Science World Journal Vol. 20(No 4) 2025 https://dx.doi.org/10.4314/swj.v20i4.30
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

Full Length Research Article

COMPARATIVE MOLECULAR PHYLOGENY OF GANODERMA LUCIDUM FROM NIGERIA, CHINA, KOREA, AND JAPAN: INSIGHTS FOR DRUG DISCOVERY

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ABSTRACT
Ganoderma lucidum, a medicinal mushroom valued for its diverse pharmacological properties, has been extensively studied in Asia, but remains underexplored in Africa. Given the influence of geographic variation on fungal evolution and metabolite production, understanding the phylogenetic relationships between Nigerian and Asian isolates is essential for both taxonomy and drug discovery. This study aimed to examine the evolutionary relationships between Nigerian and Asian *G. lucidum* isolates using molecular data. A total of 27 ITS gene sequences (seven each from Nigeria, China, and Korea, and six from Japan) were retrieved from NCBI GenBank, aligned with MUSCLE in Geneious 9.1, trimmed to uniform length, and analyzed for nucleotide composition, pairwise identity, and sequence differences; a Neighbor-Joining phylogenetic tree was then constructed using the Tamura–Nei model with 100 bootstrap replicates. The alignment yielded an average ungapped length of ~552 bp with 44.7% conserved sites and 92.8% overall pairwise identity; most Asian isolates clustered tightly (>99% identity, differing by only 1–5 nucleotides), whereas the Nigerian isolate MZ014900 was highly divergent (~250–310 nucleotide differences, 53–57% identity) and formed a distinct long branch, while other Nigerian isolates showed intermediate clustering with Asian strains. These findings reveal both conserved and divergent lineages, highlighting Nigerian isolates as potential reservoirs of conserved traits and novel bioactive compounds, though additional markers and chemical profiling are required to validate their evolutionary and pharmacological distinctiveness.

Keywords: *Ganoderma lucidum*, ITS phylogeny, Molecular divergence, Bioactive compounds, Drug discovery.

INTRODUCTION

Ganoderma lucidum, commonly known as Reishi or Lingzhi, is a highly valued medicinal fungus with a long history of therapeutic use, particularly in China, Japan, Korea, and other Asian countries (Wachtel-Galor, 2011; Ha *et al.,* 2020). Its medicinal significance is attributed to a rich repertoire of bioactive compounds, including polysaccharides, triterpenoids, proteins, and sterols, which have been linked to diverse pharmacological activities such as immunomodulatory, anti-inflammatory, antiviral, and anticancer effects (Cör Andrejč *et al.,* 2022; Pascale *et al*., 2023). Although its earliest applications are rooted in traditional Asian medicine, the therapeutic importance of *G. lucidum* is increasingly being recognized in Africa, where it is incorporated into ethnomedicine and is actively studied for its pharmacological potential (Oke *et al.,* 2022).

The global distribution of *G. lucidum* implies that its evolutionary trajectory, like that of many organisms, is shaped by geographic location and environmental factors, leading to genetic variation and potentially region-specific adaptations (Wachtel-Galor *et al.,* 2011). Such diversity suggests that regional strains of *G. lucidum* may exhibit significant genetic divergence, which can influence their biosynthetic pathways and, in turn, affect both the composition and therapeutic potential of bioactive metabolites. For instance, comparative studies have shown that only a few biosynthetic gene clusters are conserved across related fungi, indicating that secondary metabolic pathways tend to diversify or change during speciation (Wadhwa *et al,* 2024). Chemical profiling has also shown substantial variation in triterpenic acid content between Asian and European isolates of *G. lucidum* (Hennicke *et al.,* 2016), highlighting the link between geographical location, genetic divergence, and differences in metabolite profiles. Accurate species identification is critical for understanding both evolutionary patterns and medicinal applications. However, identification of *Ganoderma* species based solely on morphology has proven unreliable due to overlapping traits and phenotypic ambiguities (Wachtel-Galor, 2011; Hennicke *et al.,* 2016). For example, Haroun *et al.* (2020) demonstrated that 25% of samples originally identified as *G. lucidum* by morphology were misclassified when re-examined using DNA sequencing, underscoring the need for molecular approaches in fungal taxonomy.

Advances in DNA sequencing have transformed fungal systematics, providing higher resolution for species delimitation and phylogenetic studies (Stengel *et al.,* 2022). Among the molecular markers available, the Internal Transcribed Spacer (ITS) region is widely recognized as the universal fungal DNA barcode and was officially endorsed by the Fungal Working Group (FWG) of the Consortium for the Barcode of Life (CBOL) (Schoch *et al.,* 2011).

The ITS possesses several useful features, including a high copy number, conserved priming sites, and sufficient sequence variability to distinguish between species. In the fungal genome, the ITS region spans roughly 600 base pairs and consists of two variable segments, ITS1 and ITS2, separated by the conserved 5.8S rRNA gene (Xu, 2016), bordered by the 18S rRNA gene upstream of ITS1 and the 28S rRNA gene downstream of ITS2 (White *et al.,* 1990). Because the 18S, 5.8S, and 28S rRNA genes are highly conserved, they allow the design of universal primers capable of amplifying ITS1, ITS2, or the entire ITS region across a

wide range of fungi (Xu, 2016). Another advantage of ITS as a barcode is that haploid genomes generally contain multiple tandem repeats of the rRNA gene cluster (including ITS), enabling successful amplification even from little amounts of biological material (Xu, 2016).

While Asian strains of *G. lucidum* have been extensively profiled both molecularly and pharmacologically, African isolates remain comparatively underexplored despite their growing use in traditional medicine. This knowledge gap underscores the need for comparative molecular phylogenetic analyses to clarify evolutionary divergence and assess potential implications for medicinal properties.

Accordingly, this study aims to examine the phylo-evolutionary relationships between Nigerian and Asian *G. lucidum* by analysing ITS gene sequences from Nigeria, China, Japan, and Korea, retrieved from online repositories. The primary goal is to identify patterns of similarity or divergence that may provide insights into their evolutionary diversification. While the ITS-based analysis cannot directly reveal metabolite differences, phylogenetic clustering can highlight distinct lineages that warrant further metabolomic and pharmacological investigation for drug discovery potential.

MATERIALS AND METHODS

Data Collection

A total of 27 *G. lucidum* ITS gene sequences were retrieved from the NCBI GenBank database (https://www.ncbi.nlm.nih.gov/) for phylogenetic analysis. The dataset comprised seven sequences, each from Nigeria, China, and Korea, and six from Japan. The retrieved ITS sequences encompassed Internal Transcribed Spacer 1 (partial), the 5.8S ribosomal RNA gene (complete), Internal Transcribed Spacer 2 (complete), and part of the large subunit (28S) ribosomal RNA gene, with an average sequence length of approximately 600 bp.

Multiple Sequence Alignment

To investigate evolutionary relatedness among the isolates, all 27 ITS gene sequences were aligned using the MUSCLE algorithm with eight iterations, as implemented in Geneious version 9.1 software (Biomatters, available at http://www.geneious.com). Because the downloaded sequences varied in length, the aligned dataset was trimmed to a uniform size to remove non-overlapping regions and eliminate length discrepancies. This ensured that only homologous regions were compared across all isolates, providing a reliable basis for alignment and subsequent phylogenetic inference (Table I).

Table I. Metadata of *G. lucidum* ITS sequences
This table presents accession numbers, strains, trimmed ITS sequence lengths, and G-C content of *G. lucidum* isolates originating from Nigeria and Asia.

S/ n	Accession No	Strain	Trimmed Sequence Length (bp)	% G-C Content	Region
1	MN596944	FFUI-3	550	48.9	Nigeria
2	MZ014900	E4	501	55.3	Nigeria
3	ON394695	LAU22	560	48.6	Nigeria
4	OQ883913	EST1	559	49.8	Nigeria
5	OQ883914	EST2	557	49.2	Nigeria
6	OR164446	GF	544	47.8	Nigeria
7	PV444608	C06_09	549	45.3	Nigeria
8	JX162754	GL-1	551	47.9	China
9	JX162756	GG	551	48.1	China
10	JX162765	Jinlin G	551	48.1	China
11	JX162770	SYLZ	551	47.2	China
12	JX162769	XLLZ	558	49.5	China
13	JX162768	JHHZ	551	48.3	China
14	JX162762	Tai-1	551	47.9	China
15	KX589248	RB	551	47.9	Japan
16	AB733175	GAL-M.	552	48.8	Japan
17	AB733174	NBRC 8346	552	48.6	Japan
18	AB733122	NBRC 31863	551	48.5	Japan
19	EU021456	WD-2038	551	48.4	Japan
20	EU021455	WD-565	551	48.6	Japan
21	JQ520185	ATCC 46755	559	49.4	Korea
22	JQ520188	KCTC 16802	551	48.6	Korea
23	JQ520169	Yeongji-2	551	48.3	Korea
24	JQ520170	ASI 7074	551	48.7	Korea
25	JQ520174	IUM 0047	551	48.3	Korea
26	OM809721	15245	551	48.8	Korea
27	OP928163	JBRI-M22-038	559	49.0	Korea

Phylogenetic tree construction

A Neighbor-Joining phylogenetic tree was constructed from the aligned ITS sequences based on the Tamura-Nei genetic distance model using the Geneious version 9.1 tree builder (Biomatters) to determine the evolutionary relatedness and diversities. To assess the reliability of the clustering patterns, bootstrap resampling was performed with 100 replicates as recommended by Pattengale *et al.* (2010). A random seed of 1,000 was used to ensure the reproducibility of the bootstrap analysis. The resulting tree was interpreted such that only clades with bootstrap support values of 50% or greater were considered reliably supported, whereas branches below this threshold were considered unresolved (Efron *et al.,* 1996).

RESULTS

Multiple Sequence Alignment

The multiple sequence alignments (Figures 1–3) of 27 *G. lucidum* isolates resulted in an aligned length of 608 bp with an average ungapped length of 551.8 bp (Standard Deviation, 12.2), and sequences ranging from 501 bp (the shortest) to 584 bp (the longest). The mean molecular weight of the aligned dataset was 13.980 kDa for ssDNA and 27.804 kDa for dsDNA. Nucleotide composition analysis showed: A = 21.9% (3,267 bases), C = 24.6% (3,661 bases), G = 24.4% (3,630 bases), T = 29.1% (4,335 bases), indicating a balanced AT/GC ratio and resulting in an overall GC content of 49.0%, which is typical for fungal ITS regions (Yang *et al.,* 2018).The alignment comprised large extended stretches of conserved nucleotides interrupted by clusters of variability, most notably around positions 90–120. Out of the aligned positions, 257 were identical, representing 44.7% sequence conservation across the dataset.

The Nigerian sequences shared bases with Asian isolates, but also displayed unique substitutions. In particular, isolates MZ014900, PV444608, and ON394695 exhibited distinct indel (insertions and deletions) patterns compared to Asian strains, emphasizing their evolutionary distinctiveness. Among them, MZ014900 stood out as the most divergent, with multiple substitutions, insertions, and deletions relative to the consensus. Its inclusion reduced sequence identity by 39.7%, underscoring its genetic distance from other isolates and suggesting that this isolate may be following a separate evolutionary path.

Figure 1: Alignment view of the Sequences (from 80 to 210 nucleotides)

Figure 2: Alignment view of the Sequences (from the 260 to 400 nucleotides)

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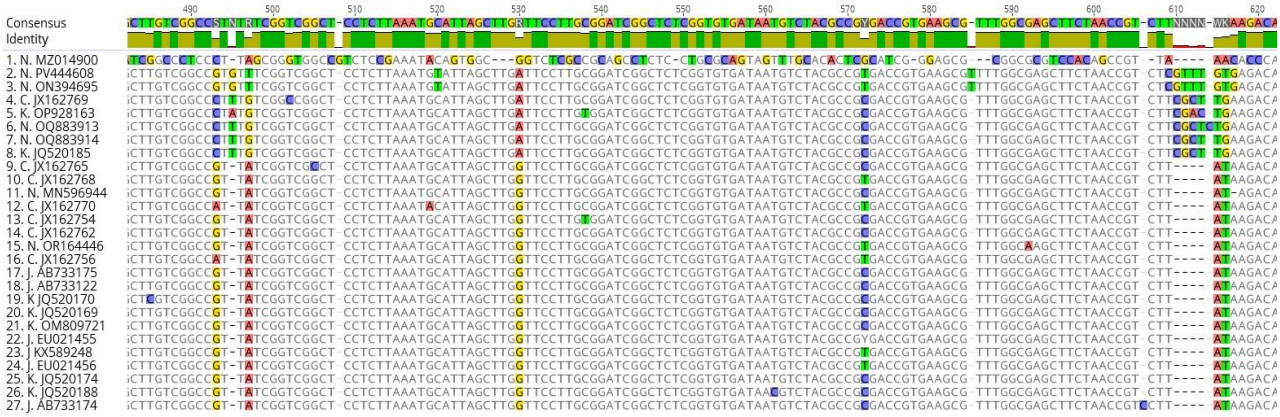

Figure 3: Alignment view of the Sequences (from 490 to 620 nucleotides)

Figure 1–3. Multiple sequence alignment of ITS regions from *G. lucidum* isolates collected from Nigeria, China, Japan, and Korea, showing conserved and variable regions

Phylogenetic Analysis

The phylogenetic tree constructed from the aligned ITS gene sequences of *G. lucidum* isolates from Nigeria and Asia (Figure.4) revealed a single ancestral root, indicating that all isolates belong to a single *G. lucidum* species. This was observed despite only 44.7% of the alignment being conserved. The tree featured 39 nodes and 27 tips, with Asian isolates clustering more closely together than their Nigerian counterparts. Over half of the isolates from various geographical regions clustered into a single group with moderate bootstrap support (58%), suggesting a degree of geo-evolutionary relatedness among these strains.

Nigerian isolates MZ014900, PV444608, and ON394695, which exhibited distinct indel patterns in the multiple sequence alignments, formed a notable sub-clade with strong bootstrap support (ranging from 57% to 99%). Additionally, Nigerian isolates OQ883913 and OQ883914 cluster with the Chinese isolate JX162769, supported by high bootstrap values (99% and 75%).

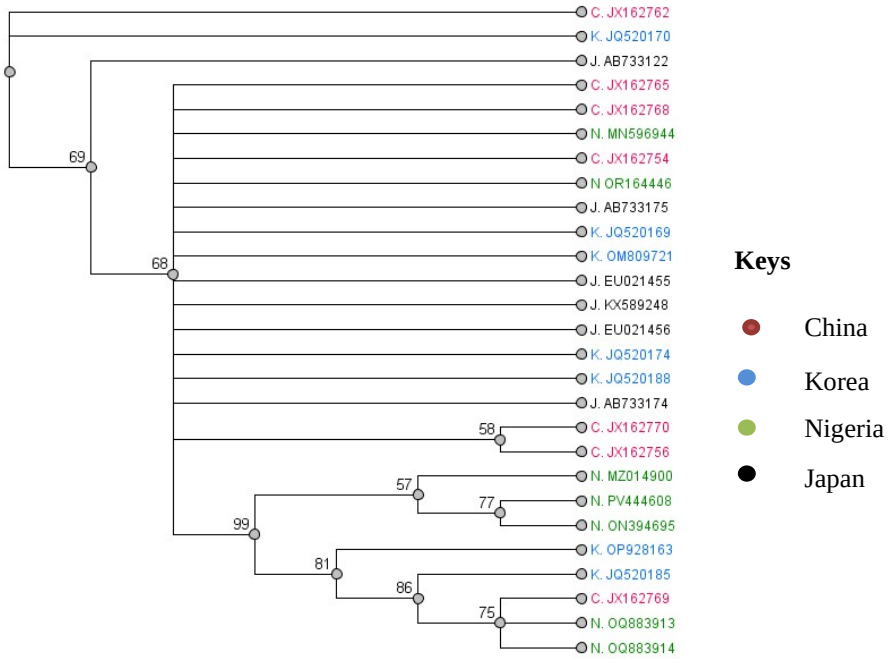

Figure 4: Phylogenetic Relationships of *G. lucidum* Isolates from Nigeria and Asia Based on ITS Sequences

Phylogenetic Distance Metrics

To evaluate the phylogenetic relatedness of Nigerian *G. lucidum* isolates to their Asian counterparts, three sequence comparison metrics based on multiple sequence alignment of the ITS gene were examined. These included nucleotide differences (number of non-identical bases; Figure. 5), percentage pairwise identity (percentage of identical bases; Figure. 6), and nucleotide identity (number of identical bases; Figure. 7). Together, these metrics

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provide a comprehensive view of genetic divergence, highlighting absolute differences, relative similarity, and shared sequence content.

In the nucleotide differences matrix (Figure. 5), many Asian isolates (China, Korea, Japan) differed by as few as 1–5 nucleotides across ~560 bp, corresponding to >99% pairwise identity in the % identity matrix (Figure. 6). For example, Japanese isolates KX589248 and EU021456 were the most closely related, showing 100% identity with zero nucleotide difference. Similarly, Japanese isolate EU02145 paired with Japanese isolates EU021456 and KX589248 at 99.9% identity (one difference) and clustered with Japanese isolate AB733174 and Korean isolates JQ520169, JQ520174, and JQ520188 at 99.7% identity (two differences). By contrast, the Nigerian isolate MZ014900 was the most divergent, differing from all other isolates by ~250–310 sites and sharing only 53–57% pairwise identity.

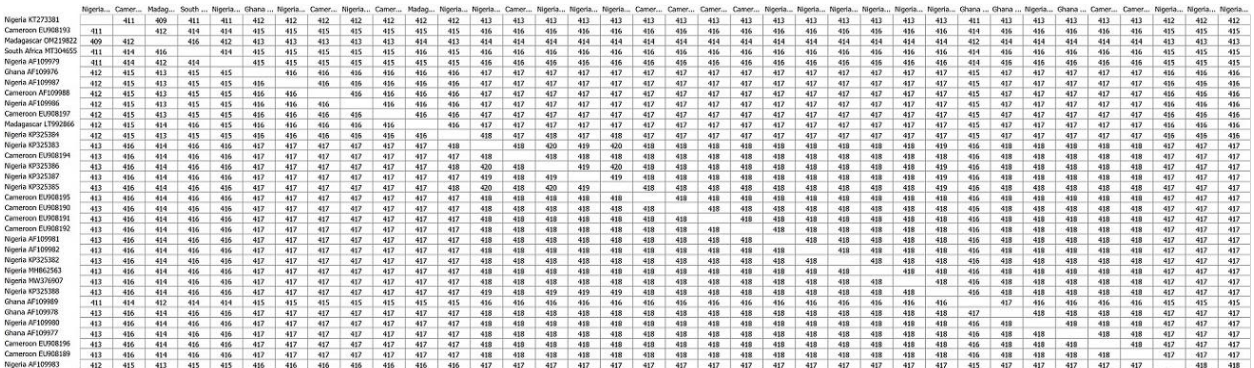

Figure: 5 Nucleotide Differences

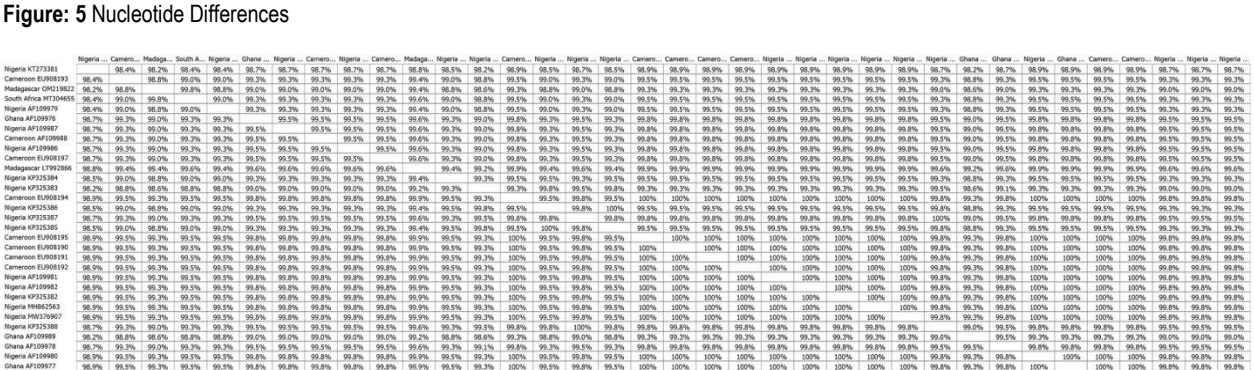

Figure: 6 Percentage Pairwise

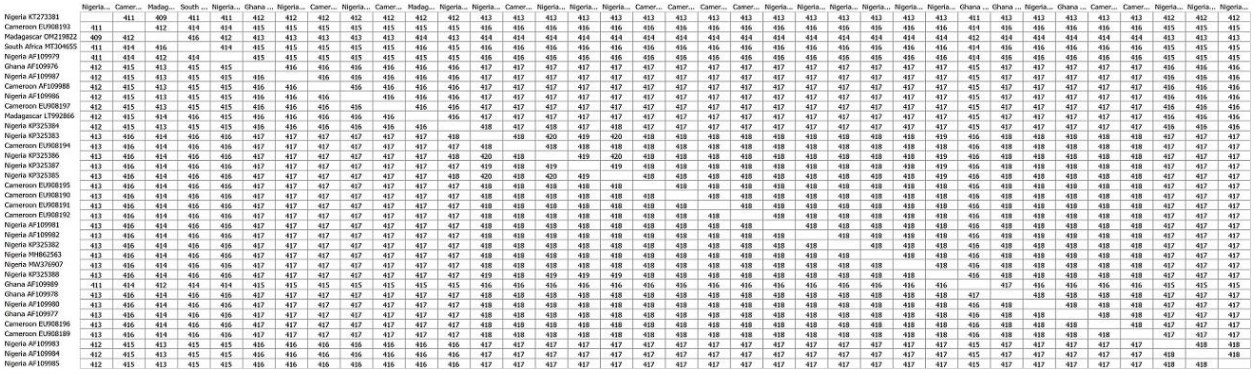

Figure: 7 Nucleotide Identity

DISCUSSION

The conserved blocks in the alignment results confirm the ITS region's utility as a reliable marker for fungal phylogenetics (Xu, 2016). Although less than half (44.7%) of the sites were fully conserved across all 27 isolates, the overall pairwise identity among all sequences remained high at 92.8%, indicating that while not all nucleotides are conserved in every sequence, the majority of the sequences exhibit significant similarity. The pronounced

divergence observed in isolates like MZ014900 suggests the presence of novel genetic and metabolic adaptations that could hold significant relevance for drug discovery efforts (Oyetayo and Yao 2010).

Furthermore, the clustering of Nigerian isolates MZ014900, PV444608, and ON394695, which exhibited distinct indel patterns in the multiple sequence alignments support by bootstrap ranging from 57% to 99%, reinforces their evolutionary distinctiveness. Given that genetic diversity often correlates with variations in secondary metabolite pathways (Wadhwa *et al.,* 2024), the observed genetic distinctiveness in these isolates suggests they may harbour unique bioactive compounds not found in their Asian counterparts. This makes them ideal targets for bio-prospecting. Additionally, clustering of Nigerian isolates OQ883913 and OQ883914 with the Chinese isolate JX162769, supported by high bootstrap values (99% and 75%), suggests possible genetic similarity and shared evolutionary history. They may also share bioactivities, making them useful for replication studies.

The near-identical sequences of many Asian isolates in the metrics reflect strong evolutionary conservation within the regional populations and explain their close clustering in the phylogenetic tree. In contrast, the high nucleotide difference and low pairwise identity of MZ014900 accounts for its distinct branch placement in the phylogenetic tree, suggesting that it may represent a cryptic species, a misidentified isolate, or a highly divergent lineage within the *Ganoderma* complex. Although ITS-based divergence alone cannot confirm functional differences, such genetic separation may indicate underlying variation that could influence secondary metabolism and, consequently, alter the bioactive compound profile.

Conclusion

This study revealed that Nigerian *G. lucidum* isolates share evolutionary relationships with Asian counterparts while also exhibiting unique diversification. Some Nigerian strains clustered closely with Asian lineages, suggesting conserved genetic traits, whereas others formed distinct clades that may represent novel evolutionary trajectories. These findings highlight the importance of Nigerian isolates as both reservoirs of conserved pharmacological potential and as candidates for metabolomic and chemical profiling to uncover novel medicinal metabolites.

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