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Full Length Research Paper

Studies on biodiversity of some mushrooms collected in Lagos State, Nigeria using biotechnological methods

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The biodiversity of mushrooms in Lagos State, Nigeria was studied using modern biotechnological method of DNA sequence analyses. Sixteen mushrooms were collected in Lagos State for 12 months. The mushrooms collected include: *Agaricus campestris, Coprinus comatus, Daldinia concetrica, Ganoderma adspersum*, *Ganoderma applanatum*, *Ganoderma lucidum*, *Mycena haematopus*, *Mycena* sp., *Pleurotus ostreatus, Pleurotus tuber-regium, Polyporus* sp., *Polyporus squamosus*, *Polyporus sulphureus, Trametes versicolor, Xylaria polymorpha,* and *Xylaria* sp. Subsequently, eight different pure mushroom mycelia were isolated using potato dextrose agar medium (PDA) from sterile tissues of *A. campestris, C. comatus, G. lucidum, P. ostreatus, P. tuber-regium, P. sulphureus, X. polymorpha*, *and Xylaria* sp. A mycelia spawn of each of the isolated mushrooms was made and kept in duplicates for DNA sequence analyses. The DNA sequence analyses of each of the eight mushroom mycelia pure isolates were carried out. No similarity was observed when a BLAST query of the mushroom DNA sequences was carried out to determine the similarity with the GenBank database previous submissions. The sequences were then submitted to the GenBank database for reference purpose. Comprehensive description given by the GenBank provides a detailed, reliable and accurate identification than visual characteristics and phenotypic properties of the mushrooms.

Key words: Biodiversity, mushroom, deoxyribonucleic acid (DNA) sequence, phylogenetic tree.

INTRODUCTION

Mushrooms are abundant in various parts of Lagos environs. While some grow in singleton like the *Ganoderma lucidum*, others grow in clusters like *Coprinus comatus and Mycena haematopus* and sometimes in layers like *Trametes versicolor*. The mushrooms showed to a large extent diverse colour variations especially of their pileus ranging from cloud/milk white; *Pleurotus ostreatus* to yellow; *Polyporus sulphureus* to red *Mycena haematopus* and some possess two or more colours; *G. lucidum*. Some grow stipes; *Agaricus campestris* while others do not; *Ganoderma adspersum* and *Daldinia concentrica*. Most of the studied mushrooms grow in humid conditions on dead logs and trees like *G. lucidum, G. adspersum, and M. haematopus* and in the soil like *A. campestris*. Basically, a mushroom is the fleshy, spore-bearing fruiting body of a fungus, typically produced above ground on soil or on its food source (substratum). Like all fungi, mushrooms are not plants and do not undergo photosynthesis. Some of the mushrooms grow fleshy fruiting bodies; *A. campestris, C. comatus*, and *Mycena* spp. while others grow woody or leathery fruiting bodies; *G. lucidum*, *T. versicolor* and *Xylaria polymorpha.* They showed varying growth patterns ranging from umbrella-like to ball-like and semi-circular forms.

Deoxyribonucleic acid (DNA) is the component containing the blueprint that specifies the biological development and composition of every form of life with no exception of mushrooms. In living organisms, DNA does not usually exist as a single molecule, but instead as a

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pair of molecules that are held tightly together (Watson and Crick, 1953; Berg et al., 2002). These two long strands entwine like vines, in the shape of a double helix. The nucleotide repeats contain both the segment of the backbone of the molecule, which holds the chain together, and a nucleobase, which interacts with the other DNA strand in the helix. A nucleobase linked to a sugar is called a nucleoside and a base linked to a sugar and one or more phosphate groups is called a nucleotide. Polymers comprising multiple linked nucleotides (as in DNA) are called a polynucleotide.

The backbone of the DNA strand is made from alternating phosphate and sugar residues (Ghosh and Bansal, 2003). One major difference between DNA and RNA is the sugar, with the 2-deoxyribose in DNA being replaced by the alternative pentose sugar ribose in RNA (Berg et al., 2002). DNA sequencing of mushroom is the process of determining the exact order of the nucleotide bases in them; Adenine (A), Thymine (T), Cytosine (C) and Guanine (G) in a piece of DNA oligonucleotide. Generating a DNA sequence for a mushroom is to determine the patterns and make up genetic traits, diversity and growth forms.

Fungal genomes contain 50 to 150 identical copies of the ribosomal DNA (rDNA), a higher copy number than in bacterial genomes. Until now, phylogenetic typing of fungi from both single cultures and mixed populations involved DNA extraction, amplification of regions of rDNA or internal transcribed sequences (ITS), and purification (or cloning) of the polymerase chain reaction (PCR) product.

Aim of the research work

The most correct and accurate method of identifying an organism is through DNA sequence analyses. The need to collect diverse mushrooms from Lagos State, Nigeria and study their biodiversity through DNA sequence analyses was the pulling force behind this research. Therefore, the aim of this research is to deposit and document the Nigerian mushroom genomic DNA sequence data in the GenBank for reference purpose.

MATERIALS AND METHODS

Sources of mushrooms

Sixteen (16) fresh and healthy mushroom samples were collected for 12 months in Lagos State ranging from Araga-poka in Epe, Lagoon front, gate and Staff Quarters all in University of Lagos (Main Campus), Owodunni suburbs of Alagbado, Ijede in Ikorodu and old Ota road in Ile-Epo. They were all collected from January to December, 2011 (Table 1).

Mushroom collection

The mushrooms were collected for twelve months; both on ground and on substratum such as trees and decaying tree logs. Their photograph and the Global positioning system (GPS) caption were taken with a Nokia device *in situ* before collection. Detailed notes of their morphological and ecological features were also recorded. A sharp sterilized knife was used to collect the whole and parts of mushrooms growing on trees and on the ground. Mushroom samples collected were free of infection or insect attack.

The earth was carefully removed from the lowest part of the stipe with knife or through dusting so as to save the fresh samples from getting too dirty. Care was also taken not to remove or damage any part of the mushroom sample. The non-fleshy mushrooms were collected, wrapped with tissue paper and kept inside a sterilized polythene bag. The heaviest and the lightest were placed on the bottom and top, respectively of the polythene bag in order to prevent damage of the samples.

Identification of the mushrooms

The mushrooms were identified based on their morphological, macroscopic, physiological and ecological features according to a previously published guide (Wei, 1979). Further identification was based on their structure, substratum they were attached to, spore growth, colour, shape, and mycology textbooks.

Mycelia isolation

Of all the sixteen mushroom samples, eight mushroom mycelia were isolated through random selection. A fresh, healthy part of each of the mushroom was cleaned with absolute alcohol to remove dirt and placed on a sterilized workbench in the ultra violet room. The mushroom was split into half longitudinally and some inside tissues at the upper part of the stipe were taken with the aid of forceps. The tissues were then sterilized in a 40% sodium hypochlorite solution after which they were rinsed thrice in separate beakers with distilled water. This is done to remove bacteria or fungal spores that might be on the mushroom tissues. The tissues were then seeded in two's with the use of a flamed forceps and inoculating needle on each Petri dish with previously prepared potato dextrose agar (PDA). Afterwards, the plates were dated and incubated at a temperature of 29 to 32°C for four days of full growth.

The mycelium growth culture was aseptically subcultured into freshly prepared PDA plates and incubated until the mycelium begins to grow. This was then followed by subsequent sub culturing for three times to ensure pure mycelia isolate. Some part of the mycelia isolated was then aseptically transferred into two sterile agar slants which had previously been prepared in McCartney bottles. The bottles were then incubated at 29 to 32°C till full mycelia growth is observed which serves as the stock culture. This procedure was repeated for all the eight mushroom mycelia isolated.

DNA extraction and analyses

The DNA extraction and analysis was carried out at the Biotechnology Centre of the Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria. Total genomic DNA was extracted with cetyl trimethyl ammonium bromide (CTAB) buffer as described by Graham et al. (1994).

Procedure for DNA extraction

Some of the isolated mycelia of respective mushrooms were taken from the incubated stock in the McCartney bottles and transferred

Table 1. Geographical points of different locations in Lagos State, Nigeria where the mushrooms were collected.

Location	Points	Mushroom samples	Latitude	Longitude	Altitude (Metres)	Altitude accuracy (Metres)	Accuracy (Metres)
Lagoon Front, University of Lagos	1	*Xylaria polymorpha*	6º31´06.08´´N	3º24´06.42´´E	19	55	25
	2	*Daldinia concentica*	6º31´07.04´´N	3º24´06.21´´E	44	150	38
	3	*Pleurotus ostrearus*	6º31´08.51´´N	3º24´06.04´´E	45	30	84
	4	*Ganoderma lucidum*	6º31´07.64´´N	3º24´06.16´´E	41	85	106
	5	*Xylaria* spp.	6º31´07.04´´N	3º24´06.25´´E	47	76	99
Ozolua road, University of Lagos	1	*Ganoderma applanatum*	6º30´49.07´´N	3º23´50.49´´E	53	56	34
	2	*Ganoderma adspersum*	6º30´53.25´´N	3º23´43.16´´E	62	50	70
Araga-poka, Epe	1	*Coprinus comatus*	6º35´19.08´´N	3º28´54.78´´E	63	32	30
	2	*Polyporus* spp.	6º35´24.05´´N	3º28´41.82´´E	77	54	61
Ijede, Ikorodu	1	*Agaricus campestris*	6º30´58.94´´N	3º23´57.03´´E	34	42	115
Old Ota road, Ile epo.	1	*Pleurotus tuber-regium*	6º33´51.09´´N	3º25´42.54´´E	55	60	75
	2	*Mycena haematopus*	6º33´35.74´´N	3º25´73.25´´E	57	69	88
	3	*Mycena* spp.	6º33´37.46´´N	3º25´71.33´´E	82	45	91
Owodunni, Alagbado	1	*Trametes versicolor*	6º38´03.67´´N	3º27´20.09´´E	58	38	77
	2	*Polyporus squamosus*	6º38´03.67´´N	3º27´20.09´´E	56	43	81
Gate (University of Lagos), Akoka	1	*Polyporus sulphurous*	6º31´33.29´´N	3º23´20.09´´E	74	47	83

into Eppendorf tubes. Five hundred microliter (500 µl) of cetyl trimethyl ammonium bromide (CTAB) buffer was added. It was later mixed for homogenisation. Afterwards, the mixture was incubated at 65°C for 15 min and allowed to cool. Ten microliter of Protenase K solution (an equal volume of a mixture of phenol: chloroform: isoamyl alcohol (25:24:1, v/v) was then added to the mixture and incubated at 37°C for 30 min. This is done in order to remove the RNA molecules in the mushroom samples.

On cooling, five hundred microliter (500 µl) of chloroform was added again to the mixture and mixed for 5 min. The resultant mixture was then centrifuged at 10,000 rpm for 10 min in order to separate each component in the homogenous mixture. The supernatant was collected into a fresh Eppendorf tube without taking the white phase. Five hundred microliter of both cold isopropanol and absolute ethanol were added to the supernatant. The mixture was kept at the temperature of -20°C inside the refrigerator for 1 h. It was later brought out for centrifugation at 10,000 rpm for 10 min in order to separate the mixture. Afterwards, the supernatant was decanted carefully. 500 µl of 70% ethanol was added to the decanted supernatant which was mixed very well to achieve a homogenous solution. This was centrifuged at 10,000 rpm for 10 min and the supernatant was discarded. The pellet left was air dried for 1 h and then re-suspended in 200 µl sterile water. This procedure was repeated for each of the eight mushroom samples for 2 days. The samples were kept in sterilized PCR tubes for subsequent DNA sequencing analysis.

Preparation of 1% agarose gel

One gram of agarose was poured into 100 ml Tris/Acetic Acid/EDTA (TAE) and was placed in the microwave oven at 50°C for 3 min. On cooling, 3 µl of ethidium bromide was added to 100 ml agarose gel. The mixture was allowed to solidify after 10 min. 3 µl of loading dye was added to the extracted DNA samples. This mixture and marker were then loaded on the electrophoresis gel machine which was allowed to run at 110 V for 1 h. Genomic DNA was visualized in a Gel Documentation System LG 2020 (Hangzhou Langqi, Inco., China) under ultra violet light source (Plate 2).

Amplification of regions of rDNA, PCR analysis and DNA sequencing

The voucher extracted genomic DNA samples in properly labelled eight different PCR tubes tagged BPO; 1 to 8 were sent to Macrogen Incorporation, Washington, U.S.A for subsequent DNA sequence analysis. Procedure – terms and conditions was applied (Macrogen Inc., Washington, U.S.A.).

Basic local alignment search (BLAST) sequence

The basic local alignment search tool (BLAST) of both the ITS 1 and ITS 4 sequences of each of the eight mushrooms was carried out to find the region of local similarity between sequences. The BLAST search was also carried out to compare a query sequence of the eight genomic DNA mushroom sequences with the library database of the GenBank (http://www.ncbi.nlm.nih.gov) and identify library sequences that resemble the query sequence above a certain threshold. This was achieved through the online analysis tool provided by the GenBank. The BLAST program was carried out

as described by Altschul et al. (1990).

Submission of genomic DNA nucleotide of the eight mushrooms

Registration was made online on the GenBank portal prior to submission of the genomic DNA FASTA sequences of the eight mushrooms. The FASTA sequences of the eight mushrooms were submitted using BankIt facility provided by the GenBank (http://www.ncbi.nlm.nih.gov). This was done to authenticate the genomic DNA sequence of the eight mushrooms for reference purpose and also for proper identification.

RESULTS

Identity of the mushrooms

A total sixteen (16) mushrooms were collected. The mushrooms collected showed varying morphological characteristics (Plate 1).

FASTA sequences of the studied mushrooms genomic DNA

The blast sequence query showed that *Issatchenkia orientalis* (FM199965.1) and *Pichia kudriavzevii* (HM771638.1) has the maximum identity (of 90%) with the genomic DNA sequence of *A. campestris* at ITS 1 (Figure 1) while at ITS 4 (Figure 2) no similarity of the *A. campestris* sequence was discovered with that in the GenBank library database (htpp://www.ncbi.nlm.nih.gov).

The blast sequence query showed that at ITS 1 (Figure 3) and ITS 4 (Figure 4) no similarity of the *P. sulphureus* sequence was discovered with that in the GenBank library database (htpp://www.ncbi.nlm.nih.gov).

The blast sequence query showed that *Lasiodiplodia pseudotheobromae* (GQ471832.1) has the maximum identity (of 100%) with the genomic DNA sequence of *G. lucidum* at both ITS 1 (Figure 5) and ITS 4 (Figure 6) with that in the GenBank library database (htpp://www.ncbi.nlm.nih.gov).

The blast sequence query showed that at ITS 1 (Figure 7) and ITS 4 (Figure 8) no similarity of the *X. polymorpha* sequence was discovered with that in the GenBank library database (htpp://www.ncbi.nlm.nih.gov).

The blast sequence query showed that *Lasiodiplodia theobromae* (EF622075.1) and *Botryosphaeria rhodina* (AY343481.1) has the maximum identity (of 84%) with the genomic DNA sequence of *C. comatus* at ITS 1 (Figure 9) while at ITS 4 (Figure 10) no similarity of the *C. comatus* sequence was discovered with that in the GenBank library database (htpp://www.ncbi.nlm.nih.gov).

The blast sequence query showed that *Schizosaccharomyces pombe* (U41410.1) has the maximum identity (of 86%) with the genomic DNA sequence of *Xylaria* spp. at ITS 1 (Figure 11) while at ITS 4 (Figure 12) no similarity of the *Xylaria* spp. Genomic DNA sequence was discovered with that in the GenBank library database (htpp://www.ncbi.nlm.nih.gov).

The blast sequence query showed that at ITS 1 (Figure 13) and ITS 4 (Figure 14) no similarity of the *P. tuber-regium* genomic DNA sequence was discovered with that in the GenBank library database (htpp://www.ncbi.nlm.nih.gov).

The blast sequence query showed that *L. pseudotheobromae* (GQ471832.1) has the maximum identity (of 99%) with the genomic DNA sequence of *P. ostreatus* at both ITS 1 (Figure 15) and ITS 4 (Figure 16) with that in the GenBank library database (htpp://www.ncbi.nlm.nih.gov).

Therefore, of all the BLAST query of the eight mushroom genomic DNA sequences carried out, it was discovered that there were no similarity with the exact sequences that were queried with the GenBank library database (htpp://www.ncbi.nlm.nih.gov). This may be attributed to the virtual absence of the various genomic mushroom DNA sequences in the GenBank database (htpp://www.ncbi.nlm.nih.gov).

Further efforts were then made to submit the eight genomic DNA sequence in the GenBank library through the BankIt facility through documentation for reference purpose. The phylogenetic relationship of each of the eight mushrooms provided by the database (Figure 17) showed that the *X. polymorpha* and *Xylaria* spp. were of the division Ascomycotina while others (*A. campestris*, *G. lucidum, P. tuber-regium, P. ostreatus, C. comatus, P. sulphureus*) belong to the division Basidiomycotina. *A. campestris, C. comatus, P. tuber-regium* and *P. ostreatus* belong to the order *Agaricales* while *X. polymorpha* and *Xylaria* spp. belongs to the order *Xylariales. A. campestris* and *C. comatus* belong to the family *Agaricaceae* while the duo of *P. tuber-regium* and *P. ostreatus* belongs to the family *Pleurotaceae. G. lucidum* and *P. sulphureus* belong to the Order *Polyporales* and of the family *Ganodermataceae*.

DISCUSSION

The biodiversity of some mushrooms have been studied in Lagos State through DNA sequence analyses. The mushrooms were collected in and around the Lagos environs for the eight marked bands indicated the mushroom RAPD/PCR products when viewed under the ultraviolet light source as shown in Plate 2. DNA sequencing of eight isolated mushroom mycelia. Also, a blast query of the mushrooms genomic DNA sequence was carried out to detect if there would be any similarity with the sequences that might have been submitted by any researcher previously in the GenBank database.

It was discovered that some of the mushroom genomic DNA sequences showed maximum identity with other fungi that were not mushrooms while others did not show any similarity with previous submission in the GenBank

Plate 1. *Mushroom samples collected in Lagos State, Nigeria. (a) Polyporus* spp. *(B) Ganoderma lucidum (C) Daldinia concentric* (D) *Xylaria polymorpha* (E) *Ganoderma applanatum.* (F) *Pleurotus ostreatus.* (G) *Coprinus comatus* (H) *Trametes versicolor* (I) *Agaricus campestris* (J) *Pleurotus tuber-regium* (K) *Ganoderma adspersum* (L) *Polyporus sulphurous* (M) *Polyporus squamosus* (N) Mycena *haematopus* (O) Xylaria spp.(P) *Mycena* spp.

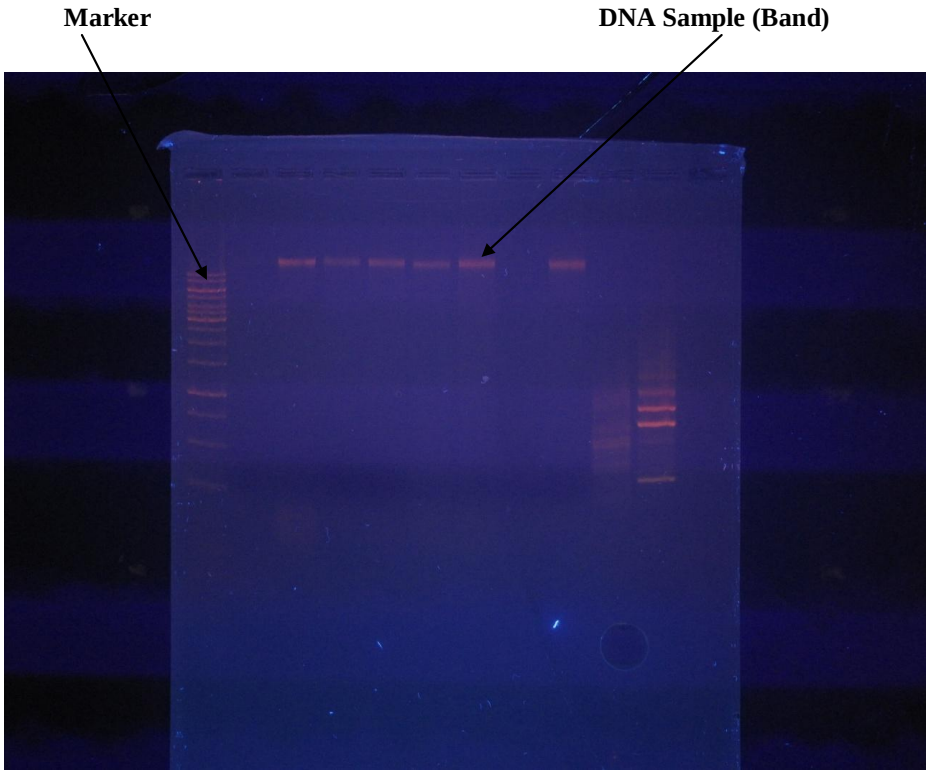

Plate 2. Agarose gel electrophoresis of RAPD/PCR products with eight identifying mushroom DNA bands viewed under the ultra-violet light.

>110810-11_E09_BPO-1-ITS1.ab1 488

TGAGTCGTTTTTGTTTCTCGGTGGAGGGGTGGGGCGGCCAAGCGGCCCCGGAAAAAAGTCTACTTCGCTCGGCCACCTTCT
CGCCCTTTCAGGGAAGTCCAGCTCCCACCTCTTTACACGTCCTCCGCTCGGCTCCCCCAACTCTGCGCACGCGCGAGAGGGA
AACGACGCTCAGACGAGCATGCCCCCCGGAAGTGGAAGGCGCAATGTGCGTTCAAGAACTCGATGATTCACATGGCTGCA
ATTCACACTAGGTATCGCATTTCGCTGCGCTCTTCATCGATGCGAGAACCAAAGATCCGTTGTTGAAAGTGTTGTTTGTGTT
TTCCGAGATTTCTCTTGTCCACTATATGCTATATTCCACATTTTAAGTGTTGTTGGTTTCCTTCCGCTCACCCCGTGTAGAAT
AAATCACAATAATGATCCTTCCCGCGGCCCTTCGGAAA

Figure 1. FASTA sequence of *Agaricus campestris* (90% homologous maximum identity with *Issatchenkia orientalis*).

>110810-11_G13_BPO-1-ITS4.ab1 1131

ACTTGTTGCCCGAGATAATCAGTGGTGGTGGTGGTGGTGCTCGAGTGCGGCCGCAGAGTCGACACCACTACCGCTACCGCC
CTGCTTGTCGGCCATGATATAACGTTGTGGCTGTTGTAGTTGTACTCCAGCTTGTGCCCCATGATGTTGCCGTCCTCCTTGAA
GTCGATGCCCTTCAGCTCTGTGCGGTTCACCAGGTGTCGCCCTCGAACTTCACCTCGGCCCGGGTCTTGTATTGCCGTCGTC
CTTGAAAAAATGGTGCGCTCCTGGACGTAACCTTCGGGCATGGCGGACTTGAAGAAGTCGTGCTGCTTCATGTGGTCGGGG
TAGCGGCTGAAGCACTGCACGCCGTAGGTCAGGGTGGTCACGAGGGTGGGCCAGGGCACGGGCAGCTTGCCGGTGGTGCA
GATGAACTTCAGGGTCAGCTTGCCGTATGTGGCATCGCCCTCGCCCTCGCCGGACACGCTGAACTTGTGGCCGTTTACGTCG
CCGTCCAGCTCGACCAGGATGGGCACCACCCCGGTGAACAGCTCCTCCCCTTGCTCACATGGTGATGATGGTGGATGGGTG
ATGTTCTTTCATATGCTATTATGTATATCTCCTTCTTAAAGTTAGACAAAATTATTTCTACAGGGGGAATTGTTATCCGCTCA
CAATTCCCCTATAGTGAGTCGTATTAATTTCGCGGGGATCAGATCGATCTCGATCCTCTACCCCGGACGCATCTGGCCGGCA
TCACCGGGCCCAGGTGCGTGCTGGCCCTATATCCCAATCCCAGGAATCAGCTCGCCACTTTCGGCTCCTGACGCTTGTTCGC
GGGGTGGTGGGCCCGGCCGGAAGAACTGTTGAGCATCGCTGGATGCCAATCTGGGGGACGGAAAGGCCTTCACTCACGCT
GGTTCAAGGAAAGATCTCGAAGGAAAGCAAGTATCCTGACATCCAACCTTGTGAAACCCAAAAATATTTTTTGGCAAGCTT
CAAAGTCCTGCTTC

Figure 2. FASTA sequence of *Agaricus campestris* (90% homologous maximum identity with *Issatchenkia orientalis*)

>110810-11_G09_BPO-2-ITS1.ab1 490

GGCTGAGGCGGCGCTCCCTTTTTTTGTGTCGTGCAGTCGGTGTTGGCCGCCAATCCCCCTCAAAAAGGACTTCTTTCGCCCC
CCCCTGCTCCTCCCTTTTGACAGATTCCTAGTTCCACCCTTTTTTACCGTCCGTCTCTTCCCTCCCCAACTTCTCGGAAGGGC
GAGAGGAAAAGCGCGACTCGTGTGATGGCCAACTTAGGGACCCTACAAGCCTGTGAGTCCAAAACCCCCGAATGCCCAAC
GGGGGCAATTAAAACTAGGAACCGCATTTCTTTGCCCTCTTCAATTCCGAAAACCAAAAAACCCCCTGTGGAAGTTTTTTTT
GGTTTTTCCGAAATTTTCTCTTGTCCATAATTTCCAATTTTCCCCATTTAAGTTTTTGTTGTTTTCTTCCCCCCCACCCGGGGA
GTATTAAATAAAAATAATGATCCTTCCGCGTGTACGTACCCATAAAG

Figure 3. FASTA sequence of *Polyporus sulphureus* (no homologous identity with previous submissions in the GenBank).

>110810-11_I13_BPO-2-ITS4.ab1 1138

CGTCTGTTAGCACGGATCTCACTGCTGGTGGTGGTGGTGCTGCACTGCCGCCCCACACTCGACACCACTACCGCCACCTACC
TGCTTGTCGGCCATGATATATACGTTGTGGCTGTTGTACTTGTACTCCATCTTGTGCCCCACCATGTTCCCCTCCTCCTTGAA
GGCTATGCCCTTCCTCGCAGTGCCGTTCCCCCCGGTGTCCCCCTCGGACTTCTGTTCGTCCCAAATTTGAAGATTCACTGAAT
TTTGAATTAATTTCACTTAGCCAATTTCGATGCTTCTTCTGGCATGCTTTCCTTAAAGCCTTTTTGTGGTCGGGGTTTATCCTT
GAAGCTTTAGACGTCAGGGTATGCTGACCGAATTTGGGGGCCCTTTGGCATTTTGCCGTGGTGCAAATGCTTCATGCTCAAC
GGGCCCCCGGCCTCGTCCTCACCCTCAACGAACACATAATCCTGCCGATGTTTTACGTCGGAGTCCACCTGGACCAGGATG
GCCACCACCCCGGTGAACAGTTCCTCCCCTTGCTCACATGACTTCACCGATGGTGATGATCGTTCTTCGCCATTTCGGGATC
TAGATCTCCTTCTTCGGTTAGAAAAAAGTAGCTCTACAGGGAGAATTGATACGGTTGGGGTTCCCCTATATTGAGGCGTATT
TCCTTGCGGGGATGGAGCTCGATCGCATCCTCTACTGGACGCAGCTTTCCACGGCTCGACTCCATCAGGGAGAGCGTGCGA
GTTCCCAATCCCTAATTGCCCAAGCGATTATCAGTATGCCATGATAGGCCTGAACGTCTCCTCGGTGATGAGAGCCAGCCC
CGCGCGTATAACAGGTCGCACTTTGCCAGGGTGTATGCTCGGTTCGGAGGAAGCCATCAACTCGTTGGGGGGATAGGGGA
AAAAATCGAAACGAGAGAATAATTACCCACTGGCGCACGATACTGTGGGAAACGCCGCAAAATAGCTTCCCTCCCTGGTC
CCCCCGCCTCCATGGTCACTTATTCTCCTC

Figure 4. FASTA sequence of *Polyporus sulphureus* (no homologous identity with previous submissions in the GenBank).

>110810-11_I09_BPO-3-ITS1.ab1 519

ATTCGGGCTTCGGCTCGACTCTCCCACCCTTTGTGAACGTACCTCTGTTGCTTTGGCGGCTCCGGCCGCCAAAGGACCTCCA
AACTCCAGTCAGTAAACGCAGACGTCTGATAAACAAGTTAATAAACTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCA
TCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTG
CGCCCCTTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTACAACCCTCAAGCTCTGCTTGGAATTGGGCACCGTCCTC
ACTGCGGACGCGCCTCAAAGACCTCGGCGGTGGCTGTTCAGCCCTCAAGCGTAGTAGAATACACCTCGCTTTGGAGTGGTT
GGCGTCGCCCGCCGGACGAACCTTCTGAACTTTTCTCAAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGC
ATATCAGGGAGGAA

Figure 5. FASTA sequence of *Ganoderma lucidum* (100% homologous maximum identity with *Lasiodiplodia pseudotheobromae*).

>110810-11_K13_BPO-3-ITS4.ab1 517

CAGATCCGAGGTCACCTTGAGAAAAGTTCAGAAGGTTCGTCCGGCGGGCGACGCCAACCACTCCAAAGCGAGGTGTATTCT
ACTACGCTTGAGGGCTGAACAGCCACCGCCGAGGTCTTTGAGGCGCGTCCGCAGTGAGGACGGTGCCCAATTCCAAGCAG
AGCTTGAGGGTTGTAATGACGCTCGAACAGGCATGCCCCCCGGAATACCAAGGGGCGCAATGTGCGTTCAAAGATTCGAT
GATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGT
TGAAAGTTTTAGTTTATTAACTTGTTTATCAGACGTCTGCGTTTACTGACTGGAGTTTGGAGGTCCTTTGGCGGCCGGAGCC
GCCAAAGCAACAGAGGTACGTTCACAAAGGGTGGGAGAGTCGAGCCGAAGCCCGAAAACTCGGTAATGATCCTTCCGCAA
GGCACCTATACGAAA

Figure 6. FASTA sequence of *Ganoderma lucidum* (100% homologous maximum identity with *Lasiodiplodia pseudotheobromae*).

CGCGCCCTTTTGTGTCCGCACGCGGTGGCGCCCCCTGGATCGGAAAAAAACCCTTTTCGATCGCAGCTTCCCCCTTGTTGGA
GTTAGCCGCTCCCCCTCTCTTATCAACTCCTCATCTTCTCTTCCCCCCCTGCTCGGCAACGCGGGAGAAGGAAACCACGCAA
AGACAGGTGTGCCCCCGAGAAATCCGAGGGGCTGTATGTGCACTTTAAAACCCATTGACTCCCGACGCCTGCCATTGATCT
ATCTTCTTATTTACCAAAAGTTAGGTTCGTGAAAACCTAAATCCCCCGTGGGAAGAAAATTCAGAGATTTCCGAAATATCC
CTTTGGCCCTGAATTCTAAGGTCCCCCCTTTATGTTTTGGTTGTTTTCTTCCGCCCCCCCCCCGGGATGAATAAATAATCCAC
ATGATTATCCTGCGGCAACGTACCGATACAGAATAATTAGCATTTATTAATGTCTCTGGAAGCTTTGGAACTAGCTCACGCA
ACAGACGTATATCTTTCGGGTGCTTAAACAATCGTTTCGGTAGGAGTATGACATCCGAGTTTCACTTGTATAAGAAACATG
AGGTTACTACAGGTAGACATGCTTAATCTCTGGGAAGATGAAGTAAAGAAACTATTCACTCTATTGCGACGCCCATGCCCC
TAGCTGTCTGGACGGGGATGCCAACATATTAATACAATGACCTCGTAGGCCGTGTAGGAATTCCAGCAATTCCAGATAACA
CTATATAATAAAAACGCTGAGCTAATAAATAAGTAATAGATGTTAGCTTAACCGGAGTCCTTATATAATAGCGAGACTATT
AGCTAGGTATATAGGTAAATAGTAAACGAGACTTTCTCTCCTCTGCCACAT

Figure 7. FASTA sequence of *Xylaria polymorpha* (no homologous similarity with previous submissions in the GenBank).

>110810-11_M13_BPO-4-ITS4.ab1 511

TTACTTGATAATAGTGCTGGTGGTGGTGGTGCTCCCTGCTGCCCCACACAAACCCACGGCCTCCACCGACCTGCTTGTCCGT
GCTGAGCTCGTTCAGGCTGACGCGAACTCTCAATGGAGAGCCCCCCCCAAGTTTCTGGCTAGCGTGAGGGCTATGCCCACAC
TGGATGTGCCCTTCCCCCCTGAGTGGCCCCAGGACCCCTGTGCCTCCAAAAACTTGATGATTCACGGAATTCTGAAATTAAT
TTTACTTAGCCCAATTCGATGTTGTCTTCTACCATGATAACCCAGAGATCCATTGTTAAAGCTTTTAGTTTATTAACTTGTGA
TCTGACGTCTGCCTTTGCTGACTCTAATTTGGAGGTGGTTTGGCATCCGTTGCCTTCCCGGACTAGGGTTCCTTCTCAAAGTG
TGTGTGGTCATACTCACTAAAACGGAAGATAATGCTCCCAATTGCCGCCGCCGCCGGCT

Figure 8. FASTA sequence of *Xylaria polymorpha* (no homologous similarity with previous submissions in the GenBank).

>110810-11_M09_BPO-5-ITS1.ab1 908

CTGCTCCCCATTTTGTGCCGCACTCTGTCGCTTTGGCGCCTCCTGCCGCCGGAGGACCTCCGACTCCCCACCCCAACTGCCC
CTCTGATAAACGACTTAACTTAATATAACATTAAACAATTGATTTTTTGGTTCTGGCATCGATGAACAAGGCACGAAATGC
GATAAGTTATGAGAATTGCACACCCCGAAAAATCATCGAATCTATGAACGCACAAAGAGCCCATTGATATTCCAGGGGGC
CTGCCTGCTCGAGCGTCATTACAACCCTCAGGCTCTGCTTGGAATTGGGTACCGCCCTCACTGCGGACGCGCCTCAAAGAC
CCCGACGGAGGCCGGTCATCCCTCAAGCGTGCTCCAATACCCCTTTTTTTGTAGTGGCTGGCGTCCCCCGCCGGACGAACCT
TCTGAACTATCCTCAATGTTGACCTCGGATCAGGTAGGGAAACCCGATGAACTTAAGCATATAATGTGCCGGAGGACCGAC
GAAGTTAGTTCACGAATTGATATTTTATCGTCTTCGGTTTCAGAATCAATCTCATCGGCAAGTTCCTGTTCTTTGACGTATCC
TTCAACAGATTTTGTCATTTTTGGAAAGAAGAGCTTGCTTAAAATATTTGAATGGAGATAGAAAGTTACATTACACAAATTT
ACTGTTATGAGCTTTTGAACCTGTCTTCCGTATGCCAATTTGATGTTATCCATGACAGTTCCCAAGTATGCTTCCTCTATACA
TAGATATAAGTAAGTTCAAATCTCTCGCTAACTAAATACGAAAGTATATGGAGATGTTGTGTTATGTATTAACAGTATGCAT
TCACAAGAGACAAACGAATCATCGCCTCAAACAAACAAT

Figure 9. FASTA sequence of *Coprinus comatus* (84% homologous maximum identity with *Botryosphaeria rhodina*).

>110810-11_O13_BPO-5-ITS4.ab1 515

TTCTGTGTTTGAGAAGTTCAGAAGGTTCGTCCGGCGGGCGACGCCAACCACTCCAAAGCGAGGTGTATTCTACTACGCTTG
AGGGCTGAACAGCCACCGCCGAGGTCTTTGAGGCGCGTCCGCAGTGAGGACGGTGCCCAATTCCAAGCAGAGCTTGAGGG
TTGTAATGACGCTCGAACAGGCATGCCCCCCGGAATACCAAGGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAA
TTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTAT
TTTATTAACTTGTTTATCAGACGTCTGCGTTTACTGACTGGAGTTTGGAGGTCCTTTGGCGGCCGGAGCCGCCAAAGCAACA
GAGGTACGTTCACAAAGGGTGGGAGAGTCAGCCGAAGCCCGAAAACTCTGTAATGATCCTTCCGCAAGGTTCACCCTACCG
GA

Figure 10. FASTA sequence of *Coprinus comatus* (84% homologous maximum identity with *Botryosphaeria rhodina*).

>110810-11_O09_BPO-6-ITS1.ab1 918

GTTGGCTTGCTCGTGCATATAAAATACTGCACTAGAAGGCTTGACCCCCAAGACACTTGGGCTTATCTATGTGGCTTACGTT
TGCCTGTGGCTTGGTGAAGTTCTCTGATCTGCATCTCCGTCTCTTCCATCCATGCCGCCACTCAATGGAGTGTTACCAGGTG
CACGGCCCTCCCTTGAAGCATCTATTGAAAACCAAAGTAATGGTATACGGAATTCTACTGTCACAAAAGACCGTGTAGAAA
TACTACTGCGGATGTCTCTCAATCCCAAACCATTGAAACATCTGGACCTTCTAACGAAGTTCCTACTGCTCAACCGGATGCT
TGTGCTGTAGCCTACATTTTTATTGGACTTTCTAACTGCCATTCCCAAGTTTTGATCGTTGAAAGACGATTTGTCCTCAGGAA
AAAATGATGAAAATCACGAATAATTTCCCTACCCTAGCCAATCTTTTTTTGATAATATTAGTTGTGAAAGTAAAGAGAAGG
GTATGGAAGCTGCAGATAGAAGAGCTCGTTTAAACTGGATGGCGGCGTTAGTATCGAATCGACAGCAGTATAGCGACCAG
CATTCACTTACGATTGACGCATGATATTACTTTCTGCGCACTTAACTTCCATCTGGGAAGGGACGATTCAGAAACTTACTAT
AATTTCGACGGCAATGTCCAAGCTGTCATACAGCGATGCCAAGATGTTGTGACAGCACCACCCAGGCCGAGGTTGATTTCC
GGCAATCACAGAGATAACATATATAATTAGAAACTCTCACTATTTAAAGTAAAGATAAAGAGAATGTTATAATTCCTTAAA
AGGAGCTCATTCAAAGATAAGAGAGACTATTTAATCCGACCCAAGGTAGATAAGTAA

Figure 11. FASTA sequence of *Xylaria* sp. (86% homologous maximum identity with *Schizosaccharomyces pombe*).

>110810-11_A15_BPO-6-ITS4.ab1 1229

CTGGACGGGCTTTCGCGAATGGAGGTGGTGGTGGGGGGTTGCGGACTGCAATTGGTCATTGGCCCCCCCGGTAGCCGGGCA
TTTGGTGTGACGATCCACCAACGCTCACTGGTAAACTACCTGGCCGATCCCCCAATAATGAATTGAAAAAGTGCATGGTCT
CGTCGCGCATGCAGCCCCGAAAGTGGTTTGGCCCATGGGGATCTTGAGAACTCGGATGAGCACGAGGGCTGACCTCTGGTT
GATTTGGGTTCAAATGAGGCGCTCTCTGGAGGATTTGAACAAAACGGGGCGGTTCATAATGTTGAGGTTGCGTTTCCATTT
GTTGCTCATGGCGAAGCTGTGCTATCTCCCACGAGATGGAGGGTGTTTTCTTTTCGGACGTTCCACAATATACTCTCATAGT
AATGATCCGCCCTAAGAACATCCTCATCACTCACCAACATCACCATCGTACTCCCCAACGTCACCATCACAGTCACCTACAT
CACCATCGTTCCAACGTCGCCATCATATTCGCCAACGTCATGATGATAGTCGCCACGTCCCTCGTATTCTCCAAGGGGTCCA
TCGTATTCACCAACGGGCCTTCCTACTAACTCACGGCCCCAAGCAATCCCCCTACGGGGTCCTTCTTATTCTCCTACGGATC
CATCATATGGTCCTACGGCACCGAGTTACGATCCCAGGGGCACCAGTTGCGGCCCAACGGGGTCCACCCTATTCCCCATCA
CCCACCAAGTTACTAGTCCTAAAACGCCATCATCCTCTTCACGCTACTATCCCTTTTCCCCAACCACCCTTCCTACTCTCCCC
GGCGCCCAAAAAGCTCGACTGCATTGTAACGCCCAACTCGAGGTACGCGCAAGTCCAGTATTCGCAGAACAAACAAACAT
ATGAAAAGGAAAATTCCCAAGAATACCATTCCTCCTGACCTATTTCCGTTTAATAAAGTCCAAAATAAGTATGTCTCANGTT
ATTTTTGGATCCTAACAANACCGTCTACAAGAAACCCTTATTTGTACTCCATTAGGGGGGGACGGGAACGCATACCTATTTT
TTCGCCAAAGAGTGGTAAAACCCGGGGAAAAT

Figure 12. FASTA sequence of *Xylaria* sp. (86% homologous maximum identity with *Schizosaccharomyces pombe*).

>110810-11_A11_BPO-7-ITS1.ab1 54

TATGCTAGGTCGATTTTTTTCACCCCG

Figure 13. FASTA sequence of Pleurotus tuber-regium (no similarity with previous submissions in the GenBank).

>110810-11_C15_BPO-7-ITS4.ab1 5

GATCGATTTTCAGCTTAGTAAAGCAAA

Figure 14. FASTA sequence of Pleurotus tuber-regium (no similarity with previous submissions in the GenBank).

Database (http://www.ncbi.nlm.nih.gov). This might have been as a result of no previous submissions of the mushroom genomic DNA sequences by any researcher in the GenBank database. Furthermore, the genomic NA

>110810-11_C11_BPO-8-ITS1.ab1 518

TCGTTCGGCTCGACTCTCCCACCCTTTGTGAACGTACCTCTGTTGCTTTGGCGGCTCCGGCCGCCAAAGGACCTCCAAACTC
CAGTCAGTAAACGCAGACGTCTGATAAACAAGTTAATAAACTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGAT
GAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCC
CTTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTACAACCCTCAAGCTCTGCTTGGAATTGGGCACCGTCCTCACTGC
GGACGCGCCTCAAAGACCTCGGCGGTGGCTGTTCAGCCCTCAAGCGTAGTAGAATACACCTCGCTTTGGGAGTGGTTGGCG
TCGCCCGCCGGACGAACCTTCTGAACTTTTCTCAAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATAT
CATGGAAGGAAA

Figure 15. FASTA sequence of *Pleurotus ostreatus* (99% homologous identity with *Lasiodiplodia pseudotheobromae*).

>110810-11_E15_BPO-8-ITS4.ab1 517

GCCCTGATCAGGTCACCTTGAGAAAAGTTCAGAAGGTTCGTCCGGCGGGCGACGCCAACCACTCCAAAGCGAGGTGTATTC
TACTACGCTTGAGGGCTGAACAGCCACCGCCGAGGTCTTTGAGGCGCGTCCGCAGTGAGGACGGTGCCCAATTCCAAGCAG
AGCTTGAGGGTTGTAATGACGCTCGAACAGGCATGCCCCCCGGAATACCAAGGGGCGCAATGTGCGTTCAAAGATTCGAT
GATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGT
TGAAAGTTTTAGTTTATTAACTTGTTTATCAGACGTCTGCGTTTACTGACTGGAGTTTGGAGGTCCTTTGGCGGCCGGAGCC
GCCAAAGCAACAGAGGTACGTTCACAAAGGGTGGGAGAGTCGAGCCGAAGCCCGAAAACTCGGTAATGATCCTTCCGCAA
GGTCCTTACGGAAG.

Figure 16. FASTA sequence of *Pleurotus ostreatus* (99% homologous identity with *Lasiodiplodia pseudotheobromae*).

sequences (Figures 1 to 17) were deposited in the GenBank for academic and reference purpose through D the BankIt Online tool provided by the web portal. This provides a detailed and comprehensive identification ofthe mushrooms through the description given by the GenBank in form of phylogenetic relationship/tree (Figure 17).

The phylogenetic tree (Figure 17) given by the GenBank further confirms the assertion that mushrooms falls into two fungal divisions of Basidiomycotina and Ascomycotina. The result of this research shows the genome rDNA sequences of the mushroom samples which is more accurate and reliable in phylogenetic typing and identification of mushrooms than the conventional means. Identification of mushrooms is mainly by morphological description of the fruiting bodies, host specificity and geographical distribution (Seo and Kirk, 2000). In most cases, morphological characteristics have their limitation in allowing a reliable distinction of intraspecific characteristics. It has been noted that the genus *Ganoderma* presently represents taxonomic chaos (Ryvarden, 1991). Molecular techniques could be used to adequately characterize and identify intra and inter species (Zakaria et al., 2009).

The ability to accurately and reproducibility identify fungi; both yeasts and molds has been greatly enhanced through comparative DNA sequencing (Hall et al., 2003). Fungal taxonomists have used DNA sequences for many years as a basis for reclassification of all fungal taxa and have more recently moved to ITS sequencing.

In the past three to four decades, research findings had pointed to mushrooms as important sources of pharmacologically important bioactive compounds that can improve health. Hence, correct identification procedure of medicinal mushrooms is required for quality control of functional health-aid preparations as well as nutritional supplements (Lee et al., 2006). Gene sequences also serve as a basis of molecular tools for sensitive and incisive identification of mushrooms. Highly conserved genes such as ribosomal RNA (rRNA) genes provide information on the general properties of the organism based on the properties of their known relatives. In addition, analysis of the rDNA sequences has the advantage that it not only enables species identification but also permits phylogenetic analysis.

In conclusion, it was demonstrated that DNA sequencing is an accurate identification measure of mushroom samples than the conventional macroscopic, ecological and geographical means. More research should be carried out on sequencing the DNA of mushroom samples of specific medicinal, edible, toxic, psychoactive properties.

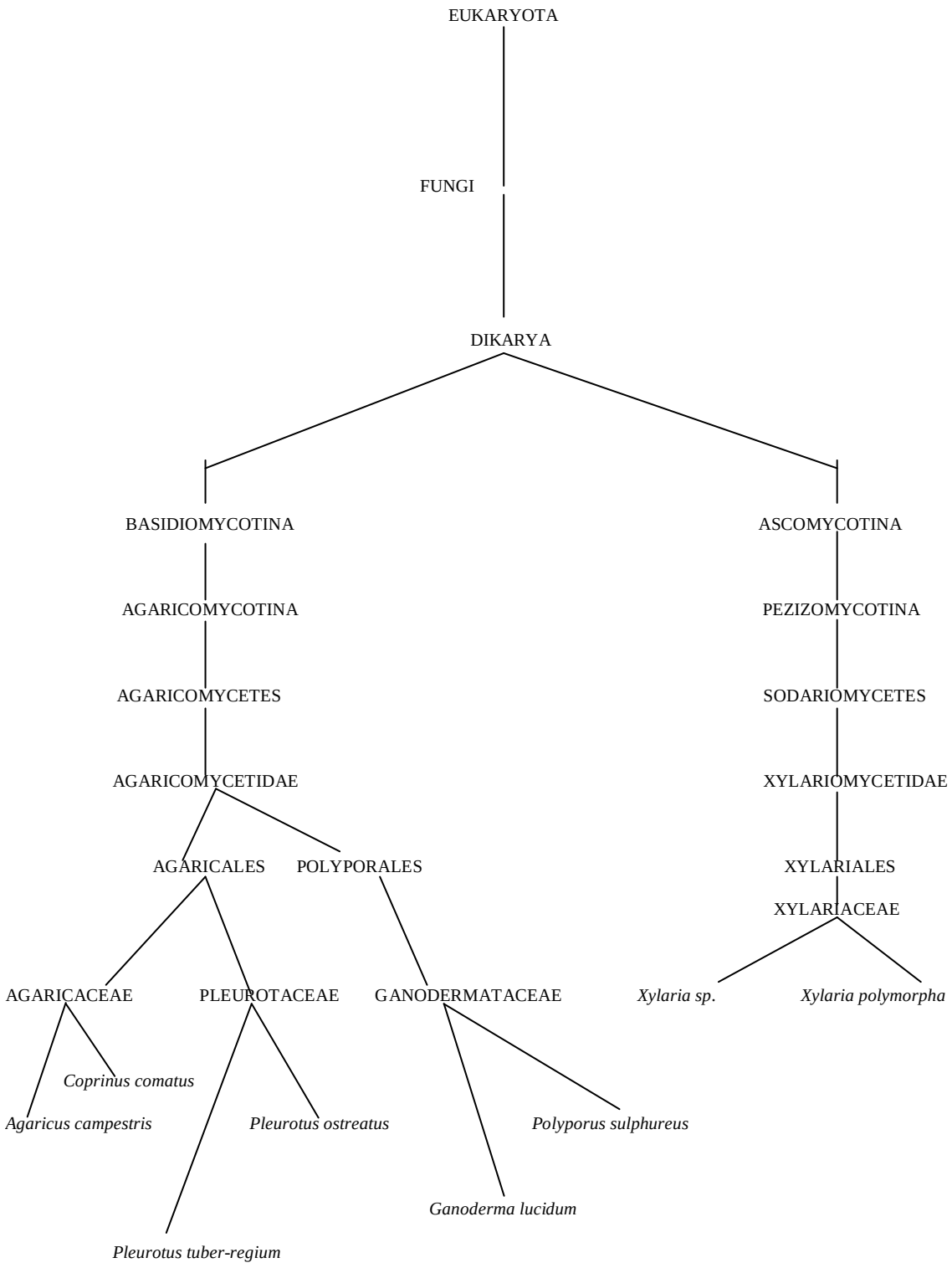

Figure 17. Phylogenetic relationship/tree of the studied mushrooms.

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