Review

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Review

The Medicinal Mushroom *Ganoderma*: A Review of Systematics, Phylogeny, and Metabolomic Insights

Gideon Adotey [1,*], Abraham Quarcoo [1], Mohammed Ahmed Gedel [1], Paul Yerenkyi [1], Phyllis Out [1], Abraham K. Anang [2], Laud K. N. Okine [3], Winfred S. K. Gbewonyo [3], John C. Holliday [4] and Vincent C. Lombardi [5,*]

[1] Science Laboratory Department, Accra Technical University, Accra P. O Box GP 561, Ghana
[2] Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana
[3] Department of Biochemistry, Cell and Molecular Biology (BCMB), University of Ghana
[4] Aloha Medicinals Inc., Carson City, Nevada, USA
[5] Department of Microbiology and Immunology, University of Nevada, Reno, School of Medicine, Reno, NV 89523, USA
* Correspondence: gadotey@atu.edu.gh (G.A.); vlombardi@med.unr.edu (V.C.L.)

Abstract

Ganoderma is a genus of fungi that has been utilized in traditional Eastern medicine and has gained global interest in recent years. Current research has focused on the molecular underpinnings of *Ganoderma* taxonomy, phylogenetic diversity, and biochemical composition. In this review, we examine the current methods for the molecular identification and classification of the various *Ganoderma* species, with an emphasis on internal transcribed spacer (ITS) sequencing as well as other molecular barcoding techniques. These methods have improved species delineation, overcoming the limitations traditionally imposed by methods that are morphological in nature. This review also highlights advancements in metabolomics, especially in the identification and quantification of pharmacologically relevant compounds such as triterpenes and polysaccharides. Next, take a closer look at how tools like high-performance liquid chromatography (HPLC) and mass spectrometry (MS) are being used to profile analytes and support quality control efforts. To build a more holistic picture, we draw on insights from systematics, phylogenetics, and metabolomics, bringing together multiple disciplines to propose a more consistent approach for classifying and evaluating the pharmacological potential of *Ganoderma*. Notably, we highlight regions that have received less research attention, especially parts of Africa, where the full extent of species diversity is still largely unknown.

Keywords: *Ganoderma*; molecular taxonomy; phylogenetics; ITS sequencing; molecular barcoding; metabolomics; triterpenes; polysaccharides; pharmacological potential; reishi; lingzhi; Africa; species diversity; quality control

1. Introduction

Ganoderma is a genus of medicinal fungi historically used in traditional Eastern medicine, and is particularly common in China and Japan, where it is esteemed for its immunomodulatory, hepatoprotective, anticancer, and antiinflammatory properties [1,2]. Among its bioactive constituents, triterpenes, polysaccharides, glucans, and glycoproteins are consistently associated with these pharmacological effects [2,3]. Increasing global demand for complementary and alternative therapies has driven the incorporation of *Ganoderma*-derived compounds into nutraceuticals, functional foods, and pharmaceuticals [4].

Research on *Ganoderma* has significantly increased, over the last few decades, well beyond its therapeutic potential to encompass its genetic, taxonomic, and metabolic diversity. Accurate species identification and characterization have become critical to ensuring the consistency, efficacy, and safety of *Ganoderma*-based products. In the past, scientists classified species within the *Ganoderma*

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genus primarily by looking at physical traits, like the size of the basidiospores, or the shape and structure of the cap (pileus), as well as the texture of the spore walls. However, these features can vary significantly depending on factors such as the specimen's age, the type of substrate it grows on, and the surrounding environmental conditions. Because of this variability, relying solely on morphology has proven problematic when trying to accurately distinguish between species [5,6]. Consequently, ambiguity and misclassification have hindered *Ganoderma taxonomy*.

The emergence of molecular phylogenetics has significantly transformed fungal taxonomy, allowing for more accurate species identification through the use of genetic barcoding. The internal transcribed spacer (ITS) region of ribosomal RNA has become a widely recognized barcode for fungi identification because of its high variability between species and its ease of amplification [7]. In addition to ITS, other genetic markers, such as the nuclear large subunit (nLSU), small subunit (nSSU), translation elongation factor 1-alpha (EF1-α), and RNA polymerase II subunit (RPB2), have further improved phylogenetic resolution and identification due to their high interspecific variability and ease of amplification [8]. Additional genetic markers, including the nLSU, nSSU, EF1-α, and RPB2, have further enhanced phylogenetic resolution [9].

In parallel, metabolomics has become essential for elucidating the biochemical composition of *Ganoderma*. Analytical platforms such as high-performance liquid chromatography (HPLC), mass spectrometry (MS), and nuclear magnetic resonance (NMR) spectroscopy are commonly employed to identify and quantify key secondary metabolites, including ganoderic acids and β-glucans [10]. These compounds play a central role in the pharmacological activity of *Ganoderma*, contributing to its immunostimulatory, antioxidant, and anticancer effects [11,12]. Comprehensive metabolite profiling is therefore crucial for product standardization, quality assurance, and validation of therapeutic efficacy (Figure 1).

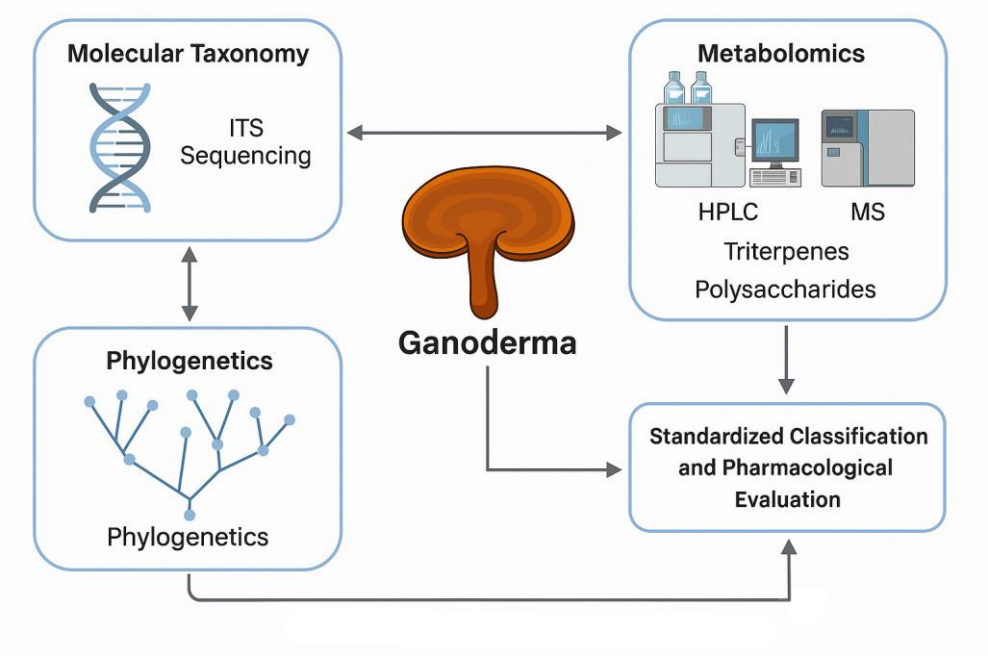

Figure 1. Integrated framework for the molecular taxonomy, phylogenetics, and metabolomic profiling of *Ganoderma* for standardized classification and pharmacological evaluation.

While recent progress has expanded our understanding of *Ganoderma*, notable gaps still exist, especially in underexplored areas like sub-Saharan Africa. While several species from this region have been described, studies into the molecular or metabolomic profiles are limited [12]. Moreover, this geographic underrepresentation has hindered global efforts to catalog biodiversity and develop regionally tailored therapeutics.

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This review provides an integrative synthesis of the state-of-the-art on *Ganoderma* systematics, phylogenetics, and metabolomics with an emphasis on the application of molecular identification techniques. Specifically, we explore the phylogenetic relationships within the genus and highlight advances in metabolite profiling using modern analytical tools. Methodological limitations, regional research gaps, and future priorities are also discussed, with particular focus on Africa, where emerging studies offer promising new directions.

2. Methodology

We used a systematic approach to identify, select and integrate peer-reviewed literature as it pertains to systematics, phylogenetics and metabolomics of *Ganoderma* species. In this review, we endeavored to ensure that a comprehensive coverage of both foundational studies and recent advancements across these thematic domains was achieved.

2.1. Data Sources and Search Strategy

Literature searches were conducted using five major academic databases: PubMed, Web of Science, Science Direct, Springer Link, and Google Scholar. These platforms were selected based on their broad coverage of molecular biology, pharmacognosy, mycology, and analytical sciences. PubMed was primarily utilized to retrieve studies on pharmacological and biomedical aspects, while Web of Science and ScienceDirect were targeted for literature on fungal taxonomy, molecular identification, and phylogenetic techniques. SpringerLink and Google Scholar were used to supplement the primary search with additional articles, reviews, and relevant book chapters.

The search was restricted to publications from 1990 to 2025, and only literature published in English was considered. Key search terms included:

- "*Ganoderma* taxonomy"
- "*Ganoderma* phylogenetics"
- "Molecular identification of *Ganoderma*"
- "ITS region in *Ganoderma*"
- "*Ganoderma* metabolomics"
- "Triterpene analysis in *Ganoderma*"
- "Bioactive compounds in *Ganoderma*"
- "Molecular barcoding of *Ganoderma* species"

Boolean operators ("AND," "OR") and truncation symbols were employed to refine and expand search results. Reference lists of relevant articles were also reviewed to identify additional sources not captured during the initial database search.

2.2. Inclusion and Exclusion Criteria

Studies were included if they met one or more of the following criteria:

- Utilized molecular techniques for species identification (e.g., ITS, nLSU, EF1-α, RPB2)
- Conducted phylogenetic analyses based on DNA sequencing
- Performed metabolomic profiling using advanced analytical platforms (e.g., LC-MS,
- HPLC, NMR)
- Investigated the pharmacological relevance of Ganoderma bioactive compounds
- Contributed data from underexplored regions, particularly sub-Saharan Africa
- Studies were excluded if they:
- Relied exclusively on morphological taxonomy without molecular validation
- Focused solely on cultivation methods without molecular or biochemical analysis
- Were not peer-reviewed (e.g., theses, conference abstracts, editorials, or opinion pieces)

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2.3. Data Extraction and Synthesis

Data from the included studies were extracted and organized into three thematic categories:

1. Systematics and Molecular Identification
2. Phylogenetic Analysis
3. Metabolomic Profiling

Each study was evaluated for its methodological approach, molecular markers utilized, analytical techniques employed, taxonomic resolution achieved, and geographical coverage. A thematic analysis was conducted to identify prevailing trends, technical challenges, and gaps in the literature—particularly those concerning African *Ganoderma* species. Methodological strengths and limitations were critically assessed, and findings were synthesized to construct an integrated narrative that informs future research priorities in *Ganoderma* systematics and pharmacological evaluation.

3. Molecular Taxonomy and Systematics

Systematics of Ganoderma with Emphasis on Molecular Identification Methods

The genus *Ganoderma*, recognized for both its medicinal significance and ecological functions, has long presented taxonomic challenges. Early classification systems relied primarily on morphological traits, including basidiospore shape and size, pileus coloration, and spore ornamentation. However, these features exhibit considerable phenotypic plasticity and are influenced by environmental conditions, developmental stage, and substrate specificity, resulting in frequent misidentifications [6]. Even basidiospore morphology—once considered diagnostic—has shown limited interspecific variation and reduced discriminatory power [13]. As a result, the taxonomy of *Ganoderma* has been in a state of considerable ambiguity.

The advent of molecular systematics has substantially improved species delineation by providing objective, high-resolution tools that are less susceptible to environmental variability. Among these, the ITS region of the nuclear ribosomal RNA operon has become the standard barcode for fungal identification, including within *Ganoderma* [8,14]. ITS sequencing has facilitated the resolution of cryptic species, clarified evolutionary relationships, and enhanced the accuracy of species-level classification.

Multiple studies have supported the use of the ITS region in conjunction with other loci, such as the nLSU and nSSU rRNA genes, to improve phylogenetic resolution [15,16]. The high interspecific variability of the ITS region has made it especially valuable for distinguishing morphologically similar taxa [17]. However, ITS-based identification has limitations; in some species complexes, ITS sequences alone do not provide sufficient resolution, necessitating supplementary genetic markers [18].

The ITS2 subregion has gained attention as a potentially superior barcode due to its shorter sequence length, ease of amplification, and high discriminatory power [19]. Despite these advantages, its widespread adoption has been limited by inconsistent amplification success and the lack of universally effective primers [20]. While the full ITS region and ITS1 subregion may offer broader coverage, ITS2 remains a useful tool for fine-scale resolution among closely related species when the full-length ITS is not available.[21].

The need for molecular tools is especially pressing in biodiverse but undercharacterized regions such as sub-Saharan Africa. In many parts of the continent, *Ganoderma* species have been identified almost exclusively through macroscopic traits, with minimal molecular confirmation [22]. For example, taxonomic surveys in Ghana have largely relied on morphology alone, raising concerns about the reliability of these identifications [12]. Incorporating molecular barcoding and phylogenetic analysis, particularly polymerase chain reaction (PCR)-based techniques, markedly improve the accuracy of species identification and biodiversity assessments in these regions.

Beyond taxonomy, molecular identification methods have practical applications in agriculture and forestry. Several *Ganoderma* species are pathogenic to economically important crops such as

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cocoa, cashew, and coffee. Molecular diagnostics provide rapid, cost-effective, and accurate tools for early detection and species-level identification, which are essential for the timely management of plant disease outbreaks [13].

In summary, while traditional morphological approaches have laid the foundation for understanding *Ganoderma* diversity, they are increasingly being supplanted by molecular methods that offer greater precision, reproducibility, and scope. The combined use of ITS, ITS2, and additional loci constitutes a robust framework for resolving taxonomic ambiguities and uncovering cryptic species. As molecular technologies become more widely accessible, they are expected to play a pivotal role in advancing both fundamental mycological research and the applied sciences related to *Ganoderma*, particularly in regions where research remains limited but potential biodiversity is high.

4. Phylogenetic Advances in Ganoderma Research

4.1. Ganoderma Phylogenetics: Molecular Insights and Advancements

Over the past several decades, phylogenetic studies of *Ganoderma* have undergone a significant transformation, driven by advances in molecular biology. Traditional taxonomy, based on macroscopic and microscopic features, has proven inadequate for resolving closely related species within the genus. As a result, researchers have increasingly adopted DNA sequencing and molecular phylogenetic tools to clarify species boundaries and evolutionary relationships.

4.2. The Role of ITS and rRNA Genes in Ganoderma Phylogenetics

One of the earliest pivotal studies in *Ganoderma* phylogeny was conducted by Moncalvo et al. [22], who analyzed sequences from the ITS and the 25S rRNA gene. Their work demonstrated that the ITS region could effectively differentiate most species, though it was insufficient to resolve the *G. tsugae* complex. The D2 domain of the 25S rRNA gene proved useful at higher taxonomic levels, and its combination with ITS sequences supported the monophyly of the subgenus *Elfvingia*. This study laid the groundwork for multilocus approaches in *Ganoderma* systematics.

Subsequent research has confirmed that ITS sequencing remains a foundational tool for species delimitation, especially when combined with multiple loci. Zhang and colleagues conducted a multilocus phylogenetic analysis on 32 collections of the *G. lucidum* complex using ITS, tef1α, rpb1, and rpb2. The study identified three distinct genetic clades (A, B, and C) within the complex, none of which corresponded strictly to geographic origin [23]. These findings underscore the extensive taxonomic complexity in *Ganoderma*, revealing that morphological classification alone often fails to accurately distinguish genetically divergent taxa due to overlapping features. A complementary ITS-focused study further demonstrated that ITS1 provided better geographic clustering than ITS2 among *G. lucidum* strains from global origins [24]. However, ITS2 alone was insufficient for fine-scale resolution, highlighting ITS limitations in detecting closely related but distinct lineages.

4.3. Multilocus Phylogenetics and Species Delimitation

Although ITS remains a core barcode for fungal systematics, it does not always provide sufficient resolution for species delimitation in complex groups. Multilocus sequencing, typically combining ITS with loci such as translation elongation factor 1-alpha (EF1-α) and RNA polymerase II subunit (RPB2), has emerged as a more robust approach.

Xing et al. employed multilocus sequencing on specimens collected in South Africa and identified a distinct lineage within the *G. lucidum* complex, ultimately describing a new species, *G. aridicola* [25]. Similarly, He et al. used multilocus phylogenetics in Yunnan Province to describe two novel species, *G. dianzhongense* and *G. esculentum*, based on their genetic divergence from known taxa [26]. These examples demonstrate the power of multilocus strategies for uncovering cryptic diversity and refining species concepts.

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4.4. Phylogenetic Tools for Product Authentication

Molecular phylogenetics has also become essential for verifying the identity of commercial *Ganoderma* products. Gunnels et al. used ITS-based phylogenetic analysis to confirm the presence of *G. lingzhi* in dietary supplements, supporting the use of this marker for quality assurance in the nutraceutical sector [27]. Liao et al. further employed DNA barcoding to assess strain-level variation in cultivated *G. lucidum* from China and Europe [28]. Their findings revealed significant genetic divergence, suggesting that some commercial *G. lucidum* may consist of multiple cryptic species, a concern with implications for product efficacy and standardization.

4.5. Regional phylogenetic studies: expanding global taxonomy

Regional molecular studies continue to expand the known diversity of *Ganoderma*, particularly in previously understudied areas. Kinge and others employed ITS and mtSSU sequencing to characterize Cameroonian isolates, identifying eight phylogenetic species, three of which were assigned to named taxa (*G. ryvardense*, *G. cupreum* and *G. weberianum*) while the remaining five lineages did not match any described species [29]. In Egypt, El-Fallal et al. identified *G. resinaceum* and a distinct lineage referred to as *Ganoderma* sp. EGDA from fig and citrus trees using ITS and 5.8S rRNA data [30]. These studies emphasize the importance of applying molecular tools globally to revise classifications and uncover undocumented fungal diversity.

4.6. Recent Advances and the Reclassification of G. lingzhi

A recent revision of *Ganoderma* systematics by Du et al. resolved a long-standing nomenclatural ambiguity by demonstrating that *G. lingzhi* is a later synonym of *G. sichuanense* [31]. Their study, based on phylogenetic comparisons of type specimens, exemplifies the need for molecular verification in taxonomic revision. Sun et al. further employed a six-locus dataset, including ITS, nLSU, EF1-α, and RPB2, to clarify relationships within the *Ganodermataceae* [16]. Their work showed that multilocus data outperform ITS alone in resolving polytomies and identifying novel species, advocating for broader use of these techniques in future studies.

In summary, molecular phylogenetics has become central to *Ganoderma* research, informing taxonomy, product authentication, and biodiversity discovery. While ITS remains a foundational marker, multilocus sequencing offers superior resolution, particularly in species complexes and cryptic groups. Continued adoption of these tools is expected to drive a comprehensive reevaluation of *Ganoderma* taxonomy worldwide.

5. Metabolomic Profiling and Bioactive Compounds

5.1. Ganoderma Metabolomics: Analytical Approaches for Characterization and Quality Control

The growing global demand for *Ganoderma*-based products has spurred interest in profiling its bioactive constituents. Metabolomics, defined as the comprehensive analysis of metabolites within a biological system, has become a critical tool for elucidating the therapeutic potential and ensuring quality control of *Ganoderma* species. Advanced analytical platforms such as liquid chromatography–mass spectrometry (LC-MS), high-performance liquid chromatography (HPLC), and nuclear magnetic resonance (NMR) spectroscopy allow for the precise identification and quantification of secondary metabolites, particularly triterpenes and polysaccharides.

5.2. LC-MS-Based Triterpene Profiling

Triterpenes, especially ganoderic acids, are among the most pharmacologically active compounds in *Ganoderma*. In 2012 Qi et al. described an LC-MS method for the simultaneous detection of 14 ganoderic acids that utilized previously described analytes as standards [32]. This targeted profiling approach is valuable for quality control in commercial production, enabling verification of triterpene composition in raw materials, extracts, and finished products. However, its focus on known

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compounds highlights the need for untargeted metabolomics capable of detecting novel triterpenoids. Adotey et al. applied LC-MS and multivariate statistical analysis to distinguish *Ganoderma* species from Ghana's Lower Volta River region [33]. Partial least squares–discriminant analysis (PLS-DA) of total ion chromatograms (TICs) separated the samples into three species: *G. enigmaticum*, *G. resinaceum*, and *G. weberianum–sichuanense*. Heatmap visualization further revealed interspecific differences in metabolite composition, suggesting potential variation in bioactivity. This study illustrates the dual utility of metabolomics for both taxonomic resolution and therapeutic evaluation.

The metabolite profile of *Ganoderma* changes significantly with developmental stage. A recent metabolomics–proteomics study of *G. lucidum* demonstrated that triterpenoid content, including ganoderenic acids E, H, and I, is highest at the budding stage, followed by a decline through maturation, implying that harvest timing critically influences therapeutic compound yield [34]. Earlier work by Chen & Chen (2003) further characterized multiple ganoderic acids (A, C, D, E, G) and ganoderenic acid D in *G. tsugae* fruiting bodies using HPLC and NMR, reinforcing the link between developmental stage and triterpenoid accumulation [35].

Zhang et al. employed ultra-high performance liquid chromatography coupled with Orbitrap high-resolution mass spectrometry (UPLC–Orbitrap–HRMS) to comprehensively profile metabolites in *G. lucidum* [36]. Their analysis identified 95 compounds, including ganoderic acids (e.g., A, B, C2, D, H, Y), ganoderenic acids (e.g., A, D, G), and additional bioactives such as kaempferol, genistein, and ergosterol. These compounds have been associated with a range of pharmacological activities, including antioxidant, antiinflammatory, and anticancer properties. This integrative approach supports structure–activity relationship studies and the development of standardized therapeutic formulations.

Expanding the geographic scope of metabolomics, Wongkhieo et al. analyzed mycelial extracts of wild *G. australe* collected in Thailand using LC-MS/MS [37]. Their chemical profiling revealed the presence of lovastatin plus tentative identification of p-coumaric acid, nicotinamide, γ-aminobutyric acid (GABA), choline, nucleosides, amino acids, and saccharides. The detection of lovastatin, a known cholesterol-lowering compound, highlights the pharmaceutical and functional food potential of this underexplored *Ganoderma* species.

In a follow-up study, Adotey et al. examined *Ganoderma* mycelial cultures from Ghana and identified four lanostanoid triterpenes, ganoderenic acid A, ganoderenic acid D, ganoderic acid C6, and ganoderic acid G, through LC-MS-based dereplication [33]. Two additional compounds, ganoderic acid AM1 and ganoderenic acid K, were tentatively identified based on retention time and MS fragmentation. These findings suggest that local *Ganoderma* strains may possess distinct metabolic signatures, offering opportunities for region-specific product development.

Indeed, metabolomic profiling has become integral to the chemical characterization and quality assurance of *Ganoderma* products. Techniques such as LC-MS and NMR not only facilitate the quantification of known compounds but also support the discovery of novel metabolites. When integrated with taxonomic and ecological data, metabolomics enhances product standardization, species authentication, and pharmacological validation. Future efforts should prioritize untargeted analyses and expanded geographic sampling to fully explore the therapeutic potential of this medicinal genus.

6. Integrative Discussion and Regional Perspectives

The integration of molecular systematics, phylogenetics, and metabolomics has significantly advanced the study of *Ganoderma*, enabling more accurate species delimitation, discovery of cryptic taxa, and detailed profiling of pharmacologically relevant metabolites. While morphology-based taxonomy laid the groundwork, it cannot meet the demands of taxonomic resolution, pharmaceutical validation, or product authentication. Molecular tools, especially ITS barcoding [8] and robust multilocus analysis using EF1-α and RPB2 alongside ITS and nLSU [13] provide essential accuracy for species-level identification.

ITS has been widely adopted as the primary fungal barcode due to its high interspecific variability, amplification success, and broad representation in public databases [8]. However, ITS

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alone often fails to resolve closely related *Ganoderma* species; supplementary markers such as EF1-α, RPB2, and nLSU significantly improve phylogenetic resolution and reduce misidentification [13]. Multilocus approaches have revealed cryptic species and enabled taxonomic revision, with implications for conservation, biotechnology, and pharmacognosy.

Phylogenetic analyses have revealed geographically structured clades within *Ganoderma*, underscoring the importance of regional molecular studies for mapping global diversity [13]. Yet, substantial taxonomic uncertainty remains in Africa, where many species, such as *G. resinaceum* and *G. weberianum* are still identified solely by morphological features. Expanding the application of molecular barcoding and multilocus phylogenetics in African contexts will enhance our understanding of *Ganoderma* evolution and distribution while supporting the sustainable development of endemic fungal resources.

Concurrently, metabolomics has emerged as a powerful tool for characterizing *Ganoderma* bioactive molecules. Triterpenes, polysaccharides, nucleosides, and other secondary metabolites, linked to immunomodulatory, antiinflammatory, and anticancer effects, have been comprehensively profiled using LC-MS, LC-MS/MS, and NMR in multiple species [33,37,38]. This biochemical diversity reinforces the therapeutic and commercial potential of *Ganoderma*, especially strains from underexplored regions.

Metabolite profiles vary with species, developmental stage, and cultivation conditions. For example, triterpenoid accumulation peaks during the primordia or budding stage and declines in mature fruiting bodies [34]. Regional metabolomics research, such as that conducted in Ghana, revealed distinct interspecific profiles among local strains, suggesting geography-dependent pharmacological variation[33].

Despite these advances, several critical challenges remain. First, the limited availability of complete genome sequences and curated metabolomic datasets for many *Ganoderma* species hampers the development of integrative identification frameworks. Second, the synergistic or antagonistic interactions among multiple bioactive compounds in crude extracts are not well understood. Although individual metabolites have demonstrated therapeutic potential, their combined effects on efficacy and toxicity require systematic study using integrated "omics" platforms and functional assays.

Third, variation in cultivation, extraction, and analytical protocols continues to impede product standardization. Environmental factors such as substrate composition, humidity, and light exposure can substantially influence metabolite production. Future work should prioritize the optimization and harmonization of these variables to ensure reproducibility and commercial quality control.

Remaining challenges include limited available genomes and curated metabolite datasets, uncharted synergy among bioactive compounds in crude extracts, and inconsistency in cultivation and analytical protocols affecting reproducibility and quality control. In particular, sub-Saharan African *Ganoderma* research is often descriptive and lacks molecular or chemical validation. Coordinated genomics and metabolomics efforts in Africa could uncover novel bioactive molecules and foster medicinal innovation. Long-term investment and international collaborative networks are essential to build research capacity.

In this review, we have endeavored to consolidate current advances in *Ganoderma* systematics, phylogenetics, and metabolomics, while identifying key priorities for future research. A unified, multidisciplinary framework that integrates molecular identification, phylogenetic analysis, and metabolite profiling is essential for unlocking the full therapeutic and commercial potential of this medicinally important genus, particularly in biogeographic regions that remain underexplored.

7. Conclusions and Future Directions

The integration of molecular identification, phylogenetic analysis, and metabolomic profiling has significantly advanced the study of *Ganoderma*, transitioning it from a genus primarily defined by morphology to one characterized by genomic and biochemical precision. Advances in DNA barcoding, particularly ITS and multilocus sequencing have enabled more accurate species delimitation and revealed

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substantial cryptic diversity. Concurrently, metabolomic analyses have provided detailed insights into the chemical complexity that underlies *Ganoderma*'s pharmacological properties.

In this review, we have attempted to synthesize these interdisciplinary developments and proposes a unified framework for taxonomic classification, biochemical characterization, and quality control of *Ganoderma* species. It underscores the essential role of molecular tools in overcoming the limitations of traditional taxonomy and highlights the utility of metabolomics in supporting both pharmacological validation and commercial standardization.

Despite substantial progress, key challenges remain, particularly in underexplored regions such as sub-Saharan Africa, where species diversity is insufficiently documented and largely unsupported by molecular or metabolomic data. Future efforts should prioritize the comprehensive genomic and metabolomic profiling of regional *Ganoderma* strains, the implementation of standardized analytical workflows, and the investigation of compound interactions that contribute to bioactivity.

Advancing these priorities through integrated, multidisciplinary research will facilitate a more complete understanding of *Ganoderma* as a medicinal resource, supporting biodiversity conservation, scientific innovation, and the development of evidence-based therapeutic applications.

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Conflicts of Interest: John C. Holliday, is the director of research and chief scientific officer of Aloha Medicinals, which produces and markets various *Ganoderma* products. The authors declare no other conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Abbreviations

The following abbreviations are used in this manuscript:

DNA – Deoxyribonucleic Acid
EF1-α – Translation Elongation Factor 1-Alpha
HPLC – High-Performance Liquid Chromatography
HRMS – High-Resolution Mass Spectrometry
ITS – Internal Transcribed Spacer
ITS1 – Internal Transcribed Spacer 1 subregion
ITS2 – Internal Transcribed Spacer 2 subregion
LC-MS – Liquid Chromatography–Mass Spectrometry
LC-MS/MS – Liquid Chromatography–Tandem Mass Spectrometry
NMR – Nuclear Magnetic Resonance

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NMIMR – Noguchi Memorial Institute for Medical Research

nLSU – Nuclear Large Subunit ribosomal RNA gene

nSSU – Nuclear Small Subunit ribosomal RNA gene

PCR – Polymerase Chain Reaction

PLS-DA – Partial Least Squares–Discriminant Analysis

rDNA – Ribosomal Deoxyribonucleic Acid

rRNA – Ribosomal Ribonucleic Acid

RPB1 – RNA Polymerase II Subunit 1

RPB2 – RNA Polymerase II Subunit 2

TIC – Total Ion Chromatogram

UPLC – Ultra-Performance Liquid Chromatography

UPLC–Orbitrap–HRMS – Ultra-Performance Liquid Chromatography–Orbitrap High-Resolution Mass Spectrometry

References

1. Andrejc, D.C.; Knez, Z.; Marevci, M.K. Antioxidant, antibacterial, antitumor, antifungal, antiviral, anti-inflammatory, and nevro-protective activity of *Ganoderma* lucidum: An overview. Front Pharmacol 2022, 13, doi:ARTN 934982 10.3389/fphar.2022.934982.
2. Wang, L.; Li, J.Q.; Zhang, J.; Li, Z.M.; Liu, H.G.; Wang, Y.Z. Traditional uses, chemical components and pharmacological activities of the genus P. Karst.: a review. Rsc Adv 2020, 10, 42084-42097, doi:10.1039/d0ra07219b.
3. Tsai, C.C.; Yang, F.L.; Huang, Z.Y.; Chen, C.S.; Yang, Y.L.; Hua, K.F.; Li, J.; Chen, S.T.; Wu, S.H. Oligosaccharide and peptidoglycan of *Ganoderma* lucidum activate the immune response in human mononuclear cells. J Agric Food Chem 2012, 60, 2830-2837, doi:10.1021/jf3000339.
4. El Sheikha, A.F. Nutritional Profile and Health Benefits of *Ganoderma* lucidum "Lingzhi, Reishi, or Mannentake" as Functional Foods: Current Scenario and Future Perspectives. Foods 2022, 11, doi:10.3390/foods11071030.
5. He, J.; Han, X.; Luo, Z.L.; Li, E.X.; Tang, S.M.; Luo, H.M.; Niu, K.Y.; Su, X.J.; Li, S.H. Species diversity of *Ganoderma* (*Ganoderma*taceae, Polyporales) with three new species and a key to *Ganoderma* in Yunnan Province, China. Front Microbiol 2022, 13, 1035434, doi:10.3389/fmicb.2022.1035434.
6. Mardones, M.; Carranza-Velazquez, J.; Mata-Hidalgo, M.; Amador-Fernandez, X.; Urbina, H. ï»¿Taxonomy and phylogeny of the genus *Ganoderma* (Polyporales, Basidiomycota) in Costa Rica. MycoKeys 2023, 100, 5-47, doi:10.3897/mycokeys.100.106810.
7. Shi, Y.; Cheng, Y.; Wang, Y.; Zhang, G.; Gao, R.; Xiang, C.; Feng, J.; Lou, D.; Liu, Y. Investigation of the fungal community structures of imported wheat using high-throughput sequencing technology. PLoS One 2017, 12, e0171894, doi:10.1371/journal.pone.0171894.
8. He, J., Luo, Z.-L., Tang, S.-M., Li, Y.-J., Li, S.-H., & Su, H.-Y. (2021). Phylogenetic analyses and morphological characters reveal two new species of *Ganoderma* from Yunnan province, China. MycoKeys, 84, 141–162. https://doi.org/10.3897/mycokeys.84.69449
9. Yang, H.D.; Ding, Y.; Wen, T.C.; Hapuarachchi, K.K.; Wei, D.P. *Ganoderma*ovisporum sp. nov. (Polyporales, Polyporaceae) from Southwest China. Biodivers Data J 2022, 10, e80034, doi:10.3897/BDJ.10.e80034.
10. Liu, C., Chen, F., Fan, X., Liu, B., Chai, X., He, S., Huang, T., Wang, X., Liu, L., Liu, H., Zeng, D., Jiang, B., Zhang, X., & Liu, M. (2024). Combined NMR and MS-based metabonomics and real-time PCR analyses reveal dynamic metabolic changes of *Ganoderma* lucidum during fruiting body growing. Food research international (Ottawa, Ont.), 180, 114056. https://doi.org/10.1016/j.foodres.2024.114056
11. Liu, Y., Ren, S., Sang, Q., Cheng, X., & Bi, Y. (2025). Potential Active Compounds of *Ganoderma* lucidum and Their Anticancer Effects: A Comprehensive Review. Food science & nutrition, 13(8), e70741. https://doi.org/10.1002/fsn3.70741

12. Obodai, M.; Mensah, D.L.; Fernandes, A.; Kortei, N.K.; Dzomeku, M.; Teegarden, M.; Schwartz, S.J.; Barros, L.; Prempeh, J.; Takli, R.K.; et al. Chemical Characterization and Antioxidant Potential of Wild *Ganoderma* Species from Ghana. Molecules 2017, 22, doi:10.3390/molecules22020196.
13. Jargalmaa, S.; Eimes, J.A.; Park, M.S.; Park, J.Y.; Oh, S.Y.; Lim, Y.W. Taxonomic evaluation of selected *Ganoderma* species and database sequence validation. PeerJ 2017, 5, e3596, doi:10.7717/peerj.3596.
14. Raja, H.A.; Miller, A.N.; Pearce, C.J.; Oberlies, N.H. Fungal Identification Using Molecular Tools: A Primer for the Natural Products Research Community. J Nat Prod 2017, 80, 756-770, doi:10.1021/acs.jnatprod.6b01085.
15. Adotey, G., Alolga, R. N., Quarcoo, A., Yerenkyi, P., Otu, P., Anang, A. K., Okine, L. K. N., Gbewonyo, W. S. K., Holliday, J. C., & Lombardi, V. C. (2023). Molecular Identification and Characterization of Five *Ganoderma* Species from the Lower Volta River Basin of Ghana Based on Nuclear Ribosomal DNA (nrDNA) Sequences. Journal of fungi (Basel, Switzerland), 10(1), 6. https://doi.org/10.3390/jof10010006
16. Sun, Y.F.; Xing, J.H.; He, X.L.; Wu, D.M.; Song, C.G.; Liu, S.; Vlasak, J.; Gates, G.; Gibertoni, T.B.; Cui, B.K. Species diversity, systematic revision and molecular phylogeny of *Ganoderma*taceae (Polyporales, Basidiomycota) with an emphasis on Chinese collections. Stud Mycol 2022, 101, 287-415, doi:10.3114/sim.2022.101.05.
17. Gunnels, T., Creswell, M., McFerrin, J., & Whittall, J. B. (2020). The ITS region provides a reliable DNA barcode for identifying reishi/lingzhi (*Ganoderma*) from herbal supplements. PloS one, 15(11), e0236774. https://doi.org/10.1371/journal.pone.0236774
18. Gazis, R., Rehner, S., & Chaverri, P. (2011). Species delimitation in fungal endophyte diversity studies and its implications in ecological and biogeographic inferences. Molecular ecology, 20(14), 3001–3013. https://doi.org/10.1111/j.1365-294X.2011.05110.x
19. Han, J.; Zhu, Y.; Chen, X.; Liao, B.; Yao, H.; Song, J.; Chen, S.; Meng, F. The short ITS2 sequence serves as an efficient taxonomic sequence tag in comparison with the full-length ITS. Biomed Res Int 2013, 2013, 741476, doi:10.1155/2013/741476.
20. Wang, X.C.; Liu, C.; Huang, L.; Bengtsson-Palme, J.; Chen, H.; Zhang, J.H.; Cai, D.; Li, J.Q. ITS1: a DNA barcode better than ITS2 in eukaryotes? Mol Ecol Resour 2015, 15, 573-586, doi:10.1111/1755-0998.12325.
21. Yang, R.H.; Su, J.H.; Shang, J.J.; Wu, Y.Y.; Li, Y.; Bao, D.P.; Yao, Y.J. Evaluation of the ribosomal DNA internal transcribed spacer (ITS), specifically ITS1 and ITS2, for the analysis of fungal diversity by deep sequencing. PLoS One 2018, 13, e0206428, doi:10.1371/journal.pone.0206428.
22. Moncalvo, J.M.; Wang, H.H.; Hseu, R.S. Phylogenetic-Relationships in *Ganoderma* Inferred from the Internal Transcribed Spacers and 25s Ribosomal DNA-Sequences. Mycologia 1995, 87, 223-238, doi:Doi 10.2307/3760908.
23. Zhou, L.W.; Cao, Y.; Wu, S.H.; Vlasak, J.; Li, D.W.; Li, M.J.; Dai, Y.C. Global diversity of the *Ganoderma* lucidum complex (*Ganoderma*taceae, Polyporales) inferred from morphology and multilocus phylogeny. Phytochemistry 2015, 114, 7-15, doi:10.1016/j.phytochem.2014.09.023.
24. Zhang, X.; Xu, Z.; Pei, H.; Chen, Z.; Tan, X.; Hu, J.; Yang, B.; Sun, J. Intraspecific Variation and Phylogenetic Relationships Are Revealed by ITS1 Secondary Structure Analysis and Single-Nucleotide Polymorphism in *Ganoderma* lucidum. PLoS One 2017, 12, e0169042, doi:10.1371/journal.pone.0169042.
25. Xing, J.H.; Song, J.; DeCock, C.; Cui, B.K. Morphological characters and phylogenetic analysis reveal a new. Phytotaxa 2016, 266, doi:10.11646/phytotaxa.266.2.5
26. He, J.; Luo, Z.L.; Tang, S.M.; Li, Y.J.; Li, S.H.; Su, H.Y. ï»¿Phylogenetic analyses and morphological characters reveal two new species of *Ganoderma* from Yunnan province, China. MycoKeys 2021, 84, 141-162, doi:10.3897/mycokeys.84.69449.
27. Gunnels, T.; Creswell, M.; McFerrin, J.; Whittall, J.B. The ITS region provides a reliable DNA barcode for identifying reishi/lingzhi (*Ganoderma*) from herbal supplements. PLoS One 2020, 15, e0236774, doi:10.1371/journal.pone.0236774.
28. Liao, B.; Chen, X.; Han, J.; Dan, Y.; Wang, L.; Jiao, W.; Song, J.; Chen, S. Identification of commercial *Ganoderma* (Lingzhi) species by ITS2 sequences. Chin Med 2015, 10, 22, doi:10.1186/s13020-015-0056-7.

 doi:10.20944/preprints202510.2225.v1

29. Kinge, T.R.; Mih, A.M.; Coetzee, M.P.A. Phylogenetic relationships among species of *Ganoderma* (*Ganoderma*taceae, Basidiomycota) from Cameroon. Australian Journal of Botany 2012, 60, 526-538, doi:https://doi.org/10.1071/BT12011.
30. El-Fallal, A.A.; El-Sayed, A.K.A.; El-Esseily, S.R. First record of two species from North East Nile Delta-Egypt. Mycosphere 2015, 6, 248-259, doi:10.5943/mycosphere/6/3/2.
31. Du, Z.; Li, Y.; Wang, X.C.; Wang, K.; Yao, Y.J. Re-Examination of the Holotype of *Ganoderma* sichuanense (*Ganoderma*taceae, Polyporales) and a Clarification of the Identity of Chinese Cultivated Lingzhi. J Fungi (Basel) 2023, 9, doi:10.3390/jof9030323.
32. Qi, Y.; Zhao, L.; Sun, H.H. Development of a rapid and confirmatory method to identify ganoderic acids in *Ganoderma* mushrooms. Front Pharmacol 2012, 3, 85, doi:10.3389/fphar.2012.00085.
33. Adotey, G.; Alolga, R.N.; Quarcoo, A.; Gedel, M.A.; Anang, A.K.; Holliday, J.C. Ultra Performance Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (UPLC-Q-TOF-MS)-based metabolomic analysis of mycelial biomass of three *Ganoderma* isolates from the Lower Volta River Basin of Ghana. J Pharm Biomed Anal 2021, 205, 114355, doi:10.1016/j.jpba.2021.114355.
34. Gao, X.; Huo, H.; Bao, H.; Wang, J.; Gao, D. Changes of Active Substances in *Ganoderma* lucidum during Different Growth Periods and Analysis of Their Molecular Mechanism. Molecules 2024, 29, doi:10.3390/molecules29112591.
35. Chen, D.H.; Chen, W.K.D. Determination of ganoderic acids in triterpenoid constituents of. J Food Drug Anal 2003, 11, 195-200.
36. Zhang, J.; Li, S.Y.; Luo, W.H.; Wang, J.; Beng, S.J.; Han, H.L.; Xiong, Q.P.; Peng, D.Y.; Peng, C. [Chemical composition analysis of *Ganoderma* lucidum based on UPLC-Orbitrap-HRMS and virtual screening of FXR activators for treatment of liver fibrosis]. Zhongguo Zhong Yao Za Zhi 2024, 49, 3804-3817, doi:10.19540/j.cnki.cjcmm.20240405.205.
37. Wongkhieo, S.; Tangmesupphaisan, W.; Siriwaseree, J.; Aramsirirujiwet, Y.; Wiriyajitsomboon, P.; Kaewgrajang, T.; Pumloifa, S.; Paemanee, A.; Kuaprasert, B.; Choowongkomon, K.; et al. In vitro cholesterol lowering activity of *Ganoderma* australe mycelia based on mass spectrometry, synchrotron Fourier-transform infrared analysis and liver-spheroid bioactivity. Sci Rep 2023, 13, 13619, doi:10.1038/s41598-023-40861-8.
38. Xu, X.; Li, C.; Wu, F.; Zhao, S.; Chen, T.; You, H.; Lin, Y.; Zou, X. Integrated Transcriptomic and Targeted Metabolomic Analysis Reveals the Key Genes Involved in Triterpenoid Biosynthesis of *Ganoderma* lucidum. J Fungi (Basel) 2025, 11, doi:10.3390/jof11010057.