THE DISTRIBUTION, GENETIC DIVERSITY AND USES OF *GANODERMA* MUSHROOMS IN OSHANA AND OHANGWENA REGIONS OF NORTHERN NAMIBIA

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ABSTRACT

Basidiomycetous fungi, including *Ganoderma lucidum*, have a variety of uses such as providing nutrition and as medical remedies. The mushroom *G. lucidum* has been used for a long time to cure liver problems, heart condition, asthma, cancer high blood pressure and arthritis. Recently, it has been associated with boosting immune systems in HIV infected persons. It is for these reasons that the mushroom has attracted a lot of attention leading to proposals of cultivating to increase supply to the Southern African market. This study was initiated with the objectives of determining the uses of *Ganoderma* by local communities; establish the distribution and genetic diversity of *Ganoderma* species. A survey was conducted in the 10% households of Ohangwena and Oshana regions of northern Namibia. All the sites where *Ganoderma* were encountered were geo-referenced and a sample was collected for DNA extraction. A questionnaire for face-to-face interviews was designed and applied to the two regions; data was analysed using SPSS software. *G. Lucidum* isolates that were found were geo-referenced to produce a distribution map. The host plants where the *Ganoderma* species were found were recorded. *Ganoderma* DNA was used for phylogenetic and genetic diversity analyses using CLUSTAL W2 program and used for phylogenetic and genetic diversity analyses using the MEGA4 programme. A total of 142 isolates of *Ganoderma* species comprising *G. lucidum, Ganoderma tsugae*, *Ganoderma neojaponicum* and *Ganoderma applanatum* were collected. The information survey revealed that *Ganoderma* species have a variety of traditional uses, including veterinary applications. Genetic diversity of 1% was observed among the collected *Ganoderma* species from the two regions and this might be explained by the diversity of the host trees recorded. *Ganoderma* species were found to be more distributed in Ohangwena region than in Oshana.

Keywords: *Ganoderma lucidum,* Medicinal uses, *Ganoderma* distribution, Ohangwena, Oshana, Genetic diversity

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ACRONYMS

RFLP	Restriction Fragment Length Polymorphism
RAMs	Random Amplified Microsatellites
ITS	Internal Transcribed Spacer
ZERI	Zero Emissions Research Initiative
DAAD	Deutscher Akademischer Austauch Dienst
AFLP	Amplified Fragment Length Polymorphism
GPS	Global Positioning System
MEGA	Molecular Evolutionary Genetics Analysis

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DEDICATION

This thesis is dedicated to my grandmother Saima Sakaria Nakatana (Pundo). Kuku, I still feel your love in my daily life, please do not let go off me, thank you for having been the best grandmother to me. May your soul rest in eternal peace; we shall meet on the day of resurrection. I love you.

DECLARATION

I, Nailoke Pauline Kadhila-Muandingi, declare hereby that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree in any other institution of higher education.

…….. Date……..

Nailoke Pauline Kadhila-Muandingi

CHAPTER 1: INTRODUCTION

1.1 General introduction

Mushrooms have long been valued as tasty, nutritious food by different societies throughout the world. In the developing world, including Africa, mushrooms are used as food and medicine in many societies. In Africa, however, the medicinal and nutritional importance of mushrooms has not been adequately studied and documented, (Munyaziza and Oldeman, 1996, p. 455). In the developed world, mushrooms now occupy a central position in natural products research such as medicine and food. For instance, there is now an increasing evidence that mushrooms have a wide range of medicinally important compounds that have anticancer and antiviral activity; offering great hope for the development of new drugs for ailments like HIV/AIDS, Avian influenza and the many cancers that afflict humanity today (Chang and Miles, 2004, p. 241).

Mushroom growing is also a source of considerable economic value to small and large scale farmers in the developed world, notably in Japan, China, Europe and the USA. Africa lags behind in this unfolding 'mushroom revolution' (Kuo, 2007, p. 1). In Namibia, the utilization of wild mushrooms as food is very common, wild edible fungi provide two main benefits to people, as source of food and income. In Namibia, it is very common to see people, particularly young women selling mushrooms by the roadside just after the start of the rainy season. *Termitomyces*

schimperi, Terfezia pfeilii (Kalahari Desert truffles) are some of the most hunted wild mushrooms in rural areas of Namibia (Mshigeni and Chang, 2000, p. 4). The awareness on wild edible mushrooms and their importance to people is generally poor and many of the collections done are for personal use (Mshigeni and Chang, 2000).

1.2 Statement of the problem

The cultivation of mushrooms in Namibia was introduced in the late 1990s and all the species being cultivated are not indigenous. The main problem is that the Namibian mushrooms are not studied and as a result, there is lack of information on basidiomycetes in the country. For instance the indigenous *Ganoderma* species are not well known and are not documented.

There is an increased demand for *Ganoderma* species world wide (Chang and Mshigeni, 2004, p.29). Knowing the distribution of *Ganoderma* species, genetic variation and uses will help researchers on mushrooms to start cultivating the *Ganoderma* species. This can lead to commercial production, thus making *Ganoderma* a source of foreign currency if exported. It is also important to know the uses, distribution and genetic diversity of *Ganoderma* species for conservation purposes. Since the *Ganoderma* species are on high demand worldwide because of their medicinal importance, there is likelihood of over-harvesting these mushrooms, so regulations can be introduced to regulate the process of harvesting.

This study was carried out to determine the distribution and genetic diversity of the *Ganoderma* species and their uses in Ohangwena and Oshana regions in Namibia. The *Ganoderma* species are of medicinal importance and they have been used in China and Japan for 4000 years as a health tonic and as folk medicine for liver problems, heart conditions, asthma, cancer, high blood pressure and arthritis (Dupler, 2001, p. 1).

1.3 Objectives of this study

a) To determine the distribution of *Ganoderma* species in Oshana and Ohangwena regions of Northern Namibia.

b) To determine the genetic diversity among *Ganoderma* species from Ohangwena and Oshana regions of Northern Namibia.

c) To determine the use of *Ganoderma* species by the local people in Ohangwena and Oshana regions of Northern Namibia.

1.4 Study questions

a) What is the distribution of *Ganoderma* species in Oshana and Ohangwena regions of Northern Namibia?

b) Is there genetic variation among *Ganoderma* species occurring in Oshana and Ohangwena regions of Northern Namibia?

c) What are the uses of *Ganoderma* species by the local people in Oshana and Ohangwena regions of Northern Namibia?

1.5 Research hypotheses

a) *Ganoderma* species are unevenly distributed in Ohangwena and Oshana regions of Namibia, because of variations in vegetation diversity within the two regions.

b) There is genetic variation among *Ganoderma* species due to variable hosts trees where they are growing.

c) There are various medicinal uses of *Ganoderma* species by local people

CHAPTER 2: LITERATURE REVIEW

2.1 Mushrooms

Mushrooms are visible reproductive structures in the form of fruiting bodies of basidiomycetes, and develop seasonally to produce and disperse spores. The mushrooms in the Basidiomycetes division have spores that are attached to basidia, which are specialized cells on the spore-bearing surface (Stamets and Wu Yao, 1999, p. 12). Basidiomycetes belong to the Kingdom Fungi. Fungi are eukaryotic organisms; resembling plants in having cell walls, but differ in lacking chlorophyll. They are heterotrophic and depend upon organic matter for nutrition and consequently live saprotrophically, parasitically, or symbiotically on or with other organisms. About 10 000 different species of mushrooms are known of which the majority are harmless, however it should be noted that there are poisonous mushrooms that can even be fatal, (Van der Westhuizen and Eicker, 1994, p. 9).

Some basidiomycetes like the Reishi (*Ganoderma lucidum*), Shiitake (*Lentinula edodes*) and Maitake (*Grifola frondosa*) are medicinally important as immuno-boosters, and are being used as remedies for cancers, diabetes, high blood pressure, and in the improvement of the conditions of HIV/AIDS victims (Mshigeni and Chang, 2001, p. 33). Medicinal mushrooms also occur in the various Namibian ecosystems and they include several *Ganoderma* species, which are the leading medicinal mushrooms in the world (Stamets and Wu Yao, 1999, p. 12).

2.2 Characteristics of *Ganoderma*

Ganoderma (Basidiomycota: Ganodermatales) is a genus of wood-inhabiting fungus on monocots, dicots, and gymnosperms. Some species are saprophytic, but several are pathogens that cause decay in roots, butts, and trunks of living trees. The name *Ganoderma* is derived from the Greek *ganos* meaning brightness or shining and *derma* meaning skin, while the specific epithet *lucidum* is Latin for shining. It is also known as Ling zhi in Chinese (Chang, 2003, p. 1202). The genus *Ganoderma* was established as early as 1881 by the Finnish botanist Karsten on the basis of its shiny epidermis, and *Ganoderma lucidum* was taken as the representative species for the genus (Gottlieb, Ferrer and Wright, 2000, p. 1033).

There are multiple species of *Ganoderma,* scientifically known to be within the *G. lucidum* species complex and mycologists are still researching the differences between species within this complex of species (Gottlieb and Wright, 1999, p. 661). It must be noted that there are poisonous mushrooms too. About 10 000 different species of mushrooms are known of which the majority are harmless. However, it should be noted that there are poisonous mushrooms that can even be fatal, (Van der Westhuizen and Eicker, 1994, p.10).

Members of the family Ganodermataceae are characterized by unique double-walled basidiospores. The shape and size of basidiospores and the texture of pileus surfaces are important characteristics that distinguish members of the Ganodermataceae. According to Moncalvo, Wang, and Hseu, (1995, p. 223), the macromorphological and micromorphological characters of *Ganoderma* are extensively variable, and

more than 250 species have been described worldwide, of which most are found in the tropics and temperate geographical regions, including North and South America, Africa, Europe, and Asia, growing as a parasite or saprotroph on a wide variety of trees (Moncalvo et al.,1995, p. 223; Ryvarden, 1991, p.235), but it is also described as the most difficult genus among the polypores to classify (Soon and Hack, 2004, p. 742).

Ganoderma species grow on dead or dying wood log of hardwood. It is recognized by its bright red colors and smooth look that darkens as it matures. Most of the species are fan or kidney shaped with zones. *Ganoderma* is a member of the Polypores, a group of fungi characterized by the presence of pores, instead of gills on the underside of the fruiting body. *Ganoderma* species contain protein-bound polysaccharides known to have medicinal properties (Dupler, 2001, p. 1). In the last 20 years, *Ganoderma* has been tested in human clinical studies and is thought to be beneficial for a wide variety of disorders, including neurasthenia, insomnia, rhinitis, and duodenal ulcers (Ying, 1987, p. 176).

Figure 1. Photo showing a distinctive basidiocap or fruiting body of *G. lucidum*

2.3 Ecological importance of fungi

Some fungi interconnect tree's microscopic root hairs, absorbing glucose in exchange for the minerals and water the fungus obtains from the soil, a symbiotic relationship called commensalism. The fungi may also create plant hormones that stimulate trees to grow. About 80% of trees depend on these mycorrhizal fungi (Van der Westhuizen and Eicker, 1994, p. 192). Examples of these are the *Terfezia pfeilii* (Kalahari Desert truffles).

Saprophytic fungi grow in dead wood or dead organic material, breaking it down, and return nutrients to the soil so that new life can flourish. This is an essential function for our ecosystems (Stamets, 1997, p. 10). One example of these fungi are the *Pleurotus* species. Other fungi like honey mushrooms and some *Ganoderma*

species are parasitic, attacking living trees and sometimes killing them, paving the way for the saprophytic species. Some parasitic fungi also become saprophytic after they kill the tree (Stamets, 1997, p. 9). This project focused on *Ganoderma* mushrooms which are also of economically and ecologically important as a source of medicine and nutriceutical products as well as their role in nutrient cycling as white rot decomposers of dead wood (Buchanan, 2001, p.27).

2.4 Genetic diversity assessment and its importance

Genetic diversity is a commonly used expression to describe the heritable variation found within biological entities and can be measured at the individual, population, and species level, (Lowe, Harris and Ashton, 2004, p. 62). Without genetic diversity, a population cannot evolve and it cannot adapt to environmental changes. Given the importance of genetic diversity for both short-term adaptation to environmental changes and long-term impact on species and communities, the preservation of genetic diversity has been a high priority in many conservation programmes (Templeton, 1996, p. 60). Genetic diversity can also be used to monitor or infer other historical or demographic processes that are essential for making wise decisions related to biodiversity management at the species level and above (Hawksworth, 2003, p. 33).

2.5 Molecular studies for basidiomycetes

Morphological data have been shown to be of limited value for fungal systematic due to their inherent simplicity, evolutionary convergence, parallelisms, and phenotypic plasticity (Hofstetter, Clemencon, Vilgalys, and Moncalvo 2002, p. 1043). In recent years, mycologists have exploited molecular techniques for the identification and study of evolutionary relationship among fungi (Thon and Royse, 1999, p. 468). Studies done on fungi fossils suggest that fruiting bodies morphology in homobasidiomycetes is evolutionarily flexible, and that in at least some cases small genetic changes can result in major morphological transformations. In contrast, the fossils suggest that certain fruiting body morphologies have remained unchanged over tens of millions of years. Taken together, the observation above suggest that homobasidiomycetes morphological evolution is characterized by periods of rapid change as well as long periods in which there is little or no morphological change (Hibbeti, Grimaldi, and Donoghue, 1997, p. 990).

Most molecular systematic studies have utilized sequences or Restriction Fragment Length Polymorphism (RFLP) data from nuclear or mitochondria genes encoding ribosomal DNA (rDNA) sometimes relying on one or the other locus for their studies (Thon and Royse, 1999, p. 468). The nuclear and mitochondrial rDNA genes are accessible and widely used among mycologists. An important characteristic of these genes is the availability of universal PCR primers that can reduce the work of obtaining large data sets (Thon and Royse, 1999, p.468).

While the functionally conserved nature of rRNA genes allows the development of highly conserved primers, this has not been possible for protein coding genes. Despite this, a review of the recent literature shows a trend among mycologists to include multiple loci in their data sets (O'Donnell, Cigelnik and Nirenberg 1998, p. 465). Undoubtedly, recent advances in PCR, automated sequencing, and sequence analysis have opened the possibility of including much larger data sets in studies. Another PCR-based technique is Random Amplified Microsatellite (RAMS), which combines several characteristics of RAPD and microsatellite analysis. In this technique, the DNA between distal ends of two closely related microsatellites is amplified (Zakaria, Kulaveraasingham, Guan, Abdullah, and Wan, 2005, p. 24).

According to Sobla, Carrera, Morales, and Roussos (2007, p.16), the collection, characterization and conservation of mushroom genetic resources have become central to scientific, biological and industrial strategy for developing new generation of commercial strains. Wild populations present not only mushrooms to be potentially cultivated, but also a prominent source of biosynthetic products and genes for genetic engineering. The capability of being able to detect, and in some cases identify, fungi from DNA sequences alone has also proved to be particularly useful for environmental studies, particularly in environments such as the soil where it is often difficult to obtain growing cultures or fruit bodies (Bridge, 2002, p. 2).

In 1995, p. 231, Moncalvo, Wang and Hseu, isolated the DNA of *Ganoderma tsugae* and *Ganoderma lucidum* and found that it was hard to tell the difference between the two species. The study applied several DNA techniques and found that, while

Ganoderma tsugae and *G.lucidum* do appear to separate with some methods. The picture is unclear, so further studies are required before reliable conclusions can be drawn (Engelbrecht and Volk, 2005, p. 3). An even more recent study by Hong and Jung (2004, p. 743), found that *G .lucidum* from Asia was in its own group, whereas *G. lucidum* from Europe and the Americas was more closely related to *G. tsugae* (Engelbrecht and Volk, 2005, p. 4). This remains a major taxonomic question to be resolved.

2.6 Molecular markers

According to Bachmann (1994, p. 404), molecular markers can label the mitochondria, chloroplast, or nuclear genomes; they can identify the organism and its taxonomic association from fragmentary remains and even where morphology cannot distinguish strains. Molecular markers can label the mitochondria, chloroplast, or nuclear genomes. Markers in the nuclear DNA are polymorphisms in the nucleotide sequence at homologous (allelic) sites. These markers can define a multilocus genotype characteristic for an individual or a clone; selected markers can be diagnostic for a population or a species.

If we consider molecular genetic DNA markers in terms of the type of information they provide at a single locus, only three main categories can be described, in increasing degrees of interest, the bi-allelic dominant, such as Random Amplification of Polymorphic (RAPD), Amplified Fragment Length Polymorphism (AFLP), the bi-allelic co-dominant, such as restriction fragment length polymorphism (RFLP), Single Stranded Conformation Polymorphism (SSCP) and the multi-allelic co-

dominant, such as the microsatellites (Vignal, A., Milan, D., Sancristobala, M., and Eggen, 2002, p. 276). Amongst others, the microsatellite DNA markers has been the most widely used due to its easy use by simple PCR, followed by a denaturing gel electrophoresis for allele size determination, and to the high degree of information provided by its large number of alleles per locus (Vigna et al, 2002, p. 276). Some molecular markers are described below in details.

2.6.1 Microsatellites

Microsatellites or Simple Sequence Repeats (SSR) are a class of PCR-based DNA markers for genetic mapping. SSR markers can be employed in the development of unique allelic profiles for establishing individual identity. They are frequent and almost randomly distributed in most eukaryotic genomes. Molecular markers based on microsatellite sequences detect extra high levels of polymorphism and can be easily assayed by PCR. The repeated sequence is often simple, consisting of two, three or four nucleotides (di-nucleotide repeat: CACACACA or tri-nucleotide repeat: ATGATGATGATG) and can be repeated 10 to 100 times (Pestsova, Ganal, and Röder, 2000, p. 690).

Simple sequence repeats often serve to modify genes with which they are associated. The influence of SSRs on gene regulation, transcription and protein function typically depends on the number of repeats, while mutations that add or subtract repeat units are both frequent and reversible (Kashi and King, (2006, p. 253). According to Bowen and Wheals, (2006, p. 634) one common example of a microsatellite is a $(CA)_n$ repeat, where n is a variable between alleles. These markers

often present high levels of inter- and intra-specific polymorphism, particularly when tandem repeats number ten or greater.

Microsatellites can be amplified for identification by the polymerase chain reaction (PCR) process, using the unique sequences of flanking regions as primers. DNA is repeatedly denatured at a high temperature to separate the double strand, and then cooled to allow annealing of primers and the extension of nucleotide sequences through the microsatellite. The process results in production of enough DNA to be visible on agarose or polyacrylamide gels; only small amounts of DNA are needed for amplification as thermocycling in this manner creates an exponential increase in the replicated segment (Hammock and Young, 2005, p. 1631). Microsatellites have proved to be versatile molecular markers, particularly for population analysis, but they are not without limitations. Microsatellites developed for particular species can often be applied to closely related species, but the percentage of loci that successfully amplify may decrease with increasing genetic distance (Hancock, and Simon (2005, p. 115). Several researchers have suggested that microsatellites and other short sequence repeats can act as 'evolutionary tuning knobs' (King, Soller, and Kashi 1997, p.37; Fondon and Garner 2004, p 18059; Verstrepen, Jansen, Lewitter, and Fink 2005, p. 986, and Vinces 2009, p. 1214).

2.6.2 Amplified fragment length polymorphism (AFLP) analysis

Amplified fragment length polymorphism (AFLP) is a highly sensitive method for detecting polymorphisms in DNA developed by Keygene and Wageningen from The Netherlands (Savelkoul, Aarts, de Haas, Dijkshoorn, Duim, Otsen., Rademaker et al.,

1999, p. 0097**)**. For AFLP analysis only a small amount of purified genomic DNA is needed. DNA first undergoes restriction enzyme digestion, and a subset of DNA fragments is then selected for PCR amplification and visualization. The AFLP technique for DNA fingerprinting enables visualization of restriction fragments without knowledge of nucleotide sequence; it also allows researchers to obtain genetic fingerprints rapidly without prior sequence information (Dehaan, Ehlke, Sheaffer, Muehlbauer, and. Wyse, 2003, p. 402). AFLP can:

a) Show the degree of genetic diversity or similarity over the whole genome and determine the genetic stability of strains.
b) It can indicate the evolutionary relationship between varieties and form the basis for trait specific genetic marker discovery.
c) It allows the comparison of future fingerprints to fingerprints that have been carried out in the past and it can result in a clear graphical representation of genetic similarities and differences (Dehaan et al, 2003, p.404).

2.6.3 ITS region and 18S rRNA

The internal transcribed spacer (ITS) region of rDNA (ITS1, 5.8S rRNA gene and ITS2) constitutes one of the most widely applied molecular markers in phylogenetic studies and species differentiation. The ITS region is part of the rDNA cistron, which consists of 18S, ITS1, 5.8S, ITS2, and 26S, and is present in several hundred copies in most eukaryotes (Hyosig and Renner, 2005, p. 581). According to Gardes and Bruns (1993, p. 113), the ITS region is now perhaps the most widely sequenced DNA region in fungi. It has typically been most useful for molecular systematics at the species level, and even within species (e.g., to identify geographic races). Because of its higher degree of variation than other genic regions of rDNA (for small and large subunit rRNA), variation among individual rDNA repeats can sometimes be observed within both the ITS regions. In addition to the standard ITS1 and ITS4 primers used by most laboratories, several taxon-specific primers have been described which allow selective amplification of fungal sequences.

In the transcribed region, ITS are found on either side of 5.8S rRNA gene and are described as ITS1 and ITS2. The length and sequences of ITS regions of rDNA repeats are believed to be fast evolving and therefore may vary**.** Universal PCR primers designed from highly conserved regions flanking the ITS and its relatively small size (600-700 bp) enable easy amplification of ITS region and this is due to high copy number (up to-30000 per cell of rDNA repeats). This makes the ITS region an interesting subject for evolutionary and phylogenetic investigations (Sharma, Rustgi, Balyan and Gupta, 2002, p. 38).

The 18S rRNA gene is a multi-copy gene that is slowly evolving and highly conserved among fungi, making it an attractive target for the detection of fungus in clinical specimen (Embong, Hitam, Yean, Rashid, Kamarudin, Khaironi et al., 2008, p. 3), The 18S rRNA is the eukaryotic nuclear homologue of 16S rRNA, it is the structural RNA for the small component of eukaryotic cytoplasmic ribosomes, and thus one of the basic components of all eukaryotic cells. The 18S rDNA data is widely used in molecular analysis to reconstruct the evolutionary history of organisms, especially invertebrates, as its slow evolutionary rate makes it suitable to reconstruct ancient divergences. The 18S rRNA in most eukaryotes is in the small ribosomal subunit, and the large subunit contains three rRNA species (the 5S, 5.8S and 28S rRNAs) (Freire, Arias, Méndez and Insua, 2009, p.1).

2.6.4 The Polymerase Chain Reaction (PCR)

The Polymerase Chain Reaction (PCR) is a technique used to amplify a particular fragment or sequence of DNA. It was invented in 1983 by Kary Mullis (Rabinow, 1996, p.1). PCR is now a common and often indispensable technique used in medical and biological research laboratories for a variety of applications (Saiki, Gelfand, Stoffel, Scharf, Higuchi, Horn et al., 1988, p. 487). These include DNA cloning for sequencing, DNA-based phylogeny, or functional analysis of genes and the diagnosis of hereditary diseases. The identification of genetic fingerprints which are used in forensic sciences and paternity testing, and the detection and diagnosis of infectious diseases (Saiki et al., 1988, p.487). PCR occurs when two oligonucleotide primers anneal to a DNA template in a proximity and orientation which allows the DNA polymerase to synthesize the DNA sequence which lies between the two primers.

PCR amplification reaction mixture is usually composed of:

a) A double-stranded DNA molecule which is the template that contains the sequence to be amplified.
b) Primers, these are single-stranded DNA molecules which can anneal or bind to a DNA sequence in the template DNA which has the complementary sequence.
c) dNTPs, this is a mixture with equal amounts of dATP, dTTP, dGTP, and dCTP which are the nucleotide subunits which will be put together to form new DNA molecules in the PCR amplification procedure.
d) *Taq* DNA polymerase, this is the enzyme which synthesizes the new DNA molecules using the dNTPs.
e) PCR buffer, this is usually stocked as a 10 concentrates. The buffer providing a suitable chemical environment for optimum activity and stability of the DNA polymerase
f) $Mg^{+}Cl_2$ (Magnesium Chloride), its concentration regulates the error rate during DNA synthesis.

The PCR usually consists of a series of 20 to 40 repeated temperature changes called cycles; each cycle typically consists of 2-3 discrete temperature steps. Most commonly PCR is carried out with cycles that have three temperature steps. The temperatures used and the length of time applied in each cycle depend on a variety of parameters. These include the enzyme used for DNA synthesis, the concentration of

divalent ions and dNTPs in the reaction, and the melting temperature of the primers (Rychlik, Spencer, and Rhoads 1990, p. 6409, Bartlett and Stirling, 2003, p. 1).

The first step consists of heating the reaction to a temperature of 94-96°C (or 98°C if extremely thermostable polymerases are used), which is held for 1-9 minutes. The second step is *Denaturation,* this step is the first regular cycling event and consists of heating the reaction to 94-98°C for 20-30 seconds. It causes the melting of DNA template and primers by disrupting the hydrogen bonds between complementary bases of the DNA strands, yielding single strands of DNA.

Annealing is the third step in which the reaction temperature is lowered to 50-65°C for 20-40 seconds allowing annealing of the primers to the single-stranded DNA template. Typically the annealing temperature is about 3-5°C below the melting temperature of the primers used. Stable DNA-DNA hydrogen bonds are only formed when the primer sequence very closely matches the template sequence. The polymerase binds to the primer-template hybrid and begins DNA synthesis.

After annealing, the next step is called extension or elongation*,* the temperature at this step depends on the DNA polymerase used; *Taq* polymerase has its optimum activity temperature at 75-80°C (Lawyer, Stoffel, Saiki, Chang, Landre, Abramson et al, 1993), and commonly a temperature of 72°C is used with this enzyme. At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTPs that are complementary to the template in 5' to 3' direction, condensing the 5'-phosphate group of the dNTPs with the 3'-hydroxyl group at the end of the nascent (extending) DNA strand. The extension time depends

both on the DNA polymerase used and on the length of the DNA fragment to be amplified. The final elongation which is a single step is occasionally performed at a temperature of 70-74°C for 5-15 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully extended. The last step is called the final hold; this step is performed at 4-15°C for an indefinite time may be employed for short-term storage of the reaction (Lawyer, Stoffel, Saiki, Chang, Landre, Abramson, 1993, p. 276).

2.7 Genetic Diversity Analysis Methods

2.7.1 Cluster analysis

The term cluster analysis was first used by Tryon in 1939; it encompasses a number of different algorithms and methods for grouping objects of similar kind into respective categories. In other words cluster analysis is an exploratory data analysis tool which aims at sorting different objects into groups in a way that the degree of association between two objects is maximal if they belong to the same group and minimal otherwise. Given the above, cluster analysis can be used to discover structures in data without providing an explanation or interpretation. Cluster analysis simply discovers structures in data without explaining why they exist (Basak, Magnuson, Niemi and Regal, 1988, p. 17). An important step in any clustering is to select a distance measure, which will determine how the similarity of two elements is calculated. This will influence the shape of the clusters, as some elements may be close to one another according to one distance and further away according to another (Basak et al, 1988, 18).

One example of clustering is Hierarchical clustering which builds (agglomerative), or breaks up a hierarchy of clusters. The hierarchical methods group data instances into a tree of clusters. There are two major methods under this category. One is the agglomerative method, which forms the clusters in a bottom-up fashion until all data instances belong to the same cluster (Clatworthy, Buick, Hankins, Weinman and Horne, 2005, p. 329). The other is the divisive method, which splits up the data set into smaller cluster in a top-down fashion until each cluster contains only one instance. Both divisive algorithms and agglomerative algorithms can be represented by dendrograms, and both methods are known for their quick termination (Clatworthy et al, 2005, p. 330).

Other advantages of these methods are that, it does not require the number of clusters to be known in advance, it computes a complete hierarchy of clusters, good result visualizations are integrated into the methods, and a flat partition can be derived afterwards by cutting through the dendrogram. Hierarchical clustering techniques use various criteria to decide at each step which clusters should be joined or split (Kotsiantis and Pintelas, 2004, p. 74).

CHAPTER 3: MATERIALS AND METHODS

3.1 Study area

The two regions are situated in the northern part of Namibia. They share the borders, though the vegetation types, soil types and the annual rainfall differ (Mendelsohn, Jarvis, Roberts, and Robertson, 2002, p. 15, 84 and 102). Ohangwena Region has an area of about 10,582 km^2 and a population of 227,728 (Central Bureau of Statistic and National Planning Commission of Namibia, 2003, p. 12) while Oshana region is about 5,290 km^2 and has a population of 161,977 (Central Bureau of Statistic and National Planning Commission of Namibia, 2003, p. 15). The people living in

Oshana and Ohangwena region speak the same dialect which is Oshiwambo.

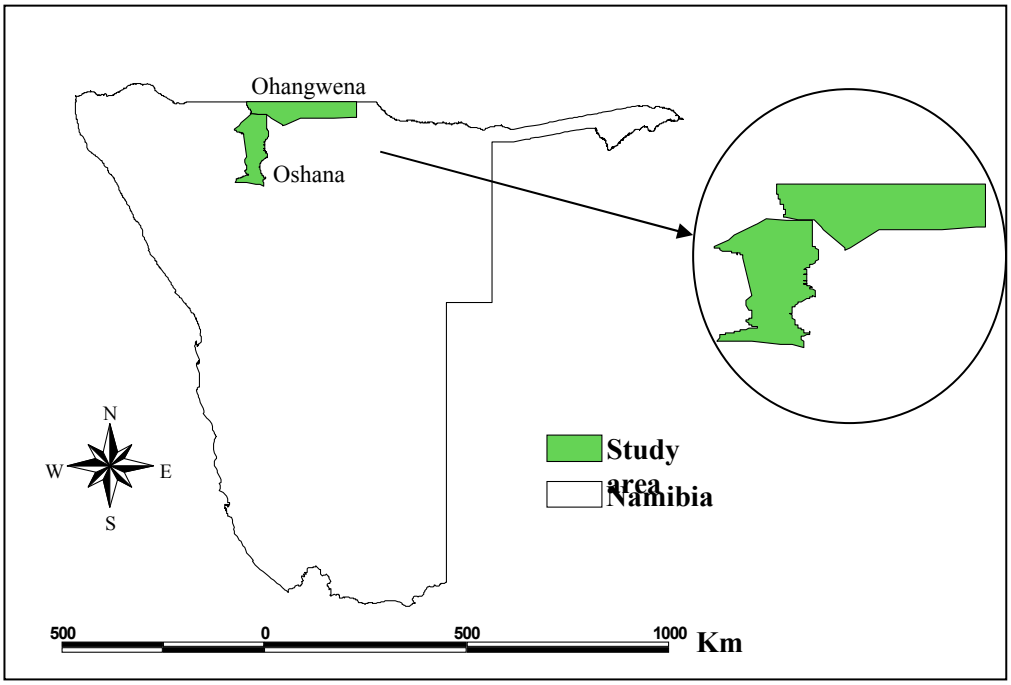

Figure 2. Map of Namibia showing the study area (Oshana and Ohangwena regions).

3.2 Mushroom collection and identification

Samples were collected from two regions; namely Oshana and Ohangwena (Fig.2) during the rainy season of the year 2008. Each of the two regions was divided into four equal parts of which four villages were to be randomly picked from each part. When in the field, there were floods in most of the Northern regions at the time of sampling, which delayed and affected sampling from the targeted villages in the two regions.

The *Ganoderma* species observed in the fields were morphologically identified using a mushroom Field Guide book for Southern Africa by Van der Westhuizen and Eicker, (1994*)*. Pictures were taken for future identification. In each field, specimen of each *Ganoderma* species observed and identified were labelled, dried and stored in stapled khaki paper bags before they were brought to the laboratory for DNA extraction to determine the genetic variation between and among the *Ganoderma* species. The locality and the substrate (if it was found growing on trees, dead log or grass) on which the mushrooms were found growing was described. The soil and the vegetation type were also described.

In Oshana region, the fields with most *Sclerocarya caffra* (marula) trees were targeted and all the trees found in the field were inspected to see if *Ganoderma* species were growing on them. The *Ganoderma* mushroom was shown to most people so that they could tell where they have seen them. They were told that if they saw them, they should not to touch them, but to inform the researcher to go and identify them. That was how most of the mushrooms were collected from the fields.

There were no specific methods to find the mushrooms, but rather to search by looking on every stump of *Sclerocarya caffra* well known marula tree stems in every sampled field. Most of the collected mushrooms were first photographed before a sample was taken. The geographic position was recorded using a handheld GPS, *Ganoderma* found growing as a clump at one site were regarded as one family and only a portion was taken using a mycologist knife to get a piece from those growing in big fruit bodies. While those with small fruiting bodies only two fruiting bodies were taken to represent those found at that specific place. In Ohangwena region, tree stumps around the cultivated fields were inspected of the *Ganoderma* species; the same method of searching used in Oshana was applied. All collected mushrooms were kept in khaki paper bags on which the village's name and region were written, care was taken not to mix the samples by stapling the papers once and by always holding them upright.

3.3 Drying of specimen

Drying was done outside in the sun and taken indoors when it started raining. All fruiting bodies were dried on top of their bags to avoid mixing and for them to dry faster than when inside the bags. The samples were brought to UNAM to the Zero Emission Research Initiative (ZERI) office where they were sorted and small tissues were cut off using sterile scalpels and placed in sterile tubes. The tubes were labelled with a permanent marker citing the letter X representing Oshana region and the letter Y representing Ohangwena region. Each letter was followed by the number representing a village in the region followed by the sample number. An example is X1.1, which meant that, the sample was from Oshana region, village one which was

Onyelelo and it was the first sample taken from that village. The fruiting bodies from where the small tissues were taken were also labelled to avoid the mixing of samples.

3.4 Questionnaire based study

Indigenous peoples around the world have sought knowledge of physical reality throughout the ages. Their understanding of the physical universe is codified in their indigenous knowledge systems. A major component of these systems is ethnobotanical knowledge, which refers to a cumulative body of traditional knowledge about the interaction between human societies and the plant kingdom, and, more specifically, how indigenous peoples perceive, manage, and utilize the plants around them. Largely oral in nature, ethnobotanical documentation is one way of capturing this body of knowledge in written and graphic form (Suminguit, 2005, p. 2).

A questionnaire was used to get information on indigenous knowledge on the uses of *Ganoderma* mushrooms. This questionnaire was administered face - to - face to the community members from 10 % of households in the selected villages. This means, if there are 100 households in the village, only 10 of these households were interviewed. The face-to face method was used because of illiteracy and communication gap within the targeted communities. The questions needed to be explained clearly to the respondents, and as a result direct questions were used. On several occasions, fresh or dry *Ganoderma* mushrooms were shown as stimuli. At the household level only elders were asked, this was done to make sure that the information collected will be trustworthy. The interviews were done in Oshiwambo,

the language which is spoken in both Ohangwena and Oshana regions. The questionnaire was meant to get information on the uses of *Ganoderma* species only, but other information on the uses of other mushrooms was also recorded (see copy of Questionnaire annex 1).

3.5 Distribution study

The location where the *Ganoderma* mushrooms were found growing was recorded using a hand held Global Positioning System receiver (GPS); the recorded points were transformed into a database and marked on the map to show the distribution of *Ganoderma* mushroom species in the two regions. No other mushrooms were found growing close or on the same stump with the *Ganoderma* mushrooms. The collected *Ganoderma* species were brought to the laboratory for DNA extraction and genetic analyses.

3.6 DNA extraction from the *Ganoderma* fruiting bodies

A total of a 101 samples were taken from one hundred and forty two samples collected from Oshana and Ohangwena regions. DNA extraction was done in the DNA laboratory at the Zoology Department of the Humbolt University in Berlin, Germany. The Qiagen kit was used and the protocol was as follows: The first step was to grind the *Ganoderma* mushroom fruit body tissue of about 1g into fine powder using a mortar and pestle. The powder was scooped out of the mortar using the tube, 360µl of ATL lysis buffer and 400 µl of buffer AL were added to each tube containing the powder, the tubes were vortexed for some seconds before incubating at 70˚C in the thermo mixer for 10 minutes before incubation, 400µl of 98% ethanol

was added before all the liquid was transferred into DNeasy Min Spin Colum tubes. The tubes were centrifuged for 1 minute at 8000 revolution per minute (rpm) to separate the liquid from the tissue.

The Min Spin Colum was transferred to new tubes and the DNA was washed with 500µl AW1 buffer, centrifuged for 1 minute at 8000 rpm to separate the liquid from the tissue, transferred to new tubes before washing with 500µl of AW2, centrifuged for 1 minute at 8000 rpm to separate the liquid from the tissue, transferred to new tubes before washing for the second time with 500µl of AW2, centrifuged for 3 minutes at full speed (14000 rpm). The Min Spin Colum was transferred for the last time to new tubes and the DNA was dissolved with 200µl of AE elution buffer, incubated at room temperature for 4 minutes, then centrifuged for 1 minute at 8000 rpm.

DNA (6µl) was mixed with the loading buffer and loaded into the gel in the electrophoresis immersed in TBE buffer; and the results were obtained after 30 minutes at 80 volts. The DNA gel was prepared with 5ml of TBE, 0.7g of agarose gel which was dissolved for two minutes or at least until completely dissolved in the microwave, 3µl of 10mg/ml ethidium bromide was added to the gel for the visibility of the DNA bands with the Utra violet light.

The PCR amplification was done using Internal Transcribed Spacers (ITS) ITS1 and ITS2 primers. It was carried out with a total volume of 35µl, comprising of 17.4µl of distilled water, 3.5µl of buffer, 1µl of dNTP, 1.5µl Mg^{2+}, 1µl ITS1 (forward primer), ITS2 (reverse primer), 0.35 µl of BSA to remove inhibitors, 7µl of Qiagen solution

to dissolve secondary structures, 0.25µl *Taq* polymerase and 2µl of DNA.The PCR amplification program was run at 96˚C for 3minutes, 96˚C for 30 seconds, 59˚C for 30 seconds, 72˚C for 1 minute, the last three were at 40 cycles. 72˚C 5 minutes and stored at 15˚C. The DNA sequencing gel was prepared with 4.5% Polyacrylamide 6M Urea powder, 18g of Urea powder, 7.5ml 30% Acrylamide solution, 6ml 10 x TBE buffer, 23ml of pure distilled water. The gel was electropholyzed at 51˚C for four hours at 2700 voltage.

Before sequencing, the DNA was precipitated using isopropanol alcohol, centrifuged for 20 minutes at 14000 rpm, purified using 78% ethanol and then, 1.5µl was loaded into the DNA sequencing machine. Primers used for 18S forward and reverse in this study are 5`-CAGGCGATGGTTCAATCAATT-3` as forward primer and 5`-ATTAGCATGGGATAATAGAATAG-3` as reverse primer. The two primers were selected carefully so that maximum results will be achieved.

Table1. Villages from Oshana region

Symbol	**Name of the village**	**Total samples from the village**	**Extracted samples**
X1	Onyelelo	5	3 (X1.1- X1.3)
X2	Oshoongela	7	5 (X2.1-X2.5)
X3	Edudhi	12	8 (X3.1-X3.8)
X4	Okalondo	2	2 (X4.1-X4.2)
X5	Okamukwa	9	6 (X5.1-X5.6)
X6	Oshamuhenye	3	3 (X6.1-X6.3)
X7	Indangungu	2	2 (X7.1-X7.2)
X8	Iilagati	10	7 (X8.1-X8.10)
X9	Ombwayanetuntu	7	5 (X9.1-X9.7)
X10	Ondombeyanamupunda	4	3 (X10.1-X10.3)

Table 2. Villages from Ohangwena region

Symbol	Name of the village	Total samples from the village	Extracted samples
Y1	Okatope	1	1 (Y1.1)
Y2	Eembaxu	33	20 (Y2.1-Y2.20)
Y3	Onghwiyu	7	5 (Y3.1-Y3.5)
Y4	Oshandi	12	10 (Y4.1-Y4.10)
Y5	Onanghulo	15	9 (Y5.1-Y5.9)
Y6	Oshela	3	3 (Y6.1-Y6.3)
Y7	Efidi	8	6 (Y7.1-Y7.8)
Y8	Ohakafiya	3	3 (Y8.1-Y8.3)

3.7 Data Analyses

Since this is the first time the Namibian *Ganoderma* species were collected for research purposes, BLAST interrogations were indeed done at the NCBI website to confirm if the mushrooms species collected are *Ganoderma*. Phylogenetic analyses and the genetic diversity calculation were conducted in Molecular Evolutionary Genetics Analysis (MEGA4). MEGA is an integrated tool for conducting automatic and manual sequence alignment, inferring phylogenetic trees, mining web-based databases, estimating rates of molecular evolution, and testing evolutionary hypotheses. The genetic diversity was calculated using the Maximum Composite Likelihood method in MEGA4 (Tamura, Dudley, Nei, and Kumar 2007, p. 1597). Phylogenetic analysis present identities and a phylogenetic tree were determined from the 18S region of the *Ganoderma* species.

The SPSS was used to analyse the data collected through face-to-face interviews. Chromas Version Chromas 1.15 which is a Windows shareware/freeware application that displays and prints chromatogram files from ABI automated DNA sequencers

was used to view DNA sequenced from ITS1 and ITS4. This program allows basecall editing and exporting of the sequence to a text file. It can view and print chromatogram, export sequence in text or FASTA format, reverse sequence and search for exact matches or redundant codes (http://www.technelysium.com.au/chromas.html, 2008).

DNA sequence editing and alignment was done using Clustal W 2, which is a general purpose multiple sequence alignment program for DNA or proteins which produces biologically meaningful multiple sequence alignments of divergent sequences, then calculates the best match for the selected sequences, It calculates the best match for the selected sequences, and lines them up so that the identities, similarities and differences can be seen (http://www.ebi.ac.uk/Tools/clustalw/index.html, 2007).

ArcView GIS 3.2 software programs was used to transform the data collected with the Global Positioning System receiver (GPS) into a database and mark them on the map to show the distribution of *Ganoderma* species in the two regions.

CHAPTER 4: RESULTS

4.1 Mushroom collection and identification

The collected *Ganoderma* mushrooms were identified using the Field guide mushrooms for Southern Africa by Van der Westhuizen and Eicker, (1994, p. 135*)*. Most of the *Ganoderma* mushrooms collected from Oshana region were found by the stem of living or stumps of *Sclerocarya caffra* (well known as marula) trees as well as in fields where there is a marula tree decomposing in the soil (Figure 3). Most of the *Ganoderma* in the fields were cut off and thrown away, because they are considered as weeds within the mahangu fields. Other trees found to have *Ganoderma* growing from their stumps are the *Colophospermum mopane* (Omusati) and *Combretum zeyheri* (Omuhama) trees. No fruit bodies were found on living mopane tree.

Figure 3. Fruiting body growing on a tree stump and on the mahangu field were a *S. caffra* (marula) tree is decomposing.

In Ohangwena region, the following tree stumps were found to be the host of *Ganoderma* species. *Colophospermum mopane*, *Combretum collinum*, *Acacia*

sieberana , *Baikiaea plurijuga, Terminalia sericea*, *Combretum frarans*, *Acacia erioloba, Grewia retinervis* and *Combretum zeyheri* (Table 3.for local names).

Table 3. Host trees and their local names in area of study

Scientific name	**Local name**	**Live tree / Stump**
Combretum collinum	Omupupwaheke	Stump
Acacia sieberana	Omutyuula	Stump
Baikiaea plurijuga	Omupupa	Stump
Terminalia sericea	Omwoolo	Stump
Colophospermum mopane	Omusati	Stump
Combretum frarans	Omushendje	Stump
Acacia erioloba	Omwoonde	Stump
Grewia retinervis	Omutoka	Stump
Sclerocarya caffra	Omwoongo	Live tree
Combretum zeyheri	Omuhama	Stump

Some fruiting bodies were found to be growing in the open field where there were no stumps or living trees, but when examined closely, it was found that there was either a decaying tree in the soil or some barks of a tree from which the mushroom were growing. Ohangwena region was found to have more *Ganoderma* fruiting bodies than Oshana region; about 81 samples were collected compared to 61 from Oshana region. There were no other mushrooms found growing in association with the *Ganoderma* in all the two regions sampled. Most of the collected *Ganoderma* species from the two regions were identified as *Ganoderma tsugae* and *Ganoderma lucidum* based on the morphological features.

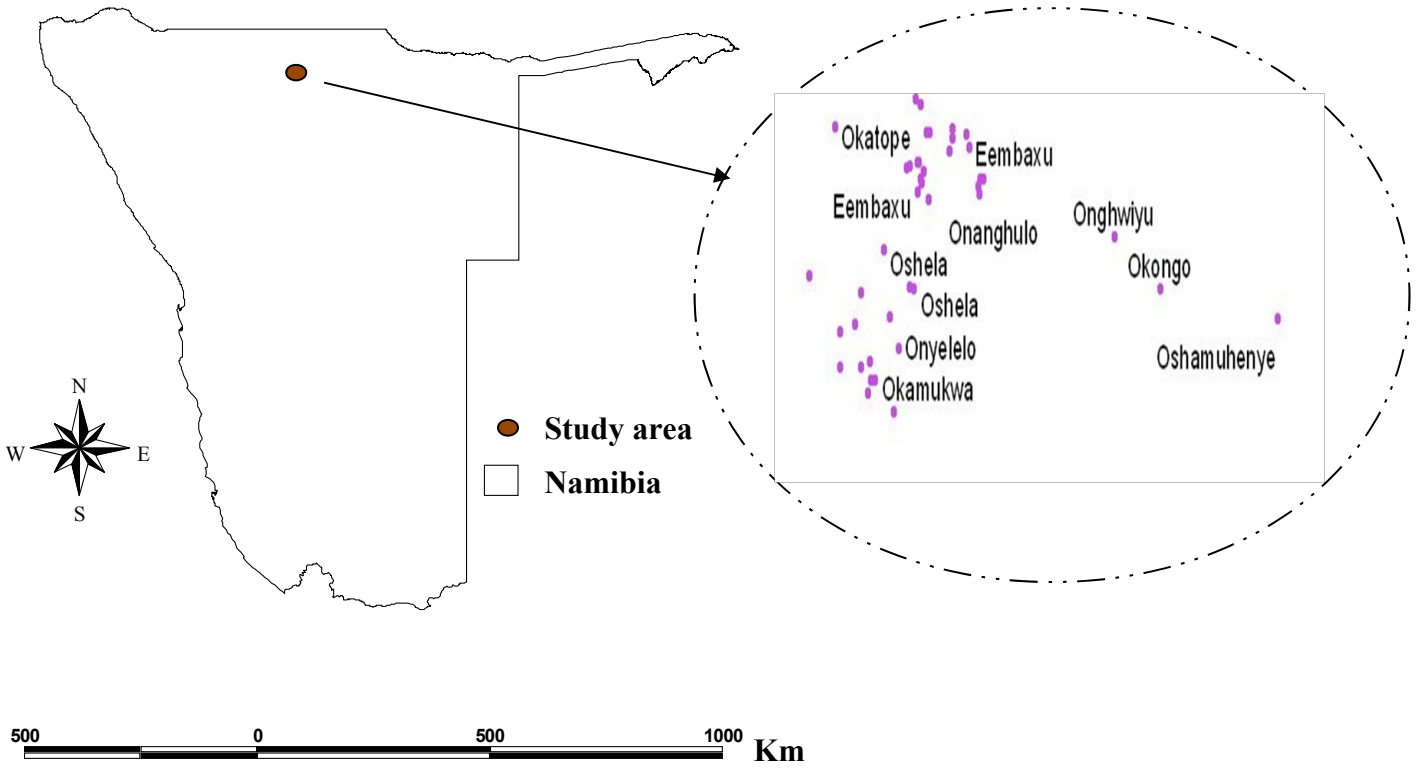

Figure 4. Map of Namibia showing the distribution of *Ganoderma* species in Oshana and Ohangwena regions.

The above map shows the villages where the *Ganoderma* samples were collected generated from the coordinates recorded while in the field. *Ganoderma* species were found to be more distributed in Ohangwena region, which was found to have more tree stumps than Oshana region, it is also having more woodland vegetation than Oshana region (Mendelsohn et al, 2000, p. 107).

4.2 Questionnaire study

The data collected through face-to-face interviews were computerized using the SPSS programme. In total, 51 questionnaires were completed during the study. The interviews were only not done in the villages where samples for *Ganoderma* mushrooms were collected, but also in some villages which were not sampled but accessible. All the people interviewed in both regions knew mushrooms especially most edible ones, but knew less about the medicinal value of the mushrooms.

In Oshana region, it was found that only those people who relocated from Ohangwena knew the medicinal uses of mushrooms, including the *Ganoderma* species. In Ohangwena region, most people interviewed knew the medicinal value of more than one mushroom species. Of all people interviewed in Oshana region, only 34% use mushrooms for medicinal purposes compared to 62% in Ohangwena region. In most households only women were found and as a result, most of the answers were given by them. The questionnaire revealed that other mushrooms which were not known or considered to be of medicinal uses by the researchers are also used for medicinal purposes, especially in Ohangwena region (Figure 6).

Table 4. Types of mushrooms found in Oshana and Ohangwena region and their uses

Local name	Scientific name	Edibility	Poisonous	Substrate	Medicinal properties	Purpose
Owowa/ Oova	*Termitomyces schimperi*	Yes	No	Termite mound	Yes	Wounds in children's heads (used in a cream form mixed with oil)
Uuhilili/ Okahauhwilili	*Termitomyces sagittiformis*	Yes	No	Humus in cultivated or open field	Yes	Wounds in children's heads (used in a cream form mixed with oil)
Omatumbula	*Terfezia pfeilii*	Yes	No	Not known, but grow underground in the soil	No	Food
Oshaamuya/ Oshihamuya	*Termitomyces reticulatus*	Yes	No	Surrounding termite moulds	No	Food
Kakalahambo	*Panaeolus papilionaceus*	Yes	No	Manure/cow dung	No	Food
Omapakululu	*Ganoderma* species	No	No	Tree stumps and poles of houses and fences (dead wood)	Yes	Stress (used in powder form)
Okapaka	*Hexagona tenuis*	No	No	Poles and stumps of different trees	Yes	Shock and trauma or stress (used in liquid form)
Omagege	*Amanita* species	No	Yes	Mostly found everywhere (field and poles)	Not known	On allows used for hunting

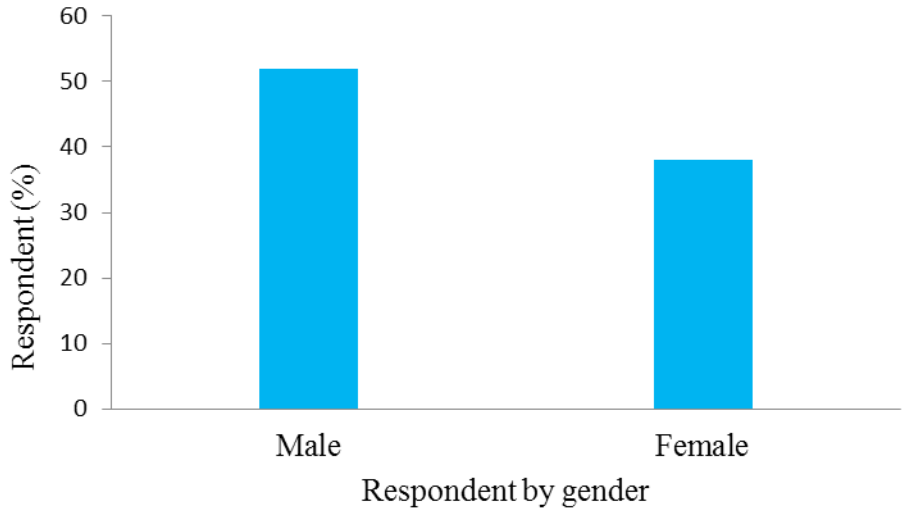

Figure 5. Response according to the gender on the use of mushrooms for medicinal purposes.

Figure 5 above shows that male respondents were more than females and this demonstrates that men know more about the medicinal use of mushrooms than their female counterparts.

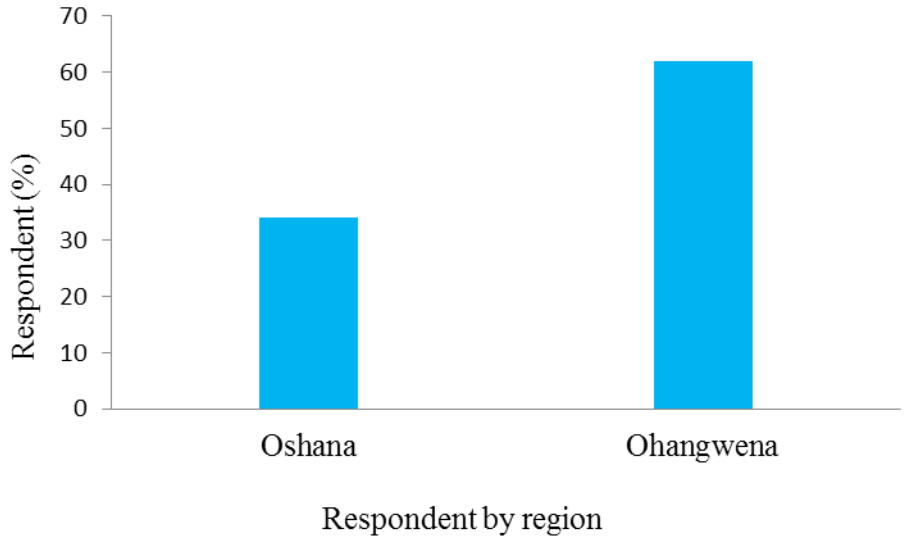

Figure 6. Responses on the use of mushrooms for medicinal purposes as per region.

Figure 6 above shows that people in Ohangwena region who were included in the study know more about the use of mushroom for medicinal purposes than those who were included in the study in Oshana region.

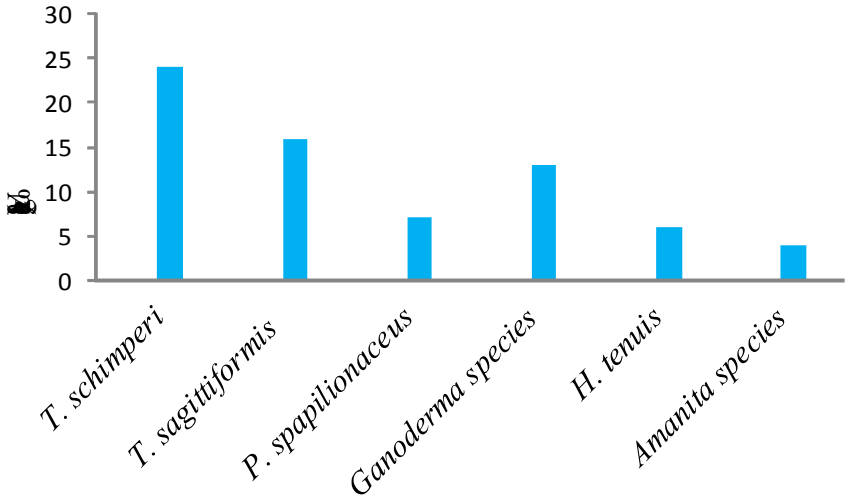

Figure 7. Types of mushrooms used for medicinal purposes.

According to the people interviewed in this study, more than six mushroom species are used for medicinal purposes in Oshana and Ohangwena regions as shown in Figure 7 above. *Ganoderma* is at the third position and the *Amanita* species which are poisonous are not said to be used that much.

4.2 DNA extraction

Among 101 samples which were ground, 80 of them gave good quality DNA which was prepared for the PCR amplification. The DNA which was not of good quality also produced poor and low quality PCR products (figure 9).

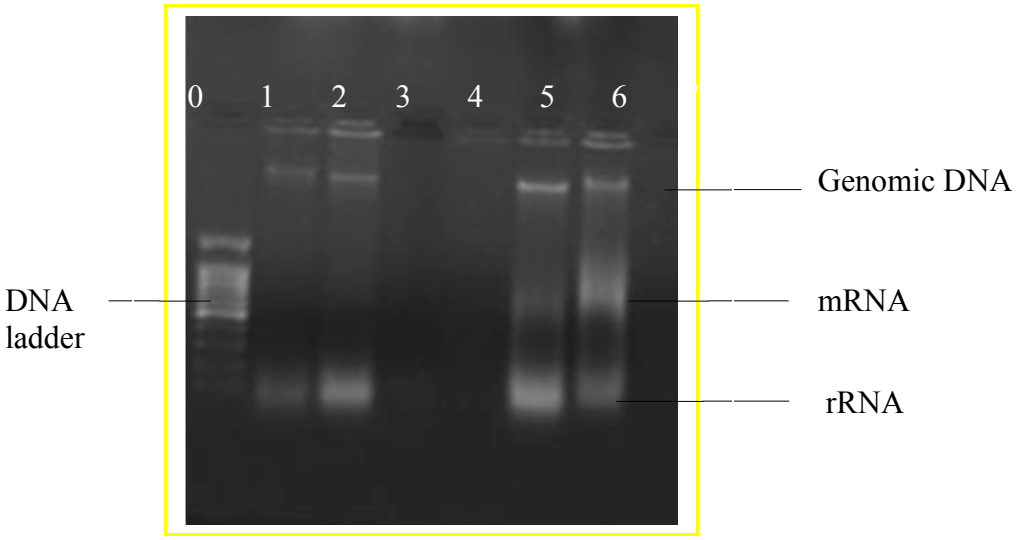

Figure 8. DNA quantification visualization on a 1% agarose gel.

It was observed that some of the bands are not showing any DNA e.g. lane 3 and lane 4 which are showing low quality DNA (Figure 8).

Figure 9. Gel electrophoresis of the amplified rDNA internal transcribed sequence 18S region of the *Ganoderma* species.

4.3 DNA sequencing

Sequencing was done using ITS1 and ITS4 primers, and it was found to be heterozygous and cloning was required in order to distinguish between the heterozygous regions. The 18S primer 5`-(CAGGCGATGGTTCAATCAATT-3`) as forward primer and 5`-(ATTAGCATGGGATAATAGAATAG-3`) as reverse primer were used for sequencing. When analysed further, it was found that the collected *Ganoderma* species are genetically diverse.

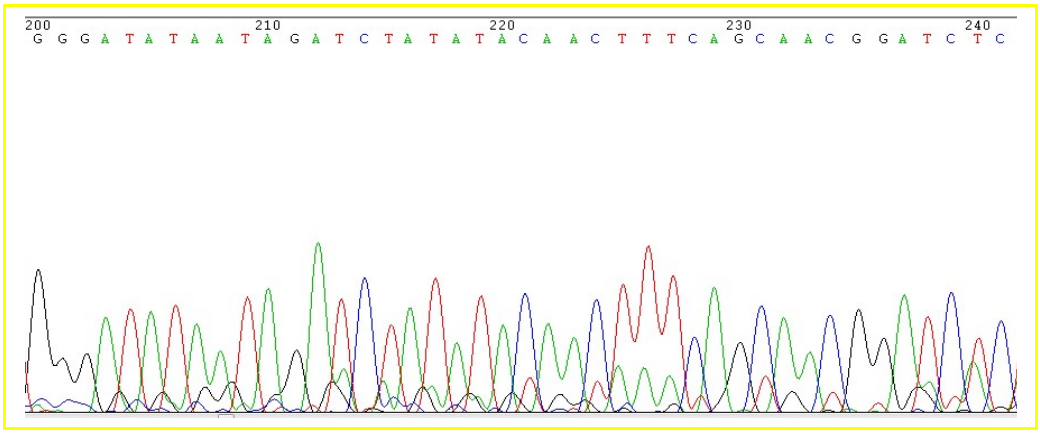

Figure 10. An example of the output generated from an automated DNA sequence (Chromas package) when ITS1 and ITS4 primers were used.

Figure 10 was obtained from direct sequencing of genomic PCR products. It was observed that the output after sequencing using ITS1 and ITS4 primers revealed heterozygosity when it was analysed further; ITS1 and ITS4 produced high frequencies that almost every sample was different from the other.

4.4 Confirmations of *Ganoderma* species

The BLAST searches alignment of the sequences revealed 96% similarities to *Ganoderma* species (Figure 11), and 90% similarities to *G. lucidum* (Figure 12). In the case of the protein coding genes, the probes used were the amino acidic sequences and the blast procedure was performed using the tblastn algorithm. In the case of the rRNA genes, the probes were nucleotide sequences and the blast protocol used was blastn.

```
Query  17   GGGNTG-AGCT-GCCTTCC-AGGCATGTGC-CGCCCTGCTCANT-CACTCTACACCTGTG 71
            ||| || |||| ||||||| |||||||||| |||||||||| | ||||||||||||||||
Sbjct  9    GGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCAATCCACTCTACACCTGTG 68

Query  72   CACTTACTGTGGGTGACNGGATCGCAAAGCGGGCTTCTTGTCCGTTATAAAGCGCATCTG 131
            |||||||||||||||| |||||||||||||| |||||||||||| ||||||||||
Sbjct  69   CACTTACTGTGGGTGAC-GGATCGCAAAGCGGGC-TCTTGTCCGTTAT-AAGCGCATCTG 125

Query  132  TGGCCTGCGTTTATCACAAACTCTTTGAAAGTACTAGAATGTAATATTGGGATATAATAG 191
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  126  TGGCCTGCGTTTATCACAAACTCTTTGAAAGTACTAGAATGTAATATTGGGATATAATAG 185

Query  192  ATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCG 251
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  186  ATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCG 245

Query  252  AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC 311
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  246  AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC 305

Query  312  TTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGGTTGAAGNGGTCATGAAATCTTCAACT 371
            |||||||||||||||||||||||||||||||| |||| | | ||||||||||||||||
Sbjct  306  TTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGA-GTG-TCATGAAATCTTCAACT 363

Query  372  TGCAACCTCTTTGCGGAGTTTGTAGGCTTGGACTTGGAGGGCTTGTCGGCCTTTAACGGT 431
            |||||||||||||||||||||||||||||||||||||||||||||| ||||||||| |||
Sbjct  364  TGCAACCTCTTTGCGGAGTTTGTAGGCTTGGACTTGGAGGGCTTGTTGGCCTTTAATGGT 423

Query  432  C  432
            |
Sbjct  424  C  424
```

Figure 11. The collected *Ganoderma* species sequence aligned against subject sequence in the Genbank database, with 96% similarity to *Ganoderma* species. The aligned sequence was obtained from the 5.8S rRNA gene using ITS2. The query is

the isolated *Ganoderma* species while the subject is the *Ganoderma* species found in the Gene bank database.

```
Query  105  ATCTGTGGCCTGCGTTTATCACAAACTCTTTGAAAGT-ACTAGAATGTAATATTGGGATA 163
            |||||| |||||||||||||||||||| | ||||| || |||||||  ||||| |||
Sbjct  160  ATCTGT-GCCTGCGTTTATCACAAACTCTAT-AAAGTAAC-AGAATGT-GTATTGCGATG 215

Query  164  TAATAGATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAAC 223
            ||| | ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  216  TAACACATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAAC 275

Query  224  GCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA 283
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  276  GCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA 335

Query  284  CGCACCTTGCGCTCCNTGG-ATTCCGAGGAGCATGCCTGTT-GAGTGGTCATGAAATCTT 341
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Sbjct  336  CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTG-TCATGAAATCTT 394

Query  342  CAACTTGCAACCTCTTTGCGGAGTTTGTAGGCTTGGACTTGGAGGGCTTGNCGGCCTTTA 401
            |||| | ||| || |||| ||  |||||||||||||||||||| |||| ||||| |||
Sbjct  395  CAACCTACAAGCT-TTTGTGG--TTTGTAGGCTTGGACTTGGAGG-CTTGTCGGCCGTTA 450

Query  402  ACGGTCGGCTCCTCTTAAATGCATTAGCTTGATTCCTTGCGGATCGGCTGTCGGTGTGAT 461
             |||||||||||||||||||||||||||| |||||||||||||||| ||||||||||
Sbjct  451  TCGGTCGGCTCCTCTTAAATGCATTAGCTTGGTTCCTTGCGGATCGGCTCTCGGTGTGAT 510

Query  462  AAAATGTCTACGCCGTGACCCGTGAAGCCGTTTGGATGAGCTTCCAACCCGTC  514
            ||  |||||||||||||||| ||||||| ||||||  ||||||| |||| |||
Sbjct  511  AA--TGTCTACGCCGTGACC-GTGAAGC-GTTTGGC-GAGCTTCTAACC-GTC  557
```

Figure 12. The collected *Ganoderma* species sequence aligned against subject sequence in the Genbank database, with 90% similarity to *G. lucidum* strain (WD-2038 voucher TFM-F 18922). The aligned sequence was obtained from the 18S ribosomal RNA gene, partial sequence; ITS1, 5.8S ribosomal RNA gene, and ITS2, complete sequence and 18S ribosomal RNA gene partial sequence.

4.5 Genetic diversity analyses

In this study, 18S primers yielded good results and when analysed further, they showed that the collected *Ganoderma* species were genetically diverse. When the overall mean genetic diversity was calculated, it was found to be 0.01. The bootstrapping tree produced half of the clades with low probability, showing values of less than 50%. All clades equal to 50% or less were classified as the same in this study (Figure 14). The table showing the calculation and estimation of evolutionary divergence between sequences also show that there is genetic diversity among the collected *Ganoderma* species (Table 5).

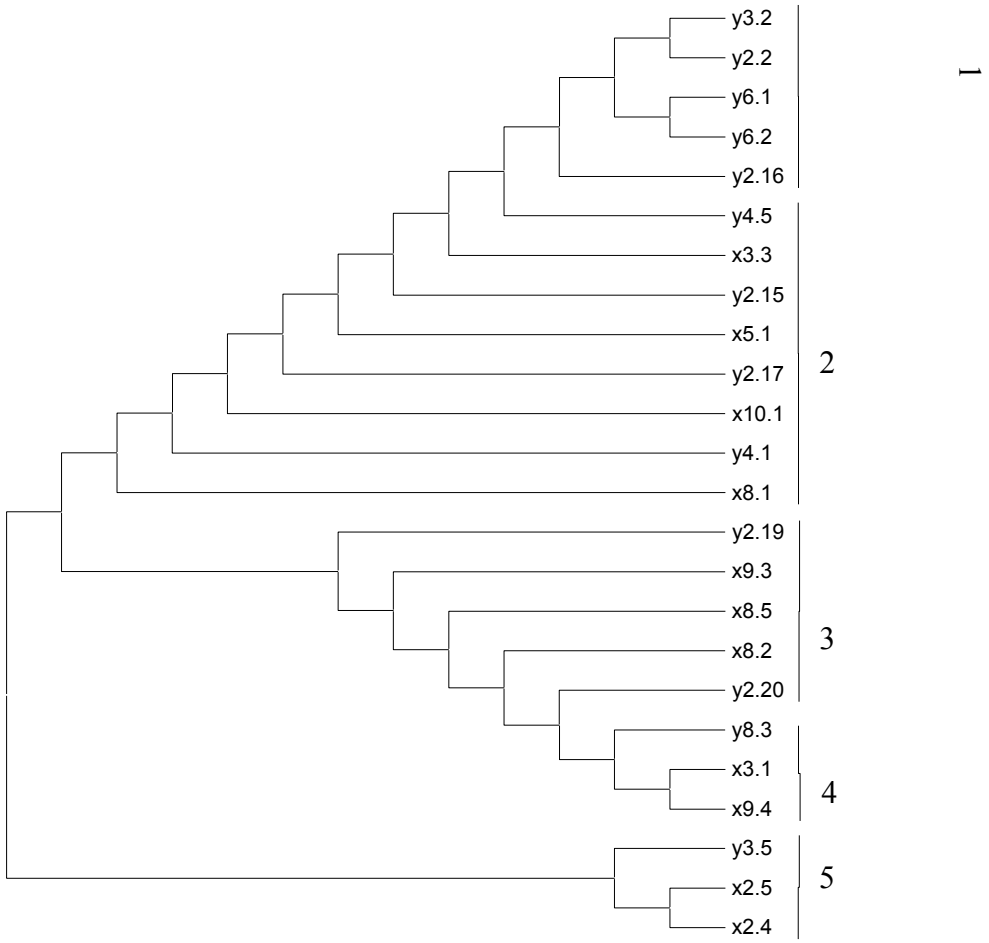

Figure 13. Phylogenetic relationships of *Ganoderma* species inferred from the sequences of the 18S region using MEGA4. Lines at the right of the figure link members of the same origin or species, forming one cluster.

Figure 13 shows the relationships of the collected *Ganoderma* species, Cluster one shows species from Ohangwena region Eembaxu and Oshela villages respectively. Cluster two show relationships of samples collected from both Ohangwena and Oshana regions, being Oshandi, Edudhi, Eembaxu, Ondombeyanamupunda and Iilagati villages resipectivery. Cluster three is also showing relationships of species

collected from both regions from Eembaxu, Ombwayanetuntu and Iilagati villages. Cluster four has species collected from Ohakafiya, Edudhi and Ombwayanetuntu villages. Cluster five has species collected from Onghwiyu and Oshoongela.

The evolutionary history was inferred using the Maximum Parsimony method. Tree 1 out of 350 most parsimonious trees (length = 17) is shown. The consistency index is 0.941176 (0.923077), the retention index is 0.972973, and the composite index is 0.915739 (0.898129) for all sites and parsimony-informative sites (in parentheses). The Maximum Parsimony tree was obtained using the Close-Neighbor-Interchange algorithm [2, pg. 128] with search level 2 [1, 2] in which the initial trees were obtained with the random addition of sequences (10 replicates). The codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset. There were a total of 360 positions in the final dataset, out of which 12 were parsimony informative. Phylogenetic analyses were conducted in MEGA4.

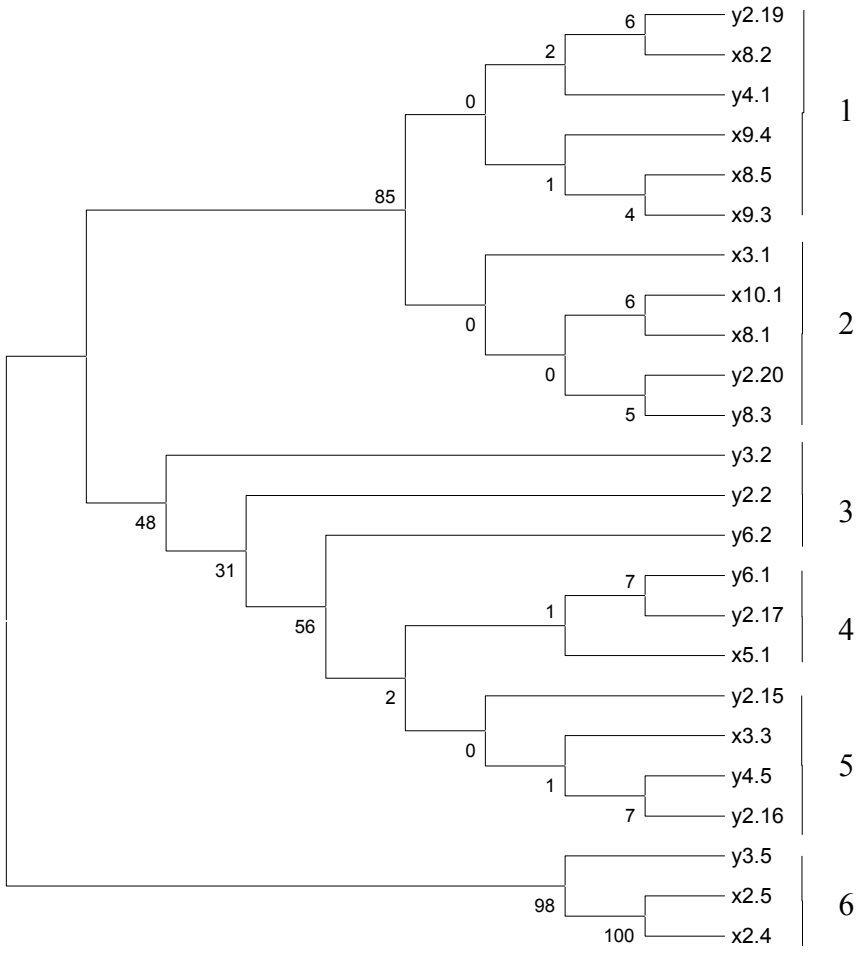

Figure 14. The bootstrapping of the *Ganoderma* species inferred from the sequences of the 18S region using Neighbor-Joining method.

Figure 14 shows the bootstrapping of the *Ganoderma* species collected from Ohangwena and Oshana regions. Low boot strap values mean that the clades supposed to collapse, an indication of low genetic variability. It can also be seen that most of the clades have values of less than 50% which are classified as same in this study. This is an indication that more molecular data might be required to support the existence of the clades. However, morphological variances have been observed among the collected *Ganoderma* species e.g. the colour and shape.

Table 5. Test of the homogeneity of Substitution Patterns between Sequences.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. y3.2		0.11	0.05	0.05	0.05	0.04	0.05	0.05	0.05	0.04	0.04	0.11	0.11	0.11	0.11	0.11
2. y8.3	0.01		0.01	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3. y2.17	0.08	0.23		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01
4. y2.16	0.10	0.21	1.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01
5. x5.1	0.07	1.00	1.00	1.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6. y2.15	0.13	0.30	1.00	1.00	1.00		0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01
7. y4.5	0.08	1.00	1.00	1.00	1.00	1.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8. y6.1	0.07	1.00	1.00	1.00	1.00	1.00	1.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9. 6.2	0.09	1.00	1.00	1.00	1.00	1.00	1.00	1.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00
10. 2.5	0.19	0.40	1.00	1.00	1.00	1.00	1.00	1.00	1.00		0.00	0.00	0.00	0.00	0.00	0.00
11. x2.4	0.20	0.40	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		0.00	0.00	0.00	0.00	0.00
12. x9.3	0.01	1.00	0.24	0.24	1.00	0.31	1.00	1.00	1.00	0.39	0.38		0.00	0.00	0.00	0.00
13. x9.4	0.01	1.00	0.22	0.21	1.00	0.31	1.00	1.00	1.00	0.35	0.40	1.00		0.00	0.00	0.00
14. x10.1	0.02	1.00	0.22	0.22	1.00	0.28	1.00	1.00	1.00	0.39	0.40	1.00	1.00		0.00	0.00
15. y2.19	0.01	1.00	0.22	0.21	1.00	0.28	1.00	1.00	1.00	0.36	0.40	1.00	1.00	1.00		0.00
16. y2.20	0.02	1.00	0.23	0.23	1.00	0.32	1.00	1.00	1.00	0.41	0.40	1.00	1.00	1.00	1.00	

The estimates of the disparity index per site are shown for each sequence pair above the diagonal. A Monte Carlo test was used to estimate the *P*-values, which are shown below the diagonal. The significant ones are marked with yellow highlights. The estimates of the disparity index per site are shown for each sequence pair above the diagonal. So as a result, it can be concluded that sequences have not evolved with the same pattern of substitution.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1. y3.2																								
2. y2.15	0.01																							
3. y4.5	0.01	0.00																						
4. y6.1	0.01	0.00	0.00																					
5. y6.2	0.01	0.00	0.00	0.00																				
6. y2.2	0.00	0.00	0.00	0.00	0.00																			
7. x3.3	0.01	0.00	0.00	0.00	0.00	0.00																		
8. x2.5	0.00	0.01	0.01	0.01	0.01	0.00	0.01																	
9. y3.5	0.00	0.02	0.02	0.02	0.02	0.01	0.02	0.00																
10. x2.4	0.00	0.01	0.01	0.01	0.01	0.00	0.01	0.00	0.00															
11. x10.1	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01														
12. y2.19	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.00													
13. y2.20	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.00	0.00												
14. x8.1	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.00	0.00	0.00											
15. x8.5	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.00	0.00	0.00	0.00										
16. x3.1	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.00	0.00	0.00	0.00	0.00									
17. x5.1	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01								
18. x8.2	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01							
19. y8.3	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00						
20. y2.16	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01					
21. y2.17	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.00				
22. y4.1	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.01			
23. x9.3	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.01	0.00		
24. x9.4	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.01	0.00	0.00	

Figure 15. Estimates of Evolutionary Divergence between Sequences.

In figure 15, the differences in base composition bias per site are shown. The substitution patterns are homogeneous among lineages, the compositional distance correlated with the number of differences between sequences.

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Oshela     TCTTCCCTATCAACTTTCGATGTTTGGGTATTGGCCAAACATGGTGGCAACGGGTAACGGAGGGTTAGGGCTCGACCCCGGAGAAGGAGCCTGAGAAACGGCTACTACATCCAAGGAAGG 120
Oshela     TCTTCCCTATCAACTTTCGATGTTTGGGTATTGGCCAAACATGGTGGCAACGGGTAACGGAGGGTTAGGGCTCGACCCCGGAGAAGGAGCCTGAGAAACGGCTACTACATCCAAGGAAGG 120
Oshand     TCTTCCCTATCAACTTTCGATGTTTGGGTATTGGCCAAACATGGTGGCAACGGGTAACGGAGGGTTAGGGCTCGACCCCGGAGAAGGAGCCTGAGAAACGGCTACTACATCCAAGGAAGG 120
Okamuk     TCTTCCCTATCAACTTTCGATGTTTGGGTATTGGCCAAACATGGTGGCAACGGGTAACGGAGGGTTAGGGCTCGACCCCGGAGAAGGAGCCTGAGAAACGGCTACTACATCCAAGGAAGG 120
Eembax     TCTTCCCTATCAACTTTCGATGTTTGGGTATTGGCCAAACATGGTGGCAACGGGTAACGGGGGTTAGGGCTCGACCCCGGAGAAGGAGCCTGAGAAACGGCTACTACATCCAAGGAAGGG 120
Eembax     TCTKCCCTATCAACTTTCGATGGTTGGGTATTGGCCAAACATGGTGGCAACGGGTAACGGRRTTAGGGCTCGAYYCCGGAGAAGGAGCCTGAGAAACGGCTACYACATCCAAGGAAGGGG 120
Eembax     TCTKCCCTATCAACTTTCGATGGTTNGGTATTGGCCAAACATGGTGGCAACGGGTAACGGRRTTAGGGCTCGAYYCCGGAGAAGGAGCCTGAGAAACGGCTACTACATCCAAGGAAGGGG 120
Onghwi     TCTGCCCTATCAACTTTCGACGGCTAGGTCTTGGCCAGCCGTGGTGACAACGGGTAACGGAGGGTTAGGGCTCGACCCCGGAGAAGGAGCCTGAGAAACGGCTACTACATCCAAGGAAGG 120
Ondomb     TCTGCCCTATCAACTTTCGATGGTAAGGTATTGGCTTACCATGGTTTCAACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGG 120
Eembax     TCTGCCCTATCAACTTTCGATGGTAAGGTATTGGCTTACCATGGTTTCAACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGG 120
Eembax     TCTGCCCTATCAACTTTCGATGGTAAGGTATTGGCTTACCATGGTTTCAACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGG 120
Ohakaf     TCTGCCCTATCAACTTTCGATGGTAAGRTATTGGCYTWCCATGGTTTCAACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGG 120
Ombway     TCTGCCCTATCAACTTTCGATGGTAGGATAGTGGCCTACCATGGTTTCAACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGG 120
Ombway     TCTGCCCTATCAACTTTCGATGGTANGATANTGGCNTACCATGGTWTCAACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGG 120
Oshoon     TCTGCCCTATCAACTTTCGATGGTAGGATAGTGGCCTACCATGGTGGCAACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGG 120
Oshoon     TCTGCCCTATCAACTTTCGATGGTAGGATAGTGGCCTACCATGGTGGCAACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGG 120
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Oshela     CAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAATACTGATACAGGGCTCTTTTGGGTCTTGTAATCGGAATGAGTACAATTTAAATCCCTTAACGAGGAAC 240
Oshela     CAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAATACTGATACAGGGCTCTTTTGGGTCTTGTAATCGGAATGAGTACAATTTAAATCCCTTAACGAGGAAC 240
Oshand     CAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAATACTGATACAGGGCTCTTTTGGGTCTTGTAATCGGAATGAGTACAATTTAAATCCCTTAACGAGGAAC 240
Okamuk     CAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAATACTGATACAGGGCTCTTTTGGGTCTTGTAATCGGAATGAGTACAATTTAAATCCCTTAACGAGGAAC 240
Eembax     CAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAATACTGATACAGGGCTCTTTTGGGTCTTGTAATCGGAATGAGTACAATTTAAATCCCTTAACGAGGAAC 240
Eembax     CAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAATACTGATACAGGGCTCTTTTGGGTCTTGTAATYGGAATGAGTACAATTTAAATCCCTTAACGAGGAAC 240
Eembaxu    CAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAATACTGATACAGGGCTCTTTTGGGTCTTGTAATYGGAATGAGTACAATTTAAATCCCTTAACGAGGAAC 240
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Onghwi    CAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAATACTGATACAGGGCTCTTTTGGGTCTTGTAATCGGAATGAGTACAATTTAAATCCCTTAACGAGGAAC 240
Ondomb    CAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAATACTGATACAGGGCTCTTTTGGGTCTTGTAATTGGAATGAGTACAATTTAAACCTCTTAACGAGGAAC 240
Eembax    CAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAATACTGATACAGGGCTCTTTTGGGTCTTGTAATTGGAATGAGTACAATTTAAACCTCTTAACGAGGAAC 240
Eembax    CAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAATACTGATACAGGGCTCTTTTGGGTCTTGTAATTGGAATGAGTACAATTTAAACCTCTTAACGAGGAAC 240
Ohakaf    CAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAATACTGATACAGGGCTCTTTTGGGTCTTGTAATTGGAATGAGTACAATTTAAAYCYCTTAACGAGGAAC 240
Ombway    CAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAATACTGATACAGGGCTCTTTTGGGTCTTGTAATTGGAATGAGTACAATTTAAATCYCTTAACGAGGAAC 240
Ombway    CAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAATACTGATACAGGGCTCTTTTGGGTCTTGTAATTGGAATGAGTACAATTTAAAYCYCTTAACGAGGAAC 240
Oshoon    CAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAATACTGATACGGGGCTCTTTTGGGTCTCGTAATTGGAATGAGTACAATCTAAATCCCTTAACGAGGAAC 240
Oshoon    CAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAATACTGATACGGGGCTCTTTTGGGTCTCGTAATTGGAATGAGTACAATCTAAATCCCTTAACGAGGAAC 240
          ************************************************************************************************************************

Oshela    AATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGTGGTTAAAAAGCTCGTAGTTGAACCTTGGGCCTGGCTGGCCGGTCCGCCT 360
Oshela    AATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGTGGTTAAAAAGCTCGTAGTTGAACCTTGGGCCTGGCTGGCCGGTCCGCCT 360
Oshand    AATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGTGGTTAAAAAGCTCGTAGTTGAACCTTGGGCCTGGCTGGCCGGTCCGCCT 360
Okamuk    AATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGTGGTTAAAAAGCTCGTAGTTGAACCTTGGGCCTGGCTGGCCGGTCCGCCT 360
Eembax    AATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGTGGTTAAAAAGCTCGTAGTTGAACCTTGGGCCTGGCTGGCCGGTCCGCCT 360
Eembax    AATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGYGGTTAAAAAGCTCGTAGTTGAACCTTGGGCCTGGCTGGCCGGTCCGCCT 360
Eembax    AATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGYGGTTAAAAAGCTCGTAGTTGAACCTTGGGCCTGGCTGGCCGGTCCGCCT 360
Onghwi    AATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGCCTGGCTGGCCGGTCCGCCT 360
Ondomb    AATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAAACTTGGGCCTGGCTGGCAGGTCCGCCT 360
Eembax    AATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAAACTTGGGCCTGGCTGGCAGGTCCGCCT 360
Eembax    AATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACTTGGGCCTGGCTGGCAGGTCCGCCT 360
Ohakaf    AATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAAMCTTGGGCCTGGCTGGCMGGTCCGCCT 360
Ombway    AATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGCCTGGCTGGCCGGTCCGCCT 360
Ombway    AATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGCCTGGCTGGCCGGTCCGCCT 360
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Oshoon     AATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGTCTGGCTGGCCGGTCCGCCT 360
Oshoon     AATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGTCTGGCTGGCCGGTCCGCCT 360
           ************************************************************************************************************************
Oshela     CACCGCGTGCACTGGTCCGGCCGGGCCTTTCCCTCTGCGGAACCCCATGCCCTTCACTGGGTGTGGCGGGGAAACAGGACTTTTACTTTGAAAAAATTAGAGTGCTCAAGGCAGGCCTAT 480
Oshela     CACCGCGTGCACTGGTCCGGCCGGGCCTTTCCCTCTGCGGAACCCCATGCCCTTCACTGGGTGTGGCGGGGAAACAGGACTTTTACTTTGAAAAAATTAGAGTGCTCAAGGCAGGCCTAT 480
Oshandi    CACCGCGTGCACTGGTCCGGCCGGGCCTTTCCCTCTGCGGAACCCCATGCCCTTCACTGGGTGTGGCGGGGAAACAGGACTTTTACTTTGAAAAAATTAGAGTGCTCAAGGCAGGCCTAT 480
Okamuk     CACCGCGTGCACTGGTCCGGCCGGGCCTTTCCCTCTGCGGAACCCCATGCCCTTCACTGGGTGTGGCGGGGAAACAGGACTTTTACTTTGAAAAAATTAGAGTGCTCAAGGCAGGCCTAT 480
Eembax     CACCGCGTGCACTGGTCCGGCCGGGCCTTTCCCTCTGCGGAACCCCATGCCCTTCACTGGTGTGGCGGGGAAACAGGACTTTTACTTTGAAAAAATTAGAGTGNGCANNNNNNNNNNNNN 480
Eembax     CACCGCGTGCACTGGTCCGGCCGGGCCTTTCCCTCTGCGGAACCCCATGCCCTTCACTGGGTGTGTTGGGGAACCAGGACTTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTT 480
Eembax     CACCGCGTGCACTGGTCCGGCCGGGCCTTTCCCTCTGSGGAACCCCATGCCCTTCACTGGGTGTGGCGGGGAAACAGGACTTTTACTTTGAAAAAATTAGAGTGCTCAAGGCAGGCCTAT 480
Onghwi     CACCGCGTGCACTGGTCCGGCCGGGCCTTTCCCTCTGGGGAGCCGCATGCCCTTCACTGGGCGTGTCGGGGAACCAGGACGTTTACTGTGAACAAATCAGATCGCTTAAAGAAGGCCTAT 480
Ondomb    CACCGCGTGTACTTGTCCGGCCGGGCCTTTCCTTCTGGAGAACCTCATGCCCTTCACTGGGTGTGTTGGGGAACCAGGACTTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTT 480
Eembax     CACCGCGTGTACTTGTCCGGCCGGGCCTTTCCTTCTGGAGAACCTCATGCCCTTCACTGGGTGTGTTGGGGAACCAGGACTTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTT 480
Eembax     CACCGCGTGTACTKGTCCGGCCGGGCCTTTCCTTCTGGRGAACCTCATGCCCTTCACTGGGTGTGTTGGGGAACCAGGACTTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTT 480
Ohakaf     CACCGCGTGTACTKGTCCGGCCGGGCCTTTCCTTCTGGRGAACCTCATGCCCTTCACTGGGTGTGTTGGGGAACCAGGACTTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTT 480
Ombway     CACCGCGTGTACTGGTCCGGCCGGGCCTTTCCTTCTGGRGAACCNCATGCCCTTCACTGGGCGTGTNGGGGAACCAGGACTTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTT 480
Ombway     CACCGCGTGTACTKGTCCGGCCGGGCCTTTCCTTCTGGNGAACCNCATGCCCTTCACTGGGNGTGTNGGGGAACCAGGACTTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTT 480
Oshoon     CACCGCGAGTACTGGTCCGGCTGGACCTTTCCTTCTGGGGAACCTCATGGCCTTCACTGCTGTGTNGGGGAACCAGGACTTTTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTT 479
Oshoon     CACCGCGAGTACTGGTCCGGCTGGACCTTTCCTTCTGGGGAACCTCATGGCCTTCACTGGCTGTGGGGGGAACCAGGACTTTTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTT 479
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Figure 16. Nucleotide multiple sequences Alignment sequences in 18S region of *Ganoderma* species. Alignment was done using CLUSTAL 2.0.8.

CHAPTER 5: DISCUSSION

5.1 Mushroom collection

In this study, the distribution of *Ganoderma* species was carried out in Ohangwena and Oshana regions. It was found that there were more *Ganoderma* fruiting bodies in Ohangwena region than in Oshana region. This might be explained by many reasons, for example, there are more vegetation types in Ohangwena region compared to Oshana region (Mendelsohn et al., 2000, p. 101). There were not many tree stumps found in Oshana compared to those found in Ohangwena. The stumps in Oshana were dug out of the soil, probably to use as fire wood, something not observed in Ohangwena region. Rainfall might be another factor to consider, because the above normal rainfall was received in the northern regions during 2008 (Mweti, 2008). Rainfall is stated as a factor because the logs or stumps where these mushrooms where growing needed to be well moisten for the mushroom to start germinating (Engelbrecht and Volk, 2005, p. 224).

Although most G*anoderma* were found in Ohangwena, not all the villages surveyed were found to have them. Villages like Omhedi and Ondombe were some of the villages in which hours were spend, but no *Ganoderma* fruiting bodies were found. This might be due to the fact that there were probably no spores for the *Ganoderma* species in the area. In other villages like Efidi and Ohakafiya, more than three hours were spent before any *Ganoderma* fruiting bodies were observed. Other villages like

Eembaxu and Onanghulo, though bigger than others, they were also found to have more fruiting bodies (Table 2).

In Oshana region, 70% of the collected fruiting bodies were found growing on dead *S. caffra* (Marula) trees. These are the trees found in most of the fields. Other types of trees like *C. mopane* (mopane) which also seem to be most in the region are outside the fields and are harvested for use as fire wood, so fewer stumps were observed and only 20% fruit bodies were harvested from them. The 10% came from *C. zeyheri* and *A. sieberana.* In Ohangwena region, about 40% of the fruit bodies were found growing on *C. collinum* tree stumps, this tree was found to be abundant in the region and as a result most stumps observed were of that tree. The remaining 60% were from other trees like *B. plurijuga, T. sericea, C. mopane, C. frarans, A. erioloba,* and *G. retinervis.*

Sclerocarya caffra tree locally known as omugongo in Oshiwambo and commonly known as marula were the only trees among all other host trees found to have fruiting bodies growing on the roots and stem of living trees. Other hosts trees were either dead or just the remaining stumps. This observation can support the fact that some species of the *Ganoderma* have been reported to be pathogenic on oil palm (*Elaeis guineensis* Jacq), coconut, betal palm, rubber, tea, coffee, cocoa, and forest trees in different countries such as Indonesia and Malaysia (Pilotti, Sanderson, Aitken, and Armstrong, 2004, p.252; Utomo, Werner, Niepold, and Deising, 2005, p.159; and Buchanan, 2001, p.1).

In this study the *Ganoderma* fruiting bodies were found on the stumps for most trees found in the regions. It seemed they have no specific preference of growing to certain tree types only, but any dead tree or stump available. This might be implying that they might grow on any woody substrate as long as it has to be well soaked.

5.2 Indigenous knowledge on the uses of *Ganoderma* mushrooms

Indigenous knowledge of medicinal mushroom use is linked to local culture and history. It should therefore be regarded as a body of knowledge that has continually developed without the outside interference of formal science (Opige, Kateyo, and Olila 2006, p.325). A major component of these systems is ethnobotanical knowledge, which refers to a cumulative body of traditional knowledge about the interaction between human societies and the plant kingdom, and, more specifically, how indigenous peoples perceive, manage, and utilize the plants around them (Suminguit, 2005, p.2).

The main component of this study was to determine the use of *Ganoderma* species as a medicinal mushroom in Ohangwena and Oshana regions of Northern Namibia.
No studies have been conducted to measure the cultural significance of fungi in Namibia. For this reason this study was carried out to learn more on the traditional mushroom knowledge of the people living in Ohangwena and Oshana regions. Mushroom knowledge in both regions was extensive.

This study was designed to document traditional knowledge on the use of *Ganoderma* mushrooms but it also generated more information on the uses of other mushroom species. The study obtained detailed information about several aspects of traditional mushroom knowledge (Table 4). According to the information obtained from the questionnaire, it can be said that *Ganoderma* mushrooms have been playing a role in the daily lives of the local communities for a very long time, because it is reported to have been used in relieving stress when sniffed as ash mixed with tobacco, calming of nerves when put in water, used as a drink and healing of cold and flu symptoms when its smoke is inhaled and applied to infected skin to treat the wounds on children's heads. The latter has also been confirmed in another study in Cameroon where they mixed the crushed *G. lucidum* with ash to be used as ointment to treat skin infections (Yongabi, Agho, and Martinez, 2004, p. 35).

This mushroom is also said to have history in treating animal diseases, especially cattle when suffering from lung diseases and goats when having skin rush. It is said that the cattle herders crush the fruiting bodies of these mushrooms, add water and mix well before giving the animals to drink. The animals are said to stop coughing after taking the mixture for some days of which the caretaker could not specify as to how many. Unfortunately, no previous studies were done to confirm this information.

Opige et al (2006, p.324) reported that most of the mushrooms for medicinal use in Uganda are identified localy with their association to particular tree species where they grow from. In Namibia, this was not the case, the local people interviewed in this study refer to the mushrooms according to how they look like, for example

"omapakululu" something that looks like an elephant's ear. Opige et al (2006, p.329) also reported that these mushrooms are normally found growing on either the roots or tree stumps, which is the same case in the two regions suveyed in northern Namibia. They stated that the patients are treated by inhaling the smoke or steam in a boiled mixture of water and mushrooms to treat measles and body pain. This study revealed the same findings in Ohangwena region. Those using *Ganoderma* as medicine are said to inhale smoke for the relief of flu and body aches.

Traditional knowledge from Oshana and Ohangwena regions show that until today, in different places of the country, the indigenous people in these regions also use other mushrooms both for food and for medicinal purposes. Of course, it is not really known how important mushroom use as medicine is. For example, it appears to be very important and well known by the people included in this study from Ohangwena region, and not so known or maybe important to those included in this study from Oshana region (Figure 6).

Although *Ganoderma* mushrooms did not make it to the top of the six listed mushroom used for medicinal purposes in the two regions as expected (figure 6), this study confirmed that it is used for medicinal purposes in Namibia as reported by previous authors in other countries (Munyanziza and Oldeman 1996, p. 454; Mshigeni and Chang, 2000, p. 16; Stamets and Wu Yao, 1999, p. 12). These findings prove that there is a need for more research to be done on all indigenous mushrooms in order to document all the information about all mushrooms found in Namibia.

5.3 Genetic diversity

This study also assessed the genetic diversity of the *Ganoderma* species collected from the two regions and it was found that the species are genetically diverse. This was confirmed when the overall mean genetic diversity was calculated and showed that there is a diversity of 1%. It was supported by the bootstrapping tree which produced half of the clades with low probability figures showing values less than 50% which are classified as same in this study (Figure 14). The table showing the calculation and estimation of evolutionary divergence between sequences (Figure 14); also confirm that there is diversity as well as the homogeneity of substitution patterns between Sequences found in (Table 5).

Although it is suggested that the ITS region of nuclear ribosomal DNA (rDNA) is a suitable molecular marker for phylogenetic analysis of species within the genus *Ganoderma* (Moncalvo et al., 1995, 227), it was not so in this study. As reported by Wang and Yao in 2005, p. 116, heterozygous was found in the *Ganoderma* species sequenced in this study when ITS1 and ITS4 primers were used (Figure 10). Cloning was required in order to remove the heterozygous regions DNA to reach the conclusion. Since cloning was not done, it was not possible to determine the alleles occurring in the heterozygous sequences in this study. There might be alleles occurring in the study area which have to be identified. Ancient hybridization or gene duplication or low concerted evolution might explain the ITS heterogeneity present in the *Ganoderma* species (Wang and Yao, 2005, p. 119). Different ITS types shared by strains/species are often cited for the detection of hybridization (Okabe and Matsumoto 2003, p. 165; Widmer and Baltisberger 1999, p. 1283).

A trial with 18S yielded good results and when analysed further revealed that the collected *Ganoderma* species are genetically diverse. Although good results were obtained, most of the DNA was of low quality (Figure 6) not giving good PCR product to be sequenced (Figure 7). This might be due to the fact that the DNA was extracted from the tissue unlike in other reports where the tissue was first grown on agar medium (Soon and Hack, 2004, p. 742; Huseu; 1996, p. 130; Wang and Yao, 2005, p. 114).

Although the majority of the samples are positioned with respect to their geographical origin in the phylogenetic relationships trees, there are also some samples clustering together which are not of the same geographical origin (Figure 13), these samples might be possibly of the same species as suggested by Moncalvo et al. (1995, p. 230), or they might be due to the fact that samples were collected from different host trees recorded in the two regions (Table 3).

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study revealed that *Ganoderma* species are used for medicinal purposes in Oshana and Ohangwena regions. These mushrooms are not only used in treating human ailments, but are also used in treating animals, for example their use in cattle for lung diseases, goats for skin diseases and chickens. The study also revealed that there is a 1% genetic diversity among the *Ganoderma* species collected from Ohangwena and Oshana regions. *Ganoderma* species are more distributed in Ohangwena region than in Oshana.

This study will provide a foundation for future studies on mushrooms of Namibia.

The documentation of medicinal mushrooms and their social as well as cultural practices associated with their use as medicine practice is a necessity. It is from such fundamental information where applied research can be carried out to develop the cultivation and improve on the use of both medicinal and edible mushrooms in Namibia.

6.2 Recommendations

It is recommended that another study should be done to analyse the genetic diversity among the *Ganoderma* species based on the host plants. Heterozygousity is reported in this study when ITS1 and ITS4 primers were used, it is therefore recommended that further sequencing using ITS1 and ITS4 primers should be done followed by cloning in order to determine the regions of heterozygousity within the species. It is also recommended that DNA extraction should be done using mycelium instead of fruiting bodies in order to obtain good PCR products. Future research should be done on the possibility to grow the *Ganoderma* mushrooms and on the active ingredients found in them, this will help in understanding and promoting the medicinal uses of *Ganoderma* species in Namibia. Free access of these mushrooms may lead to commercial harvesting which may lead to unsustainable harvesting due to poor collection practices; therefore it is recommended that conservationists should be concerned with the amounts collected and the picking methods that might lead to over-harvesting.

CHAPTER 7: REFERENCES

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ANNEX 1: THE QUESTIONNAIRE USED IN THIS STUDY

Indigenous knowledge of medicinal mushroom use is linked to local culture and history. It should therefore be regarded as a body of knowledge that has continually developed without the outside interference of formal science (Opige et al., 2006). No studies have been conducted to measure the cultural significance of fungi in Namibia. For this reason this study was done to learn more on the traditional mushroom knowledge of the people living in Ohangwena and Oshana regions.

The questionnaire used to gather information on the uses of *Ganoderma* species comprised of the following questions:

Region …….

Name of the village …….

Sex of the respondent: Female ☐ Male ☐

1. Do you know mushrooms?

Yes ☐

No ☐

If yes, what type of mushroom?

……

……

……

……

1. Do you eat mushrooms?

Yes ☐

No ☐

If yes, which type of mushrooms do you eat?

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3. How often are mushrooms collected and who collects them?

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……

……

4. Do you collect mushrooms for sale or just for own consumption?

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5. Do you use mushrooms for any other purposes other than food?

Yes ☐

No ☐

If yes, what purposes?

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……

……

6. Do you use mushrooms for medicinal purposes?

Yes ☐

No ☐

If yes which mushrooms?

……

……

……

7. Do you know this mushroom? (*Ganoderma* mushrooms will be shown)

Yes ☐

No ☐

8. Do you use these mushrooms as food?

Yes ☐

No ☐

If no, what do you use it for?

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9. If you use it for treatment, what type of diseases do you use it for?

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10. When used for treatment, in what form do you use the mushroom? (e.g.powder or soaked in water)

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11. In which month do you collect *Ganoderma* mushrooms?

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……

12. How do you preserve the *Ganoderma* mushroom?

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13. How often does one need to use the mushroom before they get healed?

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14. Which other mushrooms do you use for medicinal purposes?

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ANNEX 2: BLAST SEARCH RESULTS

Below are the collected *Ganoderma* species sequence aligned against subject sequence in the Genbank database, with percentage similarity to *Ganoderma* species. The aligned sequence was obtained from the 5.8S rRNA gene using ITS2. The query is the isolated *Ganoderma* species used in this study, while the subject is the *Ganoderma* species found in the Gene bank database.

```
>emb|AM773630.1|  Ganoderma sp. DK10 ITS1, 5.8S rRNA gene and ITS2, isolate
DK10

Length=424
 Score =  686 bits (371),  Expect = 0.0
 Identities = 405/421 (96%), Gaps = 10/421 (2%)
 Strand=Plus/Plus
Query  17   GGGNTG-AGCT-GCCTTCC-AGGCATGTGC-CGCCCTGCTCANT-CACTCTACACCTGTG 71
            ||| || |||| ||||||| |||||||||| |||||||||| | ||||||||||||||||
Sbjct  9    GGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCAATCCACTCTACACCTGTG 68
Query  72   CACTTACTGTGGGTGACNGGATCGCAAAGCGGGCTTCTTGTCCGTTATAAAGCGCATCTG 131
            |||||||||||||||| |||||||||||||||| |||||||||||| ||||||||||||
Sbjct  69   CACTTACTGTGGGTGAC-GGATCGCAAAGCGGGC-TCTTGTCCGTTAT-AAGCGCATCTG 125
Query  132  TGGCCTGCGTTTATCACAAACTCTTTGAAAGTACTAGAATGTAATATTGGGATATAATAG 191
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  126  TGGCCTGCGTTTATCACAAACTCTTTGAAAGTACTAGAATGTAATATTGGGATATAATAG 185
Query  192  ATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCG 251
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  186  ATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCG 245

Query  252  AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC 311
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  246  AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC 305
Query  312  TTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGGTTGAAGNGGTCATGAAATCTTCAACT 371
            |||||||||||||||||||||||||||||||| |||| | | ||||||||||||||||
Sbjct  306  TTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGA-GTG-TCATGAAATCTTCAACT 363
Query  372  TGCAACCTCTTTGCGGAGTTTGTAGGCTTGGACTTGGAGGGCTTGTCGGCCTTTAACGGT 431
            |||||||||||||||||||||||||||||||||||||||||||||| ||||||||| |||
Sbjct  364  TGCAACCTCTTTGCGGAGTTTGTAGGCTTGGACTTGGAGGGCTTGTTGGCCTTTAATGGT 423
Query  432  C  432
            |
Sbjct  424  C  424


>gb|DQ425010.1|  Ganoderma lucidum isolate GL166 internal transcribed spacer
1,
partial sequence; 5.8S ribosomal RNA gene, complete sequence;
and internal transcribed spacer 2, partial sequence
Length=550

 Score =  449 bits (243),  Expect = 1e-122
 Identities = 313/344 (90%), Gaps = 19/344 (5%)
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 Strand=Plus/Minus

Query  73   AAGACGGTTGGAAGCTCATCCAAACGCTTCACGGTCACGGCGTAGACATTTTATCACACC 132
            ||||||||| |||||||  |||||||||||||||| ||||||||||  ||||||||||
Sbjct  532  AAGACGGTTAGAAGCTC-GCCAAACGCTTCACGGTCGCGGCGTAGACA--TTATCACACC 476

Query  133  GACAGCCGATCCGCAAGGAATCAAGCTAATGCATTTAAGAGGAGCCGACCGTTAAA-GGC 191
            || ||||||||||||||||||||||||||||||||||||||||||||||||||  ||| |||
Sbjct  475  GAGAGCCGATCCGCAAGGAATCAAGCTAATGCATTTAAGAGGAGCCGACCGACAAAGGGC 416

Query  192  CGACAAGCCCTCCAAGTCCAAGCCTACAAACTCCGCAAAGAGGTTGCAAGTTGAAGATTT 251
            ||||||| ||||||||||||||| |||||| |||||||| | ||| | ||||||||||
Sbjct  415  CGACAAG-CCTCCAAGTCCAAGCCCACAAAC-CCGCAAAG-GCTTGTAGGTTGAAGATTT 359

Query  252  CATGACACTCAAACAGGCATGCTCCTCGGAATACCAAGGAGCGCAAGGTGCGTTCAAAGA 311
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  358  CATGACACTCAAACAGGCATGCTCCTCGGAATACCAAGGAGCGCAAGGTGCGTTCAAAGA 299

Query  312  TTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCG-ATTTCN-T-CGT-CTTC- 366
            |||||||||||||||||||||||||||||||||||||||| ||||| | ||| ||||
Sbjct  298  TTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCA 239

Query  367  TCG-T-CGAGA-CCA-GAGATCCGTN--TGAAAGTTG-ATATAG  403
            ||| | ||||| ||| |||||||||   ||||||||| ||||||
Sbjct  238  TCGATGCGAGAGCCAAGAGATCCGTTGCTGAAAGTTGTATATAG  195


>gb|DQ425010.1|  Ganoderma lucidum isolate GL166 internal transcribed spacer
1,
partial sequence; 5.8S ribosomal RNA gene, complete sequence;
and internal transcribed spacer 2, partial sequence
Length=550

 Score =  449 bits (243),  Expect = 1e-122
 Identities = 313/344 (90%), Gaps = 19/344 (5%)
 Strand=Plus/Minus

Query  73   AAGACGGTTGGAAGCTCATCCAAACGCTTCACGGTCACGGCGTAGACATTTTATCACACC 132
            ||||||||| |||||||  |||||||||||||||| ||||||||||  ||||||||||
Sbjct  532  AAGACGGTTAGAAGCTC-GCCAAACGCTTCACGGTCGCGGCGTAGACA--TTATCACACC 476

Query  133  GACAGCCGATCCGCAAGGAATCAAGCTAATGCATTTAAGAGGAGCCGACCGTTAAA-GGC 191
            || ||||||||||||||||||||||||||||||||||||||||||||||||||  ||| |||
Sbjct  475  GAGAGCCGATCCGCAAGGAATCAAGCTAATGCATTTAAGAGGAGCCGACCGACAAAGGGC 416

Query  192  CGACAAGCCCTCCAAGTCCAAGCCTACAAACTCCGCAAAGAGGTTGCAAGTTGAAGATTT 251
            ||||||| ||||||||||||||| |||||| |||||||| | ||| | ||||||||||
Sbjct  415  CGACAAG-CCTCCAAGTCCAAGCCCACAAAC-CCGCAAAG-GCTTGTAGGTTGAAGATTT 359

Query  252  CATGACACTCAAACAGGCATGCTCCTCGGAATACCAAGGAGCGCAAGGTGCGTTCAAAGA 311
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  358  CATGACACTCAAACAGGCATGCTCCTCGGAATACCAAGGAGCGCAAGGTGCGTTCAAAGA 299

Query  312  TTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCG-ATTTCN-T-CGT-CTTC- 366
            |||||||||||||||||||||||||||||||||||||||| ||||| | ||| ||||
Sbjct  298  TTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCA 239

Query  367  TCG-T-CGAGA-CCA-GAGATCCGTN--TGAAAGTTG-ATATAG  403
            ||| | ||||| ||| |||||||||   ||||||||| ||||||
Sbjct  238  TCGATGCGAGAGCCAAGAGATCCGTTGCTGAAAGTTGTATATAG  195
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>emb|AM773630.1|  Ganoderma sp. DK10 ITS1, 5.8S rRNA gene and ITS2, isolate
DK10
Length=424

 Score =  634 bits (343),  Expect = 2e-178
 Identities = 385/406 (94%), Gaps = 12/406 (2%)
 Strand=Plus/Plus

Query  35   TTCC-AGG-ATGT-CACG-NCTG-TCANT-CACTC-CACCTGTGCACTTACTGTGGGTG  87
            |||| ||| |||| ||||  ||| ||| | ||||| |||||||||||||||||||||||
Sbjct  24   TTCCGAGGCATGTGCACGCCCTGCTCAATCCACTCTACACCTGTGCACTTACTGTGGGTG 83

Query  88   ACGGATCGCAAAGCGGGCTTCNTGTCCGTTATAAAGCGCATCTGTGGCCTGCGTTTATCA 147
            |||||||||||||||||| || |||||||||| |||||||||||||||||||||||||||
Sbjct  84   ACGGATCGCAAAGCGGGC-TCTTGTCCGTTAT-AAGCGCATCTGTGGCCTGCGTTTATCA 141

Query  148  CAAACTCTTTGAAAGTACTAGAATGTAATATTGGGATATAATAGATCTATATACAACTTT 207
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  142  CAAACTCTTTGAAAGTACTAGAATGTAATATTGGGATATAATAGATCTATATACAACTTT 201

Query  208  CAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCCGAAATGCGATAAGTAA 267
            |||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct  202  CAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGC-GAAATGCGATAAGTAA 260

Query  268  TGTGAATTGCAGGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCNTGGT 327
            |||||||||||| |||||||||||||||||||||||||||||||||||||||||| ||||
Sbjct  261  TGTGAATTGCAG-AATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGT 319

Query  328  ATTCCGAGGAGCATGCCTGNTTGAGTGGNCATGAAATCTTCAACTTGCAACCTCTTTGCG 387
            |||||||||||||||||| |||||||  ||||||||||||||||||||||||||||||||
Sbjct  320  ATTCCGAGGAGCATGCCTGTTTGAGTGT-CATGAAATCTTCAACTTGCAACCTCTTTGCG 378

Query  388  GAGTTTGTAGGCTTGGACTTGGAGGGCNTGTCGGCCTTTAACGGTC  433
            |||||||||||||||||||||||||| ||| ||||||||| ||||
Sbjct  379  GAGTTTGTAGGCTTGGACTTGGAGGGCTTGTTGGCCTTTAATGGTC  424


emb|AM773630.1|  Ganoderma sp. DK10 ITS1, 5.8S rRNA gene and ITS2, isolate
DK10
Length=424

 Score =  521 bits (282),  Expect = 1e-144
 Identities = 345/377 (91%), Gaps = 18/377 (4%)
 Strand=Plus/Plus

Query  59   TCCGAGGGCATGTGCACGCCTCNGCTCAGTCCACTCTACACCTGTGCACTTACTGTGGGG 118
            ||||| |||||||||||||| | ||||| |||||||||||||||||||||||||||| |||
Sbjct  25   TCCGA-GGCATGTGCACGCC-CTGCTCAATCCACTCTACACCTGTGCACTTACTGT-GGG 81

Query  119  TGACGGATCGCAAAGCGNGGCNTCTATGTCCGNTATAAAGCCGCATCTGNGGCCTGCGNT 178
            |||||||||||||||| ||| ||| |||||| ||| ||| |||||||| |||||||| |
Sbjct  82   TGACGGATCGCAAAGCG-GGC-TCT-TGTCCGTTAT-AAG-CGCATCTGTGGCCTGCGTT 136

Query  179  TATCACAAACTCTTTGAAAGTACTAGAATGTAATATTGCGATATAATAGATCNATATACA 238
            |||||||||||||||||||||||||||||||||||| |||||||||||| |||||||
Sbjct  137  TATCACAAACTCTTTGAAAGTACTAGAATGTAATATTGGGATATAATAGATCTATATACA 196

Query  239  ACTTTCAGCAACGGATCTCTTGGCTCNCGCATCGATGATGAACGCAGCGAAATGCGATAN 298
            |||||||||||||||||||||||| |||||||||| ||||||||||||||||||||
Sbjct  197  ACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAA 256

Query  299  GTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCNT 358
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  257  GTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTT 316
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Query  359  GA-ATNCCGAG-AGCATGCCTGTTT-AGTG-CATGAAN-CTTCA-CTTG-AACCTCTTTG 411
            |  || ||||| |||||||||||||| |||| ||||||  ||||| |||| ||||||||||
Sbjct  317  GGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAACTTGCAACCTCTTTG 376

Query  412  CGG-GTTTG-AG-CTTG  425
            ||| ||||| || ||||
Sbjct  377  CGGAGTTTGTAGGCTTG  393
```

>gb|DQ424976.1| Ganoderma lucidum isolate GL23 type 2 internal transcribed spacer
1, partial sequence; 5.8S ribosomal RNA gene, complete
sequence; and internal transcribed spacer 2, partial sequence
Length=554

```
 Score =  364 bits (197),  Expect = 3e-97
 Identities = 317/375 (84%), Gaps = 28/375 (7%)
 Strand=Plus/Plus
Query  59   TCCGAGGGCATGTGCACGCCTCNGCTCAGTCCACTCTACACCTGTGCACTTACTGTGGGG 118
            ||||| |||||||||||||| | ||||| ||||||||||||||||||||||||||||| |||
Sbjct  30   TCCGA-GGCATGTGCACGCC-CTGCTCA-TCCACTCTACACCTGTGCACTTACTGT-GGG 85

Query  119  TGACGGA--TCGCAAAGCGNGGCNTCTATGT-CC--G-NTATAAAGCCGCATCTGNGGCC 172
            |  |  |  ||| |||||| | | ||| | | ||  |  | || ||| || ||||  |||
Sbjct  86   TTTCAAACGTCGTAAAGCGAGTC-TCT-T-TACCGAGCTTGTAGAGCGGCGTCTG-TGCC 141

Query  173  TGCGNTTATCACAAACTCTTTGAAAGTACTAGAATGTAATATTGCGATATAATAG-ATCN 231
            |||| ||||||||||||| | |||||| ||||||||  ||||||||| |||  | |||
Sbjct  142  TGCGTTTATCACAAACTCTAT-AAAGTATTAGAATGT-GTATTGCGATGTAA-CGCATCT 198

Query  232  ATATACAACTTTCAGCAACGGATCTCTTGGCTCNCGCATCGATGATGAACGCAGCGAAAT 291
            |||||||||||||||||||||||||||||||| |||||||||| ||||||||||||||
Sbjct  199  ATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAAT 258

Query  292  GCGATANGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGC 351
            |||||| |||||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct  259  GCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTCGAACGCACCTTGC 318

Query  352  GCTCCNTGA-ATNCCGAG-AGCATGCCTGTTT-AGTG-CATGAAN-CTTCA-CTTG-A-A 403
            ||||| ||  || ||||| |||||||||||||| |||| ||||||  ||||| |||  | |
Sbjct  319  GCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAACTTACAGA 378

Query  404  CCTCTTTGCGGGTTT  418
            ||| || ||||||||
Sbjct  379  CCT-TT-GCGGGTTT  391
```

>emb|AM773630.1| Ganoderma sp. DK10 ITS1, 5.8S rRNA gene and ITS2, isolate
DK10
Length=424

```
 Score =  612 bits (331),  Expect = 1e-171
 Identities = 372/390 (95%), Gaps = 13/390 (3%)
 Strand=Plus/Plus

Query  31   ACG-CCTG-T-AAT-CACTC-ACACCTGTGC-CTTTGTGGGTGACGGATCGCAAAGC 82
||| |||| | ||| ||||| |||||||||| ||| ||||||||||||||||||||||
Sbjct  39   ACGCCCTGCTCAATCCACTCTACACCTGTGCACTTACTGTGGGTGACGGATCGCAAAGCG 98

Query  83   GGCTTCTTGTCCGTTATAAAGCGCATCTGTGGCCTGCGNTTATCACAAACTCTTTGAAAG 142
            ||| |||||||||||| |||||||||||||||||| ||||||||||||||||||||||
Sbjct  99   GGC-TCTTGTCCGTTAT-AAGCGCATCTGTGGCCTGCGTTTATCACAAACTCTTTGAAAG 156
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Query  143  TACTAGAATGTAATATTGGGATATAATAGATCTATATACAACTTTCAGCAACGGATCTCT 202
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  157  TACTAGAATGTAATATTGGGATATAATAGATCTATATACAACTTTCAGCAACGGATCTCT 216

Query  203  TGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATT 262
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  217  TGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATT 276

Query  263  CAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCNTGGTATTCCGAGGAGCATGCC 322
            |||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||
Sbjct  277  CAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCC 336

Query  323  TGGTTGAAGTGGTCAATGAAATCTTCAACTTGCAACCTCTTTGCGGAGTTTGTAGGCTTG 382
            || |||| ||| ||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  337  TGTTTGA-GTG-TCA-TGAAATCTTCAACTTGCAACCTCTTTGCGGAGTTTGTAGGCTTG 393

Query  383  GACTTGGAGGGCTTGTCGGCCTTTAACGGT  412
            ||||||||||||||| ||||||||| |||
Sbjct  394  GACTTGGAGGGCTTGTTGGCCTTTAATGGT  423
```

>emb|AM773630.1| Ganoderma sp. DK10 ITS1, 5.8S rRNA gene and ITS2, isolate DK10
Length=424

```
 Score =  590 bits (319),  Expect = 8e-165
 Identities = 351/368 (95%), Gaps = 9/368 (2%)
 Strand=Plus/Plus

Query  52   CACTC-NCACCTGTGC-CTT-CTGTGGGTGACGGATCGCAAAGCGGGCTTCTTGTCCGTT 108
            |||||  ||||||||| ||| ||||||||||||||||||||||||| ||||||||||
Sbjct  54   CACTCTACACCTGTGCACTTACTGTGGGTGACGGATCGCAAAGCGGGC-TCTTGTCCGTT 112

Query  109  ATAAAGCGCATCTGTGGCCTGCGTTTATCACAAACTCNTTGAAAGTACTAGAATGTAATA 168
            || ||||||||||||||||||||||||||||||||| ||||||||||||||||||||||
Sbjct  113  AT-AAGCGCATCTGTGGCCTGCGTTTATCACAAACTCTTTGAAAGTACTAGAATGTAATA 171

Query  169  TTGGGATATAANAGATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGA 228
            |||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  172  TTGGGATATAATAGATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGA 231

Query  229  TGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAA 288
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  232  TGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAA 291

Query  289  TCTTTGAACGCACCTTGCGCTCCNTGGTATNCCGAGGAGCATGCCTGTTTGAGTG-CATG 347
            ||||||||||||||||||||||| |||||| |||||||||||||||||||||| |||| 
Sbjct  292  TCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATG 351

Query  348  AAANCTTCAACTTG-AACCTCTTTGCGGAGTTTGTAGGCTTGGACTTGGAGGGCNTGC-G 405
            ||| |||||||||| ||||||||||||||||||||||||||||||||||||| ||  | 
Sbjct  352  AAATCTTCAACTTGCAACCTCTTTGCGGAGTTTGTAGGCTTGGACTTGGAGGGCTTGTTG 411

Query  406  GC-TTTAA  412
            || |||||
Sbjct  412  GCCTTTAA  419
```

>gb|EU021456.1| Ganoderma lucidum strain WD-2038 voucher TFM-F 18922 18S ribosomal
RNA gene, partial sequence; internal transcribed spacer
1, 5.8S ribosomal RNA gene, and internal transcribed spacer
2, complete sequence; and 25S ribosomal RNA gene, partial
sequence

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Length=637

 Score =  484 bits (262),  Expect = 4e-133
 Identities = 419/492 (85%), Gaps = 46/492 (9%)
 Strand=Plus/Plus

Query  118  ATCTGTGGCCTGCGTTTATCACAAACTCNT-TGAAAGT-ACTAGAATGTAATATTGGGAT 175
            |||||| |||||||||||||||||||| | | ||||| || |||||||  ||||| |||
Sbjct  160  ATCTGT-GCCTGCGTTTATCACAAACTC-TAT-AAAGTAAC-AGAATGT-GTATTGCGAT 214

Query  176  ATAANAGATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAA 235
             ||| | ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  215  GTAACACATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAA 274

Query  236  CGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGA 295
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  275  CGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGA 334

Query  296  ACGCACCTTGCGCTCCNTGGTATNCCGAGGAGCATGCCTGTTTGAGTG-CATGAAANCTT 354
            |||||||||||||||| |||||| |||||||||||||||||||||||| ||||||| |||
Sbjct  335  ACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTT 394

Query  355  CAACTTG-AACCTCTTTGCGGAGTTTGTAGGCTTGGACTTGGAGGGCNTG-CGGCT-TTA 411
            |||| |  || || |||| ||  |||||||||||||||||||| | || ||||  |||
Sbjct  395  CAACCTACAAGCT-TTTGTGG--TTTGTAGGCTTGGACTTGGAGG-CTTGTCGGCCGTTA 450

Query  412  ACGG-CG-CTCCTCTTAA-TGCATTAGCTTG-TTCCTTGCGGATCG-CTGNCGNTGTGTA 466
             ||| || |||||||||| |||||||||||| |||||||||||||| ||  || |||| |
Sbjct  451  TCGGTCGGCTCCTCTTAAATGCATTAGCTTGGTTCCTTGCGGATCGGCTCTCGGTGTG-A 509

Query  467  AAATG-CTAC-CCGTG-CCGTGA-GCGTT-GGATGANNTCC-A-CCG-CNTGNTCAAGAC 518
             |||| |||| ||||| |||||| ||||| ||  ||  | | | ||| | |  | |||||
Sbjct  510  TAATGTCTACGCCGTGACCGTGAAGCGTTTGGC-GAGCTTCTAACCGTCTTA-T-AAGAC 566

Query  519  A-CTTTTAT-ANC-CTGNC-TCAA-TCAGG-AG-ACT-CCCG-TGA-CTTAAGCA-ATCA 567
            | |||| || | | ||| | |||| ||||| || ||| |||| ||| |||||||| ||||
Sbjct  567  AGCTTT-ATGACCTCTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCA 625

Query  568  ATA-GCGG-GGa  577
            ||| |||| |||
Sbjct  626  ATAAGCGGAGGA  637


>gb|AY593867.1|  Ganoderma neojaponicum AS5.541 type 2 18S ribosomal RNA
gene,
partial sequence; internal transcribed spacer 1, 5.8S ribosomal
RNA gene, and internal transcribed spacer 2, complete sequence;
and 28S ribosomal RNA gene, partial sequence
Length=670

 Score =  734 bits (397),  Expect = 0.0
 Identities = 533/597 (89%), Gaps = 29/597 (4%)
 Strand=Plus/Plus

Query  44   TTCCNAGGC-TGTGCACGCCCTGCTCAGT-CACTCTACACCTGTGCACTTACTGTGGGTG 101
            |||| |||| |||||||||||||||| | | ||||||||||||||||||||||||||||
Sbjct  84   TTCCGAGGCATGTGCACGCCCTGCTCA-TCCGCTCTACACCTGTGCACTTACTGTGGGTT 142

Query  102  ACGGATCGCAAAGCGGGCTTCTTGTCCGTTATAAAGCGCATCTGTGGCCTGCGTTTATCA 161
            | ||||||| | |||||| | |||||||   |  ||||| ||||| ||||||||||||||
Sbjct  143  ATGGATCGCGAGGCGGGC-TGTTGTCCG---TCGAGCGCGTCTGT-GCCTGCGTTTATCA 197
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Query  162  CAAACTCTTTGAAAGTACT-AGAATGTAATATTGGGATATAATAG-ATCTATATACAACT 219
            |||||||||| ||||||| | |||||||  ||||| ||| |||  | ||||||||||||||
Sbjct  198  CAAACTCTTTAAAAGTA-TCAGAATGT-GTATTGCGATGTAA-CGCATCTATATACAACT 254

Query  220  TTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTA 279
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  255  TTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTA 314

Query  280  ATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCNTGGT 339
            |||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||
Sbjct  315  ATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGT 374

Query  340  ATNCCGAGGAGCATGCCTGGTTGAGTGGTCATTGAAATCTTCAACTTGCAACCTCTTTGC 399
            || |||||||||||||||| ||||||| |||| |||||||||||| ||||| || |||
Sbjct  375  ATTCCGAGGAGCATGCCTGTTTGAGTG-TCAT-GAAATCTTCAACCTGCAAGCTTTTTTT 432

Query  400  G-GAGTTTGTAGGCTTGGACTTGGAGG-GCTTGNCGGCCTTTAAC-GGNCGGCTCCTCTT 456
            | | ||||| |||||||||||||||| | ||| ||||||||||  || ||||||||||
Sbjct  433  GTG-GTTTGCAGGCTTGGACTTGGAGGTGTTTGTCGGCCTTTAAATGGTCGGCTCCTCTC 491

Query  457  AAATGCATTAGCTTGATTCCTTGCGGATCGGCTGNCGGTGTGATAAAATGTCTACGCCGT 516
            |||||||||||||||||||||||||||||||| |||||||||||  ||||||||||||
Sbjct  492  AAATGCATTAGCTTGATTCCTTGCGGATCGGCTGTCGGTGTGATAA--TGTCTACGCCGC 549
Query  517  GACCCGTGAAGCCGT--TTGGATGAGCTTCCAACCGTCAT-GCTTCAAAGACAACTTTTT 573
            |||| ||||||| ||  ||| | |||||||||||||||| | |||||| ||||||||||
Sbjct  550  GACC-GTGAAGC-GTCCTTGAACGAGCTTCCAACCGTC-TCGCTTCAGAGACAACTTTT- 605

Query  574  ATGACCTCTGACCTCAAATCAGGTAGGACTACCCGCTGA-CTTAAGCATATCANTAA  629
            |||||||||||||||||||||||||||||||||||||| |||||||||||||| |||
Sbjct  606  ATGACCTCTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAATAA  662
```

>gb|DQ425010.1| Ganoderma lucidum isolate GL166 internal transcribed spacer 1,
partial sequence; 5.8S ribosomal RNA gene, complete sequence;
and internal transcribed spacer 2, partial sequence
Length=550
 Score = 398 bits (215), Expect = 5e-107
 Identities = 309/358 (86%), Gaps = 17/358 (4%)
 Strand=Plus/Plus

```
Query  45   TGTAGCTGGCCNTCCGAGGCATGTGCACGCCCTGCTCAGTCCACTCTACACCTGTGCACT 104
            |||||||||| ||||||||| |||||||||||||||| ||||||||||||||||||||
Sbjct  18   TGTAGCTGGCCTTCCGAGGCACGTGCACGCCCTGCTCA-TCCACTCTACACCTGTGCACT 76

Query  105  TACTGTGGGTGACGGATC-GCAAAGCGNGCTTCTTGTC--CG-NT-ATAAAGCGCATCTG 159
            |||||||||  | |||| |  ||||| ||| |||| |   |  |  | |||||| ||||
Sbjct  77   TACTGTGGGTTTCAGATCTGTGAAGCGTGCTCCTTG-CGGGGCTTCGTGAAGCGCGTCTG 135

Query  160  TGGCCTGCGTTTATCACAAACTCNTTGAAAGTACTAGAATGTAATATTGGGNTATAATAG 219
            | |||||||||||||||||||  | |||||| ||||||||  ||||| | | |||  |
Sbjct  136  T-GCCTGCGTTTATCACAAACTCCAT-AAAGTATTAGAATGT-GTATTGCGATGTAA-CG 191

Query  220  -ATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCNCGCATCGATGAAGAACGCAGN 278
             |||||||||||||||||||||||||||||||||||| ||||||||||||||||||||
Sbjct  192  CATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGC 251

Query  279  GAANTGCGATAAGTAATGTGAATTGCAGAATTCAGNGAGTCATCGAANCTTTGAACGCAC 338
            ||| |||||||||||||||||||||||||||||| || |||||||| ||||||||||||
Sbjct  252  GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCAC 311

Query  339  CTTG-GCTCCATGG-ATNCCGAGGAGCATGCCTGTT-GAGTG-CATGANN-CTTCAAC  391
            |||| ||||| ||| || |||||||||||||||||| ||||| |||||   |||||||
Sbjct  312  CTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAAC  369
```

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>gb|EU021456.1|   Ganoderma lucidum strain WD-2038 voucher TFM-F 18922 18S
ribosomal
RNA gene, partial sequence; internal transcribed spacer
1, 5.8S ribosomal RNA gene, and internal transcribed spacer
2, complete sequence; and 25S ribosomal RNA gene, partial
sequence
Length=637

 Score =  446 bits (241),  Expect = 2e-121
 Identities = 342/391 (87%), Gaps = 26/391 (6%)
 Strand=Plus/Plus

Query  121  ATCTGTGGCCNGCGTNTATCACAAACNCTTTGAAAGT-ACTAGAATGTAATATTGGGATA 179
            |||||| ||| |||| |||||||||| || | ||||| || |||||||  ||||| |||
Sbjct  160  ATCTGT-GCCTGCGTTTATCACAAACTCTAT-AAAGTAAC-AGAATGT-GTATTGCGATG 215

Query  180  TAATAGATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCNCGCATCGATGAAGAAC 239
            ||| | |||||||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct  216  TAACACATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAAC 275

Query  240  GCAGCCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGA 299
            |||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  276  GCAG-CGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGA 334

Query  300  ACGCACCTTGCGCTCCNTGGNATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTT 359
            |||||||||||||||| ||| ||||||||||||||||||||||||||||||||||||||
Sbjct  335  ACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTT 394

Query  360  CAACTTGCAACCTCTTTGCGGAGTTTGTAG-CNTG-ACTTGGAGGCTTG-CG-CCTTTAA 415
            |||| | ||| || |||| ||  ||||||| | || |||||||||||||| || || |||
Sbjct  395  CAACCTACAAGCT-TTTGTGG--TTTGTAGGCTTGGACTTGGAGGCTTGTCGGCCGTTAT 451

Query  416  CGG-CGGCTCCTCTTANATGC-TTAGCTTG-TTCCTTGCG-ATCG-CTGNCG-TGTN-TA 468
            ||| |||||||||||| |||| |||||||| ||||||||| |||| ||  || |||  ||
Sbjct  452  CGGTCGGCTCCTCTTAAATGCATTAGCTTGGTTCCTTGCGGATCGGCTCTCGGTGTGATA 511

Query  469  ANTG-CTAC-CCGT-ACCGTTGA-GCGTTTG  495
            | || |||| |||| ||||| || |||||||
Sbjct  512  A-TGTCTACGCCGTGACCGT-GAAGCGTTTG  540


>gb|DQ425010.1|  Ganoderma lucidum isolate GL166 internal transcribed spacer
1,
partial sequence; 5.8S ribosomal RNA gene, complete sequence;
and internal transcribed spacer 2, partial sequence
Length=550

 Score =  630 bits (341),  Expect = 4e-177
 Identities = 483/548 (88%), Gaps = 31/548 (5%)
 Strand=Plus/Plus

Query  17   GGGNTG-AGCT-GCCTTCC-AGGCATGTGC-CGCCCTGCTCANT-CACTCTACACCTGTG  71
            ||| || |||| ||||||| ||||| |||| |||||||||| | ||||||||||||||||
Sbjct  14   GGGTTGTAGCTGGCCTTCCGAGGCACGTGCACGCCCTGCTCA-TCCACTCTACACCTGTG  72

Query  72   CACTTACTGTGGGTGACNGGATC-GCAAAGCGGGCTTCTTGTC--CG-TT-ATAAAGCGC  26
            |||||||||||||  |  |||| |  ||||| ||| |||| |   | ||  | ||||||
Sbjct  73   CACTTACTGTGGGTTTC-AGATCTGTGAAGCGTGCTCCTTG-CGGGGCTTCGTGAAGCGC 130

Query  127  ATCTGTGGCCTGCGTTTATCACAAACTCTTTGAAAGTACTAGAATGTAATATTGGGATAT 186
             ||||| ||||||||||||||||||||  | |||||| ||||||||  ||||| ||| |
Sbjct  131  GTCTGT-GCCTGCGTTTATCACAAACTCCAT-AAAGTATTAGAATGT-GTATTGCGATGT 187
```

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Query  187  AATAG-ATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAAC 245
            ||   | ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  188  AA-CGCATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAAC 246

Query  246  GCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA 305
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  247  GCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA 306

Query  306  CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGGTTGAAGNGGTCATGAAATCT 365
            |||||||||||||||||||||||||||||||||||| |||| | | ||||||||||||
Sbjct  307  CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGA-GTG-TCATGAAATCT 364

Query  366  TCAACTTGCAACCTCTTTGCGGAGTTTGTAGGCTTGGACTTGGAGGGCTTGTCGGCC-TT 424
            ||||| | ||| | |||||||| |||||| |||||||||||||||| |||||||||| ||
Sbjct  365  TCAACCTACAAGC-CTTTGCGG-GTTTGTGGGCTTGGACTTGGAGG-CTTGTCGGCCCTT 421

Query  425  TAACGGTCGGCTCCTCTTAAATGCATTAGCTTGATTCCTTGCGGATCGGCTGTCGGTGTG 484
            |  ||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||
Sbjct  422  TGTCGGTCGGCTCCTCTTAAATGCATTAGCTTGATTCCTTGCGGATCGGCTCTCGGTGTG 481

Query  485  ATAAAATGTCTACGCCGTGACCCGTGAAGCGTTTGGATGAGCTTCCAACCCGTCNT-GCT 543
            ||||  |||||||||||| |||| |||||||||||||  ||||||| |||| ||| | |||
Sbjct  482  ATAA--TGTCTACGCCGCGACC-GTGAAGCGTTTGGC-GAGCTTCTAACC-GTCTTCGCT 536

Query  544  TNCAAGAC  551
            |  |||||
Sbjct  537  TG-AAGAC  543
```

>emb|AM773630.1| Ganoderma sp. DK10 ITS1, 5.8S rRNA gene and ITS2, isolate DK10
Length=424

```
 Score =  686 bits (371),  Expect = 0.0
 Identities = 405/421 (96%), Gaps = 10/421 (2%)
 Strand=Plus/Plus

Query  17   GGGNTG-AGCT-GCCTTCC-AGGCATGTGC-CGCCCTGCTCANT-CACTCTACACCTGTG  71
            ||| || |||| ||||||| |||||||||| |||||||||| | ||||||||||||||||
Sbjct  9    GGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCAATCCACTCTACACCTGTG  68

Query  72   CACTTACTGTGGGTGACNGGATCGCAAAGCGGGCTTCTTGTCCGTTATAAAGCGCATCTG  31
            |||||||||||||||| |||||||||||||||| |||||||||||| ||||||||||||
Sbjct  69   CACTTACTGTGGGTGAC-GGATCGCAAAGCGGGC-TCTTGTCCGTTAT-AAGCGCATCTG  25

Query  132  TGGCCTGCGTTTATCACAAACTCTTTGAAAGTACTAGAATGTAATATTGGGATATAATAG  91
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  126  TGGCCTGCGTTTATCACAAACTCTTTGAAAGTACTAGAATGTAATATTGGGATATAATAG  85

Query  192  ATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCG  51
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  186  ATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCG  45

Query  252  AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC  11
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  246  AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC  05

Query  312  TTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGGTTGAAGNGGTCATGAAATCTTCAACT  71
            |||||||||||||||||||||||||||||||| |||| | | ||||||||||||||||
Sbjct  306  TTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGA-GTG-TCATGAAATCTTCAACT  63

Query  372  TGCAACCTCTTTGCGGAGTTTGTAGGCTTGGACTTGGAGGGCTTGTCGGCCTTTAACGGT  31
            |||||||||||||||||||||||||||||||||||||||||||||||| ||||||||| |||
Sbjct  364  TGCAACCTCTTTGCGGAGTTTGTAGGCTTGGACTTGGAGGGCTTGTTGGCCTTTAATGGT  23
```

```
Query  432  C  432
            |
Sbjct  424  C  424
```

>gb|DQ424973.1| Ganoderma lucidum isolate GL12 internal transcribed spacer 1,
partial sequence; 5.8S ribosomal RNA gene, complete sequence;
and internal transcribed spacer 2, partial sequence
Length=542

```
 Score =  616 bits (333),  Expect = 1e-172
 Identities = 475/539 (88%), Gaps = 32/539 (5%)
 Strand=Plus/Plus

Query  12   TTG-CCGGGNTG-AGCT-GCCTTCC-AGGCATGTGC-CGCCCTGCTCANT-CACTCTACA  65
            ||| ||||| || |||| ||||||| |||||||||| |||||||||| | |||||||||
Sbjct  8    TTGACCGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCA-TCCACTCTACA  66

Query  66   CCTGTGCACTTACTGTGGG-TGACNGGATCGCAAAGCGGGCTTCTTGT-CC--G-TTATA  20
            |||||||||||||||||| |  |  ||| || | ||  || |||| | ||  | ||
Sbjct  67   CCTGTGCACTTACTGTGGGCT-TC-AGATTGCGAGGCACGC-TCTT-TACCGGGCTTGCG  22

Query  121  AAGCGCATCTGTGGCCTGCGTTTATCACAAACTCTTTGAAAGT-ACTAGAATGTAATATT  79
             |||  |||||| |||||||||||||||||||| | ||||| || |||||||  ||||
Sbjct  123  GAGCATATCTGT-GCCTGCGTTTATCACAAACTCTAT-AAAGTAAC-AGAATGT-GTATT  78

Query  180  GGGATATAATAGATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATG  39
            | ||| ||| | ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  179  GCGATGTAACACATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATG  38

Query  240  AAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATC  99
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  239  AAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATC  98

Query  300  TTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGGTTGAAGNGGTCATG  59
            |||||||||||||||||||||||||||||||||||||||||||| |||| | | |||||
Sbjct  299  TTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGA-GTG-TCATG  56

Query  360  AAATCTTCAACTTGCAACCTCTTTGCGGAGTTTGTAGGCTTGGACTTGGAGGGCTTGTCG  19
            |||||||||| | ||| || |||| ||  |||||||||||||||||||| |||||||
Sbjct  357  AAATCTTCAACCTACAAGCT-TTTGTGG--TTTGTAGGCTTGGACTTGGAGG-CTTGTCG  12

Query  420  GCCTTTAACGGTCGGCTCCTCTTAAATGCATTAGCTTGATTCCTTGCGGATCGGCTGTCG  79
            ||| ||| |||||||||||||||||||||||||||| ||||||||||||||||| |||
Sbjct  413  GCCRTTATCGGTCGGCTCCTCTTAAATGCATTAGCTTGGTTCCTTGCGGATCGGCTCTCG  72

Query  480  GTGTGATAAAATGTCTACGCCGTGACCCGTGAAGCGTTTGGATGAGCTTCCAACCCGTC  538
            |||||||||  |||||||||||||||| |||||||||||||  ||||||| |||| |||
Sbjct  473  GTGTGATAA--TGTCTACGCCGTGACC-GTGAAGCGTTTGGC-GAGCTTCTAACC-GTC  526
```

>gb|AY593867.1| Ganoderma neojaponicum AS5.541 type 2 18S ribosomal RNA gene,
partial sequence; internal transcribed spacer 1, 5.8S ribosomal
RNA gene, and internal transcribed spacer 2, complete sequence;
and 28S ribosomal RNA gene, partial sequence
Length=670

```
 Score =  497 bits (269),  Expect = 5e-137
 Identities = 404/473 (85%), Gaps = 30/473 (6%)
 Strand=Plus/Plus
```

```
Query  68   CGAGNGCATGTGCACCGCTCTGCTCAGTCCACTCTACACCTGTGCACTTACTGTGGGTGA 127
            |||| ||||||||| ||| ||||||| ||| ||||||||||||||||||||||||||| |
Sbjct  87   CGAG-GCATGTGCA-CGCCCTGCTCA-TCCGCTCTACACCTGTGCACTTACTGTGGGTTA 143

Query  128  CGGATCGCAAAGCGGGCNTCNTGTCCGTTATAAGCGCATCTGTGGCCNGCGTNTATCACA 187
             ||||||| | |||||| |  |||||| |   ||||| ||||| ||| |||| |||||||
Sbjct  144  TGGATCGCGAGGCGGGC-TGTTGTCCG-T-CGAGCGCGTCTGT-GCCTGCGTTTATCACA 199

Query  188  AACTCTTTGAAAGTACT-AGAATGTAATATTGNGATATAATAG-ATCTATATACAACTTT 245
            |||||||| |||||| | |||||||  ||||| ||| |||  | ||||||||||||||||
Sbjct  200  AACTCTTTAAAAGTA-TCAGAATGT-GTATTGCGATGTAA-CGCATCTATATACAACTTT 256

Query  246  CAGCAACGGATCTCTTGGCNCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAAT 305
            |||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||
Sbjct  257  CAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAAT 316

Query  306  GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCNTGN-AT 364
            |||||||||||||||||||||||||||||||||||||||||||||||||||| ||  ||
Sbjct  317  GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTAT 376

Query  365  TCCGAGGAGCATGCCTGTT-GAGTGTCATGANANCTTCAACNTG-AACCTCTTTGCG-GA 421
            |||||||||||||||||| |||||||||| | ||||||| || || || |||  | |
Sbjct  377  TCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAACCTGCAAGCTTTTTTTGTG- 435
Query  422  GTT-G-AG-CNTG-ACNTGGAGGC-TT-G-CG-CCTTTAAC-GGTCG-CNCCTCTTAAAT 471
            ||| | || | || || ||||||  || | || |||||||  ||||| | ||||| ||||
Sbjct  436  GTTTGCAGGCTTGGACTTGGAGGTGTTTGTCGGCCTTTAAATGGTCGGCTCCTCTCAAAT 495

Query  472  GCATTAGCTTGAN-CCTTGCGGANCG-CTGTCGGNGNGANCAATGTCTACGCC  522
            |||||||||||  ||||||||| || ||||||| | ||  ||||||||||||
Sbjct  496  GCATTAGCTTGATTCCTTGCGGATCGGCTGTCGGTGTGAT-AATGTCTACGCC  547
```

>gb|AY593867.1| Ganoderma neojaponicum AS5.541 type 2 18S ribosomal RNA gene,
partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Length=670

```
 Score =  640 bits (346),  Expect = 7e-180
 Identities = 519/602 (86%), Gaps = 39/602 (6%)
 Strand=Plus/Plus

Query  17   GCT-GCCNTCC-AGG-ATGT-CACGCCCTG-T-ANT-CACTC-ACACCTGTGC-CTTACT  67
            ||| ||| ||| ||| |||| ||||||||| | | | | ||| |||||||||| ||||||
Sbjct  77   GCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCA-TCCGCTCTACACCTGTGCACTTACT  35

Query  68   GTGGGTGACGGATCGCANA-GCGGGCNTCTTGTCCGTTATAAAGCGCATCTGTGGCCTGC  26
            |||||| | |||||||  | |||||| | |||||||   |  ||||| ||||| ||||||
Sbjct  136  GTGGGTTATGGATCGC-GAGGCGGGC-TGTTGTCCG---TCGAGCGCGTCTGT-GCCTGC  89

Query  127  GTTTATCACAAACTCTTTGAAAGTACT-AGAATGTAATATTGGGATATAATAG-ATCTAT  84
            |||||||||||||||||| |||||| | |||||||  ||||| ||| |||  | ||||||
Sbjct  190  GTTTATCACAAACTCTTTAAAAGTA-TCAGAATGT-GTATTGCGATGTAA-CGCATCTAT  46

Query  185  ATACAACTTTCAGCAACGGATCTCTTGGCTCNCGCATCGATGAAGAACGCAGCCGAAATG  44
            |||||||||||||||||||||||||||||| |||||||||||||||||||| |||||||
Sbjct  247  ATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAG-CGAAATG  05

Query  245  CGATAAGTAATGTGAATTGCAGGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGC  04
            |||||||||||||||||||| |||||||||||||||||||||||||||||||||||||
Sbjct  306  CGATAAGTAATGTGAATTGCA-GAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGC  64
```

```
Query  305  GCTCCTTGGTATTCCGAGGAGCATGCCTGGTTGAGTGGCATGAAATCTTCAACTTGCAAC  64
            ||||||||||||||||||||||||||||| ||||||| ||||||||||||||| |||||
Sbjct  365  GCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAACCTGCAAG  24

Query  365  CTCTTTGCG-GAGTTTGTAGGCTTGGACTTGGAGG-GCTTGNCGGCCTTTAAC-GGTCGG  21
            || |||  | | ||||| |||||||||||||||| | ||| ||||||||||  ||||||
Sbjct  425  CTTTTTTTGTG-GTTTGCAGGCTTGGACTTGGAGGTGTTTGTCGGCCTTTAAATGGTCGG  83

Query  422  CTCCTCTTAAATGCATTAGCTTGATNCCTTGCGGATCGGCTGTCGGTGTGATAAAATGTC  81
            ||||||| ||||||||||||||||| ||||||||||||||||||||||||||||  ||||
Sbjct  484  CTCCTCTCAAATGCATTAGCTTGATTCCTTGCGGATCGGCTGTCGGTGTGATAA--TGTC  41

Query  482  TACGCCGTGACCCGTGAAAGCCGT--TTGGATGAGCTTCCA-CCGTCTTGCNTCAAAGAC  38
            ||||||| |||| ||||| || ||  ||| | ||||||||| |||||| || ||| ||||
Sbjct  542  TACGCCGCGACC-GTGAA-GC-GTCCTTGAACGAGCTTCCAACCGTCTCGCTTCAGAGAC  98

Query  539  AACTTTTTTATGACNNCNGNCCTCAAATCGGGAAGGACNACCCGTTGA-CTnaagcanat  97
            |||||||  |||||  | | ||||||||| || ||||| ||||| ||| || ||||| ||
Sbjct  599  AACTTTT--ATGACCTCTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATAT  56

Query  598  ca  599
            ||
Sbjct  657  CA  658
```

>gb|DQ425010.1| Ganoderma lucidum isolate GL166 internal transcribed spacer 1,
partial sequence; 5.8S ribosomal RNA gene, complete sequence;
and internal transcribed spacer 2, partial sequence
Length=550

```
 Score =  449 bits (243),  Expect = 1e-122
 Identities = 313/344 (90%), Gaps = 19/344 (5%)
 Strand=Plus/Minus

Query  73   AAGACGGTTGGAAGCTCATCCAAACGCTTCACGGTCACGGCGTAGACATTTTATCACACC 132
            |||||||| |||||||  |||||||||||||||| ||||||||||  ||||||||||
Sbjct  532  AAGACGGTTAGAAGCTC-GCCAAACGCTTCACGGTCGCGGCGTAGACA--TTATCACACC 476

Query  133  GACAGCCGATCCGCAAGGAATCAAGCTAATGCATTTAAGAGGAGCCGACCGTTAAA-GGC 191
            || ||||||||||||||||||||||||||||||||||||||||||||||||  ||| |||
Sbjct  475  GAGAGCCGATCCGCAAGGAATCAAGCTAATGCATTTAAGAGGAGCCGACCGACAAAGGGC 416

Query  192  CGACAAGCCCTCCAAGTCCAAGCCTACAAACTCCGCAAAGAGGTTGCAAGTTGAAGATTT 251
            ||||||| |||||||||||||| |||||| |||||||| | ||| | ||||||||||
Sbjct  415  CGACAAG-CCTCCAAGTCCAAGCCCACAAAC-CCGCAAAG-GCTTGTAGGTTGAAGATTT 359

Query  252  CATGACACTCAAACAGGCATGCTCCTCGGAATACCAAGGAGCGCAAGGTGCGTTCAAAGA 311
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  358  CATGACACTCAAACAGGCATGCTCCTCGGAATACCAAGGAGCGCAAGGTGCGTTCAAAGA 299

Query  312  TTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCG-ATTTCN-T-CGT-CTTC- 366
            |||||||||||||||||||||||||||||||||||||||| |||||  | ||| ||||
Sbjct  298  TTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCA 239

Query  367  TCG-T-CGAGA-CCA-GAGATCCGTN--TGAAAGTTG-ATATAG  403
            ||| | ||||| ||| |||||||||   ||||||||| ||||||
Sbjct  238  TCGATGCGAGAGCCAAGAGATCCGTTGCTGAAAGTTGTATATAG  195
```

>emb|AJ608709.1| Ganoderma applanatum 18S rRNA gene (partial), ITS1, 5.8S rRNA
gene, ITS2, and 26S rRNA gene (partial), specimen voucher E3795
Length=663

```
 Score =  468 bits (253),  Expect = 3e-128
 Identities = 316/344 (91%), Gaps = 19/344 (5%)
 Strand=Plus/Minus

Query  74   AGACGGTTGGAAGCTCAT-CCAAACGCTTCACGGTCACGGCGTAGACATTTTATCACACC 132
            |||||||| ||||||  | |||||||||||||||| ||| |||||||  ||||||||||
Sbjct  593  AGACGGTTAGAAGCT--TGCCAAACGCTTCACGGTCGCGGAGTAGACA--TTATCACACC 538

Query  133  GACAGCCGATCCGCAAGG-AATCAAGCTAATGCATTTAAGAGGAGCCGACCGTTAAAGGC 191
            |||||||||||||||| |||||||||||||||||||||||||||||| ||||||||
Sbjct  537  GACAGCCGATCCGCAAGGAAATCAAGCTAATGCATTTAAGAGGAGCCGACCRTTAAAGGC 478

Query  192  CGACAAGCCCTCCAAGTCCAAGCCTACAAACTCCGCAAAGAGGTTGCAAGTTGAAGATTT 251
            ||||||| ||||||||||||||||||| |||||||||| ||| ||||||||||||||
Sbjct  477  CGACAAG-CCTCCAAGTCCAAGCCTACAAACCCCGCAAAGAGCTTGTAAGTTGAAGATTT 419

Query  252  CATGACACTCAAACAGGCATGCTCCTCGGAATACCAAGGAGCGCAAGGTGCGTTCAAAGA 311
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  418  CATGACACTCAAACAGGCATGCTCCTCGGAATACCAAGGAGCGCAAGGTGCGTTCAAAGA 359

Query  312  TTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCG-ATTTCN-T-CGT-CTTC- 366
            |||||||||||||||||||||||||||||||||||||||| |||||  | ||| ||||
Sbjct  358  TTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCA 299
Query  367  TCG-T-CGAGA-CCA-GAGATCCGTN--TGAAAGTTG-ATATAG  403
            ||| | ||||| ||| |||||||||   ||||||||| ||||||
Sbjct  298  TCGATGCGAGAGCCAAGAGATCCGTTGCTGAAAGTTGTATATAG  255


>emb|AJ608709.1|  Ganoderma applanatum 18S rRNA gene (partial), ITS1, 5.8S
rRNA
gene, ITS2, and 26S rRNA gene (partial), specimen voucher E3795
Length=663
 Score =  558 bits (302),  Expect = 1e-155
 Identities = 369/401 (92%), Gaps = 13/401 (3%)
 Strand=Plus/Minus

Query  70   CAAACGCNTNNACGGTCACGGCGTAGACATTTTATCACACCGACAGCCGATCTGCAAGG- 128
            ||||||| |  |||||| ||| |||||||  |||||||||||||||||||| ||||||
Sbjct  575  CAAACGC-TTCACGGTCGCGGAGTAGACA--TTATCACACCGACAGCCGATCCGCAAGGA 519

Query  129  AATCAAGCTAATGCATTTAAGAGGAGCCCGACCCGTTAAAGGCCGACAAGCCCTCCAAGT 188
            |||||||||||||||||||||||||| |||| || ||||||||||||||| |||||||||
Sbjct  518  AATCAAGCTAATGCATTTAAGAGGAG-CCGA-CCRTTAAAGGCCGACAAG-CCTCCAAGT 462

Query  189  CCAAGCCTACAAACTCCGCAAAGAGGTTGCAAGTTGAAGATTTCATGACACTCAAACAGG 248
            |||||||||||||| |||||||||| ||| ||||||||||||||||||||||||||||||
Sbjct  461  CCAAGCCTACAAACCCCGCAAAGAGCTTGTAAGTTGAAGATTTCATGACACTCAAACAGG 402

Query  249  CATGCTCCTCGGAATACCAAGGAGCGCAAGGTGCGTTCAAAGATTCGATGATTCACTGAA 308
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  401  CATGCTCCTCGGAATACCAAGGAGCGCAAGGTGCGTTCAAAGATTCGATGATTCACTGAA 342

Query  309  TTCTGCAATTCACATTACTTATCGCATTTCGCTGCGGTCNTCATCGATGCGAGAGCCAAG 368
            |||||||||||||||||||||||||||||||||| || ||||||||||||||||||||
Sbjct  341  TTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAG 282

Query  369  AGATCCGTTGCTGAAAGTTGTATATAGAT-CTATTATATCCCAATATTACATTCTAGTAC 427
            |||||||||||||||||||||||||||| |  ||| ||| |||||  |||||||| |||
Sbjct  281  AGATCCGTTGCTGAAAGTTGTATATAGATGCG-TTACATCGCAATAC-ACATTCTAATAC 224

Query  428  TTTCAAAGAGTTTGTGATAAACGCAGGCCACA-GATGCGCT  467
            ||| |  |||||||||||||||||||| ||| | ||||||
Sbjct  223  TTT-ATGGAGTTTGTGATAAACGCAGGCAACAAG-TGCGCT  185
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>gb|FJ982798.1| Ganoderma lucidum isolate TVK1 18S ribosomal RNA gene, partial
sequence; internal transcribed spacer 1, 5.8S ribosomal RNA
gene, and internal transcribed spacer 2, complete sequence;
and 28S ribosomal RNA gene, partial sequence
Length=603

Score = 630 bits (341), Expect = 4e-177
Identities = 389/417 (93%), Gaps = 10/417 (2%)
Strand=Plus/Minus

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Query  36   TGAGGTCAGAGGTC-NANAGCTGCTC-CANCGAGACGGTTAGAAGCTCGCCAAACGCTTC  93
            ||||||||||||||  | ||||| || ||  ||||||||||||||||||||||||||||||
Sbjct  578  TGAGGTCAGAGGTCATAAAGCTG-TCTCAAAGAGACGGTTAGAAGCTCGCCAAACGCTTC  20

Query  94   ACGGTCGCACGCGTAGACATTATCACACCCGAGAGCCCGATCCGCAGGGAACCAAGCTAN  53
            |||||||| |||||||||||||||| ||||||| |||||||||| ||||||||||||
Sbjct  519  ACGGTCGCA-GCGTAGACATTATCACA-CCGAGAG-CCGATCCGCAAGGAACCAAGCTAA  63

Query  154  TGCATTTAAGAGAAGCCGACCAAGAACGGCCGACAAGCCTCCAAGTCCAAGCCTACAAAC  13
            |||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  462  TGCATTCAAGAGAAGCCGACCAAGAACGGCCGACAAGCCTCCAAGTCCAAGCCTACAAAC  03
Query  214  CACAAANGCTCGTAGGTTGAAGATTTCNTGACACTCANACAGGCNTGCTCCTCGGAATAC  73
            |||||| |||||||||||||||||| ||||||||| |||||| ||||||||||||||||
Sbjct  402  CACAAAAGCTCGTAGGTTGAAGATTTCATGACACTCAAACAGGCATGCTCCTCGGAATAC  43

Query  274  CAAGGAGCGCAAGGTGCGTTCAAAGATTCGANGATTCACTGAATTCTGCAATTCACATTA  33
            |||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||
Sbjct  342  CAAGGAGCGCAAGGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTA  83

Query  334  CNTATCGCATTTCGCTGCGTTCNTTN-TCGGTGCCGAGGAGCCAAGAGATCCGTTGCTGA  92
            | |||||||||||||||||| ||  ||| ||| ||| ||||||||||||||||||||||
Sbjct  282  CTTATCGCATTTCGCTGCGTTC-TTCATCGATGC-GAG-AGCCAAGAGATCCGTTGCTGA  26

Query  393  NAGTTGTATATAGATGCGTTACATCGCAATACACATTCTGATACTTTAAAGNGTTTG  449
             ||||||||||||||||||||||||||||||||||||||||||||||||| || |||||
Sbjct  225  AAGTTGTATATAGATGCGTTACATCGCAATACACATTCTGATACTTTATAGAGTTTG  169
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>gb|EU021456.1| Ganoderma lucidum strain WD-2038 voucher TFM-F 18922 18S ribosomal
RNA gene, partial sequence; internal transcribed spacer
1, 5.8S ribosomal RNA gene, and internal transcribed spacer
2, complete sequence; and 25S ribosomal RNA gene, partial
sequence
Length=637

Score = 695 bits (376), Expect = 0.0
Identities = 510/570 (89%), Gaps = 30/570 (5%)
Strand=Plus/Minus

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Query  49   AAGACGGNT-GNAAGCTCATCCAAACGCTTCACGGTCACGGCGTAGACATTTTATCACAC 107
            ||||||| | | ||||||  |||||||||||||||||||||||||||||  |||||||||
Sbjct  559  AAGACGGTTAG-AAGCTC-GCCAAACGCTTCACGGTCACGGCGTAGACA--TTATCACAC 504

Query  108  CGACAGCCGATCCGCAAGGAATCAAGCTAATGCATTTAAGAGGAGCCGACCGTTAAAGGC 167
            ||| |||||||||||||| |||||||||||||||||||||||||||||| ||| |||
Sbjct  503  CGAGAGCCGATCCGCAAGGAACCAAGCTAATGCATTTAAGAGGAGCCGACCGATAACGGC 444

Query  168  CGACAAGCCCTCCAAGTCCAAGCCTACAAACTCCGCAAAGAGGTTGCAAGTTGAAGATTT 227
            ||||||| ||||||||||||||||||||  || |||| || ||| | ||||||||||||
Sbjct  443  CGACAAG-CCTCCAAGTCCAAGCCTACAAA--CCACAAA-AGCTTGTAGGTTGAAGATTT 388
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Query  228  CATGACACTCAAACAGGCATGCTCCTCGGAATACCAAGGAGCGCAAGGTGCGTTCAAAGA 287
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  387  CATGACACTCAAACAGGCATGCTCCTCGGAATACCAAGGAGCGCAAGGTGCGTTCAAAGA 328

Query  288  TTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCCGGTCNTT 347
            |||||||||||||||||||||||||||||||||||||||||||||||||||| | || ||
Sbjct  327  TTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGC-GTTC-TT 270

Query  348  CATCGATGCGAGAGCCAAGAGATCCGTTGCTGAAAGTTGTATATAGATCTATTATATCCC 407
            |||||||||||||||||||||||||||||||||||||||||||| | ||| ||| | 
Sbjct  269  CATCGATGCGAGAGCCAAGAGATCCGTTGCTGAAAGTTGTATATAGATGTGTTACATCGC 210

Query  408  AATATTACATTCTAGT-ACTTTCAAAGAGTTTGTGATAAACGCAGGCCACAGATGCGCTT 466
            ||||  ||||||| || ||||| | |||||||||||||||||||| ||||||  |||
Sbjct  209  AATAC-ACATTCT-GTTACTTT-ATAGAGTTTGTGATAAACGCAGGC-ACAGATATGCTC 154

Query  467  TATAA-C--GG-ACAAGAAGCCCGCTTTGCGATCCGTCA-CCCACAGTAAAGTGC-CAGG 520
               || |  || | |||| ||  || | || ||| |  | |||||||||| |||| ||||
Sbjct  153  CGCAAGCCCGGTA-AAGA-GCGTGCCTCGCAATCTGA-AGCCCACAGTAA-GTGCACAGG 98

Query  521  TGTAGAGTGGATTGAGCAGGGCGTGCACATGCCTCGGAAGGCCAGCTACA-CCCGGTCAA 579
            |||||||||||| |||||||||||||||||||||||||||||||||||| |||||||||
Sbjct  97   TGTAGAGTGGAT-GAGCAGGGCGTGCACATGCCTCGGAAGGCCAGCTACAACCCGGTCAA  39

Query  580  AAACTCGATAATGA-CCTTCCGCAGGTTCA  608
            || |||||||||| ||||||||||||||||
Sbjct  38   AA-CTCGATAATGATCCTTCCGCAGGTTCA  10
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