CHAPTER 4: MARINE FUNGI FROM SELECTED MANGROVES AND COASTAL AREA

4.1 Results

4.1.1 Biodiversity of lignicolous marine mangrove fungi from Malaysia.

Table 4.1 lists the species and percentage of occurrence of marine fungi collected from Peninsular Malaysia: Cape Rachado, Telok Pelandok and Morib. In Table 4.2, marine fungi are categorised as most common (>10%) and common (5%-10%) accordingly.

Cape Rachado

Sixty-eight fungal taxa were identified from 187 wood samples (out of 202 samples examined), comprising 48 ascomycetes, 18 anamorphic fungi and 2 basidiomycetes. Percentage colonization of fungi was 92.6%, with an average number per sample of 1.054. Five fungi were common (5-10%): *Aigialus grandis* (Figure 4.12), *A.parvus* (Figure 4.5), *Dactylospora haliotrepha* (Figure 4.2), *Kallichroma tethys* (Figure 4.3) and *Halocyphina villosa* (Figure 4.21). There were no species that can be categorized as the most common species (>10%) recorded in this study.

Telok Pelandok

From 154 wood samples examined, 71 marine fungal taxa were identified (colonizing 127 samples) which comprised of 56 ascomycetes, 14 anamophic fungi and 1 basidiomycete. Percentage colonization of fungi was 83% and the average number per sample was 1.23. *Dactylospora haliotrepha* was found to be the only very common species (>10%), *Halorosellinia oceanica* and *Kallichroma tethys* were common (5–10%).

Morib

Forty fungal taxa were identified from 123 wood samples (out of 180 samples examined), comprising 37 ascomycetes, 6 anamorphic fungi and 1 basidiomycetes. Percentage colonization of fungi was 68%, with an average number per sample of 1.09. Seven fungi were common (5-10%): *Aigialus grandis*, Asco MB sp.20, *Dactylospora haliotrepha, Haloresellinia oceanica, Morosphaeria ramunculicola, Morosphaeria velatispora* and *Verruculina enalia.*

Summary for all study sites: Cape Rachado, Telok Pelandok and Morib

Out of 536 samples from all sites examined for the occurrence of marine fungi, 437 samples (81.5%) were colonized by fungi: 105 ascomycetes, 29 anamorphic fungi and 2 basidiomycetes. The average number of fungi per sample was 1.00. Three fungi were very common in this study, namely *Dactylospora haliotrepha*, *Haloresellinia oceanica* and *Kallichroma tethys*; they occurred in all 3 study sites. Table 4.3 lists the species richness, evenness (equitability) and diversity indices of marine fungi for the seven study sites. The greatest diversity (Shannon-Wiener diversity index) was recorded in Cape Rachado, followed by Telok Pelandok and Morib. Species richness was the highest in Telok Pelandok, followed by Cape Rachado and Morib. Species evenness was highest in Cape Rachado followed by Telok Pelandok and Morib. Sorenson's similarity index is shown in Table 4.4. The similarity was low between the study sites based on the indices ranging from 0.30 to 0.37 which are below than 0.5.

Table 4.1 Marine fungi isolated from Cape Rachado, Telok Pelandok and Morib.

Fungi	No. of Sites	Sampling Sites						Total	
		Cape Rachado		Telok Pelandok		Morib			
		No.	%	No.	%	No.	%	No	%
Ascomycota (105)									
Acrocordiopsis patilii Borse & K.D. Hyde	1	1	0.505					1	0.6
Aigialus grandis Kohlm.et Schatz	3	14	7.071	3	1.974	11	5.946	28	17.7
Aigialus mangrovis Borse	1	4	2.020					4	2.5
Aigialus parvus S. Schatz et Kohlm.	3	11	5.556	2	1.316	6	3.243	19	12.0
Antennospora quadricornuta (Cribb et J.W. Cribb) T.W. Jonson	1	2	1.010					2	1.3
Anthostomella sp.	1			3	1.974			3	1.9
Asco CR sp.01	1	1	0.505					1	0.6
Asco CR sp.02	1	1	0.505					1	0.6
Asco CR sp.03	1	2	1.010					2	1.3
Asco CR sp.04	1	1	0.505					1	0.6
Asco CR sp.05	1	1	0.505					1	0.6
Asco CR sp.06	1	1	0.505					1	0.6
Asco CR sp.07	1	1	0.505					1	0.6
Asco CR sp.08	1	1	0.505					1	0.6
Asco CR sp.09	1	1	0.505					1	0.6
Asco CR sp.10	1	1	0.505					1	0.6
Asco CR sp.11	1	1	0.505					1	0.6
Asco CR sp.12	1	1	0.505					1	0.6
Asco CR sp.13	1	1	0.505					1	0.6
Asco CR sp.14	1	1	0.505					1	0.6
Asco CR sp.15	1	1	0.505					1	0.6
Asco CR sp.16	1	2	1.010					2	1.3

Asco MB sp. 17	1			1	0.541	1	0.6
Asco MB sp. 18	1			1	0.541	1	0.6
Asco MB sp. 19	1			2	1.081	2	1.3
Asco MB sp. 20	1			11	5.946	11	7.0
Asco MB sp. 21	1			1	0.541	1	0.6
Asco MB sp. 22	1			2	1.081	2	1.3
Asco MB sp. 23	1			2	1.081	2	1.3
Asco MB sp. 24	1			1	0.541	1	0.6
Asco MB sp. 25	1			1	0.541	1	0.6
Asco MB sp. 26	1			1	0.541	1	0.6
Asco TP sp.17	1	1	0.658			1	0.6
Asco TP sp.19	1	1	0.658			1	0.6
Asco TP sp.27	1	2	1.316			2	1.3
Asco TP sp.28	1	1	0.658			1	0.6
Asco TP sp.29	1	1	0.658			1	0.6
Asco TP sp.30	1	1	0.658			1	0.6
Asco TP sp.31	1	1	0.658			1	0.6
Asco TP sp.32	1	1	0.658			1	0.6
Asco TP sp.33	1	1	0.658			1	0.6
Asco TP sp.34	1	1	0.658			1	0.6
Asco TP sp.35	1	1	0.658			1	0.6
Asco TP sp.36	1	1	0.658			1	0.6
Asco TP sp.37	1	1	0.658			1	0.6
Asco TP sp.38	1	1	0.658			1	0.6
Asco TP sp.39	1	1	0.658			1	0.6
Asco TP sp.40	1	1	0.658			1	0.6
Asco TP sp.41	1	1	0.658			1	0.6
Asco TP sp.42	1	1	0.658			1	0.6

Asco TP sp.43	1			1	0.658			1	0.6
Asco TP sp.44	1			1	0.658			1	0.6
Asco TP sp.45	1			1	0.658			1	0.6
Asco TP sp.46	1			1	0.658			1	0.6
Asco TP sp.47	1			1	0.658			1	0.6
Asco TP sp.48	1			1	0.658			1	0.6
Asco TP sp.49	1			1	0.658			1	0.6
Asco TP sp.50	1			1	0.658			1	0.6
Asco TP sp.51	1			1	0.658			1	0.6
Ascocratera manglicola Kohlm.	2	4	2.020			1	0.541	5	3.2
Cryptosphaeria mangrovei K.D. Hyde	1			1	0.658			1	0.6
Dactylospora haliotrepha (Kohlm. et E. Kohlm.) Hafellner	3	12	6.061	16	10.526	13	7.027	41	25.9
Dactylospora mangrovei E.B.G. Jones, Alias, Abdel Wahab et Hsieh	1			1	0.658			1	0.6
Eutypa bathurstensis K.D. Hyde & Rappaz	1	2	1.010					2	1.3
Eutypa sp. 1	1	1	0.505					1	0.6
Eutypa sp. 2	1	1	0.505					1	0.6
Eutypa sp. 3	1			2	1.316			2	1.3
Eutypella naqsii K.D. Hyde	2	1	0.505					1	0.6
Eutypella sp.	1			1	0.658			1	0.6
Haloresellinia oceanica (Schatz) Whalley, E.B.G Jones, K.D. Hyde et Laessoe	3	8	4.040	11	7.237	19	10.270	38	24.1
Halosarpheia minuta W.F. Leong	2	1	0.505			3	1.622	4	2.5
Hypoxylon CR sp. 1	1	1	0.505					1	0.6
Hypoxylon CR sp. 2	1	2	1.010					2	1.3
Hypoxylon TP sp. 3	1			5	3.289			5	3.2
Hypoxylon TP sp. 4	1			1	0.658			1	0.6
Hypoxylon TP sp. 5	1			1	0.658			1	0.6
Julella avicenniae (Borse) K.D. Hyde	3	1	0.505	5	3.289	2	1.081	8	5.1
Kallichroma glabrum (Kohlm.) Kohlm. et Volkm. -Kohlm.	1					2	1.081	2	1.3

Kallichroma tethys (Kohlm. et E. Kohlm.) Kohlm. et Volkm. Kohlm.	3	10	5.051	14	9.211	9	4.865	33	20.9
Leptosphaeria australiensis (Cribb & J.W. Cribb) G.C. Hughes	2	4	2.020	1	0.658			5	3.2
Linocarpon appendiculatum K.D. Hyde	1					3	1.622	3	1.9
Lulworthia fucicola G.K. Sutherl	1					1	0.541	1	0.6
Lulworthia grandispora Meyers	1					4	2.162	4	2.5
Lulworthia sp.1	1	1	0.505					1	0.6
Lulworthia sp.2	1					2	1.081	2	1.3
Marinosphaeria mangrovei K.D. Hyde	1			1	0.658			1	0.6
Massarina sp.1	1					1	0.541	1	0.6
Morosphaeria ramunculicola (K.D. Hyde) Suetrong, Sakay., E.B.G. Jones et C.L. Schoch	2	1	0.505			12	6.486	13	8.2
Morosphaeria velatispora (K.D. Hyde et Borse) Suetrong, Sakay., E.B.G. Jones et C.L. Schoch	3	5	2.525	3	1.974	17	9.189	25	15.8
Nais inornata Kohlm.	1	2	1.010					2	1.3
Natantispora retorquens (Shearer et J.L. Crane) J. Campb., J.L. Anderson et Shearer	2	2	1.010	1	0.658			3	1.9
Oceanitis cincinnatula (Shearer & J.L. Crane) J. Dupont & E.B.G. Jones	2	1	0.505					1	0.6
Oceanitis viscidula (Kohlm.) J. Dupont et E.B.G. Jones	1			1	0.658			1	0.6
Phaeosphaeria sp. 1	1	1	0.505					1	0.6
Phaeosphaeria sp. 2	1			1	0.658			1	0.6
Pleospora TP sp.1	1			1	0.658			1	0.6
Pyrenographa xylographoides Atproot	3	1	0.505	1	0.658	6	3.243	8	5.1
Quintaria lignatilis (Kohlm.) Kohlm. et Volkm. Kohlm.	3	3	1.515	10	6.579	3	1.622	16	10.1
Rhizophila marina K.D. Hyde et E.B.G. Jones	2			1	0.658	5	2.703	6	3.8
Saagaromyces abonnis (Kohlm.) K.L Pang et E.B..G. Jones	3	6	3.030	1	0.658	2	1.081	9	5.7
Saagaromyces ratnagiriensis (Patil et Borse) K.L Pang et E.B.G. Jones	3	1	0.505	1	0.658	1	0.541	3	1.9
Savoryella lignicola E.B.G Jones et R.A Eaton	3	5	2.525	6	3.947	2	1.081	13	8.2
Savoryella paucispora (Cribb et J.W. Cribb) Koch	1			2	1.316			2	1.3
Trematosphaeria mangrovis Kohlm.	1	4	2.020					4	2.5
Verruculina enalia (Kohlm.) Kohlm. et Volkm.-Kohlm.	3	8	4.040	3	1.974	13	7.027	24	15.2

Anamorphic fungi (29)									
Cirrenalia sp.	1	1	0.505					1	0.6
Deuteromycete 01	1	1	0.505					1	0.6
Deuteromycete 02	1	1	0.505					1	0.6
Deuteromycete 03	1	2	1.010					2	1.3
Deuteromycete 04	1	1	0.505					1	0.6
Deuteromycete 05	1	1	0.505					1	0.6
Deuteromycete 06	1	1	0.505					1	0.6
Deuteromycete 07	1	1	0.505					1	0.6
Deuteromycete 08	1	3	1.515					3	1.9
Deuteromycete 09	1					2	1.081	2	1.3
Deuteromycete 10	1					1	0.541	1	0.6
Deuteromycete 11	1					3	1.622	3	1.9
Deuteromycete 12	1			1	0.658			1	0.6
Deuteromycete 13	1			1	0.658			1	0.6
Deuteromycete 14	1			1	0.658			1	0.6
Deuteromycete 15	1			1	0.658			1	0.6
Deuteromycete 16	1			1	0.658			1	0.6
Deuteromycete 17	1			1	0.658			1	0.6
Deuteromycete 18	1			1	0.658			1	0.6
Dictyosporium sp.	1	1	0.505					1	0.6
Humicola alopallonella (Meyers et R.T. Moore) Kohlm. et Volkm.-Kohlm.	2	4	2.020	2	1.316			6	3.8
Matsusporium tropicale (Kohlm.) E.B.G. Jones et K.L. Pang	1			1	0.658			1	0.6
Periconia prolifica Anastasiou	1	1	0.505					1	0.6
Phoma glomerata (Corda) Wollenw. et Hochapfel	1	4	2.020					4	2.5
Phoma sp. 3	3	7	3.535	3	1.974	9	4.865	19	12.0
Phoma suaedae Jaap.	3	2	1.010	2	1.316	1	0.541	5	3.2
Trichocladium achrasporum (Meyers et R.T. Moore) M. Dixon ex Shearer et J.L. Crane	3	5	2.525	1	0.658	3	1.622	9	5.7

Trichocladium melhae E.B.G. Jones, Abdel-Wahab & Vrijmoed	2	3	1.515	3	1.974			6	3.8
Trichocladium sp. 1	2	3	1.515	1	0.658			4	2.5
Basidiomycota (2)									
Halocyphina villosa Kohlm. et E.Kohlm.	3	14	7.071	4	2.632	5	2.703	23	14.6
Nia vibrissa R.T. Moore et Meyers	1	3	1.515					3	1.9
Total no. of occurrence		198		152		185		535	
Total no. of species		68		71		40		136	
No. of samples examined		202		154		180		536	
Samples colonized by fungi		187		127		123		437	
Samples with no fruiting body		15		27		17			
Empty perithecia		14		12		12			
% colonization		92.6		83.0		68.0		81.7	
Ave. no. of fungi per sample		1.054		1.230		1.090		1.001	

4.1.2 Descriptive statistics [Shannon-Weiner index (H'), H'evenness, Species richness (Margalef)]

(Results of all descriptive statistics were shown in tables as below)

Table 4.2 Most common (10% occurrence) and common species (5–10% occurrence) at the three study sites.

Study Sites	Most Common (>10%)	Common (5-10%)
Cape Rachado		*Aigialus grandis* *Aigialus parvus* *Dactylospora haliotrepha* *Kallichroma tethys* *Halocyphina villosa*
Telok Pelandok	*Dactylospora haliotrepha*	*Haloresellinia oceanica* *Kallichroma tethys*
Morib		*Aigialus grandis* Asco MB sp. 20 *Dactylospora haliotrepha* *Haloresellinia oceanica* *Morosphaeria ramunculicola* *Morosphaeria velatispora* *Verruculina enalia*

Table 4.3 Diversity indices in marine fungal communities collected from Cape Rachado, Telok Pelandok, Bachok, Jambongan Island, Mandidarah Island and Malawali Island (see Figure 1 for locations).

	Cape Rachado	Telok Pelandok	Morib
Shannon-Wiener Diversity Index, H'	3.79	3.77	3.25
Simpson's Index of Diversity (1 - D)	0.97	0.96	0.95
Evenness	0.898	0.883	0.880
Richness	68	71	40
Total abundance	198	152	185

Table 4.4 Sorenson's similarity indices between the study sites in Malaysia (see Figure 1 for locations of sites).

	Cape Rachado	Telok Pelandok	Morib
Cape Rachado	-	-	-
Telok Pelandok	0.302	-	-
Morib	0.370	0.342	-

4.1.3 Light micrographs of selected marine fungi from Malaysia (Ascomycota, Anamorphic Fungi and Basidiomycota). Overview of the decaying wood samples collection with the presence of ascomata, asci and its ascospores.

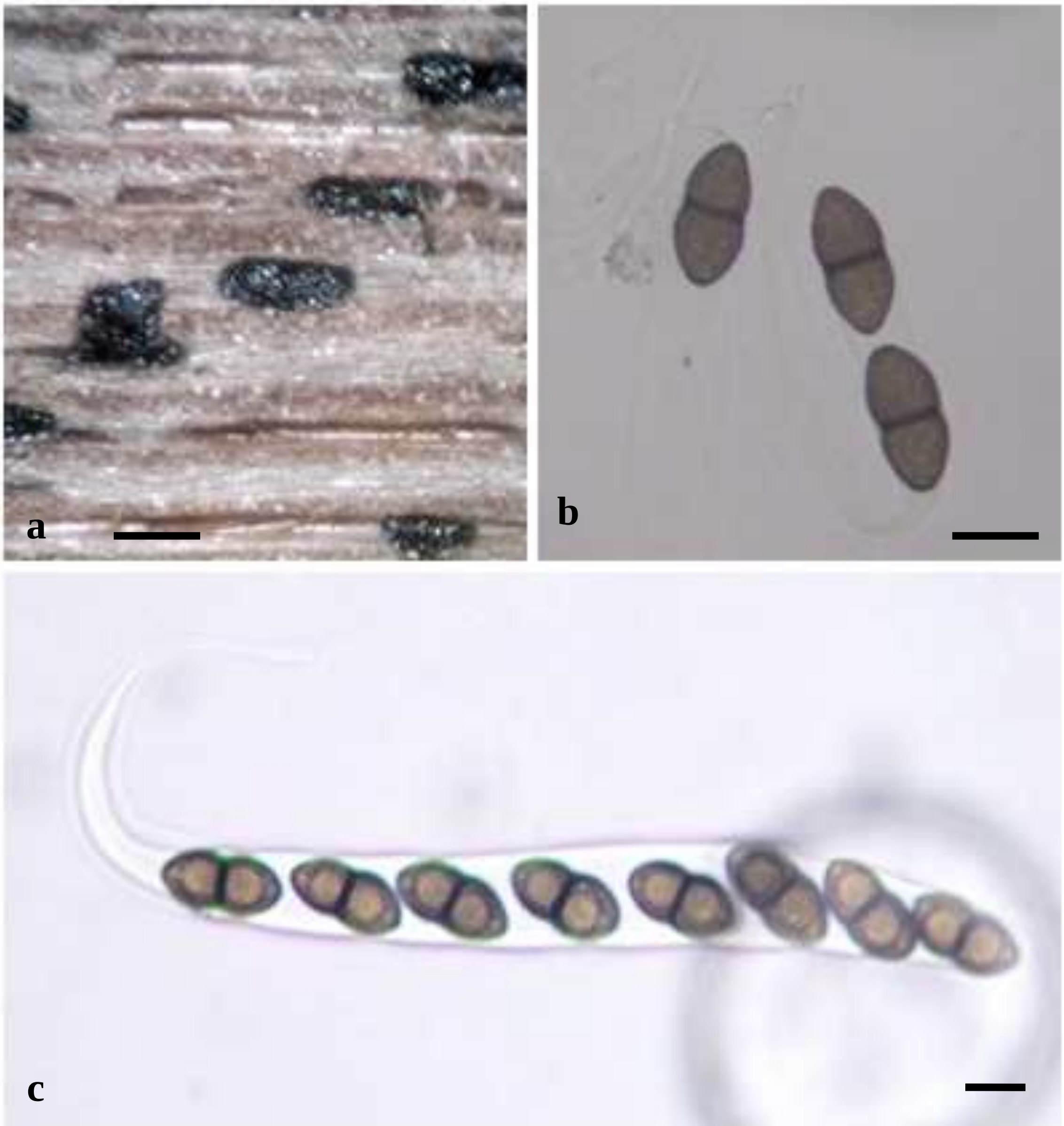


Figure 4.1 *Verruculina enalia*. a) Carbonaceous, black, partially-immersed ascomata b) Dark brown, verrucose and constricted at the septum ascospore with one septate (17-22.5 x 8.5-10 µm) c) Obliquely uniseriate with eight ascospores in ascus. Asci is pedunculate, cylindrical, bitunicate and without apical apparatus. a= 150 µm, b-c= 10 µm.

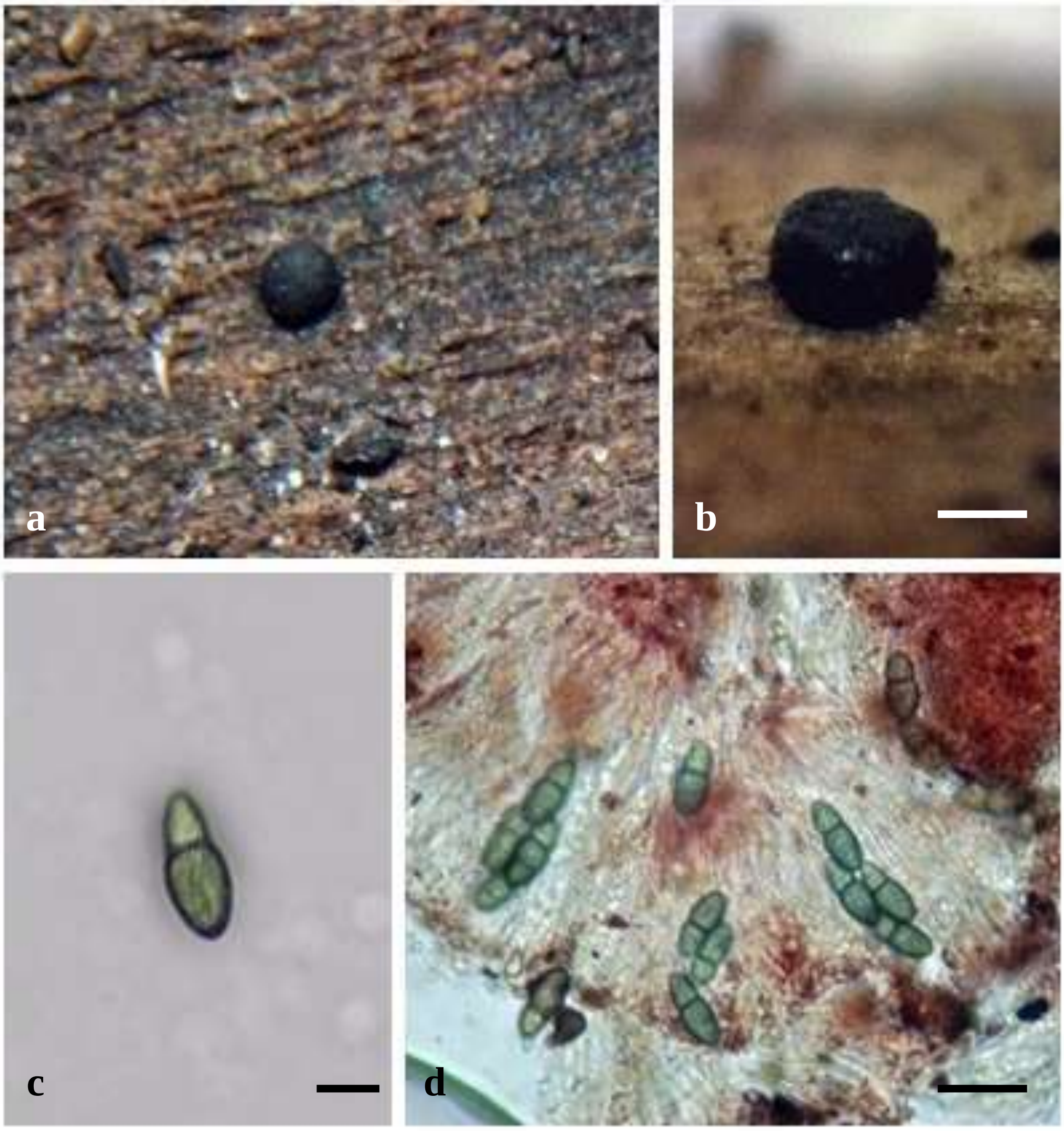


Figure 4.2 *Dactylospora haliotrepha*. a) Aerial view of ascomata showing coriaceous dark reddish brown to black fruiting body b) Side view of the superficial ascomata; subglobose or flat or convex, discoid, apothecia like c) Grayish green to brownish with delicate longitudinal striations with 1-septate ascospore and constricted at the septum (17-25 x 8-11) d) Ascospores attached to pseudoparaphyses. b= 250 µm, c= 10 µm, d= 30 µm.

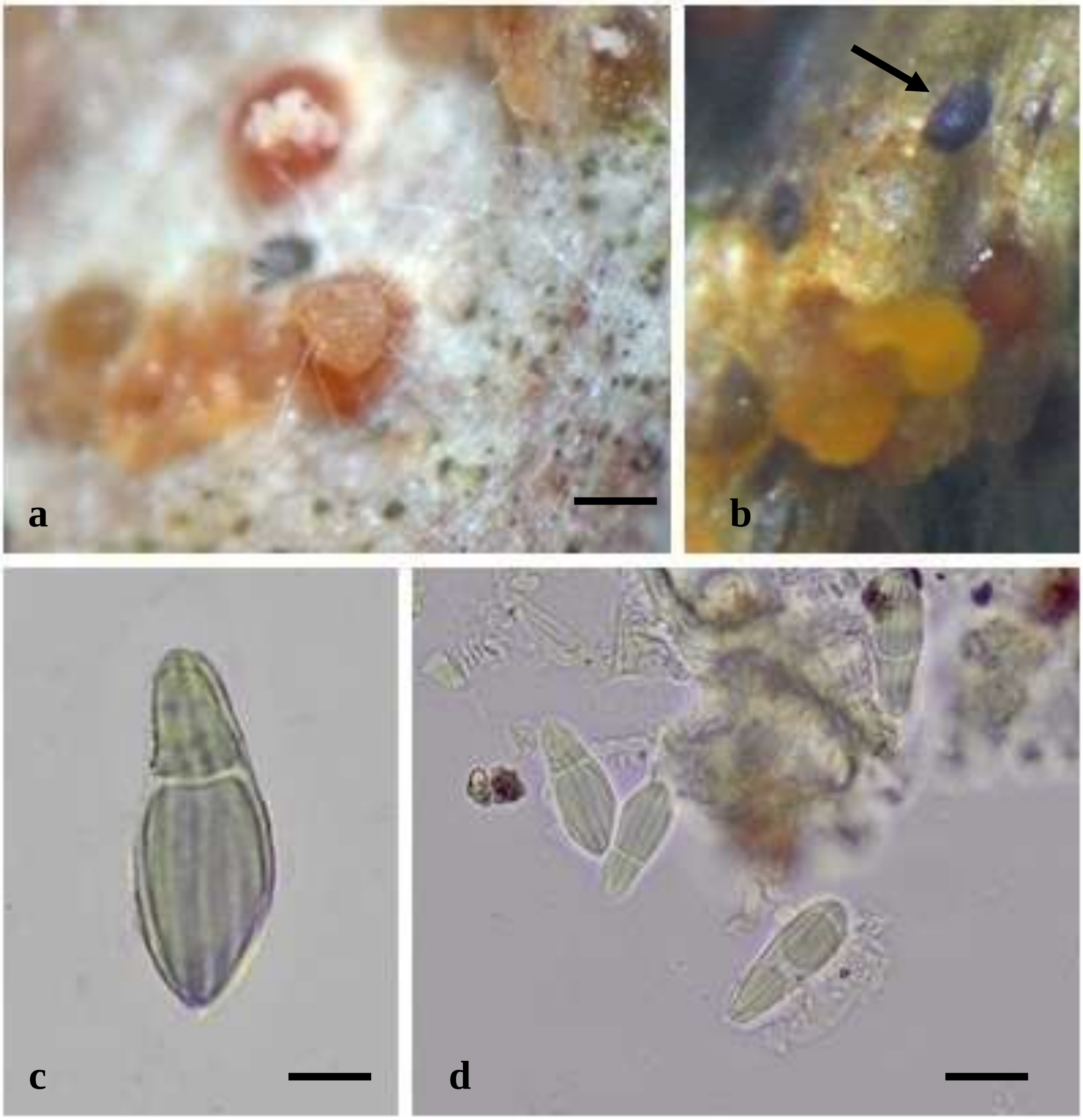


Figure 4.3 *Kallichroma tethys*. a) Coriaceous orange yellowish ascomata, gregarious, subglobose or depressed ellipsoidal b) The presence of fruing bodies of another marine fungi species (arrow) c-d) Hyaline ascospores with one septate that slightly constricted at the septum, longitudinal ridges from one pole to the other, ellipsoidal or ovoid (16-24 x 8-12 µm). a= 300 µm, c= 5 µm, d= 10 µm.

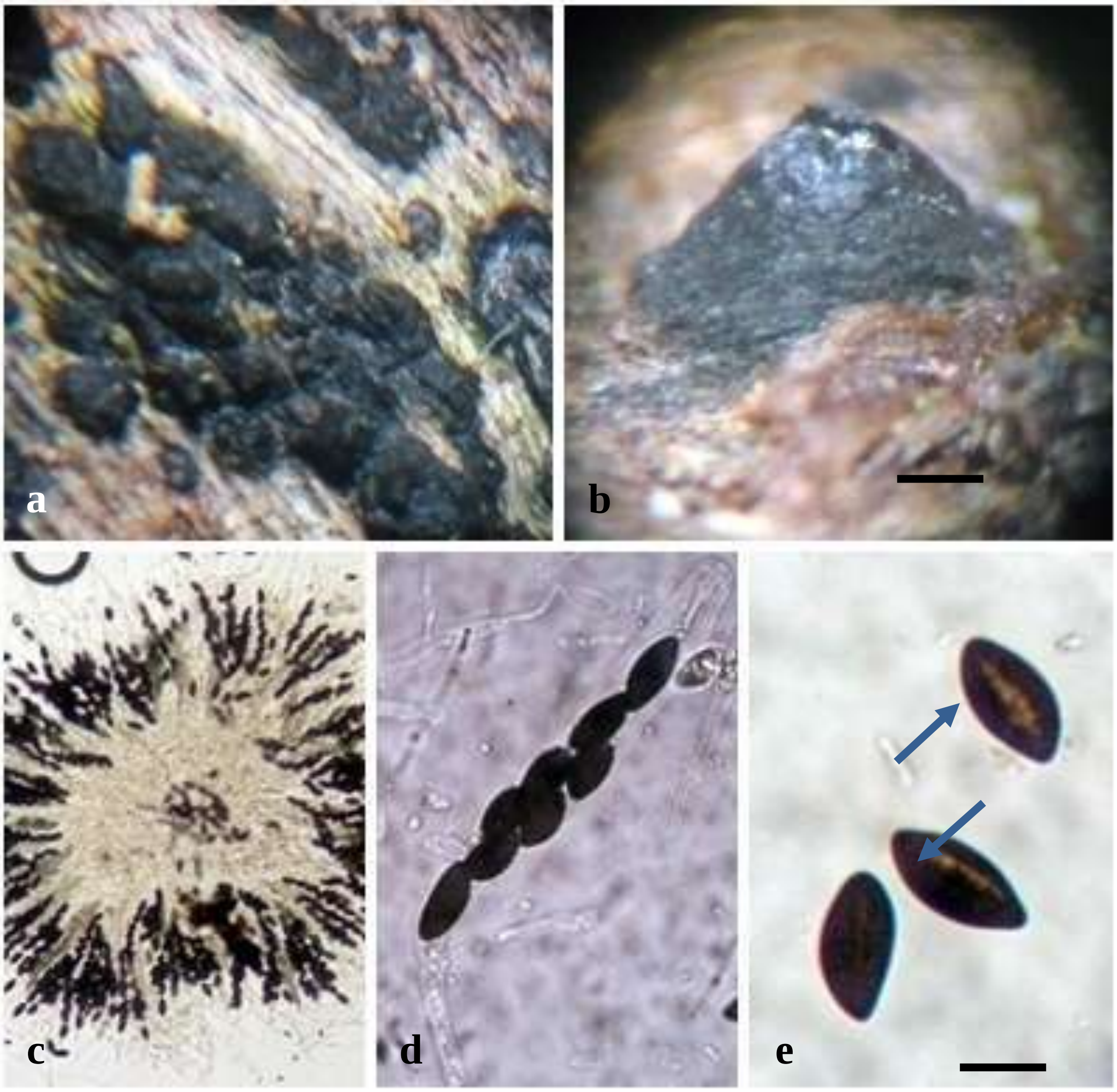


Figure 4.4 *Halorosellinia oceanica* a) Black carbonaceous ascomata on *Rhizophora apiculata* b) Side view of the subglobose ascomata c) Paraphyses persistent, broad, 2-2.5 µm diam. d) Brown, one-celled, subglobose to broadly elliptic with a straight germ slit, monostichously arranged in the ascus (19-24.5 x 8-12.5 µm) e) germ slit presence (arrow). b= 96 µm, e= 11 µm.

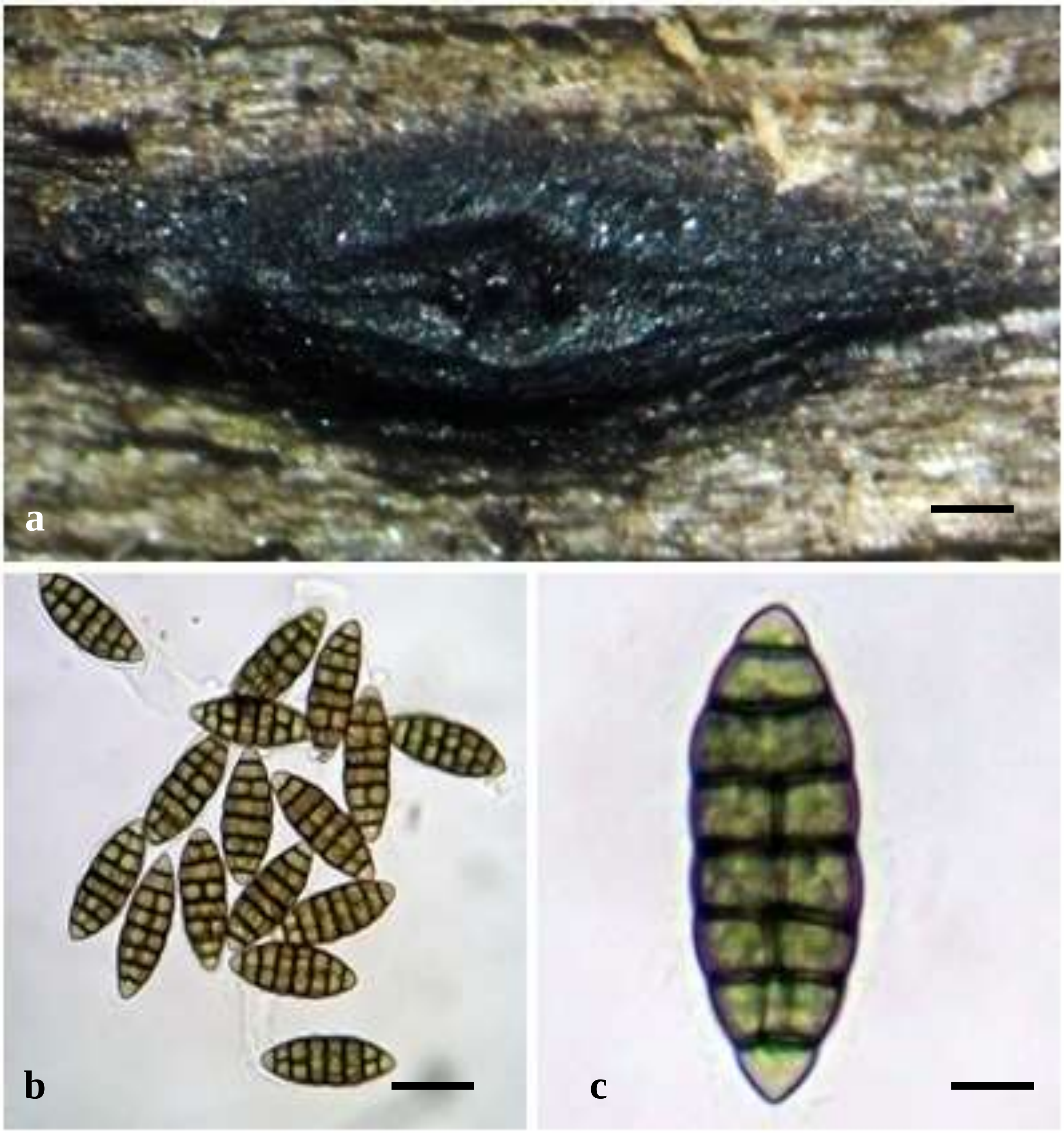


Figure 4.5 *Aigialus parvus* a) Aerial view; Black carbonaceous to coriaceous ascomata, three-fourths immersed in a black stroma with a longitudinal furrow at the top, ostiolate, gregarious b) Ascospores are yellow-brown with hyaline to light brown for its apical cells c) Ascospore with (9-) 10-11 (-12) transverse septa and 1-3 longitudinal septa in all but the end cells, slightly constricted at the septa (45-60 x 16-23 µm). a= 15 µm, b= 30 µm, c= 3 µm.

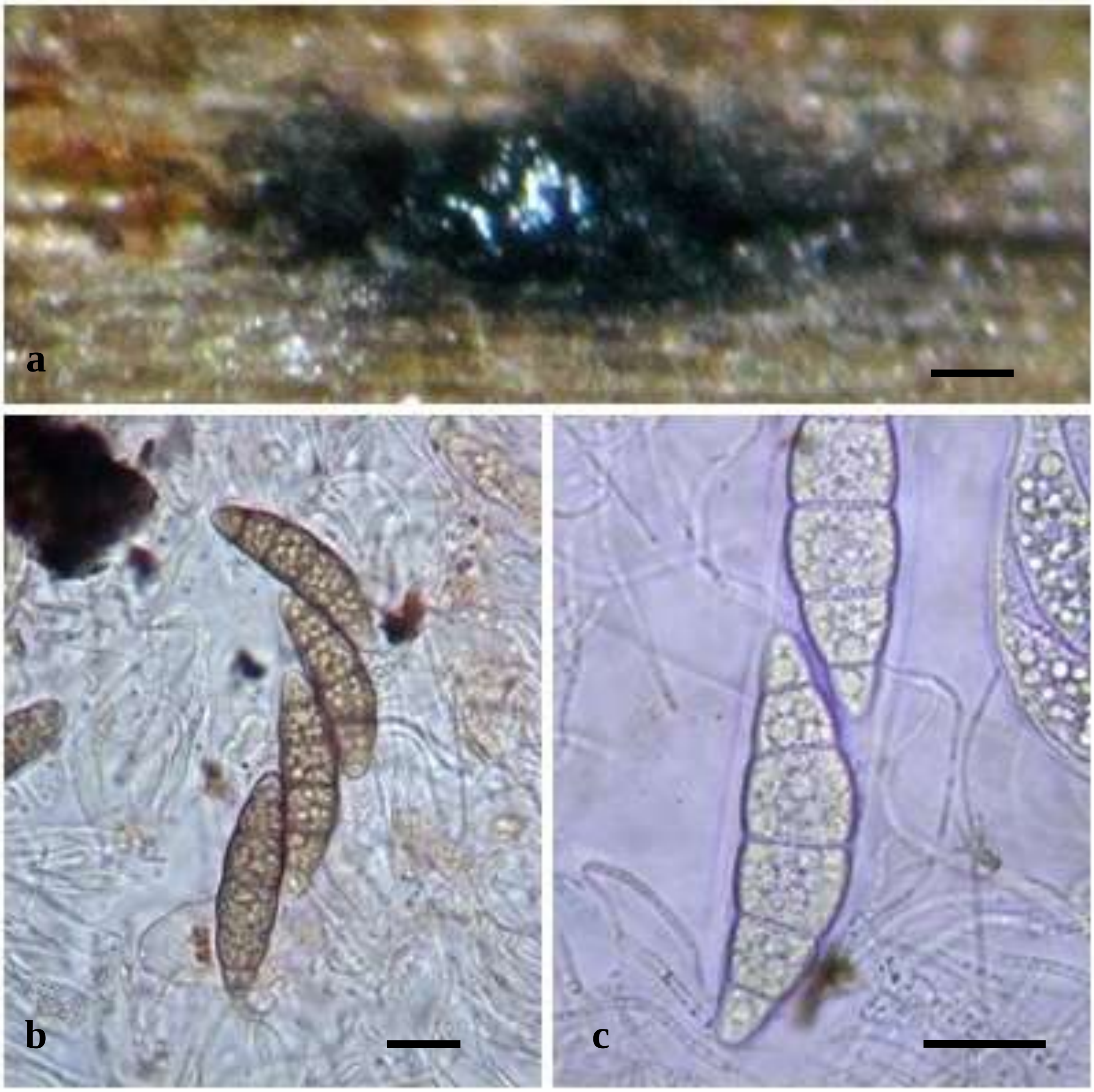


Figure 4.6 *Phaeospharia* sp.1 a) Black membranous ascomata, partially-immersed, solitary b) Light brown and becoming darker in age, biseriate, fusiform and slightly curved c) Young ascospores with 5 septate, strongly constricted at the septum. a= 120 µm, b= 25 µm, c= 30 µm.

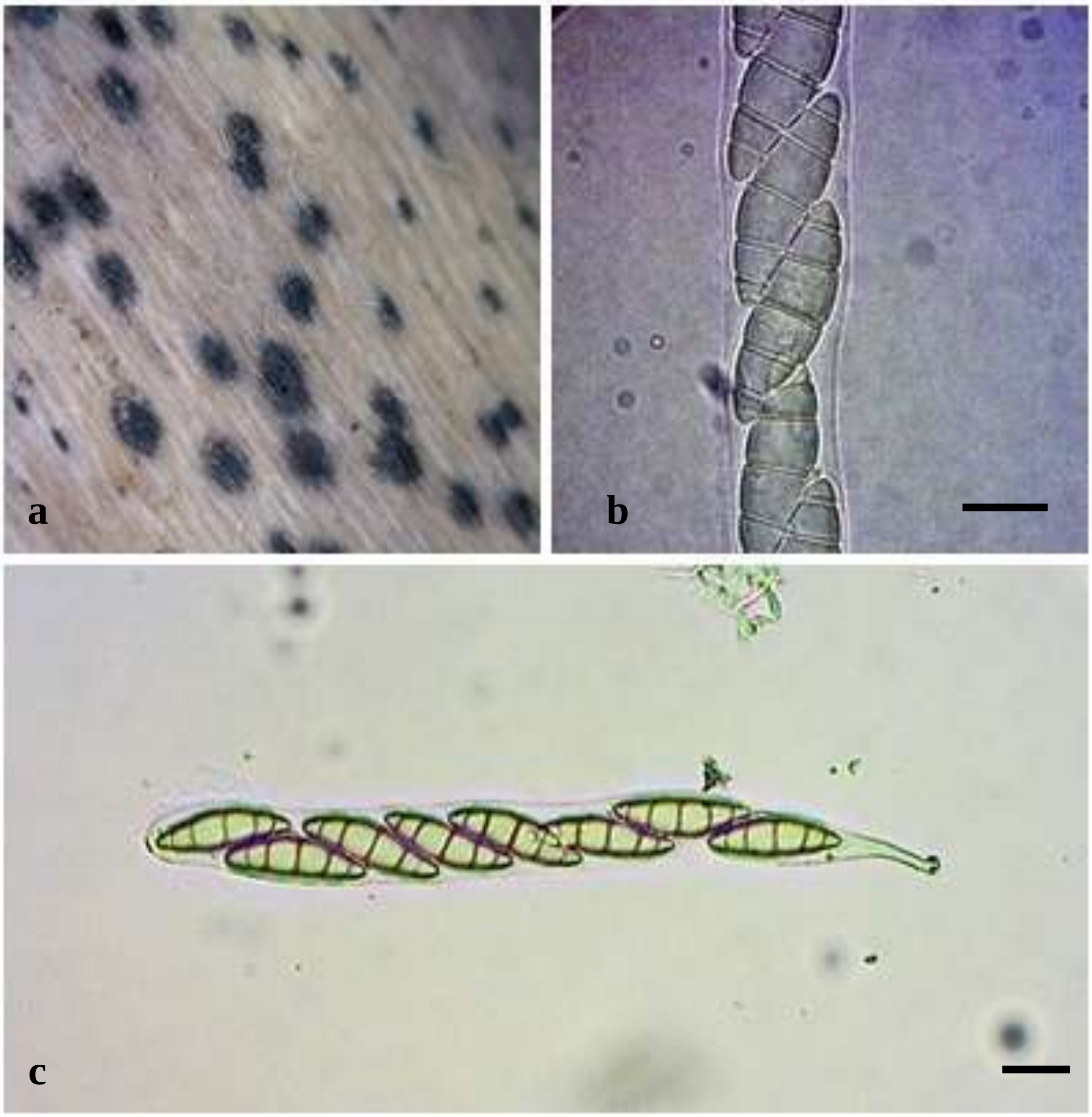


Figure 4.7 *Quintaria lignatilis* a) Ascomata; Black, carbonaceous to coriaceous, immersed ostiolate, papillate, gregarious. b) Hyaline 5-septate ascospores, fusiform, slightly constricted at septa, smooth-walled (50-76 x 14-20 µm) c) Ascus cylindrical, pedunculate, thick-walled, bitunicate with 8 biseriate ascospores. b,c= 20 µm

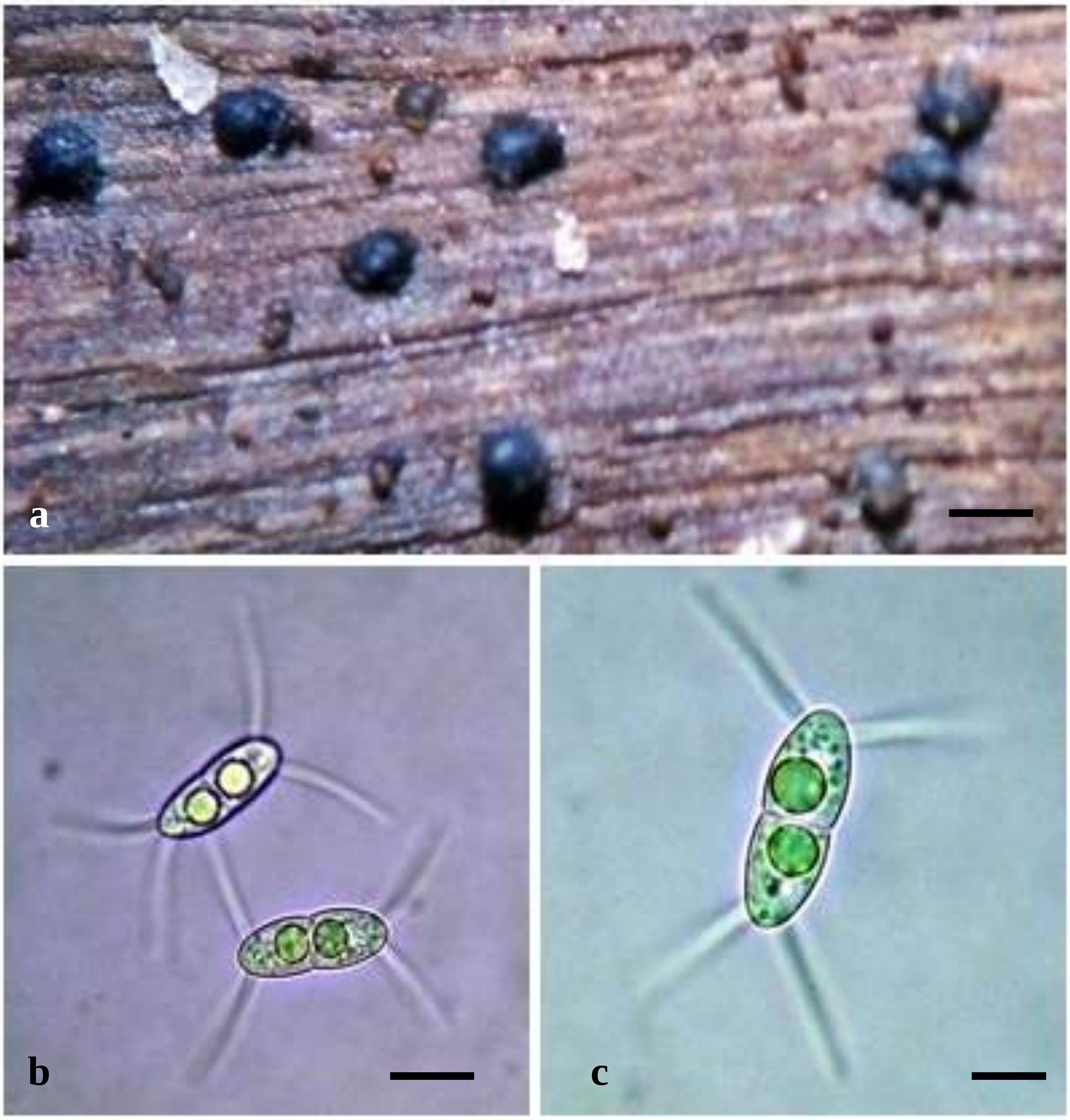


Figure 4.8 *Antennospora quadricornuta* a) Black, immersed or becoming exposed ascomata, coriaceous to carbonaceous, gregarious. b-c) Hyaline ascospores (20-35 × (6-)8-12 µm) with one septate, slightly constricted at the septum, cylindrical appendages at both ends in juxtaposition , stiff appendages (20-37 µm long, 1-2 µm diam.) a= 200 µm, b= 15 µm, c= 10 µm.

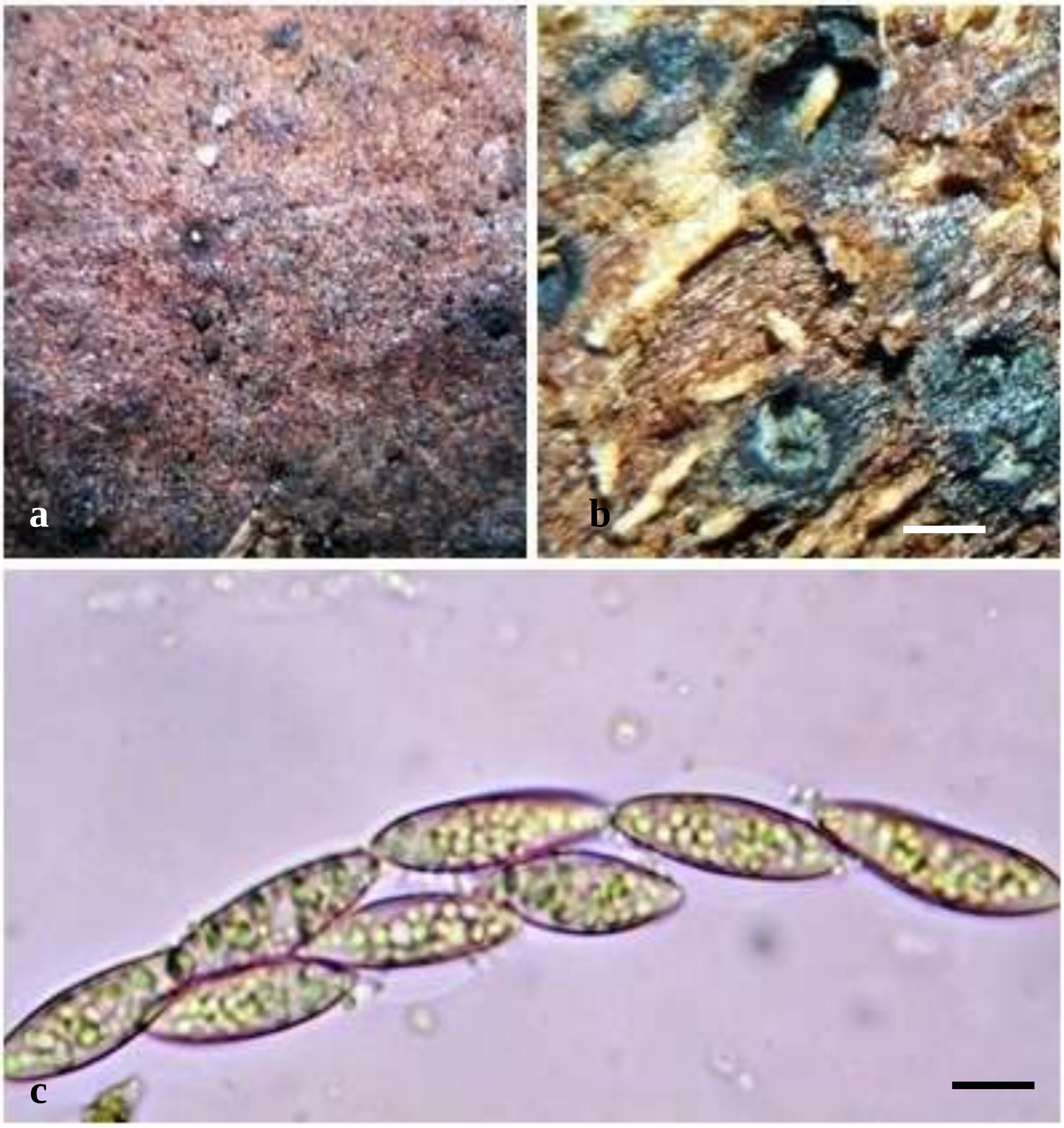


Figure 4.9 *Rhizophila marina* a) Black ascomata and completely immersed in the substrate b) Aerial view after wood surface were sliced; Coriaceous, globose to subglobose, ostiolate, papillate, gregarious ascomata c) Yellowish to yellowish brown at maturity, hyaline when immature, one celled, ellipsoidal to fusiform (20-32 x 6-10 µm). b= 600 µm, c= 9 µm.

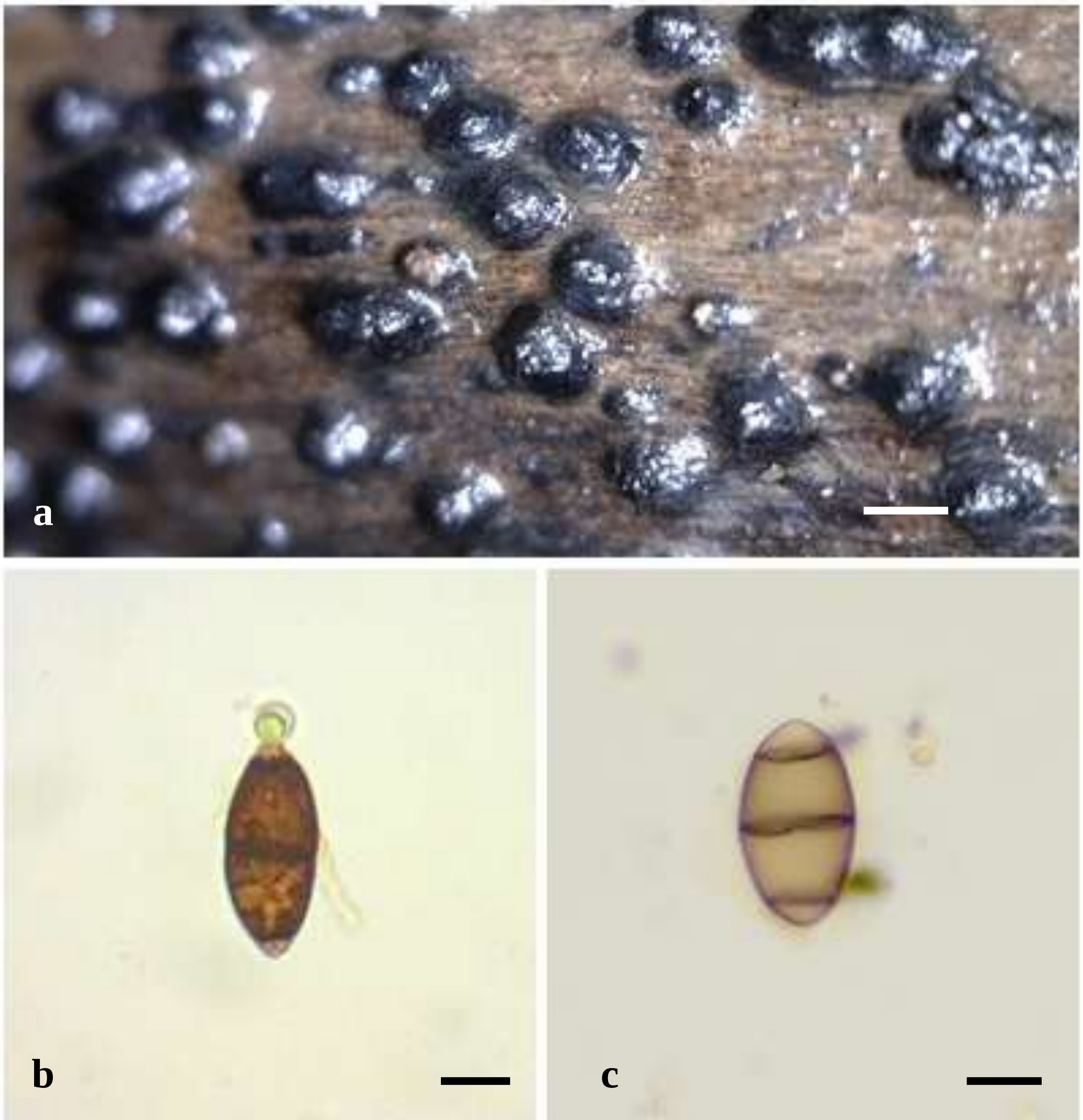


Figure 4.10 *Savoryella lignicola* a) Ascomata; black, partially-immersed to superficial, ostiolate, papillate, membranaceous b) Mature ascospore; Ellipsoidal, 3-septate and slightly constricted at the septa, large central cells are brown (10-6-16 μm), with small hyaline apical cells (2.6-6 μm), ascospore 24-36 × 8-12 μm c) Young ascospore. a= 200 μm, b,c= 10 μm.

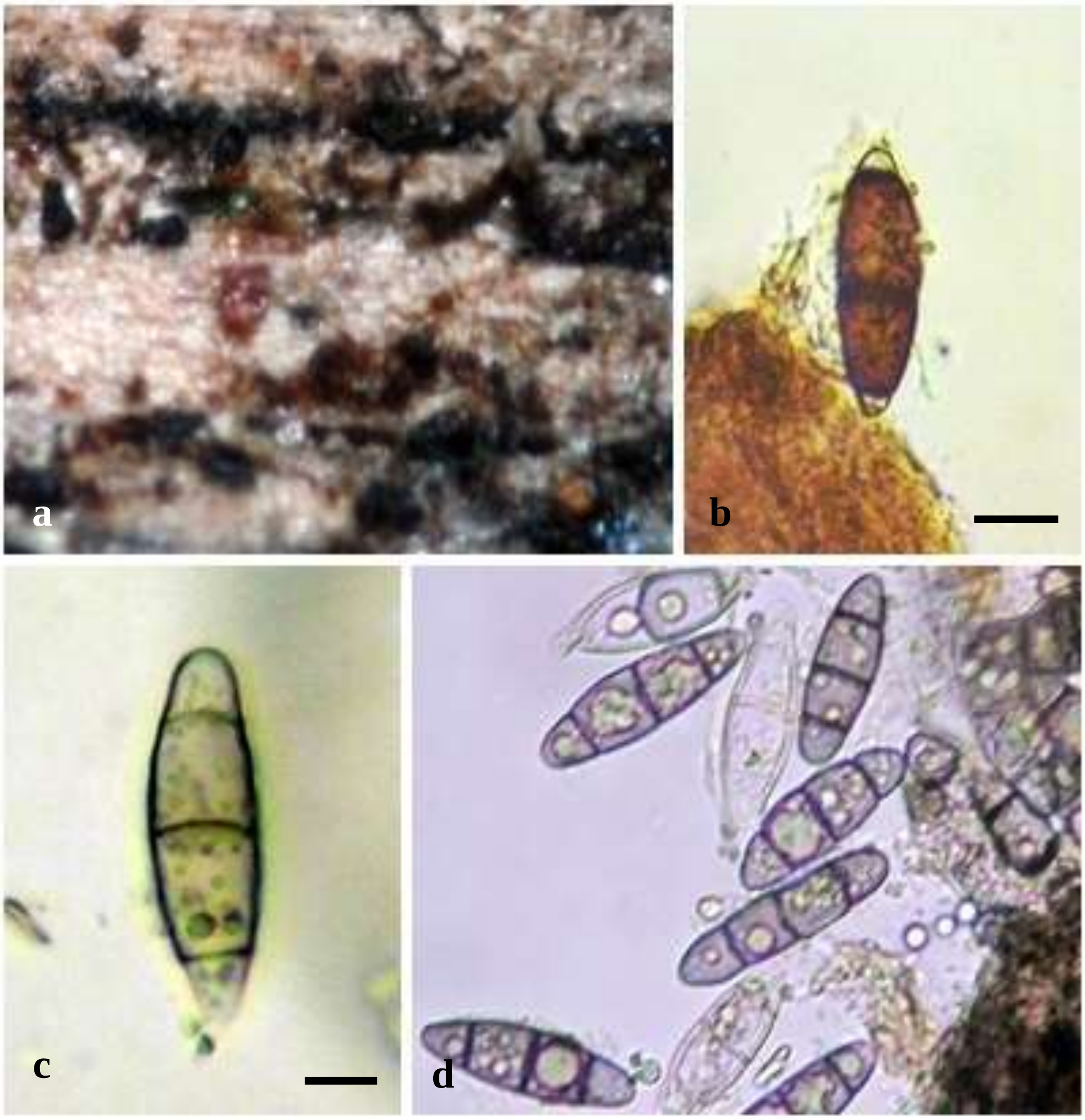


Figure 4.11 *Savoryella paucispora* a) Ascomata immersed, ostiolate, papillate, membranaceous, dark brown, gregarious b) Mature ascospore; Fusoid-ellipsoidal, three-septate, slightly constricted at the septa, central cells brown, apical cells hyaline without appendages, ascospores (36-)44-50(-60) × 12-16.5 μm c-d) Young ascospores. b= 15 μm, c= 10 μm.

Figure 4.12 *Aigialus grandis* a) Ascomata subglobose, partially immersed in a black stroma, carbonaceous to coriaceous, black, gregarious b) Ascus cylindrical, fissitunicate, thick walled with 8-spored, biseriate c) Ascospores ellipsoidal to broadly fusiform, with 14-17 (-18) transverse septa and 1-3 longitudinal septa in all but the end cells, yellow-brown except for the hyaline or light brown apical cells (76.0-102.0 x 17.0-22.5 µm); Germ tube presence (arrow). a= 315 µm, b= 45 µm, c= 20 µm.

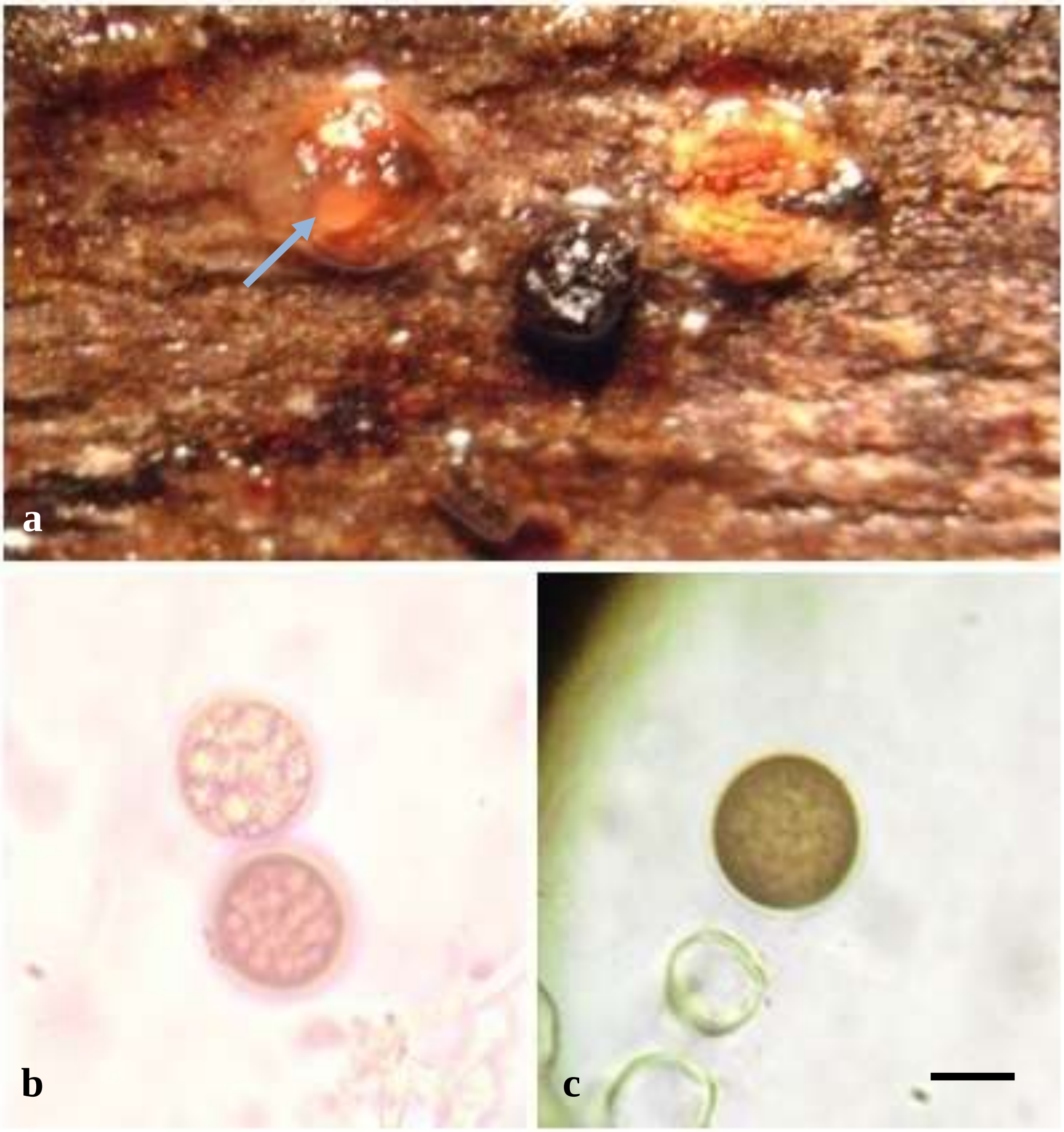


Figure 4.13 *Periconia prolifica* a) Conidiophores cylindrical, septate, simple or branched, hyaline, often forming pustules on the surface of the substrate (arrow) b-c) Conidia one-celled, subglobose or ovoid, smooth, thick-walled, light brown with a reddish tint or dark brown, developing basipetally, catenulate, cells finally separating (6-11.5 µm diam.) c= 5 µm

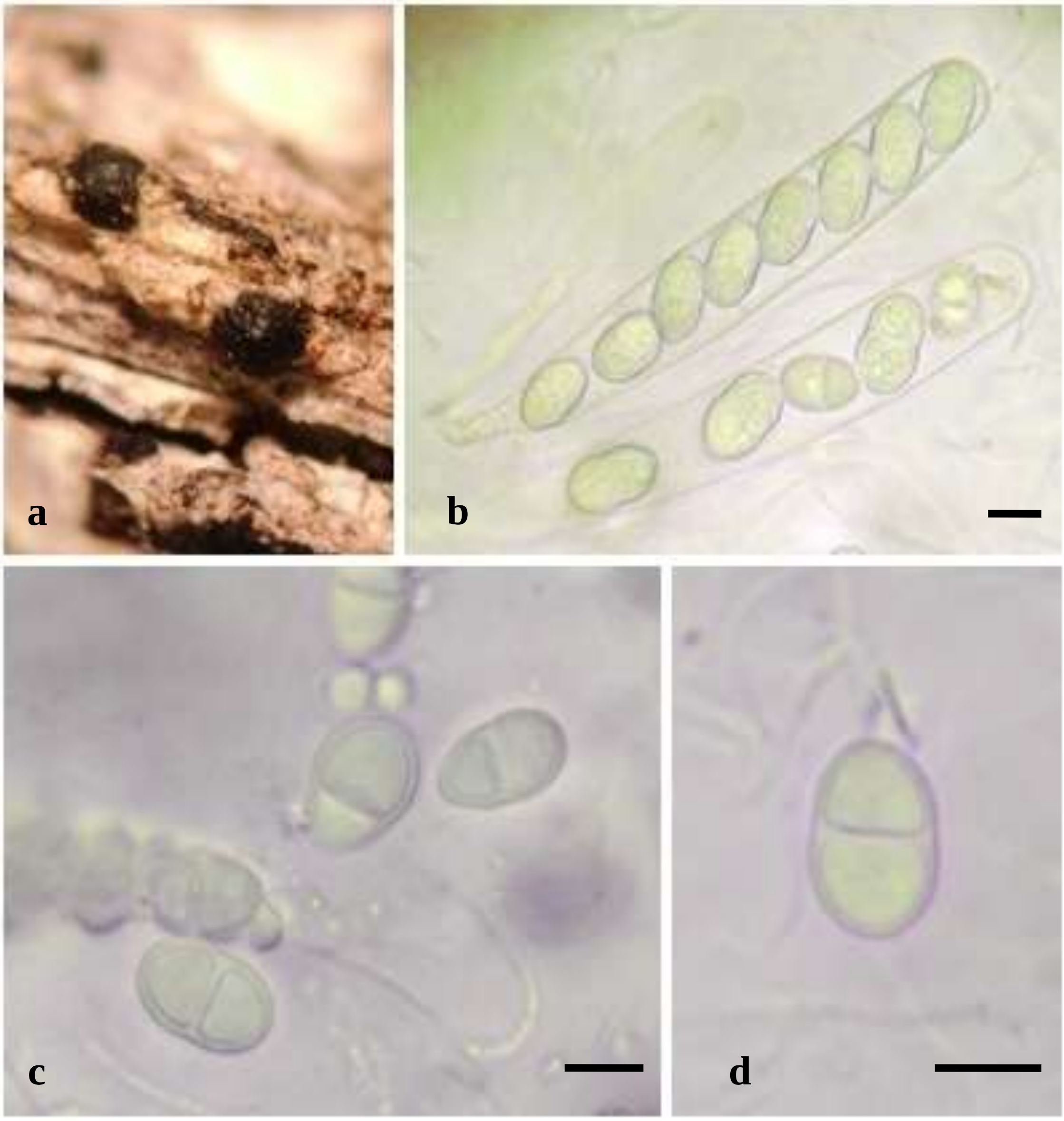


Figure 4.14 *Acrocordiopsis patilii* a) Black, carbonaceous, conical ascomata seated on a black stromata b) Ovoidal or ellipsoidal ascospores in cylindrical asci, bitunicate c-d) Ascospores; Hyaline to yellowish, 1-pseudoseptate ascospores, lacking a sheath or appendages. b,c,d= 10 µm

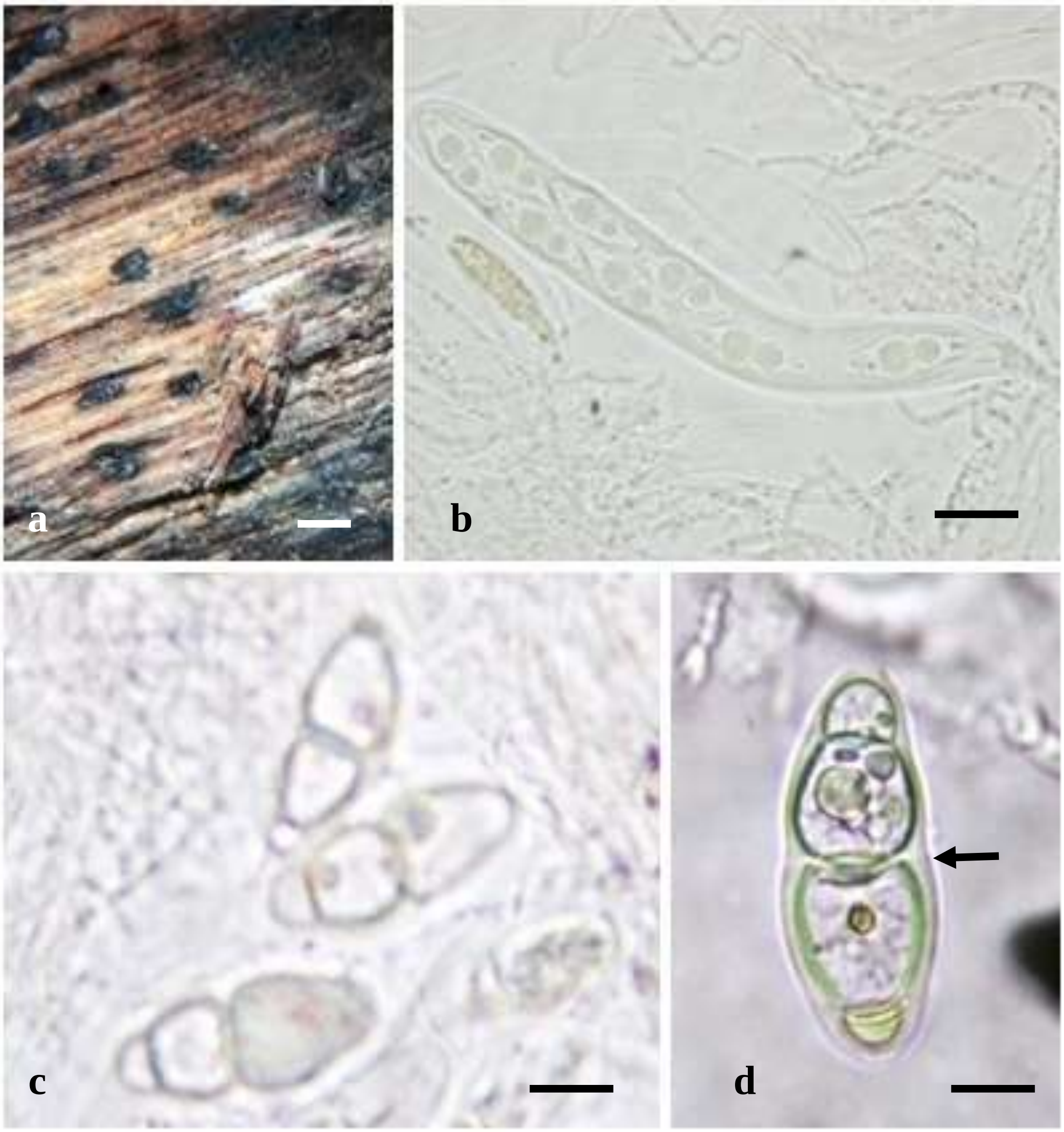


Figure 4.15 *Morosphaeria velatospora* a) Ascomata; Immersed, becoming erumpent, subglobose or depressed, ostiolate, papillate, coriaceous, brown to black, gregarious b) Asci; 8-spored, cylindrical, short pedunculate, bitunicate, thick-walled c-d) Ascospores; Hyaline, fusiform to ellipsoidal, initially 1-septate, later becoming 3-septate, central cells larger, surrounded by mucilaginous sheath (arrow) and constricted at the central septum (41-50 x 14-17 µm). a= 134 µm, b= 35 µm, c= 15 µm, d= 12 µm.

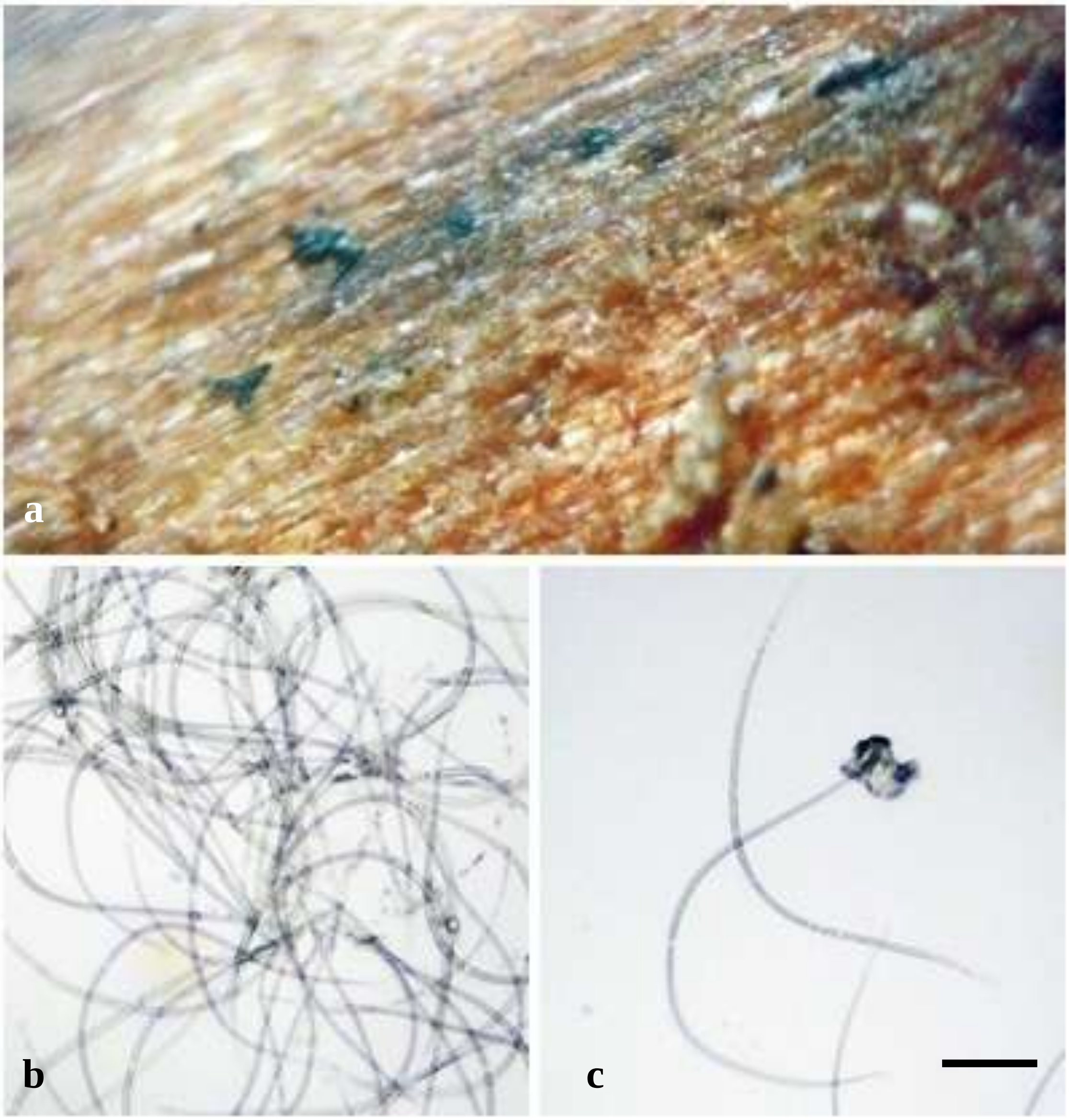


Figure 4.16 *Lulworthia grandispora* a) Ascomata immersed, brown to black, gregarious, subglobose to pyriform b-c) 530-650 x 3-5 mm (including apical chambers), filamentous, curved, hyaline, tapering at each end into an elongate, conical apical chamber, 5-10 mm long, acute or rounded, filled with mucus. c= 100 µm.

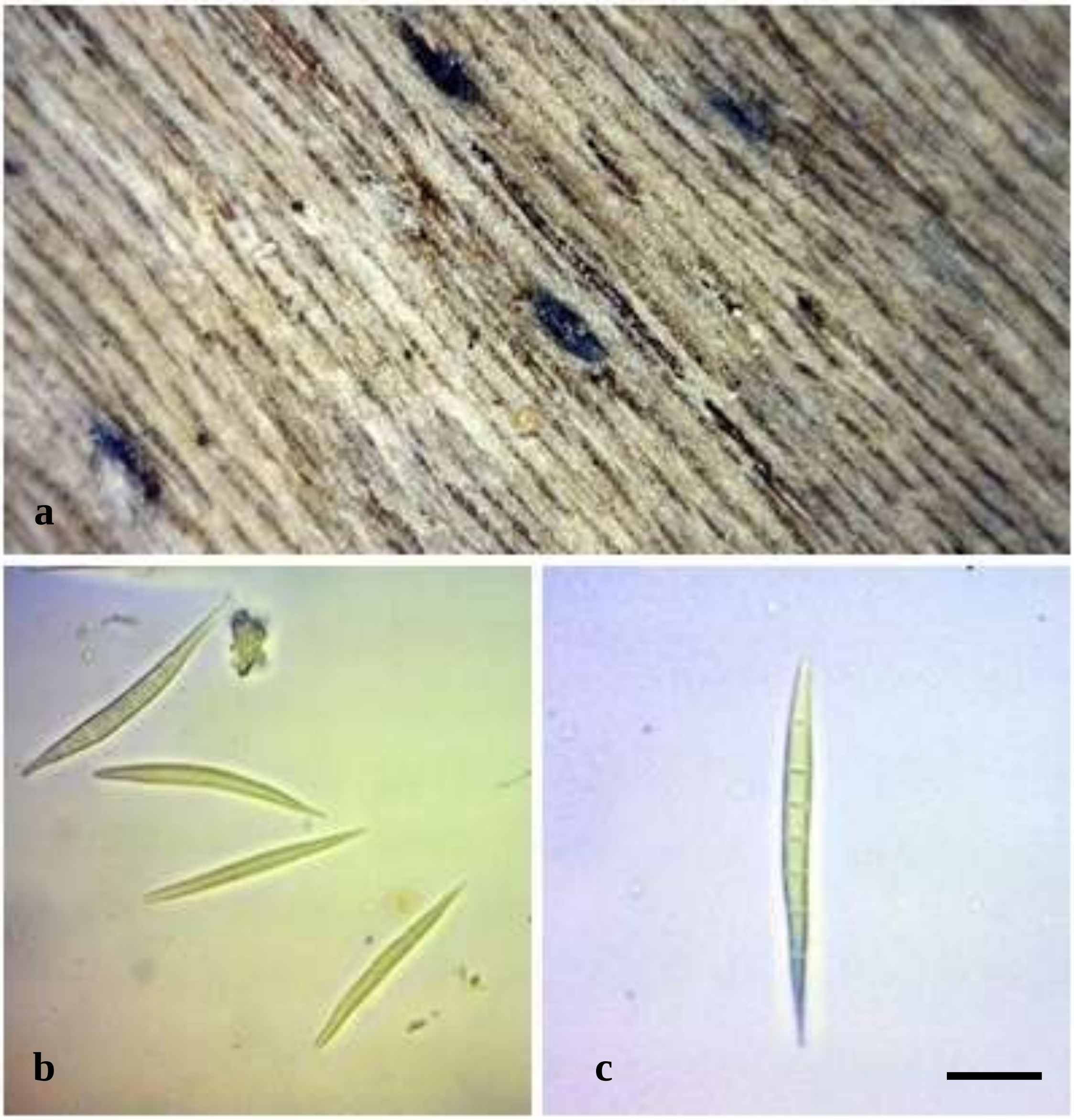


Figure 4.17 *Linocarpon* sp. a) Ascomata immersed developing below the clypeus, black, ostiolate, periphysate b-c) Ascospores hyaline, fasciculate, needle-shaped, tapering to a narrow point at the base, with refringent septum-like bands (3-7 septa) 50-72 x 4-7.5 µm. c= 23 µm.

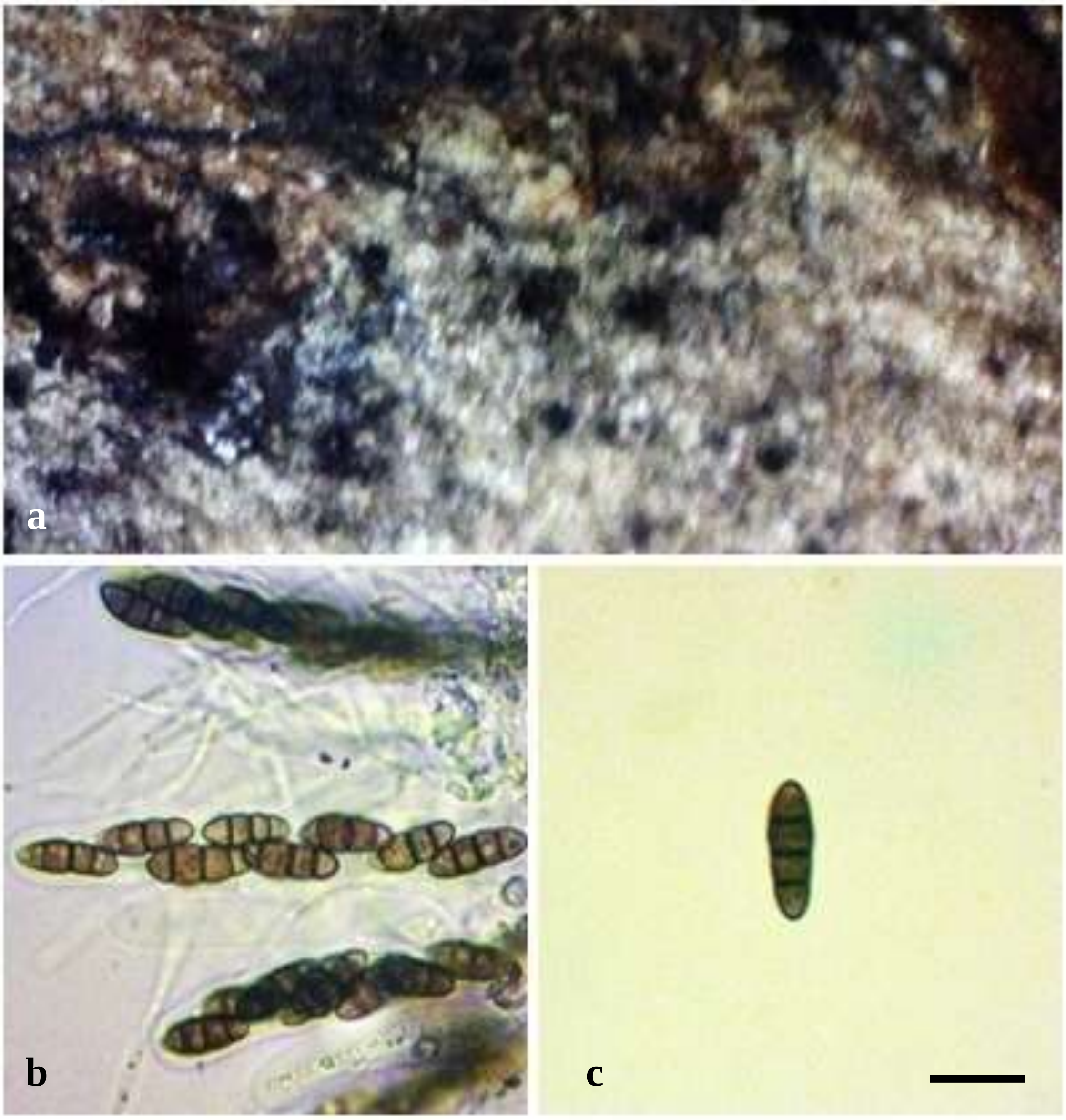


Figure 4.18 *Phaeosphaeria* sp.2 a) Ascomata; Coriaceous, black, immersed, papillate, ostiolate, obpyriform, gregarious b) Asci; 8-spored, pedunculate, clavate-fusiform, indistinctly bitunicate c) Ascospores; light to dark brown when mature, cylindrical-ellipsoidal, 3-septate, constricted at the septa, second cell from the top largest (18-24 x 5-7 µm). c= 14 µm.

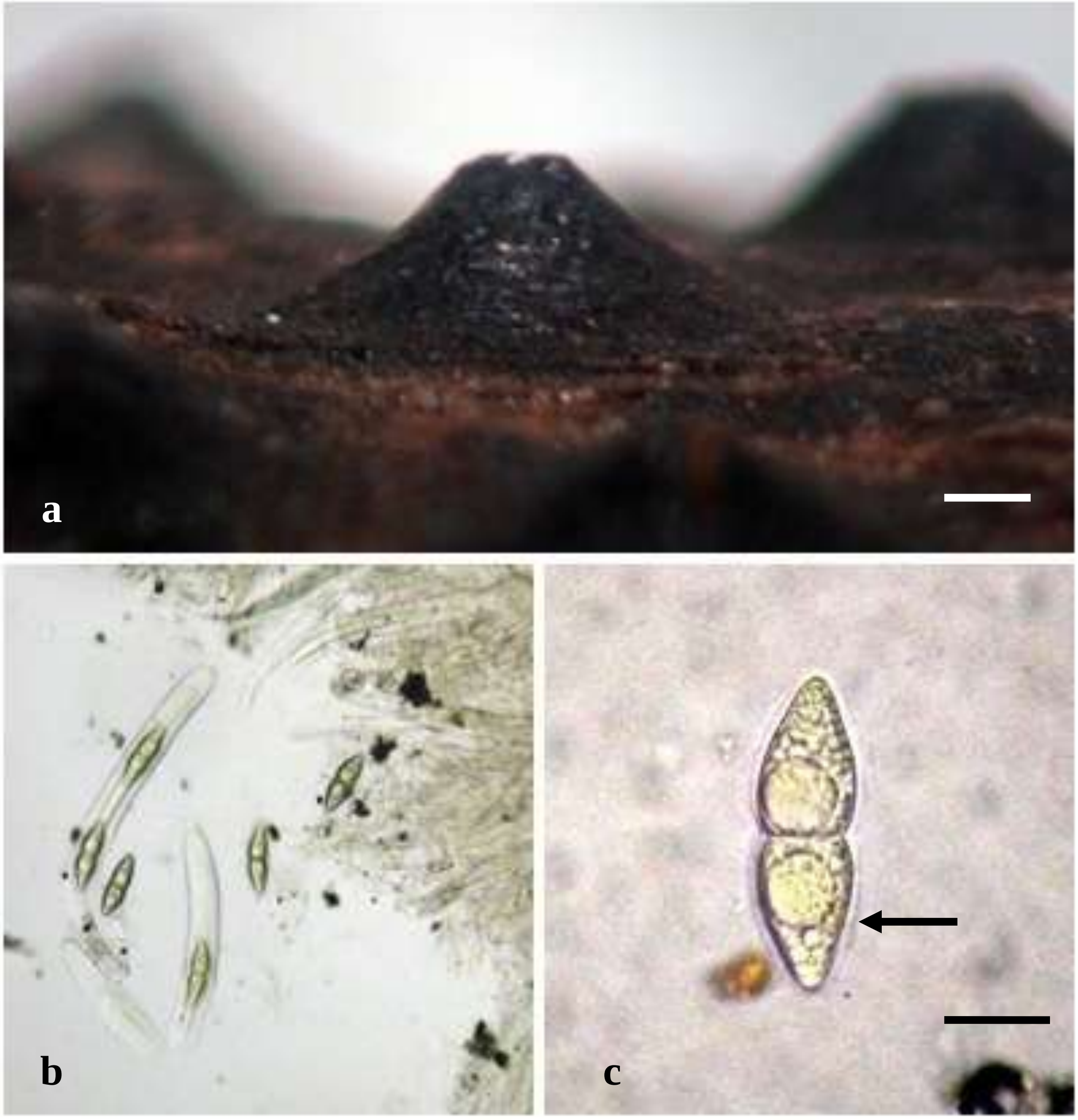


Figure 4.19 *Ascocratera manglicola* a) Ascomata; Carbonaceous, black, conical with a flat base, erumpent from the substrate and superficial when mature, seated on a thin stroma, ostiolate, periphysate, epapillate, gregarious or two or more attached to each other b) Asci; eight-spored, cylindrical, pedunculate, thick-walled, fissitunicate c) Ascospores; Hyaline, ellipsoidal, initially one-septate and later becoming three-septate, constricted at the central septum, surrounded by a gelatinous evanescent sheath (arrow). a= 150 µm, c= 20 µm.

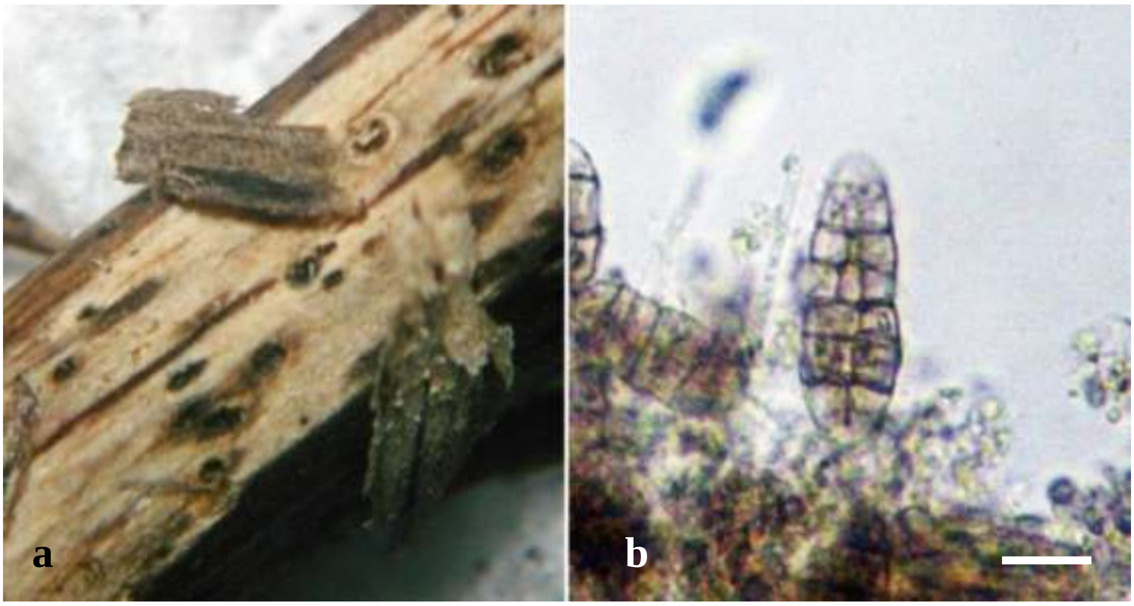


Figure 4.20 *Julella avicenniae* a) Ascomata; Immersed, globose or subglobose, membranous and ostiolate b) Ascospores; Brown, with (6-) 7 transverse septa when mature, 3 longitudinal septa, constricted at septa particularly at the central septum, ellipsoidal (28-36 x 12-16 µm). b= 10 µm.

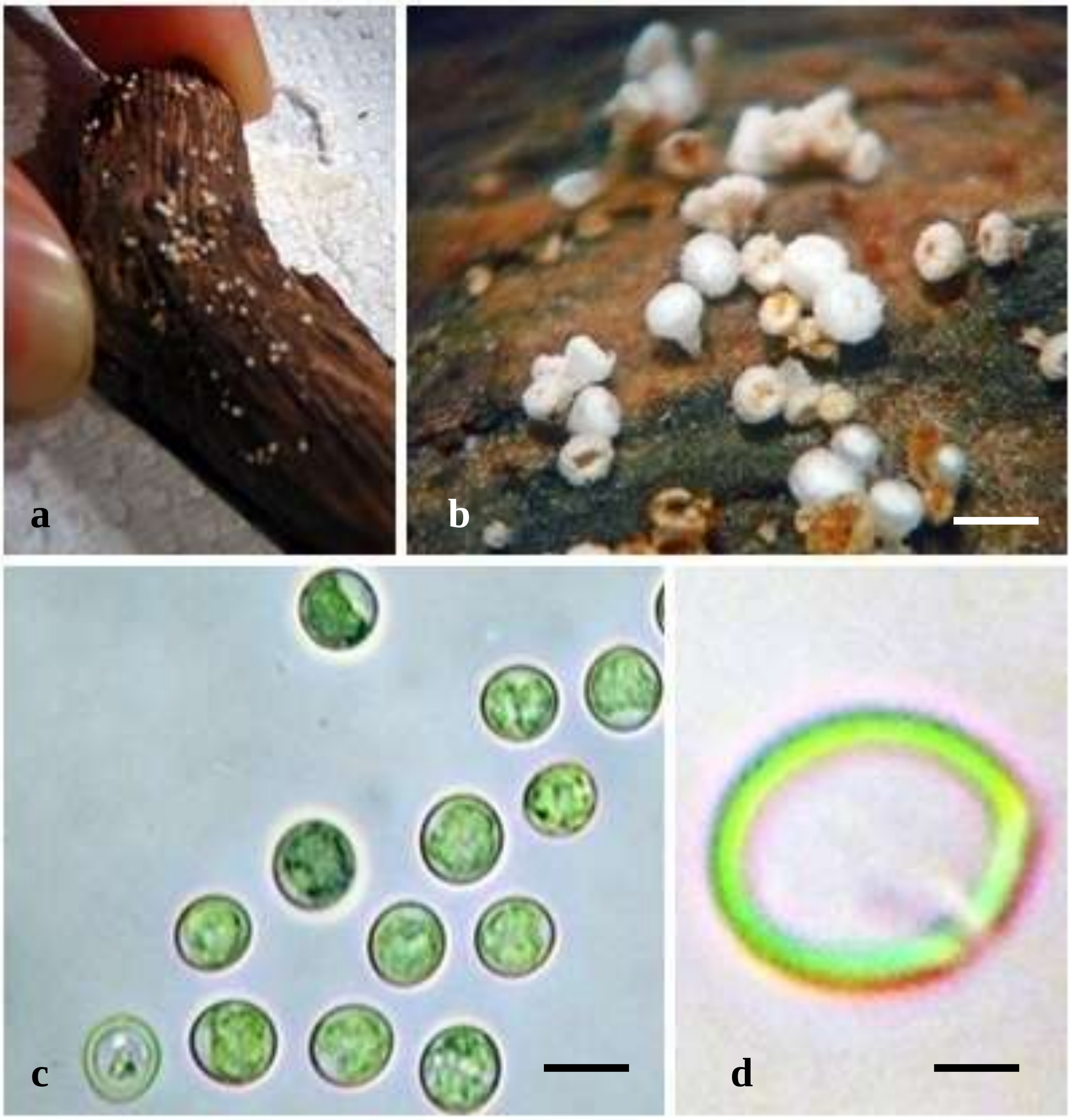


Figure 4.21 *Halocyphina villosa* a-b) Aerial view of the basidiomata on a *Rizophora apiculata* wood. b) Basidiomata; Whitish, 330-540 µm high, 260-410 µm diameter at the apex, turbinate or clavate, cyphelloid, , pedunculate, soft, thin-walled, eventually funnel shaped or cupulose, superficial, tomentose, gregarious c) Mature basidospores; 1-celled, hyaline, subglobose, smooth, non-amyloid, accumulating at maturity in the opening of the basidiocarp (7.5-9 x 6.5-7.5 µm) d) Young basidiospore. b= 475 µm, c= 8 µm, d= 2 µm.

Figure 4.22 *Nia vibrissa* a-b) Basidiomata; Whitish to olive brown, 1-3 mm in diameter, subglobose, superficial, soft, thin-walled, villous, gregarious c) Basidospore; 8-14 μm long 6-10 μm, hyaline, one-celled, ellipsoidal, appendages; hyaline, flexible attenuate, 3-5 subterminal radiating appendages around the base. a= 4 µm, b= 2 µm, c= 7 µm.

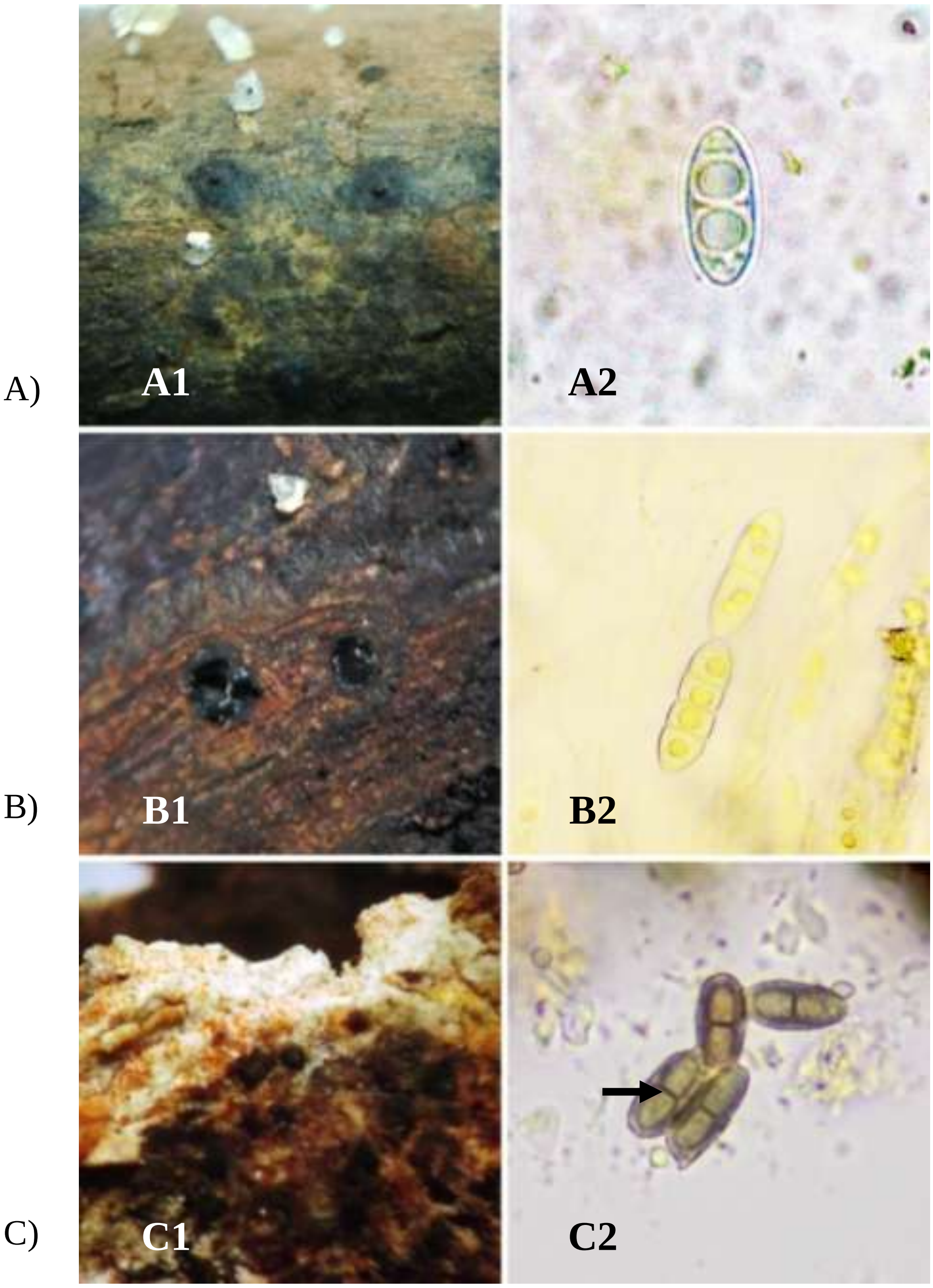


Figure 4.23 A) Asco CR sp.03, A1) Ascomata; Completely immersed with ectostromata, papillate, ostiolate A2) Ascospores; Hyaline, 2-cells, fusiform to ovoidal. B) *Leptosphaeria australiensis*, B1) Ascomata; immersed, globose, ostiolate, papillate, coriaceous, solitary or gregarious. B2) Ascospores; Hyaline. fusiform or clavate-fusiform or ellipsoidal, 3- septate, constricted at the septa (19-26 x 5-6 µm). C) Asco CR sp.16, C1) Ascomata;dark brown to black, superficial on wood C2) Ascospores; olive green, 1-septate constricted at the septum, surrounded by mucilaginous sheath (arrow).

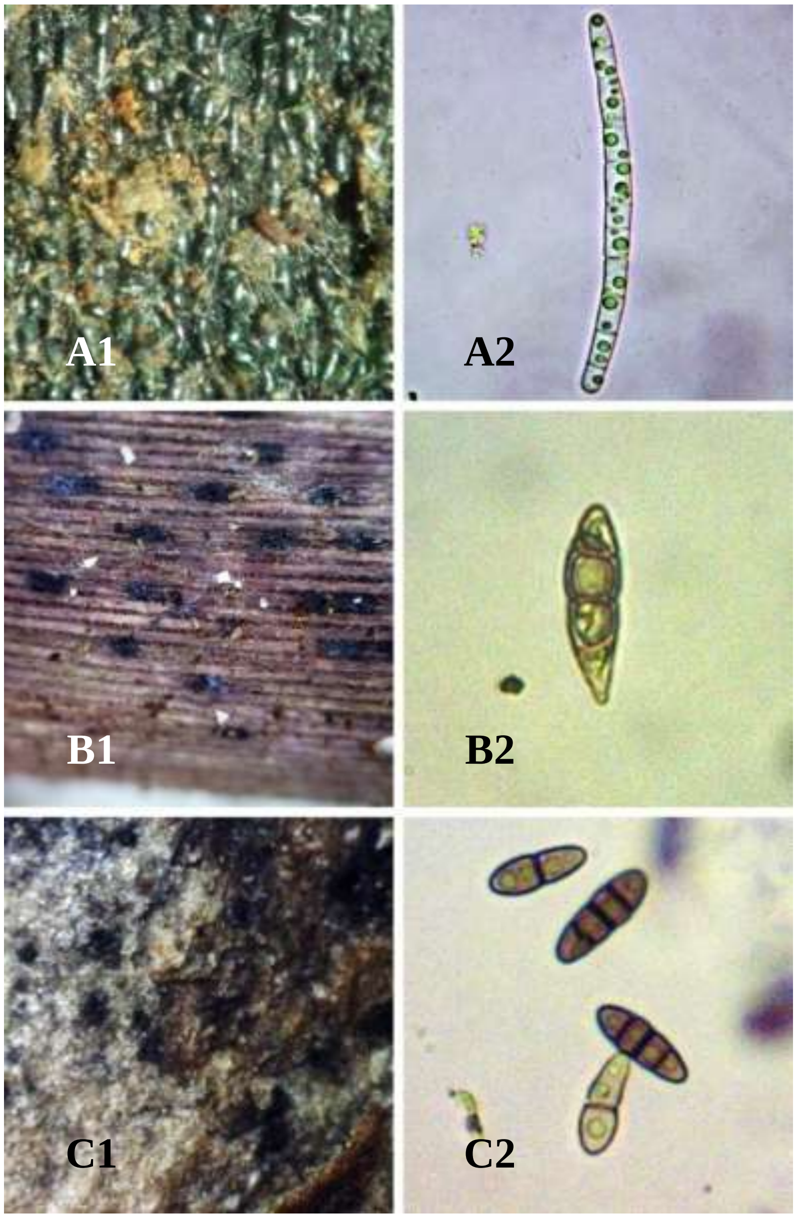


Figure 4.24 A) *Bathyascus* sp., A1) Ascomata; gregarious, ellipsoidal, superficial, ostiolate, papillate, coriaceous, thin-walled A2) Ascospores; Hyaline. elongate-filiform, rounded at each apex, straight or slightly curved, 8-10 septate, slightly constricted at the septum, without appendages. B) Asco MB sp. 19 B1) Ascomata; Immersed, becoming erumpent, subglobose or depressed ellipsoidal, ostiolate, papillate, coriaceous, brown to black, gregarious B2) Ascospores; Hyaline, fusiform to ellipsoidal, 3-septate, constricted at the septum (32-41 x 10-14.5 µm) C) *Leptosphaeria peruviana* C1) Ascomata; Immersed, lenticular-subglobose, immersed, ostiolate, black C2) Ascospores; cylindrical-ellipsoidal, obtusely rounded at both ends, three-septate, darken septate when mature, constricted at the septa (13-15 × 4.5-5 µm).

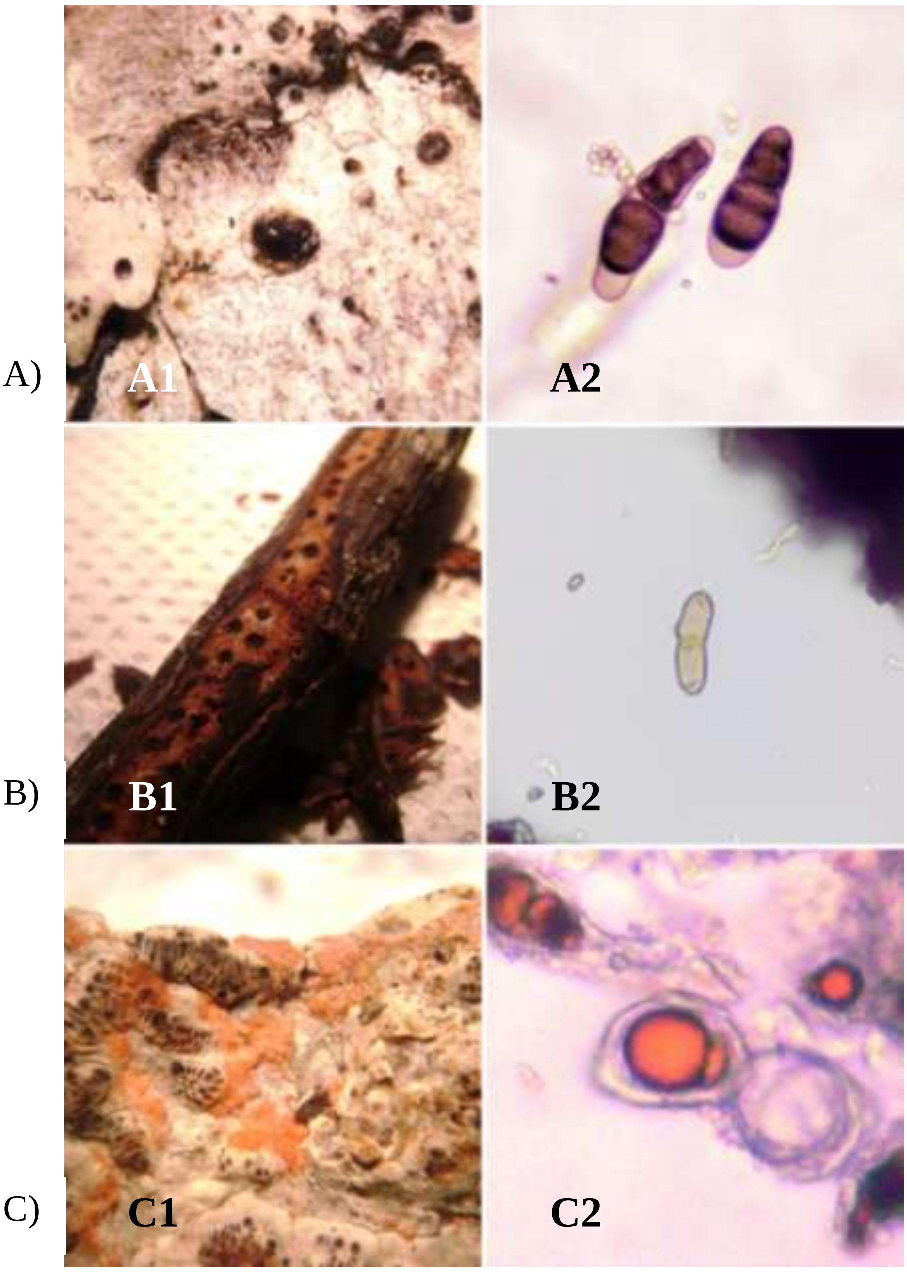


Figure 4.25 A) Asco TP sp.27, A1) Ascomata; Black, partially immersed, carbonaceous to coriaceous, globose to subglobose A2) Ascospores; Dark brown when mature, 1-septate slightly constricted at septum, ellipsoidal to fusiform, straight or curved, B) Asco MB sp.22, B1)Acomata; Immersed, globose, carbonaceous to coriaceous, gregarious B2) Ascospores; Hyaline, cylindrical with rounded end at each apex or elongate-ellipsoidal, 1-septate and slightly constricted at septum, straight or slightly curved C) Deuteromycete sp.08 C1) Colonies sporodochial, orange C2) conidia solitary, subglobose to obpyriform, 1-2 cells, second cell smaller, orange (15-29.5 $\times$ 14.5-17 µm)

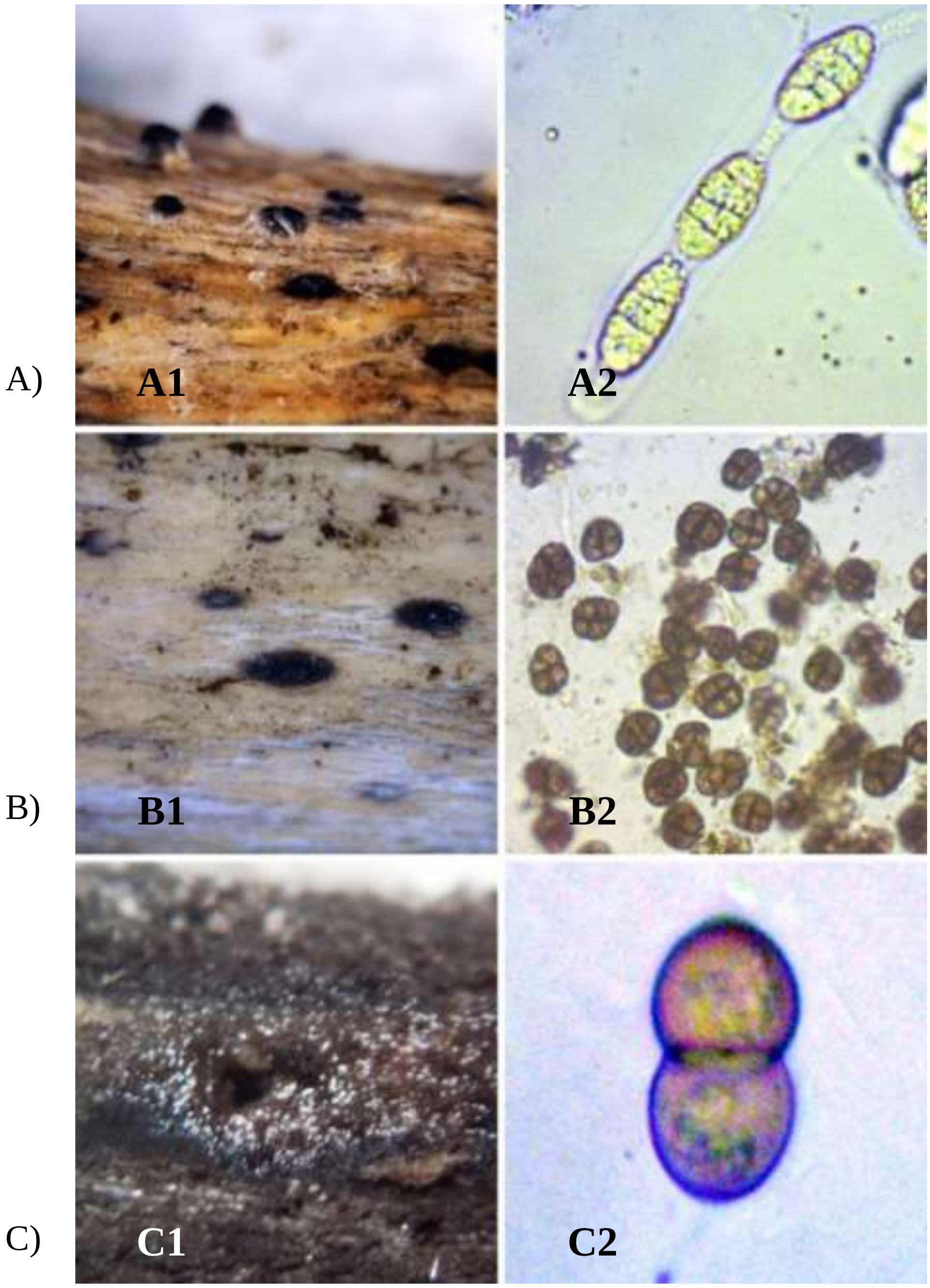


Figure 4.26 A) Asco MB sp.20, A1) Ascomata; Black, partially-immersed, carbonaceous, gregarious, subglobose A2) Ascospores; elongate-ellipsoidal with rounded end at each apex, 2-3-transverse septa, not constricted at the septa, hyaline. B) *Monodicyts pelagica*, B1-B2) Conidia; 22-35 x 18-30 µm, dark brown, composed of 4-25 cells. C) Asco TP sp.27, C1) Ascomata; completely immersed, membranous, forming pustules on the surface of substrate C2) Ascospores; Light brown, 1-septate constricted at septum, ellipsoidal, with no appendages.

A) B) C)

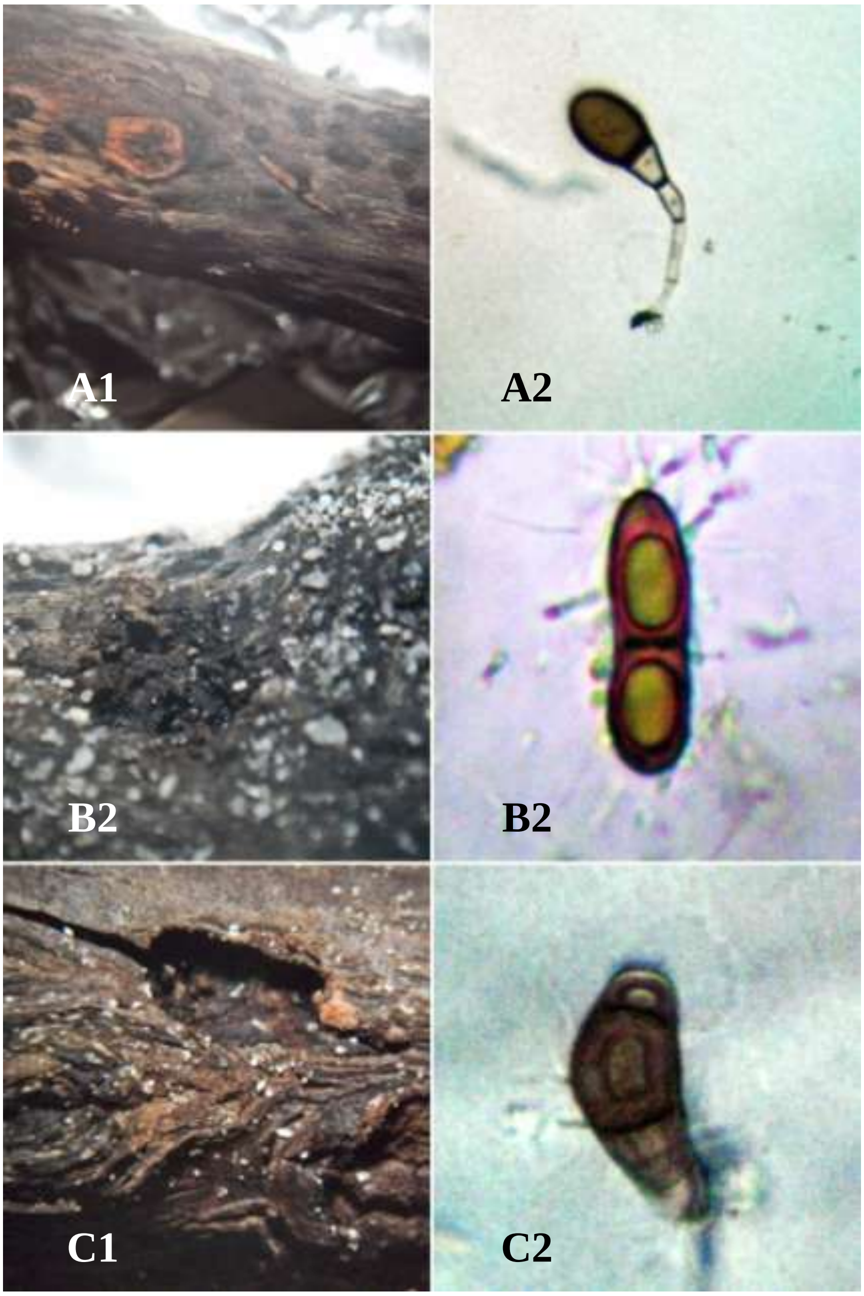


Figure 4.27 A) *Bactrodesmium linderi*, A1-A2) Conidia; Dark brown to black, solitary, subglobose to obpyriform, 1-2-septate, not constricted at the septa (20-33.6 × 14.5-20.5 µm). B) Asco MB sp.23, B1) Ascomata; Immersed, brown to black, solitary, subglobose to pyriform B2) Ascospore; Ellipsoidal, 1-pseudoseptate with large oil globules, not markedly constricted at the septa, light brown (32-45 × 6.5-9 µm), C) Asco TP sp.30, C1) Ascomata; Immersed, black, solitary or gregarious, subglobose. C2) Ascospore; Two-septate, central cell dark brown, apical cells light brown, mostly curved, fusoid-ellipsoidal (38-50 x 15-18.5 µm).

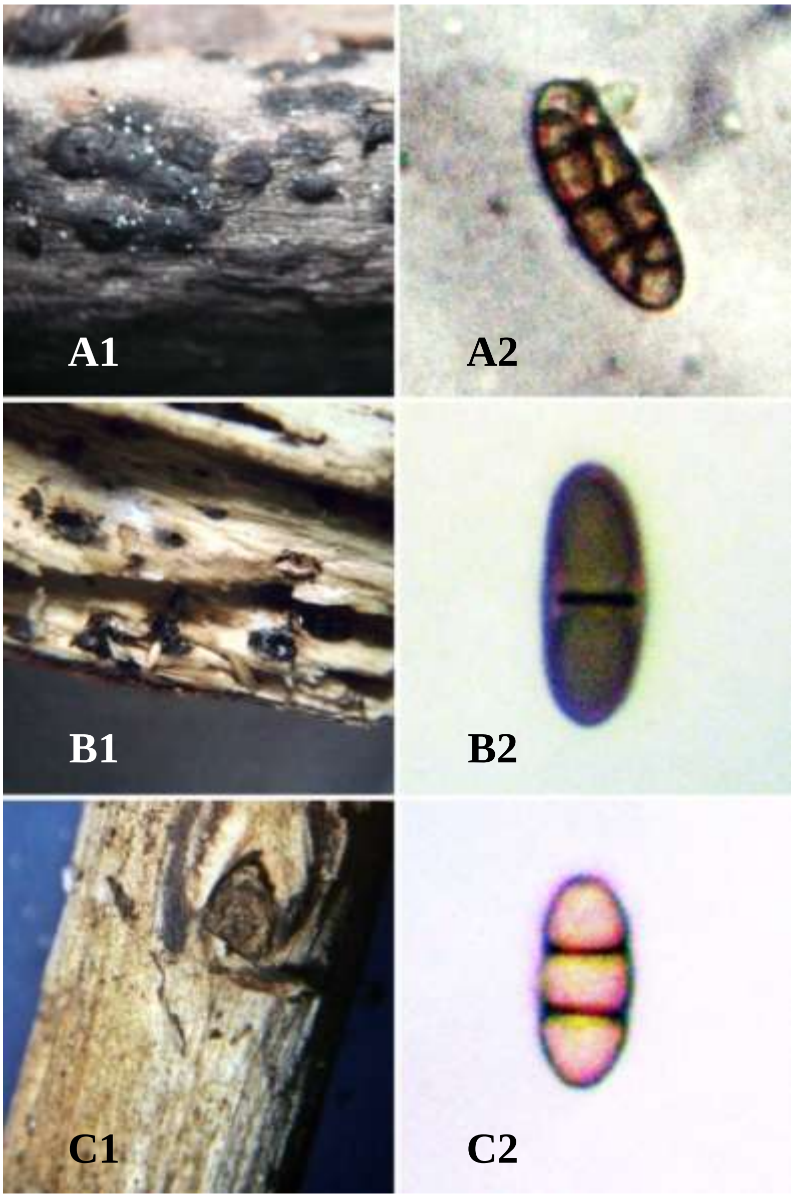
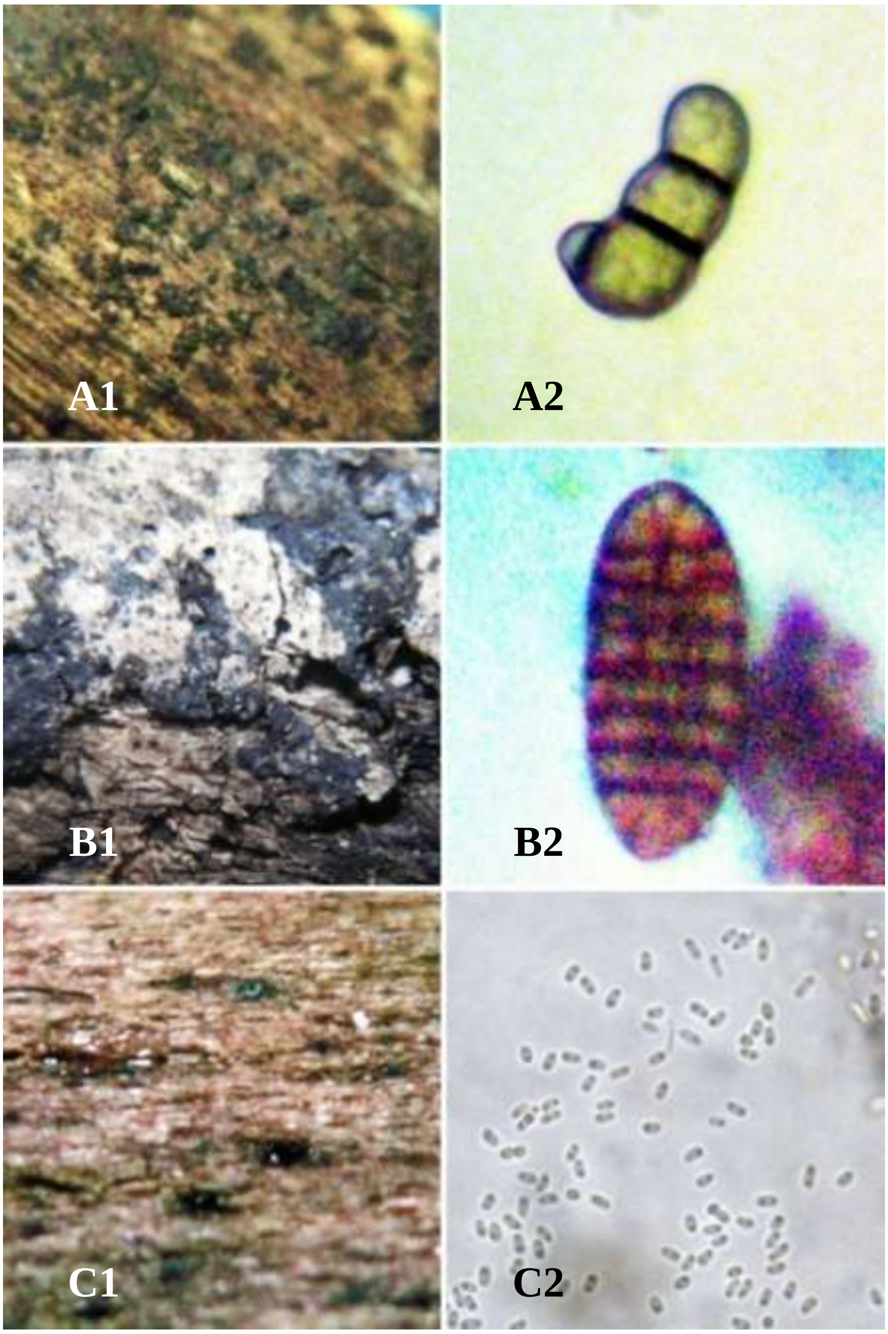


Figure 4.28 A) Asco TP sp. 36, A1) Ascomata; Immersed in pseudostroma, subglobose to hemispherical, carbonaceous, black, ostiolate, papillate A2) Ascospore; Light brown, darken septate, 3-4 trans-septa, 2-longi-septa, cylindrical-ellipsoidal, B) Asco TP sp.38 B1) Ascomata; Partially-immersed, globose to subglobose, black, carbonaceous to coriaceous, gregarious B2) Ascospore; Ellipsoidal, 1-pseudoseptate with large oil globules, not markedly constricted at the septa, light brown (32-45 × 6.5-9 µm), C) Asco TP sp.41, C1) Ascomata; Immersed, subglobose to pyriform, ostiolate, papillate, brown to black, solitary C2) Ascospore; Cylindrical-ellipsoidal, obtusely rounded at both ends, two-septate, constricted at septum, olive-brown (11-14.5 × 4-5.5 µm).



A)
B)
C)

Figure 4.29 A) Asco TP sp. 42, A1) Ascomata; Immersed, black, coriaceous, subglobose, gregarious A2) Ascospore; Hyaline, 2-septate constricted at septum, cylindrical, rounded at both ends. B) *Mauritiana* sp. (Asco TP sp.49) B1) Ascomata; Immersed, globose, ovoid. B2) Ascospore; fusiform, with rounded ends, dark brown, thick septa, 6-7 distoseptate, not constricted at septum, lacking mucilaginous sheath. C) Phoma sp.3, C1) Conidiomata; Immersed, ostiolate, unilocular, papillate, dark coloured, gregarious C2) obovoid or ellipsoidal, 1-celled, hyaline, without appendages or gelatinous sheath (2-3 x 1 µm).

4.2 Discussion

4.2.1 Species occurrence

Biodiversity studies are much related with the methods used for identification. The available method for identification keys are based on the propagules of marine fungi (Kohlmeyer and Volkmann-Kohlmeyer, 1991). Hence, some species might not be detected by traditional microscope-based techniques especially the non-sporulating fungi. Even though mycologists are making significant advances in species discovery; many fungi are still remained to be discovered. Species documented so far are only those that sporulate on selected substrata: mangroves (woody tissue, leaves, prop roots and pneumatophores), seaweeds, sea grasses and salt marshes plants (Jones, 2011b).

As for this study, three locations of mangrove forest in Malaysia were selected. 105 Ascomycota, 29 anamorphic fungi and 2 Basidiomycota were obtained. *Dactylospora haliotrepha*, *Haloresellinia oceanica* and *Kallichroma tethys* were the common manglicolous fungi species found. These are not the total number of biodiversity of marine mangrove fungi from Malaysia. The numbers differ for each study that was done before and it usually depends on the number of samples collected. Alias *et al.,* 2010 reported 115 Ascomycota, 2 Basidiomycota, 22 anamorphic fungi with another 200 species approximately that await identification. Another biodiversity study done in Malaysia reported 58 ascomycetes, two Basidiomycetes and 17 anamorphic fungi (Pang *et al.,* 2010).

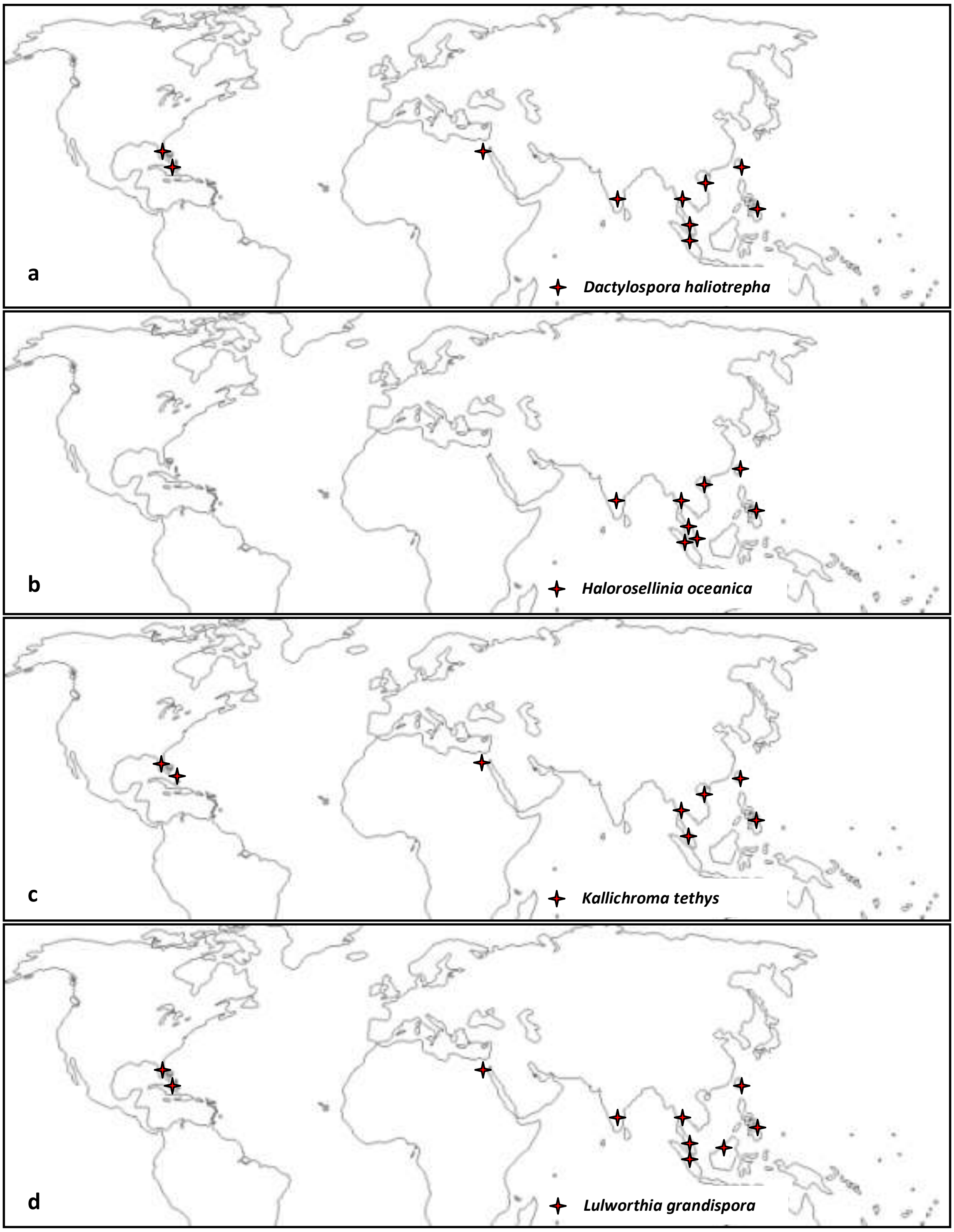
Schmit and Shearer (2003) listed 625 mangrove fungi. However, it comprises the obligate fungi, facultative fungi and fungi exclusively from terrestrial that grows on aerial parts of mangrove trees. Collecting materials from mangrove ecosystem can be tricky if one needs to focus on the marine fungi group derived from mangrove trees as there is an extreme spatial variability in the estuarine area. Thus, mangroves are known to be highly salt tolerant where salinity can be between 0.2 to 25.6 psu (Patel *et al.,* 2010). Therefore, samples that were collected need to be below the water line to assure that the substrate have been in contact with the surrounding water. The samples were then incubated with sterile seawater upon arrival to the laboratory as the major principle that defines the marine fungi group is its ability to germinate and to form mycelium under marine environment.

All three study sites selected in this study are dominated by mangrove forest. Cape Rachado, Telok Pelandok and Morib are coastal mangroves ecosystem. The total number of occurrence for all three study sites is 535. Cape Rachado has the highest total number of occurrence with 198 followed by Morib with 185 and Telok Pelandok 152. The total numbers of occurrences are related with the total number of samples colonized by fungi. So, the higher the number of samples examined, the higher the number of occurrences. It is all based on the effort of the researcher itself. Biodiversity studies have shown a wide variation in the frequency and abundance of mangrove fungi. The figures were 154 species found from Thailand (Sakayaroj *et al.*, 2004), 139 species from Malaysia (Alias *et al.*, 2010), 131 species from India (Vittal and Sarma, 2006), 128 species from Hong Kong (Jones and Vrijmoed, 2003) and 91 species from India (Maria and Sridhar, 2003). Figure 4.1- 4.22 shows species that were among the core mangrove fungi found in Malaysia and also throughout the tropics and sub-tropic countries. There are also species that were partially identified and unidentified species found in this study (Figure 4.23-4.29).

The Ascomycota are the most numerous and common taxonomic group in mangroves with the Basidiomycota the least frequently collected (Alias and Jones, 2008) and it is shown in this study as well. Ascomycota were the dominant group in comparison with the anamorphic fungi and Basidiomycota (Table 3.3). The common species recorded from Cape Rachado, Telok Pelandok and Morib are similar with previous studies done in Peninsular Malaysia. Species like *Dactylospora haliotrepha*, *Kallichroma tethys, Halocyphina villosa, Verruculina enalia* and *Morosphaeria ramunculicola* are common species for mangrove fungi and can be defined as the core group of mangrove fungi in Malaysia similarly with the study done in the east coast of India where two location sites were observed; Godovari Delta and Krishna Delta. *V. enalia* were found to be dominant in both sites and were relatively frequent in both *Avicennia* spp. and *Rhizophora apiculata* host in comparison with other fungi species. However, from the figure it shows that it is significantly more common in *Avicennia* spp. (Sarma *et al.*, 2001).

Common species might be different for studies done in other countries but these species are available across the globe. Figure 4.30 (a-f) shows some selected species of manglicolous fungi distribution around the world based on data from published literature. Fungal communities is said to be more similar within a single ocean basin than between ocean basins, but mangrove trees that are close phylogenetically do not necessarily harbour fungal communities that are distinctly different from less closely related hosts. These theories are based on the meta-analysis employing ordination analysis on published data of fungal hosts and fungal distribution (Schmit and Shearer, 2004).

Mangrove associated fungi from 14 locations in the Atlantic Ocean including Bahamas have been reported with the number of fungi ranging from 12-47 with the mean figure of 25.6. This is considerably lower than the mean figures of 42.9 and 44 for the Indian and Pacific Oceans (Schmit and Shearer, 2004). However, this could due to low number of collections that correlate with the availability of potential substrates which could also explain the lower figures recorded for Atlantic Ocean. Nevertheless, a separate study done in Bahamas illustrates higher number of marine fungi when intensive collections were done (Jones and Abdel-Wahab, 2005). This also reflects for


a
Dactylospora haliotrepha
b
Halorosellinia oceanica
c
Kallichroma tethys
d
Lulworthia grandispora

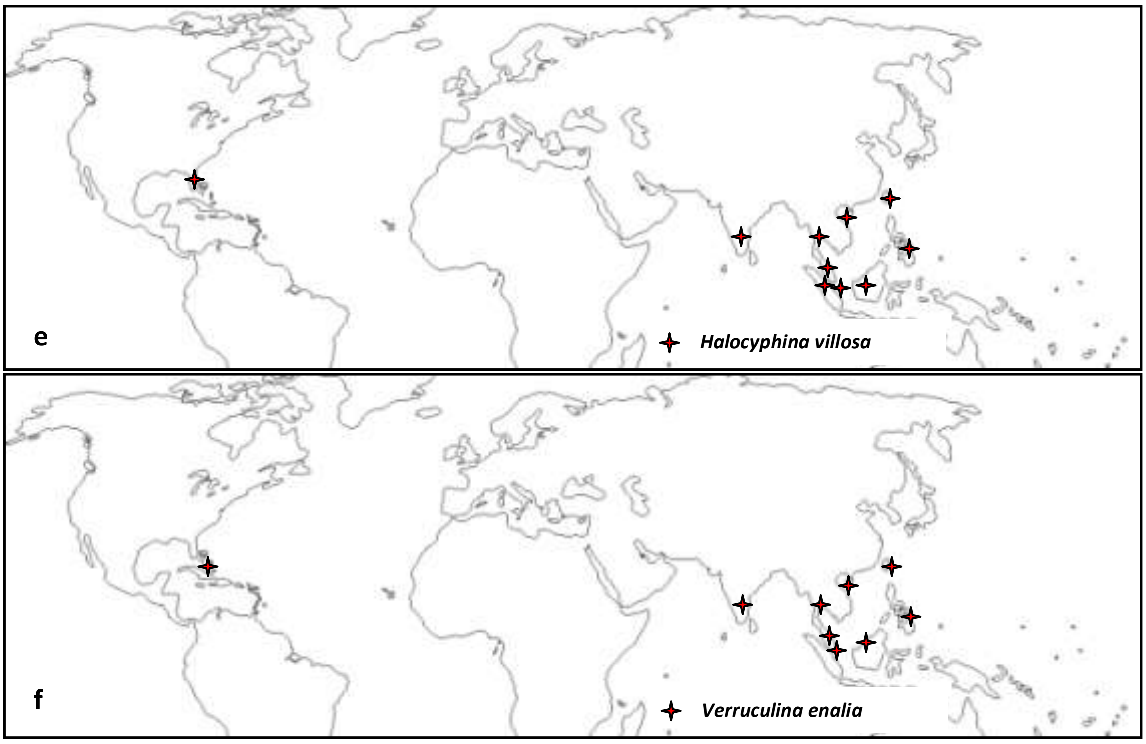


Figure 4.30 a) Distribution of *Dactylospora haliotrepha* b) Distribution of *Halorosellinia oceanica* c) Distribution of *Kallichroma tethys* d) Distribution of *Lulworthia grandispora* e) Distribution of *Halocyphina villosa* f) Distribution of *Verruculina enalia*. (a-f) Species distribution of 6 common marine mangrove fungi. Data based on published literature by Besitulo *et al.,* 2010 (Phillipines), Sakayaroj *et al.*, 2011 (Thailand), Jones and Puglisi, 2006 (Florida, USA), Pang *et al.,* 2011 (Taiwan), Hyde, 1988 (North Sumatra, Indonesia), Nambiar and Raveendran, 2009 (Kerala, India), Abdel Wahab, 2005 (Egypt), Hyde, 1988 (Brunei), Jones and Abdel Wahab, 2005 (Bahamas), Tan *et al.,* 1989; Leong *et al.*, 1991 (Singapore) and Jones and Vrijmoed, 2003 (Hong Kong).

the tropics where more diversity of fungi is recorded due to the number of studies conducted (Jones and Puglisi, 2006; Jones and Abdel-Wahab, 2005).

When mycologist began to explore tropical locations particularly the mangrove ecosystem somewhere in 80's and 90's, there was a rise in the number of described species for about 290 taxa (Jones, 2011b). Current figure of obligate marine fungi as for 2011 is 537 with 5 new genera and 7 new species (Jones, 2011b). However, this figure does not include few groups: unidentified species on a range of substrata, planktonic fungi, deep sea fungi, non-culturable fungi, marine derived fungi from soils, sands and water and fungi from unexplored area.

4.2.2 Diversity index, Species richness and Evenness

When assessing biological systems, diversity may also be seen as species richness (Kratochwil, 1999). Indo-Western Pacific region is known to have highest overall marine diversity which also illustrates the richness of its species. This region comprises waters off the coast of Asia, Southeast Africa, Northern Australia and the Pacific Islands (Hoffman and Parsons, 1991) and Malaysia is located at the centre of rich marine biodiversity.

From the result, Telok Pelandok recorded the highest number of species with a total of 71 species while Cape Rachado is following with 69 species. Even though Telok Pelandok has the highest number of species recorded, it does not correlate with the diversity index. Diversity indices calculations using Shannon-Weiner Diversity Index (H’) and Simpson’s Index of Diversity (1-D) revealed that Cape Rachado has the highest diversity index when evenness and total number of species occurrence are taken into account (Table 4.3). Species richness is the total number of species recorded.

However, overall Diversity Indices (H’) recorded in this study are higher from those recorded by Pang *et al.*, 2010 which also include Cape Rachado and Morib as study sites. This could be due to the lower number of samples examined. So we can conclude that the number of samples examined does correlate with the diversity index. The H’ Diversity Indices in this study were also higher than the recent study in Thailand except in one of their study sites where four locations in the offshore of Southern Thailand were selected (Sakayaroj *et al.*, 2011). Ao Tok, Koh Taen (09°22’15.68” N, 99°57’11.80”E) recorded the highest diversity index in comparison with the present study. The location is covered by healthy mangrove trees that surrounded by species like *Bruguiera gymnorrhiza*, *Rhizophora apiculata*, *R. mucronata*, *Sonneratia griffithii* and *Xylocarpus granatum* where salinity ranges from 30-33‰.

There could be many reasons that affect the diversity and abundance of mangrove fungi. The diversity of mangrove tree species may also be important in influencing the distribution of the Mycota (Volkmann - Kohlmeyer and Kohlmeyer, 1993). Mangrove forests are available exclusively in tropical and subtropical regions. It is known that the marine ecosystem of mangrove vegetations exhibit marine fungi that is distinctive to the ecosystem and only a few species may occur outside its niche. The comparison of tropical and subtropical biodiversities showed similarities which signify a wide geographic distribution of marine manglicolous fungi (Vrijmoed *et al.,* 1994; Hyde and Lee, 1995). The availability of substrata, the competition between fungi as well as the physical conditions such as salinity and tidal changes are some of the factor that affects fungal availability.

Mangrove is a complex ecosystem which consist marine, freshwater and terrestrial organisms. It has a wide range of salinity and the nature of marine fungi as to whether it is obligate or facultative is still under debate. Mycologist has been lurking on ambiguities on whether all fungi regarded as obligate is a truly marine species (Jones, 2011b). Can a species collected from mangrove wood that is not subject to inundation by seawater be regarded as marine?

The mangrove ecosystems itself are ecologically sensitive to anthropogenic perturbation, including the intense aquaculture trade, which has worsen in recent years (Sulong *et al.,* 2001; 2002). The estimated loss of mangroves recorded for just the state of Kelantan, Malaysia was 139.3 ha. It is due to aquaculture farm, sediment accretion and other physical phenomena, including rural settlements. The analyses were done based on satellite images between 1988 and 2000 and ground truthing data (Kasawani *et* *al.*, 2006). With the alarming rate of mangrove loss, biodiversity study of marine mangrove fungi species need to be done comprehensively across the country.

As species richness and evenness increase, so does the diversity indices. Simpson's Index (D) where the D value ranges between 0 and 1. With this index, 0 represents infinite diversity and 1, no diversity. However, Simpson's Index of Diversity (1 – D) was used to present the data. The value of this index is still ranges between 0 and 1 but now the higher the value, the higher the diversity. There are many ways in presenting the data. Some papers prefer to use the Simpson dominance ratio, 1/D (Kumar and Hyde, 2004; Shankar and Shashikala, 2010) and if one need to compare the results, similar statistical analysis should be chosen. There have not been many papers on biodiversity of marine fungi that calculate the Simpson's Index of Diversity (1 – D). The most recent paper that applied the same statistical analysis is a study done on diversity of entomopathogenic fungi in the rainforest of Thailand (Aung *et al.*, 2008) where the indices calculated are lower than the present study. The 1-D indices calculated in the present study do correlate with the Shannon-Weiner H' indices. Two formulas were used to compare the statistical analysis for diversity index.

Evenness indices showed how equal the community is numerically. Figure 4.31 gives an overview of the evenness and also the species abundance in the 3 location sites. Species-abundance distributions (Figure 4.31) showed that only a very few species dominated at each site (percentage abundance ≥ 10%). Among the 3 sites, the most abundant taxa were *Dactylospora haliotrepha* that present in all three sites (25.95% of the total occurrence from all sites), followed by *Haloresellinia oceanica* (24.05%) that present in all sites as well. There was a moderate overlap of common species among the sites. All sites had many rare taxa that occurred only once. There were also taxa with intermediate abundance (5-9% of the total occurrence of fungi) in all sites.

The evenness index range between 0-1, where the higher the number, the even the species composition in that ecosystem would be. The highest evenness recorded is in Cape Rachado (0.898) followed by Telok Pelandok (0.883) and Morib (0.880). All study sites are relatively even where the range recorded is for 0.880 to 0.898 which is near to 1. Figure 4.31 reflects the evenness figure calculated in this study whereby, only a few species that were categorized as dominant and the rest of the species relatively have an even number of occurrence.

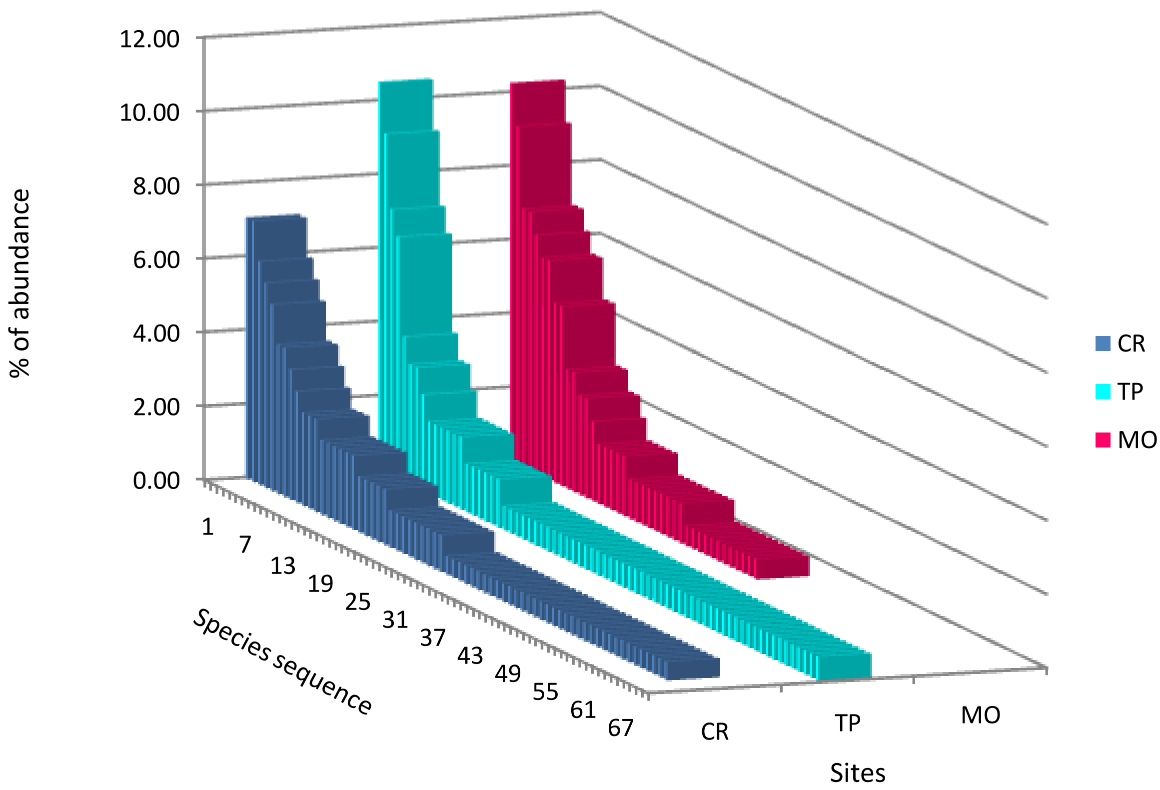


Figure 4.31 Species abundance distribution of marine fungi collected from 3 study sites. CR= Cape Rachado, TP= Telok Pelandok and MO= Morib (Species sequence are in decreasing order of % of abundance).

4.2.3 Similarity index

The Sørensen index, also known as Sørensen's similarity coefficient, is a statistic used for comparing the similarity of two samples (Sørensen, 1957) or in present study, it is between two study sites. In this study, all indices calculated between sampling sites were below 0.5. This indicates that the species composition for each study sites is unique. The highest number of index recorded is between Morib and Cape Rachado with 0.370 where both are muddy mangrove forest while the lowest are 0.302 for Telok Pelandok-Cape Rachado (Table 4.4). The uniqueness of each study sites are believed to have been influenced by the type of vegetations in every study sites. Some species could have host specificity as have been proved by several studies.

A comprehensive database system needs to be developed in order to get the total enumeration of marine fungi biodiversity in Malaysia. Studies on biodiversity are actively done and it is time to compile it to make it accessible to the public. Systematic Marine Biodiversity Inventory System (SyMBiosiS), a national inventory site is another platform that is ready and could fulfil the objective.

4.3 Conclusion

The research presented is intended to highlight the wide occurrence of filamentous fungi in mangrove ecosystems in Malaysia. The study mainly focused on the lignocellulosic material of mangroves. One hundred and thirty six fungal taxa were recorded out of a total of 536 samples collected from three study sites. It comprises 105 Ascomycota, 29 anamorphic fungi and 2 Basidiomycota. Sixty nine species of Ascomycota and 22 species of anamorphic fungi were partially identified species. All species recorded were the core group of mangrove fungi that are common on *Rhizophora* spp. and *Avicennia* spp. mangrove trees. Further taxonomic identification of the isolates presented within this study may uncover many new fungal taxa.