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## Some Physciaceae lichens from Pakistan

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This study aims to update the number of Physciaceae lichens from Pakistan. Several new records have been added to the lichen flora of Pakistan, collected from different biomes. However, the lichen specimens for this study were collected from the country's cold temperate and humid subtropical climate eco-zones. A taxonomic treatment, including a description based on the newly collected material, is provided along with molecular phylogenetic analysis. *Physcia biziana*, *Physciella nepalensis* and *Physconia perisidiosa* are three new records for the country. *Physcia aipolia* and *Physconia muscigena* are being reported for the first time from new localities, and *Physconia enteroxantha* is only being collected for the second time from the country.

Keywords: new record, Pakistan, phylogeny, *Physcia*, Physciaceae, *Physciella*, *Physconia*

As a part of ongoing studies on the determination of diversity and the phylogenetic relationship among lichen taxa of Pakistan, we reported several Physciaceae that are new to Pakistan (Aptroot and Iqbal 2012). Exploring different humid subtropical areas (Parachinar, Upper Kurrum, KP) and cold and temperate areas (Kalam, Swat, KP) of Pakistan from 2018 to 2020, Physciaceae was found to be the second most dominant family after Parmeliaceae. So far, 39 species of this family are reported from Pakistan. Several genera (*Coscino-cladium* Kunze, *Heterodermia* Trevis., *Hyperphyscia* Müll. Arg. *Leucodermia* Kalb, *Mischoblastia* A.Massal. *Mobergia* H. Mayrhofer & Sheard, *Oxnerella* S.Y.Kondr., *Polyblastidium* Kalb and *Rinodinella* H. Mayrhofer & Poelt) that belong to this family are undiscovered yet in this region. In this study, we report *Physciella* Essl. for the first time in Pakistan. Furthermore, with the exception of *Physcia vitti* (Firdous et al. 2022b), complete taxonomic descriptions, pictures and molecular sequences are presented for all Pakistani Physciaceae species, whether they are previously reported from the country or not, and submitted in the Genbank.

## Material and methods

### Morpho-anatomical study

The present study is based on a field trip to different sites in Khyber Pakhtunkhwa, Pakistan. All possible morphological features and field notes were taken and documented. The chemistry was analyzed using spot tests, i.e. K, KC and C. Free hand sections of thallus were mounted in water and observed at different magnifications for anatomical characterization and measurements. TLC was performed using standard methods (Culberson and Hale 1973).

### DNA extraction, PCR and sequencing

Total genomic DNA was extracted using the 2% CTAB method (Cubero et al. 1999). Tissue was frozen in liquid nitrogen and mechanically disrupted using a combination of a mini-pestle and a cell homogenizer with CTAB buffer.

The most universal primers were used in this study. The complete ITS plus 5.8S rDNA was amplified from six thalli using primers ITS1 and ITS4 (White et al. 1990). All PCRs were initiated with a denaturation period of 5 min. The annealing temperature was based on the  $T_m$  of the primers used in the specific reaction (approximately 5°C below the lower value). Likewise, the extension time was variable, based on the expected product size (around 1 min  $kb^{-1}$ , but not less than 30 s). The PCR products were sent for sequencing to BGI, China.

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## Phylogenetic analysis

Bidirectional sequences (ITS1 and ITS4) were reassembled using BioEdit software (Hall 1999). For phylogenetic analysis, ITS sequences were retrieved using the Basic Local Alignment Search Tool (BLAST) network service of the National Centre for Biotechnology Information (NCBI) (Altschul et al. 1990). The closest matching sequences were downloaded from GenBank for subsequent phylogenetic analysis. Online MAFFT ver. 7.0 with default setting was used to align (Katoh and Standley 2013). Our new sequences with the sequences retrieved from NCBI GenBank. The alignments were trimmed the 5' and 3' ends to the borders of ITS using BioEdit software. The phylogeny was constructed by software MEGA X (Kumar et al. 2018). The evolutionary history was retrieved by constructing maximum likelihood (ML) methods using Kimura-2 parameter model. The model was selected by searching the best DNA model for ML analysis in MEGA X (Kumar et al. 2018). One thousand rapid bootstrap replicates were run to test the robustness of the resulting phylogenetic hypothesis.

## Results

### Phylogenetic analysis of Physciaceae members

In this study, DNA was sequenced from all six Physciaceae species to add molecular data in the Genebank of Pakistani collections. The ITS region was selected as it is the most widely sequenced DNA region in phylogenetic studies of lichens and has been recommended as the universal fungal barcode sequence. Only four genera, *Phaeophyscia*, *Physciella*, *Physconia* and *Physcia* were involved in this phylogenetic study of this family (Fig. 1). Phylogeny of these members made six clades which do not represent the proper hierarchy of the Physciaceae as not all the members were incorporated. *Phaeophyscia* and *Physciella* share a common node in this tree; only three *Physciella* sequences were present in Genbank. However, we are presenting here the first genetic record of *Physciella nepalensis*. The phylogenetic tree might be influenced by limited number of available sequences and the topology is likely to change somewhat with inclusion of missing taxa. Except for *Physciella nepalensis*, the other five species, *Physcia biziana*, *Physcia aipolia*, *Physconia perisidiosa*, *Physconia muscigena* and *Physconia enteroxantha* are well-matched with the previously reported species sequences from different parts of the world.

## Taxonomic notes

*Physcia aipolia* (Ehrh. ex Humb.) Frnr., Naturhist. Topogr. Regensburg (Regensburg) 2: 249 (1839) (Fig. 2)

Thallus type: foliose.

Description: 4–6 cm across, compactly attached to the substratum, grey to silver-grey, lobulate; lobes: 1.0–1.5 cm × 0.2–0.4 mm, somewhat imbricated, orbicular to radiating, incurved; upper surface: brown-black dots all over the

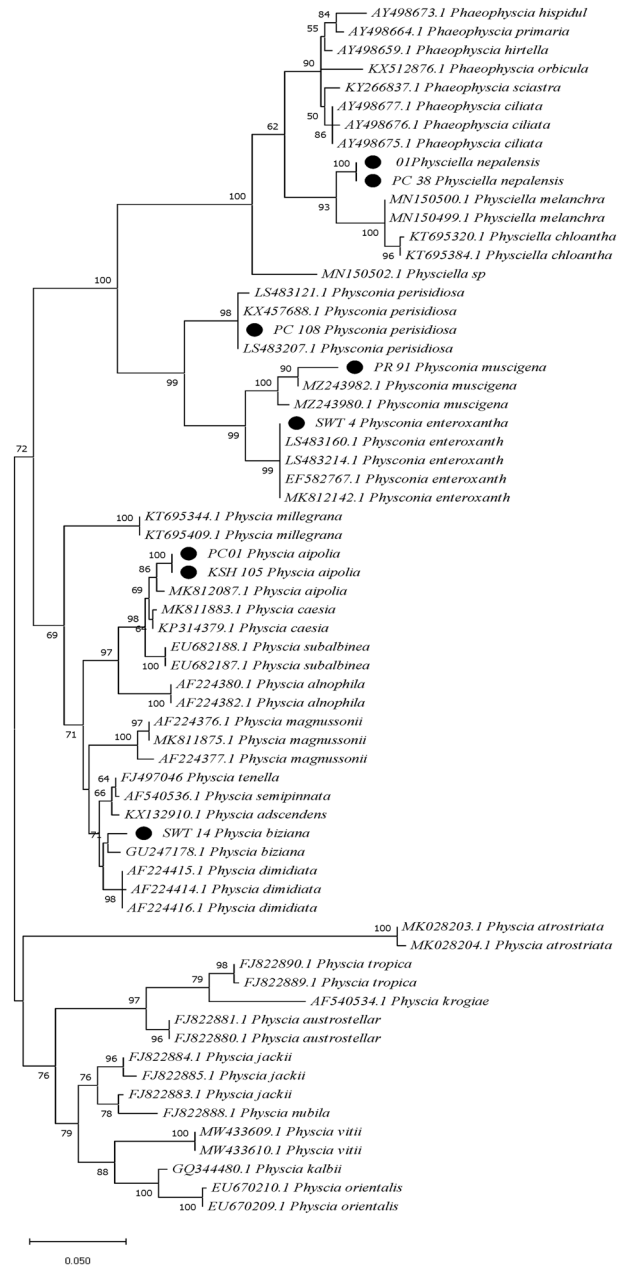


Figure 1. Molecular phylogenetic analysis of *Physciaceae* members by the maximum likelihood method based on rDNA sequences, including ITS1, 5.8S and ITS2. Numbers below branch node represent ML bootstrap (> 50%) based on 1000 replicates. Sequences generated from Pakistani collections are marked with black dots (●).

surface, slightly maculated of white patches, e-ciliate; thallus section: heteromeric; upper cortex: colourless or whitish, 21–26 µm, algal layer: green, 31–35 µm; medulla: 60–70 µm, light brown; lower cortex: 25–35 µm, brown rhizines, 50–60 × 15–19 µm; lower surface: yellowish-brown, rhizinate; rhizines: whitish brown, moderately thin, unbranched to branched.

Apothecia: 0.1–0.4 mm diameter, grey to silver grey margins, moderately thin, large disc, grey mostly, dark brown also found, e-pruinose to somewhat pruinose; apothecia section: lecanorine, epihymenium: yellow-brown to brown,

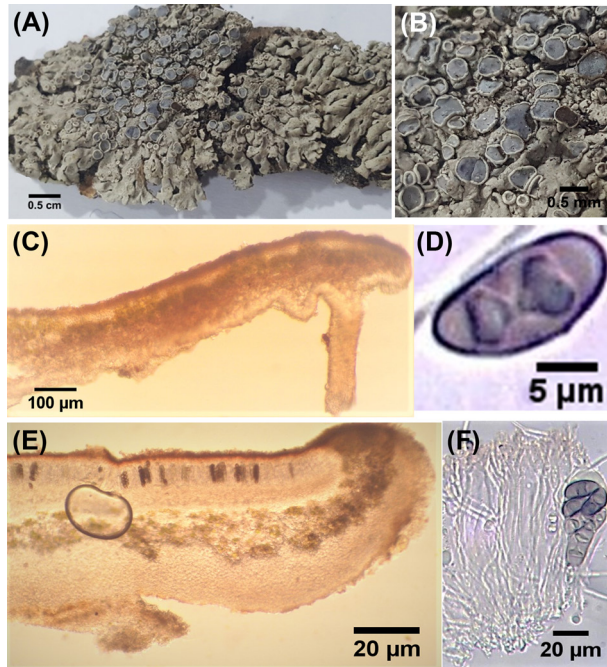


Figure 2. (A–F) *Physcia aizolia*. (A) Thallus, (B) apothecia, (C) section of thallus, (D) ascospore, (E) section of apothecium, (F) ascus surrounded by paraphysis.

15–20  $\mu\text{m}$ ; hymenium: light brown, 55–65  $\mu\text{m}$ ; hypohymenium: 72–87  $\mu\text{m}$ , golden yellow to brown to dark brown; asci: Thalline exciple: 80–110  $\mu\text{m}$  wide, dark greyish black outer margin. Paraphyses: aseptate, 1–3  $\mu\text{m}$  wide, branched and septate apically, capitate, apex swollen 2.5–4.0  $\mu\text{m}$  wide. Asci: hyaline, clavate, 8-spored, 70–90  $\times$  18–24  $\mu\text{m}$ . Ascospores: brown, ellipsoid, septate, *Physcia*-type, having 2 locules, 15.0–24.0  $\times$  8.0–10.5  $\mu\text{m}$ .

Spot test: cortex: K+, turns yellow, C–, KC–, medulla: K+, turns bright green, C–, KC–; TLC: Atranorin and Zeorin were seen with TLC.

#### Material examined

Pakistan, Parachinar forest, FATA, 1705 m a.s.l., 22 Jul 2018, Qudsia Firdous & Abdul Nasir Khalid (ANK-271; PC-01). Pakistan, Azad Jammu & Kashmir: Neelam Valley, Sharda; 34°47'35.16"N, 74°11'34.8"E; 22 Jul 2019; A. N. Khalid and Q. Firdous. KSH-105.

#### Substrate and ecology

Growing on the bark of *Quercus baloot* Griff., Itin. Not. 328. 1848 in Parachinar forest, FATA, Pakistan. It was also observed and collected from Sharda valley, Kashmir, Pakistan growing on *Pinus* L. tree bark.

#### Pakistan distribution

The species has been reported from Kalam (Swat), Sharan (Kaghan), Loon Bagla (Muzaffarabad), Dungagali, Mukshpuri (Murree), Miranjani, Baragali, Naran, Kaghan, Lalazar (Aproot and Iqbal 2012).

#### *Physcia biziana* (A. Massal.) Zahlbr., *Öst. Bot. Z.* 51: 349 (1901) (Fig. 3)

Thallus type: foliose.

Description: 3–5 cm across, irregular to orbicular mostly, compactly attached to the substratum from the center while edges are loosely connected, lobulate; lobes: radiating, truncate with open sinuses, 0.5–1.0 mm long and 0.4–0.7 mm wide, upper surface: grey, incurved appearance, smooth to strongly foveolate, soredia and isidia absent; lower surface: creamy white, smooth, rhizinate; rhizines: whitish-grey, smooth, unbranched, 0.1–0.3 mm long, eciliate. Thallus section: heteromeric; upper cortex: hyaline, 12–20  $\mu\text{m}$ , algal layer: green, 40–53  $\mu\text{m}$ , medulla: hyaline, 70–82  $\mu\text{m}$ ; lower cortex: brown to dark brown at the edges, 11–15  $\mu\text{m}$ .

Apothecia: 1–2 mm diameter, abundant greyish green, margins swollen, thick, sessile disc: somewhat pruinose, dark grey to black, flat. Apothecia section: lecanorine; hymenium: light brown to hyaline, 118–134  $\mu\text{m}$ , hypohymenium: hyaline, 180–190  $\mu\text{m}$ , epihymenium: dark brown, 22–25  $\mu\text{m}$ ; asci: 40–50  $\times$  12–15  $\mu\text{m}$ , clavate, apical dome 8–10  $\mu\text{m}$  length viz; ascospores: brown-black, 1-septate, 13–20  $\times$  6.5–8.5  $\mu\text{m}$ ; paraphysis: branched and anastomosing, hyaline.

Pycnidia: numerous, immersed; conidia: subcylindrical to cylindrical, 5.0–7.0  $\times$  0.7–1.0  $\mu\text{m}$ .

Spot tests: K+, yellow, C–, KC–; TLC: Atranorin spotted with TLC.

#### Material examined

Pakistan, Khyber Pakhtunkhwa, Swat, Kalam, 2000 m a.s.l., 21 Aug 2020, A. N. Khalid, SWT-14.

#### Substrate and ecology

Growing on the bark of a tree in a moist temperate forest.

#### Pakistan distribution

This study reports it as a new record of the lichen biota of Pakistan.

#### *Physciella nepalensis* (Poelt) Essl., *Mycologia* 78(1): 96 (1986) (Fig. 4)

Thallus type: foliose.

Description: greenish-grey to silver-grey, dry, rough surface, 5–8 cm across, loosely attached to the substratum, muscicolous, lobulated; lobes: 1.5  $\times$  0.5 cm across, wavy dentate margins, slightly imbricate; upper surface: e-pruinose to somewhat pruinose at certain part; lower surface: whitish, rhizinate; rhizines: abundant, white to off-white, simple, moderately thick, unbranched, up to 0.1 mm. Thallus section: heteromeric thallus; upper cortex: hyaline, 15–20  $\mu\text{m}$ , algal layer: 43–50  $\mu\text{m}$ ; medulla: hyaline, 30–40  $\mu\text{m}$ ; lower cortex: whitish, 30–35  $\mu\text{m}$ .

Apothecia: scarce, 0.1–0.4 mm in diameter, sessile; margins: greyish-green, swollen, entire; disc: flat, dark brown to black. Apothecia section: lecanorine; epihymenium: yellow to orange, 10–13  $\mu\text{m}$ ; hymenium: hyaline, 90–112  $\mu\text{m}$ ; hypohymenium: hyaline, 150–170  $\mu\text{m}$ ; asci: 55–65  $\times$



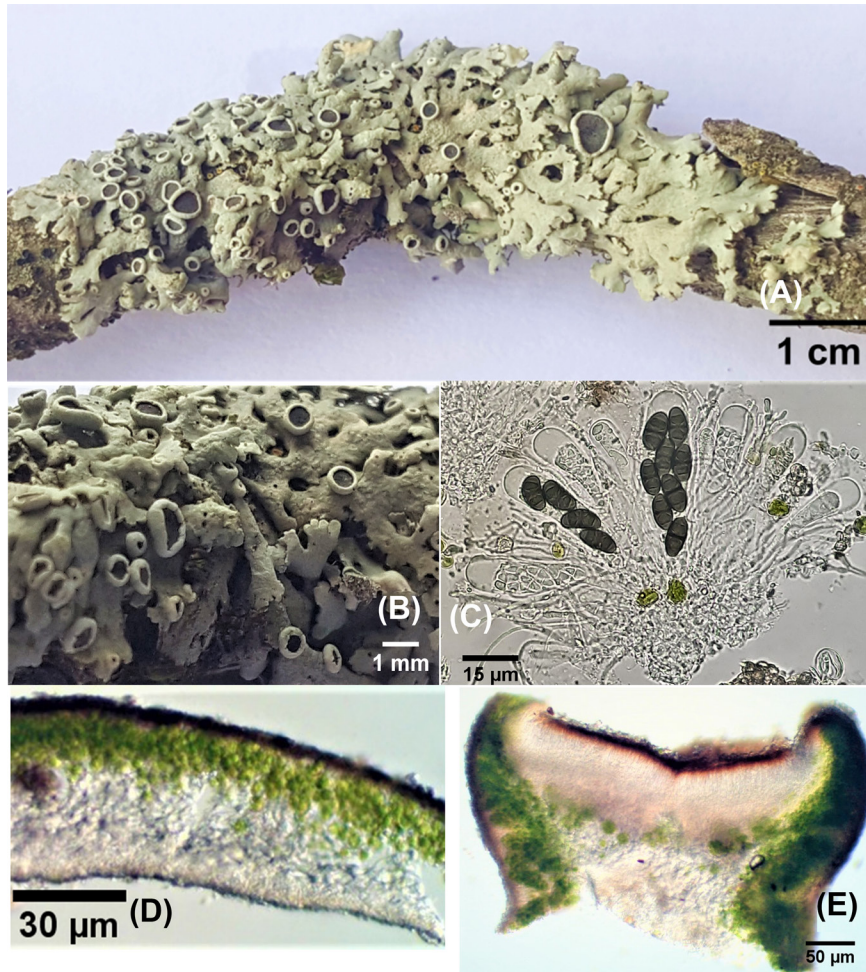


Figure 3. (A–E) *Physcia biziana*. (A) Thallus, (B) apothecia, (C) ascus surrounded by paraphysis, (D) section of thallus, (E) section of apothecium.

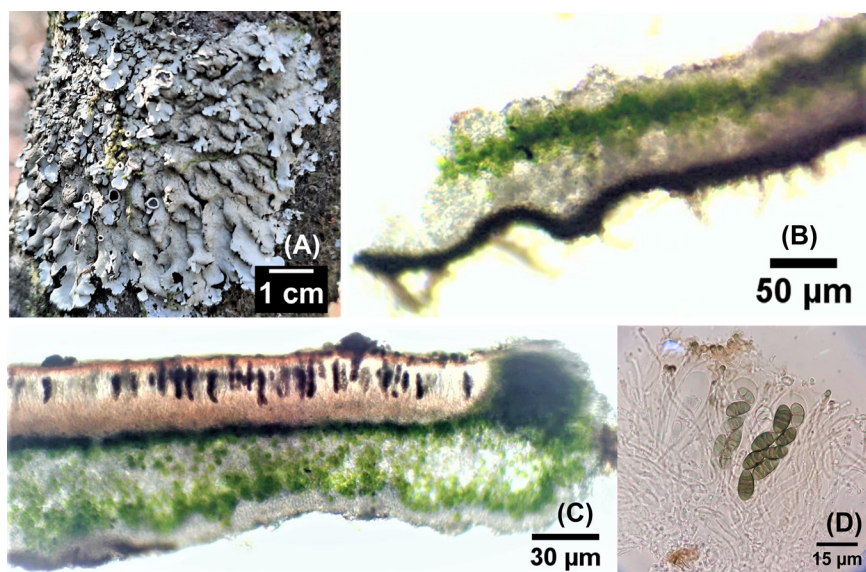


Figure 4. (A–D) *Physciella nepalensis*. (A) Thallus (habitus), (B) section of thallus, (C) section of apothecium, (D) ascus surrounded by paraphysis.

10–12  $\mu\text{m}$ , somewhat clavate; ascospores: 17–20  $\times$  7–10  $\mu\text{m}$ , ellipsoidal, uni-septate.

Spot test: K+, yellow, C–, KC+ yellow; TLC: No compound was detected with TLC.

### Material examined

Pakistan, Khyber Pakhtunkhwa; Kurram district, Parachinar; 33°53'51"N, 70°06'00"E; Jul 2018; Q. Firdous & A. N. Khalid, PC-38 & 01.

### Substrate and ecology

Growing on the bark of a tree in a moist temperate forest.

### Pakistan distribution

It is the first record from the country.

*Physconia enteroxantha* (Nyl.) Poelt, Nova Hedw. 12(1+2): 125 (1966) (Fig. 5)

Thallus type: foliose.

Description: 5–8 cm across, compactly attached to the substratum, sometimes pulvinate, lobulate; lobes: 0.7–1.5 cm  $\times$  1.0–2.0 mm, somewhat imbricated, orbicular to radiating, flat to concave; upper surface: greenish-grey to grey when dry, bright green when wet, slightly maculated; thallus

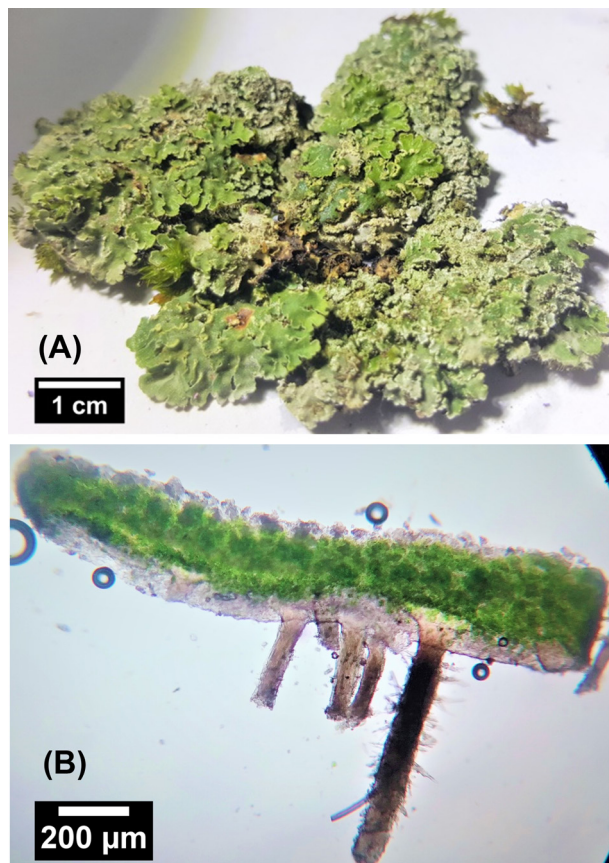


Figure 5. *Physconia enteroxantha*. (A) Thallus, (B) section of thallus.

section: heteromerous with differentiated upper- and lower cortex, thick algal layer and thin medulla; upper cortex: 10–20  $\mu\text{m}$ , yellow to light brown, paraplectenchymatous cells. Algal layer: green, 200–250  $\mu\text{m}$ ; medulla: 90–100  $\mu\text{m}$ , whitish-yellow; lower surface: off-white to creamy white, rhizinate, blackish-brown rhizines, lower cortex: indistinct, whitish brown rhizines, moderately thin, highly branched; apothecia: not seen.

Spot test: Cortex: K+ yellow, C+ slightly green, KC+ yellow; Medulla: K+ bright green, C–, KC+ green.

### Material examined

Pakistan, Khyber Pakhtunkhwa, Swat, Kalam, 2000 m a.s.l., 21 Aug 2020, A. N. Khalid, (SWT-04).

### Substrate and ecology

Growing on the bark of a tree in a moist temperate forest.

### Pakistan distribution

It is the second report from the country.

*Physconia muscigena* f. *alpina* (Nádv.) J.C. Wei & Y.M. Jiang, [Lichens of Xizang] (China): 113 (1986) (Fig. 6)

Thallus type: foliose.

Description: irregular, 8–10 cm wide, tomentose, imbricate, moderate to loosely attached, lobate; lobes: loosely attached to ascending, dense and turf-like, linear, plane to concave, 1.0–2.5 mm wide, brownish at the tips, rounded to flabellate; upper surface: whitish gray to grayish brown when dry, parrot green to leafy green when wet; heteromerous, up to 370  $\mu\text{m}$  thick; upper cortex: paraplectenchymatous, hyaline to gray, 20–60  $\mu\text{m}$  thick, cell 12–20  $\mu\text{m}$  in diam; algal layer: continuous, 55–65  $\mu\text{m}$  high; photobiont: trebouxoid, cell globular to sub globular, 7–15  $\mu\text{m}$  in diam.; medulla: hyaline, medullary layer 120–185  $\mu\text{m}$  tall, hyphae 2.0–5.5  $\mu\text{m}$  wide; lower cortex: hyaline, prosoplectenchymatous, 15–30  $\mu\text{m}$  thick; lower surface: yellow to yellowish-brown at tips, dark brown to black toward the center, dull, densely rhizinate; rhizines: black, squarrosely branched, pointed to frayed.

Apothecia: not found; pycnidia: not seen.

Spot tests: all negative.

### Material examined

Pakistan, Khyber Pakhtunkhwa; Kurram district, Parachinar; 33°53'51"N, 70°06'00"E; Jul, 2020; Q. Firdous & A. N. Khalid, PC-91.

### Substrate and ecology

Musicolous, found over mosses in the humid subtropical climatic forest that is mild with no dry season, constantly moist.



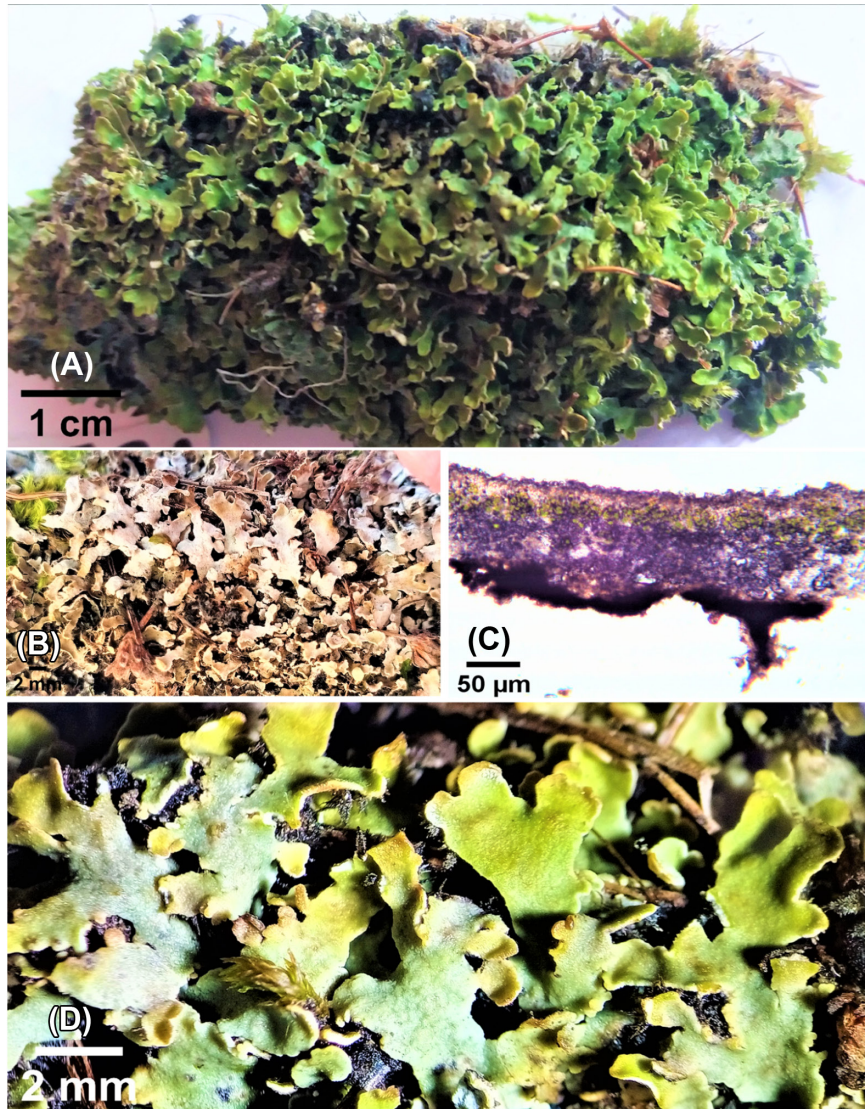


Figure 6. (A–D) *Physconia muscigena* f. *alpina*. (A–B) Habitus of thallus, (C) section of thallus, (D) lobes.

### Pakistan distribution

This taxon has been reported from Lalazar (Kaghan) by Poelt (1961) and Bhatti et al. (1978).

### *Physconia perisidiosa* (Erichsen) Moberg, *Symb. Bot. Upsal.* 22(no. 1): 90 (1977) (Fig. 7)

Thallus type: foliose.

Description: up to 5 cm across, orbicular to irregular, sorediate, compactly attached to substratum, lobate; lobes: linear 1.7 mm wide, plane to concave, discrete, lobe surfaces pruinose, at least at the tips; soredia: marginal soralia become elongate, straight to curved, rarely laminal, laminal soralia appeared in older thallus parts, soredia granular; upper surface: green when wet, yellowish-green to green when dry; upper cortex: 25–45 µm; grey-brown from the outer side, hyaline from inner side; medulla: white, 110–150 µm, prosoplectenchymatous, 1.0–3.5 µm wide; algal layer:

31–70 µm, continuous, even; photobiont: chlorococcoid, globose to subglobose, 15–20 µm in diam.

Spot tests: K–, C–, KC–.

### Material examined

Pakistan, Khyber Pakhtunkhwa; Kurram district, Parachinar; 33°53'51"N, 70°06'00"E; Jul 2020; Q. Firdous & A. N. Khalid, PC-91.

### Substrate and ecology

Corticolous, this species is found abundantly on barks of *Quercus ilex*, *Q. dilatata* of Zaryan Forest in Parachinar. This species is also found growing on rocks, epilithic in Kaghan valley, at an elevation above 1705 m a.s.l., on shaded rocks, mean max. and min. Temperature: 28 and –2°C, respectively, the climate is warm and temperate, with annual rain varying between 700 and 1500 mm.

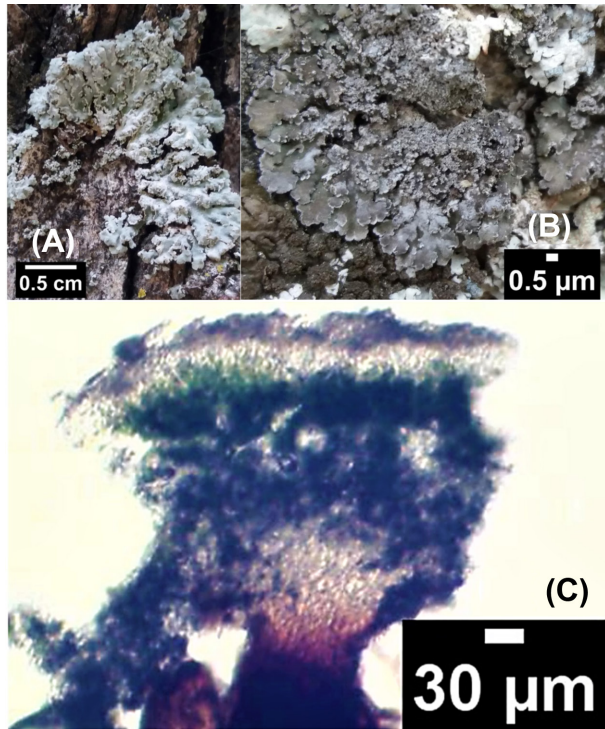


Figure 7. *Physconia perisidiosa*. (A–B) Morphology of thallus, (C) section of thallus.

### Pakistan distribution

It is a new record for Pakistan.

### Discussion

Our knowledge of the lichens and allied fungi in Pakistan has been considerably advanced in recent years. Currently ca 400 taxa of lichenized fungi are recorded for Pakistan (Habib et al. 2021, Firdous et al. 2022a). This study focused only on Physciaceae members of lichens of Pakistan. Six Physciaceae species from cold and temperate climates, as well as a location that has never been examined for lichens, are covered in this paper. *Physcia biziana* and *Physconia enteroxantha* were found in Kalam, Swat KP. Four species were discovered in Parachinar, Upper Kurram, KP, including *Physcia aipolia*, *Physciella nepalensis*, *Physconia muscigena* and *Physconia perisidiosa*. Nine species of *Physcia* (Schreb.) Michx. are reported from Pakistan previously (Aptroot and Iqbal 2012). *Physcia vittii* Nád. v., Stud. Bot. Českoslov., a species recently added to the Pakistan record, was found in Parachinar (Firdous et al. 2022b). This is the first time DNA sequences are reported for this species. *Physcia aipolia* is a cosmopolitan species, and from Pakistan, it is reported from Kalam (Swat), Sharan (Kaghan), Loon Bagla (Muzaffarabad), Dungagali, Mukshpuri (Murree), Miranjani, Baragali, Naran, Kaghan, Lalazar (Aptroot and Iqbal 2012). This is the first time *Physcia biziana* has been collected from Pakistan.

Two species of the genus *Physconia* Poelt are recorded for the second time from the country in this study. *Physconia enteroxantha* is being recorded for the second time from Swat (Bhatty et al. 1978); *Physconia muscigena* was previously

reported by Poelt (1961) and Bhatty et al. (1978) from Lalazar (Kaghan) growing on a rock while in this study we found it growing on moss and collected from a different climate zone (Parachinar). *Physconia perisidiosa* also collected from Parachinar, growing on the bark, is being reported for the first time from the region. Linear to irregular to rounded lobes of foliose thallus with pruinose and sorediate surfaces are the characterizations that matched well of our collection with that of previously reported North America, Europe, Asia, Africa and South America specimens (Kondratyuk 2021).

Until this study, no record of the genus *Physciella* Essl. was recorded from Pakistan. In this study, we report *Physciella nepalensis* from the Parachinar forest. Grey to grey-brown, epruinose surface of the foliose thallus, elongate and discrete to imbricate lobes, and white lower surface of thaliform are the main distinguishing characteristics of this species. Most of its distribution is found in North America and Eurasia (Esslinger 1986, Yearsley 1993, Moberg 1995, Liu and Hur 2019).

### Data availability statement

There are no additional data for this paper.

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