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Some new lichen records from Pakistan

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Abstract: During a survey of the lichens in the state of Azad Jammu and Kashmir, many specimens were collected from the Jhelum and Neelum valley and characterized using morpho-anatomical, molecular and chemical test methods. Two taxa new for Pakistan, i.e., *Physciella chloanta* and *Xanthoparmelia protomatrae* s. l., were found in the collection while *Physconia enteroxantha* represent range extensions within Pakistan. Morpho-anatomical descriptions, ecology and distribution are provided.

Keywords: Chikar, lichen biota, mycobiont, phycobiont

INTRODUCTION

Pakistan is well known for its geographical and climatic variations which is linked with rich biodiversity (IUCN, 2006). The lichen diversity in this region is probably very high but little known due to the lack of surveys in many areas (Aptroot & Iqbal, 2012). So far, up to 400 lichens have been reported from Pakistan (e.g. Aptroot & Iqbal, 2012; Fatima et al., 2021; Habib et al., 2021; Kousar et al., 2021; Fayyaz et al., 2022; Nadeem et al., 2022).

During our exploration of the lichen diversity of Pakistan, new collections were made from Jhelum and Neelum valley, Azad Jammu and Kashmir, Pakistan. Using molecular analysis in addition to phenotype and chemical characters we were able to confirm the presence of three foliose species which are presented here. Two of them were not previously known from Pakistan.

MATERIAL AND METHODS

Study area

Pakistan is located in western South Asia between 24–37°N latitude and 62–75°E longitudes. Three specimens were collected from the regions Azad Jammu and Kashmir, in the year 2021, during fieldwork focused on increasing knowledge of the lichen biota of Pakistan. The Azad Jammu and Kashmir state falls within the Himalayan orogenic belt and the topography is mainly hilly and mountainous, and composed of rugged undulating terrain intersected by deep ravines. The northern districts (Neelum, Muzaffarabad, Hattian, Bagh, Haveli, Poonch, and Sudhnoti) are generally mountainous while the southern districts (Kotli, Mirpur, and Bhimber) are of comparatively low relief (Abasi et al., 2019). The collected specimens were deposited in the herbarium of the Institute of Botany, University of the Punjab, Lahore (LAH).

Morphological and chemical characterization

The specimens were examined macro- and micromorphologically under a stereomicroscope (Meiji Techno, EMZ–5TR, Japan) and a compound microscope (SWIFT M4000–D) with a 9MP camera system, respectively. For anatomical investigation, sections of thallus were made by hand and examined in water. A minimum of twenty measurements in water were made for each diagnostic feature from the three specimens. The secondary chemistry was analyzed by spot tests using KOH (10%; K) and sodium hypochlorite solution (C). Thin Layer Chromatography was carried out using Solvent System G, following standard methods (Orange et al., 2010).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted directly from a portion of thallus with apothecia from each specimen using a modified 2% CTAB method (Gardes & Bruns, 1993). The primer pair ITS1F (Gardes & Bruns, 1993) and ITS4 (White et al., 1990) were used to amplify the internal transcribed

spacer (ITS) region under PCR conditions used by Khan et al. (2018). PCR products were sent to BGI, China and sequenced from both directions. Sequences were assembled using BioEdit (Hall, 1999). BLAST analysis was used to retrieve highly similar sequences. The maximum query coverage and percent identity with related taxa were noted. Sequences retrieved from Genbank and suggested by published literature were used for an initial alignment, which was trimmed and then realigned using web-PRANK with default settings (Löytynoja & Goldman, 2010).

RESULTS & DISCUSSION

PHYSCONIA ENTEROXANTHA (Nyl.) Poelt, Nova Hedwigia 12(1+2): 125 (1966) (Fig. 1)

Thallus foliose, up to 3 cm across, effuse, irregular to orbicular, entire, in section 170–230 µm thick, sorediate, lobate, partly pruinose. Lobes discrete to contiguous, rarely imbricate, digitate, initially flat becoming concave, 0.5–1.7 mm wide, surfaces pruinose, smooth. Color grey to pale brown when dry, dark green when wet. Soredia marginal, elongated to rounded, somewhat curved appearing weakly labriform or cuff–shaped, laminal at older parts, laminal soralia often rounded, soredia coarsely granular. Lower surface off white to pale brown at the peripheral lobes, dark brown to blackish brown toward the center, smooth, somewhat glossy, densely rhizinate. Rhizines black, 1–2 mm in length, squarrosely branched. Upper cortex grey brown to colourless toward center, 30–45µm thick, paraplectenchymatous, of textura globularis, cells rounded, 6–10 µm in diameter. Algal layer even, continuous, 60–70 µm thick. Photobiont cells chlorococcoid, cells globose to subglobose, 10–18 µm in diameter. Medulla pale to white, prosoplectenchymatous, 55–100 µm thick, hyphae 2–3 µm wide. Lower cortex hyaline, irregularly prosoplectenchymatous, 20–30 µm thick. Pycnidia not found.

Secondary chemistry: K+, C–, KC+ (yellow). Secalonic acid detected by TLC.

Specimen examined: Pakistan: Azad Jammu & Kashmir: Neelum Valley, Sharda (34.7931°N, 74.1930°E), 1615 m alt., on bark, 22 July 2019, A. N. Khalid & K. Habib (KM–14) (LAH36706), ITS GenBank accession number ON329736.

Taxonomic Remarks

Physconia Poelt is a small genus of lichen-forming fungi of about 30 species (Poelt, 1965). It is characterised by a foliose thallus, which is grey-brown to brown, usually with a whitish pruina, at least on the lobe ends and a characteristic thick-walled-spore type. In Pakistan it is represented by 6 species, namely *P. detersa* (Nyl.) Poelt, *P. distorta* (With.) J.R. Laundon, *P. enteroxantha* (Nyl.) Poelt, *P. farrea* (Ach.) Poelt, *P. grisea* (Lam.) Poelt and *P. muscigena* (Ach.) Poelt (Aptroot & Iqbal, 2012). *P. enteroxantha* has been reported for Pakistan in the past from Swat. This study reports it from the Neelum Valley, Azad Jammu and Kashmir on a molecular basis. The Pakistani collection of *Physconia enteroxantha* (KM–14) is nested in a branch of specimens from Spain (AY368122, AY368120, LS483214, AF540522), USA (AY368121, LS483160), Russia (EF582767, EF582768) and Norway (MK811939, MK811812, MK811840) with strong bootstrap value.

PHYSCIELLA CHLOANTHA (Ach.) Essl., Mycologia 78(1): 94 (1986) (Fig. 1)

Thallus foliose, irregularly orbicular, up to 3 cm in diameter, loosely adnate with ascending lobes. Lobes up to 1 mm, short and rounded to sometimes elongate, broad, flat, ascending at the end. Upper surface greenish brown to brown, sometimes paler at the margins (due to the white underside), shiny, epruinose, sorediate. Lower side white with few, pale rhizinae. Soralia labriform at the apex of the lobes, irregular in shape, with powdery to granular soredia. Upper cortex paraplectenchymatous. Photobiont cells globose, 8–10 × 4–8 µm in diameter. Lower cortex prosoplectenchymatons. Pycnidia not found.

Secondary chemistry: Thallus K–, C–, KC–. No substances detected by TLC.

Specimen Examined: Pakistan: Khyber Pakhtunkhwa, Kaghan valley (34°30'N, 73°18'E), 2,500 m.a.s.l, on rock, 30 August 2021, N. S. Afshan & A. R. Niazi, (SG–2) (LAH37127), ITS GenBank accession number ON329735.

Taxonomic Remarks

The species is described and discussed by Moberg (1978, 1983) and Esslinger (1978 as *Physcia luganensis* and 1986 as *Physciella chlontha*); the species is characterized by labri-

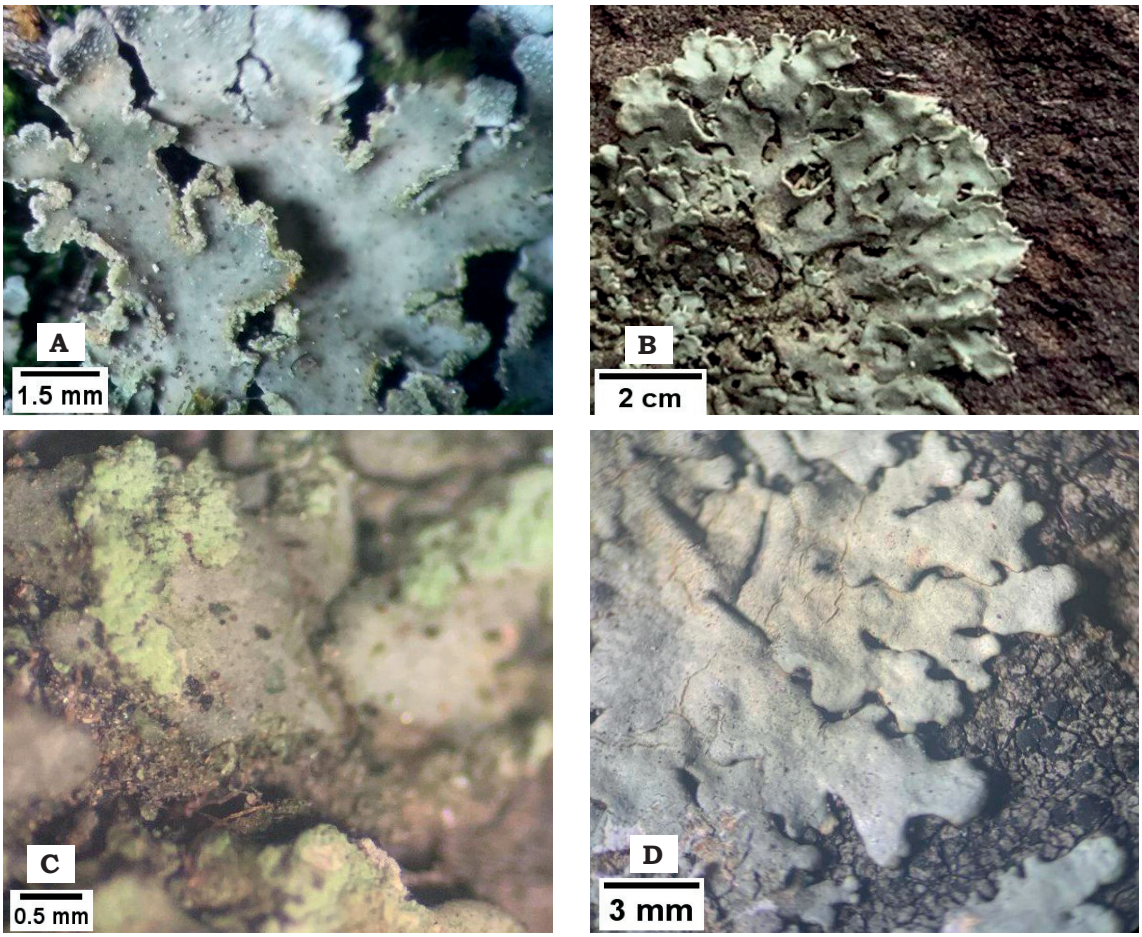

Fig. 1. Thallus of different foliose lichens A – *Physconia enteroxantha* (Nyl.) Poelt. dry thallus, lobes with pruina and soredia on margin, B – C *Physciella chloantha* (Ach.) Essl. foliose thallus with lobes and soredia, C – *Xanthoparmelia protomatrae* (Gyeln.) Hale. foliose thallus with lobes.

form soralia (Moberg, 1995). In the phylogenetic analysis, the ITS sequence of our collection of *Physciella chloantha* (BLP-61) clustered with ITS sequences of *Physciella chloantha* reported from Canada (KT695320) and Spain (GU247166, GU247162, GU247163).

Morphological comparison also confirms its identity as *Physciella chloantha* (Moberg, 1995). There are three nucleotide differences between Pakistani and Spain specimens (GU247166, GU247163). This is the first record from Pakistan, supported by ITS barcoding data.

Xanthoparmelia protomatrae (Gyeln.) Hale s. l., Phytologia 28(5): 488 (1974) (Fig. 1)

Thallus foliose, adnate to loosely adnate with ascending lobes, irregular, forming rosettes up to 4 cm in diameter. Lobes elongate, plane to slightly convex, sometimes black–rimmed, separate to weakly imbricate, up to 3 mm wide, pale green to yellow–green, smooth, shiny, epruinose and emaculate. Lower surface black, with simple to furcate, black rhizines. Upper cortex dark brown, 16–19 µm thick, paraplectenchymatous. Algal layer continuous, 22–26 µm thick. Photobiont cells chlorococcoid, 15–18 µm in diameter.

Medulla white Lower cortex: dark brown, 20–30 µm. Pycnidia not found.

Secondary chemistry: Thallus K–, C–, KC+ (yellow). Usnic acid detected by TLC, fumarprotocetraric acid.

Specimen Examined: Pakistan: Azad Jammu and Kashmir, Garhi Dupatta (34–36°N, 73–35°E), 817 m alt., on rock, 02 October 2021, N. S. Afshan & A. R. Niazi (CKT–36) (LAH37128), ITS GenBank accession number ON326553

Taxonomic Remarks

The species was discussed by, Hale (1990) and Louwhoff et al. (2009). It superficially resembles *X. conspersa* but consistently lacks isidia and has more crowded, convex lobes (especially in older thalli) (Louwhoff et al., 2009). Furthermore, *X. protomatrae* resembles *X. stenophylla* (Ach.) Ahti & D. Hawksw. in lacking soredia and isidia. The two species differ in secondary chemistry. *Xanthoparmelia protomatrae* is characterized by fumarprotocetraric acid whereas salazinic acid is the major substance in *X. stenophylla* (Elix & Thell, 2011). The morphological features of the Pakistani collections agree with the published description of *X. protomatrae* (Giordani et al., 2002) except by having black lower surface (vs. pale to dark brown lower surface) and white medulla (vs. yellow brown medulla). In the phylogenetic analysis, the ITS sequence of our sample (CKT–36) clustered with ITS sequences of *Xanthoparmelia protomatrae* (MK629940, MK629938, MK629943) from China. Presence of fumarprotocetraric acid further confirms its identity as *Xanthoparmelia protomatrae* s. l. (Louwhoff et al., 2009). This is the first record from Pakistan, supported by ITS barcoding data.

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