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Occurrence of Marine Fungi from the Straits of Malacca, Malaysia, with First Record of *Mauritiana rhizhoporae*

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Abstract The inventory of marine fungi along the Straits of Malacca was carried out in July, 2004 and December, 2007. Three locations were investigated for their marine fungal diversity: Lalang Island, Jarak Island and Rumbia Island. Samples collected from these islands were submerged wood and driftwood entrapped in between rocks, embedded in sand and washed ashore along the coastal areas. A total of 51 species of marine fungi were recorded from 274 samples collected and their frequency of occurrence is discussed. *Mauritiana rhizophorae* is reported as the first record in Malaysia.

Abstrak Inventori kulat marin di sepanjang Selat Melaka telah dijalankan pada Julai, 2004 dan Disember, 2007. Tiga lokasi telah dijalankan bagi kajian kepelbagaian kulat laut: Pulau Lalang, Pulau Jarak dan Pulau Rumbia. Sampel-sampel yang dikutip daripada pulau-pulau ini terdiri daripada kayu-kayu yang terendam dan kayu terampai yang terperangkap di antara batu-batu besar, terbenam di dalam pasir dan yang terampai di sepanjang pesisiran pantai. Sejumlah 51 spesis kulat laut telah direkodkan daripada 274 sampel-sampel yang dikutip dan kekerapan kehadirannya dibincangkan.

(marine fungi, Straits of Malacca, frequency of occurrence, diversity, driftwood)

INTRODUCTION

The Straits of Malacca is a well-known trade route that links the Indian and Pacific Oceans. It is the shortest sea route between three of the world's most populous countries, India, China and Indonesia. It contains many narrow channels, shallow reefs and thousands of tiny islands. These tiny islands are beautiful and serene but most are unknown due to their distant location from the mainland. Many tiny islands in the Straits of Malacca are yet to be explored for their richness and diversity of terrestrial and marine flora and fauna. Therefore, the expedition carried out by the group from the University of Malaya in 2004 and 2007 has become the crucial moment to document and inventorize the flora and fauna of these islands in Malaysia.

Until recently, the marine flora and fauna of Lalang and Rumbia Islands in the Straits of Malacca had never received interest from scientists in Malaysia and no records of their biological resources had been documented so far. However, the fauna and flora of Jarak Island had been studied before by foreign scientists. Audy [1] found two mammal, several bird and four reptile species, while Wyatt-Smith [2] recorded 109 species of flora including ferns, herbs, climbers and palms. However, the fungi of this island are yet to be reported.

To date, the collection data of marine fungi in Malaysia were recorded by Jones and Tan [3], Jones and Kuthubutheen [4], Tan and Leong [5], Alias et al. [6] and Zainuddin and Alias [7]. The marine fungi reported previously are mostly those from mangrove trees. In the present study, samples collected were mainly driftwood and mangrove-associated trees. Some species found on the collected driftwood showed similarity with the mangrove-derived species. Therefore, our survey to the Straits of Malacca will contribute new data of marine fungi from remote locations in Malaysian waters, which will further enhance research interest on marine fungi in Malaysia.

MATERIALS AND METHODS

Lignicolous marine fungi were randomly collected from submerged wood and decaying driftwood at three locations in the Straits of Malacca: 1) Lalang Island (4.009N, 100.548E). The island has a short white sandy beach. The other parts of the island is

Table 1. Marine fungi collected on submerged and driftwood at Straits of Malacca

Collection site	Lalang Island		Jarak Island		Rumbia Island		Total		Total
	No	%	No	%	No	%	No	%	
Ascomycota (48)									
Aigialus grandis Kohlm. & Schatz	-	-	-	-	2	2.5	2	1.0	1
Aigialus parvus Schatz & Kohlm.	1	2.0	-	-	-	-	1	0.5	1
Aniptodera mangrovei K. D. Hyde	-	-	-	-	2	2.5	2	1.0	1
Arenariomyces trifurcatus Höhnk	4	8.0	10	14.5	3	3.7	17	8.5	3
Acrocordiopsis patilii Borse & K. D. Hyde	1	2.0	-	-	-	-	1	0.5	1
Ascocratera manglicola Kohlm.	7	14.0	-	-	3	3.7	10	5.0	2
Corollospora colossa Nakagiri & Tokura	-	-	2	2.9	-	-	2	1.0	1
Cryptovalsa mangrovei Abdel-Wahab et Inderb.	-	-	-	-	1	1.2	1	0.5	1
Cryptosphaeria mangrovei K.D. Hyde	-	-	3	4.3	-	-	3	1.5	1
Dactylospora haliotrepha (Kohlm. et E. Kohlm.) Hafellner	-	-	-	-	1	1.2	1	0.5	1
Eutypella naqsii K. D. Hyde	-	-	1	1.4	1	1.2	2	1.0	2
Eutypa sp.	-	-	-	-	4	4.9	4	2.0	1
Hypoxylon sp. 1	1	2.0	-	-	4	4.9	5	2.5	2
Halorosellinia oceanicum (S. Schatz) Whalley, E. B. G. Jones, K. D. Hyde & Laessoe	1	2.0	4	5.8	4	4.9	9	4.5	3
Halosarpheia fibrosa Kohlm. & E. Kohlm	-	-	3	4.3	-	-	3	1.5	1
Kallichroma glabrum (Kohlm.) Kohlm. et Volkm.-Kohlm.	-	-	-	-	1	1.2	1	0.5	1
Leptosphaeria australiensis (Cribb et J. W. Cribb) G. C. Hughes	-	-	9	13.0	4	4.9	13	6.5	2
Lignincola leaves Höhnk	1	2.0	-	-	2	2.5	3	1.5	2
Lophiostoma sp.	1	2.0	1	1.4	-	-	2	1.0	2
Lulworthia grandispora Meyers	-	-	4	5.8	-	-	4	2.0	1
Lulworthia sp.	-	-	3	4.3	2	2.5	5	2.5	2
Marinosphaera mangrovei K. D. Hyde	-	-	-	-	1	1.2	1	0.5	1
Massarina thalassiae Kohlm. et Volkm.-Kohlm.	1	2.0	-	-	-	-	1	0.5	1
Massarina sp.	2	4.0	2	2.9	-	-	4	2.0	2
Mauritiana rhizophorae Poonyth, K. D. Hyde, Aptroot & Peerally	-	-	-	-	1	1.2	1	0.5	1
Natantispora retorquens (Shearer & J. L. Crane) J. Campb., J. L. Anderson & Shearer	1	2.0	7	10.1	1	1.2	9	4.5	3
Phaeosphaeria sp.	-	-	-	-	2	2.5	2	1.0	1
Phoma sp.	-	-	-	-	5	6.2	5	2.5	1
Quintaria lignatilis (Kohlm.) Kohlm. & Volkm.-Kohlm.	-	-	-	5	7.2	-	-		5

Collection site	Lalang Island		Jarak Island		Rumbia Island		Total		Total
	No	%	No	%	No	%	No	%	
Rhizophila marina K.D. Hyde & E. B. G. Jones	2	4.0	-	-	1	1.2	3	1.5	2
Sagaromyces ratnagiriensis (S. D. Patil & Borse) K. L. Pang & E. B. G. Jones	-	-	-	-	1	1.2	1	0.5	1
Saccardoella mangrovei K. D. Hyde	1	2.0	-	-	-	-	1	0.5	1
Savoryella lignincola E. B. G. Jones & R. A. Eaton	1	2.0	1	1.4	5	6.2	7	3.5	3
Savoryella longispora E. B. G. Jones & K. D. Hyde	-	-	-	-	2	2.5	2	1.0	1
Salsuginea ramicola K. D. Hyde	-	-	-	-	2	2.5	2		
Torpedospora radiata Meyers	2	4.0	3	4.3	-	-	5	2.5	2
Verruculina enalia (Kohlm.) Kohlm. & Volkm.-Kohlm.	7	14.0	-	-	9	11.1	16	8.0	2
Xylaria sp.	6	12.0	-	-	-	-	6	3.0	1
Aigialus sp.	-	-	-	-	2	2.5	2	1.0	1
Ascomycete LI sp. 1	2	4.0	-	-	-	-	2	1.0	1
Ascomycete LI sp. 3	1	2.0	-	-	-	-	1	0.5	1
Ascomycete LI sp. 5	1	2.0	4	5.8	4	4.9	9	4.5	3
Ascomycete LI sp. 9	4	18.2	3	4.3	-	-	7	3.5	2
Ascomycete LI sp. 10	1	2.0	-	-	-	-	1	0.5	1
Ascomycete LI sp. 11	1	2.0	-	-	-	-	1	0.5	1
Ascomycete sp. 21	-	-	1	1.4	6	7.4	7	3.5	2
Ascomycete sp. 22	-	-	-	-	1	1.2	1	0.5	1
Ascomycete sp. 23	-	-	-	-	1	1.2	1	0.5	1
Anamorphic fungi (2)									
Trichocladium achrasporum (Meyers & R. T. Moore) M. Dixon et Shearer & J. L. Crane	-	-	2	2.9	1	1.2	3	1.5	2
Trichocladium alopallonellum (Meyers & R. T. Moore)	-	-	1	1.4	1	1.2	2	1.0	2
Basidiomycota (1)									
Halocyphina villosa Kohlm. & Kohlm.	-	-	-	-	1	1.2	1	0.5	1
Number of fungal collections	50		69		81		200		
Number of samples examined	72		107		95		274		
Number of fungi per sample	0.69		0.64		0.85		0.73		
Total number of fungi	23		20		33		51		

No: number of collections; %: frequency of occurrence

dense with vegetation and rocky shores. Collection samples were limited because of the strong waves and tides that occurred on the other site of the island. Samples were collected from driftwood entrapped in sand and washed ashore; 2) Jarak Island (3.972N, 100.097E). The site was covered mostly by big boulders, intertidal rocks and vegetation. Samples were collected from submerged decaying wood and entrapped wood amongst boulders and rocks; 3) Rumbia Island (4.023N, 100.556E). One side of the island is sandy beach, while the other side was covered by rocks and dense vegetation. The whole island was not thoroughly investigated because of the difficulty to land on the island due to the strong current and waves. Sand-buried wood and washed ashore and submerged wood of trees were collected.

Samples were then incubated in sterile containers lined with moist tissue paper at room temperature. The material was examined after an incubation period of at least 2 days. Samples were kept moist by adding sterile sea water using fine aerosol. Slides of the fungi identified were prepared and are kept in the Institute of Biological Sciences, University of Malaya. Identification of marine fungi followed descriptions by Alias et al. [6] and Kohlmeyer and Volkmann-Kohlmeyer [8].

RESULTS

Marine fungi identified from Lalang Island, Jarak Island and Rumbia Island in the Straits of Malacca are listed in Table 1 with their frequency of occurrence. Two hundred and seventy four samples were examined yielding 51 species of marine fungi, including 48 ascomycetes, 2 anamorphic fungi and 1 Basidiomycetes. Marine fungi were divided into 3 groups based on their percentage of occurrence: most dominant (above 8%), frequent (4-8%) and infrequent (below 4%). *Arenariomyces trifurcatus* (8.5%) and *Verruculina enalia* (8.0%) were classified as dominant fungi in the Straits of Malacca.

1) *Lalang Island*: A total of 50 fungal collections were found from 72 samples examined. Twenty-three species (100% ascomycetes) were recorded with 85.7% sporulating fungi. Ascomycetes *Verruculina enalia* (14.0%) and *Ascocratera manglicola* (14.0%) were the most frequent fungi encountered. Most of the fungi identified were recorded/observed in at least one collection (Table 1).

2) *Jarak Island*: Twenty species (18 ascomycetes and 2 anarmorphic fungi) were recorded from 37 samples examined from 53 fungal collections. *Leptosphaeria australiensis, Natantispora retorquens* and *Arenariomyces trifurcatus* were identified as the most dominant fungi. Frequent and infrequent fungi were 14 species (4-8%) and 8 species (less than 4%), respectively (Table 1).

3) *Rumbia Island*: The total samples collected from this study influenced the highest recorded diversity of fungi from this island. Thirty-three fungal species (30 ascomycetes, 2 anarmorphic, 1 Deuteromycetes) were identified from 95 samples examined from 36 fungal collections. The percentage colonization of fungi on substrates was 85%. *Verruculina enalia* (11.1%) was the most dominant fungus. Eight fungal species (4-7.5%) were frequent while 24 were classified as infrequent (less than 4%) (Table 1).

Table 2. Margalef Diversity Index of marine fungi from Jarak, Lalang and Rumbia Islands.

Location	Margalef Diversity Index
Jarak Island	4.49
Lalang Island	5.62
Rumbia Island	7.82

Table 3. Sorenson Similarity Index of marine fungi between Jarak-Lalang, Jarak-Rumbia and Lalang-Rumbia Islands.

Locations		Sorenson Similarity Index
Jarak Island	Lalang Island	0.419
Jarak Island	Rumbia Island	0.415
Lalang Island	Rumbia Island	0.357

The Margalef Diversity Index was calculated for the three islands (Table 2). The highest diversity index was shown by Rumbia Island at 7.82. This was followed by Lalang Island and Jarak Island at 5.62 and 4.49, respectively.

The three islands harboured different species composition. Sorenson Similarity Index (Table 3) indicates that similarity of species compositions was low: Jarak Island – Lalang Island (0.419); Jarak Island – Rumbia Island (0.415); Lalang Island– Rumbia Island (0.357).

DISCUSSION

There are approximately 900 species of marine fungi that can be found worldwide [9]. Substrate-inhabiting fungi are not restricted to one substrate but a variety of substrates from decaying mangrove wood, driftwood, sand, salt marsh, other marine macroorganisms, algae, coral reefs and the water column [8, 10]. Although the substrates occupied by marine fungi are varied, mangrove areas are the main habitats for most scientists to record the diversity of marine fungi.

The study of marine fungi collected from mangrove areas became the major research topic in tropical countries since the 1950s. Many studies on marine mangrove fungi in tropical countries were recorded in Malaysia [3, 4, 5, 6], Brunei [11, 12], Singapore [13], Sumatera [14], Thailand [15, 16], Seychelles [17], Andaman and Nicobar Islands [19], Maldives [18] and Maharashtra [20]. To date, the number of marine fungi from mangrove areas has risen to 625 species [21]. This is more than half of the total marine fungi estimated by Jones [8]. However, in the present study, we highlighted the species of marine fungi collected on driftwood from non-mangrove areas. The definition of driftwood given by Hyde and Sarma [10] is the wood which is submerged or drifted on the sea surface or washed ashore and not from mangrove habitats. Marine fungi found on these substrates can be identified from variations in their ascospores and size using light microscopy, and the best way to obtain marine fungi is by searching for fruiting bodies or conidia directly on substrates collected from the marine environment.

In the present study, 51 species of marine fungi were recorded from 200 samples collected from these three Malaysian islands. In comparison, the number of marine fungi recorded from submerged driftwood excluding mangrove substrates in other regions are as follows: (1) Temperate areas - San Juan Island, 62 species [22]; Grönhoj, 47 species [23]; and Italy, 58 species [24]; (2) Tropical areas - West Coast of India, 88 species [25]; The South China Sea, 10 species [26]; Brunei, 46 species [11]; and Seychelles, 63 species [17]; (3) Subtropical ares - Hainan Island, 27 species [27]; and Hong Kong, 85 species [28]. The location of sampling site from different regions, type of substrates, and the number of samples collected are among the factors that determine total number of fungi from different locations in the marine environment.

Marine fungi are well distributed geographically, ranging from temperate to subtropical to tropical countries. Fungi in subtropical and tropical regions show the occurrence of similar fungi, e.g. *Antennospora quadricornuta*, *Halosphaeria salina* and *Periconia prolifica*. These fungi are also frequently collected in tropical/subtropical regions [29]. However, other studies in tropical/subtropical regions recorded *Aigialus grandis*, *Dactylospora haliotrepha*, *Halocyphina villosa* and *Verruculina enalia* as the most dominant and common fungi [17, 20, 30, 31, 32, 33, 34].

In the present study, the dominant species are different at each location. In Lalang Island, the dominant species recorded were *Verruculina enalia* and *Ascocratera manglicola*. However, in Jarak Island *Arenariomyces trifurcatus* was dominant whereas *Verruculina enalia* was the dominant species in Rumbia Island. Two fungi *Verruculina enalia* and *Arenariomyces trifurcatus* were identified as abundant in the Straits of Malacca. However, a few studies in Malaysia (Alias et al. in press), Bahamas [31], Brunei [11, 12], East Coast of India [35, 36] and Hainan Island [27] reported that *Leptosphaeria australiensis* is the most common fungi encountered in the marine ecosystem. In this study, *L. australiensis* was less abundant than *V. enalia* and *A. trifurcatus* but is still considered one of the most abundant in the Straits of Malacca. In previous studies also, *L. australiensis* was found to be common in certain locations [16, 37, 38, 39, 40, 41].

The present study reports a new record of marine fungi, namely *Mauritiana rhizophorae* in

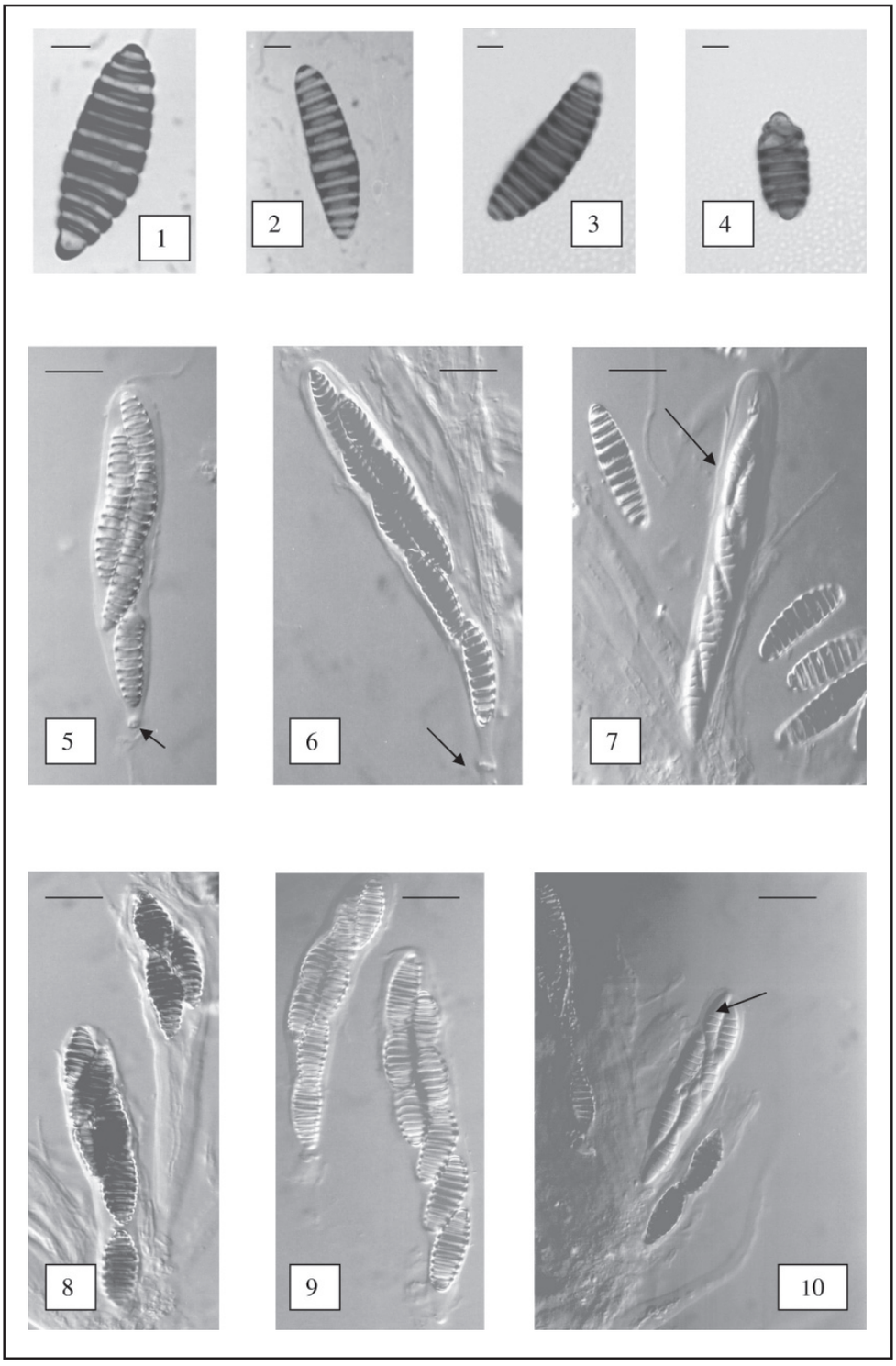

Figure 1-10. *Mauritiana rhizophorae*. Light micrographs. Figs 1-4. Mature ascospores with 6-11 distoseptate, dark brown pigmentation at the septum. Figs 7 and 10. Immature ascospores in ascus (arrowed). The septate is not developing well and smaller than the mature ascospore. Figs 5, 6, 8 and 9. Mature asci with short peduncullate (arrowed) contain 6-8 ascospores. Scale bars: 1-4 = 10µm; 5-10 = 24µm.

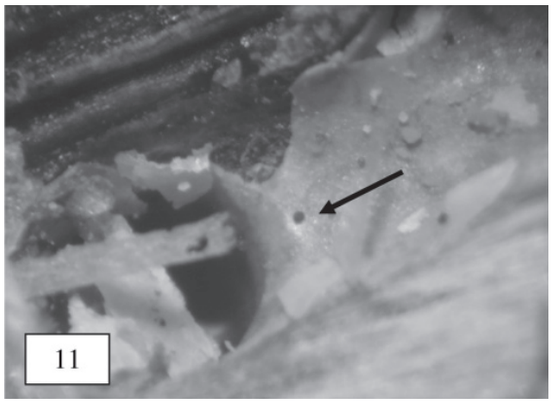

Figure 11. An empty ascomata half submerged in a shell of *Teredo navalis* (arrowed).

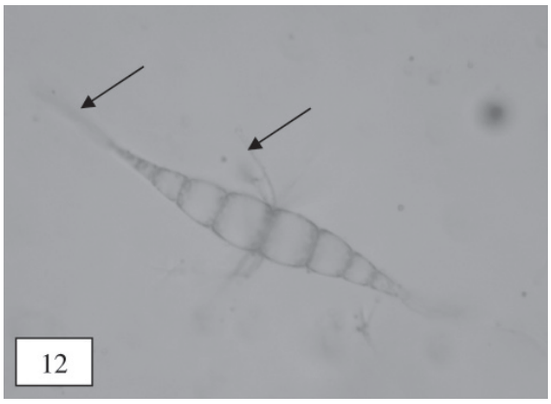

Figure 12. The ascospore of *Corollospora colossa* with polar and equatorial appendages (arrowed).

Malaysian waters (Figures 1-10). *Mauritiana* was first described by Poonyth *et al.* [42], a species with bitunicate asci. *Mauritiana rhizophorae* was previously collected from decaying mangrove wood of *Rhizophora mucronata* Lam. in Mauritius in 2000 based on light microscopy. In the present study, this fungi species was found on different substrates and from non-mangrove areas. *Corollospora collossa* was the only arenicolous fungus recorded in the present study. The fruiting body was found on the shell of *Teredo navalis* (shipworm) which was embedded in the wood (Figures 11-12).

Only four species (*Arenariomyces trifurcatus*, *Halorosellinia oceanica*, *Natantispora retorquens* and *Savoryella lignincola*) showed wide distribution in the Straits of Malacca, while the remaining species were only recorded from either one or two locations. Fungi identified in the present study were collected from at least two locations (20 species) and 31 species were collected from single collections. For example, *Aigialus parvus* and *Acrocardiopsis patilii* were only found from a single collection at one location only. The low collection of samples and a relatively short incubation period (up to 2 months only) could affect the number and species composition of marine fungi recorded in the present study. Certain species may produce fruiting bodies after a longer period of incubation in the laboratory. Other factors which may affect the occurrence of fungi include salinity, position in the intertidal region, pH and whether the substratum is driftwood, roots or branches [43].

Based on the Margalef Diversity Index, the differences in the diversity of fungi recorded could be due to the different sites of sampling and the total number of samples collected. The total number of samples collected from Jarak Island was 107, Lalang Island, 72, and Rumbia Island, 95. Collection of samples was influenced by geographical factors where only a few areas were accessible by boat in search of the samples. All three islands were predominantly covered by rocky shores, and Lalang Island was the only one with a relatively long stretch of sandy beach. Harsh and strong waves made it difficult to access the rocky shores and woody materials were mostly found between boulders near the shoreline.

Hyde and Sarma [10] and Kohlmeyer and Kohlmeyer [9] listed three major groups of fungi in the marine ecosystem: Ascomycota, Basidiomycota and Deuteromycota. In the present study, Ascomycota (93%) represents the most dominant fungi occurring in the Straits of Malacca, followed by Deuteromycota and Basidiomycota. This concurs with previous studies in tropical and subtropical countries [6, 11, 12, 20, 26, 30, 31, 32, 33, 37, 38, 43]. A study conducted by Zainuddin and Alias [7] in Langkawi Island also reported Ascomycota (86.5%) as the most common fungi collected from submerged driftwood along the coastal areas. However, the diversity of marine fungi found in Langkawi Island was lower (29 species) compared to that in the present study (43 species). This difference could be attributed to the total number of samples examined: Langkawi Island (85 samples collected), whereas the present study (105 samples

collected). The factors affecting the presence of marine fungi could be salinity and temperature [44], although this was not determined in the present study. The type of wood also plays an important role in determining the colonization of fungi in marine environment [45].

The majority of marine fungi recorded in Malaysia are from mangrove areas, particularly lignicolous marine fungi. The data collected from other substrates other than wood e.g. sand, leaf, seagrass and coral could provide more information on the the diversity and biogeography of the mycota in Malaysia. To gain more information on diversity of marine fungi, frequent samplings are required from time to time to determine their seasonal pattern of distribution. In the present study, many unidentified species were recorded and this could be new to science. Further collections of the specimens will undoubtedly confirm that their origins are from the marine milieu.

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