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The Lichen Genus *Letrouitia* (Brigantiaeaceae, Ascomycota) in China

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Abstract: Based on morphological, chemical and molecular studies, two new species of the lichen genus *Letrouitia* are newly described from China. *Letrouitia arcuata* is distinguished by its arcuate ascospores [8–10(–12)-locular, (28–)33–50(–62.5) × (8–)10–14.5 µm] and *L. sinuosa* by its ascomata with wavy margins and ascospores with lens-shaped locules [6–8-locular, (18–)19.5–32(–34) × (6.5–)8–14 µm]. In addition, *L. magenta* is reported for the first time as a new record in China, characterized by small and round ascomata. The descriptions, distribution and phylogenetic analysis of the respective species have been actualized and a key to the *Letrouitia* species known from China is provided.

Keywords: lichenized fungi; Teloschistales; taxonomy; phylogeny

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1. Introduction

The taxonomic classification of the lichen genus *Letrouitia* Hafellner & Belleme. has been controversial. In 1970, Santesson placed *Letrouitia* (as *Brigantiaea* p.p.) in the family Teloschistaceae based on the presence of anthraquinones [1]. This position remained unchanged until Hafellner and Bellemere established the genus *Letrouitia*, with *Letrouitia domingensis* (Pers.) Hafellner & Bellem. as the type species, based on anthraquinones, thick spore septa and a *Letrouitia*-type ascus [2]. *Letrouitia* was placed in the new family Letrouitiaceae, which was affiliated with the Teloschistaceae in the suborder Teloschistineae [3]. In 2022, *Letrouitia* was placed in the family Brigantiaeaceae (Teloschistales, Lecanoromycetes, Ascomycota, Fungi) [4]. To date, a total of 20 species have been reported, mostly growing epiphytically on bark and occurring mainly in the tropics and subtropics [4–10].

Letrouitia is characterized by a crustose, olive-grey to greenish thallus; round to somewhat distorted apothecia with a well-developed excipulum and a prominent margin, which is often orange in colour due to anthraquinones crystals; (1–)8-spored asci with a diffuse, I+ outer ascus wall; and hyaline ascospores with three types, normal transversely septate, muriform or screw-formed [5–7,11–13].

Prior to this study, only four species of *Letrouitia* had been reported from China. *L. subvulpina* (Nyl.) Hafellner was first reported from Taiwan [5]. Subsequently, three species were reported, *L. aureola* (Tuck.) Hafellner & Bellem., *L. parabola* (Nyl.) R. Sant. & Hafellner known from Taiwan [14] and *L. transgressa* (Malme) Hafellner & Bellem. known from Yunnan [15]. During a survey of the lichen diversity in the south of China, two new species and a new record of *Letrouitia* were discovered. The aim of this study was to provide the three species together with photographs of their external morphology and internal anatomical features. Phylogenetic trees were constructed from ITS, nuLSU and mtSSU sequences. Overall, three species of *Letrouitia* were reported here. This result greatly increased the diversity of *Letrouitia* in China and is an important addition to the study of *Letrouitia*.

2. Materials and Methods

2.1. Specimens and Morphology

The specimens were collected from Yunnan and Hainan provinces, China, and deposited in the Fungarium of College of Life Sciences, Liaocheng University (LCUF), after drying and low-temperature treatment.

A dissecting microscope (Olympus SZX16, Olympus Corporation, Tokyo, Japan) was used to observe the structure of apothecia, and a compound microscope (Olympus BX53 with OLYMPUS DP74 digital camera, Olympus Corporation, Tokyo, Japan) was used to observe microscopic characteristics. Measurements of the apothecia, epithecium, paraphysis, asci and ascospores were taken from mature vertical sections of fruit bodies mounted in water. The specific methods of anatomical study were as follows: First, well-developed ascomata were removed on white card, and the apothecia were cut longitudinally as thinly and completely as possible by hand with a blade. Second, a complete and thin section was placed on a slide moistened with sterile water, a coverslip was applied and excess water was absorbed with absorbent paper. The characteristics of the epithecium, hymenium, hypothecium, asci and ascospores were observed. Photographs and notes were made. Finally, Lugol's iodine solution (1% iodine solution) was added to check for the amyloidity of the ascospores.

2.2. Chemistry

Spot tests were performed on the thallus surface (10% KOH, saturated aqueous NaOCl and saturated p-phenylenediamine in ethanol). The lichen substances of the thallus were detected and identified by thin-layer chromatography (TLC), using solvent C [16–18]. The specific procedures were as follows: First, the solvent was prepared according to the formula (toluene/acetic acid = 200:30 mL) and the silicone glass plate was prepared; then, a small amount of thallus cortex and medulla was scraped with a blade (75% alcohol disinfectant), placed separately in centrifuge tubes and soaked with acetone for about 15 min, and the sample was spotted with the capillary according to the number, with *Lethariella cladonioides* as the partition standard sample. Second, the silicone plate was placed in a chromatography cylinder at 1 cm below the C system solvent, with the origin spots above the solvent, and the silicone resin plate was removed before the solvent reached 1 cm from the end of the chromatography plate. The solvent was dried with a dryer. Next, the silicone plate was sprayed with 10% sulfuric acid and baked at 85 °C for 10–15 min, and the colour and position of the spots were recorded under white, 365 nm and 254 nm ultraviolet light, respectively. Finally, the partitions were as follows: zone 1: between the upper and lower tangents of the chromatographic origin; zone 4 and 7: the tangent lines at the upper and lower borders of the norstictic acid and atranorin acid spots; zone 2 and 3: equal parts between zone 1 and zone 4 divided by a line; zone 5 and 6: equal parts between zones 4 and 7 divided by a line; zone 8: above zone 7. The colour and position of each spot were noted.

2.3. DNA Extraction and PCR Sequencing

Total genomic DNA was extracted from specimens collected for this study by using the Hi-DNA-secure Plant Kit (Tiangen, Beijing, China). The ITS, nuLSU and mtSSU regions were amplified using ITS1F/ITS4 [19,20], AL2R/LR6 and mtSSU1/mtSSU3R [21,22] primer pairs, respectively. Reactions were performed in a 50 µL reaction system containing 2 µL of each primer solution, 2 µL of genomic DNA, 19 µL of ddH_2O and 25 µL of 2×Taq PCR MasterMix (Tiangen, Beijing, China). The PCR conditions for ITS included an initial denaturation at 94 °C (3 min), 35 cycles of denaturation at 94 °C (30 s), annealing at 52 °C (30 s), extension at 72 °C (90 s) and a final extension at 72 °C (10 min). For nuLSU, the conditions included an initial denaturation at 94 °C (5 min), 35 cycles of denaturation at 94 °C (30 s), annealing at 58 °C (30 s), extension at 72 °C (90 s) and a final extension at 72 °C (10 min). For mtSSU, the conditions included an initial denaturation at 94 °C (5 min), 35 cycles of denaturation at 94 °C (45 s), annealing at 50 °C (30 s), extension at

72 °C (90 s) and a final extension at 72 °C (10 min). The PCR target product was confirmed by electrophoresis on 1% agarose gels and sequenced by Biosune Inc. (Shanghai, China).

2.4. Phylogenetic Analysis

Twenty-six related sequences for phylogenetic tree construction were downloaded from GenBank (Table 1). Twenty-three sequences covering the reported species of *Letrouitia* were retrieved from GenBank. According to previous research, *Lecanora contractula* AFTOL-877 was selected as an outgroup [23], while its current name is *Polyozosia contractula* (Nyl.) S.Y. Kondr., Lőkös & Farkas [24], as shown in Table 1. A multi-locus (ITS, mtSSU and nuLSU) phylogenetic analysis was performed. The combined analysis included 40 sequences representing 9 in-group taxa and 1 out-group taxon (Table 1). The alignment was performed using MAGA 11 with the MUSCLE option [25]. The three single-locus alignments were concatenated in Geneious 9.2.0. The concatenated data matrix contained 3298 nucleotide sites (ITS 863 bp, nuLSU 1459 bp and mtSSU 976 bp). To check the consistency between the three loci, an incongruence length difference (ILD) test was performed using PAUP 4.0. The P value of the ILD test was 0.7 (>0.05), so the three loci were suitable for polygenic phylogeny. Phylogenetic relationships were inferred using maximum likelihood (ML) and Bayesian inference (BI) analyses on the CIPRES Scientific Gateway portal (http://www.phylo.org/portal2/, accessed on 25 July 2023) [26]. The ML analysis was performed using RAxML-HPC BlackBox v. 8.2.12 [27], with a GTRGAMMA model and bootstrap statistics calculated from 1000 bootstrap replicates. For the BI analysis, jModelTest 2.1.6 [28] was used to determine the best fitting model for each partition. For the ITS region, we used GTR+I+G, for nuLSU, we used SYM+G, and for mtSSU, we used GTR+G. The BI analysis was performed using MrBayes on XSEDE (3.2.7a) on CIPRES with 2 independent runs, searching for 10,000,000 generations [29]. Each run included 4 independent chains and sampling every 1000 generations [30]. After discarding the burn-in, the remaining 75% was used to compute the consensus tree [27,31]. Clades with bootstrap support ≥70% under ML and posterior probabilities ≥0.95 were considered significant. The generated phylogenetic trees were plotted using FigTree v.1.4.3.

Table 1. The specimens and sequences used in the phylogenetic analysis.

Species	Specimen	Locality	ITS	nuLSU	mtSSU
Polyozosia contractula	AFTOL-877	–	HQ650604	DQ986746	DQ986898
Letrouitia arcuata	YN18225	China Yunnan	**OR395215**	**OR395220**	–
Letrouitia domingensis	AFTOL-102	–	HQ650700	AY584648	AY584619
Letrouitia domingensis	Gaya 55	Dominican Republic	JQ301673	JQ301569	JQ301505
Letrouitia domingensis	MES-3181 FLAS-F-63803	Belize	ON383441	–	–
Letrouitia flavidula	Gaya 35	Costa Rica	JQ301674	–	JQ301506
Letrouitia magenta	MFLU 18-0693	Thailand	MK499353	MK499365	–
Letrouitia magenta	YN210757	China Yunnan	**OR395216**	**OR395221**	**OR395225**
Letrouitia magenta	YN210758	China Yunnan	**OR395217**	**OR395222**	**OR395226**
Letrouitia parabola	Gaya 11	USA	JQ301675	JQ301570	JQ301507
Letrouitia sinuosa	HN19607	China Hainan	**OR395219**	**OR395224**	**OR395227**
Letrouitia subvulpina	Gaya 44	Costa Rica	JQ301676	–	–
Letrouitia subvulpina	HN19636	China Hainan	**OR395218**	**OR395223**	–
Letrouitia transgressa	MFLU 18-0689	Thailand	MK499352	MK499364	–
Letrouitia vulpina	Gaya 72	Reunion	JQ301677	JQ301571	JQ301509
Letrouitia vulpina	USE419	France	KC179452	KC179209	KC179543

Note: newly generated sequences are shown in bold.

3. Results

3.1. Phylogenetic Results

Since the topologies of the Bayesian inference tree and the maximum likelihood tree were similar, Bayesian inference posterior probabilities (BI-PPs) and maximum likelihood

bootstrap probabilities (ML-BSs) were combined and placed at the node of the BI tree (Figure 1). Within the phylogenetic tree, the new species *Letrouitia arcuata* YN18225 was clearly separated from other *Letrouitia* species. *L. sinuosa* HN19607 was shown to be sister to the clade consisting of *L. magenta* A.H. Ekanayaka & K.D. Hyde (posterior probability = 1; bootstrap = 100%). Based on the differences in phylogeny and morphology compared to other species, which were described in detail below, they were classified as two new species, as shown in bold in Figure 1.

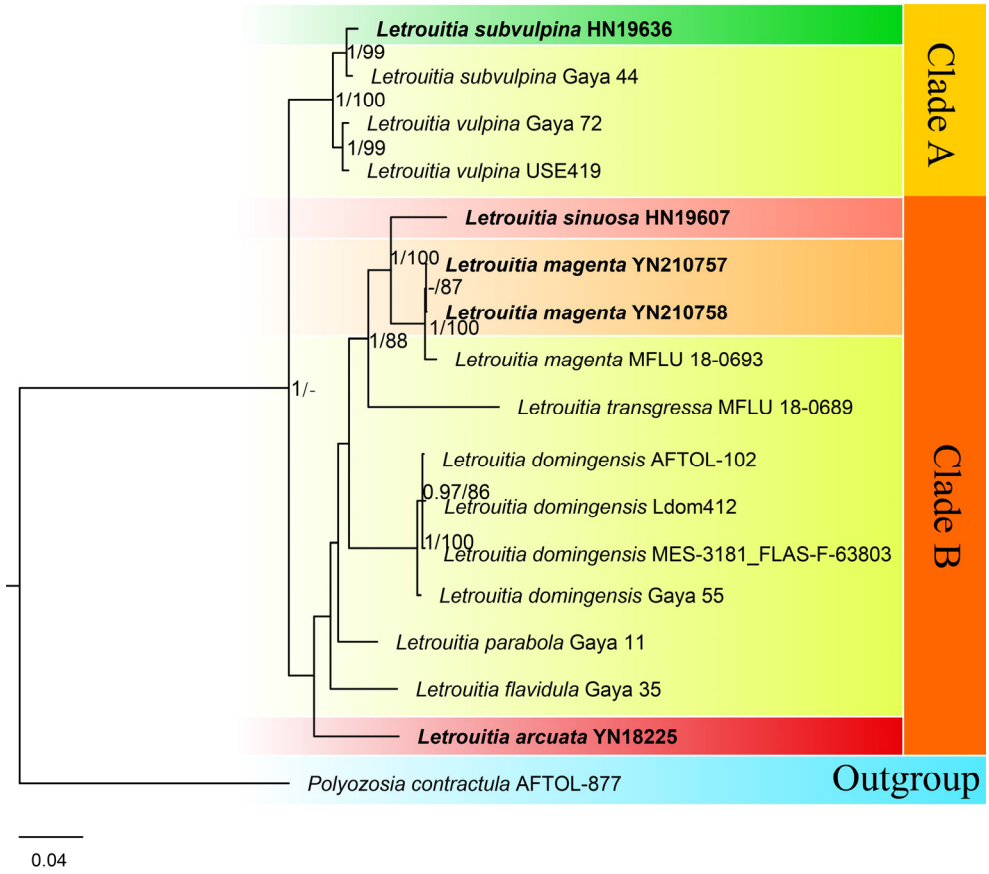

Figure 1. The Bayesian phylogenetic tree generated from the analysis of combined ITS, nuLSU and mtSSU. Bayesian posterior probabilities (PPs) and bootstrap support values of maximum likelihood (ML) are given to the right of the nodes as PP/ML. BI-PP > 0.95 and ML-BS > 70% were considered to be strongly supported. Newly generated sequences are in bold.

Two specimens of *L. magenta* from China were clustered with the material from Thailand, forming a highly supported clade (posterior probability = 1; bootstrap = 100%). In combination with the morphological similarity, we identified these specimens as *L. magenta*, which was characterized for the first time in China. A specimen of *Letrouitia* from China and *L. subvulpina* from Costa Rica were clustered together in the phylogenetic tree (posterior probability = 1; bootstrap = 99%). Based on the morphological similarity, we identified this specimen as *L. subvulpina*, which has been reported in China [5]. *Polyozosia contractula* was the out-group taxon. Bayesian posterior probabilities and ML bootstrap values are shown next to the nodes.

3.2. Taxonomy

Letrouitia arcuata C. Cui & Z.F. Jia, sp. nov., Figure 2.

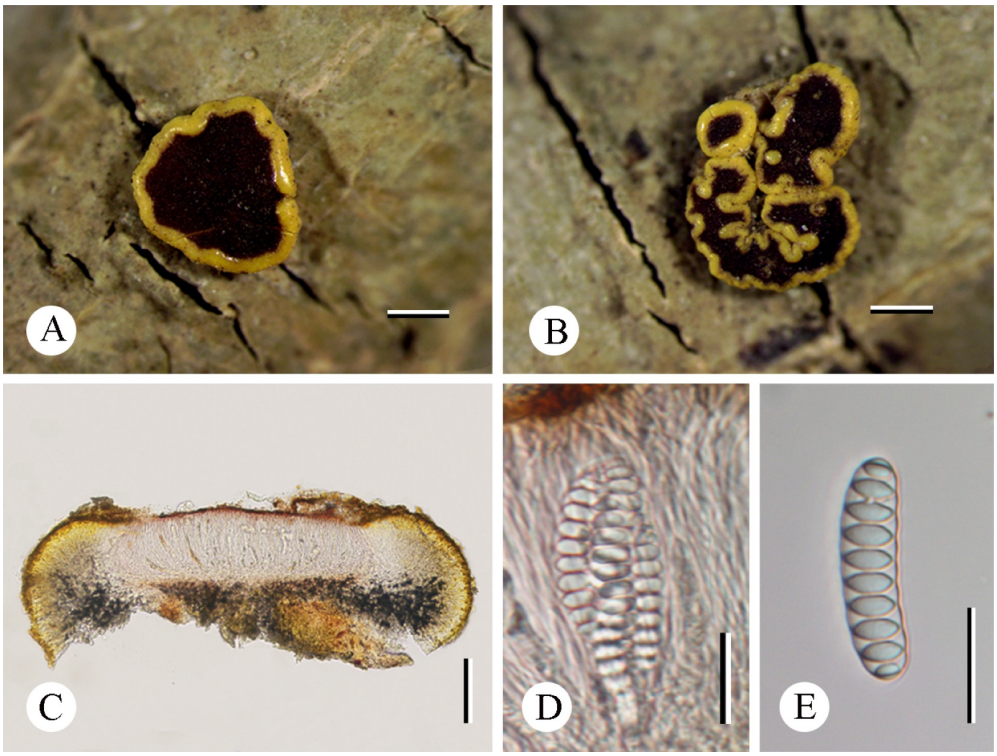

Figure 2. *Letrouitia arcuata* (holotype, YN18225): (**A**) thallus with ascoma; (**B**) thallus with ascomata; (**C**) apothecium section; (**D**) ascus with ascospores; (**E**) ascospore. Scale bars: (**A**) = 1 mm; (**B**) = 1 mm; (**C**) = 200 µm; (**D**) = 50 µm; (**E**) = 20 µm.

MycoBank: MB 852825.

Diagnosis: This species differs from *Letrouitia parabola* in having more locules and longer ascospores with a flexural shape.

Type: China. Yunnan Province: Mengla County, Rainforest Valley Xishuangbanna National Park of Tropical Rainforests, 21°54′51″ N, 101°11′28″ E, alt. 626 m, on bark, 26 January 2018, X.H. Wu YN18225 (**Holotype**, LCUF; GenBank OR395215 for ITS and OR395220 for LSU).

Etymology: The specific epithet from latin *arcuatus* refers to the arcuate ascospores. The definition of arcuate is curved like a bow.

Description: Thallus grey to greenish, smooth, crack; soredia and isidia absent. Ascomata distorted when mature, sessile, constricted at the base, 0.5–1.5 mm wide; disc reddish-brown, ±plane; margin prominent, yellow, elevated above disc; epithecium brown, 11–35 µm; hymenium hyaline, 76–142 µm; hypothecium pale brown, 35–73 µm; asci clavate, 4–6-spored, 75–80 × 25–35 µm; ascospores hyaline, flexural, transversely septate, 8–10(–12)-locular, locules lens-shaped, (28–)33–50(–62.5) × (8–)10–14.5 µm, I–. Pycnidia not seen.

Chemistry: Thallus K–, C–, KC– and P–. Disc margin K+, deep reddish brown. TLC: parietinic acid.

Ecology and distribution: Found on bark in a tropical rainforest, Yunnan, in the southwest of China. So far, this species is only known from the type locality in China, Asia.

Notes: The new species is characterized by its arcuate, transversely septate ascospores and lens-shaped locules. *Letrouitia arcuata* is easily distinguished from the three other species known in China, *L. subvulpina*, *L. parabola* and *L. transgressa*, by its transversely septate ascospores with no vertical septate (vs. muriform ascospores in *L. subvulpina* and submuriform in both *L. parabola and L. transgressa*) [5]. It differs from *L. aureola*, known in China, in that the latter has 8-spored asci and smaller ascospores of 19–27 × 5–6 µm [5]. It is similar to *L. magenta*, but the latter differs in having round and

smaller ascomata when mature (0.2–0.8 mm diam.), 6–8-spored asci and shorter ascospores sized (21–)22.5–30 × (7–)8–13 µm. It is also similar to *L. leprolyta* (Nyl.) Hafellner, but the latter differs in having short, warty or erumpent isidia, shorter ascospores (19–30 × 7–14 µm) with 6–8-locules, and the thallus and apothecia K+ purple [5].

Letrouitia sinuosa S.H. Jiang & Z.F. Jia, sp. nov., Figure 3.

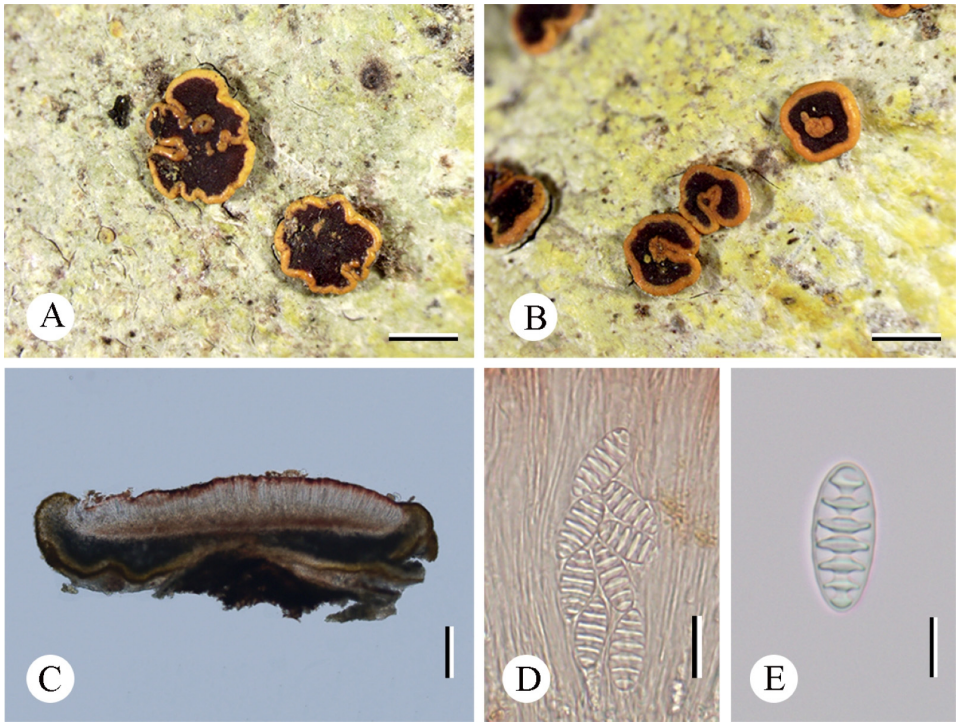

Figure 3. *Letrouitia sinuosa* (holotype, HN19607): (**A**,**B**) thallus with ascomata; (**C**) apothecium section; (**D**) ascus with ascospores; (**E**) ascospore. Scale bars: (**A**) = 1 mm; (**B**) = 1 mm; (**C**) = 200 µm; (**D**) = 20 µm; (**E**) = 10 µm.

MycoBank: MB 852828.

Diagnosis: This species differs from *Letrouitia transgressa* by distorted apothecia, smaller ascospores and lens-shaped locules at maturity.

Type: China. Hainan Province: Baoting County, Qixianling Hot Spring National Forest Park, 18°42′29″ N, 109°42′00″ E, alt. 609 m, on bark, 13 December 2019, Y.H. Ju HN19607 (**Holotype**, LCUF; GenBank OR395219 for ITS, OR395224 for LSU and OR395227 for SSU).

Etymology: The specific epithet from latin *sinuosus* refers to the wavy margin of disc.

Description: Thallus grayish yellow to grayish green, crustose, ±smooth to cracked, shiny; soredia and isidia absent. Ascomata with wavy and inward-folding margin, sessile, constricted at base, 0.5–2.5 mm diam.; disc reddish-brown, plane; margin prominent, orange, shiny, wavy and inward-folding, elevated above disc; epithecium reddish-brown, 15–35 µm; hymenium hyaline, 89–145 µm; hypothecium pale brown, 20–50 µm; asci clavate, 4–8-spored, 70–110 × 15–25 µm; ascospores hyaline, transversely septate, 6–8-locular, locules lens-shaped, no vertical septa, (18–)19.5–32(–34) × (6.5–)8–14 µm, I–. Pycnidia not seen.

Chemistry: Thallus K+ brown, C–, KC+ yellowish brown and P–. Disc margin K+, deep reddish-brown. TLC: fragilin, parietin and parietinic acid.

Ecology and distribution: Found on bark in a tropical rainforest, Hainan, in the south of China. So far, this species only known from the type locality in China, Asia.

Notes: The new species is characterized by its ascomata with a wavy and inward-folding margin, which differs from *Letrouitia magenta*. *L. sinuosa* differs from *L. subvulpina*, *L. parabola* and *L. transgressa* reported in China in that the latter have muriform or submu-

riform ascospores [5]. Although *L. sinuosa* is similar to *L. aureola in having* transversely septate ascospores, the latter differs by its narrower ascospores (19–27 × 5–6 µm) [5]. It differs from *L. arcuata* in that the latter has arcuate and larger ascospores with more locules. It resembles *L. assamana* S.Y. Kondr., G.K. Mishra & D.K. Upreti, but differs in that the latter has 4–6-spored asci, with 4(–5) spiral cells and submuriform ascospores with 1 longitudinal septum in (1–)3–4-locules [32]. It also resembles *L. muralis* Hafellner, but the latter differs in having 2–4-spored asci and submuriform ascospores, 8–10/1–4-septate [5].

Letrouitia magenta Ekanayaka & K.D. Hyde, in Ekanayaka, Jones, Zhao & Hyde, Asian Journal of Mycology 2(1): 79 (2019), Figure 4.

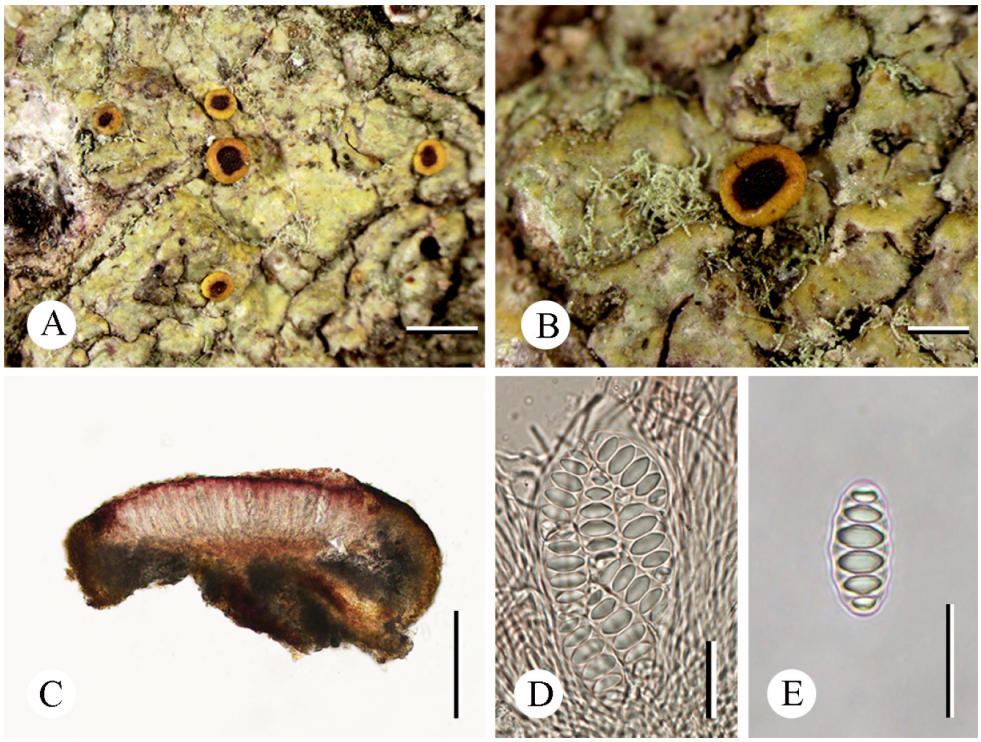

Figure 4. *Letrouitia magenta* (YN210757): (**A**) thallus with ascomata; (**B**) thallus with ascoma; (**C**) apothecium section; (**D**) ascus with ascospores; (**E**) ascospore. Scale bars: (**A**) = 1 mm; (**B**) = 0.5 mm; (**C**) = 200 µm; (**D**) = 20 µm; (**E**) = 25 µm.

Description: Thallus grey, greenish to greenish yellow, crustose, smooth, slightly shiny, crack; soredia and isidia absent. Ascomata round, sessile, constricted at base, 0.2–0.8 mm diam.; disc reddish-brown, plane to more or less convex; margin prominent, orange, elevated above disc; epithecium reddish-brown, 12–34 µm; hymenium hyaline, 72–120 µm; hypothecium pale brown, 25–42 µm; asci clavate, 6–8-spored, 60–100 × 13–30 µm; ascospores hyaline, transversely septate, 6–8 lens-shaped locules, (21–)22.5–30 × (7–)8–13 µm, I–. Pycnidia not seen.

Chemistry: Thallus K+ brown, C–, KC+ brown and P–. Disc margin K+, deep reddish-brown. TLC: fragilin, parietin and parietinic acid.

Ecology and distribution: Found on bark in tropical rainforests. Previously known only in Thailand [8] and new to China.

Specimens examined: China. Yunnan Province: Mengla County, Rainforest Valley Xishuangbanna National Park of Tropical Rainforests, 21°54′56″ N, 101°11′13″ E, alt. 640 m, on bark, 1 July 2021, X.X. He YN210757 (LCUF; GenBank OR395216 for ITS, OR395221 for LSU and OR395225 for SSU), YN210758 (LCUF; GenBank OR395217 for ITS, OR395222 for LSU and OR395226 for SSU).

Notes: The two Chinese specimens (YN210757 and YN210758) clustered with *Letrouitia magenta* from Thailand, which received a high support value (posterior probability = 1; bootstrap = 100%), as shown in Figure 1. The morphology, anatomy and chemical

characteristics of the Chinese specimens are similar to those of the type specimen [8], but the ascospores are smaller ((21–)22.5–30 × (7–)8–13 µm vs. 28–45 × 10–15 µm), and the disc colour is brown in the Chinese specimens. *L. magentas* is easily distinguished from *L. arcuata* by round and smaller ascomata when mature (0.2–0.8 mm diam.), 6–8-spored asci and shorter ascospores sized (21–)22.5–30 × (7–)8–13 µm. Although the ascospores of *L. magenta* are similar to those of *L. sinuosa*, the tree showed that they are in different clades, and the ascomata of them are different. *L. magenta* has smaller and round ascomata, whereas *L. sinuosa* has larger and distorted ascomata (0.5–2.5 mm diam.) with wavy and inward-folding margins. *L. domingensis* differs in the presence of conidia (3 × 1 µm), longer ascospores (20–40 × 10–14 µm), more locules (6–10) and K+ purple [7]. *L. aureola* differs in having 8-spored asci, narrower ascospores (19–27 × 5–6 µm) and K+ purple of the thallus [6]. *L. parabola* differs in having the thallus and apothecia K+ purple, and wider ascospores (25–35 × 12–18 µm) that are spirally septate and submuriform [5]. *L. magentas* resembles *L. muralis* with an olive-grey thallus and brown disc with an orange margin, but the latter can be distinguished by asci with 2–4 spores, ascospores submuriform at maturity and the thallus and apothecia K+ purple [5]. The ascospores of *L. magenta* are similar to those of *L. leprolytoides* S.Y. Kondr. & Elix (23–31 × 8–14 µm), but the latter has cylindrical, finger-like to coralloid-branched isidia (50–70 µm wide and 0.3–0.4 mm long), and the thallus K+ purple-violet [33].

3.3. Previously Reported Species of China

Letrouitia aureola (Tuck.) Hafellner & Bellemère, Nova Hedwigia 35 (2 & 3): 281 (1982) [1981].

≡ *Lecidea aureola* Tuck., Proc. Amer. Acad. Arts & Sci. 6: 281 (1866) [1864].

Specimens: China. Taiwan Province: Pingdong County: Kenting Forest Recreation Area, near guesthouse, alt. 200 m, 51QTE743300, on Vitex quinquefolia, 17 October 2001, Sparrius 5375 (Herb. Sparrius) [16].

Letrouitia parabola (Nyl.) R. Sant. & Hafellner, in Hafellner & Bellemère, Nova Hedwigia 35(2 & 3): 281 (1982) [1981].

≡ *Lecidea parabola* Nyl., Bull. Soc. Linn. Normandie, sér. *2* 2: 90 (1868).

Specimens: China. Taiwan Province: Pingdong County: Kenting Forest Recreation Area, near guesthouse, 200 m alt., 51QTE743300, on Ficus, 17 October 2001, Aptroot 53298 (ABL) [16].

Letrouitia subvulpina (Nyl.) Hafellner, Nova Hedwigia 35(4): 705 (1983) [1981].

≡ *Lecidea subvulpina* Nyl., Bull. Soc. Linn. Normandie, sér. 2 2: 89 (1868).

Specimens: China. Hainan Province: Baoting County, Qixianling Hot Spring National Forest Park, 18°42′29″ N, 109°42′00″ E, alt. 609 m, on bark, 13 December 2019, Y.H. Ju HN19636 (LCUF; GenBank OR395218 for ITS and OR395223 for LSU); Taiwan Province: Gaoxiong Pref., Shanping, 750 m, 6 February 1965, Kurokawa 2705 (TNS) [5].

Letrouitia transgressa (Malme) Hafellner & Bellem., in Hafellner, Nova Hedwigia 35(4): 710 (1983) [1981].

≡ *Bombyliospora domingensis* f. *transgressa* Malme, Ark. Bot. 18 (12): 5 (1923).

Specimens: China. Yunnan Province: Mengla County, Menglun Town, Rainforest Valley Xishuangbanna National Park of Tropical Rainforests, 21°55′25.1″ N, 101°15′17.3″ E, alt. 541 m, on bark of *Ficus*, 27 December 2006, a.s.l. Coll.: Hur, J.S., KoLRI-006468 (CH-060696) [17].

Additional note: *Letrouitia domingensis* was reported from China by Asahina under the name *Bombyliospora domingensis* (Pers.) Zahlbr. v. *glaucocarpa* (Nyl.) Vain. [34–36]. However, Hafellner pointed out on page 673 that *B. domingensis* reported by Asahina was not *L. domingensis* [5]. After consulting the literature and examining photographs, we found that the appearance of *B. domingensis* reported by Asahina was different from *L. domingensis* reported by Hafellner [5]. *B. domingensis* is characterized by a K– thallus, 8-spored asci, muriform ascospores, and conidia not seen, whereas *L. domingensis* is characterized by a K+ purple thallus, (6–)8-spored asci, transversely septate ascospores, and conidia present [7,34].

Therefore, *L. domingensis* is no longer found in China now, and we correct this here. A working key is provided for the Chinese species.

Key to the species of *Letrouitia* known in China

1. Ascospores submuriform or muriform....2
1. Ascospores transversely septate....4
2. Ascospores muriform; asci 1–2-spored, ascospores 25–40 × 9.5–20 µm....*L. subvulpina*
2. Ascospores submuriform....3
3. Disc orange-brown; ascospores spirally septate, locules with vertically 1–2-septate, 25–35 × 12–18 µm....*L. parabola*
3. Disc reddish-brown to brown-black; ascospores transversely septate, locules with vertically 1–3(–4)-septate, 20–52(–60) × 10–16(–22) µm....*L. transgressa*
4. Ascospores 19–27 × 5–6 µm; thallus K+ purple....*L. aureola*
4. Ascospores > 6 µm wide....5
5. Ascomata round when mature, 0.2–0.8 mm diam.; thallus grey, greenish to greenish yellow; asci 6–8-spored....*L. magenta*
5. Ascomata distorted when mature....6
6. Ascospores arcuate, (28–)33–50(–62.5) × (8–)10–14.5 µm; asci 4–6-spored; ascomata 0.5–1.5 mm diam.; disc margin yellow....*L. arcuata*
6. Ascospores straight, (18–)19.5–32(–34) × (6.5–)8–14 µm; asci 4–8-spored; ascomata 0.5–2.5 mm diam.; disc margin orange....*L. sinuosa*

4. Discussion

The chemotypes of *Letrouitia* are divided into two major groups by Johansson et al. [11]. Group 1 chemotypes contain anthraquinones, which are found in *L. aureola*, *L. bifera* (Nyl.) Hafellner, *L. corallina* (Müll. Arg.) Hafellner, *L. coralloidea* (Müll. Arg.) Hafellner, *L. domingensis*, *L. flavidula* (Tuck.) Hafellner, *L. leprolyta*, *L. muralis*, *L. parabola*, *L. pseudomuralis* Hafellner, *L. spiralis* Hafellner and *L. transgressa*. Group 2 chemotypes contain anthraquinones, dibenzofuran derivatives and/or possibly chlorodepsidones, which are found in *L. flavocrocea* (Nyl.) Hafellner & Bellem., *L. subvulpina* and *L. vulpina* (Tuck.) Hafellner & Bellem. In Figure 1, the phylogenetic tree is divided into clade A (anthraquinones, dibenzofuran derivatives and/or possibly chlorodepsidones) and clade B (anthraquinones), which is in agreement with the previous results of Johansson et al. [11]. Clade A consists of *L. subvulpina* and *L. vulpina*, which have muriform spores. Although the ascospores of *L. transgressa* and *L. parabola* are submuriform, *L. transgressa* and *L. parabola* belong to clade B. Perhaps because of their chemotypes, they differ from the other two species (*L. subvulpina* and *L. vulpina*). Clade B consists of five reported species (*L. magenta*, *L. transgressa*, *L. domingensis*, *L. parabola* and *L. flavidula*) and two new species (*L. arcuata* and *L. sinuosa*) containing only anthraquinones. Meanwhile, *L. arcuate* formed a single clade, without fragilin and parietin. Based on these facts, we speculate that there may be a relationship between the secondary chemistry and the phylogenetic evolution of *Letrouitia*. This hypothesis requires further investigation to validate.

The seven species of *Letrouitia* from China are all distributed in tropical regions, in Yunnan (Mengla), Taiwan (Pingdong and Gaoxiong) and Hainan (Baoting). Altitudes range from 200 m to 750 m, which are generally considered low or intermediate altitudes [5,14,15]. In fact, species from subtropical regions at the same level of altitude were reported as well [4–10]. The species reported from Europe were rare, possibly due to the temperate climate of Europe. Thus, the distribution pattern of *Letroitia* is more likely shaped by the climate, rather than by altitude. In tropical or subtropical regions, the abundance of *Letroitia* specimens should be higher.

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