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Internal Transcribed Spacer (*ITS*) gene as an accurate DNA barcode for identification of macroscopic fungus in Aceh, Indonesia

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Abstract. *Harnelly E, Kusuma HI, Thomy Z, Samingan. 2022. Internal Transcribed Spacer (ITS) gene as an accurate DNA barcode for identification of macroscopic fungus in Aceh, Indonesia. Biodiversitas 23: 2369-2378.* Fungal identification is crucial in determining the fungal's role and benefits and is usually carried out by morphological observation. However, this technique takes a long time and depends on the accuracy of an expert. Therefore, the present study aimed to examine the accuracy of the Internal Transcribed Spacer (*ITS*) gene to identify macroscopic fungi from Aceh, Indonesia. 23 samples were collected from the Pocut Meurah Intan Grand Forest Park in Aceh, Indonesia. Samples were extracted, amplified, electrophoresed, and sequenced. The nucleotide sequence of the amplified products was analyzed by using the Basic Local Alignment Search Tool (BLAST). 7 out of the 11 samples with good and regular peaks were continued until further analyzed using BLAST. The results showed that samples aga2, aga4, aga5, aga6, aga9, rus, and pol were identified as *Pleurocollybia* sp., *Pleurotus djamour*, *Filoboletus manipularis*, *Schyzophillum commune*, *Gymnopilus lepidotus*, *Auriscalpium vulgare*, and *Gyrodontium sacchari*, respectively. The study findings indicate that the use of the *ITS* gene as a barcode effectively separates up to the species level from the type. This is very helpful in the identification process, especially when distinguishing cryptic species clearly because of their similarity, which is difficult to distinguish morphologically.

Keywords: DNA barcoding, ITS, macrofungi, Pocut Meurah Intan TAHURA

Abbreviations: TAHURA: Taman Hutan Raya/Grand Forest Park; PMI: Pocut Meurah Intan; ITS: Internal Transcribed Spacer

INTRODUCTION

It is estimated that less than 10% of the world's fungal species have been officially described (Bass and Richards 2011; Hibbett et al. 2011; Hibbett and Taylor 2013). This is because the life phase of most fungi is dominated by inconspicuous sexual stages or only hyphae, with few phenotypic features to be detected and described, making it difficult to identify their morphology (Aime and Brearley 2012; Yahr et al. 2016). Another challenge is the existence of similar body shapes in species that are not related or in the same category but possess different morphology. Therefore, classification by using morphological characters alone is inefficient when used as the primary reference in the grouping. Furthermore, the availability of DNA sequencing data has recently increased the detection rates of the diversity of new fungi, and it is used for describing fungal taxa with an unclear taxonomy (Lindahl et al. 2013; Tripp and Lendemer 2014). Recently, DNA sequence analysis is one of the various molecular techniques employed to quickly and accurately detect fungi.

DNA barcoding is one of the methods used to identify living things based on the sequence data from their genes (CBOL 2018b). It uses short universal, and accurate sequences for identification, down to the level of the species. Furthermore, various research was carried out to support DNA barcoding as a species identification process in animals, plants, and fungi (Hebert et al. 2003; Xu 2016; Kress 2017). Yang et al. (2018) also argued that DNA barcoding is a solid framework for identifying the specimen.

Moreover, the traditional identification process of wild mushrooms based on morphology only takes a relatively long time because each type of fungus takes time to form a fruiting body and is highly dependent on the accuracy of the experts testing it. However, the introduction of DNA barcoding technology made the identification process easier, more efficient, and more accurate. This is because, unlike DNA, the morphological form in traditional identification is influenced by developmental stages and environmental conditions. Furthermore, DNA data that has been stored in GenBank is accessible to all researchers in the world (CBOL 2018a).

The most frequently used data source as a barcode for fungal groups is the *ITS* (Internal Transcribed Spacer) gene, which is a repeating area of a non-coded DNA sequence found in the coding region of ribosomal RNA molecules (Badotti et al. 2017; Schoch et al. 2012). Furthermore, it was shown that the *ITS* area can systematically identify fungal taxa until the species level (Schoch et al. 2012).

This research aimed to identify the macrofungi found in Pocut Meurah Intan TAHURA properly. Although morphological inventory studies have been conducted, identification of macrofungi from Pocut Meurah Intan

TAHURA using a molecular approach has never been made before. This, in our opinion, is important to make the use of macrofungi more efficient and to ensure the safety of those who consume these mushrooms, given that there are many poisonous mushrooms with fruit bodies that look very similar to edible mushrooms (Diaz 2016).

MATERIAL AND METHODS

Sample collection

Sampling was conducted from October 2018 to April 2019 and the specimens were collected from Pocut Meurah Intan Grand Forest Park (TAHURA), Aceh Besar District, Aceh Province, Indonesia. The exploration was carried out by following the existing hiking trail, as shown in Figure 1. Furthermore, wild mushrooms that grew on living and dead trees, litter, on the ground in groups, clusters, or only a few fruiting bodies were first photographed in their natural habitat. In addition, the character of the fungus was observed and recorded. Finally, the fruiting body was taken and cleansed using a sprayer, placed in a bottle containing 70% alcohol, and stored at 20ºC until the DNA extraction process.

DNA extraction

The fungi from the field were taken to the laboratory, and DNA extraction was carried out. The total DNA extraction involved 14 wild mushroom orders found in the Pocut Meurah Intan Tahura area. Two species were randomly selected from each order; therefore, the total sample for DNA extraction was 23. Furthermore, all DNA were extracted using the DNeasy® Plant Mini Kit (Qiagen) following factory procedures with modifications to the incubation time of 18 hours.

ITS region amplification

The entire *ITS*1-5.8S-*ITS*2 region was amplified with PCR technology using TopTaq® master mix Kit (Qiagen) following factory procedures. The primers used were *ITS*1 as a forward primer (5'-TCCGTAGGTGAACCTGCGG-3') and *ITS*4 as a reverse primer (5'-TCCTCCGCTTATTGATATGC-3'). Then, the tubes were placed in a thermal controller (SensoQuest Labcycler) for 30 cycles of amplification under the following conditions: (1) denaturation at 95ºC for 30 seconds, (2) annealing at 57ºC for 1 minute, and (3) extension at 72ºC for 1 minute.

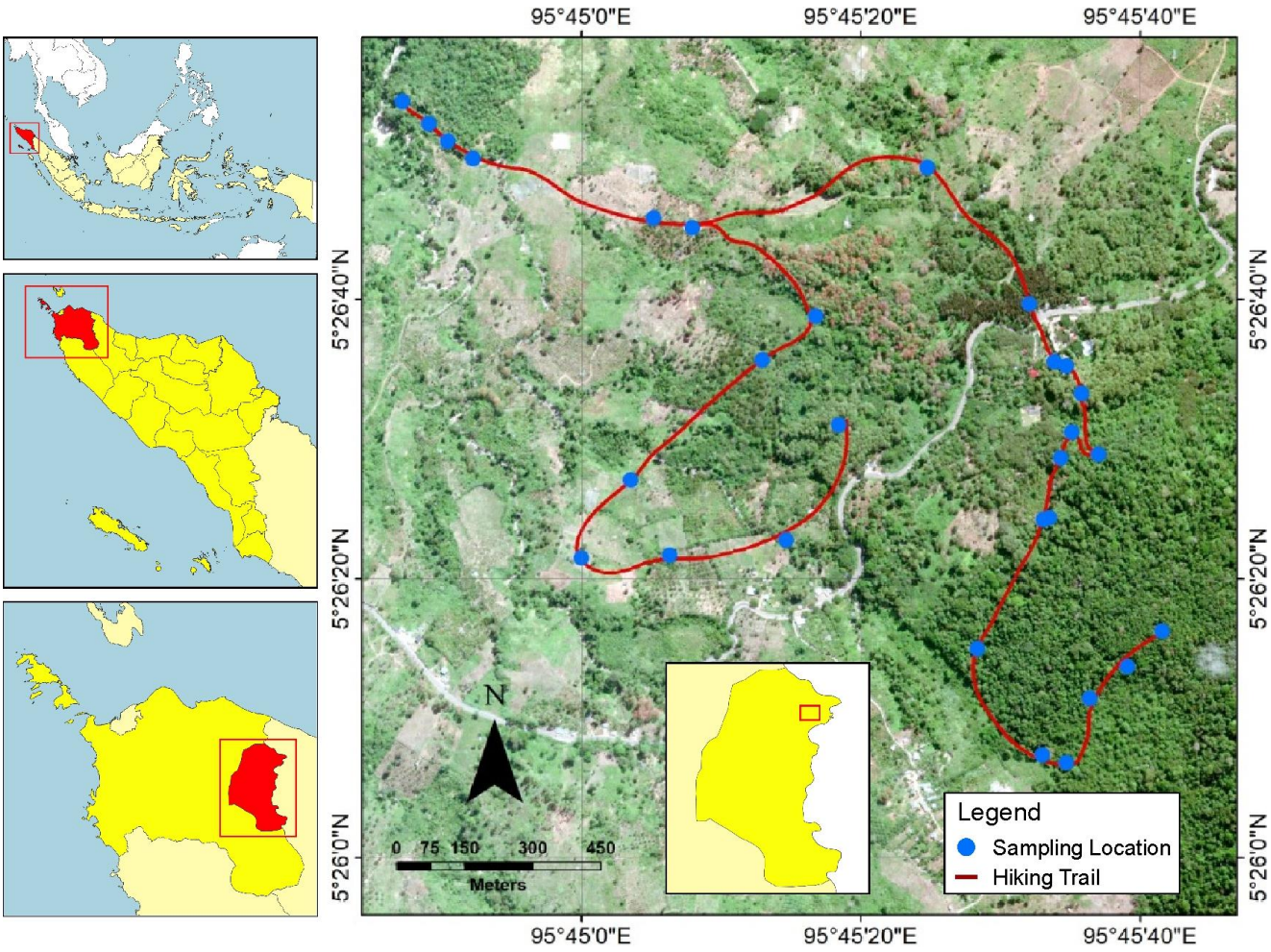

Figure 1. Map of research location in Pocut Meurah Intan Grand Forest Park (TAHURA), Aceh Besar District, Aceh Province, Indonesia

Electrophoresis of PCR products

The amplification product was electrophoresed on a 0.8% agarose gel. First, 2.4 g agarose powder was weighed and then boiled in 300 mL of 1x TAE buffer until completely dissolved to make 0.8% agarose gel. Subsequently, 1 µL of Red Gel was added to the agarose solution with a temperature ranging from 50-60ºC and briefly homogenized. Furthermore, the solution was poured into the agarose gel tray and left to turn into a solid gel, and the comb was taken with utmost care. Afterward, the tray filled with agarose gel was placed inside the electrophoresis tank, which contained a 1x TAE buffer solution. Six µL of the marker was inserted into the first well and 5 µL of the PCR products were placed in the agarose gel well. Subsequently, the cable was connected from the current source to the electrophoresis tank at 50 v for 60 minutes. Finally, the gel was removed and placed on the UV-transilluminator to observe visualized DNA bands.

Sequence analysis

The nucleotide sequence of the amplified products was identified by the Sanger method using the services of PT. Genetics Science Indonesia. The sequencing process was carried out twice in different directions (forward and backward). The data were used for molecular identification and the construction of a phylogenetic tree. The sequencing results, which were in a chromatogram with colorful peaks, were used to determine the type of nucleotides.

Furthermore, the results were edited, aligned, and combined using Bioedit software and stored in the FASTA format for BLAST analysis on gene bank (NCBI). The sequence analysis was also done using DNA Subway's open-access software (Jensen-Vargas and Marizzi 2018). According to the Neighbourhood Joining method, Phylogenetic trees were constructed using MEGA Software (Tamura et al. 2013). Subsequently, the bootstrap confidence interval (Felsenstein 1985) for each branching pattern was calculated from 1000 sampling replicates.

RESULTS AND DISCUSSION

Morphological identification

The 14 fungi included in this study were morphologically identified based on the general characteristics used to determine the pileus shape, the color of the fruiting body, type of lamellae, stem shape, stem surface, annulus, and volva. The characteristics of these mushrooms can be seen in Table 1. The appearance of some of the mushrooms included in this study can be seen in Figure 2.

Each mushroom has a character that can be used as a benchmark marker in morphological identification. Still, in some cases, because it has a fruiting body that is too similar, the identification results can be inaccurate even though it turns out that they are not related at all. In other cases, where species are closely related but not morphologically similar. Thus, the morphological classification alone cannot be used as the main reference when grouping. Therefore, the morphological identification results need to be confirmed with molecular identification to clarify the identification results accurately.

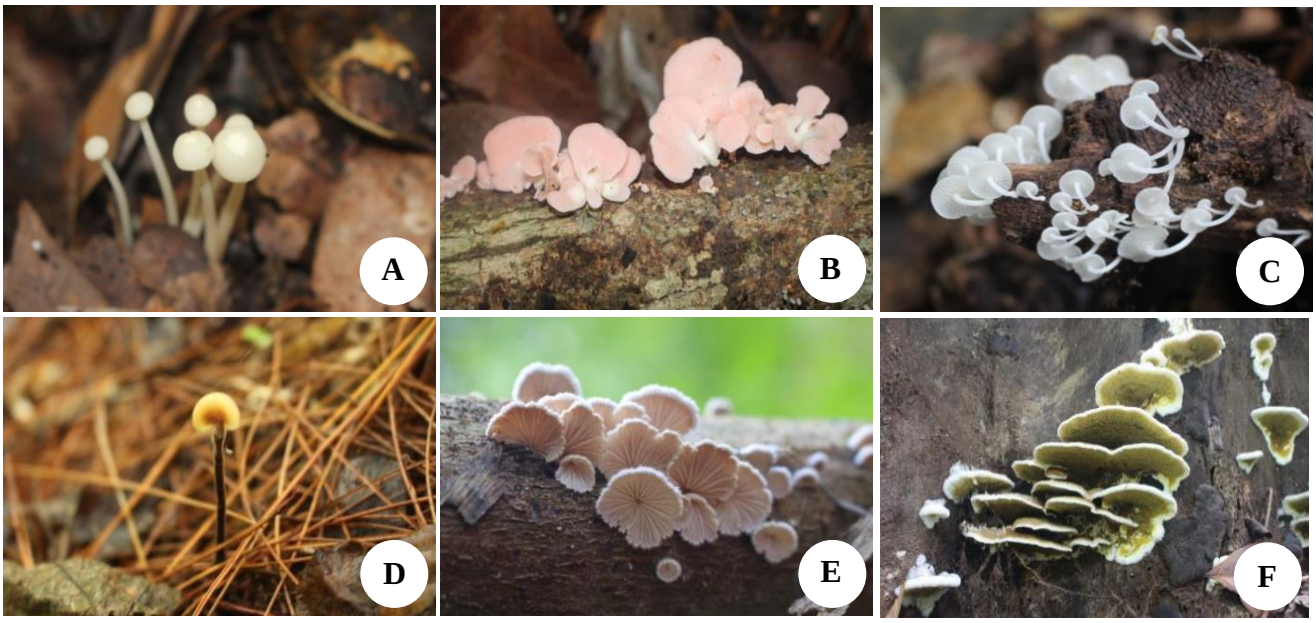

Figure 2. The morphological appearance of some wild mushrooms from Pocut Meurah Intan, Saree Grand Forest Park. A. *Hebeloma albidum*, B. *Pleurotus djamour*, C. *Filoboletus manipularis*, D. *Auriscalpium vulgare*, E. *Schyzophyllum commune*, F. *Gyrodontium sacchari*

Table 1. Characteristics of fungi and their morphological identification

Sample code	Morphological identity	Characteristics	References
Aga1	*Marasmius haematochepalus*	The fruiting body forms a small, thin, convex cap 10-40 mm diameter. The cap surface is smooth but serrated or fluted. The hood is pink, light purple, or reddish-purple. Lamellae are widely spaced, white to pink, or pale in color. Stems are long, thin, and smooth, without rings, and no volva, red-brown to dark brown.	(Hawkeswood et al. 2021)
Aga2	*Hebeloma albidum*	The fruit body is hood-shaped with a size of 2.5-7 cm. The shape of the hood is convex to convex, slightly flat or bell-shaped, sticky when very fresh, white to white. The lamellae are attached to the stem, often by notches; the lamellae are closely spaced, white when young, becoming cinnamon brown when old. Stems are 4-8 cm long, 3-7 mm thick, approximately equal in size, or often with small basal balls that lie underground. The surface of the stem is smooth to finely scaly near the apex and whitish, without annulus and volva and grows scattered or clustered on the ground.	(Kuo 2012)
Aga3	*Lycoperdon pyriforme*	The pear-shaped fruiting body is 1.5 to 4.5 cm wide by 2 to 4.5 cm high. The shape is almost round to oval at the juvenile stage, resembling an egg; the mushroom base is small and narrow. The surface of the fruiting body when young is covered with small white spines that usually fall off before the fruit body is ripe. Their color ranges from almost white to yellowish-brown. The central pores rupture in the ripe fruiting bodies, allowing wind and rain to disperse spores. Its base is attached to the wood by rhizomorphs (thick and cotton-like mycelium). It does not have stems, rings, and volva.	(Kuo 2016)
Aga4	*Pleurotus djamour*	Fruiting bodies are 3–15 cm in size, broadly kidney to fan-shaped with an extended convex surface, or nearly round when growing on a woody stem, slightly oily when young and fresh, pink to red. The margins are slightly rolled inward when young. Lamellae flows from the direction of the stem (or pseudostem), the distance between the gills is close, and the gills are crossed between long and short. Stems are usually imperfect and lateral (or nearly absent) when the fungus grows from the sides of the trunk or tree, but sometimes they are somewhat centrally positioned when growing at the top of the trunk or branches. Stems are 1-7 cm, whitish, hairy to slightly velvety. Saprophytic on dead and rotting wood.	(Guzman et al. 1993)
Aga5	*Filoboletus manipularis*	The fruiting body has a hood that is conical to convex. It becomes flattered with a protruding tip (umbonate), 1-2 cm in diameter with age. The surface of the cap is smooth but thin, and appears dimpled from the pores below, white to cream in color with a brownish tint to the middle. The pores are round and whitish. The stem is brittle, smooth brownish-white, hairy towards the base, without rings and volva. Saprophyte on wood or twigs and usually grows in clusters with the basal part attached.	(Vydryakova et al. 2014)
Pez 1	*Cookeina tricholoma*	The fruiting bodies are goblet-shaped funnel with curved edges, 1-2 cm in diameter, with slender stems 1-3 cm high, pink to bright red, have conspicuous fine hairs, stiff like toothbrush bristles, and usually 2-3 mm long, habitats on rotting wood, twigs, and tree branches.	(Ekanayaka 2016)
Rus	*Auriscalpium vulgare*	The fruiting body has a 1-3 cm cap, convex or flat. Kidney-shaped or almost circular, dry, hairy, sometimes becoming smooth with age. The color is reddish-brown to dark brown or almost black. The lower surface contains spines 1-3 mm long, white to brownish. Stem is 2-7 cm, up to 3 mm thick, and usually attached to the hood laterally. Stem stiff, reddish-brown to dark brown, hairy. Grows on pine cones.	(Das and Kayang 2010)
Aga6	*Schyzophillum commune*	Fruit bodies are 1-5 cm wide, fan-shaped when attached to the sides of the wood, and sometimes irregular to a shell-like shape. The upper surface is covered with small, dry, grayish-white, or brown hairs. The lower surface consists of split gill-like folds, whitish to grayish, without stems, rings, and volva. Grows solitary or more often in clusters on rotting wood and logs.	(Kusrinah and Kasiamdari 2017)

Tre	*Tremella foliaceae*	The 5-15 cm fruiting bodies are spherical to resemble sheets gathered like leaves attached to the same base. The surface is sticky in wet weather; otherwise, the surface is damp. Individual blades are usually wrinkled to folded, dull brown to cinnamon brown. The flesh of the body is thin, slightly translucent, and chewy. Usually, it grows on logs and hardwood twigs.	(Spirin et al. 2018)
Bol 1	*Scleroderma citrinum*	The fruiting body is almost round and slightly flattened at the top, with a width of 2.5-10 cm and a height of 2-4 cm. They are pale brown to brownish-yellow in color and covered with large, rough, flat warts. When ripe, the upper part of the fruiting body will open irregularly for spore dispersal. At the juvenile stage, the inner flesh (gleba) is firm and white, turning gray to deep black when ripe. The spore mass is a blackish-brown powder. Grows solitary or in groups on littered soil.	(Guzmán et al. 2013)
Pol	*Climacodon septentrionalis*	The fruiting body is up to 30 cm long and 5 cm thick; the surface is convex to flat, kidney-shaped, or semicircular. The surface is sticky, hairy sometimes rough. It is whitish to yellowish, sometimes with concentric zones and a smooth texture. The undersurface is 1-2.5 cm tooth-shaped, densely packed, and yellowish-white. Stems are absent, but fruiting bodies are often attached at the base and grow in large groups such as racks on trees or stumps/dead logs.	(Kuo 2010)
The	*Thelephora ganbajun*	Measuring 8-10 cm in width and 12-17 cm in height. The fruit bodies are leathery, gray-brown white, and vary in shape from coral-like tufts to slightly rose-like with stems in the middle. The surface is smooth to slightly hairy, jagged, in shades of brown. In young specimens, the margins are whitish and hairy. The underside is smooth and purplish-brown, and the stem is narrow and whitish to purple-brown. Grows under bamboo groves.	(Liu et al. 2006)
Aga 9	*Gymnopillus sapineus*	The fruiting body has a cap with a size of 2-8 cm, the shape of the hood is convex or almost flat, sometimes slightly bell-shaped. The surface is dry, downy, or scaly, yellowish or reddish-brown. The lamellae are attached to the stem; the distance between the lamellae is close; they are yellowish or whitish at first, then eventually become rusty brown in their entirety. Stem 3-7 cm long, approximately equal in size from the base (equal), smooth or downy surface, usually dark to brown from bottom to top, yellowish-white to yellowish basal mycelium. It has neither annulus nor volva, and is saprobic on dead wood and grows solitary, in groups, or in small groups.	(Holec 2005)
Gom 1	*Ramaria fennica*	The fruiting body is shaped like a coral, 5-12 cm high and 7-8 cm wide, branching repeatedly. Branches are oriented vertically and elongated, smooth surface, purplish-brown below, olive-yellow to yellow or yellowish smoky above. Stems up to 5 cm wide, white at the base, purple to brownish-purple above. It grows solitary, scattered, or in clusters and found growing on the ground under clumps of bamboo.	(Knudson 2012)

DNA quality and quantity

Fourteen samples were successfully extracted with sufficient DNA purity values. The success of extraction is influenced by the duration and the possibility of errors during sample storage, which significantly affects the DNA quality. Additionally, the composition of the cell wall and the different metabolic compounds affects the effectiveness of the buffer kit used. The concentration of the samples that are close to purity is shown in Table 2.

The extraction results were used as a template for PCR amplification, and its process was carried out with *ITS*1 and *ITS*4 primer, which was further electrophoresed using 0.8% agarose gel (Figure 3). The product ranged from 500 to ± 750 bp DNA band. Fujita et al. (2001) reported that the length of the amplified fragment using *ITS*1 and *ITS*4 on each fungus ranged from 350 to 880 bp. Meanwhile, Adeniyi et al. (2018) produced an average of about 850 bp from the PCR amplification results using *ITS*1 and *ITS*4 primers. Furthermore, the difference in band size may occur due to variations in DNA quality used in PCR. The sizes of 18S, 5.8S, and 26S rRNA genes are identical, while the length of the *ITS* region varies depending on the species.

Based on electrophoresis results, 11 of the 14 PCR samples were visualized under UV light using doc gel, while the other 3 formed a blurry band (Figure 3), which indicates a low quantity of amplified DNA. It is believed that the samples still contain contaminants in form of proteins or secondary metabolites; therefore, the polymerase enzyme is inactive when the replication chain is extended during the PCR process. It may also occur due to the inability of primer to stick to the desired area.

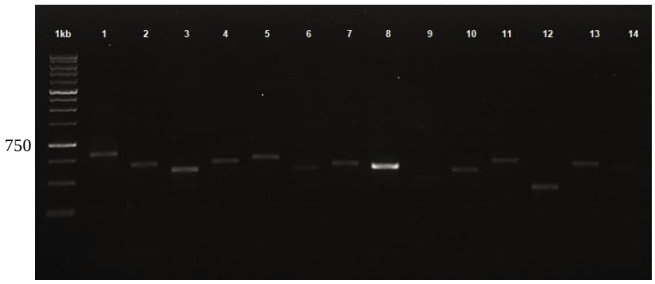

Figure 3. The electrophoresis results of PCR product on 0.8% agarose gel using the 1 kb marker. Note: 1: sample 1 (aga1), 2: sample 2 (aga2), 3: sample 3 (aga3), 4: sample 4 (aga4), 5: sample 5 (aga5), 6: sample 6 (pez1), 7: sample 7 (rus), 8: sample 8 (aga6), 9: sample 9 (tre), 10: sample 10 (bol1), 11: sample 11 (pol), 12: sample 12 (the), 13: sample 13 (aga9), 14: sample 14 (gum1)

The success of DNA amplification using PCR techniques is influenced by several factors, including the purity of the DNA template and the primer specificity. Furthermore, the purity level of the template is very important because a low DNA purity suspension affects the amplification reaction and inhibits the work of the DNA polymerase enzyme (Fatchiyah et al. 2011). Generally, the primer length ranges from 18 to 30 bases. A primer size less than 18 will result in low specificity and allow the priming in other unwanted places (mispriming), which affects the specificity and efficiency of the PCR process (Handoyo and Rudiretna 2001).

Molecular identification and DNA barcode

Seven out of the 11 samples with good and regular peaks were continued until the BLAST analysis stage. The highest percent identity score was used to determine the top BLAST result for all sequences. The result of the sample homology with the data from the GenBank NCBI showed that Aga2 has a homology percentage of 97.81% with *Pleurocollybia* sp. (Accession GenBank KY462690.1), Aga4 has 97% homology with *Pleurotus djamour* (accession GenBank KC582636.1), Aga5 has 94.20% homology with *Filobolletus manipularis* (GenBank accession KF746998.1), Rus has 93.63% homology with *Auriscalpium* sp. (Accession GenBank KY485946.1), Aga6 has 99.5% homology with *Schizophyllum commune* (accession GenBank KM458835.1), Pol has 99.14% homology with *Gyrodontium sacchari* (accession GenBank KP012932.1), and Aga9 has 100% homology with *Gymnopilus lepidotus* (accession GenBank KP764811.1).

The *ITS* region is a fungal genetic marker that is most frequently sequenced and widely used to answer research questions related to systematics and phylogeny and determine the identity of fungi down to the level of genus and species (Begerow et al. 2010). The results from the *ITS* sequence correspond to the morphological identity of fungus at the species level for samples 4 (aga4), 5 (aga5), 7 (rus), and 8 (aga6). Furthermore, The *ITS* sequence of sample 2 (aga2) showed high homology with *Pleurocollybia* sp. with a similarity percentage of 97%. However, the same specimen was morphologically identified as *Hebeloma albidum.* Also, the *ITS* sequence of sample 11 (Pol) showed high homology with *Gyrodontium sacchari* and a similarity percentage of 97%, however, the specimen was morphologically identified as *Climacodon septentrionalis.* In addition, sample 13 (Aga9) was morphologically classified as *Gymnopilus sapineus*, and the *ITS* sequence showed high similarity with *Gymnopilus lepidotus* (Table 3).

This difference occurred because these species have exhibited almost the same morphology; therefore, it is very difficult to determine the type based on only the morphological characteristics. Consequently, molecular identification is very important to obtain more reliable data on cryptic species such as wild mushrooms. Cryptic species are two or more distinct types classified as single because of their similar morphology, and many fungi show these characteristics.

Table 2. Measurement of concentration and purity of extracted DNA

Sample number	Species name (morphology)	Sample code	DNA concentration	Purity (A260/ A280)
1.	*Marasmius* sp.	Aga1	3.20 ng μL^{-1}	1.80 nm
2.	*Hebeloma albidum*	Aga2	31.35 ng μL^{-1}	1.75 nm
3.	*Lycoperdon pyriforme*	Aga3	60.30 ng μL^{-1}	1.89 nm
4.	*Pleurotus djamour*	Aga4	2.45 ng μL^{-1}	1.63 nm
5.	*Filoboletus manipularis*	Aga5	29.35 ng μL^{-1}	2.00 nm
6.	*Cookeina tricholoma*	Pez 1	38.70 ng μL^{-1}	1.82 nm
7.	*Auriscalpium vulgare*	Rus	3.20 ng μL^{-1}	1.39 nm
8.	*Schyzophillum commune*	Aga6	2.95 ng μL^{-1}	1.69 nm
9.	*Tremella foliaceae*	Tre	3.70 ng μL^{-1}	1.80 nm
10.	*Scleroderma citrinum*	Bol 1	5.35 ng μL^{-1}	1.69 nm
11.	*Climacodon septentrionalis*	Pol	17.80 ng μL^{-1}	1.78 nm
12.	*Thelephora ganbajun*	The	4.80 ng μL^{-1}	1.77 nm
13.	*Gymnopillus sapineus*	Aga 9	6.25 ng μL^{-1}	1.56 nm
14.	*Ramaria fennica*	Gom 1	13.05 ng μL^{-1}	1.46 nm

Table 3. Species identification based on DNA barcode using *ITS* from all analyzed samples

Sample number	Sample code	Morphological identification	BLASTN, GenBank			
			Barcode ID	Match (%)	Query cover (%)	E-value
2.	Aga2	*Hebeloma albidum*	*Pleurocollybia* sp.	98	96	0.0
4.	Aga4	*Pleurotus djamour*	*Pleurotus djamour*	98	83	0.0
5.	Aga5	*Filoboletus manipularis*	*Filoboletus manipularis*	99	94	0.0
7.	Rus	*Auriscalpium vulgare*	*Auriscalpium* sp.	94	85	0.0
8	Aga6	*Schyzophillum commune*	*Schyzophillum commune*	99	99	0.0
11.	Pol	*Climacodon septentrionalis*	*Gyrodontium sacchari*	99	97	0.0
13.	Aga9	*Gymnopillus sapineus*	*Gymnopillus lepidotus*	100	63	0.0

The phylogeny tree analysis of samples 2, 4, 5, 7, 8, 11, and 13 was performed using a 1000x bootstrap value, which implied 1000 repetitions of data alignment. The results showed that sample 2 forms a group with *Plurocollybia* sp. with 100% bootstrap value, sample 4 with *Pleurotus djamour* species with 87%, sample 5 with *Filoboletus manipularis* with 100%, and sample 7 with *Auriscalpium* sp. However, with a low bootstrap value of 44%, sample 8 forms a group with *Schyzophyllum commune* with 83%, sample 11 with *Gyrodontium sacchari* with 100%, while sample 13 allied with *Gymnopilus lepidotus* with 66% as the bootstrap value. The phylogenetic trees produced using the *ITS* region amplification of wild mushroom specimens from Pocut Meurah Intan Grand Forest Park are shown in Figure 4.

Additionally, the sequence data were further examined through kinship analysis with sequences of similar species found in Gene Bank. The kinship between species was seen using a phylogenetic tree topology, through which the approximate relationships between taxa (or sequences) and their *common ancestors are* determinable (Hall 2013).

The constructed phylogenetic tree revealed a close relationship between samples 2, 4, 8, and 13 to form a *clade* (clade A), a mushroom with sheet-type lamellae (*gill*). Meanwhile, the population structure described by the *ITS* region indicates that the fungi may have originated from a common ancestor. Whereas sample 5 showed closeness to samples 7 and 11 to form another *clade* (clade B), a mushroom with a unique lamella type, namely *tooth* and pore shape (sample 5, *Filoboletus manipularis*).

The visual barcode of all fungal samples tested is depicted in Figure 5, which shows that the same barcode was produced, and they are also the same species or closely related. Sample 2 (aga2) was grouped with *Pleurocollybia* sp.. They have almost the same sequence/arrangement of barcodes, indicating that these species are likely to be identical. Also, sample 4 (aga4) was grouped with *Pleurotus djamour*, and sample 5 (aga5) with *Filoboletus manipularis*. Sample 7 (rus) was grouped with *Auriscalpium vulgare*, sample 8 (aga6) with *Schyzophillum commune*, and sample 11 (pol) with *Gyrondotium sacchari*. Additionally, sample 13 (aga9) was stratified with *Gymnopillus lepidotus* and the resulting color hue equation shows correct identification via DNA barcode.

This study concluded that using the *ITS* area as a barcode has accurately identified these wild mushrooms down to the species level. Furthermore, *ITS* also shows clear kinship relationships in each fungus and groups them into separate clades. However, the extraction process to obtain fungal DNA requires more in-depth research as it is very difficult to obtain pure DNA using a universal kit in the present study. Thus, more specific tool should be employed for optimal results.

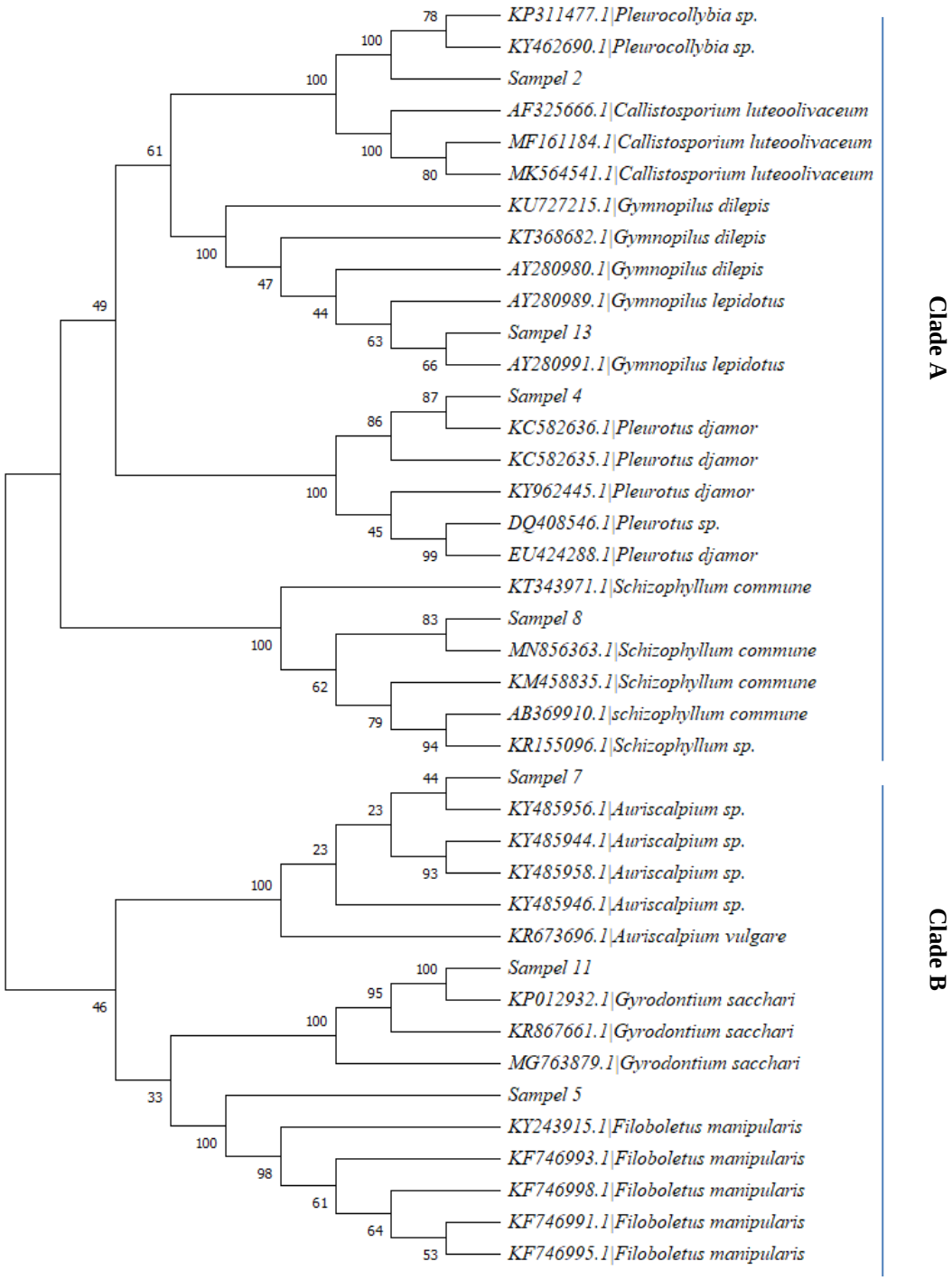

Figure 4. Results of phylogeny tree reconstruction based on *ITS*1-*ITS*2 sections using the NJ method with a 1000X bootstrap

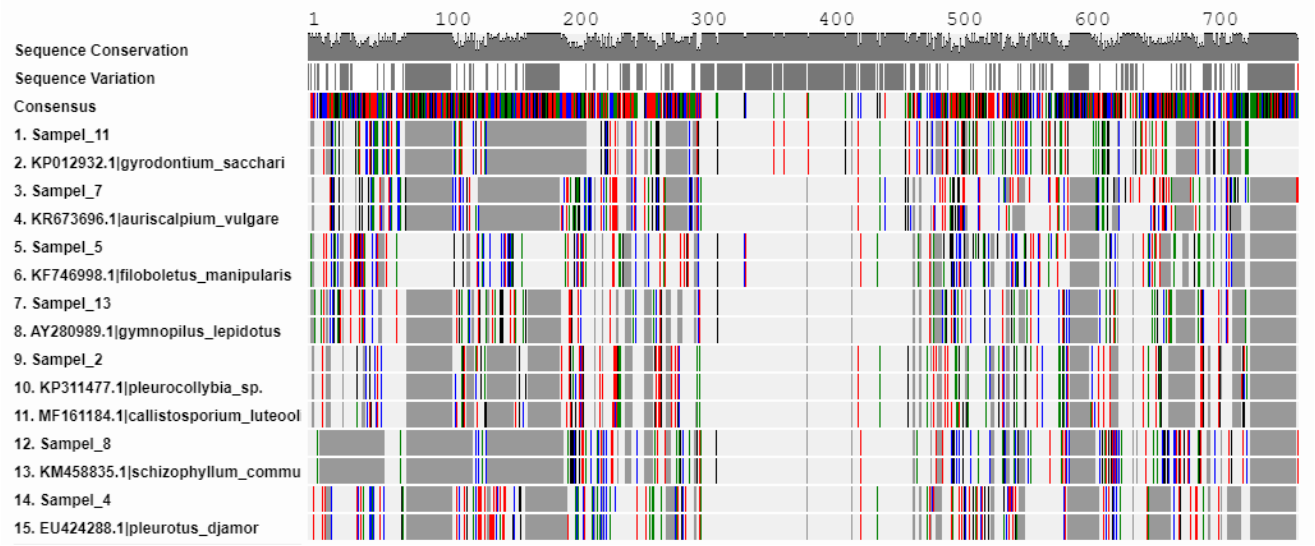

Figure 5. The consensus sample sequences (samples 2, 4, 5, 7, 8, 11, and 13) aligned with the top search results from BLASTn, using MUSCLE alignment software embedded in DNA Subway to create a visual DNA barcode. The nucleotides are color-coded with green, red, black, and blue, is representing Adenine (A), Thymine (T), Guanine (G), and Cytosine (C), respectively

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REFERENCES

Adeniyi M, Titilawo Y, Oluduro A, Odeyemi O, Nakin M, Okoh AI. 2018. Molecular identification of some wild Nigerian mushrooms using internal transcribed spacer: polymerase chain reaction. AMB Express 8 (1): 148. DOI: 10.1186/s13568-018-0661-9.

Aime MC, Brearley FQ. 2012. Tropical fungal diversity: Closing the gap between species estimates and species discovery. Biodiv Conserv 21 (9): 2177-2180. DOI: 10.1007/s10531-012-0338-7.

Badotti F, de Oliveira FS, Garcia CF, Vaz ABM, Fonseca PLC, Nahum LA, Oliveira G, Góes-Neto A. 2017. Effectiveness of ITS and sub-regions as DNA barcode markers for identifying Basidiomycota (Fungi). BMC Microbiol 17: 42. DOI: 10.1186/s12866-017-0958-x.

Bass D, Richards TA. 2011. Three reasons to re-evaluate fungal diversity 'on Earth and in the ocean'. Fungal Biol Rev 25 (4): 159-164. DOI: 10.1016/j.fbr.2011.10.003.

CBOL. 2018a. Background – iBOL. https://www.ibol.org/phase1/about-us/background [22 January 2020]

CBOL. 2018b. What is DNA Barcoding ?. https://ibol.org/about/dna-barcoding [22 January 2020]

Das P, Kayang H. 2010. Habitat preference of *Auriscalpium vulgare* gray inhabiting slash and burn affected khasi pine cones of India. Our Nat 7: 32-38. DOI: 10.3126/on.v7i1.2551.

Diaz JH. 2016. Mistaken mushroom poisonings. Wilderness Environ Med 27 (2): 330-335. DOI: 10.1016/j.wem.2015.12.015.

Ekanayaka AH. 2016. The genus *Cookeina*. Mycosphere 7 (9): 1399-1413. DOI: 10.5943/mycosphere/7/9/13.

Fatchiyah F, Arumingtyas EL, Widyarti S, Rahayu S. 2011. Biologi Molekular: Prinsip Dasar Analisis. Erlangga, Jakarta. [Indonesian]

Fujita SI, Senda Y, Nakaguchi S, Hashimoto, T. 2001. Multiplex PCR using internal transcribed spacer 1 and 2 regions for rapid detection and identification of yeast strains. J Clin Microbiol 39 (10): 3617-3622. DOI: 10.1128/jcm.39.10.3617-3622.2001.

Guzmán G, Cortés-Pérez A, Guzmán-Dávalos L, Ramírez-Guillén F, Sánchez-Jácome MDR. 2013. An emendation of Scleroderma, new records, and review of the known species in Mexico. Revista Mexicana de Biodiversidad 84: S173-S191. DOI: 10.7550/rmb.31979.

Guzman G, Montoya L, Salmones D, Bandala VM. 1993. Studies of the genus *Pleurotus* (Basidiomycotina), II. P. djamour in Mexico and in other Latin-American countries, Taxonomic confusions, distribution and semi-industrial culture. Cryptogamic Botany 3: 213-220.

Hall BG. 2013. Building phylogenetic trees from molecular data with MEGA. Mol Biol Evol 30 (5): 1229-1235. DOI: 10.1093/molbev/mst012.

Handoyo D, Rudiretna A. 2001. General principles and implementation of polymerase chain reaction. Unitas 9 (1): 17-29.

Hawkeswood T, Sommung B, Sommung A. 2021. First record of Marasmius haematocephalus (Mont.) Fr. (1838) (Basidiomycota: Marasmiaceae) from Sisaket Province, Thailand. Caloderma 940: 1-3.

Hebert PD, Cywinska A, Ball SL, deWaard JR. 2003. Biological identifications through DNA barcodes. Proc Biol Sci 270 (1512): 313-321. DOI: 10.1098/rspb.2002.2218.

Hibbett DS, Ohman A, Glotzer D, Nuhn M, Kirk P, Nilsson RH. 2011. Progress in molecular and morphological taxon discovery in Fungi and options for formal classification of environmental sequences. Fungal Biol Rev 25 (1): 38-47. DOI: 10.1016/j.fbr.2011.01.001.

Hibbett DS, Taylor JW. 2013. Fungal systematics: is a new age of enlightenment at hand?. Nat Rev Microbiol 11 (2): 129-133. DOI: 10.1038/nrmicro2963.

Holec J. 2005. The genus *Gymnopilus* (Fungi, Agaricales) in the Czech Republic with respect to collections from other European countries. Acta Musei Nationalis Pragae 61 (1-2): 1-52.

Jensen-Vargas E, Marizzi C. 2018. DNA Barcoding for identification of consumer-relevant fungi sold in New York: A powerful tool for citizen scientists? Foods 7: 87. DOI: 10.3390/foods7060087.

Knudson AG. 2012. The Genus *Ramaria* in Minnesota. [Thesis]. University Of Minnesota, Minnesota.

Kress WJ. 2017. Plant DNA barcodes: Applications today and in the future. J Syst Evol 55 (4): 291-307. DOI: 10.1111/jse.12254.

Kuo M. 2010. *Climacodon septentrionalis*. http://www.mushroomexpert.com/climacodon_septentrionalis.html. [22 January 2020]

Kuo M. 2012. *Hebeloma albidulum*. https://www.mushroomexpert.com/hebeloma_albidulum.html. [25 February 2020]

Kuo M. 2016. *Lycoperdon pyriforme*. https://www.mushroomexpert.com/lycoperdon_pyriforme.html. [25 February 2020]

Kusrinah K, Kasiamdari RS. 2017. Morphological characteristics and kinship relationship of mushroom *Schizophyllum commune* Fr. J Nat Sci Mathematic Res 1 (2): 65. DOI: 10.21580/jnsmr.2015.1.2.1620.

Lindahl BD, Nilsson RH, Tedersoo L, Abarenkov K, Carlsen T, Kjøller R, Kõljalg U, Pennanen T, Rosendahl S, Stenlid J, Kauserud H. 2013. Fungal community analysis by high-throughput sequencing of amplified markers-a user's guide. New Phytol 199: 288-299. DOI: 10.1111/nph.12243.

Liu G, Song D, Liu JH, Zhao D, Zhou Y, Ou J, Sun S. 2006. Characterization of the basidiomycetes *Thelephora ganbajun* Zang and *Termitomyces albuminosus* (Berk.) Heim by fourier transform infrared spectroscopy. Intl Soc Optics Photonics 6047: 60471U. DOI: 10.1117/12.710905.

Schoch CL, Seifert KA, Huhndorf S, Robert V, SpougeJL, Levesque CA, Chen W. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc National Acad Sci 109 (16): 6241-6246. DOI: 10.1073/pnas.1117018109.

Spirin V, Malysheva V, Yurkov A, Miettinen O, Larsson KH. 2018. Studies in the *Phaeotremella foliacea* group (Tremellomycetes, Basidiomycota). Mycol Prog 17: 451-466. DOI: 10.1007/s11557-017-1371-4.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30 (12): 2725-2729. DOI: 10.1093/molbev/mst197.

Tripp EA, Lendemer JC. 2014. Sleepless nights: When you can't find anything to use but molecules to describe new taxa. Taxon 63 (5): 969-971. DOI: 10.12705/635.1.

Vydryakova GA, Morozova OV, Redhead SA, Bissett J. 2014. Observations on morphologic and genetic diversity in populations of *Filoboletus manipularis* (Fungi: Mycenaceae) in southern Viet Nam. Mycology 5 (2): 81-97. DOI: 10.1080/21501203.2014.902402.

Xu J. 2016. Fungal DNA barcoding. Genome 59 (11): 913-932. DOI: 10.1139/gen-2016-0046.

Yahr R, Schoch CL, Dentinger BT. 2016. Scaling up discovery of hidden diversity in fungi: Impacts of barcoding approaches. Philos Trans R Soc Lond B Biol Sci 371: 1702). DOI: 10.1098/rstb.2015.0336.

Yang F, Ding F, Chen H, He M, Zhu S, Ma X, Jiang L, Li H. 2018. DNA Barcoding for the Identification and authentication of animal species in traditional medicine. Evid Based Complement Alternat Med 2018: 5160254-5160254. DOI: 10.1155/2018/5160254.