

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

Peng Hong^{1,2,*}, Ke Wang^{2,*}, Zhuo Du², Meng-Le Xie³, Di Liu¹, Tie-Zheng Wei²

¹Department of Horticulture and Landscape Architecture, College of Agriculture, Yanbian University, Yanji, 133002, Jilin, China.

²State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, 100101, China.

³School of Food Science and Engineering, Yangzhou University, Yangzhou, 225127, Jiangsu, China.

* These authors contributed equally to this work.

Corresponding Author^s:

Di Liu¹

No.977 Gongyuan Road, Yanji, Yanbian Chosenzu Aut. Prefecture, Jilin 133002, China.

Tie-Zheng Wei²

NO.1 Beichen West Road, Chaoyang District, Beijing 100101, China

Email address: liudi@ybu.edu.cn (D. Liu); weitiezheng@163.com (T.Z. Wei)

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Di Liu¹

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Tie-Zheng Wei²

NO.1 Beichen West Road, Chaoyang District, Beijing 100101, China

E-mail address: liudi@ybu.edu.cn (D. Liu); weitiezheng@163.com (T.Z. Wei)

Abstract

Two new *Cortinarius* species in subgenus *Leprocye*, *Cortinarius* ~~*henduanensis*~~ and *C. yadingensis*, are proposed based on a combination of morphological and molecular evidences. *Cortinarius henduanensis* has distinct olive tinged basidiomata, a squamulose pileus, and small, subglobose to broadly ellipsoid basidiospores. *Cortinarius yadingensis* has a squamulose pileus~~squamulose pileus and stipe, and the~~ subglobose to broadly ellipsoid coarsely verrucose basidiospores with strongly warts. Both of the two new species were found in from Southwest China. The morphological characteristics of subgenus *Leprocye* are also discussed.

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

Introduction

Cortinarius (Pers.) Gray is the largest genus in Agaricales (Basidiomycota) and is distinguished from other agarics by its arachnoid partial veil, together with rust brown and verrucose basidiospores (Moser & Horak, 1975). It is an extremely complex genus with more

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Commented [JA2]: You need to read this article and recognize that *Leprocye* is currently a subgenus in the genus *Cortinarius*, but that there are now a number of genera in the *Cortinariaceae*. Start with the family and work your way to *Cortinarius* then *Leprocye*.

than 5800 names documented in Index Fungorum (<http://www.indexfungorum.org/Names/Names.asp>), and over 2600 current species recorded in Catalogue of Life (<https://www.catalogueoflife.org>). The type locality of about 2000 *Cortinarius* species were reported from Europe and North America (data derived from Fungal Names, <https://nmdc.cn/fungalnames>) and most of them were reported from Europe and North America. However, its diversity and distribution in Asia are still poorly understood.

Moser (1969) proposed subgen. *Leprocye* M.M. Moser with five sections, sect. *Bolares* Kühner & Romagn. ex M.M. Moser, sect. *Brunneotincti* Kühner & Romagn. ex M.M. Moser, sect. *Leprocye* M.M. Moser, sect. *Limonii* M.M. Moser ex Nezdobjm. and sect. *Orellani* M.M. Moser. *Cortinarius* species with fluorescence reaction under ultraviolet light, e.g. *Cortinarius* *C. cotoneus* Fr., *C. limonii* (Fr.) Fr., *C. orellanus* Fr. and *C. venetus* (Fr.) Fr., were placed in the subgenus, and *C. cotoneus* was designated as type species respectively.

Peintner et al. (2004) found subgen. *Leprocye* is non-monophyletic based on molecular phylogenetic analysis, and a similar result was obtained by Harrower et al. (2011). Soop (2019) proposed two new sections of the subgenus, sect. *Persplendidi* Soop & Dima and sect. *Veronicae* Soop. Based on morphological and molecular data, Ammirati et al. (2021) proposed three new sections, ~~section taxa~~, sect. *Fuscotomentosi* Niskanen, Liimat. & Ammirati, sect. *Melanoti* Niskanen, Liimat. & Ammirati and sect. *Squamiveneti* Niskanen, Liimat. & Ammirati) and 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 (included three new species) from the Mediterranean, and confirmed 23 synonymies. Liimatainen et al. (2022) re-defined subgen. *Leprocye* based on result from analysis of five genes, and seven sections, such as sect. *Fuscotomentosi*, sect. *Leprocye*, sect. *Melanoti*, sect. *Persplendidi*, sect. *Squamiveneti*, sect. *Veneti* and sect. *Veronicae*, were included in the subgenus, sect. *Limonii* was placed in a new genus, *Aureonarius* Niskanen & Liimat.

In China, Keissler & Hohwag (1937) firstly reported *C. cotoneus* from Yunnan Province. Shao & Xiang (1997) documented *C. venetus* from Heilongjiang Province of Northeast China. Xie (2022) reported *Cortinarius* *C. nigrosquamosus* from Yunnan. In a survey of *Cortinarius* species in southwest of China, two undescribed species of subgenus *Leprocye* ~~was were~~ found based on morphologically and phylogenetic analysis. These species are described below with photos of morphological and microscopical characteristics. The paper described the two new species and illustrated with both macro-morphology and microscopical characteristics.

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Materials & Methods

Morphological study

Specimens were collected from Xizang Autonomous Region, Sichuan Province and Yunnan

Province, 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 ~~around~~ 45°C. A 20% KOH solution was used on fresh pileus and stipe surfaces, lamella, and context for chemical reaction. Observation of ~~the~~ basidiomata was performed under ultraviolet light at a wave length of 360 nm. The specimens are deposited in ~~housed in the~~ Fungarium, Institute of Microbiology, Chinese Academy of Sciences (also as Herbarium Mycologicum Academiae Sinicae, HMAS). Descriptions of the microscopical micro- ~~morphological~~ characters ~~was based on~~ are from dried collections. Thin sections were prepared by hand with a razor blade. ~~The s~~ Sections of dried basidiomata were mounted in 5% KOH solution. Basidiospores, basidia, tramal hyphae, context, and cutis of pileus and stipe were measured using an ocular micrometer. At least 30 basidiospores and 20 basidia 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. ~~SeventyFifty-three-eight~~ 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., 2008). 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 ~~75-58~~ ITS sequences with ~~684-692~~ 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

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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/NamesRecord.asp?RecordID=>". 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 ~~xxx~~30908; accessed on ~~xx~~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. *Leprocye* and clusters together with *C. contoneus*, *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, ~~D~~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* ~~Mast~~ and *Quercus aquifolioides* ~~Rehder et E. H. Wilson~~, 16 Aug. 2008, T.-Z. Wei, X.-Q. Zhang & F.-Q. Yu 194, HMAS 250455 (GenBank ID: KX513581).

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) ~~and~~ deviate from that of *C. flavifolius* by at least 28 substitutions and indel positions. In mixed forest with *Pinus yunnanensis* Franch., *P. densata* and *Quercus aquifolioides*.

Description:—Pileus 4.5–12.5 cm diam., hemispherical at first, later becoming convex to applanate, ~~with~~ a shallow, ~~and~~ obtuse umbo ~~at center~~ when mature, margin sometimes uplifted or undulate, ~~surface non-viscid~~, uneven, tomentose, persistently covered with small olive-brown to dark olive-brown squamules ~~in all stages~~, 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. Lamellae adnate to emarginate, 3–8 mm wide, ~~close to~~

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moderately crowded, ~~with lamellulae~~, olive to grayish green when young, brown with olive tint to rust-brown when mature, ~~edges margin~~ paler. *Stipe* 5.5–12 × 0.7–2.6 cm, cylindrical or base enlarged and up to 3 cm in diam.; surface ~~rough~~, completely covered with universal veil at first, then longitudinally fibrillose above and with conspicuous ~~finally with~~ annular girdles below; olivaceous yellow, tinged rust-brown from basidiospores; ~~at first solid, then soft to hollow~~. *Context* up to 8 mm thick at pileus center, whitish to pale when dry, olive-gray when moist, fleshy, soft; odor indistinct, taste mild. *Partial veil* arachnoid, greenish to olive, then becoming rust brown from mature basidiospores. *Universal veil* distinct and persistent, fibrillose, covering whole basidiomata at first, then forming fibrils and squamules on pileus and fibrils ~~and, strips or~~ 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) × (4.8–) 5–6 (–6.5) µm, 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 × 8–10 µm, clavate, thin-walled, mostly subhyaline, with four sterigmata. *Lamella edges* heterogeneous, with sterile cells, 20–30 × 6–9 µm, clavate, subhyaline, thin-walled. *Pleurocystidia* absent. Subhymenial layer up to 10 µm thick, of narrow and branched hyphae, hyaline, thin-walled, 2–4 µm diam. *Hymenophoral trama* regular, 80–100 µm wide, of hyaline and thin-walled hyphae, 5–20 µm diam. *Pileipellis* simplex, of subcylindrical ~~hyaline, hyphae~~ 9–15 µm diam., brownish to yellowish brown. ~~Hyphae of the cortina 3–6 µm diam., subhyaline to yellowish, thin-walled.~~ *Clamp connections* present.

Additional Specimens Examined: CHINA, Yunnan, Songming County, Aziying Town, alt. 2000 m, in mixed forest with *Pinus yunnanensis* Franch. 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). The new taxon is close to *C. flavifolius*, the latter also has a fibrillose to squamulose pileus and subglobose basidiospores (Ammirati et al., 2021). ~~However, differs~~

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from *C. hengduanensis*, *C. flavifolius* does not have any olive or greenish tint, and its basidiospores are $6.7\text{--}8.9 \times 4.8\text{--}5.9\ \mu\text{m}$ (Ammirati et al., 2021) and larger than that of *C. hengduanensis*.

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* Batalin, *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 whole basidiomata surface and subglobose to broadly ellipsoid basidiospores ($6.5\text{--}9 \times 6.3\text{--}7.6\ \mu\text{m}$). It differs from its related species by its lack of olivaceous coloration non-olive tinged basidiomata. 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 at center when mature, margin decurrent to straight, sometimes split when mature; surface pale brown, grayish brown to rust brown, densely covered with brown to dark brown fibrillose squamules, non-viscid, radially striate, silky and shining. Lamellae emarginate to adnate, up to 5 mm wide; brownish when young, rust-brown when mature; moderately crowded, with lamellulae, edges margin paler. Stipe $3.5\text{--}8 \times 0.5\text{--}1\ \text{cm}$, subcylindrical, with base up to 1.5 cm in diam.; surface brownish to pale brown, covered densely with brown to dark brown fibrillose squamules, with annular zone from partial veil, non-viscid, longitudinally striate; at first solid then soft to hollow.

Context up to 5 mm thick at pileus center, pale brown to grayish brown, fleshy, soft; odor indistinct, smell mild. Partial veil arachnoid, grayish when young, forming a rust brown ring from basidiospores, coated with basidiospores from basidiospores when mature. Universal veil persistently distinct, forming fibrillose brown to dark brown squamules at on the surface of the stipe basidiomata. Basidiospore deposit rust brown. Chemical reaction with 20% KOH dark brown to blackish brown on pileus, lamella and stipe surface, and reddish brown on at context. Fluorescence reaction under ultraviolet light bright yellow on lamellae, and weaker on surfaces of pileus and stipe.

Basidiospores $(6.5\text{--}) 7\text{--}8.5\text{--}(9) \times (6.3\text{--}) 6.5\text{--}7.2\text{--}(7.6)\ \mu\text{m}$, $Q = (1.03\text{--}) 1.08\text{--}1.2\text{--}(1.24)$ (av.=1.16); subglobose, yellow-brown, distinctly verrucose. Basidia $28\text{--}35 \times 8\text{--}10\ \mu\text{m}$, clavate, thin-walled, mostly subhyaline, with four sterigmata. Lamella edges heterogeneous, with sterile cell hyphae, $20\text{--}30 \times 6\text{--}9\ \mu\text{m}$, clavate, subhyaline, thin-walled. Pleurocystidia

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absent. Subhymenial layer up to 10 µm thick, of narrow and branched hyphae, hyaline, thin-walled, 2–4 µm diam. Hymenophoral trama regular, 80–100 µm wide, of hyaline and thin-walled hyphae, 5–20 µm diam. Pileipellis simplex, of subcylindrical hyphae, hyaline, 9–15 µm diam., brownish to yellowish brown. Hyphae of the cortina 3–6 µm diam., subhyaline to yellowish, thin-walled. Clamp connections present.

Additional Specimens Examined: CHINA, Sichuan, Litang County, road side of road 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 to brown squamulose pileus and stipe and with distinct squamules, and subglobose, to distinctly verrucose ellipsoid basidiospores (6.5–9 × 6.3–7.6 µm) with distinct warts. The species is phylogenetically close to four species of sect. *Leproclybe*, *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. However, differs from *C. yadingensis*, all 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. *Leproclybe* was re-defined as *Cortinarius* species with small- to medium-sized (occasionally large-sized) basidiomata, obvious UV fluorescent reaction, tomentose to squamulose pileus and subglobose basidiospores, and all these unique characteristics can be found in both *C. hengduanensis* and *C. yadingensis*. *Cortinarius hengduanensis* has olive coloration appearance, which was also found in most of species in sect. *Fuscotomentosi*, sect. *Leproclybe*, sect. *Melanoti*, sect. *Squamiveneti* and sect. *Veneti* (Bidaud et al., 2021; Ammirati et al., 2021). *Cortinarius yadingensis* does not have any olive tint, its pileus and stipe surfaces are covered by densely dark brown fibrillose squamules, which are found in a few *Leproclybe* species lacking olive coloration tint, such as *C. pescolanensis* (Picillo & Marchionni, 2016), but phylogenetic analysis of the present research does not support close relationship between *C. yadingensis* and *C. pescolanensis*.

Compared with a relatively richer the known diversity of *Leproclybe* with more than 10 species in North America and Europe-Mediterranean (Bidaud et al. 2021; Ammirati et al. 2021), there are only 3 species were previously reported documented in from China before.

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Cortinarius cotoneus was ~~firstly~~ 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 ~~they 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~~~~revealed~~ 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*, ~~even~~ *Cortinarius* in China.

Conclusion

Based on morphological and molecular evidence, *Cortinarius henduanensis* and *C. yadingensis*, are proposed in *Cortinarius* subgenus *Leprocyebe*. Both ~~two~~ species were reported in mixed forest of Southwest China. *Cortinarius henduanensis* has distinct olive tinged basidiomata, squamulose pileus, and small, subglobo~~idse~~ to broadly ellipsoid basidiospores. *Cortinarius yadingensis* has a squamulose pileus and stipe, subglobo~~idse~~ to broadly ellipsoid, ~~coarsely verrucose~~ basidiospores ~~with strongly warts~~. Further ~~Deeper~~ research of the *Leprocyebe* diversity in China is needed for the likely possible of ~~unknown~~~~revealed~~ new species and unanswered questions of the reported *C. cotoneus*, *C. venetus* and *C. nigrosquamosus*.

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References

- Ammirati J, Liimatainen K, Bojantchev D, Peintner U, Kuhnert-Finkernagel R, Cripps C, Dentinger B, Niskanen T. 2021. *Cortinarius* subgenus *Leprocybe*, unexpected diversity and significant differences in species compositions between western and eastern North America. *Persoonia* 46: 216–239.
- Ballara J, Mahiques R, Garrido-Benavent I. 2017. Estudi de Cortinariaceae del Parc Natural Cadi-Moixeró (IV). *Moixeró* 9: 20–49.
- Bidaud A, Carteret X, Eyssartier G, Moënné-Loccoz P, Reumaux P. 2005. Atlas des Cortinaires 15: 983–1058.
- Bidaud A, Loizides M, Armada F, de Dios Reyes J, Carteret X, Corriol G, Consiglio G, Reumaux P, Bellanger J-M. 2021. *Cortinarius* subgenus *Leprocybe* in Europe: expanded Sanger and Next Generation Sequencing unveil unexpected diversity in the Mediterranean. *Persoonia* 46: 188–215.
- Breitenbach J, Kränzlin F. 2000. Fungi of Switzerland. Vol. 5: Agarics 3rd part. Cortinariaceae. Luzern, Switzerland: Verlag Mykologia Luzern.
- Danks M, Lebel T, Vernes K. 2010. 'Cort short on a mountaintop' - eight new species of sequestrate *Cortinarius* from sub-alpine Australia and affinities to sections within the genus. *Persoonia* 24: 106–126.
- Hall TA. 1999. Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Harrower E, Ammirati J, Cappuccino A, Ceska O, Kranabetter JM, Kroeger P, Lim S, Taylor T. 2011. *Cortinarius* species diversity in British Columbia and molecular phylogenetic comparison with European specimen sequences. *Botany* 89: 799–810.
- [Horak E. 1987. Boletales and Agaricales \(Fungi\) from northern Yunnan, China I. Revision of material collected by H. Handel-Mazzetti \(1914-1916\) in Lijiang. *Acta Botanica Yunnanica* 1987\(01\): 65–80.](#)
- Keissler K, Hohweg H. 1937. Fungi in Handel-Mazzetti. *Symbolae Sinicae* 2: 1–83.
- Konrad P, Maublanc A. 1937. *Icones Selectae Fungorum* 6.
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* 35:1547–1549.
- Liimatainen K. 2014. Nomenclatural novelties. *Index Fungorum*. 196: 1–2.
- Liimatainen K, Kim JT, Pokorny L, Kirk P, Dentinger B, Niskanen T. 2022. Taming the beast: a revised classification of Cortinariaceae based on genomic data. *Fungal Diversity* 112:

89–170.

- Moser MM. 1969. *Cortinarius* Fr. Untergattung *Leprocybe* sungen. nov. Die Rauhköpfe Zeitschrift für Pilzkunde 35(3, 4): 213–248.
- Moser MM, Horak E. 1975. *Cortinarius* Fr. und nahe verwandte Gattungen in Südamerika. Beihefte zur Nova Hedwigia 52: 1–628.
- Nylander JAA. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University. ampignons de l'Équateur (Pugillus IV). Bulletin de l'Herbier Boissier 3: 53–74.
- Peintner U, Moncalvo JM, Vilgalys R. 2004. Toward a better understanding of the infrageneric relationships in *Cortinarius* (Agaricales, Basidiomycota). Mycologia 96: 1042–1058.
- Picillo B, Marchionni M. 2016. *Cortinarius pescolanensis* (Basidiomycota, Cortinariaceae), una nuova specie dal Molise (Italia). Micologia e Vegetazione Mediterranea 31: 61–74.
- Rogers SO, Bendich AJ. 1994. Extraction of DNA from plant, fungal and algal tissues. In: Gelvin, SB, Schilperoort RA (Ed.). Plant Molecular Biology Manual, Vol. D1. Boston: Kluwer Academic Publishers, pp. 1–8.
- Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.
- Shao L-P, Xiang C-T. 1997. Forest mushroom of China. Harbin, China: Northeast Forestry University Press.
- Soop K, Dima B, Cooper J A, Oertel B. 2019. A phylogenetic approach to a global supraspecific taxonomy of *Cortinarius* (Agaricales) with an emphasis on the southern mycota. Persoonia 42: 261–290.
- White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes from phylogenetics. In: Innes MA, Gelfand DH, Sninsky JS, White TJ (Ed.). PCR protocols: methods and applications. London: Academic Press, pp. 315–322.
- Xie M-L. 2022. Taxonomic, Molecular Phylogenetic and Biogeographic Studies of *Cortinarius* in China. [PhD Dissertation]. Changchun, China: Northeast Normal University.