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Characterization and diversity studies of wild *Ganoderma* species. From selected Agro Ecological Zones in Nigeria.

BY

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Abstract

Ganoderma species are macro fungi that belong to the family Polyporaceae with over 250 species. They are known to have curative properties against many diseases because they possess potent bioactive substances. This study was conducted to assess the diversity of selected wild Ganoderma species across five ecological zones in Nigeria. Fifty Ganoderma fruit bodies were collected from 5 Eco zones namely: Sudan savannah (5) Derived savannah (13), Mangrove (9), Rainforest (11), Fresh water (12) using opportunistic sampling method. For molecular characterisation and identification: DNA extraction was done using Kit extraction protocol, PCR amplification using ITS 4 and ITS 5 primers, sequencing using cycle sequencing method, and construction of phylogenetic tree using unweighted pair group method with arithmetic mean. Sixteen Ganoderma species were identified morphologically, while twelve species were molecularly identified. Three of the twelve namely; G. resinaceum, G. lucidum, and G. sanduense are existing species, while nine of twelve namely; Ganoderma sp. MT534034.1, Ganoderma sp. 1 YD-2015, Ganoderma sp. HNLS1, G. enigmaticum, G. nasalanense, G. casuarinicola, G. weixiensis, G. knysnamense CMW 47755, and G. destructans are new records in Nigeria. The species found in sudan savannah were Ganoderma sp MT534034.1, and G. resinaceum while Ganoderma lucidum, G. sp HNLSI, G. sp. MT534034.1, G. enigmaticum, and G. resinaceum were found in derived savannah. Ganoderma sanduense, G. weixiensis, G. enigmaticum, and G. lucidum were found in the mangrove area. Fresh water zone had G. nasalanense, G. casuarinicola, G. saduenses, while G. nasalanense, G. knysnamense CMW 47755, and G. destructans were found in the humid forest.

Introduction

Ganoderma species belong to the Kingdom Fungi, division Basidiomycota, class Homobasidiomycetes, order Aphyllophorales, and family Polyporaceae. (Alexopolus *et. al*, 1996; Jonathan,2019). They are potent wood and other agro-industrial waste-degrading fungi. (Jonathan *et. al.* 2008; Jonathan and Adeoyo 2011a and b). Ganoderma species are not always listed among the group of edible mushrooms because the sporophores are thick, corky, and tough and, do not have the fleshy texture characteristics of true edible fungi. (Gbolagade *et. al.*,2006;Jonathan and awotona,2010). Although Ganoderma species could not be eating directly, they have been known all over the world as highly medicinal mushrooms ( ). These polypore have attracted much attention in many countries of the world because of their wide range of pharmacological impotrance There are over 250 G*anoderma* species described worldwide (Kalliyaperumal,2013). It has been established by numerous scientific foras and high-impact journals that mushrooms including *Ganoderma* species are suitable sources of natural bioactive compounds of high and low molecular weight, especially polysaccharides, protein, sterols, and triterpenoids (Chen and Seleen, 2007;Adeoye-Isijola,2018; Chikwem et al.,,2019, Chikwem et al,2020). These compounds are known to possess extensive therapeutic and pharmacological properties, such as antibacterial, antifungal, antiviral, anticancer, antitumor, anti-inflammatory, anti-hypotensive, and antioxidative agents (Otunla et.al.,2018; Oluranti et al, 2019; Hyde *et al*., 2019)

Morphological identification methods are being supplemented with new identification methods such as Internally

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Transcribed Spacers (ITS) rDNA barcording techniques (Miller *et al*., 1999). The use of these modern techniques have helped to clarify the distribution of different species complexes in the genus *Ganoderma*, and have revealed some instances of mis-identification (Gottlieb *et al*., 1998). Despite advances in taxonomic techniques, the eco-diversity of *Ganoderma* and elsewhere in Africa have received very little attention.

In Nigeria, only exotic *Ganoderma lucidum* is cultivated commercially even though there are reports of many other *Ganoderma* species in some ecological regions. Due to the high degree of phenotypic plasticity found in *Ganoderma* species, identification of this macro fungus has been faulty because it is focused on similarity and dissimilarity of morphological characters only. It has become necessary to collect, identify, and characterize the Nigerian *Ganoderma* species using morphological and molecular markers.

In neighboring Cameroon, the following species have been reported: *Ganoderma tornatum var. tornatum, Ganoderma hildebrandii, G. lucidum, Ganoderma cf. multiplicatum, Ganoderma resinaceum, Ganoderma carocalcareus* and *Ganoderma ryvardense* (Kinge, 2012). Moncalvo and Ryvarden (1997) listed 49 *Ganoderma* species from Africa. It is therefore reasonable to state that a wealth of information in the *Ganoderma* family is waiting to be discovered. Because very little is known about the diversity of *Ganoderma* in Nigeria, there is need to examine the diversity of wild *Ganoderma* species.

Exotic *Ganoderma lucidum* is cultivated commercially even though there are reports of many other *Ganoderma* species in some ecological regions. Due to the high degree of phenotypic plasticity found in *Ganoderma* species, identification of this macro fungus has been faulty because it is focused on similarity and dissimilarity of morphological characters only. It has become necessary to collect, identify, and characterize the Nigerian *Ganoderma* species using morphological and molecular markers.

This paper is intended to carryout morphological characterization and molecular identification of selected *Ganoderma* species from some eco zones in Nigeria.

Materials and Methods

Ganoderma samples were collected randomly in the wild from 12 locations across major ecological zones in Nigeria namely, mangrove swamp, freshwater swamp, Sudan, savannah, derived savanna, and rainforest zone. The GPS Coordinates, temperature, humidity and host environment, and vegetation are recorded for each location. With the aid of thermometer and humidometer, the temperature and relative humidity of the sample location is taken. Compass software is used to take readings for the GPS. Mature fruit bodies were collected by hands, cleaned with dry cotton wool to remove soil debrises, disinfected with alcohol, and taken to the mycology lab of Botany Department, University of Ibadan for Authentication morpho metric data collection. Different samples of *Ganoderma* were identified on the basis of recording morphological characteristics using standard description of the species. Sample Identification tags were assigned to the samples before tissue isolation and Molecular identification.

A total of fifty **(50)** *Ganoderma* isolates and five (5) replicates were used for this study. Collections were taken from 12 locations in 5 ecological zones in Nigeria. For the purpose of identification and further studies, each *Ganoderma* sample collected were assigned a code based on the location were the sample is collected. GABUK=*Ganoderma* collected at BUK, Kano, GALOK = *Ganoderma* collected at lokoja, Kogi State; *Ganoderma* collected at Makurdi, Benue State; GOYO *Ganoderma* collected UI, Oyo State; GOSUN= *Ganoderma* collected in Osun state, GALAG= *Ganoderma* collected in Lagos State; GADEL = *Ganoderma* Collected in Delta State, GABAY= *Ganoderma* collected in Bayelsa State; GARIV= *Ganoderma* collected from Rivers State; GIMO= *Ganoderma* collected from Imo State. Morphological assessment, Tissue Culture isolation, and mycelia growth performance study were carried out at the Mycology lab, Department of Botany, University of Ibadan. Molecular studies for 50 samples of *Ganoderma* spp. were carried out at the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State.

Sample authentication

Samples collected were taken to the Mycology lab of Botany Department, University of Ibadan for authentication by experts in mushroom taxonomy. Morphological authentication was based on the procedure of Aleopulus *et al*.,1996 and Thatsanee *et al.* (2021) in "*Ganoderma* (Ganodermataceae, Basidiomycota) species" published in *Journal of fungi* based on qualitative and quantitative macro morphological characters climate, nutrition, vegetation, and geography.

Morphological analysis

The experiment was carried out according to the procedures described by Steyaert (1972).

With use of visual aid, qualitative data were recorded for each sample collected. Scoring were done for each macro qualitative traits under study. While flexible metre rule was used to generate data for macro quantitative traits. A total of 5 macro quatitative characters (Pileus base thickness, pileus length, pileus width, pore size) and 7 macro qualitative characters (basidiome, pileus surface, pileus shape, pileus colour, stipe shape, margin, context,) were used for this study. to examine their inter and intra relationship based on the ecological sites where these *Ganoderma* samples were collected.

Molecular Analysis

The following steps will be carried out for the molecular analysis based on the procedures described by White *et al*. (1990).

DNA extraction

According to the procedures by described by Zymo research company for kit extraction protocol.

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Extraction of fungi genomic DNA for 50 *Ganoderma* isolates is adopted using Kit extraction protocol, a modified CTAB technique. Below is the protocol used for DNA extraction; 200mg (wet weight) of *Ganoderma* isolate dry sample tissue previous crushed were added to ZR Bashing™ Lysis Tube. 750ul Lysis Solution was to the tube. The solution was secured in a bead fitted with 2 ml tube holder assembly and process at maximum speed for ≥ 5 minutes. The ZR BashingBead™ Lysis Tube was centrifuged in a microcentirifuge at > 10,000 x g for 1 minute. The base of the Zymo-Spin ™ Spin filter will be Snap off prior to use. 400 ul supernatant was transfered to a Zymo-Spin™ IV Spin Filter (orange top) in a Collection Tube and centrifuge at 7,000 x g for 1 minute. 1,200 ul of *Ganoderma* DNA Binding Buffer was added to the filterate in the Collection Tube from Step 4. 800 ul of the mixture from Step 5 was transfered to a Zymo-Spin™ IIC Column in a Collection Tube and centrifuge at 10,000 x g for 1 minute. The flow through was discarded from the Collection Tube and repeat Step 6. 200 ul DNA Pre-Wash Buffer was added to the Zymo-Spin ™ IIC Column in new Collection Tube and centrifuge at 10,000 x g for 1 minute. 500 ul of the Ganoderma DNA Wash Buffer was added to the Zymo-Spin™ IIC Column and centrifuge at 10,000 x g for 1 minute. Finally, Zymo-Spin™ IIC Column was transfered to a clean 1.5 ml microcentrifuge tube and add 100ul (35 ul minimum) DNA Elution Buffer directly to the column matrix. Centrifuge at 10,000 x g for 30 seconds to elute the DNA.

DNA Quality check

The amount of DNA were quantified by recording the absorbance at 260 nm wavelength using UV/VIS Spectrophotometer.

DNA BAND imaging: This was done by the use of Agarose Gel electrophoresis to observe the genomic DNA bands while spectrophometry will be used to ascertain the purity of the DNA. The A260/A280 ratio was 1.7 to 1.9.

PCR amplification

According to the procedures by White *et al.* (1990)

- ITS 4, reverse, (5´–TCC TCC GCT TAT TGA TAT GC– 3´)
- ITS 5, forward, (5´–GGA AGT AAA AGT CGT AAC AAG G–3´) and

Final volume of PCR cocktail is 20 µL containing: 50 ng genomic DNA, 0.5 units of Taq polymerase, 1X GoTaq Flexi Buffer, 0.2 mM dNTP, 1.5 mM MgCl2 (Promega), and 0.2 µM of each primer.

Amplifications were performed in a thermal cycler using an initial denaturation step of 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing for 1 min at 56°C and elongation for 1 min at 72°C. This was concluded with a final extension for 10 min at 72°C.

PCR products were analyzed by electrophoresis in 1% agarose gel in standard TBE 1X stained with gel red (0.4 µg mL-1) and photographed under UV. The molecular weight of the amplified DNA was estimated by comparison with a 1kb+ DNA ladder (Promega). The amplified PCR products were purified using GeneJET.

Sequencing

Cycle sequencing: Procedure was provided by Zymo Research Company for Cycle sequence mini prep. ABI 3500 sequencer was used for this analysis

A 96-well plate and the products were purified using Ethanol /EDTA precipitation technique. The cycle sequencing were performed using 25ng of the PCR output.

Reactions for 96- Well Reaction Plates or Microcentrifuge Tube: To prepare the reaction mixtures:

For each reaction the following reagents and quantity were added to a separate tube: Terminator Ready Reaction, Mix*8.0 µL, Template 5–20 ng , Primer,3.2 pmol, Deionized water, q.s.Total Volume, 20 µL.

Bio edit software was used to convert the sequence reading to Fasta format and blasted in the NCBI platform for generating the identity of the *Ganoderma* species. This act is by comparing the query sequence to the reference sequence to match the identity.

Phylogenetic and molecular evolutionary analysis were conducted using MEGA 11 (Tamura *et al*.,2007) . Binary matrices were analyzed by NTSYS-pc V2.0 and Jaccard's coefficient used to construct a dendrogram using SHAN cluster programme, selecting the unweighted pair group arithmetic mean (UPGMA). Sequence alignment was carried out using Cluster W. **B**ootstrap value was set to 1000 replications.

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Results and Discussion

Table 1A GPS, Temperature , humidity , location, host substrates of 50 *Ganoderma* isolates collected from different ecological in

Sample Pop.	Samples code	Temperature of collection(℃)	Month of collection	Location	Humidity (%)	GPS	Host environment	Eco Vegetation
8	GABAY	26±3	April	Kaiama	75 ±3	9° 36’ 22.3668”N 3° 56’ 30.9876”E	Decaying hardwood of *Tectona grandis*	Freshwater habitat
4	GABAY	26±3	June	Yenagoa	80 ±3	4° 54’ 11”N 6° 17’ 19” E	Decaying hardwood tree	Fresh water habitat
3	GARIV	28±4	July	Emohua	80 ±3	5° 10’ 0” N 6°54’ 0”E	Decaying hardwood *Magnifera indica*	Humid forest
6	GARIV	30±4	March	Mbiama	82 ±3	5° 3’ 0” N 6°27’ 0”E	Decaying hardwood, *Gmelina arborea*	Humid forest
2	GIMO	28±2	April	FUTO , Owerri	75 ±5	5° 23’ 33.6876” N 6°59’ 10.5504”E	Decaying unknown hardwood	Humid forest

Nigeria

Sample Pop.	Samples code	Temperature of collection(℃)	Month of collection	Location	Humidity (%)	GPS	Host environment	Eco Vegetation
5	GABUK	27 ±2	April	Kano municipal	67 ±5	Lat 11°57’50.5N Long. 8°26’03.9E	Roots of Hort. plants	Sudan savannah:
3	GALOK	30±2	May	Lokoja	70 ±3	07 ° 47’31.63N 06°43’5’38E	Bark of fruit trees	Derived savannah
3	GALAG	28±4	June	Badagry	75 ±3	6° 25’53.688N 2° 53’15.5184E	Roots of deciduous trees	Mangrove
3	GABEN	29±2	April	Kwande, Adikpo	65 ±3	6 0 89’21.9N 9 0 21’34.86E	Trunk of Mango trees	Derived savanna
7	GOYO	27±2	March	Botanical garden,UI	73 ±3	7° 22’39.1296N 3° 56’49.344E	Stump of decaying Hardwood tree	Derived savannah
3	GOSUN	26±2	June	Aiyetoro, Osogbo	75 ±3	7° 46’15.74N 4° 33’25.13E	Roots of deciduous trees	Lowland forest
6	GADEL	26±3	February	Patani	64 ±3	5° 13’43.86N 6° 11’29.00’E	Humus soil of dead hardwood	Mangrove

Table 1B GPS, Temperature , humidity , location, host substrates of 50 *Ganoderma* isolates collected from different ecological in Nigeria

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Plate 1: Field collections and assigned sample IDs

Table 2 Authentication results for 50 *Ganoderma* samples using face of fungi taxonomic keys, 2016

S/N	Sample Code	Authentication result	S/N	SAMPLE codes	Authentication results
1	GABUK1	*Ganoderma resinaceum*	25	GADEL3	*Ganoderma resinaseum*
2	GABUK2	*Ganoderma resinaseum*	26	GADEL4	*Ganoderma resinaseum*
3	GABUK3	*Ganoderma applanatum*	27	GADEL5	*Ganoderma orbiforme*
4	GALOK1	*Ganoderma applanatum*	28	GABAY1	*Ganoderma lucidum*
5	GALOK2	*Ganoderma applanatum*	29	GABAY2	*Ganoderma lucidum*
6	GALOK3	*Ganoderma applanatum*	30	GABAY3	*Ganoderma sichuanense*
7	GABEN1	*Ganoderma austral*	31	GABAY4	*Ganoderma lucidum*
8	GABEN2	*Ganoderma austral*	32	GABAY5	*Ganoderma sanduense*
9	GABEN3	*Ganoderma austral*	33	GABAY6	*Ganoderma sanduense*
10	GOYO1	*Ganoderma calidophilum*	34	GABAY7	*Ganoderma sanduense*
11	GOYO2	*Ganoderma calidophilum*	35	GABAY8	*Ganoderma hochiminhense*
12	GOYO3	*Ganoderma calidophilum*	36	GABAY9	*Ganoderma sanduense*

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13	GOYO4	*Ganoderma calidophilum*	37	GABAY10	*Ganoderma sanduense*
14	GOYO5	*Ganoderma resinaceum*	38	GABAY11	*Ganoderma sichuanense*
15	GOYO6	*Gaboderma lucidum*	39	GABAY12	*Ganoderma tsugae*
16	GOYO7	*Ganoderma flexipe*	40	GARIV1	*Ganoderma tsugae*
17	GOSUN1	*Ganoderma leucocontextum*	41	GARIV2	*Ganoderma tsugae*
18	GOSUN2	*Ganoderma leucocontextum*	42	GARIV3	*Ganoderma lucidum*
19	GOSUN3	*Ganoderma leucocontextum*	43	GARIV4	*Ganoderma lucidum*
20	GALAG1	*Ganoderna lucidum*	44	GARIV5	*Ganoderma sinensis*
21	GALAG2	*Ganoderma lucidum*	45	GARIV6	*Ganoderma sinensis*
22	GALAG3	*Ganoderma lucidum*	46	GARIV7	*Ganoderma lucidum*
23	GADEL1	*Ganoderma resinaceum*	47	GARIV8	*Ganoderma myanmarens*
24	GADEL2	*Ganoderma resinaceum*	48	GARIV9	*Ganoderma gibbosum*
25	GADEL 03	*Ganoderma resinaceum*	49	GIMO1	*Ganoderma sinensis*
			50	GIMO2	*Ganoderma lucidum*

Table 3: Quantitative traits of *Ganoderma* spp in different ecological zones (linear growth in cm)

Eco Zone	PBT	PW	PL	ST	PO
Sudan Sav.	2.59a	5.52b	4.33c	3.42c	4.07b
Derived Sav.	1.06b	18.23a	18.9a	5.20ba	4.27ba
Mangrove	1.95b	1.07d	3.48c	4.40bc	4.47ba
Freshwater	0.67d	3.17 c	5.36c	6.59a	4.30ba
Humid forest	1.28c	4.37cb	5.60c	6.73a	4.13ba
LSD	0.74	3.33	1.97	3.05	0.78

PBT= Pileus base thickness, PW= Plieus width, PL=Pileus length, ST=stipe length, PO= Number of Pores
Means with the same letters are not significantly different at P ≥ 0.05

Table 4: Qualitative characters of *Ganoderma* as affected by population (cm)

Population	**Ba**	**PSH**	**PSU**	**PIC**	**MAR**	**POS**	**STS**
GABUK	1.00d	1.20e	2.33bac	3.67a	1.80d	1.47c	4.00a
GALOK	1.27d	1.80dc	2.80ba	2.0c	2.67b	1.80bc	3.60ba
GABEN	1.00d	2.20bc	3.00a	2.80b	1.27e	2.60a	3.00bcd
GOYO	3.23b	1.51de	2.80ba	2.74b	2.06dc	2.17ba	2.49d
GOSUN	3.00b	2.67bc	2.40ba	2.93b	1.27e	0.93d	2.60dc
GALAG	3.91a	2.70ba	2.13bc	1.75c	2.20c	1.00d	2.50d
GABAY	3.20b	2.21bc	1.30d	4.00a	2.17dc	1.53c	3.22bc
GARIV	2.42c	1.07e	1.60dc	4.00a	1.80d	1.57c	2.53dc
GIMO	3.40b	2.90a	1.6dc	4.00a	3.4a	1.90bc	2.80dc
LSD	0.9113	0.9348	1.4166	0.8507	0.7219	0.8782	1.2646

Key: Ba= Basidiome, PSH= Pileus shape, PSU=Pileus surface, PIC= Pileus color, MAR=Margin, POS= Pore surface, STS=Stipe surface.
Means of the same letters are not significantly different at P ≥ 0.05

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Table 5: Effect of 7 Qualitative characters in different eco zones (cm)

Eco zone	Ba	PSH	PSU	PIC	MAR	POS	STS
Sudan Sav.	2.28a	2.28a	1.98b	2.9bc	1.96a	1.76a	1.92c
Derived Sav.	2.22a	2.22a	1.74cb	3.18b	2.14a	1.72a	2.24c
Mangrove	1.52b	1.52b	1.46c	3.88a	1.18b	1.74a	3.86a
Fresh water	1.36b	1.36b	3.14a	3.18b	2.14a	1.04b	2.74b
Humid forest	2.28a	2.28a	1.82cb	2.80c	2.06a	1.74a	3.62a
LSD	0.2882	0.2956	0.448	0.269	0.2283	0.2777	0.3999

Key: Ba= Basidiome, PSH= Pileus shape, PSU=Pileus surface, PIC= Pileus color, MAR=Margin, POS= Pore surface, STS=Stipe surface

Means of the same letters are not significantly different at $P \geq 0.05$

Table 6 Nanodrop readings and molecular weight of Sample DNAs

S/N	Ganoderma isolates	Nucleic Acid Unit (Ug/ul)	260/280	S/N	Ganoderma isolates	Nucleic Acid Unit (Ug/ul)	260/280
1	GABUK	104	1.89	26	GARIV	85	1.95
2	GABUK	182	1.85	27	GARIV	95.6	1.91
3	GAKOG	135.3	1.88	28	GARIV	103.9	1.90
4	GAKOG	196.9	1.88	29	GARIV	95.4	1.88
5	GABEN	105.2	1.88	30	GARIV	91.2	1.88
6	GABEN	101.2	1.89	31	GARIV	105.9	1.88
7	GOSUN	109.7	1.89	32	GARIV	102.3	1.88
8	GOSUN	190	1.94	33	GARIV	154.6	1.87
9	GOSUN	157.4	1.88	34	GARIV	93.6	1.86
10	GOYO	195.4	1.87	35	GALAG	134.1	1.86
11	GOYO	153.6	1.89	36	GALAG	108.6	1.86
12	GOYO	106.6	1.88	37	GALAG	100.1	1.85
13	GOYO	111.8	1.87	38	GABAY	97.9	1.84
14	GABAY	187.3	1.86	39	GABAY	105.5	1.84
15	GABAY	156.6	1.89	40	GABAY	93.7	1.82
16	GABAY	123.2	1.88	41	GABAY	102.3	1.82
17	GABAY	102.3	1.88	42	GABAY	99.9	1.81
18	GABAYG	150.7	1.86	43	GABAY	101.7	1.81
19	GABAY	189.7	1.89	44	GOYO	155.8	1.78
20	GADEL	125.2	1.87	45	GOYO	122.2	1.77
21	GADEL	192.3	1.85	46	GOYO	72.4	1.76
22	GADEL	191.8	1.88	47	GIMO	116.7	1.74
23	GADEL	162.4	1.86	48	GIMO	91.3	1.72
24	GADEL	141.8	1.86	49	GIMO	133.6	1.95
25	GARIV	106	1.97				

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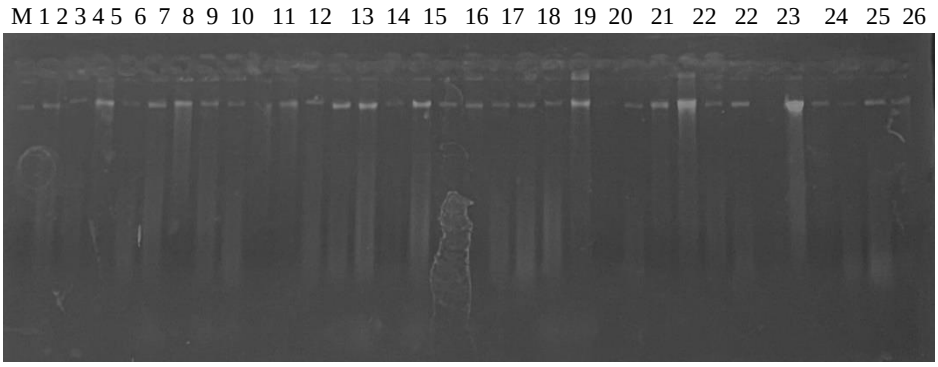

PLATE 2A Gel image for DNA extraction showing DNA band

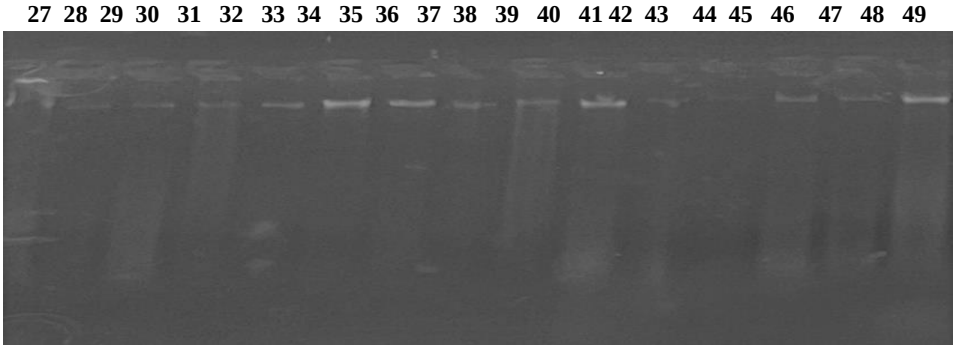

Plate 2**B**: Gel image showing Genomic DNA bands on Gel image for 1-49 wells

Table 7: Molecular Identification Result.

Sample ID	Identity	Query Cover(%)	Per. Ident(%)	Acc. Len	Accession
GABUK	*Ganoderma sp.*	26	89.02	544	MT534034.1
GABUK	*Ganoderma resinaceum*	97	90.88	641	KX055526.1
GAKOG	*Ganoderma lucidum*	8	95.65	623	GU726925.1
GAKOG	*Ganoderma sp.*	26	89.02	544	MT534034.1
GOSUN	*Ganoderma sp. 1 YD-2015*	80	77.38	644	KM229629.1
GOSUN	*Ganoderma sp. HNLS1*	61	82.13	651	KU886297.1
GOYO	*Ganoderma sp. HNLS1*	9	81.97	651	KU886297.1
GOYO	*Ganoderma sp.*	87.8	76.32	544	MT534034.1
GABEN	*Ganoderma enigmaticum*	74	86.05	652	NR_132918.1
GABAY	*Ganoderma nasalanense*	12	100.00	643	NR_164048.1
GABAY	*Ganoderma casuarinicola*	74	85.17	626	NR_158432.1
GABAY	*Ganoderma enigmaticum*	40	74.62	652	NR_132918.1
GABAY	*Ganoderma sanduense*	87	75.51	641	NR_164049.1

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GADEL	*Ganoderma weixiensis*	35	79.33	591	NR_166271.1
GADEL	*Ganoderma weixiensis*	35	79.33	591	NR_166271.1
GADEL	*Ganoderma enigmaticum*	62	76.63	652	NR_132918.1
GARIV	*Ganoderma nasalanense*	12	100.00	643	NR_164048.1
GALAG	*Ganoderma lucidum*	8	95.65	623	GU726925.1
GALAG	*Ganoderma sanduense*	98	86.89	641	NR_164049.1
GARIV	*Ganoderma knysnamense CMW 47755*	32	77.71	619	NR_165523.1
GARIV	*Ganoderma knysnamense CMW 47755*	32	77.71	619	NR_165523.1
GIMO	*Ganoderma destructans*	97	84.41	640	NR_132919.1

M 1 2 3 4 5 6 7 8 9 10 1112131415 16171819 2021 22 23 24 25 26 27 28 29 30 31 32 33

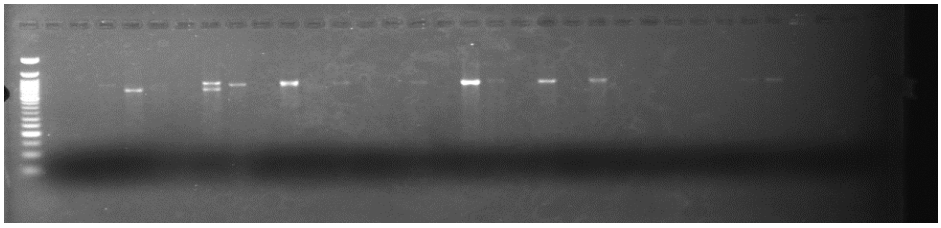

Plate 3A Gel image showing amplicons from (33 wells and a ladder)

34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 M

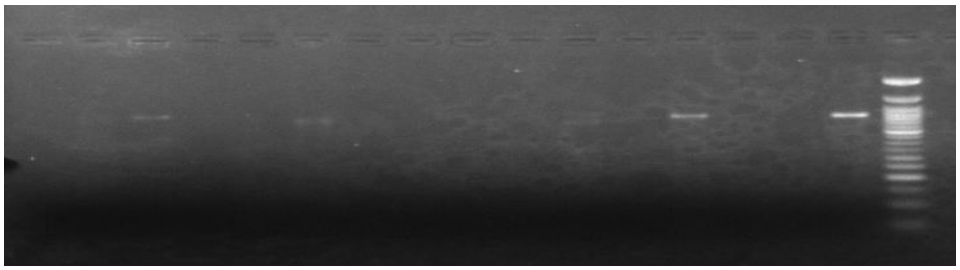

Plate 3B: Gel image for PCR results showing amplicons for sequencing

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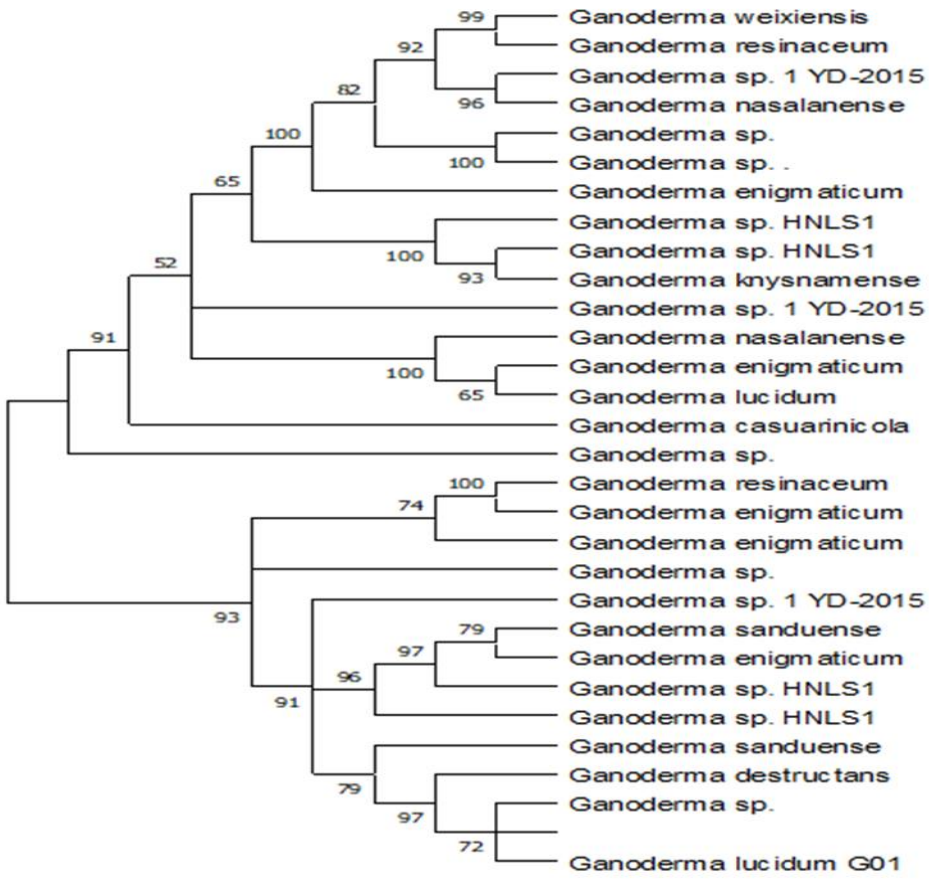

Fig 1 UPGMA Phylogenetic tree of identified *Ganoderma* species showing Cluster I & II, bootstrap at 1000 replicaters

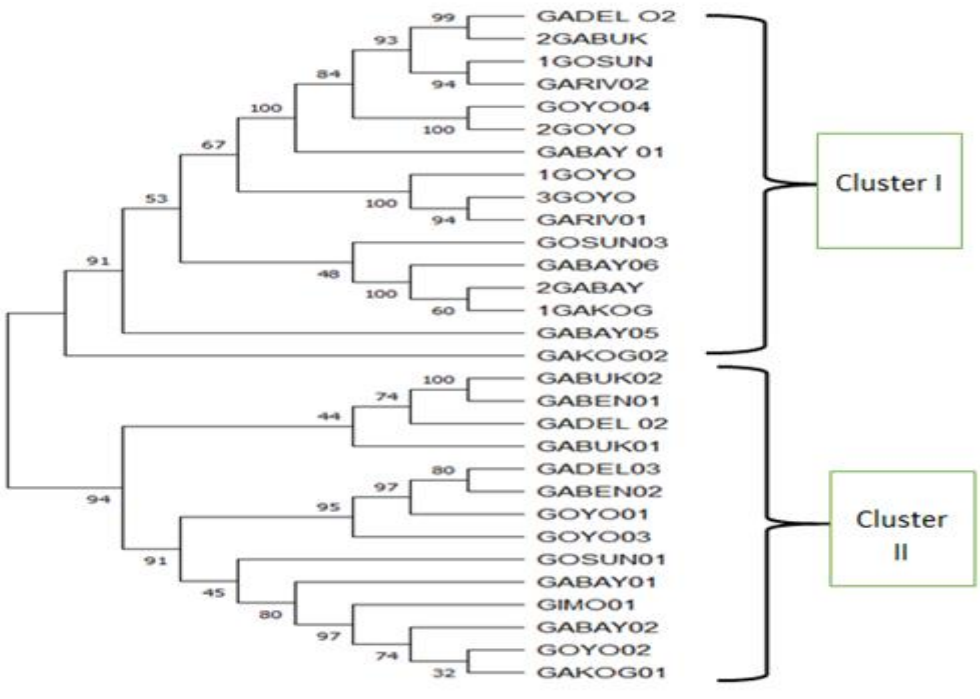

Fig 2 Phylogenetic tree showing inter/intra relationship amongst identified *Ganoderma in a monophyletic relationship*

Discussions

Morphological authentication and descriptions

From Table 1A&B and 2, *Ganoderma* samples collected in Sudan savannah are *Ganoderma resinaceum, Ganoderma resinaceum, Ganoderma applanatum.* The three (3)

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Ganoderma species were collected at the inner garden of the University Guest House, Bayero University, Kano (BUK). The sample code assign to it was GABUK. The collection was done in the month of April with room temperature to be 27℃ -29 ℃ and relative humidity of 65% - 70%. The global positioning system (GPS) reveals Lat 11˚57'50.5N Long. 8˚26'03.9E. The host substrate was roots and soils of horticultural plants while the ecological vegetation where the samples were collected is Sudan savannah and grassland shrubby region in Kano municipal, Kano State, Nigeria. The Basidiome of GABUK are dimidiate, sub-stipitate and stipitate. The Pileus base thickness is between 1.3cm - 2.9cm while the Pileus length and width are 0.6cm - 3.4cm and 3.2cm - 6.7cm respectively. The pileus shape are subdimidiate, dimidiate, and stipitate while pileus surface are strongly laccate, glossy, and sulcate. Pileus colour are homogeneous reddish-yellow. The Context are mostly soft, sometimes spongy to firm-fibrous. The stipe are central, glossy with a distinct cuticle. The margin are actively growing and smooth when young, slippery when touched from youth to maturity, and tough when broken. The pores are 4–7 in number per mm, entire to circular, cream when young, light orange to brown when mature. This is in accordance with the reports of Luangharn *et al.* (2021) on a recent review of Ganodermataceae using morphological markers, vegetation type, environmental climate, host substrate, and molecular markers.

***Ganoderma* samples collected in Guinea savannah are *Ganoderma applanatum, Ganoderma applanatum, Ganoderma applanatum*.**The three (3) *Ganoderma* strains were collected on the bark of a fruit tree along Federal University Lokoja road (LOK). The collection was done in the month of May with room temperature to be 29℃ -31 ℃ and relative humidity of 69% - 71%. The global positioning system (GPS) reveals Lat 07 ˚ 47'31.63N long. 06˚43'5'38E. The ecological vegetation where the samples were collected is Guinea savannah region in lokoja, Kogi State, Nigeria. *Ganoderma* isolates collected from LOK were assigned code GALOK 01, GALOK 02, GALOK 03 for identification and further studies. The basidiome are annual and sessile while the pileus base diameter is with 0.5 cm-1.5 cm. The pileus width and length are within 0.5cm– 4.5 cm and 1.5cm –5.8 cm respectively. The pileus shape are subdimidiate, flabelliform, usually flat and convex. The pileus surface is glabrous and non-laccate (dull) and faded from when mature to old, compact and hard when mature, woody to corky when old. Pileus color is usually homogenous with grayish-orange and yellowish-gray at the margin when mature. Context up to 0.3cm –1 cm thick at the base, mostly light brown. Stipe is sessile and broadly attached with a differentiated zone at the point of attachment. Margin up to 0.5cm thick when mature, turns light brown to brown when scratched, often slippery when wet, soft when young, thinner than the center. Number of Pores are 4–6mm. Pore surface is grayish-orange when mature. This is in accordance with the reports of Luangharn *et al.* (2021) on a recent review of Ganodermataceae using morphological markers, vegetation type, environmental climate, host substrate, and molecular markers.

Ganoderma samples collected in Derived savannah are *Ganoderma enigmaticum, Ganoderma enigmaticum, Ganoderma enigmaticum*. The three (3) *Ganoderma* strains were collected on the trunk of decaying mango tree at Adikpo town of Kwande local Government Area, Benue State. The sample code assign to it was GABEN. The collection was done in the month of April with room temperature to be 28℃ -30℃ and relative humidity of 64% - 67%. The global positioning system (GPS) reveals Lat 6˚ 89'21.9N long. 9˚ 21'34.86E. The ecological vegetation where the samples were collected is Derived savannah region in Kwande, Benue State, Nigeria. Basidiome were annual, perennial, subdimidiate, sessile. The pileus base thickness is between 1.4cm–3.2 cm thick while the pileus is 12cm–32 cm in width and 14cm–28cm in length. Pileus shape is flabelliform, spathulate and subdimidiate. The Pileus surface is corky, mostly umbonate or uneven, and non-laccate (dull). The Pileus color is brown at the base, reddish orange or almost covered with grayish-red on the upper surface. It is slight reddish-brown close to the margin. The Context is up to 0.5cm –2 cm thick near stipe. Tube is 0.4cm–1.5 cm long, brown to dark brown. The stipe is sessile and broadly attached. The margin is soft when young, slippery when fresh, blunt when mature. The number of pores are 4–6 in number per mm, subcircular to circular, sometimes angular. This is in accordance with the reports of Luangharn *et al.* (2021) on a recent review of Ganodermataceae using morphological markers, vegetation type, environmental climate, host substrate, and molecular markers.

Other *Ganoderma* samples collected in Derived Guinea savannah are *Ganoderma calidophilum, Ganoderma enigmaticum, Ganoderma resinaceum, Ganoderma lucidum, Ganoderma cupreum. Ganoderma cupreum, Ganoderma flexipe.*

The seven (7) *Ganoderma* species were collected from bark of decaying fruit trees, trunk of decaying Mango tree, stump of decaying Hardwood trees and soil of Quercus decaying tree. All collections were from three different locations namely University of Ibadan, Botany Nursery Garden, International Institute of Tropical Agriculture (IITA) Ibadan, and Botanical Garden, University of Ibadan. The collections were done in the month of March and May with room temperature to be 27℃ -29 ℃ and relative humidity of 70% - 73%. The global positioning system (GPS) reveals 7˚ 22'39.129N Latitude and 3˚ 56'49.344E longitude. The ecological vegetation where the samples were collected is derived savannah in Ibadan, Oyo State, Nigeria.

The sample code assign to it was GOYO. Basidiomes were annual, stipitate, subdimidiate 0.2–1 cm pileus base thickness 2–4 cm in pileus width and 3–7 cm in length. Pileus shape are subdimidiate to dimidiate, spathulate, stipitate, sulcate, umbonate, radial from the center extending to the margin, n, tough to break when dried, often thick at the center, slightly soft at the margin, and light in weight when dried. The pileus surface are corky, convex, furrowed, glabrous, glossy, incised, shiny, spathulate, shallow, sulcate when fresh, umbonate or uneven, laccate and glossy when mature, weakly laccate when oldThe Pileus color are usually homogenous with brownish-

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red , reddish-brown center, extending brownish-orange toward the stipe, brownish-red from the center to light brownish-orange , and usually light brown at the margin when old. The context are up to 0.2–0.6 cm thick near stipe, dry, fibrous, composed of coarse loose fibrils, brownish-orange upper layers when fresh, brown at lower layers, dark brown when dried, covered with thin crust. The stipes are 5–14 cm long, cylindrical, almost stipitate with broadly, irregularly ruptured crust overlying, strongly laccate with brown when mature, dark brown when old, and woody or corky when dried. The margin are soft when young, laccate when mature, weakly laccate to laccate when old. Pores are usually 4–5 in number per mm, subcircular to circular, sometimes angular. This is in accordance with the reports of Luangharn *et al.* (2021) on a recent review of Ganodermataceae using morphological markers, vegetation type, environmental climate, host substrate and molecular markers.

Other samples *Ganoderma* samples collected from lowland derived savannah are *Ganoderma leucocontextum, Ganoderma leucocontextum, Ganoderma leucocontextum.* Three (3) *Ganoderma* species were collected from roots of deciduous trees at Osogbo local Government Area, Osun State.The collection was done in the month of June with room temperature to be 28℃ -30℃ and relative humidity of 75% - 78%. The global positioning system (GPS) reveals Lat 7° 46'15.74N and long. 4° 33'25.13E. The ecological vegetation where the samples were collected is lowland forest area, in Aiyetoro, Osogbo area, Osun State, Nigeria. The sample code assign to it was GOSUN**.** The basidiomes are usually stipitate. The pileus base thickness are 0.5 cm thick while the Pileus width and length are 0.5cm–3 cm and 0.5cm–3.2 cm respectively. The Pileus shape are stipitate, subflabellate to flabellate, concentrically sulcate zones with tuberculate, glabrous when young to maturity, bumps when mature, often tough to break when dried. The pileus surface are shiny, smooth, and soft when young, frequently furrowed and shallow sulcate on upper surface, undulating, somewhat spathulate to uneven when mature, weakly laccate when young, and laccate when mature., and woody when old. Pileus color are usually homogenous with reddish-brown to dark brown at the center, slight to the margin from mature to old. The context is up to 0.1–0.6 cm thick at the base, very dry, brown to reddish-brown, containing fibrous pithy context and corky when old. Stipe are almost 3–12 cm in length, 0.3–1.5 in width, sub-cylindrical to cylindrical, often dark brown, and strongly laccate from mature to old. The margin soft when young, laccate when mature, some wavy, often light brown on the upper surface. The pores are 4–5 in number per mm, subcircular to circular, sometimes angular. This agrees with the reports of Luangharn *et al.* (2021) on a recent review of Ganodermataceae using morphological markers, vegetation type, environmental climate, host substrate and molecular markers.

Ganoderma samples collected from Mangrove are *Ganoderma lucidum, Ganoderma lucidum, Ganoderma lucidum.*The three (3) *Ganoderma* species were collected from roots of deciduous trees at Badagry area of Lagos State. The collection was done in the month of June with room temperature to be 28℃ -32℃ and relative humidity of 75% - 78%. The global positioning system (GPS) reveals Lat 6° 25'53.688N and long. 2° 53'15.5184E. The ecological vegetation where the samples were collected is a humid forest and derived savannah area, in Badagry, Lagos State, Nigeria. The sample code assign to it was GALAG. The Basidiomes are flabelliform, subdimidiate, stipitate. The pileus base are 1cm –3.2 cm thick while the pileus are 4cm –12 cm in width and 6cm–14 cm in length. The Pileus shape are likened to flabelliform, spathulate, stipitate, subdimid to dimidiate, umbonate/ broad and thick at the base, mostly radial from the center extending to the margin, tough to break when dried, often thick at the center, slightly soft at the margin, light in weight when dried. The pileus surface are convex, imbricate, incised, glossy, shiny, spathulate, shallow sulcate when fresh, umbonate or uneven, usually smooth layers at center when young to age. The pileus color are usually homogenous with orange and deep orange at the center toward stipe. Context are up to 0.3cm–2.4 cm thick near stipe, white context when fresh, yellowish-white when dried, soft and fibrous. Stipes are 3–10 cm in length, 4–7 cm in width. The Margin are softer, slippery when young, laccate when mature, strongly laccate when old, orange yellow to deep yellow. Pores are 4–6 in number per mm.\. This is in accordance with the reports of Luangharn *et al.* (2021) on a recent review of Ganodermataceae using morphological markers, vegetation type, environmental climate, host substrate and molecular markers.

Other *Ganoderma* samples collected from Mangrove were *Ganoderma resinaceum, Ganoderma resinaceum, Ganoderma orbiforme, Ganoderma resinaceum, Ganoderma resinaceum*. Six (6) *Ganoderma* species were collected from humus soil of dead hardwood in Patani, Delta State. The collection was done in the month of June with room temperature to be 26℃ - 29℃ and relative humidity of 64% - 69%. The global positioning system (GPS) reveals Lat 5° 13'43.86N and long. 6° 11'29.00'E. The ecological vegetation where the samples were collected is Mangrove in Patani , Delta State, Nigeria. The sample code assign to it was GADEL. The basidiomes are imbricate and stipitate. The pileus base are 0.7cm –2.7cm thick, 2–4 cm in pileus width while Pileus length are up to 2–5 cm in length. The Pileus shape are stipitate, some laterally, and flabelliform. Pileus surface are weakly laccate when present, strongly laccate and glossy when mature, weakly laccate where the new hyphae are in active development (margin), smooth layer at the center from young to age. The Pileus color are usually yellowish-red at the center and orange on upper pileus surface. Context is about 0.3cm–1.4 cm thick at the base, abundant thick-walled. Stipe are up to 8–16 cm in length, up to 0.5cm–1.8 cm in width, central stipe, cylindrical, thick with uneven at the base (up to 1.8 cm). The margin are often 0.4cm–1 cm, orange on upper surface, and reddish-yellow under surface, thin and soft than the center. This is in accordance with the reports of Luangharn *et al.* (2021) on a recent review of Ganodermataceae using morphological

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markers, vegetation type, environmental climate, host substrate and molecular markers.

Ganoderma collected from Fresh water region are *Ganoderma lucidum, Ganoderma lucidum, Ganoderma sichuanense, Ganoderma lucidum, Ganoderma sanduense, Ganoderma sanduense, Ganoderma hochiminhense, Ganoderma sanduense, Ganoderma sanduense, Ganoderma tsugae, Ganoderma sichuanense.*

Twelve (12) *Ganoderma* fruit bodies were collected from humus soil of dead hardwood, decaying hardwood of *Swietenia mahagoni*, and *Magnifera indica* in Odi, Yenagoa, and Igbogene and region of Bayelsa State. The collection was done in the month of June with room temperature to be 23℃ - 29℃ and relative humidity of 77% - 83%. The global positioning system (GPS) reveals Lat 4° 54' 11"N and long. 6° 17' 19" E. The ecological vegetation where the samples were collected is freshwater region in Odi, Yenagoa, and Mbiama region of Bayelsa State, Nigeria. The sample ID assigned to it is GABAY. The Basidiomes are annual, perennial, stipitate. The pileus base are 2 cm thick while the pileus width and length are 2cm - 6 cm and 2cm -11 cm in length respectively. The Pileus shape is shell-like (involute from margin into the center), subflabellate or reniform to circular when seen from above. The pileus shape possess undefined concentric zones at center and extend to the margin, thick at center slightly soft at margin, and leathery when aged. The pileus color are homogenous at base at the center with red, brownish-red, and dark red, extended to the margin with reddish-yellow, but do not change the color when touched. Pileus surface are laccate when young, and strongly laccate when mature to age, shiny, silky, smooth, and soft when fresh. Context are up to 0.3cm–0.5 cm thick at base, abundant thick-walled, some thin-walled. Stipe are up to 3–8 cm in length, up to 1–3 cm in width, often red to brownish-red, and dark red when mature. Margin are obtuse from the center, some wavy, slippery when wet, softer, strong laccate edged, thin than the base and soft than the center, often reddish-yellow, deep yellow , and orange to golden yellow when mature to old.

Ganoderma collected from humid forest region are *Ganoderma lucidum, Ganoderma sinensis, Ganoderma sinensis, Ganoderma sinensis, Ganoderma tsugae, Ganoderma tsugae, Ganoderma tsugae, Ganoderma myanmarens, Ganoderma gibbosum.* Seven (9) *Ganoderma* fruit bodies were collected from decaying hardwood of *Gmelina arborea* , other dead woods and the bark of Mango tree found in three areas namely, Emohua, Ahoada, Choba, all of Rivers State Nigeria. The collection was done in the month of March with room temperature to be 26℃ -34℃ and relative humidity of 79% - 85%. The global positioning system (GPS) reveals Lat 5° 3' 0" N and long.6°27' 0"E The ecological vegetation where the samples were collected is a humid forest in Ahoada,Emohua, and Choba, Rivers State, Nigeria. Morpho description of GARIV collections:The Basidiomes samples collected in Rivers State are annual , perennial, and stipitate.The Pilues base 0.2cm -2 cm thick. While the Pileus width and length are between 2cm - 10 cm and, 3cm -15 cm respectively. Pileus shape are shell-like (involute from margin into the center), subflabellate or reniform to circular when seen from above. Pileus color are usually homogenous at base at the center with red, brownish-red, and dark red, extended to the margin with reddish-yellow, but do not change the color when touched. Pileus surface are usually laccate, mildly faded or week laccate when young, and strongly laccate when mature to age, shiny, silky, smooth, and soft when fresh. Context are up to 0.3–0.5 cm thick at base. Stipe up to 3cm–8 cm in lenge, up to 1cm–3 cm in width, almost sub-cylindrical to cylindrical, often red to brownish-red, and dark red when mature. Margin are obtuse from the center, some wavy, slippery when wet, softer, strong laccate edged, thin than the base and soft than the center, often reddish-yellow , deep yellow , and orange to golden yellow when mature.

Ganoderma Collected from humid forest zone are *Ganoderma sinensis, Ganoderma lucidum* Two (2) *Ganoderma* fruit bodies were collected from decaying hardwood found in University premises, Federal University of Technology , Owerri (FUTO) Imo State of Nigeria. The collection was done in the month of April with room temperature to be 26℃ -30℃ and relative humidity of 70% - 80%. The global positioning system (GPS) reveals Lat 5° 23' 33.6876" N and long. 6°59' 10.5504"E. The ecological vegetation where the samples were collected is a Rainforest humid region in Imo State, Nigeria. Here, the basidiomes are annuals and stipitate. The Pileus base thickness are 1cm–3 cm while the Pileus width and length are 4cm–9 cm and 6cm–11 cm respectively. Pileus shape are sub-reniform to reniform, subflabellate to flabellate. Pileus surface are weakly laccate to strong laccate at center when mature to age, and faded or week laccate at active mycelial (margin). Pileus color are brownish-red at the base, slight yellowish-red at center, and light brown to brown on the upper margin surface. Context are up to 0.3cm–1.2 cm thick at the base, with walls varying in thickness. The stipe are up to 4cm–9 cm in length, up to 1cm–2.5 cm in width. Margin are often white to pale yellow . The pores are angular, 4–6 in number per mm, Pore surfaces are white when present, pale yellow to orange, turning

Eco diversity based on Morphological features

In Table 4.1, we see similarities and variability within and between species for each quantitative traits used for this study. Within the GABUK (*Ganoderma resinaceum*) population in Sudan savannah eco zone, there are variabilities for pileus base thickness(PBT), pileus length (PL), pileus width (PW) and stipe length(ST) while similarity appear for the number of pores (PO). PO could be a good taxonomic character for characterizing *G. resinaceum.* Table 4.1 showed intra species relationship for the *G. resinaceum*. *Ganoderma resinaceum* from the Mangrove eco zone of Delta state did not show better traits for PBT. This variability within and between species are traceable to the ecological factors, both biotic and abiotic. Again, the host substrate roots of horticultural plants and humus soil of dead hardwood tree) are critical to the performance of *G. resinaceum. Ganoderma enigmaticum* from derived savannah eco zone showed better feature for

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pileus length and width. There are inter and intra species variabilities for PW and PL across eco zones. This is true for Stipe length as the diversity continue to increase. *Ganoderma lucidum* from fresh water zone of Bayelsa State showed better performance for stipe length.

On the other hand, macro qualitative traits used for this study as shown in Table 4.2 revealed some similarities for basidiome. *Ganoderma resinaceum* and *Ganoderma applanatum* from Sudan savannah and Derived savannah has the same Basidiome. *Ganoderma lucidum* showed consistency in pileus color for fresh water eco zone of Odi town and humid forest area of Emohua town. Sudan savannah, derived savannah and fresh water zone showed similarities for margin. The findings from this study showed that there are morphological variabilities within and across *Ganoderma* species. This shows that morpho taxonomic keys are not sufficient for delimitation of *Ganoderma* species. This is in accordance with the observations made by Cao *et al.* (2012) and Pilotti *et al*. (2004) who reported high phenotypic variability among *Ganoderma* species.

Molecular assessment and eco diversity

Molecular identification study revealed 12 distinct species namely; *Ganoderma sp. MT534034.1*, *G. resinaceum*, *G. lucidum*, *G. sp. 1 YD-2015*, *G. sp. HNLS1*, *G. enigmaticum*, *G. nasalanense*, *G. casuarinicola*, *G. sanduense*, *G. weixiensis*, *G. knysnamense CMW 47755*, and *G. destructans.*

Three (3) of the identified *Ganoderma* species *(Ganoderma resinaceum,G. sanduense* and *G. lucidum*) are known while the other nine (9) *Ganoderma* strains; *Ganoderma sp. MT534034.1*, *Ganoderma sp. 1 YD-2015*, *Ganoderma sp. HNLS1,G. enigmaticum,G. nasalanense*, *G. casuarinicola*, *G. weixiensis,G. knysnamense CMW 47755*, and *G. destructans* were new records for Nigeria *Ganoderma* strains.This corroborates with the reports of Tonjock and Afui (2015) for new records of *Ganoderma weberianum, G. cupreum, G. steyaertanum and G. zonatum.*

From this study, *Ganoderma* species molecularly identified in the Sudan savannah are *Ganoderma sp*. MT534034.1 and *G. resinaceum.* Species found in Derived savannah are *Ganoderma lucidum, G. sp HNLSI, G. sp MT534034, G. enigmaticum,* and *G. resinaceum.* Species found in the Mangrove area are *Ganoderma sanduense, G. weixiensis, G. enigmaticum,* and *G. lucidum.* Species found in the Fresh water eco zones *are Ganoderma nasalanenses, G. casuarinicola, G. saduenses.* While species found in the Humid forest *are Ganoderma nasalanenses, G. knysnamense CMW 47755,and G.destructans.* The predominant species abundance is *Ganoderma enigmaticum* and the zone with the highest diversity are Mangrove, freshwater and Humid eco zones. This is in accordance with the report by Oyetola (2021) who identified newer species and observed high *Ganoderma* diversity in Nigeria.

From the phylogenetic tree, two distinct clusters, I & II revealed that having most *Ganoderma* strains were found in both clusters which showed their common ancestry and background inter/intra relationship among secies. However, *Ganoderma knysnamense, G. nasalanense and G. casuarinicola,* are distant relatives and peculiar strains in cluster I while, *Ganoderma sanduense* and *G. destructans* are distant relatives peculiar to cluster II. This corroborates with the report made by Mohanty *et al.* (2011) who identified two major clusters in the phylogenetic tree of *Ganoderma* sp. constructed for North India.

Conclusion

The taxonomic position of *Ganoderma* species cannot be resolved using macro morphological characters only. The Nigerian *Ganoderma* species are not different for phenotypic plasticity as observed by several fungi taxonomist. The use of molecular and morphological markers, vegetation type, host substrate and environmental climate can be a reliable thrust for understanding the phylogeny and taxonomy of *Ganoderma* species, This study has further showed that *Ganoderma* species are diverse in nature and their identification and classification can best be unravelled by molecular markers such as the ITS rDNA barcoding primers. Also, the species has shown diversity in morphology, proximate, antioxidant, phytochemical and antimicrobial properties which can be explored for domestication and sustainable utilization.New *Ganoderma* species were reported to be present in the Nigerian eco zones. This is a good development for the pharmaceutical and ethnomycological industry. The identification of these new *Ganoderma* records for Nigeria is pointer that there are many more macro fungus in Nigeria that are yet to be discovered.

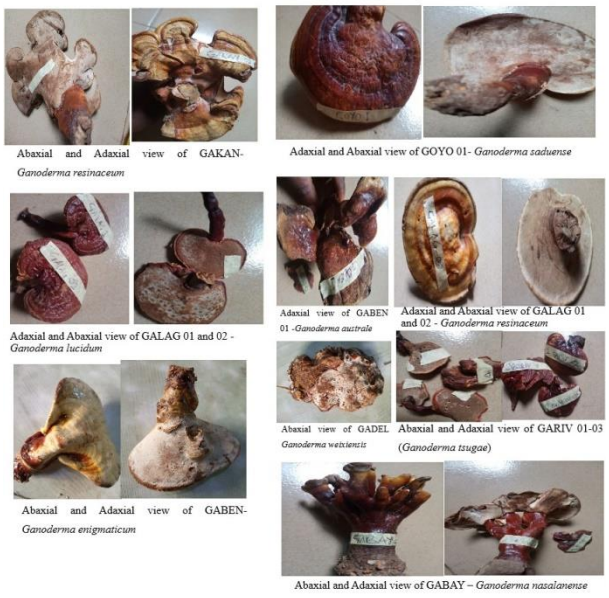

Plate 3: Pictorials of some identified *Ganoderma* species and their sample IDs

References

1. Adeoye-Isijola MO, Olajuyigbe OO, Jonathan SG, and Coopoosamy RM (2018). Bioactive compounds in ethanol extract of *Lentinus squarrosulus* mont - a Nigerian medicinal

*Corresponding Author: **Jonathan Segun Gbolagade.**

macrofungus *Afr J Tradit Complement Alternative Medicine., 15 (2): 42-50*

2. Alexopolous CJ,MimsCW and Blackwell M.(1996). Introductory Mycology (4th edition). New York John Wiley
3. Cao, Y., Wu, H. and Dai, C. 2012. Species clarification of the priced medicinal *Ganoderma* mushroom "Lingzhi". *Fungal Diversity* 56:49–62
4. John Chikwem, Gbolagade Jonathan, Anna Hull, Michael Asemoloye, Rita Omuero and Priyanka Chowdhury Tanny (2019). Comparative studies on antimicrobial potentials of *Daedalea quercina* and *Tramates pubescens. IHE: Lincoln University Journal of Science*. 8: 22-29. Available from: http://www.lugreat.com/ihe/pdfs/v8a3.pdf
5. John Chikwem, Gbolagade Jonathan;Anna Hull, Michael Asemoloye, Oluwole Osonubi and Francis Omeonu(2020) Antimicrobial Potential of *Trichaptum biforme and Bjerkandera adusta* from Pennsylvania, USA. *Journal of Natural Sciences Research*.11(16):1-8. DOI:10.1002/jpln.202000044
6. Chen, A. and Seleen, J. 2007. Potential Benefits of Ling-zhi or Reishi Mushroom *Ganoderma lucidum* (W. curt. Fr.) P. Karst. (Aphyllophoromycetideae) to breast cancer patients. *International Journal of Medicinal Mushrooms* 9: 29–38.
7. Gbolagade JS and Fasidi I.O (2005).Antimicrobial activities of some selected Nigerian mushrooms .*African Journal of Biomedical Sciences*.8 (2):83-87**)**
8. Gbolagade, J., Ajayi, A., Oku, I., and Wankasi, D. 2006. Nutritive value of common wild edible mushrooms from Southern Nigeria. *Global Journal of Biotechnology and Biochemistry* 1(1):16–21
9. Gottlieb, A.M., Wright, J.E. 1998. Taxonomy of *Ganoderma* from Southern South America: Sub genus *Ganoderma*. *Mycological Research* 103: 661–673
10. Hapuarachchi, K. K., Cheng, C. R., Wen, T. C., Jeewon, R. 2017. Therapeutic potential of *Ganoderma* species: Insights into its use as traditional medicine *Mycosphere* 8:1653–1694
11. Hapuarachchi, K. K., Karunarathna, S.C., Phengsintham, P., Yang, H.D., Kakumyan, P., Hyde, K.D., Wen, T.C. 2019. Ganodermataceae (Polyporales): Diversity in Greater Mekong Subregion countries (China, Laos, Myanmar, Thailand and Vietnam) *Mycosphere* 10: 221–309
12. Haroun, A. A., Osuji, C. E., Alhaji, A. I., Ajibade A., Onuh K., Etuk-Udo, G. A., Etim, V. A., Onyenekwe, P.C. and Abdulsalam, M. S. 2020. Molecular characterization and *in-vitro* regeneration of wild *Ganoderma lucidum* from Abuja *Nigeria Journal of Applied Life Sciences International* 23(12): 1-11
13. Hyde, K., Xu, J.C., Rapior, S., Jeewon, R., Lumyong, S., Niego, A.G.T., Abeywickrama, P.D., Aluthmuhandiram, J.V.S., Brahamanage, R.S. and Brooks, S. 2019. The amazing potential of fungi, 50 ways we can exploit fungi industrially. *Fungal Diversity*. 2019: 1–136.
14. Jonathan SG , Kigigha LT and Ohimain E. (2008).Evaluaton of the Inhibitory Potentials of Eight higher Nigerian Fungi against Pathogenic microorganisms.*African Journal of Biomedical Reaserch*.11: 197-202
15. Jonathan SG and Awotona F.E (2010).Studies on Antimicrobial Potentials of three G*anoderma species.African Journal of Biomedical Research*13(2):119-125
16. Jonathan SG and Adeoyo O.R(2011a). Collection, morphological characterization and nutrient profile of some wild mushrooms from Akoko, Ondo state, Nigeria. *Natural products*: 7(3):128-136
17. Jonathan SG and Adeoyo O.R(2011b).Evaluation of ten wild Nigerian mushrooms for amylase and cellulose activities .*Mycobiology* 39(2):103-108
18. Jonathan SG (2019).Fungi Here, Fungi There, Fungi Everywhere: Unique And Unparalleled Contributions Of Fungi To Environment, Food Production And Medicine-. *Inaugural lecture. University of Ibadan.Ibadan* .Nigeria .*ISBN978-978-8529-88-0.* (91 Pages)
19. Kaliyaperumal, M. 2013. Molecular taxonomy of *Ganoderma cupreum* from Southern India inferred from ITS rDNA sequences analysis. *Mycobiology* 41:248-251
20. Kinge, T.R. and Mih, A.M. 2012. *Ganoderma ryvardense* associated with basal stem rot (BSR) disease of oil palm in Cameroon. *Mycosphere*: 2: 179–188
21. Luangharn, T., Samantha, C., Karunarathna, S. C., Arun Kumar, D., Soumitra, P., Itthayakorn, P., Kevin, D.H, Jianchu, X., and Peter, E. M. 2021. *Ganoderma* (Ganodermataceae, Basidiomycota) Species from the Greater Mekong Subregion, *Journal of Fungi* 7: 819-824
22. Luangharn, T.; Karunarathna, S.C., Mortimer, P.E., Hyde, K.D., Xu, J.C. 2019. Additions to the knowledge of *Ganoderma* in Thailand: *Ganoderma casuarinicola*, a new record; and *Ganoderma thailandicum* sp. nov. *Mycokeys* 59: 47–65
23. Miller, M.A. Pfeiffer, W. Schwartz, T. 1999. Creating the CIPRES Science Gateway for Inference of Large Phylogenetic Trees. *Gateway Computing Environments Workshop*. 14: 1–8
24. Mohanty, P.S. Harsh, N.S.K., Pandey, A. 2011. First Report of *Ganoderma resinaceum* and *G. weberianum* from North India based on ITS Sequence Analysis and Micromorphology. *Mycosphere* 2: 469–474
25. Moncalvo, J.M.; Ryvarden, L.1997. A Nomenclatural Study of the Ganodermataceae Donk. *Fungi Flora* 10: 1–114
26. Olayinka Oluyemi Oluranti, Segun Gbolagade Jonathan, Odunayo Joseph Olawuyi

*Corresponding Author: **Jonathan Segun Gbolagade.**

27. (2019)Interactions of Extracts of Selected Macrofungi and Malaria Parasite, Plasmodium berghei berghei in BALB/c Strain Albino Mice.*Journal of Scientific Research & Reports* 23(3):1-6,
28. Otunla, C.A., **Jonathan, S. G.**, Idowu, O.O. and Olawuyi, O. J. (2018). Mycelial growth and sclerotia production of *Pleurotus tuber-regium* (Fries) singer on four sawdust types at three composting intervals. *Agronomski* 80 (2): 79-90.
29. Oyetayo, V.O, and Yao, Y.J. 2010. Identification of *Ganoderma* species Indigenous to Nigeria using ITS Region of the rDNA, *Nigerian Journal of Microbiology*. 24(1):2140-2144
30. Pilotti, C.A., Sanderson, F.R., Aitken, A.B., Armstrong, W. 2004. Morphological variation and host range of two *Ganoderma* species from Papua New Guinea. *Mycopathologia* 158, 251–265
31. Ryvarden, L 1976. Type studies in the polyporaceae species described by JM Berkeley from 1836 to 1843. *Kew Bulletin* 1: 81–103
32. Tonjock, R.K. and Afui M. M 2015. Diversity and distribution of species of *Ganoderma* in South Western Cameroon, *Journal of Yeast and Fungal Research*, 6(2):17-24
33. Wood, T., Rowaiye, A., Okwu, O., Popoola, O, Unaeze, C., AkienAlli, I., Iheka, S. and Braide, W. 2021. Phytochemical screening and antibacterial effects of wild *ganoderma* species on selected foodborne bacteria, *International Journal of Advanced Research in Biological Sciences.* 8 (1): 23-29
34. White, T., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. California: *Academic Press* 1: 315–322

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