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Checklist of Mushrooms of Mt. Cleopatra Needle Forest Reserve in Palawan Island, Philippines

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Abstract: A mushroom survey was conducted in Mt. Cleopatra Needle Forest Reserve (CNFR) northeast of Puerto Pincesa, the capital city of Palawan island, from August 2018 to September 2019. During this period, 433 fungal fruiting bodies were collected from 3 different from survey sites, Concepcion, Kalakwasan, and Tanabag Barangays. The specimens were identified based on their morphological and molecular analyses and classified into 176 species, 114 genera, 55 families, and 22 orders. The mushrooms belong to Ascomycota were classified into 20 species, 15 genera, 12 families, and 7 orders, while those belongs to Basidiomycota were classified into 156 species, 99 genera, 43 families, and 15 orders, respectively. Among these mushrooms, the families with high frequency were Polyporaceae (18.9%), Marasmiaceae (11.5%), Xylariaceae (9.7%), Agaricaceae (8.3%), Auriculariaceae (4.8%), Ganodermataceae (4.2%), Hypoxylaceae (3.2%), and Sarcoscyphaceae (3.0%), and comprised 63.7% of the total specimens identified. This report may be the first checklist of mushrooms in Mt. CNFR and could be used for developing conservation strategies of the critical habitat in Palawan island.

Key words: *Ascomycota, Basidiomycota, fungal biodiversity, Mt. Cleopatra Needle Forest Reserve (CNFR), mushroom collection and identification*

Introduction

The Palawan island is located in the southwest of the Philippines close to Borneo, to which once was connected. Palawan has received international recognition by UNESCO as a Biosphere Reserve and it contains two World Heritage Sites. Nevertheless, the island remains relatively understudies and its forests are currently diminishing quickly. Mt. Cleopatra Needle Forest Reserve (CNFR), one of the oldest and most diverse forests in the Philippines, is located at the northeastern region from Puerto Princesa, the capital city of Palawan island of Philippines. (latitude 10°15′9°00′N, longitude 118°99′4°62′E). The 38,693 ha CNFR is part of the Cleopatra's Needle Mountain, the city's highest peak with an altitude of 1,593 m. This area is a real biological resources repository as well as the last safe haven for countless endemic species. And it serve as the most important watershed of Puerto Princesa, and the area plays an important role as a corridor between the remaining forest in the southern and northern parts of the island. For this reasons, the Palawan Council for Sustainable Development (PCSD), a multi-sectoral and inter-government body, in collaboration with the Centre for Sustainability, proposed a resolution designating Mt. Cleopatra's Needle as a critical habitat according to the Republic Act 9147, which is also known as the Wildlife Resources Conservation and Protection Act. This Act defined a critical habitat as a portion of land outside a protected area that is characterized by the presence of threathened species, considering its endemicity and richness in the area as well as the presence of threats to its survival. This survey was conducted to understand fungal species divesity in this forest area, and

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thus provide the obtained information in developing the biodiversity conservation strategies for this area.

Materials and Methods

1. Survey sites

Three sites including Concepcion, Kalakwasan, Tanabag Barangays in Cleopatra Needle Forest Mountain were surveyed for mushrooms collection (Figure 1).

2. Field survey and mushroom collections

Field survey and mushroom collections were mostly conducted during wet season, from June to November, for 14 days. Whenever a mushroom was found in the survey sites, the photo was taken for recording the image of original shape, and then various information on fruiting bodies and site characteristics, such as color, single or group, substrate, habitat, etc., were recorded in the field data sheet. The collected mushroom and a serial number were wrapped with the cooking hoil and put in the collection bag. Before drying mushrooms in the portable fan-heating dryer, a small piece of tissue was taken from the fruiting body, and preserved it into 70% ethanol in a 1.5 ml microtube for molecular identification. Mushroom specimens dried for 12hrs were kept with Silicagel in Ziploc® bags labelled with collection information (date, location, coordinates, collectors, and scientific name, etc.) kept in the NIBR (National Institute of Biological Resources) specimen herbarium.

3. 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, 1958; Stunz, 1973; Imazeki and Hongo, 1989; Zhishu et al., 1993; Chandrasrikul et al., 2008, 2011; Wannathes et al., 2009; Sanoamuang 2010; Vladmir and Machiel, 2010; 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 using Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, Irvine, CA, USA). ITS (Internal Transcribed Spacer) region was amplified by using primers (ITS1F and ITS4) and PCR reactions in a Takara PCR Thermal Cycler (Takara Bio Inc., Shiga, Japan) with an initial denaturation for 3 min at 95°C, followed by 35 cycles of denaturation for 1 min at 95°C, annealing for 0.5 min at 58°C and extension for 2 min at 72°C. Standard amplicons were purified and sequenced in both directions by Macrogen Inc. (Seoul, Korea). The obtained sequences were edited using Lasergene® version 7.0 (DNASTAR, Inc., Madison, WI, USA), and compared with sequences in the NCBI GenBank database using BLAST searches to find the sequence with high similarity.

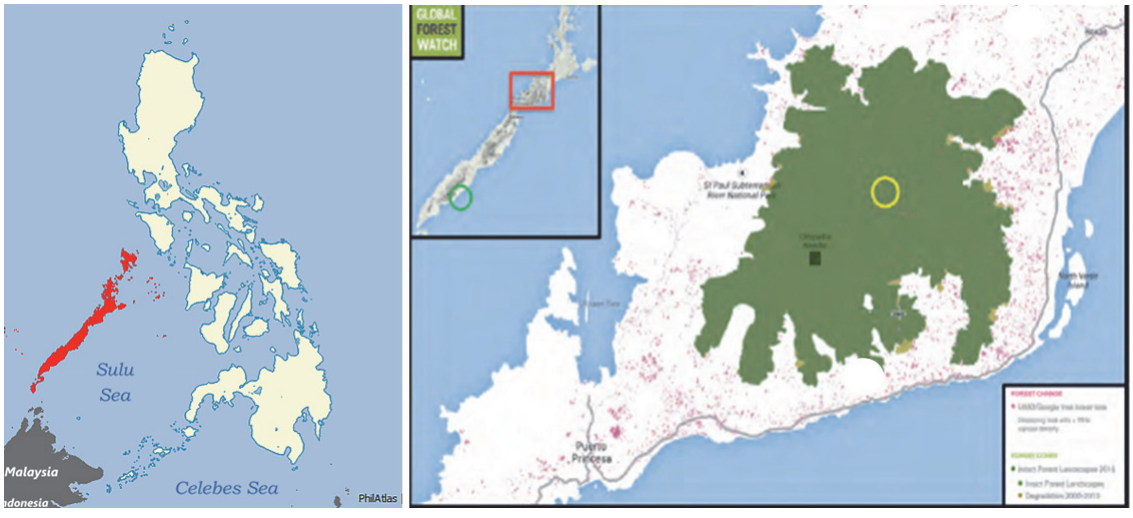

Figure 1. Map of Palawan island (red color) in Philippines and Mt. Cleopatra Needle Forest Reserve (CNFR) for mushroom diversity survey.

Results and Discussion

Four hundreds and thirty three mushroom specimens were collected from three survey sites of CNFR from 2018 to 2019, and then they were identified and classified into 176 species, 114 genera, 55 families, and 22 orders by morphological and molecular analyses (Figure 2). The mushrooms belongs to Ascomycota were classified into 20 species, 15 genera, 12 families, and 7 orders while those belongs to Basidiomycota were classified into 156 species, 99 genera, 43 families, and 15 orders, respectively (Table 1, 2). Among these mushrooms, the most frequently collected families are Polyporaceae (18.9%), Marasmiaceae (11.5%), Xylariaceae (9.7%), Agaricaceae (8.3%), Auriculariaceae

Table 1. A summary of mushroom classification collected in Mt. Cleopatra Needle Forest Reserve (CNFR) of Palawan island, Philippines.

Group	Order	Families	Genera	Species
Ascomycota	7	12	15	20
Basidiomycota	15	43	99	156
Total	22	55	114	176

Table 2. Identification results of specimens with ITS sequence similarity of accessed strain no. by DNA extraction, PCR amplification, sequencing and NCBI GenBank BLAST search.

No.	Specimen No.	Identified taxa	ITS accession No.	Similarity (%)
1	TPML20180810-068	*Gymnopus luxurians*	MN523269	100
2	TPML20180811-154	*Psatyrella candolleana*	MK247759	100
3	TPML20180810-039	*Favolaschia calocera*	DQ026239	96
4	TPML20180810-057	*Cymatoderma elegans*	JN649340	94
5	TPML20190629-115	*Cymatoderma elegans*	JN649340	98
6	TPML20180811-256	*Trametes versicolor*	KC176325	99
7	TPML20190627-002	*Trametes versicolor*	KC176325	99
8	TPML20180811-154	*Micropsalliota furfuracea*	KJ849235	99

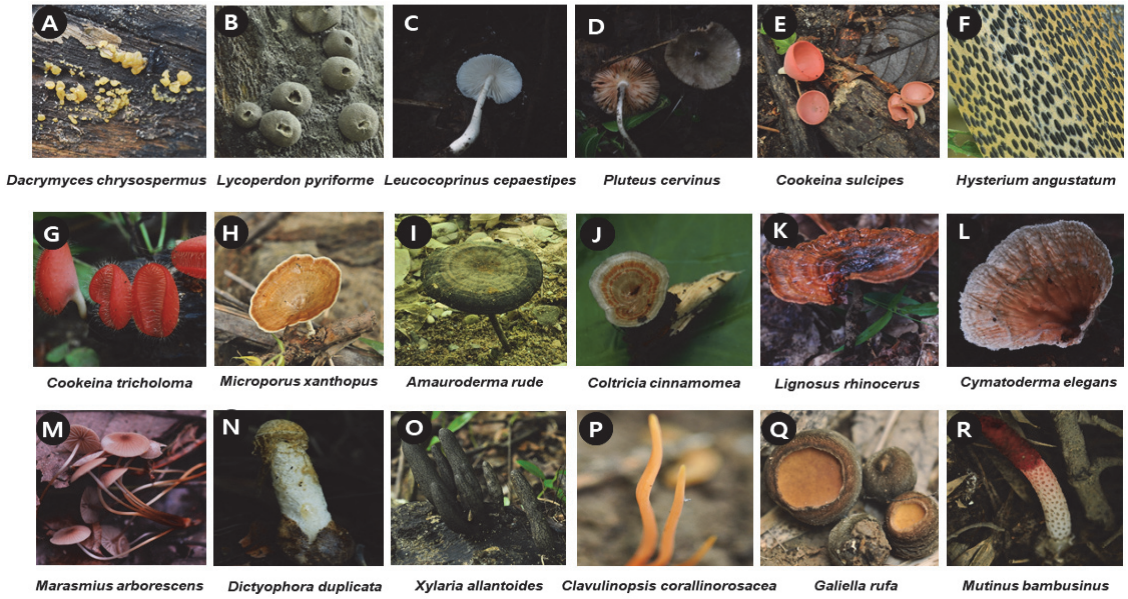

Figure 2. Diverse mushrooms with various shape collected from Mt. Cleopatra Needle Forest Reserve (CNFR) in Palawan island, Philippines. A: *Dacrymyces chrysospermus* Berk. & M.A. Curtis, B: *Lycoperdon pyriforme* Schmach, (C) *Leucocoprinus cepaestipes* (Sowerby) De Leon, Kalaw, Dulay, Undan, Alfonzo, Undan & Reyes, D: *Pluteus cervinus* (Schaeff.) P. Kumm., E: *Cookeina sulcipes* (Berk.) Kuntze, F: *Hysterium angustatum* Alb. & Schwein., G: *Cookeina tricholoma* (Mont.) Kuntze, H: *Microporus xanthopus* (Fr.) Kuntze, I: *Amauroderma rude* (Berk.) Torrend, J: *Coltricia cinnamomea* (Jacq.) Murrill, K: *Lignosus rhinocerus* (Cooke) Ryvarden, L: *Cymatoderma elegans* Jungh, M: *Marasmius arborescens* (Henn.) Beeli, N: *Dictyophora duplicata* (Bosc) E. Fisch., O: *Xylaria allantoides* (Berk.) Fr., P: *Clavulinopsis corallinorosacea* (Cleland) Corner, Q: *Galiella rufa* (Schwein.) Nannf. & Korf, R: *Mutinus bambusinus* (Zoll.) E. Fisch.

Table 3. A list of mushroom families exhibiting high species diversity.

No.	Family	No. of Genera	No. of Species	No. of Specimen	Frequency (%)
1	Polyporaceae	14	28	82	18.9
2	Marasmiaceae	7	23	48	11.5
3	Xylariaceae	1	3	42	9.7
4	Agaricaceae	9	17	36	8.3
5	Auriculariaceae	2	5	21	4.8
6	Ganodermataceae	3	4	17	3.9
7	Hypoxylaceae	2	3	14	3.2
8	Sarcoscyphaceae	2	4	14	3.2
9	Mycenaceae	3	7	12	2.8
10	Meruliaceae	3	3	12	2.8
11	Pleurotaceae	3	4	8	1.8
12	Dacrymycetaceae	3	3	8	1.8
13	Omphalotaceae	2	4	8	1.8
14	Schizophyllaceae	1	1	7	1.6
15	Tricholomataceae	4	4	6	1.4
16	Inocybaceae	2	3	6	1.4
17	Pluteaceae	2	2	6	1.4
18	Fomitopsidaceae	3	4	5	1.2
19	Psathyrellaceae	2	3	5	1.2
20	Hysteriaceae	1	1	5	1.2
21	Hymenochaetaceae	3	3	4	0.9
22	Entolomataceae	2	2	4	0.9
23	Hygrophoraceae	2	2	4	0.9
24	Amanitaceae	1	2	4	0.9
25	Geastraceae	1	2	4	0.9
26	Clavariaceae	3	3	3	0.7
27	Stereaceae	1	2	3	0.7
28	Thelephoraceae	1	2	3	0.7
29	Sclerodermataceae	1	1	3	0.7
30	Incerte sedis	1	1	3	0.7
31	Physalacriaceae	2	2	2	0.5
32	Gomphaceae	2	2	2	0.5
33	Hymenogastraceae	2	2	2	0.5
34	Phallaceae	2	2	2	0.5
35	Gloeophyllaceae	1	2	2	0.5
36	Pterulaceae	1	1	2	0.5
37	Corticiaceae	1	1	2	0.5
38	Nectriaceae	1	1	2	0.5
39	Russulaceae	1	1	2	0.5
40	Bolbitiaceae	1	1	2	0.5
41	Cortinariaceae	1	1	2	0.5
42	Geoglossaceae	1	1	1	0.2
43	Cenangiaceae	1	1	1	0.2
44	Mollisiaceae	1	1	1	0.2
45	Phacidiaceae	1	1	1	0.2
46	Helotiaceae	1	1	1	0.2
47	Pezizaceae	1	1	1	0.2
48	Pyronemataceae	1	1	1	0.2
49	Fistulinaceae	1	1	1	0.2
50	Hydnangiaceae	1	1	1	0.2
51	Strophariaceae	1	1	1	0.2
52	Coniophoraceae	1	1	1	0.2
53	Cantharellaceae	1	1	1	0.2
54	Bondarweiaceae	1	1	1	0.2
55	Ceratostomataceae	1	1	1	0.2
		114	176	433	

(4.8%), Ganodermataceae (3.9%), Hypoxylaceae (3.2%), and Sarcoscyphaceae (3.2%), and comprised 63.7% of the total specimens identified (Table 3). Mushroom diversity survey in Philippines have been mainly focused and conducted in many Luzon areas, the biggest island of the country, and numerous reports have been published since 2000 (Alzeus et al., 2018; Angeles et al., 2016; Arenas et al., 2015; 2018; De Leon et al., 2012; 2013; 2016; Lazo et al., 2015; Musngi et al., 2005; Paquit and Pampolina, 2017; Quimio, 2001; Reyes and Nair, 2016; Sibounnavong et al., 2008; Tadiosa et al., 2011; Tadiosa and Briones, 2013), but no reports on mushroom diversity except the report on ascomycetous fungi (Sydow, 1912) have been found in Palawan island so far. Thus, this report may be the first checklist of mushrooms in Mt. Cleopatra Needle Forest Reserve, and could be used for developing conservation strategies of the critical habitat in Palawan island.

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