The Lichenologist (2022), **54**, 363–369
doi:10.1017/S0024282922000226

CAMBRIDGE UNIVERSITY PRESS

Standard Paper

Lecidea glacierensis (*Lecideaceae*), a new lichen species from China revealed by morphology and molecular phylogenetics

Reyim Mamut[1], Aerguli Jiamahat[1] and Abdulla Abbas[1]
[1]College of Life Science and Technology, Xinjiang University, Urumqi 830046, China

Abstract

According to morphological and molecular data, a new species of *Lecidea* s. str., *Lecidea glacierensis*, is described from Xinjiang Province, China. It is distinctive due to its well-developed, regularly areolate to bullate thallus, and its habitat on calciferous rocks. This species was collected mostly in the area of Tianshan No.1 Glacier, which is located at an elevation of 3454 m and *c.* 118 km south-west of Urumqi and is considered to be one of the most important dryland glacier areas in Asia. An illustration and detailed description of the taxon are provided.

Key words: Bogeda Peak, *Lecidea* s. str., phylogeny, taxonomy, Tianshan No. 1 Glacier

(Accepted 13 June 2022)

Introduction

Lecideaceae Chevall. was originally introduced as a family based on crustose or squamose thalli and apothecia lacking a thalline margin. The family initially included all crustose lecideoid genera (Chevallier 1826) but it now comprises only genera with lecideine apothecia, simple hyaline ascospores and a *Lecidea*- or *Porpidia*-type ascus structure (Fryday & Hertel 2014).

The genus *Lecidea* Ach., first described by Acharius (1803), includes most of the species in the family *Lecideaceae* and over time has undergone many systematic changes. More than 1200 species with an extraordinarily wide circumscription were included in *Lecidea* by Zahlbruckner (1926) but most of these species have been moved to other genera during the past few decades (e.g. Hertel 1967, 1977, 2006; Hafellner 1984). *Lecidea* s. lat. comprises 427 species (Kirk *et al.* 2008), but only slightly more than 100 species (all saxicolous) are now included in the genus *Lecidea* s. str. on the basis of specific anatomical diagnostic characters, especially the existence of a *Lecidea*-type ascus (Hertel 1977, 2006; Hertel & Printzen 2004). Saxicolous 'lecideoid' lichens (Hertel 1984) are genera and species usually described originally under the generic name *Lecidea sensu* Zahlbruckner (1925) and comprise crustose species with apothecia lacking a thalline margin and with hyaline, non-septate ascospores (Ruprecht *et al.* 2020). Of the more than 100 taxa belonging to the genus *Lecidea* s. str., 21 species have been reported in China (Paulson 1925; Magnusson 1940; Lamb 1963; Hertel 1977; Hertel & Zhao 1982; Abbas & Wu 1998; Aptroot & Sparrius 2003; Obermayer 2004; Guo 2005; Zhang *et al.* 2010, 2012; Hu *et al.* 2014; Zhao *et al.* 2017; Jiamahat & Mamut 2019; Wei 2020), most species having been identified by morphological features and the secondary metabolites produced.

Author for correspondence: Reyim Mamut. E-mail: reyim_mamut@xju.edu.cn
Cite this article: Mamut R, Jiamahat A and Abbas A (2022) *Lecidea glacierensis* (*Lecideaceae*), a new lichen species from China revealed by morphology and molecular phylogenetics. *Lichenologist* **54**, 363–369. https://doi.org/10.1017/S0024282922000226

Processes that shape biogeographical patterns in lichens have been of long-standing interest, especially in extreme environments (Hale *et al.* 2019). Lecideoid lichen species are abundant in extreme environments in the Tianshan Mountains. During revisionary work on *Lecideaceae* in the Tianshan No.1 Glacier and Bogeda Peak, and its surroundings in the southern part of the Tianshan Mountains, one species was found to be distributed widely in the study area (Figs 1 & 2). According to the results of morphology, chemistry and molecular data, this species is recognized as a new taxon in *Lecidea* s. str.

Material and Methods

Morphological and anatomical studies

Specimens of the new species *Lecidea glacierensis* were collected from the eastern Tianshan Mountains of Xinjiang, China, at an altitude of 1700–3700 m. Climatic and environmental conditions for the sampled specimens of this species are harsh and windy, with the temperature often below freezing. Thallus sections were investigated in water. Apothecial characteristics of hand-cut sections mounted in water were studied by light microscopy and measurements of ascospores and the epihymenium, hymenium, subhymenium, hypothecium and exciple were made in distilled water using a Nikon Eclipse E200 microscope. Amyloid reactions were studied in Lugol's solution both with and without prior treatment with 10% KOH (K). The adherence and structure of the paraphyses were observed in K. The distribution of crystals in the apothecial sections was studied with 25% KOH. The specimens examined are stored at XJU (Lichen Research Center in Arid Zones of Northwest China).

Chemical studies

Spot tests were performed in K, 15% HCl (H), 50% HNO_3 (N) or 0.15% aqueous IKI. Thallus sections were investigated in water

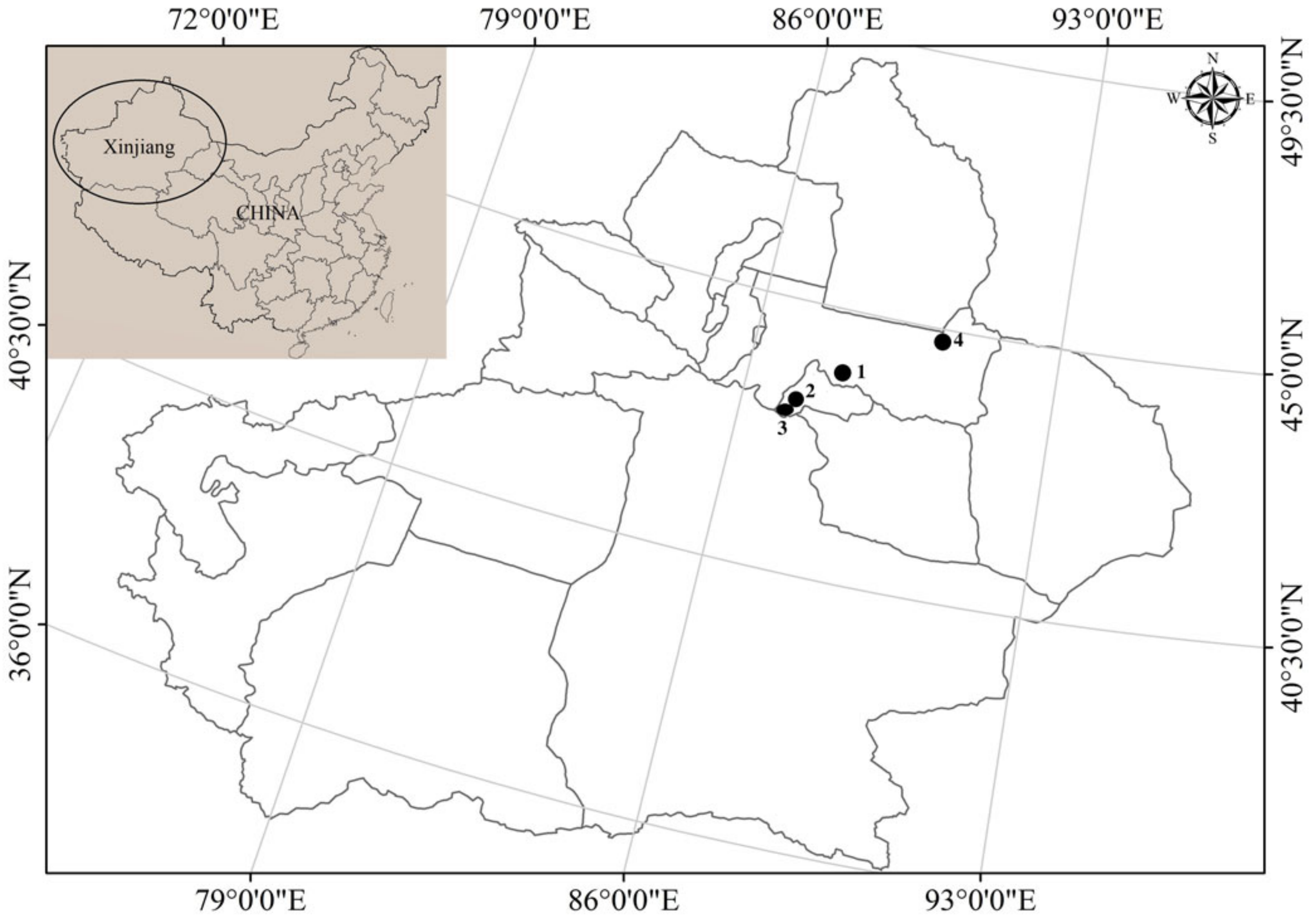

Fig. 1. Location of collection sites in Xinjiang Province, China. 1, Bogeda Peak. 2, Yingxiong Bridge. 3, Tianshan No. 1 Glacier. 4, Jiangbulake.

and K under a dissecting microscope. Acetone extracts of the thallus were analyzed by thin-layer chromatography (TLC) in solvent systems B and C (Orange *et al.* 2010) and high-performance liquid chromatography (HPLC) was also performed to identify the secondary metabolites present (Feige *et al.* 1993).

DNA isolation, PCR amplification and sequencing

Two or three apothecia were used for DNA extraction from each specimen. Whole genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. The ITS region was amplified and sequenced using the specific primers ITS1F (CTT GGT CAT TTA GAG GAA GTA A) (Gardes & Bruns 1993) and ITS4 (TCC GCT TAT TGA TAT GC) (White *et al.* 1990). The PCR procedure was as follows: 94 °C for 4 min; 34 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min; and a final extension at 72 °C for 10 min (see Supplementary Material Table S1, available online). PCR products were directly sequenced by Sangon Biotech (Shanghai).

Phylogenetic analyses

DNA was extracted and the ITS region successfully amplified and sequenced for three specimens (20081647, 20091650a, 20091652), and the sequences assembled and edited in Geneious Prime v. 2019.0.4. Additional sequences of *Lecidea* were downloaded from GenBank based on nucleotide BLAST searches (Supplementary Material Table S2, available online). Multiple sequence alignment was performed using ClustalW (Thompson *et al.* 1997) and ambiguous alignments were removed manually. Maximum likelihood (ML) and Bayesian inference (BI) approaches were used for analyses. The ML analysis was implemented in RAxML-HPC BlackBox (v. 8.2.12) at https://www.phylo.org/ (Silvestro & Michalak 2012) with the selected model of substitution, rapid ML bootstrapping, 1000 pseudoreplicates and default settings for the other parameters. The BI calculation was performed using MrBayes v. 3.1.1 (Huelsenbeck & Ronquist 2001). The prior testing procedures were used to select the Bayesian model that fitted the model choice criteria (nst = 6, rates = gamma). Four Markov chains (MCMCs) were run for 2 000 000 generations starting with a random tree and trees were sampled every 1000 generations. After discarding the first 25% of sampled trees as burn-in, the remaining trees were used to calculate the posterior probabilities (PP) for internal branches. The trees were visualized with FigTree v.1.4.0.

Results

Three new sequences of the ITS locus were generated for the three specimens (see Supplementary Material Table S2, available online). The ITS matrix used in the ITS analysis consisted of a total of 40

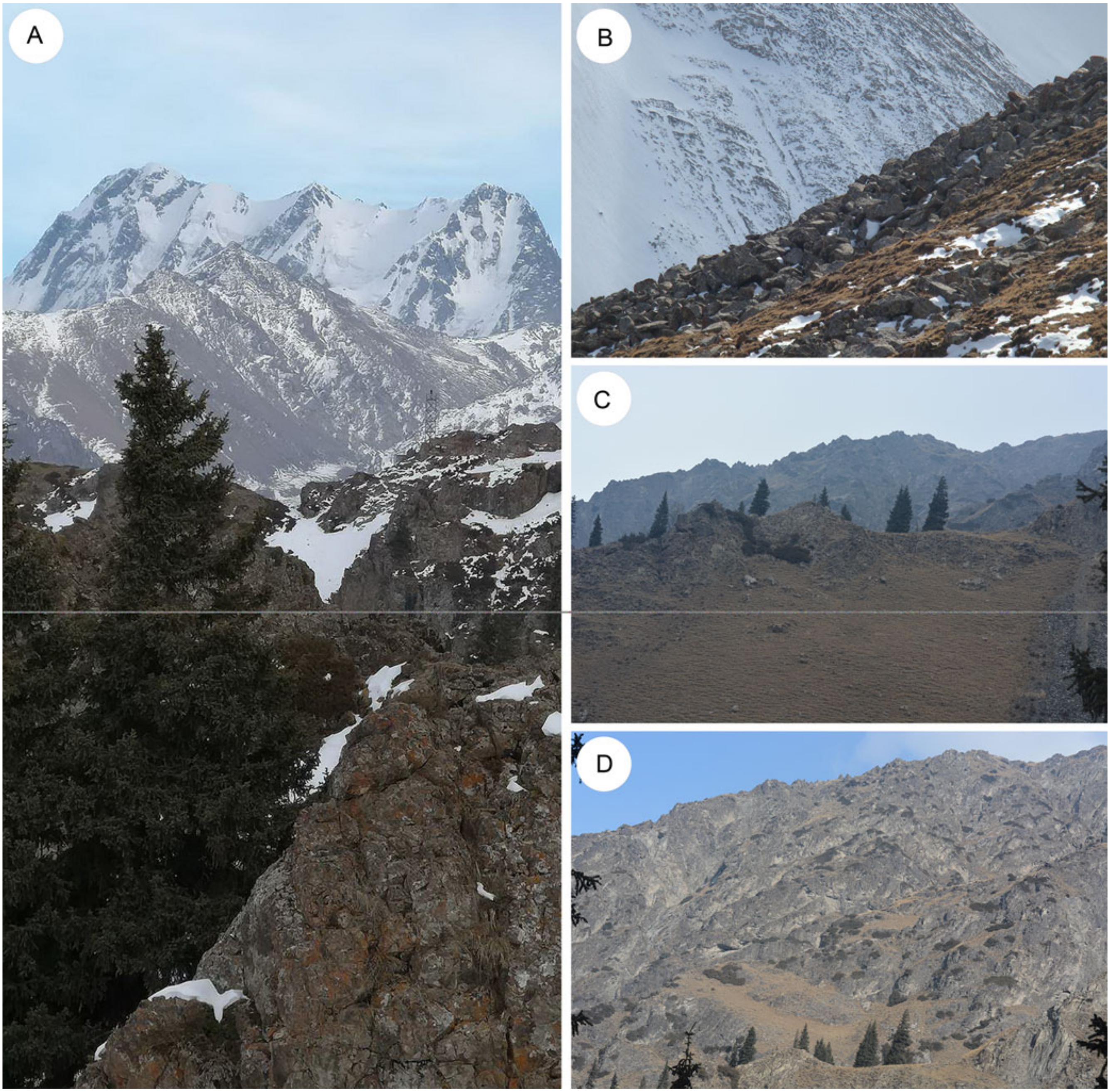

Fig. 2. Habitats of *Lecidea glacierensis*. A, south rim of Bogeda Peak in Fukang. B, alpine meadow in Yingxiong Bridge, Houxia County. C, rocky slopes adjacent to Tianshan No. 1 Glacier, Urumqi. D, steep slopes in Jiangbulake scenic spot. In colour online.

sequences representing 14 aligned taxa and 550 nucleotide positions (Supplementary Material Table S2), with *Lecidella stigmatea* (Ach.) Hertel & Leuckert selected as the outgroup. The Bayesian consensus tree had the same topology as the ML tree produced by RAxML for both datasets. In the ITS phylogram, all *L. glacierensis* sequences clustered together with strong support (Fig. 3).

Taxonomy

Lecidea glacierensis A. Abbas & R. Mamut sp. nov.

MycoBank No.: MB 836099

Thallus occurs on calcareous rock and is whitish, esorediate to farinose, with a non-amyloid medulla and persistent, thin apothecial disc margin.

Type: China, Xinjiang, Urumqi, No. 1 Glacier, 43°07.253′N, 86° 51.776′E, 2145 m alt., 5 October 2009, *R. Mamut* 20081647 (holotype—XJU). GenBank Accession numbers for the sequences of the type: MK590965 (ITS), MK591821(mtSSU), MK591017(nuLSU).

(Fig. 4)

Thallus crustose, well developed, regularly areolate to bullate, prothallus distinct, black between areoles and at thallus margin; areoles inflated, swollen, regular to relatively regular, marginal lobes

https://doi.org/10.1017/S0024282922000226 Published online by Cambridge University Press

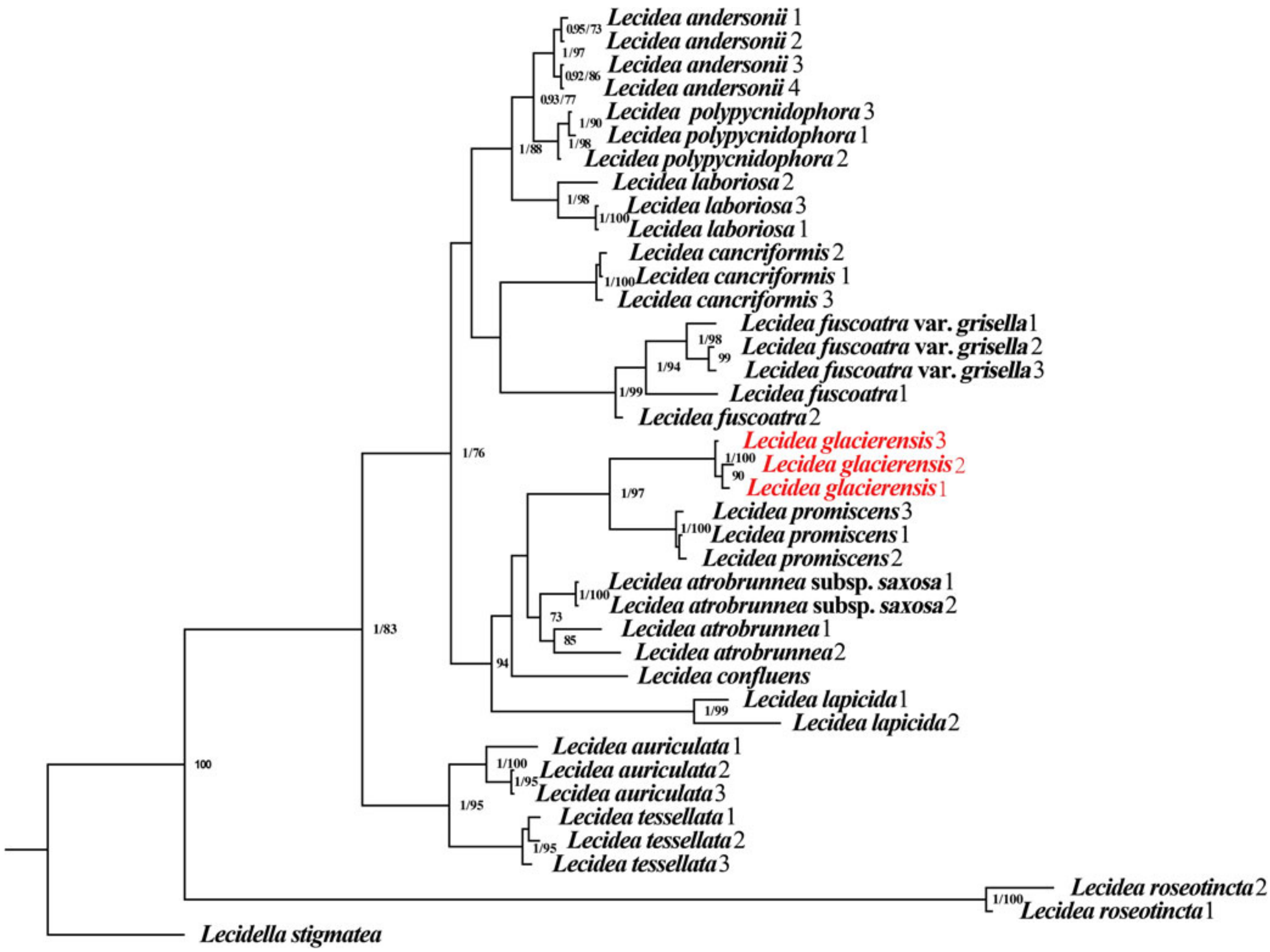

Fig. 3. Maximum-likelihood (ML) and Bayesian phylogeny of *Lecidea glacierensis* and related species inferred from the ITS loci; outgroup is *Lecidella stigmatea*. Bayesian posterior probability (PP) and ML bootstrap values are indicated next to the branches (PP/ML). In colour online.

larger than those at the centre, 0.5–1 mm thick; surface whitish grey to bluish grey, rough, mostly esorediate, rarely producing mounds of farinose, granular whitish soredia in places; margins flexuous to lacerate. *Upper cortex* distinct, with brown granules, 20 µm thick; epinecral layer 20–25 µm thick; algal layer dense, distinctly differentiated, often weakly to strongly divided by narrow, somewhat cone-like hyphal bundles, narrowing downwards to lower side at edges and tips; *photobiont* green, trebouxioid. *Medulla* white, non-amyloid.

Apothecia black, immersed between the areoles, seldom overtopping the areoles and absent from near the thallus margin, regularly round, adnate, usually scattered but sometimes clustered, 0.4–1.5 mm diam.; disc plane to slightly convex, matt when young, glossy brown, epruinose to weakly pruinose; proper margin present, 60–150 µm thick, persistent, often raised above the disc. *Exciple* greenish black in section, composed of radiating hyphae, 10–90 µm wide, cortex similar to epihymenium; *epihymenium* olive green to dark brown, *c.* 20 µm thick; *hymenium* hyaline, 40–50 µm high, I+ blue; *paraphyses* coherent, unbranched or occasionally branched, slightly swollen at the apices. *Subhymenium* hyaline to slightly brownish, 35–45 µm thick. *Hypothecium* pale yellowish brown to dirty brown, 30–100 µm high. *Asci* *Lecidea*-type, 8-spored, clavate, moderately thick-walled. *Ascospores* simple, hyaline, rarely globose to often broadly ellipsoid, with oil droplets, (9–)10–13(–16) × (6.5–)7–9 µm.

Pycnidia not seen.

Chemistry. Thallus and medulla K−, C−, P− and N−. Confluentic acid and accessories detected by TLC and HPLC.

Etymology. The epithet 'glacierensis' refers to No. 1 Glacier in the Tianshan Mountains in Xinjiang, China, where the new species was mostly found and collected.

Ecology and distribution. The new species occurs on calciferous rock, often with *Rusavskia elegans* (Link) S. Y. Kondr. & Kärnefelt and *Sporastatia testudinea* (Ach.) A. Massal. In terms of distribution, *L. glacierensis* was collected from extreme environments in the southern part of the Tianshan Mountains. The No.1 Glacier is 4.8 million years old, dating back to the Third Glacial Age. The region is characterized as a layered valley with rock ledge, basins, cirques, and 'sheep's back' stones (*roche moutonnee*) that can be seen from a distance. The No. 1 Glacier is known as the 'living fossil of glaciers' and has become the best place to observe and study modern glaciers and ancient glacier remains in China. Bogeda Peak is the highest peak in the eastern section

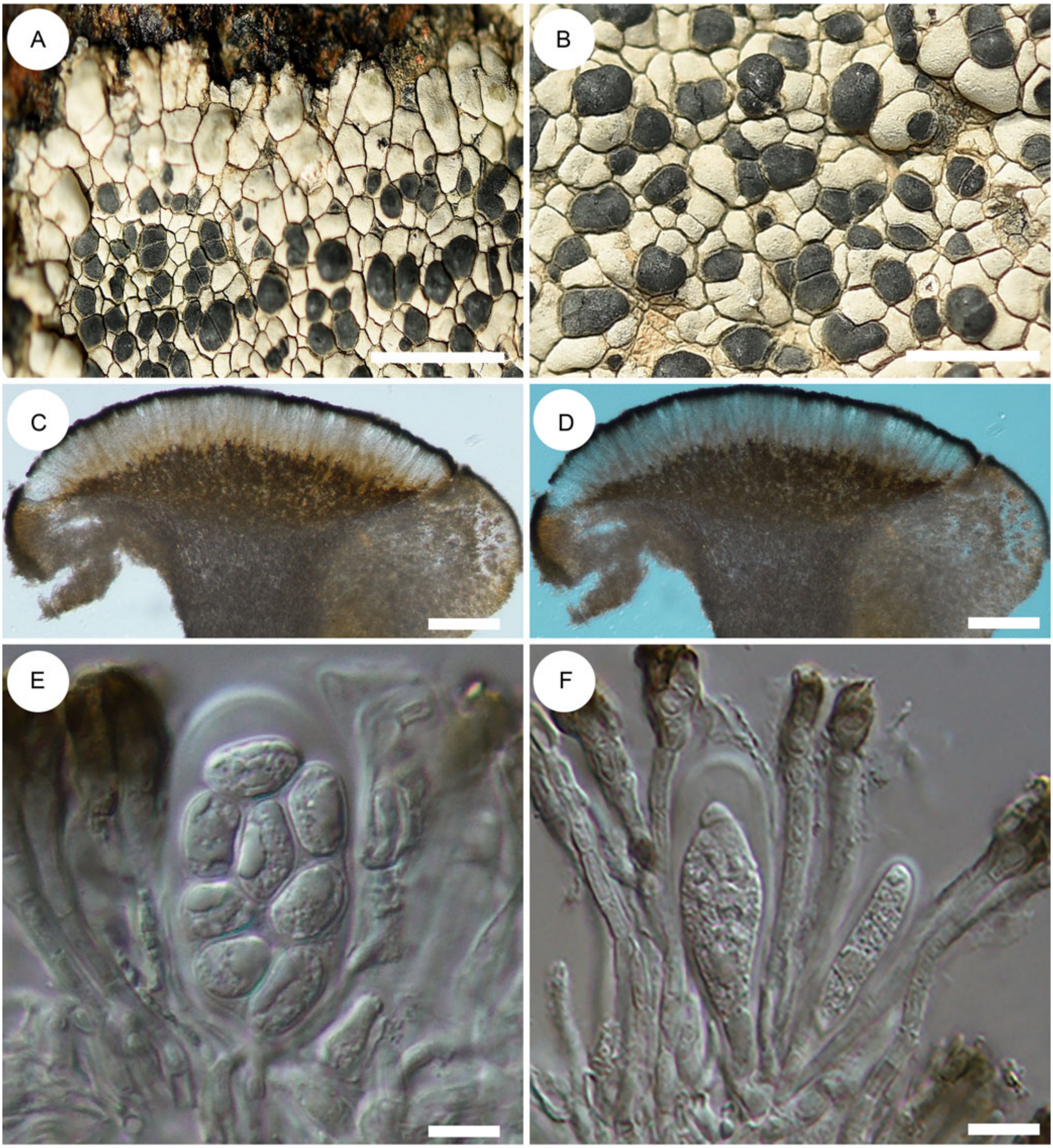

Fig. 4. *Lecidea glacierensis* (*R. Mamut* 20081647). A & B, thallus and apothecia. C, cross-section of apothecium. D, cross-section of apothecium viewed with polarized light microscopy. E, ascus. F, paraphyses. Scales: A = 6 mm; B = 3 mm; C & D = 200 μm; E = 10 μm; F = 15 μm. In colour online.

of the Tianshan Mountains, located in the hinterland of the Asian continent where the foothills are hot but the middle and high mountains are wet and cold. The peak is surrounded by desert, with Junggar Basin to the north and the Turpan Basin to the south. It is one of the youngest mountain ranges in the world, emerging from the sea during the orogenic movement 300 million years ago and from the Himalayan orogenic movement 60 000 to 70 000 years ago.

Additional specimens examined. **China:** *Xinjiang Province*: No. 1 Glacier, 1700 m, on rock, 2008, *R. Mamut* 20080704; *ibid.*, 3530 m alt., on rock, 2008, *R. Mamut* 20081012, 20081013, 20081014; *ibid.*, 2145 m, on rock, 2009, *R. Mamut* 20091647, 20092134; *ibid.*, 1830 m, on rock, 2009, *R. Mamut* 20091650-a, 20091652; Yingxiong Bridge, 3596 m, on rock, 2009, *R. Mamut* 20092298; south rim of Bogeda Peak, 3350 m, on rock, 2018, *R.*

https://doi.org/10.1017/S0024282922000226 Published online by Cambridge University Press

Mamut 20180100; Jiangbulake, 2279 m, on rock, 2018, *R. Mamut* 20180118.

Discussion

Currently, *Lecidea glacierensis* is known only from the Tianshan Mountains in Xinjiang, China. It is characterized by a well-developed, regularly areolate to bullate thallus containing confluentic acid, and by its habitat on calciferous rocks. This combination of characters renders it morphologically and anatomically similar to other *Lecidea* species and some *Porpidia* Körber species, such as *L. tessellata* Flörke and *P. speirea* (Ach.) Kremp. *Lecidea glacierensis* could be confused with *L. tessellata* owing to its light grey areolae, well-developed thallus and broadly ellipsoid ascospores (Hertel & Printzen 2004), but the new species differs by its bullate areoles and conspicuous apothecial margins. *Lecidea glacierensis* morphologically resembles *L. lapicida* (Ach.) Ach., with both species having ashy grey thalli, persistent marginated discs and ellipsoid ascospores, but *L. lapicida* has a thallus containing stictic acid and also has an amyloid (I+ violet) medulla (Hertel & Printzen 2004; Zhang *et al*. 2010). In having distinct margins, a well-developed black prothallus and sunken apothecia, *Porpidia speirea* is similar to *L. glacierensis* but can be differentiated by the occurrence of a *Porpidia*-type ascus and an amyloid medulla (Gowan 1989).

According to the phylogenetic analyses of the ITS sequences, *Lecidea glacierensis* forms a monophyletic clade and is clustered with *L. atrobrunnea*, *L. atrobrunnea* subsp. *saxosa*, *L. confluens*, *L. lapicida*, and *L. promiscens* Nyl. with high support. *Lecidea promiscens* (Hertel & Printzen 2004) is distinguished by having a thinner rimose thallus with an amyloid medulla and *L. atrobrunnea* has a reddish brown thallus. *Lecidea glacierensis* and *L. confluens* both have a well developed, whitish grey thallus, but the ascospores of *L. glacierensis* are wider (Hertel & Andreev 2003).

Acknowledgements. This research was supported by the National Natural Science Foundation of China (grants no. 31670023, 31750001, 31760052). We express our sincere thanks to Shou-Yu Guo (State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China) for his help in writing this paper. We are very grateful to Bruce Bartholomew (California Academy of Sciences) for discussion and critical comments on the manuscript, and helpful suggestions to improve this paper.

Author ORCID. Reyim Mamut, 0000-0001-8624-9584.

Supplementary Material. To view Supplementary Material for this article, please visit https://doi.org/10.1017/S0024282922000226.

References

Abbas A and Wu JN (1998) *Lichens of Xinjiang*. Urumqi: Sci-Tech & Hygiene Publishing House of Xinjiang.

Acharius E (1803) *Methodus Qua Omnes Detectos Lichenes*. Stockholm: F. D. D. Ulrich.

Aptroot A and Sparrius L (2003) New microlichens from Taiwan. *Fungal Diversity* **14**, 1–50.

Chevallier FF (1826) *Flore Géné des Environs de Paris, Selon la Métode Naturelle*. Paris: Chez Ferra Jeune.

Feige GB, Lumbsch HT, Huneck S and Elix JA (1993) Identification of lichen substances by a standardized high-performance liquid chromatographic method. *Journal of Chromatography A* **646**, 417–427.

Fryday AM and Hertel H (2014) A contribution to the family *Lecideaceae* s. lat. (*Lecanoromycetidae* inc. sed., lichenized *Ascomycota*) in the southern subpolar region; including eight new species and some revised generic circumscriptions. *Lichenologist* **46**, 389–412.

Gardes M and Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**, 113–118.

Gowan SP (1989) The lichen genus *Porpidia* (*Porpidiaceae*) in North America. *Bryologist* **92**, 25–59.

Guo SY (2005) *Lichens*. In Zhuang WY (ed.), *Fungi of Northwestern China*. Ithaca: Mycotaxon Ltd, pp. 321–382.

Hafellner J (1984) Studien in Richtung einer natürlicheren Gliederung der Sammelfamilien *Lecanoraceae* und *Lecideaceae*. *Beiheft zur Nova Hedwigia* **79**, 241–371.

Hale E, Fisher ML, Keuler R, Smith B and Leavitt SD (2019) A biogeographic connection between Antarctica and montane regions of western North America highlights the need for further study of lecideoid lichens. *Bryologist* **122**, 315–324.

Hertel H (1967) Revision einiger calciphiler Formenkreise der Flechtengattung *Lecidea*. *Beiheft zur Nova Hedwigia* **24**, 1–155.

Hertel H (1977) Gesteinsbewohnende arten der sammelgattung *Lecidea* (Lichenes) aus zentral-, ost- und südasien. *Khumbu Himal* **6**, 145–378.

Hertel H (1984) Über saxicole, lecideoide Flechten der Subantarktis. *Beiheft zur Nova Hedwigia* **79**, 399–499.

Hertel H (2006) World distribution of species of *Lecidea* (*Lecanorales*) occurring in Central Europe. In Lackovičová A, Guttová A, Lisická E and Lizoň P (eds), *Central European Lichens: Diversity and Threat*. Ithaca: Mycotaxon Ltd, pp. 19–74.

Hertel H and Andreev MP (2003) On some saxicolous lecideoid lichens of the Beringian region and adjacent areas of eastern Siberia and the Russian Far East. *Bryologist* **106**, 539–551.

Hertel H and Printzen C (2004) *Lecidea*. In Nash TH, III, Ryan BD, Diederich P, Gries C and Bungartz F (eds), *Lichen Flora of the Greater Sonoran Desert Region, Vol. II*. Tempe, Arizona: Lichens Unlimited, Arizona State University, pp. 287–309.

Hertel H and Zhao CF (1982) Lichens from Changbai Shan – some additions to the lichen flora of north-east China. *Lichenologist* **14**, 139–152.

Hu L, Zhao X, Sun LY, Zhao ZT and Zhang LL (2014) Four lecideoid lichens new to China. *Mycotaxon* **128**, 83–91.

Huelsenbeck JP and Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**, 754–755.

Jiamahat A and Mamut R (2019) Three *Lecidea* lichens new to China. *Acta Botanica Boreali-Occidentalia Sinica* **39**, 1692–1698.

Kirk PM, Cannon PF, Minter DW and Stalpers JA (2008) *Dictionary of the Fungi*, 10th Edn. Wallingford: CAB International.

Lamb IM (1963) *Index Nominum Lichenum: Inter Annos 1932 et 1960 Divulgatorum*. New York: Ronald Press.

Magnusson AH (1940) Lichens from Central Asia I. (Reports from the Scientific Expedition to the North-Western Provinces of China under the leadership of Dr Sven Hedin). *Sino-Swedish Expedition Publication 13, XI Botany* **1**, 85–133.

Obermayer W (2004) Additions to the lichen flora of the Tibetan region. *Bibliotheca Lichenologica* **88**, 479–526.

Orange A, James PW and White FJ (2010) *Microchemical Methods for the Identification of Lichens*. London: British Lichen Society.

Paulson R (1925) Lichens of Mount Everest. *London Journal of Botany* **63**, 189–193.

Ruprecht U, Fernández-Mendoza F, Türk R and Fryday AM (2020) High levels of endemism and local differentiation in the fungal and algal symbionts of saxicolous lecideoid lichens along a latitudinal gradient in southern South America. *Lichenologist* **52**, 287–303.

Silvestro D and Michalak I (2012) raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity and Evolution* **12**, 335–337.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F and Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25**, 4876–4882.

Wei JC (2020) *The Enumeration of Lichenized Fungi in China*. Beijing: China Forestry Publishing House.

White TJ, Bruns T, Lee S and Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In Innis

MA, Gelfand DH, Sninsky JJ and White TJ (eds), *PCR Protocols: a Guide to Methods and Applications*. New York: Academic Press, pp. 315–322.
Zahlbruckner A (1925) *Catalogus Lichenum Universalis, Vol. 3*. Leipzig: Verlag Bornträger.
Zahlbruckner A (1926) *Lichens*. In Engler A and Prantl K (eds), *Die Natürlichen Pflanzenfamilien*. Leipzig: Engelmann, pp. 61–270.
Zhang L-L, Wang H-Y, Sun L-Y and Zhao Z-T (2010) Four lichens of the genus *Lecidea* from China. *Mycotaxon* **112**, 445–450.
Zhang L-L, Wang L-S, Wang H-Y and Zhao Z-T (2012) Four new records of lecideoid lichens from China. *Mycotaxon* **119**, 445–451.
Zhao X-X, Zhao Z-T, Miao C-C, Ren Z-J, Zhang L-L (2017) Five *Lecidea* lichens new to China. *Mycotaxon* **132**, 317–326.