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Molecular phylogenetics of selected Ganoderma lucidum from Abuja environs: An Insight into Local and Universal Relatedness

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Abstract
Given its increasing commercial relevance in the nutraceutical market, driven by the growing demand for antitumor and immune-boosting natural products, Ganoderma lucidum has been extensively explored for its vast therapeutic potential. Its reported wide distribution globally, alongside high morphological variations, is of significant research interest. The conspicuous taxonomic ambiguity in this mushroom, owing to its species plasticity, leads to misidentification and evolutionary complexity within the species. This has given rise to various premises regarding its origin, evolution, and the influence of multiple factors on its multifaceted existence. This study investigated the preliminary evolutionary relationship and plausible origins of local Ganoderma lucidum strains from Abuja, Nigeria, using a simple phylogenetic analysis. In this work, we carried out a molecular phylogenetic analysis of ten selected specimens of G. lucidum from Abuja environs to determine their evolutionary relatedness within and among specimen sequences from other geographical locations of the world. Molecular characterization techniques were used to generate DNA sequences of the Abuja samples. Additional sequences from Nigeria, India, China, Korea, and Europe were retrieved from the NCBI. Multiple sequence alignment and phylogenetic tree construction were conducted using Clustal-W and MEGA XI, respectively. The resulting Neighbor-Joining tree revealed significant inter-regional phylogenetic clustering of the taxa, with a few overlapping clades present between them. It also revealed a strong bunching of the Nigerian-Abuja strains with their somewhat closer association to the European strains. This study provides a baseline for further molecular investigations, suggesting geographical location as a key factor in G. lucidum species variation..

Keywords: Ganoderma lucidum, ITS, NCBI, Phylogenomics, Sequences, Taxonomy

1. Introduction
The bioactive potentials of natural products, particularly those from plants and fungi, have long provided a vital pharmacologically active basis for the production of valuable therapeutic compounds (Aware et al., 2022; Nnadozie et al., 2023). They have tremendously shown promising results in the treatment and management of some complex diseases and remain indispensable in drug discovery and modern medicine (Jalil et al., 2024; Dzobo, 2022). *Ganoderma lucidum*, a widely valued medicinal mushroom known for its potent medicinal qualities, has become a subject of global interest, particularly due to the growing demand for anticancer and immune-boosting natural products for prophylaxis against diseases like COVID-19 (Ekiz et al., 2022; Gao et al., 2023; Cadar et al., 2023; Wu

et al., 2024). It is a basidiomycete fungus of the genus *Ganoderma,* with a long medicinal history (El-Sheikha, 2022). Its widely reported pharmacological potencies and widespread global distribution are significant points of relevance in its application (Oke et al., 2022; Ekiz et al., 2023).

However, its taxonomic ambiguity due to complex morphological variation and high intra-species plasticity often results in identification and classification chaos (Hapuarachchi et al., 2015; Loyd et al., 2018). This has led to the term '*G. lucidum* complexity, which describes identification ambiguity within the species owing to high morphological variation (Zhou et al., 2015; Papp, 2024). For instance, *G. lucidum* was previously known to generally exist in two growth forms, representing the two sources of the mushroom: Asia and Europe (Hennicke et al., 2016). One type is seen as sessile and large with little or no stalk, while the other is smaller with a long and narrow stalk (Hapuarachchi et al., 2015). Yet, many strains with connecting features of these two major forms exist as intermediates (Haroun et al., 2020) alongside others with very unusual morphologies, thus needing phylogenetic elucidations. An outright taxonomy and evolutionary relationships among strains of this mushroom species have not been quite explicit due to this pronounced morphological variability and inadequate molecular lucidity (Loyd et al., 2018). This is evinced by different reports of variations due to geographical location (Zhang et al., 2017), host plants, overlapping new species discoveries (Jia-Hui et al., 2016), and secondary metabolite profiles (Henicke et al., 2016), among others. The insufficient distinguishing phenotypic markers of the fruiting body morphology, vis-à-vis geographical, ecological, and host organism differences, seem to be a major focal point in the evolutionary and taxonomic complexity of this species (Du et al., 2023).

Given the distinctive nature of genes as molecular fossils that hold important organisms' genetic information over time, molecular phylogenetics has been useful in elucidating considerable epistemic knowledge far beyond morphological features alone. The morphological vagueness and concomitant identification challenges of *G. lucidum* (Loyd et al., 2018) necessitate the use of molecular techniques. Thus, the internal transcribed spacer (ITS) region of ribosomal DNA has been adopted as a standard barcoding tool in *Ganoderma* systematics for resolving most of the *Ganoderma*-associated taxonomic controversies (Hapuarachchi et al., 2018). The ITS sequence analysis method is a barcode-based species identification approach that operates on an overall similarity between the query and the reference sequence in the databases (Erasmus, 2021). Hence, a good understanding of *G. lucidum* diversity and phylogenetic relatedness with regard to their intra-species plasticity and identification chaos is crucial for conservation, stereotyping, and proper application.

This study, therefore, investigates the preliminary molecular phylogenetics of selected wild *G. lucidum* specimens from Abuja environs using molecular techniques and compares their relatedness with sequences from other regions worldwide. The objective is to investigate the local and universal evolutionary relatedness of the specimens and the influence of geographical location on their intra-specific variation.

2. Literature review

The relevance of molecular phylogenetics in modern Biotechnology is quite enormous, as it plays significant roles in modern biological taxonomy, evolution, and conservation research for studying diversity and possible migration trajectories of different organisms, including mushrooms. The production and development of mushrooms have continually steered a lot of research interests, particularly in their culinary, therapeutic, and bioremediation use (Rahman et al., 2021; Llanaj et al., 2022). Generally described as heterotrophic macro-fungi that belong to the *Basidiomycota* and *Ascomycota* divisions, mushrooms are commonly of wild origin (Elkhateeb et al., 2021; Procházka et al., 2022; Liu et al., 2022). They represent a grossly underexploited source of potent therapeutic and high-value nutritional products (Yongabi, 2019; Wang and Zhao, 2023; Singh et al., 2024). Several studies have also explicitly demonstrated their unique nutraceutical applications in the prophylaxis and treatment of important diseases like hypertension, hypercholesterolemia, blood platelet aggregation, and even viral diseases (El-Sheikha, 2022; Cör Andrejč et al., 2022).

Ganoderma lucidum, also known as Lingzhi or Reishi, in Asia and some other regions, is a polypore mushroom with widespread distribution due to its high medicinal value (Oke et al., 2022). It was given different unique names like 'special mushroom', 'king of mushrooms', and 'mushroom of Immortality' in Asian traditional medicine owing to its content of an extensive variety of exceptionally useful bioactive compounds for various medications (Loyd et al., 2018; El Mansy and Shimaa, 2019; Łysakowska et al., 2022). *G. lucidum*, which is seen in traditional medicine as a repository of bioactive compounds, is challenged by complicated identity issues (Garuba et al., 2020; Ekiz et al., 2022; Plosca et al., 2025). Complex phylogenetic relationships among strains of *G. lucidum* have been revealed, as some reports suggest the existence of multiple varieties within the species, and this constitutes the *G. lucidum* complex (Zhou et al., 2015). This also affects its relationship with species within the *Ganoderma* genus, as the different varieties of *G. lucidum* overlap with many other species of the genus. More reports of identification chaos and debate have shown that *G. lucidum* phylogenetic complexity is not yet resolved.

These include the report of Henick et al. (2016) that broadly classified *G. lucidum* into two different types of East Asian and European origins based on their anatomical structure, molecular clustering, and metabolite potentials. Also, reports of the overlapping concept in laccate *Ganoderma* species led to misidentification and misclassification of Argentinian species of *G. lucidum* as *G. resinaceum* (Moncalvo et al., 1995). Reports of Zhang et al. (2017) even classified *G. lucidum* strains into three groups, 1-3, according to geographical origin using ITS phylogenetic analysis. In the same vein, there is also a seeming uncertainty about the actual origin of the Nigerian strains of this mushroom, as there are reports of both European and Asian origins (Osuji et al., 2016). The importance of understanding the wide diversity within the *G. lucidum* complex and the significance of discovering new or overlapping species are also very crucial (Loyd et al., 2018; Hapuarachchi et al., 2019). Molecular markers, which are specific segments of DNA at precise sites, are potent tools for identifying desirable genetic characters and/or differentiating specific genetic variations (Amiteye, 2021; Hasan et al., 2021). Studies have employed different molecular marker-assisted methods to analyze *Ganoderma* phylogenetics (Pristas et al., 2023; Adotey et al., 2023).

While reports of molecular phylogenetic studies of *G. lucidum* abound (Loyd et al., 2018), there are limited reports on those of West African origin, particularly Nigeria. Thus, the need for further studies on *G. lucidum* genetic diversity and evolutionary relationships within and across geographical regions, especially in understudied areas like Nigeria, arises. Hence, this study was carried out as a preliminary molecular phylogenetic investigation to provide a valuable insight into the regional and global relatedness of *G. lucidum* from Abuja environs.

3. Research methodology

3.1. Sample collection

Wild specimens of the suspected *G. lucidum* species were collected from different locations in Abuja, the federal capital territory of Nigeria (Figure 1). Ten out of the specimens were carefully selected based on their morphological appearance (Figure 2) and thereafter taken to the Molecular Biology Unit of the Biotechnology Advanced Research Centre in Sheda Science and Technology Complex (SHESTCO), Abuja, for molecular analysis.

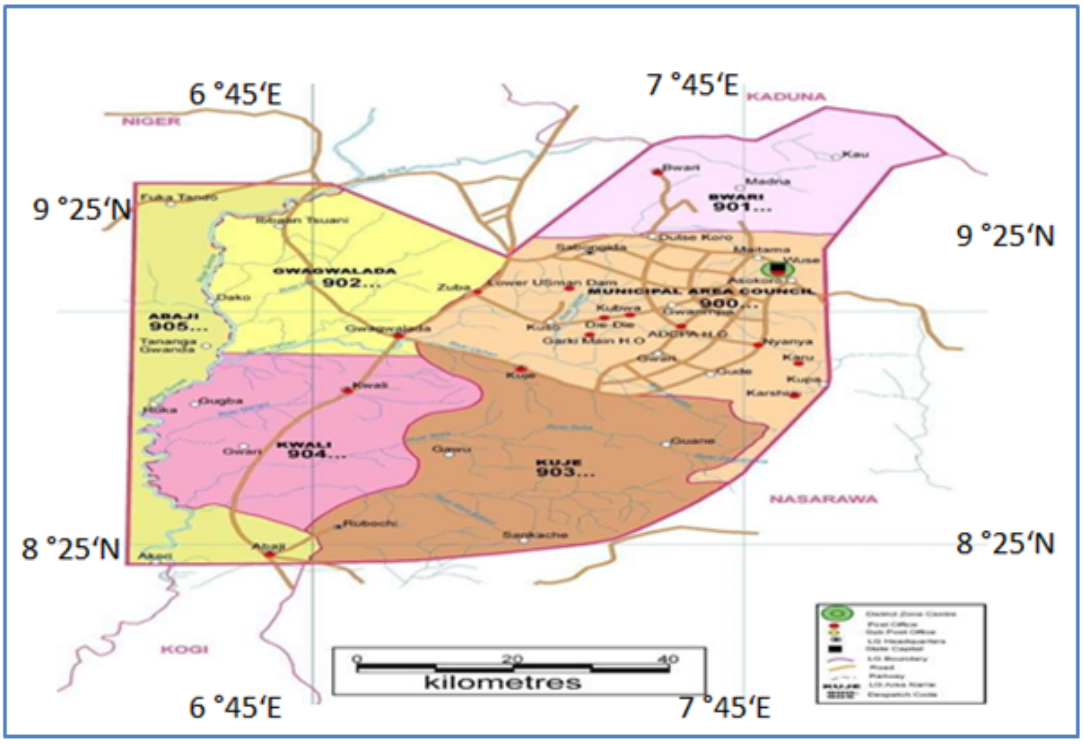

Figure 1: Map of Abuja showing the Area councils
Source: Federal Capital Development Authority, Abuja, FCDA (2020)

Figure 2: Collected wild *G. lucidum* Samples from Abuja Environs

3.2. DNA Extraction

Genomic DNA was extracted from the specimens' samples using a modified Cetyl trimethyl ammonium bromide (CTAB) DNA extraction protocol as previously described by Osuji et al., (2015). The DNA quality and quantity analyses were performed using a Nanodrop spectrophotometer and an Agarose gel electrophoresis, respectively, as displayed in Table 1 and Figure 3.

3.3. PCR Amplification

ITS primers -1 and -4 were used to amplify the rDNA sequences of the samples in an Eppendorf thermocycler model X50a following the methods previously described by Gardes and Bruns (1993). The primer sequences are: ITS1- (TCCGTAGGTGAACCTGCGG) and ITS4- (TCCTCCGCTTATTGATATGC). The optimized PCR mix was a 20 µL reaction volume containing approximately 300 ng template DNA, 0.3 µM forward, 0.3 µM reverse primer, 10µL Canvax Green-

Taq polymerase master mix, and nuclease-free water. The cycle program described by Haroun et al. (2020) was used. The PCR amplicons were later screened on a 1.2% agarose gel, stained with ethidium bromide fluorescent dye, de-stained with water, and visualized with Alpha Innotech Mini Imager Gel Documentation System as shown in Figure 4. The PCR products were cleaned with Zymo Research PCR-clean-up kits and subjected to Sanger's sequencing using Applied Biosystems International (ABI) automated sequencer model ABI13130.

3.4. Sequence Analyses

The resulting automated ABI sequence trace files from the sequencing process were edited using BioEdit software and aligned with the Clustal W algorithm. The query sequences were then used to perform multiple sequence similarity searches across sequences in the NCBI database using the Basic Local Alignment Search Tool (BLAST). Five ITS sequences each, from Nigeria, China, Korea, Europe, and India, were obtained from the database and used for the study. Multiple sequence analysis (MSA) of the entire sequences was performed using Clustal W at default parameters in MEGA XI. These ITS-aligned sequences were used to build a simple phylogenetic expression (Figure 5). The phylogenetic tree construction was done with MEGA software version XI using the Neighbor-Joining technique. This was computed according to the method previously described by Saitou and Nei (1987) and validated by Tamura et al. (2004). The phylogenetic distances were evaluated at 1000 repetitions using the standard phylogenetic computational algorithm of Maximum Likelihood in units of the number of base substitutions per site. All ambiguous positions were removed for each sequence pair in the pairwise deletion option.

4. Data analysis

The data generated from the experiment were analyzed with different software in different sections of the work. They include: BLAST, Bio-edit, Clustal W, and MEGA XI, which are standard bioinformatics data analysis software

5. Results and discussions

The overall result of this study showed the prevalent influence of geographical location on the species variation, as most of the specimens clustered significantly according to their locations. The standard molecular characterization process was used to obtain the sequences from the Abuja samples in line with researchers (Horn et al., 2020; Zhao et al., 2021). These were analyzed alongside *G. lucidum* representative reference sequences of China, Korea, India, and Europe from the NCBI. These representative sequences (depicted in Table 2) were used as secondary data in line with Zou et al. (Zou et al., 2024).

Table 1: Concentration and Quality Analyses of the DNA using Nanodrop Spectrophotometry

Sample No	Concentration (ng/µl)	Purity (OD_{260nm}/OD_{280nm})
Abuja Sample 1	407.50	1.69
Abuja Sample 2	465.00	1.78
Abuja Sample 3	447.50	1.87
Abuja Sample 4	415.00	1.82
Abuja Sample 5	350.00	1.79
Abuja Sample 6	485.20	1.78
Abuja Sample 7	474.53	1.83
Abuja Sample 8	434.00	1.82
Abuja Sample 9	481.00	1.78
Abuja Sample 10	397.50	1.85

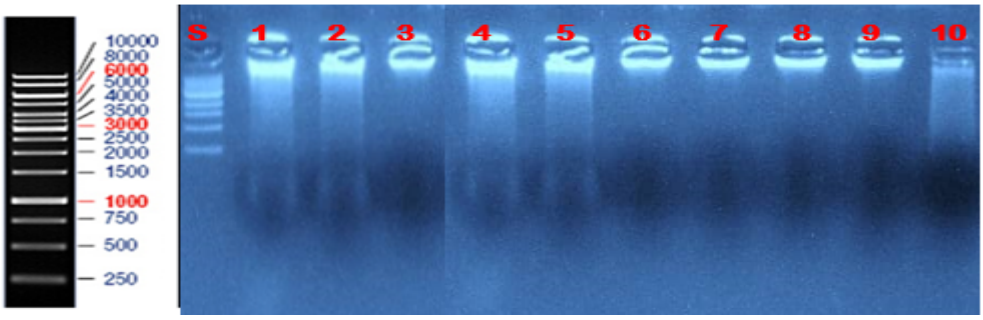

Figure 3: Agarose Gel Electrophoresis Picture of the DNA Samples
Key: S = Step ladder (1kb) from New England Biolabs,
Lanes 1 -10 represents the different samples.

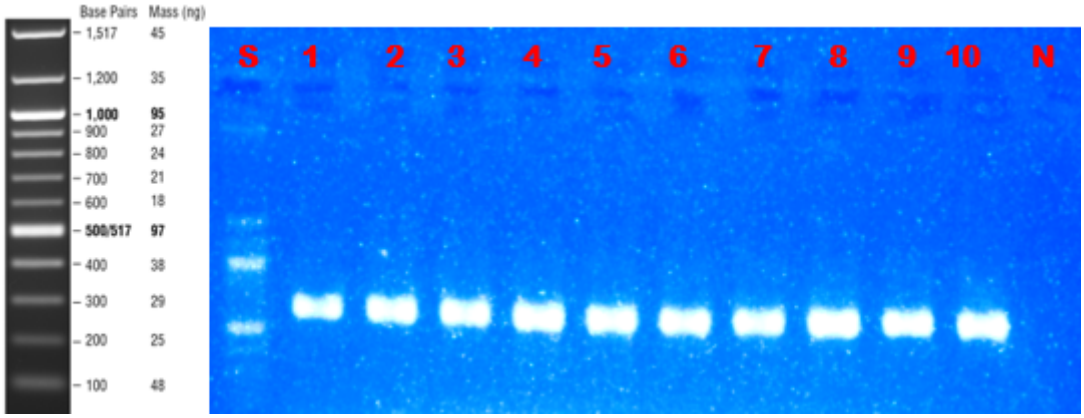

Figure 4: PCR Products Amplification with ITS1 and ITS4 Primers
Key: S = Step ladder (100bp) from New England Biolabs,
Lanes 1 -10 represents the different samples. N = Negative control

The extracted DNAs from the wild samples were of good quantities and quality, as depicted in Table 1 and Figure 3. They were amenable to PCR amplifications to give distinct bands, as shown in Figure 4. The 'amplicons' estimation size of approximately 600bp is in agreement with Usyk et al. (Usyk et al., 2017).

Table 2: Reference Table of Sequences from NCBI Database

S/N	Sequence ID	Origin	Authors	Title/Address
1	KX589250.1	China	Zhang, *et al.*, 2017	Intraspecific Variation and Phylogenetic Relationships.
2	KX589249.1			Revealed by ITS1 Analysis and SNP in Ganoderma
3	KX589248.1			Lucidum
4	KX589247.1			
5	KX589246.1			
6	HM053452.1	India	Mohanty *et al.*, 2012	Molecular phylogeny of Ganoderma lucidum isolates
7	HM053451.1			collected from northern India
8	AY636068.1			
9	GQ249885.1		Singh *et al.*, 2003	Molecular characterization of specialty mushroom.
10	GQ249884.1			germplasm of the National Mushroom Repository
11	JQ520178.1	Korea	Park *et al.*, 2012	Genetic diversity analysis of Ganoderma species and
12	JQ520168.1			development of a specific mark er for identification of
13	JQ520167.1			medicinal mushroom *Ganoderma lucidum*
14	JQ520171.1			
15	JQ520170.1			

16	MG706177.1	Europe	Fryssouli, 2017	Ganoderma species in Europe. Agricultural Iera Odos,
17	MG706176.1			Microbiology, Agricultural University of Athens,
18	MG706172.1			75, Athens 118 55, Greece
19	MG706175.1			
20	MG706171.1			
21	MZ014900.1	Nigeria	Amao *et al*., 2021	Pure and Applied Biology, Ladoke Akintola University of Technology,
22	ON394695.1			Ogbomoso., *Faculty of Pure and Applied Sciences*, Ogbomoso 210211, Nigeria
23	OR164446.1		Shaibu *et al*., 2023	Biosynthesis of silver nanoparticles using wild Ganoderma mushroom extract
				and its antimicrobial activities on food pathogens. Botany, University of Ibadan,
				university of Ibadan, Ibadan, Oyo State 234, Nigeria
24	OQ883914.1			
25	OQ883913.1		Oyeleke *et al*., 2024	Pure and Applied Biology, Ladoke Akintola University, Ogbomoso-Ilorin
				Express, Ogbomoso, Oyo 234, Nigeria

The generated molecular sequences from the Abuja samples, labelled 1-10, were used alongside the NCBI-obtained sequences for the MSA and phylogenetic tree building. Clustal W, which employs the affine gap penalty model, where gap opening and gap extension are treated separately to optimize the alignments and minimize mismatches (Chowdhury & Garai, 2017). Bootstrap values at ≥ 60% presented a strong clustering support in the phylogenetic tree for identifying and validating observed clades in line with Zhang et al. and Zou et al. (Zhang et al., 2017; Zou et al., 2024). The resulting phylogenetic tree (Figure 5) showed some distinct local and global reflective clustering patterns of evolutionary implications.

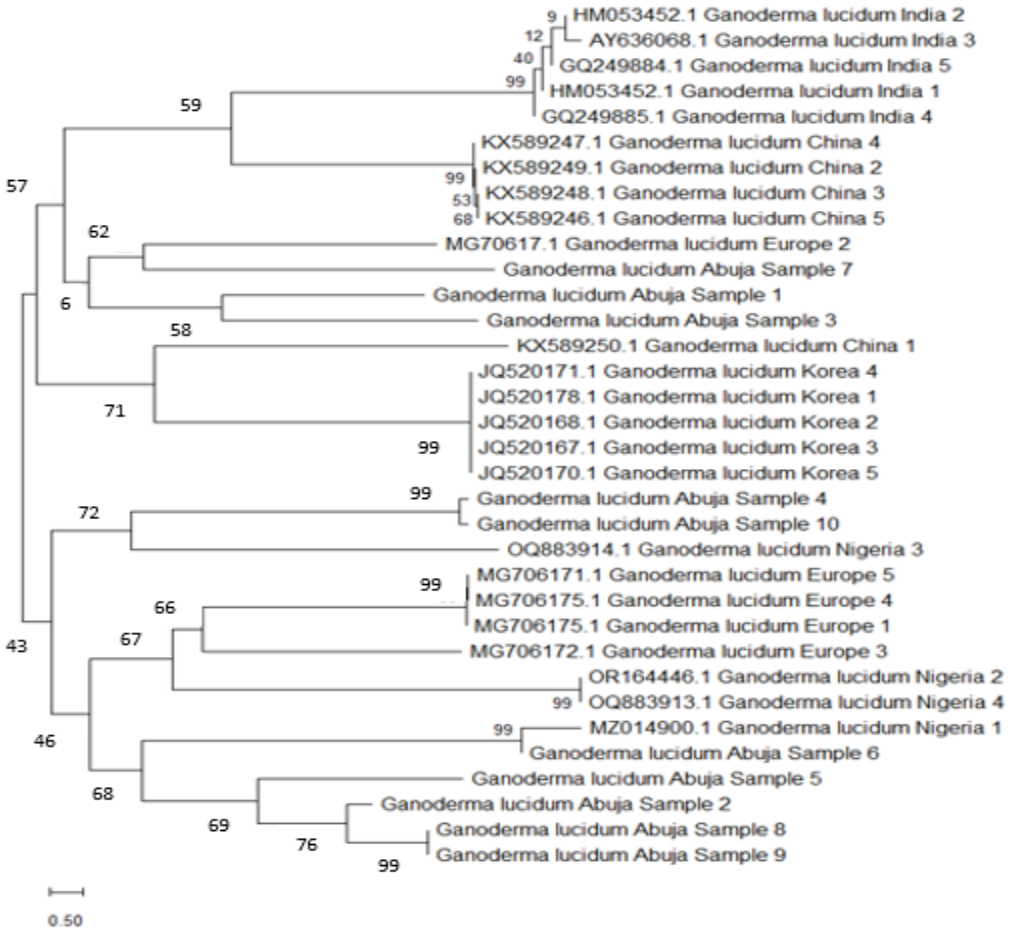

Figure 5: A simple Neighbor-Joining Phylogenetic Tree

The optimal tree (Figure 5), which involved a total of 35 genomic sequences, was inferred via the Neighbor-Joining evaluation approach. The evolutionary relationships of the taxa in the phylogenetic tree depicted the interrelationships among sequences of the collected wild samples from Abuja with other NCBI-retrieved sequences from Nigeria. It also showed their relationship with sequences from other regions of the globe to infer a simple evolutionary relatedness across the board. The Phylogenetic tree showed the various *G. lucidum* in their respective clusters. Critical evaluation of the phylogenetic expression above clearly demonstrates the clustering in terms of geographical location, which agrees with Zhang (Zhang et al., 2017). It also revealed the polyphyletic nature of the *G. lucidum* complex, with less than 20% sequence overlap among the diverse locations, in agreement with Haroun et al. (Haroun et al., 2020). The tree revealed that more than 80% clustering of the species based on geographical origin, showing distinct clades of sequences from India and China, and Korea.

Most of the Abuja samples clustered with other Nigerian strains, while demonstrating evolutionary closeness to the European taxa. Thus, the wild samples from Abuja, Nigeria, showed high uniqueness among themselves as well as a high degree of similarity to other NCBI-obtained Nigerian sequences. This intra-regional bunching suggests a localized evolutionary relatedness of the Abuja and Nigeria strains, coming from the same location. This could be attributed to likely shared ecological effects within the same geographical location, in agreement with Zhou et al. (Zhou et al., 2015).

However, the observed closer evolutionary relatedness of the Nigerian taxa with the European taxa than those of India and China suggests a common or closely related progenitors between them. It could also reflect some elements of shared ancestry, indicative of their traceable evolutionary origin, in agreement with Paterson and Hapuarachchi et al. (Paterson, 2006; Hapuarachchi et al., 2015). These observed clustering patterns buttress the impact of geographical origin on *G. lucidum* diversity. It also substantiates the stand by Zhang et al. (2023) in developing a *G. lucidum* quality control tool for geographical traceability and Wadood et al. (2023) isotopic system for Indian *G. lucidum* authentication. This mixed clustering supports the non-monophyletic nature of the Nigerian *G. lucidum* as reported by Haroun et al. (2020), highlighting the significance of molecular techniques in its taxonomic elucidations.

Interestingly, these observed phylogenetic patterns could be expounded further from a deeper anthropogenic and ecological point of view. The Nigerian *G. lucidum* could have predominantly come from exotic trees of European origin species into Nigeria for forestry and landscaping. International medicinal mushrooms trading could have also facilitated *G. lucidum* dispersal across these regions and contributed to the observed mixture. Such human-mediated transboundary dispersal for medicinal plants and other microbes has been reported by Paterson and Hapuarachchi et al. (Paterson, 2006; Hapuarachchi et al., 2015).. Also, from the biogeographical perspective comes the possibility of shared ancestry or parallel evolution in different ecological zones.

However, this present study is focused on molecular phylogenetics using molecular data; preliminary observations during field sampling of the Abuja strains provided some morphological pictures of the mushroom, as depicted in Figure 2. The macromorphological features of this mushroom have proven inadequate because its few phenotypic markers are merely centered on the fruitbody shapes and coloration. They are relatively simple and quite limited for differentiating strains (Haroun et al., 2020)

While the study investigates the local and universal evolutionary relationship of *G. lucidum* strains from Abuja environs and to ascertain the effect of geographical location on the species, it acknowledges sample size limitations and therefore emphasizes the importance of using a higher sample size and locations for further studies. Hence, this work underscores the significance of phylogenetic construction in resolving evolutionary relationships and improving taxonomic classification of complex phenotypes. It also establishes a foundation for future in-depth evolutionary studies.

6. Conclusion

The use of molecular barcodes as fossils in ascertaining the evolutionary relatedness and possible origins of species of organisms is the mainstay of phylogenomic studies. By employing a simple molecular Phylogenetics procedure, this study has determined the genetic diversities and evolutionary relatedness of the selected wild specimens of *G. lucidum* from Abuja, Nigeria, with similar specimens from other geographical locations around the world. The resulting simple phylogenetic tree was pretty fathomable, demonstrating an illative evolutionary relationship among the samples with an inkling of the probable origin of the Abuja-Nigerian strains.

This study revealed a strong intra-regional but non-homogenous phylogenetic pattern of the Nigerian strains and their notable associations with the European strains. This finding suggests that the diversity of *G. lucidum* from Nigeria is not only shaped by ecological adaptation but possible anthropological mediations, as they demonstrated notable localized and slight trans-regional trait association with the taxa from Europe. This study summarily presents a baseline molecular phylogenetic assessment and calls for continued exploration in the study of *G. lucidum* complexity. Further studies with bigger sample sizes and extensive molecular parameters are highly recommended, as they will provide a good foundation for authentication, conservation, and sustainable utilization of this medicinal mushroom.

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