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New *Kudratovia* S.Y.Kondr., Lőkös & Hur species and *Anaptychia* Körb. records from Pakistan (lichenized Ascomycota, Physciaceae Zahlbr.)

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New *Kudratovia* S.Y.Kondr., Lőkös & Hur species and *Anaptychia* Körb. records from Pakistan (lichenized Ascomycota, Physciaceae Zahlbr.)

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ABSTRACT
This study contributes to the understanding of Physciaceae Zahlbr. lichens in Pakistan by analyzing specimens collected from various regions of Gilgit-Baltistan and Khyber Pakhtunkhwa. As a result of this investigation, *Kudratovia pakistanica* U.F. Ahmad, Afshan & Khalid, sp. nov., is described here as a new species, while *Anaptychia crinalis* (Schleich.) Vězda ex J. Nowak and *A. roemerioides* Hollinger, Noell & S.D. Leav. are reported as new records for Pakistan. *Kudratovia pakistanica* U.F. Ahmad, Afshan & Khalid, sp. nov., is morphologically similar to the closely related *K. luridata* but is characterized by its thick, indeterminate thallus, plane to concave apothecial discs, a thick hypothecium forming a stipe in mature apothecia (up to 200 µm deep), larger asci (68-92 × 12-24 µm), and larger ascospores (16-19 × 10-12 µm). Furthermore, recent collections – corroborated by detailed morphological examination, molecular data, and evaluation of prior taxonomic literature – confirm *Anaptychia setifera* (Mereschk.) Räsänen as the currently accepted name for *A. kaspica* Gyeln., thereby clarifying its taxonomic status and contributing to a better understanding of its distribution within the country. A detailed taxonomic treatment, including morphological descriptions and molecular phylogenetic analysis, is provided to support these findings.

KEY WORDS
Caliciales, cryptolecanorine, foliose, Gilgit-Baltistan, maximum-likelihood, phylogeny, tomentose, symbioses, new species.

RÉSUMÉ
Nouvelles espèces de Kudratovia *S.Y.Kondr., Lőkös & Hur et nouvelles observations d'*Anaptychia *Körb. au Pakistan (Ascomycètes lichéniques, Physciaceae Zahlbr.).*
Cette étude contribue à la compréhension des lichens de la famille des Physciaceae Zahlbr. au Pakistan grâce à l'analyse de spécimens collectés dans diverses régions du Gilgit-Baltistan et du Khyber Pakhtunkhwa. À l'issue de cette étude, *Kudratovia pakistanica* U.F. Ahmad, Afshan & Khalid, sp. nov., est décrite ici comme une nouvelle espèce, tandis qu'*Anaptychia crinalis* (Schleich.) Vězda ex J. Nowak et *A. roemerioides* Hollinger, Noell & S.D.Leav. sont signalées comme de nouvelles occurrences au Pakistan. *Kudratovia pakistanica* U.F. Ahmad, Afshan & Khalid, sp. nov., présente une morphologie similaire à celle de *K. luridata*, espèce étroitement apparentée, mais se caractérise par un thalle épais et

MOTS CLÉS
Caliciales,
cryptolecanorine,
feuillu,
Gilgit-Baltistan,
méthode du maximum
de vraisemblance,
phylogénie,
tomentose,
symbioses,
espèce nouvelle.

indéterminé, des disques apothéciaux plans à concaves, un hypothécium épais formant un stipe dans les apothécies matures (profondeur jusqu'à 200 µm), des asques plus grands (68-92 × 12-24 µm) et des ascospores plus grandes (16-19 × 10-12 µm). Par ailleurs, des collectes récentes – corroborées par un examen morphologique détaillé, des données moléculaires et une analyse de la littérature taxonomique antérieure – confirment qu'*Anaptychia setifera* (Mereschk.) Räsänen est le nom actuellement accepté pour *A. kaspica* Gyeln., clarifiant ainsi son statut taxonomique et contribuant à une meilleure compréhension de sa répartition sur le territoire national. Une étude taxonomique détaillée, comprenant des descriptions morphologiques et une analyse phylogénétique moléculaire, est présentée pour appuyer ces résultats.

INTRODUCTION

The family Physciaceae Zahlbr. (Ascomycetes, Lecanorales) is a globally distributed group of lichens that includes twenty-five genera, exhibiting considerable morphological diversity and ecological adaptability (Kondratyuk *et al.* 2021; Hyde *et al.* 2024). Members of this family exhibit diverse growth forms, with some genera, such as *Anaptychia* Körb., *Phaeophyscia* Moberg, *Physcia* (Schreb.) Michx., and *Physconia* Poelt, forming fruticose or foliose thalli, while others, including *Buellia* De Not., *Kudratovia* S.Y.Kondr., Lőkös & Hur, and *Rinodina* (Ach.) Gray, are characterized by crustose or placodioid thalli (Helms *et al.* 2003). The family Physciaceae is primarily defined by its ascus and spore structure, with additional distinctions based on pycnoconidial characteristics, secondary chemistry, and cortical structures, which collectively aid in the delimitation of its genera (Lohtander *et al.* 2000). To date, around 12 genera and more than 40 species of the family Physciaceae have been recorded from Pakistan (Firdous *et al.* 2023; Niazi *et al.* 2023). Among these, the genera *Anaptychia* and *Rinodina s.l.* remain relatively unexplored in the country. While *Anaptychia* is distributed globally and has been previously reported from Pakistan, its full diversity and distribution in the region remain poorly documented, whereas *Kudratovia*, a recently segregated genus from *Rinodina s.l.*, is sparsely represented with only a single species known from the country (Aptroot & Iqbal 2012; Kondratyuk *et al.* 2021). To explore lichen diversity, field surveys were conducted in Gilgit-Baltistan and Khyber Pakhtunkhwa between 2022 and 2023. Based on the results of combined/integrated molecular, morpho-anatomical, and chemical analyses, we describe a new species of the genus *Kudratovia*, report two new records of *Anaptychia* from Pakistan, and confirm the presence of *A. setifera*, thereby extending its known distribution within the country.

MATERIAL AND METHODS

Sample collection

Lichen samples were collected from various sites in Khyber Pakhtunkhwa Province and Gilgit-Baltistan, Pakistan, during surveys conducted between July 2022 and September 2023. The first collection site, Upper Dir district (Kumrat Valley), is located in the northeast part of Pakistan, at an elevation of approximately 2439 to 3048 meters (Ahmad & Nizami 2015; Khattak *et al.* 2022). The second collection site, Abbottabad district, is located at the base of the Himalayas, and the average elevation varies from 2500 to 2700 meters above sea level (Taj *et al.* 2018). The third location, the Skardu district, lies in the Kohistan-Ladakh area of northern Pakistan. It is situated at the junction of the Indus and Shigar rivers, at an elevation of approximately 2300 meters above sea level (Hussain *et al.* 2019). Detailed information on precise collection sites for specimens can be found in the results section.

Morpho-anatomical and chemical study

The morpho-anatomical features of lichen specimens were described following the terminologies of Ryan *et al.* (2001). Freehand sections of the apothecia and thallus were prepared and mounted in water to observe the anatomical structures. Measurements of anatomical features were taken at 10× and 100× magnification, based on 20-22 ascospores per specimen, and are presented as the observed minimum-maximum range. Additionally, 5-6 sections of the thallus and apothecia were examined. The secondary chemistry of the thallus was assessed using spot tests, including standard K (5% KOH), C (commercial bleach), and KC (K followed by commercial bleach). Thin-layer chromatography was carried out using solvent system C, following standard methods (Orange *et al.* 2010). The specimens were deposited in the LAH herbarium, Institute of Botany, University of the Punjab, Lahore.

Molecular study

To conduct phylogenetic analysis, nuclear DNA was extracted from a portion of the thallus (including the apothecia, where available) using the Easypure® Plant Genomic DNA Kit following the manufacturer's instructions (TransGen Biotech Co., Ltd.). The primer pair ITS1F (forward primer: 5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS4 (reverse primer: 5′-TCCTCCGCTTATTGATATGC-3′) was utilized for amplifying the ITS (ITS1–5.8S–ITS2) region via PCR (White *et al.* 1990; Gardes & Bruns 1993), a marker widely used to resolve species-level relationships within Physciaceae. The PCR amplicons were analyzed via

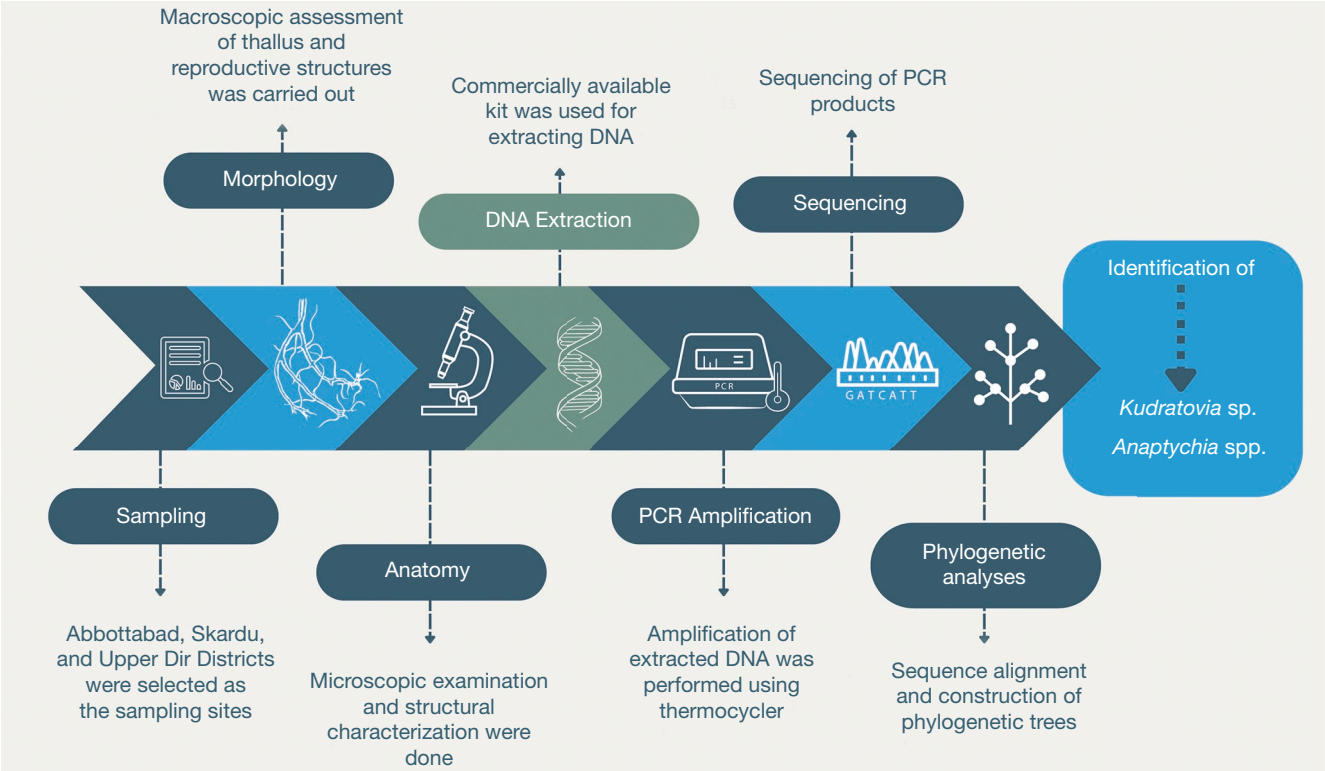

Fig. 1. — Workflow illustrating the methodological steps used in this study.

electrophoresis on a 1% agarose gel stained with ethidium bromide, followed by visualization using a gel documentation system (Sambrook & Russell 2001). Subsequently, the amplified products underwent sequencing at TsingKe, BioTech Company in Beijing, China.

Phylogenetic Study

Forward and reverse sequences were checked and assembled using BioEdit version 7.2.5 (Hall 1999). These nucleotide sequences were compared with related DNA sequences available online using the BLAST tool at NCBI (https://www.ncbi.nlm.nih.gov/guide). The ITS dataset was constructed by retrieving sequences from the GenBank database and incorporating relevant published data (Lohtander *et al.* 2008; Kondratyuk *et al.* 2021; Hollinger *et al.* 2022). Outgroup species were chosen from closely related genera outside the ingroup to provide a reliable root for each phylogenetic tree. The sequences used for phylogenetic analyses, along with their unique identifiers such as GenBank accession numbers, voucher codes, and geographic origins, are listed in Tables 1 and 2. The sequences were finally aligned using MAFFT provided by EMBL-EBI (https://www.ebi.ac.uk/jdispatcher/msa/mafft). Phylogenetic analyses were conducted via the CIPRES online portal (https://www.phylo.org/) using RAxML-HPC2 on XSEDE (8.2.10) with the GTR+GAMMA nucleotide substitution model and 1000 bootstrap replicates. The resulting Maximum Likelihood phylogenetic trees were visualized with FigTree v.1.4.2 (Rambaut 2014) and further edited using Adobe Illustrator CC 2021.

RESULTS AND DISCUSSION

Remark

Out of approximately 80 lichen samples collected from Khyber Pakhtunkhwa and Gilgit-Baltistan during 2022-2023 surveys, eight thalli exhibiting distinct phenotypic traits were tentatively identified as belonging to the family Physciaceae. These specimens were subjected to comprehensive analyses, including DNA-based phylogenetic assessments, morpho-anatomical examinations, and chemical studies (Fig. 1), as outlined below.

Family Physciaceae Zahlbr.
Genus *Kudratovia* S.Y.Kondr., Lőkös & Hur

Acta Botanica Hungarica 63 (3-4): 374 (Kondratyuk *et al.* 2021).

Diagnosis. — *Kudratovia* is characterized by a crustose thallus ranging from endolithic to epilithic and areolate to squamulose forms with a paraplectenchymatous cortex, lecanorine apothecia with or without pruina, *Bicincta*- or *Physcia*-type ascospores sometimes bearing elongated hyaline ends, bacilliform conidia, and the presence of zeorin, variolaric acid, and an unidentified fatty acid (Kondratyuk *et al.* 2021; Hyde *et al.* 2024). This genus is comprised of eight species with diverse global distributions and is typified by

TABLE 1. — Sequences used in the phylogenetic analyses of genus *Kudratovia* S.Y.Kondr., Lőkös & Hur with voucher number, accession number, country and reference.

Species	Origin	Voucher/ Isolate no	ITS	Reference
Coscinocladium gaditanum (Clemente) A.Crespo, Llimona & D.Hawksw.	Spain	MAF 9856	AY449721	Crespo *et al.* (2004)
Kudratovia bohlinii (H. Magn.) S.Y.Kondr., Lőkös & Hur	China	Hurnisa shahidin 20136744-3	MT435938	Tursun *et al.* (2020)
K. bohlinii	China	Hurnisa shahidin 20136608	MT435939	Tursun *et al.* (2020)
K. bohlinii	China	Hurnisa shahidin 20136718	MT435940	Tursun *et al.* (2020)
K. aff. *luridata* (Körb.) H.Mayrh., Schneid. & Sheard	United States	Leavitt 19047v2	MZ922199	Munger *et al.* (2022)
K. aff. *luridata*	United States	Leavitt 19060	MZ922200	Munger *et al.* (2022)
K. aff. *luridata*	United States	Leavitt 19070v2	MZ922201	Munger *et al.* (2022)
K. aff. *luridata*	United States	Leavitt 19073v4	MZ922202	Munger *et al.* (2022)
K. candidogrisea (Hafellner, Muggia & Obermayer) S.Y. Kondr., Lőkös & Hur	Germany	Hafellner 75179	JN211107	Hafellner *et al.* (2012)
K. candidogrisea	Austria	Hafellner 73530	JN211108	Hafellner *et al.* (2012)
K. candidogrisea	Austria	Hafellner 76033	JN211109	Hafellner *et al.* (2012)
K. luridata (Körb.) S.Y. Kondr., Lőkös & Hur	New Zealand	H. Mayrhofer 12122 (GZU)	DQ849304	Kaschik (2006)
K. luridata	Greece	GZU 000272660	GU553298	Nadyeina *et al.* (2010)
K. metaboliza (Vain.) S.Y. Kondr., Lőkös & Hur	China	Hurnisa shahidin 20080219	MT260861	Tursun *et al.* (2020)
K. metaboliza	China	Hurnisa shahidin 20080242	MT260862	Tursun *et al.* (2020)
K. metaboliza	China	Hurnisa shahidin 20080251	MT260863	Tursun *et al.* (2020)
K. pakistanica U.F. Ahmad, Afshan & Khalid	Pakistan	SK-12	PV687690	This paper
K. pakistanica	Pakistan	SK-30	PV687691	This paper
K. pycnocarpa (H. Magn.) S.Y. Kondr., Lőkös & Hur	China	Hurnisa shahidin 20130788	MT435947	Tursun *et al.* (2020)
K. roscida (Sommerf.) S.Y. Kondr., Lőkös & Hur	Russia	S. Kholod plot515 (GZU)	DQ849317	Kaschik (2006)
K. terrestris (J. Steiner) S.Y. Kondr., Lőkös & Hur	China	Hurnisa shahidin 183220	MT435946	Tursun *et al.* (2020)
K. straussii (Tomin) S.Y. Kondr., Lőkös & Hur	China	Hurnisa shahidin 20110228-B	MT435942	Tursun *et al.* (2020)
K. straussii	China	Hurnisa shahidin 20110231-C	MT435943	Tursun *et al.* (2020)
K. straussii	China	Hurnisa shahidin 20110231	MT435944	Tursun *et al.* (2020)
K. straussii	China	Hurnisa shahidin 20110228-a	MT435945	Tursun *et al.* (2020)

Kudratovia straussii (J. Steiner) S.Y.Kondr., Lőkös & Hur (Kondratyuk *et al.* 2021). From Pakistan, only *K. bohlinii* (H. Magn.) S.Y.Kondr., Lőkös & Hur has been documented, occurring on rocks (Aptroot & Iqbal 2012).

PHYLOGENETIC ANALYSIS AND TAXONOMIC NOTES

The phylogenetic tree was constructed using 25 ITS sequences, including 24 ingroup sequences representing nine species of *Kudratovia*, with *Coscinocladium gaditanum* (Clemente) A. Crespo (AA449721) used as an outgroup for rooting the tree. The final data matrix consists of 516 characters, of which 333 are conserved, 178 are variable, 115 are parsimony-informative, and 62 are singleton sites. The phylogenetic tree reveals two primary subgroups: the *Kudratovia straussii* and *K. roscida* groups (Hafellner *et al.* 2012; Kondratyuk *et al.* 2021). In our analysis, the Pakistani samples (SK-12 and SK-30) clustered within the *K. straussii* group, forming a distinct branch (64% ML), diverging from the clade comprising *K. bohlinii*, *K. luridata*, and *K.* aff. *luridata*. This distinct phylogenetic placement provides strong evidence supporting the recognition of the Pakistani specimens as a novel species (Fig. 2).

Kudratovia pakistanica
U.F. Ahmad, Afshan & Khalid, sp. nov.
(Fig. 3)

DIAGNOSIS. — Similar to *K. luridata* but characterized by a thick, indeterminate to determinate thallus (300-400 µm), plane to convex apothecial discs, 0.3-0.8 µm (vs plane to concave, 0.3-0.6 µm), significantly deeper hypothecium in mature asci (up to 200 µm vs 70-90 µm), larger asci (68-92 × 12-24 µm vs 50-56 × 18-22 µm), and larger ascospores (16-19 × 10-12 µm vs 11-15 × 6.5-11 µm).

MYCOBANK. — 861404.

ETYMOLOGY. — The epithet '*pakistanica*' (Latin) refers to the country of origin of this species.

TYPE MATERIAL. — **Pakistan** • Gilgit-Baltistan, Skardu District; 35°16'01"N, 75°37'48"E; 2552 m a.s.l.; on rock; 10.VII.2022; *N.S. Afshan & K. Habib leg.*; holotype: *SK-12*; GenBank: PV687690 (ITS) • *ibid.*; 35°16'10"N, 75°38'43"E; 2482 m a.s.l.; on rock; 10.VII.2022; *A.N. Khalid & K. Habib leg.*; paratype: *SK-30*; GenBank: PV687691 (ITS).

SPOT TESTS. — K–, KC–, C–. Zeorin detected through TLC.

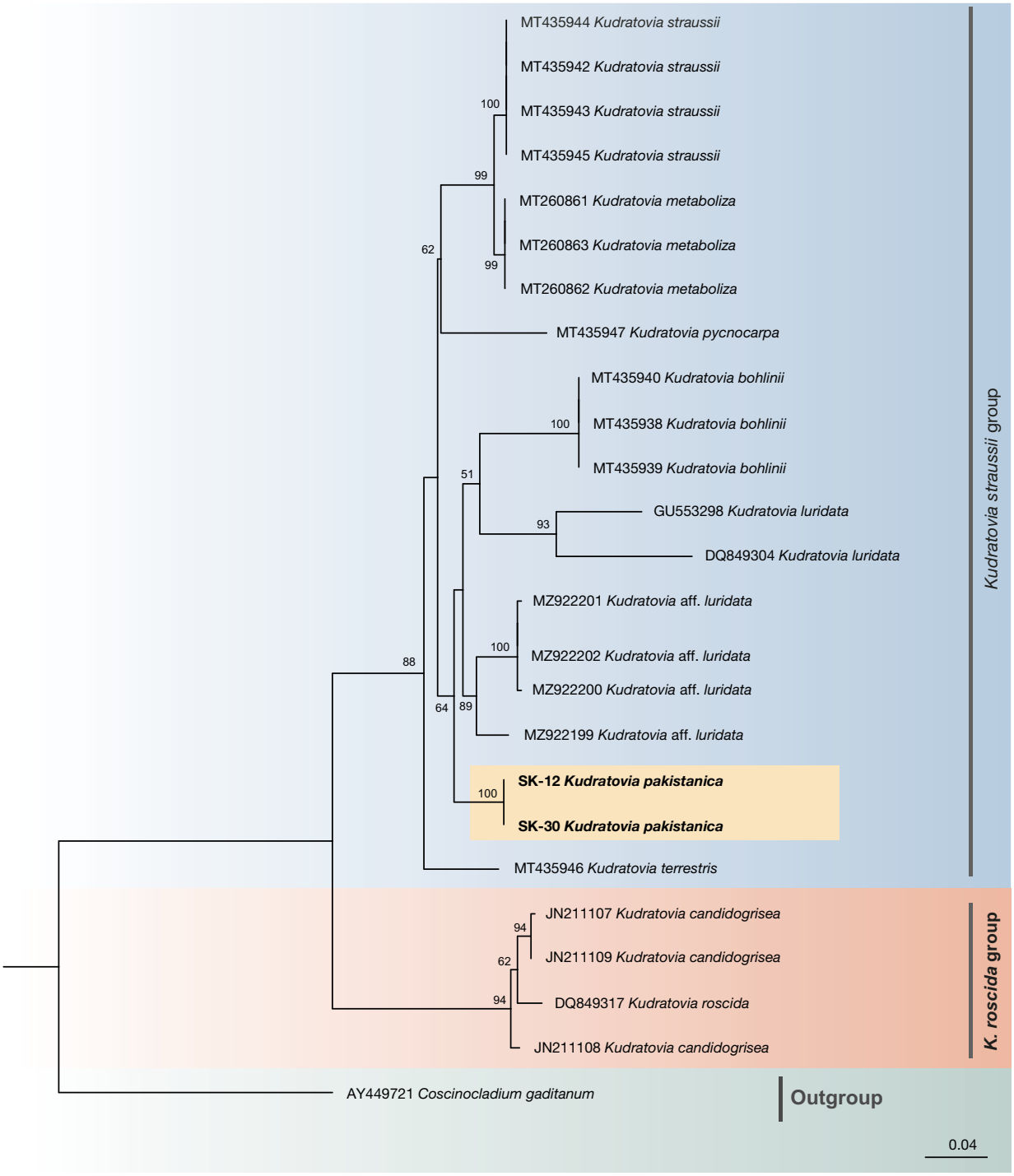

Fig. 2. — Phylogenetic tree of the genus *Kudratovia* S.Y.Kondr., Lőkös & Hur as generated by maximum likelihood (ML) analyses based on ITS sequences. Bootstrap values up to 50% based on 1000 replicates are shown at the branches. Novel sequences generated during this study are shown in **bold**.

Substrate and ecology. — Found on calcareous rock, in cold and semi-arid climate at an elevation above 2600 m, exposed to rain and sunlight, with maximum and minimum temperatures of 27°C and –10°C, respectively, and annual rainfall varying between 90-150 mm.

Description

Thallus crustose, areolate, effuse, thick, mostly indeterminate to sometimes determinate with prothallus, up to 3 cm across, 300-400 µm thick.

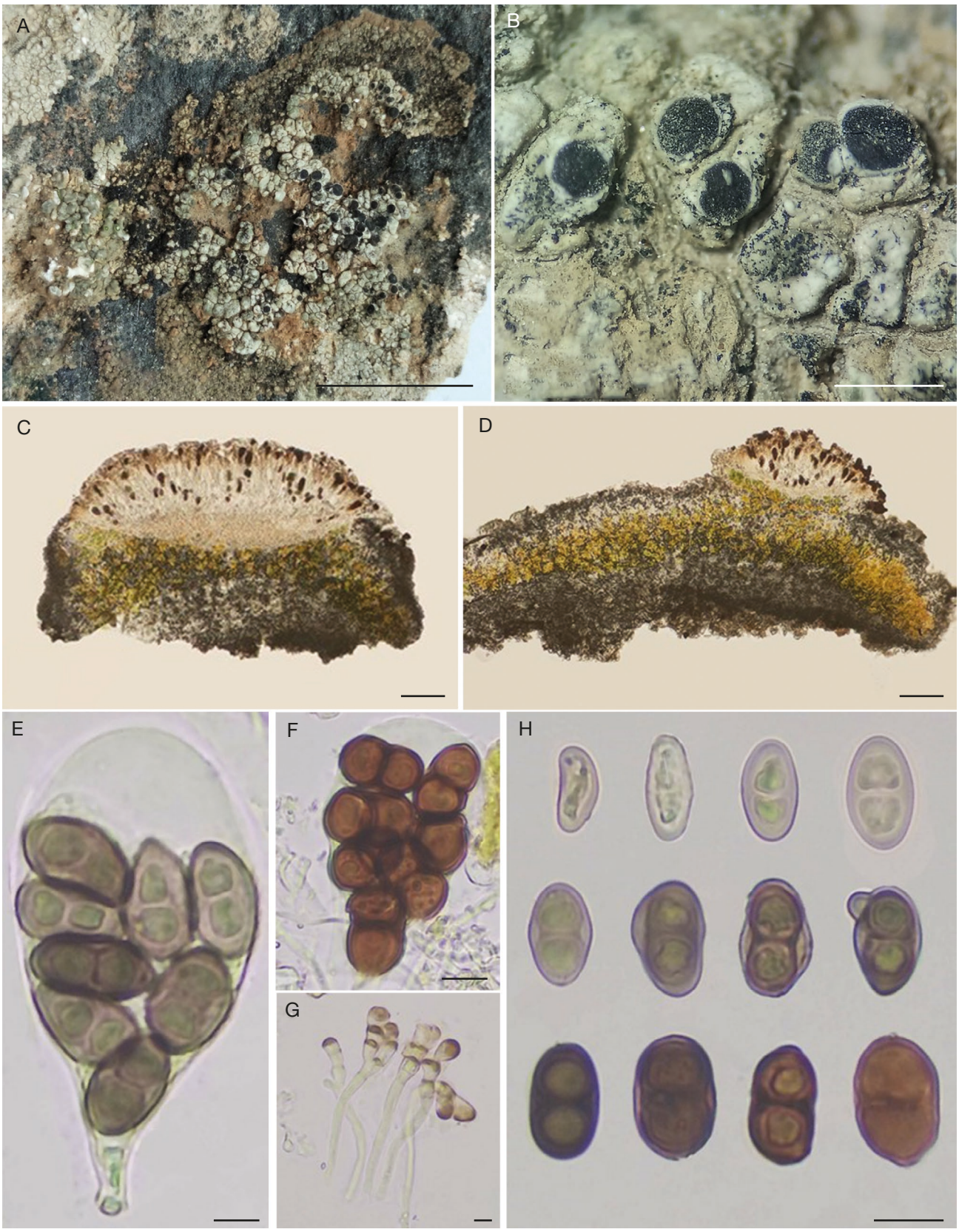

Fig. 3. — *Kudratovia pakistanica* U.F. Ahmad, Afshan & Khalid, sp. nov.: **A**, thallus; **B**, areoles and apothecia; **C**, apothecium in cross-section; **D**, cross-section of thallus showing erumpent apothecia; **E**, **F**, eight-spored asci; **G**, paraphyses; **H**, *Bicincta*-type ascospores. Scale bars: A, 1 cm; B, 1 mm; C, D, 100 µm; E, F, H, 10 µm; G, 5 µm. Photo credits: Uswa Fatima Ahmad.

TABLE 2. — Sequences used in the phylogenetic analyses of genus *Anaptychia* Körb. with voucher number, accession number, country and reference.

Species	Origin	Voucher/Isolate no	ITS	Reference
Anaptychia ciliaris (L.) Flot.	Spain	BCN-Lich 15484	GU247148	Unpublished
A. ciliaris	Spain	Dal Grande	KJ027712	Dal Grande *et al.* (2014)
A. ciliaris	Spain	Dal Grande	KJ027713	Dal Grande *et al.* (2014)
A. ciliaris	Ukraine	Kondratyuk 21326	KP747674	Kondratyuk *et al.* (2015)
A. ciliaris	Czech Republic	PRA-Vondrak2602	OQ717299	Vondrák *et al.* (2023)
Anaptychia crinalis (Schleich. ex Schaer.) Vězda	United States	Wetmore 83986 (MIN)	EF582785	Lohtander *et al.* (2008)
A. crinalis	Turkey	John exs. 131 (UPS)	EF582786	Lohtander *et al.* (2008)
A. crinalis	Switzerland	LIFU045-16	KX132954	Mark *et al.* (2016)
A. crinalis	India	JK-30	MZ836029	Unpublished
A. crinalis	United States	Henderson 34 (herb. Esslinger)	OM993335	Hollinger *et al.* (2022)
A. crinalis	Pakistan	LAH38290	PQ276732	This paper
A. crinalis	Pakistan	LAH38291	PQ276733	This paper
Anaptychia desertorum (Rupr.) Poelt	Kazakhstan	Moberg K21:12 & Nordin (UPS)	EF582783	Lohtander *et al.* (2008)
A. desertorum	Russia	Urbanavichus 5 A02 (H)	EF582784	Lohtander *et al.* (2008)
Anaptychia elbursiana (Szatala) Poelt	United States	Shevock 12350 (ASU)	AY368145	Cubero *et al.* (2004)
A. elbursiana	United States	Wetmore 81482 (MIN)	EF582782	Lohtander *et al.* (2008)
A. elbursiana	United States	Hollinger 12813 (BRY-C)	OM991841	Hollinger *et al.* (2022)
A. elbursiana	United States	Hollinger 7503 (UPS)	OM991842	Hollinger *et al.* (2022)
A. elbursiana	United States	Hollinger 9564 (BRY-C)	OM991843	Hollinger *et al.* (2022)
A. elbursiana	United States	Hollinger 11152 (BRY-C)	OM991844	Hollinger *et al.* (2022)
A. elbursiana	United States	Hollinger 11767 (H)	OM991845	Hollinger *et al.* (2022)
A. elbursiana	United States	Hollinger 12105 (UPS)	OM991846	Hollinger *et al.* (2022)
A. elbursiana	United States	Hollinger 13212 (ASU)	OM991847	Hollinger *et al.* (2022)
A. elbursiana	United States	Hollinger 16426 (NY)	OM991848	Hollinger *et al.* (2022)
A. elbursiana	United States	Hollinger 17066 (H)	OM991849	Hollinger *et al.* (2022)
A. elbursiana	United States	Hollinger 8032 (BRY-C)	OM991850	Hollinger *et al.* (2022)
Anaptychia nevadensis Hollinger, Noell & S.D.Leav.	United States	Hollinger 11768 (ASU)	OM991851	Hollinger *et al.* (2022)
A. nevadensis	United States	Hollinger 11807 (ASU)	OM991852	Hollinger *et al.* (2022)
A. nevadensis	United States	Hollinger 11942 (NY)	OM991853	Hollinger *et al.* (2022)
A. nevadensis	United States	Hollinger 15905 (NY)	OM991854	Hollinger *et al.* (2022)
A. nevadensis	United States	Hollinger 15906 (H)	OM991855	Hollinger *et al.* (2022)
A. nevadensis	United States	Hollinger 15907 (H)	OM991856	Hollinger *et al.* (2022)
A. nevadensis	United States	Hollinger 15912 (UPS)	OM991857	Hollinger *et al.* (2022)
A. nevadensis	United States	Hollinger 7747 (BRY-C)	OM991858	Hollinger *et al.* (2022)
Anaptychia roemeri Poelt	Armenia	Aptroot 73899a (BGBM)	OM993333	Hollinger *et al.* (2022)
A. roemeri	Iran	Sohrabi 1103 (UPS)	OM993334	Hollinger *et al.* (2022)
A. roemeri	Armenia	Gasparyan V-14-02a	OM993337	Hollinger *et al.* (2022)
A. roemeri	Armenia	Gasparyan V-13-3	OM993338	Hollinger *et al.* (2022)
Anaptychia roemerioides Hollinger, Noell & S.D.Leav.	United States	Leavitt 16.588 (hb. Esslinger)	OM993336	Hollinger *et al.* (2022)
A. roemerioides	United States	Hollinger 11949 (BRY-C)	OM991859	Hollinger *et al.* (2022)
A. roemerioides	United States	Hollinger 12689 (NY)/12688 (ASU)	OM991860	Hollinger *et al.* (2022)
A. roemerioides	United States	Hollinger 15523 (H)	OM991861	Hollinger *et al.* (2022)
A. roemerioides	United States	Hollinger 15910 (H)	OM991862	Hollinger *et al.* (2022)
A. roemerioides	Pakistan	LAH38294	PQ276736	This paper
A. roemerioides	Pakistan	LAH38295	PQ276737	This paper
Anaptychia setifera Mereschk. ex Räsänen	Armenia	MAF:Lich. 20421	KX457686	Unpublished
A. setifera	Iran	Sipman 55211	OM993339	Hollinger *et al.* (2022)
A. setifera	Armenia	Gasparyan D-13-1.18	OM993340	Hollinger *et al.* (2022)
A. setifera	Armenia	Gasparyan N-15-06	OM993341	Hollinger *et al.* (2022)
A. setifera	Armenia	Gasparyan T-14-04b	OM993343	Hollinger *et al.* (2022)
A. setifera	Armenia	Gasparyan D-15-19	OM993344	Hollinger *et al.* (2022)
A. setifera	Pakistan	LAH38292	PQ276734	This paper
A. setifera	Pakistan	LAH38293	PQ276735	This paper
Anaptychia ulotricoides (Vain.) Vain.	Kyrgyzstan	Rambold 6162 & Triebel	AF540494	Helms *et al.* (2003)
Kurokawia bryorum (Poelt) S.Y.Kondr., Lőkös & Hur	Austria	U383	AF250781	Grube & Arup (2001)
Kurokawia isidiata (Tomin) S.Y.Kondr., Lőkös & Hur	Korea	Hur 060480-2	EU045430	Unpublished
K. isidiata	South Korea	162011 KoLRI	OK138533	Kondratyuk *et al.* (2021)
K. isidiata	South Korea	162012 KoLRI	OK138534	Kondratyuk *et al.* (2021)
K. isidiata	South Korea	060480 KoLRI	OK138535	Kondratyuk *et al.* (2021)
K. isidiata	South Korea	121046 KoLRI	OK138536	Kondratyuk *et al.* (2021)
Kurokawia palmatula (Michx.) S.Y.Kondr., Lőkös & Hur	United States	Ahti 58054	EF582779	Lohtander *et al.* (2008)
Kurokawia runcinata (With.) S.Y.Kondr., Lőkös & Hur	Sweden	Odelvik S Karlsson 00243	EF582775	Lohtander *et al.* (2008)
Phaeophyscia ciliata (Hoffm.) Moberg	Russia	Himelbrant K-04-23 (LECB)	EF582752	Lohtander *et al.* (2008)

Areoles
Discrete, angular to mostly irregular, cracked, fissured, plane to weakly convex or concave, 0.3-1.0 mm in diameter.

Prothallus
Often present, creamy to pale brown.

Upper surface
Rough, rugose, matt, pruinose.

Color
Pale grayish-beige to creamy when dry, no change when wet.

Upper cortex
Paraplectenchymatous, 20-45 µm thick, greyish, cells globose to sub-globose, 14-20 µm in diameter.

Algal layer
Even, continuous, 68-120 µm thick, photobiont chlorococcoid, globose to sub-globose, 12-24 µm in diameter.

Medullary layer
Prosoplectenchymatous, 145-200 µm thick, hyphae white, 3-5 µm wide.

Apothecia
Frequent, cryptolecanorine, black, 1-2 per areole, usually 1 per areole.

Disc
Black, 0.3-0.8 mm in diam., weakly fissured, pruinose, dull to somewhat shiny, plane to weakly convex, rounded.

Margin
Thin, persistent, concolorous with the thallus.

Paraphyses
Hyaline, septate, 1.5-3 µm wide, terminal 3-5 cells slightly swollen, 3-6 µm wide apically, pigmented at the tip, unbranched or with few branches.

Epihymenium
Light to dark brown, 12-30 µm thick.

Hymenium
Hyaline, 80-130 µm thick.

Hypothecium
Hyaline to slightly brownish, in mature apothecia tapered into a stipe, up to 200 µm deep.

Asci
Clavate, hyaline, 8-spored, 68-92 × 12-24 µm.

Ascospores
Bicincta-type, Type A ontogeny, hyaline to light grey at first, becoming brown when mature, broadly ellipsoidal, 1-septate, pigmented band around cells is visible, young spores inflated at septum, becoming constricted when over-matured, torus absent, 16-19 × 10-12 µm.

Pycnidia
Frequent, immersed, 156-220 µm × 120-250 µm, ostiole grey to light brown.

Conidia
Bacilliform, short cylindrical, hyaline, 3-5.2 × 1 µm.

NOTES
Kudratovia pakistanica U.F. Ahmad, Afshan & Khalid, sp. nov. is closely related to *K. bohlinii*, both morphologically and phylogenetically. However, it differs by having an indeterminate, creamy to pale brown thallus (vs determinate, alutaceous thallus), a thinner upper cortex measuring 20-45 µm (vs 50-70 µm), a brown epihymenium (vs green to brown), smaller ascospores measuring 16-19 × 10-12 µm (vs 18-22.5 × 10.5-12 µm), and smaller conidia measuring 3-5.2 × 1 µm (vs 7-8 × 1 µm) (Poelt & Wirth 1968; Mayrhofer 1984).

The new species shares some morpho-anatomical features with *K. luridata* (Körb.) S.Y.Kondr., Lőkös & Hur, including a rugose, areolate thallus, erumpent apothecia, and *Bicincta*-type ascospores. However, it can be distinguished by a thicker, indeterminate thallus (vs thinner, determinate thallus), significantly deeper hypothecium in mature asci (up to 200 µm vs 70-90 µm), larger, plane to convex apothecia (0.3-0.8 mm vs plane to concave, 0.3-0.6 mm), larger asci (68-92 × 12-24 µm vs 50-56 × 18-22 µm), and larger ascospores (16-19 × 10-12à vs 11-15 × 6.5-11 µm) (Mayrhofer *et al.* 1990).

Kudratovia metaboliza (Vain.) S.Y.Kondr., Lőkös & Hur resembles *K. pakistanica* U.F. Ahmad, Afshan & Khalid, sp. nov. in spore size, but the latter has erumpent apothecia (absent in *K. metaboliza*), *Bicincta*-type ascospores (vs *Dirinaria*-type), and Type A spore development (vs Type B) (Sheard *et al.* 2017).

Kudratovia pakistanica U.F. Ahmad, Afshan & Khalid, sp. nov. also resembles *K. straussi* as both have a thick, areolate thallus and plane to convex, pruinose apothecial discs. However, *K. straussi* has a light to dark grey thallus with sublobate margins, larger apothecia (0.55-1 mm), and relatively larger ascospores (17.5-24 × 9.5-14 µm) (Sheard 2010).

NEW RECORDS AND NEW COLLECTIONS

Genus *Anaptychia* Körb.

Grundriss der Kryptogamen-Kunde: 197 (Körber 1848)

DIAGNOSIS. — This genus is characterized by a prosoplectenchymatous upper cortex, either lacking atranorin or containing it in minimal amounts (K–), a lower cortex that is either poorly developed or absent, a pale lower surface with simple rhizines, lecanorine apothecia, and thin-walled, brown, one-septate spores of *Physconia*-type (Kurokawa 1962; Poelt 1965; Poelt & Vezda 1977; Esslinger 2007; Kondratyuk *et al.* 2021; Hollinger *et al.* 2022). *Anaptychia ciliaris* (L.) Körb. ex A. Massal. is the type species of the genus *Anaptychia (*Kurokawa 1962). The genus *Anaptychia* comprises ten species

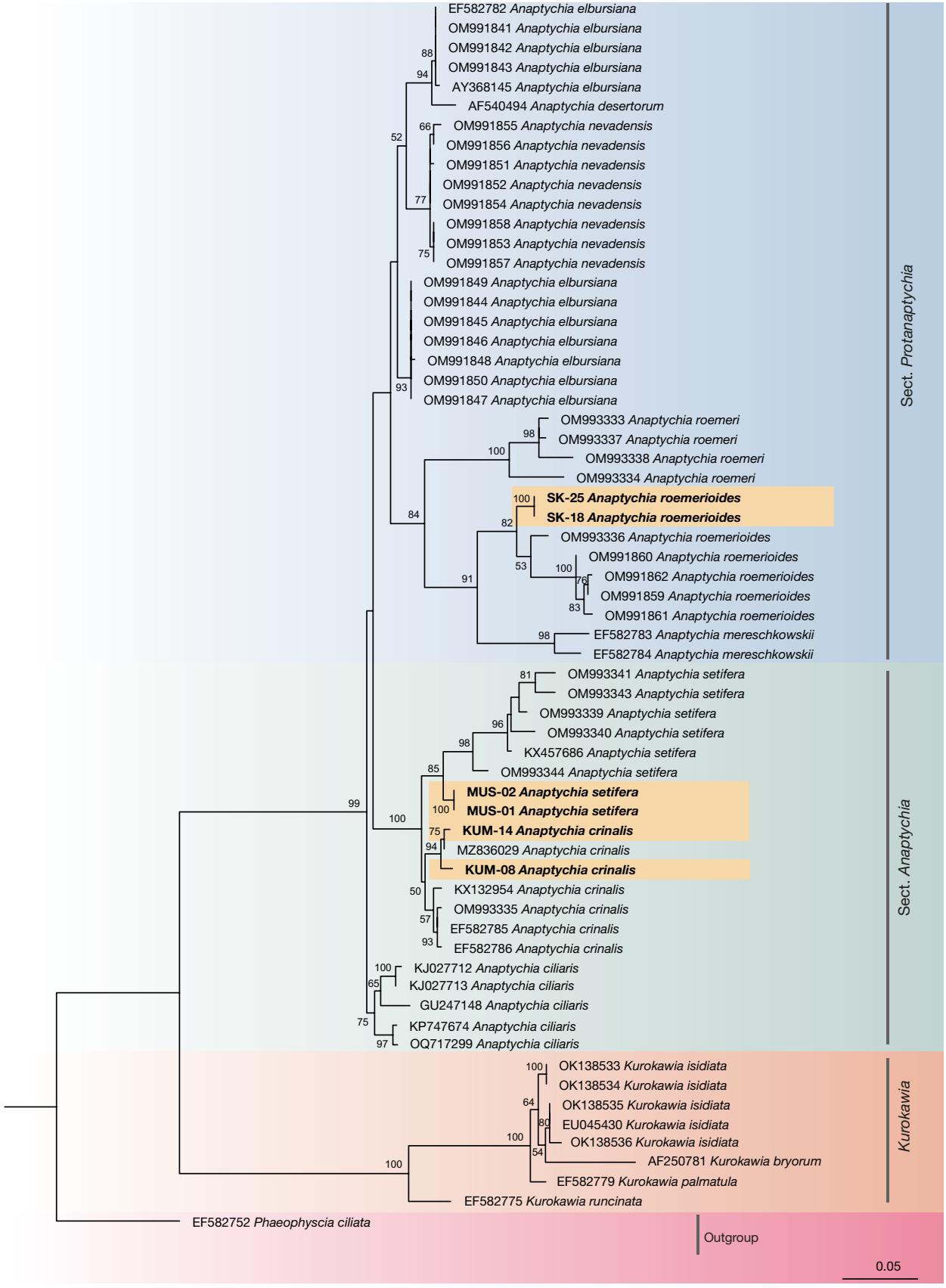

Fig. 4. — The phylogenetic tree of the genera, *Anaptychia* Körb. and *Kurokawia* S.Y.Kondr., Lőkös & Hur is generated by Maximum Likelihood (ML) method based on ITS sequences. Bootstrap values up to 50% are shown at the branches based on 1000 replicates. Sequences generated during this study are in **bold**.

according to Hollinger *et al.* (2022). From Pakistan, only *A. setifera* (syn. *A. kaspica*) has been documented, growing on tree bark and rocks (Aptroot & Iqbal 2012).

Phylogenetic analysis and taxonomic notes

The phylogenetic tree was constructed using 63 ITS sequences representing nine species of *Anaptychia* (excluding *A. ethiopica* Swinscow & Krog) and four species of *Kurokawia* S.Y.Kondr., Lőkös & Hur, including six newly generated sequences (Fig. 4). *Phaeophyscia ciliata* (Hoffm.) Moberg (EF582752) was chosen as an outgroup for rooting the tree. The final data matrix consists of 520 characters, of which 287 are conserved, 214 are variable, 160 are parsimony-informative, and 54 are singleton sites. The tree topology showed a clear separation between *Anaptychia* s.l. and *Kurokawia* and suggested the existence of two distinct sections within the genus *Anaptychia*: *Anaptychia* and *Protanaptychia*, which is consistent with previous studies (Lohtander *et al.* 2008; Kondratyuk *et al.* 2021; Hollinger *et al.* 2022).

The first group, *Anaptychia* section *Anaptychia* Körb., is characterized by fruticose thalli with hyaline spinules or tomentum on the upper surface, featuring strongly ascending, elongated branches and lacking a lower cortical layer. This group is typically found in moist, cold regions and includes species such as *A. ciliaris* (L.) Folt, *A. crinalis*, *A. ethiopica*, and *A. setifera* (Swinscow & Krog 1976; Kondratyuk *et al.* 2021; Hollinger *et al.* 2022). The second group corresponds to the section *Protanaptychia* Poelt, characterized by foliose, appressed, or mat-forming thalli with weakly developed prosoplectenchymatous lower cortex and simple rhizines. These species are commonly distributed in arid to semi-arid regions of the Northern Hemisphere, including *A. desertorum* (Rupr.) Poelt, *A. elbursiana* (Szatala) Poelt, *A. mereschkowskii* (Tomin) Kulakov, *A. nevadensis* Hollinger, Noell & S.D. Leav., *A. roemeri* Poelt, and *A. roemerioides* (Lohtander *et al.* 2008; Kondratyuk *et al.* 2021; Hollinger *et al.* 2022).

The sequences generated in this study nested within the phylogenetic branch of the genus *Anaptychia*, and confirm *A. crinalis* and *A. roemerioides* as new records for Pakistan and validates the occurrence of *A. setifera* in the country (Fig. 3). *Anaptychia crinalis* (KUM-08 & KUM-14) and *A. setifera* (MUS-01 & MUS-02) from our collections occurred in a clade with other sequences of *A. crinalis* and *A. setifera* with strong bootstrap support (100% ML). The Pakistani sequences of *A. crinalis* (KUM-8 & KUM-14) grouped with the sequences of the same taxon reported from the United States (EF582785 & OM993335), Turkey (EF582786), Switzerland (KX132954) & India (MZ836029; although deposited as *A. ciliaris* in GenBank, it clusters within the *A. crinalis* lineage, suggesting a probable misidentification), thus confirming their identification as *A. crinalis*, which is further confirmed by morpho-anatomical data. The sequences of Pakistani specimens of *A. setifera* (MUS-01 & MUS-02), clustered with the sequences of the same taxon reported from Armenia (KX457686, OM993340, OM993341 and OM993343) and Iran (OM993339), which is further validated by phenotypic characteristics. The newly generated sequences of third *Pakistani* species (SK-18 and SK-25) nested in a well-supported (91% ML) *A. roemeri* group, together with *A. mereschkowskii*, *A. roemeri* and *A. roemerioides*. Our molecular analysis supports the identification of this species (SK-18 & SK-25) as *A. roemerioides* because the Pakistani specimens formed a clade with the sequences of same taxon that originated from the United States (OM991859, OM991860, OM991861, OM991862 & OM993336), expanding its known geographical distribution.

Anaptychia crinalis
(Schleich. ex Schaer.) Vězda ex J. Nowak
(Fig. 5)

Porosty (Lichenes). 6, Physciaceae sensu stricto (Warszawa) (3): 13 (Nowak 1993).

Materials examined. — **Pakistan** • Khyber Pakhtunkhwa, Upper Dir District, Kumrat Valley; 35°32'05"N, 72°13'12"E; 2337 m a.s.l.; on bark; 21.IX.2023; *M. Usman & A.N. Khalid leg.*; U.F. Ahmad det.; KUM-14; voucher: LAH38291; GenBank: PQ276733 (ITS) • Khyber Pakhtunkhwa, Upper Dir District, Kumrat Valley; 35°33'48"N, 72°11'29"E; 2364 m a.s.l.; on bark; 22.IX.2023; *M. Usman & A.N. Khalid leg.*; U.F. Ahmad det.; KUM-8; voucher: LAH38290; GenBank: PQ276732 (ITS).

Substrate and ecology. — Found growing on barks in humid subtropical climate at an elevation above 2000 m, with an average annual precipitation varying between 1000-1200 mm. December typically has the coldest average monthly temperature, around 0.3°C, while June tends to be the warmest, reaching approximately 25°C.

Spot tests. — K–, KC–, C–.

Description

Thallus fruticose, dorsiventral, erect to bushy, epruinose, esorediate.

Color
Greenish grey when dry, green when wet. Lobes: dichotomously branched, narrow, linear, elongated, entangled, 0.1-1 mm thick.

Cilia
Marginal, rarely laminal, brown to black.

Upper surface
Dull, epruinose, smooth, tomentose.

Lower surface
White, fibrous, flat.

Upper cortex
22-85 µm thick, globose to subglobose hyaline prosoplectenchymatous cells, 8-24 µm in diameter.

Algal layer
Uneven, discontinuous, 40-82 µm thick, cells globose to subglobose, 8-22 µm in diameter.

Fig. 5. — *Anaptychia crinalis* (Schleich. ex Schaer.) Vězda ex J. Nowak (LAH38290): **A**, thallus (dry); **B**-**D**, apothecia under stereomicroscope; **E**, pycnidia under stereomicroscope; **F**, section of apothecium; **G**, section of thallus; **H**, section of pycnidium; **I**, Pycniospores; **J**, Ascospores. Scale bars: A, 1 cm; B, C, 1 mm; D, 1 mm; E, 0.5 mm; F, G, 100 µm; H, 50 µm; I, 5 µm; J 20 µm. Photo credits: Uswa Fatima Ahmad.

Medulla
Hyaline hypheae, loosely interwoven, 60-170 µm thick.

Lower cortex
Absent or poorly organized.

Apothecia
Frequent, lecanorine, laminal appearing terminal, up to 3.5 mm in diameter, stalked, dull.

Disc
Rounded, strongly concave first, remain concave or become flat with age, dark brown to black, often densely pruinose.

Margin
Concolourous with thallus, thick, persistent, weakly to strongly raised, mostly spinulate, spinules 0.2-1.2 mm long.

Epihymenium
15-30 µm thick, orange brown.

Hymenium
110-120 µm thick, hyaline.

Hypothecium
32-40 µm thick, brown.

Asci
Clavate, hyaline, 8-spored, 110-145 µm × 30-48 µm.

Ascospores
Physconia-type, 1-septate, often weakly to strongly bent, mostly with hyaline knob at one or both ends, pale brown initially, becoming dark brown at maturity, 30-50 µm × 10-24 µm.

Pycnidia
Frequent, immersed, 120-280 µm tall × 98-220 µm wide, ostiole brown to black.

Conidia
Hyaline, bacilliform, 3.5-5 µm × 0.9-1.1 µm.

NOTES
This species is characterized by a fruticose, dorsiventral, erect to bushy thallus with long, narrow and branched lobes, lecanorine apothecia with spinulate margins, *Physconia*-type 1-septate ascospores, and immersed pycnidia with hyaline bacilliform conidia (Esslinger 2007). For morphological comparison, the characteristics of the Pakistani specimen were compared with the description of *A. crinalis* given by Esslinger (2007) which shows that both of them share the same characteristics such as narrow, dorsiventral fruticose thallus and linear, highly branched lobes measuring 0.1-1 mm but the Pakistani specimen differs in having smaller apothecial discs (up to 3.5 mm vs up to 4.5 mm in diameter) and slightly larger ascospores (30-50 × 10-24 µm vs 35-46 × 17-22 µm). It is morphologically and phylogenetically similar to *A. setifera* but differs in having narrower lobes 0.1-1 mm (vs up to 1 mm) (Esslinger 2007). This study represents the first report of this species from Pakistan.

Anaptychia roemerioides Hollinger, Noell & S.D. Leav. (Fig. 6)

Bryologist 125 (4): 595 (Hollinger *et al.* 2022)

MATERIAL EXAMINED. — **Pakistan** • Gilgit-Baltistan, Skardu District; 35°17'37"N, 75°40'54"E; 2789 m a.s.l.; on rock; 10.VII.2022; *N.S. Afshan & K. Habib leg.*; U.F. Ahmad det.; SK-18; voucher: LAH38294; GenBank: PQ276736 (ITS) • Gilgit-Baltistan, Skardu District; 35°16'10"N, 75°38'43"E; 2482 m a.s.l.; on rock; 10.VII.2022; *N.S. Afshan & K. Habib leg.*; U.F. Ahmad det.; SK-25; voucher: LAH38295; GenBank: PQ276737 (ITS).

SPOT TESTS. — K–, KC–, C–.

SUBSTRATE AND ECOLOGY. — Found growing on calcareous rocks in dry temperate forest at an elevation above 2400 m, with maximum and minimum temperatures of 27°C and –10°C, respectively, and an annual rainfall varying between 90-150 mm.

DESCRIPTION
Thallus foliose, loosely adnate to the substratum, orbicular to irregular, dispersed, slightly pruinose near tips, forming discrete patches, lobate, up to 8.5 cm across, esorediate.

Lobes
Irregular, dense, branched, elongated, contiguous, imbricate, up to 1.2 mm wide.

Margins
Imbricate, black, flat to slightly upturned, ascending.

Color
Dark grey when dry, olive green with a blackish tint when wet.

Lower surface
Off-white to pale brown in young parts, dark brown towards the center, ecorticate near margins, smooth to warty, dull, moderately rhizinate.

Rhizine
Concolorous with lower surface, becoming dark brown, scattered, simple to fasciculate, 0.5-2 mm long.

Upper cortex
Brown, well-developed, prosoplectenchymatous, 25-40 µm thick, cells spherical or sub-spherical, 12-20 µm in diameter.

Algal layer
Continuous, uneven, heteromerous, 45-80 µm thick.

Photobiont
Globose to sub-globose, chlorococcoid, 10-20 µm in diameter.

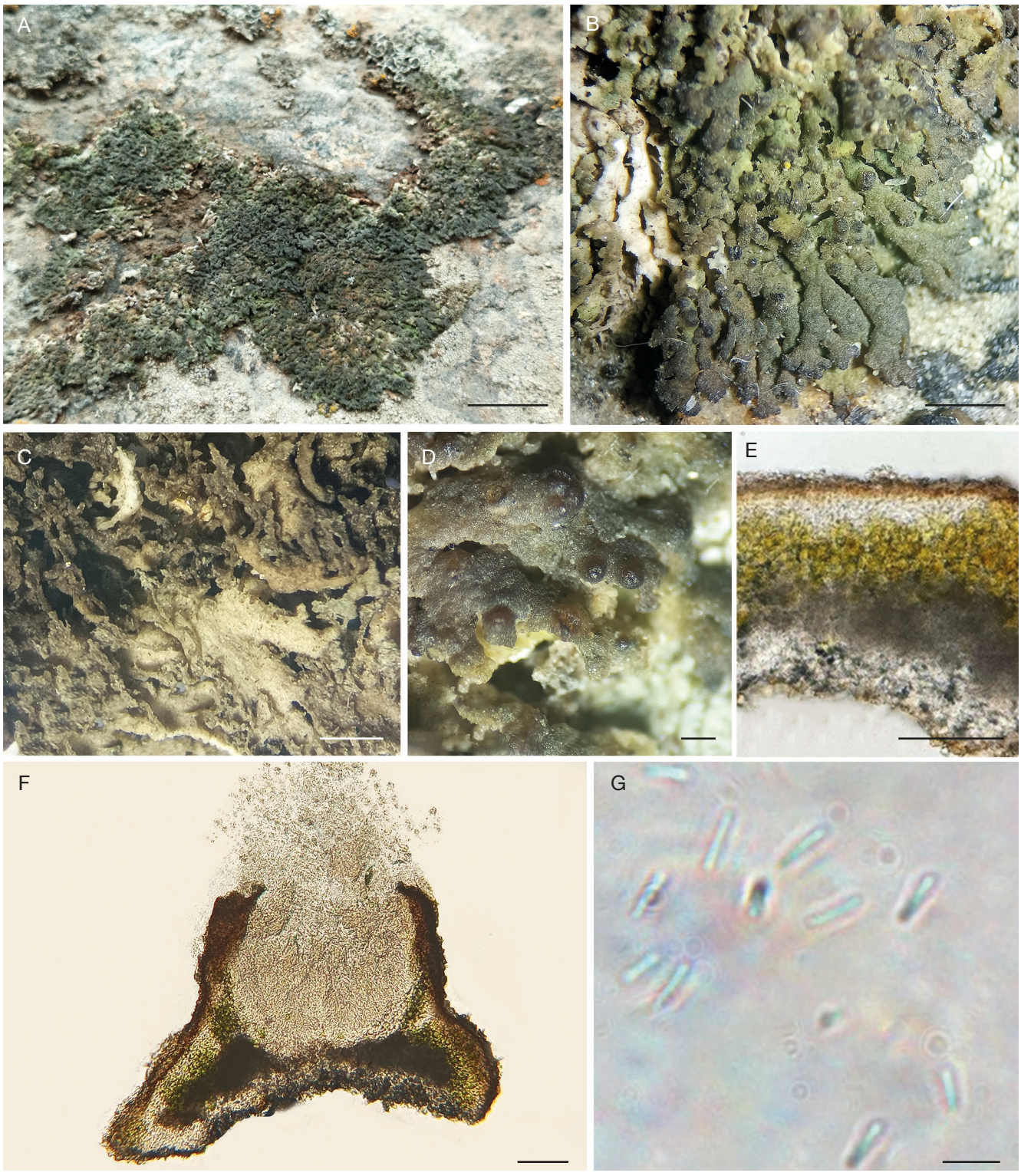

FIG. 6. — *Anaptychia roemerioides* Hollinger, Noell & S.D.Leav. (LAH38294): **A**, **B**, thallus; **C**, lower surface of the thallus; **D**, pycnidia under stereomicroscope; **E**, section of thallus; **F**, section of pycnidium; **G**, Pycniospores. Scale bars: A, 1 cm; B, C, 1.5 mm; D, 0.5 mm; E, F, 100 µm; G, 5 µm. Photo credits: Uswa Fatima Ahmad.

Medulla
Well-developed, prosoplectenchymatous, 110-150 µm thick.

Lower cortex
Prosoplectenchymatous, poorly developed.

Pycnidia
Frequent, dark brown, laminal, immersed in raised bumps, 0.2-0.5 mm in diameter.

Conidia
Hyaline, cylindrical, straight, 4.5-5.5 µm × 1 µm.

Apothecia
Not present in our collections.

NOTES
This species is characterized by delicate, imbricate lobes with ascending, crenate tips, while its marginal lobes are occasionally loosely adnate and radiating (Hollinger *et al.* 2022). For morphological comparison, the characteristics of the Pakistani sample were compared with the description of *A. roemerioides* provided by Hollinger *et al.* (2022), which shows that both of them share the same characteristics such as loosely attached, well-developed foliose thallus with dark grey upper surface and imbricate 0.5-1.2 mm wide lobes with ascending margins but the Pakistani sample differs in having slightly smaller conidia (4.5-5.5 µm vs 4.5-6.3 µm long). This is the first report of this species outside of North America. The morphological distinction between *A. roemerioides* and the closely related *A. roemeri* is extremely subtle, as their overall morphology largely overlaps and their separation has been based primarily on their disjunct distributions (Hollinger *et al.* 2022). The occurrence of *A. roemerioides* in Pakistan, adjacent to regions of *A. roemeri* in Afghanistan, suggests the possibility of sympatry between the two taxa (Mayrhofer *et al.* 2023). Therefore, additional molecular investigations on specimens from Pakistan and neighboring areas are recommended to clarify their genetic boundaries and evaluate whether *A. roemerioides* represents a distinct species or falls within the variability of *A. roemeri*.

Anaptychia setifera Mereschk. ex Räsänen (Fig. 7)

Annales Academiae Scientiarum Fennicae, Ser. A 34 (no. 4): 123 (Räsänen 1931).

MATERIALS EXAMINED. — **Pakistan** • Khyber Pakhtunkhwa, Abbottabad District; 34°03'39"N, 73°25'46"E; 2820 m a.s.l.; on bark; 24.V.2023; *A.N. Khalid leg.*; U.F. Ahmad det.; MUS-01; voucher: LAH38292; GenBank: PQ276734 (ITS) • Khyber Pakhtunkhwa, Abbottabad district; 34°03'30"N, 73°24'51"E; 2413 m a.s.l.; on bark; 24.V.2023; *A.N. Khalid leg.*; U.F. Ahmad det.; MUS-02; voucher: LAH38293; GenBank: PQ276735 (ITS).

SUBSTRATE AND ECOLOGY. — Found growing on barks in temperate forests at elevations above 2500 m a.s.l., where the mean annual temperature is approximately 11°C and the average annual precipitation is around 1500 mm, including substantial winter snowfall.

SPOT TESTS. — K–, C–, KC–.

DESCRIPTION
Thallus fruticose, dorsiventral, loosely attached, laciniate, erect to bushy, slightly pruinose, up to 8 cm across, esorediate.

Color
Pale yellowish green to greyish green when dry, green when wet.

Lobes
Irregular to dichotomously branched, narrow, linear, elongated, entangled, 0.4-1.8 mm broad.

Cilia
Marginal, brown to black.

Upper surface
Dull, tomentose.

Lower surface
White to pale yellow, fibrous, flat to weakly canaliculate.

Upper cortex
25-78 µm thick, globose to subglobose hyaline prosoplectenchymatous cells, 10-16 µm in diameter.

Algal layer
Uneven, discontinuous, 35-70 µm thick, cells globose to subglobose, 5-19 µm in diameter.

Medulla
Hyphae hyaline, loosely interwoven, 85-140 µm thick.

Lower cortex
Absent.

Pycnidia
Frequent, immersed, 230-300 µm × 150-245 µm, ostiole brown to black.

Conidia
Hyaline, bacilliform, 4-5 µm × *c.* 1 µm.

Apothecia
Not present in our collections.

NOTES
Anaptychia setifera is characterized by its relatively broad lobes, typically exceeding 1 mm in width, and the presence of spinules on the receptacle of the apothecia (Poelt & Vezda 1977; Urbanavichus 2008). This species is widely distributed across the Northern Hemisphere (Kurokawa 1962; Chen & Wang 1999). It closely resembles *A. ciliaris* in lobe width but differs by possessing spinules on the exciple, a feature absent in *A. ciliaris* (Chen & Wang 1999). Compared to the phylogenetically related *A. crinalis*, this species has noticeably broader lobes, while *A. crinalis* is characterized by its narrow and linear lobes, typically 0.2-0.6 mm wide (Esslinger 2007). In some studies, including those by Kurokawa (1962, 1973) and Aptroot & Iqbal (2012), this species has been referred to as *A. kaspica*, a name regarded by many authors as a synonym of *A. setifera* (Poelt & Vezda 1977; Esslinger 2018). However, most researchers now refer to this species as

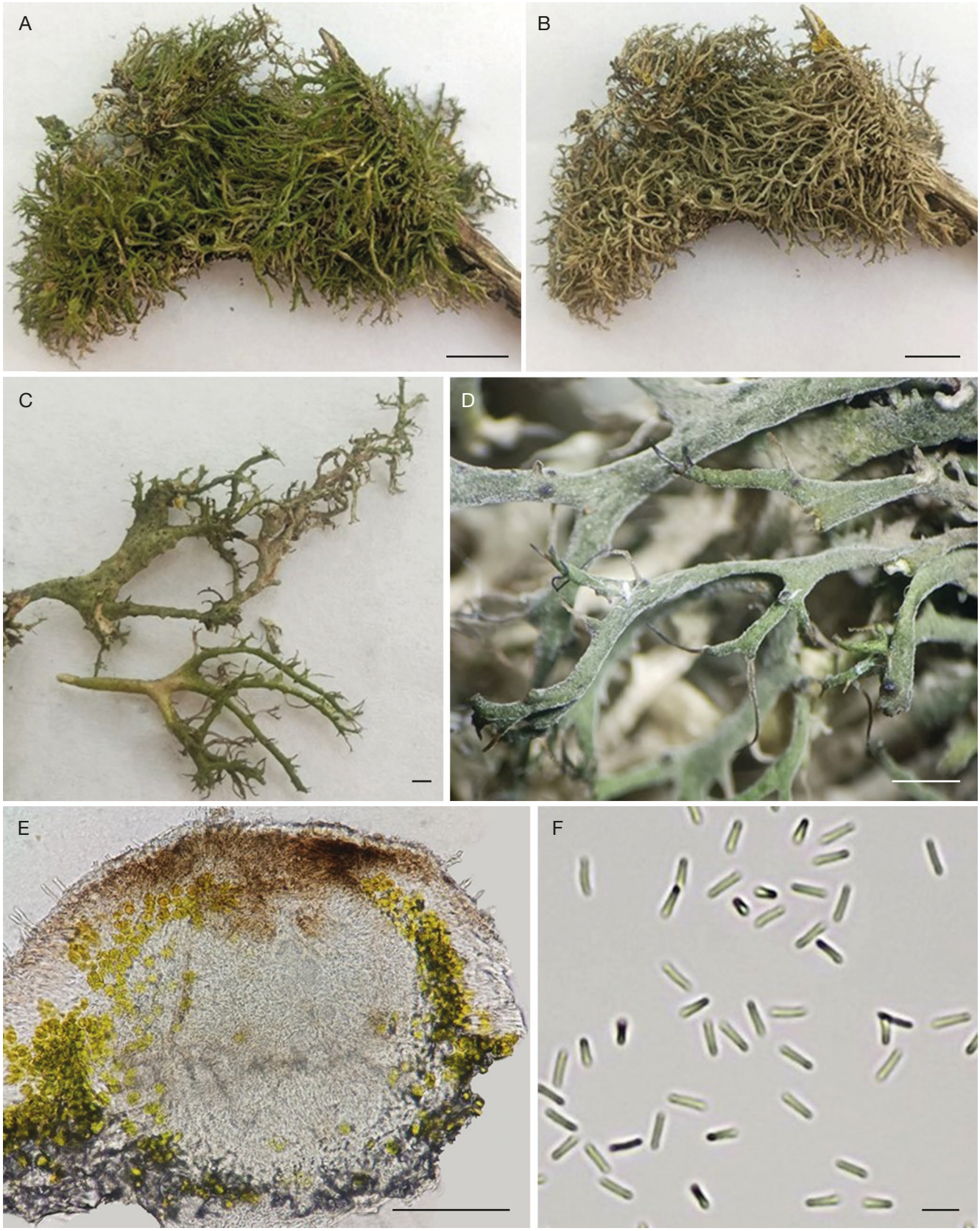

Fig. 7. — *Anaptychia setifera* Mereschk. ex Räsänen (LAH38294): **A**, **B**, thallus; **C**, lobes; **D**, lobes having pycnidia under stereomicroscope; **E**, section of pycnidium; **G**, pycniospores. Scale bars: A, B, 1 cm; C, D, , 1 mm; E, 100 µm; F, 5 µm. Photo credits: Uswa Fatima Ahmad.

A. setifera (Chen & Wang 1999; Esslinger 2007; Urbanavichus 2008; Lohtander *et al.* 2008; Esslinger 2018; Hollinger *et al.* 2022). This study provides the first molecular confirmation of *A. setifera* from Pakistan.

CONCLUSION

During field surveys, numerous lichen specimens were collected from northern regions of Pakistan, and through phenotypic and molecular analyses, this study contributes to the understanding of the family Physciaceae in the country. It led to the description of *Kudratovia pakistanica* U.F. Ahmad, Afshan & Khalid, sp. nov. as a new species, the first records of *Anaptychia crinalis* and *A. roemerioides* from Pakistan, and the confirmation of *A. setifera*, thereby expanding the known distribution of the genus in the region. Despite the infrequent occurrence of these genera in Pakistan, these discoveries suggest that additional taxa within these relatively small genera could be identified through careful observation and extensive surveys. Overall, these findings highlight the rich biodiversity of the region and emphasize the importance of continued exploration and research in unexplored regions of Pakistan.

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Data availability
Voucher specimens are available in the LAH herbarium, University of the Punjab, Lahore. DNA sequences generated in this study have been deposited in GenBank under the following accession numbers: PV687690, PV687691, PQ276733, PQ276732, PQ276736, PQ276737, PQ276734, and PQ276735.

Authors contributions
The conceptualization, data curation, methodology, formal analysis, investigation, software, and original draft writing were carried out by UFA. Funding acquisition was provided by ANK. Supervision, validation, and visualization were performed by NSA and ANK. The manuscript was reviewed by NSA and ANK.

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