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Vertical distribution of marine fungi on *Rhizophora apiculata* at Morib mangrove, Selangor, Malaysia

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Studies on the vertical distribution of marine fungi in a *Rhizophora apiculata* mangrove stand in Morib, Selangor were carried out in June 1993 and June to November 1997. Prop roots, subterranean roots and overhanging branches of *R. apiculata* were collected from three intertidal levels namely upper (high water mark), middle and lower. Fifty-three species were recorded including 39 ascomycetes, 13 deuteromycetes and one basidiomycete. The most common fungi were *Halocyphina villosa* (frequency occurrence 21%), *Kallichroma tethys* (20%), *Lulworthia grandispora* (18%), *Leptosphaeria australiensis* (16%), *Julella avicenniae* (15%) and *Massarina ramunculicola* (13%). The fungi were found to be vertically zoned, some were limited to the upper level such as *Pyrenographa xylographoides, Julella avicenniae* and *Aigialus grandis* or lower level such as *Trichocladium achrasporum* and *T. alopallonellum*, while only five species showed a broader distribution, being present at all levels: *Leptosphaeria australiensis, Halocyphina villosa, Cryptovalsa* sp., *Lulworthia grandispora* and *Lulworthia* sp. The greatest diversity of marine fungi were collected from the middle level with a Shannon Diversity Index of 5.9 while the Jaccard Similarity Index of 2.25 indicated that the upper and middle levels were the most similar in terms of species composition. Fungi with certain characteristics were also limited to particular levels, for example, carbonaceous and superficial ascomata were confined above mean tide while membranous walls and immersed ascomata were common below mean tide level.

Key Words——mangrove fungi; vertical distribution.

Prop roots of mangrove trees may be submerged continually or periodically depending on their topographic location. At the lower level, underground roots may become exposed following erosion and they are continually submerged except during low tides. At the upper levels prop roots are only submerged for short periods during high tides and a gradation of submergence and exposure will occur between these two extremes.

Zonation of epiphytic and fouling organisms has been reported along roots and stems of mangrove trees (Aleem, 1980; Kohlmeyer and Kohlmeyer, 1979). Vertical distribution of marine fungi in the intertidal region have recently been investigated by Hyde (1988, 1989a, b, c, 1990a, 1991, 1993), Hyde and Jones (1988) and Jones et al. (1988). These studies showed certain fungi showed strong affinities for certain intertidal zones.

No information is available on the vertical distribution of fungi on *Rhizophora apiculata* Blume in Malaysia. Therefore a study of the vertical distribution of marine fungi on this mangrove tree was undertaken at Morib mangrove stand in Selangor. Fungi were collected from three defined intertidal levels and their vertical distribution recorded.

Table 1. Collection level of samples.

Level	Range	Note
1	0.2–0.8 m (Above mean low water mark)	"Waterlogged" or submerged samples included exposed subterranean roots and lower tips of prop roots
2	0.8–1.8 m	Submerged daily for varying periods. Samples were from prop roots and overhanging branches
3	1.8–2.2 m	Samples were superficially dry for long periods and taken from decaying prop roots and overhanging branches

Salinity: 32.2±2.1‰
Air temperature: 29.6±1.2°C
pH: 7.0±0.18

Table 2. Percentage occurrence of fungi on dead wood of *Rhizophora apiculata*.

Species	Percentage occurrence
Ascomycota	
Agialus grandis Kohlm. & Schatz	6
Antennospora quadricornuta (Cribb & J. W. Cribb) T. W. Johnson	5
Antennospora salina (Meyers) E. B. G. Jones, Yusoff & S. T.Moss	5
Ascocratera manglicola Kohlm.	10
Ascomycete 1	0.5
Ascomycete 2	1
Ascomycete 3	1
Cirrus-like species	1
Cryptovalsa sp.	9
Dactylospora haliotrepha (Kohlm. & E. Kohlm.) Hafellner	11
Eutypa sp.	1
Halosarpheia abonnis Kohlm.	4
Halosarpheia fibrosa Kohlm. & E. Kolm.	1
Halosarpheia marina (Cribb & J. W. Cribb) Kohlm.	0.5
Halosarpheia ratnagiriensis Patil & Borse	3
Halosphaeria cucullata (Kohlm.) Kohlm.	10
Hypophloeda rhizospora K. D. Hyde & E. B. G. Jones	1.7
Halorosellinia oceanica (Schatz) Whalley, E. B. G. Jones, K. D. Hyde & Laessoe	12
Hypoxylon sp.	1
Julella avicenniae (Borse) K. D. Hyde	15
Kallichroma tethys (Kohlm. & E. Kohlm.) Kohlm. & Volkm.-Kohlm.	20
Leptosphaeria australiensis (Cribb & J. W. Cribb) G. C. Hughes	16
Lignincola laevis Höhnk	2
Lulworthia grandispora Meyers	18
Lulworthia sp.	5
Marinosphaera mangrovei K. D. Hyde	5.7
Massarina ramunculicola K. D. Hyde	13
Massarina thalassiae Kohlm. & Volkm.-Kohlm.	2
Massarina velataspora K. D. Hyde & Borse	4
Melaspelia mangrovei Vrijmoed, K. D. Hyde & E. B. G. Jones	5
Nectria-like species	2
Pleospora sp.	1
Pyrenographa xylographoides Aptroot	7
Quintaria lignatilis (Kohlm.) Kohlm. & Volkm.-Kohlm.	3
Rhizophila marina K. D. Hyde & E. B. G. Jones	7
Saccardoella rhizophora K. D. Hyde	1
Savoryella lignicola E. B. G. Jones & R. A. Eaton	2
Torpedospora radiata Meyers	5
Verruculina enalia (Kohlm.) Kohlm. & Volkm.-Kohlm.	8
Basidiomycota	
Halocyphina villosa Kohlm. & E. Kohlm.	21
Mitosporic fungi	
Cirrenalia pseudomacrocephala Kohlm.	2
Cirrenalia pygmea Kohlm.	6
Deuteromycete sp.	1
Dictyosporum pelagicum (Linder) S. Hughes	0.5
Periconia prolifica Anast.	3
Phoma sp. 1	1
Phoma sp. 2	3
Phoma sp. 3	15

Phoma suadae Jaap	15
Rhabdospora avicenniae Kohlm. & E. Kohlm.	1
Sporidesmium-like species	0.5
Trichocladium achrasporum (Meyers & Reynold) Dixon	3
Trichocladium alopallonellum (Meyers & Moore) Kohlm. & Volkm.-Kolm.	5
Samples supporting fungi	313
Samples without fungi	17
Total samples	330
Total collections	424
Empty perithecia	5
% Colonization of wood samples	97
Average number of fungi per sample	1.3
Total species	53

Material and Methods

Intertidal prop roots, subterranean roots and overhanging branches of *Rhizophora apiculata* were collected from Morib mangrove stand, Selangor during June 1993 and June to November 1997. Samples were collected from three levels and details of collections are summarized in Table 1. At the study site, 100–120 samples were collected from each level, placed in clean polythene bags and returned to the laboratory where they were gently

Table 3. Vertical distribution of intertidal fungi at Morib, Selangor showing their percentage occurrence per tidal level.

Fungi	Percentage occurrence		
	Level 3	Level 2	Level 1
Pyrenographa xylographoides	18		
Nectria-like species	6		
Massarina thalassiae	6		
Phoma sp. 2	3		
Hypoxylon sp.	4		
Pleospora sp.	3		
Ascomycete 1	1		
Ascomycete 3	3		
Ascomycete 2	1		
Julella avicenniae	15	10	
Rhizophila marina	10	14	
Halocyphina villosa	15	35	
Kallichroma tethys	10	35	
Phoma sp. 3	5	10	
Phoma sp. 1	5	4	
Hypophloeda rhizospora	3	3	
Marinosphaera mangrovei	9	13	
Savoryella lignicola	4	3	
Eutypa sp.	3	1	
Lignincola laevis	3	13	
Aigialus grandis	10	5	
Halosarpheia marina	1	8	
Halosarpheia abonnis	4	9	
Dactylospora haliotrepha	10	2	
Halorosellina oceanica	11	10	
Quintaria lignatilis	1	6	10
Leptosphaeria australiensis	10	20	4
Cryptovalsa sp.	13	10	
Halosarpheia fibrosa		1	
Rhabdospora avicenniae		3	
Saccardoella rhizophorae		3	
Phoma suadae		3	
Halosphaeria cucullata		3	
Ascocratera manglicola		10	
Halosarpheia ratnagiriensis		10	
Massarina ramunculicola		18	
Masssarina velataspora		15	
Lulworthia sp.	1	3	10
Halocyphina villosa	23	35	15
Lulworthia grandispora	6	10	43
Cirrenalia pseudomacrocephala		1	5
Periconia prolifica		3	8
Cirrenalia pygmea		5	14
Trichocladium alopallonellum		3	11
Verruculina enalia		14	13
Sporidesmium sp.			1
Dictyosporium pelagicum			1
Cirrus-like species			3
Deuteromycete sp.			4
Melaspelia mangrovei			5
Antennospora quadricornuta			5
Antennospora salina			5
Torpedospora radiata			5
Trichocladium achrasporum			10
Total species	30	35	19
Samples examined	110	100	120
Shannon Diversity Index	5.0	5.9	3.5
Jaccard Similarity Index			

Upper and lower: 0.14
Upper and middle: 2.25
Middle and lower: 0.40

washed to remove surface mud while the major fouling organisms were scraped off. The samples were then incubated for up to 6 months at room temperature in sterile plastic containers and examined as outlined by Jones and Hyde (1988).

Results

Fifty-three higher marine fungi from 330 mangrove samples were recorded (Table 2) including 39 ascomycetes, 13 deuteromycetes and 1 basidiomycete. The most common species (11% and above) were *Halocyphina villosa* (21%), *Kallichroma tethys* (20%), *Lulworthia grandispora* (18%), *Leptosphaeria australiensis* (16%), *Julella avicenniae* (15%), *Massarina ramunculicola* (13%), *Halorosellinia oceanica* (12%), *Dactylospora haliotrepha* (11%), while *Ascocratera manglicola* (10%), *Halosphaeria cucullata* (10%), and *Verruculina enalia* (8%) were classified as frequent.

Table 3 presents information on species composition and their percentage occurrence at three littoral levels. The number of fungi identified at the upper, middle and lower levels were 30, 35 and 19 species respectively. Results also showed that 27 species occurred at a single level, 21 species at two levels while only 5 species (*Leptosphaeria australiensis, Quinatria lignatilis, Lulworthia grandispora, Lulworthia* sp. and *Halocyphina villosa*) were found at all three levels, however, *Lulworthia* sp. and *L. grandispora* showed a greater affinity towards the lower level.

The Shannon Diversity Index (Ludwig and Reynolds, 1988) showed that the greatest diversity occurred in level 2, with a diversity index of 5.9, followed by the upper level (5.0) and lower (3.5). The Jaccard Index (Magurran, 1988) indicates that the upper and middle levels were the most similar in terms of species composition (2.25), followed by the middle and lower (0.4) and the upper and lower (0.14).

Species that have a greater affinity (those species occurring most frequently at levels 3 and 2) towards the upper level were *Pyrenographa xylographoides, Julella avicenniae, Aigialus grandis, Halocyphina villosa, Kallichroma tethys*, and *Rhizophila marina*. Towards the lower level species such as *Lulworthia grandispora, Cirrenalia pygmea, Trichocladium achrasporum* and *T. alopallonellum* were prevalent while *Massarina ramunculicola, Leptosphaeria australiensis*, and *Massarina velatospora* had a greater affinity for the mid level or occurred at more than one level.

Discussion

Frequency occurrence of marine fungi on *Rhizophora apiculata* Recent studies on decayed and dead timber of *Rhizophora apiculata* have provided information on host specificity, frequency of occurrence, distribution with depth and substrate and vertical distribution (Hyde 1988, 1989a, b, 1990a, b, 1991, 1993; Hyde and Jones, 1988, 1989; Leong et al. 1991). In this study the common fungi on *R. apiculata* were *Halocyphina villosa, Kallichroma tethys, Lulworthia grandispora, Leptosphaeria australiensis, Julella avicenniae, Massarina ramunculicola, Halorosellinia oceanica* and *Dactylospora haliotrepha* confirming the observations of Hyde (1993) and Hyde and Jones (1988).

The frequency occurrence of fungi on *R. apiculata* in Malaysia have been reported on by Jones and Kuthubutheen (1989) at Kampung Sementa, Selangor. For comparison, their results showed that common species in Kampung Sementa were *Verruculina enalia, Lig-*

Table 4. The most frequent fungi on *Rhizophora apiculata* mangrove trees.

Sumatra (Hyde, 1989b)	**Thailand (Hyde, 1993)**
Halosarpheia marina (22%)	*Trichocladium achrasporum* (14%)
Rhizophila marina (19%)	*Aigialus grandis* (10%)
Phoma sp. (15%)	*Lulworthia grandispora* (9%)
Lulworthia sp. (13%)	*Verruculina enalia* (8%)
Halorosellinia oceanica (12%)	*Leptosphaeria australiensis* (7%)
Singapore (Tan et al., 1989)	**India (Chinnaraj, 1993)**
Verruculina enalia (73%)	*Verruculina enalia* (14%)
Lignincola laevis (47%)	*Halocyphina villosa* (11%)
Aigialus parvus (36%)	*Marinosphaera mangrovei* (11%)
Lulworthia sp. (30%)	*Lophiostoma mangrovei* (11%)
Aigialus mangrovis (23%)	*Rhizophila marina* (7%)
Brunei (Hyde, 1990b)	**Present study (1993, 1997)**
Cirrenalia pygmea (13%)	*Halocyphina villosa* (21%)
Trichocladium cf. *opacum* (17.8%)	*Kallichroma tethys* (20%)
Lulworthia sp. (13%)	*Lulworthia grandispora* (18%)
Xylomyces sp. (13%)	*Leptosphaeria australiensis* (16%)
Leptosphaeria australiensis (11%)	*Julella avicenniae* (15%)

nincola laevis, Halorosellinia oceanica, Halosarpheia marina and *Kallychroma tethys* thus different from those reported here at Morib. At the Morib site, there was a greater diversity of species compared to Sementa (53 species Morib, 19 Sementa). However, these differences could be attributed to several factors such as: pH, salinity (Higher at Morib), origin of the substrata (attached wood at Morib, attached and predominatly driftwood at Sementa) and the total number of samples examined (53 species from 330 samples at Morib, 19 from 31 samples at Sementa). Furthermore, the Sementa site is more polluted with run off from port Klang, while Morib is relatively free from pollution. However, no one factor can be singled out to account for the differences observed.

Studies on fungi on *R. apiculata* wood have also been undertaken in Brunei (Hyde, 1988, 1990b), Singapore (Tan et al., 1989), Sumatra (Hyde, 1989b), Thailand (Hyde, 1993) and India (Chinnaraj, 1993). In Table 4, the most frequent fungi found in this study are listed along with those reported from the above-mentioned locations. It is evident that there are differences between the locations with the exception of the species *Leptosphaeria australiensis, Verruculina enalia, Lulworthia grandispora* and *Halocyphina villosa*, which were common to most studies. Several factors that affect the colonization of *R. apiculata* wood by fungi includes: seawater temperature and salinity e.g. in Sumatra (Hyde, 1989b) salinity 24 ppt and temperature 29°C; in Brunei salinity and temperature ranged from 16–25 ppt and 26–30°C respectively (Hyde, 1990b), while in this study the temperature was 29°C, with a salinity of 32 ppt. It is not possible at this stage to determine whether temperature or salinity affects individually the occurrence of the species mentioned. Such parameters do not necessarily operate independently in nature although Ritchie (1957) and others (Lorenz and Molitoris, 1992) have demonstrated that the tolerance of marine fungi to salinity is affected by temperature (Jones, 2000).

The most frequent group of fungi encountered in the present study was the Ascomycota (74%) and this is in agreement with reports from similar studies in Thailand (73%; Hyde, 1993), Brunei (72%; Hyde, 1990b) and India (78%; Chinnaraj, 1993). This supports Hyde's (1990a) view who stated that the Ascomycota have evolved to take full advantage of aquatic habitats with their small (microscopic) fruiting structures, appendaged ascospores that may aid in dispersal and attachment and in their ability to withstand fluctuating saline conditions, while the Basidiomycota with their larger, often putrescent sporophores are not well suited to aquatic habitats and few have evolved to tolerate conditions in submerged salty water (Jones, 1988).

Vertical distribution Kohlmeyer and Kohlmeyer (1979) reported that there was no distinct pattern of vertical distribution amongst intertidal mangrove fungi. However, consideration must be given to the tidal amplitude extant at mangroves under study. Aleem (1980) showed that certain fungi have a preference for specific tidal levels, e.g. *Haligena viscidula* Kohlm. & E. Kohlm., *Leptosphaeria australiensis* and *Torpedospora radiata* Meyers were common to the mid-littoral level, while *Cirrenalia tropicalis* Kohlm., *Cytospora rhizophorae* Kohlm. & E. Kohlm. and *Periconia prolifica* were more frequent in the upper littoral level. Hyde and Jones (1988), Hyde (1988, 1989c), Jones and Tan (1987), Jones et al. (1988) also reported similar observationjs, but these were general views, often not supported by quantitative data.

In this study, species that had a greater affinity towards the upper levels were *Pyrenographa xylographoides, Nectria*-like species, *Julella avincenniae, Aigialus grandis, Halorosellinia oceanica, Kallichroma tethys, Dactylospora haliotrepha* and *Rhizophila marina*. Investigations by Hyde in Brunei and Thailand (Hyde 1988, 1990b, 1993), showed the following species were most common in the upper mangrove level: *Dactylospora haliotrepha, Hypophloeda rhizospora, Halorosellinia oceanica, Marinosphaera mangrovei, Phialophoroma* cf. *littoralis* Linder, *Savoryella longispora* E. B. G. Jones & K. D. Hyde and *Quintaria lignatilis* while *Antennospora quadricornuta* and *Xylomyces* sp., were frequent at the lower level (data from Brunei).

The only species common to this study and Brunei were *D. haliotrepha* and *H. oceanica* at the upper level while at the lower level the common species which occurred were completely different. In Thailand none were the same as those found in this study. These differences can be attributed to several factors and future studies need to focus on which play the dominant role when it comes to the occurrence and distribution of marine fungi. Kohlmeyer and Volkmann-Kohlmeyer (1993) have shown that the age structure of a mangrove and the availability of mature trees yielding dead wood for colonization, greatly affects species diversity. This aspect, and other factors that affect the distribution of marine fungi are discussed by Jones (2000).

Sadaba et al. (1995) observed vertical distribution of fungi on standing plants of *Achantus ilicifolius* L. The apical portions were colonized by typical terrestrial fungi and the basal portions by marine fungi. The highest number of collections were obtained from the basal portions followed by middle and upper portions with the Deuteromycota the dominant group encountered at these levels. They attributed this to tissue type and varying degrees of exposure to tidal inundation, which are important in governing species distribution along the vertical line. Similar observations have bee made for intertidal estuarine fungi on *Phragmites communis* (L.) Trin. (Poon and Hyde, 1998).

Vertical distribution and morphology of the fungi Ascomata, asci and ascospores are morphologically and physiologically adapted to the habitat they grow in. Fungi occurring below mean tide are submerged for long periods of time, the substratum may be waterlogged and coated in silt and will have less exposure to direct sunlight. These fungi generally release their ascospores passively into the surrounding water. Examples from this study include *Antennospora quadricornuta* and *A. salina* which have ascomata that are membranous or immersed in the substratum.

Fungi growing above mean tide are exposed to harsher conditions, they may be exposed for long periods and subject to desiccation and sunlight as well as variation in salinity, during dry periods the salinity will be higher but in the monsoon season are subject to freshwater. To facilitate development in these conditions, ascomata may immersed (e.g. *Jullella avicenniae, Rhizophila marina*), or if superficial, have carbonaceous walls (e.g. *Halorosellinia oceanica, Ascocratera manglicola*).

Many Ascomycota have ascomata in which the asci are embedded in a matrix of mucilage (*Massarina* spp. and *Leptosphaeria* spp.). The mucilage helps to conserve water and thus protect the developing asci. Intertidal ascomycetes generally have forcible release of ascospores: e.g. *Halorosellinia oceanica, Jullela avicenniae, Massarina* spp. and *Aigialus grandis*. Thus mangrove fungi have developed a number of strategies for overcoming the environmental conditions they grow in.

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