

Glaucomaria rupicola: A new lichen record from Gilgit Baltistan (Pakistan), its DNA barcoding, morphology, ecology and distribution

Muhammad Shahid IQBAL^{ORCID}

Punjab University, Institute of Botany, Fungal Biology and Systematics Laboratory, Quaid-e-Azam Campus, 54590, Lahore, Pakistan
shahidmycologist112@gmail.com

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Glaucomaria rupicola: Gilgit Baltistan (Pakistan)'dan yeni bir liken kaydı, DNA barkodlaması, morfolojisi, ekolojisi ve yayılışı

Abstract: During an examination of lichen specimens in the Darel Valley, one new record of genus *Glaucomaria* was identified for the lichen flora of Pakistan. *Glaucomaria rupicola* (L.) P.F. Cannon has been collected from Gilgit Baltistan. Diagnostic characters of the species, distribution, ecology and phylogenetic analyses are given. Here, it has been reported first time on ITS-based phylogeny. It is a new record for Gilgit Baltistan, Pakistan.

Key words: *Ascomycota*, Darel, *Lecanoraceae*, phylogenetic analyses, taxonomy

Özet: Darel Vadisi'ndeki liken örneklerinin incelenmesi sırasında, Pakistan'ın liken florası için *Glaucomaria* cinsinin yeni bir kaydı tanımlandı. *Glaucomaria rupicola* (L.) P.F. Cannon, Gilgit Baltistan'dan toplandı. Türün tanısal karakterleri, dağılımı, ekolojisi ve filogenetik analizleri verilmiştir. Burada, ilk kez ITS tabanlı filogenide bildirilmiştir. Pakistan, Gilgit Baltistan için yeni bir kayıttır

Anahtar Kelimeler: *Ascomycota*, Darel, *Lecanoraceae*, filogenetik analizler, taksonomi

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1. Introduction

Maurice Gustave Benoît Choisy (1897-1966) first introduced the genus *Glaucomaria* M. Choisy in 1929. When Choisy discovered the genus *Glaucomaria* in (1929), at that time this genus consisted of three species which are ranked: *Lecanora glaucoma* (= *L. rupicola*, *L. angulosa* (= *L. carpinea* and *L. albella*). Though none of the three constituent species were formally united into *Glaucomaria*, and Choisy did not designate a type, the genus was given a description that satisfies the standards for legitimate publication. Hafellner (1984) well-defined the genus and lectotyped it with *L. rupicola*, but the requisite combination was not completed. Similarly, even though Kondratyuk et al. (2019) designated *G. rupicola* as a binomial in their summary appearance of the genus, no formal grouping was recognized. According to latest survey, the number of species of the genus *Glaucomaria* has now reached 10, whose names are classified as; *G. bicincta*, *G. carpinea*, *G. cinerella*, *G. leptoplaca*, *G. leptyodes*, *G. lojkaeana*, *G. rupicola*, *G. subcarpinea*, *G. swartzii* and *G. swartzii* subsp. *swartzii* (<http://www.indexfungorum.org/names/Names.asp>).

The genus *Glaucomaria* segregated from *Lecanora* with strongly pruinose, often semi-immersed apothecia; containing sordidone, the disc pruina C+ yellow or orange (Cannon et al., 2022). In the current study, just one species of this genus was reported and identified using comparative morpho-anatomical, molecular, and chemical investigations. *G. rupicola* has never been documented in Darel Valley, Gilgit Baltistan, Pakistan. This is the first

time it has been reported on an ITS-based phylogeny. This is a new record for Darel Valley, Gilgit Baltistan, Pakistan.

2. Materials and Method

Morpho-anatomical characteristics were examined using a stereomicroscope (Meiji Techno, EMZ-5TR, Japan) and a compound microscope (MX4300H, Meiji Techno Co., Ltd., Japan). Spot tests were conducted according to the protocol established by Orange et al. (2010). DNA was extracted from dried and purified thalli using a GF1 Plant DNA extraction kit, adhering to the manufacturer's instructions (Vivantis, Selangor Darul Ehsan, Malaysia). The cladogram was developed using MEGA 6.0 (Tamura et al., 2013). The sample referenced in this study (DR-198) clustered with *G. rupicola* (L.) P.F. Cannon and formed a sister branch with the same specimen of *G. rupicola* from Austria and the USA (AY541257, MZ243628 and MZ243629) (Fig. 1).

3. Results

Glaucomaria rupicola (L.) P.F. Cannon, in Cannon, Malíček, Ivanovich, Printzen, Aptroot, Coppins, Sanderson, Simkin & Yahr, Revisions of British and Irish Lichens 25: 75, 2022 (Fig. 2).

Thallus crustose, 0.5-1 cm broad, rimose, up to 1 mm thick, irregular. **Areoles** thin or thick, flat to convex, and slightly shiny. **Soredia** not found. **Surface** whitish grey to brownish grey, epruinose, unclear at the borders. **Prothallus** whitish at the edges. **Alga** subglobose to globose, chlorococcoid, 14-24 µm in diameter. **Apothecia** lecanorine, highly pruinose, frequent, scattered, initially immersed, and

emerged when mature. **Disc** 0.5-1.8 mm in diameter, grayish brown to greenish brown, contiguous, grey to pale grey pruinose, planar to convex. **Epiphytenium** 15-25 µm tall. **Hymenium** 95-120 µm tall. **Hypothecium** 50-75 µm tall. **Paraphyses** hyaline, simple, branching apically, 2.5-4 µm wide, apices 3 µm in diameter. **Asci** clavate, *Lecanora*-type, 60-72 × 24-34 µm broad, 8-spored. **Ascospores** 11-16×6-8 µm, simple, hyaline.

Spot tests: thallus; K+ (yellow); apothecial margin; C+

(yellow); TLC: atranorin

Ecology: The valley receives an annual rainfall ranging from 100 to 300 mm, with the majority falling as snow in the winter and early spring months.

Specimen examined: PAKISTAN: Gilgit Baltistan, Darel Valley, 35°37'N, 73°27'E, 2,000 m a.s.l., Aug. 10, 2022, Muhammad Shahid Iqbal, DR-198 (ITS genbank accession number OR751654) (LAH38347).

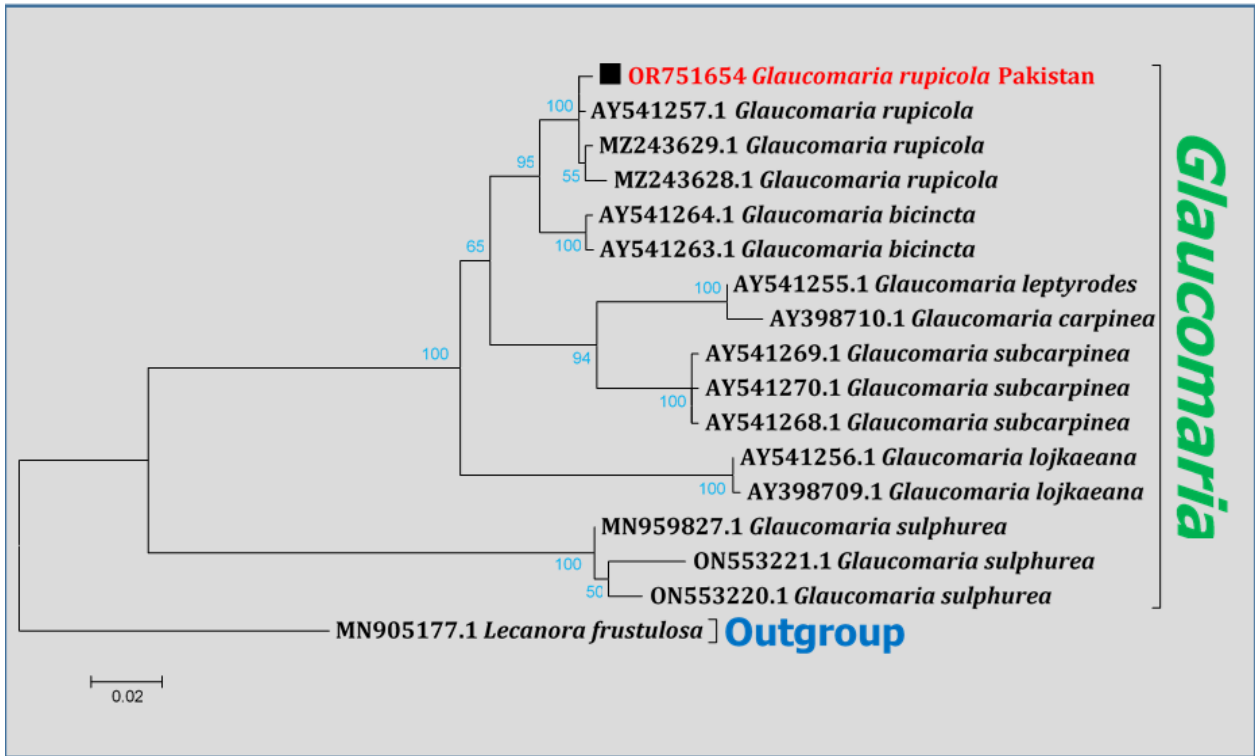


Figure 1. Molecular phylogenetic analysis of *Glaucosmaria rupicola* using the Maximum Likelihood

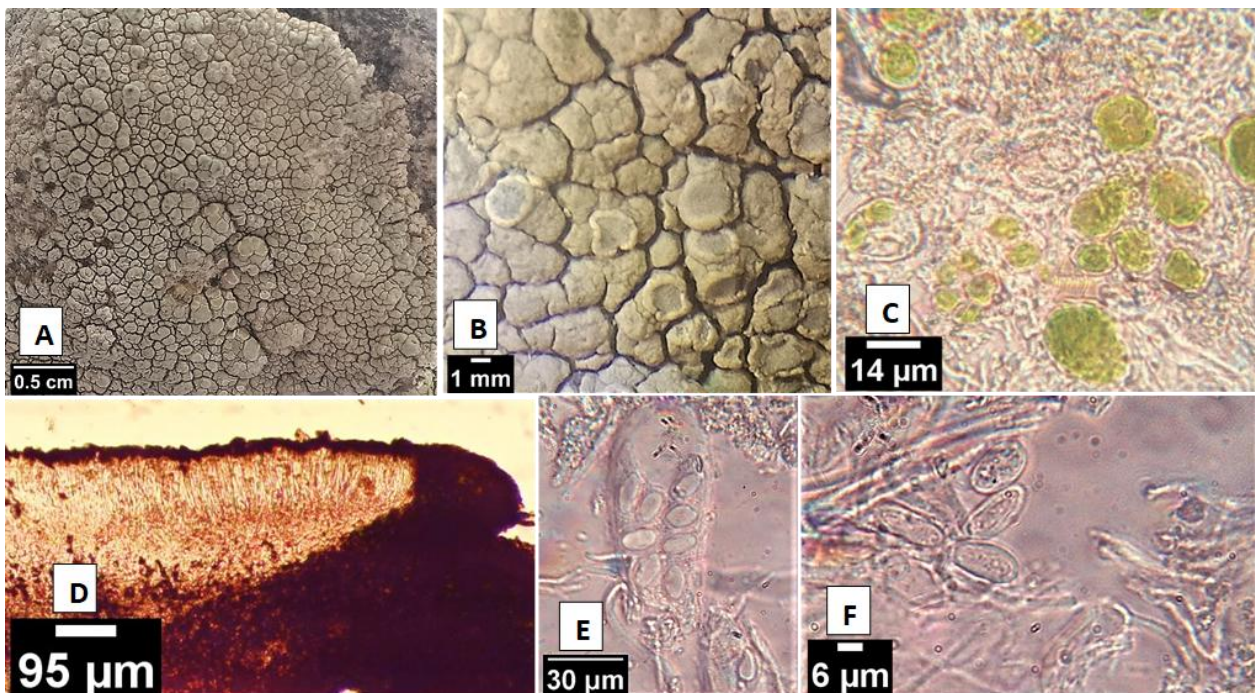


Figure 2. *Glaucosmaria rupicola*, **A:** Thallus, **B:** Apothecia, **C:** Algal cells, **D:** Cross section of apothecium, **E:** Asci with ascospores, **F:** Ascospores

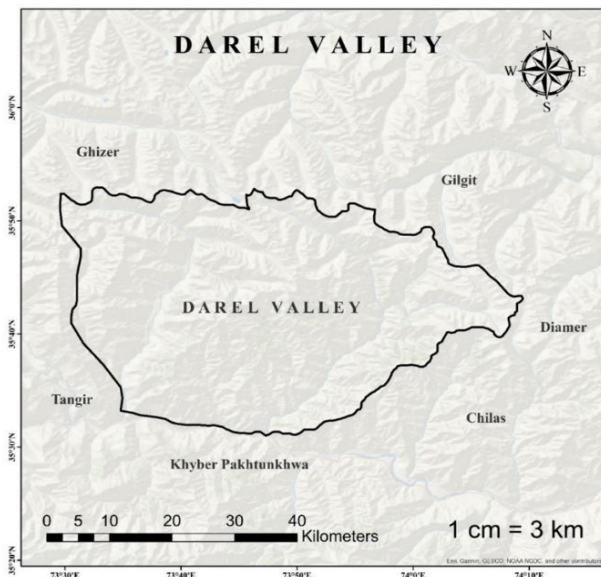


Figure.3 Map of sampling site, Darel Valley, Gilgit-Baltistan, Pakistan (Iqbal and Khalid, 2024b).

Distribution: *Glaucomaria rupicola* is discovered for the first time in Darel Valley, Gilgit Baltistan, Pakistan, growing on calcareous rocks. The species occurs in Austria, England, Ireland, and the United States. It occurs on hard exposed siliceous rocks, frequent in the xeric-supralittoral zone on coastal rocks, but also locally frequent inland, widespread in North and West Britain and Ireland, but relatively local in South and East England, where it is primarily confined to churchyards (Cannon et al., 2022).

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4. Discussions

Our species' morpho-anatomical characteristics match those of *G. rupicola* from Great Britain and Ireland, with the exception of a taller hymenium (95-120 μm vs. 80-90 μm) and larger ascospores (11-16 \times 6-8 μm vs. 9-14.5 \times 5.5-7 μm). According to ecological behaviour, the specimen *G. rupicola* found in Great Britain and Ireland grows on hard, exposed siliceous rocks. It is commonly found in the xeric supralittoral zone on coastal rocks, but it is also occasionally found inland. For some forms of the species, it has strong maritime tendencies. Additionally common throughout the aerohaline zone, however certain types are less common inland. *G. rupicola* growing on siliceous rocks and walls, widespread and fairly frequent, particularly in northern and western Britain. The species exhibits significant variation across its range, with the identification of multiple subspecies; all specimens originating from the British Isles are consistent with *Glaucomaria rupicola* (Cannon et al., 2022). *Glaucomaria rupicola* has been reported for the first time from Pakistan.

In conclusion, with the discovery of this one species, the total number of species in Darel Valley has increased to 30 (Afshan et al., 2024; Asghar et al., 2023; Aptroot and Iqbal, 2012; Din et al., 2023a,b, 2024; Fayyaz et al., 2023; Iqbal et al., 2022; 2023a, b, c; 2024a, b, c, d, e; 2025; Iqbal and Khalid, 2022, 2023, 2024a,b,c,d,e; Riaz et al., 2024; Zulfiqar et al., 2023, 2024).

Conflict of Interest

Author has declared no conflict of interest.

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