

FIRST INSIGHTS INTO THE CRUSTOSE LICHEN DIVERSITY OF MUSK DEER NATIONAL PARK, WITH NEW RECORDS TO PAKISTAN

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Abstract

Lichens are an important part of the vegetation, which plays a vital role in the environment i.e. pollution indicator. This research area is completely virgin in Pakistan, as very few studies are found in literature. Since 1965-2012, a total of 368 lichen species have been reported from Pakistan. Here, lichen diversity of an unexplored area (Musk Deer National Park) is documented. This study for the first-time reports nine lichen species from the understory area and with new records of *Acarospora socialis* and *Umbilicaria hirsuta* from Pakistan. Most of them were cosmopolitan (*Physcia dubia* and *Xanthoria elegans*), while few species had restricted distribution and were found in specific altitudinal ranges (*Dermatocarpon miniatum* and *Rhizocarpon geographicum*). Brief description of all taxa reported here is provided.

Key words: Crustose lichen; Species diversity; New records; Musk Deer National Park.

Introduction

Lichens are highly specialized mutualistic symbioses between fungal symbiont (mycobiont) hosts one or several taxa of algal symbiont (photobionts) (Grube *et al.*, 2009; Kaasalainen *et al.*, 2017) represented by approximately 20,000 species (Feuerer & Hawksworth, 2007; Lücking *et al.*, 2017). Based on different growth forms, lichens are divided into 4 classes: Crustose, Foliose, Fruticose and Squamulose (Sinha & Ram, 1994; Lücking *et al.*, 2017). Distributions of lichen species are affected by the substrate where they are found (Sevgi *et al.*, 2019). Lichens living on rocks are influenced by the characteristics of the rocks type (Sevgi & Makineci, 2005). Crustose lichens are found in almost all major terrestrial biomes ranging from the tropics to polar regions (Kaasalainen *et al.*, 2019). They also play a vital role in the field of lichenometry (Armstrong, 2016).

Few studies have been conducted on the enlisting of lichens and diversity of the epiphytic lichens, for instance, Aptroot & Iqbal (2012) reported 368 species of lichens from Pakistan. Güvenç & Öztürk (2017) identified sixty epiphytic lichen species in seven localities from urban and rural areas in Bursa province, Turkey, but no record is available focusing particularly on crustose lichen diversity. Meanwhile, literature also revealed that most crustose lichens are relatively small and detaching them from the substrate is often impossible without major damage to the thallus (Kaasalainen *et al.*, 2019). Therefore, the current study was planned to explore the unexplored area based on the crustose lichens.

Materials and Methods

Study area: Musk Deer National Park of District Neelum, Azad Jammu and Kashmir (AJ&K) is a beautiful but remote area of the Neelum valley situated in the north-east of Muzaffarabad, Kashmir (Fig. 1). The Park covers an area of 52,817 ha, lying between 2017m to 4345m elevation. The park falls under the Sharda division and Guraiz Range

of AJ&K Forest Department. It is stippled in many sub-valleys i.e. Kail, Helmat and Taubutt valleys.

Field and Lab work: Intensive surveys were carried out during 2017 to collect crustose lichens. Specimens were collected from rocks (Cobanoglu & Sevgi, 2009), sun/ air dried and placed into sealed envelopes. Each collected sample was preserved, packed and labeled with relevant information. Morpho-anatomical identification was carried out using light microscope (Fig. 2), stereomicroscope (Culberson & Kristinsson, 1970; Vinayaka *et al.*, 2012). Morphological identification was based on growth form, colour of thallus, substrate of lichen using relevant keys, monographs (Culberson, 1972; Chopta, 1934; Awasthi, 1988; Kumar *et al.*, 2011; Aptroot & Iqbal, 2012) and Flora books of the world (Clauzade & Roux, 1985). Presence of chemical substances on thalli was detected by spotting reagents that gave characteristic colour changes in UV light (Culberson, 1972). The voucher specimens were deposited in Herbarium, Department of Botany, Hazara University, Mansehra, Pakistan.

Results and Discussion

The current study for the first-time document nine crustose lichen species from of an unexplored area (Musk Deer National Park) and with new records of *Acarospora socialis* and *Umbilicaria hirsuta* from Pakistan. Most of the lichens had wide distribution (*Physcia dubia* and *Xanthoria elegans*), while few species were found very rare. *Rhizocarpon geographicum* was recorded in the highest altitudinal ranges and *Dermatocarpon miniatum* in the middle range. Nationwide, very few studies have been conducted, and to-date, the checklist documented by Aptroot & Iqbal (2012) is a baseline for Pakistani Lichenologists. For instance, since 1965-2012, a total of 368 lichen species have been reported from Pakistan. Brief description of all taxa reported here is provided.

Musk Deer National Park

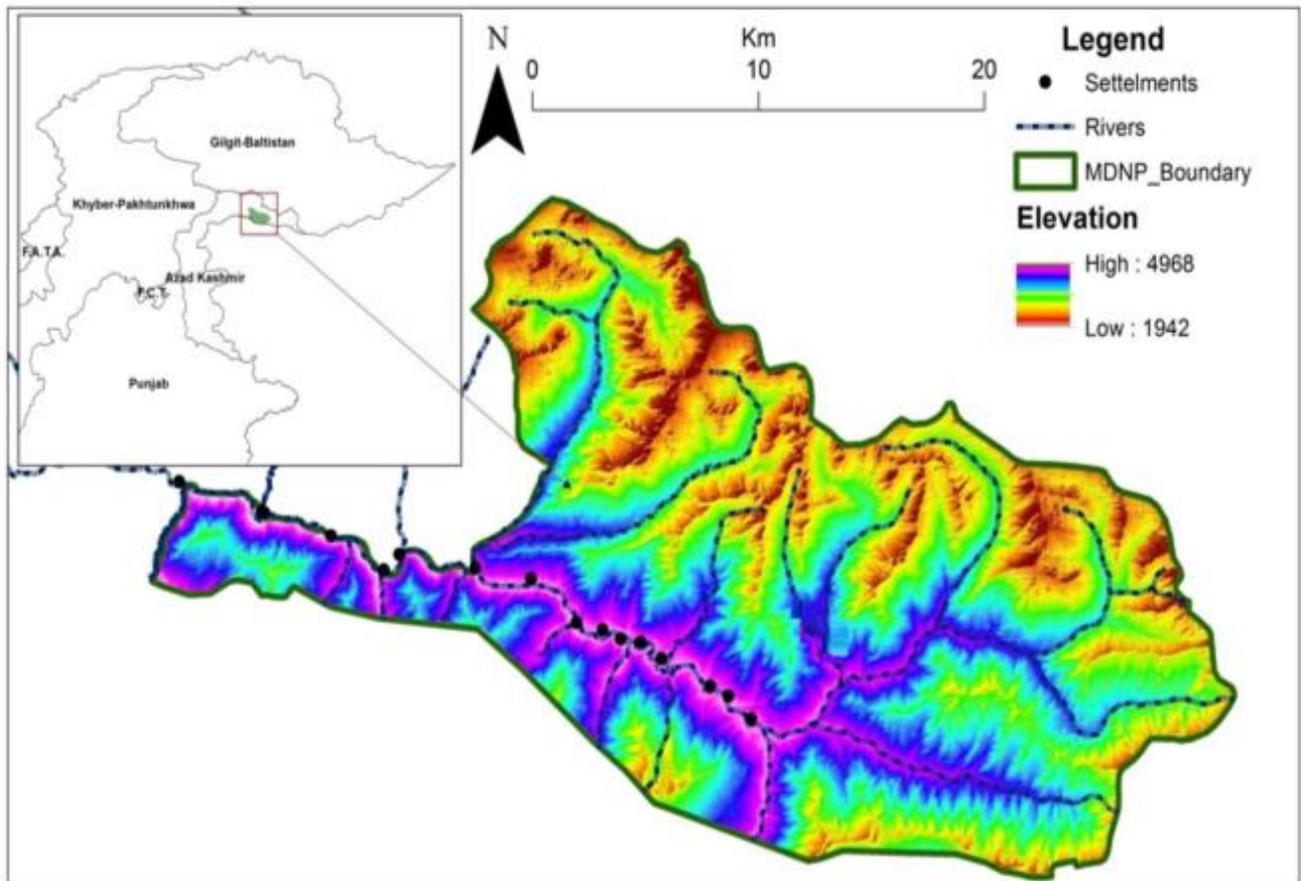


Fig. 1. Map of the study area (Musk Deer National Park, Azad Kashmir).

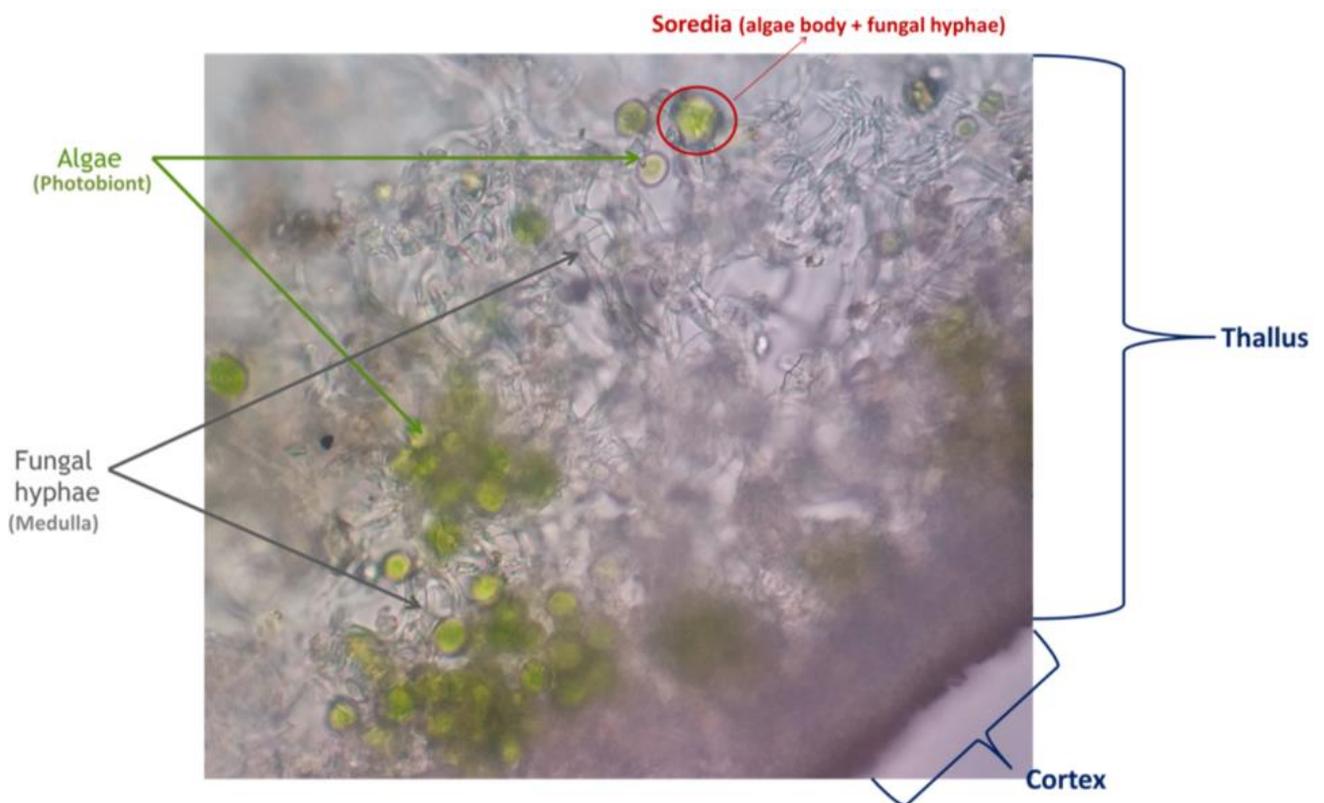


Fig. 2. Anatomical features of a typical lichen.

New Records

New records from Pakistan

Acarospora socialis H. Magn. [Syn: *Acarospora amabilis* H. Magn.]

Voucher No.: FI-K-021-HUP

Family: Acarosporaceae

Common name (English): Yellow Cobblestone Lichen

Substrate: This crustose lichen grows on sandstones, granitic rocks and outcrops on ridges.

Thallus: Thallus is areolate to squamulose. Areoles are contiguous to scattered, angular to round. Upper surface: usually glossy, convex to flat, smooth to rough. Apothecia: Lecanorine apothecia, immersed, round to angular, usually one per squamule, disc is plane to concave. Algal layer: continuous, with usually feathered or uneven lower stratum, algal cells up to 15 µm wide in thick hyphal matrix. Medulla: hyaline to dirty white.

Colour: Upper surface is greenish yellow to various yellow shades. Disc is dull and opaque, brown.

Size: Thallus is up to 10 cm wide (Fig. 3A).

Important note: *A. socialis* often continues to produce conidia production for many years, delaying sexual reproduction, and then produces multiple apothecia, dissolving the thallus plectenchyma.

Umbilicaria hirsuta (Sw. ex Westr.) Hoffm. [Syn: *Gyrophora depressa* var. *hirsuta* (Sw. ex Westr.) Schaer.]

Voucher No.: FI-TB-008-HUP

Family: Umbilicariaceae

Common name: Granulating Rocktripe Lichen

Substrate: This crustose lichen grows on siliceous rocks in sun (Fig. 3B), mostly on steeply inclined surfaces that regularly have seeping water (hygrophilic).

Thallus: Thallus is umbilicate, usually monophyllous to almost polyphyllous, lobate, soft, thin, often with a lacerated margin. upper surface: smooth initially but developing a fine network of fissures, dissolving into patchy or flake-like soredia marginally. Apothecia: not common, black, sessile to stipitate, gyrose, asci: clavate, 8-spored ascospores: simple, ellipsoid.

Colour: upper surface is light grey to greyish brown, lower surface: white to pink.

Size: Thallus 2 to 5 cm in diameter, apothecia up to 2 mm in diameter.

New reports from the study area

Aspicilia cinerea (L.) Korb. [Syn: *Gussonea cinerea* (L.) Tornab.]

Voucher No.: FI-H-031-HUP

Family: Megasperaceae

Common name (English): Cinder Lichen

Substrate: This lichen is mostly found on exposed siliceous or volcanic rocks, rarely on calciferous rocks in open habitats.

Thallus: Areolate, sometimes rimose at the edge, areoles: angular or sometimes irregular, flat to slightly convex, contiguous, separated by distinct cracks. Surface: gray to white-gray or almost white, sometimes with a yellowish tinge, dull or slightly shiny. Apothecia: aspicilioid, usually

numerous, 1-3 per areole, round to angular or irregular disc: black, rarely brown-black and usually concave. Asci: clavate, 8-spored, ascospores: hyaline, ellipsoid. pycnidia: rare to rather common, 1 per areole, rarely aggregated, conidia: filiform, straight or slightly curved.

Colour: Pale to ashy-grey and thin to thick continuous surface with black spots, discs are black (Fig. 4A).

Size: Thallus 1.5-8 cm in diameter, disc 0.4-1.2mm in diameter.

Important note: *Aspicilia cinerea* is characterized by a gray to almost white thallus with quite large apothecia, brown to olive-brown epihymenium.

Candelariella vitellina (Hoffm.) Müll.Arg.

Voucher No.: FI-H-022-HUP

Family: Candelariaceae

Common name (English) = Common Goldspeck Lichen

Substrate: This crustose lichen grows on granitic rocks in open sun (Fig. 4B).

Thallus: Thallus is areolate to subsquamulose, scattered and crowded, areoles: flat to convex, almost granular to irregular. Surface: smooth to uneven, apothecia: common, lecanorine, yellow flat crowded disc: round, flat to somewhat convex at the margin: thin to thick, entire to crenulate, Pycnidia: sparse, appear as darker yellow spots on the thallus conidia: oblong to ellipsoid.

Colour: Small yellow cushions, discs are darker yellow, epihymenium: yellow to brown, ascospores and conidia are hyaline.

Size: 0.5-1.5mm diameter. Lobes up to 0.5 mm long, 0.35-1.0 mm wide disc, 7-15 µm thick apothecia.

Important note: This is commonly distributed but frequently overlooked lichen. The chemical 'Calycin' reported in this lichen serves as a sunscreen and regulating the solar intensity at the algal layer. Its algal partner (*Trebouxia*) grows best at low light intensity.

Dimelaena oreina (Ach.) Norman [Syn: *Amphiloma oreinum* (Ach.) Körb.]

Voucher No.: FI-AK-013-HUP

Family: Caliciaceae

Common name (English): Golden Moonglow Lichen

Substrate: This crustose lichen grows on sunny siliceous rocks mostly on the steeply inclined and vertical surfaces (Fig. 4C).

Thallus: Thin to thick, placodioid, with radiate-plicate marginal lobes, areolate towards the center plane, prothallus: narrow. Abundant black discs are found on the central surface. Apothecia: frequent, adnate. Disc: black or sometimes white-pruinose, plane to slightly convex; hypothecium: hyaline asci: cylindrical, 8-spored ascospores: brown. Pycnidia: immersed, occurring singly in the center of younger areolae, pyriform conidia: hyaline, simple, bacilliform.

Colour: Greenish yellow, sometimes blackish at the edges.

Size: lobes 0.5-1mm in diameter and 1-3mm in length.

Important note: *Dimelaena oreina* is characterized primarily by the presence of usnic acid, giving the thallus a greenish yellow color.

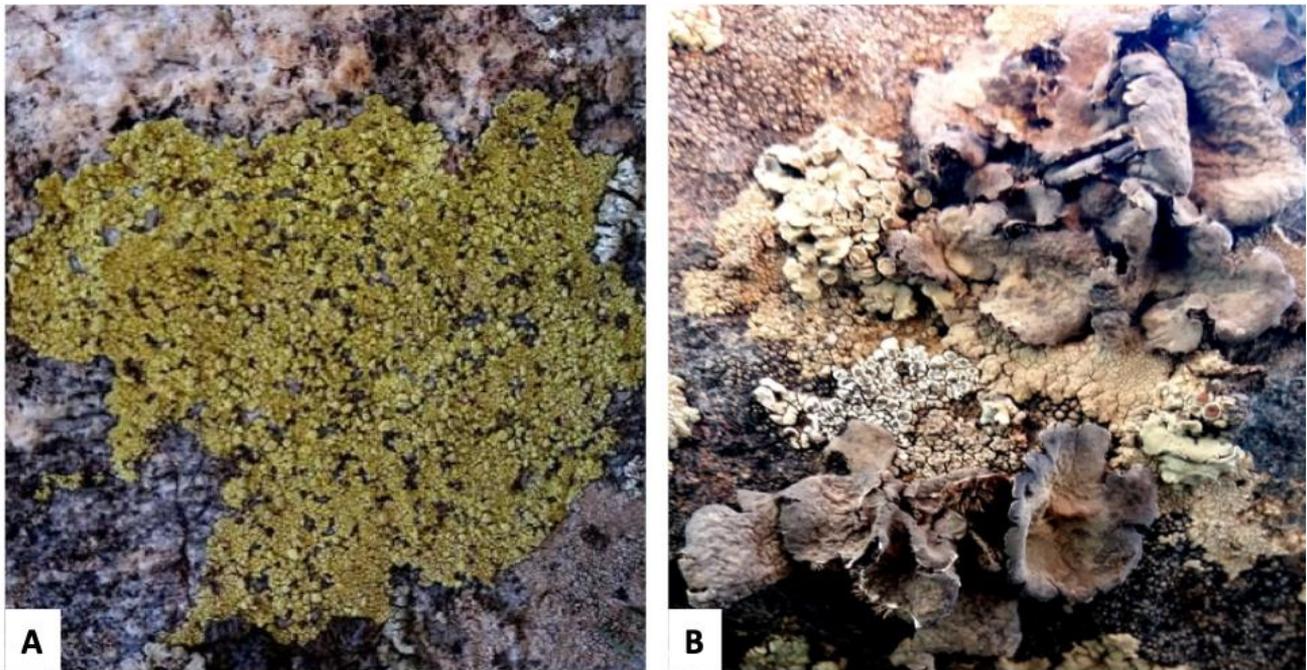


Fig. 3. New records of lichen (i.e. A: *Acarospora socialis* and B: *Umbilicaria hirsuta*) reported from Pakistan.

Dermatocarpon miniatum (L.) W. Mann [Syn: *Dermatocarpon miniatum* var. *complicatum* (Lightf.) Th.Fr.]

Voucher No.: FI-TB-010-HUP

Family: Verrucariaceae

Common name (English): Leather lichen

Substrate: This lichen commonly grows on calcareous rocks in very dry habitats with much sunlight (Fig. 4D). This is foliose lichen having grey leathery lobes growing in spherical clumps.

Thallus: Umbilicate, monophyllous or divided with many lobules close together, the lobes 1-7 cm broad, flat, reddish brown or grey-brown, grey pruinose, 0.3-0.5 mm thick; underside smooth to warty, light to dark brown. Apothecia are absent, spores 8, hyaline, elongate elliptical.

Colour: Grey leathery lobes.

Size: single spherical clump of lobe varies from 15-45mm diameter.

Important note: This lichen reproduces through fragmentation. Occasionally, the lobes breakdown, free from the rock surface and further lodged in the crevices or cracks of rock, which provides favorable growing conditions.

Physcia dubia (Hoffm.) Lettau [Syn: *Lobaria dubia* Hoffm.]

Voucher No.: FI-TB-007-HU

Family: Phyciaceae

Common name (English): Rosette Lichen

Substrate: Growing on exposed acidic rocks.

Thallus: Thallus is irregular, rarely orbicular, usually with darker lobe tips. Upper surface: mostly without pruina and maculation, soredia: sorediate, marginal soralia (mostly at the tip of the lobes in lip-shaped). Apothecia: not common, up to 2 mm diameter, margins: usually sorediate, ascospores: 1-septate, conidia: subcylindrical.

Colour: Thallus is grey to greyish brown (Fig. 4E), upper surface has whitish grey to cream-colored, and lower

surface has pale colour with white to black rhizines, medulla is whitish. Ascospores are brown,

Size: Overall thallus range up to 3 cm in diameter. Lobes are variable in width (rarely exceeding 1 mm).

Important note: This lichen mostly grows in cold temperate areas and has lip-shaped soralia.

Rhizocarpon geographicum (L.) DC. [Syn: *Rhizocarpon tinei* (Tornab.) Runem.]

Voucher No.: FI-AK-019-HUP

Family: Rhizocarpaceae

Common name: Yellow Map lichen

Substrate: This crustose lichen grows on non-calciferous siliceous rocks in open sites.

Thallus: Scattered areoles or areolate to rimose; prothallus: black, poorly to well-developed areoles: round to angular, plane to strongly convex. Surface: epruinose, smooth. Apothecia: round or angular, black dots tucked in the margins. Disc: plane or weakly convex, asci: clavate, 8-spored ascospores: submuriform or muriform.

Colour: Greenish yellow or yellowish green shiny surface (Fig. 4F), prothallus and disc are black.

Size: areoles are up to 1.4 mm in diameter, surface is up to 1 mm diameter, apothecia is up to 1 mm in diameter, hymenium 100-180 µm long.

Important note: The understory lichen is the poster child of lichenometry. To measure the age of geologic events (i.e. glaciers retreat or sloughing of gigantic rock surfaces), the lichen aging techniques (lichenometry) were used (Armstrong, 2016). It is common in some areas *Rhizocarpon* may be over 1000 years old, even 4500 years old lichen has been reported Sweden (Beschel, 1958; 1961). In literature, the radial growing rate is reported in British Columbia in 0.26-0.42 mm per annum (McCarthy, 2003).

Xanthoria elegans (Link.) Th. Fr. [Syn: *Caloplaca elegans* auct.]

Voucher No.: FI-H-020-HUP

Family: Teloschistaceae

Common name (English): Elegant Sunburst Lichen

Substrate: This foliose to crustose lichen mostly grows on rocks and prominent along the sides of river, rarely on bones.

Thallus: Foliose, forming medium-sized to large rosettes or sometimes colonies, lobate lobes: dorsiventral, concave to flattened. Upper surface: smooth, somewhat wrinkled and lower surface: wrinkled. Medulla: bundled, with elongate hyphae, apothecia: common, laminal, stipitate, up to 8 mm in diameter; margin: smooth to crenulate; disc: orange; epihymenium: brown, thick. Asci: clavate,

8-spored, ascospores: ellipsoid, hyaline. Abundant orange discs are found on the central surface, and its lower surface is whitish in colour.

Colour: Orange upper surface and white to yellow lower surface, white medulla, very closely attached lobes from pale yellowish orange to reddish orange (Fig. 4G).

Size: Over all up to 10 cm wide. Size of the lobes varies from 0.4-1mm, disc thickness 10 µm and diameter 1-3 mm.

Important note: This was the first ever lichen species travelled an interplanetary journey in order to test its possible survival rate in the harsh environment of space. After about 14 days, the result showed 100 percent survival with fully active photosynthetic rate (Sancho *et al.*, 2007).

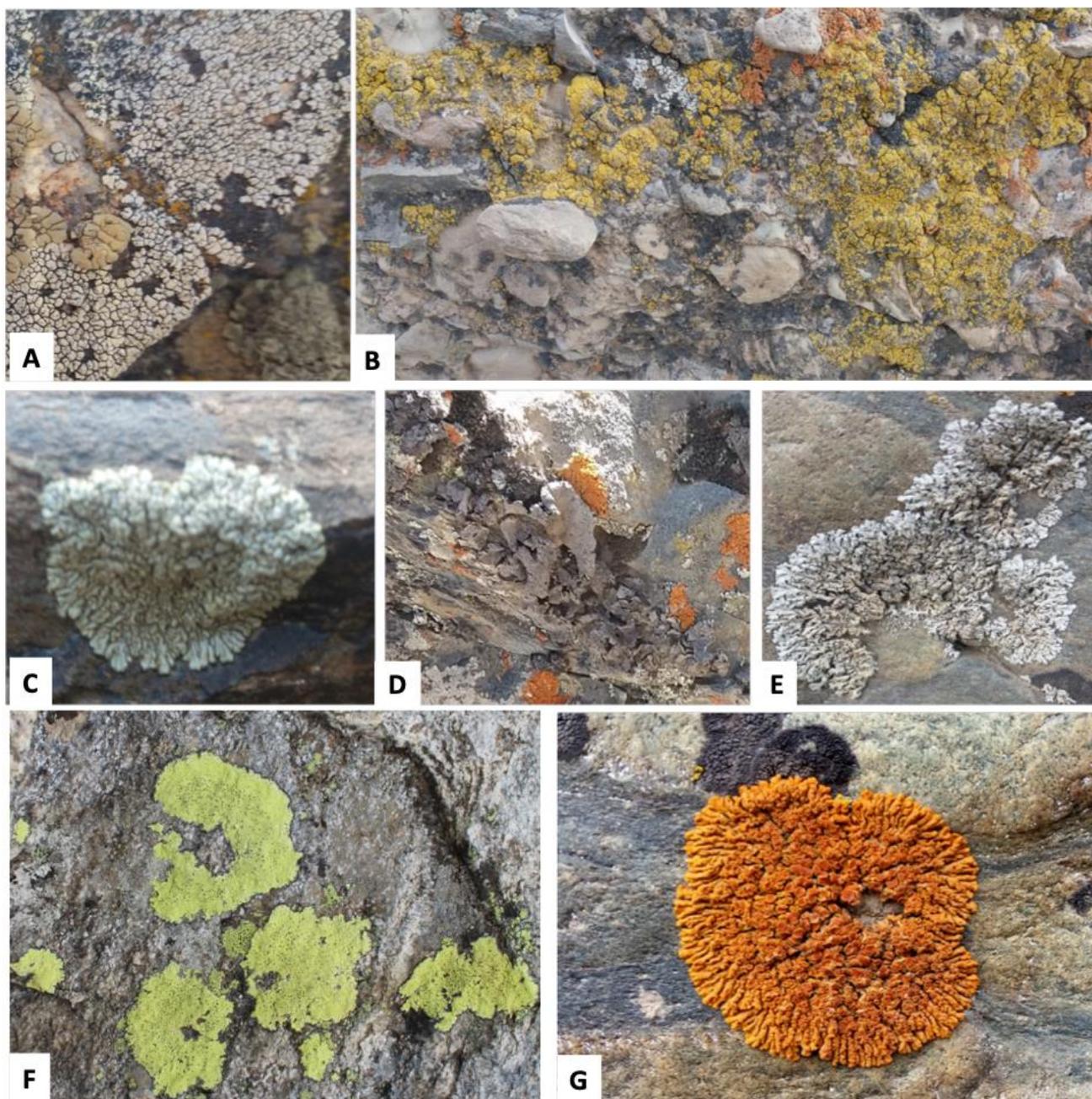


Fig. 4. New records of lichens reported for the first time from the study area: A) *Aspiciella cinerea*, B) *Candelariella vitellina*, C) *Dimelaena oreina*, D) *Dermatocarpon minutum*, E) *Physcia dubia*, F) *Rhizocarpon geographicum* and G) *Xanthoria elegans*.

Conclusion and way forward

The area is a hotspot of biodiversity and with abundance of lichen species making it an attractive site for studies of lichen communities associations with different substrates. Both biotic and abiotic variables were associated that influenced the distribution of lichen species and thereby lichens may itself act as bio indicators for assessing the air quality or pollution load index within the area. The application of Next generation sequencing technologies (NGS) and high throughput genotyping platforms in different plant species have enabled us gaining insights into the identification of genes, gene structure and functional characterization of pathways implying in the synthesis of various important metabolites. Studies using such state-of-the-art tools are missing in lichens and are warranted, as lichens are typically the first organisms to colonize bare rocks and therefore the pioneer species in plants succession. Genomic and transcriptomic studies may help in identification of useful genes for adaptation and their networks that are direly needed to address climatic resilience and climate change.

Authors contribution

FI and NA: designed the study; FI: carried out lichens sampling, microscopy, literature survey and drafted the manuscript. NA and EFA helped in designing experiments, writing, editing and review; AH, AAA analysed the overall results and critically reviewed the manuscript.

Acknowledgments

The authors would like to extend their sincere appreciation to the Researchers Supporting Project Number (RSP-2019/134), King Saud University, Riyadh, Saudi Arabia.

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