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Original article

A new species of *Lecidella* (Lecanorales, Ascomycota) from Azad Jammu and Kashmir, Pakistan

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ABSTRACT

The newly described species, *Lecidella iqbalii* sp. nov., is so far only known from Azad Jammu and Kashmir, Pakistan. A comparative morpho–anatomical study combined with a phylogenetic analysis based on the marker ITS placed this species together with two accessions from southern South America in a distinct and highly supported group at the base of the *Lecidella stigmatea* clade. The taxon is characterized by a shiny thallus, strongly convex apothecia, a thin excipulum, a dark brown epihymenium and relatively small ascospores (10-) 11-12 (-13) × (6-) 6.5-8(-9) µm.

Keywords: crustose lichen, Ganga choti, Garhi dupatta, saxicolous, lichenized ascomycetes

Introduction

The western Himalayan region of Azad Jammu and Kashmir (AJ&K) extends from 73-75°E longitude and 33-36°N latitude. The mainly mountainous landscape is characterized by fertile, green, rocky, and undulating territory (Dar *et al.* 2012). The resulting diverse environmental conditions would suggest high lichen diversity. However, little is known about how diverse the lichen flora actually is in these regions, because there has been very little exploration (Ahmad *et al.* 1997; Aptroot & Iqbal 2012).

One of the more common and widely distributed crustose lichen genera is *Lecidella* (Lecanoraceae), established by Körber (1855) and comprising about 80 species (Kirk *et al.* 2008). This genus is mainly characterized by black lecideine apothecia with a persistent proper excipulum and *Lecidella*-type asci (Hafellner 1984). It is widely distributed - from the tropics to polar regions - and occurs on various substrata, including rock, detritus, bark, wood and mosses (Zhang *et al.* 2012). This group of lichenized ascomycetous fungi is usually regarded as taxonomically difficult due to a high degree of variation in morphological characters (Hertel 1984). In such cases, the support of molecular tools has played a significant role in systematics and species delimitation (Zhao *et al.* 2015).

So far, six *Lecidella* species have been reported from Pakistan: *Lecidella carpathica*, *L. euphoria*, *L. patavina*, *L. pulveracea*, *L. stigmatea* and *L. tumidula* (Zulfiqar *et al.* 2020).

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As part of the study of the lichen flora of Azad Jammu and Kashmir, Pakistan, a new species of *Lecidella* was described using morphological and molecular methods.

Materials and methods

Collection and preservation

Study site: Two specimens of the genus *Lecidella* were collected from Azad Jammu and Kashmir, Pakistan in 2020 during fieldwork focused on an increasing floristic knowledge of the lichens of Pakistan. The Azad Jammu & Kashmir state falls within the Himalayan orogenic belt and the topography of northern districts (Neelum, Muzaffarabad, Hattian, Bagh, Haveli, Poonch, and Sudhnoti) is mainly hilly and mountainous with deep ravines, and rugged and undulating terrain, whereas the southern districts (Kotli, Mirpur and Bhimber) are of comparatively low relief (Abasi *et al.* 2019). The collected specimens were deposited in the herbarium of the Institute of Botany, University of the Punjab, Lahore (LAH).

Morphological characterization

The specimens were examined macro and micromorphologically under a stereomicroscope (Meiji Techno, EMZ-5TR, Japan) and compound microscope (SWIFT M4000-D) with a 9MP camera system, respectively. For anatomical investigation, sections of apothecia were made by hand and examined in water, KOH (10%) and Lugol's iodine solution (50%). A minimum of twenty measurements in water were made for each diagnostic feature from the two samples.

Chemical characterization

The secondary chemistry was analyzed using spot tests, which were performed using KOH (10% K), Lugol's iodine solution (50% I) and sodium hypochlorite solution (C). Thin Layer Chromatography was carried out using Solvent System G, following standard methods (Orange *et al*. 2010).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted directly from the center of the thallus with apothecia from each specimen using a modified 2% CTAB method (Gardes & Bruns 1993). The internal transcribed spacer region of the nuclear ribosomal DNA (ITS) was amplified using the primers ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al*. 1990) following the amplification protocol of Khan *et al.* (2018). PCR products were sent to Tsingke, China for sequencing.

The sequences of the marker ITS were assembled and edited using Geneious Pro 6.1.8 (www.geneious.com), aligned with MAFFT v7.017 (Katoh *et al.* 2002) and placed in context of the most recently published phylogeny of Ruprecht *et al.* (2020) based on the concept of Zhao *et al.* (2015). 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 analysis was implemented (Stamatakis 2014), using rapid bootstrapping with 1000 iterations. The resulting tree was visualised with FigTree v 1.4.3 (Rambaut *et al.* 2014).

Results

Phylogenetic analyses

The final alignment contained 46 sequences of the marker ITS with a length of 536 characters. The tree (Fig. 1) was rooted with species of the genera *Carbonea*, and *Lecanora* (see Tab. 1 for voucher details). According to Zhao *et al.* (2015) and Ruprecht *et al.* (2020), the tree shows four main clades: *Lecidella stigmatea, L. enteroleucella, L. elaeochroma* and *Lecidella* ssp. nov. The two specimens from Pakistan, described below as *L. iqbalii*, are sister to two specimens from southern South America (sSA; MK620141, MK620152), which form together a well supported group basal to the *L. stigmatea* clade.

***Lecidella iqbalii* Fayyaz, Afshan, Niazi & Khalid**
Mycobank number MB842789

Thallus crustose, areolate, whitish to grey, apothecia lecideine, disc strongly convex; asci *Lecidella*-type, ascospores hyaline, non-septate, ellipsoid, ascospores relatively small, (10-) 11-12 (-13) × (6-) 6.5-8(-9) µm.

Type:-Pakistan: Azad Jammu and Kashmir, Garhi Dupatta (34-36 °N, 73-35°E), 817 m a.s.l., on rock, 22 September 2020, N. S. Afshan and A. R. Niazi (JV-08H) (LAH37006-holotype), ITS GenBank accession number OL843364

Thallus: Crustose, well developed, shiny, granulose-rimose, areolate or rimose-areolate to verruculose-areolate, 7-8 cm in diameter, up to 0.1 mm thick, prothallus and pycnidia not found. **Areoles:** Distinct, plane, smooth, angular to irregular in outline, 0.01-0.1 mm in diameter. **Surface colour:** greyish brown to grey. **Cortex:** dark brown, 15-10 µm. **Medulla:** white, 25-30 µm**. Photobiont cells:** cells globose, 10-15 µm. **Apothecia:** frequent, scattered, shiny, lecideine, black, but sometimes becoming reddish when mature, sessile, 0.1-1.2 mm in diameter. **Disc:** black, strongly convex. **Margins:** distinct, concolorous with disc, round to irregular, thin, becoming excluded. **Exciple:** 40-60 µm thick, bluish green to dark brown in section. **Epihymenium:** dark brown, 10-15 µm. **Hymenium:** hyaline to brown, 80-95 µm, not inspersed. **Hypothecium:** hyaline, 75-150 µm K+, N+. **Paraphyses:** aseptate, hyaline, apices usually expanded to 3.0 µm, dark greenish to brown, separate in KOH. **Asci:** *Lecidella*-type, clavate, 8-spored, (40-) 45-50-53 (-55) × (18-) 20-21-22(-24) µm. **Ascospores:** simple, hyaline, thick and smooth walled, ellipsoid to ovoid, (10-) 11-12 (-13) × (6-) 6.5-8(-9) µm (Fig. 2).

Secondary chemistry: Atranorin; spot test: thallus K+, C-, KC-

Habitat and distribution: The only known specimens were collected in the sub-tropical forest of Azad Jammu and Kashmir, Pakistan. The forest is dominated by *Pinus roxburghii*, *Quercus oblongata*, *Quercus glauca* and *Pyrus pashia* and others. The maximum daily temperature of the region is 20 to 30° C during the summer and the average temperature is 4° C during the winter, and there is moderate rainfall. Additionally, the area is a beautiful wonderland in view of its transcendent Himalayan peaks, peaceful lakes, majestic glaciers and waterfalls.

Etymology: The epithet "*iqbalii*" honours the late Pakistani mycologist S.H. Iqbal for his significant contribution to the knowledge of lichen diversity in Pakistan.

Additional specimens examined: PAKISTAN. Azad Jammu and Kashmir, Ganga choti (34-36 °N, 73-35°E), 3, 675 m a.s.l on rock, 25 September 2020, N. S. Afshan and A. R. Niazi (JV-08) (LAH37007) (ITS GenBank accession number OL843365).

Discussion

The species *Lecidella iqbalii* can be distinguished from the two formerly *L. stigmatea* to assigned specimens from southern South America (sSA, UR00090, UR00107; Ruprecht *et al.* 2020) by several morphological characters. The thallus is more areolate, the apothecia are more frequent with a strongly convex disc, a small exciple and a dark brown epihymenium in contrast to the granulose to rimose thallus, dull black and partially flat apothecia, a dark green epihymenium and in one case much smaller ascospores (UR00090). The phylogenetically distinct group formed by the new species and the two specimen from sSA can be distinguished to *L. stigmatea* by smaller ascospores (Wirth *et al.* 2013), to *L. greenii* by a different chemistry (stictic acid chemosyndrome) and by a pale thallus in contrast to the mainly darker pigmented thallus of *L. siplei* (Ruprecht *et al.* 2012) (compare Figs. S1, S2; Text S1, S2; Tab. 2).

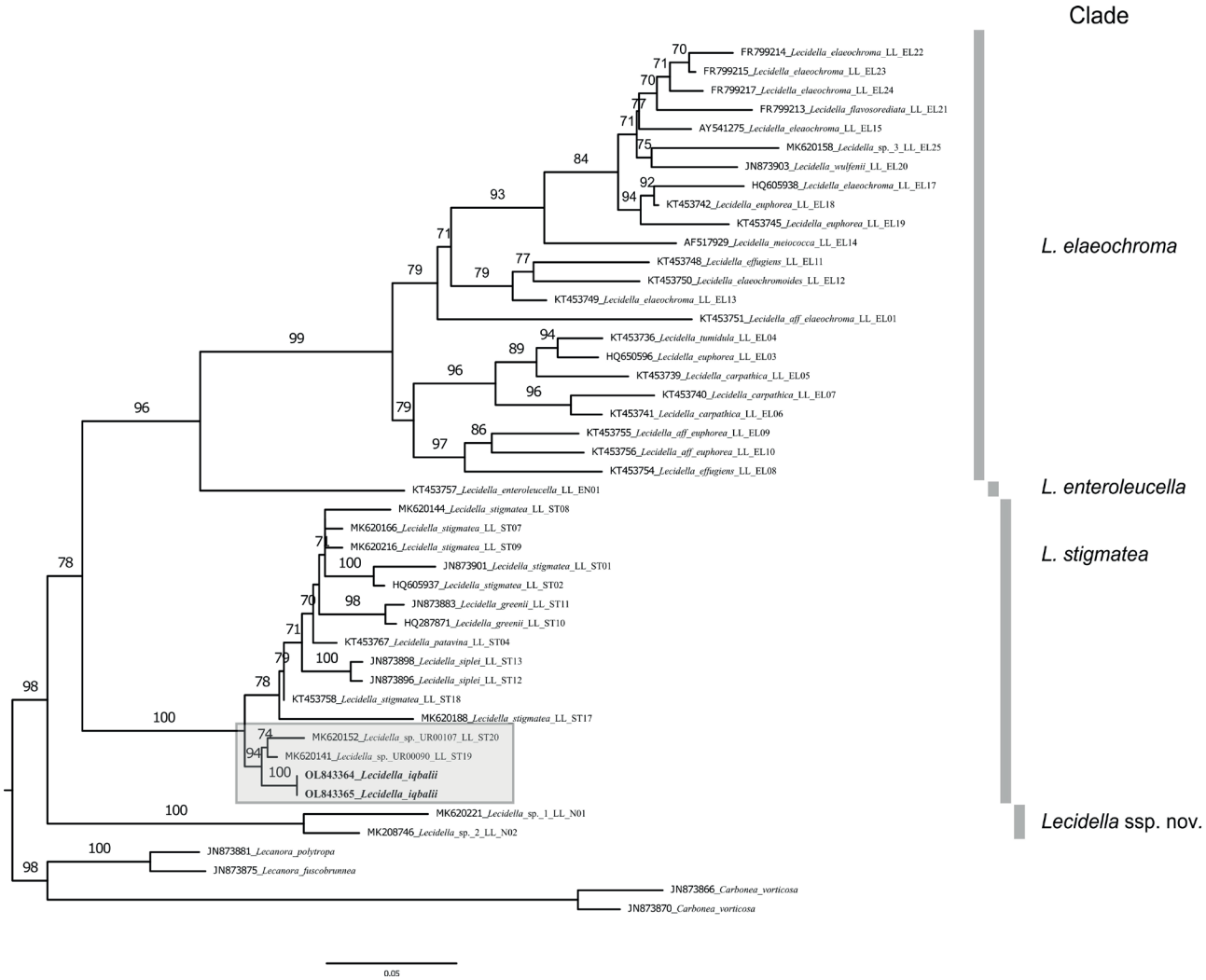

Figure 1. Phylogeny (ML) of the genus *Lecidella* with two accessions of the newly described species *Lecidella iqbalii*, based on single OTUs of the species concepts of Ruprecht *et al.* (2020) and Zhao *et al.* (2015).

Lecidella iqbalii was so far only found in Azad Jammu and Kashmir, Pakistan. It is phylogenetically clearly separated from the two closely related sSA specimens (UR00090, UR00107) which have a distinct morphology to *L. iqbalii* and to each other. However, these two accessions from sSA can be treated at least on subspecies level (Figs. S1, S2; Tab. 2). Unfortunately, there is only one specimen each available and therefore we refrain from describing these accessions as new species.

At this stage of knowledge, it is not viable to make a definite assignment regarding the state of endemism of the new species. Further investigations are required to clarify this unresolved aspect.

Table 1. Specimens used in the ITS phylogenetic analyses of *Lecidella* species. New sequences are in bold.

Name of species	Country/Origin	Voucher specimen	Genbank no.
Carbonea vorticosa	Antarctica: Ross Dependency, Brown Hills	R. Tuerk 35647 (SZU)	JN873866
Carbonea vorticosa	Norway	R. Tuerk 43429 (SZU)	JN873870
Lecanora fuscobrunnea	Antarctica: Ross Dependency, Diamond Hills	R. Tuerk 44628 (SZU)	JN873875
Lecanora polytropa	USA: California, Tranverse Range	U.C. Riverside 47815UCR1	JN873881
Lecidella carpathica	China: Inner Mongolia	ZX 20141477	KT453739
Lecidella carpathica	China: Xinjiang	ZX 20140367-2	KT453741
Lecidella carpathica	China: Xinjiang	ZX 20129069	KT453740
Lecidella effugiens	China: Yunnan	ZX YN0203	KT453754
Lecidella effugiens	China: Jilin	ZX 20141269-2	KT453748
Lecidella enteroleucella	China: Yunnan	ZX YN0201	KT453757
Lecidella aff. *Elaeochroma*	China: Jilin	ZX 20141284	KT453751
Lecidella elaeochroma	China: Xinjiang	ZX XL0395-2	KT453749
Lecidella elaeochroma	United Kingdom	_	FR799214
Lecidella elaeochroma	Turkey: Kayseri	_	HQ605938
Lecidella elaeochroma	United Kingdom	_	FR799215
Lecidella elaeochroma	_	U. Arup L98156 (Arup priv.herb.)	AY541275
Lecidella elaeochroma	United Kingdom	_	FR799217
Lecidella elaeochromoides	China: Xinjiang	ZX 20141142	KT453750
Lecidella euphoria	China: Xinjiang	ZX 20140638	KT453742
Lecidella euphoria	China: Xinjiang	ZX XL0351-2	KT453745
Lecidella aff. *Euphoria*	China: Yunnan	ZX YN0186-2	KT453755
Lecidella aff. *Euphoria*	China: Shaanxi	ZX 20114605	KT453756
Lecidella flavosorediata	United Kingdom	_	FR799213
Lecidella greenii	Antarctica: Ross Dependency, Granite Harbour	R. Tuerk 33586 (SZU)	HQ287871
Lecidella greenii	Antarctica: Victoria Land, Taylor Valley	R. Tuerk 33586 (SZU)	JN873883
Lecidella iqbalii	**Pakistan**	**LAH37006**	**OL843364**
Lecidella iqbalii	**Pakistan**	**LAH37007**	**OL843364**
Lecidella meiococca	Sweden	Ekman 3101 (BG)	AF517929
Lecidella patavina	China: Xinjiang	ZX 20140501-2	KT453767
Lecidella siplei	Antarctica: Victoria Land, Botany Bay	R. Tuerk 32991(SZU)	JN873896
Lecidella siplei	Antarctica: Quenn Maud Mountains, Mt Kyffin	R. Tuerk 33457(SZU)	JN873898
Lecidella sp. UR00090	Chile: Region de Magallanes y de la Antartica Chilena	UR00090 (SZU)	MK620141
Lecidella sp. UR00107	Chile: Region de Magallanes y de la Antartica Chilena	UR00107 (SZU)	MK620152
Lecidella stigmatea	China: Xinjiang	ZX 20141254	KT453758
Lecidella stigmatea	Argentina: Provinzia de Santa Cruz	UR00162(SZU)	MK620188
Lecidella stigmatea	Argentina: Provincia de Chubhut	UR00208 (SZU)	MK620216
Lecidella stigmatea	Argentina: Provinzia de Santa Cruz	UR00133 (SZU)	MK620166
Lecidella stigmatea	Austria: Kärnten, Gailtaler Alpen	R. Tuerk 38608 (SZU)	JN873901
Lecidella stigmatea	Turkey: Kayseri	_	HQ605937
Lecidella stigmatea	Chile: Region de Magallanes y de la Antartica	UR00095 (SZU)	MK620144
Lecidella tumidula	China: Xinjiang	ZX XL0009	KT453736
Lecidella tumidula	_	AFTOL-ID 1374	HQ650596
Lecidella wulfenii	Austria: Salzburg, Hochkönigmassiv	R. Tuerk 39666 (SZU)	JN873903
Lecidella sp. 3	Chile: Region de Magallanes y de la Antartica Chilena	UR00120 (SZU)	MK620158
Lecidella sp. 1	Argentina: Provincia de Chubhut	UR00213 (SZU)	MK620221
Lecidella sp. 2	Antarctica: Ross Dependency	MAF-GS1-64 (MAF)	MK208746

Table 2. Comparison of the non-uniform characteristics.

Characters	*Lecidella iqbalii*	*Lecidella* sp. UR00090	*Lecidella* sp. UR00107	*Lecidelaa stigmatea* (Kantvilas & Elix, 2013)	*Lecidella greenii* (Ruprecht *et al*., 2012)	*Lecidella siplei* (Ruprecht *et al*., 2012)
Thallus colour	Whitish to grey	Greyish to beige	Greyish to beige	Pale cream to grey-brown, sometimes discoloured brownish	Whitish, grey to beige.	Whitish, beige, pale gray to dark gray
Apothecia	Strongly convex	Plane, pruinose, black	Plane to strongly convex	Plane to convex	Flat to convex	Flat to convex
Exciple	40-60 µm thick, bluish green to dark brown	Up to 120 µm thick, hyaline, J-, prominent, dark-green margin.	Up to 100 µm thick, hyaline, J-, dark green margin	35-50 (-80) µm thick	Up to 200 µm thick, hyaline, dark green margin	Up to 200 µm thick, hyaline, dark green to brown margin
Ascus	40-55 x 18-24 µm	Up to 45 µm	Up to 55µm	45-60 × 13-20 µm	*Lecidella*-type, 40-50 µm	*Lecidella*-type, 40-50 µm
Ascospores	10-13 x 6-9 µm.	8-11 x 6-8 µm	10-12 x 6-8 µm	(10-) 11-13.4-15(-16) × (6-) 6.5-7.8-9(-10) µm	10-11 (-12) x 6-7 (-8) µm	(10-) 12-14 (-15) x (5-) 6-7 µm
Apical cell of paraphyses	Up to 3.0 µm	Up to 3.0 µm	Up to 4.0 µm	2.5-5 µm wide	Up to 4.0 µm	Up to 4.0 µm
Epihymenium	Dark brown	Dark-green	Dark-green		Olive to dark green	Olive to dark green
Hypothecium	Hyaline to light brown at the base,	Hyaline, inspersed with dark crystals.	Hyaline , inspersed with crystals	60-150 µm thick, colourless or occasionally pale yellow-brown at the base.	Hyaline, inspersed with brown crystals.	Hyaline, inspersed with brown crystals

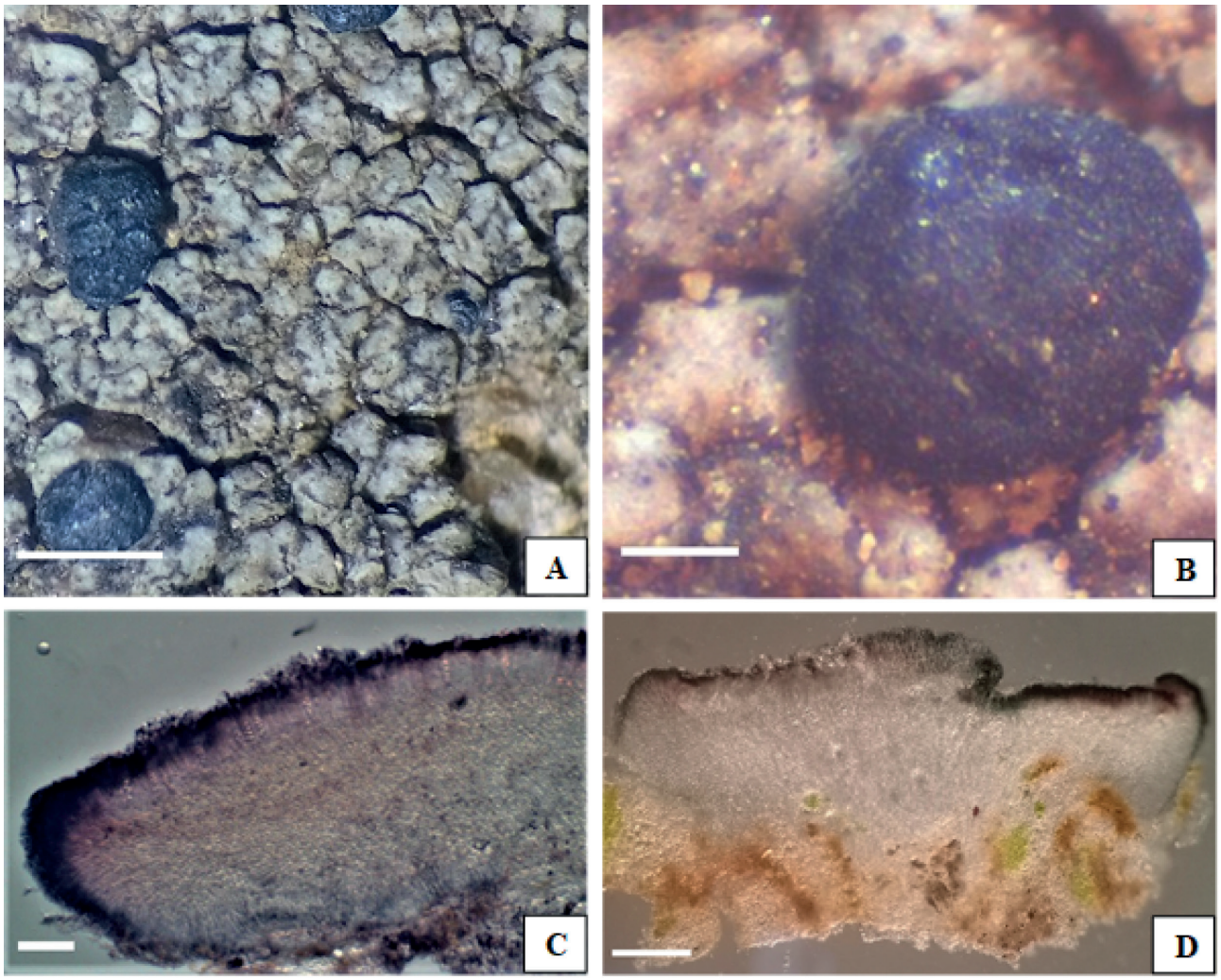

Figure 2. Diagnostic characters of *Lecidella iqbalii* (LAH37006). **A**. Typical thallus of the type specimen. **B**. Close up of Apothecia. **C**. Hand-cut apothecial section in KOH. **D**. Cross section of apothecium. Bars: A = 2 cm, B = 1 mm, C = 60 µm, D = 80 µm.

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