



Diversity and characterisation of *Ganoderma* species from the northeastern hills of India

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Abstract

The present investigation was undertaken to characterise *Ganoderma* species collected from Longleng district of Nagaland, India, using morphological, biochemical and molecular approaches. Four sporocarp samples were collected from different host trees during the 2024 rainy season and examined for their macroscopic features such as pileus shape, size, margin, context and pore characteristics, as well as microscopic features including basidiospores and hyphal structures. Two isolates (GLN1 and GLN2) were successfully cultured on potato dextrose agar (PDA), where they displayed distinct colony growth patterns, pigmentation and radial expansion rates. Biochemical profiling revealed that GLN2 contained almost double the water-soluble polysaccharide content (14.37 mg g⁻¹ dry weight) compared with GLN1 (7.74 mg g⁻¹ dry weight), while insoluble polysaccharides ranged from 2.10–2.91 mg g⁻¹ dry weight. Antioxidant assays showed that polyphenol-rich extracts, particularly from isolate GLN2, exhibited stronger 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, reaching approximately 60 % inhibition at 0.5 mg mL⁻¹ (EC₅₀ = 0.23 ± 0.05 mg mL⁻¹) compared with 36 % inhibition and a higher EC₅₀ (0.69 ± 0.02 mg mL⁻¹) for GLN1. Consistently, GLN2 also recorded higher total polyphenol content (27.9 mg g⁻¹ extract) than GLN1 (16.5 mg g⁻¹ extract), confirming its superior antioxidant potential. Molecular identification was carried out through ITS rDNA sequencing, with BLAST analysis showing 99-100 % similarity to *Ganoderma lucidum*. Phylogenetic analysis placed both isolates within the *G. lucidum* complex but in a distinct sub-clade, suggesting possible genetic divergence and local adaptation of Nagaland isolates. The combined morphological, biochemical and molecular data indicate that these isolates possess unique traits of pharmacological importance. The study enriches the understanding of *Ganoderma* diversity in Northeast India and underscores its potential for nutraceutical and community-based cultivation. Furthermore, the findings highlight the potential for developing community-based cultivation and commercialisation strategies that could benefit local farmers and promote sustainable utilisation of this medicinal mushroom.

Keywords: antioxidant activity; bioactive compounds; *Ganoderma lucidum*; ITS rDNA sequencing; medicinal mushroom; polysaccharides

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1. Introduction

Ganoderma is a group of macrofungi that usually grow on rotting wood. It is well known for causing plant diseases like white rot and stem rot. *Ganoderma* is found all over the world, but it does best in tropical and temperate climates in Africa, Asia, Europe and the Americas. There are more than 2000 known species of it. Karsten created the genus in 1881 for laccate and stipitate white rot fungi. *Ganoderma* mushrooms are not eaten because they

taste bad and feel tough and woody, but they are very useful for health, both in traditional and modern ways. *Ganoderma* has been used as medicine in East Asia for more than 2000 years (1). In China, it is called "Lingzhi," in Japan, "Reishi," and in Korea, "Youngzhi." Ancient Chinese art, such as painting, sculpture and jewellery, also showed how important it was to their culture, especially during the Yuan dynasty (2). China is the biggest producer of *Ganoderma* in the world, making up more than half of the total supply. More and more people

in India are interested in growing *Ganoderma lucidum* because there is a growing demand for Ayurvedic and herbal products. It is grown in Tamil Nadu, Kerala, Karnataka, Maharashtra, Himachal Pradesh and Uttarakhand, but not as much as it is grown around the world. Traditionally, *Ganoderma* species, especially *G. lucidum*, are grown on hardwoods like plum, mulberry, chestnut and Japanese apricot. The cap of the fruiting body is usually lacquered and glossy and shaped like a kidney or an oval. In the beginning, the cap is yellowish white or yellowish-brown, but as it grows, it turns reddish or purplish-brown. *Ganoderma lucidum* is valued for its many medicinal properties. It has β -3-glucan polysaccharides, which are strong substances that are thought to fight cancer. It also has antiviral, antibacterial, anti-inflammatory, antioxidant and immune-boosting effects. In Taoism, Lingzhi is thought to help people live longer and get closer to God (3).

There are more than 10000 scientific papers and more than 7000 related patents on *Ganoderma* right now. In China alone, more than 1000 health food products with *Ganoderma* are certified (4). Teas, soups, wines and yoghurts are some of these products. To make them work better, they are often mixed with other traditional herbs like ginseng (*Panax ginseng*) or *Panax notoginseng*. In 1997, the world made about 4300 Mt of *G. lucidum*, with China making about 3000 Mt of that.

Japan, Korea, Taiwan and other countries such as the USA, Vietnam and Sri Lanka contributed the rest. *Ganoderma*-based products, ranging from dietary supplements and herbal wines to cosmetics, are sold globally, some as prescription drugs in Asian markets and as health supplements elsewhere (5). Traditional *Ganoderma* cultivation involved inoculating full-length natural logs, often without sterilisation. Fruiting could take 6–24 months and continue for up to 5 years. In the late 1980s, however, new methods were created that use short logs (15 cm or less) and sawdust substrates. These methods are now common in China, Japan and Korea. To meet rising demand and keep things the same, people are also using mycelium- and broth-based production methods (6). *Ganoderma* cultivation is becoming more popular in India, especially in the Himalayas, where advanced methods like log culture, sawdust substrates and billet methods are used. The growing of this plant has a lot of economic potential because the dried fruiting bodies sell for Rs. 4000–5000 per kg (7).

In India, several research centres such as the National Research Centre for Mushrooms (Solun) and the Shri AMM Murugappa Chettiar Research Centre (Chennai) have optimised *G. lucidum* cultivation on diverse agro-wastes including banana leaves and sugarcane bagasse. *Ganoderma* species are chemically diverse, containing hundreds of bioactive compounds with nutritional and medicinal properties. Major components include polysaccharides, triterpenes and steroids, as well as alkaloids, fatty acids, peptides, phenols and vitamins

(8). These compounds contribute to its growing use as a functional food for preventing and managing immunological disorders (9).

The systematic cultivation and marketing of *G. lucidum*, a well-known medicinal mushroom with many health benefits and a high economic value, is a great way to help rural areas in India, especially by giving young people jobs and making them feel like they are part of the community. Mushroom farming has become very popular in the last few years all over the country. This includes both edible mushrooms and high-value medicinal mushrooms like *G. lucidum*, which are known as "Reishi" or "Lingzhi" in traditional medicine systems (10). The global demand for natural health products has skyrocketed because scientists have proven that mushrooms have pharmacological effects that boost the immune system, fight cancer, reduce inflammation and protect the liver. The northeastern state of Nagaland is a great place to grow *Ganoderma* because it has a subtropical to tropical climate, a lot of different types of plants and animals in its forests and communities that practise organic farming. The area's natural resources and ecological conditions make it a good place for the long-term growth and sale of medicinal mushrooms.

Nagaland also has a large rural youth population that, despite limited formal employment, is increasingly interested in value-added farming. With proper training, these youths can effectively engage in the cultivation, processing and marketing of *G. lucidum*, which has high market value, low input requirements and strong income-generation potential. However, scientific information on the local distribution, diversity and medicinal properties of native *Ganoderma* species in Nagaland remains limited. This study is based on the hypothesis that *G. lucidum* isolates from Longleng district possess distinct morphological, biochemical and genetic characteristics, along with strong antioxidant potential, making them suitable for nutraceutical use and community-based cultivation. Accordingly, the objectives were to: (i) collect *Ganoderma* fruiting bodies from different sites in Longleng; (ii) characterise the isolates morphologically, culturally and molecularly; (iii) assess their polysaccharide, polyphenol and antioxidant profiles; and (iv) determine their phylogenetic relationships to evaluate their potential for medicinal application and sustainable utilisation. This integrated approach enhances understanding of local *Ganoderma* diversity while emphasising their pharmacological and socio-economic importance.

2. Materials and Methods

Experimental site

Fruiting bodies of *Ganoderma* spp. were collected from various locations in Longleng District of Nagaland. The present investigation on the characterisation and biochemical analysis of the promising *Ganoderma* isolates under Nagaland conditions was conducted at the Centre of Tropical

Mushroom Research and Training (CTMRT), Department of Plant Pathology, College of Agriculture, Odisha University of Agriculture and Technology (OUAT), Bhubaneswar. The materials used and the procedures adopted during the study are detailed below.

Survey and collection of *Ganoderma* fruiting bodies

Extensive surveys were conducted in the forested regions of Longleng district, Nagaland, during the rainy season of 2024 to collect *Ganoderma* fruiting bodies. Sporocarps at different developmental stages were collected, labelled and documented with detailed field notes including date, location, host species and condition of the host tree. The specimens were wrapped in brown paper, transported to the laboratory and examined for morphological and structural characteristics.

Macromorphological features such as pileus shape, size, surface texture, colour and margin type were recorded using fresh samples. Microscopic observations were made on basidiospores, mycelial structure, clamp connections and chlamydospore formation. Morphometric parameters such as length, breadth and pore distribution were measured and representative specimens were preserved for further analysis.

Laboratory reagents and glassware

All glassware used during the investigation was of Borosil make. Culture tubes (25 × 150 mm), Petri plates (90 mm diameter), conical flasks (250 mL), beakers (500 mL) and measuring cylinders were cleaned thoroughly before use. A chromic acid cleaning solution was prepared by dissolving 80 g potassium dichromate ($K_2Cr_2O_7$) in 400 mL concentrated H_2SO_4 , diluted with 300 mL distilled water. Glassware was soaked in this solution for 4–6 hr, rinsed several times with distilled water and air-dried. Sterilisation was done in a hot air oven at 160–180 °C for 2 hr. Media and culture substrates were sterilised in an autoclave at 15 psi for 15 min. Polypropylene bags and bottles containing boiled wheat or paddy substrates for spawn production were autoclaved at 22 psi for 2 hr. Metal instruments such as inoculation loops, scalpels and forceps were flame-sterilised after dipping in 70 % ethanol.

Media preparation

Potato dextrose agar (PDA) medium (HiMedia Laboratories, Mumbai) was used for isolation and media preparation. Culturing was carried out as per the manufacturer's instructions. To prepare 1 L of PDA medium: 200 g peeled potato tubers were boiled in 500 mL distilled water, filtered and mixed with 500 mL of water containing 20 g agar and 20 g dextrose. The final volume was adjusted to 1000 mL. The medium was dispensed into flasks and tubes, plugged with cotton and sterilised at 121 °C (15 psi) for 15–20 min. Agar slants were formed by solidifying the medium in a slanted position (11, 12).

Isolation and pure culture of *Ganoderma*

Isolation was carried out using the tissue culture technique. Surface-sterilised fruiting bodies were cut into small ($\approx 3 \text{ mm}^2$) tissue bits and inoculated aseptically on PDA plates. The plates were incubated at 28 °C for 7 days. Actively growing mycelia were subcultured using the hyphal tip method to obtain pure isolates. Cultures were maintained on PDA slants and periodically subcultured at fortnightly intervals for long-term preservation (13).

Preparation of mycelial extracts

Aerial mycelia were collected, dried in an oven at 60 °C for 8 hr and ground in liquid nitrogen (N). Extraction was performed using 80 % ethanol (v/v) at 4 °C from 10 g dried biomass. The mixture was centrifuged at 1500 rpm for 15 min (three cycles). Supernatants were dried to obtain low-molecular-weight fractions. Intracellular polysaccharides were extracted from pellets by hot-water extraction at 100 °C for 3 hr, followed by centrifugation and ethanol precipitation (96 %). Crude polysaccharides were redissolved, filtered and purified chromatographically. Polysaccharide content was estimated by the anthrone method and total phenolics by the Folin–Ciocalteu assay (14, 15).

Polysaccharide fractionation

Ion-exchange chromatography of water-soluble polysaccharides used a gold high-performance liquid chromatography (HPLC) system (Beckman Coulter Inc., USA) with a Toyopearl DEAE 650S column (15 × 4.6 cm). Elution was performed isocratically using $NaHCO_3$ and NaOH gradients at 1 mL min^{-1} . Fractions containing polysaccharides were pooled, precipitated with ethanol and further purified using gel filtration chromatography with a TSKgel G3000SWXL column and distilled water (0.5 mL min^{-1}). Fractions were analysed using the anthrone method (16).

Assay for 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging

The DPPH radical scavenging assay was used to evaluate antioxidant activity. A 100 μM DPPH solution in ethanol (0.85 mL) was mixed with 0.15 mL of sample diluted in 50 mM Tris–HCl buffer (pH 7.4). After 10 min at room temperature, absorbance was measured at 517 nm. Scavenging activity (%) = $(A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100$. The EC_{50} value was derived from dose–response curves (17).

Molecular characterisation

To take out genomic DNA, we used a modified cetyltrimethylammonium bromide (CTAB) based method (18). After 3–5 days, the fungal mycelia grown in potato dextrose broth (PDB) were collected, filtered and freeze dried. 50 mg of mycelium was ground up in liquid N and put into 500 μL of extraction buffer. The mixture was kept at 60 °C for 20 min, then mixed with phenol-chloroform isoamyl alcohol (25 : 24 : 1) and spun at 10000 rpm for



10 min at 4 °C. We added RNase (10 mg/mL) to the aqueous phase and then used chloroform–isoamyl alcohol (24 : 1) to extract it. Isopropanol was used to precipitate the DNA; 70 % ethanol was used to wash it and it was then air-dried and resuspended in TE buffer (pH 8.0). A 1.5 % agarose gel was used to check the quality of the DNA. The ITS1 (5'-TCC GTA GGT GAA CCT GC GG-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') primers were used to amplify the ITS region (19). The polymerase chain reaction (PCR) reactions were done in 25 µL volumes with 50–100 ng of template DNA, 10 pmol of each primer, 2.5 mM MgCl₂, 200 µM dNTPs, 0.5 U Taq DNA polymerase and 1× buffer. The thermal cycling profile was: • Initial denaturation: 95 °C for 5 min • 39 cycles of:

- Denaturation: 95 °C for 30 sec
- Annealing: 36 °C for 45 sec
- Extension: 1 min at 72 °C
- Last extension: 72 °C for 8 min.

We used a gel documentation system to see the amplified products on a 1.5 % agarose gel (1× TAE buffer) and compared them to a 600 bp DNA ladder.

Sequencing and phylogenetic examination

We used ITS1 and ITS4 primers to sequence PCR amplicons at a commercial lab in India called Heredity Biosciences Labs. We used DNA Baser v4 to put the sequences together, CLUSTAL W (BioEdit) to line them up and BLASTn to compare them to GenBank sequences. We used MEGA v11 software to make phylogenetic trees using the neighbour-joining (NJ) method and 1000 bootstrap replications to find branch support (20, 21). We examined *Ganoderma* isolates and related reference sequences obtained from GenBank.

3. Results and Discussion

Survey and collection of *Ganoderma* fruiting bodies

Systematic surveys conducted during the 2024 rainy season in Longleng district, Nagaland, yielded four *Ganoderma* sporocarp samples from different locations and host substrates (Table 1). Fruiting bodies were collected from both dead and living trees, including *Peltophorum pterocarpum* and *Areca catechu*, indicating that the local *Ganoderma* population can colonise a range of woody hosts under humid subtropical conditions. The condition of the host (dead vs. live) and stage of sporocarp development were recorded to relate substrate status with basidiocarp morphology. Morphological traits of the specimens were documented following standard descriptions and taxonomic keys (22), providing a basis

for preliminary identification and subsequent selection of isolates for cultural and biochemical studies.

Morphological variability among field-collected basidiocarps

All four specimens exhibited typical *Ganoderma* morphology but with clear variation in pileus texture, colour and laccate character.

Isolate 1

Solitary basidiocarps were observed on a dead, decaying tree. The fruiting body was stipitate and distinctly laccate, with overlapping structures. The pileus ($\approx 15.5 \times 12.3 \times 1.0$ cm) was reddish-orange with a smooth white margin and conspicuous concentric colour zones. The disc region was deep reddish to yellowish-brown, surrounded by a broad creamy-white edge; the hymenophore was white. These features are consistent with descriptions of laccate *G. lucidum* (23) and suggest adaptation to highly decomposed woody substrates (Fig. 1A).

Isolate 2

Solitary, stipitate, laccate basidiocarps were recorded on a living *P. pterocarpum* tree. The semicircular to fan-shaped pileus ($\approx 12.0 \times 10.6 \times 2.0$ cm) had a depressed centre, dark-brown varnished surface with pronounced concentric zonation and growth rings and a thin, wavy reddish-brown margin. The hymenophore was pale with closely packed pores (Fig. 1B). The combination of a glossy cap, distinct zonation and leathery context again matched diagnostic features of *G. lucidum* (23), but on a live host, suggesting a potential pathogenic or weak parasitic association.

Isolate 3

Basidiocarps were gregarious, arising on exposed roots of a living *Areca catechu* tree. Fruiting bodies were stipitate, semicircular to irregular, with a central depression and cream to pale-yellow margin. The pileus ($\approx 7.0 \times 5.5 \times 0.5$ cm) had a varnished surface, reddish-brown centre and subtle concentric zonation; the context was leathery and the hymenophore cream-coloured with dense pores (Fig. 1C). These traits are typical of smaller, troop-forming *G. lucidum* basidiocarps under root-associated microhabitats.

Isolate 4

Solitary basidiocarps were formed on exposed above ground roots of a living tree. Fruiting bodies were arranged in overlapping, semicircular to irregularly lobed tiers. The pileus ($\approx 17.0 \times 12.0 \times 1.5$ cm) had a dull brown to cinnamon-brown, non-laccate surface with visible spore deposition, a slightly thickened white margin and a corky to woody context; the hymenophore was pale (Fig. 1D).

Table 1. Collection details of *Ganoderma* isolates from Longleng district of Nagaland

Sample No.	Date of collection	Place of collection (GPS readings)	Stage at which collected	Average weight of fruiting body(g)	Host plant	Condition of the host plant
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1	10/7/2024	Yanchem Lat 522587 °N; Long 94.723854 °E	Active growing stage	56	Not identified	Dead
2	28/7/2024	Sakshi Lat 26.406713 °N; Long 94.868189 °E	Mature	98	Peltophorump terocarpum	Live
3	4/8/2024	Longleng HQ Lat 26.486628 °N; Long 94.815882 °E	Growing	47	Areca catechu	Live
4	18/8/2024	Orangkong Lat 26.50612 °N; Long 94.800507 °E	Mature	302	Not identified	Live



Fig. 1. Morphological characteristics of *Ganoderma lucidum* isolates collected from different host substrates in Longleng District, Nagaland. **A.** Isolate 1: Stipitate, laccate basidiocarp with a reddish-orange pileus showing distinct concentric zonation and a smooth white margin, occurring on a dead, decaying tree; **B.** Isolate 2: Solitary, laccate, dark-brown basidiocarp with clear concentric zonation and a cream-coloured hymenophore, developing on a living tree; **C.** Isolate 3: Troop-forming, stipitate basidiocarps with reddish-brown central zones and cream margins, emerging on exposed roots of a living tree; **D.** Isolate 4: Non-laccate, dull brown to cinnamon-brown basidiocarp with a whitish margin and corky texture, found on above-ground roots of a living tree.

The non-laccate, dull surface contrasts with the strongly varnished pilei of isolates 1–3, suggesting substantial morphological plasticity within the local *Ganoderma* population and the possible

presence of distinct ecotypes or closely related taxa within the *G. lucidum* complex.

Overall, the variation in laccate versus non-laccate pilei, pileus size and host condition (dead

vs. living trees, stem vs. roots) points to ecological and morphological diversity characteristic of the *G. lucidum* species complex. Such variability supports the need for complementary cultural, biochemical and molecular analyses to achieve reliable identification and to link morphology with functional traits.

Cultural characteristics and implications

Of the four field-collected specimens, two isolates were successfully established in pure culture and designated GLN1 (from Isolate 1) and GLN2 (from Isolate 2) (Table 2). The successful isolation from laccate basidiocarps on both dead and living hosts suggests that these phenotypes may represent vigorous, actively growing mycelial types capable of adapting to *in vitro* conditions.

GLN1 exhibited the fastest radial mycelial growth on PDA, with a mean growth rate of 9.1 mm day⁻¹. Initially, colonies were white with yellow patches, later becoming uniformly yellow. The mycelium was dense and fluffy, forming strand-like aggregates with a smooth, non-zoned margin and a markedly thickened central region (Fig. 2A). This growth pattern indicates a highly competitive, rhizomorphic mycelium, which is advantageous for rapid substrate colonisation and makes GLN1 a suitable candidate for spawn production and biomass-based bioactive compound extraction.

By contrast, GLN2 showed a slower growth rate of 7.5 mm day⁻¹ and remained white in early stages, developing reddish-brown pigmentation later in culture. The colony texture was loosely cottony to feathery, becoming denser towards the periphery. Margins were irregular, lobate and semicircular, with distinct concentric zonation and the colony expanded asymmetrically in a fan-like pattern (Fig. 2B). The relatively slower but pigmented growth of GLN2 suggests differential regulation of secondary metabolism and possible association with higher production of low-molecular-weight phenolic compounds, which was later corroborated by its superior antioxidant activity.

Microscopic examination showed chlamydospores and clamp connections in both GLN1 and GLN2 (Fig. 3A and 3B). Scanning electron microscopy further demonstrated clustered basidiospores and the dimensional features of individual basidiospores (Fig. 4A and 4B).

The contrasting cultural phenotypes of GLN1 and GLN2- fast-growing, uniformly expanding, dense colonies versus slower-growing, zonate, asymmetrical colonies highlight intra-specific diversity in growth strategies within *G. lucidum*. From an applied perspective, GLN1 appears better suited for rapid biomass generation and polysaccharide

extraction, whereas GLN2, with its strong pigmentation and distinct growth zonation, may be more promising for the production of bioactive polyphenols and antioxidant metabolites. These observations justify the selection of both isolates for subsequent biochemical and molecular characterisation and underscore the importance of combining field morphology with *in vitro* traits when prioritising *Ganoderma* isolates for nutraceutical and cultivation-related research.

Polysaccharide isolation and separation

Water-soluble and insoluble polysaccharides were successfully extracted from aerial mycelial aggregates of isolates GLN1 and GLN2. The yield of water-soluble polysaccharides differed markedly between the isolates: GLN2 produced almost twice the amount (14.37 mg g⁻¹ dry weight) compared with GLN1 (7.74 mg g⁻¹ dry weight). Insoluble polysaccharide levels, however, were similar between the isolates (2.10 mg g⁻¹ for GLN2 and 2.91 mg g⁻¹ for GLN1), indicating that the major biochemical variation lies in the soluble fraction, which is often associated with pharmacologically active β -glucans.

Ion-exchange chromatography revealed distinct elution profiles for both isolates. GLN1 yielded two fractions (C1 and C2), corresponding to neutral (eluted with water) and weakly anionic polysaccharides (eluted with 0.1 M NaHCO₃). In contrast, GLN2 produced a single dominant anionic peak (I), suggesting a relatively homogeneous soluble polysaccharide population enriched in negatively charged molecules. The GLN1 chromatographic pattern closely resembled the profile reported for *G. lucidum* strain MZKI G97 from Slovenia (16), implying similar structural diversity (Fig. 5).

Further separation using gel-filtration chromatography showed that both C1 and C2 from GLN1 resolved into two sub-fractions, indicating the presence of multiple high-molecular-weight components. By comparison, GLN2 produced a single, distinct high-molecular-weight peak, likely comparable to the first C1 peak of GLN1. High molecular-weight β -1,3- and β -1,6-D-glucans are known contributors to immunomodulatory and antitumor activity in *G. lucidum* (24). The structural heterogeneity observed in GLN1 suggests a more complex polysaccharide mixture, while the single dominant profile in GLN2 may reflect a more specialised biosynthetic pathway.

Despite these differences, neither P-GLN1 nor P-GLN2 exhibited significant antioxidant activity in DPPH assays. This agrees with earlier reports that polysaccharides generally show weak radical scavenging properties (25, 26).

Table 2. Morphological and cultural variability among *Ganoderma* isolates on Potato dextrose agar medium (8th day)

Isolates	Mycelial	Pigmentation	Colony	Margin and	Distinctive features	Growth rate
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	colour	(in a later stage)	texture	zonation		(mm/day)
GLN1	White	Yellow coloured	dense and fluffy with strand-like aggregates	Smooth margin Zonation is absent	Fastest mycelial growth. The colony is circular with uniform radial expansion. Thickened central mycelium.	9.1 mm/day
GLN2	White	Reddish brown	Loosely cottony, Feathery, initially thin, thick towards the periphery	Irregular, lobate, semi-circular margin Distinct concentric zonation	Colony asymmetrically radiating, semicircular form, producing a fan-like expansion pattern. Its radial growth was slowest of all.	7.5 mm/day

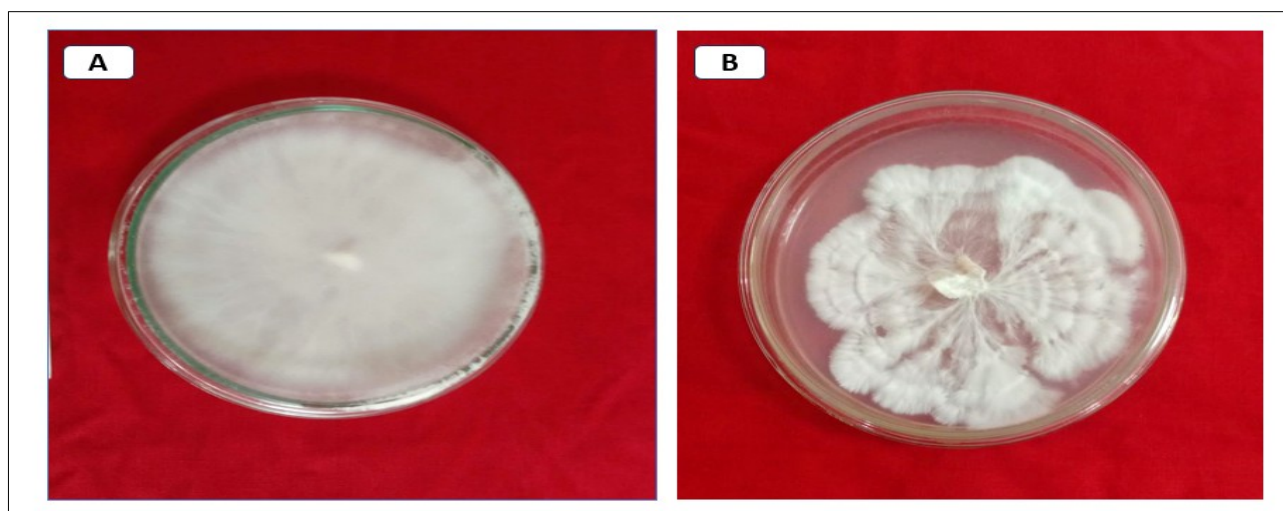


Fig. 2. Mycelial characteristics of *Ganoderma* isolates: **A.** GLN1; **B.** GLN2.

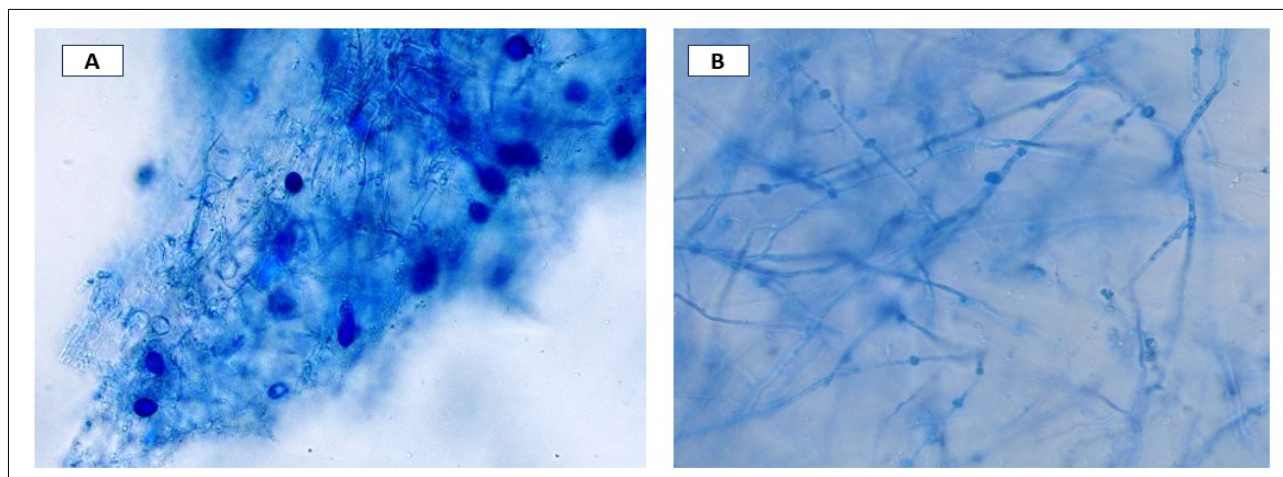


Fig. 3. Microscopic features of *Ganoderma lucidum* isolates. **A.** Presence of chlamydospores and clamp connections in isolate GLN1; **B.** Chlamydospores and clamp connections observed in isolate GLN2.

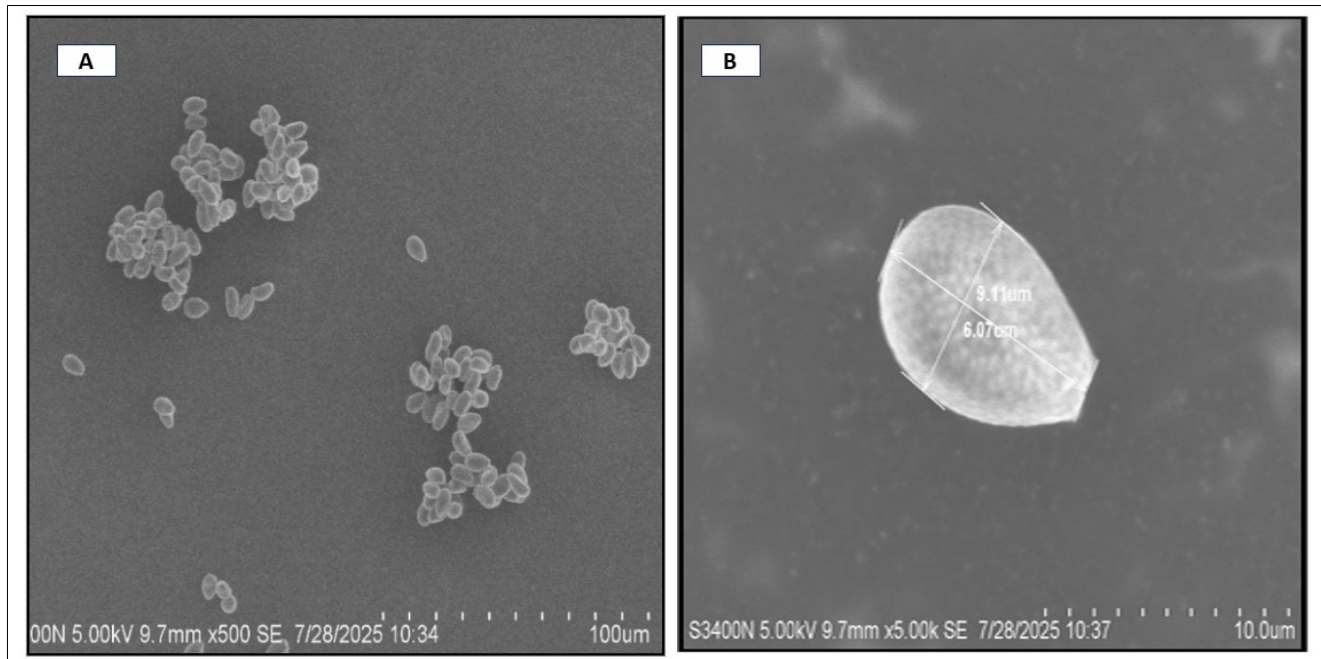


Fig. 4. Scanning electron micrograph (SEM) of *Ganoderma lucidum* basidiospores. **A.** Clustered arrangement of basidiospores observed under SEM; **B.** Dimensional characteristics of individual *Ganoderma* basidiospores as revealed under SEM.

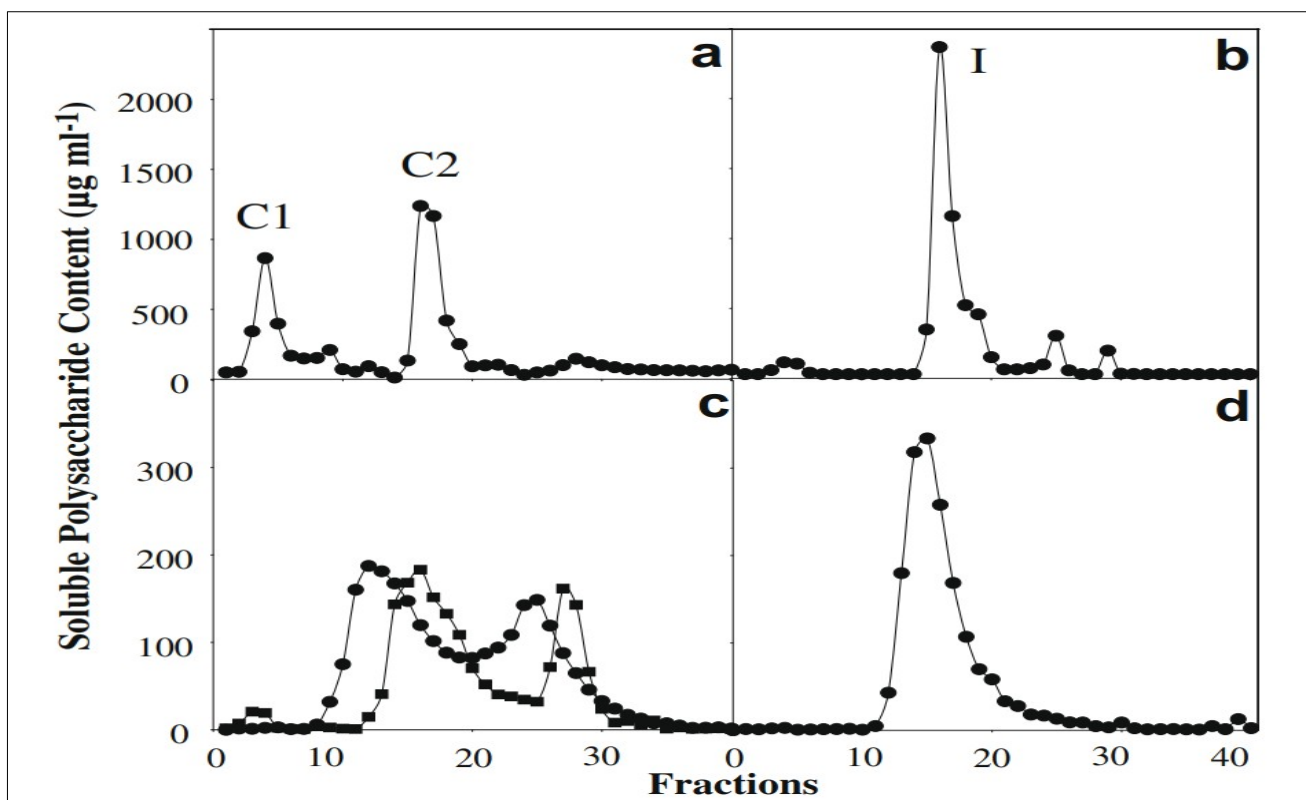


Fig. 5. Chromatographic profiles of soluble polysaccharides from *Ganoderma lucidum* isolates. Elution chromatograms from ion-exchange chromatography (DEAE) were obtained by loading 5 mg of polysaccharides from **A.** GLN1; **B.** GLN2 isolates. Elution chromatograms from gel filtration chromatography were obtained by loading 2 mg of each DEAE-polysaccharide fraction; **C.** C1 peak (box-dot) and C2 peak (dot-dot) from the GLN1 isolate and (d) I peak from the GLN2 isolate.

Low activity may also be due to incomplete solubilisation or lack of high-activity low-molecular weight components, indicating the need for further structural characterisation and purification. The observed biochemical profiles, however, remain valuable because high-molecular-weight glucans are primarily associated with immunological rather than antioxidant mechanisms (27, 28).

Activity of radical scavenging

Low-molecular-weight fractions (L-GLN1 and L-GLN2) displayed strong, concentration-dependent DPPH radical scavenging activity, with clear differences between isolates (Fig. 6). L-GLN2 consistently outperformed L-GLN1 at lower concentrations, achieving approximately 60 % inhibition at 0.5 mg mL⁻¹ compared with 36 % for L-GLN1. This trend matches the EC₅₀ values: L-GLN2 exhibited a significantly lower EC₅₀ (0.23 ± 0.05 mg mL⁻¹) than L-GLN1 (0.69 ± 0.02 mg mL⁻¹), confirming higher antioxidant potency. The stronger activity of L-GLN2 correlates with its higher total polyphenol content (27.9 mg g⁻¹ extract) relative to L-GLN1 (16.5 mg g⁻¹ extract). Correlation analysis showed strong linear associations between polyphenol content and scavenging activity ($r^2 = 0.9933$ for L-GLN2; $r^2 = 0.9878$ for L-GLN1), indicating polyphenols as the primary contributors to antioxidant effects. These findings align with reports on *Inonotus obliquus* and plant-derived polyphenolic flavonoids, where phenolic compounds showed strong antioxidant activity (29, 30). Overall, GLN2 appears to possess a more active phenolic metabolism, explaining its superior antioxidant performance and suggesting its potential suitability for nutraceutical applications targeting oxidative stress.

Molecular identification and phylogenetic analysis

Polymerase chain reaction amplification of the ITS region from GLN1 and GLN2 yielded single, clear bands of approximately 600 bp (Fig. 7). BLASTn analysis showed 99–100 % similarity to *G. lucidum* sensu lato sequences, confirming species-level identity. Both sequences were deposited in GenBank (GLN1: PX435394; GLN2: PX435396). Phylogenetic reconstruction using maximum likelihood (MEGA-11) grouped reference *G. lucidum* sequences into four major clades. Interestingly, both Nagaland isolates formed a distinct, well-supported monophyletic cluster (Group IV) with 100 % bootstrap support (Fig. 8). Their close branch proximity indicates minimal intra-regional divergence between GLN1 and GLN2, while their separation from Groups I–III suggests a geographically and genetically distinct lineage within the *G. lucidum* species complex (Fig. 8). This divergence may reflect local adaptation to the ecological conditions of Nagaland's forest ecosystems, especially substrate type, altitude and humidity. Similar region-specific lineage formation has been reported globally in *G. lucidum*, supporting the hypothesis that the Nagaland isolates represent a

unique genetic subgroup with potentially novel biochemical or therapeutic properties.

4. Conclusion

This study revealed that *Ganoderma lucidum* isolates GLN1 and GLN2 from Longleng district, Nagaland, possess distinct morphological, biochemical and molecular characteristics. GLN2 exhibited superior antioxidant activity due to its higher polyphenol and low-molecular-weight compound content, while both isolates showed unique polysaccharide profiles and formed a distinct phylogenetic sub-clade, indicating regional genetic divergence. These traits highlight their potential as valuable sources of bioactive compounds for nutraceutical and pharmaceutical use. The findings also support their suitability for sustainable cultivation, offering opportunities for rural livelihood development. Future work should focus on detailed compound characterisation, therapeutic evaluation and optimised cultivation to enable commercial utilisation and conservation of local *Ganoderma* resources.

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Authors' contributions

SKB conceived and designed the study, conducted the investigation, analysed the data and drafted the manuscript. SP¹ and SSR performed the statistical analysis and interpretation of the data. ASR contributed to molecular characterisation and sequence analysis. AA contributed to phylogenetic analysis and interpretation. AK assisted in field sampling and documentation of specimens. KR contributed to laboratory processing and maintenance of isolates. BJ contributed to literature review and taxonomic interpretation. PN contributed to data compilation and preparation of tables and figures. SP² contributed to manuscript editing and formatting. HCK provided supervision and coordinated the research activities at ICAR-KVK, Longleng. AKM provided scientific guidance and critically reviewed the manuscript. All authors read and approved the final manuscript. [SP¹ stands for Sabyasachi Pradhan and SP² stands for Subhadarsini Pradhan]



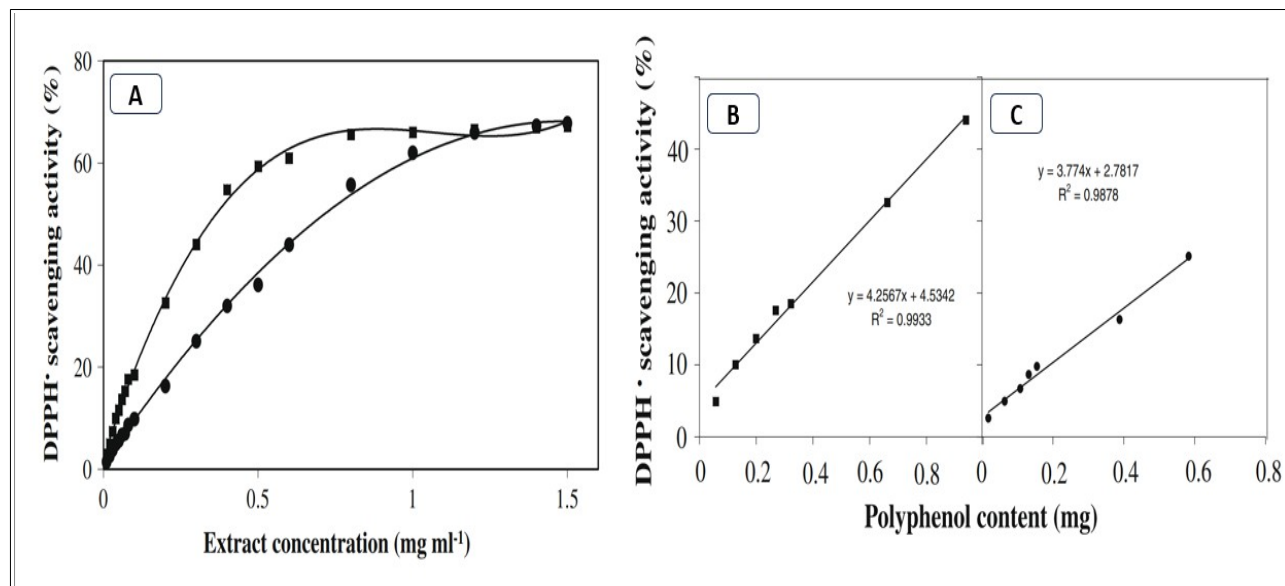


Fig. 6. Antioxidant capacity of *Ganoderma lucidum* extracts. **A.** 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of L-GLN1 (d–d) and L-GLN2 (j–j) fractions derived from GLN1 and GLN2 isolates, respectively. The graph illustrates the percentage inhibition at increasing extract concentrations; **B.** Correlation between total polyphenol content and DPPH scavenging activity of L-GLN2; **C.** L-GLN1 fractions. Each value represents the mean of three independent experiments, with deviations not exceeding 5 % of the mean.

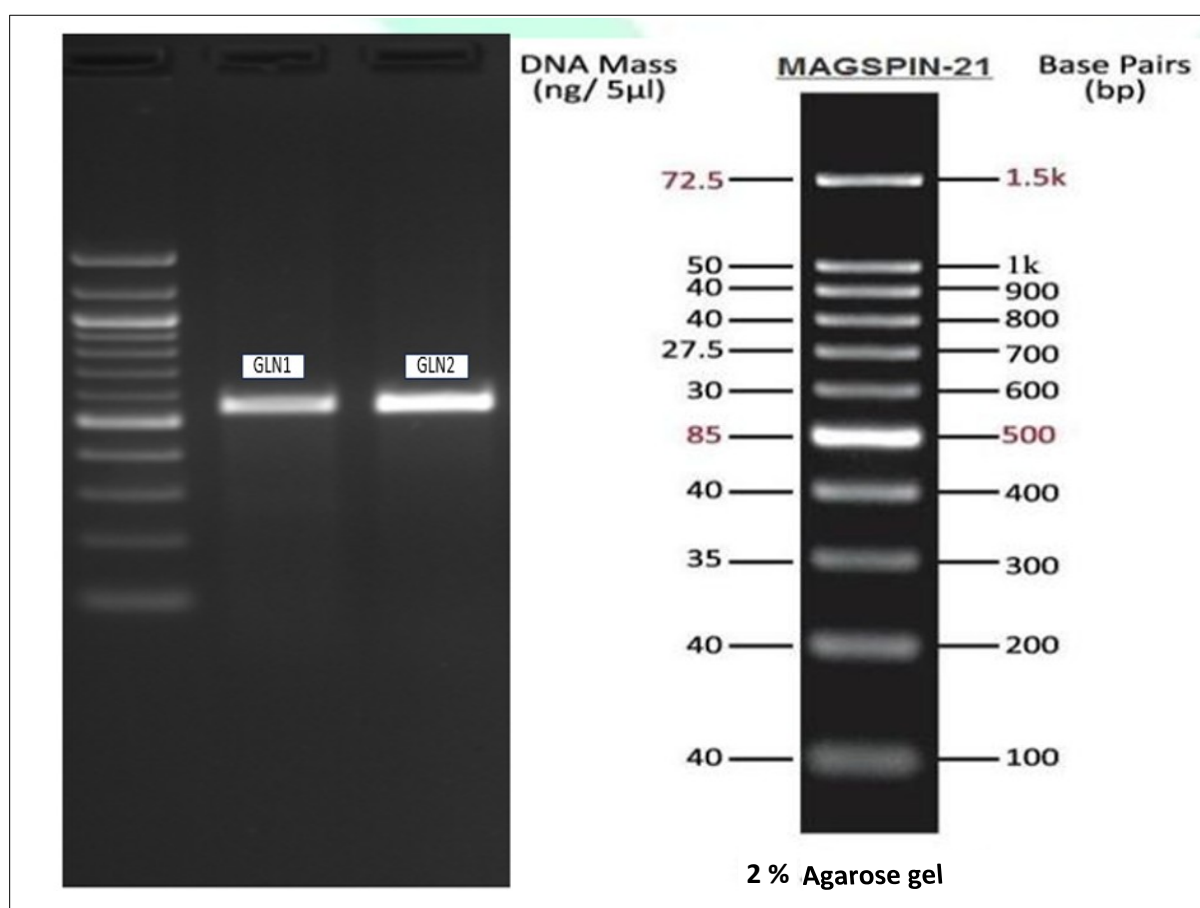


Fig. 7. 1.8 % agarose gel showing a single 600 bp of 18s amplicon.

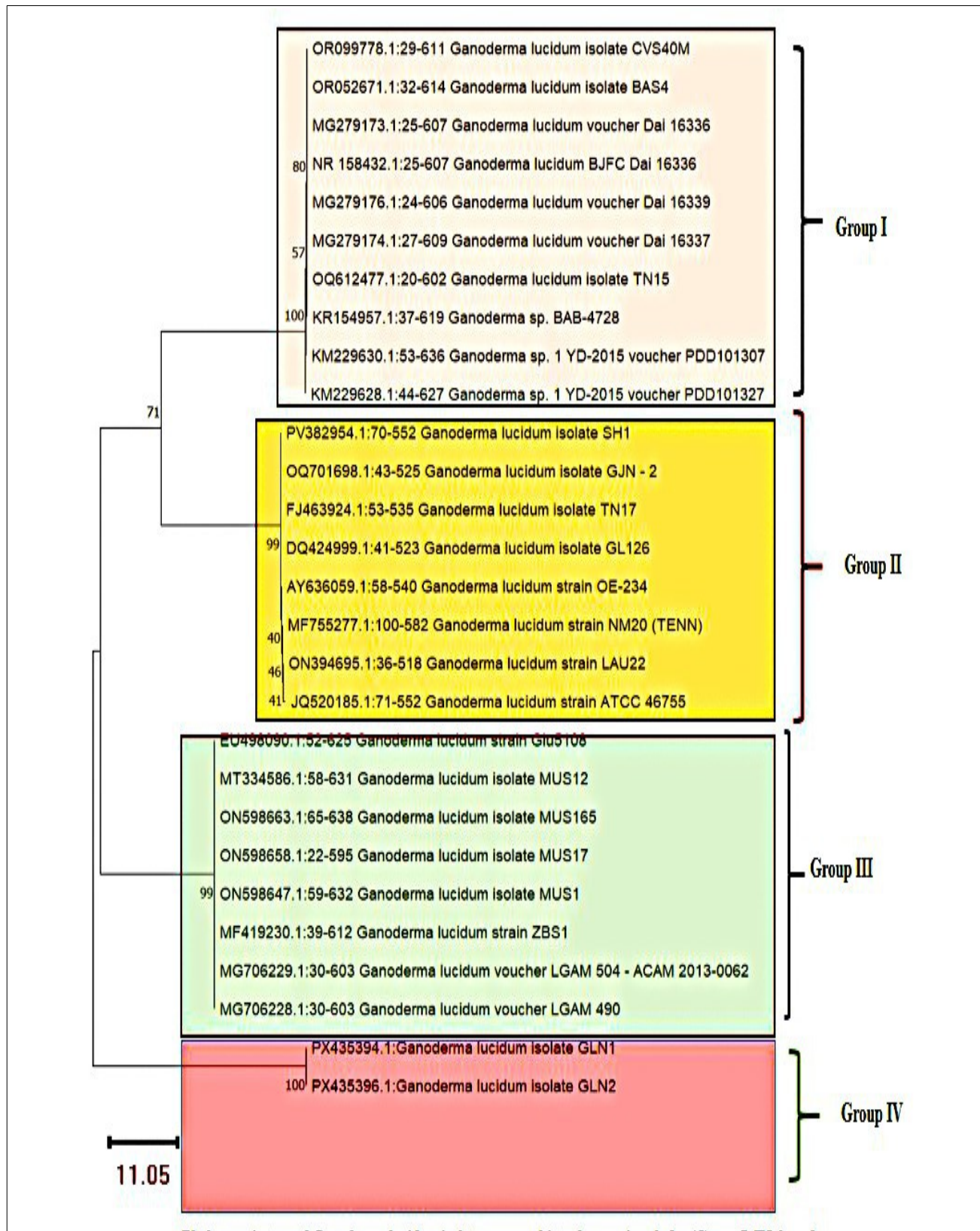


Fig. 8. Phylogenetic tree of *Ganoderma lucidum* isolates based on ITS sequences. The Nagaland isolates (GLN1 and GLN2) formed a distinct clade (Group IV) with 100 % bootstrap support.

Compliance with ethical standards

Conflict of interest

Authors do not have any conflict of interests to declare.

Ethical issues

None

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