NUCLEOTIDE SEQUENCE-BASED IDENTIFICATION OF *Lentinus* ISOLATES FROM LUZON ISLAND, PHILIPPINES

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ABSTRACT

Lentinus species (Polyporaceae) are saprophytic, wood-rotting basidiomycetous mushrooms with significant nutritional and pharmacological values. In this study, the molecular identity and variability of 15 *Lentinus* isolates collected from different areas in Luzon Island, Philippines were investigated. Genomic DNA from fresh *Lentinus* mycelia was extracted, PCR amplicons-amplified using ITS1F and ITS4BR primers were sequenced and the consensus sequences were compared against NCBI GenBank database. Phylogenetic trees were constructed using Maximum Likelihood (ML) method with Kimura-2-parameter model in Molecular Evolutionary Genetics Analysis (MEGA X). Results of BLASTn analysis of ITS consensus sequences confirmed the molecular identities of seven *Lentinus* species including *L. tigrinus*, *L. squarrosulus*, *L. sajor-caju*, *L. strigosus*, *L. swartzii*, *L. glabratus* and *Panus* (*Lentinopanus*) *conchatus* showing similarities that ranged from 97.90% to 100% (with 99% to 100% query coverage) with their corresponding GenBank reference sequences. The ML phylogenetic tree supported the distinct grouping of isolates of each *Lentinus* species, showed the separation of *L. squarrosulus* CPS5 from the other two *L. squarrosulus* isolates (LSQBot and LSQOs), and the separation of both *L. strigosus* isolates and *P. conchatus* from other *Lentinus* taxa, indicating their own separate lineage. In conclusion, ITS rDNA is a useful genetic marker for molecular identification and species differentiation of Philippine *Lentinus* isolates.

Key words: *Lentinus* species, wild edible mushrooms, phylogeny, ITS rDNA, CPS5.

INTRODUCTION

Mushrooms are important resources of functional food and bioactive metabolites.They have a wide variety of bioactive compounds, which have been shown to exhibit several biological activities including antioxidant, anticancer, antitumor, anti-inflammatory, immunomodulatory, anti-aging, anti-diabetic, anti-hypertension, anti-obesity, anti-atherosclerotic, anticoagulant, anti-hyperlipidemia, antimicrobial, antiviral, anti-hepatitis, antimalarial, anti-schistosome infection, hepatoprotective, neuroprotective, cardiovascular-protective, hypo-sexuality, anti-osteoporotic, anti-arthritic, improve metabolic syndrome, immune functions, and gastrointestinal health (Ashraf et al. 2020; Samarasinghe and Waisundara 2020; Su et al. 2020; Blumfield et al. 2020; Lin et al. 2019). Polysaccharides such as lentinan, grifolan, pleuran, ganoderan, schizophyllan, krestin, and polysaccharide peptide (PSP) isolated from *L. edodes*, *G. frondosa*, *P. ostreatus*, *G. lucidum*, *S. commune*, and *T. versicolor*, respectively,

have been well-characterized and widely reported for their medicinal properties, particularly anticancer and immunomodulatory activities (Meng et al. 2016; Rathore et al. 2017; Zhang et al. 2019; Chakraborty et al. 2021).

In the exploration and exploitation of the numerous advantages of mushrooms, accurate identity is very important. Many studies have been made to molecularly identify and distinguish variations among closely related mushrooms using different genetic barcodes. Schoch et al. (2012) reported the use of six genes namely, *RPB1, RPB2, nLSU, nSSU, mSSU,* and *TEF1* by the consortium of Assembling the Fungal Tree of Life (AFTOL). However, the International Fungal Barcoding Consortium formally recommended that the internal transcribed spacer (ITS) regions of the nuclear ribosomal RNA gene cluster be used as the primary barcode for fungal identification (Schoch et al. 2012). Analysis of ITS regions confirmed the identity of 38 macrofungal isolates from Iran belonging to 22 species, 19 genera, 10 families and 5 orders (Alimadadi et al. 2019) and *Hericium* species, *H. alpestre*, *H. coralloides* and *H. erinaceus*, collected in Italy (Cesaroni et al. 2019). Moreover, ITS1 and ITS4 sequence analysis revealed the identity of 3 *Termitomyces aurantiacus*, 8 *Tricholoma matsutake*, 2 *Tricholoma robustum*, 4 *Pleurotus ostreatus*, *Schizophyllum commune* and *Pleurotus pulmonarius* (Adeniyi et al. 2018) and non-gilled mushrooms *Auricularia polytricha, Ganoderma lucidum, Auricularia auricula-judae,* and *Trametes elegans* collected from North Western Himalayas (Singh and Tripathi 2018).

Lentinus species (Polyporaceae, Basidiomycota) are normally wood-decaying mushroomsgrowing solitary or more often in groups on water-soaked logs or trunks of trees, which are morphologicallycharacterized by firm to tough round pileus with light brown to blackish scales, saw-toothed edges, white to yellowish underside gills, and with scaly white stipe fruiting bodies (Dulay et al. 2020a). They are widely distributed in lowland and upland areas in the Philippines, mostly during the months of May to October. They have been recorded and documented in various ethnomycological and species listing studies conducted in different areas of Luzon Island, including six Aeta tribal communities in Pampanga, Zambales, Tarlac (De Leon et al. 2013), Gaddang communities in Nueva Vizcaya (Lazo et al. 2015), Bazal-Baubo Watershed, Aurora (Tadiosa et al. 2011), Mt. Bangcay, Cuyapo, Nueva Ecija (Dulay and Maglasang 2017), Central Luzon State University Campus, Science City of Muñoz, Nueva Ecija (Culala and Dulay 2018), Sitio Canding, Barangay Maasin, San Clemente, Tarlac (Dulay et al. 2020b), Mt. Makiling Forest Reserve, Los Baños, Laguna (De Castro and Dulay 2015), and Mt Palay- Palay / Mataasna Gulod Protected Landscape, Southern Luzon (Arenas et al. 2015).

The Central Luzon State University (CLSU) is one of the 27 Tuklas Lunas Development Centers (TLDC) in the Philippines, and the only one that focuses on the exploration of Philippine wild mushrooms for their medicinal properties and development of functional foods. The CLSU-TLDC has established its culture collections of successfully isolated wild mushrooms collected from the different areas of Luzon Island, Philippines. However, most of the isolated mushroom species were identified based on their morphology, which is sometimes not conclusive. Thus, the present study investigated the molecular identification of these wild mushrooms using the internal transcribed spacer (ITS) region of the rRNA genes in our intention to establish their accurate identity, which is essential for further exploration of their significant position in various applications.

MATERIALS AND METHODS

Mushroom culture. Pure cultures of the different isolates of *Lentinus* species were acquired from the culture collections of the Center for Tropical Mushroom Research and Development, and Tuklas Lunas Development Center, Department of Biological Sciences, College of Science, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines. The fruiting bodies of *Lentinus* species growing in their natural habitat are shown in Figure 1.

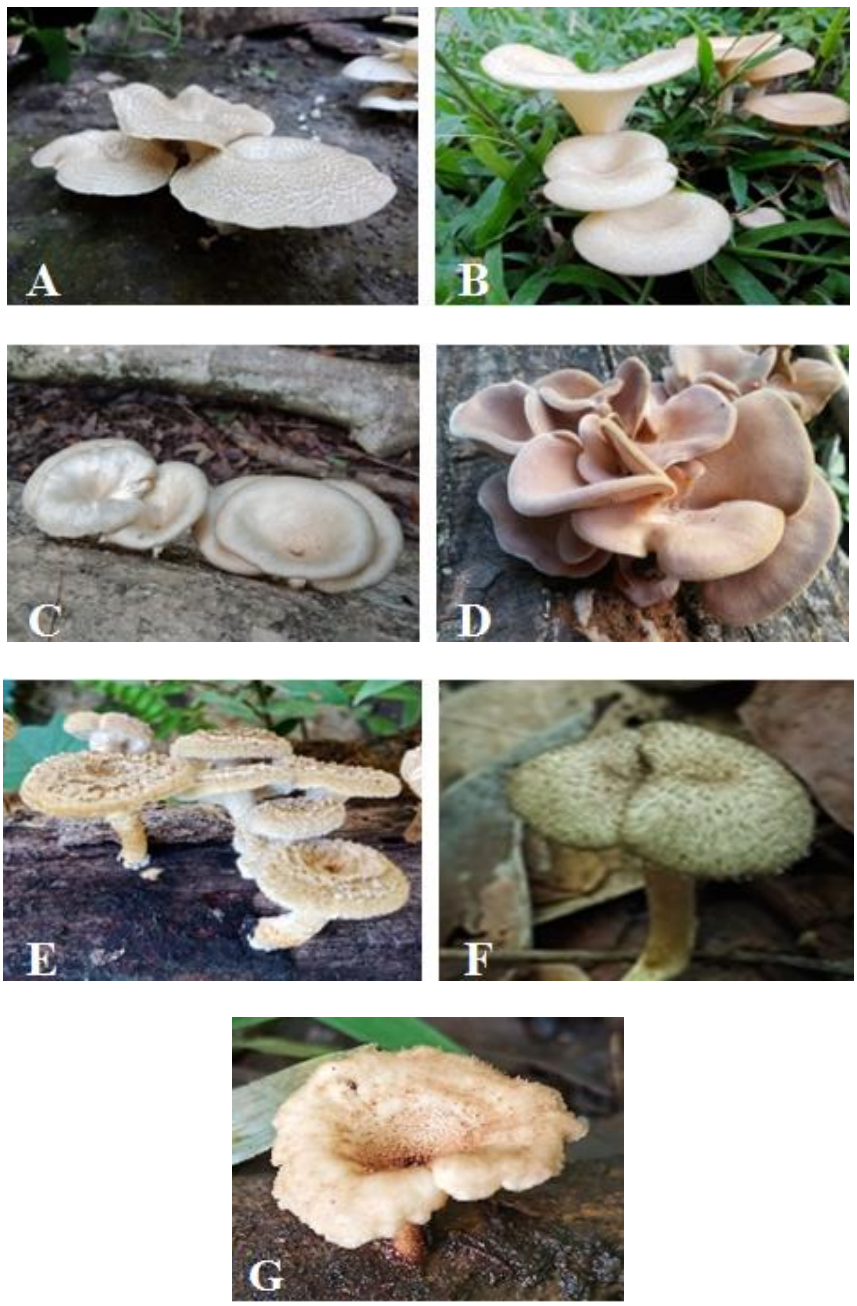

Fig. 1. Wild fruiting bodies of *Lentinus tigrinus* AS21 (A) from Poblacion, Alilem, Ilocos Sur; *L. squarrosulus* LSQOs (B), *L. sajor-caju* C005 (C), *L. strigosus* BIL1324 (D), *L. swartzii* BIL4618 (E), and *L. glabratus* CVS22 (F) from Science City of Muñoz, Nueva Ecija; and *P. conchatus* (G) from So. Canding, Maasin, San Clemente, Tarlac.

The presumptive identity based on the morphological characteristics, culture code, and place of origin of the 15 *Lentinus* isolates are summarized in Table 1. Agar blocks of mycelia were sub-cultured on potato dextrose agar (PDA) plates and incubated at 28°C, alternating light and dark

condition for seven days to allow mycelial growth. These cultures were used as source of the DNA samples for molecular identification.

Table 1. The different isolates of *Lentinus* collected from the different areas of Luzon.

Presumptive Identity*	**Isolate code**	**Place of origin**
Lentinus tigrinus	DQS75	Dolores, Quezon
	BP32	Binanuaanan, Camarines Sur
	AS21	PoblacionAlilem, Ilocos Sur
Lentinus squarrosulus	LSQBot	Botolan, Zambales
	CPS5	Patagueleg, Cagayan
Lentinus sajor-caju	LSCBot	Botolan, Zambales
	C005	Lingap Kalikasan, CLSU Campus, Muñoz, Nueva Ecija
Lentinus strigosus	BIL1324	RM Cares, CLSU Campus, Muñoz, Nueva Ecija
	CL-01	Trailer House, CLSU Campus, Muñoz, Nueva Ecija
Lentinus swartzii	CL-02	Trailer House, CLSU Campus, Muñoz, Nueva Ecija
	BIL4618	Bioassay, CLSU Campus, Muñoz, Nueva Ecija
Lentinus glabratus	CVS 22	Lingap Kalikasan, CLSU Campus, Muñoz, Nueva Ecija
	CVS 29	Lingap Kalikasan, CLSU Campus, Muñoz, Nueva Ecija
Lentinopanus conchatus	A52	So. Canding, Maasin, San Clemente, Tarlac
Lentinus sp.	LSQOs	Osmeña, CLSU Campus, Muñoz, Nueva Ecija

*Presumptive identity was based on the morphological characteristics of the 15 *Lentinus* isolates.

Genomic DNA extraction. The genomic DNA was extracted from the fresh mycelia of mushrooms. A 100 mg mycelial sample from each mushroom was crushed using mortar and pestle with liquid nitrogen, and the ground sample was transferred into 2 mL-capacity microtube. A 600 µL volume of pre-warmed 2× cetyltrimethylammonium bromide (CTAB) buffer (20 g of CTAB dissolved in 860 mL sterile double distilled water, 100 mL of 1M Tris pH 8.0, 81.82 g of NaCl, and 40 mL of 0.5M EDTA pH 8.1) and 70 µL of 20% sodium dodecyl sulfate (SDS) were added into each sample, mixed using a vortex, and incubated at 65°C for 45 min in a dry bath (LabnetAccublock™ Digital Dry Bath, USA). After cooling, 600 µL of chloroform (1 isoamyl alcohol: 20 chloroform) were added, mixed thoroughly using a vortex, and centrifuged (Centurion Scientific Refrigerated Centrifuge, UK) at 10,000 rpm for 30 min. The upper layer was transferred into 1.5 mL-capacity microtube. Then, 600 µL of ice-cold isopropanol were added and incubated overnight at -20°C. After incubation, the mixture was centrifuged at 10,000 rpm for 10 min, and the isopropanol was decanted. The pellet was washed with 500 µL of 70% ethanol and centrifuged at 10,000 rpm for 3 min. Washing was done twice. Ethanol was decanted and the microtubes were inverted on a clean paper towel for about 10 min to completely remove any traces of ethanol. The DNA pellet was re-dissolved in 100 µL 1× Tris EDTA buffer (10 mM Tris, pH 8.0 and 1 mM EDTA pH 8.0) and allowed to stand at room temperature for 3-4 hrs. To check the quality of the DNA, a mixture of 1 µL of DNA and 1 µL of 2× loading dye was prepared and loaded onto 1% agarose gel with 1 µL of FloroSafe DNA stain (Axil Scientific Pte Ltd, Singapore), run in gel electrophoresis system (Mupid® - One Electrophoresis, Japan) at 100 V for 30 min, and visualized using Labnet GDS-1302 Enduro Imaging System, USA. The fungal genomic DNA samples were quantified using spectrophotometer (Thermo Scientific™ Multiskan™ GO Microplate Reader), and the final concentration was adjusted to 100 ng/µL using 1× Tris EDTA buffer and stored at 4°C.

PCR amplification. PCR amplification of the ITS rDNA was carried out using primer pair ITS1 Forward (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4B Reverse (5'-CAGGAGACTTGTACACGGTCCAG-3') (Gardes and Bruns 1993). The ITS1F/ITS4BR primer pair has been shown to be particularly useful for detection and analysis of the basidiomycete component

(Gardes and Bruns 1993), and has been widely used for the molecular identification of several mushrooms (Adeniyi et al. 2018; Alimadadi et al. 2019). The 25 µL PCR reaction mixture contained the following: 4 ng of fungal genomic DNA, 0.4 µM of each ITS primer, 1 mM of deoxynucleoside triphosphates (dNTPs) mix, 1× PCR buffer, 1.5 mM of MgCl2, 0.5 U of KAPA Taq DNA polymerase (KAPABiosystems Manufacturing, South Africa), and 16.9 µL of nuclease-free water. The PCR Applied Biosystem® 2720 Thermal Cycler (Applied Biosystems, California, USA) conditions were programmed as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 51°C for 30 sec, extension at 72°C for 60 sec, and final cycle of extension at 72°C for 10 min. The quality of PCR amplicons were checked following the same method described in the preceding section. The expected amplicon size was approximately 850 bp.

DNA sequencing and sequence alignment. PCR amplicons were sent to Apical Scientific SdnBhd in Malaysia for outsourced DNA purification and double pass DNA sequencing. The sequence data generated using the forward primer and reverse primer were edited, aligned, and the consensus sequences were determined using BioEdit 7.2 (Hall 1999). The nucleotide sequence comparisons were performed using the standard nucleotide Basic Local Alignment Search Tool (BLASTN) against the National Center for Biotechnology Information (NCBI) GenBank database.

Phylogenetic analyses. Phylogenetic analysis was conducted in Molecular Evolutionary Genetics Analysis (MEGA) version X (Kumar et al. 2018). The nucleotide sequences were aligned using sequence alignment program MUSCLE algorithm. The ML best-fit substitution model was generated with maximum likelihood statistical method and selected based on the Bayesian Information Criteria (BIC) score. The phylogenetic tree was constructed by using maximum likelihood method with Kimura-2-parameter (K2P) substitution model (Kimura 1980). A discrete gamma distribution was used to model evolutionary rate variation among sites (5 categories). The alignment gaps were treated using Use All Sites method. Bootstrap analysis was performed with 1,000 replicates to evaluate confidence levels of the clades in the phylogenetic tree (Felsenstein 1985). Tree was rooted using *Pleurotus ostreatus* as the outgroup taxon.

RESULTS AND DISCUSSION

Mushrooms are high-valued mycoresources of nutritious food and bioactive metabolites for nutraceutical and pharmacological applications. With the high interest to the numerous benefits of mushrooms, it is imperative to establish their accurate identity especially the unrecorded and underutilized naturally occurring mushrooms. Mushrooms are usually identified based on their morphological structures and unique additional features such as annulus or ring, volva or cup, and veil present in the fruiting body. However, this method is sometimes not conclusive because of the inconsistent morphology.

DNA barcoding is a novel diagnostic tool in providing accurate identity of the species of Basidiomycota (Badotti et al. 2017). Several studies reported the use of this tool to identify wild and cultivated mushrooms, grocery-store fresh and powdered mushrooms, and mushroom-based dietary supplements, to confirm misidentified mushroom species, and to compare different genetic markers and establish the most suitable target markers for specific mushroom group (Adedokun et al. 2016; Raja et al. 2017; Badotti et al. 2017; Fernández-López et al. 2018; Li et al. 2018; Wang et al. 2019; Gunnels et al. 2020; Dulay et al. 2020c).

PCR-amplified products, ITS sequences, and BLASTn identities of *Lentinus* isolates. In the present study, the molecular identities of 15 *Lentinus* isolates collected from Luzon Island, Philippines using the ITS region of rDNAs were established. PCR amplification using the ITS1F and ITS4BR primer pair produced single bands of approximately 850 bp.

BLASTn analysis of the nucleotide sequences of the ITS rDNA amplicons generated using ITS1F and ITS4BR primers through homology search in GenBank database confirmed that the PCR-amplified products were *Lentinus* mushrooms. The morphological identities and results of the BLASTn analysis of rDNA-ITS sequences of the different isolates of *Lentinus* species are summarized in Table 2. The nucleotide sequences of all *Lentinus* isolates showed 97.90% to 100.00% similarity to their respective GenBank sequences with 99% to 100% query coverage. It can be noticed that most *Lentinus* nucleotide sequences showed <100% similarity to their GenBank accession sequence equivalent, which indicates sequence divergence between Philippine *Lentinus* and the deposited *Lentinus* sequences in the GenBank repository.

Table 2. Morphological identities and BLASTn analysis results of the rDNA-ITS sequences of the different isolates of *Lentinus* species and the reference sequences from GenBank.

Isolate / Strain Code	Morphological Identity	BLASTn Identity	Accession Code	Identity (%)	Query Cover (%)
DQS75	*L. tigrinus*	*L. tigrinus*	OM102521	99.86	100
BP32	*L. tigrinus*	*L. tigrinus*	OM102522	99.71	100
AS21	*L. tigrinus*	*L. tigrinus*	OM102523	99.29	100
LSQBot	*L. squarrosulus*	*L. squarrosulus*	OM102524	99.03	100
CPS5	*L. squarrosulus*	*L. squarrosulus*	OM102525	98.44	99
LSQOs	*Lentinus* sp.	*L. squarrosulus*	OM102526	99.03	100
LSCBot	*L. sajor-caju*	*L. sajor-caju*	OM102527	98.60	100
C005	*L. sajor-caju*	*L. sajor-caju*	OM102528	97.90	100
BIL1324	*L. strigosus*	*L. strigosus*	OM102529	99.58	100
CL-01	*L. strigosus*	*L. strigosus*	OM102530	99.58	100
CL-02	*L. swartzii*	*L. swartzii*	OM102531	99.39	100
BIL4618	*L. swartzii*	*L. swartzii*	OM102532	99.39	100
CVS 22	*L. glabratus*	*L. glabratus*	OM102533	100.00	99
CVS 29	*L. glabratus*	*L. glabratus*	OM102534	100.00	99
A52	*L. conchatus*	*P. conchatus*	OM102535	100.00	100
LE214778	-	*L. tigrinus*	KM411459.1	-	-
JZ26	-	*L. squarrosulus*	MG719283.1	-	-
MEL:2382718	-	*L. sajor-caju*	KP012899.1	-	-
LE5829	-	*L. strigosus*	KM411451.1	-	-
EB1101	-	*L. swartzii*	KT956124.1	-	-
AP8	-	*L. glabratus*	KF860882.1	-	-
UOC SIGWI S24	-	*P. conchatus*	KR818817.1	-	-

Raja et al. (2017) mentioned that it is reasonable to start with ≥97% to 100% sequence similarity (i.e., <3% sequence divergence) and ≥80% query coverage for assigning species name based on the results of BLASTn analysis in the GenBank database, since the calculated average weighted infraspecific ITS variability in fungi was 2.51±4.57% (Nilsson et al. 2008). Therefore, the percentage identities and the query coverage values of all *Lentinus* isolates obtained in the present study are within the above-mentioned values, confirming their acceptable and valid molecular identities.

Interestingly, the morphological identities of 13 *Lentinus* isolates conformed with their molecular identities. However, the LSQOs isolate, which was morphologically identified down to the genus level only (*Lentinus* sp.), was molecularly identified down to the species level as *Lentinus squarrosulus*. On the other hand, the A52 isolate, which was morphologically identified as *Lentinopanus conchatus*, showed 100% homology with *Panus conchatus*. According to the Mycobank database

(www.mycobank.org), *P. conchatus* (Bull.) Fr. 1838 (MB#160358) is synonymous to *Lentinus conchatus* (Bull.) J. Schrot. 1889 (MB#456032) and *Lentinopanus conchatus* (Bull.) Pilat 1941 (MB#333101). Accordingly, *P. conchatus* was also regarded as one of the species of *Lentinus* by some mycotaxonomists.

Previously, the molecular identities of other Philippine wild mushrooms including *Agaricus erectosquamosus*, *Agaricus haematinus*, *Agaricus parvibicolor*, *Auricularia polytricha*, *Auricularia asiatica*, *Chaetocalathus conchatus*, *Chlorophyllum molybdites*, *Clitopilus prunulus*, *Coprinopsis clastophylla*, *Cyathus crassimurus*, *Cymatoderma elegans*, *Dacryopinax spathularia*, *Deconica coprophila*, *Earliella scabrosa*, *Favolus acervatus*, *Ganoderma australe*, *Ganoderma gibbosum*, *Gymnopilus dilepis*, *Gymnopus melanopus*, *Hexagonia tenuis*, *Hohenbuehelia grisea*, *Hymenagaricus taiwanensis*, *Marasmius leveilleanus*, *Marasmius tenuissimus*, *Oudemansiella canarii*, *Panus conchatus*, *Phallus merulinus*, *Polyporus tenuiculus*, *Polyporus thailandensis*, *Pterula echo*, *Schizophyllum commune*, *Trametes hirsuta*, *Xanthagaricus taiwanensis*, *Xylaria anisopleura* and *Xylaria escharoidea* were confirmed with 97.16% to 100% similarity using ITS region of rDNA (Dulay et al. 2020c). Similarly, molecular identification using ITS region confirmed the identities of Philippine mushrooms such as *Lentinus strigosus*, *Cookeina insititia*, *Psathyrella typhae*, *Panus conchatus*, *Pleurotus pulmonarius*, *Xylaria papulis*, *Ganoderma lucidum*, *Schizophyllum commune*, *Coprinellus aureogranulatus*, *Lentinus swartzii*, and *Panaeolus foenisecii* (Ramel 2018; Undan et al. 2016; Lopez et al. 2016). The ITS region is a good DNA marker for identification of *Lentinus* spp.

Phylogeny of *Lentinus* species. Maximum likelihood tree showed the distinct clades of isolates of each *Lentinus* species with 99% to 100% bootstrap support (Fig. 2). The first clade showed the grouping of *L. tigrinus* isolates (BP32, DQS75 and AS21) with *L. tigrinus* (KM411459.1) reference sequence, which is found more related to the second clade, the group of two *L. glabratus* isolates (CVS29 and CVS22) and reference sequence (KF860882.1). The other side branch revealed the closer relationship of *L. swartzii* isolates (BIL4618 and CL-02) and *L. squarrosulus* isolates (LSQBot and LSQOs) and their GenBank reference sequences, the third and fourth clade. However, it can be noticed that *L. squarrosulus* CPS5 separated from these two *L. squarrosulus* isolates. These four clades were closely related to the group of *L. sajor-caju* isolates (LSCBot and C005) as the fifth clade. Both *P. conchatus* and the two *L. strigosus* isolates separated from the other *Lentinus* taxa. The ML phylogenetic tree rooted in *Pleurotus ostreatus*, which was used as an outgroup taxon.

The separation of CPS5 from the other two isolates of *L. squarrosulus* and the similarity of *L. squarrosulus* isolates with the two different GenBank accession codes (MG719283.1 and JQ868749.1) in the BLASTn analysis strongly suggest that there are two different strains of *L. squarrosulus* present in the isolates studied. In the alignment of sequences of *L. squarrosulus* isolates, several nucleotide variations were found including 30 positions of basepair substitutions, six positions of adenine and cytosine deletions and three positions of thymine, adenine and cytosine insertions. This shows that nucleotide variations in the ITS rDNA region exist among *Lentinus* isolates belonging to the same species. This nucleotide variation may be attributed to the differences on the climatic condition of the specific regions where the mushrooms were collected. The Philippines has four types of climates depending on the distribution of rainfall and the period of the dry season. The Provinces of Nueva Ecija and Zambales, where *L. squarrosulus* isolates LSQOs and LSQBot originated, respectively, are under climate type 1, while Cagayan Valley, where CPS5 isolate was found, is under the type 3 climate zone, which has a yearlong warm condition. Similarly, *Hericium* mushrooms, including six species and 23 isolates from different geographic origin showed nucleotide sequence variation in both ITS1 and ITS2 regions (Park et al. 2004). Oyetayo (2014) suggested that variations in the ITS nucleotide sequences might be due to the different ecological regions where the mushrooms are growing. In addition, the shared similarity can be diminished or erased after some time because of the changing environmental conditions (Konstantinidis and Stackebrandt 2013).

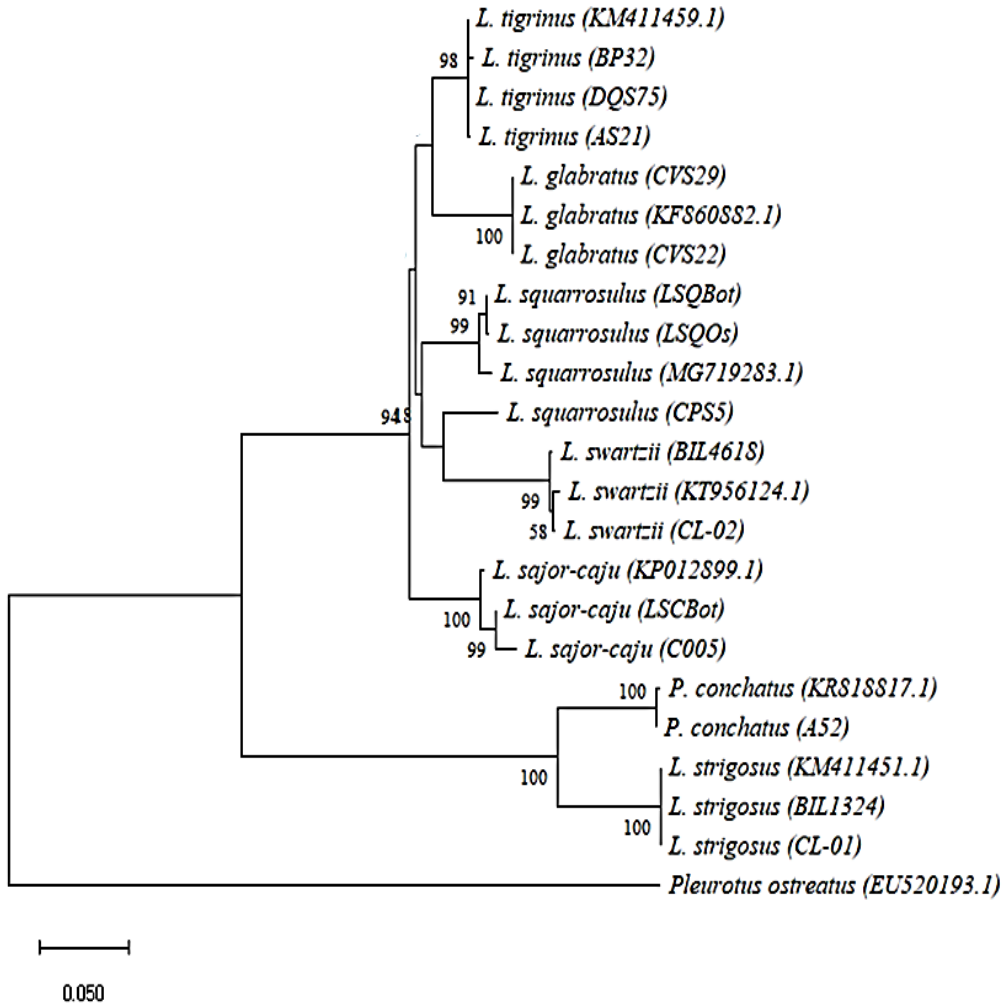

Fig. 2. Phylogenetic tree of 15 *Lentinus* isolates recovered from the Maximum Likelihood analysis using MEGA X. Bootstrap values were based on the 1000 replicates. Scale bar represents the substitution per site. *Pleurotus ostreatus* was used as an outgroup taxon.

Moreover, the separation of both *P. conchatus* and the two *L. strigosus* isolates from the other *Lentinus* taxa observed in the ML tree suggests their own separate lineage. In the BLASTn analysis, one of the equivalent GenBank sequences of *L. strigosus* isolates CL-01 and BIL1324 nucleotide sequences was *Panus lecomtei* (JQ955726.1) with 99.71% similarity and 96% query cover. Therefore, *L. strigosus* was also regarded as *Panus* species. The phylogenetic relationships between *Lentinus* and *Panus* and their allies have been controversial. On the basis of their hyphal morphology, some mycologists consider *Lentinus* and *Panus* as separate genera (Corner 1981), while other authors treat *Panus* as a subgenus of *Lentinus* (Pegler 1983). However, Binder et al. (2013) consider *Lentinus* within the core polyporoid clade and *Panus* within the residual polyporoid clade based on the phylogenetic and phylogenomic analyses.

Altogether, the molecular identities of 15 Philippine *Lentinus* isolates up to the species level and their phylogenetic relationships were established in the study.

CONCLUSIONS

ITS rDNA is a suitable genetic marker for molecular identification, characterization, and species differentiation of the 15 *Lentinus* isolates, and for the establishment of their phylogenetic relationships. With the observed degree of divergence in the ITS rDNA sequences of *Lentinus* isolates with those previously deposited GenBank sequences, there is a need to deposit the unique nucleotide type culture of indigenous Philippine *Lentinus* into the GenBank database to sufficiently load the database with Philippine mushroom sequences. The molecular identities of *Lentinus* species are very useful in the evaluation of their growth performance, biomass production and biological activities.

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