ETHNOBOTANICAL SURVEY AND MOLECULAR CHARACTERIZATION OF WILD MUSHROOMS OF BILIGIRI RANGANA (B.R.) HILLS

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PALB-2239

DEPARTMENT OF PLANT BIOTECHNOLOGY

UNIVERSITY OF AGRICULTURAL SCIENCES

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In partial fulfillment of the requirements
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Master of Science (Agriculture)

in

PLANT BIOTECHNOLGY

BANGALORE SEPTEMBER,2014

AFFECTIONATELY DEDICATED

to

My Family

Chairman

Dr. N. Earanna

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CERTIFICATE

This is to certify that the thesis entitled **"ETHNOBOTANICAL SURVEY AND MOLECULAR CHARACTERIZATION OF WILD MUSHROOMS OF BILIGIRI RANGANA (B.R.) HILLS"** submitted by **Mr. AKASH, D., ID No. PALB-2239** in partial fulfillment of the requirement for the degree of **MASTER OF SCIENCE (AGRICULTURE) in PLANT BIOTECHNOLOGY** to the University of Agricultural Sciences, Bangalore is a bonafide record of research work done by him under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

Bangalore
September, 2014

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Bangalore

September, 2014 **(Akash, D.)**

ETHNOBOTANICAL SURVEY AND MOLECULAR CHARACTERIZATION OF WILD MUSHROOMS OF BILIGIRI RANGANA (B.R.) HILLS

AKASH, D.

ABSTRACT

Evergreen forests of B.R.Hills spread over an area of 540 sq KM at the eastern most edge of Western Ghats in Karnataka. Climatic conditions are more favorable for establishment of mushrooms and complete their life cycle. In the present study, fourteen mushrooms designated as BRM-1, BRM-2, BRM-3, BRM-4, BRM-5, BRM-5, BRM-7, BRM-8, BRM-9, BRM-10, BRM-11, BRM-12, BRM-13 and BRM-14 were collected in the months of June through September with the help of Soliga tribe people who inhabited the region since many years. During collection, field information such as weather conditions, habitat, abundance, growth habit and fruiting body characters were recorded. Of the fourteen mushroom species collected, the two mushrooms viz., BRM-1 and BRM-2 were identified as *Ganoderma lucidum* and *Polyporous flabelliformis* based on their phenotypic characters. The other 12 mushrooms, were identified by using ITS/ 18S rRNA gene sequence using the sequence data available in the National centre for Biotechnological Information. Based on the sequence homology, the mushrooms were identified as *Termitomyces* sp (BRM-3)., *Auricularia delicate* (BRM-4), *Termitomyces microcarpus* (BRM-5), *Amanita* sp. *(BRM-6), Podoscypha petalodes (BRM-7), Agaricaceae* sp. *(BRM-8), Macrolepiota* sp.*(BRM-9), Calvatia holothurioides(BRM-10), Gymnopillus crociphyllus (BRM-11), Coprinus comatus (BRM-12), Gyrodontium sacchari* (BRM-13) and *Clitocybe aff fellea (BRM-14).* Among the fourteen mushrooms characterized, only three species viz., *Termitomyces* sp., *Auricularia delicate* and *Termitomyces microcarpus* was found edible. These three species when cultured on Potato dextrose agar, only *Termitomyces sp.* (BRM-3) produced mycelial growth.

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ಮನುಕುಲಸಸ್ಯಶಾಸ್ತ್ರದ ಸಮೀಕ್ಷೆ ಹಾಗು ಬಿಳಿಗಿರಿರಂಗನ ಬೆಟ್ಟದಲ್ಲಿ ದೊರೆಯುವ ಕಾಡು ಅಣಬೆಗಳ ಪ್ರಭೇಧಗಳನ್ನು ಆಣ್ವಿಕ ವಿಧಾನದಿಂದ ಪತ್ತೆಹಚ್ಚುವಿಕೆ

ಆಕಾಶ್, ಡಿ.

ಸಾರಾಂಶ

ನಿತ್ಯಹರಿದ್ವರ್ಣ ಕಾಡಾದ ಬಿಳಿಗಿರಿ ರಂಗನ ಬೆಟ್ಟ ಪಶ್ಚಿಮ ಘಟ್ಟಗಳ ಅತ್ಯಂತ ತುದಿಯಲ್ಲಿ ೫೪೦ಚದರ ಕಿಲೋಮೀಟರ್ ಪ್ರದೇಶದಲ್ಲಿ ಹರಡಿದೆ. ಅಣಬೆ ಬೆಳವಣಿಗೆ ಮತ್ತು ಅವುಗಳ ಜೀವನ ಚಕ್ರ ಪೂರ್ಣಗೊಳಿಸಲು ಈ ಹವಾಮಾನಹೆಚ್ಚು ಅನುಕೂಲಕರವಾಗಿರುತ್ತದೆ. ಪ್ರಸ್ತುತ ಅಧ್ಯಯನದಲ್ಲಿ ಜೂನ್ ನಿಂದ ಸೆಪ್ಟೆಂಬರ್ ವರೆಗೆ ಹದಿನಾಲ್ಕು ಅಣಬೆಗಳನ್ನು ಸೋಲಿಗ ಬುಡಕಟ್ಟು ಜನಾಂಗದ ಸಹಾಯದಿಂದ ಸಂಗ್ರಹಿಸಲಾಯಿತು. ಸಂಗ್ರಹಿಸುವಾಗ ಅಲ್ಲಿನ ಸ್ವಾಭಾವಿಕ ಗುಣ ಲಕ್ಷಣಗಳು , ದಿನಾಂಕ, ಋತು, ಅವಸಾ ಸ್ಥಾನಗಳನ್ನು ದಾಖಲಿಸಿಕೊಂಡು . ಈ ಹದಿನಾಲ್ಕು ಅಣಬೆಗಳ ಪೈಕಿ ಎರಡು ಅಣಬೆಗಳಾದ ಬಿಆರ್ ಎಂ-೧ ಮತ್ತು ಬಿಆರ್ ಎಂ-೨ ಅನ್ನು ಕ್ರಮವಾಗಿ *ಗ್ಯಾನೊಡರ್ಮಾ ಲುಸಿಡಂ* ಮತ್ತು *ಪಾಲಿಪೋರಸ್ ಫ್ಲಬೆಲಿಫಾರ್ಮಿಸ್* ಎಂದು ಅವುಗಳ ರೂಪರಚನೆ ಮತ್ತು ಬೀಜಾಣುಗಳ ಆಧಾರಗಳ ಮೇಲೆ ಪತ್ತೆ ಹಚ್ಚಲಾಯಿತು. ಉಳಿದ ೧೨ ಅಣಬೆಗಳನ್ನು ೧೮ಎಸ್ ರೈಬೋಸೋಮಲ್ ರೈಬೋ ನ್ಯೂಕ್ಲೆಈಕ್ ಆಮ್ಲದ ಅನುಕರಣೆ ಬಳಸಿ ಅಮೇರಿಕದಾ ರಾಷ್ಟ್ರೀಯ ಜೀವತಾಂತ್ರಿಕ ಶಾಸ್ತ್ರದಮಾಹಿತಿಕೇಂದ್ರದಲ್ಲಿಯ (ಏನ್ . ಸಿ .ಬಿ .ಐ) ದತ್ತಮಾಹಿತಿ ವಿಶ್ಲೇಷಣೆ ಆಧಾರದ ಮೇಲೆ ಗುರುತಿಸಲಾಯಿತು. ಅನುಕರಣೆ ಹೋಲಿ ಕೆ ಯನ್ನುಆಧರಿಸಿ ಈ ಅಣಬೆಗಳನ್ನು, *ಟೆರ್ಮಿಟೋಮೈಸಿಸ್* ಪ್ರಭೇಧ, *ಆರಿಕ್ಯುಲರಿಯ ಡೆಲಿಕೆಟಾ, ಟೆರ್ಮಿಟೋಮೈಸಿಸ್ ಮೈಕ್ರೋಕಾರ್ಪಸ್* , *ಅಮ್ಯಾನಿಟಾ* ಪ್ರಭೇಧ, *ಪೊಡೋಸ್ಸೈಫಾ ಪೇಟಲೋಡಿಸಿ* ,*ಅಗರಿಕೆಸೆಯ* ಪ್ರಭೇಧ., *ಮ್ಯಾಕ್ರೊಲೆಪಿಯೋಟಾ* ಪ್ರಭೇಧ, *ಕ್ಯಾಲ್ವೆಶಿಯ ಹೊಲೋತುರೀಡೆಸ್, ಜೀಮ್ನೊಪಿಲ್ಲಸ್ ಕ್ರಾಸಿಪಿಲ್ಲಸ್ ಕೊಪ್ರಿನುಸ್ ಕೋಮಾಟಾಸ್* , *ಗೈರೋಡಾಂಟಿಯಮ್ ಸಾಕಾರಿ, ಕ್ಲೈಟೋಸೈಬೆ ಅಫ್.ಫೇಲಾ* ಎಂದು ಗುರುತಿಸಲಾಗಿದೆ. ಈ ಹನ್ನೆರಡುಅಣಬೆಗಳಲ್ಲಿ, *ಆರಿಕ್ಯುಲರಿಯ ಡೆಲಿಕೆಟಾ, ಟೆರ್ಮಿಟೋಮೈಸಿಸ್ ಮೈಕ್ರೋಕಾರ್ಪಸ್, ಟೆರ್ಮಿಟೋಮೈಸಿಸ್* ಪ್ರಭೇಧ (ಬಿಆರ್ ಎಂ-೩, ಬಿಆರ್ ಎಂ-೪ ಮತ್ತು ಬಿಆರ್ ಎಂ-೫) ಖಾದ್ಯ ಅಣಬೆಗಳೆಂದು ಗುರುತಿಸಲಾಗಿದ್ದು, ಅವುಗಳನ್ನು ಆಲುಗಡ್ಡೆ ಅಗರ್ ಮಾಧ್ಯಮದಲ್ಲಿ ಅಂಗಾಂಶಕೃಷಿ ಮಾಡಿದಾಗ *ಟೆರ್ಮಿಟೋಮೈಸಿಸ್* ಪ್ರಭೇಧ ಮಾತ್ರ ಮೈಸೀಲಿಯಾವನ್ನು ಉತ್ಪತ್ತಿ ಮಾಡಿರುತ್ತದೆ . ಆದ್ದರಿಂದ *ಟೆರ್ಮಿಟೋಮೈಸಿಸ್* ಪ್ರಭೇಧ (ಬಿಆರ್ ಎಂ-3)ಅಣಬೆಯನ್ನು ಪಿಡಿಎ ಮಾಧ್ಯಮದಲ್ಲಿ ಅಂಗಾಂಶ ಕೃಷಿಯಿಂದ ಪ್ರತ್ಯೇಕಿಸಿ, ಬೆಳೆಸಿ ಭವಿಷ್ಯದ ಅಧ್ಯಯನಕ್ಕಾಗಿ ಸಂರಕ್ಷಿಸಲಾಗಿದೆ.

ಸಸ್ಯಜೈವಿಕತಂತ್ರಜ್ಞಾನ ವಿಭಾಗ — ಡಾ॥ ಏನ್, ಈರಣ್ಣ

ಯು .ಎ .ಎಸ್. ಜಿ. ಕೆ . ವಿ. ಕೆ., ಬೆಂಗಳೂರು — (ಪ್ರಧಾನ ಸಲಹೆಗಾರರು)

"Ethnobotanical Survey and Molecular Characterization of Wild Mushrooms of Biligirirangana (B.R) Hills."

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Introduction

Evergreen forest canopy of B.R.Hills spread over an area of 540sq KM at the eastern most edge of Western Ghats at the temperature of 9^0c-16^0c maximum reach up to 20^0c-38^0c with the annual average rainfall of 600mm-3000mm which is preferable climatic conditions for mushroom flora establishment . In this study wild mushrooms were collected from the B.R.Hills forest area during the monsoon months of July-august 2013 and identified using ITS/18SrRNA sequencing.

Objective

1) Ethnobotanical survey and collection of wild mushrooms
2) Identification of the wild mushrooms based on morphological and molecular characters(ITS/18S rRNA sequencing)
3) Invitro culturing of edible mushrooms

Methodology

Genomic DNA was isolated from the fruiting body using the modified CTAB method (Datta *et al.,* (1989). 18SrRNA/ITS gene was amplified by PCR using 18SrRNA primers. The amplified product was eluted and cloned into pTZ57R/T cloning vector mobilized into *E. Coli* (DH5α) cells (Sambrook *et al*., 1989). The recombinant colonies were selected by blue white screening and confirmed by colony PCR using M13 primers. The plasmid having 16SrRNA gene was got sequenced by Sci genome co. The mushrooms species were identified based on their sequence homology in NCBI. (http://www.ncbi.nlm.nih.gov/BLAST/).

Results

Fourteen mushroom species were collected from B R Hills of Karnataka, identified by using ITS/ 18SrRNA gene sequencing (Plates above). Out of 14 species, three were edible, one was medicinal and 10 mushrooms were non edible. *Termitomyces* species BR 17 was cultured on Potato dextrose agar using stipe tissue.

Discussion

To document mushroom diversity in the Western Ghats of Karnataka systematic survey is needed(Meera P and Veena SS, 2012). In the present investigation, 14 mushroom species were documented and characterized by using morphological and molecular characters(18S/ITS rRNA). Among the 14 species, three species were found edible, one was identified as medicinal and remaining were non edible. This indicated the occurrence of many more potential mushroom species in BR Hills of Karnataka from which pure culture can be isolated and used for commercial production.

Summary

Fourteen mushrooms were documented from B.R.Hills of Karnataka and identified to species level using 18SrRNA/ITS gene sequence and two were identified based on morphological characters. There were three edible mushrooms and one medicinal species.

Advisory Committee
1. Dr. N. EARANNA (Chairperson)
2. Dr. H. E. SHASHIDHAR(member)
3. Dr. ANITHA Peter(member)
4. Dr. NATARAJA N Karaba(member)
5. Dr. S SUBRAMANYA (Member)

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I. INTRODUCTION

"Without leaves, without buds, without flowers, yet they form fruit. As food, as a tonic and as medicine, mushrooms are wonderful creations of nature"

- S.T. Chang

Mushroom is broadly defined by Chang and Miles (2004) as "a macro fungus with distinctive fruiting body which can either epigeous or hypogeous and large enough to be seen with the naked eye and to be picked by hand". The magnitude of fungal diversity was estimated to be at least 1.5 million species of which 140,000 species produce fruiting bodies of sufficient size and suitable structure to be considered as macro fungi. About 7000 species are considered to possess varying degrees of edibility, and more than 3000 species are regarded as edible mushrooms. To date, only 200 of them are experimentally grown, and 100 economically cultivated. Approximately 60 commercially cultivated, and only 10 have reached an industrial scale of production in many countries (Rajaratnam and Thiagarajan, 2012). Many more potential species remained unexplored in the womb of nature.

Biligiri Rangana Hills (B.R. Hills) rich mushroom biodiversity is located in between 11° and 12° N and the ridges of the hills run in the north-south direction. It is a projection of the Western Ghats in a north-easterly direction and meets the hills of the Eastern Ghats at 78°E situated in Yelandur and Kollegal Taluks of Chamaraja nagar district of south-eastern part of Karnataka. The area is spread over 540 km² with a wide variation in mean temperature ranging from 9 to 16 °C minimum and 20 to 38 °C maximum. B.R. Hills receives 600 mm annual rainfall at the base and 3000 mm at top of the hills. A variety of mushroom species appears during rainy season. A wide range of climatic conditions along with the altitude variations made the ecosystem congenial for biodiversity. For hundreds of years, this region has been inhabited by semi-nomadic Soliga tribes. The Soligas are nature worshippers and mainly dependent on forest products including mushrooms for their livelihood.

Mushrooms are source of good quality protein, rich in vitamins, minerals and low in calorie with negligible starch, sugars and fats. In addition to their nutritive value, many edible and non-edible mushrooms have long been used for medicinal purposes. The important therapeutic properties includes, antioxidants, hypertension, cholesterol lowering, liver protection, anti-inflammatory, anti-diabetic and anti-microbial (Wasser and Weis, 1999; Hobbs, 1995). In India, only four species namely *Agaricus bisporus* (white button mushroom), *Pleurotus sajorcaju* (dhingri mushroom), *Calocybe indica* (milky mushroom) and *Volvariella volvacea* (paddy straw mushroom) are commercially cultivated. Annual production of mushrooms has been estimated to be 40000 MT. *Agaricus bisporus* contribute 80-85 per cent, *Pleurotus* species contribute 15-19 per cent and other varieties contribute 1 per cent of the total production (Sharma and Upadhyay, 1998).

Mushroom possesses ephemeral character and disappears within a day. Hence, mushroom hunting is not a simply roaming through the forest after it rains. It is an art, a

skill and meditation. Further, all mushrooms are not gilled; therefore giving a full account of their classification is difficult. Some have pores underneath (Boletus), others have spines, such as the hedgehog mushroom and other tooth fungi, and so on. Based on their external appearance and inherent properties such as odour, texture etc., mushrooms have been named as Polypores, Puffballs, Jelly fungi, Coral fungi, Bracket fungi, Stinkhorns, and Cup fungi. Thus, the term has more common application to macroscopic fungal fruiting bodies than one having precise taxonomic meaning.

Mushrooms can be identified based on their morphological and molecular characters. The phenotypic characters include the shape, size, texture, colour and odour of the mushroom. The microscopic characters include the spore size, spore shape, basidium etc., A typical mushroom consists cap, gills, stipe, annulus, and volva. Cap is an expanded portion of the mushrooms which may vary in size, shape and color. This may be thick, fleshy and membranous. Gills are situated inside the pileus, started from the apex of the stipe and expanded towards the margin. These gills bear spores on their surface. The veil covers the gill extending from the margin of the cap to the stipe. As the fruiting body matures it breaks away; some portion remaining attached to the margin of the cap while others form a ring on stipe which is known as the "annulus". Stipe or stalk gives the support to the cap. This may be centrally attached in some cases it may be laterally attached to the stalk. Its presence or absence is completely dependent up on the genera it belongs to. The stipe may be solid, hollow, fleshy etc. the shape of the stipe also varies accordingly to the genera. It may be cylindrical, spindle or club shaped. As the fruiting bodies expand the veil breaks and remain as the cap like structure known as volva which is surrounding the base of the stalk. The presence or absence of volva helps in identifying the genera of mushroom. But some other mushrooms like coral fungi, bird nest fungi, morels deviate from the above description.

Molecular tools provide more accurate methods for identification than the few characters afforded by traditional morphological features. Molecular markers, especially DNA techniques are quick and reliable to establish the identities of wild collections and are helpful in mushroom taxonomy. After the advent of cycle sequencing methodology, direct sequencing of PCR products became a routine matter at least in organelle DNA loci or repetitive nuclear DNA such as ribosomal DNAs. This technology is considered to be one of the most powerful methods for phylogenetic studies. The ribosomal RNA genes (rDNA) of fungi are located on a single chromosome and are present as repeated subunits of a tandem array of transcribed and non-transcribed stretches of DNA, which appeared highly conserved. ITS (Internal Transcribed Spacer) refers to a piece of non-functional RNA situated between structural ribosomal RNAs on a common precursor transcript. Sequence comparison of the ITS region is widely used in taxonomy and molecular phylogeny because it is easy to amplify even from small quantities of DNA and has a high degree of variation between closely related species. The ITS region/ 18S rRNA gene sequence are the most widely used techniques in molecular phylogenetics to identify the mushrooms to species level. Further, isolation of potential species into pure culture and their conservation for future experimentation is essential. Therefore, the present investigation was undertaken with the following objectives.

1. Ethno-botanical survey and collection of wild mushrooms from B.R. hills.
2. Identification of the wild mushroom based on morphological and molecular characters.
3. *In-vitro* culturing of important wild mushrooms.

II. REVIEW OF LITERATURE

Ethnobotany is the study of past and present interrelationships between human cultures and the plants, animals, mushrooms and other organisms in their environment. Since the beginning of civilization, people have used mushrooms for their food, medicines and entertainment. Since mushrooms were included with plants till recent past the term ethnobotany is applied in this context. Otherwise, ethnomycology is an appropriate term to be used. Mushrooms have been used because of their variety of properties. Nowadays their nutritional and medicinal properties are increasingly explored for human benefits. In this chapter, literature survey made on mushroom collection, identification, nutrition and medicinal value and molecular characterization are described.

Artificial cultivation of mushroom was initiated in France around 1650 AD and spread rapidly to the entire Europe. Early people believed mushrooms to be wild food but now it has become very popular and valuable food item in the modern dietary regimes because of its delicacy. The evidences for use of mushrooms as food and medicine have been found in the inscriptions and sculptures of many ancient civilizations. Mushrooms were believed by the Greek to give strength to the warrior while Romans regarded them as the 'Food for the God'.

2.1. History: Mushroom as food

Mushrooms growing in forest were picked up and consumed by mankind from the time immemorial. However, authentic records were available from the Guatemalan sculptures, dating back from 1000 B.C. to 2000 A.D. which clearly depict that the Agaricsporophores of hallucmogenic mushrooms used in ceremonies (Wason and Wason 1957). The first record on the use of Gasteromycetes as food was observed by Berkeley (1860) who reported that the *Melanogaster variegatus* fruit bodies were sold in the market under the name reci truffle. Rea (1924) mentioned all British puffballs *(Calvatia* and *Lycoperdon)* as tender, delicate in flavor and taste. Hippocrates, a Greek physician, indicated that ancient people were aware of therapeutic power of mushrooms in 500 B C (Buller, 1922). Many species of the order Aphyllophorales in hymenomycetes have edible fruit body and some of them are most sought after by mycophagists. Prance (1973) while, visiting several Indian villages and in the north west of the territory of Roraima, Brazil, found that the Waika tribes in that area were using four species of fungi as food namely, *Favolus brasiliensis, Favolus tesseleatus, Polyporus stipitatus* and *Neoclitocybe bissiseda. Laetiporus sulphureus* found in foot hills of North Western Himalayas were also quite popular because of their tastes resembling with that of a chicken Harsha and Bisht (1997).

2.2. Nutritional attributes of mushrooms

Nutritional and pharmaceutical values are attributed to the edible and medicinal mushrooms. Mushrooms are rich sources of proteins, minerals and essential amino acids besides carbohydrates and fats. Barry (1971) analyzed free carbohydrates in *Agaricus bisporous,* the cultivated Mushrooms and found four sugars fructose, glucose, mannitol and sucrose. By qualitative and quantitative analysis of mushrooms, mannitol was found

to be present in largest amount accounting for over 12 percent of the dry weight of mushroom. Mushroom tissue contained approximately 92 percent water. It was reported that the high amount of free carbohydrate, especially mannitol, may provide the osmotic potential necessary to maintain high concentration of water.

According to Crisan and Sands (1978), the mushroom carbohydrate constitutes of pentoses *viz*., methyl pentoses, hexoses, as well as disaccharides, amino sugars, sugar alcohols and sugar acids. Fresh mushroom contains relatively large amount of carbohydrates (3.28 %) and fibre (3.32%). The carbohydrate content may consist of free sugars, organic acids and variety of compounds. The dried shiitake mushroom includes D- arbitol (1.2 to 7.7 %), D-mannitol (2.2 to 6.2 %) and trehalose (3.7 to 9.4%). Organic acid content of fresh mushroom ranges from 1.1 to 1.5 per cent and includes malic, fumaric, pyroglutamic and citric acids (Yoshida *et al*., 1979)**.**

Pleurotus species were reported to contain about 90% moisture. 10% dry matter, 10-35% protein, 1-2% fat and 5-10% ash on dry weight basis (Jandaik,1986). In addition to their nutritional value, many edible large mushrooms have long been used in the Orient for medicinal purposes. Many non-edible species have also gained important medicinal usage. At present, there are at least 270 species of mushroom that are known to have various therapeutic properties (Ying *et al.*, 1987)

Bano and Rajarathnam (1988) studied the changes in distribution of low molecular weight carbohydrate and high molecular weight polysaccharides trehalose and mannitol in the fruit bodies of *Pleurotus ostreatus* in major quantities. In *Pleurotus,* carbohydrate ranges from 46.6 to 81.8 percent on dry weight basis. The absence of starch in mushrooms makes it an ideal food for diabetic patients and for persons who wish to shed excess fat from their bodies Bhal (1990). Fiber content of *Pleurotus* species ranged from 7.4 to 27.6 percent and 10.4 percent in *Agaricus bisporous* and 4 to 20 percent in *Volvariella volvacea* (Shu-Ting and Philip, 1993). Kamugisha and Sharan (2005) found that the moisture content of Milky mushroom *(Calocybe indica)* is about 88-90%. The total carbohydrate content is about 60-75% with fiber content of 3.9-7% and the protein content ranged between 18-22% on dry weight basis.

Jonathan *et al.* (2006) collected 12 wild edible Nigerian mushrooms from different locations at southern part of Nigeria for analysis of prominent and mineral element composition. Of the entire wild mushrooms tested, *Termitomyces globulus* possessed maximum protein, ash, calcium, phosphorus and iron content followed by *Termitomyces microcarpus*, *Volvariella esculanta*, *Lycoperdon gigantium and Lycoperdon pusilum.* The nutritional value of dietary mushrooms *viz*., *Pleurotus ostreatus, P. sajorcaju, P. florida and Calocybe indica* were analysed for their nutritional value. These species are rich in proteins (20-25%), fibers (13-24%), carbohydrate (37-48%) and mineral content (8-13%). Different parts such as pileus and gills showed higher protein and lipid content, whereas stipe was rich in carbohydrate and fiber content (Alam *et al.,* 2008). Six species of oyster mushrooms compared for their nutrient content indicated varied composition. Of the six, *Pleurotus sajorcaju* showed highest protein content, *Pleurotus cystidiosis* had highest lipid, maximum carbohydrate was present *in*

Table 1. Nutritional value of mushrooms as compared with important pulses

Sl. No.	Sample name	Protein (%)	Fe (µg/g)	Zn (µg/g)
1	*Agaricus bisporous*	24.85	786.60	323.76
2	*Pleurotus florida*	24.81	148.16	518.56
3	*Termitomyces* sp.	27.03	138.40	659.60
4	*Cajanus cajan*	17.24	866.06	305.30
5	*Glycine max*	37.32	875.50	286.90
6	*Vigna unguiculata*	20.07	917.63	202.83
7	*Macrotyloma uniflorus*	17.59	880.60	171.20
	SEM±	0.22	9.33	1.94
	CD@1%	0.66	28.30	5.88

Source: Earanna *et al.* (2013)

Pleurotus geesteranus, fiber content was high in *Pleurotus shighking* and *Pleurotus florida* had more ash content (Asaduzzaman *et al.*, 2008).

2.3. Medicinal properties of mushrooms

Medicinal mushrooms have become even more widely used ingredient in traditional medicine for the treatment of various diseases and related health problems. As a result of large numbers of scientific studies on medicinal mushrooms especially in Japan, China and Korea, over the past three decades, many of the traditional uses have been confirmed and new applications developed. They also offer other potentially important therapeutic properties including antioxidants, anti-hypertensive, cholesterol-lowering, liver protection, anti-fibrotic, anti-inflammatory, anti-diabetic, anti-viral and anti-microbial (Borchers *et al.,* 2004).

Pathirage and Yunman (2011) reviewed scientific information on mushrooms with regards to its anti-diabetic active compounds and/or pharmacological test results, which are commonly used as functional foods and ingredients used in the traditional medical system and which had demonstrated experimental or/and clinical anti-diabetic effectiveness and these functional food might have a big potential for the prevention or cure of diabetes.

Information on the ethnomedicinal uses of some mushrooms such as *Pleurotus tuber-regium* for headache, stomach pain fever, cold, constipation; *Lentinus squarrsulus* for mumps, heart diseases; *Termitomyces microcarpus* for gonorrhea; *Calvatia cyathiformis* for leucorrhea, barreness; *Ganoderma lucidum* for treating arthritis, neoplasia; *G. resinaceum* for hyperglycemia, liver diseases (hepatoprotector); *G. applanatum* as antioxidant had been gathered through survey (Olusegun and Oyetayo, 2011).

De Silva *et al.* (2012) reported that bioactive metabolites including polysaccharides, proteins, dietary fibres, and many other biomolecules isolated from several edible mushrooms like *Agaricus bisporus* (White button mushroom), *Agaricus campestris* (Field mushroom or meadow mushroom), *Agaricus subrufescens* (Almond mushroom), *Agaricus sylvaticus* (Sun mushroom), *Auricularea auricule-judae* (Jelly Ear mushroom or Jew's Ear), *Coprinus comatus* (Shaggy ink cap), *Cordyceps militaris, Cordyceps sinensis* (Caterpillar fungi), *Ganoderma lucidum sensulato* (Lingzhi), *Grifola frondosa* (Maitake), *Hericium rinaceus* (Lion's Mane Mushroom), *Lentinula edodes* (Shiitake), *Phellinus spp*., *Pleurotus spp*.(Oyster mushroom), *Tremella fuciformis* (Snow fungus or Silver ear fungus), *Tremella mesenterica* (Yellow brain mushroom), *Wolfiporia extense* (*Poriacocos*) (Pine tree rotting mushroom) and their cultured mycelia have been shown to be successful in diabetes treatment as biological anti hyperglycemic agents by directly acting upon the glucose metabolism and related biochemical pathways.

2.4. Collection and identification of Mushrooms

Mcllavine and Macadam (1902) listed 18 species of *Calvatia, Lycoperdon* and *Bovista* as edible in North America when their fruit bodies were young and fresh. They

also recorded the unexpanded fruit bodies (egg) of members of *Phallales* to be edible by natives of North America. Manjula (1983) reported that best collection of the fleshy agarics was achieved during the early days of the early monsoon period reported 115 genera and 538 species of *Agricoid* and *Boletoid* fungi from India and Nepal. Purkayastha and Chandra (1985) reported several edible, non-edible and poisonous mushrooms from West Bengal. Similar studies were also reported from U.P. Hills by Harsh and Bisht (1985). Several fleshy fungi belongs to Agaricales were recorded from South Indian states viz., Tamil Nadu, Kerala, Karnataka and Andhra Pradesh (Bhavani Devi and Nair, 1987).

Kumar *et al.* (1991) collected 110 genera of mushrooms from forests of Chhattisgarh and identified seventeen genera of fleshy fungi. Of these, *Volvariella diplasia, V. volvacea, Termitomyces mammiformis, T. albuminosa, and T. albigenosus* were identified and characterized as edible mushrooms. A Survey of Mandla, Jabalpur and Shahdol districts of Madhya Pradesh conducted by Harsha *et al.*(1993) reported six fleshy fungi *viz, Astraeus sp., Mycenastrum corium, Podahrella sp, Russula sp*. and *Termitomyces sp.*

According to Singh (1994) *Podaxis* and *Phellorina* were most common edible mushrooms grown on road side during rainy season and sold in local markets of Bikaner, Jodhpur, Jaisalmer, Ajmer, Jaipur etc. Edible wild mushroom *Phellorina inguinans* was also reported from Haryana and Rajasthan (Sharma *et al.,* 1994). Nineteen species of edible mushrooms were found to grow wildly on natural substrates at Tripura (Bhattachargee and Sarkar, 1994).

Kumar and Lakhanpal (1994) characterized six genera of family *Agaricaceae* from North-Western Himalayas out of these, two species *viz*., *Agaricus angustu* and *Lencoagaricus rubrotinctus* were the new records from India. Bhatt *et al.* (1995) illustrated and described four species of the genus *Russula viz*., *R. albida, R. amoenolens. R. brevipes* and *R.dissimulans* for the first time from India which were collected from Garhwal districts of Himalaya.

Sahu *et al.* (1995) characterized and described on wild edible bamboo mushroom about the pileus, gills, stipe and other morphological characteristics and identified it as *Cantharellus* species. During the survey of forest areas of Kerala, Bhavani Devi (1995) reported occurrence and distribution of 134 species of mushroom belonging to 45 genera of these, *Pleurotus cornucopiae, P. opuntiae, Volvariella volvacea, Rhodophyllus abortivus, Agaricus and Lepiota* species.

Patil *et al.* (1995) listed 231 species of fleshy fungi from Maharashtra of which 50 species were edible. Similar studies were also made by Verma *et al.* (1995) from North-Eastern hills of India. They found 95 species growing naturally and several species of fleshy fungi belonging to different families *viz., Agaricaceae, Amanitaceae, Bolbitiaceae, Boletaceae, Cantharellaceae, Coprinaceae, Cortlnanaceae, and Hygrophoraceae. Gomphidiceae, Russulaceae* and *Tricholomataceae* were reported from Himalayan region of Himachal Pradesh.

The fungi, namely *Astraeus hygromatricus. Agaricus* sp., *Calvatia cyathiformis, Cyathus sterocoreus, Cyathus limbatus, Lycoperdon pusillum, Microcarpus xanthopus, Phallus rubicandus, Termitomyces microcarpus* and *Xylaria polymorpha* were surveyed by Sahu *et al.*(1995) from Balaghat district and found *Cantharellus* sp. (Bamboo mushroom) was most frequently collected and sold by the tribal people in the local markets.

Doshi and Sharma (1997) conducted forest surveys during monsoon season in Rajasthan from 1989 to 1990 and reported 173 species of mushrooms belonging to 95 genera, out of which 18 genera were reported from class Gasteromycetes. Of these six were edible and cosmopolitan. Bhatt *et al.* (2000) studied the genus *Lactarius* and gave the detailed morphological, anatomical structures of nine species. Seventeen species of mushrooms were recorded from Assam of which *Tricholoma lascivum, Cantharellus brevipas, Lycoperdon pusillum, Marasmius areades, Pleurotus citrinopileatus, Termitomyces mammiformis* and *Tricholoma gamhosum* were edible (Gogoi *et al.*, 2000).

Kanaddas (2010) collected 126 wild mushrooms from Barsey Rhododendron sanctuary of the state Sikkim are enlisted with scientific names, common names, distribution, growing period and status of edibility. And 46 medicinal mushrooms are also high lightened with their medicinal properties.

Srivastava *et al*. (2011) identified four species of edible mushrooms from Gorakhpur forest division using morphological and phenotypical characters. Four species naming *Termitomyces heimii*, *Termitomyces clypeatus*, *Termitomyces mammiformis* and *Termitomyces microcarpus* characterized by different morphological traits i.e., shape of perforatorium, stipe length, pileus length, margin of fruiting body, colour, gills, flesh, annulus, psedorhhiza and spore print were recorded and results indicate that all the four species of *Termitomyces* shows great diversity in their morphological traits.

Gurudevan *et al*. (2011) collected 68 mushrooms from different Western Ghats regions of India during 2008-2010 of which 19 were identified morphologically. Among them a wild mushroom *Pleurotus djamor-roseus* was identified up to molecular level and *Ganoderma lucidum* and *Lentinula edodes* shows inhibition of mycelial growth by diethyl ether fraction. Segulamasaphy (2011), located several distinct populations of black morels growing in different habitats and identified that mushrooms of black ectotypes belongs to *Morchella conica* and *M. elata.*

Sandhya *et al*. (2012) studied the biodiversity of mushrooms from Amarkantak, Madhya Pradesh, India where they have collected 50 samples from July-September 2010, of which only 16 samples were identified up to species level and concluded as the forest is rich in mushroom diversity. The survey conducted by Pushpa and Purushothama (2012) from June 2007 to November 2010 in 8 different places which included scrub jungles and urban places in an around Bangalore and a total number of 90 species in 48 genera belonging to 19 families in 05 orders were recorded, 28 species were found to be recorded for the first time in India. Among the collected species *Coprinus disseminates* followed by *Coprinus fibrillosis* and *Schizophyllum communae* were abundant.

Meera and Veena (2012) documented the mushroom biodiversity of Western Ghats of Karnataka. The documented species includes important edible and medicinal mushrooms *viz*., *Pleurotus djamor, Pycnoporus cinnabarinus, Trametes spp and Ganoderma spp.* Among the documented species, the *P. djamor* has been domesticated and commercialized. Fekadualemu (2013) reported various wood decaying Basidiomyces *viz*., *Ficus sycomorus, Trametes versicolor, Gyromitra spp*. *Agaricus campestris,* from the Dilla University surroundings of Ethiopia.

2.5. Molecular characterization of mushrooms

Phylogeny is the evolutionary history of organisms. Various genes are used in molecular phylogenetic analysis. The genes encoding for 16SrRNA in prokaryotes and 18S rRNA/ITS in eukaryotes are most widely used in molecular phylogenetics. These small subunit 30S ribosomal RNA (SSU rRNA) genes have been used extensively for sequence based evolutionary analysis because they are Universally distributed, functionally constant, sufficiently conserved and have adequate length therefore, they can provide a view of evolution encompassing all living microorganisms (Madigan, *et al*., 2009).

A large and growing database of rRNA gene sequences exists. The chromosomal database project II *(RDP II: http//rdp.cme. msu.edu)* contains a selection of such sequences, numbering over 440,000 and provide a variety of analytical programs Madigan, *et al*., (2009). The 23S large subunit of r-RNA (LSU rRNA) gene is also phylogeniticaly informative with its large sequence providing additional information. However, its length makes it more costly and time consuming. Sequence comparisons of individual genes can provide valuable insight for taxonomy. The 18S rRNA/ITS gene serve as a 'golden standard' for the identification and description of new species Madigan *et al*. (2009).

Phylogenetic analysis using DNA sequencing relies heavily on the polymerase chain reaction (PCR) to obtain sufficient copies of a gene for efficient sequencing. Specific oligonucleotide primers are designed that binds to the ends of the gene of interest, allowing DNA polymerase to bind and copy the gene. The PCR product is then visualized by agarose gel electrophoresis, excised from the gel, extracted and purified from the agarose ,and then sequenced. Once the DNA sequence of a gene is obtained, the next step is sequence alignment. The web based BLAST (Basic Alignment Search Tool) of the National Institutes of Health (http://www.ncbi.nlm.nih.gov/BLAST) does this automatic ally and can help to identify genes homologous to a new sequence from among the many thousands of genes already sequenced.

Moor *et al.* (2002) showed by performing Polymerase chain reaction (PCR) analysis on the internal transcribed spacer (ITS) for identifying mushroom species of *Boletus edulis* and several closely related mushroom species (e.g. *Suillus luteus*). Singh *et al.,* (2006) collected eighteen germplasm mushrooms from Udaipur and characterized using DNA fingerprinting and ribosomal rRNA gene sequencing. Phylogenetic analysis based on RAPD profiles and nucleotide sequence 5.8 rRNA gene revealed variation among intergeneric and intra species isolates among accessions. Based on ITS region

polymorphism, seven isolates were identified as *Podaxis pistillaris*, four as *Phellinus igniarius* one as *Gymnopilus subearlei*, and six as *Phellorinia herculea.* The similarity matrix revealed very high intra species homology and significant inter generic diversity. *Gymnopilus subearlei* and *Phellorinia herculea* have been discovered as new addition to the Indian basidiomycete's biodiversity.

Muruke *et al*. (2002) determined the relatedness of mushroom fruiting bodies to the isolated mycelia of nine isolates of edible mushroom of genera *Oudemansiella, Coprinus* and *Pleurotus* by using PCR in combination with Restriction Fragment Length Polymorphism (RFLP) analysis which helped in sorting out a case of mistaken identity of *Oudemansiella* fruiting bodies which were interchanged with another mushroom specimen during packing.

Prakasam *et al.* (2011) collected two milky mushroom (*Calocybe indica)* strains-Ci (P), Ci (N), and *Tricholoma giganteum* from Coimbatore and Erode districts of Tamil Nadu. The mushroom *Tricholoma* has been partially sequenced using ITS primers 1 and 2 and it shares 91 % homology with *Tricholoma giganteum* and is given with Gen bank accession number 120872.

Rajarathnam and Thiagarajan (2012) identified a wild mushroom using ITS 1 and ITS 4 primers subjected to nucleotide sequence determination. The sequence thus determined was aligned using Jukes-Cantor Corrected Distance model. The aligned sequence (559bp) revealed 88% match score with *Perenniporia sp.* (GQ982890.1) belong to *Agaricomycetes,* which is a new addition to Indian mushroom biodiversity.

Victor *et al.* (2012) Identified 18 *Termitomyces species* collected from 2 states (Ondo and Ekiti) of Nigeria using Internal transcribed spacer region and compared with existing sequences in NCBI Gen Bank. The degree of similarity of T1 to T18 *Termitomyces sp*. obtained from NCBI ranges from 82-99 percent. A phylogenetic tree generated with ITS sequence obtained from NCBI Gen Bank revealed that T1 to T18 are more related to *Termitomyces species* indigenous to African counties such as Senegal, Congo and Gabon.

Sudip *et al.* (2013) collected eight wild edible mushrooms from eastern Chota Nagpur plateau of West Bengal, India was identified using ITS 1 and ITS 2 primers. The aligned sequence revealed identity of *Amanita hemibafpha, Amanita* sp.*, Astraeus hygrometricus, Termitomyces* sp., *Termitomyces* sp., *Volvariella volvceae*, *Termitomyces* sp. Among eight mushrooms four could be identified up to species level and the rest any be revealed to further characterization. A phylogenetic tree was conducted using neighbor- joining method showing inter relationship between or among the mushroom.

A delicious mushroom vernacularly known as heggalanabe was documented from the Theerthahalli forest area of the Shimoga district of Karnataka. This mushroom was identified by using ITS region of ribosomal DNA sequence. The sequences when subjected to BLAST matches showed 98% homology with *Termitomyces* sp. Earanna *et al.* (2013).

2.6. Pure culture isolation and preservation

Ghazalanusine *et al.* (2001) studied the effect of three different culture media they were malt extract agar medium (MEA), murashige and skoog's (MS) medium and potato dextrose agar (PDA) medium on mycelial growth of oyster and Chinese mushrooms. He observed that the mycelial growth of *Plerotus ostreatus* , *Volvariella volvacea* were maximum in MEA medium compared to potato dextrose agar, which is ended up with slowest growth.

Rehana *et al*. (2007) cultivated Oyster mushroom on different agricultural wastes due to its compatibility and produce high yield in diversified climate. Studies revealed that the joint portion of cap and stipe produced vigorous mycelium growth in minimum time. The average growth was obtained on Malt Extract Agar (MEA) than on Potato Dextrose agar (PDA) medium at 25 ºC under humid (65 – 80 RH) conditions. For the substrate, out of three types of grains viz., wheat, sorghum and oat, the sorghum was found to be the best substrate for mycelium propagation and the time period for optimum growth was 7 days.

Ahmed *et al.* (2008) observed vegetative growth of four different strains of *Hericium erinaceus*. The temperature suitable for optimal mycelial growth was 25^0C, when growth observed with extended temperature range of 20-30^0C. The different strains of this mushroom showed distinct pH requirements for their optimum vegetative growth, with the most favorable growth observed at pH 6. For mycelial growth, the different media like, PDA, YM, Hennerberg, Hamada, and Glucose peptone were found most favorable and CzapekDox, Hoppkins, Glucose tryptone, and Lilly were found most unfavorable media for these mushroom strains. With the exception of lactose, most of the carbon sources assayed demonstrated favorable vegetative growth of *H. erinaceus*. For mycelial growth, the most suitable nitrogen source was alanine and the most unsuitable was histidine. Oak sawdust medium supplemented with 10~20% rice bran was the best for mycelial growth of the mushroom.

Seven different types of substrates viz. Mango, Jackfruit, Coconut, Jam, Kadom, Mahogony, Shiris sawdust with wheat bran and $CaCO_3$ were evaluated for growth and yield of oyster mushroom. The maximum biological yield per packet was obtained with Mango sawdust (150 gm) followed by Mahogony (148 gm), Shiris (146 gm), Kadom (136 gm), Jam (114 gm), Jackfruit (97 gm) and Coconut sawdust (83 gm). The lowest yield was observed in Coconut sawdust (83 gm). However, highest return was obtained with Mango sawdust. Cost benefit analysis revealed that the Mango sawdust and Shiris sawdust were promising substrates for the growing of Oyster mushroom (*Pleurotus flabellatus*) (Islam *et al.,* 2009).

Ukoima and Ikpe (2009) studied the mycelial growth of *Volvariella volvacea, Pleurotus tuber-regium* and *Pleurotus sajor-caju* using low input and cheap substrates namely, rice bran, cassava peels, palm fiber, potato dextrose agar, Yeast agar and found that the varied preference of individual species. Among the above, the *P. sajorcaju* produced highest mycelial growth (7.8 cm) on rice bran-soil culture media followed by *P. tuber-regium* (5.8 cm) on cassava peels/soil culture media and *Volveriella volvacea*

(7.1 cm) on palm fibre soil culture medium. The least mycelial growth (1.5-4.4 cm) was observed on potato dextrose agar and yeast agar.

Influence of various culture media on the mycelial growth of *Pleurotus pulmonarius* was investigated by using Malt extract agar, Corn cob extract agar and Cassava peelings extract agar. The above substrates influenced excellent mycelial growth of *P. pulmonarius* compared to potato dextrose agar and plantain peelings extract agar under *in-vitro* condition Stanley and Nyenke (2011).

Stanley *et al.* (2013) reported highest mycelial growth (12.4mm/day) of *Pleurotus sajor-caju* (Oyster mushroom) on cassava peelings mixed with 25 ml of honey compared to plantain peelings applied with 25 ml honey (2.43 mm/day) indicating the significance of organic waste incorporation in the culture media.

Lentinus cladopus collected from the wild was cultured on different solid and liquid media. The solid media used were Malt Extract Agar, Potato Dextrose Agar, Pea Extract Agar, Milk Powder Agar, Potato Malt Agar, Yeast Extract Agar, Yeast Potato Dextrose Agar, Gram Grain Extract Agar, Dimmick Medium, Maize Grain Extract Agar, Wheat Grain Extract Agar, Czapek Agar and the broth used were Malt Broth, Potato Dextrose Broth, Czapek Solution, Glucose Asparagine Medium, Glucose Peptone Medium, Richard Solution, Dimmick Medium, Peptone Water, Maltose Peptone Medium, Bilai Medium and Koser Citrate Medium for selecting the best medium that supports vegetative growth. Among all the above, the Malt Extract Agar and Malt broth was found superior in producing better mycelial growth (Atri and Lata 2013). In this study, fourteen mushrooms were documented from the BR hills of Karnataka and identified by Phenotypic and molecular characters. Pure culture of elite species was isolated and conserved.

III. MATERIAL AND METHODS

The ethnobotanical survey was under taken for documentation and molecular characterization of wild mushrooms occur in the B.R.Hills of Karnataka. The collected mushrooms were identified by morphological and molecular characters using ITS/18S rRNA gene sequencing. The details of material used and methods followed are described below.

3.1 Ethnobotanical survey

In order to document the wild mushrooms, a ethnobotanical survey was carried out in BR Hills Forest area of Karnataka during June-September 2013. The survey was carried out with the help of native soliga tribals, and mushroom samples were collected around their settlements. During the survey details on the usage of the mushrooms by soliga tribals also collected. A small sample of the mushroom species were collected in paper bags and field notes like date, weather condition, abundance, habitat and phenotypic characters were recorded.

3.2 Morphological characters

Mushroom is fleshy, spore bearing fruiting body of fungus. Fourteen mushrooms were collected from B.R. Hill forest area at different period of the season and brought to the laboratory for further study. Phenotypic characters like colour, size of cap and stipe, stipe position, presence or absence of gills or pores, presence or absence of annulus, texture were recorded.

The pileus of the matured mushroom was cut and placed over the Black and White paper over night to collect the spore print. The spores thus collected were used to study for their shape, size and color under compound microscope. Spores were collected by using a sterile needle and placed on a microscopic slide having a drop of lacto phenol was on it. A cover slip was placed on the lacto phenol and observed under 40 x objective lens of the microscope.

3.3 Molecular characterization

3.3.1 Genomic DNA Isolation

Total genomic DNA from the cap tissue of the mushroom fungus was extracted using CTAB lysis buffer (Datta *et al.,* 1989). For this 0.2 grams of dried mushrooms sample was ground into fine powder using liquid nitrogen and sample was transferred into 2 ml of extraction buffer containing CTAB and incubated at 65°C for 30 minutes. After incubation the tubes were centrifuged at 10,000 rpm for 10 minutes. The clear supernatant was transferred into a fresh centrifuge tube and equal volume of chloroform Isoamyl alcohol (24:1V/V) was added, it was mixed by inverting the tubes and centrifuged at 10000 rpm for 10 minutes. The above step was repeated till no white interface is seen. Clear supernatant was collected and DNA was precipitated by adding 0.6 volumes of chilled Isopropanol and place in -20ºC for 2 hours. After incubation the

mixture was centrifuged and the pellet was washed with 70% ethanol, further air dried, dissolved in Tris-EDTA (10:1) buffer and stored at -20°C.

3.3.2 PCR amplification

PCR components (40 µl)

- 4.0 µl of 10 X PCR Taq. Buffer
- 4.0 µl of 10 mM dNTP's mix
- 2.0 µl of 18S rRNA/ITS primers
- 0.6 µl of Taq. DNA Polymerase
- 2.0 µl of Template DNA
- 27.4 µl of Sterile distilled water
- Sterile PCR tubes

Primers used for amplification of 18S rRNA/ITS region of the wild mushrooms.

Primers	Sequence	References
ITS 1	5' TCCGTAGGTGAACCTGCGG 3'	(Sasidara & Thiagarajan., 2012)
ITS 4	5' TCCTCCGCTTATTGATATGC 3'	
18S F	5'GTCAGAGGTGAAATTCTTGGATTTA3'	(Wolfganag *et al.*, 2001)
18S R	5' AAGGGCAGGGACGTAATCAACG 3'	

Procedure

The genomic DNA extracted was checked for purity using UV spectrophotometer at 260/280 nm wave length. Concentration was measured using nano drop (ependorf). Then 50-100 ng DNA was used for PCR amplification in a 40 µl reaction mixture mentioned above. The reaction was carried out in a Thermal Cycler (Applied biosystems). The PCR programme was standardized as follows: initial denaturation at 96°C for 3 min, 40 cycles of denaturation of 94°C for 1 min, annealing at 60°C for 30seconds and extension at 72°C for 1 min and final extension at 72°C for 10 min. The amplified products were separated by agarose gel electrophoresis.

3.3.4 Agarose gel electrophoresis

Agarose gel electrophoresis was performed to resolve the amplified product using 0.8% agarose in 1 X TAE buffer, 0.5 µg/ml of ethidium bromide, and loading buffer (0.25 % bromophenol blue in 40% sucrose). 5µl of loading dye was added to 40 µl of PCR product and loaded to the agarose gel. Electrophoresis was carried at 100V for 2 Hours. The gel was visualized under UV light and documented using Alpha innotech Gel

documentation unit. The visualized band was excised and purified by using gel extraction kit.

3.3.5 Gel elution

The Gene JET™ Gel Extraction Kit was used for rapid and efficient purification of DNA fragments from agarose gels.

Gel slice containing the DNA fragment was excised using a clean scalpel or razor blade. Gel slice was placed into a pre-weighed 1.5 ml tube equal volume of binding buffer was added. (e.g., for 100 mg of gel slice 100 µl of binding buffer was added). The mixture was incubated at 50-60°C until the gel slice was completely dissolved. Solubilized gel solution was added to the Gene JET™ purification column and centrifuged at 12,000 rpm for 60 seconds. The flow-through was discarded and the column was placed back into the same collection tube, washed by adding 700 µl of wash buffer by centrifugation at 12,000 rpm for 60 seconds. The tube was again centrifuged for 60seconds to completely remove residual wash buffer. Purification column was then transferred into a clean 1.5 ml micro centrifuge tube and 20 µl of elution buffer was added and centrifuged at 12,000 rpm for 60 seconds. The amplified DNA thus eluted was used for ligation.

3.4. Ligation, Cloning and Transformation

3.4.1 Ligation

The efficiency of ligation is known to depend greatly on purity and concentration of PCR products. If the PCR product is sufficiently clean (a homogeneous band of desired size is observed on the gel), it can be directly used in ligation reaction.

Protocol

1. The eluted 18S/ITS region was ligated using the following components of Thermo SCIENTIFIC (Ins TAclone PCR Cloning Kit,) in an 0.5ml microfuge tube.

Vector pTZ57R/T (55ng/µl)	**:**	1.5 µl
Insert DNA (50ng/µl)	**:**	3.0 µl
5X Ligation Buffer	**:**	2.5 µl
Water (nuclease-free)	**:**	2.5 µl
T4 DNA Ligase (5u/µl)	**:**	0.5 µl.

2. The above reaction mixture was incubated in 4^0C for overnight.

The vector used for cloning in this study pTZ57R/T.

3.4.2 Transformation

Transformation of cloned gene sequence of mushrooms was done by using *Escherichia coli* (DH5α) competent cells. (Sambrook *et al.,* 1989) DH5α is the most frequently used *E. coli* strain for routine cloning applications.

Protocol

The day of transformation C-medium is pre incubated at 37^0c for 30 minutes. LB (Luria Bertani) plates supplemented with ampicilin, X-gal, Isopropyl β-D-1-thiogalactopyranoside (50mg/50ml) were pre warmed in a 37^0c incubator for 20 min. T-solution A and T-solution B were mixed by 250 µl of each in a new centrifuge tube.

Overnight grown *E.coli* DH5α culture (150 ml) was added to the 1.5 ml of pre warmed C-medium and incubated in a shaker for 20 minutes at 37^0C. The bacterial cells were centrifuged at 12,000 rpm for 60 seconds and supernatant was discarded. Pellet obtained was resuspended in 300 µl of T-solution and incubated on ice for 5minutes, centrifuged at 12,000 rpm for 1 minute and supernatant was discarded. Again the pellet was resuspended in 120µl of T-solution and incubated for 5 minutes on ice. Further 50µl of prepared cells was added to each tubes containing DNA mix and incubated on ice for 5 minutes. Immediately it was spread on LB agar dispensed plates supplemented with ampiciline (100 mg/ml), X-gal (50 mg/ml), IPTG (50 mg/ml). These plates were incubated at 37^0C for overnight.

3.4.3 Confirmation of presence of recombinant gene by colony PCR

The white (transformed) colonies were selected by blue white screening and the presence of the gene is confirmed by colony PCR.

PCR components (20 µl)

- 10 X Taq buffer : 2.0 µl
- 10 mM dNTP's mix : 2.0 µl
- M13 primers(forward and reverse primers)(10µm) : 1.0 µl
- Taq. DNA Polymerase (5u/µl) : 0.3 µl
- Sample from recombinant colony : 1
- Sterile distilled water : 13.7 µl

Procedure

Master mix of above mentioned PCR components was prepared and transformed white colony was added. The PCR programme was standardized as follows: initial denaturation at 96°C for 3min, 35 cycles of denaturation of 94°C for 1 min, annealing at 60°C for 30 second and extension at 72°C for 1 min and final extension at 72°C for 10 min. The amplified products were resolved by agarose gel electrophoresis and visualized under UV Transilluminator and photographed.

3.5 Plasmid Isolation

Upon confirmation with the colony PCR, recombinant white colonies were inoculated into 5ml of LB broth with 5µl of ampicilin (100 mg/ml) and incubated in shaker for 16 hours at 37^0 C. After 16 hours plasmid was isolated using the Gen Elute™ HP Plasmid Mini Prep kit (Sigma Aldrich) following the manufacturers protocol.

Procedure:

E. coli culture having the compliment plasmid was pelleted by centrifuging at 12,000 rpm for 60 seconds. The supernatant was discarded and the pellet was resuspended in 200 µl of resuspension solution and mixed thoroughly. The resuspended cells were lysed by adding 200 µl of lysis solution and mixed by inversion (6-8 times). The cell debris was precipitated by adding 350 µl of neutralization solution and mixed by inverting the tube 4-6 times and immediately pelleted the cell debris by centrifuging at 12,000 rpm for 10 minutes. Obtained clear lysate was transferred to prepared column (Column was prepared by adding column preparation buffer) and centrifuged at 12000 rpm for 60 seconds. The flow-through was discarded. Into it 500 µl of optional wash solution was added and centrifuged at 12000 rpm for 60 seconds and flow through was discarded. Then 750 µl of diluted wash solution was added to the column and centrifuged to remove the residual salts and other contaminants. Flow through was discarded and again the empty column was centrifuged to remove excess ethanol. The column was transferred to a fresh tube and 50 µl of elution buffer was added and centrifuged at 12000 rpm for 60 seconds. The plasmid thus obtained was stored at -20^0C and used for sequencing.

3.6 Sequencing

The isolated 14 plasmid samples were got sequenced by Sci Genom Labs Private Ltd. Kerala, INDIA) using M13 forward and reverse primers.

3.6.1 Sequence analysis and homology search

Sequence results were analyzed with Vec Screen, online software from National Centre for Biotechnology Information (NCBI) for removing the vector backbone contamination. Forward and reverse primer sequences were checked against each other by generating the reverse complement of the reverse sequence using Fast PCR professional (Experimental test version 5.0.83) and aligning it with the forward sequence with the help of CLUSTALW Multiple Sequence Alignment Programme using the online software SDSC Biology Workbench (San Diego Supercompter Center). The full length PCR homology search was performed with NCBI (*http//www. Ncbi.nlm.nih.gov/BLAST/*) (Altschul *et al*., 1990).

3.7 *In-vitro* culturing and conservation of edible mushroom species

The mushrooms found growing in forest area of B.R.Hills were collected in paper bags and brought to the laboratory. Stem/Pileus tissue was cleaned and outer layer was peeled off using razor. The inner tissue which is almost free of microbes selected and washed in 3% sodium hypochorite and then serially washed with sterile water to remove the residual chemical. Then a piece of the sterilized tissue was inoculated/placed on Potato Dextrose Agar (PDA) medium dispensed plates. These plates were incubated at room temperature till the growth of the mycelium produced from the tissue. After formation of mycelial growth, the mycelium was transferred on fresh PDA slant and preserved in refrigerator.

IV. EXPERIMENTAL RESULTS

Field survey was made on the occurrence of mushrooms in forest area of B.R.hills of Chamarajanagara district of Karnataka from June to September 2013. Fourteen mushroom species was collected of which two were identified by studying their phenotypic characters and other twelve were identified using 18S rRNA/ITS gene sequence homology. Results obtained during the study are presented here under.

4.1. Identification of mushrooms by phenotypic or morphological characters

The two mushrooms designated as **BRM-1 and BRM-2** were collected in the month of august and identified based on phenotypic characters by consulting the book *Mushroom Demystified* (Arora, 1986). The mushroom bearing robust fruiting body found growing on the hard wood. It was locally known as Dodda mara anabe in Kannada. The pileus was reddish brown colour and border surrounded by white colour, embricate in shape. The diameter of cap is 14 cm and stipiate polypore (Table, 1 and Plate, 1). Hence, it was identified as *Ganoderma lucidum*. The other mushroom designated as BRM-2 was vernacularly known as Maraanabe found growing on dead wood. Sporophore is stipiate, pileus is deep brown colour, deep depression at the center (Plate, 2), basidia are clavate and gills are absent, scattered growth habbit. Thus, it was identified as *polyporus flabelliformis* by verifying the earlier record reported for indian polypores (Bakshi 1971).

4.2. Molecular identification of wild mushrooms by 18S rRNA / ITS gene sequencing

Molecular identification is a recent origin to identify the prokaryotic and eukaryotic organisms. The genes encoding for 16S rRNA in prokaryotes and 18S rRNA/ITS in eukaryotes are most widely used in molecular phylogenetics. In the present study phenotypic characters were recorded first and then proceeded for molecular identification (Plate 3. A, B, C, D, E and F) using 18SrRNA/ ITS sequence for identification of different mushroom species. Expected amplicon size is 500bp to 800bp. The full length sequence homology was searched in website, National center for biotechnology information (NCBI) (*http://www.ncbi.nlm.nih.gov/BLAST/*) (Altschul *et al*., 1990) and mushrooms were identified into species level based on their homology

BRM-3: These mushrooms vernacularly known as Dodda anabe. It grows scattered in soil, produced robust fruiting body (Plate 4). Colour of pileus is creamy white, pileus is up lifted, diameter is 12 cm. The stipe is tapering downwards, central, 9 cm length and thickness is 1cm gills are free, annulus and rhizoides are present. ITS region was amplified using ITS-1 and ITS-4 primer and sequenced. The (734bp) sequence data showed 99 per cent homology with the earlier reported *Termitemyces* sp. (Fig. 1). The mushroom is edible.

BRM-4: mushroom grows gregariously on the tree (Plate 5), it is commonly known as Mavu anabe because of its jelly texture it called as jelly fungi. Fruiting body has brown colour with the absence of cap, stipe, gills and annulus. Amplified ITS region

Table 2. Field characters of wild mushrooms collected from Biligirirangana hills

Sl. No	Mushrooms collected	Vernacular name	Date of collection	Edibility	Habitat	Abundance	Growth habit
1	BRM-1	Doda mara anabe	09/08/2013	Medicinal	Tree	Seven	Scattered
2	BRM-2	Mara anabe	26/08/2013	Non edible	Wood	Group	Scattered
3	BRM-3	Doda anabe	26/08/2013	Edible	Soil	Four	Scattered
4	BRM-4	Mavu anabe	20/07/2013	Edible	Tree	Group	Gregarious
5	BRM-5	Koli anabe	05/09/2013	Edible	Soil	Group	Gregarious
6	BRM-6		23/06/2013	Poisonous	Humus	Three	Gregarious
7	BRM-7		05/09/2013	Non edible	Soil	Group	Gregarious
8	BRM-8	Huch anabe	23/06/2013	Non edible	Soil	Three	Scattered
9	BRM-9		26/08/2013	Non edible	Soil	Two	Scattered
10	BRM-10		09/08/2013	Non edible	Soil	Two	Scattered
11	BRM-11		23/06/2013	Non edible	Dead wood	Five	Scattered
12	BRM-12		09/08/2013	Non edible	Soil	Two	Scattered
13	BRM-13	Haladi anabe	09/08/2013	Non edible	Dead wood	Four	Gregarious
14	BRM-14	Kotesutthu anabe	05/09/2013	Non edible	Soil	Group	Gregarious

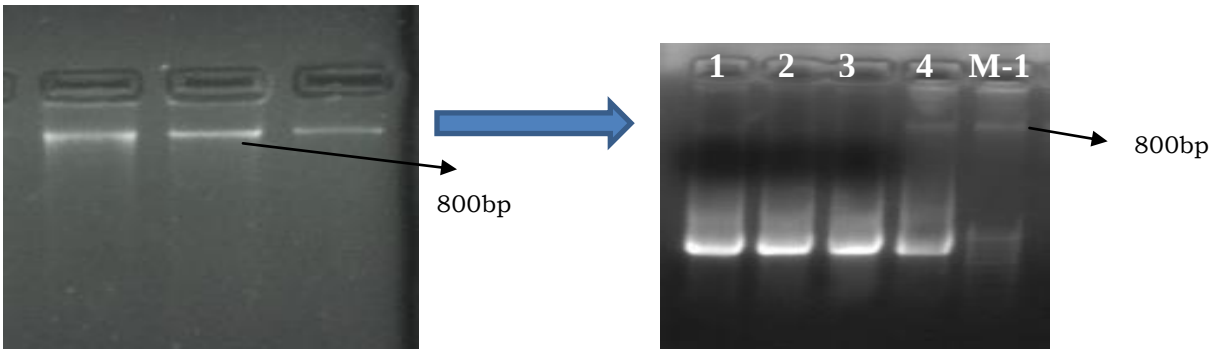
Note: BRM= Biligiri RanganaHill Mushroom; **Edibility**-known by local consumers & further confirmed on identification, **Habitat**-place were mushroom was growing; **Abundance**- number of fruiting body occur in a place.

Plate 1. *Ganoderma lucidum* (BRM-1)

Field characters	**Phenotypic characters**	**Scientific classification**
Mushroom Designation: BRM-1 Vernacular Name: Doddamara anabe Date of Collection:09/08/2013 Edibility: Nonedible Habitat : Dead wood Abundance: Group Growth habit: Scattered	Colour: Reddish brown Lacuate, shine, sporophore usually stipitate Texture : tough leathery clumps Gills : Absent Annulus : Absent	Kingdom: Fungi Division: Basidiomycota Class: Basidiomycetes Order: Polyporales Family: Ganodermataceae Genus: *Ganoderma* Species: *lucidum*

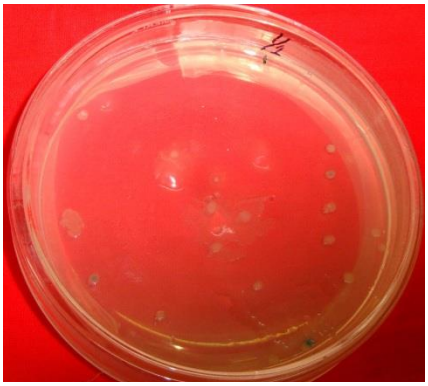
Plate 2. *Polyporus flabelliformis* (BRM-2)

Field characters	Phenotypic characters	Scientific classification
Mushroom Designation: BRM-2 Vernacular Name: Mara anabe Date of Collection:26/08/2013 Edibility: Non edible Habitat : Wood Abundance: Group Growth habit: Scattered	Colour: Upper black and pale brown colour hymenal surface Texture: Leathery, funnel shaped. Gills : Absent Annulus : Absent Sporophore stipiate, concentrically zonate.	Kingdom: Fungi Division: Basidiomycota Class: Basidiomycetes Order: Polyporales Family: Polyporaceae Genus: *Polyporus* Species: *flabelliformis*

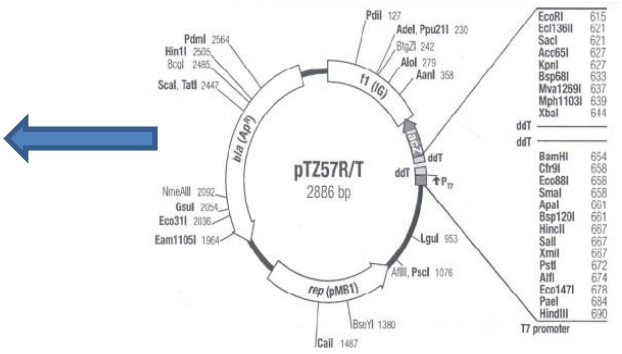


A. Genomic DNA

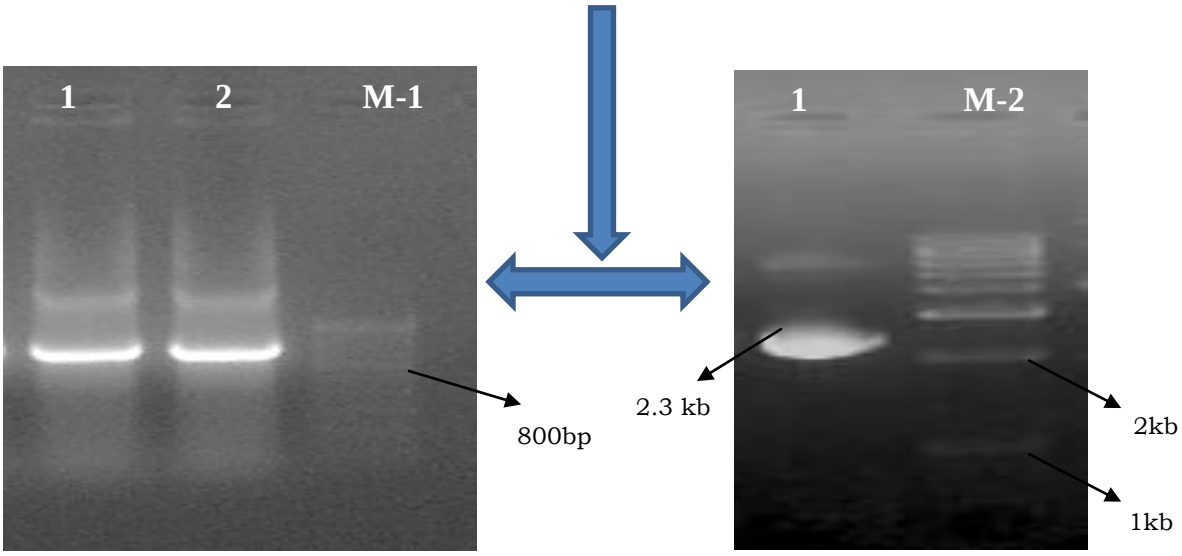
B. PCR Amplification of ITS/18S rRNA region



D. Recombinant colonies



E. T/A cloning vector



E. Amplicon of colony PCR

F. Recombinant plasmid

Plate 3. (A, B, C, D, E & F): Flow chart showing amplification of 18S rRNA/ITS region, T/A cloning vector, Transformed colonies, Colony PCR and Isolated plasmid.100bp lader (M-1) and 1kb lader (M-2).

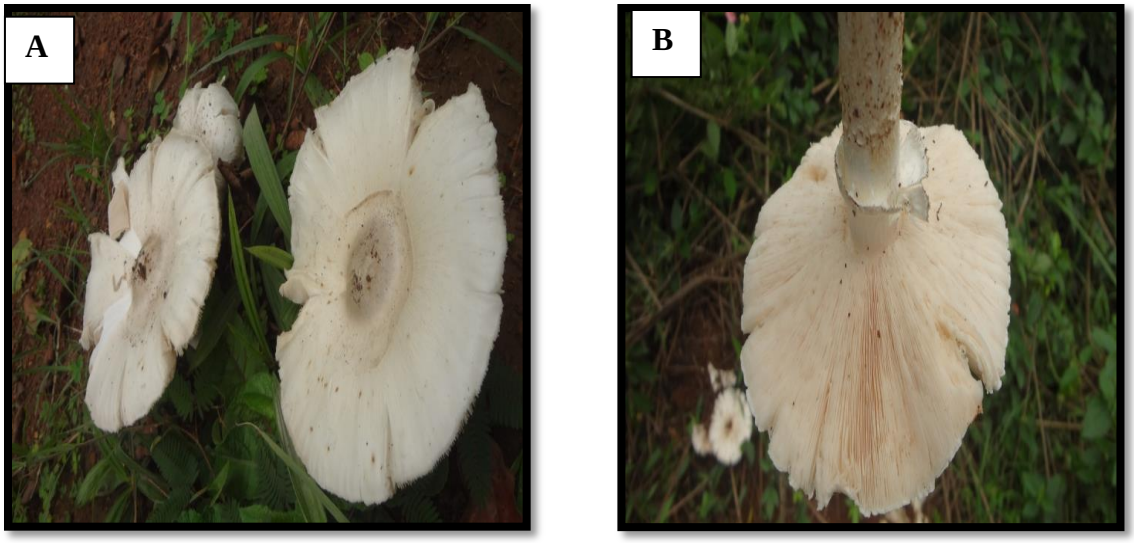


Plate 4. (A & B), *Termitomyces* sp. (BRM-3)

Field characters	**Phenotypic characters**	**Scientific classification**
Mushroom Designation: BRM-3 Vernacular Name: Dodda anabe Date of Collection:26/08/2013 Edibility: Edible Habitat: Soil Abundance: Four Growth habit: Scattered	Colour: Creamish white Shape 1)cap: Uplifted 2)Stipe: Tapering upwards Size 1) cap dia.:12cm 2)Stipe: length :9cm Thickness:1cm Texture : Crispy Stipe Position: Central Rhizods: Present Gills : Free Annulus : Present	Kingdom: Fungi Division: Basidiomycota Class: Basidiomycetes Order: Agaricales Family: Lyophyllaceae Genus: *Termitomyces* Species:

TCCTCCGCTTATTGATATGCTTAAGTTCAGCGGGTAGTCCTACCTGATTTGAG
GTCAAATGGTCAAAAGCGTCTTCCTCAACAAAGAGGAATTACGAGTTAGAAG
CAGAAAAGCCCATTAGAATAAAGTTGACTGCGCACGATGTAGATAATTATCA
CACCAGGAACAGGTCAACAAAGGGTTCCACTAATGCATTTAAGGGGAGCTGA
CTTCTTAAGAAGCCTGCAAAAACCCCCACATCCAAGCCTAAACCAACTCGCA
AAAGCTGGTTAAGGTTGAGAATTTAATGACACTCAAACAGGCATGCTCCTCG
TAATACCAAGGAGCGCAAGGTGCGTTCAAAGATTCGATGATTCACTGAATTC
TGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGC
CAAGAGATCCGTTGCTGAAAGTTGTATTTGATTAAAGGCACTAAAAAGGCCA
ATAAAAAAACATTCTAATACATTCATTACGGGGTATAAGAAGATGCATAGAC
TGAGAATGCAAGGGAAGCCGAACTTTGCAGCACAGCAAGCCCTCAAAACCG
AGGAGGGGGTTTGACCCTCGAACAGTATATATCCAAAGTCTACAAAAGGTGC
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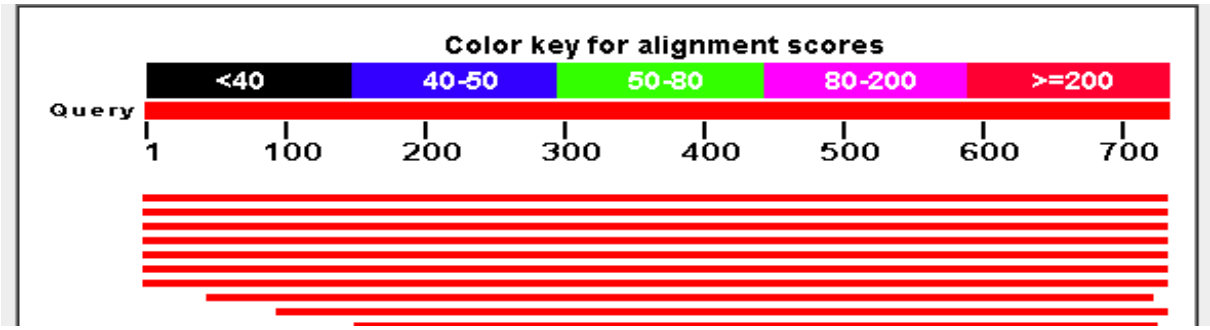
ACGGA



***Termitomyces* sp.**

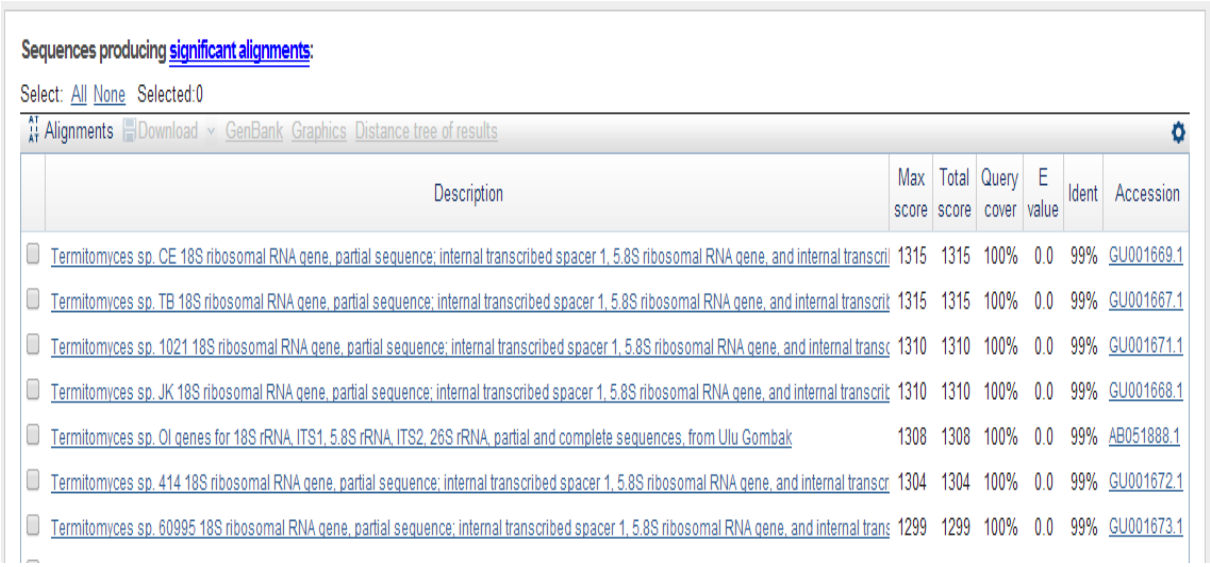


Fig 1. Full length sequence and homology search of BRM-3 (*Termitomyces* sp.)

Plate 5. ***Auricularia delicata*** **(BRM-4)**

Field characters	**Phenotypic characters**	**Scientific classification**
Mushroom Designation: BRM-4 Vernacular Name: Mavu anabe Date of Collection:20/07/2013 Edibility: Edible Habitat: Tree Abundance: Clusters Growth habit: Gregarious	Colour: Brown Shape 1)cap: Absent 2)Stipe: Absent Texture : Jelly Gills : Absent Annulus : Absent	Kingdom: Fungi Division: Basidiomycota Class: Basidiomycetes Order: Agaricales Family: Auriculariaceae Genus: *Auricularia* Species *delicata*

TCCGTAGGTGAACCTGCGGAAGGATCATTAAAGATTTTGGGCTTTTAACCCG
ATCGCTCAGCTGTGCGCCCTCCCGGGCTGCACGCTGAATCAAGACCTCACAC
CTGTGCACATTTTCGGTTGCGGCTTCGGTCGCTGCCGCTTTCAAATGCAACTA
CTCAGTCTCGAATGTCAACAAACTATAAAAAAGTAACAACTTTCAACAACGG
ATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATG
TGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCATCTTGCGCTCCT
TGGTATTCCATGGAGCATGCCTGTTTGAGTGTCACGTAAACCCTCACCCTTGC
GATGTAACAGTCGCCCGCGGTGGACTTGGACTGTGCCGTAACCGGCTCGTCTT
GAAATGCATTAGCTGGCGCTTTTAGAGTGCTGGGCGACGGTGTGATAATTAT
CTGCGCCAATGCCTTAGGCCTCTTCAGCGGTGCTGCTTACAGCCGTCCCTCTG
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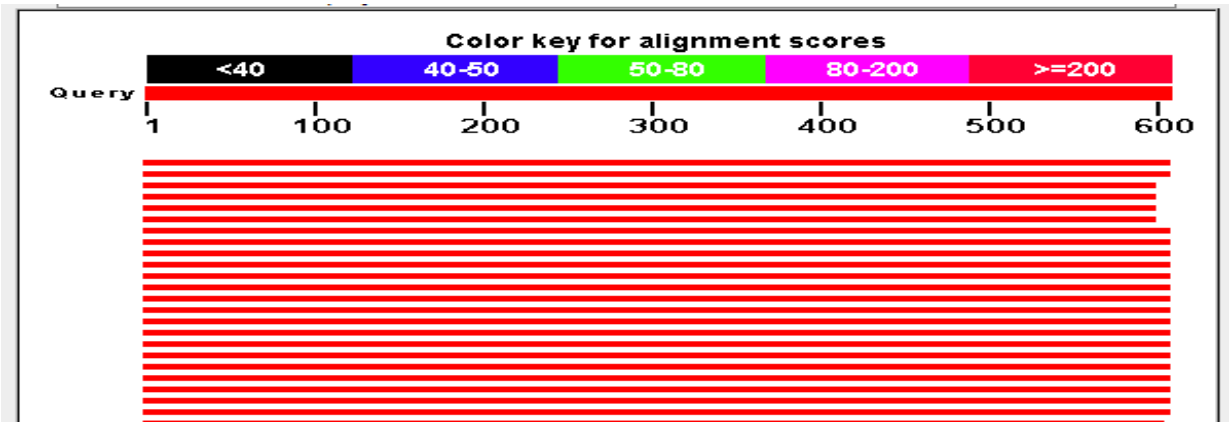
AACTTAAGCATATCAATAAGCGGAGGA



Auricularia delicata

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Auricularia delicata strain HNSD 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and inte	1094	1094	100%	0.0	99%	KF297965.1
Auricularia delicata strain GIM5.177 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and i	1094	1094	100%	0.0	99%	KF297963.1
Auricularia delicata isolate CNSBlitz0093 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene,	1062	1062	98%	0.0	99%	JX065165.1
Auricularia delicata isolate CNSBlitz0098 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene,	1062	1062	98%	0.0	99%	JX065171.1
Auricularia delicata isolate CNSBlitz0050 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene,	1057	1057	98%	0.0	99%	JX065168.1
Auricularia delicata isolate CNSBlitz0012 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene,	1055	1055	98%	0.0	99%	JX065169.1

Fig 2. Full length sequence and homology search of BRM-4 (*Auricularia delicata*)

having (604bp) showed 99 per cent homology with the earlier reported *Auricularia delicata* (Fig.2). This mushroom is edible.

BRM-5: It is a mini mushroom and is name locally as koli anabe in Kannada (Plate, 6). It grows gregariously on soil near termite nests. Fruiting body of the mushrooms is white in colour with plane pileus measures 2 cm in diameter and stipe present in central position. Length of stipe is 8 cm and thickness is 0.3 cm (Plate, 6). Gills present, annulus is absent. The blast analysis of this mushroom for amplified sequence (603bp) showed 95% homology (Fig. 3) with the earlier reported *Termitomyces microcarpus*. The mushroom is edible.

BRM-6: Pileus colour is creamy white, usually grows on humus, convex shaped pileus 5.5 cm in diameter (Plate 7). The stipe is tapering upwards the central in position, length is 15 cm and thickness is 1.2 cm. Scales are present on pileus and stipe surface. The gills are adnexed type, annulus is present. The sequence of the ITS region (614 bp) showed 99% homology with the earlier reported *Amanita* sp. (Fig. 4).

BRM-7: The mushroom grows gregariously on soil (Plate 8) phenotypically this mushroom has creamy colored petal type pileus. Stipe, gills and annulus are absent, leathery texture. The molecular characterization of this mushroom has been done using ITS primer. The sequence having (693bp) showed 99 per cent homology with the earlier reported *Podoscypha petalodes* (Fig. 5).

BRM-8: This mushroom is vernacularly called as Huch anabe in Kannada. Mushrooms exhibit scattered growth on soil (Plate 9). Pileus is creamy white, convex shaped measures 7cm in diameter and stipe is club shaped, 12cm length and 0.9 cm thickness. Gills and annulus are present. The sequence of ITS region (729bp) showed 99 per cent homology with the earlier reported *Agaricaceae* sp. (Fig. 6).

BRM-9: It produces robust fruiting body and grows scatterely in soil. Pileus is creamy white, convex shaped measures 6 cm in diameter. Pileus bears prominent scales (Plate 10). Stipe is enlarged at the bottom, 9 cm long and thickness is 0.6 cm. gills present and annulus is absent. It has soft and crispy texture. The sequence homology (726bp) found in blast analysis showed 98 per cent with the earlier reported *Macrolepiota* sp. (Fig. 7).

BRM-10: the mushroom has orange colour, cone shaped fruiting body (Plate 11). Scattered growth was observed. Pileus is 5cm long and 5.5 cm width and rhizoids are present. Gills are absent and soft textured. Blast analysis for ITS region (732bp) showed 95 per cent homology with the earlier reported *Calvatia holothurioides* (Fig. 8).

BRM-11: It is a yellow colored mushroom, grow on the dead wood (Plate, 12). Pileus is convex shaped with 3 cm in diameter. Stipe tapered upward, 4cm long and 0.7cm thickness. Gills are present, annulus is absent. Fruit body texture is soft. The blast analysis of amplified sequence having (542bp) revealed 99 per cent homology with the earlier reported *Gymnopilus crociphyllus* (Fig. 9).

BRM-12: This is a cream colored and soft textured mushroom; pileus is convex shaped with 9 cm diameter. Stipe is club shaped 14 cm in long and thickness is 1.2 cm. Gills and annulus are present (Plate 13). The ITS region sequence (822bp) in blast analysis showed 99 per cent homology with the earlier reported *Coprinus comatus* (Fig. 10).

BRM-13: Regional name of this mushroom is Haladi anabe because of its yellowish tinge. It has gregarious growth habitat on dead wood. Pileus and stipe are absent. It has corky texture (Plate 14), teeth present below the surface. The molecular characterization was done using 18S rRNA primers. The sequence having (736bp) showed 99 per cent homology with the earlier reported *Gyrodontium sacchari* (Fig. 11).

BRM-14: In kannada it is known as kote suthu anabe. Grow as cluster in soil. Pale brown colored pileus and stipe (Plate 15), annulus is absent. Gills are decurrent with soft texture. ITS region sequence having (736bp) showed 99 per cent homology with the earlier reported *Clitocybe aff.fellea* (Fig. 12).

4.3. *In-vitro* culturing of edible mushroom species

Based on molecular and morphological characterization out of fourteen mushrooms three mushrooms were identified as edible. For further studies edible mushrooms namely *Termitomyces* sp., *Auricularia delicata* and *Termitomyces microcarpus.* were cultured on PDA medium. *Auricularia delicata Termitomyces microcarpus* failed to get mycelium growth on PDA medium. *Termitomyces* sp. designated as BRM-3 (Plate-16) was successfully produced mycelium growth on PDA medium. Hence result shows that *Termitomyces* sp. shows good response to grow mycelium in PDA medium.

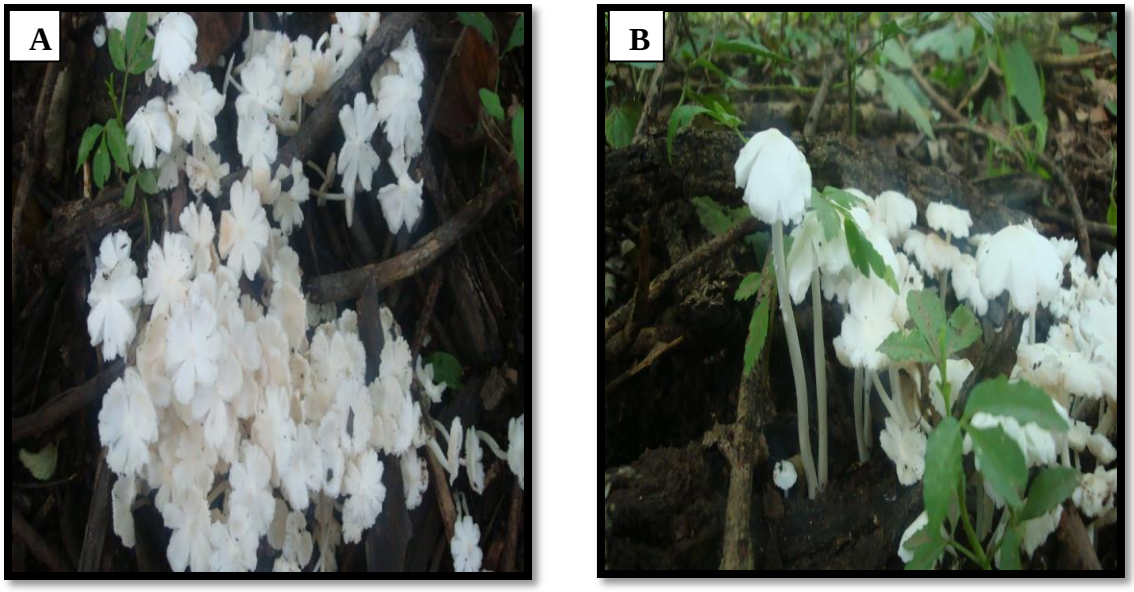


Plate 6. (A & B), ***Termitomyces microcarpus*** **(BRM-5)**

Field characters	**Phenotypic characters**	**Scientific classification**
Mushroom Designation: BRM-5 Vernacular Name: Kolianabe Date of Collection:05/09/2013 Edibility: Edible Habitat: Soil Growth habit: Gregarious	Colour: white Shape 1)Cap: Plane 2)Stipe: Tapering upwards Size 1) Cap dia.:2 cm 2)Stipe: Length: 8cm Thickness: 0.3cm Texture : Soft Stipe Position: Central Gills : Free Annulus : Absent	Kingdom: Fungi Division: Basidiomycota Class: Basidiomycetes Order: Agaricales Family: Lyophyllaceae Genus: *Termitomyces* Species: *microcarpus*

TTAAGTTCAGCGGGTATCCTACCTGATTTGAGGTCAAATGGTCAAAATGATTC
CCCCTTATAAATCCCGATGATACACGTTAAAATCAAAAAGGCCCCATTATTCA
ACCGACTGCACGCGATGTAGATAATTATCACACCACGAGCAAGTCAACAAAG
GGTTCCACTAATGCATTTAAGGGGAGCTGACTTCGAAATGAAGCCGGGGAAA
CCCCCACAATCCAAGCCTATCCAAGCTCGCAAAAGCTGGTTAGGTTGAGAAT
TTAATGACACTCAAACAGGCATGCTCCTCGGAATACCAAGGAGCGCAAGGTG
CGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCA
TTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGCTGAAAGTT
GTATTTGATTAAAGGCACCAAAGGGCCAATAACAAAACATTCTAATACATTC
TTTACGGGGTATAATAAAATGCATAGACCGGAAATGCAGGGAAAGCCGGCTG
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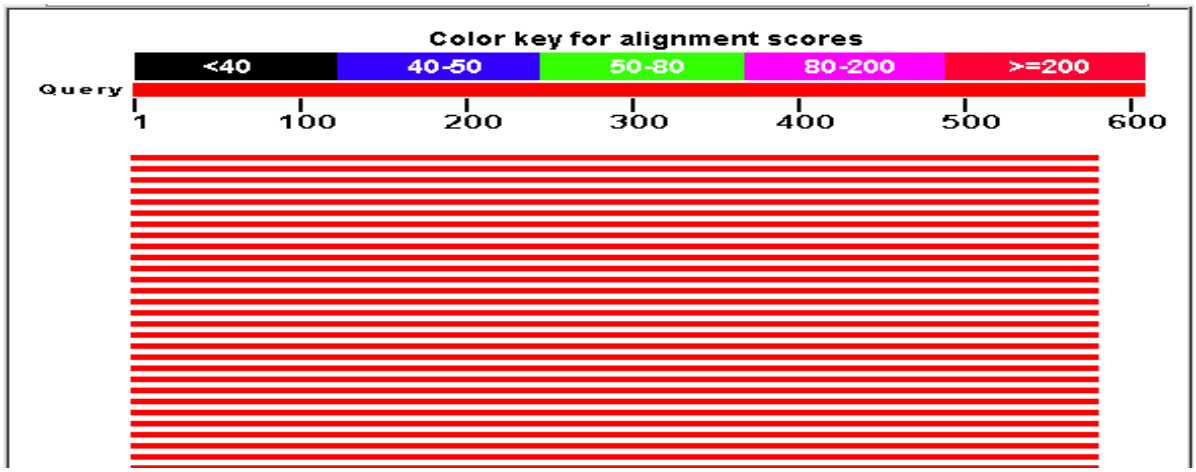


Termitomyces microcarpus

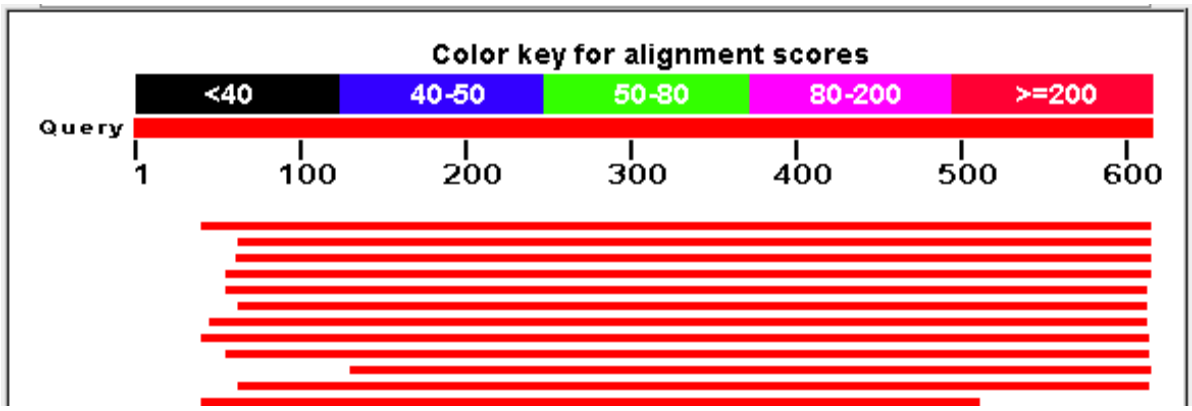
Uncultured Termitomyces clone cC09 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and inter	782	782	95%	0.0	91%	JQ306680.1
Uncultured Termitomyces clone cC10 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and inter	782	782	95%	0.0	91%	JQ306681.1
Uncultured Termitomyces clone cC14 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and inter	782	782	95%	0.0	91%	JQ306685.1
Uncultured Termitomyces clone cC07 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and inter	782	782	95%	0.0	91%	JQ306678.1
Uncultured Termitomyces clone cC27 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and inter	782	782	95%	0.0	91%	JQ306698.1
Uncultured Termitomyces clone cC01 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and inter	782	782	95%	0.0	91%	JQ306672.1
Uncultured Termitomyces clone cC02 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and inter	782	782	95%	0.0	91%	JQ306673.1
Termitomyces microcarpus 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete	780	780	84%	0.0	95%	HM230661.1

Fig 3. Full length sequence and homology search of BRM-5 (*Termitomyces microcarpus*)

Plate 7. *Amanita* sp. (BRM-6)

Field characters	**Phenotypic characters**	**Scientific classification**
Mushroom Designation: BRM-6 Date of Collection:23/06/2013 Edibility: Non edible Habitat : Humus Abundance: Three Growth habit: Scattered	Colour: Creamish white Shape 1)cap: Convex 2)Stipe: Tapering upwards Size 1) cap dia.:5.5cm 2)Stipe: Length:8cm Thickness:1.2cm Spore : Globose shape, creamish colour Texture : Soft & stingey Stipe Position: Central Gills : Adnexed Annulus : Present	Kingdom: Fungi Division: Basidiomycota Class: Agricomycetes Order: Agaricales Family: Agaricaceae Genus: *Amanita* Species:

GCTTGCATGCAGGCCTCTGCAGTCGACGGGCCCGGGATCCGATTTCCGTAGG
TGAACCTGCGGAAGGATCATTATTGAAATGAAACCTTTGGTGAAGGTTGTAT
CTGGCTCTAATAAGAGCATGTGCACACCTTTGCTATTGCTTCTTTCATTTTTCC
ACCTGTGCACCTTTTTGTAGACCTGGGTTAGAGGAGGTTACATTTTATTATGAT
ATGCCTCCTTGTTTCGAATGTGAATTTGAATTCTCTAGGTCTATGTATTTACAT
ATACACTATTTGAATGTTTATAGAATGATCATTAGTTTTGGGCTTACTACTAT
GTATGTAGCCTTTAAAACAAATAATACAACTTTCAACAACGGATCTCTTGGCT
CTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGA
ATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGA
GGAGCATGCCTGTTTGAGTGTCAGTAAATTCTCAAATGCCCCATCAGTTATTA
TTGACTGATTTTGAGGCTTTTGGAGTTTGGGAGTCTGCAGGTCACTAATTTTTT
GTGATCAGCTCTCCTCAAATATATTAGTAGA



***Amanita* sp.**

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Amanita sp. TO AV20113 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal tr	1025	1025	93%	0.0	99%	KC137253.1
Amanita manicata voucher RET 387-4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spa	987	987	89%	0.0	99%	HQ625014.1
Amanita nauseosa internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	980	980	89%	0.0	99%	AY194976.1
Amanita nauseosa voucher DPL 6117 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, a	967	967	90%	0.0	99%	HQ625013.1
Amanita armillariiformis voucher RET 266-8 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA g	666	666	90%	0.0	87%	HQ625012.1
Amanita prairiicola voucher RET 266-1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed sp	500	500	89%	5e-138	83%	HQ625015.1
Amanita silvifuga voucher SFSU HDT 4630 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA ge	471	471	91%	3e-129	80%	HQ625016.1

Fig 4. Full length sequence and homology search of BRM-6 (*Amanita* sp.)

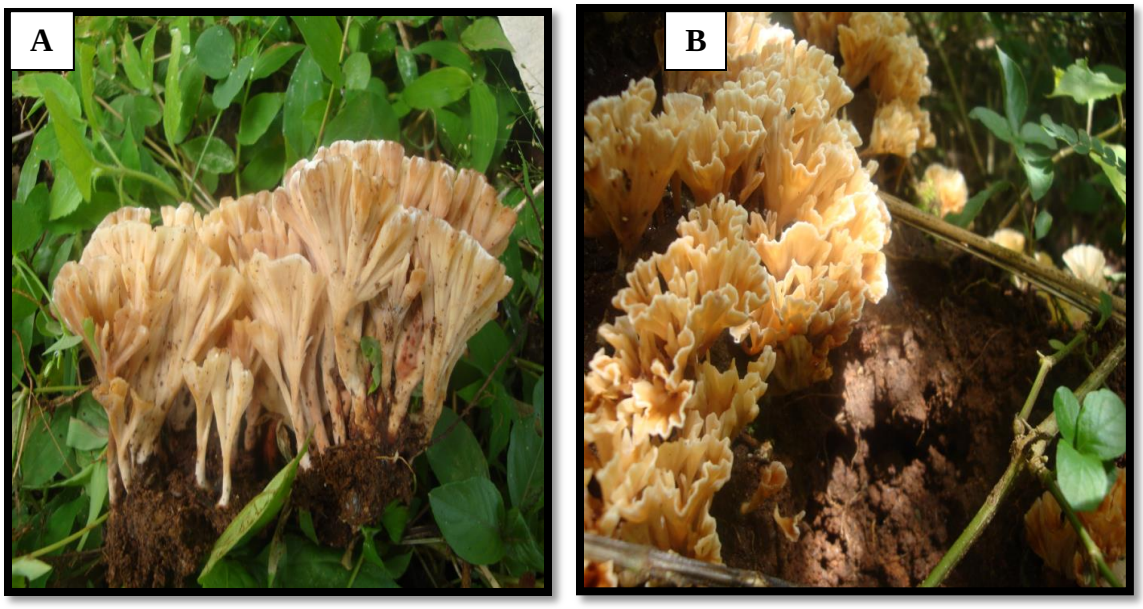


Plate 8. (A & B) *Podoscypha petalodes* (BRM-7)

Field characters	**Phenotypic characters**	**Scientific classification**
Mushroom Designation: BRM-7 Date of Collection:05/09/2013 Edibility: Non edible Habitat : Soil Abundance: More Growth habit: Gregarious	Colour: Creamish Shape 1)cap: Absence 2)Stipe: Absence Texture : Leathergy Gills : Absent Annulus : Absent	Kingdom: Fungi Division: Basidiomycota Class: Basidiomycetes Order: Polyporales Family: Meruliaceae Genus: *Podoscypha* Species: *petalodes*

TCCTCCGCTTATTGATATGCTTAAGTTCAGCGGGTAGTCCTACCTGATTTGAG
GTCAGATTTCAAAGTAAAGTGTCTGAGTCAACAGACGGGTTATAAGCATGAA
CACTTATGAAGTCAACGTTGAAACACAGCGCAGATAATTATCACACTGAGTA
ACGTGTAACTTGATTCACACTAATGCATTTAAGAGGAGCCAGCCGCCGAAGC
ACCGGCAAAAAAACCTCCAAGTCCAATTCCGAATAACAAAAGTTATTAGAATT
GAGAATACCATGACACTCAAACAGGCATACTCCTCGGAATACCAAGGAGTGC
AAGGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTT
ATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGTTG
AAAGTTGTATATAGTTTGCGTTAAACGCGGTATAACATTCTAGACTGACTGGT
ATAAGTGTATGAACCACAGAAGACTTACTGAGTTGAACTACTGAAGTCAACT
CGAGAGAGTCGACCCTTTCACCCAACTTAATAAATCGTTCTATGTAAAGTGCA
CAGAGGTATAAGAATTTGGAATGACCAAGGTGTGCACATTACCTCTCGATTG
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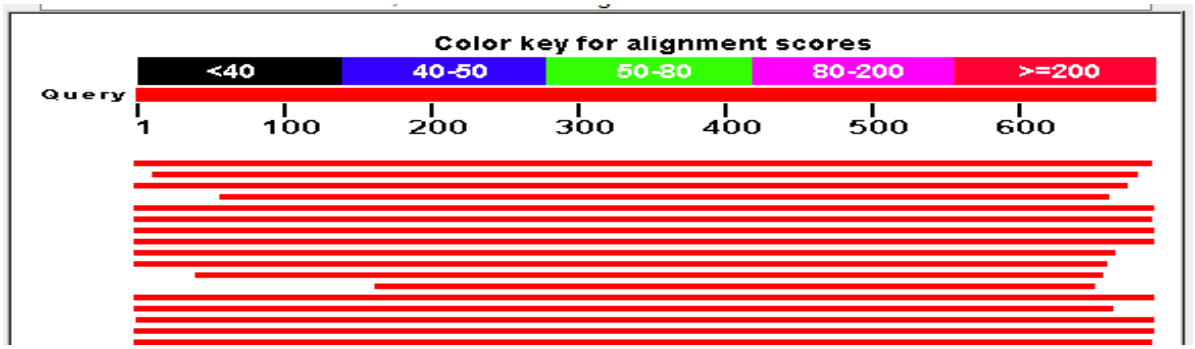
TTCACCTACGGA



Podoscypha petalodes

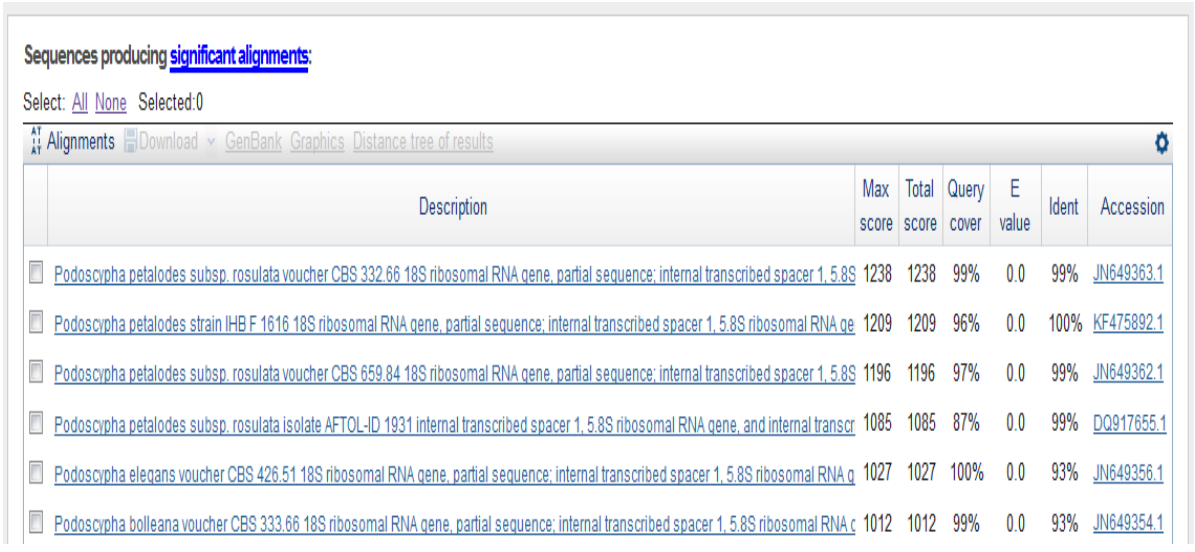


Fig 5. Full length sequence and homology search of BRM-7 (*Podoscypha petalodes*)

Plate 9. *Agaricaceae* sp. (BRM-8)

Field characters	**Phenotypic characters**	**Scientific classification**
Mushroom Designation: BRM-8 Vernacular Name: Huchanabe Date of Collection:26/03/2013 Edibility: Non edible Habitat : Soil Abundance: Three Growth habit: Scattered	Colour: Creamish Shape 1)cap: Convex 2)Stipe: Club shaped Size 1) cap dia.: 7cm 2)Stipe: length: 12cm Thickness: 0.9cm Spore : Oval shape, creamish colour Texture : Soft Stipe Position: Central Gills : Free Annulus : Present	Kingdom: Fungi Division: Basidiomycota Class: Basidiomycetes Order: Agaricales Family: Agaricaceae Genus: *Agaricus* Species:

ATGTTTTCATTAATCAAGAACGAAGGTTAGGGGATCGAAAACGATCAGATAC
CGTTGTAGTCTTAACAGTAAACTATGCCGACTAGGGATCGGGCGACCTCAAA
TTTGATGTGTTGCTCGGCACCTTACGAGAAATCAAAGTCTTTGGGTTCTGGGG
GGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGACGGAAGGATTTACTC
AAGACCGACTATTGCGAAAGCATTTGCCAAGGATGTTTTCATTAATCAAGAA
CGAAGGTTAGGGGATCGAAAACGATCAGATACCGTTGTAGTCTTAACAGTAA
ACTATGCCGACTAGGGATCGGGCGACCTCAAATTTGATGTGTCGCTCGCCAC
CTTACGAGAAATCAAAGTCTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCT
GAAACTTAAAGGAATTGACGGAATGGAGTGATTTGTCTGGTTAATTCCGATA
ACGAACGAGACCTTAACCTGCTAAATAGCCAGGCCGGCTTTCGCTGGTCCCC
GGCTTCTTAGAGGGACTGTCAGCGTCTAGCTGACGGAAGTTTGAGGCAATAA
CAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGACA
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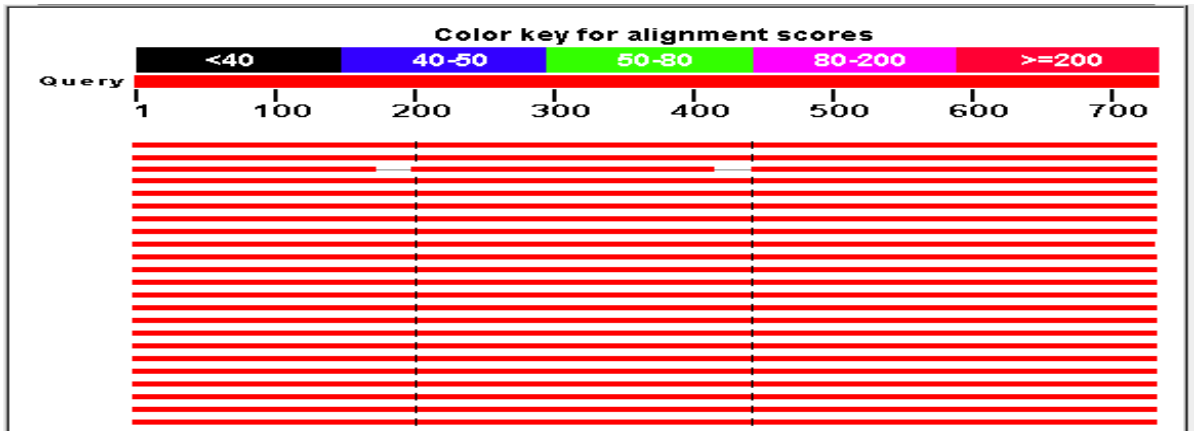

***Agaricaceae* sp.**

Alignments | Download | GenBank | Graphics | Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Agaricaceae sp. RC_Mart06_016 small subunit ribosomal RNA gene, partial sequence	523	1286	100%	8e-145	99%	HQ839744.1
Uncultured fungus clone 18s3-69 18S ribosomal RNA gene, partial sequence	523	1314	100%	8e-145	99%	EU733634.1
Mycena galericulata strain RV87/14.01 18S small subunit ribosomal RNA gene, partial sequence	523	1187	92%	8e-145	99%	DQ851576.1
Mycena plumbea isolate AFTOL-ID 1631 18S small subunit ribosomal RNA gene, partial sequence	523	1292	100%	8e-145	99%	DQ457697.1
Phaeomarasmius proximans isolate AFTOL-ID 979 18S ribosomal RNA gene, partial sequence	523	1297	100%	8e-145	99%	AY752970.1
Coprinus comatus voucher UC<USA-CA>:ECV 3198 18S ribosomal RNA gene, partial sequence >gb|AY665772.1| Coprinus comatus isolate	523	1325	100%	8e-145	99%	NG_016488.1

Fig 6. Full length sequence and homology search of BRM-8 (*Agaricaceae* sp.)

Plate 10. *Macrolepiota* sp. (BRM-9)

Field characters	**Phenotypic characters**	**Scientific classification**
Mushroom Designation: BRM-9 Date of Collection:26/03/2013 Edibility: Non edible Habitat : Soil Abundance: Two Growth habit: Scattered	Colour: Creamish white Shape 1)cap: Convex 2)Stipe: Enlarge below Size 1) cap dia.: 6cm 2)Stipe: length: 9cm Thickness:0.6cm Texture : Crispy & soft Stipe Position: Central Gills : Free Annulus : Absent	Kingdom: Fungi Division: Basidiomycota Class: Basidiomycetes Order: Agaricales Family: Lepiotaceae Genus:*Macrolepiota* Species:

TCCTCCGCTTATTGATATGCTTAAGTTCAGCGGGTAGTCCTACCTGATTTGAG
GTCAAATATTCAGTATGATTGTCCATGAAGACTGGACGATTAGCAGCTGAAC
AGACAGAGAGCGATTACACGGCGTAGATAATTATCACACCTGTGACGGATCG
CAAACGGTTCCGCTAATGCATTTCAGAGTAGCTGACCCCCTAAAGGGCCAGC
AAAAACTCCAAATCCAAGCCCCATTCACAGAAAAAACTGTGAGGGGTTGAGA
ATTTAATGACACTCAAACAGGCATGCTCCTCGGAATACCAAGGAGCGCAAGG
TGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCG
CATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGCTGAAAG
TTGTATAAGATCATTTATAGGCACAAAGGCCCATTAAAGACATTCTATAACAT
ACTATGTGGTATATGAAAACATAGACTCTGGGGCAGGGTTCACAACAACCTT
ATGTTGAGGAGAGCTGCAAAAGCATTCCCCACATCCGAGAAGAACTCGATTA
GATTGGTTACTTTCAAATCCCTAGAAGACTACAAAAAGTGCACAGGTGGATG
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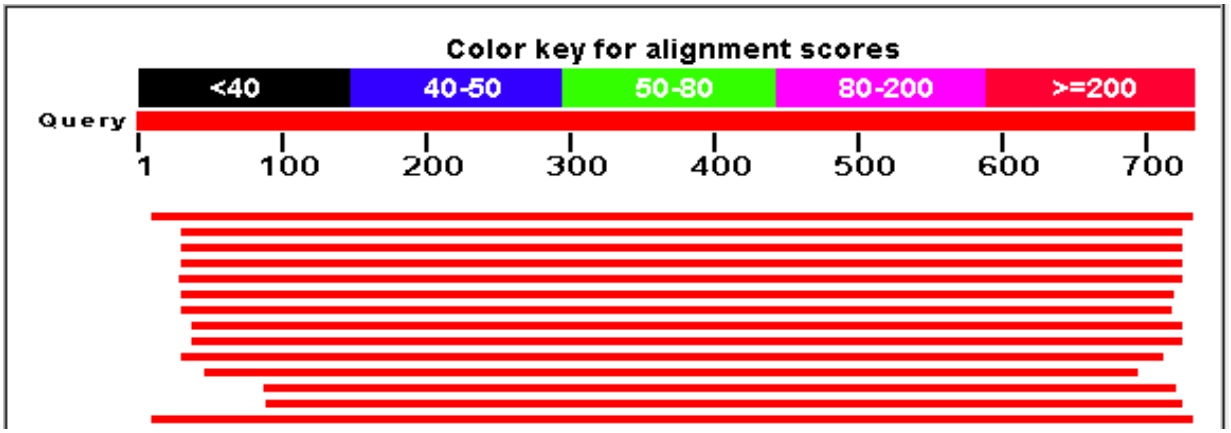

***Macrolepiota* sp.**

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Macrolepiota sp. H-603 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribe	1198	1198	98%	0.0	98%	GQ254066.1
Macrolepiota sp. TL-2011b voucher P59 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and int	1171	1171	94%	0.0	98%	JF495073.1
Macrolepiota sp. TL-2011b voucher P32 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and int	1166	1166	94%	0.0	98%	JF495077.1
Macrolepiota sp. TL-2011b voucher H0219 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and	1164	1164	94%	0.0	98%	JF495076.1
Macrolepiota sp. Oku X-2000 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal trans	1164	1164	94%	0.0	98%	AF482851.1
Macrolepiota sp. TL-2011b voucher Watling14576 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequen	1159	1159	93%	0.0	98%	JF495074.1
Macrolepiota sp. TL-2011b voucher P71 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and int	1155	1155	93%	0.0	98%	JF495075.1

Fig 7. Full length sequence and homology search of BRM-9 (*Macrolepiota* sp.)

Plate 11. *Calvatia holothurioides* (BRM-10)

Field characters	**Phenotypic characters**	**Scientific classification**
Mushroom Designation: BRM-10 Date of Collection:09/08/2013 Edibility: Non edible Habitat : Soil Growth habit: Scattered	Colour: Orange Cone shaped , fruiting body measures 5cm long and 5.5cm wide. Orangish spores round in shape. Rhizods are present Texture : Like dry paper Gills : Absent Annulus : Absent	Kingdom: Fungi Division: Basidiomycota Class: Basidiomycetes Order: Agaricales Family: Lycoperidiaceae Genus: *Calvatia* Species: *Holothurioides*

TCCTCCGCTTATTGATATGCTTAAGTTCAGCGGGTAATCCTACCTGATTTGAG
GTCAAGAGTTTCATTAAGTTGTCCGAGTAATGGACGATTAGCAGCTGAACAA
ACTAGAGAGCAGTCATCACAGCGTAGATAATTATCACACTAGTGACGGGACT
GCAAACGGTTCCGCTAATGTATTTCAGGAGAGCTGACCTCTATGAGGCCCGC
AAGCTCCCATTTCCAAGCCCTAATAGCTCACAAAAGTCATAAGGGTTGAGAA
TTTAATGACACTCAAACAGGCATGCTCCTCGGAATACCAAGGAGCGCAAGGT
GCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGC
ATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGCTGAAAGT
TGTATATGATTGTTATAGGCACAAAGTCCCATTGATCACATTCTACAACATAC
TATGTGGTATATGAATACATAGACTTGAAGGAGAGGTTTGCAAGTCACTTTG
AGAAAAGCTGTATGCTTGCGCACTCAGCATTCCCCACATCCGGAACGACAAA
TAGACGTCCGATAGTTGACCGCTCTCAACCCCCAAGACTACAAAAGGTGCAC
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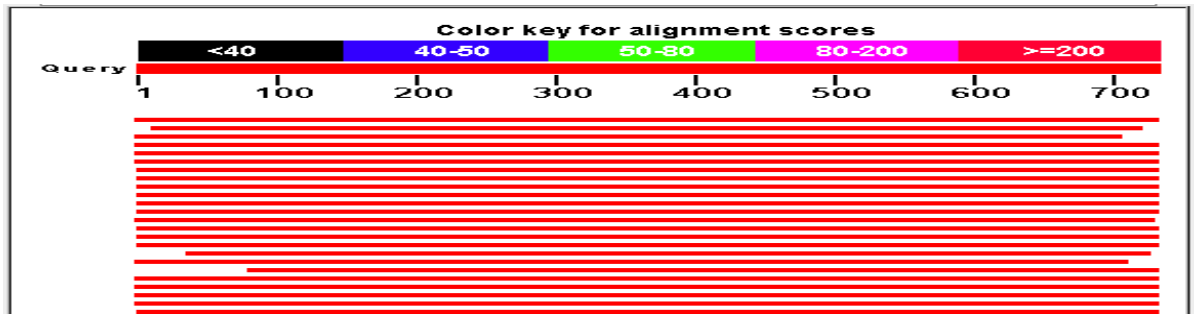
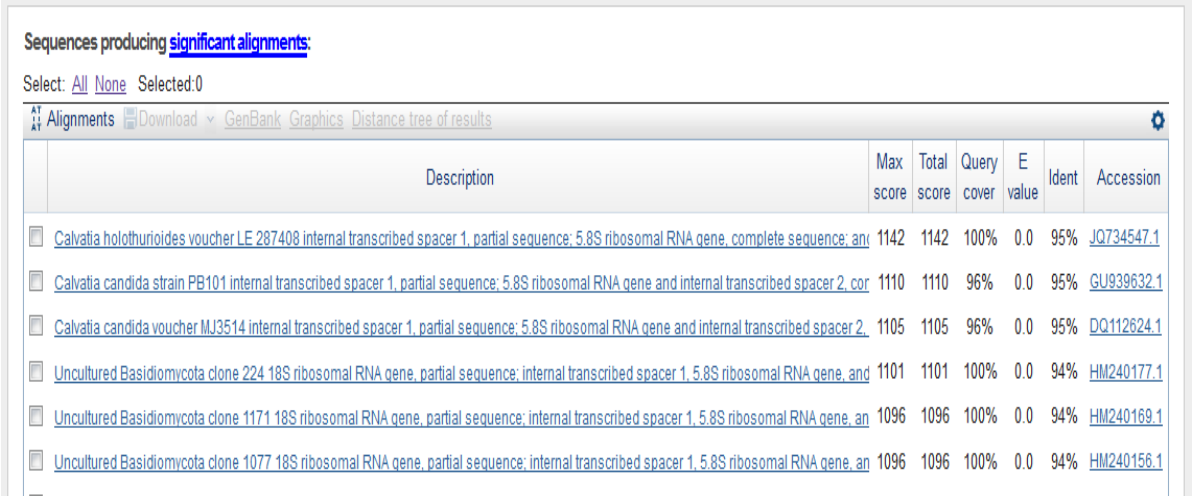
CAACCCATCAAGAATATTCAATAATGATCCTTCCGCAGGTTCCTACGGA



Calvatia holothurioides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Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Calvatia holothurioides voucher LE 287408 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; an	1142	1142	100%	0.0	95%	JQ734547.1
Calvatia candida strain PB101 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, cor	1110	1110	96%	0.0	95%	GU939632.1
Calvatia candida voucher MJ3514 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2,	1105	1105	96%	0.0	95%	DQ112624.1
Uncultured Basidiomycota clone 224 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and	1101	1101	100%	0.0	94%	HM240177.1
Uncultured Basidiomycota clone 1171 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, an	1096	1096	100%	0.0	94%	HM240169.1
Uncultured Basidiomycota clone 1077 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, an	1096	1096	100%	0.0	94%	HM240156.1

Fig 8. Full length sequence and homology search of BRM-10 (*Calvatia holothurioides*)

Plate 12. *Gymnopilus crociphyllus* (BRM-11)

Field characters	**Phenotypic characters**	**Scientific classification**
Mushroom Designation: BRM-11 Date of Collection:23/06/2013 Edibility: Non edible Habitat : Dead wood Abundance: Five Growth habit: Scattered	Colour: Yellowish orange Shape 1)cap: Convex 2)Stipe: Tapering upward Size 1) cap dia.: 3cm 2)Stipe: length: 4cm Thickness :0.7cm Texture : Soft Stipe Position: Central Gills : Free Annulus : Absent	Kingdom: Fungi Division: Basidiomycota Class: Basidiomycetes Order: Agaricales Family: Cartinariaceae Genus: *Gymnopilus* Species: *crociphyllus*

GCATCTAGATTTCCTCCGCTTATTGATATGCTTAAGTTCAGCGGGTAGTCCTA
CCTGATTTGAGGTCAAATAGTCATTAATTGTCCAAGACGGACGATTAGAAGC
AGCACATCCCATTTAATAGCAGACGTCCAACGGCGTAGATAATTATCACACC
AATAGACGGTCCACGCGGGGCACCGGCTAATGCATTTTAGGGGAGCTGACTT
CTTGCGAAACCAGCAAAAGACCCCCACTTCCAAGCCATTACATAGCTAGTAA
AAGCTAGTAAGGTTGAGAATTTAATGACACTCAAACAGGCATGCCCCTCGGA
ATACCAAGGGGCGCAAGGTGCGTTCAAAGATTCGATGATTCACTGAATTCTG
CAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCA
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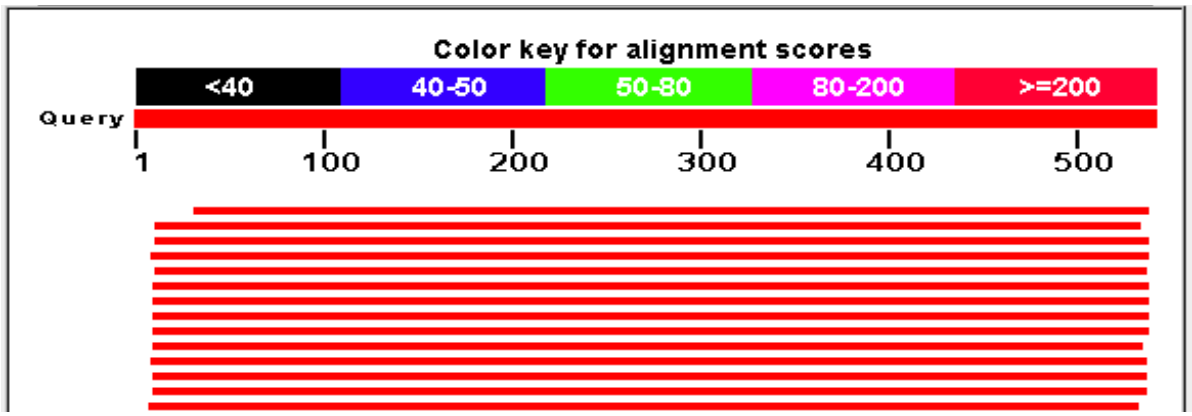

Gymnopilus crociphyllus

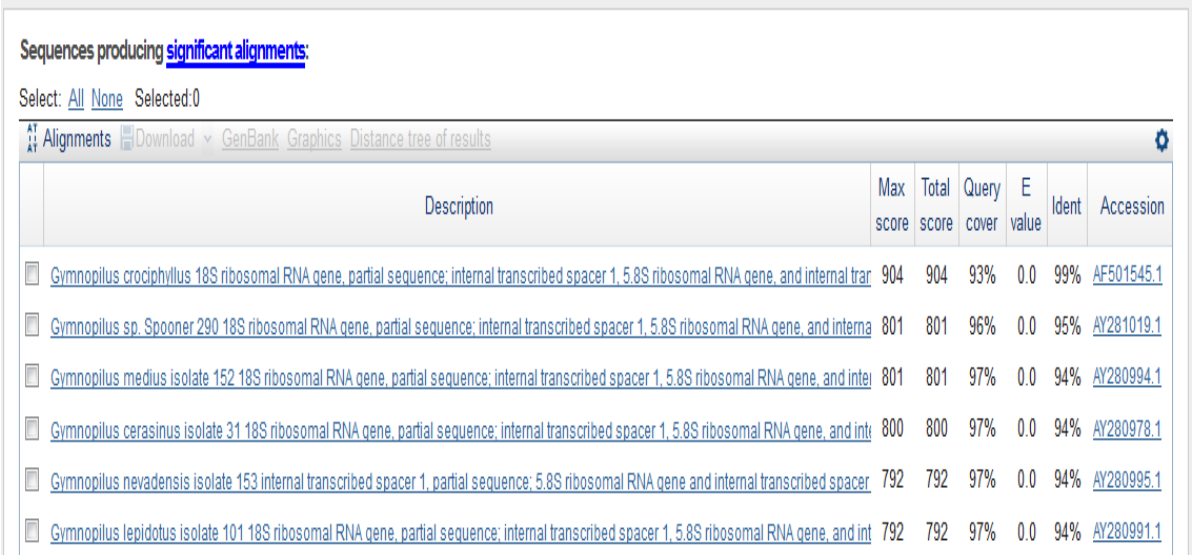

Fig 9. Full length sequence and homology search of BRM-11 (*Gymnopilus crociphyllus*)

Plate 13. *Coprinus comatus* (BRM-12)

Field characters	**Phenotypic characters**	**Scientific classification**
Mushroom Designation: BRM-12 Date of Collection:09/08/2013 Habitat : Soil Abundance: Two Growth habit: Scattered	Colour: Creamish Shape 1)cap: Convex 2)Stipe: Club Size 1) cap dia.: 9cm 2)Stip:length:14cm Thickness:1.2cm Texture : Soft Stipe Position: Central Gills : Free Annulus : Present	Kingdom: Fungi Division: Basidiomycota Class: Basidiomycetes Order: Agaricales Family: Agaricaceae Genus: *Coprinus* Species: *comatus*

AGCTTGCATGCAGGCCTCTGCAGTCGACGGGCCCGGGATCCGATTAAGGGAA
GGGACGTAATCGACGCGAGCTGATGACTCACGCTTACTAGGTATTCCTCGTTG
AAGAGCAATAATTGCAATGCTCTATCCCCAGCACGACAGAGTTTCACAAGAT
TACCCAGACCTTCCGGCCAAGGTGAAAAACTCGCTGGCTCTGTCAGTGTAGC
GCGCGTGCGGCCCAGAACATCTAAGGGCATCACAGACCTGTTATTGCCTCAA
ACTTCCGTCAGCTAGACGCTGACAGTCCCTCTAAGAAGCCGGCGACCAGCGA
AAGCCGGCCTGGCTATTTAGCAGGTTAAGGTCTCGTTCGTTATCGGAATTAAC
CAGACAAATCACTCCACCAACTAAGAACGGCCATGCACCACCACCCATAAAA
TCATGAAAGAGCTATCAATCTGTCAATCCTAGTTATGTCTGGACCTGGTGAGT
TTCCCCGTGTTGAGTCAAATTAAGCCGCAGGCTCCACACCTGGTGGTGCCCTT
CCGTCAATTCCTTTAAGTTTCAGCCTTGCGACCATACTCCCCCCAGAACCCAA
AGACTTTGATTTCTCGTAAGGTGCCGAGCGACACATCAAATTTGAGGTCGCCC
GATCCCTAATCGGCATAGTTTACTGTTAAGACTACAACGGTATCTGATCGTTG
TCGATCCCCTAACCTTCGTTCTTGATTAATGAAAACATCCTTGGCAAATGCTT
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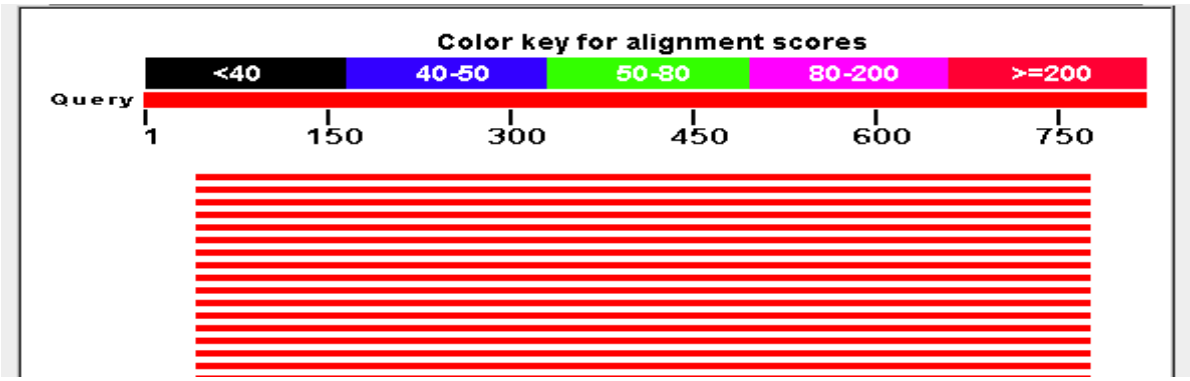
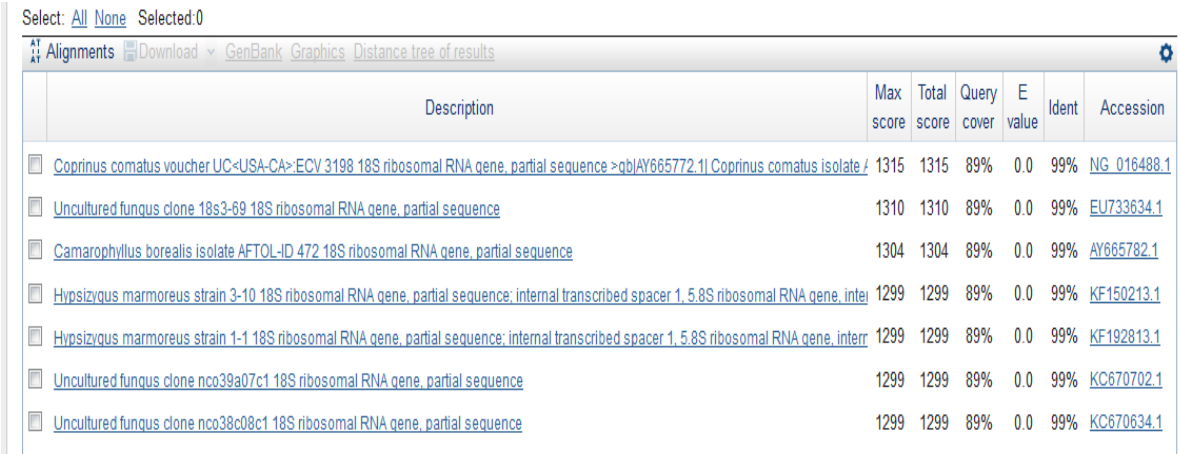


Coprinus comatus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Alignments Download GenBank Graphics Distance tree of results

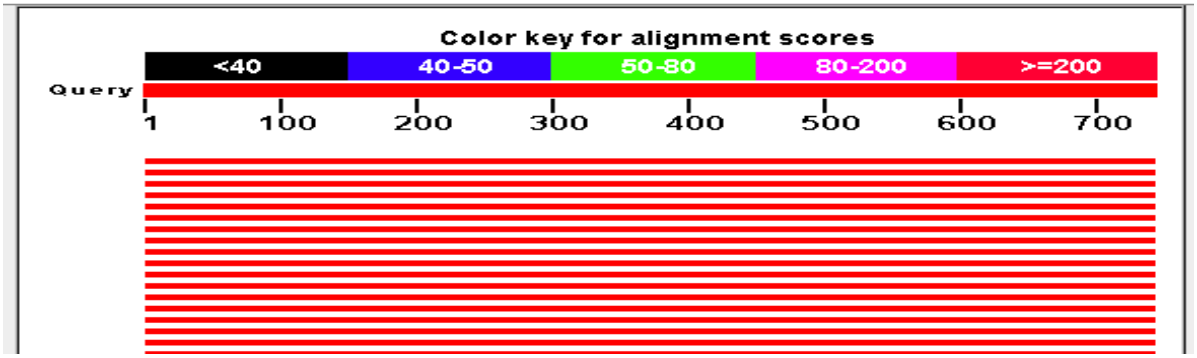
Description	Max score	Total score	Query cover	E value	Ident	Accession
Coprinus comatus voucher UC<USA-CA>:ECV 3198 18S ribosomal RNA gene, partial sequence >gb|AY665772.1| Coprinus comatus isolate /	1315	1315	89%	0.0	99%	NG_016488.1
Uncultured fungus clone 18s3-69 18S ribosomal RNA gene, partial sequence	1310	1310	89%	0.0	99%	EU733634.1
Camarophyllus borealis isolate AFTOL-ID 472 18S ribosomal RNA gene, partial sequence	1304	1304	89%	0.0	99%	AY665782.1
Hypsizygus marmoreus strain 3-10 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, inte	1299	1299	89%	0.0	99%	KF150213.1
Hypsizygus marmoreus strain 1-1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, interr	1299	1299	89%	0.0	99%	KF192813.1
Uncultured fungus clone nco39a07c1 18S ribosomal RNA gene, partial sequence	1299	1299	89%	0.0	99%	KC670702.1
Uncultured fungus clone nco38c08c1 18S ribosomal RNA gene, partial sequence	1299	1299	89%	0.0	99%	KC670634.1

Fig 10. Full length sequence and homology search of BRM-12 (*Coprinus comatus*)

Plate14. *Gyrodontium sacchari* (BRM-13)

Field characters	**Phenotypic characters**	**Scientific classification**
Mushroom Designation: BRM-13 Vernacular Name: Haladi anabe Date of Collection:09/08/2013 Edibility: Non edible Habitat : Dead wood Abundance: Four Growth habit: Gregarious	Colour: Whitish yellow Shape 1)cap: Absent 2)Stipe: Absent Texture : Corkey Teeth presnt below surface Gills : Absent Annulus : Absent	Kingdom: Fungi Division: Basidiomycota Class: Basidiomycetes Order: Boletales Family: Boletaceae Genus: *Gyrodontium* Species: *sacchari*

GTCAGAGGTGAAATTCTTGGATTTACTGAAGACTAACTACTGCGAAAGCATT
TGCCAAGGATGTTTTCATTAATCAAGAACGAAGGTTAGGGGATCGAAAACGA
TCAGATACCGTTGTAGTCTTAACAGTAAACTATGCCGACTAGGGATCGGGCA
ATCTCTTTTTTGATGTGTTGCTCGGCACCTTACGAGAAATCAAAGTCTTTGGGT
TCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGACGGAAGG
GCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACT
CACCAGGTCCAGACATGACTAGGATTGACAGATTGATAGCTCTTTCATGATTT
TATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTA
ATTCCGATAACGAACGAGACCTTAACCTGCTAAATAGCCAGGCTGGCTTTCG
CTGGTCGCCGGCTTCTTAGAGGGACTGTCAGCGTCTAGCTGACGGAAGTTTG
AGGCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCT
ACACTGACAGAGCCAGCGAGTTCTTTTTCCTTGGCCGGAAGGTCTGGGTAATC
TTGTGAAACTCTGTCGTGCTGGGGATAGAGCATTGCAATTATTGCTCTTCAAC
GAGGAATTCCTAGTAAGCGTGAGTCATCAGCTCGCGTTGATTACGTCCCTGCC
CTT



Gyrodontium sacchari

Alignments | Download | GenBank | Graphics | Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Gyrodontium sacchari MUCL:40589 18S small subunit ribosomal RNA gene, partial sequence	1317	1317	99%	0.0	99%	GU187632.1
Serpula incrassata DAOM 170590 18S small subunit ribosomal RNA gene, partial sequence	1290	1290	99%	0.0	99%	GU187652.1
Leucogyrophana olivascens CFMR:HHB-11134 18S small subunit ribosomal RNA gene, partial sequence	1290	1290	99%	0.0	99%	GU187639.1
Leucogyrophana arizonica CFMR:RLG-9902 18S small subunit ribosomal RNA gene, partial sequence	1290	1290	99%	0.0	99%	GU187636.1
Leucogyrophana mollusca strain DAOM138006 18S small subunit ribosomal RNA gene, partial sequence >gb|GU187634.1| Leucor	1287	1287	99%	0.0	99%	DQ534684.1
Uncultured eukaryote clone RU12192007A7 18S ribosomal RNA gene, partial sequence	1278	1278	99%	0.0	99%	HQ427481.1
Leucogyrophana romellii CFMR:T-547 18S small subunit ribosomal RNA gene, partial sequence	1278	1278	99%	0.0	99%	GU187635.1

Fig 11. Full length sequence and homology search of BRM-13 (*Gyrodontium sacchari*)

Plate 15. *Clitocybe aff.fellea* (BRM-14)

Field characters	Phenotypic characters	Scientific classification
Mushroom Designation: BRM-14 Vernacular Name: Kotesuthu anabe Date of Collection:05/09/2013 Edibility: Non edible Habitat : Soil Abundance: group Growth habit: Gregarious	Colour: Pale brown Shape 1)cap: Absent 2)Stipe: Absent Texture : Soft Gills : Decurrent Annulus : Absent	Kingdom: Fungi Division: Basidiomycota Class: Basidiomycetes Order: Agaricales Family: Tricholomataceae Genus: *Clitocybe* Species: *aff.fellea*

GTCAGAGGTGAATTCTTGGATTTACTCAAGACCGACTATTGCGAAAGCATTTG
CCAAGGATGTTTTCATTAATCAAGAACGAAGGTTAGGGGATCGAAAACGATC
AGATACCGTTGTAGTCTTAACAGTAAACTATGCCGACTAGGGATCGGGCAAC
CTCAAATTTGATGCGTTGCTCGGCACCTTACGAGAAATCAAAGTCTTTGGGTT
CTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGACGGAAGGG
CACCACCAGGTGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGAAAACTC
ACCAGGTCCAGACATAACTAGGATTGACAGATTGATAGCTCTTTCATGATTTT
ATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAA
TTCCGATAACGAACGAGACCTTAACCTGCTAAATAGCCAGGTCGGCTTTTGCT
GATCGCAGGCTTCTTAGAGGGACTGTCAGTGTCTAACTGACGGAAGTTTGAG
GCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTAC
ACTGACAGAGCCAGCGAGTTTCTTTTCCTTGGCCGGAAGGTCTGGGTAATCTT
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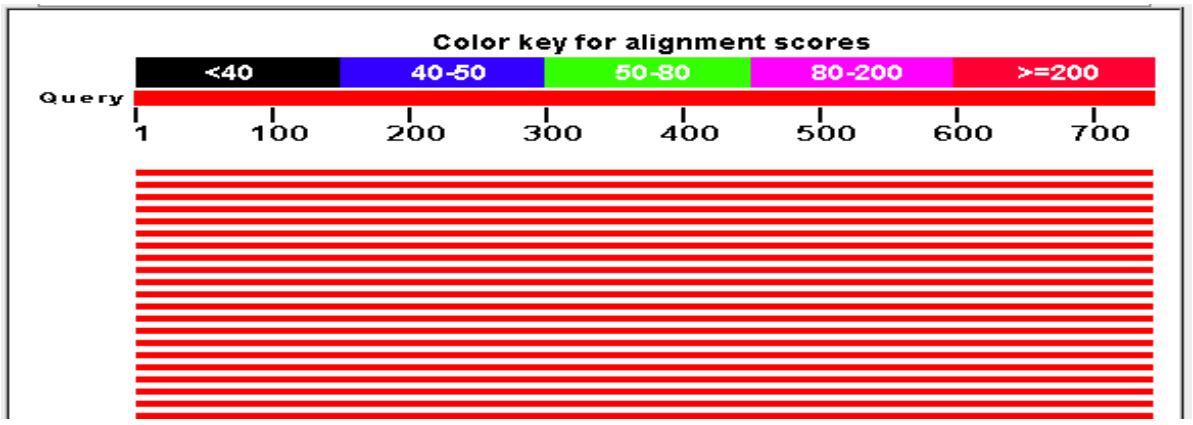
GGAATACCTAGTAAGCGTGAGTCATCAGCTTGCGTTGATTACGTCCCTGCCCT
T



Clitocybe aff.fellea

Alignments | Download | GenBank | Graphics | Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Clitocybe aff. fellea PBM3028 small subunit ribosomal RNA gene, partial sequence	1304	1304	99%	0.0	99%	HQ728535.1
Pseudoomphalina pachyphylla voucher TL-5643 (CFMR) 18S ribosomal RNA gene, partial sequence	1293	1293	99%	0.0	99%	KF291252.1
Lycoperdon pyriforme voucher CUW:DSH 96-054 18S ribosomal RNA gene, partial sequence >gb|AF026619.1| Lycoperdon sp. DSH 96-054 1	1293	1293	99%	0.0	99%	NG_013125.1
Lopharia mirabilis 18S ribosomal RNA gene, partial sequence	1291	1291	99%	0.0	99%	AY293141.1
Uncultured eukaryote clone 18S-T6-2-6B 18S ribosomal RNA gene, partial sequence	1288	1288	99%	0.0	98%	KC664496.1
Callistosporium graminicolor isolate AFTOL-ID 978 18S ribosomal RNA gene, partial sequence	1288	1288	99%	0.0	98%	AY752974.1

Fig 12. Full length sequence and homology search of BRM-14 (*Clitocybe aff.fellea*)

Table 3. List of Mushroom species identified using 18S rRNA/ ITS gene sequencing

Sl. No.	Mushroom Species Designation	Mushroom species identified	Size of amplified product (base pair)
1	BRM-3	*Termitomyces* sp.	734
2	BRM-4	*Auricularia delicata*	604
3	BRM-5	*Termitomyces microcarpus*	603
4	BRM-6	*Amanita* sp.	614
5	BRM-7	*Podoscypha petalodes*	693
6	BRM-8	*Agaricaceae* sp.	729
7	BRM-9	*Macrolepiota* sp.	726
8	BRM-10	*Calvatia holothurioides*	732
9	BRM-11	*Gymnopilus crociphyllus*	542
10	BRM-12	*Coprinus comatus*	822
11	BRM-13	*Gyrodontium sacchari*	736
12	BRM-14	*Clitocybe aff.fellea*	736

BRM= Biligiri Ranganahill Mushroom

***Termitomyces sp.* (BRM-3) cultured in PDA medium**

Plate 16. *In-vitro* culturing of edible mushroom species

V. DISCUSSION

Mushrooms are picked up and eaten by mankind from time immemorial. Ancients had well acquainted knowledge on mushrooms grown in forest area particularly of edible ones. Roman's regarded mushrooms as food of gods because of their delicacy and taste. Mushroom is a reproductive structure of fungus known as fruiting body or sphorophore. They are neither plants nor animals as they do not contain chlorophyll like green plants and manufacture their own food. So also they lack nervous system, specialized organs and mobility which are of animal characteristics. Furthermore, they reproduce by means of microscopic reproductive units called spores. Therefore, mushrooms are placed in a separate division called Eumycota (the true fungi). In eumycota, mushrooms belong to two major classes. They are Basidiomycetes and Ascomycetes. Basidiomycetes comprise most of the mushroom fungi whereas Ascomycetes includes a few (Davidson *et al*., 2012).

Wild mushrooms are gathered during rainy season as they flourish in cool and humid weather. During collection, it is essential to record field characters meticulously as it facilitates further classification and speciation. As David Arora said in his book entitled *Mushrooms Demystified* that, the mushroom hunting is not simply a matter of traipsing through the woods after it rains. It is an art, a skill, a meditation and a process. Don't just collect, but observe the mushroom and their surroundings. In this process, mushroom hunter can discover many clandestine wonders that were unaware. As observation begin in field, recording field details are very much essential. The field details such as, date, season, weather, abundance, growth habit, vegetation, substrate *etc*., should be documented. In the present study, both phenotypic and genotypic information has been used for identification of documented mushrooms from B.R. Hills of Karnataka. The results of the study are discussed here under.

5.1. Documentation and identification of wild mushrooms by Phenotypic Characters

Since, survey for mushroom is never ending process as occurrence of different types of mushrooms depends on season, climatic conditions, habitat and rainfall, mushroom hunter can discover different species. Totally fourteen mushroom species were documented from B.R. Hills with the help of information availed by Forest Department and Soliga tribe residing there. Collected mushrooms are designated as BRM (Biligiri Rangana hill Mushroom) with serial numbers. Field information was recorded while collecting mushrooms. Mushrooms were collected in paper bags and brought to the laboratory for further study.

Phenotypic characters of pileus, stipe, gills arrangement, and microscopic characters like spore shape, color and size were recorded. Based on this information while consulting the books *Mushroom Demystified* (Arora, 1984) and *The Indian Polyporaceae* (Bakshi, 1971), similarly Kumar *et al.* (1991) collected 110 genera of mushrooms from forests of Chhattisgarh and identified seventeen genera of fleshy fungi. Of these, *Volvariella diplasia, V. volvacea, Termitomyces mammiformis, T. albuminosa, and T. albigenosus* were identified and characterized as edible mushrooms. Bhatt *et al.*

(1995) illustrated and described four species of the genus *Russula viz*., *R. albida, R. amoenolens. R. brevipes* and *R.dissimulans* for the first time from India which were collected from Garhwal districts of Himalaya. In the present study two mushrooms designated as **BRM-1** and **BRM-2** were identified as ***Ganoderma lucidum*** (medicinal mushroom) and ***Polyporus flabelliformis*** (wood rotting fungi). Giri and Rana (2008) collected 29 mushrooms from Solu-Khumbu district of the north eastern region of Nepal with the traditional mycological knowledge of Sherpa community and identified 26 species as a edible, 2 species as medicinal and one species ornamental. Similarly, Meeraand Veena, (2012) reported 45 species of mushrooms from Kodagu district of Western ghats and Gurudevan*et al.,* (2011) documented 68 mushroom floras from the Anaikatti, Ataipadi, Kallar, Nilgiris, Palakkad and Siruvani regions of Western ghats.

Though, mushrooms are gathered for eating throughout the world for thousands of years, it is also likely that during that time many people became ill or died due to consumption of poisonous mushrooms inadvertently. Hence, differentiation of edible or poisonous species mainly depends on either subtle knowledge of experienced consumer in the locality or by well experienced mycologist as there are no pool proof methods available so far to detect the poisonous species. Nevertheless, mushrooms can be differentiated as edible or poisonous only after identification of the species. The wild mushroom can be identified by well experienced mushroom hunter by its morphological and microscopic characters (Arora, 1986).

5.2. Molecular characterization of wild mushrooms

The fruiting bodies of mushrooms vary greatly in detail and design. Based on the form/structure of fruiting body, mushrooms are named as gilled mushrooms, polypores, puff balls, earth stars, cup fungi, coral fungi and so on. The common name thus given based on their morphology or habitat in which they grow may not be sufficient enough to classify the organism. Hence, scientific classification and binomial nomenclature is universal and used by both naturalists and biologists. Though the classical taxonomy mainly depends on phenotypic characters, which some time leads to confusion between taxonomists. As a result, they arrive at different conclusions, therefore molecular characterization using 18S rRNA gene/ITS region sequence analysis is reliable for precise classification.

In molecular taxonomy, the gene encoding for 16SrRNA in prokaryotes and 18S rRNA/ITS in eukaryotes are most widely used. This small subunit (30S) ribosomal RNA (SSU rRNA) genes sequence have been extensively used for sequence based evolutionary analysis because, they are Universally distributed, functionally constant, sufficiently conserved and have adequate length therefore, they can provide a view of evolution encompassing all living microorganisms (Madigan *et al*., 2009). The 23S large subunit of r-RNA (LSU rRNA) gene is also phylogeniticaly informative with its large sequence providing additional information. However, its length makes it more costly and time consuming. Taxonomists opine that 18S rRNA/ITS gene serve as a ‘golden standard’ for the identification and description of new species (Madigan *et al*., 2009).

A molecular taxonomist must invariably familiar with the morphology and habitat of the mushroom as this acumen helps in recognition of mushrooms precisely by looking at its fruiting body structure when he confront them in field. Thus, phenotypic as well as molecular characters would equally play an important role in mushroom classification. Otherwise, it would be a blind man's experience of the day. However, it is often difficult to distinguish certain species of mushrooms particularly during their young stages by morphological features. Therefore in such circumstance molecular tools provide more accurate results for identifying the species (Rajarathnam and Thiagarajan, 2012).

Literatures on the use of 18S rRNA / ITS region for identification of mushrooms are limited as mushrooms are most of the time identified based on Phenotypic and microscopic characters. However, modern taxonomists use molecular approaches for classification of mushrooms and have found its advantages over phenotypic characterization. Moor *et al.* (2002) identified *Boletus edulis* and several closely related mushroom species using ITS region sequence. Phylogenetic analysis based on RAPD profiles and nucleotide sequence 5.8 rRNA gene revealed variation among inter generic and intra species isolates among accessions (Singh *et al.,* (2006).

Ranjaratnam and Thiagarajan (2012) identified *Perenniporia* sp. by amplification of ITS region and aligned by using Jukes-Cantor Corrected Distance model. Similarly, eighteen species of Termitomyces collected from Ondo and Ekiti States of Nigeria were identified using ITS region of rDNA (Oyetayo, 2012).

Sudip *et al.* (2013) collected eight wild edible mushrooms from eastern Chota Nagpur plateau of West Bengal, India and was identified using ITS 1 and ITS 2 primers. The aligned sequence revealed identity of *Amanita hemibafpha, Amanita* sp., *Astraeus hygrometricus, Termitomyces* sp., *Termitomyces* sp., *Volvariella volvceae, Termitomyces* sp. Among eight mushrooms four could be identified up to species level In the present study, twelve mushrooms designated as BRM-3, BRM-4, BRM-5, BRM-6, BRM-7, BRM-8, BRM-9, BRM-10, BRM-11, BRM-12, BRM-13 and BRM-14 were identified up to species level by using ITS/ 18SrRNA gene sequence in addition to phenotypic characters.

The PCR amplification of genomic DNA of the 12 mushrooms in the present study yielded amplified product sizes varying from 542 bp to 822 bp (Table,3) which are corresponding to almost full length gene sequence of ITS/ 18SrRNA. The sequence homology of the 12 species ranged from 95-99% when aligned with the sequences present in NCBI. BRM-3 has 99 % homology with *Termitomyces* sp., BRM-4 has 99% homology with *Auricularia delicata*, BRM-5 showed 95% homology with *Termitomyces microcarpus*, BRM-6 showed 99% homology with *Amanita* sp, BRM-7 with 99% homology for *Podo scypha petalodes*, BRM-8 with 99% homology for *Agaricaceae* sp, BRM-9 with 98% homology for *Macrolepiota* sp, BRM-10 showed 95% homology with *Calvatia holothurioides*, BRM-11 had 99% homology with *Gymnopilus crociphyllus*, BRM-12 showed 99% homology with *Coprinus comatus*, BRM-13 had 99% homology with *Gyrodontaium sacchari*, and BRM-14 showed 99% homology with *Clitocybe aff. fellea.*

Thus, the above 12 species have been identified based on the homology with the respective species sequences found in NCBI. Similarly, a delicious mushroom documented from Western ghats of Karnataka was identified using ITS region of ribosomal DNA sequence as *Termitomyces* sp. (Earanna *et al*., 2013).

The 18S rRNA/ ITS flanking sequence is highly conserved in eukaryotes. Primer developed for flanking sequence of 18S r RNA/ ITS can be used as a universal primer for all eukaryotic species. In the present study, mushrooms characterized by using 18S r RNA/ ITS primers showed variation in the amplicon size. The ampicon size varied from 542-822bp for different mushroom species. This could be due to insertion, deletion, duplication and translocation. Further, it may also due to species variation (Zhihhong *et al*., 2003).

5.3. Isolation and conservation of pure culture of wild mushroom

Since, mushroom fungi are important source of food and medicine. There is a need to explore and conserve potential species for further application in future. Besides edibility, many non-edible mushrooms are very good decomposer of organic matter which is essential for bioremediation. Many traditional uses of mushrooms have been confirmed and new applications have been developed as mushrooms possess potentially important therapeutic compounds including antioxidants, anti-hypertensive, cholesterol-lowering, liver protection, anti-fibrotic, anti-inflammatory, anti-diabetic, anti-viral and anti-microbial etc.,(Borcher*set al.,* 2004). Therefore, one of the intentions of this study was to conserve the elite species documented from the wild. Pure culture can be obtained from tissue/spore of the sphorophore by using different culture media.

Rehana *et al.* (2007) isolated pure culture of *Pleurotus sajor-caju* by using joint portion of cap and stipe tissue that produced vigorous mycelial growth on malt extract agar at 25ºC. Similarly, *L. cladopus* produced profuse vegetative growth on malt extract agar (Atri and Lata, 2013). Influence of various culture media on the mycelial growth of *Pleurotus pulmonarius* was investigated by using Malt extract agar, Corn cob extract agar and Cassava peelings extract agar. The above substrates influenced excellent mycelial growth of *P. pulmonarius* compared to potato dextrose agar and plantain peelings extract agar under *in-vitro* condition Stanley and Nyenke (2011).

Stanley *et al.* (2013) reported highest mycelial growth (12.4mm/day) of *Pleurotus sajor-caju* (Oyster mushroom) on cassava peelings mixed with 25 ml of honey compared to plantain peelings applied with 25 ml honey (2.43 mm/day) indicating the significance of organic waste incorporation in the culture media. In the present study, three edible mushrooms namely, *Termitomyces microcarpus , Auricularia delicata* and *Termitomyces* sp. were cultured using pileus/stipe tissue on Potato Dextrose Agar (PDA). Among the three, only *Termitomyces* sp. produced mycelium on PDA and *A. delicata* and *T. microcarpus* were failed to grow. This may need further experimentation on use of different media and growth conditions to obtain the growth. Thus, isolated pure culture of *Termitomyces* sp. was preserved for further study.

VI. SUMMARY

B.R. Hills located in Chamarajnagar district of Karnataka. It is a confluence of Western ghats and Eastern ghats. B.R. Hills receives 600 mm annual rainfall at the base and 3000 mm at the top of the hills. A wide range of climatic conditions along with the altitude variations made the ecosystem congenial for biodiversity. A variety of mushroom species appears during rainy season. In the present study, ethnobotanical survey of mushroom flora was carried out during June to September 2013 with the help of Soliga tribes inhabited in the area for hundreds of years. The main aim of the study was to document, characterize and explore the potential edible species.

Fourteen species of mushrooms were collected in a paper bags during field survey with the help of Soliga tribes and designated them as BRM-1, BRM-2, BRM-3, BRM-4, BRM-5, BRM-6, BRM-7, BRM-8, BRM-9, BRM-10, BRM-11, BRM-12, BRM-13 and BRM-14. Field observation like, place, date, abundance, growth habitat etc., were recorded during collection. Collected mushrooms were brought to the laboratory, and morphological characters like colour, size of cap and stipe, stipe position, presence or absence of gills or pores, presence or absence of annulus, texture and microscopic characters like spore shape and color were recorded. The two polypores designated as BRM-1 and BRM-2 were identified as *Ganoderma lucidum and Polyporous flabelliformis* respectively based on phenotypic /fruiting body characters.

The other twelve species of mushrooms designated as BRM-3, BRM-4, BRM-5, BRM-6, BRM-7, BRM-8, BRM-9, BRM-10, BRM-11, BRM-12, BRM-13 and BRM-14 were identified by using ITS/18S rRNA gene sequence data base deposited in National Centre for Biotechnology Information (NCBI). The genomic DNA of the mushrooms was amplified using 18S rRNA/ITS primers, cloned into T/A vector and transferred into *E.coli* DH5α cells. Recombinant plasmid from *E.coli* was isolated and sequenced. The sequence alignment was made with NCBI data base revealed 95-99% homology. BRM-3 has 99% homology with *Termitomyces* sp., BRM-4 has 99% homology with *Auricularia delicata*, BRM-5 has 95% homology with *Termitomyces microcarpus*, BRM-6 has 99% homology with *Amanita* sp, BRM-7 showed 99% homology with *Podoscypha petalodes*, BRM-8 with 99% homology for *Agaricaceae* sp, BRM-9 showed 98% homology with *Macrolepiota* sp, BRM-10 with 95% homology for *Calvatia holothurioides*, BRM-11 has 99% homology with *Gymnopilus crociphylus*, BRM-12 showed 99% homology *Coprinus comatus*, BRM-13 has 99% homology with *Gyrodontium sacchari*, BRM-14 showed 99% homology with *Clitocybe aff. fellea*.

Of the fourteen species of mushrooms documented, three species namely, *Termitomyces* sp., *Auricularia delicata,* and *Termitomyces microcarpus* were found to be edible. Three edible species were attempted to cultured *in-vitro* on Potato dextrose agar. Of these three mushrooms, only *Termitomyces* sp. was successfully produced mycelial growth on potato dextrose agar but *Auricularia delicata* and *Termitomyces microcarpus* did not produce mycelial growth. This needs further experimentation.

Future line of work:

1) More and more survey needed for the collection of different mushroom species and characterization.

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APPENDIX-I

1. Composition of various microbiological media

1) Potato dextrose agar media

INGREDIENTS	GRAMS/LITRE
Peeled potato slices	200
Dextrose	20
Agar	15

Boil the potato slices till it smoothens, filter the potato fluid, add dextrose and make up the volume to 1000ml with distilled water.

2) Composition of LB-broth and LB-agar

- Tryptone - 10.0gm
- Yeast extract - 5.0 gm
- NaCl - 5.0gm
- Distilled water - 1000 ml

Before sterilization, pH adjusted to 7.00 with 0.2N sodium hydroxide and as and when needed 15 grams of bacteriological grade agar was added to prepare Luria agar. The content was autoclaved at 15 lbs/sq inch for 20 minutes

2. Genomic DNA isolation (stock solutions)

EXTRACTION BUFFER

STOCK	REQUIRED CONC.	Required volume
1M TrisHCl (pH 8)	0.1M	28ml
0.5 M EDTA	0.02M	10ml
5M NaCl	1.4M	4ml
CTAB 2%	2%	2g

Make up the volume to 100 ml with Distilled water.

0.2% β- mercaptoethanol

1% PVP

Note : β- mercaptoethanol and PVP must be added before starting the DNA isolation.

Choloroform : Isoamylalcohol – 24:1 (V/V)

70 % chilled Ethanol.

Add 30 ml of distilled water to 70 ml of ethanol

TE Buffer

1ml of 1M Tris HCl plus 200µl of 0.5M EDTA and make up the volume to 100ml with distilled water.

Ribonuclease A

Use at concentration of 10µg/ml.

Agarose gel electrophoresis

TAE buffer (50 X)

Tris base	- 242 g
Glacial acetic acid	- 57.1 ml
EDTA 0.5 M (pH 8.0)	- 1000 ml

Loading dye (5 X)

Bromophenol blue	- 0.25%
Xylene cyanol	- 0.25%
Glycerol	- 30%