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Mushroom diversity in the Biligiri rangana hills of Karnataka (India)

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Abstract: Evergreen forests of Biligiri rangana hills (BR hills) spread over an area of 540 sq. KM over eastern most edge of Western Ghats in Karnataka. Climatic conditions are more favorable for establishment of mushrooms and complete their life cycle. In this study, fourteen mushroom species were collected from BR hills regionduringmonsoon (June through September 2013) with the help of Solega tribe inhabited the region since many years. Of the fourteen mushrooms, two mushrooms were identified as *Ganoderma lucidum* and *Polyporous flabelliformis* based on their phenotypic characters. The other 12 mushrooms were identified by ITS (Internal Transcribed spacer)region sequence homology as *Termitomyces* sp. (BRM-3)., *Auricularia delicate* (BRM-4), *Termitomyces microcarpus* (BRM -5), *Amanita* sp. (BRM-6), *Podoscypha petalodes* (BRM-7), *Agaricaceae* sp. (BRM-8), *Macrolepiota* sp.(BRM-9), *Calvatia holothurioides* (BRM-10), *Gymnopillus crociphyllus* (BRM-11), *Coprinus comatus* (BRM-12), *Gyrodontium sacchari* (BRM-13) and *Clitocybeafffellea* (BRM-14). Among the fourteen mushrooms, three species viz., *Termitomyces* species, *Auricularia delicate* and *Termitomyces microcarpus* were edible. The others were non edible/ poisonous species. This study reports the diverse mushroom species as addition to biodiversity at BR hills.

Keywords: Biligiri rangana hills, Documentation, ITS region, Mushroom diversity

INTRODUCTION

BR Hills are located in between 11°47'-12°9' N and 77°0'-77° 16'E. The ridges of hills run in the north-south direction. It is a projection of Western Ghats in a north-eastern direction and meets the hills of Eastern Ghats at 78°E situated in Yelandur and Kollegal Taluks of Chamaraja nagar district of south-eastern part of Karnataka (Sreenivasan and Prashanth, 2005). There is a variation in mean temperature ranging from 9 to 16 °C minimum and 20 to 38 °C maximum. The region receives 600 mm annual rainfall at the base and 3000 mm at top of the hills (Gireesha and Raju, 2013). The wide range of climatic conditions along with altitude made an ecosystem congenial for bio diversity. The Solega tribes inhabited the region over hundreds of years are nature worshippers and mainly dependent on forest products for their livelihood. Mushrooms are sources of good quality protein, vitamins and minerals. They are low in calorie with negligible starch, sugars and fats. In addition, many edible and non-edible mushrooms have been used for medicinal purposes (Wasser and Weis, 1999; Hobbs, 1995). Mushrooms appear during rainy season when the soil is moist and weather conditions are cool and humid. Therefore, mushroom hunting in such seasons is essential.

Mushrooms can be identified based on their morphological characters such as shape, size, texture, colour and odour of the fruiting body. The microscopic characters viz., spore size, spore shape, basidium structure etc. Despite the above, use of molecular tools to identity wild mushrooms have become quick and reliable methods in recent years. The ribosomal RNA genes (rDNA) of fungi are located on a single chromosome and are present as repeated subunits of a tandem array of transcribed and non-transcribed stretches of DNA, which appeared highly conserved. The ITS region/ 18S rRNA gene sequence are the most widely used techniques in molecular phylogenetics to identify the mushrooms up to species level (Rajaratnam and Thiagarajan, 2012). Victor *et al.* (2012) Identified 18 *Termitomyces species* collected from 2 states (Ondo and Ekiti) of Nigeria using ITS region and compared with existing sequences in NCBI Gen Bank. Several workers from elsewhere reported molecular identification of mushrooms using ITS 1 and ITS 2 primers (Oyetayo, 2012; Sudip *et al.,* 2013). The BR hills as reserve forest area, various flora, animals and birds diversity was reported with help of forest and ecology department. This study, reports the mushroom diversity of the protected area.

MATERIALS AND METHODS

Collection and documentation of mushrooms: Mushrooms were collected during rainy season from June to September 2013 with the help of tribal (Solega)

people in the agricultural field bunds, forest anddomicile area of the Soligas, in a paper bags and were named as BRM-1, BRM-2, BRM-3, BRM-4, BRM-5, BRM-5, BRM-7, BRM-8,
BRM-9, BRM-10, BRM-11, BRM-12, BRM-13and BRM-14(BRM=Biligiri Rangana hills Mushroom).Field information for all the mushrooms during collection was recorded.

Molecular characterization:

Genomic DNA Isolation: Total genomic DNA from cap/ stipe tissue of the mushrooms was extracted using CTAB method (Sambrook *et al.,* 1989). The DNA concentration was measured using Nanodrop (Eppendorff) and then PCR amplification was carried out in 40µl reaction mixture containing 4.0µl of 10 X PCR Taq-Buffer,4.0 µl of 10mM dNTP's mix, 2.0 µl of ITS primers (ITS1-5'TCCGTAGGTGAACCTGCGG3'and ITS4-5' TCCTCCGCTTATTGATATGC 3', 0.6 µl of Taq. DNA polymerase,2.0 µl of Template DNA (~50 ng) and 27.4 µl of sterile distilled water.

PCR amplification: The PCR reaction was carried out in a Thermal Cycler (Applied Bio systems) programmed as initial denaturation at 96°C for 3min, 40 cycles of denaturation of 94°C for 1 min, annealing at 60°C for 30sec and extension at 72°C for 1 min and final extension at 72° C for 10 min. The amplified products were separated by agarose gel electrophoresis. The gel was visualized under UV light and documented using Alpha Innotech Gel documentation unit. The amplified product was eluted using Gene JET™ Gel Extraction Kit (Thermo Scientific) following manufacturer protocol.

Cloning, transformation and sequencing: The eluted product was cloned into pTZ57R/T cloning vector using InsT/A clone PCR product cloning kit [MBI, Fermentas Life Sciences, USA (#K1214)] after determining the appropriate vector: insert ratios (Sambrook *et al.,*1989). The ligation reaction was performed in a 10µl reaction volume at 16°C overnight. The ligated product was transformed in to *Escherichia coli* (DH5α) cells using heat shock method (Sambrook *et al.,* 1989) and plated on Luria Berton (LB) agar medium containing antibiotic (ampicillin, 100 µg/ml). The recombinant clones were initially screened for blue white selection, followed by colony PCR using M13 forward and reverse primers (Sambrook *et al.,* 1989). Then the transformed colony was multiplied in LB broth containing 100 µlampicillin for overnight and the recombinant plasmid was isolated using GenElute^{TM}HP Plasmid MiniPrep Kit (Sigma, USA) following the manufactures protocol. The isolated plasmid was sequenced at Sci Genome Labs Private Ltd. Kerala, INDIA using M13 forward and reverses primers.

Sequence analysis and homology search: Sequence results were analyzed with Vec Screen online software from National Centre for Biotechnology Information (NCBI) for removing the vector contamination. Forward and reverse primer sequences were checked against each other by generating the reverse complement of the "reverse" sequence using Fast PCR Professional (Experimental test version 5. 0. 83) and aligning it with the "forward" sequence with the help of CLUSTAL W Multiple Sequence Alignment Programme using online software SDSC Biology Workbench (San Diego Supercomputer Center). The full-length gene homology search was performed with blast programme of NCBI (Altschul *et al*., 1990).

RESULTS AND DISCUSSION

In the present study two mushrooms (BRM-1 and BRM-2) collected in the month of August 2012 were identified based on phenotypic characters. The BRM-1 bearing robust corky fruiting body was found growing on the hard wood. The pileus was reddish brown colour and border surrounded by white colour, embricate in shape. The diameter of cap was 14 cm and stipiate polypore (Table, 1 and Fig.1). The other mushroom (BRM-2) vernacularly known as Maraanabe was found growing on dead wood. Sporophore was stipiate, pileus was deep brown colour with depression at the center (Fig.2). Basidiaclavate, gills were absent, scattered growth habit. After verifying these characters with the keys described for classification of polyphores by Bakshi (1971), they were identified as *Ganoderma lucidum* (BRM-1) and *Polyporus flabelliformis,*(BRM-2).

Molecular identification:Molecular identification was done for other 12 mushrooms using ITS region sequence. The gene sequence analysis (BLAST) was made with the sequences available at NCBI Gen Bank. The Fig.3 represents the full length homology search of BRM-13 (*Gyrodontium sacchari*), (BLAST search of other mushrooms not shown). The sequence length (base pair) of 12 mushrooms was given in Table 2.

BRM-3: Vernacularly known as Doddaanabe due to its robust fruiting body, scattered growth habit (Fig. 4a). Thepileus up lifted creamy white, 12 cm in diameter. The stipe tapered downward, central and 9 cm in length. Gills were free, annulus and rhizoids present. The ITS region with 734bp sequence showed 99 per cent homology with *Termitomyces* sp.

BRM-4: Vernacular name of this mushroom was Mavuanabe. Because of its jelly texture it was known as jelly fungi. Fruiting body was brown in colour(Fig.4b). Amplified ITS region (604bp) showed 99 per cent homology with *Auricularia delicata*.

BRM-5: It is a mini edible mushroom and locally named as Kolianabe, collected near termite nests. Fruiting body was white colour with plane pileus (Fig. 4c). Stipe present in central position. Gills present and annulus absent. The blast analysis of 603bp sequence showed 95% homology with *Termitomyces microcarpus*.

BRM-6: Pileus was creamy white, convex shaped (Fig. 4d). Stipe tapered upward and central in position, length was 15 cm. Scales present on pileus and stipe surfaces. Gills were adnexed, annulus present. The

Table 1. Field information of wild mushrooms collected from Biligirirangana hills.

Sl. No	Mushrooms collected	Vernacular Name	Date of collection	Edibility	Habitat
1	BRM-1	Dodamaraanabe	09/08/2013	Medicinal	Tree
2	BRM-2	Mara anabe	26/08/2013	Non edible	Wood
3	BRM-3	Dodaanabe	26/08/2013	Edible	Soil
4	BRM-4	Mavuanabe	20/07/2013	Edible	Tree
5	BRM-5	Kolianabe	05/09/2013	Edible	Soil
6	BRM-6	Not Known	23/06/2013	Poisonous	Humus
7	BRM-7	Not Known	05/09/2013	Non edible	Soil
8	BRM-8	Huchanabe	23/06/2013	Non edible	Soil
9	BRM-9	Not Known	26/08/2013	Non edible	Soil
10	BRM-10	Not Known	09/08/2013	Non edible	Soil
11	BRM-11	Not Known	23/06/2013	Non edible	Dead wood
12	BRM-12	Not Known	09/08/2013	Non edible	Soil
13	BRM-13	Haladianabe	09/08/2013	Non edible	Dead wood
14	BRM-14	Kotesutthuanabe	05/09/2013	Non edible	Soil

BRM= BiligiriRanganaHill Mushroom

Table 2. List of Mushroom species identified using ITS gene sequencing.

S. N.	Mushroom sample	Mushrooms identified	Size of amplified product (bp)	Homology (%)
1	BRM-3	*Termitomyces* sp.	734	99
2	BRM-4	*Auricularia delicata*	604	99
3	BRM-5	*Termitomyces microcarpus*	603	95
4	BRM-6	*Amanita* sp.	614	99
5	BRM-7	*Podoscypha petalodes*	693	99
6	BRM-8	*Agaricaceae* sp.	729	99
7	BRM-9	*Macrolepiota* sp.	726	98
8	BRM-10	*Calvatiaholo thurioides*	732	95
9	BRM-11	*Gymnopilus crociphyllus*	542	99
10	BRM-12	*Coprinus comatus*	822	99
11	BRM-13	*Gyrodontium sacchari*	736	99
12	BRM-14	*Clitocybeaff. fellea*	736	99

BRM= BiligiriRanganaHill Mushroom; bp= base pair

sequence of the ITS region (614 bp) showed 99% homology to *Amanita* sp. available at NCBI Gen Bank.

BRM-7: ThePileus creamy, petaloid with leathery texture (Fig. 4e). Stipe, gills and annulus were absent. The ITS sequence (693bp) showed 99 percent homology with *Podoscyphapetalodes*.

BRM-8: Themushroom was vernacularly known as Huchanabe in Kannada.Pileus creamy white, convex shaped (Fig. 4f). Stipebulged at the bottom 12cm length. Gills and annulus present. The sequence of ITS region (729bp) showed 99 per cent homology with *Agaricaceae* sp.

BRM-9: Large fruiting body with soft and crispy texture; scattered growth habit. Convex shaped pileuswith prominent scales on surface (Fig. 4g).Stipe enlarged at the bottom.Gills present and annulus absent. The sequence homology (726bp) of blast analysis showed 98 per cent homology with *Macrolepiota* sp.

BRM-10: Orangecoloured fruiting body with cone shape (Fig. 4h).Scattered growth habit; rhizoids present. Gills absent, soft textured. Sequence analysis ofITS region (732bp) showed 95 per cent homology with *Calvatiaholo thurioides* available at NCBI Gen Bank.

BRM-11: Itwas found on dead wood (Fig. 4i).Pileus was yellow and convex shaped. Stipe tapered upward, 4cm long.Gills present, annulus absent. Fruit body texture was soft. The analysis of amplified ITS sequence (542bp) showed 99 per cent homology with *Gymnopilus crociphyllus*.

BRM-12: Soft textured white mushroom,convex shaped pileus (Fig. 4j) with 9 cm diameter. Stipe was club shaped 14 cm long.Gills and annulus present. The ITS region sequence (822bp) in blast analysis showed 99 per cent homology with *Coprinus comatus*.

BRM-13: Vernacularname of the mushroom wasHaladianabedue to itsyellowish tinge (Fig. 4k). Pileus and stipe absent, corky textured, teeth present below the surface. The sequence (736bp) showed 99 per cent homology with *Gyrodontium sacchari*.

BRM-14: The mushroom was known as Kotesuthuanabe. Found in clusters, Pale brown colored pileus, stipe and annulus absent (Fig. 4l).Gills decurrent with soft texture. ITS region sequence of 736 bp showed 99 per cent homology with *Clitocybeaff fellea.*

Mushroom hunting is an art which needs skill and experience to differentiate edible mushroom from poisonous species as there are no pool proof methods available so far. However, by knowing the species after taxonomic identification, one can ensure precise identity. Therefore,

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

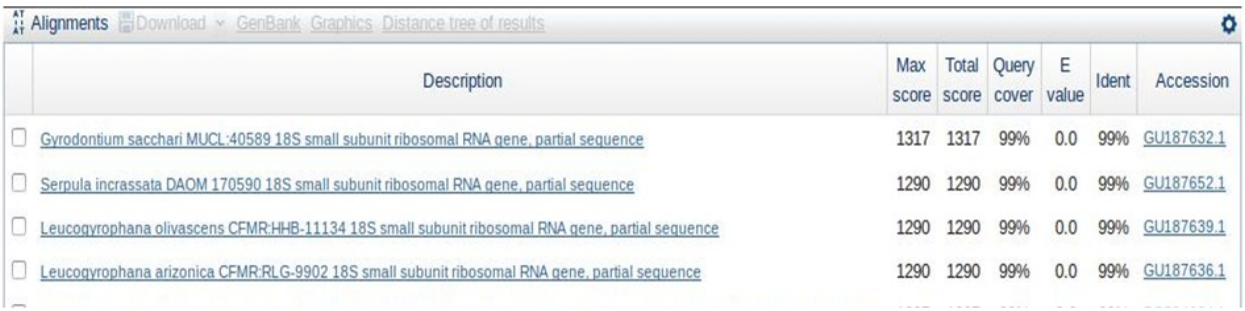
Fig.2. *Polyporus flabelliformis (BRM-2)*

scientific identification is very much essential in this context. Mushroom hunting is a never ending process as occurrence of different species depends on season, climate, habitat and rainfall (Arora, 1986). In this study, totally fourteen species of mushrooms were documented from BR Hills with the help of Forest Department and Solegatribe inhabiting the area. The observations recorded for phenotypic characters of pileus, stipe, gills arrangement and microscopic characters like spore shape, color, the two mushrooms,BMR-1 and BMR-2 were identified as *Ganoderma lucidum* and *Polyporus flabelliformis*, respectively (Bakshi, 1971). In classical taxonomy, identification of mushrooms is being done on phenotypic characters (Arora, 1986; Bhatt *et al.,* 1995). Meera and Veena, (2012) collected 45 species of mushrooms and identified by morphological characters from Kodagu district of Western Ghats, Karnataka.

Classical taxonomy depends on phenotypic characters, which some time leads to confusion between taxonomists and it may not useful for identification of younger fruit bodies. In such circumstances, the molecular identification using 18S rRNA gene/ITS region sequence is essential. In molecular taxonomy, the gene encoding for 16SrRNA in prokaryotes and 18S rRNA/ITS in eukaryotes are most widely used (Gross *et al*. 2001). The small subunit (30S) ribosomal RNA (SSU rRNA) genes sequence have been extensively used for sequence based evolutionary analysis because, they are universally distributed, functionally constant, sufficiently conserved and have adequate length and therefore, providing a view of evolution encompassing all living organisms (Madigan *et al*., 2009).

The 18S rRNA/ ITS flanking sequence are highly conserved in eukaryotes. Primer developed for flanking sequence of 18S r RNA/ ITS can be used as a universal

GTCAGAGGTGAAATTCTTGGATTTACTGAAGACTAACTACTGCGAAAGCATTT-
GCCAAGGATGTTTTCATTAATCAAGAACGAAGGTTAGGGGATCGAAAACGATCAGATACCGTTGTAGTCTTAACA
GTAAACTATGCCGACTAGGGATCGGGCAATCTCTTTTTGATGTGTTGCTCGGCACCTTACGAGAAATCAAAGTCTT
TGGGTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAG
CCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACATGACTAGGATTGACAGATTGATAGCT
CTTTCATGATTTTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTATCCGATAACGA
ACGAGACCTTAACCTGCTAAATAGCCAGGCTGGCTTTCGCTGGTCGCCGGCTTCTTAGAGGGACTGTCAGCGTCT
AGCTGACGGAAGTTTGAGGCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGA
CAGAGCCAGCGAGTTCTTTTTCCTTGGCCGGAAGGTCTGGGTAATCTTGTGAAACTCTGTCGTGCTGGGGATAGA
GCATTGCAATTATTGCTCTTCAACGAGGAATTCCTAGTAAGCGTGAGTCATCAGCTCGCGTTGATTACGTCCCTGC



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

Description	Max score	Total score	Query cover	E value	Ident	Accession
Gyrodontium sacchari MUCL:40589 18S small subunit ribosomal RNA gene, partial sequence	1317	1317	99%	0.0	99%	GU187632.1
Serpula incrassata DAOM 170590 18S small subunit ribosomal RNA gene, partial sequence	1290	1290	99%	0.0	99%	GU187652.1
Leucogyrophana olivascens CFMR:HHB-11134 18S small subunit ribosomal RNA gene, partial sequence	1290	1290	99%	0.0	99%	GU187639.1
Leucogyrophana arizonica CFMR:RLG-9902 18S small subunit ribosomal RNA gene, partial sequence	1290	1290	99%	0.0	99%	GU187636.1

Fig. 3. *Full length sequence and homology search of BRM-13 (Gyrodontium sacchari).*

Contd.

Fig. 4. *(a)Termitomyces sp. (BRM-3), (b)Auricularia delicata (BRM-4), (c)Termitomycesmicrocarpus (BRM-5), (d) Amanita sp. (BRM-6), (e)Podoscypha petalodes (BRM-7), (f)Agaricaceae sp. (BRM-8), (g) Macrolepiota sp. (BRM-9), (h)Calvatiaholo thurioides (BRM-10), (i)Gymnopilus crociphyllus (BRM-11), (j)Coprinus comatus (BRM-12), (k)Gyrodontium sacchari (BRM-13), (l)Clitocybeaff.fellea (BRM-14).*

primer for all eukaryotic species (Rajaratnam and Thiagarajan, 2012). We identified 12 mushrooms by using ITS primers showed variation in the amplicon size (Table 2). Amplicon size of the ITS region varied from 542-822bp for different mushroom species in this study. This could be due to insertion, deletion, duplication and translocation. Further, it may also be due to species variation (Zhihong*etal*., 2003). Sudip*et al.* (2013) collected eight wild edible mushrooms from eastern Chota Nagpur plateau of West Bengal, India and was identified using ITS 1 and ITS 2 primers. The aligned sequence revealed identity of *Amanita hemibafpha, Amanita* sp*., Astraeushygrometricus, Termitomyces*sp*., Termitomyces* sp*., Volvariellavolvceae, Termitomyces* sp.Thus, molecular identification has become faster and precise method of classification compared to classical taxonomy. However, molecular taxonomy will be blind without knowing morphological identity to mushroom hunter. Out of fourteen mushrooms collected in this survey, three mushrooms *viz.*, *Termitomyces* sp.,*Auricularia delicata,* and *Termitomyces microcarpus* belonged to edible species and the*A. delicate* being a cultivable mushroom can be exploited for commercial production. The other species are good decomposers of organic matter.

Conclusion

The BR hills are the hot spot of biodiversity. Various flora and fauna including birds are documented by the Department of Ecology and Environment of Karnataka. This study adds the mushroom resources to the biodiversity. Further, the study revealed that the diversity of mushroom flora (edible, poisonous and medicinal species) which are of scientific importance besides for commercial exploitation.

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