

Standard Paper

Phaeophyscia kashmirensis sp. nov. (Lecanorales, Physciaceae) from Azad Jammu and Kashmir, Pakistan

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Abstract

A new species in the genus *Phaeophyscia* is described from Azad Jammu and Kashmir, Pakistan, supported by nrITS sequences, morphology and chemistry. The taxon is characterized by a green to greyish green thallus, usually narrow (0.5–2 mm), flat to convex lobes with abundant marginal soralia, black, dense small rhizines, small *Physcia*-type ascospores 18–22 × 8–10 μm and an absence of pycnidia. Differences from related species are discussed.

Key words: moist temperate forest, molecular studies, Muzaffarabad, phylogenetics, siliceous rocks

(Accepted 21 July 2022)

Introduction

The foliose lichen genus *Phaeophyscia*, first described by Moberg, was originally included in the genus *Physcia* (Schreb.) Michx. and comprises c. 50 species worldwide. This group occurs on diverse substrata, including bark, wood, bryophytes, rock and soil, in a wide range of habitats (Moberg 1977; Aptroot & Sipman 1991). *Phaeophyscia* is characterized by a foliose thallus, white or orange-red medulla, paraplectenchymatous upper and lower cortex, lecanorine apothecia with *Physcia* or *Pachysporaria*-type ascospores, ellipsoid conidia, and an absence of atranorin (Moberg 1977; Aptroot & Sipman 1991).

Numerous authors have conducted taxonomic studies of *Phaeophyscia* around the world, from North America (Esslinger 1978a, b; Brodo & Sharnoff 2001), East Africa (Moberg 1983), China (Moberg 1995; Hu & Chen 2003; Li & Zhao 2006), Japan (Kashiwadani 1975, 1984a, b; Harada 2016) and Europe (Moberg 1974, 1977, 1978, 1994; Poelt 1974; Hale 1983) to Russia (Moberg 1995). Little attention has been paid to the phylogenetics of this group, although some molecular studies of several species have been conducted as part of studies on *Physconia* Poelt, *Physcia* (Schreb.) Michx., *Anaptychia* Körb., *Heterodermia* Trevis. and other related groups (Helms *et al.* 2003; Cubero *et al.* 2004; Lücking *et al.* 2007; Lohtander *et al.* 2008; Gaya *et al.* 2012).

Previously, eight species of *Phaeophyscia* have been reported from Pakistan (for instance, see Table 1), of which half were added by Ahmad (1965), viz. *Phaeophyscia ciliata* (Hoffm.) Moberg, *P. endococcina* (Körb.) Moberg, *P. hispidula* (Ach.) Essl. and *P. orbicularis* (Neck.) Moberg. Bhatti & Iqbal (1978) added *P. sciastra* (Ach.) Moberg and the remaining three species were reported by Aptroot & Iqbal (2012), viz. *P. exornatula* (Zahlbr.)

Kashiw., *P. melanchra* (Hue) Hale and *P. primaria* (Poelt) Trass. In this study, we combined morphology, chemistry and phylogenetic analysis with the aim of clarifying the relationships among *Physciaceae* and investigating support for a potential new species.

Materials and Methods

Study area and collection

A survey of the Jhelum Valley in Azad Jammu and Kashmir, undertaken in 2021, focused on increasing the biotic knowledge of the lichens of Pakistan. Azad Jammu and Kashmir, which lies in the north-eastern part of Pakistan, comprises an area of 13 297 km², with mountains ranging from 360 to 6325 m in altitude, and is characterized by siliceous rocks and undulating terrain (Dar *et al.* 2012). The collected samples were deposited in the herbarium of the Institute of Botany, University of the Punjab, Lahore (LAH).

Morphological characterization

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, hand-cut sections of thallus and apothecia were mounted in water and potassium hydroxide (K). A minimum of 20 measurements in water were made for each diagnostic feature from two samples; length × width of ascus and dimensions of ascospores were measured. The dimensions of ascospores and ascus are presented as smallest value recorded–largest value recorded.

Chemical characterization

Chemistry was analyzed using standard spot tests. These were performed with 10% K, 5% para-phenylenediamine in ethanol (P) and calcium hypochlorite solution (C). Secondary chemistry was

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Cite this article: Fayyaz I, Afshan NS and Khalid AN (2022) *Phaeophyscia kashmirensis* sp. nov. (Lecanorales, Physciaceae) from Azad Jammu and Kashmir, Pakistan. *Lichenologist* 54, 355–361. <https://doi.org/10.1017/S0024282922000275>

Table 1. Comparison of morphological characters of selected *Phaeophyscia* species recorded in Pakistan and which are similar to *P. kashmirensis*.

Characters	<i>P. ciliata</i>	<i>P. hispidula</i>	<i>P. kashmirensis</i>	<i>P. orbicularis</i>	<i>P. primaria</i>
Thallus colour	greyish brown to dark brown	grey	green to greyish green	grey to pale brown or dark brown	greenish grey to grey
Lobes	mostly flat	flat to concave	usually flat to convex	usually flat	concave
Soralia	absent	present, laminal	present, marginal	mostly orbicular, laminal sometimes marginal	absent
Apothecia	abundant	rare	rare	uncommon	rare
Apothecial disc	–	–	flat to slightly convex	–	flat to concave
Ascus	–	–	50–60 × 15–17 µm	–	67–75 × 14–18 µm
Ascospores	16–27 × 7–13 µm	18–25 × 7–12 µm	18–22 × 8–10 µm	17–26 × 7–11 µm	16.5–18.5 × 6.9–8.5 µm
Pycnidia	abundant	not seen	not seen	frequent	common
References	Moberg 1995	Esslinger 2004; Liu & Hur 2019	This paper	Esslinger 2004; Edwards & Coppins 2009	Liu & Hur 2019

analyzed using thin-layer chromatography (TLC) using solvent system C, 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 ITS-nrDNA region (internal transcribed spacer of the nrDNA) was amplified using the primer pair ITS1F (forward primer) (Gardes & Bruns 1993) and ITS4 (reverse primer) (White *et al.* 1990), following the amplification protocol of Khan *et al.* (2018). PCR products were visualized on a 1% agarose gel with ethidium bromide (Sambrook & Russel 2001). PCR products were sent to Tsingke, China for sequencing. Sequences were assembled using BioEdit (Hall 1999). BLAST analysis was used to retrieve highly similar sequences of the nrITS region. The maximum query coverage and percent identity of the sequences, along with related taxa, were noted. Sequences retrieved from GenBank and sourced from relevant literature were used in an initial alignment, which was trimmed and then realigned using web-PRANK with default settings (Löytynoja & Goldman 2010). On the CIPRES Portal (Miller *et al.* 2010), the HYK + G + I model was selected using jModelTest (Posada 2008). Using RAXML-HPC2 v.8.1.11 on CIPRES, a maximum likelihood (ML) analysis was also implemented (Stamatakis 2014), with 1000 bootstraps used for rapid bootstrapping. FigTree v. 1.4.3 (Rambaut *et al.* 2014) was used for visualizing the phylogeny from the ML analysis.

Results

Molecular phylogenetic analyses

ITS sequences of the holotype of the new species and of the collections CKR-03 and CKR-4 were identical. The final ITS dataset consisted of 42 sequences, including *Polyblastidium japonicum* (M. Satô) Kalb (DQ337322) as outgroup (Liu & Hur 2019) (see Table 2 for voucher details). The aligned ITS1-5.8S-ITS2 region comprised 542 sites, of which 289 were conserved and 241 variable; 186 sites were parsimony-informative. In the analysis of the ITS region (Fig. 2), three newly generated sequences formed a separate clade in a strongly supported sister relationship with the *Phaeophyscia hispidula* group, justifying the recognition of an undescribed species which is presented here.

Taxonomy

Phaeophyscia kashmirensis Fayyaz, Afshan & Khalid sp. nov.

Mycobank No.: MB 844145

The species is characterized by a green to greyish green thallus, usually narrow (0.5–2 mm), flat to convex lobes with abundant marginal soralia, black, dense small rhizines, small *Physcia*-type ascospores 18–22 × 8–10 µm and an absence of pycnidia.

Type: Pakistan, Azad Jammu and Kashmir, Garhi Dupatta, 34°36'N, 73°35'E, 817 m alt., on siliceous rock, 2 October 2021, I. Fayyaz & N. S. Afshan (CKR-23) (LAH37160—holotype).

(Fig. 1)

Thallus foliose, green when fresh but greyish green when dry, loosely attached to substratum, very variable in size, 5–10 cm diam., orbicular to irregular. *Lobes* irregularly or dichotomously branched, without pruina, lobe tips creamy to paler, flat to slightly convex, imbricate, usually upturned near the tips, 0.5–2 mm wide. *Soralia* frequently present, marginal. *Lobules* absent. *Cortex* dark brown, paraplectenchymatous, 15–20 µm thick. *Algal layer* 25–30 µm thick. *Photobiont cells* globose, 10–15 µm diam. *Medulla* white. *Lower surface* black, usually becoming pale to brown towards lobe tips, rhizinate. *Rhizines* dense, black, simple, 1–2 mm long. *Lower cortex* paraplectenchymatous, blackish brown, 22–35 µm thick.

Apothecia rather rare, up to 0.5 mm diam., sessile to very short stipitate, the margin entire or becoming irregularly crenate, lacking cortical hairs, epruinose. *Disc* flat to slightly convex. *Epithemium* light brown, 20–24 µm. *Hymenium* hyaline, 70–80 µm. *Hypothecium* hyaline to light brown, 35–40 µm. *Ascus* cylindrical to clavate, 50–60 × 15–17 µm. *Ascospores* dark brown, ellipsoid, *Physcia*-type, 18–22 × 8–10 µm. *Paraphyses* hyaline, branched and anastomosing, 2–3 µm thick.

Pycnidia not found.

Chemistry. Thallus K–, C–, KC–, P–; no substances detected by TLC.

Etymology. The specific epithet '*kashmirensis*' (Latin) refers to the type locality of Azad Jammu and Kashmir.

Table 2. Voucher information and GenBank Accession numbers of sequences used in the ITS phylogenetic analysis of *Phaeophyscia* and related species. New sequences are in bold.

Name of species	Country/origin	Voucher specimen	GenBank no. (ITS)
<i>Anaptychia isidiata</i>	Russia	<i>Himmelbrant</i> K-04-10	EF582780
<i>A. palmulata</i>	USA	<i>Ahti</i> 58054	EF582779
<i>Hyperphyscia adglutinata</i>	–	<i>Moberg</i> 12045	AF224361
<i>H. adglutinata</i>	Germany	<i>P. Dornes</i> 411b (Dornes)	AF540521
<i>Phaeophyscia adiastrata</i>	Canada	BIOUG24047-D11	KT695334
<i>P. adiastrata</i>	Canada	BIOUG24047-D10	KT695398
<i>P. ciliata</i>	–	<i>Tehler</i> 7892b	AF224455
<i>P. endococcinodes</i>	South Korea	130163	MN150503
<i>P. exornatula</i>	–	<i>J. B. Chen</i> 22251	AY498669
<i>P. exornatula</i>	–	<i>Hur</i> 060117	EU670224
<i>P. hispidula</i>	India	CUPVOUCHER-HP-41L-2018-PH-1	OK577921
<i>P. hispidula</i>	India	CUPVOUCHER-HP-54L-2018-PH-1	OK577928
<i>P. hispidula</i>	–	<i>J. B. Chen & G. R. Hu</i> 22061	AY498673
<i>P. hispidula</i>	–	–	AY303138
<i>P. kashmirensis</i>	Pakistan	LAH37160	ON640614
<i>P. kashmirensis</i>	Pakistan	LAH37161	ON640615
<i>P. kashmirensis</i>	Pakistan	LAH37162	ON640616
<i>P. orbicularis</i>	Germany	<i>M. Schultz</i> 9808.021 (Dornes)	AF540528
<i>P. orbicularis</i>	–	–	AF250799
<i>P. primaria</i>	–	<i>M. R. Huang</i> 636	AY498660
<i>P. primaria</i>	–	<i>M. R. Huang</i> 660	AY498664
<i>P. pyrrophora</i>	–	<i>Hur</i> 060299	EU670227
<i>P. pyrrophora</i>	South Korea	162409	MN150492
<i>P. rubropulchra</i>	South Korea	141271	MN150493
<i>P. rubropulchra</i>	South Korea	162431	MN150495
<i>P. rubropulchra</i>	South Korea	141402	MN150494
<i>P. trichophora</i>	–	<i>Hur</i> 041160	EU670215
<i>P. trichophora</i>	–	<i>Hur</i> 041524	EU670216
<i>Physcia austrostellaris</i>	Australia	CBG: <i>Elix</i> 38829	GU074409
<i>P. integrata</i>	USA	<i>H. Sipman & R. Welz</i> 44890 (B)	AF540533
<i>P. krogiae</i>	USA	<i>H. Sipman & R. Welz</i> 44672 (B)	AF540534
<i>Physciella chloantha</i>	–	BCN-Lich 17032	GU247163
<i>P. chloantha</i>	–	BCN-Lich 15525	GU247166
<i>P. chloantha</i>	Canada	BIOUG24047-E05	KT695384
<i>P. chloantha</i>	Canada	BIOUG24047-F01	KT695320
<i>P. melanchnra</i>	South Korea	170625	MN150499
<i>P. melanchnra</i>	South Korea	171426	MN150500
<i>P. melanchnra</i>	–	<i>J. B. Chen & G. R. Hu</i> 21437	AY498666
<i>P. melanchnra</i>	South Korea	171449	MN150501
<i>P. melanchnra</i>	–	<i>Hur</i> 040625	EU670211
<i>Physconia subpulverulenta</i>	Spain	MAF-Lich 14116	DQ862489
<i>P. thorstenii</i>	Spain	MAF-Lich 14120	DQ862494
<i>P. venusta</i>	Spain	MAF-Lich 14124	DQ862498
<i>Polyblastidium japonicum</i>	USA	15132c	DQ337322

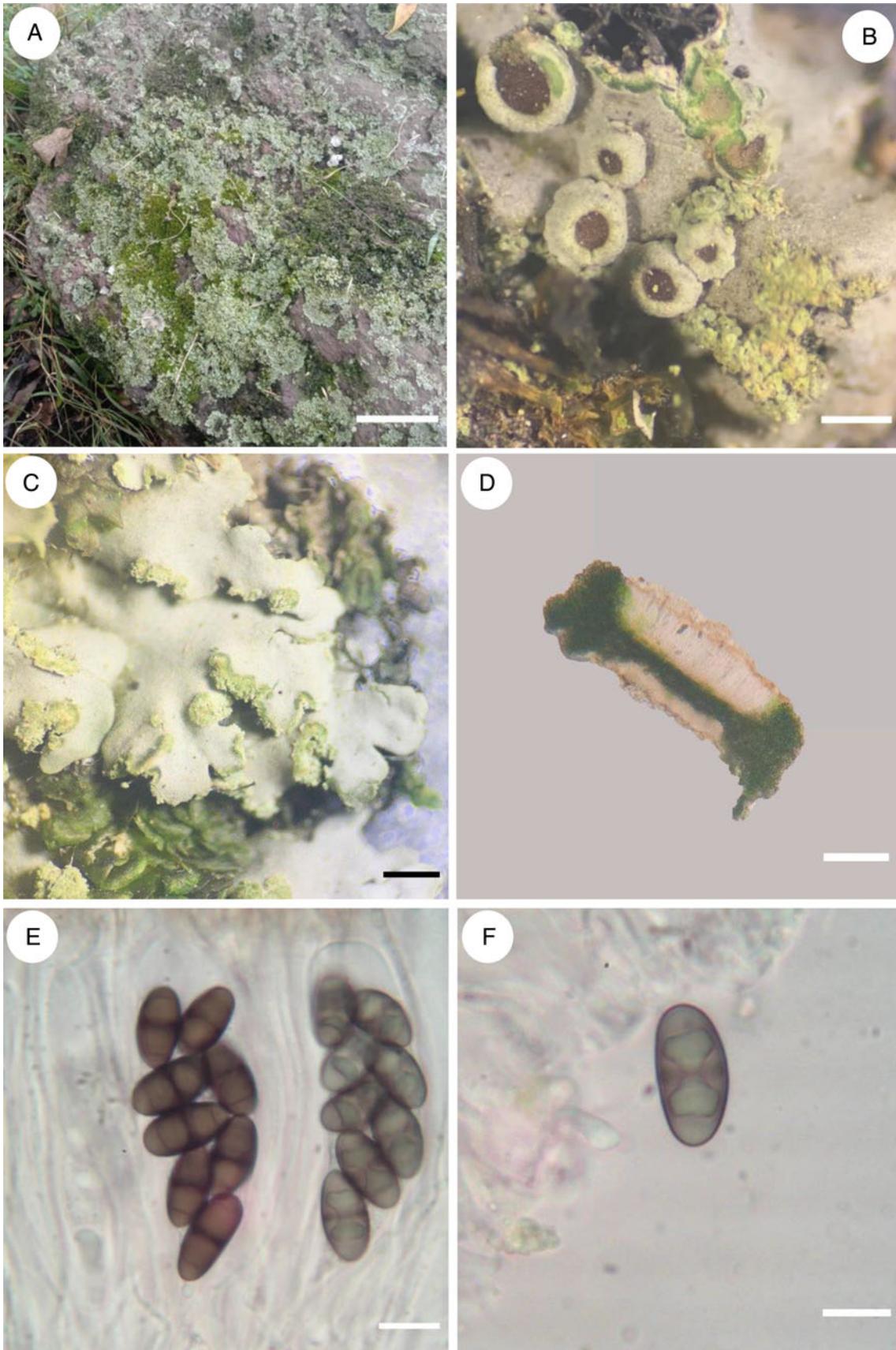


Fig. 1. *Phaeophyscia kashmirensis*. A, foliose thallus. B, apothecia. C, lobe with rhizines. D, section of an apothecium. E, ascus. F, ascospore. Scales: A = 2 cm; B = 0.5 mm; C = 2 mm; D = 75 μ m; E = 16 μ m; F = 8 μ m. In colour online.

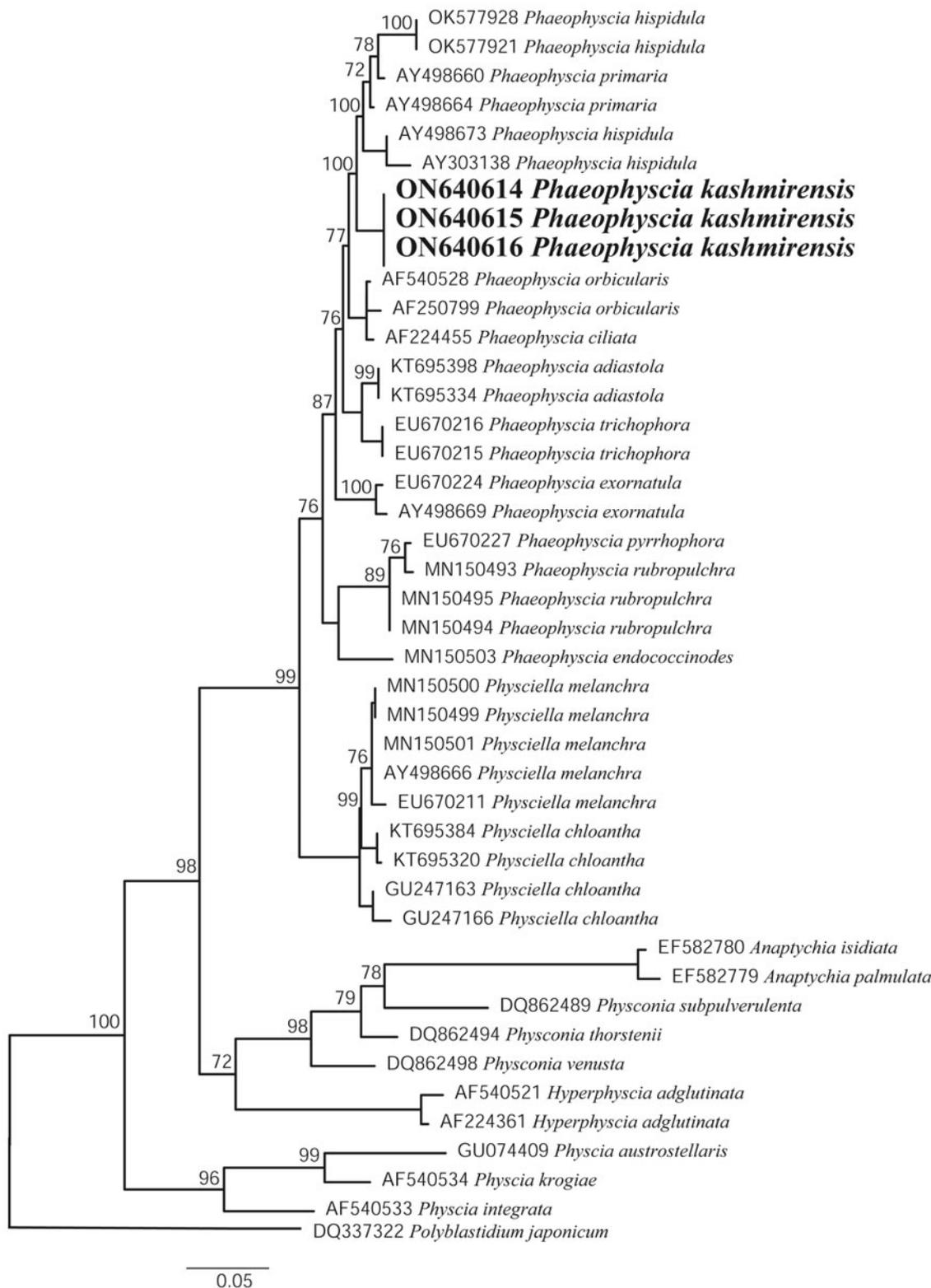


Fig. 2. Phylogeny of *Phaeophyscia* and related species based on a maximum likelihood (ML) analysis of the ITS region. The tree was rooted using *Polyblastidium japonicum*. The new species, *Phaeophyscia kashmirensis*, is shown in bold.

Habitat and distribution. The known collections of the new species are from Himalayan moist temperate forest in Azad Jammu and Kashmir. The specimens were found on siliceous rocks. The coniferous forest is dominated by species such as *Pinus*

roxburghii Sarg., *Pyrus pashia* L., *Quercus oblongata* D. Don and *Q. glauca* Thumb. The maximum daily temperature of the region varies from 30–32 °C during the summer, with an average winter temperature of 4 °C, and there is moderate rainfall.

Additional specimens examined. Pakistan: Azad Jammu and Kashmir: Chikar (34°36'N, 73°35'E), 1607 m alt., on rock, 2021, I. Fayyaz & N. S. Afshan (CKR-03); *ibid.*, 2021, I. Fayyaz & N. S. Afshan (CKR-04).

Discussion

Three collections of this taxon were made from Garhi Dupatta and Chikar (Muzaffarabad District, Azad Jammu and Kashmir). The two sites vary significantly in terms of elevation, vegetation and climatic conditions. However, phylogenetic analysis confirmed that the three collections of *Phaeophyscia kashmirensis* form a monophyletic clade with good support (Fig. 2).

In the phylogeny, *Phaeophyscia kashmirensis* appeared as sister species to the *P. hispidula* group, comprising the sorediate *P. hispidula* and the non-sorediate *P. primaria*. The new taxon has a green to greyish green thallus and usually narrow, flat to convex lobes (0.5–2 mm wide). *Phaeophyscia hispidula* differs in having a grey thallus and broad, flat to concave lobes 1–3 mm wide, while *P. primaria* differs in the greenish grey to grey thallus, broad, concave lobes 2–8 mm wide, absence of soralia, and smaller ascospores (16.5–18.5 × 6.9–8.5 µm) (Liu & Hur 2019).

Phaeophyscia orbicularis differs in the mostly laminal (to marginal) soralia and the often presence of skyrin (a yellow-orange pigment) in the uppermost part of medulla (Edwards & Coppins 2009). *Phaeophyscia ciliata* differs in the darker (grey-brown to dark brown) thallus and absence of soralia (Moberg 1995). These differences, together with the phylogenetic analysis, confirm the novelty of this species.

Acknowledgements. We are highly grateful to the University of the Punjab, Lahore, Pakistan for providing project no. D/72/EST-I (dated 14/01/2022) to conduct this research work. We also wish to thank the anonymous reviewers for their valuable comments that greatly improved the manuscript.

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