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Standard Paper

A new species and new combination of *Lecanora* s. str. (*Lecanoraceae*) from China

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Abstract

A new lichen species, *Lecanora zeorina* Li J. Li & Printzen is described here from the south-west of China. *Lecanora zeorina* is characterized by its somewhat areolate-squamulose thallus, zeorine to lecanorine apothecia, an epihymenium without crystals around expanded paraphyses tips, an amphithecium with large calcium oxalate crystals and the production of atranorin. A new combination, *Lecanora crystalliniformis* (B.G. Lee & Hur) Li J. Li & Printzen, is based on *Protoparmeliopsis crystalliniformis* B.G. Lee & Hur, which was described as a new species from South Korea. Collections from China are almost identical in morphology and chemistry, and are phylogenetically closely related. Phylogenetic reconstructions based on ITS and mtSSU suggest that these two lecanoroid species belong in *Lecanora* s. str. where they form a sister group to sorediate species such as *L. barkmaniana* Aptroot & Herk and *L. variolascens* Nyl. Detailed descriptions, discussions, distributions and phylogenetic trees, based on multiple collections, are presented.

Keywords: chemistry; *Lecanora subfusca* group; lichens; phylogeny; taxonomy

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Introduction

The lichen genus *Lecanora* Ach. s. lat. is a widespread and very diverse genus consisting of *c.* 600 species worldwide, and broadly circumscribed as including species with lecanorine apothecia, simple, hyaline ascospores, green-algal photobionts and *Lecanora*-type asci (Zahlbruckner 1926; Ryan *et al.* 2004; Bungartz *et al.* 2020). Species of *Lecanora* in its broadest sense are currently assigned to several different genera or species groups, based on phenotypic characters and/or phylogenetic analyses (Eigler 1969; Zhao *et al.* 2016), for example *Bryonora* Poelt (Poelt 1983); *Palicella* Rodr. Flakus & Printzen (Rodriguez Flakus & Printzen 2014); *Lecanora* s. str. (Brodo 1984; Lumbsch 1994; Guderley 1999; Papong & Lumbsch 2011); *Lecanoropsis* M. Choisy (=*L. saligna* group) (Ivanovich *et al.* 2021); *Polyozosia* A. Massal. (=*Myriolecis* Clem., *L. dispersa* group) (Clements 1909; Zhao *et al.* 2016); *Protoparmelia* M. Choisy (Choisy 1929); *Protoparmeliopsis* M. Choisy (=*L. muralis* group) (Zhao *et al.* 2016); *Pulvinora* Davydov *et al.* (Davydov *et al.* 2021); *Tephromela* M. Choisy (Choisy 1929); *Vainionora* Kalb (Kalb 1991). Even so, there are still some species that do not fit into existing genera or species groups, partly because no conclusive systematic framework currently exists on how to separate the remainder of *Lecanora* species into more natural units.

The core of *Lecanora* s. str. (i.e. the *Lecanora subfusca* group) is the species related to the type species *L. allophana* Nyl. (Brodo & Vitikainen 1984), which is characterized by a crustose thallus, the production of atranorin, the presence of calcium oxalate crystals in the amphithecium, ellipsoid to broadly ellipsoid spores, and filiform conidia (Brodo 1984; Lumbsch 1994). The presence and size of crystals in the epihymenium and amphithecium (when checked in polarized light, crystals shine brightly), their solubility, pigmentation of the epihymenium, and the structure and development of the amphithecial cortex, are important diagnostic characters in this group (Kofler 1956; Brodo 1984; Miyawaki 1988; Lumbsch 1994; Guderley 1999). Brodo (1984) summarized and revised these different diagnostic characters based on previous studies, and published a valuable monograph of North American species that has been used as an important reference for the study of *Lecanora* s. str.

In addition, detailed regional revisions of *Lecanora* s. str. exist for Australasia (Lumbsch 1994), South and Central America (Guderley 1999), the Czech Republic (Malíček 2014), India (Upreti 1998), Thailand (Papong & Lumbsch 2011) and Japan (Miyawaki 1988). From China, 119 species of *Lecanora* s. lat. have so far been reported (Zahlbruckner 1930; Wei 1991; Mamut *et al.* 2019; Lü *et al.* 2020), including several newly described species and new country records published in recent decades (Lü *et al.* 2008, 2009*a*, *b*, 2012, 2013; Wang *et al.* 2013; Lü & Zhao 2017*a*, *b*). Among them, *c.* 25 species belong to *Lecanora* s. str. (Han *et al.* 2009; Lü *et al.* 2013). However, molecular data are largely lacking and no comprehensive systematic revision of the group has so far been published.

This study is part of the ongoing project 'Lecanomics' (https://lecanomics.org), which aims to develop new ways to identify and delimit species of the genus *Lecanora*. Among the collections from south-west China, there were two distinctive, closely related

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taxa. *Lecanora zeorina* Li J. Li & Printzen constitutes a new species, which we describe below. Another species was identified as *Protoparmeliopsis crystalliniformis* B. G. Lee & Hur., for which we suggest the new combination *Lecanora crystalliniformis* (B. G. Lee & Hur) Li J. Li & Printzen. Phenotypic characters, geographical distribution and phylogenetic relationships of the two species are presented and discussed below.

Materials and Methods

The specimens in this study are deposited in the Lichen Herbarium, Kunming Institute of Botany, Chinese Academy of Sciences (KUN), and the Herbarium Senckenbergianum Frankfurt/M. (FR) (duplicates).

External morphological characters were studied on air-dried material under a stereomicroscope (Zeiss Stemi SV11). Anatomical features were studied using a light microscope (Zeiss Axioskop 2 plus) on transverse sections of apothecia and thalli prepared with a freezing microtome (Zeiss HYRAX KS 34) and mounted in water or lactophenol cotton blue. Measurements are presented in the following way: (minimum–)($\bar{x}$ - SD)–($\bar{x}$ + SD)(–maximum), where $\bar{x}$ is the arithmetic mean and SD is the standard deviation. Crystals in apothecia were observed in polarized light (POL); their solubility was studied in 20% HNO_3 (N) and 10% KOH (K).

Spot tests were conducted with K and a saturated aqueous solution of NaClO (C). Thin-layer chromatography (TLC) was performed in solvents A, B′ and C to identify lichen chemical compounds, generally following standardized methods (Culberson 1972; Arup *et al.* 1993).

Total DNA was extracted from dried samples using the GeneOn Plant DNA Extraction Kit (GeneOn BioTech, Changchun, China) with the magnetic bead method. The fungal internal transcribed spacer (ITS) regions of the rDNA were amplified via polymerase chain reaction (PCR) using the primers ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990), and the mitochondrial small subunit (mtSSU) of ribosomal RNA using the newly designed primers 16F (5′–3′: CAGCAA CTTGGAGGAATG) and 972R (5′–3′: ATGATGACTTGTCTT AGTCC). Polymerase chain reactions (PCR) were performed in 25 µl volumes using Illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare Life Sciences, Little Chalfont, Buckinghamshire, UK) containing 5 µl of DNA extract and 1 µl (10 pmol μl^{-1}) of each primer. Cycling conditions included initial denaturation at 94 °C for 5 min, followed by four cycles of 94 °C for 30 s, 54 °C (53 °C for mtSSU) for 30 s, and 72 °C for 60 s; 30 cycles of 94 °C for 30 s, 48 °C for 30 s, and 72 °C for 60 s; with a final extension at 72 °C for 10 min. The PCR products were sequenced by Macrogen Europe (Amsterdam, The Netherlands).

We used the newly generated nrITS and mtSSU sequences and additional sequences downloaded from GenBank (Table 1) to reconstruct a phylogenetic tree. Sequences were assembled and edited with Geneious Prime v. 2021.0.3. Each gene dataset was aligned using the MAFFT algorithm on the GUIDANCE2 web server (http://guidance.tau.ac.il/; Sela *et al.* 2015), simultaneously removing poorly or ambiguously aligned regions with the default parameter settings. Congruence between different gene datasets was checked before concatenating. The final concatenated alignment included 972 bp and 80 taxa, and had the following dimensions: 77 sequences, 296 bp for ITS; 72 sequences, 676 bp for mtSSU.

A maximum likelihood (ML) phylogenetic tree with simultaneous inference of the optimal partitioning scheme and substitution models for each data partition was inferred using the online version of IQ-TREE (Trifinopoulos *et al.* 2016) with automated substitution model selection, suggesting four initial partitions (ITS1, 5.8S, ITS2, mtSSU). The best-fit model for each partition was selected according to the Bayesian information criterion (BIC) as follows: K2P + G4 for ITS1 and ITS2, K3P + R2 for 5.8S, and TPM2u + F + I + G4 for mtSSU. Branch support was estimated with 1000 ultrafast bootstrap (UFBoot) replicates; UFBoot ≥ 95% indicates well-supported clades (Minh *et al.* 2013). Bayesian reconstructions of phylogenies were performed with MrBayes v. 3.2.6 (Ronquist *et al.* 2012) to infer phylogenetic trees applying the models inferred by PartitionFinder v. 2.1.1 (Lanfear *et al.* 2017), because most of the models inferred by IQ-TREE are not implemented in MrBayes. The substitution models for MrBayes were: K80 + G for ITS1 and ITS2, K80 + I for 5.8S, and HKY + I + G for mtSSU. All model parameters were unlinked among partitions and we used the default distributions for priors. Two parallel runs with four chains each were run for 10 million generations, in which the initial 50% of sampled data was discarded as burn-in. Bayesian posterior probabilities (PP) ≥ 0.95 were visualized on the ML tree.

Results and Discussion

The taxon sampling comprised 76 taxa of the *Lecanoraceae*, with two taxa of *Ramboldiaceae* (*Ramboldia brunneocarpa* Kantvilas & Elix and *R. stuartii* (Hampe) Kantvilas & Elix) and two taxa of *Parmeliaceae* (*Parmotrema reticulatum* (Taylor) M. Choisy and *P. tinctorum* (Despr. ex Nyl.) Hale) used as the outgroup (Fig. 1). Newly generated sequences were deposited in GenBank.

The phylogenetic tree shows most major genera and species groups within *Lecanoraceae*. Among them, *Protoparmeliopsis* (=*Lecanora muralis* group), *Rhizoplaca* Zopf, *Polyozosia* (=*Lecanora dispersa* group), the *Lecanora polytropa* group and the *Lecanora saligna* group fall into one strongly supported clade (UFBoot = 100%, PP = 0.97). Selected sequences of the species of *Lecanora* s. str. cluster together, but the clade is poorly supported.

Sequences of '*Protoparmeliopsis*' *crystalliniformis*, proposed as a new species by Lee & Hur (2021) from South Korea, are nested among sequences of an unidentified taxon collected from China with strong support (UFBoot = 100%, PP = 1.00). Its systematic position is distant from the genus *Protoparmeliopsis*, instead falling within *Lecanora* s. str. in our phylogenetic tree. This, together with its phenotypic characteristics being similar to *Lecanora* s. str., leads us to suggest the new combination *Lecanora crystalliniformis* (B. G. Lee & Hur) Li J. Li & Printzen, with a detailed discussion following below. The new species *Lecanora zeorina* Li J. Li & Printzen is closely related to *L. crystalliniformis* and the sister group relationship is strongly supported (UFBoot = 98%, PP = 0.97). Both species share the characteristics of an areolate to squamulose thallus, large POL+ crystals in the amphithecium, the production of atranorin, and filiform conidia, characters in agreement with typical members of *Lecanora* s. str.

These two species form a strongly supported (UFBoot = 100%, PP = 1.00) sister group to a group consisting of two sorediate species (*L. barkmaniana* Aptroot & Herk and *L. variolascens* Nyl.) and two species with pruinose apothecia (*L. fulvastra* Kremp. and *L. flavidomarginata* B. de Lesd.). Further sorediate species (*L. darlingiae* Lendemer & E. Tripp and *L. alboflavida* Taylor) and a species with a dark hypothecium (*L. flavoviridis* Kremp.) cluster into one clade with strong support (UFBoot = 100%,

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Table 1. Specimens used for the phylogenetic analyses including collection information and GenBank Accession numbers for nrITS and mtSSU sequences. Newly obtained sequences are in bold; species names are followed by a Lecanomics ID, which refers to the 'Lecanomics' project (https://lecanomics.org).

Species name	Voucher details	Country	ITS	mtSSU
Japewia aliphatica	*Maliček* 9764	Czech Republic	MN547352	MN547343
J. tornoensis	*Printzen* s. n.	Canada	HQ650656	HQ660559
Lecanora albella	*Maliček* 7336	Czech Republic	KY548048	KY502423
L. alboflavida 0	*Coppins* s. n.	United Kingdom	KY548045	KY502428
L. alboflavida 1	*Flakus* 29124	United Kingdom	OL604044	OL604124
L. allophana 0	*Maliček* 9491	Finland	KY548051	KY502416
L. allophana 1	*Maliček* 9626	Russia	KY548050	KY502421
L. argentata	*Maliček*-7	Czech Republic	KT630245	KT630264
L. barkmaniana	*Maliček* 10602	Russia	MK778605	MK778530
L. caesiorubella	*Lumbsch* 19094a	USA		JQ782666
L. campestris	*Arup* U225	Sweden	AF159930	
L. carpinea	*Kondratyuk S.* 21337	Ukraine	MK672827	MK693683
L. cenisia	*Maliček* 5869	Austria	KY548047	KY502425
***L. crystalliniformis* L768**	*Wang* et al. 19-63013	China	**ON807164**	**ON807171**
***L. crystalliniformis* L825**	*Wang* et al. 17-56082	China		**ON807172**
***L. crystalliniformis* L830**	*Wang* et al. 17-56085	China	**ON807163**	**ON807173**
***L. crystalliniformis* L831**	*Wang* et al. 17-56554	China	**ON807162**	
***L. crystalliniformis* L1201**	*Tang* & *Lui* L09	China		**ON807174**
L. darlingiae	*Lendemer* 46644	USA		MH481360
L. expersa	*Maliček* 9624	Russia	KY548053	KY502420
L. flavidomarginata 0	*Flakus* 29951	Bolivia	OL604056	OL604135
L. flavidomarginata 1	*Flakus* 28943	Bolivia	OL603996	OL604077
L. flavoviridis	*Papong* 6539	Thailand	JQ782711	JQ782675
L. fulvastra	*Flakus* 26717	Bolivia		OL604089
L. gangaleoides	*Lumbsch* 19923a	USA	MG554660	JQ782676
L. glabrata	*Lubeck* & *Kukwa* 17811	Poland	MN387101	
L. helva	*Papong* 6444	Thailand	JQ782716	JQ782679
L. horiza		Spain	KT453772	KT453821
L. hybocarpa	*Lumbsch* s. n.	Spain	EF105412	EF105417
L. intricata	*Flakus* 29565b	Bolivia	OL604030	OL604112
L. phaeocardia	*Papong* 3492	Thailand	JQ782724	JQ782687
L. pulicaris 0	*Maliček* 10262	Russia	MK778611	MK778539
L. pulicaris 1	*Maliček* 10263	Russia	MK778612	MK778540
L. rugosella	*Arup* L97561	Sweden	AY398712	
L. saligna	*Dolnik* 4234	Germany	MT938982	MT939209
L. subimmersa	*Lumbsch* 19103b	Australia	JQ782733	JQ782697
L. subintricata	*Printzen* 15562	Japan	MT939010	MT939239
L. subrugosa	*Arup* L98188	Austria	AY398711	
L. substerilis	*Maliček* 202	Slovakia	KT630243	KT630254
L. tropica	*Papong* 6440	Thailand	JN943720	JQ782699
L. vainioi	*Papong* 7120	Thailand	JN943716	JQ782702
L. variolascens	*Maliček* 8422	Austria	KY548038	KY502445

(*Continued*)

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Table 1. (*Continued*)

Species name	Voucher details	Country	ITS	mtSSU
***L. zeorina* L740**	*Wang* et al. 19-63051	China	**ON807167**	**ON807168**
***L. zeorina* L744**	*Wang* et al. 19-63070	China	**ON807166**	**ON807169**
***L. zeorina* L801**	*Wang* et al. 19-62893	China	**ON807165**	**ON807170**
Lecidella carpathica	*Zhao* 20140367-2	China	KT453741	KT453831
L. meiococca	*Ekman* 3101	Sweden	AF517929	AY300893
Miriquidica gyrizans	*Fryday* 10175	USA	MN483126	MN508282
M. leucophaea	*Kossowska* 1354	Thailand	KP822311	KP822516
Palicella glaucopa 0	*Flakus* 2539	Argentina	KJ152486	KJ152471
P. glaucopa 1	*Flakus* 2504b	Argentina	KJ152485	KJ152472
Parmotrema reticulatum	*Moon* 14834	Japan	KP942516	KP942523
P. tinctorum	TNS-L-Y.O. 5947	Japan	KF129464	KF129521
Polyozosia altunica	*Xahidin* 20071910	China	MH698406	MH698407
P. contractula	*Brodo* 31501	USA	HQ650604	DQ986898
P. perpruinosa	*Arup* U176	Sweden	AF070025	
Protoparmeliopsis achariana	*Arup* U155	Sweden	AF070019	DQ787342
'*P. crystalliniformis* 0'	*Lee* 2020-000098	South Korea	MW832796	MW832802
'*P. crystalliniformis* 1'	*Lee* & *Kim* 2020-000149	South Korea	MW832797	MW832803
P. garovaglii 0	*Leavitt* 089	USA	KT453728	KT453818
P. garovaglii 1	*Haji Moniri* s. n.	Iran	MK672841	MK693689
***P. garovaglii* L722**	*Wang* et al. 18-59770	China	**ON807161**	**ON807175**
***P. garovaglii* L1192**	*Wang* et al. 19-63467	China	**ON807160**	**ON807176**
P. kopachevskae	KoLRI 040276	South Korea	MK672847	
P. muralis 0	SK 765	Romania	KP059048	KP059054
P. muralis 1	*Schmull* s. n.	Germany	HQ650653	HQ660556
P. nashii	*Knudsen* 19030		ON447553	ON367825
P. peltata		USA	KT453722	KT453860
***P. peltata* L44**	*Sohrabi* et al. 14619	Iran	**ON807159**	**ON807177**
P. zareii	SK 480	Iran	KP059049	KP059055
Pulvinora stereothallina 0	*Davydov* 14817	Russia	MW257118	MW257159
P. stereothallina 1	*Davydov* 14820	Russia	MW257112	MW257152
Ramboldia brunneocarpa	*Elix* 36756	Australia	EU075542	EU075528
R. stuartii	*Elix* 28664	Australia	EU075549	EU075535
Rhizoplaca chrysoleuca 0	*Leavitt* 8698	USA	KU934619	
R. chrysoleuca 1	BRY 55000	USA	HM577233	KT453856
R. melanophthalma 0	*Vivas* & *Rico* 16805	Spain	JX948232	
R. melanophthalma 1	*Sohrabi* 014624	Iran	JX948275	KT453858
R. pachyphylla	*Wang* et al. 18-59561	China	MK778050	MN192154

PP = 1.00); this seems related to the above clades but the relationship is poorly supported in this analysis. Malíček *et al.* (2017) mentioned that *L. barkmaniana* and *L. variolascens* do not belong to the *L. subfusca* group in the narrow sense but form an isolated clade. Our results show that these species sit on relatively long divergent branches and display some phenotypic characters (soredia, pigmented hypothecium, more or less squamulose thallus and zeorine apothecia) not usually found in species of the *L. subfusca* group s. str. (with a mostly non-sorediate thallus, hyaline hypothecium and lecanorine apothecia). However, the majority of characters are in accordance with the circumscription of *Lecanora* s. str. Additional studies are necessary to fully understand the circumscription of these species and we prefer to retain them in *Lecanora* s. str. here.

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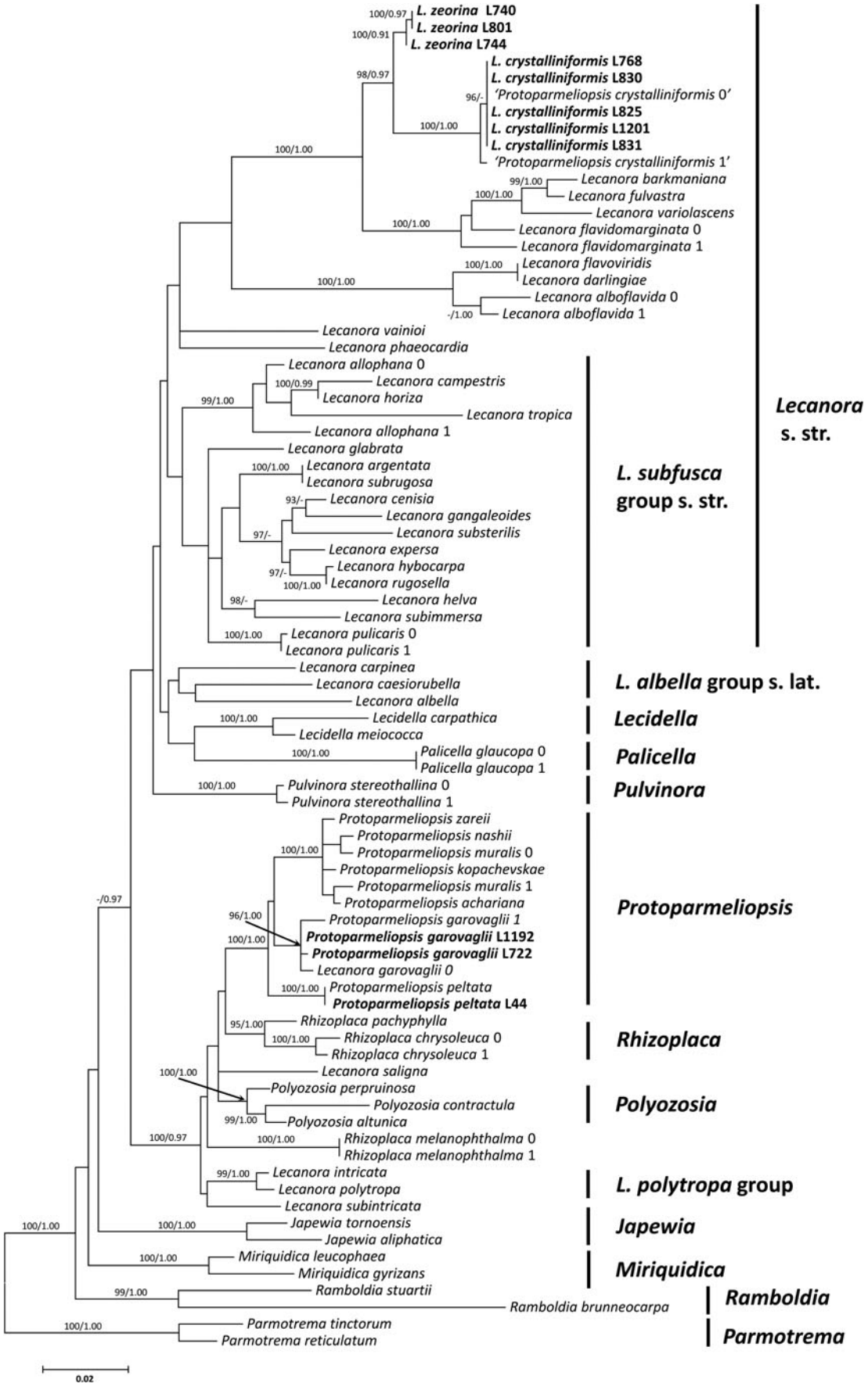

Figure 1. Maximum likelihood (ML) phylogeny of representative genera and species groups of the family *Lecanoraceae* based on concatenated nrITS and mtSSU sequences. Ultrafast bootstrap values (UFBoot) ≥ 95% and posterior probabilities (PP) ≥ 0.95 are shown above internal branches. Newly generated sequences are in bold; species names are followed by a Lecanomics ID, which refers to the 'Lecanomics' project (https://lecanomics.org). All other sequences were downloaded from GenBank (Table 1).

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Taxonomy

Lecanora crystalliniformis (B. G. Lee & Hur) Li J. Li & Printzen comb. nov.

MycoBank No.: MB 847550

Protoparmeliopsis crystalliniformis B. G. Lee & Hur, *MycoKeys* **84**, 163–183 (2021); type: South Korea, 2020, BDNA-L-0000349 (KBA—holotype), MB 839183.

(Fig. 2A–I)

Thallus crustose, areolate to squamulose, reduced to scattered granules or areolae, or almost squamulose, sometimes forming a continuous areolate crust, margin indeterminate, areoles irregular in outline, smooth to irregularly wrinkled; upper surface pale greyish or pale grey-green to whitish, dull or slightly glossy; cortex within small crystals, POL+, soluble in K.

Apothecia lecanorine, numerous, rounded, dispersed or densely aggregated, arising singly on areoles when young, then becoming sessile, (0.3–)0.5–1.0(–1.3) mm diam.; *disc* plane to slightly convex, reddish to deep reddish brown, somewhat shiny, epruinose; *margin* persistent or rarely excluded, level with disc or prominent, entire or slightly crenate, concolorous with the thallus, smooth, with a parathecial ring; *amphithecium* with numerous algal cells and large crystals (POL+) insoluble in K, soluble in N; cortex distinct, gelatinous or interspersed, filled with minute crystals soluble in K but remaining in N, 15–20(–25) µm thick; *parathecium* hyaline, 15–20 µm thick, consisting of thick-walled anastomosing hyphae, with fine crystals mostly in the uppermost layer, dissolving in K but remaining in N; *epihymenium* orangish brown, 10–20 µm high, pigment soluble in K (or fading to paler brown), changing to orangish in N, with fine crystals dispersed mostly between paraphyses in upper part of the hymenium, soluble in K but remaining in N; *hymenium* colourless, 80–100 µm high; *paraphyses* slightly anastomosing, septate, simple, *c.* 1.5–2.0 µm thick, not or slightly expanded at tips; *hypothecium* colourless, composed of anastomosing hyphae; *asci* clavate, *Lecanora*-type, 40–60 × 11–13 µm, 8-spored; *ascospores* simple, hyaline, ellipsoid to broadly ellipsoid, (8.0–)9.0–13.0(–14.0) × (5.0–)5.5–7.0(–7.5) µm.

Pycnidia immersed in thallus, wall brown to olive-brown; *conidia* filiform, straight to curved, 15–26 × 1 µm.

Chemistry. Thallus K+ yellow, KC−, C−; atranorin and an unidentified compound at R_f class 4–5 (A), 5–6 (B′), 5–6 (C), after charring yellow to orange-brown with orange halo in daylight and yellow to orange or brown with greenish halo under long-wave UV light by TLC. The South Korean collections of '*Protoparmeliopsis crystalliniformis*' have been reported to contain atranorin and rhizocarpic acid (Lee & Hur 2021).

Distribution and ecology. This saxicolous species grows on sandstone at elevations of 250–1524 m in China. The majority of collections examined in this study were collected from a Tulin ('Earth Forest') formation in the south-west of China. The two collecting localities in South Korea are near the coast, at elevations of 5–31 m (Lee & Hur 2021).

Notes. This species is mainly characterized by the somewhat squamulose thallus, large crystals in the amphithecium, fine crystals in the epihymenium and the presence of atranorin. It was first included in the genus *Protoparmeliopsis* because it nested within this genus in the ITS and mtSSU phylogenetic tree of Lee & Hur (2021). However, only data from the genus *Protoparmeliopsis* were selected for this phylogenetic reconstruction. Our results, including sequences from most major species groups and genera of *Lecanoraceae*, showed (with high support) that this species belongs to *Lecanora* s. str. rather than *Protoparmeliopsis* (Fig. 1). Morphological characters support this result. The genus *Protoparmeliopsis* is mainly characterized by placodioid or umbilicate thalli containing usnic, placodiolic or pseudoplacodiolic acids and adnate to distinctly sessile lecanorine apothecia (Zhao *et al.* 2016; Bungartz *et al.* 2020). The distinctly squamulose thallus of *L. crystalliniformis* looks similar to that of members of the genus *Protoparmeliopsis*, but the species differs by having large crystals in the amphithecium as well as the presence of atranorin and absence of dibenzofurans. These two characters correspond to *Lecanora* s. str. rather than *Protoparmeliopsis*.

Protoparmeliopsis ertzii Bungartz & Elix is morphologically similar to *Lecanora crystalliniformis* in having large crystals in the amphithecium, a brown epihymenium with minute crystals soluble in K, and the absence of lichen substances, which is unique in the genus *Protoparmeliopsis* (Bungartz *et al.* 2020). We suspect it is a member of *Lecanora* s. str. related to *L. crystalliniformis*, but without any available molecular data there is currently no support for this hypothesis. The species is so far known only from Isabela Island, Ecuador, geographically distant from East Asia.

Specimens examined. **China:** *Sichuan Province*: Xichang Co., Huanglian Earth Forest, 27°40′41.85″N, 102°12′14.25″E, 1451 m elev., on rock, 2019, *Lisong Wang* et al. 19-63013 (KUN-L-66483, FR-0183007). *Yunnan Province*: Chuxiong Yi Autonomous Prefecture, Yuanmou Co., along the roadside between Yuanmou to Yongren, 25°58′41.76″N, 101°43′49.39″E, 1524 m elev., on rock, 2017, *Lisong Wang* et al. 17-56085 (KUN-L-59521, FR-0183008), 17-56554 (KUN-L-59990, FR-0183009); Chuxiong Yi Autonomous Prefecture, Yuanmou Co., Yuanmou Earth Forest park, 25°50′47.48″N, 101°45′37.67″E, 1068 m elev., on rock, 2017, *Lisong Wang* et al. 17-56071 (KUN-L-59507, FR-0183010), 17-56082 (KUN-L-59518, FR-0183011); Chuxiong Yi Autonomous Prefecture, Yuanmou Co., Liangshan town, 25°43′53.69″N, 101°57′40.47″E, 1124 m elev., on rock, 2013, *Lisong Wang* et al. 13-39702 (KUN-L-20687, FR-0183012); Chuxiong Yi Autonomous Prefecture, Yuanmou Co., Wumao Earth Forest, 25°50′58.78″N, 101°45′31.46″E, 1124 m elev., on rock, 2013, *Lisong Wang* et al. 13-39770 (KUN-L-20755, FR-0183013). *Hong Kong*: Lung Fu Shan Country Park, Pokfulam, 22°27′84.84″N, 114°13′81.37″E, *c.* 250 m elev., on rock, 2017, *Alvin Tang* & *Henry Lui* L09 (KUN-L-61450).

Lecanora zeorina Li J. Li & Printzen sp. nov.

MycoBank No.: MB 846334

Distinguished from other species of *Lecanora* s. str. by the zeorine to lecanorine apothecia.

Type: China, Sichuan Prov., Huili Co., on the way from Huili to Jiaopingdu, rocky slopes beside the provincial highway 213, 26° 21′38.36″N, 102°19′19.85″E, 1860 m elev., on sandstone, 2019, *Lisong Wang* et al. 19-62893 (KUN-L-66434—holotype; FR-0183014—isotype).

(Fig. 3A–I)

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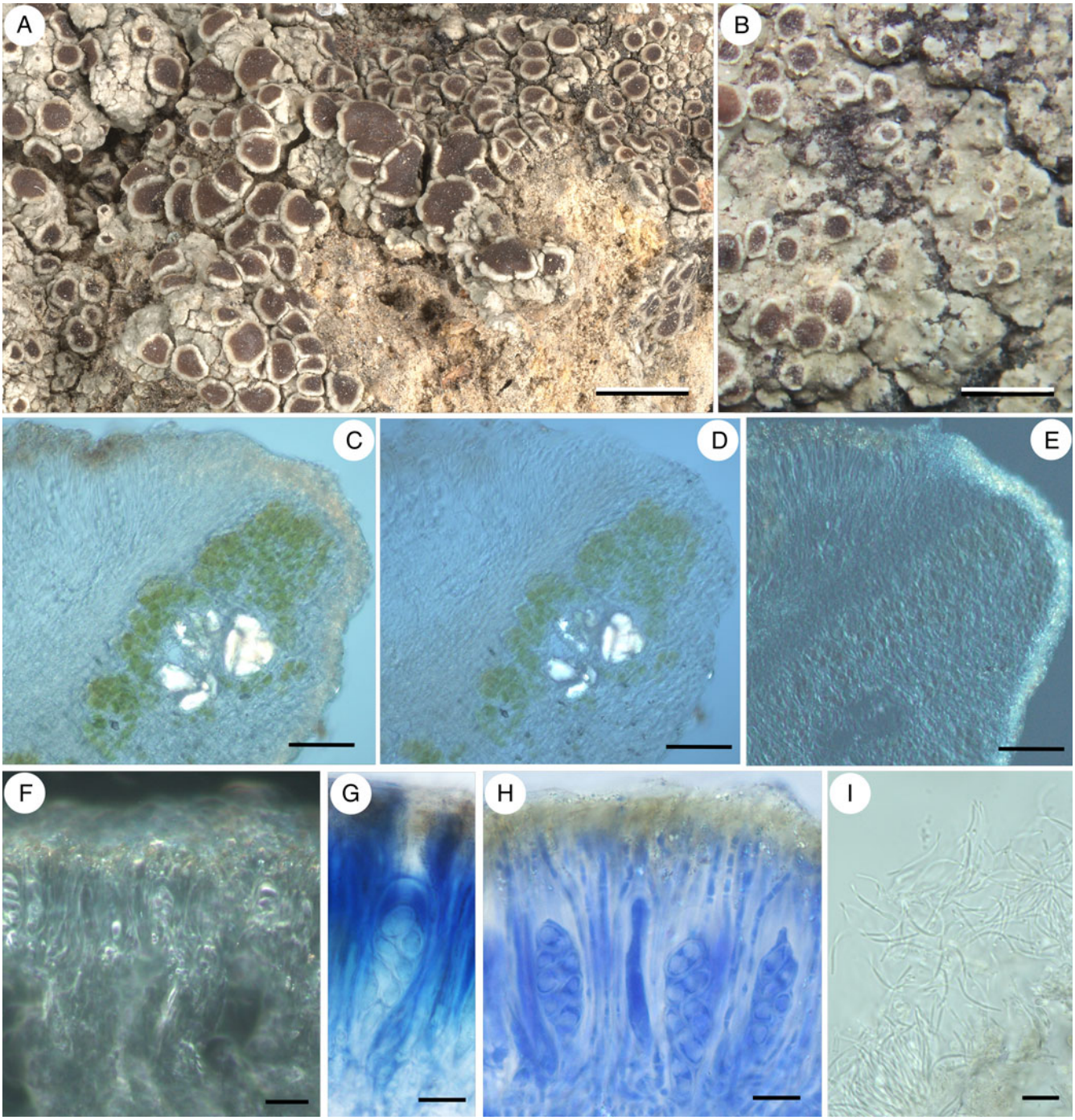

Figure 2. *Lecanora crystalliniformis* (FR-0183007). A & B, lichen thallus habit and lecanorine apothecia. C–E, vertical sections of apothecia in polarized light. C, in water, small crystals in epihymenium and amphithecial cortex, and large crystals in amphithecial medulla POL+, epihymenium with brown pigment. D, in K, cortical and epihymenial small crystals dissolved, amphithecial large crystals persistent, epihymenial pigment dissolved or paler. E, in N, cortical and epihymenial small crystals persistent, amphithecial large crystals dissolved, epihymenial pigment turned orangish. F, details of epihymenial small crystals in polarized light. G, *Lecanora*-type asci, in Lugol's iodine. H, 8-spored, clavate asci in lactophenol cotton blue, paraphyses weakly anastomosing. I, filiform conidia. Scales: A & B = 1 mm; C–E = 50 μm; F–I = 10 μm. In colour online.

Thallus crustose, dispersed verrucose to areolate, or bullate areolate, sometimes with poorly developed marginal squamules, irregular in outline, upper surface pale greyish to whitish, somewhat cracked; cortex within small crystals, POL+, dissolving in K.

Apothecia zeorine to lecanorine, numerous, rounded to undulate, dispersed or very densely aggregated to coalescent, sometimes deformed by mutual pressure, subimmersed (when young) to sessile, 0.3–1.0 mm diam.; *disc* plane to moderately convex, deep reddish brown to blackish brown, somewhat shiny, epruinose; *margin* of two layers, the inner, slightly prominent, glossy and more or less concolorous with the disc, the outer persistent or excluded, even or prominent, entire or flexuose, and concolorous with the thallus; *amphithecium* present, with

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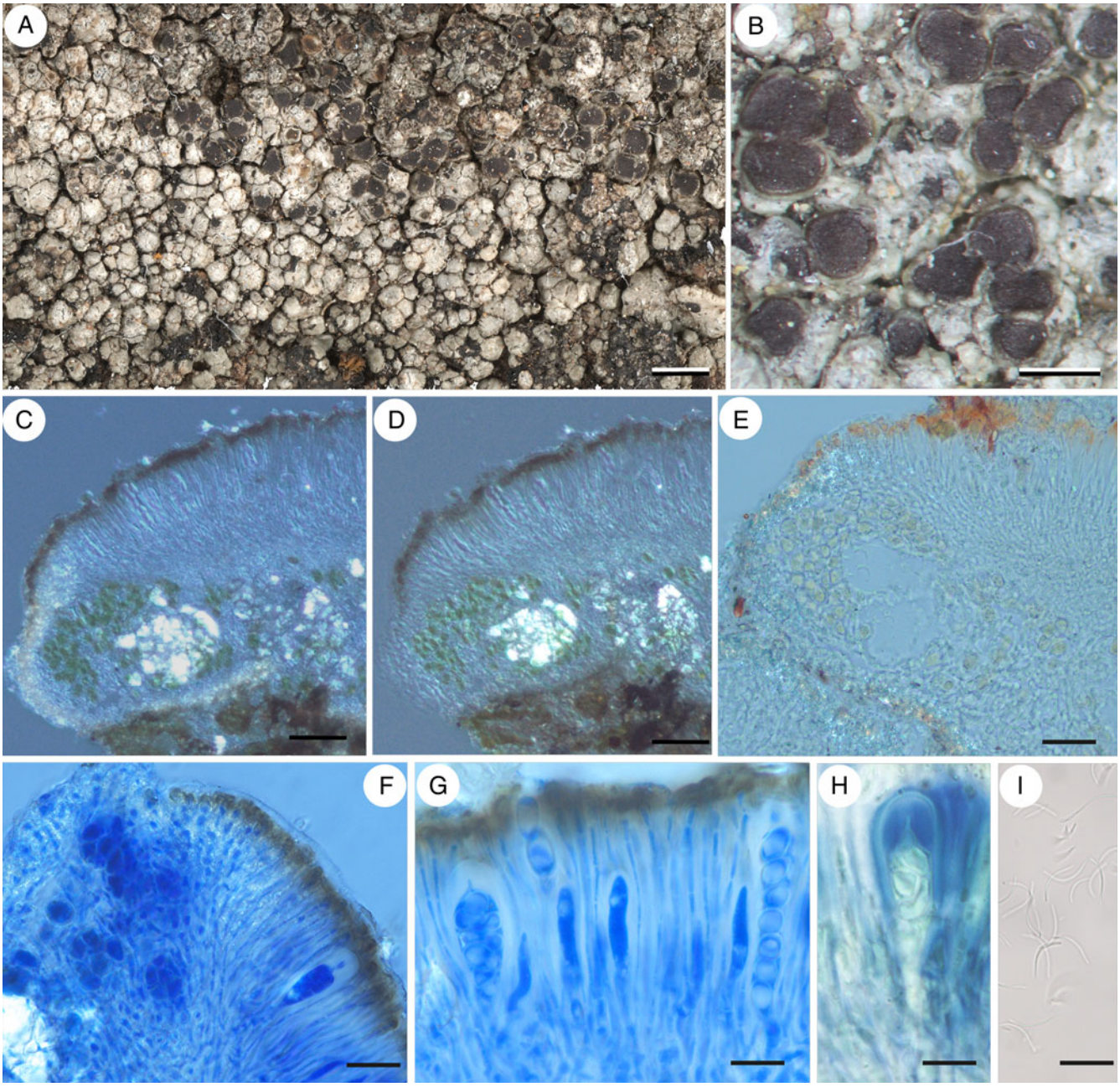

Figure 3. *Lecanora zeorina* (FR-0183014-isotype). A & B, lichen thallus habit and zeorine apothecia. C–E, vertical sections of apothecia in polarized light. C, in water, small crystals in amphithecial cortex, and medulla with large crystals, POL+, epihymenium with deep orange-brown pigment but no crystals, POL−. D, in K, cortical small crystals dissolved, amphithecial large crystal persistent, epihymenial pigment persistent. E, in N, cortical small crystals persistent, amphithecial large crystals dissolved, epihymenial pigment orange intensifying. F, proper margin (parathecium) with small crystals in lactophenol cotton blue. G, 8-spored, clavate and narrowly clavate asci in lactophenol cotton blue, paraphyses weakly anastomosing. H, *Lecanora*-type asci, in Lugol's iodine. I, filiform conidia. Scales: A & B = 1 mm; C–E = 50 μm; F–I = 10 μm. In colour online.

numerous algal cells and large crystals (POL+) insoluble in K (or occasionally partly dissolved in K, usually broken into several small pieces), soluble in N; cortex distinct, gelatinous or interspersed, filled with fine crystals soluble in K but remaining in N, 15–20(–25) μm thick; *parathecium* hyaline, consisting of thick-walled, apically somewhat radiating hyphae, 15–25(–40) μm thick, with fine crystals mostly in the outermost part which are soluble in K and insoluble in N; *epihymenium* deep orangish brown to dark brown, 10–20 μm high, with pigment not dissolving in K (sometimes becoming slightly more dark brown), orange intensifying in N, without crystals (POL−), sometimes capped with a layer of hyaline gel, *c.* 5 μm thick; *hymenium* colourless, 60–90 μm high; *paraphyses* septate, simple, *c.* 2 μm thick, tips expanded to 4 μm, surrounded by orangish brown pigment; *hypothecium* colourless, composed of anastomosing hyphae; *asci* clavate to narrowly clavate, *Lecanora*-type, 40–60 × 12–16 μm, 8-spored; *ascospores* simple, hyaline, ellipsoid to ovoid, (8.0–)9.0–13.0(–15.5) × (4.5–)5.5–7.5(–8.0) μm.

Pycnidia immersed, wall brown to olive-brown; *conidia* filiform, straight to curved, 20–25 × 1 μm.

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Chemistry. Thallus K+ yellow, KC−, C−; atranorin and an unidentified compound at R_f class 4–5 (A), 5–6 (B′), 5–6 (C), after charring yellow to orange-brown with orange halo in daylight and yellow to orange or brown with greenish halo under long-wave UV light by TLC.

Etymology. The specific epithet refers to its zeorine apothecia.

Distribution and ecology. This species occurs on sandstone and is currently known only from Sichuan Province in the south-west of China.

Notes. This species is characterized by its areolate to squamulose thallus, zeorine apothecia, large calcium oxalate crystals in the amphithecium and an epihymenium without crystals, a unique combination of characters within *Lecanora* s. str.

The zeorine apothecia of this species have a distinct proper margin protruding from the disc, and a thalline margin, which together look like two 'rims' around the apothecial disc (particularly evident in the holotype specimen). We also observed some lecanorine apothecia without a distinct protruding proper margin but a wide parathecium can always be seen in section. The term parathecium has been used as a synonym for 'true exciple' or 'proper exciple', as the inner apothecial margin in the phenotypic study of *Teloschistaceae* by Vondrák *et al.* (2013); it is a thin layer consisting of relatively thick-walled, radiating fungal hyphae lacking photobiont cells, adjacent to the hymenium and subhymenium, from which it can easily be distinguished by its negative iodine reaction (Miyawaki 1988; Guderley 1999).

Lecanora zeorina and *L. crystalliniformis* form a strongly supported group in the phylogenetic tree. Both have similar morphological characters (a somewhat areolate to squamulose thallus, large crystals in the amphithecium) and produce the same secondary metabolites. The new species differs in its zeorine apothecia, an epihymenium without crystals, a pigment insoluble in K and expanded paraphyses tips.

Additional specimens examined. **China:** *Sichuan Province*: Huili Co., on the way from Huili to Jiaopingdu, 26°21′39.11″N, 102°19′19.20″E, 1875 m elev., on sandstone, 2019, *Lisong Wang* et al. 19-63051 (KUN-L-66521, FR-0183015); Huili Co., on the way from Huili to Jiaopingdu, 26°21′39.11″N, 102°19′19.20″E, 1880 m elev., on sandstone, 2019, *Lisong Wang* et al. 19-63070 (KUN-L-66540, FR-0183016).

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