

Lecanora darelensis, a new species of Lecanoraceae from Darel Valley, Gilgit Baltistan, Pakistan

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Abstract

A new species, *Lecanora darelensis* sp. nov. is described here from Darel Valley, Gilgit Baltistan, Pakistan. A comparative morpho-anatomical, chemical study and ITS-based molecular analyses confirmed the position of new species within the genus *Lecanora*. The new taxon is phylogenetically closely related to *L. argopholis*. The former can be distinguished from latter in having pale yellowish green upper surface (vs yellowish white), larger apothecial disc 0.5–1.5 mm (vs c. 1.2 mm), brown, plane to convex, pruinose to rarely epruinose apothecial disc (vs deep yellowish brown, plane or slightly concave, epruinose), taller epihymenium 25–35 μm (vs c. 20 μm), taller hymenium 110–150 μm (vs 70–75 μm), larger ascospores 14–18 \times 5–8 μm (vs c. 12.7 \times 7.3 μm), atranorin is detected by TLC (vs atranorin, epanorin, zeorin) and growing on calcareous rocks (vs rock surfaces, detritus, mosses, sloping siliceous and calcareous rocks, boulders, gravel).

Introduction

Lecanora Ach. (Lecanoraceae) is a large group of crustose lichens that is distributed worldwide (LaGreca & Lumbsch 2001; Lücking *et al.* 2017). It is defined by its hyaline, simple oval spores, *Lecanora*-type asci, thalline amphithecial cortex, Lecanorine apothecia and crustose habit (Hafellner 1984; Henssen & Jahns 1974). This lichen genus serves as the type for one of the largest orders of euascomycetes, the Lecanorales, and it has been reported for *Lecanora sensu lato* that includes well over 1,000 species (Zahlbruckner 1926). The region of Gilgit-Baltistan, formerly known as the 'Northern Areas', forms part of northern Pakistan amidst the Karakoram, Greater Himalayas, Pamir, and Hindu Kush mountain ranges. It shares international borders with Afghanistan to the northwest, China to the northeast, and India in the east. Administratively, the region is divided into seven districts – Astore, Diamer, Ghanche, Ghizer, Gilgit, Hunza-Nagar and Skardu with the administrative capital in Gilgit city (Khan *et al.* 2013).

Sampling was undertaken in the Darel Valley, Gilgit Baltistan. Darel Valley is dominated by barren mountains with patches of fertile land with pastures. The samples were collected in a cold, semi-arid climate at an altitude of 2,000 m a.s.l. The climate has typically cold desert characteristics, with severe winters (usually with moderate to heavy snowfall) and dry summers. Average annual precipitation in the valley is 100–300 mm, mostly occurring during winter and early spring in the form of snow. The vegetation of area falls in dry temperate coniferous forest containing the natural

Muhammad Shahid Iqbal,
Abdul Nasir Khalid (2024)
Lecanora darelensis, a new
species of Lecanoraceae from
Darel Valley, Gilgit Baltistan,
Pakistan. *Telopea* 27: 117–124.
[doi:10.7751/telopea17708](https://doi.org/10.7751/telopea17708)

Received: 26 September 2023
Accepted: 27 May 2024
Published: 2 July 2024

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forests which mainly consist of *Cedrus deodara* (Roxb.) G.Don, *Pinus wallichiana* A.B.Jacks., *Abies pindrow* (Royle ex D.Don) Royle, *Fraxinus xanthoxyloides* (G.Don) Wall. ex DC. and *Pinus gerardiana* Wall. ex D.Don (Khan *et al.* 2013).

To date, 32 species of *Lecanora* have been reported from Pakistan (Aptroot & Iqbal 2010). All previously recognised species of *Lecanora* in Pakistan have been identified solely on morphological characters, so this is the first study of this genus to include a phylogenetic analysis. A new species *Lecanora darelensis* sp. nov. from Darel Valley has been described in this paper. The present work contributes molecular data on the genus *Lecanora* from Darel Valley and adds to our knowledge of *Lecanora* in Pakistan. Phylogenetic analyses assist in determining relationships of a *Lecanora* species collected from Pakistan, with those collected from other geographical areas of the world. Moreover, this research provides a baseline for future investigation on *Lecanora* taxonomy and diversity in Pakistan.

Materials and Methods

Morphological and chemical studies

Surveys were carried out in August and October 2022 in Darel Valley, Gilgit Baltistan, as a component of the corresponding author's PhD study project. Iqbal & Khalid (2024) provide additional details of the sample location. A stereomicroscope (Meiji Techno, EMZ-5TR, Japan) was used to study the morphological features of the thallus and apothecial discs. A compound microscope (MX4300H, Meiji Techno Co., Ltd., Japan) was used to measure a free-hand sections of the apothecia on a water-mounted glass slide. Standard microscopy and secondary chemistry was analysed using spot tests, which were performed using KOH (10%; K) and sodium hypochlorite solution (C). Thin Layer Chromatography was carried out using Solvent System C, following standard methods (Orange *et al.* 2010).

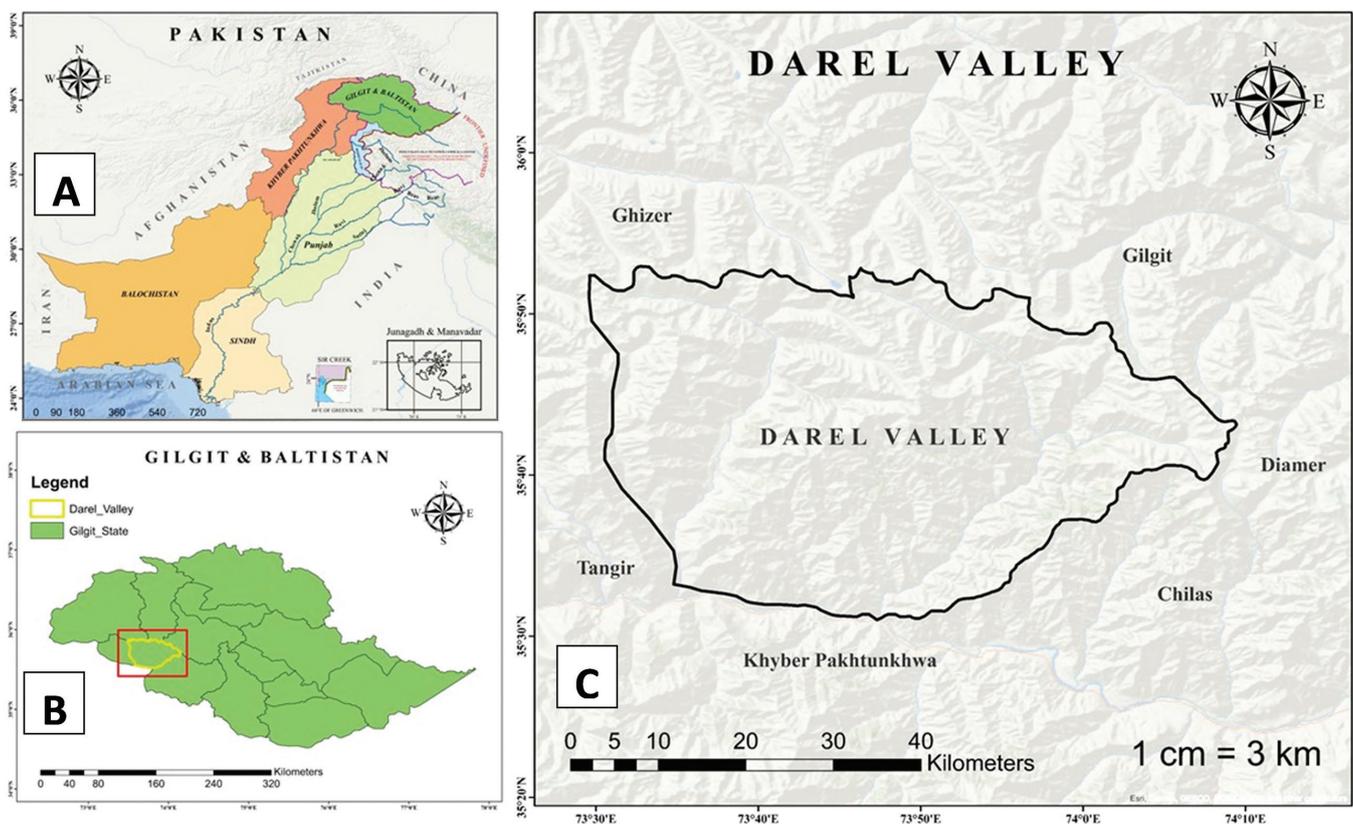


Fig. 1. Maps of sampling site. **A.** Pakistan. **B.** Gilgit Baltistan. **C.** Darel Valley.

Molecular Characterization

Nuclear DNA was extracted in the Fungal Biology and Systematics Research Laboratory, Institute of Botany, University of the Punjab from air dried and cleaned thalli using a GF1 Plant DNA extraction kit, as directed by the manufacturer (Vivantis, Selangor Darul Ehsan, Malaysia). For qualitative examination of total extracted DNA, 1% agarose gel electrophoresis was employed (Voytas 2000). PCR amplification of the ITS region was performed on a Bio-RAD T100 thermal cycler. Primers used during amplifications were ITS1F 50 -CCT GGT CAT TTA GAG GAA GT A-A-3' and ITS4 50 -TCC GCT CTA TTG ATA TGC-30 for

the ITS region (White *et al.* 1990; Gardes & Bruns 1993). Usman *et al.* (2021) adopted the PCR conditions used by Gardes & Bruns (1993) and these modified PCR conditions were used here. PCR products were purified using a QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA) and then submitted to TsingKe, China for Sanger sequencing utilizing the aforesaid ITS1F, ITS4 amplicons for forward and reverse sequencing. To reconstruct forward and reverse sequences, the BioEdit sequence alignment editor was utilized (Hall 1999). The nucleotide sequence comparison was carried out using the National Centre for Biotechnology Information's (NCBI) Basic Local Alignment Search Tool (BLAST; Altschul *et al.* 1990). MAFFT v.7 was used

for the multiple sequence alignment, with all parameters set to default levels (Kato & Standley 2013).

For phylogenetic analyses, the ITS gene was amplified and sequenced successfully from two Pakistani *Lecanora* collections (voucher no. LAH38235 & LAH38236) that yielded a fragment length of 600–650 base pairs each. Phylogenetic relationships were investigated using Maximum Likelihood bootstrapping, as implemented in RAxML-HPC2 v. 8.1.11 (Stamatakis 2014), hosted on the CIPRES Science Gateway (Miller *et al.* 2010). Analyses used rapid bootstrapping with 1,000 replications, and Kimura 2 (+G) parameter model. FigTree v 1.4.3 (Rambaut *et al.* 2014) was used for visualising trees from the ML analysis and preparing the figure. *Protoparmeliopsis garovaglii* (Körb.) Arup, Zhao Xin & Lumbsch was selected as an outgroup, for rooting the tree.

Results

Phylogenetic analysis

A total of 43 ITS nrDNA sequences were analysed, including two from Pakistan. The data matrix had 606 unambiguously aligned nucleotide positions among which 305 were constant, 280 variable sites, 246 were parsimony-informative and 32 were singleton variants. In our phylogram, our novel Pakistani samples (OR539699 & OR539700) formed a well-supported (74%) sister relation to *L. argopholis* (Ach.) Ach. from Austria, China and Russia (MN905177, MW257122 & MH512978).

Table 1. Comparison of characters between *Lecanora* species.

Characters	<i>Lecanora darelensis</i> sp. nov.	<i>L. argopholis</i>	<i>L. frustulosa</i>	<i>L. expallens</i>	<i>L. paramerae</i>	<i>L. substerilis</i>
Upper surface	pale yellowish green	yellowish white	pale cream yellow to yellowish white	mostly green or pale-yellow	whitish grey to greenish grey	grey
Apothecia	0.5–1.5 mm	1.2 mm	1.0 mm	1.0 mm	1.5–2.5 mm	1 mm
Apothecial Disc	brown, plane to convex, pruinose to rarely epruinose	deep yellowish brown, plane or slightly concave, epruinose	dark brown, blackish brown, plane or slightly concave, epruinose	yellowish, rarely distinctly convex	pale brown, flat to concave, epruinose	brown, epruinose
Epihymenium	25–35 µm	20 µm	15–20 µm	-	-	-
Hymenium	110–150 µm	70–75 µm	50–70 µm	40–45 µm	60–70 µm	60–80 µm
Ascospores	14–18 × 5–8 µm	12.7 × 7.3 µm	12 × 5.3 µm	10–14 × 3.4–4.5 µm	12–15 × 7.5–10 µm	10–14 × 7–11 µm
Chemistry	atranorin	atranorin, epanorin, zeorin	acetone	usnic acid and zeorin	atranorin, 2'-O-methylperlatolic acid, and psoromic acid, chloroatranorin, and 2'-O-demethylpsoromic acid	atranorin and roccelic acid
Ecology	calcareous rocks	rock surfaces, detritus, mosses, sloping siliceous and calcareous rocks, boulders, gravel	siliceous and sub-calcareous rocks	bark of trees	on old <i>Juniperus thurifera</i> trees	forests, woodlands, <i>Fagus sylvatica</i> , <i>Abies alba</i>
References	this paper	Vänskä 1984	Vänskä 1984	Bajpai <i>et al.</i> 2012	Martínez <i>et al.</i> 1999	Malíček <i>et al.</i> 2017

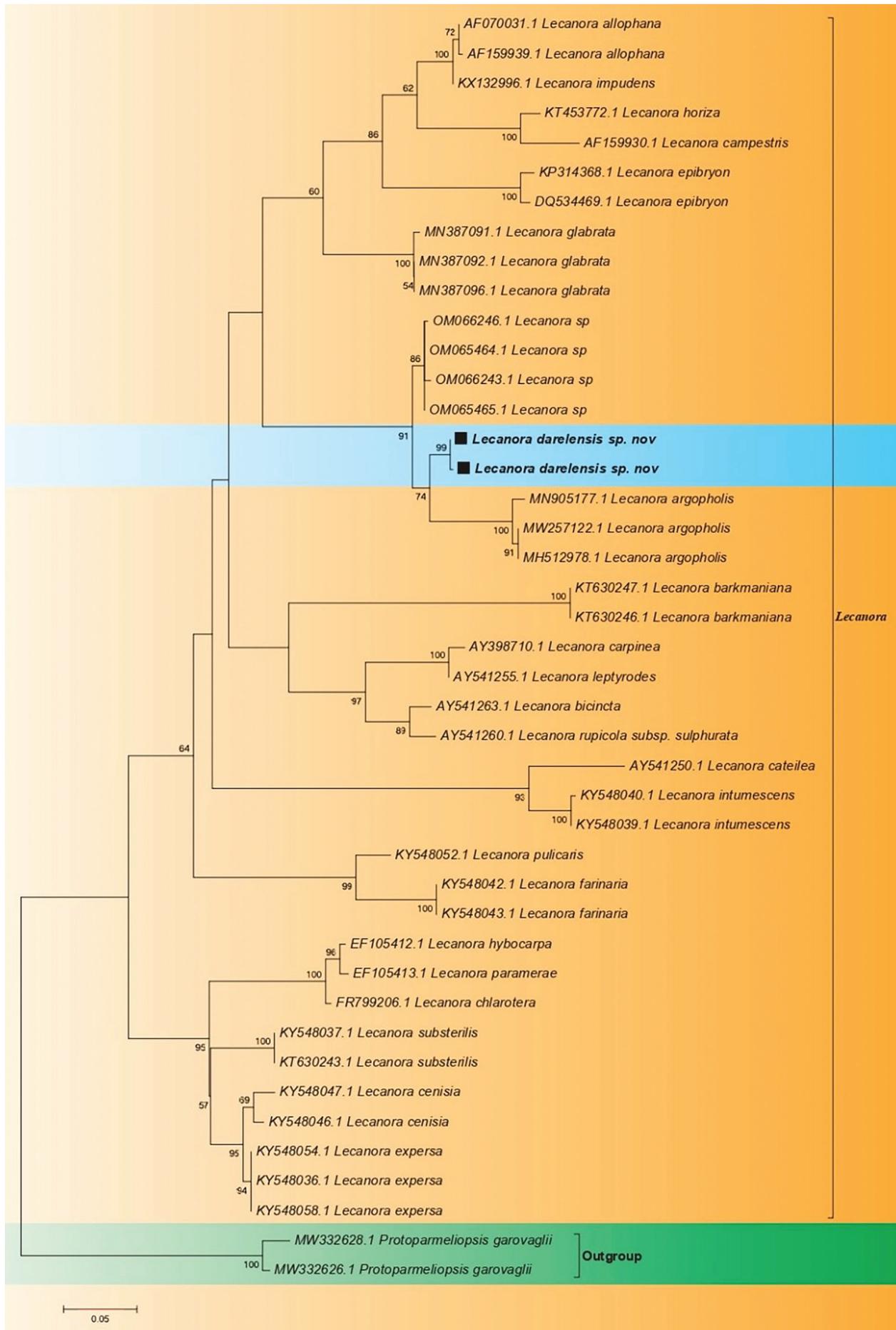


Fig. 2. Molecular phylogenetic analysis of *Lecanora* species using maximum likelihood methods, based on ITS1, 5.8S and ITS2 data. Numbers below branch node represent ML bootstrap (> 50%) based on 1,000 replicates. Sequences generated from Pakistani collections are marked with black box (■).

Taxonomy

Lecanora darelensis M.S.Iqbal & Khalid, *sp. nov.*

MYCOBANK # MB 849954

Holotype: PAKISTAN: Gilgit Baltistan, Darel Valley, 35°37'N, 73°27'E, 1,900 m, on rocks, 10 Aug, 2022, *Muhammad Shahid Iqbal DR-20* [ITS GenBank accession number OR539699] (holotype: LAH 38235).

Thallus: crustose, saxicolous, areolate, (0.5–)1–3(–5) cm across, (0.5–)0.8(–1) mm thick, loosely adnate to tightly adnate, pruinose, esorediate. **Areoles:** usually contiguous, plane to convex, flattened, smooth, matte, 0.2–0.7(–1) mm diam. **Upper surface:** pale yellowish green. **Cortex:** reddish brown to greyish reddish, (19–)25–45(–48) μm ; crystals: hyaline, granular, dark brown, (3–)5.5(–7.5) \times (2.5–)4(–5) μm , hyphae 2–3 μm diam. **Medulla:** 0.2–0.7(–1.0) μm thick. **Algal layer:** simple, countinuous, (45–)50–110(–130) μm thick. **Algal cells:** *Trebouxia* type, cells subglobose to globose, (10–)14–22(–26) μm in diam. **Apothecia:** frequent at centre, dispersed, lecanorine. **Disc:** smooth, pruinose to rarely epruinose, plane to convex, glossy, brown, discrete to contiguous, (0.3–)0.5–1.5(3.5–) mm in diam. rounded to irregular with age. **Margins:** smooth, uneven, discontinuous, concolorous to thallus. **Thalline exciple:** containing algal cells. **Ephymenium:** light brown to dark greyish brown in KOH, (18–)25–35(–45) μm high. **Hymenium:** hyaline, (80–)110–150(–170) μm tall. **Hypothecium:** (45–)60–75(–90) μm tall, hyaline to light brown, containing algal cell in the lower part. **Paraphyses:** (1.5–)2.5–3.5(–4.5) μm thick, apically branched with capitate apices, (2.5–)3.7–4.5(–5) \times (2–)2.8–3.2(–4) μm . **Asci:** clavate, *Lecanora*-type, (45–)55–65(–80) \times (12–)16–21(–28) μm wide, 8-spored. **Ascospores:** aseptate, simple, hyaline, ellipsoid to sub-ellipsoid, (11–)14–18(–22) \times (3–)5–8(–11) μm . **Conidiomata:** pycnidial, hyaline to pale brown walls, immersed, (8–)10–23(–27) μm thick. **Conidia:** simple, filiform, hyaline, bacilliform, (14–)17(–19.5) μm \times 0.3 μm .

Chemistry: thallus and apothecial margin; K⁺ (bright yellow), C⁻, KC⁻. TLC: atranorin.

Diagnosis: The new taxon is phylogenetically closely related to *L. argopholis*. The former can be distinguished from latter in having pale yellowish green upper surface (vs yellowish white), larger apothecial disc 0.5–1.5 mm (vs 1.2 mm), brown, plane to convex, pruinose to rarely epruinose apothecial disc (vs deep yellowish brown, plane or slightly concave, epruinose), taller ephymenium 25–35 μm (vs 20 μm), taller hymenium 110–150 μm (vs 70–75 μm), larger ascospores 14–18 \times 5–8 μm (vs 12.7–7.3 μm), atranorin is detected by TLC (vs atranorin, epanorin, zeorin) and growing on calcareous rocks (vs rock surfaces, detritus, mosses, sloping siliceous and calcareous rocks, boulders, gravel).

Habit and Habitat: found on calcareous rocks, collected from cold semi-arid climate at an altitude of 2,000 m a.s.l. The climate has typically cold desert characteristics, with severe winters (usually with moderate to heavy snowfall) and dry summers. Average annual precipitation in the valley is 100–300 mm, mostly occurring during winter and early spring in the form of snow. Common associated species are trees and shrubs, including *Pinus gerardiana*, *Cedrus deodara*, *Pinus wallichiana* and *Fraxinus xanthoxyloides*.

Additional specimen examined: PAKISTAN: Gilgit Baltistan, Darel Valley 34°36'N, 72°26'E, 1,843 m, on rocks, 10 Oct, 2022, *Muhammad Shahid Iqbal DR-26* [ITS GenBank accession number OR539700] (LAH 38236).

Etymology: The epithet *darelensis* refers to the type locality Darel, GB, Pakistan.

Discussion

Lecanora darelensis is phylogenetically closely related to *L. argopholis* (Ach.) Ach. The former can be distinguished from latter in having pale yellowish green upper surface (vs yellowish white), larger apothecial disc 0.5–1.5 mm (vs 1.2 mm), brown, plane to convex, pruinose to rarely epruinose apothecial disc (vs deep yellowish brown, plane or slightly concave, epruinose), taller ephymenium 25–35 μm (vs c. 20 μm), taller hymenium 110–150 μm (vs 70–75 μm), larger ascospores 14–18 \times 5–8 μm (vs 12.7 \times 7.3 μm), atranorin is detected by TLC (vs atranorin, epanorin and zeorin) and growing on calcareous rocks (vs rock surfaces, detritus, mosses, sloping siliceous and calcareous rocks, boulders, gravel) (see Vänškä 1984).

The new taxon differs from *L. frustulosa* (Dicks.) Ach. in having pale yellowish green upper surface (vs pale cream yellow to yellowish white), larger apothecial disc 0.5–1.5 mm (vs c. 1.0 mm), brown, plane to convex, pruinose to rarely epruinose apothecial disc (vs dark brown, blackish brown, plane or slightly concave, epruinose), taller ephymenium 25–35 μm (vs 15–20 μm), taller hymenium 110–150 μm (vs 50–70 μm), larger ascospores 14–18 \times 5–8 μm (vs c. 12 \times 5.3 μm), atranorin is detected by TLC (vs acetone) and growing on calcareous rocks (vs siliceous and sub-calcareous rocks) (see Vänškä 1984).

Our new species can be distinguished from *L. expallens* Ach. in having pale yellowish green upper surface (vs mostly green or pale-yellow), larger apothecial disc 0.5–1.5 mm (vs 1.0 mm), brown, plane to convex, pruinose to rarely epruinose apothecial disc (vs yellowish, rarely distinctly convex), taller hymenium 110–150 μm (vs 40–45 μm), larger ascospores 14–18 \times 5–8 μm (vs 10–14 \times 3.4–4.5 μm), atranorin is detected by TLC (vs usnic acid and zeorin) and growing on calcareous rocks (vs bark of trees) (Bajpai *et al.* 2012).

Similarly, our new taxon is clearly differentiated from the *L. paramerae* in having pale yellowish green upper surface (vs whitish grey to greenish grey), smaller apothecial disc 0.5–1.5 mm (vs 1.5–2.5 mm), brown, plane to convex, pruinose to rarely epruinose apothecial disc (vs pale brown, flat to concave, epruinose), taller hymenium 110–150 μm (vs 60–70 μm), larger ascospores 14–18 \times 5–8 μm (vs 12–15 \times 7.5–10 μm) and growing on calcareous rocks (vs on old *Juniperus thurifera* trees) (see Martínez *et al.* 1999).

The new species also differs from *L. substerilis* Malíček & Vondrák in having pale yellowish green upper surface (vs grey), larger apothecial disc 0.5–1.5 mm (vs 1 mm), brown, plane to convex, pruinose to rarely epruinose apothecial disc (vs brown, epruinose), taller hymenium 110–150 μm (vs 60–80 μm), larger ascospores 14–18 \times 5–8 μm (vs 10–14 \times 7–11 μm) and growing on calcareous rocks (vs forests, woodlands, *Fagus sylvatica*, *Abies alba*) (see Malíček *et al.* 2017).

Acknowledgements

We would like to express our gratitude to Mr. Javed Chaudhary & Mr. Tahir Chaudhary for accompanying us on field visits and

helping with the collection. Constructive comments from an anonymous reviewer significantly improved the manuscript.

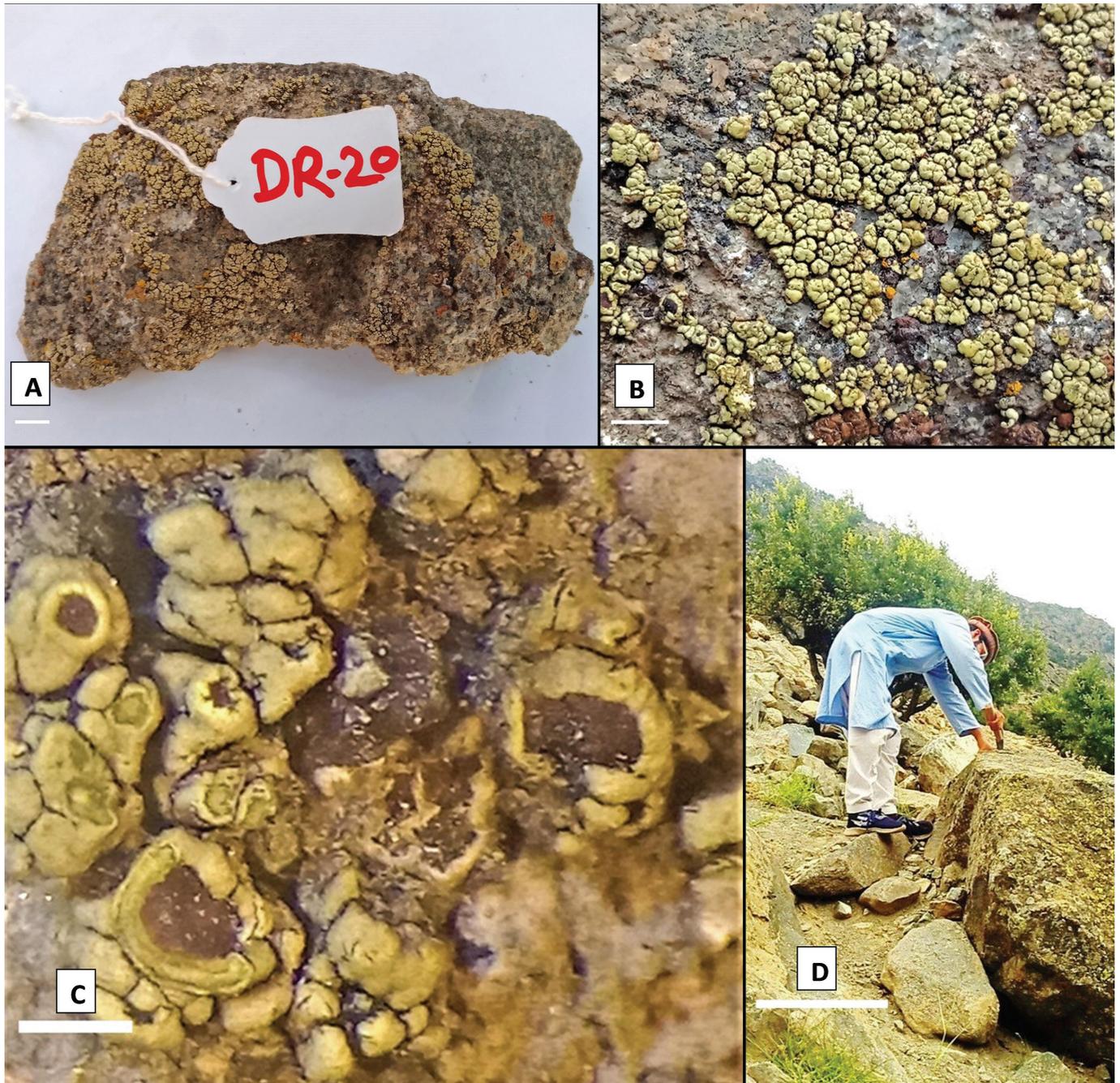


Fig. 3. *Lecanora darelensis*, sp. nov. **A, B.** Thallus. **C.** Apothecia. **D.** Author collecting lichens on rocks in the Darel Valley. Voucher: M.S. Iqbal DR-20 (holotype: LAH 38235). Scale bars: A, B = 1 cm; C = 1.5 mm; D = 6 cm.

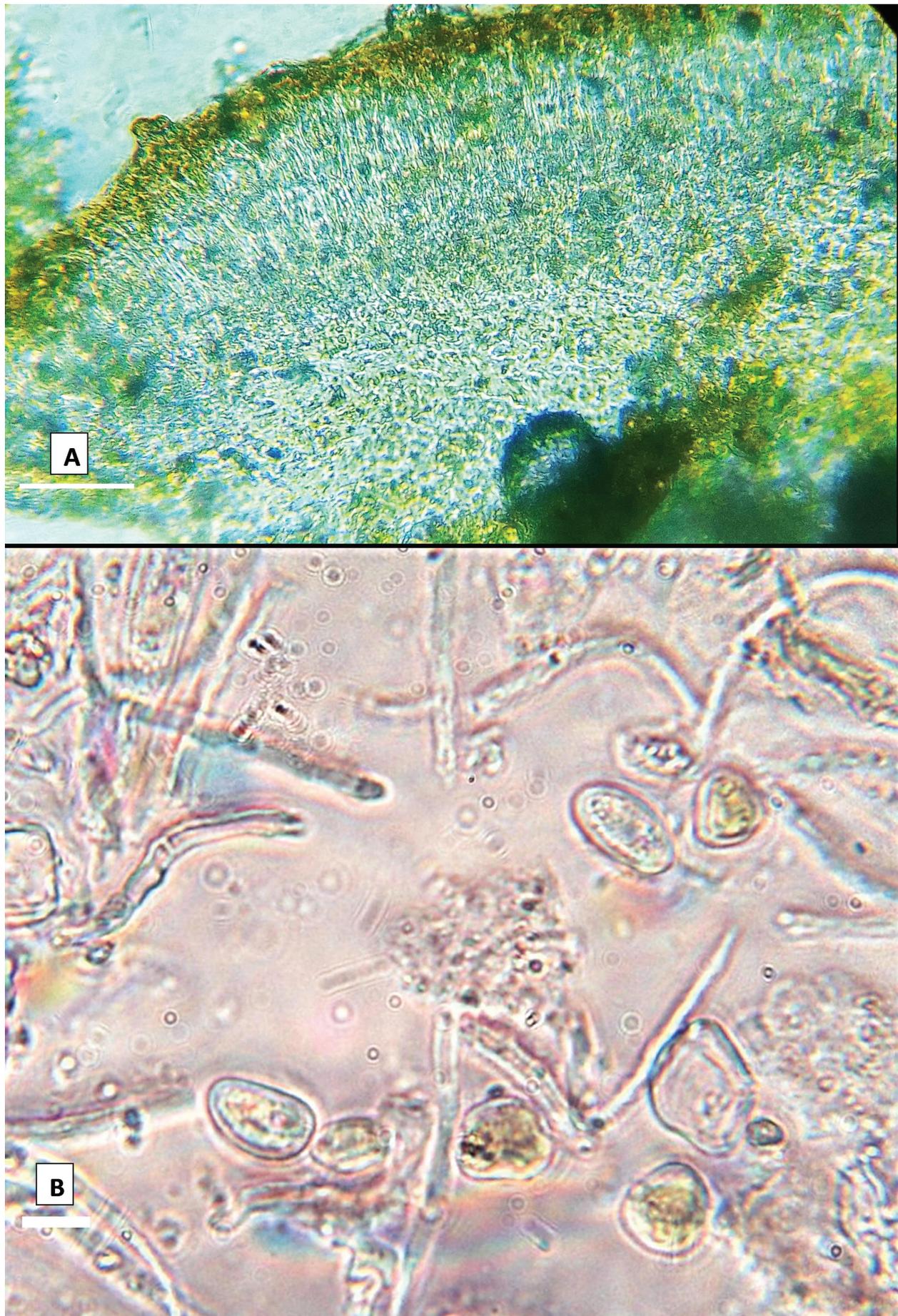


Fig. 4. *Lecanora darelensis*, sp. nov. **A.** Cross section of apothecium at 40 ×. **B.** Ascospores. Voucher: M.S. Iqbal DR-20 (holotype: LAH 38235). Scale bars: A = 110 µm; B = 8 µm.

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