# Two new *Cortinarius* species in subgenus *Leprocybe* from Southwest China (#92333)

First submission

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

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

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# Two new Cortinarius species in subgenus Leprocybe from

## 2 Southwest China

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# 20 Two new Cortinarius species in subgenus Leprocybe

## 21 from Southwest China

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### 40 Abstract

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

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49 **Keywords:** Basidiomycota Lina; phylogeny; new taxa; taxonomy

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### Introduction

52 Cortinarius (Pers.) Gray is the largest genus in Agaricales (Basidiomycota) and distinguished



- 53 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 than 5800 54
- names documented in Index Fungorum (http://www.indexfungorum.org/Names/Names.asp), and 55
- most of them were reported from Europe and North America. However, its diversity and 56
- 57 distribution in Asia are still poorly understood.
- Moser (1969) proposed subgen. Leprocybe M.M. Moser with five sections, sect. Bolares Kühner 58
- 59 & Romagn. ex M.M. Moser, sect. Brunneotincti Kühner & Romagn. ex M.M. Moser, sect.
- Leprocybe M.M. Moser, sect. Limonii M.M. Moser ex Nezdojm. and sect. Orellani M.M. Moser. 60
- 61 Cortinarius species with fluorescence reaction under ultraviolet light, e.g. C. cotoneus Fr., C.
- limonius (Fr.) Fr., C. orellanus Fr. and C. venetus (Fr.) Fr., were placed in the genus, and C. 62
- cotoneus was designated as type species. Peintner et al. (2004) found subgen. Leprocybe is non-63
- monophyletic base on molecular phylogenetic analysis, and similar result was obtained by 64
- 65 Harrower et al. (2011). Soop (2019) proposed two new sections of the subgenus, sect.
- Persplendidi Soop & Dima and sect. Veronicae Soop. Base on morphological and molecular data, 66
- Ammirati et al. (2021) proposed three new sections (sect. Fuscotomentosi Niskanen, Liimat. & 67
- Ammirati, sect. *Melanoti* Niskanen, Liimat. & Ammirati and sect. *Squamiveneti* Niskanen, 68
- Liimat. & Ammirati) and 11 new species from North America. The paper also validated sect. 69
- 70 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 71
- 72 of the subgenus (included three new species) from Mediterranean, and confirmed 23 synonymies.
- Liimatainen et al. (2022) re-defined subgen. Leprocybe base on result from analysis of five genes, 73
- 74 and seven sections, such as sect. Fuscotomentosi, sect. Leprocybe, sect. Melanoti, sect.
- 75 Persplendidi, sect. Squamiveneti, sect. Veneti and sect. Veronicae, were included in the subgenus,
- 76 sect. Limonii was placed in a new genus, Aureonarius Niskanen & Liimat.
- In China, Keissler & Hohwag (1937) firstly reported C. cotoneus from Yunnan Province. Shao & 77
- Xiang (1997) documented C. venetus from Heilongjiang Province. Xie (2022) reported 78
- 79 Cortinarius nigrosquamosus from Yunnan. In a survey of Cortinarius species in southwest of
- 80 China, two undescribed species of subgenus *Leprocybe* was no and base on morphology and
- phylogenetic analysis. The paper exscribed the two new species and illustrated with both macro-81
- morphology and microscopic characteristics. 82

## **Materials & Methods**

Morphological study 84

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- Specimens were collected from Xizang Autonomous Region, Sichuan Province and Yunnan 85
- 86 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. 87



- 88 A 20% KOH solution was used on fresh pileus and stipe surfaces, lamella, and context for
- 89 chemical reaction. Observation of the basidiomata was performed under ultraviolet light at a
- wave length of 360 nm. The specimens are housed in the Fungarium, Institute of Microbiology,
- 91 Chinese Academy of Sciences (also as Herbarium Mycologicum Academiae Sinicae, HMAS).
- 92 Description of the micro-morphologica aracters was based on it decilections. Thin sections
- were prepared by hand with a razor blade. The sections of dried basidiomata were mounted in 5%
- 84 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
- 96 collection were measured.
- 97 DNA extraction, amplification and sequencing
- 98 Genomic DNA was extracted from dried specimens using standard protocol (Rogers &
- 99 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
- 102 following conditions: initial denaturation for 3 min at 95 °C, followed by 30 cycles, at 95 °C for
- 103 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.
- 104 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.
- 107 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., 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 ITS sequences with 681 nucleotide sites is available from
- TreeBASE under S30908 (http://purl.org/phylo/treebase/phylows/study/TB2:S30908).
- 115 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
- 119 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
- 121 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 "



- 124 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
- 126 Central SCIE, and CLOCKSS.

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### Results

- 129 *Molecular phylogeny*
- 130 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;
- accessed on xx 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.
- 135 Cortinarius hengduanensis has a close relationship with C. flavifolius Peck, C. yadingensis nests
- in a clade of sect. Leprocybe and clusters together with C. contoneus, C. hughesiae Ammirati,
- 137 Matheny, Liimat. & Niskanen, C. selinolens Bidaud & Bellanger and C. subcotoneus Bidaud.
- 138 Taxonomy
- 139 Cortinarius hengduanensis P. Hong, K. Wang, Z. Du, M.L. Xie, D. Liu & T.Z. Wei, sp. nov.
- 140 Figure 2.
- 141 **Index Fungorum:** IF901373.
- 142 Holotype:—CHINA, Yunnan, Shangri-La County, Haba Mountains Nature Reserve, alt. 3000
- m, in mixed forest with *Pinus densata* Mast. and *Ouercus aquifolioides* Rehder et E. H. Wilson,
- 144 16 Aug. 2008, T.-Z. Wei, X.-Q. Zhang & F.-Q. Yu 194, HMAS 250455 (GenBank ID:
- 145 KX513581).
- 146 **Etymology:—**"hengduan", Chinese, referring Hengduan Mountains of Southwest China, the
- locality of the type collection.
- 148 **Diagnosis:**—Cortinarius hengduanensis has olive tinged basidiomata, squamulose pileus, and
- small subglobose to broadly ellipsoid basidiospores (5.5–7.5  $\times$  5–6.5  $\mu$ 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
- 152 *yunnanensis* Franch., *P. densata* and *Ouercus* aguifolioides.
- 153 **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
- center. Lamellae adnate to emarginate, 3–8 mm wide; close to moderately crowded, with



- lamellulae, olive to grayish green when young, brown with olive tint to rust-brown when mature,
- margin paler. Stipe  $5.5-12 \times 0.7-2.6$  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
- 163 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.
- 166 Universal veil distinct and persistent, fibrillose, covering whole basidiomata at first, then
- forming fibrils and squamules on pileus and fibrils, 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.
- 172 Basidiospores (5.2–) 5.5–7 (–7.5) × (4.8–) 5–6 (–6.5)  $\mu$ m, Q= (1.08–) 1.1–1.2 (–1.23) (av.=1.15);
- subglobose to broadly ellipsoid, yellowish brown, moderately to distinctly verrucose. *Basidia*
- $28-35 \times 8-10 \,\mu m$ , clavate, thin-walled, mostly subhyaline, with four sterigmata. Lamella edges
- heterogeneous, with sterile cells,  $20-30 \times 6-9 \mu m$ , clavate, subhyaline, thin-walled.
- 176 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 hyalin 178 μm
- diam., brownish to yellowish brown. Hyphae of the cortina 3–6 µm diam., subhyaline to
- 180 yellowish, thin-walled. *Clamp connections* present.
- 181 Additional Specimens Examined: CHINA, Yunnan, Songming County, Aziying Town, alt.
- 182 2000 m, in mixed forest with *Pinus yunnanensis* Franch. and *Quercus aquifolioides*, 5 Aug. 2005,
- 183 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 *O. aquifolioides*, 19 Aug.
- 185 2008, T.-Z. Wei, X.-Q. Zhang & F.-Q. Yu 258, HMAS 250509 (GenBank ID: KX513583);
- 186 **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 &
- 188 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).
- 190 **Notes:** Cortinarius hengduanensis is characterized by its distinct olive tinged basidiomata,
- fibrilloso-squamulose pileus and subglobose to broadly ellipsoid basidiospores  $(5.2-7.5 \times 4.8-$
- 192 6.5 μm). The new taxon is close to *C. flavifolius*, the latter also has fibrillose to squamulose
- pileus and subglobose basidiospores (Ammirati et al.,2021). However, differs from C.
- 194 hengduanensis, C. flavifolius does not have any olive or greenish tint, and its basidiospores are



- 195 6.7–8.9  $\times$  4.8–5.9  $\mu$ m (Ammirati et al.,2021) and larger than that of *C. hengduanensis*.
- 196 Cortinarius yadingensis P. Hong, K. Wang, Z. Du, M.L. Xie, D. Liu & T.Z. Wei, sp. nov.
- 197 Figure 3.
- 198 **Index Fungorum:** IF901374.
- 199 Holotype:—CHINA, Sichuan, 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
- 201 Wei, L-H Sun, Z-X Wu & R-C Zhang, 7168, HMAS 254819 (GenBank ID: OR538892).
- 202 **Etymology:**—"yading", Chinese, referring Yading Scenic Spot of Sichuan Province, China, the
- 203 locality of the type collection.
- 204 **Diagnosis:**—Cortinarius yadingensis has distinct brown squamules on whole basidiomata
- surface and subglobose to broadly ellipsoid basidiospores  $(6.5-9 \times 6.3-7.6 \,\mu\text{m})$ . Figure 1205 from its
- 206 related species by its monolive tinged basidiomata. ITS sequences of the new species (GenBank
- 207 ID: OR53889–OR538893) are distinct from other members of sect. *Leprocybe* and deviating
- from them by at least 11 substitutions and indel positions. In mixed forest with *Abies* sp., *Larix*
- 209 potaninii, Picea sp. and Q. aquifolioides.
- 210 **Description:**—*Pileus* 2–6 cm diam., hemispherical at first, later becoming convex to applanate,
- 211 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 th
- brown to dark brown fibrillose squamules, non-viscid, radially striate, silky and shining.
- 214 Lamellae emarginato-adnate, up to 5 mm wide; brownish when young, rust-brown when mature;
- 215 moderately crowded, with lamellulae, margin paler. Stipe  $3.5-8 \times 0.5-1$  cm, subcylindrical, with
- base up to 1.5 cm in diam.; surface brownish to pale brown, densely with brown to dark brown
- 217 fibrillose squamules, with annular zone from partial veil, non-viscid, longitudinally striate; at
- 218 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, form
- rust brown ring from basidiospores when mature. *Universal veil* persistently distinct, form
- fibrillose brown to dark brown squamules at the face of basidiomata. Basidiospore deposit rust
- brown. Chemical reaction with 20% KOH dark brown to blackish brown on pileus, lamella and
- stipe surface, and reddish brown at context. Fluorescence reaction under ultraviolet light bright
- vellow on lamellae, and weaker on surfaces of pileus and stipe.
- 225 Basidiospores (6.5–) 7–8.5 (–9) × (6.3–) 6.5–7.2 (–7.6)  $\mu$ m, Q= (1.03–) 1.08–1.2 (–1.24)
- (av.=1.16); subglobose, yellow-brown, distinctly verrucose. Basidia  $28-35 \times 8-10 \mu m$ , clavate,
- 227 thin-walled, mostly subhyaline, with four sterigmata. Lamella edges heterogeneous, with sterile
- 228 eells 21–30 × 6–9 μm, clavate, subhyaline, thin-walled. *Pleurocystidia* absent. *Subhymenial*
- 229 layer up to 10 μm thick, of narrow and branched hyphae, hyaline, thin-walled, 2–4 μm diam.
- 230 Hymenophoral trama regular, 80–100 μm wide, of hyaline and thin-walled hyphae, 5–20 μm



- diam. *Pileipellis* simplex, of subcylindrical hyalin—15 μm diam., brownish to yellowish
- brown. *Hyphae of the cortina* 3–6 µm diam., subhyaline to yellowish, thin-walled. *Clamp*
- 233 connections present.
- 234 Additional Specimens Examined: CHINA, Sichuan, Litang County, side of road to Daocheng
- 235 County, alt. 3937 m, in conifer forest with *Abies* sp. and *Picea* sp., 17 Aug. 2016, T-Z Wei, L-H
- 236 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*.
- 238 aquifolioides, 18 Aug. 2016, T-Z Wei, L-H Sun, Z-X Wu & R-C Zhang, 7169, HMAS 254820
- 239 (GenBank ID: OR538893); 7123, HMAS 280697 (GenBank ID: OR538890); 7127, HMAS
- 240 280698 (GenBank ID: 254819).
- Notes: Cortinarius yadingensis is characterized by its grayish brown to brown pileus and stipe
- with distinct squamules, and subglobose to ellipsoid 3 sidiospores (6.5–9 × 6.3–7.6 µm) with
- 243 distinct warts. The species is phylogenetically close to four species of sect. Leprocybe, C.
- 244 cotoneus (Breitenbach & Kränzlin 2000, Soop, 2018), C. hughesiae Ammirati, Matheny, Liimat.
- 245 & Niskanen (Ammirati et al., 2021), C. selinolens Bidaud & Bellanger (Bidaud et al., 2021) and C.
- 246 subcotoneus (Bidaud et al., 2005). Compared with C. yadingensis, all the four related species
- 247 have similar finely tomentose to squamulose pileus and strongly verrucose basidiospores.
- 248 However, differs from C. yadingensis, all the related species have olive tinged basidiomata.
- 249 Phylogenetically, C. yadingensis can be distinguished from its sister species by at least 11
- 250 substitutions and indels in ITS.

### Discussion

- According to Bidaud et al. (2021), Ammirati et al. (2021) and Liimatainen et al. (2022), subgen.
- 253 Leprocybe was re-defined as Cortinarius species with small- to medium-sized (occasionally
- 254 large-sized) basidiomata, obvious fluorescent reaction, tomentose to squamulose pileus and
- 255 subglobose basidiospores, and all these unique characters can be found in both of C.
- 256 hengduanensis and C. yadingensis. Cortinarius hengduanensis has olive appearance, which was
- 257 also found in most of species in sect. Fuscotomentosi, sect. Leprocybe, sect. Melanoti, sect.
- 258 Squamiveneti and sect. Veneti (Bidaud et al., 2021; Ammirati et al., 2021). Cortinarius yadingensis
- does not has any olive tint, its pileus and stipe surfaces are covered by densely dark brown
- 260 fibrillose squamules, which are found in few *Leprocybe* species lacking olive tint, such as C.
- 261 pescolanensis (Picillo & Marchionni, 2016), but phylogenetic analysis of the present research
- does not support close relationship between C. vadingensis and C. pescolanensis.

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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.
- 274 Persoonia 46: 216–239.
- Ballara J, Mahiques R, Garrido-Benavent I. 2017. Estudi de Cortinariaceae del Parc Natural
- 276 Cadi-Moixero (IV). Moixero 9: 20–49.
- 277 Bidaud A, Carteret X, Eyssartier G, Moënne-Loccoz P, Reumaux P. 2005. Atlas des Cortinaires
- 278 15: 983–1058.
- 279 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.
- 282 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.
- 287 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.
- 290 Harrower E, Ammirati J, Cappuccino A, Ceska O, Kranabetter JM, Kroeger P, Lim S, Taylor T.
- 291 2011. *Cortinarius* species diversity in British Columbia and molecular phylogenetic
- comparison with European specimen sequences. Botany 89: 799–810.
- 293 Keissler K, Hohwag H. 1937. Fungi in Handel-Mazzetti. Symbolae Sinicae 2: 1–83.
- 294 Konrad P, Maublanc A. 1937. Icones Selectae Fungorum 6.
- 295 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–
- 297 1549.
- 298 Liimatainen K. 2014. Nomenclatural novelties. Index Fungorum. 196: 1–2.
- 299 Liimatainen K, Kim JT, Pokorny L, Kirk P, Dentinger B, Niskanen T. 2022. Taming the beast: a



300	revised classification of Cortinariaceae based on genomic data. Fungal Diversity 112: 89-
301	170.
302	Moser MM. 1969. Cortinarius Fr. Untergattung Leprocybe sungen. nov. Die Rauhköpfe
303	Zeitschrift für Pilzkunde 35(3, 4): 213–248.
304	Moser MM, Horak E. 1975. Cortinarius Fr. und nahe verwandte Gattungen in Südamerika.
305	Beihefte zur Nova Hedwigia 52: 1–628.
306	Nylander JAA. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology
307	Centre, Uppsala University. ampignons de l'Équateur (Pugillus IV). Bulletin de l'Herbier
308	Boissier 3: 53–74.
309	Peintner U, Moncalvo JM, Vilgalys R. 2004. Toward a better understanding of the infrageneric
310	relationships in Cortinarius (Agaricales, Basidiomycota). Mycologia 96: 1042-1058.
311	Picillo B, Marchionni M. 2016. Cortinarius pescolanensis (Basidiomycota, Cortinariaceae), una
312	nuova specie dal Molise (Italia). Micologia e Vegetazione Mediterranea 31: 61-74.
313	Rogers SO, Bendich AJ. 1994. Extraction of DNA from plant, fungal and algal tissues. In:
314	Gelvin, SB, Schilperoort RA (Ed.). Plant Molecular Biology Manual, Vol. D1. Boston:
315	Kluwer Academic Publishers, pp. 1–8.
316	Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed
317	models. Bioinformatics 19: 1572–1574.
318	Shao L-P, Xiang C-T. 1997. Forest mushroom of China. Harbin, China: Northeast Forestry
319	University Press.
320	Soop K, Dima B, Cooper J A, Oertel B. 2019. A phylogenetic approach to a global supraspecific
321	taxonomy of Cortinarius (Agaricales) with an emphasis on the southern mycota. Persoonia
322	42: 261–290.
323	White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal
324	ribosomal RNA genes from phylogenetics. In: Innes MA, Gelfand DH, Sninsky JS, White
325	TJ (Ed.). PCR protocols: methods and applications. London: Academic Press, pp. 315–322.
326	Xie M-L. 2022. Taxonomic, Molecular Phylogenetic and Biogeographic Studies of Cortinarius
327	in China. [PhD Dissertation]. Changchun, China: Northeast Normal University.
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# Table 1(on next page)

ITS sequences of subgen. Leprocybe used in phylogenetic analysis.

Note: \* means from this study.



Table 1. ITS sequences of subgen. Leprocybe used in phylogenetic analysis.

Table 1. ITS seque	nces of subgen. <i>Leproc</i>	<i>ybe</i> used in	i phylogenetic a	nalysis.
Name	Voucher	Locality	GenBank ID	References
Cortinarius clandestinus	B. Dentinger 325	USA	MW009208	Ammirati et al. 2021
C. clandestinus	SMI200A	Canada	FJ039583	Harrower et al. 2011
C. clandestinus	SAT03-137-02	USA	FJ717552	Harrower et al. 2011
C. clandestinus	SMI24	Canada	FJ157136	Harrower et al. 2011
C. clandestinus	JFA 10285	USA	MW009201	Ammirati et al. 2021
C. clandestinus	T. Niskanen 11-454	USA	MW009204	Ammirati et al. 2021
C. clandestinus	JFA 13263	USA	MW009203	Ammirati et al. 2021
C. clandestinus	JFA 8986	USA	MW009202	Ammirati et al. 2021
C. clandestinus	M. Moser 1995-165a	USA	MW009206	Ammirati et al. 2021
C. cotoneus	PML 5260	France	MW010117	Bidaud et al. 2021
C. cotoneus	PML 5429	France	MW010116	Bidaud et al. 2021
C. cotoneus	CFP1032	Sweden	MW009216	Ammirati et al. 2021
C. flavifolius	TENN:068695 (epitype)	USA	MW009217	Ammirati et al. 2021
C. flavifolius	256	USA	MW009238	Ammirati et al. 2021
C. flavifolius	T. Niskanen 14-227	USA	MW009218	Ammirati et al. 2021
C. fuscoflavidus	JFA 11644	USA	MW009221	Ammirati et al. 2021
C. fuscoflavidus	T. Niskanen 09-158	USA	MW009222	Ammirati et al. 2021
C. fuscoflavidus	DBB41055	USA	MW009223	Ammirati et al. 2021
C. hengduanensis	HMAS250455 (holotype)	China	KX513581	*
C. hengduanensis	HMAS145537	China	KX513582	*
C. hengduanensis	HMAS250509	China	KX513583	*
C. hengduanensis	HMAS272520	China	OR538887	*
C. hengduanensis	HMAS270305	China	OR538888	*
C. hughesiae	JFA 13086	USA	MW009224	Ammirati et al. 2021
C. hughesiae	TENN:068689	USA	MW009225	Ammirati et al. 2021
C. leproleptopus	R. Henry 8409	France	MW009226	Ammirati et al. 2021
C. leproleptopus	ST40	Italy	MW010181	Bidaud et al. 2021
C. leproleptopus	AB 08-10-395	France	MW010171	Bidaud et al. 2021
C. leproleptopus	AB 08-10-320	France	MW010166	Bidaud et al. 2021
C. leproleptopus	AB 08-09-215	France	MW010165	Bidaud et al. 2021
C. leproleptopus	AB 08-09-199	France	MW010164	Bidaud et al. 2021
C. leproleptopus	GE01-049	France	MW010150	Bidaud et al. 2021
C. leproleptopus	PML 5420	France	MW010133	Bidaud et al. 2021
C. leproleptopus	FR2312003	France	MW010065	Bidaud et al. 2021
C. leproleptopus	GS9	Germany	MW010100	Bidaud et al. 2021
C. leproleptopus	GS8	Germany	MW010099	Bidaud et al. 2021
C. leproleptopus	GS4	Germany	MW010095	Bidaud et al. 2021
C. leproleptopus C. lutescens	GS1 f1781	Germany USA	MW010092	Bidaud et al. 2021 Ammirati et al. 2021
C. lutescens	H:7000893	Canada	MW009228	Ammirati et al. 2021
C. pescolanensis	MCVE 29054 (holotype)	Italy	MW009229 NR 153070	Picillo & Marchionni 2016
C. pescolanensis	BP13/291	Italy	KX010945	Picillo & Marchionni 2016
C. pescolanensis	JB-8114/13	Spain	KY657256	Ballara et al. 2017
C. pescolanensis	JMB2013092404	France	MW010107	Bidaud et al. 2021
C. pescolanensis	PML 3262	France	MW010107 MW010129	Bidaud et al. 2021
C. pescolanensis	PAM02082506	France	MW010129	Bidaud et al. 2021
C. pescolanensis	JMB2013092503	France	MW010109	Bidaud et al. 2021
C. pescolanensis	PML 5449	France	MW010140	Bidaud et al. 2021
C. pescolanensis	PML 5426	France	MW010135	Bidaud et al. 2021
C. pescolanensis	PML 5448	France	MW010139	Bidaud et al. 2021
C. selinolens	MPU1116858	France	MW010172	Bidaud et al. 2021
C. selinolens	FR2013185	Tunisia	MW010072	Bidaud et al. 2021
C. subcotoneus	PML 2143 (holotype)	France	MW010122	Bidaud et al. 2021
C. subcotoneus	AB 08-10-331	France	MW010167	Bidaud et al. 2021
C. subcotoneus	AB 08-10-387	France	MW010168	Bidaud et al. 2021
C. subcotoneus	AB 08-10-394	France	MW010170	Bidaud et al. 2021
C. subcotoneus	GS7	Germany	MW010098	Bidaud et al. 2021
C. subcotoneus	GS15	Germany	MW010106	Bidaud et al. 2021
C. veneto-occidentalis	T. Niskanen 11-051	USA	MW009243	Ammirati et al. 2021
C. veneto-occidentalis	T. Niskanen 11-053	USA	MW009246	Ammirati et al. 2021
C. veneto-occidentalis	T. Niskanen 11-281	Canada	MW009248	Ammirati et al. 2021
C. veneto-occidentalis	T. Niskanen 11-258	USA	MW009245	Ammirati et al. 2021





C. veneto-occidentalis	T. Niskanen 11-106	USA	MW009244	Ammirati et al. 2021
C. venetus	CFP112	Sweden	MW009250	Ammirati et al. 2021
C. venetus	PC:245	France	MW009252	Ammirati et al. 2021
C. venetus	AB 12-09-62	France	MW010178	Bidaud et al. 2021
C. venetus	GH20100927	Germany	MW010090	Bidaud et al. 2021
C. venetus	GS13	Germany	MW010104	Bidaud et al. 2021
C. yadingensis	HMAS254811	China	OR538889	*
C. yadingensis	HMAS280697	China	OR538890	*
C. yadingensis	HMAS280698	China	OR538891	*
C. yadingensis	HMAS254819 (holotype)	China	OR538892	*
C. yadingensis	HMAS254820	China	OR538893	*
C. veronicae	PDD68468 (holotype)	New Zealand	KC017355	Bidaud et al. 2021
C. veronicoides	MEL2120747	Australia	GQ890324	Danks et al. 2010

Note: \* means from this study.

1

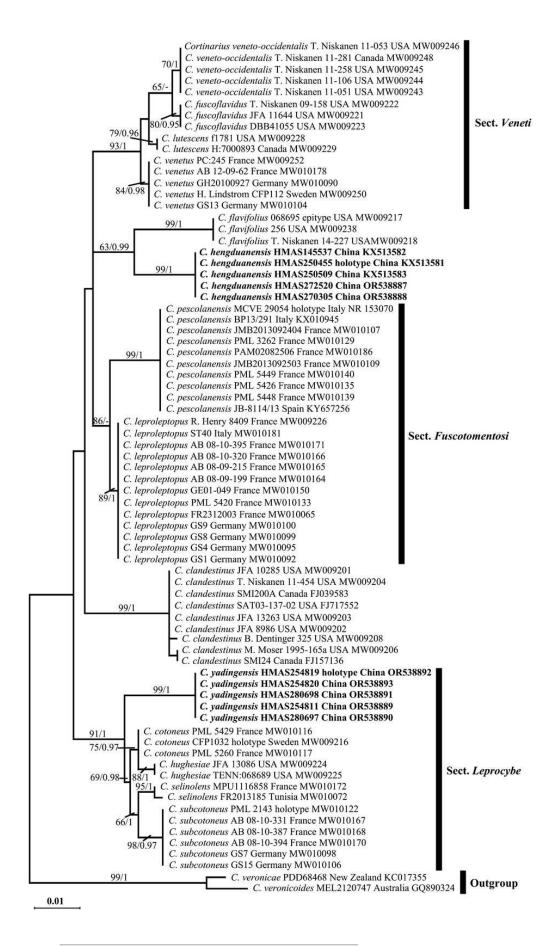


# Figure 1

ML phylogram inferred from the ITS dataset of *Cortinarius* species.

The ML bootstrap values (ML) $\geq$ 50% and Bayesian posterior probabilities (BPP) $\geq$ 0.95 are shown on the branches (BPP/ML). New species are marked in black bold font.





# Figure 2

Cortinarius hengduanensis.

A-B. HMAS 250455 (holotype). A. Basidiomata (scale bar=5 cm); B. basidiospores (scale bar=10  $\mu$ m). C-D. HMAS 250509. C. Basidiomata (scale bar=5 cm); D. basidiospores (scale bar=10  $\mu$ m).



# Figure 3

Cortinarius yadingensis.

A-B. HMAS 254819 (holotype). A. Basidiomata (scale bar=3 cm); B. basidiospores (scale bar=10  $\mu$ m). C-D. HMAS 254811. C. Basidiomata (scale bar=2 cm); D. basidiospores (scale bar=10  $\mu$ m).

