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Diversity of Foliicolous Fungi in Kashmir

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Abstract

The fungus kingdom encompasses an enormous diversity of taxa with varied ecologies, life cycle strategies, and morphologies ranging from unicellular aquatic chytrids to large mushrooms. Studying fungal diversity is vital if we want to shed light on terrestrial ecosystem functioning. However, there is still poor understanding of fungal diversity and variation given that fungi are highly diversified and that most of fungal species remain uncultured. In this study we explored diversity across the Kashmir region using the basic morphotaxonomic tools. In total we explored 12 sites and collected the fungal species in vast numbers. Fungal beta-diversity patterns were significantly explained by the environmental conditions, and were very consistent for abundant/rare and fungal/unidentified species confirming the ecological significance of rare/unidentified species, and therefore the existence of a fungal rare biosphere. This study shows that a beta-diversity estimation based on morpho taxonomy is robust enough to support ecological studies in the region. Additionally, our results suggest that rare fungal species harbour ecological information. Thus the fungal rare biosphere may be important for ecosystem dynamics and resilience in the region. Further, the mycotaxonomic characterizations revealed ample amount of genetic divergences probably due to the persistent extremely harsh environmental conditions in the surveyed area. Being deciduous nature of flora, the fungal growth may have encountered the extremes of harsh weather viz. prolonged chilly snow periods prevailing in the region which might have result in variations in fungus genotype as well as phenotype.

Keywords: Ecologies; Chytrids; Beta-Diversity; Genetic Divergences; Phenotype

Introduction

Fungi formed a part of human life from thousands of years. They have been utilized as food, in preparing alcoholic beverages and also as medicines. A new era of fungal research actually started with the discovery of Penicillin by Fleming in 1928. Addition to the knowledge of fungi has continuously been taking place with the urge to explore new organisms for unknown commercially exploitable, bioactive metabolites.

Jammu and Kashmir occupies a prime and unique place in the variety and galaxy of macro-fungi as well as micro-fungi due to wide agro climatic variations, diverse physiography and undulating topography. Although, the fragmentary work in this field from different parts of state has already been done by some researchers. But understanding the micro-fungal flora of the Kashmir is still in an exploratory or pioneer stage and undoubtfully there are many more species to be recorded (Watling & Abrahim, 1992) [1]. Watling and Gregony, (1980) [2] recorded 119 taxa of macro-fungi from Kashmir. The list has been extended to 145 species (Beig, *et al.*, 2008) [3] 150 species (Dar, *et al.*, 2009a) from Kashmir and 250 from whole Jammu and Kashmir state (Dar, *et al.*, 2009b; Dar, et al., 2014, 2015, 2016) [4-6]. Much work has been done on macro fungal diversity and there is thorough need to exploit the micro fungal diversity as well, especially the foliicolous mycoflora as the prevailing state is only state of India which produces all most all types of fruits and there is an urgent need to combat the possible economic losses due to wide spread of fungal diseases. The climate of Jammu and Kashmir varies greatly owing to its rugged topography. In the south around Jammu, the climate is typically monsoonal, though the region is sufficiently far west; an average of 40 to 50 mm (1.6 to 2 inches) of rain has been recorded between January and March. Annual precipitation is only around 100 mm (4 inches) per year and humidity is very low. The usual temperature range is between -10°C and 37°C while annual rain fall varies between 500 mm and 700 mm, of which over 40% is in the form of snow during winter. The Mean monthly rainfall (mm), humidity (%) and air temperature (°C) for South Kashmir for year 2012-2015 is depicted in (Figure 1 and Table 1). Forests are one of the most important resources of Jammu and Kashmir. Spread over 2,236 Km^2 of demarcated forest area, it accounts for 20% of the total geographical area of the state. The Kashmir Himalaya harbors a rich angiospermic flora, 152 species of which are endemic exclusively to Kashmir region. Total alien flora of the Kashmir Himalayas is represented by 571 plant species, belonging to 352 genera and 104 families. It constitutes a relatively

higher (29%) proportion of the total flora of the region. Over 19,236 Km^2 is under coniferous softwood (Pine) and another 946 Km^2 is under non coniferous softwood. In the coniferous category, Fir accounts for 3355 Km^2 whereas Kail for about 1874 Km2, Chir accounts for around 1773 Km^2 and finally Deodar for 1122 Km2 (Table 2). However, scrub forests do occur in regions, where rainfall is less than 100 cm. The valley of Kashmir has deciduous vegetation with different Kashmir forest divisions (Table 3).

Months	Max. Temp.°C	Min. Temp.°C	R. Humidity (0830 Hrs)	R. Humidity (1730 Hrs)	Rain fall (mm)
January	9.45	-0.55	91.00	64.50	35.30
February	10.40	0.95	88.00	60.00	105.60
March	18.35	5.75	71.50	43.00	105.20
April	20.85	8.50	75.00	50.50	77.50
May	24.00	10.95	73.00	52.00	47.50
June	26.20	13.25	71.50	53.50	82.40
July	29.40	17.30	73.00	51.00	16.60
August	29.95	18.35	78.00	54.50	75.10
September	27.60	12.60	75.50	51.00	54.70
October	23.35	6.20	74.50	48.00	8.60
November	16.55	1.35	85.00	56.50	0.20
December	11.00	-2.25	90.00	61.50	8.50

Table 1: Meteorological Data for South Kashmir (2012-15).

Type of forests		Area (estimated)	
	Million hectares	Sq. Kms.	% of total forest area
Subtropical deciduous	0.081	807	4
Subtropical pine	0.273	2732	14
Subtropical evergreen	0.069	690	3
Himalayan dry temperate	0.137	1371	7
Himalayan moist temperate		8914	44
Dry alpine	0.571	5716	28
Total	**2.023**	**20230**	**100**

Table 2: Forest Cover in different Forest Type Groups

Region	Forest Circle	Forest Division		Area in Ha.
Kashmir		Budgam/Tangmarg		76588
	Srinagar Circle	Sindh		37956
		Bandipora		199396
		Total		**313934**
		Shopian	Kulgam	81270
		Anantnag		96016
	South circle			
		Lidder		106591
		Total		**283877**
		Langate		35495
		Kamraj		71146
	North circle	Kehmil		62472
		Baramulla		45906
		Total		**215019**
	Central circle	Leh		2937
		Kargil		651
		Total		**3588**
	Total Kashmir			816418

Table 3: Different Kashmir Forest divisions

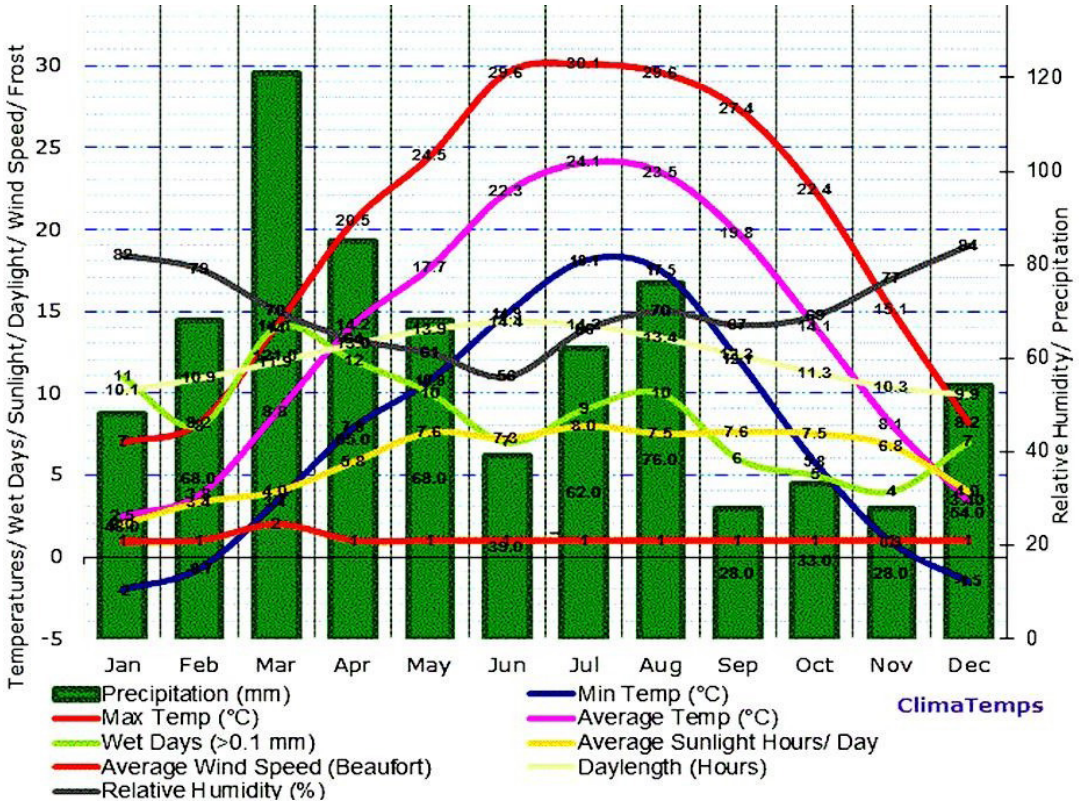

Figure 1: Mean monthly rainfall (mm), humidity (%) and air temperature (C) for South Kashmir (2012 -15).

Materials and Methods

All the necessary information regarding distribution and type of forest, rest house, occurrence of rivers, rivulets, flowing seasonal streams, approach etc. were taken from the official source of the Chief Conservator of Forest. In addition, some minor but essential minute specific information concerning a rough composition of the vegetation were also noted. The course and flow of streams, temporary water reservoirs, swamps, hide of wild animals and other hazards were collected from the individual forest range staff and local inhabitants. All the queries were made prior to proceed for the collection. Local names of the forest trees were asked from the local people or the forest staff. This was done just to be free from any problem and for maximum collection in minimum time. Frequent periodic extensive and intensive surveys of selected forest vegetation and other areas were made (composed of different plant communities such as grassland, forests, nurseries, orchards, gardens, crop fields and even the waste lands). The fungi may have different types of habits such as foliicolous, fruiticolous, corticolous; lignicolous etc. and they may be parasites or saprophytes. Infected plant parts were collected and brought to the laboratory for further study in polythene bags. During all the visits to forest area articles like magnifying lens, polythene bags, rubber bands, scissor, secateurs, knife, tagged labels, field diary, pencil, herbarium press, blotters, vasculum etc. were carried. The scrap mounts and free hand sections of infected tissues were prepared in lacto phenol cotton blue. The semi-permanent prepared slides were studied carefully under the compound microscope so as to have a clear picture of the important morphotaxonomic characters. The fungi were identified tentatively with the help of available literature and a list of host parasite was prepared. Some of the fungi have been identified right up to the species level while some fungi only up to the level of genera. The remaining few fungi which have not been identified even up to the generic level were assigned to their next higher ranks in hierarchy. The processing of rapidly perishable succulents and rare specimens was started right in the camps of collection tours. A fairly good number of specimens of each type of sample were pressed on the blotting sheets bearing a collection number and date. The whole stock of such blotters were pressed and dried under herbarium plant press. The pressing and drying were done with maximum care by changing their blotting sheets at short intervals in the first week and subsequently at successively longer intervals till the specimens were completely pressed and dried. The fully dried specimens thereafter were sprayed with 0.1% $HgCl_2$ aqueous solution to avoid the microbial decomposition (saprophytes, insects etc.). The duly pressed and dried specimens were transferred into two separate sets of envelops. The fore flap of envelop was circumscribed with collection number, date, place, name of the host and the taxonomic identity of the fungus and other noteworthy hints, if any.

Results

Separate mounts of scraps and free hand sections of infected tissues were prepared in lacto phenol cotton blue (LPCB) mixture or lacto fuschin and glycerin. The slides were made semi-permanent by ringing them with nail polish and preserved for further study as required. The slides were critically studied under the compound microscope in different combination of eye piece (5X, 8X, 10X, 12.5X and 15X) and objectives (10X, 40X, 45X, 100X and oil immersion). The desired camera lucida drawings of the interesting

forms were made showing maximum diagnostic features available in the morphology and ontogeny of reproductive propagules and their measurements along with microphotographs taken directly from the slides showing maximum variability of structure and characters of taxonomic importance. On the basis of these observations, the identities of fungi were reaffirmed with the help of relevant monographs, authoritative books and research papers published in reputed journals. A comprehensive list of the species with associated host and place/date of collection has been tabulated (Table 5) with different sites selected and surveyed at different intervals of time (Table 4).

Name of site	Division	Elevation (m)	Latitude	Longitude	Aspect	Slope (Degree)
Kuthar	Anantnag	2824	33°39'41.30"N	75°16'27.10"E	S	30-40
Khiram	Lidder	2331	33°52'20.63"N	75°06'47.78"E	NW	15-20
Thamankote	Anantnag	2538	33°29'51.20"N	75°17'19.60"E	N	05-10
Kulgam	Kulgam	1761	33°37'28.50" N	75°01'30.23" E	NW	10-15
Kokernag	Anantnag	2119	33°34'51.50" N	75°15'47.29" E	NW	10-15
Tral	Lidder	2304	34°03'18.78" N	75°06'47.15" E	SW	15-20
Pahloo	Kulgam	1714	33°37'50.34" N	75°03'06.45" E	NW	05-10
Mattan	Lidder	2008	33°45'33.24" N	75°13'41.65" E	SW	10-15
Pahalgam	Lidder	2364	34°03'49.40" N	75° 16'15.10 E	NE	15-20
Varinag	Anantnag	2642	33°28'54.12"N	75°17'29.20"E	SW	20-25

S→South, N→North, NW→North West, SW→South West, NE→North East

Fungus Host Date and place of collection

Table 4: The general description about the selected sites of the surveyed area

Fungus	Host	Date and place of collection
Alternaria K.	Juglans regia L.	Kuthar, Forests, 2014
Alternaria allii N.	Allium cepa L.	Shopian, Agricultural farms, 2014
Alternaria alternata K.	Solanum tuberosum L.	Anantnag, Agricultural farms, 2015
Alternaria arborescens	Lycopersicon esculentum M.	Anantnag, Agricultural farms, 2013
Alternaria brassicae B.		
Brassica oleracea L.	Shopian, Agricultural farms, 2014	
Alternaria cucumerina E.		
Cucurbita L.	Kulgam, Agricultural fields, 2014	
Alternaria dauci J.		
Daucus carota L.	Shopian, Agricultural farms, 2013	
Alternaria kashmeriana sp.nov.	Platanus orientalis L.	Dialgam, Agricultural farms, 2015
Alternaria melongenae R.	Solanum melonogena L	Anantnag, Agricultural farms, 2013
Alternaria solani S.	Solanum tuberosum L.	Shopian, Agricultural farms, 2013
Alternaria solani S.		
Lycopersicon esculentum M.	Anantnag, Agricultural farms, 2014	
Alternaria somnifera	Papaver somniferum L.	Pahalgam, Agricultural farms, 2013
Alternaria sp.	Iris kashmiriana B.	Daksum forests 14/K, 2013
Alternaria sp.	Prunus armeniaca L.	Dachigam National park, 2014
Alternaria sp.	Phaseolus vulgaris L.	Anantnag, Agricultural farms, 2014
Alternaria sp.	Salix alba L.	Pulwama, Forests, 2014
Alternaria sp.	Pyrus malus L.	Dialgam apple orchards, 2013
Alternaria sp.	Zea mays L.	Kulgam, Agricultural fields, 2014
Alternaria sp.	Brassica oleracea var. aquatica F.	Anantnag, Agricultural farms, 2014
Alternaria sp.	Phaesolus mungo L.	Pahalgam, Agricultural farms, 2014
Alternaria sp.	Datura stramonium L.	Pulwama, Forests, 2014
Alternaria sp.	Acacia sp.	Pampore, Forests, 2014
Alternaria sp.	Allium cepa L.	Pampore, Forests, 2014
Alternaria sp.	Eunanymus sp.	Dachigam National park, 2014
Alternaria sp.	Berginia ligulata W.	Daksum forests 14/K, 2013
Aspergillus flavus L.	Allium cepa L.	Pahalgam, Agricultural farms, 2014

Cladosporium sp.		
Juglans regia L.	Batpora, Agricultural fields, 2014	
Cercospora penniseti C.	Pennisetum glaucum L.	Batpora, Agricultural fields, 2014
Cercospora sp.	Zea mays L.	Batpora, Agricultural fields, 2014
Cercospora sp.	Cichorium L.	Herbal Garden, Daksum, 2013
Cercospora sp.	Spinacia oleracea L.	Kulgam, Agricultural fields, 2014
Cladosporium sp.	Acacia sp.	Pulwama, Forests, 2014
Cladosporium sp.	Eunanymus sp.	Pulwama, Forests, 2014
Cladosporium sp.	Populus alba L.	Pulwama, Forests, 2014
Cladosporium sp.	Populas album L.	Kuthar forests, 2013
Cladosporium sp.	Allium cepa L.	Batpora, Agricultural fields, 2014
Cladosporium sp.	Ulmus americana L.	Kulgam, Agricultural fields, 2014
Cladosporium sp.	Prunus armeniaca L.	Botanical garden Srinagar, 2014
Cladosporium sp.	Salix alba L.	Kuthar forests, 2013
Cladosporium sp.	Populas alba L.	Pampore, Forests, 2014
Cladosporium sp.	Prunus avium L.	Botanical garden Srinagar, 2014
Colletotrichum coccodes W.	Solanum tuberosum	Batpora, Agricultural fields, 2014
Colletotrichum fragariae A.	Fragaria ananassa D.	Agricultural fields of Sangam, 2013
Colletotrichum lillacola sp.nov.	Bergenia ligulata L.	Herbal Garden, Daksum, 2013
Colletotrichum lindemuthianum	Phaseolus vulgaris L.	Agricultural fields of Sangam, 2013
Colletotrichum lindemuthianum	Phaseolus vulgaris L.	Agricultural fields of Sangam, 2013
Colletotrichum sp.	Saussuria lappa D.	Herbal Garden, Daksum, 2013
Colletotrichum sp.	Berginia ligulata L.	Pampore, Forests, 2014
Curvelaria sp.	Podophyllum hexandrum L.	Daksum forests 14/K, 2013
Curvularia penniseti B.	Penniseti sp.	Kulgam, Agricultural fields, 2014
Curvularia penniseti B.	Zea Mays L.	Batpora, Agricultural fields, 2014
Drechslera dematioidea B.	Triticum aestivum L.	Pampore, Forests, 2014
Drechslera dematioidea B	Pearl millets L.	Agricultural fields of Sangam, 2013
Erysiphe heraclei D.	Pierohiza kyroj L.	Botanical garden Srinagar, 2014
Erysiphe heraclei D.	Juglans regia L.	Kulgam, Agricultural fields, 2014
Erysiphe heraclei D.	Prunus persica L.	Kulgam, Agricultural fields, 2014
Erysiphe heraclei D.	Saussuria lappa C.	Botanical garden Srinagar, 2014
Erysiphe heraclei D.	Arnebia benthamil F.	Daksum forests 14/K, 2013
Erysiphe polygoni	Populus alba L.	Dialgam local fields, 2015
Erysiphe polygoni D.	Salix alba L.	Pampore, Forests, 2014
Fusarium moniliforme	Saccharum officinarum L.	Kokernag, Agricultural fields, 2014
Fusarium oxysporum	Oryza sativa L.	Kokernag, Agricultural fields, 2014
Fusarium oxysporum	Lycopersicon esculentum M.	Kokernag, Agricultural fields, 2014
Fusarium oxysporum	Pisum sativum L.	Kokernag, Agricultural fields, 2014
Fusarium oxysporum	Cucurbita L.	Kokernag, Agricultural fields, 2014
Glomerella cingulata	Salix alba L.	Daksum forests 14/K, 2013
Glomerella cingulate S.	Pyrus malus L.	Shopian apple orchards, 2013
Gnomonia leptostyla C.	Juglans regia L.	Batpora, Agricultural fields, 2014
Ioidium euonymi-japonici L.	Eunanymus sp.	Kuthar forests, 2013
Melampsora sp.	Populus alba L.	Dachigam National park, 2014
Meliola sp.	Populas alba L.	Pampore, Forests, 2014
Meliola sp.	Salix alba L.	Dachigam National park, 2014
Peronospora sp.	Lavatera cashmeriana L.	Daksum forests 14/K, 2013
Phyllosticta penicillariae	Pearl millet L.	Kulgam, Agricultural fields, 2014
Podosphaera fuliginea S.	Cucurbita pepo L.	Kulgam, Agricultural fields, 2014

Podosphaera leucotricha	Pyrus malus L.	Dialgam apple orchards, 2013
Erysiphe heraclei D.	Rosa alba L.	Botanical garden of Kokernag, 2014
Erysiphe heraclei D.	Plantago major L.	Batpora, Agricultural fields, 2014
Erysiphe polygoni	Salix alba L.	Dachigam National park, 2014
Erysiphe polygoni	Cucumis sativus L.	Kulgam, Agricultural fields, 2014
Erysiphe heraclei D.	Aquilegia nivalis L.	Daksum forests 14/K, 2013
Erysiphe heraclei D.	Lycopersicon esculentum M.	Dachigam National park, 2014
Erysiphe polygoni	Platanus orientalis L.	Dachigam National park, 2014
Pyricularia oryzae	Oryza sativa L.	Kulgam, Agricultural fields, 2014
Sirosporium celtidis B.	Celtis australis L.	Botanical garden Srinagar, 2014
Cladosporium sp.	Celtis australis L.	Dialgam local fields, 2015
Cladosporium sp.	Hedera helix L.	Dachigam National park, 2014
Cladosporium sp.	Rheam emodi L.	Daksum forests 14/K, 2013
Cladosporium sp.	Rosa alba L.	Botanical garden Srinagar, 2014
Cladosporium sp.	Cucurbita pepo L.	Dachigam National park, 2014
Stigmina carpophila E.	Platanus orientalis L.	Dachigam National park, 2014
Stigmina carpophila E.	Prunus sp.	Kulgam, Agricultural fields, 2014
Trichothecium kashmeriana sp.nov.	Pyrus malus L.	Botanical garden Srinagar, 2014
Tricothecium sp.	Berginia ligulata L.	Pampore, Forests, 2014
Tricothecium sp.	Rosa alba L.	Dachigam National park, 2014
Trichothecium roseum	Rosa alba L.	Daksum forests 14/K, 2013
Ulcocladium sp	Allium cepa L.	Kulgam, Agricultural fields, 2014
Uncinula necator B.	Vitis vinifera L.	Kulgam, Agricultural fields, 2014
Venturia inequalis	Pyrus malus L.	Dialgam apple orchards, 2013

Table 5: List of fungal species collected and identified

Conclusions

The study reveals that much work has been done on macro fungal diversity and there is still thorough need to exploit the micro fungal diversity as well, especially the foliicolous myco flora as the prevailing state (Jammu & Kashmir) is only state which produces all most all types of cash fruits and there is urgent need to combat the possible economic losses due to the wide spread of fungal diseases. Further, the mycotaxonomic characterizations of *Alternaria kashmeriana* sp. Nov., *Colletotrichum lillacola* sp. Nov., *Meliola mangiferae*_KY623717, *Sirosporium celtidis*_KY656465, *Stigmina carpophila*_ KY661359, *Trichothecium kashmeriana* sp.nov. & *Venturia inaequalis*_KY661360 revealed ample amount of genetic divergences probably due to the persistent extremely harsh environmental conditions in the surveyed area. Being deciduous nature of flora, the fungal growth may have encountered the extremes of harsh weather viz. prolonged chilly snow periods prevailing in the region which might have result in variations in fungus genotype as well as phenotype.

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