

# Diversity of mushrooms in Dry Dipterocarp forest at Phuphan National Park, Sakon Nakhon Province

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## ABSTRACT

The purposes of this study were, 1) to study the variety of mushrooms grown in the Dry Dipterocarp forest during the year 2008-2009 by Releve method, 2) to study the relationship between *Shorea siamensis* Miq. and Ectomycorrhizal of the Amanitaceae and the Boletaceae families, and 3) study the sequences of DNAs in some types of mushrooms in the Amanitaceae and the Boletaceae families by the PCR method. The findings of the study were as follows: First, they were totally 34 types of mushrooms found in Dry Dipterocarp forest at the Phuphan National Park during the studying period, 2008-2009. There were 26 types found in both years *Amanita hemibapha* subsp. *javanica* Corner & Bas, *A. princeps* Corner & Bas, *A. umbrinolutea* (Secr. ex Gillet) Bataille, *Termitomyces microcarpus* (Berk. & Br.) R. Heim, *T. perforans* Heim, *T. striatus* f. *griseus* Heim, *Boletellus ananas* (M. A. Curtis) Murrill, *B. grisei-purpureus* Cor., *B. edulis* Bull. ex Fr., *B. luridus* Schaeffer ex Fr., *Alpovatrappii* Fogel, *Cantharellus cibarius* Fries, *Craterellus aureus* Berk. & Curis, *Astraeus hygrometricus* (Pers.) Morgan, *Lactarius aquifluus* Peck, *L. glaucescens* Crossl., *L. piperatus* (Scop. ex Fr.) S. F. Gray, *L. vellereus* (Fr.) Fr., *L. virescens* Fr., *R. densifolia* (Secr.) Gill, *R. emetica* (Schaeff. & Fr.) S. F. Gray, *R. xelampelina* (Schaeff.) Fr., *R. foetens* (Pers.) Fr., *R. rosacea* (Pers. ex Secr.) Fries, *R. violeipes* Qué., *R. virescens* (Schaeff.) Fries. and those found only in 2009 were *Boletus curtisii* M. A. Curtis, *B. nobilis* Peck, *Russula alboboreolata* Hongo, *R. cyanoxantha* (Schaeff. ex Secr.) Fr. The mushrooms that had been found had significantly relations to the surrounding physical conditions. Second, there was a relationship between the *Shorea siamensis* Miq. and *Amanita princeps* Cor. & Bas., *Amanita hemibapha* (Berk. & Br.) Sacc. subsp.

*javanica*. Cor. & Bas., *Boletus chrysenteron* Bull., *Boletus griseipurpureus* Cor. and *Heimiellares-tipora* (Pat & Bek.) Boedijn. at the intensity of spores 500, 5000, 50,000 per each. From the SPSS for ANOVA analysis, the *Shorea siamensis* Miq. had no relations to *Amanita princeps* Cor. & Bas., *Amanita hemibapha* (Berk. & Br.) Sacc. subsp. *javanica*. Cor. & Bas., *Boletus chrysenteron* Bull., *Boletus grisei-purpureus* Cor. and *Heimiellares-tipora* (Pat & Bek.) Boedijn. in height and circumference growths of *Shorea siamensis* Miq. seedlings at the intensity of spores 500, 5000 and 50,000 per each *Shorea siamensis* Miq. at 95% level of confidence. Finally, by using the BLASTN computer program to examine, compare and separate the differences 4 types of mushrooms' DNA in the Amanitaceae and the Boletaceae families subjected to the PCR method, there were 4 findings i.e. 1342 DNA sbp. of *Amanita princeps*, 880 DNAs pb. of *Boletus nobilis* Peck, 1381 DNAs pb. of *Boletus edulis* Bull. ex Fr. and 758 DNAs bp. of *Heimiellaresipora* (Pat & Baker).

**Keywords:** The Diversity; Mushroom; The Dry Dipterocarp Forest; The Phuphan National Park

## 1. INTRODUCTION

There are several edible well known mushrooms naturally growing in the dry Dipterocarp forest at the Phuphan National Park whilst a few types are poisonous. Most of the mushrooms are in the Actomycorrhizal family which growing as saprobes and parasites. [1] Ectomycorrhizal fungi (ECM) are vital component of forest biodiversity and play a part in forest restoration. Those in the symbiotic type which growing around many plants' roots provide water, minerals to their host plants, moreover, they help protect the hosts from many pathogens. Up until now, there are still less studies on mushroom

morphological characters which are inadequate to obviously classify types of mushrooms, so molecular techniques studies are needed to classify mushrooms' species, those of the *Amanita* and *Boletus* families in particularly.

As the result, it remains less explanation and knowledge about mushrooms made published, whether it be the relationship between mushrooms and other plants, the physical factors are favourable to mushrooms' growth, and etc.

## 2. MATERIALS AND METHODS

### 2.1. Collecting Sites

The mushrooms were collected from Phuphan National Park village forest in Sakon Nakhon Province. The site was in Dry Dipterocarp forest.

### 2.2. Collecting Methods

The samples were collected randomly from every stage of ages in two experimental areas sized  $100 \times 100 \text{ m}^2$  and  $1000 \times 10 \text{ m}^2$  during January-December in 2008-2009. Young mature and old individual fruiting bodies were collected with chisel soil were removed with a fine brush and placed in brown paper bags in a basket, while data of associated host, temperature humidity and intensity of light at the study were recorded. The mushroom collected in study sites and roadside markets nearby the study sites were noted and some interest species were also bought for examination. The tissue of all specimens was kept in 1.5 ml microcentrifuge tube poured in 300  $\mu\text{l}$  2X CTAB buffer at  $-20^\circ\text{C}$ . Specimens were dried at  $45^\circ\text{C} - 50^\circ\text{C}$  overnight and kept in plastic boxes with silica gel to keep out humidity.

### 2.3. Taxonomy Methods

The mushrooms were tentatively identified by a conventional morphology method presented by [1,2] and chemical methods using the keys that provided by [3-5] was employed to classify their chemical characteristics. The latter method was used as the main way to assemble mushrooms in the Basidiocarp family, physical features and basidiospore.

### 2.4. Culture and Edibility Information of Some Edible and Wild Boletes

The fresh sporocarps of mushroom especially the edible species were cultured for vegetative mycelium. A pure culture of each collection was isolated from the issue (small pieces that cut from section between the pileus and strip) using half strength Potato Dextrose Ager (PDA) plate. The pure culture was incubated at room temperature ( $28^\circ\text{C} \pm 2^\circ\text{C}$ ) [6].

### 2.5. DNA Extraction

A small tissue from the flesh section between the pileus and stipe of fruit body was cut with a sterilized knife and placed into a 1.5 ml centrifuge tube containing 300  $\mu\text{l}$  2X CTAB buffer and kept in  $-20^\circ\text{C}$  for DNA extraction. The specimens were ground with 200 mg of sterilized quartz sand then 2X CTAB extraction buffer were added for adjusted to 600  $\mu\text{l}$ . Contents were then incubated at  $60^\circ\text{C}$  in a water bath for 30 min with gentle swirling. The solution was then extracted two or three times with an equal volume of chloroform: isoamyl (24:1) at 13,000 rpm for 30 min until no interface was visible. The supernatant phase containing the DNA was precipitated by addition of 2.5 volumes of absolute ethanol and kept at  $-20^\circ\text{C}$  overnight. The DNA pellet was washed (70% ethanol) 2 times, dries (under vacuum), and resuspended in TE buffer (1 mM EDTE, 10 mM Tris-HCL, pH 8) and mixed together with RNase A (1  $\text{mg}/\text{ml}^{-1}$ ). In addition some tissues specimens were extracted for DNA by using DNA extract Kits (NucleoSpin<sup>®</sup> Plant II, Macherey Nagel, Catalog no, 740770.50) following manufacturer's protocol.

### 2.6. PCR Amplification Anf Sequencing of 28S rDNA and ITS

Approximately 900 nucleotides at the 5' end of the nuclear large ribosomal subunit gene (28S rDNA) were amplified by OPERON primer pairs LROR (5'-ACCC GCTGAAGTAAAGC-3') and LR5 (5'-TCCTGAGGGA AACTTCG-3') [7]. A portion of the ITS region was performed by using a pair of universal primer ITS5 (5'-GGAAGTAGTCGTAACAAGG-3') and ITS4 (5'-TCCT CCGCTTATTGATATGC-3') (OPERON) [8].

Genomic DNA 3  $\mu\text{l}$  was used in a standard 50  $\mu\text{M}$  PCR mixture (25 mM  $\text{MgCl}_2$ , 10 MG-free buffer, 2.5  $\mu\text{M}$  dNTPs, 1.5  $\mu\text{M}$  primers, and 1.5 unit of Taq DNA Polymerase-BioLabs M0267-S) under the following thermal condition:  $94^\circ\text{C}$  for 30 s, 35 cycles of  $94^\circ\text{C}$  for 30 s,  $60^\circ\text{C}$  for 30 s, and  $72^\circ\text{C}$  for 1.5 min. Amplicons were checked on 1% agarose gels stained with ethidium bromide under UV light. Negative control reaction omitting DNA were included in all sets of amplifications to monitor potential contamination by exogenous DNA. PCR products were purified using NucleoSpin<sup>®</sup> Extract II PCR clean-up Kit (Macherey Nagel, Catalog no. 740609.50) following manufacturer's protocol. The amplified 28S rDNA and ITSs fragments were directly sequenced. Sequencing reaction was performed and sequences determined automatically by Macrogen Company in Korea using PCR primer mentioned upper part.

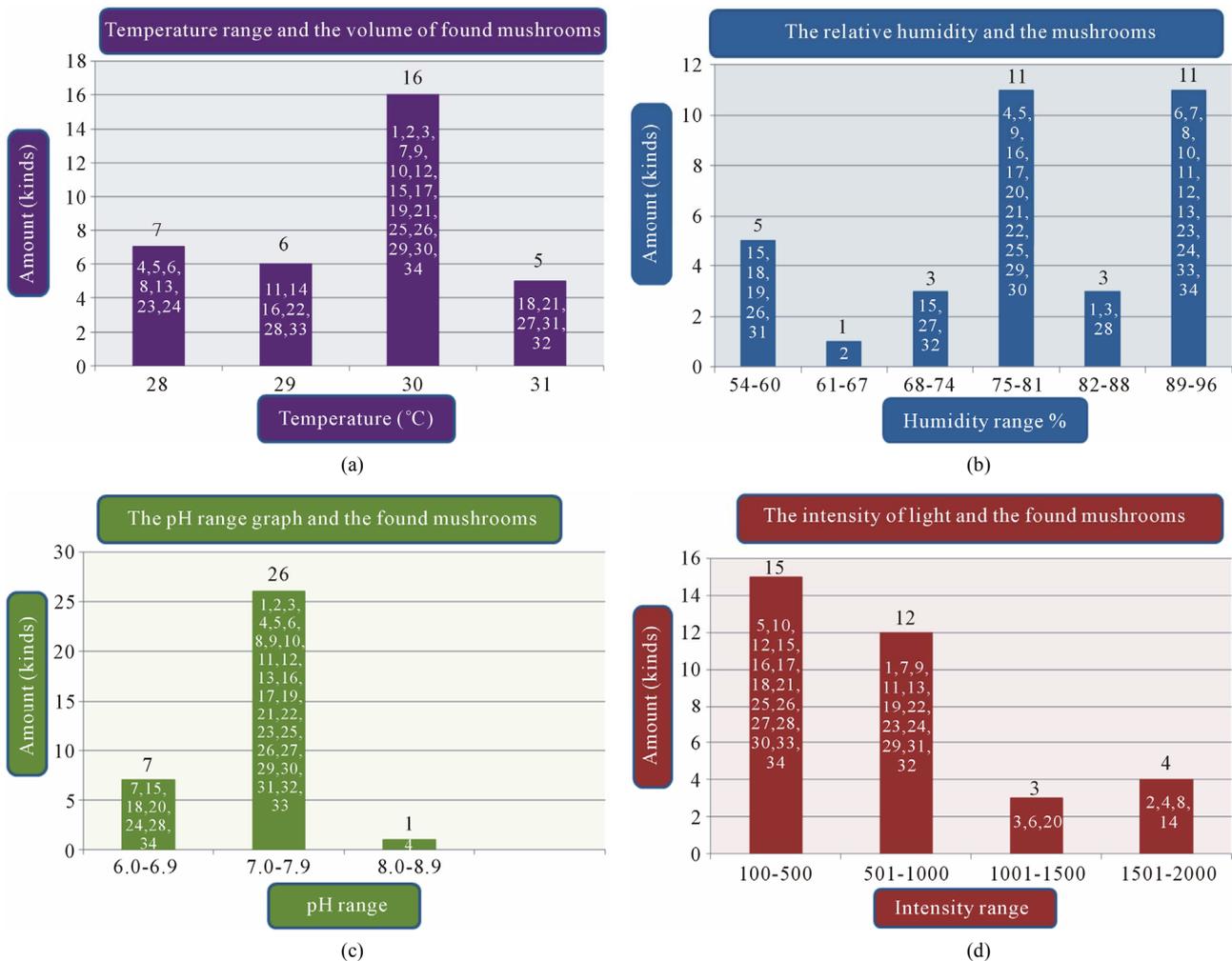
## 3. RESULTS

Knowledge of Mushroom diversity is important

**Table 1.** Species classifications and distribution of the identified mushroom present.

Scientific name	Physical and Biological Characteristic							
	Humidity (%)	Temp. (°C)	Light (Lux)	pH	Weight (g)	Wide (cm)	High (cm)	Month
<i>Amanita hemibapha</i> subsp. <i>javanica</i> Corner & Bas	85	30	770	7.2	40	6.5	7	Jan-Sep
<i>Amanita princeps</i> Corner & Bas	67	30	1600	7.2	40	7	10	Jan-Sep
<i>Amanita umbrinolutea</i> (Secr. ex Gillet) Bataille	85	30	1030	7	20	4	8	Apl-Nov
<i>Termitomyces microcarpus</i> (Berk. & Br.) R. Heim	80	28	1550	7	30	9.7	73.2	May-Dec
<i>Termitomyces perforans</i> Heim	75	28	450	7	14	1.77	15	May-Sep
<i>Boletellus ananas</i> (M. A. Curtis) Murrill	92	30	700	6	70	8	9.27	May-Sep
<i>Boletus campestris</i> A. H. Smith & Thiers	93	28	1552	7	40	6.62	8.7	Jul-Aug
<i>Boletus curtisii</i> M. A. Curtis	80	30	789	7	50	2.5	5.5	Jul-Oct
<i>Boletus edulis</i> Bull. ex Fr.	92	30	201	7	108	7.5	5.4	Jul-Oct
<i>Boletus luridus</i> Schaeffer ex Fr.	90	29	1000	7.2	30	1.77	5	Jan-Oct
<i>Boletus nobilis</i> Peck	94	30	150	7	15	2.5	1	Sep-Oct
<i>Heimiella retispora</i> (Pat. & Bak.) Boedijn	96	28	950	7.1	60	5.7	3.77	Jul-Aug
<i>Boletus griseipurpureus</i> Cor.	54	29	1800	8.9	90	5	5	Jul-Oct
<i>Russula emetic</i> (Schaeff. & Fr.) S. F. Gray.	77	30	800	7	20	6.7	3.77	Mar-Aug
<i>Russula xelempeina</i> (Schaeff.) Fr.	75	30	450	7.1	60	4.5	3.0	Jul-Aug
<i>Russula foetens</i> Fr.	60	31	460	7.2	40	5.77	3.5	Jul-Aug
<i>Russula rosacea</i> (Pers. ex Secr.) Fries	69	31	1000	7.1	30	5.77	5.5	Jul-Sep
<i>Russula virescens</i> (Schaeff.) Fries	92	29	350	7	30	14.6	3.77	Jul-Aug
<i>Russula violeipes</i> QuéL.	90	30	376	6	60	4.0	2.78	Jul-Aug
<i>Alpova trappei</i> Fogel	74	30	197	6.5	15	2.5	1	Jul-Aug
<i>Cantharellus cibarius</i> Fries	78	29	200	7.2	55	5.27	4	Apl-Nov
<i>Craterellus aureus</i> Berk. & Curis	76	30	150	7.1	25	2.5	4.7	Apl-Nov
<i>Astraeus hygrometricus</i> (Pers.) Morgan	60	31	395	6.5	15	1.5	2.13	Apl-Nov
<i>Lentinus polychrorus</i> Lév.	57	30	750	7	26	3.2	1	Apl-Nov
<i>Microporus xanthopus</i> (Fr.) Kuntze	78	31	1200	6	45	4.4	3	Apl-Nov
<i>Lactarius aquifluus</i> Peck	79	30	470	7.1	30	3	2.87	Jul-Aug
<i>Lactarius glaucescens</i> Crossl.	79	29	700	7.1	30	2.5	3	Apl-Nov
<i>Lactarius piperatus</i> (Scop. ex Fr.) S. F. Gray	90	28	512	7	50	3.7	2.4	Jan-Sep
<i>Lactarius vellereus</i> (Fr.) Fr.	70	31	350	7.1	50	7	1.77	Jul-Aug
<i>Lactarius virescens</i> Fr.	93	28	713	6	50	3.7	2.4	May-Nov
<i>Russula alboareolata</i> Hongo	75	30	450	7	50	7	1.77	Jan-Sep
<i>Russula cyanoxantha</i> (Schaeff. ex Secr.) Fr.	55	30	370	7.2	30	3.7	2	Jul-Aug
<i>Russula densifolia</i> (Secr.) Gill	86	29	400	6.5	40	4.0	3	Jul-Aug

Jan = January; Feb = February; Mar = March; Apl = April; May = May; Jun = June; Jul = July; Aug = August; Sep = September; Oct = October; Nov = November; Dec = December.



**Figure 1.** (a) Temperature range and the volume of found mushrooms; (b) The relative humidity and the mushrooms in the dry dipterocarp forest; (c) The pH range graph and the found mushrooms; (d) The intensity of light and the found mushrooms. 1 = *Amanita hemibapha* (Berk. et Broome) sacc Subsp. *javanica* Corner et Bas; 2 = *Amanita princeps* Corner et Bas; 3 = *Amanita umbrinotulata*; 4 = *Termitomyces microcarpus* (Berk. et Broome) Hiem; 5 = *Termitomyces perforans* Heim; 6 = *Termitomyces straiatus* Heim F. griseus Heim; 7 = *Boletellus ananus* (M.A. Curtis) Murrill a; 8 = *Boletus campestris*; 9 = *Boletus curtisii* Curtis; 10 = *Boletus edulis* Bull. ex Fr; 11 = *Boletus luridus* Schaeffer; 12 = *Boletus nobilis* Peck; 13 = *Heimiella retispora* (Pat. & Baker) Boedijn; 14 = *Tylopilus plumbeoviolaceus* (Snell & Dick); 15 = *Russula emetic* (Schaeff. & Fr.) S. F. Gray; 16 = *Russula fageticola* (Melzer); 17 = *Russula foetens* Fr.; 18 = *Russula rosacea* Pers Ex S.F. Gray; 19 = *Russula virescens* (Schaeff.) Fr; 20 = *Russula violeipes* Quel; 21 = *Alpova trappei* Fogel; 22 = *Cantharellus cibarius* Fr.; 23 = *Cantharellus aureus* Berk. & Curt; 24 = *Astraeus hygrometricus* (Pers.) Morgan; 25 = *Lentinus polychrlus* Lev.; 26 = *Microporus xanthopus* (Fr.) Kuntze; 27 = *Lactarius aquifluus* Peck.; 28 = *Lactarius glaucescens* Crossl.; 29 = *Lectavius Piperatus* (Scop. ex. Fr) S. F. Gray.; 30 = *Lectavius virescens* Fr.; 31 = *Russula adusta* (Pers ex Fr.) Fries.; 32 = *Russula albidula* Peck; 33 = *Russula alboareolata* Hongo.; 34 = *Russula densifolia* (Secr.) Gill.

because of their major roles in natural and managed ecosystems as ectomycorrhizal fungi. The fungal group becomes an important factor for reforestation program worldwide. Furthermore, they are important as food source for human beings and animals [9]. In addition fungal diversity as well as mushroom can also be used as a bio-indicator of environmental quality. Studies on the diversity and taxonomy (base on morphological characters and molecular analyses) of mushroom and other macro and other macro-fungi of Thailand are interested and need more investigation.

This study aims to complete the study of diversity and molecular relations of some stripe-tubulate mushroom collected from reserved rainforest in The Phuphan National Park, Sakhon Nakhon Province.

### Diversity of Mushroom in Phuphan National Park, Sakhon Nakhon Province

The result of this study provided overviews of the diversity and molecular phylogeny of ectomycorrhizal mushroom in The Phuphan National Park, Sakhon Nak-

hon Province. Approximately 34 collections were recorded in The Phuphan National Park, Sakhon Nakhon Province over a two-year period from 2008 to 2009 during wet and cool dry season (January to November). Species classifications and distribution of the identified mushroom present in **Table 1**.

The study found that the first, there are a variety of mushrooms in dry dipterocarp forest on 2008-2009 at Phuphan national park, found 34 species in total. There were 26 types found in both years *Amanita hemibapha* subsp. *javanica* Corner & Bas, *A. princeps* Corner & Bas, *A. umbrinolutea* (Secr. ex Gillet) Bataille, *Termitomyces microcarpus* (Berk. & Br.) R. Heim, *T. perforans* Heim, *T. striatus* f. *griseus* Heim, *Boletellus ananas* (M. A. Curtis) Murrill, *B. griseipurpureus* Cor., *B. edulis* Bull. ex Fr., *B. luridus* Schaeffer ex Fr., *Alpova trappei* Fogel, *Cantharellus cibarius* Fries, *Craterellus aureus* Berk. & Curis, *Astraeus hygrometricus* (Pers.) Morgan, *Lactarius aquifluus* Peck, *L. glaucescens* Crossl., *L. piperatus* (Scop. ex Fr.) S.F. Gray, *L. vellereus* (Fr.) Fr., *L. virescens* Fr., *R. densifolia* (Secr.) Gill, *R. emetica* (Schaeff. & Fr.) S.F. Gray., *R. xelempeina* (Schaeff.) Fr., *R. foetens* (Pers.) Fr., *R. rosacea* (Pers. ex Secr.) Fries, *R. violeipes* Qué., *R. virescens* (Schaeff.) Fries. The mushroom types found only in 2008 were *Boletus campestris* A.H. Smith & Thiers, *Heimiella restipora* (Pat. & Bak.) Boedijn, *Lentinus polychlorus* Lév., *Microporus xanthopus* (Fr.) Kuntze and those found only in 2009 were *Boletus curtisii* M. A. Curtis, *B. nobilis* Peck, *Russula alboareolata* Hongo, *R. cyanoxantha* (Schaeff. ex Secr.) Fr. The mushrooms that had been found had significantly relations to the surrounding physical conditions, 22 species were found at the temperature range 29°C - 30°C (min 28°C, max 31°C, average 29°C) as shown in **Figure 1(a)**, most mushrooms (28 species) were found at the relative humidity range between 68% - 96% (min 54%, max 96%, the average relative 75%) as shown in **Figure 1(b)**, 26 species found at pH range 7.0 - 7.9 (min 6, max 8.9, average 7) as shown in **Figure 1(c)**, and 16 species found at the light intensity range of 150 - 500 lux (min 150, max 1800, average 970) as shown in **Figure 1(d)**.

The second, there is a relationship between the *Shorea siamensis* Miq. and *Amanita princeps* Cor. & Bas., *Amanita hemibapha* (Berk. & Br.) Sacc. subsp. *javanica* Cor. & Bas., *Boletus chrysenteron* Bull., *Boletus griseipurpureus* Cor. and *Heimiella restipora* (Pat & Bek.) Boedijn. at the intensity of spores 500, 5000, 50,000 per each—From the SPSS for ANOVA analysis, the *Shorea siamensis* Miq. had no relations to *Amanita princeps* Cor. & Bas., *Amanita hemibapha* (Berk. & Br.) Sacc. subsp. *javanica* Cor. & Bas., *Boletus chrysenteron* Bull., *Boletus griseipurpureus* Cor. and *Heimiella restipora* (Pat & Bek.) Boedijn. in height and circumference growths of *Shorea siamensis* Miq. seedlings at the intensity of

spores 500, 5000 and 50,000 per each *Shorea siamensis* Miq. at 95% level of confidence.

The third, by using the BLASTN computer programme to examine, compare and separate the differences 4 types of mushrooms' DNA in the Amanitaceae and the Boletaceae families subjected to the PCR method, there were 4 findings *i.e.* 1342 DNAs bp. of *Amanita princeps*, 880 DNAs pb. of *Boletus nobilis* Peck, 1381 DNAs pb. of *Boletus edulis* Bull. ex Fr and 758 DNAs bp. of *Heimiella resipora* (Pat & Baker). The modern science can effectively identify types of mushrooms of the *Amanita* family as well as the difference levels of the molecule of each type of mushrooms [10-16].

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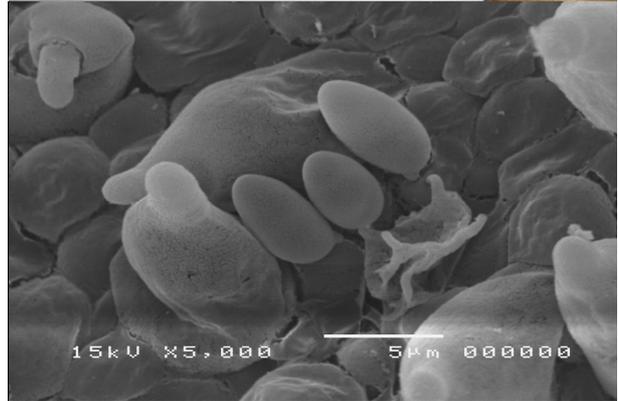
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APPENDIX



*Amanita hemibapha* (Berk. et Broome) sacc. Subsp. *javanica* Corner et Bas.



*Boletus edulis* Bull. ex Fr.



*Boletus campestris*.



*Boletus luridus* Schaeffer.



*Boletus nobilis* Peck.



*Tylopilus plumbeoviolaceus* (Snell & Dick).



*Heimiella retispora* (Pat. & Baker) Boedijn.



*Russula densifolia* (Secr.) Gill.