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# Sequence Analysis and rRNA Secondary Structure Prediction of 28S rRNA Genes of Two *Ganoderma* Mushrooms from Similipal Biosphere Reserve, Odisha, India

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**Abstract:** Two *Ganoderma* mushrooms (*Ganoderma* sp.M1 & *Ganoderma* sp.M3) were collected from Similipal Biosphere Reserve, Odisha, India. Ethnomedicinally these mushrooms are known to have medicinal value used in curing asthma and having wound healing capability. The objective of present study is to evaluate the morphological and molecular characteristics of two *Ganoderma* mushrooms with a view to identify them. Based on their morphological characteristics, the studied mushrooms were identified as member of genus *Ganoderma*. Further confirmation of identity of these mushrooms was made by molecular study based on LSU rDNA sequences and RNA secondary structure prediction. The results obtained from molecular phylogenetic tree revealed that the newly collected *Ganoderma* sp.M1 shown 82 % homology with *Ganoderma applanatum*, hence may be identified as *Ganoderma applanatum*. But the other isolated strain of *Ganoderma* sp.M3 shown less similarity with same species of *Ganoderma,* hence no species is assigned to them. The results of 28S rRNA sequence analysis shown a weak similarity between both the new strains (*Ganoderma* sp.M1 & *Ganoderma* sp.M3) which is also reflected at their secondary structural folding patterns observed from their minimum free energy based predicted secondary structures.

**Key words**: *Ganoderma* sp., LSU primer, 28S rDNA, RNA secondary structure.

## Introduction

*Ganoderma* are the most common medicinal mushrooms which have been used in folk-medicine in China and Japan from ancient time to treat several diseases [13]. Each type of *Ganoderma* has its own biological properties. The commonly used medicinal *Ganoderma* include *G. lucidum*, *G. tsugae*, *G. capense* and *G. applanatum*. Some of the physiological effects and distinctive properties of *Ganoderma* are strain dependent [18]. Besides that, several reports has been made for their distinctive biological properties like antibacterial, anti oxidant, anti inflammatory, anti proliferative, anti cancer, anti tumor, cytotoxic, anti-HIV, hypo cholesterolemic, anti diabetic, hepatoprotective etc.[20,26]. Polysaccharides and triterpenes of *Ganoderma* are the major source of its pharmacological active constituents. *Ganoderma* species are difficult to identify and have traditionally been defined by the morphological characters of their highly variable sporocarps. Over 300 species have been named, many on the basis of single collections with narrow or poorly defined species concepts [23]. A large number of synonyms exist. For example, the Mycobank database lists eight obligate and 26 taxonomic synonyms for *G. applanatum* (Pers.) Pat., which is listed as a taxonomic synonym of *G. lipsiense* (Batsch) G.F.Atk.

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Index fungorum, however, lists *G. lipsiense* as a synonym of *G. applanatum* [2]. In contrast, *G. lucidum* may represent a complex of six or more species [9]. Ryvarden [21] went so far as to propose a 10-year moratorium on the description of new *Ganoderma* species. Molecular characteristics of *Ganoderma* are increasingly being used as additional taxonomic criteria in classification or to resolve controversies in taxonomic position of taxa. A wide range of molecular methods has been introduced, especially with the rapid development of polymerase chain reaction (PCR)-based techniques. Recently, a PCR-based method was developed for the identification of several decay fungi from the wood of broad leaved trees [7]. Comparative analysis of coding and non-coding regions of ribosomal DNA has become a popular tool for construction of phylogenetic trees of many organisms including mushrooms. Mushrooms rRNAs, like other fungal rRNAs, are generally composed of 5S, 5.8S, and 18S small subunits (SSU) and a 25S~28S large subunit (LSU). Since rRNA structural genes have been known to be well conserved at the genus or species levels, most molecular phylogenetic studies have been focused on the internal transcribed spacer (ITS) region located between rRNA structural genes in the rDNA cistron [15,19]. However, LSU rRNA gene has been revealed to have some variable regions with sequence divergency: D1, D2 and D3. These are enough to infer phylogenetic relationships between species [8,27].

Molecular techniques such as those used to detect and identify basidiomycete wood rots including *Ganoderma* [5] have not been applied to the fungi of Similipal Biosphere Reserve, Odisha, India for identification of *Genoderma* species. In the present study, an attempt has been made for molecular characterization, identification and study of evolutionary divergence of two *Ganoderma* species collected from Similipal Biosphere Reserve.

## Materials and methods

### *Collection of mushroom*

Two different samples of mushroom were collected from the forest of Similipal Biosphere Reserve, Odisha, India in the month between September and November of 2011 (Fig. 1a and b). Both the mushrooms were found to attach with decaying trunk of *Shorea robusta*. Morphological characters such as laccate and non-laccate, type of basidiocarp (stipitate/sessile/dimidiate, imbricate, concave, number of concentric zones, etc.), margin shape (lobed, fertile/sterile and rounded/acute) and colour (brown, white, reddish etc.), pores (colour, pores per $mm^{-1}$, angular/rounded), pore diameter, dissepiments and axes; tube size and colour, context, and spore characters, which are confirmative with the species of *Ganoderma* as described by various authors [1,6,22]. Further, the collected samples (fresh and dried) were preserved in 4 % formaldehyde and in polythene bags respectively for future use. Each of the collected samples were also wrapped in wax paper and brought to the laboratory for identification purposes. All the specimens were deposited in the Herbarium of Department of Biotechnology, College of Engineering and Technology, Bhubaneswar, Odisha, India.

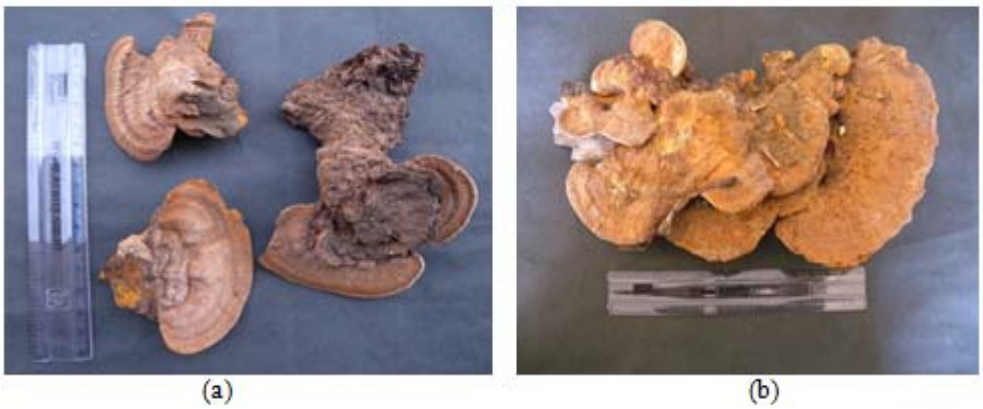


**Figure 1.** Fruitning body of *Ganoderma* sp.M1 (a) and *Ganoderma* sp.M3 (b)

**Isolation of total genomic DNA**

Total genomic DNA was extracted from fruiting body of mushroom according to the CTAB method described by Zhou *et al*.[28] with minor modifications. Mushroom samples (0.1 g/sample) were homogenized in liquid nitrogen, transferred to a 15 mL tube containing 5 mL of 2X CTAB Buffer (1.4 mol/L NaCl, 100 mmol/L Tris-HCl (pH 8.0), 20 mmol/L EDTA, 2 % CTAB), and 0.2 % β-mercaptoethanol and mixed gently. The mixture was placed on a water bath at 65°C for 30 min. The homogenate was extracted with equal volume of chloroform:isoamyl alcohol (24:1). The homogenate was pelleted by centrifugation at 10,000 rpm for 10 minutes at 4°C and the aqueous phase was removed. Then 2/3 volume of cold iso-propanol was added. Samples were incubated over night at 4°C. After incubation the pellet was washed with 70 % ethanol, dried, and re-suspended in 50 µL TE buffer. The DNA was stored at -20°C for further use.

**PCR amplification**

DNA was quantified and diluted to 30 ng DNA/µL. The 25S~28S region was amplified using universal primers LR0R (ACCCGCTGAACTTAAGC) and LR3 (GGTCCGTGTTTCAAGACGG) described by Kim *et al*.[12]. PCR reaction were carried out in 25 µL of reaction mixture containing 7.5 µL DNAse-RNase free water, 12.50 µL 1X PCR master mix (MBI Fermentas), 1.00 µL LROR primer (10 pmole/µL), 1.00 µL LR3 primer (10 pmole/µL), 3.0 µL of diluted genomic DNA. Amplification was performed in Eppendorf Thermal Cycler with the following protocol: (95°C, 2 min) × 1 cycle, (94°C, 30 sec; 48°C, 30 sec; 72°C, 90 sec) × 30 cycles and (72°C, 10 min) × 1 cycle. To confirm the targeted PCR amplification, 5 µL of PCR products were electrophoresed in a 0.8 % agarose gel. Amplified PCR product was purified using QIA quick PCR purification kit (QIAGEN, UK) according to the manufacturer's protocol.

**DNA sequencing**

All these PCR products were sequenced at Xcelris Labs Ltd., Ahmedabad, India by using ABI 373xl Genetic Analyzer (Applied Biosystems, USA). Sequencing was carried out using BigDye Terminator v3.1 Cycle Sequencing Kit (Sangon Co., Shanghai, China) according to the manufacturer's protocol. For sequencing same primers were used.

**Phylogenetic analysis and RNA secondary structure prediction**

The 28S rRNA genes sequenced from two strains (*Ganoderma* sp. M1 & *Ganoderma* sp. M3) of genus *Ganoderma* were subjected for molecular evolutionary analysis purpose. From their forward and reverse sequences, the consensus sequences were constructed for both the strains using RevComp tool (http://www.bioinformatics.org/sms/rev_comp.html) and Prob Cons: Probabilistic Consistency-based multiple sequence alignment tool (http://toolkit.tuebingen.Mpg.de/Probcons) [4]. The consensus sequences of both strains were submitted in the European Nucleotide Achieve Database (http://www.ebi.ac.uk/ena/) published with accession numbers: HF545883 and HF545884 respectively. As morphological study suggested both the strains of mushroom belong to genus *Ganoderma,* hence sequence homology search was conducted against all species of Ganoderma for both the strains using BLAST algorithm (http: //blast.ncbi.nlm.nih.gov/Blast.cgi). The Multiple Sequence Alignment (MSA) was performed using Clustalw2 tool (http://www.ebi.ac.uk/Tools/msa/clustalw2/) of EMBL-EBI web server. The MSA was performed again using ClustalX 2.0 tool [14] and results were further used in phylogenetic tree construction using MEGA 5.05 [17,24]. Minimum energy model was used for RNA secondary structure prediction [25]. Secondary structure of *Ganoderma* sp.M1 & *Ganoderma* sp.M3 28S have been constructed by using RNA Fold web Server (http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi) [16].

**Results**

Both the studied wild mushrooms (*Ganoderma* sp.M1 & *Ganoderma* sp.M3) were collected from dead wood trunk of *Shorea robusta* on the ground in the forest of Similipal Biosphere Reserve and considered as saprophyte. The mushrooms were normally found between September and Novem-

ber during the post monsoon season. The morphological characteristics of studied mushrooms are given below.

**Morphological characteristics**

***Ganoderma sp.M1***

Basidiocarp is sessile, woody to corky, applanate, up to 20 cm diameter and 1.5-5 cm thick at base. Upper surface is pale grey to dark brown, crustose with concentric zonation, sulcate, covered with layer of chocolate and brown spore appearing dusty. Margin is 1.0 mm to 10.0 mm thick, sterile, rounded and turning brown on drying. Pore surface is whitish, milky to coffee and rough. Pores are 4-5 per mm and spherical to ovoid. Tubes are multi layer and 4-8 layers in perennial specimen separated by layer. Context is thick, purplish brown and shining. Cutis type is trichodermis. Hyphal system is trimitic. Generative hyphae are 3.3-4.1 ìm diameters and pale yellow with clamp connection; skeletal hyphae are 5.8 to 6.6 µm diameter and dark brown; binding hyphae are 7.5 µm diameter and dark brown. Basidiospore size is 7-8×4-6 µm and pale yellow in color.

**Ganoderma sp. M3**

Basidiocarp is hard, dimidiate, medium, applanate woody and up to 15 cm diameter. Upper surface is slightly zonate, pulverulent glabrous, tuberous, rugose solitary, crust, rigid, up to 1 mm thick and reddish grey or cinnamon. Margin is hard, obtuse, slightly thick and lobate, cinnamon to grayish white or slightly yellowish. Pore surface is milky coffee. Pores are minute and 5-6 per mm. Tubes are unstratified concolourous to pileus and 4-13 mm long. Context is reddish brown, sub ferrugineous to coccoa coloured, corky, tough, thin and up to 30 mm wide. Cutis type is trichodermis. Hyphal system is trimitic. Generative hyphae are 3.3 µm diameter and yellow; skeletal hyphae are 5 µm diameter and brown; binding hyphae are 5.8 µm diameter and brown. Basidiospore size is 6-10×4-6 µm, ovoid to broadly ellipsoid.

**DNA extraction and PCR amplification**

The total genomic DNA was isolated from two different mushrooms strain of *Ganoderma* by adopting cTAB DNA extraction method. The quantity of extracted genomic DNA was found satisfactory and determined by taking absorbance at 260 nm and 280 nm. The OD value was found to be in range of 140-180 µg/µl. The quality of extracted genomic DNA was determined by performing Agarose gel electrophoresis and the genomic DNA band at ~350 bp was observed (Fig. 2). The genomic DNA obtained was very good quality and yielded the expected PCR products using suitable primers (LR0R and LR3). The PCR products sizes were approximately ~600 bp (Fig. 3).

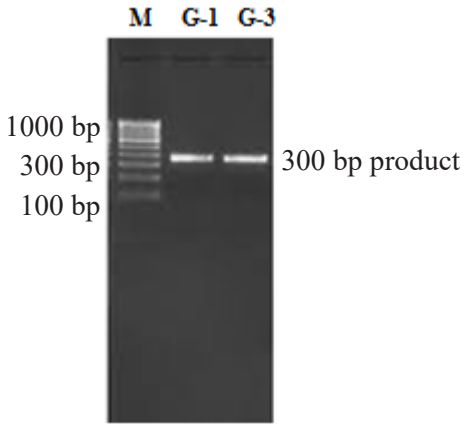


**Figure 2.** Genomic DNA

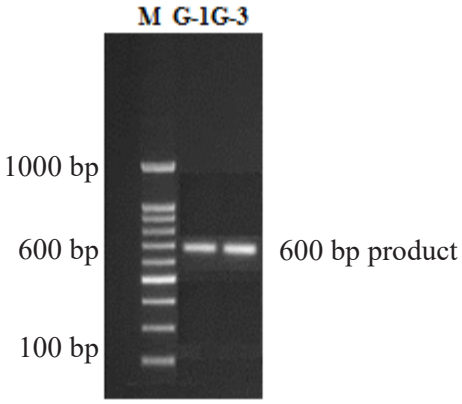


**Figure 3.** PCR amplified product

**Phylogenetic analysis**

From all of the BLAST hits obtained for 28S rRNA genes of *Ganoderma* sp.M1, seventeen homologous sequences **(Table 1)** of genus *Ganoderma* were retained on the basis of good sequence identity (80 % to 82 %), lowest e-value and query coverage (81 % and 100 % respectively). A total of nineteen 28S rRNA genes in-

**Table 1. Homologous sequences resulted from BLAST search for 28S rRNA gene of *Ganoderma* sp.M1 (ENA: HF545883)**

| No. | Accession | Description | Query coverage | E value | Identity |
|---|---|---|---|---|---|
| 1 | EU232303.1 | *G. applanatum* strain BC\ RC 36235, 28S LSU rRNA gene | 100% | 2e-158 | 82% |
| 2 | X78776.1 | *G. lucidum* gene for 25S rRNA | 100% | 5e-145 | 81% |
| 3 | X78777.1 | *G. boninense* gene for 25S rRNA | 100% | 2e-143 | 81% |
| 4 | AY333807.1 | *G. australe* strain:Wu9302-56, 28S rRNA gene, partial sequence | 98% | 9e-143 | 81% |
| 5 | DQ208411 | *G. lucidum* strain IUM01122 large subunit ribosomal RNA gene, partial sequence | 99% | 9e-143 | 81% |
| 6 | EU232274 | *G. applanatum* strain BCRC 36091 28S large subunit ribosomalRNA gene, partial sequence | 100% | 1e-142 | 81% |
| 7 | X78779.1 | *G. microsporum* gene for25S rRNA | 100% | 1e-141 | 81% |
| 8 | DQ208413 | *G. lucidum* strain C-2 LSUe | 99% | 4e-141 | 81% |
| 9 | X78778.1 | *G. tsugae* gene for 25S rRNA rRNA gene, partial sequenc | 100% | 1e-140 | 80% |
| 10 | AY515339 | *G. applanatum* strain ATCC44053 28S ribosomal RNA gene, partial sequence | 99% | 5e-140 | 81% |
| 11 | DQ208412 | *G. lucidum* strain C-1 large subunit ribosomal RNA gene, partial sequence | 99% | 2e-139 | 80% |
| 12 | AY684163.1 | *G. tsugae* isolate AFTOL-ID 771 25S *ribosomal* RNA gene,partial sequence | 98% | 2e-138 | 80% |
| 13 | AB733303.1 | *G. gibbosum* gene for 28S rRNA, partial sequence, strain:KUT0805 | 90% | 5e-135 | 81% |
| 14 | AM269836.1 | *G. resinaceum* partial 28S rRNA gene, isolate FGR5 | 87% | 7e-129 | 81% |
| 15 | AM269832.1 | *G. pfeifferi* partial 28S rRNA gene, isolate G2/11 | 87% | 3e-127 | 81% |
| 16 | AM269829.1 | *G. adsperum* partial 28S rRNA gene, isolate FGA1 | 86% | 2e-124 | 81% |
| 17 | AB368069.1. | *G. tsunodae* gene for 28S rRNA partial sequence, strain:WD2034 | 81% | 7e-119 | 81% |
| 18 | HF545884 | *Ganoderma* sp. M3 partial 28S rRNA gene, strain M3 | 23% | 8e-44 | 87% |

cluding two isolated mushroom strains (M1 and M3) were subjected for MSA and phylogenetic analysis. The resulted phylogram guide tree from ClustalW2 tool based on distance is given in Fig. 4. Further, Maximum Parsimony (MP) tree was constructed using the close-neighbor interchange algorithm with search level 1 in which the initial trees were obtained with the random addition of sequences (10 replicates). Bootstrapping was performed for 10000 replicates. The resultant bootstrap consensus parsimonious tree was shown in Fig. 5. The BLAST result obtained for *Ganoderma* sp.M1 shown highest similarity with *Ganoderma applanatum* strain BCRC 36235, 28S LSU rRNA gene (EU232303.1) with support of query coverage 100 %, identity 8 2% and e-value 2e-158 which implicates *Ganoderma* sp.M1 is one of the close homologous of *Ganoderma*

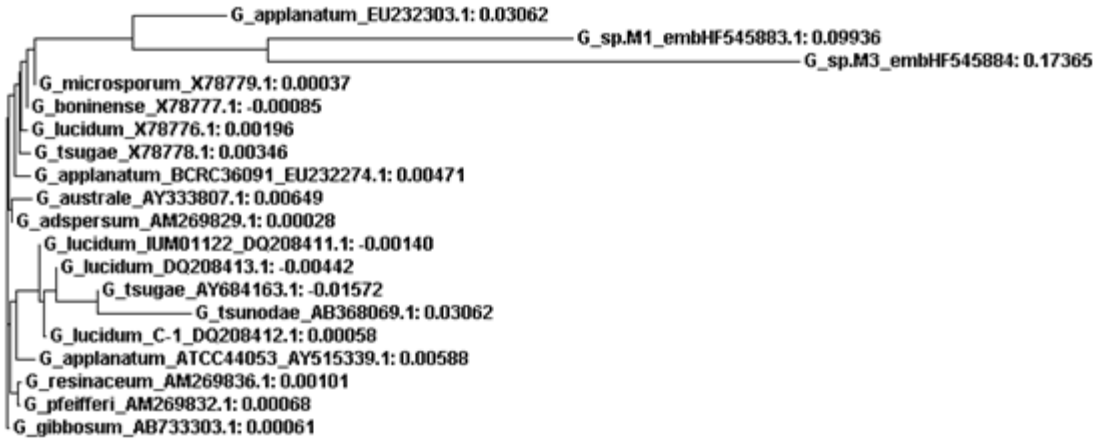


**Figure 4.** Phylogram guide tree constructed using ClustalW 2.1 of EMBL-EBI server based on homologous sequences of *Ganoderma* sp.M1 (ENA: HF545883). The digits on the right side of the tree represent the distance based on which the associated taxa clustered together from multiple sequence alignment

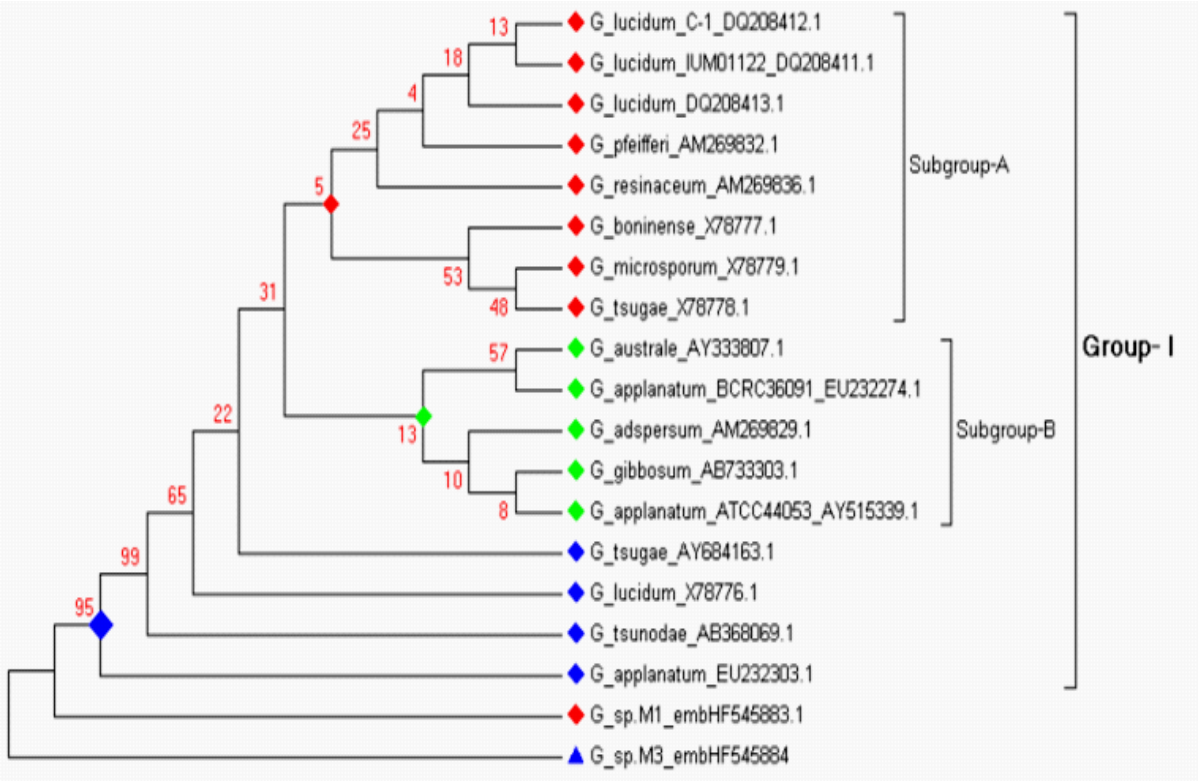


**Figure 5.** Maximum Parsimonious tree (boot strap consensus) constructed using MEGA 5.05 based on homologous sequences of *Ganoderma* sp.M1 (ENA: HF545883). The digits on the left side of the tree represent the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates)

*applanatum*. The phylogram guide tree constructed using ClustalW 2.1 of EMBL-EBI server based on homologous sequences of *Ganoderma* sp.M1 (ENA: HF 545883) also suggested that both strains *Gano-derma* sp.M1 and *Ganoderma* sp.M3 close to each other but *Ganoderma* sp.M1 is closer to *Gano-derma applanatum* (Table 1). The boot strap consensus MP tree was resulted

using MEGA 5.05 for seventeen strains of genus G*anoderma* were clustered together in one group (Group I). Again Subgroup-A (indicated by red diamond) and Subgroup-B (indicated by green diamond) of Group-I contains eight numbers and five numbers of different strains of genus G*anoderma* respectively clustered together with the support of bootstrap value 31. The *Ganoderma* sp.M1 (ENA: HF 545883) clustered with *Ganoderma applanatum* strain (EU232303.1) of genus *Ganoderma* as a taxon in Group -I supported by a good bootstrap value 95. This also supported the fact that *Ganoderma* sp.M1 (ENA: HF545883) is a close homologous of *Ganoderma applanatum* (EU 232303.1). The second strain *Ganoderma* sp.M3 (ENA: HF545884) which showed 23% of query coverage and 87 % of identity with *Ganoderma* sp.M1 (ENA: HF545883) from the BLAST search diversified from Group-I cluster of MP tree like an out group taxon which implicates poor homology with the members of Group I but somewhat similar to *Ganoderma* sp.M1 (ENA: HF545883).

**RNA Secondary structure prediction**

Predicted 28S rDNA secondary structural features of *Ganoderma* sp.M1 and *Ganoderma* sp.M3 were reconstructed (Fig. 6 and 7) with the lowest negative free energy which provides the basic information for phylogenetic analysis. Length of 28S sequences of *Ganoderma* sp.M1 and *Ganoderma* sp.M3 were 675 bp and 641 bp respectively. The G+C content for *Ganoderma* sp.M1 was 50.17 % and for *Ganoderma* sp.M3 was 47.56 %. The RNA secondary structures of the 28S gene regions were analyzed on the basis of conserved stems and loops. The observed structural similarities in the predicted secondary structure are further reflected at the energy level. The optimal secondary structure with minimum free energy (MFE) has been calculated for *Ganoderma* sp.M1 (ENA: HF545883) as -225.00 kcal/mol and for *Ganoderma* sp.M3 (ENA: HF545884) as -259.90 kcal/mol using RNA Fold Web Server. The secondary structure analysis reveals presence of external loop, multi loop, bulge loop, hairpin loop and interior loop. The analysis of secondary structure revealed a partial conservation in RNA folding pattern found in *Ganoderma* sp.M1 (ENA: HF545883) and *Ganoderma* sp.M3 (ENA: HF545884) labeled as region A, C and D in Fig. 6 and 7. In order of preference of conservation, one external loop followed by three interior loops connected by stems observed in both RNA secondary structures (region A and C). The folding patterns in both *Ganoderma* sp.M1 and *Ganoderma* sp.M3 labeled as region D are quite similar with only variation of a small extended

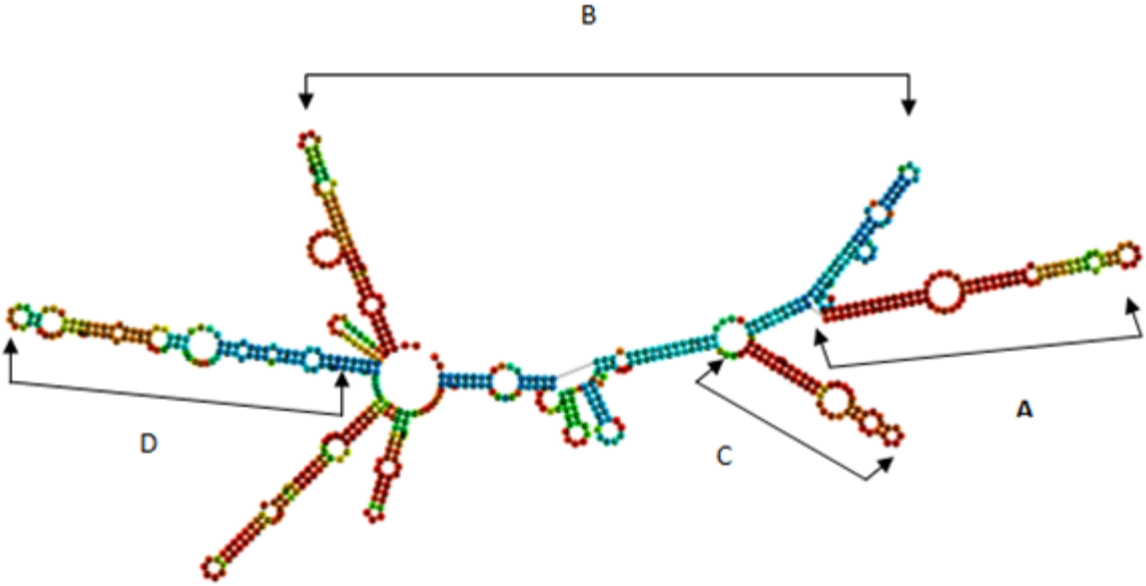


**Figure 6.** Predicted 28S rDNA secondary structures for *Ganoderma* sp. M1 from Similipal Biosphere Reserve, Odisha, India

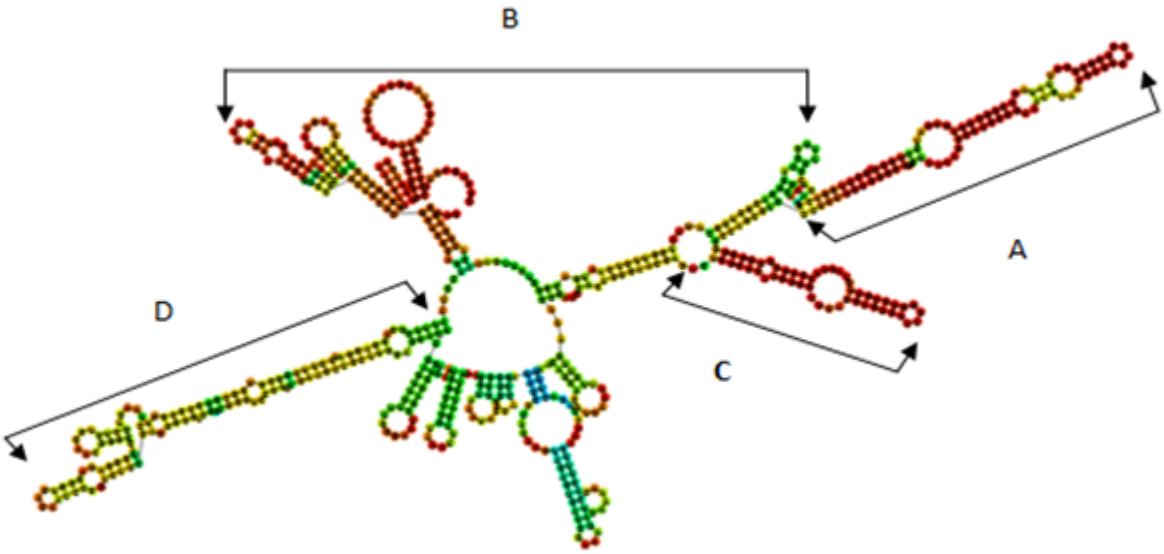


**Figure 7.** Predicted 28S rDNA secondary structures for *Ganoderma* sp .M3 from Similipal Biosphere Reserve, Odisha, India

branch found in case of *Ganoderma* sp.M3 (Fig. 7). The noticeable variations have been observed between both structures (Fig. 6 and 7) particularly in region B with presence of different looping and branching patterns. Furthermore the compatible base pairs are dark red where hue shows the numbers of different types C-G, G-C, A-U, U-A, G-U or U-G of compatible base pairs and sequence conservation of the base pairs. The saturation decreases with the number of incompatible base pairs which indicates the structural conservation of the base pair. Prediction of the consensus structure is much higher in accuracy than the secondary structure prediction from single sequences. However, ribosomal RNA secondary structure of *Ganoderma* sp.M1 & *Ganoderma* sp.M3 differ from each other with respect to their external loop, multi loop, bulge loop, hairpin loop and interior loop.

**Discussion**

The identification of natural herbs used for medicinal purpose is very important in order to obtain the desired species. For a long time, natural herbs were examined with the naked eye based on morphological characteristics, but it was impossible to distinguish between genetically related species by this method. Recently molecular approach has been introduced to detect the biological source or origin of natural herbs. The molecular procedure is convenient, rapid, accurate and requires only a small amount of sample. Furthermore this method can be employed even with sample in the form of powders or extracts in mixed drug formulas. However, this procedure does not directly reflect the pharmacological activity of the specimen in question.

The identification procedure of medicinal mushrooms is also required for quality control of functional health-aid preparations as well as nutritional supplements. However in our present study, morphological characteristics and molecular analysis of nuclear rDNA was attempted to identify the biological identity of two wild mushrooms (*Ganoderma* sp.M1 & *Ganoderma* sp.M3) collected from Similipal Biosphere Reserve. Morphologically the studied mushrooms were found similar with genus of *Ganoderma* described by Bhosle *et al*. [1]. To confirm the identity of the mushrooms, molecular study was done based on LSU rDNA sequences, because nuclear LSU rRNA gene have been revealed to have some variable regions with sequence divergency: D1, D2, D3 [8,27].

With the support of macroscopic and microscopic study, the homologous were obtained from sequence homology search using BLAST algorithm shown a good similarity against *Ganoderma* species. The results obtained from MSA performed using ClustalW2 tool and ClustalX 2.0

tool followed by reconstruction of phylogenetic tree supported the fact that *Ganoderma* sp.M1 (ENA: HF545883) are closely related to *Ganoderma applanatum* (EU232303.1) belonging to order Polyporales of the Basidiomycotina which are evolutionary close on the basis of phenotypic characteristics. Further the second strain *Ganoderma* sp.M3 (ENA: HF545884) which showed less homology with the members of Group-I but somewhat homologous to *Ganoderma* sp.M1 (ENA: HF545883) may be due to environmental effects or evolution [10]. Therefore more extensive investigations are needed to clarify the phylogenetic relatedness of *Ganoderma* sp.M1 and *Ganoderma* sp.M3 or *Ganoderma* sp.M3 with other species of *Ganoderma.* Further we evaluate an alternative method applicable to ribosomal RNA (rRNA) genes that increases information content without addition of nucleotides. As non-coding RNA fragments of the genome, the rRNA gene is generally capable of folding into a secondary structure. In most cases, these structures are necessary for cell function and are thus evolutionarily conserved. Accordingly, structural information may be treated as a conserved marker. Secondary structures of ribosomal RNA therefore offer an additional source of information for tree reconstruction. In particular this is a major advantage in cases where secondary structures are much conserved, yet mutations of nucleotides occur frequently [11]. 28S RNA secondary structure of *Ganoderma* sp.M1 & *Ganoderma* sp.M3 have been constructed by using RNA Fold web Server [16]. Though the results of 28S rRNA sequence analysis revealed that both mushrooms (*Ganoderma* sp.M1 & *Ganoderma* sp.M3) were 87% identical, but it showed low query coverage percentage (23 %) which implicates the weak similarity between them. RNA secondary structure analysis also showed great variation among them with respect to their external loop, multi loop, bulge loop, hairpin loop and interior loop. Remarkably, these secondary structure were similar despite the fact that nucleotide sequences of rRNA themselves exhibit a low degree of similarity. Apparently, evolution is acting at the level of rRNA secondary structure, not rRNA nucleotide sequence [3].

## Conclusion

The studied mushrooms were found similar with genus of *Ganoderma* based on their morphological characteristics. On the basis of morphological study, followed by cluster analysis, it was revealed that the newly identified strain *Ganoderma* sp.M1 (ENA: HF545883) is a close homologous of *Ganoderma applanatum* species which is one of the common medicinal mushrooms. Again another identified strain *Ganoderma* sp.M3 (ENA: HF545884) shown less similarity with other species of *Ganoderma* but closer to *Ganoderma* sp.M1 might be due to same geographical location. The results of 28S rRNA sequence analysis revealed that both mushrooms (*Ganoderma* sp.M1 & *Ganoderma* sp.M3) having weak similarity with each other, which is also supported by observation of most variable regions at their secondary structural state. Therefore, it is concluded both the mushrooms (*Ganoderma* sp.M1 & *Ganoderma* sp.M3) having strong divergence at their sequence and structural level.

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