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RESEARCH ARTICLE

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Two New Species of *Placolecis* (Lichenized Ascomycota) from China

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ABSTRACT

Two new species of the lichen genus *Placolecis* are discovered in China, namely *P. kunmingensis* An. C. Yin & Li S. Wang and *P. sublaevis* An. C. Yin & Li S. Wang. The new combination *P. loekoesiana* (S.Y. Kondr., Farkas, J.J. Woo & Hur) An. C. Yin is proposed. *Placolecis kunmingensis* is characterized by having simple, spherical or ellipsoid, hyaline spores, and pear-shaped pycnidia; while *P. sublaevis* can be distinguished by its thallus forming larger aggregations with slightly flattened lobes at the thallus margin, and urn-shaped pycnidia. Descriptions, a phylogenetic tree and a key are provided for all the known *Placolecis* species in China.

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Lichen; taxonomy; Catillariaceae; Yunnan Province

1. Introduction

The genus *Placolecis* Trevis. was established by Trevisan (1857) with two species: *Placolecis balanina* (Fr.) Trevis. and *P. plumbea* (Lightf.) Trevis [1]. Later on, Hafellner synonymized one more species *Lecidea opaca* Dufour into *Placolecis*, as *P. opaca* (Dufour) Hafellner, based on the morphological characters and secondary metabolites [2,3]. In recent studies, *P. balanina* was merged into *P. opaca* [4,5], and *P. plumbea* was treated as synonym of *Pectenia plumbea* (Lightf.) P. M. Jørg. [6]. After this study, four species (*Placolecis kunmingensis*, *P. loekoesiana, P. opaca*, and *P. sublaevis*) are recognized in this genus.

The genus *Placolecis* is mainly distributed in the Mediterranean region, and has been infrequently reported from Algeria, Russia, South Korea, and India, and so forth [7–14]. Species of this genus frequently occur on limestone in open and sunny habitats [9]. *Placolecis opaca* (≡*Lecidea opaca* var. *crocea* (B. de Lesd.) Zahlbr.) was first reported from China by Handel-Mazzetti based on collections from Sichuan Province, however, few studies were carried out [15,16]. Recently, during a field survey in Yunnan Province, some specimens of *Placolecis* were collected, and morphological, anatomical, chemical, and molecular phylogenetic analyses based on the internal transcribed spacer regions (ITS) were performed in order to determine species composition. A key to *Placolecis* species worldwide is provided.

2. Materials and methods

2.1. Materials and morphological observation

The specimens in this study are deposited in the Lichen Herbarium, Kunming Institute of Botany (KUN), and Korean Lichen Research Institute (KoLRI). Anatomical descriptions are based on observations under a NIKON Eclipse 50i microscope, and photographs were taken using NIKON digital camera head DS-Fi2. Spot tests were conducted by K (a 10% aqueous solution of potassium hydroxide), C (a saturated solution of aqueous sodium hypochlorite), and P (a saturated solution of *p*-phenylenediamine in 95% ethyl alcohol). Secondary metabolites were detected by thin-layer chromatography (TLC) as described by Orange et al. [17] in solvent C (toluene: acetic acid =170:30).

2.2. DNA isolation, PCR and phylogenetic analysis

Genomic DNA was extracted from dried materials using AxyPrep Multisource Genomic DNA Miniprep Kit50-prep (Qiagen, Hilden, Germany) according to the manufacturer's instructions. ITS was amplified via polymerase chain reaction (PCR) using the primers ITS1F [18] and ITS4 [19]. Amplifications were performed in a 25 µL volume comprising 12.5 µL of 2 × MasterMix, 0.5 µL of each primer, 10.5 µL distilled H_2O, and 1 µL of DNA.

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PCR amplifications program were performed as following: initial denaturation at 94 °C for 4 min, 34 cycles each composed of 94 °C for 1 min, 62 °C for 1 min, 72 °C for 1.5 min, and a final extension at 72 °C for 10 min. PCR products were sequenced by TsingKe biological technology company (Kunming, China).

The ITS dataset consists of 6 newly generated sequences and 17 relevant ITS sequences from GenBank (Table 1). All raw sequences were assembled and edited using SeqMan 7.0 (DNAstar packages), then aligned with MAFFT version 7 [20] with the default parameters. Ambiguous regions were excluded using Gblocks [21] with default settings. Maximum likelihood (ML) was generated using the RAxML-HPC2 on XSEDE (8.2.10) in the CIPRES Science Gateway Platform (http://www.phylo.org/portal2/) using GTRGAMMA model, fast bootstrap analyses of 1000 pseudoreplicates were completed [22]. Phylogenetic trees were visualized using the program FigTree 1.4.0 [23]. The Bayesian analysis was conducted with MrBayes v.3.2.6 to evaluate Bayesian posterior probabilities by Markov Chain Monte Carlo sampling (MCMC). Bayesian inference was conducted using four chains and run for one million generations. *Lecidea tessellata* Flörke, *L. atrobrunnea* (DC.) Schaer., *Teloschistes flavicans* (Sw.) Norman, *T. exilis* (Michx.) Vain. were used as outgroup [24].

3. Results and discussion

Topologies were established based on 23 ITS sequences including related species of family Catillariaceae and four species for outgroup (Table 1). The tree topology obtained from the maximum likelihood is used to represent phylogenetic relationships. A phylogenetic analysis using ITS sequences revealed 19 species, including *P. kunmingensis*, *P. sublaevis* new to science (Figure 1). Molecular phylogenetic analyses strongly supported the current taxonomic delimitation of species within the genus of *Placolecis*, which is in accordance with the taxonomic study based on morphological and chemical characters.

Placolecis is characterized by saxicolous, crustose to effigurate, dark brown or yellowish brown or yellowish thallus, areolate in the center; Lobes contiguous, flat to convex, usually branched. Cortex paraplectenchymatous; medulla reddish orange in the upper portion and white in the lower portion. Apothecia lecideine, 0.3–1.3 mm across, black, sessile, with a flat to convex disc and a persistent proper margin. Hymenium colourless, I + blue; paraphyses capitate, the apical cells with an internal pigment cap. Asci 8-spored, narrowly clavate, *Catillaria*-type (with a prominent amyloid tholus, lacking any internal differentiation, I + blue). Ascospores simple, hyaline, ellipsoid, (7.5-)10-14 × (4-)5-7.5 µm. Pycnidia frequent, immersed or slightly protruding. Conidia hyaline, bacilliform, straight. Chemistry: medulla with anthraquinones [2,3,12,16].

Although these species have similar habit, colour reaction, thallus section, *Catillaria*-type ascus and simple hyaline ascospores, and so forth, they can be distinguished by the thallus morphology. The thallus of *P. opaca* usually dark brown or yellowish brown, areolate in the center, forming regular to irregular rosettes to 4 cm diameter; lobes are elongate, contiguous, flat to slightly convex, radiating from center toward to periphery, often with secondary lobes as branches of main lobules, in having yellow-ochre-orange medulla [1,2,7,25]. The new species *P. kunmingensis* has a crustose thallus with rather few lobes,

Table 1. Sequence information used in this study.

Species	Collection No.	GenBank No.	Locality
Placolecis loekoesiana	041238 (KoLRI)	**MN052962**	South Korea
Placolecis sublaevis	19-62675(KUN)	**MK995874**	China, Yunnan
Placolecis kunmingensis	18-58078(KUN)	**MK995884**	China, Yunnan
Placolecis kunmingensis	56795(KUN)	**MK995879**	China, Yunnan
Placolecis opaca	Inv.Nr.8763	**MK995882**	Spain
Placolecis opaca	Inv.Nr.8764	**MK995885**	Spain
Solenopsora liparina	Liparina-3-CZ (Herb. SAV)	KF689879	Czech Republic
Solenopsora cesatii	Cesatii-19-FR (Herb.J. Malíček, Sedlčany 5338)	KF689850	France
Solenopsora vulturiensis	Vulturiensis-4-FR (Herb. SAV)	KF689897	France
Solenopsora grisea	Grisea-9-AL (Herb. BP)	KF689875	Albania
Solenopsora olivacea	Olivacea-3-ME (Herb. O 11378)	KF689889	Montenegro
Solenopsora marina	Marina-4-FR (Herb. SAV)	KF689883	France
Austrolecia sp1		JX036089	Antarctica
Austrolecia sp2		JX036083	Antarctica
Austrolecia sp3		JX036047	Antarctica
Austrolecia sp		JX036104	Antarctica
Austrolecia sp		JX036103	Antarctica
Catillaria scotinodes	O: L 161161	MG925964	Norway
Catillaria corymbosa	Hur ANT050798	DQ534457	Antarctica
Lecidea tessellata	UR00138	KX120213	Argentina
Lecidea atrobrunnea	UR00200	KX120206	Argentina
Teloschistes flavicans	03.22.03-13A (DUKE)	JQ301685	Costa Rica
Teloschistes exilis	D. Hillis 07-726 (DUKE)	JQ301684	USA

Newly generated sequences were in bold.

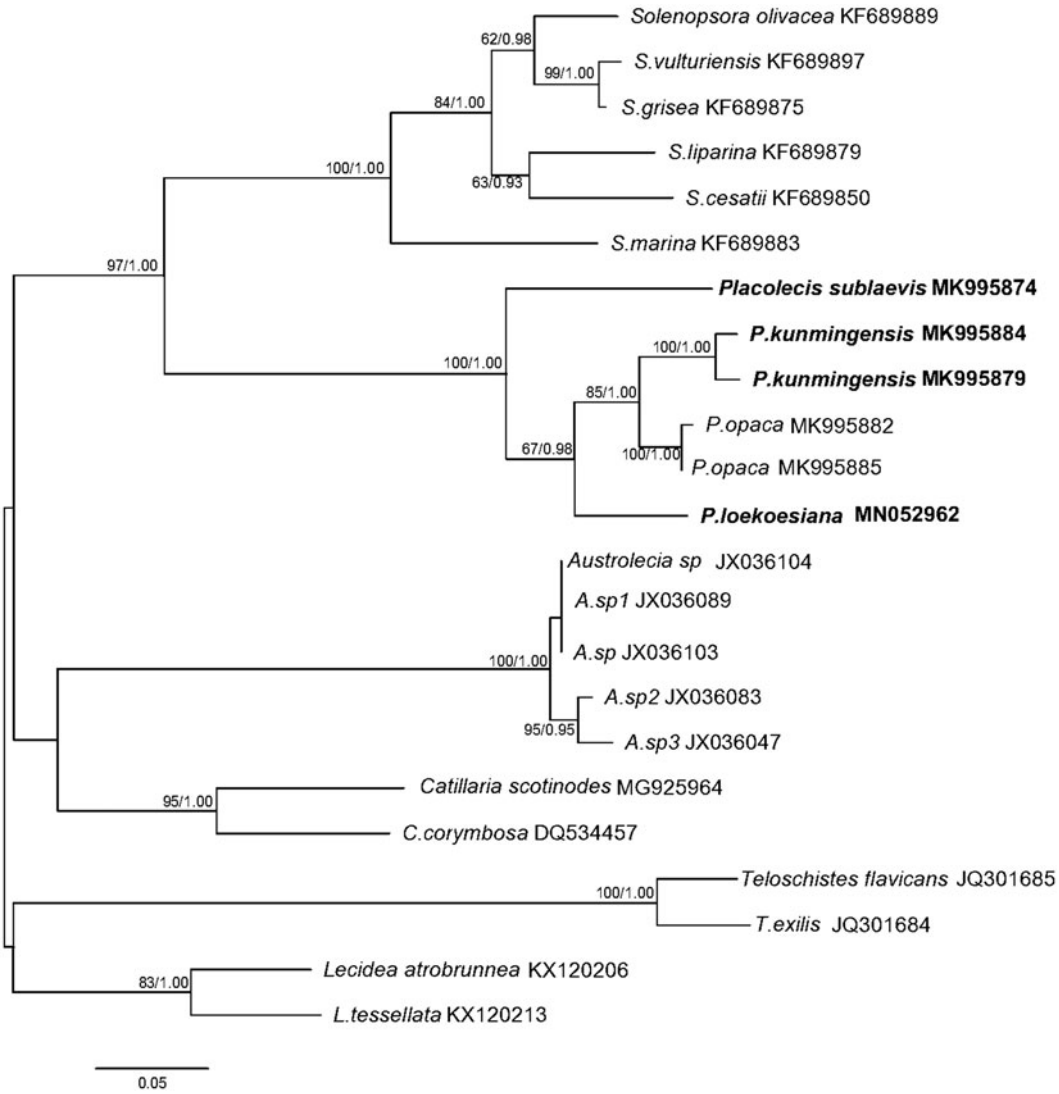

Figure 1. Phylogenetic relationships of the family Catillariaceae with the genus *Placolecis* inferred by Bayesian analysis of the ITS sequences. New species and the new combination are shown in bold. Bootstrap support values (MLBS) and posterior probabilities (PP) are shown above or below the branches.

areolate-squamulose in the center, forming irregular patches or clumps 10–50 mm wide; upper surface dark brown, uneven; rarely with radiating shorter lobules at the thallus margin, sometimes difficult to delimitate secondary lobules and main lobes. *P. sublaevis* is similar to *P. kunmingensis* in having the same crustose thallus in the center, differs in having larger aggregations of lobes at the thallus margin, in having distinct secondary lobules as branches of main lobes, slightly subflattened at the apex, in having pear-shaped pycnidia and elongated conidia. All four species contain unidentified anthraquinones by TLC and the same compounds: emodin, erythrin, fragilin, 7-chloro-emodin, 2-chloro-derivatives [26,27].

4. Taxonomy

4.1. New species

Placolecis kunmingensis An. C. Yin & Li S. Wang, **sp. nov.**

Holotype: CHINA: Yunnan Province, Baiyi village, 25°21′N, 102°50′E, 2130 m, on rock, April 12, 2018. Li S. Wang et al. 18-58078 (KUN-L).

MycoBank no. MB: 831234.

Description (Figure 2)**: Thallus** saxicolous, crustose to effigurate, areolate-squamulose in the center, forming irregular patches or clumps 10–50 mm wide; rarely with radiating shorter lobules at the thallus margin; lobes to 0.5–1 mm long and 0.2–0.3 mm wide in the middle part and distinctly widened towards the tips, 0.35–0.5 mm wide, dark brown, swollen at the apex, equal dichotomous branching, sometimes difficult to delimitate secondary lobules and main lobes. **Upper surface** dark brownish, uneven. **Upper cortex** 25–30 µm thick, paraplectenchymatous. **Algal layer** uniform, 50–70 µm thick, photobiont cells *Trebouxia*-like, 7–12.5 µm diameter **Medulla** 180–250 µm thick, reddish orange in the upper portion and white in the lower portion. **Lower cortex** absent. **Apothecia**

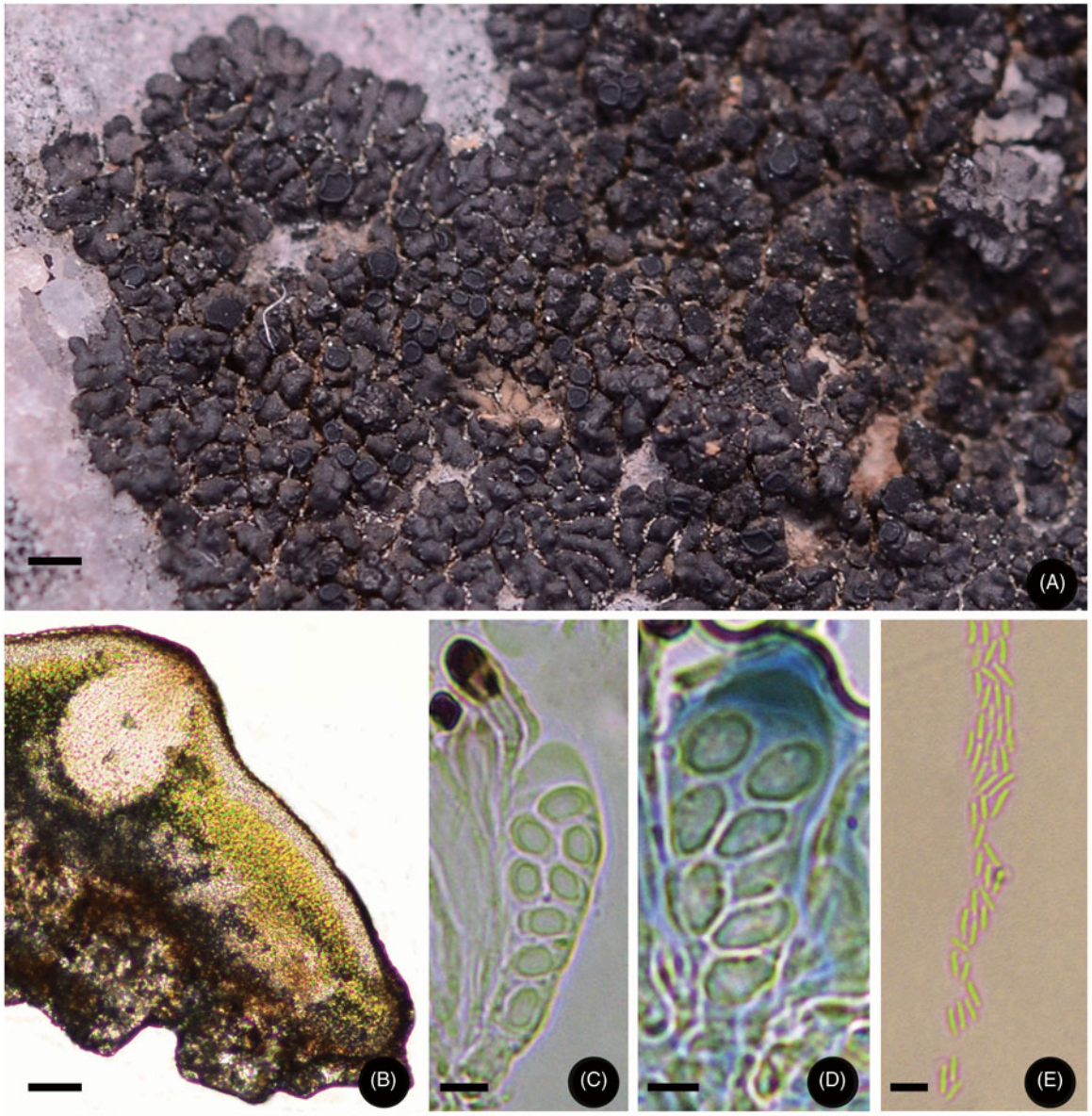

Figure 2. *Placolecis kunmingensis*, holotype. (A) Thallus and habit; (B) cross-section of thallus and a pycnidium; (C) ascus with ascospores; (D) I + blue of ascus; (E). conidia. Scale bars: A = 1 mm; B = 50 µm; C, D = 10 µm; E = 5 µm.

0.3–1.2 mm diameter, sessile, lecideine, numerous, scattered to crowded, margin well developed, black. **Exciple** 40–60 µm thick at the sides, black. **Epihymenium** 7–15 µm thick, brown. **Hymenium** hyaline, 50–75 µm thick, I + blue. **Hypothecium** 75–120 µm thick, brown. **Asci** clavate to cylindrical, *Catillaria-type* with amyloid tholus, I + blue, 8-spored. **Paraphyses** simple or sparsely branched, with dark brown cap at the apex. **Ascospores** hyaline, simple, spherical or ellipsoid, 5–10 × 4–6 µm, with smooth 0.5–1 µm thick wall. **Pycnidia** numerous, is not totally immersed (ca. 2/3 immersed), 150–190 × 100–130 µm, pear-shaped, ostiole slightly black. **Conidia** bacilliform, 3–5 µm.

Chemistry: Medulla K + violet in the upper yellow portion, P−, C−; containing fragilin and anthraquinone (detected by TLC).

Ecology and distribution: This species occurs on limestone in sunny slopes, and is only found in Kunming and surrounding areas in the high mountains in Yunnan (SE China).

Notes: *Placolecis kunmingensis* is characterized by a thallus areolate-squamulose in the thallus center, forming irregular patches or clumps 10–50 mm wide and ellipsoid or globose ascospores with slightly thickened wall. This species is similar to *P. opaca* in having similarly dark brownish thallus, but the latter has a thallus forming regular or irregular rosettes, areolate in the center, with longer lobes radiating in the periphery of the thallus. It differs from *P. loekoesiana* in having areolate-squamulose thallus center, in having brown (vs. yellow) thallus colour, somewhat shorter thalline lobes at the thallus margin.

Etymology: The epithet "*kunmingensis*" refers to the type locality.

Further specimens: Yunnan Prov. Kunming City: Xiaohe village, Songhuaba, 25°11′54″N, 102°48′26″E,

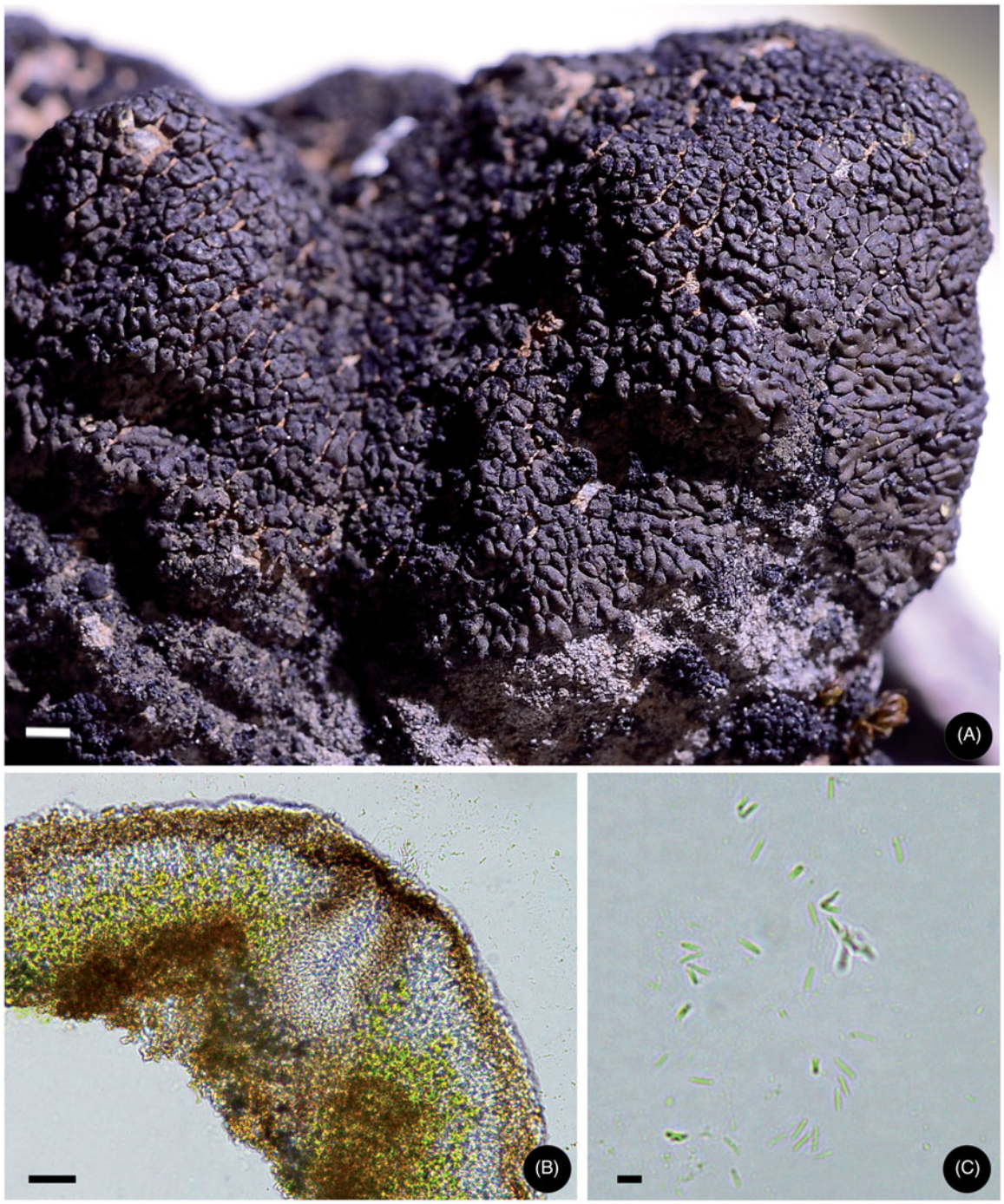

Figure 3. *Placolecis sublaevis*, holotype. (A) Thallus and habit; (B) cross-section of thallus and a pycnidium; (C) conidia. Scale bars: A = 1 mm; B = 25 µm; C = 5 µm.

2,000 m, on rock, 10 December. 2001. Li S. Wang 01-21029; Lunan Co., near Shilin and Stone Forest. 24°43′56″N, 103°20′33″E, 1,900 m, on limestone, October 27, 2002, A. Aptroot. 56795 (in KUN-L).

Placolecis sublaevis An. C. Yin & Li S. Wang, **sp. nov.**

Holotype: CHINA: Yunnan Province, Lijiang City, on the way from Lijiang to Ninglang, 26°58′54.38″N, 100°24′57.24″E, 1,902 m, on limestone, April 9, 2019. Li S. Wang et al. 19-62675 (KUN-L).

MycoBank no. MB: 831362.

Description (Figure 3)**: Thallus** saxicolous, crustose to effigurate, areolate-squamulose in the center, forming irregular patches or clumps, pseudopycnidia numerous, immersed into thallus; lobes distinctly forming larger aggregations at the thallus margin: to 2–3 (–4) mm long and 0.1–0.3 mm wide in the middle part and slightly widened towards the tips to 0.15–0.4 mm wide, dark brown, slightly subflattened at the apex; often secondary lobules as branches of main lobes to 1–2 mm long observed. **Upper surface** dark brownish, uneven. **Upper cortex** 12.5–20 µm thick, paraplectenchymatous. **Algal layer** diffuse, 25–40 µm thick, photobiont cells *Trebouxia*-like, 6–12 µm diameter **Medulla** 60–140 µm thick, reddish orange in the upper portion and white in the

lower portion. **Lower cortex** absent. **Apothecia** none. **Pycnidia** numerous, immersed into thallus, 75–95 × 40–60 µm, urceolate, ostiole black. **Conidia** bacilliform, 5–6 µm.

Chemistry: Medulla K + violet in the upper yellow portion, P−, C−; fragilin, solorinic acid and anthraquinone substances detected by TLC.

Ecology and distribution: This species occurs on exposed steep slopes with limestone boulders near the stream, in the high mountain area in Yunnan and Sichuan (SE China).

Notes: The variety *Lecidea opaca* var. *crocea* (B. de Lesd.) Zahlbr. was described from France and reported later also from China [28,29]. According to Handel-Mazzetti's specimen information, *L. opaca* var. *crocea* was collected in Sichuan Yanyuan. We visited the original locality and collected samples from similar habitats, in order to compare with the European specimens (L-76218, L-203661, L-622068, L-622018, L-676136, L-685879) in UPS. According to the ITS phylogenetic tree, the morphological descriptions, and the WU online data query, we believe that the specimens Handel-Mazzetti collected in Sichuan in 1914 were misidentified as *L. opaca* var. *crocea* [15,16], they are in fact a new species in *Placolecis*, *P. sublaevis*. It differs from *P. opaca* in having areolate-squamulose in the thallus center, in having somewhat shorter and somewhat narrower thalline lobes, especially towards the tips (0.15–0.4 mm wide versus to 0.4–0.6 mm wide), medulla yellow in the upper portion and white in the lower portion (vs. yellow-brown), *Trebouxia* algae normally have smaller size cells (6–12 µm vs. 10–15 µm), in having elongated conidia (5–6 µm vs. 3–5 µm). The species *P. sublaevis* is similar to *P. kunmingensis* in having similar small crustaceous thallus center, but it is different in having larger aggregations of lobes at the thallus margin (2–3 (–4) mm vs. 0.5–1 mm), in having distinct secondary lobules as branches of main lobes (vs. equal dichotomous branching), in having thinner algal layer (diffuse, not uniform, 25–40 µm vs. uniform, 50–70 µm), in having pear-shaped pycnidia (vs. urn-shaped), elongated conidia (5–6 µm vs. 3–5 µm).

Etymology: The epithet *sublaevis* refers to the shape of lobes, slightiy subflattened at the apex.

4.2. New combination

Placolecis loekoesiana (S.Y. Kondr., Farkas, J.J. Woo & Hur) An. C. Yin, **comb. nov.**

Basionym: *Astroplaca loekoesiana* S.Y. Kondr., Farkas, J.J. Woo & Hur, in Kondratyuk et al., Acta Bot. Hung. 59(1/2): 139 (2017).

Type: Republic of Korea. Gangwon-do: Jeongseon-gun, Jeongseon-eup, Aesan-ri, limestone rocky wall along river, on calcareous rocks. Lat.: 37°22′18.66″N; Long.: 128°40′27.76″E; Alt.: 325 m a.s.l. Coll.: Kondratyuk, S. Y. and Lőkös, L. (163000), 16.09.2016 (KoLRI 041238 – holotype!).

MycoBank no. MB: 831252.

Notes: Baglietto established the genus *Astroplaca* based on *Lecidea opaca* Dufour in 1858, which was published later than *Placolecis* [1]. The genus *Astroplaca* is synonymized with *Placolecis* [1,2,4,30–34], our phylogenetic study supported this view, and this species *A. loekoesiana* is now synonymized as *P. loekoesiana*.

4.3. Key to the Placolecis species worldwide

1. Thallus center areolate-squamulose, rather few lobes; in high mountain 2
1. Thallus center areolate, elongated lobes; in lower elevations 3
2. Thallus dark brown, algal layer diffuse, not uniform, 25–40 µm; apothecia none, pycnidia urn-shaped, 75–95 × 40–60 µm, conidia 5–6 µm . . . . . ***P. sublaevis***
2. Thallus dark brown, algal layer uniform, 50–70 µm; apothecia numerous, ascospores spherical or ellipsoid, pycnidia pear-shaped, 150–190 × 100–130 µm, conidia 3–5 µm . . . . . ***P. kunmingensis***
3. Thallus yellow, thalline lobes shorter and narrower, medulla yellow; apothecia plane, rare, ascospores ellipsoid, 10–14 × 5–6 µm . . . . . ***P. loekoesiana***
3. Thallus dark brown or yellowish brown, thalline lobes elongated and contiguous, medulla ochre to reddish orange; apothecia convex, rather rare, ascospores ellipsoid, 9–11 × 4–5 µm . . . . . ***P. opaca***

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Disclosure statement

No potential conflict of interest was reported by the authors.

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