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# A Checklist of Mushrooms of Phousabous National Protected Area (PNPA) of Lao PDR

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## Abstract

Mushroom survey and collection were conducted in the Phousabous National Protected Area (PNPA), which is located at the Northeastern area 230km from the capital city of Lao-PDR, Vientiane, from April 2017 to September 2018. During this periods, mushroom specimens were collected from 8 different locations, and then they were identified and classified into 284 species, 142 genera, 56 families, 20 orders and 7 classes by morphological and molecular analyses. The mushrooms belongs to Ascomycota were classified into 10 species, 5 genera, 5 families, 4 orders and 3 classes, while those belongs to Basidiomycota were classified into 274 species, 137 genera, 51 families, 16 orders, and 4 classes, respectively. Among these mushrooms, the most species-rich families are Boletaceae (16.06%), Polyporaceae (10.12%), Russulaceae (9.94%), Amanitaceae (7.68%), Agaricaceae (4.54%), Marasmiaceae (4.36%), Cortinariaceae (3.66%), Entolomataceae (3.66%), Mycenaceae (2.79%), Xylariaceae (2.44%), Physalacriaceae (2.09%), Omphalotaceae (2.09%), Hydnangitaceae (2.09%), Lyophyllaceae (1.92%), Tricholomataceae (1.75%) and comprised 75.19% of the total specimens identified.

**Key Words:** fungal biodiversity, mushroom collection and identification, Phousabous National Protected Area (PNPA), ascomycota, basidiomycota

## Introduction

Phousbous National Protected Area (PNPA) is located at northeast direction 230km from Vientiane, the capital city of Lao-PDR (latitude 19°43'25.57"N, longitude 103°29'16.90"E-103°40'56.17"E). It was designated as a Biodiversity Conservation Area in 2011. This area covers 1,490 km$^2$ in Xiangkhouang Province. The elevation ranges above 800 m. PNPA has monsoonal climate similar to the rest of central Lao-PDR (Lucas et al. 2013). The rainy season is starting from May and lasting through to October, and distinct dry season is from November to April. PNPA has diverse forest types including mixed deciduous and coniferous forests, and is known for its high biodiversity. Thus there are many species of flora and fauna including mammals, birds, reptiles, as well as trees and orchids in the well-conserved forest areas, wetlands, falls and streams. However, forests in this region have been affected by human activities such as forest encroachment, illegal logging, shifting cultivation, wildfire and wildlife hunting, and consequently lead to habitat loss and biodivesity decrease. Mushroom survey in this area was conducted for getting information on the diversity of higher fungi in tropical rain forests.

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## Materials and Methods

### *Survey sites*

Mushroom survey and collections were conducted at 8 areas including Ban Tha (North), Ban Tha (South), Vieng Kham, Souan Mone Village, Nam Chak Village, Ban Naxay, Ban Nong Oln, and Gnod Lieng located in PNPA (Fig. 1).

### *Field survey and mushroom collections*

Field survey and mushroom collections were conducted from at the end of April to September, for 28 days during wet season. Whenever the mushroom was found in the survey sites, the photo was first taken for recording the image of original shape, and then various informations such as color, single or group, substrate, habitat, and so on, were recorded in the field data sheet. The collected mushroom was wrapped with the serial number in cooking hoil and put in the collection bag. Before drying mushroom in the portable mushroom drier, the small piece of tissue was taken from the inside of fruiting body, and put it in 70% ethanol for molecular identification by DNA extraction, PCR amplification, sequencing, and NCBI BLAST search. Dried mushrooms for 12hrs were kept with Silicagel in Ziploc® bags labelled with collection informations (date, location, coordinates, collectors, and scientific name, etc.) in the NIBR (National Institute of Biological Resources) specimen herbarium.


**Fig. 1.** Location map of Phousabous National Protected Area (PNPA) in Lao-PDR.

### *Mushroom identification*

The collected mushrooms were identified by morphological and molecular analyses. Morphological identification was done by observing dried specimens and photos according to the identification key in the illustrated mushroom books (Lowy 1951; Stunz 1973; Imazeki and Hongo 1989; Zhishu et al. 1993; Phillips 2005; Tan et al. 2007; Chandrasrikul et al. 2008; Tan et al. 2009; Wannathes et al. 2009; Sanoamuang 2010; Vladmir and Noordeloos 2010; Chandrasrikul et al. 2011; Lee et al. 2012; Whalley et al. 2012; Lee et al. 2015; Kim et al. 2017; Lee et al. 2017) and Index Fungorum system (www. indexfungorum.org). For the molecular identification, total DNA was extracted from mushroom tissue preserved in ethanol, and then ITS (Internal Transcribed Spacer) and/or LSU (Large Sub Unit) regions were amplified by using primers and PCR. The amplified products were sequenced, and the results were BLAST-searched from NCBI GenBank to find the sequence with high similarity.

## Results and Discussion

Five hundreds and seventy three mushroom specimens were collected from 8 survey sites of PNPA from 2017 to 2018, and then they were identified and classified into 284 species, 142 genera, 56 families, 20 orders and 7 classes by morphological and molecular analyses (Fig. 2). The mushrooms belongs to Ascomycota were classified into 10 species, 5 genera, 5 families, 4 orders and 3 classes, while those belongs to Basidiomycota were classified into 274 species, 137 genera, 51 families, 16 orders, and 4 classes, respectively (Table 1). Among these mushrooms, the most

**Table 1.** A summary of mushroom classification collected in PNPA of Lao PDR

| Group | Class | Order | Families | Genera | Species |
|---|---|---|---|---|---|
| Ascomycota | 3 | 4 | 5 | 5 | 10 |
| Basidiomycota | 4 | 16 | 51 | 137 | 274 |
| Total | 7 | 20 | 56 | 142 | 284 |


**Fig. 2.** Mushrooms collected from Phousabous National Protected Area (PNPA) in Lao-PDR.

species-rich families are Boletaceae (16.06%), Polyporaceae (10.12%), Russulaceae (9.94%), Amanitaceae (7.68%), Agaricaceae (4.54%), Marasmiaceae (4.36%), Cortinariaceae (3.66%), Entolomataceae (3.66%), Mycenaceae (2.79%), Xylariaceae (2.44%), Physalacriaceae (2.09%), Omphalotaceae (2.09%), Hydnangitaceae (2.09%), Lyophyllaceae (1.92%), Tricholomataceae (1.75%) and are comprised of 75.19% among the total specimens identified (Table 2, Supplementary Material 1).

## Acknowledgements

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**Table 2.** A list of mushroom families with high species diversity

| Families | No. of Genera | No. of Species | No. of Specimen | % |
|---|---|---|---|---|
| Boletaceae | 16 | 32 | 92 | 16.06 |
| Polyporaceae | 12 | 23 | 58 | 10.12 |
| Russulaceae | 3 | 25 | 57 | 9.94 |
| Amanitaceae | 1 | 17 | 44 | 7.68 |
| Agaricaceae | 8 | 13 | 26 | 4.54 |
| Marasmiaceae | 5 | 15 | 25 | 4.36 |
| Cortinariaceae | 1 | 14 | 21 | 3.66 |
| Entolomataceae | 1 | 8 | 21 | 3.66 |
| Mycenaceae | 5 | 9 | 16 | 2.79 |
| Xylariaceae | 1 | 5 | 14 | 2.44 |
| Physalacriaceae | 3 | 7 | 12 | 2.09 |
| Omphalotaceae | 5 | 5 | 12 | 2.09 |
| Hydnangitaceae | 2 | 5 | 12 | 2.09 |
| Lyophyllaceae | 4 | 6 | 11 | 1.92 |
| Tricholomataceae | 7 | 7 | 10 | 1.75 |
| Inocybaceae | 2 | 5 | 10 | 1.75 |
| Ganodermataceae | 2 | 4 | 10 | 1.75 |
| Hygrophoraceae | 2 | 5 | 8 | 1.4 |
| Auriculariaceae | 2 | 4 | 8 | 1.4 |
| Psathyrellaceae | 4 | 5 | 7 | 1.22 |
| Sclerodermataceae | 2 | 4 | 7 | 1.22 |
| Stereaceae | 2 | 2 | 7 | 1.22 |
| Ophiocordycepitaceae | 1 | 2 | 7 | 1.22 |
| Gyroporaceae | 1 | 3 | 6 | 1.05 |
| Hymenochaetaceae | 4 | 4 | 5 | 0.87 |
| Gomphaceae | 3 | 4 | 5 | 0.87 |
| Meruliaceae | 3 | 4 | 4 | 0.7 |
| Hydnaceae | 3 | 4 | 4 | 0.7 |
| Strophariaceae | 3 | 3 | 4 | 0.7 |
| Fomitopsidaceae | 3 | 3 | 4 | 0.7 |
| Pleurotaceae | 2 | 2 | 4 | 0.7 |
| Clavariaceae | 2 | 2 | 4 | 0.7 |
| Hymenogastraceae | 2 | 3 | 3 | 0.52 |
| Incerte sedis | 1 | 3 | 3 | 0.52 |
| Schizophyllaceae | 1 | 1 | 3 | 0.52 |
| Bankeraceae | 1 | 1 | 3 | 0.52 |
| Paxillaceae | 2 | 2 | 2 | 0.35 |
| Dacrymycetaceae | 2 | 2 | 2 | 0.35 |
| Tremellaceae | 1 | 2 | 2 | 0.35 |
| Suillaceae | 1 | 2 | 2 | 0.35 |
| Leotiaceae | 1 | 2 | 2 | 0.35 |
| Hypoxylaceae | 1 | 1 | 2 | 0.35 |
| Trixhosporonaceae | 1 | 1 | 1 | 0.17 |
| Pterulaceae | 1 | 1 | 1 | 0.17 |
| Pluteaceae | 1 | 1 | 1 | 0.17 |
| Pezizellaceae | 1 | 1 | 1 | 0.17 |

**Table 2.** Continued

| Families | No. of Genera | No. of Species | No. of Specimen | % |
|---|---|---|---|---|
| Lentariaceae | 1 | 1 | 1 | 0.17 |
| Lachnaceae | 1 | 1 | 1 | 0.17 |
| Geoglossaceae | 1 | 1 | 1 | 0.17 |
| Geastraceae | 1 | 1 | 1 | 0.17 |
| Fistulinaceae | 1 | 1 | 1 | 0.17 |
| Cortiaceae | 1 | 1 | 1 | 0.17 |
| Clavariaceae | 1 | 1 | 1 | 0.17 |
| Bondarzewiaceae | 1 | 1 | 1 | 0.17 |
| Bolbitiaceae | 1 | 1 | 1 | 0.17 |
| Albatrellaceae | 1 | 1 | 1 | 0.17 |
| *56* | 142 | 284 | 573 | 100 |

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