



Melanoleuca subgriseoflava and *M. substridula*—two new *Melanoleuca* species (Agaricales, Basidiomycota) described from China

Yue Qi¹, Cai-Hong Li¹, Yu-Meng Song¹, Ming Zhang², Hong-Bo Guo³ and Xiao-Dan Yu¹

¹ College of Biological Science and Technology, Shenyang Agricultural University, Shenyang, Liaoning, China

² Guangdong Academy of Sciences, Guangdong Institute of Microbiology, Guangzhou, Guangdong, China

³ College of Life Engineering, Shenyang Institute of Technology, Fushun, Liaoning, China

ABSTRACT

Two new *Melanoleuca* species, *Melanoleuca subgriseoflava* and *M. substridula*, are originally reported and described in China based on both morphological and molecular methods. *Melanoleuca subgriseoflava*, collected in Liaoning province, is mainly characterized by its greyish-brown to yellowish-grey pileus, creamy to light orange lamellae, greyish-yellow context, round and warted basidiospores and fusiform hymenial cystidia. *Melanoleuca substridula*, discovered in Sichuan province, is mainly characterized by its light brown to dark brown pileus, whitish lamellae, light brown to greyish-brown stipe, round and warted basidiospores and lack of any forms of cystidia. The phylogenetic relationships as well as divergence-time estimation were analyzed using the combined data set (ITS-nrLSU-RPB2), and the results showed that the two *Melanoleuca* species formed two distinct lineages. Based on the combination of morphological and molecular data, *M. subgriseoflava* and *M. substridula* are confirmed as two new species to science. A theoretical basis is provided for the species diversity of *Melanoleuca*.

Subjects Biodiversity, Molecular Biology, Mycology, Plant Science, Taxonomy

Keywords *Melanoleuca*, Agaricales, Morphology, Multiple-genes phylogeny

Submitted 28 April 2022

Accepted 7 July 2022

Published 19 September 2022

Corresponding author

Xiao-Dan Yu, yuxd126@126.com

Academic editor

Héctor Mora-Montes

Additional Information and
Declarations can be found on
page 10

DOI 10.7717/peerj.13807

© Copyright
2022 Qi et al.

Distributed under
Creative Commons CC-BY 4.0

OPEN ACCESS

INTRODUCTION

Melanoleuca Pat. is distributed worldwide, containing around 423 validly published names (Index Fungorum, <http://www.indexfungorum.org/>, accessed on 7 April 2022), 12 of which were considered as edible by *Dai et al. (2010)*, including *Melanoleuca arcuata* (Bull.) Singer, *Melanoleuca brevipes* (Bull.) Pat., *Melanoleuca cognata* (Fr.) Konrad & Maubl etc. Recently, many new species of *Melanoleuca* have been reported throughout the world (*Vizzini et al., 2010; Vizzini et al., 2011; Sánchez-García, Cifuentes-Blanco & Matheny, 2013; Antonín et al., 2014; Antonín et al., 2017; Antonín et al., 2021; Yu et al., 2014; Nawaz, Jabeen & Khalid, 2017; Xu et al., 2019; Pei et al., 2021*). *Melanoleuca* is a taxonomically complicated genus because many species in the genus are very similar in macroscopical characteristics and only present subtle differences (*Bon, 1991; Boekhout, 1999; Vizzini et al., 2011*). The genus is typified by a dull-colored pileus; amyloid and warted basidiospores; two types of cystidia

(urticiform or fusiform to lageniform) and all hyphae without clamp connections ([Singer, 1986](#); [Bon, 1991](#); [Boekhout, 1988](#); [Vizzini et al., 2011](#)).

Despite considerable evidence that the *Melanoleuca* genus belongs to a monophyletic group, the infrageneric classification system of the genus has always been controversial. [Singer \(1986\)](#) divided the genus into four sections circumscribed only by pileus color and stipe ornamentations, *i.e.*, sect. *Alboflavidae* Singer, sect. *Humiles* Singer, sect. *Oreinae* Singer and sect. *Melanoleuca* Pat. [Bon \(1978\)](#), the first to take micro-morphological characters into consideration, divided the genus into seven sections. As [Boekhout \(1988\)](#) emphasized the crucial role of cystidia, the genus was divided into three subgenera according to the absence/presence and shape of cystidia, *i.e.*, subgen. *Macrocytis* Boekhout, subgen. *Melanoleuca* Pat. and subgen. *Urticocystis* Boekhout. However, these taxonomical units are not supported by molecular data. [Vizzini et al. \(2011\)](#), using a large number of ITS sequences to construct phylogenetic relationships of *Melanoleuca*, defined only two subgenera, *i.e.*, subgen. *Urticocystis* Vizzini and subgen. *Melanoleuca* Vizzini. Then, follow-up studies on species of *Melanoleuca* support the classification opinion proposed by [Vizzini et al. \(2011\)](#) ([Yu et al., 2014](#); [Kalmer, Acar & Dizkirici, 2018](#); [Xu et al., 2019](#)).

Only 31 species of *Melanoleuca* have been reported in China ([Bau & Li, 1999](#); [Zhang, Li & Song, 2001](#); [Chen, 2007](#); [Mao, 2009](#); [Sun et al., 2012](#); [Wang, 2013](#); [He et al., 2014](#); [Yu et al., 2014](#); [Zhao et al., 2014](#); [Wei, Fan & Yan, 2015](#); [Du et al., 2016](#); [Tian et al., 2018](#); [Xu et al., 2019](#); [Pei et al., 2021](#)). Although China has a complex climate and geographical conditions, species resources of *Melanoleuca* remain scarce. This study reports and describes two *Melanoleuca* species collected from Liaoning province and Sichuan province in China from 2019 to 2020. In order to confirm the two collections as new to science, both morphological and method analyses were conducted. The morphological similarities and differences between the two species and other related species are also discussed.

MATERIAL AND METHODS

Specimens and morphological description

Fresh basidiomata were photographed in the field. Specimens were dried with an electric drier and deposited with silica gel. Dried specimens were preserved in the Fungal Herbarium of Shenyang Agricultural University (SYAU-FUNGI), Liaoning, China and Herbarium of Microbiology Institute of Guangdong (GDGM), Guangdong, China. Tissue blocks were removed from the inner part of the dried specimens for DNA analyses. Color abbreviations followed [Kornerup & Wanscher \(1963\)](#). Methods for morphological observation followed [Pei et al. \(2021\)](#). For observation of surface of the basidiospores, SEM microphotographs were performed using a scanning electron microscope (REGLUS 8100; Hitachi, Tokyo, Japan).

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,

Table 1 Primers used in this study.

Regions	Primer	Sequence (5'–3')	Reference
ITS	ITS5	GGAAGTAAAAGTCGTAACAAGG	<i>White et al. (1990)</i>
	ITS4	TCCTCCGCTTATTGATATGC	<i>White et al. (1990)</i>
nLSU	LROR	GTACCCGCTGAACTTAAGC	<i>Michot, Hassouna & Bachelierie (1984)</i>
	LR5	ATCCTGAGGGAAACTTC	<i>Michot, Hassouna & Bachelierie (1984)</i>
RPB2	b7.1R	TGGGGYATGGTNTGYCCYGC'	<i>Matheny et al. (2007)</i>
	b6F	CCCATRGCYTGYYTMMCCCATDGC	<i>Matheny et al. (2007)</i>

new names contained in this work have been submitted to MycoBank from where they will be made available to the Global Names Index. The unique MycoBank number can be resolved and the associated information viewed through any standard web browser by appending the MycoBank number contained in this publication to the prefix “<http://www.mycobank.org/MycoTaxo.aspx?Link=T{&}Rec=>”. The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central SCIE, and CLOCKSS.”

Phylogenetic construction

Genomic DNA was extracted from the dried specimens using the CTAB method (*Doyle & Doyle, 1987*). PCR protocol and sequencing were conducted as described by *Wang et al. (2019)*. Primer pairs used in this study are listed in [Table 1](#). The newly generated sequences were submitted to GenBank.

Representative sequences of *Melanoleuca* species in former studies (*Sánchez-García, Cifuentes-Blanco & Matheny, 2013; Yu et al., 2014; Antonín et al., 2014; Antonín et al., 2015; Antonín et al., 2017; Nawaz, Jabeen & Khalid, 2017; Xu et al., 2019; Antonín et al., 2021; Pei et al., 2021*) were retrieved from GenBank. These sequences were aligned with those obtained from this study using Bioedit v7.0.9 (*Hall, 1999*) and MAFFT v7.313 (*Katoh & Standley, 2013*). The data partition homogeneity test (*Farris et al., 1995*) performed in PAUP (*Swofford, 2003*) allowed combining three regions (ITS, nrLSU and RPB2) (P -value 0.43). A combined data set was then completed with *Pluteus romellii* as an outgroup. The data matrix includes a total of 2,073 characters of 71 samples. Bayesian Inference (BI) and Maximum Likelihood (ML) were performed as previously described in *Pei et al. (2021)*. Specifically, the combined data set was run for 2 million generations under the GTR+I+G mode using MrBayes v.3.2.6 (*Ronquist et al., 2012*). RAxML–8.2.10-WIN was performed under the GTR-GAMMA model of evolution (*Stamatakis, 2014*). The resulting files were viewed using Figtree v1.4.4 (*Rambaut, 2018*) and were compiled in Adobe Illustrator CC.

Divergence time estimation within *Melanoleuca*

Divergence time was estimated using BEAST v2.6.3 (*Bouckaert et al., 2014*). BEAUTI v2.6.3 was used to construct an XML file. ModelFinder (*Kalyaanamoorthy et al., 2017*) was used to infer the best substitution model. The clock model and substitution model were chosen following *Pei (2021)* and *Zhao et al. (2016)*. On the calibrated nodes, the offset ages of 98 and 110 Ma were set for the genus *Melanoleuca* and *Pluteus*, respectively (*Pei, 2021*). We

Table 2 The sequenced *Melanoleuca* species analyzed in this study.

Species	Voucher collection	Origin	GenBank accession No.		
			ITS	nLSU	RPB2
<i>M. subgriseoflava</i>	SYAU-FUNGI-073	Shenyang City, Liaoning Province, China	ON262573	ON262569	ON220896
<i>M. substridula</i>	GDGM 84648	Jiuzhaigou valley, Sichuan Province, China	ON262575	ON262571	ON220898
<i>M. subgriseoflava</i>	SYAU-FUNGI-074	Shenyang City, Liaoning Province, China	ON262574	ON262570	ON220897
<i>M. substridula</i>	GDGM 84683	Jiuzhaigou valley, Sichuan Province, China	ON262576	ON262572	ON220899

ran four independent Monte Carlo Markov Chains (MCMC) of 50 million generations, logging states every 5,000 generations. The checking for convergence was completed in Tracer v1.6 (Rambaut, 2018). TreeAnnotator v.1.8 was used for summarizing tree files. The resulting files were viewed and compiled in Figtree v1.4.4 (Rambaut, 2018) and Adobe Illustrator CC, respectively.

RESULTS

Phylogenetic analyses

The GenBank accession numbers of the sequences, determined in this study, are from ON262569 to ON262573 and ON220896 to ON220899 (Table 2). Maximum likelihood (ML) and Bayesian Inference (BI) showed almost identical topologies and the BI tree was selected for display (Fig. 1). The phylogenetic result suggested that the *Melanoleuca* should belong to a monophyletic group (PP = 1, BS = 100), which is consistent with the previous studies (Yu et al., 2014; Vizzini et al., 2011). A total of five clades (Clade A to Clade E) can be recognized within *Melanoleuca*, which is in line with the result of Pei et al. (2021). Additionally, the collections (SYAU-FUNGI-073 and SYAU-FUNGI-074) named *M. subgriseoflava* formed an independent lineage with strong statistical support (PP = 1.00, BS = 100), located within clade E. And these specimens are closely related to a clade containing sequences of <http://www.indexfungorum.org/names/Names.asp?strGenus=Melanoleuca> *griseoflava* X.D. Yu & H.B. Guo, *M. arcuata* (Bull.) Singer and *M. heterocystidiosa* (Beller & Bon) Bon. In clade A, the collections (GDGM 84648 and GDGM 84683) named *M. substridula* group together with well support (PP = 1.00, BS = 100) and far away from the other species in *Melanoleuca*.

Maximum Clade Credibility (MCC) tree for *Melanoleuca* (Fig. 2) generated a topology similar to those of the phylogenetic analyses. Two new species of *Melanoleuca* also formed two separate clades with high support (PP = 1.00).

Taxonomy

Melanoleuca subgriseoflava X.D. Yu & H.B. Guo, sp. nov.

MycoBank No. MB843803 (Fig. 3)

Etymology The epithet “*subgriseoflava*” refers to the greyish-brown color of the pileus, which is similar to the species *Melanoleuca griseoflava*.

Diagnosis: Pileus convex to applanate to depressed at center, greyish-brown to yellowish-grey pileus; lamellae adnate to sinuate, creamy to light orange; stipe yellowish-brown to

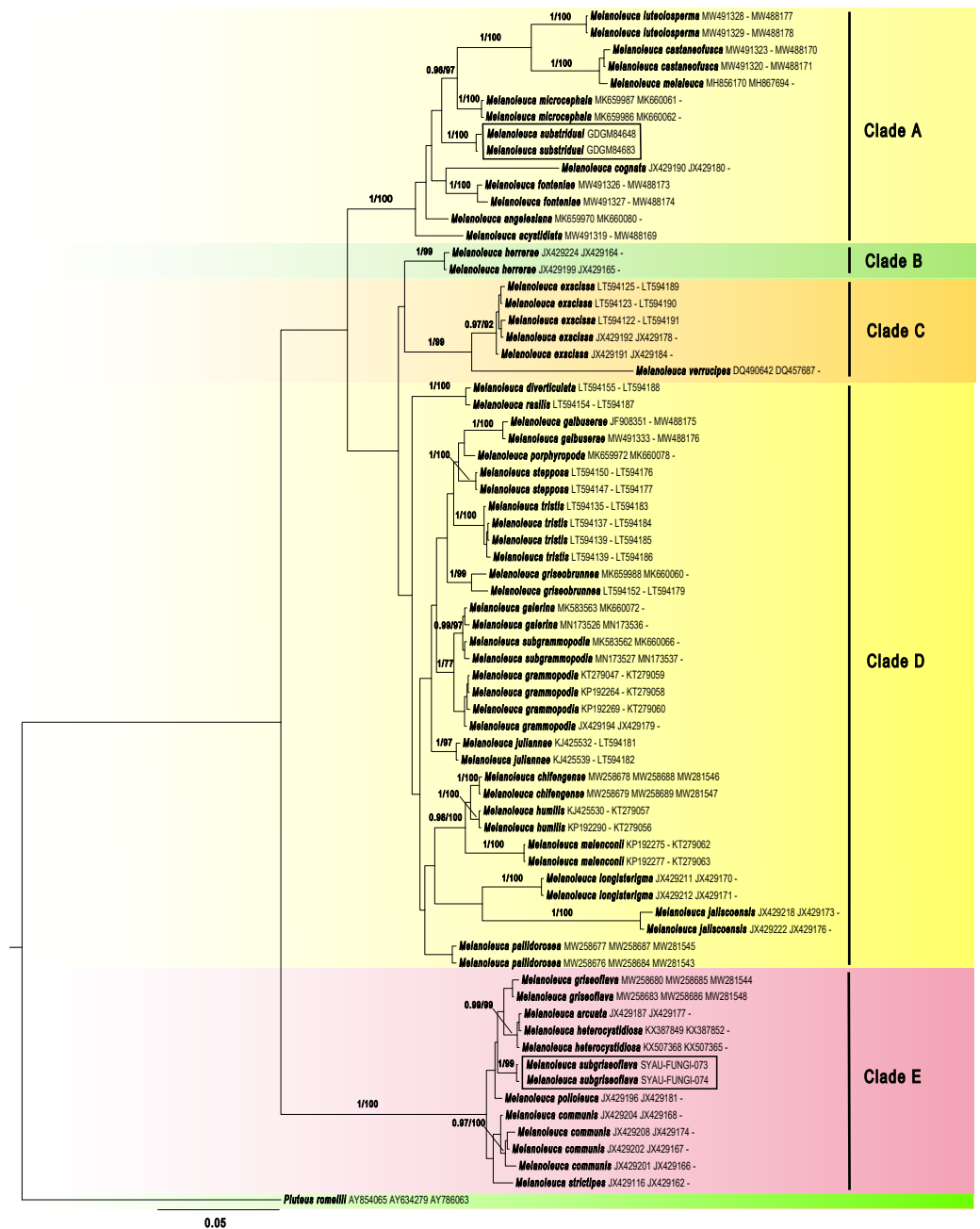


Figure 1 Phylogenetic positions of the two new *Melanoleuca* species, inferred from the combined regions (ITS-nrLSU-RPB2) using MrBayes. The lineages with new species were shown in boxes. PP \geq 0.95 and BS \geq 75% were indicated around the branches. Accession numbers in GenBank (ITS, nrLSU, RPB2) follow the fungal names.

Full-size DOI: 10.7717/peerj.13807/fig-1

brown; context greyish-yellow; basidiospores with round and scattered warts and hymenial cystidia fusiform.

Holotype: CHINA. Liaoning Province: Shenyang City, Shenyang Agricultural University, on the soil in meadows, 2 Sep 2020, X.D. Yu (holotype: SYAU-FUNGI-073).

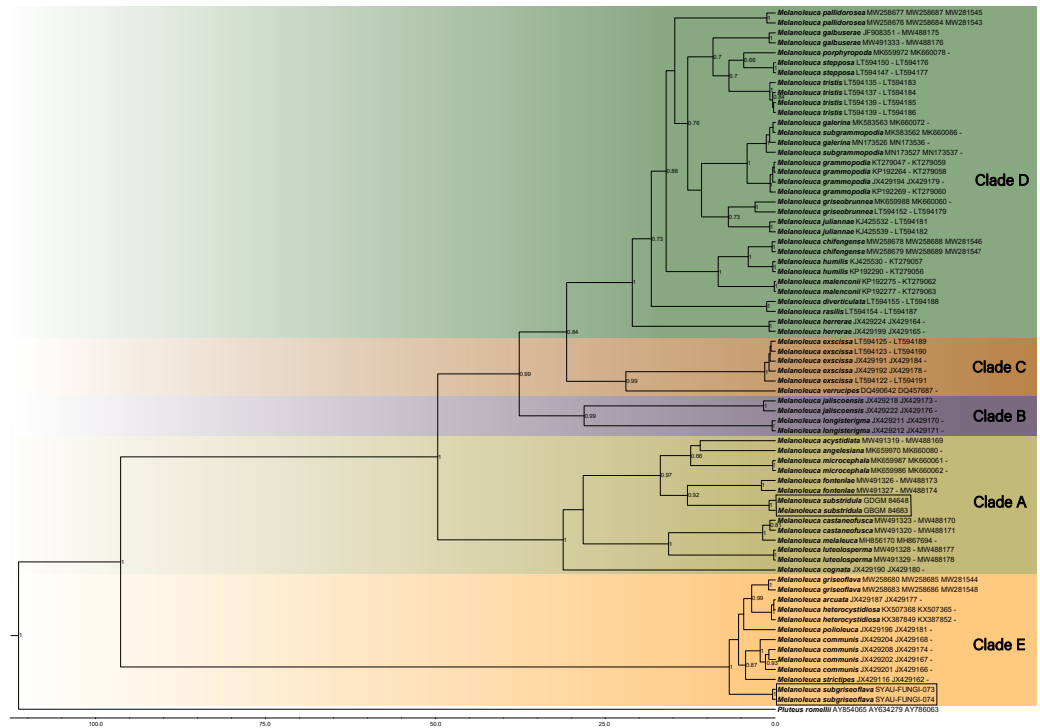


Figure 2 Maximum Clade Credibility tree of *Melanoleuca* based on ITS, nrLSU, and RPB2 genes sequences with the outgroup *Pluteus*. The lineages with new species were shown in boxes. PP ≥ 0.60 are annotated at the internodes.

Full-size DOI: [10.7717/peerj.13807/fig-2](https://doi.org/10.7717/peerj.13807/fig-2)

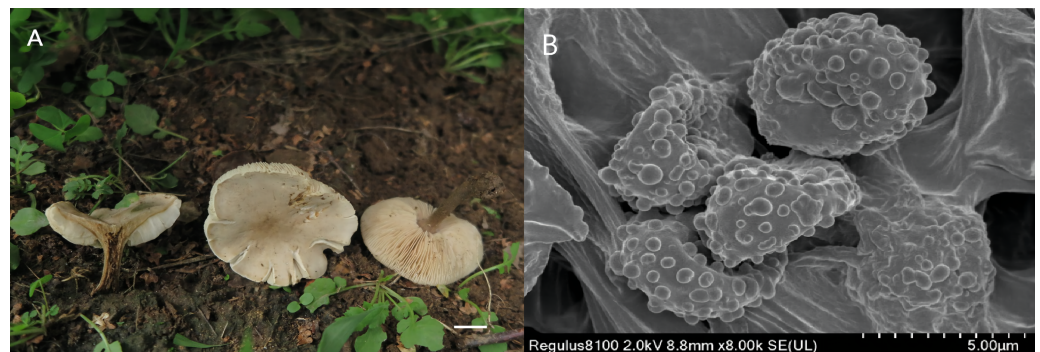


Figure 3 *Melanoleuca subgriseoflava* (Holotype, SYAU-FUNGI-073). (A) macroscopic habitat (B) surface of basidiospores. Scale bars: 1 cm (A); 5 μm (B).

Full-size DOI: [10.7717/peerj.13807/fig-3](https://doi.org/10.7717/peerj.13807/fig-3)

Description: Basidiomata medium-sized. Pileus 24–70 mm diam., convex to plano-convex at first, then gradually appanate, becoming depressed at center when mature; margin entire at first, and slightly lacerate when mature; surface greyish-brown (6D3 to 6F3) at first, then gradually becoming greyish-orange, greyish-yellow, yellowish-grey (5B3 to 5B5, 4B2 to 4B5, 4B2) when mature and dry, brownish-orange at centre (5C5 to 5C6). Lamellae adnate to sinuate, 3.0–7.0 mm broad, white to greyish-orange (5A1, 5B3) at first,

becoming creamy, orange-white, light orange (4A3, 5A2, 5A3) when mature, often deeper at margin, crowded, with lamellulae of two or four lengths, edge entire. Stipe cylindrical and somewhat broadened downwards, 25–55 mm long \times 2.0–7.0 mm diam., central, solid, light brown to yellowish-brown (5D7 to 5D8) in upper part, often becoming brown (5F8 to 6E8) towards base, with whitish flocculose apex, striate. Pileus context up to 10 mm thick near stipe attachment, thinner at margin, greyish-yellow (4B3 to 4B6), unchanging when exposed. Smell slightly farinaceous, taste mild. Spore deposit creamy.

Basidiospores (88/10/8) (5.0) 7.0–8.0 (8.5) \times 4.0–5.0 (6.0) μm , av. 7.6 \times 4.9 μm , $Q = 1.55$, broadly ellipsoid, some obovate, ornamentation of small to large warts, some warts with irregular ridges, amyloid. Basidia (40/10/8) (18) 23–27 (28) \times (7.0) 8.0–10.0 (11.0) μm , av. 26 \times 8.7 μm , clavate to subclavate, hyaline, four-spored. Cheilocystidia lageniform, fusiform to conical cystidia, (30/10/8) (40) 45–49 (50) \times (9.0) 10.0–11.0 (12.0) μm , found both at the edge of lamellae, most thin-walled, less thick-walled without distinct upper part. Pleurocystidia have a small amount, similar to cheilocystidia. Trama hyphae thin-walled, regular, 4.0–10.0 μm wide, inamyloid. Pileipellis hyphae cylindrical, with numerous branched, thin-walled, up to 10.5 μm wide. Stipitipellis hyphae in parallel, 4–14.0 μm wide, thin-walled, somewhat slightly thick-walled. Caulocystidia absent. Clamp connections absent in all tissues.

Habitat and distribution: Solitary or in small groups, saprotrophic on the soil, on the grass, on roadsides, in woods. Known from north-eastern China.

Additional material studied: CHINA. Liaoning Province: Shenyang City, Dongling Park, on the soil in meadows, 21 Jul 2019, H.B. Guo (SYAU-FUNGI-074); CHINA. Liaoning Province: Shenyang City, Dongling Park, on the grass in woods, 21 Jul 2019, X.D. Yu (SYAU-FUNGI-075); CHINA. Liaoning Province: Shenyang City, on the campus of Shenyang Agricultural University, on roadsides, 2 Sep 2020, X.D. Yu (SYAU-FUNGI-076); CHINA. Liaoning Province: Shenyang City, on the campus of Shenyang Agricultural University, on the soil in meadows, 8 Sep 2019, X.D. Yu (SYAU-FUNGI-077); CHINA. Liaoning Province: Shenyang City, on the campus of Shenyang Agricultural University, on roadsides, 8 Sep 2019, H.B. Guo (SYAU-FUNGI-078).

Remarks: The main features of *M. subgriseoflava* are its greyish-brown to yellowish-grey pileus, creamy to light orange lamellae, greyish-yellow context, basidiospores with scattered warts and fusiform hymenial cystidia. On account of the pileus color, *M. subgriseoflava* is closely related to *M. griseoflava* originally described in northeastern China by [Pei et al. \(2021\)](#). Nevertheless, *M. griseoflava*, differs from *M. subgriseoflava* by its adnate to adnexed and white lamellae. Besides, *M. griseoflava* differs by the presence of whitish tomentum at the stipe base ([Pei et al., 2021](#)). Micromorphologically, *M. griseoflava* is also distinct from *M. subgriseoflava* by its almost reticulate surface ornamentations of basidiospores and the presence of caulocystidia ([Pei et al., 2021](#)).

***Melanoleuca substridula* M. Zhang & X.D. Yu, sp. nov.**

Mycobank No. MB 843804 ([Fig. 4](#))

Etymology: The epithet “*substridula*” refers to the ochre brown color of the pileus, which is similar to the species *Melanoleuca stridula*.

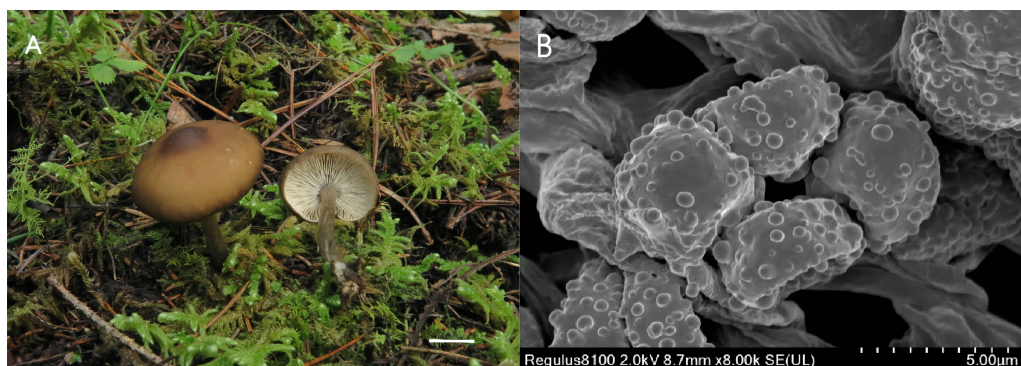


Figure 4 *Melanouca substridula* (Holotype, GDGM 84648). (A) Macroscopic habitat; (B) surface of basidiospores. Scale bars: 1 cm (A) 5 μ m (B).

Full-size DOI: [10.7717/peerj.13807/fig-4](https://doi.org/10.7717/peerj.13807/fig-4)

Diagnosis: Basidiomata slightly small; pileus umbonate, brown to dark brown; lamellae sinuate to adnate, white; stipe light brown in upper part and grey-brown in lower part; basidiospores with round and scattered warts and lack of any forms of cystidia.

Holotype: CHINA. Sichuan Province: Jiuzhaigou valley reserve, on the soil in meadows, 19 Sep 2020, Ming Zhang (GDGM 84648).

Description: Basidiomata slightly small-sized. Pileus 21–38 mm diam.; umbonate at first; margin first slightly inflexed, soon becoming straight, depressed when mature and dry; surface glabrous, light brown at first (5D5 to 5D7), becoming brown to dark brown (7E8 to 7F8) when mature, often darker at margin, with a conspicuous dark brown (6F8 to 7F8) umbo at centre. Lamellae crowded, sinuate to adnate, 3.0–4.0 mm broad, white, edge entire and concolorous, with lamellulae of two or four lengths. Stipe cylindrical and somewhat broadened downwards, 26–38 mm long \times 4.0–5.0 mm diam., central, solid, light brown (6D4 to 6D8) in upper part, becoming grey-brown (5E3 to 6E3) towards base, with whitish flocculose apex, longitudinally striate, with whitish basal tomentum. Context up to 30–50 mm thick near stipe attachment, thinner at margin, white. Odor faint. Spore deposit whitish.

Basidiospores (86/6/2) 7.0–8.0 (9.0) \times 5.0–6.0 (6.5) μ m, av. 7.4 \times 5.2 μ m, Q = (1.40) 1.42–1.45 (1.50), obovate to ellipsoid, subhyaline, ornamentation of somewhat regular warts, less warts with ridges, amyloid. Basidia (43/6/2) (25) 27–30 (31) \times (6.0) 7.0–9.0 (10.0) μ m, av. 30 \times 8.0 μ m, clavate, subhyaline. All types of cystidia absent. Lamella edge sterile. Trama hyphae thin-walled, regular, 5.5–16.5 μ m wide. Pileipellis hyphae cylindrical, with numerous branched, thin-walled, up to 7.5 μ m wide. Stipitipellis hyphae in parallel, with few branches, 3.5–8.0 μ m wide, thin-walled. Caulocystidia absent. Clamp connections absent in all tissues.

Habitat: Solitary or in small groups, saprotrophic on the soil, on the grass in woods. Known from south-western China.

Material studied: CHINA. Sichuan Province: Jiuzhaigou valley reserve, 19 Sep 2020, Ming Zhang (GDGM 84683).

Remarks: The most distinctive characteristics of *Melanoleuca substridula* are its slightly small-sized basidiocarp, light brown to dark brown pileus with a prominent umbo, sinuate to adnate lamellae, light brown to greyish-brown stipe and lack of cystidia. On account of the pileus color, *M. substridula* is closely related to *M. stridula* originally described by Singer (1943). However, *M. stridula* is featured by a slightly larger pileus (15–60 mm in diameter) (Singer, 1943). Additionally, *M. stridula* is often characterized by a pileus with a center depression, which differs by the umbonate pileus of *M. substridula*. Microscopically, *M. stridula* can be distinguishable from *M. substridula* by the presence of subcylindrical cystidia-like cells at the apex of the stipe (Metrod, 1949).

DISCUSSION

Two new species, *Melanoleuca subgriseoflava* and *Melanoleuca substridula*, discovered and collected in Liaoning province and Sichuan province respectively, were originally reported and described in this study. Morphologically, the most distinctive features of *M. subgriseoflava* are a grey-brown to yellowish-grey pileus, creamy lamellae, fusiform hymenial cystidia and warted basidiospores. According to the classification system of Boekhout (1988), *M. subgriseoflava* should belong to the section *Strictipedes* in the subgenus *Macrocystidia* because of the presence of grey-brown pileus and fusiform hymenial cystidia (Boekhout, 1988). Amongst the section *Strictipedes*, *M. subgriseoflava* mainly differs from the other species by its lack of caulocystidia, including *M. turrita* (Fr.) Sing, *M. polioleuca* (Fr.: Fr.) Kühn. & Maire, *M. atripes* Boekhout and *M. albifolia* Boekhout and (Boekhout, 1988). Furthermore, *M. albifolia* Boekhout differs from *M. subgriseoflava* by white lamellae. *M. polioleuca* (Fr.: Fr.) Kühn. & Maire can be distinguishable from *M. subgriseoflava* by a longer stipe, with a length of around 35–85 mm (Boekhout, 1988).

Melanoleuca substridula is easily recognized by its light brown to dark brown pileus, prominent umbo, adnate to sinuate lamellae, light brown to grey-brown stipe and acystidiate micromorphology. For lack of any forms of cystidia, *M. substridula* belongs to the subgenus *Melanoleuca* (Boekhout, 1988). Within the subgenus *Melanoleuca*, *M. graminicola* (Velen.) Kühner & Maire, *M. melaleuca* (Pers.: Fr.) Murrill, *M. stridula* (Fr.) Metrod and *M. striimarginata* Metrod are characterized by adnate to subdecurrent lamellae and a depressed pileus center, making them easily distinguishable from *M. substridula* (Boekhout, 1988). The latter three species also differ by their larger basidiomata, with a pileus diameter of 35–65 mm. Moreover, *M. striimarginata* differs by a striate margin of the pileus (Metrod, 1942).

Both the phylogenetic relationships and the divergence-time estimation, based on three regions (ITS-nrLSU-RPB2), showed that there are five clades in the genus *Melanoleuca* (Figs. 1 and 2), which was corroborated by Pei et al. (2021). According to the phylogram, *M. subgriseoflava* is closely related to the other three species with high support in clade E, i.e., *M. arcuata*, *M. heterocystidiosa* and *M. griseoflava*. *Melanoleuca arcuata* differs by its brick-red pileus and decurrent lamellae (Fries, 1821). *Melanoleuca heterocystidiosa* can be easily separated from *M. subgriseoflava* based on its smaller basidiomata, with a pileus diameter of 15 mm (Singer, 1939; Bon, 1984). *Melanoleuca griseoflava* differs from *M.*

subgriseoflava as elaborated above. In clade A, with the exception of *M. microcephala*, *M. substridula* is far away from the other species of *Melanoleuca*. However, *M. microcephala* can easily distinguish from *M. substridula* by its longer stipe with a length of 105 mm. Besides, caulocystidia in groups can be observed in *M. microcephala*, but not any forms of cystidia in *M. substridula* (Antonín *et al.*, 2021).

In the present study, five clades can be recognized in both the BI tree (Fig. 1) and the MCC tree (Fig. 2). However, using phylogenetic analyses, species of *Melanoleuca* were divided into two clades in former research (Vizzini *et al.*, 2011; Yu *et al.*, 2014; Nawaz, Jabeen & Khalid, 2017; Xu *et al.*, 2019). The species of *Melanoleuca* within each clade have lacked uniform characteristics to work in identification. In order to clarify the infrageneric classification, taxonomic treatments should be performed based on additional materials and complete morphological descriptions in later studies. Two new *Melanoleuca* species have been confirmed and a key for further studies on the *Melanoleuca* genus has been provided in this study.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

This study was supported by the National Natural Science Foundation of China (No. 31770014) and the Science and Technology Plan Project of Liaoning Province (2020-MZLH-33). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:

National Natural Science Foundation of China: 31770014.

Science and Technology Plan Project of Liaoning Province: 2020-MZLH-33.

Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Yue Qi performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Cai-Hong Li performed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Yu-Meng Song analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Ming Zhang performed the experiments, prepared figures and/or tables, and approved the final draft.
- Hong-Bo Guo conceived and designed the experiments, prepared figures and/or tables, and approved the final draft.
- Xiao-Dan Yu conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:

GenBank accession numbers [ON262569](#) to [ON262573](#) and [ON220896](#) to [ON220899](#).

New Species Registration

The following information was supplied regarding the registration of a newly described species:

MycoBank No. MB843803

MycoBank No. MB 843804

Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.13807#supplemental-information>.

REFERENCES

- Antonín V, Rimóczi I, Benedek L, Papp V, Szarkándi JG, Dima B, Nagy LG, Papp T, Ďuriška O, Tomšovský M. 2014.** *Melanoleuca juliannae* (Basidiomycota, Tricholomataceae), a new species from subgen. *Urticocystis*. *Phytotaxa* **170**:13–23 DOI [10.11646/phytotaxa.170.1.2](#).
- Antonín V, Ďuriška O, Gafforov Y, Jančovičová S, Para R, Tomšovský M. 2017.** Molecular phylogenetics and taxonomy in *Melanoleuca excissa* group, (Tricholomataceae, Basidiomycota) and the description of *M. griseobrunnea* sp. nov.. *Plant Systematics and Evolution* **303**:1181–1198 DOI [10.1007/s00606-017-1430-y](#).
- Antonín V, Ďuriška O, Jančovičová S, Tomšovský M. 2015.** Identity of *Agaricus brevipes* Bull. (*Melanoleuca brevipes*, Tricholomataceae, Basidiomycota). *Mycological Progress* **14**:e107 DOI [10.1007/s11557-015-1125-0](#).
- Antonín V, Ševčíková H, Para R, Ďuriška O, Kudláček T, Tomšovský M. 2021.** *Melanoleuca galbuserae*, *M. fontenlae* and *M. acystidiata*, three new species in subgen. *Urticocystis*, and comments to *M. castaneofusca* and related species. *Journal of Fungi* **7**(3):e191 DOI [10.3390/jof7030191](#).
- Bau T, Li Y. 1999.** Species diversity of macrofungi studies in Daqinggou nature reserve. *Journal of Jilin Agricultural University* **3**:36–45.
- Boekhout T. 1988.** Notulae ad Floram Agaricinam Neerlandicam—XVI, New taxa, new combinations in *Melanoleuca* Pat. and notes on rare species in the Netherlands. *Persoonia* **13**:397–431.
- Boekhout T. 1999.** *Melanoleuca* Pat. In: Bas C, ThW Kuype, Noordeloos ME, Vellinga EC, eds. *Flora agaricina neerlandica*. vol 4. Brookfield, Rotterdam: A. A. Balkema, 153–165.
- Bon M. 1978.** Tricholomataceae de France et d'Europe occidentale (Sous-famille Leucopaxilloidae (Singer) Bon). (*DOC*) *Mycology* **9**(33):1–79.
- Bon M. 1984.** Combinaisons et taxons nouveaux. *Documents Mycologiques* **14**(53):1–6.
- Bon M. 1991.** Flore mycologique d'Europe 2, Les Tricholomes et ressemblants. *Documents Mycologiques: Mémoire Hors Série* **2**:1–154.

- Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu CH, Xie D, Suchard MA, Rambaut A, Drummond AJ. 2014.** BEAST 2: a software platform for Bayesian evolutionary analysis. *PLOS Computational Biology* **10**(4):e1003537 DOI [10.1371/journal.pcbi.1003537](https://doi.org/10.1371/journal.pcbi.1003537).
- Chen YQ. 2007.** Studies on the Macrofungal Diversity in Laoyeling Mountain of Jilin. Master's thesis, Jilin Agricultural University, Jilin, China.
- Dai YC, Zhou LW, Yang ZL, Wen HA, Bau T, Li TH. 2010.** A revised checklist of edible fungi in China (in Chinese). *Mycosystema* **29**(1):e29 DOI [10.13346/j.mycosystema.2010.01.022](https://doi.org/10.13346/j.mycosystema.2010.01.022).
- Doyle JJ, Doyle JL. 1987.** A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* **19**:11–15.
- Du MH, Gao LX, Li JZ, Chai CY, Jia S, Wang SM. 2016.** Preliminary investigation of diversity of macrofungi in Henan Funiu mountain nature reserve. *Edible and Medicinal Mushrooms* **24**:306–311.
- Farris JS, Källersjö M, Kluge AG, Bult C. 1995.** Constructing a significance test for incongruence. *Systematic Biology* **44**:570–572 DOI [10.2307/2413663](https://doi.org/10.2307/2413663).
- Fries EM. 1821.** Systema mycologicum. *Monograph* **1**:1–520 DOI [10.5962/bhl.title.5378](https://doi.org/10.5962/bhl.title.5378).
- 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.
- He LP, Ma CS, Zhang SY, Wang JH, Gu ZD, Jin QY. 2014.** Macrofungi resources in Chagangliang Provincial Nature Reserve of Gansu. *Journal of Gansu University of Technology* **4**:46–53.
- Kalmer A, Acar I, Dizkirici A. 2018.** Phylogeny of some *Melanoleuca* species (Fungi: Basidiomycota) in Turkey and identification of *Melanoleuca angelesiana* A.H. Sm. As a first record. *Kastamonu University Journal of Forestry Faculty* **18**:314–326 DOI [10.17475/kastorman.499076](https://doi.org/10.17475/kastorman.499076).
- Kalyaanamoorthy S, Minh BQ, Wong TKF, Haeseler AV, Jermiin LS. 2017.** ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* **14**:587–589 DOI [10.1038/nmeth.4285](https://doi.org/10.1038/nmeth.4285).
- Katoh K, Standley DM. 2013.** MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**:772–780 DOI [10.1093/molbev/mst010](https://doi.org/10.1093/molbev/mst010).
- Kornerup A, Wanscher JH. 1963.** *Methuen handbook of colour*. London: Methuen and Co. Ltd, 242.
- Mao XL. 2009.** *Mushroom in China*. Beijing: Science Press.
- Matheny PB, Wang Z, Binder M, Curtis JM, Lim YM, Nilsson RH, Hughes KW, Hofstetter V, Ammirati JF, Schoch CL, Langer E, Langer G, McLaughlin DJ, Wilson AW, Frøslev T, Ge ZW, Kerrigan RW, Slot GC, Yang ZL, Baroni TJ, Fischer M, Hosaka K, Matsuura K, Seidl MT, Vauras J, Hibbett DS. 2007.** Contributions of *rpb2* and *tef-1* α to the phylogeny of mushrooms and allies (Basidiomycota, Fungi). *Molecular Phylogenetics and Evolution* **43**:430–451 DOI [10.1016/j.ympev.2006.08.024](https://doi.org/10.1016/j.ympev.2006.08.024).
- Metrod G. 1942.** Sur le genre *Melanoleuca*. *Revue de Mycologie* **94**:89–96.

- Michot B, Hassouna N, Bachellerie J. 1984.** Secondary structure of mouse 28S rRNA and a general model for the folding of the large rRNA in eucaryotes. *Nucleic Acids Research* **12**:4259–4279 DOI [10.1093/nar/12.10.4259](https://doi.org/10.1093/nar/12.10.4259).
- Nawaz F, Jabeen S, Khalid AN. 2017.** New and noteworthy *Melanoleuca* (Pluteaceae) from Pakistan. *Phytotaxa* **311**(2):175–184 DOI [10.11646/phytotaxa.311.2.5](https://doi.org/10.11646/phytotaxa.311.2.5).
- Pei Y. 2021.** Phylogenetic reconstruction on *Melanoleuca* and allied genera using divergence times (In Chinese). Dissertation for Master's Degree.
- Pei Y, Guo HB, Liu TZ, Qin WQ, Zhao D, Qi XJ, Yu XD. 2021.** Three new *Melanoleuca* species (Agaricales, Basidiomycota) from north-eastern China, supported by morphological and molecular data. *MycKeys* **80**:133–148 DOI [10.3897/mycokeys.80.64369](https://doi.org/10.3897/mycokeys.80.64369).
- Rambaut A. 2018.** FigTree v1.4.4: Tree figure drawing tool.
- Ronquist F, Teslenko M, Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012.** MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**:539–542 DOI [10.1093/sysbio/sys029](https://doi.org/10.1093/sysbio/sys029).
- Sánchez-García M, Cifuentes-Blanco J, Matheny PB. 2013.** Revisión taxonómica del género *Melanoleuca* en México y descripción de especies nuevas. *Revista Mexicana de Biodiversidad* **84**:S111–S127 DOI [10.7550/rmb.31569](https://doi.org/10.7550/rmb.31569).
- Singer R. 1943.** Das System der Agaricaldes, III. *Annales Mycologici* **41**(1–3):1–189.
- Singer R. 1986.** *The Agaricales in modern taxonomy*. 4th edn. Koenigstein: Koeltz Scientific Books, 981 pp.
- Stamatakis A. 2014.** RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**:1312–1313 DOI [10.1093/bioinformatics/btu033](https://doi.org/10.1093/bioinformatics/btu033).
- Sun LH, Song G, Wang LY, Yun XF. 2012.** Ecological diversity of macro fungi in Helan Mountain. *Journal of Anhui Agricultural Sciences* **40**:2219–2222.
- Swofford DL. 2003.** *PAUP*. phylogenetic analysis using parsimony (*and Other Methods), Version 4*. Sunderland: Sinauer Associates.
- Tian HM, Liu TZ, Tian YC, Ren XQ, Qiu YL, Li XD. 2018.** List of macrofungi in the xilamulun river basin I. *Journal of Chifeng University* **34**:26–29.
- Vizzini A, Consiglio G, Setti L, Murat C. 2010.** The agaricoid genus *Kinia* is a new member of the Pluteoid clade subordinate to *Melanoleuca*. *Mycosphere* **1**(2):141–145.
- Vizzini A, Para R, Fontenla R, Ghignone S, Ercole E. 2011.** A preliminary ITS phylogeny of *Melanoleuca* (Agaricales) with special reference to European taxa. *Mycotaxon* **118**:361–381 DOI [10.5248/118.361](https://doi.org/10.5248/118.361).
- Wang JR. 2013.** Biodiversity of Macrofungi in Shandong Province. dissertation, Jilin Agricultural University, Jilin.
- Wang SY, Guo HB, Li JJ, Li W, Wang Q, Yu XD. 2019.** Evaluation of five regions as DNA barcodes for identification of *Lepista* species (Tricholomataceae, Basidiomycota) from China. *Peer Journal* **7**:e7307 DOI [10.7717/peerj.7307](https://doi.org/10.7717/peerj.7307).
- Wei J, Fan YJ, Yan W. 2015.** A preliminary report on macrofungi from Wulashan area of Yin mountain. *Guangdong Agricultural Sciences* **9**:149–153.
- 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, eds. *PCR protocols: methods and applications*. London: Academic Press, 315–322 DOI [10.1016/B978-0-12-372180-8.50042-1](https://doi.org/10.1016/B978-0-12-372180-8.50042-1).

Xu JZ, Yu XD, Lu MZ, Hu JJ, Moodley O, Zhang CL, Gong L, Li Y. 2019. Phylogenetic analyses of some *Melanoleuca* species (Agaricales, Tricholomataceae) in Northern China, with descriptions of two new species and the identification of seven species as a first record. *Frontiers in Microbiology* **10**:e2167 DOI [10.3389/fmicb.2019.02167](https://doi.org/10.3389/fmicb.2019.02167).

Yu XD, Lv SX, Ma D, Li FF, Lin Y, Zhang L. 2014. Two new species of *Melanoleuca* (Agaricales, Basidiomycota) from north-eastern China, supported by morphological and molecular data. *Mycoscience* **55**:456–461 DOI [10.1016/j.myc.2014.01.007](https://doi.org/10.1016/j.myc.2014.01.007).

Zhang WM, Li TH, Song B. 2001. General situation of macrofungi in Guangdong Province. *Ecologic Science* **4**:48–58.

Zhao RL, Zhou JL, Chen J, Margaritescu S, Sánchez-Ramírez S, Hyde KD, Moncalvo JM. 2016. Towards standardizing taxonomic ranks using divergence times –a case study for reconstruction of the *Agaricus* taxonomic system. *Fungal Diversity* **78**:239–292 DOI [10.1007/s13225-016-0357-x](https://doi.org/10.1007/s13225-016-0357-x).

Zhao XH, Chi JL, Zhong LJ, Zhang QH, Zhu WW, Guan YL. 2014. Investigation of macrofungi resources in Western Liaoning. *Edible Fungi* **3**:16–18.