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SOIL FUNGAL COMMUNITY ASSOCIATED WITH PEAT IN SARAWAK IDENTIFIED USING 18S rDNA MARKER

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ABSTRACT

Fungi are principal decomposing microorganisms in acidic environment of peatlands. A useful tool for molecular screening of soil fungal communities using the 18S rDNA primer has been proven capable of identifying a broad range of fungi species within Ascomycota, Basidiomycota, Zygomycota and Chytridiomycota. Currently, very little information is available on fungal communities in deep peat of Sarawak, Malaysia. In this study, we have isolated the fungi from soil samples taken in deep peat forests and oil palm cultivated areas. The fungal identity was undertaken using 18S rDNA primer which is EF4-F/ fung5-R. The microscopic structures were conducted to confirm the identity of the isolates. Based on this study, the fungal division most commonly found in deep peat is the Ascomycota. Aspergillus fumigatus *was the most common species and more dominant in oil palm cultivated areas and logged-over forest than in primary forest. In the primary forest, the dominant species was the* A. flavus*, while* Hypocrea atroviridis *was commonly associated with oil palm cultivated areas and logged-over forest. Other species of fungi isolated in peat primary forests were* Penicillium chrysogenum*,* Trichoderma *sp*., Phanerochaete *sp*., Mortierella chlamydospora, A. niger, A. alliaceus, etc. *The in-depth difference in the fungal communities for the different sites will be further investigated using the next generation sequencing technology.*

Keywords: fungal community, peat soil, 18S rDNA.

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INTRODUCTION

Tropical peatland ecosystems are unique environments developed from prolonged accumulation of plant organic matter as a results of incomplete decomposition under specific geological conditions (Kanokratana *et al*., 2011). Most tropical peatlands are found in South-east Asia (Malaysia, Brunei, Indonesia and southern Thailand), where high rainfall and poor drainage conditions contribute to slow organic matter decomposition rates resulting in the accumulation of deep layers of peat (Ambak and Melling, 2000; Dommain *et al*., 2014). Peat forests play a significant role as one of the largest terrestrial organic carbon sinks and it is very important in the global carbon cycle.

Peat soil ecosystems provide excellent habitats for a large number of species of organisms from all domains of life including archaea, bacteria, fungi, protists, animals and plants (Nuyim, 2000). It has been suggested that fungi are the dominant microbes in many peatland ecosystems which act as plant pathogens, mycorrhizal symbionts and most importantly as the principal decomposers of organic materials. About 601 species of fungi have been identified globally from peatlands (Thormann and Rice, 2007). Ascomycetes are the largest group with 276 species (46%), followed by Basidiomycetes (243

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species, 40%), Zygomycetes (55 species, 9%), and Chytridiomycetes (26 species, 4%) (Thormann and Rice, 2007). These groups of fungi play an important role in carbon cycle and interact with plants through exchange of organic and inorganic compounds (Tiwari *et al*., 2008).

Peat is defined as soil containing organic matter with the minimum cumulative thickness of 50 cm within 100 cm of the soil surface. Tropical peat is characterised based on the nature of the organic soil materials in the sub-surface tier (50-100 cm), presence or absence and stage of decomposition of wood within 100 cm of the soil surface, and the nature of the underlying mineral substratum (Paramananthan, 2010). Peat is formed from the product of degraded organic matter or partially degraded for over long period in water-logged or formerly water-logged areas. Peat soil comprises basically of partially decomposed plant materials in addition to some mineral fractions such as clay, sand and silt. The soil also consists of decayed biomass and develops in depressions or wet coastal areas when the rate of biomass production from adapted vegetation is greater than the rate of decomposition (Ywih *et al*., 2009).This is due to the presence of high water table that prevents aerobic decomposition of plant debris.

Molecular techniques have been used extensively in identifying broad range of organisms such as 18S rDNA PCR (Atkins and Clark, 2004). These molecular approaches are rapid, sensitive, easy to interpret, and provide a useful alternative to culture-based detection and identification methods. The 18S rDNA-based approaches are a useful tool for initial screening of fungal communities, and that they represent a more comprehensive picture of the community than plate culturing (Hunt *et al*., 2004). The fungi-specific primer pair of EF4/fung5 has been initially developed for identification of the fungal diversity (Smit *et al*., 1999). This primer has been used to identify a broad range of fungi species of Ascomycota, Basidiomycota, Zygomycota and Chytridiomycota (Smit *et al*., 1999; Hunt *et al*., 2004). Besides, this primer is highly specific for fungal 18S rDNA sequences and no non-fungal sequence was retrieved from the soil libraries (Hunt *et al*., 2004). Knowledge on the diversity of soil fungal communities in tropical peat remains limited. Therefore, this study focused on the identification of fungi from tropical deep peat using conventional and 18S rDNA approach.

MATERIALS AND METHODS

Locations and GPS Points of Peat Soil Sampling

The sites of sampling for this study were Pelitanah 2 (Pelitanah Forest, Pelitanah Clean-cleared, Pelitanah Planted and Naman Plantation at Sibu, Sarawak, Malaysia), Maludam National Park, Cermat Ceria logged over forest and Durafarm Plantation at Sri Aman, Sarawak, Malaysia. Ten GPS sampling points were determined for each sites (*Table 1*). The peat soil was sampled at different depths, namely, topsoil (A), midway between topsoil and water table (B), water table (C) and below water table (D) using peat auger.

Isolation of Culturable Fungi

Soil samples were serially diluted to dilutions of 10^{-8}. Potato dextrose agar (PDA) and malt extract agar (MEA) were used for fungal isolation. All the cultures were incubated at 30°C for two to three days. Different fungi were selected and sub-cultured onto PDA to obtain pure fungal isolates.

Fungal DNA Extraction

The fungal genomic DNA was extracted by using fungi DNA isolation kit (Norgen Biotek Corporation). The fungal colonies were scraped from the PDA and transferred into beads tube provided in the kit with 500 µl lyses solutions. The mixtures were spun vigorously for 10 min to lyse the fungal cells. Then, the samples were incubated in a water bath at 65°C for 15 min. Fungal DNA from this lysate was recovered according to the provided protocol. The extracted DNA was stored at -20°C for further analysis.

Fungal DNA Amplification

The fungal DNA was amplified with a thermal cycler using a set of primer EF4-F (5'-GGAAGGG[G/A]TGTATTTATTAG-3') and fung5-R (5'-GTAAAAGTCCTGGTTCCCC-3') (Smit *et al*., 1999). The 25 µl reaction mixture containing 50 mM Tris (pH 8.3) buffer, 1.0 µl or 400 nM each primer, 250 mM dNTPs, 0.3 µl Taq polymerase, 2.5 µl or 2.5 mM $MgCl_2$, 1.0 µl of 500 mg ml^{-1} BSA, 1.0 µl DNA (3 to 8 ng) template and 15.2 µl deionised water was prepared. PCR reaction was set with initial denaturation at 94°C for 3 min, followed by 30 cycles in a series of denaturation at 94°C for 1 min, annealing at 48°C for 1 min, and extension at 72°C for 1 min, with a final step of one cycle at 72°C for 5 min to final extension. The products were gel electrophoresed on a 1.5% agarose gel with 1X TBE buffer (8.9 mMTris-borate, 0.2 mM EDTA) and analysed after staining with ethidium bromide.

Purification of DNA and Sequence Analysis

The agarose gel that contained the positive band of the PCR products were excised and purified using Gel Purification Kit (Qiagen, USA). The

TABLE 1. LOCATION OF SAMPLING POINTS STUDIED IN DEEP PEAT, SARAWAK

No.	Estates	Sampling points	Location on GPS
1.	Pelitanah Forest	1	N, 2°33′ 49.70083″ E, 111°59′ 20.82349″
		2	N, 2°33′ 49.74448″ E, 111°59′ 21.60867″
		3	N, 2°33′ 49.74448″ E, 111°59′ 22.52463″
		4	N, 2°33′ 50.40209″ E, 111°59′ 21.39658″
		5	N, 2°33′ 50.69863″ E, 111°59′ 21.41331″
		6	N, 2°33′ 52.5665″ E, 111°59′ 20.76283″
		7	N, 2°33′ 52.8402″ E, 111°59′ 20.94537″
		8	N, 2°33′ 53.45389″ E, 111°59′ 20.38395″
		9	N, 2°33′ 54.16472″ E, 111°59′ 20.26925″
		10	N, 2°33′ 54.91013″ E, 111°59′ 20.63004″
2.	Pelitanah (clean cleared)	1	N, 2°33′ 58.80795″ E, 111°58′ 3.83025″
		2	N, 2°33′ 58.90221″ E, 111°58′ 5.51199″
		3	N, 2°33′ 59.34559″ E, 111°58′ 3.8354″
		4	N, 2°33′ 59.31421″ E, 111°58′ 5.44331″
		5	N, 2°33′ 0.09518″ E, 111°58′ 5.48516″
		6	N, 2°33′ 0.03417″ E, 111°58′ 3.87299″
		7	N, 2°33′ 58.08519″ E, 111°58′ 3.89269″
		8	N, 2°33′ 58.11211″ E, 111°58′ 5.46769″
		9	N, 2°33′ 57.77412″ E, 111°58′ 5.45351″
		10	N, 2°33′ 57.8725″ E, 111°58′ 3.84324″
3.	Pelitanah (planted)	1	N, 2°33′ 25.16557″ E, 111°59′ 12.67154″
		2	N, 2°33′ 33.95301″ E, 111°59′ 8.88645″
		3	N, 2°33′ 40.21324″ E, 111°59′ 11.43377″
		4	N, 2°33′ 44.03304″ E, 111°59′ 13.60258″
		5	N, 2°33′ 45.16517″ E, 111°59′ 6.26114″
		6	N, 2°33′ 48.77943″ E, 111°59′ 6.26114″
		7	N, 2°33′ 54.61519″ E, 111°59′ 8.52343″
		8	N, 2°33′ 26.08133″ E, 111°59′ 8.5631″
		9	N, 2°33′ 26.21618″ E, 111°59′ 10.65928″
		10	N, 2°33′ 25.11598″ E, 111°59′ 8.88799″
4.	Maludam National Park	1	N, 1°37′ 43.15897″ E, 111°02′ 22.12806″
		2	N, 1°37′ 43.14684″ E, 111°02′ 22.10784″
		3	N, 1°37′ 43.13676″ E, 111°02′ 22.10172″
		4	N, 1°37′ 43.11048″ E, 111°02′ 22.09380»
		5	N, 1°37′ 43.09068″ E, 111°02′ 22.08912″
		6	N, 1°37′ 43.08420″ E, 111°02′ 22.08408″
		7	N, 1°37′ 47.89410″ E, 111°02′ 23.28638″
		8	N, 1°37′ 48.31230″ E, 111°02′ 22.79459″
		9	N, 1°37′ 48.40347″ E, 111°02′ 22.83173″
		10	N, 1°37′ 48.52168″ E, 111°02′ 22.79459″
5.	Cermat Ceria (logged over forest)	1	N, 1°23′ 58.85626″ E, 111°24′ 08.61675″
		2	N, 1°23′ 58.29805″ E, 111°24′ 08.52634″
		3	N, 1°23′ 57.46257″ E, 111°24′ 13.68159″
		4	N, 1°23′ 56.50727″ E, 111°24′ 17.37404″
		5	N, 1°23′ 56.11111″ E, 111°24′ 21.85227″
		6	N, 1°23′ 55.39797″ E, 111°24′ 27.35565″
		7	N, 1°23′ 54.86120″ E, 111°24′ 31.39397″
		8	N, 1°23′ 52.45833″ E, 111°24′ 39.79472″
		9	N, 1°23′ 42.87224″ E, 111°24′ 41.60620″
		10	N, 1°23′ 53.80569″ E, 111°24′ 43.18166″

TABLE 1. LOCATION OF SAMPLING POINTS STUDIED IN DEEP PEAT, SARAWAK (continued)

No.	Estates	Sampling points	Location on GPS
6.	Durafarm	1	N, 1°23′ 50.63697″ E, 111°24′ 50.59624″
		2	N, 1°23′ 48.99708″ E, 111°24′ 49.62261″
		3	N, 1°23′ 49.35106″ E, 111°24′ 49.30407″
		4	N, 1°23′ 51.21340″ E, 111°24′ 50.47918″
		5	N, 1°23′ 51.40201″ E, 111°24′ 50.03203″
		6	N, 1°23′ 49.36877″ E, 111°24′ 48.74209″
		7	N, 1°23′ 49.55455″ E, 111°24′ 48.16225″
		8	N, 1°23′ 51.60689″ E, 111°24′ 49.45209″
		9	N, 1°23′ 51.97572″ E, 111°24′ 49.26872″
		10	N, 1°23′ 49.72718″ E, 111°24′ 47.85654″
7.	Naman Plantation	1	N, 2°09′ 47.03580″ E, 111°55′ 22.32516″
		2	N, 2°09′ 47.04192″ E, 111°55′ 22.33812″
		3	N, 2°09′ 47.04564″ E, 111°55′ 22.34604″
		4	N, 2°09′ 47.06316″ E, 111°55′ 22.38024″
		5	N, 2°09′ 47.06568″ E, 111°55′ 22.38708″
		6	N, 2°09′ 47.06712″ E, 111°55′ 22.39356″
		7	N, 2°09′ 47.06820″ E, 111°55′ 22.40076″
		8	N, 2°09′ 47.06821″ E, 111°55′ 22.40616″
		9	N, 2°09′ 47.07072″ E, 111°55′ 22.40580″
		10	N, 2°09′ 47.07288″ E, 111°55′ 22.40148″

purified PCR products were sent out to Firstbase Laboratories for sequencing. Sequence similarity searches on the sequence data were conducted using the nucleotide-nucleotide Basic Logic Alignment Search Tool (BLAST) service of the NCBI GenBank database through website http://www.ncbi.nlm.nih.gov/ to identify the nearest relatives of the partially sequenced genes of excised dominant bands.

Mycological Slide Method

Mycological slide was prepared as documented in literature (Fischer and Dott, 2002). A small amount of spores and conidia were taken up using sterile loop and placed on a droplet of sterile PDA broth onto a clean sterile slide. A sterile coverslip was used to cover the broth and the slide was placed on a sterile and damp filter paper inside a sterile petri dish. The prepared slides were incubated at room temperature for three days before the microscopic photos were taken with standard light microscope.

RESULTS AND DISCUSSION

A total of 42 isolates of fungi were obtained from Pelitanah (forest, 29: clean-cleared, 7; planted, 6) while 22, 50, 80 and 43 isolates of fungi were obtained from Maludam, Cermat Ceria, Durafarm and Naman respectively. Based on the 18S rDNA techniques, the fungi in deep peat Sarawak were identified as in *Table 2*. There were 24 different species of fungi isolated and identified. Overall, the most common fungi identified in deep peat is *Hypocrea atroviridis* (79 isolates) where Durafarm showed the highest number of isolate (58 isolates). *Hypocrea* are teleomorphs of *Trichoderma* which themselves have *Hypocrea* as anamorphs (Samuels and Gary, 2006). *Trichoderma* sp. have been isolated widely from agricultural soils (Rahbaek *et al*., 2000) including peat soil. These fungi occur naturally in soils and favour the abundance of plant roots. Apart from *Aspergillus* sp., *Trichoderma* sp. was also considered as the most versatile fungi from soil and this is due to its high adaptability to its environment where it may reproduce both sexually and asexually, depending on the environmental conditions (Harman *et al*., 2008).

The second most common fungal species identified was *Aspergillus fumigatus* (69 isolates) followed by *Aspergillus flavus* (42 isolates). Both of these fungi have been isolated from all of the sampling sites. *Aspergillus fumigatus* is a well-known soil fungus that occurs throughout the world (O'Gormann, 2011). This species fits well in the soil niche as the fungal nature allows it to obtain moisture and nutrients without the sun. This fungus adapts well in a wide range of environment especially in air and soil (McNeill *et al*., 2006). The same results were also documented elsewhere, where *A. fumigatus* and *A. flavus* had been isolated from peat soil (Thormann and Rice, 2007).

The fungi prevalence in deep peat of Sarawak according to sampling sites is shown in *Table 3*. The most common fungi found in Pelitanah were *A. fumigatus* followed by *A. flavus*. Others fungi

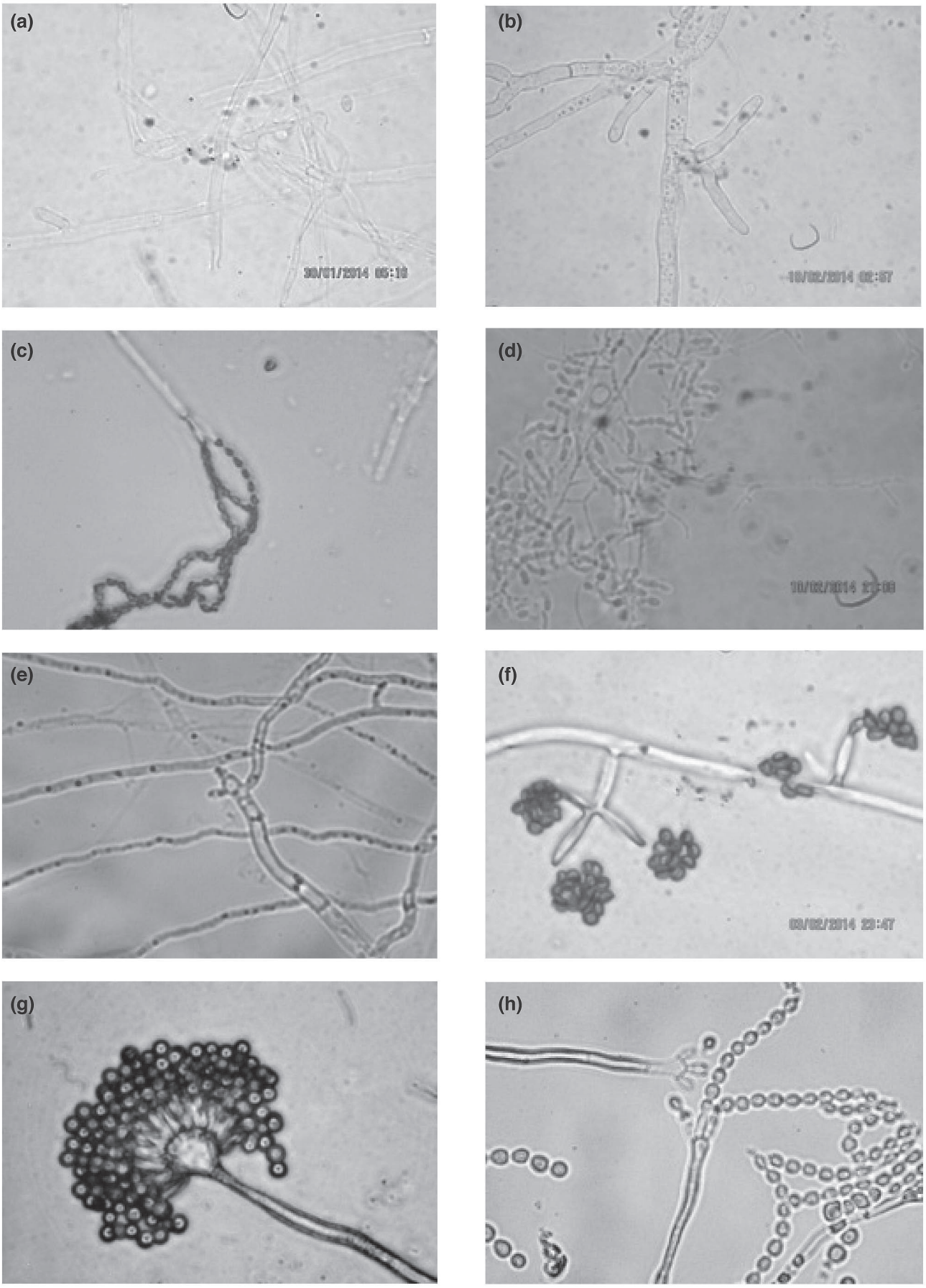

Figure 1. Microscopic photographs of selected fungi in deep peat, Sarawak: (a) Apiospora sinensis, *(b)* Phanerochaete *sp., (c)* Penicillium chrysogenum, *(d)* Taphrina farlowii, *(e)* Mortierella chlamydospora, *(f)* Hypocrea atroviridis, *(g)* Aspergillus fumigatus *and (h)* Aspergillus flavus *(magnification 1000X).*

TABLE 2. FUNGI PREVALENCE IN DEEP PEAT IDENTIFIED BY USING 18S rDNA PRIMER

No.	Accession No.	Fungi species and strain/isolate	Frequency	Frequency according to sites
1	JX242484.1	*Hypocrea atroviridis* strain SMF-H08	79	C (7) (1B, 1C, 2B, 3B, 4A, 6D, 7B) D (58) (10A, 1A, 1B, 1C, 1D, 2A, 2B, 2C, 3C, 4A, 4B, 4C, 4D, 5A, 5B, 5C, 6B, 6C, 6D, 7A, 7B, 7C, 8A, 8D, 9A, 9B, 9D) N (8) (1A, 1B, 2A, 3A, 3B, 3C, 7D, 9D) PF (6) (2B, 2C, 3A, 6B, 7B, 7C)
2	FJ560718.1	*Aspergillus fumigatus* strain YA-14	69	C (22) (10A, 10C, 10D, 1A, 1B, 1C, 3A, 3B, 3C, 4D, 6A, 7B, 7C, 7D, 8A, 8B, 8D) D (2) (6B, 7D) M (8C) N (27) (1B, 1D, 2A, 2B, 2D, 3A, 3C, 4A, 4C, 4D, 5A, 5B, 5C, 6A, 6B, 7B, 8B, 8C, 8D, 9C, 9D) PF (9) (10C, 1B, 1D, 2A, 2B, 5D, 7B, 7C, 8C) PT (5) (3B, 5C, 7C, 9D) UP (5C, 5D, 8C)
3	KF691806.1	*Aspergillus flavus* isolate EFB2.3A	42	C (13) (2A, 2D, 3A, 3C, 4B, 7A, 8A, 8C, 9D) D (2) (10B, 9D) M (13) (10D, 10A, 2A, 2D, 3A, 4C, 5D, 6A, 8A, 8B, 9B, 9D) N (4) (1C, 2B, 6C, 9D) PF (6) (1B, 1D, 2A, 3A, 5C, 6B) PT 10B UP (3) (10C, 3B, 4B)
4	JN935058.1	*Hypocrea* sp. W63	14	D (10) (6D, 7A, 7C, 7D, 8A, 8C, 8D) M 3C PF (1C, 5D, 7C)
5	HM590651.1	*Aspergillus* sp. MJ1-3	5	D 7D M (1C, 4A) N 5D UP 2B
6	KJ470706.1	*Aspergillus tamarii* strain ZJUT ZQ013	4	C (3C, 3D, 8B, 9B)
7	KF803346.1	*Aspergillus niger* strain SSA071	3	N (4A, 5C, 9C)
8	JQ045856.1	*Aspergillus nomius* isolate DSF11 NIOT	2	M (1A, 1B)
9	HG798649.1	*Meyerozyma guilliermondii* strain K7	2	D (3B, 7D)
10	KF913249.1	*Aspergillus fumigatus* strain Salmanfa	2	C (1B 6B)
11	AY083815.1	*Apiospora sinensis*	1	PF (1C)
12	KF018469.1	*Aspergillus alliaceus* strain 21.1	1	M (5C)
13	KF758784.1	*Aspergillus niger* strain W1102	1	M (2C)
14	JX121089.1	*Hypocrea rufa* strain ZY-01	1	D (5A)
15	HQ667508.1	*Mortierella chlamydospora* strain CBS 120.34	1	M (6A)
16	KF466870.1	*Ophidiomyces ophiodiicola* strain UAMH 6688	1	D (3C)
17	KF849480.1	*Penicillium chrysogenum* strain SKPM	1	PF (9A)
18	GU190189.1	*Phanerochaete* sp. enrichment culture clone NJ-F8	1	PF (4B)
19	AY249515.1	*Pichia guilliermondii* AjvM12	1	D (8A)
20	AB000950.1	*Taphrina farlowii*	1	PF (9A)
21	EF060538.1	*Trichocomaceae* sp. LM192	1	PF (1C)
22	KJ467778.1	*Trichoderma* sp. fP6	1	C (9A)
23	AB923813.1	*Trichoderma* sp. SM-2014-A	1	C (4A)
24	JQ242230.1	Uncultured eukaryote clone C07_PNG60-1-16	1	D (7C)

Note: Letters outside the bracket are sites P - Pelitanah Forest, UP - Pelitanah unplanted, PT - Pelitanah planted, C - Cermat Ceria, M - Muladam, D - Durafarm and N - Naman. Number in the bracket is the sampling point number and letter in the bracket are depth of sampling A - topsoil, B - midway between topsoil and water table, C - at water table and D - below water table.

TABLE 3. FUNGI PREVALENCE ACCORDING TO SITE IN DEEP PEAT, SARAWAK

Sites	Accession No.	Fungal species	Frequency	Sampling point and soil level
Pelitanah Forest	FJ560718.1	*Aspergillus fumigatus* strain YA-14	9	PF (10C, 1B, 1D, 2A, 2B, 5D, 7B, 7C, 8C)
	JX242484.1	*Hypocrea atroviridis* strain SMF-H08	6	PF (2B, 2C, 3A, 6B, 7B, 7C)
	KF691806.1	*Aspergillus flavus* isolate EFB2.3A	6	PF (1B, 1D, 2A, 3A, 5C, 6B)
	JN935058.1	*Hypocrea* sp. W63	3	PF (1C, 5D, 7C)
	AY083815.1	*Apiospora sinensis*	1	PF 1C
	KF849480.1	*Penicillium chrysogenum* strain SKPM	1	PF 9A
	GU190189.1	*Phanerochaete* sp. enrichment culture clone NJ-F8	1	PF 4B
	AB000950.1	*Taphrina farlowii*	1	PF 9A
	EF060538.1	*Trichocomaceae* sp. LM192	1	PF 1C
Pelitanah (planted)	FJ560718.1	*Aspergillus fumigatus* strain YA-14	5	PT (3B, 5C, 7C, 9D)
	KF691806.1	*Aspergillus flavus* isolate EFB2.3A	1	PT 10B
Pelitanah (unplanted)	FJ560718.1	*Aspergillus fumigatus* strain YA-14	3	UP (5C, 5D, 8C)
	KF691806.1	*Aspergillus flavus* isolate EFB2.3A	3	UP (10C, 3B, 4B)
	HM590651.1	*Aspergillus* sp. MJ1-3	1	UP 2B
Maludam	KF691806.1	*Aspergillus flavus* isolate EFB2.3A	13	M (10D, 10A, 2A, 2D, 3A, 4C, 5D, 6A, 8A, 8B, 9B, 9D)
	HM590651.1	*Aspergillus* sp. MJ1-3	2	M (1C, 4A)
	JQ045856.1	*Aspergillus nomius* isolate DSF11 NIOT	2	M (1A, 1B)
	JN935058.1	*Hypocrea* sp. W63	1	M 3C
	FJ560718.1	*Aspergillus fumigatus* strain YA-14	1	M 8C
	KF018469.1	*Aspergillus alliaceus* strain 21.1	1	M 5C
	KF758784.1	*Aspergillus niger* strain W1102	1	M 2C
	HQ667508.1	*Mortierella chlamydospora* strain CBS 120.34	1	M 6A
Cermat Ceria	FJ560718.1	*Aspergillus fumigatus* strain YA-14	22	C (22) (10A, 10C, 10D, 1A, 1B, 1C, 3A, 3B, 3C, 4D, 6A, 7B, 7C, 7D, 8A, 8B, 8D)
	KF691806.1	*Aspergillus flavus* isolate EFB2.3A	13	C (13) (2A, 2D, 3A, 3C, 4B, 7A, 8A, 8C, 9D)
	JX242484.1	*Hypocrea atroviridis* strain SMF-H08	7	C (7) (1B, 1C, 2B, 3B, 4A, 6D, 7B)
	KJ470706.1	*Aspergillus tamarii* strain ZJUT ZQ013	4	C (3C, 3D, 8B, 9B)
	KF913249.1	*Aspergillus fumigatus* strain Salmanfa	2	C (1B 6B)
	KJ467778.1	*Trichoderma* sp. fP6	1	C 9A
	AB923813.1	*Trichoderma* sp. SM-2014-A	1	C 4A
Durafarm	JX242484.1	*Hypocrea atroviridis* strain SMF-H08	58	D (10A, 1A, 1B, 1C, 1D, 2A, 2B, 2C, 3C, 4A, 4B, 4C, 4D, 5A, 5B, 5C, 6B, 6C, 6D, 7A, 7B, 7C, 8A, 8D, 9A, 9B, 9D)
	JN935058.1	*Hypocrea* sp. W63	10	D (6D, 7A, 7C, 7D, 8A, 8C, 8D)
	FJ560718.1	*Aspergillus fumigatus* strain YA-14	2	D (6B, 7D)
	KF691806.1	*Aspergillus flavus* isolate EFB2.3A	2	D (10B, 9D)
	HG798649.1	*Meyerozyma guilliermondii* strain K7	2	D (3B, 7D)
	HM590651.1	*Aspergillus* sp. MJ1-3	1	D 7D
	JX121089.1	*Hypocrea rufa strain* ZY-01	1	D 5A
	KF466870.1	*Ophidiomyces ophiodiicola* strain UAMH 6688	1	D 3C
	AY249515.1	*Pichia guilliermondii* AjvM12	1	D 8A
	JQ242230.1	*Uncultured eukaryote* clone C07_PNG60-1-16	1	D 7C
Naman	FJ560718.1	*Aspergillus fumigatus* strain YA-14	27	N (1B, 1D, 2A, 2B, 2D, 3A, 3C, 4A, 4C, 4D, 5A, 5B, 5C, 6A, 6B, 7B, 8B, 8C, 8D, 9C, 9D)
	JX242484.1	*Hypocrea atroviridis* strain SMF-H08	8	N (1A, 1B, 2A, 3A, 3B, 3C, 7D, 9D)
	KF691806.1	*Aspergillus flavus* isolate EFB2.3A	4	N (1C, 2B, 6C, 9D)
	KF803346.1	*Aspergillus niger* strain SSA071	3	N (4A, 5C, 9C)
	HM590651.1	*Aspergillus* sp. MJ1-3	1	N 5D

Note: Letters outside the bracket are sites P - Pelitanah Forest, UP - Pelitanah unplanted, PT - Pelitanah planted, C - Cermat Ceria, M - Muladam, D - Durafarm and N - Naman. Number in the bracket is the sampling point number and letter in the bracket are depth of sampling A - topsoil, B - midway between topsoil and water table, C - at water table and D - below water table.

recorded were *Penicillium chrysogenum, Trichoderma* sp., *Hypocrea* sp., *Phanerochaete* sp., *Taphrina farlowii* and *Apiospora sinensis* (*Figure 1*). For Maludam National Park, about 59.09% of the isolates obtained were *A. flavus*. *A. fumigatus* (48.0%) was the predominant fungal species found in Cermat Ceria followed by *A. flavus* (26.0%).

Other fungi obtained were *Hypocrea atroviridis*, *Aspergillus tamari* and *Trichoderma* sp. which had been isolated on peat soil (Dickinson and Boardmann, 1970). In Durafarm Oil Palm Plantation, most common fungi found were *Hypocrea atroviridis* (72.5%) followed by *Hypocrea* sp. (15.0%). *Aspergillus flavus, Aspergillus fumigatus, Aspergillus* sp. *Ophidiomyces ophiodiicola* and *Pichia guilliermondii* were amongst fungi found in Durafarm. Whearas, in Naman Oil Palm Plantation, *Aspergillus fumigatus* was the commonly isolated fungus (62.79%) followed by *Hypocrea atroviridis* (18.6%). In all study sites examined, the predominantly isolated fungi were from the genus *Aspergillus* spp. This particular observation is not surprising, as other study has indicated that the *Aspergillus* spp. can be isolated in a wide variety of environments (Klich, 2002).

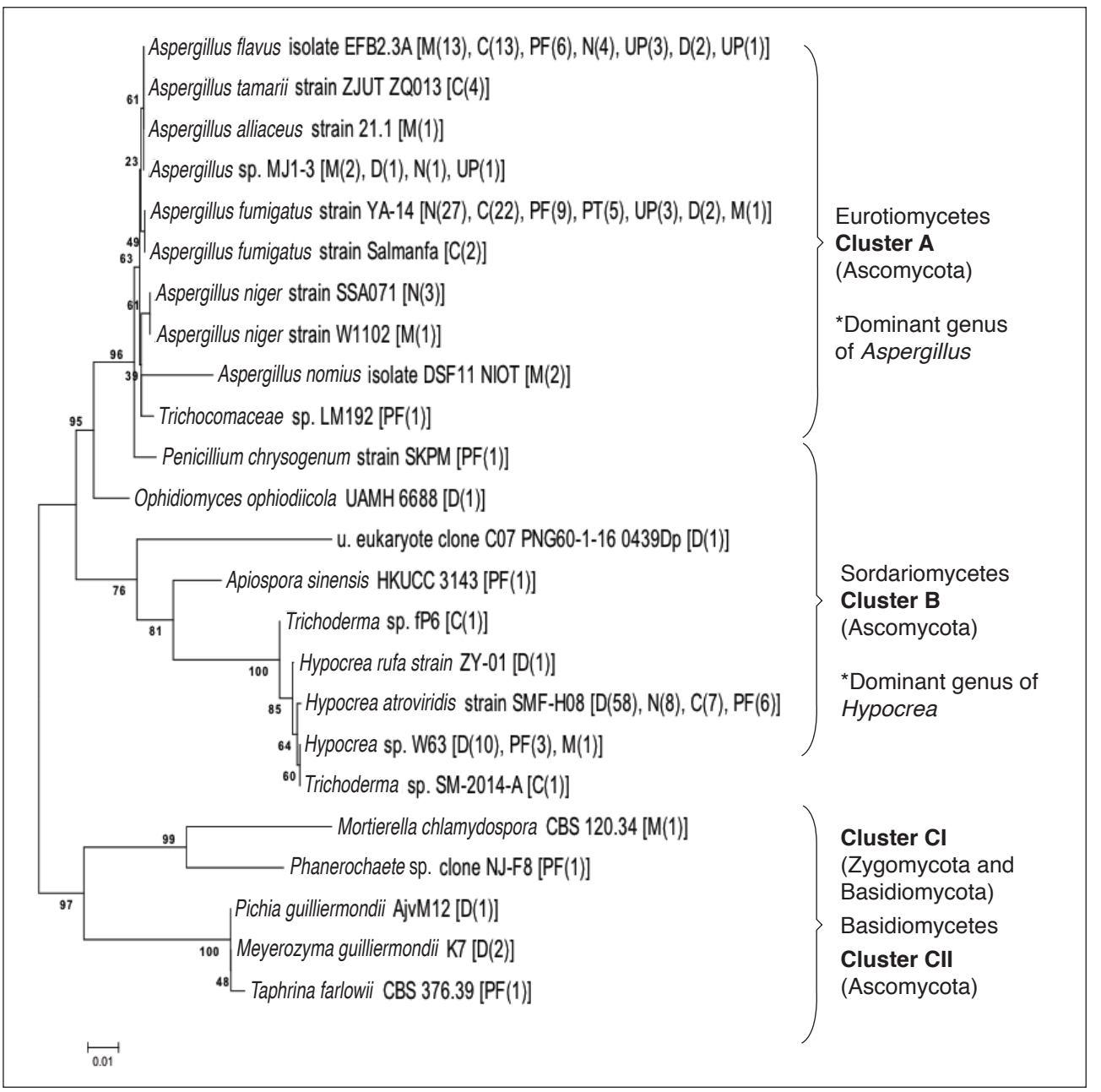

Figure 2. Evolutionary relationships of 24 fungus taxonomy from seven sites of deep peat soil in Sarawak sampled in September/October 2011. The evolutionary history was inferred using the Neighbour-Joining method and the evolutionary distances were computed using the Kimura 2-parameter method. Phylogenetic analyses were conducted in MEGA4. Capital letter and number in bracket represent the sites and frequency of appearance of the fungus. M - Maludam National Park, N - Naman, C - Cermat Ceria, D - Durafarm, PF- Pelitanah Forest, PT - Pelitanah (planted) and UP - Pelitanah (clean cleared).

Analysis of the phylogenetic tree was conducted by using MEGA 4 software (*Figure 2*). The evolutionary history was inferred using the Neighbour-Joining method and the evolutionary distances were computed using the Kimura 2-parameter method. The figure mainly showed three major clusters (A, B and C). Major cluster A was the dominant cluster which consisted of 24 different fungi from division Ascomycota. The most prevalent genus in this dominant cluster was *Aspergillus* spp. with nine different species/strain. Major cluster B consisted of seven fungi species where the dominant genus was Hypocrea/Trichoderma. For major cluster C, there were two sub-clusters namely C1 and C2. Sub-cluster C1 contained two division of fungi which were Basidiomycota (*Phanerochaete* sp.) and Zygomycota (*Mortierella chlamydospora*), whereas subcluster C2 fungi were under division Ascomycota.

Table 4 shows the recorded moisture content and pH reading for samples taken at different sites at different depths. The soil moisture of the Pelitanah ranged from 64.09% to 88.04% while the pH ranged from 3.47 to 3.91. For peat soil sampled at Maludam National Park, the moisture content recorded ranged from 152.64% to 277.78% and the pH ranged from 3.20 to 3.51. As for soil taken at Cermat Ceria, the moisture content went from 45.10% to 64.07% with the pH ranged from 3.69 to 3.87. As for the soil taken at Durafarm, the moisture content ranged from 72.03% to 131.39% and the pH ranged from 3.30 to 3.33. The soil moisture of the Naman ranged from 210.49% to 294.75% while the pH readings were 3.03 to 3.19. The moisture content and pH for each site are recorded in *Table 4*. The range of readings for moisture content seems lower compared to other studies. Tropical peat soil has surface soil temperature ranging from 27°C to 30°C with the moisture content from 338% to 398% (Firdaus *et al*., 2011). It is the favourable range for most of Ascomycetes (Smit *et al*., 1999), although Kolay and Aminur (2011) documented that moisture content and pH, of 598% and 3.75, respectively could support Ascomycetes. The large difference in the

TABLE 4. MOISTURE CONTENT, pH AND RAINFALL OF DEEP PEAT SOIL IN SARAWAK

Sites		Moisture content (w) (%)	pH	Total rainfall (mm)
Pelitanah Forest	L1	88.04 ± 44.66	3.74 ± 0.36	
	L2	77.86 ± 9.13	3.52 ± 0.33	347.5
	L3	74.72 ± 14.40	3.68 ± 0.26	
	L4	68.27 ± 17.21	3.53 ± 0.29	
Pelitanah (clean-cleared)	L1	67.05 ± 9.61	3.91 ± 0.14	
	L2	65.55 ± 9.28	3.75 ± 0.25	347.5
	L3	64.09 ± 10.91	3.83 ± 0.22	
	L4	64.33 ± 14.89	3.86 ± 0.23	
Pelitanah (planted)	L1	70.07 ± 9.16	3.52 ± 0.30	
	L2	70.18 ± 9.20	3.47 ± 0.35	347.5
	L3	69.35 ± 10.64	3.61 ± 0.45	
	L4	65.10 ± 11.25	3.65 ± 0.25	
Maludam National Park	L1	277.78 ± 269.72	3.51 ± 0.28	
	L2	199.58 ± 224.54	3.28 ± 0.12	263.5
	L3	187.57 ± 226.96	3.22 ± 0.14	
	L4	152.64 ± 154.67	3.20 ± 0.09	
Cermat Ceria Forest	L1	64.07 ± 9.20	3.81 ± 0.10	
	L2	58.10 ± 24.88	3.79 ± 0.16	No data
	L3	45.10 ± 22.82	3.87 ± 0.34	
	L4	49.18 ± 20.60	3.69 ± 0.19	
Durafarm Plantation	L1	72.03 ± 36.65	3.33 ± 0.22	
	L2	84.07 ± 59.24	3.30 ± 0.13	476.0
	L3	131.39 ± 79.68	3.28 ± 0.12	
	L4	97.70 ± 53.11	3.31 ± 0.16	
Naman Plantation	L1	210.49 ± 182.09	3.19 ± 0.55	
	L2	273.30 ± 289.08	3.03 ± 0.16	398.0
	L3	251.93 ± 252.24	3.05 ± 0.11	
	L4	294.75 ± 322.80	3.11 ± 0.14	

moisture content maybe due to the different water holding capacity for the different types of deep peat. The reduced water content is also caused by water evaporation and lower soil organic content (Firdaus *et al*., 2010). Water retention of the peat soil may also be reduced because of the removal of vegetation and soil compaction. The pH readings obtained, however, did not differ much with the results of others (Abat *et al*., 2012). Soil pH has been identified as one of the most influential factors controlling microbial diversity and community composition across soils types (Fierer and Jackson, 2006). However, fungal community composition was less strongly affected by pH (Rousk *et al*., 2010).

CONCLUSION

Tropical peat soil offers a wide range of soil fungi that may be used for various applications. The commonly isolated fungal species for peat ecosystems in Sarawak were *A. fumigatus*, *A. flavus*, *Hypocrea atroviridis*, and *Hypocrea* spp. In the drained peat ecosystems, *Trichoderma* spp. or *Hypocrea* spp. were more dominant as compared to the primary peat forest. However, drained cultivated peat situated at Rajang River which is often flooded has almost similar fungal community as the primary peat forest. With the utilisation of 18S rDNA marker, molecular approach can be a promising alternative for fungal identification and it is better supported with conventional approaches. Information on fungal species associated with tropical peat ecosystems is lamentably low. Therefore, further research on microbial biodiversity in tropical peat need to be performed using more primers and the latest state-of-the-art for conservation and utilisation purposes.

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