IDENTIFICATION OF TANZANIAN SAPROPHYTIC EDIBLE MUSHROOMS BY AMPLIFICATION AND SEQUENCING OF ITS/LSU REGIONS OF RIBOSOMAL RNA OPERON

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ABSTRACT

In this study, ten wild saprophytic edible mushrooms samples, collected from Tanzania natural forests and planted trees, and their two domesticated forms were characterized by in-vitro/in-vivo amplification and sequencing of ITS/LSU regions. Mushroom genomic DNA was extracted by ZR Fungal/Bacterial DNA MniPrep Kit. ITS and LSU regions were amplified using ITS-4/ITS-5 and LR16/LROR primers, respectively and sequenced. The amplicons with messy sequences were cloned. For analyzing recombinant E. coli DH5α cells, colony PCR and sequencing were done using M13-F/M13-R primers. The studied mushrooms were identified as Amylosporus sp. IJ-2014, Polyporales sp., Polyporus tenuiculus, Pleurotus cystidiosus, Laetiporus sp. IJ-2014, Lentinus sajor-caju, Favolus roseus and Auricularia polytricha. The ITS-based phylogeny inferred by Neighbor-Joining method accommodated six genera under bootstrap support values of 100% with each genus consisting mushrooms of a single species. The LSU-based phylogeny inferred by Maximum Likelihood method accommodated nine genera with bootstrap support of ≥ 66% with some genera consisting mushrooms of different species. From these results, it is clear that both ITS and LSU markers successfully discriminated wild saprophytic edible mushrooms to their respective genera but ITS marker demonstrated the higher resolving power at the species level than LSU marker.

Keywords: Saprophytic edible mushrooms, ITS, LSU, RNA operon.

INTRODUCTION

Mushroom taxonomy is the science that includes description, identification, nomenclature and classification of mushroom (Simpson, 2010). The presence of edible and poisonous mushrooms, while some of them do resemble much, entails the good scientific mushroom taxonomy in order to avoid misidentification of mushrooms which may lead into death (Tibuhwa, 2013). Mushroom taxonomy can be done on the basis of morphological features (conventional methods) and molecular methods (modern techniques) (Tibuhwa, 2011). Conventional methods involve observation of macro- and micro-morphological features, a task often complicated by inconsistencies, morphological plasticity between organisms of the same species and existence of convergent evolution (Muruke et al., 2002). In addition to that, conventional methods cannot be applied in cases where there is only a small amount of biological material available for examination or a sample contains biological material from different species mixed together (Pereira et al., 2008). Nowadays, conventional methods are being complemented with molecular methods such as DNA sequencing methods. The most frequently sequenced genetic markers for fungi are the internal transcribed spacer

(ITS) region and nuclear ribosomal large subunit (LSU) of ribosomal RNA operon (Muruke et al., 2002). ITS/LSU regions possess two salient features which make them valuable as barcoding regions. The first feature is possession of highly conserved segments (common to all fungi) which enables researchers to design universal PCR primers that can amplify a wide range of fungi. The second feature is possession of variable/highly variable regions (unique sequences) which serve as signatures for different species (Pereira et al., 2008). DNA sequencing methods offer data for identification that is more accurate and reproducible which portray phylogenetic relationships among organisms (Hibbett et al., 1997). The advent of gene cloning had enabled DNA sequencing methods to discriminate and identify biological materials from different species mixed in the same sample (Pereira et al., 2008). Three main steps involved in any gene cloning process are ligation, transformation, and analysis of recombinant clones (Russell and Sambrook, 2001; Brown, 2006).

Though Tanzania harbors natural forests rich in different species of wild edible mushrooms, few studies on mushroom taxonomy had been done and most of them are based on conventional methods (Tibuhwa et al., 2010) as well as described single genus such as *Cantharellus* (Tibuhwa et al., 2008) and *Sacorscypha* (Tibuhwa, 2011). A comprehensive study on Tanzanian edible and harmful mushrooms was done on the basis of conventional taxonomy by Härkönen et al. (1995; 2003). Few molecular taxonomy conducted includes identification of mushroom mycelia by PCR-RFLP of the ITS region (Muruke et al., 2002), molecular study of the genus *Afrocantharellus* by PCR-DNA sequencing of LSU, 5.8S-ITS2 and ATP6 (Tibuhwa et al., 2012) and molecular phylogeny of some saprophytic edible mushrooms by Hussein et al. (2014). This study complements the work by Hussein et al. (2014) and it aimed at characterizing more wild/domesticated saprophytic edible mushrooms by combining morphological method and *in-vitro*/*in-vivo* amplification and sequencing of ITS/LSU regions of ribosomal RNA operon.

MATERIALS AND METHODS

Sample collection

The study was carried out in natural forests of Lutindi, Shume-Magamba and Kieti in Tanga region, Kazimzumbwi forest in Pwani region and some planted trees at University of Dar es Salaam main campus in Dar es Salaam (DSM) region (Figure 1). Eight wild edible saprophytic mushroom morpho-species collected during rainy seasons (March− May and September− December, 2011/2012) with their two domesticated counterparts were used in this study. Preliminary morphological identification was done in the field as per Tibuhwa et al. (2010). The identified wild edible saprophytic mushrooms were subjected to domestication trials as comprehensively reported by Juma et al. (2015). The wild and successfully domesticated mushrooms were dried at 50°C to constant weight and then preserved in silica gel for antioxidant potential determination, as broadly reported by Juma et al. (2016), and molecular characterization. Other fruitbodies were dried at 55°C for herbarium deposits at the University of Dar es Salaam.

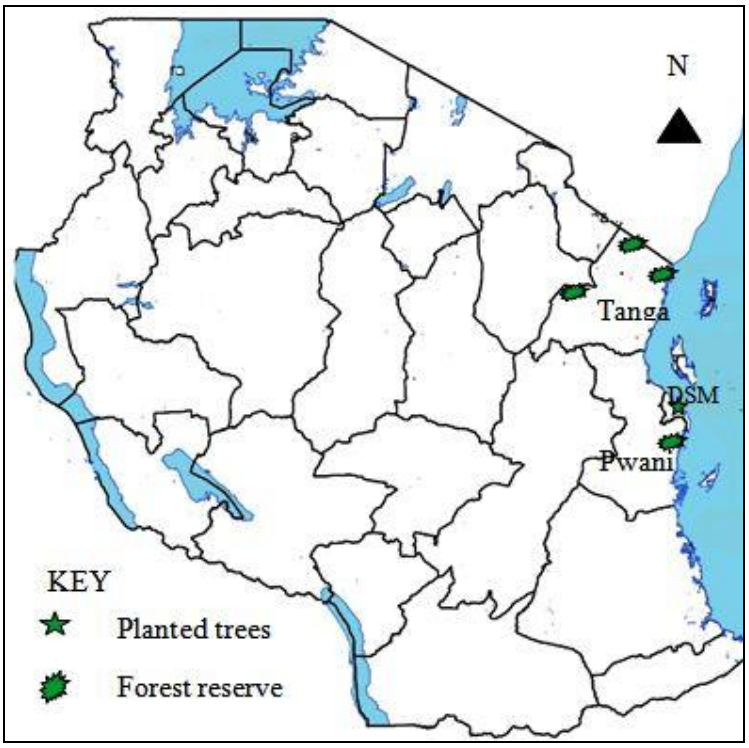

Figure 1: A map of Tanzania showing studied **sites**

DNA extraction and quality/quantity check up

DNA was extracted from dried fruiting bodies by ZR fungal/bacterial DNA MiniPrep Kit (ZYMO RESEARCH) according to manufacturer's instructions. Concentration and cleanliness of genomic DNA were assayed by nanodrop spectrophotometer(THERMO SCIENTIFIC NanoDrop 2000c) whereas DNA quality was checked by gel electrophoresis on 1% agarose gel stained with GelRed using λ DNA Marker (Góes -Neto et al., 2011).

***In-vitro* ITS/LSU amplification and sequencing**

PCR−amplification of ITS and LSU regions was performed in thermocycler (APPLIED BIOSYSTEMS® GENEAMP® PCR SYSTEMS 9700) using ITS-4/ITS-5 (White et al., 1990) and LROR/LR16 (Vilgalys and Hester, 1990) primer pairs, respectively. PCR reactions were carried out in 20µL reaction volume using PCR master mix (ACCUPOWER® TAQ PCR PREMIX, BIONEER). Preparation of PCR reaction mixtures and amplification were done according to AccuPower® Taq PCR PreMix protocol with slight modifications. The reactions were heated in an initial step of 95 °C for 5 min and then subjected to 35 cycles of the following program: 95 °C for 30 seconds, 60 °C for 30 seconds, and 72 °C for 1 min. After the final cycle, the temperature was maintained at 72 °C for 10 min and finally chilled at 10 °C to stop reaction. PCR products were analyzed by electrophoresis whereby 1.8% agarose gel prepared in 0.5X Tris-Borate EDTA buffer with addition of 2.5 µL gel red was used together with DNA

ladder (THERMO SCIENTIFIC GENERULER 1Kb+ DNA LADDER). Amplicons were purified using Thermo Scientific GeneJET PCR purification kit #K0701 following the manufacture's protocol. Concentration of purified amplicons was determined by nanodrop spectrophotometer (THERMO SCIENTIFIC NANODROP 2000C) at absorbance (A 260/280). Sequencing was done at at SegoliP Unit of BecA− ILRI Hub using capillary sequencer (ABI PRISM 3730 GENETIC ANALYZER, APPLIED BIOSYSTEMS) according to the manufacturer's instructions.

***In-vivo* ITS/LSU amplification (Gene Cloning) and sequencing**

Some mushroom DNA sequences portrayed messy chromatograms thus, their purified PCR products were cloned by using THERMOSCIENTIFIC InsTA CLONE PCR CLONING KIT #K1214, following manufacturer's protocol. After ligation and transformation processes, analysis of recombinant clones of *E. coli* DH5α cells was done using colony PCR and sequencing methods. Colony PCR reaction was performed in 20µL reaction volume of ACCUPOWER® TAQ PCR PREMIX tubes. Preparation of colony PCR reaction mixture and amplification was done according to ACCUPOWER® TAQ PCR PREMIX protocol with some amendments. 0.8 µL of each M13F (forward primer) and M13R (reverse primer) were pipette into 18.4 µL of nuclease free water in ACCUPOWER® TAQ PCR PREMIX 20 µL reaction tubes and a small amount of the bacterial colony from representative white clones was added and mixed thoroughly. Amplification was carried out in the thermocycler (APPLIED BIOSYSTEMS® GENEAMP® PCR SYSTEMS 9700). The reaction conditions were initial step of 95 °C for 3 min and then subjected to 35 cycles of the following program: 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min. After the final cycle, the temperature was maintained at 72 °C for 15 min and finally chilled at 10 °C to stop reaction. Analysis of colony PCR products and processing for sequencing were carried out as explained earlier in the previous subsection.

Data analysis

After quality control of sequence datasets using CLC Main Workbench 6.9.1 as per manufacture's protocol, the LSU and ITS sequences were interrogated on NCBI GenBank database and then assembled with some reference sequences (Table 1) using same software, CLC Main Workbench 6.9.1. The sequence alignments were imported separately in MEGA6 (Tamura et al., 2013) that analyzed the ITS and LSU datasets and phylogenetic relationships were analyzed using Neighbor-Joining (Sitou and Nei, 1987) and Maximum Likelihood (Kimura, 1980) methods, respectively, based on Kimura 2-parameter model with a bootstrap analysis involving 1000 replication rounds.

RESULTS AND DISCUSSION

***In-vitro*/*In-vivo* ITS/LSU amplification and sequencing**

ITS and LSU regions were selectively amplified from extracted genomic DNA of twelve mushroom samples. Gel electrophoresis (Figure 2) revealed that the size of ITS and LSU fragments ranged between 600 and 800bp. This finding is in agreement with the study by Maeta et al. (2008) who suggested that the expected fragment size for this ribosomal RNA operon region to be 600-800bp.

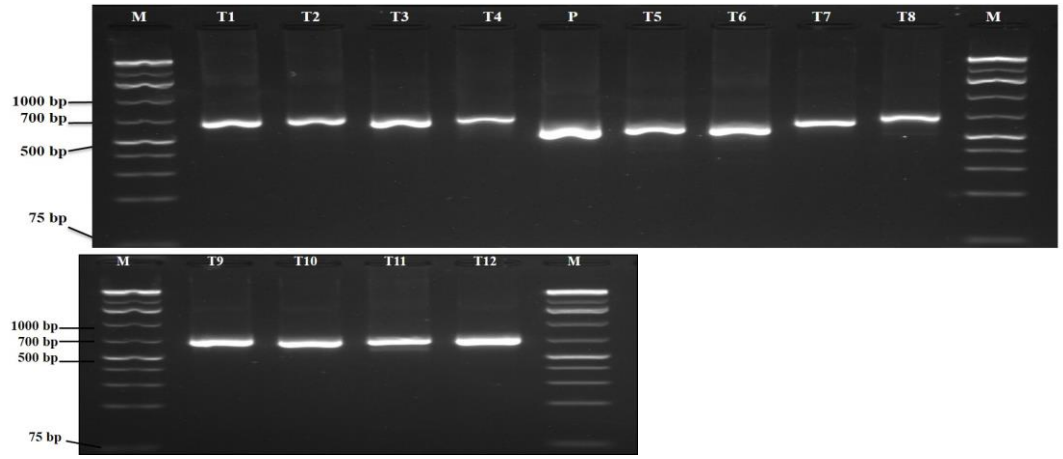

Figure 2: Electropherogram showing purified ITS PCR amplicons as representative of PCR amplification products; M: 1Kb^{+} DNA Ladder, T1: *Polyporus tenuiculus*, T2: *Laetiporus sp. IJ-2014*, T3: *Lentinus sajor-caju*, T4: *Auricularia polytricha*, T5: *Favolus roseus*, P: Positive control, T6: *Pleurotus cystidiosus*, T7: *Pleurotus cystidiosus*, T8: *Pleurotus cystidiosus*, T9: *Amylosporus sp. IJ-2013* **(W)**, T10: *Polyporales sp.* **(W)**, T11: *Polyporales sp.* **(D)**, T12: *Amylosporus sp. IJ-2013* **(D)**.

After sequencing, some mushroom ITS/LSU sequences portrayed clear chromatograms while others portrayed chromatograms with more amplicons. These profiles resulted from co-amplification of ITS/LSU from yeast/mould that contaminated mushroom samples. Following gene cloning, after analyzing recombinant clones by colony PCR and sequencing, the clear sequences were obtained and identified. The mushroom sequences generated in this study were submitted to NCBI GenBank database under accession numbers shown in Table 1.

Table 1: Information of ITS and LSU sequences used in this study

No	Species	Voucher	Collection no.	ITS-Accession #	LSU-Accession #
1	*Pleurotus cystidiosus*	IJV6	Ibrahim 6.2012	***KM593877***	***KM593885***
2	*Pleurotus cystidiosus*	IJV35	Ibrahim 35.2012	***KM593878***	***KM593886***
3	*Pleurotus cystidiosus*	IJV51	Ibrahim 51.2013	***KM593883***	***KM593891***
4	*Amylosporus sp. IJ-2014* **(W)**	IJV29-1	Ibrahim 29-1. 2012	***KM851314***	***KM593892***
5	*Amylosporus sp. IJ-2014* **(D)**	IJV29-2	Ibrahim 29-2. 2013	***KM851315***	***KM593893***
6	*Auricularia polytricha*	IJV46	Ibrahim 46.2013	***KM593881***	***KM593889***
7	*Laetiporus sp. IJ-2014*	IJV38	Ibrahim 38.2013	***KM593880***	***KM593888***
8	*Favolus roseus*	IJV4	Ibrahim 4.2012	***KM593876***	***KM593884***
9	*Polyporus tenuiculus*	IJV34	Ibrahim 34.2012	***KM593879***	***KM593887***
10	*Lentinus sajor-caju*	IJV50	Ibrahim 50.2013	***KM593882***	***KM593890***
11	*Polyporales sp.* **(W)**	IJV40-1	Ibrahim 40-1. 2012	–	***KM593895***

12	*Polyporales sp.* **(D)**	IJV40-2	Ibrahim 40-2. 2013	–	***KM593894***
13	*Auricularia polytricha*	strain AP10158	Fan X. et al.	*KF297985*	–
14	*Laetiporus sp.*	RV4A	Vasaitis R. et al.	*EU840662*	–
15	*Laetiporus sp.*	RV2A	Vasaitis R. et al.	*EU840664*	–
16	*Laetiporus sp.*	RV3A	Vasaitis R. et al.	*EU840665*	–
17	*Laetiporus sp.*	6693	Gao S. et al.	*EU840678*	–
18	*Pleurotus tuber-regium*	voucher DMC 173	Douanla-Meli C and Langer E.	–	*EU908175*
19	*Pleurotus tuber-regium*	strain ACCC 50657	Gao S.et al.	–	*EU365676*
20	*Polyporus tenuiculus*	strain ML284	Ota Y and Hattori T.	*Q409357*	–
21	*Polyporus tenuiculus*	isolate HE2934	Sun X. et al.	*KC505555*	–
22	*Polyporus tenuiculus*	strain: WD1576	Sotome K. et al.	*AB587633*	–
23	*Lentinus sajor-caju*	isolate TFB11739	Grand EA. et al.	*GU207308*	–
24	*Lentinus sajor-caju*	isolate 11739	Grand EA.	–	*AY615989*
25	*Auricularia polytricha*	strain HBME	Fan X. et al.	*KF297976*	–
26	*Auricularia polytricha*	strain SN111	Fan X. et al.	*KF297977*	–
27	*Hericium coralloides*	isolate HHB-9082-Sp.	Guglielmo F.et al.	–	*AM269840*
28	*Hericium erinaceum*	isolate 654	Guglielmo F.et al.	–	*AM269839*
29	*Hericium erinaceum*	isolate JHO-62-149-Sp.	Guglielmo F.et al.	–	*AM269841*
30	*Auricularia polytricha*	strain APTJ6101	Fan X. et al.	–	*KF298022*
31	*Auricularia delicata*	strain GIM5.177	Fan X. et al.	–	*KF297998*
32	*Auricularia cornea*	strain AU141	Fan X. et al.	–	*KF297996*
33	*Laetiporus sulphureus*	strainTFRI1092	Chou TH. et al		*EU232302*
34	*Laetiporus sulphureus*	isolate CT-1	Lindner DL and Banik MT.	–	*EU402532*
35	*Laetiporus sulphureus*	isolate GR-12	Lindner DL and Banik MT.	–	*EU402534*
36	*Favolus emerici*	voucher: TFM: F 21697	Sotome K. et al.	–	*AB735951*
37	*Polyporus tenuiculus*	strain WD1576	Sotome K. et al.	–	*AB587622*
38	*Lentinus sajor-caju*	isolate 11736	Grand EA.	–	*AY615988*
39	*Lentinus sajor-caju*	isolate 11731	Grand EA.	–	*AY615990*
40	*Aspergillus penicillioides*	strain ALI 231	Zeng QY. et al.	–	*AY386182*
41	*Favolus emerici*	voucher: TFM:F-21697	Sotome K. et al.	*AB735972*	–
42	*Favolus roseus*	voucher: TFM:F-20589	Sotome K. et al.	*AB735975*	–
43	*Lentinus sajor-caju*	isolate TFB11736	Grand EA.	*GU207309*	–
44	*Pleurotus ostreatus*	isolate NW449	Yu Z.	*EU520110*	–
45	*Pleurotus smithii*	strain ATCC 46391 clone 4	Zervakis GI. et al	*AY315784*	–
46	*Pleurotus cystidiosus*	subsp. abalonus strain Blao clone A	Truong BN.	*DQ882570*	–
47	*Pleurotus cystidiosus*	strain: ATCC 28599	Neda H	*AB115036*	–

48	*Laetiporus* sp.	6692	Vasaitis R. et al.	*EU840677*	–
49	*Cylindrocladium quinqueseptatum*	isolate FCQ-341	Pandey A. et al.	*JQ347275*	–

NCBI GenBank database Interrogation and Sequence alignment

The BLAST search results on the NCBI database showed that the studied mushroom sequences have ≥ 97% identity for ITS and ≥ 85% identity for LSU. The nuclear ribosomal LSU sequence matrix contained 30 sequences (12 newly generated in this study) whereas the ITS sequence matrix contained 28 sequences (8 newly generated in this study). The few taxa on ITS sequence matrix were due to failure of the two ITS sequences of *Amylosporus sp. IJ-2014*, the wild and cultivated forms, from aligning with other ITS sequences. Another reason was the failure of isolating two reproducible ITS sequences from the wild and cultivated forms of *Polyporales sp.* The ITS alignment demonstrated high percentage of imprecisely aligned sites which needed intensive manual correction compared to the LSU alignment. After trimming of non-overlapping start/end positions of sequences as well as excluding ambiguously aligned regions, there were a total of 499 and 519 positions in the final datasets of LSU and ITS, respectively.

Phylogenetic relationships

The phylogenetic tree using LSU datasets revealed two major clades. The first clade grouped eight genera; *Pleurotus*, *Amylosporus*, *Hericium*, *Auricularia*, *Polyporales*, *Laetiporus*, *Favolus* and *Polyporus* whereas the last major clade contained the genus *Lentinus* (Figure 3). Likewise, the phylogenetic tree using ITS datasets revealed two major clades. The first clade grouped five genera; *Polyporus*, *Favolus*, *Lentinus*, *Pleurotus* and *Auricularia* while the second clade contained the genus *Laetiporus* (Figure 4).

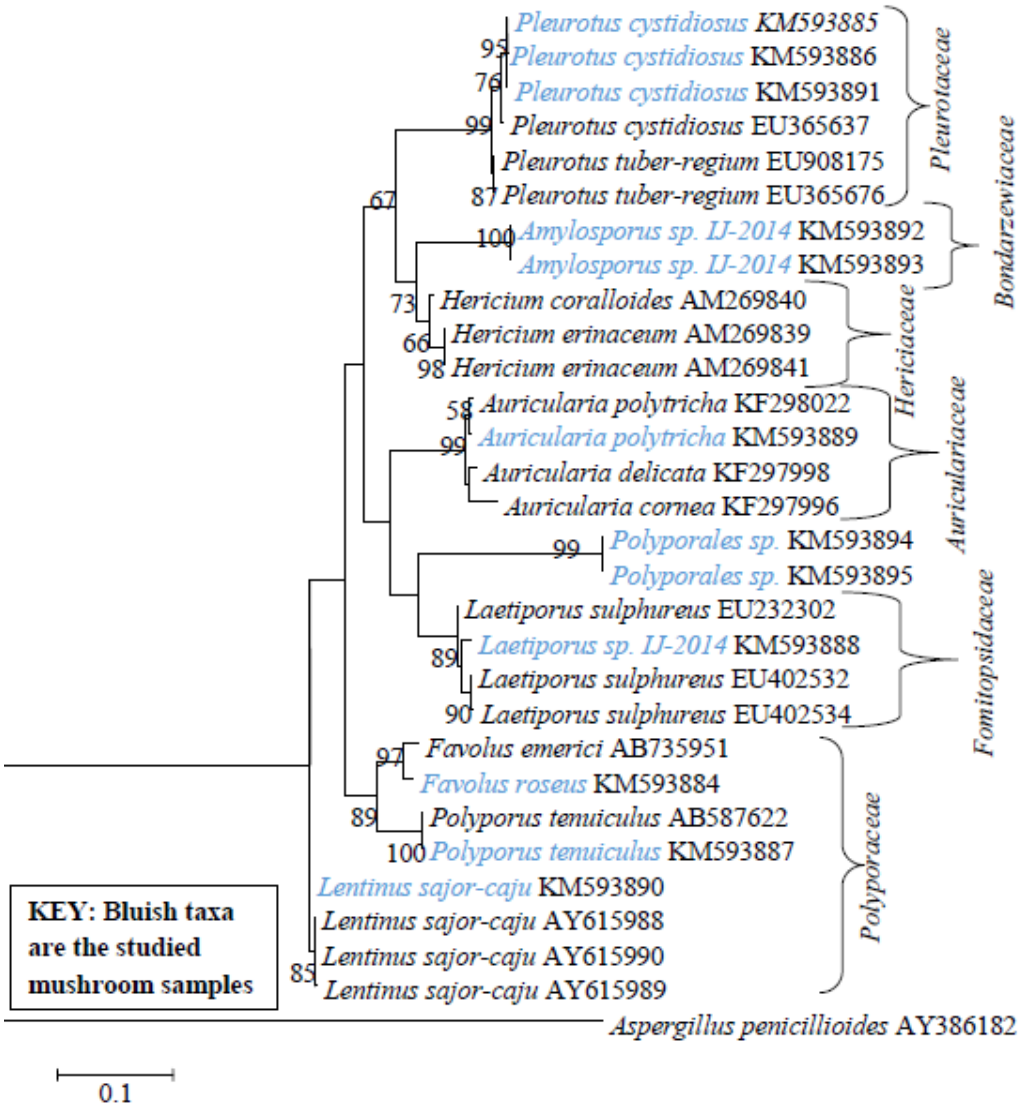

Figure 3: Evolutionary relationships of taxa inferred from the maximum likelihood method based on Kimura 2-parameter model using nuclear ribosomal LSU data. Bootstrap support value above 50% is shown in each node. The tree is rooted with *Aspergillus penicillioides* (Ascomycota: Trichomaceae).

The LSU-based phylogeny grouped *Favolus roseus* |KM593884| with *Favolus emereci* |AB735951| under a bootstrap support of 97% whereas the ITS-based phylogeny grouped *Favolus roseus* |KM593876| with *Favolus roseus* |AB735975| under a bootstrap value of 99%. This is due to the least resolving power of LSU at the species

level discrimination of fungi compared to the ITS as reported earlier by Varga et al. (2007) and also noted by Hussein et al (2014).

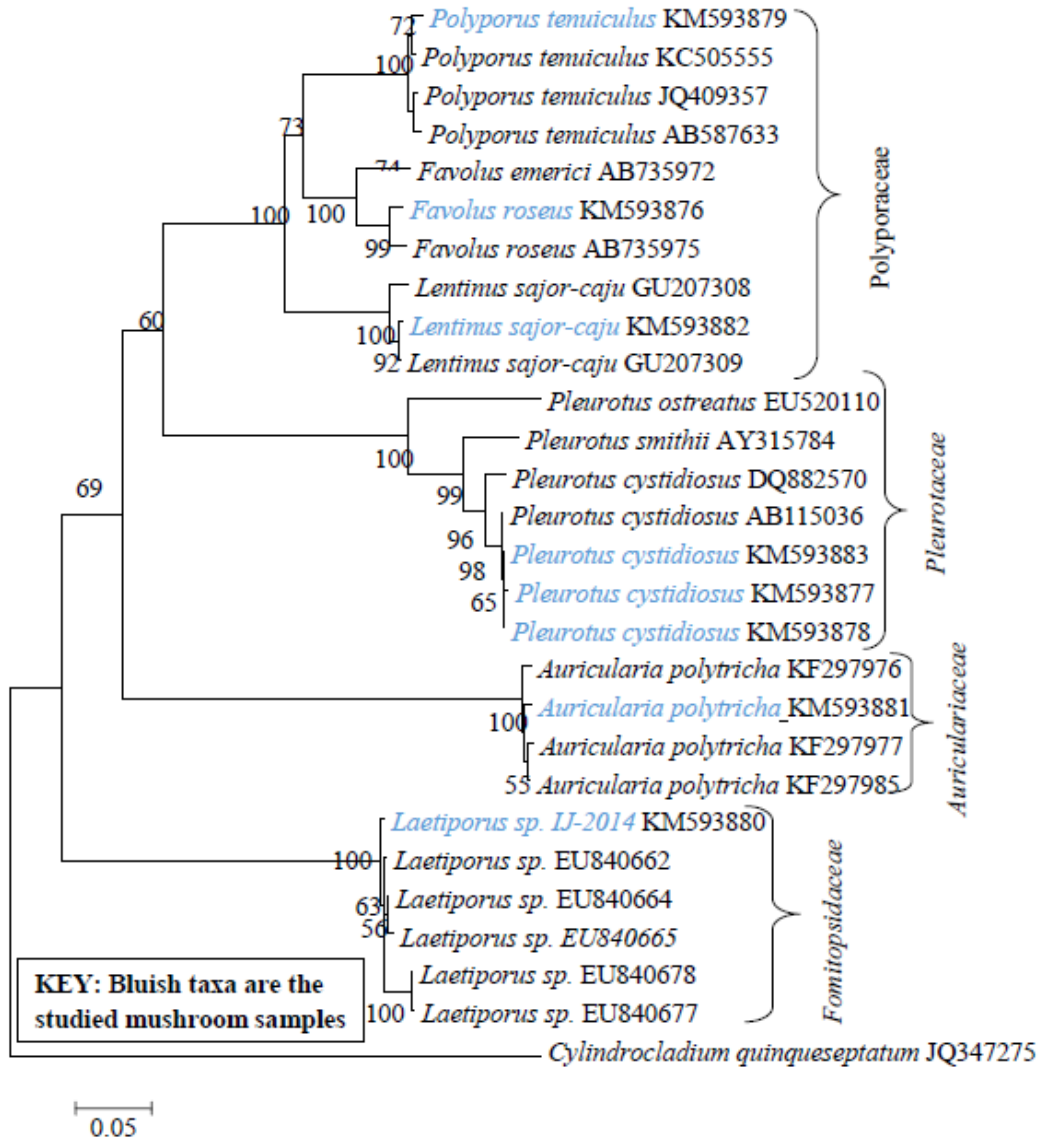

Figure 4: Evolutionary relationships of taxa inferred from the Neighbor-Joining method using ITS data. Bootstrap support value above 50% is shown in each node. The tree is rooted with *Cylindrocladium quinqueseptatum* (Ascomycota: Nectriaceae).

The LSU-based phylogeny grouped *Polyporus tenuiculus* |KM593887| with *Polyporus tenuiculus* |AB587622| under a bootstrap support of 100%. For the ITS-based phylogeny, *Polyporus tenuiculus* |KM593879| was resolved as the first diverging taxon of the genus *Polyporus* comprised of strains of *Polyporus tenuiculus*. Both phylogenies suggest that species belonging to *Polyporus* and *Favolus* genera share the most recent common ancestry as previously reported by Sotome et al. (2013). This study has contributed towards understanding the taxonomic ambiguities which is prevailing on some species of *Favolus* and *Polyporus* genera. For example, *Polyporus tenuiculus*, characterized by white to cream basidiocarps with hexagonal to radially elongated pores, has been considered as a synonym of *Favolus roseus*, characterized by a small-sized pileus and radially elongated pores (Ryvarden 1989, Núñez and Ryvarden 1995a). The taxonomic study of *Favolus and Neofavolus gen. nov.* segregated from *Polyporus, conducted by* Sotome et al. (2013), commented that multiple species of the P. *tenuiculus* complex are found in Africa, and thus preferred leaving 'P. *tenuiculus*' as an ambiguous name until a study of the type can be made. By considering morphological and phylogenetic characters, the present study sides with Härkönen et al. (2003) who claimed that *Favolus roseus* and *Polyporus tenuiculus* found in Tanzania are two distinct species. On the other hand, mushroom species belonging to *Polyporus*, *Favolu*s and *Lentinus* genera formed a monophyletic subclade on the ITS-based phylogeny. This is because all of them belong to the same family, *Polyporaceae* (Ryvarden 1989, Hibbett et al. 1997, Sotome et al. 2013).

With respect to the LSU-based phylogeny, the three strains of *Pleurotus cystidiosus* with accession numbers |KM593885|, |KM593886| and |KM593891| grouped together under a bootstrap support of 95% in the genus *Pleurotus*. These strains appeared to be closer to *Pleurotus cystidiosus* |EU365637| compared to *Pleurotus tuber-regium*. In ITS-based phylogeny, those stains of *Pleurotus cystidiosus* with accession numbers |KM593877|, |KM593878| and |KM593883| grouped together under a bootstrap support of 65%. All strains of *Pleurotus cystidiosus* appeared to share the recent common ancestor with *Pleurotus smithii*. Furthermore, *Pleurotus* subclade is clearly resolved from a subclade containing *Amylosporus and Hericium* species on LSU-based phylogeny. This is because *Amylosporus and Hericium* species belong to the same order, *Russulales* (Kirk et al., 2008), while *Pleurotus* species belong to the distinct order, *Agaricales*, which consists of the gilled-mushrooms (Kirk et al., 2008). Likewise, on the ITS-based phylogeny *Pleurotus* subclade is clearly resolved from a subclade containing *Polyporus, Favolus and Lentinus* genera. This is because *Polyporus, Favolus and Lentinus* species belong to the family *Polyporaceae* that encompasses bracket fungi belonging to the Basidiomycota (Corda, 1839) while *Pleurotus* species belong to the distinct family *Pleurotaceae* composed of small to medium-sized mushrooms which have white spores (Thorn et al., 2000; Kirk et al., 2008).

For the *Laetiporus sp. IJ-2014*, it was positioned with members of *Laetiporus sulphureus* under the bootstrap support less than 50% in LSU-based phylogeny. The low bootstrap value was due to fact that *Laetiporus sp. IJ-2014* and *Laetiporus sulphureus* are two distinct species. In ITS-based phylogeny, *Laetiporus sp. IJ-2014* was resolved, as the first diverging taxon in the genus *Laetiporus* comprised of *Laetiporus* sp. under the bootstrap value greater than 50%. This is another case in this study of which the ITS portrayed higher

resolution at the species level discrimination of fungi than LSU (Varga et al., 2007).

The wild and domesticated *Amylosporus sp. IJ-2014* clustered together while clearly resolved from other genera studied on the LSU-based phylogeny. This phylogeny suggests that the genus *Amylosporus* is somehow closer to the genus *Hericium* in evolutionary history as they have 73% identity matrix in their LSU marker. This similarity comes from the fact that *Amylosporus and Hericium* species belong to the same order, *Russulales* (Kirk et al., 2008). During this study, there was no LSU (28S rRNA gene) reference sequences of *Amylosporus* sp. on NCBI GenBank database for comparison with the one generated in this study. The ITS sequences of the wild and domesticated forms of *Amylosporus sp. IJ-2014* failed to align with other ITS sequences thus were excluded from ITS alignment dataset and hence ITS-based phylogeny. This observation further supports the idea that ITS marker is highly divergent and thus can sometimes fail to accommodate distantly related mushrooms in molecular phylogeny which may otherwise be accommodated by LSU marker (Hussein et al., 2014).

For *Polyporales sp.*, the wild and domesticated forms clustered distinctively from other genera studied using the LSU-based phylogeny. These results suggest that these taxa could be novel species and distinct studies on the identity of this mushroom are under way. The LSU-based phylogeny further suggests that the genus to which this novel species belongs is somehow closer to the genus *Laetiporus* in evolutionary history than it is to other mushroom genera so far reported though they are morphologically highly dissimilar.

Auricularia polytricha |KM593889| clustered together with other members of genus *Auricularia* including A. *delicata* and A. *cornea* under a bootstrap support of 58% in the LSU-based phylogeny. Contrary to that, the ITS-based phylogeny grouped *Auricularia polytricha* |KM593881| with strains of its species under a bootstrap support of 100%. This is due to the fact that there is much variation in ITS, that facilitates to differentiate mushrooms to species level. This findings support the fact that both ITS and LSU can perform similarly as DNA barcode but ITS data set are generally superior to LSU in species discrimination (Varga et al., 2007; Tibuhwa et al., 2012; Hussein et al., 2014).

CONCLUSION

Conventional taxonomy alone is prone to erroneous identification and classification of mushrooms unless it is complemented with molecular taxonomy. On the other hand, molecular taxonomy alone may sometimes fail to attain correct mushroom identification especially for the species whose ITS/LSU sequences are missing in public GenBank databases. The findings from this study disclosed efficacy of gene cloning in isolation and identification of mushroom DNA from yeast/mould contaminated samples. Furthermore, the study findings revealed that both LSU and ITS markers can distinctly show the fungal phylogeny but ITS marker is superior, to discriminate mushrooms to the species level and even within species.

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