

Article

Morphological and Phylogenetic Studies of Three New Species of *Calocybe* (Agaricales, Basidiomycota) from China

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Abstract: Three species, *Calocybe lilacea*, *C. longisterigma*, and *C. subochraceus*, were newly discovered in Inner Mongolia, Hunan, and Liaoning provinces of China. *Calocybe lilacea* is mainly characterized by its small-sized basidiocarp, brownish-orange pileus, lilac gray to dull violet stipe, and noncellular epicutis. The main characteristics of *C. longisterigma* are its light brown to brownish-orange pileus, long sterigmata, and noncellular epicutis. The main morphological features of *C. subochraceus* are its small-sized basidiocarp, pale-orange pileus, adnexed lamellae, tortuous stipe, and noncellular epicutis. These morphological features confirmed that the three species of *Calocybe* all belonged to Sect. *Carneoviolaceae*. Phylogenetic analysis based on a combined dataset (ITS–nrLSU–RPB2) determined that the three species belong to the genus *Calocybe* and form a distinct lineage. The morphological differences between the three new species and other related species of *Calocybe* are also discussed.



Citation: Qi, Y.; Xu, A.; Zhou, Y.; Bi, K.; Qin, W.; Guo, H.; Yu, X.

Morphological and Phylogenetic Studies of Three New Species of *Calocybe* (Agaricales, Basidiomycota) from China. *Diversity* **2022**, *14*, 643. <https://doi.org/10.3390/d14080643>

Academic Editors: Michael Wink, Changlin Zhao and Zai-Wei Ge

Received: 26 June 2022

Accepted: 7 August 2022

Published: 11 August 2022

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Keywords: Lyophyllaceae; morphology; phylogeny; taxonomy

1. Introduction

Calocybe Kühner ex Donk (1962: 42), a well-known genus, was published as a valid name by Donk [1]. It was considered as a section of the genus *Lyophyllum* P. Karst at first [2,3]. On account of the brighter pileus of *Calocybe* species, Singer later proposed that *Calocybe* is an independent genus of Lyophyllaceae [4]. The species of *Calocybe* have characteristics of white to light-colored pileus, small spores, and pileipellis of a cutis or cellular type. On the basis of pileus color, spore surface, and hyphae of the epicutis, Singer divided *Calocybe* into four sections [4]. However, the classification opinion proposed by Singer has not been fully supported by molecular data. In recent years, many phylogenetic investigations on Lyophyllaceae indicated that *Calocybe* belongs to a monophyletic group [5–14]. However, the infrageneric classification system of *Calocybe* remains unclear. For instance, *C. naucoria* and *C. chrysenteron* are assigned to the same section, namely, Sect. *Carneoviolaceae* [4], but the two species belong to two distinct clades according to phylogenetic results [11,12,14].

The species of *Calocybe* have a widespread distribution all over the world. Currently, over 116 records have been listed in Index Fungorum (Available online: www.indexfungorum.org, accessed on 8 June 2022). In China, 16 species of *Calocybe* have been reported, most of which were discovered and described in the northern China [11–20]. In addition, several of these species are treated as edible by Dai et al. [21], including *C. carnea* (Bull.) Donk, *C. gambosa* (Fr.) Singer, and *C. ionides* (Bull.) Donk. To identify whether the three newly collected species of *Calocybe* are new to science, phylogenetic analysis and morphological feature determinations were conducted and discussed in the present study.

2. Material and Methods

2.1. Specimens and Morphological Study

All the collections used in this study were photographed in the field and deposited in the Fungal Herbarium of Shenyang Agricultural University (SYAU-FUNGI), with voucher collection numbers of SYAU-FUNGI-066 to SYAU-FUNGI-071 (Table 1). Color abbreviations followed Kornerup and Wanscher [22]. Methods for morphological observation followed Li et al. [11] and Pei et al. [23].

Table 1. Species names, voucher numbers, and GenBank accession numbers of the *Calocybe* specimens analyzed in the study.

Species	Collection	GenBank Accession Numbers		
		ITS	nrLSU	RPB2
<i>Agaricus bisporus</i>	CCBAS306	LN714517	-	LN714623
<i>Asterophora lycoperdoides</i>	CBS170.86	AF357037	AF223190	-
<i>Asterophora parasitica</i>	CBS683.82	AF357038	AF223191	-
<i>Calocybe aurantiaca</i>	SYAU-FUNGI-005	KU528828	KU528833	-
<i>Calocybe aurantiaca</i>	SYAU-FUNGI-006	NR156304	NG058937	-
<i>Calocybe badiofloccosa</i>	HMJU00098	MN172332	MN172334	-
<i>Calocybe buxea</i>	EB20140228	KP885633	KP885625	-
<i>Calocybe carnea</i>	CBS552.50	AF357028	AF223178	-
<i>Calocybe chrysenteron</i>	AMB17092	KP885639	KP885628	-
<i>Calocybe chrysenteron</i>	L05-87	KP885640	KP885629	-
<i>Calocybe coacta</i>	HMJU269	OK649907	OL687156	-
<i>Calocybe convexa</i>	SYAU-FUNGI-007	KU528826	KU528830	-
<i>Calocybe convexa</i>	SYAU-FUNGI-008	NR156303	NG058936	-
<i>Calocybe decolorata</i>	SYAU-FUNGI-003	KU528824	KU528834	-
<i>Calocybe decolorata</i>	SYAU-FUNGI-004	NR156302	NG058938	-
<i>Calocybe decurrens</i>	HMJU382	MT080028	MW444857	-
<i>Calocybe erminea</i>	HMJU00100	MN172331	MN172333	-
<i>Calocybe fulvipes</i>	HMJU3027	OK649910	OK649880	-
<i>Calocybe fulvipes</i>	HMJU317	MT071590	OK649878	-
<i>Calocybe gangraenosa</i>	Hae251.97	AF357032	AF223202	DQ367427
<i>Calocybe gambosa</i>	HC78/64	AF357027	AF223177	-
<i>Calocybe ionides</i>	H77/133	AF357029	AF223179	-
<i>Calocybe lilacea</i>	SYAU-FUNGI-070	OM203538	OM341407	OM281286
<i>Calocybe lilacea</i>	SYAU-FUNGI-071	OM203539	OM341409	OM281287
<i>Calocybe longisterigma</i>	SYAU-FUNGI-066	OM203542	OM341406	OM281286
<i>Calocybe longisterigma</i>	SYAU-FUNGI-067	OM203543	OM341408	OM281287
<i>Calocybe naucoria</i>	AMB17094	KP885642	KP885630	-
<i>Calocybe naucoria</i>	HC80/103	AF357030	AF223180	-
<i>Calocybe obscurissima</i>	HC79/181	AF357031	AF223181	-
<i>Calocybe onychina</i>	CAON-RH19-563	MW084664	MW084704	-
<i>Calocybe onychina</i>	CL121115-07	KP885644	KP885632	-
<i>Calocybe persicolor</i>	HC80/99	AF357026	AF223176	-
<i>Calocybe subochraceus</i>	SYAU-FUNGI-068	OM203540	OM341410	OM281288
<i>Calocybe subochraceus</i>	SYAU-FUNGI-069	OM203541	OM341411	OM281289
<i>Calocybe vinacea</i>	HMJU5135	OK649908	OK649876	-
<i>Calocybe vinacea</i>	HMJU5160	OK649909	OK649877	-
<i>Calocybella pudica</i>	AMB15994	KP858000	KP858005	-
<i>Calocybella semitale</i>	EL187-09	HM572552	-	-
<i>Calocybella semitale</i>	HC85/13	AF357049	AF042581	-
<i>Gerhardtia borealis</i>	AMB15993	KP858004	KP858009	-
<i>Lyophyllum favrei</i>	HAe234.97cp	AF357034	AF223183	-
<i>Lyophyllum favrei</i>	IE-BSC-HC 96cp4	EF421102	AF223184	-
<i>Lyophyllum ochraceum</i>	BSI94.cp1	AF357033	AF223185	-
<i>Ossicaulis lignatilis</i>	D604	DQ825426	AF261397	-
<i>Tephrocycbe boudieri</i>	BSI96/84	AF357047	DQ825430	-
<i>Tephrocycbe gibberosa</i>	CBS328.50	AF357041	AF223197	-
<i>Tephrocycbe tylicolor</i>	BSI92/245	AF357040	AF223195	-
<i>Tricholomella constricta</i>	HC84/75	DQ825429	AF223188	-

Note: The newly obtained sequences in this study are marked in bold.

2.2. DNA Extraction, PCR Amplification, and Sequencing

Total DNA was extracted from fresh tissue blocks using the cetyltrimethylammonium bromide (CTAB) method [23]. The universal primer pairs ITS5/ITS4 [24], LR0R/LR5 [25],

and b6F/b7.1R [26] were used for amplification of the ITS region, nrLSU region, and RPB2, respectively. The PCR protocol followed that in Li et al. [11]. The products of PCR amplification were examined on a 1% agarose gel and visualized by the Gel Imaging System (Bio-Rad, SYSTEM GelDoc XR+ IMAGELAB, CA). The fragments used for analysis in this study were provided by BGI Co, Ltd., Beijing, China.

2.3. Phylogenetic Analyses

BLAST (Available online: www.blast.ncbi.nlm.nih.gov, accessed on 13 May 2022) was used to search for the related species of the three new species. Then, high-quality and representative sequences of *Calocybe* and related genera in former phylogenetic studies [7,11–14,27] were downloaded from GenBank in order to reconstruct the phylogenetic relationships of the genus *Calocybe*. The newly generated sequences from this study were aligned with those retrieved from GenBank using MAFFT v7.313 [28]. A data partition homogeneity test [29] was implemented in PAUP 4.0b4a [30]. The result detected no conflicts among ITS, nrLSU, and RPB2 regions (p -value = 0.8), suggesting that the three regions can be combined. ModelFinder [31] and PartitionFinder 2 [32] were used for the selection of the best-fitting evolution mode. Maximum likelihood (ML) and Bayesian inference (BI) analyses were performed following the method of Pei et al. [23]. Both BI and ML was conducted under GTR mode, and the BI of the combined datasets was run for 8,000,000 generations. The best tree was viewed in FIGTREE v1.4.4 [33] and was compiled in Adobe Illustrator CC.

3. Results

Molecular Phylogenetic Inference

The dataset for phylogeny analysis consisted of three gene regions from 46 samples representing 33 species, and *Agaricus bisporus* (J.E. Lange) Imbach was selected as an outgroup in this study. All the sequences used in this study are listed in Table 1. ML and BI resulted in almost identical tree topologies, and the BI tree was selected for display (Figure 1). The results suggested that the species of *Calocybe* formed a distinct group supported by a strong value (1.00 PP and 100% BS), corroborated by former research [5–14]. A total of five clades can be viewed within species of *Calocybe*, which is in line with Li et al. [11]. *Calocybe lilacea* and *C. subochraceus* formed an independent lineage supported by a strong value (1.00 PP and 100% BS), located within clade II. Moreover, the two new species gathered in a separate lineage with high support (1.00 PP and 100% BS). In clade I, *C. longisterigma* formed a single clade with high support (1.00 PP and 100% BS), sister to the clade containing *C. decolorata* (0.86 PP and 99% BS).

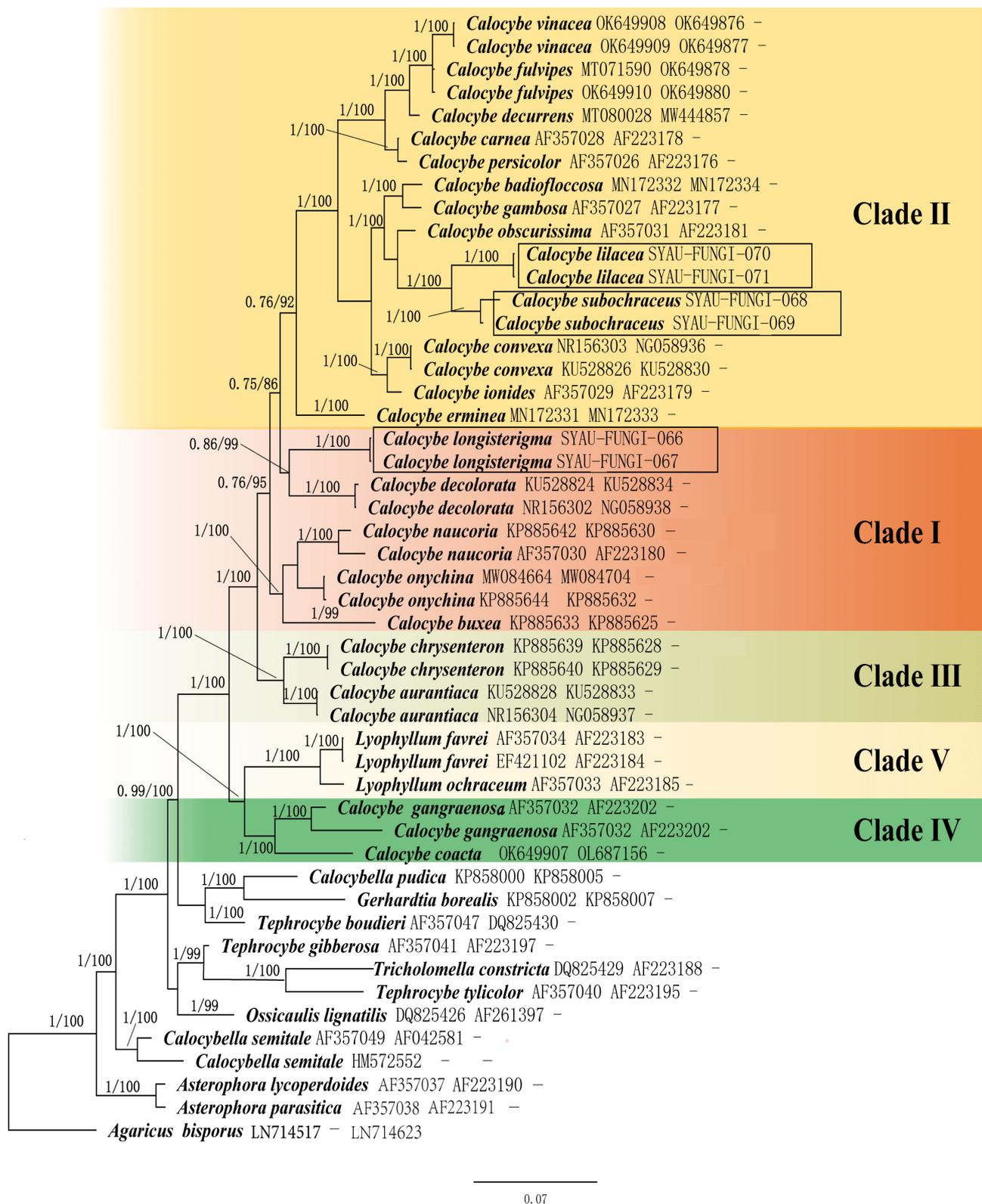


Figure 1. Bayesian phylogenetic analysis of *Calocybe* based on the combined ITS–nrLSU–RPB2 sequences. Node support (PP ≥ 0.90 and BS ≥ 75%) is annotated on the branches. The three new species and corresponding voucher numbers are shown in boxes.

4. Taxonomy

Calocybe lilacea X.D. Yu, Y. Zhou & W.Q. Qin, sp. nov. MycoBank No. MB 843876 (Figure 2A–E).

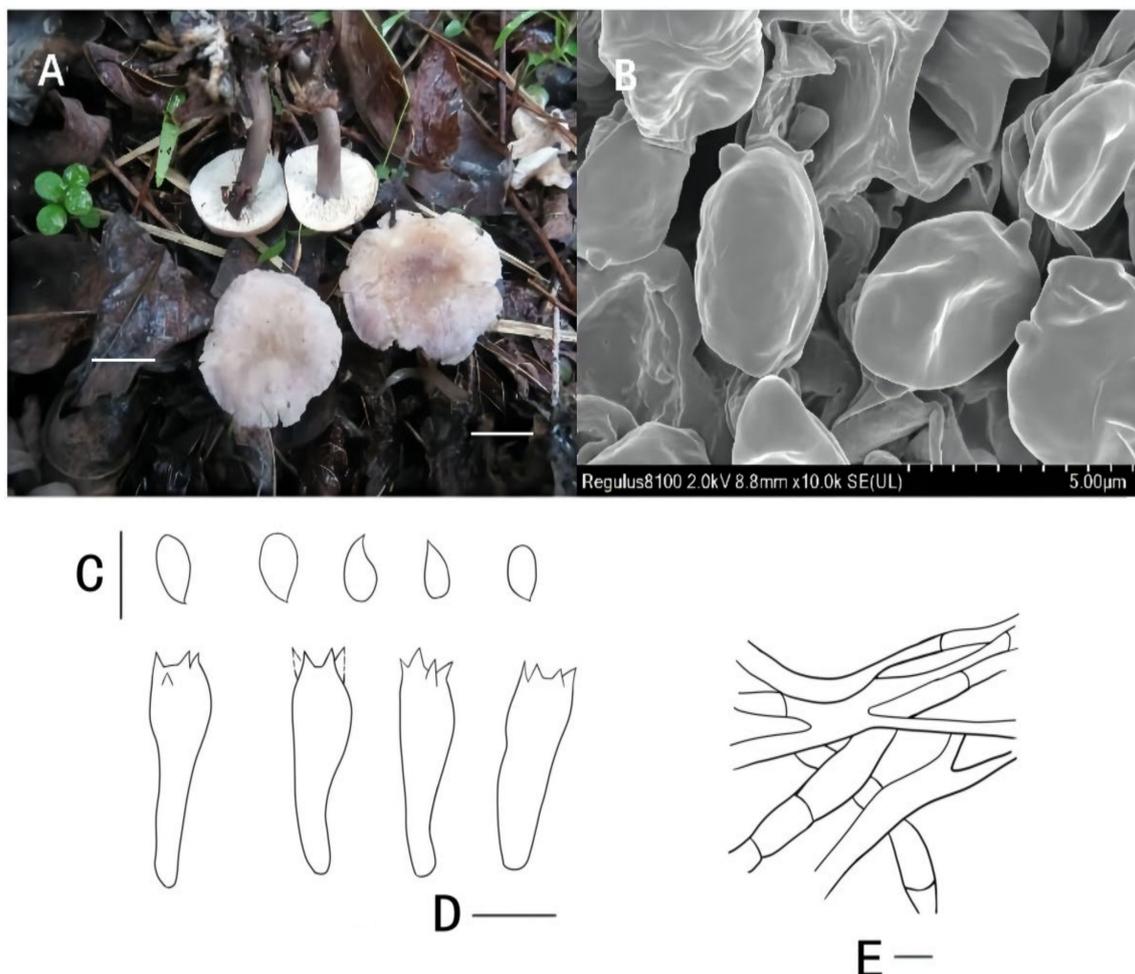


Figure 2. *Calocybe lilacea* (holotype, SYAU-FUNGI-070): (A) macroscopic habitat and basidiomata; (B) surface of basidiospores; (C) basidiospores; (D) basidia; (E) pileipellis. Scale bars: (A) 1 cm; (B–E) 5 μ m.

Etymology: The epithet “*lilacea*” refers to the lilac color of the stipe.

Holotype: China. Hunan Province: Zhangjiajie, Jishou University, on the soil, on 26 November 2019, W.Q. Qin (holotype: SYAU-FUNGI-070).

Description: Basidiomata slightly small-sized. Pileus 1.5–3.0 cm in diameter, convex at first, soon becoming plano-convex, surface hygrophanous, light brown (7D4 to 7D5) at center, becoming paler toward the margin, margin brownish orange (7C3) to reddish gray (7B2), slightly incurved at first, soon becoming extended and sometimes irregular. Lamellae 1.0–2.0 mm broad, moderately crowded, adnate, white, with lamellulae, edges concolorous and denticulate. Stipe 1.5–2.5 cm long \times 0.3–0.5 cm diameter, central, stuffed, equal or slightly attenuated upward, surface lilac gray to dull violet (16C2 to 17E3). Pileus context 0.2–0.3 cm thick, white to creamy. Odor faint. Spore deposit white.

Basidiospores (3.1) 3.4–4.5 (4.9) \times (2.7) 3.2–3.7 (4.1) μ m, $Q = (1.37) 1.40$ –1.51 (1.56) μ m ($n = 30$), average $Q = 1.44$, subglobose, hyaline, inamyloid, smooth. Basidia (17.0) 18.0–20.5 (21.0) \times (4.0) 4.5–7.0 (8.0) μ m, clavate, four-spored, hyaline, siderophilous granules observed. Hymenial cystidia absent. Hymenophoral trama 90–130 μ m thick, regular, consisting of 2.0–5.0 μ m wide, thin-walled, hyaline hyphae. Subhymenium 18–26 μ m thick. Pileipellis and cutis of dense, subparallel, repent branched hyphae, hyphae 2.4–4.8 μ m

wide, thin-walled, pigmented. Stipitipellis hyphae 3.4–8.6 μm wide, thin-walled, hyaline. Caulocystidia absent. Clamp connections absent.

Ecology and distribution: Saprophytic on soil by forests or roads. Known from Hunan province.

Additional specimen examined: CHINA. Hunan Province: Zhangjiajie, Jishou University, on the soil, on 26 November 2019, W.Q. Qin (SYAU-FUNGI-071).

Remarks: *Calocybe lilacea* is mainly characterized by its small basidiomata, brownish orange pileus, adnate and white lamellae, lilac gray to dull violet stipe, smooth basidiospores, and noncellular epicutis. On the basis of Singer's taxonomical opinion and these morphological characteristics, *Calocybe subochraceus* is assigned to Sect. *Carneoviolaceae* [4]. The lilac gray to violet stipe also appears in other three *Calocybe* species of the section, i.e., *C. africana*, *C. cyanella*, and *C. ionides*. However, *Calocybe lilacea* features a brownish pileus, which is different from the three species with purple tones pileus [34–36]. Furthermore, *C. decurrens* is similar to *C. lilacea* in having a violet stipe when young [14]; however, *C. decurrens* is characterized by a much larger pileus up to 105 mm in diameter and much longer spores up to 9.3 μm in length [14].

Calocybe longisterigma X.D. Yu, Y. Zhou & H.B. Guo, sp. nov. MycoBank No. MB 843877 (Figure 3A–E).

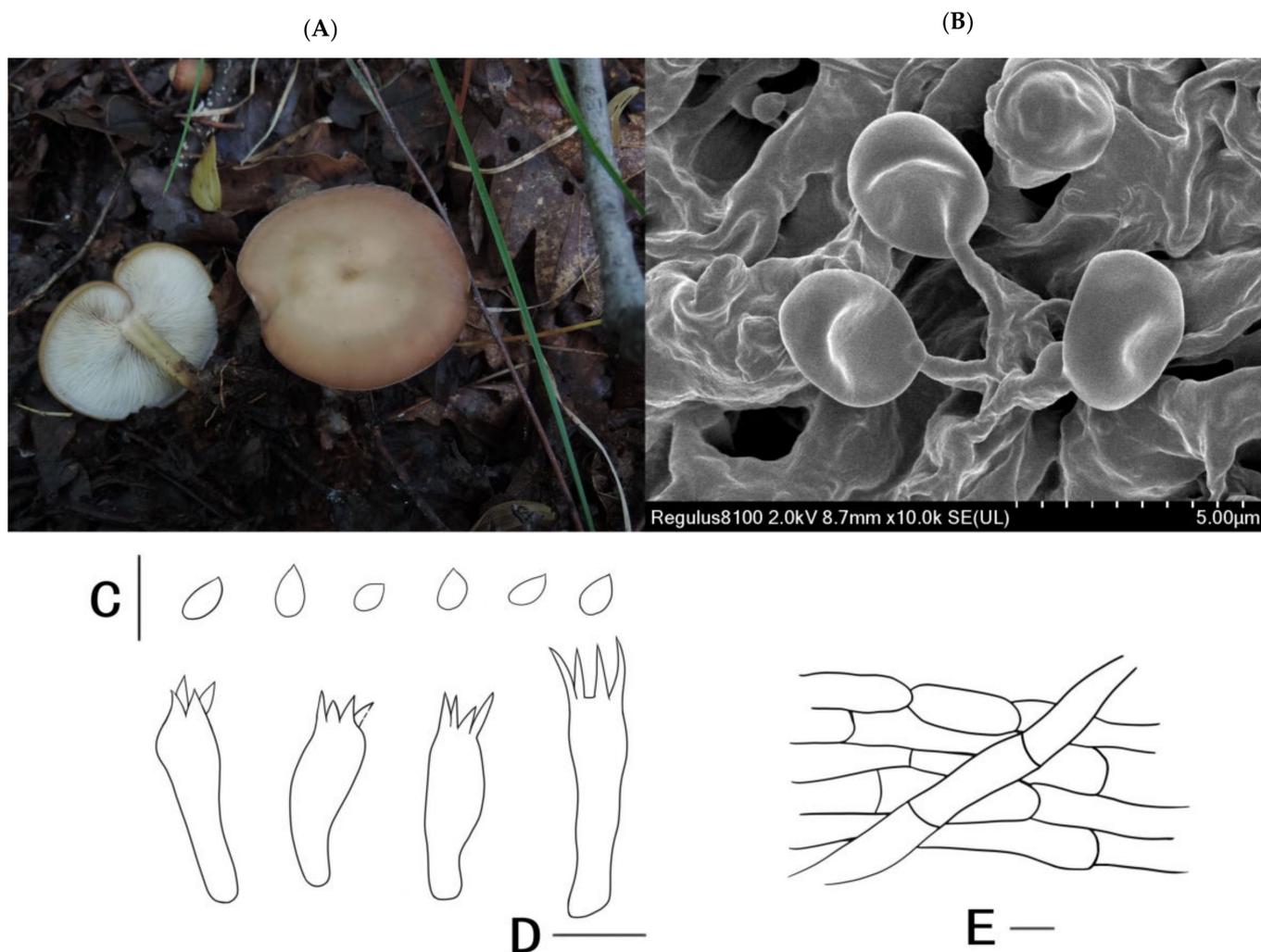


Figure 3. *Calocybe longisterigma* (holotype, SYAU-FUNGI-066): (A) macroscopic habitat and basidiomata; (B) surface of basidiospores; (C) basidiospores; (D) basidia; (E) pileipellis. Scale bars: (A) 1 cm; (B–E) 5 μm .

Etymology: The epithet “*longisterigma*” refers to the species characterized by long sterigmata.

Holotype: China. Inner Mongolia: Chifeng, Linxi County, Xinlin Town, Dauran Village, on the soil, on 22 July 2017, X.D. Yu (holotype: SYAU-FUNGI-066).

Description: Basidiomata medium-sized. Pileus 2.5–6.5 cm broad, convex to plano-convex at first, then gradually becoming flat, surface silky, hygrophanous, brownish orange (5C4 to 6C3) to grayish orange (6B3), often becoming light brown (7D4 to 7D5) at margin; edge entire. Lamellae 1.0–3.0 mm broad, adnate to decurrent, rather crowded, white, with lamellulae; edge entire, concolorous with the sides. Stipe 2.0–3.5 cm long × 0.3–0.5 cm diameter, central, stuffed, equal or and slightly attenuated downward; surface glabrous, non-striate or indistinctly striate, white at apex and light brown at base when young, brownish orange (5C4 to 5C5) at apex and brown at base (6D4 to 6D5) when mature, with whitish basal tomentum. Pileus context up to 0.3–0.6 cm thick, white to cream. Odor faint. Spore deposit white.

Basidiospores (2.1) 2.6–3.4 (3.9) × 1.7–2.6 (3.2) μm, Q = (1.39) 1.42–1.49 (1.52) μm ($n = 30$), average Q = 1.47, subglobose to ellipsoid, hyaline, inamyloid, smooth. Basidia (12) 13.0–16.0 (18.0) × (3.3) 3.6–5.4 (6.0) μm, clavate, four-spored, long sterigmata up to 3.8–8.2 μm, hyaline, siderophilous granules observed. Any type of cystidia absent. Hymenophoral trama 190–220 μm thick, regular, consisting of 2.0–4.0 μm wide, thin-walled, hyaline hyphae. Subhymenium 24–35 μm thick. Pileipellis and cutis of dense, subparallel, repent branched hyphae, hyphae 3.0–5.5 μm wide, thin-walled, sometimes pigmented. Stipitipellis hyphae 4.4–8.5 μm wide, thin-walled, hyaline. Caulocystidia absent. Clamp connections absent.

Ecology and distribution: Saprophytic on the grass. Known from Inner Mongolia.

Additional specimen examined: China. Inner Mongolia: Chifeng, Linxi County, Xinlin Town, Dawulan village, on the grass in woods, on 22 July 2017, H.B. Guo (SYAU-FUNGI-067).

Remarks: The main characteristics of *Calocybe longisterigma* are brownish orange to grayish orange pileus, adnate to decurrent lamellae, small basidiospores, long sterigmata, and non-cellular epicutis. According to Singer’s concept of infrageneric classification [4], it should be classified in Sect. *Carneoviolaceae*. In this section, *Calocybe longisterigma* resembles *C. carnea*, *C. decurrens*, and *C. fulvipes* because these members of this section all have carneous or brownish pileus. The long sterigmata up to 8.2 μm and small basidiospores (2.6–3.4 × 1.7–2.6 μm) of *C. longisterigma* can distinguish it from these three species. Moreover, *C. decurrens* can be differentiated by its pastel violet stipe at first [14]. *Calocybe carnea* differs from *C. longisterigma* by its adnexed gills and white stipe [37]. *Calocybe fulvipes* has adnexed to emarginate lamellae, while *C. longisterigma* features adnate to decurrent lamellae, which can separate the two species. In addition, *Calocybe fulvipes* produces grayish-orange lamellae when bruised, which is absent in *C. longisterigma* [12].

Calocybe subochraceus X.D. Yu, Y. Zhou & H.B. Guo, sp. nov. MycoBank No. MB 84387 (Figure 4A–E).

Holotype: China. Liaoning Province: Shenyang, Shenyang Agricultural University, on the grass in woods, on 21 July 2020, X.D. Yu (holotype: SYAU-FUNGI-068).

Etymology: The epithet “*subochraceus*” refers to the light-yellow color of the pileus, which is similar to the species *Calocybe ochraceus*.

Description: Basidiomata small-sized. Pileus 1.0–3.0 cm broad, convex to plano-convex at first, then becoming applanate, even slightly depressed at center with age; surface pale orange (5A3 to 5A5), often becoming paler orange/white (5A2) at margin; margin entire at first, then expanding to uplifted and flexuous with age. Lamellae 0.4–1.0 mm broad, adnexed, moderately close, white, with lamellulae; edge denticulate, concolorous with the sides. Stipe 1.6–2.3 cm long × 0.4–0.7 cm diameter, subcylindrical to flexuous, central, hollow, light brown to grayish brown (7D3 to 7D4) at first, then gradually becoming light orange to orange white to grayish orange (5A3, 5A2, 5B2 to 5B5), generally deeper in upper part, with whitish basal tomentum. Pileus context 0.3–0.5 cm thick, white. Odor faint. Spore deposit white.

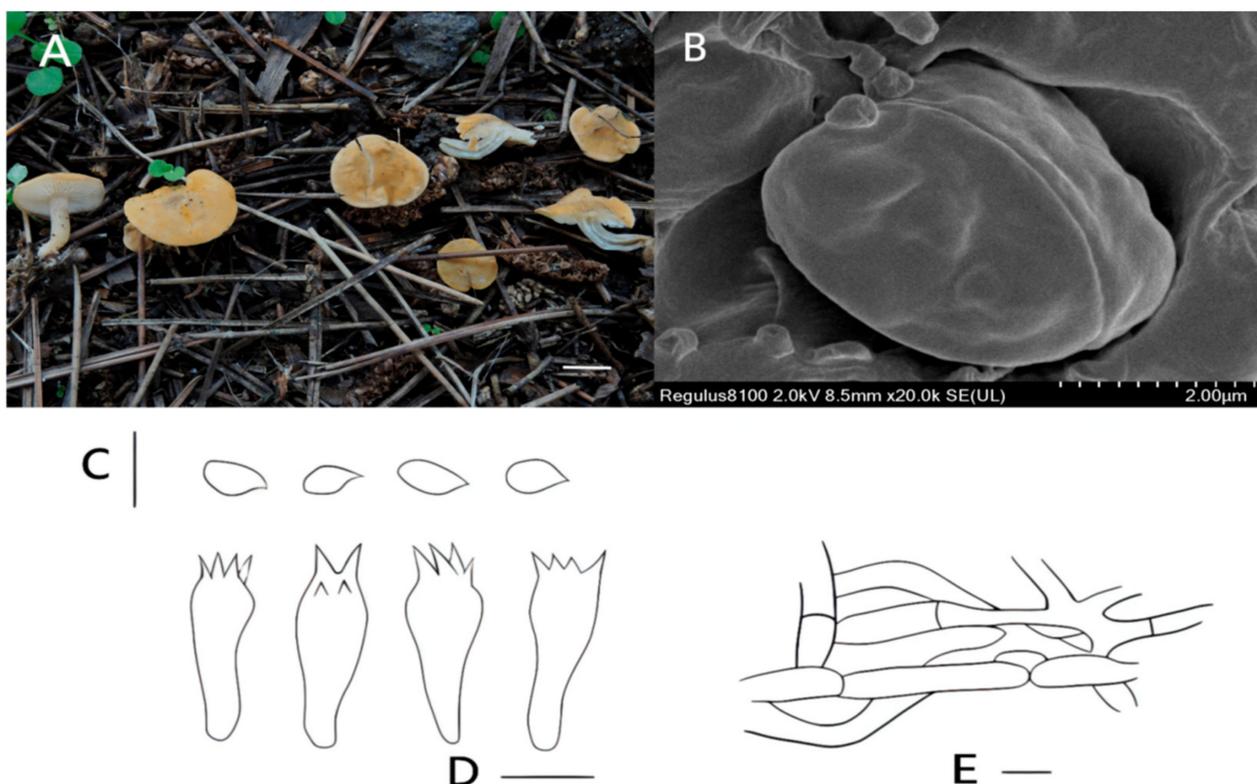


Figure 4. *Calocybe subochraceus* (holotype, SYAU-FUNGI-068): (A) macroscopic habitat and basidiomata; (B) surface of basidiospores; (C) basidiospores; (D) basidia; (E) pileipellis. Scale bars: (A) 1 cm; (B) 2 μ m; (C–E) 5 μ m.

Basidiospores (3.1) 3.3–4.5 (4.9) \times (2.4) 2.9–3.6 (3.9) μ m, $Q = (1.49) 1.56$ –1.62 (1.64) μ m ($n = 30$), average $Q = 1.59$, subglobose to elongate ellipsoid, hyaline, inamyloid, smooth. Basidia (15.5) 16.0–19.0 (20.0) \times (5.0) 5.5–7.5 (8.0) μ m, clavate, four-spored, subhyaline, siderophilous granules observed. Hymenial cystidia absent. Hymenophoral trama 100–160 μ m thick, regular, consisting of 2.0–5.0 μ m wide, thin-walled, hyaline hyphae. Subhymenium 17–24 μ m thick. Pileipellis and cutis of dense, subparallel, repent branched hyphae, hyphae 2.6–4.9 μ m wide, thin-walled, pigmented. Stipitipellis hyphae 2.6–7.8 μ m wide, thin-walled, hyaline. Caulocystidia absent. Clamp connections absent.

Ecology and distribution: Saprophytic in small groups on soil in forests. Known from Liaoning province.

Additional specimen examined: China. Liaoning Province: Shenyang, Shenyang Agricultural University, on the grass, on 21 July 2020, X.D. Yu (SYAU-FUNGI-069).

Remarks: The main features of *Calocybe subochraceus* are its small basidiomata, pale orange pileus, white and adnexed lamellae, tortuous stipe, and noncellular epicutis. These key features suggest that *C. subochraceus* belongs to Sect. *Carneoviolaceae* [4]. Amongst this section, several members have similar orange pileus, including *C. alpestris*, *C. carnea*, and *C. coniceps*. However, *C. alpestris* (6.2–6.8 \times 2.7–3.0 μ m), *C. coniceps* (6.5–8.5 \times 4.5–5.8 μ m), and *C. carnea* (more than 5 μ m long) are characterized by larger basidiospores [34,36], making them distinguishable from *C. subochraceus*. Furthermore, *C. aurantiaca*, *C. convexa*, and *C. decolorata* resemble *C. subochraceus* in having orange to yellow pileus. However, *C. aurantiaca* and *C. decolorata* are distinct from *C. subochraceus* in having decurrent gills. *Calocybe convexa* and *C. decolorata* have fusiform hymenial cystidia, which is absent in *C. subochraceus*.

5. Discussion

Calocybe lilacea, *C. longisterigma*, and *C. subochraceus*, newly discovered in Inner Mongolia, Hunan, and Liaoning provinces of China, respectively, were originally reported and

described in detail in this study. *Calocybe lilacea* is mainly characterized by small-sized basidiomata and lilac gray to dull violet stipe. The main characteristics of *C. longisterigma* are brownish orange pileus, decurrent lamellae, small basidiospores, and long sterigmata. The key features of *C. subochraceus* are small basidiomata, pale orange pileus, white lamellae, and tortuous stipe. On account of the Singer's infrageneric classification system, the three species of *Calocybe* are all assigned to the Sect. *Carneoviolaceae* [4]. Morphologically, unique characteristics of the three species of *Calocybe* can readily distinguish them from other related species, as elaborated above.

Phylogenetically, species of *Calocybe* appears to form a monophyletic group, which is consistent with previous studies [5–14]. Additionally, the three new species determined in this study occupy an independent position in the phylogenetic tree. *Calocybe lilacea* clusters in a single clade sister to the clade of *C. longisterigma*; however, the two species described in the study can be easily delimited using morphological methods. *Calocybe lilacea* has a smaller pileus and a lilac gray to dull violet stipe, but *C. longisterigma* has a slightly larger pileus and a light-brown stipe. Microscopically, the two *Calocybe* species can also be separated; *Calocybe longisterigma* is characterized by smaller basidiospores and much longer sterigmata, which is absent in *C. lilacea*. *Calocybe subochraceus* occupies an independent lineage sister to the clade containing *C. decolorata*, originally reported in northeastern China by Li et al. [11]. *Calocybe decolorata* has decurrent and blue lamellae when injured, which differs from *C. subochraceus* that has adnexed and unchanged lamellae when bruised. Additionally, *C. decolorata* has fusiform hymenial cystidia, which is absent in *C. subochraceus*.

In the phylogenetic analysis (Figure 1), five clades and 25 species were recognized within the genus *Calocybe*. The morphologically based infrageneric classification of *Calocybe* species by Singer [4] could not be fully supported by the results of our phylogenetic analyses. Except for *C. onychina* with an amaranthine pileus and *C. longisterigma* with a carneose pileus [38], the species of Clade I are characterized by a white to yellow pileus. The pileus color within Clade II varies, including white, yellow, brown, and violet shades [34,39]. In Clade III, *C. chrysenteron* and *C. aurantiaca* are yellow. *Calocybe coacta* and *C. gangraenosa*, located in Clade IV, have a white pileus. Clade V consists of *C. ochracea* and *C. favrei*, which have a gilded pileus [40]. Therefore, it is not appropriate to consider pileus color as the only criterion of the infrageneric classification of *Calocybe*, but it could provide taxonomic basis to some extent. The species of *Calocybe* within each phylogenetic clade lack uniform characteristics to work in identification. Further investigations and additional materials are still needed to reveal the infrageneric classification of *Calocybe* species.

Until now, a total of 19 species of *Calocybe* have been reported from China. On the basis of observations and the literature [11–14,18,39,40], a key for the *Calocybe* species from China is provided in Table 2.

Table 2. A key to the Chinese species of *Calocybe*.

Key to Chinese Species of <i>Calocybe</i>	
1. Basidiomata medium to large, pileus usually more than 6 cm diam., stipe more than 3.5 cm long.	2
1'. Basidiomata small, pileus usually less than 6 cm diam., stipe less than 3.5 cm long.	7
2. Lamellae decurrent.	3
2'. Lamellae not decurrent.	5
3. Lamellae grayish orange when bruised.	<i>C. coacta</i>
3'. Lamellae unchanged when bruised.	4
4. Stipe pastel violet when young, sterigmata less than 4 µm long.	<i>C. decurrens</i>
4'. Stipe light brown when young, sterigmata more than 4 µm long.	<i>C. longisterigma</i>
5. Pileus blue when bruised, basidiospores surface warted.	<i>C. gangraenosa</i>
5'. Pileus unchanged when bruised, basidiospores surface smooth.	6
6. Lamellae grayish orange when bruised.	<i>C. fulvipes</i>
6'. Lamellae unchanged when bruised.	<i>C. gambosa</i>

Table 2. Cont.

Key to Chinese Species of <i>Calocybe</i>	
7. Pileus with yellowish tones.	8
7'. Pileus without yellowish tones.	15
8. Lamellae decurrent.	13
8'. Lamellae not decurrent.	9
9. Lamellae yellow.	10
9'. Lamellae white.	11
10. Basidiospores more than 5 µm long.	<i>C. naucoria</i>
10'. Basidiospores less than 5 µm long.	<i>C. chrysenteron</i>
11. Pileus yellow, stipe white, basidiospores less than 5 µm long.	12
11'. Pileus pink, stipe pink, basidiospores more than 5 µm long.	<i>C. carneae</i>
12. Pileus convex when mature, cystidia present.	<i>C. convexa</i>
12'. Pileus applanate when mature, cystidia absent.	<i>C. subochraceus</i>
13. Stipe with white pubescence at base.	<i>C. badiofloccosa</i>
13'. Stipe without white pubescence at base.	14
14. Lamellae yellow, blue when bruised.	<i>C. decolorata</i>
14'. Lamellae white, unchanged when bruised.	<i>C. aurantiaca</i>
15. Pileipellis cellular.	<i>C. erminea</i>
15'. Pileipellis noncellular.	16
16. Stipe with violet tones.	17
16'. Stipe without purple tones.	18
17. Pileus with purple tones.	<i>C. ionides</i>
17'. Pileus with brown tones.	<i>C. lilacea</i>
18. Pileus with reddish tones, lamellae with yellow tones.	<i>C. vinacea</i>
18'. Pileus with violet tones, lamellae with taupe tones.	<i>C. obscurissima</i>

Author Contributions: Conceptualization, X.Y. and A.X.; methodology, Y.Z. and Y.Q.; software, Y.Z. and Y.Q.; validation, X.Y. and Y.Q.; formal analysis, Y.Q.; investigation, W.Q., K.B. and H.G.; resources, H.G. and W.Q.; data curation, Y.Z.; writing—original draft preparation, Y.Z. and Y.Q.; writing—review and editing, Y.Q. and X.Y.; visualization, Y.Z. and Y.Q.; supervision, X.Y.; project administration, X.Y.; funding acquisition, X.Y. and A.X. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Science and Technology Plan Project of Liaoning Province (2020-MZLH-33), the National Natural Science Foundation of China (No. 31770014), and research and development of wild macrofungal germplasm resources collection and preservation technology in Tibet Germplasm Resource Bank (ZDZ×2018000017).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data can be found within the manuscript.

Acknowledgments: The authors kindly thank Xin-Yu Ma and Jian-Xuan Hou for modifying the pictures in this study.

Conflicts of Interest: The authors declare no conflict of interest.

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