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FIRST REPORT OF GENUS PARMELIELLA MÜLL. (PELTIGERALES; LECANOROMYCETES; ASCOMYCOTA) FROM PAKISTAN

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When studying lichens in Pakistan, we came across a crustose species with small to moderate squamulose on a thin blackish hypothallus with a dry, rough, gray-brown to the black upper surface. The standard chemical tests integrated with conventional to modern taxonomic tools were used to name the specimen. Consequently, with minor differences in the morphology, and no difference in nucleotides, the lichen species was baptized *Parmeliella thriptophylla* (Ach.) Müll. Arg. The descriptive taxonomy and n-ITS-based phylogeny of this species with its habitus are presented in this study. No previous record of this species, genus, or family was found in Pakistan.

Keywords: *Parmeliella thriptophylla*; phylogeny; geography; Azad Jammu & Kashmir; ITS rDNA

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Project details. This study is part of the Ph.D. dissertation of Ms. Qudsia Firdous. Different surveys have been made all around the Northern areas of Pakistan to collect, identify, and generate a database of Pakistan's flora of lichens.

CONFLICT OF INTEREST:

There exists no conflict of interest for publishing this manuscript in IJIST.

Author's Contribution. Qudsia Firdous designed and conducted all of the experiments and wrote the manuscript. Prof Dr. Abdul Nasir Khalid conducted the survey and supervised and overviewed the manuscript, while Dr. Arslan Ali helped in the spot tests and its interpretation of the species. All the authors have read and approved the final manuscript.

INTRODUCTION

Parmeliella Müll. is categorized as a lichen genus having crustose-squamulose to foliose thallus spread on a cottony prothallus, biatorine apothecia, with or without thalline margins; hymenium I^+ persistent blue; asci with an apical amyloid plug that is unique and hyaline and simple ascospores [1]. It has three forms; *Parmeliella* Müll. Arg. 1862, *Parmeliella* sect. Austroparmeliella P.M. Jørg. 2004 and *Parmeliella* sect. *Parmeliella* Müll. Arg. 1862 (http://www.indexfungorum.org/Names/Names.asp). A recent estimate places 100 species in this genus [2]. It belongs to the family Pannariaceae, order Peltigerales [3]. The family has a widespread distribution, but its species are especially ubiquitous in southern temperate regions [4]. Wedin & Wiklund, (2004)[5] treated this family under the monophyletic suborder Peltigerineae having approximately 27 genera reported from different parts of the world. From Pakistan, we found *P. thriptophylla* (Ach.) Müll. Arg., also known as *Parmeliella triptophylla* (Ach.)[6], is a basionym of *Lecidea thriptophylla* Ach. 1808, representing the first record for the country. The study illustrates morpho-anatomical diagnostic characters and molecular confirmations in the phylogenetic tree. Comparisons with previous descriptions and data on their distributions are briefly discussed. It is observed that this species has a minimal distribution range and has disjunctive dissemination.

Material and Methods.

Collection site and morphology

The sample for this study was collected during a lichen survey of different sites of Azad Jammu and Kashmir, Pakistan, in 2018 (Figure 2.). Morphological characters were observed under a stereomicroscope (Meiji Techno, EMZ-5TR, Japan). Standard microscopy and spot tests [7] were used for further identification. Measurements were made from freehand sections of thallus mounted in water on glass slides. The microscopic features were observed under a compound microscope (MX4300H, Meiji Techno, Japan).

DNA extraction and PCR amplification

DNA from the thallus was extracted using a 2% CTAB protocol [7]. Molecular data was generated for the internal transcribed spacer (ITS) region. The primer pair ITS1F [8] and ITS4 was used to amplify the ITS region under the PCR conditions used by Khan et al. (2018)[9]. PCR products were visualized in a 1% agarose gel [10] and sent to BGI Hong Kong for sequencing.

Phylogenetic analysis

The ITS locus was amplified and sequenced for the lichen specimen. The BioEdit sequence alignment editor was used to reassemble forward and reverse sequences [11]. The nucleotide sequence comparison was performed using the Basic Local Alignment Search Tool (BLAST) of the National Centre for Biotechnology Information (NCBI) [12]. The closely matching sequences were downloaded from GenBank for subsequent phylogenetic analysis (Table 1). Multiple sequence alignment was performed using MAFFT v 7.0 with all parameters set to default values [13]. The aligned sequences were trimmed from both 5' and 3' ends at conserved sites. Maximum Likelihood analysis was performed using the software MEGA v 7.0 [14]. One thousand rapid bootstrap replicates were run to infer the evolutionary history of the species using the Kimura 3-parameter model. The length of the final aligned file was 1304 nucleotides, of which 296 sites were conserved, 418 variables, 203 parsimony-informative and 141 were singletons. *Degelia plumbea* (Lightf.) P.M. Jørg. & P. James (AF429265) was chosen as an outgroup.

Results and discussion.

Parmeliella thriptophylla was growing on exposed sedimentary rocks and soil in moist and shady sites in a mountainous landscape characterized by fertile, green, rocky, and undulating territory, Azad Jammu and Kashmir [15]. The taxon is maybe not the easiest to recognize due to its appearance and pattern of distribution. It has small, brown to blue-grey squamules [16]. We also described morphological descriptions accompanied by colored photographs of the thallus and microscopic structures (Table 3). In comparison, a few morphological differences were observed (Plate 1). The color of thallus from America is blue-brown [17]; from Norway, it is reported as dark blue-black while brown-black in our findings. Lobes and lobules were also seen in previous studies from Europe and the South of Nordland [13]; our specimen was non-lobulated. It is also different in having non-branched isidia in our case vs. branched isidiate from the previously reported species [14]. Except for a bit of difference in the sizes of macroscopic and microscopic structures, it was more or less similar to other world collections. The similar features, squamulose thallus, granular and marginal isidia, absence of lower cortex, and negative spot tests revealed the taxon identity. Phylogenetically, our lichen collection (MW255137) was 100% matched with *P.thriptophylla*, reported from the different parts of the world (Table 1). Our sequence clustered in the tree with accession numbers HM448804 and KC618727 submitted from Sweden (Figure 1). It showed robust bootstrap (100%) value for the phylogenetic support. *P. nigrocincta* (KC618724) and *P. thysanot* (KC618726) made sister clades to the *P.thriptophylla*. This fork-shaped structure is further divided to form another clade with different lengths of branches for each specific species. The first clade is further extended for *P. parvula* and then developed with two sister clades containing other species. The branching pattern in a phylogenetic tree reflects ancestor-descendant relationships, having more common ancestors. Black nodes represent our node species. The geographical comparison showed the species had been reported from temperate and tropical areas, primarily in cool-temperate, widespread in the Northern Hemisphere (Table 2)

It is not known with certainty from the African continent; however, present in Macaronesia [10], [14], Andes and Central America [14] and Russia [18], Kodagu district and India [19] for which detail taxonomic descriptions are not available. However, in this study, we describe the species for the first time from the warm and temperate areas of Sharda Valley, Azad Jammu, and Kashmir, Pakistan, as a first record.

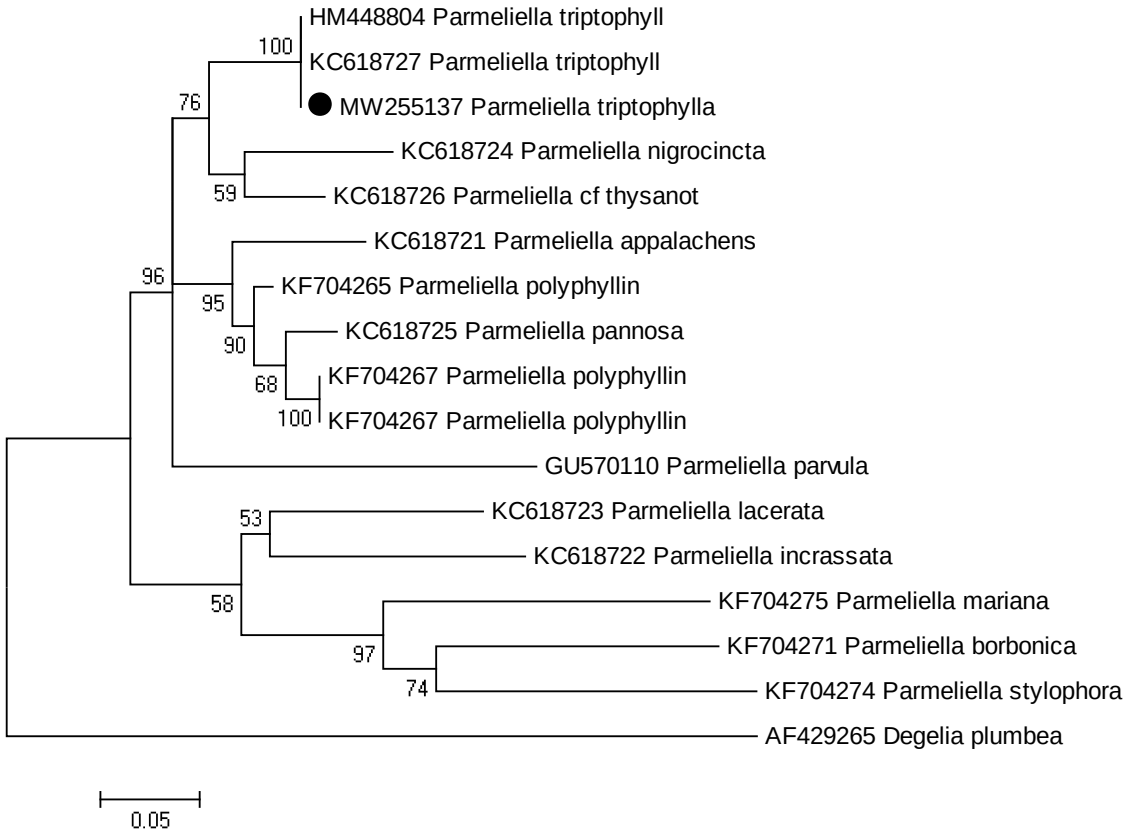

Figure 1. Phylogenetic analysis of *Parmeliella thriptophylla* species based on nrITS–rDNA regions. This tree is based on maximum likelihood method using Tamura 3-parameter model. The bootstrap values are given above branches. Species collected from Pakistan have been labeled with a box (●).

Table 1. *Parmeliella* species with their accession numbers of ITS sequences used in this study

Accession Numbers	Species Names	Country	References
MW255137	*Parmeliella thriptophylla*	Pakistan	Current Study
HM448804	*Parmeliella thriptophylla*	Sweden	Unpublished
KC618727	*Parmeliella thriptophylla*	Sweden	Unpublished
KC618726	*Parmeliella cf thysanota*	Sweden	Unpublished
KF704265	*Parmeliella polyphyllina*	Belgium	Magain & Sérusiaux 2014
KC618724	*Parmeliella nigrocincta*	Sweden	Unpublished
KF704267	*Parmeliella polyphyllina*	Belgium	Magain & Sérusiaux 2014
KC618721	*Parmeliella appalachensis*	Sweden	Unpublished
KC618725	*Parmeliella pannosa*	Sweden	Unpublished

KC618723	*Parmeliella lacerate*	Sweden	Unpublished
KC618722	*Parmeliella incrassate*	Sweden	Unpublished
KF704267	*Parmeliella polyphyllina*	Belgium	Magain & Sérusiaux 2014
KF704271	*Parmeliella borbonica*	Belgium	Magain & Sérusiaux 2014
GU570110	*Parmeliella parvula*	Norway	Unpublished
KF704275	*Parmeliella mariana*	Belgium	Magain & Sérusiaux 2014
KF704274	*Parmeliella stylophora*	Belgium	Magain & Sérusiaux 2014
AF429265	*Degelia plumbea*	Norway	Ekman & Jørgensen 2002

Table 2. ITS sequences present in Genebank for *Parmeliella thriptophylla* overall

Countries	Accession Numbers	Geography of the areas	Climatic Conditions
Sweden	HM448804 KC618727	*Parmeliella thriptophylla* *Parmeliella thriptophylla* voucher Wedin 7037 (UPS)	Temperate climate
South America	MH802366 MH802347 MH887519	*Parmeliella thriptophylla* voucher NK-278 *Parmeliella thriptophylla* voucher NK-278	Tropical rain forest
Alaska, USA	MN437620 MN508285 MN483131 MN483097 MN483096 MN460220	*Parmeliella thriptophylla* voucher Spribille	Frigid winters and short, cool summers
Norway	AF429269 MK812457 MH802418	*Parmeliella thriptophylla* voucher Ekman 3203 (BG) *Parmeliella thriptophylla* voucher O-L--207999 *Parmeliella thriptophylla* voucher NK-278	Marine climate, with comparatively cool summers, mild winters

Table 3. Morphological notes of *Parmeliella thriptophylla* (Ach.) Müll.Arg. from Pakistan

Lichen Features	Description
Morphological features of thallus	Small to moderate size squamulose, resting on a thin blackish hypothallus that contributes to the dark color of this species; Squamules: up to 1 mm wide, incised; Upper surface: dry, rough, gray-brown to black, isidiate but not sorediate; Isidia: coralloid, digitate to granular mostly, marginal, sometimes obscuring the squamules; Pycnidia: not seen; Apothecia: Not present
Thallus section	Thick upper cortical layer present, 16-18 um high, hyaline; Cortical cells: two rows of cells can be seen easily, square to irregular to multiangular, 3-6 um long
Photobiont layer	Yellowish green, very thick, 45-55 um high; Lower layer: thin, darker (blackish), only up to 9 to 11 um high
Spot test	Cortex: K-, C-, KC-

Substrate and Ecology	On rocks in a Himalayan moist temperate forest, at an altitude of 1,943 m.a.s.l., shaded, maximum and minimum temperature of 28°C and -2 °C, respectively, annual rainfall varying between 800–1200 mm.
Material examined	PAKISTAN. Azad Jammu & Kashmir: Neelam Valley, Sharda; 34.7931° N, 74.1930° E; July 25, 2019; A. N. Khalid and Q. Firdous. KSH-13, LAH36782

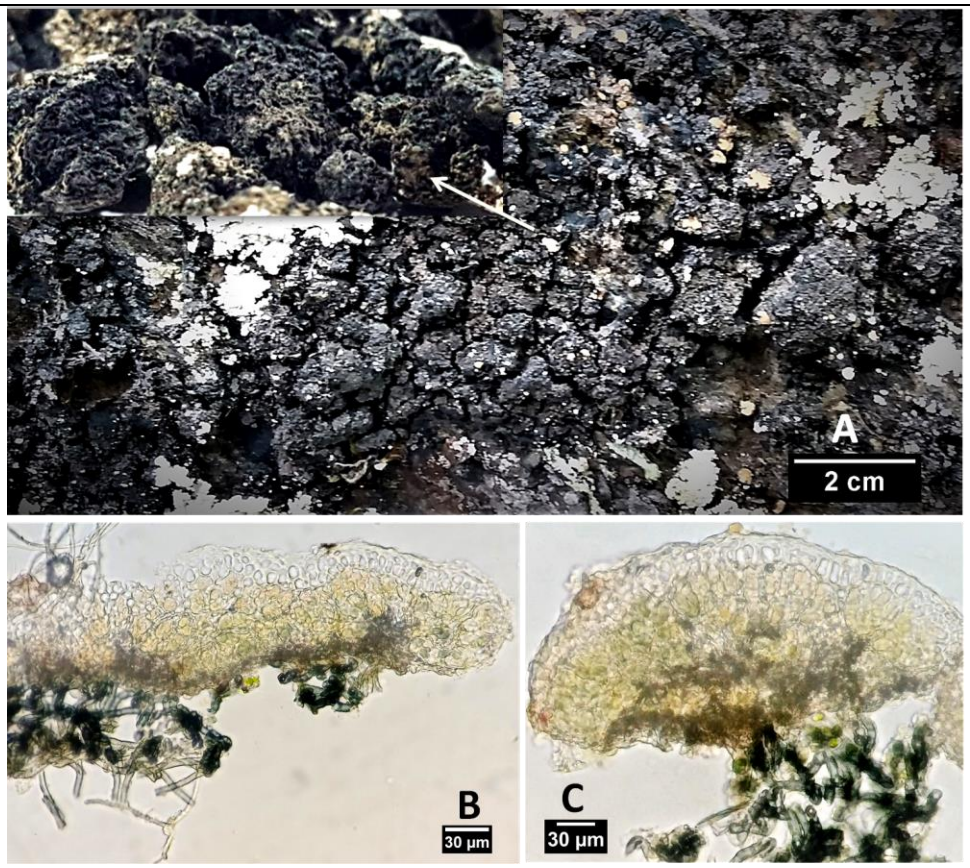

Plate 1. ***Parmeliella thriptophylla*** A: Thallus morphology along with the substrate B & C: Sections of thallus

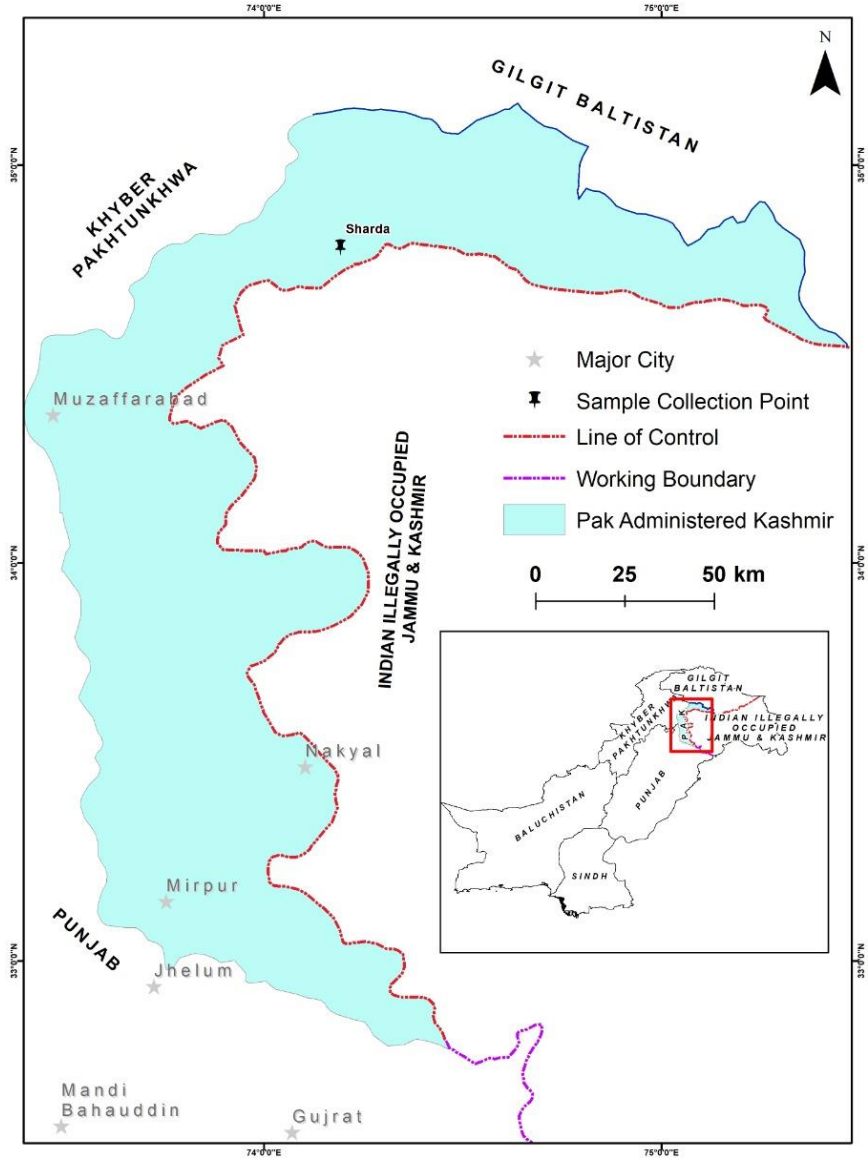

Figure 2. Map of the sampling site

CONCLUSION

In this study, we present the molecular taxonomy of *Parmeliella thriptophylla* and its family for the first time from Pakistan. The detailed macroscopy and microscopy of the species strongly supported its classification and proof of phylogeny. Here, we mentioned the geographical positions and compared the climatic conditions to see their effect on the specimen's morphology. It has been established that an area's physical conditions influence the outlook of living organisms but have little or no effect on the nucleotide of the specimen.

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