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Identification of Common Edible and Medicinal Mushrooms by DNA Barcoding

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ABSTRACT

Objective: To identify common edible and medicinal mushrooms using the internal transcribed spacer (ITS) region.
Method: A total of eighty-five samples belonging to forty-three species were used in this study. ITS regions were amplified and sequenced. All ITS regions were analyzed using BLAST and MEGA 5.10 methods.
Results: Forty species were identified correctly via BLAST method whereas forty-one species were distinguished via Neighbor joining (NJ) tree. All the species could be distinguished by BLAST combined with NJ tree and ITS sequence characteristics.
Conclusion: Forty-three common species of edible and medicinal mushrooms were effectively identified using DNA barcoding technology based on ITS region.
Keywords: Edible mushroom, Medicinal mushroom, ITS, DNA Barcoding
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INTRODUCTION

Edible and medicinal mushrooms, whose history can be traced back to 4000 years, are treasured as a health food because of their unique edible and medicinal values[1]. Studies have shown that edible mushrooms are low in calories and fat, but rich in proteins, amino acids, polysaccharides, dietary fiber, and mineral elements[2-4]. Edible mushrooms also have anti-tumor, anti-viral, blood lipid reducing, and immunity promoting effects[5-8]. In recent years, increasing attention has been given to edible and medicinal mushrooms because of their special nutritional and medicinal attributes. The United Nations Food and Agriculture Organization proposed that a rational diet should be "meat, vegetable, and mushroom every meal". China has abundant edible and medicinal mushrooms resources. Reports have stated that more than 1000 wild species have been found in China. More than 100 species have also been cultivated and more than 80 species can be bought in markets as common edible and medicinal mushrooms[9]. Common edible and medicinal mushrooms include the *Lentinula edodes*, *Flammulina velutipes*, *Auricularia auricula-judae*, *Hericium erinaceum*, *Tricholoma matsutake,* and so on. The special nutritional value and growing market demand for mushrooms, has resulted in higher prices, particularly for rare and wild species. Therefore, other fungi species are usually used to adulterate wild rare edible mushrooms. Unfortunately, species cannot be identified solely on morphological characteristics and fruiting body. Synonyms and homonyms are also so widespread that it could confuse consumers and disrupt the market. For example, adulterants of *Ophiocordyceps sinensis* included *Cordyceps gunnii*, *Cordyceps militaris, Cordyceps cicadae*, and so on[10]. In addition, a significant number of poisoning through the accidental ingestion of wild mushroom pose a significant threat to food and drug safety[9,11,13]. Currently, studies on fungal identification have focused mainly on traditional morphological identification. Molecular marker methods, such as Restriction Fragment Length Polymorphism and random amplified polymorphism DNA, are used mostly in strain breeding and identification. Unfortunately, these methods fail to achieve accurate and efficient identification results[14,15]. Hence, exploring a novel and powerful identification method for common edible and medicinal mushrooms to ensure food and drug safety is necessary.

DNA barcoding is a novel and powerful identification method, which has been applied widely to species identification[16-20]. Cytochrome *c* oxidase subunit 1 (COI) region has been used as a universal barcode for animals[21]. Internal transcribed spacer 2 region complemented with *psbA-trnH* has been proposed as a candidate DNA barcode for identification of medicinal plants[22,23]. Internal transcribed spacer (ITS) region has also been suggested as a candidate barcode for fungal identification and has been used in the successful identification of many edible and medicinal mushrooms at the species level, such as *O. sinensis, Boletus roseoflavus,* and *B. brunneissimus*[10,24,25]. Therefore, in this paper, ITS region was used as a universal barcode to identify common edible and medicinal mushrooms.

Table 1. Information on the samples

Samples						
Order	**Family**	**Species**	**Sample no.**	**Voucher ID**	**GenBank ID**	**Sample source**
Agaricales	Agaricaceae	*Calvatia gigantea*	1	ZJ0001MG01	KU836552	Jilin Province
		Calvatia utriformis (*Handkea utriformis*)	2	ZJ0002MG01-02	KU836553-54	Neimeng Province
		Lycoperdon perlatum	2	ZJ0004MG01-02	KU836556-57	Kangding City of Sichua Province
		Coprinus comatus	1	ZJ0006MG01	KU836594	Sichuan Province
	Boletaceae	*Suillus granulatus*	3	ZJ2001NGJ01-03	KU855375-77	Yunnan Province
	Cortinari-aceae	*Cortinarius claricolor*	2	ZJ0004SMJ01-02	KU836523-24	Kangding City of Sichua Province
	Hygrophor-aceae	*Hygrophorus russula*	4	ZJ0002LS01-03	KU836531-33	Sichuan Province
				ZJ0002LS04	KU836534	Jilin Province
	Lyophyll-aceae	*Hypsizygus marmoreus*	6	ZJ0001LZS01-06	KU836539-44	Markets
		Lyophyllum connatum	1	ZJ0002LZS01	KU836558	Tibet
		Lyophyllum shimeji	1	ZJ0004LZS01	KU836561	Yunnan Province
	Marasmi-aceae	*Gymnopus confluens*	1	ZJ0002XPS01	KU836528	Kangding City of Sichua Province
	Physalacri-aceae	*Armillaria tabescens*	1	ZJ0001PHJ01	KU836590	Jilin Province
		Flammulina velutipes	1	ZJ0002PHJ01	KU836596	Markets
	Pleurotaceae	*Pleurotus citrinopileatus*	5	ZJ0001CE01-04	KU836565-68	Heilongjiang Province
				ZJ0001CE05	KU836569	Northeast of China
		Pleurotus eryngi	2	ZJ0002CE01	KU836570	Fujian Province
				ZJ0002CE02	KU836571	Markets
		Pleurotus nebrodensis	2	ZJ0003CE01-02	KU836572-73	Markets
		Pleurotus ostreatus	1	ZJ0004CE01	KU836574	Tibet
	Pluteaceae	*Volvariella volvacea*	2	ZJ0001GBJ01-02	KU836588-89	Yunan Province
	Schizophyll-aceae	*Schizophyllum commune*	3	ZJ0001LZJ01-03	KU836575-77	Jilin Province
	Strophari-aceae	*Hypholoma capnoides*	1	ZJ0002QGG01	KU836535	Kangding City of Sichua Province
		Pholiota squarrosa	2	ZJ0006QGG01-02	KU836563-64	Tibet
		Pholiota microspora (*Pholiota nameko*)	1	ZJ0005QGG01	KU836562	Heilongjiang Province
		Stropharia rugosoannulata	1	ZJ0001QGG01	KU836595	Sichuan Province
	Tricholomat-aceae	*Infundibulicybe gibba* (*Clitocybe gibba*)	3	ZJ2001BM01-03	KU855378-80	Kangding City of Sichua Province
		Laccaria proxima	1	ZJ0011BM01	KU836593	Kangding City of Sichua Province
		Lepista nuda	1	ZJ0009BM01	KU836551	Yunan Province
		Leucopaxillus giganteus	1	ZJ0010BM01	KU836592	Heilongjiang Province
		Tricholoma matsutake	4	ZJ0002BM01	KU836579	Yunan Province
				ZJ0002BM02, 03	KU836580, 81	Linzhi City of Tibet
				ZJ0002BM04	KU836582	Tibet
		Tricholoma populinum	3	ZJ0003BM01-03	KU836583-85	Neimeng Province
		Tricholoma squarrulosum	1	ZJ0004BM01	KU836586	Kangding City of Sichua Province
		Tricholoma virgatum	1	ZJ0001BM01	KU836578	Kangding City of Sichua Province
Auriculari-ales	Auriculari-aceae	*Auricularia auricula-judae*	2	ZJ2001MR01-02	KU855346-47	Qinling of Shanxi Province
		Auricularia polytricha	2	ZJ2002MR01	KU855348	Yunan Province
				ZJ2002MR02	KU855349	Shanxi Province
Pezizales	Tuberaceae	*Tuber indicum*	2	ZJ2001KJ02-03	KU855365, 66	Panzhihua City of SichuanProvince
	Morchell-aceae	*Morchella eximia*	2	ZJ2001YDJ01	KU855368	Hainan Province
				ZJ2001YDJ02	KU855369	Yunan Province
Russulales	Hericiaceae	*Hericium erinaceum*	2	ZJ2001HTJ01-02	KU855350-51	Neimeng Province
	Russulaceae	*Russula rosea*	2	ZJ2001HG01-02	KU855370-71	Jilin Province
		Russula turci	1	ZJ2003HG01	KU855374	Kangding City of Sichua Province
		Russula vesca	2	ZJ2002HG01-02	KU855372-73	Panzhihua City of SichuanProvince

Table 1. (Continued)

Samples						
Order	**Family**	**Species**	**Sample no.**	**Voucher ID**	**GenBank ID**	**Sample source**
Polyporales	Polypor-aceae	*Lentinula edodes*	6	ZJ1001DKJ01-04 ZJ1001DKJ05 ZJ1001DKJ06	KU863030-33 KU863034 KU863035	Yunan Province Markets Panzhihua City of SichuanProvince
		Neolentinus lepideus	1	ZJ1004DKJ01	KU863047	Heilongjiang Province
		Polyporus umbellatus	1	ZJ1008DKJ01	KU863055	Eastern of China
	Fomitopsid-aceae	*Laetiporus sulphureus* var. *miniatus*	1	ZJ1029NCKJ01	KU863098	Jilin Province

MATERIALS AND METHODS

1. Materials

Eighty-five samples representing forty-three species from five orders were collected from wild areas and markets (Table 1). The fifteen representative species of common edible and medicinal mushrooms are shown in Supplemental Figure S1.

All samples were identified by morphology by Professor Xiaolan Mao from the Institute of Microbiology of the Chinese Academy of Sciences. Voucher samples were deposited at the Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences. All ITS sequences were submitted to GenBank (Table 1).

2. Methods

2.1 DNA extraction

Total genomic DNA was extracted from 20–40 mg dry internal tissue of eighty-five samples following the protocol of the Plant Genomic DNA Kit (Tiangen Biotech Co.) and previous studies[26]. The materials were mashed for 200 s at 50 HZ by a DNA Extraction Grinder (Sceintz Biotech Co., China). Then, 800 μL nucleus separation liquid (100 mmol•L^{-1} Tris-HCl (pH 8.0), 20 mmol•L^{-1} EDTA (pH 8.0), 0.7 mol•L^{-1} NaCl, 2% PVP-40, 0.4% β-mercaptoethanol) was added once or several times to clean the materials until they were colorless.

2.2 PCR amplification and sequencing

Primers of ITS region: ITS5F (5′-GGAAGTAAAAGTCGTAACAAGG-3′), ITS4R (5′-TCCTCCGCTTATTGATATGC-3′). PCRs were performed in 25 μL reaction mixtures containing 4 μL of genomic DNA, 12.5 μL of 2×Taq PCR Master Mix (Aidlab Biotech Co., China), 1 μL of each primer (2.5 μmol•L^{-1}). The mixtures were adjusted to 25 μL with distilled deionized water. Reaction conditions were as follows: 94 °C 5 min; 94 °C 1 min, 50 °C 1 min, 72 °C 90 s, 30 cycles; 72 °C 7 min. After obtaining the PCR products, electrophoresis (1.0% agarose gel in 0.5×TBE purified buffer), purification, and sequencing (3730 XL Sequencer, Applied Biosystems) were performed.

2.3 Data analysis

Trimming and assembling trace files were performed using CondonCode Aligner V 3.0 (CondonCode Co., USA). The ITS sequences were obtained by annotation based on the Hidden Markov model. All sequences were blasted in the NCBI database, then aligned through Muscle and ClustalW analysis and analyzed by MEGA 5.10 (Center for Evolutionary Medicine and Information, USA). The neighbor joining (NJ) trees were constructed based on the Kimura 2-Parameter model.

RESULTS

1. DNA extraction and PCR amplification

Total genomic DNA of eighty-five samples was extracted by an optimal DNA extraction method. Eighty-five available ITS sequences were obtained after amplification and sequencing and subsequently submitted to GenBank (Table 1).

2. Sequence characteristics analysis

The sequence characteristics are shown in Table 2. All ITS sequences were more than 500 bp in length except for *H. russula* (464 bp). The longest sequence was that of *A. tabescens* (751 bp). The ITS sequences belonging to the same genus were similar in length, such as *P. citrinopileatus* (581 bp), *P. eryngi* (589 bp) and *P. ostreatus* (587 bp) of *Pleurotus* genus; *T. populinum* (618 bp), *T. squarrulosum* (608 bp) and *T. virgatum* (614 bp) of *Tricholoma* genus; *A. auricula-judae* (514 bp) and *A. polytricha* (520 bp) of *Auricularia* genus.

The lowest GC content was 37.2% (*H. russula*) and the highest was 59.4% (*V. volvacea*). The GC contents of four species were lower than 40%, including *H. russula* (37.2%), *L. giganteus* (37.8%), *L. edodes* (38.4%), and *I. gibba* (39.0%). GC contents of six species were higher than 50%, including *V. volvacea* (59.4%), *S. granulatus* (53.4%), *F. velutipes* (50.2%), *A. auricula-judae* (50.1%), *M. eximia*, (51.5%) and *L. sulphureus* var. *miniatus* (51.7%). In addition, the GC contents of thirty-three species ranged from 40% to 50%. The average GC content of thirty-one species belonging to Agaricales order was 44%. Four species of Russulaceae order had GC contents in close range, including *H. erinaceum* (48.4%), *R. rosea* (49.5%), *R. turci* (48.2%) and *R. vesca* (49.5%).

The ITS sequences of mushrooms belonging to the same genus had similar lengths and GC contents. For example, *Tricholoma* genus: *T. populinum* (618 bp; 43.2%), *T. squarrulosum* (608 bp; 43.4%) and *T. virgatum* (614 bp; 42.0%); *Auricularia* genus: *A. auricula-judae* (514 bp; 50.1%) and *A. polytricha* (520 bp; 49.4%).

For ITS sequences, ten species *(S. granulatus, C. claricolor, P. eryngi, P. nebrodensis, V. volvacea, I. gibba, T. indicum,*

Table 2. ITS sequence characteristics of common edible and medicinal mushrooms

Species	Sample no.	Sequence length/bp	Average GC contents/%	No. of intra-specific variable sites	Intra-specific distance (mean)	Inter-specific distance (mean)
C. gigantea	1	661	44.9	—	—	0.137-0.560 (0.323)
C. utriformis	2	646	45.0	1	0.002	0.095-0.537 (0.320)
L. perlatum	2	638-655	43.9	3	0.028	0.095-0.589 (0.327)
C. comatus	1	587	43.4	—	—	0.213-0.587 (0.316)
S. granulatus	3	573	53.4	0	0	0.325-0.621 (0.391)
C. claricolor	2	612	42.3	0	0	0.201-0.527 (0.302)
H. russula	4	464	37.2	2	0-0.004 (0.002)	0.165-0.512 (0.276)
H. marmoreus	6	606	45.7	4	0-0.067 (0.003)	0.088-0.516 (0.272)
L. connatum	1	579	46.6	—	—	0.088-0.487 (0.279)
L. shimeji	1	626	45.7	—	—	0.106-0.565 (0.287)
G. confluens	1	728	42.6	—	—	0.299-0.567 (0.366)
A. tabescens	1	751	44.9	—	—	0.288-0.619 (0.364)
F. velutipes	1	642	50.2	—	—	0.323-0.609 (0.399)
P. citrinopileatus	5	581	46.1	3	0-0.021 (0.008)	0.179-0.548 (0.324)
P. eryngi	2	589	44.1	0	0	0.066-0.584 (0.331)
P. nebrodensis	2	677	44.0	0	0	0.046-0.571 (0.312)
P. ostreatus	1	587	43.3	—	—	0.046-0.551 (0.299)
V. volvacea	2	633	59.4	0	0	0.337-0.643 (0.441)
S. commune	3	568	47.2	2	0-0.004 (0.002)	0.287-0.616 (0.375)
H. capnoides	1	615	40.8	—	—	0.110-0.631 (0.314)
P. squarrosa	2	611	40.3	1	0.012	0.164-0.629 (0.320)
P. microspora	1	637	40.7	—	—	0.190-0.578 (0.315)
S. rugosoannulata	1	590	40.7	—	—	0.110-0.597 (0.300)
I. gibba	3	616	39.0	0	0	0.159-0.542 (0.296)
L. proxima	1	605	42.5	—	—	0.184-0.587 (0.298)
L. nuda	1	602	40.7	—	—	0.134-0.550 (0.260)
L. giganteus	1	643	37.8	—	—	0.192-0.635 (0.323)
T. matsutake	4	598	44.1	1	0-0.002 (0.001)	0.136-0.565 (0.297)
T. populinum	3	618	43.2	3	0-0.005 (0.003)	0.101-0.558 (0.291)
T. squarrulosum	1	608	43.4	—	—	0.101-0.524 (0.260)
T. virgatum	1	614	42.0	—	—	0.115-0.559 (0.301)
A. auricula-judae	2	514	50.1	1	0.002	0.237-0.604 (0.402)
A. polytricha	2	520	49.4	2	0.012	0.237-0.627 (0.402)
T. indicum	2	521	44.1	0	0	0.432-0.654 (0.519)
M. eximia	2	631	51.5	0	0	0.471-0.667 (0.565)
H. erinaceum	2	548	48.4	0	0	0.255-0.558 (0.323)
R. rosea	2	665	49.5	0	0	0.216-0.667 (0.421)
R. turci	1	608	48.2	—	—	0.216-0.599 (0.420)
R. vesca	2	587	49.5	1	0.002	0.313-0.625 (0.384)
L. edodes	6	655	38.4	8	0-0.011 (0.005)	0.256-0.591 (0.329)
N. lepideus	1	550	47.6	—	—	0.244-0.579 (0.331)
P. umbellatus	1	599	44.6	—	—	0.263-0.654 (0.387)
L. sulphureus var. *miniatus*	1	543	51.7	—	—	0.293-0.536 (0.384)

M. eximia, H. erinaceum, R. rosea) had no variable site. Five species (*C. utriformis, P. squarrosa, T. matsutake, A. auricula-judae* and *R. vesca*) had one variable site. Three species *(H. russula, S. commune* and *A. polytricha*) had two variable sites. Three species (*L. perlatum, P. citrinopileatus* and *T. populinum*) had three variable sites. *H. marmoreus* had four variable sites and *L. edodes* had eight variable sites. On the whole, the ITS sequences of common edible and medicinal mushrooms were conservative.

3. Intra- and inter-specific distance analysis

The intra- and inter-specific K2P distances of ITS sequences are listed in Table 2. The maximum intra-specific K2P distances of twenty-three species were less than that of the minimum inter-specific distances, indicating that these twenty-three species could be distinguished from others by distance method. The other twenty species only had one sequence. The minimum inter-specific K2P distance of *F. velutipes* was 0.323, seven species (*C. comatus*, *G. confluens*, *A. tabescens*, *R. turci*, *N. lepideus*, *P. umbellatus*, *L. sulphureus* var. *miniatus*) were from 0.2 to 0.3, ten species (*C. gigantea*, *L. shimeji*, *H. capnoides*, *P. microspora*, *S. rugosoannulata*, *L. proxima*, *L. nuda*, *L. giganteus*, *T. squarrulosum*, *T. virgatum*) were from 0.1 to 0.2, *L. connatum* and *P. ostreatus*'s were less than 0.1.

4. BLAST analysis

In this study, the highest similarity species of forty species were the species themselves after being blasted in the NCBI database using ITS sequence. The sequence identities were higher than 99%. Therefore, forty species could be successfully identified at the species level by BLAST method in the NCBI website (http://blast.ncbi.nlm.nih.gov/). However, the

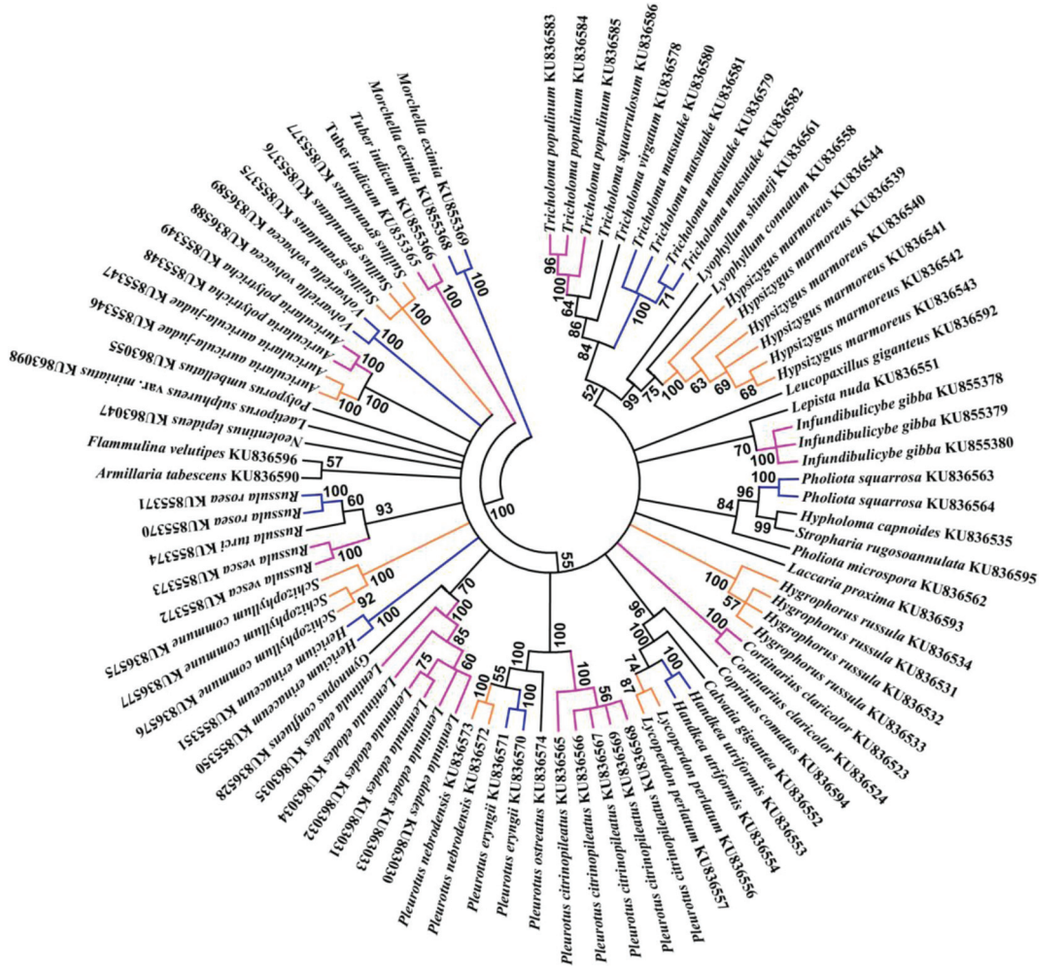

Figure 1. Neighbor-joining (NJ) tree based on ITS sequences of common edible and medicinal mushrooms (Note: The bootstrap values (1000 replicates) were showed (≥50%) for each branch)

highest similarity species of three species were not themselves after being blasted in NCBI database using ITS sequence. The highest similarity species of *P. ostreatus* were species in the same *Pleurotus* genus (*P. cornucopiae*, *P. pulmonarius* and *P. sapidus*) with 100% sequence identities. The highest similarity species of *L. giganteus* was *L. paradoxus* with 99% sequence identities, which was higher than its own 97% sequence identities. The highest similarity species of *L. proxima* was itself, with 83% sequence identities.

5. NJ tree analysis

Two NJ trees were constructed using eighty-five ITS sequences of the forty-three species. But the NJ tree based on Muscle analysis exhibited better topology than the NJ tree based on ClustalW analysis (Figure 1). Forty-one species were formed into separate branches with high bootstrap scores. Moreover, species from the same family clustered together. Species of Lyophyllaceae (*H. marmoreus*, *L. connatum* and *L. shimeji*), Agaricaceae (*C. Gigantea*, *C. utriformis*, *L. perlatum* and *C. comatus*), Strophariaceae (*H. capnoides*, *S. rugosoannulata* and *P. squarrosa*) and Physalacriaceae (*A. tabescens* and *F. velutipes*) family clustered together with bootstrap values of 99%, 96%, 84% and 57%, respectively. Species from the same genus also clustered together. Species from *Auricularia* (*A. auricula-judae* and *A. polytricha*), *Pleurotus* (*P. citrinopileatus*, *P. eryngi*, *P. nebrodensis* and *P. ostreatus*), *Russula* (*R. rosea*, *R. turci* and *R. vesca*) and *Tricholoma* (*T. matsutake*, *T. populinum*, *T. squarrulosum* and *T. virgatum*) genus clustered together with high bootstrap values of 100%, 100%, 93%, and 84%, respectively. However, two ITS sequences of *H. capnoides* and *S. rugosoannulata* of the Strophariaceae family formed into a single branch with high bootstrap value of 99%, and hence requires combining with other methods for authentication.

DISCUSSION

Edible and medicinal mushrooms play an important role in fungi resources because of its high economic value. Hence, accurately authenticating edible and medicinal mushrooms is highly important to ensure the safety in its use. DNA barcoding technology based on ITS region was used to identify common edible and medicinal mushrooms in our study.

Plant Genomic DNA kit was used to extract total genomic DNA. Samples were cleaned with 75% ethanol because the DNA is easy to pollute. The samples were then cleaned

once or several times using the nucleus separation liquid (100 mmol•L^{-1} Tris-HCl (pH 8.0), 20 mmol•L^{-1} EDTA (pH 8.0), 0.7 mol•L^{-1} NaCl, 2% PVP-40, 0.4% β-mercaptoethanol) until the liquid layer became lightly colored or colorless. The total genomic DNA of eighty-five samples was successfully extracted by the modification of a Plant Genomic DNA kit protocol.

Agaricales order, thirty-one species of which were included in this study, was an important taxon of edible and medicinal mushrooms. The results showed that the variable sites of most species were less than three, especially for cultivated mushrooms, such as *A. polytricha* (two variable sites), *A. auricula-judae* (one variable site), *P. eryngi* (no variable site) and *V. volvacea* (no variable site). Li Li also found that the *A. auricula-judae* originated from the same area were with a narrow genetic basis by analysing the ITS and IGS regions[27]. The results further indicated that ITS sequences of cultivated mushrooms were conservative, because of mature cultivation techniques and stable strains[28].

The sequence characteristics combined with BLAST and NJ tree method were used to estimate the capability of distinguishing edible and medicinal mushrooms using DNA barcoding based on ITS region. The results showed that the highest similarity species of forty species were themselves after being blasted in the NCBI database using ITS sequence, while three species, including *P. ostreatus, L. giganteus,* and *L. proxima* were not. Fortunately, *P. ostreatus*, *L. giganteus,* and *L. proxima* could be distinguished from the other forty species by NJ tree method. Furthermore, forty-one species were clearly identified by NJ tree. *H. capnoides* and *S. rugosoannulata* of Strophariaceae family formed into a single branch with high bootstrap value of 99%. This result indicated that the two species has a considerably close genetic relationship. These species could be successfully identified at the species level by BLAST method. Tian Enjing reported a close genetic relationship between *Hypholoma* and *Stropharia* using Bayesian analysis on the basis of rDNA-ITS sequences, which was consistent with our results[29].

In this study, some species only had one ITS sequence respectively. However, despite the limited sequences, the common edible and medicinal mushrooms could still be identified using the combined methods with ITS sequence characteristics, NJ tree and BLAST methods. Both *A. tabescens* and *F. velutipes*, which had only one ITS sequence, were good cases. *A. tabescens* and *F. velutipes* formed into a single branch with a bootstrap value of 57%, but they could be identified by the BLAST method. Because of the difficulty in collecting abundant samples, enough ITS sequences could not be obtained in the present study. Further research on common edible and medicinal mushrooms identification using DNA barcoding could be performed after the supplement of samples.

In conclusion, forty-three common species of edible and medicinal mushrooms could be identified effectively using DNA barcoding technology based on the ITS region. The study also estimated the capability of DNA barcoding technology to authenticate fungi based on the ITS region and established a powerful tool for fungal identification.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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Supplemental Figure S1. Morphological characters of fifteen common species of edible and medicinal mushrooms