



117°27'31.98"), Mandidarah Island (N 6°55'17.75", E 117°19'40.29") and Malawali Island (N 7°3'10.39", E 117°18'10.43"). Jambongan Island is the largest island of all three islands and has the widest mangrove area. Mangroves can be seen along the beach all around Jambongan Island and a large zonation of mangrove can be seen covering the bay from the west to the north of the island. Mandidarah Island is the second largest island,

covering an area of 2.5 km<sup>2</sup> with formation of sandy and rocky shores and with patches of mangrove stand. Malawali Island is the smallest island with rocky shores constructed mainly from ultrabasic rocks [28]. As silt tends to accumulate between rocks, mangroves are more noticeable on rocky shores. Small patches of mangrove were available and this island is surrounded by coral reef.



**Figure 1.** Map of the study sites.

*Collection of samples and identification of fungi*

Due to constraints on time and accessibility to some areas of the sites, the sampling strategy was mostly opportunistic. Decaying mangrove driftwood, prop roots, pneumatophores, subterranean roots, rhizomes, overhanging branches and twigs of mangrove trees were randomly collected at the three islands. Samples were then placed in polythene

Ziploc bags. Samples were incubated in a damp chamber to prevent dehydration and examined periodically over a period of 3 months (Figure 2). Slides were prepared for identification on a differential interference contrast (DIC) microscope to record characteristics of the fungi, including morphology of asci, ascospores and ascospore appendages (Figure 3). Fungi were identified using the identification key by Kohlmeyer and Volkmann-

Kohlmeyer [29] and Sarma and Hyde [30]. Axenic cultures from these isolates were then deposited into the culture collection at the Environmental Microbiology Laboratory at the Institute of Ocean and Earth Sciences, University of Malaya.

*Statistical analysis*

Data of the samples collected were analyzed using univariate and multivariate methods:

Univariate methods:

- i) Percentage of species occurrence  
(Total collection of each species) / (Total number of samples examined) × 100
- ii) Percentage of colonization  
(Number of samples colonized by fungi) / (Number of samples examined) × 100
- iii) Average number of fungi per sample  
(Total number of fungi occurrence) / (Total number of samples examined) × 100
- iv) Sorenson's Similarity Index

$$S = \frac{2J}{(A+B)}$$

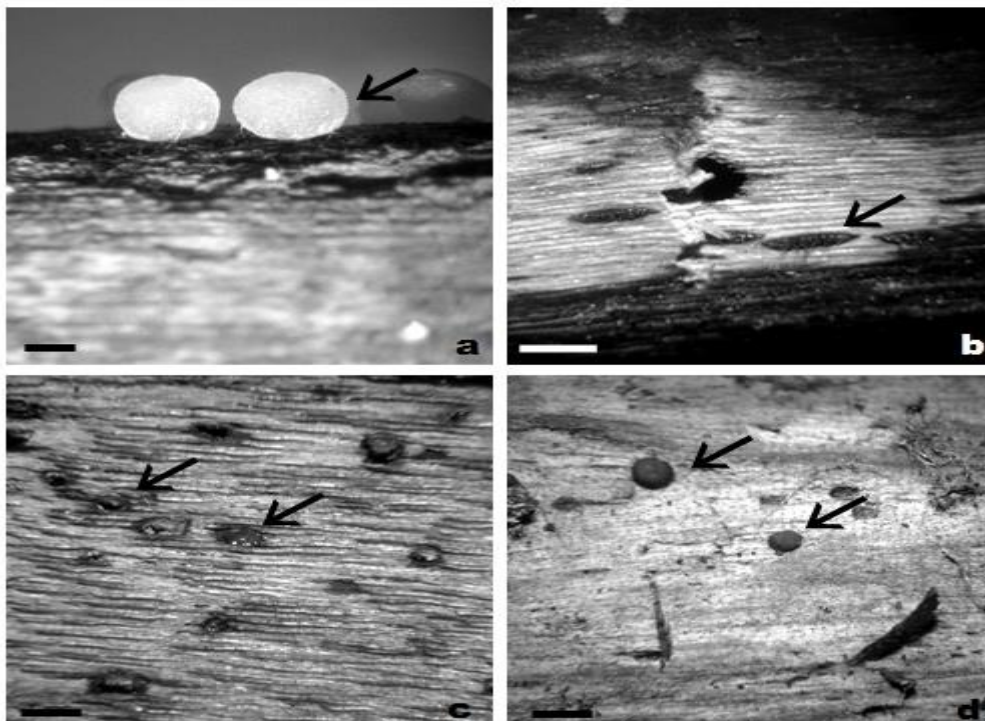
where,

- S = Associated comparison between zone A and zone B
- J = Number of similar species that found in zone A and zone B
- A = Number of species found in zone A
- B = Number of species found in zone B
- v) Shannon Weiner Diversity Index,  $H' = -\sum(pi)(\ln(pi))$   
 $pi$  = proportion of individuals belonging to the  $i$ th of S species
- vi) Evenness,  $E = H'/\ln(S)$ , range from 0 (not even) to 1 (completely even)
- vii) Species richness ( $S'$ )

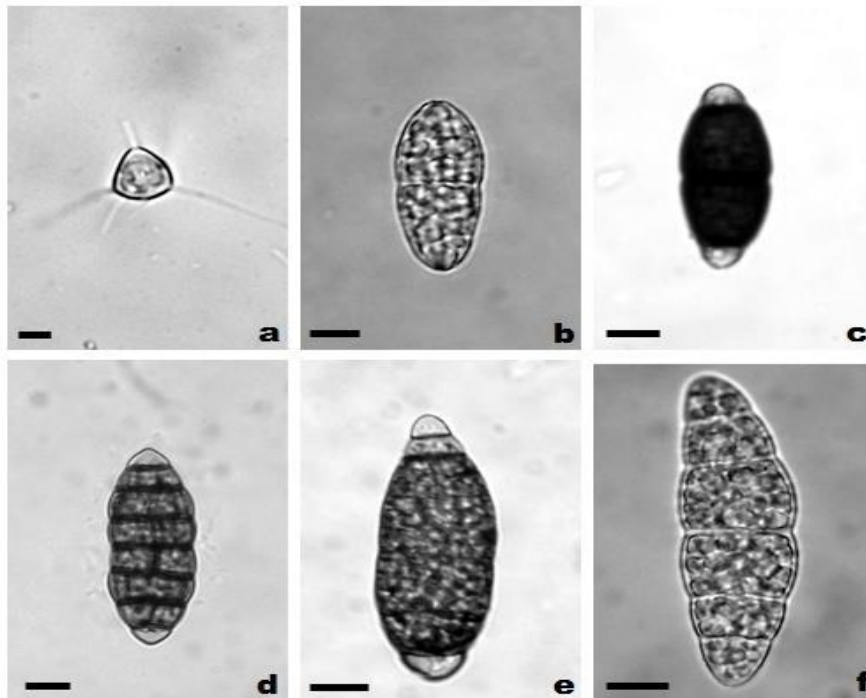
Multivariate method:

Principal Component Analysis (PCA)

PCA was performed to examine and visualize the distribution of marine fungal species on different sites using the software CANOCO 3.1 [31].



**Figure 2.** The presence of fruiting body of marine fungi on decaying mangrove wood. (a) *Nia vibrissa*. (b) *Aigialus mangrovei* (arrowed). (c) *Quintaria lignatilis* (arrowed). (d) *Dactylospora haliotrepha* (arrowed). Scale bars: a = 2mm; b-d = 0.5mm.



**Figure 3:** Light micrographs of marine manglicolous fungi in East Malaysia. (a) *Nia vibrissa*. (b) *Kallischroma tethys*. (c) *Savoryella lignicola*. (d) *Aigialus mangrovei*. (e) *Aigialus parvus*. (f) *Quintaria lignatilis*. Scale bars: a-c = 5µm; d-f = 10µm.

### RESULTS

The marine manglicolous fungi species found at Jambongan, Mandidah and Malawali Islands of Sabah are listed in Table 1. A total of 173 collected samples yielded 78 taxa of marine fungi representing 60 ascomycetes, 17 anamorphic fungi and one basidiomycete. 39 taxa of marine fungi were recorded for Jambongan Island, followed by 34 taxa from Malawali Island and 33 taxa documented from Mandidah Island. The highest percentage of colonization was recorded at Malawali Island (91%), followed by Jambongan Island (86.2%) and Mandidah Island (85%). The average number of fungi per sample at Jambongan Island, Mandidah Island and Malawali Island was 1.71, 0.67 and 1.47, respectively.

Based on Shannon-Weiner Diversity Index (Table 1), highest diversity was recorded at Jambongan Island (3.15) followed by Malawali Island (2.95) and Mandidah Island (2.87). Evenness between species from all the study sites was low: Jambongan Island (0.85), Mandidah Island (0.84) and Malawali Island (0.79).

Species were categorized as very frequent (>10%), frequent (5-10%) and less frequent (<5%) (Table 2).

Very frequent species recorded in Jambongan Island were *Aniptodera* sp. (27.6%), *Sphaerulina orae-maris* and (24.1%), *Rhizophila marina* (17.2%), whereas *Trichocladium alopallonellum* (33.3%), *Pleospora* sp. 1 (13.3%) and *Sphaerulina orae-maris* and (11.7%) were very frequent species encountered in Mandidah Island. *Savoryella lignicola* (36.4%) and *Halorosellinia oceanica* (20.0%) were very frequent species in Malawali Island.

There are 12 new records of marine fungal species from these three islands and another 29 unidentified species in the list that could be new to science. The 12 newly recorded species for Malaysia are *Bicrouania maritima*, *Camarosporium roumegueri*, *Chaetomastia typhicola*, *Gnomonia salina*, *Heleococcum japonense*, *Leptosphaeria orae-maris*, *Lophiostoma acrostichi*, *L. asiana*, *Oceanitis cincinnatula*, *Phaeosphaerianeomaritima*, *Sphaerulina orae-maris* and *Wettsteinina marina*.

Sorenson's Similarity Index (Table 3), between all pairwise comparisons of the sampling sites was recorded between 0.31 to 0.36. Principal Component Analysis showed that the fungal distribution between study sites were significantly different (Figure 4).

Figure 2. Occurrence of marine fungi in Jambongan Island (JI), Mandararah Island (MDI) and Malawali Island (MI).

SPECIES	SAMPLING SITES							
	JI	%	MDI	%	MI	%	Total	%
<b>Ascomycota (60)</b>								
<i>Acrocordiopsis patilii</i> Borse et. K.D. Hyde					1	1.8	1	0.6
<i>Aigialus mangrovis</i> Borse	2	3.5					2	1.2
<i>Aigialus parvus</i> S. Schatz et Kohlm.			1	1.7			1	0.6
<i>Aniptodera chesapeakeensis</i> Shearer et M.A. Miller	1	1.7					1	0.6
<i>Aniptodera</i> sp.	16	27.6	1	1.7	2	3.6	19	11
<i>Anthostomella</i> sp.					1	1.8	1	0.6
<i>Ascocratera manglicola</i> Kohlm.	1	1.7					1	0.6
<i>Astrosphaeriella asiana</i> (K.D. Hyde) Aptroot et K.D. Hyde					1	1.8	1	0.6
<i>Astrosphaeriella striatospora</i> (K.D. Hyde) K.D. Hyde	1	1.7					1	0.6
Unidentified Ascomycete sp. 1			1	1.7			1	0.6
Unidentified Ascomycete sp. 2			1	1.7			1	0.6
Unidentified Ascomycete sp. 3			1	1.7			1	0.6
Unidentified Ascomycete sp. 4			1	1.7			1	0.6
Unidentified Ascomycete sp. 5	2	3.5					2	1.2
Unidentified Ascomycete sp. 6	1	1.7					1	0.6
Unidentified Ascomycete sp. 7	1	1.7					1	0.6
Unidentified Ascomycete sp. 8					1	1.8	1	0.6
Unidentified Ascomycete sp. 9			1	1.7			1	0.6
Unidentified Ascomycete sp. 10	1	1.7					1	0.6
<i>Bicrouania maritima</i> (P. Crouan et H. Crouan) Kohlm. et Volkman. Kohlm.*			1	1.7			1	0.6
<i>Ceriosporopsis</i> sp.					1	1.8	1	0.6
<i>Chaetomastia typhicola</i> (P. Karst.) M.E. Barr*	1	1.7					1	0.6
<i>Dactylospora haliotrepha</i> (Kohlm. et Kohlm.) Hafellner	5	8.6	4	6.7			9	5.2

Table 1. Continued

SPECIES	SAMPLING SITES									
	JI	%	MDI	%	MI	%	Total	%		
<i>Eutypa bathurstensis</i> K.D. Hyde et Rappaz	1	1.7			4	7.3	5	2.9		
<i>Eutypella naqsii</i> K.D. Hyde					1	1.8	1	0.6		
<i>Gnomonia salina</i> E.B.G. Jones*			1	1.7			1	0.6		
<i>Halorosellinia oceanica</i> (S. Schatz) Whalley et al.	2	3.5	3	5	11	20	16	9.2		
<i>Heleococcum japonense</i> Tubaki*			1	1.7			1	0.6		
<i>Hypoxylon</i> sp.	1	1.7					1	0.6		
<i>Kallichroma glabrum</i> (Kohlm.) Kohlm. et Volk. -Kohlm.	1	1.7					1	0.6		
<i>Kallichroma tethys</i> (Kohlm. et Kohlm.) Kohlm. et Volk. -Kohlm.	3	5.2					3	1.7		
<i>Leptosphaeria australiensis</i> (Cribb et J.W. Cribb) G.C. Hughes	2	3.5	1	1.7	3	5.5	6	3.5		
<i>Leptosphaeria avicenniae</i> Kohlm. et E. Kohlm.	1	1.7					1	0.6		
<i>Leptosphaeria orae-maris</i> Linder*	1	1.7			4	7.3	5	2.9		
<i>Lophiostoma acrostichi</i> (K.D. Hyde) Aptroot & K.D. Hyde*	1	1.7			1	1.8	2	1.2		
<i>Lulworthia</i> sp.1	1	1.7	1	1.7			2	1.2		
<i>Lulworthia</i> sp.2	2	3.5					2	1.2		
<i>Mangrovispora pemphii</i> K.D. Hyde et Nakagiri					1	1.8	1	0.6		
<i>Massarina thalassiae</i> Kohlm. et Volk. -Kohlm.			1	1.7			1	0.6		
<i>Massarina lacertensis</i> Kohlm. et Volk. -Kohlm.	1	1.7					1	0.6		
<i>Morosphaeria ramuncolica</i> (K.D. Hyde) Suetrong et al.			1	1.7			1	0.6		
<i>Morosphaeria velatipora</i> (K.D. Hyde et Borse) Suetrong et al.					1	1.8	1	0.6		
<i>Nais inornata</i> Kohlm.	1	1.7					1	0.6		
<i>Neptunella longirostris</i> (Cribb et J.W. Cribb) K.L. Pang et E.B.G. Jones			1	1.7			1	0.6		
<i>Oceanitis cincinnatula</i> (Shearer et J.L. Crane) J. Dupont et E.B.G. Jones*					1	1.8	1	0.6		
<i>Panorbis viscosus</i> (I. Schmidt) J. Campb. et al.			1	1.7			1	0.6		
<i>Phaeosphaeria neomaritima</i> (R.V. Gessner et Kohlm.) Shoemaker et C.E. Babc.*	2	3.5	1	1.7			3	1.7		
<i>Phaeosphaeria</i> sp.					1	1.8	1	0.6		

Table 1. Continued

SPECIES	SAMPLING SITES									
	JI	%	MDI	%	MI	%	Total	%		
<i>Platyostomum scabridisporum</i> Abdel-Wahab et E.B.G. Jones	1	1.7			1	1.8	2	1.2		
<i>Pleospora</i> sp.1	3	5.2	8	13.3	3	5.5	14	8.1		
<i>Pleospora</i> sp. 2			1	1.7	1	1.8	2	1.2		
<i>Quintaria lignatilis</i> (Kohlm.) Kohlm. et Volk.-Kohlm.	2	3.5					2	1.2		
<i>Rhizophila marina</i> K.D Hyde et E.B.G. Jones.	10	17.2	1	1.7	1	1.8	12	6.9		
<i>Rimora mangrovei</i> (Kohlm. et Vittal) Kohlm. et al.	2	3.5					2	1.2		
<i>Savoryella lignicola</i> E.B.G. Jones et R.A. Eaton			1	1.7	20	36.4	21	12		
<i>Savoryella longispora</i> E.B.G. Jones et K.D. Hyde					1	1.8	1	0.6		
<i>Sphaerulina orae-maritima</i> Linder *	14	24.1	7	11.7	4	7.3	25	14		
<i>Trematosphaeria mangrovis</i> Kohlm.					1	1.8	1	0.6		
<i>Verruculina enalia</i> (Kohlm.) Kohlm. et Volk.-Kohlm.	1	1.7	1	1.7			2	1.2		
<i>Wettsteinina marina</i> (Ellis et Everh.) Shoemaker et C.E. Babc.*	1	1.7					1	0.6		
<b>Anamorphic fungi (17)</b>										
<i>Alternaria</i> sp.			2	3.3			2	1.2		
<i>Bactrodesmium linderi</i> (J.L. Crane et Shearer) M.E. Palm et E.L. Stewart*					1	1.8	1	0.6		
<i>Camarosporium roumegueri</i> Sacc.					1	1.8	1	0.6		
Anamorphic fungi sp. 1	5	8.6			2	3.6	7	4		
Anamorphic fungi sp. 2			1	1.7	1	1.8	2	1.2		
Anamorphic fungi sp. 3			1	1.7			1	0.6		
Anamorphic fungi sp. 4					1	1.8	1	0.6		
Anamorphic fungi sp. 5	1	1.7					1	0.6		
Anamorphic fungi sp. 6	1	1.7					1	0.6		
Anamorphic fungi sp. 7			1	1.7	1	1.8	2	1.2		

Table 1. Continued

SPECIES	SAMPLING SITES									
	J1	%	MDI	%	MI	%	Total	%		
Anamorphic fungi sp. 8			1	1.7			1	0.6		
Anamorphic fungi sp. 9					1	1.8	1	0.6		
<i>Periconia prolifica Anastasiou</i>					1	1.8	1	0.6		
<i>Humicola alopallonella</i> Meyers et R.T. Moore	2	3.5	20	33.3	2	3.6	24	14		
<i>Kohlmeyer medullaris</i> (Kohlm. et al.) Klaubauf et al.			1	1.7			1	0.6		
<i>Trichocladium melhae</i> E.B.G. Jones et al.			3	5	3	5.5	6	3.5		
<i>Sporidesmium salinum</i> E.B.G. Jones*	3	5.2					3	1.7		
<b>Basidiomycota (1)</b>										
<i>Nia vibrissa</i> R.T Moore et Meyers	1	1.7					1	0.6		
Total no. of occurrence	99		40		81		253			
Total no. of species	39		33		34		78			
No. of samples examined	58		60		55		173			
Samples colonized by fungi	50		51		50		151			
Samples with no fruiting body	8		9		5		22			
Empty perithecia	7		5		9		21			
% colonization	86.2		85		91		87.3			
Ave. no. of fungi per sample	1.7069		0.6666		1.4727		1.4624			
Shannon Index	3.15		2.87		2.95					
Evenness	0.85		0.79		0.84					
Species richness	39		33		34					

\*New record species



**Table 2.** Frequent marine manglicolous fungi of the East Malaysian mangroves.

<b>Location</b>	<b>Very Frequent (&gt;10%)</b>	<b>Frequent (5-10%)</b>
Jambongan Island	<i>Sphaerulina orae-marais</i> <i>Aniptodera sp.</i> <i>Rhizophila marina</i>	<i>Kallichroma tethys</i> <i>Pleospora sp. 1</i> <i>Sporidesmium salinum</i> <i>Dactylospora haliotrepha</i> Anamorphic fungus 1
Mandidah Island	<i>Humicola alopallonella</i> <i>Pleospora sp.1</i> <i>Sphaerulina orae-marais</i>	<i>Halorosellinia oceanica</i> <i>Trichocladium melhae</i> <i>Dactylospora haliotrepha</i>
Malawali Island	<i>Haloresellinia oceanica</i> <i>Savoryella lignicola</i>	<i>Leptosphaeria australiensis</i> <i>Pleospora sp.1</i> <i>Trichocladium melhae</i> <i>Eutypa bathurstensis</i> <i>Leptosphaeria orae-marais</i> <i>Sphaerulina orae-marais</i>

**Table 3.** Similarity among the species of marine lignicolous fungi from mangrove between study sites.

<b>Pairs of beaches compared</b>	<b>Number of species from the beaches compared</b>	<b>Common species</b>	<b>Community coefficient of Sorenson</b>
JI-MDI	39 - 33	11	0.29
MDI-MI	33 - 34	12	0.35
MI-JI	34 - 39	12	0.26

(JI: Jambongan Island, MDI: Mandidah Island and MI: Malawali Island)

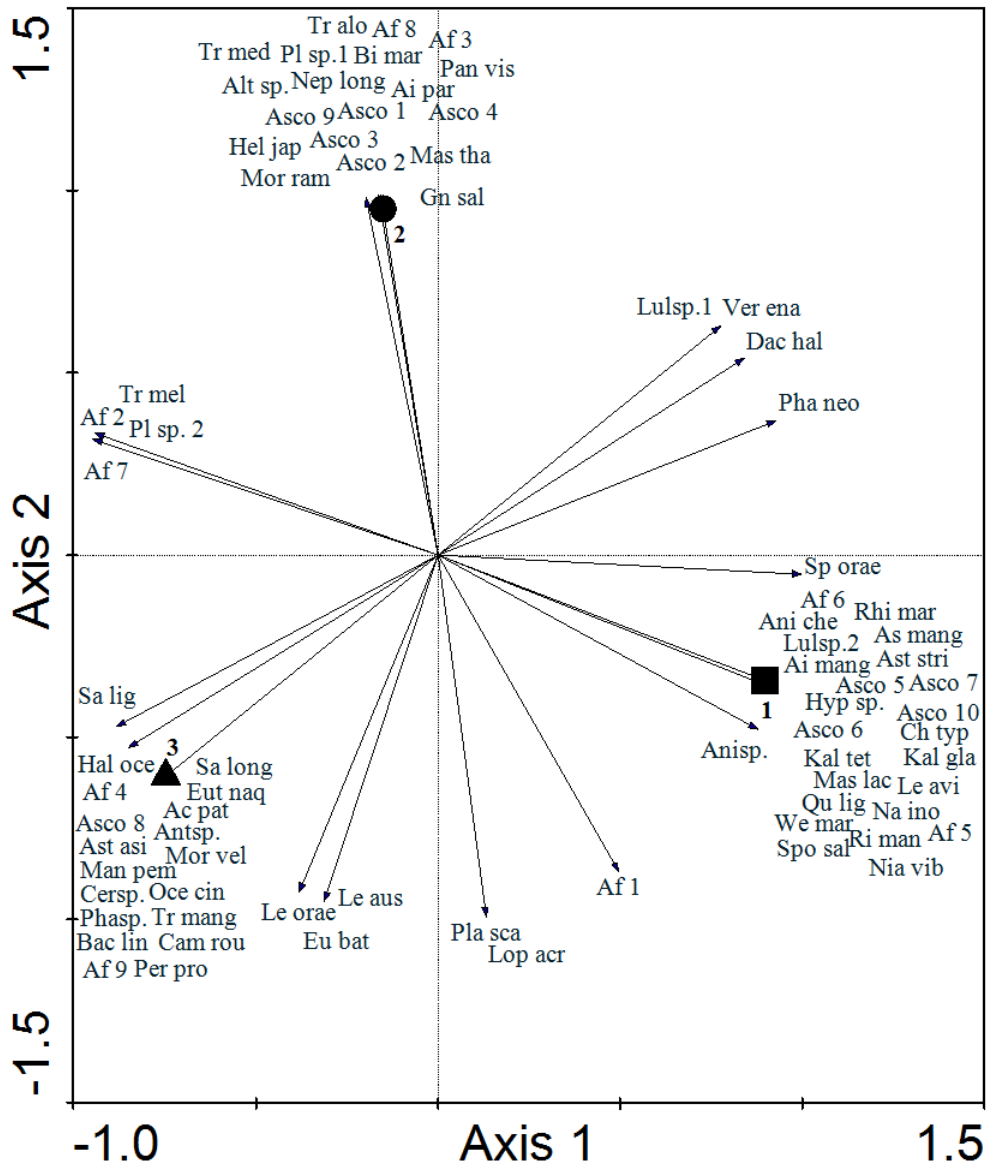
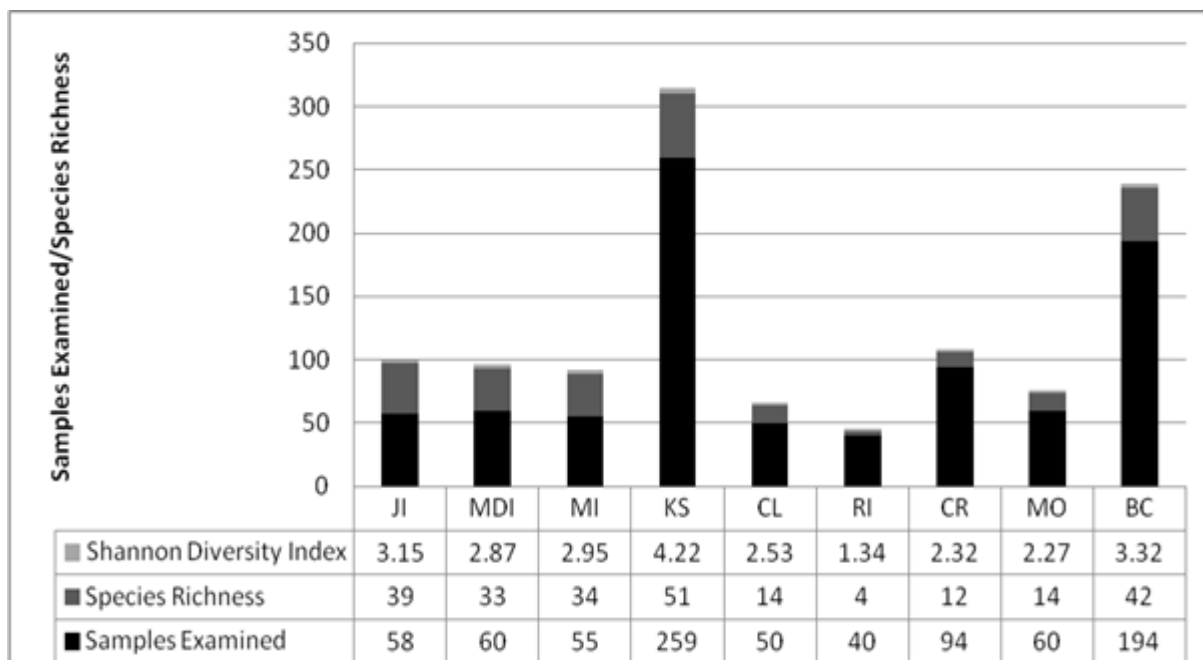
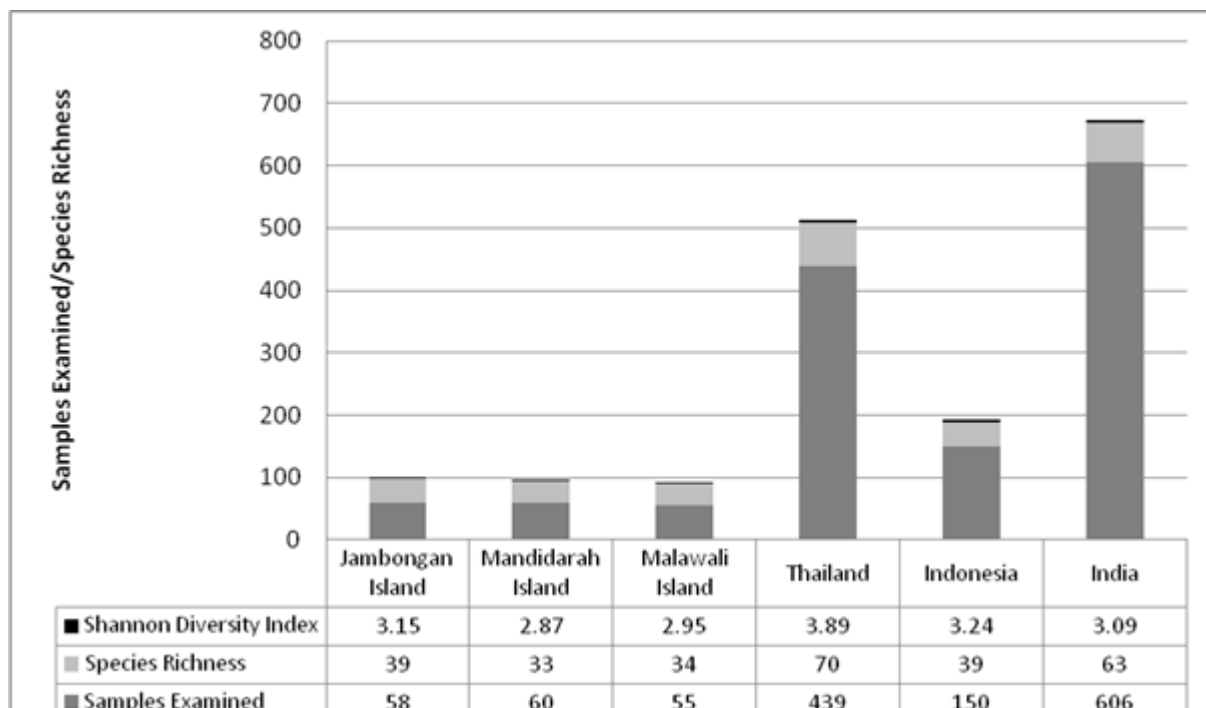


Figure 4. Principal Component Analysis (PCA) of marine fungal species recorded from Jambongan Island, Mandarah Island and Malawali Island.



**Figure 5.** Comparison of species diversity between the present study sites and previous studies in Malaysia (JI: Jambongan Island, MDI: Mandidarah Island, MI: Malawali Island, KS: Kuala Selangor, CL: Cape Layak-Layak, RI: Redang Island, CR: Cape Rachado, MO: Morib, BC: Bachok).



**Figure 6.** Comparison of species diversity of marine fungi between the present study sites and previous studies in Thailand, Indonesia and India.

## DISCUSSION

Extensive reports on corals, reef organisms and marine mammals are available from the Sulu-Sulawesi marine eco-region [24, 25, 32], but not on marine fungi. Findings in this study provide integral baseline information on the species of manglicolous marine fungi that can be found on Jambongan Island, Mandidarah Island and Malawali Island in the Sulu Sea. The majority of the marine fungi isolated were ascomycetes (60) and only one basidiomycete, a result concordant with other studies [33]. Species richness and diversity index between the three sites were found to be comparable (between 33-39 and 2.87-3.15, respectively). However, very frequent and frequent fungi species recorded in the three study sites (Table 2) were different. The only overlapping fungi that occurred in all the sites were *Sphaerulina orae-maris* and *Pleospora* sp. 1. *Dactylospora haliotrepha*, *Halorosellinia oceanica*, *Savoryella lignicola*, *Kallichroma tethys*, *Trichocladium alopallonellum* and *Verrulina enalia* were among the very frequent and frequent species found in the study sites that were consistent with previous studies [14, 15, 23]. This differences in fungal dominance could be due to the composition of mangrove tree species at different sites, bias in the type of substrate (twigs, seedling, branches, root, etc.) collected at different sites or/and the difference in the length of incubation for the different samples [14, 34, 35].

Outcome from Sorenson's Similarity Index as shown in Table 3 indicated a low level of correlation between the sites. Only five out of the 88 fungal species found reoccurred in all three sites including *Aniptodera* sp., *Leptosphaeria australiensis*, *Sphaerulina orae-maris* and *Trichocladium alopallonellum*. Further analysis through principal component analysis (PCA) also suggested that the fungal communities among the sites were significantly different. Jones [36] and Hughes [37] stated that location, different mangrove stands, type of mangrove wood and availability of substrates play an important role in the diversity of fungi. While Jambongan Island has a larger mangrove area providing more substrates for colonization, Mandidarah and Malawali Islands have smaller areas of mangrove stand. The higher number of fungi recorded may also be related to higher mangrove diversity at the particular location [14, 38]. Kohlmeyer [39], Jones [36] and Kis-Papo [40] stated that geographical distribution and the temperature of the sea are key factors that

govern the diversity of marine fungi. The present study is the first attempt to study fungal diversity in the Sulu-Sulawesi marine eco-region that is widely known to hold some of the world's highest concentrations of marine biodiversity. Hence, such samples would significantly contribute to future studies on ecosystem functions or adaptive response to climate change.

A comparison on the diversity of marine fungi from this study with previous studies in Malaysia is shown in Figure 5: Kuala Selangor, Bachok, Cape Layak-Layak, Redang Island, Cape Rachado and Morib [22, 23]. Diversity index and species richness of the three sites in this study are comparable with Bachok but slightly lower than Kuala Selangor. However, marine fungi in Kuala Selangor have been studied extensively while those in the Sulu-Sulawesi area have not, hence more intensive sampling in the future may result in the discovery of higher diversity. Comparison with data from Cape Layak-Layak, located in mainland of Sabah, 166 km south-west from the study sites [23] shows that the diversity and species richness there were much lower than in our three study sites. These results may be reflecting the different physical attributes of our study sites in the type, age and density of mangrove stand and the type of substrate collected from the sites [34, 36, 38]. In addition, Kis-Papo [40] and Jones [36] stated that fungal diversity may differ from one mangrove to another within the same region. Overall, there is no doubt that these sites harbour an interesting diversity of fungi and potentially a greater number of species should more studies be done.

Only a few species were observed as dominant in the present study (frequency of occurrence  $\geq 5\%$ ). This may be due to the presence of many rare species of the fungi that occurred only once and was never found again [41]. A larger sample size showed more intermediate species occurring, as seen by a study done in Kuala Selangor that had the largest samples examined (259 samples) and showed less distinction between dominant, intermediate and rare occurring fungi [22]. In conclusion, sample size and the single collection event contributed to the high distinction of dominant and rare fungi of the three study sites. Further collections in the study sites will give better view of the frequency occurrence pattern of dominant and rare fungi in the Sulu-Sulawesi area.

Species diversity comparisons between the study

sites and countries neighboring Malaysia including Thailand [42], Indonesia [43] and India [44] is shown in Figure 6. The difference in diversity index and the species richness between the present study and India is low but high when compared with Indonesia and Thailand. However, the number of samples examined in this study was much lower, which may suggest a higher diversity when more samples are examined.

This study showed that the diversity of manglicolous marine fungi from Jambongan, Mandidarah and Malawali Islands of the Sulu-Sulawesi region was different from other regions in Malaysia. Many taxa were unidentified and could be new to science. Studying this region is therefore beneficial not only for the inventory of marine fungi of Malaysia, but also as a vital step in understanding some of the main players in the decomposer communities that inhabit this productive biodiverse region. The results obtained from this study only represent a fraction of the diversity of marine mangrove fungi, thus further studies are required to collect a wider range of substrates from a wide geographical area in order to better understand the distribution and composition of marine fungi in the region. The axenic cultures of fungi are important for the study of fungi, be it for genetic conservation and as a basis for studies to investigate adaptive response to climate change. Marine fungi is a good model candidate, since the marine environment and organisms are now under threat from stresses associated with climate change.

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