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Molecular identification of four wild higher basidiomycetes collected in Mt. Mingan, Gabaldon, Nueva Ecija, Philippines

Chenny Dhanryll M. Guzman, Marisol M. Baltazar, Aiza Jhane I. Sanchez, Mariel G. Linsangan, Rich Milton R. Dulay*

Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines

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Abstract

This paper reports the molecular identification of the four mushrooms collected in Mt. Mingan, Gabaldon, Nueva Ecija, Philippines. The DNA samples from the fresh fruiting bodies of mushrooms were extracted, isolated, purified, PCR amplified (using ITS-1F and ITS4 primers), and sequenced (using Capillary Electrophoresis Sequencing). The DNA sequences were trimmed and cleaned-up before sequence alignment using MEGA7 software and the phylogenetic affiliations of the ITS genes were determined by alignment to the NCBI non-redundant database using the BLASTn algorithm. The four mushrooms were identified as *Stereum hirsutum* (95%), *Micropus xanthopus* (99%), *Pleurotus tuberregium* (99%) and *Trametes elegans* (99%). To our knowledge, this is the first report on the collection and molecular identification of these wild mushrooms in Mt. Mingan.

***Corresponding Author:** Rich Milton R. Dulay ✉ richmiltondulay@clsu.edu.ph

Introduction

Mushrooms are basidiomycetous fungi which are naturally found growing on lignin-cellulosic substrates in the temperate and tropical regions. This group has been reported to be beneficial to humans. Wild mushrooms are seasonally and traditionally collected in the forest and/or any areas with lignin-cellulosic substrates and consumed by farmers and mushroom hunters. This is due to their nutritional as well as their therapeutic values (Rahi and Malik, 2016). They contain proteins, fibers, carbohydrates, and minerals (Amabye and Bezabh, 2015) and use for the development of medicines, pharmaceutical products such as drugs, dietary supplements, and healthy cosmetic products (Tang, 2004).

The Philippines, being one of the mega diverse countries in the world, is rich in mycological resources that remain in the wild and underutilized. Several studies have been conducted on the taxonomic identification and listing of wild macrofungi in the Philippines. For instance, in Mt. Malinao, Albay, 9 Tricholomataceae, 3 Coprinaceae, 2 Pluteaceae, and 1 Auriculariaceae species were identified (Daep and Cajuday, 2003) while Mt. Apo in Mindanao had 25 genera and 87 species of basidiomycetes (Biadnes and Tangonan, 2008). Moreover, Militante and Tadiosa (2005) identified 27 different wood-decaying species of Basidiomycetes belonging to 15 genera whereas 30 species classified under 18 families and 21 genera were listedin agro-ecosystem of Brgy. Bambanaba, Cuyapo, Nueva Ecija (Dulay and Maglasang, 2017).

However, identification and classification of these wild mushrooms are basically morphological which is sometimes leading to misidentification of the species. Therefore, it is indeed necessary to use molecular approach in order to have accurate and reliable fungal taxonomic studies.

This present work molecularly identified the four wild mushrooms collected in Mt. Mingan, Gabaldon, Nueva Ecija, Philippines.

Materials and methods

Collection of samples

The wild fruiting bodies of mushrooms were collected in Mt. Mingan, Gabaldon, Nueva Ecija Philippines during the month of February 2018. The collected samples were either carefully handpicked or with the use of knife and were placed in paper bag.

DNA extraction

Individual mushroom was placed in a sterile petridish and were cut into tiny pieces using sterile surgical blade. The DNA extraction was seemly applied using ZYMO DNA extraction kit. Briefly, samples were mixed with ZR bashing beads, genomic lysis buffer and bashing bead buffer. There were rigorously mix for 2 min and centrifuged at 10,000 xg for 1 min.

The supernatant was then filtered from columns following manufacturer protocol. From the column, the DNA was then wash with washing buffer and eluted from the column using elution buffer. The DNA was then transferred in a 1.5 ml tube and stored at -20°C.

PCR amplification and sequencing

Amplification was performed in a BioRad T100 thermal cycler using the Fungal ITS region of isolates, primers ITS-1F and ITS-4 (Martin and Rygiewicz, 2005; Manter and Vivanco, 2007). The PCR cycles consist of an initial heating step for 5 min at 95°C, 35 cycles of 95°C for 1 min, with annealing temperature of 55°C for 1 min, and extension of 72°C for 1 min, and a final extension for 6 min at 72°C were used. The PCR products were loaded into a 1.5% agarose gels and run in 1X TAE at 120V for 30 min. These were purified using gel purification kit prior sequencing. The purified products were sequenced using capillary electrophoresis sequencing (Sanger Sequencing) using biochemical method at Macrogen Laboratory, Korea.

Data analysis

The obtained DNA sequences were trimmed and cleaned-up before sequence alignment using MEGA7

software (https://www.megasoftware.net) and were refined manually by visual inspection prior structural analysis. Phlyogenetic affiliation of the ITS genes was determined by alignment to the NCBI non-redundant database (www.ncbi.nlm.nih.gov) using the BLASTn algorithm. Phylogenetic analyses were determined under MEGA software using maximum likelihood (Jukes Cantor Model).

Results and discussion

Many of the important mycological resources which have promising benefits to human are still in the wild. They can be valuable sources of food and medicines. In this study, wild mushrooms from Mt.

Mingan, Gabaldon, Nueva Ecija were documented in their natural habitat as shown in Figure 1, collected, and molecularly identified. DNAs of four mushrooms were successfully extracted, amplified, purified.

Sequenced, and analyzed. Table 1 presents the result of molecular identification.

Table 1. BLAST results of different samples using the ITS sequence fragment.

Sample Code	Sequence Length (bp)	% Identity (Accession No.)	Sample Name
Sample 1	568	95% (FJ810148.1)	*Stereum hirsutum*
Sample 2	643	99% (JX290074.1)	*Micropus xanthopus*
Sample 3	1582	99% (AF109972.1)	*Pleurotus tuberregium*
Sample 4	618	99% (KU752345.1)	*Trametes elegans*

The four mushrooms were identified as *Stereum hirsutum* (95%), *Micropus xanthopus* (99%), *Pleurotus tuberregium* (99%) and *Trametes elegans* (99%). To our knowledge, this is the first report on the collection and molecular identification of wild mushrooms from Mt. Mingan, Gabaldon, Nueva Ecija.

Fig. 1. Fruiting bodies of (A) *Stereum hirsutum*, (B) *Micropus xanthopus*, (C) *Pleurotus tuberregium* and (D) *Trametes elegans* on their substrate.

The phylogenetic analysis was also determined in this study (Figure2). A total of 44 sequences including the ITS sequence of Sample 1 supported by 68% bootstrap value using the 568 bp amplified fragment from the sample revealed that Sample 1 (180514-R01_M20_1_F-ITS1f) was *Stereum hirsutum* (FJ810148.1). From 52 related sequences, 180514-R01_O20_2_F-ITS1f was identified as *Micropus xanthopus* (JX290074.1) supported 102% bootstrap value using the 643 bp length of the amplified fragment. On the other hand, 180517-R01_I21_3_F-ITS1f was identified as *Pleurotus tuberregium* (AF109972.1) with 95% bootstrap value based on the 1582 bp sequence of the sample using 53 related sequences. Sample 180517-R01_K21_4_F-ITS1f was identified as *Trametes elegans* (KU752345.1) using 618 bp sequences and found to have 94% bootstrap value using the 61 related sequences.

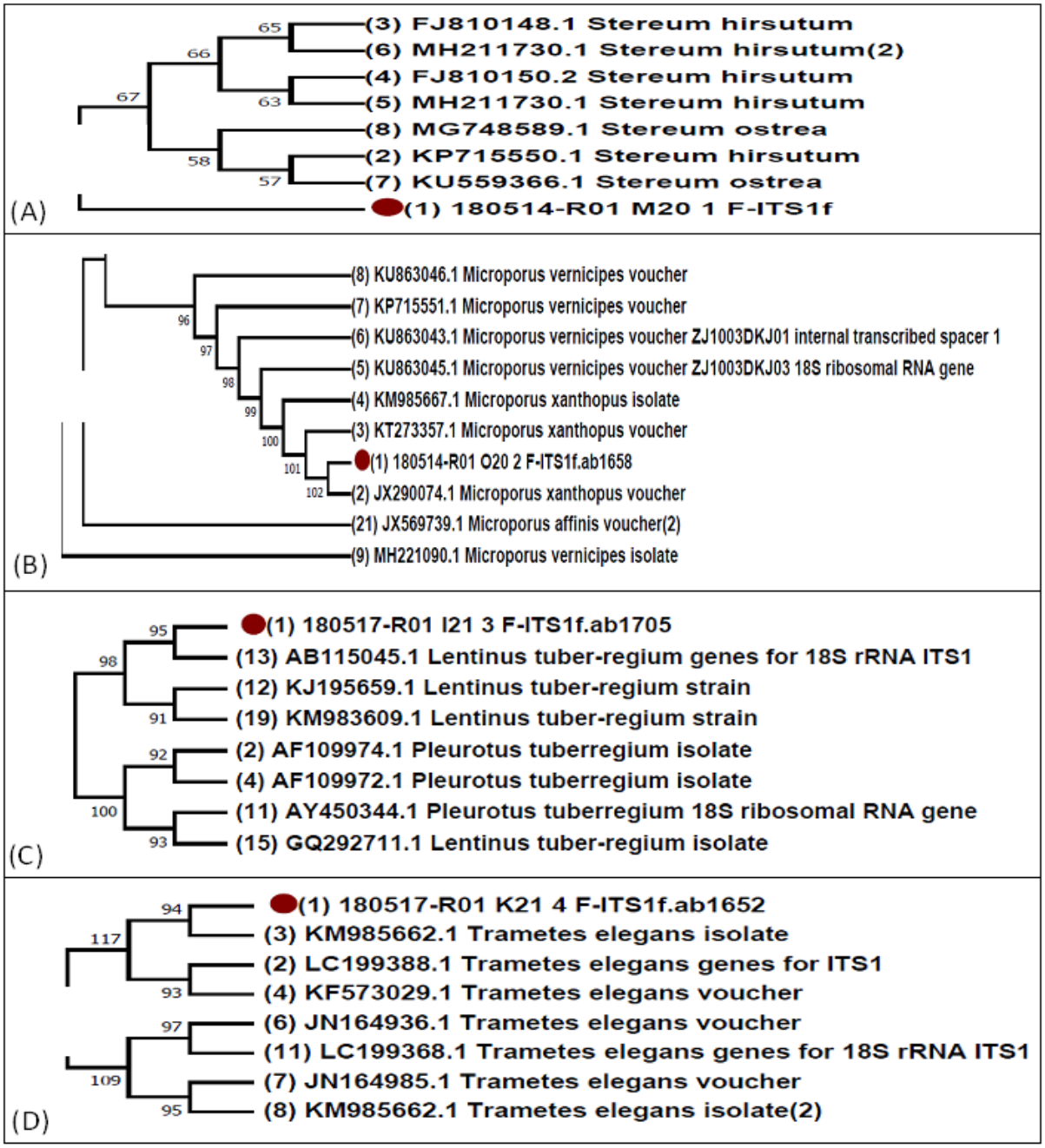

Fig. 2. Phylogenetic relationship of (A) *Stereum hirsutum*, (B) *Micropus xanthopus*, (C) *Pleurotus tuberregium* and (D) *Trametes elegans* inferred from the ITS sequence fragment.

Mushrooms are reported to have medicinal potentials. *Ganoderma lucidum*, for instance, is used to treat chronic hepatitis, hypertension, arthritis, insomnia, bronchitis, asthma, gastric ulcer, diabetes and cancer.

It possesses anti-tumor activity and has also been found to inhibit platelet aggregation and to lower blood pressure, cholesterol and blood sugar (Borchers *et al*., 2004). Moreover, *Pleurotus* species have been reported to exhibit various functional activities including anti-tumour, immuno-modulatory, anti-oxidant, anti-inflammatory, anti-hyperglycemic, and antimicrobial (Hu *et al*., 2006; Gu and Sivam, 2006; Wang *et al*., 2005; Yang *et al*., 2002; Bobek *et al*., 2001; Periasamy, 2005). *Polyporus grammocephalus* is found to have rich bioactive metabolites, and its ethanolic extract exhibits radical scavenging activity and cytotoxic effect (Aquino *et al*., 2018).

The acetonitrile and hexane extracts of *Lentinus tigrinus* and *Pleurotus djamor* exhibit radical scavenging activity and acetonitrile extracts of both mushrooms showed inhibitory activity against *Staphylococcus aureus* (Dulay *et al*., 2017).

Therefore, these four mushrooms collected in the present study could be sources of nutritious food and could exhibit functional and pharmacological properties.

Their cell lines must be rescued and domesticated in the laboratory for their cultivation towards utilization as food and potential medicine.

Conclusion

In conclusion, four wild mushrooms namely; *Stereum hirsutum*, *Micropus xanthopus*, *Pleurotus tuberregium* and *Trametes elegans* from Mt. Mingan, Nueva Ecija, Philippines were documented, collected, and successfully identified using molecular approach.

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