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Rediscovery of Five *Rinodina* Species Originally Described from Southwest China and One New Species

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Abstract: *Rinodina* is a lichenized fungal genus belonging to the Physciaceae, with c. 300 species worldwide. Nearly a century ago, Zahlbruckner described five species of the genus *Rinodina* from Southwest China. The type collections were the only records for these species. In the present study, new records for four of these species: *Rinodina cornutula*, *R. globulans*, *R. handelii*, and *R. setschwana*, and a recently described species, *R. pluriloculata*, are documented based on specimens collected from the holotype localities. Furthermore, one new species was discovered: *Rinodina hengduanensis*, characterized by areolate to subsquamulose thallus, jigsaw-like areoles, lecanorine apothecium, and *Dirinaria*-type ascospores. *Rinodina setschwana* is transferred to the genus *Buellia* based on its morphology, chemistry, and phylogeny and proposed as *Buellia setschwana*. We provide detailed morphological descriptions, pictures, and molecular phylogenetic analyses.

Keywords: lichenized Ascomycetes; new species; Physciaceae; taxonomy

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1. Introduction

Among lichenized fungi with a Holarctic distribution, *Rinodina* (Ach.) Gray is one of the better understood genera. It has been studied in the Iberian Peninsula [1], Scandinavia [2], South Europe [3,4], the Old World [5], North America [6,7], and Northeast Asia [8]. However, molecular data have shown that *Rinodina* is a polyphyletic genus [9–12]. Species-level concepts within *Rinodina* are becoming better established, with clear outlines of species groups emerging in conjunction with a refined understanding of crustose microlichen transitions [8,12]. However, there remains a substantial knowledge gap for East Asia, especially for Southwest China, a region that has played an important role in the evolution of higher plants [8,13–16]. The inclusion of specimens and molecular data from East Asia is vital for the generation of testable hypotheses regarding lichen biogeography [8].

A total of 51 *Rinodina* species have been reported from China [5,17–38]. In the early 20th century, five of these species were described from Southwest China by Zahlbruckner [19]. The chemistry of their lichen substances was not recorded. More recently, Aptroot and Sparrius described a new species, *R. pluriloculata* Aptroot and Sparrius, from the same area, including the chemistry of its lichen substances but not molecular data [29]. Since the initial publication of these species, no further studies have been conducted. Inter-specific comparisons of chemical compounds are lacking, and the phylogenetic position of these species remains unclear.

During the Second Tibetan Plateau Scientific Expedition and Research Program (STEP), we conducted a thorough survey of the lichen diversity of western China, with a particular focus on the Qinghai-Tibetan Plateau. With the addition of specimens that we previously collected in Yunnan Province, over 150 specimens that were confirmed as *Rinodina* species have been stored in the lichen Herbarium at the Kunming Institute of Botany (KUN). We surveyed the relevant literature, examined the morphology of type specimens, and compared these to our newly collected materials from the type localities. This confirmed that we had obtained fresh collections of the species, which were originally described from southwestern China nearly a century ago [19] and twenty years ago [29]. In addition, we discovered one new species of *Rinodina*. In the present paper, we characterize these species using morphological, chemical, and molecular evidence and provide a key to the species included in this study.

2. Materials and Methods

2.1. Morphological and Chemical Study

All the new materials from this study have been deposited in the Herbarium of the Kunming Institute of Botany (KUN). Observations of specimens were made using a Nikon SMZ 745T dissecting microscope (Nikon Corp., Tokyo, Japan). Vertical sections of apothecia and thalli were cut with a razor blade, then mounted in GAW (glycerol:ethanol:water = 1:1:1) and examined under a Nikon Eclipse 50i stereomicroscope. Measurements and descriptions of the sections and ascospores were conducted under the microscope and stereomicroscope at either $\times$10 or $\times$40 magnification to an accuracy of 10 μm and 2.5 μm, respectively. Photographs were all taken using a Nikon digital camera head, the DS-Fi2. Secondary metabolites were identified by spot testing and by thin-layer chromatography (TLC), following the methods of Culberson [39] and Orange et al. [40], using solvent system C (toluene:acetic acid = 85:15).

2.2. DNA Extraction, Purification, and Sequencing

Total genomic DNA was extracted from 26 specimens using DNAsecure Plant Kits (Tiangen Biotech, Beijing, China), following the manufacturer's protocol. Three loci were amplified: the mitochondrial SSU gene with primer pairs mtSSU1 and mtSSU3R [41], the internal transcribed spacer region (nrITS) with ITS1F [42] and ITS4 [43], and the 5′ portion end of the 28S, including the D1–D2 domain [44], with LR0R [45] and LR5 [46]. PCR amplification was performed in 25 μL reactions containing 12.5 μL of 2 $\times$ MasterMix (0.1 units/μL TaqDNA polymerase, 4 mM $MgCl_2$, and 0.4 nM dNTPs; Aidlab Biotechnologies Co. Ltd., Beijing, China), 0.5 μL of each primer, 9.5 μL of ddH_2O, 2 μL of each primer, and 1 μL of DNA, using the PCR settings and primer profile issued by Zhao et al. [47]. Polymerase chain reaction (PCR) products were sequenced by TsingKe Biological Technology (Kunming, China).

2.3. Phylogenetic Analysis

The DNA sequences were aligned using MAFFT v. 7.107 in GENEIOUS v. 8.0.2 with the following parameters: algorithm = auto; scoring matrix = 200 PAM/k = 2; gap open penalty = 1.53; offset value = 0.123 [48]. A 3-locus (nrITS, mtSSU, and nrLSU) concatenated matrix was generated using GENEIOUS v. 8.0.2. This matrix contained both *Rinodina* species and species belonging to related genera in the Physciaceae and Caliciaceae. In addition to our newly generated sequences, other related sequences were downloaded from GenBank and added to the matrix (Table 1). Subsequently, a maximum likelihood (ML) tree and a Bayesian inference (BI) tree were constructed. Based on the lowest Bayesian information criterion (BIC), the partitioned analysis was selected using ModelFinder in IQ-TREE v.1.6.12 for the subsequent ML and BI analyses [49,50]: In ML analysis, GTR + F + I + G4 is for ITS1 and ITS2, TNe + I + G4 for 5.8S rRNA and nrLSU, and TPM3u + F + I + G4 for mtSSU; in BI analysis, GTR + F + I + G4 is for ITS1 and ITS2, SYM + I + G4 for 5.8S rRNA and nrLSU, and HKY + F + I + G4 for mtSSU. The ML analyses were performed by the IQ-TREE web server [51–53]. The BI analyses were performed by MrBayes v. 3.2.7 [54], using three Markov chains running for 10 million generations for the concatenated dataset. The trees were sampled every 100 generations, and the first 25% of the trees were discarded as burn-ins. Posterior probabilities (PPs) were obtained from the 95% majority rule consensus tree of all saved trees. The tree files were visualized with FigTree 1.4.2 [55].

Table 1. Specimens and sequences used for phylogenetic analyses. Newly generated sequences are in bold.

Species	Locality	Voucher Specimens	GenBank Numbers			References
			nrITS	mtSSU	nrLSU	
Acolium karelicum	unknown	UPS Hermansson 16472	KX512897	na	KX512879	[56]
Acroscyphus sphaerophoroides 1	unknown	UPS Obermayer 6077	KX512898	KX512984	na	[56]
A. sphaerophoroides 2	unknown	TNS Shimizu	AY450562	na	na	[57]
Allocalicium adaequatum		UPS Spribille 14143	KX512906	KX512986	KX512859	[56]
Amandinea punctata 1	Japan, Tokyo	TNS YO6843	LC669587	na	na	[58]
A. punctata 2	Japan, Tokyo	TNS YO6855	LC669588	na	na	[58]
Anaptychia bryorum	Bhutan, Thimphu	Soehting 8378	EF582777	EF582825	na	[59]
A. ciliaris	Spain	MAF-Lich 17758	KC559095	na	na	[60]
Baculifera remensa		S Prieto	na	KX512962	KX512881	[56]
Buellia disciformis 1	Jämtland, Klaxåsen	Wedin 6155	AY143392	AY143401	na	[61]
B. disciformis 2	Sweden	UPS A. Nordin 4429	AF540498	na	na	[9]
B. elegans	unknown	S Hansen	KX512901	KX512993	na	[56]
B. erubescens	unknown	S Wetmore 95879	KX512902	KX512969	KX512874	[56]
B. frigida	unknown	S Westberg	KX512903	KX512992	KX512852	[56]
B. polita 1	China, Sichuan	KUN-L XY20-341	**OP526814**	**OP526788**	**OP526772**	
B. polita 2	China, Yunnan	KUN-L 15-47741	**OP526792**	**OP526774**	**OP526763**	
B. polita 3	China, Sichuan	KUN-L 19-62813	**OP526800**	**OP526780**	**OP526768**	
B. polita 4	China	KUN-L 14-43546	**MN615679**	na	na	
B. polita 5	China	KUN-L 15-48012	**MN615678**	na	na	
B. polita 6	China	KUN-L 13-41328	**MN615677**	na	na	
B. setschwana 1	China, Yunnan	KUN-L 15-48032	**OP526796**	na	na	
B. setschwana 2	China, Yunnan	KUN-L 15-47744	**OP526794**	na	na	
B. setschwana 3	China, Yunnan	KUN-L 15-47743	**OP526793**	**OP526775**	**OP526764**	
B. tesserata	unknown	S Tehler 7323	KX512904	na	KX512885	[56]
Calicium abietinum	unknown	UPS Tibell 25061	KX512905	KX512971	KX512872	[56]
C. corynellum	unknown	S Prieto	KX512908	KX512985	KX512855	[56]
Coscinocladium gaditanum 1	Spain	MAF 9855	AY449720	AY464073	na	[62]
C. gaditanum 2	Spain	MAF 9856	AY449721	AY464074	na	[62]
Culbersonia nubila 1	South Africa	ALV14224	MH121317	na	MH121319	[63]
C. nubila 2	South Africa	ALV14225	MH121318	na	MH121320	[63]
Dimelaena oreina	unknown	S Lendemer 4193	KX512922	KX512976	KX512867	[56]
Diplotomma alboatrum	unknown	S Prieto 3034	KX512924	KX512966	KX512877	[56]
Heterodermia diademata	Bolivia, Depto. Beni	B K. Bach, M. Kesseler and Portugal 389	AF540518	na	na	[9]
H. vulgaris	unknown	DUKE AFTOL-320	HQ650704	na	DQ883798	[64,65]
Hyperphyscia adglutinata	unknown	BCN-Lich 17031	AF250795	GU247189	na	[66]
H. crocata	South Korea	120413	MN150490	na	na	[67]
Kashiwadia orientalis 1	Eastern Asia	Hur 040044	na	KM397366	na	[68]
K. orientalis 2	Eastern Asia	Hur 040164	na	KM397365	na	[68]

Table 1. *Cont.*

Species	Locality	Voucher Specimens	GenBank Numbers			References
			nrITS	mtSSU	nrLSU	
Mobergia calculiformis 1	Mexico	Moberg 10412	AF224359	na	na	[69]
M. calculiformis 2	unknown	M231	AF250796	na	na	[66]
Oxnerella safavidiorum	Iran	KW-L 70300	KM410153	KM410156	na	[70]
Phaeophyscia endococcinodes	South Korea	130163	MN150503	na	na	[67]
P. sciastra	Norway, Sor-Trondelag	O-L-196352	MK812372	na	na	[71]
Phaeorrhiza nimbosa	China, Xizang	KUN-L 19-65695	MW133637	MW133652	MW133660	[72]
P. sareptana	China, Gansu	KUN-L 18-59809	MW133625	MW133640	MW133654	[72]
Physcia adscendens	Norway, Vestfold	O-L-198947	MK812201	na	na	[71]
P. tenella 1	Gästrikland	S Odelvik and Hellstrom 0827	KX512932	KX512974	KX512869	[56]
P. tenella 2	Finland	Lohtander 650	AF224425	EF582800	na	[59]
Physciella chloantha 1	Iberian Peninsula	BCN-Lich 15525	GU247166	GU247200	na	
P. chloantha 2	Iberian Peninsula	BCN-Lich 17033	GU247164	GU247198	na	
Physconia enteroxantha	Norway, Akershus	O-L-196328	MK812142	na	na	[71]
P. grisea	Morocco, Imli	Staro192	LS483208	na	na	[71]
Pseudothelomma ocellatum 1	unknown	S Tehler 8063	KX512934	KX512957	KX512862	[56]
P. ocellatum 2	unknown	UPS Hermansson 18662	KX512935	KX512952	KX512891	[56]
Pyxine himalayensis	China, Yunnan	KUN-L 12-36055	KY611881	KY751388	na	[73]
P. subcinerea	China, Taiwan	KUN-L 15-49012	KY611867	KY751374	na	[73]
Rinodina cana	unknown	Sipman 63008	MN587029	na	na	[74]
R. conradii	China, Yunnan	KUN-L 13-40531	**OP526791**	**OP526773**	na	
R. cornutula 1	China, Yunnan	KUN-L XY20-3571	**OP526815**	**OP526789**	na	
R. cornutula 2	China, Yunnan	KUN-L XY20-3572	**OP526816**	**OP526790**	na	
R. gennarii	Netherlands, Utrecht	B H. Sipman 44435	AJ544187	na	na	[9]
R. globulans 1	China, Yunnan	KUN-L 15-47871	**OP526795**	**OP526776**	na	
R. globulans 2	China, Yunnan	KUN-L XY20-171	**OP526807**	**OP526785**	**OP526770**	
R. globulans 3	China, Yunnan	KUN-L XY20-117	**OP526805**	na	na	
R. globulans 4	China, Sichuan	KUN-L XY20-279	**OP526810**	na	na	
R. globulans 5	China, Sichuan	KUN-L XY20-280	**OP526811**	na	na	
R. handelii 1	China, Yunnan	KUN-L XY20-224	**OP526808**	na	na	
R. handelii 2	China, Yunnan	KUN-L XY20-238-1	**OP526809**	na	na	
R. mniaroea 1	USA, Idaho	GZU Spribille 15242	KX015687	KX015706	na	[12]
R. mniaroea 2	USA, Montana	GZU Spribille 20391	KX015692	KX015711	na	[12]
R. mniaroea 3	Norway, Svalbard	TROM_L_565871	MK812098	na	na	[71]
R. mniaroea 4	unknown	M249	AF250811	na	na	[66]
R. mniaroeiza	Canada	GZU V. Wagner 15.07.06/1	KX015691	KX015710	na	[12]
R. moziana 1	Australia, Queensland	GZU H. Mayrhofer 11742	DQ849306	na	na	[75]
R. moziana 2	New Zealand, Nelson	GZU M. Lambauer 0214	DQ849305	na	na	[75]
R. oxydata 1	Australia, Queensland	GZU H. Mayrhofer 11790	DQ849311	na	na	[75]
R. oxydata 2	Australia, Queensland	GZU H. Mayrhofer 11406	DQ849313	na	na	[75]

Table 1. *Cont.*

Species	Locality	Voucher Specimens	GenBank Numbers			References
			nrITS	mtSSU	nrLSU	
R. oxydata 3	New Zealand, Nelson	GZU M. Lambauer 0206	DQ849310	na	na	[75]
R. oxydata 4	Austria, Steiermark	GZU H. Mayrhofer 13.930	AF540548	na	na	[9]
R. oxydata 5	Austria, Styria	GZU H. Mayrhofer 15761	DQ849312	na	na	[75]
R. pluriloculata 1	China, Yunnan	KUN-L XY20-162	**OP526806**	**OP526784**	na	
R. pluriloculata 2	China, Yunnan	KUN-L 20-66417	**OP526802**	**OP526782**	na	
R. sophodes 1	Austria, Styria	GZU 000272661	GU553304	GU553321	na	[10]
R. sophodes 2	Austria, Steiermark	GZU P. Bilovitz 968	AF540550	na	na	[9]
R. orientalis 1	South Korea	KBA BDNA-L-0000284	MW832807	na	na	[76]
R. orientalis 2	South Korea	KBA BDNA-L-0000653	MW832808	na	na	[76]
R. salicis 1	South Korea	KBA BDNA-L-0000558	MW832810	na	na	[76]
R. salicis 2	South Korea	KBA BDNA-L-0000560	MW832811	na	na	[76]
R. hengduanensis 1	China, Sichuan	KUN-L 20-66506	**OP526803**	**OP526783**	na	
R. hengduanensis 2	China, Yunnan	KUN-L XY20-44	**OP526804**	na	na	
R. hengduanensis 3	China, Sichuan	KUN-L XY20-290	**OP526813**	**OP526787**	na	
R. hengduanensis 4	China, Sichuan	KUN-L XY20-287	**OP526812**	**OP526786**	**OP526771**	
R. zeorina 1	South Korea	KBA BDNA-L-0000642	MW832812	na	na	[76]
R. zeorina 2	South Korea	KBA BDNA-L-0000646	MW832813	na	na	[76]
Rinodinella controversa 1	unknown	M281	AF250814	na	na	[66]
R. controversa 2	Greece, Kreta	GZU Mayrhofer and Ertl 13.74	AJ421423	na	na	[77]
Tetramelas pulverulentus	unknown	UPS Nordin 6368	KX512940	KX512983	KX512860	[56]
Texosporium sancti-jacobi	unknown	UPS Rosentreter and De Bolt 6514	KX512941	KX512981	KX512863	[56]
Thelomma mammosum	unknown	UPS Tibell 23775	KX512942	KX512954	KX512888	[56]
T. santessonii	unknown	UPS Nordin 4011	KX512944	KX512951	KX512889	[56]
Tholurna dissimilis	unknown	UPS Wedin 6330	AY143397	AY143407	KX512893	[56]
Tornabea scutellifera 1	Spain	AFTOL-ID 1061	JQ301698	na	DQ973037	[11,65]
T. scutellifera 2	unknown	UPS Tibell 23833	KX512946	KX512970	KX512873	[56]
Xanthoria aureola	Sweden, Bohuslän	E. Gaya etc. (BCN)	JQ301690	JQ301526	JQ301585	[12]
Xanthoria parietina	Sweden, Bohuslän	E. Gaya etc. (BCN)	JQ301691	JQ301530	JQ301589	[12]

3. Results and Discussion

By re-collecting these species from their holotype localities and conducting morphological and phylogenetic analyses, we confirmed the identities of *Rinodina cornutula*, *R. globulans*, *R. handelii*, *Buellia setschwana* (≡ *R. setschwana*), and *R. pluriloculata*. Detailed figures of morphology and spores, together with information regarding chemical compounds, have been provided. Furthermore, the phylogenetic positions of these species have been confirmed. An additional species has been described as new to science: *R. hengduanensis*, for which morphological descriptions and molecular data have been provided.

The sequence alignment comprised 105 terminals, of which 25 were newly generated sequences from this study (Table 1). Phylogenetic trees that were based on datasets generated from either ML or BI analyses had almost identical topologies, with minimal differences in the level of statistical support. The phylogenetic analysis showed that the families Physciaceae and Caliciaceae both formed monophyletic clades. *Rinodina cornutula*, *R. globulans*, *R. handelii,* and *R. pluriloculata* belong to the Physciaceae, while *Buellia setschwana* (syn. *R. setschwana*) belongs to the Caliciaceae.

Our results indicate that *R. pluriloculata* is phylogenetically close to *R. conradii*. Both species have 3–8-septate ascospores, but *R. pluriloculata* can be distinguished by its pale to olive green thallus, punctiform, discrete, orbicular, convex to flattened soralia, bluish, granular soredia, and submuriform-type ascospores. *R. cornutula* is phylogenetically closely related to *R. oxydata*. The morphological characters of these two species are largely similar: both are characterized by *Mischoblastia*-type spores and a thallus with atranorin in the cortex. They can be separated by their thallus color and habitat. The molecular data available for *R. oxydata* showed that this species is not monophyletic, so further research is required based on samples from its type locality. *R. handelii* appears close to *R. orientalis* and *R. zeorina* in the phylogenetic tree, but the three species can be separated by their ascospore morphology and their chemistry. *R. handelii* lacks secondary metabolites, and it has *Milvina*- to *Mischoblastia*-type ascospores (up to 27 μm), which are longer than the spores of the other two species (both less than 20 μm). *R. orientalis* has *Physcia*-type spores, whereas *R. zeorina* contains zeorin and has *Dirinaria*-type ascospores. *R. globulans* forms a well-supported clade (100/100/1.00), which is also supported by its morphological, chemical, and geographic characteristics. It differs from other species by its round, capitate, and scattered thallus areoles covered with soralia. Interestingly, *Buellia setschwana* (syn. *R. setschwana*) forms a monotypic lineage that is close to *B. polita*. These two species can be distinguished by the characters of their apothecia: *B. polita* has a reddish brown hypothecium and immersed lecideine apothecia, whereas *B. setschwana* has a hyaline hypothecium and cryptolecanorine apothecia. The apothecia characters of *B. setschwana* are identical to those of other species within *Rinodina*, which was probably the basis for its previous placement within the genus *Rinodina*.

The new species *Rinodina hengduanensis* formed a single clade, represented by a SH-aLRT support of 100, an ultrafast bootstrap support of 100, and a posterior probability of 1 for the branch (Figure 1). Its species status is further supported by its distinctive morphological, chemical, and geographic characteristics.

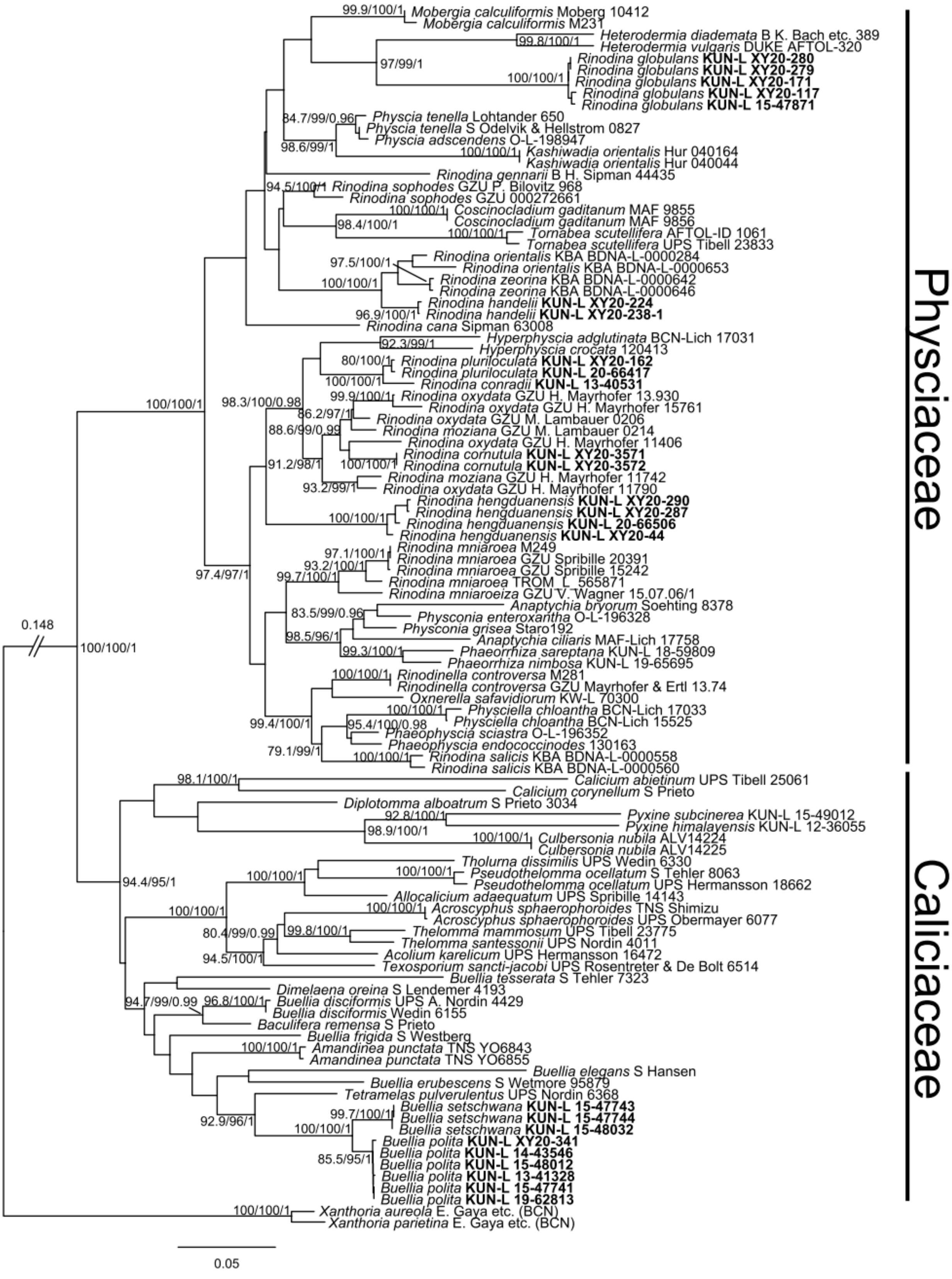

Figure 1. Phylogenetic tree generated from maximum likelihood (ML) analysis based on combined nrITS, mtSSU, and nrLSU sequences. SH-aLRT support (%) for ML greater than 80%, ultrafast bootstrap support (%) greater than 95%, and Bayesian posterior probabilities (PPs) greater than 0.95 are given above the nodes. Newly generated sequences are indicated in bold.

4. Taxonomy

Rinodina cornutula Zahlbr., in Handel-Mazzetti, Symb. Sinic. 3: 233 (1930), Figure 2.

Type: China, Yunnan Prov., Manhao nahe der Grenze von Tonkin, Tonschieferfelsen am Flußufer in der tr. St., 200 m, 2 March 1915, Handel-Mazzetti no. 5858 (WU, holotype!).

Description:*Thallus* crustose, thin, areolate, continuous at the central part, scattered near the margins, margins not distinct; areoles irregular in shape, 0.2–0.6 mm wide; upper surface flat, brown to olive brown, mottled, dull, non-pruinose; prothallus absent or delimiting the thallus as a black outline; vegetative propagules absent. *Apothecia* common, usually abundant and contiguous, lecanorine (becoming lecideine), broadly attached to slightly innate, 0.2–0.6 mm in diam.; disc black, plane and roundish, non-pruinose; margin concolorous with thallus, entire, persistent, usually becoming carbonized and concolorous with the disc when mature; exciple hyaline to dark brown, with algal cells; hymenium hyaline, colorless, not inspersed, 75–125 µm high; paraphyses simple, conglutinate, apices expanded, light brown; epihymenium pale brown to dark brown; hypothecium hyaline, colorless, 35–75 µm deep; asci 8-spored, *Lecanora*-type; ascospores 1-septate, Type A development, *Mischoblastia*-type, pale brown, lumina triangle- or heart-shaped, walls thickened at both ends and the septum, 18–25 × 7.5–12.5 µm, torus indistinct. *Pycnidia were* not seen.

Chemistry: Cortex K+ yellow; KC+ yellow or orange; C−, P+ faint yellow; medulla I+ blue. Containing atranorin (confirmed by TLC).

Ecology and distribution: This species is distributed across subtropical to temperate regions, growing on siliceous rocks, rhyolite, or basalt, at elevations between 950 and 2500 m. Endemic to China, previously reported in Sichuan and Yunnan [19,36].

Notes: *Rinodina cornutula* is characterized by its flat, areolate, ochre to olive thallus, broadly attached to slightly innate apothecia, *Mischoblastia*-type ascospores, and containing atranorin. This species was originally collected from Manhao town, Yunnan province, China. The specimens that we collected from the same area were compared to type specimens and confirmed as identical to the holotype. This species is similar to *Rinodina cana* (Arnold) Arnold in morphology, but *R. cana* lacks atranorin (spot tests are all negative), and its apothecia are usually entirely innate, with *Milvina*-type ascospores. It might be confused with *Rinodina moziana* (Nyl.) Zahlbr., but the latter species can be distinguished by its verruciform or granular thallus and its constricted ascospores when mature. *R. oxydata* (A. Massal.) A. Massal differs from this species by its gray to ochraceous thallus and shorter ascospores (<18 µm long). *R. cornutula* would key out at couplet 9 in Lee and Hur's paper, as it has a plane thallus, *Mischoblastia*-type, 18–25 × 7.5–12.5 µm ascospores, and is rarely swollen at the septum [76].

Specimens examined: China, Yunnan Prov.: Lijiang Co., Yufeng Temple, 2500 m, on rock, August 15, 1982, Wang Lisong 82-1099; Lijiang City, Yulong Naxi Autonomous Co., on the way from Lijiang to Ninglang, Jinsha River side, 1846–1883 m, on rock, April 9, 2019, Wang Lisong et al. 19-62703, 19-62705, 19-62706; Honghe prefecture, Lvchun Co., Niukong Vil., 972 m, on rock, December 17, 2020, Wang Xinyu et al. XY20-3571, XY20-3572.

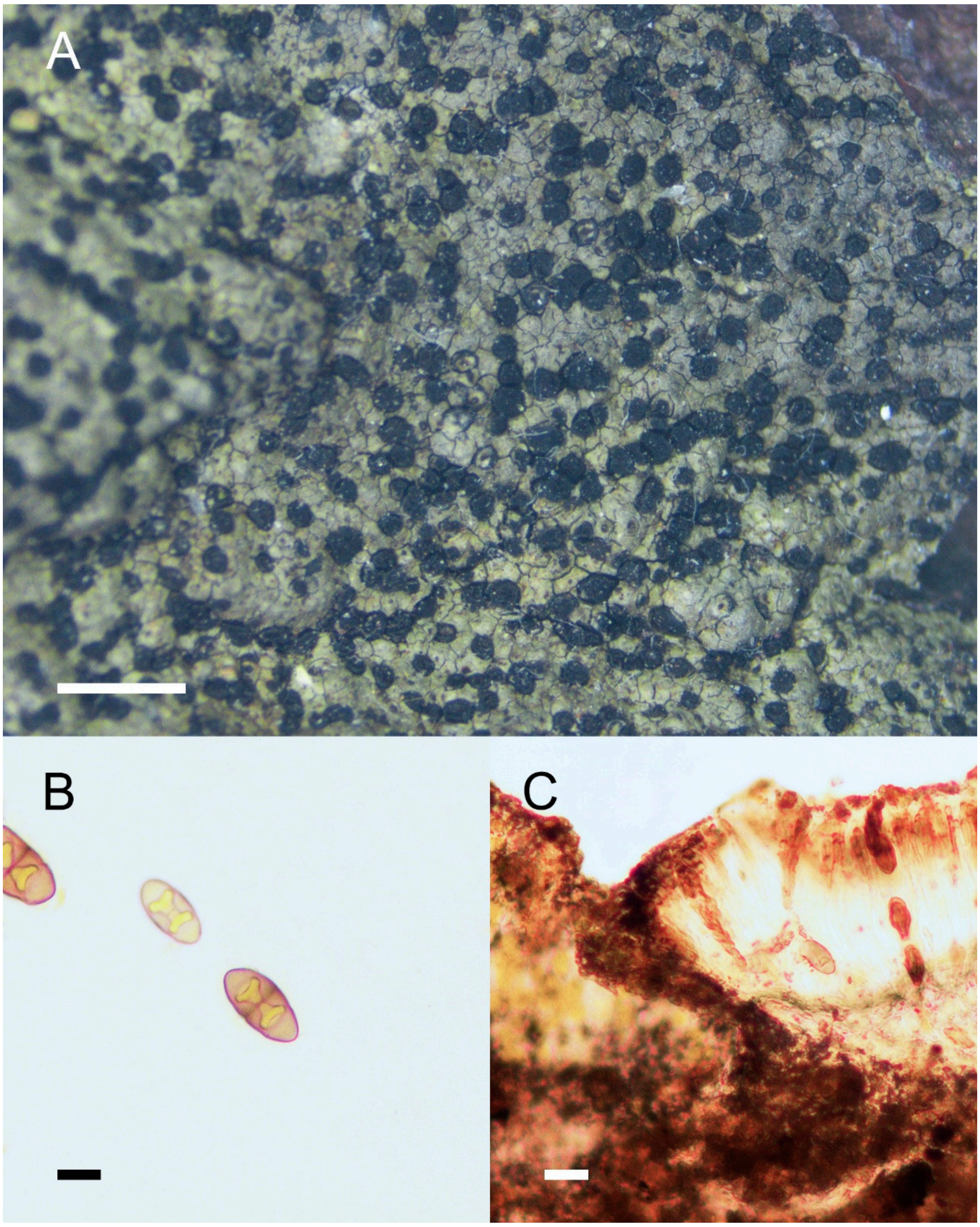

Figure 2. Morphology and anatomy of *Rinodina cornutula*. (**A**) Thallus and apothecia. (**B**) *Mischoblastia*-type ascospores. (**C**) Section of apothecia. Scale bars: 2 mm (**A**); 10 µm (**B**); 20 µm (**C**).

Rinodina globulans Zahlbr., in Handel-Mazzetii, Symb. Sinic. 3: 233 (1930), Figure 3.

Type: China, Sichuan Prov., Eisenschüssiger Sandstein in der tp. St. Um den Paß Dsiliba im Daliangschan e von Ningyüen, ±3000 m, 26 April 1914, Handel-Mazzetti no. 1771 (WU, holotype!).

Description: *Thallus* crustose, round, capitate to areolate, scattered; areoles 0.1–0.4 mm in diam.; surface pale green (greyish white to pale after storage in herbarium), with white to greyish white pruina, covered by abundant soralia, globular, pale green; prothallus prominent, black, persistent; *Apothecia* lecanorine, usually scattered, rarely contiguous, sessile, 0.6–0.8 mm in diam.; disc brown to dark brown, concave, round, non-pruinose; margin concolorous with thallus, thick, distinctly raised, entire, persistent; exciple hyaline to pale brown, with algal cells, persistent; hymenium hyaline, colorless, not inspersed, 80–130 µm high, I+ blue; paraphyses simple or merely branched at the apices; epihymenium olive to greyish brown; hypothecium hyaline, colorless, 40–65 μm deep; asci 8-spored, *Lecanora*-type; ascospores 1-septate, Type A development, *Mischoblastia*- to *Pachysporaria*-type, brown, lumina triangle- or trapezoid-shaped; walls thicken at both ends and at the septum, 15–25 × 7.5–12.5 μm; torus absent with oil droplets when over-mature. *Pycnidia* were not seen.

Chemistry: Cortex and medulla K+ yellow, KC−, C−, P−. Atranorin, zeorin, and stictic acid are detected by TLC.

Ecology and distribution: This species is saxicolous and often grows on sandstone at elevations between 2000 and 3600 m. It is endemic to China, previously reported in Sichuan province [19,36]. This is a new record for Yunnan Province, China.

Notes: *R. globulans* is a unique species with numerous, scattered, globular, pale green soralia and a prominent black prothallus, which can be reliably distinguished from other *Rinodina* species in China. Apothecia are rare in this species, and the ascospores are *Mischoblastia*-*Pachysporaria*-type. A recently published species, *Rinodina punctosorediata* Aptroot and Sparrius, is highly similar to this species, with identical external and anatomic morphology [29]. However, *R. punctosorediata* grows both on trees (*Castanopsis* sp.) and rocks, whereas the holotype of *R. globulans* and all our collections grew on rocks. Furthermore, *R. punctosorediata* has smaller ascospores (<18 μm long) than the *Milvina*-type. *R. globulans* would be keyed out at couplet 2 in Lee and Hur's paper because it has scattered thallus areoles covered with abundant, globular, pale green soralia [76].

Specimens examined: China, Sichuan Prov.: Liangshan Yi Autonomous Prefecture, Yanyuan Co., Weicheng Town, 2816–2831 m, on sand rock, 28 2020, Wang Xinyu et al. XY20-280, XY20-279. Yunnan Prov.: Diqing Tibetan Autonomous Prefecture, Shangri-La City, Jiantang Town, Pudacuo National Park, Bita, 3496 m, on rock, 25 July 2020, Wang Xinyu et al. XY20-117; Lijiang City, Ninglang Yi Autonomous Co., Xichuan Vil., next to Lining Highway, 2509 m, on rock, 26 July 2020, Wang Xinyu et al. XY20-171; Dali Prefecture, Nanjian Co., Leqiu Vil., Awuhe Vil., Dayao Reservoir, 2095 m, on rock, 2 July 2015, Ye Xin and Wang Weicheng 15-47871; Luquan Co., Jiaozi Snow Mt., 3600 m, on rock, September 18, 2003, Wang Lisong 03-22628; Dali Co., Cangshan Mt., Zhonghe Temple, 2580 m, on rock, August 30, 2005, Wang Lisong et al. 05-25172.

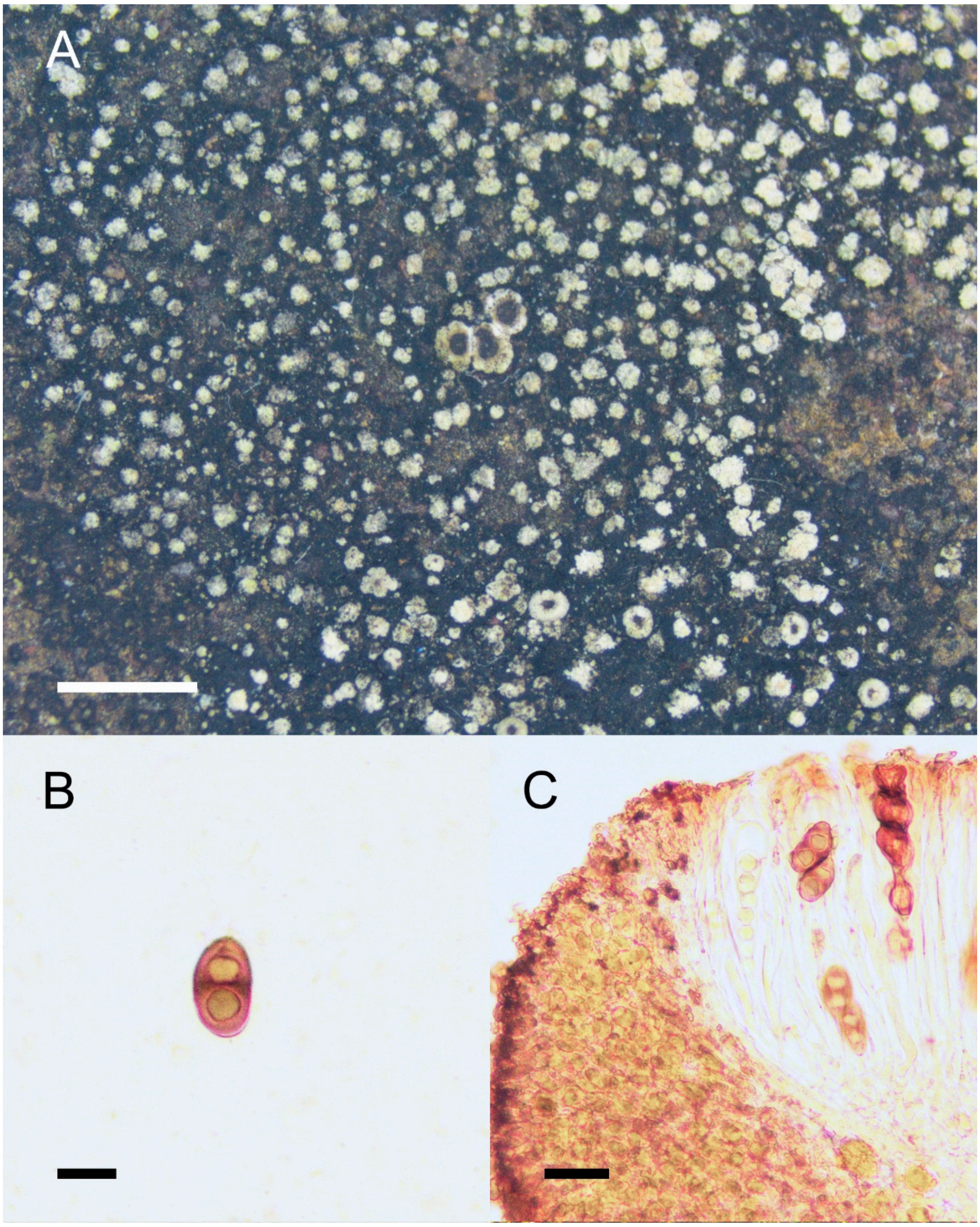

Figure 3. Morphology and anatomy of *Rinodina globulans*. (**A**) Thallus and apothecia. (**B**) *Pachysporaria*-type ascospores. (**C**) Section of apothecia. Scale bars: 2 mm (**A**); 10 µm (**B**); 20 µm (**C**).

Rinodina handelii Zahlbr., in Handel-Mazzetti, Symb. Sinic. 3: 232 (1930),

Type: China, Yunnan Prov., Lebende Rinde von Pflaumenbäumen in Gärten der wtp. St. in Yünnanfu, 1920 m, February 24, 1914, Handel-Mazzetti no. 275 (WU, holotype; US, isotype).

Description: *Thallus* crustose, thin, rimose to continuous, somewhat verrucose; areoles 0.1–0.2 mm wide; upper surface rough to verrucous, grey, brown, or pale green, dull, non-pruinose; prothallus absent; vegetative propagules absent. *Apothecia* common, scattered or contiguous, lecanorine, broadly attached, 0.2–0.6 mm in diam.; disc ochre brown to dark brown, plane to convex, round, non-pruinose; margin concolorous with thallus, thin, entire, disappeared when mature; exciple hyaline, with abundant algal cells; hymenium hyaline, colorless, not inspersed, 50–100 µm high, I+ blue; paraphyses simple, somewhat conglutinate, apices expanded, dark pigmented; epihymenium dark brown; hypothecium hyaline, colorless or pale brown, 50–100 μm deep; asci 8-spored, *Lecanora*-type; ascospores 1-septate, Type A development, pale brown to brown, *Milvina*- to *Mischoblastia*-type, 20–27 × 7–12.5 μm, torus present, always inspersed with oil droplets when mature. *Pycnidia were* not seen.

Chemistry: Spot tests were all negative; no metabolites were detected by TLC.

Ecology and distribution: This species is corticolous, growing on bark or branches of *Pinus*, *Quercus*, *Rhododendron*, etc., in coniferous forest or mixed coniferous broad-leaved forest, at elevations between 2700 and 3600 m. It has only previously been reported from Yunnan province, China [19,36]. This is a new record for Sichuan province.

Notes: *R. handelii* is a corticolous species with a thin, rimose to continuous, rough thallus; sessile apothecia; a dark brown disc; characterized by ascospores with torus, always inspersed with oil droplets when mature; a size of 20–27 × 7–12.5 μm. It could be confused with other corticolous species such as *Rinodina bolanderi* and *R. subminuta*, but *R. bolanderi* can be distinguished by containing atranorin and zeorin, and *Teichophila*-type ascospores, and *R. subminuta* can be distinguished by containing zeorin and *Physcia*-type ascospores. *R. handelii* also resembles *Rinodina subleprosula* Jatta in external morphology, which is a corticolous species previously described by Jatta from China [17]. However, *R. subleprosula* has larger ascospores (27.5–30.5 × 12–15 μm). Another corticolous species, *Rinodina cinereovirens* (Vain.), has similar ascospores to *R. handelii*, but it can be distinguished by its medulla, which is UV + blue-white and contains sphaerophorin. *R. handelii* would key out at couplet 60 in Lee and Hur's paper because the margins of the apothecia are not radially cracked and there are *Milvina*- to *Mischoblastia*-type ascospores [76].

Specimens examined: China, Sichuan Prov.: Liangshan Prefecture, Yanyuan Co., Weicheng Town, 3210 m, on *Pinus densata*, 28 July 2020, Wang Lisong et al. 20-66526. Yunnan Prov.: Diqing Prefecture, Shangri-La City, Jiantang Pudacuo National Park, Bita, 3540 m, on Quercus bark, Wang Xinyu et al. XY20-134; Gongshan Co., Yeniu Valley, 2700 m, on bush, 30 May 2000, Wang Lisong 00-19328; Lijiang City, Ninglang Co., Hongqiao Vil., c. 3100 m, on branch, 27 July 2020, Wang Xinyu et al. XY20-224, XY20-238-1. Figure 4.

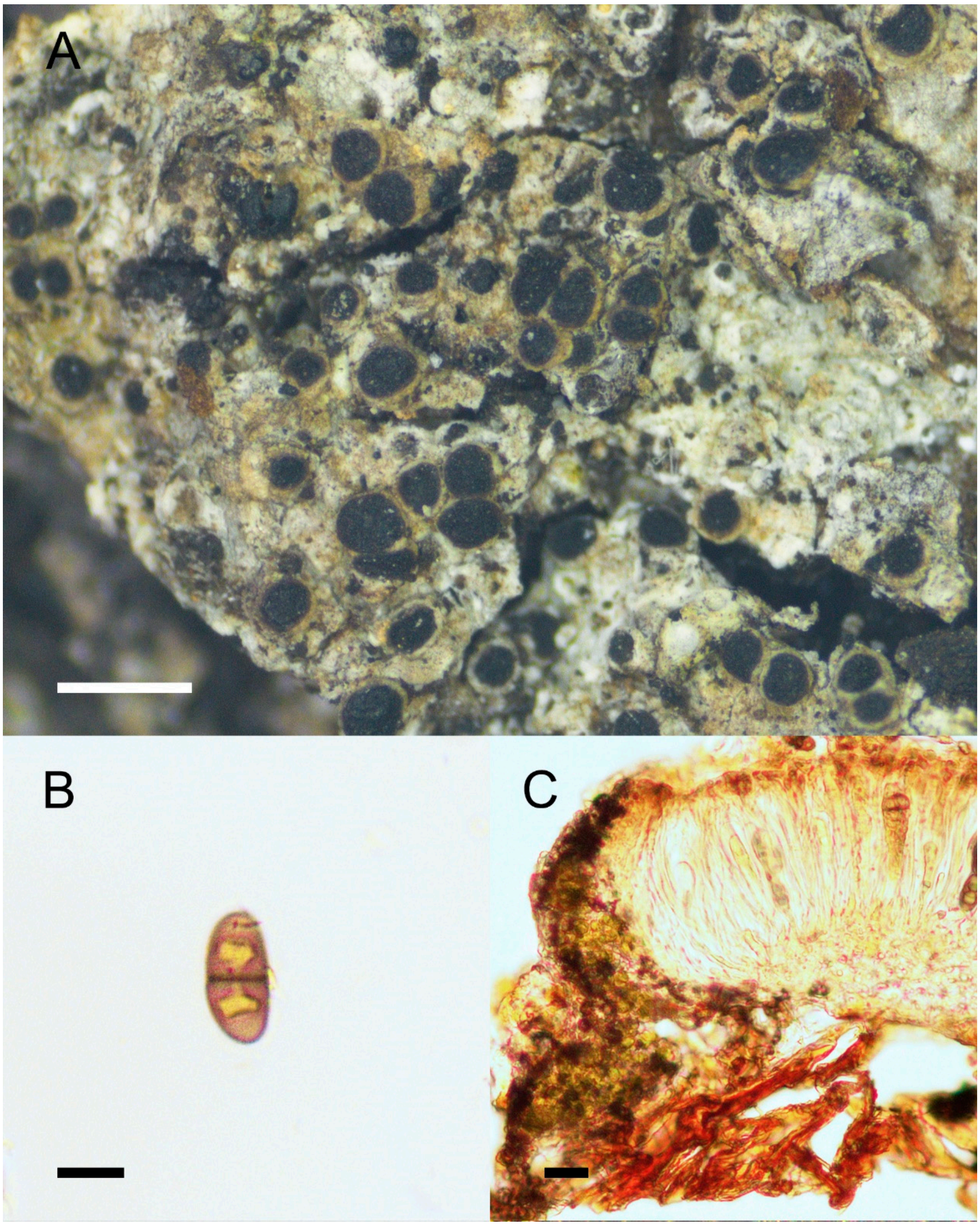

Figure 4. Morphology and anatomy of *Rinodina handelii*. (**A**) Thallus and apothecia. (**B**) *Mischoblastia*-type ascospores. (**C**) Section of apothecia. Scale bars: 1 mm (**A**); 10 µm (**B**); 20 µm (**C**).

Rinodina pluriloculata Aptroot and Sparrius, Fungal Diversity 14: 42 (2003), Figure 5.

Type: CHINA, Yunnan Prov., 5 km W of Kunming, just outside of the city, 1750 m alt., 48RTN604714, on bark of tree base of living *Eucalyptus globulus*, 16 October 2002, Aptroot no. 55505 (B, holotype; KUN isotype).

For a detailed description, see [29].

Notes: *R. pluriloculata* is characterized by its continuous, verrucous, pale green to olive thallus; punctiform, discrete, orbicular, convex to flattened soralia; bluish, granular soredia; lecanorine, sessile apothecia; and 3–8-septate, *Submuriform*-type, pale brown ascospores 27–35 × 15–17.5 µm. Spot tests: cortex and medulla K− or + yellow, KC−, C−, P−; containing skyrin and ± atranorin by TLC. This species was previously reported in Yunnan, growing on rock or bark at elevations around 2500 m. According to Aptroot and Sparrius, the holotype of this species grows on the tree trunk base of *Eucalyptus globulus*, with an additional cited specimen growing on soil and shale [29]. The specimens that we collected all grew on rocks. Similar to this species, *Rinodina conradii* Körb also has 3–8-septate ascospores, but it can be differentiated by its brown thallus, lack of vegetative propagules, and Type B development, *Conradii*-type ascospores. *R. pluriloculata* would key out at couplet 20 in Lee and Hur's paper because it has Type A development ascospores (apical wall thickening after septum formation) and contains skyrin [76].

Specimens examined: China, Yunnan Prov.: Lijiang City, Ninglang Yi Autonomous Co., Xichuan Vil., beside Lining Highway, 2504–2520 m, on rock, 26 July 2020, Wang Xinyu et al. XY20-162, 20-66417.

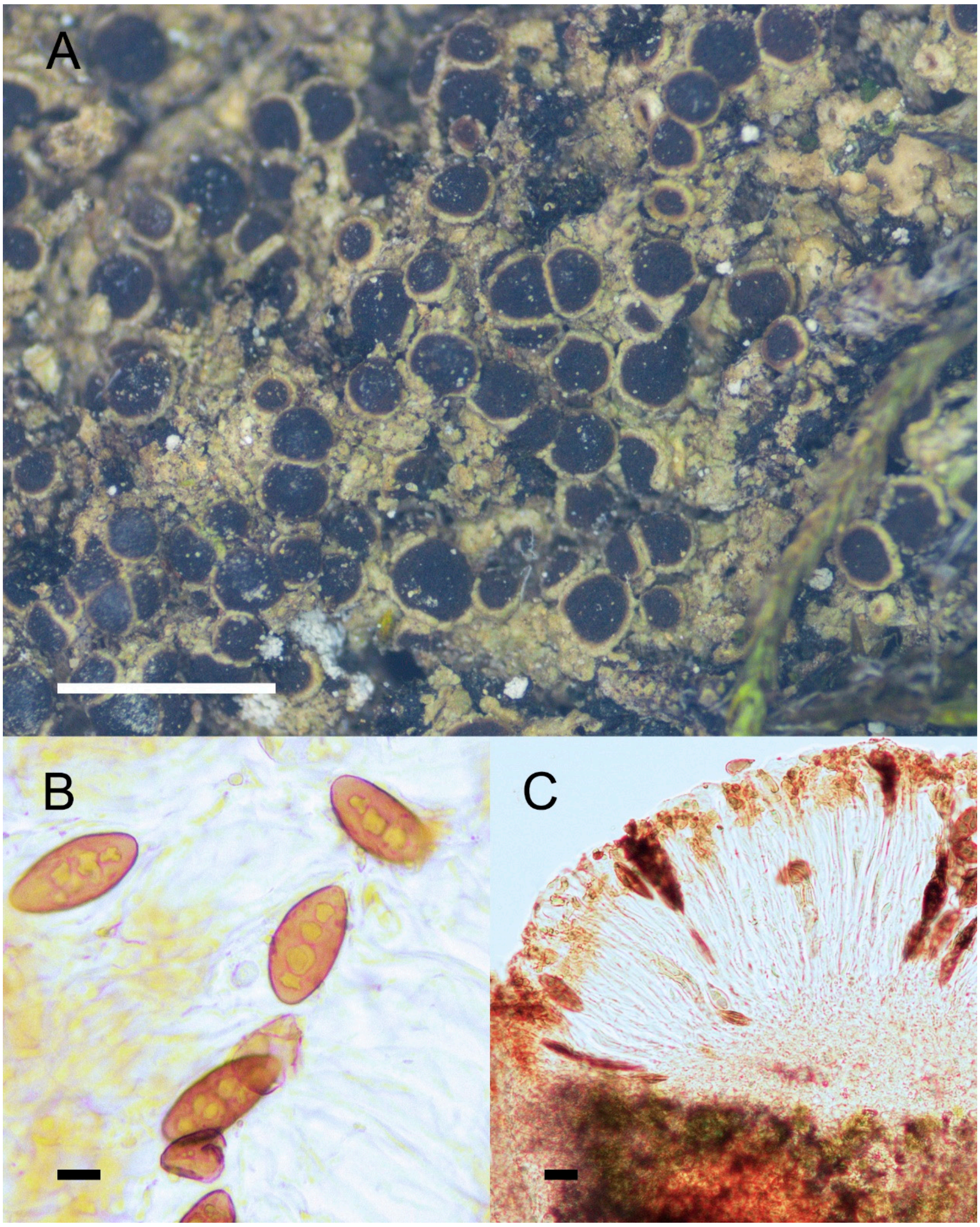

Figure 5. Morphology and anatomy of *Rinodina pluriloculata*. (**A**) Thallus and apothecia. (**B**) *Submuriform*-type ascospores. (**C**) Section of apothecia. Scale bars: 2 mm (**A**); 10 µm (**B**); 20 µm (**C**).

Buellia setschwana (Zahlbr.) Q. Y. Zhong and Xin Y. Wang, comb. nov., Figure 6.
= *Rinodina setschwana* Zahlbr., in Handel-Mazzetti, Symb. Sinic. 3: 231 (1930)

Type: China, Sichuan Prov., Sandsteinfelsen in der str. St. bei Dötschang im Pjientschang, 1450 m, 4 April 1914, Handel-Mazzetti no. 1171 (WU, holotype; US, isotype).

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Description: *Thallus* crustose, thin, plane, closely attached to the substrate, rimose; upper surface pale gray to pale yellowish brown, dull, non-pruinose; prothallus black, prominent; vegetative propagules absent. *Apothecia* common, usually scattered or sometimes contiguous, cryptolecanorine to lecideine, innate, 0.3–0.5 (0.7) mm in diam.; disc black, plane, round, or somewhat irregular; margin concolorous with thallus, entire, distinct when young, sometimes becoming carbonized when mature; exciple prominent, brown; hymenium colorless, not inspersed, 80–90 μm high; paraphyses simple, contiguous; apices expanded and septate, brown pigmented; epihymenium brown; hypothecium hyaline, colorless or pale brown, 50–60 μm deep; asci 8-spored, *Bacidia*-type; ascospores 1-septate, *Buellia*-type, brown, 12.5–18 × 5–7.5 μm; mature spores with obtuse ends, slightly constricted at septum; torus absent. *Pycnidia* common, dark brown to black, immersed; ostioles brown pigmented; conidia hyaline, bacilliform, 4–6 × 1 μm.

Chemistry: Cortex K+ yellow; KC+ yellow or orange; C−, P−; medulla I+ blue. Atranorin, arthothelin, norstictic acid, and sometimes stictic acid are detected by TLC.

Ecology and distribution: This species grows on rocks at elevations between 1100 m and 2000 m. It has previously been reported from Sichuan Province, China [19]. This is a new record for Yunnan Province.

Notes: *Buellia setschwana* is characterized by its thin, rimose thallus; black and prominent prothallus; innate cryptolecanorine to lecideine; black apothecia; and containing atranorin and norstictic acid as main compounds. It resembles *Buellia polita* in its external morphology and chemical compounds but could be distinguished by the hyaline hypothecium and cryptolecanorine apothecia. Both *B. setschwana* and the globally distributed *Buellia halonia* contain the secondary metabolite arthothelin. This could lead to confusion between the two species. However, *R. halonia* has exciples with typical aeruginose pigments (HNO_3+ purple), a reddish brown hypothecium, and adnate apothecia (innate in *B. setschwana*). Furthermore, *R. halonia* is always maritime, whereas *B. setschwana* is continental.

Rinodina setschwana was originally collected in Sichuan Province, Dechang County. It was placed in the genus *Rinodina* based on its cryptolecanorine apothecia and hyaline hypothecium [19]. After the collection of fresh specimens from the same locality and comparison with the type specimen, we confirmed that our collections were morphologically identical to the holotype. However, phylogenetic analyses demonstrated that this species should be placed within the genus *Buellia* rather than *Rinodina*. We consider that the type specimens were previously misassigned as species of *Rinodina*. Based on the morphology, chemistry, and phylogeny of specimens from the type locality, a new combination is proposed here, *Buellia setschwana*.

Specimens examined: China, Sichuan Prov.: Liangshan Prefecture, Huili Co., on the way from Dechang to Huili, beside Beijing-Kunming Railway, 1879 m, on rock, April 11, 2019, Wang Lisong et al. 19-62862, 19-62864; Jinyang Co., Dashaba Vil., 640 m, on rock, 12 May 2017, Wang Lisong et al. 17-55142. Yunnan Prov.: Xinping Co., Mopanshan Mt., 2540 m, on rock, 20 December 2008, Wang Lisong et al. 08-29962; Kunming Ci., Yinmin Town, 1100 m, on rock, 16 May 2014, Wang Lisong et al. 14-43770; Nanjian Co., Zhonghua Vil., Kongquedu Port, 2799 m, on rock, 4 July 2015, Ye Xin et al. *15-47743*, *15-47744*.

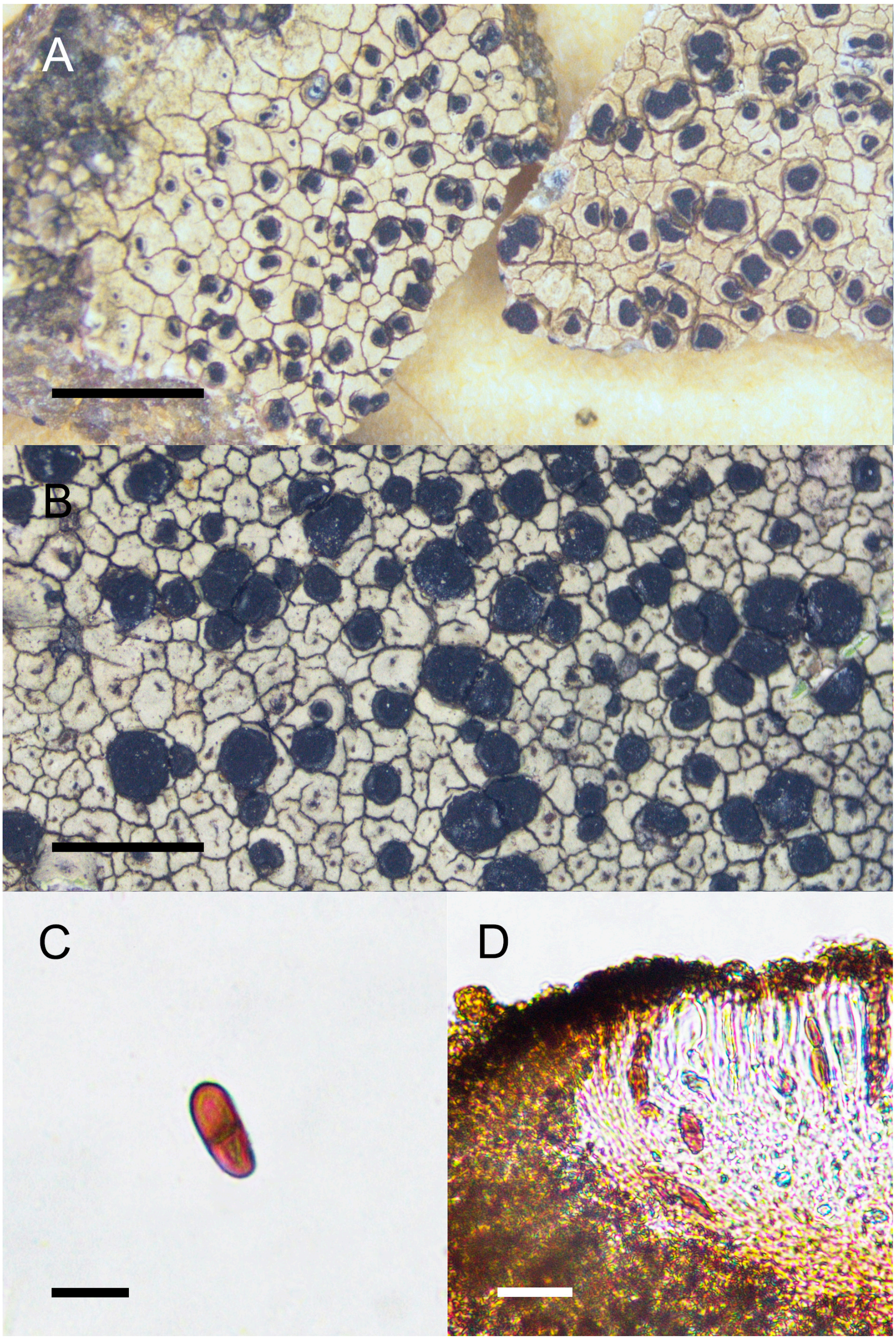

Figure 6. Morphology and anatomy of *Buellia setschwana*. (**A**) Thallus and apothecia of the holotype (W-1171). (**B**–**D**) Specimen collected from the type locality (KUN 15-47744). (**B**) Thallus and apothecia. (**C**) *Buellia*-type of ascospores. (**D**) Section of apothecia. Scale bars: 2 mm (**A**,**B**); 10 µm (**C**); 20 µm (**D**).

New species

Rinodina hengduanensis Q. Y. Zhong and Xin Y. Wang, sp. nov., Figure 7.

Type: China, Sichuan Prov., Liangshan Yi Autonomous Prefecture, Yanyuan Co., Weicheng Town, on sandstone, 2819 m elev., 27°30′ N, 101°41′ E, 28 July 2020, Wang Xinyu, Zhong Qiuyi, and Wang Luting no. XY20-290 (KUN, holotype).

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Characterized by saxicolous, subsquamulose, greyish brown to olivaceous thallus, lecanorine apothecium, and *Dirinaria*-type ascospores.

Etymology: The epithet '*hengduanensis*' refers to its distribution, which is mainly in the Hengduan Mountains region.

Description: *Thallus* crustose and areolate in the center, areoles jigsaw-like, 0.2–0.4 mm wide, closely attached to the substrate, continuous to scattered, margins prominent with extending squamules, appearing subsquamulose; upper surface flat, greyish brown to olive green, slightly glossy, non-pruinose; prothallus sometimes present (when thallus scattered), black; vegetative propagules absent. *Apothecia* common, usually scattered, lecanorine, broadly attached, 0.5–0.8 mm in diam.; disc dark brown to black, plane or convex, irregular; margin concolorous with the thallus, crenulate, persistent; exciple hyaline, hyphae parallel, with algal cells; hymenium colorless, hyaline, not inspersed, 100–125 μm high; paraphyses simple, contiguous, apices expanded and septate, brown pigmented; epihymenium ochre brown; hypothecium hyaline, colorless, not inspersed, 80–100 μm deep; asci 8-spored, *Lecanora*-type; ascospores 1-septate, brown or dark, Type A development, *Dirinaria*-type, 20–24 × 7.5–12.5 μm, swollen at the septum. Swelling becomes more obvious upon treatment with KOH. Usually with oil inspersed when mature, torus present. *Pycnidia* were not seen.

Chemistry: Spot tests for cortex were all negative; medulla I− contained traces of gyrophoric acid and skyrin, confirmed by TLC.

Ecology and distribution: This species grows on rocks at approximately 2800 m elevation. It is reported from Sichuan and Yunnan provinces in China.

Notes: *R. hengduanensis* is characterized by subsquamulose, greyish-brown to olivaceous thallus, jigsaw-like areoles, and *Dirinaria*-type ascospores. *Rinodina gennarii* Bagl. is a saxicolous species with the same type of ascospores that has been reported from China [26,27,29,78]. It differs from this new species by having a whitish thallus, crowded brown apothecia, and smaller ascospores (length < 17.5 μm). *Rinodina pycnocarpa* H. Magn. was originally reported from China. It also has a subsquamulose thallus and saxicolous habit, but it can be differentiated by its *Bicincta*-type and smaller ascospores (<18 μm). Three other species of *Rinodina* have similar subsquamulose thalli: *R. dolichospora* Malme can be differentiated by its *Pachysporaria*-type I spores and lack of substances; *R. intermedia* Bagl. can be recognized by its 3-septate to submuriform ascospores with type A development; whereas *R. willeyi* Sheard and Giralt can be separated by having soredia on the areole margin (sublabriform soralia) and its pannarin and zeorin content. *R. hengduanensis* would key out at couplet 4 in Lee and Hur's paper because it has a continental distribution and has *Dirinaria*-type, larger (length > 20 μm) ascospores [76].

Specimens examined: China, Sichuan Prov.: Liangshan Prefecture, Yanyuan Co., Weicheng Town, 2817–2865 m, on sandy rock, 28 July 2020, Wang Lisong et al. 20-66506, XY20-290, XY20-287. Yunnan Prov.: Diqing Prefecture, Shangri-La City, Luoji Vil., Niru Vil., 2774–2804 m, on rock, 21 July 2020, Wang Xinyu et al. XY20-44, XY20-41.

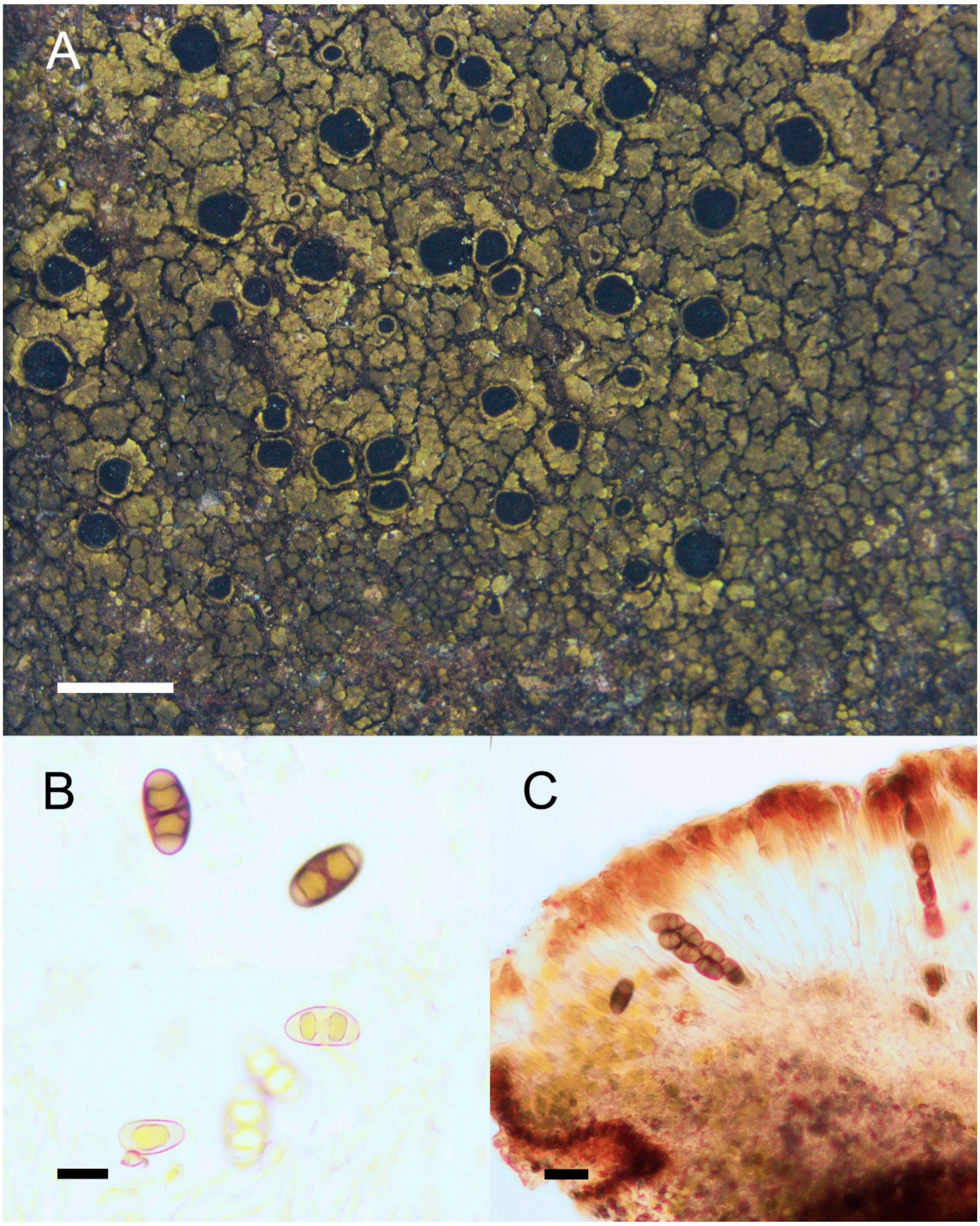

Figure 7. Morphology and anatomy of *Rinodina hengduanensis*. (**A**) Thallus and apothecia. (**B**) *Dirinaria*-type ascospores. (**C**) Section of apothecia. Scale bars: 2 mm (**A**); 10 µm (**B**); 20 µm (**C**).

5. Conclusions

In the present study, using morphological, chemical, and phylogenetic methods, new records for the five endemic species *Rinodina cornutula*, *R. globulans*, *R. handelii*, *R. setschwana*, and *R. pluriloculata* from Southwest China were documented based on specimens collected from the holotype localities. *Rinodina setschwana* was transferred to the genus *Buellia* and proposed as *Buellia setschwana*. One new species was discovered: *Rinodina hengduanensis*. Photographs, chemical compounds, and sequences support the circumscription of these species. However, such details are lacking for many of the previously proposed species of *Rinodina*. The absence of genetic material means that no molecular phylogeny can yet be constructed that includes all species currently assigned to the genus. For those species lacking genetic material, further new collections are required from the type localities.

Currently available keys based on morphology and chemistry are either outdated, limited in their geographic scope, or do not include the recently described *Rinodina* spp. A full revision of *Rinodina* is required in order to produce a key that should incorporate all described species across its full global distribution.

Author Contributions: Conceptualization, X.W. and L.W.; methodology, X.W.; software, Q.Z. and M.A.; validation, F.R.W., A.Y. and Q.Z.; formal analysis, Q.Z. and M.A.; investigation, Q.Z., M.A. and X.W.; resources, M.A. and A.Y.; data curation, Q.Z. and M.A.; writing—original draft preparation, Q.Z.; writing—review and editing, F.R.W. and X.W.; visualization, Q.Z. and A.Y.; supervision, X.W. and L.W.; project administration, X.W. and L.W.; funding acquisition, X.W., Y.J. and L.W. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Not applicable.

Data Availability Statement: Publicly available datasets were analyzed in this study. This data can be found from here: https://www.ncbi.nlm.nih.gov/ (accessed on 24 September 2022).

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Conflicts of Interest: The authors declare no conflict of interest.

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