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Three new species of *Herpothallon* (Lichenized *Ascomycota*) from Southern China

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Abstract

This paper describes three species of *Herpothallon* new to science from southern China: *H. glaucescens*, *H. lilacinum* and *H. tomentosum*. The three species all possess a non-pigmented thallus, hypothallus and prothallus. *Herpothallon glaucescens* has a white, whitish grey to greyish green thallus and swollen, subglobose to ± vermiform pseudisidia, rounded at the top. *Herpothallon lilacinum* has subglobose or irregularly cushion-shaped, fluffy-felty pseudisidia, white at the base, lilac to lilac grey at their tips. *Herpothallon tomentosum* has globular pseudisidia, felty with many projecting hyphae, sometimes containing a central pycnidium. Detailed descriptions for all three new species are provided with an updated key to the genus *Herpothallon* in China. Additionally, a phylogenetic tree based on Bayesian and ML analyses of mtSSU data shows the position of the new species in *Herpothallon*.

Keywords: Arthoniaceae, byssoid thalli, sterile lichens, taxonomy, mtSSU

Introduction

Herpothallon Tobler (1937: 446) is a widespread genus in tropical and subtropical regions, growing mostly in humid and sheltered habitats within forests. The lichen genus is recognized by the usually loosely appressed thallus with a byssoid hypothallus and prothallus that is often a different colour compared to the upper, algal-containing part of the thallus; pseudisidia or pseudisidioid structures are typically present, and the different species contain a diverse array of lichen secondary metabolites (Aptroot *et al*. 2009). The genus was first established by Tobler (1937), subsequently treated as a synonym of *Cryptothecia* Stirton (1876 [1877]: 164), but reinstated by Aptroot *et al*. (2009), recognizing 29 species worldwide. So far approximately 50 species are known world-wide, eight have been reported from China (Jagadeesh Ram *et al*. 2009; Jagadeesh Ram & Sinha 2009; Frisch *et al*. 2010; Jagadeesh Ram & Sinha 2011; Cheng *et al*. 2012; Bungartz *et al*. 2013; Frisch *et al*. 2014; Jagadeesh Ram 2014; Aptroot *et al*. 2017; Sipman 2018; Aptroot & Souza 2021; Chen *et al*. 2022; Lendemer & James C. 2022). Almost all *Herpothallon* species are sterile (only *H. fertile* Aptroot & Lücking (2009: 40) and *H. inopinatum* Frisch & G. Thor (2014: 64) are known to produce ascospores), therefore species boundaries within the genus are mainly based on morphological and chemical characteristics (Frisch *et al*. 2014).

Due to its ecological and climatic preferences, the genus *Herpothallon* presents its highest diversity in tropical and subtropical regions. Here we focused on lichen surveys in Southern China, which is characterized by a subtropical and tropical, warm and humid monsoon climate. During our survey, three new species were found and are now described, using mitochondrial small subunit (mtSSU) rDNA data, to also construct a phylogenetic tree showing the position of these new species, supporting the delimitation of the new taxa.

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Material & methods

Investigation of lichen specimens:—The specimens studied were collected in Fujian, Guizhou, Zhejiang Provinces and Guangxi Zhuang Autonomous Region, China, and are preserved in the Lichen Section of the Botanical Herbarium, Shandong Normal University, Jinan, China (SDNU).

Morphology and Chemistry:—The morphological and anatomical characters were examined under a stereomicroscope (COIC XTL7045B2) and a polarizing compound microscope (Olympus CX41). For identification thallus and medulla were tested with the spot test reagents K (a 10% aqueous solution of potassium hydroxide), C (a saturated solution of aqueous sodium hypochlorite), P (a saturated solution of p-phenylenediamine in 95% ethyl alcohol), I (a 3% solution of Lugol's iodine). Polarized light microscopy (pol) was used to locate crystals in thallus sections. H_2SO_4 (a 10% solution of sulfuric acid) was then applied to tests if the characteristic needle shaped crystals formed when calcium oxalate is present in the thallus. Lichen secondary metabolites were identified using standardized thin-layer chromatography (TLC) with solvent systems A, B' and C (Orange *et al.* 2010). Photos were taken in the Olympus SZX16 and BX61 microscope with a DP72 camera system.

DNA Extraction:—Molecular materials were extracted directly from the clean growing portions of the thallus (e.g.,prothallus hyphae, pseudisidia, pseudisidioid structure) of *Herpothallon* specimens, pigmented or carbonized portions were removed as far as possible. Genomic DNA was extracted using the Sigma-Aldrich REDExtract-N-Amp Plant PCR Kit (St. Louis, Missouri, U.S.A.) following the manufacturer's instructions, except only 30 µl of extraction buffer and 30 µl dilution buffer were used.

PCR and Sequencing:—The following primers were used for PCR amplification: mrSSU1 and mrSSU3R (Zoller *et al*. 1999). The 50 µl PCR mixture consisted of 2 µl DNA, 2 µl of each primer, 25 µl 2 × Taq PCR MasterMix (Taq DNA Polymerase [0.1 unit/µl]; 3 mM $MgCl_2$; 100 mM KCl; 0.5 mM dNTPs; and 20 mM Tris-HCl [pH 8.3]) (Tiangen, Beijing, China) and 19 µl dd H_2O. PCR cycling conditions were 94 ℃ for 10 minutes, followed by 34 cycles of 95 ℃ for 45 seconds, 50 ℃ for 45 seconds, and 72 ℃ for 90 seconds, followed by a final extension at 72 ℃ for 10 minutes. Sequencing was performed by the company of BioSune Biological Technology (Shanghai).

Sequence alignments:—The mtSSU sequences were assembled and edited using SeqMan v.7.0 (DNAstar packages). Sequences were aligned using the online version of MAFFT v.7.0.26 and MEGA v.7.0. The algorithm of MAFFT choose Auto (FFT-NS-1, FFT-NS-2, FFT-NS-i or L-INS-i; depends on data size). The species *Cryptothecia austrocoreana* J.J. Woo *et al.* (2017: 341) was chosen as outgroup (Woo *et al*. 2017).

Phylogenetic analyses:—The alignments were analyzed using maximum likelihood (ML) and Bayesian approaches. The ML analyses were performed using the program MEGA v.7.0. support values were assessed using the'rapid bootstrapping' option with 1000 replicates. For the Bayesian analysis, the best substitution models were estimated using jModelTest 2.1.7 (Darriba *et al*. 2012). Based on the result, we used TVM+G model for mtSSU. Four Markov chains were run with 2 million generations for this dataset. Trees were sampled every 100 generations, with the first 25% of trees discarded as burn-in. Stationarity of analysis was determined by examining the standard deviation of split frequencies (< 0.01). Posterior probabilities above 0.9 and bootstrap support above 70% were considered significant supporting values. Generated phylogenetic trees were visualized with FigTree v. 1.4.2 (Rambaut 2012).

Results & Discussion

Molecular phylogeny:—A total of 9 sequences of mtSSU were newly generated from 4 specimens, and 5 sequences downloaded from NCBI (Table 1). The aligned mtSSU region comprised 929 sites.

The phylogenetic trees obtained from maximum likelihood (ML) and Bayesian inference analysis (BI) exhibited the same topology; we therefore present only the ML tree, with bootstrap support ≥ 70% for the ML analysis and posterior probabilities ≥ 0.95 for the Bayesian analysis (Fig. 1).

Through evolutionary distances and support, the taxonomic positions of those three new species can be confirmed, they indeed belong to *Herpothallon*. And we can clearly see that there are two main clades in *Herpothallon*: the three new taxa and *H. echinatum* Aptroot *et al*. (2009: 38) belong to clade A, *H. inopinatum*, *H. kigeziense* Frisch & G. Thor (2014: 303) and *H. rubrocinctum* (Ehrenb.) Aptroot, Lücking & G. Thor (2009: 61) belong to clade B. The four species belong to clade A without chiodectonic acid, the three species belong to clade B all possess chiodectonic acid; In clade A, *H. glaucescens* and *H. lilacinum* clusters with *H. echinatum*, they all possess psoromic acid as major substance, and *H. tomentosum* which contains confluentic acid as major substance forms a separate lineage, indicating that secondary metabolites are valuable in the classification of *Herpothallon*. Additional studies are necessary, including extended sampling of more variable loci and material to confirm these preliminary results.

TABLE 1. Taxon, locality, GenBank accession numbers and voucher for the specimen of *Herpothallon* included in the phylogenetic analysis. Newly generated sequences are in bold. * = outgroup.

Taxon	Locality	GenBank accession no.	Voucher
***H. echinatum* 1**	China	OQ676528	**20220048 (SDNU)**
***H. echinatum* 2**	China	OQ676537	**20220494 (SDNU)**
***H. echinatum* 3**	China	OQ676534	**20220455 (SDNU)**
H. glaucescens	China	OQ676531	**20220069 (SDNU)**
H. inopinatum	Mexico	KJ850964	Rudolphi 12 (UPS)
H. kigeziense	Uganda	KF707644	Frisch 11/Ug26 (UPS)
***H. lilacinum* 1**	China	OQ676532	**20220090 (SDNU)**
***H. lilacinum* 2**	China	OQ676529	**20220240 (SDNU)**
H. rubrocinctum	Mexico	KF707643	Rudolphi 5 (UPS)
H. rubrocinctum	America	GU327693	Nelsen 4006 (F)
***H. tomentosum* 1**	China	OQ676538	**20220565 (SDNU)**
***H. tomentosum* 2**	China	OQ676533	**20220463 (SDNU)**
***H. tomentosum* 3**	China	OQ676539	**20222313 (SDNU)**
Cryptothecia austrocoreana*	South Korea	MF769374	KoLRI No.041892

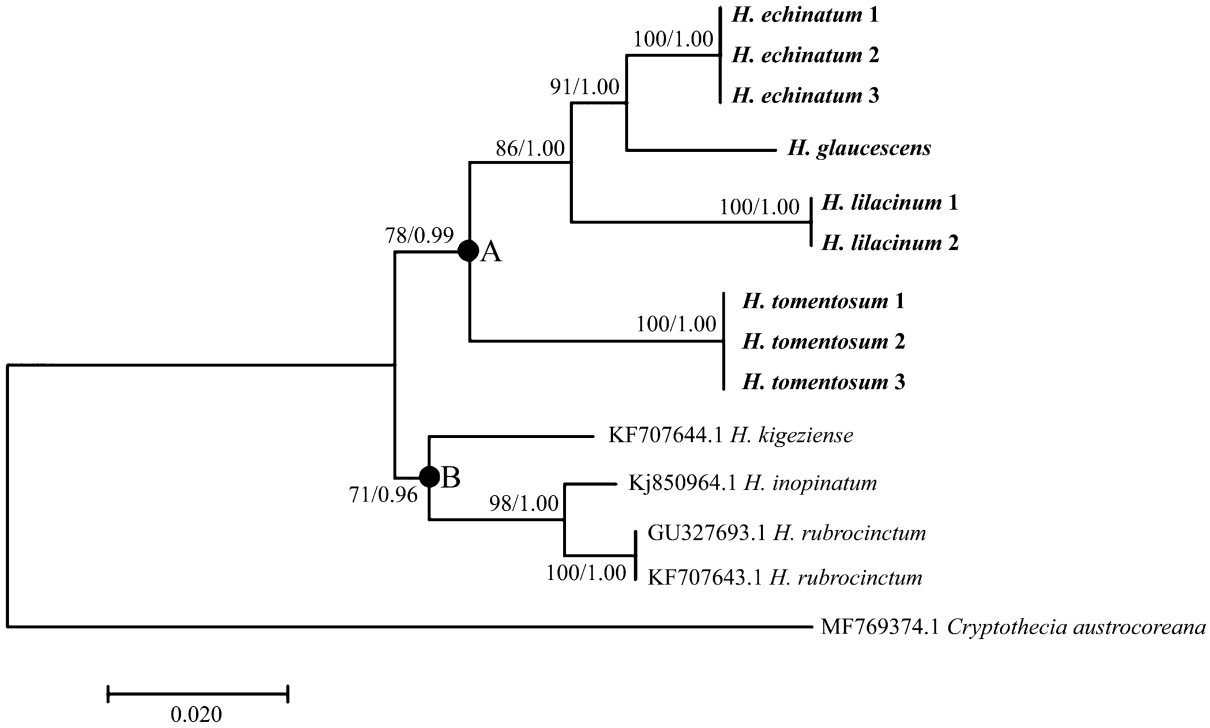

FIGURE 1. Phylogenetic tree constructed from Maximum Likelihood analysis of *Herpothallon* species, based on the mtSSU dataset. Maximum likelihood (ML) bootstrap value ≥ 70, and Bayesian posterior probabilities (PP) value ≥ 0.95 are shown above the branches. Bootstrap values are shown in the order of ML, PP in the tree. Newly described species are marked in bold. Scale = 0.02 substitution per site.

New species

Herpothallon glaucescens L.L. Liu & Lu L. Zhang, *sp. nov.* Mycobank number: 843998 (Fig. 2)

Type:—CHINA. Zhejiang Province: Lishui City, Jingning County, Baiyun Protection Station. 1131.3 m elev., 27°72′57.76″ N, 119°64′12.09″ E, on bark of *Cunninghamia* R. Br., 2 December 2020, C.G. Zhao & Lu L. Zhang 20211617 (Holotype in SDNU).

Thallus corticolous, up to 3 cm across, suborbicular, sometimes flaking off, loosely to firmly appressed to the substrate, felty to byssoid, dull, white in the center, whitish grey to greyish green along the margin, in section up to 200 µm thick, with abundant calcium oxalate crystals throughout the thallus (insoluble in KOH, dissolving and recrystallizing as colourless, needle-shaped crystals in 10% H_2SO_4), with 1–2 µm wide hyphae. Hypothallus whitish, byssoid, composed

of 1–2 µm wide hyphae. Prothallus up to 2 mm broad, whitish, indistinct, byssoid, composed of interwoven and radiating hyphae. Pseudisidia numerous, sparse to dense, dispersed or 2–3 in coralloid aggregations, often branched, swollen, subglobose to ±vermiform, rounded at the top, of the same colour as the thallus, compact with few projecting hyphae, 0.15–0.18 mm in diam., or 0.22–0.3 × 0.11–0.18 mm. Photobiont trentepohlioid, single or a few cells aggregated; cells yellowish, 10–12.5 × 7–8 µm. Asci and pycnidia not seen.

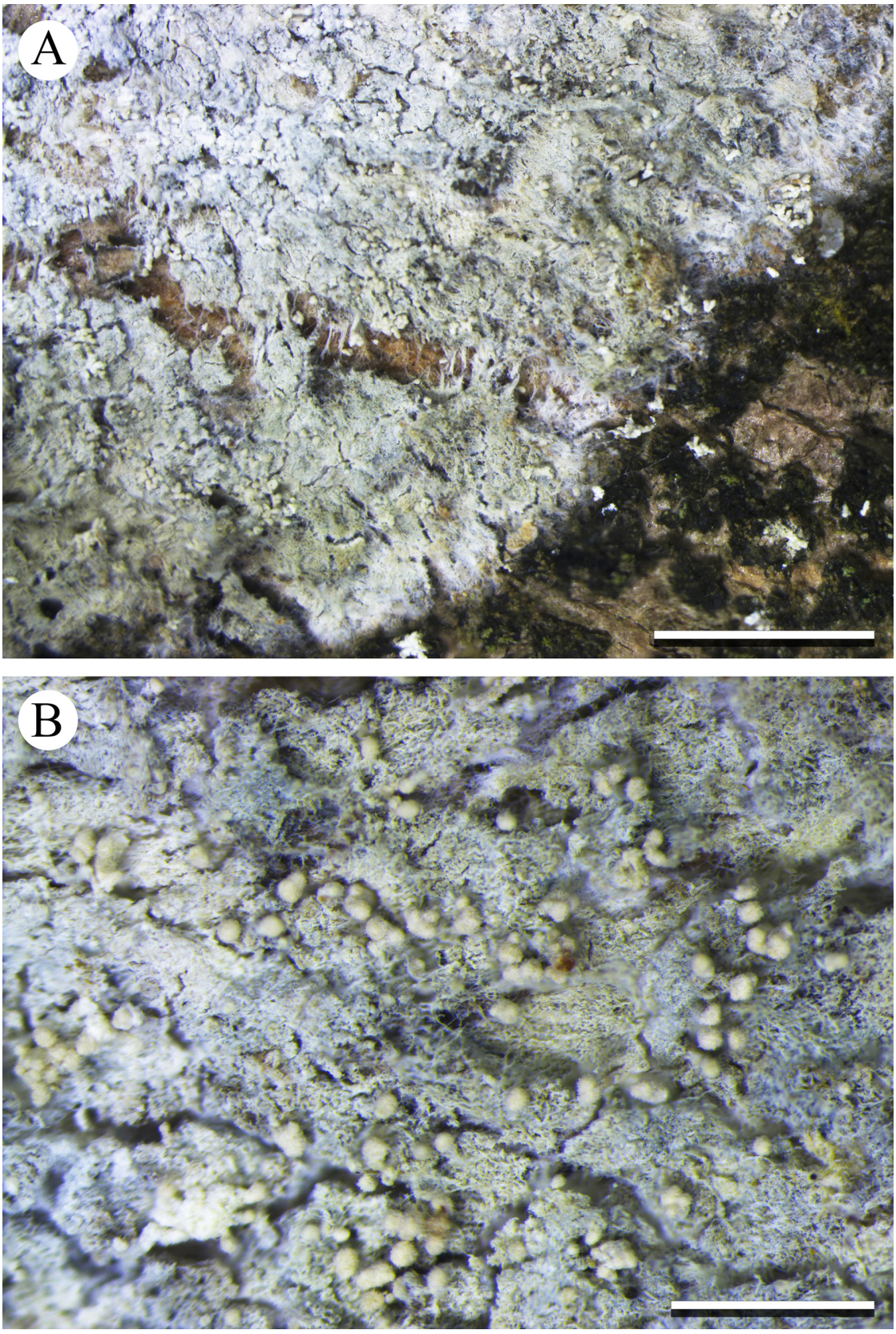

FIGURE 2. *Herpothallon glaucescens* (holotype, SDNU 20211617). **A.** Thallus and prothallus. Scale = 2 mm. **B.** Pseudisidia. Scale = 600 µm.

Chemistry and spot tests: Thallus and prothallus K−, C−, P+ bright yellow, UV−, I+ blue in medulla. TLC: psoromic acid (major), 2'-O-demethylpsoromic acid (minor).

Etymology: The epithet "*glaucescens*" refers to the whitish grey colour of the thallus and the pseudisidia.

Ecology and distribution: The new species was found growing on bark of *Cunninghamia* R. Br. at the Baiyun Protection Station in the Zhejiang Province and the bark of other trees in the Daming Mountain National Nature Reserve of the Guangxi Zhuang Autonomous Region.

Note: This species is characterized by a thallus with swollen, compact, subglobose to ±vermiform, often branched pseudisidia, with few projecting hyphae, and the presence of psoromic and 2'-O-demethylpsoromic acids. Although *H. globosum* G. Thor (2009: 42) also has globose pseudisidia and contains psoromic acid, it lacks calcium oxalate crystals, has a red hypothallus and prothallus, the upper parts of its pseudisidia are dark red and often with black spots (Aptroot *et al*. 2009). *Herpothallon biacidum* Frisch, Elix & G. Thor (2010: 286) possesses a loosely attached thallus with abundant calcium oxalate crystals, globular to short cylindrical pseudisidia, up to 0.30 × 0.12 mm, in small coralloid aggregations, but it has a brown to blackish brown hypothallus and produces gyrophoric and norstictic acids (Frisch *et al*. 2010). *Herpothallon coralloides* Jagadeesh (2014: 40) is also similar to *H. glaucescens* because of its firmly to loosely appressed thallus and white prothallus, cylindrical pseudisidia that are simple to irregularly branched and coralloid, but the pseudisidia of *H. coralloides* are much larger, up to 1.0 × 0.1 mm. The two species also differ in chemistry, *H. coralloides* containing confluentic and norstictic acids (Jagadeesh Ram 2014).

Phylogenetically, *H. glaucescens* is the sister taxon to *H. echinatum* (Fig. 1), they are similar in containing psoromic acid as a major secondary metabolite, but *H. echinatum* has a much softer thallus, often seemingly farinose due to loose soredioid fragments, and its pseudisidia are cylindrical, more elongated, up to 0.5 × 0.1 mm, and are mostly unbranched, felty with projecting hyphae.

Additional specimen examined: CHINA. Guangxi Province: Nanning City, Wuming County, Daming Mountain National Nature Reserve, 592.0 m elev., 23°30′12.336″ N, 108°26′08.231″ E, on bark of a tree, 30 December 2020, X. Zhang *et al*. 20211618 (SDNU).

Herpothallon lilacinum L.L. Liu & Lu L. Zhang, *sp. nov.* Mycobank number: 845790 (Figs 3, 4)

Type:—CHINA. Guizhou Province: Tongren City, Yang Jia Ao township, Bi Er Tang village. 874 m elev., 27°52'27.59" N, 107°58'10.71" E, on rock, 10 June 2022, L.L. Liu, Y.X. Bi, Z.H. Jiang & D.C. Yan 20220232 (Holotype in SDNU).

Thallus corticolous or saxicolous, up to 3.5 cm across, irregular shaped, sometimes flaking off, loosely appressed to the substrate, rather soft, felty, often seemingly farinose, dull, white to cream white, in section up to 200 µm thick, with many calcium oxalate crystals throughout the thallus (insoluble in KOH, dissolving and recrystallizing as colourless, needle-shaped crystals in 10% H_2SO_4), with 1–2 µm wide hyphae. Hypothallus whitish, byssoid, composed of 1–2 µm wide hyphae. Prothallus up to 0.8 mm broad, whitish, indistinct, byssoid to cottony, composed of interwoven and radiating hyphae. Pseudisidia numerous, unbranched, soft, whitish subglobose or irregularly cushion-shaped, fluffy-felty with many projecting hyphae, basally of the same colour as the thallus, upper parts often lilac to lilac grey, 0.1–0.45 mm in diam.. Photobiont trentepohlioid, in short, irregular threads; cells yellowish green, 10–15 × 5–8 µm. Asci and pycnidia not seen.

Chemistry and spot tests: Thallus and prothallus K−, C−, P+ bright yellow, UV−, I+ blue in medulla, the lilac to lilac grey parts K+ black blue, C−. TLC: psoromic acid (major), an unknown substance (minor), 2'-O-demethylpsoromic acid (minor).

Etymology: The epithet "*lilacinum*" refers to the lilac to lilac grey pseudisidia.

Ecology and distribution: The new species was found growing on rock wall by the roadside and bark of a tree in Guizhou Province.

Notes: This species is characterized by the subglobose or irregularly cushion-shaped, lilac to lilac grey, fluffy-felty pseudisidia, 0.1–0.45 mm in diam., the psoromic and 2'-O-demethylpsoromic acids, and an unknown substance chemistry. *Herpothallon lilacinum* is most similar to *H. weii* Yuliang Chen & Haiying Wang (2012: 440): both contain psoromic acid and the similar unknown substance, but *H. weii* has a tightly appressed thallus, an I− medulla, a distinct prothallus, pinkish and larger, and *not* whitish subglobose pseudisidia, up to 1 × 0.5 mm (Cheng *et al*. 2012). *Crypthonia albida* (Fée) Frisch & G. Thor (2010: 290) also has fluffy-felty pseudisidia and contains psoromic acid as its major substance, but has loosely byssoid, whitish pseudisidia, up to 1.0 × 1.0 mm (Frisch & G. Thor, 2010). *Herpothallon himalayanum* Jagadeesh & Sinha (2009: 40) and *H. capilliferum* Pengfei Chen & Lulu Zhang (2022: 02) are also similar to *H. lilacinum* in producing fluffy-felty pseudisidia, but they differ in secondary chemistry: *Herpothallon himalayanum* contains gyrophoric acid as its major substance (Jagadeesh & Sinha, 2009), and *H. capilliferum* only contains norstictic acid (Chen *et al*. 2022).

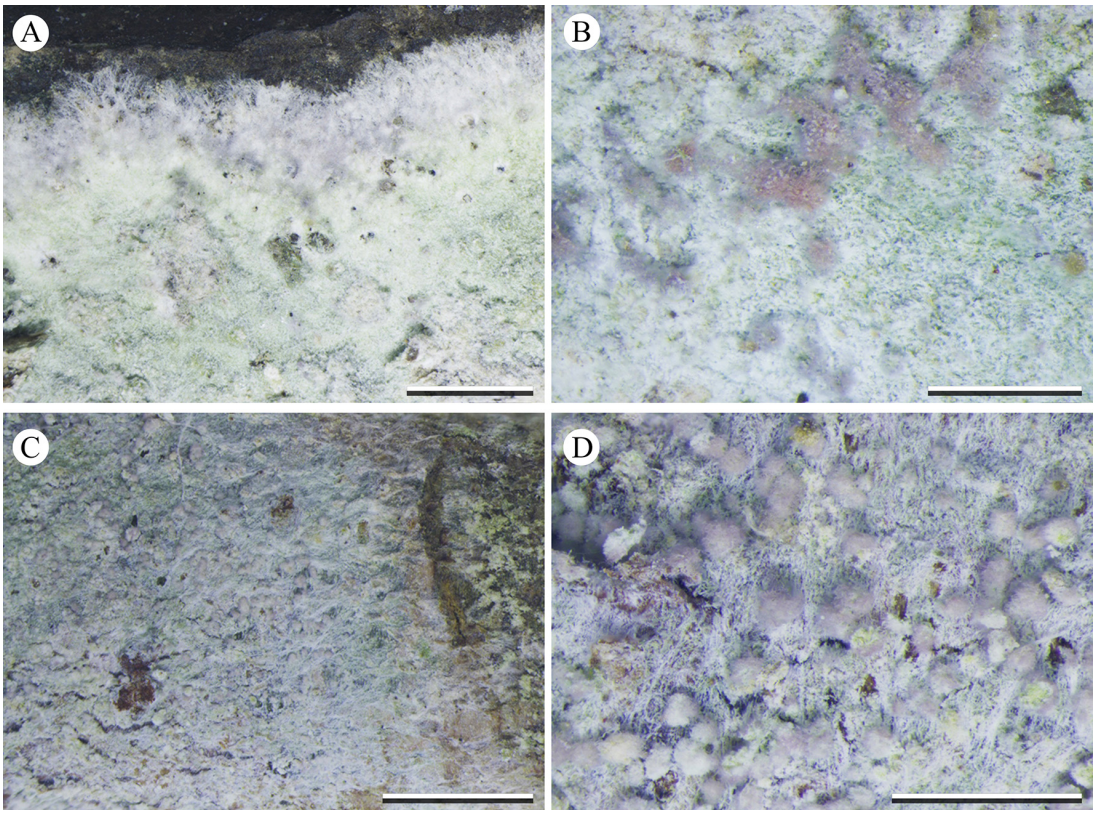

FIGURE 3. A–B. *Herpothallon lilacinum* growing on rock, (holotype, SDNU 20220232). **A.** Thallus and prothallus. Scale = 1 mm.. **B.** Pseudisidia. Scale = 400 µm. **C–D.** The new species *H. lilacinum* growing on bark, (paratype, SDNU 20220090). **C.** Thallus. Scale = 1.5 mm. **D.** Pseudisidia. Scale = 600 µm.

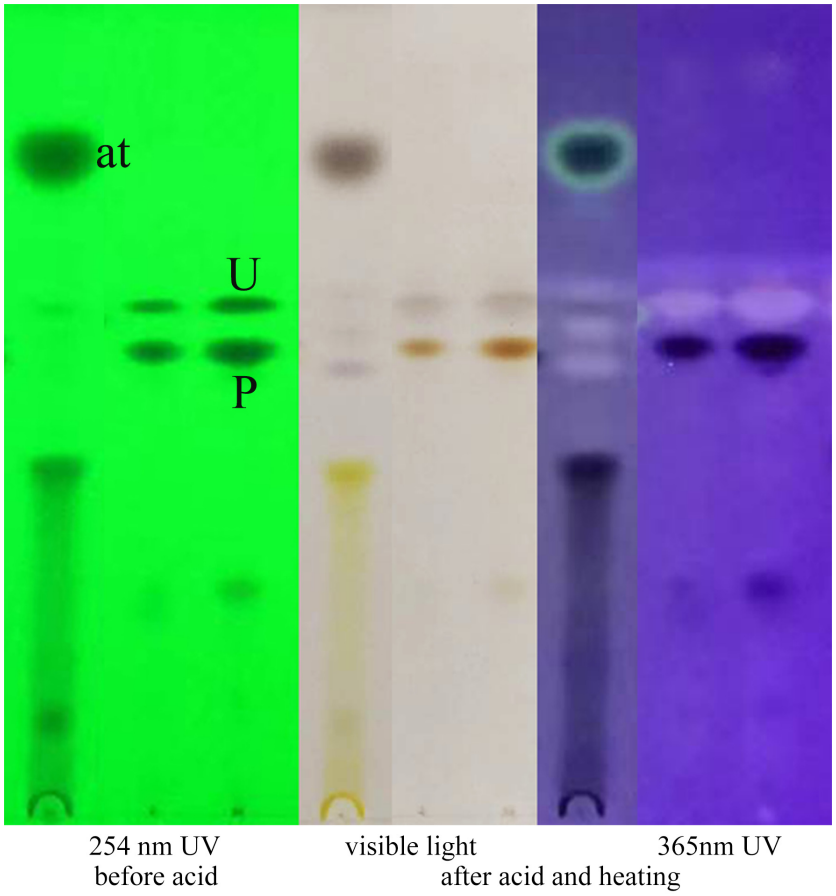

FIGURE 4. TLC of *H. lilacinum* with C system (**at**: atranorin; **U**: the unknown substance; **P**: psoromic acid).

Phylogenetically, *H. lilacinum* clusters with *H. echinatum* and *H. glaucescens* (Fig. 1), they all possess psoromic acid as major, but *H. echinatum* and *H. glaucescens* have subglobose or cylindrical pseudisidia, all without the unknown substance.

Additional specimen examined: CHINA. Guizhou Province: Tongren City, Yang Jia Ao township, Bi Er Tang village. 874 m elev., 27°52'27.59" N, 107°58'10.71" E, on rock, 10 June 2022, L.L. Liu, Y.X. Bi, Z.H. Jiang & D.C. Yan 20220231, 20220237, 20220238, 20220239, 20220240, 20220253 (SDNU); Guizhou Province: Tongren City, Xu Jia Ba town, Zhang Jia Gou village, along the stream. 851 m elev., 27°55'33.38" N, 108°1'59.13" E, on bark of a tree, 11 June 2022, L.L. Liu, Y.X. Bi, Z.H. Jiang & D.C. Yan 20220090 (SDNU).

Herpothallon tomentosum L.L. Liu & Lu L. Zhang, *sp. nov.* Mycobank number: 845791 (Fig. 5)

Type:—CHINA. Fujian Province: Longyan City, Dongxiao National Forest Park, Barbecue field. 450 m elev., 24°58'25.33" N, 117°0'56.67" E, on bark of a tree, 12 July 2022, L.L. Liu, J.X. Xue & L. Wang 20220468 (Holotype in SDNU).

Thallus corticolous, up to 2 cm across, suborbicular to sometimes irregular, not flaking off, loosely to firmly appressed to the substrate, soft, minutely felty, dull, blue green to greenish grey, in section up to 120 µm thick, with few calcium oxalate crystals throughout the thallus (insoluble in KOH, dissolving and recrystallizing as colourless, needle-shaped crystals in 10% H_2SO_4), with 1–2 µm wide hyphae. Hypothallus whitish, byssoid, composed of 1–2 µm wide hyphae. Prothallus up to 0.9 mm broad, whitish, distinct, byssoid, composed of interwoven and radiating hyphae. Pseudisidia numerous, unbranched, globular, of the same colour as the thallus, soft, felty with many projecting hyphae, 0.06–0.12 mm in diam.. Photobiont trentepohlioid, single or a few cells aggregated; cells yellowish green, 12.5–15 × 5–10 µm. Asci not seen. Pycnidia embedded in the tips of some pseudisidia, opening with a apical pore, pigmentation occasionally extending along the pycnidial wall. Conidia simple, hyaline, short bacilliform, 3–4 × 1–1.5 µm.

Chemistry and spot tests: Thallus and prothallus K−, C−, P−, UV−, I− in medulla. TLC: confluentic acid (major), 2'-O-methylmicrophyllinic acid (minor).

Etymology: The epithet "*tomentosum*" refers to the pseudisidia felty with many projecting hyphae.

Ecology and distribution: The new species was found growing on bark of trees beside a stream of Dongxiao National Forest Park and on bark of trees beside a mountain path of Tianzhu Mountain Forest Park of Fujian Province.

Notes: This species is characterized by the globular pseudisidia each containing a pycnidium, 0.06–0.16 mm in diam., and the presence of confluentic and 2'-O-methylmicrophyllinic acids. *Herpothallon tomentosum* is most similar to *H. cinereum* G. Thor (2009: 34) in its minutely felty thallus, the white, byssoid-felty prothallus and the presence of confluentic and 2'-O-methylmicrophyllinic acid in its thallus. However, *H. cinereum* has a loosely appressed thallus, up to 200 µm thick, and cylindrical pseudisidia up to 0.5 × 0.1 mm, without pycnidia at their tips. *Herpothallon tomentosum* has the same chemistry as *H. confluenticum* Aptroot & Lücking (2009: 36); both have pycnidia at the tips of their pseudisidia, but the latter has a rather firm thallus delimited by a dirty whitish prothallus, on a whitish to brownish hypothallus, and cylindrical pseudisidia that are partly cauliflower-like at the tips, up to 0.6 × 0.2 mm (Aptroot *et al*. 2009). The specimens of *H. echinatum* that Bungartz *et al*. collected from Ecuador (2013: 752) also have a non-pigmented thallus, prothallus and hypothallus, globular pseudisidia with pycnidia at the tips, and bacilliform conidia (3–4 × 1–1.5 µm), but they contain psoromic acid (Bungartz *et al*. 2013). Two other morphologically similar species are *H. biacidum* and *H. subglobosum* Pengfei Chen & Lulu Zhang (2022: 07): both have a minutely felty thallus and globular pseudisidia, but differ in chemistry: *H. biacidum* contains gyrophoric and norstictic acids (Frisch *et al.* 2010), whereas *H. subglobosum* contains gyrophoric, lecanoric and umbilicaric acids (Chen *et al*. 2022).

Phylogenetically, *H. tomentosum* belongs into a different monophyletic clade from the other species in clade B (Fig. 1), demonstrating that it is a distinct species. As part of our survey, we collected multiple specimens of the new species in two areas. These specimens are similar in morphological and anatomical characters except that some of the specimens lack pycnidia. In our phylogenetic analysis this material appears on the same branch, with a relatively close evolutionary distance, strongly supported (BS=100, PP=1.00).

Additional specimen examined: CHINA. Fujian Province: Longyan City, Dongxiao National Forest Park, Barbecue field. 450 m elev., 24°58'25.33" N, 117°0'56.67" E, on bark of a tree, 12 July 2022, L.L. Liu, J.X. Xue & L. Wang 20220477 (SDNU); Fujian Province: Longyan City, Dongxiao National Forest Park, Bajiao forest to Suoluo group. 558 m elev., 24°58'21.42" N, 117°1'1.71" E, on bark of a tree, 12 July 2022, L.L. Liu, J.X. Xue & L. Wang 20220565, 20220582, 20220587 (SDNU); Fujian Province: Xiamen City, Tianzhu Mountain Forest Park, No. 3 branch road near the air monitoring station. 183 m elev., 24°35'46.33" N, 117°54'31.06" E, on bark of a tree, 11 July 2022, L.L. Liu, J.X. Xue & L. Wang, 20220442, 20220443, 20220462, 20220463 (SDNU).

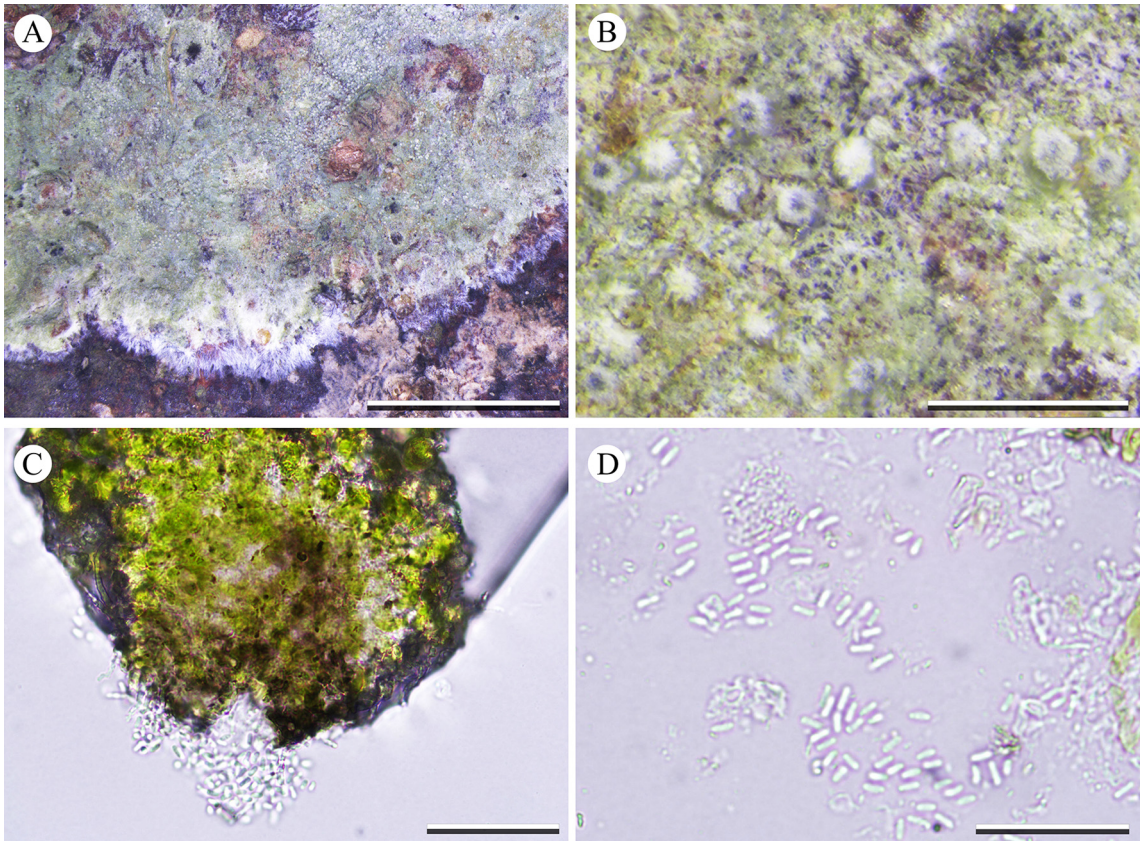

FIGURE 5. *Herpothallon tomentosum* (holotype, SDNU 20220468), **A.** Thallus and prothallus. Scale = 3 mm. **B.** Pseudisidia. Scale = 200 µm. **C.** Pycnidia. Scale = 40 µm. **D.** Conidia. Scale = 20 µm.

Key to the species of *Herpothallon* known from China

1. Prothallus and pseudisidia with red pigment, K+ purple in pigmented parts ..... ***H. rubrocinctum***[1]
Prothallus and pseudisidia without red pigment (rarely pinkish or lilac pigments present in pseudisidia) **2**

2. Thallus C+ red; gyrophoric acid major, lecanoric acid and some other substances minor **3**
Thallus C–; gyrophoric and lecanoric acids absent **5**

3. Thallus K+ yellow; an unknown substance present (RF close to atranorin in solvent C) present; pseudisidia cylindrical (0.2 × 0.1 mm) ..... ***H. viridi-isidiatum*** P.F. Chen & L.L. Zhang (2022: 07)
Thallus K–; the unknown minor substance absent; pseudisidia globular or cylindrical **4**

4. Pseudisidia globular (0.1 × 0.1 mm) ..... ***H. subglobosum***
Pseudisidia cylindrical (1.0 × 0.1 mm) ..... ***H. philippinum*** (Vain.) Aptroot & Lücking (2009: 43)

5. Thallus K+ yellow or K+ yellow then red, P+ orange-red; stictic or norstictic acids present **6**
Thallus K–, P– or P+ yellow; stictic and norstictic acids absent **7**

6. Thallus K+ yellow then red; norstictic acid present; pseudisidia irregularly cushion-shaped (0.4 × 0.2 mm) ..... ***H. capilliferum***
Thallus K+ yellow; stictic acid present; pseudisidia cylindrical (0.3 × 0.1 mm) ..... ***H. polyisidiatum*** P.F. Chen & L.L. Zhang (2022: 02)

7. Thallus P–; psoromic acid absent **8**
Thallus P+ yellow; psoromic acid present **9**

8. Confluentic acid major; globular pseudisidia (0.06–0.12 mm in diam.) ..... ***H. tomentosum***
Perlatolic acid major; minute, irregular, soredia-like granular pseudisidia (0.05 × 0.05 mm in diam.) ..... ***H. granulare*** (Sipman) Aptroot & Lücking (2009: 43)

9. Pseudisidia cylindrical (0.5 × 0.1 mm) ..... ***H. echinatum***
Pseudisidia subglobose to irregularly cushion-shaped **10**

1 Wei J.C. (2020) The Enumeration of Lichenized Fungi in China. In: Beijing, China Forestry Publishing House, China, 44–47. Specimen examined: CHINA. Guizhou Province: Leishan County, Leigongshan National Forest Park, 892 m elev., on bark of a tree, 2 Nov. 2009, Q. Tian, 20102819 (SDNU). Discussion: The identification of this material as *Herpothallon rubrocinctum* appears problematic, because its prothallus is an orange to red (rather than bright scarlet red), the thallus lacks secondary metabolites and the pseudisidia of the specimen are granular. Molecular data could not successfully be obtained from the material. The report of *H. rubrocinctum* may thus refer to a still undescribed species, which needs to be further investigates, when fresh specimens are collected.

10. Pseudisidia without pinkish or lilac pigments; subglobose (0.22–0.3 × 0.11–0.18 mm).....***H. glaucescens***
Pseudisidia with pinkish or lilac pigments; subglobose to mostly irregularly cushion-shaped **11**
11. Thallus tightly appressed, medulla I–; pseudisidia irregularly cushion-shaped, pinkish, large (1 × 0.5 mm).....***H. weii***
Thallus loosely appressed, medulla I+ blue; pseudisidia whitsh subglobose or irregularly cushion-shaped, basally white, upper parts often lilac to lilac grey, small (0.1–0.45 mm in diam.).....***H. lilacinum***

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