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Revision of *Dimelaena* Norman (Caliciaceae, Ascomycota) species containing usnic acid reveals a new species and a new combination from China

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Abstract

Dimelaena tibetica is described as a species new to science, characterized by a grayish yellow surface, usually with a covering of white pruina, a crustose thallus, areolate at center, radiate-plicate at margin, adnate apothecia and lacking gyrophoric acid. It differs from other usnic acid-containing *Dimelaena* species by its plane marginal lobes and adnate apothecia. Based on the study of fresh specimens from the environment of the type locality, *Dimelaena altissima* is proposed as a new combination. A fresh specimen of *Dimelaena oreina* (type species of the genus *Dimelaena*) was collected in the Swiss Alps, in the vicinity of the area where the type material of the species presumably was collected, and a sequence was generated to confirm the phylogenetic position of this genus. All three species share common characters: usnic acid content and a yellowish green upper surface. Descriptions of these species were based on a combination of morphological and phylogenetic analyses. A key and phylogram are provided for these three *Dimelaena* species which produce usnic acid.

Keywords: lichenized fungi, nuITS-nuLSU-mtSSU, phylogeny, taxonomy

Introduction

Norman (1852: 20) described the genus *Dimelaena*, distinguishing it from *Rinodina* (Ach. 1810: 344) Gray (1821: 448) primarily on the basis of its radiate-plicate thallus margins. The genus was later included in *Rinodina* by Zahlbruckner (1926). *Dimelaena* tended to be treated as a separate genus from the 1960s onwards (Poelt 1969). It was re-circumscribed and formally treated as containing seven species (Hale & Culberson 1970). Sheard (1974) redefined *Dimelaena* as possessing the following characteristics: brown spores with a single septum, unthickened walls and a radiate-plicate thallus margin. Eight more species of the genus *Dimelaena* have since been published: *D. australiensis* (H Mayrhofer & Sheard 1984: 247) and *D. weberi* (Sheard 1984: 246), *D. somaliensis* (Alstrup, Huneck & Aptroot 1994: 206) (which is a synonym for *D. tenuis* (Müll. Arg. 1881: 510) H. Mayrhofer & Wippel (1996: 304), *D. elevata* (Elix, Kalb & Wippel 1996: 298), *D. lichenicola* (K. Knudsen, Sheard, Kocourk. & H. Mayrhofer 2013: 259), *D. subsquamulosa* (Giralt, H. Mayrhofer, van den Boom & Elix 2014: 82), *D. ewersii* (Elix 2017: 38), and *D. mayrhoferiana* (Aptroot & M. Cáceres 2018: 360). Currently, eleven species are accepted worldwide. Three species of *Dimelaena* have previously been recorded in China, including the usnic acid content species *D. oreina* (Wei 2021).

Dimelaena oreina (Ach.) Norman (1852: 231) is the type species of its genus. Seven distinct chemotypes have been recognized within this species (Sheard & Ahti 1975, Obermayer *et al.* 2004). Sheard & Ahti (1975) stated that until it could be proved whether these chemotypes are either homogenous, or morphologically distinct, there was no basis for attributing taxonomic status to the chemotypes of *D. oreina*. No chemical or morphological associations among the different chemotypes were found at that time.

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In this study, we examined the chemical and morphological characteristics of over 200 specimens of the genus *Dimelaena* collected from the Tibetan Plateau during the Second Tibetan Plateau Scientific Expedition and Research Program (STEP). We constructed a phylogenetic tree based on nuITS, nuLSU and mtSSU sequences. The aim of this study was to clarify the phylogeny of *Dimelaena* species from China and to examine whether the chemotypes of *D. oreina* should have separate taxonomic status. The results show that the various chemotypes of *D. oreina* have no taxonomic value within *Dimelaena*. However, there were distinct morphological differences, which could support species delimitation. *Dimelaena altissima* is separated from *D. oreina* as a new combination, and a new species *Dimelaena tibetica* is described.

Materials and methods

Morphological and chemical study

Dimelaena specimens were mainly collected during expeditions across the Tibetan Plateau from 2018 to 2020. Most specimens were deposited in the lichen herbarium, Kunming Institute of Botany (KUN), with the exception of one specimen stored by Prof. Christoph Scheidegger at the Swiss Federal Institute for Forest, Snow and Landscape Research, Switzerland (WSL), and two deposited in the herbarium of Ningxia Agricultural College (NXAC). All these specimens were examined using light microscopy. External morphology was observed under a dissecting microscope (Nikon SMZ 745T). Anatomical characteristics were described at 400× magnification under a compound microscope (Nikon Eclipse Ci-S). Measurements are presented as the range between the smallest and largest values. Photographs were taken using a NIKON digital camera head (Nikon DS-Fi2). Secondary metabolites were tested using spot tests and TLC with solvent C (White & James 1985, Orange *et al.* 2001).

DNA extraction, purification and sequencing

DNA was extracted using DNA Secure Plant Kits (TIANGEN) according to the manufacturer's instructions. Subsequently, the following regions were amplified: nuITS, nuLSU and mtSSU, for which the primers used were respectively ITS1F & ITS4 (White *et al.* 1990, Gardes & Bruns 1993), LR0R & LR5 (Vilgalys & Hester 1990, Rehner & Samuels 1994) and mtSSU1 & mtSSU3R (Zoller *et al.* 1999). PCR amplifications were performed using 1.1 × T3 Super PCR Mix (TSINGKE) in a 25 μL volume, containing 1 μL of genomic DNA, 1 μL of a 10 mM solution for each primer and 22 μL of 1.1 × Taq PCR Mix. The PCR program was: initial denaturation at 98°C for 3 min, followed by 35 cycles of 98°C for 10 s, 54–56°C for 10 s, 72°C for 15 s, followed by a final extension at 72°C for 2 min. The PCR products were sequenced with the same amplification primers using Sanger technology by Tsingke Biotechnology Co., Ltd. (Kunming).

Phylogenetic analysis

The raw sequences were edited using Geneious v8.0.2, together with representative sequences of Caliciaceae Chevall. and Physciaceae downloaded from GenBank (Table 1). All sequences were aligned using MAFFT v7 (Katoh *et al.* 2005) with the following parameters: algorithm = auto; scoring matrix = 200; PAM / k = 2; gap opening penalty = 1.53; offset value = 0.123. Ambiguous regions were excluded using Gblocks (Talavera & Castresana 2007) with the default settings. The final matrix was submitted to TreeBASE with accession number TB2: S29477. Bayesian inference (BI) and maximum likelihood (ML) were employed to reveal the phylogenetic relationships. In this phylogenetic tree, all sequences belong to Physciaceae and Caliciaceae. In Caliciaceae, several representative monophyletic genera were selected, including *Amandinea* M. Choisy ex Scheid. & H. Mayrhofer, *Buellia* s. str. De Not., *Tetramelas* Norman, *Thelomma* A. Massal., *Diplotomma* A. Massal. and *Pyxine* Fr. Several Physciaceae species were selected as the outgroup.

ML analyses were performed via RAxML v8.2.12 (Stamatakis 2006). Based on the lowest Bayesian information criterion (BIC), the best-fit partition substitution models were selected using Partition Finder 2 (Guindon *et al.* 2010, Lanfear *et al.* 2012, 2017): GTR+I+G for ITS1, nuLSU and mtSSU; GTR+G for ITS2 and 5.8S. Bootstrap frequencies were estimated from the consensus tree built with 1000 trees obtained from nonparametric bootstrapping pseudoreplicates (Hall 2017). Bootstrap support values (MLBS) were obtained from the 70% majority rule tree of all saved trees.

BI analyses were performed with the same model as ML via MrBayes v3.2.6 (Ronquist *et al.* 2012). Markov Chain Monte Carlo algorithms with 4 incrementally heated chains were run for 2 million generations to implement

the BI analyses. Trees were sampled every 100 generations during the run. The stop rule was the average standard deviation of split frequencies < 0.01. The first 25% of trees were discarded as burn-in. Bayesian posterior probabilities (BPP) were obtained from the 95% majority rule consensus tree of all saved trees. The tree was visualized by FigTree v1.4.0 (Rambaut 2012).

TABLE 1. Specimens used in this study, with the taxon name, voucher, locality and GenBank accession number. Newly obtained sequences are in bold font.

Taxon	Voucher	Locality	Accession number		
			nuITS	nuLSU	mtSSU
Acolium inquinans	Wedin 6352 (UPS)	SWE	AY450583	AY453639	AY143404
A. karelicum	Hermansson 16472 (UPS)	-	KX512897	KX512879	-
Amandinea coniops	Nordin 6113 (UPS)	-	-	KX512865	KX512978
Am. frigida	Westberg (S)	-	KX512903	KX512852	KX512992
Am. punctata 1	AFTOL 1306	-	HQ650627.1	DQ986756.1	-
Am. punctata 2	**18-60759 (KUN)**	Xizang, CHN	**OL467351**	-	-
Buellia arborea	ANT050881 (KoLRI)	CHE	KX132975	-	-
B. disciformis 1	EDNA09-01524	UK	FR799139	-	-
B. disciformis 2	EDNA09-02095	UK	FR799136	-	-
B. disciformis 3	EDNA09-02116	UK	FR799138	-	-
B. muriformis	Nordin5336a (UPS)	USA	AF540501	-	-
B. penichra	Nordin5322 (UPS)	USA	AF540503	-	-
Calicium chlorosporum	Tibell 25012 (UPS)	-	-	KX512892	KX512956
C. nobile 1	Tibell 21968 (UPS)	-	KX512913	KX529070	KX512988
C. nobile 2	Tibell 23396 (UPS)	IND	KX512914	KX529071	KX512987
Dimelaena altissima	**19-63935 (KUN)**	Xizang, CHN	**MZ229869**	-	-
D. altissima	**18-58543 (KUN)**	Gansu, CHN	**MZ229883**	-	-
D. altissima	**18-58855 (KUN)**	Gansu, CHN	**MZ229884**	-	-
D. oreina	**18-58843 (KUN)**	Gansu, CHN	**MZ229874**	**OL444786**	-
D. oreina	**18-59460 (KUN)**	Gansu, CHN	**MZ229875**	**OL444787**	-
D. oreina	**18-59552 (KUN)**	Gansu, CHN	**MZ229879**	-	-
D. oreina	**18-58641 (KUN)**	Gansu, CHN	**MZ229880**	-	-
D. oreina	**Scheidegger 11363**	Valais, CHE	**OK569798**	-	-
D. oreina	Nordin 4800	-	AF224352	-	-
D. oreina	Mayrhofer 13.737 (GZU)	AUT	AJ421417	-	-
D. tibetica	**20-68977 (KUN)**	Xizang, CHN	**MZ229868**	-	**OL467283**
D. tibetica	**18-59122 (KUN)**	Qinghai, CHN	**MZ229878**	-	-
D. tibetica	**19-65539 (KUN)**	Xizang, CHN	**MZ229871**	-	-
D. tibetica	**18-58119 (KUN)**	Qinghai, CHN	**MZ229885**	-	-
D. tibetica	**16-50799 (KUN)**	Xizang, CHN	**MZ229889**	-	-
Diplotomma alboatrum 1	**18-60034 (KUN)**	Yunnan, CHN	MN615696	**OL444781**	**OL467286**
Di. alboatrum 2	**18-60448 (KUN)**	Yunnan, CHN	**MZ224658**	**OL444782**	**OL467287**
Di. alboatrum 3	Prieto 3034 (S)	-	kx512924	KX512877	KX512966
Di. venustum 1	**18-58557 (KUN)**	Gansu, CHN	**OL467349**	**OL444779**	**OL467284**
Di. venustum 2	**18-58102 (KUN)**	Qinghai, CHN	**OL467350**	**OL444780**	**OL467285**

.....continued on the next page

TABLE 1. (Continued)

Taxon	Voucher	Locality	Accession number		
			nuITS	nuLSU	mtSSU
Di. venustum 3	**XY19-252 (KUN)**	Xizang, CHN	**OL467353**	**OL444784**	**OL467290**
Di. venustum 4	Westberg 10-176 (S)	-	KX512925	-	KX512968
Heterodermia speciosa	Wetmore (S)	-	KX512927	KX512868	KX512975
H. vulgaris	Frisch 11/Ug1226 (UPS)	-	KX512928	KX512857	KX512989
Pyxine coccoes	Prieto (S)	-	KX512936	-	KX512964
P. sorediata 1	-	-	-	DQ973036.1	-
P. sorediata 2	Wetmore 91254 (S)	-	KX512937	KX512870	KX512973
P. subcinerea	-	-	HQ650705	DQ883802	-
Phaeophyscia ciliata	Prieto (S)	-	KX512929	KX512886	KX512958
Ph. orbicularis	Prieto 3012 (S)	-	KX512930	KX512876	KX512967
Physcia aipolia	Wedin 6145 (UPS)	-	KX512931	AY300857	AY143406
Phy. tenella	Odelvik & Hellström 0827 (S)	-	KX512932	KX512869	KX512974
Thelomma mammosum 1	Tibell 23775 (UPS)	-	KX512942	KX512888	KX512954
T. mammosum 2	Hernández *et al.* 2002 (UPS)	-	KX512943	KX512851	KX512953
T. santessonii 1	Nordin 4011 (UPS)	-	KX512944	KX512889	KX512951
T. santessonii 2	Nash 38262 (UPS)	-	KX512945	KX512890	KX512950
Tetramelas chloroleucus	Westberg 10-001 (S)	-	KX512938	KX512875	-
Te. geophilus 1	**18-60217 (KUN)**	Yunnan, CHN	MN615682	**OL444783**	**OL467288**
Te. geophilus 2	**20-67496 (KUN)**	Yunnan, CHN	**OL467354**	**OL444785**	**OL467291**
Te. pulverulentus	Nordin 6368 (UPS)	-	KX512940	KX512860	KX512983

Results

The 3-locus (nuITS, nuLSU and mtSSU) concatenated matrix comprised 114 terminals, which included 36 newly generated sequences (Table 1). The phylogenetic analyses showed that the genus *Dimelaena* is clustered within Caliciaceae with high support (100% ML and 1.00 PP, Fig. 1). Furthermore, within *Dimelaena*, three clades were formed. Firstly, a sequence of *Dimelaena oreina* (type species of *Dimelaena*) was newly generated from Switzerland, forming a single clade with other sequences of this species, with high support (97% ML and 1.00 PP), confirming the phylogenetic position of this genus for the first time. Secondly, the proposed new combination *D. altissima* (H. Magn.) M. Ai & Xin Y. Wang also formed a monophyletic clade with high support (99% ML and 1.00 PP). Thirdly, *D. tibetica* M. Ai & Xin Y. Wang *sp. nov.* formed another distinct monophyletic clade with strong support (100% ML and 1.00 PP). All three species were supported by distinct morphological and chemical characteristics.

Seven chemotypes had previously been detected in *D. oreina*, five of which were reported from Asia (Obermayer *et al.* 2004). Four of these chemotypes were detected in our materials (Ⅱa, Ⅲ, Ⅴ and Ⅶ). We did not detect fumarprotocetraric acid (chemotype I) in our materials, although it had previously been reported from Tibetan specimens by Obermayer *et al.* (2004). Gyrophoric acid and stictic acid (chemotype Ⅶ) were detected in only one specimen (*18-58641*, KUN) of *D. oreina* from Gansu Province. Gyrophoric acid (chemotype Ⅱa) was detected in *D. oreina* and *D. altissima*. Stictic acid and hypostictic acid (chemotype Ⅴ) were detected in *D. altissima* and *D. tibetica*. Usnic acid without additional substances (chemotype Ⅲ) was detected in *D. tibetica* (Table 2).

Discussion

Species delimitation of Dimelaena

In this study, three distinct lineages were shown within the genus *Dimelaena*, representing three species. *Rinodina altissima* previously was treated as a synonym of *D. oreina* (Sheard & Ahti 1975). However, phylogenetic analysis

of materials from the type localities (Gansu Province, China) showed that these two species are sister groups. Thus, they should be treated as two separate species within the genus *Dimelaena*. The separation of these two species was supported by significant morphological differences: *D. oreina* has convex marginal lobes, pycnidia are numerous and apothecia are symmetrically round with well-defined margins. In contrast, *D. altissima* has a flat-lobed thallus margin, tightly attached to the substrate, and innate apothecia, irregularly rounded and lacking prominent margins. The proposed new species *D. tibetica* forms a separate clade, which is morphologically distinguished by possessing convex apothecia, pruinose thallus surface, lacking black pigmentation around the areole margins and lacking gyrophoric acid.

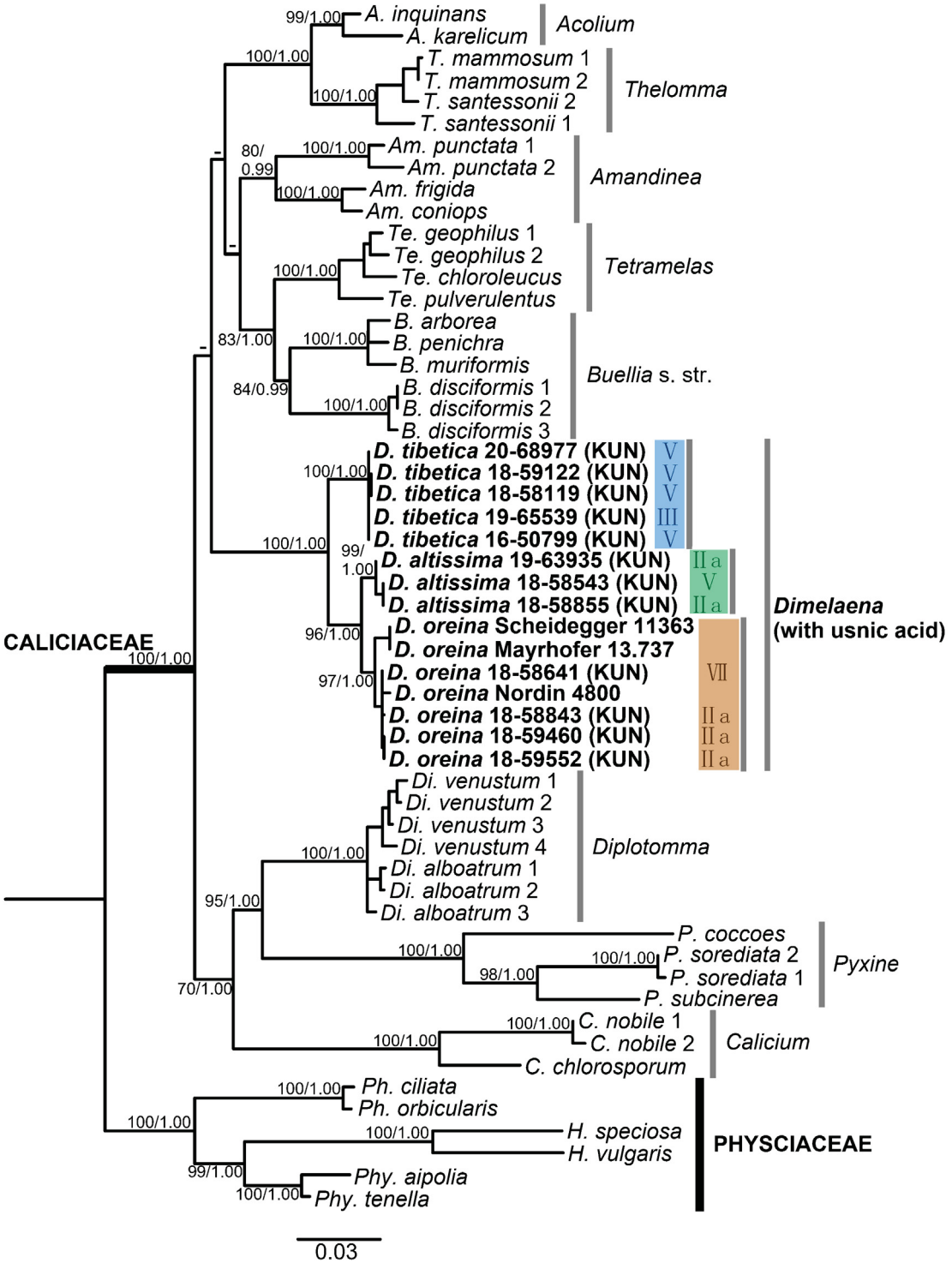

FIGURE 1. Phylogenetic tree generated from maximum likelihood (ML) analysis based on nuITS-nuLSU-mtSSU concatenated sequence matrix. Bootstrap support values for ML and Bayesian posterior probabilities for BI are given near the nodes.

TABLE 2. Species of examined Chinese *Dimelaena* specimens and their chemical compounds. Main distinguishing chemical compounds are in bold font.

Species	Chemical compounds	Corresponding 'Chemotype'
D. oreina	a. usnic acid, **gyrophoric acid**, lecanoric acid (trace)	II a
	b. usnic acid, **gyrophoric acid, stictic acid**	VII
D. altissima	a. usnic acid, **gyrophoric acid**, lecanoric acid (trace)	II a
	b. usnic acid, **stictic acid, hypostictic acid**, [no norstictic acid], menegazziaic acid (trace), cryptostictic acid (trace), constictic acid (trace), hypoconstictic acid (trace)	V
D. tibetica	a. usnic acid [no further substances]	III
	b. usnic acid, **stictic acid, hypostictic acid**, norstictic acid, menegazziaic acid (trace), cryptostictic acid (trace), constictic acid (trace), hypoconstictic acid (trace)	V

Many previous synonyms of *D. oreina* (e.g., *Rinodina altissima*) were assigned to different chemotypes (Sheard & Ahti 1975). However, our phylogenetic and chemical analyses show that in China these 'chemotypes' belong to more than one clade. None of the chemotypes included in this study are monophyletic. In other words, chemotype characteristics do not correspond to the molecular clades. Thus morphology, not chemistry, should be the basis for differentiating between these species, and anatomical differences were not detected.

Phylogeny and genus concept for Dimelaena

As shown in the phylogenetic tree, the three *Dimelaena* species with usnic acid form a monophyletic lineage with high support, clustering within Caliciaceae, which is consistent with previous results based on 5-locus phylogenetic analysis (Prieto & Wedin 2017). All the species in the '*Dimelaena*' clade produce usnic acid, whereas species lacking usnic acid (e.g., *Dimelaena radiata*) form a separate clade according to previous studies (Gaya *et al.* 2012, Prieto & Wedin 2017). Therefore, the genus concept might require revision, with such species lacking usnic acid being excluded from *Dimelaena*. However, redefinition of this genus needs further study, requiring fresh material with matching molecular data from other *Dimelaena* species.

Taxonomy

Key to Dimelaena species with usnic acid:

1. Marginal lobes convex, loosely attached to the substrate; edges of the areolae usually dark; pycnidia numerous ..... *D. oreina*
- Marginal lobes plane and closely attached to the substrate; edges of the areolae rarely dark; pycnidia rare 2
2. Apothecia adnate and apothecia margins obvious, upper surface grayish-yellow, with whitish pruina ..... *D. tibetica*
- Apothecia innate and apothecia margins absent or not obvious, upper surface yellowish-green, without pruina ..... *D. altissima*

Dimelaena altissima (H. Magn.) M. Ai & Xin Y. Wang, *comb. nov.* MycoBank no. 841532

Rinodina altissima H. Magnusson (1940: 155).

Type:—CHINA. Gansu Province: Taben-Buluk, 4000 m, 1931, Birger Bohlin 35a (S, holotype!).

Rinodina altissima var. *exalbescens* H. Magnusson (1940: 156); *Dimelaena oreina* var. *exalbescens* (H. Magn.) Wei (1991: 89).

Type:—CHINA. Gansu Province: Ehr-tao-ch'uan (Nan-shan), at about 4200 m, on rock facing the South, 5 January 1932, Birger Bohlin 74b (S, holotype**!**).

(Fig. 3)

Thallus crustose, thick or thin, closely attached to the substrate, areolate at center and radiate-plicate at margin, areolae 0.55–0.90 mm wide, sometimes darkened around the areolae; marginal lobes flat and narrow, < 3.00 mm long, c. 1.50 mm wide, tightly attached to the substrate, sometimes darkened around the tips; thallus yellowish green with usnic acid in cortex but sometimes lighter to pale yellowish white, medulla white, without the lower cortex.

Apothecia cryptolecanorine to lecanorine, immersed, often touching each other, irregularly rounded, 0.18–0.63 mm in diam., disc black, disc margin not obvious or absent; *epihymenium* brown; *hymenium* hyaline, 60–80 μm high, inspersed with oil droplets; *hypothecium* hyaline, 80–135 μm high; *asci* clavate, *Bacidia*-type, 8-spored; *ascospores* brown, 1-septate, thin walled, 8.5–13 × 4.5–7 μm, markedly constricted at septum when mature.

Pycnidia rare, immersed; *conidia* bacilliform, 6 × 1 µm.

Chemistry: Usnic and gyrophoric acids, sometimes with hypostictic, stictic and norstictic acids.

Ecology and distribution: This species usually grows on exposed siliceous rock, at elevations of 2737–4805 m. Distributed in Sichuan, Xizang, Qinghai and Gansu provinces in China (Fig. 2).

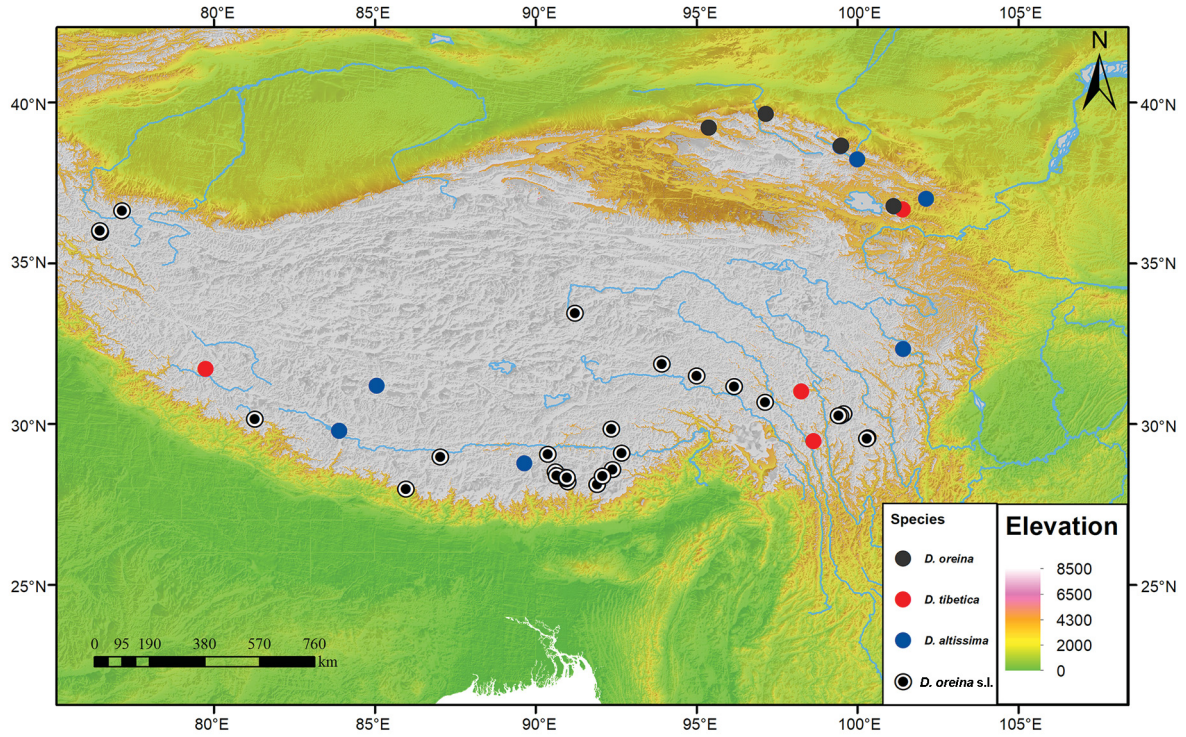

FIGURE 2. Distribution of species of *Dimelaena* containing usnic acid in the Tibetan Plateau. Distribution of *D. oreina* s.l. is from Obermayer *et al.* (2004).

Notes: Dimelaena altissima is characterized by having a plane lobed thallus margin, tightly attached to the substrate, innate and irregularly rounded apothecia. It differs from *D. oreina* by lacking convex marginal lobes and well-defined apothecia margins. Sheard & Ahti (1975) regarded *Rinodina altissima* as a synonym for *D. oreina* due to similar anatomical features, and because morphological characters did not correlate with chemical ones. Obermayer *et al.* (2004) agreed with this treatment. Now, however, morphological differences, supported by molecular data, lead to the division of these species. There is no reason to subsume several chemotypes within a species. Gyrophoric acid was detected in *D. altissima.* In addition, stictic acid and hypostictic acid were detected in some specimens.

Additional specimens examined. CHINA. Gansu Province: Sunan Co., on the route from Sunan to Qilian, 3479 m, 38°39′16.58″N, 99°29′30.87″E, 30 May 2018, *Wang Lisong et al. 18-59774*; Sunan Co., on the route from Sunan to Qilian, 3928 m, 38°37′45.45″N, 99°28′31.76″E, 30 May 2018, *Wang Lisong et al. 18-58855*; Subei Co., Nanshan Mt., near 29th Mengke Glacier, 3392 m, 39°13′52.53″N, 95°24′18.40″E, 23 May 2018, *Wang Lisong et al. 18-58543.* Qinghai Province: Huangyuan Co., on the route to Qinghai Lake, 2737 m, 36°45′54.72″N, 101°08′05.79″E, 18 May 2018, *Wang Lisong et al. 18-59138*; Huzhu Co., beside Weibei road, 3060 m, 37°00′26.52″N, 102°08′16.81″E, 31 May 2018, *Wang Lisong et al. 18-59930*; Qilian Co., on the route from Sunan to Qilian, 2807 m, 38°13′44.92″N, 99°59′48.79″E, 30 May 2018, *Wang Lisong et al. 18-59878*; Zaduo Co., 4090 m, 32°52′43.78″N, 95°20′28.55″E, 20 September 2020, *Wang Lisong et al. 20-68622.* Sichuan Province: Rangtang Co., Haizishan, 4237 m, 32°20′02.40″N, 101°25′00.23″E, 9 June 2020, *Wang Lisong et al. 20-66696*. Xizang Province: Cuoqin Co., 4805 m, 31°11′37.70″N, 85°03′32.37″E, 20 July 2019, *Wang Lisong et al. 19-65370;* Kangma Co., Nanni Vil., 4136 m, 28°46′16.29″N, 89°38′48.64″E, 24 July 2019, *Wang Xinyu et al. xy19-1416*; Zhongba Co., Zhuzhu Vil., 4647 m, 29°47′17.16″N, 83°54′09.76″E, 25 July 2019, *Wang Lisong et al. 19-63935*; Zhongba Co., Zhuzhu Vil., 4650 m, 29°47′17.29″N, 83°54′09.74″E, 25 July 2019, *Wang Lisong et al. 19-63938;* Zhongba Co., Zhuzhu Vil., 4639 m, 29°47′16.57″N, 83°54′09.61″E, 25 July 2019, *Wang Lisong et al. 19-63927;* Kangma Co., Nanni Vil., 4120 m, 28°46′14.32″N, 89°38′48.78″E, 24 July 2019, *Wang Xinyu et al. XY19-2343;* Yadong Co., Kangbu Vil., 4039 m, 27°45′41.83″N, 88°58′58.41″E, 27 July 2019, *Wang Xinyu et al. XY19-1719.*

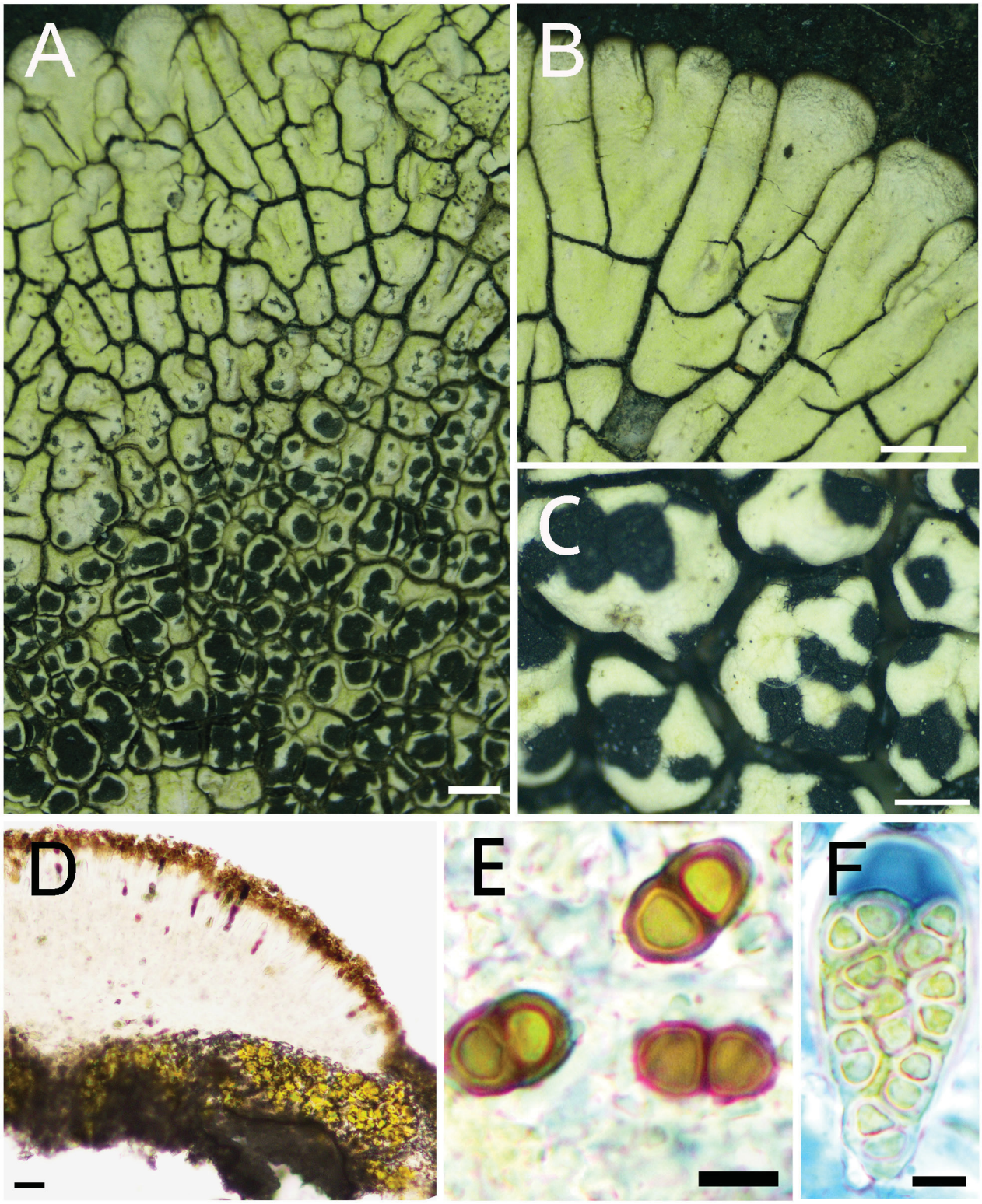

FIGURE 3. Morphology and anatomy of *Dimelaena altissima* (*18-58855*, KUN). **A.** Thallus of *Dimelaena altissima*. **B.** Straight and plane lobes of radiate-plicate margin. **C.** Cryptolecanorine apothecia. **D.** Section of apothecium. **E.** *Buellia*-type ascospores, constricted at septum. **F.** *Bacidia*-type ascus-apex. Scale bars: 1 mm (A, B); 0.5 mm (C); 25 µm (E); 5 µm (E, F).

Dimelaena oreina (Ach.) Norman (1852: 231) MycoBank no. 384375

Lecanora straminea var. *oreina* Acharius (1810: 432).

Type:—SWITZERLAND. 'In alpibus Helvetiae', Schleicher, 1018A (H-ACH, lectotype!).

For further synonyms see Sheard & Ahti (1975).

(Fig. 4)

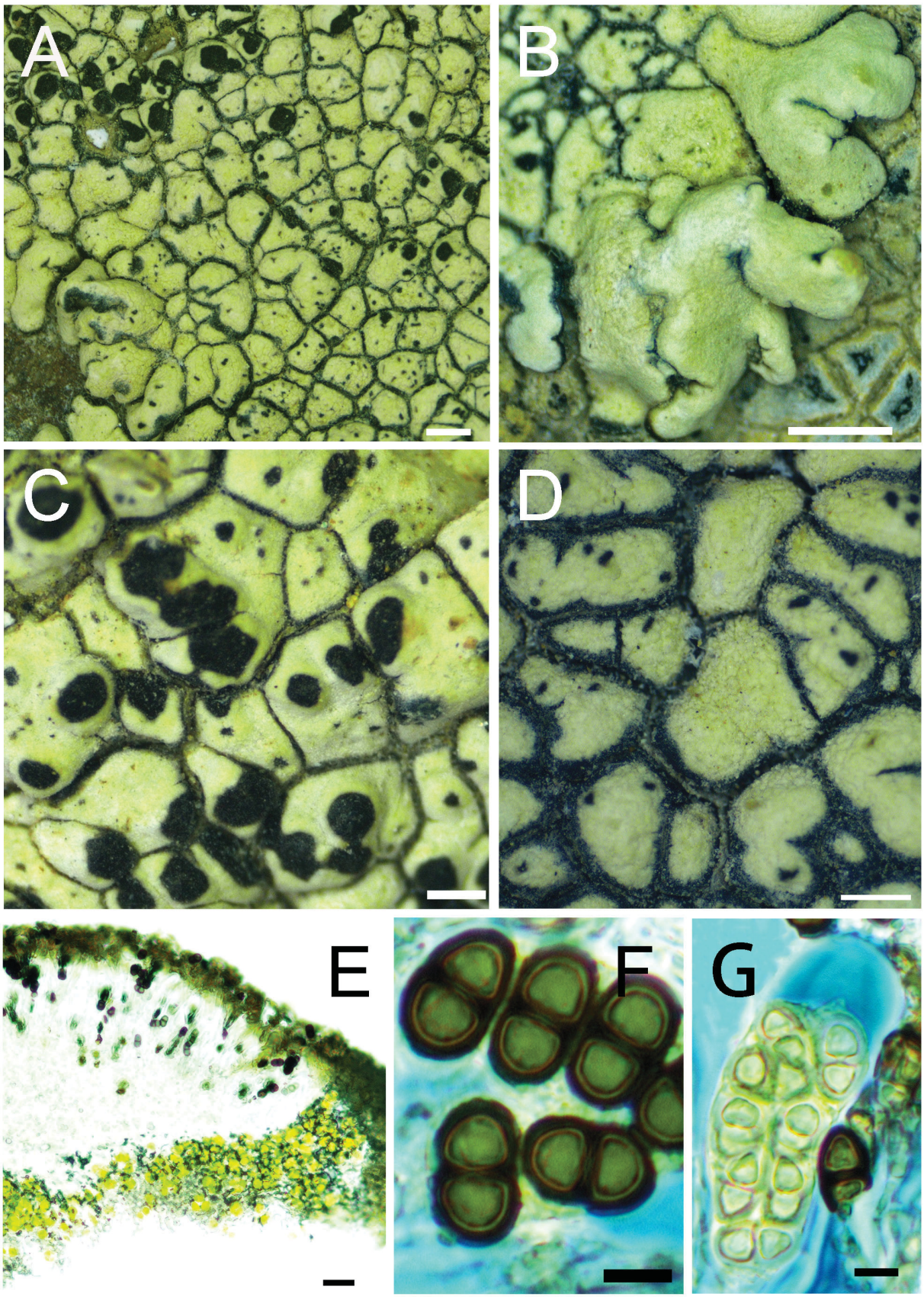

FIGURE 4. Morphology and anatomy of *Dimelaena oreina* (18-59460, KUN). **A.** Thallus of *Dimelaena oreina*. **B.** Convex non-linear lobes of radiate-plicate margin. **C.** Cryptolecanorine apothecia. **D.** Darkened margin around the areolae. **E.** Section of apothecium. **F.** *Buellia*-type ascospores, constricted at septum. **G.** Typical *Bacidia*-type ascus-apex. Scale bars: 1 mm (A, B); 0.5 mm (C, D); 25 µm (E); 5 µm (F, G).

Thallus crustose, thick or thin, loosely attached to the substrate, areolate at center and radiate-plicate at margin, areolae large, up to 2.00 mm long, 1.15 mm wide, irregular in shape, usually darkened around the areolae and surface margin; marginal lobes narrow, < 2.00 mm long, c. 1.50 mm wide, thick, convex and usually darkened around the tips; thallus yellowish green with usnic acid in cortex but sometimes lighter to pale yellowish white, medulla white, without the lower cortex.

Apothecia cryptolecanorine to lecanorine, innate to adnate, often touching each other, roundish, 0.18–1.20 mm in diam., disc black, disc margin concolorous with thallus; *epihymenium* brown; *hymenium* hyaline, 80–100 μm high, inspersed with oil droplets; *hypothecium* hyaline, 80–125 μm high; *asci* clavate, *Bacidia*-type, 8-spored; *ascospores* brown, 1-septate, thin walled, 8.5–13 × 4.5–7 μm, markedly constricted at septum when mature.

Pycnidia numerous, immersed; *conidia* bacilliform, 3–6 × 0.7–1 μm.

Chemistry: Usnic and gyrophoric acids, occasionally with stictic acid in our examined specimens from China.

Ecology and distribution: This species usually grows on exposed siliceous rock, at elevations of 2798–3942 m. Distributed in North America, South America (rare), Europe, Asia and South Africa (rare) (Sheard & Ahti 1975, Mayrhofer *et al.* 1996).

Notes: Dimelaena oreina is the type species of *Dimelaena*. One specimen from Switzerland (where *D. oreina* was originally described) has a newly generated molecular profile to confirm the phylogenetic position of the genus *Dimelaena*. Specimens of *D. oreina* from China cluster with those from Europe in the phylogenetic tree. Furthermore, the specimens in this clade contain gyrophoric acid, which is consistent with spot test results from the type specimen (K–; KC–). The species still needs further study by molecular and chemical analysis including all the five chemotypes from Europe.

Additional specimens examined. CHINA. Gansu Province: Subei Co., Nanshan Mt., near 29th Mengke Glacier, 3942 m, 39°12′42.51″N, 95°23′00.50″E, 23 May 2018, *Wang Lisong et al.* 18-59460; Subei Co., Nanshan Mt., near 29th Mengke Glacier, 3909 m, 39°12′43.08″N, 95°22′59.77″E, 23 May 2018, *Wang Lisong et al.* 18-59432; Subei Co., Nanshan Mt., near 29th Mengke Glacier, 3942 m, 39°12′43.93″N, 95°23′00.32″E, 23 May 2018, *Wang Lisong et al.* 18-59450; Subei Co., Nanshan Mt., near 29th Mengke Glacier, 3885 m, 39°12′40.74″N, 95°23′03.95″E, 23 May 2018, *Wang Lisong et al.* 18-58504; Sunan Co., on the route from Sunan to Qilian, 3492 m, 38°39′16.51″N, 99°29′29.16″E, 30 May 2018, *Wang Lisong et al.* 18-58843; Yumen Ci., Yuerhong Vil., 3332 m, 39°38′13.77″N, 97°09′15.57″E, 27 May 2018, Wang Lisong *et al.* 18-58641; Yumen Ci., Yuerhong Vil., 3291 m, 39°38′14.84″N, 97°09′15.94″E, 27 May 2018, *Wang Lisong et al.* 18-59552. Qinghai Province: Huangyuan Co., rocky slope beside G315, 2798 m, 36°46′53.65″N, 101°07′21.77″E, 18 May 2018, *Wang Lisong et al.* 18-58134.

Dimelaena tibetica M. Ai & Xin Y. Wang, *sp. nov.* MycoBank No. 841524

Characterized by a grayish yellow surface, usual covered in white pruina, and by a plane, radiate-plicate thallus margin, a crustose, areolate thallus, adnate apothecia and numerous pycnidia.

Type:—CHINA. Qinghai Prov.: Huangyuan Co., 36°39′55.26″N, 101°24′32.23″E, 2430 m, on siliceous rock, 18 May 2018, Wang Lisong *et al.* 18-58092 (KUN, holotype!).

Etymology: The epithet ‘tibetica’ refers to its distribution being mainly in the Tibetan Plateau.

(Fig. 5)

Thallus crustose, rarely bulging and verrucous, thick or thin, tightly attached to the substrate, areolate at center and plane, radiate-plicate at margin, cracks obvious, areolae large, 2.00–3.00 mm wide; marginal lobes slim and long, up to 4.00 mm long, 1.00 mm wide, tightly attached to the substrate, apex lobes multilobed; thallus grayish yellow with usnic acid in cortex, usually a covering of white pruina near the thallus tip; medulla white, without the lower cortex.

Apothecia cryptolecanorine to lecanorine, one or more apothecia in each areola, sessile, scattered, roundish, 0.20–0.60 mm in diam., disc black, disk margin obvious or concolorous with thallus; *epihymenium* brown; *hymenium* hyaline, 50–75 μm high, inspersed with oil droplets; *hypothecium* hyaline, 75–100 μm high; *asci* clavate, *Bacidia*-type, 8-spored; *ascospores* brown, 1-septate, thin walled, 8–12 × 4–7 μm, markedly constricted at septum when mature.

Pycnidia rare, immersed; *conidia* bacilliform, 6 × 1 μm.

Chemistry: Usnic, stictic, norstictic and hypostictic acids.

Ecology and distribution: This species usually grows on exposed siliceous rock, at elevations of 1366–4232 m. Distributed in Xizang, Qinghai and Ningxia provinces in China (Fig. 2).

Notes: The new species *D. tibetica* is similar to *D. oreina*, but with a plane, radiate-plicate thallus margin, and the apothecia of the new species are raised, the upper surface has a grayish covering of white pruina, and the margins of the areoles are not darkened. The apothecia of *D. oreina* are innate to adnate, the upper surface is bright yellowish-green

without pruina, the edge of the areolae is darkened and pycnidia are numerous. Furthermore, gyrophoric acid has not been reported in *D. tibetica* while *D. oreina* in China always contains this compound. Obermayer *et al.* (2004, Figure 5) displayed two types of apothecia in *Dimelaena oreina* s.l. Based on phylogenetic analysis, these should belong to distinct species: 5A is similar to *D. tibetica* and 5B is *D. altissima*.

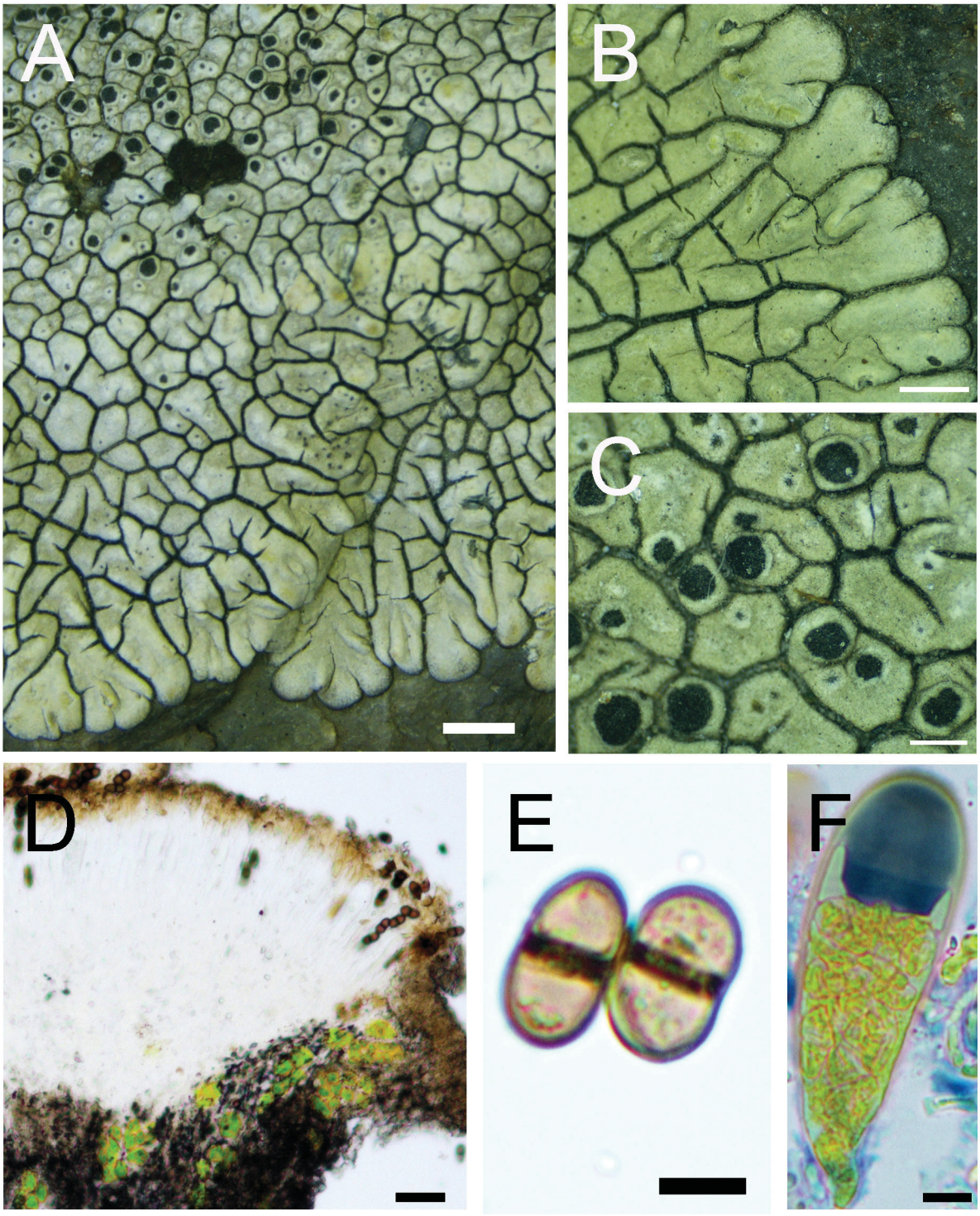

FIGURE 5. Morphology and anatomy of *Dimelaena tibetica* (18-58092, KUN). **A.** Thallus margin of *Dimelaena tibetica*. **B.** Straight and plane lobes of radiate- plicate margin. **C.** Isolated cryptolecanorine apothecia (disc margin obvious). **D.** Section of apothecium. **E.** *Buellia*-type ascospores, constricted at septum. **F.** *Bacidia*-type ascus-apex. Scale bars: 1 mm (A, B); 0.5 mm (C); 25 µm (D); 5 µm (E, F).

Additional material studied. CHINA. Ningxia Province: Helan Mt., dakouzi, 1366 m, 38°35′32″N, 105°56′31″E, 24 September 2010, *Niu Dongling 10-0259* (NXAC); Helan Mt., Xiangshuigou, 2189 m, 38°46′30.9″N, 105°54′48.8″E, 10 June 2017, *Niu Dongling 17-0417* (NXAC). Qinghai Province: Huangyuan Co., by the side of the Qinghai Lake,

2441 m, 36°39′57.41″N, 101°24′32.03″E, 18 May 2018, *Wang Lisong et al. 18-59122*; Huangyuan Co., 2430 m, 36°39′55.26″N, 101°24′32.23″E, 18 May 2018, *Wang Lisong et al. 18-58092*; Huangyuan Co., on the route to Qinghai Lake, 2473 m, 36°39′57.15″N, 101°24′32.28″E, 18 May 2018, *Wang Lisong et al. 18-58119*. Xizang Province: Gongjue Co., Xiangpi Vil., 3578 m, 31°00′46.41″N, 98°15′24.14″E, 24 September 2020, *Wang Lisong et al. 20-68977;* Mangkang Co., Bangda Town beside the G318 national road, 3690 m, 29°27′46.30″N, 98°38′26.57″E, 20 September 2016, *Wang Lisong et al. 16-50799*; Zhada Co., Tuolin Town, 4232 m, 31°43′00.68″N, 79°44′56.99″E, 23 July 2019, *Wang Lisong et al. 19-65539;* Gongjue Co., Xiangpi Vil., 3590 m, 31°00′45.79″N, 98°15′24.00″E, 24 September 2020, *Wang Lisong et al. 20-67410*.

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