Spatial and regional directory of tropical *Auricularia* mushrooms in Southwest, Nigeria

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Abstract

Bioremediation of wastelands and dumpsites in Africa is fast declining due to reduced mushroom populations. In the past, the forest of Africa was teaming with mushrooms, but nowadays; mushrooms are severely exploited, resulting in gradual drift to extinction. Mushrooms have the tendency to degrade recalcitrant wastes and absorb heavy metals (Bio-accumulation). Unless concerted efforts are made to rejuvenate or rescue the surviving mushroom population, Africa will one day be overshadowed by wastes. The mushroom diversity in Southwest, Nigeria was determined by both morphological and molecular markers, 14 primers (OPB-11, OPB-12, OPB-15, OPB-20, OPB-21, OPH-3, OPH-5, OPH-10, OPH-15, OPT-1, OPT-5, OPT-7, OPT-10 and OPT-19) produced polymorphism with the test samples under electrophoresis gel (PCR and RAPD). Using standard morphological markers, *Auricularia auricula* was found to be evenly distributed across 8 locations in Ekiti and Osun, 6 locations in Ogun, 5 locations in Oyo and 4 locations in Lagos. There was none identified in Ondo. *Auricularia polytricha* was found in abundance in all the locations in Ondo. Lagos only had 3 out of its outline Stations graced with the presence of *A. polytricha*, whereas, Ogun, Ekiti, Osun and Oyo had no records of *A. polytricha.* From the genetic dissimilarity chart, 6 clusters of mushroom, sub-characterized into 3 distinct species (*Auricularia polytricha*, *A. auricula* and an unrelated *Auricularia* outlier species) and 5 cultivars were obtained in the region of Southwest, Nigeria. The population of all the *Auricularia* mushrooms currently present in Southwest, Nigeria was effectively captioned (Location, type and identity) by this research.

Keywords: *Auricularia* Mushrooms; Southwest, Nigeria; Species speciation; Regional diversity; RT-PCR; RAPD; DNA markers.

Word Count: 250

1.0 Introduction

Mushroom foraging was once considered as a prominent means of generating income for the locals of West Africa and it was also a pertinent raw material in folklore and traditional medicine (Guissou *et al.*, 2008). In the past, West Africa was regarded as a major hotspot of mushroom diversity (Hawksworth, 2004), and as such, the implementation of mushrooms in the bioremediation of wastelands and dumpsites was considered as a lucrative and environmentally friendly approach, because mushrooms have the tendency to degrade ligno-cellulosic wastes within a short period of time and even possess the capacity to bio-accumulate and harness a vast array of heavy metals from the soil (Adenipekun *et al*., 2015). Mushrooms are majorly found in the wild (Osemwegie *et al.*, 2014), or close to human settlements (Crous *et al.*, 2006).

Mushrooms are currently threatened by extinction due to the massive destruction of their natural habitat (The wild) by natural disasters e.g. bush fire, earth quake, tornado etc., or by human intervention for their selfish interest e.g. construction of schools, hospitals, industries, houses, sport centres etc. (Gateri *et al.*, 2004) and overgrazing by animals/humans. Mushroom domestication was the first line of action taken by ancient man to secure, safeguard, preserve and conserve mushroom species from extinction. One of the most economically important genera of the mushroom family is "*Auricularia* mushroom", they are mostly edible mushroom of global repute with only about 17% global production, currently ranked 3rd as the most cultivated mushroom genus after *Lentinula* (22%) and *Pleurotus* (19%) (Royse *et al.*, 2017; Bandara *et al*., 2019).

The genus *Auricularia* belongs to the family *Auriculariaceae*, class *Agaricomycetes*, phylum *Basidiomycota* and kingdom *Fungi* (Moore *et al*., 2001; Moore 2013). Several species exist within

the fungi order "*Auriculariales"* that are useful as both edible and medicinal mushrooms (Chang and Hayes, 2013). *Auricularia* mushrooms are widespread throughout the temperate and sub-tropical zones of the world, and can be found across Europe, North America, Asia, and Australia (Conte and Laessoe, 2008). It has been reported that there are only 15-20 species of *Auricularia* worldwide with 8 species identified in China (Chang and Miles, 2004). Among these species, *A. auricula and A. polytricha* are the most popular and the most cultivated around the world (Chang and Miles, 2004). *Auricularia auricula* commonly known as wood ear mushrooms is native to Kenya and occurs in Kakamega forest in Western Kenya. In other parts of Africa, the wood ear mushrooms have been reported in Nigeria where it is being conserved through cultivation on palm substrates (Osemwegie and Okhuoya, 2009). In Kenya, the wood ears have not been previously cultivated because they are protected by wildlife conservation laws.

According to Osemwegie *et al.* (2014), proper inventory of wild or domesticated edible mushrooms with high medicinal values, sold in local markets is required for the development of a mushroom genetic resource or germplasm, a database for differentiating between toxic and edible mushrooms, and the cultivation of species yet uncultivated. Above all, the safety of life and a decline in the cases of "Mycotoxicosis" resulting in human and wildlife casualty is of paramount global interest. The dearth of information regarding the domestication and cultivation of mushrooms (Mushroom technology) became the major cause for dependence on mushroom hunting/foraging, practiced by the indigenous people of Africa. The common way to identify different *Auricularia* species was based on morphological characters such as size, shape and colour of the fruiting body etc. Musngi *et al*. (2004) effectively classified various strains of *Auricularia* spp in the Philippines by simply using phenotypic characters.

The use of morphological markers only for characterization of *Auricularia* species found in Southwest, Nigeria is largely unreliable, misleading and ineffective because it has several limitations which abound majorly due to the adverse effects and intricate influence of environmental factors (Etaware *et al*., 2020) on the phenotype or physical appearance of similar mushrooms species grown under different environmental conditions. Therefore, the use of molecular markers (PCR and RAPD) to determine the genetic diversity and variation among large genomic entity of *Auricularia* mushrooms with similar gene pool is an added advantage, a more reliable and valuable tool that can detect the slightest trace of genetic variability, which is the basis for characterization and classification of these species into a more organized taxa (Al-Gabbiesh *et al*., 2006).

Finally, a comprehensive knowledge of the varietal differences that exist within the genus, species and sub-species of the *Auricularia* mushroom can be exploited by the use of molecular markers, which will inferably serve as sources of cell lines for researchers and in-breeding programs within Africa and around the world (Pei-Sheng and Chang, 2004). Inferably without an aorta of doubt, one can surmise that the inability to effectively distinguish between poisonous and edible mushroom may also have accounted for the visible underdevelopment in global mushroom cultivation which has undermined the commercial scale production of edible and medicinal mushrooms for decades unending leading to low priority export or impact on foreign exchange earnings.

2.0 Materials and Methods

2.1 Spatial grouping (Stereotype and Catalogue) of *Auricularia* sp.

A total of fifty four (54) sample stations was setup across Southwest, Nigeria for the sole purpose of efficiency and accuracy in the catalogue of wild *Auricularia* mushrooms in the tropical, sub-

tropical, and rain forest region of Southwest, Nigeria (See Table 1-6 for comprehensive details). Field assessment was conducted regularly between September 2011 and July 2012. The geographical view of Southwest, Nigeria was described in Fig 1. The coordinates and geographical identity of each sample station was referenced in Table 1-6. Prospective *Auricularia* samples were identified at the Department of Botany, University of Ibadan, Ibadan, Oyo State, Nigeria, based on the recommended phenotypic behaviours (colour, shape, texture, and fruit body) given by Musngi *et al*. (2004).

Fig 1. **The geographical positioning of the States that comprises Southwestern Nigeria**

Table 1: The coordinates and location of sample stations in Ogun State, Southwest-Nigeria

S/N	Stations	Code	Local Govt. Area	Town	State	Latitude	Longitude
1	01	OG1	Abeokuta North	Abeokuta	Ogun	7.1475°N	3.3619°E
2	02	OG2	Ewekoro	Itori	Ogun	6.9530°N	3.2181°E
3	03	OG3	Ifo	Ifo	Ogun	6.8192°N	3.1930°E
4	04	OG4	Ijebu Ode	Ijebu Ode	Ogun	6.8300°N	3.9165°E
5	05	OG5	Ikenne	Ikenne	Ogun	6.8717°N	3.7105°E
6	06	OG6	Shagamu	Shagamu	Ogun	6.8322°N	3.6319°E
7	07	OG7	Odeda	Odeda	Ogun	7.2328°N	3.5281°E
8	08	OG8	Odogbolu	Odogbolu	Ogun	6.8365°N	3.7689°E

State Code: OG→Ogun → No *Auricularia* specimen found in that region

Table 2: The coordinates and location of sample stations in Lagos State, Southwest-Nigeria

S/N	Stations	Code	Local Govt. Area	Town	State	Latitude	Longitude
1	09	LA1	Agege	Ikeja	Lagos	6.6180°N	3.3209°E
2	10	LA2	Ojo	Ojo	Lagos	6.4579°N	3.1580°E
3	11	LA3	Apapa	Ikeja	Lagos	6.4553°N	3.3641°E
4	12	LA4	Badagry	Badagry	Lagos	6.4316°N	2.8876°E
5	13	LA5	Epe	Epe	Lagos	6.6055°N	3.9470°E
6	14	LA6	Shomolu	Shomolu	Lagos	6.5392°N	3.3842°E
7	15	LA7	Ikorodu	Ikorodu	Lagos	6.6194°N	3.5105°E
8	16	LA8	Mushin	Ikeja	Lagos	6.5273°N	3.3414°E

State Code: LA→Lagos → No *Auricularia* specimen found in that region

Table 3: The coordinates and location of sample stations in Oyo State, Southwest-Nigeria

S/N	Stations	Code	Local Govt. Area	Town	State	Latitude	Longitude
1	17	OY1	Akinyele	Moniya	Oyo	7.5249°N	3.9152°E
2	18	OY2	Egbeda	Egbeda	Oyo	7.3796°N	3.9675°E
3	19	OY3	Ido	Ido	Oyo	7.5077°N	3.7194°E
4	20	OY4	Iseyin	Iseyin	Oyo	7.9765°N	3.5914°E
5	21	OY5	Ogbomosho North	Ogbomosho	Oyo	8.1227°N	4.2436°E
6	22	OY6	Oluyole	Idi Ayunre	Oyo	7.2247°N	3.8732°E
7	23	OY7	Oyo	Oyo	Oyo	7.8430°N	3.9368°E
8	24	OY8	Olorunsogo	Igbeti	Oyo	8.7699°N	4.1104°E
9	52	OY9	Akinyele	Ojo	Oyo	7.5503°N	3.9470°E
10	53	OY10	Ibadan North	Bodija	Oyo	7.4351°N	3.9143°E

State Code: OY→Oyo → No *Auricularia* specimen found in that region

Table 4: The coordinates and location of sample stations in Ekiti State, Southwest-Nigeria

S/N	Stations	Code	Local Govt. Area	Town	State	Latitude	Longitude
1	25	EK1	Ado Ekiti	Ado Ekiti	Ekiti	7.6124°N	5.2371°E
2	26	EK2	Ilejemeje	Iye	Ekiti	7.9591°N	5.2371°E
3	27	EK3	Ikole	Ikole	Ekiti	7.7983°N	5.5145°E
4	28	EK4	Oye	Oye	Ekiti	7.7979°N	5.3286°E
5	29	EK5	Irepodun	Igede	Ekiti	7.7313°N	5.2476°E
6	30	EK6	Ikere	Ikere	Ekiti	7.4991°N	5.2319°E
7	31	EK7	Ijero	Ijero Ekiti	Ekiti	7.8120°N	5.0677°E
8	32	EK8	Emure	Emure Ekiti	Ekiti	7.4317°N	5.4621°E

State Code: EK→Ekiti → No *Auricularia* specimen found in that region

Table 5: The coordinates and location of sample stations in Ondo State, Southwest-Nigeria

S/N	Stations	Code	Local Govt. Area	Town	State	Latitude	Longitude
1	33	OD1	Idanre	Idanre	Ondo	7.0914°N	5.1484°E
2	34	OD2	Ilaje	Igbokoda	Ondo	6.2585°N	4.7692°E
3	35	OD3	Ile Oluji	Ile Oluji	Ondo	7.2825°N	4.8521°E
4	36	OD4	Odigbo	Ore	Ondo	6.7519°N	4.8780°E
5	37	OD5	Okitipupa	Okitipupa	Ondo	6.5025°N	4.7795°E
6	38	OD6	Ose	Ifon	Ondo	6.9235°N	5.7774°E
7	39	OD7	Owo	Owo	Ondo	7.1989°N	5.5932°E
8	40	OD8	Ifedore	Igbara Oke	Ondo	7.3877°N	5.0807°E
9	54	OD9	Akure South	Akure	Ondo	7.2571°N	5.2058°E

State Code: OD→Ondo → No *Auricularia* specimen found in that region

Table 6: The coordinates and location of sample stations in Osun State, Southwest-Nigeria

S/N	Stations	Code	Local Govt. Area	Town	State	Latitude	Longitude
1	41	OS1	Bolunduro	Ota Aiyebaju	Osun	7.5912°N	4.7329°E
2	42	OS2	Ejigbo	Ejigbo	Osun	7.9045°N	4.3052°E
3	43	OS3	Ifedayo	Oke Ila Orangun	Osun	7.9946°N	4.9974°E
4	44	OS4	Ifelodun	Ikirun	Osun	7.9227°N	4.6347°E
5	45	OS5	Ila	Ila Orangun	Osun	8.0121°N	4.8988°E
6	46	OS6	Irepodun	Ilobu	Osun	7.9021°N	4.5315°E
7	47	OS7	Iwo	Iwo	Osun	7.6292°N	4.1872°E
8	48	OS8	Obokun	Ibokun	Osun	7.8019°N	4.7692°E
9	49	OS9	Irewole	Ikire	Osun	7.3700°N	4.1872°E
10	50	OS10	Oriade	Ilesha	Osun	7.6395°N	4.7588°E
11	51	OS11	Oriade	Ipetu Ijesha	Osun	7.4273°N	4.9091°E

State Code: OS→Osun → No *Auricularia* specimen found in that region

2.2 Morphological characterization of prospective *Auricularia* sp.

The basidiocarps were rehydrated by soaking in water for 10 minutes before characterization. Qualitative characters such as colour, shape, and presence of hymenia was evaluated by physical observation while texture was determined by touching the back and top surfaces (Onyango *et al*., 2011). For microscopic characters, free hand transverse sections of approximately 0.1 mm thick were made from rehydrated basidiocarps with the aid of a sharp surgical blade. The sections were immersed in a diluted solution of methylene blue stain and left for 10 minutes. Thin sections were selected and placed on glass slides, fitted with cover slips and the anatomy of each basidiocarp was studied. The characterization of *Auricularia* spp based on morphological markers (Colour, Shape, and Texture etc. of both the mycelia and entire mushroom body) was described in Table 7.

Table 7: Classification of Auricularia species using morphological markers

	External Mushroom Features			Mycelia Features		Inference	
Case	**Colour**	**Shape**	**Texture**	**Colour**	**Type**	**Genus**	**Species**
1	Dark brown	Discoid	Gelatinous	White	Cottony	*Auricularia*	Unknown
2	Yellow brown	Auriform	Leathery	Off white	Cottony	*Auricularia*	*auricula*
3	Brown	Flattened	Rubbery	Off white	Scanty	*Auricularia*	*Unknown*
4	Dark brown	Discoid	Gelatinous	White	Cottony	*Auricularia*	*polytricha*

A modified protocol adapted from Onyango *et al*. (2011)

2.3 Molecular characterization of prospective *Auricularia* sp.

2.3.1 Tissue preparation and DNA Extraction

The modified DNA extraction protocol of Chen *et al*. (2010), involving the use of Cetyltrimethylammonium Bromide (CTAB), was used for DNA isolation. Tissues from the pileus of each mushroom specimens was aseptically detached using a sterile scalpel and 200mg was weighed out prior to DNA extraction. The tissues was pulverized with 800ml of CTAB buffer (20 mM EDTA, 1.4 mM NaCl, 100 mM Tris-HCl pH 8.0, SDS (1.25%, 2% CTAB and 0.2% β-mercaptoethanol (v/v)), incubated at 65°C for 15 min using water bath with intermittent homogenization, allowed to cool for approximately 1 min before adding equal volume of phenol, chloroform and iso-amyl alcohol at the ratio of 25:24:1.

The mixture was further centrifuged at 12,000 revolutions per minute (rpm) for 15 minutes; the supernatant was transferred to clean sterile tubes without unsettling the pellets. About 400 µl of ice-cold isopropanol was added to the supernatant and mixed by inverting the tubes 2-5 times to

precipitate the DNA and subsequently kept at -80°C for 1hr. The DNA sediment was pelleted by centrifugation at 12,000 rpm for 10 min and the dried DNA pellets obtained were re-suspended in 100 µl of Grand Island Biological Company (GIBCO) water (Invitrogen, Carlsbad, CA, USA) and 2 µl of 10 mg/ml RNase (Qiagen Valencia, CA, USA) was added to each of the samples and kept at 4°C for 30 minutes to remove fragments of RNA strands.

2.3.2 DNA Sequencing

The extracted DNA fragments from each Auricularia mushroom specimen was sequenced using the high-throughput Sequencing (HTS) or Next Generation Sequencing (NGS) Technique. A total of 2.5µl of the stock DNA samples were loaded on 1.5% agarose gel for electrophoresis and visualized under UV light (Model-2, Upland, CA, USA) to check the quality of the extracted DNA samples. Following the high level of concentration of the extracted DNA samples, dilution of each DNA sample was uniformly made to 100ng/uL DNA.

2.3.3 DNA Purification and Quantification

The sequenced DNA fragments were quantified using Nano-Drop spectrophotometer (ND-1000). About 2µl of the extracted DNA sample was used to obtain a unique ration of 1.8:2.0 at OD 260/280 absorbance level and concentration through which dilution samples were prepared for polymerase chain reaction (PCR).

Mathematically,

$$\text{The Optical Density (OD)} = \text{Log}_{10} \frac{Intensity\ of\ Incident\ Light}{Intensity\ of\ transmitted\ Light}$$

$$\text{OD 260/280 } (A_{260/280}) = \text{Log}_{10} \frac{IIL}{ITL}$$

2.3.4 DNA/RNA primer selection and buffer preparation

A total of twenty five (25) primers were subjected to screening for polymorphism with the prospective *Auricularia* species (i.e. OPB-1, OPB-2, OPB-3, OPB-4, OPB-5, OPB-6, OPB-7, OPB-8, OPB-9, OPB-10, OPB-11, OPB-12, OPB-15, OPB-20, OPB-21, OPH-3, OPH-5, OPH-10, OPH-15, OPT-1, OPT-5, OPT-7, OPT-10, OPT-19 and OPD-18) out of which fourteen (14) primers were polymorphic (OPB-11, OPB-12, OPB-15, OPB-20, OPB-21, OPH-3, OPH-5, OPH-10, OPH-15, OPT-1, OPT-5, OPT-7, OPT-10 and OPT-19) as shown in Table 8**.** The fourteen (14) arbitrary RAPD decamer primers (Table 8) obtained from Operon Technology (Alameda, CA, USA) were used for PCR amplification.

Table 8: Primers used for DNA amplification during molecular analysis

S/N	RAPD primer	DNA/RNA Primer Sequence	Melting Point (Tm°C)
1	OPB-11	5l GTAGACCCGT 3l	34
2	OPB-12	5l CGTTGACGCA 3l	34
3	OPB-15	5l GGAGGGTGTT 3l	32
4	OPB-20	5l GGACCCTTAC 3l	34
5	OPB-21	5l CGACCCTTAC 3l	34
6	OPH-3	5l AGACGTCCAC 3l	34
7	OPH-5	5l AGTCGTCCCC 3l	32
8	OPH-10	5l CCTACGTCAG 3l	32
9	OPH-15	5l GCTTCGTCAG 3l	34
10	OPT-1	5l GGGCCACTCA 3l	34
11	OPT-5	5l GGGTTTGGCA 3l	32
12	OPT-7	5l GGCAGGCTGT 3l	34
13	OPT-10	5l CCTTCGGAAG 3l	32
14	OPT-19	5l GATGCCAGAC 3l	32

Operon Technology, Alameda, California, USA

2.3.5 PCR Analysis (DNA Amplification and Fingerprinting)

The required capacity for PCR amplification was 25µl i.e. 2.0µl of 100ng DNA, 2.5µl of 10 x Buffer (Bioline), 1.25µl of 50mM $MgCl_2$ (Bioline), 2.0µl of 2.5mM dNTPs (Bioline), and 0.2µl 500U *Taq* DNA polymerase (Bioline), 1.0µl DMSO (dimethyl sulfoxide), 1.0µl of 10uM of each primer and 16.05µl of 500ml DEPC-treated water (Invitrogen Corporation). PCR amplifications were performed using Applied Bio-systems thermo-cycler with a cycling profile of an initial step of 94°C for 2 minutes, 40 cycles of 94°C for 20 s, 72°C for 1min, and 54°C for 2 mins., and a 5-min final extension at 72°C.

2.3.6 RAPD profiling using electrophoresis gel

Amplified fragments were separated by electrophoresis on 1.5% (w/v) agarose (Sigma Aldrich, USA) gels with 1X TBE (Tris-Boric acid-EDTA) buffer and stained with ethidium bromide (0.5mg/ml). The molecular fragments were estimated using 100-bp step DNA marker (Bio-labs, New England).

2.4 Statistical Analysis

Data matrix generated from the RAPD sequence for fragments of similar molecular weight from each individual mushroom specimens were scored as present (1) or absent (0). The data obtained from scoring the RAPD bands were used to determine the genetic dissimilarity matrix using Jaccard's similarity coefficient (Jaccard 1908 Standard Protocol). Phylogenetic relations were determined by cluster analysis using UGPMA (un-weighted pair-group method with arithmetic averages) aided by the NTSYS-pc software version 2.02 (Rohlf 1998 Preferred Protocol). Phylogenetic characterization into multivariate groups was done using principal component analysis

(PCA) with Darwin software version 5.0.0.157 while polymorphic information content (PIC) was calculated using the method of Botstein *et al*. (1980). The data obtained were analyzed using a one way analysis of variance (ANOVA) aided by SPSS v20. Significantly different means were separated using Tukey test at $P<0.05$

3.0 Results

3.1 Geo-mapping of *Auricularia* sp. in Southwest, Nigeria

A total of 31 samples of *Auricularia auricula* were identified and geo-tagged at several strategic locations within Southwest, Nigeria (See Table 9-14 for more details). *Auricularia auricula* was evenly distributed across 8 sample Stations in Ekiti (Table 12) and Osun (Table 14) States, 6 locations in Ogun State (Table 9), 5 Stations in Oyo State (Table 11) and 4 locations in Lagos State (Table 10). There was none identified in Ondo State (Table 13) as at the time of filing this reports. *Auricularia polytricha* was found in abundance in Ondo State i.e. it was evenly distributed around 8 strategic locations within the State (Table 13). Lagos State only had 3 out of its outline Stations graced with the presence of *A. polytricha* (Table 10). Ogun, Ekiti, Osun and Oyo States had no records of *A. polytricha* i.e. the mushroom was not found within their forest domain prior to the compilation of this report. About 5 species from the genus *Auricularia* found in Ogun (Table 9), Lagos (Table 10) and Oyo (Table 11) States were not identified to their species level due to discrepancy in their morphological status.

Table 9: Geo-mapping of *Auricularia* species in Ogun, Nigeria based on morphological characters

Tag		Location		GPS Coordinate		Status	
Station	**Code**	**Local Govt. Area**	**Town**	**Latitude**	**Longitude**	**Genus**	**Species**
01	OG1	Abeokuta North	Abeokuta	7.1475°N	3.3619°E	*Auricularia*	Unknown
02	OG2	Ewekoro	Itori	6.9530°N	3.2181°E	*Auricularia*	Unknown
03	OG3	Ifo	Ifo	6.8192°N	3.1930°E	*Auricularia*	*auricula*
04	OG4	Ijebu Ode	Ijebu Ode	6.8300°N	3.9165°E	*Auricularia*	*auricula*
05	OG5	Ikenne	Ikenne	6.8717°N	3.7105°E	*Auricularia*	*auricula*
06	OG6	Shagamu	Shagamu	6.8322°N	3.6319°E	*Auricularia*	*auricula*
07	OG7	Odeda	Odeda	7.2328°N	3.5281°E	*Auricularia*	*auricula*
08	OG8	Odogbolu	Odogbolu	6.8365°N	3.7689°E	*Auricularia*	*auricula*

State Code: OG→Ogun → No *Auricularia* specimen found in that region

Table 10: Geo-mapping of *Auricularia* species in Lagos, Nigeria based on morphological characters

Tag		Location		GPS Coordinate		Status	
Station	**Code**	**Local Govt. Area**	**Town**	**Latitude**	**Longitude**	**Genus**	**Species**
09	LA1	Agege	Ikeja	6.6180°N	3.3209°E	*Auricularia*	*auricula*
10	LA2	Ojo	Ojo	6.4579°N	3.1580°E	*Auricularia*	*auricula*
11	LA3	Apapa	Ikeja	6.4553°N	3.3641°E	*Auricularia*	*auricula*
12	LA4	Badagry	Badagry	6.4316°N	2.8876°E	*Auricularia*	*auricula*
13	LA5	Epe	Epe	6.6055°N	3.9470°E	*Auricularia*	Unknown
14	LA6	Shomolu	Shomolu	6.5392°N	3.3842°E	*Auricularia*	*polytricha*
15	LA7	Ikorodu	Ikorodu	6.6194°N	3.5105°E	*Auricularia*	*polytricha*
16	LA8	Mushin	Ikeja	6.5273°N	3.3414°E	*Auricularia*	*polytricha*

State Code: LA→Lagos → No *Auricularia* specimen found in that region

Table 11: Geo-mapping of *Auricularia* species in Oyo, Nigeria based on morphological characters

Tag		Location		GPS Coordinate		Status	
Station	**Code**	**Local Govt. Area**	**Town**	**Latitude**	**Longitude**	**Genus**	**Species**
17	OY1	Akinyele	Moniya	7.5249°N	3.9152°E	*Auricularia*	*polytricha*
18	OY2	Egbeda	Egbeda	7.3796°N	3.9675°E	*Auricularia*	*auricula*
19	OY3	Ido	Ido	7.5077°N	3.7194°E	*Auricularia*	Unknown
20	OY4	Iseyin	Iseyin	7.9765°N	3.5914°E	*Auricularia*	Unknown
21	OY5	Ogbomosho North	Ogbomosho	8.1227°N	4.2436°E	*Auricularia*	*auricula*
22	OY6	Oluyole	Idi Ayunre	7.2247°N	3.8732°E	*Auricularia*	*auricula*
23	OY7	Oyo	Oyo	7.8430°N	3.9368°E	*Auricularia*	*auricula*
24	OY8	Olorunsogo	Igbeti	8.7699°N	4.1104°E	*Auricularia*	*auricula*
52	OY9	Akinyele	Ojo	7.5503°N	3.9470°E	None	None
53	OY10	Ibadan North	Bodija	7.4351°N	3.9143°E	None	None

State Code: OY→Oyo → No *Auricularia* specimen found in that region

241 **Table 12**: Geo-mapping of *Auricularia* species in Ekiti, Nigeria based on morphological characters

Tag		Location		GPS Coordinate		Status	
Station	**Code**	**Local Govt. Area**	**Town**	**Latitude**	**Longitude**	**Genus**	**Species**
25	EK1	Ado Ekiti	Ado Ekiti	7.6124°N	5.2371°E	*Auricularia*	*auricula*
26	EK2	Ilejemeje	Iye	7.9591°N	5.2371°E	*Auricularia*	*auricula*
27	EK3	Ikole	Ikole	7.7983°N	5.5145°E	*Auricularia*	*auricula*
28	EK4	Oye	Oye	7.7979°N	5.3286°E	*Auricularia*	*auricula*
29	EK5	Irepodun	Igede	7.7313°N	5.2476°E	*Auricularia*	*auricula*
30	EK6	Ikere	Ikere	7.4991°N	5.2319°E	*Auricularia*	*auricula*
31	EK7	Ijero	Ijero Ekiti	7.8120°N	5.0677°E	*Auricularia*	*auricula*
32	EK8	Emure	Emure Ekiti	7.4317°N	5.4621°E	*Auricularia*	*auricula*

242 State Code: EK→Ekiti → No *Auricularia* specimen found in that region

243 **Table 13**: Geo-mapping of *Auricularia* species in Ondo, Nigeria based on morphological characters

Tag		Location		GPS Coordinate		Status	
Station	**Code**	**Local Govt. Area**	**Town**	**Latitude**	**Longitude**	**Genus**	**Species**
33	OD1	Idanre	Idanre	7.0914°N	5.1484°E	*Auricularia*	*polytricha*
34	OD2	Ilaje	Igbokoda	6.2585°N	4.7692°E	*Auricularia*	*polytricha*
35	OD3	Ile Oluji	Ile Oluji	7.2825°N	4.8521°E	*Auricularia*	*polytricha*
36	OD4	Odigbo	Ore	6.7519°N	4.8780°E	*Auricularia*	*polytricha*
37	OD5	Okitipupa	Okitipupa	6.5025°N	4.7795°E	*Auricularia*	*polytricha*
38	OD6	Ose	Ifon	6.9235°N	5.7774°E	*Auricularia*	*polytricha*
39	OD7	Owo	Owo	7.1989°N	5.5932°E	*Auricularia*	*polytricha*
40	OD8	Ifedore	Igbara Oke	7.3877°N	5.0807°E	*Auricularia*	*polytricha*
54	OD9	Akure South	Akure	7.2571°N	5.2058°E	None	None

244 State Code: OD→Ondo → No *Auricularia* specimen found in that region

245 **Table 14**: Geo-mapping of *Auricularia* species in Osun, Nigeria based on morphological characters

Tag		Location		GPS Coordinate		Status	
Station	**Code**	**Local Govt. Area**	**Town**	**Latitude**	**Longitude**	**Genus**	**Species**
41	OS1	Bolunduro	Ota Aiyebaju	7.5912°N	4.7329°E	*Auricularia*	*auricula*
42	OS2	Ejigbo	Ejigbo	7.9045°N	4.3052°E	*Auricularia*	*auricula*
43	OS3	Ifedayo	Oke Ila Orangun	7.9946°N	4.9974°E	*Auricularia*	*auricula*
44	OS4	Ifelodun	Ikirun	7.9227°N	4.6347°E	*Auricularia*	*auricula*
45	OS5	Ila	Ila Orangun	8.0121°N	4.8988°E	*Auricularia*	*auricula*
46	OS6	Irepodun	Ilobu	7.9021°N	4.5315°E	*Auricularia*	*auricula*
47	OS7	Iwo	Iwo	7.6292°N	4.1872°E	*Auricularia*	*auricula*
48	OS8	Obokun	Ibokun	7.8019°N	4.7692°E	*Auricularia*	*auricula*
49	OS9	Irewole	Ikire	7.3700°N	4.1872°E	None	None
50	OS10	Oriade	Ilesha	7.6395°N	4.7588°E	None	None
51	OS11	Oriade	Ipetu Ijesha	7.4273°N	4.9091°E	None	None

246 State Code: OS→Osun → No *Auricularia* specimen found in that

3.2 Molecular characterization of Prospective *Auricularia* sp.

3.2.1 DNA Purification (Quality) and Quantification

The prospective *Auricularia* specimens marked out from forty eight (48) locations within Southwest, Nigeria were further subjected to molecular test in order to ascertain and fully establish the genomic differences that exist among the mushroom specimens based on the influence of the environment and geographical boundaries, and further enhance the characterization made in this research based on morphological markers (Table 9-14). The first step was to extract and sequence their DNA materials. The extracted DNA from each prospective *Auricularia* Mushroom was tested for impurities; the purity of the extracted DNA samples was determined by UV light Absorbance at 260/280nm ratio using a spectrophotometer prior to PCR and RAPD analysis (See Table 15-20 for more details).

Majority of the DNA samples extracted for use in this experiment were pure ($A_{260/280}$ ~ 1.8) i.e. high-quality DNA extracts, with the exception of OG2 ($A_{260/280}$ = 1.78), OG6 ($A_{260/280}$ = 1.76) (Table 15), EK2 ($A_{260/280}$ = 1.74), EK3 ($A_{260/280}$ = 1.75) (Table 18), OD2 ($A_{260/280}$ = 1.67), OD8 ($A_{260/280}$ = 1.62) (Table 19), and OS4 ($A_{260/280}$ = 1.75) (Table 20), with little protein and RNA contaminants found in their DNA extracts. It was observed that *Auricularia* specimen collected from Station 8 in Ogun State had the highest quantity of pelleted DNA sample with 1,548.2ng/μL of pure concentrated nucleic acid (Table 15). The least quantity of DNA extracts was obtained from *Auricularia* mushroom samples within Station 6 in Ondo State (56.5ng/μL of Nucleic acid concentration) (Table 19).

Table 15: Qualitative assessment of nucleic acid extracted from *Auricularia* species in Ogun State

Station	Code	Town	Latitude	Longitude	Nucleic Acid Conc. (ng/µL)	$A_{260/280}$
01	OG1	Abeokuta	7.1475°N	3.3619°E	190.6	2.03
02	OG2	Itori	6.9530°N	3.2181°E	79.70	1.78
03	OG3	Ifo	6.8192°N	3.1930°E	250.0	1.87
04	OG4	Ijebu Ode	6.8300°N	3.9165°E	118.0	1.95
05	OG5	Ikenne	6.8717°N	3.7105°E	107.9	1.87
06	OG6	Shagamu	6.8322°N	3.6319°E	189.0	1.76
07	OG7	Odeda	7.2328°N	3.5281°E	92.10	2.02
08	OG8	Odogbolu	6.8365°N	3.7689°E	1548.2	1.89

State Code: OG→Ogun

Table 16: Qualitative description of nucleic acid extracted from *Auricularia* species in Lagos State

Station	Code	Town	Latitude	Longitude	Nucleic Acid Conc. (ng/µL)	$A_{260/280}$
09	LA1	Ikeja	6.6180°N	3.3209°E	79.00	2.08
10	LA2	Ojo	6.4579°N	3.1580°E	282.3	2.14
11	LA3	Ikeja	6.4553°N	3.3641°E	309.1	2.12
12	LA4	Badagry	6.4316°N	2.8876°E	137.8	2.13
13	LA5	Epe	6.6055°N	3.9470°E	890.0	1.83
14	LA6	Shomolu	6.5392°N	3.3842°E	96.90	2.11
15	LA7	Ikorodu	6.6194°N	3.5105°E	94.00	2.11
16	LA8	Ikeja	6.5273°N	3.3414°E	187.7	2.03

State Code: LA→Lagos

Table 17: Qualitative description of nucleic acid extracted from *Auricularia* species in Oyo State

Station	Code	Town	Latitude	Longitude	Nucleic Acid Conc. (ng/µL)	$A_{260/280}$
17	OY1	Moniya	7.5249°N	3.9152°E	110.6	2.06
18	OY2	Egbeda	7.3796°N	3.9675°E	1507.5	1.92
19	OY3	Ido	7.5077°N	3.7194°E	96.70	2.07
20	OY4	Iseyin	7.9765°N	3.5914°E	1118	1.96
21	OY5	Ogbomosho	8.1227°N	4.2436°E	543.5	1.99
22	OY6	Idi Ayunre	7.2247°N	3.8732°E	193.8	2.10
23	OY7	Oyo	7.8430°N	3.9368°E	490.7	2.01
24	OY8	Igbeti	8.7699°N	4.1104°E	239.3	2.10

State Code: OY→Oyo

Table 18: Qualitative description of nucleic acid extracted from *Auricularia* species in Ekiti State

Station	Code	Town	Latitude	Longitude	Nucleic Acid Conc. (ng/μL)	$A_{260/280}$
25	EK1	Ado Ekiti	7.6124°N	5.2371°E	867.5	2.04
26	EK2	Iye	7.9591°N	5.2371°E	87.30	1.74
27	EK3	Ikole	7.7983°N	5.5145°E	80.80	1.75
28	EK4	Oye	7.7979°N	5.3286°E	120.3	1.95
29	EK5	Igede	7.7313°N	5.2476°E	100.6	2.11
30	EK6	Ikere	7.4991°N	5.2319°E	450.2	1.99
31	EK7	Ijero Ekiti	7.8120°N	5.0677°E	125.0	2.09
32	EK8	Emure Ekiti	7.4317°N	5.4621°E	138.0	2.39

State Code: EK→Ekiti

Table 19: Qualitative description of nucleic acid extracted from *Auricularia* species in Ondo State

Station	Code	Town	Latitude	Longitude	Nucleic Acid Conc. (ng/μL)	$A_{260/280}$
33	OD1	Idanre	7.0914°N	5.1484°E	190.6	2.03
34	OD2	Igbokoda	6.2585°N	4.7692°E	107.9	1.67
35	OD3	Ile Oluji	7.2825°N	4.8521°E	92.10	2.02
36	OD4	Ore	6.7519°N	4.8780°E	105.0	2.08
37	OD5	Okitipupa	6.5025°N	4.7795°E	309.1	2.12
38	OD6	Ifon	6.9235°N	5.7774°E	56.50	1.83
39	OD7	Owo	7.1989°N	5.5932°E	94.00	2.11
40	OD8	Igbara Oke	7.3877°N	5.0807°E	1507.5	1.62

State Code: OD→Ondo

Table 20: Qualitative description of nucleic acid extracted from *Auricularia* species in Osun State

Station	Code	Town	Latitude	Longitude	Nucleic Acid Conc. (ng/μL)	$A_{260/280}$
41	OS1	Ota Aiyebaju	7.5912°N	4.7329°E	543.5	1.99
42	OS2	Ejigbo	7.9045°N	4.3052°E	490.7	2.01
43	OS3	Oke Ila Orangun	7.9946°N	4.9974°E	867.5	2.04
44	OS4	Ikirun	7.9227°N	4.6347°E	87.30	1.75
45	OS5	Ila Orangun	8.0121°N	4.8988°E	80.80	1.85
46	OS6	Ilobu	7.9021°N	4.5315°E	120.3	1.95
47	OS7	Iwo	7.6292°N	4.1872°E	100.6	2.11
48	OS8	Ibokun	7.8019°N	4.7692°E	193.8	2.10

State Code: OD→Ondo

3.2.2 Genetic diversity (speciation) of the sequenced *Auricularia* sp.

The major allele frequency, number of alleles, genetic diversity and polymorphic information content (PIC) of the sequenced DNA extracts from all the *Auricularia* mushroom specimens geo-tagged within Southwest, Nigeria was presented in Table 21. The allele frequency ranged from 0.3542 (OPB-15) to 0.6042 (0PH-15), while the genetic diversity was from 0.5930 (0PH-15) to 0.7977 (OPB-12) and the polymorphic information content was from 0.5594 (OPH-15) to 0.7819 (OPB-12). The percentage polymorphic amplicons varied from 55.9 (OPH-15) - 78.2% (OPB-12). Therefore, OPB-12 RAPD primer gave the highest level of polymorphism (78.2%) while OPH-15 gave the least level of polymorphism (55.9%) as represented in Table 21. Nevertheless, the polymorphisms revealed by the 14 decamer primers indicate that they are good and reliable for genetic diversity assessment in Mushroom and there is a high degree of diversity in the species studied.

Table 21: Genetic diversity of the sequenced *Auricularia* specimens from Southwest, Nigeria

DNA Primers	Major Allele Freq.	No. of Allele	Genetic diversity	PIC	Polymorphic Amplicons (%)
OPB-11	0.44	14.0	0.78	0.76	76.2
OPB-12	0.40	13.0	0.80	0.78	78.2
OPB-15	0.35	11.0	0.79	0.76	76.4
OPB-20	0.44	14.0	0.78	0.76	76.3
OPB-21	0.54	16.0	0.69	0.68	67.9
OPH-03	0.46	12.0	0.75	0.74	73.6
OPH-05	0.56	6.0	0.63	0.60	60.1
OPH-10	0.44	5.0	0.72	0.68	67.9
OPH-15	0.60	5.0	0.59	0.56	55.9
OPT-01	0.46	11.0	0.74	0.71	71.3
OPT-05	0.54	8.0	0.65	0.62	62.0
OPT-07	0.46	7.0	0.72	0.69	68.7
OPT-10	0.46	16.0	0.76	0.75	75.4
OPT-19	0.52	14.0	0.70	0.69	68.7
Average	0.48	10.9	0.72	0.70	70.0

Sample size (n = 48)

3.2.3 DNA fingerprinting and nucleotide polymorphism

The DNA marker OPB-21 had the highest number of polymorphic nucleotide (45/48) formed at 900bp (Fig 6 and Table 22), while DNA markers OPB-11 and OPB-15 at a joint highest record of polymorphic nucleotide units (46/48 each) at 100bp (Table 22). Majority of the *Auricularia* samples profiled on electrophoresis gel had no polymorphic nucleotides formed between 800-900bp units for the DNA markers OPB-11 (Fig 2), OPB-12 (Fig 3), OPB-15 (Fig 4), OPH-3, OPH-5 (Table 22), OPH-10 (Fig 7), OPH-15, OPT-1, OPT-5, OPT-7, OPT-10 (Table 22), and OPT-19 (Fig 8). A breakdown of the electrophoresis gel analysis for all base pair units was as follows: OPT-5 marker had 44/48 polymorphic nucleotides at 200bp, OPH-5 had 36/48 at 300bp, OPT-19 had 41/48 at 400bp, OPT-1 had 44/48 at 500bp, OPT-10 had 41/48 at 600bp, OPB-20 had 43/48 at 700bp and 46/48 at 800bp respectively as shown in Table 22 (only the highest number of polymorphic nucleotide was captioned).

Also, it was noted that OPH-5 DNA marker expressed polymorphism in 3 bands only (100, 200 and 300bp, respectively) making it the least effective marker for this experiment, OPH-10, OPH-15 and OPT-5 all expressed nucleotide polymorphism in 4 bands each (100, 200, 300 and 400bp, respectively), whereas, OPH-3 and OPT-7 showed nucleotide polymorphism at 100, 200, 300, 400 and 500bp, respectively (i.e. 5 bands each). Furthermore, OPB-15, OPT-1 and OPT-19 expressed polymorphism in 6 band units each (100, 200, 300, 400, 500 and 600bp, respectively), OPB-11, OPB-12 and OPT-10 each expressed nucleotide polymorphism in 7 bands (100, 200, 300, 400, 500, 600 and 700bp, respectively), and OPB-20 (shown in Fig 5) had 8 band units of polymorphic nucleotides (100, 200, 300, 400, 500, 600, 700 and 800, respectively). Finally, the highest band expression for polymorphic nucleotides was found in the DNA marker OPB-21 with 9 band units

(100, 200, 300, 400, 500, 600, 700, 800 and 900bp, respectively) thus, making it the most efficient marker for determining genetic variation with the earmarked *Auricularia* species in Southwest, Nigeria (Table 22).

Table 22: The number of polymorphic nucleotide amplified by different RAPD markers

	Polymorphic Nucleotide Count/bp Units								
Markers	**900bp**	**800bp**	**700bp**	**600bp**	**500bp**	**400bp**	**300bp**	**200bp**	**100bp**
OPB-11	00	00	06	13	13	06	33	38	46
OPB-12	00	00	22	02	31	37	24	32	32
OPB-15	00	00	00	32	26	21	10	42	46
OPB-20	00	46	43	02	35	16	02	32	44
OPB-21	45	09	12	37	17	09	01	09	39
OPH-3	00	00	00	00	36	27	14	27	40
OPH-5	00	00	00	00	00	00	36	35	04
OPH-10	00	00	00	00	00	03	21	31	07
OPH-15	00	00	00	00	00	03	04	07	05
OPT-1	00	00	00	09	44	28	09	38	33
OPT-5	00	00	00	00	00	02	10	44	17
OPT-7	00	00	00	00	02	02	04	26	31
OPT-10	00	00	38	41	15	12	05	04	26
OPT-19	00	00	00	30	38	41	16	12	05

Fig 2. RAPD profiling of 24 *Auricularia* mushroom specimens using OPB-11 marker

Fig 3. RAPD profiling of 24 *Auricularia* mushroom specimens using OPB-12 marker

Fig 4. RAPD profiling of 20 *Auricularia* mushroom specimens using OPB-15 marker

Fig 5. RAPD profiling of 20 *Auricularia* mushroom specimens using OPB-20 marker

Fig 6. RAPD profiling of 10 *Auricularia* mushroom specimens using OPB-21 marker

Fig 7. RAPD profiling of 24 *Auricularia* mushroom specimens using OPH-10 marker

Fig 8. RAPD profiling of 24 *Auricularia* mushroom specimens using OPT-19 marker

3.2.4 The Level of polymorphism within the *Auricularia* sp.

It was observed from the records in Table 23 that the DNA markers OPB-11, OPB-15, OPB-20, OPB-21, OPT-1 and OPT-5 was able to effectively detect 90-99% polymorphism in the DNA strands of the *Auricularia* specimen profiled on electrophoresis gel (Table 23), as such they were listed as the best markers for this research. Other DNA markers such as OPH-3, OPT-10 and OPT-19 showed an impressive 80-89% variability within the *Auricularia* population in Southwest, Nigeria, while OPB-12 and OPH-5 were able to give between 70-79% variation in the examined *Auricularia* mushroom population. DNA markers OPH-10 and OPT-7 each gave between 60-69% variation, while OPH-15 was only able to detect between 10-19% variation at maximum in the Auricularia mushroom population of Southwest, Nigeria (Table 23).

Table 23: The percentage variation (polymorphism) that exist among the selected *Auricularia* spp

	DNA Polymorphism (%)								
Marker	**900bp**	**800bp**	**700bp**	**600bp**	**500bp**	**400bp**	**300bp**	**200bp**	**100bp**
OPB-11	0.0	0.0	12.5	27.1	27.1	12.5	68.8	79.2	95.8
OPB-12	0.0	0.0	45.8	4.2	64.6	77.1	50.0	66.7	66.7
OPB-15	0.0	0.0	0.0	66.7	54.2	43.8	20.8	87.5	95.8
OPB-20	0.0	95.8	89.6	4.2	72.9	33.3	4.2	66.7	91.7
OPB-21	93.8	18.8	25.0	77.1	35.4	18.8	2.1	18.8	81.3
OPH-3	0.0	0.0	0.0	0.0	75.0	56.3	29.2	56.3	83.3
OPH-5	0.0	0.0	0.0	0.0	0.0	0.0	75.0	72.9	8.3
OPH-10	0.0	0.0	0.0	0.0	0.0	6.3	43.8	64.6	14.6
OPH-15	0.0	0.0	0.0	0.0	0.0	6.3	8.3	14.6	10.4
OPT-1	0.0	0.0	0.0	18.8	91.7	58.3	18.8	79.2	68.8
OPT-5	0.0	0.0	0.0	0.0	0.0	4.2	20.8	91.7	35.4
OPT-7	0.0	0.0	0.0	0.0	4.2	4.2	8.3	54.2	64.6
OPT-10	0.0	0.0	79.2	85.4	31.3	25.0	10.4	8.3	54.2
OPT-19	0.0	0.0	0.0	62.5	79.2	85.4	33.3	25.0	10.4

3.2.5 Grouping of *Auricularia* sp. in Nigeria based on genotype

The population of all the *Auricularia* mushrooms currently present in the six (6) States of Southwest, Nigeria were effectively classified into six (6) clusters on the genetic dissimilarity chart (Fig 9) using PCR and RAPD markers on representative samples collected during field survey. The six (6) clusters of mushroom categories were effectively characterized into three (3) distinct species and further sub-classified into five (5) cultivars (sub-species). The genetic relatedness of all the *Auricularia* mushrooms' population in Southwest, Nigeria was represented in Fig 9 and as such, classified thus:

Species 1: *Auricularia polytricha*

→ Cultivar I (Group I): OD1, OD8, OY1, OG1, OG2, LA6, LA7, LA8

→ Cultivar II (Group II): OD2, OD3, OD4, OD5, OD6, OD7

→ Cultivar III (Group IV): LA5

Species 2: *Auricularia auricula*

→ Cultivar I (Group III): OG3, OG4, OG5, OG6, OG7, OG8, OS1, OS2, OS3, OS4, OS5, OS6, OS7, OS8, EK1, EK2, EK3, EK4, EK5, EK6, EK7, EK8

→ Cultivar II (Group V): OY2, LA1, LA2, LA3, LA4, OY5, OY6, OY7, OY8

Species 3: Unrelated *Auricularia* specimen (Outliers)

OY3, OY4

Note: *The farther apart the Auricularia mushrooms on the genetic dissimilarity tree, the more related the species.*

Fig 9. Genetic dissimilarity among the population of *Auricularia* spp in Southwestern Nigeria

4.0 Discussion

The morphological markers used in this study was able to identify thirty one (31) locations in Southwest, Nigeria where *Auricularia auricula* can be found, and twelve (12) locations where *Auricularia polytricha* thrived better within the region under survey. It was noted also that six (6) out of the earmarked fifty four (54) locations had no *Auricularia* mushrooms present within their domain. These could be as a result of severe foraging (wild mushroom exploit) or destruction of their natural habitats by man. Samples of *Auricularia auricula* was evenly distributed in Ekiti, Osun, Ogun, Oyo and Lagos States; but there was none identified in Ondo State as at the time of filing this reports. There are limited scientific explanations to this observation since the region enjoys a seemingly even distribution of rainfall and sunshine as do other States in Southwestern Nigeria. *Auricularia polytricha* was found in abundance in Ondo and Lagos States only, but none was found in Ogun, Ekiti, Osun and Oyo States. Ironically, a scientific explanation is imminent. Therefore, more research work is recommended in this field and with regards to the observations outlined by this research in order to fully address the questions raised. The findings were in line with the reports of Onyango *et al.,* 2010) who identified three (3) main strains (brown, dark brown and yellow brown) of *Auricularia* mushrooms occurring in the forest region of Africa using morphological markers. Also, Li *et al*. (2011) reported that similar clustered patterns, reveals that all the tested strains could be divided into three distinct groups, each of which was correlated with different geographical regions.

In order to ascertain and fully establish the genomic differences that exist among the mushroom specimens based on the influence of the environment and geographical boundaries, and further enhance the characterization made in this research based on morphological markers. The

mushrooms were further subjected to molecular testing using PCR and RAPD techniques. Molecular markers such as rDNA sequencing, Restriction fragment length polymorphism (RFLP), Random amplified polymorphic DNA (RAPD) and genotyping have been used to discriminate mushroom species or strains of *Agaricus, Auricularia, Ganoderma, Lentinula, Stropharia*, and *Volvariella.* All of these technologies provided data for mushroom strain identification and protection (Chandra *et al.*, 2010).

The DNA marker "OPH-5" was the least effective marker for this experiment, while OPB-21 was the most efficient marker for determining genetic variation with the earmarked *Auricularia* species in Southwest, Nigeria. This was in agreement with the research of Khan *et al.*, (2011) who conducted molecular characterization of Oyster mushroom *(Pleurotus* spp.) using 14 RAPD primers and obtained the highest polymorphism by primers OPL-3 (72.70 %) and OPL-11 (70%). Two species (P-56 and P-17) were found to be genetically similar having a similarity value of 86%. The population of all the *Auricularia* mushrooms currently present in the six (6) States of Southwest, Nigeria were effectively classified into six (6) clusters on the genetic dissimilarity chart using PCR and RAPD markers on representative samples collected during field survey. The six (6) clusters of mushroom categories were effectively characterized into three (3) distinct species and further sub-classified into five (5) cultivars (sub-species). The result obtained in this study also agrees with the report of Ravash *et al.,* (2009) who used RAPD markers to confirm the similarity or dissimilarity of genetic relationship of *Pleurotus* spp.

Conclusion

The use of morphological markers only for characterization of *Auricularia* species found in Southwest, Nigeria was pragmatic as it produced a very good result but the best option was a combination of both morphological and molecular markers (PCR and RAPD) to determine the genetic diversity and variation within the large genomic entity of *Auricularia* mushroom population in Southwest, Nigeria.

Ethical Statement

This is to confirm that:

Prof. Clementina O. Adenipekun, Dr. V. S. Ekun, Dr. P. M. Etaware and Dr. Omena B. Ojuederie declares that they have no conflict of interest and that they actively participated in the research both in the field and in the procurement of materials for morphological and molecular analysis.

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Peter M. Etaware (Ph.D.)

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Conflict of Interest

All the authors declare that there is no competing interest.

Ethical Approval

'Not applicable'.

Consent to Participate

All the authors gave their consent to participate in this research.

Consent for Publication

All the authors unanimously agreed that this article should be published

Availability of Data and Material

All data and material are present in this publication.

Authors' Contributions

E.V.S. and A.C.O conceptualized and designed the experiment. E.V.S conducted the research and E. P. M. wrote the draft manuscript. E. P. M. and O.O.B. reviewed the manuscript. All authors approved the final version of the manuscript.

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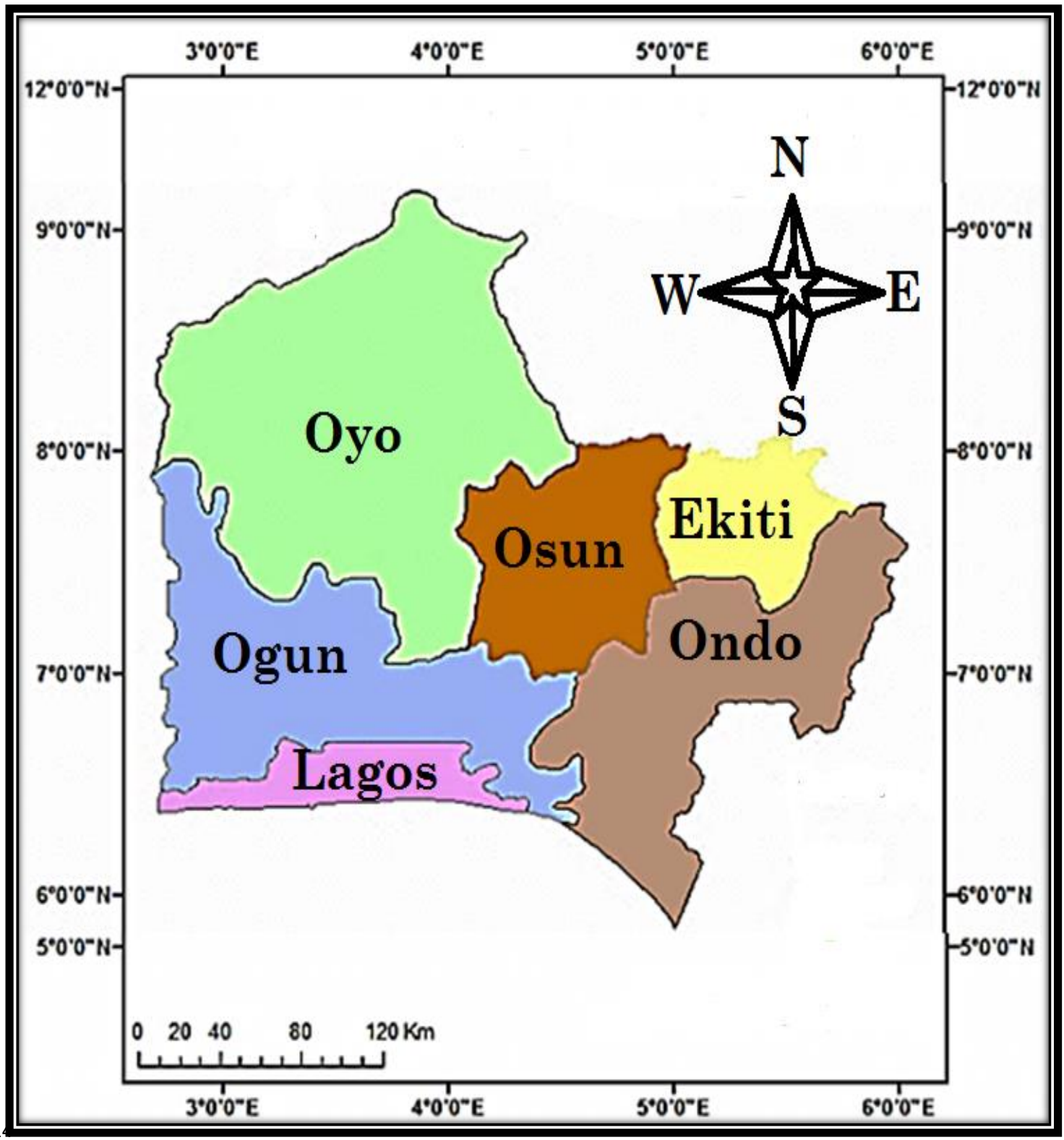
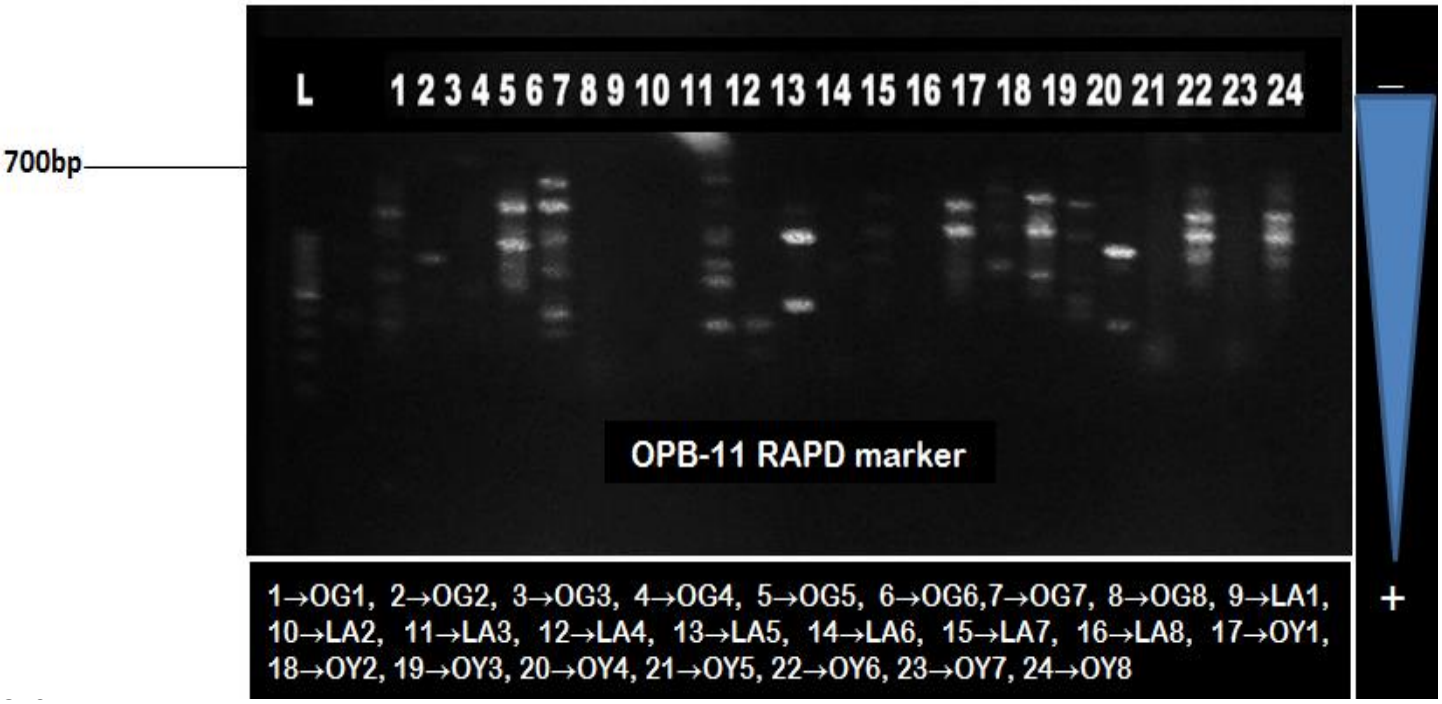


Fig 1



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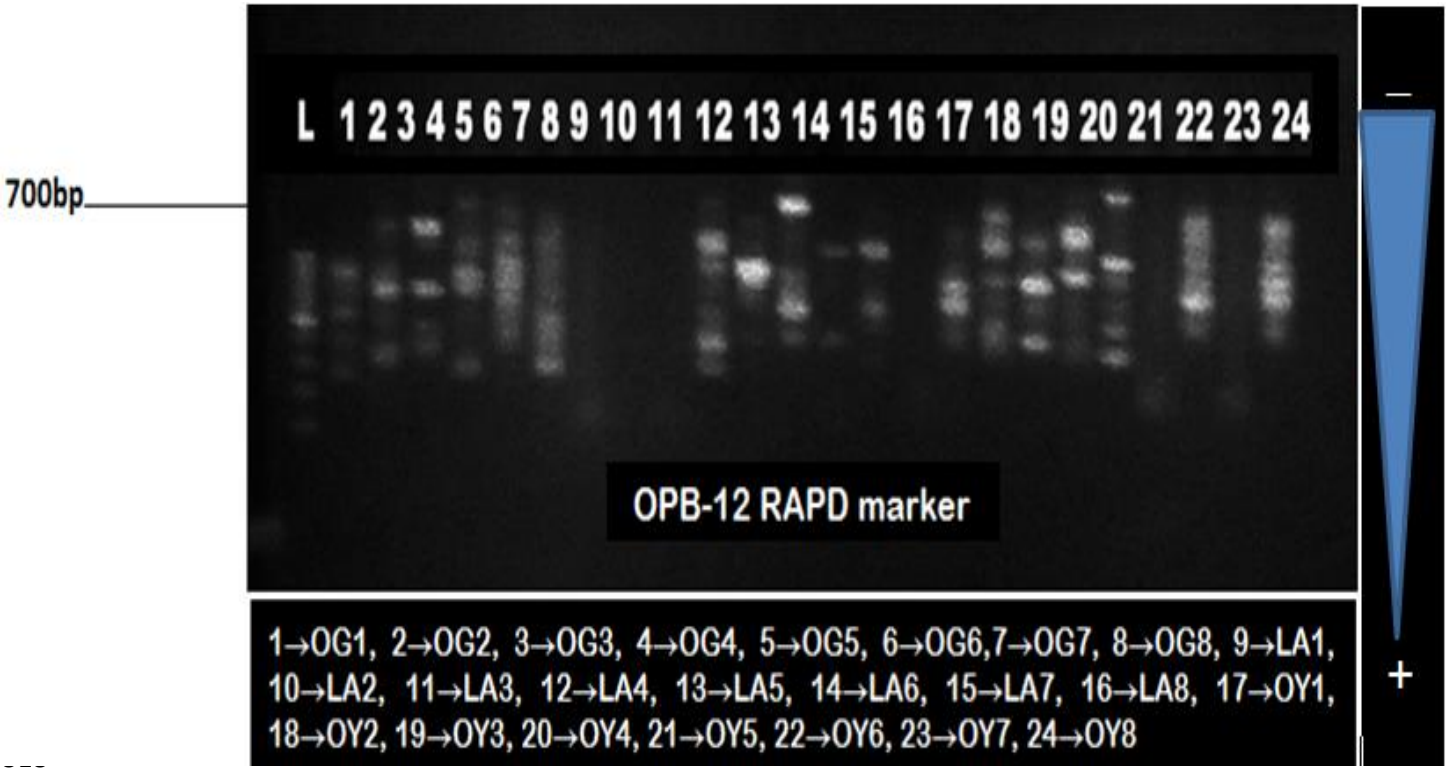

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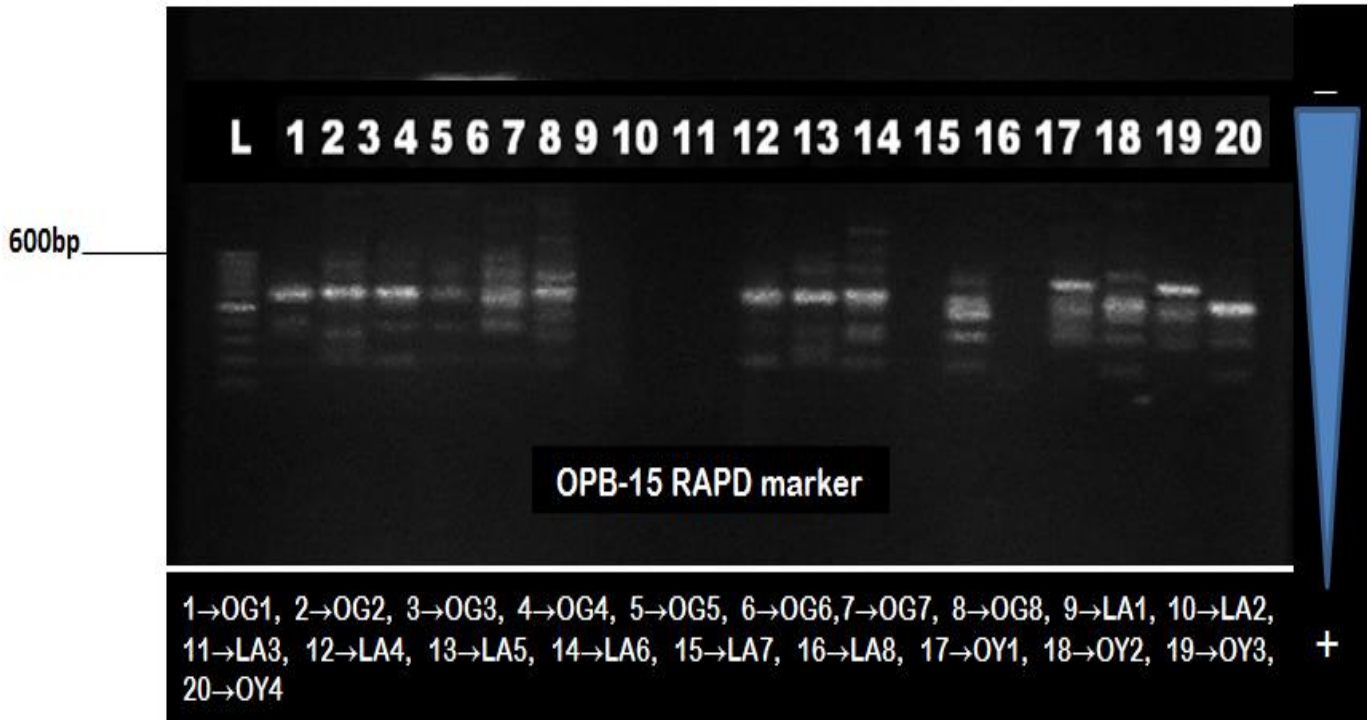

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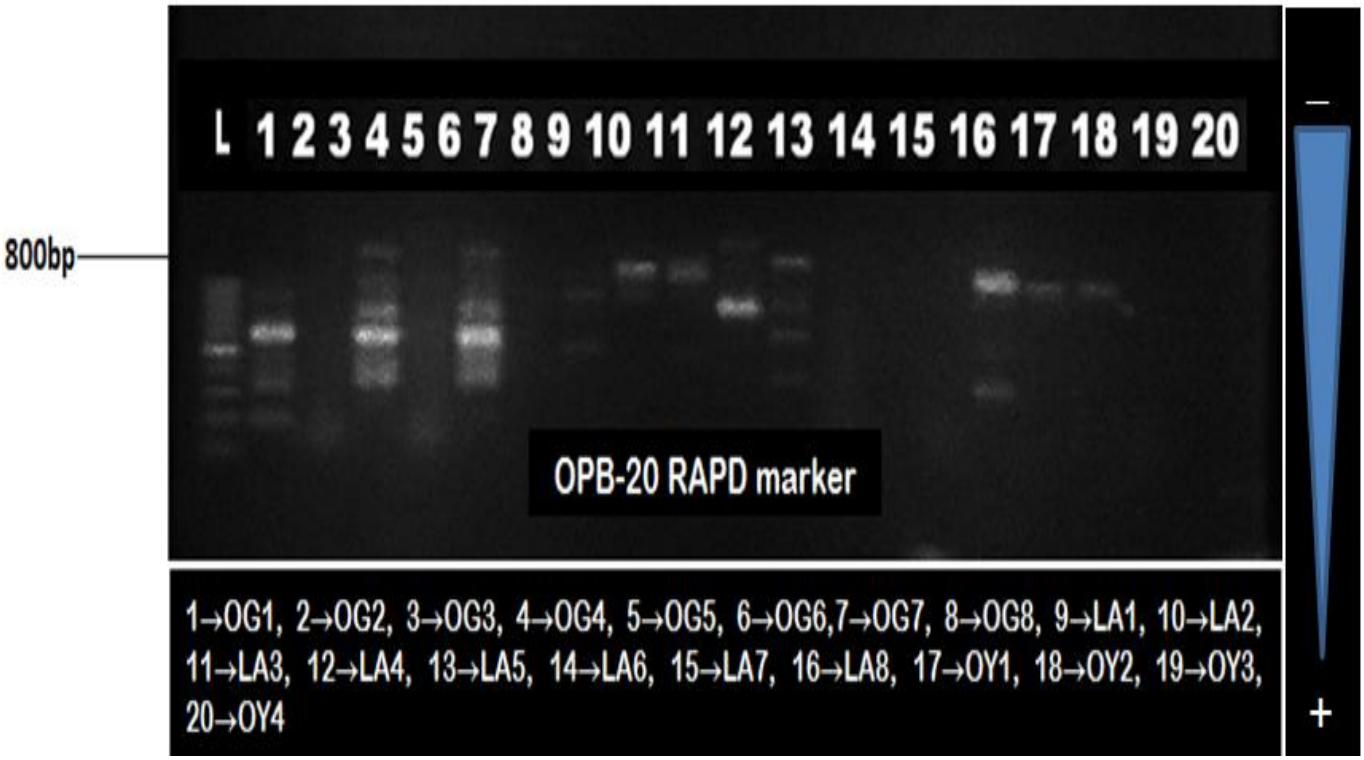

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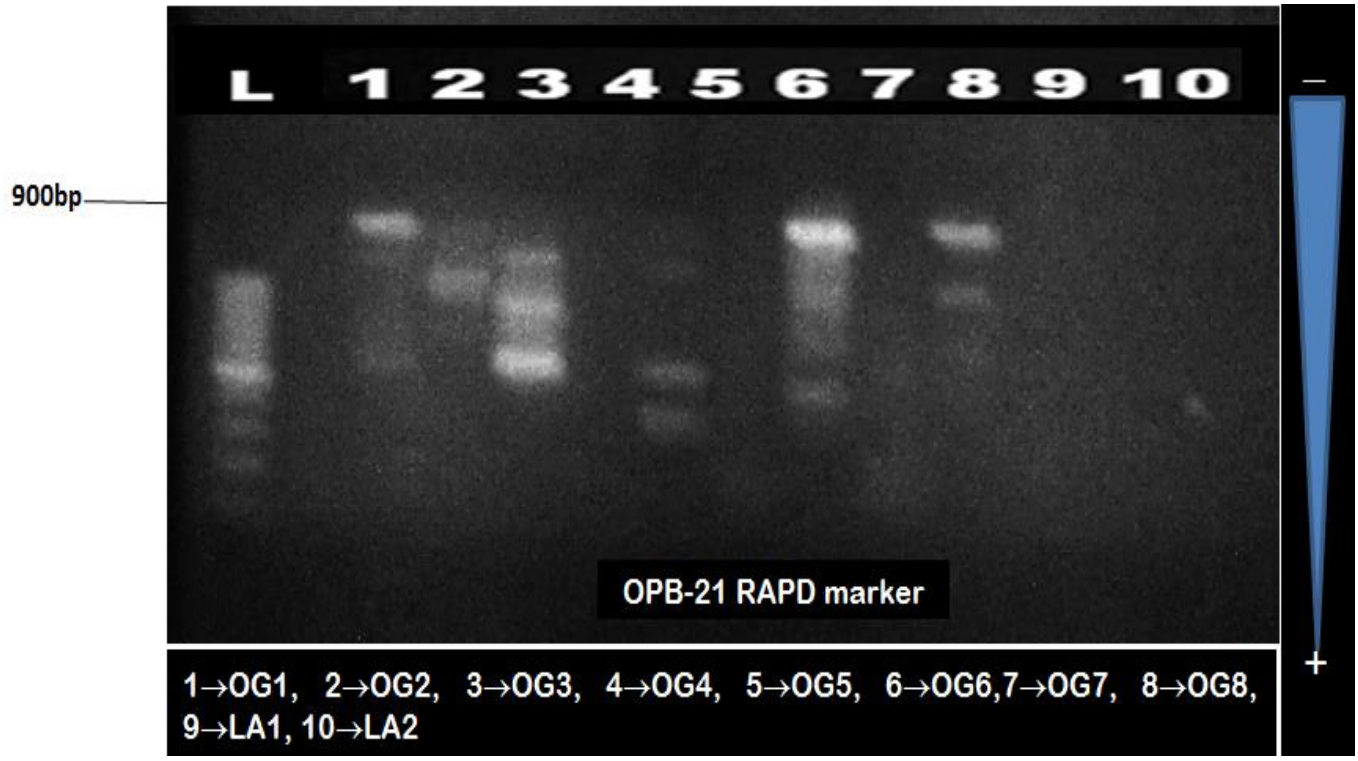

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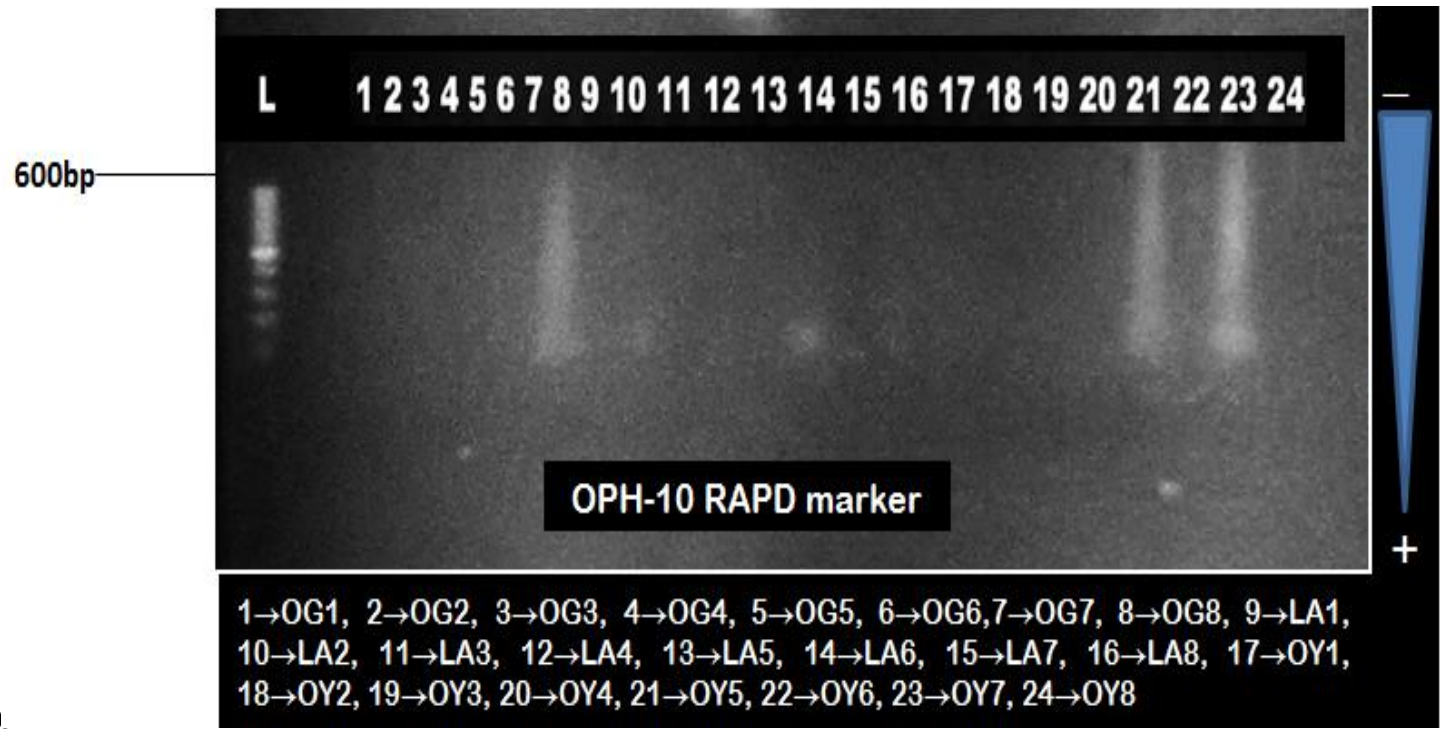

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527 **Fig 7**

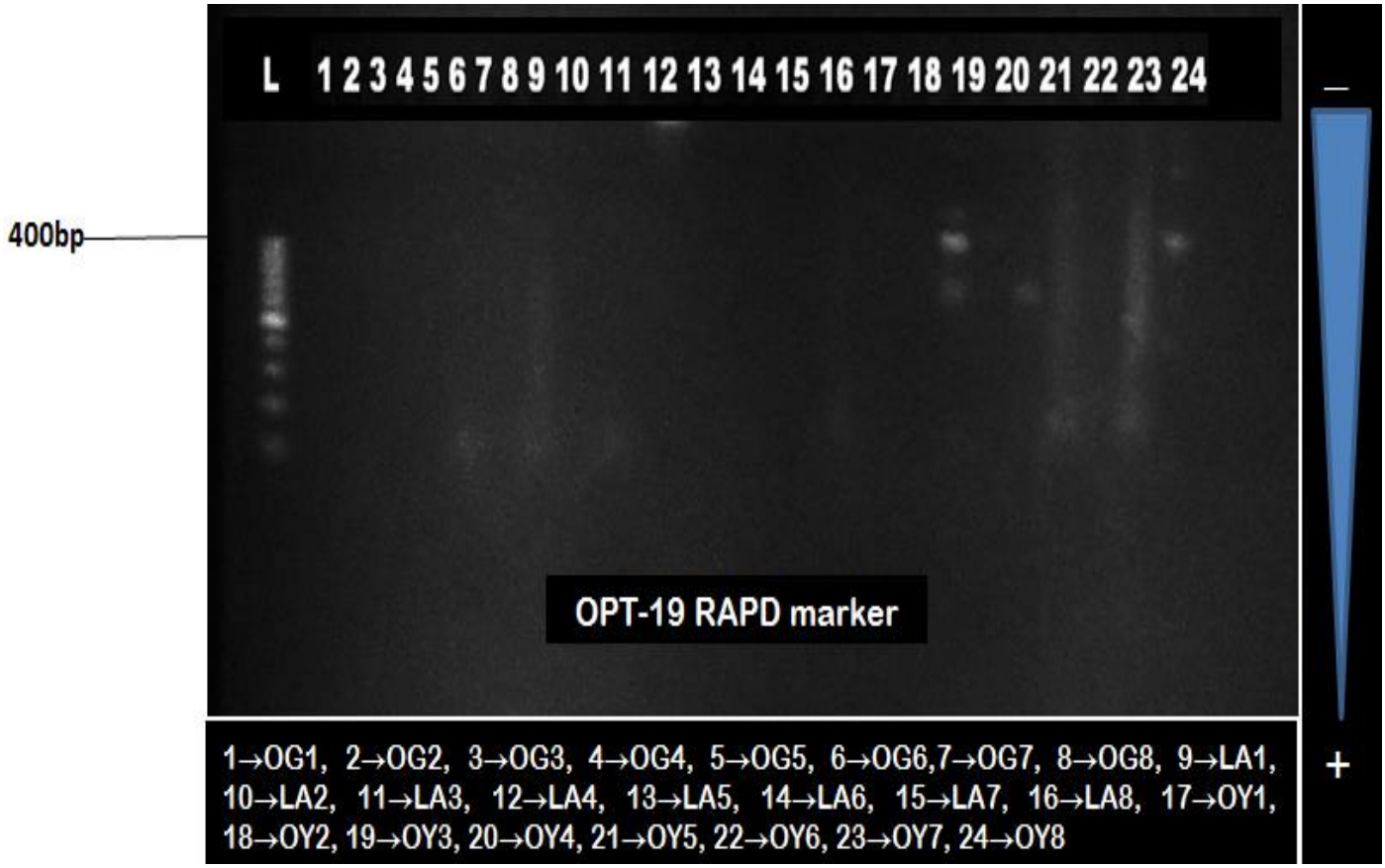
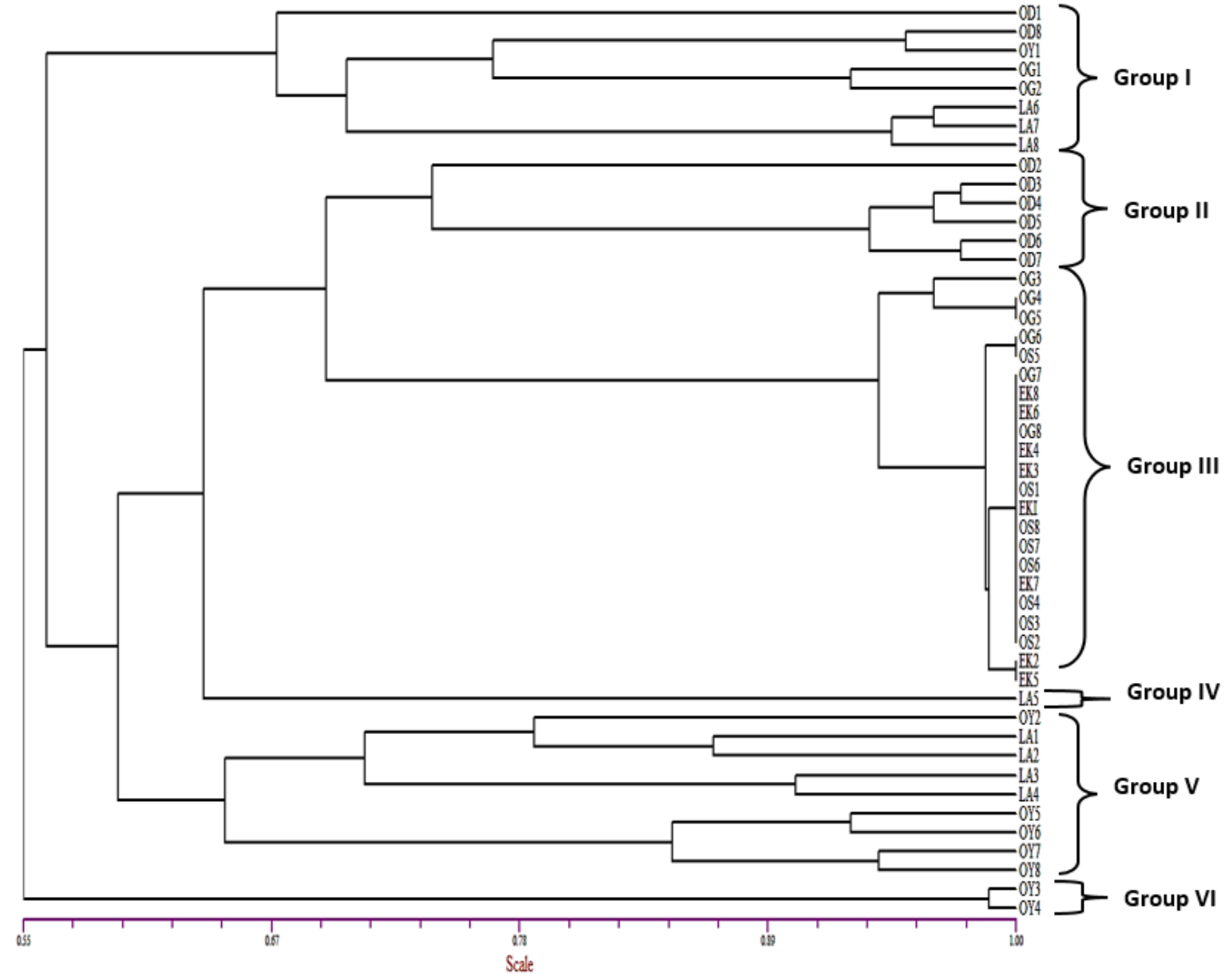


Fig 8



531 **Fig 9**