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BOTANY

Pachypeltis phoenicopta, an Interesting Lichenicolous Lichen and *Flavoplaca flavocitrina* (Lichenized Ascomycetes: Teloschistaceae) from Pakistan

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Abstract—*Flavoplaca flavocitrina* and *Pachypeltis phoenicopta* are reported as new to Pakistan. The genus *Pachypeltis* is the first report for Pakistan. The descriptions for both species based on the taxonomic examinations of the Pakistan's specimens are provided along with ITS-based phylogenetic analysis and notes on ecology and distribution.

Keywords: distribution, Kohistan, morphology, phylogeny, taxonomy

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INTRODUCTION

Teloschistaceae is a species rich lichen family (Gaya et al., 2008), comprising 65 genera and 755 species according to most recent classification of lichenized fungi by Lücking et al., (2017). The family is diverse in morphology and occupying various terrestrial habitats (Vondrák et al., 2019). Most members of Teloschistaceae are characterized by polardiblastic ascospores and yellow to orange colour due to anthraquinone pigments in the cortex (Arup et al., 2013). As Søchting and Lutzoni (2003) pointed out that the delimitation among genera included within the Teloschistaceae is highly artificial and in need of revision, especially for the closely related species (Gaya et al., 2008).

From Pakistan, 86 species of family Teloschistaceae have been reported so far from various localities (Aptroot and Iqbal, 2012; Nadeem et al., 2022). The lichen mycota of Pakistan is still poorly studied therefore this study will contribute the understanding of lichen mycota of the country. In this paper the authors focus on two lichen species of Teloschistaceae, *Flavoplaca flavocitrina* and *Pachypeltis phoenicopta,* with their ITS rDNA data. The aim of this study is to provide the descriptions and the molecular phylogenetic results based on the specimens collected from Pakistan.

MATERIALS AND METHODS

Morphological and Chemical Studies

Collections were made during lichen surveys of different areas of Kohistan District and Gilgit Baltistan Province in year 2020 and 2021 respectively. All voucher specimens are housed in the herbarium of University of the Punjab (LAH). Morphological characters were observed under a stereomicroscope (Meiji Techno, EMZ-5TR, Japan). Secondary metabolites were detected by thin-layer chromatography (TLC) in solvent C following the method of Orange et al., (2001). Measurements of anatomical features were made from free hand sections of apothecia mounted in water on a glass slide. The sections were observed using a compound microscope (MX4300H, Meiji Techno Co., Ltd., Japan).

DNA Extraction and PCR Amplification

Genomic DNA was extracted directly from a portion of thallus with apothecia from each specimen using a modified 2% CTAB method of Gardes and Bruns (1993). PCR amplification of the ITS nrDNA was performed using ITS1F forward primer (5' CTTGGTCATTTAGAGGAAGTAA 3') (Gardes and Bruns, 1993) and ITS4 reverse primer (5' TCCTCCGCTTATTGATATGC 3') (White et al., 1990). The PCR products were visualized with the help of 1% agarose gel using ethidium bromide through Gel documentation system (Sambrook and Russel, 2001), and sequenced at TSINGKE Biotechnology Co., Ltd. (China).

Phylogenetic Analysis

The forward and reverse sequences of ITS region were reassembled using BioEdit sequence alignment editor (Hall, 2005). The multiple sequence alignment

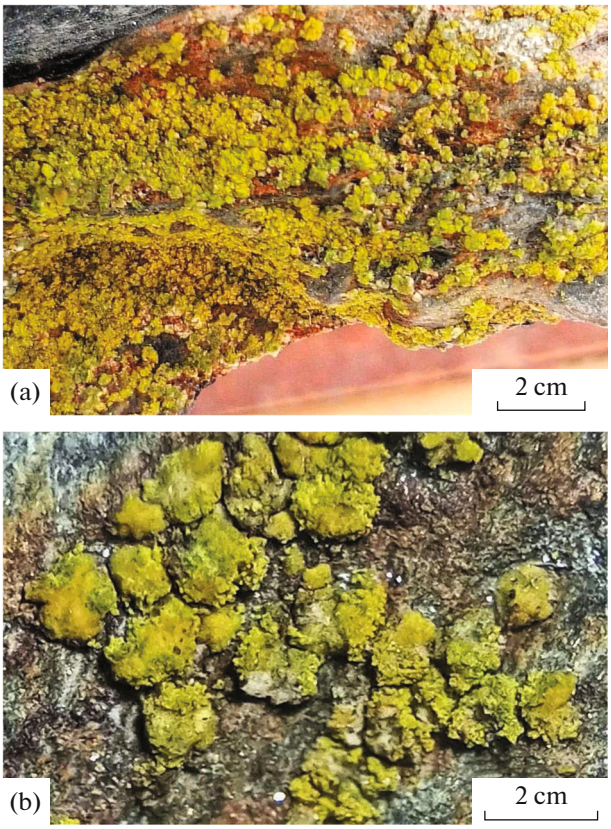

Fig. 1. *Flavoplaca flavocitrina* (KOH–37). (a) Habit; (b) squamules with soredia.

was performed using MAFFT ver. 7 with default parameter settings (Katoh et al., 2019). All gaps were treated as missing data. Maximum Likelihood analysis was performed with MEGAX (Kumar et al., 2018) based on Kimura 2–parameter. One thousand rapid bootstrap replicates were run to infer the evolutionary history of each species.

RESULTS

The sequences used in the phylogenetic analysis of *Flavoplaca* spp. were retrieved from GenBank based on similarity (Fig. 2). The length of the final alignment was 546 sites, among which 384 were conserved, 158 were variable, 101 were parsimony-informative and 57 were singleton variants. *Xanthoria elegans* (Link) Th. Fr. was used as an out group for the rooting purpose of tree. In the ITS phylogram (Fig. 2), the Pakistani sequence of *F. flavocitrina* (KOH-37) clustered with sequences of the same taxon (LC669629, LC669630, LC669631) reported from Japan, with a strong bootstrap (100%) and formed a sister branch relation with *F. dichroa* (MF595932, MF595937) reported from UK (Orange, 2018).

The sequences included in the phylogenetic analysis of *Pachypeltis* spp. were retrieved from GenBank (Fig. 4). The length of the final aligned file was 531 sites, among which 417 were conserved, 113 were variable, 38 were parsimony-informative and 74 were singleton variants. *Xanthomendoza trachyphylla* (Tuck.) Frödén, Arup & Søchting was used as an out group for the rooting purpose of tree. The ITS sequence of Pakistani *Pachypeltis phoenicopta* (BT-10) forms a monophyletic clade together with those of *P. phoenicopta* (MZ244094, MZ244095, MZ244096, MG954218) reported from USA and Russia respectively.

TAXONOMY

Flavoplaca flavocitrina (Nyl.) Arup, Frödén & Søchting, *Nordic J. Bot.* 31(1): 45 (2013).

Thallus crustose, squamulose, pale orange when dry, greenish yellow when wet, up to 8 cm across, effuse, thin, 150–200 μm thick. **Squamules** contiguous to dispersed, 0.3–0.7 mm in diameter, plane to flexuous, rounded to irregular, almost entirely covered with soralia. **Soralia** marginal, 0.5–1 mm in diameter, yellowish green. **Upper cortex** paraplectenchymatous, with many small orange-brown crystals, 10–15 μm thick, cells rounded, 5–10 μm in diameter. **Algal layer** even, continuous, 50–70 μm thick; photobiont chlorococcoid, 5–9 μm in diameter. **Medulla** 60–90 μm thick, hyphae hyaline, 3–4 μm wide. **Apothecia** not found.

Chemistry: thallus K+ red, C–, KC–. **TLC:** parietin, teloschistin.

Habitat and ecology: saxicolous (on calcareous rocks), in dry temperate climate, in an open situation exposed to sun and rain. Temperature typically varies from –8 to 28°C and is rarely below –14°C or above 31°C with an annual rainfall varying between 700 and 800 mm. The topography of the area has extreme variations in elevation. This species was found at an elevation of 841 m. a.s.l.

Distribution: cosmopolitan. It was reported in Europe (UK, Italy, Finland, Denmark, Sweden, Norway) (Arup 2006), European Russia (Vondrák et al., 2009; Muchnik et al., 2014; Himelbrant et al., 2015), North America (Brodo et al., 2013), Hawaii (Vondrák et al., 2009), Siberia (Vondrák et al., 2016), and Asia including China, Turkey, Russia and Japan (Vondrák et al., 2019; Ohmura et al., 2022). Here, this species is reported for the first time from Pakistan. Consequently, the genus *Flavoplaca* in Pakistan is now three species in addition to other two known species, *F. citrina* from Kotli (Ahmad 1965, as *Caloplaca citrina*) and *F. oasis* (A. Massal.) Arup, Frödén & Søchting from Kalam (Swat) (Poelt and Hinteregger, 1993, as *C. oasis*).

Notes: *Flavoplaca flavocitrina* is easily recognized by the areolate thallus with marginal soredia (Arup, 2006). Morphological comparison confirms the identity of Pakistani taxon (KOH-37) as *F. flavocitrina* (Arup, 2006) except the absence of apothecia in Paki-

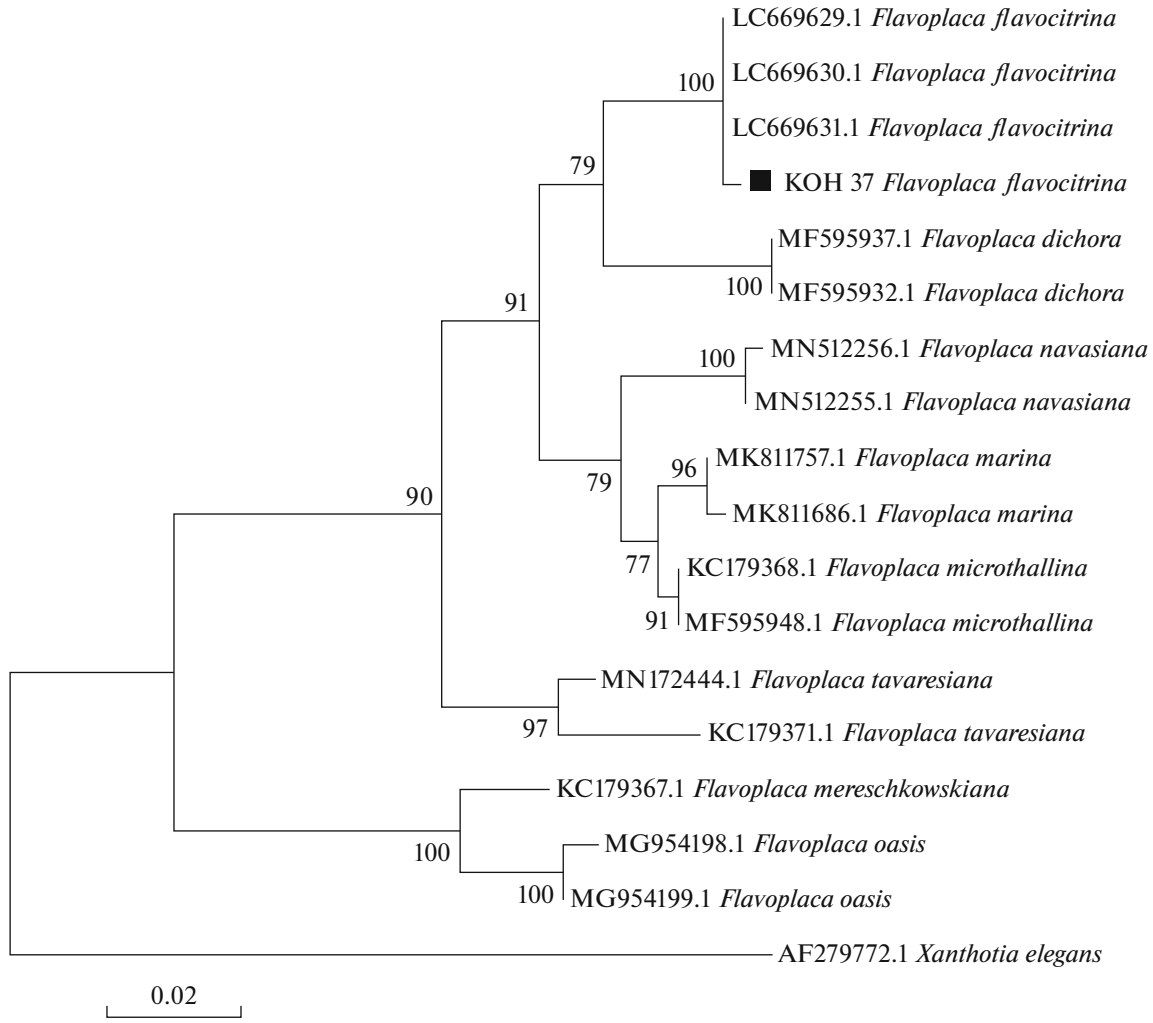

Fig. 2. Phylogenetic analysis of *Flavoplaca* spp. inferred using maximum likelihood method. The bootstrap values based on 1000 replicates are shown below the branches. Sequence generated from local collection is marked with black-painted square (■).

stani collection which is consistent with one of the Japanese specimens (Ohmura et al., 2022). Two nucleotide differences have been found between Pakistani and Japanese samples.

Specimen examined: Pakistan. Khyber Pakhtunkhwa Province, Kohistan: Dassu, on calcareous rocks, 841 m a.s.l, 35°35′ N, 73°37′ E, Sep 9, 2020; K. Habib & A.N. Khalid; KOH–37 (LAH37536) (GenBank accession number: OP010194).

Pachypeltis phoenicopta (Poelt & Hinter.) Vondrák, in Vondrák, Frolov, Davydov, Yakovchenko, Malíček, Svoboda & Kubásek, *Phytotaxa* 396(1): 29 (2019).

Thallus crustose-areolate, yellowish orange to orange, epruinose, up to 4 cm across, 150–200 μm thick in section; areoles dispersed to crowded, rounded to irregular, flat to convex, 0.3–0.5 mm in diameter; prothallus absent. **Upper cortex** paraplectenchymatous, with many small orange-brown crystals, 15–25 μm thick; cells rounded, 8–12 μm in diameter. **Algal layer** even, continuous, 40–70 μm thick; photobiont chlorococcoid, rounded, 7–9 μm in diameter. **Medulla** 90–120 μm thick; hyphae hyaline, 3–4 μm wide. **Apothecia** not found.

Chemistry: K+ red, C–, KC+ red. **TLC:** parietin, teloschistin, emodin.

Habitat and ecology: lichenicolous on *Aspicilia* sp. which grew on siliceous rocks at an elevation of 1265 m a.s.l. The habitat is in cold semi-arid climate, hilly topography exposed to sun and rain, average temperature 32°C with average annual rainfall about 250 mm.

Distribution: widely distributed in USA (Sierra Nevada) (Leavitt et al., 2021), Europe (Spain, Greece, Russia, Czech Republic) (Vondrák et al., 2019) and Asia (Nepal) (Poelt and Hinteregger, 1993). This species and the genus are the first report for Pakistan.

Notes: *Pachypeltis phoenicopta* was originally described as a lichenicolous lichen parasitized on

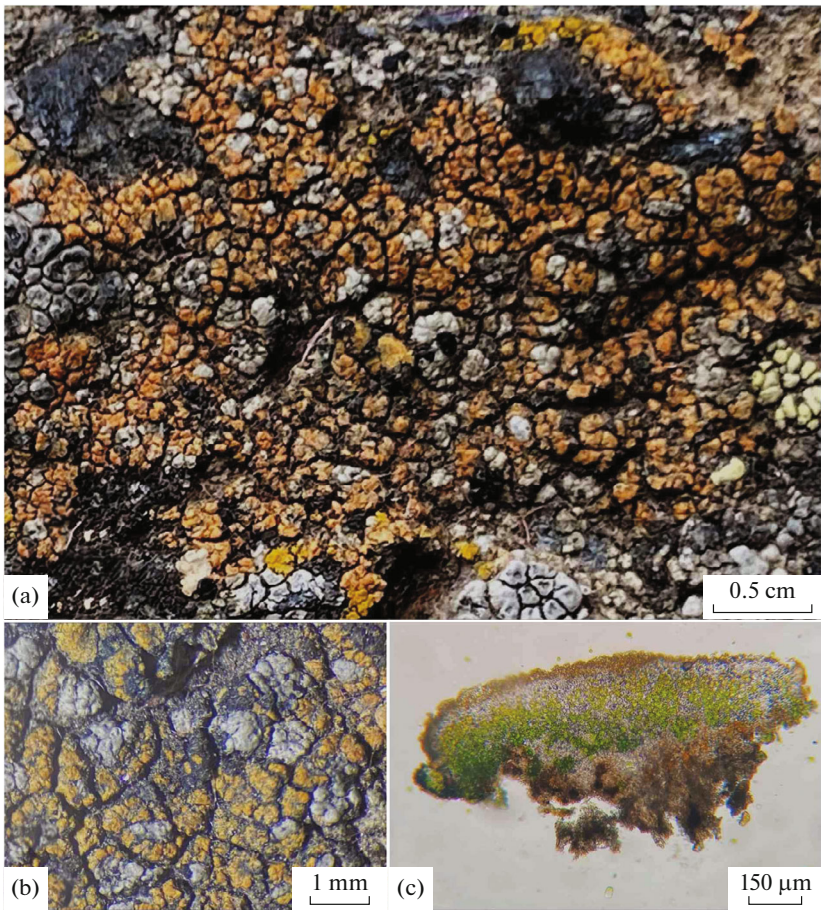

Fig. 3. *Pachypeltis phoenicopta* (BT-10). (a) Habit; (b) areoles; (c) cross section of thallus.

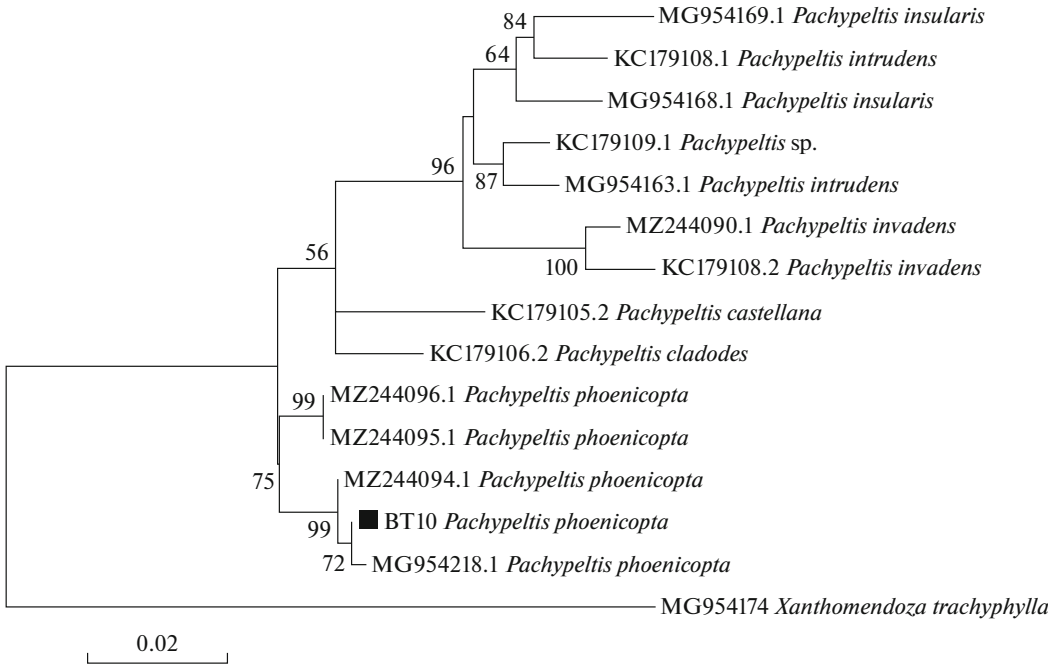

Fig. 4. Phylogenetic analysis of *Pachypeltis* spp. inferred using maximum likelihood method. The bootstrap values based on 1000 replicates are shown below the branches. Sequence generated from local collection is marked with black-painted square (■).

Caloplaca demissa with a diagnostic contrast between pale ochraceous thallus and brown red apothecia (Poelt and Hinteregger, 1993). The species was found from various hosts including the following species such as *Aspicilia* sp., *Calogaya biatorina*, *Lecidella* sp., *Lobothallia* sp., *Rhizocarpon* cf. *disporum*, and *Rinodina* sp. (Vondrák et al., 2019). The Pakistani collection of *Pachypeltis phoenicopta* (BT-10) was found on *Aspicilia* sp. It is morphologically similar to Russian *P. phoenicopta* reported from Altai Sayan region (Vondrák et al., 2019) except the presence of pruina in Pakistani material.

Specimen examined: Pakistan. Gilgit Baltistan: Diamer, Deong Basti; on siliceous rocks; 35°19′ N, 74°17′ E; 1926 m a.s.l; Oct 12, 2021, A.N Khalid & K. Habib; BT-10 (LAH37499) (GenBank accession number: OP030724).

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflicts of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

AUTHOR CONTRIBUTIONS

Material preparation, data collection and the first draft of the manuscript was done by Rizwana Zulfiqar. Phylogenetic analysis was performed by Fatima Razzaq. Samples collection, field study and morphology was done by Kamran Habib. Supervision and final corrections in the manuscript was done by Dr. Abdul Nasir Khalid. All authors read and approved the final manuscript.

ADDITIONAL INFORMATION

Samples analysed during this study has been deposited in the LAH Herbarium, University of the Punjab, Lahore (https://vymaps.com/ PK/LAH-Herbarium-Department-of-Botany-University-of-the-Punjab-Pakistan-334248940-395482/). Sequences generated during this study has been deposited in NCBI, GenBank data repository (https://www.ncbi.nlm.nih.gov/genBank/).

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