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Research Article

Phylogenetic Analysis of Pleurotus Tuber-Regium Across Selected African Regions

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ABSTRACT

Pleurotus tuber-regium, the King Tuber Oyster mushroom, is an edible and medicinal macrofungus widely distributed across tropical and subtropical Africa. Despite its nutritional and therapeutic significance, limited molecular data exist on its phylogenetic diversity across the continent. This study investigated the evolutionary relationships of P. tuber-regium isolates from selected African regions using Internal Transcribed Spacer (ITS) gene sequences retrieved from the NCBI GenBank database. A total of thirty-six sequences were analysed, representing isolates from Nigeria, Ghana, Cameroon, Madagascar, and South Africa. Multiple sequence alignment was performed in Geneious version 9.1 using the MUSCLE algorithm, and phylogenetic reconstruction was conducted with the Neighbour-Joining method under the Tamura-Nei model. The aligned dataset had a total length of 423 bp, with 93.6% conserved sites and an overall pairwise identity of 99.5%, indicating high genetic stability. The phylogenetic tree comprised 41 nodes and 36 tips, revealing minimal genetic divergence among most isolates. Strains from West and Central African formed two tightly clustered subclades, whereas isolates from Madagascar and South Africa appeared as earlydiverging lineages, suggesting minor regional differentiation potentially linked to ecological adaptation. Overall, the results demonstrate low intra-continental variation and a single, broadly distributed African lineage of P. tuberregium. The strong sequence conservation across diverse ecological zones implies potential similarity in metabolite composition and mycochemical profiles. However, the scarcity of genomic and complementary molecular data from Eastern and Southern Africa highlights the need for expanded sampling and multi-loci phylogenetic studies to refine understanding of the species' evolutionary and biochemical diversity.

Keywords: Genetic diversity; Geo-evolutionary divergence; Internal Transcribed Spacer (ITS); Molecular phylogeny; *Pleurotus tuber-regium*

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INTRODUCTION

Pleurotus tuber-regium, often referred to as the King Tuber Oyster mushroom, is an important edible and medicinal fungus distributed throughout tropical and subtropical zones of Africa, Asia, and Australasia. Its use is widespread in West and Central Africa, including Nigeria, Ghana, Cameroon, and the Democratic Republic of Congo, as well as in Eastern

and Southern Africa, including Burundi, Madagascar, Tanzania, and South Africa (Afolabi *et al.*, 2024; Jonathan and Fasidi, 2003). In these regions, this mushroom is consumed both as food and in traditional medicine (Afolabi *et al.*, 2024); while its potential value in modern medicine is being increasingly investigated globally (Akpaja *et al.*, 2003).

Unlike other Pleurotus mushrooms, P. tuber-regium develops both fruiting bodies and underground sclerotia, which serve as nutrient-rich survival structures and are consequently widely consumed for their nutritional value. Like many fungi, its morphology and biochemical composition vary in response to environmental and ecological conditions. Comparative studies have revealed considerable variation in morphological features and nutrient composition among P. tuber-regium and other Pleurotus isolates collected from different environments (Sher et al., 2010; Anyakorah et al., 2015). Similarly, environmental factors geography play significant roles in shaping fungal evolution, driving genetic differentiation that may lead to region-specific adaptations. Hennicke et al. (2016) demonstrated that such geographical variation is associated with genetic divergence and corresponding shifts in fungal metabolite profiles. This diversity may alter biosynthetic pathways, thereby influencing the nutritional content and therapeutic properties of P. tuber-regium.

As the species gains greater recognition for its nutritional and pharmacological significance, it becomes essential to determine whether P. tuberregium populations across Africa represent genetically uniform strains or regionally distinct lineages. Recent progress in DNA sequencing technologies has greatly advanced fungal taxonomy, allowing researchers to achieve finer resolution in distinguishing species and reconstructing evolutionary relationships (Stengel et al., 2022). Among the molecular markers used for such analyses, is the Internal Transcribed Spacer (ITS) region which stands out as the marker of choice for fungal phylogenetic investigations because it combines a high copy number with conserved primer sites and adequate sequence variation to discriminate between closely related taxa. The ITS region has been formally designated as the universal fungal DNA barcode by the Fungal Working Group under the Consortium for the Barcode of Life (CBOL) (Schoch et al., 2011). In addition, ITS sequences represent the most extensively generated and publicly available fungal nucleotide data, forming the largest single locus dataset in databases such as NCBI GenBank. This abundance of data makes ITS exceptionally valuable for large-scale comparative and evolutionary genomic studies (Nilsson et al., 2019).

In this study, the evolutionary relationships of P. tuber-regium populations across Africa were explored using Internal Transcribed Spacer (ITS) gene sequences representing four major ecological regions: West Africa (Nigeria and Ghana), Central Africa (Cameroon), East Africa (Madagascar), and Southern Africa (South Africa). Sequence data obtained from public repositories were analyzed to uncover patterns of genetic relatedness and divergence that may reflect continent-wide evolutionary dynamics. While ITS data alone cannot directly capture metabolic or nutritional variability, the resulting phylogenetic groupings provide an essential framework for recognizing distinct genetic lineages that could be associated with metabolic diversity, thereby informing future studies in fungal evolution and metabolomics.

MATERIALS AND METHODS

Data Collection

For this study, all available and verified African *P. tuber-regium* ITS sequences were retrieved from the NCBI GenBank database (https://www.ncbi.nlm.nih.gov/) for phylogenetic analysis. In total, thirty-six sequences were obtained, representing isolates from Nigeria (19), Cameroon (10), Ghana (4), Madagascar (2), and South Africa (1). Each sequence included ITS1, the complete 5.8S rRNA gene, ITS2, and a segment of the 28S rRNA gene, with lengths ranging from 430 to 719 bp (Table 1).

Table 1. Metadata of P. tuber-regium ITS sequences

S/N	Accession number	Strain	Sequence Length (bp)	% G-C Content	Region

1	EU908189	DMC 187	440	43.0	Cameroon
2	EU908190	DMC 183	441	43.1	Cameroon
3	EU908191	DMC 711c	701	43.4	Cameroon
4	EU908192	DMC 711a	715	43.3	Cameroon
5	EU908193	DMC 172	627	44.2	Cameroon
6	EU908194	DMC 185	423	43.0	Cameroon
7	EU908195	DMC 173	443	43.1	Cameroon
8	EU908196	DMC 186	438	43.2	Cameroon
9	EU908197	DMC 711b	675	43.4	Cameroon
10	AF109988	PtWat	630	43.3	Cameroon
11	AF109978	PTV2	626	43.3	Ghana
12	AF109989	PTR-1	640	43.6	Ghana
13	AF109977	PTR-3	616	43.8	Ghana
14	AF109976	PTR-5	619	43.3	Ghana
15	MH862563	CBS:850.95	685	43.6	Nigeria
16	MW376907	CBS 850.95	636	43.1	Nigeria
17	KT273381	WRRIPtS	626	43.4	Nigeria
18	KP325388	Pt180	663	43.3	Nigeria
19	KP325387	Pt150	664	43.5	Nigeria
20	KP325386	Pt120	670	43.4	Nigeria
21	KP325385	Pt90	673	43.4	Nigeria
22	KP325384	Pt60	663	43.4	Nigeria
23	KP325383	Pt30	682	43.4	Nigeria
24	KP325382	Pw 18S	666	42.9	Nigeria
25	AF109983	Pt1	638	43.7	Nigeria
26	AF109981	Pt2	617	43.6	Nigeria
27	AF109982	Pt3	660	43.8	Nigeria
28	AF109985	Pt4	610	43.6	Nigeria
39	AF109986	Pt8	640	44.6	Nigeria
30	AF109987	Pt9	629	43.7	Nigeria
31	AF109979	PtW1	617	43.6	Nigeria
32	AF109980	PtW4	618	43.4	Nigeria
33	AF109984	PtW6	610	43.8	Nigeria
34	OM219822	K(M)206672	648	42.9	Madagascar
35	LT992866	-	687	43.3	Madagascar
36	MT304655	M. van der Walt:VDW1544	613	43.4	South Africa

Multiple Sequence Alignment

Multiple sequence alignment was performed to evaluate genetic relatedness among the 36 ITS sequences using the MUSCLE algorithm (eight iterations) implemented in Geneious version 9.1 (Biomatters; http://www.geneious.com). Since the sequences differed in length, the alignment was trimmed to a consistent size by removing non-overlapping regions. This step ensured that only comparable parts of the sequences were analyzed, providing a reliable foundation for subsequent phylogenetic analysis.

Phylogenetic Tree Construction

Phylogenetic tree was construction using Geneious version 9.1 (Biomatters). The Neighbor-Joining (NJ) algorithm under the Tamura—Nei substitution model was used to estimate genetic distances among the aligned ITS sequences. The robustness of the inferred relationships was evaluated through bootstrap resampling with 100 replicates, as recommended by Pattengale *et al.* (2010), while a random seed value of 1,000 was applied to ensure reproducibility. Only clades with bootstrap support values of ≥50% were considered reliably resolved, whereas those below

this threshold were regarded as poorly supported or unresolved (Efron *et al.*, 1996).

RESULTS

Multiple Sequence Alignment

The multiple sequence alignment (Figures 1–3) of 36 isolates of *P. tuber-regium* produced an aligned length of 423 bp with an average ungapped length of 418.3 bp (standard deviation = 0.7), and sequence lengths ranging from 417 to 421 bp. The mean molecular weights were estimated at 129.110 kDa for ssDNA and 258.426 kDa for dsDNA. Nucleotide composition analysis revealed A = 25.3% (3,808)

bases), C = 21.5% (3,234 bases), G = 21.6% (3,260 bases), and T = 31.5% (4,750 bases), yielding an overall GC content of 43.1%, which aligns with reported values for fungal ITS regions (Yang *et al.*, 2018). Of the aligned positions, 396 were identical, corresponding to 93.6% sequence conservation across the dataset, with an overall pairwise identity of 99.5%. **Figure 1–3.** Shows multiple sequence alignment of ITS regions from *P. tuber-regium* isolates originating from Nigeria, Ghana, Cameroon, Madagascar, and South Africa, showing extensive sequence conservation across all populations.



Figure 1. Alignment view of the Sequences (from 250 to 370 nucleotides)



Figure 2. Alignment view of the Sequences (from 380 to 500 nucleotides)

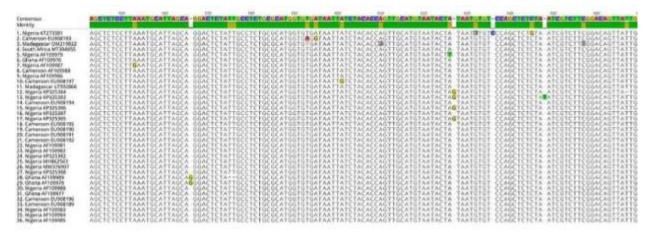


Figure 3. Alignment view of the Sequences (from 550 to 660 nucleotides)

Phylogenetic analysis

The Neighbor-Joining phylogenetic tree constructed from ITS gene sequences of 36 isolates of *P. tuber-regium* (Figure 4) featured 41 nodes and 36 tips, indicating moderate branching complexity within the dataset. Bootstrap support values ranged from 67% to 91%, reflecting consistent but moderately supported clustering patterns. Overall, the topology showed three principal clades with limited genetic distance among isolates, consistent with the high pairwise sequence identity (99.5%) and 93.6% conserved sites observed in the multiple sequence alignment.

Phylogenetic Distance Metrics

To assess the genetic relationships among African *P. tuber-regium* isolates, three comparative indices derived from the multiple sequence alignment of the ITS region were analyzed. These included nucleotide variation (count of differing bases; Figure. 5), pairwise identity percentage (proportion of matching bases;

Fig. 6), and total nucleotide identity (number of identical bases; Figure. 7). Collectively, these parameters offer an integrated overview of sequence divergence, illustrating both the extent of variation and the degree of conservation among the isolates. In the nucleotide differences matrix (Figure. 5), most P. tuber-regium isolates differed by only nucleotides across approximately 420 corresponding to a pairwise identity greater than 99.5%. The greatest observed divergence was ten nucleotides, occurring between the Nigerian isolate KT27338 and the Madagascar isolate OM2119822. Isolate KT27338 also exhibited relatively higher variation (6–9 nucleotide differences) compared with several other sequences, in contrast to the generally low pairwise differences among the remaining isolates. In the percentage identity matrix (Figure. 6), Isolates from Nigeria and Cameroon were the most similar, sharing 100% sequence identity and zero nucleotide difference.

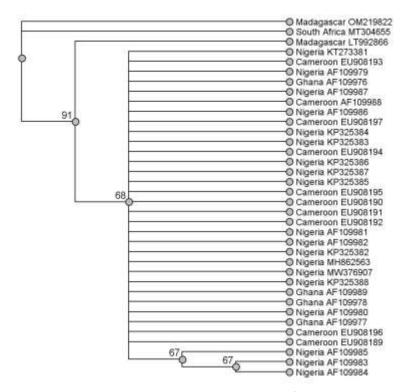


Figure 4. Neighbor-Joining phylogenetic tree of *P. tuber-regium* isolates from Nigeria, Ghana, Cameroon, Madagascar, and South Africa based on ITS sequences

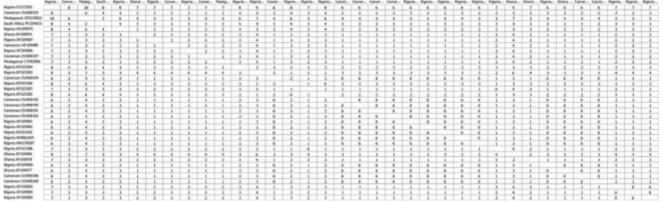


Figure 5. Nucleotide Differences

	fign's .	Orient.	Petigs.	both to	lights.	(Figure -	Name .	Orient.	Name .	Lines.	Name .	Ngara-	Turn.	Canen.	Name .	Sport.	ters.	lmes.	Deeper.	Connec.	Open.	Algebra	Tipers .	Name -	Ages.	Nyers	Tigme:	tives	Shine -	North-	Own-	Caroni.	Camera-	States -	Sylva	hiprin
without duty 1255		30.00	35.7%	35.6%	35.5%	8.7%	38.75	1.1675	M.75	95.7%	39.0%	95,75%	.91.75	LMSA	1675	0.88,7%	30.0% 20.0%	10,000		36.0%	360%	90,0%	38.0%	39,7%	190,0%	367%	16.7%	96.0%	35.7%	35.7%	39.75	1.9979	39.75	8.75	9175	1.79.05
Witnesser III DERCES					LMD.	91.7%	. M.M.	H-7%	MI. (%)	95.7%	_195.KM.	900%	.94251	LMM.	.9875.	_B.0s.	TRUN.	.79U%	U.S.O.	.mm.	1.99.0%	L PLOS	LMPA.	_19.0°s.	.,500	LHD.	_95,796_	. M.Oh.	_90.0%	J.M.Ph.	, MUST	LHD.	70 (%)	_B.Es.	.91/51	LRIP
NAMED AND DESCRIPTIONS		19.Mb		MM.	SEP4	30.0%	, more.	_HM54;	M.99.	HPh.	39.0%	JAPA.	MATS.	. MUN.	. M.Ms.	19476	98,876	JHJ76.	. 19.7%	90.0h	190/Flx	J. M.Ph.	39.7h	_MUN.	- M.M.	MJM.	16 PK	men.	NPA.	JRIN.	. 19.7%	_ M2%	M/N.	mes.	30.7%	.90375
SHIP WHAT PERSON	36,4%	10,1%	70.0%		36254	35.7h.	95.7%	19,7%	15 /St.	94.7%	96/8%	55.0°h	.91.7%	.9U%	10.7%	91.7%	300%	50.5%		2017h	10.5%	35.7%	9175	26.5%	(9.5%	_16.5h;	16.2%	30,6%	96.7%		55.5%	. 99.7h	30.8%	35.7%	99.7%	36.7%
Rypnia Afrontolini	No.	10.1%	16.P6	95/75		10.7%	94.Pb	\$6,7%	10.7%	PL75	190.4%	79.FW	84.6%	100,004	10.07a	9.75	49.074	10.7%	10.00	90.256	10.1/h	10.7%	10:0%	10.176	100.010	1 99.25s	10.74	IR.Ch.	31.7%	20.1%	10.116	10.75	10.1/4	In.m.	19. Po	10.7h
Des 2/1000	94.79-	99,7%	85.0%	91.7h	98,0%		100,000	40.Fb	66.0%	91.2%	90,856	15.7%	84.0%	40.0%	10.7%	PLT's	90.7%	MARK	100.00	40.0%	69.80	HIP.	10.0%	79.8%	10.0%	24.5%	90.0%	88.0%	19:09	90.904	49.00	1 Hi Ph	96.6%	99.0%	10.7%	PLP9:
NUMBER OF STREET	96.7%	96.7%	85,2%	98.3%	96.2%	96,570		MCE's.	19879	98.2%	96/374	98.7%	44.7%	96.8%	95.7%	NO.	90.0%	MUSE.	1889	96.0%	90,3%s	H.O.	95.0%	20,0%	180.0%	96.8%	16.1%	IR.Ph.	depts.	90,0%	30,00	9425	96,8%	HL76.	93.7%	900096
Commence Advanced	MICPS.	M.Ph								M/h	30.6%	75.7%	78.00	Life physical	19.7%	nin	9.74 90% 90% 91% 91% 91%	MARK	T. March	M1056	10.6%	TOOL	TO STATE	16.6%	man.	1666	69.6%	MIN'S	nn.	30.8%	man	LH45	95.8%	99.0%	mn.	36.6%
Rysis of GREE	95.7%	49.7%	10 PA	95.5%	36.5%	H/Fe	92.7%	- 60 fts		98.5%	1000%	10.7%	- Birth.	- 50.0%	16.74	9.7%	99.7%	MAN.	24.6%	90.0%	294.0%	mes.	96.0%	30.0%	Alten.	10.0%	19.5%	95.0%	10.7%	20.0%	81.0%	30.0%	55.6%	93.7%	10.7%	90.0%
Carpenson Statistical	86.7%	88.7%	10,7%	95.7%	18.0N	MUTW	96.2%	90.5%	99,7%		90.6N	(0.7h	98.0%	90.8h	10.7%	03.2%	90,IN	30.0%	- 10,0%	60.0%	10.00m	20.0%	94.6%	10.0%	10.0%	99.0%	60.5%	83.0%	195.5%	30.0%	10,00	36.0%	66.27h	10,7%	195,356	90,5%
Hedgeve [Tel 601	86.07%	10.0%	86.66	min.	96.6%	W1476	94.00kg	90.40%	04.6%	94.0%		98.6%	94,7%	William.	MI CO	· minim	91.7% 91.7% 91.7% 91.7% 91.7%	161.070	100.00	961995	100.00%	99.0%	80.0%	90.00s	10.00	96/975	00.459	95,7%	M1.0%	00.00g	84.00	Marthy.	80.954	94,0%	90.0%	90,450
Wylera RECESSES	86.2%	96.2%	98.8%	H25	99.0%	60.0%	30.86	96.2%	99.29.	R.26	99-6%		H.Ph.	90.1%	99.2%	94.7%	90.0%	99.0%	180.0%	40.5%	99.2%	100.0%	95.7%	99.0%	95,0%	40.5%	99.2%	9.75	90.256	49.3%	90,0%	94.0%	99-274	99.7b	99-756	96.7%
Nigery OFESSER	96.7%	98.2%	96.6%	Mah	54.0%	952%	M/76	862%	86.0%	9475	96/2%	16, Pa		36.3%	95,8%	98.0%	90.8%	10.7h	18.7%	96.0%	96.7%	95.7%	99.7%	45.7%	61.76	- 86.8W	96.0%	98.676	96.1%	95.7%	35.76	26.7%	96.3%	95.7%	90,0%	95.2%
Circles on Multiple	Milita	69,7%	84.7%	80.1%	90.7%	Million	Million	80.6%	16.4%	man.	10.0%	HiPh	H.Pa		89.7%	0.75	90.0%	100%	188%	10Fb	100%	1889	1879	180%	110%	100%	16.4%	85.7%	95.6%	100%	1000	100	stiffs.	10.0%	90.0%	MIN.
Name of LEGIS	80.7%	10.7%	M.Ph.	M/h	98,004	70.Ph	76.Ph	99,7%	95,7%	94.7%	99,4%	20,0%	188,670	99.27%		n.m.	100%	10,17%	38.6%	190.0%	10.0%	95.6%	99.5%	10.5%	38,9%	96.7%	10.5%	95,7%	95,754	99.5%	85,570	975	99,7%	95.7%	99.7%	19.7%
Signiful UP EM 3851	99.7%	HL2%	95.0%	94.7h	99,0%	P5.07to	91.25	46,7%	B179	Pk2%	70.47%	95,0%	88.7%	- WLIN-	IN Ph		90.0% 100% 100%	BLE's	01.6%	91.25	98.6Pe	19.0%	85.6%	70 Ph	91.0%	160,0%	100%	mo.	96.0%	the Phys	95,0%	1925	10.35	99.7%	96.5%	91.150
Nagery offernation	44.7%	99,5%	MAPS.	Males.	25.7%	31.7h	94.7%	99,7%	86.76	94.7%	99.0%	94,676	64,8%	60.67q	100%	68.6%	975 975 975 975	88.0%	11179	Mit High	89.4/9.	95.0%	162%	194,670	84.079	99 Fly	99.474	HLPS.	96.7%	99.0%	64,650	96 (Fb)	100,674	86.7%	96.7%	36,1%
Carraminoi SLESSATSII	56.7%	66.8%	16.7%	94.75	96.6%	MAPS.	30.6h	(6.8%)	65,8%	H-Ph	30,8%	91.0%	44.7%	100%	60.0%	19.65	RON.		100%	3045	10099	180%	100%	100%	100%	100%	10.8%	PS-2%	75.6%	100%	100%	00%	19594	INFh.	10.6%	10.8%
Carterment RURRING BO	86,0%	10.Dy	85.7%	96.5%	90.5%	50,6%	36.9%	10.0%	95,8%	24.6%	10.6%	86,6%	44.7%	100%	88.0%	05-055	10.1%	100%		258%	100%	13679	207%	.100fa	100%	25%	16.6%	96.7%	20.0%	100%	20%	250%	1075	25.6%	95.8%	30,0%
Correspond (E-600031)	55.5%	59.57a	90,5%	95.7%	60.5%	81.6%	161,879.	10 Pa	91,6%	mm.	99:5%	91.0%	.61.0%	100%	98,676	100,000	90.5%	100%	189%		150%	13079	20079	100%	18879	1000	96,8%	81.7%	99.5%	180%	100%	107%	100%	88,6%	10.0%	50,570
Carriorous (S. Stein 6)	94.5%	99.50a.	99.2%	39.2%	90.5%	94,0%	94.5%	99,956	84,6%	Hith.	90.0%	96,0%	64.7%	100%	69,176	94,6%	90.1%	100%	180%	307%		12079	200%	100%	C 1007w	307%	66,8%	81.79	96.0%	100%	100%	10%	100%	HUPS.	W-2%	99.3%
Argenta AFTERTREE	8E.9%	50.654	91.2%	99.27%	00.0%	91.0'h	91.8%	39.8%	19.6%	95.0%	-90,074	81,1%	38.2%	20(%	20.0%	94.0%	80.2%	500fe	1339%	3045	1075		200%	100%	120%	20%	15.6%	95.70.	86.8%	100%s	330%	20%	100%	HLPS	96.8%	90.37e
Bryania officiality.	86.7%	46 Fig.	66.5%	95.7%	90.8%	81.6%	90.6%	40.8%	85,690	96,8%	90-294	81.0%	18.7%	100%	60.0%	19.6%	90.0%	100%	1889	20079	100%	Links		190%	1000	100%	10.6%	99.70	96.6%	100%	18859	1979.	150%	89.6%	10.0%	60.57%
Rigera (PEXE)	80.076	60.57%	85,7%	90.0%	90.8%	91.6%	90.0%	99.6%	99.6%	95.6%	90:0%	99.5%	10.7%	200%	69.5%	91.6%	815 815 815 815 815 815	100%	189%	300%	100%	150%	100%		100%	107%	99.8%	13.7h	196.8%	100%	100%	100%	100%	mes.	59.8%	20.6%
Suproa Introdutibili	90.5%	99.37b	81.7%	89.7%	90.0%	20.0%	33.6%	10.5%	98,8%	0.05	90.0%	101.00kg	38.7%	207%	19.7%	0.05	90.5%	.100%	1989	200%	100%	1207%	200%	100%		200%	10.7%	84.7%	19.5%	.100%	38%	12079	100%	mes.	10.5%	10 E/a
Regardy MANA ROBIT	96.755	69.70+	98.7%	95.8%	90.5%	NP	36.6%	90.Ph	93.8%	96.6%	90.8%	10.0%	44.5%	320 hr	99,176	95,8%	90.8%	100%	130%	208 fe-	100%	180%	100%	100%	C.100%		96.8%	90.7%	90.0%	. 100%s	100%	337%	100%	95.0%	96.8%	William.
Rights 1P327389	96.7%	96.75s	95.0%	96.7%	96.7%	98.7%	36.0%	96.2%	100.0%	96.6%	-90354	10.7%	48.7%	90.2%	19.4%	1875	90.0% 86.0% 90.0% 90.0%	MAPs.	44.8%	HIGHs.	91.6%	HARA.	95.6%	66.8Pa.	64.0%	WAN.		394794	46.8%	90.0%	BLDW	H-2%	90.0%	95.9%	95.7%	90.0%
Share Michigan	M.75.	10.7%	10.4%	Marin.	26.0%	Milita	BUT.	Miles.	81176	Milh.	(8.74)	16.6°a	96.6%	HU7s	15.6%	91.6%	MidN-	16.7b	10.7%	95.76	10.7%	96.7%	16,7%	Hi,Pa	M39.	66,7%	doorle		70.0%	16.74	49.76	16.7h	16:7%	39.0%	th the	in its
Day HURCS	196,7%	00.7%	86.0%	00,70	\$6.5%	35.0%	88.7%	dir (file.	86.5%	04.5%	90,074	93,7%	86.7%	60,7%	85.7%	RES	\$10.7%	10.6%	191.0%	65,7%	10.5%	0.0%	86.0%	70.8%	91.6%	26.0%	88.5%	81.7%		30.3%	30.05	10.0%	69.9%	88.5%	98.7%	99.5%
Nagery ATSURES	30.7%	60,754	16.7h	90.7%	90.5%	10.0°m	36.6%	40.5%	80.0%	98.6%	30.3%	80,0%	94.7%	100%	85,3%	inch.	90,1%	100%	110%	100%	100%	130%	15%	100%	100%	1976	10.8%	81.7%	94.5%		10000	185	10%	HUPS.	10.0%	90,0%
Draw 91/200617																																				
Certaino S/MINE	M-2%	99.5%	19.7%	60.7%	99.5%	men.	44.8%	10.0%	100 Fb.	H.Ps.	95.0%	MA79a	94.7%	200%	10076	SLHL.	90.0%	100%	189%	2019	14%	189%	100%	100%	1899	100m	10.6%	86,7%	85.8%	180%	3376		100%	86.0%	9.0	10 Ph
Determine (A.Print St.)	36.5%	99.3/A	10.7%	25.75	36:5%	30.6k	50.0%	100.604	80.00	96.8%	10070	30.57m	.04 PM	1079	10.1/4	25,7%	100,076	200/m	18976	100%	150%	15004	100%	190fa	100%	1000	10.3%	95,7%	20.054	1900s	300°w	. 50%		93.0%	10.0%	30.354
Standard (Printer)	86.7%	MI, (%)	86,6%	94.3%	99.7%	Mars	m.m	80.374	66,7%	900	90474	84.5%	84.0%	100,000	68,7%	188,550	100,074	30.5%	High-	301/ba	99,0%	man.	99,0%	30.8%	man.	90.0%	66 Ph.	MACO.	90.0%	30.5%	more	meth.	00.00a		200	100%
Signia artificiani	46.7%	99,7%	95,7%	99,791	26.2%	49,5%	99.7%	99,5%	95,7%	94.7%	99,076	ML7%	88.5%	99,5%	16.7%	19,7%	90.7% 90.7% 90.7% 90.7%	99.8%	B.Fh	90.0%	10.6%	94.0%	95,0%	191,870	Mark.	99,5%	99,5%	99,6%	95,750	81.8%	80.00	19.7h	99.8%	100%		100%
Name of Street	96.7%	16.7%	98.104	Fi.2%	06:2%	46.0%	94.7%	391,37hr	98.0%	1925	99.454	95.7%	100%	96(35)	101,754	19.7%	-06.0%	DUFFE	111/0	80.35	10.0%	men.	96.6%	the phy.	94,0%	96.25v	18.7%	391.170	TED.	m Pa	91.05	16.2%	36.8%	100%	20%	

Figure 6. percentage pairwise

	Marks.	Deen.	Noting-	limith .	Storie	- Illyelds -	. Hjeru	Control	. Myeria.	Correc-	State.	Marrie	Signite.	Giren.	Mer-	Nonia.	. Algorita.	Camp	. Jane.	fares.	Carry	Migris.	Herr.	. Hyris.	Marris	Marre.	Harris.	Shire.	Charte	. Nigetie-	Dista.	Down.	Carrier,	Figures.	Ngets.	None.
Miguel ATTYTOM:		440	40	403	463	413	433	411	411	411	44.1	462	462	46.0	465	-50	465	40	40	431	40	411	411	411	441	441	41.0	41	462	462	962	460	40	40	411	413
Carrieron Extinctivi	800		407	104	-101	465	60	889	1.01	1.00	446	468	165	186	456	50	mA -	HA	168	46	886	484	5.48	68	1.00	141:	186	186	104	466	669	466	HIA.	.85	4/5	- 65
Pelapoir (PERC)	401	42		400	.40	413	411	941	413	412	494	412	-416	-46.0	404	464	694	44	404	414	414	414	414	494	-498	414	414	-462	-46.6	498	664	404	494	407	401	413
Sod-Mila Historia	401	405	500		1611	40	10.40	1.48	0.00	310	400	-115	-160	168	198	706	106	466	496	48	486	196	5.386	486	-516	444	496	-515	1.106	-10b	159	106	r68	160	491	485
Najerky Art (Retrie	40	464	40	404	E.	40	400	40	400	405	411	463	495	469	406	45	408	406	496	49	435	496	486	496	440	460	188	486	468	409	409	458	456	40	423	40
Marrie M1900/6	-61	-81	913	411	411		196	. 946	414	446	446	446	467	967	198	-83	407	407	425	-617	417	917.	417	417	-447	417	117	462	167	1.960	-61	407	42	100	446	410
Nipole SFEDRED	40	45	80	45	415	.49		430	410	416	486	486	187	465	48	40	467	407	407	401	417	497	467	1.40	447	467	457	465	- ar	465	47	407	47	65	486	456
Concessor Mysteries	407	45	40	400	400	410	400		430	995	400	400	Agir	46.0	467	407	467	407	407	401	407	417	415	417	447	417	417	462	40	-467	-467	407	407	406	400	416
Stephole AFE (STAGE)	40	44.	400	401	411	480	440	140		110	446	486	145	965	m/	-60	407	407	417	407	1.80	447	441	MAT	1487	447	-817	105	160	-91	40	40	407	466	400	100
Consum EPRETT	462	45	401	415	415	416	425	111	411	11:11	400	466	97	-97	407	47	407	40	407	417	407	417	417	417	417	447	417	465	-85	407	-87	407	407	415	416	416
Pedigenow (1000866)	402	45	414	406	410	425	410	916	416	840	100	405	ALF	467	1-407	-62	407	40	407	411	-917	417	411	411	1.40	411	1417	965	447	-407	-407	407	467	408	49	436
Mysel STYTTSA	401	985	#0	401	401	486	440	400	636	144	446		160	467	466	40	100	46	401	40	447	467	411	867	107	417	117	105	167	460	481	44	40	69	69	400
Migratio HP125780	402	460	494	416	419	417	417	947	417	40	41.7	463	10.0	198	400	-88	488	408	419	419	418	489	4.0	418	-111	410	445	100	405	-108	-608	-418	409	407	427	-417
Companion Elyfrethire	463	488	404	456	400	417	417	991	41	447	441	467	196		-108	48	468	103	40	40	48	418	410	488	-416	411	160	196	468	+08	-108	-68	400	407	411	417
Mysele (POZZINE)	463	466	614	6.6	400	447	447	947	447	445	407	440	180	968		41	100	40	446	40	488	444	448	434	446	440	160	104	168	494	-94	10.8	48	407	401	447
Majoria 10752/5/807	402	-66	404	406	400	407	E-411	847	417	1-411	467	-617	490.	-408	-600			10.0	416	439	6.00	438	440	410	-110	410	141	104	-418	468	468	-68	466	407	407	407
MpH4-9732589	40	406	64 64	61 61	459	407	407	417	407	401	407	446	495	49 40	-51 -61 -61	49 61		45	416	40	4.0	486	430	486	486	445	485 485	***	40.	468	-51	48	48	407	407	467
Grown BRRIS	40.0	460	404	400	1456	40	441	447	417	-417	417	.467	-409	469	406	43	408		416	439	419	460	446	418	-111	411	-112	444	468	-106	400	408	48	407	401	40
Central Horses	163	-min	69	400	400	407	1.40	467	447	947	417	111	468	-168	108	-68	108 108 108 108	45	100	40	440	446	846	416	166	400	160	166	168	168	108	100	48	697	407	617
Camerion KONSERS	40.	400	404	439	401	417	60	417	467	411	417	401	469	400	468	48	409	409	438		439	488	430	411	418	488	460	405	469	408	409	408	419	407	427	407
Coverner D/988181	46.0	966	414	Mid.	496	-412	441	947	411	-115	err.	.167	- 000	168	- 100	108	108	nier.	416	655		450	+10	411	-110	488	310	199	100	008	98	108	100	AIT	401	917
Registe And Direct	40.	965	414	496	466	447	447	447	447	447	117	447	-140	448	868	46	108	100	400	616	486	Dec.	416	498	1666	444	146	444	148	168	666	-68	84	427	407	467
Myerke AF1204KE	903	406	919	416	436	417	0.0	417	417	411	417	41.5	- 19.6	168	168	400	408	108	410	436	919	418	Array Control	418	418	148	188	166	468	468	40.0	108	448	400	40	417
Nyosi sississi	963	-000	619	468	400	917	01	146	411	443	SEE	188	188	100	48	65 65	48 68 88	48 12 15	465	400	100	410	446		-110	488	140	198	10.0	408	168	-68	168	ADT	401	417
Stones IMMERCHED	400	466	604	416	466	467	61	945	411	441	167	485	460	198	000	46	- 600	466	488	410	10	410	9.00	466	1	110	440	444	168	466	468	- 68	46	407	447	40
Myete MVS70007	903	408	401	406	400	417	417	607	617	411	417	467	100	PER	108	-61	108	43	938	400	408	438	416	1 434	418		418	111	-108	1 468	408	408	408	40	407	437
Nijeri AFTEETOE:	863	-00	.61	400	410	917	1.00	417	107	100	467	617	160	168	- 86	.03	48	MR	48	62	100	886	110	121	411	446		198	104	100	100	-04	100	407	407	447
Disco-M'LITREE	411	994	407	104	414	41)	411	90	913	411	415	481	485	190	098	49	106	40	404	410	436	636	446	411	444	444	186	110.00	10.5	468	969	406	46	401	410	415
Own H1000W	603	-68	404	100	46	917	417	607	417	417	417	137	168	758	168	48	908	48	418	488	100	120	- 00	438	418	468	100	467	1100	468	168	908	608	42	907	917
transa Arazenso	401	440	414	440	438	447	3 417	441	485	111	441	447	100	100	- 40	-41		MR	488	910	416	444	9.00	411	110	111	111	194	10.0		-48	418	MR	407	401	417
Pero Milesto	903	min.	101	N/A	- 111	417		917	607	FET	467	937	488	106	968	49	600	400	401	400	100	410	198	198	116	+11	188	100	168	1 966		460	400	407	497	917
Common BURDELW	40	466	814	466	46	447	407	967	107	107	485	SET	160	166	168		600	48	446	401	- 68	439	110	414	110	111	460	166	- 111	100	144		48	467	467	467
Centeron Full State	403	166	404	406	438	407	417	987	417	117	481	411	166	-109	408	48	100	48			419	488	410	438	100	411	111	100	-409	108	-63	100		with	401	417
Angula: AF\$ (MISS)	953	98	401	401	440	100	100	936	636	110	110	110	HE	or.	80	160	907	407	40	60	417	607	107	- 60	417	407	-0.1	165	167	1.040	967	- 118 - 107	407		400	410
Mgmin 8/120000	RI.	465	40	465	45	816	430	. 100	856	240	480-	486	467	487	467	407	467	467	407	467	417	407	417	417	467	467	417	105	465	467	462	467	467	4:8	12.5	440
Separation of Edition (443	40.5	411	400	433	840	436	446	638	940.	440	446	417	-467	401	407	407	407	407	627	80	617	417	411	441	447	138	100	463	401	- MIT	407	407	638	400	-

Figure 7. Nucleotide Identity

DISCUSSION

The alignment of *P. tuber-regium* sequences revealed predominantly conserved nucleotide regions, interrupted by very few insertions or substitutions. This high level of conservation (93.6% sequence identity and 99.5% overall pairwise similarity) confirms the ITS region's reliability as a molecular marker for fungal phylogenetics (Xu, 2016).

The phylogenetic tree revealed that isolates from Madagascar and South Africa formed an early-diverging clade, suggesting a degree of geographical differentiation from the West and Central African populations, possibly reflecting ecological isolation or regional adaptation. In contrast, most Nigerian, Cameroonian, and Ghanaian isolates clustered tightly into two closely related subclades with bootstrap values between 67% and 68%, suggesting a shared ancestral lineage within West–Central Africa.

Despite the broad geographic spread of sampling locations, the overall phylogenetic topology indicates limited genetic divergence across the continent. This is in consistent with the reports of Isikhuemhen *et al.* (2000) and Vizzini *et al.*, (2019) who reported minimal ITS sequence variation within the African clade of *P. tuber-regium* Ilineages. The slight divergence observed in isolates from Madagascar and South Africa may represent early-stage regional adaptation rather than distinct speciation.

However, limited availability of *P. tuber-regium* genomic data (Villani *et al.*, 2025), particularly ITS sequences from Eastern and Southern Africa, together with the scarcity of complementary molecular markers and functional gene data in public database constrains the scope of comparative phylogenetic analysis in this study.

CONCLUSION

The strong genetic similarity across isolates suggests that *P. tuber-regium* populations in Africa are genetically stable and may share comparable metabolic and nutritional characteristics. This high level of genetic uniformity across diverse ecological zones suggests substantial evolutionary stability and ecological adaptability, implying that *P. tuber-regium* populations across Africa may share comparable metabolite profiles and mycochemical compositions.

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