

# Two new *Cortinarius* species in subgenus *Leprocybe* from Southwest China

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## Abstract

Two new *Cortinarius* species in the subgenus *Leprocybe*, *C. henduanensis* and *C. yadingensis*, are proposed based on a combination of morphological and molecular evidence. *Cortinarius henduanensis* has distinctly olive tinged basidiomata, a squamulose pileus, and small, subglobose to broadly ellipsoid basidiospores, and the ITS sequence differs from that of *C. flavifolius* by at least 28 substitutions and indel positions. *Cortinarius yadingensis* has a brownish basidiomata, squamulose pileus and subglobose to broadly ellipsoid, coarsely verrucose basidiospores, and the ITS sequence differs by at least 11 substitutions and indel positions from other members of the section *Leprocybe*. Both new species were found in the mixed forest of Southwest China.

**Keywords:** Basidiomycota; Cortinariaceae; China; phylogeny; new taxa; taxonomy

## Introduction

The family Cortinariaceae as currently defined, contains ten genera, *Cortinarius*,

*Phlegmacium*, *Thaxterogaster*, *Calonarius*, *Aureonarius*, *Cystinarius*, *Volvanarius*, *Hygronarius*, *Mystinarius*, and *Austrocortinarius*. In this study *Cortinarius* was divided into several subgenera, including the subgenus *Leprocybe*, which has recently been studied by several investigators. Soop (2019) proposed two new sections of the subgenus, sect. *Persplendidi* Soop & Dima and sect. *Veronicae* Soop. Ammirati et al. (2021) proposed three new sections, *Fuscotomentosi* Niskanen, Liimat. & Ammirati, sect. *Melanoti* Niskanen, Liimat. & Ammirati and sect. *Squamiveneti* Niskanen, Liimat. & Ammirati as well as 11 new species from North America based on morphological, ecological, and molecular data. The paper also validated sect. *Veneti* Bellanger, Niskanen, Ammirati & Liimat., which was proposed by Konrad & Maublanc (1937), and designated neo- or epitypes for four species. Bidaud et al. (2021) reported 11 species of the subgenus (including three new species) from the Mediterranean and confirmed 23 synonymies. Liimatainen et al. (2022) based on result from analysis of five genes proposed seven sections in subgenus *Leprocybe*, *Fuscotomentosi*, *Leprocybe*, *Melanoti*, *Persplendidi*, *Squamiveneti*, *Veneti* and *Veronicae*. A few *Leprocybe* species have previously been reported from China. Keissler & Hohwag (1937) reported *C. cotoneus* Fr. from Yunnan Province, Shao & Xiang (1997) documented *C. venetus* (Fr.) Fr. from Heilongjiang Province of Northeast China, and Xie (2022) reported *C. nigrosquamosus* Hongo from Yunnan. In a survey of *Cortinarius* species in southwest China, two undescribed species of *Leprocybe* were found based on morphological and phylogenetic analyses. These species are described below with photos of morphological and microscopical characteristics.

## Materials & Methods

### *Morphological study*

Specimens were collected from Xizang Autonomous Region, Sichuan and Yunnan Provinces, China. The fresh basidiomata were photographed after collecting from the field and the macro-morphological characters were recorded in detail before drying in an oven at 45°C. A 20% KOH solution was used on fresh pileus and stipe surface, lamella, and context for chemical reaction. Observation of basidiomata was performed under ultraviolet light at a wavelength of 360 nm. The specimens are deposited in Fungarium, Institute of Microbiology, Chinese Academy of Sciences (also as Herbarium Mycologicum Academiae Sinicae, HMAS). Descriptions of the microscopical characters are from dried collections. Thin sections were prepared by hand with a razor blade. Sections were mounted in 5% KOH solution. Basidiospores, basidia, and hyphae were measured using an ocular micrometer. At least 30 basidiospores of each mature collection were measured.

### *DNA extraction, amplification and sequencing*

Genomic DNA was extracted from dried specimens using standard protocol (Rogers & Bendich, 1994). The DNA extracts were used as templates for PCR. Amplification reactions were performed to obtain sequences of nuclear ribosomal internal transcribed spacer region (ITS) using primer pairs ITS5/ITS4 (White et al., 1990). The amplification was carried out

under the following conditions: initial denaturation for 3 min at 95 °C, followed by 30 cycles, at 95 °C for 30 s, at 55 °C for 45 s, at 72 °C for 60 s, and a final elongation step at 72 °C for 10 min.

#### *Phylogenetic analysis*

The newly generated ITS sequences were submitted to GenBank. The ITS sequences for the phylogenetic analyses were selected based on results of BLASTn (>94% identity) in GenBank. Two species, *C. veronicae* (KC017355) and *C. veronicoides* (GQ890324), were chosen as outgroup. Seventy-three sequences (Table 1) were aligned and edited with BioEdit 7.2.2 (Hall, 1999). Bayesian Inference (BI) and Maximum Likelihood (ML) methods were implemented to analyses in this study. MrModeltest 2.3 was used to calculate the best model for BI analysis (Nylander et al., 2004). The BI analysis was performed with MrBayes 3.2.6 (Ronquist & Huelsenbeck, 2003), and the ML analysis was conducted in MEGA X (Kumar et al., 2018). The matrix contained 75 ITS sequences with 681 nucleotide sites is available from TreeBASE under S30908 (<http://purl.org/phylo/treebase/phyloids/study/TB2:S30908>).

#### *Nomenclature*

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants, and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. In addition, new names contained in this work have been submitted to Index Fungorum from where they will be made available to the Global Names Index. The unique Index Fungorum number can be resolved, and the associated information viewed through any standard web browser by appending the Index Fungorum number contained in this publication to the prefix "<https://www.indexfungorum.org/Names/Names.asp>". The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed, Central SCIE, and CLOCKSS.

## **Results**

#### *Molecular phylogeny*

The ITS matrix for phylogenetic analyses included 73 sequences, representing 15 species. The resulting alignments were deposited at TreeBASE (<http://www.treebase.org>; submission ID 30908; accessed on 1 November 2023). The BI and ML trees showed similar topologies, and the ML tree was selected as the representative phylogeny (Figure 1). According to the phylogenetic tree, the independences of *Cortinarius hengduanensis* and *C. yadingensis* were well-surppoted. *Cortinarius hengduanensis* has a close relationship with *C. flavifolius* Peck, *C. yadingensis* nests in a clade of sect. *Leprocycbe* and clusters together with *C. cotoneus*, *C. hughesiae* Ammirati, Matheny, Liimat. & Niskanen, *C. selinolens* Bidaud & Bellanger and *C. subcotoneus* Bidaud.

### Taxonomy

***Cortinarius hengduanensis*** P. Hong, K. Wang, Z. Du, M.L. Xie, Di Liu & T.Z. Wei, sp. nov.  
Figure 2.

**Index Fungorum:** IF901373.

**Holotype:**—CHINA, Yunnan Province, Shangri-La County, Haba Mountains Nature Reserve, alt. 3000 m, in mixed forest with *Pinus densata* and *Quercus aquifolioides*, 16 Aug. 2008, T.-Z. Wei, X.-Q. Zhang & F.-Q. Yu 194, HMAS 250455 (GenBank ID: KX513581 ITS).

**Etymology:**—“hengduan”, Chinese, referring Hengduan Mountains of southwest China, the locality of the type collection.

**Diagnosis:**—*Cortinarius hengduanensis* has olive tinged basidiomata, squamulose pileus, and small subglobose to broadly ellipsoid basidiospores ( $5.5\text{--}7.5 \times 5\text{--}6.5\ \mu\text{m}$ ). ITS sequences of the new species (GenBank ID: KX513581–KX513583, OR538887 and OR538888) deviate from that of *C. flavifolius* by at least 28 substitutions and indel positions. In mixed forest with *Pinus yunnanensis*, *P. densata* and *Quercus aquifolioides*.

**Description:**—*Pileus* 4.5–12.5 cm diam., hemispherical at first, later becoming convex to applanate, with a shallow, obtuse umbo when mature, margin sometimes uplifted or undulate, surface uneven, tomentose, persistently covered with small olive-brown to dark olive-brown squamules, margin radially striate, olive-green with brownish tint at first, then brownish olive, olive-brown to dull brown with paler margin, sometimes darker at the center. *Context* up to 8 mm thick at pileus center, whitish to pale when dry, olive-gray when moist, fleshy, soft; odor indistinct, taste mild. *Lamellae* adnate to emarginate, 3–8 mm wide, close to moderately crowded, olive to grayish green when young, brown with olive tint to rust-brown when mature, edges paler. *Stipe* 5.5–12  $\times$  0.7–2.6 cm, cylindrical or base enlarged and up to 3 cm in diam., surface completely covered with universal veil at first, then longitudinally fibrillose above and with conspicuous annular girdles below; olivaceous yellow, tinged rust-brown from basidiospores, at first solid, then soft to hollow. *Partial veil* arachnoid, greenish to olive, then becoming rust brown from mature basidiospores. *Universal veil* distinct and persistent, fibrillose, covering the basidiomata at first, then forming fibrils and squamules on pileus and fibrils and girdles on stipe surface, pale with olive tint at first, brownish with olive tint to olive-brown when mature. *Basidiospore deposit* rust brown. *Chemical reaction with 20% KOH* dark reddish brown on pileus and lamella, brown on stipe and reddish brown on context. *Fluorescence reaction under ultraviolet light* distinctly bright yellow on lamellae, and weaker on surfaces of pileus and stipe.

*Basidiospores* (5.2–) 5.5–7 (–7.5)  $\times$  (4.8–) 5–6 (–6.5)  $\mu\text{m}$ , Q= (1.08–) 1.1–1.2 (–1.23) (av.=1.15), subglobose to broadly ellipsoid, yellowish brown, moderately to distinctly verrucose. *Basidia* 28–35  $\times$  8–10  $\mu\text{m}$ , clavate, thin-walled, mostly subhyaline, with four sterigmata. *Lamella edges* heterogeneous, with sterile cells, 20–30  $\times$  6–9  $\mu\text{m}$ , clavate, subhyaline, thin-walled. *Pileipellis*: epicutis well developed, hyphae 9–15  $\mu\text{m}$  wide,

subcylindrical, colorless, yellowish to brownish, thin-walled, smooth; hypocutis poorly developed, hyphae 3–8 µm wide, irregular, with yellowish intracellular pigment. *Clamp connections* present.

**Additional Specimens Examined:** CHINA, Yunnan Province, Songming County, Aziying Town, alt. 2000 m, in mixed forest with *Pinus yunnanensis* and *Quercus aquifolioides*, 5 Aug. 2005, T.-Z. Wei & F.-Q. Yu Gm1082, HMAS 145537 (GenBank ID: KX513582); Deqin County, Baima Mountains, alt. 3100 m, in mixed forest with *P. densata* and *Q. aquifolioides*, 19 Aug. 2008, T.-Z. Wei, X.-Q. Zhang & F.-Q. Yu 258, HMAS 250509 (GenBank ID:

KX513583); Xizang, Nyingchi County, Bayi Town, 29°38'02.58"N, 94°23'44.72"E, alt. 3360 m, in mixed forest with *P. densata* and *Q. aquifolioides*, 14 Sept. 2014, T.-Z. Wei, J.-Y. Zhuang, X.-Y. Liu & H. Huang 5281, HMAS 270305 (GenBank ID: OR538888); Lulang Town, alt. 3481 m, 22 Sept. 2014, W-L Lu & Q-M Wang, 3017, HMAS 272520 (GenBank ID: OR538887).

**Notes:** *Cortinarius hengduanensis* is characterized by its distinct olive tinged basidiomata, fibrillose-squamulose pileus and subglobose to broadly ellipsoid basidiospores (5.2–7.5 × 4.8–6.5 µm). It is close to *C. flavifolius*, the latter also has a fibrillose to squamulose pileus and subglobose basidiospores but does not have any olive or greenish tint, and its basidiospores are 6.7–8.9 × 4.8–5.9 µm (Ammirati et al., 2021). The new species is morphologically similar to *C. venetus*. The latter has olive basidiomata, subglobose basidiospores and an ephemeral veil (Bidaud et al., 2005). *Cortinarius melanotus* Kalchbr. has olive or olive tinged basidiomata, fibrillose squamulose pileus and smaller subglobose basidiospores (Breitenbach & Kränzlin, 2000).

***Cortinarius yadingensis*** P. Hong, K. Wang, Z. Du, M.L. Xie, D. Liu & T.Z. Wei, sp. nov.  
Figure 3.

**Index Fungorum:** IF901374.

**Holotype:**—CHINA, Sichuan Province, Daocheng County, Yading Scenic Spot, alt. 4034 m, in mixed forest of *Abies* sp., *Larix potaninii*, *Picea* sp. and *Q. aquifolioides*, 18 Aug. 2016, T-Z Wei, L-H Sun, Z-X Wu & R-C Zhang, 7168, HMAS 254819 (GenBank ID: OR538892).

**Etymology:**—"yading", Chinese, referring Yading Scenic Spot of Sichuan Province, China, the locality of the type collection.

**Diagnosis:**—*Cortinarius yadingensis* has distinct brown squamules on the basidiomata surface and subglobose to broadly ellipsoid basidiospores (6.5–9 × 6.3–7.6 µm). It differs from related species by its lack of olivaceous coloration. ITS sequences of the new species (GenBank ID: OR538889–OR538893) are distinct from other members of sect. *Leprocye* and deviating from them by at least 11 substitutions and indel positions. In mixed forest with *Abies* sp., *Larix potaninii*, *Picea* sp. and *Q. aquifolioides*.

**Description:**—*Pileus* 2–6 cm diam., hemispherical at first, later becoming convex to applanate, mostly with a shallow and obtuse umbo when mature, margin decurrent to straight,

sometimes split when mature, surface pale brown, grayish brown to rust brown, covered brown to dark brown fibrillose squamules, radially striate, silky and shining. *Context* up to 5 mm thick at pileus center, pale brown to grayish brown, fleshy, soft; odor indistinct, smell mild. *Lamellae* emarginate-adnate, up to 5 mm wide, brownish when young, rust-brown when mature, moderately crowded, edges margin paler. *Stipe* 3.5–8 × 0.5–1 cm, subcylindrical, with base up to 1.5 cm in diam., surface brownish to pale brown, covered with brown to dark brown fibrillose squamules, with annular zone from partial veil, longitudinally striate; at first solid then soft to hollow. *Partial veil* arachnoid, grayish when young, forming a rust brown ring from basidiospores when mature. *Universal veil* forming fibrillose brown to dark brown squamules on the surface of the stipe. Basidiospore deposit rust brown. *Chemical reaction with 20% KOH* dark brown to blackish brown on pileus, lamella and stipe surface, and reddish brown on context. *Fluorescence reaction under ultraviolet* light bright yellow on lamellae, and weaker on surfaces of pileus and stipe.

*Basidiospores* (6.5–) 7–8.5 (–9) × (6.3–) 6.5–7.2 (–7.6)  $\mu\text{m}$ , Q= (1.03–) 1.08–1.2 (–1.24) (av.=1.16); subglobose, yellow brown, distinctly verrucose. *Basidia* 28–35 × 8–10  $\mu\text{m}$ , clavate, thin-walled, mostly subhyaline, with four sterigmata. *Pileipellis*: epicutis well developed, hyphae 9–15  $\mu\text{m}$  wide, subcylindrical, colorless, yellowish to brownish, thin-walled, smooth; hypocutis poorly developed, hyphae 3–8  $\mu\text{m}$  wide, irregular, with yellowish intracellular pigment in. *Hyphae of the cortina* 3–6  $\mu\text{m}$  diam., subhyaline to yellowish, thin-walled. *Clamp connections* present.

**Additional Specimens Examined:** CHINA, Sichuan, Litang County, road side to Daocheng County, alt. 3937 m, in conifer forest with *Abies* sp. and *Picea* sp., 17 Aug. 2016, T-Z Wei, L-H Sun, Z-X Wu & R-C Zhang, 7117, HMAS 254811 (GenBank ID: OR538889); Daocheng County, Yading Scenic Spot, alt. 4034 m, in mixed forest of *Abies* sp., *Larix potaninii*, *Picea* sp. and *Q. aquifolioides*, 18 Aug. 2016, T-Z Wei, L-H Sun, Z-X Wu & R-C Zhang, 7169, HMAS 254820 (GenBank ID: OR538893); 7123, HMAS 280697 (GenBank ID: OR538890); 7127, HMAS 280698 (GenBank ID: 254819).

**Notes:** *Cortinarius yadingensis* is characterized by its grayish brown squamulose to brown pileus and stipe and subglobose, distinctly verrucose basidiospores (6.5–9 × 6.3–7.6  $\mu\text{m}$ ). The species is phylogenetically close to four species of sect. *Leprocye*, *C. cotoneus* (Breitenbach & Kränzlin 2000, Soop 2018), *C. hughesiae* Ammirati, Matheny, Liimat. & Niskanen (Ammirati et al., 2021), *C. selinolens* Bidaud & Bellanger (Bidaud et al., 2021) and *C. subcotoneus* (Bidaud et al., 2005). Compared with *C. yadingensis*, all the four related species have similar finely tomentose to squamulose pileus and strongly verrucose basidiospores. Unlike *C. yadingensis*, all of the related species have olive tinged basidiomata. Phylogenetically, *C. yadingensis* can be distinguished from its sister species by at least 11 substitutions and indels in ITS.

## Discussion

According to Bidaud et al. (2021), Ammirati et al. (2021) and Liimatainen et al. (2022), subgen. *Leprocyebe* was re-defined as *Cortinarius* species with small- to medium-sized (occasionally large-sized) basidiomata, yellow UV fluorescent reaction, tomentose to squamulose pileus and subglobose basidiospores, characteristics found in both *C. hengduanensis* and *C. yadingensis*. *Cortinarius hengduanensis* has olive coloration, which is also found in most of species in sect. *Fuscotomentosi*, sect. *Leprocyebe*, sect. *Melanoti*, sect. *Squamiveneti* and sect. *Veneti* (Bidaud et al., 2021; Ammirati et al., 2021). *Cortinarius yadingensis* lacks olive tints, its pileus and stipe surfaces are covered by densely dark brown fibrillose squamules, which are found in a few *Leprocyebe* species lacking olive coloration, such as *C. pescolanensis* (Picillo & Marchionni, 2016), but phylogenetic analysis does not support a close relationship between *C. yadingensis* and *C. pescolanensis*.

Compared with the known diversity of *Leprocyebe* in North America and Europe (Bidaud et al. 2021; Ammirati et al. 2021), only 3 species were previously reported from China.

*Cortinarius cotoneus* was reported by Keissler & Hohwag (1937) in Yunnan according to a collection of 1914. Horak (1987) reexamined the specimen and confirmed its morphological identification. Shao & Xiang (1997) reported *C. cotoneus* and *C. venetus* but there are lacking specimen citations. Sequences from materials of China and East Asia belonging to this lineage with doubtful annotations in GenBank were also included in the phylogenetic analysis (GenBank ID: HM105543, KX444284, KX513578, KX513580, KX513584-KX513586, LC373240), these sequences formed multiphyletic clades near *C. cotoneus* and *C. venetus*, which may represent some unknown new species. Unfortunately, the sequenced Chinese materials (e.g., HMAS260331, HMAS254210, HMAS274611, HMAS274352 and HMAS268596) are dried specimens without any in situ photos, which cannot provide sufficient morphological characteristics for the introduction of new species. Xie (2022) reported *Cortinarius nigrosquamosus* and identified it as a member of *Leprocyebe* for its morphological evidence of yellowish to olivaceous basidiomata with black squamules. Although the ITS sequence of the studied specimens is the same, there are some morphological variations among the three specimens. Due to the unanswered questions on all the three *Leprocyebe* species historically reported in China, more samplings are still needed to accumulate sufficient data to clarify the diversity of *Leprocyebe* in China.

## Conclusion

Based on morphological and molecular evidence, *Cortinarius henduanensis* and *C. yadingensis*, are proposed as new species in *Cortinarius* subgenus *Leprocyebe*. Both species occur in mixed forest of Southwest China. *Cortinarius henduanensis* has distinct olive tinged basidiomata, squamulose pileus, and small, subglobose to broadly ellipsoid basidiospores. *Cortinarius yadingensis* has a brownish squamulose pileus and stipe, subglobose to broadly ellipsoid, coarsely verrucose basidiospores. Further research of the *Leprocyebe* diversity in China is needed for the likely possible of unrevealed new species and unknown questions of

the reported *C. cotoneus*, *C. venetus* and *C. nigrosquamosus*.

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