



Two new species of *Lecaimmeria* (lichenized *Ascomycota*, *Lecideaceae*) from Asia

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Abstract

Two new species of the lichen genus *Lecaimmeria* are described and illustrated from Pakistan, namely, *Lecaimmeria asiatica* and *L. crispula*. The former taxon is characterized by pale brown to dark brown thallus, up to 2-mm large areoles, flat to strongly convex apothecial disc, taller hymenium 120–150 µm, apically branched, not anastomosing paraphyses, large conidia 5–7 × 1.1–1.4 µm, with respect to ecology, and the absence of secondary metabolites, whereas *L. crispula* can be characterized by having a light brown to chocolate brown thallus, apothecia having flat to slightly concave discs with wavy margins that curl inward, a tall hymenium 150–180 µm, branched and anastomosing paraphyses, large ascospores 25–33 × 12–17 µm, and absence of secondary metabolites. A phylogenetic analysis is provided based on nrITS and nrLSU sequences that support the separation of both novel species. Comparative analyses with related species of *Lecaimmeria* are provided to confirm the status of these species.

Keywords Khyber Pakhtunkhwa · Phylogeny · Taxonomy · *Lecideaceae* · Lichens · Sharan

Introduction

The lichen genus *Lecaimmeria* C.M. Xie, Lu L. Zhang & Li S. Wang has recently been erected and segregated from *Immersaria* Rambold & Pietschm., using multigene phylogenetic analysis (nrITS, nrLSU, *RPB1*, *RPB2*, and mtSSU) (Xie et al. 2022). The genus is represented by 11 species from Asia and Europe of which seven species are distributed in alpine areas, high-latitude steppe, or high-altitude desert-steppe areas of China (Xie et al. 2022). Previously, only one species of *Lecaimmeria* was reported from Pakistan, viz., *L. pakistanica* K.Habib, R.Zulfiqar & Khalid (Habib et al. 2022).

Lecaimmeria is characterized by its waxy glossy, orange or red-brown thallus with an amyloid medulla, immersed apothecia with a crypto-thalline margin, orange

epihymenium, thallus with an epinecral layer, and its *Porpidia*-type asci with eight halonate, non-amyloid ascospores (Xie et al. 2022). Species of the genus frequently grow on granite or sandstone, with the exception of one species, *L. tuberculosa* C.M. Xie & Xin Y. Wang, which invades on jade.

In this paper, we describe two new species of *Lecaimmeria*, *L. asiatica* H.S Asghar, Oryakhil, Naseer & Khalid and *L. crispula* Niazi, Nadeem & Afshan sp. nov. *Lecaimmeria asiatica* grows on calcareous rock in the dry temperate pine forests of the Himalaya, whereas *L. crispula* grows in moist temperate forest, near a waterfall on enriched siliceous rock in Khyber Pakhtunkhwa Province. We present a brief diagnosis, an extensive description, illustrations, and a phylogenetic analysis based on two loci.

Materials and methods

Morphological and chemical studies

Collections were made during a lichen survey of Chitral and Kaghan Valley (Khyber Pakhtunkhwa) Pakistan in 2021–2022 and from Afghanistan in 2022. The macro- and

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micro-morphology of the specimens was examined using a stereomicroscope (Meiji Techno, EMZ-5TR, Japan) and a compound microscope (SWIFT M4000-D) with a 9MP camera system, respectively. For anatomical investigation, hand-made sections of apothecia were examined in water and KOH (10%). A minimum of twenty measurements in water were made for each diagnostic feature from four specimens. The collected specimens were deposited in the herbarium of the Institute of Botany, University of the Punjab, Lahore (LAH). The secondary chemistry was analyzed by spot tests with KOH (10%; K) and sodium hypochlorite solution (C) and Thin Layer Chromatography with the Solvent System C following standard methods (Orange et al. 2001).

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted directly from a portion of thallus with apothecia from each specimen using slightly modified 2% CTAB method (Gardes and Bruns 1993). The ITS region (Internal Transcribed Spacer of the nrDNA) was amplified using the primer pair ITS1F (forward primer) (Gardes and Bruns 1993) and ITS4 (reverse primer) (White et al. 1990). The nrLSU region was also amplified by using the LROR as forward and LR5 as reverse primer (Vilgalys and Hester 1990) following the amplification protocol of Khan et al. (2018). PCR products were sent to BGI (Beijing Genomics Institute), China, where both strands were sequenced.

Sequences were assembled using BioEdit (Hall 1999). BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) analysis was used for an initial verification of their identities and to retrieve highly similar sequences of the ITS region. The newly generated sequences and additional sequences retrieved from GenBank were used in an initial alignment, which was then trimmed and realigned using web-PRANK with default settings (Löytynoja and 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 Portal, maximum likelihood analysis (ML) was also implemented (Stamatakis 2014), using 1000 bootstraps for rapid bootstrapping. FigTree v 1.4.3 (Rambaut 2014) was used for displaying the phylogeny reconstruction from the ML analysis.

Results

Phylogenetic analyses

The final dataset of ITS consisted of 35 sequences including *Poeltidea perusta* (Nyl.) Hertel & Hafellner (MK620112) as an outgroup (Xie et al. 2022) (see Table 1 for voucher details). The aligned ITS1-5.8S-ITS2 region comprised 569

sites, of which 378 were conserved and 179 variable; 156 were parsimony-informative (Fig. 1). *Lecaimmeria asiatica* formed a separate independent clade outside a group composed of *L. qinghaiensis* C.M. Xie and Li S. Wang and *L. tuberculosa* C.M. Xie and Xin Y. Wang. The analysis confirms the distinctness of the new taxon from other species. The sequences of *Lecaimmeria crispula* were recovered in a clade together with *L. mongolica* C.M. Xie & Lu L. Zhang, *L. tuberculosa* C.M. Xie & Xin Y. Wang, and *L. qinghaiensis* C.M. Xie & Li S. Wang demonstrating their status as independent species.

The nrLSU final dataset is composed of 18 sequences including *Romjularia lurida* (Ach.) Timdal (KF683107) as an outgroup (Xie et al. 2022). In the tree resulting from analysis of the LSU region (Fig. 2), *L. crispula* formed a well-supported lineage sister to *L. mongolica* C.M. Xie & Lu L. Zhang. The molecular phylogenetic analysis strongly supported the taxonomic delimitation of the new species.

Taxonomy

Lecaimmeria asiatica H.S Asghar, Oryakhil, Naseer & Khalid, sp. nov. (Fig. 3A–E).

Mycobank number **MB850362**.

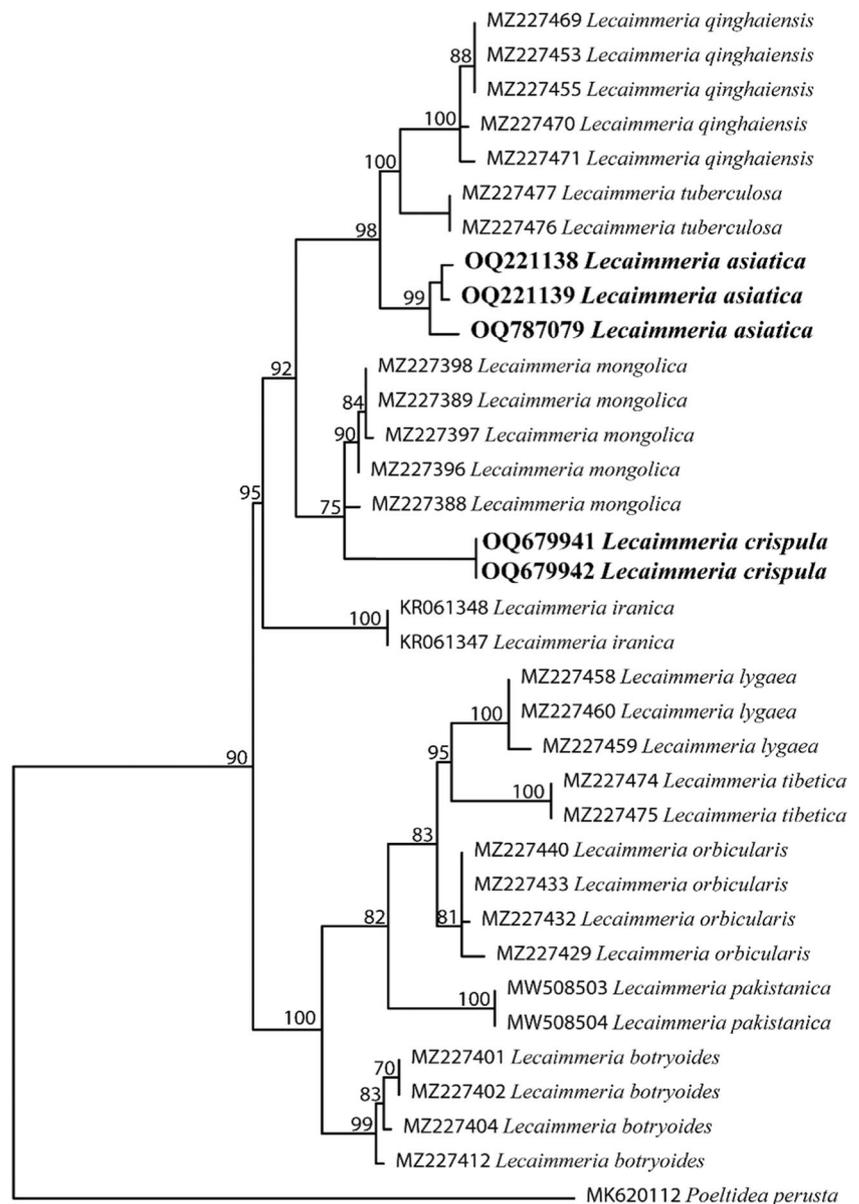
Holotype: Pakistan, Khyber Pakhtunkhwa, Chitral, Garam Chashma (35° 59' 50" N and 71° 33' 50" E), 2550 m a.s.l., on calcareous rocks, November 14, 2021, M. Usman & G. Qadir. (GC06B) (LAH38008-Holotype) (GenBank accession number OQ221138) and Isotype (GC09, LAH38009) (GenBank accession number OQ221139).

Etymology: The specific epithet “*asiatica*” refers to the Asian continent where the species have been found.

Diagnosis: Distinguished from the morphologically analogous species, *L. qinghaiensis*, by its pale brown to dark brown thallus, up to 2-mm large areoles, flat to strongly convex apothecial disc, taller hymenium 120–150 µm, apically branched paraphyses and large conidia 5–7 × 1.1–1.4 µm and absence of secondary metabolites.

Description: Thallus: crustose, areolate, c. 7 cm wide, in Sect. 220–270 µm thick, upper surface pale brown to dark brown, becomes greenish when wet. **Areoles** separate, flat to weakly convex, irregular/angular, densely pruinose near the margin, without fissures, 0.5–2 mm across, often with whitish margins/rim. **Prothallus:** visible between areoles, black **Cortex:** 2-layered, ca 40–60 µm thick, paraplectenchymatous, cells 8–12 µm in diam., upper layer dark brown, 15–20 µm thick, lower layer hyaline, 25–35 µm thick, epinecral layer distinct, up to 15 µm high. **Algal layer:** 85–105 µm thick, chlorococcus, cells globose to subglobose, 10–20 µm in diam. **Medulla:** white, hyphal bundle hyaline. **Apothecia:** lecanorine, 1–3 per areole, immersed initially slightly raised when mature surrounded by the areole, becoming confluent. **Disc:** contiguous to separate, flat to strongly convex,

Fig. 1 Phylogenetic relationships of *Lecaimmeria* spp. based on a maximum likelihood analysis of the ITS region. Sequences from Pakistan are in bold



0.03

chocolate brown, rounded at first becoming irregular, sometimes surrounded by a white rim, 0.5–1.7 mm in diam., epruinose. **Proper exciple:** thin, poorly differentiated, reduced, hyaline, 10–25 μm thick. **Epihymenium:** 30–50 μm brown. **Hymenium:** hyaline, 120–150 μm tall. **Paraphyses:** apically branched, not anastomosing, 1–3 μm wide, apical slightly swollen, apices 2–4 μm wide. **Hypothecium:** 60–19 μm tall, light brown, containing algal cells. **Asci:** *Porpidia*-type, clavate, 60–90 \times 15–20 μm , 8-spored. **Ascospores:** hyaline, ellipsoid, 10–15 \times 5–8 μm . **Conidiomata:** frequent,

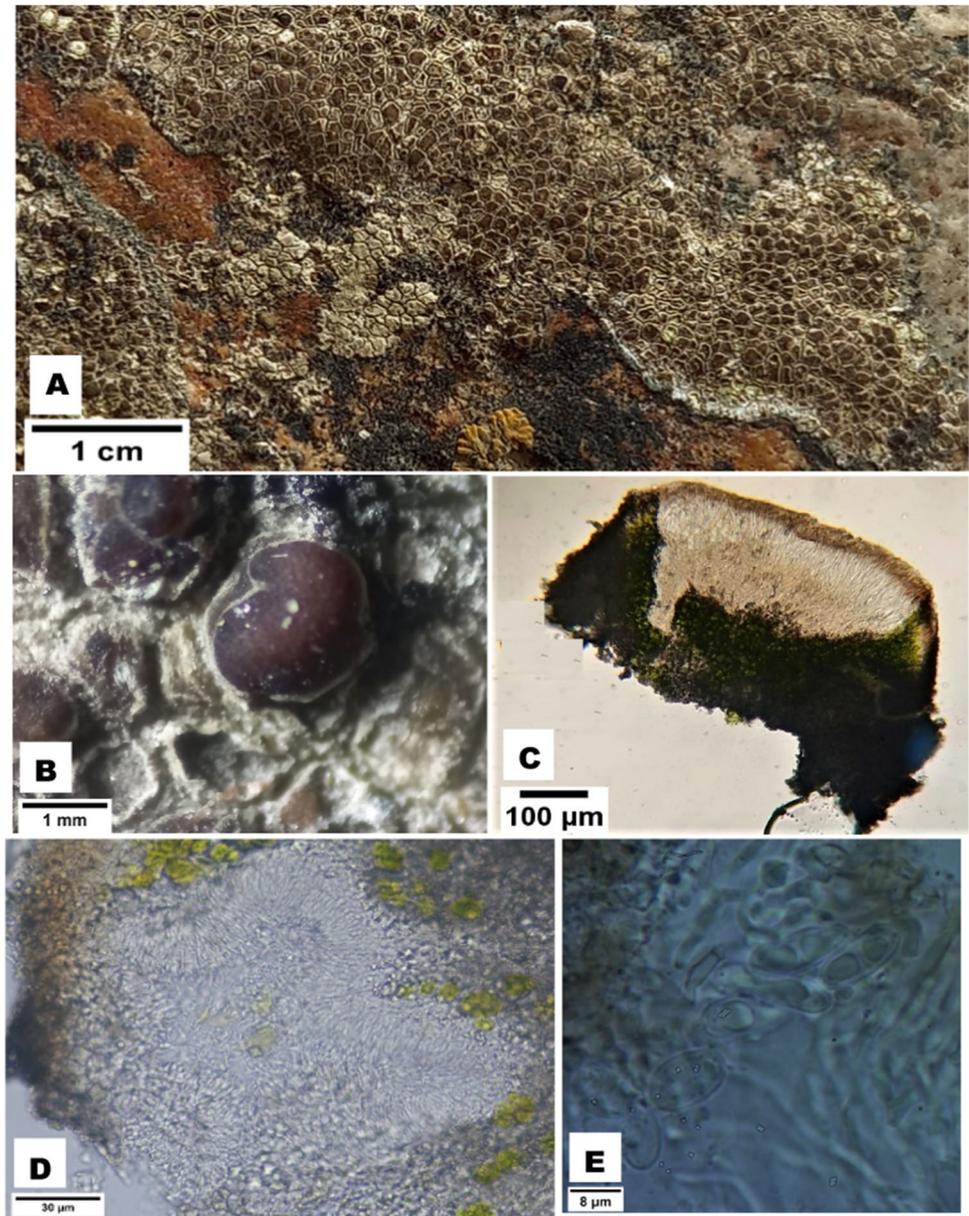
immersed, linear, rarely tuberculiform, black, margin pruinose. **Conidia** 5–7 \times 1.1–1.4 μm , bacilliform.

Chemistry: thallus K–, C–, KC–, no lichen substance detected by TLC.

Additional specimen examined (paratype): Afghanistan: Kabul, Paghman, at 1800 m a.s.l., 34.543896° N and 69.160652° E; on rock; 14 June 2022, Naqibullah, PGM-3D (LAH38007) (GenBank accession number OQ787079).

Ecology: Saxicolous on calcareous rocks in a dry temperate climate at an altitude of 2100–2550 m in

Fig. 3 *Lecaimmeria asiatica* sp. nov. (-LAH38008-Holotype). **A** Thallus. **B** Apothecia. **C** Apothecium section. **D** Pycnidium section with conidia. **E** Ascospores



150–180 µm tall. **Paraphyses** apically branched, anastomosing, 1.5–2.5 µm wide, apically slightly swollen, apices 3–4 µm wide. **Hypothecium**: 70–120 µm tall, hyaline. **Asci**: *Porpidia*-type, clavate, 90–150 × 30–45 µm, amyloid, 8-spored. **Ascospores**: hyaline, ellipsoid to broadly ellipsoid, 25–33 × 12–17 µm. **Conidiomata** not found. **Conidia** not observed.

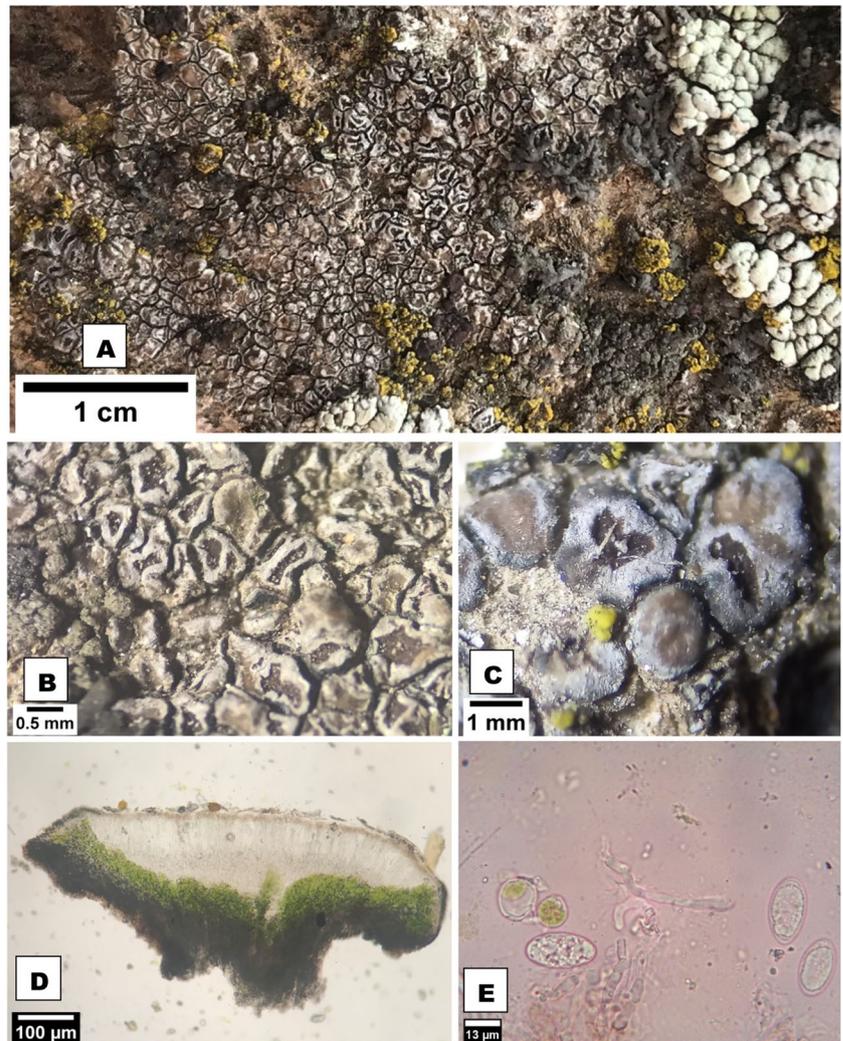
Chemistry: cortex and medulla K–, C–, KC–; medulla IKI + blue; no lichen substance detected by TLC.

Additional specimen examined: Pakistan, Khyber Pakhtunkhwa, Kaghan Valley (Babusar Pass) (35.1462° N, 74.0482° E), 4155 m a.s.l., on siliceous rock; August 24, 2022, M. Nadeem, A. R. Niazi & N. S. Afshan (LAH37799), ITS (GenBank accession OQ679942).

Ecology: Growing on rain and sun exposed siliceous rocks in a humid continental climate, at an altitude of 4170 m a.s.l., hilly topography, which receive heavy snowfall in winters, with maximum and minimum temperature of 15 °C and – 8 °C, respectively, annual rainfall 893 mm.

Comments: During current explorations of lichens from Kaghan Valley in Pakistan, we observed several specimens that could not be readily assigned to any known species. A morpho-anatomical and phylogenetic analysis of the ITS and nrLSU regions confirmed their position within the genus *Lecaimmeria* and showed their distinctness from other known species of the genus. We therefore describe these specimens as a new species, *Lecaimmeria crispula*.

Fig. 4 *Lecaimmeria crispula* (LAH37798-Holotype). **A, B** Crustose thallus with abundant apothecia. **C** Apothecia with curled margins. **D** Cross section of apothecium. **E** Ascospores



Lecaimmeria crispula is superficially similar to *L. mongolica* (Xie et al. 2022). The comparison of the new species with its close relatives is presented in Table 2. Our species can be distinguished from *Lecaimmeria mongolica* in having light brown to chocolate brown thallus (vs. orange thallus), larger ascospores $25.0\text{--}33.0 \times 12.0\text{--}17.0 \mu\text{m}$ (vs. small $10.0\text{--}17.5 \times 6.0\text{--}7.5 \mu\text{m}$), and absence of secondary metabolites (vs. gyrophoric acid) (Xie et al. 2022).

Another phylogenetically close taxon, *Lecaimmeria tuberculosa*, differs from the new taxon in having a red-brown thallus (vs. light brown to chocolate brown), small areoles $0.5\text{--}1.3 \text{ mm}$ (vs. large areoles 2 mm across), small apothecia $0.3\text{--}0.6 \text{ mm}$ in diameter (vs. large 1 mm), smaller ascospores $6.0\text{--}12.5 \times 3.0\text{--}5.0 \mu\text{m}$ (vs. larger $25\text{--}33 \times 12\text{--}17 \mu\text{m}$), and paraphyses are unbranched and not anastomosing (vs. apically branched and anastomosing) (Xie et al. 2022).

The new taxon differs from *Lecaimmeria qinghaiensis* in having light brown to chocolate brown thallus (vs. yellow thallus), larger areoles up to 0.2 mm (vs. smaller areoles $0.5\text{--}1.5 \text{ mm}$), taller hymenium $70\text{--}120 \mu\text{m}$ (vs. $52\text{--}63 \mu\text{m}$), and larger ascospores $25.0\text{--}33.0 \times 12.0\text{--}17.0 \mu\text{m}$ (vs. small $10.0\text{--}17.5 \times 6.0\text{--}7.5 \mu\text{m}$) (Xie et al. 2022).

Conclusions

Two novel species based on nrITS and nrLSU sequences support the separation of the species from already reported species of the genus named *Lecaimmeria asiatica* and *L. crispula*. Further molecular data on the Asian members of genus *Lecaimmeria* as well as data on several new monophyletic branches including Asian representatives of the *Lecideaceae* will be presented elsewhere in the nearest future.

Appendix

Table 1 Sequences used in the ITS phylogenetic analysis of *Lecaimmeria* species. New sequences are in bold

Name of species	Country/origin	Voucher specimen	GenBank accession number (nrITS)	GenBank accession number (nrLSU)
<i>Lecaimmeria botryoides</i>	China: Qinghai	KUN 20–66765	MZ227412	MZ227053
<i>Lecaimmeria botryoides</i>	China: Sichuan	KUN 20–66711	MZ227404	MZ227048
<i>Lecaimmeria botryoides</i>	China: Sichuan	KUN 20–66706	MZ227401	MZ227046
<i>Lecaimmeria botryoides</i>	China: Sichuan	KUN 20–66707	MZ227402	MZ227047
<i>Lecaimmeria asiatica</i> sp. nov.	Pakistan	LAH38008	OQ221138	–
<i>Lecaimmeria asiatica</i> sp. nov.	Pakistan	LAH38009	OQ221139	–
<i>Lecaimmeria asiatica</i> sp. nov.	Afghanistan	LAH38007	OQ787079	–
<i>Lecaimmeria crispula</i> sp. nov.	Pakistan	LAH37798	OQ679941	OQ679940
<i>Lecaimmeria crispula</i> sp. nov.	Pakistan	LAH37799	OQ679942	–
<i>Lecaimmeria iranica</i>	China	SDNU20117623	KR061348	–
<i>Lecaimmeria iranica</i>	China	SDNU20117663	KR061347	–
<i>Lecaimmeria lygaea</i>	China: Tibet	KUN 20–69054	MZ227458	MZ227075
<i>Lecaimmeria lygaea</i>	China: Tibet	KUN 20–69070	MZ227459	MZ227076
<i>Lecaimmeria lygaea</i>	China: Tibet	KUN 20–69072	MZ227460	MZ227077
<i>Lecaimmeria mongolica</i>	China: Inner Mongolia	SDNU20190350	MZ227388	MZ227037
<i>Lecaimmeria mongolica</i>	China: Inner Mongolia	SDNU20117851	MZ227396	–
<i>Lecaimmeria mongolica</i>	China: Inner Mongolia	SDNU20117613	MZ227397	–
<i>Lecaimmeria mongolica</i>	China: Inner Mongolia	SDNU20190354	MZ227389	MZ227038
<i>Lecaimmeria mongolica</i>	China: Inner Mongolia	SDNU20117399	MZ227398	–
<i>Lecaimmeria orbicularis</i>	China: Qinghai	KUN 20–66935	MZ227440	MZ227071
<i>Lecaimmeria orbicularis</i>	China: Qinghai	KUN 20–66899	MZ227433	MZ227066
<i>Lecaimmeria pakistanica</i>	Pakistan	LAH-36674	MW508503	–
<i>Lecaimmeria pakistanica</i>	Pakistan	LAH-36675	MW508504	–
<i>Lecaimmeria qinghaiensis</i>	China: Qinghai	KUN 20–3127	MZ227471	–
<i>Lecaimmeria qinghaiensis</i>	China: Qinghai	KUN 20–3115	MZ227470	–
<i>Lecaimmeria qinghaiensis</i>	China: Qinghai	KUN 20–68687	MZ227453	–
<i>Lecaimmeria qinghaiensis</i>	China: Qinghai	KUN 20–68698	MZ227455	–
<i>Lecaimmeria qinghaiensis</i>	China: Qinghai	KUN 20–849	MZ227469	MZ227078
<i>Lecaimmeria tibetica</i>	China: Tibet	KUN XY19-1288i	MZ227474	MZ227039
<i>Lecaimmeria tibetica</i>	China: Tibet	KUN XY19-1288A	MZ227475	MZ227040
<i>Lecaimmeria tuberculosa</i>	China: Gansu	KUN 18–58856	MZ227476	MZ227041
<i>Lecaimmeria tuberculosa</i>	China: Gansu	KUN 18–58857	MZ227477	MZ227042
<i>Poeltidea perusta</i>	Chile	UR00039	MK620112	–
<i>Romjularia lurida</i>	Spain	S. Pérez-Ortega 1372	–	KF683107

Table 2 Comparison of characters of some *Lecanimeria* species

Characters	<i>L. asiatica</i> sp. nov.	<i>L. crispata</i> sp. nov.	<i>L. qinghaiensis</i>	<i>L. tuberculosa</i>	<i>L. mongolica</i>	<i>L. botryoides</i>	<i>L. pakistanica</i>
Prothallus	Distinct, black	Black, distinct	Distinct black	Indistinct	Not distinct, black	Distinct, black	Distinct black
Thallus (color)	Pale brown to dark brown	Light brown to chocolate brown	Yellow–brown, rusty	Red–brown	Orange	Red–brown	Yellow brown
Areole size (mm)	Up to 2	Up to 2 mm across	0.5–1.5 across	0.5–1.3 across	0.4–0.8 across	0.2–1.0	Up to 1.5 across
Apothecium disc size (mm) and shape	0.5–1.7	Up to 1.5	0.2–1.3	0.3–0.6	0.2–0.8	0.2–1.3	Up to 0.8
Hymenium (μm)	Flat to strongly convex	Flat to slightly concave	Flat	Concave	Flat to slightly convex	Flat or concave	Flat to concave
Paraphyses	120–150 tall	150–180	52.0–63.0	55–83	62.0–83.0	67.0–100.0	130–160
Size of ascospores (μm) and shape	Apically branched, not anastomosing	Apically branched, anastomosing	Unbranched, not anastomosing	Unbranched, not anastomosing	Unbranched, not anastomosing	Only branched at the top, not anastomosing	Apically branched, anastomosing
Conidiomata	10–15 \times 5–8	25–33 \times 12–17 μm	8.0–15.0 \times 5.0–7.5	6.0–12.5 \times 3.0–5.0	10.0–17.5 \times 6.0–7.5	7.5–8.0 \times 4.0–6.0	20–32 \times 10–16
Conidia (μm)	Distinctly halonate	Not halonate	Not distinctly halonate	Halonate	Distinctly halonate	Halonate	Not halonate
Chemistry	Frequent, linear rarely tuberculiform	Absent	Rare stellate, graphioid once mature	Stellate, tuberculiform	Oblate, rarely ellipsoid	Absent	Absent
Substrate	5–7 \times 1.1–1.4	Not observed	Not seen	3.0–4.5 \times 1.0	5.0 \times 1.0	Absent	Absent
References	No substance	No substance	No substance	No substances	Gyrophoric acid	Gyrophoric acid	No substance
	Calcareous rock	Siliceous rock	Sandstone	Sandstone	Granite	Granite	Limestone
	This paper	This paper	(Xie et al. 2022)	(Xie et al. 2022)	(Xie et al. 2022)	(Xie et al. 2022)	(Habib et al. 2022)

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11557-024-01954-4>.

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Author contribution All authors contributed to this study's conception and design. Material preparation, data collection, and analysis were performed by Hafiza Simab Asghar, Iram Fayyaz, and Najam-ul-Sehar Afshan. The first draft of the manuscript was written by Hafiza Simab Asghar, and all authors commented on previous versions of the manuscript. Dr. Abdul Rehman Khan Niazi and Muhammad Nadeem also helped in the collection of the specimens from Pakistan that are used in this study. Naqibullah Oryakhil and Arooj Naseer helped in the collection from another site that is Afghanistan. Iram Fayyaz helped in the molecular analysis of this manuscript. Prof. Dr. Abdul Nasir Khalid supervised and validated the manuscript. All authors read and approved the final manuscript.

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Data availability Samples analyzed during this study have 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-334248940395482/>). Sequences generated during this study have been deposited in NCBI, GenBank data repository (<https://www.ncbi.nlm.nih.gov/genbank/>). For list of accession numbers and voucher information, please see Appendix Table 2. All the alignments of phylogenies are available as supplementary information.

Declarations

Competing interests The authors declare no competing interests.

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