

# Ethnomycological survey and molecular identification of Macrofungi utilized by Bicolano community in Camarines Sur, Southern Luzon, Philippines

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## ABSTRACT

In the Philippines, mushrooms are one of the most important foods of the local communities situated near the mountainous region. However, they lack knowledge on how they fully benefit from these important resources found in the area. In this study, the survey and documentation on the traditional knowledge of Bicolanos on the utilization of mushroom species was conducted. Qualitative survey was done using interviews with the help of survey questionnaires for the documentation of data and followed by collection of mushroom species using purposive sampling. The collected mushrooms were placed in the sterile properly labelled brown paper bag for molecular identification and for germplasm preservation. The results showed that there were seven mushroom species utilized mainly as food by the Bicolanos. During the collection, five species were collected and were initially identified using its morphology and verified using molecular approach. The collected mushroom PCO8 has 100% sequence similarity to *Schizophyllum commune* (MK732122.1), the mushroom sample PC01 has 99.59% sequence similarity to *Auricularia polytricha* (MN523226.1), the mushroom sample PC04 has 99.09% sequence similarity to *Coprinopsis cinerea* (KX468975.1), the mushroom sample PC05 has 99.84% sequence similarity to *Pleurotus djamor* (KY328723.1) and the mushroom sample PC16 has 100% sequence similarity to *Volvariella volvacea* (MG280838.1). People in the community still practice the traditional beliefs and knowledge acquired from their ancestors on mushroom utilization and cultivation. These practices left by their ancestors were traditionally passed on verbally from generation to generation.

**Key words :** *Bicolano community, Molecular identification, Mushroom utilization, Traditional knowledge, Ethnomycological survey*

## Introduction

Various indigenous people in the Philippines are known for their utilization of mushroom species as

food and medicine. The Bicolanos are a large community situated in Mt. Isarog and is located in Southern Luzon, a region comprising part of the Bicol Peninsula in the Philippines. During the 20<sup>th</sup>

century, they are the fifth largest ethnolinguistic group in the Philippines numbering about 4,070,000. Earlier to Spanish colonization, the Bicolano people believed in an indigenous pantheon of deities. These deities were honored in feasts and everyday ways of life (NCCA, 2021).

Mushrooms are valuable assets for the welfare of humans as they have tremendous medicinal food, drugs and mineral values (Wani *et al.*, 2010). These have been incorporated into health tonics, tinctures, teas, soups, and healthful food dishes, as well as herbal formulas (Chang, 1996). They contain numerous substances including glycoproteins, glyconutrients, lectins, among others. They also contain compounds that have been classified under the Host Defense Potentiators (HDP) which can have immune system enhancement properties, which is one of the explanations that are currently used as adjuncts to cancer treatments in Japan and China (Prasad *et al.*, 2015). Currently, an increasing number of researchers within the Philippines are paying particular attention to the medicinal benefits of mushrooms.

Many indigenous people within the Philippines are known for their utilization of various mushroom species: Aeta communities (De Leon *et al.*, 2016), Ayta in Bataan (Tantengco and Ragrario, 2018) and Ifugao community (De Leon *et al.*, 2018). However, reports on the utilization of the ethnic communities (Ilocano, Kapangpangan, Bicolano etc.) are scarce. Wild edible mushrooms are an important source of food for the local people in Southern Luzon, Philippines. To date, there is still no documentation and report on the use of Bicolanos on mushroom species present in Camarines Sur situated in Southern Luzon, Philippines. The ethnic community lacks knowledge on how they will fully benefit from these important resources. Thus, the study was conducted to document the use of mushrooms, their utilization and the traditional knowledge of Bicolanos. In addition, preservation efforts of these mushroom species collected in their area were initiated for future research such as assessment of its medicinal potential.

## Materials and Methods

### Ethnomycological survey and collection

The survey and collection were conducted in Barangay Binanuaanan, in the municipality of Pili, Camarines Sur in Southern Luzon, Philippines (Fig-

ure 1). The collection site was selected based on the presence of ethnic communities in the area.

A qualitative survey was done with seventeen respondents. The actual interview with the community was conducted with questionnaires adopted elsewhere (De Leon *et al.*, 2012), with minor modifications to suit this study. It was a scheme in the collection of information of traditional knowledge and the importance of mushroom species in their daily lives. The purposive collection was done with the assistance of some environmental officers in the area. The mushrooms utilized by the community were collected and photographed in their habitat and properly labelled and then placed in a brown paper bag to avoid excess humidity. All important information in the collection was recorded. For fleshy mushroom, it was carefully dug up to its base while bark scraping was done to the bracket mushroom. The collected samples were submerged into CTAB and transported in the laboratory for molecular identification and germplasm preservation.

### Molecular identification of mushroom species utilized by the Bicolanos

The genomic DNA was extracted from the collected fruiting bodies and using the cetyltrimethylammonium bromide (CTAB) method. About 5-10 mg of fruiting body samples were homogenized using sterilized mortar and pestle with CTAB buffer. After grinding, samples were added with 750  $\mu$ l of pre-warmed 2x CTAB buffer and 50  $\mu$ l of 20% SDS was also added prior to incubation at 65 °C for 45 min. After the incubation, samples were briefly cooled down before the addition of 750  $\mu$ l chloroform-isoamyl (24:1) alcohol and was spun in

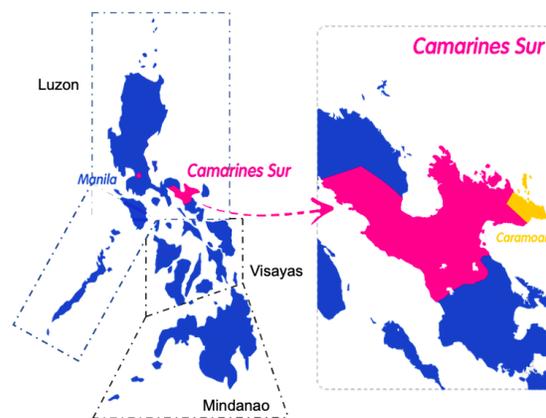


Fig. 1. Map of the Philippine island, showing the Camarines Sur in Southern Luzon.

a centrifuge at 10,000 rpm for 30 min. Samples were incubated at -20 °C overnight after the addition of 600 µl ice-cold isopropanol for the precipitation of DNA. To get the DNA, samples were spun in a centrifuge at 10,000 rpm for 10 min. Pelleted samples were then washed twice with 70% ethanol, air-dried and then dissolved in 100 µl TE buffer. A 2µl stock DNA mixed with 1 µl loading dye was loaded into 1% agarose gel then run in the Enduro gel electrophoresis system at 100 V for 30 min for DNA quality checking.

The ITS gene fragment was amplified forward primer ITS and reverse primer ITS4 (White et al., 1990). The amplified PCR product was verified in agarose gel and was sent to First Base Laboratory in Malaysia for PCR purification and sequencing.

**Data Analysis**

The data on the socio-demographic profile were arranged and tabulated in order to provide basic information regarding the Bicolano communities. All the information gathered in the questionnaires was determined, correlated and compared with the collected specimens.

**Results and Discussion**

**Socio-demographic profile**

The respondents are mostly 46 years old and above or 64.71%. The survey was conducted in the middle of the day wherein the males are out for work, thus most of the respondents are females (64.71%) this is similar to the interview done to the Gaddang community in Nueva Vizcaya (Lazo et al., 2015). The religion of the respondents is mainly Catholic with 94.12%, while 64.71% are married. All the respondents have blue-collar jobs consisting of: farmers, vendors, local community worker, carpenter, butcher and technician which has an average annual income ranging from Php 20,000 to 40,000 and Php 50,000 to 70,000 with 5 to 8 family members in the

family (Table 1).

Most of the respondents did not finish college due to poverty and resulted in having no stable job. On the other hand, the Philippine Statistics Authority (PSA, 2018) reported that a family of five need no less than PhP 7,337.00 which is the amount for food threshold to meet the family’s basic food needs for a month. On the other hand, not less than PhP 10,481 on average was needed to meet both basic food and non-food needs. Thus, they are living below the poverty line nevertheless most of them reached high school level in their education.

**Traditional Knowledge on mushroom utilization**

Bicolano generally called mushroom “tubo” and “kabute”. Other indigenous groups in the Philippines such as Aeta in Central Luzon called it “kuwat” (De Leon et al., 2012), “tarulok” and “uong” by the Gaddang community (Lazo et al., 2015) and “bagel” and “buo” by Kalanguya tribe (De Leon et al., 2016) and utilized it mainly as food. Matilla et al., (2001) stated that the higher fungi have been used as a source of food thousands of years ago due to their chemical composition which is attractive from the nutrition point of view. All of the respondents stated that mushrooms appear during the rainy season in decaying logs, soil and dried leaves (Table 2). However, few of them believed that mushrooms also appeared during cold months.

In collecting mushroom, they identified the edible mushroom based on the color, absence of ring and substrate where it grows and provided local names to the edible mushroom for the transfer of knowledge to the next generation which is a bit similar to the *Benna* and *Hehe* ethnic groups in Tanzania (Chelela et al., 2014).

Bicolano utilized mushrooms mostly as food. They sautéed mushrooms with other vegetables, similar to the way of cooking of the Aeta and Kalanguya tribe (De Leon et al., 2016; De Leon et al., 2012). Moreover, the Ayta community in Bataan sautéed mushrooms with garlic, onion and eaten

**Table 1.** Socio demographic profile of Bicolano community in Southern Luzon, Philippines (n=17).

Age			Sex		Educational Attainment					Religion		Job		Annual income		No. of family members				
12-25	26-45	46 >	M	F	EL	EG	HL	HG	V	RC	DD	BC	WC	50-70K	20-40K	NA	2-4	5-8	9-11	NA
2	4	11	6	11	1	5	5	4	1	16	1	17	0	8	8	1	4	7	1	2

M- Male, F- Female, EL- Elementary Level, EG- Elementary Graduate, HL- High School Level, HG- Highschool Graduate, V-Vocational, RC- Roman Catholic, DD- Dating daan, BC- Blue collar, WC-White collar, NA- No Answer

with rice<sup>6</sup>. Another Bicolanos way of cooking mushrooms was boiled with coconut milk and seasoned with salt and pepper. This is expected since Bicolanos are known for their dishes with coconut milk. In India, the *Kaani* tribe in the Kanyakumari district also cooked mushrooms by boiling and adding grated coconut and seasoned with spices, salt and green chilies to add flavour and aroma (Davidson *et al.*, 2012). Moreover, the Khasi tribe cooked mushrooms by frying, together with salt, butter, black pepper, chilli and a little garlic paste (Khaund and Joshi, 2013) while in Cameroon the indigenous people of Kilum-Ijim mountain forest consumed mushroom as raw, boiled with water and stir-fried (Teke *et al.*, 2018). Another interesting way of mushroom cuisine by the male Bicolano in the community was boiled, added with calamansi extract, onion and garlic then served as “pulutan” an appetizer taken with alcoholic drinks.

**Indigenous beliefs and practices on mushroom cultivation and utilization**

All respondents recognized mushroom as food and stated seven edible species (Table 3). During the collection, only five documented edible mushrooms were collected during the collection period (October 2019) (Figure 2). Interestingly, these mushrooms are also utilized as food in India (Khaund and Joshi, 2013), Nigeria (Okhuoya *et al.*, 2018), Nepal (Aryal and Budathoki, 2013) and Cameroon (Teke *et al.*, 2018). In the study of Shin *et al.* (2007), *Schizophyllum*

*commune* and, *Volvariella* sp., are some of the edible wild mushrooms consumed by the natives of Sabah in Malaysia. Additionally, within the Philippines, reported that some indigenous people in the Philippines utilized these mushroom species as food as well (De Leon *et al.*, 2016; Tantengco and Ragrario, 2018; De Leon *et al.*, 2018; De Leon *et al.*, 2012; Lazo *et al.*, 2015). Moreover, male in their community stated that kabuteng saging (*Volvariella volvacea*) was utilized as an appetizer that matches with alcoholic beverages. In China, Chang *et al.* (2018), processed a new ready-to-eat spicy *V. volvacea*. A total of 27 volatile components were detected in fresh *V. volvacea*, and 24 volatile flavor components in the processed product. This product of *V. volvacea* was characterized by moderate saltiness and strong umami taste.

Indigenous beliefs are fundamental to those of the indigenous group in the Philippines. Their knowledge was inherited from their great ancestors through verbal statements (Balangcod and Balangcod, 2009). The respondents said they are still practising the indigenous beliefs left by their ancestors and was traditionally passed on to the next generation verbally. In the mushroom collection, they refused to collect those with rings on its stipe, yellow and green external color, mushrooms with insects on its cap and those that grow in animal dung because they believed that these are all poisonous mushrooms and can give dizziness to anyone who attempts collecting it. In addition to this, they ask

**Table 2.** Practical knowledge of the respondents on mushrooms (n=17).

Do you know mushrooms?		Local term for mushroom?		When do mushrooms appear?			Where do mushrooms appear?		
Yes	No	Tubo	Kabute it's raining	When it's hot	When it's cold	When logs	Decaying	Soil leaves	Dried
17	0	17	17	17	0	3	17	17	17

**Table 3.** Indigenous beliefs of the respondents on mushroom utilization and cultivation of Bicolanos in Camarines Sur in Southern Luzon, Philippines

Indigenous knowledge about mushrooms?		Rituals before collecting mushrooms?		Rituals performed		Did you know that mushrooms can be cultivated?	How do you utilize mushrooms?		
Yes	No	Yes	No	Asking permission to the hiding spirits	None	Yes	No	Food	Medicine
14	3	12	5	12	5	5	12	17	1

permission from the hiding spirits by saying “*tabi-tabi po*” before the collection, for the certainty that mushrooms are sometimes surrounded by dwarfs and spirits. This belief was also exercised by the Aeta communities in selected areas of Central Luzon (De Leon *et al.*, 2012).

Most of the respondents lack ideas about mushroom cultivation (Table 3). Still, some of them practiced the cultivation they learned from their ancestors. They grow mushrooms by decaying the base of a sugar cane after cropping, logs and corn cob, after several days Kuratding (*S. commune*) will grow in decayed sugar cane and logs while kabuteng mais (*Coprinopsis cinerea*) appeared in a corn cob and kabuteng saging (*V. volvacea*) in dried banana leaves. A study by Belewu and Belewu, (2005) and Obodai *et al.* (2003), revealed the potential use of banana leaves as a good substrate for the cultivation of *V. volvacea*. Bicolano also named the mushroom according to their appearances such as *Taingang daga* named from its similarity to rodent’s ear and the substrate where it grows. For instance, Kabuteng mais grows on corn cob, Kabuteng kawayan on Bamboo stem and decayed logs, Kabuteng saging on dried banana leaves, Kabuteng punso on termite/ant hill and Kabuteng dayami on rice straw this is similar to the Ayta communities in Bataan. They usually named the mushrooms based on the substrates where the mushrooms were actually found (Tantengco and Ragrario, 2018). An example is *V. volvacea* which is locally known as “kwat-saging” because it grows in decomposing banana pseudostem and leaves, “Kwat kawayan” was the local names of *Schizophyllum commune* and *Stereum* sp. and they also named based on their morphological characteristics such as the *Auricularia auricula-judae* and *Auricularia polytricha* which is locally

known as “tengang-daga” because of its similarity in the ears of rodents.

Another indigenous cultivation of *kabuteng mais* performed is the decomposition of corn cob tossed with ashes on top. The cultivation of white-rot edible fungus using corn cob as a basal substrate (Naraian *et al.*, 2009). In addition, oyster mushroom (*Pleurotus pulmonarius*) was successfully cultivated resulting from supplemented corn cob (Stanley *et al.*, 2011). Thus, the corncob is considered as one of the good substrates in mushroom cultivation. All of the respondents recognized mushrooms as food, and one stated that mushrooms can also be medicinal.

#### Identification of mushroom species utilized by Bicolanos

The identity of mushroom samples that were utilized by the Bicolano’s and collected during the study was resolved, the geographical coordinates where the mushrooms collected was also provided (Table 4).

The collected mushroom PC01 locally known as Taingang daga was found to have 99.59% sequence similarity with *Auricularia polytricha* (MN523226.1). The PC04 with local name Kabuteng mais has 99.09% sequence similarity with *Coprinopsis cinerea* (KX468975.1), while PC05 sample with local name Kabuteng kawayan has 99.84% sequence similarity with *Pleurotus djamor* (KY328723.1). The PC08 sample known as Kuratding has 100% sequence similarity to *Schizophyllum commune* (MK732122.1).

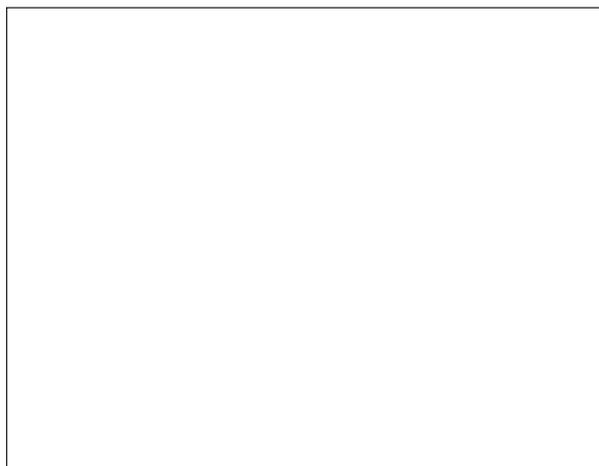
#### Conclusion

Mushroom is a vital resource for the Bicolano as it serves mainly as food. Five mushrooms out of seven (7) utilized by the Bicolanos as foods were collected

**Table 4.** Molecular identification of collected mushrooms utilized by Bicolano using the ITS gene sequence and its geographical coordinates.

Local names	Code	Scientific name	Percentage similarity	Accession number	Geographical coordinates	
					Latitude	Longitude
Taingang daga	PC01	<i>Auricularia polytricha</i>	99.59%	MN523226	13°37'01.2"	123°18'53.1"
Kabuteng mais	PC04	<i>Coprinopsis cinerea</i>	99.09%	KX468975	13°37'03.1"	123°18'55.7"
Kabuteng kawayan	PC05	<i>Pleurotus djamor</i>	99.84%	KY328723	13°37'01.5"	123°18'53.4"
Kuratding	PC08	<i>Schizophyllum commune</i>	100.00%	MK732122	13°37'11.9"	123°19'02.2"
Kabuteng saging	PC16	<i>Volvariella volvacea</i>	100.00%	MG280838	13°37'20.1"	123°19'04.2"
Kabuteng punso	nd	nd	nd	nd	nd	nd
Kabuteng dayami	nd	nd	nd	nd	nd	nd

nd=no data



**Fig. 2.** Mushrooms collected in the study that were utilized by the Bicolanos as food, (a) *Schizophyllum commune*, (b) *Auricularia polytricha*, (d) *Coprinopsis cinerea*, (D) *Pleurotus djamor*, (e) *Volvariella volvacea*

during the collection date. The collected mushrooms were identified in their local name and were properly identified using a molecular technique using the ITS gene marker. Traditional beliefs were surveyed such as adhering to the rituals by some of the locals during the collections of mushrooms. Traditional local knowledge of the people and the collected identified wild mushroom species provide additional knowledge for future scientific research in optimization and cultivation of its beneficial properties for health, culinary and food production. The documentation also promotes education about the different mushrooms found in the Philippines.

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### Conflict of Interest

The author declares no conflict of interest in publishing the paper.

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