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Morphological and molecular characterization of some mushrooms in Kashmir Himalayan Forests

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ABSTRACT

Mushrooms comprise an important, yet less explored, part of the Himalayan biodiversity. Hence, intensive field surveys were carried out in the coniferous forests of Yusmarg, Gulmarg, Mammer, Kellar and Pahalgam during the growing seasons of 2016-17 for collection of wild mushrooms. Overall 25 species of mushrooms were collected including the species namely Morchella esculenta, Coprinus comatus, Fomes fomentiarus, Ganoderma lucidum, Neolentinus sp.,Suillus sibiricus, Suillus granulates, Lactarius deliciosus, Russula atropurpurea, Russula aurea, Calvatia sp., Lycoperdon sp., Agaricus bisporus, Cantharellus cibarius, belonging to order Pezizales, Agaricales, Polyporales, Gloephyllales, Boletales, Russulales, Cantharellales and families Coprinaceae, Ganodermataceae, Gloeophyllaceae, Cantharellaceae, Agaricaceae. These were found growing mostly on leaf-litter except Coprinus comatus, Ganoderma and Fomes found on lignicolous habitat. The detailed morphometric measurements were undertaken. All these species are being molecularly characterized and their therapeutic potential is being worked out.

Keywords: *Biodiversity, Himalayan, Mushrooms, Species*

I. INTRODUCTION

Nature has bestowed Kashmir with special geographical settings, climatic conditions and forest cover, apt for sustaining bewildering diversity of mushrooms. Inadequate exploration and lack of proper identification is a major bottleneck in the way of fair assessment of their extent of diversity[1], calling for an urgent exploration for documentation and characterization. The present work is a part of our broad approach on assessment of the mushroom diversity of the Kashmir valley using molecular approaches. It was a gap –filling attempt to generate passport data to also identify different species on the basis of morphometric analysis.

II. MATERIALS AND METHODS

2.1 MORPHOLOGICAL CHARACTERIZATION:

Extensive field surveys were conducted in the coniferous forests of district Anantnag , Baramullah ,Budgam, Ganderbal, and Pulwama of Kashmir valley during 2016-2017 growing seasons. For the collection of sporocarps standard methods were followed [2]. Different mushroom species were collected in suitable collection bags. Photographs were taken by Nikon D5300 DSLR Camera with a zoom lens of 18 – 140 VR. Passport data and the micro-habitat characteristics of collected species were recorded in the field book (TABLE 1). Sample specimens of each type were properly labelled, given a voucher number and carried to laboratory for

detailed morphometric examination[3].Collected specimens were identified by keen observation of structures like pileus, stipe , their shape , structure , gill attachment etc using standard keys (eg .Mycokey, Index fungoram etc) and field guides. Varoius parameters like cap diameter , cap shape, cap color, cap margin, stipe length, stipe diameter, stipe color, thickness of gills, gill attachment, gill spacing, presence /absence of scales, presence /absence of annulus etc. were recorded .

2.2 MOLECULAR CHARACTERIZATION:

The molecular characterization of sporocarps involved the sequencing of internal spacer (ITS) region of the nuclear ribosomal genes (rDNA). [4]

2.2.1 DNA EXTRACTION

Genomic DNA was extracted from fresh sporocarps by manual CTAB method (cetyl trimethyl ammonium bromide). For each species DNA was isolated from five sporocarps and the samples were processed separately. [5] 200–250 mg of material was weighed and grand into fine powder with the aid of liquid nitrogen. 5 ml pre-warmed CTAB buffer (1 M TrisHCl pH 8.0, 5 M NaCl, 0.5 M EDTA pH 8.0, CTAB, 2 % b-Mercaptoethanol) was added to this powder. This mixture was subjected to various steps like addition of chloroform, iso-propyl alcohol, phenol, isoamyl alcohol ribonuclease and finally the DNA pellet was kept in 50 µl TE buffer at -20 C^0. The purified DNA was separated in a 1 % agarose gel stained with ethidium bromide and the concentration was estimated by comparison with known length standards. [6]

. **2.2.2 PCR ANALYSIS**

The ITS region of rDNA was amplified by polymerase chain reaction (PCR) using ITS1 and ITS4 primers in Applied Biosystems 2720 Thermal Cycler. The 50 µl reaction mixture for PCR amplification contained 2 µl template DNA, 5 µl PCR buffer, 5 µl of 2 mM DNTps, 1 µl of each primer, and 0.4 µl of Taq polymerase,4 µl $MgCl_2$ and 31.6 millique water. Amplification program started with an initial denaturation step of 94 ^{0}C for 5 min followed by 35cycles of 94 ^{0}C for 40 secs , with an annealing step of 54 ^{0}C for 30 secs, and 72 ^{0}C for 2 min, and a final extension of 72 ^{0}C for 10 min. The purified PCR products of the ITS amplified region were directly sequenced in both directions using the ITS1 and ITS4 pair of amplification primers.[I]

2.2.3 DNA SEQUENCE ASSEMBLY AND ALIGNMENT

Finally the sequenced PCR amplicons were BLAST (Basic Local Alignment Search Tool) searched using the National Center for Biotechnology Information (NCBI),USA data-base for comparison of sequences .The initial alignment of all sequences were made directly using Clustal X multiple alignment program (Higgins et al 1992)(Fig 1 and Fig 2)

III. RESULTS:

Extensive field surveys were conducted in coniferous forests of District Ganderbal, Anantnag, Pulwama , Baramullah and Budgam (TABLE 2) areas of Kashmir Region[7][8] . A total of 25 different types of mushrooms have been collected so far (TABLE 3) . A few of them are unknown.

- In general mushrooms were found abundantly in moist and shady habitats of coniferous forests which are least disturbed .[9]
- Most of mushrooms form mycorrhizal associations with coniferous trees like Pinus wallchiana , Abies pindrow, Piceae smithiana. Few of them like Ganoderma applantum and Fomes have lignocolous growth habit.[10]
- In the present study 25 species of mushrooms belonging to 16 genera and 13 families were recorded. Conspectus of species distribution revealed that Agaricaceae , Coprinaceae and Russulaceae were the dominant families .
- The basidiomycetes constituted the major proportion i.e; 22 species while Ascomycetes constituted only 3 species.
- Majority of mushrooms collected belong to gilled fungi while as species of Boletaceae were porous fungi. Puffballs and cup fungi also lack gills.
- Species of Coprinus, Flammulina, Peziza were found in clusters while as other species occur in scaterred patches.
- Mushrooms belonging to genus Suillus and Neolentinus have been characterized molecularly. The sequenced result of two specimens have been shown in the form of a chromatogram.(Fig 3 and 4)

IV. FIGURES AND TABLES:

TABLE1. PASSPORT DATA OF *Cantharellus cibarius*

S.NO	SCIENTIFIC NAME	LOCATION	HABITAT	SEASON	DESCRIPTION
1.	*Cantharellus cibarius*	Gulmarg	Dense forest area with mixed plantation found on moist soil under shrubs	Summer	Length of stipe =5.5 cm Diameter of cap =5.3 cm Stipe diameter =0.7 cm Gill spacing = 0.1 cm Cap shape : Infundibuliform Cap color : yellowish cream Cap margin :Wavy and inrolled Thickness of gills : Venticose Stipe base : Unswollen Attachment of gills :Decurrent Stipe attachment : Central Gill branching : Dichotomous Gill margin : Smooth and entire

					Stipe shape :Cylindrical Stipe interior : Solid

TABLE 2. STUDY AREA FOR THE COLLECTION OF MUSHROOMS

Site Name	Altitude masl	Latitude	Longitude	Site Characteristics	District
Kellar	1630	33 °46' N	74°46' E	Open coniferous forests	Pulwama
Gulmarg	2703m	34°03' N	74°23' E	Dense forest area with mixed plantation	Baramulla
Yusmarg	2400m	33°50' N	74°38' E	Open forests and some grassy forest fields	Budgam
Pahalgam	2740 m	34°01N	74°31' E	Open Coniferous forests	Anantnag
Mammer	2400 m	34°14N	75°01' E	Open forest areas with coniferous trees	Ganderbal

TABLE 3: LIST OF COLLECTED MUSHROOMS

S no .	Family	Genus	Species
1.	Agaricaceae	*Agaricus*	*1. Agaricus campestris* *2. Agaricus bisporus*
		Lycoperdon	*3.Lycoperdon pyriforme* *4.Lycoperdon perlatum*
2.	Boletaceae	*Suillus*	*5.Suillus granulatus* *6.Suillus sibiricus*
3.	Cantharellaceae	*Cantharellus*	*7 .Cantharellus cibarius*
4 .	Coprinaceae	*Coprinus*	*8. Coprinus disseminatus* *9. Coprinus comatus* *10. Coprinus atramentaria*
5	Fomitopsidaceae	*Fomitopsis*	11 .*Fomitopsis rosea*
6	Ganodermataceae	*Ganoderma*	*12.Ganoderma applanatum*
7.	Gloeophyllaceae	*Neolentinus*	*13.Neolentinus lepideus*

8.	Morchellaceae	*Morchella*	*14.Morchella esculenta*
9.	Pezizomycetes	*Peziza*	*15.Peziza vesiculosa*
10.	Physalacriaceae	*Flammunila*	*16 .Flammulina velutipes*
11.	Polyporaceae	*Fomes*	*17 .Fomes fomentarius*
		Trametes	*18.Trametes versicolor* *19.Trameteshirsuta*
12.	Russulaceae	*Russula*	*20.Russula firmula*
		Lactarius	*21.Russula aurea* *22 .Lactarius deliciosus* *23 .Lactarius scrobiculatus*
13.	Sclerodermataceae	*Scleroderma*	*24 .Scleroderma citrinum*

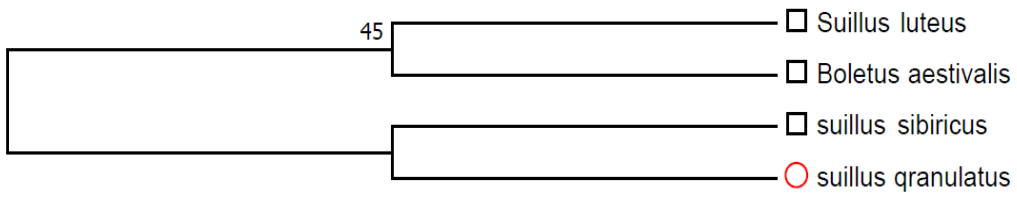

Figure1. Phylogenetic tree of ***Suillus sp.***

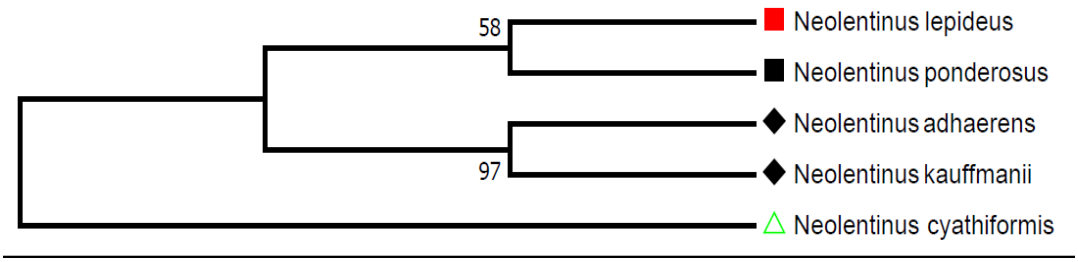

Figure. 1 Phylogenetic tree of ***Neolentinus sp.***

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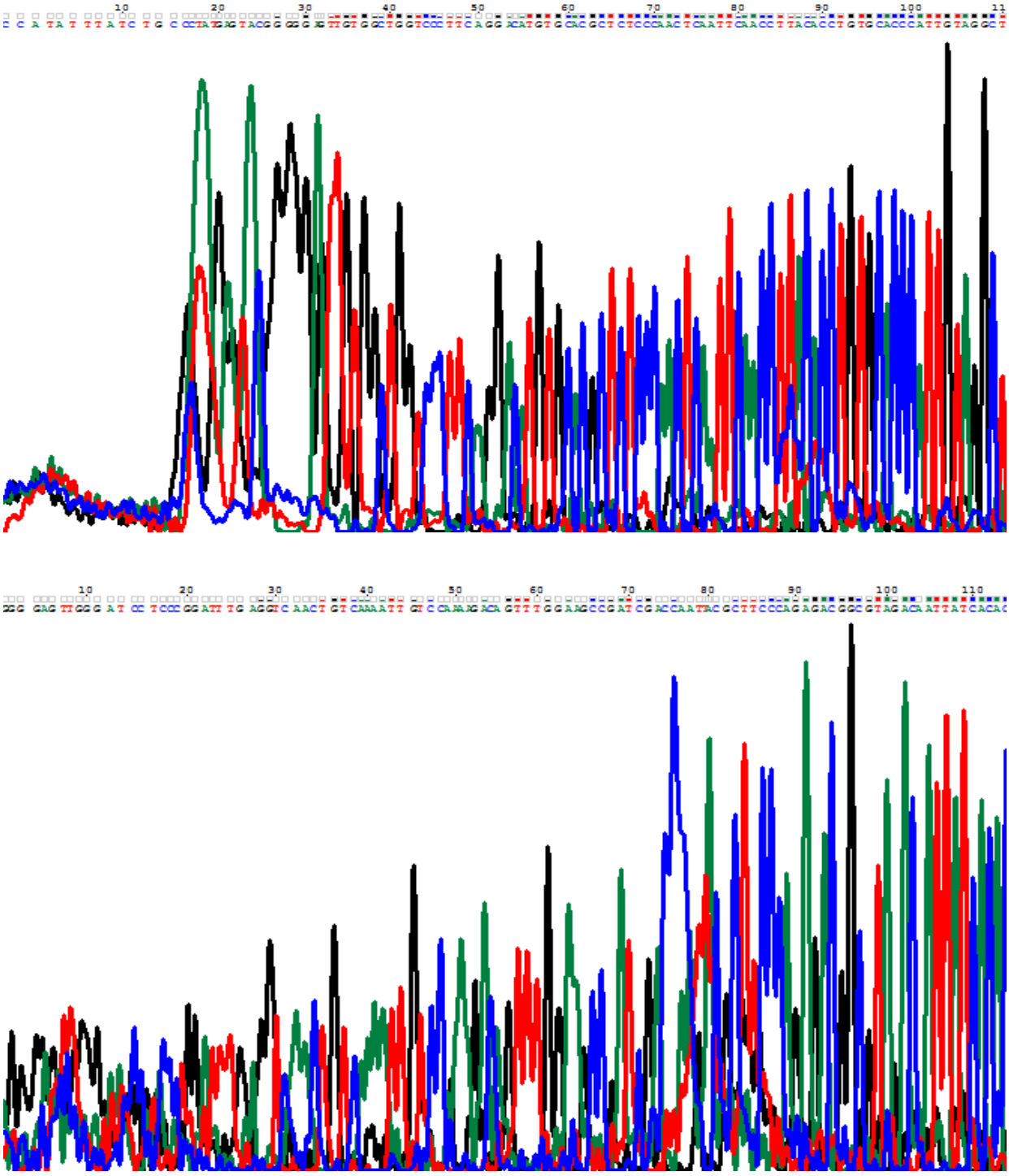

Figure 3 and 4 :Chromatograms showing sequenced results B17-ITS 1and B17-ITS 4 *(Neolentinus lepideus)*

V.CONCLUSIONS

The present work enabled us to assess the diversity of mushrooms in the different regions of Kashmir Himalaya. By using different keys we have done the morphometric analysis of mushrooms that gives us an insight that

even species belonging to same genus vary in certain traits .Furthermore, since there are many indiscrepancies in the accurate identification of mushrooms, therefore, applying the molecular approaches along with morphological approaches authenticated their taxonomic status and we able to identify them upto species level. This work will also lead us to discover new species as the fungal diversity is very huge .

Paxillus involutus ***Lactarius deliciocus*** ***Neolentinus lepideus***

Suillus sibiricus ***Coprinus comatus*** ***Russula atropurpurea***

Amanita pantherina ***Ganoderma applanatum*** ***Fometopsis pinicola***

VI.ACKNOWLEDGEMENT

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REFRENCES

Journal Papers:

[1]Rahi,D.K & Malik, D. (2016).Diversity of mushrooms and their metabolites of nutraceutical and therapeutic significance.*Journal of Mycology*,*2016*.

[2] Gateri, M. W., Ndung, U. B., Muriuki, A. W., Rauwl, V., & Kabacia, S. (2014).Collection, identification and morphological characterization of indigenous mushrooms in coastal Kenya.In *Proceedings of 8th International Conference on Mushroom Biology and Mushroom Products (ICMBMP8), New Delhi, India, 19-22 November 2014.Volume I & II* (pp. 17-23).

[3] Itoo, Z. A., Reshi, Z. A., Majeed, S. T., Basharat, Q., & Andrabi, K. I. (2016).Morphological and Molecular Characterization of Ectomycorrhizal Amanita Species Associated with Pinus wallichiana AB Jacks. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *86*(4), 985-994.

[4] Ojwang**,** D. O. (2014).*Molecular Characterization of the Wild Edible Mushrooms of the Pleurotus species in Kenya* (Doctoral dissertation).

[5] Pushpa, H., and Purushothama, K. B. (2014). Taxonomic studies and molecular characterisation of Tricholoma giganteum and Calocybe indica isolates from Bangalore. *Journal of Biochemical Technology*, *3*(5), 218-220.

[6] Aamir, S., Sutar, S., Singh, S. K., & Baghela, A. (2015). A rapid and efficient method of fungal genomic DNA extraction, suitable for PCR based molecular methods. *Gene*, *42*(2), 169-173.

[7]Dar,G. H.,Ganai, N. A., Beigh, M. A., Ahanger, F. A., & Sofi, T. A. (2010). Biodiversity of macro-fungi from conifer dominated forests of Kashmir, India. *J Mycol Pl Pathol*, *40*(2), 169-171.

[8]Dar, G. H., Beig, M. A.,& Ganai, N. A. (2009).Diversity of ectomycorrhizal fungi of various forests in District Baramulla (Jammu & Kashmir). *Indian Journal of Forestry*, *32*(1), 137-140.

[9] Dar, G. H., Beig, M. A., & Ganai, N. A. (2010). Biodiversity of ectomycorrhizal fungi of Pahalgam forests (Jammu & Kashmir). *Indian Journal of Forestry*, *33*(3), 373-376.

[10] Watling, R., & Abraham, S. P. (1992).Ectomycorrhizal fungi of Kashmir forests. *Mycorrhiza*, *2*(2), 81-87.

Thesis:

[I] Itoo Z.A (2014).*Molecular characterisation of some mushrooms of Kashmir Himalaya and their screening for anticancer bioactive molecules*,Department of Botany,University of Kashmir