

Chapter I

Introduction

1.1 Background to the thesis

Marine lignicolous fungi have been known for six decades as the result of studies by Barghoorn and Linder (1944), who described ten new genera and twenty-five species. Since then marine mycological research has flourished and greatly expanded our knowledge of marine fungi. There are approximately 444 species of higher filamentous marine fungi reported; the majority are ascomycetes with 360 species in 177 genera (Hyde *et al.*, 2000). Most of these belong in the Halosphaeriales, the largest order (with one-fourth of the marine ascomycetes), with many genera showing adaptations to the marine environment. Moreover, there are ascomycetes whose classification remains uncertain (*Ascomycota incertae sedis*) and have not yet been assigned with confidence to any taxonomic position.

The main criterion to differentiate between taxa depends on the morphological characteristics of various reproductive structures, such as the ascomata, asci and ascospores. Generally, the genera in the Halosphaeriales are well delineated. However, the classification of some genera and species remain problematic since their morphological features have been subjected to convergence through evolutionary time, e.g. *Lulworthia* species. Moreover, the placements of some genera

have been assigned because no other taxonomic position was suitable or no further taxonomic information available.

The advent of DNA sequencing techniques has been commonly used in fungal systematics, from a single locus to multi loci. It provides a powerful tool to resolve problems in fungal taxonomy and their evolutionary relationships that cannot be determined using morphological characters alone.

There have been the attempts to clarify the phylogeny of the Halosphaeriales by examining selected genera and related taxa. For example, Spatafora *et al.* (1995, 1998) revealed that halosphaeriaceous taxa evolved from a terrestrial ancestor. Subsequent studies have been undertaken by many authors in order to resolve the phylogenetic relationships among several genera such as *Halosarpheia* Kohlm. and E. Kohlm., *Corollospora* Werderm., *Lignincola* Höhnk, *Nais* Kohlm. (Campbell, 1999; Kong *et al.*, 2000; Anderson *et al.*, 2001; Pang, 2001). These studies showed the polyphyletic origin of some of these genera and resulted in newly described taxa such as *Natantispora* J. Campb., J. L. Anderson and Shearer, *Magnisphaera* J. Campb., J. L. Anderson and Shearer, *Saagaromyces* K. L. Pang and E. B. G. Jones, *Halosarpheia unicellularis* Abdel-Wahab and E. B. G. Jones (Abdel-Wahab *et al.*, 2001b; Campbell *et al.*, 2003; Pang *et al.*, 2003). Moreover, the marine ascomycetes *Lulworthia* G. K. Sutherl., *Lindra* I. M. Wilson, have been transferred to the new order, the Lulworthiales (Kohlmeyer *et al.*, 2000). Molecular evidence also shows that the genera *Kohlmeyeriella* E. B. G. Jones, R. G. Johnson and S. T. Moss and *Spathulospora* A. R. Caval and T. W. Johnson nestle within the Lulworthiales (Inderbitzin *et al.*, 2004). Only a few genera of marine Ascomycota have been studied at the molecular level. Therefore, this thesis furthers this theme in order to develop a

more natural classification of the marine Halosphaeriales and Ascomycota *incertae sedis*.

Marine fungi were first reported for Thailand in 1984 (Kohlmeyer, 1984) and since then a number of publications have been published (Koch, 1986; Hyde, 1989a, 1992; Hyde and Jones, 1992; Hyde *et al.*, 1990, 1993; Pilantanapak, 2003). This thesis also documents collections of marine fungi for Thailand.

1.2 Outline of the thesis

The major aim for this thesis was to study the molecular phylogeny of selected marine ascomycetes. Some species required for the study are temperate in their distribution and most of the axenic cultures were available to me for study. They were obtained from various culture collections mainly from City University of Hong Kong, Portsmouth University and from Dr. Lai Ka Pang. Some cultures have been isolated from various coastal areas in Thailand and other countries (China, Denmark, Guam (Micronesia, USA), England and Wales, UK). All isolates are conserved in the BIOTEC Culture Collection.

All region of the ribosomal RNA gene (small subunit: SSU, large subunit: LSU and ITS1-5.8S-ITS2) were sequenced for Ascomycota *incertae sedis*, and the LSU region sequenced for the Halosphaeriales taxa. Both DNA strands were sequenced at City University of Hong Kong and Bio Service Unit (BSU), BIOTEC. All sequences were aligned along with other sequences retrieved from the GenBank database (<http://www.ncbi.nlm.nih.gov>). Phylogenetic softwares used in this study include: Clustal W (Thompson *et al.*, 1994), BioEdit 5.0.6, 6.0.7 (Hall, 2001; 2004),

Se-AI v1.0a1 (Rambaut, 1999) and PAUP*4.0b10 (Swofford, 2002). Different phylogenetic assumptions were employed: maximum parsimony, neighbour joining, maximum likelihood, and bayesian inference. Finally, a number of taxonomic conclusions were drawn based on the obtained molecular results and morphological observations.

1.3 Benefits of this thesis

1.3.1 Currently only 29% of the described marine fungi have been documented for Thailand, compared to the worldwide figure (Jones, *et al.*, unpublished). The collections in this study will contribute towards a database for fungal diversity in Thailand and the production of a check-list of higher marine fungi.

1.3.2 Cultures isolated from various regions and substrata are conserved in the BIOTEC Culture Collection for further researches, for example the screening for new bioactive compounds, enzymes, fine chemicals and other genetic studies.

1.3.3 The sequences obtained from this study have been deposited in the GenBank. This will help to increase the fungal sequences in this international database, which represents only 16% of the currently known species worldwide (Hawksworth, 2004).

1.3.4 The natural classification of marine ascomycetes will help us to understand the fungal lineages that invaded the sea. Moreover, their biology, ecology and physiology can be estimated based on their known relatives. Many marine ascomycetes are important in producing potentially useful bioactive compounds. Therefore, their true nature may provide a clue in screening for future activities.