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Molecular phylogeny of saprophytic wild edible mushroom species from Tanzania based on ITS and nLSU rDNA sequences

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Abstract

Tanzania is endowed with diversified topographical features rich in indigenous forests which harbor many different saprophytic wild edible mushrooms (SWEM). Few studies have been carried out on characterizing these mushrooms, and those have used conventional methods whereby taxa were characterized using micro- and macromorphological features which are subtle and sometime fail to delimit closely related taxa. In this study, eight SWEM taxa were characterized using two molecular markers—the Internal transcribed spacer (ITS) and the nuclear large subunit (nLSU). The studied sequences were analyzed together with an additional of 19 GenBank sequences of related taxa in the genera *Lentinus*, *Polyporus*, *Panus*, *Macrolepiota* and *Auricularia* with maximum likelihood and *Aspergillus niger* as an outgroup. The BLAST search results on the NCBI database showed that the studied SWEM have ≥ 92% identity for ITS and ≥ 97 % identity for LSU. The phylogenetic tree constructed using the ITS data set revealed two major distinct clades with bootstrap support of 77% and 90% and five sub-clades supporting the five genera. The bootstrap support were 94% for *Lentinus*, 100% for *Polyporus*, 98% for *Panus*, 98% for *Macrolepiota* and 90 % for *Auricularia*, while the nLSU data set revealed the same two major distinct clades but with higher bootstrap support of 91% and 100%. The five subclades again supporting the five genera were 100% for *Lentinus*, 100% for *Pluteus*, 100% for *Panus*, 99% for *Macrolepiota* and 100% for *Auricularia*. From these results, it is clear that both ITS and LSU delineated the SWEM taxa to the six genera. However, the obtained support values showed that ITS sequences have the highest possibility of successful delineating the studied SWEM to species level than LSU. Moreover, the result also showed the genus *Panus* forming a monopyletic clade with *Lentinus* and *Polyporus*, thus contributing towards a better understanding of its problematic taxonomic ambiguities.

Key words – Indigenous forest – ITS – LSU – Saprophytic – SWEM

Introduction

Classification system for gilled fungi and their allies (Basidiomycota) is increasingly relying on molecular data. Morphological information has been shown to be of limited value for fungal systematic due to their inherent simplicity, evolutionary convergence, parallelisms, and phenotypic plasticity (Hofstetter et al. 2002). Few wild mushrooms are deadly poisonous and many more are mildly poisonous. Morphologically some deadly poisonous mushrooms look like edible species, for example *Volvariella volvacea* (edible) may be confused with *Amanita phalloides*, which is deadly

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poisonous (Tibuhwa 2013). Therefore, correct mushroom identification is very important to avoid harm that may be a result of eating poisonous mushrooms. In different part of the world, information on how to recognize and differentiate between edible and none edible mushroom is largely based on culture traditions, and vernacular naming systems "folk taxonomy" are used. Folk taxonomy is distinguished from scientific taxonomy in that it remains within social relations and hence un-universal (Tibuhwa 2012, 2013).

Taxonomic and phylogenetic studies of Basidiomycota have been based mainly on the analysis and comparison of morphological characters like the shape, size, and color of caps and gills (Lee et al. 2006, Tibuhwa et al. 2012). These methods for identifying mushrooms using morphological characteristics are subtle hence unreliable (Lian et al. 2008). With current advances in biotechnology, molecular genetic markers have been used for rapid identification of different mushrooms (Frøslev et al. 2005, Moreau et al. 2006). These tools offer a more accurate and reliable method for identification than the traditional method (Fonseca et al. 2008). The techniques for analyzing the structures of nucleic acids (DNA and RNA) (Guglielmo et al. 2010) have made additional characters available to systematic mycology. Nucleic acid technologies have provided new insights into the evolutionary relationships and the diversity among mushrooms (Francis & Bougher 2003).

Comparative analysis of coding and non-coding regions of ribosomal DNA has become a widely used tool for construction of phylogenetic trees of many organisms including mushrooms. Internal Transcribed Spaces (ITS) are the proposed standard bar-coding marker for fungi (Schoch et al. 2012). The ITS region is perhaps the most extensively sequenced DNA region in fungi. This region has higher degree of variation than other genetic regions of rDNA (Gardes & Bruns, 1993) and are polymorphic thus provide sequence variability that allows distinguishing among different species or strains of mushrooms (Martin et al. 2004).

In Tanzania, few studies have been conducted on identification of wild edible mushrooms. With the exception of those done by Muruke et al. (2002) who used molecular markers on mushroom mycelia; Tibuhwa et al. (2012) and De crop et al. (2013) who used both morphological and molecular markers for the genera *Cantharellus* and *Lactarius*, other studies based on macro-micromorphological characters (Härkönen et al. 2003, Magingo et al. 2004, Tibuhwa et al. 2008, Tibuhwa et al. 2010, Tibuhwa 2011, 2012, 2013). This study therefore used nucleotide sequence data from the internal transcribed spacer region of the nuclear ribosomal (ITS) and the nuclear ribosomal large subunit gene (nLSU) to characterise selected Tanzanian SWEM isolates.

Materials & Method

Study site and sample collection

The study was conducted within natural forests of Lutindi-vue, Shume Magamba, Shaghayu, Kieti and Kunga in Tanga region, and Kazimzumbwi forest near Kisarawe town in the Coast region in Tanzania (Figure 1). These natural forests contain natural vegetation which provides favorable conditions for mushrooms to grow. The study samples were collected during rainy seasons from April to May and November to December 2011/12. The collected fresh fruit bodies were examined in fresh conditions as explained in Tibuhwa et al. (2008). Photographs of the fruit bodies were taken before and after picking from their substrates; features of the habitat and some features of the specimen including color of the cap, stem and gills and mode of attachment were recorded. Some features like diameter of the gills and length of the stem were also measured. Fruit bodies were preserved in silica gel for molecular characterization and some were dried at 50°C over night for herbarium deposit (DSM) at the University of Dar es Salaam.

DNA extraction

DNA was extracted from dried fruit body specimens using a modified cetyl-trimethyl ammonium bromide (CTAB) extraction method as detailed in Muruke et al (2002). This method has proven to be useful for DNA extraction of herbarium specimen, field collected specimen and woody

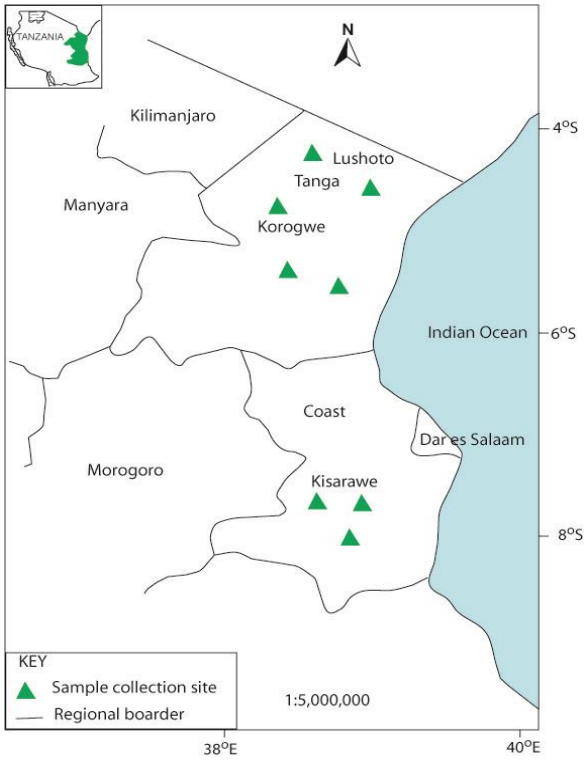


Fig. 1 The Map of Tanzania showing the sampling sites

basidiomata bearing high content of secondary metabolite and high polysaccharide content (Góes-Neto et al. 2011). The mushroom samples that were previously collected and preserved in silica gel were crushed to powder using a motor and pestle with liquid nitrogen added to aid the process. About 30-50 mg of powered mushroom samples were added to 1.5 mL eppendorf tubes and labeled accordingly. To each sample 450 µL of preheated (65°C) extraction buffer (100 mM Tris-HCl [pH 8], 1.4 M NaCl, 20 mM EDTA, CTAB [2.5% w/v], Dithiol threitol solution (DTT) [1% v/v] and polyvinyl pyrolidone (PVP) 1% w/v) were added. The samples were incubated in a water bath at 65°C for 45 minutes. An equal volume (450 µL) of chloroform-isoamylalcohol (24:1) were added to each sample and the tubes were inverted twice to mix. The mixtures were centrifuged at 12000 rpm (eppendorf centrifuge 5424) for 15 minutes. A fixed volume of 400 µL of supernatants was transferred to new 1.5 mLeppendorf tubes. To precipitate DNA 0.7 volumes (280 µL) of cold isopropanol (stored at -20 °C) was added to the samples and inverted twice to mix to aid precipitation. The samples were incubated at -20 °C for three hours and then centrifuged at 12000 rpm (eppendorf centrifuge 5424R) for 15 minutes at -4°C. The supernatants were decanted and the pellets (crude DNA) were air dried for 30 minutes. Samples were washed twice with 70% ethanol and centrifuged at 12000 rpm (eppendorf centrifuge 5424R) for 15 minutes at -4°C and supernatants were decanted. The pellets were suspended in 200 µL of low-salt TE (10 mM Tris, 0.1 mM EDTA [pH 8]). Three (3) µL of RNAse (10 mg/mL) was added to each sample and followed by incubation in a water bath at 37 °C for 30 minutes. After incubation, 450 µL of chloroform-isoamylalcohol (24:1) were added to each sample and the tubes inverted twice to mix. The samples were centrifuged at 12000 rpm (eppendorf centrifuge 5424R) for 15 minutes at -4°C. A fixed volume of 150 µL of the supernatant layer was transferred to the fresh-labeled eppendorf tubes 1.5 mL. Purification was done by adding 315 µL of µL ethanol-acetate solution (30mL EtOH, 1.5mL 3 M NaOAc [pH 5.2]) to each sample and kept in –20°C for three hours. Samples were centrifuged at 12000 rpm (eppendorf centrifuge 5424R) for 15 minutes at -4°C. The supernatants were decanted and the pellets were washed with 100 µL of 70% ethanol. The samples were centrifuged at 12000 rpm (eppendorf centrifuge 5424R) for 15 minutes at -4°C, the supernatants were decanted, and pellets were air-dried for 30 minutes. They were finally suspended in 100 µL of low-salt TE (10 mM Tris, 0.1 mM EDTA [pH 8]).

DNA quality evaluation

Evaluation of DNA quality was performed according to Góes-Neto et al. (2011) with some modifications. Five parameters were used to evaluate the quality of DNA namely: (i) DNA condition, (ii) color, (iii) spectral absorbance ratio (A260/280), (iv) Final concentration (ng DNA/µL purified DNA), and (v) Purified PCR amplification products of ITS and nLSU. Quality of the amplified DNA was examined by agarose gel (0.8%) prepared in 0.5X Tris-Borate EDTA buffer, the DNA stained with 2.5 µL gel red while the marker used was Lambda DNA ladder (20 ng/ µL). An equal proportion of 3 µL of DNA and loading dye were loaded to the wells. The gel was set to run at 100V for 45 minutes. The DNA condition was categorized as (1) high molecular weight DNA, no degradation, (2) somewhat degraded, but still showing a band with high molecular weight DNA, or (3) highly degraded and/or with a low molecular weight DNA band. The color of DNA solutions was classified, as (1) hyaline, sub-hyaline or whitish, (2) colored (yellowish) but still clear, (3) dark, opaque (Góes-Neto et al. 2011). Concentration of DNA was determined by nanodrop spectrophotometer (Thermo Scientific NanoDrop 2000) with absorbance (A 260/280).

PCR amplification of extracted DNA

PCR reactions were performed in a Techne TC plus Thermocyler by using PCR master mix of Bioneer kit (Accu Power ®, Taq PCR premix). To each well of Bioneer kit, a total volume of 20 µL of reaction mixture containing 2 µL of diluted template DNA (20ng/ µL), 0.8 µL of each primer and 16.4µL milli Q water was added (Vellinga 2004). A positive control of known mushroom DNA was used, and negative control contained all reagents except DNA template was done to test the presence of contamination. The cycling system was as described in Tibuhwa et al. (2012) with slight modification whereby the reaction started with 5 min initial denaturation at 95 °C, followed by 35 cycles of 30 seconds denaturation at 95 °C, 30 seconds annealing at 60 °C, 1 min extension at 72 °C with a final extension step at 72 °C for 10 minutes. Reaction was stopped by chilling to 10 °C. PCR products were analyzed by electrophoresis whereby agarose gel 1.8% prepared in 0.5X Tris-Borate EDTA buffer with addition of 2.5 µL gel red was used together with Thermo Scientific GeneRuler 100 bp DNA Ladder 100-1000 bp. Amplicons were purified using Thermo Scientific Gene JET PCR purification kit following the manufacture's protocol. Concentration of purified amplicons was determined by nanodrop spectrophotometer (Thermo Scientific NanoDrop 2000) at absorbance (A 260/280). Sequencing was done at BeCA ILRI-Hub Segoli Unit using Applied BioSystems sequencer (ABI Prism 3730 Genetic Analyzer, Applied BioSystems) according to the manufacturer's instructions.

Data analyses

A total of 54 sequences, 14 generated in this study, 40 from the GenBank were involved in the analysis as summarized in Table 1. Sequences were assembled and manually examined for errors using CLC Workbench software and the amplified regions were aligned using CLUSTAL W (Thompson et al. 1994) in MEGA version 6 (Tamura et al. 2013) with default settings. Phylogenetic relationships were analyzed using the Maximum Likelihood method and Neighbor joining based on the Kimura 2-parameter model (1980) with a Bootstrap analysis involving 1000 replication rounds. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pair wise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. Identity matrixes were calculated by using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/).

Results

This study examined eight SWEM species from indigenous forest in Tanzania. The electropherogram of genomic DNA, PCR products of ITS and LSU regions are shown in Figure 2. Results of quality of extracted DNA and PCR products which showed high variation are summarized in Table 2.

Table 1 Specimens and sequences used in this study, with their respective voucher information. GenBank accession numbers in italics represent sequences published here for the first time

No	Specie	Voucher	Collection no.	ITS-Accesion #	LSU - Accession #
1	*Pluteus umbrosus*	JMH32	Hussein 32.2013		*KM267732*
2	*Polyporus tenuiculus*	JMH33	Hussein 33.2013	*KM267725*	
3	*Lentinus sajor-caju*	JMH36	Hussein 36.2013	*KM267726*	*KM267733*
4	*Lentinus squarrosulus*	JMH41	Hussein 41.2013	*KM267727*	*KM267734*
5	*Macrolepiota procera*	JMH42	Hussein 42.2013	*KM267728*	*KM267735*
6	*Auricularia polytricha*	JMH43	Hussein 43.2013	*KM267729*	*KM267736*
7	*Panus conchatus*	JMH44	Hussein 44.2013	*KM267730*	*KM267737*
8	*Auricularia auricula-judae*	JMH45	Hussein 45.2013	*KM267731*	*KM267738*
9	*Pluteus umbrosus*	DAOM197235	Moncalvo et al.	–	AF261580.1
10	*Pluteus primus*	isolate JB94/	Moncalvo et al.	–	AF042610.1
11	*Pluteus leoninus*	voucher AJ212	Justo, et al.	–	HM562234.1
12	*Polyporus tenuiculus*	strain ML284	Ota & Hattori	Q409357.1	–
13	*Polyporus tenuiculus*	isolate HE2934	Sun et al.	KC505555.1	–
14	*Polyporus tenuiculus,*	strain: WD1576	Sotome et al.	AB587633.1	–
15	*Lentinus sajor-caju*	isolate TFB11739	Grand et al.	GU207308.1	–
16	*Lentinus sajor-caju*	strain VKGJ02	Johnsy & Kaviy.	JQ428820.1	
17	*Lentinus sajor-caju*	isolate 11739	Grand	–	AY615989.1
18	*Lentinus sajor-caju*	isolate 11731	Grand	–	AY615990.1
29	*Lentinus sajor-caju*	isolate 11736	Grand		AY615988.1
20	*Lentinus squarrosulus*	strain S3016	Grand	JQ868749.1	
21	*L. squarrosulus*	strain 7-4-2 18S	Cao & Bao	GU001951.1	JN710579.1
22	*L. squarrosulus*	DMC 178	Douanla et al.		EU908178.1
23	*L. squarrosulus*	strain C500 l	Moncalvo et al.	–	AF261563.1
24	*L. squarrosulus*	strain C500 l	Douanla et al.	–	EU908176.1
25	*Macrolepiota neomastoidea*	–	Vellinga et al.	AF482845.1	–
26	*Lepiota cf. atrodisca*	ecv3265	Vellinga & Balsley	GU903302.1	–
27	*Macrolepiota sp*	P 36	Lebel	JF495071.1	
28	*Lepiota sp.*	MB56	Matheny et al.	–	EF561634.1
29	*Lepiota procera*	strain DSH 96-038	Hibbett & Binder	–	AF518628.1
30	*Lepiota neophana*	–	Vellinga et al.	–	HM488785.1
31	*Auricularia polytricha*	Voucher Cui6110		FJ617300.1	
32	*A. polytricha*	voucher Cui6113	Du	FJ617301.1	–
33	*A.polytricha*		Bolsenbroek	AJ537388.1	–
34	*A. polytricha*	strain APTJ6101	Fan et al.	–	KF298022.1
35	*A. polytricha*	strain APXW6621	Fan et al.	–	KF298019.1
36	*A. polytricha*	strain AP910	Fan et al.	–	KF298017.1
37	*Panus strigellus*	INPA243940	Isla et al.		JN710579.1
38	*Panus conchatus*	isolate X1234	Miettinen et al.	JN710579.1	
39	*Panus conchatus*	isolate 6254 l	Grand	–	AY616004.1
40	*Panus conchatus*	isolate X1234 i	Miettinen et al.	–	JN710579.1
41	*Panus conchatus*	isolate 4314	Grand	–	AY616003.1
42	*Auricularia auricula-judae*	strain M26	Liu et al.	HQ388377.1	–
43	*A. auricula-judae*	strain Sw	Liu et al.	HQ388388.1	–
44	*A. auricula-judae*	strain XK8	Liu et al.	HQ388364.1	–
45	*A. auricula-judae*	strain 5L0114	Fan et al.	–	KF297993.1
46	*A. auricula-judae*	strain AU110 1	Li et al.	–	JN712676.1
47	*A. auricula-judae*	Strain HW5D31	Fan et al.	–	KF297994.1

Results of genomic DNA isolated from dried samples

The bands were of different quality due to different concentrations obtained after purification of PCR amplicons as shown in Electropherogram Figure 2. Using ITS5 and ITS4 primers, single amplified product was observed that corresponded to expected rDNA target region fragment sized

Table 2 Quality and quantity of extracted DNA and PCR product

Sample ID.	Species Nomenclature	DNA[a] condition	Color[b]	$A_{260/280}$[c]	Conc. (ng/µL) [d]	Purified PCR Product [e] Conc. (ng/µL)	
						ITS	nLSU
32	*Pluteus umbrosus*	2/2	2/2	2/2	2/2	3/3	2/2
33	*Polyporus tenuiculus*	2/2	2/2	2/2	2/2	3/3	2/2
36	*Lentinus sajo-caju*	3/3	2/2	2/2	1/1	3/3	2/2
36-1	*Lentinus sajo-caju*	1/1	1/1	3/3	3/3	1/1	1/1
41	*Lentinus squarrolosus*	3/3	2/2	1/1	2/2	2/2	1/1
42	*Macrolepiota procera*	2/2	1/1	2/2	2/2	2/2	2/2
43	*Auricularia polytricha*	3/3	3/3	3/3	3/3	1/1	2/2
44	*Panus conchatus*	1/1	2/2	1/1	2/2	2/2	2/2
45	*Auricularia judae*	3/3	3/3	3/3	3/3	3/3	2/2

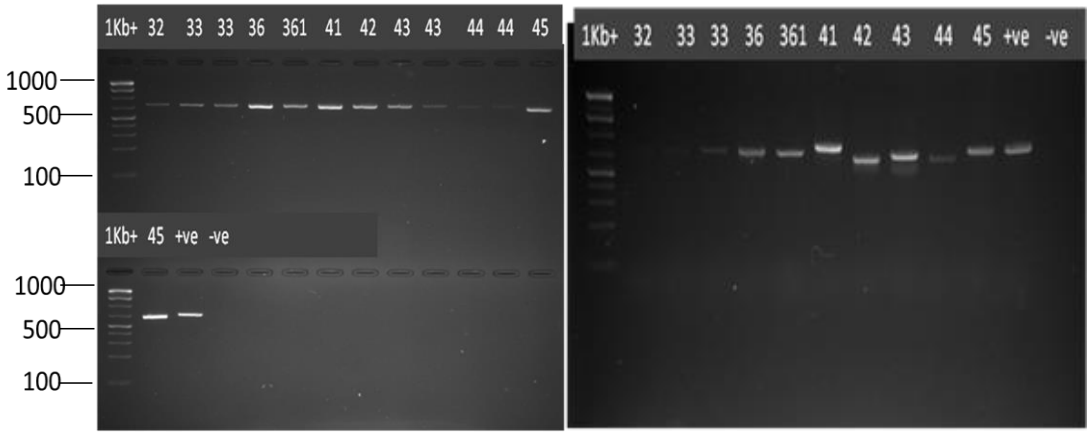
(1) High molecular weight DNA; no degradation, very good DNA condition, (2) somewhat degraded, but still showing a band with high molecular weight DNA, good DNA condition, (3) highly degraded and/or with low molecular weight DNA band, poor DNA condition.

a- (1) Hyaline, sub-hyaline or whitish, (2) colored (yellowish), but still clear, (3) dark, opaque.

b- (1) $A_{260/280}$>1.8, (2) 1.5< $A_{260/280}$<1.8, (3) 1.2< $A_{260/280}$<1.5.

c- Concentration of genomic DNA: Cc >500ng/ µL, (2) 100ng/ µL <Cc<500ng/ µL, (3) Cc<100ng/ µL,

d- Concentration of Purified PCR product: Cc >40ng/ µL, (2) 20ng/ µL <Cc<40ng/ µL, (3) Cc<20ng/ µL.



(i) (ii)

Fig. 2 – Electropherogram showing purified PCR amplification products of (i) ITS region and (ii) nLSU. (32) *Pluteus umbrosus* (33) *Polyporus tenuiculus* (36) *Lentinus sajo-kaju* (36-1) *Lentinus sajo-kaju* (Domesticated) (41) *Lentinus squarrosulus* (42) *Macrolepiota procera* (43) *Auricularia polytricha* (44) *Panus conchatus* (45) *Auricularia judae*

600-800 bp. The obtained DNA fragments are in accord with that of Gardes & Bruns (1993) observed in other basidiomycetes.

Phylogenetic analysis

The ITS and nLSU sequences of eight wild edible saprophytic mushroom species were interrogated in NCBI. The lowest BLAST hit of eight wild edible saprophytic mushroom species has ≥ 92 for ITS and 97 % identity for LSU data set. The length of the sequence used ranged from 640-698 bp. The phylogenetic tree using ITS data revealed two major clades and five sub-clades. The first clade contained four genera; *Lentinus, Polyporus, Panus* and *Macrolepiota* while the second clade grouped the genus *Auricularia* species (Figure 3). The phylogenetic tree obtained by using LSU data set revealed two major clades with higher bootstrap support of 92% and 100 and the seven subclades supported the five genera: 100% *Lentinus*, 100% *Pluteus*, 100% *Panus*, 99% *Macrolepiota* and 100% *Auricularia* (Figure 4).

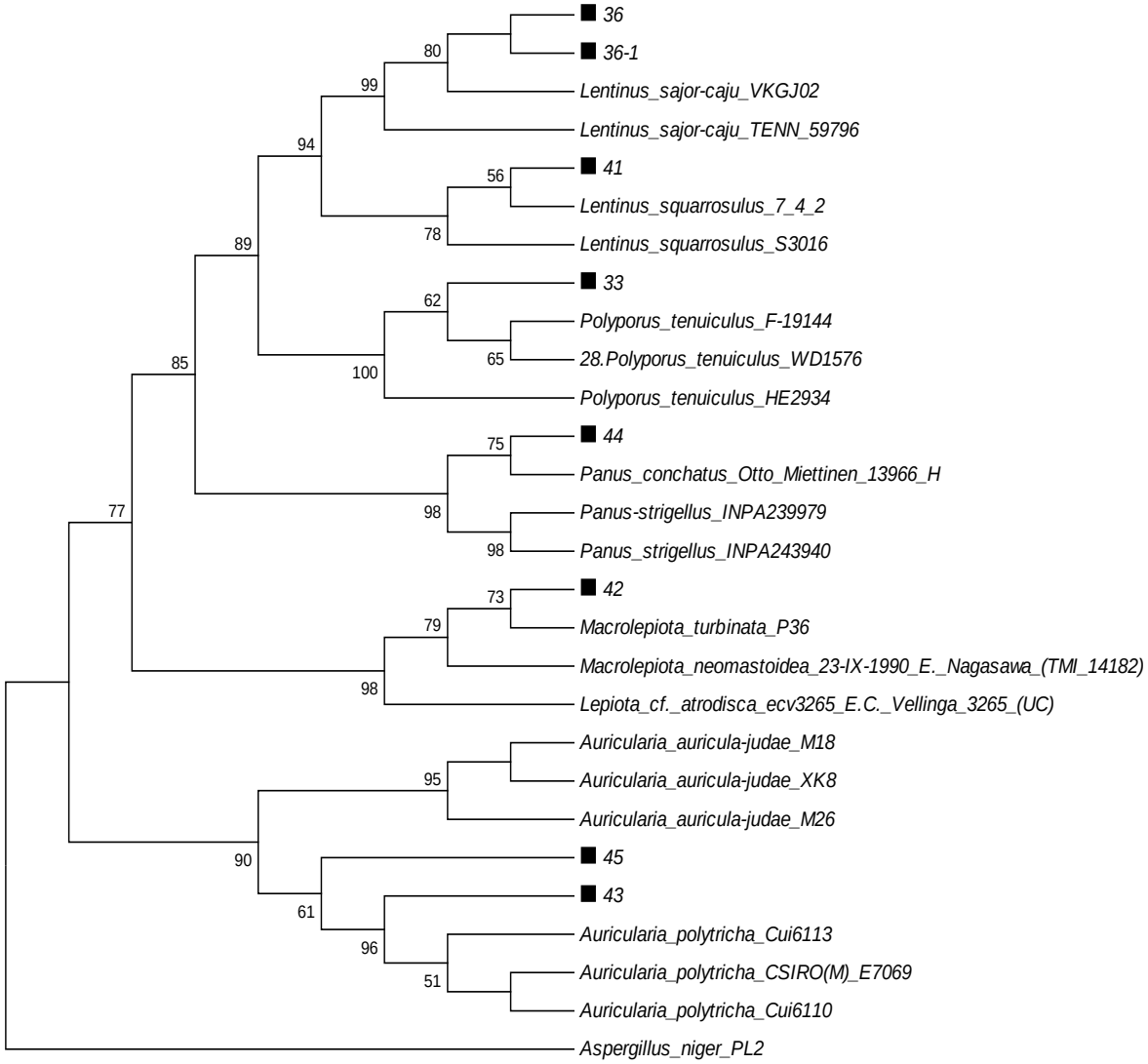


Fig. 3 – Phylogenetic analysis showing the evolutionary history inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model of ITS alignments of nuclear DNA coding. Bootstrap support value above 50 is shown in each node. The tree is rooted with *Aspergillus niger*.

Discussion

The results of purified PCR product of ITS LSU region show the bands that vary between 650-850 bp which is in consistency with that of Gardes & Bruns (1993) and Maeta et al. (2008) who suggested the expected fragment size for this rDNA target region of 600-800 bp. Blaalid et al. (2013) explained that fungal ITS region varies roughly, with some exceptions, between approximately 450 and 750 base pairs (bp) in length. All these studies support the results of this study. The sequence results were used for interrogation in NCBI and the names of species were identified as in Table 2 above.

The phylogetic tree obtained from ITS and nLSU data set with Maximum likelihood method shows two major clades and six sub clades (Figure 3 and 4). Mushroom species belonging to genera *Lentunus, Polyporus, Panus, Pluteus and Macrolepiota* form a monophyletic clade composing of four and three distinct subclades for ITS and LSU data set, respectively. This is because they belong to the same class Agaricomycetes. However in LSU analysis the genera *Lentinus*, *Polyporus* and *Panus*, grouped together with low support (55) while *Pluteus and Macrolepiota* formed a different subclade with high support (99). The obtained result of *Panus* and *Lentinus* forming a monophyletic clade with *Polyporus* species regardless of it having gills, support earlier findings and suggestions by

Kuo (2005) that it has evolved with them. Likewise, Hibbett et al. (1993) established that the two genera of *Lentinus* and *Panus* have free gills and they both belong to the same family Polyporaceae with genus *Polyporus* which has no free gills. This study has thus contributed towards understanding the taxonomic ambiguities, which have prevailed for quite sometimes on genus *Panus*. For example, *Panus* has been considered as a subgenus of *Lentinus* by Pegler (1983) whereas Corner (1981) maintained *Panus* as independent genus. Singer (1986) placed *Panus* and *Lentinus* in the Polyporaceae, regardless of the fact that *Panus* and *Lentinus* are gilled mushrooms. The second clade contains two subclades of *Macrolepiota* and *Pluteus* (Figure 4) which belong to the same order of Agaricales (http://www.gbif.org, Vellinga et al. 2003). The genus *Auricularia* is grouped separate from the other group because it belongs to a different class of Heterobasidiomycetes (Kuo 2002).

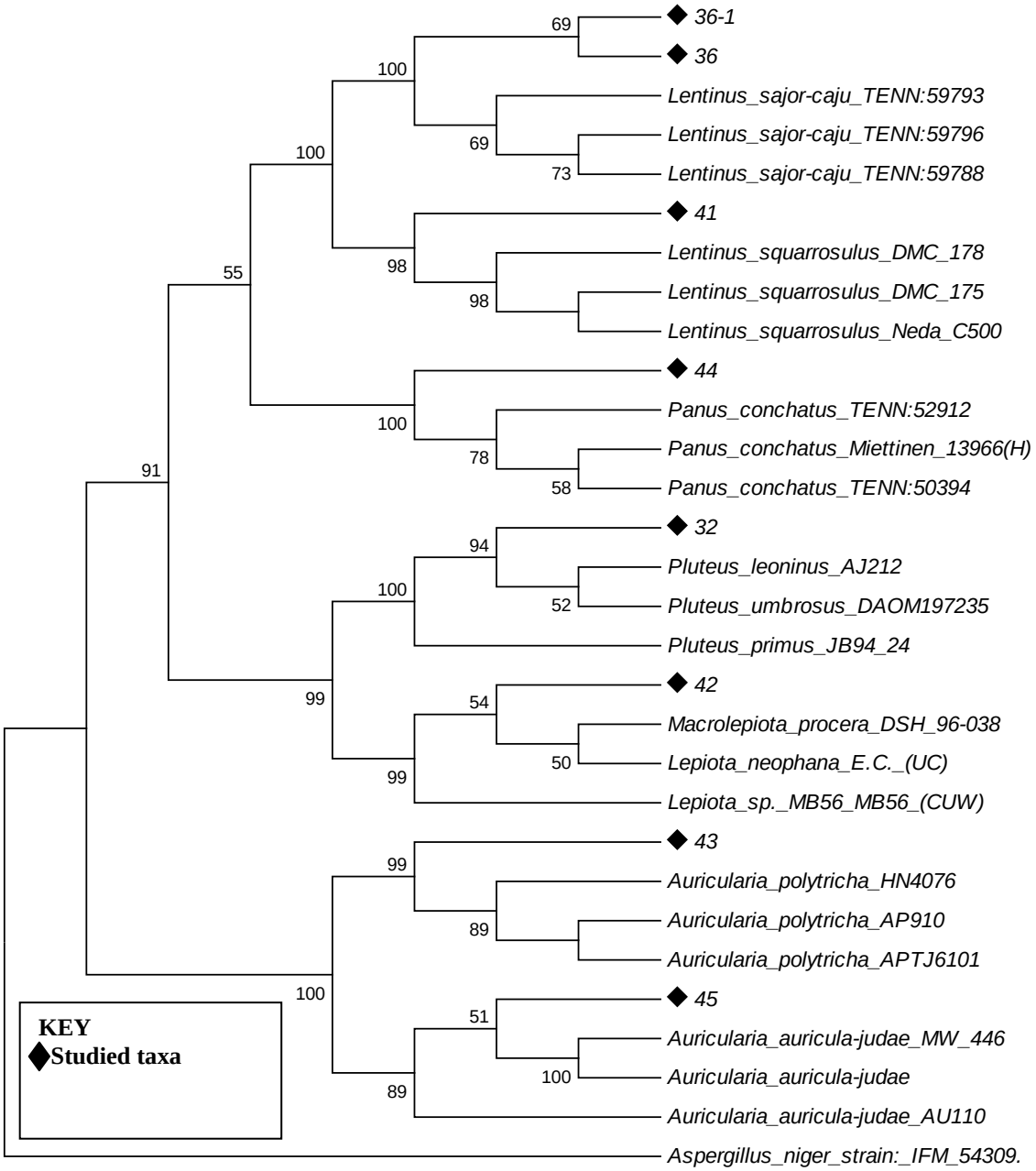


Fig. 4 – Phylogenetic analysis showing the evolutionary history inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model of nLSU alignments of nuclear DNA coding. Bootstrap support value above 50 is shown in each node. The tree is rooted with *Aspergillus niger*.

The phylogenetic tree of ITS data set obtained by Maximum likelihood method based on the Kimura 2-parameter model grouped *Auricularia judae* (45) and *Auricularia polyrticha* (43) in one clade (Figure 3) with less bootstraps value support of 90. However, the analysis by nLSU data set grouped *A. judae* (45) and *A. polyrticha* (43) into two different sub-clades well supported by bootstraps value of 100 (Figure 4). This is due to the fact that there is much variation in ITS, that facilitates to differentiate even closely related species. Likewise *Lentinus sajor-caju* (36) relate to *Lentinus squarrolosus* (41) by 85.6% based on identity matrix whereas nLSU data show the relation among the above mentioned species to be 97.5%. Also the same results were observed for *A. polytricha* (43) which relate to *A. auricularia-judae* (45) by 79.1% whereas in nLSU data show the relation is by 97.8%. This finding support the fact that both ITS and nLSU can perform similar work as DNA barcode but ITS data set are generally superior to LSU in species discrimination (Tibuhwa et al. 2012). Similar results have been also reported by Schoch et al. (2012) who also noted the ITS region to have the highest probability of successful identification for the broadest range of fungi, with the most clearly defined barcode gap between inter- and intraspecies variation. Nevertheless, they found nuclear ribosomal large subunit giving superior species resolution in some taxonomic groups, such as the early diverging lineages and the Ascomycete yeasts, but slightly inferior to the ITS. This study thus seconds the observation by Schoch et al. (2012) that for identification of mushrooms and other fungi, a combination of two markers (ITS and LSU) is best.

Conclusion

Depending merely on morphological characters in the identification of wild mushrooms may be misleading due to their high plasticity in morphological features. Combining morphological and molecular data is evidently the best approach to make progress in the study of mushroom identification rather than uniform morphology where few characters are available for morphological study. The study result reveals that ITS sequences have the highest possibility of successful delineating the studied SWEM to species level than LSU.

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