CHAPTER 1: INTRODUCTION

This research comprise 2 phase of studies. The first phase is mainly focused on the biodiversity of marine fungi from 3 locations in Peninsular Malaysia (Cape Rachado, Telok Pelandok and Morib). The second phase is the study on the molecular phylogeny of one selected species namely *Verruculina enalia* (Figure 1.1), a common mangrove fungi species from Malaysia. This species is selected due to its availability across the tropics and sub-tropics (Pang *et al.*, 2011), its ability to sporulate on incubated substrates (Grasso *et al.*, 1985; Peterson and Koch, 1997) and the ability to form abundant ascomata in pure culture (Tan *et al.*, 1995). Marine fungi are mostly saprobes growing on various cellulosic materials including woody tissues, leaves, fruits, seagrasses, algae and seaweeds and some marine fungi are also reported on animal exoskeletons, keratinaceous substrates, sediments, sea foam and seawater (Vrijmoed, 2000). However, this present study will focus on the lignocellulosic (wood) material of mangroves from Peninsular Malaysia.

1.1 The Pioneer Studies in Marine Mycology

The first publication that influenced the growth of marine mycological research is the classic publication “Marine fungi: Their taxonomy and Biology” by Barghoorn and Linder (1944). They proved that there are an indigenous marine mycota that showed growth and reproduction on submerged wood after defined period of time. Published

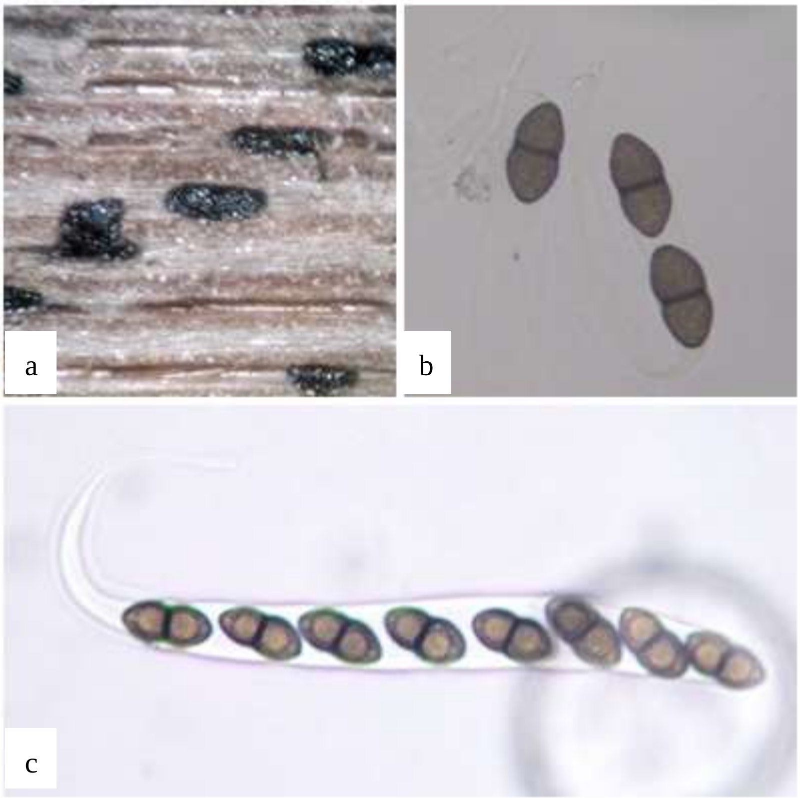

Figure 1.1 The morphology of *Verruculina enalia*; a) Ascoma of *V.enalia* on sliced mangrove wood, the fruiting body is partially embedded b) Dark brown and verrucose ascospores of *V.enalia* c) Ascus of *V.enalia* containing 8 ascospores.

papers on marine fungi from 1950-1970 were focus on woody substrata. This is in contrast to the earlier studies that were done mainly on fungi from seaweeds (Cotton, 1909; Sutherland, 1915; 1916a; b; Sparrow, 1936). From 1970-2000, we have seen more descriptive studies on marine fungi and a few phylogenetic studies were carried out (Davidson, 1974; Kohlmeyer, 1977; Steinke and Jones, 1993; Kohlmeyer *et al.,* 1995; Koch and Petersen, 1996; Kowalchuk *et al.,* 1997; Jones *et al.,* 1998). From the beginning of year 2000 till present, there is a rise of using molecular approach to study the diversity, ecology and phylogeny of marine fungi (Möhlenhoff *et al.,* 2001; Buchan *et al.,* 2002; Osherov *et al.,* 2002; Zuccaro *et al.,* 2003; Inderbitzin *et al.,* 2004). This present study will be the first initiative work in marine mycology on observing the genetic diversity among *V. enalia* from different biogeographical isolates based on its ITS gene sequence. It is hoped that the result will be significant and will be a breakthrough for other researchers to investigate more on genetic diversity of marine fungi across the globe and to better understand its ecology.

1.2 Marine fungi in South East Asia region and neighbouring countries

Over the last two decades, biodiversity studies on marine fungi in Asia have been carried out in various countries: Brunei (Hyde, 1988), China/Hong Kong/Macau (Vrijmoed *et al.*, 1994; Jones and Vrijmoed 2003; Pang *et al.*, 2008), Taiwan (Pang *et al.*, 2011), India (Ananda and Sridhar, 2002; Prasannarai and Sridhar,2001; Sarma and Vittal, 2001; Maria and Sridhar, 2003; Raveendran and Manimohan 2007), Indonesia (Hyde 1989b), Japan (Nakagiri 1993), Malaysia (Jones, 2007; Alias and Jones, 2008; Alias *et al.*, 2010), Mauritius (Poonyth *et al.*, 1999; 2001), Philippines (Jones *et al.*, 1988; Besitulo *et al.*, 2010), Singapore (Tan and Leong 1990, Leong *et al.*, 1991) and Thailand (Sakayaroj *et al.*, 2004; Sakayaroj *et al.*, 2011). The abundance of works done on investigating the biodiversity of marine fungi shows that there are worldwide effort in compiling a series of checklist of marine fungi available in the nature. It also gives an idea that it is important to study the ecology of marine fungi in order for us to know better about this ecologically defined group of organisms. The most recent figure on the number of marine fungi is 530 (in 321 genera): Ascomycota 424 species (in 251 genera), anamorphic fungi 94 species (in 61 genera) and Basidiomycota 12 species (in 9 genera) (Jones *et al.,* 2009). These numbers are undoubtedly will increase in years to come based on the recent estimation that there as many as 5.1 million fungal species exist (Blackwell, 2011). This estimation is based on high-throughput sequencing methods. Molecular methods have been a tool in discovering new knowledge and information of fungi. DNA sequencing grants a multitude of new characters for analysis. Ten phyla have been revealed so far as members of monophyletic kingdom Fungi. The present study on the biodiversity of marine fungi from Malaysia will be a small contribution to the efforts in updating the species checklist. Fundamental studies like this may seem straightforward but it is equally important with other studies as well.

1.3 Marine mangrove fungi from Malaysia

Malaysia has around 650,000 hectares (ha) of land area or 6,424sq km of mangrove forests (Spalding *et al.,* 1997). Most mangroves are found in Sabah (57%) and Sarawak coasts with 26% mainly along the Rajang and Trusan-Lawas rivers, while Peninsular Malaysia supports only 17% of mangroves (Spalding *et al.,* 1997). Matang Forest Reserve, Perak State comprising largely *Rhizophora* species is the largest mangrove stand in Malaysia with a total area of 34,769 ha (Saenger *et al.,* 1983). Aquaculture development, land reclamation and deforestation are the major threats to mangroves in

Malaysia (Ong, 1995). Tomlinson (1986) listed 114 species of true mangrove trees and mangrove associates. In Malaysia, some of the true mangrove species available are *Rhizophora apiculata, Rhizophora mucronata, Avicennia marina, Avicennia alba, Sonneratia ovata, Lumnitzera racemosa, Xylocarpus granatum* and *Bruguiera gymnorrhiza*. *R. apiculata* is dominant in mangroves of Peninsular Malaysia while the main mangrove tree in the East Malaysia is *A. marina*.

Marine fungi recorded previously from Malaysia were often associated with mangrove and mangrove-associate (Alias and Jones, 2008). There are 22 manglicolous fungi species from Malaysia that are new to science have been described with the majority are from *Nypa fruticans* substrates (Hyde, 1993; Alias *et al.*, 1994; Hyde, 1994; Hyde & Alias, 1999; Hyde *et al.*, 1999).

Mangrove fungi can be quite distinct from those that occurring in deep sea and coastal waters. Examples of species that occur in coastal and oceanic area are *Arenariomyces* sp., *Corollospora* sp. and *Torpedospora radiata*. While for mangrove fungi the common species in Malaysia are *Halorosellinia oceanica, Kallichroma tethys* and *Leptosphaeria australiensis* (Jones and Hyde, 1990).

This is a revisiting study for Morib while Telok Pelandok and Cape Rachado are the new locations selected. Previous study done in Morib was mainly focused on the vertical species distribution on a *Rhizophora apiculata* mangrove stand (Alias and Jones, 2000). While this study focused on species occurrence for fungi that grows below the water line.

1.4 Phylogeography of marine fungi

Phylogeography is a multi-discipline study that combines the phylogenetic and geography and also the historical events that cause the current distribution of species (Avice, 2000; Knowles and Maddison, 2002). Vicariance and dispersal are the two main theories related with the distribution of a species. In vicariance theory, a species evolved around a certain period of time and regional species diversity resulted from *in situ* "diversification" after continental drift (Wiley, 1988). While dispersal or also known as the centre of origin hypothesis, asserts that a particular species originates from one locality and subsequently disperses to other parts of the world (Bremer, 1992).

Most phylogeographical studies of fungi have been restricted to terrestrial taxa and vicariance is mostly inferred to be the cause of the current distribution (Coetzee *et al.*, 2000; Hibbett *et al.*, 1998; Thon and Royse, 1999; Murillo *et al.*, 2009; Muggia, 2008). A terrestrial origin of marine Ascomycota has been suggested (Spatafora *et al.*, 1998) but knowledge on the phylogeography of aquatic fungi is still lacking. So what is the reason causing the current distribution of marine mangrove fungi? In this study, the nuclear ITS (internal transcribed spacer) regions of various geographical isolates of the marine mangrove fungi, *Verruculina enalia* were sequenced to infer the phylogenetic relationships between these isolates, i.e. to explain the possible historical events (vicariance and dispersal) leading to the current distribution of *V. enalia.*

Verruculina enalia (Figure 1.1) is characterised by immersed, clypeate, carbonaceous, black ascomata, presence of trabeculate pseudoparaphyses, thick-walled, fissitunicate, cylindrical asci without an apical apparatus but with an ocular chamber and ellipsoidal, 1-septate, dark-brown, verrucose ascospores (Kohlmeyer and Volkmann-Kohlmeyer, 1991). This is one of the most common marine fungus in mangrove environment worldwide and is also widely distributed in Malaysia (Alias *et al.,* 1995; Alias and Jones, 2000; Alias and Jones, 2008; Pang *et al.,* 2010), therefore, isolates from various geographical locations were selected for a phylogeographical study.

The nuclear small subunit rRNA gene is the most conserved part of the gene cluster and its sequence has been used to delineate taxa at taxonomical ranks above family, while large subunit rRNA is more variable and it can be used to differentiate taxa at family or genus levels. ITS regions are extremely variable in length and nucleotide composition, so it is a suitable marker for instraspecific variation (White *et al.,* 1990). In fungi, ITS is the default marker for species level studies, with the notable exception of the yeasts, where the LSU became the standard for identification. ITS has been used widely to answer specific questions at genus or species levels, e.g. oomycetes (Cooke *et al.,* 2000 for *Phytophthora*, Lévesque and de Cock 2004 for *Pythium*), agarics (Aanen and Kuyper

2004 for *Hebeloma*, Frøslev *et al.*, 2007 for *Cortinarius*, Kretzer *et al.*, 1996 for *Suillus*) and lichens (Stenroos et al. 2002, for *Cladonia*).

The multicopy nature of the rDNA repeat makes the ITS regions easy to amplify from small, dilute or highly degraded DNA samples. Studies have demonstrated that the ITS region is often highly variable among the morphologically distinct fungal species (Gardes *et al.*, 1991; Gardes and Bruns, 1993; Baura *et al.*, 1992; Chen *et al.*, 1992; Lee and Taylor, 1992) with one known exception that the intraspecific variation is low in Laccaria species (Gardes *et al.*, 1991). Therefore, it is a good gene marker to study intraspecific variation between isolates of the same species, as in the current study.

1.5 Objectives of the study

- To record the occurrence, species richness and distribution of marine fungi in Peninsular Malaysia: Cape Rachado, Morib and Telok Pelandok.
- To determine the diversity and species composition that include evenness, similarity and richness indexes for all study sites by using statistical analysis.
- To determine if different geographical of *Verruculina enalia* correlate with their ITS rRNA gene phylogeny by using biogeographical isolates from Malaysia, Philippines, Hong Kong and Taiwan.
- To examine if phylogeographic structure of *V. enalia* follows the vicariance pattern of terrestrial taxa or if *V. enalia* is able to disperse over long distance through the ocean.