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Standard Paper

One new species and three new records in the genus *Porpidia* from China

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Abstract

Four species of *Porpidia* are newly reported from China, including one species new to science (*Porpidia crystallina*) and three records (*Porpidia umbonifera*, *P. seakensis* and *P.* cf. *contraponenda*) new to China. *Porpidia crystallina* is characterized by a *macrocarpa*-type exciple containing crystals, a Cinereorufa-green epihymenium, large ascospores and a lack of secondary metabolites. Morpho-anatomical, chemical and phylogenetic analyses were carried out to elucidate the placement of the species and to support the delimitation of the new taxon. Detailed taxonomic descriptions, ecological and chemical characters, and illustrations are provided for each species. A key to all known Chinese *Porpidia* species is also provided.

Keywords: ITS; *Lecideaceae*; lichens; phylogeny; taxonomy

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Introduction

The cosmopolitan crustose genus *Porpidia* Körb. (*Lecideaceae*) is estimated to comprise *c.* 50 saxicolous species (Ruprecht *et al.* 2016, 2020; Zhao *et al.* 2016; Elix & McCarthy 2018; Kondratyuk *et al.* 2019; Spribille *et al.* 2020; Fayyaz *et al.* 2022). It is characterized by crustose, thick to inconspicuous, grey, white or occasionally orange thalli, dark brown to black lecideine apothecia, thick hymenia, darkly pigmented hypothecia, a usually moderately to heavily pigmented exciple, *Porpidia*-type, 8-spored asci, and simple, colourless, ellipsoid, halonate ascospores (Rambold 1989; Fryday 2005). Based on molecular systematics and anatomical characteristics, the genus *Porpidia* can be divided into three main infrageneric groups: the *Porpidia albocaerulescens* group, the *Porpidia macrocarpa* group and the *Porpidia speirea* group (Buschbom & Mueller 2004; Fryday 2005).

Although the genus is one of the most studied of the segregates of *Lecidea*, species delimitation in *Porpidia* has proved to be very difficult because of the problems in recognizing species-level characteristics (Hertel 1975; Hertel & Knoph 1984; Schwab 1986; Gowan 1989*a*, *b*; Gowan & Ahti 1993; Buschbom & Mueller 2004, 2006; Fryday 2005; Coppins & Fryday 2006; Jabłońska 2009; Osyczka & Olech 2011; Wang *et al.* 2012; Li *et al.* 2013; Fryday & Hertel 2014; Hu *et al.* 2014; Lendemer & Harris 2014; Orange 2014; Zhang *et al.* 2015; Ruprecht *et al.* 2016; Zhao *et al.* 2016; Elix & McCarthy 2018; Kondratyuk *et al.* 2019; Spribille *et al.* 2020; Fayyaz *et al.* 2022). In recent years, molecular studies of the genus have greatly enhanced our understanding of the infrageneric relationships, but there are also many unidentified collections that probably represent undescribed taxa.

During our research into *Porpidia* in China, we identified one new species (*P. crystallina* Q. J. Zuo & Lu L. Zhang) and three species that have not previously been reported from China (*P. umbonifera* (Müll. Arg.) Rambold, *P. seakensis* Fryday and *P.* cf. *contraponenda*). All four species are described below, and a phylogenetic analysis based on the internal transcribed spacer region of the ribosomal DNA (nrITS) supports the placement of *P. crystallina* in *Porpidia* and its status as a new taxon within that genus. A key to all known Chinese *Porpidia* species is also provided.

Materials and Methods

Morphology and anatomy

The specimens studied are preserved in SDNU (Lichen Section of the Botanical Herbarium, Shandong Normal University) and KUN (Kunming Institute of Botany, Chinese Academy of Sciences). Morphological and anatomical characters were examined under a stereomicroscope (COIC XTL7045B2) and a polarizing microscope (Olympus CX41).

Spot tests were carried out on the thallus and medulla with K (a 10% aqueous solution of potassium hydroxide), C (a saturated solution of aqueous sodium hypochlorite), I (Lugol's iodine), N (a 50% aqueous solution of nitric acid) and P (a saturated solution of p-phenylenediamine in 95% ethyl alcohol). Lichen substances were identified using standardized thin-layer chromatography techniques (TLC) with systems A, C and G (Orange *et al.* 2010). Nomenclature of apothecial pigments follows Meyer & Printzen (2000).

A minimum of 20 measurements from material mounted in water were made for each diagnostic feature from these samples.

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The dimensions of ascospores and the ascospore length/width ratio are presented as (minimum) mean ± SD (maximum); *n* = the number of measurements. Images were captured using a dissecting microscope (Olympus SZX16) and a compound light microscope (Olympus BX61), with a DP72 camera system. High-resolution images of type specimens were obtained from the *Global Plants* website (https://plants.jstor.org/).

DNA extraction, PCR amplification and sequencing

DNA was extracted from recently collected or frozen specimens. The Sigma-Aldrich REDExtract-N-Amp Plant PCR Kit (St Louis, Missouri, USA) was used to isolate DNA, following the manufacturer's instructions, except only 30 µl of extraction buffer and 30 µl dilution buffer were used. The internal transcribed spacer region of the ribosomal DNA (nrITS) was amplified using the primers ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990).

The 50 µl PCR mixture consisted of 2 µl DNA, 2 µl of each primer, 25 µl 2× Taq PCR MasterMix (Taq DNA Polymerase [0.1 unit], 3 mM $MgCl_2$, 100 mM KCl, 0.5 mM dNTPs and 20 mM Tris-HCl [pH 8.3]) (Tiangen, Beijing, China) and 19 µl dd H_2O. Conditions for PCR of nrITS were set for an initial denaturation at 94 °C for 10 min, followed by 34 cycles of denaturation at 95 °C for 45 s, annealing at 50 °C for 45 s, extension at 72 °C for 90 s, and a final extension at 72 °C for 10 min. Sequencing was performed by BioSune Biological Technology (Shanghai).

Sequence alignment and phylogenetic analysis

A BLAST search was carried out to identify similar sequences in GenBank. The raw sequences were assembled and edited using SeqMan v. 7.0 (DNASTAR packages). Sequences extracted from new material were aligned with the additional sequence data from GenBank, using MEGA v. 7.0 and an online version of MAFFT v. 7.0.26. The algorithm of MAFFT chose Auto (FFT-NS-1, FFT-NS-2, FFT-NS-i or L-INS-i; depending on data size). *Farnoldia jurana* was chosen as outgroup based on previous phylogenetic analyses (Ruprecht *et al.* 2016).

Phylogenetic relationships were inferred using Bayesian inference (BI) and maximum likelihood (ML). ML and BI were performed using the CIPRES Science Gateway (http://www.phylo.org/portal2/) (Miller *et al.* 2010). ML analyses were performed with RaxML-HPC v. 8.2.6 (Stamatakis 2014), using the default parameters as implemented on CIPRES, and support values were based on 1000 non-parametric bootstrap pseudoreplicates. For the Bayesian analysis, the best substitution models were estimated using jModelTest v. 2.1.7 (Darriba *et al.* 2012). Based on the results, we used the GTR + I + G model for nrITS. Four Markov chains were run with 2 million generations for this dataset. Trees were sampled every 100 generations, with the first 25% of trees discarded as burn-in. Stationarity of analysis was determined by examining the standard deviation of split frequencies (< 0.01). Bootstrap support (BS) ≥ 70% and posterior probabilities (PP) ≥ 0.9 were considered significant supporting values. The phylogenetic trees generated were visualized with FigTree v. 1.4.2 (Rambaut 2012).

Results and Discussion

A total of 10 sequences of nrITS were newly generated from 10 specimens, and 25 sequences were downloaded from GenBank (Table 1). The aligned ITS1-5.8S-ITS2 region comprised 640 sites.

The phylogenetic trees obtained from maximum likelihood (ML) and Bayesian inference (BI) analysis exhibited the same topology; we therefore present only the ML tree, with BS ≥ 70% for the ML analysis and PP ≥ 0.9 for the Bayesian analysis (Figure 1).

Although it would be unwise to draw any phylogenetic conclusions from our single-gene phylogeny, our results are consistent with the hypothesis that there are three main groups in *Porpidia*: the *Porpidia albocaerulescens* group, the *P. macrocarpa* group and the *P. speirea* group (Buschbom & Mueller 2004; Fryday 2005). Through evolutionary distance and support, it is not difficult to see that the new species belongs to *Porpidia* and exists on the branch of the *P. macrocarpa* group. The new species *P. crystallina*, described and discussed below, is supported (BS = 100, PP = 1.00) as sister to *P. macrocarpa*, *P. crustulata* and *P. thomsonii*.

Taxonomy

Porpidia crystallina Q. J. Zuo & Lu L. Zhang sp. nov.

MycoBank No.: MB 845976

The species is characterized by having a *macrocarpa*-type exciple containing crystals, a Cinereorufa-green epihymenium, large ascospores ((16–)18.8 ± 1.6(–22) × (7–)8.6 ± 0.9(–10) µm) and a thallus lacking secondary metabolites.

Type: China, Sichuan Province, Huili Co., Mt Longzhou, 26°47′22.89″N, 102°12′18.58″E, 3548 m alt., on rock, 23 April 2021, *Q. J. Zuo* 20210851 (SDNU—holotype).

(Fig. 2)

Thallus crustose, epilithic, continuous to irregularly rimose, sometimes areolate, thin to moderately thick, grey-white to pale greyish orange-yellow, with crystals in the medulla, surface wrinkled, occasionally shallowly papillate, margin usually distinct; *prothallus* sometimes present, especially between contiguous thalli, thin, black; *medulla* I−; *soredia* and *isidia* absent; *photobiont* with globose green cells, 9–14 µm.

Apothecia scattered or clustered in small groups, sessile, 0.5–0.7 mm diam., round to irregular; *disc* black, shallowly concave, margin 0.1–0.17 mm wide, occasionally weakly pruinose; *pruina* whitish. *Exciple* with brown pigment, at margin with dense pigment which is dark green to blackish, dilute to moderately dense within, 85–100(–125) µm wide, containing crystals partly dissolving in K, hyphae 3–5(–6) µm wide; *hymenium* hyaline, (80–)90–100–125 µm tall; *paraphyses* richly anastomosing and branched in upper part, conglutinate; *epihymenium* aeruginose (Cinereorufa-green); *subhymenium* 25–35 µm tall, colourless; *hypothecium* brown to dark brown, without crystals. *Asci* clavate, 8-spored, *Porpidia*-type; *ascospores* simple, colourless, ellipsoid, halonate, (16–)18.8 ± 1.6(–22) × (7–)8.6 ± 0.9(–10) µm, length/width ratio (1.8–)2.2 ± 0.3(–2.6); *n* = 24.

Conidiomata usually present, sometimes frequent, black, immersed or slightly raised, orbicular to somewhat elongate, with raised and white margin; *conidia* simple, colourless, bacilliform, 7–11 × 0.8–1.2 µm.

Chemistry. Spot test: cortex and medulla K−, C−, KC−. TLC: no substance detected.

Etymology. The specific epithet refers to the crystals in the exciple and thallus.

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Table 1. Voucher information and GenBank Accession numbers of *Porpidia* specimens used in the phylogenetic analyses in this study. Newly generated sequences are in bold. * = outgroup.

			GenBank Accession number
Taxon	Locality	Voucher specimens	nrITS
P. albocaerulescens 1	Japan	YO 0775 (TNS)	LC669678
P. albocaerulescens 2	China	*Q. J. Zuo* 20200372 (SDNU)	**OP594902**
P. albocaerulescens 3	China	*Q. J. Zuo* 20211604 (SDNU)	**OP594901**
P. cinereoatra 1	Wales	*Orange* 20432 (NMW)	KJ162305
P. cinereoatra 2	Ireland	*Orange* 21426 (NMW)	KJ162307
P. contraponenda 1	Austria	*Orange* 21127 (NMW)	KJ162298
P. contraponenda 2	Wales	*Orange* 20447 (NMW)	KJ162297
P. crustulata 1	China	*L. F. Han* 2021060488	OM065418
P. crustulata 2	China	*Q. J. Zuo* 20210562 (SDNU)	**OP594908**
P. crustulata 3	China	*Q. J. Zuo* 20210063 (SDNU)	**OP594909**
P. crystallina 1	China	*Q. J. Zuo* 20210843 (SDNU)	**OP594906**
P. crystallina 2	China	*Q. J. Zuo* 20210826 (SDNU)	**OP594907**
P. crystallina 3	China	*Q. J. Zuo* 20210851 (SDNU)	**OP594905**
P. degelii 1	Carolina	*Tripp* 2503 (NY)	KJ653479
P. degelii 2	Tennessee	*Tripp* 2161 (NY)	KJ653478
P. flavicunda 1	Austria	US12413 (US)	MK620268
P. flavicunda 2	Norway	*Orange* 18971 (NMW)	KJ162332
P. hydrophila 1	Wales	*Orange* 17598 (NMW)	KJ162319
P. hydrophila 2	Wales	*Orange* 16313 (NMW)	KJ162318
P. hypostictica 1	China	*L. Hu* 20141375 (SDNU)	KR069080
P. hypostictica 2	China	*L. Hu* 20141111 (SDNU)	KR069079
P. irrigua 1	Wales	*Orange* 16321 (NMW)	KJ162299
P. irrigua 2	Wales	*Orange* 18014 (NMW)	KJ162302
P. macrocarpa 1	China	*Q. J. Zuo* 20210947 (SDNU)	**OP594910**
P. macrocarpa 2	Austria	*R. Türk* 39740	EU263923
P. macrocarpa 3	-	UR00411 (SZU)	MK620258
P. melinodes 1	Norway	*Orange* 19234 (NMW)	KJ162331
P. melinodes 2	Norway	*Orange* 19209 (NMW)	KJ162327
P. rugosa 1	Faroe Islands	*Orange* 17159 (NMW)	KJ162320
P. rugosa 2	Alaska	*McCune* 34894a	KY800511
P. thomsonii 1	China	*Q. J. Zuo* 20210023 (SDNU)	**OP594903**
P. thomsonii 2	China	*Q. J. Zuo* 20210018 (SDNU)	**OP594904**
P. umbonifera	China	*C. X. Wang* 20181546 (SDNU)	MW520053
P. tuberculosa	Ireland	*Orange* 18291 (NMW)	KJ162322
*Farnoldia jurana**	Austria	*R. Türk* 39660	EU263920

Ecology and distribution. The species is found in southern China; on siliceous rocks (HCl−), exposed, in an upland region.

Notes. The new species, characterized by its exciple with a dark cortex and paler medulla, thick excipular hyphae (3–5(–6) µm wide) and lack of secondary metabolites, clearly belongs to the *Porpidia macrocarpa* group. This is consistent with the results of our phylogenetic analysis. It can be separated from other species of the *macrocarpa*-group by the Cinereorufa-green epihymenium, and the thallus and exciple containing crystals. *Porpidia hydrophila* (Fr.) Hertel & A. J. Schwab also has a Cinereorufa-green epihymenium and lacks secondary metabolites but the exciple of that species has a hyaline medulla with thinner hyphae, placing it in the *P. albocaerulescens* group. In addition, *P. hydrophila* lacks crystals in the exciple medulla and thallus, and occurs on damp rocks. In our phylogenetic

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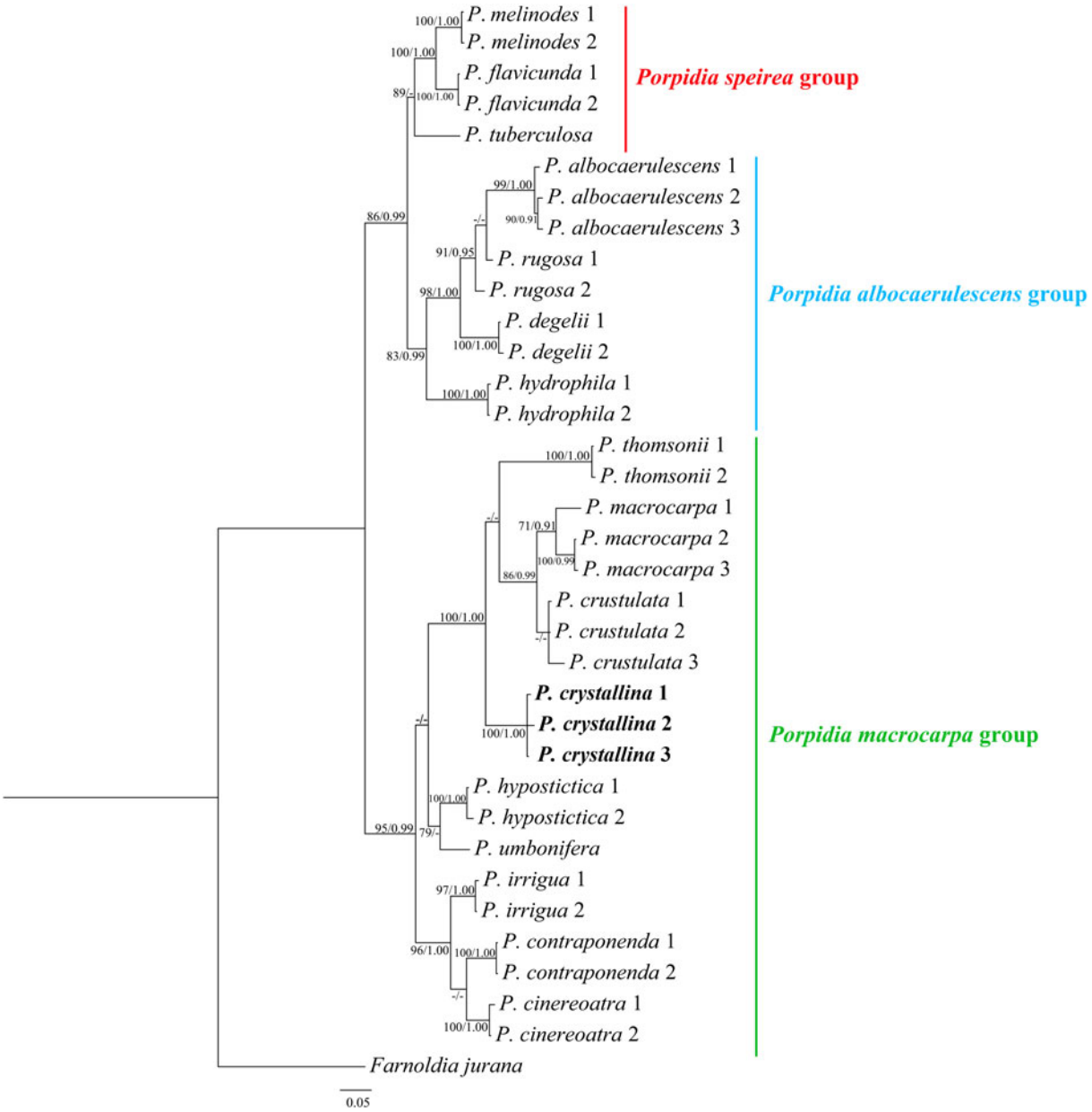

Figure 1. Phylogenetic tree constructed from a maximum likelihood (ML) analysis of species in the genus *Porpidia*, based on the nrITS dataset. Bootstrap support (BS) ≥ 70 for ML and posterior probabilities (PP) ≥ 0.9 for Bayesian methods are indicated above or below the branches (BS/PP). '-/-' indicates low support. The newly described species is marked in bold. Scale = 0.05 substitutions per site. In colour online.

tree, *Porpidia crystallina* clustered into a different monophyletic clade to other species, demonstrating that it is a distinct species. We also conducted a comprehensive comparative analysis of the main morphological characters of the species that have been reported in China that have a Cinereorufa-green epihymenium and no substances found by TLC (Table 2) in the thallus. Through the course of our research, we collected multiple specimens of the new species. These specimens vary greatly in colour and thickness of the thallus but are similar in anatomical characters, belong to the same branch in the phylogenetic analyses and have a relatively close evolutionary distance with high support (BS = 100, PP = 1.00) in our phylogenetic analysis. However, there are also differences in morphology and the location of the crystals is slightly different between specimens collected in the same place. We suggest that this may be caused by differences in the growing environment, such as aspect and composition of rocks.

Additional specimens examined. **China:** *Sichuan Province*: Huili Co., Mt Longzhou, 26°47′22.89″N, 102°12′18.58″E, 3548 m alt., on rock, 2021, *Q. J. Zuo* et al. 20210843, 20210826 (SDNU).

Porpidia seakensis Fryday

Lichenologist **52**, 116 (2020).

(Fig. 3)

Thallus crustose, pale rusty yellowish to greyish white, epilithic to mostly endolithic, usually thin; *prothallus* absent; *soredia* absent; *medulla* I−.

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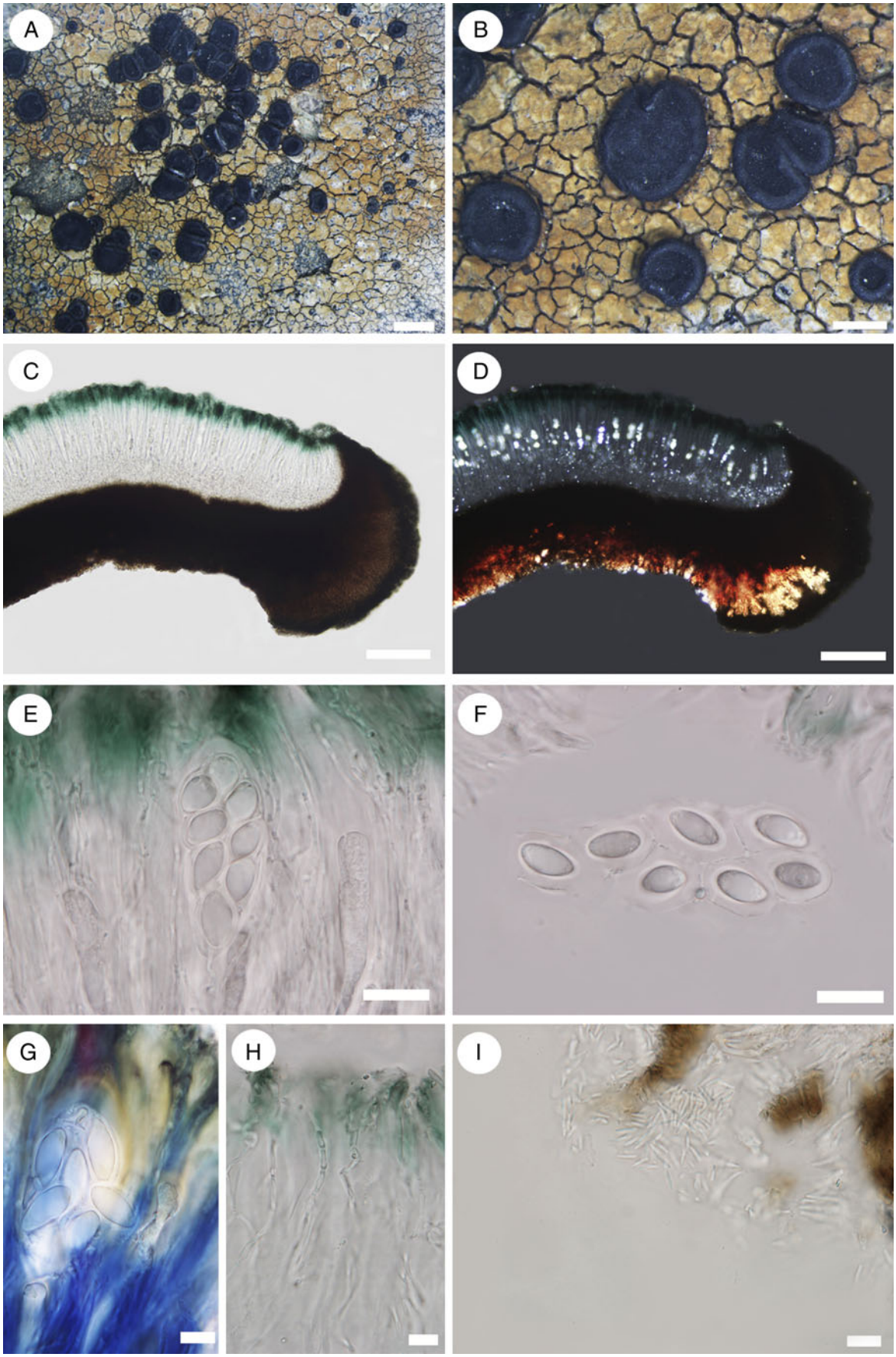

Figure 2. *Porpidia crystallina* (holotype! *Q. J. Zuo* 20210851, SDNU). A, thallus. B, apothecia. C, apothecium section. D, crystals. E, ascus. F, ascospores. G, amyloid reaction of ascus. H, paraphyses. I, conidia. Scales: A = 1 mm; B = 200 μm; C & D = 100 μm; E & F = 20 μm; G–I = 10 μm. In colour online.

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Table 2. Comparison of the morphological characters of *Porpidia* species in China that have a Cinereorufa-green epihymenium and no substances found by thin-layer chromatography (TLC) in the thallus (*P. crystallina*, *P. hydrophila*, *P. shangrila*) (Fryday 2005; Wang *et al.* 2012).

Species	Thallus	Crystals	Hyphae (μm)	Ascospores (μm)	Substratum
P. crystallina	rimose to areolate	in exciple	3–6	(16–)18.8 ± 1.6(–22) × (7–)8.6 ± 0.9(–10)	siliceous rocks
P. hydrophila	rimose to areolate	none	2–4	(14–)17–20(–22) × 6–8(–9)	siliceous rocks
P. shangrila	endolithic	none	6–8	13–15–18 × 5–7	calcareous rocks

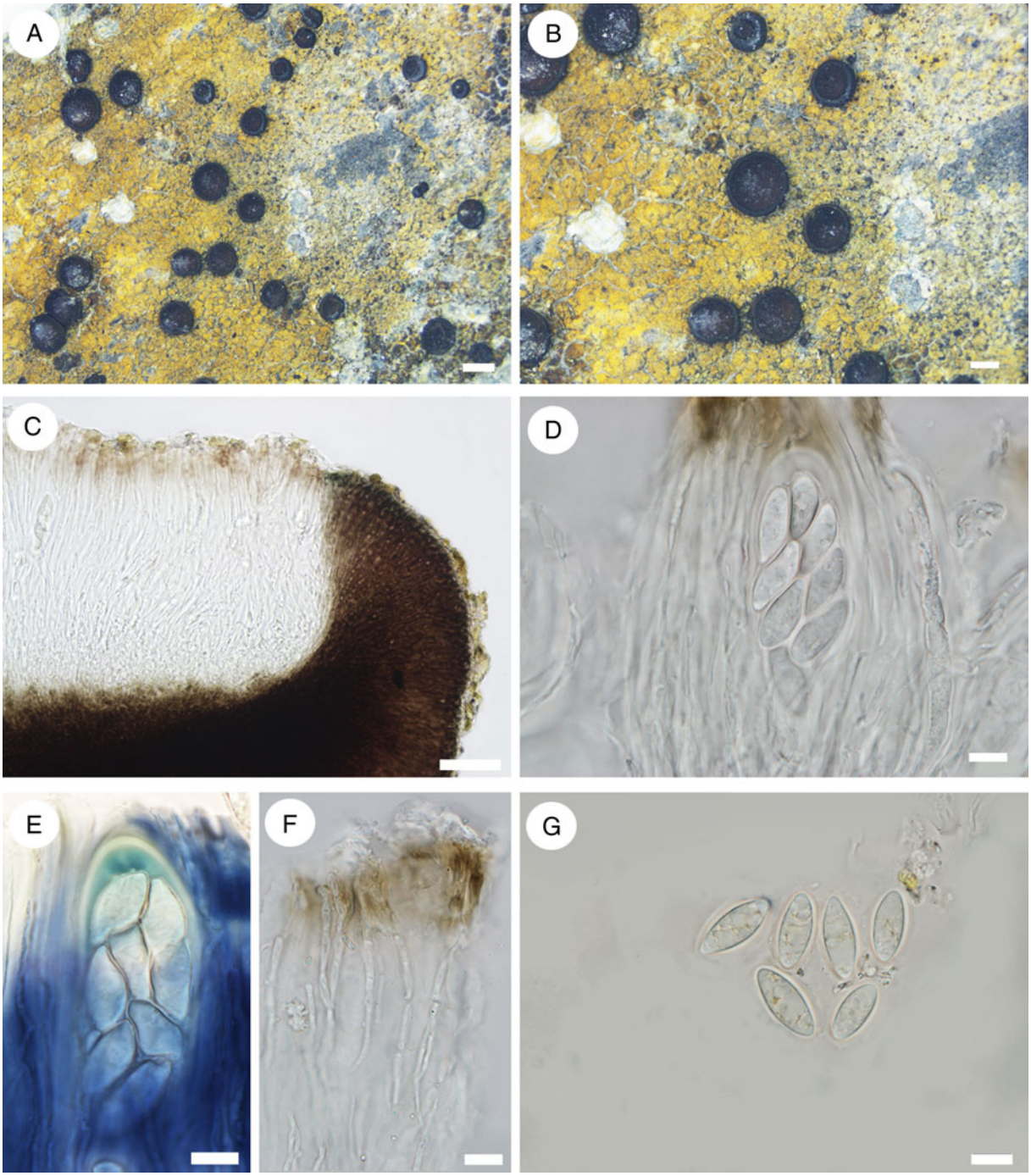

Figure 3. *Porpidia seakensis* (*L. S. Wang* & *X. Y. Wang* 15-49296, KUN). A, thallus. B, apothecia. C, apothecium section. D, ascus. E, amyloid reaction of ascus. F, paraphyses. G, ascospores. Scales: A = 1 mm; B = 500 μm C = 40 μm; D–G = 10 μm. In colour online.

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Apothecia scattered, broadly sessile, 0.5–0.9(–1.1) mm diam. when mature; *disc* red-brown to brownish black, plane to shallowly concave, margin moderately thick, 0.06–0.08 mm wide, rarely very weakly pruinose; *pruina* whitish. *Exciple* dark brown to brownish black at exciple cortex, pale brown to red-brown within, 67.5–92.5 µm wide, without crystals, composed of radiating hyphae (4–)5–6–7(–8) µm wide; *hymenium* hyaline, 87.5–100–125 µm tall, I+ blue; *paraphyses* strongly branched and anastomosing, slightly enlarged at the apical part; *epihymenium* dilute brown, (12.5–)17.5–22.5 µm thick; *subhymenium* 20–32.5 µm thick; *hypothecium* brown to dark brown. *Asci* clavate, 8-spored, *Porpidia*-type; *ascospores* simple, colourless, ellipsoid, halonate, (17–)20.8 ± 2.8(–25) × (7–)9.3 ± 1.3(–11) µm, length/width ratio (1.8–)2.2 ± 0.2(–2.5); $n = 18$.

Conidiomata not observed.

Chemistry. Spot test: cortex and medulla K+ yellow, C–, KC–. Stictic acid was detected by TLC.

Ecology and distribution. *Porpidia seakensis* has previously been reported only from Alaska, USA (Spribille *et al.* 2020) and is new to China; on siliceous rocks (HCl–), exposed, in upland regions.

Notes. *Porpidia seakensis* is characterized by a thin to endolithic thallus, strongly constricted apothecia with a brown pruinose disc, large ascospores and a *macrocarpa*-type exciple. The Chinese material closely matches the description in Spribille *et al.* (2020), except that the thallus is tinged with a yellowish pigment, which may be due to the extreme environment. As a member of the *P. macrocarpa* group, it has similar ascospores to *P. superba* (Körb.) Hertel & Knoph, but that species has a thallus composed of thick, bullate areoles and an orange-brown exciple (Superba-brown), and usually occurs on slightly basic rocks.

Specimen examined. **China:** *Taiwan Province*: Nantou Co., Mt Hehuanshan, Wuling, 24°08.365′N, 121°17.253′E, 3139 m alt., on rock, 2015, *L. S. Wang* & *X. Y. Wang* 15–49296 (KUN).

Porpidia umbonifera (Müll. Arg.) Rambold

Biblioth. Lichenol. **34**, 296 (1989)

(Fig. 4)

Thallus crustose, irregularly rimose to rimose-areolate, even to weakly verruculose, thin to moderately thick, areoles angular to irregularly shaped, medium grey to greyish white; *prothallus* between areas of the thallus, black, thin; *soredia* absent; *medulla* I+ pale violet-black.

Apothecia clustered, soon becoming sessile, 0.4–0.8 mm diam.; *disc* black, flat to shallowly concave, even umbonate, margin distinct, 0.07–0.1 mm wide, epruinose. *Exciple* at surface with dense pigment, black to carbonaceous, pale brown to red-brown within, 100–120(–130) µm wide, without crystals, hyphae 4–5–6 µm wide; *hymenium* (75–)85–100 µm tall, colourless, I+ blue; *paraphyses* strongly anastomosed and apically branched, slightly enlarged at the apex; *epihymenium* brown to olive-brown, 10–15 µm thick; *subhymenium* 15–25(–30) µm; *hypothecium* brown to dark brown. *Asci* clavate, 8-spored, *Porpidia*-type; *ascospores* simple, colourless, ellipsoid, halonate, (12–)16.5 ± 2.2(–20) × (5–)7.3 ± 1.4(–9) µm, length/width ratio (1.8–)2.2 ± 0.2(–2.8); $n = 18$.

Conidiomata not observed.

Chemistry. Spot test: cortex and medulla K–, C–, KC–. No substances detected by TLC.

Ecology and distribution. *Porpidia umbonifera* has previously been reported only from Australia (Rambold 1989) and is new to China; on siliceous rocks (HCl–), exposed.

Notes. According to Rambold (1989), *Porpidia umbonifera* is characterized by sessile apothecia that are constricted at the base, an exciple with a black-brown (ectal zone) to pale brown (inner zone) pigment, no substances detected by TLC and a medulla that reacts I+ violet. The Chinese material closely matches this description except that the medulla is only I+ light violet and finally fades away. *Porpidia umbonifera* resembles *P. speirea* (Ach.) Kremp. and *P. grisea* Gowan in having an I+ medulla and similar ecological requirements. However, both *P. grisea* and *P. speirea* contain confluentic acid as the major substance and have a very dark exciple, even in thin sections. In addition, the thick excipular hyphae and lack of secondary metabolites of *Porpidia umbonifera* indicate that it belongs to the *P. macrocarpa* group, whereas *P. grisea* and *P. speirea* belong to the *P. speirea* group.

Specimens examined. **China:** *Yunnan Province*: Shangri-La County, Mt Hongshan, 28°07′55.99″N, 99°54′06.68″E, 4361.9 m alt., on rock, 2018, *C. X. Wang* et al. 20181546 (SDNU); 28°07′54.66″N, 99°54′09.08″E, 4459.4 m alt., on rock, 2018, *C. X. Wang* et al. 20181672 (SDNU); 28°07′55.66″N, 99°54′13.58″E, 4503.1 m alt., on rock, 2018, *C. X. Wang* et al. 20181734 (SDNU).

Porpidia cf. *contraponenda*

(Fig. 5)

Thallus crustose, continuous to irregularly rimose, pale cream-white to greyish white, occasionally tinged rusty orange, surface occasionally shallowly papillate, thick, tartareous, margin thinner than thallus centre; *prothallus* between areoles, black, thin; *soredia* absent; *medulla* I–.

Apothecia clustered, half-immersed when young, occasionally more or less sessile or remaining sunken when mature, (0.3–)0.5–1 mm diam.; *disc* shallowly concave at first then flat to subconvex, black or less commonly dark brown, margin smooth, moderately thick to thin, usually thinning in old or convex apothecia, 0.07–0.11 mm, epruinose or rarely very weakly pruinose; *pruina* greyish white. *Exciple* at surface with dense pigment that is dark bluish green to olive-brown, moderately to heavily dark brown within, (65–)80–90–110(–125) µm wide, without crystals, hyphae 3.5–4.5 µm wide; *hymenium* (75–)85–97.5–110 µm tall, colourless, I+ blue; *paraphyses* richly anastomosing and branched in upper section, conglutinate; *epihymenium* dull olive green to dull greenish brown, without crystals; *subhymenium* 12.5–20 µm; *hypothecium* brown to reddish brown, pale aeruginose pigment occasionally present in medullary region around apothecial base. *Asci* clavate, 8-spored, *Porpidia*-type; *ascospores* simple, colourless, ellipsoid, halonate, (15–)17.6 ± 1.7(–21) ×

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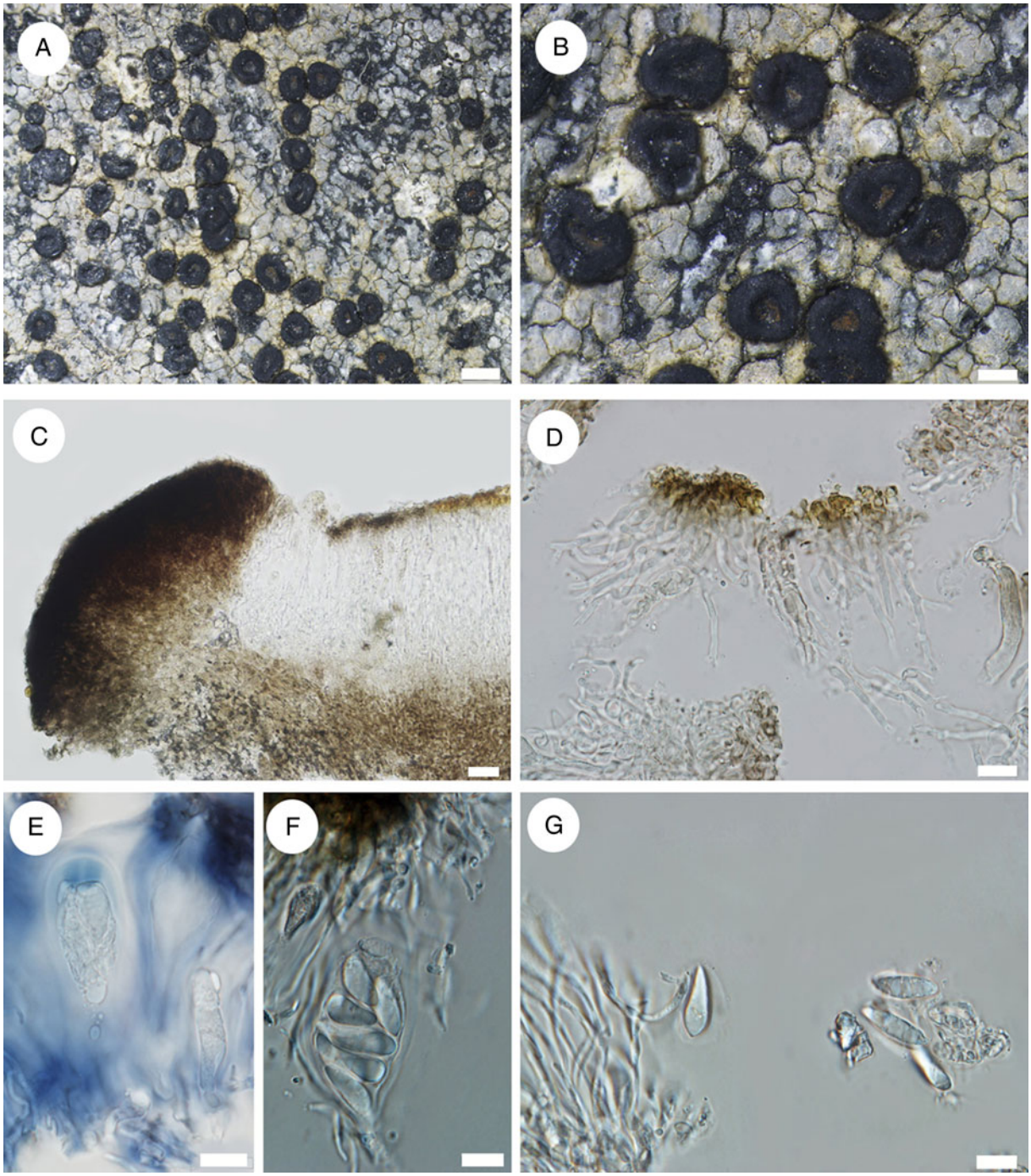

Figure 4. *Porpidia umbonifera* (*C. X. Wang* et al. 20181546, SDNU). A, thallus. B, apothecia. C, apothecium section. D, paraphyses. E, amyloid reaction of ascus. F, ascus. G, ascospores. Scales: A = 500 µm; B = 200 µm; C = 20 µm; D–G = 10 µm. In colour online.

(7–)8.9 ± 1.5(–11.5) µm, length/width ratio (1.6–)2.1 ± 0.3(–2.5); $n = 18$.

Conidiomata not observed.

Chemistry. Spot test: cortex and medulla K–, C–, KC–. Methyl 2′-*O*-methylmicrophyllinate and 2′-*O*-methylmicrophyllinic acid (trace) were detected by TLC.

Ecology and distribution. *Porpidia contraponenda* has been reported from North America and Europe (Hertel & Knoph 1984; Fryday 2005; Orange 2014), and is new to China; on siliceous rocks (HCl–), exposed, in upland regions.

Notes. *Porpidia contraponenda* is characterized by having an epilithic thallus, apothecia at least partly sunken in the thallus, an olive green to dull greenish brown epihymenium, and a thallus containing a second unidentified depside as a major compound in addition to methyl 2′-*O*-methylmicrophyllinate. The specimens found in China are very similar to those described previously in the literature (Gowan 1989*a*; Fryday 2005; Orange 2014), but they have a shorter hymenium (75–110 µm vs 100–170 µm

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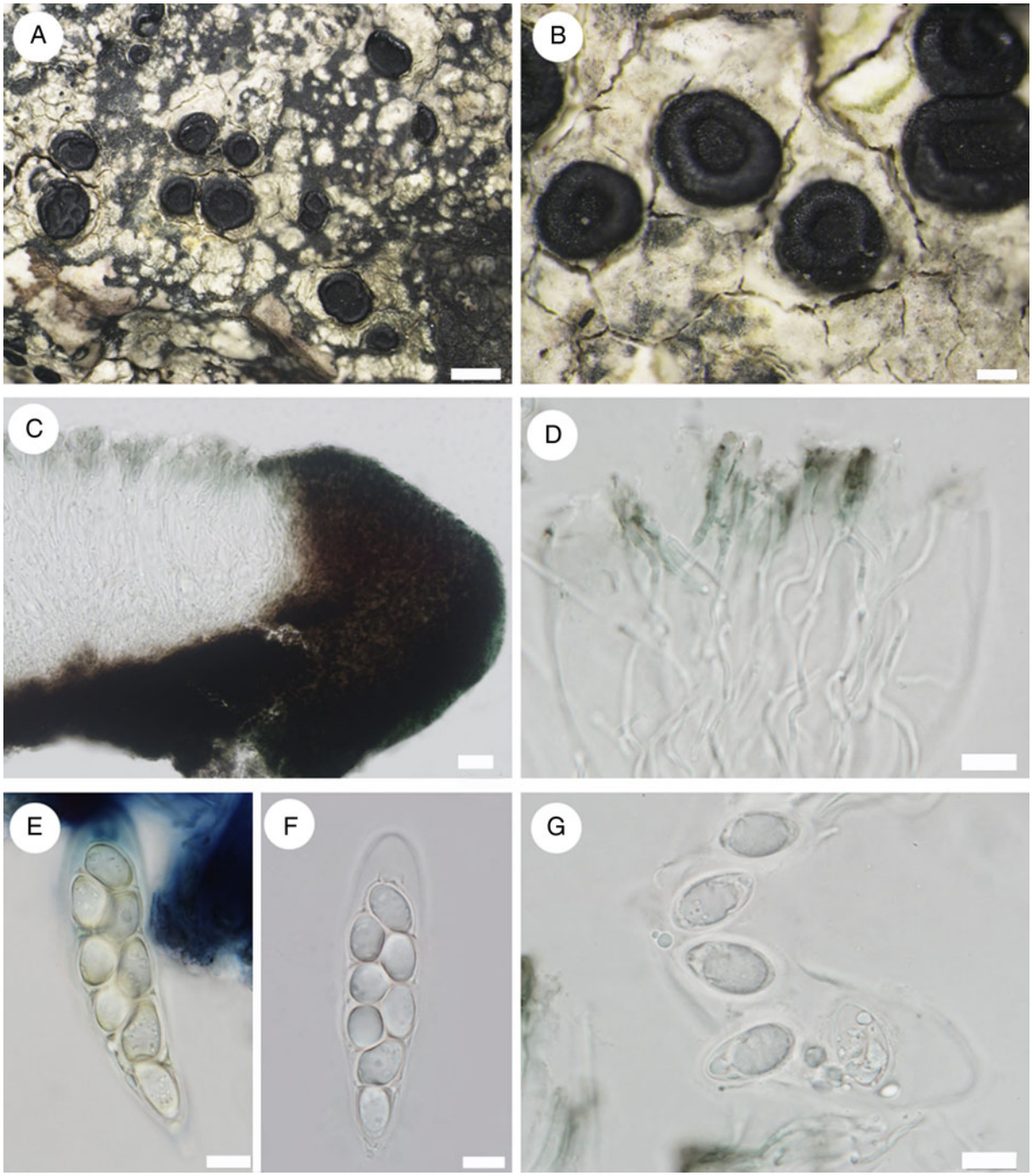

Figure 5. *Porpidia* cf. *contraponenda* (*Q. J. Zuo* et al. 20210986, SDNU). A, thallus. B, apothecia. C, apothecium section. D, paraphyses. E, amyloid reaction of ascus. F, ascus. G, ascospores. Scales: A = 500 μm; B = 100 μm; C = 20 μm; D–G = 10 μm. In colour online.

high) and methyl 2′-*O*-methylmicrophyllinate as the only major constituent. Through standardized thin-layer chromatography techniques (with systems A, C and G), we did not find a second unidentified depside as a major compound, as described by Orange (2014), only methyl 2′-*O*-methylmicrophyllinate. These specimens are also similar to *Porpidia irrigua* Orange in secondary metabolite content, but the latter has apothecia which are sessile from a very early stage and wider hyphae in the exciple (3.5–8.0(–12) μm). Our specimens might represent a new species, but we do not have enough material and molecular data to support this. Therefore, they are identified here as *Porpidia* cf. *contraponenda* and further research will be carried out in the future.

Specimens examined. **China:** *Taiwan Province*: Nantou Co., Mt Hehuanshan, Wuling, 24°08.182′N, 121°17.366′E, 3174 m alt., on rock, 2015, *L. S. Wang* & *X. Y. Wang* 15-49210 (KUN). *Sichuan Province*: Puge Co., Mt Luoji, Dahaizi, 27°34′44.07″N, 102°22′28.52″E, 3554 m alt., on rock, 2021, *Q. J. Zuo* et al. 20210986 (SDNU).

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Key to the species of *Porpidia* occurring in China

1 Thallus subsquamulose, brownish yellow, lacking secondary products; epihymenium blue-green (Cinereorufa-green) . . . . . **P. squamosa**
Thallus crustose; thallus upper surface, chemistry and epihymenium colour various 2

2(1) Thallus with soredia; apothecia present or absent 3
Thallus without soredia; apothecia present 4

3(2) Medulla I+ blue, confluentic acid present . . . . . **P. tuberculosa**
Medulla I−, stictic acid present . . . . . **P. soredizoides**

4(2) Exciple of apothecia in section with ±hyaline medulla, usually composed of thin filamentous hyphae 2–4 µm wide (*albocaerulescens*-type) 5
Exciple of apothecia with pigmented medulla, usually composed of thicker pseudoparenchymatous hyphae 3–8 µm wide; if hyphae thinner then medulla pigmented 9

5(4) Epihymenium vivid aeruginose (Cinereorufa-green) . . . . . **P. hydrophila**
Epihymenium brown or olivaceous (Macrocarpa-green) 6

6(5) 2′-*O*-methylperlatolic acid and confluentic acid present; apothecia epruinose; hypothecium K+ red . . . . . **P. chungii**
2′-*O*-methylsuperphyllinic acid, stictic acid, or norstictic acid present; apothecia often pruinose 7

7(6) 2′-*O*-methylsuperphyllinic acid present . . . . . **P. carlottiana**
2′-*O*-methylsuperphyllinic acid absent, stictic acid (K+ yellow, Pd+ orange) or norstictic acid (K+ red) present 8

8(7) Thallus and apothecium exciple containing stictic acid (K+ yellow); apothecia usually innate, and often heavily pruinose . . . . . **P. albocaerulescens** var. **albocaerulescens**
Thallus and apothecium exciple containing norstictic acid (K+ red); apothecia usually innate, and often slightly pruinose . . . . . **P. albocaerulescens** var. **polycarpiza**

9(4) Medulla I+ blue 10
Medulla I− 12

10(9) No substances detected by TLC . . . . . **P. umbonifera**
Confluentic acid present 11

11(10) On ±basic rocks; thallus white; apothecia innate . . . . . **P. speirea**
On siliceous rocks; thallus grey; apothecia usually sessile . . . . . **P. grisea**

12(9) Thallus orange, orange-brown or yellowish brown, usually containing confluentic acid . . . . . **P. flavicunda**
Thallus grey or endolithic, sometimes partly oxidized, chemistry various 13

13(12) Epihymenium and exciple with only orange-brown pigment (Superba-brown); thallus white, usually bullate, but occasionally smoother; usually on basic rocks; ascospores usually > 20 µm long . . . . . **P. superba**
Epihymenium and/or exciple cortex with olivaceous (Macrocarpa-green), dark bluish green (Cinereorufa-green) or brown (Arnoldiana-brown) pigments (N+ reddish) 14

14(13) On basic rocks; thallus endolithic; epihymenium dark bluish green (Cinereorufa-green, K−, N+ rose pink), containing traces of norstictic acid . . . . . **P. shangrila**
On siliceous rocks 15

15(14) Exciple dark, with paler medulla apparent only in thin section; thallus epilithic, containing confluentic acid, methyl 2′-*O*-methylmicrophyllinate or unidentified triterpenes 16
Exciple not uniformly dark in section; thallus often endolithic, containing stictic acid, norstictic acid, or no substances (K+ red or yellow or K−) 18

16(15) Apothecia usually innate, becoming convex, with thin (*c.* 0.05 mm) barely raised margin; thallus thick, cracked-areolate, usually continuous, containing confluentic acid (K+ numerous 'oil droplets' in section) . . . . . **P. cinereoatra**
Apothecia sessile with thick (*c.* 0.1 mm) raised, persistent margin; thallus thinner, often composed of ±dispersed areoles, containing methyl 2′-*O*-methylmicrophyllinate or confluentic acid 17

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17(16) Thallus containing confluentic acid (K+ numerous 'oil droplets' in section); apothecia often pruinose . . . . . **P. lowiana**
Thallus containing methyl 2′-*O*-methylmicrophyllinate (K−); apothecia epruinose or rarely pruinose . . . . . **P.** cf. **contraponenda**

18(15) Proper margin thin and barely raised, < 0.08 mm wide; thallus thin, usually containing stictic acid (K+ yellow, Pd+ orange) . . . 19
Proper margin thick and raised, > 0.1 mm wide 21

19(18) Epihymenium dilute brown, hymenium 90–125 µm high; ascospores 17–25 µm long . . . . . **P. seakensis**
Epihymenium light to dark brown to olive-brown, or olive-ochre, hymenium 60–90 µm high; ascospores 12–16(–19) µm long 20

20(19) Hypothecium brown to dark brown, K+ yellow or K− . . . . . **P. crustulata**
Hypothecium light brown to medium brown, K+ carmine-violet . . . . . **P. cervinopungens**

21(18) Hypostictic acid present; thallus white to grey-white, with yellow, oxidized patches near the margin; apothecia sessile, up to 2–3 mm diam.; ascospores 17.5–25.0 µm long . . . . . **P. hypostictica**
Hypostictic acid absent 22

22(21) Thallus grey-white to pale greyish orange-yellow; epihymenium aeruginose, exciple with crystals, no substances; ascospores (16–)19(–22) µm long . . . . . **P. crystallina**
Exciple without crystals 23

23(22) Thallus white, containing norstictic acid (K+ red), rarely with stictic acid (K+ yellow); apothecia densely pruinose; medulla of exciple very pale . . . . . **P. platycarpoides**
Thallus grey to whitish, sometimes rust coloured, containing stictic acid or no substance present (K+ yellow or K−); apothecia not or only slightly pruinose; medulla of exciple pale brown 24

24(23) Apothecia < 1.5 mm diam. (usually < 1.1 mm), proper margin *c.* 0.1 mm wide; exciple composed of swollen elongate cells 5–8 (–10) µm wide . . . . . **P. thomsonii**
Apothecia up to 3.0 mm diam., margin up to 0.2 mm wide; exciple composed of cells 4–6(–8) µm wide 25

25(24) Exciple reacting K+ crimson . . . . . **P. macrocarpa** f. **nigrocruenta**
Exciple not reacting K+ crimson . . . . . **P. macrocarpa** f. **macrocarpa**

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