

**DOCUMENTATION AND MOLECULAR
CHARACTERIZATION OF WILD MUSHROOMS
FROM WESTERN GHATS OF KARNATAKA**

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PALB 3066

**DEPARTMENT OF PLANT BIOTECHNOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
BENGALURU**

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AUGUST, 2017




*Affectionately dedicated to
My Guide
My Family
and
My Friends*

**DEPARTMENT OF PLANT BIOTECHNOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
GKVK, BENGALURU - 560065**

CERTIFICATE


This is to certify that the thesis entitled "DOCUMENTATION AND MOLECULAR CHARACTERIZATION OF WILD MUSHROOMS FROM WESTERN GHATS OF KARNATAKA" submitted by Mr. PRABHU, K. N., I.D. No. PALB 3066, for the award of degree of DOCTOR OF PHILOSOPHY (AGRICULTURE) in PLANT BIOTECHNOLOGY to the University of Agricultural Sciences, GKVK, Bengaluru, is a record of research work done by him during the period of his study in this University under my guidance and supervision, and the thesis has not previously formed the basis for the award of any other degree, diploma, associateship, fellowship or any other similar titles.

BENGALURU
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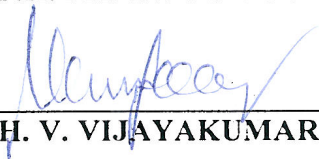
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(Prabhu, K. N.)

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KARNATAKA**

PRABHU K N

THESIS ABSTRACT

Western Ghats of Karnataka is one of the world's biodiversity hot spot and has four major forest types, 23 floristic types and unique high altitude grasslands. The cool and humid climate make it ideal habitat for different kinds of mushrooms. In the present study, ethno-mycological survey was made in and around Chikkamagaluru, Dakshina Kannada, Hassan and Shivamogga districts of Karnataka for mushroom flora during June to October (2014-2016) with the help of local peoples and Adivasi tribes. Totally fifty mushrooms collected during the above period and identified. Out of fifty mushrooms, ten mushrooms were identified by morphological characters and forty mushrooms were identified by using Internal Transcribed Sequence (ITS) homology available in the National Centre for Biotechnology Information (NCBI) USA. The sequence alignment was made with NCBI data base revealed 85-100 % homology. The mushrooms viz., *Schizophyllum commune*, *Tremella fuciformis*, *Lentinus sajor-caju* (WGM-35), *Lentinus sajor-caju* (WGM-36), *Lentinus polychrous*, *Lentinus strigosus*, *Termitomyces eurhizus*, *Pleurotus pulmonarius*, *Pluteus petasatus* and *Polyporus tenuiculus* were cultured using stipe tissue on Potato dextrose agar (PDA) and the *Lentinus sajor-caju* was cultivated on paddy straw and sawdust as substrate as it exhibited good mycelial growth. The highest bio-efficiency (65.25 %) was recorded in 50 % each Paddy straw and sawdust mixed substrate. The least bio-efficiency (38 %) was recorded in paddy straw substrate alone. The protein, fat and fiber content were in the range of 22.33-34.46 %, 1.8-4.0 % and 5-12 % respectively. Minerals viz., Ca, Mg, Fe, Zn and P were in the order of 48.45-143.96 µg/g, 14.78-59.49 µg/g, 8.44-33.32 µg/g, 0.58-3.4 µg/g and 107.33-505.66 µg/g respectively.

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ಕರ್ನಾಟಕದ ಪಶ್ಚಿಮ ಘಟ್ಟಗಳಲ್ಲಿನ ಕಾಡು ಅಣಬೆಗಳ ದಾಖಲಾತಿ ಮತ್ತು ಆಣ್ವಿಕ ವಿವರಣೆ

ಪ್ರಭು, ಕೆ. ಎನ್.

ಪ್ರಬಂಧದ ಅಮೂರ್ತ

ಕರ್ನಾಟಕದ ಪಶ್ಚಿಮ ಘಟ್ಟಗಳು ವಿಶ್ವದ ಜೀವವೈವಿಧ್ಯತೆಯ ಕೇಂದ್ರ ಬಿಂದುಗಳಲ್ಲಿ ಒಂದಾಗಿದೆ ಮತ್ತು ನಾಲ್ಕು ಪ್ರಮುಖ ಅರಣ್ಯ ಪ್ರದೇಶವನ್ನು, ಇಪ್ಪತ್ತಮೂರು ಹೂವಿನ ರೀತಿಯ ಮತ್ತು ಗಿರಿಗಳ ಎತ್ತರದಲ್ಲಿ ಹುಲ್ಲು ಪ್ರದೇಶವನ್ನು ಹೊಂದಿದೆ. ತಂಪಾದ ಮತ್ತು ಆರ್ಧ್ರ ವಾತಾವರಣವು ವಿಭಿನ್ನ ರೀತಿಯ ಅಣಬೆಗಳಿಗೆ ವಾಸಸ್ಥಾನವಾಗಿದೆ. ಪ್ರಸ್ತುತ ಅಧ್ಯಯನವನ್ನು, ಸ್ಥಳೀಯರು ಮತ್ತು ಆದಿವಾಸಿ ಬುಡಕಟ್ಟು ಜನಾಂಗದವರ ಸಹಾಯದಿಂದ ಜೂನ್ ತಿಂಗಳಿಂದ ಅಕ್ಟೋಬರ್ ತಿಂಗಳವರೆಗೆ (೨೦೧೪-೨೦೧೬) ಚಿಕ್ಕಮಗಳೂರು, ದಕ್ಷಿಣ ಕನ್ನಡ, ಹಾಸನ ಮತ್ತು ಶಿವಮೊಗ್ಗ ಜಿಲ್ಲೆಗಳಲ್ಲಿ ಅಣಬೆಗಳ ಸಮೀಕ್ಷೆಯನ್ನು ಮಾಡಲಾಯಿತು. ಒಟ್ಟಾರೆ ಐವತ್ತು ಅಣಬೆಗಳನ್ನು ಸಂಗ್ರಹಿಸಿ, ಅವುಗಳಲ್ಲಿ ಹತ್ತು ಅಣಬೆಗಳನ್ನು ಅವುಗಳ ರೂಪರಚನೆಗಳ ಆಧಾರದ ಮೇಲೆ ಯಾವಯಾವ ಪ್ರಭೇದಗಳೆಂದು ಗುರುತಿಸಲಾಯಿತು. ಉಳಿದ ನಲವತ್ತು ಅಣಬೆಗಳನ್ನು ಆಂತರಿಕ ಲಿಪ್ಯಂತರ ಸರಣಿಗಳ ಆಧಾರದ ಮೇಲೆ, ಅಮೇರಿಕದ ರಾಷ್ಟ್ರೀಯ ಜೈವಿಕ ತಂತ್ರಜ್ಞಾನ ಮಾಹಿತಿ ಕೇಂದ್ರದಲ್ಲಿ ಶೇಖರಿಸಿದ ಗುಣಾಣು (ವಂಶವಾಹಿನಿ) ಸರಣಿಗಳೊಂದಿಗೆ ಹೋಲಿಸಿ ಗುರುತಿಸಲಾಯಿತು. ರಾಷ್ಟ್ರೀಯ ಜೈವಿಕ ತಂತ್ರಜ್ಞಾನದ ಮಾಹಿತಿ ಅನುಕ್ರಮ ಜೋಡಣೆಯು ಶೇಕಡ ೮೫ ರಿಂದ ೧೦೦ ರಷ್ಟು ಸಮಾನತೆ ತೋರಿಸುತ್ತದೆ. ಹತ್ತು ಅಣಬೆಗಳಾದ *ಸ್ಕಿಜೋಫಿಲ್ಲಮ್ ಕಮ್ಯೂನ್*, *ಟ್ರಿಮೆಲ್ಲ ಫ್ಯೂಸಿಫಾರ್ಮಿಸ್*, *ಲೆಂಟಿನಸ್ ಸಜೋರ್-ಕಾಜು* (ಡಬ್ಲ್ಯು ಜಿಎಂ-೩೫), *ಲೆಂಟಿನಸ್ ಸಜೋರ್-ಕಾಜು* (ಡಬ್ಲ್ಯು ಜಿಎಂ-೩೬), *ಲೆಂಟಿನಸ್ ಪಾಲಿಕ್ರಾಸ್*, *ಲೆಂಟಿನಸ್ ಸ್ಟ್ರಿಗೋಸಸ್*, *ಟರ್ಮಿನಾಟೋಸಸ್ ಯೂರಿಹಿಯುಸ್*, *ಪ್ಲೆರೋಟಿಸ್ ಪೆಲೊನಾರಿಯುಸ್*, *ಫ್ಲೆಟಿಯುಸ್ ಪೆಟಾಸಾಟಿಸ್* ಮತ್ತು *ಪಾಲಿಫೊರಸ್ ಟೆನ್ಯುವಿಕಸ್*ಗಳನ್ನು ಅವುಗಳ ಕಾಂಡಗಳನ್ನೂ ಉಪಯೋಗಿಸಿ ಆಲೂಗಡ್ಡೆ ಡೆಕ್ಲೋಸ್ ಅಗರ್ (ಪಿಡಿಎ) ಮೇಲೆ ಅಂಗಾಂಶ ಕೃಷಿ ಮಾಡಲಾಯಿತು. ಇವುಗಳ ಪೈಕಿ, ಲೆಂಟಿನಸ್ ಸಜೋರ್-ಕಾಜು ಪ್ರಭೇದವು ಉತ್ತಮ ಬೆಳೆವಣಿಗೆಯನ್ನು ಹೊಂದಿದ್ದು, ಈ ಅಣಬೆಯನ್ನು ಕೃಷಿ ಮಾಡಲಾಯಿತು. ಈ ಅಣಬೆಯು ಶೇ. ೫೦ ರಷ್ಟು ಭತ್ತದ ಹುಲ್ಲು ಮತ್ತು ಶೇ. ೫೦ ರಷ್ಟು ಮರದ ಹೊಟ್ಟನ್ನು ಬಳಸಿದಾಗ ಹೆಚ್ಚಿನ (ಶೇ. ೬೫.೨೫ ರಷ್ಟು) ಮಧ್ಯಮ ಪರಿವರ್ತನೆಯನ್ನು ಹೊಂದಿದ್ದು, ಕೇವಲ ಮರದ ಹೊಟ್ಟನ್ನು ಬಳಸಿದಾಗ ಕನಿಷ್ಠ ಮಧ್ಯಮ ಪರಿವರ್ತನೆ ಕಂಡುಬಂದಿದೆ. ಸಸಾರಜನಕ, ಕೊಬ್ಬಿನ ಅಂಶ ಮತ್ತು ನಾರಿನ ಅಂಶ ಪೌಷ್ಟಿಕಾಂಶವು ಕ್ರಮವಾಗಿ ಶೇ. ೨೨.೩೩-೩೪.೪೬, ೧.೮-೪.೦ ಮತ್ತು ೫-೧೨ ರಷ್ಟು ಕಂಡುಬಂದಿದೆ. ಖನಿಜಗಳಾದ ಕ್ಯಾಲ್ಸಿಯಂ, ಮೆಗ್ನೀಸಿಯಮ್, ಕಬ್ಬಿಣ, ಸತು ಮತ್ತು ರಂಜಕಗಳು ಪ್ರತಿ ಗ್ರಾಂಗೆ ಕ್ರಮವಾಗಿ ೪೮.೪೫-೧೪೩.೯೬, ೧೪.೭೮-೧೪.೭೮-೫೯.೪೯, ೮.೪೪-೩೩.೩೨, ೦.೫೮-೩.೪ ಮತ್ತು ೧೦೭.೩೩-೫೦೫.೬೬ ಮೈಕ್ರೋಗ್ರಾಂಗಳಷ್ಟು ಕಂಡುಬಂದಿದೆ.

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I INTRODUCTION

Mushrooms grown in wild are picked up and eaten by mankind is a tradition from the time immemorial. The ancient Egyptians called mushrooms “sons of the gods” and “plants of immortality” that provides strength for soldiers in battle, Romans regarded as “Food of the Gods”; the Chinese considered them as “the life elixir”, while shiitake mushroom was highly prized by the Japanese emperor (Chang and Buswell, 1996). Mushrooms were served as main dish in parts of Russia and South Africa. Thus, mushrooms were famed due to their taste, delicacy, flavour and nutritional quality. There are about 69,000 known mushroom species of which 2000 species are regarded as prime edible mushrooms. But, a few species are being cultivated commercially around the world (Chang and Miles, 2004).

Mushrooms are grouped as edible, poisonous and medicinal. The edible mushrooms are rich in proteins (20-35% on dry wt. basis), vitamins and minerals. They are poor in fats and carbohydrates content. The mushroom proteins are considered to be of high quality containing all the essential amino acids required for an adult individual. They are rich in lysine and tryptophan, the two essential amino acids that are deficient in cereals. They are also known to be excellent source of riboflavin, nicotinic acid and pantoic acid. Minerals such as calcium, phosphorous and potassium are present along with fair quantity of copper and iron which are essential for bones, teeth and eye sight. Potassium/ Sodium ratio is very high in mushrooms, which is desirable for patients of hypertension. Medicinal mushrooms contain potential pharmaceutical compounds which are effective against cancer, cholesterol reduction, stress, insomnia, asthma, allergies and diabetes (Wani *et al.*, 2010). Non edible/poisonous mushrooms have scientific curiosity as they possess various toxins. They can also be used for various other purposes such as bioremediation/ biodegradation.

Mushrooms grow in almost all types of soil, on decaying organic matter, wood stumps, termite nests etc. Majority of mushrooms are saprophytes and some are associated with plant roots which are referred to as mycorrhizal mushrooms. Mushrooms emanate well at relative humidity levels around 95-100 % and substrate moisture levels at

50 to 75 %. Since, mushrooms are ephemeral in nature; their documentation needs constant survey and collection during appropriate seasons. In 1990 the magnitude of fungal diversity was estimated to be at least 1.5 million species of which 140,000 species produce fruiting bodies of sufficient size and suitable structure to be considered as macro fungi. About 7000 species are considered to possess varying degrees of edibility, and more than 3000 species are regarded as edible mushrooms. To date, only 200 of them are experimentally grown, 100 economically cultivated, approximately 60 commercially cultivated, about 10 have reached an industrial scale of production in many countries (Rajaratnam and Thiagarajan, 2012). Many more potential species remained unexplored in the womb of nature.

Mushrooms are good source of quality protein, rich in vitamins, minerals and low in calorie with negligible starch, sugars and fats. In addition to their nutritive value, many edible and non-edible mushrooms have long been used for medicinal purposes. The important therapeutic properties includes, antioxidants, hypertension, cholesterol lowering, liver protection, anti-inflammatory, diabetic and anti-microbial (Wasser and Weis, 1999; Hobbs, 1995). In India, only four species namely *Agaricus bisporus* (white button mushroom), *Pleurotus sajorcaju* (dhingri mushroom), *Calocybe indica* (milky mushroom) and *Volvariella volvacea* (paddy straw mushroom) are commercially cultivated. Annual production of mushrooms has been estimated to be 40000 MT. *Agaricus bisporus* contribute 80-85 per cent, *Pleurotus* species contribute 15-19 per cent and other varieties contribute one per cent of the total production (Sharma and Upadhyay, 1998). Mushroom possesses ephemeral character and disappears within a day. Hence, mushroom hunting is not a simply roaming through the forest after it rains. It is an art, a skill and meditation. Further, all mushrooms are not gilled; therefore giving a full account of their classification is difficult. Some have pores underneath (*Boletus*), others have spines, such as the hedgehog mushroom and other tooth fungi, and so on. Based on their external appearance and inherent properties such as odor, texture etc., mushrooms have been named as polypores, puffballs, jelly fungi, coral fungi, bracket fungi, stinkhorns, and cup fungi. Thus, the term has more common application to macroscopic fungal fruiting bodies than one having precise taxonomic meaning.

Western Ghats is also known as Sahyadris (in Sanskrit) 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 with localized extremes touching 9,000 mm. The eastern region of the Western Ghats which lies in the rain shadow, receive far less rainfall averaging about 1,000 mm bringing the average rainfall figure to 2,500 mm 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, Chikmagalur, 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 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 life cycle of mushroom starts with germination of spore that produces a primary mycelium. This mycelium continues to grow in branches and forms mycelial network. When two sexually complementary hyphal networks intercepts one another and make contact, the resulting mycelium formed is dikaryotic. This mycelium is fertile and capable of producing fruit bodies. Characterization of mushroom species requires basic knowledge on the structure of the fungi. The Phenotypic characters used for identification of mushroom species are shape, size, texture, colour and odour of the fruiting body.

However, in recent years, molecular tools occupied important position in mushroom taxonomy. Molecular markers, particularly DNA based techniques are quick and reliable to establish identities of wild mushrooms. After the advent of cycle sequencing methodology, direct sequencing of PCR products became a routine subject at least in organelle DNA loci or repetitive nuclear DNA such as ribosomal DNA.

Ribosomal RNA genes exist in genome as multiple copies arranged in tandem repeats along with one or more chromosomes. The Internal Transcribed Spacer (ITS) region/ 18S rRNA gene sequence are the most widely used techniques in molecular phylogenetics of mushroom as these sequences are conserved irrespective of life history and evolution.

Internal transcribed spacer (ITS) refers to the spacer DNA (non-coding DNA) situated between the small-subunit of ribosomal RNA (rRNA) and large-subunit of rRNA genes in the chromosome or the corresponding transcribed region in the polycistronic rRNA precursor transcript. The 5' to 3' of polycistronic rRNA precursor transcript contains 5' External Transcribed Spacer (ETS)-18S rDNA-ITS1-5.8S rDNA-ITS2-28S rDNA-3' ETS. Internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) has been widely used in phylogenetics of mushroom at both species and genus level characterization because, it is 1) easy to amplify from small quantities of DNA, 2) due to high copy number of rRNA genes and 3) high degree of variation between closely related species. Therefore, the present study was intended to explore the mushroom diversity in the Western ghats of Karnataka with the following objectives.

1. Ethnomycological survey, collection and documentation of wild mushrooms in the Western Ghats of Karnataka.
2. Identification of the mushrooms based on morphological (phenotypic) and molecular characters.
3. Pure culture isolation of elite species by tissue or spore and evaluation for cultivation.
4. Nutritional analysis of important edible mushrooms and conservation of elite species.

II REVIEW OF LITURATURE

Fungi are eukaryotic, heterotrophic, absorptive organisms that develop a rather diffuse, branched, tubular body and reproduce by means of spores. The two important work the “Methodica Fungorum” by Persoon and “Systema Mycologicum” by Fries describes the fungal taxonomy. Mushrooms belong to the *Eumycota* (true fungi) that comprises five subdivisions viz., i) Ascomycotina, characterized by producing meiospores within a sac-like structure called ascus, ii) Basidiomycotina, where meiospores produced externally on cells called basidia, iii) Mastigomycotina, recognized by the production of motile, flagellate meiospores; iv) Zygomycotina, an heterogeneous group produces non septate hyphae and thick-walled meiospores called zygosporoes, and v) Deuteromycotina, which are also known as fungi imperfecti, they lack sexual stage (Manoharachary *et al.*, 2005).

Mushrooms are macro fungi with distinctive fruiting bodies that can either be epigeous or hypogeous and large enough to be seen by naked eye (Chang and Miles, 1992). Mushrooms were found on ancient sculpture of Roman around 1000BC to 200AD. The soma rasa of the Rig Veda consist of hallucinogenic mushroom *Amanita muscaria*, still it was using by the shamans of remote tribes in Siberia (Dobberstein, 1995).

Theophrastus (372-287 BC) wrote the food values of mushrooms. The diversity of fungi and their natural beauty occupy a prime place in biological world and India has been a cradle for these species. Humid weather and high rainfall during monsoon is congenial atmosphere for the establishment of many kinds of mushroom flora which includes edible, medicinal and poisonous types. 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 *et al.*, 2006). Only a fraction of total fungal wealth has been subjected to scientific scrutiny and mycologists continue to unravel the hidden wealth of fungi. One third of fungal diversity of the globe exists in India and of this only 50 % have been characterized so far (Manoharachary *et al.*, 2005).

2.1 Mushroom life cycle

The mushroom life cycle starts with germination of spore. The germinated spore form a thread like structure called mycelium. Three types of mycelia were involved in the life cycle of Basidiomycota. Primary mycelium formed by germination of basidiospore and consists of hyphae. Secondary mycelium consists of dikaryotic hyphae and tertiary mycelium consists of specialized dikaryotic hyphae which participate in the form of basidia (Dube, 2013). The matured mycelia fuse and form a network that leads to formation of small pinheads. The pin heads rapidly enlarge into button stage. Typical mushrooms form an umbrella-like structure and finally the gills underneath. At the edges of gills, special cells are formed where two nuclei eventually fuse and double their chromosomes. These cells are called basidia. The diploid nuclei undergo meiosis and produce four meiotic nuclei. These four haploid nuclei migrate outside the basidial cell and form basidiospores.

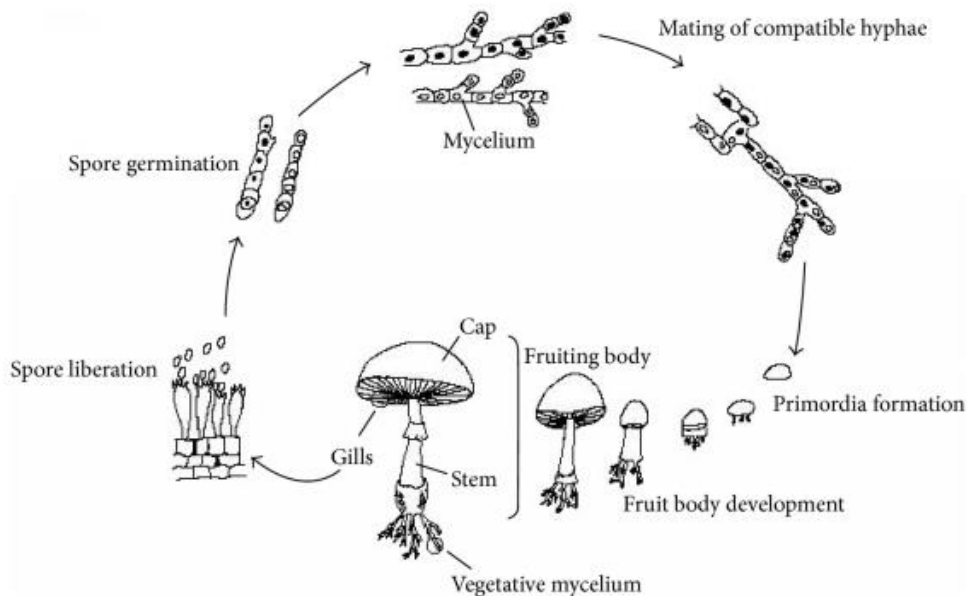


Fig. 1: Life cycle of mushroom (Lull *et al.*, 2005)

The new spore develops inside the cap fully expands at maturity and disseminate in soil. The mycelium from germinating spore is called primary mycelium. It is uninucleate and haploid. Mycelia from different spore tend to ramify and fuse to form

secondary mycelium which is septate. The aggregation of mycelium gives rise to fruiting stage of the visible mushroom thus completing the life cycle.

2.1.2 Myths about Mushrooms

Local people of Lumle area in Nepal thought that, both youngsters and elders eat edible mushrooms up to Shrawana (middle of August according to Hindu calendar) and after this month only elderly people eat and youngsters are not allowed to consume. According to their beliefs eating mushrooms after Shrawana by youths may cause some sort of unwanted events in their home. Rainy season was the ideal time for mushrooms collection. The 'Purnima', 'Aunsi' and 'Domasey' were the best 'Tithis' in which local mushroom hunters prefer to go for hunting because, during these days the moisture content of the atmosphere raises creating favourable condition for mushroom growth (Adhikari, 1999; Adhikari *et al.*, 2006). In Nepal the people believed that if a person hits a mushroom with leg, he/she would have lame children. These superstitious beliefs gradually faded away as science advanced.

The different Aeta sub-tribes were governed by different indigenous beliefs in mushroom collection, utilization, and cultivation. (i) They do not cook mushrooms together with yellow or red vegetables or with shrimps, fish, and snails as eating these cooked food could cause fatal sickness. (ii) A large *Termitomyces* species was believed to be guarded by supernatural beings. Thus, before collecting this mushroom, sub-tribe (Mag-Indi) of Aeta community performs ritual dancing or asks permission from the spirits and kisses the ground. (iii) Sub-tribe Zambal simply thanks their local deity "Apo Namalyari" for the abundance of their collected mushrooms. (iv) Sub-tribes Mag-Antsi in Tarlac forbids combing their hair and singing while cooking mushrooms. They believed this could attract lightning to strike the person. (v) Sub-tribe Zambal also mentioned that they should not observe the development of mushrooms on the ground as the mushroom will not continue to grow. (vi) All Aeta sub-tribes believed that spontaneous lightning causes the growth of mushrooms (Leon *et al.*, 2012).

The mythology and the traditional knowledge is equally controlling factor to be aware of poisonous mushrooms. In Nepal, people break the fruit body, smell, taste and

then say whether or not it is edible (Adhikari, 2005). They believed that the species with annulus nearby cap were poisonous and species with more curved pileus with annulus were also poisonous. Species turning to white after touching is poisonous whereas species turning black after touching are said to be edible. Further, species found on the fodder plants are generally edible and species which peeled off easily are edible. However, there is no fool proof method available so far to detect mushrooms about their edibility in the laboratory.

2.2 Major Significance of mushrooms

Mushrooms can be grouped as edible, medicinal and poisonous types. Many mushrooms are hallucinogenic which were used in the antiquity. Edible mushrooms have significant amount of proteins, minerals and lower amounts of carbohydrates and fat. Thus edible mushrooms play an important role in human dietetics. Many species of medicinal mushrooms have potential pharmaceutical properties.

2.2.1 Nutritional value of Mushrooms

Agaricus bisporus, *Agaricus bitorguis*, *Lentinula edodes*, and *Pleurotus ostreatus* were found to be good sources of vitamin B₂, niacin, and folates. The content of these mushrooms varied in the ranges 1.8-5.1, 31-65, and 0.30-0.64 mg/100 g dry weights respectively. Potassium, Phosphorus, Zinc, and Copper contents varied from 26.7-47.3 g/kg, 8.7-13.9 g/kg, 47-92 mg/kg, and 5.2-35 mg/kg respectively (Mattila *et al.*, 2001). *Ramaria botrytis* was found to be most delicious and highly preferred species in all the sites of Kathmandu valley (Adhikari, 2005).

The indoor grown *L. eddoes*, *Ganoderma lucidum* and *Grifola frondosa* mushrooms on 6-8 hours of sunlight exposure of stimulated the production of vitamin D from low levels of 134-46000 IU, 66-2760 IU and 469-31900 IU, respectively. The most vitamin D was produced in *L. edodes*, whose spore-producing lamellae were exposed to the sun. Dried mushrooms also elicited vitamin D production subsequent to sunlight exposure. The sunlight-activated biosynthesis of vitamin D from ergosterols within mushrooms has enriches the health of the humans (Stamets, 2005).

The macronutrient profile of cap, stalk, and tuber of *Pleurotus tuber-regium* was separately analyzed. Crude protein was highest (4.1 to 13.8 g/100 g) in cap and total carbohydrates ranged from 34.0 to 56.2, while the crude fat and ash contents were low. Potassium was the most abundant nutritive element found in highest concentration in stalk (3.3 mg/g) while copper was found in trace amounts in all the parts of the fruiting body. Cyanide, phytate and tannin content were present below harmful level (Akindahunsi and Oyetayo, 2006).

Pleurotus ostreatus, *Pleurotus sajorcaju*, *Pleurotus florida* and *Calocybe indica* that were very popular among the cultivated mushrooms in Bangladesh. These mushrooms were rich in proteins (20-25 %) and fibers (13-24 % in dry samples) and contained a lower amount of lipid (4 to 5 %). The carbohydrate contents ranged from 37 to 48 % and rich in mineral contents (8-13 %). The cap and gills were rich in protein and lipid while stripe was rich in carbohydrate and fiber. The moisture content of mushrooms ranged from 86 to 87.5 %. This study indicated that mushrooms are rich in nutritional value (Alam *et al.*, 2008).

Gencelep *et al.* (2009) collected thirty wild-grown common edible mushrooms from Erzurum province, Turkey. The mineral content of mushrooms were for Mg (0.90-4.54), Ca (0.17-8.80), K (12.6-29.1), Na (0.03-4.85) and P (0.64-4.49), while minimum and maximum micronutrient contents of mushrooms were determined as mg/kg for Zn (26.7-185), Fe (50.1-842), Cu (9.23-107) and Mn (5.54-135). The potassium content was found to be higher than those of the other minerals in all the mushrooms.

The wild mushrooms (*Laccaria laccata*, *Leucopaxillus giganteus*, *Russula rosea*, *Cantharellus cibarus*, *Tricholoma saponaceum*, *Agaricus arvensis*, *Boletus edulis*, *Clavulina rugosa*, *Hydnum repandum*, *Cantharellus tubaeformis* and *Lepista nuda*) of East Black Sea region, Turkey were contains potassium (21800-39800 µg/g), Phosphorus (2590-14000 µg/g) Calcium (268-1600 µg/g), Magnesium (561-1210 µg/g), Iron(74-829 µg/g), Copper (11.2-321 µg/g), Zinc(36.2-241 µg/g), Manganese (14.1-76.5 µg/g) and Cobalt (0.13-2.85 µg/g) in dry weight basis. *Laccaria laccata* contained a heavy metal Arsenic about 145 µg/g. These results showed that the mushrooms could be a useful

component (mineral) for human diets and toxic due to presences of heavy metals (Faik *et al.*, 2011).

Lignolytic fungi *Pleurotus pulmonarius* degrades crude oil and palm kernel sludge in the soil with increased in organic carbon, organic matter, nitrogen content in crude oil contaminated soil at 5% from 2.17 % - 2.40 %; 3.79 % - 4.16 % and 0.22 % - 0.24 % respectively. Heavy metal Lead decreased at 2.5 %, 20 %, 40 % and Copper at 1 %, 40 % palm kernel sludge contamination after 60 days of incubation. The improvement of nutrient content and bioaccumulation of heavy metals at all levels of crude oil concentrations with inoculation of *P. pulmonarius*. It implies that the mycoremediation of crude oil and palm kernel sludge polluted soil (Adenipekun and Lawal, 2011).

Celestine *et al.* (2013) compared the nutrient contents of five wild mushroom species found in Nigeria. Vitamins A, C, E and niacin were found to be relatively higher in *Agaricus bisporus*. Thiamin was also, relatively higher in Coral mushroom, followed by *Agaricus bisporus*, *Pleurotus ostreatus*, *Auricularia polytricha* and *Lentinus sajor*. Vitamins A and E were only found in *Agaricus bisporus*. The mushroom species were good sources of vitamin C, B1 and niacin, which could be considered as ideal supplements for low vitamin diets.

A mushroom *Pleurotus citrinopileatus* from Kakamega forest of Western Kenya contained 22.1 % protein, 1.32 % crude lipid and 20.78 % fibre. Mineral content of the mushroom was found that 2.28 % Potassium, 0.0002 % Copper, 0.0015 % Zinc and 0.01 % Iron. Leucine, Valine, Threonine, Lysine, Phenylalanine, Isoleucine, Methionine and Tryptophan amino acids were in decreasing order of abundance. Nicotinic acid, Pantothenic acid and Riboflavin vitamin and minerals were present in mushrooms (Musieba *et al.*, 2013). Protein content of the *Termitomyces* species was found 27 % which was more than the cultivated mushrooms (Earanna *et al.*, 2013).

Das and Das (2014) conducted the food habitat survey and suggested that mushroom consumption was 18 % of the total food consumption in Chota Nagpur platue of West Bengal. They reported that *Astraeus* sp., *Volvariella* sp. *Lentinus* sp. and

Termitomyces sp. were sold in local markets of West Bengal. They also conducted histopathological studies of heart, liver, lung kidney and toxicity test for *Termitomyces* and *Lentinus* species, on Wistar rats. Rana *et al.* (2013) analyzed amino acid composition of two edible mushrooms. The dried *Pleurotus* sp and *Agaricus bisporus* contained 12.2 % and 33.3 % of protein, respectively. The essential amino acid Lysine was found in high concentration (1.87 %) in dry and fresh fruiting bodies as well. Chittaragi *et al.* (2014) revealed nutrient value of wild mushrooms which contained 83.6-90.43 % moisture, 4.76-11.46 % dry matter, 25.71-36.51 % protein, 4.45-10.29 % ash, 1.4-2.79 % lipid, 3.77-11.44 % fiber and 37.38-48.63 % carbohydrates. Nutritionally they are valuable source of healthy food.

Wild edible mushroom species viz., *Macrolepiota dolichaula*, *Macrolepiota procera*, and *Macrolepiota rhacodes* contained 16.45 - 19.95 % protein, 2.9 - 3.4 % fat, 2.5 - 5.1 % crude fiber, 56.2 - 68.19 % carbohydrates, 1.93 - 7.3 % ash and 7.8 - 8.8 % moisture. The minerals content of these species are as follows: 5 - 28 mg/100g Ca, 143-254 mg/100g Mg, 5 - 9 mg/100g Cu, 241 - 276 mg/100g Fe, 1- 5 mg/100g Mn, 0.06 - 0.09 mg/100g Zn, 0.06 - 0.10 mg/100g Se, 0.062 - 0.087 mg/100g Hg, 0.0014 - 0.0019 mg/100g Cd and 0.074 mg/100g As. Mushrooms were found to be good sources of bioactive compounds like phenolics (5.9-16.81 mg/g), flavonoids (1.36-1.76 mg/g), alkaloids (0.048 - 0.103 mg/g), β -carotene (0.12 - 0.29 μ g/gm) and lycopene (0.05-0.12 μ g/gm) (Kumari *et al.*, 2014).

2.2.2 Medicinal value of mushrooms

Mushrooms have been valued as medicine by man since times immemorial. The traditional knowledge about the bio-resources, which is the outcome of experiences passed on to successive generations, was disappearing with the passage of time due to lack of interest in traditional system.

The mushroom *Inonotus obliquus* has been used as a folk medicine in Russia and western Siberia (Saar, 1991). *Ganoderma lucidum* has been used since 4th century A.D. and is well known in Chinese medicine due to its beneficial effects and nontoxic nature (Liu, 1999).

The PL-1 heteroglucan had a backbone consisting of 1-4-linked α -D-glucopyranosyl residues and 1-6-linked β -D-galactopyranosyl residues with branches at *O*-6 of glucose residues and *O*-2 of galactose residues, composed of terminal glucose, 1-6-linked glucosyl residues and terminal rhamnose. PL-3 was highly branched glucan composed of 1-3-linked β -D-glucopyranosyl residues substituted at *O*-6 with 1-6-linked glucosyl residues. PL-4 was comprised of 1-3, 1-4 and 1-6-linked β -D-glucopyranosyl residues and 1-6-linked β -D-mannopyranosyl residues. These polysaccharides enhanced the proliferation of B- lymphocytes and T-lymphocytes *in vitro* to varying contents and PL-1 exhibited an immune-stimulating activity in mice (Bao *et al.*, 2002).

Polysaccharides of *Ganoderma lucidum* possess the hypoglycemic effect on normal mice. One mechanism is through its insulin releasing activity due to facilitation of Ca^{2+} inflow to the pancreatic beta cells (Zhang and Lin, 2004). Berger *et al.* (2004) reported the cholesterol lowering properties of *Ganoderma lucidum*. Organic reactions containing oxygenated lanosterol derivatives inhibited cholesterol synthesis in T9A4 hepatocytes. In hamsters, 5% *Ganoderma lucidum* did not affect low-density lipoprotein; but decreased total cholesterol 9.8%, and high-density lipoprotein 11.2%. *G. lucidum* (2.5 and 5%) had effects on several fecal neutral sterols and bile acids. *G. lucidum* doses reduced hepatic microsomal ex-vivo HMG-CoA reductase activity. In mini pigs, 2.5 *G. lucidum* decreased total cholesterol, low-density lipoprotein and high-density lipoprotein cholesterol 20, 27, and 18%, respectively ($P < 0.05$); increased fecal cholestanol and coprostanol; and decreased cholate. *G. lucidum* had potential to reduce LDL cholesterol *in vivo* through various mechanisms.

A complex polysaccharide (Glucuronoxylomannan) from the fruiting bodies of *Tremella fuciformis* exhibited a significant dose-dependent hypoglycemic activity in normal mice and also showed significant activity in streptozotocin-induced diabetic mice by intraperitoneal administration (Miles and Chang, 2004). Akpaja *et al.*, 2005 reported the ethnomycology and indigenous uses of mushrooms among the Bini-speaking people of Nigeria. *Daldinia concentrica* (Bolton) was used for curing stomach upset, skin diseases, stomach ulcer, whooping cough, and prevention of excessive growth of the foetus for easy delivery. *Calvatia* sp. was used for stopping chronic hiccups and for

curing barrenness in women as well. *Pleurotus tuberregium* ingredients were used for making gunpowder, apart from being used as food and medicine.

Hyodo *et al.* (2005) conducted nationwide survey on complementary and alternative medicine (CAM) for cancer patients in Japan. They found most frequently used Complementary and Alternative Medicine (CAM) product was mushrooms. *Exobasidium butleri* was squeezed during sunny days as alternative to water to make throat wet and called this mushroom as “Pani Pokey Chyau”. (Adhikari *et al.*, 2005). *Grifola frondosa* and *Ramaria botrytis* were used to get relief from muscles pain (Adhikari *et al.*, 2005).

Beta-glucans and oligosaccharides of *Agaricus blazei* both showed anti-hyperglycemic, anti-hypertriglyceridemic, anti-hypercholesterolemic, and anti-arteriosclerotic activity indicating overall anti-diabetic activity in diabetic rats, oligosaccharides had about twice the activity of β -glucans with respect to anti-diabetic activity (Kim *et al.*, 2005). Zhu *et al.* (2006) expressed *Vitreoscilla* hemoglobin (VHb) gene in *Tremella fuciformis* to increase cell density in submerged fermentation by enhancing oxygen uptake. They suggested that the CaMV35S promoter was capable of driving the expression of heterologous genes in *T. fuciformis*. *Ganoderma lucidum*, *Phellinus rimosus*, *Pleurotus florida* and *Pleurotus pulmonaris* (Ajith and Janardhanan, 2007).

Anti-diabetic activities of the exopolysaccharides (EPS) produced by submerged mycelial culture of *Tremella fuciformis* in *ob/ob* mice were investigated by Cho *et al.* (2007). They suggested that EPS was exhibited considerable hypoglycemic effect and improved insulin sensitivity possibly through regulating PPAR-gamma-mediated lipid metabolism. These results indicated that *Tremella fuciformis* has potential oral hypoglycemic effect as a functional food for the management of diabetes mellitus.

Hypoglycemic effects of *Ganoderma applanatum* exo-polymer (GAE) and *Collybia confluens* exo-polymer (CCE) were achieved in streptozotocin (STZ)-induced diabetic rats by administration at a level of 100 mg/kg body weight (BW) daily for 3

weeks. The administration of GAE and CCE substantially reduced the plasma glucose levels by as much as 22 % and 25.9 %, respectively, when compared to control group. The GAE and CCE also lowered the plasma total cholesterol and triglyceride levels by 20.3 % and 22.5 %, and by 22.7 % and 25.5 %, respectively. The results strongly demonstrate the potential of GAE and CCE in combating diabetes in experimental animals (Yang *et al.*, 2007). Similarly, anti-hyperglycemic, anti-lipid peroxidative and antioxidant effects of *Inonotus obliquus* in alloxan-induced diabetic mice was observed by Sun *et al.* (2008).

The *Cordyceps* species of fungus had pharmacological and therapeutic property and it has been known for ethno-pharmacological uses. Mushroom contains a compound called Cordycepin (30deoxyadenosine) which has a potent anti-cancer, antioxidant, anti-inflammatory activities (Kunwar *et al.*, 2010). The *Phellinus* fruiting body showed potential antimicrobial activities against *Pseudomonas aeruginosa* with maximum inhibition zone of 42 mm from 200 mg of aqueous extract and minimum of 5 mm at 50 mg of methanol extract (Balakumar *et al.*, 2011).

The high beta glucan content was observed in the mushroom species viz., *Pycnoporus cinnabarinus*, *P. coccineus*, and *P. sanguineus* growing in highland dry evergreen forest habitat. High beta glucan content of mushrooms were correlated with specific habitat characteristics of high altitude, high crown cover percentage and high rainfall (Boonyanuphap and Hansawasdi, 2011). The protein extracts of *Calvatia lilacina*, *Pleurotus ostreatus* and *Volvariella volvacea* exhibited therapeutic efficacy against human colorectal adenocarcinoma cells and human monocytic leukemia cells. The protein extracts of *Pleurotus ostreatus* induced apoptosis in SW480 cells partially through ROS production, GSH depletion and mitochondrial dysfunction (Wu *et al.*, 2011).

Secondary metabolites of Basidiomycota represents a largely uncharacterized source of pharmaceutically relevant natural products. Terpenoids were the primary class of bioactive compounds isolated from mushrooms. *Omphalotus olearius* was identified 50 years ago as a prolific producer of anticancer illudin sesquiterpenoids. The draft

genome sequence of *O. olearius*, revealed diverse network of sesquiterpene synthases and two metabolic gene clusters associated with illudin biosynthesis (Wawrzyn *et al.*, 2012). *Tremella fuciformis* and *Auricularia auricula* have long been studied for their medicinal purposes such as anti-oxidant, anti-tumour, anticoagulant, hypoglycaemic activity and memory improvement (Khamlue *et al.*, 2012).

The enzyme Tyrosinase catalyses the O-hydroxylation of monophenols (monophenolase activity) and the oxidation of o-phenols to reactive O-quinones (diphenolase activity), both reactions using molecular oxygen. In fungi, tyrosinases are associated with melanin production, which constitute a mechanism of defense to stress such as UV radiation, free radicals, gamma rays, dehydration and extreme temperatures and contribute to the fungal cell-wall resistance against hydrolytic enzymes, avoiding cellular lysis. The production and characterization of tyrosinase from *P. sanguineus* CCT-4518 were investigated by Duarte *et al.* (2012) and the enzyme was stable at 45 °C for 15 minutes while higher temperatures decreased tyrosinase activity.

Wild mushrooms were used in traditional healthcare system to treat wide spectrum of diseases like scalds, burns, cold, leucoderma, headache, fever, abdominal pain, eczema, constipation, hypertension, leucorrhoea, baldness, mumps, diabetes, arthritis, liver diseases, rheumatism, asthma, and respiratory infections in Himalayas of Kashmir (Pala *et al.*, 2013).

Willis *et al.* (2013) conducted an experiment to evaluate the production performance, blood parameters and natural coccidiosis oocyst excretion in floor reared broiler chickens by feeding of four medicinal mushrooms Shiitake (*Lentinula edodes*), Reishi (*Ganoderma lucidum*), Oyster (*Pleurotus ostreatus*) and Cordyceps (*Cordyceps inensis*) myceliated grain. They found that Eimeria oocyst counts, blood differential percentages and both bursa and body weights differed significantly ($P \leq 0.05$) between treatment groups. Five percent Oyster produced the highest average body weight at 3.21 kg, while 10 % Cordyceps produced the lowest at 2.33 kg. A higher oocyst count and increased heterophil percentage was observed at 5 % Reishi. There were some basic trends in the patterns of heterophil and lymphocyte percentage elevation at 10% Shiitake.

Mushrooms were influences body weight of broiler chickens and show some immune enhancement via the bursa of Fabricius. *Ganoderma tsugae*, *Tremella mesentrica* and *Microglossum virde* were used in traditional medicine in Vindyan ranges of Madhya Pradesh (Vyas *et al.*, 2014). Ethno-medicinal uses of wild edible mushrooms among different tribes of Orissa were recorded. *Astraeus hygrometricus* spore mass blended with mustard seed oil used as salve against burns. *Geastrum species* used to reduce bleeding and swelling and *Termitomyces reticulates*, *Lactarius sp.*, *Volvariella sp.* and *Volvariella glandiformis* were used to lower high blood pressure (Panda and Tayung, 2015).

Termitomyces albuminosus and *Termitomyces clypeatus* syrup was used for the remedy of measles and yellow fever. *Termitomyces badius* and *Termitomyces robustus* syrup were used as remedy for constipation, laziness, indolence and inactiveness. *Termitomyces eurhizus* used as a lotion for skin diseases with mixing the herb (*Cynodon doctylon*). *Termitomyces globulus* fresh extract powder or paste of fruiting bodies was used to heal wounds. *Termitomyces heimii* and *Termitomyces palpensis* were used to cure jaundice and diarrhoea. *Termitomyces letestui* and *Termitomyces arghakhanchensis* soup were used for remedy of inappetence, abdominal disorder, indigestion and stomach ache. *Termitomyces mammiformis* was used to strengthen the body, for increase in body healing ability and its powder was taken as one table spoon with hot water after dinner till recovery is evident. *Termitomyces microcarpus* fruiting bodies decoction was given as tonic for stimulating power. *Termitomyces schimperi* was used to cure cut wound and skin diseases. *Schulzeria umkowaan* powder was used in mouth-wash for buccal cavity infection, and relief for arthritics pain (Aryal and Budathoki, 2016).

2.3 Documentation and identification of mushrooms

Adhikari *et al.* (2005) reported that the *Cantharellus cibarius*, *Grifola frondosa*, *Laccaria laccata*, *Lactarius volemus*, *Laetiporus sulphureus* and *Termitomyces clypeatus* were good edible species of Lumle region. *Ramaria botrytis* was considered as the best edible mushroom in Kathmandu. *Hericium erinaceus*, *Oudemansiella radicata*, *Ramaria flaccida* and *Russula chloroides* were considered to be tasty ones. *Auricularia auricula-*

judae, *Clavulinopsis fusiformis*, *Exobasidium butleri* and *Lactarius piperatus* are edible but not tasty according to their survey.

Esan tribes of Nigeria were collect *Pleurotus tuberregium*, *Lentinus squarrosulus*, *Volvariella* sp., *Agaricus* sp., *Auricularia* sp., *Termitomyces* sp., *Schizophyllum commune* and *Coprinus* sp. during early monsoon. These mushrooms were used as meat substitute, sold fresh in local markets, or dried for use during the summer. The elderly women lead the younger women and children to collect and identify the edible mushrooms from poisonous ones (Osagualekhor and Okhuoya, 2005).

Ju and Hsieh (2007) collected nine species of *Xylaria* from Taiwan from nests of *Odontotermes formosanus*, the only known macrotermite in Taiwan. These include six newly described species viz., *X. acuminatitongissima*, *X. atrodivaricata*, *X. brunneovinosa*, *X. griseosepiacea*, *X. intraflava* and *X. ochraceostroma*, and three previously known species, *X. cirrata*, *X. escharoidea* and *X. nigripes*. Almomany and Saleh (2009) collected eight wild *Agaricus* mushrooms from different forests, field and coastal plains of North Cyprus. *Agaricus placomyces* was suspect poisonous, while edibility of *Agaricus bresadolianus* was not known and remaining six mushrooms viz. *Agaricus bernardii*, *Agaricus campestris*, *Agaricus porphyrocephalus*, *Agaricus silvicola*, *Agaricus vaporarius* were edible.

Karwa and Rai (2010) collected 153 species of mushroom in Melghat forest, Amravati District in the State of Maharashtra, India. Ten *Agaricus* species were recorded from different locations of the Melghat forest of these, seven species namely *Agaricus bitorquis*, *A. subrufescens*, *A. augustus*, *A. placomyces*, *A. essettei*, *A. basioanolosus* and *Agaricus* sp. were reported for the first time from the region.

The mushroom *Coprinopsis cinerea* was a classic experimental model for multicellular development in fungi because it grows on defined media which completes its life cycle in 2 weeks, produces some 108 synchronized meiocytes, and can be manipulated at all stages in development by mutation and transformation. The 37-megabase genome of *C. cinerea* was sequenced and assembled into 13 chromosomes.

Meiotic recombination rates vary greatly along the chromosomes, and retrotransposons were absent in large regions of the genome with low levels of meiotic recombination. The chromosome assembly of *C. cinerea* helps in understanding the evolution of multicellularity in the fungi (Stajich *et al.*, 2010).

Kamat *et al.* (2010) found *Pleurotus ostreatus* on logs of wild *Mangifera indica* in Goa region. Among many cultures BA-50/GUOMS strain was selected for cultivation under natural outdoor conditions. Spawn prepared on wheat grains was produced within 11 days and was used to ramify sterilized paddy straw as substrate using standard polybag technique. Spawn run was initially slow and took 52 days. Mushrooms fruiting bodies identical to the original wild type were produced in eight flushes at intervals of 8 to 13 days for each flush over a period of four months. These had pleasant odour, better shelf life, tougher texture, resistance to insects, fungi and bacteria, exhibited photosensitivity by producing brownish pigment in direct sunlight and responded well to outdoor conditions for fruit body initiation with ambient temperature ranging from 23-35°C.

Thiribhuvanamala *et al.* (2011) conducted survey for mushrooms in the Anaikatti, Attapadi, Palghat, Siruvani, Nilgiris and Kallar regions of the Western Ghats of India and recorded 68 mushroom flora belonging to 19 genera viz., *Agaricus* sp., *Amanita* sp., *Auricularia polytricha*, *Calocybe*, *Ganoderma lucidum*, *Lentinula edodes*, *Lycoperdon esculentum*, *Mycena* sp., *Macrolepiota* sp., *Pleurotus cystidiosus*, *P.djamor*, *P. pulmonarius*, *Polyporus versicolor*, *Ramaria* sp., *Russula* sp., *Ramella* sp., *Schizophyllum commune*, *Termitomyces*, *Trametes versicolor*, *Tricholoma giganteum*, and *Volvariella volvacea*. They reported that the seasonal occurrence of mushrooms viz. *Volvariella* from June-September; *Calocybe* from February-September; *Pleurotus* from June-July and November-January; *Auricularia*, *Lentinus*, *Agaricus* from October-January; *Tricholoma* during June; *Ganoderma*, *Polyporus*, *Trametes* from September-January and June-August; *Lycoperdon* during October; *Termitomyces* from July-October; *Tramella*, *Mycena* and *Rusulla* from January; *Ramaria* from October-November; *Schizophyllum* from June-October and January; *Amanita* from November-December and *Macrolepiota* during December.

Arun and Eyini (2011) collected 130 wild Basidiomycetes fungi. The polycyclic aromatic hydrocarbons degradation of *Phellinus* sp. and *Polyporus sulphureus* were higher in liquid state fermentation and the efficiency of the organisms declined in solid state fermentation and in soil treatment. *Phellinus* sp. showed better degradation in solid state fermentation and in soil. *Phellinus* sp. which had higher polycyclic aromatic hydrocarbons and lignin degradation showed higher biosurfactant production than other organisms. Manganese peroxidase was the predominant enzyme present in *Phellinus* sp. while lignin peroxidase (Lip) was predominant in *P. sulphureus*.

Mycophilic people were those, which demonstrate special interest towards fungi food or cultural activity. Nagaland ethnic group were considered to be mycophilic and have extensive mycological knowledge. *Termitomyces eurrhizus*, *Tricholoma giganteum*, *Tricholoma virgatum*, *Agaricus silvaticus*, *Agaricus campestris*, *Lentinus conatus*, *Lentinus cladopus*, *Pleurotus ostreatus*, *Pleurotus flabellanus*, *Schizophyllum commune*, *Lycoperdon* sp., *Clavtia gigantea* and *Auricularia delicate* were documented in Nagaland. *Termitomyces eurrhizus*, *Lentinus conatus*, *Schizophyllum commune*, *Tricholoma giganteum*, *Tricholoma virgatum* and *Pleurotus ostreatus* were marketing in Kohima district of Nagaland (Tanti *et al.*, 2011).

Srivastava *et al.* (2011) collected four different *Termitomyces* from the Gorakhpur forest Division of Uttar Pradesh. They identified mushrooms as *Termitomyces heimii*, *Termitomyces clypeatus*, *Termitomyces mammiformis* and *Termitomyces microcarpus* by using phenotypic characters like shape of perforatorium, stipe length, pileus length, margin of fruit body, colour of fruit body, gills, flesh, annulus, pseudorrhiza and spore print. They found all the four species of *Termitomyces* were great diversity in their morphological characters. The highest average weight of fruiting body was found in *Termitomyces heimii* (11.60 gm) followed by *Termitomyces clypeatus* (11.53 gm), *Termitomyces mammiformis* (11.20 gm) and *Termitomyces microcarpus* was 10.30 gm. They found *Termitomyces mammiformis* yield was higher than those of other three species.

Pandey and Veena (2012) documented Forty five mushrooms species from Shivamogga region and thirty from Kodagu region of Karnataka. They found Polypore members were predominant followed by gilled agarics in both the region. *Termitomyces medius*, *Dictyophora indusiata*, *Pleurotus djamor*, *Russula emetic* and *Auricularia spp.* were prominent edible mushrooms in Shivamogga region. Edible mushrooms *Tremella fuciformis*, *Auricularia polytricha*, *A. auricula*, *Termitomyces spp.* And *Pleurotus spp.* Were prominent edible mushrooms reported in Kodagu region. Other species like *Amanita pachycolea*, *Hygrocybe spp.*, *Stereum hirsutum*, *Phellinus spp.*, *Bjerkendrea spp.*, *Phaeolus spp.*, *Ramaria spp.*, *Clavaria spp.* and medicinal fungi *Ganoderma lucidum*, *Phellinus spp.*, *Pycnoporus cinnabarinus*, *Trametes spp.* were reported in these regions. *Termitomyces spp.* was called by different local names as 'Beru Anabae' in Shivamogga, 'Alande Kumi' in Kodagu and 'Kanda almi' in Konkani regions.

Selvam *et al.* (2012) collected and indentified wood rot fungi in Tamilnadu and coorg region of India. *Polyporus sp.*, *Daldenia sp.*, *Ganoderma lucidum*, *Polyporus hirsutus*, *Daedalea flavida*, *Schizophyllum commune*, *Phellinus sp.*, *Ganoderma sp.*, *Lenzites eximia*, *Ganoderma sp.*, *Ganoderma sp.* were documented from Kovai Kutralam. *Polyporus sp.*, *Trametes sp.*, *Poria sp.*, *Ganoderma sp.*, *Polyporus grammacephallus*, *Phellinus sp.*, *Phellinus sp.*, *Lenzites sp.*, *Ganoderma sp.* were collected from Aliyar. *Fomes sp.*, *Tricholomopsis sp.*, *Ganoderma sp.* and *Phellinus sp.* were from Kallar. *Ganoderma sp.*, *Daldinea sp.*, *Schizophyllum sp.*, *Phellinus sp.* and *Daldenia concentrica* occurrence were reported from Ooty. *Lenzites sp.* and *Ganoderma sp.* from Coonor. *Stereum hirsutum*, *Trametes gibbosa*, *Lepiota sp.*, *Crepidotus sp.*, *Trametes serialis*, *Microporus sp.*, *Ganoderma applanatum*, *Lenzites sp.*, *Poria sp.*, *Poria sp.*, *Irpex sp.*, *Phellinus sp.*, and *Gymnopilus sp.* were documented in Coorg and *Daldenia sp.* from Gudallur. *Polyporus hirsutus*, *Daedalea flavida* and *Phellinus sp.* were found high lignin degrading macro fungi.

Thirty-eight different mushrooms were utilized by the Aeta communities in three provinces of Central Luzon, Northern Philippines: 21 species in Pampanga province, 10 species in Tarlac province and 19 species in Zambales province. *Auricularia auricula*, *A. polytricha*, *Calvatia sp.*, *Ganoderma lucidum*, *Lentinus tigrinus*, *L. sajor-caju*, *Mycena*

sp., *Pleurotus* sp., *Schizophyllum commune*, *Termitomyces clypeatus*, *T. robustus*, *Termitomyces* sp. 1, *Termitomyces* sp. 2, and *Volvariella volvacea* species were collected and identified based on their morphological characters. Twelve of the identified mushrooms were consumed as food while *Mycena* sp. and *Ganoderma lucidum* were used as medicine and house decoration, respectively (Leon *et al.*, 2012). Saadatm and Azarboeyeh (2012) recorded *Amanita virosa*, *Russula vesca* and *Russula persicina* for the first time from the forest of Talesh in Iran.

Termitomyces lives in a mutualistic symbiosis with termites of the subfamily Macrotermitinae. *Termitomyces clypeatus*, *T. umkowaani*, *T. aurantiacus*, *T. striatus*, *T. saggitiformis*, *T. mammiformis*, *T. titanicus*, *T. microcarpus*, *T. microcarpus* and *T. errhizus* were recorded in Tanzania. The scanning electronic microscopy revealed that *Termitomyces aurantiacus* and *Termitomyces striatus* had rough basidiospore. *T. saggitiformis*, *T. titanicus* and *Pearce* species are reported for the first time in the country record and key to the studied species is supplied (Tibuhwa, 2012).

Tadosa and Briones (2013) documented 75 species from Taal Volcano Protected Landscape, Southern Luzon, Philippines. *viz.* *Lactarius trivialis*, *Lactarius* sp. *Russula* sp., *Clitocybe* sp., *Omphalina* sp., *Tricholomopsis* sp., *Agrocybe* sp., *Schizophyllum commune*, *Volvariella volvacea*, *Pleurotus porrigens*, *Mycena* sp., *Calvatia* sp., *Hygrocybe* sp., *Lepiota* sp., *Lepiota cristata*, *Coprinus micaceus*, *Agaricus campestris*, *Auricularia auricula-judae*, *Auricularia cornea*, *Auricularia fusco-succinea*, *Auricularia mesenterica*, *Auricularia polytricha*, *Auricularia* sp., *Cantharellus aureus*, *Dacryopinax spathularia*, *Octospora humosa*, *Octospora humosa*, *Cookeina tricholoma*, *Galiella rufa*, *Dictyophora duplicate*, *Lenzites* sp, *Trametes corrugate*, *Trametes versicolor*, *Ganoderma applanatum*, *Ganoderma lucidum*, *Cymatoderma elegans*, *Daedalea quercina*, *Daedaleopsis confragosa*, *Fomes gilvus*, *Microporus affinis*, *Microporus vernicipes*, *Microporus xanthopus*, *Polyporus pinsitus*, *Pycnoporus sanguineus*, *Stereum insignatum*, *Daldinia concentrica*, *Xylaria cornu-damae*, *Xylaria filiformis*, *Xylaria filiformis*, *Xylaria longiana*, *Xylaria longipes* and *Xylaria polymorpha*. They found Russulaceae, Tricholomataceae, Agaricaceae, Coriolacea, Xylariaceae, Auriculariaceae

and Polyporaceae were 3 %, 3 %, 4 %, 5 %, 7 %, 10 % and 22 % abundant respectively, which covers the 8% of the entire Philippine mushrooms.

Atri (2013) collected wild six *Lentinus* species from different localities of North West India. Both morphological and molecular characterizations were used to identify *L. sajor caju*, *L. connatus*, *L. torulosus*, *L. cladopus*, *L. squarrosulus* var. *squarrosulus* and *L. squarrosulus* var. *mangoensis*. Kaur and Kaur (2013) reported the occurrence of *Psilocybe tampanensis*, *Conocybe pulchella* and *Panaeolus tropicalis* for the first time in India. Doshi (2013) observed that species of *Ganoderma*, *Schizophyllum commune*, *Pleurotus*, *Auricularia*, *Polypores*, *Innonotus*, *Favolus canadensis*, *Ceriporia*, *Phanerochaete*, *Leucoagaricus*, *Tricholoma*, *Macrocybe* and *Calocybe* were associated with the decline of arid zone trees of southern and northwestern Rajasthan, India.

Dorjey *et al.* (2013) documented six *Coprinus* mushrooms (*Coprinus comatus*, *C. disseminatus*, *C. micaceus*, *C. plicatilis*, *Coprinus sp.1*, *Coprinus sp.2* and *Coprinus sp.3*) from the cold desert of Ladakh region in Jammu and Kashmir. Vrinda *et al.* (2013) collected *Lentinus giganteus* mushroom from Kerala. The mushroom was cultivated in lab and confirmed the edibility of the mushroom which is suitable for commercial cultivation. Kotwal *et al.*, (2013) recorded *Morchella angusticeps*, *M. crassipes*, *M. deliciosa*, *M. elata*, *M. rotunda*, *M. semilibera*, *M. tomentosa* and *Verpa conica* from Jammu region of North-West Himalaya and these mushrooms belongs to Morchellaceae of order Pezizales. Pradeep *et al.* (2012) documented the *Pluteus cervinus* mushroom from Kerala. Earanna *et al.* (2013) reported the occurrence of *Termitomyces* mushroom which was locally called as Heggalanbe in the forests of Theertahalli, Shivamogga district of Karnataka. The mushroom was identified based on internal transcribed spacer (ITS) region of ribosomal DNA.

The genus *Auricularia* comprises 10 to 15 recognized species worldwide, and most are considered to have intercontinental to cosmopolitan distributions. Looney *et al.* (2013) recorded *A. fuscusuccinea*, *A. auricula-judae*, *A. mesenterica*, *A. polytricha*, and *A. delicate* in the southeastern United States. . Das *et al.* (2013) documented three species

of *Russula* from West district of Sikkim in the Eastern Himalaya viz. *Russula sharmae*, *R. dubdiana* and *R. sikkimensis*.

Kumar *et al.* (2013) collected five wild macrofungi *Coprinus comatus*, *Ganoderma lucidum*, *Pleurotus sp.*, *Schizophyllum commune* and *Volvariella volvacea* from the forest area of Gurdaspur and Ludhiana Districts. These were found on lignicolous habitat except *Volvariella volvacea* growing on leaf-litter. Bhalerao *et al.* (2013) recorded sixteen wild mushrooms from Sahayadri ranges, Vidharba and Konkan region of Maharashtra. Among the collected mushrooms *Pleurotus sp.*, *Tricholoma sp.*, *Termitomyces sp.*, *Polyporus sp.*, *Boletus sp.*, *Clitocybe sp.* and Tuber sp. were found to be edible.

Aryal and Budathokio (2013) recorded 30 species of mushrooms under 26 genera belonging to 18 families of Basidiomycetes at Karhiya Community Forest, in Terai region of western Nepal. The mushrooms includes *Amanita caesarea*, *Amanita chepangiana*, *Amanita pantherina*, *Agaricus sylvicola*, *Armillariella mellea*, *Auricularia auricular-judae*, *Bjerkandera adusta*, *Bovista nigrrescens*, *Cantharellus cibarius*, *Cerrena unicolor*, *Clavulinopsis helvola*, *Coprinus disseminates*, *Coriolus hirsutus*, *Flammulina velutipes*, *Fomitopsis vinosa*, *Ganoderma lucidium*, *Grifola frondosa*, *Guepinia spathularia*, *Laetiporus sulphureus*, *Macrolepiota procera*, *Macrolepiota rhacodes*, *Marasmius oreade*, *Psathyrella candolleane*, *Pycnoporus cinnabarinus*, *Ramariopsis kunzei*, *Russula nigricans*, *Termitomyces clypeatus*, *Termitomyces eurhizeus* and *Tyromyces sambucens*. The litter fall of *Shorea robusta* favors the temperature regulation in the soil which helps in the growth of mushrooms. Conservation of natural habitat of mushroom diversity was necessary for the sustainable development.

Kumar and Awasthi (2013) conducted survey for the wild edible/medicinal mushrooms in gardens and forest areas of Sultanpur, Bahraich, Gonda, Faizabad, Basti, and Jaunpur districts of Uttar Pradesh. A total of 20 mushrooms were collected, out of which three were *Termitomyces* species, two species of *Volvariella* and one each of *Ganoderma*, *Calocybe indica*, *Calocybe gambosa*, *Coprinus comatus*, four *Pleurotus* species and one *Polyporus sp.* Out of these mushrooms *Termitomyces* and *Volvariella*

were most common and occasionally available in the local market of Uttar Pradesh during rainy season.

Senthilarasu (2014) collected 13 species of mushrooms from Pune and Western Ghats of Mahabaleshwar and Mulshi (Maharashtra). The mushrooms viz., *Amanita flavofloccosa*, *Leucocoprinus fragilissimus*, *Leucocoprinus birnbaumii*, *Parasola plicatilis*, *Pleurotus cystidiosus*, *Trogia infundibuliform*, *Hygrocybe alwisii*, *Hygrocybe astatogala*, *Laccaria fraternal*, *Lactocollybia epia*, *Lentinus sajor-caju* and *Lepista sordida* were documented from Maharashtra for the first time.

Vyas *et al.* (2014) collected wild mushrooms from Patharia forest in Vindhyan ranges of Madhya Pradesh. They identified 18 mushrooms viz. *Vascellum pretense*, *Lycoperdon pyriform*, *Coniophora puteana*, *Clitocybe geotropa*, *Ganoderma tsugae*, *Microglossum virde*, *Panaeolus sphinctrinus*, *Pleurotus cornucopiae*, *Fomes fomentarius*, *Tyromyces lacteus*, *Lenzites Betulina*, *Hypholoma elongatum*, *Pholiota highlandensis*, *Serpula lacrymans*, *Tremella mesenterica*, *Lepista nuda*, *Collybia butyracea* and *Omphalina ericetorum*. *Lepista nuda* and *Clitopilus prunulus* were used for culinary uses.

Kunttu *et al.* (2014) documented nineteen species of macrofungi in Finland viz. *Amylocorticium subsulphureum*, *Antrodiella parasitica*, *Ceraceomyces sulphurinus*, *Clavaria atroumbrina*, *Clavaria rosea*, *Gloeophyllum carbonarium*, *Hyphodontia flavipora*, *Junghuhnia fimbriatella*, *Lindtneria chordulata*, *Odonticium septocystidia*, *Peniophorella guttulifera*, *Perenniporia tenuis*, *Postia immitis*, *Repetobasidium vile*, *Resinicium pinicola*, *Sidera vulgaris*, *Tomentella coerulea*, *Trechispora laevis* and *Xylodon pruni*. Govindaraj *et al.* (2014) documented and identified mushroom *Hygrocybe calyptriformis* (Berk.) belonging to family Hygrophoraceae from the tea estates of Anamalai hills in Valparai taluk of Coimbatore, Tamil Nadu. It was the first report from the Southern Asia. In European countries, conservation of *H. calyptriformis* was listed in red lists of threatened fungi.

Das and Das (2014) collected seventy different fruiting bodies of mushrooms from Munsiry and its adjoining areas forest of Uttarakhand. Forty five specimens were identified and all of them were belonged to the subdivision Basidiomycotina. Among the indentified mushrooms, twelve species belong to the family Agaricaceae followed by eight members of Russulaceae, four of Boletaceae, three each of *Tricholomataceae*, *Cantharallaceae* and *Polyporaceae*, two each of *Amanitaceae* and *Marasmiaceae* and one each of the family *Mycenaceae*, *Entolomataceae*, *Cortinariaceae*, *Hydnangiaceae* and *Ganodermataceae*. Many of the mushrooms were mycorrhizal association with the members of the plant family Pinaceae.

Usha and Janardhana (2014) documented 135 species of macrofungi belonging to 56 genera and 34 families from forests of Chamarajnar, Chickmagalur, Hassan, Kodagu and Mysore. They found family Agaricaceae was predominant in these regions. Twelve species of *Russula* viz. *Russula aciculocystis*, *Russula adusta*, *Russula atropurpurea*, *Russula cinerella*, *Russula congoana*, *Russula delicula*, *Russula hygrophytica*, *Russula luteotacta*, *Russula mariae*, *Russula martinica*, *Russula michiganensis* and *Russula periglypta* and white coloured latex exuding *Lactarius nebulosus* were recorded from the Western Ghats of Kerala. All the Russulaceae members exhibit an ectomycorrhizal association with tree species like *Hopea ponga*, *Hopea parviflora*, *Myristica malabarica*, *Vateria indica* and *Calophyllum apetalum* (Mohan, 2014).

Senthilarasu (2014) collected 13 species of macrofungi from Pune and Western Ghats of Mahabaleshwar and Mulshi viz. *Leucocoprinus birnbaumii*, *Leucocoprinus fragilissimus*, *Amanita flavofloccosa*, *Laccaria fraternal*, *Hygrocybe alwisii*, *Hygrocybe astatogala*, *Lactocollybia epia*, *Trogia infundibuliformis*, *Pleurotus cystidiosus*, *Pleurotu. djmor*, *Lentinus sajor-caju*, *Parasola plicatilis* *Lepista sordida*. *Amanita flavofloccosa* was reported for the first time from Western Ghats. Senthilarasu and Kumaresan (2016) were documented 15 agaric fungi from the forests of Western Ghats of Karnataka. *Cyptotrampa asprata*, *Hygrocybe acutoconica*, *Hygrocybe alwisii*, *Oudemansiella furfuracea*, *Hypholoma subviride* and *Lactocollybia epia* were reported for the first time from Karnataka.

Chittaragi *et al.* (2014) collected *Clavaria rosea*, *Ganoderma* sp., *Geastrum triplex*, *Hygrocybe parvula* and *Scleroderma bermudense* from the Western Ghats of Shivamogga (Karnataka). Das *et al.* (2014) collected sixteen wild edible mushrooms collected from tropical dry deciduous forest of West Bengal and morphologically characterized up to genus level as *Amanita* sp., *Astraeus* sp., *Termitomyces* sp., *Volvariella* sp., *Agaricus* sp., *Clavatia* sp., *Lentinus* sp., *Russula* sp.

Lazo *et al.* (2015) conducted ethnomycological survey of mushrooms utilized by Gaddang communities in Nueva Vizcaya, Philippines. *Auricularia auricula*, *Auricularia fuscosuccinea*, *Schizophyllum commune*, *Volvariella volvacea*, *Lentinus* sp., *Pleurotus* sp. and *Polyporus* sp. Gaddang communities had indigenous beliefs that the occurrence of spontaneous lightning induces mushroom growth and they ask permission of spirits before collecting mushrooms. They believed that mushrooms with rings on the stipe and bad odor were poisonous while those without rings were edible. This method of identification was inherited from their ancestors.

Chelela *et al.* (2015) reported 7 edible species (Russulaceae-4 and Cantharellaceae-3), 11 inedible species (Boletaceae-4, Polyporaceae-3, Russulaceae-2, Suillaceae-1 and Agaricaceae-1), 2 deadly poisonous (both Amanitaceae) and 4 of unknown edibility (Russulaceae-2, Suillaceae-1 and Boletaceae-1) wild mushrooms from Iringa and Njombe regions in the Southern Highlands of Tanzania. The rare *Inocybe insignis* mushroom was documented by Kuo and Matheny (2015) in eastern North America which belongs to Inocybaceae, Agaricales. *I. insignis* has stellate basidiospores, marginate stipe base, entirely pruinose stipe, rimose pileus, and strong distinctive spermatic odor.

Amandeep *et al.* (2015) reported eleven species of *Termitomyces* mushrooms viz. *Termitomyces aurantiacus*, *T. bulborhizus*, *T. clypeatus*, *T. entolomoides*, *T. eurrhizus*, *T. globulus*, *T. heimii*, *T. mammiformis*, *T. microcarpus*, *T. striatus* and *T. tylerianus* species of mushrooms were distributed in China. Aryal and Budathokio (2015) reported occurrence of *Termitomyces aurantiacus*, *T. badius*, *T. le-testui*, *T. microcarpus f. santalensis* and *T. schimperi* in reserve forest of Nepal.

Panda and Tayung, (2015) were documented 24 different mushrooms from the three district of Northern Orissa. Out of which eighteen species were edible macro fungi belong to eleven families. They reported that agaricales were dominated in this region. Among the edible mushroom collected, they found thirteen species from forest soil, three species from decaying paddy straw, two species from termite nests, and one species from wood log. Govindaraj *et al.* (2016) reported the dog stinkhorn mushroom *Mutinus caninus* (Huds.) Fr. under bamboo tree, decomposed wood and leaf debris in soil inside the Loyola college campus and some other places in Chennai city during the northeast monsoon. The immature buds of the fungus were abundantly dispersed in soil. The unique unpleasant smell can sensible when crossing across their habitat. Stinkhorn attracts the insects from their odour and helping in pollination of nearby flowering plants.

Aryal and Budathoki (2016) were collected *Termitomyces albuminosus*, *T. aurantiacus*, *T. badius*, *T. clypeatus*, *T. eurhizus*, *T. fuliginosus*, *T. globulus*, *T. heimii*, *T. le-testui*, *T. mammiformis*, *T. microcarpus*, *T. robustus*, *T. schimperi*, *T. straitus*, *Schulzeria umkowaan*, *Termitomyces palpensis* and *T. arghakhanchensis* from Tarai, Siwalik and Midhill regions of Nepal. Bhatt *et al.* (2016) recorded fifteen species of wild mushrooms from higher (2000-3500 mean sea level) elevations in the Garhwal Himalaya-I of India. They are *Cantharellus cibarius*, *Cantharellus minor*, *Lactarius subindigo*, *Morchella esculenta*, *Boletus edulis*, *Grifola frondosa*, *Hydnum repandum*, *Boletus regineus*, *Strobilomyces floccopus*, *Craterellus cornucopioides*, *Russula virescens*, *Russula brevipes*, *Lactifluus hygrophoroides*, *Marasmius oreades*, *Lactarius deliciosus*,

2.4 Molecular identification of mushrooms

The genes encoding for 16S rRNA in prokaryotes and 18S in eukaryotes are most widely used in molecular phylogenetics. The Internal transcribed spacer refers to the spacer DNA situated between the small-subunit of ribosomal RNA (rRNA) and large-subunit of rRNA genes in the chromosome. The ITS region is the most widely sequenced DNA region in molecular ecology of fungi and has been recommended as the universal fungal barcode sequence.

Ko *et al.* (2001) investigated the phylogeographic divergences of four coprinoid species *viz.*, *Coprinus comatus*, *Coprinellus disseminatus*, *Coprinellus micaceus* and *Coprinopsis lagopus* based on nuclear ITS sequences. Each taxon showed genetic variation that corresponds with the geographic origins of collections. The species of *Cookeina* was encountered in tropical as well as subtropical areas around the world. Morphologically the species were distinguished by combinations of several features including ascospore shape, surface relief, presence and origin of apothecial hairs and presence or absence of gelatinous material within the cortical layer of the excipular tissue. The hymenium Colour was attributed to carotenoid pigments particularly variable in *C. speciosa*. The phylogenetic analyses of *Cookeina* were analysed by rDNA ITS and rDNA LSU sequences and genus was shown to be monophyletic with several well-supported lineages. These analyses generally support the established, morphologically distinguished taxa within a monophyletic genus *Cookeina* (Weinstein *et al.*, 2002).

Muruke *et al.*, (2002) analysed the phylogeographic divergences of nine edible mushroom mycelia of genera *Oudemansiella*, *Coprinus* and *Pleurotus* species collected from East Asia, Hawaii, Japan, Korea, France and England by using ITS sequence. Using the molecular technique it was possible to sort out a case of mistaken identity of *Oudemansiella* fruiting bodies. Molecular method was fast, accurate and could be used to screening of edible mushrooms.

Geml *et al.* (2004) conducted Phylogenetic analyses of 42 *Agaricus* mushrooms and related secotioid species based on sequence data of the internal transcribed spacers and partial large subunit of ribosomal DNA. Analysis showed that the most morphological features likely have evolved through independently during evolution.

Iotti *et al.* (2005) isolated pure culture of *Boletus edulis*, *Boletus aestivalis*, *Boletus luridus*, *Amanita muscaria* and *Hebeloma radicosum* which were identified by ITS region sequence of nuclear ribosomal RNA genes. The phylogenetic relation of the isolated mycelia were evaluated by comparison of their ITS sequences. Leonardi *et al.* (2005) discriminate the species of *Boletus edulis*, *Boletus aestivalis*, *Boletus pinophilus* and *Boletus aereus* based on internal transcribed spacer of nuclear rDNA region. *Boletus*

species were the most frequently eaten mushrooms among those harvested in natural conditions in Europe. Mello *et al.* (2006) analyzed the sequence of ITS region from 28 different *Boletus* species. The sequence similarity indicated that the allergenic caused by *Boletus violaceofuscus* intermixed with edible *Boletus edulis*.

Singh *et al.* (2006) collected 18 mushrooms from Udaipur district of Rajasthan and characterized the mushrooms based on DNA fingerprinting and ribosomal DNA sequencing. Based on ITS sequence polymorphism, seven isolates were identified as *Podaxis pistillaris*, four as *Phellinus igniarius*, one as *Gymnopilus subearlei* and six as *Phellorinia herculea*. The similarity matrix revealed very high intra-species homology (99.5-100 %) and significant inter-generic diversity (21.2-37.7 %).

Chen *et al.* (2006) isolated and identified medicinal mushroom as *Cordyceps sinensis* based on using ITS sequence homology of the related fungi. The phylogenetic tree of ITS sequence showed that isolated wild *C. sinensis* strain was clustered together with *C. sinensis*, *Tolypocladium cylindrosporum* and *Tolypocladium inflatum* in the phylogenetic tree.

Lee *et al.* (2006) collected four medicinal mushrooms from Korea and subjected to molecular identification. The amplified gene products of nuclear large subunit rDNA of mushrooms were cloned and sequenced. The sequence of all the mushrooms were showed more than 97 % homology to database sequences and mushrooms identified as *Ganoderma lucidum*, *Coriolus versicolor*, and *Fomes fomentarius*.

The genetic diversity and phylogenetic analysis of different strains of *Ganoderma australe* collected from southern India were done by using ITS1 and ITS2 of rDNA. The high bootstrapping clearly represents the presence of two biological species of *G. australe* in southern India (Kaliyaperumal and Kalaichelvan, 2007).

The ITS primers, such as ITS1-F, were hampered with a high proportion of mismatches relative to the target sequences, and most of them appeared to introduce taxonomic biases during PCR. ITS1-F, ITS1 and ITS5, were biased towards amplification of Basidiomycetes, whereas ITS2, ITS3 and ITS4, were biased towards Ascomycetes.

The assumed Basidiomycetes specific primer ITS4-B only amplified a minor proportion of Basidiomycetes ITS sequences, even under relaxed PCR conditions. ITS primers have to be selected carefully, when used for high-throughput sequencing of environmental samples (Bellemain *et al.*, 2010).

Junior *et al.*, (2010) identified mushrooms based on morphological characters of the fruiting body and sequencing of the Large Sub Unit (LSU) rDNA gene as well. Out of the three, two species were identified as *Pleurotus ostreatus* and one as *Pleurotus djamor*. Lian and Fang (2011) identified wild mushroom *Volvariella volvacea* based on morphologically as well as by using ITS sequence amplification using ITS4 and ITS5 primers. Prakasam *et al.* (2011) collected *Tricholoma* mushroom in Coimbatore and Erode districts of Tamil Nadu. The mushroom DNA was sequenced using ITS 1 and ITS 2 primers which shares 91 % homology with *Tricholoma giganteum* and is given with Gen bank accession number 120872.

Oyetayo (2011) collected eighteen *Termitomyces* species from Ondo and Ekiti States of Nigeria. These mushrooms were identified by using Internal Transcribed Spacer (ITS) region sequence were compared with existing sequences in the NCBI GenBank. The results of the ITS sequence analysis discriminated between all the *Termitomyces* species and degree of similarity was 82 to 99 percent obtained from NCBI. Phylogenetic tree generated with ITS sequences obtained from NCBI GenBank data revealed that a total of five clades were observed. Clade 2 was made up of a *Termitomyces* species from Gabon and 12 *Termitomyces* species from Nigeria; this implies that the *Termitomyces* species from Nigeria and Gabon may be from the same ancestral source.

Rajaratnam and Thiagarajan (2012) identified the wild mushrooms by amplification of genomic DNA using internal transcribed spacers (ITS1 and ITS4) primers. The sequence was aligned using Jukes-Cantor Corrected Distance model. Aligned sequence revealed 88 % homology with *Perenniporia* sp. which belongs to Agaricomycetes and it was a new addition to the mushroom biodiversity of India.

Dung *et al.* (2012) isolated three different strains of oyster mushroom and then identified by morphological and molecular methods. The morphological characterization was based on hyphae and spore characters and molecular characterization using ITS 1 and ITS 4 primers. The sequence results in NCBI Blast showed two strains of white oyster mushrooms as *Pleurotus floridanus* and one strain as *Pleurotus cystidiosus*. The species of *Tricholoma* and *Calocybe* were distinguished by using ITS region of rDNA sequence analysis in NCBI database. This technique is used to overcome the misidentification of macro fungi by classical identification.

Manikprabhu and Lingappa (2013) isolated fifty nine fungi from soil of Gulbarga and identified by using ITS/18S rRNA sequence homology. The internal transcribed spacers (ITS) region present in the nuclear rRNA genes of all eukaryotes could serve as DNA bar-code and a marker for phylogeny reconstruction of the species. The ITS1 and ITS2 regions on the either side of 5.8S rRNA gene were shows length and nucleotide variation which helps in assigning of an organism into a specific taxon. As ITS1 and ITS2 region were flanked by conserved rRNA genes, universal primers could be synthesized for amplification (Yadav, 2013).

Molecular phylogenies of nuclear ribosomal ITS (ITS 1-2 and 5.8S), gene regions and information on ecology, morphology and pigment chemistry were used in a partial revision of the agaric family Hygrophoraceae. The phylogenetically supported genera of Hygrophoraceae based on previous analyses were *Acantholichen*, *Ampulloclitocybe*, *Arrhenia*, *Cantharellula*, *Cantharocybe*, *Chromosera*, *Chrysomphalina*, *Cora*, *Corella*, *Cuphophyllus*, *Cyphellostereum*, *Dictyonema*, *Eonema*, *Gliophorus*, *Haasiella*, *Humidicutis*, *Hygroaster*, *Hygrocybe*, *Hygrophorus*, *Lichenomphalia*, *Neohygrocybe*, *Porpolomopsis* and *Pseudoarmillariella*. A new genus *Chromosera* was described as *Gloioxanthomyces*. Revisions were made at the ranks of subfamily, genus and subgenus. Species of *Chromosera*, *Gliophorus*, *Humidicutis* and *Neohygrocybe* were often treated within the genus *Hygrocybe* (Lodge *et al.*, 2014).

Anand and Chowdhry (2013) collected a mushroom from lower Shivalik range of moist temperate conifer forest of Rajouri and identified on the basis of morphological and

molecular characters. Internal transcribed spacers (ITS) were used in amplification of target region. The target rDNA sequence, when aligned with GenBank by performing BLAST, showed 100% homology with *Verpa bohemica*. This species was reported for the first time from Rajouri District of Jammu and Kashmir. The five new species of macrofungi *Scleroderma citrinum*, *Psilocybe subtropicalis*, *Ganoderma applanatum*, *Cyptotrama asprata* and *Entoloma serrulatum* were documented from this region (Anand and Chowdhry, 2013).

Yanyi (2014) identified *Schizophyllum commune* using ITS region and 18S rDNA. The mushroom was able to produce the polysaccharide known as schizophyllan from glucose. schizophyllan was the alternate carbon source for survival of the mushroom. Wu *et al.* (2014) examined *Auricularia auricula-judae* and related species based on using ITS and ribosomal RNA gene regions and morphology. They showed that the wild and cultivated samples previously identified as *Auricularia auricula-judae* in China are different from those from Europe. They identified and described the new species- *Auricularia heimuer*. *Auricularia auricula-judae* was not found in China, and most probably has a distribution in Europe only. In addition, *A. americana* and *A. villosula* were the first time reported in China. Both *A. heimuer* and *A. villosula* grow on angiosperm wood, while *A. americana* is found on gymnosperm wood exclusively.

The Phylogenetic analysis of *Volvariella volvacea* was characterized by using Internal Transcribed Spacer region and 5.8S rRNA gene region direct sequencing revealed the interspecific variations of the new isolate with other *Volvariella* isolates deposited in NCBI Gene Bank database (Raju *et al.*, 2014). Olusegun (2014) isolated the DNA of *Trametes* by CTAB lysis method and amplified the ITS region. Phylogenetic tree generated from *Trametes* ITS sequences from Nigeria and sequences obtained from NCBI GenBank revealed *Trametes* from Nigeria were placed in 3 separate clades out of 7 clades. The closest *Trametes* species indigenous to Nigeria were *T. lactinea* and *T. polyzona* with 99% of similarity.

Mi *et al.* (2016) analysed ITS sequence of 232 fruiting bodies of *Trogia venenata* and confirmed that all the isolates were belongs to same species. Wiriya *et al.* (2014)

identified five *Termitomyces* species based on morphological and ITS sequence homology.

2.5 Pure culture isolation and conservation

The mycelium can be obtained either from spores or from a piece of the specific mushroom tissue. The pure culture isolation and preservation of potential species is necessary for investigating the possibility for using the wild strain for commercial cultivation. Thiribhuvanamala *et al.* (2011) collected a wild strain of *Pleurotus djamor roseus* and isolated pure culture for commercial use. It was found suitable for commercial cultivation with bioefficiency of 132 percent and cost benefit ratio of 1:2.9.

Mushroom cultures could be preserved safely at 8°C on sorghum grains for more than one year without any changes in growth and morphology. The most significant advantage of this method was its suitability to conserve mushrooms like *Calocybe indica* and some isolates of *Ganoderma lucidum* cultures (Veena and Pandey, 2010). The mycelia of wild strain *Volvariella volvacea* grew on banana leaves (26.4 %) better than on other raw materials like paddy straw (23.76 %) and pseudo stem sheath (17.11 %). The utilization of banana wastes for cultivation of wild strains showed good growth (Lian and Fang, 2011). The *Tricholoma giganteum* could be best cultivated using paddy straw substrate throughout the year at 30-35 °C temperature with 70 % relative humidity. Taste of the mushroom was excellent, with good odour and aroma. The mushroom had shelf life of 3-4 days under room temperature and 5-6 days under refrigerated conditions (Prakasam *et al.*, 2011).

The molecular genetics of the mating type system of *V. volvacea* was found similar to bipolar system of basidiomycetes, suggesting that it was secondary homothallism. Sensitivity to low temperatures could be due to the lack of initiation of biosynthesis of unsaturated fatty acids, trehalose and glycogen biosyntheses in this mushroom. Genome sequencing of *V. volvacea* enabled in understanding biological characteristics related to degradation of agricultural wastes, the sexual reproduction mechanism, and low temperatures sensitivity at molecular level (Bao *et al.* 2013).

Wiriya *et al.* (2013) tested the growth of *Termitomyces* on different carbon, nitrogen and solid substrates. They found that the malt extract agar was the best medium for mycelial growth. Sucrose and fructose were the best carbon sources for these fungi. Peptone was the best nitrogen source for enhancing radial growth of myceli. Pure culture isolation of edible species from the wild collection is essential to explore the possibility to bring them in to commercial cultivation.

III MATERIAL AND METHODS

The present investigation was undertaken for documentation and molecular characterization of wild mushrooms occurring in the Western Ghats regions of Karnataka. The field information was recorded during collection. The samples were brought to laboratory and identified by using morphological and molecular characterization (ITS gene and 5.8S rRNA gene sequencing). Pure culture was isolated from edible mushrooms and cultivation was carried out in the mushroom laboratory, University of Agricultural Sciences, Bengaluru, Karnataka. The materials used and methods followed are described below.

3.1 Ethno mycological survey of wild mushrooms in the Western Ghats of Karnataka

In order to document the wild mushrooms, Extensive survey was carried out in the Western Ghats forest area of Karnataka including Bababundungiri Hills, Darmasthala, Managundi, Nettana, Sakaleshpur, Sringeri, Sulya and Theerthahalli for four months (June-September) during rainy season of the years 2014, 2015 and 2016. The survey was carried out with the help of information provided by tribal communities like Adivasis, Halakkivokkals and Siddis in the locality during the visits as they were familiar with mushroom types and time/season of appearance. The places were selected based on the presence of timber research centers. In this region mushroom diversity will be more due to the spores migration along with the timber from the forest. The mushroom samples were collected in paper bags. During collection, weather condition, abundance, habitat and phenotypic characters were recorded. The fruiting body of the mushrooms was photographed *in-situ* using “Sony digital still camera DSC-W170”.

3.2 Morphological characterization of wild mushrooms

Morphological characters like colour, size of cap and stipe, stipe position, presence or absence of gills or pores, presence or absence of annulus, texture (soft, watery, spongy, brittle, tough, leathery, corky and woody) and odour of the fleshy fruit bodies were recorded (Arora, 1986).

Color is one of the noticeable features of mushrooms, Size is most important for comparison in terms of small, medium and large which represents the key of average size ranges. Gills or lamellae features like mode of attachment to the stalk, spacing, thickness were noted. The spore print from the cap was collected by placing lower portion of cap on black and white paper over night. Thus collected spores were observed for their shape, size and colour under compound microscope. Pure culture from edible mushroom species were isolated on Potato dextrose agar and stored for further use.

3.3 Molecular Identification of wild mushrooms using ITS region sequence

The molecular identification was done by extracting the genomic DNA and amplifying was done using ITS primers given below (White *et al.*, 1990; Rajaratnam and Thiagarajan, 2012).

Primer Name	Sequences
ITS 1	5' TCCGTAGGTGAACCTGCGG 3'
ITS 4	5' TCCTCCGCTTATTGATATGC 3'

3.3.1 Genomic DNA Isolation

Total genomic DNA from the cap/stipe tissue of the mushroom fungus was extracted using Cetyl Trimethyl Ammonium Borate (CTAB) method (Doyle and Doyle, 1987). Dried tissue (0.2 g) of the mushroom sample was ground into fine powder using pestle and mortar and transferred into 2 ml 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 (24:1V/V) was added and mixed by inverting the tubes. These tubes were centrifuged at 10000 rpm for 10 minutes. The above step was repeated till no white interface was seen. Clear supernatant was collected and DNA was precipitated by adding 0.6 volumes of chilled Isopropanol and place in -20°C 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.

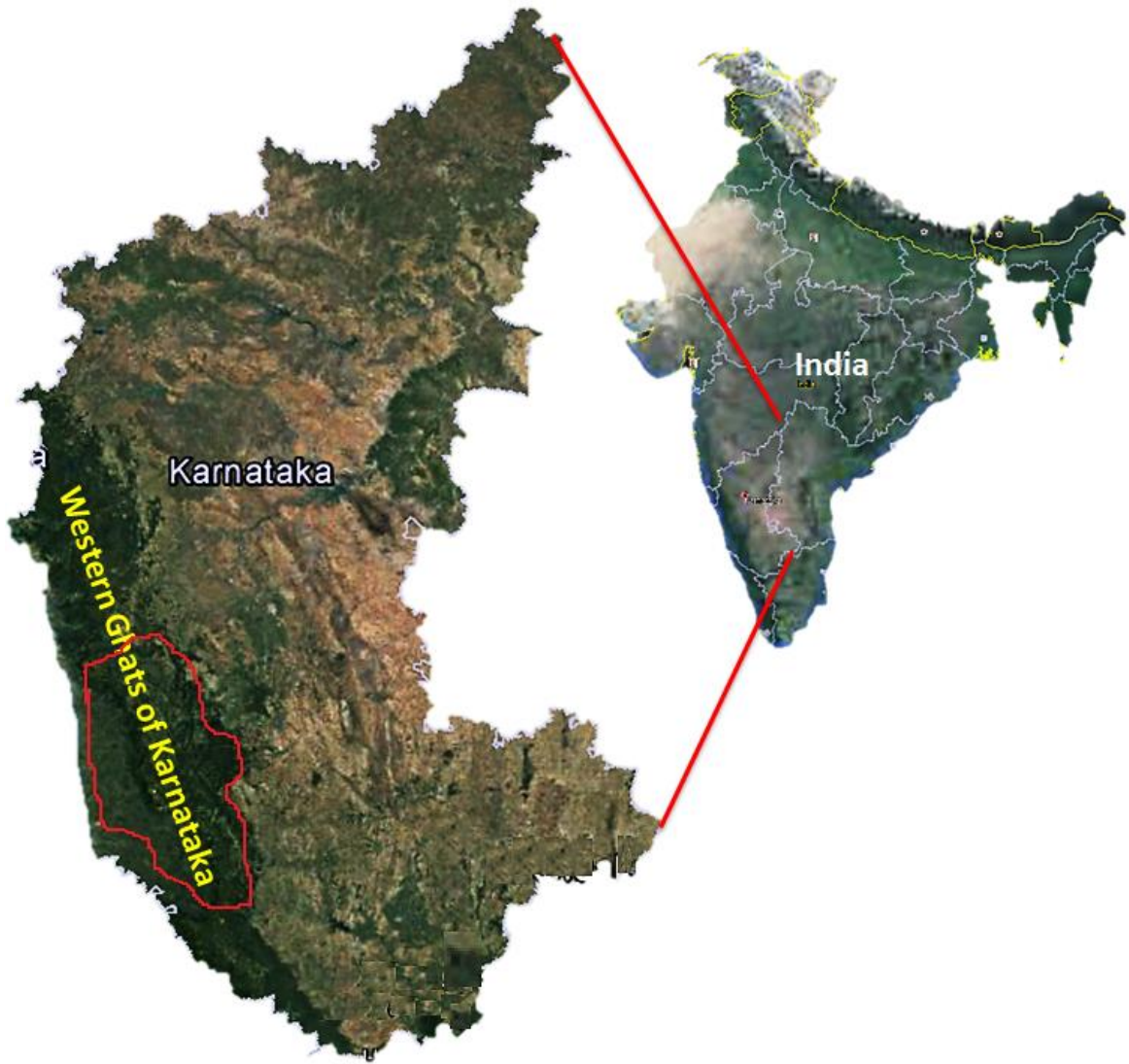


Fig. 2: Map showing experimental site in Western Ghats of Karnataka (India)

3.3.2. PCR amplification

PCR components (in 40 µl reaction mixture)

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

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 3 min, 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.

3.3.3 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 100 V for 2 hours. The gel was visualized under UV light and documented using Alpha Inno Tech Gel documentation unit. The visualized band was excised and purified by using gel extraction kit.

3.3.4 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 placed into a clean 1.5 ml micro centrifuge tube and 20 µl of elution buffer was added to the column. It was centrifuged at 12,000 rpm for 60 seconds. The amplified DNA thus eluted was used for sequencing.

3.4 ITS region sequencing

Concentration of the eluted DNA were measured at 260 nm using nano drop and sent for sequencing to Sci genome Pvt., Kerala, India. The sequence data received from the company was analysed for molecular identification.

3.4.1 Sequence analysis and homology search

Sequence results were analysed with online software of National Centre for Biotechnology Information (NCBI), USA. The BLAST (Basic Local Alignment Search Tool) search gave the partial length sequence homology with NCBI data (<http://www.Ncbi.nlm.nih.gov/BLAST/>) (Altschul *et al.*, 1990).

3.5 Phylogenetic analysis

Phylogenetic analyses were performed to know the relationship between identified and other mushroom species. Preliminary pairwise and multiple alignments

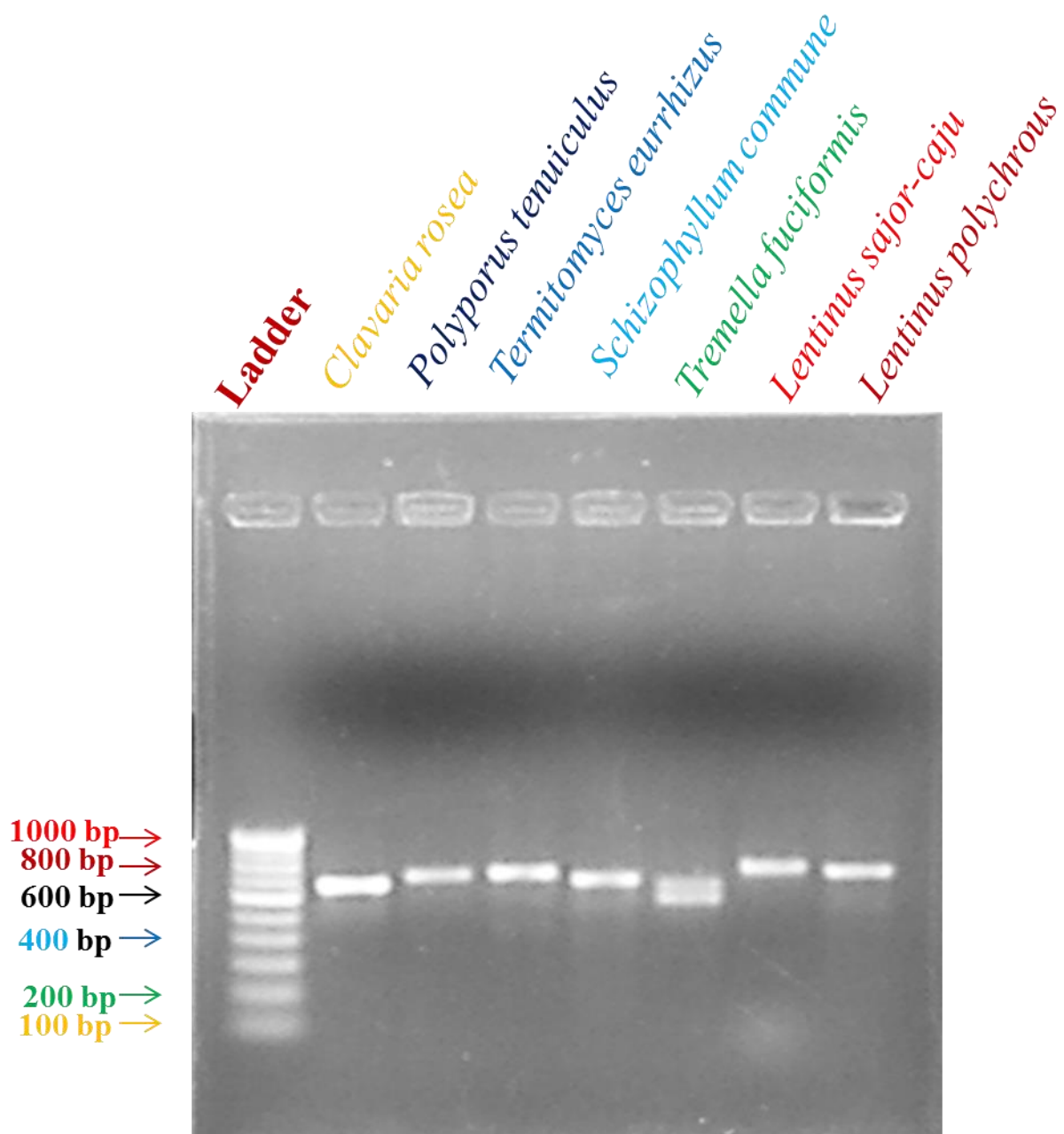


Plate 1: Agarose gel profile showing bands of ITS region in the range of 600 to 800bp

were performed using Clustal W for all twenty mushroom sequence data independently. Phylogenetic analyses were performed using MEGA.6 software (www.megasoftware.net/) and phylogenetic trees were generated using the neighbour-joining algorithm (Tamura *et al.*, 2013).

3.6 Isolation of pure culture of edible mushrooms

The mushrooms were brought to the laboratory and stored in -20 refrigerator for pure culture isolation. The inner tissue of the stipe was most preferable for *in vitro* culturing and gills as well. The inner tissue which is almost free of microbes selected and washed in 70 % alcohol and then serially washed with sterile water to remove the residual chemical. Then, the piece of sterilized tissue were inoculated on Potato Dextrose Agar (PDA) medium (Appendix) dispensed plates and incubated at 25 °C temperature till the growth of the mycelium generated from the tissue.

3.7 Cultivation of isolated edible Mushroom

3.7.1 Spawn Production

Spawn for mushroom cultivation was prepared by following the standard procedure given by Krishnamoorthy (2003). The uninfected sorghum grains were washed in tap water for three to four times and boiled for 30 minutes in a pressure cooker. The moisture content of half boiled grains was adjusted to 50 - 55 % by air drying. Then half boiled grains were mixed with 2 per cent of calcium carbonate and 2 per cent of calcium sulphate. After mixing, grains were filled into polypropylene bags (10 x 16 cm in size with 250 gauge). It was filled only one third portion of the bag to have proper aeration. Mouth of the polypropylene bags was closed with a rubber band to avoid entry of moisture upon sterilization. The bags were sterilized in an autoclave at 121 °C and 15 psi for 22 minutes. After sterilization, the Sorghum grains were cooled and inoculated with the pure culture maintained in the refrigerator. The inoculated bags incubated at 25 °C for 15 days. The fully grown mycelium (cottony growth) covered grains in the bag after incubation were used for mushroom cultivation.

3.7.2 Preparation of substrate

Paddy straw was used for the cultivation of *Lentinus sojar-caju* mushroom. The substrates were chopped into small pieces (4 inches) and then chopped substrates were soaked in water for 10 hours in a container. The excess water was allowed to drain off and substrate was sterilized in a autoclave at 121°C, 15lb pressure/ Sq. Inch for 15 minutes. The sterilized substrate was spread on a disinfected cement floor inside the room and cooled to room temperature.

The substrate was filled into polythene bags of 150 gauge thickness and having 30 cm x 45 cm size. The substrate was filled in the bag layer by layer by adding about 50 g spawn in each layer. When the bag was filled to 3/4th, the mouth of the polythene bag was closed with a rubber band and two small holes were made at the corner of the bottom of the bag to drain out excess water if any in the substrate. Few small holes were made all over the bag to create aeration for growing mushroom mycelium during spawn run stage. These bags were incubated on racks in mushroom cultivation room. The temperature (25-30 °C) and humidity (70-80 per cent) was maintained in the cropping room till the harvest. The mushroom fruit bodies formed were harvested before maturation. The bioefficiency of was calculated by using the formula given by Chang and Miles (1989).

$$\text{Bio efficiency (\%)} = \frac{\text{Fresh weight of mushrooms (g)}}{\text{Dry weight of substrate (g)}} \times 100$$

3.8 Nutrient analysis of wild edible mushrooms

3.8.1 Estimation of Protein content

The nitrogen estimation was carried out by Micro-Kjeldhal method (Koch, 1924). Oven dried mushroom sample was ground in to fine powder using pestle and mortar. One gram of ground powder were added in to the digestion mixture consisting 5 g K₂ SO₄ and 0.5 g CuSO₄ and digested in 15 ml of concentrated sulphuric acid in Pelican Kelplus-KES 08LR AL digester. The digested samples were distilled by adding 15 ml of 40% NaOH in Pelican Kelplus-Supra LX VA distillation apparatus. The ammonia liberated was trapped in 25 ml of 4% boric acid containing 2 to 3 drops of mixed indicator

(appendix). The ammonium borate thus formed was titrated against 0.1 N H₂SO₄. The per cent nitrogen was calculated by using the formula given below. The crude protein content was estimated by multiplying the percent nitrogen with 6.25.

$$\% \text{ N} = \frac{\text{Titre value of H}_2\text{SO}_4 \times \text{N of H}_2\text{SO}_4 \times 0.014}{\text{Weight of the sample digested}} \times 100$$

3.8.2 Estimation of phosphorus (P)

Phosphorus content of mushrooms was estimated by calorimetrically following the Vanadomolybdo phosphoric acid yellow colour method (Jackson, 1973). The oven dried mushroom samples (0.5 g) were digested in a conical flask containing 10 ml di-acid mixture (Nitric acid and perchloric acid in the ratio 9:4 v/v) clear precipitate forms on hot plate. Volume of the digested samples were made up to 100 ml after passing through Whatman No.1 filter paper using double distilled water. Ten ml of the aliquot was taken in 50 ml capacity volumetric flask and 10 ml of vanadomolybdate solution was added in to it. Then the volume was made up to 50 ml by adding distilled water and allowed for 20 minutes to develop yellow colour. After 20 minutes, optical density (OD) was measured at 430 nm wave length using spectrophotometer. The OD values thus obtained was compared with standard graph developed for different P concentrations. The per cent P was calculated by using the formula given below.

$$\% \text{ P} = \frac{\text{Graph ppm} \times \text{Volume of digested sample} \times \text{vol. Made up}}{10^6 \times \text{Weight of sample} \times \text{Aliquot taken}} \times 100$$

3.8.3 Estimation of Calcium, Iron, Magnesium and Zinc

The mushroom samples were washed with deionized water. Then the samples were dried at 60 °C overnight and ground in to fine powder using pestle and mortar. From the ground sample 0.5g powder was digested with concentrated di-acid mixture (Nitric acid and Perchloric acid in the ratio of 9:4 respectively) on a hot plate. Digested samples were filtered and volume was made up to 50 ml using deionized double distilled water. Metal ion concentrations were determined as three replicates by Atomic absorption spectrometer. The absorption measurements of the elements were performed

under the conditions recommended by the manufacturer protocol (Thermo Scientific, USA) at Fisheries research and information center (Inland) Hebbal, Bengaluru.

3.8.4 Determination of crude fat and fiber

Crude fat was determined by extracting 2 g dried mushroom sample with petroleum ether in a Soxhlet extraction heating flask on sandbar for about 6 h till a drop taken from the drippings left no greasy stain on the filter paper. After boiling with petroleum ether, it was filtered using Whatman no. 41 filter paper and the filtrate was evaporated in a pre- weighed beaker. Increased in weight indicated the crude fat content (Nagaraj *et al.*, 2013). Fiber content was analyzed in Fisheries research and information center (Inland) Hebbal, Bengaluru.

3.9 Stastical analysis

The statistical analysis of the data obtained was done by Duncan's new multiple range test using WASP: 2.0 (Web Agri Stat Package) statistical tool (www.icargoa.res.in/wasp/index.php).

3.10 Conservation of pure culture of edible mushrooms

Important edible mushroom species, *In vitro* cultured mycelium was transferred on fresh Potato Dextrose Agar (PDA) slant and incubated at 25 °C for 7 days. After growth of mycelium the PDA slant were preserved in refrigerator by sub culturing in PDA slants once in every 3 months. Similarly, the spores of the mushroom were conserved in viols at room temperature.

IV RESULTS AND DISCUSSION

Mushrooms occur under various ecological conditions from desert to forest. They comprise a large heterogeneous group of fungi with different shapes, sizes, colour and edibility. 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 odor of the fruiting body. During collection, it is essential to record field character and observe the surroundings to facilitate further classification and speciation. The field details such as date, season, weather, abundance, growth habit, vegetation, substrate *etc.*, are documented. In this study, genotypic information has been used for identification of documented mushrooms in the districts of Chikkamagaluru, Dakshina Kannada, Hassan and Shivamogga.

The Central Western Ghats of India in Karnataka ranges from 12° to 14° covering the area of Dakshina Kannada, Chikkamagaluru, Hassan, Kodagu Shivamogga and Uttara Kannada districts. Dakshina Kannada, Hassan, Chikkamagaluru, Shivamogga districts are 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' N latitude and between 74°37' and 75°52' E longitude in about the mid-South Western part of the Karnataka State. These districts receive an average annual rainfall of 2869 mm (Bhat, *et al.*, 2012). The cool and humid climate of this region make it ideal habitat for different kinds of mushrooms.

4.1 Ethnomycological survey

The diversity of fungi and their natural beauty occupy prime place in the biological world and the Western Ghats of India have 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 *et al.*, 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 this study, survey was carried out for documentation and molecular characterization of wild mushrooms from Western Ghats of Karnataka. Field survey was made on the occurrence of wild mushrooms from July-October 2014-2016. Fifty mushroom species were collected of which forty were identified using Internal Transcribed Spacer (ITS) rRNA gene sequence homology and ten mushrooms identified by studying their phenotypic characters. Mushrooms are designated as WGM-(Western Ghats Mushroom) and results obtained during the study are presented and discussed here under.

4.2 Identification of mushrooms by phenotypic/morphological characters

The collected mushrooms had different growth habits like scattered, clustered and gregarious. These mushrooms also showed varied morphological characters and colors. These characters were being used as bench mark for classification of mushrooms in traditional methods (Arora, 1986). In the present study, ten mushrooms designated as WGM-22, WGM-23, WGM-24, WGM-25, WGM-26, WGM-27, WGM-28, WGM-29, WGM-33 and WGM-34 were identified based on morphological characters. Others are identified based on ITS sequence homology available at NCBI GenBank. Identified mushrooms were grouped into three categories according to their utilization. They are inedible mushrooms, medicinal mushrooms and edible mushrooms.

4.2.1 Inedible mushrooms

These mushrooms are not palatable to human consumption and may cause allergic and sometimes to the extent of death due to it's poisoning. However, these mushrooms can have a benefit to the environment as the mushroom mycelia acts as a major role in soil formation and degradation of waste materials including dead flora and fauna. There are 29 species of such mushrooms, which were documented in this study and identified by ITS region sequence homology.

4.2.1.1 WGM-01

This mushroom was documented in Hassan (latitude 12°55'16.97"N and longitude 75°45'17.15"E, at an altitude of 3187 ft from mean sea level) and also seen in

Dakshina Kannad district. The surface of the cap is shiny due to the moisture and fine texture. The phenotypic description is as under.

Pileus: Thin-fleshed, fan to funnel shape, alternatively brown and dark brown zonation and there is peach yellow color at the edge of the cap (Plate 02). *Pores*: Pores are white in color. *Stem*: short stem with tapering at the middle of the stalk, broad at the attachment of the substrate. Stem is peach colour and white at the top. *Spores*: spores are white as observed the spore print. The mushroom was found on dead wood in groups.

ITS region amplified using ITS-1 and ITS-4 primer having sequence of 613 bp showed 99 % homology with the earlier reported *Microporus xanthopus* (Fig.03). The phylogenetic tree showed that WGM-01 is closely related to Russian *Microporus xanthopus* (KC503506.1), *Microporus xanthopus* from Mizoram (KM985667.1) and China (JK290074.1) with bootstrap support 22 and grouped under clade V (Fig.04). This species was reported from the subtropical dry forests of Thailand (Boonyanuphap and Hansawasdi, 2011), Taal Volcano Protected Landscape in Philippines (Tadiosa and Briones, 2013), Attaipadi and Kallar region of Western Ghats (Thiribhuvanamala *et al.* 2013) and Kodagu district of Karnataka (Usha and Janardhana, 2014).

4.2.1.2 WGM-02

The mushroom was documented in Western Ghats of Hassan district (latitude 12°55'16.20"N and longitude 75°45'16.54"E, altitude: 3184 ft.).

Phenotypic description: Pileus is light apricot to light orange in colour; conical in shaped measures 5.5 cm in diameter, surface of the cap is smooth (Plate 03). Gills are attached, greyish brown in colour. The stalk is thin, light yellowish white in color and delicate, the stalk position is central and cylindrical in shape, 7 cm length and 0.7 cm thickness, Rhizoids and annulus are absent, produced brown coloured spores. The fruiting bodies of this species were found grow in coffee plantation.

ITS region sequence showed 86 % homology with the earlier reported *Psathyrella candolleana* (DQ389720.1) (Fig.05). Phylogenetic analysis showed that WGM-02 is

closely related to *Psathyrella candolleana* reported from Tamil Nadu, India (KT000344.1), New Delhi (AM237456.1) and China (KU194325.1) and grouped under clade IV (Fig.06). Kaya and Bag (2010) reported this species from *Populus* tree stump in Adiyaman region of Turkey. The species was also recorded in the Pune region by Senthilarasu (2014). Usha and Janardhana (2014) reported this species from Heggadadevana kote forest region of Karnataka, India.

4.2.1.3 WGM-03

The mushroom was found on dead and decaying wood (latitude 12°57'09.84"N, longitude 75°49'36.83"E and an altitude of 3145 ft. from mean sea level). Appearance of the mushrooms was observed in Hassan, Chikkamagaluru, Shivamogga and Dakshina Kannada districts of Karnataka. It is saprotrophic fungus vernacularly known as 'fairy inkcap' or 'trooping crumble cap'. The fruiting bodies were disappears within 2-3 days and washes away by heavy rain. The cap is convex in shape and diameter of the cap is 3 cm. The texture is soft, gills present on hymenium and widely attached to stipe. The stipe is cylindrical, slender and hollows which measures 4-4.5 cm in length and 0.50-0.75 cm in diameter. The spores are black, elliptical and measured $9 \times 5 \mu\text{m}$. The mushroom produces gregarious and large troops of fruiting bodies (Plate 04).

ITS region sequence showed 99 % homology with the earlier reported *Coprinellus disseminates* (JQ922133.1) (Fig.07). The phylogentic tree of *Coprinellus disseminates* showed that of WGM-03 is slightly close to Ecuador and Slovakian *Coprinellus disseminates* in clade V (Fig.08). Kaya and Bag (2010) reported that this species was found on damp wood debris in Adiyaman region of Turkey. The species was also recorded in the Pune region (India) by Senthilarasu (2014). Usha and Janardhana (2014) reported this species from Heggadadevana kote forest, Kodagu, Chikkamagaluru and Chamarajanagara districts.

4.2.1.4 WGM-04

It is a spatula-shaped jelly fungus with 1-2 cm tall and 0.5-0.3 cm wide. Color of the fruiting body is bright golden yellow. This mushroom was documented in Western



Plate 2: WGM-01 (*Microporus xanthopus*)



Plate 3: WGM-02 (*Psathyrella candolleana*)



Plate 4: WGM-03 (*Coprinellus disseminates*)



Plate 5: WGM-04 (*Dacryopinax spathularia*)

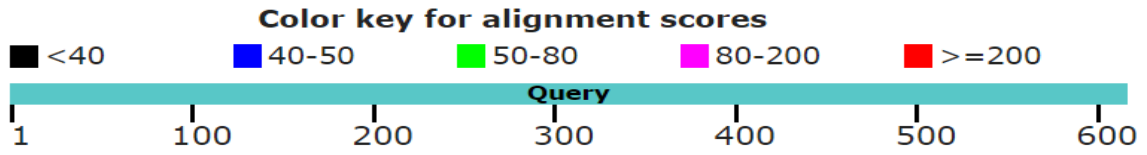


Plate 6: WGM-05 (*Corioloopsis polyzona*)



Plate 7: WGM-06 (*Cookeina tricholoma*)

GTTGTAGCTGGCCTTCCGAGGCATGTCGCACGCCCTGCTCAATCCACTCTACAC
 CTGTGCACTTACTGTAGGTTTCTTGGTTCGCGTTGGGTTCCGTTACTGGGGCTCG
 ACAAAGCCGAGGGGCTTATGTTTTACTACAACTATAAAGTAACTGAATGTATA
 CCGCGTCTAACGCATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGC
 ATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGT
 GAATCATCGAATCTTTGAACGCACCTTGCCTCCTTGGTATTCCGAGGAGCATG
 CCTGTTTGAGTGTTCATGAAATCTCAACCTACAAGCCTTTTCGGAGGTCTTTGTA
 CGGCTTGGACTTGGAGGATCATTGTCGGCAGCGATGTCGGCTCCTCTTAAACGC
 ATTAGCTAGTTCTCGCGGAACGGCTCTCGGTGTGATAATTGTCTACGCCGTGGT
 CGTGCCGGGTACATGGACAAGCTTCTAAACCGGTCACTCCTTGTGAGAGACACA
 TATCTTGACATCTGACCTCAAAACAGGTAGGACTACCCGCTGAACTTAAGCATA
 TCAATAAGCCGGAGGGAA



Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/> Microporus xanthopus isolate BPSM30 internal transcribed spacer 1, partial sequence: 5.8S ribosc	1045	1045	97%	0.0	99%	KM985667.1
<input type="checkbox"/> Microporus xanthopus voucher Cui8242 internal transcribed spacer 1, partial sequence: 5.8S ribos	1045	1045	100%	0.0	98%	JX290074.1
<input type="checkbox"/> Microporus vernicipes voucher UOC-DAMIA-D16 18S ribosomal RNA gene, partial sequence: inte	1032	1032	100%	0.0	98%	KP715551.1
<input type="checkbox"/> Microporus sp. BAB-5195 internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA ger	1027	1027	100%	0.0	98%	KT188608.1

Fig. 3: Partial sequence length and homology search of WGM-01 (*Microporus xanthopus*)



Fig. 4: Phylogenetic relationship between *Microporus xanthopus* (WGM-01) and other *Microporus xanthopus* species

ATCTATGGCGTTGGTTGTAGCTGGCTCTTAGGAGCATTFTTGTGCACGCCCGTCA
 TTCATATCATCTTTCCACCTGTGAACTATGTGTAGGCCTGGATACCCCTCGCTTT
 GGCAACAAGGCGGATGCAAGGATTGCTGTGTCGACAAAGGCCCGGTTCTCTTTG
 AATTTCCAGGTCTATGGCTTTTACACCAACCCATTTGAAGGATTTAAAATGTATC
 AATGGGCTTTTCTGGCCAATAAAAAACAATCCACTTTCCACAAAGGAACCCCTG
 GGTCCCCCATTCAAGAAAAAACCCACGGAATGGCAATAAGAATGGGAATTGGC
 AAAATCCGGGGATTCTTCAATTCTTTGACCGACCCTGGCCTTCCTGGGTATCCC
 AAGGACATGGCCGGTTGAA TGGCCTTAAATTCCCCCCCCCCCCCAGTTTGGACCA
 AAATGGTGGAAAGGTTGGAAAGGGGGGGTTTGGCAGGTTGCTTAAGGCTGGCCTC
 TCCCCTGAAATGCCTTACCGACTCTTATTGAGCTCCGTCTATTGGTGTGATAATT
 ATCTACGCCGTGGATTGGACTCATGCTTGTCTTACCGTCCGCAAGGACAATTT
 ACTTGACCAATTTGACCTCAAA TCAGGTAGGACTACCCGCTGAACTTAAGCATA
 TCATAGCGAGA

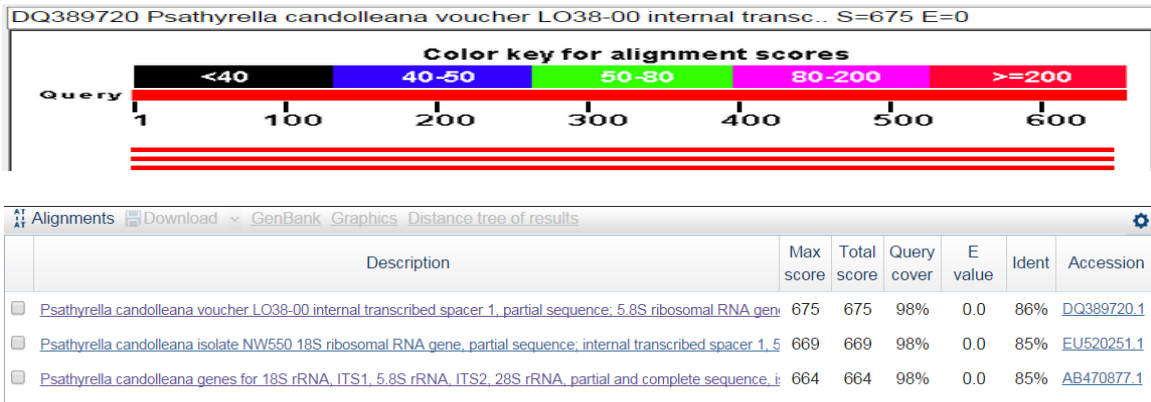


Fig. 5: Partial sequence length and homology search of WGM-02 (*Psathyrella candolleana*)

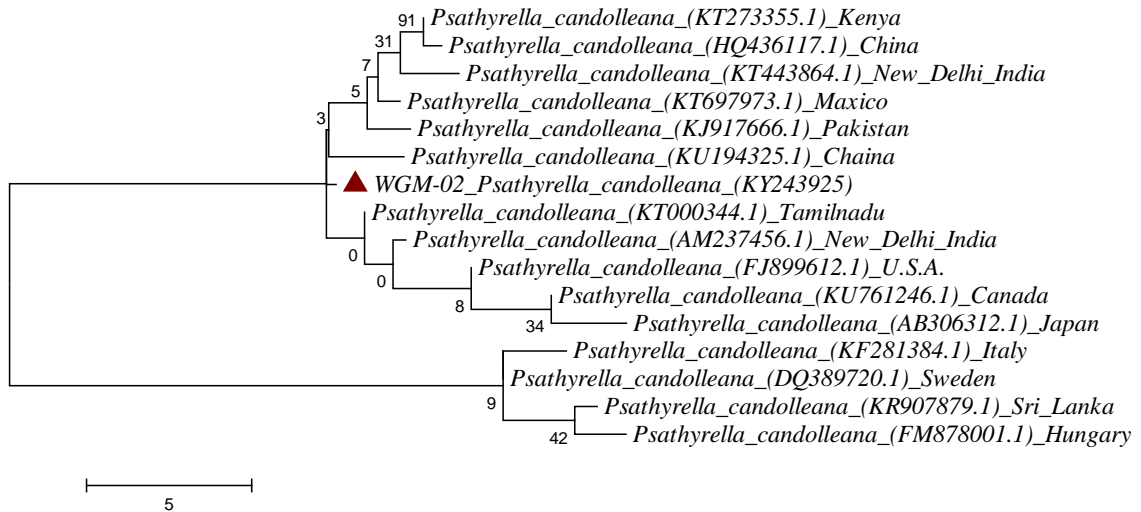


Fig. 6: Phylogenetic relationship between *Psathyrella candolleana* (WGM-02) and other *Psathyrella candolleana* species

CGGCATCGATACTATGGTGTCTTGGTTGTCGCTGGCTCCTCGGAGCATCGTGCA
 CGCCCGCCATTTTATCTATCCACCTGTGCACCGAATGTAGGTCTGGATGACTC
 TCGCCTTCGGGCGGATGCGAGGTTTGGCTTTCGCGTTCGAGCGCTCTCCTCGA
 ATTTCCAGGCTCTACGTCTCTTTACACACCCCAAACGTATGATGCAGAATGTAG
 TCAATGGGCTTTACAAGCCTATAAAACACTATACAACTTTCAGCAACGGATCT
 CTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATT
 GCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCCTCCTTGGTATT
 CCGAGGAGCATGCCTGTTGAGTGTCAATAAATCTCAACCTCACCGGTTTTCT
 GAACCGTTCTCCGAGGCTTGGATGTGGGGGTCTGTGCAGGTTCGCTCAGCGCG
 GTCTGCTCCCCTGAAATGCATTAGCGAGATTCATTCTGGACCTCCGTCTATCGG
 TGTGATAATTATCTACGTTCGTTGACTTGGTTCGGACTCGCTTCTAACCGTCCGC
 GAGGACAACATACTTGACAAATTTGACCTCAAATCAGGTAGGACTACCCGCTGAA
 CTTAAGCATATCAATAAGCGGGAGGAAGAGGT

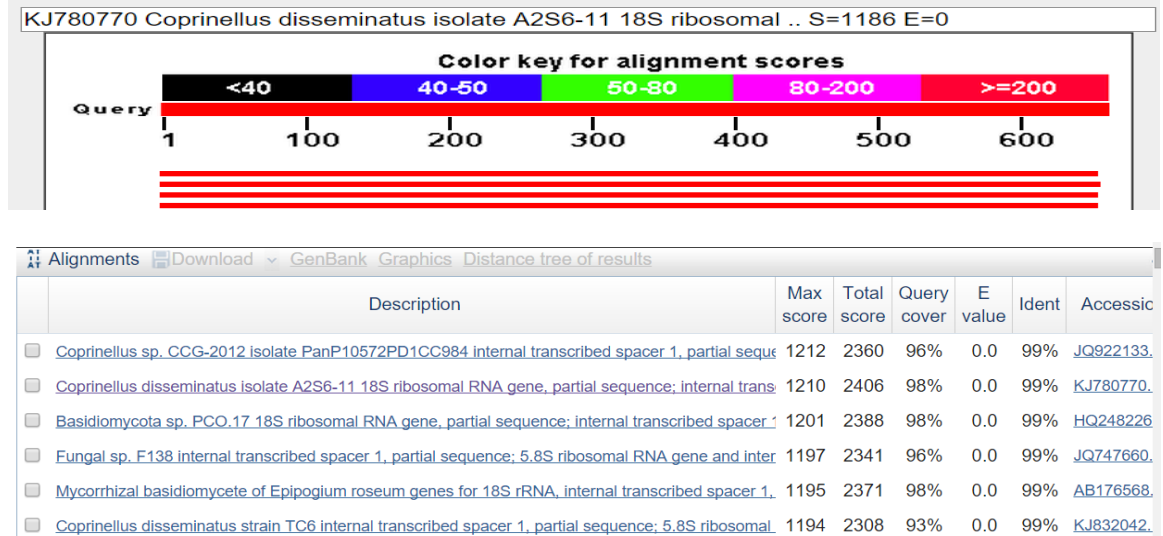


Fig. 7: Partial sequence length and homology search of WGM-03 (*Coprinellus disseminates*)

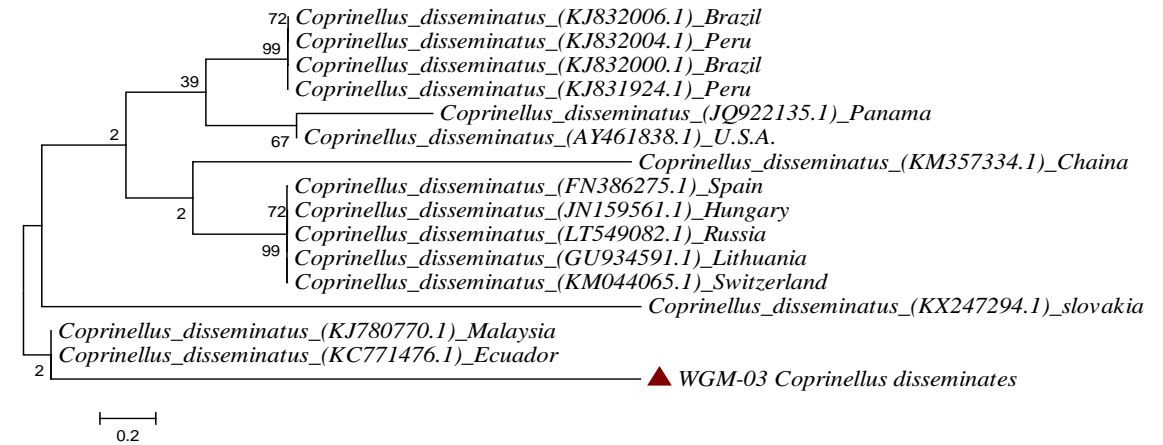


Fig. 8: Phylogenetic relationship between *Coprinellus disseminates* (WGM-03) and other *Coprinellus*

Ghats of Hassan, Shivamogga and Dakshina Kannada at latitude 12°43'32.28"N, longitude 75°33'17.20"E, and an altitude of 372 feet from mean sea level. The pileus was bright golden yellow, stalked, tough jelly club, usually with a flattened petal-like top and 1.5 cm tall (Plate 05). Spores are ellipsoid with smooth-surface and translucent. Average size of the spore was $8 \times 3 \mu\text{m}$. It was found growing on wooden logs gregariously in clusters.

The ITS sequence of 447 bp showed 96 % homology with *Dacryopinax spathularia* (Fig.09). Phylogenetic tree constructed showed that the WGM-04 is closely related to Japan and Indonesian *Dacryopinax spathularia* (AB712473.1 and AJ537397.1) and grouped into clade III (Fig.10). This species was reported to be edible in some parts of Sikkim (Das, 2010). Tadiosa and Briones (2013) recorded that the distribution of this species in Taal Volcano Protected Landscape of Philippines.

4.2.1.5 WGM-05

It is a polypore mushroom with woody texture and there is a presence of zonation on the mushroom. This was collected from Dakshina Kannada (latitude 12°43'41.82"N and longitude 75°32'55.89"E, altitude at 436 ft from the mean sea level). The pileus of the fruit body was yellow with many green and light orange zones (Plate 06). The fruit body had hard texture and grows in half circular manner. Stalk is sessile, spore were white in colour and found growing solitary on dead wood.

The sequence ITS region showed 99 % homology with the earlier reported *Coriolopsis polyzona* (Pers.) (Fig.11). This mushroom species was very close to Kenyan *Coriolopsis polyzona* (FJ904854.1) (Fig.12). *Coriolopsis polyzona* could be a good choice for olive oil mill waste waters decolourisation and better effluent decolourisation was obtained under lignin peroxidase induction condition (Jaouani *et al.*, 2006). The immobilized system showed better chemical oxygen demand decreases during three consecutive batches without remarkable loss of performances of the *Coriolopsis polyzona* (Neifar *et al.*, 2012).

4.2.1.6 WGM-06

The fungus fruiting body is cup shaped with an in rolled margin, slender stipe. The mushroom was collected in Nettana (latitude 12°43'49.06"N and longitude 75°33'13.12"E, at an altitude of 372 ft.). The pileus is goblet to funnel-shaped with an in rolled margin, 1-2 cm in diameter, with slender stipe that are 1-3 cm tall, hair like structures are present on pileus. Hairs are stiff, bristle-like and 2-4 mm long (Plate 07). Stem is short and position at the center. The ascospores are pointed-ellipsoid, surface with fine, longitudinal ridges measured $22 \times 11 \mu\text{m}$. It was grown on fallen trees and dead wood in group.

ITS sequence showed 99 % homology with the earlier reported *Cookeina tricholoma* (AF394020, 1) (Fig.13). The phylogenetic tree showed that WGM-06 is closely related to *Cookeina tricholoma* (AF394024, AF394019.1 and AF394026.1) (Fig.14). *Cookeina tricholoma* was reported in China, Venezuela, Puerto Rico, Thailand and Malaysia (Weinstein *et al.*, 2002). Ascospores of this species were ornamented with 22-24 longitudinal ridges. This species produces high number of fruiting bodies on wood (Lopez-Quintero *et al.* (2012). Enow *et al.* (2013) reported from Mount Cameroon Region. Tadosa and Briones (2013) recorded the distribution of this species in Taal Volcano Protected Landscape of Philippines. Moreno *et al.* (2016) showed the significant inhibition of neurogenic pain by β -D-Glucans from *Cookeina tricholoma*.

4.2.1.7 WGM-07

A coral fungus was appeared in moist soil under the shrubs in Nettana (latitude 12°43'35.69"N and longitude 75°33'18.39"E, altitude: 377 ft.). The mushroom produces ash like powder which sticks to hand while picking (Plate 08). The fruiting body of the fungus was cylindrical. Asci with eight ascospores arranged in uniseriate manner. The ascospores were brown to dark brown, unicellular, ellipsoid, in-equilateral, usually minutely pinched at ends, $6-7 \times 2.5 \times 3-4 \mu\text{m}$. The mushroom was found growing in group (30-40 fruiting bodies) under the herbs and shrubs.

ACCTGTACATGCCCTTCGGGGTAACACACAAACTCTAGTGTTGTCTATGTATGT
 CTAGTTATTCATAACAAGTATAACTTTCAACAACGGATCTCTTGGCTCTCGCATC
 GATGAAGAACGCAGCGAAA TCGGATAAGTAATGTGAATTGCAGAATAGTGAATC
 ATCGAATCTTTGAACGCACCTTTCGCCCCGACGGGGCATGCCGGTTTGAGCGC
 CTGTTCACTCCTGCACTAGTGGATTCTTCTATTAGAGCGATGTGAGTGTGCTGG
 TCTTACCAGCTCGCTCTGAATGCATTAGCAGCAGTTAGGCTTGTGACAACGTGA
 TAAGTCGTCGTTGAAGCAATGCTGAGCCGCCGCTCCTAATCGTCTTTCGGACAAT
 AACCTAATGCTAGGCATCAAAGCGGTAGGACTACCCGCTGAACTTAAGCATATC
 AATAAGCGGAGGAAG

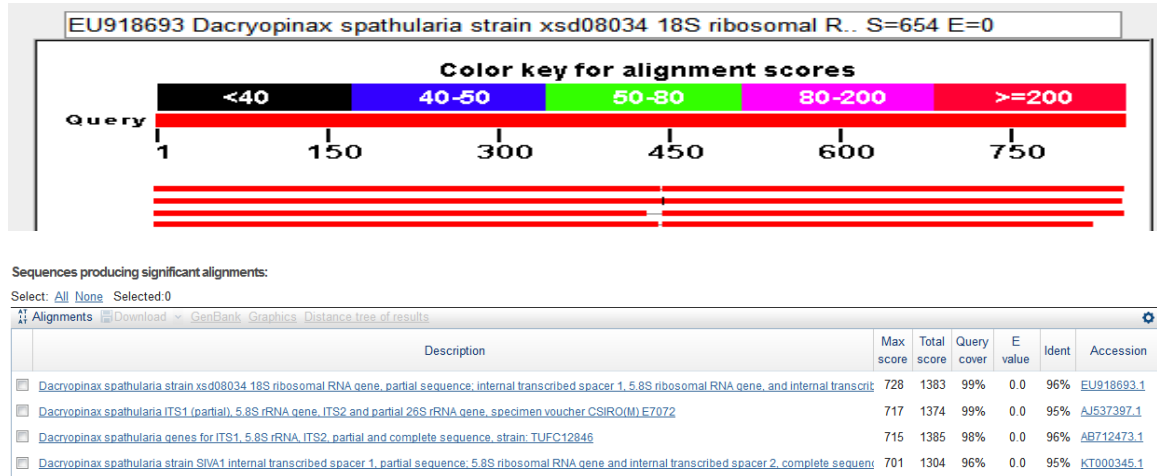


Fig. 9: Partial sequence length and homology search of WGM-04 (*Dacryopinax spathularia*)

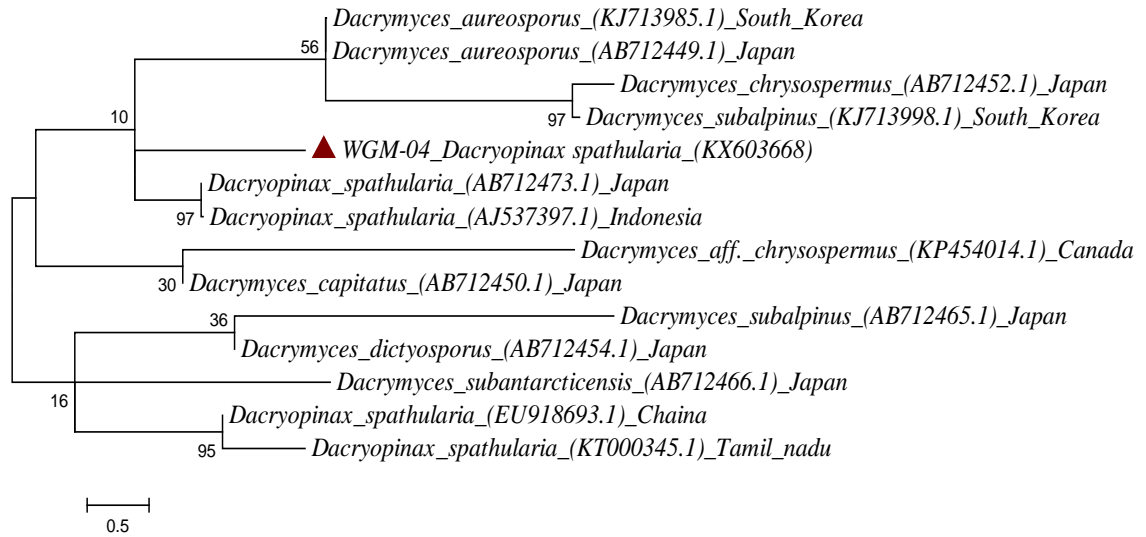


Fig. 10: Phylogenetic relationship between *Dacryopinax spathularia* (WGM-04) and other *Dacryopinax spathularia* species

GGCCCTCGGGCTTTGACGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACACCC
 TGCTCATCCACTCTACACCTGTGCACTTACTGTAGGTTGGCGTGGGCTTCGGAC
 CTCCGGGTTTCGAGGCATTCTGCCGGTCTATGTACTACTACAACTCCGAAGTAAC
 AGAATGTAACGCGTCTAACGCATCTTAATACAACCTTTCAGCAACGGATCTCTT
 GGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCA
 GAATTCAGTGAATCATCGAATCTTTGAACGCACCTTTCGCTCCTTGGTATTCCG
 AGGAGCATGCCTGTTTGTGATGTCATGGAATTCTCAACCCATAGATCCTTGTGGT
 CTACGGGCTTGGATTTGGAGGCTTGCCTGGCCCTTACACGGGGTTCGGCTCCTCTT
 GAATGCATTAGCTTGATTCCGTGCGAATCGGCTCTCAGTGTGATAATTGTCTAC
 GCTGTGGCCGTGAAGCGTTTGGCGAGCTTCTAACTGTCCGTTAGGACAACCTCT
 TGACATCTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAAT
 A

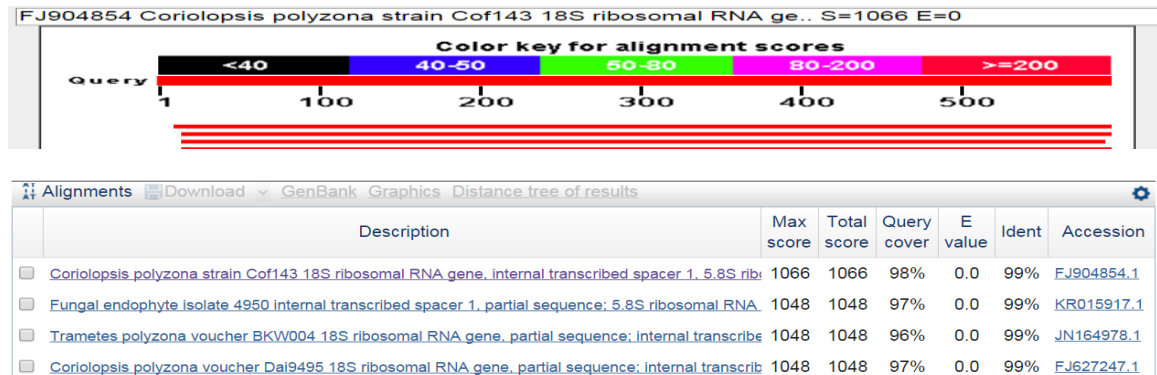


Fig. 11: Partial sequence length and homology search of WGM-05 (*Coriolopsis polyzona*)

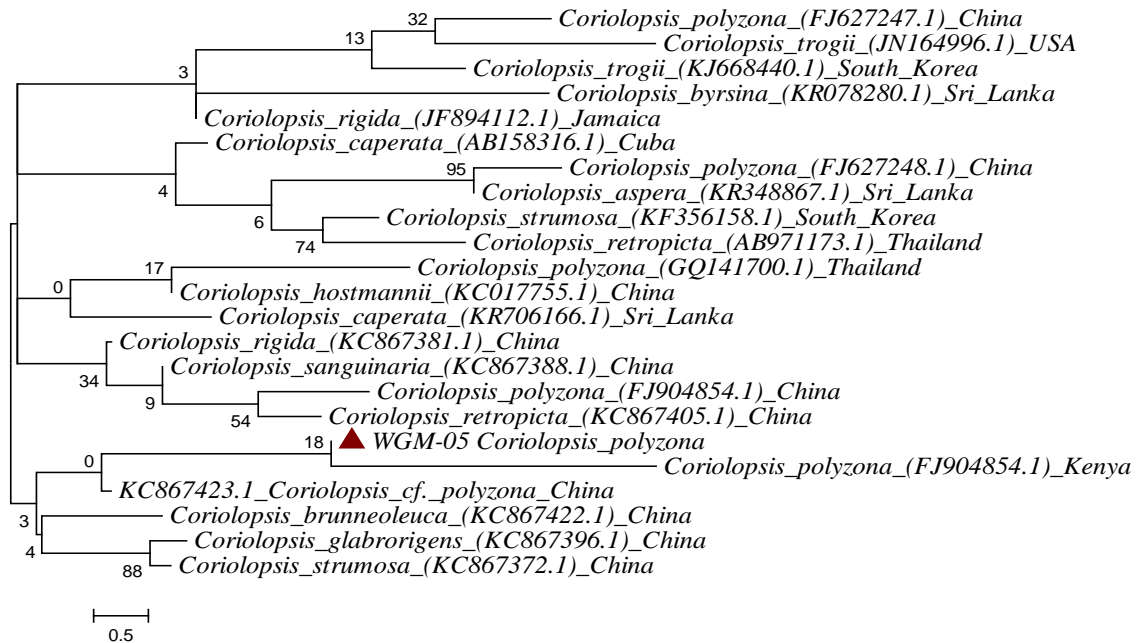


Fig. 12: Phylogenetic relationship between *Coriolopsis polyzona* (WGM-05) and other *Coriolopsis* species



Plate 8: WGM-07 (*Xylaria cirrata*)



Plate 9: WGM-08 (*Hygrocybe persistens*)



Plate 10: WGM-09 (*Gymnopus brunneigracilis*)



Plate 11: WGM-10 (*Filoboletus manipularis*)



Plate 12: WGM-11 (*Agaricus trisulphuratus*)

GGGCAGGGGCGAACGATCCGACGGCTGGGGCTTACCTGATGTTTGACTTGCTC
 CCAGACGACCCACCCCGAGCTGTCCTAGGCTGTACCGCGACGTCGCTGATCG
 CAACAATCGGCGCTGTGTTGTCAATTTCTTCTGAGACTCTTAAATACCTGGGCAT
 ATCAATAAGCGGACGAGCTTCTGGCTACGATGAACAACGCAGCGAAAATGCGAT
 AAGTAATGTGAATTGCAGAAATCAGTGAATCATCGAATCTTTGAACGCACATTG
 CGCCCATTAGCATTCTAGTGGGCATGCCTGTTGAGCGTCAATTTCAACCCTTAA
 GCCCTGTTGCTTAGTGTGAGAGGCTACGGCACGCCGTACCTCTCGAAAGTCA
 TTGGCGGAGCCGACTTCATGCTCTAGACGTAGTAAATTCATCTCGTCTGCAGCT
 GGGCCGTCCCCTGCCATAAAACACATACTTTTTTCAGGTTGACCTCGGATCAGG
 TAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA

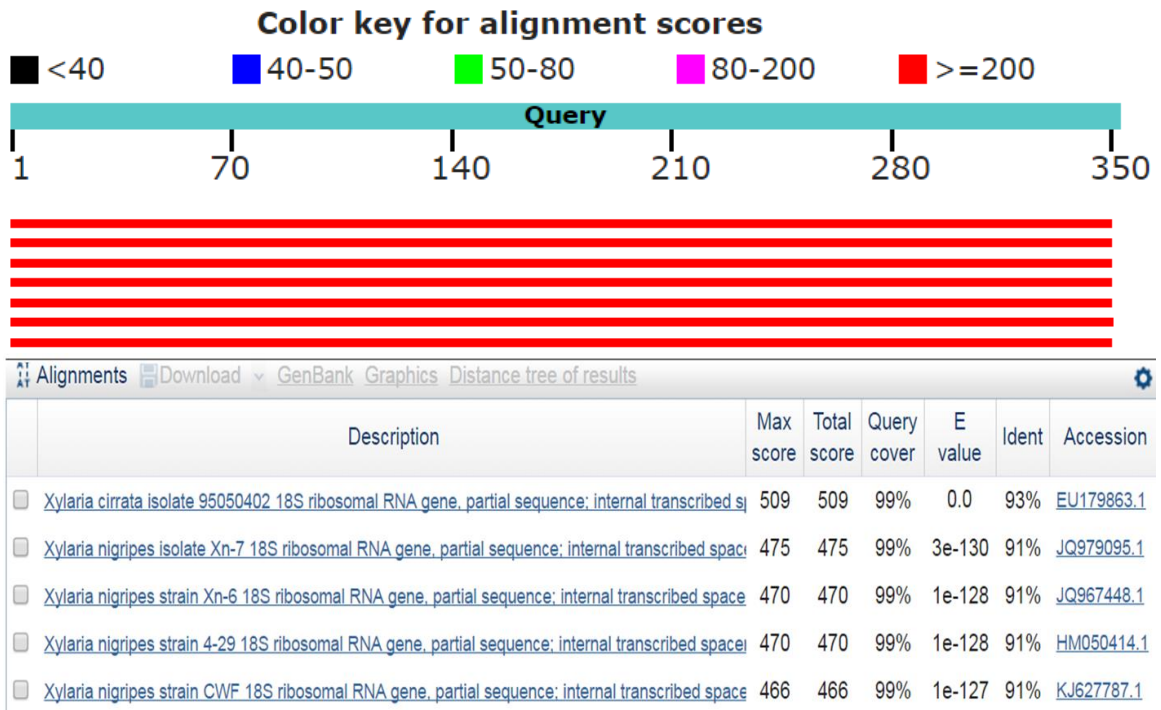


Fig. 15: Partial sequence length and homology search of WGM-07 (*Xylaria cirrata*)

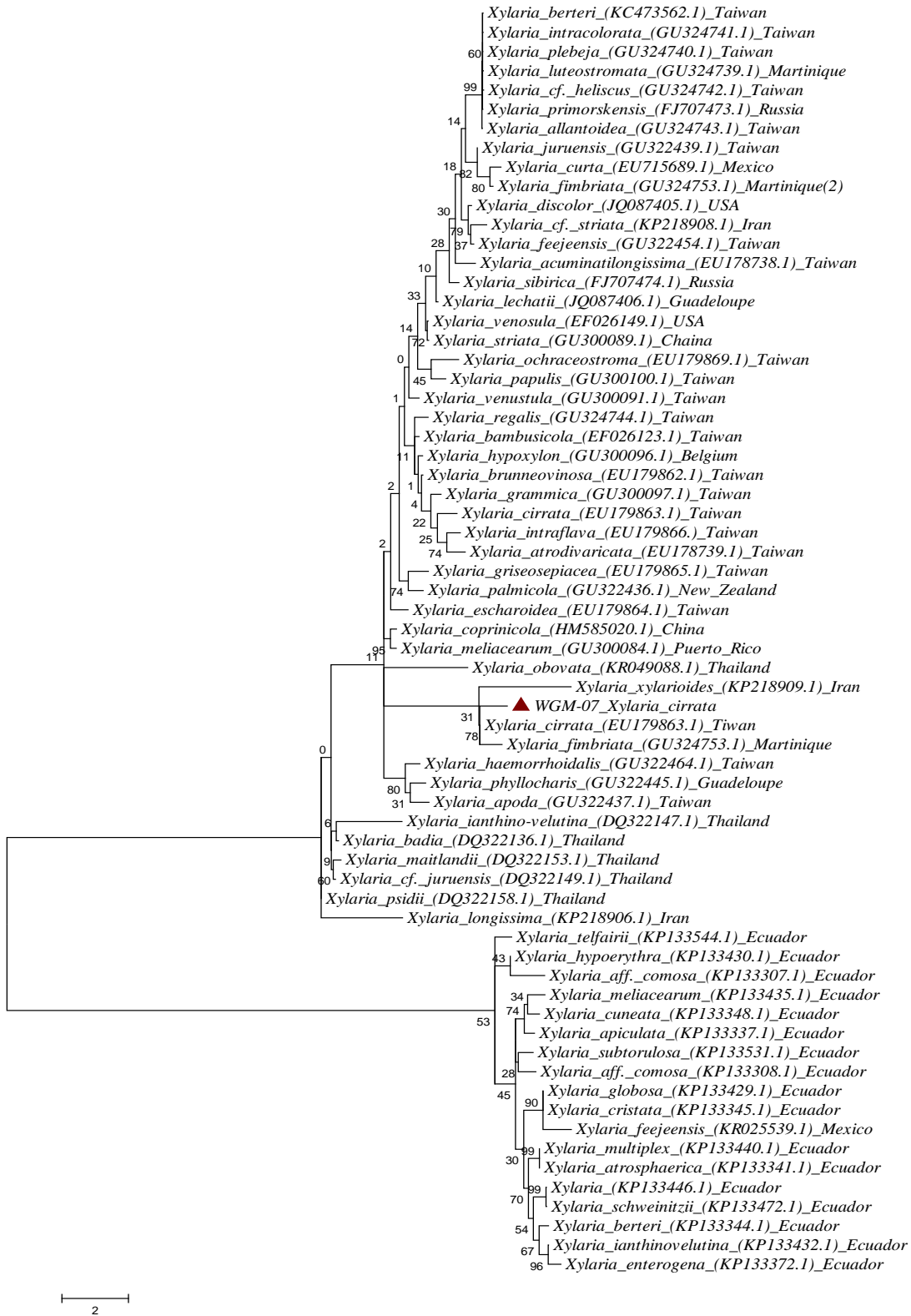


Fig. 16: Phylogenetic relationship between *Xylaria cirrata* (WGM-07) and other *Xylaria* species

The sequence data showed 93 % homology with the earlier reported *Xylaria cirrata* (Fig.15). Phylogenetic tree of genus *Xylaria* was closely related to *Xylaria cirrata* (EU179863.1) which was earlier reported from Tiwan (Fig.16). *Xylaria cirrata* was associated with nests of *Odontotermes formosanus* (Ju and Hsieh, 2007).

4.2.1.8 WGM-08

The mushroom is collected from Dakshina Kannada (latitude 12°49'32.99"N and longitude 75°25'27.11"E, altitude: 377 ft.) and it contains orange colour pigment. Pileus was bright orange colour, conical in shape with smooth texture (Plate 09). Gills and stem are white and orange yellow combination. It was found growing on grassland during onset of monsoon in group of three to four.

ITS region was sequence showed 83 % homology with the earlier reported *Hygrocybe persistens* (Fig.17). The phylogenetic tree showed that WGM-08 is grouped in Clade IV (Fig.18). Polemis *et al.* (2011) documented *Hygrocybe persistens* in bare moist land and forests of Greece. *H. persistens* was also reported from Mt Sakar of southeastern part of the Balkan Peninsula by Lacheva (2015).

4.2.1.9 WGM-09

Basidiocarp is bright orange coloured with waxy texture and is documented in the Managundi of Dakshina Kannada district (latitude 12°43'48.09"N and longitude 75°25'26.42"E, altitude: 431 ft.). Cap is uplifted in wave like arrangement, peach-orange color, Cap diameter is up to 12 cm, smooth to medium texture (Plate 10). Gills are peach color and broadly placed. Stalk is 12-14 cm; cylindrical. This mushroom are grows gregariously in cluster of 10 to 15 fruiting bodies on humus soil.

ITS region sequence (684bp) showed 99 % homology with the earlier reported *Gymnopus brunneigracilis* (AY263434.1) (Fig.19). Phylogenetic tree showed that the identified *Gymnopus brunneigracilis* (Corner.) is very close to *Gymnopus brunneigracilis* of U.S.A. (Fig.20) Antonin *et al.* (2014) reported this species from region of Java.

4.2.1.10 WGM-10

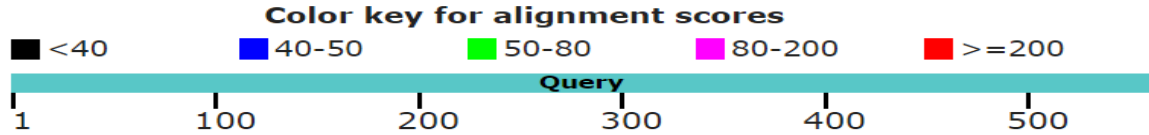
The mushroom is documented from Western Ghats of Dakshina Kannada district (latitude 12°49'36.60" N and longitude 75°25'26.41" E; altitude at 410 ft.). Pileus is white with peach colour towards the central which is knob like structure. Pore structure (interlinking gills) can be seen from the top of the cap due to translucent in nature (Plate 11). Pileus is convex to umbonate structure and measures about 2-3.5 cm in diameter. Pores are adanate, 1-3 per mm and symmetrically arranged. Stipe position is at central and the length is about 5-6 cm with 0.2-0.5 cm diameter. Spore print is white (Plate 31a), smooth, subglobose to almost globose, hyaline, amyloid, thin-walled and spores measures about $4 \times 6 \mu\text{m}$ (Plate 31b). Basidium is about $15-25 \times 6.5-8 \mu\text{m}$, clavate and bearing four sterigmata up to $7 \mu\text{m}$ long. This mushroom grows gregariously on rotting wood during rainy season (July to September).

The ITS sequence data showed 100 % homology with the earlier reported *Filoboletus manipularis* (Berk.) (Fig.21). Phylogenetic tree showed that WGM-11 is closely related to Vietnam *Filoboletus manipularis* (KF746998.1, KF746991.1, and KF746995.1,) with bootstrap support 100 and grouped in clade II (Fig.22). This mushroom was reported emitting yellowish green light on stipe and hymenophore, sometimes on pileus (Chew *et al.*, 2015).

4.2.1.11 WGM-11

This mushroom (Plate 12) was collected from Thirthahalli of Shivamogga district (latitude 13°40'35"N and 75°13'49.82"E, altitude 2063 ft.). Orange coloured cap with presence of scales which looks like cotton. Scales will fall or stick to fingers when disturbed. Stipe is neon carrot to orange colour, presence of cottony scales, presence of annulus; Stalk is tapering downwards and measures 5 cm in length. Lamellae are free, pale pinkish, becoming dark vinaceous brown the cheilocystidia are clavate to cylindrical. Spores are ellipsoid, adaxially applanate, fairly thick-walled, brown in colour; $5-6 \times 3-4 \mu\text{m}$. mushrooms grows in a group on forest soil near acacia plantation during the monsoon

AGATGATGGGCAAAAAAAAAAGCTCATCCTGTTTTAGGAGCAATAGCTACTTGA
 GCAGCCCTCTGACATAGAAAAAGCTATCAATGCCAAAGGAGCATACTATCACTT
 TGCTAATACGTTTCAAAGGAGCTGAACCAAAGGGTCCAGCACAAACCCCAAGA
 TCCAAAAACAAGAGAGAGATCTCCTTGTGGTATAGATTAATGACACTCAA
 ACAGGCATGCCCTCGGAATACCAAGAGGGCGCAAGGTGCGTTCAAAGATTGCA
 TGATTACGGAATTCTGCAATTCACATTAAGTATCGCATTTCGCTGCGTTCTTCA
 TCGATGCGAGAGCCAAGAGATCCATTGTTGAAAGTTGTTTCAATTAATTTTTA
 ATTTAAGCCAACAACATTCAAAAGCAATGCGTTGCATGTGTGGTACATAGGACA
 AGCACCAAAAAACCCGAACAGGTGAGCAGGAGCCGCCTACAGGATGTGCACAA
 GGGTGTGATGGAGATTGTGGAGTTGTTTTGAGGAGTGCACGTTGCCTCGATGA
 GAGGCCAGCAACA TCCCCAAAATCACTTCAGTAATGATCCTCCGCAGGTTTAC
 CTACGGAAAGAGGTTCACTTACTGAAAGTGATTTTGGGGATGTTGCTGGCCTCTCA
 TCGAGGCAACGTGCACTCCTCAGAACA



Description	Max score	Total score	Query cover	E value	Ident	Accession
Hygrocybe persistens var. konradii 18S rRNA gene (partial), ITS1, 5.8S rRNA gene, and ITS2, iso	1043	1136	100%	0.0	99%	FM208893.1
Uncultured basidiomycete clone M2A7 18S ribosomal RNA gene, partial sequence, internal transcr	403	535	89%	3e-108	84%	FJ456975.1
Hygrocybe acutoconica f. japonica voucher CFMR JP-2 internal transcribed spacer 1, partial sequ	401	484	95%	1e-107	77%	KF291161.1

Fig. 17: Partial sequence length and homology search of WGM-08 (*Hygrocybe persistens*)

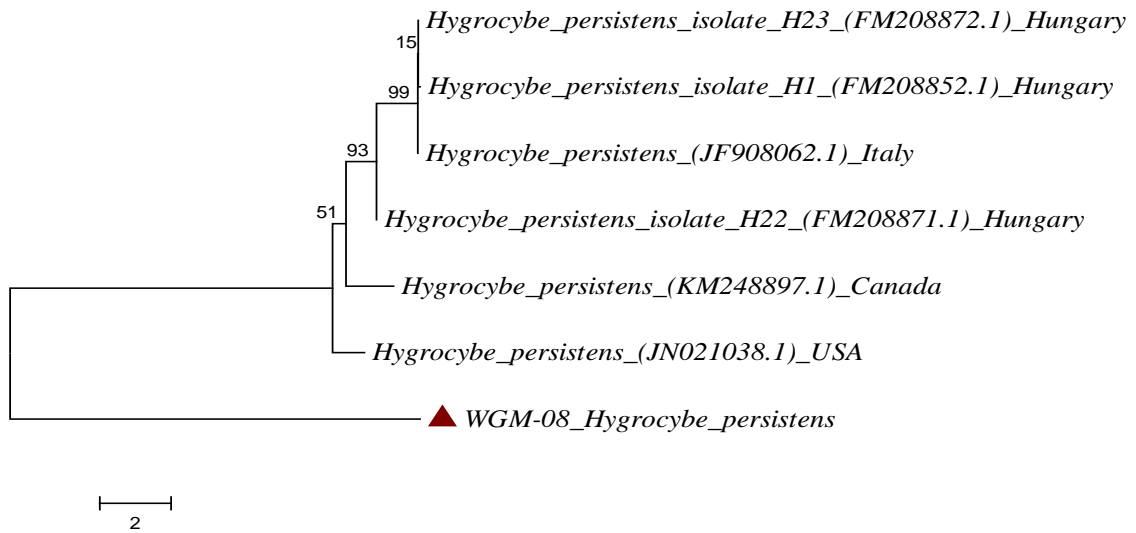
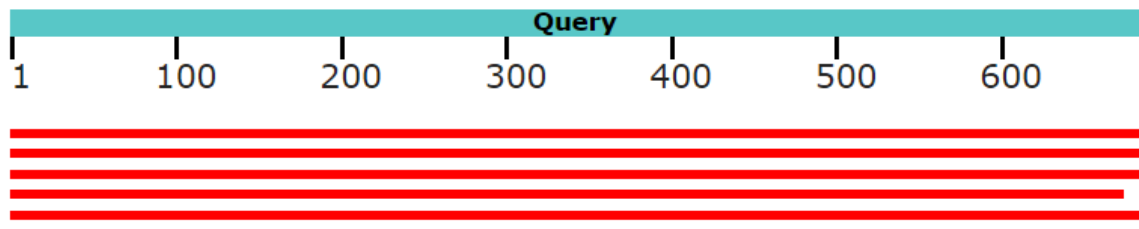


Fig. 18: Phylogenetic relationship between *Hygrocybe persistens* (WGM-08) and other *Hygrocybe persistens* species

CATGTGCACGTA CTTCTAATCTATTCATCCACCTGTGCATTTTTTTGTAAAAGTTC
 TTTCAGGTTGGCTTGCAAGGTCCTTTGGACATTGTTAATGGCTAGCATGCGAGG
 CTTCTATGTCCTTACAAACCAAGTTAAGTATGTTTAAAATGTGTTGTATTGGGACT
 TCATTGACCCATTA AACTATACAACCTTCAGCAACGGATCTCTTGGCTCTCCCAT
 CGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAATTCAGTGA
 ATCATCGAATCTTTGAACGCACCTTGCGCCCTCTGGTATTCCGGAGGGCATGCC
 TGTTTGAGTGT CATTAAATCTCAACTTCAATAATTTGTAATGAACTAATGAAGC
 TTGGATGTGGAGGTTTTGCTGGCGTCTTAATATGTTCCGGCTCCTCTTAAATGCA
 TTAATGGAACCCGTTTGTGGACTATCCATAGTGTGATAATTATCTACGCTTTGG
 TTGATTCAATGAACTCTATGGACTTACTTCAGAATTGCTTAAGCATTCTGGTGTG
 GGTATTTGTACTTAATGCTTTACCTGGTTCAATTAGAGTTGGGACATCTGCTCTCT
 AACTGTCTGTATATGCGGACAATATCTTGCTATTTGACCTCAAATCAGGTAGGA
 CTACCCGCTGAACTTAAGCATATCAATAA

Color key for alignment scores

■ <40 ■ 40-50 ■ 50-80 ■ 80-200 ■ >=200



Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/> Gymnopus brunneigracilis AWW01 internal transcribed spacer 1, partial sequence: 5.8S ribosom	1198	1198	100%	0.0	99%	AY263434.1
<input type="checkbox"/> Gymnopus luxurians voucher KUC20080725-28 18S small subunit ribosomal RNA gene, partial s	1088	1088	100%	0.0	96%	KM496469.1
<input type="checkbox"/> Gymnopus luxurians 18S ribosomal RNA gene, partial sequence: internal transcribed spacer 1_5	1088	1088	100%	0.0	96%	AY256709.1
<input type="checkbox"/> Uncultured Gymnopus clone HATFD14-44 18S ribosomal RNA gene, partial sequence	1059	1059	98%	0.0	95%	KU712543.1
<input type="checkbox"/> Gymnopus gibbosus AWW66 18S ribosomal RNA gene, partial sequence: internal transcribed sp	1052	1052	100%	0.0	95%	AY263437.1

Fig. 19: Partial sequence length and homology search of WGM-09 (*Gymnopus brunneigracilis*)

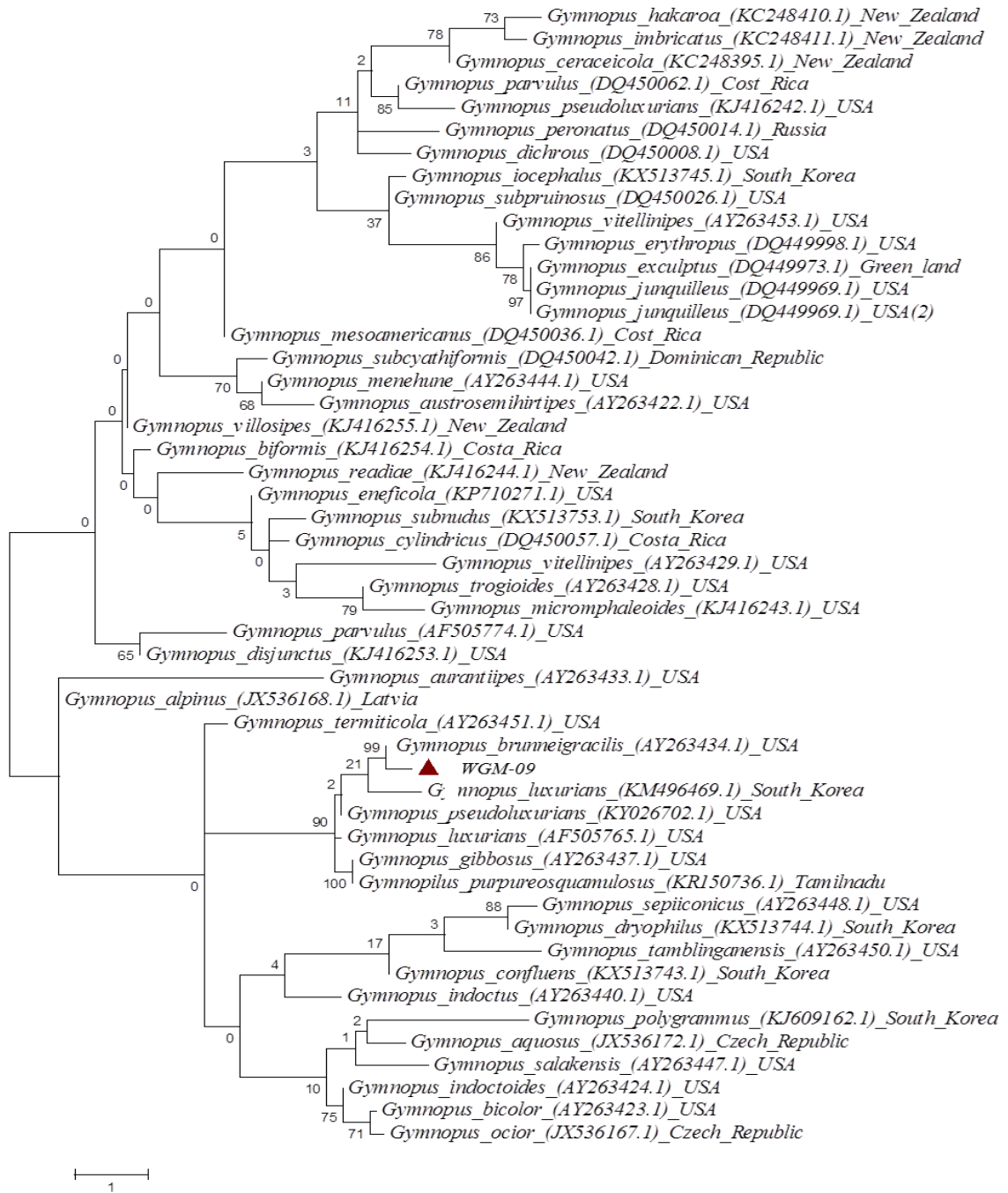
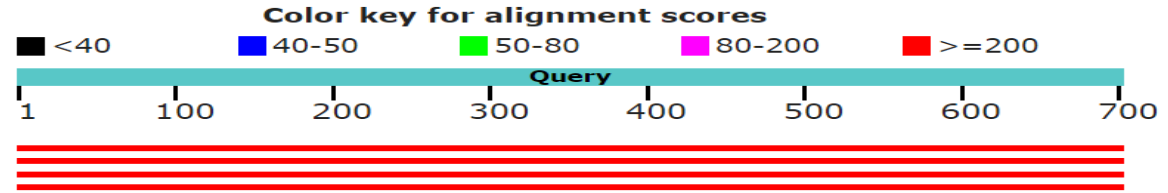


Fig. 20: Phylogenetic relationship between *Gymnopus brunneigracilis* (WGM-09) and other *Gymnopus* species

CTGATGCTGGCTCTTTACTGAGCATGTGCTCGTGTCTCTATTTATCTTCTCTTG
 TGCACATTTTGTAGTCAGTGAATGGAGACTTGCGTGCTTTCATTAGTGCGGTCT
 AGGGGTTTGTTCAAACCTTACCCCTGTTCCACTGCGCAGACTTTCATTAGTCTT
 GCGGTCTGGGAGTTTGTAAACCTTACTCCTTGTCTCACTGACTATGTTTTTCATA
 TACCCTTTATAAAGTTACAGAATGTCTTTTAACTTGATTGCGCTTGTCTGATGTC
 GTAAACCTATACAACCTTTCAGCAACGGATCTCTTGGCTCTCCTATCGATGAAG
 AACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGA
 ATCTTTGAACGCACCTTGCGCCCTTGGTATTCCGAAGGGCATGCCTGTTTGAG
 TGTCAATAAATTATCAACCTTAAAGACTTGCGTCTTCAAGGCTTGGATGTGAGG
 GCTTGCTGGCTTCCTTCAGTGGACGGTCTGCTCCCTTTAAATACATTAGTGGGA
 TCTCTTGTGGACCGTCACTTGGTGTGATAAATTATCTATGCCACTTGACTTTGAAA
 CAACTAAATGGGAACCTGCTTATAACCGTCCCTTCGGGACAACTTATTGACAAT
 TTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAATA



Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value
<input type="checkbox"/> Filoboletus manipularis strain G2 18S ribosomal RNA gene, partial sequence; internal transcribed s	1286	1286	100%	0.0
<input type="checkbox"/> Filoboletus manipularis strain G6 18S ribosomal RNA gene, partial sequence; internal transcribed s	1279	1279	100%	0.0
<input type="checkbox"/> Filoboletus manipularis strain G30 18S ribosomal RNA gene, partial sequence; internal transcribed	1279	1279	100%	0.0
<input type="checkbox"/> Filoboletus manipularis strain G11 18S ribosomal RNA gene, partial sequence; internal transcribed	1279	1279	100%	0.0
<input type="checkbox"/> Filoboletus manipularis strain G7B 18S ribosomal RNA gene, partial sequence; internal transcribed	1273	1273	100%	0.0

Fig. 21: Partial sequence length and homology search of WGM-10 (*Filoboletus manipularis*)

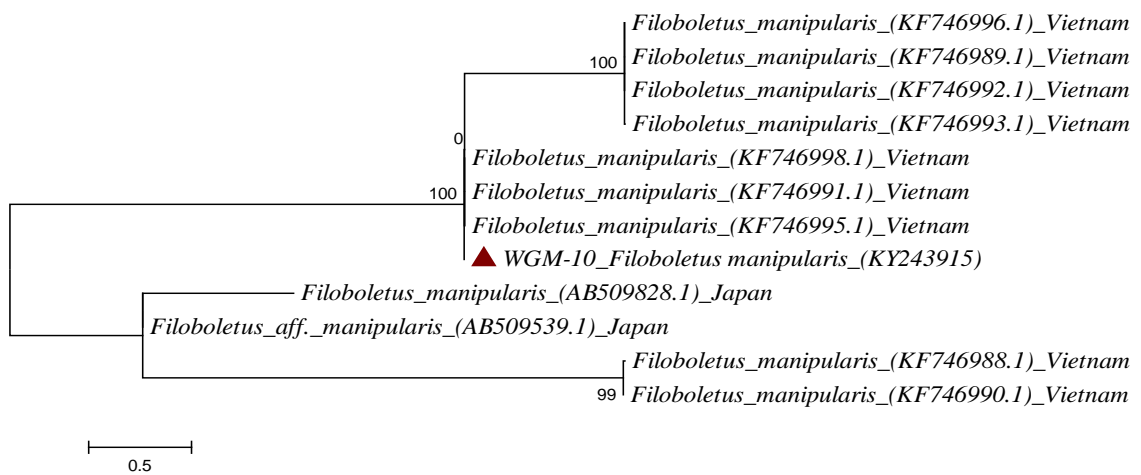


Fig. 22: Phylogenetic relationship between *Filoboletus manipularis* (WGM-10) and other *Filoboletus manipularis* species.

TTTGAGGTCAGCATTTCAGAAAAATTGCTTTTGCAGACGATTGGAAGCTGA
 ACAACACAGAGCAATCCCCCTCGCTAGTGTAGATAGTTATCACACTTGTG
 GCAGATCGCAAACGGTTCCGCTAATGCATTTTCAGGGGAGCTGGCCTCAC
 AAAGTGGCCAGCACACCCCCACAATCCAAGCCCTCCTTTACCGCAAAAG
 TATTGGAGAGTTGAGAAATTAATGACACTCAAACAGGCATGCTCCTCGG
 AATACCAAGGAGCGCAAGATGCGTTCAAAGATTCGATGATTCACTGAAT
 TCTGCAATTCACTTACTTATCGCATTTTCGCTGCGTTCTTCATCGATGCG
 AGAGCCAAGAGATCCGTTGCTGAAAGTTGTATTATAGTTTTCATAGGCTA
 GGCCCATGTAAAGACATTCGATAAACATTTCTATAGGGTATATGAATGACAT
 AGACTCCGACAGGAAGACGATTCGTTGGTTCGCATAGAGAACAGCAGTGC
 TTGCACACCACAGGTCCTCAAATCCAGCAAGTGCTGATAGGCCGACCAC
 TTCCTCAAAATCCGACGAAGACTACAAAAGGTGCACAGGTGGATGAAAA
 TGAAGTCCGAACAAGCGTGCACATGCTCCGAGGAGCCAGCTACAACCA
 TCCGGAAAACATAATTCAATAATGATCCTTCCGCAGGTTACCTACGGAA
 G

Agaricus trisulphuratus voucher LAH.No.8672011 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1 and 5.8S ribosomal RNA gene	1245	1315	99%	0.0	99%	KU170545.1
Uncultured fungus genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA gene, clone 3-174	1242	1311	97%	0.0	99%	FN812771.2
Agaricus sp. BAB-5233 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2	1236	1294	98%	0.0	99%	KT186165.1
Uncultured fungus genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA gene, clone 3-100	1236	1305	97%	0.0	99%	FN812762.2
Uncultured fungus genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA gene, clone 3-112	1236	1305	97%	0.0	99%	FN812764.1
Uncultured fungus genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA gene, clone 3-21	1234	1304	97%	0.0	99%	FN812759.2

Fig. 23: Partial sequence length and homology search of WGM-11 (*Agaricus trisulphuratus*)

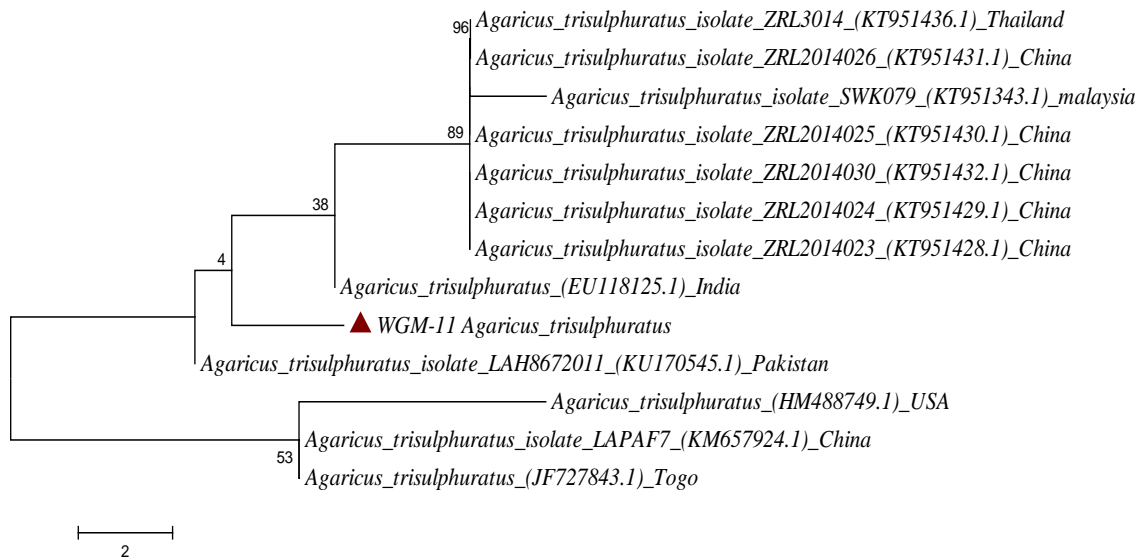


Fig. 24: Phylogenetic relationship between *Agaricus trisulphuratus* (WGM-11) and other *Agaricus trisulphuratus* species

The ITS region sequence data showed 99 % homology with the earlier reported *Agaricus trisulphuratus* Berk. (Fig.23). WGM-11 is grouped in Clade III in the phylogenetic tree and is closely related to Indian *Agaricus trisulphuratus* (EU118125.1) (Fig. 24). *A. trisulphuratus* is common in forests or grasslands in tropical and subtropical areas. This species was distributed in Chennai (Natarajan and Manjula, 1981), Malappuram, Thiruvananthapuram, Alapuzha, Palakkad, Kollam (Pradeep and Vrinda, 2007), Villupuram, Pichandikulam Forest, Oorani Forest (Mani and Kumaresan, 2009), Bengaluru (Pushpa and Purushothama, 2012), Kolkata (Pradhan *et al.*, 2013), Mangalore (Ghate *et al.*, 2014), Mulshi (Senthilarasu 2014). Tibuhwa and Mwangi (2014) reported this species in Tanzania. This mushroom also distributed in Ratnagiri (Borkar *et al.*, 2015) and Konaje (Greeshma *et al.*, 2015).

4.2.1.12 WGM-12

This mushroom is documented in the Managundi of Dakshina Kannada (latitude 12°49'30.98"N and longitude 75°25'29.63"E, altitude 450 ft.). Cap is convex when young, later flat, colour is bright pastel red to carmine red and measure up to 10 cm in diameter (Plate 13). Gills are white, brittle, and occasionally with a red edge at the rim of the cap. Stem is hollowing in the middle and pure white in colour. Spores are pale cream in colour (Plate 31c). This species grows scattered in soil.

ITS region sequence data showed 97 % homology with the earlier reported *Russula rosea* (Fig. 25). Phylogenetic tree showed that WGM-13 is grouped in clade II with Chinese *Russula rosea* (KF002750.1) (Fig. 26). Ayaz *et al.* (2011) collected and characterized the *Russula rosea* from the East Black Sea region of Turkey. Usha and Janardhana (2014) reported this species from Aanekaadu forest of Kodagu and Chamarajanagara region. Panda and Tayung (2015) reported *Russula rosea* from the forests of Northern Odisha, India during June - September.

4.2.1.13 WGM-13

Mushroom was collected from Dakshina Kannada (latitude 12°49'34.95" N and longitude 75°25'28.40" E, altitude: 439 ft.). Pileus is 2.5-6 cm across; oval when young,

becoming broadly conical, broadly convex, or bell-shaped; dry; powdery to scaly, the margin lined or grooved nearly to the center; bright white in colour (Plate 14). Gills are free from the stem. Stem was 8 cm long; 2-5 mm thick with slightly swollen base. Spore print was white, spores ellipsoid and were measured about $6-8 \times 5-6 \mu\text{m}$. This species found in a group of two to three fruiting bodies. The sequence data showed 99 % homology with the earlier reported *Leucocoprinus cretaceus* (Fig. 27). Phylogenetic tree showed that identified *Leucocoprinus cretaceus* (WGM-28) is closely related to Malaysian *Leucocoprinus cretaceus* (AF482861 and AY176447.1) with bootstrap support 43 and grouped in clade I (Fig. 57). *Leucocoprinus cretaceus* grows on manure bed and documented from Punjab (Amandeep *et al.*, 2015).

Leucoagaricus cretaceus is distributed in tropical regions and adjacent temperate regions in North America and Europe (Birkebak, 2010). It has been recorded from Africa, Asia, Europe, North America, and South America (Meijer, 2006; Rother *et al.*, 2009; Sobestiansky, 2005, Wartchow *et al.*, 2008). *Leucocoprinus cretaceus* is easily recognized by its fleshy white basidiomata covered by floccose squamules that are easily removed when touched and by a fusoid stipe inflated toward the base (Niveiro *et al.*, 2013).

4.2.1.14 WGM-14

Grey to black coloured mushroom with presence of volva and white scales on the stipe (Plate 15). The mushroom was documented from Nelyadi (latitude $12^{\circ}49'36.23''$ N and longitude $75^{\circ}25'27.61''$ E; altitude: 424 ft.). Pileus is blackish-grey colour with presence of straight lines on cap from the half center of the cap to edge of the cap. Spores are elliptical, $5 \times 4-4.5 \mu\text{m}$ (Plate 31d). White scales are present on the stalk. Fruiting bodies are grown in group of 3-5 fruiting bodies.

Volva is white in colour. ITS region sequenced. The sequence data showed 99 % homology with the earlier reported *Amanita pseudovaginata* Hongo (Fig. 29). Phylogenetic tree showed that identified WGM-15 (*Amanita pseudovaginata*) is closely related to Russian *Amanita pseudovaginata* (KM658285.1) with boot strap support 23



Plate 13: WGM-12 (*Russula rosea*)



Plate 14: WGM-13(*Leucocoprinus cretaceus*)



Plate 15: WGM-14 (*Amanita pseudovaginata*)



Plate 15: WGM-16 (*Amanita hemibapha*)

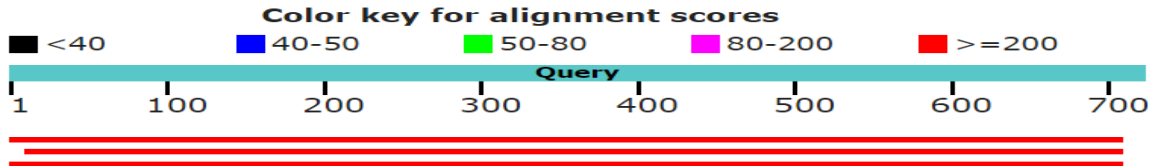


Plate 17: WGM-16 (*Polyporus arcularius*)



Plate 18: WGM-17 (*Pluteus chrysaegis*)

GGGACTGGCTGTCTACCGATTTGAGGTCAGGTTTTAACTCCGGCCTGGATCCAA
 AAGGGTAAAAACCAAATCCCAAACAACCGTGGCAAACCCGAAACGTAAAAACAT
 CCTAACACGTCCAGGGTTGGCAAACCAAACCTAATAATCCATTTAAGGGGAGC
 TGGGTTCCAGTGGGGGCCGCAATCCCAAAATCCAAACCTTTCTCCGGTGAAAAA
 CCCAAAAAGGGTTAAAGAAGGTCACCAAACCTCCAACCGGTGGGCCCCCGGAA
 TTTCCAGGGGCCCCAGGGGGGTTCCAAGAATCCA TGAATCCCTGGATTCCGGC
 ATTTCCATTAACTAACCCATTTCCCTGCCTTCTTTCTCCATGCCAAAACCCAGAA
 ATCCCTTGGTTAAAGTTGGAA TGGTTGGCTAATAACCCAAATAATAACACCTTT
 CAACCCTGGCGTGGGTCCAAGTAAAAACCCAATGGCCCTTTTCAAATGAACC
 CAAAGGAAAACGCCACCCCGTGAAGCCCCAAAAGGGGTGAAATGGAATTGGA
 AAAAAACCGAAGCCTGGACCAAAGTTAACGACCGGCCCCCGCACCTCTGGTTTA
 CCATAATGATCCTTCCGCAAGGTCCCCTACCGAAAGGGGGGCCTTTTCGGAAAA
 AGAAGTGCCGGGGGTGGCCTTATTTTGGGGCCCCCGGGGTCTCTCCAATCCA
 ATCCCCTTTTGGATCCCCGGGGGTTTCTTT



Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Russula rosea voucher HKAS 78360 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1	1252	1252	97%	0.0	99%	KF002750.1
Russula rosea 2-1121IC75 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1	1094	1094	96%	0.0	94%	AY061715.1
Uncultured Russula clone yii1T4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1	1077	1077	97%	0.0	94%	KC679825.1
Uncultured Russula clone bi5A4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1	1077	1077	97%	0.0	94%	KC679824.1
Russula peckii strain BPL270 18S small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1	1074	1074	97%	0.0	94%	KT933970.1

Fig. 25: Partial sequence length and homology search of WGM-12 (*Russula rosea*)

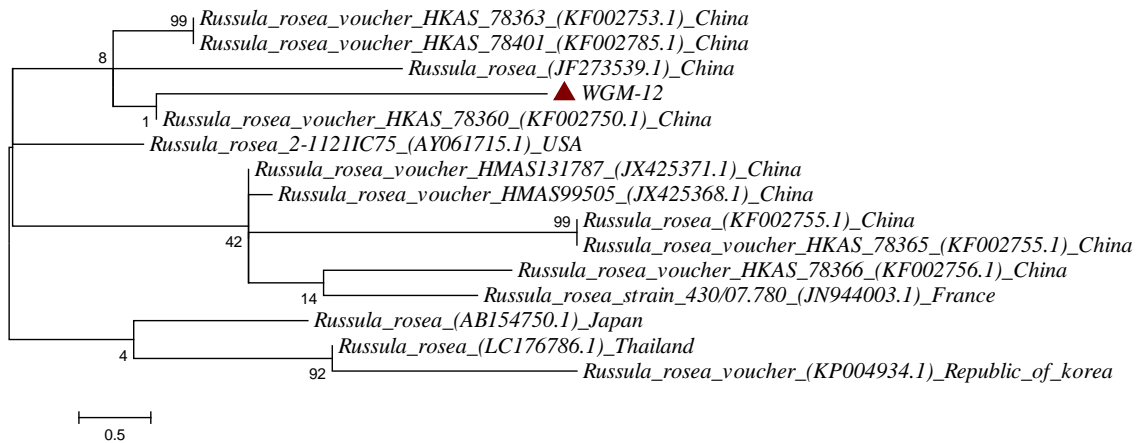
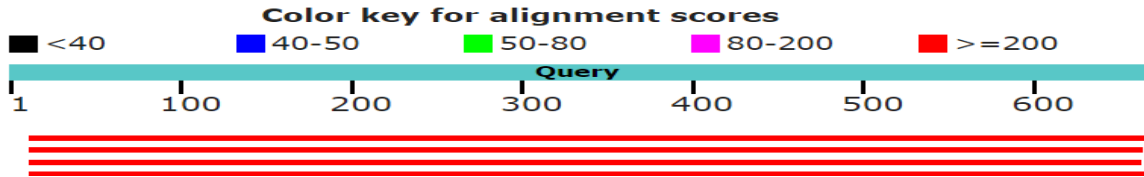


Fig. 26: Phylogenetic relationship between *Russula rosea* (WGM-12) and other *Russula rosea* species

TGATGGCTATCGACGTGATTTGAGGTCTTATGTTTCAGTAAAGTTTGTCCCGAGGG
 ACGGTTGGAAGCTGAACAGACAGAGAGCGATCCACTGGCGTAGATAATTATCA
 CACCTGTGACGGAGTCGCAAACGGTTCCGCTAATACATTTTCAGAGGAGCCGACC
 TTTGAAAAGGCCCGCAAACCTCCACATCCAAGCTCCGACTAGCAAAGGCTAGT
 GGAGTTGAGAAATTTACTGACACTCAAACAGGCATGCTCCTCGGAATACCAAGGA
 GCGCAAGGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTA
 CTTATCGCATTTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGC
 TGAAGTTGTATAAGGTTTTTATAGGCACAAAGGCCATTGAATACATTCTGCA
 ACATACTACATGGTTTATGAATAAAGACATAGTCCACCGAGAGCCAGAGAGGTG
 CCGCACTTTCGCGCTGCATCCCTCACATCCGGTCTTGCCGACCGACTACTCTCG
 AATGAGACTACAATAGGTGCACAGGTGGATGAATAGAGTCAAGACAGGCGTGC
 ACATGCTCCGAGGAGCCAGCTACAACCCATCGAGAATATTCAATAATGATCCTT
 CCGCAGGTTACCTACG



Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/> Leucocoprinus sp. BAB-5025 18S ribosomal RNA gene, partial sequence; internal transcribed spa	1171	1257	97%	0.0	99%	KR002883.1
<input type="checkbox"/> Leucocoprinus sp. BAB-4735 18S ribosomal RNA gene, partial sequence; internal transcribed spa	1168	1252	97%	0.0	99%	KR154964.1
<input type="checkbox"/> Leucocoprinus cretaceus strain LCJ 164 18S ribosomal RNA gene, partial sequence; internal trans	1164	1250	97%	0.0	99%	KF383135.1
<input type="checkbox"/> Uncultured fungus clone WJ1 18S ribosomal RNA gene, partial sequence	1158	1248	97%	0.0	99%	KU931434.1

Fig. 27: Partial sequence length and homology search of WGM-13 (*Leucocoprinus cretaceus*)

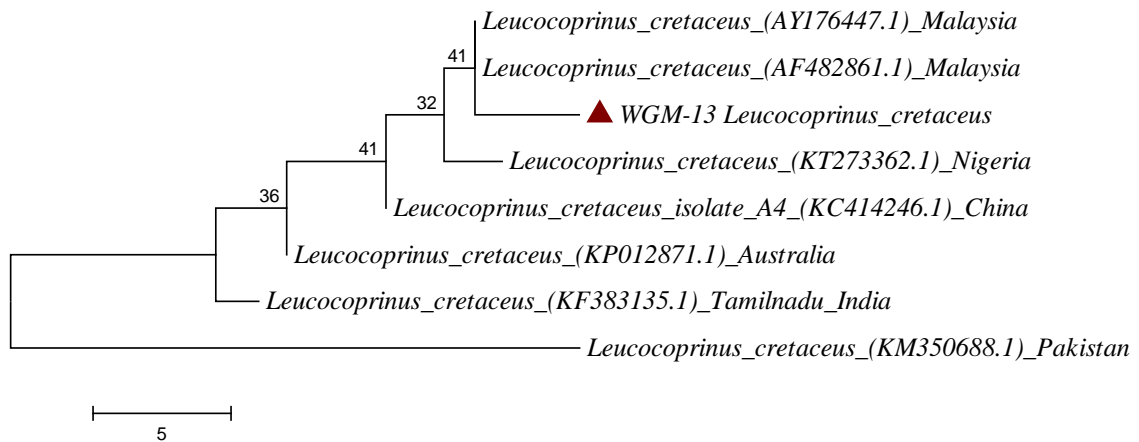
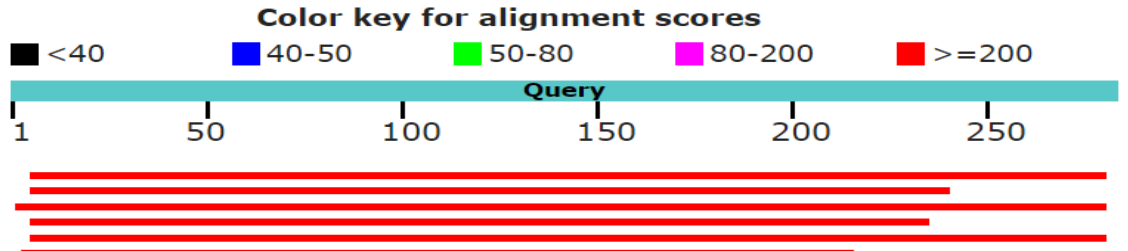


Fig. 28: Phylogenetic relationship between *Leucocoprinus cretaceus* (WGM-13) and other *Leucocoprinus cretaceus* species

CAGAA**T**CCCAA**A**GTCCAAA**A**CCAC**G**ATA**A**AA**T**AAG**A**TGT**G**CTTT**G**ATA
GTT**T**AA**T**AA**C**ACT**C**AA**A**CAG**G**CAT**G**CT**C**CT**C**GG**A**AT**A**CC**A**AG**G**AG**C**G**C**A
AG**A**T**G**CG**T**T**C**AA**A**G**A**TT**C**CA**T**G**A**TT**C**ACT**G**G**A**TT**C**T**G**CA**A**TT**C**AC**A**TT**A**C
TT**A**T**C**AC**A**TT**T**CG**T**GT**G**TT**C**TT**C**AT**C**G**A**T**G**CG**A**G**A**G**C**CA**A**G**A**G**A**T**C**CG**T**
TGT**T**G**A**AG**T**T**G**T**A**AAAA**T**TT**A**T**A**T**C**AT**T**TT**A**C**A**CA**A**G**A**C**A**T**A**CA**A**T**A**TT
TAA**A**GT**A**C**A**T**G**CA**A**AA**C**AT**G**T**C**ATA**A**AA**C**AC**A**G**C**A



Alignments Download GenBank Graphics Distance tree of results

	Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/>	Amanita pseudovaginata voucher LE<RUS>:216819 18S ribosomal RNA gene, partial sequence: int	928	928	89%	0.0	99%	KM658285.1
<input type="checkbox"/>	Amanita sp. 'subnigra' voucher RET_369-5 18S ribosomal RNA gene, partial sequence: internal tran	847	847	85%	0.0	98%	KT006761.1
<input type="checkbox"/>	Amanita sp. 'subnigra' voucher RET_155-9 18S ribosomal RNA gene, partial sequence: internal trans	847	847	85%	0.0	98%	KP711843.1

Fig. 29: Partial sequence length and homology search of WGM-14 (*Amanita pseudovaginata*)

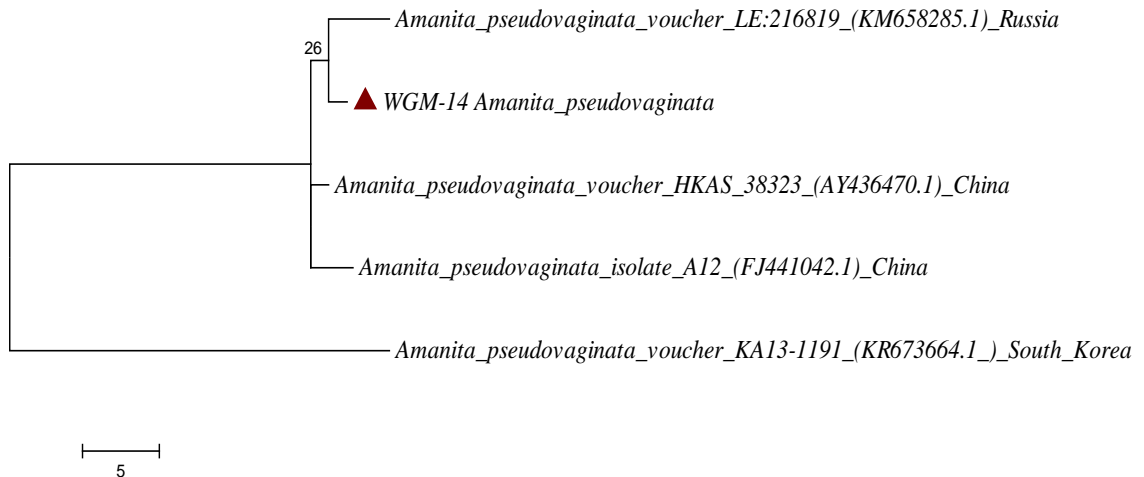


Fig. 30: Phylogenetic relationship between *Amanita pseudovaginata* (WGM-14) and other *Amanita pseudovaginata* species

and grouped in clade I (Fig. 30). Deng *et al.* (2014) also reported this species in southern part of China.

4.2.1.15 WGM-15

This species is a fairly large and beautiful mushroom (Plate 16) characterized by its orange red to yellowish orange cap with a distinctly tuberculate-striated margin; pastel yellow to light yellow lamellae. This mushroom was documented during the onset of rainy season in July at Theerthalli of Shivamogga district (latitude 13°40'47.78"N and longitude 75°12'50.65"E; Altitude: 2023 ft.). This area is very close to the Tunga River in the Western Ghats of Karnataka.

The cap is 8-10 cm wide, it is hemispheric at first, it becomes convex and then planar with or without a central depression. At first the color is pastel orange to red. With age and exposure, the cap becomes deep orange and the margin distinctly more yellow. The gills are not attached to stalk, crowded, creamy white at first and pastel yellow to light yellow in mature fruiting body, they are up to 1.2 cm broad. Short gills occur in at least three different lengths. The stem is 7.0-15.0 × 0.6-1.2 cm, covered with zigzag, finely ornamented yellowish fibrils to small squamules above and below annulus; context white to light lemon yellow, soft, stuffed first with white, cottony material, hollowing with age. Annulus is superior, persistent and yellow colour. Volva is saccate, membranous, fleshy and white in colour. Spore print is white.

The ITS region/5.8S rRNA gene sequence data showed 98 % homology with the earlier reported *Amanita hemibapha* (Fig. 31) at NCBI. Phylogenetic tree showed that identified WGM-16 (*Amanita hemibapha*) is closely related with *Amanita hemibapha* strain RET from Kerala with bootstrap support 77 and grouped in clade IV (Fig. 32). We have found more than 50 fruiting bodies in a 100 square meter. Boonyanuphap and Hansawasdi (2011) documented this species in subtropical dry forests of Thailand. Bhatt *et al.* (2016) reported that *Amanita hemibapha* in Uttarakhand, Rudraprayag and Baniyakund which was consumed by local peoples of this area.

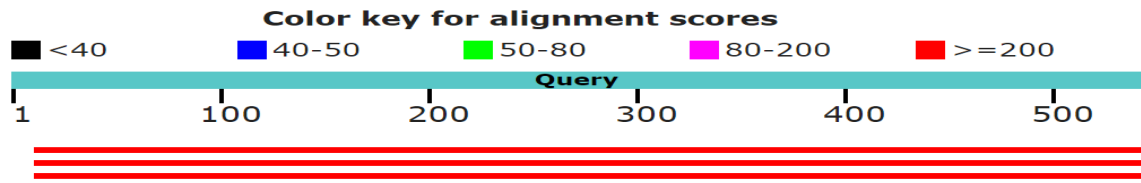
But there is no such information was found during this study. *Amanita hemibapha* is a fairly large and beautiful mushroom characterized by its orange to orange red, yellowish orange cap with a distinctly tuberculate-striated margin; pastel yellow to light yellow lamellae; stipe finally fibrillose, yellowish; annulus orangish yellow to pale yellow, large, pendant and volva thick, white, saccate, and broadly ellipsoid spores.

4.2.1.16 WGM-16

Small sized fruiting body with fairly large pores and presence of hairs on cap grows in group on hard wood. This mushroom was collected from Sakleshpur (latitude 12°57'25.98" N and longitude 75°25'29.63"E, at an altitude of 3125 ft.). Pileus is light to brown, convex to plane with a deep central depression, often covered with small darker-tan flattened scales in a concentric pattern, margin is lined with very fine hairs, leathery, thin-fleshed, dries very tough and cap measures up to 5 cm (Plate 17). Pores are light brown, large, coffin-shaped (polygonal) with ragged edges. Stem is brown, central, cylindrical, short, tough, smooth or slightly velvety; measure up to 5 x 0.3 cm. Spores are white, cylindrical, smooth, 8 x 3.5 µm. Found in a wide variety of habitats, particularly drier vegetation communities, disturbed areas and gardens. This species was grown solitary or in small groups on dead hard wood. This mushroom is easily distinguishable by its large polygonal pores, fringed margin, concentric scales and leathery texture.

The sequence data showed 95 % homology with the earlier reported *Polyporus arcularius* (KF179532.1 and KP283489) (Fig. 33). Phylogenetic tree showed that the identified *Polyporus arcularius* (WGM-16) is closely related to *Polyporus arcularius* isolate M51 from Tamilnadu and *Polyporus auricularius* isolate L50 from China and grouped in clade VII (Fig. 34). Jegatheesan and Eyini (2014) showed that nearly threefold increase in laccase production (28.30 IU/ml) over the control experiments in basal salt medium could be obtained earlier at 15 days of incubation by increasing the content of the nitrogen source, yeast extract to 0.5 g/l, and by adding 250 µm CuSO₄ to the basal salt medium.

AGAAGGGTCTATCATGCGTGATTGCGGTCAACTGTAAACATAACAAGGGACTAC
TACTTGTGGCATAACAATTATCATACACCAACATTTTCGTCCTCAAAGGAATT
TAAGGAGAGCTGAGCTTCGACAGCTGCCAGCAAATGACTCCCAATACCCAAAAA
CCAAGTGTTTTGGAGAGATTAATGACACTCAAACAGGCATGCTCCTCGGAATAC
CAAAGAGCGCAAGATGCGTTCAAAGATTTCGATGATTCACTGAATTCTGCAATTC
ACATTACTTATCGCATTTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATC
CATTGTTGAAAGTTGTATAATATTATACACATTCATTCATTCACAAGGTTATATA
TGATATAATAAATCATAGACAGGCCAAGCCTGTCTACAAGTAGTGCACAGGGGA
GGGGAAAAGAAATAGACAAAAGGCGTGACATTGCCAGTGGGCCAGCAACAGC
CTCTTGCTATAGATTTCAATTACAATAATGATCCTTCCGCAGGTTACCTACGGAA
GCA



Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Amanita hemibapha genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, strain: MS-1	913	913	77%	0.0	98%	LC056764.1
Amanita hemibapha subsp. hemibapha genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, specimen voucher: C	874	874	77%	0.0	96%	AB458888.1
Amanita cf. princeps Nan-M5 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence	874	874	77%	0.0	96%	AB451970.1
Amanita princeps genes for ITS1, 5.8S rRNA, ITS2, partial and complete sequence, strain: MRNo327	867	867	73%	0.0	98%	LC068793.1
Amanita hemibapha strain RET-342-8 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene, complete sequence, and internal trans	822	822	65%	0.0	90%	JX844716.1
Amanita cf. hemibapha TRTC-150422 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene, complete sequence, and internal trans	795	795	67%	0.0	98%	JX844715.1

Fig. 31: Partial sequence length and homology search of WGM-15 (*Amanita hemibapha*)

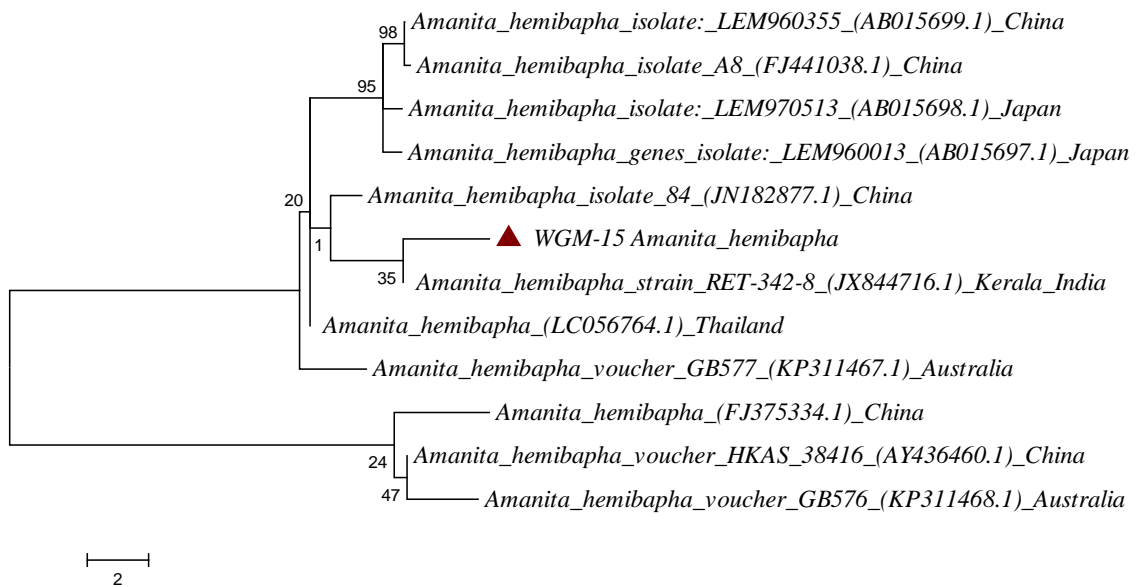
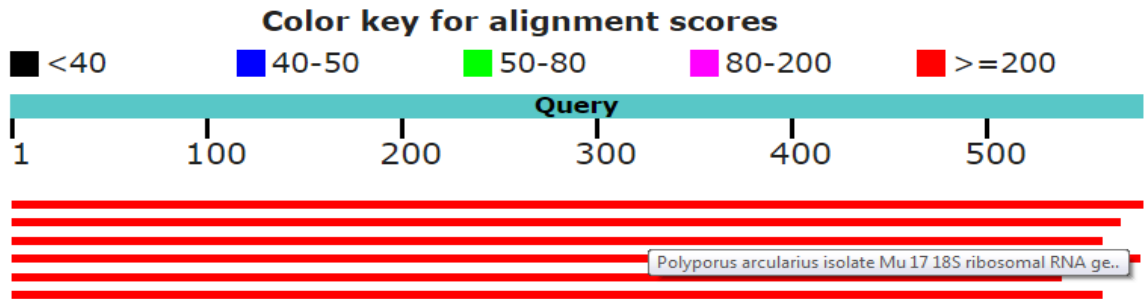


Fig. 32: Phylogenetic relationship between *Amanita hemibapha* (WGM-15) and other *Amanita hemibapha* species

ATGCTCATCCACTCTACACCTGTGCACCTTACTGTGGGTCTCAGGAGCTTCGAAG
 CGAGGGTTTAACCGCTCTCGCCGAGTTGTTACTGGGCCCTACGTTTATCACAAAC
 TCTTAAAAGTATCAGAATGTAAACGCGTCTAACGCATCTATATACAACTTTCAGC
 AACGGATCTCTTGGCTCTCGCATCGATGAAAGAACGCAGCGAAATGCGATAAGTA
 ATGTGAATTGCAGAAATTCAGTGAATCATCGAATCCTTTGAACGCACCTTGCGCTC
 CTTGGTATTCGGAGGAGCATGCCTGTTTGAGTGTTCATGAAATTTCTAACCTAAC
 AAGTTTTTAACGGGACTTGCTTAGGCTTGGACTTGGAGGCTTGTGGCTCTTTA
 GCAGTCGGCTCCTCTCAAATGCATTAGCTTGGTTCCTTGGGATCGGCTCACGG
 TGTGATAATTATCTGCGCCGCGACCGTTGAAGCGTTTAAATGGGCCACTTTCTAT
 TCGCTTCTGGCAAACAAGTTTTCTTCAAATTCGGACCTCAATTCAGGAAGAATT
 ACCCGTTGACCTTAGCCTTATCATTAACGGAAGAA



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
<input type="checkbox"/>	Polyporus arcularius isolate Mu 17 18S ribosomal RNA gene, partial sequence; internal transcribed s	884	884	97%	0.0	95%	KF179532.1
<input type="checkbox"/>	Polyporus arcularius voucher DSH92132 18S ribosomal RNA gene, partial sequence; internal transc	879	879	96%	0.0	95%	KP283489.1
<input type="checkbox"/>	Polyporus arcularius isolate JZBWD2100003 18S ribosomal RNA gene, partial sequence; internal tre	874	874	99%	0.0	94%	JQ283965.1
<input type="checkbox"/>	Polyporus sp. E7355 5.8S rRNA gene, 26S rRNA gene (partial), ITS1 (partial) and ITS2, specimen v	870	870	92%	0.0	96%	AJ542518.1
<input type="checkbox"/>	Polyporus arcularius isolate DO82 internal transcribed spacer 1, partial sequence; 5.8S ribosomal Ri	868	868	96%	0.0	95%	KP050637.1

Fig. 33: Partial sequence length and homology search of WGM-16 (*Polyporus arcularius*)

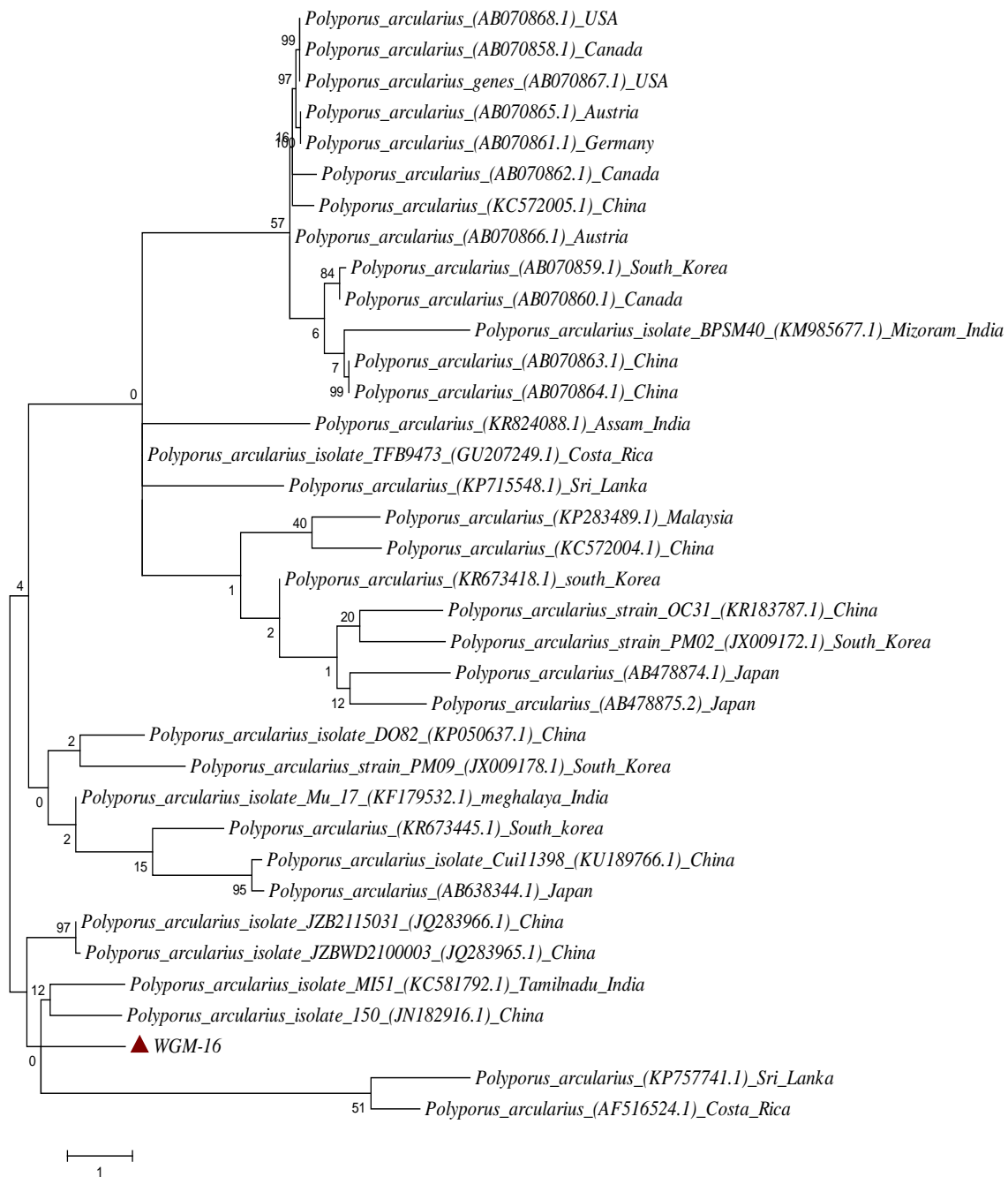


Fig. 34: Phylogenetic relationship between *Polyporus auricularius* (WGM-16) and other *Polyporus auricularius* species

4.2.1.17 WGM-17

This mushroom species was collected from Dakshina Kannada (latitude 12°49'34.10"N and longitude 75°25'31.67"E, altitude: 456 ft.). Basidiocarps are yellow in colour; grows in group during the rainy season. Pileus is 2-5 cm in diameter, convex, becoming plano-convex, chrome yellow to butter yellow with olive brown striate up to half way (Plate 18). Gills are free, pale orange colour, up to 6 mm wide, crowded with lamellae of different lengths. Stipe is central, cylindrical, equal with a slightly broad base, yellowish white, fibrillose striate; up to 2 mm thick.

Spores are 4.5- 6 × 4.5- 5 µm, subglobose, thick-walled. Basidia 22-26 × 5-6 µm; four spored. Cheilocystidia 21-56 × 6-15 µm, thin-walled.

The sequence data showed 99 % homology with the earlier reported *Pluteus chryzaegis* (JN603206.1) (Fig. 35). Phylogenetic tree of *Pluteus* species showed that identified mushroom *Pluteus chryzaegis* is closely related with *Pluteus chryzaegis* (JN603206) from India with bootstrap support 91 with clade IV (Fig. 36). The chrome yellow cap and mucronate dermatocystidia of *Pluteus chryzaegis* was a unique set of characters (Pradeep and Vrinda, 2006). Pradeep *et al.* (2012) reported the similar morphological characters of the mushroom documented from Kerala.

4.2.1.18 WGM-18

Small basidiocarp, medium texture, grows scattered on hard wood during the rainy season. Snails were feeding on this mushroom (Plate 19), but not fit for human consumption. This mushroom was documented from Dakshina Kannada district (latitude 12°43'45.91"N and longitude 75°32'57.56"E, elevation at 439 ft.). Pileus is 2-3 cm in diameter, convex, yellowish orange and maroon towards the center. Lamellae are attached parallel to the stipe, pale orange colour. Stipe is central, cylindrical, equal, yellowish orange colour.

The sequence data of ITS region/5.8S rRNA gene showed 99 % homology with the earlier reported *Gymnopilus lepidotus* (KX035108.1) (Fig. 37). Phylogenetic tree showed that identified mushroom (WGM-18) closely related with *Gymnopilus lepidotus*

(KP764811.1) from Sri Lanka with clade II (Fig. 38). Lechner *et al.* (2006) reported this species from Iguazu National Park of Misiones. *Gymnopilus lepidotus* (Hesler.) also found in Sri Lanka (Fernando *et al.* 2015). Munguia *et al.* (2006) reported this species from Mexico.

4.2.1.19 WGM-19

Medium sized white mushroom growing scatter on hard wood and documented from Western Ghats of Dakshina Kannada (latitude 12°43'43.17"N and longitude 75°32'56.53"E, Altitude: 438 ft.). Pileus is white colour, convex shape (Plate 20). Gills are large sized and decurrent. Spore print is white. Stipe is cylindrical, white, thick and firm and 4-6 cm in length.

The ITS region / 5.8S rRNA gene sequence data showed 85 % homology with the earlier reported *Clitopilus prunulus* (FJ770389.1) (Fig. 39). Phylogenetic tree showed that identified *Clitopilus prunulus* (WGM-19) as closely related to *Clitopilus prunulus* (KR261096.1) from Netherlands with bootstrap support 95 and grouped in clade I with *Clitopilus prunulus* from Switzerland (KJ425345.1), Mexico (KT697952.1), United Kingdom (FJ770405.1) and United States of America (DQ202272.1) (Fig. 40). Angelini *et al.* (2012) recorded this species from Collestrada forest ecosystems in Perugia of Italy. *Clitopilus prunulus* is an edible mushroom (Pui *et al.*, 2013) and also using for culinary purpose. Vyas *et al.* (2014) documented this species from Patharia forest of Sagar in Madhya Pradesh.

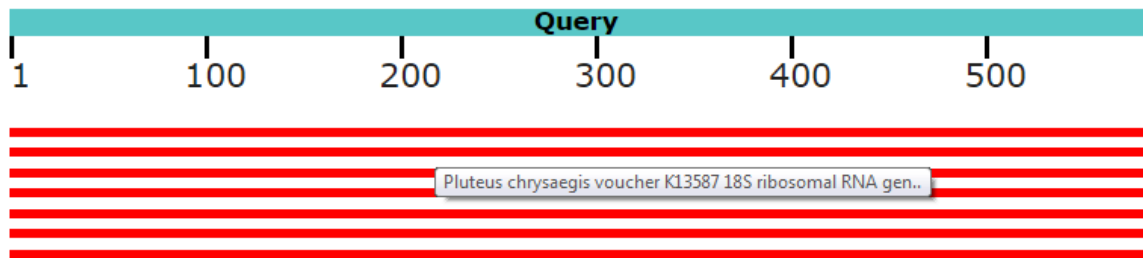
4.2.1.20 WGM-20

The mushroom was documented from Hassan district and also found in Dakshina Kannada district (altitude 450-3220 ft.). Pileus is brown colour, flexible, deeply depressed funnel shaped, looks like waxy paper (Plate 21). Gills are running briefly down stipe, short length gills are towards the edge of the cap, lamellae are distantly spaced. Spore is 4- 6 × 2.5- 3µm. Stipe is cylindrical, dark brown with velvety texture. Slightly bulge at attachment of the gills and attachment of the substrate as well. This mushroom grows on decaying wood in mixed vegetation in group.

GGTGTGGTTGCTGCTGGCCCTTTCCTAGGGCATGTGCACACCCGCCATTTGTT
 TCATCCTTCTTCCCACCTGTGCACTTCTGTAGGACACCCAGCCAAAGGCCTTCC
 TGGCATGCAGCTGGAGCAAAGTGTCTACGTTTTCTCATCAGCTCCAATGAATGT
 GTAGAGTGTAGAAAACAAGGTCCCTTCATGGCCTTATAAACTAATACAACCTTCA
 ACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAG
 TAATGTGAATTGCAGAAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGC
 TCCTTGGTATTCCGAGGAGCATGCCTGTTTGAAGTGCATTGAATCATCAACCCC
 CACTTCCTTGTGGAGTCGGGTGCTTGGATATGGAGACTGCAGGCCCTCTTGGGT
 CAGCTTCTCTGAAATGCATTAGCAGAGCCCATCCTGTGCATCTGTGGCTTGGTG
 TGATAGTATCTACGCCTAGCGATGTTGTGCATTTCGAATCTGCTGCCAGTCGTCC
 ACCTTCTCTGGACACTTATTGACAATTTGACCTCAA

Color key for alignment scores

<40
 40-50
 50-80
 80-200
 >=200



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
<input type="checkbox"/>	Pluteus chrysaegis voucher K13587 18S ribosomal RNA gene, partial sequence; internal transcr	1014	1014	100%	0.0	99%	JN603206.1
<input type="checkbox"/>	Pluteus conizatus var. africanus voucher Thoen 5250 18S ribosomal RNA gene, partial sequenc	980	980	100%	0.0	98%	HM562142.1
<input type="checkbox"/>	Pluteus roseipes voucher UC 1861251 18S ribosomal RNA, partial sequence; internal transcribe	796	796	100%	0.0	91%	KC147681.1
<input type="checkbox"/>	Pluteus leoninus voucher Halling6546 18S ribosomal RNA gene, partial sequence; internal trans	796	796	100%	0.0	91%	HM562071.1

Fig. 35: Partial sequence length and homology search of WGM-17 (*Pluteus chrysaegis*)

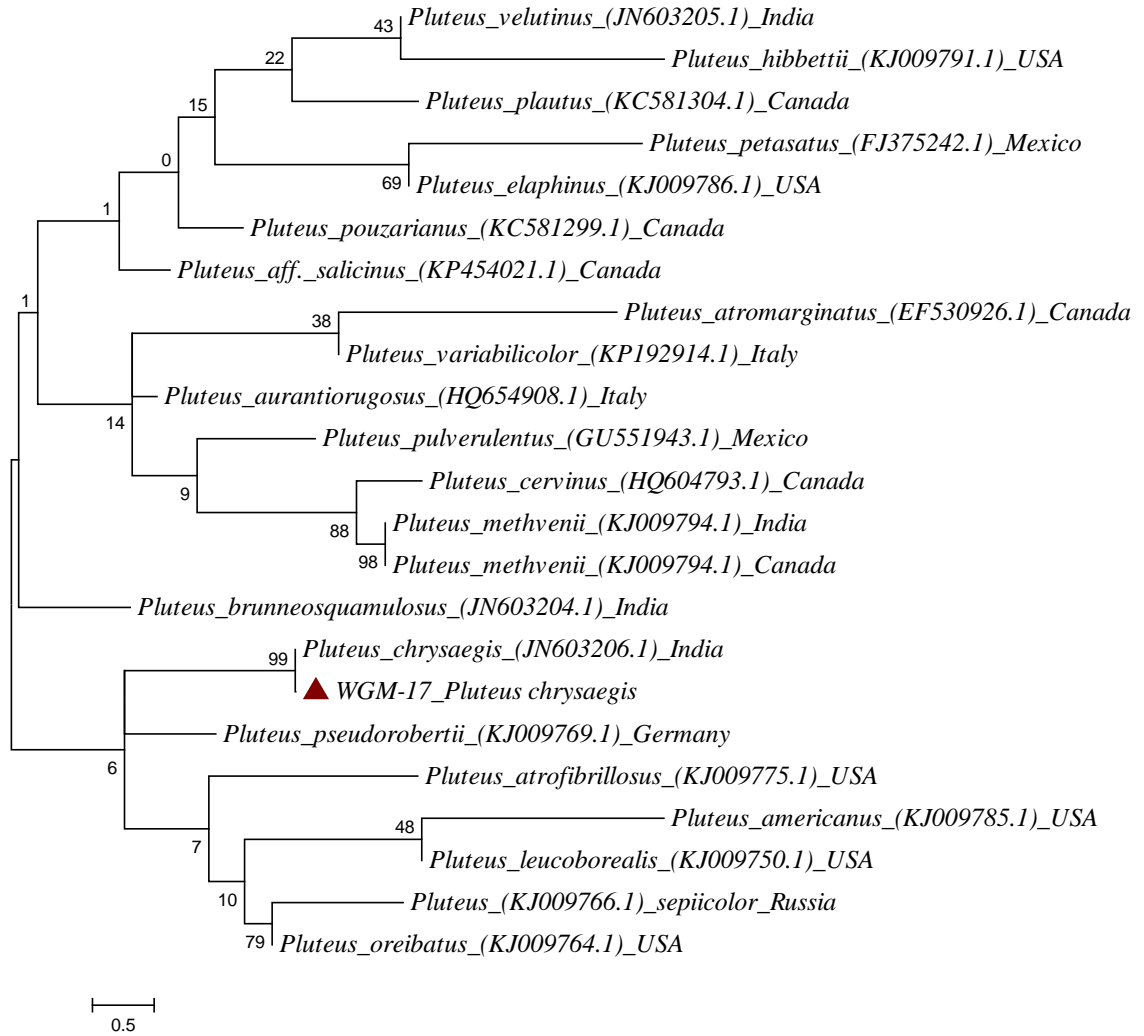


Fig. 36: Phylogenetic relationship between *Pluteus chrysaegis* (WGM-17) and other *Pluteus chrysaegis* species



Plate 19: WGM-18 (*Gymnopilus lepidotus*)



Plate 20: WGM-19 (*Clitopilus prunulus*)



Plate 21: WGM-20 (*Panus similis*)



Plate 22: WGM-21 (*Clavaria rosea*)



Plate 23: WGM-22 (*Phallus indusiatus* /
Dictyophora indusiata)

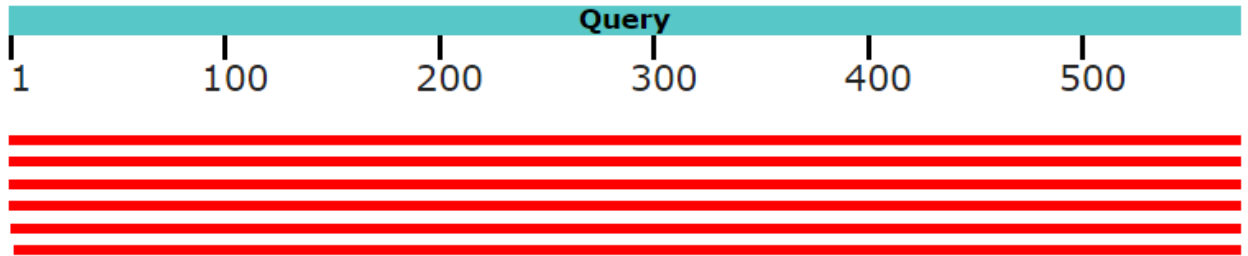


Plate 24: WGM-23 (*Chlorophyllum molybdites*)

TGGCGTGGTTGAGCTGACTCTCTCGGGAGTATGTGCTCGCTCGTCATCTTTATC
 TTTCCACCTGTGCACTTTTTGTAGATTTGGATGTAACCTTCTGAGGCAACTCAGT
 TGGGAGGAATGCTATTTTCGATGGCTTTCCTTGTATGTCCAAGTCTATGTTTTCAT
 AACTCCAAGTATGTAACAGAATGTATCACTGGGCCTTGTGCCATAAACCTTA
 TACAACCTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGA
 AATGCGATAAGTAA TGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAAC
 GCACCTTGCGCCCTTGGTATTCCGAGGGGCATGCCCTGTTTGAGTGTCAATAAA
 TTCTCAACCTTACTAGCTTTTGCGAAGTAATGGTTTGGATGTGGGGGTCTTTTG
 CTGGTTTCGAAAGAAATCTGCTCCCCTTAAATGCATTAGCCGGTGCCTCCGCGTG
 GACCGTCTATTGGTGTGATAATTATCTACGCCGTTAGACGTCTGCTATTAAATG
 GGATTGCGCTGCTTCTAATCGTCCCCT

Color key for alignment scores

<40
 40-50
 50-80
 80-200
 >=200



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
<input type="checkbox"/>	Gymnopilus lepidotus isolate E140524 internal transcribed spacer 1, partial sequence: 5.8S ribosom	1018	1018	100%	0.0	99%	KX035108.1
<input type="checkbox"/>	Gymnopilus suberis voucher EGB11 internal transcribed spacer 1, partial sequence: 5.8S ribosomal	1018	1018	100%	0.0	99%	KT273354.1
<input type="checkbox"/>	Gymnopilus sp. 2 KK-2015 voucher 374 18S ribosomal RNA gene, partial sequence: internal transcri	1018	1018	100%	0.0	99%	KP075002.1
<input type="checkbox"/>	Gymnopilus lepidotus isolate 1 18S ribosomal RNA gene, partial sequence: internal transcribed spac	1018	1018	100%	0.0	99%	AY280989.1

Fig. 37: Partial sequence length and homology search of WGM-18 (*Gymnopilus lepidotus*)

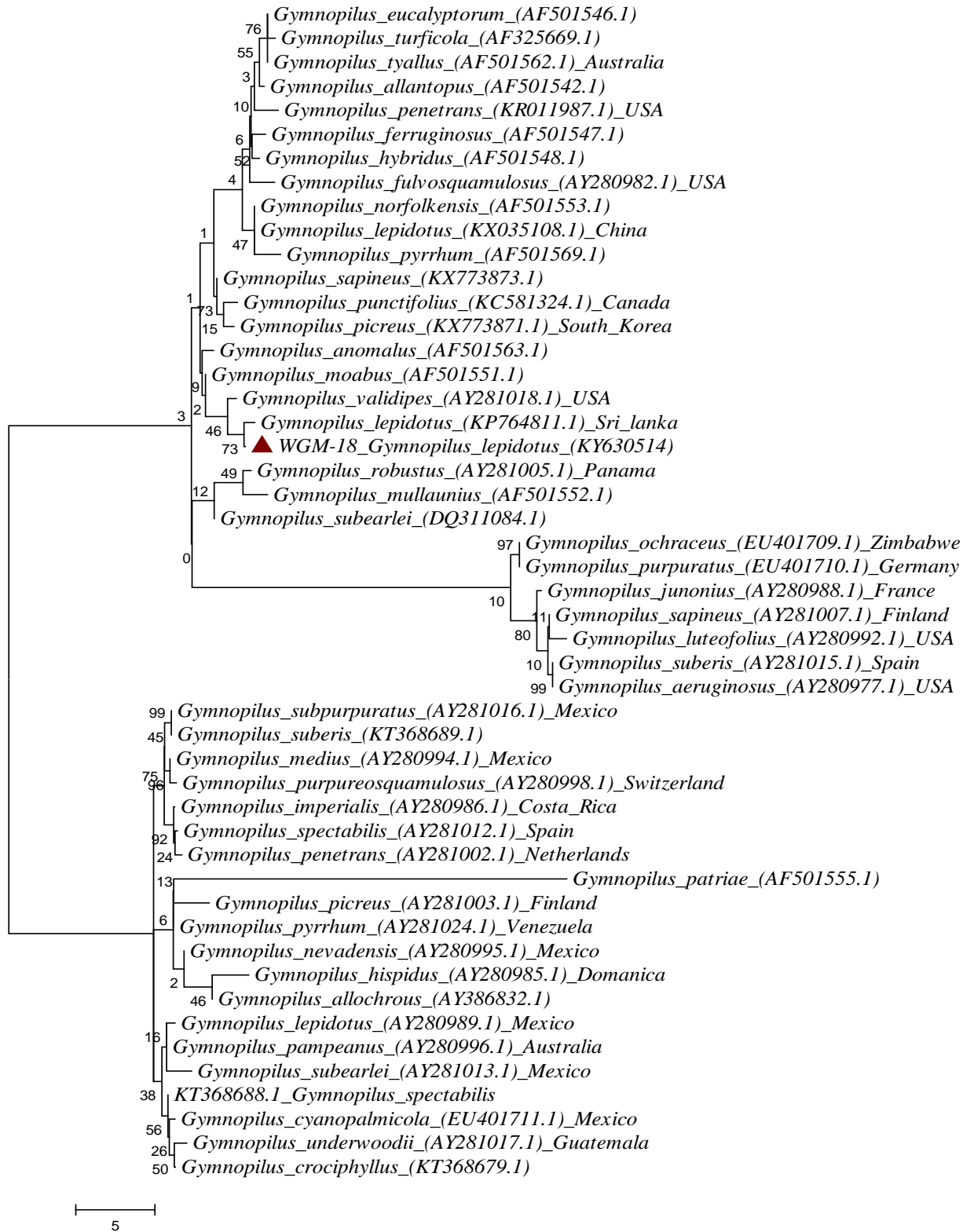
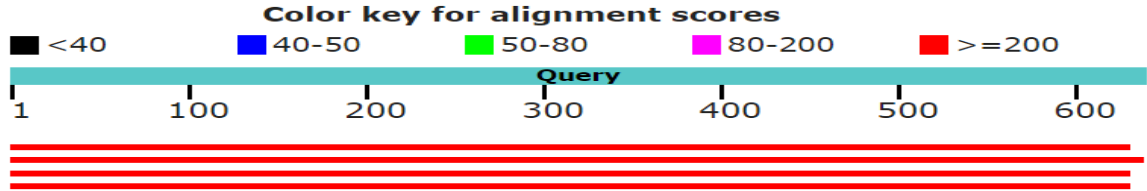


Fig. 38: Phylogenetic relationship between *Gymnopilus lepidotus* (WGM-18) and other *Gymnopilus* species

TGTTTCGGTTGTTGCTGGCCCCTTGGGGCATGTGCACGCCTGCCAACAGTTTTCA
 ACCACCTGTGCACTTCATCTAGGACCTAGAGATGTATTTTCGAGGCCAAAACCTCG
 GTTTTGAGGACTGCTGTGCGCAAAGTCAGCTCTCTTCTGTGTCTTTTCGGTTCTAT
 GTATTTGTTATATACACCCCTCAATGCATGTATAAGAATGTGTTGTTAGGCTATTA
 GTGCCTGTAAC TCAAATACAAC TTTCAAACAACGGATCTCTTGGCTCTCGCATCG
 ATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAAT
 CATCGAATCTTTGAACGCACCTTGCCTCCTTGGTATTCCGAGGAGCATGCCTG
 TTTGAGTGTCAATTAATTTCTCAACCATAACAAGTTGTATCACCAGCCTGTATGGCT
 TGGATCTGGGATGTTGCGGGCTTATAACCAGTCAGCTGTCCTTAAATGCATTAG
 CAAAGCGTTTGTGCCCCAAATCTCTGGTGTGATAAATTAATCTACGCCATTGAGG
 CTACTTCGAGGCTTTGCTTCCAATTTGTCCTGTAAGGGACGACATCTGATAATCT
 GACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAA



Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/> Clitopilus prunulus strain CBS_129.42 internal transcribed spacer 1, partial sequence: 5.8S riboso	661	661	100%	0.0	85%	FJ770389.1
<input type="checkbox"/> Clitopilus cystidiatus 18S ribosomal RNA gene, partial sequence: internal transcribed spacer 1.5.	659	659	99%	0.0	85%	KC885964.1
<input type="checkbox"/> Clitopilus prunulus 18S ribosomal RNA gene, partial sequence: internal transcribed spacer 1.5.8:	657	657	98%	0.0	85%	KC885965.1
<input type="checkbox"/> Clitopilus giovanellae strain S.F.14368 18S ribosomal RNA gene, partial sequence: internal transcr	655	655	98%	0.0	85%	EF413030.1
<input type="checkbox"/> Clitopilus prunulus 18S ribosomal RNA gene, partial sequence: internal transcribed spacer 1.5.8:	654	654	99%	0.0	85%	AY228348.1

Fig. 39: Partial sequence length and homology search of WGM-19 (*Clitopilus prunulus*)

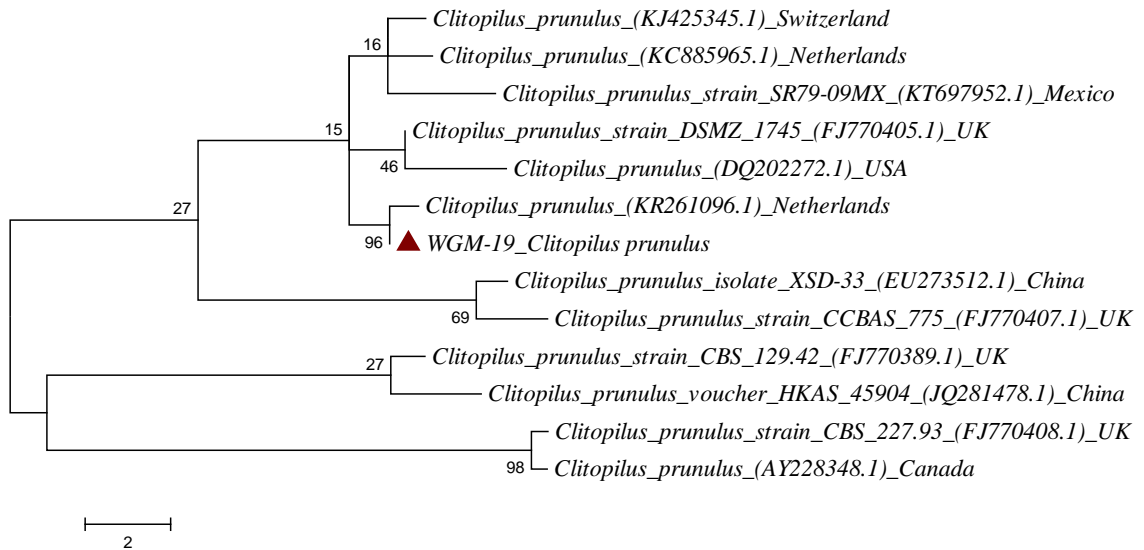


Fig. 40: Phylogenetic relationship between *Clitopilus prunulus* (WGM-19) and other *Clitopilus prunulus* species

The sequence data showed 93 % homology with the earlier reported *Panus similis* (Berk. and Broome.) (KM411466.1)(Fig.41). Phylogenetic tree showed that identified *Panus similis* (WGM-21) closely related with *Panus similis* (KR818820.1) from Sri Lanka with bootstrap support 62 and grouped with clade I (Fig. 42). Bolhassan *et al.* (2012) documented this species from Peninsular Malaysia. Njouonkou *et al.* (2013) documented *Panus similis* from Cameroon and recorded the similar features of this mushroom as robust basidiocarp with finely glabrescent velutinate cap with striated to cannelated and lamellae are distantly spaced.

4.2.1.21 WGM-21

This beautiful pinkish red coloured mushroom resembles like leg of hen in colour and is vernacularly called as ‘kolikalanbe (Chicken leg mushroom in Kannada)’. This mushroom looks like ornamental (Plate 22) and it is documented in Dakshina Kannada (latitude 12°43’47.47”N and longitude 75°32’56.84”E, at an altitude of 442 ft. from mean sea level) and Shivamogga district. The fruiting body is a long, straight, fragile, cylindrical tapering downward, with no branches; it has a size of 5-8 cm in height and 0.2-0.3 cm in width. It tends to be small at base and grows bigger toward the end. The surface is smooth, as it grows bigger in the top part end is rounded and red or dark pink. The color is clear red, shiny and glassy. Inner fresh is firm, moist, and gelatin-like. The spores are ellipsoid in shape with an average size of 6 × 3.5 microns (Plate 31d). The fruiting bodies of this mushroom stay together in a cluster.

The ITS sequence showed 82 % homology with *Clavaria rosea* (KP257133.1) (Fig.43). Phylogenetic tree showed that the WGM-21 is grouped under clade VII in the genus *clavaria* (Fig.44). Chittaragi *et al.* (2013) collected *Clavaria rosea* from Haniya. This species was recorded in Finland (Kunttu *et al.* 2014).

4.2.1.22 WGM-22

Immature fruit bodies are initially enclosed in an egg-shaped to roughly spherical subterranean structure encased in a peridium. The "egg" measures up to 6 cm in diameter, and usually has a thick mycelial cord attached at the bottom. As the mushroom matures,

the pressure caused by the enlargement of the internal structures cause the peridium to tear and the fruit body rapidly emerges from the "egg". The mature mushroom is up to 28 cm tall and girded with a net-like structure called the indusium (a "skirt" like structure) that hangs down from the conical to bell-shaped cap (Plate 23).

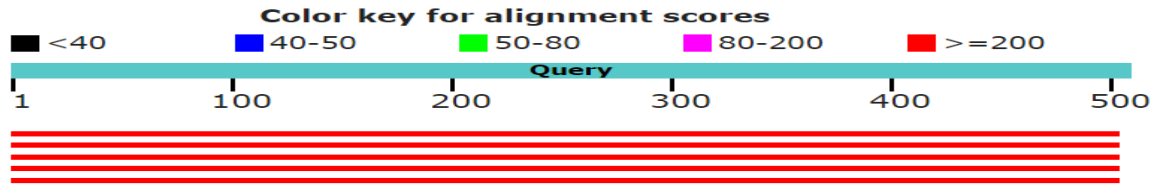
The net like opening of the indusium is polygonal with the internal pores. Well-developed specimens have an indusium that reaches to the volva and flares out somewhat before collapsing on the stalk. The cap is 1.5-4 cm wide and its reticulated (pitted and ridged) surface is covered with a layer of greenish-brown and foul-smelling slime. The top of the cap has a small hole. The stalk is 9-28 cm long, and 1.5-3 cm thick. The hollow stalk is white, roughly equal in width throughout its length, sometimes curved, and spongy. The ruptured peridium remains as a loose volva at the base of the stalk. The indusium within an hour and they are short-lived, typically lasting no more than a few days. At that point the slime has usually been removed by insects, leaving the pale off-white, bare cap surface exposed.

Spores are thin-walled, smooth, elliptical or slightly curved, translucent and measure 2-3 X 1-1.5 μm . This mushroom was also recorded by Desjardin *et al.*, (2015) from West Africa and this species is called as *Phallus indusiatus* / *Dictyophora indusiata* (Vent. and Pers.). Dash *et al.* (2010) recorded this species from Eastern Ghats of India.

4.2.1.23 WGM-23

Mushroom is documented from the Western Ghats of Dakshina Kannada district during the onset of monsoon. Twenty to thirty basidiocarps were seen in one square meter. Pileus is white with brown scales in concentric rings becoming more concentrated towards the centre, which is often solid brown, globular when immature expanding to become convex to plane with a raised point (umbel) at maturity (Plate 24). Lamellae are white when immature, quickly becoming dull grey-green to green as spores mature, free. Stipe is dull white becoming pale grey-brown, smooth, central, cylindrical with swollen base, hollow. Has a thick white, double-movable ring high on the stem; stipe is about 22-28 cm x 2-2.5 cm in length. Spore print is olive-green colour, smooth, broadly elliptical, truncated at one end by germ pore, thick walled, 10 x 8 μm . This species was found in

TGTCCGAGGACGATTAGAAGCAGAAACATAAGAGTTTTTGCAATGCAACAACAG
 CGTAGACAATTATCACACTGAGGTTGACAAAGCAATATTCCTGCTAATGTATTT
 CAGAGGAGCCGGCACGCCATTGGCATGGATGCCAGCAATCACCTCCACATCCA
 ATCCATCCAAATCCGCAAAGATTTAGAGAAATTGAGAAATACCATGACACTCAAAC
 AGGCATGCTCCTCGGAATACCAAGGAGCGCAAGGTGCGTTCAAAGATTGATG
 ATTCACTGAATTTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATC
 GATGCGAGAGCCAAGAGATCCGTTGCTGAAAGTTGTATAAGATGCGTTATAAAT
 ACGCTGTTTACATTCTGATACTATAGATGTGTTTGTAGAGAACCACAGGCAGGG
 TCAAAGCTCAGTCACCCCATATGCGGGCAACCAGGCAACAACCAAACCCATGT
 TAAGTGCACAGAGGTGTGTAAA



Sequences producing significant alignments:

Select: All None Selected:0

	Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/>	Panus similis voucher LE287548 18S ribosomal RNA gene, partial sequence: internal transcribe	902	902	99%	0.0	99%	KM411466.1
<input type="checkbox"/>	Panus similis voucher UOC SIGWI S38 18S ribosomal RNA gene, partial sequence: internal trar	818	818	99%	0.0	96%	KR818820.1
<input type="checkbox"/>	Lentinus sp. WCR1104 18S ribosomal RNA gene, partial sequence: internal transcribed spacer	814	814	99%	0.0	96%	KT956126.1
<input type="checkbox"/>	Panus sp. MEL_2382698 internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA ge	807	807	99%	0.0	96%	KP012877.1
<input type="checkbox"/>	Panus sp. MEL_2382967 internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA ge	778	778	99%	0.0	95%	KP012827.1

Fig. 41: Partial sequence length and homology search of WGM-20 (*Panus similis*)

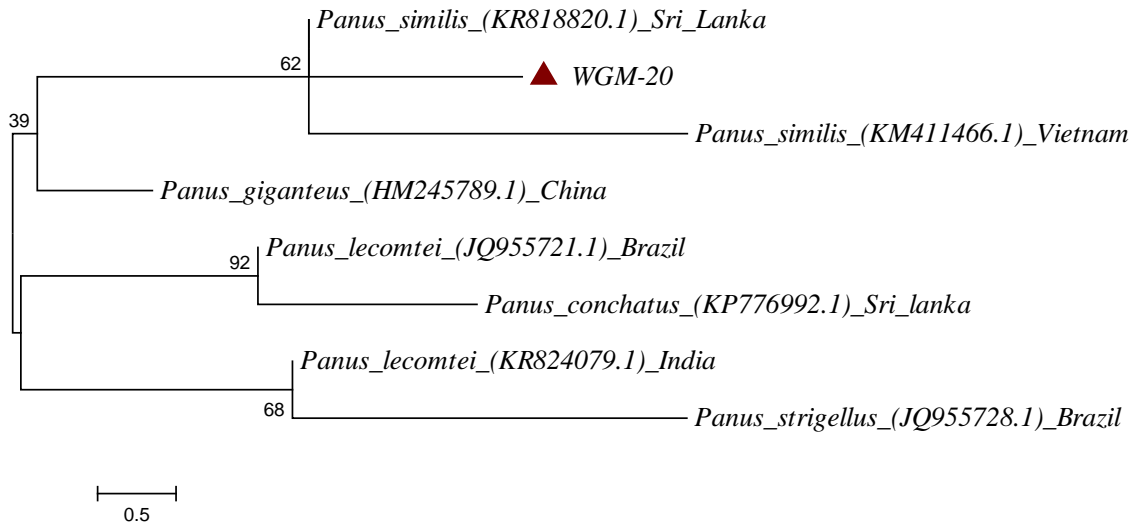


Fig. 42: Phylogenetic relationship between *Panus similis* (WGM-20) and other *Panus* species

CCCTCTCAAATCTCATTACCTGTGCACTCATGATGGGCATTGTGAGGAAGACC
 CAATGTGGTTTTTTCAAATCTTGCCGTGTCGATTTACCTTACACACCCAAGAAAC
 TATGATGTATTTGATTTTGAATCTTAACAACCTTCAACAATGGATCTCTTGGTTC
 TCGCATCGATGAAGAACGCAGCGAATTGCGATATGTAATGTGAATTGCAGAATT
 CTGTGAATCATCGAATCTTTGAACGCACATTGCACCCTCTGGCATTCCAGAGGG
 TATGCCTGTTTGAGTGTCTAATCACCTCAATGCAAAGATATTCTTTTGCAAT
 TGGATGTTGAAGGACTTGCTGGTGCCTTTGATGCTTCCCAGCTCCTTTCAAATG
 CATTAGCAGGAGTTTCTCTGACCAGCTTTCAGTTTGATACATTTAAGCTATCTGA
 GCTGTTACTGGGGGAAAGAGTCTTTATGGTCTTCTGCTCCTTGAGCTGTCTGTA
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 ACCCGCTGAACCTAAGCATATCAATAAGGCC

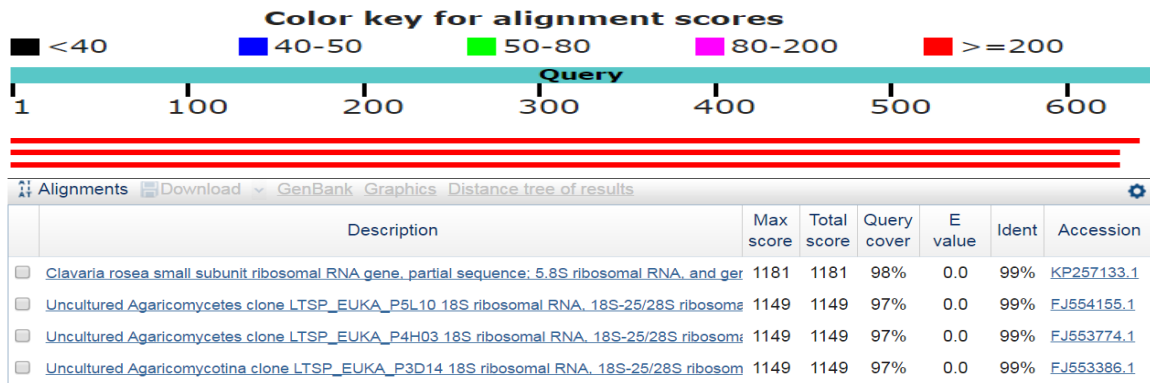


Fig. 43: Partial sequence length and homology search of WGM-21 (*Clavaria rosea*)

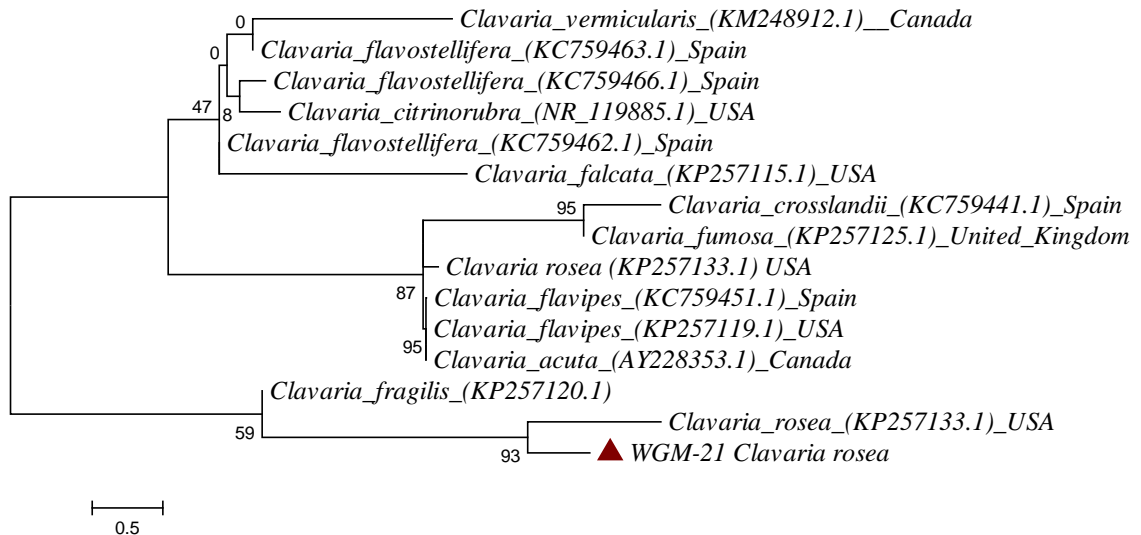


Fig. 44: Phylogenetic relationship between *Clavaria rosea* (WGM-21) and other *Clavaria rosea* species

grassy areas in soil where there has been some kind of disturbance. This mushroom can be easily recognized as *Chlorophyllum molybdites* by its size, scaly cap with darker center, light grey-green gills and spores as it matures. Stains red when damaged and this mushroom were poisonous (Megersa *et al.*, 2016).

4.2.1.24 WGM-24

Small sized bird nest fungi, fruiting bodies are tiny funnel or barrel-shaped, 0.6-1.5 cm tall, 0.4-0.8 cm wide at the mouth, sometimes short-stalked, golden brown to blackish brown in matured fruiting body. Wall of nest is tough, three layered. The mushroom is exactly look like the bird nest and hence it is commonly called as “Bird nest fungi” (Plate 25a). This species was found on decaying twigs and branches. grows gregariously in group.

Spores are spherical and relatively large, with typical dimensions of 20-35 x 20-25 μm . The spores are growing directly from the surface of the basidium, without attachment via sterigmata, and are separated from the basidia after it collapses and gelatinizes. This is accompanied by the gelatinization of the inner walls of the peridiole (Plate 25b). The morphological characters are closely related to *Cyathus striatus* (Huds.) (Cruz *et al.*, 2014). Cyath- means "cup." Striat-means "finely furrowed" or "lined" (striate) in reference to the grooved inner surface. Common name of this fungi are splash cups and fluted bird's nest, bird nest fungi. When an egg is ejected, the trailing cord (funiculus) helps it adhere to an object it encounters.

4.2.1.25 WGM-25

Small slightly translucent mushroom (Plate 26) is documented from Dakshina Kannada during the onset of monsoon (June). Pileus is Pale surface covered with minute yellow squamules along the radial ridges with darker-yellow central disc, convex, becoming plane, very thinly fleshed (almost translucent particularly at margins), delicate, margins are striate, covered with fine powdery scales. 3-4 cm. Gills are free, subdistant, white colour and thin.

Stipe is yellow, fragile, central, cylindrical, slightly tapering upwards, hollow and has a small membranous yellow ring midway up stem which measures 5.5-8 cm x 0.3 cm. Spore print is white, elliptical and smooth; spores measure about 11 x 7 μm . This mushroom grows in a habitat of variety of forest types, occasionally in disturbed areas and found scatteredly in small troops on substrate leaf litter. This mushroom is easily identified by its fine delicate form and pale yellow colour. Fruit bodies are extremely fragile, often collapsing if not handled with care. *Leucocoprinus fragilissimus* (Berk.) was widely distributed in the tropics and subtropics (Halling and Mueller, 2005).

4.2.1.26: WGM-26

Mushroom surface is pink and raspberry pink towards the substrate. Grows gregariously on hard wood, when cut open this mushroom there is a concentric rings of alternative white black (Plate 27). The irregularly spherical to cushion-like fruiting bodies of this fungus are commonly encountered on dead but not yet decorticated trunks and on branches of acacia tree. At first red-brown but later becoming black (hence the commonly called as carbon balls), fruiting bodies are 2-4 cm across and quite hard to the touch. When sectioned, the conspicuous concentric zones present on the interior of the fruiting body. Spore print is black; spores are human eye shape which measures 10 x 4 μm (Plate 31f).

This mushroom is *Daldinia concentrica* belongs to a large and diverse group of ascomycetes that decay the wood of living and dead broadleaf trees (Hall *et al.*, 2003). *Daldinia concentrica* contains a unique secondary metabolite called concentricol, all other investigated *Daldinia* spp. were found devoid of concentricol (Stadler *et al.*, 2001).

4.2.1.27 WGM-27

Polypore mushroom with sessile stalk with bracket like fruiting bodies attached to the hard wood in the Western Ghats. Pileus is dark red-brown, rounded growing edge light lilac to pinky mauve, zoned, variable to fan shaped bracket, concentrically ridged, covered by coarse flattened hairs, flesh dull rusty to dark brown, adhering closely to substrate at times forming a crust or thick mat on the wood surface, tough leathery to



Plate 25: WGM-24 (*Cyathus striatus*)



Plate 26: WGM-25 (*Leucocoprinus fragilissimus*)

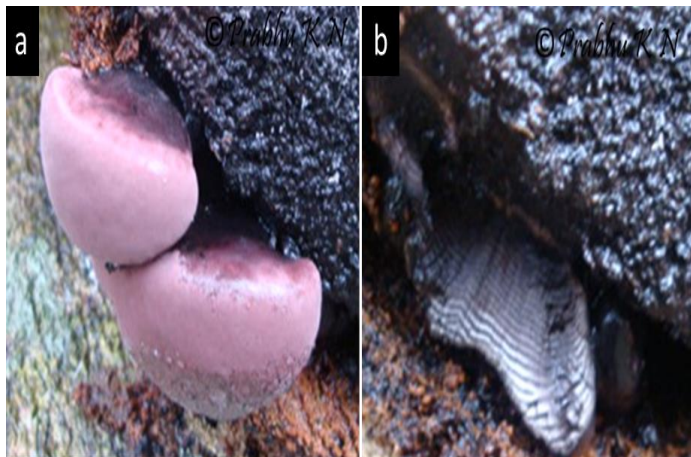


Plate 27: WGM-26 (*Daldinia concentrica*)



Plate 28: WGM-27 (*Fomitopsis lilacinogilva*)



Plate 29: WGM-28 (*Marasmius haematocephalus*)



Plate 30: WGM-29 (*Hygrocybe miniata*)

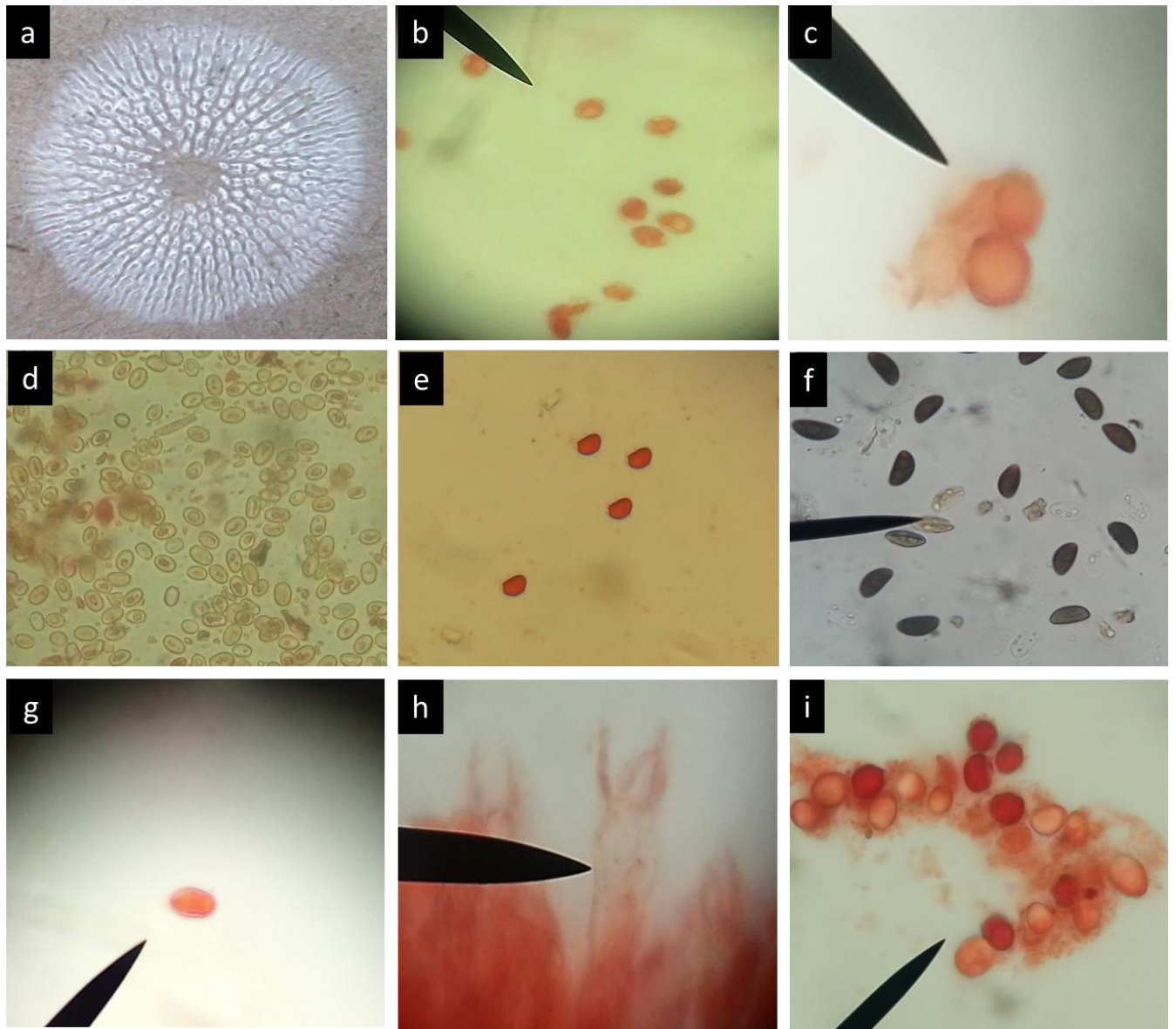


Plate 31: Microscopic characters of wild mushrooms: a- spore print of *Filoboletus manipularis*; b-spore structure of *Filoboletus manipularis*; c- spore structure of *Russula rosea*, d- spore structure of *Amanita pseudovaginata*; e- spore structure of *Clavaria rosea* f- spore structure of *Daldinia concentrica*; g- spore structure of *Marasmius haematocephalus*; h- Basidia of *Hygrocybe miniata* and i- spore structure of *Hygrocybe miniata*.

woody texture; Up to 20 cm wide (Plate 28). Pores are pink, mauve-pink to lilac, round to angular to slightly labyrinthiform. Stipe is Sessile but broadly laterally attached. Spores print is white, smooth, broadly elliptical, $7-9 \times 2.5-3 \mu\text{m}$. Habitat is mixed vegetation, grows on dead stumps and logs, construction timber; this species grows solitary or gregarious clusters.

This species has wide variation in the size of basidiospores from $5-12 \times 2.5-5 \mu\text{m}$, as observed by Ryvarden and Johansen (1980), Carranza and Gilbertson (1986) and Silva *et al.* (2015). This mushroom is *Fomitopsis lilacinogilva* Berk. (Fomitopsidaceae). Bala *et al.* (2011) documented this mushroom from Fraser Island, Central Coast of Australia.

Montoya *et al.* (2012) reported the occurrence of *Fomitopsis lilacinogilva* from the Camanti Marcapata Biological Corridor of south-eastern Peru. *Fomitopsis* mycelium, extracts and derivatives were blends of medicinal mushroom species and are useful in preventing and treating viruses including Poxviridae and Orthopox viruses (Stamets, 2014). Gibertoni *et al.* (2015) recorded the mushroom from Brazilian Amazonia. The Eucalyptus wood sheet piling in a new housing estate in Harkstede, province of Groningen, turned out to be subject to decay by *Fomitopsis lilacinogilva*. This fungus is not native in Europe, and probably was already infecting the imported wood used for the construction work (Keizer, 2016).

4.2.1.28 WGM-28

This delicate fungus has a thin, dark pink, conical cap, which is scalloped at the edges to 1.5 mm diameter, margins ribbed. (Plate 29) The mushroom is documented from Aanebailu near Agumbe. Gills are light colour with widely spaced. Cheilocystidia are in the form of broom-cells, $11-21 \times 6-9 \mu\text{m}$, clavate, sub-cylindrical and thin-walled with slightly thick-walled, coloured projections. Pleurocystidia are $35-63 \times 7-13 \mu\text{m}$, clavate, subcylindrical, thin-walled. is dark pink, gills pale pink to white, stem dark. Stipe is thin, dark brown to black, 5-6 cm long, 0.5 mm diameter, cylindrical, hollow, smooth, lustrous, reddish brown to dark brown, with paler apex and with white tomentose basal mycelium. Spore print is white, slightly ellipsoid, $10-12 \times 4-5 \mu\text{m}$, double walled (Plate 31g). fusoid, clavate-fusoid, thin-walled, hyaline. Basidia are $28-33 \times 7-8 \mu\text{m}$, 4-spored,

clavate. Fruiting bodies of this species were appears on gregariously dead twigs, leaves and small branches.

This mushroom exactly matches to the *Marasmius haematocephalus* (Fr.) (Hasselquist *et al.*, 2011). *Marasmius haematocephalus* is a very easily identifiable species by having a pileus coloured in various tinges of purple and pink, distant lamellae, a brown or fuliginous stipe at base, long and slender basidiospores and welldeveloped pleurocystidia; it lacks caulocystidia. (Antonin and Buyck, 2006). This mushroom was recorded from Thailand, Republic of Korea (Antonin *et al.*, 2012)

4.2.1.29 WGM-29

The mushroom is collected from the Dakshina Kannada (latitude 12°49'32.99"N and longitude 75°25'27.11"E, altitude: 377 ft.) and it contains red colour pigment. Pileus is red in colour, convex in shape with smooth texture, 1.5 to 2 cm diameter (Plate 30). Lamellae is distant, broadly adnate to decurrent; combination of white and orange; basidia is four spore (Plate 31h). Stem is cylindrical, orange to red colour, 30-50 × 3-4mm. Spore print is white, 5.5-6 × 4 μm, ovoid (Plate 31i). It occurs in group of four to six on grass land.

Pigmentation and the waxy mushroom indicated that this mushroom is belongs to Genus *Hygrocybe* and from above characters we can clearly say that this species is *Hygrocybe miniata* Fr. Ronikier and Borgen (2010) recorded this species from Poland, cap was 1.0-3.5 cm in diameter, first hemispherical, then expanded with slightly depressed center, dry, squamulose, orange-yellow, orange-reddish, paler on drying, brownish orange (Halling and Mueller, 2005). It was characterized by subdeliquescent basidiomata and a membranous, yellowish-white pileus with small yellow squamules (Pegler 1983, Rother *et al.*, 2009).

4.2.2 Medicinal Mushrooms

Medicinal Mushrooms are comparable to “medicinal plants” and can be defined as macroscopic fungi, mostly higher Basidiomycetes and some Ascomycetes, which are used in the form of extracts or powder for prevention, alleviation, or healing of diseases,

and/or in providing a balanced healthy diet. According to the definition of “herbal drugs,” dried fruit bodies, mycelia, or spores are considered “mushroom drugs” or “fungal drugs.” Analogous to “phytopharmaceuticals” or “herbal preparations,” the resulting preparations should be considered as “mushroom pharmaceuticals” or “mushroom preparations (Lindequist, 2013). Mushrooms also have potential medicinal properties. Pharmaceuticals worth 700 million dollars 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).

4.2.2.1 WGM- 30

This mushroom is easily recognized by its colour and which forms splitting gills (Plate 30). It is dehydrated in dry condition and can rehydrate after rain. It causes white rot in wood and very good lignin degrading fungi. It is found predominantly from spring to autumn on dead wood. This mushroom is found in Western Ghats of Hassan (latitude 12°57'20.61"N and longitude 75°50'53.62"E, 3182 ft elevation from mean sea level), Dakshina Kannada, Shivamogga and Chikkamagaluru. The cap is shell-shaped to convex. It is often wavy and lobed, with a rigid margin when old. It is tough, and hairy, and slippery when moist. It is greyish white and up to 4 cm in diameter and thickness about 0.5 cm. The gills are pale silver or grey, very narrow with a longitudinal split edge which becomes in-rolled when dry, the only known fungus with radiating type of gills that is capable of retracting by movement. Spore print is white to pale pink, cylindrical, smooth and measures 6 x 3 µm. This mushroom decomposing wood like tree stumps, logs and fallen branches.

The sequenced data showed 99 % homology with the earlier reported *Schizophyllum commune* Fr. (KM985685.1) (Fig.45). Phylogenetic tree showed that WGM- 30 is very close to Thailand *Schizophyllum commune* (KT844679.1) with boot strap support 59 and grouped into clade III (Fig.46). Anti-candida, anti-tumor and anti-

viral properties, antitumor, anticancer and immunomodulating activities (Kidd, 2000 and Wasser, 2002).

Tadosa and Briones (2013) recorded the distribution of this species in Taal Volcano Protected Landscape in Philippines. Thiribhuvanamala *et al.* (2011) reported the distribution of this species in Coimbatore. This species was edible and has been used as for culinary purpose; has religious/ cultural values in Rupandehi District of Nepal (Aryal and Budathoki, 2013). This mushroom also had been utilized as edible by Aeta community in Central Luzon, Philippines (Leon *et al.*, 2013). Lazo *et al.* (2015) collected and identified the *Schizophyllum commune* based on their morphological characteristics which was utilized by Gaddang communities in Philippines. This species was also reported from Kovai Kuttralam (Selvam *et al.*, 2012). The species was also recorded in the Pune region by Senthilarasu (2014). Collected by the local people during the season not only for their own consumption and but also for sale (Tanti *et al.*, 2011). Usha and Janardhana (2014) reported this species was grown on wood in Aanekaadu forest of Kodagu, Heggadadevana kote forest and Cchamarajanagara region. Giri *et al.* (2012) reported this mushroom species in the hilly regions of West Bengal.

Schizophyllan is derived from *Schizophyllum commune* and obtains β -1, 6-branched- β -1, 3-glucan, which decreases inflammation, irritation and other damage due to UV and toxic environment exposure of the skin (Kumari *et al.* 2008). The product Sulwhasoo Hydroaid contains extract of *Schizophyllum commune* which serves as Hydrating cream promoting clear, radiant skin. Alqvimia Eternal Youth Cream Facial Maxima Regeneration a cosmetic product contains Anti-aging and lifting property (Hyde *et al.*, 2010).

4.2.2.2 WGM- 31

This mushroom grows single or scattered pattern and is documented from Sullia of Dakshina Kannada district (latitude 12°29'55.19"N and longitude 75°33'13.95"E) at an altitude of 494 ft. from mean sea level which is warm and humid climate. This jelly mushroom is predominantly distributed in this region which is very close to west coast of India. Pileus is gelatinous, white and glassy or translucent, erect with branched fronds,



Plate 32: WGM-30 (*Schizophyllum commune*)



Plate 33: WGM-31 (*Tremella fuciformis*)



Plate 34: WGM-32 (*Auricularia polytricha*)



Plate 35: WGM-33 (*Auricularia delicata*)



Plate 36: WGM-34 (*Ganoderma applanatum*)

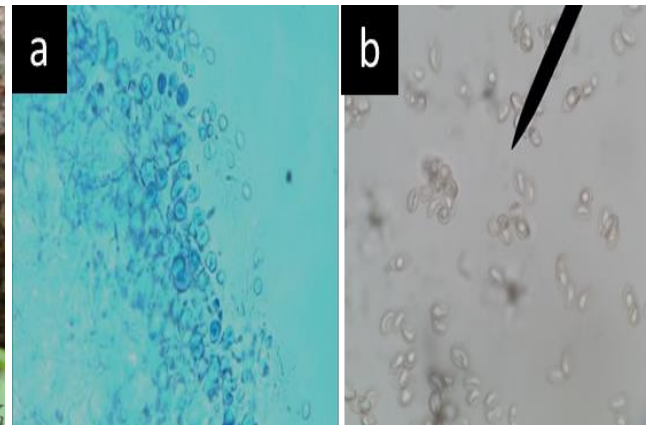
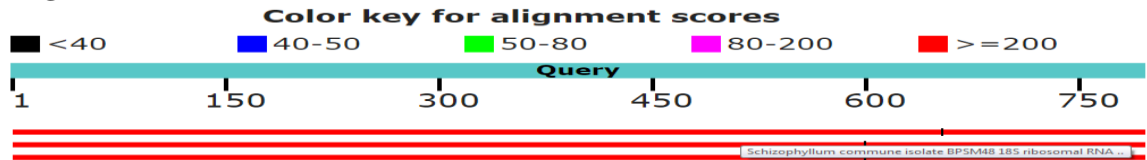


Plate 37: Spore structures a- *Tremella fuciformis* and b- *Ganoderma applanatum*

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 GACGGTTGGAAGCAGACTCCTATTGAACTGACTAGGTCAGCCCCGAGATGGT
 CAACGACGTAGAAATTATCACATCGGAGACGCGATCCCCGCAAGGGAAATCCGC
 TAATACATTTGAGAGGAGCTGGCTCCGTTAGGCTCCAGCAGACCTCCACTTCCA
 AGCCACTCTCGAGACCGAAGTCAAAAAGAGGGTTGATGGTATTTAATGACACTCA
 AACAGGCATGCCCTCGGAATACCAAAGGGCGCAAGGTGCGTTCAAAGATTG
 ATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTC
 ATCGATGCGAGAGCCAAGAGATCCGTTGTTCGAAAGTTGTATTAACTTTTAGGG
 TCTGTCAAGACCATGATTACATTCGTTAACATACTTTAAGGTGTGAGGGTAGACG
 TAGTCAACCGCCGCCCGTGAAGGCTTTGGGACTACATAAGGTGCACAGGATCA
 GAACAAGATGAACTTGTGTTGATTCGTTAATGATCCTTCCGCAGGTTACCCTAC
 GGAAGGATCAGTAACGAATCAGACAAGTTTCATCTTGTCTGATCCTGTGCACTT
 ATGTA



Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Schizophyllum commune isolate BPSM48 18S ribosomal RNA gene, partial sequence, internal transcribed space	1115	1421	99%	0.0	99%	KM985685.1
Schizophyllum sp. BAB-4410 18S ribosomal RNA gene, partial sequence, internal transcribed space	1063	1293	99%	0.0	99%	KR154901.1
Schizophyllum sp. BAB-4418 18S ribosomal RNA gene, partial sequence, internal transcribed space	1059	1290	99%	0.0	99%	KT186214.1
Schizophyllum commune isolate Vijaya-ahmedabad-03 18S ribosomal RNA gene, partial sequence	1058	1281	99%	0.0	99%	JN049907.1

Fig. 45: Partial sequence length and homology search of WGM-30 (*Schizophyllum commune*)

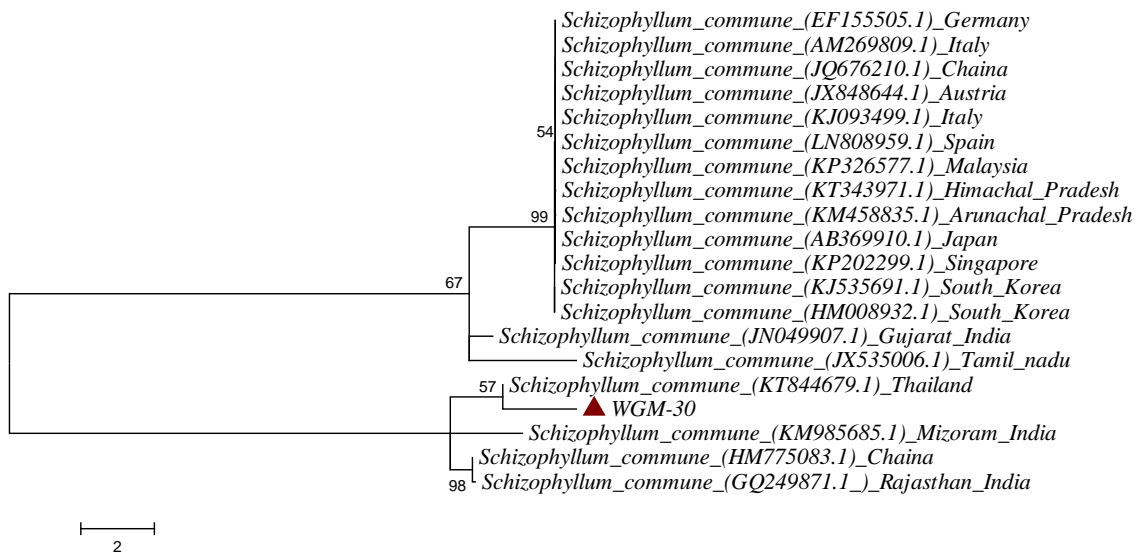


Fig. 46: Phylogenetic relationship between *Schizophyllum commune* (WGM-30) and other *Schizophyllum commune* species

diameter of the fruiting body ranged from 3.5 to 9.5 cm (Plate 31). The spores are ellipsoid, smooth, measured 8 X 6 µm (Plate 37a).

This species is predominantly occurs in Sullia region of Dakshina Kannada and the mushroom is called as snow fungus. The hyphae are clamped and occur in a dense gelatinous matrix. Haustorial cells arise on the hyphae, basidia with oblique to vertical septa. Based on these phenotypic characters the mushroom was identified as the genus *Tramella*. Further the mushroom species was identified using ITS region sequence. The sequence of the fungus when blast searched showed 99 % homology with *Tremella fuciformis* (AF042409.1) available at NCBI GenBank (Fig.47). Phylogenetic tree showed the WGM- 31 is grouped into clade III (Fig.48).

Tremella fuciformis is an edible mushroom as well as medicinal uses and its various traditional indications including clearing heat and dryness, nourishing the brain and enhancing beauty. *T. fuciformis* is rich in polysaccharides suggested the main bioactive component. *T. fuciformis* has been reported for its medicinal purposes such as anti-oxidant (Wu *et al.*, 2008), hypoglycemic (Cho *et al.*, 2007), memory improvement and anti-aging (Kim *et al.*, 2007). *Tremella fuciformis* was one of predominantly occurring edible mushroom in the Western Ghats of Kodagu region (Pandey and Veena, 2012) and Aanekaadu forest (Usha and Janardhana, 2014).

Tremella fuciformis extract was used in cosmetic product La prairie advanced marine biology night solution which had functions like Moisturizer which nourishes, revitalizes and hydrates skin. A mushroom extract from *Tremella fuciformis* fruiting body holds up to 500 times its own weight of water. It was used in skin care product like Aquamella cream (Hyde *et al.*, 2010).

4.2.2.3 WGM-32

This brown jelly mushroom documented in Dkshinakannada (latitude 12°43'39.71"N and longitude 75°32'57.96"E, at an altitude of 433ft from mean sea level). Pileus is gelatinous, dark brown in colour, basidia are cylindrical, hyaline, tri-septate, Pileus is exactly look like animal's ear and it also called as cloud ear fungus

(Plate 32). Stipe is short and laterally attached to the dead wood. Spores are hyaline, $15 \times 4-5 \mu\text{m}$. This mushroom occurs in small group on acacia tree

It is a jelly mushroom is belongs to genus *Auricularia* and further identification was done by blast search of ITS sequenced region showed 89 % homology with the *Auricularia polytricha* sequence data available at NCBI (Fig.49). Thus, the mushroom was identified as *A. polytricha*. The phylogenetic tree showed that WGM- 32 is very close to Indonesian *Auricularia polytricha* (AJ537388.1) with bootstrap support 67 and grouped into Clade VII (Fig.50).

Tadosa and Briones (2013) recorded the distribution of this species in Taal Volcano Protected Landscape in Philippines. *Auricularia polytricha* had been utilized as edible by Aeta community in Central Luzon, Philippines (Leon *et al.*, 2012). *Auricularia polytricha* was one of predominantly occurring edible mushroom in the Western Ghats of Kodagu region (Pandey and Veena, 2012).

The polysaccharide (1, 3- β -glucan, 1, 4- α -glucan, and 1, 3- α -glucan backbone with a single α -D-glucopyranosyl side-branching in O-6 of 1, 3- α -glucan residue) from *Auricularia polytricha* was able to significantly inhibit the growth of Sarcoma-180 in mice which can be used to promotion of food-based therapies (Song and Du, 2012).

4.2.2.4 WGM- 33

Fruiting body is gelatinous when fleshy. However, when dry their texture becomes hard, brittle and returning to their original consistency when exposed to water. Mushroom grows gregariously on the dead tree stem, gelatinous in nature (Plate 33), documented from Western Ghats of Dakshina Kannada, Hassan and Shivamogga, it commonly known as Mavuanabe. Pileus made up of densely compacted gelatinised hyphae with cuticular hairs with rounded tips; basidia $42 \times 4 \mu\text{m}$, cylindrical, with 3 transverse septa; prominent ridges at the dorsal side / Basidioma with reticulate hymenial which is a main features of *Auricularia delicata*.(Alvarenga *et al.*, 2015). Spores are allantoid with 2-3 prominent oil globules, $11 \times 5 \mu\text{m}$.

GTGCTTGCATCCGGGAGCAGGCCCTTCCAACACCTGTGCACATCGGACC
GCGCCTCCGGGCCGGGCCGCTTCACACAAACATATGTCAAGAACGTAA
TGCATCATAACATGAAACAACCTTCAACAACGGATCTCTTGGCTCTCGCA
TCGATGAAGAACGCAGCGAATTGCGAAAAGTAATGTGAAATTGCAGAATT
CAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCTTTTGGTATTCCGA
AAGGCATGCCTGTTTGAAGTGCATGTAGACTCAACCCCCCGGGTTTCTG
ACCCGGCGGTGTTGGATTTGGGCCCTGCCTCTCTGGCTGGCCTTAAATG
CGTTAGTGGTTTCACGCAGACGTCGTAAGTTACGCGTCGACTGTGGGCC
GCTCACAAACCCCTTTACTTTTGCCTCTGGCCTCAAATCAGGTAGGGCT
ACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA

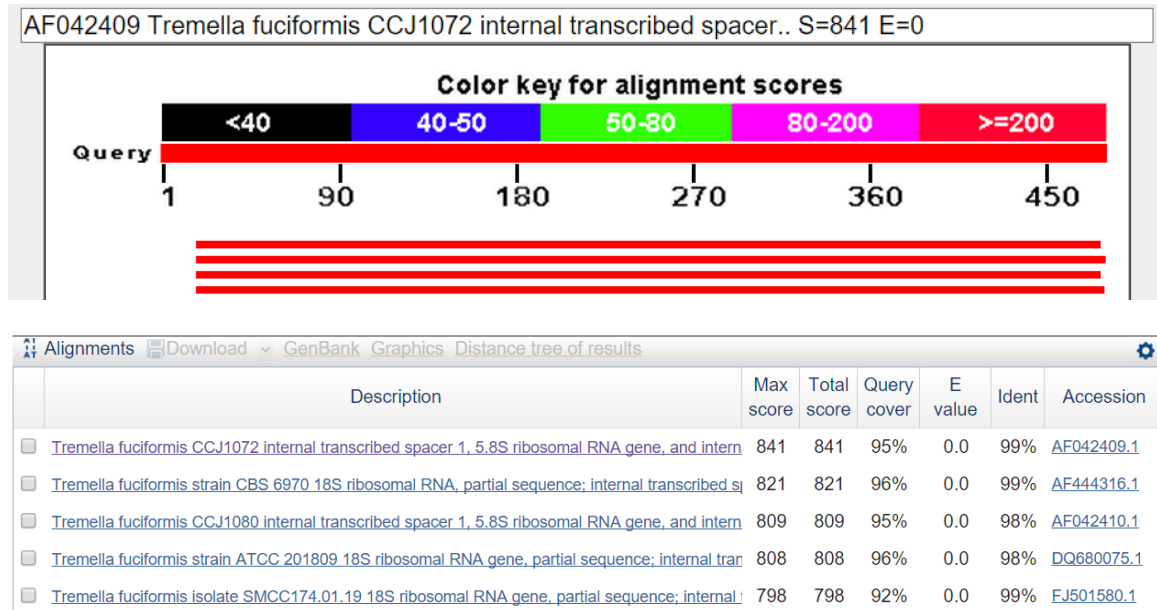


Fig. 47: Partial sequence length and homology search of WGM-31 (*Tremella fuciformis*)

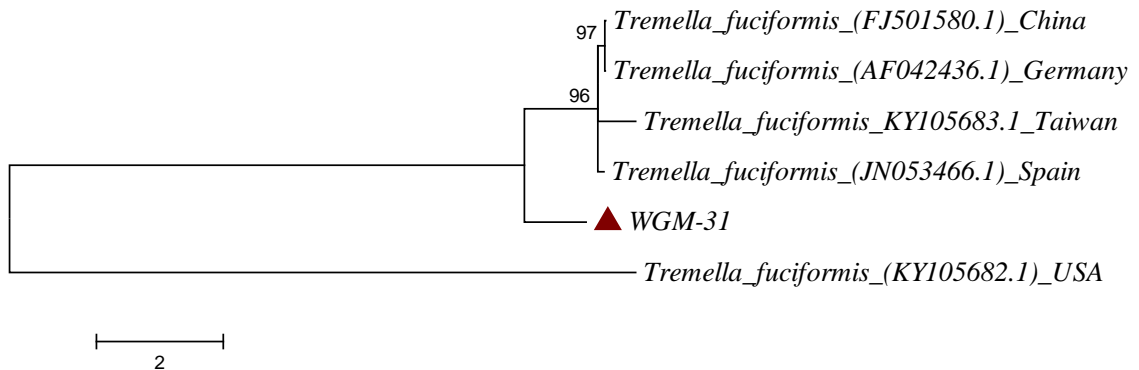
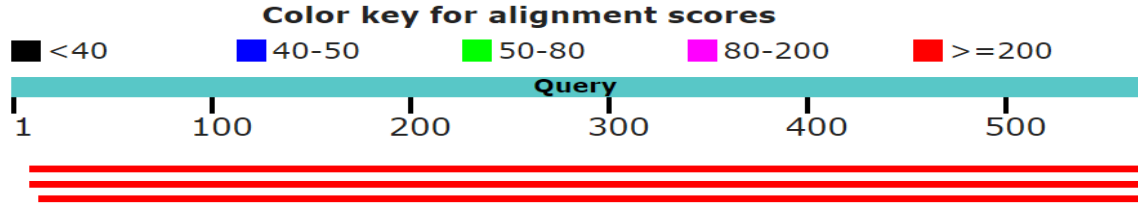


Fig. 48: Phylogenetic relationship between *Tremella fuciformis* (WGM-31) and other *Tremella fuciformis* species

TGAATGGTGTCTACTGATTTGAGGCCAAGCTTTAAAAAATTGGTGGTCCCAGAGG
 GGACGGCGGTAACCACCACCGCTGAAAAAGCCTAAGGCATGGGCGAAAAATATT
 TATAACCCCGTCGCCAGCATTCTAAAAACCGCCGCTTAAGGCCTTTTCAGAACAA
 GCCGGTTACGGCCTGTCCCAAGTCCCCCGCGAGCGACGGTTACTTCGCAGGGG
 TGAGGGTTTACGTGACACTCAAACAGGCAGGCTCCAGGGGAAAACCAAGGACCG
 CAGAATGCGTTCAAAAAATCAATGATTCCTTGAATTCTGCATTTCACTTTACTTA
 TCGAATTTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAAAAATCCGTTGTTGAA
 ATTTGTACTTTTTAGGGTTTTGTTACCATTCAAGACTTGATTTGTTGCTTTTAA
 AAGCGGCAGCGACCGAACCCGCAACCGAAAAAGGTGCACAGGTGTGGGGTCTGTG
 CTCCAGCGTGCACCCCGGTA AAAAGGCGCACAGCTGAACGATCGGTTTAAAAGC
 CAAAAATCTTTAATGATCCTTCCGC



Sequences producing significant alignments:

Select: All None Selected:0

	Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/>	Auricularia sp. BAB-4720 18S ribosomal RNA gene, partial sequence; internal transcribed spacer	688	1182	98%	0.0	89%	KR154949.2
<input type="checkbox"/>	Auricularia sp. BAB-5206 18S ribosomal RNA gene, partial sequence; internal transcribed spacer	688	1403	98%	0.0	89%	KT186177.1
<input type="checkbox"/>	Auricularia sp. BAB-5200 18S ribosomal RNA gene, partial sequence; internal transcribed spacer	671	1165	97%	0.0	89%	KT186175.1
<input type="checkbox"/>	Auricularia sp. BAB-5001 18S ribosomal RNA gene, partial sequence; internal transcribed spacer	671	1165	97%	0.0	89%	KR155089.1
<input type="checkbox"/>	Auricularia polytricha strain AP3 18S ribosomal RNA gene, partial sequence; internal transcribed	660	1163	98%	0.0	88%	KF297984.1
<input type="checkbox"/>	Auricularia polytricha strain SN111 18S ribosomal RNA gene, partial sequence; internal transcrib	660	1163	98%	0.0	88%	KF297977.1

Fig. 49: Partial sequence length and homology search of WGM-32 (*Auricularia polytricha*)

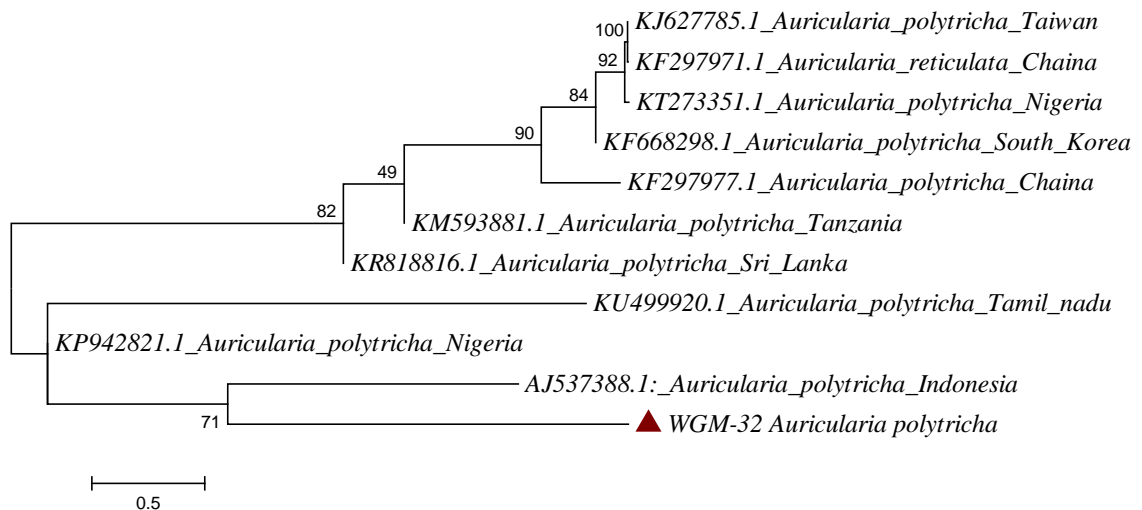


Fig. 50: Phylogenetic relationship between *Auricularia polytricha* (KWGM-32) and other *Auricularia polytricha* species

This is a jelly mushroom and found to be an edible and also used to stop bleeding (Sarma *et al.*, 2010) Sarma *et al.*, (2010) documented this mushroom on dead branch of *Semecarpus sp.* in western Assam. Fierroslopez, (2006) reported that mushroom sporophore was associated with *Scaphisoma jaliscanum*. *Auricularia delicate* was one of the wild edible mushroom species consumed and sold in local markets of Tanzania (Tibuhwa, 2013).

4.2.2.5 WGM- 34

The mushroom is documented in the Western Ghats of Sakleshpur, Dakshina Kannada and Chikkamagaluru. Mushroom is reddish brown colour with white spore surface which is used to do the artistic work (when the fresh white pore surface is rubbed or scratched with a sharp implement, dark brown tissue under the pores is revealed, resulting in visible lines and shading that become permanent once the fungus is dried), this mushroom also called as artist's bracket, artist's fungi (Plate 34). Lamellae are shorter towards the edge. Spore print is brown, ellipsoid, rough texture, $5-6 \times 4.5-5 \mu\text{m}$ (Plate 53f). Mushroom grows solitary or in group.

The fruiting body margin is narrower. Pore surface is softer and depressible with a thumb or finger whilst, this mushroom is *Ganoderma applanatum* (Overall, 2016). This mushroom is also used for therapeutic purpose along with coconut oil to cure the blockage in ear. *G. applanatum* have antioxidant, hypoglycemic and anti hypertension activity (Oyetayo, 2011).

G. applanatum extracts was the strongest 1,1-Diphenyl-2-picrylhydrazyl scavengers, i.e. $EC_{50} < 0.1 \text{ mg/mL}$ and had the highest chelating ability of ferrous ion ($EC_{50} = 3.58 \text{ mg/mL}$), inhibition of lipid peroxidation ($EC_{50} = 2.07 \text{ mg/mL}$) and had the highest reducing power, $EC_{50} = 0.18$ than the other medicinal mushrooms like *Ganoderma lucidum*, *Lentinus edodes* and *Trametes versicolor*. The extracts contained a mixture of polysaccharides, proteins and polyphenols, still present after hot water treatment, ethanol precipitation and dialysis. Glucose was the prevalent monosaccharide and contained both α - and β -glucans. Total glucan contents was 35 % for *G. applanatum* and 83 % for *G. lucidum* (Kozarski *at al.*, 2012)

Beta glucan act on several immune receptors including Dectin-1, complement receptor (CR3) and TLR-2/6 and trigger a group of immune cells including macrophages, neutrophils, monocytes, natural killer cells and dendritic cells. As a consequence, both innate and adaptive response can be modulated by β -glucans and they can also enhance opsonic and nonopsonic phagocytosis (Chan *et al.*, 2009). Manganese Peroxidases from *Ganoderma applanatum* degrade β -Carotene under alkaline conditions which may replace common bleaching agents to produce environmentally more compatible detergent formulations (Lanfermann *et al.*, 2015).

Ganoderma applanatum used as antioxidant and for lowering blood sugar level, as well as antihypertensive (Oyetayo, 2011). *Ganoderma applanatum* is extremely valuable mushroom, with strong antimicrobial potential. Depending on the method of extraction it is possible to obtain different kinds of extracts that could act as microbistatic or microbicidal agents against certain species and strains of microorganisms (Klaus *et al.*, 2016).

The three medicinal mushrooms have been identified based on the homology with the respective species sequences found in NCBI. WGM-30 showed 99 % sequence homology with *Schizophyllum commune*; WGM-31 showed 89 % sequence homology with *Tremella fuciformis* and WGM-32 showed 89 % sequence homology with *Auricularia polytricha*. Among these medicinal mushroom, *Auricularia delicate*, *Auricularia polytricha*, *Schizophyllum commune* and *Tremella fuciformis* were known to be edible (Han *et al.*, 2005).

Manjunathan *et al.* (2011) studied the solvent based effectiveness of antibacterial activity of edible mushroom *Lentinus tuberregium* (Fr.). *In vitro* antimicrobial properties of *L. tuberregium* culture filtrate extracted using four different solvent systems (Hexane, dichloromethane, chloroform and ethyl acetate) were the most active to inhibit the growth of *S. aureus*, *Micrococcus luteus*, *E. coli*, *Salmonella typhi* and *Shigella flexneri*.

The variation of climatic conditions within the continent was directly reflected on the prevalence of mushrooms in different geographical regions. Accordingly, medicinal

applications may differ not only between different countries, but also between different ethnic groups inhabiting the same country. Mushrooms belonging to species of *Termitomyces*, *Pleurotus*, *Lentinus*, *Lenzites*, *Trametes*, *Ganoderma*, *Pycnoporus*, *Coriolopsis*, and *Calvatia* have been reported to be used in folk medicine in Nigeria (Oso, 1977, Oyetayo, 2011 and Ezekiel *et al.*, 2013).

The body usually needs endogenous sources to fulfill its antioxidant requirements. Mushrooms used as dietary source can help to surpass this lack since they contain high amounts of bioactive compounds displaying antioxidant activity. These dietary sources of antioxidants such as ascorbic acid, tocopherol, beta-carotene, vitamin K, flavonoids, phenolic acids, selenium and zinc tend to maintain a balance to control oxidative stress (Carocho and Ferreira, 2013).

4.2.3 Edible Mushrooms

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.

4.2.3.1 WGM-35

This Mushroom was documented in the Western Ghats of Sakleshpur (latitude 12°57'04.41"N and longitude 75°49'05.60"E) at an altitude of 3223ft from mean sea level during the rainy season. Fruiting body appears up to 40-60 feet from the ground on dead tree (Plate 38). Flies are the common visitor of these fruiting bodies and they are sucking the juice content in the edge of the cap. Pileus is white in color and depressed at the center, Diameter of the cap is 7-12 cm, Medium in texture. Gills are decurrently attached to the stalk. Stipe is equal which measures 4-6 cm in length, 2-3mm in thickness,

white in colour, stipe position is central, Presences of annular ridge. The Spore print is cremish white colour and structure of the spore is ovule, which are doubled layered and measures about $5-6 \times 2-2.5 \mu\text{m}$. It grows on dead wood gregariously with 30 to 50 fruiting bodies.

The sequence of the ITS region (594 bp) showed 100 % homology with the earlier reported *Lentinus sajor-caju* Fr. (Fig. 51). Phylogenetic tree showed that the identified *Lentinus sajor-caju* is closely related to *Lentinus sajor-caju* (KT818506) documented from West Bengal with bootstrap support 43 and grouped under clade V (Fig. 52). This mushroom also had been utilized as edible by Aeta community in Central Luzon, Philippines (Leon *et al.*, 2014).

4.2.3.2 WGM-36

Fruiting bodies appears up to 40-60 feet from the ground on dead tree. This species was collected from Nettana village (latitude $12^{\circ}43'57.69''$ N and longitude $75^{\circ}32'06.14''$ E at an altitude of 540 ft. from mean sea level) during the rainy season. Cap is peach to light brownish in color and depressed at the center (Plate 39), Diameter of the cap is 5-10 cm, Medium in texture. Structure of the cap cuticle is filamentous. Gills are decurrently attached to the stalk. Stipe is equal which measures 6-7 cm in length, 2-3mm in thickness, white in colour Stipe Position was central, Annulus was absent. The Spore print is white colour and structure of the spore is ovule, which are doubled layered (Plate 54a). This mushroom grown on dead standing trees and dead wood. Two to forty fruiting bodies can be seen in a dead tree.

Sequenced data showed 98 % homology with the earlier reported *Lentinus sajor-caju* (Fig. 53). The phylogenetic tree showed that the identified *Lentinus sojar-caju* (WGM-36) closely related to *Lentinu sajor-caju* (KM985672.1) of Mizoram, with bootstrap support and grouped under clade IX (Fig.4). De Leon *et al.* (2013) found this mushroom growing on the live bamboo tree and also reported that this mushroom species was utilized as food by the Aeta communities as well as the lowlanders of Philippines. Gulati *et al.* (2011) documented this mushroom in the North West region of India. Ao *et al.* (2016) observed this mushroom was growing on dead stumps of trees like Oak in



Plate 38: WGM- 35 (*Lentinus sajor-caju*)



Plate 39: WGM-36 (*Lentinus sajor-caju*)



Plate 40: WGM-37 (*Lentinus polychrous*)



Plate 41: WGM-38 (*Lentinus strigosus*)



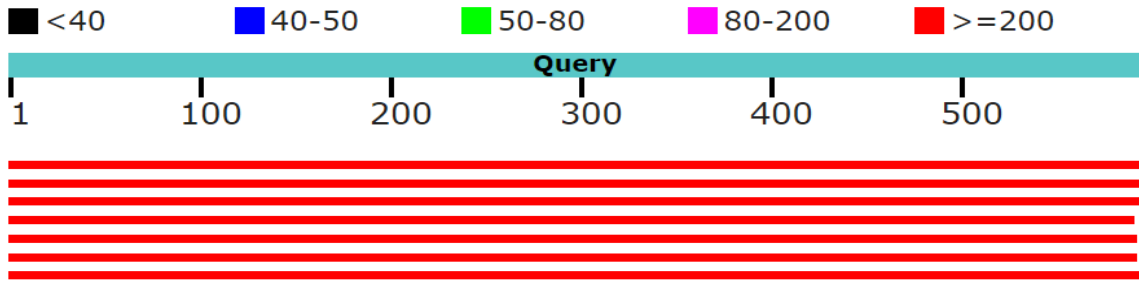
Plate 42: WGM-39 (*Lentinus tigrinus*)



Plate 43: WGM-40 (*Lentinus tuberregium*)

CTCATCCACTCTACACCTGTGCACCTTACTGTGGGTTTCAGGAGCTTCGAA
 AGCGGAGGACCTTTGCGGGTTTTTCGTTATTAGTTGTGACTGGGCTCAT
 GTCCACTACAACTCTTATAAAGTAACAGAATGTGTATTGCGATGTAACG
 CATCTATATACAACCTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGA
 AGAACGCAGCGAAAATGCGATAAGTAATGTGAAATTGCAGAAATTCAGTGAA
 TCATCGAAATCTTTGAAACGCACCTTGCCTCCTTGGTATTCCGAGGAGCAT
 GCCTGTTTGAGTGTTCATGAAATTCTCAACCTGACGGGTTCTTAACGGAG
 CTTGGTTTCAGGCTTGGACTTGGAGGCTTGTTCGGCTTGCTTTGTCGAGTC
 GGCTCCTCTCAAATGCATTAGCTTGGTTCTTTGCGGATCGGCTCACGGT
 GTGATAATTGTCTACGCCGCGACCGTTGAAGCGTTTGAATGGGCCAGCT
 TATAGTCGTCTCCATCGCGAGACAACATTTTCATCGAACTCTGACCTCAA
 TCAGGTAGGACTACCCGCTGAACTTAAGCATATCATAAAGCGGGAGGAAA
 G

Color key for alignment scores



Sequences producing significant alignments:

Select: [All](#) [None](#) Selected: 0

Alignments						
Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/> Lentinus sajor-caju strain EB1001 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, partial sequence	1070	1070	100%	0.0	99%	KT956122.1
<input type="checkbox"/> Pleurotus giganteus strain Torpido0811 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, partial sequence	1062	1062	99%	0.0	99%	KT956125.1
<input type="checkbox"/> Lentinus sajor-caju voucher FRI62056 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, partial sequence	1053	1053	97%	0.0	99%	KP283492.1
<input type="checkbox"/> Lentinus sajor-caju voucher MEL-2382718 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, partial sequence	1048	1048	99%	0.0	98%	KP012899.1
<input type="checkbox"/> Lentinus sajor-caju voucher SNP24989 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, partial sequence	1040	1040	97%	0.0	99%	KP283493.1
<input type="checkbox"/> Polyporus squamosus internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, partial sequence	1027	1027	99%	0.0	97%	KR824090.1

Fig. 51: Partial sequence length and homology search of WGM-35 (*Lentinus sajor-caju*)

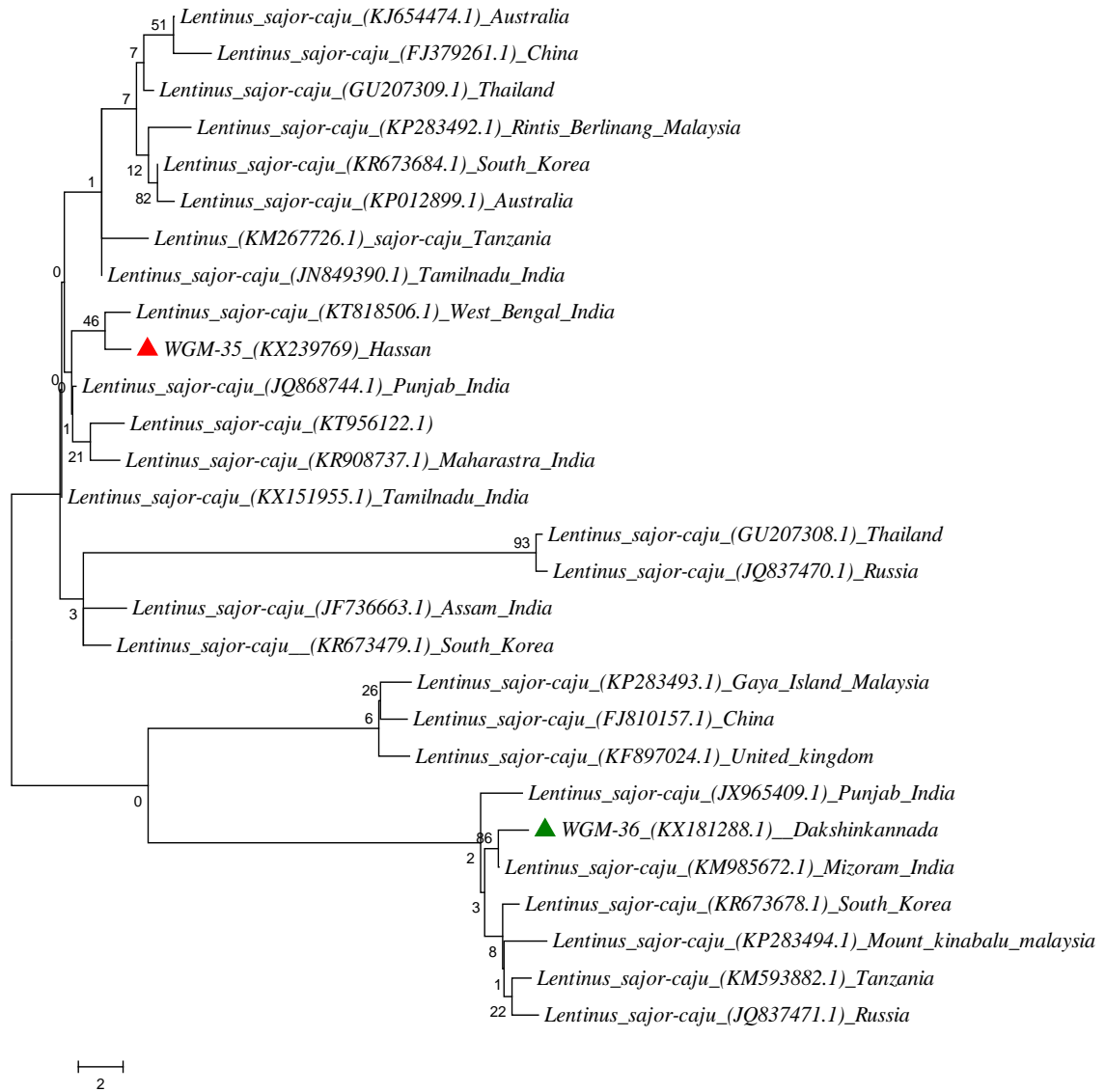
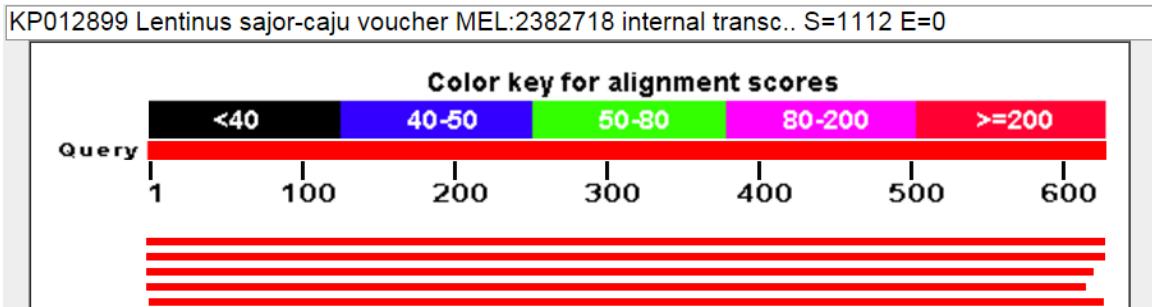


Fig. 52: Phylogenetic relationship between *Lentinus sajor-caju* (WGM-35 and WGM-36) and other *Lentinus sajor-caju* species

ACGGGTTGTAGCTGGCCTTCGAGGCATGTGCACGCCCTGCTCATCCACT
 CTACACCTGTGCACTTACTGTGGGTTTCAGGAGCTTCGAAAGCGAAGGC
 CTTTGCCGGCTTTTTTCGTTATTAGTTGTGACTGGGCTCATGTCCACTACA
 AACTCTTATAAAGTAAACAGAATGTGTATTGCGATGTAACGCATCTATATA
 CAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAG
 CGAAATGCGATAAGTAAATGTGAATTGCAGAATTCAGTGAATCATCGAAT
 CTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTG
 AGTGTGATGAAATTCTCAACCTGACGGGTCTTAACGGAGCTTGGTTCA
 GGCTTGGACTTGGAGGCTTGTTCGGCTTGCTTTGTTCGAGTCGGCTCCTCT
 CAAATGCATTAAGCTTGGTTCTATGCGGATCGGCTCACGGTGTGATAATT
 GTCTACGCCGCGACCGTTGAAGCGTTTGAATGGGCCAGCTTATAGTCGT
 CTCCATCGCGAGACAACATTTTCATCGAACTCTGACCTCAAAATCAGGTAG
 GACTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA



Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/> Lentinus sajor-caju voucher MEL:2382718 internal transcribed spacer 1, partial sequence: 5.8S ribos	1112	1112	100%	0.0	98%	KP012899.1
<input type="checkbox"/> Lentinus sajor-caju voucher FR162056 18S ribosomal RNA gene, partial sequence: internal transcrib	1107	1107	98%	0.0	99%	KP283492.1
<input type="checkbox"/> Lentinus sajor-caju voucher SNP24989 18S ribosomal RNA gene, partial sequence: internal transcri	1103	1103	97%	0.0	99%	KP283493.1
<input type="checkbox"/> Polyporus squamosus internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA gene an	1070	1070	99%	0.0	97%	KR824090.1
<input type="checkbox"/> Fungal endophyte isolate 1629 18S ribosomal RNA gene, partial sequence: internal transcribed spac	1062	1062	100%	0.0	97%	KR015073.1

Fig. 53: Partial sequence length and homology search of WGM-36 (*Lentinus sajor-caju*)

Nagaland during May to June. *Lentinus sajor-caju* is also reported from Dja Biosphere Reserve of Cameroon (Njouonkou *et al.*, 2013), *Lentinus sajor-caju* can easily be recognized in the field by its large, thin, whitish to cream pileus with pale brown disc, lacking or with small squamules at the center, short stipe with annulus or annular ridge (Senthilarasu, 2014).

4.2.3.3 WGM-37

This mushroom is documented in Sringeri of Chikkamagaluru district (latitude 13°25'27.24"N and longitude 75°14'47.07"E at an altitude of 2197ft from mean sea level). Funnel like woody mushroom (Plate 40). Cap color is grey and hard texture, skeleto-ligative hyphae. Gills are in brown color, attached decurrently to the stipe. Stalk position is eccentric and is about 2 cm in length and wood texture. Spore size is 6-9 × 2.5-3 µm. Fruiting bodies are scattered growth which grows on wooden log.

ITS region/rRNA gene amplified using ITS-1 and ITS-4 primer revealed 100 % homology with the earlier reported *Lentinus polychrous* (KX239770.1) (Fig. 54). The phylogenetic tree showed that identified WGM-37 (*Lentinus polychrous*) is closely related to *Lentinus polychrous* (KP283487.1) of Thailand with bootstrap support of 52 and grouped in clade I (Fig.55). This mushroom was reported from Ernakulam and Thiruvananthapuram district of Kerala, India (Manimohan *et al.*, 2004). *Lentinus polychrous* is one of the edible mushrooms in Northeast and Northern part of Thailand (Thanonkeo *et al.*, 2010). Thetsrimuang *et al.* (2011) showed cytotoxic effects of crude polysaccharide of *Lentinus polychrous* against the human breast adenocarcinoma cell line (MCF-7). *Lentinus polychrous* is a local edible mushroom in Thailand and traditionally used for the treatments of fever and inflammation due to snake or scorpion envenomation (Fangkrathok *et al.*, 2013).

4.2.3.4 WGM-38

The mushroom was documented in Dakshina Kannada district (latitude 12°43'45.23"N and longitude 75°33'08.98"E, elevation at 412 ft. from mean sea level) during the rainy season (August-September). Color of the mushroom is antique brass.

Smooth texture with presence of small hair like structure on cap funnel shaped cap which measures about 4-5 cm in diameter (Plate 41). Gills color is antique white. Stem is short, eccentric, hispid stipe presence of hair like structures. Spores are ellipsoid measuring 4-6 x 2-3.5 μm (Plate 54b). Fruiting bodies were grown in group of four to six fruiting bodies on dead wood.

The sequence data of ITS region (742bp) showed 97 % homology with the earlier reported *Lentinus strigosus* Fr. (KR824089.1) (Fig. 56). The phylogenetic tree showed that WGM-38 is closely related to *Lentinus strigosus* (KR824089.1) with bootstrap support 98 and grouped under clade II (Fig. 57). This mushroom was also documented in Waynad and Kannur district of Kerala, India.

Souza-fagundes *et al.* (2010) isolated hypnophilin and panepoxydone (terpenoids) from *Lentinus strigosus* and showed the differential cytotoxic effect of these terpenoids on the proliferation of human peripheral blood mononuclear cells (PBMC) stimulated by phytohemagglutinin (PHA) and on the immunophenotyping of normal PBMC. These compounds also had a significant antiparasitic effect against *Trypanosoma cruzi* amastigotes (AMA) and AMA-like forms of *Leishmania (Leishmania) amazonensis* and also reported that suggest that hypnophilin from *L. strigosus*, is a potential prototype for Chagas disease and leishmaniasis chemotherapy.

4.2.3.4 WGM-39

The mushroom (Plate 42) was collected from the Western Ghats of Hassan (latitude 12°57'02.77"N and longitude 75°48'59.90"E, altitude: 3044 ft.). Pileus is white and scales are present, medium texture. Gills are decurrent. Stipe position is at central to the Pileus, equal 4-6 x 3-5 cm. Spore print is white, 5 x 3 μm . mushroom grows gregariously in groups on decomposing wood, branches.

The sequence data showed 99 % homology with the earlier reported *Lentinus tigrinus* (KF860881.1) (Fig. 58). Phylogenetic tree showed that identified mushroom (WGM-39) closely related with *Lentinus tigrinus* (JQ428822.1) from Tamilnadu with bootstrap support 81 with clade III (Fig. 59). The acetonitrile extracts of *L. tigrinus* could

AGCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCATCCACTCTACACCTGTGCA
 CTTACTGTGGGCTTCAGGAGCTTCGAAAGCGAGAAGGGCCCCCTTGC GGGGTCA
 GTCTCGTCGTAGTAGTGA CTGGGCCACGTTTACTATAAACTCTTAAAAGTATC
 AGAATGTGTATTGCGATGTAACGCATCTATATACAACTTTCAGCAACGGATCTC
 TTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTG
 CAGAAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTC
 CGAGGAGCATGCCTGTTTGAGTGT CATGAAATCTCAACCTAACGGGTCTTAA
 CCGGACTTGCTTAGGCTTGGACTTGGAGGCTTTGTGGCTTGCTTATGTCGAGT
 CGGCTCCTCTCAAATGCATTAGCTTGGTTCCCTTTGCGGATCGGCTCACGGTGTG
 ATAATTGTCTACGCCGCGACCGTTGAAAGCGTTTAAATGGCCAGCTTCTAATCGT
 CTCTTGCGAGAGACCACTTTCCTCAATCTCTGACCTCATCTCGGGTAAGAATAC
 CCGGAGATCTTACATATATATATAAGGCGGAAGA

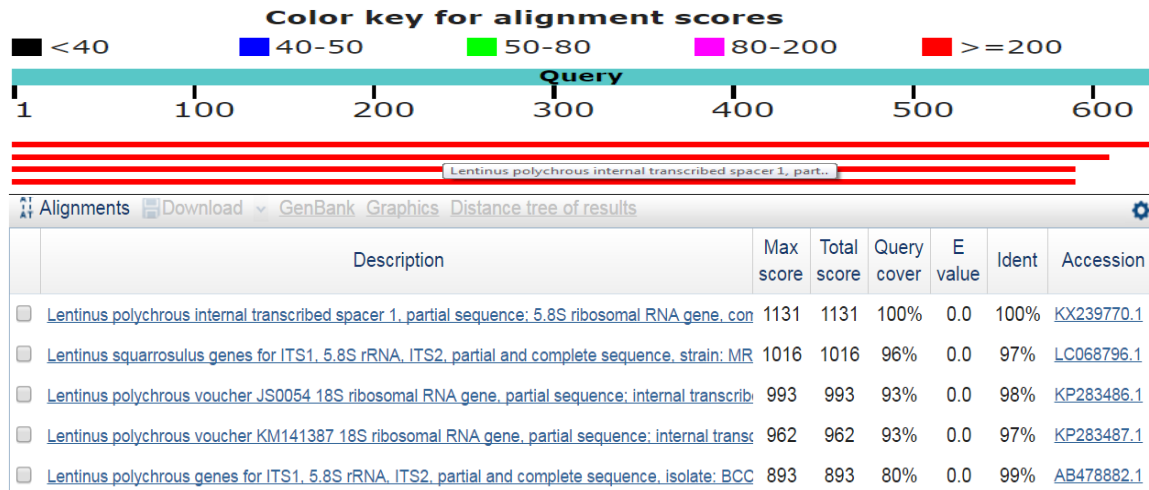


Fig. 54: Partial sequence length and homology search of WGM-37 (*Lentinus polychrous*)

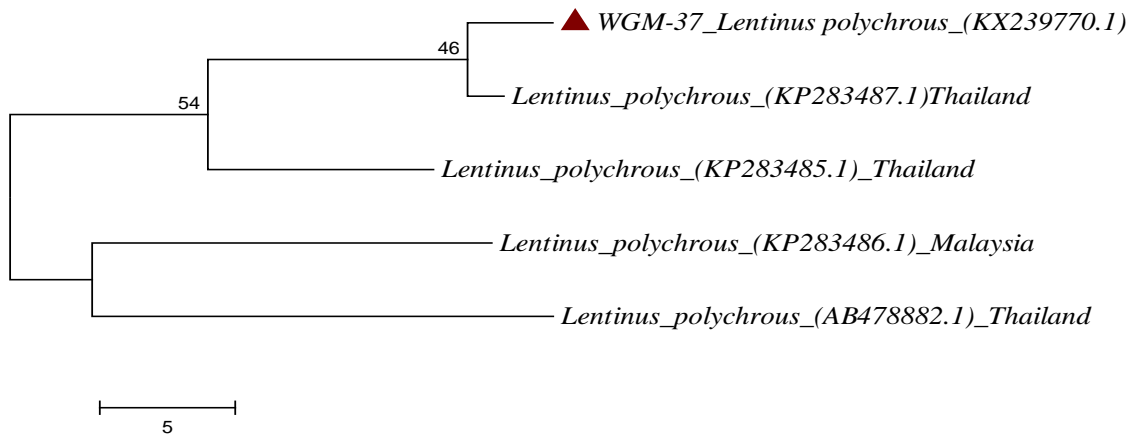


Fig. 55: Phylogenetic relationship between *Lentinus polychrous* (WGM-37) and other *Lentinus polychrous* species

AGGTTGTAGCTGGCCCTATCCGGGCATGTGCACGCCTTGCTCATTCCAATTCTT
 ACACCTCTGTGCACCTAACATGGGCTGGTTCGTAGGCTTTTGTCTTGCTTCACTG
 TGAGACGGGCTTTGACCTGCCTGTGGTTACTCTACAAACACTTTAAAGTATTAG
 AATGTAACATCGCGGATAATAAACGCATCTTATACAACCTTTCAGCAACGGATCT
 CTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATT
 GCAGAAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATT
 CCGAGGAGCATGCCTGTTTGTGAGTGTTCATGGTATTCTCAATTCTCTAAATTTTTGC
 GGATTTGGATGAATTGGATGTGGAGGTTTATTGCTGGCGACTATCCTTCTGGAT
 CTGTGTCCGGCTCCTCTGAAATAAATTAGCAGGAATGTTGCCGTGCCAACCTCA
 GTGTGATAATTTATCTGCGCTGTTGTTGCTCAGCAAAAATATATGTTCTTGCTTCTA
 ATCGTCTTCGGACAAATTTCTTGACATCTGACCTCAAATCAGGTAGGACTACCCG
 CTGAACTTAAGCATATCAATAAGCGGAGGAACTATACTGAATTTATGACAAGGT
 TGTAACTGGCCCTATCCGGCATGTGCACGCCTTGCTCATTCCAATTCTTACACC
 TCTGTGCACTTACATGAGCTGGTTCGTAAGCTTTTGTCTTGTTCCTGTGAGAGG
 GCTTTGACCTGCTGTGTTTACTCTCCACACTTTAAGTATAAAAAGGAACATCGG
 GAAAAAA

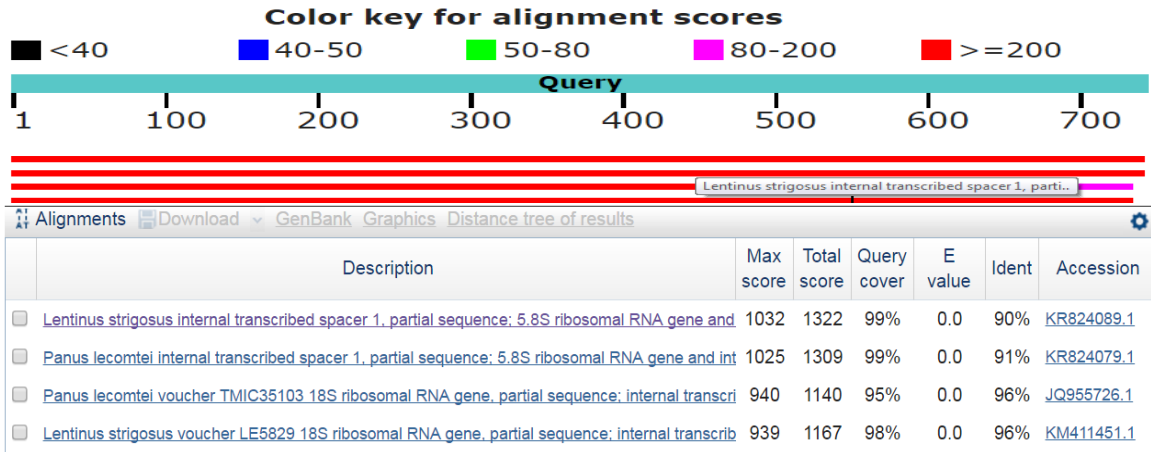


Fig. 56: Partial sequence length and homology search of WGM-38 (*Lentinus strigosus*)

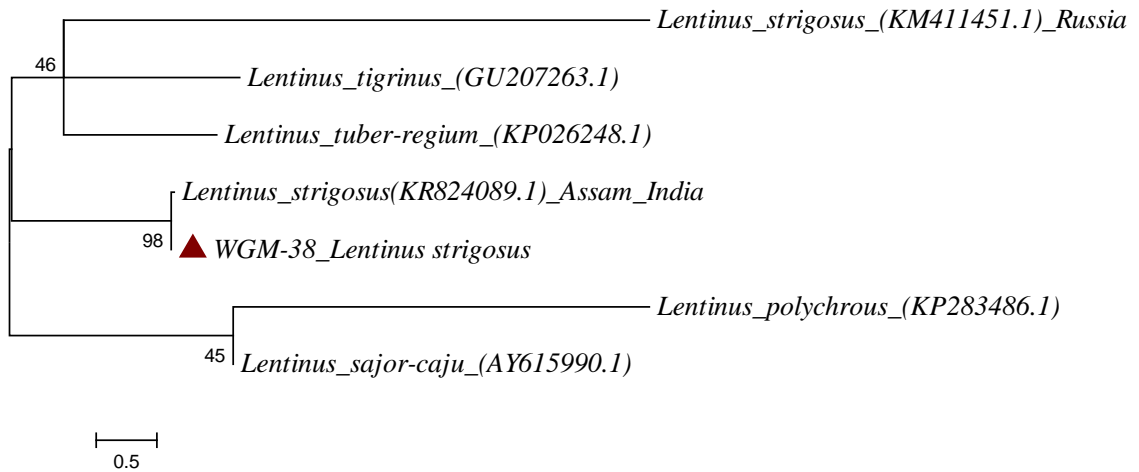
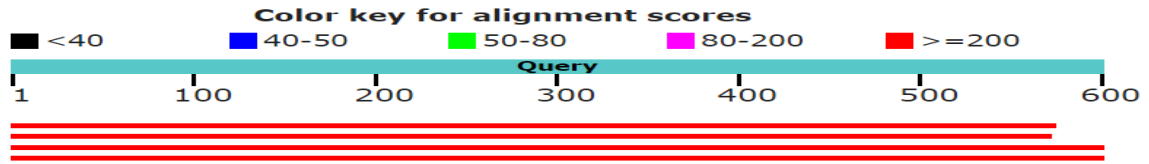


Fig. 57: Phylogenetic relationship between *Lentinus strigosus* (WGM-38) and other *Lentinus strigosus* species

GGGCAATTCGGGCTCTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACG
 CCCTGCTCATCCACTCTACACCTGTGCACTTACTGTGGGTTTCAGGAGCTTCGA
 AGGCGTTTCTTACGCTGGTAGTCGTGACTGGGCCCATGTTTACCACAAACACTT
 ATAAGTATCAGAAATGTGTATTGCGATGTAACGCATCCATATACAACTTTCAGCA
 ACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAA
 TGTGAATTGCAGAAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCC
 TTGGTATTCCGAGGAGCATGCCTGTTTGAAGTGTGATGAAATCTCAACCTAACG
 GGTTCTTAACGGGACTTGCTTAGGCTTGGACTTGGAGGCTTTGTGGCTTGCTT
 GTCGAGTCGGCTCCTCTCAAATGCATTAGCCTTGGTTCTTTGCGGATCTGGCTC
 ACGGTGTGATAATTGTCTACGCCGCGACCTTTGAAGCGTTTGAATGGACTGGCT
 TCTAATCGTCTCCTTGCAGACAAGCATTTCATCGAACTCTGACCTCAAATCAGG
 TAGTGGCCTGCAAGATAAAAAACCAGGAAAGGTTAAAAATCA



Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/> Lentinus tigrinus strain AP8 internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA gen	1020	1020	95%	0.0	99%	KF860881.1
<input type="checkbox"/> Lentinus glabratus strain TP7 internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA g	1014	1014	95%	0.0	99%	KF860882.1
<input type="checkbox"/> Lentinus tigrinus voucher MMRD-04 18S ribosomal RNA gene, partial sequence: internal transcribed	953	953	100%	0.0	96%	KC797150.1
<input type="checkbox"/> Lentinus tigrinus LE(BIN)0861 SBI 5 18S ribosomal RNA gene, partial sequence: internal transcribec	944	944	100%	0.0	95%	AF516518.1

Fig. 58: Partial sequence length and homology search of WGM-39 (*Lentinus tigrinus*)

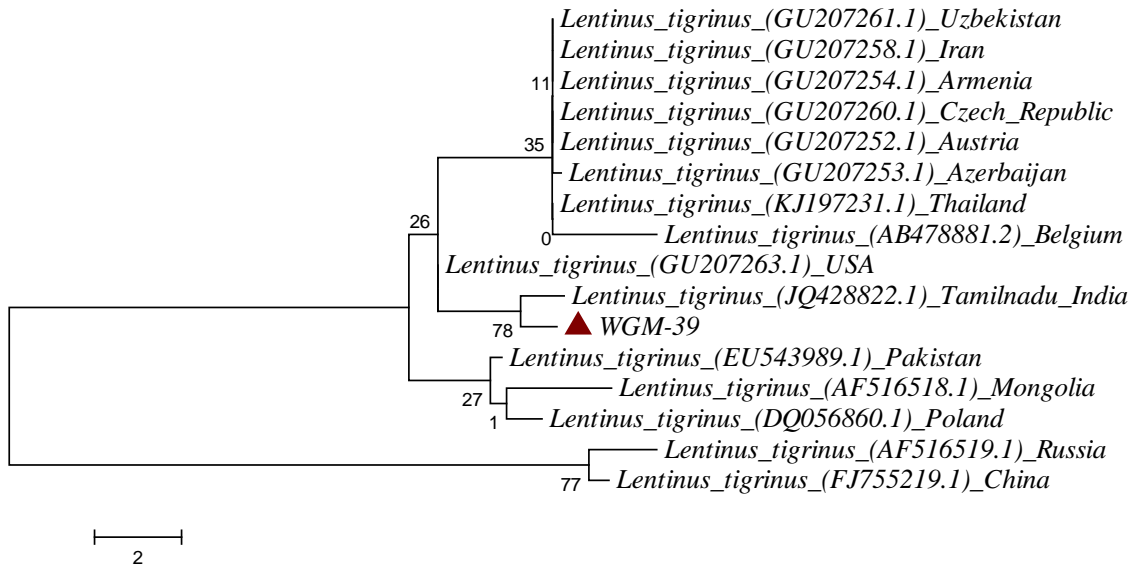


Fig. 59: Phylogenetic relationship between *Lentinus tigrinus* (WGM-39) and other *Lentinus tigrinus* species

be valuable resource of antioxidant and anti-bacterial (*Staphylococcus aureus*) compounds (Dulay *et al.*, 2017).

4.2.3.5 WGM-40

The mushroom was collected from Chikkamagaluru (latitude 13°28'11.74"N and longitude 75°16'39.06"E, at an altitude of 2522 ft.). Basidiocarp grows gregariously in group on hard wood during August to September. Stipe is central to eccentric, 2.5-5 cm × 1.7-2.5 cm, slightly tapering downwards, solid, hard, woody velvety, pale yellow (Plate 40). Spores measures 6.5 - 8.5 × 3 - 3.5 µm.

The sequence data showed 100 % homology with the earlier reported *Lentinus tuberregium* (GQ292711.1) (Fig. 60). Phylogenetic tree showed that identified mushroom (WGM-40) closely related with *Lentinus tuberregium* (KP325386.1) from Nigeria, *Lentinus tuberregium* (EU908197.1) from Germany, *Lentinus tuberregium* (AB115045.1) and *Lentinus tuberregium* (KP734199.1) from Sri Lanka with clade I (Fig. 61). In Rupandehi District of Nepal this species was used for edible but not regularly (Aryal and Budathoki, 2013). This mushroom also had been utilized as edible by Aeta community in Central Luzon, Philippines (Leon *et al.*, 2013). Kaya and Bag (2010) reported that this species was growing on *Populus* sp. in Adiyaman region of Turkey. *Lentinus tuberregium* is characterized by large robust pale yellow to yellow hard basidiocarp with stout stipe arising from sclerotia and moreover the context is thin at margin and thick at centre. The spores are cylindrical and hyaline. This species was usually found on the mango tree substrate in Tamilnadu, India (Kumar and Kaviyarasan, 2012).

4.2.3.6 WGM-41

The fruiting body exactly looks like goblet glass (Plate 44) which helps in holding of rainwater which maintains the temperature and growth of the fruiting body. This mushroom is documented from Dakshina Kannada district (latitude 12°43'44.03"N and longitude 75°33'07.48"E at an elevation of 392ft from mean sea level). It can be seen during July and August in this region. The mushroom color is pale red-violet, medium. Gills color is pale pink and decurrent and gill mycelium is shown on Plate 54c. Stalk is in

4-5 cm diameter and 6 cm long positioned at center of the head of the mushroom, hair like structure are present on the stipe, hard texture (Plate 54d). Mushroom grows in a group of 4-5 fruiting bodies on decaying wood.

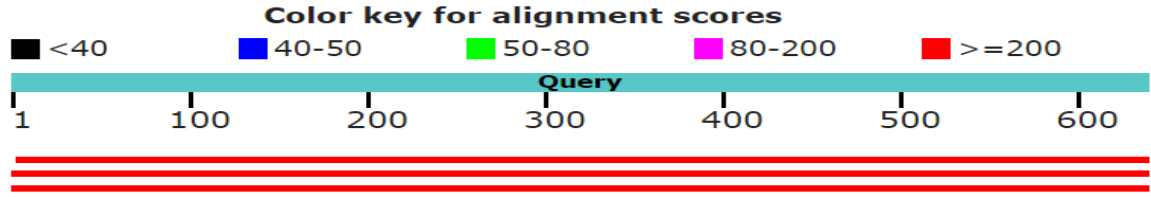
The sequence data (627bp) revealed 99 % homology with the earlier reported *Lentinus velutinus* Fr. (GQ849478.1) (Fig. 62). The phylogenetic tree showed that WGM-41 is closely related to *Lentinus velutinus* of Tamilnadu with bootstrap support 57 and grouped under clade IV (Fig. 63). Bolhassan *et al.* (2012) recorded this species in Langkawi region of peninsular Malaysia.

4.2.3.7 WGM-42

This mushroom is edible and considered as delicacy, smooth texture and attracts insects. Local people were collect this delicious mushroom in early hours of the day to avoid or minimize the insects contamination in their food. This mushroom was documented in Western Ghats of Hassan district (latitude 12°57'45.36"N and longitude 75°49'34.27"E, at an altitude of 3081ft mean sea level) during the monsoon season (Plate 47). Cap is Conic to flat and slightly umbonate with a peach-white color on the surface measures about 8-11 cm in diameter, fleshy. This mushroom produces long-stalk about 7 cm from the ground level with the thickness of 1.6 cm near the cap. Stem is creamy-white, somewhat tough, bulbous base, solid above ground, hollow below the soil, penetrating the soil to some distance, with a persistent apical annulus. Stipe is slightly tapering upward and positioned at center of the pileus and presence of rhizoids as well. Pseudorrhiza is about 6-11 × 1.5 cm, dark brown in colour, solid, cylindrical with a cartilaginous and widening at certain depth. Gills are free which is not attached to the stalk. Basidia is clavate, 4-spored, 11.66 × 6.9 µm, Cheilocystidia is usually napiform, Spore print is peach colour, Spores are large, hyaline, ellipsoid, smooth, non-amyloid, thin-walled, 6.0-8.0 × 4.0-5.0 µm. This mushroom was appeared solitary in grass land near the lake

The sequence data (659bp) showed 98 % homology with the earlier reported *Termitomyces eurhizus* Berk. (KC414235.1, KJ620056.1 and AB073529.1) (Fig.64). the identified *Termitomyces eurhizus* was closely related to mushroom from Republic of the

CTTGAGCTGTTGCTGGCCTCTAGGGGCATGTGCACGCTTCATCAGTCCATTCAA
 CCACCTGTGCACTTTTTGTAGATCA TTGAAGTCATCTCTCAGGTCATTAGTGACT
 TGGATGTCGGGAGGTTCTTATACCTTCTGGCTGACTCTCAGTGATCTAATTTAC
 ACACCCTAATGTATGTTAATGAATGTCGTTTATCTTTGGGCCATGTGCCTATAAA
 CCTAATAACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGC
 AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
 TGAACGCACCTTGCGCCCTTGGTATTCGAGGGGCATGCCTGTTTGAGTGTC
 TAAAATCTCAACCTATAAAGGCTTTTGTGAAGTCATTATAGGCTTGGATTGTT
 GGGGGCTGCTGGCTTGTGAGAGTCAGCTCTCCTTAAATGCATTAGCAGGACTCT
 ATTGCCTCTGCGCATGGTGTGATAATTATCTACACCAGTTGCATGTAATACTATA
 ATGTGTCCAGCTCTCTAA TCGTCTTCGGACAATTATTGAACATTTGACCTCAAAT
 CAGGTAGGACTACCCGCTGAACCTTAAGCATATCAATAAGCG



Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/> Lentinus tuber-regium isolate VKMK01 internal transcribed spacer 1, partial sequence: 5.8S ribo	1150	1150	99%	0.0	100%	GQ292711.1
<input type="checkbox"/> Lentinus tuber-regium voucher UOC-DAMIA-D18 18S ribosomal RNA gene, partial sequence: in	1148	1148	100%	0.0	99%	KP734199.1
<input type="checkbox"/> Pleurotus tuberregium 18S ribosomal RNA gene, partial sequence: internal transcribed spacer 1	1144	1144	100%	0.0	99%	AY450344.1
<input type="checkbox"/> Pleurotus sp. HT04 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complet	1142	1142	100%	0.0	99%	AB971704.1

Fig. 60: Partial sequence length and homology search of WGM-40 (*Lentinus tuberregium*)

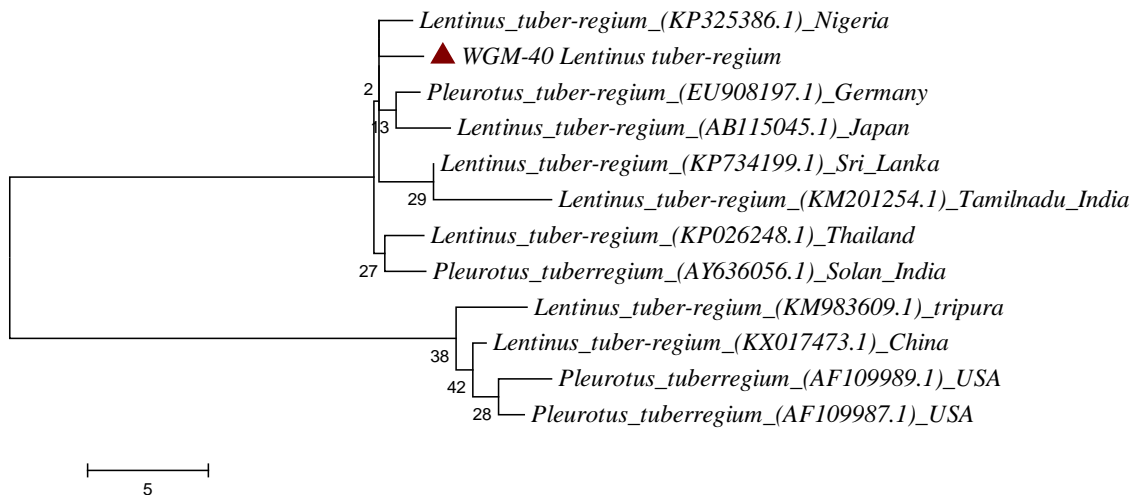


Fig. 61: Phylogenetic relationship between *Lentinus tuberregium* (WGM-40) and other *Lentinus tuberregium* species



Plate 44: WGM-41 (*Lentinus velutinus*)



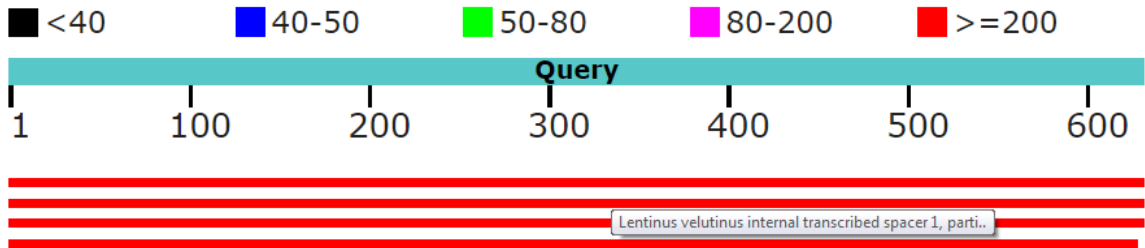
Plate 45: WGM-42 (*Termitomyces eurrhizus*)



Plate 46: WGM-43 (*Termitomyces* sp.)

TGTAGCTGGCCCTATCCGGGCATGTGCACGCCTTGCTCATTCCAATTCTACACCTCTGTG
 CACTTAACATGGGTTTGGTTGTTGCCTGGTTGCCCGCATATGGGGGTGACTGAGCTTTG
 ACCCTGCCTGTGGTTCTCTACAAACACATCTATAGTATCAGAATGTAAACAGCGTATTTA
 TAACGCATCTTATACAACCTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACG
 CAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAAC
 GCACCTTGCCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGTGATGTCATGGTATTCTCA
 ATTCTCTAAATCTTTGCGGATTTGGATGGATTGGATGTGGAGGTGATTGCTGGCATCCAT
 GCCAATGGCGTGCCGGCTCCTCTGAAATACATTAGCAGGAAATTGCTTTGTCAACCTCA
 GTGTGATAATTGTCTACGCTGTTGTTGCATTGCAAAAACCTTTATGTTTCTGCTTCTAAT
 CGTCCTCGGACAATTTCTTGACATCTGACCTCAAATCAGGTAGGACTACCCGCTGAACTT
 AAGCATATCAATAAGCGGAGGAAAGAAA

Color key for alignment scores



Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/> Lentinus velutinus 18S ribosomal RNA gene, partial sequence: internal transcribed spacer 1, 5.8	1136	1136	100%	0.0	99%	GQ849478.1
<input type="checkbox"/> Panus similis voucher UOC SIGWI S38 18S ribosomal RNA gene, partial sequence: internal tran	1048	1048	100%	0.0	97%	KR818820.1
<input type="checkbox"/> Panus sp. MEL 2382698 internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA ge	1020	1020	100%	0.0	96%	KP012877.1
<input type="checkbox"/> Panus sp. MEL 2382967 internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA ge	998	998	100%	0.0	96%	KP012827.1

Fig. 62: Partial sequence length and homology search of WGM-41 (*Lentinus velutinus*)

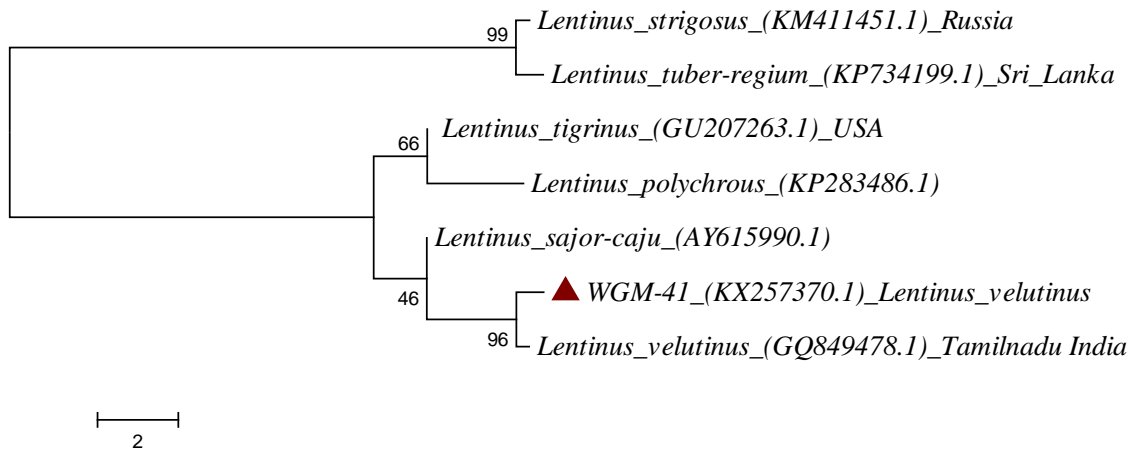
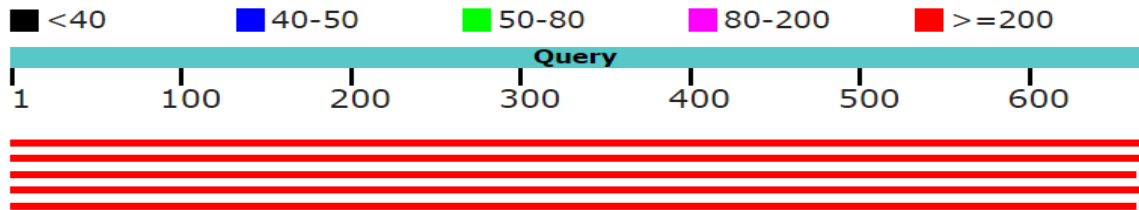


Fig. 63: Phylogenetic relationship between *Lentinus velutinus* (WGM-41) and other *Lentinus velutinus* species

TTGTTGCTGGCCTCTAGAGGCATGTGCACGCCTGCCATCGTTTTCAACCACCTG
 TGCACCTTTTGTAGACTTTTGGATACCTTTCCGGGGGGTCAAACCTCCCGGCTTC
 GAGGGCTGCCGCTTGGCTTTCCTCGCATTTCCGGTCTATGCATTTTCATACACTC
 CGTAAAGAATGTATTAGAATGTCTTGTATTGGCCTCAGTGCCTTTAATCAAATA
 CAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAA
 TGCGATAAGTAATGTGAATTGCAGAAATTCAGTGAATCATCGAATCTTTGAACGC
 ACCTTGCCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGTGAGTGTCAATTAATT
 CTCAACCTAACCAGCTTTTGTGAGCTTGGGATAGGTTTGGATTGTGGGGGTTTC
 TTGCGGGCTTCATCTAGAGGTCGGCTCCCCCTTAAAAGCATTAGTGGAAACCTTTT
 GTTGACCTGTTTCTGGTGTGATAATTATCTACATCGTGTGCAGTCAGCTATCA
 ACAGGTTTCTGCTTCCAACCTCGTATCACCCCTTTACTTGGGGGACGCTTTGACC
 ATTTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAATAAGC
 CGGAGGAAAA

Color key for alignment scores



Alignments							
Download GenBank Graphics Distance tree of results							
	Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/>	Termitomyces eurhizus isolate D13 internal transcribed spacer 1, partial sequence: 5.8S ribosomal F	1124	1124	99%	0.0	98%	KC414235.1
<input type="checkbox"/>	Uncultured Agaricales clone Ter8 18S ribosomal RNA gene, partial sequence: internal transcribed sp	1124	1124	99%	0.0	98%	JN000931.1
<input type="checkbox"/>	Termitomyces eurhizus strain BA2 internal transcribed spacer 1, partial sequence: 5.8S ribosomal R	1121	1121	99%	0.0	98%	KJ620056.1
<input type="checkbox"/>	Termitomyces sp. Group8 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and compl	1131	1131	100%	0.0	98%	AB073529.1

Fig. 64: Partial sequence length and homology search of WGM-42 (*Termitomyces eurhizus*)

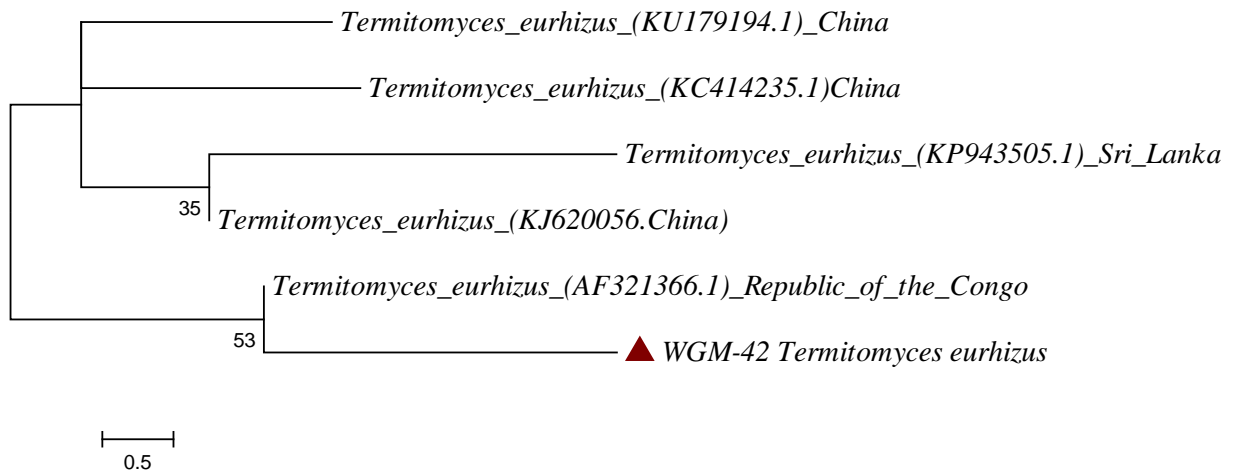


Fig. 65: Phylogenetic relationship between *Termitomyces eurhizus* (WGM-42) and other *Termitomyces eurhizus* species

Congo (Fig. 65). Tanti *et al.* (2011) reported that *Termitomyces eurhizus* was been collected by tribes of Nagaland during the rainy season for their consumption and marketing as well. Usha and Janardhana (2014) reported the *Termitomyces eurhizus* from Kodagu. Giri *et al.* (2012) reported this edible mushroom species in the hilly regions of West Bengal. The reason for the higher market price of *Termitomyces eurhizus* was due to its delicious taste, the demand, and its lesser availability in the forest.

Boonyanuphap and Hansawasdi (2011) documented this species in subtropical dry forests of Thailand. Aryal and Budathoki (2016) reported the occurrence of *Termitomyces eurhizeus* in Nepal and it has been used as edible and a lotion for skin diseases with mixing the herb (*Cynodon doctylon*) as well.

4.2.3.8 WGM-43

This mushroom is edible and considered as delicacy, smooth texture and attracts insects. This mushroom was documented from Nettana (latitude 12°43'46.81"N and longitude 75°32'58.47"E, at an altitude of 436ft mean sea level) during the monsoon season. Termites are consuming this mushroom from the ground (Plate 46). Pileus is white and light peach colour with flat and slightly umbonate at the middle which is grey colour, basidiocarp measures about 10- 12.5 cm in diameter, fleshy. Long-stalk which is 7-10 cm above the ground level and thickness is 2.5 cm. Stipe is white, somewhat tough, penetrating the soil to some distance, with a persistent apical annulus. Stipe is cylindrical and positioned at center of the pileus and presence of rhizoids as well which is tapering down and is about 12-18 cm, dark brown in colour, solid, cylindrical. Gills are free which is not attached to the stalk Basidia is clavate, 4-spored, 10-12 × 6-7 µm, Cheilocystidia is napiform, Spore print is light peach colour; Spores are large, hyaline, ellipsoid, smooth, non-amyloid, thin-walled, 6.0-8.0 × 4.0 µm. This species grows solitary in soil of mixed forest.

The sequence data showed 99 % homology with the earlier reported *Termitomyces* sp. (KC848201.1, KX646696.1 and GU001671.1) (Fig. 66). Phylogenetic tree showed that identified mushroom (WGM-43) is closely related with *Termitomyces aurantiacus* (GU594650.1) with clade II (Fig. 67). *Termitomyces* sp. is edible mushroom

which is a good source of protein. *Termitomyces* symbionts and the fungus-farming termites have an exceptional relationship which benefits of both the organisms. However, the existing problem of classifying fungal species of the genus *Termitomyces* remains unresolved (Kuja *et al.* 2014). Makonde *et al.* (2013) observed the more than one *Termitomyces* strains per mound, since mounds can contain different termite colonies each fungal strain is farming by different colonies of termites. Apart from the food serving to the termites, *Termitomyces* also helps in maintaining the humidity of the colony (Dayal *et al.*, 1965).

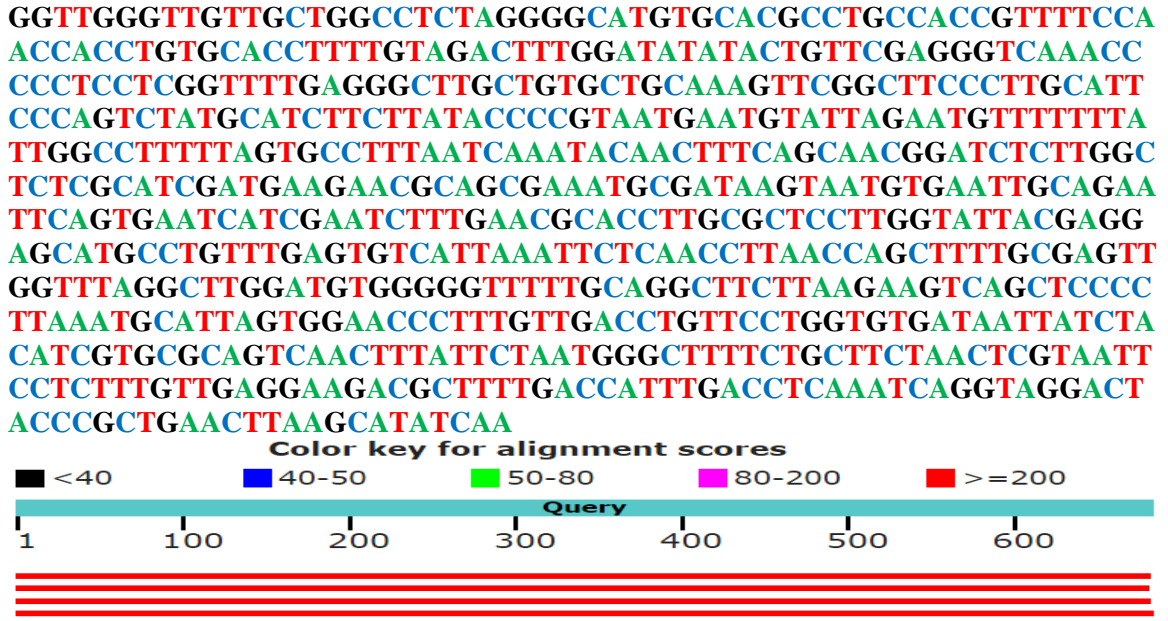
4.2.3.9 WGM-44

This species is one of the largest edible mushrooms documented in the Western Ghats region of Sakleshpur (latitude 12°56'11.52"N and longitude 75°48'38.34"E). Local peoples were collecting these mushrooms during the early hours of the day in monsoon as it appears (Plate 47). This particular species is very much delicious to make samber out of it. Cap of the mushroom measures about 23 cm in diameter. Stalk is measures about 18 cm X 3-4 cm of from the ground level. Spore print is cremish white. Mushroom grows solitary on soil near termite mount in coffee plantation during August to September

The sequence data showed 97 % homology with the earlier reported *Termitomyces* sp. (AB073529.1) (Fig.68). The phylogenetic tree showed that the WGM-44 is close to *Termitomyces aurantiacus* and *Termitomyces eurhizus* which are grouped into clade I (Fig.69). Oyetayo (2011) collected the *Termitomyces* species from Ondo and Ekiti states and they constructed the phylogenetic tree based on ITS region which confirms that the mushrooms were more related to African species.

4.2.3.10 WGM-45

This mushroom was grown gregariously in group on fallen tree at initial stage of decomposition. It is an edible mushroom but consists of many insect larvae between the gills of Baidiocarp. It was appeared after the heavy rain in the mouth of August and September in S Sakleshpur of Hassan district (latitude 12°55'14.01"N and longitude 75°45'15.46"E). Pileus is brownish white color with convex in shape and measured 5-11



Sequences producing significant alignments:

Select: All None Selected:0

	Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/>	Termitomyces sp. 22T4 internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA gene, c	1209	1209	99%	0.0	99%	KC848201.1
<input type="checkbox"/>	Termitomyces sp. 22T3 internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA gene, c	1207	1207	99%	0.0	99%	KC848200.1
<input type="checkbox"/>	Termitomyces sp. isolate 93T4 18S ribosomal RNA gene, partial sequence: internal transcribed spac	1202	1202	99%	0.0	99%	KX646696.1
<input type="checkbox"/>	Termitomyces sp. 1021 18S ribosomal RNA gene, partial sequence: internal transcribed spacer 1, 5:	1195	1195	100%	0.0	99%	GU001671.1

Fig. 66: Partial sequence length and homology search of WGM-43 (*Termitomyces* sp.)

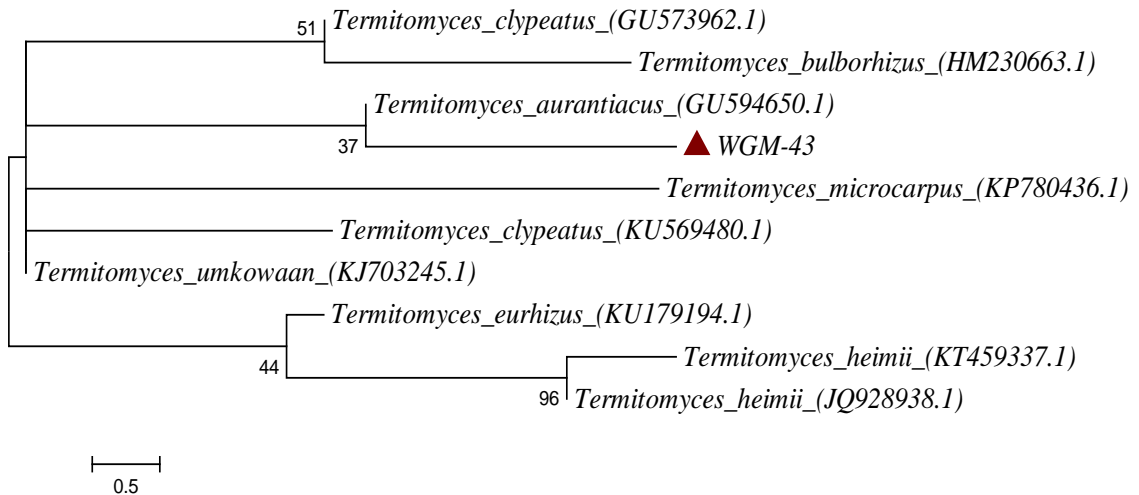


Fig. 67: Phylogenetic relationship between *Termitomyces* sp. (WGM-43) and other *Termitomyces* species

cm, Smooth Texture (Plate 09). Gills are white in colour; gills are decurrently to the stipe. Stipe is tapering downwards, length 2-3 cm and thickness 2.3 cm, position of the stalk is eccentric and annulus is absent.

The sequence data showed 94 % homology with the earlier reported *Pleurotus pulmonarius*. (KJ865840.1) (Fig. 70). Phylogenetic tree showed that WGM-45 showed closely related to South Korean *Pleurotus pulmonarius* (KR673648.1) with boot strap support 48 and grouped under clade V (Fig. 71). Smiderle *et al.* (2008) showed anti-inflammatory and analgesic properties of a (1→3), (1→6)-linked β -glucan isolated from *Pleurotus pulmonarius*. Oral administration of *Pleurotus pulmonarius* two week in advance could markedly inhibit the incidence and size of tumor (Huh7 liver cancer cells) with an inhibition rate of 93.1 % in nude mice and also this mushroom can be used as an adjuvant in the chemotherapy of liver cancer (Xu *et al.*, 2014).

4.2.3.11 WGM-46

This mushroom was collected from Kokkada village in Dakshina Kannada district (latitude 12°49'36.43"N and longitude 75°25'26.40"E, altitude: 411ft.) which grows in a group on dead tree. Pileus is convex at first, becoming broadly convex or nearly flat, tacky when fresh but soon dry; bald when young, becoming finely scaly over the center; 5-14 cm in diameter; whitish and very pale brown at center (Plate 26). Gills are free not attached to stalk, close or crowded, short-gills frequent, white, cystidia are long. Stem is 3.5-12 cm long, 1-1.6 cm thick, more or less equal and with a slightly enlarged base, finely fibrillose, discoloring brownish white to brown below; basal mycelium is white. Spore print is peach in color and spores measures 5-7 x 3.5-5 μ m in ellipsoid structure. It is one of the edible mushrooms. The sequence data showed 100 % homology with the earlier reported *Pluteus petasatus* Fr. (Fig. 72). Phylogenetic tree showed that WGM-46 very closely related to *Pluteus petasatus* (KJ009730.1) of Papua New Guinea with bootstrap support 34 and grouped in clade III (Fig. 73).

4.2.3.12 WGM-47

This mushroom was documented in the Nelyadi of Dakshina Kannada District (latitude 12°49'32.53"N and longitude 75°25'31.45"E, Altitude: 436 ft.). Fruiting body is without ring and volva, small to medium, convex grows on well decayed wood (Plate 39). Pileus is convex at first and then become plane, greyish brown colour at center and lighter towards margin and measured about 5-6 cm in diameter. Gills are light orange, crowded, free. Stipe is white and greyish brown in lower parts. Spore print is light brown; spores are broadly ellipsoid, 6-8 × 4-5 µm. this species was grown on well decaying wood in a group.

The sequence data showed 93 % homology with the earlier reported *Pluteus spinulosus* (KJ009777.1) (Fig. 74). Phylogenetic tree showed that identified *Pluteus spinulosus* (WGM-48) closely related with *Pluteus spinulosus* (KR261096.1) from Bolivia with bootstrap support 35 and grouped with clade V (Fig. 75). *Pluteus spinulosus* is recognized by its dark gill edge together with presence of dimorphic facial cystidia and clam connections (Lee *et al.*, 1992). This mushroom was also recorded from Bolovia (Justo *et al.* 2014).

4.2.3.13 WGM-48

Large sized mushroom with a long stalk (Plate 24) and found in Dakshina Kannada (latitude 12°49'31.48"N and longitude 75°25'28.55" E, altitude 444 ft.). Pileus is ovoid to hemispherical when young, becoming convex to plano-convex with age cap is umbonate (convex with bump at center), white to peach colour with presence of light brown colour scales and the bump like structure at the center of the cap which is light brown in colour, diameter of the cap is 10 cm. Lamellae is free, moderately crowded, white when young, white to cream colored when mature, up to 1 cm in height, thin, with lamellae, sometimes with brown spots on the lamellae. Stipe is cylindrical and measures 18 cm from the ground and 2 cm width, peach color, Annulus is present and is cottony like texture. Annulus is ascending, whitish, membranous, complex, big, with brownish patchy squamules on the underside, movable when mature. Spore print (Plate 53a) is



Plate 47: WGM-44 (*Termitomyces* sp.)



Plate 48: WGM-45 (*Pleurotus pulmonarius*)



Plate 46: WGM-46 (*Pluteus petasatus*)



Plate 50: WGM-47 (*Pluteus spinulosus*)

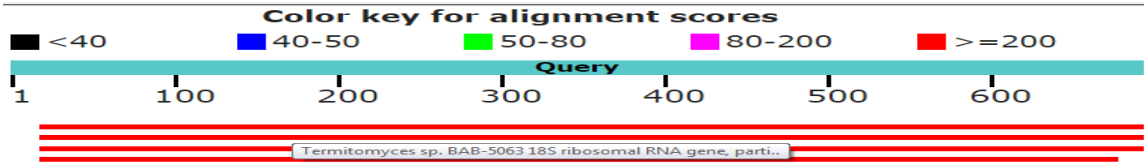


Plate 51: WGM-48 (*Macrolepiota detersa*)



Plate 52: WGM-49 (*Tricholoma giganteum*)

GGAAAGGATTAGCTGGGTCCGGGTTGTTGCTGGCCTCAAGGGGCAGGGGCACGC
CGGCATTTCGTTTCAACCCCCTGTGCACCTTTTGGAAACTTTTGGAAATACCTTCC
GGGGGTCCACCCTCCCGGTTCCAAGGCTGGCCGTTGGGTTTTCCTTCGAAATTTCC
GGTTTAGGCTTTTCA TACCCTCCGAAAAGATTCGTATTA AAAAGGCCCTGGTAATG
GGCCTCAGGGCCTTTATCCAATAACACTTTTCGGCACCGAACCCCTGGGTTCTC
GTTCAATGAAAACCCACCAAATGGCAAAAAGAATGGGGATTGGCAAAAATTC
GGGAATCTTCAAACCTTTGAACGCCCTTGCCTCCTGGGATTCCCAAGAAGCA
TGCCTGTTTGAGGGCCTTAAAATCCCCACCCTACCCAGTTTGGGAACCTGGGA
TAGGCTTGGATTGTGTGGGGTTTCTTTGCGGGCTTCATCTCAGAGGTCCGGCTCC
CCTAAAAGCATTAGTGGAACTTCTGCTCGACCTGTTTCTTGGTGTGATAATT
ATCTACATCGTGTGCAGTCAGCTA TCTAACAGGTTTCTGCTTCCAACTTGTATC
ACCCTTTTTTACTTGGGGGGGAACGCTTTGACCAATTTGACCTCAAATCAGGTTAG
GACTACCCGCTGAACTTAAGCATATCATTAAAGCGGAGGAAA



Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Termitomyces sp. Group8 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence	678	678	97%	0.0	85%	AB073529.1
Uncultured Termitomyces clone ZZ-Z70 18S ribosomal RNA gene, partial sequence, and ITS1, 5.8S ribosomal RNA	664	664	97%	0.0	85%	KF302106.1
Uncultured Termitomyces clone ZZ-Z71 18S ribosomal RNA gene, partial sequence, ITS1 and 5.8S ribosomal RNA	664	664	97%	0.0	85%	KF302104.1
Termitomyces eurhizus strain BA2 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and ir	658	658	99%	0.0	84%	KJ620056.1

Fig. 68: Partial sequence length and homology search of WGM-44 (*Termitomyces* sp.)

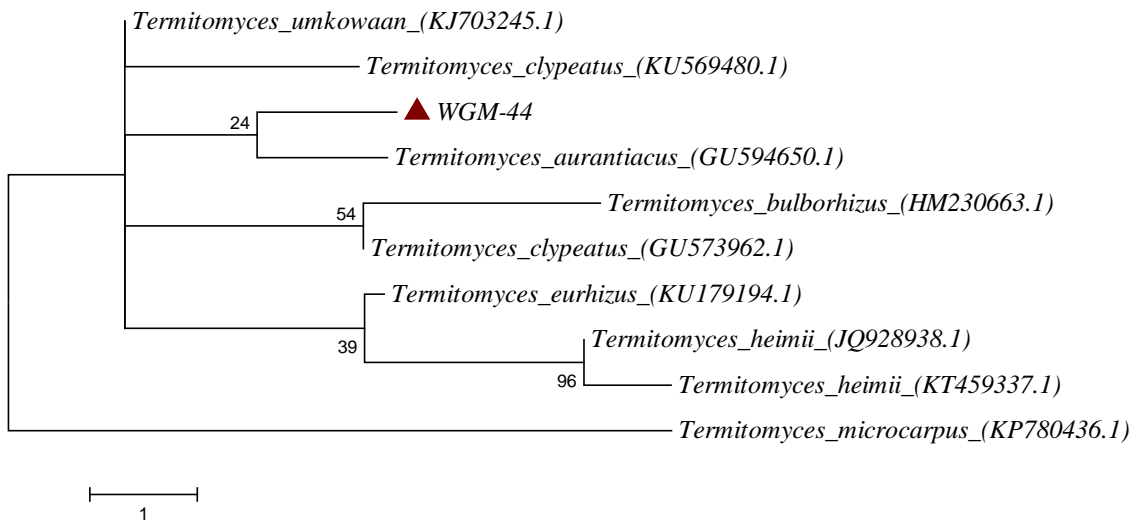


Fig. 69: Phylogenetic relationship between *Termitomyces* sp. (WGM-44) and other *Termitomyces* sp. species

CTAGACTTTCAACCACCTGTGAACTTTTTTATAGATCTGTGAAGTCGCCTTCAAG
 TCGTCAGACTTGGTTTGCTGGGATTTAACGTCTCGGGTGACAACGCAGTCTATT
 TACTTAACACACCCCAAATGGTGTCTACTAATGAATATAATGGGTCTTGTGCCT
 AATAACCATAATACAACCTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAA
 TAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCATGAATCATCGA
 ATCTTTGAACGCACCTTGCGCCCTTGGTATCCGAGGGGCATGCCTGTTGAGT
 GTCATTAAAATTCTCAAACCTCACATTTATTTGAGATGTTTGGATTGTTGGGGTTG
 CTGGTTTTAACAGTCGGTCCCTCTTAAATGCATTATCAGGACTTCTCATTGCCTC
 TGCGCATGATGTGAAACTCTCACTCATCAATTCACGCATGAATAGAGCCAGCTC
 TCTATCGCCGCAAGGACAATTTGACATTTGACCTCAAACAGGAGGACTACCCGC
 TGAATTAGCATATCAATAACGGAGGA

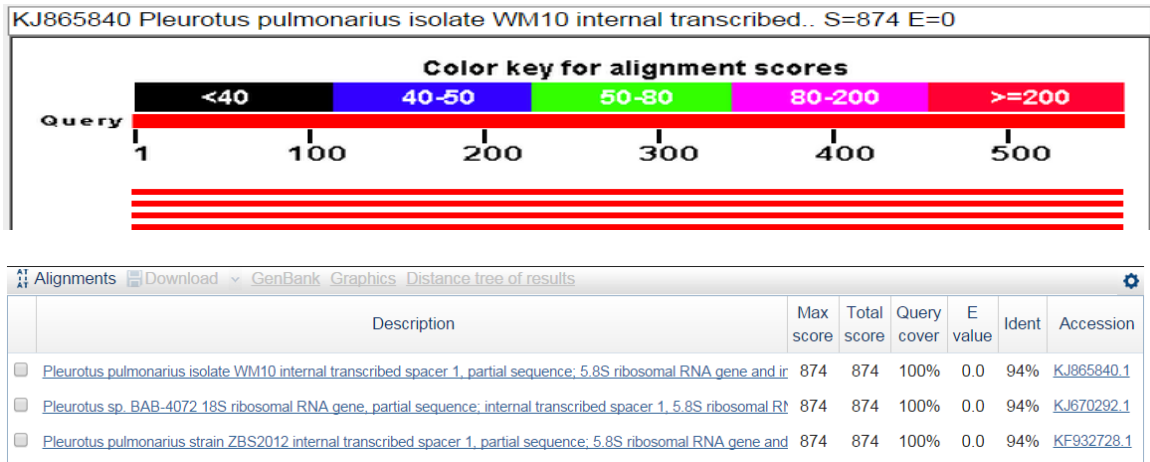


Fig. 70: Partial sequence length and homology search of WGM-45 (*Pleurotus pulmonarius*)

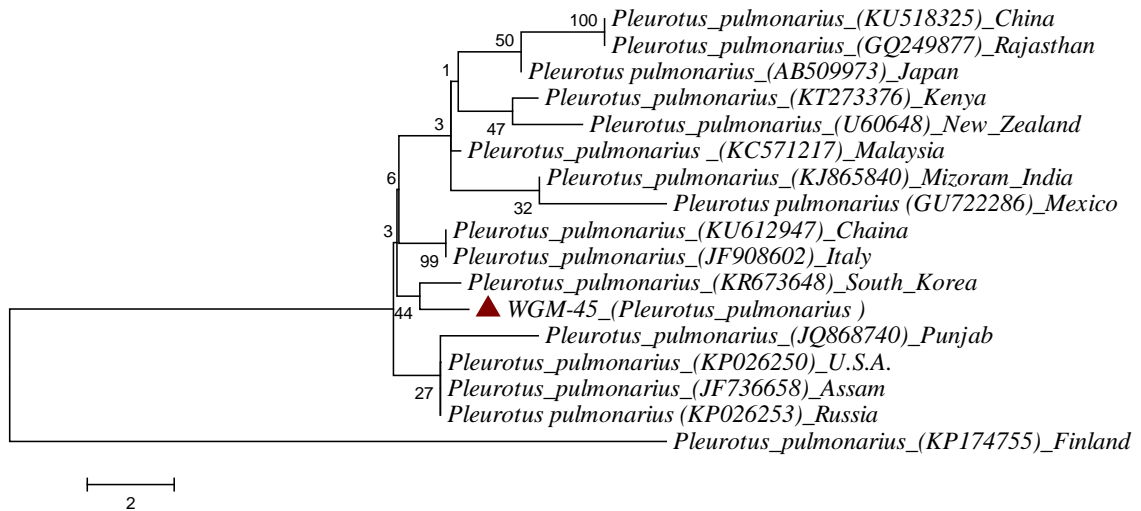
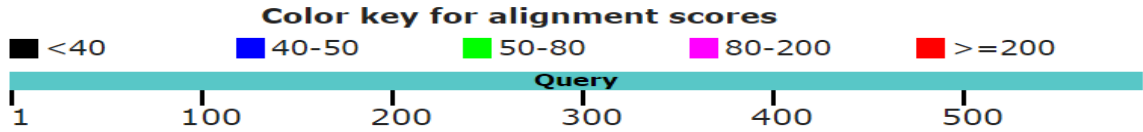


Fig. 71: Phylogenetic relationship between *Pleurotus pulmonarius* (WGM-45) and other *Pleurotus pulmonarius* species

ATGTCAGATGACAGTTGGAAGCAGTTGTGCACAATAAGTACAATGTATAGATAT
ATATCATACAAAGCACAGATGGCATAATATCCTGCTAATGTTTTTAAGGAGAGC
TGAACAAGCCAGCAAGCCCCAAAATCCAATCACCCAGCAACACAAGATTGTGG
GGATTGAGAAATTAATGACACTCAAACAGGCATGCTCCTCGGAATGCCAAGGAG
CGCAAGGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTAC
TTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGTT
GAAAGTTGTATATGTTTTAAAGGCTAAAAGCCCAAAGCACATTCTATTACATTCA
TATGGTGTGATATAAAACATAGACCTGCATACCCAAGAAGAGCTTGTCCAATG
GAACAAGCAACTCCTCTCAACCAAAGCCTAGTAAAAGACTTGAAAATAGCATACA
GATCTACAGATAGTGCACAGGTGGGAAGATAGAATGAAACATTGGCAGGCCTG
CACATACCGAGAAGGCCAGCAACCTGCACCTTGTTTATTTACTAATGATCCTT
CCGCAGGTTCACCCTACGGAAGT



Select: [All](#) [None](#) Selected: 0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/> Pluteus petasatus isolate P65 18S ribosomal RNA gene, partial sequence: internal transcribed spacer	1125	1125	98%	0.0	99%	KF306019.1
<input type="checkbox"/> Pluteus cervinus isolate P57 18S ribosomal RNA gene, partial sequence: internal transcribed spacer	1125	1125	98%	0.0	99%	KF306014.1
<input type="checkbox"/> Pluteus petasatus voucher Ammiranti3103 18S ribosomal RNA gene, partial sequence: internal transcribed spacer	1125	1125	98%	0.0	99%	HM562084.1
<input type="checkbox"/> Pluteus petasatus voucher MuellerStrack1 18S ribosomal RNA gene, partial sequence: internal transcribed spacer	1125	1125	98%	0.0	99%	HM562070.1
<input type="checkbox"/> Pluteus petasatus voucher AJ143 18S ribosomal RNA gene, partial sequence: internal transcribed spacer	1125	1125	98%	0.0	99%	HM562065.1

Fig. 72: Partial sequence length and homology search of WGM-46 (*Pluteus petasatus*)

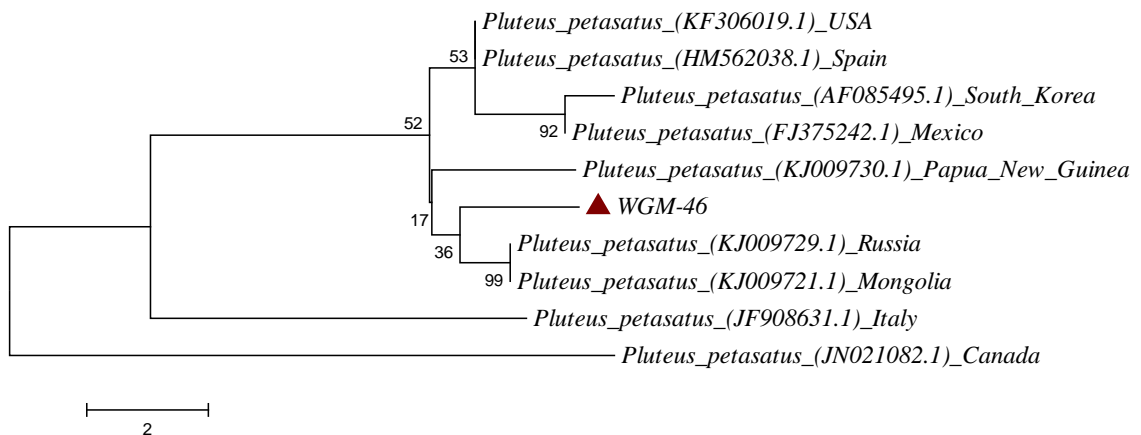
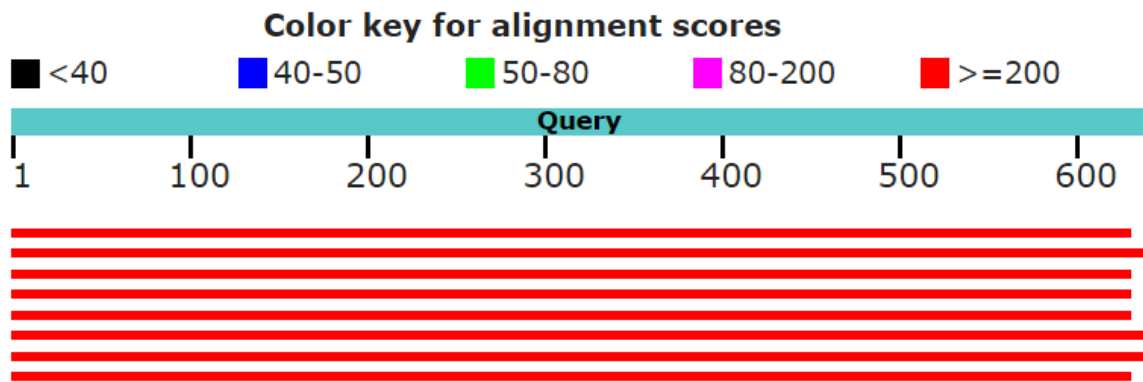


Fig. 73: Phylogenetic relationship between *Pluteus petasatus* (WGM-46) and other *Pluteus petasatus* species

TGCTGGCCTTTTTGGCATGTGCACGTCCACCAGTGTTTCATTCTTTTCTTCTCCA
 CCTGTGCATCTTTTGTAGGTCGTGTATGTTATCTTTCAGGCCCTTTGTGGCTTGG
 CCTAGGGAATTGCCTGCTCACACAGGCTCTTCTTGGACATGCTGGCCTATGTTT
 TACATCAACACCCAGACGAATGTAATTGAATGTATTTGCAGGCCCTTTGTGCCT
 TTAAATTTATATACAACCTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAG
 AACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAATTCAGTGAATCATCGA
 ATCTTTGAACGCACCTTGCCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGGAG
 TGTCAATTAATTTCTCAATCCTCAGCAGCTTTGTGTTGTTGGGTCAATTGGATTGTG
 GGAGCTGCTGGCTATGTCCAGCTCTCCTCAAAAGCATTAGCAGGGCAACAATGC
 TGTCTGTCACCTTGGTATGATATTTATCTATGCCTTGGACTGATGGTGTTCACTT
 GCTTCTAATAGTCTCTGTGGGACATTTCAATTTTTTGACCCTTGACCTCAAATCAG
 GTAGGATTACCCGCTGAACTTAAGCATATCAATAAGG



Sequences producing significant alignments:

Select: [All](#) [None](#) Selected:0

Alignments Download v GenBank Graphics Distance tree of results ⚙							
	Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/>	Pluteus spinulosus isolate FrancoMolano367 internal transcribed spacer 1, partial sequence: 5.8S	922	922	98%	0.0	93%	KJ009777.1
<input type="checkbox"/>	Pluteus pellitus voucher LE 217548 18S ribosomal RNA gene, partial sequence: internal transcrib	612	612	99%	3e-171	83%	FJ774078.1
<input type="checkbox"/>	Pluteus leucoborealis isolate LE289399 internal transcribed spacer 1, partial sequence: 5.8S ribos	603	603	98%	2e-168	83%	KJ009741.1

Fig. 74: Partial sequence length and homology search of WGM-47 (*Pluteus spinulosus*)

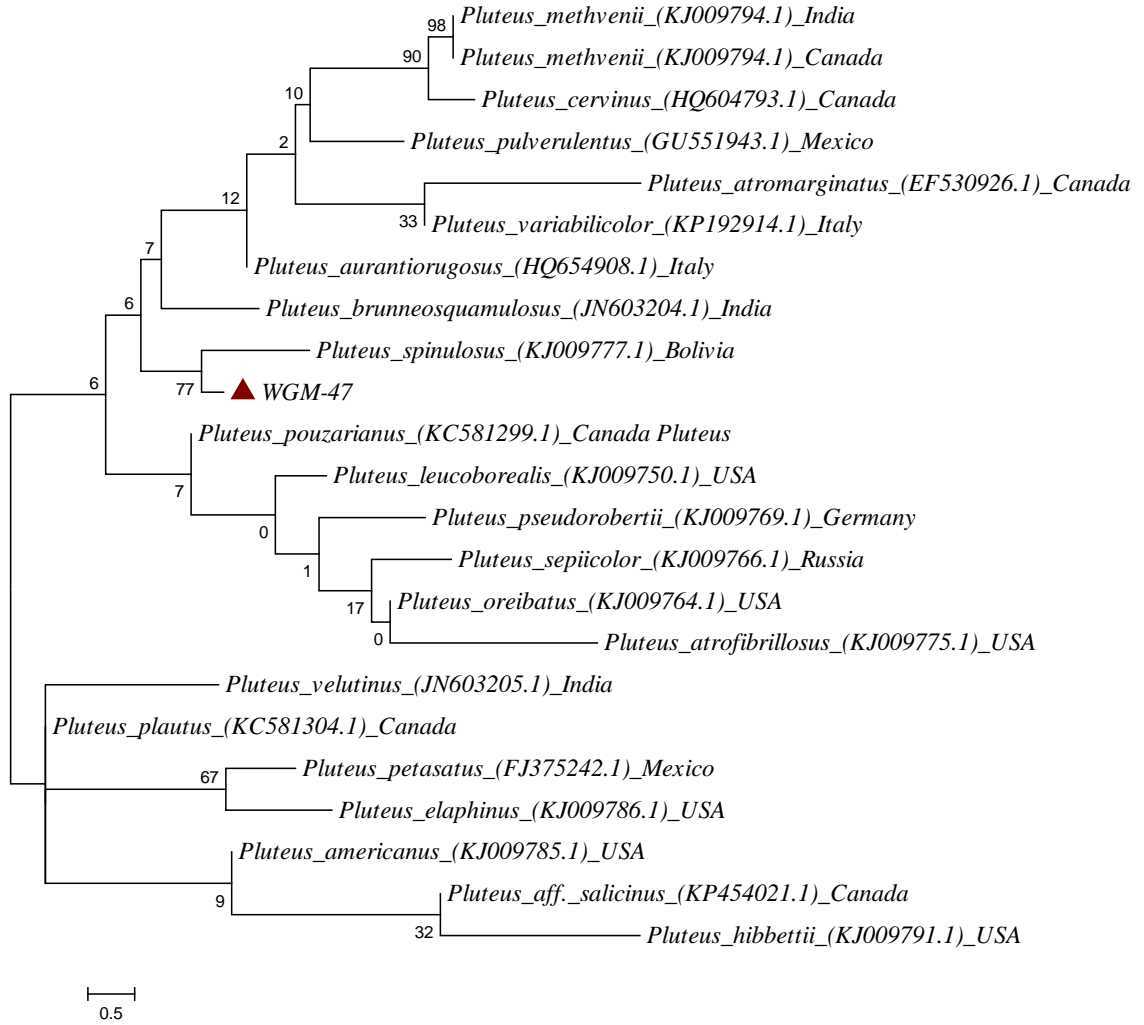


Fig. 75: Phylogenetic relationship between *Pluteus spinulosus* (WGM-47) and other *Pluteus* species

cremish white colour; spores are double layer (Plate 53b). Fruiting body of this mushroom grows scatteredly in humus soil.

The sequence data (668bp) showed 98 % homology with the earlier reported *Macrolepiota deters* (Fig. 76). The phylogenetic tree showed that WGM-48 is grouped in clade IV (Fig. 77). The “*detersa*” refers to the easily detachable squamules on the pileus. *Macrolepiota detersa* was characterized by the combination of scattered, reflexed, easily detachable, brown squamules on the white pileal background, a relatively big membranous annulus, and clavate to broadly clavate to pyriform cheilocystidia and this species was reported in China (Ge *et al.*, 2010). *Macrolepiota detersa* was edible mushroom and the fructification of *M. detersa* occurs only once a year during rainy season in the month of June - August (Rizal *et al.*, 2014).

4.2.3.14 WGM-49

White basidiocarp grows gregariously in soil of Managundi (latitude 12°49'36.48"N and longitude 74°25'26.42"E, at an altitude 446 ft.) of Dakshina Kannada. This mushroom is edible and there are many insects like dipteran flies had been visiting this mushroom which lay their maggots between the gills. These maggots complete their larval stage in this fruiting body of the mushroom. The fruiting body was soft and pileus was convex in shape, diameter of the cap 6-10.5 cm (Plate 23). Gills are attached to stipe. Stipe of the fruiting body was measured about 9 cm in length; the stipe position was at eccentric and tapering upwards. Annulus is absent. The spore is globose in shape, white color and measures about 2 X 2 µm. It grows gregariously in small group on humus soil.

The sequence data showed 100 % homology with the earlier reported *Tricholoma giganteum* (JN006792.1) (Fig. 78). The phylogenetic tree showed that WGM-49 very closely related to *Tricholoma giganteum* (JN006792.1) with bootstrap support 99 and grouped in clade I (Fig.79). Thiribhuvanamala *et al.* (2013) reported the distribution of this species in Coimbatore Giri *et al.* (2012) reported this edible mushroom species in the hilly regions of West Bengal.

4.2.3.15 WGM-50

The fruiting bodies are stipited with a poroid himenium. The pileus is whitish to yellowish when fresh and becomes darker when dry, the surface is smooth, and can be found growing on woods in Sakleshpur and Dakshina Kannada (latitude 12°57'22.84"N and longitude 75°49'59.77"E, altitude: 450-3085 ft.) in high humid condition. Insects are visiting this mushroom to suck the sap from the fruiting body. Pileus is white colour with smooth texture (Plate 41). Gills are interlinked to form medium sized pores. Stipe is very small, tapering up wards, lateral position. Spore print is white. It grows gregariously on decaying wood in mixed vegetation.

The sequence data showed 93 % homology with the earlier reported *Polyporus tenuiculus* (JQ409357.1, KC951263.1, KC505555.1, KM267725.1 and AB587633.1) (Fig. 80). Phylogenetic tree showed that identified *Polyporus tenuiculus* (WGM-50) closely related with *Polyporus tenuiculus* (KC505555.1) from China with clade II (Fig. 81). *Polyporus tenuiculus* also called as *Favulus tenuiculus*. *Polyporus tenuiculus* was observed being consumed by the land snail in Italy (Gibertoni *et al.* 2006).

Thus, totally fifty mushrooms have been documented and characterized in this study using both phenotypic and molecular tools. Morphological characters alone used in the classification are often inadequate for exact strain identification particularly when immature fruiting bodies are collected. Therefore, in such circumstances molecular tools play a prime role in identification of the mushrooms. Further, resolving systematic and evolutionary relationships, the molecular tools are very much essential to obtain precise information. Molecular markers have been used to discriminate mushroom lineages at the species level. The internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) has been widely used for identification of mushrooms at both the species and genus level (Sanchez-Ballesteros *et al.*, 2000). The ITS region fragment composed of ITS1, 5.8S rDNA and ITS2. The size usually ranges between 450bp to 700bp (Bellemain *et al.*, 2010).

Internal transcribed spacer (ITS) region has the highest probability of successful identification for the broadest range of the fungi, with the most clearly defined gap

GCTGGCTCTCTGGAGCATTGTGCACGCCTGTCTTGTTTTTTTAATTCATCCACCTG
 TGCACTTTTTGTAGTCTTCTAGGGATTTGAAAATAACCAATCTAATCGAGTTCTT
 CTCGGATGTGGGGAATGCTTTTGCAGCTCTCCTCAACATAAGGTTGTTGTGAAC
 CCTGCCCTAGAGTCTATGTTTTTCATATAACCACATAGTATGTTATAGAATGTCTTT
 AATGGGCCTTTGTGCCTATAAATGATCTTATACAACCTTTCAGCAACGGATCTCTT
 GGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCA
 GAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCCTCCTTGGTATTCCG
 AGGAGCATGCCTGTTTGAGTGTCAATAAATCTCAACCCCTCACAGTTTTTTCTG
 TGAATGGGGCTTGGATTTGGAGTTTTTGTGGCCCTTAGGGGGTTCAGCTACTC
 TGAATGCATTAGCGGAACCGTTTGGATCCGTACAGGTGTGATAAATTATCTA
 CGCCGTGTAATCGCTCTCTGTCTGTTTCAAGCTGCTAATCGTCCAGTCTTCATGGA
 CAATCATATTGAATATTTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAA
 GCATATCAATAAGGC

Color key for alignment scores



Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/> Macrolepiota sp. H-603 internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA gene, c	1119	1119	99%	0.0	98%	GQ254066.1
<input type="checkbox"/> Macrolepiota detersa voucher MFLU121784 18S ribosomal RNA gene, partial sequence: internal tra	1115	1115	98%	0.0	98%	KJ524560.1
<input type="checkbox"/> Macrolepiota turbinata voucher P59 internal transcribed spacer 1, partial sequence: 5.8S ribosomal	1092	1092	95%	0.0	98%	JF495073.1
<input type="checkbox"/> Macrolepiota turbinata voucher P71 internal transcribed spacer 1, partial sequence: 5.8S ribosomal	1090	1090	95%	0.0	98%	JF495075.1

Fig. 76: Partial sequence length and homology search of WGM-48 (*Macrolepiota detersa*)

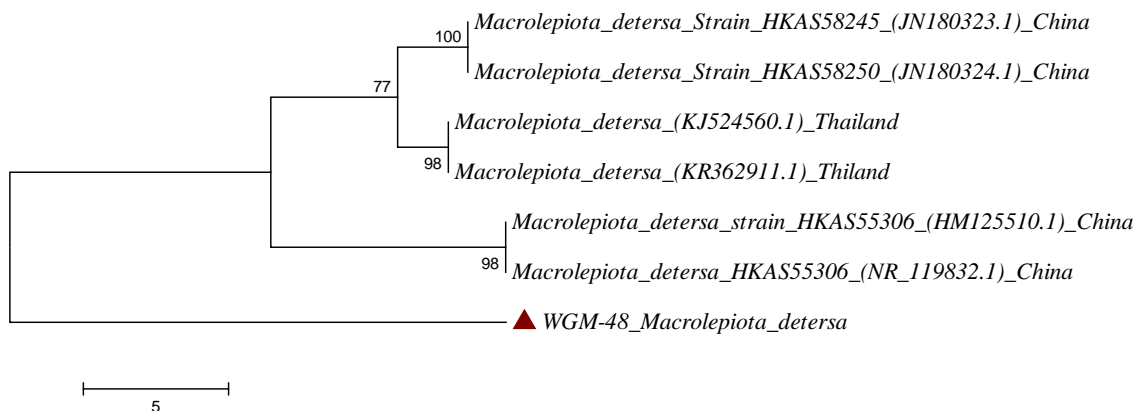
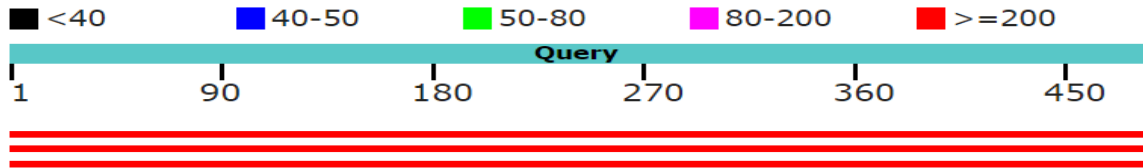


Fig. 77: Phylogenetic relationship between *Macrolepiota detersa* (WGM-48) and other *Macrolepiota detersa* species

TGGTCAGTTGTTGCTGGCCCTTTCAGAGCATGTGCACGCTTGGCTATTGTTTCT
TAAACCACCTTGTGCACCTTTTGTAGACTTTGGGTAAAGTTTTGAGTCGAGAGTG
ATCTTGGCCCTTATACTCCAAAAGTCTATGTCTTTTCATATCATTTACTCTATGTA
TAAGAATGTTTTCTAAGGCATTTCTTTGATGCCTTTAAATCATATACAACCTTCA
ACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAG
TAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGC
TCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTTCATGAAATTCCAACCTT
TGTTACTTTTTTGTTCAAAAGTGTGGATGTGGGAGTTGCTGGCTTTTTGTGTT
CAAAGAGTCAGCTCTTCTGAAATACATTAAGTGGGACCCATCGTTG

Color key for alignment scores



Alignments							
Download GenBank Graphics Distance tree of results							
	Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/>	Tricholoma giganteum internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA gene	866	866	100%	0.0	100%	JN006792.1
<input type="checkbox"/>	Macrocybe gigantea isolate MGNL internal transcribed spacer 1, partial sequence: 5.8S ribosom	859	859	100%	0.0	99%	KJ463732.1
<input type="checkbox"/>	Macrocybe gigantea isolate MGNBH internal transcribed spacer 1, partial sequence: 5.8S riboso	859	859	100%	0.0	99%	KJ463731.1
<input type="checkbox"/>	Macrocybe gigantea genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S	856	856	100%	0.0	99%	LK932287.1

Fig. 78: Partial sequence length and homology search of WGM-49 (*Tricholoma giganteum*)

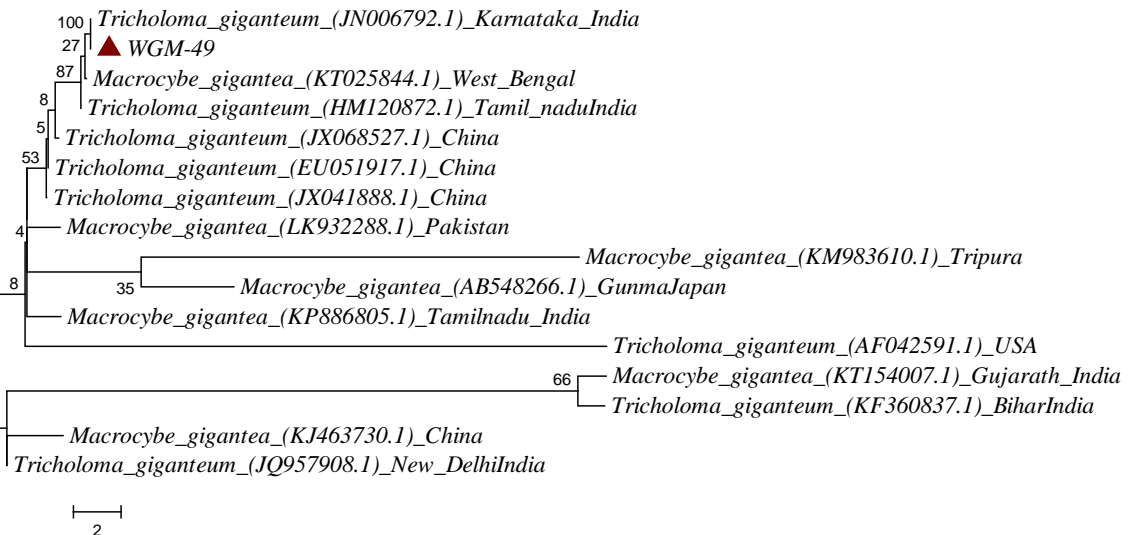
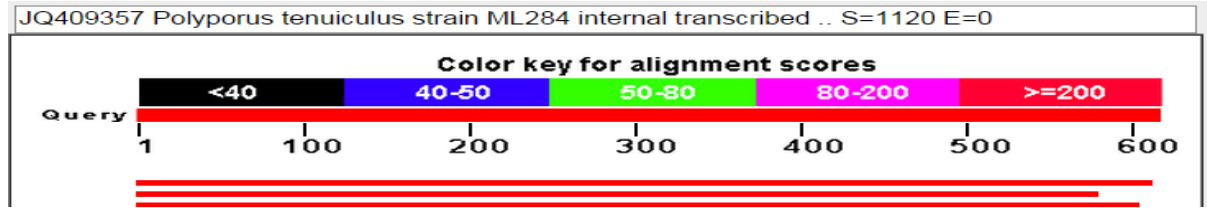


Fig. 79: Phylogenetic relationship between *Tricholoma giganteum* (WGM-49) and other *Tricholoma giganteum* species



Plate 53: WGM-50 (*Polyporus tenuiculus*)

GGTTGTAGCTGGCCTTACGAAGGCATGTGCACGCCCTGCTCAATCCACTCTACA
 CCTGTGCACTTACTGTGGGTCTTCGAGCAAATGGGGGTTTATAATTTCTTATAAG
 CCTTCGTTTGAAGCCCACGTTTACACACAAACACTTATAAAGTAAAAGAACGTG
 TATTGCGATGTAACGCATCCATAATACAACCTTTCAGCAACGGATCTCTTGGCTC
 TCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATT
 CAGTGAATCATCGAATCTTTGAACGCACCTTGCCTCCTTGGTATTCCGAGGAG
 CACGCCCTGTTTGAGTGTCTGTAAATCTCAAACCTTACCCGTCTTTGCGGATGGT
 GTAAGGCTTGGATGTTGGAGGTCTATTGTCGGTTTGTAAACGAACCGGCTCCTCT
 TAAATGCATTAGCTCAGTCCCTTTGTGGATCGGCTCCCAGTGTGATAATTATCTG
 CGCTGCGACCGTGAAGCGTTTTATTATGGCGAGCTTCTAATGGTCTCTTCAATG
 AGACAAGCAACACTTTGACAATCTGACCTCAAATCAGGCGGGACTACCCGCTGA
 ACTTAAGCATATCAATAAGCCGGAGGGAAATG



Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/> Polyporus tenuiculus strain ML284 internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA gene and in	1120	1120	99%	0.0	99%	JQ409357.1
<input type="checkbox"/> Polyporus tenuiculus strain Mu_1 internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA gene and int	1081	1081	94%	0.0	99%	KC951263.1
<input type="checkbox"/> Polyporus tenuiculus isolate HE2934 internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA gene and	1064	1064	97%	0.0	98%	KC505555.1
<input type="checkbox"/> Polyporus tenuiculus voucher JMH33 18S ribosomal RNA gene, partial sequence: internal transcribed spacer 1, 5.8	1055	1055	95%	0.0	98%	KM267725.1
<input type="checkbox"/> Polyporus tenuiculus genes for ITS1, 5.8S rRNA, ITS2, partial and complete sequence, strain: WD1576	948	948	83%	0.0	99%	AB587633.1
<input type="checkbox"/> Polyporus aff. alveolaris 249-402 gene for 5.8S ribosomal RNA, internal transcribed spacer 2 and 28S ribosomal R	643	643	57%	0.0	98%	AB509741.1
<input type="checkbox"/> Polyporus aff. alveolaris 249-539 gene for 5.8S ribosomal RNA, internal transcribed spacer 2 and 28S ribosomal R	627	627	57%	8e-176	98%	AB509821.1

Fig. 80: Partial sequence length and homology search of WGM-50 (*Polyporus tenuiculus*)

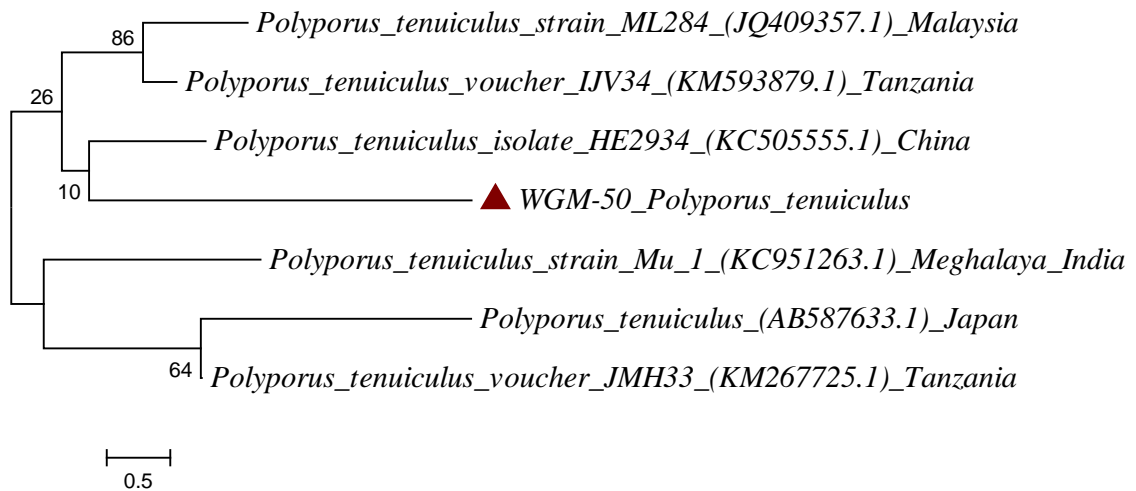


Fig. 81: Phylogenetic relationship between *Polyporus tenuiculus* (WGM-50) and other *Polyporus tenuiculus* species

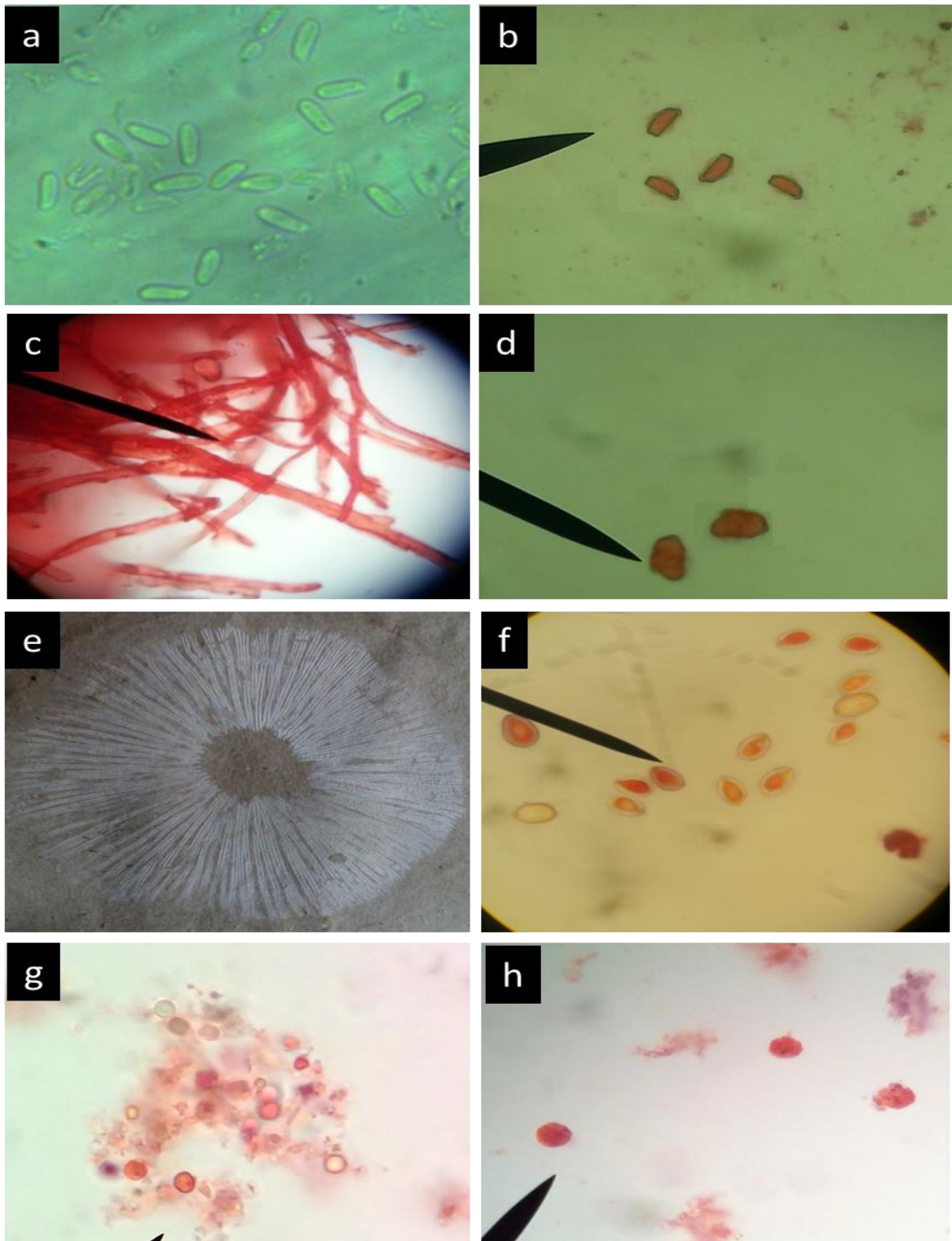


Plate 54: Spore print and spore structure of edible mushrooms: a-spore structure of *Lentinus sajor-caju*; b- spore structure of *Lentinus strigosus*; c- Mycelia of *Lentinus velutinus*; d- spore structure of *Lentinus velutinus*; e-spore print of *Macrolepiota detersa*; f-spore structure of *Macrolepiota detersa*; g- spore structure of *Tricholoma giganteum* and h- spore structure of *Polyporus tenuiculus*.

between inter- and intra specific variation (Schoch *et al.*, 2012). Rajarathnam and Thiagarajan (2012) extracted genomic DNA from the wild mushroom and subjected to nucleotide sequencing. The aligned sequence (559 bp) showed 88 % homology with *Perennispora* sp. (GQ982890.1).

Among the documented mushrooms stipe was found highest in *Chlorophyllum molybdites*, *Phallus indusiatus*, *Termitomyces* sp., *Macrolepiota detersa*, *Amanita hemibapha* and *Tricholoma giganteum* (Fig. 82). In some species like *Coriolopsis polyzona*, *Daldinia concentrica*, *Fomitopsis lilacinogilva* and *Ganoderma applanatum* stipe is sessile/very short. Mushrooms having large stalk can be seen by far distance which may attracts insects, it also helps in spore disperstion through wind. Scales were present in the stalk of *Amanita pseudovaginata*, *Agaricus trisulphuratus* and *Leucocoprinus cretaceous*.

Cap size of the mushrooms is varying among the species and across the family. The diameter of the cap of *Termitomyces* species was found highest among the document mushroom (230 mm in daiiameter). Cap size of the mushrooms is inversely proportional to the number of fruiting body. This kind of relation may balance the populstion in a habitat due balance in the number of spores which present in the lamellae/porse of the piles (Fig. 83).

Internal transcribed spacer (ITS) region has the highest probability of successful identification for the broadest range of fungi, with the most clearly defined gap between inter- and intra-specific variation (Schoch *et al.*, 2012). Rajarathnam and Thiagarajan (2012) extracted genomic DNA from the wild mushroom and subjected to nucleotide sequencing. The aligned sequence (559bp) showed 88 % homology with *Perennispora* sp. (GQ 982890.1).

Based on the sequenced data phylogenetic tree was constructed and which showed clear cut picture between the family and division (Fig. 84). Lentinaceae, Polyporaceae and coriolaceae are grouped into same clade which are belongs to order Polyporales. Pleurotaceae, Mycenaceae, Agaricaceae, Psathyrellaceae, Pluteaceae,

Amanitaceae, Omphalotaceae, Clavariaceae, Hygrophoraceae, Schizophyllaceae, Cortinariaceae, Entolomataceae and Cathelasmataceae are belongs to order Agaricales.

Species of Lyophyllace Family is comes under order Agaricales which is grouped into different clade and shares some similarity with Russulaceae of order Russulales. Order Agaricales, order Polyporales, order Auriculariales are grouped into Class Agaricomycetes of Basidiomycota. Sarcoscyphaceae and Xylariaceae are belongs to order Pezizomycetes and Sordariomycetes respectively which comes under division Ascomycota (Appendix-II).

Color of the fruiting bodies is varying from pure white, yellow to dark brown. Diverse color was seen on cap of the mushroom than stalk. Very few mushrooms gills are coloured and remaining mushrooms were white coloured (Table 1).

Lentinus polychrous and *Lentinus tuberregium* are the two species only found in the Western Ghats of Chikkamagaluru. *Lentinus sajor-caju* (WGM-36), *Termitomyces* sp., *Gymnopus brunneigracilis*, *Phallus indusiatus*, *Pluteus petasatus*, *Pluteus chrysaegis*, *Pluteus spinulosus*, *Russula rosea*, *Cookeina tricholoma*, *Tremella fuciformis*, *Filoboletus manipularis*, *Xylaria cirrata* were observed in Dakshina kannada district only. *Lentinus sajor-caju* (WGM-35), *Termitomyces eurhizus*, *Pleurotus pulmonarius* and *Psathyrella candolleana* were observed only in Western Ghats region of Hassan district (Sakleshpur) only. *Amanita hemibapha* and *Marasmius haematocephalus* were observed only in Tirthalli taluk of Shivamogga district.

Panus similis was documented from both Dakshina Kannada and Hassan (Sakleshpur). *Lentinus tigrinus* was observed in Hassan (Sakleshpur) and Shivamogga (Tirthahalli). *Macrolepiota detera*, *Auricularia polytricha*, *Clavaria rosea*, *Hygrocybe miniata*, *Cyanthus striatus* and *Daldania concentrica* were documented from Dakshina Kannada and Shivamogga. *Leucocoprinus cretaceous*, *Auricularia delicate*, *Dacryopinax spathularia*, *Ganoderma applanatum*, *Polyporus arcularius*, *Coprinellus disseminates* and *Schizophyllum commune* were found in Chikkamagaluru, Dakshina Kannada, Hassan and Shivamogga as well.

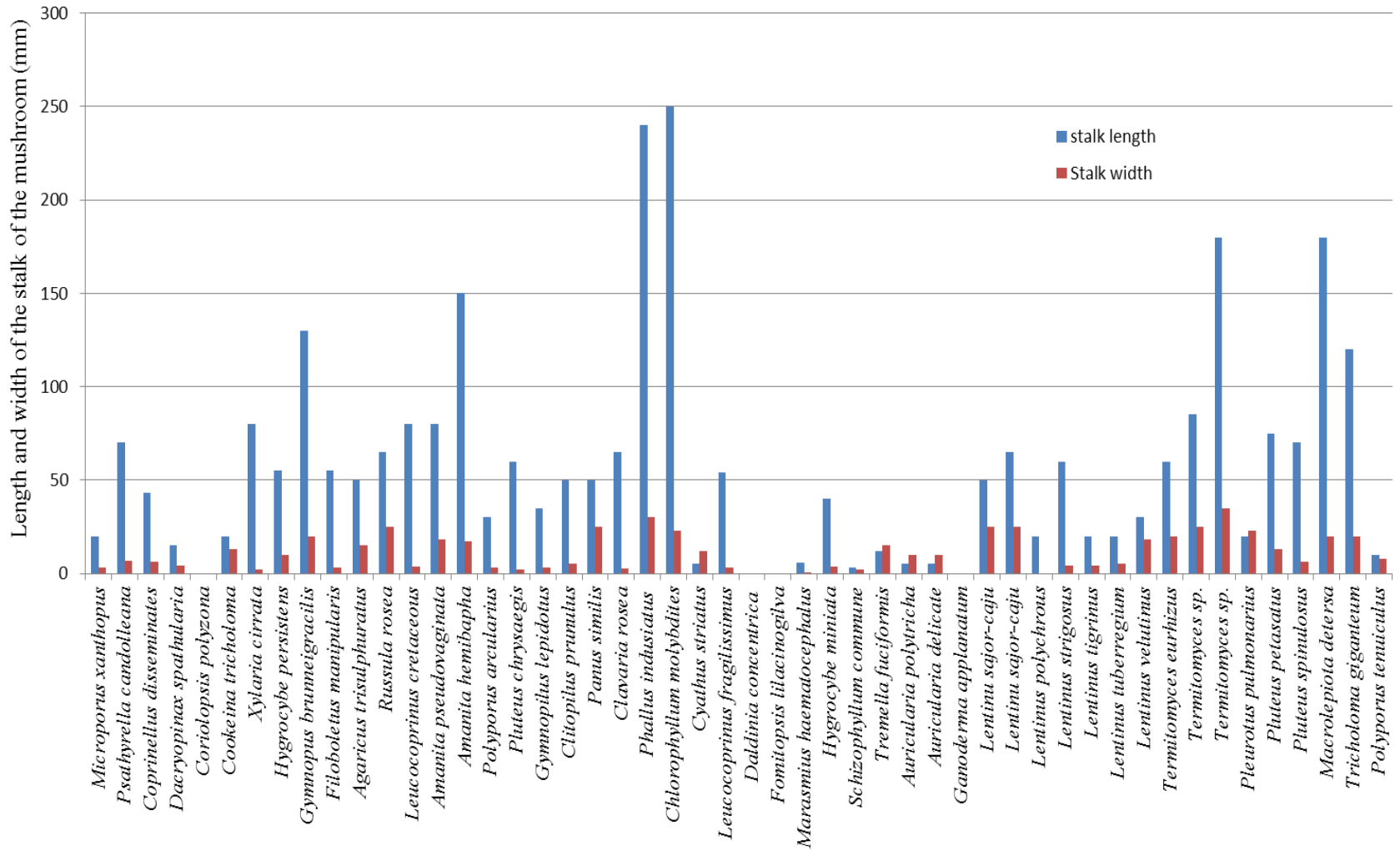


Fig. 82: Diversity of mushroom stipe (length and width) in the Western Ghats of Karnataka

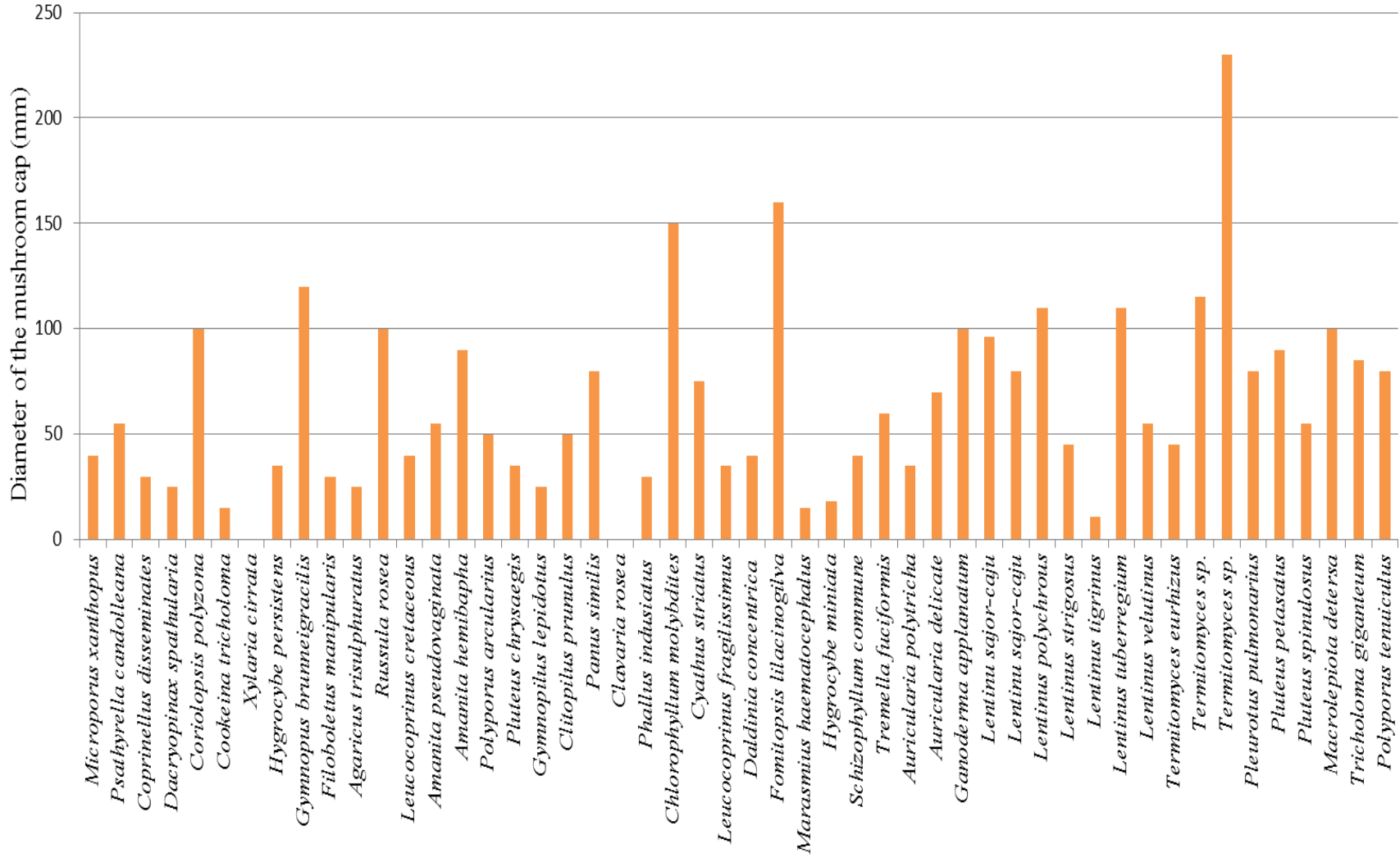


Fig. 83: Diversity of mushroom cap size in the Western Ghats of Karnataka

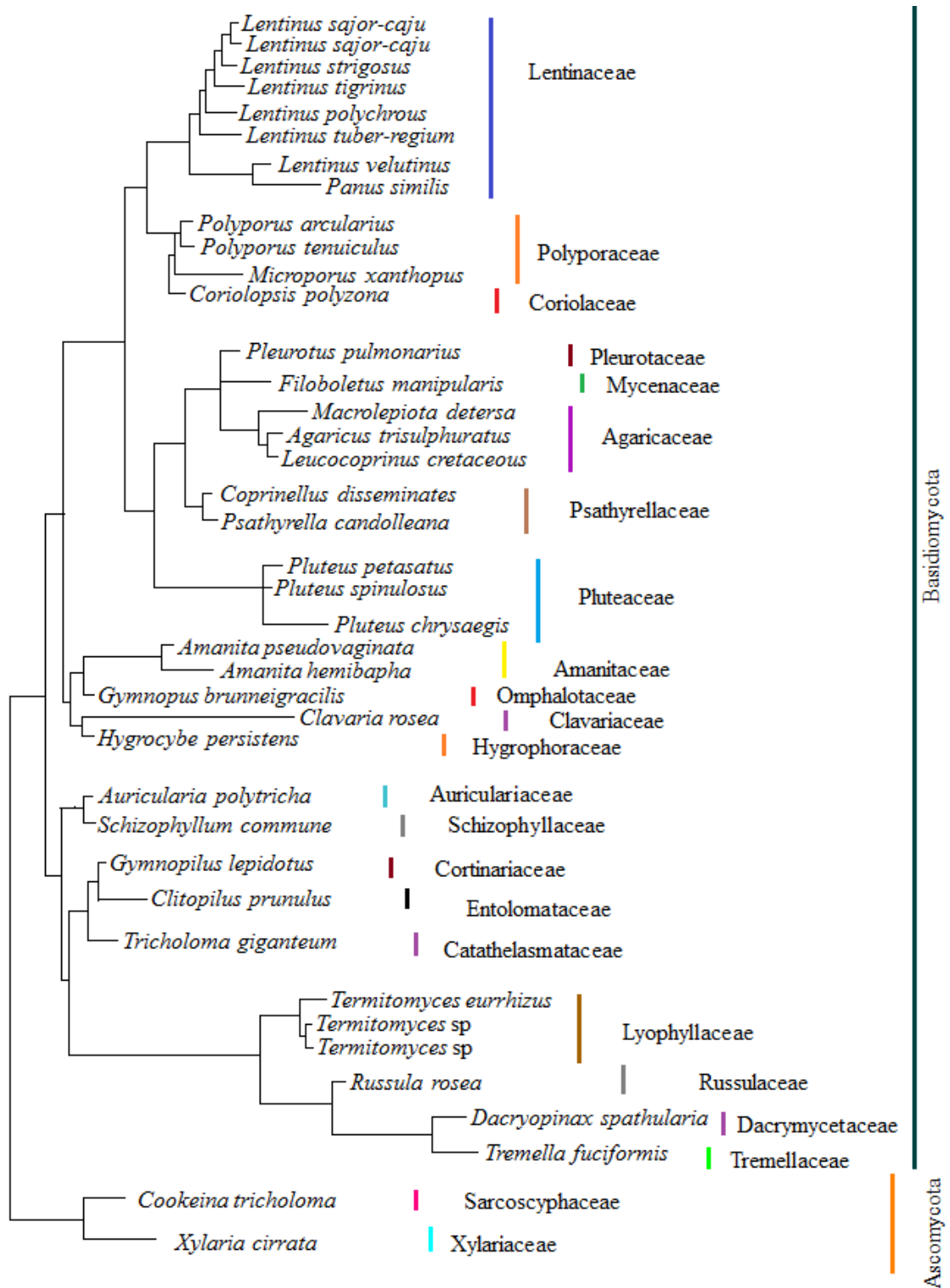


Fig. 84: Phylogenetic relationship between identified mushrooms based on ITS region

Table 1: Colour of cap, stalk, gills/pores and spores of the mushroom

Mushroom designation	Mushrooms	Cap	Stalk	Gills/pores	Spore
WGM-01	<i>Microporus xanthopus</i>	Dark Red	Yellow	Yellow	
WGM-02	<i>Psathyrella candolleana</i>	Orange	Yellow		
WGM-03	<i>Coprinellus disseminates</i>	Grey	Grey	Grey	
WGM-04	<i>Dacryopinax spathularia</i>	Yellow	Yellow		
WGM-05	<i>Corioloopsis polyzona</i>	Orange			
WGM-06	<i>Cookeina tricholoma</i>				
WGM-07	<i>Xylaria cirrata</i>	Grey	Grey		
WGM-08	<i>Hygrocybe persistens</i>	Orange	Yellow	Yellow	
WGM-09	<i>Gymnopus brunneigracilis</i>	Orange	Light Orange	Light Orange	
WGM-10	<i>Filoboletus manipularis</i>				
WGM-11	<i>Agaricus trisulphuratus</i>	Orange	Orange		
WGM-12	<i>Russula rosea</i>	Red			
WGM-13	<i>Leucocoprinus cretaceus</i>				
WGM-14	<i>Amanita pseudovaginata</i>	Grey	Grey		
WGM-15	<i>Amanita hemibapha</i>	Orange	Light Orange	Light Orange	
WGM-16	<i>Polyporus arcularius</i>	Orange			
WGM-17	<i>Pluteus chrysaegis</i>	Yellow	Yellow	Yellow	
WGM-18	<i>Gymnopilus lepidotus</i>	Red			
WGM-19	<i>Clitopilus prunulus</i>				
WGM-20	<i>Panus similis</i>	Orange	Dark Brown	Orange	
WGM-21	<i>Clavaria rosea</i>	Red	Red		
WGM-22	<i>Phallus indusiatus</i>	Dark Brown			
WGM-23	<i>Chlorophyllum molybdites</i>	Grey			
WGM-24	<i>Cyathus striatus</i>	Dark Brown	Dark Brown		
WGM-25	<i>Leucocoprinus fragilissimus</i>				
WGM-26	<i>Daldinia concentrica</i>	Pink	Black	Black	Black
WGM-27	<i>Fomitopsis lilacinogilva</i>	Red			
WGM-28	<i>Marasmius haematocephalus</i>	Red	Dark Red		
WGM-29	<i>Hygrocybe miniata</i>	Red	Yellow	Orange	

Mushroom designation	Mushrooms	Cap	Stalk	Gills/pores	Spore
WGM-30	<i>Schizophyllum commune</i>				
WGM-31	<i>Tremella fuciformis</i>				
WGM-32	<i>Auricularia polytricha</i>				
WGM-33	<i>Auricularia delicate</i>				
WGM-34	<i>Ganoderma applanatum</i>				
WGM-35	<i>Lentinu sajor-caju</i>				
WGM-36	<i>Lentinu sajor-caju</i>				
WGM-37	<i>Lentinus polychrous</i>				
WGM-38	<i>Lentinus strigosus</i>				
WGM-39	<i>Lentinus tigrinus</i>				
WGM-40	<i>Lentinus tuberregium</i>				
WGM-41	<i>Lentinus velutinus</i>				
WGM-42	<i>Termitomyces eurhizus</i>				
WGM-43	<i>Termitomyces sp.</i>				
WGM-44	<i>Termitomyces sp.</i>				
WGM-45	<i>Pleurotus pulmonarius</i>				
WGM-46	<i>Pluteus petasatus</i>				
WGM-47	<i>Pluteus spinulosus</i>				
WGM-48	<i>Macrolepiota detersa</i>				
WGM-49	<i>Tricholoma giganteum</i>				
WGM-50	<i>Polyporus tenuiculus</i>				





-  Nonedible mushroom
-  Edible and medicinal mushroom
-  Nonedible and medicinal mushroom
-  Edible mushroom

Table 2: Colour coding of the mushroom based on the number of fruiting body and study site

Family	Mushrooms	Study site (district)			
		Chikkamagaluru	Dakshina Kannada	Hassan	Shivamogga
Agaricaceae	<i>Macrolepiota detersa</i>		1-2		1-2
	<i>Agaricus trisulphuratus</i>				1-2
	<i>Leucocoprinus cretaceous</i>	1-2	1-2	1-2	1-2
	<i>Chlorophyllum molybdites</i>		>60		
	<i>Leucocoprinus fragilissimus</i>		1-2		1-2
Amanitaceae	<i>Amanita pseudovaginata</i>		2-6		
	<i>Amanita hemibapha</i>				>60
Auriculariaceae	<i>Auricularia polytricha</i>		2-6		2-6
	<i>Auricularia delicate</i>	2-6	2-6	7-15	1-2
Catathelasmataceae	<i>Tricholoma giganteum</i>		2-6		
Clavariaceae	<i>Clavaria rosea</i>		31-60		31-60
Coriolaceae	<i>Coriolopsis polyzona</i>		1-2		
Cortinariaceae	<i>Gymnopilus lepidotus</i>		2-6		
Dacrymycetaceae	<i>Dacryopinax spathularia</i>	>60	>60	>60	>60
Entolomataceae	<i>Clitopilus prunulus</i>		1-2		
Fomitopsidaceae	<i>Fomitopsis lilacinogilva</i>		>60		
Ganodermataceae	<i>Ganoderma applanatum</i>	1-2	1-2	1-2	1-2
Hygrophoraceae	<i>Hygrocybe persistens</i>		2-6		
	<i>Hygrocybe miniata</i>		2-6		2-6
Lentinaceae	<i>Lentinus velutinus</i>		2-6		
	<i>Lentinus strigosus</i>		7-15		
	<i>Lentinus sajor-caju</i>			>60	
	<i>Lentinus sajor-caju</i>		>60		
	<i>Lentinus polychrous</i>	2-6			
	<i>Lentinus tigrinus</i>			7-15	2-6

Number of Fruiting body

1-2

2-6

7-15

16-30

31-60

>60

Family	Mushrooms	Study site (district)			
		Chikkamagaluru	Dakshina Kannada	Hassan	Shivamogga
Lentinaceae	<i>Lentinus tuber-regium</i>	2-6			
	<i>Panus similis</i>		2-6	1-2	
Lyophyllaceae	<i>Termitomyces eurrhizus</i>			1-2	
	<i>Termitomyces</i> sp		2-6		
	<i>Termitomyces</i> sp		1-2		
Marasmiaceae	<i>Marasmius haematocephalus</i>				>60
Nidulariaceae	<i>Cyathus striatus</i>		>60		>60
Omphalotaceae	<i>Gymnopus brunneigracilis</i>		7-15		
Phallaceae	<i>Phallus indusiatus</i>		7-15		
Pleurotaceae	<i>Pleurotus pulmonarius</i>			31-60	
Pluteaceae	<i>Pluteus petasatus</i>		1-2		
	<i>Pluteus chrysaegis</i>		2-6		
	<i>Pluteus spinulosus</i>		2-6		
Polyporaceae	<i>Microporus xanthopus</i>		7-15	2-6	2-6
Polyporaceae	<i>Polyporus arcularius</i>	2-6	2-6	2-6	2-6
	<i>Polyporus tenuiculus</i>		7-15	31-60	2-6
Psathyrellaceae	<i>Psathyrella candolleana</i>			1-2	
	<i>Coprinellus disseminates</i>	>60	>60	>60	>60
Russulaceae	<i>Russula rosea</i>		2-6		
Sarcoscyphaceae	<i>Cookeina tricholoma</i>		>60		
Schizophyllaceae	<i>Schizophyllum commune</i>	>60	>60	>60	>60
Tremellaceae	<i>Tremella fuciformis</i>		>60		
Tricholomataceae	<i>Filoboletus manipularis</i>		7-15		
Xylariaceae	<i>Xylaria cirrata</i>		7-15		
	<i>Daldinia concentrica</i>		2-6		2-6

Number of Fruiting body



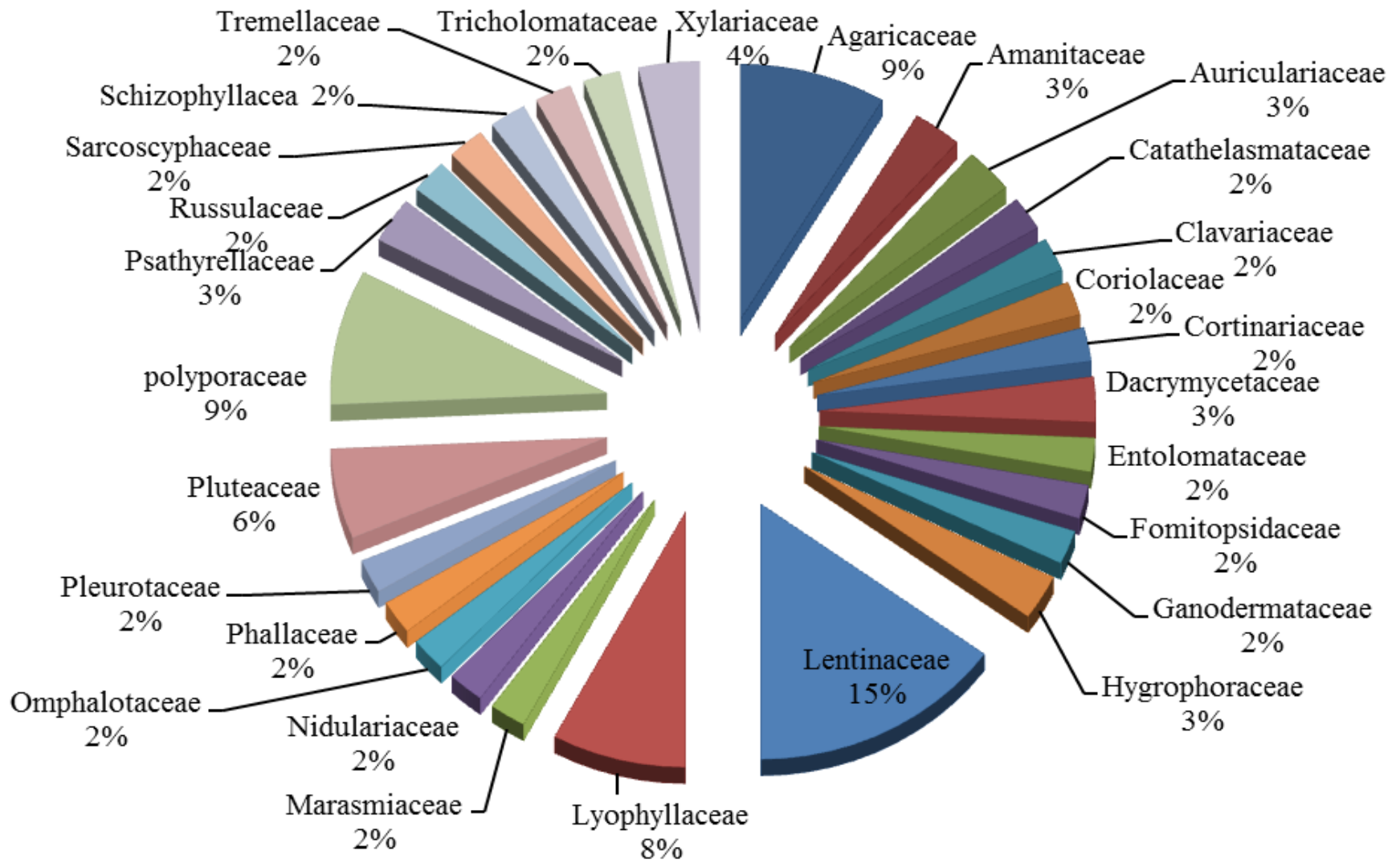


Fig. 85: Diversity of mushrooms in the Western Ghats of Karnataka (in the present study area)



Plate 55: Photograph showing hair like structures on Scolytidae insects which helps in mushroom spore dispersion

Psathyrella candolleana, *Xylaria cirrata*, *Hygrocybe persistens*, *Gymnopus brunneigracilis*, *Agaricus trisulphuratus*, *Russula rosea*, *Leucocoprinus cretaceous*, *Amanita psuedovaginata*, *Amanita hemibapha*, *Clavaria rosea*, *Phallus indusiatus*, *Chlorophyllum molybdites*, *Leucocoprinus fragilissimus*, *Termitomyces eurrhizus*, *Termitomyces sp.*, *Termitomyces sp.*, *Macrolepiota deters* and *Tricholoma giganteum* were produced fruiting body on soil. Other type of mushrooms was grown on dead wood/twigs.

Presence of annulus is observed only in the family Agaricaceae and Amanitaceae. Volva is present only in, *Amanita psuedovaginata*, *Amanita hemibapha* and *Phallus indusiatus*. Rhizoids are observed in species of Lyophyllaceae family which are associated with termite mount.

Among the fifty different species documented 15 % of the species belongs to lentinaceae family. Agaricaceae and Lyophyllaceae family accounts 9 %; Polyporaceae and Plutaceae family were 7 %. Xylariaceae is 4%; Hygrophoraceae, Auriculariaceae, Psathyrellaceae, Dacrymycetaceae accounts 3%, remaining family accounts 2 % each (Fig. 85).

There are many insects which feeds on mushrooms and spend part of there life cycle in the fruiting body of the mushroom. Insects like fruit flies, flea beetles, ants, bark beetles (Bark beetles were identified up to family level at ICAR- niche of Excellence on Taxonomy of Insects and Mites, Bengaluru) were found during this study. Snails also feeds mushroom. Some predators like praying mantids, lady bird beetles and spiders were found near the mushrooms. Mushrooms are one of the components in the food web in a habitat. These insects carry the mushroom spores through hair like structure on their body (Plate 55). In the present study, few edible species were documented and some of the species were isolated to pure culture to conserve the elite species for commercial production.

4.3 Pure culture isolation and evaluation for cultivation

Mushrooms are recognised for their nutritional value and medicinal potentialities. Besides edible species, many non-edible mushrooms are efficient decomposers of organic matter those can be used in bioremediation (Asghar *et al.*, 2007). Therefore, conservation of elite species is essential for further use. In the present study, ten mushrooms namely *Schizophyllum commune* (WGM-30), *Tremella fuciformis* (WGM-31), *Lentinus sajor-caju* (WGM-35), *Lentinus sajor-caju* (WGM-36), *Lentinus polychrous* (WGM-37), *Lentinus strigosus* (WGM-38), *Termitomyces eurrhizus* (WGM-42), *Pleurotus pulmonarius* (WGM-45), *Pluteus petasatus* (WGM-46) and *Polyporus tenuiculus* (WGM-50) were cultured (Plate 56a-j) using stipe tissue on Potato dextrose agar (PDA). The mycelium produced from the tissue was preserved for further study (Plate 56k). Among the cultured fungi *Lentinus sajor-caju* (WGM-35), *Lentinus sajor-caju* (WGM-36), *Lentinus strigosus*, *Termitomyces eurrhizus*, *Pleurotus pulmonarius*, *Tremella fuciformis*, *Pluteus petasatus* and *Polyporus tenuiculus* are edible. *Tremella fuciformis* and *Schizophyllum commune* have the medicinal value and edible as well.

Amongst isolated species, the *Lentinus sajor-caju* was only produced the good quality of spawn (Plate 57) and the same was attempted to cultivate (Plate 58) by using different substrate combination *viz.* Paddy straw alone, Straw dust alone and 50 % Paddy + 50 % saw dust. Maximum mushroom yield (520 g/bag) was recorded in 50 % Paddy + 50 % saw dust used as substrate, followed by saw dust substrate alone (425 g/bag) and minimum yield (200 g/bag) was recorded in paddy straw substrate alone (Plate 59). Similarly, highest bio-efficiency (65.25 %) was recorded in 50 % Paddy + 50 % saw dust substrate, followed by saw dust substrate alone (46.6 %) and minimum bio-efficiency (38 %) was recorded in paddy straw substrate alone. Fruiting bodies were fully grown within 4-5 days after the formation of bud (plate 60).

However, further study is required to screen various substrates in the controlled condition as variation in yield depends on substrate preference, temperature, relative humidity of the mushroom (Desai, 1982).

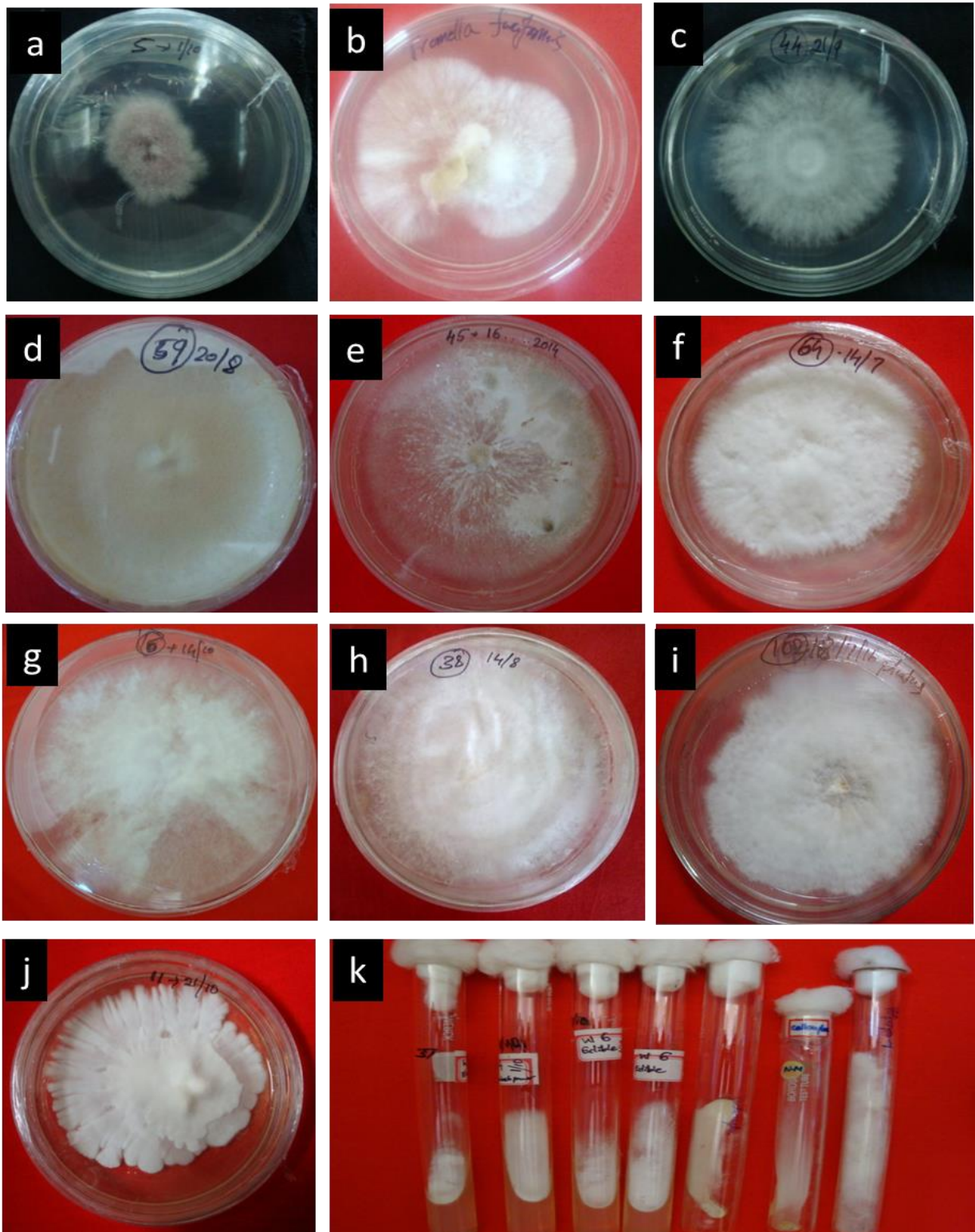


Plate 56: Pure culture of wild mushrooms: a- *Schizophyllum commune*; b- *Tremella fuciformis*; c- *Lentinus sajor-caju* (WGM-35); d-*Lentinus sajor-caju* (WGM-36); e- *Lentinus polychrous*; f- *Lentinus strigosus*; g- *Termitomyces eurrhizus*; h-*Pleurotus pulmonarius*; i- *Pluteus petasatus*; j- *Polyporus tenuiculus* and k- : Pure cultures are maintained in PDA slant.

Table 3: Effect of different substrate and substrate combination on yield and Bioefficiency of *Lentinus sajor-caju*

Sl. No.	Substrate	Yield (g/bag)	Bioefficiency (%)
1.	Paddy straw alone	200	38
2.	Straw dust alone	425	46.6
3.	50 % Paddy + 50 % saw dust	550	65.25

4.4 Nutritional analysis of edible mushrooms and conservation of elite species

Mushrooms have a long association with humankind and provide profound biological and economic impact. From ancient times, wild mushrooms have been consumed by man with delicacy, for their taste and pleasing flavor (Das, 2010). They have rich nutritional value with high content of proteins, Minerals and low in calories (Wani *et al.*, 2010). In the present study, protein, fat and fiber content of seven important mushrooms viz., *Tricholoma giganteum*, *Polyporus tenuiculus*, *Lentinus strigosus*, *Termitomyces eurrhizus*, *Lentinus sajor-caju*, *Lentinus tuberregium* and *Tremella fuciformis* were analysed (Table-4).

Table 4: Protein, Fat and Fiber content of edible mushrooms collected from Western Ghats of Karnataka

Sl. No.	Mushroom	Protein (%)	Fat (%)	Fiber (%)
1.	<i>Tricholoma giganteum</i>	34.46 ^a	3.6 ^b	12.0 ^b
2.	<i>Polyporus tenuiculus</i>	29.46 ^b	4.0 ^a	8 ^c
3.	<i>Lentinus strigosus</i>	20.53 ^e	3.2 ^{bc}	7 ^c
4.	<i>Termitomyces eurrhizus</i>	29.30 ^b	3.3 ^b	11 ^b
5.	<i>Lentinus sajor-caju</i>	25.50 ^c	3.0 ^c	8 ^c
6.	<i>Lentinus tuberregium</i>	28.33 ^{bc}	1.8 ^d	14 ^a
7.	<i>Tremella fuciformis</i>	23.70 ^d	3.8 ^{ab}	5 ^d

Note: Means followed by same superscript in the column do not differ significantly at $p < 0.05$ by Duncan Multiple Range Test.

Maximum protein content was observed in *Tricholoma giganteum* (34.5 %), this was followed in the order *Termitomyces eurrhizus* (29.3 %), *Polyporus tenuiculus* (29.46 %), *Lentinus sajor-caju* (29.3 %), *Lentinus sajor-caju* (cultivated) (25.5 %), *Tremella fuciformis* (23.7 %) and *Lentinus strigosus* (20.53 %). The *Termitomyces* contained highest protein compared other mushrooms. This metabolic character of the different mushrooms and the substrate they utilize. The nutritional values of 10 edible mushrooms from Western Ghats of Kanyakumari district have been analyzed by Johnsy *et al.* (2011) and reported that edible mushrooms are highly valued as a good source of protein ranged from 28.93 to 39.1 % of dry weight. Singdevsachan *et al.* (2013) reported the nutrient values of two wild mushrooms (*Lentinus sajor-caju* and *Lentinus torulosus*) from Similipal Biosphere Reserve, Odisha, India.

Amongst the seven mushrooms, the fat content was found highest in *Polyporus tenuiculus* followed by *Tremella fuciformis*, *Tricholoma giganteum*, *Termitomyces sp.*, *Lentinus strigosus*, *Lentinus sajor-caju* and *Lentinus tuberregium* (Table-4). Similarly, fiber content was found highest in *Lentinus tuberregium* followed by *Tricholoma giganteum*, *Polyporus tenuiculus*, *Termitomyces sp.*, *Lentinus sajor-caju*, *Polyporus tenuiculus*, *Lentinus strigosus* and *Tremella fuciformis*. These results are in agreement with the report of Wang *et al.* (2014) who reported less crude fat content (1.0 % to 6.7 %) and more protein, essential minerals and fiber content in many wild grown mushrooms.

Mineral content of wild edible mushrooms

Minerals are important in everyday life. Calcium, Magnesium and several other minerals contribute to healthy bones and teeth. A single mineral may serve many unrelated functions. Magnesium alone participates in many enzyme reactions as co-factor. Ca^{2+} in blood is maintained by the concentrated action of the calcitrophic hormones, parathyroid hormone, 1, 25-dihydroxy vitamin D and calcitonin (Prentice *et al.*, 2003). Inadequate dietary calcium intake leads to impair bone development in adolescence, bone loss and osteoporosis in adult (Flynn, 2003). Hence, the mushrooms are the good source of minerals like greens and vegetables. In the present study, seven



Plate 57: Spawns are produced on sorghum with 2% of Calcium carbonate and 2% of Calcium sulphate.



Plate 58: Mycelium growth on substrate (Cultivation)

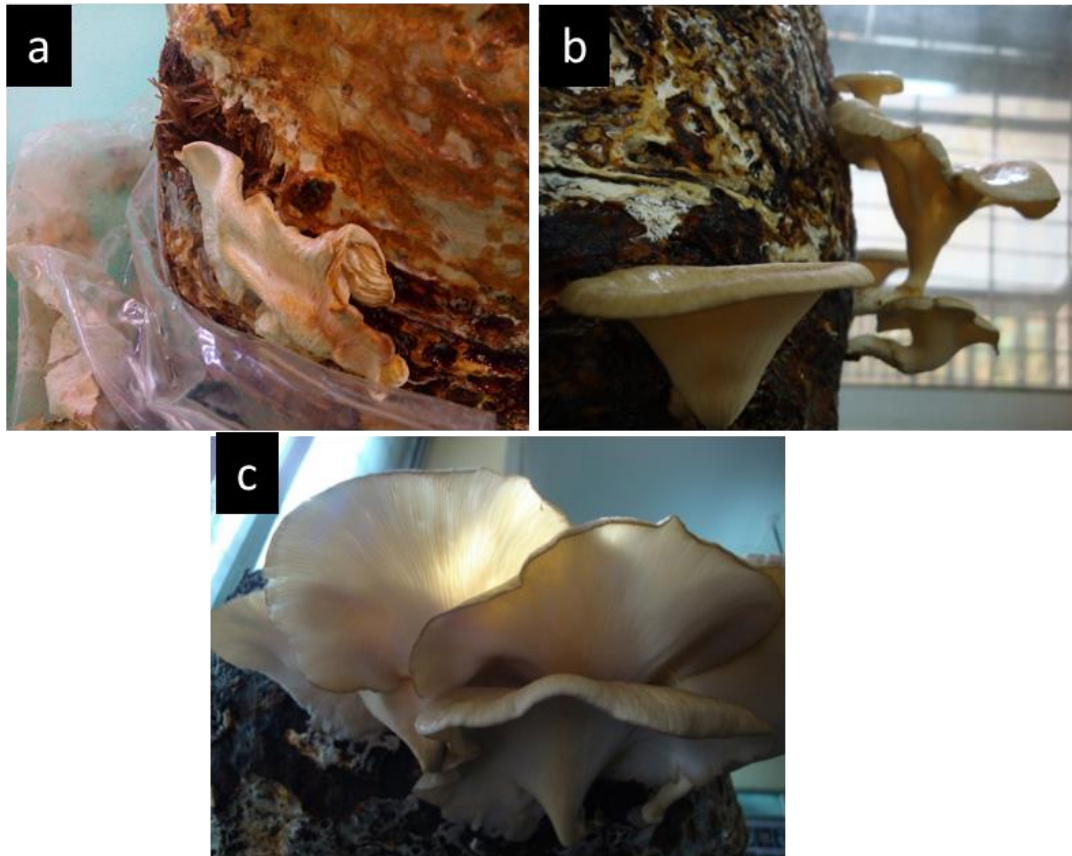


Plate 59: Cultivation of mushroom; a-Paddy straw alone, b- swadust alone and c- combination of 50% paddy straw + 50% sawdust.

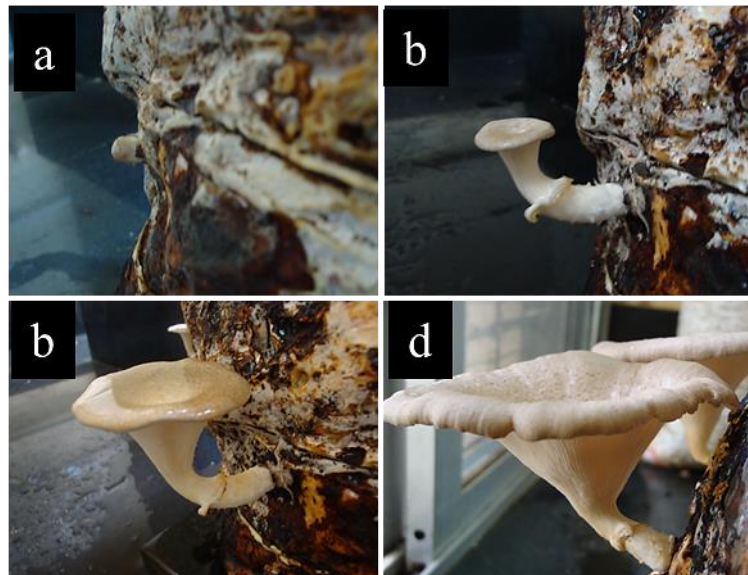


Plate 60: Blooming of mushroom fruiting body of *Lentinus sajor-caju* on sawdust and paddy straw (1:1); a –bud formation, b- second day after bud formation, c- third day after bud formation, d- fourth day after bud formation

mushrooms were analysed for Ca, Mg, Fe, Zn and P content using Atomic Absorption Spectrophotometer.

Table 5: Mineral content of wild mushrooms collected from Western Ghats of Karnataka

Sl. No.	Mushroom species	Calcium (µg/g)	Magnesium (µg/g)	Iron (µg/g)	Zinc (µg/g)	Phosphorus (µg/g)
1.	<i>Tricholoma giganteum</i>	73.25 ^d	14.99 ^e	19.28 ^c	0.58 ^e	280.00 ^d
2.	<i>Polyporus tenuiculus</i>	143.96 ^a	40.86 ^b	33.32 ^a	3.40 ^a	200.00 ^e
3.	<i>Lentinus strigosus</i>	32.15 ^g	17.29 ^d	30.74 ^b	2.40 ^b	107.33 ^f
4.	<i>Termitomyces eurrhizus</i>	66.72 ^e	14.78 ^e	17.44 ^d	1.83 ^d	505.66 ^a
5.	<i>Lentinus sajor-caju</i>	90.63 ^c	17.43 ^d	15.93 ^d	0.87 ^e	200.66 ^e
6.	<i>Lentinus tuberregium</i>	48.45 ^f	15.06 ^e	17.73 ^d	1.95 ^e	338.33 ^b
7.	<i>Tremella fuciformis</i>	132.49 ^b	59.49 ^a	08.44 ^f	1.85 ^d	306.00 ^c

Note: Means followed by same superscript in the column do not differ significantly at $p < 0.05$ by Duncan Multiple Range Test.

In this study, the mineral content of different mushrooms varied with individual minerals as it depends on species characters. Ca, Fe and Zn content were highest in *Polyporus tenuiculus*, Mg content is highest in *Tremella fuciformis* and P content is in *T. eurrhizus* (Table 5) indicating they are the good sources of these minerals. Similar results were obtained by Mattilla *et al.* (2001) and Henry and Rajakumar (2014) for different mushrooms. However, nutritional status of many wild edible mushrooms still remained unexplored for health benefits. If discovered, some of them may serve as valuable supplements for human diet.

V SUMMARY

Mushrooms are epigeous and hypogeous fleshy fruiting bodies of fungi belonging to Basidiomycetes and certain Ascomycetes. They are the most important components of ecosystem. Their edibility, poisonous nature psychotropic properties, medicinal properties are makes mushroom economically important. High humidity during monsoon period provides ideal conditions for their growth and development.

Western Ghats of Karnataka is one of the world's ten hottest biodiversity hot spots 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, ethno-mycological survey was made in and around Chikkamagaluru, Dakshina Kannada, Hassan and Shivamogga districts of Karnataka for mushroom flora during June to October (2014-2016) with the help of local people and adivasi 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.

Fifty mushrooms were collected in paper bags during field survey with the help of local peoples and tribes inhabited in the area and these mushrooms were designated as WGM-1 to WGM-50. 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 basidia, spore shape and color were recorded.

Forty mushrooms were identified by using Internal Transcribed Sequence (ITS) region/ 5.8 SrRNA genes 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; the sequence alignment was made with NCBI data base revealed 85-100 % homology. WGM-01 showed 99 % sequence homology with

Microporus xanthopus; WGM-02 had 86 % sequence homology with *Psathyrella candolleana*; WGM-03 had 99 % sequence homology with *Coprinellus disseminates*; WGM-04 showed 96 % sequence homology with *Dacryopinax spathulari*; WGM-05 showed 99 % sequence homology with *Coriolopsis polyzona*; WGM-06 showed 98 % sequence homology with *Cookeina tricholoma*; WGM-07 showed 99 % sequence homology with *Xylaria cirrata*; WGM-08 showed 99 % sequence homology with *Hygrocybe persistens*; WGM-09 showed 99 % sequence homology with *Gymnopus brunneigracilis*; WGM-10 showed 100 % sequence homology with *Filoboletus manipularis*; WGM-11 with 99 % sequence homology with *Agaricus trisulphuratus*; WGM-12 with 99 % sequence homology for *Russula rosea*; WGM-13 with 99 % sequence homology for *Leucocoprinus cretaceous*; WGM-14 showed 99 % sequence homology with *Amanita pseudovaginata*; WGM-15 had 96 % sequence homology with *Amanita hemibapha*; WGM-16 had 95 % sequence homology with *Polyporus auricularius*; WGM-17 showed 99 % sequence homology with *Pluteus chryzaegis*; WGM-18 showed 99 % sequence homology with *Gymnopilus lepidotus*; WGM-29 showed 85 % sequence homology with *Clitopilus prunulus*; WGM-20 showed 99 % sequence homology with *Panus similis* and WGM-21 showed 99 % sequence homology with *Clavaria rosea*

WGM-30 showed 99 % sequence homology with *Schizophyllum commune*; WGM-31 showed 89 % sequence homology with *Tremella fuciformis* and WGM-32 showed 89 % sequence homology with *Auricularia polytricha*. These three mushrooms belong to medicinal mushrooms.

WGM-35 showed 99 % sequence homology with *Lentinus sajor-caju*; WGM-36 showed 98 % sequence homology with *Lentinus sajor-caju*; WGM-7 showed 98 % sequence homology with *Lentinus polychrous*; WGM-38 showed 96 % sequence homology with *Lentinus strigosus*; WGM-39 showed 99 % sequence homology with *Lentinus tigrinus*; WGM-40 showed 100 % sequence homology with *Lentinus tuberregium*; WGM-41 showed 99 % sequence homology with *Lentinus velutinus*; WGM-42 with 98 % sequence homology with *Termitomyces eurhizus*; WGM-43 showed 99 % sequence homology with *Termitomyces* sp.; WGM-44 with 85 % sequence

homology for *Termitomyces* sp.; WGM-45 with 94 % sequence homology for *Pleurotus pulmonarius*; WGM-46 showed 99 % sequence homology with *Pluteus petasatus*; WGM-47 showed 93 % sequence homology with *Pluteus spinulosus*; WGM-48 showed 98 % sequence homology with *Macrolepiota detersa*; WGM-49 showed 100 % sequence homology with *Tricholoma giganteum*; and WGM-50 showed 99 % sequence homology with *Polyporus tenuiculus*. All the forty sequences were submitted to GenBank in NCBI public database and obtained accession numbers (Appendix-I).

Ten mushrooms *Phallus indusiatus* (WGM-22), *Chlorophyllum molybdites* (WGM-23), *Cyathus striatus* (WGM-24), *Leucocoprinus fragilissinus* (WGM-25), *Daldinia concentrica* (WGM-26), *Fomitopsis lilacinogilva* (WGM-27), *Marasmius haematocephalus* (WGM-28), *Hygrocybe miniata* (WGM-29), *Auricularia delicate* (WGM-33) and *Ganoderma applanatum* (WGM-34) were identified by using morphological characteristics.

Of the fifty documented mushrooms, *Lentinus sajor-caju* (WGM-35), *Lentinus sajor-caju* (WGM-36), *Lentinus polychrous*, *Lentinus strigosus*, *Lentinus tigrinus*, *Lentinus tuberregium*, *Lentinus velutinus*, *Termitomyces eurhizus*; *Termitomyces* sp. (WGM-43), *Termitomyces* sp. (WGM-44), *Pleurotus pulmonarius*, *Pluteus petasatus*, *Pluteus spinulosus*, *Macrolepiota detersa*, *Tricholoma giganteum* and *Polyporus tenuiculus* were reported to be edible. *Auricularia delicate*, *Auricularia polytricha* and *Tremella fuciformis* were used as medicinal and edible species as well.

In the present study, ten mushrooms namely *Schizophyllum commune*, *Tremella fuciformis*, *Lentinus sajor-caju* (WGM-35), *Lentinus sajor-caju* (WGM-36), *Lentinus polychrous*, *Lentinus strigosus*, *Termitomyces eurhizus*, *Pleurotus pulmonarius*, *Pluteus petasatus* and *Polyporus tenuiculus* were cultured using stipe tissue on Potato dextrose agar (PDA). Among all the cultured species only *Lentinus sajor-caju* produced the good quality of spawn and the same was tried for cultivation. The highest bio-efficiency (65.25 %) was recorded in 50 % Paddy + 50 % saw dust substrate, followed by saw dust substrate alone (46.6 %) and minimum bio-efficiency (38 %) was recorded in paddy straw substrate alone.

Nutrient estimation revealed that protein, fat and fiber content were in the range of 22.33-34.46 %, 1.8-4.0 % and 5-12 % respectively. Calcium, Magnesium, Iron, Zinc and Phosphorus were found in the range of 48.45-143.96 µg/g, 14.78-59.49 µg/g, 8.44-33.32 µg/g, 0.58-3.4 µg/g and 107.33-505.66 µg/g respectively.

Many wild grown mushrooms are having ecological as well as economic value. Due to extensive human activity in the forest area leads to loss of vegetation and change in micro climate which is essential to growth and existence of mushroom species. The documentation and conservation of the mushroom species is very much essential to overcome the extension of the species.

Future line of works

1. Substrate formulation and commercial cultivation of wild edible mushrooms like *Termitomyces* species has to be standardized.
2. Antiviral property of the mushroom has to be analysed
3. Chemical compounds responsible for the Medicinal value in the mushroom has to be extracted and utilized in a proper way.

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APPENDIX-I

List of Mushroom species identified by using ITS region/5.8S rDNA sequence and their accession number. (Submitted to NCBI Genbank)

Sl. No.	Mushroom sample Designation	Mushroom species identified	Size of amplified product (base pair)	Accession No.
1.	WGM-01	<i>Microporus xanthopus</i>	613	KY243926
2.	WGM-02	<i>Psathyrella candolleana</i>	659	KY243925
3.	WGM-03	<i>Coprinellus disseminatus</i>	678	KX243924
4.	WGM-04	<i>Dacryopinax spathularia</i>	447	KX603668
5.	WGM-05	<i>Coriolopsis polyzona</i>	594	KY243921
6.	WGM-06	<i>Cookeina tricholoma</i>	583	KX610963
7.	WGM-07	<i>Xylaria cirrata</i>	525	KY243920
8.	WGM-08	<i>Hygrocybe persistens</i>	674	KY243919
9.	WGM-09	<i>Gymnopus brunneigracilis</i>	684	KY243918
10.	WGM-10	<i>Filoboletus manipularis</i>	702	KY243915
11.	WGM-11	<i>Agaricus trisulphuratus</i>	691	KY243913
12.	WGM-12	<i>Russula rosea</i>	730	KY243912
13.	WGM-13	<i>Leucocoprinus cretaceous</i>	663	KY288021
14.	WGM-14	<i>Amanita pseudovaginata</i>	350	KY288022
15.	WGM-15	<i>Amanita hemibapha</i>	545	KY288023
16.	WGM-16	<i>Polyporus arcularius</i>	576	KY628941
17.	WGM-17	<i>Pluteus chrysaegis</i>	576	KY630513
18.	WGM-18	<i>Gymnopilus lepidotus</i>	569	KY630514
19.	WGM-19	<i>Clitopilus prunulus</i>	641	KY630515

Sl. No.	Mushroom sample Designation	Mushroom species identified	Size of amplified product (base pair)	Accession No.
20.	WGM-20	<i>Panus similis</i>	507	KY630517
21.	WGM-21	<i>Clavaria rosea</i>	475	KY243922
22.	WGM-30	<i>Schizophyllum commune</i>	650	KY243923
23.	WGM-31	<i>Tremella fuciformis</i>	479	KX603666
24.	WGM-32	<i>Auricularia polytricha</i>	562	KX603667
25.	WGM-35	<i>Lentinus sojar-caju</i>	594	KX239769
26.	WGM-36	<i>Lentinus sojar-caju</i>	627	KX181288
27.	WGM-37	<i>Lentinus polychrous</i>	627	KX239770
28.	WGM-38	<i>Lentinus strigosus</i>	620	KY243939
29.	WGM-39	<i>Lentinus tigrinus</i>	634	KY630511
30.	WGM-40	<i>Lentinus tuberregium</i>	640	KY630512
31.	WGM-41	<i>Lentinus velutinus</i>	627	KX257370
32.	WGM-42	<i>Termitomyces eurrhizus</i>	659	KY243929
33.	WGM-43	<i>Termitomyces sp.</i>	677	KY643773
34.	WGM-44	<i>Termitomyces sp.</i>	645	KY243928
35.	WGM-45	<i>Pleurotus pulmonarius</i>	569	KY243927
36.	WGM-46	<i>Pluteus petasatus</i>	618	KY243914
37.	WGM-47	<i>Pluteus spinulosus</i>	636	KY630516
38.	WGM-48	<i>Macrolepiota detersa</i>	668	KY243916
39.	WGM-49	<i>Tricholoma giganteum</i>	480	KY243917
40.	WGM-50	<i>Polyporus tenuiculus</i>	625	KY659206

APPENDIX-II

Mushrooms of different families recorded during the study

Division	Class	Order	Family	Mushrooms
Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	<i>Macrolepiota detersa</i>
				<i>Agaricus trisulphuratus</i>
				<i>Leucocoprinus cretaceous</i>
				<i>Chlorophyllum molybdites</i>
				<i>Leucocoprinus fragilissinus</i>
			Amanitaceae	<i>Amanita pseudovaginata</i>
				<i>Amanita hemibapha</i>
			Catathelasmataceae	<i>Tricholoma giganteum</i>
			Clavariaceae	<i>Clavaria rosea</i>
			Entolomataceae	<i>Clitopilus prunulus</i>
			Hygrophoraceae	<i>Hygrocybe persistens</i>
				<i>Hygrocybe miniata</i>
			Lyophyllaceae	<i>Termitomyces eurrhizus</i>
				<i>Termitomyces</i> sp
				<i>Termitomyces</i> sp
			Marasmiaceae	<i>Marasmius haematocephalus</i>
			Nidulariaceae	<i>Cyathus striatus</i>
			Omphalotaceae	<i>Gymnopus brunneigracilis</i>
			Cortinariaceae	<i>Gymnopilus lepidotus</i>
			Pleurotaceae	<i>Pleurotus pulmonarius</i>
Pluteaceae	<i>Pluteus petasatus</i>			
	<i>Pluteus chrysaegis</i>			
	<i>Pluteus spinulosus</i>			

Division	Class	Order	Family	Mushrooms	
			Psathyrellaceae	<i>Psathyrella candolleana</i>	
			Schizophyllaceae	<i>Schizophyllum commune</i>	
			Mycenaceae	<i>Filoboletus manipularis</i>	
		Auriculariales	Auriculariaceae		<i>Auricularia polytricha</i>
					<i>Auricularia delicate</i>
		Phallales	Phallaceae		<i>Phallus indusiatus</i>
		Polyporales		Coriolaceae	<i>Coriolopsis polyzona</i>
				Fomitopsidaceae	<i>Fomitopsis lilacinogilva</i>
				Ganodermataceae	<i>Ganoderma applanatum</i>
		Basidiomycota	Agaricomycetes	Polyporales	Lentinaceae
<i>Lentinus strigosus</i>					
<i>Lentinus sajor-caju</i>					
<i>Lentinus sajor-caju</i>					
<i>Lentinus polychrous</i>					
<i>Lentinus tigrinus</i>					
<i>Lentinus tuber-regium</i>					
<i>Panus similis</i>					
Polyporaceae	<i>Microporus xanthopus</i>				
	<i>Polyporus arcularius</i>				
	<i>Polyporus tenuiculus</i>				
Russulales	Russulaceae		<i>Russula rosea</i>		
Dacrymycetes	Dacrymycetales	Dacrymycetaceae	<i>Dacryopinax spathularia</i>		
Tremellomycetes	Tremellales	Tremellaceae	<i>Tremella fuciformis</i>		
Ascomycota	Pezizomycetes	Pezizales	Sarcoscyphaceae	<i>Cookeina tricholoma</i>	
	Sordariomycetes	Xylariales	Xylariaceae	<i>Xylaria cirrata</i>	
				<i>Daldinia concentrica</i>	

Molecular Identification of the *Lentinus* Mushroom Documented from the Western Ghats of Karnataka

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ABSTRACT

The study was conducted with an objective of documenting and conservating the wild edible mushroom of Western Ghats of Karnataka. Mushroom species were collected during the monsoon season from the Nettana forest, Dakshina Kannada district and field data was recorded. Mushroom DNA was isolated and amplified by using ITS1 and ITS4 primers and sequenced. The sequence alignment was made with sequence available in the National Centre for Biotechnological Information (NCBI). The Blast search analysis indicated 98 per cent homology with the *Lentinus sajorajju* Fr.). Pure culture (mycelium) of the mushroom was obtained through tissue culture on potato dextrose agar (PDA) and attempts were made to cultivate the mushroom on paddy straw and sawdust mix in the ratio of 1:1(w/w). Mycelium produced fruiting body at 60 days after spawning. Further, the fruiting body of the mushroom contained 25.55 per cent crude protein.

WESTERN GHATS of Karnataka (India) constitute a mountain range along the western coast of India. It is one of the eight hotspots of biological diversity in the world. Humid weather and high rainfall during monsoon is a congenial atmosphere for the establishment of many kinds of mushroom flora which includes edible, medicinal and poisonous types. One third of fungal diversity of the globe exists in India and Only a fraction of this fungal wealth has been subjected to scientific scrutiny and mycologists continue to unravel the unexplored and hidden wealth. (Manoharachary *et al.*, 2005). There is a need for concerted effort to document the wealth of mushroom biodiversity so that a national database can be developed, which can ultimately help in building up of a global mushroom database of India (Pandey and Veena, 2012). Mushroom contains a substantial amount of dietary fibers, proteins, vitamins and minerals, a good nutritional compound in dietary system (Shirur and Shivalingegowda, 2015).

Mushroom was collected during the rainy season (August 2015) and field information of the mushroom such as habitat, abundance, size, shape, color of the fruiting body, arrangement of the gills were recorded (Arora, 1986). DNA was extracted from the mushroom tissue by using CTAB lysis buffer. The tissue (100 mg) was ground in to fine powder using liquid nitrogen and the sample was transferred into

1.5ml of extraction buffer and incubated at 65°C for 45 minutes. Equal volumes of chloroform: Isoamylalcohol (24:1 v/v) was added and mixed by inverting by tubes. These tubes were centrifuged at 10000 rpm for 10 minutes. Clear supernatant was collected and DNA was precipitated by adding chilled isopropanol. The pellet was washed with 70 per cent ethanol and dissolved in TE buffer. The concentration and purity were measured by using 280 nm and 260 nm / 280nm, respectively in biospectrometer (Eppendorf).

Amplification of 5.8S rRNA gene and ITS region was done by using ITS1 (Forward primer) 5'TCCGTAGGTGAACCTGCGG3' and ITS4 (Reverse primer) 5'TCCTCCGCTTATTGATATGC3' (Rajaratnam and Thiagarajan, 2012). Polymerase chain reaction was performed in a 40µl reaction volume containing 1x Taq buffer with MgCl₂ (1.5mm), dNTPs (200µM), forward and reverse primers (0.5 µM each), Taq DNA polymerase (3U) and template DNA (50ng). Amplification was carried out with an initial denaturation at 95°C for 4 minutes, followed by denaturation at 94°C for 1minute, annealing at 59°C for 30 seconds and extension at 72°C (these three steps for 35 cycles) and a final extension at 72°C for 10 minutes. Amplicon were separated on agarose gel (1%) and documented using gel documentation unit (Alpha Innotech). Amplicon was isolated and purified

by using Genjet Elution kit™ (Thermo Scientific), sequenced (Scigenom Pvt. Ltd., Kerala) and compared with sequence in National Centre for Biotechnology Information (NCBI).

Spawn production was done on sorghum with 2 per cent of each calcium carbonate and calcium sulphate. Mushroom was grown on 1:1 (w/w) proportion of sawdust and paddy straw. One gram of dried sample was digested in 15ml of H₂SO₄ containing 5 gm of K₂SO₄ and 0.5mg of CuSO₄ at 180°C for 15min, 250°C for 30 min, 380°C for 30 min and 450°C for 90 min. The digest was distilled using 40 per cent NaOH and liberated ammonia was trapped in 4 per cent boric acid. It was titrated against 0.1N H₂SO₄ and then protein content was determined by multiplying the nitrogen value with 6.25 (Raghuramulu *et al.*, 2003).

The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model (Nei and Kumar, 2000). The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). Initial tree for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. The analysis involved ITS sequence of *L. sajor-caju* and other 15 different species of the genus *Lentinus*. Evolutionary analyses were conducted in MEGA6 (Tamura *et al.*, 2013).

The Fig. 1a revealed that the Habitat of the mushroom is on dead wood, the growth habit is scattered; Colour of the mushroom cap is light Brown and stem is white; Cap is depressed at the center, Cap diameter was 7-12 cm, Stipe is equal which measures 4-6 cm in length, 2-3mm in thickness; Medium in texture; Stipe Position was central; Gills were Decurrent towards the stalk, Annulus was absent, spore print, was white in color; Structure of the cap cuticle is filamentous. The Fig. 1b revealed that Spore were rod like structure, which were white in color

The sequence data was showed 98 per cent homology for *Lentinus sajor-caju* Fr. in the NCBI

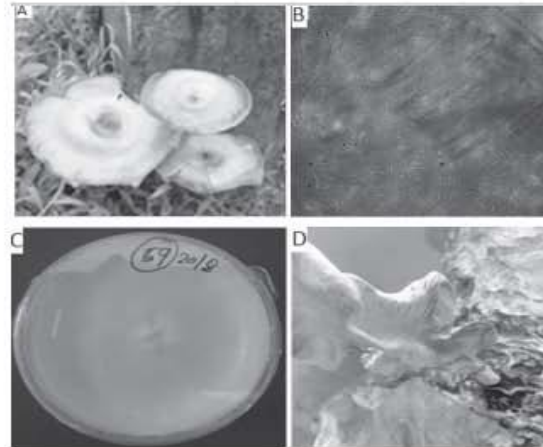


Fig. 1. A) Mushroom grown naturally on the stem of the dead tree; B) Microscopic view of the spores (400 × magnification); C) Pure culture of *Lentinus sajor-caju* on potato dextrose agar medium; d) cultivation of mushroom on 50% paddy straw and 50% sawdust.

nucleotide blast. Therefore, the mushroom was confirmed as *L. sajor-caju* and it belongs to the family polyporaceae of the order polyporales in the class Basidiomycets. We got the accession number KX181288 for the sequence.

Pure culture of the mushroom was obtained on Potato dextrose agar medium (Fig. 1C) which further used for the spawn production. This cultivated

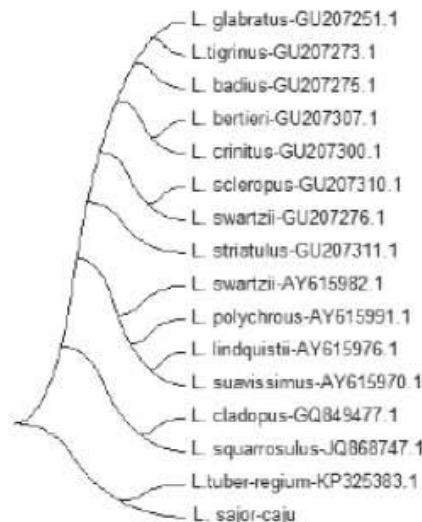


Fig. 2. Molecular Phylogenetic analysis of *L. sajor-caju* with fifteen different species of the same genera by Maximum Likelihood method.

mushroom produced the fruiting body on 60 days after spawing (Fig. 1d). and contained 25.55 per cent of protein.

The Fig.2 revealed that there were eight branches in genus *Lentinus* in which *Lentinus sajor- caju* (marked with dark triangle in Fig.2) is closely evolved with *Lentinus regium*. *L. glabratus* and *L.tigrinus* were distinctly evolved from the *L. sajor-caju*.

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Jelly Mushrooms Documented from Western Ghats of Karnataka (India)

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Western Ghats are one of the biodiversity hotspots of the world which stretches parallel to west coast of India. Climatic conditions are congenial for establishment of diverse mushroom flora. In this study, five Jelly mushroom species were collected during the monsoon season of 2015 from the Western Ghats region of Karnataka (India). Of the five Jelly fungi collected, two species were identified as *Auricularia delicata* (brown strain) and *A. delicata* (white strain) based on phenotypic characters. The other three Jelly fungi were identified using Internal Transcribed Spacer (ITS) region. The sequence alignment made with available data base in the National Centre for Biotechnological Information (NCBI) showed 89%, 99% and 96% homology with *Auricularia polytricha*, *Tremella fuciformis* and *Dacryopinax spathularia* respectively.

Keywords: Jelly mushroom, western ghats, ITS region.

Western Ghats is a mountain range that runs parallel to the western coast of the peninsular India starting from Maharashtra, Goa, Karnataka, Kerala and ending at Kanyakumari in Tamilnadu which covers about 1,60,000 square kilometers. Western Ghats is the one of the eight hottest hotspots in terms of numbers of endemics and endemic species/area ratios for both plants and vertebrates, and habitat loss (Myers *et al.*, 2000). The region receives ample of its rain from south-west monsoon which makes wettest season from June to October. Annual rainfall on Western Ghats averages 2500 mm. However, at Agumbe (place) rainfall exceeds 7600 mm (Dahanukar *et al.*, 2004). The central Western Ghats of Karnataka, known as 'Sahyadri', represents a long mountain chain along the west coast of India and encompass the

districts namely Chikmagalur, Shivamogga, Udupi, Dakshina Kannada, Uttara Kannada, Hassan and Coorg (Kumar *et al.*, 2014). Humid weather and high rainfall during monsoon is congenial atmosphere for the establishment of many kinds of mushroom flora which includes edible, medicinal and poisonous types.

Mushroom is fascinating fleshy fruiting body structure of a fungus belongs to class Basidiomycetes and Ascomycetes (Arora, 1986). The species diversity of fungi and their natural beauty occupy prime place in the biological world and the western ghats of India is a cradle of 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 *et al.*, 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

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et al., 2005). This study reports five Jelly mushrooms from Western ghats of Karnataka.

MATERIALS AND METHODS

The Jelly mushrooms were collected from Western Ghat districts of Karnataka viz., Hassan, Shivamogga and Dakshinakannada during the monsoon 2015. Field information such as habitat, abundance and phenotypic characters like size, shape, color of the fruiting body were recorded (Arora, 1986). The genomic DNA of mushrooms was extracted from tissue by using CTAB lysis buffer (Doyle and Doyle, 1987). Stipe (stem) tissue of mushroom (0.2 g) was ground into fine powder using liquid nitrogen and the sample was transferred into 1.5ml of extraction buffer and incubated at 65°C for 45 minutes. Equal volumes of chloroform: Iso-amylalcohol (24:1 v/v) was added and mixed by inverting by tubes. These tubes were centrifuged at 10000 rpm for 10 minutes. Clear supernatant was collected by removing jelly layer at the top and DNA was precipitated by adding chilled isopropanol. The pellet was washed with 70% ethanol and dissolved in Tris-EDTA buffer. The concentration and purity were measured by using spectrometer (Eppendorf).

Amplification of ITS region was done by using ITS1 (Forward) 5'TCCGTAGGTGAAC CTGCGG3' and ITS4 (Reverse) 5'TCCTCCGCT TATTGATATGC3' primers (Rajaratnam and Thiagarajan, 2012). Polymerase chain reaction was performed for 40µl reaction mixture containing 4µl of 1x Taq buffer with MgCl₂, 4µl of dNTPmix, 1µl each of forward and reverse primers, 0.6µl of 3U Taq DNA polymerase, 1µl (50ng) of template DNA and 28.4µl sterile water. Amplification was carried out with an initial denaturation at 94°C for 4 minutes, followed by 35cycles of denaturation at 94°C for 1minute, annealing at 59°C for 30 seconds and extension at 72°C and a final extension at 72°C for 10 minutes. Amplified product was separated by 1% agarose gel electrophoresis and documented using gel documentation unit (Alpha Innotech). The amplified band is isolated by using Genjet Elution kit™ (Thermo Scientific). The purified PCR product was sequenced (Sci genom Pvt. Ltd., Kerala). The BLAST search for sequence homology was performed with the sequence data available at National Centre for Biotechnology

Information (NCBI) for identification of the three mushrooms.

RESULTS AND DISCUSSION

The white jelly fungus was collected from forest of Sakaleshpur, Hassan district and the brown fungus was from Dakshinakannada district of Karnataka. Fruiting bodies of the two jelly mushrooms was soft, rubbery/gelatinous; translucent. One strain was white in colour and the other was dark brown (Fig. 1a and 1c). The fruit body was sessile to substipitate and reniform to semicircular measuring from 6 to 10 cm diameter. The pileus with translucent hairs at the dorsal side, hymenium conspicuously meruloid to porose reticulate with veins (Fig. 1b) and pale hyaline cream colour. The mushroom grows gregariously on decaying wood. Pileus made up of densely compacted gelatinised hyphae with cuticular hairs with rounded tips. The basidia were cylindrical 42 × 4µm in size with 3 transverse septa. The spores are allantoid with 2 – 3 prominent oil globules



Fig. 1. Jelly mushrooms of Western Ghats of Karnataka- a) *Auricularia delicata* (White strain), b) Dorsal portion of the *Auricularia delicata* showing reticulate like structure, c) *Auricularia delicata* (Brown strain), d) *Auricularia polytricha*, e) *Tremella fuciformis* and f) *Dacryopinax spathularia*

measured 11 × 5 µm. Based on the above phenotypic characters both the strains of mushrooms were identified as *Auricularia delicata* white and brown strains (Sarma et al.,2010). Fruiting body of the *cloud ear* fungus

GGCTTGATTTGGGCTTTTACCCGATCGTTCAGCTGTGCGCCTTTTACCGGGCTGCACG
 CTGGAGCAAAGACCCACACCTGTGCACCTTTTCGGTTGCGGCTTCGGTCGCTGCCGC
 TTTCAAATGCAACAACCTCAAGCCCCGAAGGTTACCAAAACCTTAAAAATTAACACTT
 TTCAACAACGAATCTCTGGGTTCTCCCACCAATAAAAAACCCACC AAATGGCAATA
 ATTAAGGGGAAATTGCAAAAATTCATGGAATCACCAAAACCTTTGAACGCACCTGGCCCT
 CCTGGGAAATTCAGGAACCAGGCCGGTTTGATGGTACAGTAACCCCCCCCCCGGCA
 AGGTACCATCCCCTCGCGGGAACTGGAACCTGGGCGGAACCCGGTTCCGCTTGAAA
 GGCATTACCTGGCCCTTTTAAAGGGCTGGGCAACGGGGGATAATTATCGGCCCA
 AGGCCTTAGGCCTCTTACCGGGGCTGCTTACAGCCGCCCTCTGTGAACACTTTTTT
 TTAACTTTTTGCTCTCATCTCGGGTAAGACTACCCTCTGAACTTACATATATCATAAG
 GGAGG

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
<input type="checkbox"/> Auricularia sp. BAB-4720 18S ribosomal RNA gene, partial sequence, internal transcribed spacer	688	1182	98%	0.0	89%	KR154949.2
<input type="checkbox"/> Auricularia sp. BAB-5209 18S ribosomal RNA gene, partial sequence, internal transcribed spacer	688	1403	98%	0.0	89%	KT186177.1
<input type="checkbox"/> Auricularia sp. BAB-5200 18S ribosomal RNA gene, partial sequence, internal transcribed spacer	671	1165	97%	0.0	89%	KT186175.1
<input type="checkbox"/> Auricularia sp. BAB-5001 18S ribosomal RNA gene, partial sequence, internal transcribed spacer	671	1165	97%	0.0	89%	KR155089.1
<input type="checkbox"/> Auricularia polytricha strain AP3 18S ribosomal RNA gene, partial sequence, internal transcribed	660	1163	98%	0.0	88%	KF297984.1
<input type="checkbox"/> Auricularia polytricha strain SN111 18S ribosomal RNA gene, partial sequence, internal transcrib	660	1163	98%	0.0	88%	KF297977.1

Fig. 2. Partial sequence and homology search of *Auricularia polytricha* ITS region (575bp)

GTGCTTGCATCCGGGAGCAGGCCCTTCCAACACCTGTGCACATCGGACCGCGCCTCC
 GGGCCGGGCCCGCCTTTCACACAAACATATGTCAA GAACGTAATGCATCATAACATGA
 AACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAATT
 GCGAAAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCT
 TGCGCCTTTTGGTATTCCGAAAGGCATGCCTGTTTGAGTGTATGTAGACTCAACCC
 CCGGGTTTTCTGACCCGGCGGTGTTGGATTTGGGCCCTGCCTCTCTGGCTGGCCTTAA
 ATGCGTTAGTGGTTTCACGCAGACGTCGTAAGTTACGCGTCGACTGTGGGCCGCTCA
 CAACCCCTTTACTTTTGCACCTCTGGCCTCAAATCAGGTAGGGCTACCCGCTGAACT
 TAAGCATATCAATAAGCGGAGGA

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/> Tremella fuciformis CCJ1072 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and intern	841	841	95%	0.0	99%	AF042409.1
<input type="checkbox"/> Tremella fuciformis strain CBS 6970 18S ribosomal RNA, partial sequence, internal transcribed sp	821	821	96%	0.0	99%	AF444316.1
<input type="checkbox"/> Tremella fuciformis CCJ1080 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and intern	809	809	95%	0.0	98%	AF042410.1
<input type="checkbox"/> Tremella fuciformis strain ATCC 201809 18S ribosomal RNA gene, partial sequence, internal tran	808	808	96%	0.0	98%	DQ680075.1
<input type="checkbox"/> Tremella fuciformis isolate SMCC174.01.19 18S ribosomal RNA gene, partial sequence, internal	798	798	92%	0.0	99%	FJ501580.1

Fig. 3. Partial sequence and homology search of *Tremella fuciformis* ITS region (479bp)

documented from Dkshinakannada region of Karnataka was gelatinous (Fig.1d), laterally attached to the dead wood. Stipe is short and the fruiting body was dark brown. Basidia cylindrical, hyaline, tri - septate, spores are hyaline, reniform to allantoid, size was $15 \times 4-5$ μ m. Pileus was exactly look like animal's ear (Sarma *et al.*, 2010). Amplified product of ITS region sequenced and blast searched at NCBI GenBank (Fig.2) showed 89% homology with *Auricularia polytricha*. Thus, the mushroom was identified as *A. polytricha*.

The fruit body of Snow fungus documented from Shivamogga district was gelatinous, white and glassy or translucent (fig.1e). Diameter of the fruiting body ranged from 3.5 to 9.5 cm. The fruiting body was erect with branched fronds. Hyphae are clamped and occur in a dense gelatinous matrix. Haustorial cells arise on the hyphae, basidia with oblique to vertical septa. The spores are ellipsoid, smooth, measured 8×6 μ m. Based on these phenotypic characters the mushroom was identified as the genus *Tremella* (Moore and O'Sullivan, 2014). Further the mushroom species was identified by using ITS region sequence. The sequence of the fungus when blast searched with NCBI GenBank showed 99% homology with *Tremella fuciformis* (Fig.3). Thus, the mushroom was confirmed as *T. fuciformis*. Similarly, Spatula-shaped yellow jelly fungus (Fig.1f) was collected from

Dkshinakannada and identified. Spores of this fungus were ellipsoid with smooth-surface and translucent. Average size of the spore was 8×3 μ m, grow in clusters. The ITS sequence (Fig.4) of 447 bp showed 96% homology with *Dacryopinax spathularia* of the class Dacrymyetes. The sequences of the three mushrooms were submitted to NCBI GenBank and accession numbers (*Auricularia polytricha* KX603667, *T. fuciformis*. KX603666; *D. spathularia* KX603668) were obtained.

There are reports on documentation of mushrooms from forests of Western ghats but limits the record of Jelly fungi (Pandey *et al.*, 2012). Usha *et al.*, (2014) reported the occurrence of *T. fuciformis* from Kodagu region of Karnataka. *Tremella fuciformis* has both medicinal and culinary uses. The polysaccharides and steroids it contains reportedly have antitumor and anti-inflammatory properties. The white jelly fungus can be included in desserts and added to soups and other dishes (Hall *et al.*, 2003) and the fruiting bodies of *A. polytricha* are useful adsorbent to remove emulsified oil from water (Yang *et al.*, 2014). However, occurrence of Jelly mushrooms were reported to be one percent of the total mushrooms (Kishnappa *et al.*, 2014). Therefore, there is a need to explore more and more species of jelly fungi, culture and conserve them for further studies as mushrooms are fascinating creatures in nature.

ACCTGTACATGCCCTTCGGGGTAACACACAAACTCTAGTGTGGTCTATGTATGTCTA
GTTATTCATAACAAGTATAACTTTCAACAACGGATCTCTTTGGCTCTCGCATCGATGA
AGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAATAGTGAATCATCGAAT
CTTTGAACGCACCTTGGCCCCGACGGGGCATGCCGGTTTGAGCGCCTGTTTCACTCT
GCACTAGTGGATTCTTCTATTAGAGCGATGTGAGTGTGCTGGTCTTACCAGCTCGCT
CTGAATGCATTAGCAGCAGTTAGGCTTGTGACAACGTGATAAGTCGTCGTTGAAGCA
ATGCTGAGCCGCCGCTCCTAATCGTCTTCGGACAATAACCTAATGCTAGGCATCAAA
GCGGTAGGACTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAG

Sequences producing significant alignments:

Select All (1/1) Selected 0

Alignments

Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/> <i>Dacryopinax spathularia</i> strain re20024.183 ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, strain: re20024.183	728	1383	99%	0.0	96%	EU918893.1
<input type="checkbox"/> <i>Dacryopinax spathularia</i> (ITS1) partial 5.8S rRNA gene, ITS2 and partial 28S rRNA gene, specimen voucher C590301.81272	717	1374	99%	0.0	95%	AF522282.1
<input type="checkbox"/> <i>Dacryopinax spathularia</i> genes for ITS1, 5.8S rRNA, ITS2, partial and complete sequence, strain: TUC12846	715	1385	98%	0.0	96%	AB732473.1
<input type="checkbox"/> <i>Dacryopinax spathularia</i> strain D541 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, strain: D541	701	1304	96%	0.0	95%	KI100245.1
<input type="checkbox"/> <i>Dacryopinax spathularia</i> isolate (mucosulphu) 2 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, strain: mucosulphu	701	1304	96%	0.0	95%	KR155742.1
<input type="checkbox"/> <i>Dacryopinax spathularia</i> isolate of TOL-40-404.183 ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, strain: TOL-40-404.183	660	1313	95%	0.0	95%	AF54070.1

Fig.4. Partial sequence and homology search of *Dacryopinax spathularia* ITS region (447 bp)

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