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# *Rusavskia dasanensis* (Teloschistaceae), a new record for Pakistan

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*Rusavskia dasanensis* (Teloschistaceae), which was previously found in Norway, the Svalbard Islands, Ny-Ålesund, Haloya Island, the Korean Arctic station and Europe, is being reported as a new record from Darel Valley, Gilgit Baltistan, Pakistan. A description of the species is provided together with details on its range, habitat and phylogeny.

Keywords: Darel, Gilgit Baltistan, ITS, taxonomy, phylogeny

## Introduction

Despite the fact that Pakistan is widely recognized for its geographic and climatic features, which are linked to a broad range of biodiversity (IUCN 2006), the nation has not been well investigated for lichens. The lack of surveys in many areas means that the lichen diversity in this area is probably extremely great. Up to 400 lichens from Pakistan have so far been identified. ([Aptroot and Iqbal 2012](#), [Fatima et al. 2021](#), [Habib et al. 2021](#), [Kousar et al. 2021](#), [Fayyaz et al. 2022](#), [Nadeem et al. 2022](#), [Iqbal et al. 2023](#)). During our exploration of the lichen diversity of Pakistan, a collection of lichen genus *Rusavskia* S.Y. Kondr. & Kärnefelt was made from Darel Valley, Gilgit Baltistan, Pakistan. The morphological and chemical traits along with molecular characterization of this newly collected specimen allowed us to confirm the existence of *Rusavskia dasanensis* S.Y. Kondr., I. Galanina & Hur from Pakistan, which was previously reported from Norway, the Svalbard Islands, Ny-Ålesund, Haloya Island, the Korean Arctic station and Europe.

## Material and methods

### Morphological and chemical studies

Lichens were collected in 2022 while surveying the various regions of Gilgit Baltistan, Pakistan. The morphological properties were examined using a stereomicroscope. Thin

layer chromatography (TLC) and spot tests were used to identify the lichen substances (Hale 1979). For the observation of microscopical characters, free-hand sections of the thallus were placed on a water-mounted glass slide and measurements made with a compound microscope.

DNA extraction, PCR amplification and sequencing

Genomic DNA was directly isolated using a modified 2% CTAB procedure from a section of each specimen's thallus with apothecia (Gardes and Bruns 1993). Extracted DNA was used for PCR amplification of the ITS nrDNA marker using a pair of primers: ITS1F forward primer (5'CTTGGTCATTTAGAGGAAGTAA3') and ITS4 reverse primer (5'TCCTCCGCTTATTGATATGC 3') (White et al. 1990). The amplified DNA fragments (PCR results) were observed on a 1% agarose gel using an ethidium bromide-based gel documentation system (Sambrook and Russell 2001). The amplified products were subsequently sequenced by a commercial company.

Phylogenetic analysis

High similarity sequences were retrieved using the BLAST search of Genbank (www.ncbi.nlm.nih.gov/genbank), taking maximum query coverage and the percentage of related taxonomic identities in consideration. The MAFFT ver. 7 tool was used to prepare a multiple sequence alignment with all settings set to default values (Katoh and Standley 2013). The start and end of alignments were trimmed at a conserved site. Gaps were treated as missing data. Phylogenetic analysis was performed in MEGA ver. 6.0 by creating maximum likelihood (ML) trees at 1000 Bootstraps based on the General reversible model (Tamura et al. 2013) Table 1, Fig. 1–2.

Taxonomic treatment

*Rusavskia dasanensis* S.Y.Kondr., Galanina and Hur, Acta Bot. Hung. 55: 360 (2013), Fig. 3

Thallus: foliose, saxicolous, 4–6 cm across, smooth, shiny, adnate to loosely adnate, lobate, isidiate. Lobes: narrow to branched, 3–5 mm long, tips 0.4–1.0 mm wide, plane to convex, rotund to truncate, ramified, with 3–6 isidium-like secondary lobules. Lobes and lobules: less separated to aggregated, somewhat flat, sometimes with whitish pruina. Isidia: concolorous to the thallus, frequent at the centre of the thallus, globose to marginal or somewhat laminal, 0.2–0.4 mm long, irregular, simple. Color: bright orange when dry, becoming yellowish orange when wet.

Table 1. Data of the ITS alignment for *Rusavskia dasanensis*

Total no. of aligned sites	584
No. of constant characters	281
No. of variables	294
No. of parsimony-informative characters	256
No. of singleton characters	38

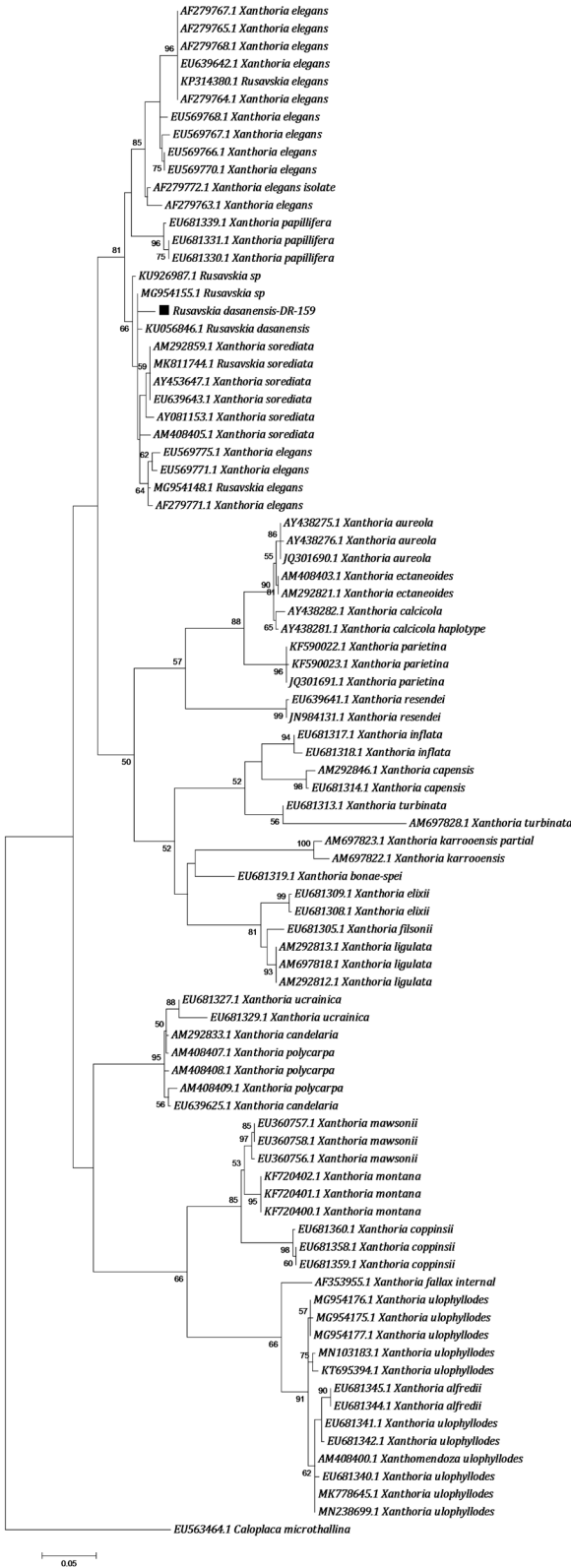


Figure 1. Molecular phylogenetic analyses by maximum likelihood analysis of *Rusavskia dasanensis*. The new sequence generated from the Pakistan collection is marked with a bold black box.

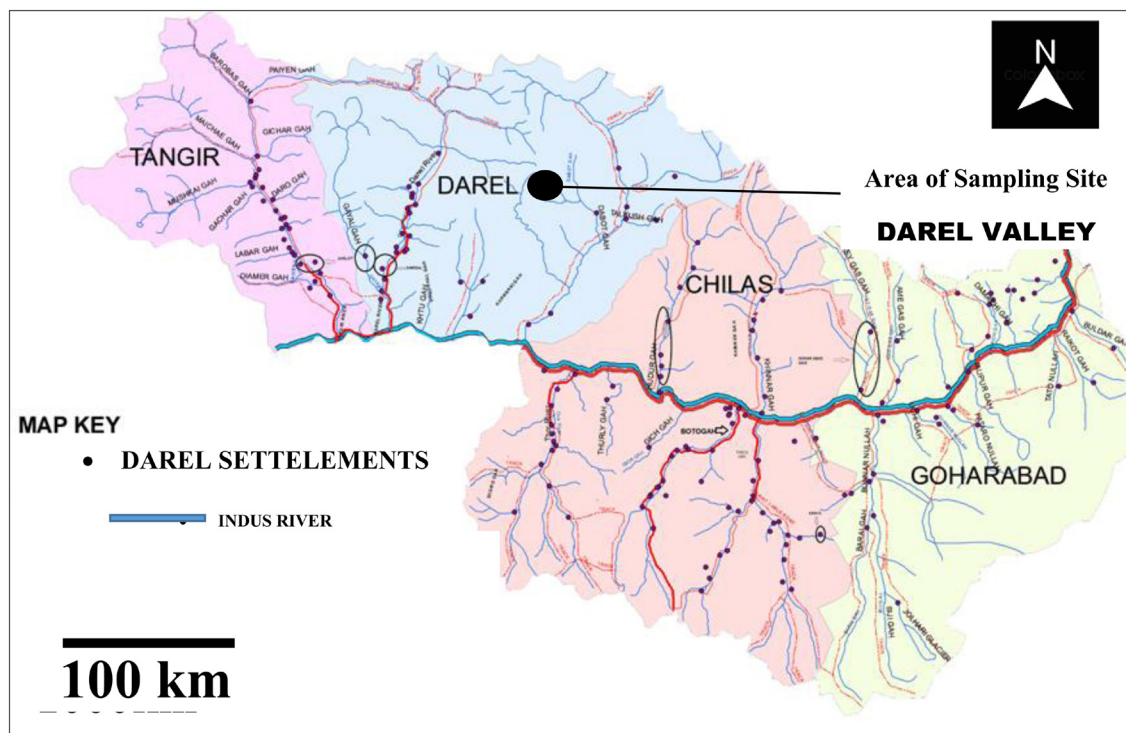


Figure 2. Situation of the Darel Valley, Gilgit-Baltistan, Pakistan. Source: (<https://pamirtimes.net/2018/08/03/miscreants-torch-more-than-a-dozen-schools-in-darel-valley-of-diamer>).

Upper cortex: orange to yellowish brown, thin to thick, 25–45  $\mu\text{m}$  thick, paraplectenchymatous, cells globose, 5–11  $\mu\text{m}$  in diam. Algal layer: continuous, 50–110  $\mu\text{m}$  tall, photobiont chlorococcoid, cells subglobose to globose, 10–16  $\mu\text{m}$  in diam. Medullary layer: 45–100  $\mu\text{m}$  thick, hyphae white, elongate, 2–4  $\mu\text{m}$  wide, with hapters. Lower cortex: inside hyaline, outside brown, paraplectenchymatous, isodiametric, 20–40  $\mu\text{m}$  thick.

**Apothecia:** not found. **Pycnidia:** not found.

**Spot tests:** K+ (purple), C–, KC–.

**TLC:** parietin (major) and teloschistin

**Ecology:** *Rusavskia dasanensis* is quite common and widespread in Norway, Svalbard Islands, Ny-Ålesund, Haloya Island, Korean Arctic station and Europe. (Kondratyuk et al. 2013). It is a new record for Pakistan and is reported from Gilgit Baltistan.

**Material examined:** Pakistan: Gilgit Baltistan, Diamer District, Darel Valley 35°37'N, 73°27'E, elev. 2000 m a.s.l., on rock, 21 Oct. 2022, Muhammad Shahid Iqbal DR-159 (ITS GenBank Accession no OR030381).

## Discussion

*Rusavskia dasanensis* is characterized by very narrow and richly branched lobes and 0.3–0.5 mm long, more or less horizontal, numerous, cylindrical isidia (richly ramified, 3–7 mm long and 0.3–0.4 mm wide lobes with 5–7 secondary lobules according to Kondratyuk et al. 2013). The ITS sequence of Pakistani *R. dasanensis* (DR-159) is exactly similar to the one from China (accession no. KU056846) indicating that they belong to the same species. In our phylogram, the sequences of *R. dasanensis* form a sister clade to *R. sore-diata*. Morphologically, both are similar but differ in having a crustose-foliose smooth thallus (versus horizontal foliose thallus), and the presence of fallacinal, emodin, teloschistin and parietinic acid in *R. sore-diata*. (Mishra et al. 2020). The morphological features of the Pakistani collection agree with the published description of *R. dasanensis*, reported from Europe (Kondratyuk et al. 2013) except for the larger lobes 0.4–1.0 mm (versus 0.3–0.4 mm) and smaller isidia, 0.2–0.4 mm (versus 0.3–0.5 mm). *Rusavskia dasanensis*



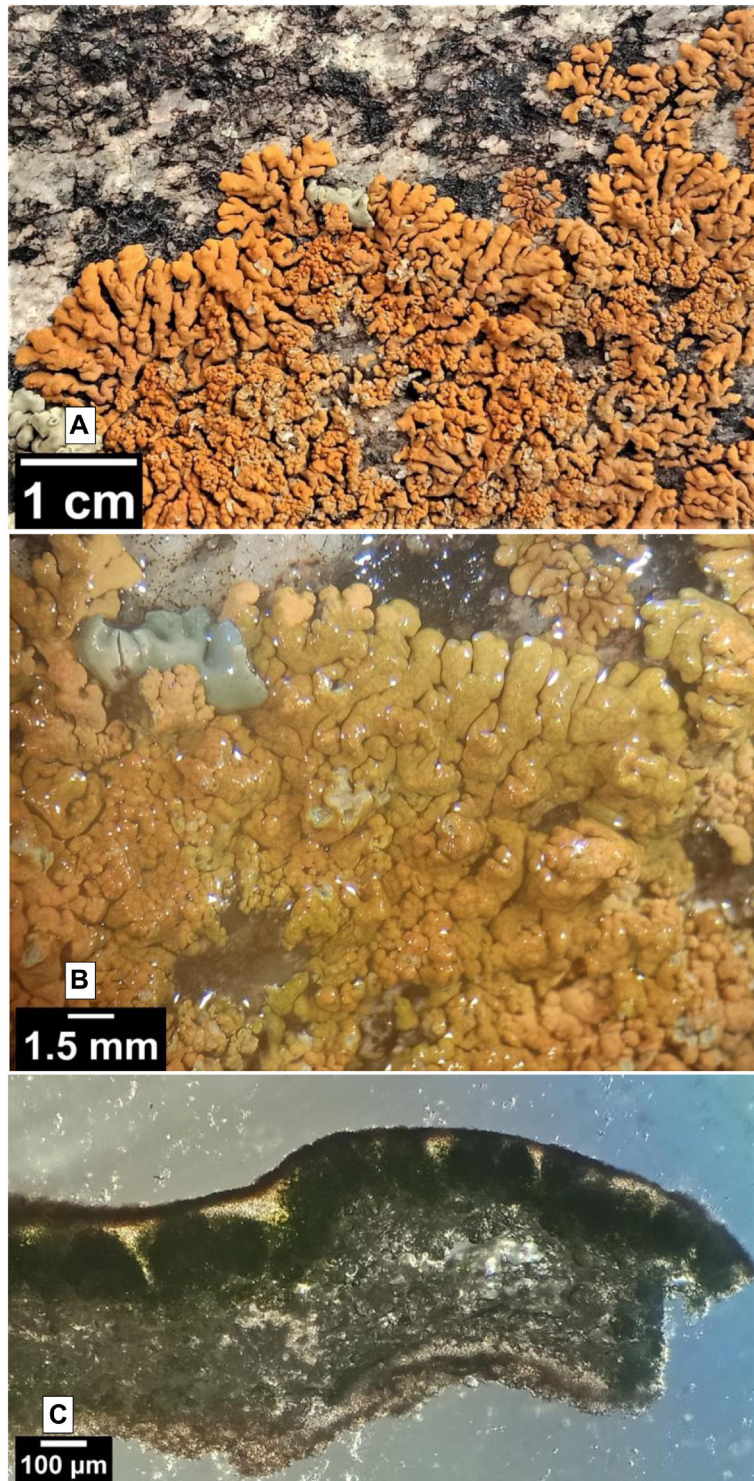


Figure 3. (A) Habitus of *Rusavskia dasanensis*, (B) narrow to branched lobes, (C) cross section of thallus.

was originally described from northern Norway, Svalbard Islands, Ny-Alesund, Haloya Island, Korean Arctic station, Europe, and was later reported from China and Russia (Kondratyuk et al. 2015). Here it is reported from Pakistan for the first time (Fig. 3).

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## Author contributions

The study's inception and design were contributed by all authors. Muhammad Shahid Iqbal gathered samples and morpho-anatomical data. MSI and ANK were in charge of material processing and analysis. Muhammad Shahid Iqbal wrote the initial draft of the manuscript. The final manuscript was reviewed and approved by all authors..

## Data availability statement

There are no additional data for this paper.

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