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Species listing, distribution, and molecular identification of macrofungi in six Aeta tribal communities in Central Luzon, Philippines

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Abstract

The species of macrofungi found in ancestral domains and resettlement areas of Aetas in three provinces of Luzon are presented in this paper. A total of 76 species of macrofungi were collected from May to October 2011. Fifty-three of the macrofungi were identified up to species level while 23 were identified up to genus level only. The macrofungi belonged to 23 taxonomic families. Some of the collected macrofungi were recorded only in either the ancestral domain (*Auricularia polytricha*) or in resettlement areas (*Ganoderma sinense* and *Pleurotus sajor-caju*). The majority of the fungi were recorded during the rainy season. Many of the collected fungi were not also utilized by the Aeta communities. This is the first comparative report of macrofungi in ancestral domains and resettlement areas of the Aetas in Central Luzon.

Key words – ancestral domain – indigenous communities – mushroom – resettlement area

Introduction

The Philippines has about 110 indigenous tribes scattered all around the country (Waddington, 2002). These indigenous tribes constitute 10-15% of the population of the country (UNDP, 2010). Among the earliest indigenous people in the Philippines are the Aetas, particularly the Aetas in Northern Philippines. According to the National Commission on Indigenous People (NCIP, 2009), the Aetas are divided into seven sub-tribes according to their local dialect and are scattered in different parts of Central Luzon. These sub-tribes are as follows: (1) Mag-Indi (Pampanga), (2) Mag-Antsi (Tarlac), (3) Zambal (Zambales) (4) Ambala (Bataan), (5) Kabayukan (Bataan), (6) Kaunana (Bataan), and (7) Magbekin or Magbukon (Bataan). In spite of modernization and contact with lowlanders, the Aetas still followed many of their social beliefs and traditional practices including their faith in their local deity, *Apu Namalyari*, whom they believed lives in Mt. Pinatubo, Zambales.

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Mt. Pinatubo played an important element in the Aetas ethnic identity. Their folklore spoke of the mountain as the abode of their local deity *Apu Namalyari*. In essence, the mountain stands as their last stronghold, the central cradle for their communities who live along its ranges. When Mt. Pinatubo erupted in June 1991, many of the Aetas were forced to evacuate and resettled in areas allotted to them by government agencies (Gobrin & Andin, 2002). However, their life in the resettlement areas was totally different from their way of life in their ancestral domain. They have encountered a lot of difficulties, like for example, in Kalangitan resettlement, the local leaders reported that the school could not hold regular classes because no teacher was willing to travel the rough and steep terrain going to their resettlement area (Gobrin & Andin, 2002). Also, they could not do the same practices they do when they were still in their ancestral domain. Nevertheless, the Aetas in these areas continued with their collection of edible plants and wild mushrooms for their food.

The number of macrofungi in Asia including the Philippines is relatively high. Mueller *et al.* (2007) estimated the species of macrofungi in tropical Asia to be between 10,000 and 25,000 species. In Burma, Thaung (2007) reported 24 orders, 56 families, 117 genera and 176 species of macrofungi. On the other hand, Swapna *et al.* (2008) reported a total of 778 species of macrofungi belonging to 43 families and 101 genera in India. Li *et al.* (2011) stated that there were 275 species of macrofungi classified into 122 genera and 52 families in China while Bolhassan *et al.* (2012) identified 60 species of macrofungi from five families in Peninsular Malaysia. In the Philippines, most taxonomic work on macrofungi focused on general descriptions of *Basidiomycota* (Musngi *et al.* 2005), though several researchers documented the different macrofungi found in many mountainous areas of the country. Daep and Cajuday (2003) studied the mushroom diversity of Mt. Malinao, Albay and documented nine species of *Tricholomataceae*, three species of *Coprinaceae*, two species of *Pluteaceae* and one species of *Auriculariaceae*. Biadnes and Tangonan (2003) assessed the basidiomycetous fungi in Mt. Apo in Mindanao and recorded 87 species representing 25 genera. In Mt. Makiling, Laguna, Quimio (1996) surveyed the *Agaricales*. Musngi *et al.* (2005) also described four species of *Auricularia* from the campus of Central Luzon State University in Muñoz, Nueva Ecija. In addition, Sibounnavong *et al.* (2008) reported 8 species of macrofungi in Puncan, Carranglan.

In the present study, we aimed to collect, characterize, identify (using morphological and molecular methods) and assess the distribution of the macrofungi that are found in the Aeta communities in both ancestral domain (AD) and resettlement area (RA). Then, we reported a checklist of macrofungi present in these six Aeta communities in Central Luzon covering the provinces of Pampanga, Tarlac, and Zambales. It is hoped that through this study, some species of edible and/or medicinal macrofungi not utilized by the Aetas can be documented for future possible utilization. Furthermore, the identities of selected macrofungi in this study were confirmed using gene sequence analysis.

Methods

The Study Site

Six sites in three provinces in Central Luzon served as our study sites. In each province, one of the study communities surveyed was the ancestral domain (AD) of the Aetas while the other site was their resettlement area (RA). Those in the resettlement areas were displaced by the eruption of Mt. Pinatubo in 1991. The study sites and the Aeta sub-tribes were as follows: (1) Floridablanca, Pampanga: Brgy. Mawacat (site 1: 14°58'26" N, 120°26'9" E, AD) and Brgy. Nabuclod (site 2: 15°1'1" N, 120°26'36" E, RA), sub-tribe Mag-indi, (2) Capas, Tarlac: Brgy. Yeyoung (site 3: 15°19'32" N, 120°25'1" E, AD) and Brgy. Kalangitan (site 4: 15°18'46" N, 120°31'11" E, RA), sub-tribe Mag-antsi, and (3) Botolan, Zambales: Brgy. Bucao (site 5: 15°15'28" N, 120°1'51" E, AD) and Brgy. Bihawo (site 6: 15°19'8" N, 120°2'46" E, RA), sub-tribe Zambal.

Collection and preservation of macrofungal specimens

All visibly present basidiomata were collected during the rainy season from the months of May to October 2011. Specimens were initially photographed in their habitat. Then, representative specimens were collected. The fruit bodies in soil and ground litter were dug carefully so as not to damage their bases. For wood-rotting mushrooms, these were cut off from the bark of trees where they were attached. All collected specimens were then labeled, wrapped in wax paper, placed in paper bags, and brought to the laboratory for identification. To preserve the specimens, fleshy mushrooms were soaked in 95% ethanol while dried mushroom specimens, particularly the polypore fungi, were air-dried and prepared as herbarium specimens. These specimens were deposited in the Center for Tropical Mushroom Research and Development (CTMRD), Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines.

Identification of collected macrofungi

Morphological Characterization – All collected macrofungi were identified based on their macroscopic (fruiting bodies) and microscopic (spore and hyphal morphologies) features. Morphometric data collected for each of the specimens were the different features of the cap, gills, and stalk of the mushrooms. A spore print was also prepared from fleshy mushrooms while tissues sectioning was done for non-fleshy mushrooms. This is to observe for microscopic features such as spore color, shape, and size. Identification was made by comparing these morphologies with published literature, *e.g*., Quimio (2001), Lodge *et al*. (2004), and Tadiosa (2011). Taxonomic classification was based on the works of Kuo (2011) and Quimio (2001). Data obtained from the study were used to prepare a checklist of the macrofungi present in the six Aeta communities. The list of macrofungal specimens were then compared with the list of macrofungi utilized by the Aeta communities as previously reported by De Leon *et al*. (2012) to determine which species were utilized or not utilized in the study areas. In this study, the occurrences of these macrofungi were also assessed in relation to their collection sites (ancestral domains, resettlement areas) and sampling months (May to October).

Molecular Identification – Three species of wild macrofungi, *Lentinus tigrinus* (ZB12MF03, ZB12MF04, ZBMF05, ZBMF06, and ZBMF07), *Lentinus squarrosulus* (ZB12MF02), and *Polyporus grammocephalus* (ZB12MF01) were selected for confirmation of their identities using molecular methods. These species were claimed as edible by the Aeta indigenous tribes, and thus, confirmation of their identities using gene sequence analysis is important prior to any mass production or *in vitro* culture. Collected fruiting bodies of *Lentinus* and *Polyporus* were initially tissue cultured on basal medium (PDA). Then, the genomic DNA of the macrofungi was extracted using modified CTAB method. Mycelia of the species of *Lentinus* and *Polyporus* grown on PDA culture plates were scrapped off and placed in 1.5 ml tube containing 600 µl CTAB buffer. With a sterilized pestle, the mycelia were ground to release the DNA, and then, incubated at 65 ^{o}C for 15 minutes in a heatblock machine. Afterwards, 600 µl chloroform:isoamyl alchohol (C:IAA 24:1) was added, mixed by inverting the tube slowly for more than 50 times, and then, centrifuge at 13,000 rpm for 15 minutes at 25 ^{o}C. The upper aqueous phase was pipetted to a new tube and to this, 300 µl cold isopropanol was added and later kept in the freezer (-20 ^{o}C) for 20 minutes. The tubes were again centrifuged at 13,000 rpm for 5 minutes at 4 ^{o}C. The supernatant was discarded and the DNA pellets were washed with 70% EtOH, and then, air dried until EtOH has totally evaporated. Finally, the DNA was diluted in 50 µl TE buffer.

The genomic DNA of the selected macrofungi were then subjected to PCR to amplify the ITS regions of the nuclear ribosomal DNA using two primers: ITS 1 (5′TCCGTAGGTGAA CCTTGCGG 3′) and ITS 4 (5′TCCTCCGCTTATTGATATGC3′) (White *et al.* 1990). The PCR reaction included 1 x PCR buffer, 2.5 µM $MgCl_2$, 200 µM dNTP, 0.5 µM of each primer, 1 U Taq DNA polymerase, and 50-100 ng extracted genomic DNA and nanopure water to make a volume of 50 µl. The PCR conditions are as follows: 94 ^{o}C for 3 min, followed by 30 cycles at 94 ^{o}C for 1 min, 52 ^{o}C for 50 sec and 72 ^{o}C for 1 min, with a final extension step of 72 ^{o}C for 10 min

(Karunarathna *et al.* 2011). The PCR products were then purified using QIAGEN purification kit following the manufacturer's instructions and the purified PCR products were sent to Macrogen, South Korea for outdoor DNA sequencing. Related gene sequences for each of the macrofungal specimens were obtained from NCBI GenBank and then, automatically aligned using ClustalW program incorporated in BioEdit v. 7.1.9 (Hall 2004). Manual sequence alignments were then performed using Bioedit to allow maximum sequence similarity. Finally, phylogenetic tree was constructed based on maximum parsimony using PAUP v. 4.0 b10 (Swofford, 2002).

Results

Species list and distribution of macrofungi in the six Aeta tribal communities

Seventy-six species of macrofungi were accounted in six Aeta communities in Central Luzon, Philippines. Of these, only 53 were identified up to the species level while 23 could only be identified up to the genus level. The collected macrofungi belonged to 23 families, 41 genera, and 76 species. A checklist of the macrofungi found in the six Aeta communities is presented below. The specimens were arranged alphabetically by their taxonomic families. Included were information about their locality, substrate, growth habit, and whether the specimen is edible or not edible. Relevant notes on some species were also reported in the checklist. The codes for each of the localities were as follows: Brgy. Mawacat (MA) and Brgy. Nabuclod (NA) in Pampanga, Brgy. Yeyoung (YE) and Brgy. Kalangitan (KA) in Tarlac, and Brgy. Bucao (BU) and Brgy. Bihawo (BI) in Zambales.

Agaricaceae

Agaricus sp.

Location: YE — Substrate: soil (grassland)
Growth habit: solitary — Edibility: not edible

Agaricus trisulphuratus (Berk.) Singer

Location: BI — Substrate: soil (termite mound)
Growth habit: solitary — Edibility: not edible

Macrolepiota procera (Scop.exFr.) Sing.

Location: NA — Substrate: soil
Growth habit: solitary — Edibility: not edible

Macrolepiota rhacodes (Vittad.) Singer.

Location: KA — Substrate: soil
Growth habit: solitary — Edibility: not edible

Macrolepiota sp.

Location: KA — Substrate: soil (grassland)
Growth habit: solitary to gregarious — Edibility: not edible

Amanitaceae

Amanita cokeri (E.-J. Gilbert & Kühner) E.-J. Gilbert

Location: KA — Substrate: soil
Growth habit: solitary — Edibility: not edible

Auriculariaceae

Auricularia auricula (Hook.) Underw.

Location: NA, YE, KA, BU, BI — Substrate: wood of living trees
Growth habit: gregarious — Edibility: edible

This species is utilized as food by the six Aeta communities as reported by De Leon *et al.* (2012).

Auricularia mesenterica (Dicks.) Pers.

Location: KA — Substrate: wood of living trees
Growth habit: gregarious — Edibility: not edible

Auricularia polytricha (Mont.) Sacc.

Location: MA, YE, BU — Substrate: wood of dead trees

Growth habit: gregarious — Edibility: edible

A. polytricha is reported as edible food by the Aeta communities living in Pampanga and Zambales (De Leon *et al*. 2012).

Auricularia tenuis (Lév.) Farl.

Location: KA — Substrate: wood of living trees

Growth habit: gregarious — Edibility: edible

Auricularia sp.

Location: MA — Substrate: wood of living trees

Growth habit: gregarious — Edibility: not edible

Bankeraceae

Phellodon niger (Fr.) P.Karst.

Location: NA, YE, BI — Substrate: wood of living trees

Growth habit: solitary — Edibility: not edible

Bolbitaceae

Agrocybe sp.

Location: KA — Substrate: soil in grassland

Growth habit: gregarious — Edibility: edible

This species of fungi grows only in the month of May and is locally known as "*kuwat mayo*" by the Aeta communities.

Panaeolus sp.

Location: YE — Substrate: carabao dung

Growth habit: Solitary — Edibility: not edible, hallucinogenic

Panaeolus papilionaceous (Bull.) Fr.

Location: YE, KA — Substrate: soil (grassland)

Growth habit: solitary to gregarious — Edibility: not edible, hallucinogenic

Cariolaceae

Hexagonia tenuis (Hook.) Fr.

Location: BU — Substrate: wood of living trees

Growth habit: solitary to gregarious — Edibility: not edible

Trametes cervina (Schwein.) Bres.

Location: BU — Substrate: decaying wood

Growth habit: gregarious — Edibility: not edible

Trametes versicolor (L.:Fr.) Pilat.

Location: NA, BU — Substrate: wood of living trees

Growth habit: solitary — Edibility: not edible

Trametes pubescens (Schum.:F.) Pilat

Location: BU — Substrate: decaying wood

Growth habit: gregarious — Edibility: not edible

Coprinaceae

Coprinus disseminatus (Pers.:Fr.) S.F. Gray

Location: MA, NA, KA, BU, BI — Substrate: wood of living trees, soil

Growth habit: gregarious — Edibility: not edible

Psathyrella sp.

Location: BU — Substrate: wood of living trees

Growth habit: gregarious — Edibility: edible

Psathyrella sp. is reported as edible food by the Aeta communities living in Zambales and it is locally known as *kuwat kunyayabi*.

Dacrymycetaceae

Dacryopinax spathularia (Schwein.) G.W. Martin

Location: NA, YE, BU, BI Substrate: decaying wood
Growth habit: gregarious Edibility: not edible

Ganodermataceae

Ganoderma applanatum (Pers.) Pat.
Location: NA, YE, BI Substrate: wood of living trees
Growth habit: solitary Edibility: medicinal
This species is not utilized as food by the six Aeta communities, however, Paterson (2006) reported it with medicinal properties.

Ganoderma lucidum (Leys.) Karst
Location: NA, YE, KA, BU, BI Substrate: wood of living trees
Growth habit: gregarious Edibility: with medicinal properties
The Aeta communities in Zambales utilized this fungus as household decoration (De Leon *et al*. 2012).

Ganoderma neo-japonicum Imazeki
Location: BU Substrate: wood of living trees
Growth habit: gregarious Edibility: medicinal
This species is not utilized as food by the six Aeta communities (De Leon *et al*. 2012). Paterson (2006) reported this species to be medicinal.

Ganoderma sinense J.D. Zhao, L.W. Hsu & X.Q. Zhang
Location: NA, BI Substrate: decaying wood
Growth habit: gregarious Edibility: not edible

Lycoperdaceae

Calvatia gigantea (Batsch ex Pers.) Lloyd
Location: YE Substrate: soil (grassland)
Growth habit: solitary to gregarious Edibility: edible
This species is not utilized as food by the five Aeta communities, however, the Aetas in Brgy. Yeyoung in Tarlac reported it as edible (De Leon *et al*. 2012).

Lycoperdon perlatum Pers.
Location: MA, BI Substrate: soil
Growth habit: gregarious Edibility: not edible

Vascellum pratense (Pers.: Pers.) Kreisel
Location: NA Substrate: soil
Growth habit: gregarious Edibility: not edible

Marasmiaceae

Marasmiellus ramealis (Bull.) Singer
Location: YE Substrate: decaying wood
Growth habit: gregarious (2-3 in group) Edibility: not edible

Marasmius androsaceus (Linn.) Fr.
Location: YE Substrate: decaying wood
Growth habit: gregarious Edibility: not edible

Marasmius rotula (Scop.) Fr.
Location: MA, KA Substrate: decaying leaves
Growth habit: gregarious Edibility: not edible

Marasmius siccus (Schweinitz) Fries.
Location: YE Substrate: decaying wood
Growth habit: gregarious Edibility: not edible

Marasmius sp. 1
Location: MA Substrate: decaying grasses
Growth habit: gregarious Edibility: not edible

Marasmius sp. 2
Location: YE Substrate: decaying wood

Growth habit: gregarious | Edibility: not edible

Marasmius sp. 3

Location: YE | Substrate: decaying wood

Growth habit: gregarious | Edibility: not edible

Marasmius sp. 4

Location: YE | Substrate: decaying leaf litter

Growth habit: gregarious | Edibility: not edible

Marasmius sp.5

Location: BU | Substrate: decaying betel nut

Growth habit: gregarious | Edibility: not edible

Nidulariaceae

Cyathus striatus (Huds.) Hoffm.

Location: YE | Substrate: wood of living trees

Growth habit: gregarious | Edibility: not edible

Sphaerobolus stellatus (Tode) Persoon

Location: YE | Substrate: carabao dung

Growth habit: gregarious | Edibility: not edible

This species is the first record of this fungus in the province of Tarlac.

Paxillaceae

Paxillus sp.

Location: BU | Substrate: soil

Growth habit: gregarious | Edibility: not edible

Phallaceae

Dictyophora duplicata (Bosc) E. Fisch.

Location: BI | Substrate: soil

Growth habit: solitary to gregarious | Edibility: not edible

Mutinus caninus (Huds.) Fr.

Location: KA | Substrate: decaying leaf litter

Growth habit: gregarious | Edibility: not edible

Phallus duplicatus Bosc

Location: YE | Substrate: soil

Growth habit: solitary to gregarious | Edibility: not edible

Phallus multicolor (Berk. & Broome) Cooke

Location: BI | Substrate: soil

Growth habit: solitary to gregarious | Edibility: not edible

Pleurotaceae

Pleurotus porrigens (Pers. ex Fr.) Kühn. & Romagn.

Location: YE | Substrate: decaying wood

Growth habit: gregarious | Edibility: edible

Pleurotus pulmunarius (Fr.) Quél.

Location: BI | Substrate: wood of living trees

Growth habit: gregarious | Edibility: edible

This mushroom species was utilized as food by the six Aeta communities, as well as by the lowlanders (De Leon *et al*., 2012).

Pleurotus sajor-caju (Fr.) Singer

Location: NA, BI | Substrate: wood of living trees

Growth habit: gregarious | Edibility: edible

Pleurotus sp. 1

Location: YE | Substrate: decaying wood

Growth habit: gregarious | Edibility: edible

This fungus is not utilized by the six Aeta communities but is utilized by the lowlanders.

Pleurotus sp. 2

Location: BU, BI — Substrate: wood of living trees

Growth habit: gregarious — Edibility: edible

This fungus is not utilized by the six Aeta communities but is utilized by the lowlanders.

Pleurotus sp. 3

Location: YE, BI — Substrate: wood of living trees

Growth habit: gregarious — Edibility: edible

This fungus is not utilized by the six Aeta communities but is utilized by the lowlanders.

Pluteaceae

Volvariella volvacea (Bull.) Singer

Location: MA, BI — Substrate: decaying trunk and leaves of banana (*Musa sapientum* L.)

Growth habit: gregarious — Edibility: edible

This fungal species is utilized as food by the six Aeta communities as well as by the lowlanders (De Leon *et al*. 2012).

Polyporaceae

Daedaleopsis confragosa (Bolt.:Fr.) Schroet.

Location: BI — Substrate: wood of living trees

Growth habit: solitary to gregarious — Edibility: not edible

Lentinus cladopus Lev.

Location: KA — Substrate: decaying wood

Growth habit: gregarious — Edibility: edible

This mushroom is not utilized as food by the six Aeta communities (De Leon *et al*. 2012). However, Labarere and Gemini (2000) reported it as food and with nutritional properties.

Lentinus sajor-caju (Fr.) Fr.

Location: BU — Substrate: living bamboo trunks

Growth habit: gregarious — Edibility: edible

This mushroom species was utilized as food by the six Aeta communities, as well as the lowlanders (De Leon *et al*. 2012).

Lentinus squarrosulus (M.) Singer*

Location: MA, BI — Substrate: decaying wood

Growth habit: gregarious — Edibility: edible

This species is utilized as food by the Aeta communities in Zambales as reported in De Leon *et al.* (2012). The identity of one specimen (ZB12MF02) of this mushroom was confirmed using gene sequence analysis.

Lentinus tigrinus (Bull.) Fr.*

Location: MA, NA, BI — Substrate: decaying wood

Growth habit: gregarious — Edibility: edible

This fungal species is utilized as food by the six Aeta communities as well as by the lowlanders (De Leon *et al*. 2012). Five specimens of this species (ZB12MF03, ZB12MF04, ZBMF05, ZBMF06, and ZBMF07) were also identified using molecular methods.

Lentinus sp. 1

Location: BU — Substrate: wood of living trees

Growth habit: gregarious — Edibility: edible

The Aeta communities in Zambales utilized this fungus as food (De Leon *et al*. 2012).

Lentinus sp. 2

Location: BU — Substrate: wood of living trees

Growth habit: gregarious — Edibility: edible

The Aeta communities in Zambales utilized this fungus as food (De Leon *et al*. 2012).

Polystictus xanthopus Fr.

Location: BI — Substrate: wood of living trees

Growth habit: gregarious Edibility: not edible

Pycnoporus sanguineus (L. ex Fr.) Murr.

Location: MA, BI Substrate: decaying wood

Growth habit: gregarious Edibility: not edible

Polyporous grammocephalus Berk.*

Location: BU, BI Substrate: decaying wood

Growth habit: gregarious Edibility: edible

The Aeta communities in Zambales utilized this fungus as food. Molecular method was used to confirm identity of one specimen (ZB12MF01) of this species.

Schizophyllaceae

Schizophyllum commune Fr.

Location: MA, NA, YE, KA, BU, BI Substrate: decaying wood

Growth habit: gregarious Edibility: edible

This fungal species is utilized as food by the Aeta communities as well as by the lowlanders (De Leon *et al*. 2012).

Sclerodermataceae

Scleroderma citrinum Persoon

Location: BI Substrate: soil

Growth habit: solitary to gregarious Edibility: not edible

Strophariaceae

Pholiota sp.

Location: MA Substrate: decaying wood

Growth habit: solitary Edibility: not edible

Stropharia semiglobata (Batsch) Quél.

Location: YE Substrate: carabao dung

Growth habit: solitary Edibility: not edible

Tricholomataceae

Collybia sp.

Location: NA Substrate: decaying wood

Growth habit: gregarious Edibility: not edible

Mycena sp. 1

Location: NA, BU Substrate: decaying leaf litter

Growth habit: gregarious Edibility: not edible

Mycena sp. 2

Location: MA Substrate: decaying grass

Growth habit: gregarious Edibility: not edible

Mycena sp. 3

Location: MA, NA Substrate: decaying grass

Growth habit: gregarious Edibility: not edible

Termitomyces clypeatus R. Heim

Location: MA, NA, KA, BI Substrate: soil

Growth habit: solitary to gregarious Edibility: edible

This fungal species is utilized as food by the six Aeta communities as well as by the lowlanders (De Leon *et al*. 2012).

Termitomyces robustus (Beli) Heim.

Location: BU Substrate: soil

Growth habit: gregarious Edibility: edible

This fungal species is utilized as food by the six Aeta communities as well as by the lowlanders (De Leon *et al*. 2012).

Termitomyces sp. 1

Location: BU Substrate: soil (termite mound)

Growth habit: gregarious Edibility: edible

This fungal species is utilized as food by the six Aeta communities as well as by the lowlanders (De Leon *et al*. 2012).

Termitomyces sp. 2

Location: BI Substrate: soil

Growth habit: gregarious Edibility: edible

This fungal species is utilized as food by the six Aeta communities as well as by the lowlanders (De Leon *et al*. 2012).

Xylariaceae

Daldinia concentrica (Bolton ex Fries) Cesati & Notaris

Location: NA, YE, KA, BU Substrate: decaying wood

Growth habit: gregarious Edibility: not edible

The number of macrofungi per taxonomic family was also assessed in each of the six Aeta communities (Table 1). Of the 76 species collected, 15 were reported from Brgy. Mawacat (AD) while 20 species were reported in Brgy. Nabuclod (RA) in Pampanga. Twenty six were recorded in Brgy. Yeyoung (AD) while only 15 were noted in Brgy. Kalangitan (RA) in Tarlac. In Zambales, 23 and 25 species of macrofungi were found in Brgy. Bucao (AD) and Brgy. Bihawo (RA), respectively.

We also evaluated whether the collected macrofungal species were found only in the ancestral domain (AD) or in resettlement area (RA). Many of our collected fungi were found in both AD and RA (Table 1). For example, *Coprinus disseminatus*, *Lentinus tigrinus*, *Mycena* sp. 3, *Scutellinia scutellata,* and *Termitomyces clypeatus* were recorded in Pampanga. *Panaeolus papilionaceous* and *Daldinia concentrica* were recorded in Tarlac while *Dacryopinax spathularia*, *Pleurotus* sp. 2, *Lentinus* sp. 1, and *Polyporous grammocephalus* were recorded in Zambales. Several species were recoded in both AD and RA of two or three provinces: *Coprinus disseminatus* in Pampanga and Zambales, *Auricularia auricula* and *Ganoderma lucidum* in Tarlac and Zambales, and *Schizophyllum commune* in Pampanga, Tarlac, and Zambales. We also observed species found only in specific Aeta communities. *Auricularia polytricha* was found in AD in the three provinces while *Agaricus trisulphuratus, Macrolepiota procera, Macrolepiota rhacodes, Macrolepiota* sp., *Amanita cokeri, Auricularia mesenterica, Ganoderma sinense, Vascellum pratense, Dictyophora duplicata, Mutinus caninus, Phallus multicolor, Pleurotus pulmunarius, Pleurotus sajor-caju, Daedaleopsis confragosa, Lentinus cladopus, Polystictus xanthopus, Scleroderma citrinum, Collybia* sp.*, Mycena* sp. 2, and *Termitomyces* sp. 3 were collected only in the RA.

In the present study, the number of collected macrofungi in each of the sampling months from May to October was also evaluated. We wish to see which month when most of the fungi occurred and correlated it with the utilization of the Aetas. Our results showed that more species of macrofungi were observed during the later part of the rainy season, *i.e.,* from the months of August to October (Table 2).

Molecular phylogeny of selected macrofungi

ITS sequence data of seven wild, edible macrofungi were analyzed. PCR amplifications yielded ca. 740bp of ITS rDNA. Maximum parsimony analysis resulted in 16 most parsimonius trees. These trees were 100 steps and had a consistency index (CI) of 0.717 and a retention index (RI) of 0.744. One of the most parsimonius trees is shown in Fig. 1. *Pleurotus ostreatus* was designated as an outgroup. Phylogenetic analyses showed that *L. tigrinus* (ZB12MF03) had a 93% bootstrap support with *L. tigrinus* (ZB12MF07). On the other hand, *L. squarrosulus* (ZB12MF02) had a 71% bootstrap support with *L. tigrinus* (ZB12MF04). *Polyporus grammocephalus* also had a lower bootstrap value (68%) with *P. grammocephalus* sequences obtained from GenBank.

Macrofungal species utilized and not utilized by the Aeta communities

Comparison of the macrofungi found in the Aeta communities in Pampanga, Tarlac and Zambales showed that there were generally more species of macrofungi that were not utilized by the Aetas (Table 3). These species were particularly recorded in the early part of the rainy season, *i.e.,* from months of May until July. We then looked closely at the species recorded in the study sites and assessed whether these were utilized by other communities in Central Luzon, e.g. by lowlanders.

Discussion

All macrofungi in the ancestral domains and resettlement areas in six Aeta tribal communities in Central Luzon, Philippines were accounted in this study. Of the macrofungal species collected, 64 were reported from Brgy. Mawacat, Brgy. Yeyoung, and Brgy. Bucao, all were located in the ancestral domains, while 60 species were reported in resettlement areas in Brgy. Nabuclod, Brgy. Kalangitan, and Brgy. Bihawo (Table 1). It is interesting to note that more species of macrofungi were recorded in the ancestral domain. This is understandable since most macrofungi have mycorrhizal associations needed for their growth and this could only be achieved in the areas with abundant trees. Also, many factors are important for the efficient growth of the macrofungi such as humidity, temperature, soil type which are found most likely in the forested areas. More macrofungal species was reported from the family Polyporaceae (Table 1).

Tadiosa *et al.* (2011) also reported higher number of Polyporaceae in the province of Aurora. Our finding is not surprising since these species are known wood-inhabiting fungi. Their commonality in the area is expected since the communities where the Aetas live are in mountainous areas where trees are abundant. In addition, their abundance in these areas could be attributed to their role in nature, *i.e.*, as wood decomposers. However, these fungal species also caused economic losses as they are responsible for wood decay in standing trees, logs, and timbers (Bolhassan *et al*. 2012).

The collected macrofungal species were also evaluated whether these were found only in the ancestral domain (AD) or in resettlement area (RA). Eleven species of our collected macrofungi were found in both AD and RA (Table 1). Four species were recorded in both AD and RA of two or three provinces. We also observed species found only in specific Aeta communities. One species, *Auricularia polytricha,* was found in AD in the three provinces while 20 were collected only in the RA (Table 1). A study by Tayamen *et al.* (2004) also reported *Auricularia polytricha, Schizophyllum commune, Volvariella volvacea, Coprinus* sp., *Mycena* sp. and *Termitomyces* sp. in Mt. Nagpale in Abucay, Bataan. This area is also home to some tribes of Aetas. Unfortunately, no other studies were reported on the macrofungi found in AD or RA of other indigenous tribes so comparisons cannot be made.

Although the classification and identification of macrofungi is traditionally based on morphological characteristics, recently, gene sequences, e.g., parts of the nuclear ribosomal DNA locus, have been extensively used as essential phylogenetic and taxonomic tools (Hibbett *et al.* 2007). For example, the phylogenetic relationships of *Amanita* based on ribosomal DNA sequences from both the internal transcribed spacer (ITS) region and the large subunit (nLSU) of nuclear ribosomal DNA, were previously studied to shed new light on the taxonomy and biogeography of species of *Amanita* in eastern Asia (Zhang *et al*. 2004). Singh *et al.* (2006) also characterized eighteen mushrooms using DNA fingerprinting and ribosomal rRNA gene sequencing and reported their genetic diversity. Furthermore, Hyeon-Su *et al.* (2007) studied the diversity of *Pleurotus eryngii* using RAPD and its sequence analysis, and observed that grouping based on physiological parameters was closely related to RAPD based grouping. Stajic *et al.* (2005) used randomly amplified polymorphic DNA technique to asses the genetic diversity among 37 *Pleurotus* species. In another study, RAPD- polymerase chain reaction (PCR) amplification was used to

Table 1 Number of macrofungi reported per family in the ancestral domain (AD) and resettlement areas (RA) of the six Aeta communities.

Family	Pampanga		Tarlac		Zambales		Total
	Brgy. Mawacat (AD)	Brgy. Nabuclod (RA)	Brgy. Yeyoung (AD)	Brgy. Kalangitan (RA)	Brgy. Bucao (AD)	Brgy. Bihawo (RA)	
Agaricaceae	0	1	1	2	0	1	**5**
Amanitaceae	0	0	0	1	0	0	**1**
Auriculariaceae	2	1	3	2	2	1	**11**
Bankeraceae	0	1	1	0	0	1	**3**
Bolbitaceae	0	0	2	2	0	0	**4**
Cariolaceae	0	1	0	0	4	0	**5**
Coprinacea	1	1	0	1	2	1	**6**
Dacrymycetaceae	0	1	1	0	1	1	**4**
Ganodermataceae	0	3	2	1	2	3	**11**
Lycoperdaceae	1	1	1	0	0	0	**3**
Marasmiaceae	2	0	6	1	1	0	**10**
Nidulariaceae	0	0	2	0	0	0	**2**
Paxillaceae	0	0	0	0	1	0	**1**
Phallaceae	0	0	1	1	0	2	**4**
Pleurotaceae	0	1	3	0	1	4	**7**
Pluteaceae	1	0	0	0	0	1	**2**
Polyporaceae	3	1	0	1	4	6	**15**
Pyronemataceae	1	1	0	0	0	0	**2**
Schizophyllaceae	1	1	1	1	1	1	**6**
Sclerodermataceae	0	0	0	0	0	1	**1**
Strophariacea	1	0	1	0	0	0	**2**
Tricholomataceae	2	5	0	1	3	2	**13**
Xylariaceae	0	1	1	1	1	0	**4**
Total	**15**	**20**	**26**	**15**	**23**	**25**	**124**

Table 2 Number of macrofungi reported per family in each of the collection months.

Family	May	Jun	Jul	Aug	Sept	Oct	Total
Agaricaceae	4	0	0	1	1	0	**6**
Amanitaceae	0	1	0	0	0	0	**1**
Auriculariaceae	3	3	1	3	3	1	**14**
Bankeraceae	1	0	0	1	1	1	**4**
Bolbitaceae	1	0	0	1	1	0	**3**
Cariolaceae	0	1	0	1	2	2	**6**
Coprinacea	1	1	1	1	0	1	**5**
Dacrymycetaceae	0	1	1	1	1	1	**5**
Ganodermataceae	0	2	2	2	3	3	**12**
Lycoperdaceae	3	0	0	0	0	0	**3**
Marasmiaceae	0	2	0	3	4	1	**10**
Nidulariaceae	0	2	0	0	0	0	**2**
Paxillaceae	0	0	1	0	0	0	**1**
Phallaceae	0	0	2	0	1	1	**4**
Pleurotaceae	1	2	1	3	4	2	**13**
Pluteaceae	0	0	1	1	0	0	**2**
Polyporaceae	1	3	2	2	3	4	**15**
Pyronemataceae	0	0	1	0	1	1	**3**
Schizophyllaceae	1	1	1	1	1	1	**6**
Sclerodermataceae	0	1	0	0	0	0	**1**
Strophariacea	0	0	0	1	0	1	**2**
Tricholomataceae	1	1	1	4	2	3	**12**
Xylariaceae	0	0	1	1	0	1	**3**
Total	**17**	**21**	**16**	**27**	**28**	**24**	**133**

evaluate the genetic diversity among 45 *Pleurotus* strains and found that this technique was better than morphological analysis in assessing their genetic diversity. In this study, phylogenetic analyses were also done on some species of wild, edible macrofungi. It is necessary to confirm the identity of the macrofungi prior to their *in vitro* culture for mass production of their fruiting bodies. The species of *Lentinus* and *Polyporus* sequenced in this paper were reported edible and consumed by the Aeta tribes. Results showed close phylogenetic relationships between the specimens of *L. tigrinus* and *L. squarrosulus,* and thus, could not fully be resolved. This was in contrast with their morphological characterization which identified the specimens belonging to these two distinct species. Bruns (2001) noted that the ITS region was very variable and not the best region to use for phylogenetic inference on macrofungi. A multi-gene phylogeny of *Lentinus* species is needed and should be based on a sampling of a wide range of species (Karunarathna *et al.* 2011). However, a close relationship has long been observed between *Lentinus* and other polypores (Corner 1981; Pegler 1983; Singer 1986). *Lentinus* has been grouped under the family Polyporaceae based on the presence of dimitic and amphimitic hyphal systems (Moser 1978; Kühner 1980; Pegler 1983; Singer 1986). Moreover, hyphal pegs, fascicles of sterile hyphae coming out from the hymenium surface, are some of the common features present in some genera of the Polyporaceae and in *Lentinus* subg. *Lentinus* (Pegler 1983; Corner 1981).

The collected specimen identified as *P. grammocephalus* in this study grouped with obtained sequences of *P. grammocephalus* species, and thereby, confirming its identity. However, the phylogenetic position and relationship of *Polyporus* were not clear so far. Many mycologists included it in the order Aphyllophorales as it has gymnocarpic basidiocarp development and complex hypal systems (Ryvarden & Gilbertson, 1994). On the other hand, Corner (1984) and Singer (1986) preferred to place it in the order Agaricales due to the similarities of *Polyporus* and gilled agaric genera *Lentinus, Panus,* and *Pleurotus* in basidiocarp consistency. We proposed that a more detailed study on the phylogenetic relationship of different species of *Lentinus* and *Polyporus* will be done based on multi-loci gene sequence analysis in order to confirm their taxonomic position.

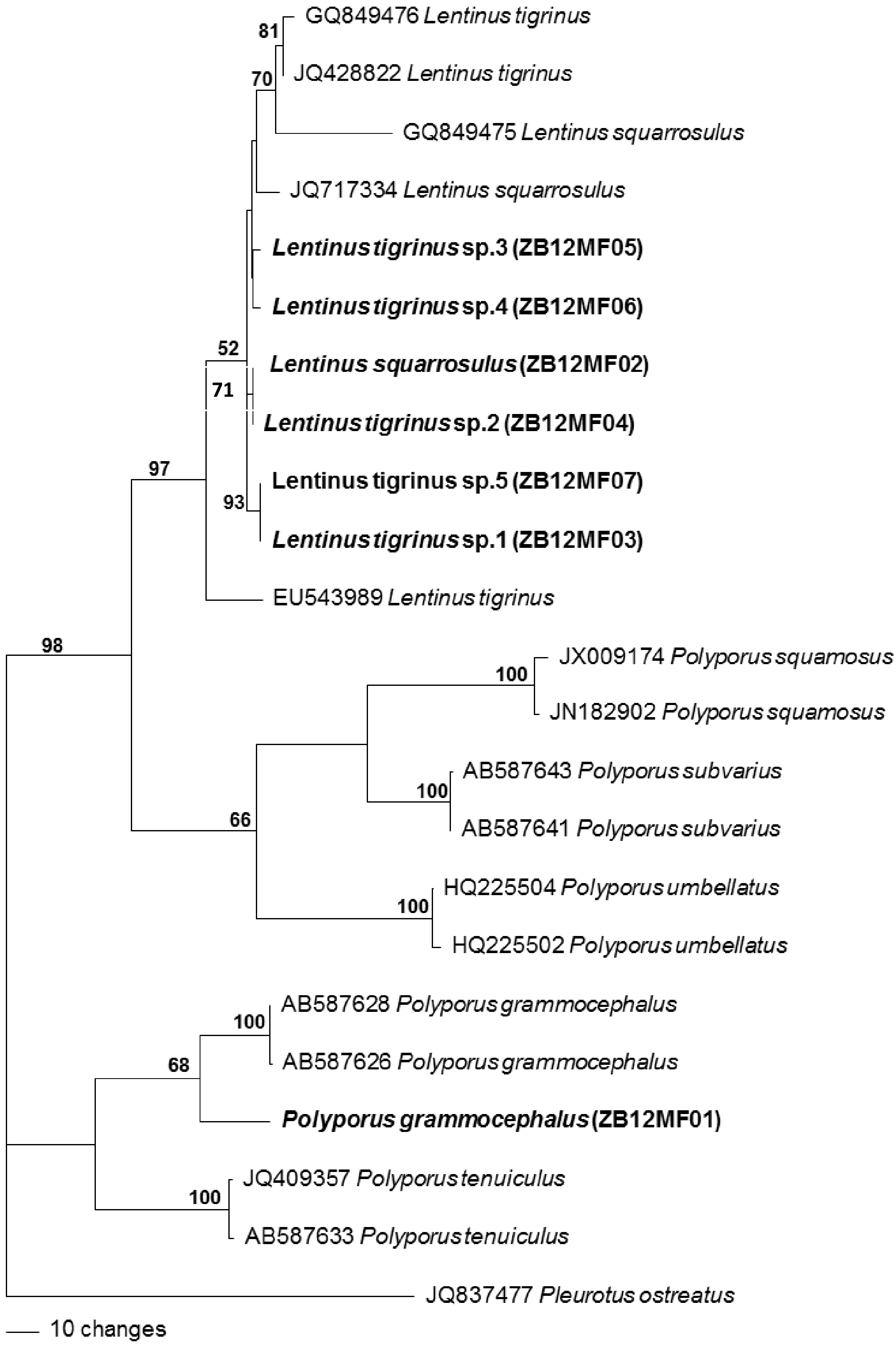

Fig. 1 – Phylogenetic tree based on maximum parsimony showing the relationship of *Lentinus* species and *Polyporus* species.

Table 3 Number of mushrooms utilized and not utilized by the Aeta communities in Central Luzon, Northern Philippines.

Sites		May	Jun	Jul	Aug	Sept	Oct	Total
Pampanga	Utilized	3	2	1	5	6	3	**20**
	Not Utilized	6	3	4	4	4	5	**26**
	Total	**9**	**5**	**5**	**9**	**10**	**8**	**46**
Tarlac	Utilized	5	1	1	2	4	1	**14**
	Not Utilized	3	8	3	8	11	2	**35**
	Total	**8**	**9**	**4**	**10**	**15**	**3**	**49**
Zambales	Utilized	ND[a]	6	4	7	6	6	**29**
	Not Utilized	ND[a]	4	6	6	4	15	**35**
	Total	**ND[a]**	**10**	**10**	**13**	**10**	**21**	**64**

[a] ND = no data obtained

Indigenous people are known to utilize macrofungi for various purposes, *i.e.*, as food and medicine, as materials for their societal rituals and traditional practices, and to some extent, as household decoration. Recently, De Leon *et al.* (2012) reported the utilization of 38 macrofungi by the Aetas based on survey questionnaires and interviews of community elders. *Auricularia auricula*, *A. polytricha*, *Calvatia* sp., *Lentinus tigrinus*, *L. sajor-caju*, *Mycena* sp., *Pleurotus* sp., *Schizophyllum commune*, *Termitomyces clypeatus*, *T. robustus*, *Termitomyces* sp. 1, *Termitomyces* sp. 2, and *Volvariella volvacea* were all used as food while *Ganoderma lucidum* was used as a household decoration. Comparing the collected macrofungi with this previously presented paper (De Leon *et al*. 2012), many more fungi were recorded in this study from these Aeta communities. Comparison of the macrofungi found in the Aeta communities in Pampanga, Tarlac and Zambales showed that there were generally more species of macrofungi that were not utilized by the Aetas (Table 3). These species were particularly recorded in the early part of the rainy season, *i.e.*, from months of May until July. Note though that many of the edible mushrooms reported in De Leon *et al.* (2012) were collected during the later months of the rainy season (August to October). We then looked closely at the species recorded in the study sites and assessed whether these were utilized by other communities in Central Luzon, e.g. by lowlanders. It is interesting to note that one edible species of mushroom, *Lentinus cladopus*, was not utilized as food by the Aetas. Labarere and Gemini (2000) reported this species as edible and with nutritional properties. Two species of macrofungi, *Ganoderma applanatum* and *Ganoderma neo-japonicum*, were also used as medicines (Paterson, 2006), though the Aetas again do not utilize these fungi. Since we do not wish to influence or change the beliefs of our indigenous tribes, perhaps, on their own time in the future, the Aetas will consider using these fungi.

In summary, seventy six species of macrofungi were accounted in the six Aeta communities in Central Luzon. These macrofungi belonged to 23 families and 41 genera. Majority of the collected macrofungi were identified as belonging to family Polyporaceae. Some species were recorded only in either of the ancestral domains (AD) or resettlement areas (RA). However, there were macrofungi found in both AD and RA. Majority of the macrofungi were also recorded during the late rainy season. It can be noted though that many of the species of mushrooms found in the areas were not utilized by the Aeta communities. Three species, in particular, were reported to be either edible or with medicinal properties but were not used as such by the Aeta communities.

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