

A NEW SPECIES IN THE LICHEN GENUS *CIRCINARIA* (MEGASPORACEAE)
FROM PAKISTAN BASED ON MORPHOLOGY AND DNA SEQUENCE DATA

Muhammad S. Iqbal, Alla U. Din, Abdul N. Khalid, and Abdul R. Niazi

*Fungal Biology and Systematics Research Laboratory
Institute of Botany, University of the Punjab
Qaid-e-Azam Campus, Lahore 54590, PAKISTAN
Correspondence: mshahidiqbal012@gmail.com*

ABSTRACT

Circinaria terrenoides sp. nov. was collected in Balochistan and Gilgit Baltistan. Comparative morpho-anatomical research, chemical analysis, and ITS-based DNA analyses all support its placement within the genus *Circinaria*. *Circinaria terrenoides* sp. nov. is distinguished from the closely related species *C. affinis* in having a crustose, areolate to rimose-areolate thallus (vs. subfruticose, verrucose-areolate), earthy brown to greenish gray upper surface (vs. earthy gray to gray-green), smaller conidia $12\text{--}18 \times 0.4\text{--}0.9 \mu\text{m}$ (vs. $16\text{--}20 \times 1\text{--}1.2 \mu\text{m}$), pycnidia black (vs. black to brownish) and growing on calcareous sedimentary rock (vs. stony steppes).

RESUMEN

Circinaria terrenoides sp. nov. se recolectó en Baluchistán y Gilgit Baltistán. La investigación morfoanatómica comparativa, el análisis químico y los análisis de ADN basados en ITS apoyan su inclusión en el género *Circinaria*. *Circinaria terrenoides* sp. nov. se distingue de la especie estrechamente relacionada *C. affinis* por tener un talo costroso, areolado a rimoso-areolado (frente a subfruticosa, verrucosa-areolada), superficie superior marrón terroso a gris verdoso (frente a gris terroso a gris verdoso), superficie superior marrón terroso a gris verdoso (frente a gris terroso a gris verdoso) y superficie superior marrón terroso a gris verdoso (frente a gris verdoso), gris terroso a gris verdoso, conidios más pequeños de $12\text{--}18 \times 0,4\text{--}0,9 \mu\text{m}$ (frente a $16\text{--}20 \times 1\text{--}1,2 \mu\text{m}$), picnidios (frente a negros a parduscos) y que crecen en rocas sedimentarias calcáreas (frente a estepas pedregosas).

KEY WORDS: Balochistan, Gilgit Baltistan, Taxonomy, Phylogeny

INTRODUCTION

Darel Valley has a structure of barren mountains with patches of fertile land with pastures. The samples were collected on calcareous sedimentary rocks in cold semi-arid climate at an altitude of 2,000 m a.s.l. The climate has typically cold desert characteristics, with severe winters (usually with moderate to heavy snowfall) and dry summers. Average annual precipitation in the valley is 100–300 mm, mostly occurring during winter and early spring in the form of snow. The climax vegetation of area belongs to dry temperate coniferous forest which mainly consist of *Cedrus deodara* (Roxb. ex D. Don) G. Don, *Pinus wallichiana* A.B. Jackson, *Abies pindrow* Royle and *Pinus gerardiana* Wall. ex D. Don (Khan et al. 2013).

The district Killa Saifullah, area-wise ranks 15 (ranking order: smallest to the largest) in Balochistan and has an area of 6,831 square kilometers. It lies between $67^{\circ}17'37''\text{--}69^{\circ}22'54''$ East longitudes and $30^{\circ}30'35''\text{--}31^{\circ}37'10''$ North latitudes consisting of 3 Tehsils and 15 Union Councils. The district is located in the north-east part of Balochistan, sharing its boundaries in the west with Afghanistan and Sindh Province. Awaran and Gawadar Districts are on the west and Khuzdar District is in the north. The climate of Killa Saifullah, generally elevated at 1,500–2,200 m above sea level, is semi-arid. It can be placed in “warm summer and cool winter” temperature region. The summer is warm with mean temperatures ranging from 21°C to 32°C. The winter is cool and longer than summer. It lasts for about 7 months (October–April). In winter, the mean temperature is below 10°C. Geographically the district is mountainous with valleys at varying elevations above sea level (Gob & Unicef 2011).

The genus *Circinaria* Link (Megasperaceae) is closely related to *Megaspora* Clauzade & Cl. Roux and includes sphaerothalloid, umbilicate and crustose species with diverse substrate preferences, found predominantly on rock (outcrops, stones, pebbles) but some species grow on soil and others are without stable

substrate. *Circinaria* species are devoid of secondary substances or contain aspicilin, norstictic or stictic acid (Cannon et al. 2023) and have usually a reduced number of spores per ascus, 4–6 instead of 8. The spores are broadly ellipsoid to globose. Many species are endemic in arid and semi-arid regions of the northern hemisphere (Sohrabi 2012). According to Speciesfungorum.com, *Circinaria* is currently represented by 40 species worldwide, of which only six are reported from Pakistan, namely: *C. contorta* (Hoffm.) A. Nordin, Savić, & Tibell, *C. caesiocinerea* (Nyl. ex Malbr.) A. Nordin, Savić, & Tibell, *C. darelensis* M.S. Iqbal & Khalid, *C. thorstenii* R. Zulfiqar & Khalid, *C. pakistanica* Fayyaz, M.S. Iqbal, Afshan, & Khalid, and *C. russia* S. Riaz, M.S. Iqbal, Nadeem, A.R. Niazi, & Khalid (Aptroot & Iqbal 2012; Din et al. 2024; Iqbal et al. 2023d; Riaz et al. 2024; Zulfiqar & Khalid 2023). The samples were collected from Darel Valley (Gilgit Baltistan) and Killa Saifullah (Balochistan).

This work uses morphological and anatomical study to describe *Circinaria terrenoides* sp. nov. in detail. Additional evidence for the classification of this taxon as a new species comes from an ITS-based phylogenetic analysis.

MATERIAL AND METHODS

Morphological and Chemical Studies.—Lichens were collected in 2022, while surveying the various regions of Balochistan and Gilgit Baltistan. Every thallus component's morphological properties were examined using a stereomicroscope (Meiji Techno, EMZ-5TR, Japan). Measurements were made with a compound microscope (MX4300H, Meiji Techno Co., Ltd., Japan), using free-hand sections of pycnidia in water mounts. The collected specimens were deposited in the Herbarium of the Institute of Botany, University of the Punjab, Lahore (LAH37685, LAH37682, LAH37684, LAH37681, LAH37680 & LAH37683). The secondary chemistry was analyzed using spot tests which were performed using KOH (10%; K) and sodium hypochlorite solution (C). Thin Layer Chromatography was carried out using Solvent System C, following standard methods (Orange et al. 2010).

Molecular Characterization.—For molecular analysis, DNA was extracted from air dried and cleaned thalli using a GF1 Plant DNA extraction kit, following the instructions of the manufacturer (Vivantis, Selangor Darul Ehsan, Malaysia). For qualitative examination of total extracted DNA, 1% agarose gel electrophoresis was employed (Voytas 2000). A thermal cycler (Bio-RAD T100) was used to amplify rDNA ITS region. Primers used during amplifications were ITS1F and ITS4 for the ITS region (Gardes & Bruns 1993; White et al. 1990). PCR products were purified using a QIAquick PCR Purification Kit (Qiagen, Valencia, CA, and USA) and then submitted to TsingKe, China for sequencing utilizing ITS1F, ITS4 amplicons for forward and reverse sequencing. To reconstruct forward and reverse sequences, the BioEdit sequence alignment editor was utilized (Hall 1999). The nucleotide sequence comparison was carried out using the National Centre for Biotechnology Information (NCBI) (<https://www.ncbi.nlm.nih.gov/>) Basic Local Alignment Search Tool (BLAST) (<https://blast.ncbi.nlm.nih.gov/>) (Altschul et al. 1990). MAFFT v.7 (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) was used for the multiple sequence alignment, with all parameters set to default levels (Katoh & Standley 2013). The phylogenetic tree (Fig. 1) was created utilizing the MEGA 6.0 program (Tamura et al. 2013) and the ML approach based on the Kimura 2-parameter model. The “rapid bootstrapping” option with 1,000 repetitions was used to assess nodal support. For rooting purposes of the tree, *Lobothallia praeradiosa* (Nyl.) Hafellner (MK347502) was selected as an outgroup. Newly generated sequences were deposited in GenBank (<https://submit.ncbi.nlm.nih.gov/subs/genbank/>) (see Table 1).

RESULTS

Phylogenetic analysis.—The newly generated ITS nrDNA sequences were compared with a large selection of representative sequences of the genus *Circinaria*, taken from Genbank. In all, 68 ITS rDNA sequences were examined. The alignment file contained 706 characters, 409 of which were preserved, 231 variables, 185 parsimony informative, and 46 singleton variations. The new sequences (PP034177, OQ376375, OQ376374, OQ359391, OQ359392 & OQ376376) appeared in the ITS phylogram all together on a separate clade, sister to

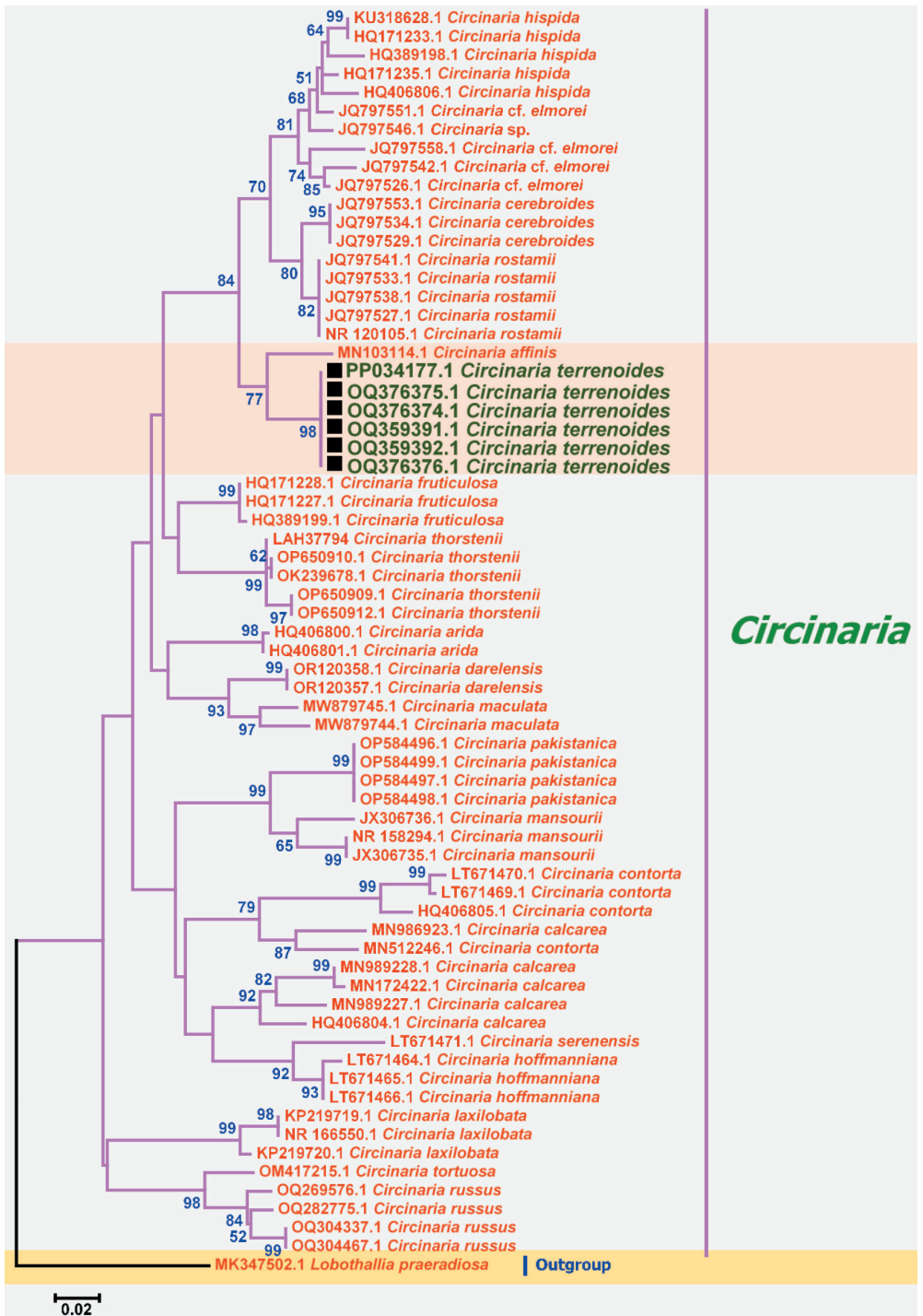


Fig. 1. Phylogenetic analysis of *Circinaria* Link members based on ITS sequences by the maximum likelihood method. Sequences generated from Pakistani collections are marked with ■.

TABLE 1. Voucher specimens and NCBI GenBank accession numbers of the sequences used in the phylogenetic analysis. New species are indicated in **bold** text.

Species names	ITS Genbank Accession Numbers	Voucher name	Origin
<i>Circinaria affinis</i>	MN103114	10-0195	China
<i>Circinaria arida</i>	HQ406801	Knudsen 2046 (UPS)	USA
<i>Circinaria arida</i>	HQ406800	Owe-Larsson 8759 (UPS)	USA
<i>Circinaria calcarea</i>	MN989228	Sipman & Raus 63719 (B 60 0203232)	Greece
<i>Circinaria calcarea</i>	MN172422	Sipman & Raus 63332 (B 60 0202736)	Greece
<i>Circinaria calcarea</i>	MN989227	Sipman & Raus 63710 (B 60 0203223)	Greece
<i>Circinaria calcarea</i>	HQ406804	Nordin 5914 (UPS)	Sweden
<i>Circinaria calcarea</i>	MN986923	1006475	Algeria
<i>Circinaria cerebroides</i>	JQ797553	Ringel 5184 (H)	Kyrgyzstan
<i>Circinaria cerebroides</i>	JQ797529	Ringel & Jaschhof 5180 (H)	Kyrgyzstan
<i>Circinaria cerebroides</i>	JQ797534	Ringel 5138 (H)	Kyrgyzstan
<i>Circinaria cf elmorei</i>	JQ797542	Sohrabi 10128 (IRAN)	Iran
<i>Circinaria cf elmorei</i>	JQ797526	Sohrabi 10405C (IRAN)	Iran
<i>Circinaria cf elmorei</i>	JQ797558	Sochting 11187 (hb. M. Sohrabi)	Iran
<i>Circinaria cf. elmorei</i>	JQ797551	Vondrak 5671B (CBFS)	Ukraine
<i>Circinaria contorta</i>	MN512246	Gavalas IRGA-473 (B 60 0202378)	Greece
<i>Circinaria contorta</i>	HQ406805	Tibell 23702 (UPS)	Sweden
<i>Circinaria contorta</i>	LT671470	UPS L-204675	Sweden
<i>Circinaria contorta</i>	LT671469	UPS L-163190	Sweden
<i>Circinaria darelensis</i>	OR120358	LAH37882	Pakistan
<i>Circinaria darelensis</i>	OR120357	LAH37883	Pakistan
<i>Circinaria fruticulosa</i>	HQ171228	Markus Lange 5186 (H)	Kazakhstan
<i>Circinaria fruticulosa</i>	HQ171227	V. G. Kulakov 1408 Herb. V. Jhon (9913)	Russia
<i>Circinaria fruticulosa</i>	HQ389199	Vondrak 5188 (GBFS)	Ukraine
<i>Circinaria hispida</i>	HQ406806	Candan 11 (ANES)	Turkey
<i>Circinaria hispida</i>	HQ171235	N. N. Ochirova s.n. 2003 (LE)	Russia
<i>Circinaria hispida</i>	HQ389198	Rosentreter 16233 & Cochrane (SRP)	USA
<i>Circinaria hispida</i>	KU318628	1006477	Spain
<i>Circinaria hispida</i>	HQ171233	Sohrabi 15099 (Herb. M. Soharbi)	Iran
<i>Circinaria hoffmanniana</i>	LT671465	UPS L-163189	Sweden
<i>Circinaria hoffmanniana</i>	LT671464	UPS L-154684	Sweden
<i>Circinaria hoffmanniana</i>	LT671466	UPS L-204669	Sweden
<i>Circinaria laxilobata</i>	KP219720	Ismayil & Abbas 20111049	China
<i>Circinaria laxilobata</i>	KP219719	Ismayil & Abbas 20111099	China
<i>Circinaria laxilobata</i>	NR166550	HMAS:Abbas 20111049	China
<i>Circinaria maculata</i>	MW879745	Q. Ren 2971	China
<i>Circinaria maculata</i>	MW879744	Q. Ren 2966	China
<i>Circinaria mansourii</i>	JX306736	Sohrabi 10097	Iran
<i>Circinaria mansourii</i>	NR158294	IRAN:MS015088	Iran
<i>Circinaria mansourii</i>	JX306735	Sohrabi 15077	Iran
<i>Circinaria rostamii</i>	JQ797541	Sohrabi 9364 (IRAN)	Iran
<i>Circinaria rostamii</i>	JQ797538	Sohrabi 10212 (IRAN)	Iran
<i>Circinaria rostamii</i>	NR120105	Sohrabi 10212 (IRAN)	Iran
<i>Circinaria rostamii</i>	JQ797527	Sohrabi 10212 (IRAN)	Iran
<i>Circinaria rostamii</i>	JQ797533	Sohrabi 10095 (IRAN)	Iran
<i>Circinaria ruscus</i>	OQ269576	LAH37655	Pakistan
<i>Circinaria ruscus</i>	OQ282775	LAH37656	Pakistan
<i>Circinaria ruscus</i>	OQ304337	LAH37672	Pakistan
<i>Circinaria ruscus</i>	OQ304467	LAH37673	Pakistan
<i>Circinaria serenensis</i>	LT671471	UPS L-205589	France
<i>Circinaria sp</i>	JQ797546	Sohrabi 9347 (IRAN)	Iran
<i>Circinaria terrenoides</i>	OQ376375	LAH37682	Pakistan
<i>Circinaria terrenoides</i>	OQ359392	LAH37680	Pakistan
<i>Circinaria terrenoides</i>	OQ359391	LAH37681	Pakistan
<i>Circinaria terrenoides</i>	OQ376376	LAH37683	Pakistan
<i>Circinaria terrenoides</i>	OQ376374	LAH37684	Pakistan
<i>Circinaria terrenoides</i>	PP034177	LAH37685	Pakistan
<i>Circinaria thorstenii</i>	–	LAH37794	Pakistan
<i>Circinaria thorstenii</i>	OP650912	LAH37797	Pakistan
<i>Circinaria thorstenii</i>	OP650910	LAH37793	Pakistan

TABLE 1. continued

Species names	ITS Genbank Accession Numbers	Voucher name	Origin
<i>Circinaria thorstenii</i>	–	LAH37796	Pakistan
<i>Circinaria thorstenii</i>	OK239678	Wahab 20170	Pakistan
<i>Circinaria tortuosa</i>	OM417215	20216046	China
<i>Circinaria pakistanica</i>	OP584496	LAH36028	Pakistan
<i>Circinaria pakistanica</i>	OP584497	LAH36029	Pakistan
<i>Circinaria pakistanica</i>	OP584498	LAH37576	Pakistan
<i>Circinaria pakistanica</i>	OP584499	LAH37577	Pakistan
<i>Lobothallia praevalidiosa</i>	MK347502	Paukov 3026	Russia

C. affinis (MN103114), with high bootstrap support (77% BS), so confirming the status of the novel species that is detailed below.

TAXONOMIC TREATMENT

Circinaria terrenoides M.S. Iqbal, Alla Ud Din, Khalid, & Niazi, **sp. nov.** (Fig. 2). TYPE: PAKISTAN: Gilgit Baltistan, Darel Valley 35°37'N, 73°27'E, 2,000 m a.s.l., on rocks, 10 Aug 2022, Muhammad Shahid Iqbal, DR-995 (HOLOTYPE: LAH37685) (ITS GenBank accession number PP034177).

Mycobank No.—MB847428.

Diagnosis.—*Circinaria terrenoides* sp. nov. distinguished from closely related species *C. affinis* in having crustose areolate to rimose-areolate thallus (vs. subfruticose, verrucose-areolate), earthy brown to greenish gray upper surface (vs. earthy gray to gray-green), smaller conidia 12–18 × 0.4–0.9 µm (vs. 16–20 × 1–1.2 µm), pycnidia black (vs. black to brownish) and growing on calcareous sedimentary rock (vs. stony steppes).

Description.—Thallus: crustose, saxicolous, 6 cm across, areolate to rimose areolate, thick, marginate, mostly black spotted, thick, slightly epruinose. Areoles: up to 1 mm wide and 0.6 mm thick, discrete to contiguous, smooth, flat to concave. Upper surface: earthy brown to greenish gray. Prothallus: absent. Pseudocypbellae: absent. Upper cortex: 25–45 µm thick, smooth, grayish brown, paraplectenchymatous, cells 12–18 µm in diam. Algal layer: continuous, 80 to 140 µm thick. Photobiont cells: rounded to subglobose, 15–35 µm wide. Medulla: 70–130 µm thick, hyphae prosoplectenchymatous, 1–4 µm wide. Apothecia: not found. Pycnidia: frequent, immersed, black, 0.2 mm. Conidiophores: 11–17 × 1–3.5 µm. Conidia: filiform, straight, hyaline, 12–18 × 0.4–0.9 µm.

Etymology.—The epithet 'terrenoides' refers to the earthy color of the thallus.

Spot tests.—K–, C–, KC–, I–, P–.

TLC.—no substance detected.

Additional specimens examined:—**PAKISTAN. Balochistan:** District Killa Saifullah, Muslim Bagh, 30°50'N, 67°44'25"E, 1787 m a.s.l., on rocks, 18 Aug 2022, Alla Ud Din, MB-32, MB-40, MB-14, MB-21 & MB-45 (LAH37682, LAH37684, LAH37681, LAH37680, & LAH37683), (ITS GenBank accession numbers OQ376375, OQ376374, OQ359391, OQ359392, OQ376376).

Distribution.—*Circinaria terrenoides* sp. nov. is reported here for the first time from Darel Valley (Gilgit Baltistan) and Killa Saifullah (Balochistan).

Discussion.—*Circinaria terrenoides* sp. nov. is distinguished morphologically from the phylogenetically closest species, *C. affinis* (Eversm.) Sohrabi, in having a crustose areolate to rimose-areolate thallus (vs. subfruticose, verrucose-areolate), earthy brown to greenish gray upper surface (vs. earthy gray to gray-green), smaller conidia 12–18 × 0.4–0.9 µm (vs. 16–20 × 1–1.2 µm), black pycnidia (vs. black to brownish) and its substrate, calcareous sedimentary rock (vs. stony steppes) (Sohrabi et al. 2013). The new species differs from *C. fruticulosa* (Eversm.) Sohrabi by having an areolate to rimose-areolate thallus (vs. vagrant, subfruticose), earthy brown to greenish gray upper surface (vs. blackish olive, grayish brown, earthy, sometimes rusty red), frequent, immersed pycnidia (vs. rare, usually at tips of branchlets), larger conidia 12–18 × 0.4–0.9 µm (vs. 8–12 × 1–1.3 µm) and as substrate calcareous sedimentary rock (vs. loamy or salty soil) (Sohrabi et al. 2013).



FIG. 2. *Circinaria terrenoides* sp. nov. (HOLOTYPE: LAH37685). Crustose thallus.

The new taxon shows resemblance with *C. hispida* (Mereschk.) A. Nordin, Savić, & Tibell, both have absence of apothecia and presence of black pycnidia, but can be distinguished by their crustose, saxicolous thallus (vs. subfruticose), earthy brown to greenish gray upper surface (vs. gray to green-gray), larger conidia $12\text{--}18 \times 0.4\text{--}0.9 \mu\text{m}$ (vs. $10\text{--}12 \times 0.8\text{--}1.2 \mu\text{m}$) and as substrate calcareous sedimentary rock (vs. calciferous soil in arid steppe) (Sohrabi et al. 2013). The new taxon differs from *C. arida* Owe-Larss., A. Nordin, & Tibell by its areolate to rimose-areolate thallus (vs. areolate to verrucose), earthy brown to greenish gray upper surface (vs. brown to olive-brown or gray-brown), thick thallus (vs. thin), flat to concave areoles (vs. \pm convex), absence of secondary metabolites (vs. aspicilin) and growing on calcareous sedimentary rock (vs. siliceous rocks) (Owe-Larsson et al. 2011). Our species differs from *C. thorstenii* R. Zulfiqar & Khalid in having earthy brown to greenish gray upper surface (vs. whitish gray), flat to concave areoles (vs. plane to convex), larger conidia $12\text{--}18 \times 0.4\text{--}0.9 \mu\text{m}$ (vs. $11\text{--}15 \times 1 \mu\text{m}$) and growing on calcareous sedimentary rocks (vs. siliceous rocks) (Zulfiqar & Khalid 2023). The new species is distinct from *C. darelensis* M.S. Iqbal & Khalid in having earthy brown to greenish gray upper surface (vs. light brown to grayish-brown), flat to concave areoles (vs. flat to slightly convex), and frequent, immersed pycnidia (vs. absent) (Din et al. 2024).

CONCLUSIONS

In conclusion, we here suggest adding a new species to the genus *Circinaria* from Pakistan, taking into account all of the distinctive morphological and phylogenetic characteristics. The finding of the new species *C. terrenoides* emphasizes the importance of the Darel Valley in Pakistan as a source of lichen biodiversity. This contribution to lichen taxonomy will serve as a foundation for future research in Darel Valley. Several new records and new species have already been reported from this area (Iqbal et al. 2022a; 2023a, b, d; 2024a,b; Iqbal & Khalid 2022b; 2023c; 2024), which evidently merits further attention.

ACKNOWLEDGMENTS

We would like to express our thanks to Usman Qadir and Hassan Mohayyodin for accompanying us on field visits and helping with the collection. We are highly thankful to the anonymous reviewer for his valuable comments and reviewing article that greatly improved the manuscript.

DECLARATIONS

Conflict of interest.—There are no conflicts of interest for the authors of this study.

FUNDING

The authors received no funding from any organization for the work they submitted.

REFERENCES

- ALTSCHUL, S.F., W. GISH, & W. MILLER. 1990. Basic local alignment search tool. *J. Molec. Biol.* 215:403–410.
- APTROOT, A. & S.H. IQBAL. 2010. Annotated checklist of the lichens of Pakistan, with reports of new records. *Herzogia* 25:211–229.
- CANNON, P., A. NORDIN, B. COPPINS, A. APTROOT, N. SANDERSON, & J. SIMKIN. (2023). Pertusariales: Megasporaceae, including the genera *Aspicilia*, *Aspiciliella*, *Circinaria*, *Lobothallia*, *Megasporea* and *Sagedia*. *Revisions of British and Irish lichens* 34:1–15.
- DIN, A.U., M.S. IQBAL, A.N. KHALID, & A.R. NIAZI. 2024. *Chlorangium ahmadii* sp. nov. and *Circinaria darelensis* sp. nov. two new species of lichenized Ascomycetes from Pakistan. *Pl. Syst. Evol.* 310:13.
- GARDES, M., & T.D. BRUNS. 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molec. Ecol.* 2(2):113–118.
- GOB & UNICEF. 2011. District development profile of Killa Saifullah, Government of Balochistan (Planning and Development Department) and United Nations Children's Fund (Provincial Office Balochistan), Quetta, Pakistan. Pp. 3–117.
- HALL, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In: *Nucleic acids symposium series* 41:95–98.
- IQBAL, M.S., A. ASHRAF, H.S. ASGHAR, & A.N. KHALID. 2024a. A new species and a new record of the genus *Candelariella* (Candelariaceae, lichenized Ascomycota) from Pakistan. *Nova Hedwigia*, [https://doi: 10.1127/nova_hedwigia/2024/0942](https://doi.org/10.1127/nova_hedwigia/2024/0942)
- IQBAL, M.S., M. NADEEM, A.N. KHALID, & A.R. NIAZI. 2024b. Some new records of lichens from Pakistan. *Nova Hedwigia*, [https://doi: 10.1127/nova_hedwigia/2024/0975](https://doi.org/10.1127/nova_hedwigia/2024/0975)
- IQBAL, M.S. & A.N. KHALID. 2024. *Diploschistes iqbalii* sp. nov. (Ascomycota: Graphidaceae), a new lichen species from Darel Valley, Gilgit Baltistan, Pakistan. *Nova Hedwigia*, [https://doi: 10.1127/nova_hedwigia/2024/0936](https://doi.org/10.1127/nova_hedwigia/2024/0936)
- IQBAL, M.S., M. USMAN, K. HABIB, & A.N. KHALID. 2023a. *Oxneriaria pakistanica* sp. nov. (Megasporaceae, Pertusariales, Ascomycota) from Darel Valley, Gilgit Baltistan, Pakistan. *Phytotaxa* 579:125–131. <https://doi.org/10.11646/phytotaxa.579.2.6>
- IQBAL, M.S., H.S. ASGHAR, & A.N. KHALID. 2023b. A new species and a new record of family Ramalinaceae (Lichenized Ascomycetes) from Pakistan. *Biol. Bull.* 50:1187–1194. <https://doi.org/10.1134/S1062359023602203>
- IQBAL, M.S. & A.N. KHALID. 2023c. *Rusavskia dasanensis* (Teloschistaceae), a new record for Pakistan. *Lindbergia* 2023(1). <https://doi.org/10.25227/linbg.025179>
- IQBAL, M.S., I. FAYYAZ, N.U.S. AFSHAN, F. IFTIKHAR, & A.N. KHALID. 2023d. A new species of *Circinaria* (Pertusariales, Megasporaceae) from Pakistan. *Bot. Serbica* 47:235–239. <https://doi.org/10.2298/BOTSERB2302235I>
- IQBAL, M.S., M.Z. ABBAS, & A.N. KHALID. 2022a. A new species of lichen genus *Acarospora* (Acarosporaceae, Lichen Forming Ascomycota) from Darel Valley, Gilgit Baltistan, Pakistan. *Biol. Bull.* 49:545–550. <https://doi.org/10.1134/S1062359022150110>
- IQBAL, M.S. & A.N. KHALID. 2022b. *Acarospora sultanii* sp. nov. (Acarosporaceae, Lichen Forming Ascomycota) from Darel Valley, Gilgit Baltistan, Pakistan. *Biol. Bull.* 49:540–544. <https://doi.org/10.1134/S1062359022150122>
- KATO, K. & D.M. STANDLEY. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molec. Biol. Evol.* 30:772–780.

- KHAN, M.Z., B. KHAN, S. AWAN, G. KHAN, & R. ALI. 2013. High-altitude rangelands and their interfaces in Gilgit-Baltistan, Pakistan: Current status and management strategies. In High-altitude rangelands and their interfaces in the Hindu Kush Himalayas. International Centre for Integrated Mountain Development, Nepal, India. Pp. 66–77.
- ORANGE, A., P.W. JAMES, & F.J. WHITE. 2010. Microchemical methods for the identification of lichens. British Lichen Society, London, UK.
- OWE-LARSSON, B., A. NORDIN, L. TIBELL, & M. SOHRABI. 2011. *Circinaria arida* sp. nova and the '*Aspicilia desertorum*' complex. *Biblioth. Lichenol.* 106:235–246.
- RIAZ, S., M. NADEEM, M.S. IQBAL, A.R. NIAZI, & A.N. KHALID. 2024. *Circinaria russus* sp. nov. (Lichenized Ascomycota: Megasporaceae) from Pakistan. *Biol. Bull.* 50:1–6.
- SOHRABI, M. 2012. Taxonomy and phylogeny of the manna lichens and allied species (Megasporaceae). PhD Thesis, Univ. Helsinki, Finland.
- SOHRABI, M., S. STENROOS, L. MYLLYS, U. SØCHTING, T. AHTI, & J. HYVÖNEN. 2013. Phylogeny and taxonomy of the 'manna lichens'. *Mycol. Progr.* 12:231–269.
- TAMURA, K., G. STECHER, D. PETERSON, A. FILIPSKI, & S. KUMAR. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molec. Biol. Evol.* 30(12):2725–2729.
- VOYTAS, D. 2000. Agarose gel electrophoresis. *Curr. Protoc. Molec. Biol.* 51:2–5.
- WHITE, T.J, T. BRUNS, S.J.W.T. LEE, & J. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols. Academic Press, Cambridge, MA, U.S.A. Pp. 315–322.
- ZULFIQAR, R. & A.N. KHALID. 2023. A novel saxicolous species of *Circinaria* (Megasporaceae, Ascomycota) from Pakistan. *Pl. Syst. Evol.* 309:21.