CHAPTER 2: LITERATURE REVIEW

2.1 Definition of marine fungi

Johnson and Sparrow (1961) and Tubaki (1969) defined marine fungi based on its ability to grow at certain seawater concentrations while Kohlmeyer and Kohlmeyer (1979) stated that marine fungi can be categorized into two groups, namely the obligate marine fungi which are able to grow and sporulate exclusively in marine and estuarine habitat; and facultative marine fungi which originated from freshwater and terrestrial habitats but are able to grow and possibly sporulate in marine environment. This definition has been used by many and inspired mycologists to initiate studies on the effect of salinity tolerance of the fungi (Nakagiri *et al.* 1996; Leano *et al.,* 1998).

Kohlmeyer and Kohlmeyer (1979) also suggest that a valid criterion for the definition of a marine fungus is the ability for the spores to germinate and to form mycelium under natural marine environment. Thus, marine fungi are not a taxonomically, but rather an ecologically and physiologically defined group (Hyde and Jones, 1988; Hyde *et al.*, 1998). Marine fungi may also be categorised into ecological groups based on their ability to occupy specific habitats and substrata (Kis-Papo, 2005).

Fungi from marine ecosystems are distinct from those from the terrestrial and freshwater environments but there are species that can occur in both seawater and freshwater habitats (e.g. *Sarvoyella lignicola*). The term “marine” is used to describe fungi occurring in a marine or marine-related environment but often they are labelled as oceanic, manglicolous etc. The terms were based on their habitats or substrata.

2.2 Estimation of fungi available worldwide

Hawksworth (1991) estimated that there are 1.5 million species of fungi in the biosphere and only 5% among these fungi were described and inventoried. However, the estimation number has been increased to 5.1 million species based on high-throughput sequencing methods (Blackwell, 2011). Meanwhile, the number of marine fungi is estimated over 10,000 (Jones, 2011b).

The estimated number of marine fungi available worldwide has been increasing in time since the first estimation by Kohlmeyer and Kohlmeyer (1979) who predicted less than 500 marine fungi in the ocean. Since then, there have been an increase number of marine fungi described from various substrata, but mainly on decaying mangrove wood. There are several papers that studied the diversity of marine fungi (Abdel-Wahab and Jones, 2000; Maria and Sridhar, 2003; Pilantanapak *et al.*, 2005; Hyde and Sarma, 2006; Jones and Alias, 2007; Figuera and Barata, 2007; Besitulo *et al.*, 2010; Pang *et al.*, 2011). Jones and Mitchell (1996) estimated that there are some 1500 species compared with 200,000 marine animals and 20,000 marine plants. Jones (2011a) believed that the documentation of marine fungi is still at the inventory stage and many new taxa await to be discovered.

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). However, Jones (2011b) stated that the figures are based on a narrow interpretation of “marine species” where other groups of fungi like yeasts and chytrids were not included. The estimated figure of fungi will be as many as 10,000 taxa if these groups are considered as marine species (Jones, 2011a).

2.3 Distribution of marine fungi

Phylogeography of marine fungi can be considered an unexplored discipline in marine mycology as there are no studies done on it yet. However there are records of study and review study on the traditional biogeography done by marine mycologists (Hughes, 1986; Volkmann-Kohlmeyer and Kohlmeyer, 1993). Biogeography is a study that looks into the distribution of species and also its ecosystem and can be divided into ecological biogeography and historical biogeography without looking on the pattern of the genetic variation of species (Hughes, 1986).

Jones *et al.* (2009) documented 530 obligate marine fungi and these fungi fall into distinct geographical (cold water, temperate, tropical) and ecological (oceanic waters, mangroves, salt marshes, algicolous, arenicolous) groups. Booth and Kenkel (1986), used ordination analysis to describe a distribution model of marine fungi based on salinity and temperature regimes and concluded that temperature is the dominant factor influencing the worldwide distribution of marine fungi. Hughes (1974, 1986) supported the major effect of temperature in their geographical distribution and produced a series of maps based on isocrymal/isothermal lines.

Physiological studies of selected fungi have indicated that tropical fungi have a higher temperature requirement, when compared with their temperate counterparts (Boyd and Kohlmeyer 1982; Bebout *et al.*, 1987; Panebianco 1994). On the other hand, a number of species appear to adapt to a wider range of habitats and geographical distribution and are cosmopolitan in distribution, e.g. *Aniptodera chesapeakensis, Arenariomyces trifurcatus, Ceriosporopsis halima, Corollospora maritima, Lignincola laevis, Savoryella lignicola, Trichocladium alopallonellum* and *Zalerion maritimum.*

What is the main historical cause for the above distribution of marine fungi? Fossil evidence for eukaryotic organisms dated around 2000 million years ago while the oldest known fossilised record of terrestrial fungi dated back in between 480 and 460 million years ago (MA) (Heckman *et al.,* 2001). The oldest fossilised fungi found preserved in marine phosphorite of the Doushantuo Formation dated back in between 551 and 635 MA at Weng-an, Southern China. This fossil revealed that the fungus formed a symbiotic partnership with photoautotrophs before the evolution of vascular plants (Yuan *et al.,* 2005). Fossilized marine manglicolous fungi were also found on lignite beds of west coast of India (Kumaran *et al.,* 2004), but the age of the sample was not known.

Spatafora *et al.* (1998) suggested a terrestrial origin of marine Ascomycota, but do these fungi follow the vicariance theory like other terrestrial fungi in relation to phylogeography? Can marine fungi disperse through the ocean? To date, there is no information on the relationship between different isolates of the same species of marine fungi to infer vicariance or dispersal theories.

2.4 Factors affecting the availability of marine fungi

There are several factors that affect the availability of marine fungi in the environment. All factors that are known affecting the availability are believed to have a certain degree of correlation with one another and it is impossible to name only one factor that can explain the occurrence. In most cases there are combinations of several factors which play an important role to the existence of fungi.

2.4.1 Temperature

Sea temperature is one of the important factors that influence the occurrence of a species as seen in a case study of *Digitatispora maritima* in Portsmouth, England. The fungus appeared on a test block when sea temperature dropped to 10°C and stop producing fruiting bodies when the temperature rises above 10°C (Byrne and Jones, 1974).

Mycogeographic zones as proposed by Hughes (1974) are divided into 4 zones separated by the sea surface temperature which then led other researchers to map the distribution of marine fungi to give general idea on marine fungi geographical distribution (Kohlm, 1983, 1984; Jones, 1993; Kohlmeyer and Volkmann-Kohlmeyer, 1993; Hyde and Lee, 1995; Jones and Alias, 1997; Whalley *et al.*, 2000). The zones are temperate, subtropical, tropical and arctic.

2.4.2 Climate

Climate and sea temperature are correlated. The following paragraphs are intended to discuss about species from different climate zones and to discuss on the cosmopolitan fungi species with broad mycogeographic zones.

Marine fungi recorded in tropical waters are distinct from those in temperate areas. Species that commonly found in tropics are *Adomia avicenniae*, *Antennospora quadricornuta* and *Massarina acrostichi* (Hyde, 1989a; Jones, 1993; Panebianco, 1994). While the temperate common fungi are *Ceriosporopsis trullifera*, *Lindra inflata* and *Ondiniella torquata* (Kohlmeyer and Kohlmeyer, 1979; Jones, 1985; Jones *et al.*, 1998). These species can only be found in their respective zones.

There are also the cosmopolitan species which can be found all over the region in the world. It can be found in tropics, sub-tropics and temperate zones. Examples of common cosmopolitan species are *Ceriosporopsis halima* Linder, *Corollospora maritima* Werdermann, *Leptosphaeria australiensis* (Cribb and Cribb) G.C. Hughes, *Savoryella lignicola* Jones et Eaton, *Aniptodera chesapeakensis* Shearer et Miller and *Corollospora colossa* Nakagiri et Tokura (Shearer and Crane, 1980; Vrijmoed, *et al.*, 1997; Prasannarai and Sridhar, 2001; Sarma and Vittal, 2001a,b). All of these species mentioned above has been recorded found in Malaysia except for *Ceriosporopsis halima* (Alias *et al.*, 1995; Zainuddin and Alias, 2005).

Bebout *et al.* (1987) suggested that cosmopolitan species may form distinct geographical races based on physiological responses to temperature. Eight years later, Roberts *et al.* (1995) confirms the theory by Bebout *et al.* when he sequenced 18S rRNA gene of 7 isolates of *Corollospora maritima* and found that 5 isolates group together and subtropical isolates formed a separate group.

2.4.3 Antagonistic germination

Interference competition between marine species does exist. The behaviour was observed on media agar (Miller *et al.*, 1985) and small test blocks (Strongman *et al.*, 1986). The dominant fungi might have produced metabolites affecting the growth of the other fungi an act as the anti-fungal agent. Panebianco (1991) suggested extending the experiment to monitor colonization at molecular level and assess if secondary metabolites are produced.

The ability to sporulate was again tested by Tan *et al.* (1995). The researchers chose to study the interaction between *Aigialus parvus*, *Lignincola laevis* and *Verruculina enalia*. Sporulation was proved affected when in mixed cultures where sporulation of *L. laevis* was suppressed by *A. parvus* and/or *V. enalia*. While, *V. enalia* have greater ascomata formation when co-cultured with *L. laevis*. However, all the 3 species formed abundant ascomata within 6-12 weeks in pure culture.

2.4.4 Salinity

Requirement for sodium chloride is the earliest scope that was looked into by several researchers. This physiological studies were done by Jones and Jennings (1964); Meyers (1968); Jones *et al.* (1971); Byrne and Jones (1975); Jones and Harrison (1976) and Jennings (1983, 1986). Later studies done by Nakagiri *et al.* (1996) and Leano at al. (1998) have shown that there are species that exhibit a wide tolerance to salinity in nature and controlled environment. It shows that they are well adapted in mangrove ecosystem that is known to have wide range of salinity.

The higher marine fungi as observed by Jenning (1983) and Jenning (1986) do not appear to have sodium requirement at macronutrient level. They can tolerate concentration of ions present in seawater and prefer the alkaline pH of seawater. This is proven and there is a genetic basis for salinity tolerance as found in *Dendryphiella salina* (Stanley *et al.,* 1995; Clement *et al.*, 1999). Although some freshwater fungi are able to grow at salinities as high as $18^{o}/_{oo}$, they were not physiologically adapted to, nor morphologically active in highly saline environment (Harrison and Jones, 1975).

Hyde (1992a) reported species diversity at a mangrove site with brackish water salinity range between 3-24 $^{o}/_{oo}$ was much higher compared with the samples from the open ocean site. However, species recorded include those from terrestrial.

High salinities may reduce the production of cellulases which are the lignocellulolytic enzymes used to degrade wood material (Pointing *et al.*, 1999). This is one of the factors that affect the availability of fungi in marine environment.

2.4.5 Habitat and niche

Some marine fungi live only on exclusive substrata such as the digestive tracts of living marine amphipods, decapods and isopods. They are the commensals species to the host (Lichtwardt, 1976). A wide range of substrata need to be studied in order to get a better picture of marine fungi that are available in the nature.

Ecological factors that determined the presence of marine fungi are the host specificity or substrates preference and the dissolved oxygen while the vertical distribution of marine fungi appears to be controlled by hydrostatic pressure (Kohlmeyer, 1983) and it is believed that the distribution is affected by the ultraviolet (UV) light as well (Alias and Jones, 2000).

Ascomycetes from mangrove able to survive these conditions because of the developing asci are embedded in mucilage in the centrum or the ascomata are immersed deeply within the wood (Au *et al.,* 1999). Carbonaceous wall of manglicolous fungi ascomata were also believe to protect the spore from UV light (Alias and Jones, 2000).

This explains why some marine fungi are rare in the ecosystem because marine environment like mangrove and coastal areas are a dynamic ecosystem where parameters of its ecology usually fluctuate in certain range. Some species only grow on a specific host that is seasonal. Hence, the availability of the fungus also becomes seasonal. The biology of marine fungi itself differs between another. Without the right pH and organic nutritive content may also affect the availability and concurrently its biogeographic dispersion.

Biogeographical studies of marine fungi is hoped to explain the impact of different environment to the selected species and will provide theories about how these fungi and their hosts have co-evolved. Data gathered from these studies will also contribute towards a check list of Malaysia diversity and increase our knowledge of fungi in the coastal waters of Malaysia.

2.4.6 Interrelation among factors

Most studies on salinity were conducted in fixed temperature manner. Ritchie (1957) however, demonstrate an interrelationship between temperature and salinity. Two selected marine fungi *Dendryphiella marina* and *Zalerion maritimum* were observed and indeed there are linear connection between salinity and temperature. Optimum salinity becomes higher with the increasing of seawater temperature (Molina and Hughes, 1982; Lorenz and Molitoris, 1992; Torzili, 1997).

2.5 Marine fungi based on its substrata and ecology

Fungi were grouped together based on their occurrence on various substrata from wood to sediments, muds, soils, sand, algae, corals, calcareous tubes of mollusks, decaying leaves of mangroves, intertidal grasses and living animals, to those growing in the guts of crustaceans (Kohlmeyer and Kohlmeyer, 1979; Jones and Hyde, 1988; Hyde, 1996). They are also labelled according to its ecological characteristic. Several researchers have observed host preference by observing test blocks and proved that certain fungi prefer to grow on a specific host (Jones, 1968; Byrne and Jones, 1974; Grasso *et al.*, 1985; Peterson and Koch, 1997).

2.5.1 Algicolous

The term is used for algae-derived fungi group. The first obligate marine fungus was discovered on a rhizome of a seagrass. *Halotthia posidoniae* was found on the rhizomes of *Posidonia oceanica* (Durieu and Montagne, 1869).

One third of the fungi described from marine environment occur on algae (Kohlmeyer and Kohlmeyer, 1971). However, algicolous group received comparatively less interests among mycologists when they mostly study saprophytic fungi living on wood and other cellulosic substrates in the sea (Kohlmeyer, 1973).

Algicolous is an interesting group of marine fungi as there are various species that act as saprophytic or perthophytic and parasitic and symbiotic with the host. Occurrence on various host substrates were studied by several mycologist in the recent years (Kohlmeyer, 1975; Kohlmeyer and Demoulin, 1981; Raghukumar, 1987; Li Wei *et al.*, 2010)

2.5.2 Arenicolous

Arenicolous fungi can be considered as unique as their ascomata attach to sand grains or calcareous shell fragments associated with wood from which they derive their nutrients. They can tolerate extreme conditions like high temperature, exposure to UV light, abrasion of waves, dessication and fluctuation of salinity.

Jones and Mitchell (1996) recorded 35 species of marine fungi from sand grains and 20 species from hard calcareous material like coral. As for Malaysia, there are 44 arenicolous fungi recorded where 11 species were new records for the country (Sundari *et al.*, 1996). Some of the species are *Arenariomyces parvulus, Carbosphaerella leptosphaerioides, Lulworthia crassa* and *Tetraploa aristata*. Study of arenicolous fungi in Malaysia was done by Sundari *et al.* (1996) and no further study was done on this particular group after that.

2.5.3 Foliicolous

Foliicolous fungi grow on leaves. There is no study on this particular group that has been done in Malaysia. Studies that were done before focus on the fungi of temperate plant species like *Juncus roemerianus* and *Spartina* sp. and mangrove trees leaves (Fell and Master, 1973; Bremer; 1995; Kohlmeyer and Kohlmeyer, 2000; Ananda *et al.*, 2008). *Juncus roemerianus* and *Spartina* sp. can be found and collected in salt marshes. Senescent leaves or the culms of standing marsh plants were cut at the base close to rhizome and stored in plastic bag and put in a refrigerator or freezer until examined or air-dried. The fungal structures are usually immersed in the plant tissue, the culms and leaves are cut in length-wise and scanned under microscope for embedded fruiting bodies of ascomycetes and coelomycetes. However, facultative marine and halotolerant fungi species can be found up until the tip of the plant (Kohlmeyer *et al.*, 2004).

2.5.4 Lignicolous

This is one of the groups that received relatively wide attention from various mycologists in Malaysia and worldwide (Jones *et al.,* 1988; Alias *et al.,* 1995; Kohlmeyer *et al.,* 1995; Abdel-Wahab and Jones, 2000; Kane *et al.,* 2002). Many species were inventoried from this particular group of fungi. The lignocellulosic substrata support typically marine mangrove fungi (Vrijmoed *et al.*, 1996; Jones and Vrijmoed, 1997). Lignocellulosic parts of mangroves such as its branches, twigs and roots were observed and fungal species found were inventoried by mycologists (Hyde, 1988; Alias *et al.*, 1995; Alias and Jones, 2000; Ananda and Sridhar, 2002). It is also encompass drift woods, submerged woods and fallen branches from mangrove trees. Wood in the open ocean tends to favour the growth of members of the Halosphaeriales whose species have deliquescing asci, passive ascospores release and variously appendaged ascospores which are involved in attachment (Jones, 1994).

2.5.5 Manglicolous

Marine fungi which are found on any parts of mangroves are called manglicolous fungi (Kohlmeyer, 1969; Jones and Hyde, 1988, 1990; Jones and Kuthubutheen, 1989). Manglicolous fungi are mangrove–derived fungi which the availability of the host is restricted in tropics and subtropics only. Mangroves are rich in substrata with abundance of leaf litter and dead wood. Mangrove leaf litter is also an important substratum as it supports different fungal community (Awang and Gan, 1989; Jones, 2000). A series of studies done by Hyde in 1990, 1992 and 1993 have shown the host specificity and determined the marine fungi that can only grow and found to a specific mangrove trees. (e.g. *Caryospora mangrovei* on *Xylocarpus granatum*, *Aigialus mangrovis* on *Avicennia alba*, *Rhizophila marina* on *Rhizophora* spp.). However, driftwood shows that it supports a greater diversity compare those on exposed test blocks (Jones and Vrijmoed, 1997).

In mangrove habitats, loculoascomycetes are common on decaying wood in the intertidal zone where they can eject their spores during low tides (Hyde, 1989b). Loculoascomycetes have the ability to forcibly eject their ascospores, and the ascospores generally have sheaths rather than appendages.

Schmit and Shearer (2003) listed 625 mangrove fungi. Species recorded in the study include those from terrestrial as well as from marine substrata and many of these species may not be restricted to marine environments. Marine fungi species extracted from the recorded list of species in the study are as follows: 175 Ascomycota, 35 anamorphic species, 5 Basidiomycota. 14 Oomycetes, 3 Chytridiomycota, 2 myxomycetes, 8 Thraustochytriales and 12 Zygomycota with a total of 230 fungi and 24 other taxa.

There are also vegetations that were recognized as mangrove-associate, for example, *Nypa fruticans*. Several studies on the biodiversity of fungi on the intertidal brackish water palm *Nypa fruticans* have been undertaken (Hyde and Alias 2000; Pilantanapak *et al.,* 2005, Hyde and Sarma, 2006). Pilantanapak *et al.* (2005) collected 81 species on the palm in Thailand while Hyde and Sarma (2006) documented 46 species from the Tutong River, Brunei. Most common species from *Nypa fruticans* are *Linocarpon appendiculatum*, *L. nypae*, *Oxydothis nypae* and *Astrosphaeriella striatispora* (Hyde 1992a; b; Hyde and Alias, 2000; Besitulo *et al.,* 2002; 2010; Pilantanapak *et al.* 2005; Hyde and Sarma, 2006). *Nypa* may be a host that specific for species like *Aniptodera nypae, Helicascus nypae*, *Linocarpon nypae*, *Tirisporella beccariana* and *Vibrissea nypicola* (Jones, 2011a).

Mangrove forests can be considered as the main “source” of marine fungi. Many species were found in this ecological niche. The biodiversity of fungi associated with mangroves are affected by the nature of its ecosystem biologically and physically. Mangroves provide a wide variety of substrates for fungi to grow i.e. sediments or peat material, leaves, twigs, pneumatophores, fruits and roots. The important environmental gradients, such as salinity and tidal changes may provide further opportunities for niche differentiation.

2.5.6 Deep sea

It is the largest ecosystem on earth and the deep sea floor is an extreme environment. Pressure is high, temperature is low and food input is small. It has been characterized as a physically stable environment (Sanders, 1968). " It is the very remoteness of the deep sea and the difficulties encountered in its exploration that have resulted in it being one of the least understood environment on earth" (Damare, 2006).

The presence of fungal hyphae in deep sea sediment is proven but they are in low abundance because of the poor detection of the fungi due to its cryptic presence in macroaggregates. Fungal biomass carbon from different core sections of deep-sea sediments from ∼5000 m depth was estimated based on direct microscopic detection of fungal mycelia. However, treatment with ethylenediamine tetra-acetic acid (EDTA) onto the sediment samples enabled more frequent detection and significantly higher biomass than in samples without such treatment (Damare and Raghukumar, 2008).

Deep water fungi are unique and different from other marine fungi. They have build up special ability to adapt to low temperature that range between 2-2.4^{o}C, high pressure and the lack of substrata like wood. Living in such extreme condition makes them distinct morphologically and have different host preference (Kohlmeyer and Kohlmeyer, 1979) and fungal diversity is likely to be low in this area.

2.5.7 Other substrates

There is not much known about fungal propagules in pelagic seawater. It is clear that it is an area of research that requires further studies. First observation on fungal propagules was initiated by Fazzani and Jones (1977). They recorded eight spores of marine fungi per litre. Storm that generated sea foams may yielded a number of propagules which were recorded from sediments. However, the existence of marine fungi in open ocean has not been demonstrated convincingly as the free living state is a barren condition for fungi to germinate where only yeast and lower fungi were may be found attach to planktonic organisms or pelagic animals (Kohlmeyer and Kohlmeyer, 1979). The method of enumeration of spores was designed by Kirk (1983) and *Arenariomyces trifurcatus* and *Corollospora maritima* were common in this observation.

2.6 Evidence for the vicariance of fungi

Although an investigation of the genetic relationship of cosmopolitan marine fungi from different geographical regions is lacking, several terrestrial mycota have been examined. Most studies on geographical differences of fungi are restricted to basidiomycetes, e.g. *Armillaria* (Coetzee *et al.,* 2000), *Lentinula* (Hibbett *et al.,* 1998; Thon and Royse, 1999), *Rhizoctonia solani* Kuhn (Salazar *et al.,* 1999) and Flammulina (Hughes *et al.,* 1985). Coetzee *et al.* (2000) found that different populations of *Armillaria mellea* are genetically distinct possibly by geographical isolation, i.e. they may have undergone speciation. Some *Lentinula* populations were discovered to be narrowly distributed; some had a wide distribution and some with an overlapping distribution (Hibbett *et al.,* 1998).

An isolate of *Flammulina velutipes* from British Columbia has been demonstrated to be more closely related to that from China than those from North America due to gene exchange or migration events (Hughes *et al.*, 1985). Phylogenetic analysis of nuclear ITS rDNA sequences corroborated with chemical characters also revealed the presence of four distinct lineages of *Rhytidhysteron rufulum* complex isolated from Costa Rica. Re-examination of its morphology showed differences in ascomatal, ascal and ascospore size between the lineages which were once regarded as intraspecific variations. This study had contradicted the presence interpretations of one morphologically variable species (Murillo *et al.*, 2009).

Study on endolithic *Caloplaca* lichens from Italy also revealed a high genetic diversity but with low morphological variability. A phylogenetic analysis of its nuclear ITS which includes the available *Caloplaca* sequence data revealed that these lichens form a monophyletic group within the genus (Muggia *et al.*, 2008).

These studies give evidence of a possible vicariance event. A species was split into sub-populations and these populations were separated by physical barriers. Mutation accumulated over time and each population speciates. To the best of my knowledge, there is no study that looks into the phylogeography of marine fungi, i.e. to infer vicariance or dispersal theories of marine fungi.

However, a few studies have provided evidence for infraspecific physiological and genetic variations (Bebout *et al.*, 1987; Roberts, 1996). The growth of *Corollospora maritima* isolates, a cosmopolitan species were examined at various temperatures and reported that they grew best at the temperature they were isolated from (Bebout *et al.*, 1987; Jones, 1993). Based on the 18S rRNA gene sequence, *C. maritima* isolates formed separate group according to the climate of their localities. Temperate and subtropical isolates formed a different group (Roberts *et al.*, 1995). These studies indicated that there are distinct “races” within a single species but there is a lack of research to investigate the extent of these variations (morphological, physiological, phylogenetic).

2.7 Dispersal of marine fungi

Although no direct evidence is available on possible dispersal of marine fungi, propagules of marine fungi could have been dispersed by association with hosts, movement of ocean and human-mediated transfer.

2.7.1 Dispersal by ocean

Kohlmeyer and Kohlmeyer (1979) stated that it is impossible for a delicate ascospore to serve transoceanic dispersal, let alone its fruiting bodies that need an optimum condition for it to emerge. However, ascospores of marine fungi are mostly equipped with large oil globules and appendages to remain suspended in the sea water (Jones *et al.*, 2009). Mechanism used by fungi to survive in marine environment comes from the ability of its spore to maintain the osmotic gradient across the fungal cell membrane that will balance the water transport into the cell (Hooley *et al.*, 2003). Fungi may also disperse through woods and not by the spore itself. Hyphae of marine fungi growing inside driftwood or fruits of marine plants can possibly transported over a long distance.

2.7.2 Dispersal with hosts

The Old Mangroves ecosystems were estimated to exist possibly just after the first angiosperms about 80 million years ago (Duke, 1992). It appears along the coastline in the intertidal zones across the tropics and subtropics region worldwide (Chapman, 1976; Duke, 1992).

Mangroves are said to evolve from terrestrial or fresh water plant species rather than marine plants but are able to tolerate a large range of salinities under natural conditions (Suarez *et al.*, 1998). The break-up of continental land masses that take place million years ago may provide conditions that are favourable for the growth of mangroves that inhabit the intertidal zones (Kathiresan and Bingham, 2001; Shan *et al.,* 2008).

Scientists theorize that the earliest mangrove species originated in the Indo-Malayan region, where there are far more mangrove species than anywhere else in the world. Their unique floating propagules and seeds enable these early mangrove species to spread westward, borne by ocean currents, to India and East Africa, and eastward to the Americas, arriving in Central and South America between 66 million years ago (upper Cretaceous period) and 23 million years ago (lower Miocene epoch) (Ellison *et al.,* 1999).

Study of the origins of mangroves support the vicariance hypothesis based on five evidence tested which are the 1) mangrove fossil record, 2) by comparing eight genera of gastropods with high fidelity to mangrove ecosystem, 3) by describing species-area relationship with respect to area of available habitat, 4) analyzing patterns of nestedness of individual plant and gastropod communities and 5) by the nestedness of individual plant and gastropod species (Ellison *et al.,* 1999).

There is general agreement that modern genera arose on the eastern shores of the Tethys Sea, diversified in what is now the Indo West Pacific (IWP) and dispersed eastward across the Pacific Ocean. Mangrove occurred primarily in Southeast Asia/Malaysia by restricting most mangrove taxa to the IWP because of poor dispersal abilities and closure to the Tethys connection to the Atlantic by the Mid-Tertiary (Aubréville, 1964; Chapman, 1976; Tomlinson, 1986; Ricklefs and Latham, 1993).

Southeast Asia was once a huge landmass known as the ancient continent of Sundaland. Ancient continent of Sundaland was broken up into the present day archipelago by rising sea levels. The rising sea level at the end of Ice Age 15,000-7000 years ago were the main force shaping the modern diversity in the region. Around half of land area of Sundaland continent was lost to the sea between 15,000-7000 years ago with a concomitant doubling of the length of coastline as the resulting archipelago was formed (Bird *et al.,*2005; Sathiamurthy and Voris, 2006).

This event suggests vicariance biogeography is taken into place in the distribution of mangroves and also in building a new set of human populations and its placement in the Island South East Asia (ISEA). It had an important role in shaping subsequent life in the region. The larger coastal lines exhibit genetic drift of mangroves and eventually settled in the new found area. So do marine fungi evolved and disperse worldwide with their hosts?

2.7.3 Human-mediated dispersal

Anthropogenic activities might cause dispersal of marine fungi. There are two reasons on why human dispersals are related with the biogeography of marine mangrove fungi. First of all, mangrove forest can only grow on coastal lines and ancient humans are known to live near coastal areas especially those countries that were in Southeast Asia. They are well known as maritime countries. Thus, human dispersals could probably associate with the dispersal of marine mangrove fungi. They might come from the same origin locality. Another possible factor is that human travel on wooden rafts/ boats/ships in the ancient days. They might accidently carried mycelia embedded in the wood or ballast water that contain fungal propagules.

2.7.4 Colonisation and establishment of new habitats

Marine fungi could disperse from one area to another but it has to adapt to the environment of the new area. Factors like host availability or substrates, salinity and temperature are among the important factors that affect the presence of marine fungi in a particular area (Jones, 2000).

2.8 Role of fungi in the ecosystem

The role of marine fungi in the ecosystem has previously been discussed (Kohlmeyer and Kohlmeyer, 1979; Hyde and Lee, 1995; Hyde *et al.,* 1998; Pang *et al.,* 2010). Some higher marine fungi are known as parasite on animals and plants. Nevertheless, marine fungi are major decomposers of woody and herbaceous substrates. It is also important in the degradation of dead animals. Besides the saprophytic manners, marine fungi also may form symbiotic relationship with other organism i.e. lichens (a symbiotic association of fungi and algae) and commensals in digestive tracts of marine animals (Lichtwardt, 1976).

Fungi from coastal and marine ecosystem contributes a significant part of marine biodiversity but there are no in depth study yet. Fungi in general are able to degrade a wide range of recalcitrant biological molecules and particularly in coastal ecosystem. Fungal activity also may be critical in the early stages of biodegradative pathways (Hyde and Lee, 1995 and Hyde *et al.,* 1998)z.

Marine fungi also play an important part in biological aspect, agronomic, medical and bio-industry (Sugiyama, 1998). It is also been known that fungi can reduce the concentration of heavy metals in the estuarine area (Babich and Stotzky, 1983). Fungi from marine samples are also useful as microbial resources in the search for new bioactive compounds (Liberra and Lindequist, 1995; Masuma *et al.,* 2001; Lin *et al.,* 2002a, b; Colegate and Molyneux, 2007, Blunt *et al.,* 2008). Organisms that live in extreme environment tend to produce valuable secondary metabolites which are useful as anti-microbial and anti-cancer.

Marine fungi are also known to produce lignin degrading enzymes that can be used to decolourized bleach plant effluent from pulp and paper mills, effluent from textile and dye making industries and molasses spent wash from alcohol distilleries (Pointing *et al.* 1998, Raghukumar *et al.* 1999, Raghukumar 2002, 2008). Raghukumar

(2002) has discussed some of the problems related to bioremediation of water-borne pollutants and dyes by fungi. There has been no commercial application although various fungi have been shown the ability to decolorize a wide range of dyes and effluents. However, the rate of the reactions is low for industrial scale. Still, marine fungi have a great potential use in bioremediation especially in cases of oil spills in coastal waters (Sadaba and Sarinas, 2010; Jones, 2011a).

In Malaysia, previous studies on marine fungi gives highlight more on the ecology, taxonomy, biodiversity and ultrastructure study (Alias *et al.*, 1995; Jones, 1995; Alias, 2007; Jones and Alias, 2007; Zainuddin *et al.*, 2007). There are no records of studies on the molecular phylogeny aspects of marine fungi in Malaysia.

2.9 Approach in studying marine fungi

Samples collected need to be examined immediately upon arrival to the laboratory (Jones, 1971). 70% of the samples produced fruiting bodies after 6 months of incubation (Prasannarai and Sridhar, 1997). This must be taken into account when examining driftwood (Jones, 2000). The present enumeration studies on marine fungi were mainly focus on those that sporulate on incubated substratum which subsequently underestimate the real diversity because fungi may present only as mycelium and may not be able to sporulate with the presence of other fungi. Molecular techniques need to be use for detection, identification and enumeration for such situation (Liew *et al.*, 1998; Jones, 2000).

Pang and Mitchell (2005) describe molecular approaches suitable for assessing fungal diversity in marine substrata. A range of molecular techniques are now available to study the fungal diversity in natural environments that are not restricted to the culturable species only. Baseline data need to be established in order to build a model of fungal community dynamics that covers species redundancy, species spatial and temporal distribution, and nutrient cycling.

Phylogeny and taxonomy of marine fungi are a complex studies where extensive research available now can be divided into three major studies: 1. Resolution in the delineation of species and genera 2. Higher order taxonomic placement of marine fungi and application to ecological studies 3. To identify sterile endophyte/cryptic species cultures and unculturable isolates (Jones, 2011a)

As for Malaysia, to date no studies on molecular phylogeny has been done on any marine fungal strains from Malaysia. This would be a pioneer studies that would characterize the strains that we have here.

A study will be incomplete without statistical analysis. Many mycologists have incorporated statistical analysis in order to get an overview of species abundance patterns (Prasannarai and Sridhar, 2001; Maria and Sridhar, 2002; Fryar *et al.,* 2004; Figuera and Barata, 2007; Pang *et al.,* 2010). Shannon-Weiner diversity index (H') and evenness was calculated for each site along with Margalef species richness. Calculations were carried out according to Magurran (1988). For multivariate analysis, Detrended Correspondence Analysis (DCA) was used to detect differences in species abundance patterns between sites. Basic data that is needed for univariate and bivariate statistical analysis are: No. of species (S), No. of individuals (N), No. species per branch, No. branches with no fungi, No. of species of Ascomycota/ Basidiomycota/ Anamorphic fungi.