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## A taxonomic and phylogenetic study of some *Lecidella* species from Pakistan

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**Abstract.** In this study, a number of species of the genus *Lecidella* were collected from different areas of Pakistan and characterized using morpho-anatomical and molecular techniques. The present work revealed that collected specimens belong to four species of *Lecidella*. Among which *L. tumidula* is a new record for Pakistan while *L. carpathica*, *L. patavina* and *L. stigmatea* are being reported here from new localities, from Pakistan, representing their wider distribution. Complete morpho-anatomical descriptions, ecology and distribution, along with ITS-based molecular analysis is provided.

**Keywords:** Khyber Pakhtunkhwa, Lecanoraceae, *Lecidella*, phylogeny, Pakistan.

### INTRODUCTION

The genus *Lecidella* Korb. (Lecanoraceae), established by Korber in 1855, is comprised of 80 species (Zhao *et al.* 2015). This genus of crustose lichens is mainly characterized by black leciadiene apothecia with persistent proper excipulum and *Lecidella*-type asci (Zhao *et al.* 2015). It is a cosmopolitan genus, frequently found in temperate latitudes on wood, bark or rock (Kantvilas and Elix 2013). This group of lichenized ascomycetous fungi is usually regarded as taxonomically difficult due to a high degree of variation in morphological characters. In such cases, molecular study has played a significant role in systematics and species delimitation (Zhao *et al.* 2015).

Pakistan is located in western South Asia between 24–37° N latitude and 62–75° E longitudes. The country is well known for its geographical and climatic variations which is linked with rich biodiversity (IUCN 2006). The lichen diversity in this region is probably very high but little known due to the lack of surveys in many areas (Ahmad *et al.* 1997; Aptroot and Iqbal 2012). So far, 375 lichen species have been reported from Pakistan (Ahmad 1965; Aptroot and Iqbal 2012; Habib *et al.* 2017; Khan *et al.* 2018; Habib and Khalid 2019). In the past, attempts were made to describe the lichen diversity of the country using morpho-anatomical techniques (Ahmad 1965; Iqbal *et al.* 1978; Aptroot and Iqbal 2012). Recently, molecular techniques have also

been used to study lichen flora of Pakistan (Habib et al. 2017; Khan et al. 2018; Habib and Khalid 2019).

From Pakistan, five species of *Lecidella* have been reported so far, viz; *L. carpathica* Korb., *L. euphorea* (Florke) Hertel, *L. pulveraceae* (Florke) Th.Fr., *L. pata-vina* (A.Massal.) Knoph & Leuckert, and *L. stigmatea* (Ach.) Hertel & Leuckert (Aptroot and Iqbal 2012). In the present study, different species of *Lecidella* have been collected from different areas of Pakistan. Use of morpho-anatomical techniques along with phylogenetic analysis led to identification of four different species. *L. tumidula* (A.Massal.) Knoph & Leuckert has been collected and described for the first time from Pakistan which made an addition to the lichen flora of this country. Now, the number of *Lecidella* species reported from Pakistan has been raised from five to six.

## MATERIALS AND METHODS

### *Morphological and chemical studies*

Collections were made during a lichen survey of Chikar, Muzaffarabad (Azad Jammu and Kashmir), Parachinar, and Fairy Meadows (Gilgit Baltistan) in 2017 and 2018. Morphological characters were observed under a stereomicroscope (Meiji Techno, EMZ-5TR, Japan). Standard microscopy and spot tests (Hale 1979) were used for identification. Measurements were made from free hand section of apothecia mounted in water on a glass slide. The sections were observed using a compound microscope (MX4300H, Meiji Techno Co., Ltd., Japan). Minimum twenty measurements in water were made for each diagnostic feature.

### *DNA extraction and PCR amplification*

We used thallus material along with apothecial material to extract fungal DNA using a 2% CTAB protocol (Gardes and Bruns 1993). The primer pair ITS1F (Grades and Bruns 1993) and ITS4 (White et al. 1990) was used to amplify the internal transcribed spacer (ITS) region under PCR conditions used by Khan et al. (2018). PCR products were visualized in a 1 % agarose gel using ethidium bromide (Sambrook and Russell 2001). PCR products were sequenced from BGI, China.

### *Phylogenetic analysis*

The ITS regions of all specimens were amplified and sequenced. Bio-edit sequence alignment editor was

used to reassemble forward and reverse sequences (Hall 2005). Sequences of other *Lecidella* species based on initial BLAST searches and those used in a study on phylogeny of *Lecidella* by Zhao et al. (2015) were used in phylogenetic analysis (Table 1). The multiple sequence alignment was performed using MAFFT v7 with all parameters set to default values (Kato and Standley 2013). The ends of the alignment were trimmed to nearly an equal number of sites for all sequences. All gaps were treated as missing data. Maximum Likelihood analysis was performed with MEGA6 using a GTR model for bootstrapping (Tamura et al. 2013). One thousand rapid bootstrap replicates were run to infer the evolutionary history of each species.

The length of the final aligned file was 541 nucleotides, of which 355 sites were conserved, 181 were variable, 156 were parsimony informative and 25 were singleton, *Rhizoplaca porterii* and *R. parilis* (HM577376, HM577309) were chosen as an outgroup (Zhao et al. 2015).

## RESULTS

*Lecidella tumidula* (A.Massal.) Knoph & Leuckert, *Bibliotheca Lichenol.* 68: 131. 1997. (Figure 1, A-E; Figure 5; A-D).

### *Description*

Thallus crustose, continuous, up to 3 cm in diameter, up to 0.6 mm thick, granulose to rimose. Colour: light greyish green to light olive green, dull to weakly glossy. Apothecia: rounded, semi-immersed, sessile, 0.5–1 mm, frequently present, strongly constricted at base. Margins: thin, continuous, concolorous to disc, distinct when young, indistinct when mature. Disc: black, smooth, glossy, pruinose, flat to convex. Exciple: black, 40–50  $\mu\text{m}$  thick. Epihymenium: blackish, 30–36  $\mu\text{m}$  tall. Hymenium: hyaline, 80–86  $\mu\text{m}$  tall. Hypothecium: dark reddish brown, 30–40  $\mu\text{m}$  tall. Paraphyses: septate, hyaline, rarely anastomosing or branched, apex swollen, 2–4  $\mu\text{m}$  wide. Asci: clavate, 8-spored, 58–78  $\mu\text{m}$   $\times$  10–14  $\mu\text{m}$ . Ascospores: simple, hyaline, narrowly ellipsoid to ovoid, 10–12  $\mu\text{m}$   $\times$  5–7  $\mu\text{m}$ .

### *Spot Tests*

All negative (diploicin and lichexanthone reported according to literature but not examined in our specimen).

### *Substrate and ecology*

*L. tumidula* was found on bark of *Quercus incana* W. Bartram in dry temperate forest, at an altitude of 1,705

**Table 1.** Specimens used in phylogenetic analysis of *Lecidella* species.

ITS Accession No-	Specimen Name	Country	Voucher No-	ITS Accession No-	Specimen Name	Country	Voucher No-
KT453736	<i>Lecidella tumidula</i>	China	ZX XL0009 (Zhao <i>et al.</i> 2015)	MK970672	<i>Lecidella greenii</i>	Antarctica	T48787 (Wagner <i>et al.</i> 2019)
KT453737	<i>Lecidella tumidula</i>	China	ZX 20129166-2 (Zhao <i>et al.</i> 2015)	HQ605934	<i>Lecidella patavina</i>	Turkey	574971 (Basaran <i>et al.</i> 2014)
HQ650596	<i>Lecidella tumidula</i>	USA	404720 (Schmull <i>et al.</i> 2011)	KT453767	<i>Lecidella patavina</i>	China	ZX 20140501-2 (Zhao <i>et al.</i> 2015)
KT453750	<i>Lecidella elaeochromoid</i>	China	ZX 20141142 (Zhao <i>et al.</i> 2015)	MK620163	<i>Lecidella stigmatea</i>	Argentina	UR00128 (Ruprecht <i>et al.</i> 2019)
KT453746	<i>Lecidella elaeochromoid</i>	China	ZX 20114966-2 (Zhao <i>et al.</i> 2015)	MK620136	<i>Lecidella stigmatea</i>	Argentina	UR00080 (Ruprecht <i>et al.</i> 2019)
KT453748	<i>Lecidella effugiens</i>	China	ZX 20141269-2 (Zhao <i>et al.</i> 2015)	KT453764	<i>Lecidella stigmatea</i>	China	ZX 20140086-2 (Zhao <i>et al.</i> 2015)
KT453747	<i>Lecidella effugiens</i>	China	ZX 20141148-2 (Zhao <i>et al.</i> 2015)	KT453765	<i>Lecidella stigmatea</i>	China	ZX 20140045-2 (Zhao <i>et al.</i> 2015)
AF517929	<i>Lecidella meiococca</i>	Sweden	Ekman 3101 (BG) (Ekman & Tonsberg, 2002)	KT453760	<i>Lecidella stigmatea</i>	China	ZX 20140519-2 (Zhao <i>et al.</i> 2015)
KT453743	<i>Lecidella euphorea</i>	China	ZX XL0387 (Zhao <i>et al.</i> 2015)	KT453763	<i>Lecidella stigmatea</i>	China	ZX 20140507-2 (Zhao <i>et al.</i> 2015)
KT453742	<i>Lecidella euphorea</i>	China	ZX 20140638 (Zhao <i>et al.</i> 2015)	KT695322	<i>Lecidella carpathica</i>	Canada	BIOUG24047-H06 (Telfer <i>et al.</i> 2015)
KX132994	<i>Lecidella flavosorediata</i>	Switzerland	980812 (Mark <i>et al.</i> 2016)	KT695353	<i>Lecidella carpathica</i>	Canada	BIOUG24047-E02 (Telfer <i>et al.</i> 2015)
KX132965	<i>Lecidella flavosorediata</i>	Switzerland	980812 (Mark <i>et al.</i> 2016)	JN873899	<i>Lecidella siplei</i>	Antarctica	Tuerk 35895 (Ruprecht <i>et al.</i> 2012)
JN873903	<i>Lecidella wulfenii</i>	Austria	Tuerk 39666 (Ruprecht <i>et al.</i> 2012)	JN873896	<i>Lecidella siplei</i>	Antarctica	Tuerk 32991 (Ruprecht <i>et al.</i> 2012)
MN387031	<i>Lecidella elaeochroma</i>	Poland	272987 (Singh <i>et al.</i> 2019)	JN873898	<i>Lecidella siplei</i>	Antarctica	Tuerk 33457 (Ruprecht <i>et al.</i> 2012)
MN387029	<i>Lecidella elaeochroma</i>	Poland	272987 (Singh <i>et al.</i> 2019)	JN873897	<i>Lecidella siplei</i>	Antarctica	Tuerk 33449 (Ruprecht <i>et al.</i> 2012)
HQ287871	<i>Lecidella greenii</i>	Antarctica	Herbarium Tuerk 43015 (Lumbsch <i>et al.</i> 2011)	HM577376	<i>Rhizoplaca porterii</i>	USA	55145 (BRY-C) (Leavitt <i>et al.</i> 2011)
JN873884	<i>Lecidella greenii</i>	Antarctica	Tuerk 33612 (Ruprecht <i>et al.</i> 2012)	HM577309	<i>Rhizoplaca parilis</i>	USA	55078 (BRY-C) (Leavitt <i>et al.</i> 2011)

m, dominant vegetation including *Pinus gerardiana* Wall. ex D. Don, *Quercus ilex* L., *Juniperus macropoda* Boiss. and *Picea smithiana* Boiss, temperature ranges between 6–16 °C, average annual rainfall 300–500 mm.

#### Distribution

Temperate areas of Asia including China (Zhao *et al.* 2015), have also been reported from Europe and North America (Nash *et al.* 2004). Here it is been reported for the first time from Pakistan.

#### Material examined

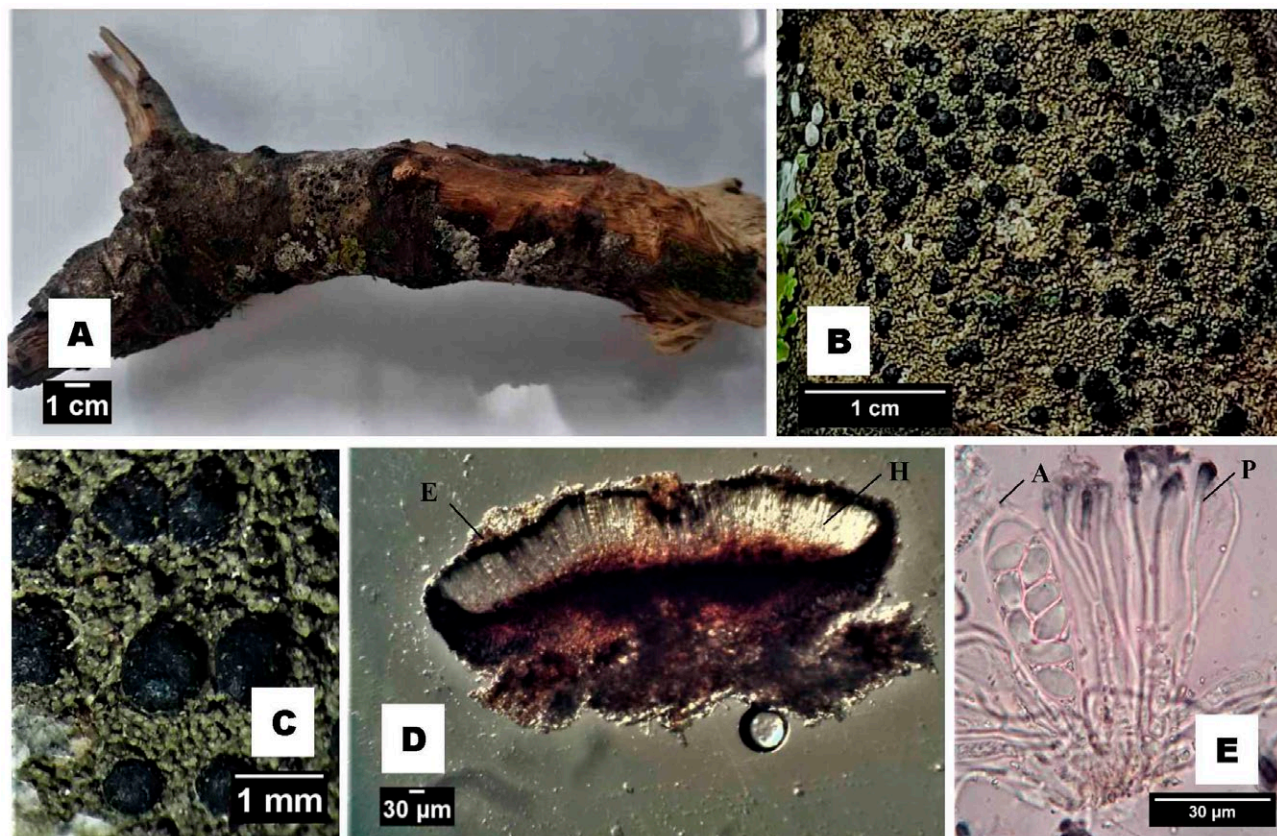
**PAKISTAN:** Khyber Pakhtunkhwa Province: Kurram District, Parachinar: 33°90' N, 70°08' E; 1,705 m; on bark of *Quercus incana* W. Bartram, 18 August 2018,

A.N. Khalid & K. Habib; LAH36399; (GenBank accession no. MT139651).

*Lecidella carpathica* Korb., Parerga lichenol. (Breslau) 3: 212. 1861. (Figure 2, A-F; Figure 5; E-H).

#### Description

Thallus crustose, effuse, rimose areolate to subsquamulose, dull, up to 5 cm wide, up to 300 µm thick. Areoles: scattered to crowded, smooth or rough, up to 0.5 mm in diameter, irregular in outline. Colour: pale green to grey green. Apothecia: sessile, semi-immersed, constricted at base, scattered to crowded, up to 1 mm in diameter. Disc: black, plane, flat to moderately con-



**Figure 1.** *Lecidella tumidula*. (A & B) showing crustose thallus and abundant apothecia (C) pruinose apothecia; (D) Cross section of an apothecium (H: Hymenium; E: Epihymenium); (E) showing ascus and paraphyses (A: Ascus; P: Paraphyses).

vex, pruinose. Margins: distinct, entire or flexuose, up to 0.9 mm wide. Exciple: bluish green to black, 20–30 µm thick. Epihymenium: pale brown to dark brown, 15–20 µm tall. Hymenium: hyaline, 65–90 µm tall. Hypothecium: pale brown to brown, 30–45 µm tall. Paraphyses: aseptate, hyaline, rarely anastomosing or branched, sometimes slightly swollen apically, up to 2 µm wide. Asci: clavate, 45–60 x 13–15 µm, 8-spored. Ascospores: simple, hyaline, ellipsoid to ovoid, 10–14 x 6–8 µm.

#### Spot tests

K+ yellow, C–, KC–, P–

#### Substrate and ecology

*L. carpathica* was found on rock (saxicolous), in moist temperate forest at an altitude of 234 m, temperature ranges between –2–37 °C, average annual rainfall 1,500–1,600 mm, with dominant tree species *Pinus wallichiana* A.B. Jacks., *Picea smithiana* Boiss., *Abies pindrow* Royle., *Quercus incana* W. Bartram and *Q. dilatata* Lindl. ex Royle.

#### Distribution

Widespread including Africa, Europe, Australia, Macaronesia, New Zealand (Smith et al. 2009), UK, USA, Ukraine (Oxner 1968; Kondratyuk et al. 1998; 2003), temperate parts of Asia (Nash et al. 2004), China (Zhao et al. 2015), India (Singh & Sinha 2010) and Pakistan (Nasim et al. 2004).

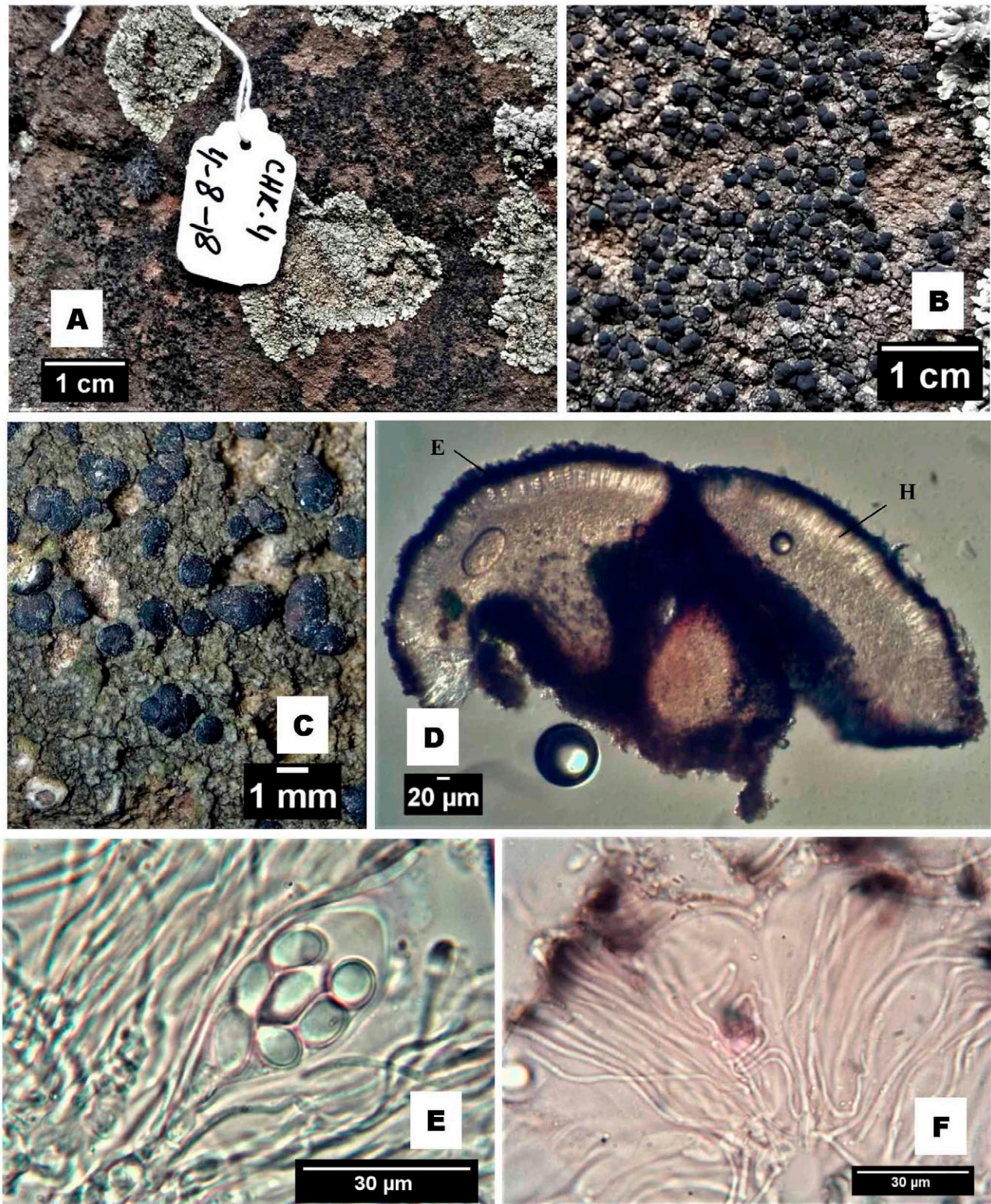
#### Material examined

**PAKISTAN:** Azad Jammu & Kashmir: Chikar, 34° 9' N, 73° 41' E, 234 m; on rock; 18 August 2018, *K. Habib*; LAH36400; (Genbank accession no. MT139649).

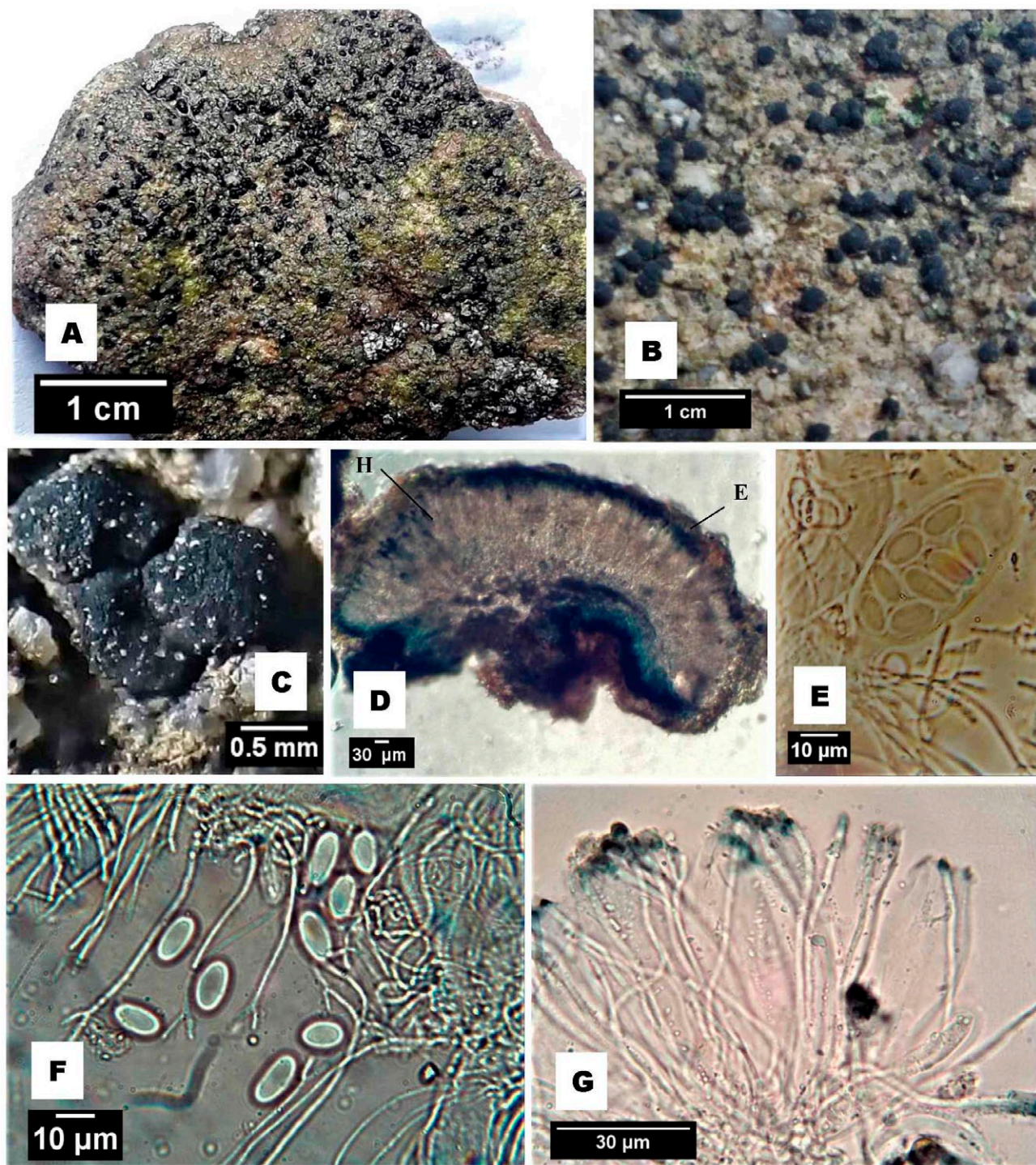
*Lecidella patavina* (A.Massal.) Knoph & Leuckert, in Knoph, *Bibliotheca Lichenol.* 36: 116. 1990. (Figure 3, A-G; Figure 6; A-D).

#### Description

Thallus indistinctly crustose, inconspicuous, intermingled with rock particles, up to 4 cm in diameter.



**Figure 2.** *Lecidella carpathica*. (A & B) Crustose thallus showing abundant apothecia; (C) black, pruinose apothecia; (D) Cross section of an apothecium (H: Hymenium; E: Epihymenium); (E) Ascus; (F) Paraphyses.



**Figure 3.** *Lecidella patavina*. (A & B) Crustose thallus showing abundant apothecia; (C) black, pruinose fascicle of apothecia; (D) Cross section of an apothecium (H: Hymenium; E: Epihymenium); (E) Ascus; (F) Ascospores; (G) Paraphyses.

Colour: light green to dark green. Apothecia: rounded to irregular, 0.2–1.5 mm in diameter, sessile, constricted at base, frequent, separate, rarely fascicle. Margins: thin,

continuous, concolorous to disc, distinct when young, indistinct when mature. Disc: black, flat to strongly convex, smooth and slightly glossy, slightly pruinose. Exci-

ple: bluish green, 40–50  $\mu\text{m}$  thick. Epithymenium: dark blue with blackish tint, 20–30  $\mu\text{m}$  tall. Hymenium: hyaline to grey, 110–120  $\mu\text{m}$  tall, interspersed. Hypothecium: hyaline to brown, 90–110  $\mu\text{m}$  tall. Paraphyses: hyaline, aseptate, apically branched, not anastomosing, apex slightly swollen, 1–2  $\mu\text{m}$  wide. Asci: clavate, 8-spored, 30–67  $\mu\text{m}$   $\times$  12–20  $\mu\text{m}$ . Ascospores: hyaline, simple, narrowly ellipsoid to ovoid, 9–13  $\mu\text{m}$   $\times$  5–6  $\mu\text{m}$ .

#### Spot Test

K+ (Slight yellow), C–, KC–, P– (atranonin or lichexanthone might be predicted).

#### Substrate and ecology

*L. patavina* was found on nutrient enriched siliceous rocks near water falls, in moist temperate forest at an altitude of 1,705 m, temperature ranges between –2–37 °C, average annual rainfall 1,500– 1,650 mm, with dominant tree species *Cedrus deodara* (Roxb. ex Lambert) G. Don, *Pinus wallichiana* A.B. Jacks., *Picea smithiana* Boiss., *Abies pindrow* Royle., *Quercus incana* W. Bartram, *Q. dilatata* Lindl. ex Royle. and *Q. semecarpifolia* Sm.

#### Distribution

Mainly arctic-alpine, UK, N.W. Scotland (Smith et al. 2009), Africa, Antarctica, Europe, USA, (Knoph and Leuckert 2004; Knudsen and Kocourková 2012), Ukraine (Vondrak et al. 2010), temperate parts of Asia (Nash et al. 2004), China (Zhao et al. 2015) and Pakistan (Aptroot and Iqbal 2012).

#### Material examined

**PAKISTAN:** Khyber Pakhtunkhwa Province: Kurram District, Parachinar: 33°90' N, 70°08' E; 1705 m; on rock; 18 August 2018, A.N. Khalid & K. Habib; LAH3640; (GenBank accession no. MT139652).

***Lecidella stigmatea*** (Ach.) Hertel & Leuckert, Willdenowia 5: 375. 1969. (Figure 4, A-G; Figure 6; E-H).

#### Description

Thallus crustose, areolate or rimose-areolate to verruculose-areolate, 7–8 cm in diameter, up to 0.2 mm thick, rarely thick up to 0.6 mm. Areoles: indistinct to distinct, flat to slightly convex, angular to irregular in outline, 0.2–0.6 mm in diameter. Surface: finely granulate, rough, dull. Colour: greyish to grey to brownish grey. Apothecia: lecideine, black, sessile, 0.4–0.9 mm in diameter. Disc: black, flat to somewhat convex, pruinose. Margins: distinct, thin, becoming excluded. Exciple:

78–88  $\mu\text{m}$  thick, bluish green to brown. Epithymenium: dark brown, 12–16  $\mu\text{m}$  high. Hymenium: hyaline, 75–80  $\mu\text{m}$  tall, not interspersed. Hypothecium: hyaline to brown, 65–75  $\mu\text{m}$  tall. Paraphyses: aseptate, hyaline, rarely anastomosing or branched, slightly swollen apically, up to 1.2  $\mu\text{m}$  wide. Asci: lecanoral-type, clavate, 8-spored, 40–57  $\times$  14–18  $\mu\text{m}$ ; Ascospores: simple, hyaline, thick and smooth wall, ellipsoid to ovoid, 9–14  $\times$  5–8  $\mu\text{m}$ .

#### Spot tests

K+ yellow, C–, KC–, P– (atranonin, zeorin, lichexanthone or norlichexanthone might be predicted).

#### Substrate and ecology

*L. stigmatea* was found on bark of *Pinus wallichiana* A.B. Jacks., in moist temperate forest, with dominant tree species *Cedrus deodara* (Roxb. ex Lambert) G. Don, *Pinus wallichiana* A.B. Jacks., *Picea smithiana* Boiss., *Abies pindrow* Royle., *Quercus incana* W. Bartram, *Q. dilatata* Lindl. ex Royle. and *Q. semecarpifolia* Sm. at an altitude of 3,300 m, temperature ranges between –2–37 °C, average annual rainfall 1650 mm.

The second collection of *L. stigmatea* was found on a rock (saxicolous), close to water falls, in moist temperate forest at an altitude of 2,900 m, temperature ranges between –2–37 °C, average annual rainfall 1,500– 1,650 mm.

#### Distribution

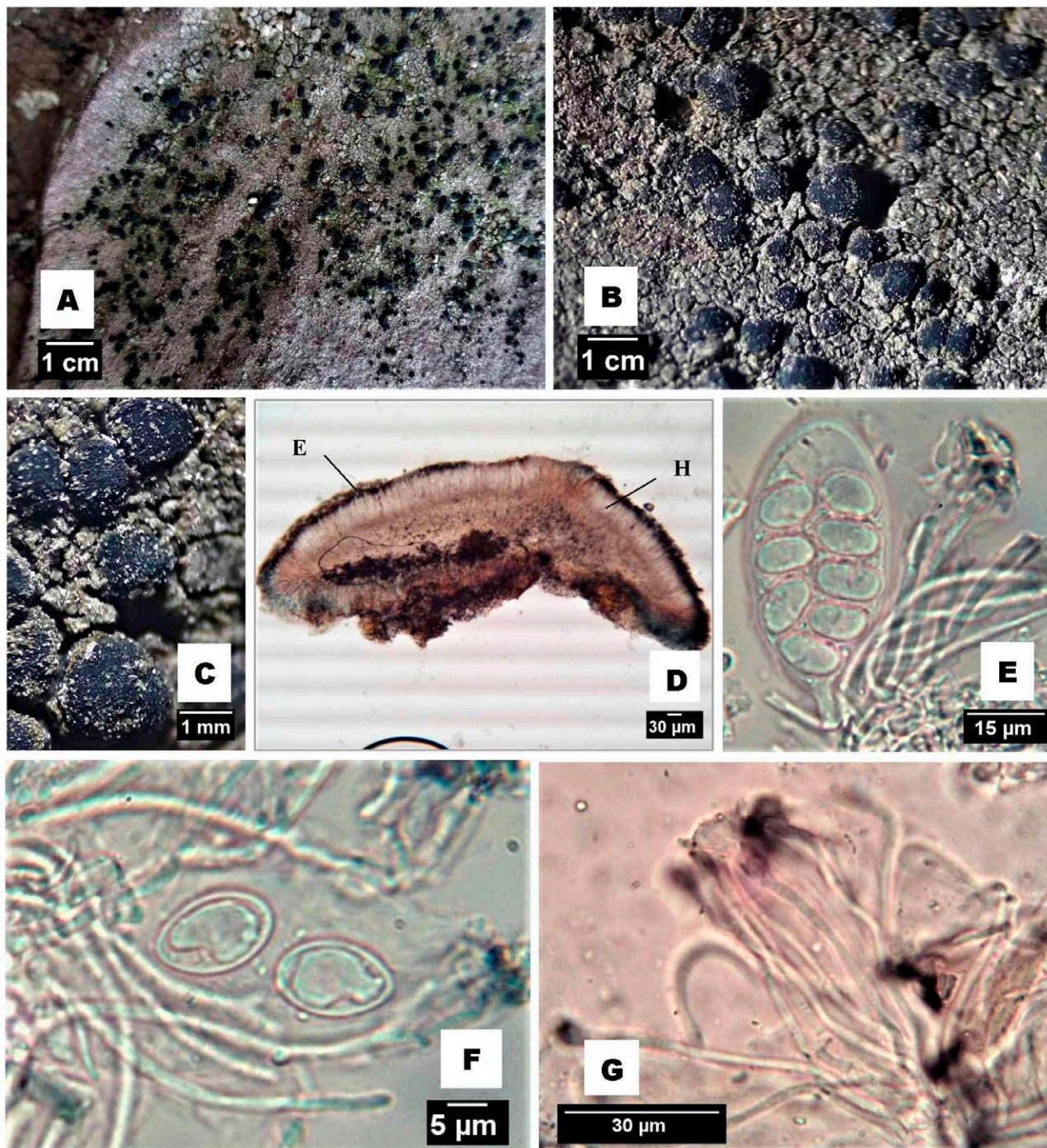
Probably cosmopolitan, including UK, USA, Antarctica, Australia (Smith et al. 2009), Ukraine (Oxner 1968, Kondratyuk et al. 1998, 2003), temperate parts of Asia (Nash et al. 2004), China (Zhao et al. 2015), India (Singh & Sinha 2010) and Pakistan (Ahmad 1965).

#### Material examined

**PAKISTAN:** Gilgit Baltistan, Fairy Meadows: 35°23' N, 74°35' E; 3300 m; on bark of *Pinus wallichiana* A.B. Jacks., 18 July 2017, A.N. Khalid & M. Khan; LAH36402; (GenBank accession no. MT139653); Azad Jammu & Kashmir: District Muzaffarbad, Peer Chanasi: 34°2' N, 73°33' E; 2900 m; on rock; 18 August 2018, K. Habib; LAH36403; (Genbank accession no. MT139650).

#### Key to Genus *Lecidella* in Pakistan

- 1a. On Bark..... *L. tumidula*
- 1b. On rock, Cortex K–, C..... 2
- 1c. On rock, or on bark, Cortex K+, C ..... 3



**Figure 4.** *Lecidella stigmatea*. (A & B) Crustose thallus showing abundant apothecia; (C) black, pruinose disc of apothecia; (D) Cross section of an apothecium (H: Hymenium; E: Epihymenium); (E) Ascus; (F) Ascospores; (G) Paraphyses.

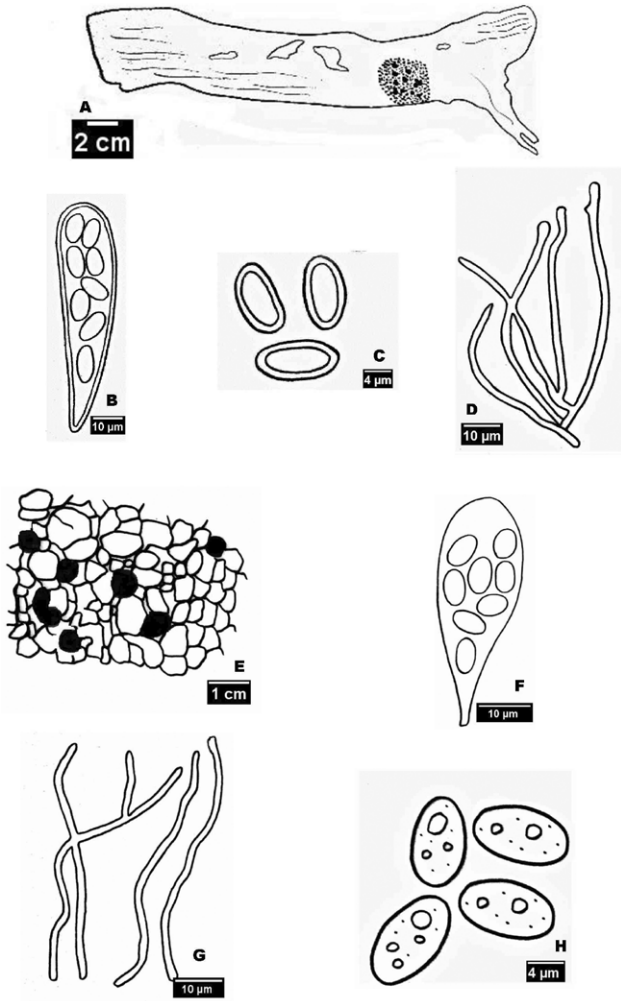
1d. On rock, Cortex K+, C..... 4

2a. Thallus crustose, areolate, epihymenium 15–20 µm tall, hypothecium brownish grey, on rock.....*L. carpathica*

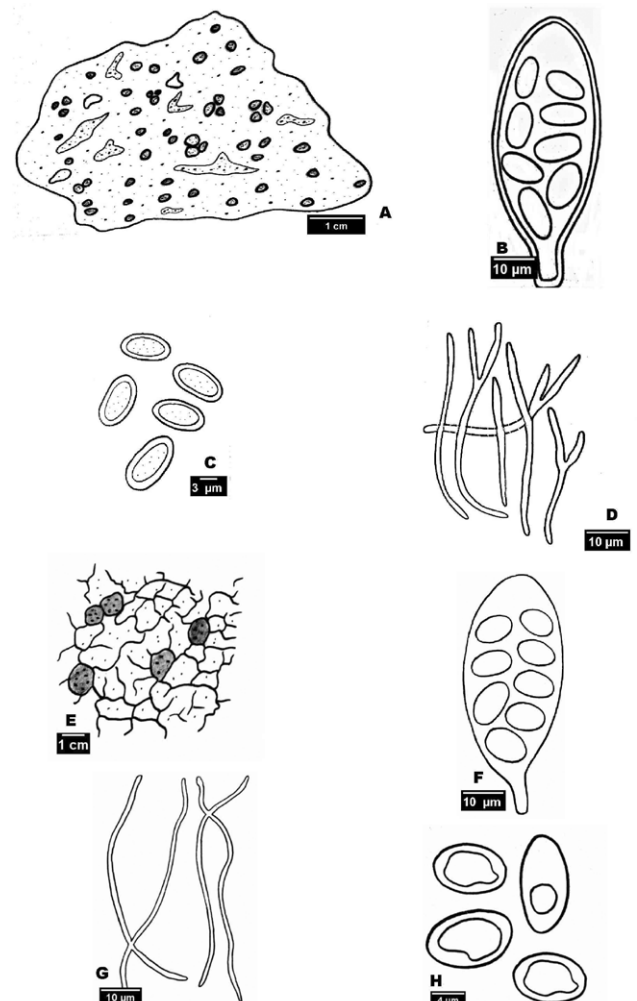
2b. Thallus crustose, granulate, epihymenium 30–36 µm tall, hypothecium dark reddish brown, on bark.....*L. tumidula*

3a. Thallus rimose-areolate, paraphyses rarely branched, exciple greenish black, hymenium not interspersed... *L. stigmatea*





**Figure 5.** (A-D): Line drawings of *Lecidella tumidula* A: Granulose thallus; B: Ascus; C: Ascospores; D: Paraphyses; (E-H): Line drawings of *Lecidella carpathica* E: Crustose-areolate thallus; F: Ascus; G: Paraphyses; H: Ascospores.



**Figure 6.** (A-D): Line drawings of *Lecidella patavina* A: crustose thallus B: Ascus; C: Ascospores; D: Paraphyses; (E-H): Line drawings of *Lecidella stigmatea* E: Rimose-areolate thallus; F: Ascus; G: Paraphyses; H: Ascospores.

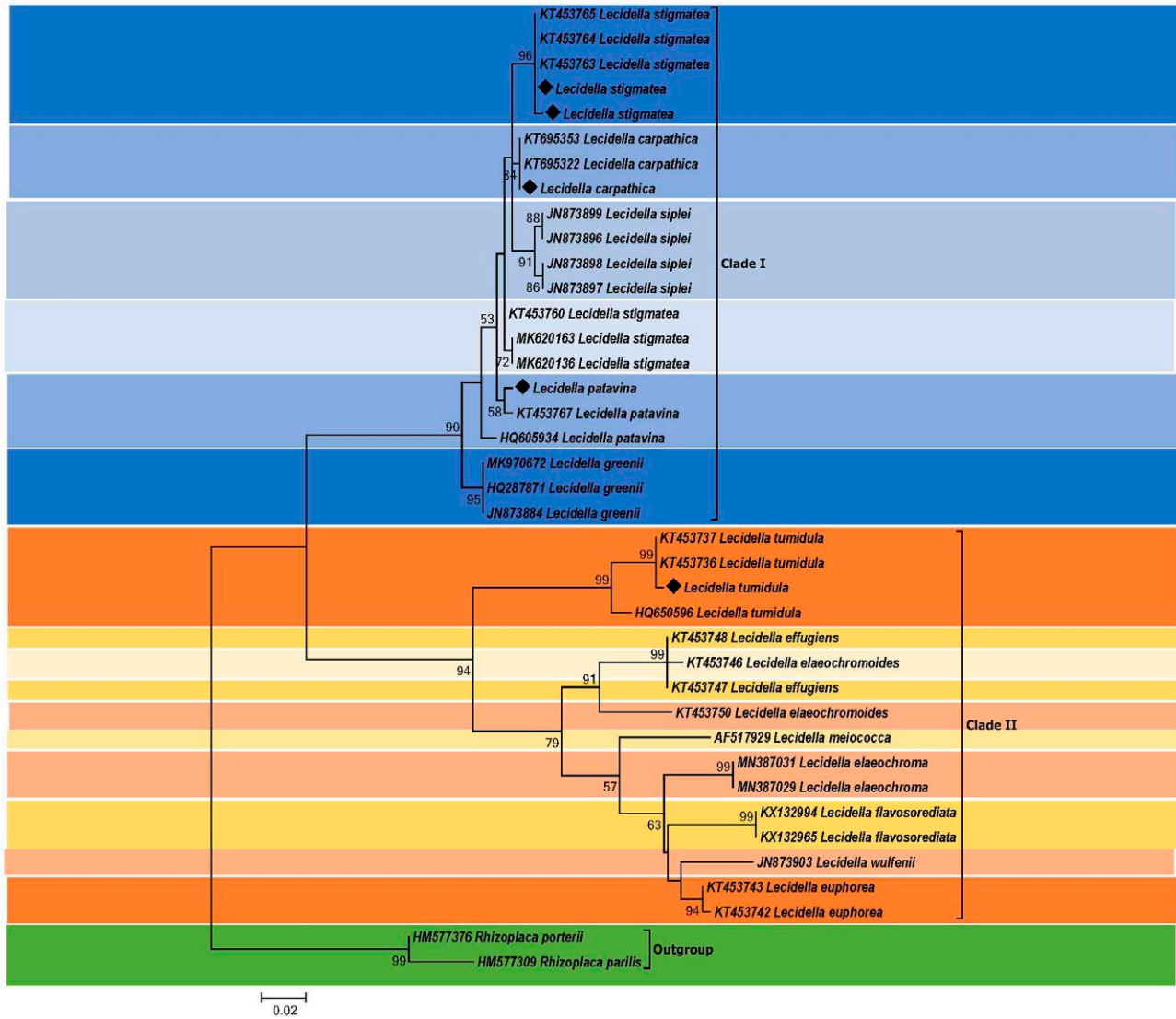
- 3b. Thallus inconspicuous, paraphyses mostly branched, exciple bluish green, hymenium interspersed.....*L. patavina*
- 4a. Thallus crustose, granulose to rimose-areolate, hymenium 55–100 µm tall.....*L. euphorea*
- 4b. Thallus sorediate, farinose, hymenium 60–70 µm tall,.....  
.....*L. pulveraceae*

*Discussion*

The present study of the genus *Lecidella* from northern Pakistan revealed one new record for the lichen flora of Pakistan while other *Lecidella* species have been collected from new localities thus indicating their wide distributional range in Pakistan.

In the ITS-based phylogenetic analysis, the Pakistani collection of *L. tumidula* (PR-112) clustered with specimens of the same taxon reported from China (Accession no. KT453736, KT453737) with strong support (99%, Fig. 7) and formed a sister group relationship with *L. tumidula* collected from USA (Accession no. HQ650596) that was wrongly reported in GenBank (Schmull et al. 2011), but after re-examination it was *L. tumidula* (Zhao et al. 2015).

Morphological comparison also confirms its identity as *L. tumidula* (Nash et al. 2004) except the presence of a pruinose disc in the specimen collected from Pakistan. There is only one nucleotide difference between the Pakistani collection and the Chinese *L. tumidula* specimen. It is also the second report of this taxon from Asia after China (Zhao et al. 2015).



**Figure 7.** Phylogenetic analysis of species of *Lecidella*, comprised of 39 sequences. This tree has been inferred using maximum likelihood method. The bootstrap values based on 1000 replicates are shown below the branches. Sequences generated from local collection are marked with ◆.

In the phylogenetic analysis, the ITS sequence of *Lecidella carpathica* (CHK-04) clustered with ITS sequences of *L. carpathica* collected from Canada (KT695322, KT695353). Morphological comparison also confirms its identity as *L. carpathica* (Nash *et al.* 2004). There is only one nucleotide difference between Pakistani and Canadian *L. carpathica* (KT695322, KT695353). Previously, it was reported from dry temperate forest of Kalam (Swat), Pakistan at an elevation of about 2,001 m. a.s.l. The new collection is from moist temperate forest of AJK state, found at an elevation of 2,900 m. a.s.l.

The ITS sequence of *Lecidella patavina* (PR-11) clustered with a Chinese collection of *L. patavina* (Accession

no. KT453767) in the phylogenetic analysis and made a sister branch with *L. stigmatea* (Ach.) Hertel & Leuckert. Morphologically, both *Lecidella* species are similar in having the same chemistry, a crustose epilithic thallus with a black disc of apothecia (Basaran *et al.* 2014) but differ due to the presence of the conspicuous and thicker thallus in the latter.

There are also two nucleotide difference between Pakistani and Chinese collection of *L. patavina* (KT453767). From Pakistan, it was previously reported but locality was not recorded. Here it is described from high elevation in Parachinar i.e. 1,705 m. a.s.l., where it is widespread in warm and temperate climates.

The ITS sequence of *Lecidella stigmatea* (MKF-7 and PC-34), clustered with *L. stigmatea* reported from China (KT453763, KT453764, KT453765). The *L. stigmatea* (MK620163, MK620136) in our tree formed separate subclade. Results from phylogenetic analysis of *L. stigmatea* requires revision between specimens identified as *L. stigmatea* from Argentina, are distantly related to samples of our clade. Morphologically our specimen is similar to the Sonoran *L. stigmatea*, in having rimose-areolate thallus and lecideine apothecia (Nash et al. 2004). From Pakistan, this taxon has previously been reported from dry temperate forest of Kalam (Swat), Pakistan, at an elevation of about 2,001 m. a.s.l. but recent collections are from moist temperate forests of Azad Jammu & Kashmir and Gilgit Baltistan, at high elevations 2,900 m and 3,300 m. a.s.l. respectively.

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