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Chaenothecopsis xishuiensis sp. nov. to Science and *Lecanora pseudargentata* Newly Reported from China

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Abstract: In order to provide data for lichenologists studying taxonomy, *Chaenothecopsis xishuiensis* is supported and proposed as a new species from China based on phenotypic, molecular, and metabolite data. It is characterised by leprose thallus, single, conical to hemispherical apothecia, nonbranching stipe, cylindrical, eight-spored asci, and nonseptate and brown spores, and this lichenised fungus contains atranorin and zeorin in the thallus. In addition, *Lecanora pseudargentata* is reported for the first time as a new record from China. This species is characterised by red-brown to dark brown apothecial discs, eight-spored asci, nonseptate, hyaline spores, and the presence of atranorin and gangaleoidin. The biological activity of its lichen substances is discussed.

Keywords: lichenised fungi; Mycocaliciaceae; Lecanoraceae; taxonomy

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1. Introduction

Lichen is a mutually beneficial symbiotic complex of fungi and algae (or cyanobacteria) [1]. It is the pioneer of the Earth [2]. They can grow in extreme environments and adapt to their habitat mainly in two main ways: genes and metabolites. For example, some species that grow at high latitudes or altitudes have cold resistance genes, and some species that grow in deserts have drought tolerance genes, which is their genetic function. On the other hand, secondary metabolites and lichen polysaccharides have enormous medicinal properties, such as anticancer, antibacterial, antioxidant, human immunomodulatory properties, and so on. More and more values are being unlocked [3–5]. They are important resources for drug discovery and development. However, due to the characteristics of limited lichen resources and slow growth, scientists have rarely studied and exploited them, and many species are still unknown. There is a strong need for taxonomic studies on lichens to provide a basis for their further application. Therefore, we carried out taxonomic studies on some species belonging to *Chaenothecopsis* and *Lecanora* based on phenotypic, molecular, and metabolite data.

Lichenicolous fungi are highly parasitic organisms that develop on lichens [6,7]. They obtain fixed carbon from living lichens by forming haustoria [7,8]. To date, approximately 2000 obligate lichenicolous species and more than 60 facultative lichenicolous species have been identified [7]. These species are found in different classes and genera [7]. Although they have been studied for centuries, only 50 lichenicolous fungi have been reported from China, which means that many new species collected in China are waiting to be discovered [6].

Chaenothecopsis Vain., belonging to Sphinctrinaceae, Mycocaliciales, Mycocaliciomycetidae, Eurotiomycetes, Ascomycota, was established by Vainio in 1927 and has a cosmopolitan distribution [9–13]. The genus is characterised by the sessile or stalked ascomata, mostly obovoid to lenticular capitulum, cylindrical to subclavate, less than 60 µm long, and eight-spored asci with strongly thickened apex penetrated by a thin channel, and nonseptate or

one-septate, nearly hyaline to brown spores [9,14]. A number of *Chaenothecopsis* species are lichenicolous fungi, growing as parasites or commensals on lichens, or lichenised fungi, while others are parasitic on algae, conifer resins, or exudates of vascular plants. And many species are highly specialised, producing ascomata only on specific substrates [3,9,14,15]. More than 50 species of *Chaenothecopsis* have been reported worldwide, 20 of which were found in China prior to this report [10,16,17].

Lecanora Ach. is a genus of lichenised fungi belonging to Lecanoraceae, Lecanorales, Lecanoromycetes, Ascomycota [10,18] with a worldwide distribution [19]. The genus is characterised by its crustose to scaly shell-shaped thallus, lecanorine or biatorine ascomata, lecanora-type and 8–32-spored asci, nonseptate and hyaline spores, the presence of atranorin and usnic acid, and green algae as photobionts [19–22], growing on bark, rock, wood, soil or detritus [19]. *Lecanora* comprises about 1000 species worldwide [18,19,23]. Previously, 119 species were known from China [24–26]. However, due to the large variation in morphological and chemical characteristics of the genus, the traditional concept of *Lecanora* corresponds to blurred species boundaries [22,27,28]. Since the 20th century, the concept of this genus has been somewhat stabilised by the introduction of molecular data [22,28–33]. The importance of combining morphological, chemical, and molecular data to delineate species within the genus has been highlighted [22,28,34]. In 2023, Santos et al. confirmed that the phylogenetic tree constructed from ITS sequences was a good method for classifying the species in the genus *Lecanora* [34].

2. Materials and Methods

2.1. Materials Specimens

Three *Chaenothecopsis* specimens were collected from Xishui National Nature Reserve, and one *Lecanora* specimen was collected from Sanqing Mountain. All the specimens are deposited in the Fungarium of the College of Life Sciences, Liaocheng University, China (LCUF).

2.2. Morphological Study

The external morphology of the thallus and apothecia was observed and measured under a dissecting microscope (OLYMPUS SZX16) while the characteristics and data were recorded. The observations included the growth type, colour and texture of the thallus, the shape, colour, size, mode of attachment, and disc situation of the apothecia.

2.3. Anatomical Study

Apothecia were cut longitudinally and filmed under a dissecting microscope (OLYMPUS SZX16), and their internal structures were later observed under a compound microscope (OLYMPUS BX53). The operation steps are as follows:

1. Moisten the selected apothecia: Apply an appropriate amount of sterile water to the well-developed apothecia with a rubber-tipped dropper and remove the ascospores after the apothecia have absorbed the water and become soft.
2. Slice: Using a single-sided blade, cut the selected apothecia longitudinally so that the slices are as thin and complete as possible.
3. Production: Pick up the section with the tip of a needle on a slide moistened with sterile water, cover it with a coverslip and absorb excess water with absorbent paper.
4. Observation and recording: Observe the internal structure of the apothecia under a compound microscope (OLYMPUS BX53), including the colour and thickness of exciple; the colour and thickness of epithecium, hymenium, and hypothecium; the shape, size of asci, and the number of ascospores contained in them; the colour, size, and type of ascospores. Take photographs and record the relevant information.
5. Colour development: Stain the prepared mount with iodine solution and observe the amyloplastic reflection of the ascospores.

2.4. Methods for the Determination of Lichen Secondary Metabolites

The metabolites of lichens were determined using chemical chromogenic reaction assays (CT) and thin layer chromatography (TLC) assays [3,4,35–37].

For CT, aqueous solutions of 10–25% potassium hydroxide (K), saturated aqueous solution of calcium hypochlorite (C), and 4% ethanolic solution of p-phenylenediamine (P) reagents were added dropwise to the cortex of the thallus using capillary tubes, and the colour reactions were observed and recorded.

TLC was performed according to the method of Culberson and Kristinsson, slightly modified by Jia and Wei, using *Lethariella cladonioides* (Nyl.) Krog as the standard sample and the C-solvent system (toluene:acetic acid = 200:30 mL) as the spreading agent [3,4,35–37]. The operation steps are as follows:

1. Prepare the glass silicone adhesive board. Use a precoated glass silicone backing sheet (20 cm long, 10 cm wide, 0.25 mm thick). Using a pencil, carefully draw a straight line 1.5 cm from the bottom of the glass silicone board. Mark a point every 1 cm on the straight line, which will be the sample point.
2. Prepare the solvent. Mix 20 mL of toluene and 3 mL of acetic acid, add to a rectangular TLC developing tank, and place in a fume cupboard.
3. Prepare the samples. Take an appropriate quality of the thallus to be examined and place them separately in small centrifuge tubes. Add a suitable volume of acetone to each small centrifuge tube until the acetone covers the sample. After 10 min, the samples can be placed in order.
4. Spot sampling: Use microcapillary tubes to sample separately according to the position of the sampling points on the glass silicone board. The left, right, and centre sampling points are brushed with *Lethariella cladonioides* to facilitate the use of split standard samples, while the remaining sampling points are sampled sequentially for testing.
5. Exposure layer: After sampling, place the silicone board in a chromatography cylinder and place it 1 cm below the solvent level so that the sample origin is approximately 0.5 cm from the solvent level and make the origin line parallel to the liquid level line. When the leading edge of the solvent moves from the origin to about 1.5 cm from the top of the silicone board, remove the silicone board and dry the solvent on the board surface with a hair dryer.
6. Colour rendering: Spray the silicone board with 10% sulphuric acid and observe if there are any grease spots when it is wet. Then, heat it in an oven at 94 °C for about 10–15 min until the chromatography develops well. Observe and record the colour and position of the spots under white and ultraviolet light, respectively.
7. Partition: Draw tangents at one point above and one point below the chromatographic origin for colour display. The area between the top and bottom tangents is the first zone. Using the same method, draw the fourth and seventh zones of the atranorin and norstictic acid stains, respectively. Then, draw a median line between the first and fourth zones, dividing them equally into the second and third zones; draw the fifth and sixth zones between the fourth and seventh zones using the same method.
8. Identify metabolites with reference to the information on the chromatography of lichen metabolites presented by Culberson 1972 and Orange et al. 2001 [35–37].

2.5. DNA Extraction, PCR Amplification, and Sequencing

Collect some apothecia and thallus into 1.5 mL centrifuge tubes using a sterilised blade and tweezers. DNA was extracted using the Hi-DNAsecure Plant Kit according to the procedure in the slightly modified instructions, as follows:

1. Collect apothecia and thallus in microcentrifuge tubes and add beads for thorough grinding. Add 400 µL FGA buffer and 6 µL RNaseA (10 mg/mL), vortex for 1 min, and leave for 10 min at room temperature.
2. Add 130 µL LP2 buffer, mix thoroughly and vortex for 1 min.

3. Centrifuge at 12,000 rpm (~13,400× *g*) for 5 min and transfer 300 µL of supernatant to a new tube.
4. Add 1.5 times the volume of buffer LP3 and immediately vortex for 15 s.
5. Add a total of 750 µL of the solution obtained in the previous step and the flocculated precipitate to an adsorbent column CB3, centrifuge at 12,000 rpm (~13,400× *g*) for 30 s, pour off the waste solution and place the column CB3 in a collection tube.
6. Add 600 µL of rinsing solution PW to column CB3, centrifuge at 12,000 rpm (~13,400× *g*) for 30 s, pour off the waste solution, and place column CB3 in the collection tube. Repeat this step twice.
7. Place the column CB3 back into the collection tube and centrifuge at 12,000 rpm (~13,400× *g*) for 2 min, discarding the waste solution. Leave the column CB3 at room temperature for a few minutes to thoroughly dry the residual rinse from the adsorbent material.
8. Transfer the adsorbent column CB3 to a clean centrifuge tube, add 90 µL of elution buffer TB dropwise to the centre of the adsorbent membrane in suspension, allow to stand at room temperature for 2–5 min, centrifuge at 12,000 rpm (~13,400× *g*) for 2 min, and collect the solution in the centrifuge tube. Aspirate the solution from the centrifuge tube, drop it back onto the centre of the adsorbent membrane, leave at room temperature for 2–5 min, centrifuge at 12,000 rpm (~13,400× *g*) for 2 min, and collect the solution in a centrifuge tube. This solution contains the extracted genomic DNA.

The internal transcribed spacer (ITS) region of ribosomal DNA was amplified using ITS1F/ITS4 primers [38,39]. Polymerase chain reactions (PCR) were reacted in a volume of 50 µL mixture containing 25 µL 2 × PCR Master Mix, 17 µL ddH_2O, 2 µL of each primer, and 4 µL DNA template. PCR thermal cycles were performed with the following reaction conditions: initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, extension at 72 °C for 1.5 min, and a final extension at 72 °C for 10 min. The PCR products were purified and sequenced at Biosune Biotechnology Company (Jinan, China).

2.6. Alignment and Phylogenetic Tree Construction

ITS sequences were generated from the *Chaenothecopsis* specimen and *Lecanora* specimen. According to the results of BLAST on the NCBI website (https://www.ncbi.nlm.nih.gov/ (accessed on 27 April 2023)) and related research, other similar taxa sequences from different genera of Sphinctrinaceae were downloaded from GenBank (Table 1) [19,40,41]. *Talaromyces acaricola* Visagie, N. Yilmaz & K. Jacobs and *Protoparmelia ochrococca* (Nyl.) P.M. Jørg., Rambold & Hertel were used as outgroups, respectively. The selected ITS sequences, together with newly generated sequences, were aligned in BioEdit using the ClustalW method [42,43].

Phylogenetic relationships were inferred using maximum likelihood (ML) and Bayesian inference (BI) on the CIPRES Scientific Gateway portal (http://www.phylo.org/ (accessed on 24 April 2023)). ML analyses were performed using RAxML-HPC2 on XSEDE (8.2.12) with 1000 replicates as bootstrap analysis. BI analyses were performed using MrBayes on XSEDE (3.2.7a) based on the GTR+I+G model, with 2 independent analysis runs for 1 million generations. Each run included four chains, parameters were sampled every 1000 generations and 25% were discarded as burn-in. The remaining 75% were used to calculate the consensus tree [40,44–46]. Bootstrap support above 70% and posterior probabilities above 0.9 were considered significant support values. Trees were visualised in FigTree v.1.4.2 (http://tree.bio.ed.ac.uk/software/figtree (accessed on 27 April 2023)) and MEGA11 [47]. Phylogenetic trees are shown in Figures 1 and 2.

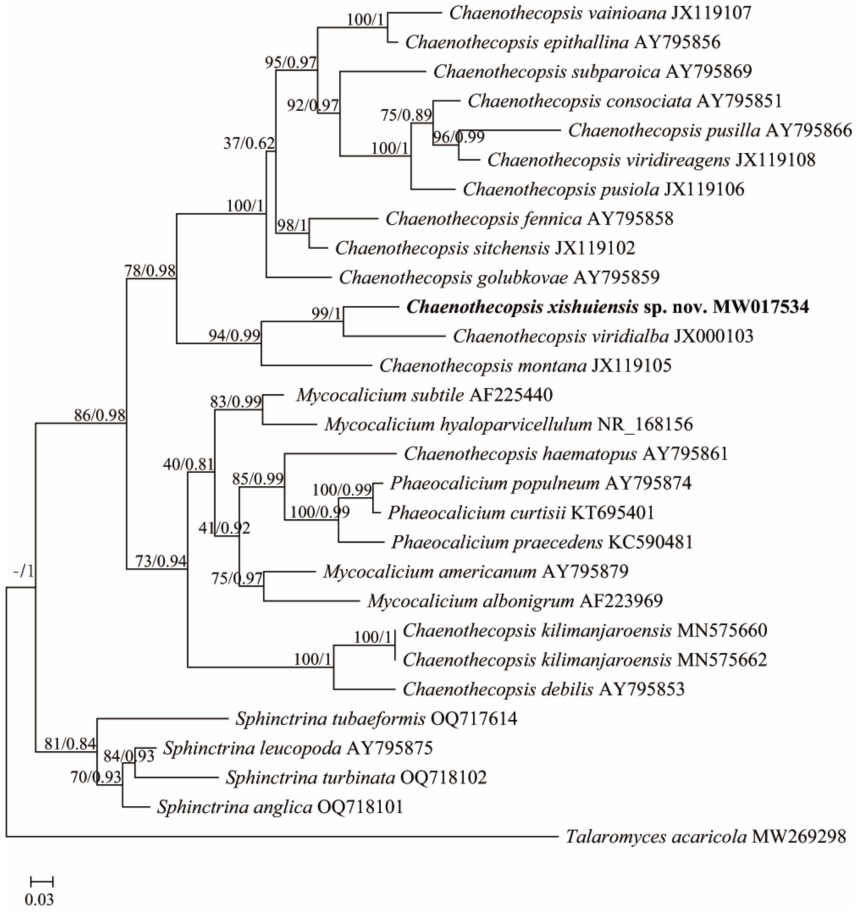

Figure 1. Maximum likelihood tree of *Sphinctrinaceae* s. str. based ITS sequences with *Talaromyces acaricola* as outgroup. ML-BS > 70% (**left**) and BI-PP > 0.9 (**right**) are considered to be strongly supported. Terminal in bold indicates newly generated sequence.

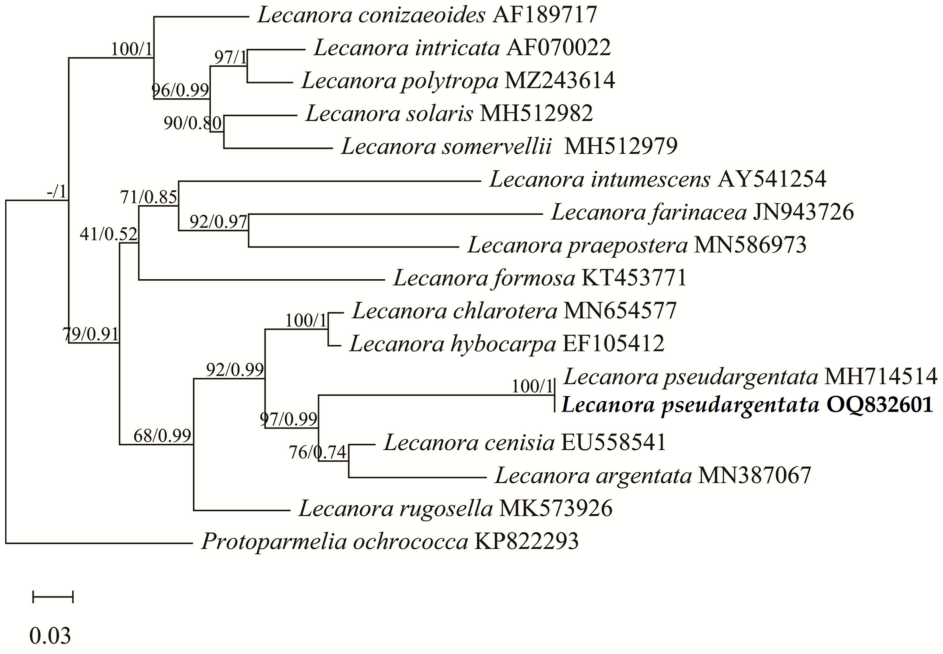

Figure 2. Maximum likelihood tree of *Lecanora* based on ITS sequences with *Protoparmelia ochrococca* as outgroup. ML-BS > 70% (**left**) and BI-PP > 0.9 (**right**) are considered to be strongly supported. Terminal in bold indicates newly generated sequence.

Table 1. Taxa and their GenBank accession numbers used to construct phylogenetic trees. Information on new species and records are indicated in bold.

Species	GenBank Accession Numbers
Chaenothecopsis consociata (Nádv.) A.F.W. Schmidt	AY795851
Chaenothecopsis debilis (Sm.) Tibell	AY795853
Chaenothecopsis epithallina Tibell	AY795856
Chaenothecopsis fennica (Laurila) Tibell	AY795858
Chaenothecopsis golubkovae Tibell & Titov	AY795859
Chaenothecopsis haematopus Tibell	AY795861
Chaenothecopsis kilimanjaroensis Temu & Tibell	MN575660
Chaenothecopsis kilimanjaroensis Temu & Tibell	MN575662
Chaenothecopsis montana Rikkinen	JX119105
Chaenothecopsis pusilla (Ach.) A.F.W. Schmidt	AY795866
Chaenothecopsis pusiola (Ach.) Vain.	JX119106
Chaenothecopsis sitchensis Rikkinen	JX119102
Chaenothecopsis subparoica (Nyl.) Tibell	AY795869
Chaenothecopsis vainioana (Nádv.) Tibell	JX119107
Chaenothecopsis viridialba (Kremp.) A.F.W. Schmidt	JX000103
Chaenothecopsis viridireagens (Nádv.) A.F.W. Schmidt	JX119108
Mycocalicium albonigrum (Nyl.) Fink	AF223969
Mycocalicium americanum (R. Sant.) Tibell	AY795879
Mycocalicium hyaloparvicellulum Daranag. & K.D. Hyde	NR_168156
Mycocalicium subtile (Pers.) Szatala	AF225440
Phaeocalicium curtisii (Tuck.) Tibell	KT695401
Phaeocalicium populneum (Brond. ex Duby) A.F.W. Schmidt	AY795874
Phaeocalicium praecedens (Nyl.) A.F.W. Schmidt	KC590481
Sphinctrina anglica Nyl.	OQ718101
Sphinctrina leucopoda Nyl.	AY795875
Sphinctrina tubaeformis A. Massal.	OQ717614
Sphinctrina turbinata (Pers.) De Not.	OQ718102
***Chaenothecopsis xishuiensis* Z.F. Jia**	**MW017534**
Talaromyces acaricola Visagie, N. Yilmaz & K. Jacobs	MW269298
Lecanora argentata (Ach.) Malme	MN387067
Lecanora cenisia Ach.	EU558541
Lecanora chlarotera Nyl.	MN654577
Lecanora conizaeoides Nyl. ex Cromb.	AF189717
Lecanora farinacea Fée	JN943726
Lecanora formosa (Bagl. & Carestia) Knoph & Leuckert	KT453771
Lecanora hybocarpa (Tuck.) Brodo	EF105412
Lecanora intricata (Ach.) Ach.	AF070022
Lecanora intumescens (Rebent.) Rabenh.	AY541254
Lecanora somervellii Paulson	MH512979
Lecanora praepostera Nyl.	MN586973
***Lecanora pseudargentata* Lumbsch**	**OQ832601**
Lecanora pseudargentata Lumbsch	MH714514
Lecanora rugosella Zahlbr.	MK573926
Lecanora solaris L.S. Yakovchenko & E.A. Davydov	MH512982
Lecanora polytropa (Ehrh.) Rabenh.	MZ243614
Protoparmelia ochrococca (Nyl.) P.M. Jørg., Rambold & Hertel	KP822293

3. Results

3.1. Phylogenetic Analysis

The *Sphinctrinaceae* s. str. phylogenetic tree dataset included 1 newly generated ITS sequence submitted to GenBank under accession number MW017534, plus 28 sequences downloaded from GenBank (Table 1). The *Lecanora* phylogenetic tree data set included 1 newly generated ITS sequence submitted to GenBank under accession number OQ832601, plus 16 sequences downloaded from GenBank (Table 1). The *Sphinctrinaceae* s. str. dataset and *Lecanora* dataset were used to construct phylogenetic trees, respectively. Since the topologies of the maximum likelihood tree and Bayesian Inference tree are congruent,

maximum likelihood bootstrap probabilities (ML-BS) and Bayesian inference posterior probabilities (BI-PP) are combined and placed at the node of the maximum likelihood tree. ML-BS are on the left, and BI-PP are on the right. The results are shown in Figures 1 and 2, respectively.

Within the *Sphinctrinaceae* s. str. phylogenetic tree, the newly offered *Chaenothecopsis* specimen forms a sister group with *Chaenothecopsis viridialba* (Kremp.) in an independent clade with other species (Figure 1). Based on the differences in phylogeny and morphology compared to other species, which are described in detail below, it is classified as a new species named *Chaenothecopsis xishuiensis*.

In Figure 2, the ITS sequences of the newly offered *Lecanora* specimen and *Lecanora pseudargentata* Lumbsch downloaded from GenBank are clustered together in the phylogenetic tree. Combined with the morphological similarity, we identified this specimen as *Lecanora pseudargentata*, which is a new record in China.

3.2. Taxonomy

Chaenothecopsis xishuiensis Z.F. Jia, sp. nov., Figure 3.

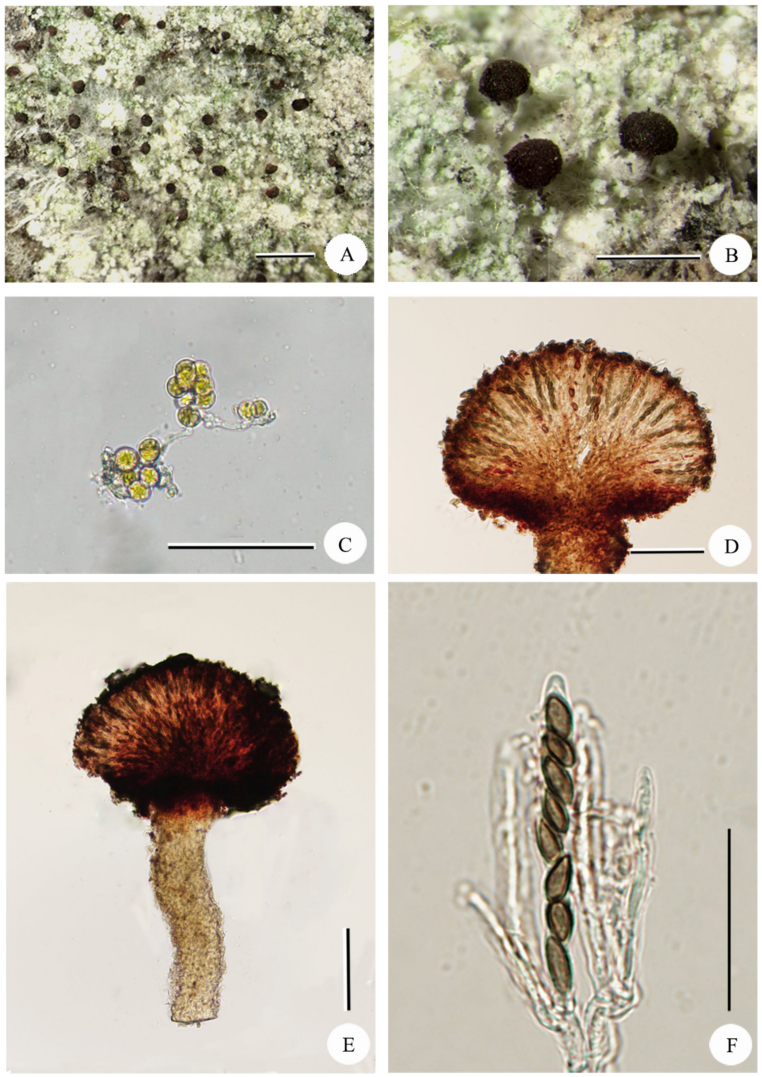

Figure 3. *Chaenothecopsis xishuiensis* (holotype, M.L. Zhu GZ19513 LCUF). (**A**) Thallus and ascomata; (**B**) thallus and ascomata; (**C**) mycelia and algal cells of thallus; (**D**) cross section of ascomata; (**E**) cross section of ascomata and stalk; (**F**) an ascus containing ascospores. Scales: (**A**) = 1.5 mm; (**B**) = 0.5 mm; (**C**) = 50 μm; (**D**) = 50 μm; (**E**) = 100 μm; (**F**) = 20 μm.

It differs from *Chaenothecopsis kilimanjaroensis* mainly by its single apothecia, wider and nonseptate ascospores.

TYPE: China. Guizhou: Zunyi City, Xishui County, Sanchahe Town, Chinese Danxia Valley, 28°33′ N 106°24′ E, alt. 1040 m, on bark, 17/XI/2019, M. L. Zhu GZ19513 (LCUF, holotype).

MycoBank No: 848ht5588

ETYMOLOGY: The specific epithet *xishuiensis* is derived from the type locality, *xishui*, and *–ensis*, Latin.

THALLUS: corticolous, leprose, irregular, and its surface is whitish-green to yellowish-green.

APOTHECIA: brownish black, single, stalked, conical to flattened flabelliform to hemispherical, 0.1–0.25 mm in diameter, epruinose; STIPE: brown, with pruina, nonbranching, 0.2–0.3 mm high, 31–77 µm wide, K−, in section the central part composed of irregularly intertwined hyphae; STIPE HYPHAE: intertwined, elongated, hyaline to lightly brown, 2.2–4.4 µm wide. EXCIPLE: dark brown, 20–23 µm thick; EPITHECIUM: dark brown, 10–12 µm tall; HYMENIUM: 52–65 µm tall, hyaline to brownish, oil droplets absent, K+ yellowish green, I–; PARAPHYSES: hyaline, filiform; HYPOTHECIUM: brown, convex, 33.5–43.5 µm tall. ASCI: cylindrical with a thickened apex penetrated by a fine canal, 39–45 × 5.6–7.3 µm, eight-spored. ASCOSPORES: uniseriate, brown, fusiform, nonseptate, 6.5–8 × 2.9–4.5 µm, I–. PYCNIDIA not observed.

CHEMISTRY: Thallus K+ yellow, C−, P+ blue-grey. Atranorin and zeorin were detected in the thallus by TLC.

ECOLOGY AND DISTRIBUTION: on bark in forests and are only known from the type locality.

ADDITIONAL SPECIMEN EXAMINED: China. Guizhou: Zunyi City, Xishui County, Sanchahe Town, Chinese Danxia Valley, alt. 1040 m, on bark, 17/XI/2019, M. L. Zhu GZ19514, GZ19515 (LCUF).

REMARKS: *Chaenothecopsis xishuiensis* is characterised by leprose thallus, single, conical to hemispherical apothecia, nonbranching stipe, cylindrical, eight-spored asci, nonseptate and brown spores, and it is a lichenised fungus containing atranorin and zeorin in the thallus.

The closest species phylogenetically are *Chaenothecopsis viridialba* and *Chaenothecopsis montana* (Figure 1). However, these species are morphologically clearly different. *C. viridialba* has whitish pruina or granules on longer-stalked (2–3 mm) apothecia and ellipsoid spores (6–10 × 2.5–3.5 µm) [9,48], whereas *C. montana* has narrower asci (3.5–5.5 mm), ellipsoid to ovoid ascospores, and all parts of apothecium K− [49].

Morphologically, the new species is similar to *Chaenothecopsis kilimanjaroensis* Temu & Tibell and *Chaenothecopsis pusiola* (Ach.) Vain., but the latter two are far from the new species in the phylogenetic tree. Moreover, *C. kilimanjaroensis* has 2–5 or single aggregated capitula, one-septate, and narrower ascospores (2.1–2.6 µm wide) [41]; *C. pusiola* has black and higher stalks (0.2–0.6 mm), one-septate spores, and lichenicolous on species of *Chaenotheca* (Th. Fr.) Th. Fr. [14].

As some species of *Chaenothecopsis* are described based on phenotype only, no sequence data are available. Therefore, we compare the new species with other available phenotypic information in the *Chaenothecopsis* genus species, with the result that the similar species are *C. ochroleuca* and *C. ussuriensis*, which share the short stalks and small, simple ascospores. But *C. ochroleuca* is distinguished from the new species by its larger apothecia (0.2–0.6 mm), longer asci (43–47 µm), black stalks with thick white pruina, and stalks are KOH+ red and green simultaneously [14,50–52]. *C. ussuriensis* is distinguished from the new species by its larger apothecia (0.34– 0.52 mm), smaller asci (30.8–33.7 × 3.0–3.9 µm), shorter ellipsoidal spores with rounded apices (4.0–4.8 × 2.3–3 µm) [53].

Lecanora pseudargentata Lumbsch, J. Hattori Bot. Lab. 77: 127 (1994)., Figure 4.

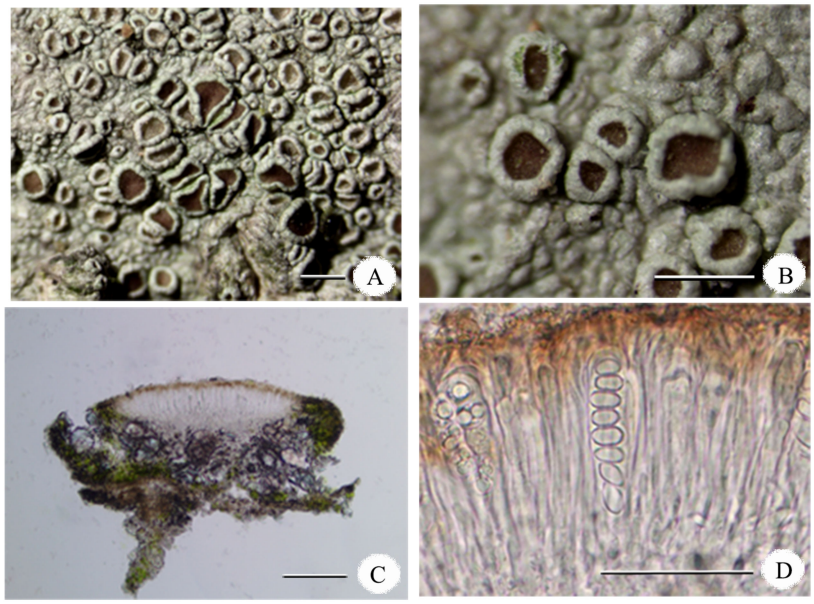

Figure 4. *Lecanora pseudargentata* (M. Li JX19079 LCUF). (**A**) Thallus with apothecia; (**B**) apothecia; (**C**) cross section of apothecium; (**D**) an ascus containing ascospores. Scales: (**A**) = 1 mm; (**B**) = 1 mm; (**C**) = 50 μm; (**D**) = 100 μm.

THALLUS: corticolous, crustose, thin, yellowish white to whitish grey surface, continuous to verrucose.

APOTHECIA: lecanorine, sessile, and constricted at base, 0.3–1.0 mm diameter, margins concolorous with thallus, entire, verrucose; DISCS: red-brown to dark brown, epruinose to slightly pruinose; EXCIPLE: 50–98 μm thick; EPITHECIUM: red-brown, 15–29 μm tall, with granules; HYMENIUM: hyaline, 80–132 μm tall, I+ blue; HYPOTHECIUM: hyaline to pale grey, 27–62 μm tall. ASCI: cylindrical with amyloid apex, 35–55 × 10–15 μm, eight-spored. ASCOSPORES: ellipsoid, 7.5–10.5 × 4.5–7 μm, I–.

CHEMISTRY: Thallus K+ yellow, C−, P−. It contains atranorin, gangaleoidin, chloroatranorin, and norgangaleoidin.

In addition to *Lecanora pseudoargentata*, atranorin, gangaleoidin, chloroatranorin, and norgongaleoidin have also been isolated from various other lichen species and have been shown to have different biological activities [3,54]. For example, atranorin has been reported to have antimicrobial, antioxidant, anti-inflammatory, and anticancer properties [3]. It is also used in traditional medicine to treat skin conditions, wounds, and inflammation and is being investigated for its potential as a natural food and cosmetic preservative [55]. Gangaleoidin is a scavenger of hydroxyl and superoxide anion radicals. It has therefore been investigated for its potential as an anti-inflammatory and antioxidant agent [56]. It has also been shown to have antifungal and antibacterial activities. Chloroatranorin has been shown to have antibacterial activity against various bacteria and fungi [57]. It has also been investigated for its potential as a natural food preservative and anti-inflammatory agent. However, there are no clear reports on the specific bioactive function of norgangaleoidin. And it is worth noting that although some compounds have shown promising results in some studies, more research is needed to fully understand the potential uses and limitations of the lichen metabolites found and being discovered.

SUBSTRATE: Bark.

ECOLOGY AND DISTRIBUTION: Pantropical area [21] including Thailand, Argentina, Australia, Brazil, Colombia, Costa Rica, Paraguay, Puerto Rico, America, Uruguay, and Venezuela [21,58]. New record to China.

SPECIMEN EXAMINED: China. Jiangxi: Shangrao City, Yushan county, Entrance to Sanqing Mountain, alt. 395 m, on bark, 17/III/2019, M. Li JX19079 (LCUF).

REMARKS: *Lecanora pseudargentata* is characterised by its crustose, yellowish white to whitish grey thallus, lecanorine ascomata, red-brown to dark brown discs, eight-spored asci, nonseptate and hyaline spores, and the presence of atranorin and gangaleoidin. The species is similar to *Lecanora argentata* (Ach.) Röhl. and *Lecanora subrugosa* Nyl., but *L. argentata* differs in having nongranular epithecium, larger ascospores (11–15 × 6.5–9 µm), and the absence of atranorin [21,58,59], and *L. subrugosa* has epruinose discs, nongranular epithecium, and atranorin is a constant component in *Lecanora* s. str. and also present in *L. argentata*, which does not contain gangaleoidin, chloroatranorin, and norgangaleoidin [60].

4. Discussion

In Figure 1, the phylogenetic analysis dataset includes 29 ITS sequences representing four relative genera and an outgroup of *Talaromyces*, which is based on 28 species from 29 specimens. The phylogenetic results showed that two individuals of *Chaenothecopsis kilimanjaroensis* were strongly supported as monophyletic (ML = 100% PP = 1); the other species were not grouped with any other species in the tree. The genus of *Chaenothecopsis* was resolved as polyphyletic, and the genera of *Phaeocalicium* and *Sphinctrina* were resolved as monophyletic clades, which is consistent with the previous findings of Thiyagaraja et al. [13]. In addition, the results showed that the genus *Mycocalicium* is not a monophyletic lineage, which is consistent with the results of Tuovila et al. and Rikkinen et al. [17,40]. The ITS phylogeny revealed the new species located in a well-supported clade of *Chaenothecopsis* s. str. (ML = 78% PP = 0.98) (*C. vainioana*, *C. epithallina*, *C. subparoica*, *C. consociate*, *C. pusilla*, *C. viridireagens*, *C. pusiola*, *C. fennica*, *C. sitchensis*, *C. golubkovae*, *C. xishuiensis*, *C. viridialba*, and *C. montana*). Although blast searches of ITS sequences indicated that *C. xishuiensis* has close affinities with *C. montana* (83% identity) and *C. viridialba* (87% identity), the phylogenetic analyses strongly support that *C. xishuiensis* is not grouped with these two species in the phylogenetic tree. Although *C. xishuiensis* occupies a close position to *C. viridialba* and *C. montana*, which three together form a supported phylogenetic clade (BS = 94%, PP = 0.99), the phylogenetic analyses strongly support that *C. xishuiensis* is separated from two species in the tree, appearing as sister to *C. viridialba* with a high support value (BS = 99%, PP = 1) and in a completely different clade from *C. montana* (Figure 1). Moreover, morphologically *C. viridialba* and *C. montana* differ from *C. xishuiensis* in that *C. viridialba* has whitish pruina or granules on longer-stalked (2–3 mm) apothecia and ellipsoid spores (6–10 × 2. 5–3.5 µm) [9,48], whereas *C. montana* has narrower asci (3.5–5.5 mm), ellipsoid to ovoid ascospores, and all parts of the apothecium K− [49]. We, therefore, describe it as a new species of *C. xishuiensis*, which is characterised by leprose thallus, single, conical to hemispherical apothecia, nonbranching stipe, cylindrical, eight-spored asci, nonseptate and brown spores, and it is a lichenised fungus containing atranorin and zeorin in the thallus.

Within the *Lecanora* phylogenetic tree (Figure 2), the molecular phylogeny based on the 16 ITS of *Lecanora* shows a well-supported monophyletic lineage with *Protoparmelia ochrococca* as an outgroup. The tree shows that the specimens collected in China and South Africa are monophyletic with a high support value (BS = 100%, PP = 1.0). This result confirms the hypothesis that the specimen belongs to *L. pseudargentata* on the basis of obvious phenotypic and metabolic characteristics, where *L. pseudargentata* is characterised by its crustose, yellowish white to whitish grey thallus, lecanorine ascomata, red-brown to dark brown discs, eight-spored asci, nonseptate and hyaline spores, and the presence of atranorin and gangaleoidin [21,58]. Phylogenetically *L. pseudargentata* is closely related to the species *L. cenisia* and *L. argentata*, together with *L. cenisi* and *L. argentata* forming a lineage with good support (BS = 97%, PP = 0.99), in which *L. pseudargentata* is revealed as sister to the clade consisting of *L. cenisia* and *L. argentata*. In addition, *L. cenisia* differs in epruinose thallus, 0.5–2 (2.5) mm apothecia, and the absence of gangaleoidin. *L. argentata* differs in having nongranular epithecium, larger ascospores (11–15 × 6.5–9 µm), and the absence of atranorin [21,58,59].

In this paper, our results suggest that *Chaenothecopsis xishuiensis* is a new species to science, and *Lecanora pseudargentata* is a new record for China based on phenotypic,

molecular, and metabolite data. We have provided a detailed description and discussion of the *Chaenothecopsis xishuiensis* and *Lecanora pseudargentata*, together with photos of external morphology and internal anatomical features, and provided phylogenetic trees constructed from ITS sequences of some species. We also provided data for lichen classification research. However, we have only roughly identified lichen secondary metabolites and have not further investigated their medicinal value. Next, we plan to conduct a meta-analysis of reported articles on the medicinal value of lichens, screen compounds with high reliability of application value, and conduct preclinical studies to provide data to support their clinical applications.

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