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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 early-diverging 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. tuber-regium*. 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 and 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. tuber-regium* 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.

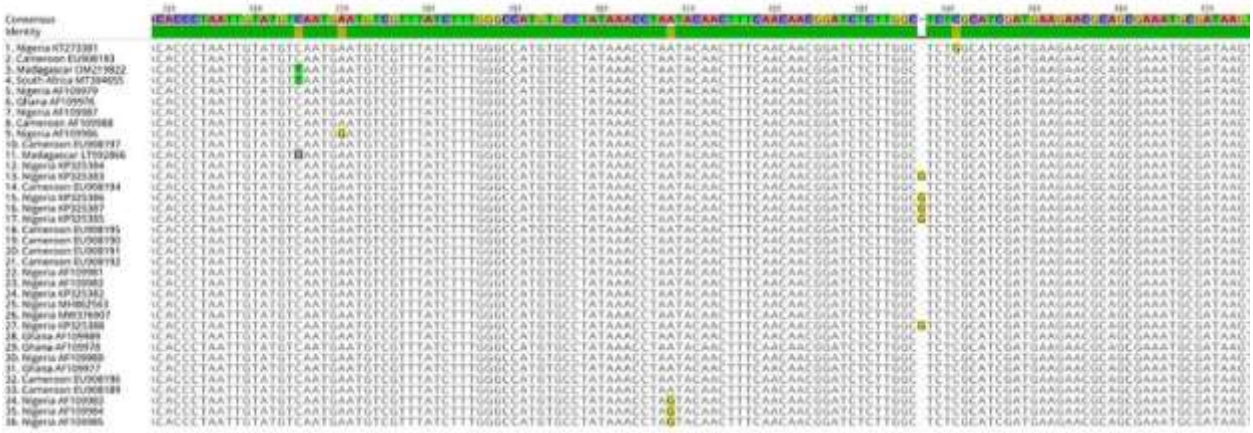

Figure 1. Alignment view of the Sequences (from 250 to 370 nucleotides)

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

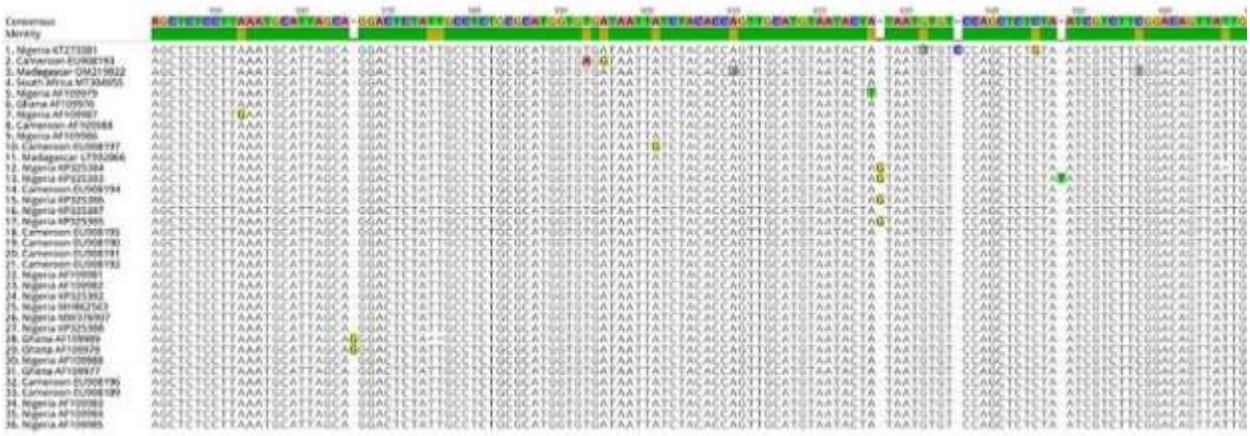

Figure 3. Alignment view of the Sequences (from 550 to 660 nucleotides)

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 1–5 nucleotides across approximately 420 bp, 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) when 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.

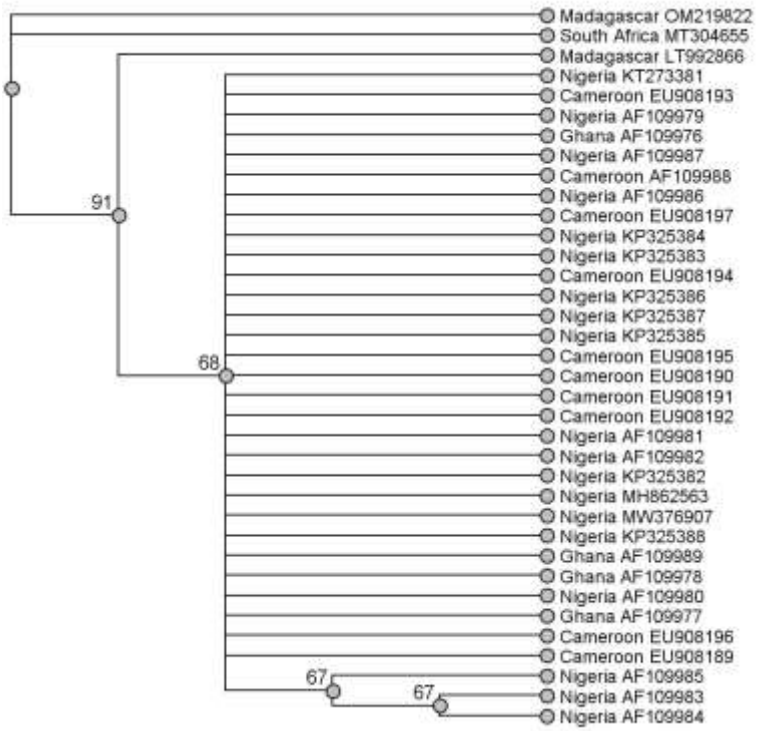

Figure 4. Neighbor-Joining phylogenetic tree of *P. tuber-regium* isolates from Nigeria, Ghana, Cameroon, Madagascar, and South Africa based on ITS sequences

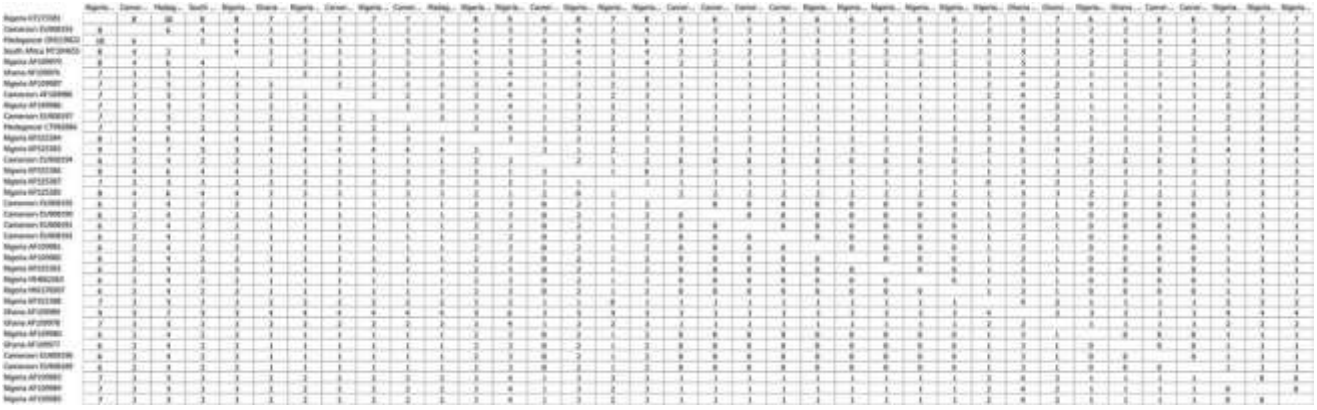

Figure 5. Nucleotide Differences

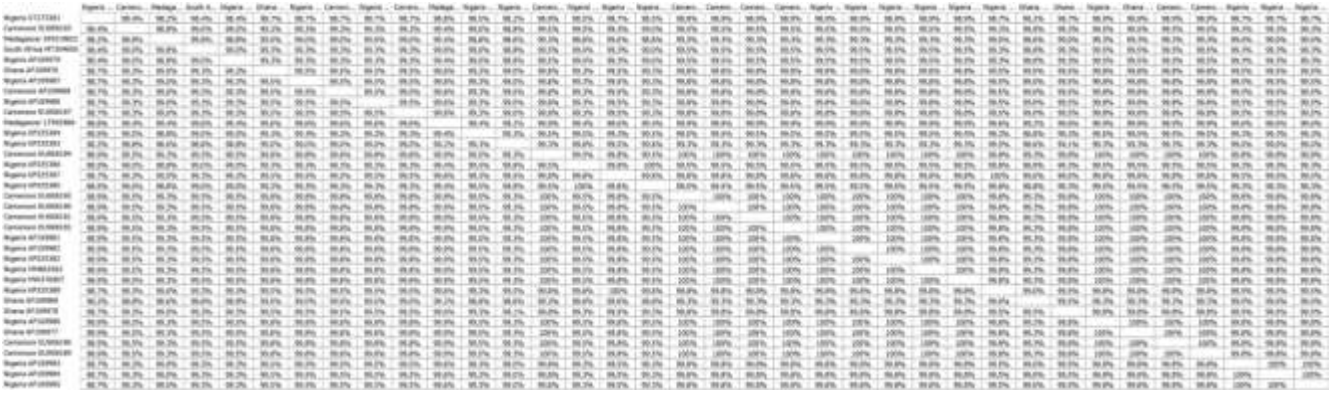

Figure 6. percentage pairwise

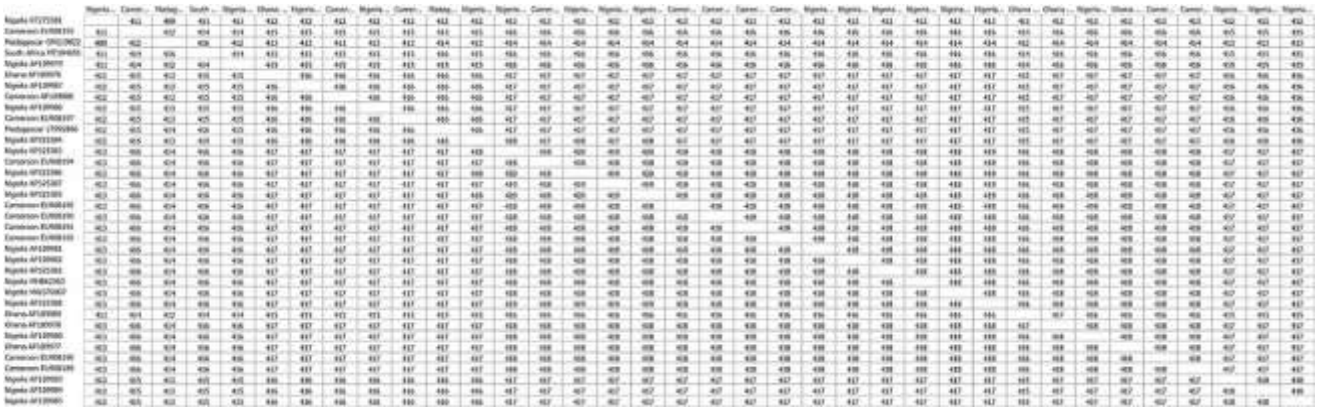

Figure 7. Nucleotide Identity

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* llineages. 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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