

**PHYSICIA VITII NADV. NEW TO SOUTH ASIA - MOLECULAR DATA****QUDSIA FIRDOUS<sup>1</sup>, KAMRAN HABIB<sup>1</sup>, ABDUL NASIR KHALID<sup>1</sup> AND ANDRÉ APTROOT<sup>2</sup>**<sup>1</sup>Department of Botany, University of the Punjab, Lahore 54590, Pakistan<sup>2</sup>Laboratório de Botânica / Liquenologia, Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, Avenida Costa e Silva s/n, Bairro Universitário, CEP 79070-900, Campo Grande, MS, BrazilCorresponding author's E-mail: [qudsiafirdous26@gmail.com](mailto:qudsiafirdous26@gmail.com)**Abstract**

A species in the genus *Physcia* was collected from dry temperate ecozones of Pakistan. The critical morpho-anatomical comparison to the known species of the genus revealed it to be *P. vitii*. Its existence here indicates that it is first report from South Asia and also here been described for the first time on the basis of ITS marker. A taxonomic treatment including a description based on the newly collected material is provided along with a molecular phylogenetic analysis.

**Key words:** New record, Pakistan, Phylogeny, Physciaceae.**Introduction**

Morpho-anatomical characters have been used for the identification and systematics placement of fungi over the last 300 years (Seifert & Roberts, 2007). It is very difficult to do identification of lichens using phenotypic parameters due to nonexistence of distinct characters (Schoch *et al.*, 2012). Moreover, the morphological features have also limitations that results in incorrect identification, which leads to parallel evolution, homoplasy and phenotypic plasticity (Judd *et al.*, 2002), but molecular data have, gained impact in lichen systematics (Divakar & Crespo, 2015).

The genus *Physcia* is represented by 80 species worldwide (Lücking *et al.*, 2017). Ten species of this genus were so far known to occur in Pakistan i.e., *P. undulata* Moberg, *P. adscendens* (Th.Fr.) H.Olivier, *P. aipolia* (Ehrh. ex Humb.) Fürnr., *P. caesia* (Hoffm.) Fürnr., *P. dubia* (Hoffm.) Lettau., *P. labrata* Mereschk., *P. leptalea* (Ach.) DC., *P. stellaris* (L.) Nyl., *P. wainioi* Räsänen and *P. karakorina* Poelt (Aptroot & Iqbal, 2012).

As a part of ongoing studies on determination of diversity and the phylogenetic relationship among lichen taxa of Pakistan, we report *P. vitii* Nadv. for the first time on the basis of molecular data and first record of this species in South Asia.

**Material and Methods**

The present study is based on the results of a field trip to Parachinar, Khyber Pakhtunkhwa and Hunza, Gilgit-Baltistan, Pakistan. All possible morphological features along with field notes were taken and documented. The chemistry was analyzed using spot tests (with potassium hydroxide KOH (10%) and calcium hypochlorite C-test reagents) and thin layer chromatography (with solvents C) according to the method proposed by Orange *et al.* (2001). Free hand sections of thallus were mounted in water and observed at different magnifications for anatomical characterization and measurements. Minimum twenty measurements were made for each diagnostic feature.

**DNA extraction, PCR, and sequencing:** The DNA was extracted by 2% CTAB method (Gardes & Bruns, 1993). The markers used in the present study are shown in Table 1. The ITS and LSU loci of nrDNA were amplified from 3 thalli following the amplification protocol of Khan *et al.*, (2018) and then sequenced commercially.

**Table 1. Primer sequences used in this study.**

Sr. No.	Name	Primer sequence
1.	ITS1F	CTGGTCATTTAGAGGAAGTAA
2.	ITS4	TCCTCCGCTTATTGATATGC

**Morphological notes**

***Physcia vitii* Nadv., Stud. Bot. Čechoslov. 8(2-4): 94 (1947): (Fig 1). Thallus:** Foliose, loosely adnate, circular at first become irregular, up to 18 cm diam., highly sorediate, lobate; **Lobes:** discrete to imbricate, often ascending, 1.5–3.5 mm wide, fan-shaped, plan to often tubular eciliate. **Upper surface:** smooth, soft, epruinose, whitish grey to bluish grey. **Soredia:** granulose, whitish green in color, mostly continuous throughout marginal, soralia lip-shaped or crater like. **Lower surface:** whitish at lobes brown toward center, rhizinate; **Rhizines:** white, up to 2 mm, simple to rarely branched. **Thallus in section:** 120–200 µm. **Upper cortex:** above brown to dark brown, below hyaline, paraplectenchymatous, 10–22 µm thick. **Algal layer:** continuous, uneven, 25–50 µm high, photobiont trebouxoid, globose to subglobose, 10–18 µm in diam. **Medullary layer:** 25–70 µm thick, prosoplectenchymatous, hyphae white, moderate to loosely interwoven. **Lower cortex:** hyaline, paraplectenchymatous, cell rounded to elongate.

Fig. 1. **A & B:** View of the host tree *Quercus baloot* from locality Parachinar **C:** Transverse section of thallus **D:** *Physcia vitii* thallus with substrate **E:** Sorilia on thallus surface.

**Spot test:** Cortex: C-, K+ yellow Medulla: C-, K+ yellow

**Habitat and ecology:** growing on the bark of *Quercus baloot* Griff and coniferous trees in dry temperate forest at an altitude of 1,705 and 2700 m.a.s.l., maximum and minimum temperature 30°C to -6°C respectively.

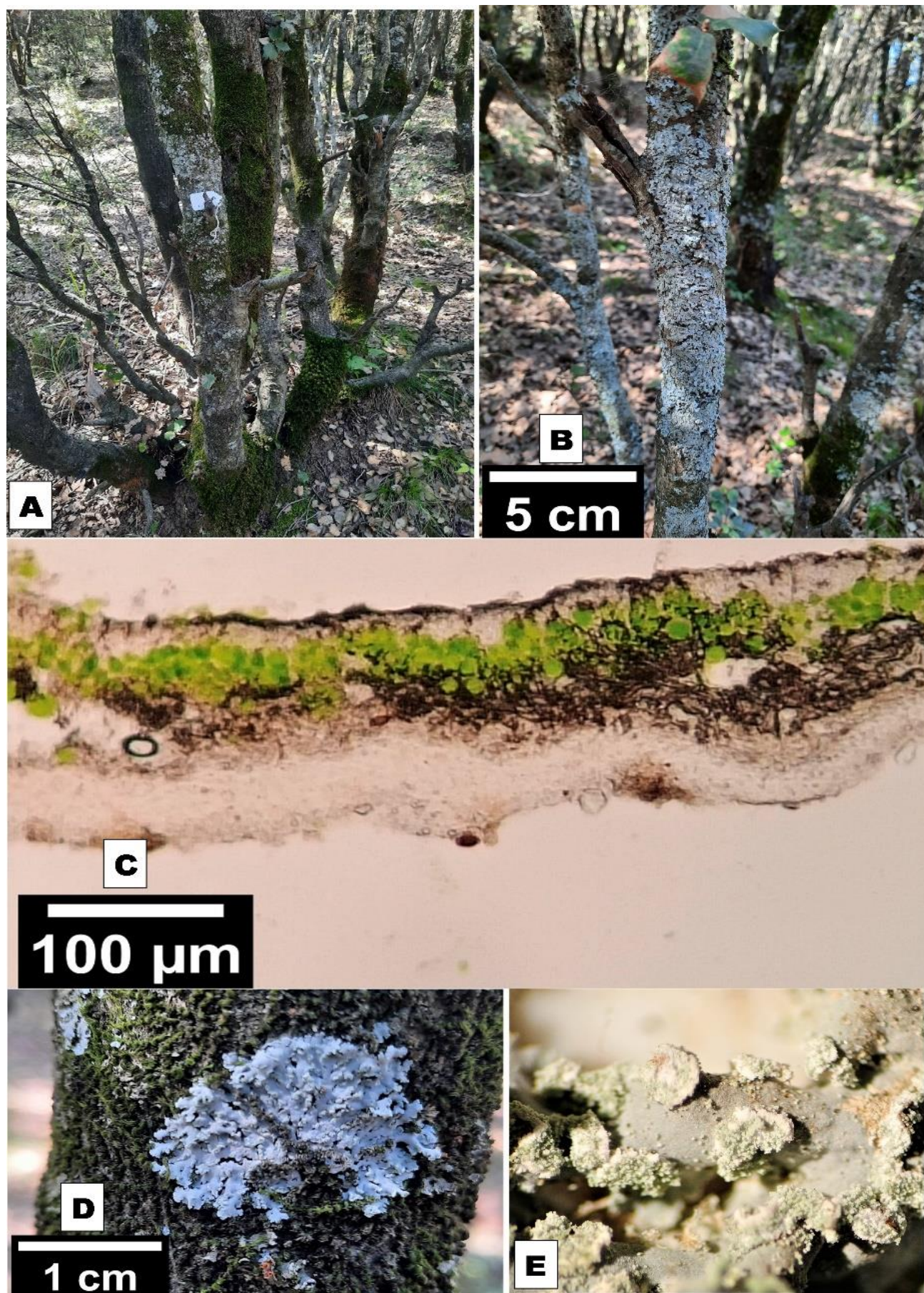


Fig. 1. A & B: View of the host tree *Quercus baloot* from locality Parachinar C: Transverse section of thallus D: *Physcia vitti* thallus with substrate E: Sorilia on thallus surface.

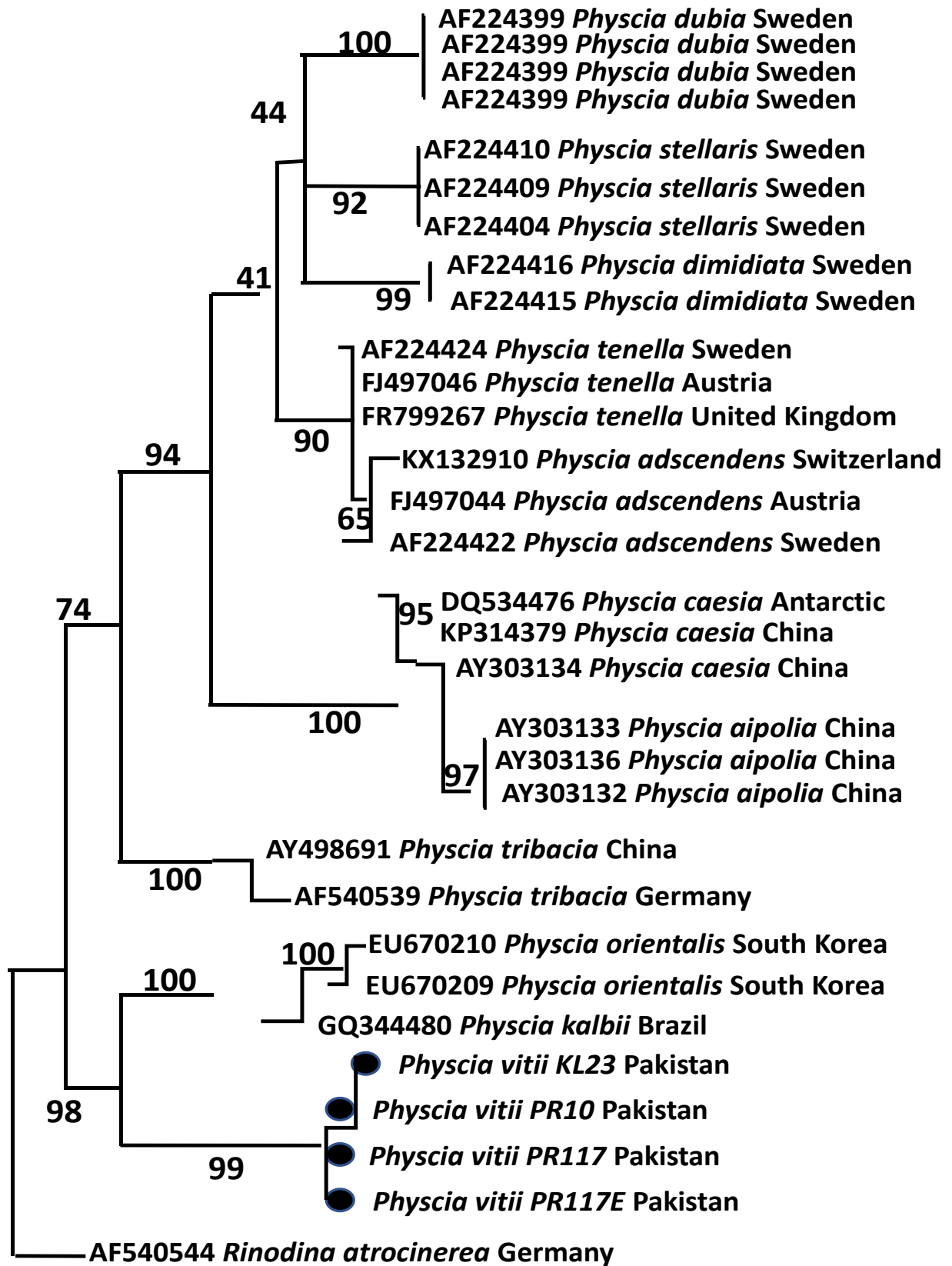


Fig. 2. Maximum likelihood Phylogenetic analysis (ITS-rDNA) of *Physcia* species based Tamura 3-parameter model. The bootstrap values are given above branches. The sequence of local specimens are marked with black circle.

**Table 2. The sequences used in the phylogenetic analysis.**

Species names	Accession numbers	Voucher number	Countries	References
<i>Physcia vittii</i> PR10	MW433609	LAH36784	Pakistan	From this study
<i>Physcia vittii</i> KL23	MW433610	LAH36785	Pakistan	From this study
<i>Physcia orientalis</i>	EU670210	Hur 040164	South Korea	Unpublished
<i>Physcia orientalis</i>	EU670209	Hur 040044	South Korea	Unpublished
<i>Physcia tribacia</i>	AY498691	PLN	China	Unpublished
<i>Physcia kalbii</i>	GQ344480	CEM01L	Brazil	Unpublished
<i>Physcia tenella</i>	FJ497046	PLN	Austria	Unpublished
<i>Physcia adscendens</i>	FJ497044	PLN	Austria	Unpublished
<i>Physcia adscendens</i>	KX132910	PLN	Switzerland	Unpublished
<i>Physcia caesia</i>	DQ534476	PLN	Antarctic	Unpublished
<i>Physcia caesia</i>	AY303134	PLN	China	Unpublished
<i>Physcia caesia</i>	KP314379	PLN	China	Unpublished
<i>Physcia tenella</i>	FR799267	PLN	United Kingdom	Unpublished
<i>Physcia tenella</i>	AF224424	PLN	Sweden	Unpublished
<i>Physcia adscendens</i>	AF224422	PLN	Sweden	Unpublished
<i>Physcia stellaris</i>	AF224409	PLN	Sweden	Unpublished
<i>Physcia dubia</i>	AF224403	PLN	Sweden	Unpublished
<i>Physcia stellaris</i>	AF224410	PLN	Sweden	Unpublished
<i>Physcia dimidiata</i>	AF224416	PLN	Sweden	Unpublished
<i>Physcia dimidiata</i>	AF224415	PLN	Sweden	Unpublished
<i>Physcia stellaris</i>	AF224404	PLN	Sweden	Unpublished
<i>Physcia aipolia</i>	AY303133	PLN	China	Unpublished
<i>Physcia dubia</i>	AF224400	PLN	Sweden	Unpublished
<i>Physcia dubia</i>	AF224402	PLN	Sweden	Unpublished
<i>Physcia dubia</i>	AF224399	PLN	Sweden	Unpublished
<i>Physcia tribacia</i>	AF540539	PLN	Germany	Unpublished
<i>Physcia aipolia</i>	AY303136	PLN	China	Unpublished
<i>Physcia aipolia</i>	AY303132	PLN	China	Unpublished
<i>Rinodina atrocineria</i>	AF540544	PLN	Germany	Unpublished

**Material examined 1:** PAKISTAN. Khyber Pakhtunkhwa; Kurram district, Parachinar; 33° 53' 51" North, 70° 6' 0" East; July, 2018 & 2020; A. N. Khalid, PR 117, PR 10; LAH36783, LAH36784.

**Material examined 2:** PAKISTAN. Gilgit-Baltistan; Hunza, 40°N/74 degrees 54'E, 20th August, 2015; S. Wali, J. Alam, K. Habib, KL23; LAH36785.

**TLC Results:** Using solvent 'c' for *Physcia vittii*, two compounds Olivetoric acid (Rf<sub>25</sub>) and Diploicin (Rf<sub>67</sub>) were spotted on the plate.

**Phylogenetic analysis:** All sequences of the collected specimens (voucher # PR 117, PR 117E, PR 10, PR KL 23) showed 93.52% similarity with *Physcia kalbii* Moberg (GQ344480) having 90% query cover and 0.0 E-value in BLAST search. Homology search was done at NCBI using BLAST tool, sequences with closest identity were downloaded. Nodal support was evaluated using the "rapid bootstrapping" option with 1,000 replicates.

A total of 31 sequences have been analyzed (Table 2). The data matrix had 560 positions of which 404 were conserved, 139 variable sites, 96 parsimony sites and 43 singletons sites. *Rinodina atrocineria* (AF540544) Out group for this tree was The sequences of *Physcia vittii* formed a strongly supported sister clade to *P. kalbii* Moberg and *P. orientalis* Kashiw. (96% BS) (Fig. 2).

## Discussion

*Physcia vittii*, also known as a finger moss, is fairly rare species from the *Physcia tribacia* group (Aptroot *et al.* 2003). It was described by Nádvorník (1947) from Bohemia, Czech Republic. In Europe, it is reported from central and southern regions (Aptroot *et al.*, 2003; Giordani & Incerti, 2008; Roux, 2012; Wirth *et al.*, 2013). The species is reported from Austria (Hafellner & Türk, 2001), Germany (Aptroot *et al.*, 2003), Italy (Nimis, 1993), Portugal (<https://www.gbif.org>), Spain (Gómez-Bolea, 1985), the Netherlands (van der Kolk, 2017), and Switzerland (Frey, 1963). In Asia, it is reported from Japan (East Asia), Mongolia (Golubkova, 1981), and the Russian Federation (Northern Asia). The species is here been reported for the first time from South Asia (Pakistan).

The type material was collected in lowland while the European countries collections were made in lowland and montane areas as well (Aptroot *et al.*, 2003; Nimis, 1993). Wirth (1995) documented that it is a montane species, growing in areas rich in precipitation, with a temperate to cold climate, on rarely or fully sun exposed sites, on moderately acid to sub-neutral cortex, on bark of tree trunks in roadside, in woodlands margins, in brookside stands, on relatively mineral-rich, but hardly eutrophicated bark. Aptroot *et al.*, (2003) reported on the twigs and bark of *Acer pseudoplatanus* L.

Our collections are from dry temperate climates of Parachinar and Hunza respectively. It was growing on the bark of *Q. baloot* above and Intermix with mosses. The specimen has been identified by morpho-anatomical characters and by lichen taxonomy expert Prof. Dr. André Aptroot. It is characterized by somewhat brownish lobes, K+ yellow upper cortex, pseudoparenchymatic lower cortex and absence of cilia. Morphological it is very close to *P. tribacia* (Ach.) Nyl. but differs in having broad lobes up to 4 mm (vs. up to 1 mm wide), thallus thin and soft (vs. thallus not thin and soft), rarely crenulate margin (vs. mostly crenulate margins) and distant position in the phylogenetic analyses. In the ITS-based phylogenetic analysis *P. vitii* formed sister species relationship with *P. kalbii*, from which it is easily recognized by the presence of soralia and pseudoparenchymatic lower cortex. The only distinguished character is K+ spot test of our collection with the already reported species of *P. vitii*. Moreover, despite of a few similar morphological characters of *P. vitii* with *Phaeophyscia orbicularis*, *Physcia dubia* and *Physcia tenella*, it falls very far making a complete distinctive clade in the phylogenetic trees, showing a distinctive nucleotide data for species *P. vitii*.

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(Received for publication 11 February 2021)