Malaysian Journal of Science **35** (2): 290- 303 (2016)

DIVERSITY AND DISTRIBUTION OF MICROFUNGI FROM DIPTEROCARP FORESTS IN SARAWAK, BORNEO ISLAND (MALAYSIA).

Keanekaragaman dan Distribusi mikrofungi dari hutan dipterokarpa di Sarawak, Pulau Borneo (Malaysia).

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Received: 22 Mac 2016 Revise: 22 Aug 2016 Accepted:19 Sept 2016

ABSTRACT South-east Asian dipterocarp forests contain plant species that are vulnerable and at risk of extinction. Microfungi, as important decomposers and sources of useful metabolites, have not been well studied in regards to their diversity and distribution in the South-east Asian dipterocarp forests. This study reports the microfungal species associated with dominant and vulnerable plant species in two dipterocarp forests, namely Gunung Gading and Kubah National Parks, in Sarawak on the Borneo Island. Green leaves and litters of five host plants, namely *Baccaurea* sp., *Macaranga triloba* (Thunb.) Müll.Arg., *Macaranga* sp., *Shorea macrophylla* (de Vr.) Ashton and *Syzygium* sp., were incubated on water agar and malt extract agar. A total of 171 fungal taxa were recorded on the five host plants, during two visits, each to both forests. *Syzygium* sp. had the highest microfungal diversity of 84 taxa followed by 80 taxa on *Macaranga* sp., 53 taxa on *Baccaurea* sp., 43 taxa on *M. triloba* and the lowest, 35 taxa, on *Shorea macrophylla. Pestalotiopsis* spp., *Oidiodendron* spp., *Graphium* spp., and *Mycelia sterilia* were the most frequently isolated microfungal species from all the five host plants. There was a high microfungal similarity between *Syzygium* sp. and *Macaranga* sp. with 34 taxa common to both plants. The lowest similarity was recorded between *S. macrophylla*, *M. triloba* and *Baccaurea* sp. with only 13 taxa common between them. This is the first report on microfungi associated with dominant and vulnerable plant species in South-east Asian dipterocarp forests; an important record of the fungal diversity on these plants and an exposition on microfungi in Sarawak, Borneo Island, Malaysia.

Keywords: Microfungi, *Macaranga, Shorea, Baccaurea, Syzygium*

ABSTRAK Selatan-timur hutan dipterokarpa Asia berisi spesies tanaman yang rentan dan terancam punah. Mikrofungi, sebagai dekomposer penting dalam ekosistem dan sumber metabolit yang berguna belum diteliti dengan baik dalam hal keragaman dan distribusinya di hutan dipterokarpa Asia Tenggara. Penelitian ini mengungkapkan spesies mikrofungi terkait dengan spesies tanaman yang dominan dan rentan dalam dua hutan dipterokarpa, yaitu Gunung Gading dan Taman Nasional Kubah, di Sarawak di pulau Borneo. Daun hijau dan Serasah dari lima tanaman inang diinkubasi pada agar air dan ekstrak malt agar. Sebanyak 171 taksa mikrofungi dicatat pada lima tanaman inang, yaitu *Baccaurea* sp., *Macaranga triloba*, *Macaranga* sp., *Shorea macrophylla* dan *Syzygium* sp. selama empat kunjungan ke kedua hutan. *Syzygium* sp. telah keragaman mikrofungal tertinggi dari 84 taksa diikuti oleh 80 taksa pada *Macaranga* sp., 53 taksa pada *Baccaurea* sp., 43 taksa di *M. triloba* dan terendah, 35 taksa, pada *Shorea macrophylla*. *Pestalotiopsis* spp., *Oidiodendron* spp., *Graphium* spp., dan miselia steril yang spesies mikrofungal yang paling sering diisolasi dari semua lima tanaman inang. Ada kesamaan microfungal tinggi antara *Syzygium* sp. dan *Macaranga* sp. dengan 34 taksa umum untuk kedua tanaman. Kesamaan terendah tercatat antara *S. macrophylla*, *M. triloba* dan *Baccaurea* sp. dengan hanya dengan 13 taksa umum untuk ketiganya. Ini adalah laporan pertama pada mikrofungi terkait dengan spesies tanaman rentan dan dominan di hutan dipterokarpa Asia Tenggara; catatan penting dari keragaman mikrofungi didukung oleh tanaman ini dan dalam pameran mikrofungi di Sarawak, pulau Borneo, Malaysia.

Kata kunci: Mikrofungi, *Macaranga*, *Shorea*, *Baccaurea*, *Syzygium*

INTRODUCTION

The forest ecosystem is home to a vast diversity of plants, animals and microorganisms (Engelbrecht, 2012) including fungi. Forests are essential to the long-term wellbeing of local populations, national economies, and the earth's biosphere as a whole (Santiago Declaration, 1995). Dipterocarp forests, mainly found in the South-east Asian region (Whitmore, 1975), are

unique and have been noted for their high diversity of living organisms. These dipterocarp forests got their name from the plant family (Dipterocarpaceae) which dominates the forests' plant species. Among the major plant families found in this type of forest are the Dipterocarpaceae, Phyllanthaceae, Myrtaceae and Euphorbiaceae. In Malaysia, the dipterocarp forest is one of the major forest types in the country, occupying about 80% of the total natural forests in the Malaysian state of Sarawak on the Borneo Island (Sarawak Forestry, 2015).

Microfungi are known for their various importance to human life, for example, as natural decomposers, source of novel drugs, biological control of pests and diseases, in industrial production of chemicals and also as part of our ecosystem diversity. Microfungi on different plant species of the dipterocarp forests on the Borneo Island have not been well documented. Also, there is insufficient report on the microfungal communities associated with many plant families, including the Dipterocarpaceae, Phyllanthaceae, Myrtaceae and Euphorbiaceae which are found in the dipterocarp forests in the tropics and in other forest types across the globe. Few studies on microfungi associated with plants in dipterocarp forests include that of Osono et al., (2009) on *Shorea obtusa*, Bettucci & Roquebert (1995) on *S. robusta* and recently Harahap, Rahayu, & Hidayat (2013) on litters of *Shorea* spp.

Understanding and monitoring the diversity and distribution of fungi in our forests and on various plant families are necessary for the proper functioning of our ecosystem in addition to being sources of sustainable and renewable raw materials for human uses. This study was carried out in order to understand the diversity and distribution of microfungi associated with five host plants in two dipterocarp forests in Sarawak, Borneo Island, Malaysia. The result from this study will be a valuable contribution to the global fungal diversity in reference to their distribution on plants and their occurrence in dipterocarp forests and in Sarawak, Borneo Island.

MATERIALS AND METHODS

The Sample Collection Sites

The sample collection sites were Kubah National Park and Gunung Gading National Park located in the seventh division of Sarawak, East Malaysia. The two Parks are covered with a lowland mixed dipterocarp forest, with the family Dipterocarpaceae dominating the plant species and are characterised by a tropical climate with an average annual temperature of 27 °C and an average annual rainfall of 4095 mm (Climate-Data, 2015).

Sampling Design

Leaf samples were collected from five host plants namely *Syzygium* sp., *Baccaurea* sp., *Shorea macrophylla*, *Macaranga triloba*, and *Macaranga* sp. on four collection trips to both sampling sites; two trips to Kubah National Park in March, 2014 and September, 2014, likewise, two trips to Gunung Gading National Park in November, 2014 and February, 2015. The host plants studied were selected based on their availability at the site since the vegetation at each site is natural, and not planted, thus the plants grow at random points. The latitudinal and longitudinal readings at each collection point were recorded with a handheld Global Positioning System (GPS) equipment (Table 1).

Table 1. Details of the host plants sampled in this study

	Plant collected	Collection site	GPS co-ordinate	Elevation
1.	*Macaranga* triloba (Family Euphorbiaceae)	KNP	N 01^o 36 081 E 110^o 11 204	410
2.	*Shorea macrophylla* (Family Dipterocarpaceae).	KNP	N 01^o 36 757 E 110^o 11 750	175 m
3.	*Baccaurea* sp. (Phyllanthaceae)	GNP	N 01^o 41 510 E 110^o 50 704	635 m
4.	*Macaranga* sp. (Family Euphorbiaceae)	GNP	N 01^o 41 510 E 109^o 50 704	635 m
5.	*Syzygium* sp. (Family Myrtaceae)	GNP	N 01^o 41 625 E 109^o 50 573	657 m

KNP=Kubah National Park
GNP= Gunung Gading National Park

For each plant sampled, leaves were cut from different parts of the plant, mostly matured leaves while leaf-litters were picked directly around the plant on the ground. The samples were put in plastic bags and transported to the laboratory for processing.

Processing of Leaf Samples and Data analysis

Upon return to the laboratory, the leaves were processed within 24 hrs for endophytic and
saprophytic microfungal isolation as described in Lateef, Sepiah, & Bolhassan (2015). Endophytic fungi were isolated from leaves which were washed under running tap water to remove superficial dust and debris on their surface, cut into 1 cm^2 and then surface sterilized with 70% ethanol for one minute, 10 % hydrogen peroxide (H_2O_2) for five minutes, rinsed with 70% ethanol for one minute and finally rinsed with deionized sterile distilled water. For saprophytic microfungi isolation, the leaf samples were washed with double sterilized distilled water, cut into 1 cm^2 and washed again with sterile distilled water. The leaf segments were allowed to dry fully under sterile conditions in laminar flow before incubating on the media. Four hundred leaf segments were used from each plant species except for *S. macrophylla* in which only 200 leaf segments were incubated without any antibiotics on water agar and malt extract agar. Identification of the microfungi were made to genus level, and wherever possible, to species level based on conidia types and colony appearance using various identification guides (Carmichael, Kendrick, Connors, & Sigler, 1980; Ellis, 1971, 1976; Kendrick & Carmichael, 1973; Morris, 1963; Seifert, Morgan-Jones, Gams, & Kendrick, 2011).

Data on the occurrences of the microfungi on the leaf segments were recorded and the presence of a microfungus on each leaf segment was recorded as one occurrence. The frequency of isolation of each microfungal taxa was determined according to Hata & Futai (1995) and Osono (2008) as follows:

$$\text{Frequency of isolation (I. F.)} = \frac{\text{the total number of leaf segments from which a fungal taxa was present}}{\text{the total number of leaf segments observed}} \times 100$$

Diversity index such as the Shannon's diversity index as well as species accumulation curves and species richness were estimated with two richness estimators – Abundance-base Coverage Estimator (ACE) and Chao1 using the EstimateS diversity software (http://purl.oclc.org/estimates) version 9.1.0 released June 2013 (Colwell, 2013).

RESULTS AND DISCUSSION

Microfungal Diversity

One hundred and seventy-one microfungal taxa were recovered, which included 29 ascomycetes, seven basidiomycetes, 21 coelomycetes, 102 hyphomycetes, five zygomycetes and seven sterile forms from a total of 1800 leaf fragments of the five host plants namely *M. triloba*, *Macaranga* sp. *S. macrophylla*, *Baccaurea* sp. and *Syzygium* sp. (Table 2). The highest number of microfungal species, eighty-four (84) taxa, was recorded on the host plant *Syzygium* sp. followed by 80 taxa on *Macaranga* sp., 53 taxa on *Baccaurea* sp., 43 on *M. triloba* and the lowest, 35 taxa, on *Shorea macrophylla* (Table 3).

Table 2. Percentage (%) occurrence and host plant distribution of fungal taxa on leaves of plants from Kubah and Gunung Gading National Parks in Sarawak ("-" means absent).

		Host plant				
	Fungal species	*Syzygium* sp.	*Baccaurea* sp.	*Shorea macrophylla*	*Macaranga triloba*	*Macaranga* sp.
1	*Acremonium* spp.	-	0.25	2	8.5	7
2	*Anthostomella* sp.	1.5	-	-	-	-
3	*Arthrinum* sp.	0.25	-	-	-	-
4	Ascomycete 1	-	-	-	-	0.5
5	Ascomycete 17	-	-	2.5	-	-
6	Ascomycete 2	-	-	-	-	0.25
7	Ascomycete 26	-	-	0.5	-	-
8	Ascomycete 3	-	-	0.5	-	-
9	Ascomycete 4	-	0.25	-	-	-
10	*Aspergillus niger*	0.25	1.5	-	0.25	0.5
11	*Aspergillus* sp.	0.5	-	-	1.25	-
12	*Aureobasidium pullulans*	-	-	-	0.25	1
13	Basidiomycete 2	-	-	-	-	0.25
14	Basidiomycete 3	5	0.25	-	-	-
15	*Basipetospora* sp.	0.75	-	-	-	-
16	*Beltrania rhombica*	0.25	3.75	-	0.5	5.25
17	*Beltraniopsis* sp.	-	-	-	-	1.5
18	*Bipolaris* sp.	-	-	-	-	0.75
19	*Bispora* sp.	-	0.25	-	1	-
20	Black *Mycelia sterilia* 1	4.5	7.75	14	12.5	13.25
21	Black *Mycelia sterilia* 2	-	-	-	1.75	0.75

22	*Blakeslea* sp.	-	-	-	-	0.75
23	*Botryodiplodia* sp.	0.25	-	0.5	0.75	5.5
24	*Botryodiplodia theobromae*	-	-	2	-	6.75
25	*Botrytis* sp.	-	-	2.5	-	-
26	*Brachyphoris* sp.	-	-	-	-	0.5
27	Brown *Mycelia sterilia*	0.5	1	-	-	0.75
28	*Campylospora* sp.	0.25	0.25	-	-	-
29	*Cercospora* sp.	1.25	-	-	1.25	-
30	*Ceriospora polygonacearum*	1.25	-	-	-	-
31	*Chaetomium* sp.	-	-	1.5	-	1.25
32	*Chaetopsina* sp.	-	-	-	0.25	-
33	*Chaetospermum artocarpi*	0.75	1.75	-	-	-
34	*Chaetospermum* spp.	0.25	0.25	-	-	2
35	*Chaetosphaeria* sp.	11.75	-	-	-	1
36	*Chloridium* sp.	-	-	-	-	2.75
37	*Circinotrichum fertile*	0.25	-	-	0.5	-
38	*Circinotrichum* sp. 2	2.5	-	-	-	-
39	*Circinotrichum* sp. 3	1.5	-	-	-	-
40	*Cladosporium* sp.	4	0.75	-	-	6
41	*Codinae* sp.	1.75	3.25	-	0.5	-
42	Coelomycete 1	-	-	-	0.25	-
43	Coelomycete 2	-	-	-	2.5	-
44	Coelomycete 3	-	0.5	-	-	-
45	Coelomycete 39	-	0.75	-	-	-
46	Coelomycete 54	1.25	-	-	-	-
47	*Colletotrichum acutatum*	1.25	-	4.5	4.5	-
48	*Colletotrichum gloeosporioides*	12.75	5.5	9	8.25	-
49	*Colletotrichum* sp.	0.5	-	-	-	-
50	*Conidiobolus*-like sp.	-	-	-	-	1.25
51	*Cordana* sp.	0.5	-	-	-	-
52	*Cryptophiale kakombensis*	-	-	-	0.5	-
53	*Cryptophiale minor*	-	-	-	1	-
54	*Cryptophiale* sp.	-	-	-	0.25	-
55	*Cryptophiale udagawae*	0.25	-	-	0.75	-
56	*Curvularia* sp.	-	-	-	-	0.75
57	*Cylindrocarpon obtusisporum*	-	-	-	0.5	-
58	*Cylindrocarpon* sp.	0.5	-	-	-	-

59	*Cylindrocladium* sp. 3	4.25	-	0.5	-	1.25
60	*Dactylaria ciliata*	2.5	-	-	0.75	1.75
61	*Dactylaria obtriangularia*	1.75	2	-	-	-
62	*Dactylaria* sp. 1	-	-	-	-	8.75
63	*Dactylaria* sp. 2	-	0.25	-	-	4
64	*Dictyosporium* sp. 1	-	-	-	-	3
65	*Dictyosporium* sp. 2	-	0.75	-	-	-
66	*Didymella* sp. 3	-	-	-	-	4.5
67	*Diplococcium* sp.	-	-	-	1	-
68	*Diplodia* sp.	1.75	1.25	-	-	1.25
69	*Fusariella* sp.	-	0.5	-	-	1.25
70	*Fusarium* spp.	1	-	0.5	6	3.75
71	*Gelasinospora* sp.	1.25	-	-	-	-
72	*Gliocladium* sp.	2	-	-	-	-
73	*Glomerella* sp. (Sexual state of *Colletotrichum*)	0.75	-	-	-	1.25
74	*Gnomonia* sp.	6.75	3.25	11	7	-
75	*Gonatobotrys* sp.	0.5	-	-	-	-
76	*Grahium pennicillioides*	16	22.25	8	2.5	6.5
77	*Graphium* sp. 2	5.5	-	-	-	-
78	*Guemannomyces graminis*	-	0.5	-	-	9.25
79	*Hansfordia* sp.	4.5	-	-	1.5	2.5
80	*Helicomyces* sp.	1.5	2.5	-	-	4.5
81	*Helicosporium* sp.	0.5	-	-	-	1
82	*Humicola grisea*	0.25	8.75	0.5	-	-
83	*Humicola* sp.	0.75	-	-	-	0.5
84	Hyphomycete 1	-	-	-	2	-
85	Hyphomycete 3	-	-	1.5	-	-
86	Hyphomycete 4	0.5	-	-	-	-
87	Hyphomycete 49	-	-	-	-	0.75
88	Hyphomycete 54	-	-	-	-	3
89	Hyphomycete 7	0.25	-	-	-	-
90	*Idriella lunata*	-	-	-	-	0.25
91	*Isthmolongispora minima*	1	-	-	-	-
92	*Isthmotricladia laeensis*	1.25	-	-	-	-
93	*Kinochaeta* sp.	1.25	-	-	1.5	1.25
94	*Kinochaeta*-like sp.	0.75	-	-	-	-
95	*Kylindria* sp.	0.25	-	-	-	1.5
96	*Lateriramulosa uniinflata*	-	-	-	-	0.25

97	*Leptospahaeria horniaraensis*	-	0.5	4	-	3.25
98	*Leptosphaeria* sp. 2	-	0.5	-	-	-
99	*Lophiostoma* sp.	-	-	-	-	1
100	*Lophiostoma* sp. 2	-	-	-	-	1.5
101	*Menispora* sp.	0.5	-	-	-	-
102	*Minimedusa* sp.	2.25	0.5	-	-	3.5
103	*Monacrosporium* sp.	1.5	-	-	-	6.75
104	*Monilinia* sp.	-	-	2.5	-	0.75
105	*Monodictys* sp.	-	-	-	-	0.25
106	*Mucor* sp.	1	-	9.5	12.5	-
107	*Mucor* sp. 2	-	-	-	2.75	-
108	Myxomycete 1	-	-	-	1	-
109	*Neopestalotiopsis* sp.	-	-	-	-	2.25
110	*Neottiosporella* sp.	-	2	-	-	-
111	*Oidiodendron* sp.	11.75	12.5	2.5	12.5	4.75
112	*Ojibwaya*-like sp.	-	-	-	-	0.5
113	*Paecilomyces* sp.	0.75	-	-	-	1
114	*Penicillium* sp.	1.75	10.25	1	-	0.5
115	*Penicillium* sp. 2	1.5	-	-	-	-
116	*Periconia* sp.	0.25	-	-	-	-
117	*Pestalotiopsis humus*	-	-	5	-	-
118	*Pestalotiopsis malayana*	3	-	-	-	-
119	*Pestalotiopsis* sp.	5.5	45.25	13.5	-	15
120	*Pestalotiopsis* sp. 15	-	-	3.5	-	-
121	*Pestalotiopsis* sp. 16	-	-	0.5	-	-
122	*Pestalotiopsis* sp. 17	-	-	1	-	-
123	*Pestalotiopsis* sp. 7	-	0.25	-	-	-
124	*Pestalotiopsis* sp. 9	-	-	10.5	-	-
125	*Phalangispora* sp.	3	-	-	-	-
126	*Phialophora* sp.	-	0.25	-	-	-
127	*Phomopsis* sp.	0.75	4.25	-	-	0.75
128	*Physarum* sp.	-	-	-	-	1
129	Pink *Mycelia sterilia*	-	0.25	-	-	1.75
130	*Pseudopestalotiopsis kubahensis*	-	-	-	3.25	-
131	*Ramularia* sp.	-	0.25	-	0.25	-
132	*Rhinocladiella cristaspora*	3.25	3.5	-	-	5
133	*Rhinocladiella* sp.	-	-	-	1.75	-
134	*Rhinocladium* sp.	1.5	0.25	-	-	-

135	*Sartorya* sp.	-	0.75	-	-	-
136	*Scolecobasidium* sp.	2.25	-	1.5	-	15
137	*Septonema* sp.	-	-	-	-	1.25
138	*Speiropsis pedatospora*	2.25	-	-	-	0.25
139	*Sporidesmium* sp.	-	-	-	1	-
140	*Sporidesmium* sp. 2	-	-	-	-	0.25
141	*Sporochisma nigroseptata*	-	0.25	-	-	-
142	*Stachybotrys* sp.	0.75	-	-	-	-
143	*Stachybotrys* sp. 1	-	-	2.5	2.5	-
144	*Stachybotrys* sp. 2	-	0.5	-	-	-
145	*Stibella* sp.	-	-	-	-	6.75
146	*Suttoniella* sp.	-	-	-	-	1.25
147	*Sympodiella* sp.	-	2.25	-	-	0.5
148	*Thielavia* sp.	0.5	-	1-	-	-
149	*Thozetella* sp.	0.25	-	1	-	-
150	*Torula* sp.	-	0.25	-	-	3.5
151	*Torula* sp. 2	-	-	-	-	1.25
152	*Trichoderma koningii*	-	-	-	-	-
153	*Trichoderma* sp.	8.75	5.25	22	0.25	-
154	*Trichoderma viride*	0.5	-	-	-	-
155	*Triscelophorus* sp.	0.25	-	-	-	1.5
156	*Ulocladium* sp.	-	-	-	-	3.5
157	*Ulocladium* sp. 2	-	0.5	-	-	-
158	Unidentified 3	-	-	-	-	2.5
159	*Venturia* sp.	-	-	-	3.25	-
160	*Verticillium* sp.	7	0.75	-	0.5	21.25
161	*Verticillium* sp. 3	0.5	-	-	-	-
162	*Volutella ciliata*	1	-	-	-	-
163	*Volutella* sp.	0.25	-	-	-	-
164	White *Mycelia sterilia* 1	7.75	10.5	7	0.25	11.25
165	White *Mycelia sterilia* 3	-	0.75	-	-	-
166	White *Mycelia sterilia* 7	-	-	-	-	0.25
167	*Wiesneriomyces javanicus*	3	2.25	-	-	0.25
168	*Wiesneriomyces*-like sp.	-	-	-	-	5.75
169	*Xylaria* sp.	0.5	0.5	-	-	-
170	Yellow *Mycelia sterilia*	0.25	1.75	0.5	-	-
171	*Zygosporium* sp.	-	-	-	-	0.25

Table 3. Diversity indices and species estimate of microfungi based on host plant.

Name of Plant host	No. of isolates	Species observed	Singletons	Doubletons	ACE (SE*)	Chao1(SE*)	% SC*	Shannon Index
Syzygium sp.	749	84	17	13	93.7	93.7	89.65	3.79
Baccaurea sp.	707	53	14	9	62.09	62.09	85.35	2.91
S. macrophylla	319	35	8	3	41.98	41.98	83.37	3.04
M. triloba	447	43	8	6	46.99	46.99	91.50	3.12
Macaranga sp.	993	80	10	7	85.62	85.62	93.43	3.81

SC*=Sample completeness
SE*=Species estimate.

This study is the first detailed investigation of microfungi associated with several plant species in a dipterocarp forests in Borneo Island, Malaysia. The use of two different growth media in this study enhanced the isolation of different microfungal taxa. Using different media for microfungal diversity studies as earlier reported by Bills & Polishook (1991) and Paulus, Kanowski, Gadek, & Hyde (2006) helps to isolate both the slow growing and fast growing microfungi. In the present study, more microfungal taxa were isolated from *Syzygium* sp. from Gunung Gading National Park as compared to the other plant species. This is in line with previous reports that plant species in the family Myrtaceae, which *Syzygium* sp. belongs support high microfungal diversity (Cheewangkoon et al., 2009) which can be attributed to their possession of certain oils and other substances which favour occurrence of diverse microfungal communities (Carnegie, Burgess, Beilharz, & Wingfield, 2007; Crous, Mohammed, Glen, Verkley, & Groenewald, 2007).

Macaranga sp. and *M. triloba* are dominant plant species in disturbed forests in Southeast Asia (Whitmore, 1975) and in this study, they were more abundant in Kubah National Park compared to Gunung Gading National Park. Eighty and forty-three microfungal taxa were recorded from *Macaranga* sp. from Kubah and *M. triloba* from Gunung Gading respectively. The difference in the microfungal diversity observed from these two plant species of the same genus can be attributed to the different environmental factors in the two sites, and this phenomenon has been earlier reported by Paulus (2004) that site environmental characteristics have influence on microfungal diversity on closely related plant species.

With regards to *Baccaurea* sp. and *S. macrophylla*, inventory of microfungal species on the leaves of these plants has not been reported. *Shorea macrophylla* is presently a vulnerable species at risk of extinction and is mainly found in the South-east Asian forests as a source of quality timber. Documenting the microfungi on this vulnerable plant species at risk of biological extinction is important with many implications. Similar studies on fungal diversity on close relatives of *S. macrophylla* were carried out on *S. obtusa*, *S. robusta* and *Shorea* spp. by Osono et al., (2009), Bettucci & Roquebert (1995) and recently by Harahap et al., (2013), respectively in which low water content and presence of certain phenolic compounds were suggested to be responsible for the low fungal colonization on *Shorea* spp.

The Chao 1 estimates of species richness on each plant species using the diversity software EstimateS ranged from 93.7 taxa on leaves of *Syzygium* sp. to 41.98 taxa on leaves of *S. macrophylla* (Table 3). The Chao 1 species estimate for all the plant hosts studied indicated that some microfungal species were

missed during the isolation period; though this is usual in most microfungal surveys as the Chao 1 and ACE species richness prediction are always higher than the observed species richness (Mohamed & Martiny, 2011; Muthukrishnan, 2012; Zhao et al., 2015).

Species accumulation curves (SACs) of each plant species did not reach an asymptote (Figure 1), suggesting that there are more species to be discovered from these plants. However, the sampling completeness ranged from 83.37 % for *S. macrophylla* to as high as 93.43 % for *Macaranga* sp. (Table 3) showing that the samples collected were enough to recover majority of the microfungal species present on the leaves of the studied plant species, though the sampling was not totally exhaustive.

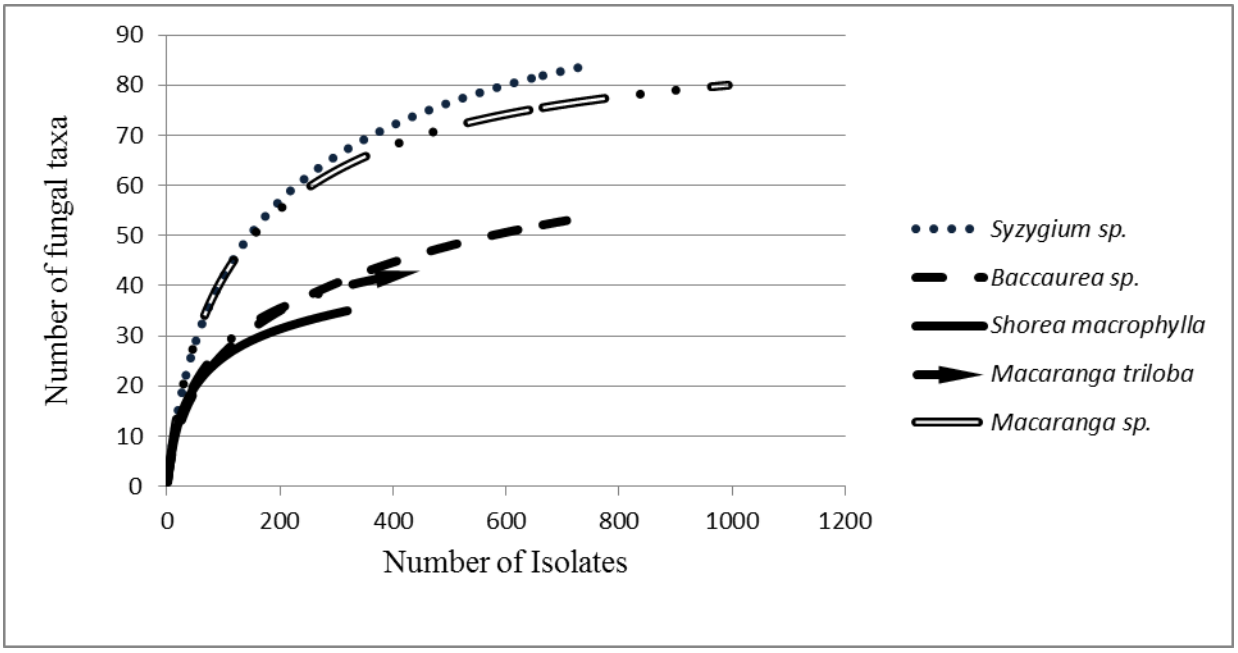

Figure 1. Rarefied species accumulation curves (SACs) of the five host plant studied

Microfungal Distribution and similarities between the host plants

Out of the 171 fungal taxa recorded, only four taxa were isolated from all the five host plants, six taxa from four host plants while majority of the microfungi were recorded only on one host plant. Majority of the microfungal taxa (67%) that were recorded only on one host plant were isolated from *Syzygium* sp. Four fungal taxa (Black *Mycelia sterilia*, *Oidiodendron* sp., white *Mycelia sterilia* and *Graphium penicillioides*) were common on all the five plant species studied. *Mycelia sterilia* (both white and black forms) are known to be generalist fungi found on a wide range of host plants (Allegrucci, Bucsinszky, Arturi, & Cabello, 2015; Kumar, 2013; Sepiah, Roha, & Laman, 2006) while *Oidiodendron* sp. and *Graphium penicillioides* have also been isolated from a variety of host plants. This result shows that these microfungal species can be recovered from most plant species.

The most frequently isolated taxa from the host plants belonged to the genera *Pestalotiopsis*, *Oidiodendron*, *Graphium*, *Mycelia sterilia*, *Trichoderma*, *Colletotrichum*,

Verticillium, *Gnomonia*, *Mucor*, *Scolecobasidium*, *Acremonium*, *Penicillium*, *Chaetosphaeria*, *Rhinocladiella*, *Fusarium* and *Cladosporium* (Figure 2). *Pestalotiopsis* spp. were isolated from 85% of all the five host plants' leaves while *Colletotrichum* spp. were recorded on 55% of all the five host plants' leaves. *Pestalotiopsis* spp. are known common plant saprophytes and endophytes (Maharachchikumbura, Hyde, Groenewald, Xu, & Crous, 2014). Together with *C. gloeosporioides* and *Trichoderma* spp., *Pestalotiopsis* spp. have been reported as one of the most frequently occurring microfungi on different host plant (Cannon & Simmons, 2002; Sepiah et al., 2006). Other species have also been reported as frequent species such as *Penicillium* sp. on 15 medicinal plants in India and bamboo leaf respectively (Kumar, 2013; Shankar, Shashikala, & Krishnamurthy, 2008), *Acremonium* sp., *Verticillium* sp., *Fusarium* sp. (Kumar, 2013; Shankar et al., 2008). However, *Gnomonia* sp., *Scolecobasidium* sp. and *Rhinocladiella* sp. were also isolated frequently in this study, which were not common in previous studies.

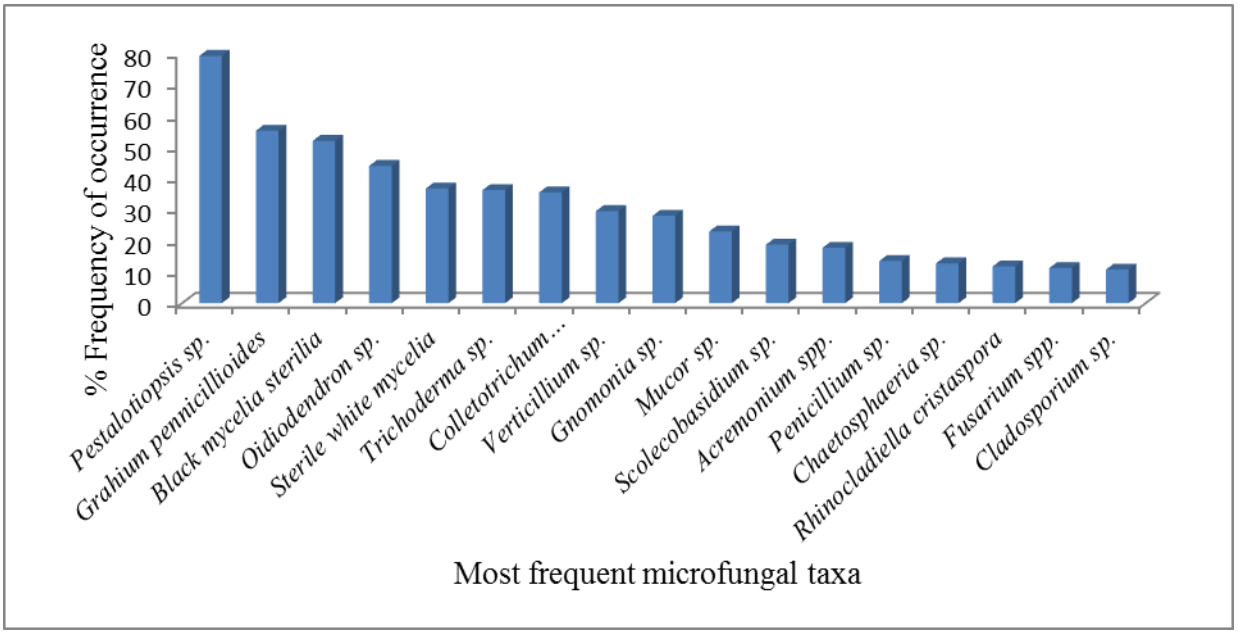

Figure 2. The most frequently observed fungal taxa from all the five host plants studied.

Table 4. Shared microfungal species/overlap between different host plants.

Host plant	*Syzygium* sp.	*Macaranga* sp.	*Baccaurea* sp.	*M. triloba*	*S. macrophylla*
Syzygium sp.	0				
Macaranga sp.	34	0			
Baccaurea sp.	30	26	0		
M. triloba	22	15	14	0	
S. macrophylla	19	15	13	13	0

In terms of similarity of microfungal communities on the host plants, the highest microfungal similarities were between *Syzygium* sp. and *Macaranga* sp. in which they shared 34 taxa in common (Table 4).

However, there were low similarities between the microfungal communities on *S. macrophylla, Macaranga* sp. and *Baccaurea* sp. with only 13 taxa in common. The high microfungal community similarity between *Syzygium* sp. and *Macaranga* sp., both from Gunung Gading National Park signifies that they both share some similar fungal species. This could be a site-dependent occurrence, as the same high similarities were observed between all the host plants in Gunung Gading National Park. On the other hand, there was a low overlap in the microfungal communities on host plants from Kubah National Park suggesting that each host plant species in that site has its own adapted environment for its microfungal species. Furthermore, this also suggests that Gunung Gading's environment is still more natural than that of Kubah National Park which has been highly open to many human interactions as evidenced by the high human activities observed during our sample collection in Kubah National Park.

CONCLUSION

The microfungal communities found on *Syzygium* sp., *M. triloba, Macaranga* sp., *Baccaurea* sp., and *S. macrophylla* represent valuable resources about the mycoflora associated with these plants as well as Kubah and Gunung Gading National Parks in Borneo Island, Sarawak. The present fungal discoveries represent new records of microfungi from dominant plant species as well as vulnerable plant species from the South-east Asian dipterocarp forests. The results obtained in this study from each plant species can be compared with other host plants in the same plant family and with other plant species thereby increasing our understanding of the diversity and distribution of microfungi in the tropics.

ACKNOWLEDGEMENTS

The first author is grateful to Universiti Malaysia Sarawak and the Sarawak government for the Zamalah scholarship awarded to him and permission to collect samples from the National Parks respectively.

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