Faculty of Resource Science and Technology

MORPHOLOGICAL CHARACTERIZATION AND IDENTIFICATION OF BOLETACEAE COLLECTED IN KUBAH-MATANG NATIONAL PARK

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Morphological Characterization and Identification of Boletaceae Collected in Kubah-Matang National Park

ZAZEVIA ANAK FRANK CLIFTON

A report submitted in partial fulfillment of the requirement for the degree of Bachelor of Science with Honours

(Plant Resource Science and Management)

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This Final Year Project 2017 is based on my original work except for quotation and citation which have been acknowledged. I also declared that it has not been submitted previously or currently to any other degree at Universiti Malaysia Sarawak or any other institution.

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LIST OF ABBREVIATIONS

%	Percent
µl	microlitre
µm	micrometer
Bp	base pair
ddH_2O	Double Distilled Water
DNA	Deoxyribonucleic Acid
ECM	Ectomycorrhizal Fungi
EtBr	Ethidium Bromide
G	gram
ITS	Internal Transcribed Spacer
KOH	Potassium Hydroxide
kb	Kilo base pair
M	Molar
MgCl	Magnesium Chloride
Ml	milliliter
Mm	Millimeter
NaCl	Sodium Chloride
NH_4OA_C	Sodium Acetate
NH_4OH	Ammonium Hydroxide
ºC	Degree Celcius
PCR	Polymerase Chain Reaction
Rpm	rotation per minute
TAE Buffer	Tris Acetate-EDTA buffer
TE Buffer	Tris-EDTA buffer
UNIMAS	Universiti Malaysia Sarawak
UV	Ultraviolet
V	Volt
v/v	Volume of Solute / Volume of Solution

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ABSTRACT

Documentation of Boletaceae in Sarawak, mushrooms with pores is poorly reported. The aim of this research is to identify the species under the Boletaceae family collected at Kubah National Park including Matang Wildlife Centre. Collections of the samples were carried out in October (2016), November (2016) and March (2017). A total of 36 samples of the mushrooms were collected and identified on the basis of the macroscopic and microscopic characteristics. The mushroom collected representing ten genera. Then, 21 samples were identified to species level. Genera of the Boletaceae in this study were *Tylopilus, Gyroporus, Austroboletus, Boletus, Xerocomus, Fistulinella, Aureoboletus, Phylloporus, Bolettelus dan Pulveroboletus.* Out of 36 samples, 6 species were first time recorded in Sarawak which was *Tylopilus alboater, Boletus mirabalus, Boletellus ananas, Boletus aurisporus, Boletus nanus* and *Fistulinella viridis*. Molecular study also had been carried out in this study. Although most of the PCR products showed single band after the gel was viewed and photographed with UV light transilluminator, the result for sequence blasting was unsuccessful. Detailed morphological and microscopic characteristics of the Boletaceae recorded were documented in this study.

Keywords: Boletaceae, Boletaceae in Kubah Matang National Park, morphological characteristics of Boletaceae, mushrooms in Kubah National Park.

ABSTRAK

Dokumentasi Boletaceae di Sarawak, cendawan dengan liang adalah kurang dilaporkan. Kajian ini bertujuan untuk mengenal pasti spesies di bawah keluarga Boletaceae yang dikumpul di Taman Negara Kubah termasuk Pusat Hidupan Liar Matang. Koleksi sampel telah dijalankan pada bulan Oktober (2016), November (2016) dan Mac (2017). Sejumlah 36 sampel cendawan telah dikumpul dan dikenalpasti berdasarkan ciri-ciri makroskopik dan mikroskopik. Cendawan yang dikumpul mewakili sepuluh genera. Kemudian, 21 sampel telah dikenalpasti sehingga peringkat spesies. Genera Boletaceae dalam kajian ini adalah Tylopilus, Gyroporus, Austroboletus, Boletus, Xerocomus, Fistulinella, Aureoboletus, Phylloporus, Bolettelus dan Pulveroboletus. Daripada 36 sampel, 6 spesies didapati pertama kali direkodkan di Sarawak iaitu Tylopilus alboater, Boletus mirabalus, Boletellus ananas, Boletus aurisporus, Boletus nanus dan Fistulinella viridis. Kajian molekular juga telah dijalankan dalam kajian ini. Walaupun kebanyakan produk PCR menunjukkan satu band selepas gel dilihat dengan transilluminator cahaya UV, hasil penyejajaran sekuens tidak berjaya didapatkan. Ciri-ciri morfologi dan mikroskopik Boletaceae telah direkodkan dan didokumentasikan dalam kajian ini.

Kata Kunci: Boletaceae, Boletaceae di Taman Negara Kubah, ciri-ciri morfologi Boletaceae, cendawan di Taman Negara Kubah

1.0 INTRODUCTION

Malaysia is the home to the world's oldest rainforest and one of the twelve mega biodiverse countries, places a lot of emphasis on the conservation and sustainable utilization of this natural heritage (Malaysia's National Policy on Biological Diversity, 1998). The diversity of Malaysian plants and animals is well illustrated in the general books by Rubeli (1986) and Dawson (1997). However, there is no book that is devoted exclusively to the diversity of Malaysian fungi due to poor documentation (Jones et al., 2007). Early Malaysian fungal checklists were concerned with pathogenic species primarily listing their hosts (Chipp, 1920; Thompson & Johnson, 1953; Singh, 1973).

The Boletaceae is a monophyletic family of mushroom (Binder, 1999; Binder & Besl, 2000) with ephemeral basidiomata, whose hymenial, or spore-bearing tissue positioned in pores on the underside of the pileus or cap. The boletes are an important part of any forest ecosystem because they always almost form ectomycorrhizae with a variety of green plant symbionts (Halling et al., 2007). Many members of the boletes have also been identified as rearing sites and food sources for insects (Bruns, 1984). Some of these boletes have great economic, dietary, and health value. For example, *Boletus edulis* Bull. sensu lato (Porcini) is a gourmet mushroom, highly prized in many parts of the world (Feng et al., 2012).

Boletaceae have been recorded with 162 species in 18 genera in Malaysia (Lee et al., 2012). In comparison, 787 species of boletes in 35 genera have been described worldwide (Kirk et al., 2008). Like the polypores, the boletes records for Malaysia are largely due to the pioneering research of E.J.H. Corner who described 100 new species out of 140 compiled in his 'Boletus in Malaysia' (Corner, 1972). He remarked that 300 species of boletes may be found in Malaysia in view of the large area of forests that have yet to be explored mycologically. For

example, only three species of *Heimiosporus* are presently known and described from Malaysia, while a minimum of at least 6 species are likely to be present (Halling et al., 2007).

1.1 PROBLEM STATEMENTS

In the past, Boletaceae have not been well documented in Malaysia especially in Kubah National Park. There are lacks of descriptions for both macroscopic and microscopic features for guidance in identification of the members of these mushrooms (Halling et al., 2007; Jones et al., 2007). This has caused difficulty in the identification of the specimen of these mushrooms. Thus, documenting the Boletaceae collected from Kubah National Park will be the crucial part for the better documentation.

Problems are encountered when documenting their diversity in tropical forests due to the seasonality of fruiting, short lived and solitary fruiting bodies (Corner, 1972; Tan et al., 2007). Different species may produce fruiting bodies at different season. Some genus only produces fruiting body once in 50 years (Corner, 1972). Thus, it is important to document those species.

1.2 OBJECTIVES

Hence, the objectives of this research are:

- to identify the Boletaceae collected in Kubah National Park based on morphological and molecular technique.
- to document the occurence of Boletaceae in Kubah National Park.

2.0 LITERATURE REVIEW

2.1 Boletaceae

Boletaceae is a major family of Boletineae (Boletales: Agaricomycetidae: Agaricomycetes), of which the fruiting bodies possess two conspicuous parts, namely pileus and stipe (Nuhn et al., 2013). Boletes are often called fleshy pore fungi because of the presence of the tubulose and their pores (Thiers, 1975). They are mainly characterized by fleshy context and a tubulose, rarely lamellate or loculate hymenophore (Wu et al., 2014). The fruiting bodies are usually terrestrial, short lived (putrescent) and usually have a well-developed stipe (Desjardin et al., 2015).

2.1.1 Classifications of Boletaceae

Corner (1972) suggested that there are only four genera of Boletaceae in Malaysia which are *Boletus, Gyroporus, Heimiella*, and *Strobilomyces*. Based on spore morphology, Pegler and Young (1981) introduced a new classification of the boletes that recognized 35 genera in 6 families. Singer (1981, 1986) presented an overview of bolete genera in one family in which he recognized 25 genera worldwide. However, in 2008, 50 genera and 800 species have been identified in this family (Kirk et al., 2008).

2.1.2 Boletaceae in Malaysia

In 1970 and 1972, Corner documented the Malaysian boletes diversity with 140 species from Peninsular Malaysia (Corner, 1970; 1972). In 1974, Corner described another 20 additional species from Borneo (Corner, 1974). At present time, 18 genera of Boletaceae have been

reported from Malaysia by Lee et al., (2012) as shown in Table 1. It is expected that there are new records of boletes present in Malaysia which need further search and discovery.

Table 1. Genera of boletes reported from Malaysia (Lee et al., 2012).

i. *Austroboletus*	ii. *Boletellus*
iii. *Boletochaete*	iv. *Boletus*
v. *Fistulinella*	vi. *Gyroporus*
vii. *Heimioporus*	viii. *Leccinum*
ix. *Mycoamaranthus*	x. *Phaeogyroporus*
xi. *Phylloporus*	xii. *Pulveroboletus*
xiii. *Rossbeevera*	xiv. *Spongiforma*
xv. *Strobilomyces*	xvi. *Suillus*
xvii. *Tylopilus*	xviii. *Xerocomus*

The genus *Austroboletus* is differentiated microscopically with spores that are pitted (Kuo, 2005). Spore colour range from lilac- or pinkish brown to wine coloured (Halling & Mueller., 2006). Pore and tubes are whitish (Wu et al., 2014). Lacks clamp connection in the hyphae (Zhishu et al., 1993). For *Boletellus*, the annual fruit body grows on wood. Stipe, centrally placed, solid, white and not reticulates (Murill, 1909). Spore, oblong to ellipsoid, smooth and rust- coloured. According to Murill (1909), the tubes on the underside of the cap are angular, depressed, yellowish and covered with a partial veil.

The fruiting bodies of *Boletochaete* will turn blue when bruise or expose to air (Kirk et al., 2008). *Boletus* usually has large pileus reaching 5 cm or more in diameter, plane to convex with decurved margin (Smith & Thiers, 1971; Corner, 1972). As the fruiting body getting older, it may be cracked into rimose or finely aerolate appearance. All *Boletus* comprised masses of soft, moist and detachable tubes at the hymenophoral layer. At the open ends of the tubes, there are circular or angular pores radiating from the stipe (Smith & Thiers, 1971; Corner, 1972).

Fistulinella have basidiospores that are smooth, elongate-fusoid, and pink-brown, cinnamon-brown to purple brown in deposit. Besides, Fistulinella have a basidioma that is viscid to glutinous, and a white hymenophore that is pink to pink-gray at maturation (Watling, 2008). According to Kuo (2013), *Gyroporus* is easily recognized by their stems, which are brittle and hollow and their yellow spore prints. *Heimioporus* have spore that is ornamented, lack of an adaxial path which distinguished it from *Strobilomyces* (Corner, 1972). For genus *Leccinum*, their stems are punctuated with scabers which typically become brown or black when the mushroom is mature, though in a few species the scabers are light in color, reddish, or nearly invisible to the naked eye (Singer & Williams, 1992). The spores of this genus are smooth and subfusiform.

According to Saisamorn et al. (2003), *Mycoamaranthus* is a truffle-like fungi. *Phaeogyroporus* have similarity in appearance to species in the genus *Gyrodon*, but distinguished by its olive-brown to brown spore print, its stem which is never hollow, and it have smooth spore which are brownish when viewed with a light microscope (Singer, 1986). *Phylloporus* is the only genus genera in Boletaceae that have gills instead of pores. According to Corner (1972), it has smooth spores and lamellate hymenophore. Murill (1909) stated that

Pulveroboletus having a cap and stem ‘clothed with a conspicuous sulphur-yellow, powdery tomentum, context white, fleshy, tubes adnate, yellowish, covered with a large veil, spores oblong-ellipsoid, stipe solid, annulate and not reticulate.

According to Orihara et al. (2012), *Rossbeevera* have ellipsoid to spindle-shaped spores, with 3-5 longitudinal ridges, bluish-gren to deep blue fruit body staining reaction, and a thin whitish peridium. *Spongiforma* is a gasteroids boletes and have a sponge-like shape and texture, astipitate, epigeous basidiome with large exposed locules and a strong coal tar odor, and rugulose, reddish brown basidiospores with an apical pore (Desjardin et al., 2009). *Strobilomyces* have the caps and stipe that are covered in soft hairy or wooly scales. Spore roughly spherical and prominently ornamented, dry fibrous appearance and resistant todecay whereas most mushrooms in the Boletaceae are soft and decompose notoriously rapidly (Binder & Hibbett, 2006).

Kirk et al. (2008) stated that *Suillus* have cylindrical stipe, cap, soft flesh and tubular hymenium. The presence of dark staining, slimy and sticky cap cuticle when moist, clustered and spores usually cinnamon brown in colour are often used to identify this genus (Singer, 1986). According to Snell (1942), *Tylopilus* are distinguished by their pinkish pore surfaces. There is no partial veil, and the spore print is usually pinkish brown to reddish brown, or in a few cases chocolate brown (Halling et al., 2007). They have stout stipe, which do not have ring. Lastly, the genus of *Xerocomus*. Spore surface of this genus is bacilitate, hymenophoral trama, phylloporoid type with nongelatinous lateral strata (Husbands et al., 2013). Pileipellis, initially a trichoderm, are never encrusted. It also have lateral stipe stratum which is never gelatinous.

2.2 Ecology and Distribution

In most cases, basidiomata of boletes are found in forest and woodland communities where they have been implicated or proven to be ectomycorrhizal symbionts (Singer, 1986; Osmundson et al., 2007). Ecologically, most species of Boletaceae are important ectomycorrhizal (ECM) fungi in the ecosystem, forming ECM relationships with plants of more than 10 families (Thoen & Bâ, 1989; den Bakker et al., 2004; Husbands et al., 2013). Since the majority of boletes are assumed to be ectomycorrhizal, they would then play a significant role in forest health and maintenance by facilitating water and nutrient uptake as has been documented associated with Malaysian dipterocarp trees (Lee & Alexander, 1994). According to Hongo (1984), the boletes also are among the principle components of Japanese evergreen oak forests.

2.2.1 Mycorrhizal Associations

The members of the Boletaceae form one of the largest symbiotic mycorrhizal communities throughout the temperate and tropical forest ecosystems of the world (Halling et al., 2007). This family is one of the fungal families that form the ectomycorrhizal associations. According to Futai et al. (2008), ectomycorrhizal fungi form a symbiotic relationship through the formation of a sheath around the root tip. The fungi then penetrate the root along the middle lamellae between cell wall by inwards growth of the hyphae, thereby forming a Hartig net. It is a side of nutrient exchange between the fungi and host plant (Peterson et al., 2014). The fungi will obtain carbon and other essential organic substances from the tree. In return, the fungus will help the tree take up water, mineral salts and metabolites. The plant growth and vigour was enhanced as the fungal hyphae in mycorrhizal roots increase the ability to absorb

nutrient (Hatch, 1937). Dipterocarpaceae serves as the dominant host trees for the growth of *Boletus* in Malaysia (Lee et al., 2003; Watling & Lee, 1995).

2.3 Morphological Characters

2.3.1 Macromorphological Structures of Boletaceae

2.3.1.1 Pileus and Context (Trama)

The pileus of a boletes is similar with most large agaric genera (Corner, 1972). Bessette et al., (2012) described that the pileus diameters of the boletes vary from 2 cm to 20 cm or more, though most boletes fall within the 6 cm to 12 cm range. Pileus size in relation to stipe length and thickness may result in a stature that is tall and thin or short and stout, or various other combinations. There is a wide range of colours of the stipe. Mostly shades or blends of brown, gray, black, white, yellow, orange, red or purple. The colour is an obvious feature, but it also can be tricky. Not only there is some colour variation within species, but also pileus colour can change because of age, exposure to light and other environmental influences (Bessette et al., 2010).

Surface texture of a pileus may be glabrous, tomentose, scaly, fibrillose, or glutinous. The colour of the context is usually white or yellow but is pinkish or orange-buff in some species. Some of the fruiting bodies of the Boletaceae cracked into rimose or finely aerolate appearance when getting older (Smith & Thiers, 1971; Corner, 1972). Species of *Gyroporus* have a context that remains relatively hard and brittle (Bessette et al., 2010).

The context and tubes of many boletes change colour when exposed to the air or damaged (Thiers, 1975). The characteristic blue discoloration, or bluing, seen in a number of species

may result from the production of pigments and other metabolites such as the oxidation of variegatic acids and xerocomic acids (Singer, 1986; Alexopoulos et al,. 1996; Webster & Weber, 2007; Chan, 2010).

2.3.1.2 Hymenophore

The most distinctive feature of boletes is the hymenophore. This feature consists of soft, moist and putrescent tubes. The inner lining of these tubes is known as the hymenium (Thiers, 1975). The pores of the tubes are mostly angular, varying from almost square to rectangular, and range in size from 0.5-2 mm in diameter (Chan, 2010). According to Thiers (1975), the hymenophore of most of the boletes separates readily and cleanly from the pileus.

2.3.1.3 Stipe

Majority of boletes have central, straight stipes with exceptions such as *Gyrodon merulioides*, which has an eccentric stipe, and *Boletus longicurvipes*, which is often curved at the base (Bessette et al., 2010). The overall shape of the stipe is equal to subclavate or clavate. Most are solid but a few such as *Suillus cavipes* and species of *Gyroporus* have hollow or pithy centers (Thiers, 1975; Bessette et al., 2010). The colour of the boletes stipe range from white to yellow to pink to darker colour, such as red, brown or almost black. The context of the stipe is usually the same colour as the cap context, but sometimes, especially toward the base, the colours are intensified or altogether different (Bessette et al., 2010). According to Thiers (1975), the appearance of the fruiting body of a Boletaceae is similar to the typical mushroom except that, in Boletaceae, tubes have replaced lamellae, on the under surface of the cap (Figure 1).

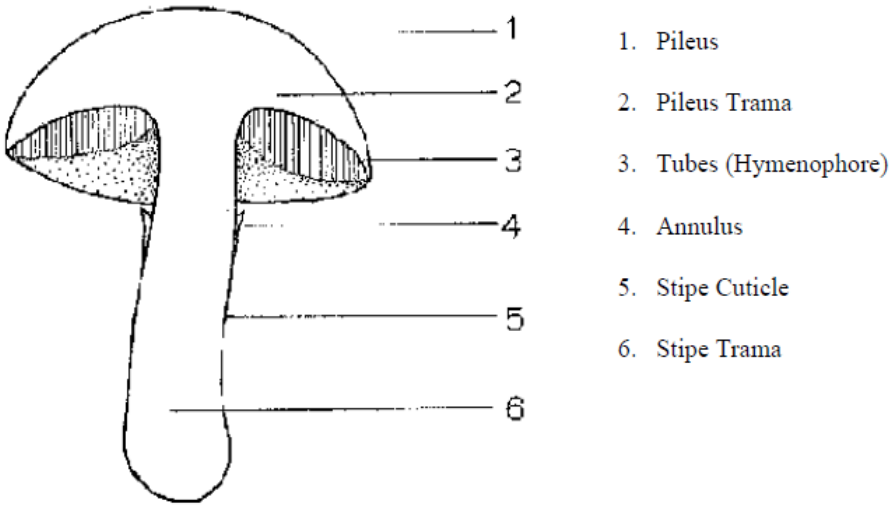

Figure 1. Fruiting body of a Boletaceae (Thiers, 1975).

2.3.2 Micromorphological Structures

Numerous microscopic structures such as spores, cystidia, basidia, clamp connection, hyphae and others are commonly used to differentiate species and genera (Bessette et al., 2010)

2.3.2.1 Basidiospores

According to Chan (2010), the basidiospores are of diagnostic value in the taxonomy and systematics of the boletes. Prints of the spores in mass are of significance for identification, and spore prints are often necessary. The spore print varies in colour from a shade of brown to yellow or dark pink. Generally, the spores are elongate and cylindric to fusoid or ellipsoid in face view. The spore length averages from 9 – 15 µm and the width from 4 – 5 µm. The variation of spore size is dependent upon its basidiocarp size, ecology and also the host plant (Kauserud et al., 2008). When mounted in Melzer's reagent, the colour may either remain unchanged or colour changes may occur. The change to rusty brown, bright rust red or a tawny