ETHNOBOTANICAL SURVEY AND MOLECULAR CHARACTERIZATION OF WILD MUSHROOMS FROM THE PARTS OF WESTERN GHATS REGION OF KARNATAKA

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PALB-2253

DEPARTMENT OF PLANT BIOTECHNOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
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IN

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BANGALORE **APRIL, 2015**

Affectionately dedicated to

My Family members

DEPARTMENT OF PLANT BIOTECHNOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES, BANGALORE-560065

CERTIFICATE

This is to certify that the thesis entitled, **"ETHNOBOTANICAL SURVEY AND MOLECULAR CHARACTERIZATION OF WILD MUSHROOMS FROM THE PARTS OF WESTERN GHATS REGION OF KARNATAKA"** submitted by **Mr. SANTHOSH, D. B., ID, No PALB-2253,** in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (AGRICULTURE) in PLANT BIOTECHNOLOGY** to the University of Agricultural Sciences, GKVK- Bengaluru. This 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
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April, 2015 **(SANTHOSH, D. B)**

ETHNOBOTANICAL SURVEY AND MOLECULAR CHARACTERIZATION OF WILD MUSHROOMS FROM THE PARTS OF WESTERN GHATS REGION OF KARNATAKA

SANTHOSH, D. B.

ABSTRACT

Western Ghats of Karnataka is one of the richest biodiversity hotspot in India, which receives an average rainfall of 2869 mm and provides congenial atmosphere for the growth of variety of mushrooms. In the present study eleven mushrooms were collected from Siddapura, Theertha halli and Agumbe forest area during monsoon season (June-September) with the help of Siddi, Adivasi and Alakki tribal community. During collection, the field information was recorded and the samples were designated as WGM-1, WGM-2, WGM-3, WGM-4, WGM-5, WGM-5, WGM-7, WGM-8, WGM-9, WGM-10 and WGM-11. Further, these mushrooms were identified by ITS region sequence homology using NCBI data base. The mushrooms identified based on sequence homology are *Lentinus squrossulus* (WGM-1), *Pleurotus salmoneostramenius* (WGM-2), *Termitomyces* sp. (WGM-3), *Termitomyces* sp. (WGM-4), *Leucoagaricus purpureolilacinus* (WGM-5), *Tricholosporum porphyrophyllum* (WGM-6), *Agrocybe pediades* (WGM-7), *Leucocoprinus birnbaumii* (WGM-8), *Podoscypha petalodes* (WGM-9), *Xylaria* sp. (WGM-10) and *Antrodia serialis* (WGM-11). Among the eleven mushrooms identified, WGM-1, WGM-2, WGM-3, WGM-4 and WGM-5 were belonging to edible species. The two mushrooms viz., *Lentinus squrossulus* (WGM-1) and *Termitomyces* sp. (WGM-4) was cultured *in-vitro* on Potato Dextrose Agar and conserved.

April, 2015

Department of Plant Biotechnology
GKVK, UAS, Bengaluru

N. Earanna
Major Advisor

ಮನುಕುಲಸಸ್ಯಶಾಸ್ತ್ರದ ಸಮೀಕ್ಷೆ ಹಾಗೂ ಕರ್ನಾಟಕದ ಪಶ್ಚಿಮ ಘಟ್ಟದ ವಲಯಗಳ ಭಾಗಗಳಲ್ಲಿ ದೊರೆಯುವ ಕಾಡು ಅಣಬೆಗಳ ಪ್ರಭೇದಗಳನ್ನು ಆಣ್ವಿಕ ವಿಧಾನದಿಂದ ಪತ್ತೆ ಹಚ್ಚುವಿಕೆ

ಸಂತೋಷ್, ಡಿ. ಬಿ.

ಪ್ರಬಂಧದ ಸಾರಾಂಶ

ಕರ್ನಾಟಕದ ಪಶ್ಚಿಮ ಘಟ್ಟಗಳಲ್ಲಿ ಸರಾಸರಿ ೨೮೬೯ ಮಿಮೀ ಮಳೆ ಸುರಿಯುತ್ತದೆ ಮತ್ತು ವಿವಿಧ ಅಣಬೆಗಳ ಬೆಳವಣಿಗೆಗೆ ಸರಿಹೊಂದುವ ವಾತಾವರಣ ಒದಗಿಸುವ ಭಾರತದ ಅತ್ಯಂತ ಶ್ರೀಮಂತ ಜೀವವೈವಿಧ್ಯತೆಯ ಪ್ರದೇಶಗಳಲ್ಲಿ ಒಂದಾಗಿದೆ. ಪ್ರಸ್ತುತ ಅಧ್ಯಯನದಲ್ಲಿ ಹನ್ನೊಂದು ಅಣಬೆಗಳನ್ನು ಸಿದ್ದಿ, ಆದಿವಾಸಿ ಮತ್ತು ಅಲಕ್ಕಿ ಬುಡಕಟ್ಟು ಸಮುದಾಯದ ಸಹಾಯದಿಂದ ಮುಂಗಾರಿನ (ಜೂನ್ ಸೆಪ್ಟೆಂಬರ್) ಅವಧಿಯಲ್ಲಿ ಸಿದ್ದಾಪುರ, ತೀರ್ಥಹಳ್ಳಿ ಮತ್ತು ಆಗುಂಬೆ ಅರಣ್ಯ ಪ್ರದೇಶದಲ್ಲಿ ಸಂಗ್ರಹಿಸಲಾಯಿತು. ಸಂಗ್ರಹ ಸಂದರ್ಭದಲ್ಲಿ ಕ್ಷೇತ್ರ ಮಾಹಿತಿ ದಾಖಲಿಸಿಕೂಂಡು, ಅವುಗಳನ್ನು ಡಬ್ಲ್ಯುಜಿಎಮ್ -೧, ಡಬ್ಲ್ಯುಜಿಎಮ್ -೨, ಡಬ್ಲ್ಯುಜಿಎಮ್ -೩, ಡಬ್ಲ್ಯುಜಿಎಮ್ -೪, ಡಬ್ಲ್ಯುಜಿಎಮ್ -೫, ಡಬ್ಲ್ಯುಜಿಎಮ್ -೬, ಡಬ್ಲ್ಯುಜಿಎಮ್ -೭, ಡಬ್ಲ್ಯುಜಿಎಮ್ -೮, ಡಬ್ಲ್ಯುಜಿಎಮ್ -೯, ಡಬ್ಲ್ಯುಜಿಎಮ್ -೧೦ ಮತ್ತು ಡಬ್ಲ್ಯುಜಿಎಮ್ -೧೧ ಎಂದು ಗುರುತಿಸಲಾಯಿತು. ನಂತರ ಈ ಅಣಬೆಗಳನ್ನು ಅಮೇರಿಕದಾ ರಾಷ್ಟ್ರೀಯ ಜೀವತಾಂತ್ರಿಕ ಶಸ್ತ್ರದ ಮಾಹಿತಿ ಕೇಂದ್ರದಲ್ಲಿಯ (ಎನ್. ಸಿ. ಬಿ. ಐ) ದತ್ತಮಾಹಿತಿ ವಿಶ್ಲೇಷಣೆ ಆಧಾರದ ಮೇಲೆ ಗುರುತಿಸಲಾಯಿತು. ಅನುಕರಣೆ ಹೋಲಿಕೆಯನ್ನು ಆಧರಿಸಿ, ಈ ಅಣಬೆಗಳನ್ನು ಲೆಂಟಿನಸ್ ಸ್ಕ್ವರಸುಲಸ್ (ಡಬ್ಲ್ಯುಜಿಎಮ್ -೧), ಪ್ಲ್ಯುರೊಟಸ್ ಸಾಲಮ್ನಿಯೊಸ್ಟ್ರಾಮಿನಸ್ (ಡಬ್ಲ್ಯುಜಿಎಮ್ –೨), ಟರಮಿಟೊಮ್ಯೆಸಿಸ್ ಎಸ್ಪಿ (ಡಬ್ಲ್ಯುಜಿಎಮ್ –೩), ಟರಮಿಟೊಮ್ಯೆಸಿಸ್ ಎಸ್ಪಿ. (ಡಬ್ಲ್ಯುಜಿಎಮ್ –೪), ಲ್ಯುಕ್ಯಾಅಗಾರಿಕಸ್ ಪರಪ್ಯೆರಿಯೊಲಿಲೆಸಿನಸ್ (ಡಬ್ಲ್ಯುಜಿಎಮ್ –೫), ಟ್ರೈಕಲಸ್ಪೊರಂ (ಡಬ್ಲ್ಯುಜಿಎಮ್ –೬) ಅಗ್ರೋಸೈಬ ಪಿಡಿಯೆಡ್ಸ್ (ಡಬ್ಲ್ಯುಜಿಎಮ್ –೭), ಲ್ಯುಕೊಕೊಪ್ರಿನಸ್ ಬಿರ್ನೊಬೋಮ್ಸಿ (ಡಬ್ಲ್ಯುಜಿಎಮ್ –೮), ಪೊಡೊಸ್ಯೆಪ ಪೆಟಾಲೊಡ್ಸ್ (ಡಬ್ಲ್ಯುಜಿಎಮ್ –೯), ಕ್ಲೆಲೆರಿಯ ಎಸ್ಪಿ. (ಡಬ್ಲ್ಯುಜಿಎಮ್ –೧೦) ಮತ್ತು ಅನ್ಯೊಡಿಯ ಸಿರಿಯಾಲಿಸ್ (ಡಬ್ಲ್ಯುಜಿಎಮ್ –೧೧) ಎಂದು ಪತ್ತೆಹಚ್ಚಲಾಯಿತು. ಈ ಹನ್ನೊಂದು ಅಣಬೆಗಳಲ್ಲಿ ಲೆಂಟಿನಸ್ ಸ್ಕ್ವರಸುಲಸ್, ಪ್ಲ್ಯುರೊಟಸ್ ಸಾಲಮ್ನಿಯೊಸ್ಟ್ರಾಮಿನಸ್, ಟರಮಿಟೊಮ್ಯೆಸಿಸ್ ಎಸ್ಪಿ, ಟರಮಿಟೊಮ್ಯೆಸಿಸ್ ಎಸ್ಪಿ ಮತ್ತು ಲ್ಯುಕ್ಯಾಅಗಾರಿಕಸ್ ಪರಪ್ಯೆರಿಯೊಲಿಲೆಸಿನಸ್ ಖಾದ್ಯ ಪ್ರಭೇದಗಳಿಗೆ ಸೇರಿವೆ. ಇವುಗಳಲ್ಲಿ ಲೆಂಟಿನಸ್ ಸ್ಕ್ವರಸುಲಸ್ (ಡಬ್ಲ್ಯುಜಿಎಮ್ –೧) ಮತ್ತು ಟರಮಿಟೊಮ್ಯೆಸಿಸ್ ಎಸ್ಪಿ. (ಡಬ್ಲ್ಯುಜಿಎಮ್–4) ಎರಡು ಅಣಬೆಗಳನ್ನು ಆಲೂಗಡ್ಡೆ ಅಗಾರ್ ಮಾಧ್ಯಮದಲ್ಲಿ ಅಂಗಾಂಶ ಕೃಷಿ ಮಾಡಿದಾಗ ಮೈಸೀಲಿಯಾವನ್ನು ಉತ್ಪತ್ತಿ ಮಾಡಿರುತ್ತವೆ ಮತ್ತು ಇವುಗಳನ್ನು ಸಂರಕ್ಷಿಸಲಾಗಿದೆ.

ಆಪ್ರೀಲ್, ೨೦೧೫
ಜೈವಿಕ ತಂತ್ರಜ್ಞಾನ ವಿಭಾಗ
ಕೃಷಿ ವಿಶ್ವವಿದ್ಯಾಲಯ, ಜಿ.ಕೆ.ವಿ.ಕೆ,
ಬೆಂಗಳೂರು–೬೫

ಎನ್. ಈರಣ್ಣ
ಮುಖ್ಯ ಸಲಹೆಗಾರರ

"Ethnobotanical survey and molecular characterization of mushrooms from Western Ghats of Karnataka".

Santhosh D.B ., Earanna .N and Nandini .k
Department of Plant Biotechnology, GKVK, Bengaluru.

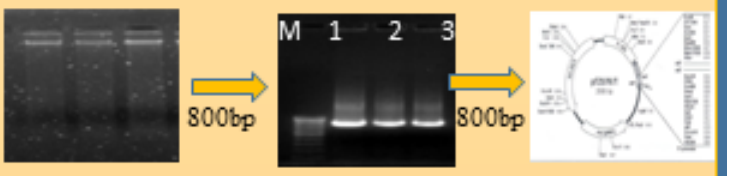
Introduction: Western Ghats mountain ranges cover a length of around 1600 km. This bioregion is highly rich with flora and fauna and is considered as one of the 34 biodiversity hot spots of the world. The stretch of Central Western Ghats ranges from 12° to 14° covering areas of Coorg, Hassan, Chikmangalur, Shimoga up to south of Uttara Kannada (Bhat., *et al.*, 2012).

Objective

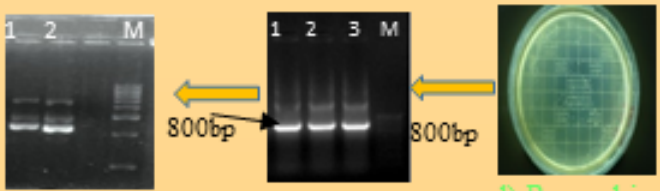
1) Ethnobotanical survey and collection of wild mushrooms
2) Identification of the wild mushrooms based on morphological and molecular characters(ITS/18S rRNA sequencing)
3) Invitro culturing of edible mushrooms

Materials and methods:
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



a) Genomic DNA b) PCR Amplification of ITS Sequence c) T/A Cloning Vector



f) Plasmid e) Colony PCR To Confirm Colonies d) Recombinent Colonies

Discussion:
To document mushroom diversity in the Western Ghats of Karnataka systematic survey is needed (Meera P and Veena SS, 2012). Among the 14 species 3 are identified as edible mushrooms and remaining are non edible mushrooms. This study shows large number of mushroom diversity available in western ghats region of Karnataka. Edible mushrooms were cultured and cultivated for commercial production.

Summary: Fourteen mushrooms were documented from western ghats of Karnataka and identified to species level using 18SrRNA/ITS gene sequence . There were three edible mushrooms and remaining are non-edible mushroom species.

Advisory committee:
Chairman
Dr. N. Earanna

Members
Dr. K.M Harini kumar
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Dr. Mallesh

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I. INTRODUCTION

"The only source of knowledge is experience"

-Albert Einstein

Western Ghats -also known as Sahyadris is one of the world's ten hottest biodiversity hotspots and has four major forest types, 23 floristic types and unique high altitude grasslands. It also contains more than 30 percent of India's species diversity (Daniel, 1997). The Western Ghats mountain ranges cover a length of around 1600 km through the states of Maharashtra, Goa, Karnataka, Tamil Nadu, Kerala ending at Kanyakumari and situated in 8° N to 21° N latitudes and 73° E to 77° E longitudes. The range runs north to south along the western edge of the Deccan Plateau, and separates the plateau from a narrow coastal plain, called Konkan, along the Arabian Sea. Mean temperature range from 20 °C (68 °F) in the south to 24 °C (75 °F) in the north. During the monsoon season from June to September, the unbroken Western Ghats chain acts as a barrier to the moisture laden clouds. Rainfall in this region averages 3,000–4,000 mm (120–160 in) with localized extremes touching 9,000 mm (350 in). The eastern region of the Western Ghats which lie in the rain shadow, receive far less rainfall averaging about 1,000 mm (40 in) bringing the average rainfall figure to 2,500 mm (150 in), and this bioregion is highly rich with flora and fauna (Bhat, *et al.*, 2012).

The stretch of Central Western Ghats ranges from 12° to 14° covering areas of Coorg district, Hassan, Chikkamangalur, Shimoga up to south of Uttara Kannada. Shimoga district is situated in the heart of the Western Ghats region, which is one of the 'hot-spots of biodiversity' in India. This district comes under south-eastern transitional zone and situated between 13°27' and 14°39' latitude and between 74°37' and 75°52' E longitude in about the mid-South Western parts of the Karnataka State. The district receives an average annual rainfall of 2869 mm (Bhat, *et al*., 2012). The cool and humid climate made ideal habitat for different kinds of mushrooms.

Mushrooms are epigeous and hypogeous fleshy fruiting bodies of fungi belonging to Basidiomycetes and certain Ascomycetes. Mushrooms have been used as food and medicine by the ancient Egyptian, Greek, Roman and Chinese civilizations. These fungi had attained the status of a regular crop in France and China by 17^{th} and 19^{th} centuries, respectively, spreading gradually to the other countries in few years. There are about 69 thousand known mushroom species of which 2000 species from more than 30 genera are regarded as prime edible mushrooms but 80 of them are grown experimentally and around 20 are cultivated commercially (Chang and Miles, 1991). Today, mushrooms are liked all over the world due to their delicate taste, flavor and health promoting properties. Mushrooms contain good quality protein, unsaturated fatty acids, minerals and vitamins (Wahid *et al.*, 1988). These are low in fat, carbohydrates, salts and rich source of dietary fiber (Genders, 1990).

The species diversity of fungi and their natural beauty occupy prime place in the biological world and India has been a cradle for these species. Defining the number of fungi on earth has been a point of discussion and several studies have focused on

enumerating the World fungal diversity (Crous, 2006). Only a fraction of total fungal wealth has been subjected to scientific scrutiny and mycologists continue to unravel the unexplored and hidden wealth. One third of fungal diversity of the globe exists in India and of this only 50 % are characterized so far (Manoharachary *et al.*, 2005).

Mushrooms are ephemeral and disappear within a day. Therefore, documentation of mushrooms needs constant survey during appropriate season. Mushrooms can be identified based on their morphological and molecular characters. The Phenotypic characters include the shape, size, texture, colour and odour of the fruiting body. 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 or membranous. Gills are situated below 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 upon 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 mushroom 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 DNA. 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. In the Western Ghats of Karnataka, even though different kinds of mushrooms are available; very few mushrooms have been documented (Meera pandey and Veena, 2012). However, there are plenty of mushrooms (both edible and medicinal) yet to be documented. Further, isolation of potential species into pure culture and their conservation for future

experimentation is essential for commercialization. Therefore, the present investigation was undertaken with the fallowing objectives.

1. Ethno-botanical survey and collection of wild mushrooms from some parts of Western Ghats region of Karnataka.
2. Identification of mushrooms based on morphological and molecular characterization (ITS).
3. *In-vitro* culturing and conservation of edible mushroom species.

II. REVIEW OF LITERATURE

The term mushroom is most often used to describe the reproductive structure of the fungus which is also known as fruiting body or sphorophore. The mushrooms belong to the classes Ascomycetes and Basidiomycets. The fruiting body of the ascomycetes and basidiomycetes vary greatly in detail and design. Therefore, based on the structure and design of their fruiting body they are named as gilled mushrooms, polyphores, earth stars, cup fungi, puff balls, coral fungi etc., and each kind of mushroom reproduce within a certain spectrum of humidity and temperature.

Man has been hunting the wild mushrooms for food since antiquity. Thousands of years ago, fructifications of higher fungi have been used as a source of food due to their attractive flavor and taste. During the early days of civilization, mushrooms were consumed mainly for their palatability and unique flavors (Rai, 1997).Mushrooms are efficient degraders of lignocelluloses, hence they found vital role in biodegradation. In addition, many kinds of mushroom have pharmaceutical value such as antimicrobial, anticancer, antioxidants which are known as medicinal mushrooms.

Rolfe and Rolfe (1925) mentioned that the mushrooms like *Agaricus campestris*, *Morchella esculenta*, *Helvella crispa*, Hydnum *coralloides*, *Hypoxylon vernicosum* and *Polyporus mylittae* were used as food much earlier in India. Gupta (1986) reported that the mushroom can substantiate malnutrition as they contain protein, vitamins and essential amino acids. Mushrooms can be produced in large quantities in a short time and provide more protein per unit area than other crops. However, thousands of mushroom species known worldwide, only about 20 species were cultivated commercially (Chang, 1990).

Mushrooms are recognized as an alternative source of good quality protein and are capable of producing highest quantity of protein per unit area and time from the worthless agro-wastes (Chadha and Sharma, 1995). Mushroom was used as food and food supplements from ancient times. They are increasingly being recognized as one of the important food items for their significant roles in human health, nutrition and diseases (Chang and Buswell, 1996).

2.1 Nutritional value of mushrooms

Mushrooms are good source of high quality protein. They contains 20-35% protein (dry wt. basis) which is higher than vegetables and fruits and is of superior quality. They are rich in lysine and tryptophan, the two essential amino acids that are deficient in cereals. Mushrooms are also called white vegetables or "boneless vegetarian meat". Protein is an important constituent of dry matter of mushrooms. The fruiting bodies of mushrooms are characterized by a high level of well assimilated mineral elements. Major mineral constituents in mushrooms are K, P, Na, Ca, Mg and trace elements like Cu, Zn, Fe, Mo, Cd (Chang and Buswell,1996).

Bano *et al.* (1963) determined the nutritive value of *Pleurotus flabellatus* as 0.974% ash, 1.084% crude fibre, 0.105% fat, 90.95% moisture, 0.14% non-protein

nitrogen and 2.75% protein. Bano (1976) suggested that food value of mushrooms lies between meat and vegetables. Orgundana and Fagade (1981) indicated that an average mushroom is about 16.5% dry matter out of which 7.4% is crude fibre, 14.6% is crude protein and 4.48% is fat and oil.

Edible mushrooms were highly nutritional and compared favorably with meat, egg and milk (Gruen and Wong, 1982). Verma *et al.* (1987) reported that mushrooms are very useful for vegetarian because they contain some essential amino acids which are found in animal proteins. The digestibility of *Pleurotus* mushroom proteins is as that of plants and meat. (Bano and Rajarathnam, 1988).

Nanba (1993) reported that due to high amount of proteins, they can be used to bridge the protein malnutrition gap. Mushrooms as functional foods, used in nutrient supplement to enhance immunity. Due to low starch content and low cholesterol, they suit diabetic and heart patients. One third of the iron in the mushrooms is in available form.

Buswell (1996) reported that mushrooms can provide balancing diet in sufficient quantities for human nutrition, and contain medicinal compounds. They are rich in crude fibre and protein. In fact, mushrooms also contain low fat, low calories and good vitamins and many mushrooms possess multi-functional medicinal properties.

Mushrooms are low in calories, carbohydrates, calcium and sodium. They contain high proportion of unsaturated fat but virtually no harmful lipid or cholesterol. They contains a large amount of vitamins such as Thiamine 1.4-2.2 mg (%), Riboflavin 6.7-9.0 mg (%), Niacin 60.6-73.3 mg (%), Biotin, Ascorbic acid 92-144 mg (%), Pantothenic acid 21.1-33.3 mg (%) and Folic acid 1.2-1.4 mg/100g in dry weight basis (Hossain *et al.*, 2007).

Alam *et al*., (2007) reported that minerals that found in mushroom are Calcium, Iron, Manganese, Magnesium, Zinc and Selenium. Mushroom has thousands of variety to enrich the food basket of human health. Mushroom is an alternative rich source of meat, fish, vegetables, fruits. Mushroom's nutritional value proves to have many health benefits. Mushrooms contains more protein, in comparison to other animal and plant food, low carbohydrate, and that too is not in the form of starch rather than in the form of glycogen, zero fat and adequate vitamins and minerals which is higher than vegetables and fruits and is of superior quality. They are good source of high quality fibers and low calorie food (Kakon *et al*., 2012).

2.2. Medicinal value of mushrooms

Mushrooms offer tremendous applications as they can be used as food and medicines besides their key ecological roles. They represent as one of the world's greatest untapped resources of nutrition and palatable food of the future. Mushrooms have been found effective against cancer, cholesterol reduction, stress, insomnia, asthma, allergies and diabetes (Bahl, 1983). Tam *et al*., (1986) reported that aqueous extracts of *Pleurotus sajorcaju* proved good in correction of renal failure. The first successful

research discovered that the antitumor effect of the hot water extracts from several mushrooms (Ikekawa *et al*., 1969).

Mushrooms have been used in medicine since from the Neolithic and Palaeolithic eras (Samorini, 2001). Although mushrooms as medicine have been used in China since 100 A.D. (Gunde-Cimmerman, 1999), but it was only in 1960 that scientists investigated the basic active principles of mushrooms which are health promoting. Their polysaccharide content is used as anticancer drug. Even, they have been used to combat HIV effectively (King, 1993). Mushrooms have been used in health care for treating simple and age old common diseases like skin diseases to present day complex and pandemic disease like acquired immunodeficiency syndrome (AIDS). Mushrooms in the twentieth century are well known to people all over Asian countries as an important bio-source of novel secondary metabolites. In India, particularly the alternative systems of medicine, utilize the curative properties of mushrooms. The secondary metabolites of these mushrooms are chemically diverse and possess a wide spectrum of biological activities, which are explored in traditional medicines (Rai *et al*., 2005).

Numerous compounds have been isolated from mushrooms and have great potential for development as mushroom nutraceutical and pharmaceutical products. Among these compounds, water soluble polysaccharides and proteoglycans, proteins, and various constituents of small molecular mass are considered to have immunomodulatory potential by regulating several types of immune cells that function in antitumor or antimicrobial activities, including dendritic cells, macrophages, cytolytic T cells, and NK cells (Yu *et al*., 2009).

Caglarirmak (2011) reported that edible mushrooms are having essential compounds and functional substances for human health. Mushrooms also contain bioactive components including β-glucans and chitin.

2.3. Antioxidant activity of mushrooms

Antioxidants are chemical compounds that protect cells from the damage caused by unstable molecules known as free radicals. Free radicals are powerful oxidants and those chemical entities that contain unpaired electrons. They are capable of randomly damaging all components of the body, viz. lipids, proteins, DNA, sugars and are involved in mutations and cancers (Przybytniak *and Ambroz*, 1999). Extracts from fruiting bodies and mycelia of *Ganoderma lucidum, Phellinus rimosus* and several *Pleurotus sp.* occurring in South India were found to possess antioxidant activity with high free radical scavenging activity (Jones and Janardhanan, 2000; Ajith and Janardhanan, 2001; Lakshmi et al., 2003).

The nascent oxygen is trapped by enzymes like superoxide dismutase, catalase and glutathione peroxidase. Over production of free radicals creates oxidative stress. The antioxidants are an important defense of the body against free radicals and the mushrooms were found rich sources of these antioxidants (Mau *et al*., 2004; Puttaraju *et al.,* 2006; Ferreira *et al*., 2007; Oyetayo *et al.,* 2012).

Mushrooms are known to be rich sources of various bioactive substances like antibacterial, antifungal, antiviral, antiparasitic, antioxidant, anti-inflammatory, anti-proliferative, anticancer, anti-tumour, cytotoxic, anti-HIV, hypo-cholesterolemic, antidiabetic, anticoagulant, hepato-protective compounds, among others (Lindequist et al., 2005; Ajith and Janardhanan, 2007).

Antioxidant and phytochemical properties of ethanolic extracts from the wild edible mushroom *Termitomyces reticulatus* and their individual parts (Cap and Stipe) were evaluated through the reducing power, β-carotene bleaching, ABTS and DPPH radicals scavenging activity methods. Antioxidant components like total phenol, flavonoid, β-carotene and lycopene were also determined and the amount of phenol was correlated with the antioxidant property. All the extracts showed potent antioxidant activities, in which the entire mushroom extract showed more antioxidant property than other two mushroom extracts compared (Loganathan *et al*. 2010). Antioxidant activity and bioactive compounds from six wild mushrooms (*Lycoperdon perlatum, Clavaria vermiculris, Ramaria formosa, Marasmius oreades,*) of Western Ghats of Karnataka, India were reported by Ramesh and Pattar (2010).

Kumari *et al*. (2011) investigated the antioxidant activity of *Cantharellus friessi, Cantharellus subcibarius, Cantharellus cinerius* and *Pleurotus florida* collected from North-Western Himalayan region of India including their bioactive compounds such as phenol, flavonoid, ascorbic acid and β-carotene. Among them *C. friessi* showed significantly higher antioxidant activity through β-carotene bleaching method and high phenol content (16.80 mg/g) than the other mushroom species.

2.4. Biodegradation

Lignin is the most abundant natural aromatic polymer on earth and degradation of this recalcitrant aromatic polymer is caused in nature by white rot fungi through a process that was defined as an enzymatic combustion (Kirk and Farrell (1987). The ligninolytic system is an extracellular enzymatic complex that includes peroxidases, laccases and oxidases responsible for the production of extracellular hydrogen peroxide (H_2O_2) (Ruiz-Duenas and Martinez, 2009).

Cho *et al*. (2009) reported that Basidiomycete's species are considered to be a very interesting group of fungi given their exceptional adjustment abilities to accommodate detrimental conditions of the environment where they continue to act as natural lignocellulose destroyers and include very different ecological groups such as white rot, brown rot, and leaf litter fungi. Doriv Knop *et al*., (2014) reported that the versatile peroxidases and manganese peroxidases (MnPs) are components of the complex ligninolytic system found in different *Pleurotus sp.* as well as other white-rot fungi.

2.5. Ethanomycological survey and documentation

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 of members of *Phallales* to be edible by

natives of North America. Manjula (1983) reported that the best collection of 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).

North-Western Himalaya has been the centre of intensive research on higher fungi since the 1950s. An edible species of *Agaricus,* namely *Kbasianulosus* was first reported from Punjab of North-West Himalaya by Paracer and Chahal (1962). Purkayastha and Chandra (1974) collected two edible mushroom species from West Bengal namely *Calocybe indica* and *Termitomyces eurhizus.* Ten species of *Calvatia and Lycoperdon* have been incorporated in the list of edible fungi. Out of the 10 species described, 7 were edible in their immature stage. Among seven edible mushrooms, 3 species belong to *Calvatia,* 2 belong to *Lycoperdon* and one each to *Geastrum* and *Bovista* (Gupta *et al*., 1974).

Collection and scientific study of mushrooms in India really began during the 19th century and continued till date (Kaul, 2002). The period can be divided into three phases. The first phase lasted from 1825 to 1899 and in addition to Berkeley and Montagne, recorders during this phase included Fries, Leveille, Currey, Cooke, Massee, Watt and Lloyd (Sathe, 1979; Natarajan, 1995). The second phase (1900-1969) started with Paul Henning's significant contributions which have described another 32 genera and 68 species from India (Natarajan, 1995). A significant feature of the second phase was the involvement, besides European and American workers, of a number of Indian workers in research on higher fungi (Sathe, 1979).

The number of species of *Coprinus, Morchella, Pleurotus, Lycoperdon, Calvatia* and *Helvella* were studied by Professor Watling from Edinburgh, UK, who, besides providing constant guidance to these workers, published a list of 119 species of higher fungi from the Kashmir valley, based on his personal collection (Watling and Gregory, 1980).

Special mention should be made of the work on Indian fungi by E.J. Butler at Pusa (Bihar) in the post of Imperial Mycologist who has produced the first authoritative list, *Fungi of India,* in collaboration with G.R. Bisby (Butler and Bisby, 1931). Berkeley in 1876 was probably the first to report higher fungi from the Kashmir valley. Later, T. N. Kaul and his group provided fragmentary records on higher fungi in the late 1960s working at the Regional Research Laboratory, Srinagar and Kashmir. Due to sustained work carried out by Kaul, Kapoor and Abraham from Northern India, 262 higher fungi have been recorded from Kashmir valley, among which 226 taxa were agarics (Abraham, 1991).

Several reports on higher fungi and mushroom have been conducted from northern India which includes North Western region, Eastern Himalaya proper and North-Eastern hilly areas. North western region of India includes Punjab, Haryana,

Chandigarh and Gujarat while the Eastern Himalaya proper includes the northern parts of Assam, the whole of Arunachal Pradesh and Sikkim, and North-Eastern covers the hilly states of Nagaland, Meghalaya, Manipur, Mizoram and Tripura (Khoshoo, 1992).

Singh, (1994) reported that a total of 173 species belonging to 95 genera were recorded from north India. Most of the genera were Gasteromycetes or Aphyllophoroid taxa. Special mention should be made of two edible Gasteromycetes, *Phellorinia inquinans* Berk and *Podaxis pistillaris*, tonnes of which can be collected from desert areas. *P. inquinans*is associated with sand dunes in the area.

In India the total recorded mushrooms are approximately 850 species (Deshmukh, 2004). There are references to the use of mushrooms as food and medicine in India in the ancient medical treatise, Charaka Samhita (3000±500 BC). However, the scientific study of mushrooms in India started with the identification and description of *Podaxis pistillaris* L. Pers, by Linnaeus in the 18th century which was collected and sent by Koening from Tamil Nadu State. Later, Sir J.D. Hooker made extensive collection mostly from Assam, Darjeeling, Sikkim and Khasi hills which led to the publication of a series of papers by an English mycologist, Revd M.J. Berkeley between 1850 and 1882 (Natarajan, 1995).

The collections were made from 12 agro climatic zones of India in four monsoon seasons and revealed the presence of 134 species of mushrooms (including 14 Gasteromycete species) belonging to 45 genera. Edible fruiting bodies included species of *Termitomyces*, *Volvariella*, *Pleurotus*, *Macro lepiota*, *Boletus* and *Calvatia*. *Tuber magnatum* (Ascomycotina), a highly prized truffle, is regularly collected and consumed by tribal people in the forest area of the southern part of this state (Bhavani Devi, 1995).

Pradeep *et al.* (1998) reported the genus of *Volvariella* for the first time from Kerala. Out of ten species of *Volvariella* collected, *Volvariella nigrodisca, Volvariella taylori, Volvariella apalotricha* and *Volvariella gandiformis* are described and illustrated.

A survey of macro fungi diversity has been conducted by Swapna *et al*., (2008) in semi-evergreen and in moist deciduous forest of Shivamogga District of Karnataka, during 2005 to 2007. They collected 778 species of macro-fungi belonging to 43 families, 101 genera were enumerated of which 242 species were identified to genus level and 73 were identified to species level.

Bhosle *et al*., (2010) reported 15 species and 3 varieties of *Ganoderma lucidum* (of which one variety remains unidentified) from the Western parts of Maharashtra (India) and in their study, only 9 valid *Ganoderma* species have been reported from India.

Karwa and Rai (2010) also surveyed six different zones of Melghat forest of Amravati District, Maharashtra State from July 2005 to December 2008 for the availability of wild edible and medicinal mushrooms. In their study, out of total 153 species, ten species of *Agaricus* were recorded from different localities. Of these, seven species namely *Agaricus bitorquis, A. subrufescens, A. augustus, A. placomyces, A.*

essettei, A. basioanolosus and *Agaricus* sp. are being reported for the first time from this region.

Srivastava *et al.,* (2011) from Gorakhpur forest division collected mushrooms belonging to four species of *Termitomyces* namely, *Termitomyces heimii, Termitomyces clypeatus, Termitomyces mammiformis and Termitomyces microcarpus* and characterized by different morphological traits such as shape of stipe, pileus, margin of fruit body, colour of fruit body, gills, flesh, annulus, pseudorrhiza and spore print.

Biodiversity of mushrooms from Amarkantak Biosphere Reserve, Madhya Pradesh have been reported 52 mushroom samples from Amarkantak region forests which were belonging to different genera out of which only 14 mushroom samples were identified up to species level and others were identified only up to the genus level (Dwivedi *et al*., 2012).

Pushpa and Purushothama (2012) have studied the biodiversity of mushrooms in and around Bangalore (Karnataka), India and recorded 90 species in 48 genera belonging to 19 families in 5 orders. Among them, 28 species were found to be recorded for the first time in India.

Different genera of wild mushrooms were collected from various locations that were grow naturally in different area and at summer season in Dilla University and the collected wild mushrooms were identified to genus and species level on the basis of morphology. These fungi have significant role in recycling of nutrient through decaying (rotting) mechanisms of fallen wood tree and solid waste of agricultural products (Fekadu Alemu., 2013).

2.6. Molecular characterization of mushrooms

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. All DNA markers other than RFLPs are based in some way or other upon the PCR. 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 tools for phylogenetic studies.

Bruns *et al*., (1991) reported that nuclear rDNA and particularly the internal transcribed spacer (ITS) regions are good targets for the phylogenetic analysis of fungi. The ITS regions are often highly variable between isolates of the same species (O' Donnell *et al*. 1988).

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 and 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. This ITS region is most widely used to sequence the DNA region in fungi. It has typically been most useful for molecular systematic at the species level and within the species (Wipf *et al*., 1999).

Singh *et al*., (2003) reported that molecular characterization of specialty mushrooms collected from germplasm accessions from Rajasthan using DNA fingerprinting and rRNA gene sequencing and added two new additions to the Indian Basidiomycetes biodiversity. This is the first kind of report documented from India on molecular characterization of specialty mushrooms.

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) were made a new addition to the Indian mushroom biodiversity by identifying 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).

Oyetayo (2012) Identified 18 *Termitomyces species* collected from 2 states (Ondo and Ekiti) of Nigeria using Internal transcribed spacer (ITS) 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.

Pawlik *et al*., (2012) applied AFLP method for genomic fingerprinting of 21 *Pleurotus* isolates of Asian and European origin. Using one PstI restriction endonuclease and four selective primers in AFLP assay, 371 DNA fragments were generated, including 308 polymorphic bands. AFLP profiles were found to be highly specific and distinguished 21 *Pleurotus* species. They suggest the possible applicability of the AFLP-PstI method is effective identification and molecular characterization of *Pleurotus* sp.

Das *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. A phylogenetic

tree was constructed using neighbor- joining method showing inter relationship between or among the mushroom.

Earanna *et al., (*2013) reported an edible mushroom from the Theerthahalli forest area of Western Ghats of Shimoga district of Karnataka and identified using ITS region of ribosomal DNA sequences. Sequences subjected to BLAST matches showed 98% homology with the *Termitomyces* sp.

2.7. Pure culture isolation

Mushroom cultivation technology is the most suitable technology for creating wealth out of plant, animal and industrial waste. They are abundantly available all over the globe. Mushroom cultivation is the gaining momentum worldwide because of dietary food protein and it is unique in the sense that it is the most efficient and economically valuable technology for conservation of lignocellulose material into high quality protein. Mushroom cultivation could subsidies income of the millions of farmers.

Thakur (1995) reported that Mushrooms are amongst the most popular food items accepted world over. The increased consumer demand over the years has resulted in production of mushrooms in large proportions through cultivation which is highly efficient method for recycling the agricultural residues so as to produce nutritious food (Chang *et al.* 1998).

Mushroom culture involves several different operations, each of which must be carefully performed. Substrate preparation, inoculation, incubation, and production conditions depend on the mushroom species to be cultivated. The first stage involves obtaining pure mycelium of the specific mushroom strain. The mycelium can be obtained from spores and tissue. The mycelium impregnated on cereal grain, e.g., wheat, sorghum, rye, or millet is usually called as "spawn" (Chang and Hayes., 1978). The purpose of using mycelium-coated grain is to facilitate rapid colonization on substrate. The success of mushroom production depends in great part on the quality of "spawn", which must be prepared under sterile conditions to remove contamination of the substrate. Several studies have been done to improve the quality and develop new techniques for its production (Beetz and Kustudia, 2004; Pathmashini *et al*. 2008).

Production of *G. frondosa* is usually on a substrate contained in polypropylene bottles or bags. A common substrate used for production is composed of sawdust supplemented with rice bran or wheat bran. It has also been produced on a substrate consisting of oak sawdust, wheat bran, millet, and rye. This formula gave the highest yields, best quality, and shortest crop cycle time (12 weeks). For bottle production, the containers are filled with moistened substrate and sterilized or pasteurized prior to inoculation. For production in bags, the moistened substrate is filled into micro filtered polypropylene bags and sterilized. After cooling (16 to 20 h), the substrate is inoculated, and the bags are heat-sealed and shaken to uniformly distribute the spawn throughout the substrate. Spawn runs last about 30 to 60 days depending on strain and substrate formulation. After primordia formation, two holes usually are cut in the bags exposing the developing primordia that tend to develop around the outside perimeter of the

substrate surface. The top of the bag is then folded over, exposing only the developing primordia to the fruiting environment (Royse, 2007).

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, different media like, PDA, YM, Henner berg, Hamada, and Glucose peptone were used. The Czapek Dox, Hoppkins, Glucose tryptone, and Lilly media were found most unfavorable for growth.

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 *et al.*, (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 fibre, potato dextrose agar, Yeast agar and found varied preference of individual species. Among the above, the *P. sajor caju* 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).

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).

III. MATERIAL AND METHODS

The ethnobotanical survey was under taken for documentation and molecular characterization of wild mushrooms occur in the Western Ghats region of Karnataka. The collected mushrooms were identified by using ITS gene sequence. The details of material used and methods followed are described in this chapter.

3.1. Ethnobotanical survey

In order to document the wild mushrooms, Extensive survey was carried out in the Western Ghats forest area of Karnataka including Shimogha, Siddapura, Agumbe and Theerthahalli for four months (June-September 2013) during the rainy season as rainy season provides congenital climatic conditions for growth and development of mushrooms so as to get maximum number of different kinds of mushrooms species. The survey was carried out with the help of information provided by tribal communities like Adivasis, Halakki vokkals and Siddis in the locality during the visits as they are familiar with mushroom types and time/season of their appearance. The mushroom samples were collected in paper bags and field notes like date, weather condition, abundance, habitat and phenotypic characters were recorded.

3.2. Morphological characters

Mushrooms are fleshy, spore bearing fruiting body of fungus. Thirteen mushrooms were collected from Western Ghats forest area including Shimoga, Siddapura, Agumbe and Theertha halli 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.

3.3. Molecular characterization

Genomic DNA Isolation

Total genomic DNA from the cap tissue of the mushroom fungus was extracted using CTAB lysis buffer. A dried tissue (0.2 g) of the mushroom sample was ground into fine powder using liquid nitrogen and sample was transferred into 2ml 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: Iso-amyl alcohol (24:1 V/V) was added and mixed by inverting and the tubes. These tubes were 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.

PCR amplification

PCR components (40 µl)

- 4.0µl of 10 X PCR Taq. Buffer
- 4.0µl of 10 mM dNTP's mix
- 2.0 µl of 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 ITS region of the wild mushrooms.

Primers	Sequence	Reference
ITS1 ITS4	5' TCCGTAGGTGAACCTGCGG 3' 5' TCCTCCGCTTATTGATATGC 3'	Sasidara and Thirunalasundari (2012)

Procedure

The genomic DNA extracted was checked for purity using UV spectrophotometer at 260/280nm wave length. Concentration was measured using nano drop (eppendorff). Then 50-100ng DNA was used for PCR amplification in a 40µl 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 3min, 40 cycles of denaturation of 94°C for 1 min, annealing at 60°C for 30sec 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.

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). Five µl 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.

Gel elution

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

Gel slice containing the DNA fragment was excised using a clean 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 100µl 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. Empty tube was again centrifuged for 60 seconds 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 12,000rpm 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 the purity and concentration of the 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 the ligation reaction.

Protocol

The eluted ITS region was ligated using the following components of Thermo Scientific (TA clone PCR Cloning Kit) in 0.5ml microfuge tube.

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

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

3.4.2 Transformation

Transformation of cloned gene sequence of mushrooms was done by using *Escherichia coli* competent cells (DH-5α). DH-5α is the most frequently used *E. coli* strain for routine cloning.

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 (5bromo-4chloro-3indolyl-β-D-galacto pyranoside) an analog of lactose, IPTG (Iso propyl thio galactoside) were pre warmed in a 37^0C incubator for 20 min. T-solution A and T-solution B were mixed by 250µl of each in a new centrifuge tube.

Overnight grown *E. coli* DH5α cells (150µl) were 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 re-suspended in 300 µl of T-solution and incubated on ice for 5 minutes, centrifuged at 12,000 rpm for 1 minute and supernatant was discarded. Then 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 spreads on LB agar dispensed plates supplemented with ampicillin (100mg/ml), X-gal (50mg/ml), IPTG (50mg/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 (20µl)

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

Procedure

Master mix of the 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 30sec 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 Trans-illuminator and photographed.

3.5 Plasmid Isolation

Upon confirmation with the colony PCR, recombinant white colonies were inoculated into 5 ml of LB broth with 5µl of ampicilin (100mg/ml) and incubated in shaker for 10 hours at 37^0C. After 10 hours plasmid was isolated using the Gen Elute^{TM} HP Plasmid Mini Prep kit (Sigma Aldrich) following the manufacturers protocol.

Procedure:

Three ml of recombinant *E.coli* culture was pelleted by centrifuging at 12,000 rpm for 60 seconds. The supernatant was discarded and the pellet was re-suspended in 200 µl of re-suspension solution and mixed thoroughly. The re-suspended cells were lysed by adding 200µl of lysis solution and mixed gently by inversion (6-8times). The

cell debris was precipitated by adding 350µl 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 500µl of optional wash solution was added and centrifuged at 12000 rpm for 60 seconds and flow through was discarded. Then 750µl 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 pre washed column was centrifuged to remove excess ethanol. The column was transferred to a fresh tube. Into it 50µl 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 11 plasmid samples were got sequenced by Sci-Genome 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 Super computer 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 Western Ghats region were collected in paper bags and brought to the laboratory. Stem/Pileus tissue was cleaned and outer layer was peeled off using razor/sharp knife. 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, the piece of sterilized tissue was inoculated/placed on Potato Dextrose Agar medium (PDA medium) dispensed plates. These plates were incubated at room temperature till the growth of the mycelium produced from the tissue. After mycelial growth formation, the mycelium was transferred on fresh PDA slant and incubated for 7 days for growth. PDA slants were preserved in refrigerator.

IV. EXPERIMENTAL RESULTS

Field survey was made to document the wild mushrooms in forest area of Shimoga district of Karnataka from June to September 2013. Eleven mushroom species were documented and identified by using Internal Transcribed Sequence (ITS) region. Results obtained during the study are presented here under.

4.1. Molecular identification of wild mushrooms by ITS gene sequencing

Molecular identification tool is one of the recent origins to identify the prokaryotic and eukaryotic organisms. In the present study molecular identification was done using ITS region sequence for identification of different mushroom species. Expected amplicon size is 500bp to 800bp. The full length sequence homology was searched in website, National Centre for Biotechnology Information (NCBI) (*htpp://www.ncbi.nlm.nih.gov/BLAST*) (Altschul *et al*., 1990) and mushrooms were identified into species level based on their homology.

WGM-1: This mushroom was collected from Siddapura forest area of Shimogha district and it is vernacularly known as Mara anabe. The fruiting body was grown scatterly on dead and decaying wood. The cap was brown colour, funnel shaped and the stipe is white in colour. The length of the stem was 6 cm and diameter of cap was 3.5 cm. Gills are decurrent, annulus are absent and hard textured. ITS region was amplified using ITS-1 and ITS-4 primer and sequenced. The sequence data showed 97% homology with the earlier reported *Lentinus squrossulus* (Fig. 1). Thus it was identified as *Lentinus squrossulus* (fig-1). It is an edible species.

WGM-2: The mushroom was collected from Theerthahalli forest area of the Shimogha district. Vernacular name of the mushroom is Henne anabe, it was growing gregariously on the decaying branch of a tree. Pelius was white in colour, soft textured. The diameter of the cap was 11 cm, gills were adnate, annulus absent. The amplified sequence of ITS region with ITS-1 and ITS-4 primer showed 99% homology with the earlier reported *Pleurotus salmoneostramenius* (Fig. 2). Based on the homology, this mushroom was confirmed as *P. salmoneostramenius.*

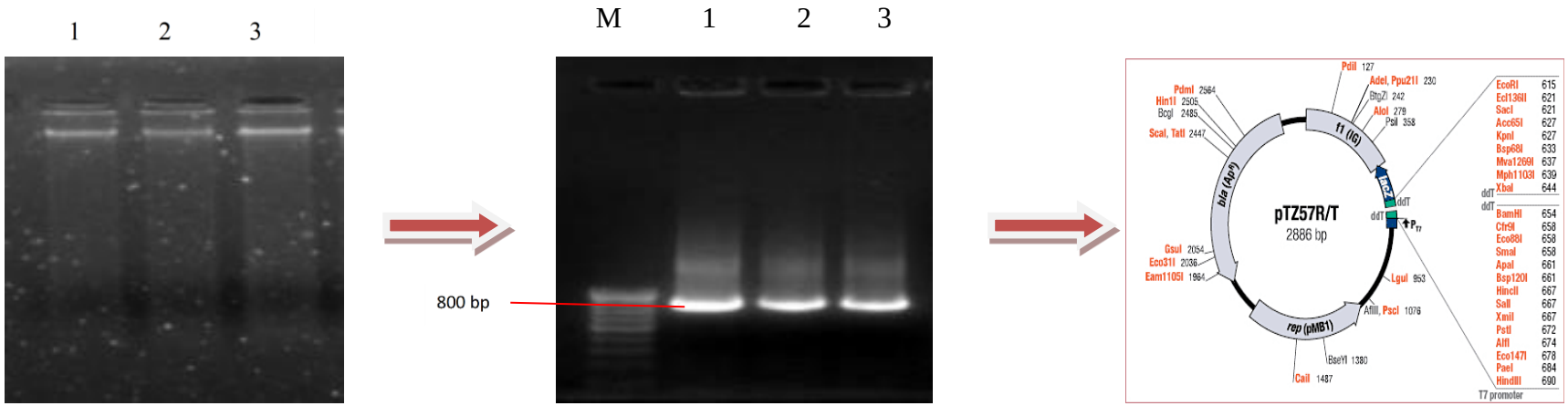
WGM-3: This mushroom was collected from Siddapura forest area of Shimoga district. The mushroom was popularly called as Beru anabe by rural folk. It grows gregariously on highly humus soil and near the termite mounds. The fruiting body was soft and crispy. It produces long stipe (15-25cm). The stipe position was central and annulus present. ITS region amplified with ITS-1 and ITS-4 primer showed 99% homology with the earlier reported *Termitomyces species* (Fig. 3). Hence, it is confirmed as *Termitomyces species*, it is an edible mushroom.

WGM-4: The fruiting body of mushroom was collected from Agumbe Ghat of Shimoga district. It is an edible mushroom. In Kannada, it is commonly called as Huthada anabe. July is the best month for this mushroom. It has a large cap with rusty brown colour. The mushroom was very big and grows vigorously on highly fertile soil. Stipe position is central and length 12cm. Gills are free and annulus present. ITS region

Table 1: Field characters of mushrooms collected from parts of Western Ghats region of Karnataka

Sl. No.	Mushroom collected	Place of collection	Vernacular name	Date of collection	Edibility	Habitat	Abundance	Growth habit
1	WGM-1	Siddapura, Shimoga district.	Mara anabe	22/09/2013	Edible	Tree	Seven	Scattered
2	WGM -2	Theerthahalli Shimogha district.	Henne anabe	18/09/2013	Edible	Branch of tree	Group	Gregarious
3	WGM -3	Siddapura Shimoga district	Beru anabe	17/09/2013	Edible	Soil	Four	Scattered
4	WGM -4	Agumbe forest of Shimoga district	Huthada anabe	22/09/2013	Edible	Termite mound	Group	Scattered
5	WGM -5	Siddapura of Shimoga district	Thuppada anabe	22/10/2013	Edible	Soil	Group	Scattered
6	WGM -6	Agumbe forest of Shimoga district	Nayee anabe	22/10/2013	Non edible	Decomposing litter	Three	Gregarious
7	WGM -7	Siddapura, Shimoga district	Chikka anabe	17/08/2013	Non edible	Soil	Group	Scattered
8	WGM -8	Agumbe forest of Shimoga district	Hucha anabe	19/08/2013	Non edible	Soil	Single	Solitary
9	WGM -9	Siddapura, Shimoga district	Mara anabe	10/10/2013	Non edible	Black soil	More	Gregarious
10	WGM -10	Theerthahalli Shimoha district.	Kaddi anabe	28/09/2013	Non edible	Humus soil	More	Gregarious
11	WGM -11	Agumbe forest Shimoha district	Mara anabe	26/08/2013	Non edible	Decay wood	Four	Gregarious

Note: WGM= Western Ghats Mushroom, **Edibility is** known by local consumer and further confirmed after identification, **Habitat**- Place where mushroom were grown, **Abundance**- number of fruiting body occur in a place.



(a) Genomic DNA (b) PCR Amplification of ITS region (c) T/A Cloning vector

(f) Plasmid (e) Colony PCR (d) Recombinant colonies

Plate 1: (A, B, C, D, E & F) Flow chart showing amplification of ITS region, T/A cloning vector, Transformed colonies, Colony PCR and Isolated plasmid.

Plate 2. *Lentinus squrossulus* (WGM-1)

Field characters	**Phenotypic characters**	**Scientific classification**
Mushroom Designation: WGM-1 Location: Siddapura Vernacular Name: Mara anabe Date of Collection:22/09/2013 Edibility: Edible Habitat: Dead wood Growth habit: Scattered	Colour: White cap and white stem Shape 1)Cap: Funnel shaped 2)Stipe: Equal Size 1) Cap dia.:3-4 cm 2)Stipe: Length: 16-7cm Thickness:2-3mm Texture : Hard Stipe Position: Central Gills : Decurrent Annulus : Absent	Kingdom: Fungi Division: Basidiomycota Class: Agaricomycetes Order: Polyporales Family: Polyporaceae Genus: *Lentinus* Species: *squrossulus*

TCCTCCGCTTATTGATATGCTTAAGTTCAGCGGGTAGTCCTACCTGATTTGAGGTCAG
AGTTCGATGATTATTGTCTCGTAAAGAGACGACTAGAAGCTGGCCTATAAAAACGCT
TCAACGGTCGCGGCGAAAACAATTATCACACCGTGAGCCGATCCGCACAGGAACCA
AGCTAATGCATTTAAAAGGAGCCGACTTGACATTGAAGCAAGCCGACAAAAACCTC
CAAGTCCAAGCCTAAAGCAAGTCCCGTTAAAAACCCGTTAGGTTGAGAATTTCGTGA
CACTCAAACAGGCATGCTCCTCGGAATACCAAGGAGCGCAGGGTGCGTTCAAAGATT
CTATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCAT
CGATGCGAGAGCCAAGAGATCCGTTGCTGAAAGTTGTATATAGATGCGTTACATCGC
AATACACATTCTGATACTTTATAAGTGTTTGTAGTGAAACGTAGGCCCAGTAACAAC
TAGGCAAGAAAAAAGCCCGTGAAGGCCCCTTTTCTCGCTTTCGAAGCTCCTGAAACC
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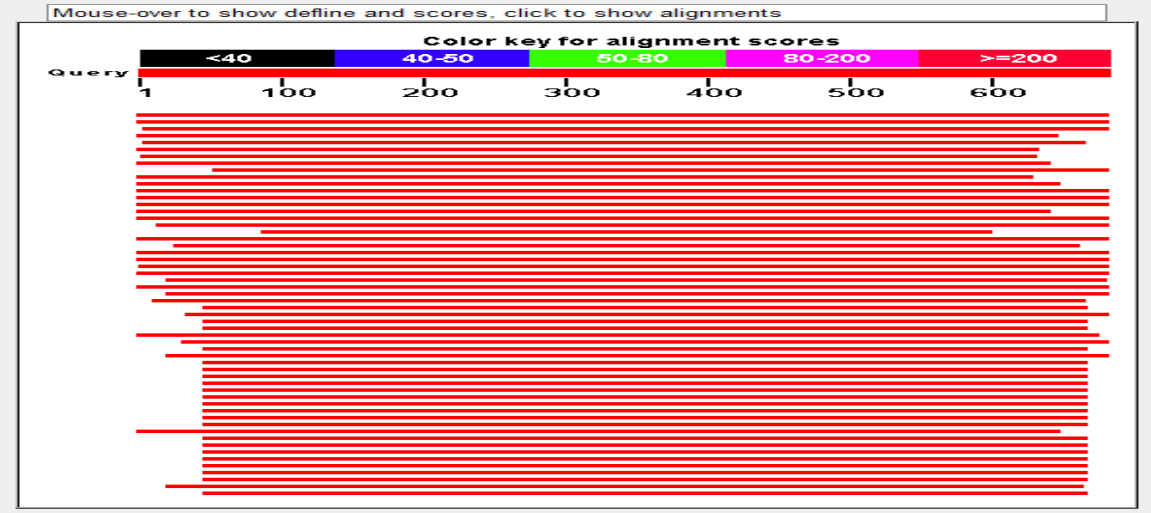

Lentinus squrossulus

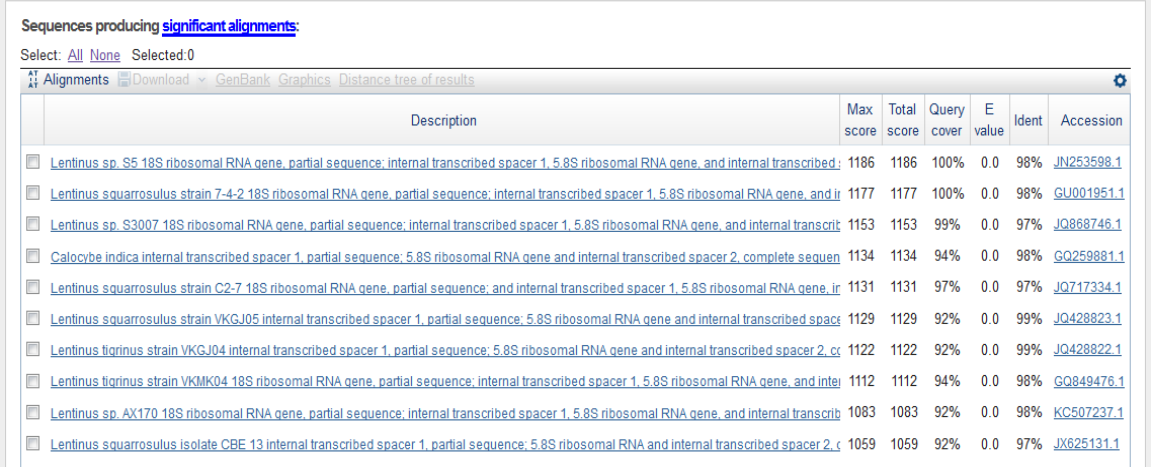

Fig. 1: Full length sequence and homology search of WGM-1(*Lentinus squrossulus*)

Plate 3. ***Pleurotus salmoneostramenius*** **(WGM-2)**

Field characters	**Phenotypic characters**	**Scientific classification**
Mushroom Designation: WGM-2 Location: Theertha halli Vernacular Name: Henne anabe Date of Collection:18/09/2013 Edibility: Edible Habitat: Grows on decaying wood Abundance: Clusters Growth habit: Gregarious	Colour: White Shape 1)cap: Plane 2)Stipe: Sessile Size 1) Cap: 10-11 cms 2) Stipe: sessile Texture : Soft Gills : Adnate Annulus : Absent	Kingdom: Fungi Division: Basidiomycota Class: Agaricomycetes Order: Agaricales Family: Agaricaceae Genus: *Pleurotus* Species: *salmoneostramenius*

GTCAGAGGTGAAATTCTTGGATTTACTCAAGACCAACTACTCGCGAAAGCATTTGCC
AAGGATGTTTTCATTAATCAAGAACGAAGGTTAGGGGATCGAAAACGATCAGATAC
CGTTGTAGTCTTAACAGTAAACTATGCCGACTAGGGATCGGGCAAACTCAAACATGA
TGTGTTGCTCGGCACCTTACGAGAAATCAAAGTCTTTGGGTTCTGGGGGGAGTATGG
TCGCAAGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGTGTGGAGCCT
GCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACATAACTAGGATT
GACAGATTGATAGCTCTTTCATGATTTTATGGGTGGTGGTGCATGGCCGTTCTTAGTT
GGTGGAGTGATTTGTCTGGTTAATTCCGATAACGAACGAGACCTTAACCTGCTAAAT
AGCCAGGCCGGCTTTGGCTGGTCGCCGGCTTCTTAGAGGGACTGTTGGCGTCTAGCT
GACGGAAGTTTGAGGCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCAC
GCGCGCTACACTGACAGAGCCAGCGAGTTTTTTTCCTTGGCCGGAAGGTCTGGGTAA
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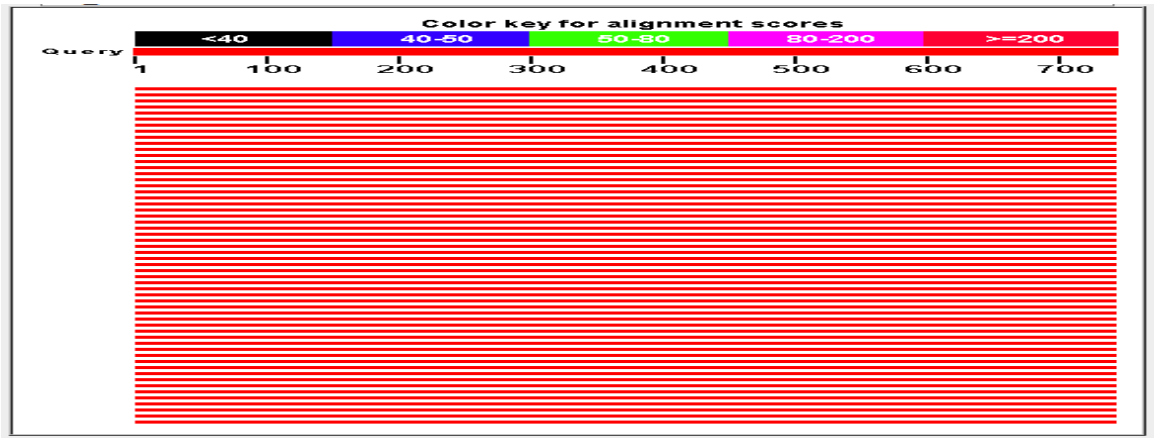
GAATACCTAGTAAGCGTGAGTCATCAGCTCGCGTTGATTACGTCCCTGCCCTT



Pleurotus salmoneostramenius

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Pleurotus salmoneostramineus strain TH 18S ribosomal RNA gene, partial sequence	1343	1343	99%	0.0	99%	FJ379285.1
Uncultured fungus clone nco62d08c1 18S ribosomal RNA gene, partial sequence	1321	1321	99%	0.0	99%	KC672540.1
Pleurotus eryngii strain X-102 18S ribosomal RNA gene, partial sequence	1321	1321	99%	0.0	99%	FJ379286.1
Pleurotus ostreatus strain Po-13 18S ribosomal RNA gene, partial sequence	1321	1321	99%	0.0	99%	FJ379284.1
Pleurotus cystidiosus strain P-24 18S ribosomal RNA gene, partial sequence	1321	1321	99%	0.0	99%	FJ379283.1
Lentinus sajor-caju strain GIM5.82 18S ribosomal RNA gene, partial sequence	1321	1321	99%	0.0	99%	FJ379278.1
Pleurotus nebrodensis strain BL-1 18S ribosomal RNA gene, partial sequence	1321	1321	99%	0.0	99%	FJ379276.1
Hohenbuehelia tristis strain RV95/214 18S small subunit ribosomal RNA gene, partial sequence	1321	1321	99%	0.0	99%	DQ851573.1
Pleurotus ostreatus voucher TENN:53662 18S ribosomal RNA gene, partial sequence >gb|AY657015.1| Pleurotus ostreatus isolate AFTOL-ID	1321	1321	99%	0.0	99%	NG_013165.1
Laccocephalum mylittae strain L66 18S ribosomal RNA gene, partial sequence	1315	1315	99%	0.0	99%	AY944218.3
Laccocephalum mylittae strain L66 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, complete sequence; and 5.8S r	1315	1315	99%	0.0	99%	EU157728.1
Uncultured fungus clone nco63f05c1 18S ribosomal RNA gene, partial sequence	1310	1310	99%	0.0	99%	KC672652.1
Pleurotus tuberregium 18S small subunit ribosomal RNA gene, partial sequence	1306	1306	99%	0.0	99%	U59091.1
Pleurotus tuberregium 18S ribosomal RNA gene, partial sequence	1306	1306	99%	0.0	99%	AF026595.1

Fig. 2: Full length sequence and homology search of WGM-2

(*Pleurotus salmoneostramenius*)

Plate 4. *Termitomyces* sp. (WGM-3)

Field characters	**Phenotypic characters**	**Scientific classification**
Mushroom Designation: WGM-3 Location: Siddapura Vernacular Name: Beru anabe Date of Collection: 17/09/2013 Edibility: Edible Habitat: Humus soil Growth habit: Gregarious	Colour: White Shape 1)Cap: Plane 2)Stipe: Tapering downwards Size 1) Cap dia.:4-5cm 2) Stipe: Length: 9-18cm Thickness:9-12mm Texture : Soft and Crispy Stipe Position: Central Gills : Free Annulus : Present	Kingdom: Fungi Division: Basidiomycota Class: Basidiomycetes Order: Agaricales Family: Lyophyllaceae Genus: *Termitomyces*

TCCTCCGCTTATTGATATGCTTAAGTTCAGCGGGTAGTCCTACCTGATTTGAGGTCAA
ATGGTCAAAAGCGTCTTCCTCAACAAAGAGGAATTACGAGTTAGAAGCAGAAAAGC
CCATTAGAATAAAGTTGACTGCGCACGATGTAGATAATTATCACACCAGGAACAGGC
CAACAAAGGGTTCCACTAATGCATTTAAGGGGAGCTGACTTCTTAAGAAGCCTGCAA
AAACCCCCACATCCAAGCCTAAACCAACTCGCAAAAGCTGGTTAAGGTTGAGAATTT
AATGACACTCAAACAGGCATGCTCCTCGTAATACCAAGGAGCGCAAGGTGCGTTCAA
AGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTT
CTTCATCGATGCGAGAGCCAAGAGATCCGTTGCTGAAAGTTGTATTTGATTAAAGGC
ACTAAAAAGGCCAATAAAAAAACATTCTAATACATTCATTATGGGGTATAAGAAGAT
GCATAGACTGGGAATGCAAGGGAAGCCGAACTTTGCAGCACAGCAAGCCCTCAAAA
CCGAGGAGGGGGTTTGACCCTCGAACAGTATATATCCAAAGTCTACAAAAGGTGCAC
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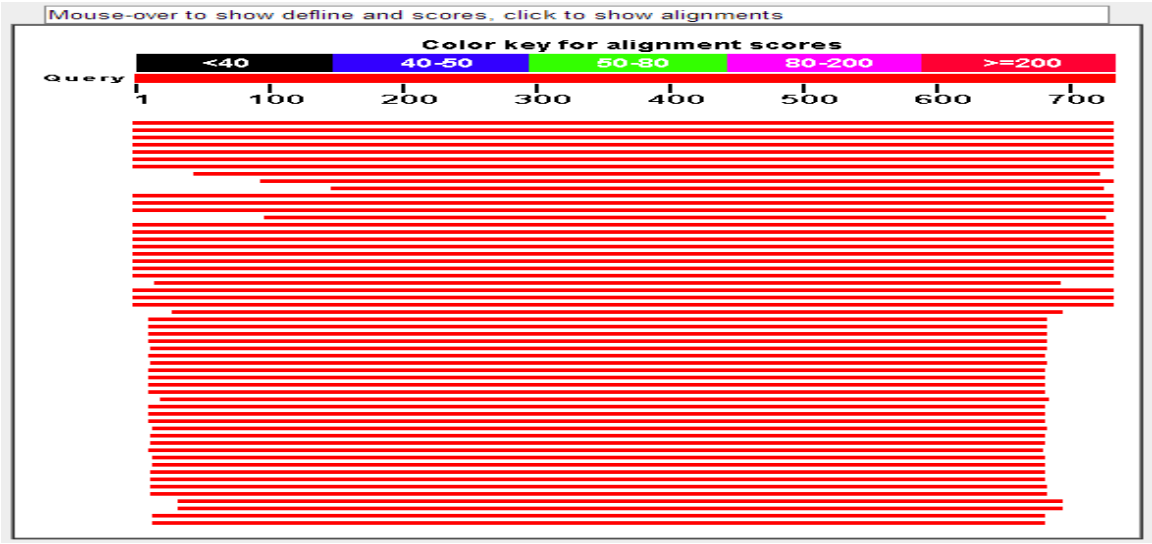

***Termitomyces* sp.**

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
Termitomyces sp. TB 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcrit	1283	1283	100%	0.0	99%	GU001667.1
Termitomyces sp. 1021 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transc	1279	1279	100%	0.0	99%	GU001671.1
Termitomyces sp. CE 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcri	1279	1279	100%	0.0	99%	GU001669.1
Termitomyces sp. JK 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcrit	1274	1274	100%	0.0	99%	GU001668.1
Termitomyces sp. 414 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcr	1272	1272	100%	0.0	99%	GU001672.1
Termitomyces sp. OI genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 26S rRNA, partial and complete sequences, from Ulu Gombak	1270	1270	100%	0.0	99%	AB051888.1
Termitomyces sp. 60995 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal trans	1269	1269	100%	0.0	99%	GU001673.1
Termitomyces sp. T.si 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete seque	1169	1169	92%	0.0	99%	HQ339953.1
Termitomyces heimii strain JMleg.MUIDs.n. internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene, complete sequence and i	1029	1029	86%	0.0	96%	AF357022.2
Termitomyces heimii 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequer	1003	1003	78%	0.0	99%	HM230662.1

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

Plate 5. *Termitomyces* sp. (WGM-4)

Field characters	**Phenotypic characters**	**Scientific classification**
Mushroom Designation: WGM-4 Location: Agumbe Vernacular Name: Huthada anabe Date of Collection:22/09/2013 Edibility: Edible Habitat: Humus soil Growth habit: Solitary	Colour: Brown cap and white stem Shape 1)Cap: Bell shaped 2)Stipe: Tapering downwards Size 1) Cap dia.:6-9cm 2) Stipe: Length: 12cm Thickness: 1cm Texture : Crispy Stipe Position: Central Gills : Free Annulus : Present	Kingdom: Fungi Division: Basidiomycota Class: Basidiomycetes Order: Agaricales Family: Lyophyllaceae Genus: *Termitomyces*

TGGTCAAAGCGTTCCCCCCCAAGTAGAAAGGGTGATACAAGTTGGAAGCACAAACC
TGTTACATAGCTGACTGCTCACGATGTAGATAATTATCACACCAGGAAACAGGTCAA
CGGAAGGTTCCACTAATGCTTTTAAGGGGAGCCGACCTCTGAGATGAAGCCCGCAAA
GAAAACCCCCACAATCCAAGCCTATCCCAGCTCACAAAAGCTGGTTAGGTTGAGAAT
TTAATGACACTCAAACAGGCATGCTCCTCGGAATACCAAGGAGCGCAAGGTGCGTTC
AAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCG
TTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGCTGAAAGTTGTATTTGATTAAAG
GCACTGAGGCCAATAACAAGACATTCTAATACATTCTTTACGGAGTGTATGAAATGC
ATAGACCGGAAATGCGAGGGAAGCCAAGCGGCAGCCCTCGAAACCGAGAGTGTGAC
CCCCCGAAAGATATCCAAAAGTCTACAAAAGGTGCACAGGTGGTTGAAAACGATGG
CAGGCGTGCACATGCCCCTAGAGGCCAGCAACAACCCGACCAGGGTTTAATTCAATA
ATGATCCTTCCGCAGGTTCACCTACGGAAATCGGATCCCGGGCCCGTCGACTGCAGA
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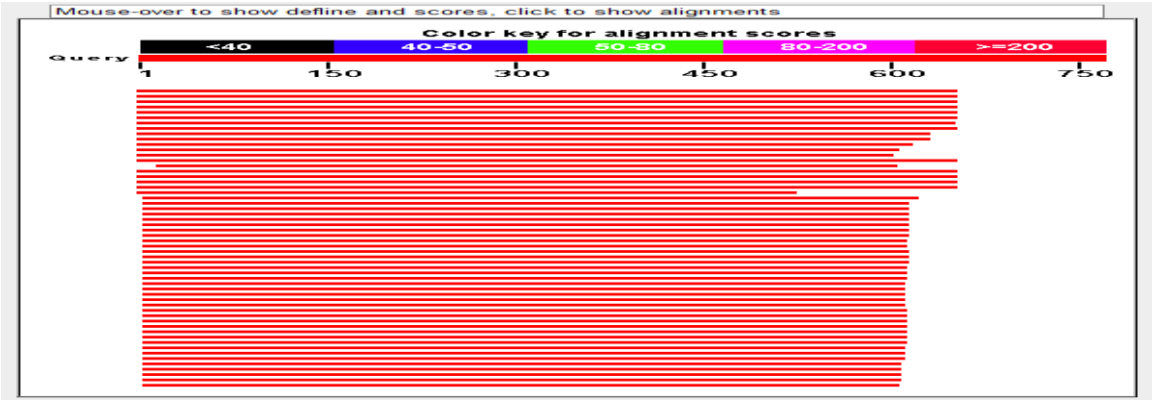

***Termitomyces* sp.**

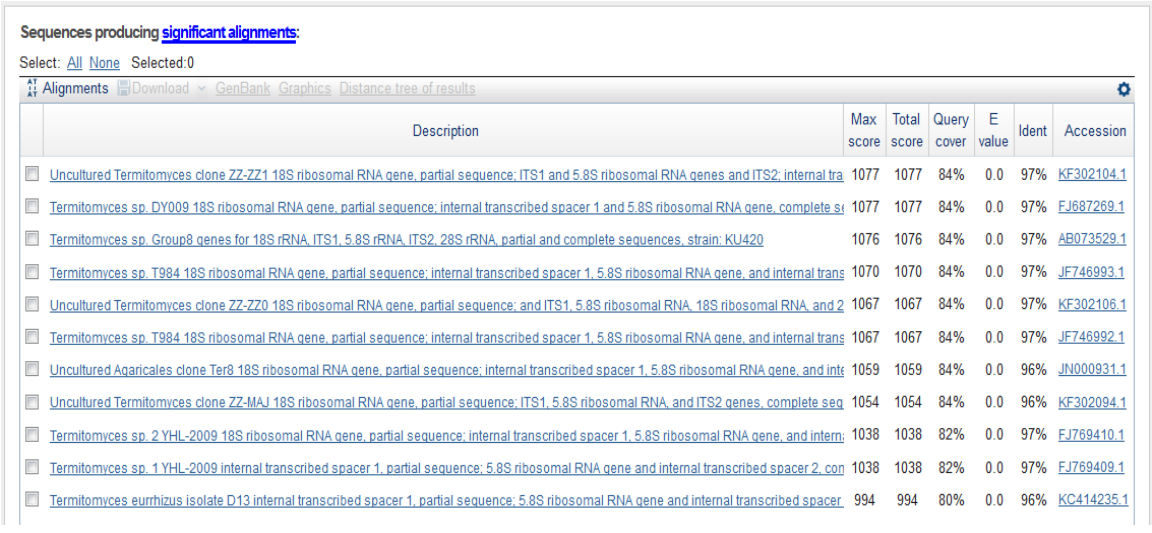

Fig. 4: Full length sequence and homology search of WGM-4 (*Termitomyces* sp.)

was amplified using ITS-1 and ITS-4 primer and sequenced. The sequence data showed 99% homology with the earlier reported *Termitomyces species* (Fig.4).

WGM-5: This mushroom is vernacularly known as Thuppada anabe and it was collected from Siddapura evergreen forest area. It occurred during August and September months. A small creamish white coloured mushroom, grows scatterly on the termite mound. Stipe is tapering upwards, gills are free and annulus absent. ITS region amplified using ITS-1 and ITS-4 primer revealed 94% homology with the earlier reported *Leucoagaricus purpureolilacinus* (Fig.5).

WGM-6: It is a purple coloured mushroom, found in Agumbe forest area in highly humus soil. Pileus, stipe and gills all are purple coloured. A convex shape of pileus measures 6cm in diameter and stipes are present with 7cm in length and 0.5cm thickness. Annulus was absent on the stem. The molecular characterization of this mushroom has been done using ITS primer and sequenced. The sequence data showed 87% homology with the earlier reported *Tricholosporum porphyrophyllum* (Fig-6). This mushroom was grouped under non-edible category.

WGM-7: The mushroom was collected from Siddapura forest area and vernacularly known as chikka anabe in Kannada. Fruiting body of the mushroom was small and grows scatterely on humus soil. The mushroom was creamy white in colour, pileus is convex to flat shaped, two centi meters in diameter and the stipe is enlarged at the bottom. Gills were narrowly attached, annulus absent and soft in texture. The molecular characterization of this mushroom was done by using ITS primer and sequenced. The sequence showed 99% homology with the earlier reported *Agrocybe pediades* (Fig-7).

WGM-8: This mushroom was collected from Agumbe Ghat evergreen forest area and it is vernacularly known as Haladi anabe. The cap was pale yellow colored and cone shaped. Its growth was solitary in red humus soil. Fruiting body possessed 5.5cm long, rhizods. Gills present freely without attachment. The sequence data of the amplified product using ITS primers1 and 4 showed 99% homology with the earlier reported *Leucocoprinus birnbaumii* (Fig-8).

WGM-9: The mushroom was documented from Siddapura evergreen forest area of the Shimogha districts of Karnataka. In Kannada, it is known as Mara anabe. This mushroom found between July and September month occupies prime place in beautification of surrounding nature. Morphologically, mushroom is creamy white in colour and it grows on the dead and decaying wood. Usually grows in black soil and it had leathery structure. The stipe, rhizoids, and annulus are absent. The sequence data of the amplified product using ITS primers1 and 4 showed 99% homology with the earlier reported *Podoscypha petalodes* (Fig-9).

WGM-10: This mushroom was documented from Theerthhalli forest area. Locally this mushroom was named as Kaddi anabe by tribal people. This mushroom grows in groups. Soil is its habitat. The colour of the cap was white, stipe was straight

Table 2: List of Mushroom species identified using ITS region sequence

Sl. No	Mushroom Species Designation	Mushroom species identified	Size of amplified DNA(base pair)
1	WGM-1	*Lentinus squrossulus*	684
2	WGM-2	*Pleurotus salmoneostramenius*	737
3	WGM-3	*Termitomyces* sp.	735
4	WGM-4	*Termitomyces* sp.	773
5	WGM-5	*Leucoagaricus purpureolilacinus*	716
6	WGM-6	*Trycholosporum porpyrophyllum*	719
7	WGM-7	*Agrocybe pediades*	729
8	WGM-8	*Leucocoprinous bimbaumii*	764
9	WGM-9	*Podoscypha petalodes*	693
10	WGM-10	*Xylaria* sp.	455
11	WGM-11	*Antrodia serialis*	735

WGM- Western Ghats Mushroom.

Plate 6. ***Leucoagaricus purpureolilacinus*** **(WGM-5)**

Field characters	**Phenotypic characters**	**Scientific classification**
Mushroom Designation: WGM-5 Location: Siddapura Vernacular Name: Thuppada anabe Date of Collection:22/10/2013 Edibility: Edible Habitat: Humus soil Growth habit: Scattered	Colour: Creamish white Shape 1)Cap: Plane 2)Stipe: Tapering downwards Size 1) Cap dia.:2-3cm 2) Stipe: Length: 3-4cm Thickness: 7mm Texture : Soft Stipe Position: Central Gills : Free Annulus : Absent	Kingdom: Fungi Division: Basidiomycota Class: Agaricomycetes Order: Agaricales Family: Agaricaceae Genus: *Leucoagaricus* Species: *purpureolilacinus*

TCCGTAGGTGAACCTGCGGAAGGATCATTATTGAATATACTTGATGGGTTGTTGCTG
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GTAGTCTCTGAGGGCTGGGATTGGTAGTTGGCCCATCAGCTGAAAAGCTGGATAAGA
AGGTTGCTGCTGCTTTCTTCTGCAACAGGCCATGATCTCCCTTGGGGCCTATGTATCT
TTCATAAACCATGTAGCATGTTAAGAATGTAATCAATAGGCCTTTGTGTCTATAAAA
CCTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGC
GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG
CACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCAGTAAATTCTC
AATCCCATCTAGCTTTTGCGGTTGGGACTTGGATAGTGGAGGTATTTGCAGGCGCCTT
GTGAGGTGTCAGCTCCTCTGAAATACATTAGCGGAACCGTTTGCAATCCGTTACAGG
TGTGATAATTATCTACACCGCGTGGGGTTGCTCTCTGAGTGTTCAGCTTCCAACTGTC
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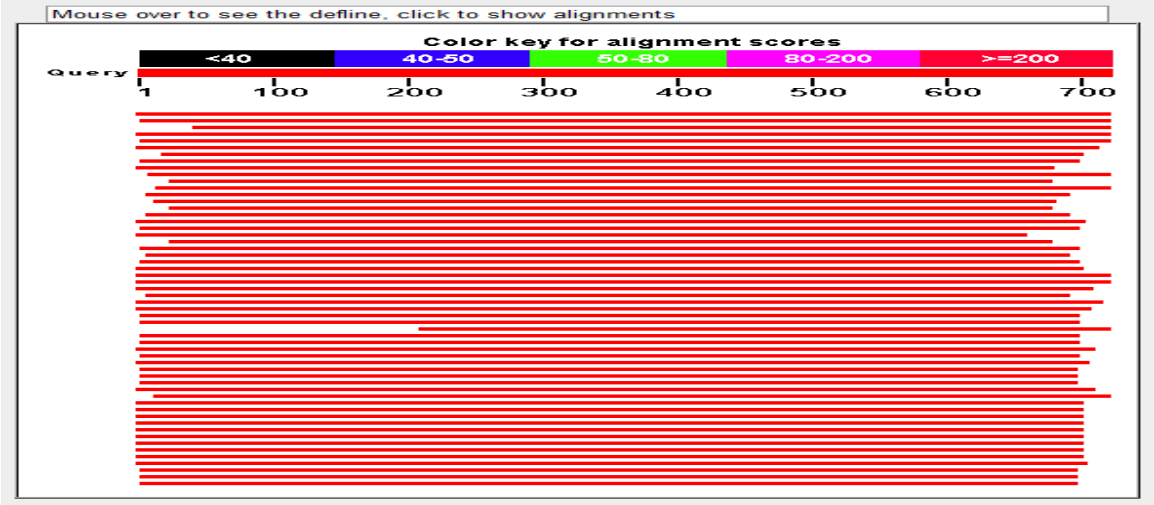
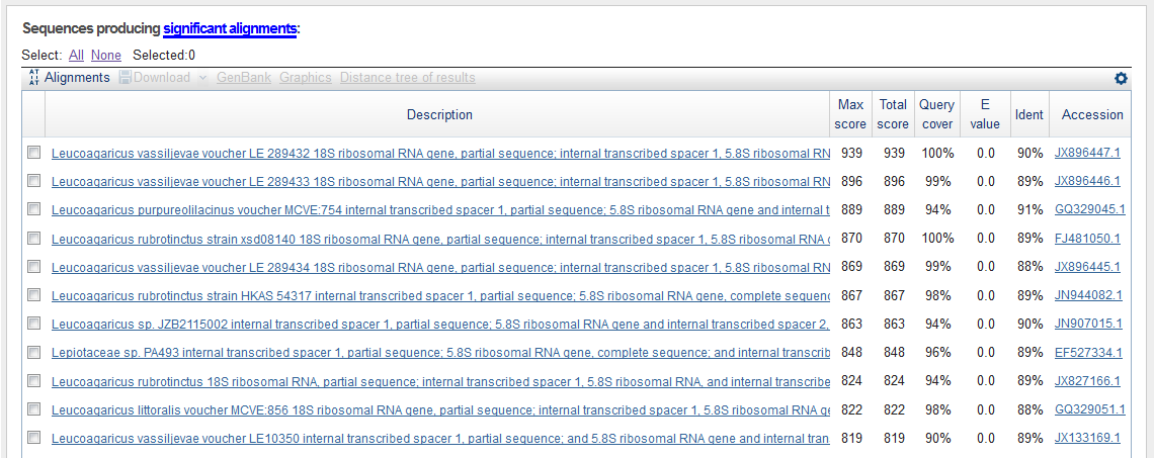


Leucoagaricus purpureolilacinus


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
Leucoagaricus vassilievae voucher LE 289432 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RN	939	939	100%	0.0	90%	JX896447.1
Leucoagaricus vassilievae voucher LE 289433 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RN	896	896	99%	0.0	89%	JX896446.1
Leucoagaricus purpureolilacinus voucher MCVE:754 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal t	889	889	94%	0.0	91%	GQ329045.1
Leucoagaricus rubrotinctus strain xsd08140 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA (	870	870	100%	0.0	89%	FJ481050.1
Leucoagaricus vassilievae voucher LE 289434 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RN	869	869	99%	0.0	88%	JX896445.1
Leucoagaricus rubrotinctus strain HKAS 54317 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequen	867	867	98%	0.0	89%	JN944082.1
Leucoagaricus sp. JZB2115002 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2,	863	863	94%	0.0	90%	JN907015.1
Lepiotaceae sp. PA493 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcrib	848	848	96%	0.0	89%	EF527334.1
Leucoagaricus rubrotinctus 18S ribosomal RNA, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA, and internal transcribe	824	824	94%	0.0	89%	JX827166.1
Leucoagaricus littoralis voucher MCVE:856 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA ge	822	822	98%	0.0	88%	GQ329051.1
Leucoagaricus vassiljevae voucher LE10350 internal transcribed spacer 1, partial sequence; and 5.8S ribosomal RNA gene and internal tran	819	819	90%	0.0	89%	JX133169.1

Fig. 5: Full length sequence and homology search of WGM-5

(*Leucoagaricus purpureolilacinus*)

Plate 7. *Tricholosporum porphyrophyllum* (WGM-6)

Field characters	**Phenotypic characters**	**Scientific classification**
Mushroom Designation: WGM-6 Location: Agumbe forest Vernacular Name: Nayee anabe Date of Collection:22/10/2013 Edibility: Non-edible Habitat: Humus soil Abundance: 2-3in number Growth habit: Scattered	Colour: Violet Shape 1)cap: Convex 2)Stipe: Enlarged below Size 1) cap dia.: 2-3cm 2)Stipe: Length : 3-4cm Thickness: 5-8mm Texture : Soft Stipe Position: Central Rhizoids: Absent Gills : Adnexed (Narrowly attached) Annulus : Absent	Kingdom: Fungi Division: Basidiomycota Class: Agaricomycetes Order: Agaricales Family: Tricholomataceae Genus: *Tricholosporum* Species: *porphyrophyllum*

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AAGGATTCTAAGCAAAAGGCGTAGATAATTATCACACCAAAAGCTTTGTATCCACAA
AGTCTAGCTAATGCTTTTTTAGAAGAGCTGACTATAAAAGCCTGCAACTCCCATAATC
CAATACTAACTTTTGTTCAATAAAAACAAAAGCAGATTGAGAAATTTAATGACACTC
AAACAGGCATGCTCCTCGGAATACCAAGGAGCGCAAGGTGCGTTCAAAGATTCGAT
GATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGAT
GCGAGAGCCAAGAGATCCATTGTTGAAAGTTGTATTTTATTACAAGTAACCCAAACA
TTCAGTTACATTCGTGATATAATATAATACATAGATACCCCAGAGAGAGAAATTGAA
TAAAGGAAAGCTGACTTTCGCACAGCAAACCTTCAACTCAGGCGCATATATGCCTGA
AATAATAAATCCATCTAAGGAGATATCTACAGATAAGTGCACAAGTGGTAATTGGAA
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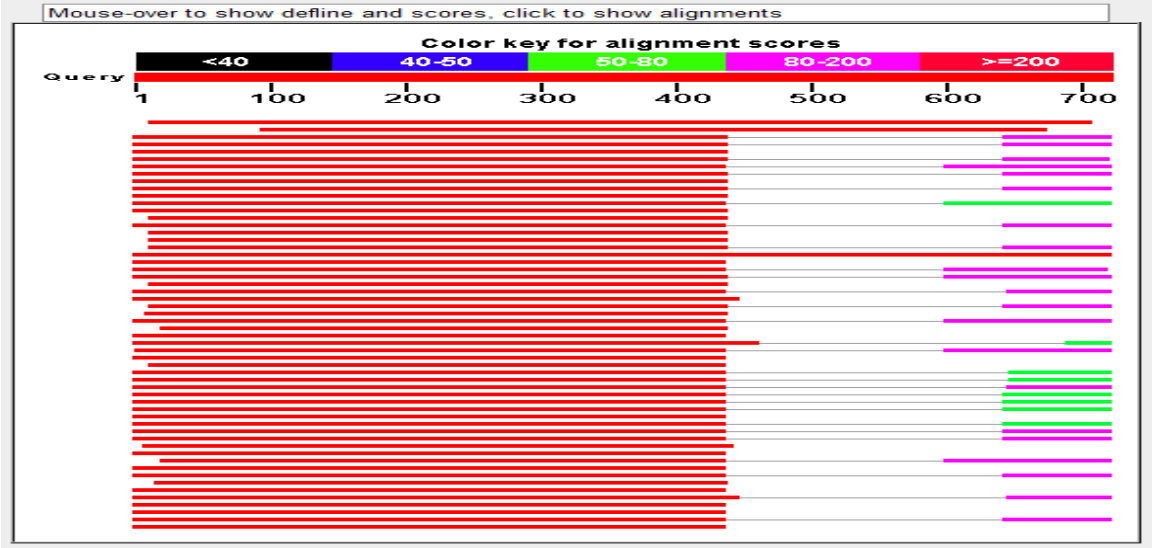
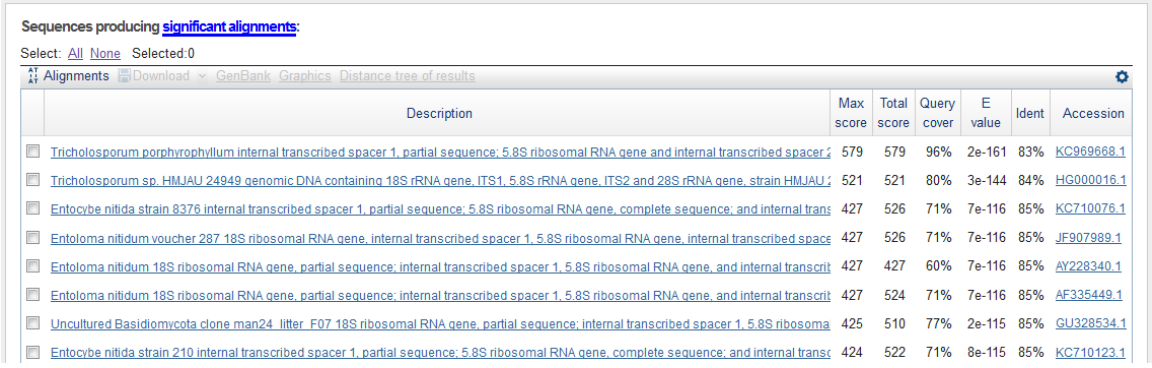


Tricholosporum porphyrophyllum



Description	Max score	Total score	Query cover	E value	Ident	Accession
Tricholosporum porphyrophyllum internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2	579	579	96%	2e-161	83%	KC969668.1
Tricholosporum sp. HMJAU 24949 genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene, strain HMJAU 2	521	521	80%	3e-144	84%	HG000016.1
Entocybe nitida strain 8376 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal trans	427	526	71%	7e-116	85%	KC710076.1
Entoloma nitidum voucher 287 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal RNA gene, internal transcribed space	427	526	71%	7e-116	85%	JF907989.1
Entoloma nitidum 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcrit	427	427	60%	7e-116	85%	AY228340.1
Entoloma nitidum 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcrit	427	524	71%	7e-116	85%	AF335449.1
Uncultured Basidiomycota clone man24_litter_F07 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosoma	425	510	77%	2e-115	85%	GU328534.1
Entocybe nitida strain 210 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transc	424	522	71%	8e-115	85%	KC710123.1

Fig. 6: Full length sequence and homology search of WGM-6

(*Tricholosporum porphyrophyllum*)

Plate 8. ***Agrocybe pediades*** **(WGM-7)**

Field characters	**Phenotypic characters**	**Scientific classification**
Mushroom Designation: WGM-7 Location: Siddapura Vernacular Name: Chikka anabe Date of Collection:17/08/2013 Edibility: Non-edible Habitat: Red soil (Grass land) Abundance: More in number Growth habit: Scattered	Colour: Creamish white Shape 1) Cap: 2-3cm 2) Stipe: 5-6cm Size 1) Cap dia.: 2-3cm 2) Stipe: Length : 5-6cm Thickness:2-3mm Texture :Soft Stipe Position: Central Rhizoids: Absent Gills : Adnexed (narrowly attached) Annulus : Absent	Kingdom: Fungi Division: Basidiomycota Class: Basidiomycetes Order: Agaricales Family: Strophariaceae Genus: *Agrocybe* Species: *pediades*

GGTGAATTCTTGGATTTACTCAAGACCAACTATCTGCGAAAGCATTTGCCAAGGATG
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TCTTAACAGTAAACTATGCCGACTAGGGATCGGGCGACCTCAACTATGATGTGTCGC
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CTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGTGTGGAGCCTGCGGCTTA
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GATTTGTCTGGTTAATTCCGATAACGAACGAGACCTTAACCTGCTAAATAGCCAGGC
CGGCTTTTGCTGGTCGCCGGCTTCTTAGAGGGACTGTCAGCGTCTAGCTGACGGAAG
TTTGAGGCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTA
CACTGACAGAGCCAGCGAGTTTTTTTCCTTGACCGGAAGGTCTGGGTAATCTTGTGA
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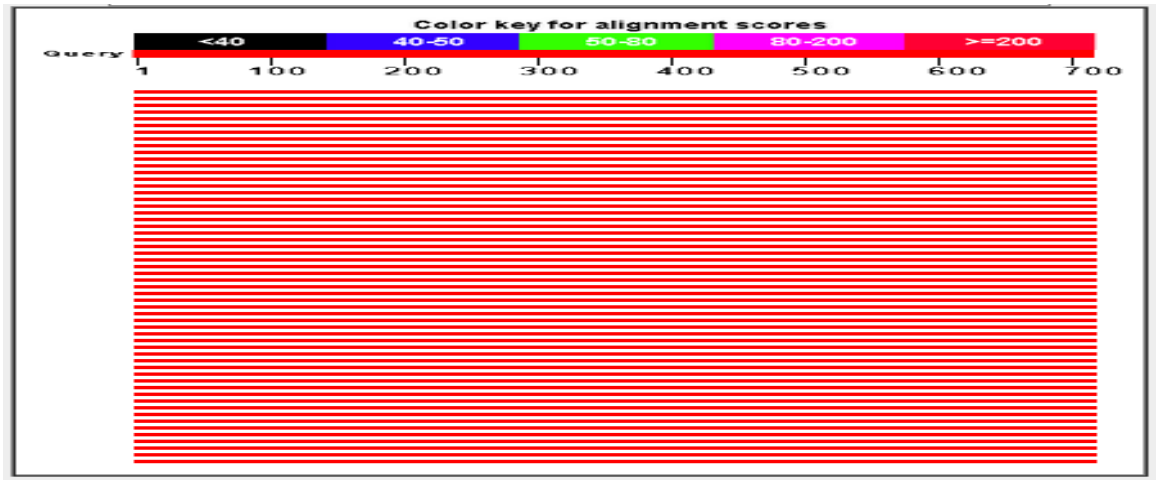

Agrocybe pediades

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

Description	Max score	Total score	Query cover	E value	Ident	Accession
Agrocybe pediades isolate AFTOL-ID 1493 18S ribosomal RNA gene, partial sequence	1332	1332	100%	0.0	99%	DQ113915.1
Pholiota multicingulata voucher PBM3124 small subunit ribosomal RNA gene, partial sequence	1327	1327	100%	0.0	99%	HQ832430.1
Pholiota squarrosa isolate AFTOL-ID 1627 18S ribosomal RNA gene, partial sequence	1327	1327	100%	0.0	99%	DQ466337.1
Nivatogastrium nubigenum isolate AFTOL-ID 1600 18S small subunit ribosomal RNA gene, partial sequence	1327	1327	100%	0.0	99%	DQ459373.1
Agrocybe smithii isolate AFTOL-ID 1494 18S ribosomal RNA gene, partial sequence	1327	1327	100%	0.0	99%	DQ115779.1
Pachylepyrium carbonicola voucher AHS66056 small subunit ribosomal RNA gene, partial sequence	1321	1321	100%	0.0	99%	HQ832426.1
Pachylepyrium carbonicola voucher AHS44809 small subunit ribosomal RNA gene, partial sequence	1321	1321	100%	0.0	99%	HQ832427.1
Hypholoma subviride voucher PBM2954 small subunit ribosomal RNA gene, partial sequence	1321	1321	100%	0.0	99%	HQ832424.1

Fig. 7: Full length sequence and homology search of WGM-7 (*Agrocybe pediades*)

Plate 9. *Leucocoprinus birnbaumii* (WGM-8)

Field characters	**Phenotypic characters**	**Scientific classification**
Mushroom Designation: WGM-8 Location: Agumbe forest Vernacular Name: Hucha anabe Date of Collection:19/08/2013 Edibility: Non-edible Habitat: Humus soil Abundance: Single Growth habit: Solitary	Colour Cap: Pale yellow Stipe: Yellowish Shape 1) Cap: Bell shaped 2) Stipe: Tapering upward Size 1) cap : 5cm 2)Stipe: Length : 7.5cm Thickness:8mm Texture : Soft and Crispy Stipe Position: Central Rhizoids: Present Gills : Free Annulus : Present	Kingdom: Fungi Division: Basidiomycota Class: Agaricomycetes Order: Agaricales Family: Agaricaceae Genus: *Leucocoprinus* Species: *birnbaumii*

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AGAGCGACCCACTAGGCGTAGATAATTATCACACCAGTTGACGGATCGCAAACGGTT
CCGCTAATGCATTTCAGAGGAGCCGACCCATGCGGGCCCGCAAGACCTCCACATCCA
AGCCCCAGTCAGCCACAAAAGCTGAAGGAGTTGAGAATTTACTGACACTCAAACAG
GCATGCTCCTCGGAATACCAAGGAGCGCAAGGTGCGTTCAAAGATTCGATGATTCAC
TGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGA
GCCAAGAGATCCGTTGCTGAAAGTTGTATAAGATTTTATAGGCACAAAGGCCCATTG
AATACATTCTGGCAACATACTACATGGTTTGTGATATAAGACATAGCCTCGAAGCTT
TCCAAAAGGCAACGGTTCCGCGGCTTTTGCAAACAGAAGAGAGCCAAGTCTTTCGAC
CAAAGCAATCCTCAAATCCGGCCTTTCGACCGAGCAGCCGTTTCCTCAATAAGCCCT
CAGAAAGTCATTCAAGACTACAAAAGGTGCACAGGTGGATGAATAGAGTCAAGACA
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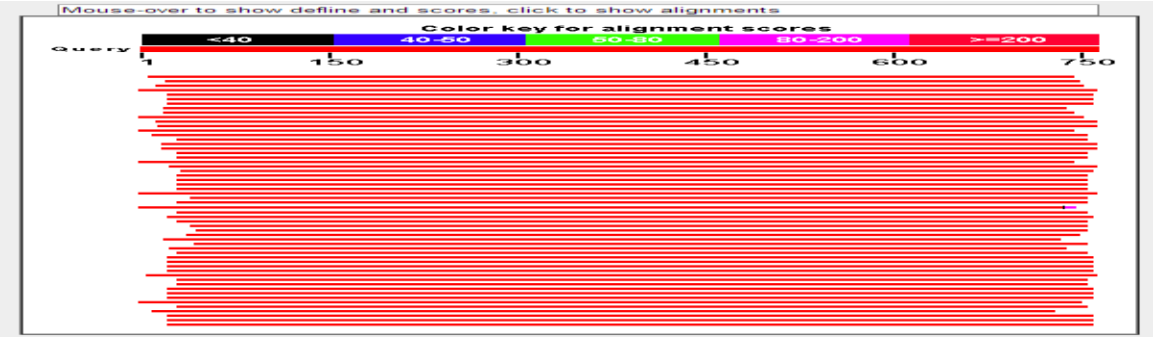

Leucocoprinus birnbaumii

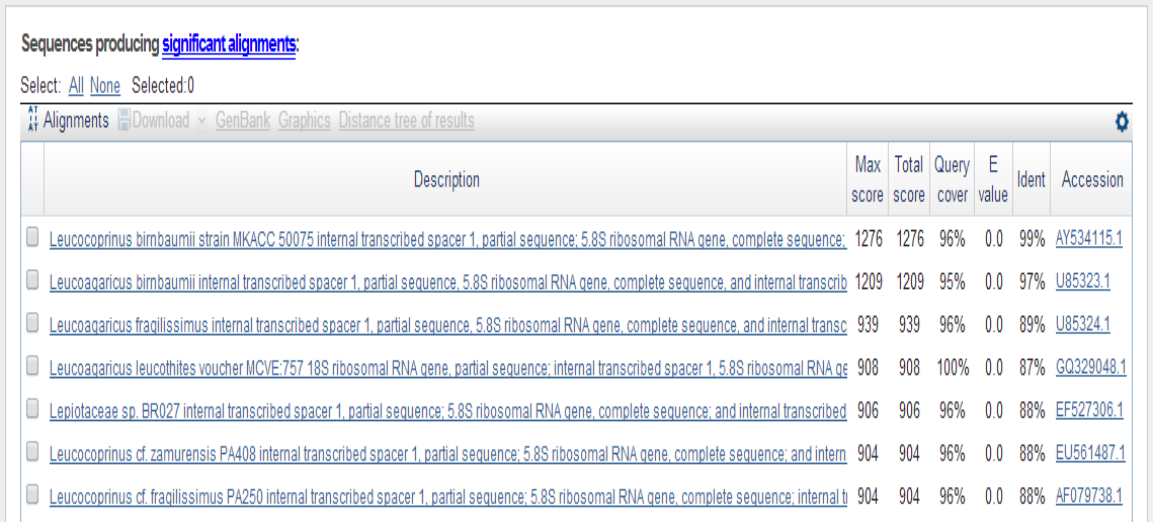

Fig. 8: Full length sequence and homology search of WGM-8

(*Leucocoprinus birnbaumii*)

Plate 10. ***Podoscypha petalodes*** **(WGM-9)**

Field characters	**Phenotypic characters**	**Scientific classification**
Mushroom Designation: WGM-9 Location: Siddapura Vernacular Name: Mara anabe Date of Collection:10/10/2013 Edibility: Non-edible Habitat: Black soil Abundance: More in number Growth habit: Gregarious	Colour: Creamish white Shape 1) Cap: Absent 2) Stipe: Absent Size 1) Cap dia.: Absent 2) Stipe: Absent Texture : Leathery Rhizoids: Absent Gills : Free Annulus : Absent	Kingdom: Fungi Division: Basidiomycota Class: Agaricomycetes Order: Polyporales Family: Meruliaceae Genus: *Podoscypha* Species: *petalodes*

TCCTCCGCTTATTGATATGCTTAAGTTCAGCGGGTAGTCCTACCTGATTTGAGGTCAG
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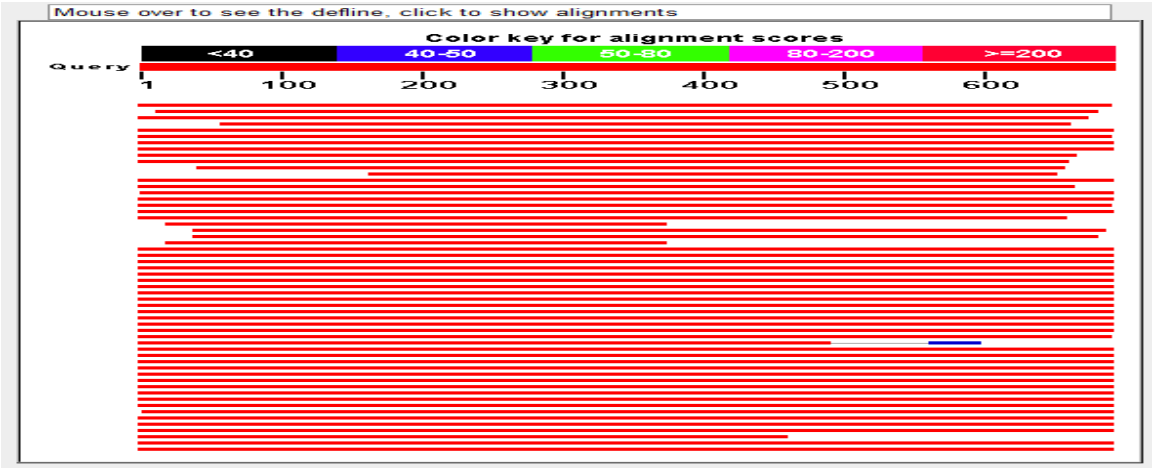

Podoscypha petalodes

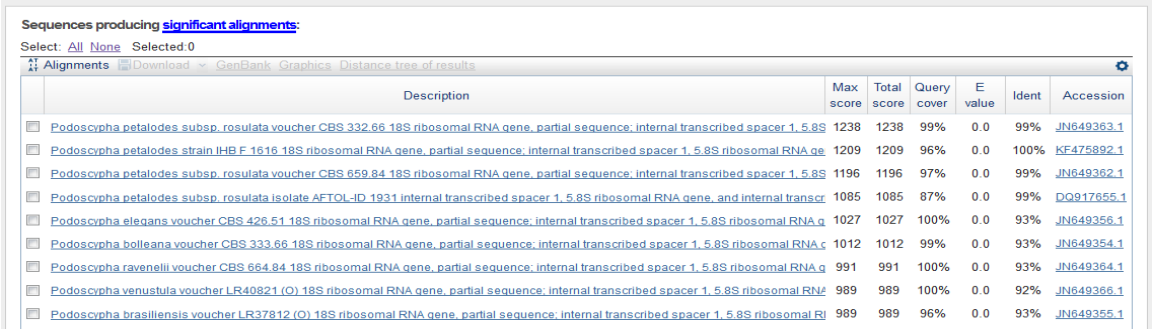

Fig. 9: Full length sequence and homology search of WGM-9

(*Podoscypha petalodes*)

Plate 11. ***Xylaria*** **sp. (WGM-10)**

Field characters	**Phenotypic characters**	**Scientific classification**
Mushroom Designation: WGM-10 Location: Theertha halli Vernacular Name: Kaddi anabe Date of Collection:28/09/2013 Edibility: Non-edible Habitat: Humus soil Abundance: More number Growth habit: Gregarious	Colour: Creamish white Shape 1) Cap: Absent 2) Stipe: Straight and curved Size 1) Cap dia.: Absent 2) Stipe: Length : 5-7cm Thickness:3-5mm Texture : Hard Stipe Position: Sessile Rhizoids: Absent Gills : Absent Annulus : Absent	Kingdom: Fungi Division: Basidiomycota Class: Sordariomycetes Order: Xylariales Family: Xylariaceae Genus: Xylaria

CATCTAGATTTCCGTAGGTGAACCTGCGGAGGGATCATTAAAGAGTTTACAACACTC
CCCAACCCATGTGAACGTTCCCTTCTGTTGCCCCGCAGGCCGAGGCGCGACACTGCT
CCCGCCAGCTCCCCCCCTGCCGGCTGCCCTGAAAACTCTGTTTCCTACATGAATTCGGA
ATCCATAACTAAATACTTTAAAACTTTCAACTTTCGATCTCTTGGTTCTGGCATCTAT
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GAATCTTTGAACGTTGATTGCGCCCGTCAGCATTCTGGCGGCCATGGCTGTTCGAGG
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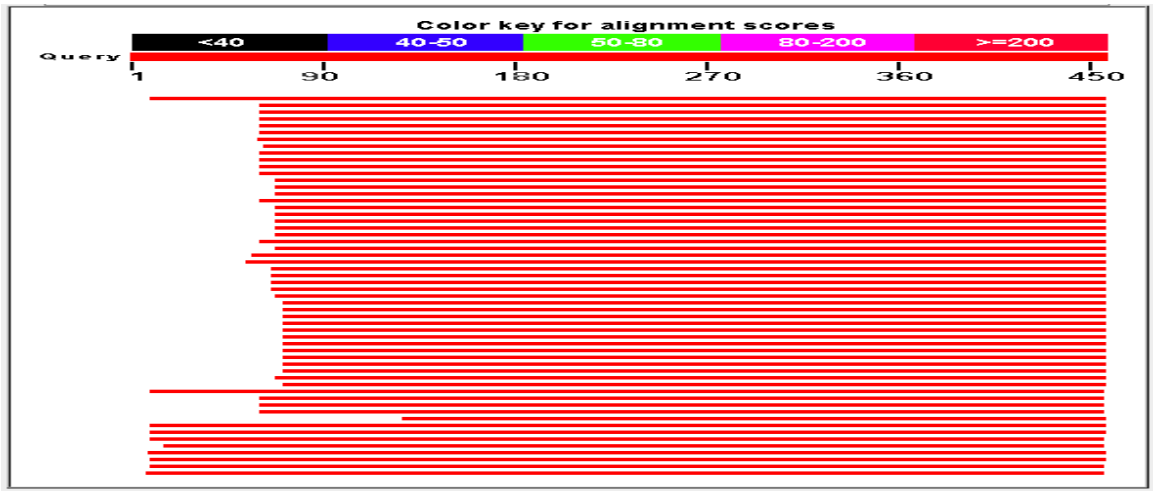
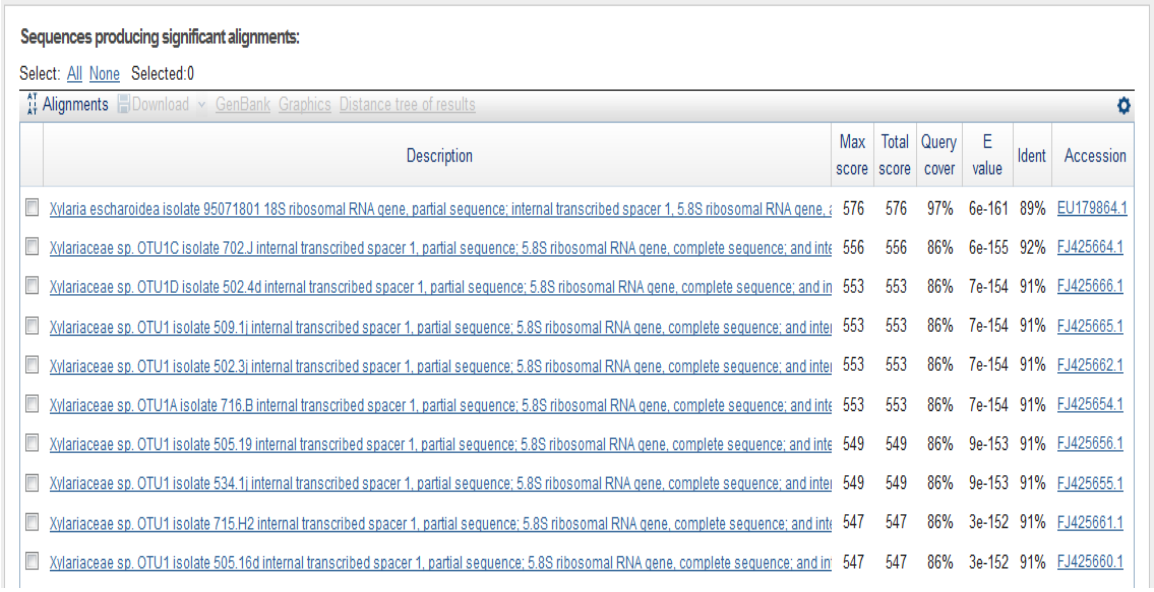


***Xylaria* sp**



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
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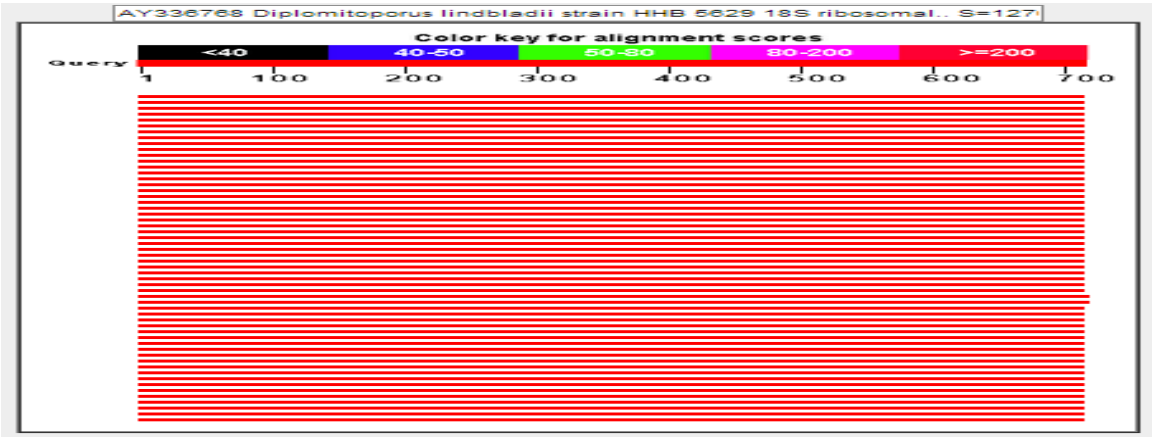
Xylariaceae sp. OTU1C isolate 702.J internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and inte	556	556	86%	6e-155	92%	FJ425664.1
Xylariaceae sp. OTU1D isolate 502.4d internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and in	553	553	86%	7e-154	91%	FJ425666.1
Xylariaceae sp. OTU1 isolate 509.1j internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and inte	553	553	86%	7e-154	91%	FJ425665.1
Xylariaceae sp. OTU1 isolate 502.3j internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and inte	553	553	86%	7e-154	91%	FJ425662.1
Xylariaceae sp. OTU1A isolate 716.B internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and inte	553	553	86%	7e-154	91%	FJ425654.1
Xylariaceae sp. OTU1 isolate 505.19 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and inte	549	549	86%	9e-153	91%	FJ425656.1
Xylariaceae sp. OTU1 isolate 534.1j internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and inte	549	549	86%	9e-153	91%	FJ425655.1
Xylariaceae sp. OTU1 isolate 715.H2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and inte	547	547	86%	3e-152	91%	FJ425661.1
Xylariaceae sp. OTU1 isolate 505.16d internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and in	547	547	86%	3e-152	91%	FJ425660.1

Fig. 10: Full length sequence and homology search of WGM-10 (X*ylaria* sp)

Plate 12. ***Antrodia serialis.*** **(WGM-11)**

Field characters	**Phenotypic characters**	**Scientific classification**
Mushroom Designated: WGM-11 Vernacular Name: Mara anabe Date of Collection: 26/08/2013 Edibility: Non-edible Habitat: Grows on decayed wooden logs Abundance: 3-4 in number Growth habit: Gregarious	Colour: Creamish white Shape 1) Cap: Absent 2) Stipe: Sessile Size 1) Cap dia.: Absent 2) Stipe: Thickness: 1cm Texture : Corky Rhizoids: Present Gills : Free Annulus : Present	Kingdom: Fungi Division: Basidiomycota Class: Agaricomycetes Order: Polyporales Family: Formitopsidaceae Genus: *Antrodia* Species: *serialis*

AAGGGCAGGGACGTAATCAACGCGAGCTGATGACTCACGCTTACTAGGAATTCCTCG
TTGAAGAGCAATAATTGCAATGCTCTATCCCCAGCACGACAGGGTTTCACAAGATTA
CCCAGACCTTTCGGTCAAGGAAAAAAACTCGCTGGCCCTGTCAGTGTAGCGCGCGTG
CGGCCCAGAACATCTAAGGGCATCACAGACCTGTTATTGCCTCAAACTTCCGTCAGC
TAGACGCTGACAGTCCCTCTAAGAAGCCGGCGACCAGCAAAAGCCGGCCTGGCTATT
AAGCAGGTTAAGGTCTCGTTCGTTATCGGAATTAACCAGACAAATCACTCCACCAAC
TAAGAACGGCCATGCACCACCACCCATAAAATCATGAAAGAGCTATCAATCTGTCAA
TCCTAGTCATGTCTGGACCTGGTGAGTTTCCCCGTGTTGAGTCAAATTAAGCCGCAGG
CTCCACTCCTGGTGGTGCCCTTCCGTCAATTCCTTAAAGTTTCAGCCTTGCGACCATA
CTCTCCCCAGAACCCAAAGACTTTGATTTCTCGTAAGGTGCCGAGCGACACATAAAT
TTGAGGTCGCCCGATCCCTAGTCGGCATAGTTTACTGTTAAGACTACAACGGTATCTG
ATCGTTTTCGATCCCCTAACCTTCGTTCTTGATTAATGAAAACATCCTTGGCAAATGC
TTTCGCAGTAGTTAGTCTTCAGTAAATCCAAGAATTTCACCTCTGAC



Antrodia serialis

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident
Antrodia serialis strain FP 105599 18S ribosomal RNA gene, partial sequence	1285	1285	99%	0.0	99%
Antrodia sp. 20 OG-2012 small subunit ribosomal RNA gene, partial sequence	1279	1279	99%	0.0	99%
Antrodia variiformis strain FP 89848R 18S ribosomal RNA gene, complete sequence	1279	1279	99%	0.0	99%
Antrodia variiformis strain FP 90100SP 18S ribosomal RNA gene, complete sequence	1279	1279	99%	0.0	99%
Ganoderma boninense 18S small subunit ribosomal RNA gene, partial sequence	1279	1279	99%	0.0	99%
Uncultured marine fungus clone NB16_BASS small subunit ribosomal RNA gene, partial sequence	1278	1278	99%	0.0	99%
Unclassified marine fungus clone JJ14_BASS small subunit ribosomal RNA gene, partial sequence	1278	2455	99%	0.0	99%
Fomitopsis cajanderi strain BCRC 35447 18S ribosomal RNA gene, partial sequence	1276	1276	99%	0.0	99%
Ganoderma applanatum strain BCRC 36091 18S ribosomal RNA gene, partial sequence	1276	1276	99%	0.0	99%

Fig. 11: Full length sequence and homology search of WGM-11 (*Antrodia serialis*)

Mycelial growth of edible mushroom cultured *In-vitro*

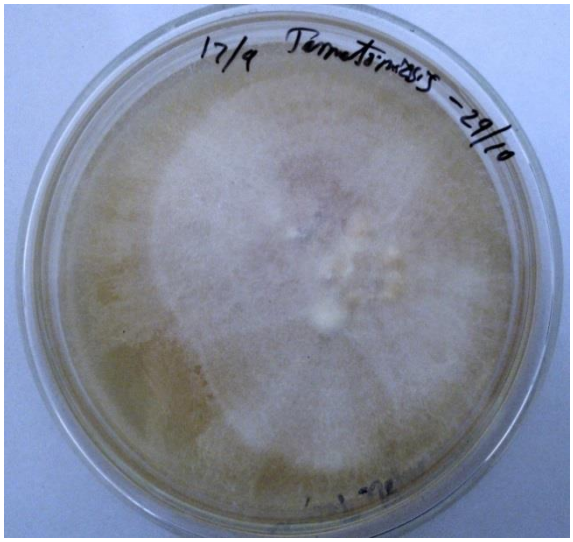


Plate 13: ***Lentinus squrossulus*****.(WGM-1)**

Plate 14: ***Termitomyces*** **sp.(WGM-4)**

and curvey, pileus, gills and annulus are absent. The molecular characterization of this mushroom has been done using ITS primers (ITS 1 and ITS 4) and sequenced. The sequence data showed 89% homology with the earlier reported *Xylaria sp.* (fig-10). The mushroom is grouped under non-edible species.

WGM-11: The fruiting body was collected from Agumbe Ghat evergreen forest area. This mushroom was known as Mara anabe in Kannada. The fruiting body was white and corky textured. It was growing abundantly on decayed wooden logs. Gills are free, rhizoids and annulus are present. The molecular characterization of the mushroom was done by using ITS primer (ITS 1 and ITS 4) and sequenced. The sequence data revealed 95% homology with the earlier reported *Antrodia serialis* (fig-11).

4.2. Mycelial growth of edible mushroom cultured *in-vitro*

To obtain pure culture for conservation, four edible mushrooms namely, *Lentinus squrossulus, Pleurotus salmoneostramenius*, and two *Termitomyces species* were cultured using stipe tissue on Potato Dextrose Agar (PDA). Among the four, two mushrooms namely, *Lentinus squrossulus* (WGM-1), (Plate-13) and one *Termitomyces* species (WGM-4), (Plate-14) produced mycelium on PDA. The other two mushrooms did not produce mycelium.

V. DISCUSSION

Man has been hunting wild mushrooms for food since antiquity. Thousands of years ago, fructifications of higher fungi have been used as a source of food due to their attractive flavor and taste. During the early days of civilization, mushrooms were consumed mainly for their palatability and unique flavors (Rai, 1997). Mushrooms are placed in a separate division called Eumycota (the true fungi). The fungal class, Basidiomycetes comprises larger group of mushroom fungi compared to Ascomycetes. Mushrooms have been used as food and medicine by the ancient Egyptian, Greek, Roman and Chinese civilizations. These fungi had attained the status of a regular crop in France and China by 17^{th} and 19^{th} centuries, respectively, spreading gradually to the other countries in few years. There are about 69 thousand known mushroom species of which 2000 species from more than 30 genera are regarded as prime edible mushrooms but 80 of them are grown experimentally and around 20 are cultivated commercially (Chang, 1991).

Mushrooms occur under various ecological conditions from desert to forest. They comprises a large heterogeneous group of fungi with different shapes, sizes, colour and edibility. Mushrooms are the good sources of high quality proteins and contain 20-35% protein on dry weight basis which is higher than in vegetables and fruits (Pathak et al. 2013). They are rich in lysine and tryptophan, the two essential amino acids that are deficient in cereals. Mushrooms are also rich in vitamins and minerals. They contain good amount of vitamin C and B complex and minerals viz., potassium, phosphorus and sodium. In mushrooms, potassium: sodium ratio is very high which is ideal for patients of hypertension. They are low calorie food with very little fat and sugars and without starch and cholesterol.

Mushrooms also have potential medicinal properties. Pharmaceuticals worth $ 700 million are produced annually in Japan from *Lentinus, Coriolus, Schizophyllum* and *Ganoderma.* The compounds extracted from mushrooms have antiviral, antibacterial and antifungal properties. Besides these mushroom extract also have anti-tumor activity useful in cancer treatment. Mushrooms are capable of agro-waste degradation and grown on organic substrates (Pathak *et al*., 2013).

As mushrooms are ephemeral in nature and disappear within a day, constant survey during appropriate season is essential to know the species diversity. Mushrooms can be identified based on their morphological and molecular characters. The phenotypic characters include the shape, size, texture, colour and odour of the fruiting body. During collection it is essential to record field character and observes the surrounding to facilitate further classification and speciation. The field details such as, date, season, weather, abundance, growth habit, vegetation, substrate *etc*., should be documented. In this study, genotypic information has been used for identification of documented mushrooms in the Shimoga district of Karnataka.

The Central Western Ghats of Karnataka ranges from 12° to 14° covering the area of Coorg, Hassan, Chikmagalur, Shimoga districts up to south of Uttara Kannada.

Shimoga district is situated in the heart of the Western Ghats region, which is one of the 'hot-spots of biodiversity' in India. This district comes under south-eastern transitional zone and situated between 13°27' and 14°39' latitude and between 74°37' and 75°52' E longitude in about the mid-South Western part of the Karnataka State. The district receives an average annual rainfall of 2869 mm (Bhat, *et al*., 2012). The cool and humid climate made ideal habitat for different kinds of mushrooms.

The diversity of fungi and their natural beauty occupy prime place in the biological world and India has been a cradle for these species. Defining the number of fungi on the earth has been a point of discussion and several studies have focused on enumerating the World fungal diversity (Crous, 2006). Only a fraction of total fungal wealth has been subjected to scientific scrutiny and mycologists continue to unravel the unexplored and hidden wealth. One third of fungal diversity of the globe exists in India and of this only 50 % are characterized so far (Manoharachary *et al.,* 2005). In the present study, ethnomycological survey was under taken in some parts of Shimoga district and eleven mushroom species were documented and characterized by using the sequence of ITS region.

5.1. Documentation of mushrooms

Ethnomycology refers to the study of the relationship between man and fungi. To understand the occurrence, abundance, locality or habitat and edibility of the mushrooms, traditional knowledge of the tribal and rural folks of the specific region is very much essential. Therefore, in the present study, villager's knowledge and their company were used during surveying and collection of mushrooms. Eleven species of mushrooms were collected and field information like, place, habitat, soil type and growth characters were recorded.Those mushroom fruit bodies were brought to the laboratory and morphological characters (cap, stipe, gills arrangement, volva) were recorded. The mushrooms were morphologically different though they grew in same climatic conditions. The habitats were also varied from soil to tree stump and leaf litter. This is due to the versatility of the forest ecosystem which provides diversified niche for different types of mushrooms under same umbrella. Dwivedi *et al*., (2012) reported 52 mushroom species from Amarkantak Biosphere Reserve of Madhya Pradesh. Those mushrooms belonged to different genera out of which only 14 mushroom samples were identified up to species level. A delicious *Termitomyces* species known as Heggalanabe by the local people of Shimoga district is reported by Earanna *et al*., (2013).

5.2. Molecular identification of mushrooms

Identification of mushrooms based on their morphology or habitat in which they grew may not be sufficient enough to classify the fungus. Therefore, use of molecular tools for identification of mushrooms is essential for precise speciation. Molecular techniques are quick and reliable to establish the identities of wild mushroom collections and are helpful in mushroom taxonomy, therefore molecular characterization using 18S rRNA gene/ITS region sequence analysis is reliable for precise classification.

In molecular taxonomy, 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 (Rajarathnam and Thiagarajan, 2012) and 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. This ITS region is most widely used to sequence the DNA region in fungi. It has typically been most useful for molecular systematic at the species level and within the species (Wipf *et al*., 1999). Amplification of the ITS region of 5.8S rDNA was done using ITS 1 and ITS 4 primers (Rajaratnam and Thiagarajan, 2012). The length and sequence of ITS region are believed to be fast evolving and perhaps the most widely sequenced DNA region in fungi (Peay *et al*., 2008).

Literatures on molecular characterization of mushrooms are limited, as earlier classification of mushroom was done only on the basis of morphological and phenotypic characters that will leads to confusion in identifying mushrooms with in the same species. However, in the 20^{th} century scientist identified mushrooms species by using 18s RNA/ITS genes. 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 mushrooms were pure cultured from the cap using tissue culture method and maintained on Potato Agar slants and stored. Then they isolated genomic DNA from the pure culture and sequencing was done using ITS-1(forward) and ITS-4 (reverse primer), the nucleotide sequence were performed using Blast Multiple Alignment Tool (BLAST) network sequence against the National Centre for Biotechnology Information (NCBI) database shows 91% homology with *Tricholoma giganteum* and is given with Gene bank accession number 120872.

Identification of wild mushrooms is difficult by considering only visual or metabolic approaches. Molecular markers, especially DNA markers are quick and reliable to establish the identities of wild collections and are helpful in mushroom taxonomy. Hence an attempt was made in the study to characterize the wild mushrooms by molecular methods. Fungal material used in the present study is the fruiting body of wild mushrooms.

In this present study, eleven mushrooms designated as WGM-1, WGM-2, WGM-3, WGM-4, WGM-5, WGM-6, WGM-7, WGM-8, WGM-9, WGM-10 and WGM-11 were identified up to species level by using ITS region sequence in addition to phenotypic characters.

The PCR amplification of genomic DNA of the 11 mushrooms in the present study yielded amplified product sizes varying from 455 bp to 773bp (Table, 2) which are corresponding to almost full length gene sequence of ITS. The sequence homology of the 11 species ranged from 83-99% when aligned with the sequences present in NCBI. WGM-1 has 98% homology with *Lentinus squrossulus,* WGM-2 has 99 % homology

with *Pleurotus salmoneostramenius*. WGM-3 has 99% homology with *Termitomyces* sp. WGM-4 showed 97% homology with *Termitomyces* sp. WGM-5showed 90% homology with *Leucoagaricus purpureolilacinus*, WGM-6 with 83% homology with *Triholosporum porphyrophyllum*, WGM-7 with 99% homology for *Agrocybe pediades*, WGM-8 with 99% homology for *Leucocoprinus birnbaumii*, WGM-9 showed 99% homology with *Pedoscypha petalodes*, WGM-10 had 89% homology with *Xylaria* sp. and WGM-11 had 99% homology with *Antrodia serialis*. Thus, the above 11 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).

5.3. Pure culture isolation and conservation

Since, mushroom fungi are important source of food and medicine from time immemorial, there is a need to explore and conserve potential species for further application in future. Many of the non-edible mushrooms contains anti-oxidant and medicinal value, they will cure cancer, diabetes and skin diseases etc., (Thakur *et al.*, 2009). Besides edibility, many non-edible mushrooms are very good decomposers of organic matters which is essential for bioremediation. Therefore, one of the main intentions of this study was to conserve the elite wild species documented from the Western Ghats.

Pure culture can be obtained from tissue/spore of the fruiting body by using different culture media. Asghar *et al.*, (2007) isolated pure culture of *Pleurotussajor-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). In the present study, five edible mushrooms namely, *Lentinus squrossulus, Pleurotus salmoneostramenius*, two *Termitomyces species* and *Leucoagaricus purpureolilacinus* were cultured using stipe and cap tissue on Potato Dextrose Agar (PDA). Among the five, two mushrooms namely, *Lentinus squrossulus*, (WGM-1) and *Termitomyces* species (WGM-4) produced mycelium on PDA, indicating that they could be grown *In-vitro* on culture medium. The other three species may need further experimentation.

VI. SUMMARY

Western Ghats also known as Sahyadris is one of the world's ten hottest biodiversity hotspots and has four major forest types, 23 floristic types and unique high altitude grasslands. It also contains more than 30 percent of India's species diversity. Mean temperature range from 20 °C (68 °F) in the south to 24 °C (75 °F) in the north. The cool and humid climate made ideal habitat for different kinds of mushrooms. In the present study, ethnobotanical survey was made in and around Shimoga district of Karnataka for mushroom flora during June to September 2013 with the help of Adivasis, Halakki vokkals and Siddis 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.

Eleven mushrooms were collected in a paper bags during field survey with the help of tribes inhabited in the area and these mushrooms were designated as WGM-1, WGM-2, WGM-3, WGM-4, WGM-5, WGM-6, WGM-7, WGM-8, WGM-9, WGM-10 and WGM-11. 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 and shape of cap and stipe, presence or absence of gills or pores, presence or absence of annulus, texture and microscopic characters like spore shape and color were recorded.

The mushrooms were identified by using Internal Transcribed Sequence (ITS) region and identified by using the sequence data deposited in National Centre for Biotechnology Information (NCBI).The genomic DNA of the mushrooms was amplified using 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.WGM-1 has 98% homology with *Lentinus squrossulus*, WGM-2 has 99 % homology with *Pleurotus salmoneostramenius*, WGM-3 has 99% homology with *Termitomyces* sp, WGM-4 showed 97% homology with *Termitomyces* sp., WGM-5 showed 90% homology with *Leucoagaricus purpureolilacinus*, WGM-6 with 83% homology with *Tricholosporum porphyrophyllum*, WGM-7 with 99% homology for *Agrocybe pediades*, WGM-8 with 99% homology for *Leucocoprinus birnbaumii*, WGM-9 showed 99% homology with *Podoscypha petalodes*, WGM-10 had 89% homology with *Xylaria* sp and WGM-11 had 89% homology with *Antrodia serialis.*

Of the Eleven mushrooms documented, five mushrooms namely, *Lentinus squrossulus, Pleurotus salmoneostramenius*, *Termitomyces* sp (WGM-3), *Termitomyces* sp (WGM-4), and *Leucoagaricus purpureolilacinus*, were edible. Four edible species were cultured *In-vitro* on Potato dextrose agar. Of the four, *Lentinus squrossulus*, and *Termitomyces* sp (WGM-4), were produced mycelia and *Pleurotus salmoneostramenius* and *Termitomyces* sp (WGM-3), did not produce mycelial growth.

Future line work

1. Cultivation techniques for the cultured species need to be developed for commercial exploitation.
2. Nutritional value of those cultured ones required to be analyzed.
3. Medicinal property of mushroom required to be analyzed.

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APPENDIX

Composition of microbiological media

1) Potato dextrose agar media

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

Boil the potato slices in water and filter the potato boiled extract, 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

p^H adjusted to 7.00

(15 grams of bacteriological grade agar was added to prepare Luria agar.)

The content was autoclaved at 15 lbs/sq inch for 20 minutes

3) 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- mercaptaethanol

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)

Bomophenol blue - 0.25%

Xylene cyanol - 0.25%

Glycerol - 30%