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Characterization and Identification of Polypore Fungi Collected from Forests in Sandakan, Sabah Based on the Macro- and Micro-morphology

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ABSTRACT Polypore is a group of fungi from the order polyporales that form fruiting bodies with pores or tubes on the underside. Although tropical rainforests are one of the global biodiversity hotspots, but the diversity of polypore fungi has been little examined especially in Sandakan, Sabah. In addition, polypore fungi probably could be exploited as bio-control agents against phytopathogens, and they should be identified. Thus, the objectives of this study were to (i) investigate the macro- and micro-morphological characteristics of polypore fungi; (ii) identify the polypore fungi based on their macro- and micro-morphologies. Fruiting bodies of polypore fungi were collected from Rainforest Discovery Centre (RDC) and Sandakan Rainforest Park (SRP), located in Sandakan, Sabah. In addition, few samples of polypore fungi were obtained from mushroom farms and oil palm estates. A total 35 polypore fungi were collected, their macro-morphologies were characterized based on 47 characteristics of the fruiting bodies, and then isolated using potato dextrose agar (PDA). Moreover, the *in-vitro* micro-morphologies of the fungi were characterized based on 37 characteristics of the pure cultures. Dendrograms were generated using unweight pair group of arithmetic averages (UPGMA). The macro-morphological characteristics exhibited high variation (20% to 90%) among the fungi. The fungi were categorized into 29 groups at 90% similarity of their macro-morphologies. While for micro-morphology, the fungi were categorized into 32 groups at 100% similarity. The isolates for micro-morphology showed less than 33% dissimilarity among the fungi. These results indicated that most polypore fungi collected are from family Polyporaceae and Ganodermataceae. Among these family, there are about 10 different genera were identified based on their macro- and micro-morphological characteristics. Either the family, genus, or species of 25 out of 35 polypore fungi (71%) were identified and others remain unidentified based on their macro- and micro-morphological characteristics. Further research should be conducted to study the potential uses of the polypore fungi, such as in bio-control against phytopathogens.

KEYWORDS: Morphology characteristics; Polypore fungi; Mushrooms; Dendrogram; Basidiomycetes
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INTRODUCTION

Polypore are large group of terrestrial fungi of the phylum Basidiomycota (Jordan, 2004). Usually many of these fungi have high distribution in North America, Europe, and Asia. The name "polypore" translates to "many pores" that located on the underside of the cap and usually the pores are very tiny and small. Most are woody or leathery (though some are soft and fleshy) and make it as inedible. Some of polypore fungi are annual; while others are perennial, adding new layers each year. Polypore fungi can be either as saprophytes, those that breakdown dead material and release the nutrients back into the ecosystem for reuse by other living organisms or parasites, and those that take nourishment from living organisms (Susan & Van, 1992). Polypores are much more diverse in old natural forests with abundant dead wood than in younger managed forests or plantations.

Determining the exact name of any given sample of polypore fungi requires some identification and characterization of their macro- and micro- morphologies. Therefore, there is a need to identify polypore fungi as the diversity of polypore fungi in Malaysia are poorly known especially in Sandakan, Sabah, and at the same time polypore fungi could be exploited as biological control agents against phytopathogen. Thus, the objectives of this study were to investigate the macro- and micro- morphological characteristics of polypore fungi, and to identify the polypore fungi based on the characterization.

MATERIALS AND METHODS

Macro-morphology Characterization of Polypore Fungi

A total of 35 polypore fungi basidiocarps were collected randomly from forests in Sandakan, Sabah, which were Sandakan Rainforest Park (SRP) and Rainforest Discovery Centre (RDC). In addition, four samples of polypore fungi were obtained from mushroom farms and oil palm estates were included in this study.

The macro-morphology characterization of the basidiocarps were based on 47 characteristics, where the length of body, width of body, pileus texture, pileus shape, pileus surface texture, stipe thickness, pileus margin, pileus colour (using Munsell Soil Colour Chart), and their habitat were recorded (Lodge *et al.*, 2004).

In-vitro micro-morphology characterization of Polypore Fungi

The polypore fungi were isolated from the basidiocarps on potato dextrose agar (PDA). The pure cultures were obtained then used for *in-vitro* micro-morphology characterization based on 37 characteristics as described by Rakib *et al.*, (2014). Mycelia plug from seven-days-old active culture was transferred onto the center of a standard 9 cm PDA plate, and then incubated for 14 days in darkness at room temperature (25±35 °C) (Idris, 2000). The experiment was conducted in three replications for each isolates, and arranged in a completely randomized design (CRD). The radius of the mycelia on PDA plate was measured daily, and the number of days required to fully cover the plate was recorded. The colony radius (on day 7), mycelia density, mycelia texture, mycelia surface texture, colony concentric ring, margin, surface colour and reverse pigmentation colour (using Munsell Soil Colour Charts) were recorded on the seventh day after incubation.

Data Analysis

The qualitative data of basidiocarp macro-morphology and *in-vitro* micro-morphology characteristics were transformed into codes that correspond to the morphological features, and the codes were then converted to binary matrix. The binary matrix was subjected to cluster analysis using multivariate statistical package (MVSP version 3.13). A dendrogram was generated using the unweighted pair group method of arithmetic average (UPGMA) (Pilotti *et al.*, 2004).

RESULTS AND DISCUSSION

Few examples of basidiocarps macro-morphology characteristics in this study were shown in Figure 1. Pileus texture of the basidiocarps that mostly found in this study was concentric texture (have ring-shaped like on the surface), some basidiocarps have smooth texture, while others were wrinkled. Based on Yajima *et al.* (2013), there were three types of pileus shape, namely, kidney-shaped, horse shoe-shaped and gingko-leaf shaped. Stipe is present in most of the fleshy mushroom and most of *Ganoderma* and *Polyporus* species attached with bark of tree (Aminuzzaman, 2017). Pileus margin is referred to the boarder of the fruiting body, where most of the samples were having entire, undulated and curled pileus margin of polypore fungi. Morphologically, most of the samples were dark yellowish brown and dark reddish brown based on Munsell Soil Colour Chart.

Figure 1. Basidiocarps macro-morphology characteristics of polypore fungi. Pileus texture: (a) concentric, (b) smooth, (c) wrinkled; pileus shape: (d) kidney shaped, (e) horse-shoe shaped, (f) gingko-leafed shaped; stipe thickness: (g) no stipe, (h) thin, (i) thick; pileus margin: (j) entire, (k) undulated, (l) curled; pileus colour: (m) dark brown, (n) dark reddish brown, (o) light grey.

The dendrogram of the basidiocarp macro-morphology characteristics (Figure 2) showed that the polypore fungi exhibited high range of variation (20% to 90%). The polypore fungi were grouped into 29 groups at 90% similarity. At the 90% similarity, 6 pairings of samples were considered as similar, which were between IOI01 and SRP07, RDC23 and SRP22, SRP06 and RDC06, SRP31 and SRP29, RDC26 and SRP21, and RDC15 and SRP08. The 10% dissimilarity between the mentioned samples could be due to differences in pileus colour, size, and pileus margin. In many species of macrofungi, those dissimilarity characteristics usually lost when the basidiocarp are dried (Lodge *et al.*, 2004).

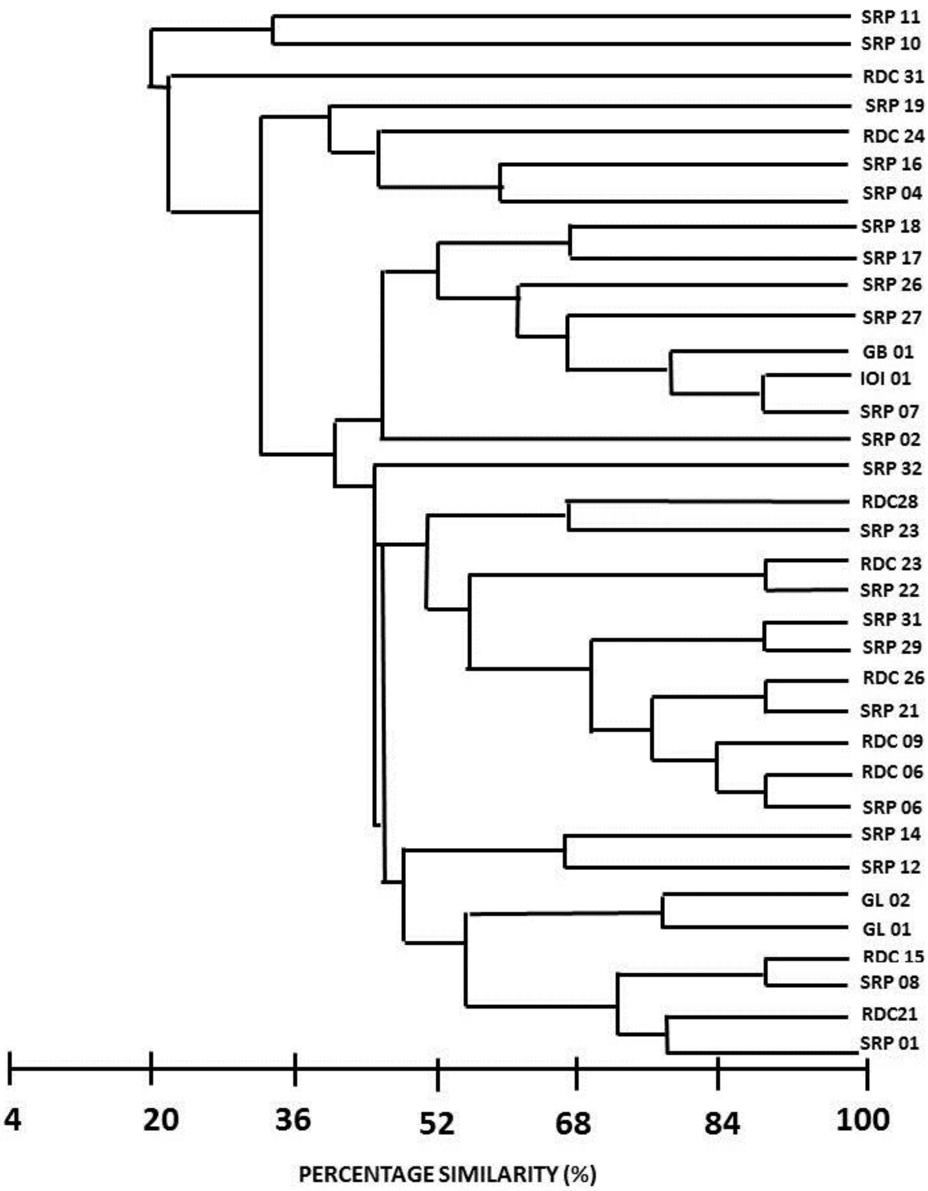

Figure 2. Dendrogram (UPGMA) generated from 47 basidiocarps macro-morphology characteristics of polypore fungi.

Few examples of *in-vitro* cultural micro-morphology characteristics in this study were shown in Figure 3. Mycelia density has three categories where some of isolate have thin mycelia, some have dense mycelia and also a few isolate dense at center. While, mycelia texture referred to texture of mycelia at the border where some isolate have very smooth mycelia texture, some were wrinkled and concentric texture. For mycelia surface texture referred to the top surface where some mycelia have adpressed surface texture, moderately wavy and some mycelia have strongly wavy surface texture (Rakib *et al*., 2014). Lastly, for mycelia margin mostly the mycelia having filamentous, undulated and lobate shaped at the border. *In-vitro* cultural micro-morphology characteristics of basidiomycetes are affected by various environmental factors such as light, aeration, temperature, humidity and nutritional condition (Flood *et al*., 2000). Among these light was found to be the most critical single factor in determining the density and texture of the mycelia culture of the fungus. Dark incubation led to more rapid radial growth when compared to the light incubation. Light might have exerted some degree of inhibitory effects on mycelial growth rate (Shrestha *et al*., 2006).

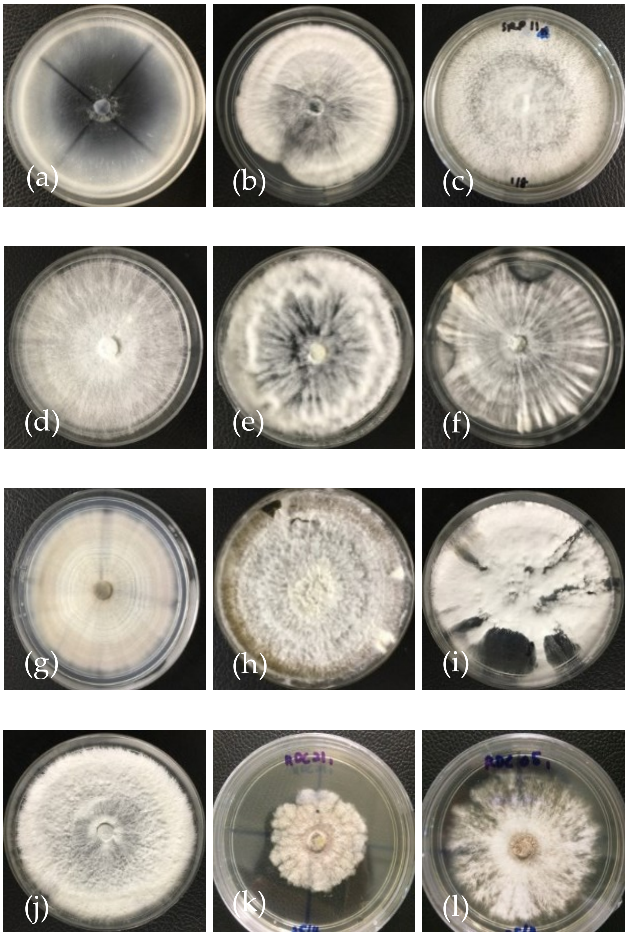

Figure 3. *In-vitro* cultural micro-morphology characteristics of polypore fungi. Mycelia density: (a) thin, (b) dense, (c) dense at centre; mycelia texture: (d) smooth, (e) wrinkled, (f) concentric; mycelia surface texture: (g) adpressed, (h) moderately wavy, (i) strongly wavy; mycelia margin: (j) filamentous, (k) undulated, (l) lobate.

The dendrogram of the *in-vitro* cultural micro-morphology characteristics (Figure 4) showed that the polypore fungi exhibited range of variation between 33% to 100%. Micro-morphological characters of colonies have been used to confirm the identity of fungi in pure culture (Brundrett *et al.,* 1996). The polypore fungi were grouped into 32 groups at 100% similarity. The mycelium in the basidiomycota is composed of septate hyphae and usually the morphology is not similar within the group (Taylor *et al*., 2014). However, at the 100% similarity, 2 pairings of samples were considered as similar, which were between SRP02 and RDC06, and SRP01 and RDC09. One of the factor similarities of these pairing was maybe due to their mycelia density and mycelia surface texture. These findings indicated that there was no association found between the macro- and micro-morphology characteristics.

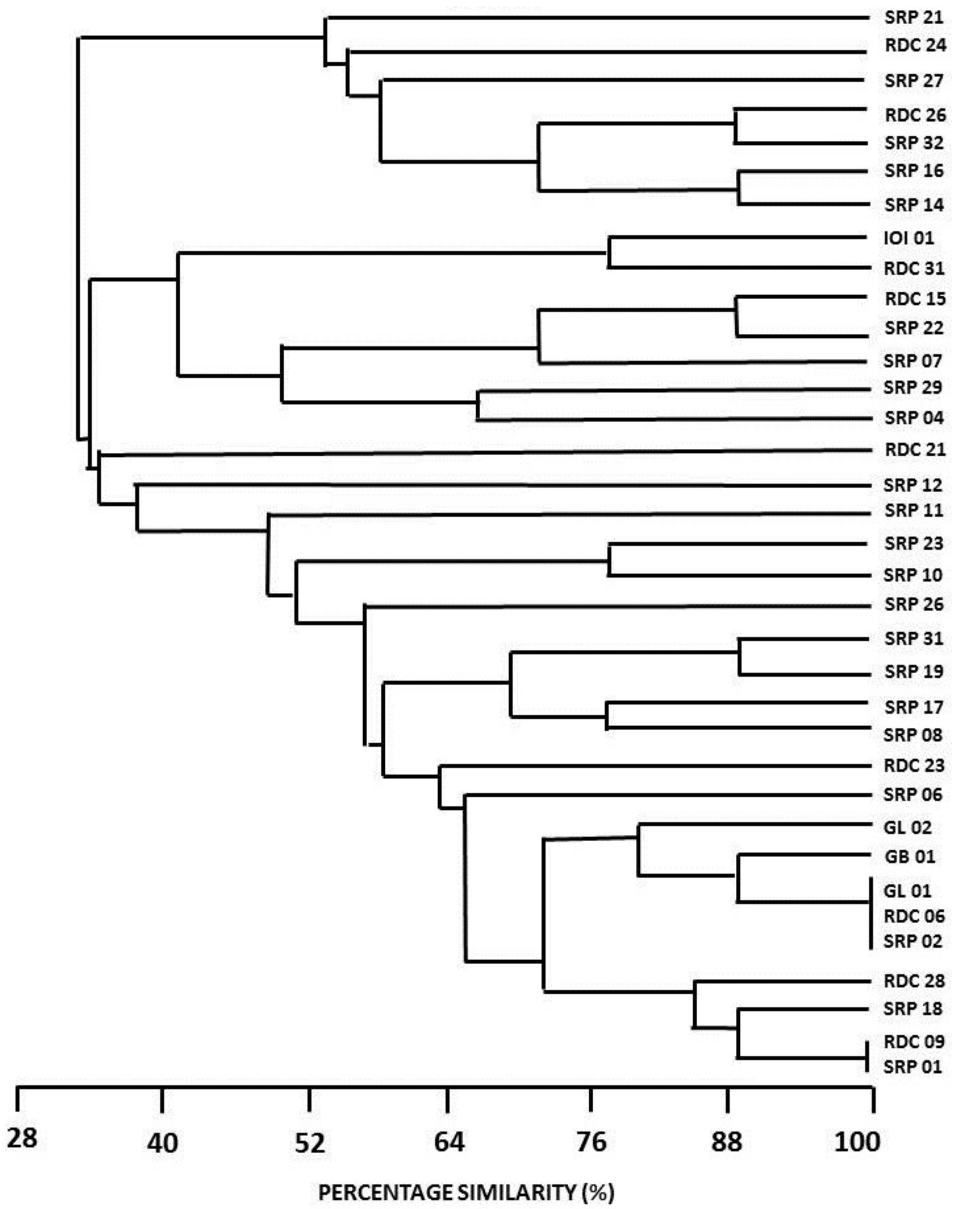

Figure 4. Dendrogram (UPGMA) generated from 37 *in-vitro* cultural micro-morphology characteristics of polypore fungi.

Based on this morphology characteristics of the polypore fungi, 10 genera were found in this study, which were *Ganoderma sp*, *Earliella sp*., *Laetiporus sp*., *Trametes* sp., *Coltricia sp*., *Lenzites sp*., *Microporus sp*., *Fomes sp*., *Hydnum sp*., *Pycnoporus sp*. (Table 1 and Figure 5). Based on Cannon & Kirk (2007), these results indicated that most polypore fungi collected were from the same family which were Polyporaceae and Ganodermataceae while a few samples of polypore fungi were from family Hydnaceae, Hymenochaetaceae, Steccherinaceae and Fomitopsidaceae. However, all these identified family were still under the same order which is order Polyporales. About 71% where 25 out of 35 of polypore fungi were identified based on the family and genus while others remain unidentified based on their macro- and micro-morphological characteristics. Based on FRIM (2014), most of the unidentified fungi in their study was related due to their tiny size and dull colour, while those with bright and brilliant colour usually could be identified easily.

Table 1. Identity of polypore fungi.

Isolate code	Family	Genus
SRP01	Ganodermataceae	*Ganoderma sp.*
SRP02	Polyporaceae	Unidentified
SRP04	Unidentified	Unidentified
SRP06	Polyporaceae	*Pycnoporus sp.*
SRP07	Ganodermataceae	*Ganoderma sp.*
SRP08	Ganodermataceae	*Ganoderma sp.*
SRP10	Polyporaceae	Unidentified
SRP11	Ganodermataceae	*Fomes sp.*
SRP12	Unidentified	Unidentified
SRP14	Ganodermataceae	*Ganoderma sp.*
SRP16	Unidentified	Unidentified
SRP17	Hydnaceae	*Hydnum sp.*
SRP18	Unidentified	Unidentified
SRP19	Polyporaceae	*Trametes sp.*
SRP21	Polyporaceae	Unidentified
SRP22	Polyporaceae	*Microporus sp.*
SRP23	Hymenochaetaceae	*Coltricia sp.*
SRP26	Polyporaceae	*Lenzites sp.*
SRP27	Ganodermataceae	*Ganoderma sp.*
SRP29	Polyporaceae	Unidentified
SRP31	Polyporaceae	Unidentified
SRP32	Steccherinaceae	*Nigroporus sp.*
RDC06	Polyporaceae	Unidentified
RDC09	Fomitopsidaceae	*Pycnoporellus sp.*
RDC15	Ganodermataceae	*Ganoderma sp.*
RDC21	Ganodermataceae	*Ganoderma sp.*
RDC23	Polyporaceae	Trametes sp.
RDC24	Polyporaceae	*Laetiporus sp.*
RDC26	Polyporaceae	*Earliella sp.*
RDC28	Ganodermataceae	*Ganoderma sp.*
RDC31	Ganodermataceae	*Ganoderma sp.*
IOI01	Ganodermataceae	*Ganoderma sp.*
GL01	Ganodermataceae	*Ganoderma sp.*
GL02	Ganodermataceae	*Ganoderma sp.*
GB01	Ganodermataceae	*Ganoderma sp.*

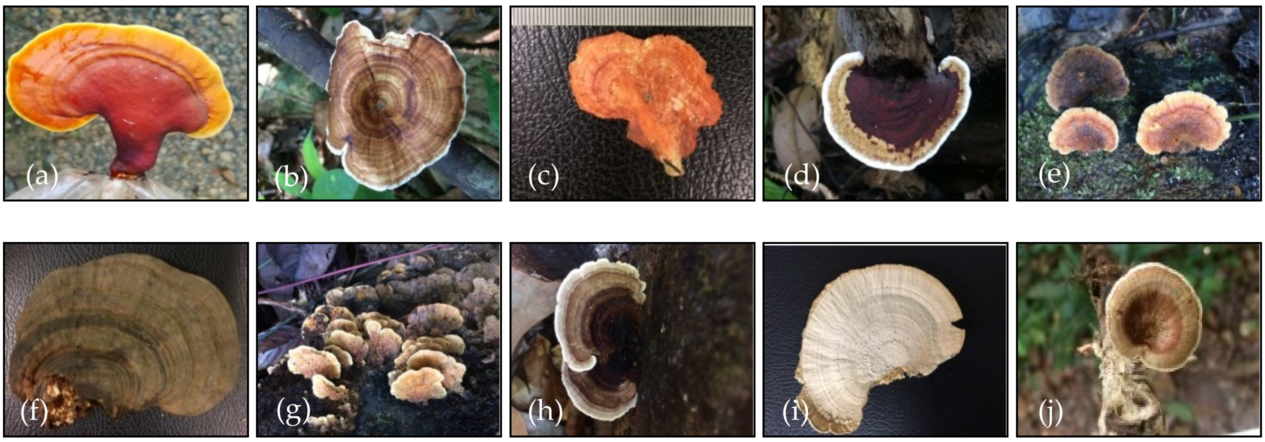

Figure 5. Identity of polypore fungi. (a) *Ganoderma* sp.; (b) *Coltricia* sp.; (c) *Pycnoporus* sp.; (d) *Earliella* sp.; (e) *Pycnoporellus* sp.; (f) *Fomes* sp.; (g) *Laetiporus* sp.; (h) *Trametes* sp.; (i) *Hydnum* sp. And; (j) *Microporus* sp.

CONCLUSION

The present study provides some information on polypore fungi diversity in the forests of Sandakan in terms of their macro- and micro-morphologies. The macro-morphological characteristics exhibited 20% to 90% of similarity, while for micro-morphological showed 33% to 100% of similarity among the polypore fungi. About 71% of fungi successfully identified at the genera level, while other 29% were unidentified. As for recommendation, further research should be conducted to study the potential uses of the polypore fungi, such as in biological control agent against phyto-pathogens as polypore fungi have their own potential that worth to be discovered.

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