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Original Article

Diversity of higher marine fungi at Hat Khanom-Mu Ko Thale Tai National Park, Southern Thailand

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Abstract

Species occurrence and distribution of higher marine fungi at Hat Khanom-Mu Ko Thale Tai National Park, Nakhon Si Thammarat Province, Southern Thailand was investigated. Four collections from four sites yielded 112 higher marine fungi representing 79 ascomycetes, 3 basidiomycetes, 30 anamorphic fungi, while others await further identification. Average percentage occurrence of fungi recorded ranged from 0.05-11.3%. Species diversity, richness, and evenness were greatest at Site IV, Ao Tok, Koh Taen. The most frequently encountered taxa for all sites (>10%) were *Corollospora maritima* and *Lindra thallasiae*. *Lindra thallasiae* and *Swampomyces aegyptiacus* were recorded for the first time in Thailand.

Keywords: Gulf of Thailand, Khanom, mangrove, marine fungi

1. Introduction

Higher filamentous marine fungi are those, which have the ability to germinate and form mycelia under natural marine conditions (Kohlmeyer and Kohlmeyer, 1979). They have been known for decades as the result of studies by Barghoorn and Linder (1944), who described ten new genera and twenty-five species from woody substrata. Since then, marine mycological research has flourished and our knowledge of marine fungal diversity has greatly expanded. Schmit and Shearer (2003) listed 625 fungi associated with mangroves, but this figure includes those species from soil, sediments and on standing mangrove trees. Recently, in the monograph of the higher marine fungi, Jones *et al.* (2009) list 530 species belonging to Ascomycota, Basidiomycota and anamorphic taxa.

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Marine mangrove fungi documented for Thailand total 154 (116 Ascomycota, 28 anamorphic fungi, 3 Basidiomycota and 7 Straminopiles) while 30 taxa await further identification (Jones *et al*., 2006b). Thailand, located in the tropical zone, is a country rich in biodiversity (Jones and Hyde, 2004); however, only 34% of the described marine fungi have been documented for Thailand (Jones *et al*., 2006b). Early studies on marine fungi in Thailand were by Kohlmeyer (1984) and Koch (1986). Over the past 15 years further studies of marine fungal diversity and their ecology have been undertaken by Hyde (1988a,b; 1989a,b; 1992), Hyde *et al.* (1990, 1993), Chalermpongse (1991), Hyde and Jones (1992), Jones *et al.* (2006a,b), Ito *et al*. (2001), and Chatmala *et al*. (2004). Studies of marine fungi in Thailand have been restricted to sandy beaches, sea shores, mangroves and estuarine *Nypa* palm forests of the mainland (Pilantanapak, 2003; Pilantanapak *et al*., 2005; Jones *et al*., 2006b). In contrast, occurrence and distribution of marine fungi from the Thai islands have been less studied, with the exception of a few studies at Mu Ko Chang National Park, Trat Province, Eastern Thailand (Jones *et al*., 2006b; Jones *et al*., 2008; Suetrong *et al*., 2010).

As part of an ongoing Khanom Marine Biodiversity Project at Hat Khanom-Mu Ko Thale Tai National Park, the present study was undertaken to investigate the diversity, occurrence and distribution of higher marine fungi in this area. The major aim was to develop a baseline research of marine microorganisms, in order to encourage awareness to the conservation of bioresources in the local marine protected area.

2. Materials and Methods

2.1 Description of collecting sites

Hat Khanom-Mu Ko Thale Tai National Park is a marine protected area, located offshore in Khanom District, Nakhon Si Thammarat Province, Southern Thailand. It covers several islands, with Koh Taen being the largest island in the National Park (7.5 km^2). Four collecting sites were chosen for the present study and details of each collecting site are given below and in Figure 1.

Site I: Headquarter nature trail (09°12′59.96″N, 99°51′10.18″E): this site is located along Khanom Canal, part of the mainland area in the national park, and dominated by mature and young mangrove tree species e.g. *Bruguiera gymnorrhiza, Rhizophora apiculata, R. mucronata* and *Xylocarpus granatum*. The salinity varies between 28-32‰.

Site II: Khanom Canal (09°13′04.60″N, 99°50′37.32″E): it is located as part of mainland area, and connected to the mouth of the Gulf of Thailand. This is a healthy mangrove stand along Khanom Canal, with the salinity varying between 28-32‰. Mangrove trees include *B. gymnorrhiza, R. apiculata, R. mucronata* and *X. granatum*.

Site III: Ao Tok, Koh Taen (09°22′15.68″N, 99°57′ 11.80″E): this site is located to the west of Koh Taen, and is covered by healthy mangrove trees, including *B. gymnorrhiza, R. apiculata, R. mucronata, Sonneratia griffithii* and *X. granatum*. Seawater salinity varies from 30-33‰.

Site IV: Ao Ok, Koh Taen (09°22′19.33″N, 99°56′ 19.21″E): a sandy beach to the east of Koh Taen, with salinity varying from 30-33‰. Substrata collected include driftwood, trapped wood, decaying coconut trunks, seaweeds, and seagrasses.

2.2 Sampling procedure and isolation of fungi

Sampling was carried out four times from October 2006 to July 2007. Over 1,900 samples of decaying mangrove wood, leaf blades, seaweeds, seagrasses, fruits and seeds of mangrove plants were randomly collected. Samples were washed using sterile seawater and examined immediately after returning to the laboratory and up to three weeks after incubation in a damp chamber at 25°C. Sterile seawater was sprayed periodically to maintain the moisture in the incubation chambers.

All fungi were isolated using single spore technique (Choi *et al.* 1999) and maintained on seawater cornmeal agar (CMA). Axenic cultures were deposited at the BIOTEC Culture Collection (BCC), herbarium material and voucher slides at the BIOTEC Bangkok Herbarium (BBH), Thailand.

2.3 Data analysis

The following data were recorded for each site:

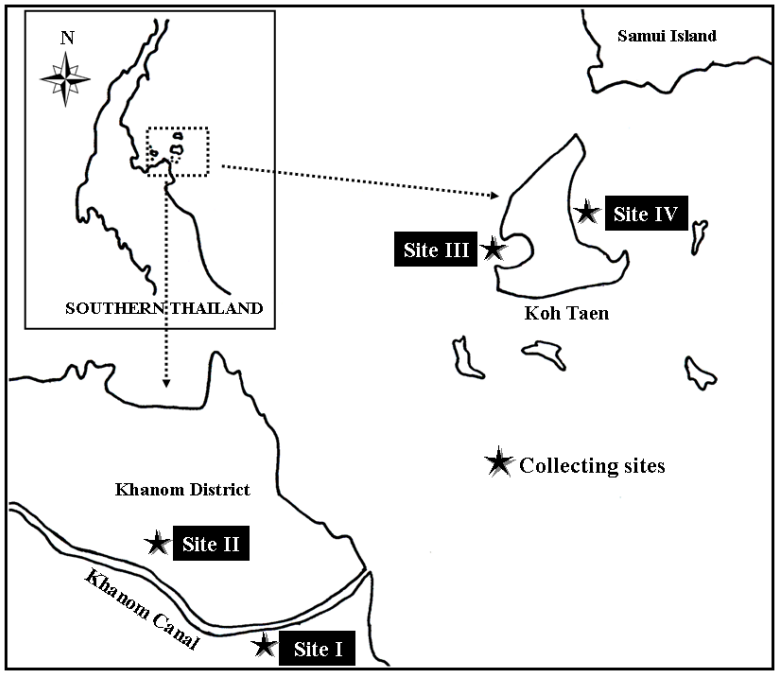

Figure 1. Map showing collecting sites at Hat Khanom-Mu Ko Thale Tai National Park.

1) List of species and number of collections observed.

2) Percentage occurrence of each taxon (total number of collections of particular taxon encountered × 100 divided by total number samples examined.

3) Percentage colonization (number of samples supporting fungi × 100 divided by the number of samples examined).

4) Average number of fungi per sample (total number of fungal isolates divided by total number of substrata supporting fungi).

5) The diversity of fungi at each collecting site was assessed based on the diversity indices: species diversity index (Shannon-Wiener, Simpsons D), species evenness (Equitability J) and species richness (Margalef D) (Henderson and Seaby, 1998; Ludwig and Reynolds, 1998; Magurran, 1998).

3. Results

3.1 Frequency of occurrence

Nine hundred and forty-seven (947) fungal collections from the four sites yielded 112 higher marine fungi representing 79 ascomycetes (70.5%), 3 basidiomycetes (2.7%), and 30 anamorphic fungi (26.8%) (Table 1). Forty eight taxa (42.9%) were identified to species level, 48 (42.9%) to generic level. Ten and nine species tentatively identified as ascomycetes (7.1%) or anamorphic fungi (8%), respectively, and await further identification.

At Site I, headquarter nature trail, a total of 406 samples were examined, which yielded 37 marine fungi (138 fungal collections) including 28 ascomycetes, 2 basidiomycetes and 7 anamorphic fungi. Percentage colonization was 30.8%, and the average number of fungi per sample was 0.3.

At Site II, Khanom Canal, 803 samples were examined with 296 samples supported growth of fungi (36.9%), which included 44 ascomycetes, 2 basidiomycetes and 11 anamorphic fungi. A total of 302 fungal collections were made with the average number of fungi per sample 0.4.

Of the 439 samples examined at Site III, Ao Tok, Koh Taen, 218 fungal collections were recorded, yielding 51 ascomycetes, 2 basidiomycetes and 17 anamorphic fungi. Percentage colonization was 53.1% and the average number of fungi per sample was 0.5.

At Site IV, Ao Ok, Koh Taen yielded the highest relative percentage colonization and the average number of fungi per sample at 71.5% and 1.0, respectively. Of the 284 samples examined, 289 fungal collections were recorded 11 ascomycetes, 1 basidiomycete, and 9 anamorphic fungi.

3.2 Statistical analysis

Species diversity, species evenness, and species richness of fungi at each collecting site are summarized in Table 1. Shannon-Wiener and Simpson Diversity Indices, Equitability Species Evenness, and Margalef's Species Richness were greatest at the two mangroves: Site III, Ao Tok, Koh Taen, and Site II, Khanom Canal, respectively, while the lowest diversity value was at Site IV, Ao Ok, Koh Taen.

3.3 Common species

The most common species (1-5%) found at each collecting site were: *Calathella mangrovei, Dactylospora*

Table 1. Percentage occurrence of higher marine fungi at Hat Khanom-Mu Ko Thale Tai National Park.

Fungi	Percentage occurrence				
	Site I	Site II	Site III	Site IV	Total
Ascomycetes (79 taxa)					
Acrocordiopsis patilii Borse & K.D. Hyde	-	0.1	-	-	0.05
Aigialus sp.	-	-	0.7		0.20
Aigialus grandis Kohlm. & S. Schatz	-	1.0 *	0.2	0.7	0.60
Aigialus cf. *mangrovei*	-	-	0.2	-	0.05
Aniptodera longispora K.D. Hyde	-	0.1	-	-	0.05
Aniptodera mangrovei K.D. Hyde & E.B.G. Jones	0.2	0.1	-	-	0.10
Antennospora quadricornuta (Cribb & J.W. Cribb) T.W. Johnson	-	-	0.2	0.4	0.10
Argentinomyces-like sp.	-	0.1	0.2	-	0.10
Ascocratera manglicola Kohlm.	3.0 *	0.1	-	0.4	0.70
Astrosphaeriella striatispora (K.D. Hyde) K.D. Hyde	-	0.1	-	-	0.05
Corollospora maritima Werderm.	-	-	-	11.3 ***	1.70
Cryptovalsa sp.	-	-	0.2	-	0.05
Cucullosporella mangrovei (K.D. Hyde & E.B.G. Jones) K.D. Hyde & E.B.G. Jones	-	-	0.5	-	0.10
Dactylospora haliotrepha (Kohlm. & E. Kohlm.) Hafellner	0.7	2.0 *	4.1 *	1.4 *	2.10

Table 1. (Continued)

Fungi	Percentage occurrence				
	Site I	Site II	Site III	Site IV	Total
Eutypella sp.	-	1.5 *	1.6 *	-	1.00
Fasciatispora-like sp. 1	-	-	1.1 *	-	0.30
Fasciatispora-like sp. 2	-	-	0.2	-	0.05
Fasciatispora-like sp. 3	-	-	0.5	-	0.10
Haiyanga salina K.L. Pang & E.B.G. Jones	-	-	0.2	-	0.05
Halorosellinia oceanica Whalley, E.B.G. Jones, K.D. Hyde & Laessøe	2.0 *	1.6 *	0.2	0.4	1.20
Halorosellinia-like sp.	0.5	4.1 *	-	-	1.80
Halosarpheia kandeliae Abdel-Wahab & E.B.G. Jones	-	0.1	1.1 *	-	0.30
Halosarpheia minuta Leong	0.2	0.6	-	-	0.30
Helicascus kanaloanus Kohlm.	2.7 *	0.7	0.2	-	0.90
Hysterium-like sp. 1	0.2	0.6	0.5	-	0.40
Hysterium-like sp. 2	0.5	0.1	-	-	0.20
Juella avicenniae (Borse) K.D. Hyde	-	-	0.2	-	0.05
Kallichroma glabrum (Kohlm. & E. Kohlm.) Kohlm. & Volkm.-Kohlm.	-	0.1	-	-	0.05
Kallichroma tethys (Kohlm. & E. Kohlm.) Kohlm. & Volkm.-Kohlm.	0.2	0.6	2.1 *	-	0.80
Leptosphaeria sp.	-	0.1	-	-	0.05
Lignincola laevis Höhnk	-	0.1	-	-	0.05
Lindra thallasiae Orpurt, Meyers, Boral & Simms §	-	-	0.9	75.4 ***	11.3
Lineolata rhizophorae (Kohlm. & E. Kohlm.) Kohlm. & Volkm.-Kohlm.	-	-	1.1 *	-	0.30
Linocarpon sp.	-	1.0 *	1.4 *	-	0.70
Rimora mangrovei Kohlm. & Vittal	2.0 *	2.6 *	-	-	1.50
Lophiostoma sp. 1	-	-	2.3 *	-	0.50
Lophiostoma sp. 2	1.2 *	0.2	-	-	0.40
Lulworthia grandispora Meyers (>800 mm)	4.2 *	2.9 *	-	-	2.10
Lulworthia sp. 1 (very long neck, 500 mm)	-	1.0 *	0.9	-	0.60
Lulworthia sp. 2 (250-350 mm)	-	-	1.1 *	-	0.30
Mangrovispora pemphi K.D. Hyde & Nakagiri	-	-	0.5	-	0.10
Marinosphaera mangrovei K.D. Hyde	-	0.4	1.1 *	-	0.40
Massarina sp. 1	-	0.1	-	-	0.05
Massarina sp. 2	0.2	-	-	-	0.05
Massarina sp. 3	1.0 *	-	-	-	0.20
Morosphaeria cf. *velataspora*	0.2	0.1	-	-	0.10
Morosphaeria velataspora K.D. Hyde & Borse	0.7	0.5	1.6 *	-	0.70
Halomassarina thallasiae Kohlm. & Volkm.-Kohlm.	1.0 *	0.9	0.9	-	0.80
Massariosphaeria-like sp.	-	0.1	-	-	0.05
Phaeosphaeria sp. 1	-	-	0.2	-	0.05
Phaeosphaeria sp. 2	-	-	0.2	-	0.05
Phaeosphaeria-like sp.	-	0.1	-	-	0.05
Pleospora sp.	-	-	0.2	-	0.05
Pyrenographa xylographoides Aptroot	0.2	-	0.9	1.8 *	0.50
Pyrenula sp.	-	-	0.7	-	0.20
Quintaria cf. *lignatilis*	-	-	0.7	-	0.20
Quintaria lignatilis (Kohlm.) Kohlm. & Volkm.- Kohlm.	0.2	0.7	2.1 *	1.1 *	1.00
Rhizophila marina K.D. Hyde & E.B.G. Jones	0.5	0.5	0.7	-	0.50
Saagaromyces abonnis (Kohlm.) K.L. Pang & E.B.G. Jones	0.2	1.5 *	2.1 *	-	1.10
Saagaromyces glitra (J.L. Crane & Shearer) K.L. Pang & E.B.G. Jones	0.5	0.7	-	-	0.40
Saagaromyces ratnagiriensis (Patil & Borse) K.L. Pang & E.B.G. Jones	0.2	0.7	0.9	-	0.60
Saccardoella mangrovei K.D. Hyde	-	-	0.7	-	0.20
Saccardoella rhizophorae K.D. Hyde	-	0.1	1.1 *	-	0.30
Savoryella lignicola E.B.G. Jones & R.A. Eaton	-		0.5	-	0.10
Savoryella longispora E.B.G. Jones & K.D. Hyde	0.2	0.5	-	-	0.30

Table 1. (Continued)

Fungi	Percentage occurrence				
	Site I	Site II	Site III	Site IV	Total
Swampomyces aegyptiacus Abdel-Wahab, El-Sharouney & E.B.G. Jones ♣	-	-	0.7	-	0.20
Tirisporella beccariana (Ces.) E.B.G. Jones, K.D. Hyde & Alias	-	-	1.1 *	-	0.30
Torpedospora radiata Meyers	-	-	0.2	3.5 *	0.60
Verruculina enalia (Kohlm.) Kohlm. & Volkm.- Kohlm.	1.5 *	0.6	1.4 *	0.7	1.00
Unidentified ascomycetes (10 taxa)	#	#	#	#	#
Anamorphic fungi (30 taxa)					
Acremonium sp.	-	-	-	0.4	0.05
Cirrenalia cf. *fusca*	0.2	-	”	-	0.05
Cirrenalia pygmea Kohlm.	0.2	-	0.2	-	0.10
Cirrenalia sp. 1	0.2	-	-	-	0.05
Cirrenalia sp. 2	-	-	-	0.4	0.05
Diplodiplodia sp.	-	-	0.2	-	0.05
Epicoccum sp.	-	0.1		-	0.05
Halenospora varia (Anastasiou) E.B.G. Jones	0.2	0.1	-	1.1 *	0.30
Periconia prolifica Anastasiou	-	0.1	0.2	-	0.10
Periconia-like sp.	-	-	0.2	-	0.05
Pestalotiopsis sp.	-	-		0.4	0.05
Phoma sp.	-	0.2	0.2	0.4	0.20
Phoma-like sp.	0.7	0.1	0.2	-	0.30
Sporidesmium-like sp.	-	-	0.5	-	0.10
Trichocladium achrasporum (Meyers & R.T. Moore) Dixon	-	0.4	0.2	-	0.20
Trichocladium sp. 1	-	-	1.1 *	-	0.30
Trichocladium sp. 2	-	0.1	-	-	0.05
Trichocladium sp. 3	-	-	0.2	-	0.05
Varicosporina ramulosa Meyers & Moore	-	0.1	-	0.7	0.20
Zalerion cf. *maritimum*	-	-	-	0.4	0.05
Zalerion-like sp.	-	-	0.2	”	0.05
Unidentified Coelomycetes (5 taxa)	#	#	#	#	#
Unidentified Hyphomycetes (4 taxa)	#	#	#	#	#
Basidiomycetes (3 taxa)					
Calathella mangrovei E.B.G. Jones & Agerer	2.7 *	3.5 *	1.4 *	-	2.30
Halocyphina villosa Kohlm. & E. Kohlm.	2.5 *	1.4 *	1.1 *	-	1.30
Nia vibrissa R.T. Moore & Meyers	-	-	-	0.4	0.05
Total number of collections	138	302	218	289	947
Number of samples examined	406	803	439	284	1932
Number of samples colonized	125	296	233	203	857
% colonization	30.8	36.9	53.1	71.5	48.1
Average number of fungi per sample	0.3	0.4	0.5	1.0	0.6
Total number of taxa	37	57	70	21	112
Ascomycota	28 (75.7%)	44 (77.2%)	51 (72.9%)	11 (52.4%)	79 (70.5%)
Basidiomycota	2 (5.4%)	2 (3.5%)	2 (3.8%)	1 (4.8%)	3 (2.7%)
Anamorphic fungi	7 (18.9%)	11 (19.3%)	17 (24.3%)	9 (42.8%)	30 (26.8%)
Species diversity index (Shannon-Wiener)	3.139	3.440	3.894	1.140	-
Species diversity index (Simpson)	19.449	22.748	43.883	1.781	-
Species evenness index (Equitability)	0.665	0.729	0.825	0.241	-
Species richness index (Margalef's)	7.1245	9.8066	12.815	3.5296	-

data not presented; * common fungi (1-5%); *** very frequent fungi (>10%); ♣ new record.

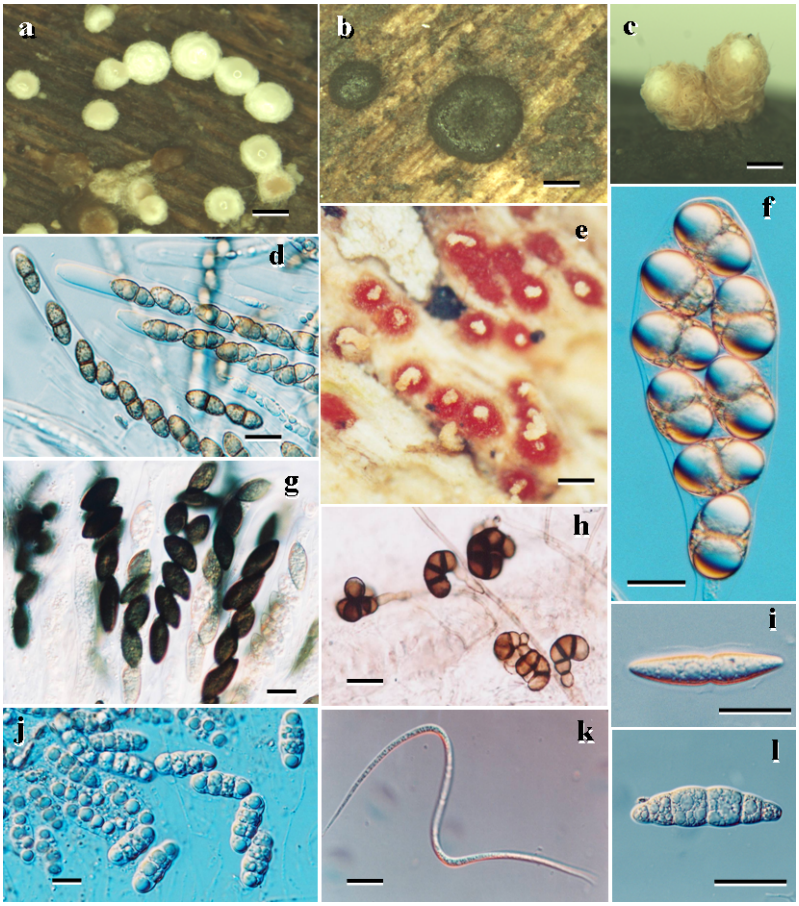

Figure 2. Common and new records of marine fungi found at Hat Khanom-Mu Ko Thale Tai National Park: a. *Halocyphina villosa*, b. *Dactylospora haliotrepha*, c. *Calathella mangrovei*, d. *Verruculina enalia*, e. *Kallichroma tethys*, f. *Saagaromyces glitra*, g. *Halorosellinia oceanica*, h. *Halenospora varia*, i. *Rimora mangrovei,* j. *Swampomyces aegyptiacus**, k. *Lindra thalassiae**, l. *Quintaria lignatilis*. Bars: a, e = 300 mm, b = 500 mm, c = 400 mm, d = 15 mm, f = 25 mm, g-j = 10 mm, k, l = 20 mm. * New records for Thailand.

haliotrepha, Halocyphina villosa, Halorosellinia oceanica, Eutypella sp., *Rimora mangrovei, Quintaria lignatilis,* and *Verruculina enalia* (Figure 2). *Corollospora maritima* (11.3%) and *Lindra thallasiae* (75.4%) were the most frequently encountered taxa from Site IV, Ao Ok, Koh Taen (Table 1). Moreover, the results of this study revealed two new records for Thailand, *Lindra thallasiae* and *Swampomyces aegyptiacus*.

4. Discussion

This study on the occurrence and diversity of marine fungi was part of a larger project into the diversity of marine organisms in the Hat Khanom-Mu Ko Thale Tai National Park. This survey has extended our knowledge of marine fungi in Thai mangroves and islands. Currently, 157 marine fungi have been documented for Thailand, while Suetrong *et al.* (2007 and unpublished data) have reported a further five species from Mu Ko Chang National Park (Trat Province), Hat Ban Krut (Prachuab Kiri Khun Province), Kungkraben Bay Development Center (Chantaburi Province) and Laem Talumpuk (Nakhon Si Thammarat Province). This figure (162 species) is comparable to those reported from mangroves in other neighboring countries e.g. Brunei, 95 (Hyde, 1988a, b), Hong Kong, 128 (this figure includes marine derived fungi) (Jones and Vrijmoed, 2003), India, 91 (Maria and Sridhar, 2002, 2003), Malaysia, 82 (Alias, 2007) and 295 (Jones, 2007).

The fungi reported here were similar to those reported in Ranong and Phang-nga Province, Thailand (Hyde, 1989a; Hyde *et al.*, 1990; Chalermpongse, 1991 and Hyde *et al.*, 1993), but differ in the overall percentage occurrence 0.1 to 16.4 compared to 0.05 to 11.3 in the current study. The core-group of fungi (>10%) reported by earlier studies included *Savoryella lignicola*, *Caryosporella rhizophorae*, *Leptosphaeria australiensis*, *Lulworthia grandispora*, *Marinosphaera mangrovei,* and *Cirrenalia pygmea*, while in this study *Corollospora maritima* (11.3%) and *Lindra thallasiae* (75.4%) were the most frequently reported ones. The dominance of *Corollospora maritima* and *Lindra thallasiae* may be accounted by their occurrence on seaweeds and sea-

grasses at Site IV. Ao Ok, Koh Taen.

Many factors determine the diversity and abundance of mangrove fungi and these have been discussed by Jones (2000) and Sarma and Hyde (2001). These include: the differences in mangrove community, the availability of substrata for colonization, differences in the quality of substrata, the length of time the substrata exposed to conditions conducive to fungal colonization, the competition between fungi and the physical factors such as temperature, rainfall, salinity, tidal schedule, moisture and aeration.

The results of this study give an insight into the distribution and diversity of fungi present in Thai mangroves. Greater species diversity, richness and evenness, especially at Site IV, Ao Tok, Koh Taen, is generally thought to indicate a more complex and healthier community. Because of a greater variety of species may allow for more species interactions, hence greater ecosystem stability, and may indicate good environmental conditions. However, there is no baseline for determining if the numbers reported are typical of a healthy ecosystem. Most studies are of mangroves that are deemed to be healthy, while there are few from polluted or degraded sites. Hyde (1989b) reported that the presence of hydrocarbons reduced the diversity and numbers of saprophytic fungi on intertidal mangrove wood. For example, only 23 species were identified at Seria mangrove, Brunei (contaminated with natural sewage from the soil) compared with 45 marine fungi at Kpg. Serassa mangrove, considered a healthy mangrove. Conversely, Poonyth *et al.* (1999) documented 36 manglicolous fungi at Beau Champ, Mauritius, which were contaminated with oil compared to 30 species in nearby undisturbed mangroves. Therefore, there is a need to develop a baseline for viability in marine ecosystems, as well as fungal succession, the interference competition and biotechnological potential for fungi in mangrove ecosystem.

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