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Cover Page Footnote

The first author is grateful to Universiti Malaysia Sarawak (UNIMAS) for the Zamalah scholarship awarded. We are also grateful to the Sarawak government and to Sarawak Forestry Co-operation (SFC) for permission to collect samples from the National Park.

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Microfungi on Leaves of *Licuala bidentata* (Arecaceae) from Sarawak, Malaysia

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Abstract

A microfungal survey was carried out on the living leaves and litters of *Licuala bidentata* in Kubah National Park in Sarawak, Malaysia. A total of 400 leaf segments (200 segments for each leaf type) were plated on two isolation media (water agar and malt extract agar) for endophytic and saprophytic fungal isolation. Forty-three microfungal species were obtained from both leaf types, 31 species identified from living leaves and 18 species from litters. Only six species were common to both leaf types, with 25 and 12 species exclusively identified from living leaves and litters, respectively. New records of fungi from this host plant and for the genus *Licuala* include *Isthmotricladia laeensis*, *Chloridium* sp., *Mucor* sp., *Oidiodendron* sp., *Kinochaeta* sp., *Cryptophiale* sp., *Chrysosporium merdarium* and *Circinotrichum fertile*. This study constitutes the first report on microfungal community on *L. bidentata*. Implications of this new report in comparison with the microfungal species on other plant species in the genus *Licuala* are discussed.

Abstrak

Mikrofungi pada Daun *Licuala bidentata* (Arecaceae) dari Serawak, Malaysia. Sebuah survei microfungal telah dilakukan pada daun dan serasah daun *Licuala bidentata* di Taman Nasional Kubah, Sarawak. Studi mengenai spesies microfungal pada spesies tumbuhan *L. bidentata* yang merupakan tanaman asli Asia Tenggara belum pernah dilakukan sebelumnya. Segmen daun yang diletakkan pada water agar dan malt ekstrak dilakukan untuk memperoleh fungi endofit dan saprofit. Sebanyak 43 spesies microfungal diperoleh dari kedua jenis daun, 31 spesies dari daun, dan 18 spesies dari serasah daun. Hanya 6 spesies yang ditemukan pada kedua jenis daun, sedangkan sebanyak masing-masing 25 spesies dan 12 spesies yang eksklusif pada daun dan serasah daun. Catatan baru mikrofungi dari tanaman inang ini dan untuk genus *Licuala* termasuk *Isthmotricladia laeensis*, *Chloridium* sp, *Mucor* sp, *Oidiodendron* sp, *Kinochaeta* sp, *Cryptophiale* sp, *Chrysosporium merdarium* dan *Circinotrichum fertile*. Pada laporan ini dilakukan perbandingan spesies mikrofungal yang diperoleh dari hasil studi ini dengan spesies mikrofungal pada spesies tanaman lain dalam genus *Licuala*.

Keywords: leaves, Licuala bidentata, litters, microfungi

Introduction

Microfungi are important components of our ecosystem that are involved in the breakdown of organic matter and recycling of nutrients for plants' use. They are also important in other aspects of human life such as drug production, biocontrol agents, and production of industrial chemicals [1]. The diversity and distribution of microfungi are still not fully understood, especially in the tropical regions of the world, where there are many unstudied areas, substrates and host plants. Adequate knowledge of global fungal diversity and distribution will not only enrich our biodiversity database, it also will improve the accessibility, usage, and conservation of our fungal natural endowments. This also will assist policy makers in prioritizing conservation programs in relation to specific areas and specific plant species.

Licuala bidentata is a member of the palm family Arecaceae, the only family in the order Arecales. Plant species in the genus *Licuala* are highly diverse in the forests of southeast Asia [2], with 141 species mainly found in the Malesian region [3]. However, this plant genus is not well studied for their microfungal communities, especially in Malaysia. *Licuala bidentata* is native to Asia and one of the palm species found in Kubah National Park in Sarawak, Malaysia, with characteristic leaves that split into very narrow leaflets.

Previous studies on *Licuala longicalycata* from Thailand isolated 358 fungal taxa from a variety of this plant's samples including submerged materials, dry materials, petioles, trunks, and leaves [4]. On studies using another palm species, *Eleiodoxa conferta*, 462 fungal taxa were found[5]. Also, from the work of Mckenzie *et al*. [6] on two species of *Nikau* palms, 197 fungal taxa were isolated from different types of leaf materials. This study aimed to assess the fungal communities on living leaves and litters of *L. bidentata* from Kubah National Park in Sarawak. The outcome of this study will be a contribution to the microfungal communities from the palm family Arecaceae, to the genus *Licuala*, and also to Sarawak.

Materials and Methods

Sample collection and processing. Living leaves and leaf litters were collected from Kubah National Park in Sarawak (N 01^o 36' 721", E 110^o 11' 743 ") at an elevation of 163 m above sea level in March 2014 and September 2014. Kubah National Park is located 22 km from Kuching, the capital city of the state of Sarawak [7]. The park is covered with lowland mixed dipterocarp forest, a hilly terrain, and an elevation ranging from 20 m to 799 m above sea level. Each sample collection was recorded, noting the date of collection and type of leaf collected, either living leaves or leaf litters. The samples were put in plastic bags and transported to the laboratory for processing.

Isolation and identification of fungi. The leaves were prepared for the isolation of both endophytic and saprophytic fungi and plated on two different types of isolation media, water agar (WA) and malt extract agar (MEA).

The protocol for endophytic fungal isolation using the method of Rakotoniriana *et al.* [8] was followed with little modifications whereby hydrogen peroxide was used as a disinfectant instead of calcium hypochlorite. The leaves were then plated on WA and MEA in petri dishes with 5 segments equidistant to each other. The plates were sealed with parafilm and incubated at room temperature under alternating light and dark conditions. Observation and isolation of the growing fungi start from the third day of incubation on MEA and four weeks on WA.

For isolation of saprophytic fungi, the leaf samples were washed with double sterilized distilled water [9], cut into 1 cm^2 into a 500 mL conical flask, washed again with sterile distilled water 5 times, and then blotted on sterilized filter paper. The leaf segments were then plated as done for the endophytic fungi. A total of 400 leaf segments were plated, 200 each for living leaves and leaf litters.

Data on the occurrence of fungi on the leaf segments were recorded. The presence of microfungi on each leaf segment was recorded, observing the characteristics of each microfungus using a Motic stereo microscope model MZ-168 and an Olympus compound microscope model CX31. Micrographs were taken with a Samsung handheld camera model ES91. Identification of the observed microfungi were made to the genus level and, wherever possible, to species level based on the morphological characters. The fungal isolates were preserved as pure cultures and semipermanent slide specimens and deposited in the Mycology laboratory culture collections at Universiti Malaysia Sarawak.

Data analysis. Multiple colonies of the same fungal taxa growing on a single leaf segment were counted as one occurrence. The frequency of occurrence of each microfungal taxa observed was calculated and the frequency of isolation was determined according to [10,11] as follows:

$$Frequency\ of\ isolation\ (IF) = \frac{total\ number\ of\ leaf\ segments\ from\ which\ a\ fungal\ taxa\ was\ present}{total\ number\ of\ leaf\ segments\ observed} x100$$

Species diversity indices were calculated for each microfungal assemblage based on leaf type. Shannon's diversity index (H), Simpson's diversity index (1/D), and Bray-Curtis's similarity index were calculated for the microfungal communities.

Species accumulation curves (SACs) were plotted and species richness estimated with the nonparametric estimators, abundance-based coverage estimator (ACE), and Chao1 for microfungal abundance data using the EstimateS diversity software version 9.1.0 released June 2013 [12].

Results and Discussion

A total of 43 fungal taxa were identified from 400 leaf segments observed both on living leaves and leaf litters of *L. bidentata*, comprising 3 Ascomycetes, 1 Basidiomycete, 1 Zygomycetes, 5 Coelomycetes, and 29 Deuteromycetes and 4 sterile mycelia (Table 1). Thirty-one fungal species were identified on living leaves, and 18 species were on litters (Figure 1). The Shannon and Simpson's diversity indices for fungal community on living leaves, 2.88 and 13.43, respectively, were higher than that of the litters, 2.48 and 9.1, respectively (Table 2).

The higher number of fungal taxa observed from leaves as compared to litters indicates that the leaves contain more supporting nutrients for the microfungal communities, which depletes rapidly on decomposition of

Table 1. Percentage Dominance of Microfungal Species Identified from Green Leaves and Leaf Litters of *L. bidentata*

S/N	Microfungal species name	Green leaves (%)	Leaf litters (%)
1	*Anthostomella* sp.	2.46	0
2	Ascomycete sp. 26	1.23	0
3	*Sordaria fimicola*	0.82	0
4	*Xylaria* sp.	2.05	0
5	*Botryodiplodia* sp.	0.41	0
6	*Pestalotiopsis concolorous*	8.20	0
7	*Pestalotiopsis* sp.	5.74	0
8	*Thielavia* sp.	0	9.40
9	*Colletotrichum gloeosporioides*	0	1.34
10	*Isthmotricladia laeensis Matsushima*	2.46	20.13
11	*Graphium penicillioides*	10.25	2.68
12	*Cercospora* sp.	9.43	1.34
13	*Beltraniella* sp.	0	13.42
14	*Rhinocladium* sp.	6.15	0
15	*Gliocladium* sp.	0	6.71
16	*Neottiosporella* sp.	4.10	0
17	*Cladosporium* sp. 2	0	5.37
18	*Sporidesmium* sp.	2.87	0
19	*Bispora betulina*	2.46	0
20	*Menispora* sp.	0	4.03
21	*Trichoderma* sp.	0	4.03
22	Hyphomycetes sp. 14	0	2.68
23	*Sporidesmium* sp.	0	2.68
24	*Chloridium* sp.	1.23	0
25	*Codinae* sp.	1.23	0
26	*Diplodia* sp.	1.23	0
27	*Basipetospora* sp. 2	0	1.34
28	*Botrytis* sp.	0	1.34
29	*Chrysosporium merdarium*	0.41	0.67
30	*Circinotrichum fertile*	0.82	0
31	*Cryptophiale udagawae*	0.82	0
32	*Fusarium* spp.	0	1.34
33	Hyphomycetes sp. 11	0.82	0
34	*Idriella* sp.	0.82	0
35	*Cryptophiale* sp.	0.41	0
36	*Dactylaria* sp. 2	0.41	0
37	*Kionochaeta* sp.	0.41	0
38	*Oidiodendron* sp.	0.41	0
39	Black sterile mycelia	11.07	5.37
40	White sterile mycelia	13.93	0
41	Brown sterile mycelia	0.82	0
42	Yellow sterile mycelia	0.41	0
43	*Mucor* sp.	6.15	16.11

the leaf. This is also evident from the feeble nature of the litters of *L. bidentata*.

The number of fungal taxa recovered from both leaf types was lower than those recorded from other palm species [4-6]. This could be attributed to the fact that only living leaves and litters were used in this study while other different plant tissues, such as trunks, petioles, and rachides were used in other studies. The most dominant microfungi on both leaf types include *Isthmotricladia laeensis*, black sterile mycelia, white sterile mycelia, *Graphium penicillioides*, *Cercospora* sp., *Pestalotiopsis* sp. 2, *Beltraniella* sp., *Rhinocladium* sp., *Pestalotiopsis* sp. and *Thielavia* sp. (Figure 2).

On living leaves, the 5 most frequently observed taxa were white sterile mycelia, black sterile mycelia, *Graphium penicillioides*, *Cercospora* sp., and *Pestalotiopsis* sp. 2, while on leaf litters, *Isthmotricladia laeensis*, *Beltraniella* sp., *Thielavia* sp., *Gliocladium* sp., and black sterile mycelia were the most frequently observed.

Only 6 species were observed to be common both on leaves and litters with a Bray-Curtis value of 0.183. The species common to both leaf types are *Isthmotricladia laeensis*, black sterile mycelia, *Graphium penicillioides*, *Cercospora* sp., *Mucor* sp., and *Chrysosporium merdarium.* Species observed only on leaf litters include *Beltraniella* sp., *Thielavia* sp., *Gliocladium* sp., *Cladosporium* sp. 2, *Menispora* sp., *Trichoderma* sp., Hyphomycetes sp. 14, *Sporidesmium* sp. 2, *Colletotrichum gloeosporioides*, *Basipetospora* sp. 2, *Botrytis* sp., and *Fusarium* spp., while *Botryodiplodia* sp., *Cryptophiale* sp., *Dactylaria* sp. 2, *Kinochaeta* sp., *Oidiodendron* sp., yellow sterile mycelia, *Sordaria fimicola*, *Circinotrichum fertile*, *Cryptophiale udagawae*, Hyphomycetes sp. 11, *Idriella* sp., brown sterile mycelia, Ascomycete sp. 26, *Chloridium* sp., *Codinae* sp., *Diplodia* sp., *Xylaria* sp., *Anthostomella* sp., *Bispora betulina*, *Sporidesmium* sp. 1, *Pestalotiopsis* sp., *Rhinocladium* sp., *Pestalotiopsis* sp. 2, and white sterile mycelia were only observed on living leaves. The low similarity between microfungal assemblages from the two leaf types and the high

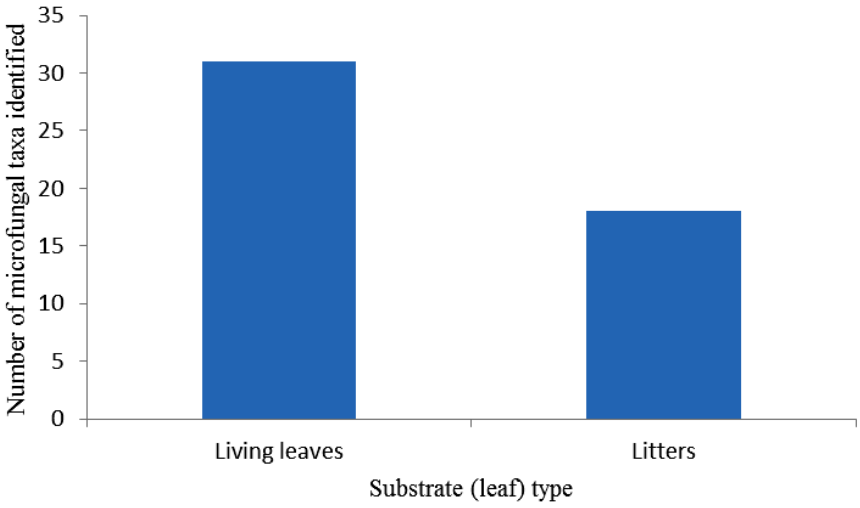

Figure 1. Microfungal Taxa Identified on Living Leaves and Litters of *L. bidentata*

Table 2. Diversity Indices and Similarity Index of Microfungal Communities on Green Leaves and Leaf Litters of *L. bidentata*

Diversity measurements	Living leaves	Leaf litters
Number of Isolates	244	149
Number of species observed	31	18
Number of Singletons	7	1
Number of Doubletons	6	5
ACE species estimate	33.99	18
Chao 1 species estimate	33.99	18
Shannon Index	2.88	2.48
Simpson Inv Index	13.43	9.1
Bray-Curtis similarity index	0.183	

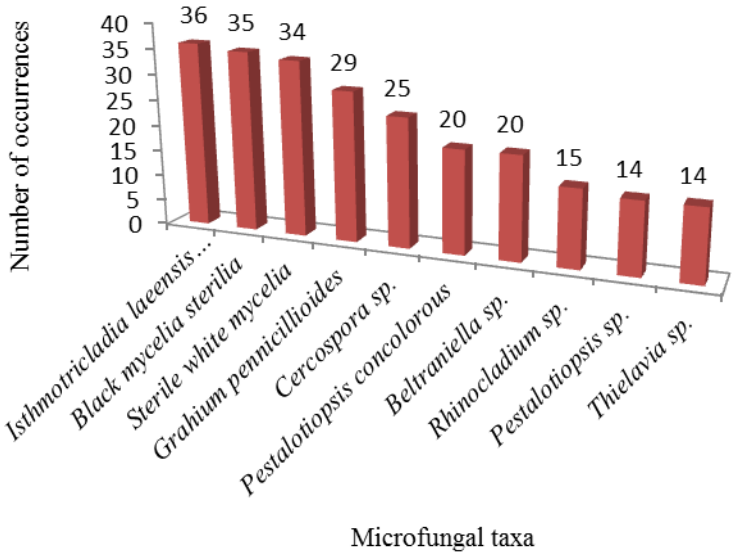

Figure 2. Most Dominant Microfungal Taxa on Leaves of *L. bidentata*

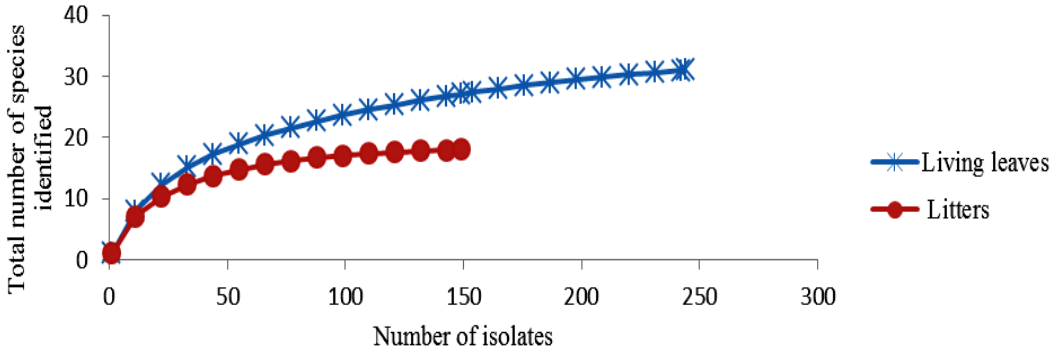

Figure 3. Species Accumulation Curve of Microfungi on Living Leaves and Litters of *L. bidentata*

specificity show *L. bidentata* to support a wide variety of microfungi. This result represents new records of microfungal occurrence from *L. bidentata* from Sarawak as this is the first report of microfungi on the leaves of this plant species. However, some of the identified species have been isolated earlier from other *Licuala* host plants. For example, *Pestalotiopsis* spp. has been recorded from *Licuala ramsayi* [13,14], *L. spinosa* [15], *Elaeis guineensis* [15], and *Licuala* sp. in Brunei [13]; *Gliocladium* sp. and *Trichoderma* sp. from *L. longicalycata* in Thailand [4], *L. spinosa* [15], and *Licuala* sp. from Brunei [13]; and *Beltraniella* sp., *Sporidesmium* sp., *Colletotrichum gloeosporioides*, *Cladosporium* sp., *Anthostomella* sp., *Botryodiplodia* sp., *Dactylaria* sp., *Fusarium* spp., *Idriella* sp., *Cercospora* sp., and *Xylaria* sp. also have been reported for the genus *Licuala*. Additional new reports of species from this study thus constitute new records for microfungi on host plants from the genus *Licuala*. For example, *Isthmotricladia laeensis*, which is found on both green leaves and leaf litters of *L. bidentata*, was first isolated and described from decaying leaves of *Cocos nucifera* (also a palm species) from Papua New Guinea [16].

In conclusion**,** 43 microfungal taxa were identified from both leaves and litters of *Licuala bidentata* with living leaves harboring more taxa than the leaf litters. *Isthmotricladia laeensis* was one of the dominant species on both leaf types, which indicates a microfungal similarity with other palm host plants. New records of microfungi occurring on *L. bidentata* and on palm species were recorded for the first time. The result from this study contributes to knowledge of fungal diversity and distribution occurring on leaves of *L. bidentata* and on palms (family Arecaceae).

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