relationships, except for the monophyly of *Phaeonectriella* and *Tirispora* which possess an apical pore, apical plate and retraction of the

plasmalemma (Figure 35). Ascus apical structures were thought to be characteristic of terrestrial ascomycetes that serve as an aide for active discharge of ascospores (Ingold, 1975). Within terrestrial ascomycetes they can be quite distinctive at the ordinal/family level, e.g. J+ and well developed rings in the Xylariales and J- with huge rings in the Annulatasceae (Wong *et al.*, 1998).

3.2.3 Ascospore and appendage morphology

Jones *et al.* (1986) and Jones (1995) suggested that ascospore shape and ascospore appendage ontogeny were of primary importance as taxonomic characters for genera in the Halosphaeriales. Halosphaerialean ascospores are usually hyaline, (with the exception of *Phaeonectriella*, *Carbosphaerella*, *Magnisphaera* and *Nereiospora*), with ascospore morphology varying from ellipsoidal (*Morakotiella*, *Remispora*, *Neptunella*), cylindrical to ellipsoidal (*Haligena*), long cylindrical or fusiform (*Ascosalsum*, *Magnisphaera*). Ascospore septation is also variable: unicellular (*Nautosphaeria*), 1-septate (*Morakotiella*, *Remispora*, *Neptunella*), or multi-septate (*Haligena*, *Ascosalsum*, *Magnisphaera*). Therefore, there is no clear pattern for ascospore morphology within the Halosphaeriales.

A great diversity of ascospore appendage developments has been observed for a wide range of genera. As I mentioned earlier in section 3.1, different wall layers and pattern of appendage ontogenies arose many times within the order (Figure 33). There is no clear correlation pattern or stability of appendage ontogeny that can be used in separating families within the order. Ascospore appendage diversity of the halosphaerialean taxa is a unique adaptive character resulting from convergent evolution to aquatic habitats.

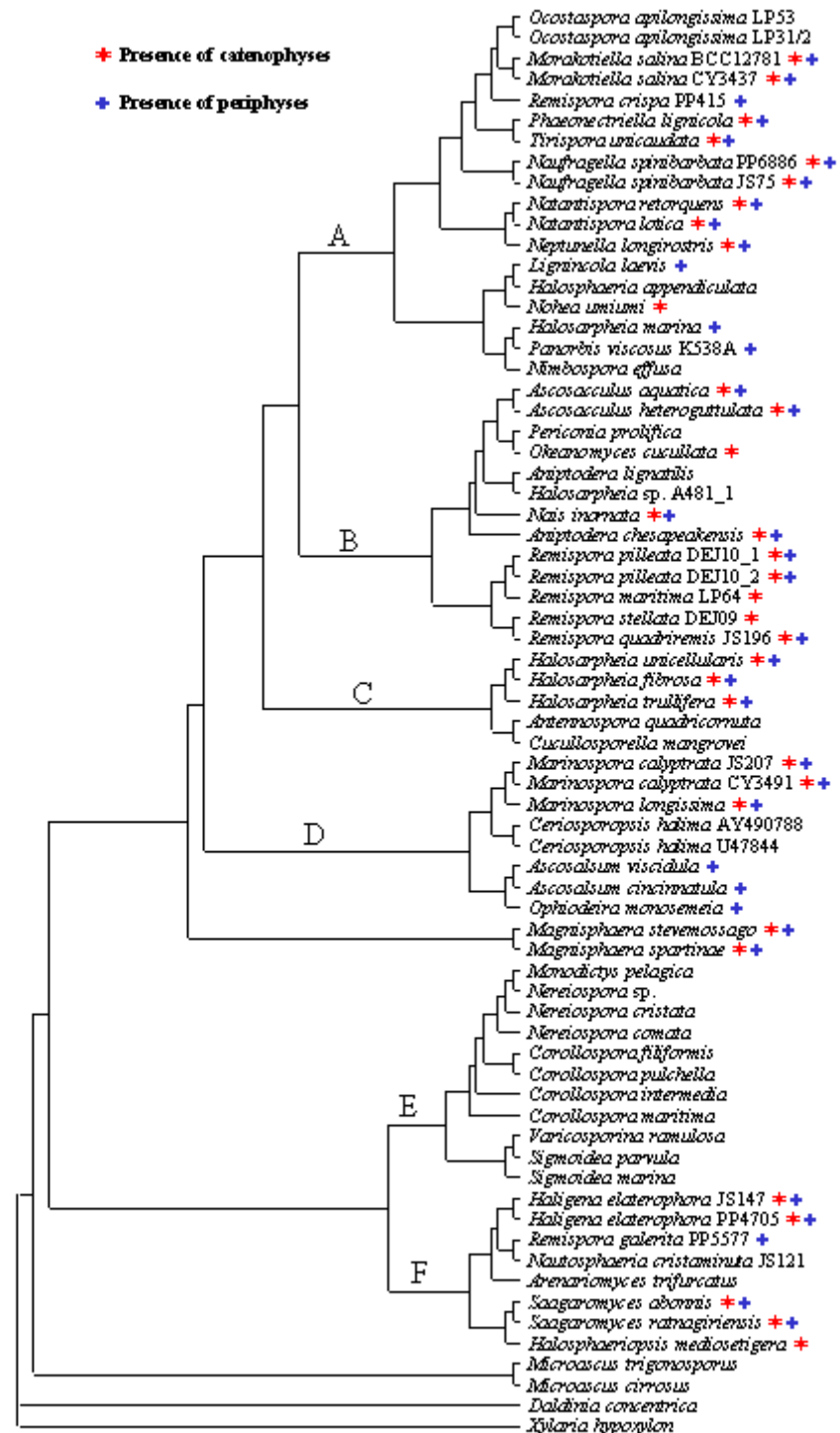


Figure 34. One of three MPTs inferred from LSU rRNA sequences of all halosphaeriales taxa, generated with maximum parsimony analysis, with presence or absence of catenophyses and periphyses indicated.

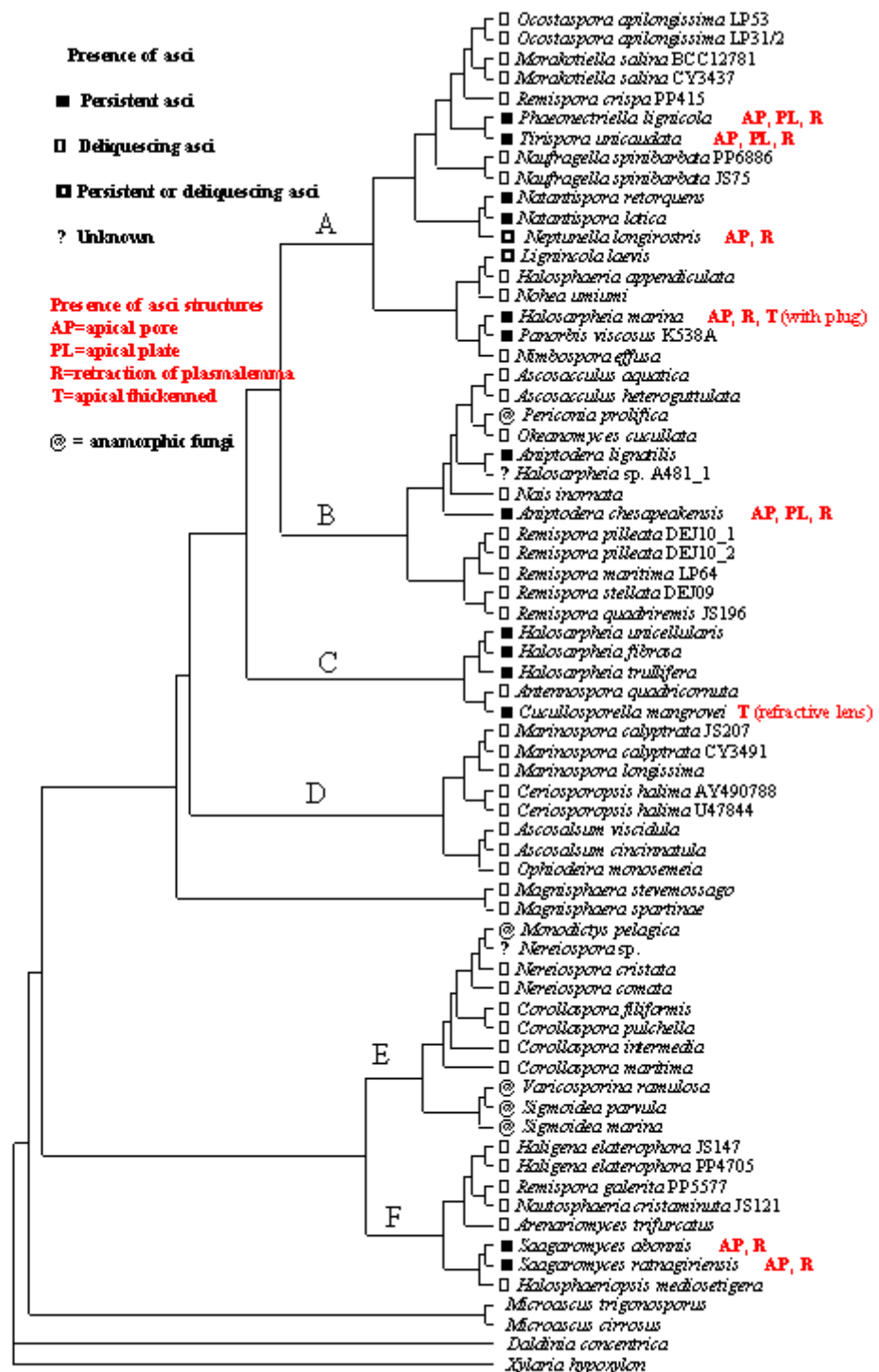


Figure 35. One of three MPIs inferred from LSU rRNA sequences of all halosphaerales taxa, generated with maximum parsimony analysis, with presence of asci and ascus structures indicated.

3.3 Can ascospore appendage morphology be used in delineation selected genera?

Ascospore appendage morphology of the Halosphaeriales is diverse and no clear trends in phylogenetic relationships can be inferred. In this thesis, I demonstrate the polyphyletic origin of selected genera including *Haligena* and *Remispora*.

The polar long unfurling appendages of *Haligena* (*H. elaterophora*, *H. salina*), although morphologically similar, are not homologous. Therefore, a new genus is proposed for *H. salina* based on the difference in the nature and dimension of appendages, and support from molecular evidence.

Sequence data indicates the polyphyletic nature of the genus *Remispora*, and an analysis of ascospore appendage development shows that they are heterogenous. Thus appendage development and ontogeny as proposed by Johnson *et al.* (1984) cannot be used in the delineation of this genus, and other of characters must be evaluated for separation the species to new genera. Differences in the nature of appendages and the arrangement of appendage fibers may be criteria for transferring *R. crispa* and *R. galerita* to new genera. This will be proposed when more genes are sequenced.

The only group that shows stability in ascospore appendage development is those with polar appendages that arise as an outgrowth of the spore wall and with secondary appendages formed by fragmentation of an exosporium (*Corollospora*), or with appendages that arise as outgrowth of the spore wall and elaboration of the exosporium (*Marinospora*, *Ceriosporopsis*). Moreover, the genera *Bovicornua* and *Limacispora* need further study to determine if they are phylogenetically related to the *Ceriosporopsis/Marinospora* clade.

Another group, the *Halosarpheia* complex, share morphological features with apical ascospore appendages that are initially hamate and then uncoil to form thin, thread-like appendages that are very sticky. However although morphologically similar, molecular data indicates they are not congeneric (Campbell *et al.*, 2004). Taxa are assigned to *Halosarpheia sensu stricto* possess large, ellipsoidal or subglobose, ostiolate ascomata; long, cylindrical, periphysate necks; presence of catenophyses; asci that are thin-walled, clavate, and lacking of apical pore; broadly ellipsoidal ascospores with bipolar coiled hamate appendages that are exuded through a pore (Campbell *et al.*, 2004).

Additionally, morphological characters in the Halosphaeriales can not be used singly in the delineation of genera. Some features are considered to be less important (e.g. periphysate necks, presence or absence of catenophyses, ascospore color or septation), but using a combination of other characters may give more taxonomic resolution.

Comparison of selected orders in the Sordariomycetes indicates a greater variation in morphological characters in the Halosphaeriales, than in others. For example, infinite variation in the ascospores (septation, color, appendage morphology) of the Halosphaeriales, while in the Xylariales they are predominantly unicellular, ellipsoidal, and dark in color with a longitudinal slit (Table 10). This wide variation in the morphology of the Halosphaeriales is interesting and warrants further investigation.

Table 10. Comparison of the morphological characters used for delineation the fungi in the orders Sordariales, Xylariales, Hypocreales (Bionectriaceae) and Lulworthiales (Alexopoulos *et al.*, 1996; Samuels and Blackwell, 2001; Rossman *et al.*, 1999)

| | Halosphaeriales | Sordariales | Xylariales | Hypocreales (Bionectriaceae) | Lulworthiales |
|--------------------------|--|---|--|--|---|
| Trophic condition | Saprotrophs on decaying wood | Saprotrophs on dung, old plant material, wood, leaf litter, rarely pathogenic | Saprotrophs on wood, leaves, dung, rarely pathogenic | Saprotrophs on dead plant or other fungi, rarely pathogenic | Saprotrophs on decaying wood |
| Ascoma position | Superficial or immersed, stromatic absent | Superficial and rarely stromatic, sometime embedded in a subiculum | Superficial, mostly stromatic | Superficial or rarely immersed, stromatic absent , often hardly papillate | Superficial or immersed |
| Ascomata | Perithecial, membranous, carbonaceous, hyaline, black, presence or absence of periphysate necks, centrum cavity initially filled with deliquescing thin-walled parenchymatous cells, presence or absence of catenophyses | Perithecial, cleistothecial, membranous, black, periphysate necks | Perithecial, carbonaceous, black, periphysate necks | Perithecial, membranous, pale yellow, orange or brown, periphysate necks | Perithecial, coriaceous to carbonaceous, brown, black, the ascomatal cavity initially filled with deliquescing thin-walled parenchymatous cells |

Table 10. (Continued)

| | Halosphaeriales | Sordariales | Xylariales | Hypocreales (Bionectriaceae) | Lulworthiales |
|------------------------------|--|---|--|---|---|
| Paraphyses | Absent | Present | Present | Present | Absent |
| Hamathecium formation | - | Paraphyses are either sparsely formed, scattered among mature asci and broader at the base than the tip, deliquescing early | Paraphyses are abundant, typically narrowly cylindrical and persistent among mature asci, grow upward from the base and inward from the sides of the ascocarp wall | Apical paraphyses, growing from a meristem at the top of the locule and grow downward, persistent | - |
| Asci formation | Unitunicate, formed in a basal hymenium, persistent or deliquescing early | Unitunicate, formed in a basal hymenium | Unitunicate, formed in a basal hymenium | Unitunicate, formed in a basal hymenium | Unitunicate, formed in a basal hymenium, deliquescing early |
| Apical apparatus | Presence or absence of apical structures e.g apical ring, apical plate, apical thickening, retraction of plasmalemma | Apical ring is shallow, J- apical ring | Apical ring is variable in shape, from inconspicuous or rarely absent to massive, J+ apical ring | J- apical ring | Absent |

Table 10. (Continued)

| | Halosphaeriales | Sordariales | Xylariales | Hypocreales (Bionectriaceae) | Lulworthiales |
|-------------------------------|---|--|---|--|---|
| Ascus shape | Varied in shape e.g. clavate, fusiform, ellipsoidal, saccate, clavate- broadly fusoid, clavate to ellipsoidal, cylindrical, persistent or deliquescing early | Ovoid to cylindrical, persistent, may be evanescent in some species | Subglobose, club-shaped, most frequently cylindrical | Cylindrical, thin- walled | Cylindrical to fusiform, thin- walled |
| Ascospores | Typically haline, but also brown- colored, unicellular, one- septum to many septate | Typically black, ellipsoidal and unicellular or cylindrical and bent in the middle, germ pores are common, germ slits are found | Typically are ellipsoidal, unicellular, less frequently bicellular, typically brown or black, less often are hyaline, with germ slits | Varied in shape, usually transversely septate, not disarticulate, hyaline to yellow | Long, predominantly filiform, often many septate |
| Appendages/ sheath | Present or absent with different types | Present in some species | Absent | Ornamented sometimes, without appendages or sheath | Absent but with end chambers releasing mucilages |
| Examples | <i>Halosphaeria</i> , <i>Remispora</i> , <i>Halosarpheia</i> | <i>Sordaria</i> , <i>Chaetomium</i> , <i>Neurospora</i> | <i>Xylaria</i> , <i>Hypoxylon</i> , <i>Daldinia</i> | <i>Bionectria</i> , <i>Hydropisphaera</i> , <i>Nectriopsis</i> | <i>Lindra</i> , <i>Lulworthia</i> , <i>Kohlmeriella</i> |

3.4 The evolution of monotypic genera

Thirty-two genera assigned to the Halosphaeriales are monotypic, of which 14 have been sequenced (*Ocostaspora*, *Morakotiella*, *Remispora crispa*, *Neptunella*, *Halosphaeria*, *Nohea*, *Panorbis*, *Okeanomyces*, *Cucullosporella*, *Ophiodera*, *Haligena*, *R. galerita*, *Nautosphaeria*, *Halosphaeriopsis*), while 13 monotypic genera await for confirmation at the molecular level (Table 11). However, more species in the genera e.g. *Phaeonectriella*, *Tirispora*, *Naufragella*, *Lignincola*, *Nais*, need to be sequenced.

Jones (1995) suggested that the large number of monotypic genera in the Halosphaeriales might reflect their recent evolution. To this can be added the great variation in morphology and in particularly ascospore appendages of genera in the order again implying plasticity and recent evolution. Although a number of monotypic genera remain to be studied, the phylogenetic relationship of the 19 generic sequences available place most of these in clades A, B, C, D, and some are present in clade F (Figure 33). Most of the monotypic taxa are located in clades that appear to be more recently evolved. In addition, other monotypic genera in the basal clade (e.g. *Halosphaeriopsis*, *Nautosphaeria*, *R. galerita*, and *Haligena*) may have evolved earlier or have given rise to other halosphaerialean taxa. This aspect requires further study employing a wide range of genes.

4. Multiple invasion of ascomycete lineages into the sea

Kohlmeyer and Kohlmeyer (1979) and Kohlmeyer (1986) proposed that marine ascomycetes to be evolved from a marine ancestor. However Spatafora *et al.*

(1998), using molecular evidence of ribosomal DNA sequences analysis, concluded they have evolved from a terrestrial ancestor. A plausible explanation of the origin of marine ascomycetes could be the migration routes from terrestrial habitats to freshwater and brackish water, then to marine environments (Shearer, 1993; Jones, 1995). This gradual transition may bring about morphological changes in response to environment conditions. Adaptation to aquatic habitats may include deliquescent asci, lack of apical ascus structure, passive release of ascospores, modification of pseudoparenchymatous tissue (catenophyses) and presence of appendaged ascospores (Shearer, 1993). Presence of appendaged ascospores may enable ascospores to stick onto substrata and remain attached often under turbulent water movement (Shearer, 1993; Jones, 1994).

The monophyly of the Halosphaeriales has been confirmed and my results are concordant with other studies (Spatafora *et al.*, 1998; Chen *et al.*, 1999; Kohlmeyer, *et al.*, 2000; Kong *et al.*, 2000), with the order closely related to the terrestrial Microascales (Figures 32, 36). Members of the Halosphaeriales and Microascales share certain features in common e.g. perithecial ascomata, evanescent asci and passively discharged ascospores (Spatafora *et al.*, 1998). Unlike the Halosphaeriales, however, the Microascales possess insect-dispersed ascospores. The terrestrial to marine adaptation in the common ancestor of the Halosphaeriales may have been accompanied by the loss of arthropod ascospore dispersal (Spatafora *et al.*, 1998).

Another marine order, the Lulworthiales, comprises the genera *Lulworthia* and *Lindra*, which are distantly placed from the Halosphaeriales, but to which it was previously assigned. Phylogenetic analysis revealed that this order is not derived from a halosphaerialean ancestor (Figure 36), but has independently evolved from a

terrestrial ancestor. Thus marine ascomycetes have invaded the sea more than once (Spatafora *et al.*, 1998; Kohlmeyer *et al.*, 2000).

Other marine lineages within the Ascomycota have been documented and studied at the molecular level. These include the genera: *Kallichroma tethys*, *K. glabrum* and *Heleococcum japonense* that have been linked to the Hypocreales by the molecular data (Rossman *et al.*, 1999). They share a common ancestor with the Bionectriaceae (Figure 36), which are saprophytic on wood and mycoparasitic (Rossman *et al.*, 1999; 2001). Thus, the routes of evolution from terrestrial to marine habitats may imply the loss of host-association or mycoparasitism, to saprophytism in brackish and marine habitats.

Our study of the genera *Torpedospora* and *Swampomyces* indicates another lineage derived from a terrestrial ancestor. The lineage of *Marinosphaera mangrovei* is unclear, however, it does not have affinities with the Phyllachorales, or with *Swampomyces/Torpedospora*. It is located between the Halosphaeriales and Microascales without any closely related taxa (Figure 36). The true affinities of this fungus may not be resolved at this time due to lack of other ascomycete taxa for comparison. However, morphological features of *M. mangrovei* indicates it may share morphological characters with a terrestrial ancestor, such as, long bushy necks, and persistent asci with an apical structure. This fungus may have evolved from a recent common ancestor from terrestrial counterparts.

Although the resolution of *Bathyascus* sp. and *Pedumispora rhizophorae* phylogenies cannot be advanced at this time. *Bathyascus* sp. and *P. rhizophorae* share the same clade with the Magnaporthaceae (freshwater) and Xylariales (terrestrial), respectively (Figure 36). These two genera possess long filiform ascospores, which

may enhance entanglement with substrata in aquatic habitats (Shearer, 1993). This adaptation is found commonly in many freshwater and marine ascomycetes (e.g. *Ophioceras*, *Pseudohalonestria*, *Gaumannomyces*, *Plagiosphaeria*, *Lulworthia*, *Lindra*) (Shearer, 1993).

Figure 37 indicates that marine unitunicate ascomycetes have evolved from terrestrial ancestors, and that this has occurred several times. How these fungi invaded marine habitats, whether as pathogens, endophytes or saprophytes of wetland aquatic plants, remains unresolved.

The marine lineage of bitunicate ascomycetes has not been addressed although many terrestrial genera with marine species are documented (e.g. *Leptosphaeria*, *Massarina*, *Pleospora*). This is an aspect that requires further study.

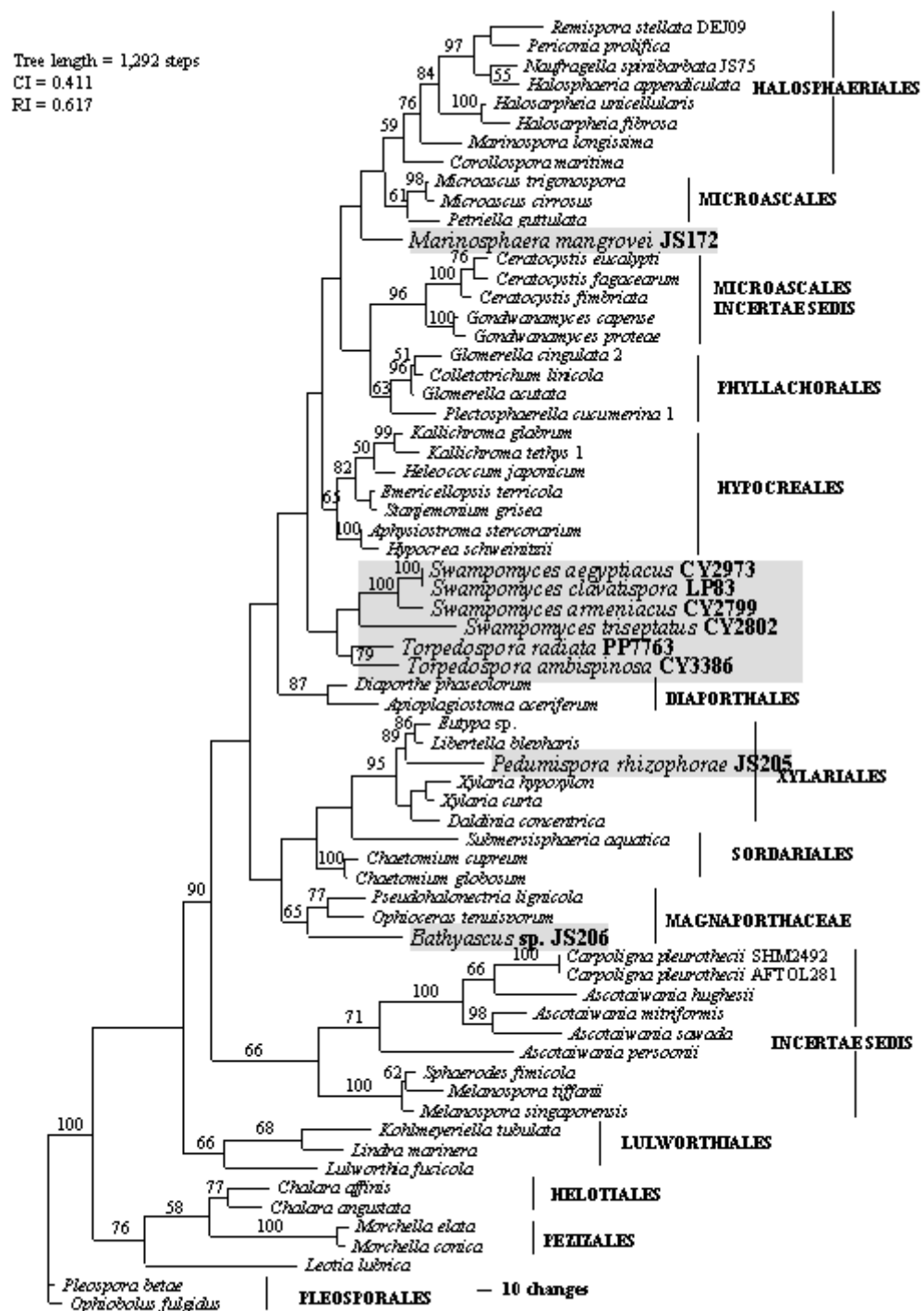


Figure 36. One of three MPTs inferred from LSU rRNA sequences of different marine ascomycete lineages, generated with maximum parsimony analysis. Bootstrap values higher than 50% are given above branches. Scale bar indicates 10 character state changes.

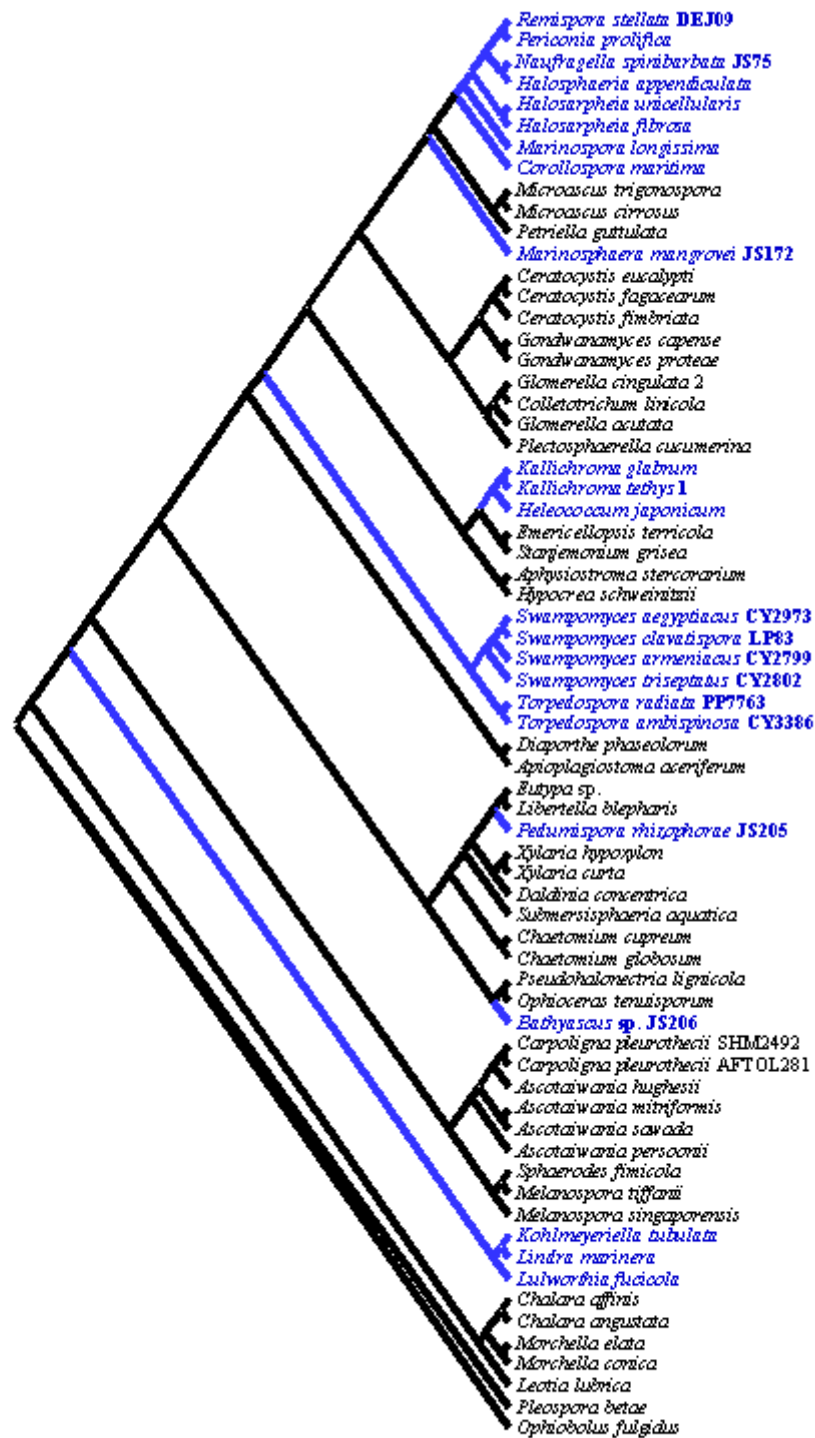


Figure 37. A cladogram inferred from LSU rRNA sequences of marine ascomycetes, representing lineages in terrestrial and marine, generated with maximum parsimony analysis.

■ Marine ■ Terrestrial

5. Further studies

This thesis has elucidated the taxonomic affinities of a wide range of species but further studies are required to resolve specific taxonomic problems:

5.1 Sequencing of other genes:

- *Torpedospora*, *Swampomyces* (RPB2, EF1- α , mitochondrial SSU).
- *Marinosphaera mangrovei* (ITS1-5.8S-ITS2, beta-tubulin, RPB2, EF1- α , mitochondrial SSU)
- *Pedumispora rhizophorae* (SSU rRNA, ITS1-5.8S-ITS2, beta-tubulin, RPB2, EF1- α , mitochondrial SSU)

5.2 Species/strains were not available or insufficient for study, thus more taxa need to be found/described/sequenced. This applies to the resolution of the ordinal status of *Bathyascus* species.

5.3 One hundred and thirty seven species are included in the Halosphaeriales (Pang, 2002; Campbell *et al.*, 2003; Eriksson *et al.*, 2004; Pang *et al.*, 2004), but only 62 have been sequenced and deposited in the GenBank (46%) (data retrieved 15 March 2004). Table 12 lists some of the Halosphaeriales that await confirmation at the molecular level. Moreover, a number of unitunicate Ascomycota await assignment at the family or order level (Table 11).

Table 11. Marine unitunicate Ascomycota that await family and ordinal assignment at the molecular level (Jones *et al.*, unpublished)

| | Genus, species |
|----|---|
| 1 | <i>Abyssomyces hydrozoicus</i> Kohlm. |
| 2 | <i>Adomia avicenniae</i> S. Schatz |
| 3 | <i>Aquamarina speciosa</i> Kohlm., Volkm.-Kohlm. and O.E.Erikss. |
| 4 | <i>Aropsichus junci</i> Kohlm. and Volkm.-Kohlm. |
| 5 | <i>Banhegyia setispora</i> Zeller and Tóth |
| 6 | <i>Biflua physasca</i> J. Koch and E. B. G. Jones |
| 7 | <i>Chaetomastia typhicola</i> (P. Karst.) Barr |
| 8 | <i>Crinigera maritima</i> I. Schmidt |
| 9 | <i>Dactylospora canariensis</i> Kohlm. and Volkm.-Kohlm. |
| 10 | <i>D. haliotrepha</i> (Kohlm. and E. Kohlm.) Hafellner |
| 12 | <i>D. mangrovei</i> E.B.G. Jones, Alias, Abdel-Wahab and S.Y. Hsieh |
| 13 | <i>Dryosphaera navigans</i> J. Koch and E. B. G. Jones |
| 14 | <i>D. tropicalis</i> Kohlm. and Volkm.-Kohlm. |
| 15 | <i>Eiona tunicata</i> Kohlm. |
| 16 | <i>Etheiophora bijubata</i> Kohlm. and Volkm.-Kohlm. |
| 17 | <i>E. blepharospora</i> (Kohlm. and E. Kohlm.) Kohlm. and Volkm.-Kohlm. |
| 18 | <i>E. unijubata</i> Kohlm. and Volkm.-Kohlm. |
| 19 | <i>Eutypa bathyurstensis</i> K. D. Hyde and Rappaz |
| 20 | <i>Gaeumannomyces medullaris</i> Kohlm., Volkm.-Kohlm. and O.E. Erikss. |
| 21 | <i>Halonectria milfordensis</i> E.B.G. Jones |
| 22 | <i>Hapsidascus hadrus</i> Kohlm. and Volkm.-Kohlm. |
| 23 | <i>Mangrovispora pemphi</i> K.D. Hyde and Nakagiri |
| 24 | <i>Marisolaris ansata</i> J. Koch and E. B. G. Jones |
| 25 | <i>Nipicola carbonispora</i> K.D. Hyde |
| 26 | <i>N. selangorensis</i> K.D. Hyde |
| 27 | <i>Oceanitis scuticella</i> Kohlm. |
| 28 | <i>Orcadia ascophylli</i> G.K. Sutherl. |
| 29 | <i>Papulosa amerospora</i> Kohlm. and Volkm.-Kohlm. |
| 30 | <i>Phomatospora acrostichi</i> K.D. Hyde |
| 31 | <i>P. bellaminuta</i> Kohlm., Volkm.-Kohlm. and O.E.Erikss. |
| 32 | <i>P. kandela</i> K.D. Hyde |
| 33 | <i>P. nypae</i> K.D. Hyde |
| 34 | <i>P. nypicola</i> K.D. Hyde and Alias |
| 35 | <i>P. phragmiticola</i> O.K. Poon and K.D. Hyde |
| 36 | <i>Polystigma apophlaeae</i> Kohlm. |
| 37 | <i>Pontogeneia calospora</i> (Pat.) Kohlm. |
| 38 | <i>P. codicola</i> (M.L. Dawson) Kohlm. and E. Kohlm. |
| 39 | <i>P. cubensis</i> (Har. and Pat.) Kohlm. |
| 40 | <i>P. enormis</i> (Har. and Pat.) Kohlm. |
| 41 | <i>P. erikae</i> Kohlm. and Demoulin |
| 42 | <i>P. padinae</i> Kohlm. |
| 43 | <i>P. valoniopsidis</i> (Cribb and J.W. Cribb) Kohlm. |
| 44 | <i>Rhizophila marina</i> K.D. Hyde and E.B.G. Jones |

Table 11. (Continued)

| | Genus, species |
|----|--|
| 45 | <i>Saccardoella marinospora</i> K.D. Hyde |
| 46 | <i>S. rhizophorae</i> K.D. Hyde |
| 47 | <i>S. mangrovei</i> K.D. Hyde |
| 48 | <i>Savoryella appendiculata</i> K.D. Hyde and E.B.G. Jones |
| 49 | <i>S. lignicola</i> E.B.G. Jones and R.A. Eaton |
| 50 | <i>S. longispora</i> E.B.G. Jones and K.D. Hyde |
| 51 | <i>S. melanospora</i> M.A. Abdel-Wahab and E.B.G. Jones |
| 52 | <i>S. paucispora</i> (Cribb and J.W. Cribb) Jørgen Koch |

Table 12. Members of the Halosphaeriales that await confirmation of their ordinal status at the molecular level (Pang, 2002; Eriksson *et al.*, 2004)

| | Genus, species |
|----|---|
| 1 | <i>Aniptodera fusiformis</i> Shearer* |
| 2 | <i>A. haispora</i> Vrijmoed, K.D.Hyde and E.B.G.Jones |
| 3 | <i>A. inflatiscigera</i> K.M.Tsui, K.D.Hyde and Hodgkiss |
| 4 | <i>A. lignicola</i> K.D.Hyde, W.H.Ho and K.M.Tsui |
| 5 | <i>A. limnetica</i> Shearer* |
| 6 | <i>A. longispora</i> K.D.Hyde |
| 7 | <i>A. mangrovei</i> K.D.Hyde and E.B.G.Jones |
| 8 | <i>A. margaritum</i> Shearer* |
| 9 | <i>A. mauritaniensis</i> K.D.Hyde, W.H.Ho and K.M.Tsui* |
| 10 | <i>A. megalospora</i> K.D.Hyde, W.H.Ho and K.M.Tsui* |
| 12 | <i>A. nypae</i> K.D.Hyde |
| 13 | <i>A. intermedia</i> K.D.Hyde and Alias |
| 14 | <i>A. palmicola</i> K.D.Hyde, W.H.Ho and K.M.Tsui* |
| 15 | <i>A. salsuginosa</i> Nakagiri and Ito |
| 16 | <i>Anisostagma rotundatum</i> K.R.L. Petersen and Jørgen Koch |
| 17 | <i>Antennospora salina</i> (Meyers) Yossoff, E.B.G.Jones and S.T.Moss |
| 18 | <i>Appendichordella amicta</i> (Kohlm.) E.B.G.Jones, R.G.Johnson and S.T.Moss |
| 19 | <i>Arenariomyces majusculus</i> Kohlm. And Volkm.-Kohlm. |
| 20 | <i>A. parvulus</i> J. Koch |
| 21 | <i>A. triseptatus</i> Kohlm. |
| 22 | ? <i>Argentinomyces naviculisporus</i> Peña and Arambarri |
| 23 | <i>Bathyascus avicenniae</i> Kohlm. |
| 24 | <i>B. grandisporus</i> K.D.Hyde and E.B.G.Jones |
| 25 | <i>B. mangrovei</i> Ravikumar and Vittal |
| 26 | <i>B. tropicalis</i> Kohlm. |
| 27 | <i>B. vermispurus</i> Kohlm. |
| 28 | <i>Bovicornua intricata</i> J.Koch and E.B.G.Jones |
| 29 | <i>Buxetrolidia bisaccata</i> K.R.L Petersen and J.Koch |
| 30 | <i>Carbosphaerella pleosporoides</i> I.Schmidt |
| 31 | <i>C. leptosporioides</i> I.Schmidt |
| 32 | <i>Ceriosporopsis caduca</i> E.B.G.Jones and Zainal |
| 33 | <i>C. cambrensis</i> I.M. Wilson |
| 34 | <i>C. capillaceae</i> Kohlm. |
| 35 | <i>C. tubulifera</i> (Kohlm.) P.W.Kirk |
| 36 | <i>Chadefaudia balliae</i> Kohlm. |
| 37 | <i>C. corallinarum</i> Müller and von Arx |
| 38 | <i>C. gymnogongri</i> (G. Feldmann) Kohlm. |
| 39 | <i>C. marina</i> G. Feldmann |
| 40 | <i>C. polyporolithi</i> (Bonar) Kohlm. |
| 41 | <i>C. schizymeniae</i> Stegenga and Kemperman |
| 42 | <i>Corallicola nana</i> Volkm-Kohlm. and Kohlm. |
| 43 | <i>Corollospora armoricana</i> Kohlm. and Volkm-Kohlm. |
| 44 | <i>C. besarispora</i> Sundari |

* freshwater ascomycetes, highlighted areas = monotypic genera

Table 12. (Continued)

| | Genus, species |
|----|---|
| 45 | <i>C. californica</i> Kohlm. and Volkm-Kohlm. |
| 46 | <i>C. cinnamomea</i> J.Koch |
| 47 | <i>C. fusca</i> Nakagiri and Tokura |
| 48 | <i>C. gracilis</i> Nakagiri and Tokura |
| 49 | <i>C. indica</i> Prasannarai, Ananda and Sridhar |
| 50 | <i>C. novofusca</i> Kohlm. and Volkm-Kohlm. |
| 51 | <i>Fluviatispora reticulata</i> K.D.Hyde |
| 52 | <i>F. tunicata</i> K.D.Hyde |
| 53 | <i>Halosarpheia aquatica</i> K.D.Hyde* |
| 54 | <i>H. aquadulcis</i> S.-Y.Sieh, H.S.Chang and E.B.G.Jones* |
| 55 | <i>H. bentotensis</i> J. Koch |
| 56 | <i>H. culmiperda</i> Kohlm., Volkm.-Kohlm. and O.E.Erikss. |
| 57 | <i>H. hamata</i> Höhnk c.f |
| 58 | <i>H. minuta</i> W.F.Leong |
| 59 | <i>H. phragmiticola</i> Poon and K.D.Hyde |
| 60 | <i>Iwilsoniella rotunda</i> E.B.G.Jones |
| 61 | <i>Lautisporopsis circumvestita</i> E.B.G.Jones, Yosoff and S.T.Moss |
| 62 | <i>Lignincola nypae</i> K.D.Hyde and Alias |
| 63 | <i>Limacospora sundica</i> J.Koch and E.B.G.Jones |
| 64 | <i>Luttrellia estuarina</i> Shearer |
| 65 | <i>Moana turbinulata</i> Kohlm. and Volkm-Kohlm. |
| 66 | <i>Nais aquatica</i> K.D.Hyde |
| 67 | <i>Naufragella delmarensis</i> Kohlm. and Volkm-Kohlm. |
| 68 | <i>Nimbospora bipolaris</i> K.D.Hyde and E.B.G.Jones |
| 69 | <i>N. octonnae</i> Kohlm. |
| 70 | <i>Ondiniella torquata</i> (Kohlm.) E.B.G.Jones, R.G.Johnson and S.T.Moss |
| 71 | <i>Phaeonectriella appendiculata</i> K.D.Hyde, W.H.Ho and K.M.Tsui* |
| 72 | <i>Thalassogena sphaerica</i> Kohlm. and Volkm-Kohlm. |
| 73 | <i>Tirispora mandoviana</i> Sarma and K.D.Hyde |
| 74 | <i>Trailia ascophylli</i> G.K.Sutherl. |
| 75 | <i>Trichomaris invadens</i> Hibbits, G.C.Hughes and Sparks |
| 76 | <i>Tunicatispora australiensis</i> K.D.Hyde |

* freshwater ascomycetes, highlighted areas = monotypic genera

In concluding remark, a wide range of taxonomic fungal groups has evolved in the sea, with a great variation in morphology. Not only the Halosphaeriales, but also other ascomycetes that have smartly adapted morphological features for dispersal and survival in the marine habitats. This thesis has expanded the knowledge of the phylogeny of marine fungi. However, further studies (collection, isolation, DNA sequencing) are required to resolve taxonomical and phylogenetical relationships of some genera.