

Morphological characteristics and phylogenetic analyses revealed *Melanogaster sinensis* sp. nov. (Paxillaceae, Boletales) from Northeast China

XIN YANG^{1,2,6}, XIN GAO^{2,7}, SIMIAO LI^{2,8}, RONGHUA WANG^{5,9}, CHANGLIN (CHARLIE) ZHAO^{1,2,4,10*} & SHAN SHEN^{3,11*}

¹Key Laboratory of Forest Disaster Warning and Control in Yunnan Province, Southwest Forestry University, Kunming 650224, P.R. China

²College of Forestry, Southwest Forestry University, Kunming 650224, China

³College of Biological Science and Food Engineering, Southwest Forestry University, Kunming 650224, China

⁴Department Microbial Drugs (MWIS), Helmholtz-Centre for Infection Research, Braunschweig 38124, Germany

⁵State Key Laboratory of Supramolecular Structure and Materials, College of Chemistry, Jilin University, Changchun 130012 China

⁶✉ fungixiny@163.com;  <https://orcid.org/0009-0009-7755-0581>

⁷✉ fungigaoxin@163.com;  <https://orcid.org/0009-0002-0836-9315>

⁸✉ fungils@163.com;  <https://orcid.org/0009-0009-0396-3244>

⁹✉ wangrh1223@mails.jlu.edu.cn;  <https://orcid.org/0009-0004-9661-1355>

¹⁰✉ fungichanglinz@163.com;  <https://orcid.org/0000-0002-8668-1075>

¹¹✉ shanfungi@163.com;  <https://orcid.org/0000-0002-7114-092X>

*Corresponding author: C.L. Zhao, ✉ fungichanglinz@163.com; S. Shen, ✉ shanfungi@163.com

Abstract

A new species, *Melanogaster sinensis* (Paxillaceae, Boletales), from Jilin Province in Northeast China, is described and illustrated based on morphological observations and molecular evidence. It is characterized by hypogeous to semi-hypogeous basidiomata, a single-layered peridium, 8spored basidia (10.5–16.0 × 6.0–9.0 µm), and fusiform basidiospores (4.8–6.2 × 3.0–3.8 µm). Phylogenetic analysis of the ITS dataset placed our collection within *Melanogaster* as *M. sinensis* and recovered a monophyletic lineage. A full description, illustrations, and phylogenetic analysis results of the new species are provided. In addition, the new species is compared with closely related taxa.

Key words: hypogeous fungi, new species, phylogeny, taxonomy

Introduction

The genus *Melanogaster* Corda (Paxillaceae, Boletales) was established in the early nineteenth century (Corda 1831), with *M. tuberiformis* Corda as the type species. It was historically placed within Melanogastraceae (Hymenogastrales) (Zeller 1949), but recent molecular phylogenetic studies have transferred the genus to the Paxillaceae within the Boletales (Basidiomycota) (Binder & Hibbett 2006, Lacheva 2015). Morphologically, *Melanogaster* is characterized by gasteroid, hypogeous to semi-hypogeous basidiomata with a prosenchymatic peridium that varies from yellowish to brownish, sometimes exhibiting basal or superficial mycelial strands (Xu *et al.* 2022). The gleba consists of gelatinous locules separated by whitish to yellowish sterile veins; the basidiospores are yellow to dark brown and variable in shape (globose, ellipsoid, pyriform, or fusiform) (Castellano *et al.* 1986, Pegler *et al.* 1993, Moreau *et al.* 2011; Alvarado *et al.* 2021; Xu *et al.* 2022, Yuan *et al.* 2024). Ecologically, *Melanogaster* species are significant ectomycorrhizal (EcM) fungi that associate with broadleaved and coniferous hosts, such as members of Betulaceae, Cistaceae, Fagaceae, Pinaceae, and Salicaceae (Comandini *et al.* 2006, Türkoğlu & Castellano 2013). The genus is predominantly distributed in the Northern Hemisphere (Europe, North America, and Asia); rare occurrences, such as *M. quercus* L., have been reported in the Southern Hemisphere (Trappe *et al.* 2009).

Currently, *Melanogaster* encompasses approximately 36 species (Hyde *et al.* 2024). Taxonomically, *Melanogaster* is distinct from related sequestrate Paxillaceae genera like *Alpova* C.W. Dodge, *Neoalpova* Vizzini, and *Paralpova* Cabero & P. Alvarado, primarily by its peridium structure. *Melanogaster* has a peridium composed of prostrate or

interwoven hyphae, whereas related genera exhibit a pseudoparenchymatous structure with inflated hyphae (Yuan *et al.* 2024). It also differs from the false-truffle genus *Rhizopogon* Fr. (Rhizopogonaceae) by gel-filled locules and pigmented basidiospores (de la Fuente *et al.* 2021). Phylogenetic analyses consistently recover *Melanogaster* within Paxillaceae Lotsy, alongside with *Gyrodon* Opat., *Paxillus* Fr., and *Alpova* (Binder & Hibbett 2006, Vizzini *et al.* 2010, Moreau *et al.* 2011, 2013, Alvarado *et al.* 2021).

In China, studies on *Melanogaster* date back to 1915, when the first specimen was collected from the Jinshajiang Valley in Yunnan Province. It was originally identified as *M. variegatus* (Vittad.) Tul. & C. Tul., but was later described as a novel species, *M. ovoidisporus* Y. Wang (Wang *et al.* 1995). A second significant specimen, collected in Beijing in 1958 and initially determined as *M. ambiguus* (Vittad.) Tul. & C. Tul., was subsequently republished as *M. spinisporus* Y. Wang (Teng 1963, Wang *et al.* 1995). Early contributions by Liu *et al.* (1989) and Wang *et al.* (1995) suggested that China harbors substantial *Melanogaster* diversity, with several new taxa proposed based solely on morphology. However, because these early studies lacked molecular verification, molecular data have yet to confirm the taxonomic status of some of these species. More recently, molecular approaches have revolutionized the taxonomy of the genus in the region. Xu *et al.* (2022) revised *Melanogaster* in China based on the morphology and ITS phylogenetic analyses, recognizing ten taxa, including four new species (*M. minobovatus* L. Fan, X.Y. Yan & Y.Y. Xu, *M. panzhihuaensis* L. Fan, X.Y. Yan & Y.Y. Xu, *M. quercicola* L. Fan, X.Y. Yan & Y.Y. Xu, and *M. tomentellus* L. Fan, X.Y. Yan & Y.Y. Xu). Furthermore, Yuan *et al.* (2024) described three additional species from southwestern China (*M. cyaneus* T.J. Yuan, Shu H. Li & Raspé, *M. diqingensis* T.J. Yuan, Shu H. Li & Raspé, and *M. truncatisporus* T.J. Yuan, Shu H. Li & Raspé) and noted that traditional sections appear non-monophyletic, challenging the current infrageneric classification.

During recent field investigations of *Melanogaster* in China, we collected specimens that, although morphologically resembling *M. panzhihuaensis*, could not be assigned to any known species due to preliminary differences in basidiospore dimensions. Based on morphological characters and molecular phylogenetic analysis, we herein describe this distinct lineage as a new species, *Melanogaster sinensis*.

Materials and methods

Sample collection and examination

Fresh basidiomata growing in the soil beneath *Tilia* sp. were collected from Jilin Province. The samples were photographed *in situ* using a Xiaomi 13, and macroscopic characteristics, including peridium color, along with associated metadata, were recorded (Rathnayaka *et al.* 2025). Photographs were focus-stacked and merged using Helicon Focus Pro 7.7.5 software. Specimens were dried in an electric food dehydrator at 40 °C (Yang *et al.* 2024, 2025), sealed in envelope bags, and deposited in the herbarium of Southwest Forestry University (SWFC), Kunming, Yunnan Province, P.R. China.

Morphological observations

The macromorphological descriptions were based on field notes and photos captured in the field and laboratory and followed the color terminology of Petersen (1996). Micromorphological data were obtained from the dried specimens following observation under a light microscope (Yang *et al.* 2025). Sections were studied at up to 1000× magnification using a Nikon E80i microscope with phase-contrast illumination (Nikon, Tokyo, Japan). Line drawings were produced using a fungus plotter. The ultrastructure of basidiospores was examined by Scanning Electron Microscopy (SEM) with a Field Emission Scanning Electron Microscope (FESEM) GeminiSEM 360. Measurements and drawings were obtained from slide preparations stained with Cotton Blue (0.1 mg aniline blue dissolved in 60 g pure lactic acid). Sections were mounted in 2% phloxine B dye (C₂₀H₂Br₄Cl₄Na₂O₅). For spore measurements, 5% of values from each end of the range were excluded, as indicated in parentheses. Sixty basidiospores were randomly selected from two specimens for measurement. Results are presented as L, W, and Q, representing the extreme values of length, width, and length-to-width ratio, respectively. Mean values are reported as L_m = L ± S.D., W_m = W ± S.D., and Q_m = Q ± S.D.

Genomic DNA was extracted from dried fungal specimens using the CTAB rapid fungi genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd., Beijing, China), following the manufacturer’s instructions (Gu *et al.* 2025, Yuan *et al.* 2025). The DNA was stored at –20 °C for long-term preservation. Three molecular markers were analyzed, including the internal transcribed spacer (ITS). The nuclear ribosomal ITS region was amplified using the ITS5 and ITS4 primer pair (White *et al.* 1990). The PCR protocol for ITS consisted of an initial denaturation at 94 °C for 3 minutes, followed by 35 cycles of 94 °C for 60 seconds, 56 °C for 60 seconds, and 72 °C for 1 minute, with a final extension at 72 °C for 10 minutes (Yang *et al.* 2024). The PCR products were purified and sequenced at Kunming Tsingke Biological Technology Limited Company (Yunnan Province, China). All newly generated sequences were deposited in NCBI GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) (Table 1).

Phylogenetic analyses

Sequences generated in this study were aligned with additional sequences obtained from GenBank. Alignment was performed using MAFFT 7 (<https://mafft.cbrc.jp/alignment/server/>), with nucleotide sequence direction adjusted according to the first sequence and the G-INS-i iterative refinement method selected (Katoh *et al.* 2019). Manual adjustments to the alignment were made using AliView version 1.27 (Larsson 2014). The dataset was initially aligned, after which the ITS sequences were combined using Mesquite v. 3.51. The ITS sequence dataset was then used to infer the phylogenetic position of the new species within the genus *Melanogaster*. Sequences generated in this study were sourced from GenBank and a recent publication (Xu *et al.* 2022). Phylogenetic trees were constructed using randomized accelerated Maximum Likelihood (ML) and Bayesian Inference (BI) analyses.

Maximum Likelihood (ML) and Bayesian Inference (BI) analyses were conducted on the ITS dataset in accordance with previous studies (Zhao & Wu 2017; Zhao *et al.* 2023). Phylogenetic reconstructions utilized ML tools available in the PhyloSuite v1.2.3 platform (Xiang *et al.* 2023; Zhang *et al.* 2020). ML phylogenies were generated using IQ-TREE v2.2.0, employing 5,000 ultrafast bootstrap replicates and 1,000 SH-aLRT tests to evaluate branch support (Nguyen *et al.* 2015). BI was performed via the Cipres Science Gateway (<https://www.phylo.org/portal2/login!input.action>) with two independent runs, each consisting of 0.5 million replicates and sampling one tree every 1,000 generations. The initial 25% of sampled trees were discarded as burn-in, and the remaining trees were used to construct a majority-rule consensus and to calculate Bayesian posterior probabilities (BPP) for the clades (Miller *et al.* 2010).

All trees were viewed in FigTree v. 1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>). The ML bootstrap support values ≥ 70 % and Bayesian Posterior Probabilities ≥ 0.95 were presented on topologies from ML analyses, respectively. The sequence alignments were deposited in figshare (DOI: 10.6084/m9.figshare.31146655).

TABLE 1. List of species, specimens, and GenBank accession numbers of sequences used in this study.

Species	Voucher	Country	GenBank ITS	Reference
<i>Melanogaster ambiguus</i>	B-1613	Hungary	AJ555513	Unpublished
<i>Melanogaster ambiguus</i>	Ch12	Poland	KX438335	Unpublished
<i>Melanogaster anatolicus</i>	OKA-TR142441 *	Turkey	OP548645	Crous <i>et al.</i> 2023
<i>Melanogaster anatolicus</i>	OKA-TR142442	Turkey	OP548646	Crous <i>et al.</i> 2023
<i>Melanogaster broomeanus</i>	OTU_718s	UK	MT095837	Arraiano-Castilho <i>et al.</i> 2021
<i>Melanogaster broomeanus</i>	OTU_719s	UK	MT095838	Arraiano-Castilho <i>et al.</i> 2021
<i>Melanogaster coccolobae</i>	375 ITCV	Mexico	MZ098622	de la Fuente 2021
<i>Melanogaster coccolobae</i>	378 ITCV	Mexico	MZ098623	de la Fuente 2021
<i>Melanogaster cyaneus</i>	TJ75_1 *	China	ON427476	Yuan <i>et al.</i> 2024
<i>Melanogaster cyaneus</i>	TJ75_2	China	ON427477	Yuan <i>et al.</i> 2024
<i>Melanogaster diqingensis</i>	WXH_9068 *	China	ON427482	Yuan <i>et al.</i> 2024
<i>Melanogaster eurypermus</i>	OSC158339 JLF1044	USA	MN984311	Unpublished
<i>Melanogaster eurypermus</i>	OSC158325 JLF1129	USA	MN984312	Unpublished

.....continued on the next page

TABLE 1. (Continued)

Species	Voucher	Country	GenBank ITS	Reference
<i>Melanogaster intermedius</i>	RBG Kew K(M)130202	England	EU784372	Brock <i>et al.</i> 2009
<i>Melanogaster intermedius</i>	MT48	Germany	KX168661	Genbank
<i>Melanogaster luteus</i>	S328/PAM09082801	France	HQ714780	Moreau <i>et al.</i> 2011
<i>Melanogaster luteus</i>	S407/Mon06	Montenegro	HQ714794	Moreau <i>et al.</i> 2011
<i>Melanogaster macrosporus</i>	B-2254	USA	AJ555528	Unpublished
<i>Melanogaster minobovatus</i>	BJTC FAN911 *	China	NR_186967	Xu <i>et al.</i> 2022
<i>Melanogaster natsii</i>	OSC82168 JMT7491	USA	MN984331	Unpublished
<i>Melanogaster natsii</i>	OSC158336 MES297	USA	MN984332	Unpublished
<i>Melanogaster obovatus</i>	BJTC FAN808	China	MW598539	Xu <i>et al.</i> 2022
<i>Melanogaster obovatus</i>	BJTC FAN809	China	MW598540	Xu <i>et al.</i> 2022
<i>Melanogaster panzihuaensis</i>	HMAS 81915 *	China	NR_186968	Xu <i>et al.</i> 2022
<i>Melanogaster quercicola</i>	BJTC FAN1091*	China	MW598532	Xu <i>et al.</i> 2022
<i>Melanogaster quercicola</i>	BJTC FAN1208	China	MW598535	Xu <i>et al.</i> 2022
<i>Melanogaster rivularis</i>	S190/PAM08090514	France	HQ714731	Moreau <i>et al.</i> 2011
<i>Melanogaster rivularis</i>	S285/PAM08090514	France	HQ714767	Moreau <i>et al.</i> 2011
<i>Melanogaster shanxiensis</i>	HMAS81910 *	China	MW598550	Xu <i>et al.</i> 2022
<i>Melanogaster sinensis</i>	CLZhao 48944 *	China	PX919889	This study
<i>Melanogaster sinensis</i>	CLZhao 48945	China	PX919890	This study
<i>Melanogaster spinisporus</i>	BJTC FAN941-A	China	MW598548	Xu <i>et al.</i> 2022
<i>Melanogaster spinisporus</i>	BJTC FAN941-B	China	MW598549	Xu <i>et al.</i> 2022
<i>Melanogaster subglobisporus</i>	HMAS83329 *	China	MW598534	Xu <i>et al.</i> 2022
<i>Melanogaster tomentellus</i>	BJTC FAN539 *	China	MW598538	Xu <i>et al.</i> 2022
<i>Melanogaster truncatisporus</i>	TJ83	China	ON427478	Yuan <i>et al.</i> 2024
<i>Melanogaster truncatisporus</i>	TJ87 *	China	ON427479	Yuan <i>et al.</i> 2024
<i>Melanogaster tuberiformis</i>	JC110130BT	Spain	MN594289	Alvarado <i>et al.</i> 2021
<i>Melanogaster variegatus</i>	JC180617BT	Spain	MN594290	Unpublished
<i>Paxillus involutus</i>	Can12.1	France	KF261368	Jargeat <i>et al.</i> 2014

* Indicates type material (holotype); the new species are in bold.

Results

Phylogeny of *Melanogaster*

A total of 40 internal transcribed spacer (ITS) sequences were utilized to construct phylogenetic trees of *Melanogaster*. The ITS dataset included 40 specimens representing 25 taxa of *Melanogaster*, with *Paxillus involutus* designated as the outgroup.

RAxML analysis of the combined dataset yielded the best-scoring tree (Figure 1), with a final ML optimization likelihood of -4473.692. The alignment contained 756 bp. The matrix had 373 distinct alignment patterns. Estimated base frequencies; A = 0.217, C = 0.232, G = 0.263, T = 0.288; substitution rates AC = 1.29834, AG = 3.37969, AT = 1.00000, CG = 1.29834, CT = 4.96428, GT = 1.000000. The best BI model for the ITS dataset was GTR+I+G; the average standard deviation of split frequencies in the Bayesian analyses was 0.009003, and the effective sample size (ESS) across the two Bayesian runs was double the average ESS (avg ESS) = 368.5.

BLAST searches of the ITS sequence of *Melanogaster sinensis* retrieved *M. broomeanus* and *M. rivularis* as the closest matches among the top ten, with *M. rivularis* showing the highest similarity (Maximum record descriptions: Max score 1247; Total score 1247; Query cover 100%; E value 0; Ident 93.70%). Although BLAST searches indicated sequence similarity to *M. broomeanus* and *M. rivularis*, the phylogenetic analysis revealed a different topology. *Melanogaster sinensis* forms a distinct, highly supported monophyletic lineage (ML = 100, BPP = 1). In

the ITS phylogeny, it clusters with a larger clade comprising *M. broomeanus*, *M. anatolicus*, *M. variegatus*, and *M. truncatisporus*; however, the statistical support for this relationship is relatively low and lacks Bayesian support (ML = 83, BPP = 0.73). Furthermore, *M. sinensis* is phylogenetically distant from *M. rivularis*.

The ITS-based phylogenetic topology indicated that this new species grouped within the genus *Melanogaster*, with bootstrap support for ML and BI $\geq 70\%$ and 0.95, respectively, as shown in Figure 1, and then grouped with *Melanogaster truncatisporus*.

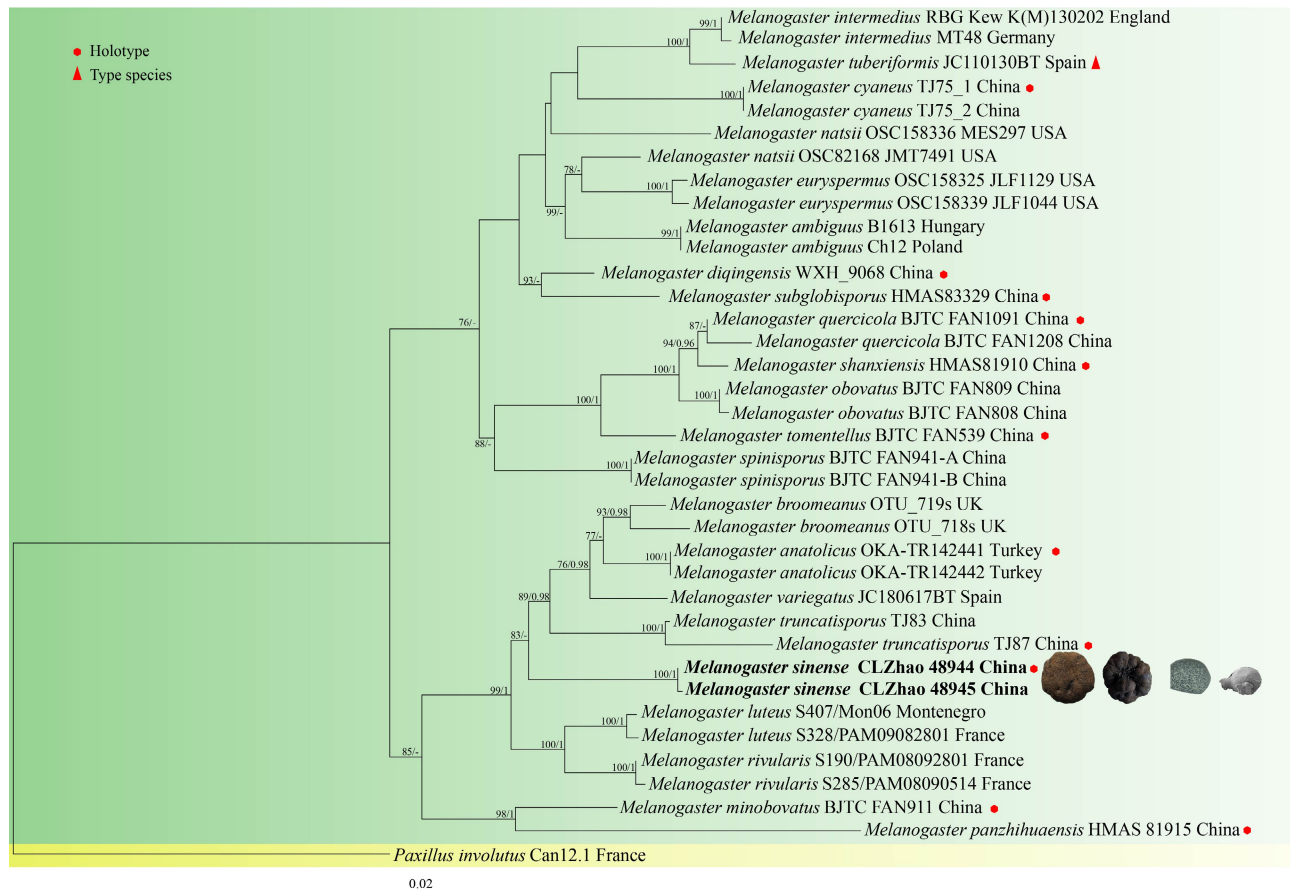


FIGURE 1. Maximum Likelihood tree illustrating the phylogeny of *Melanogaster* and related species based on the ITS sequence dataset. Branches are labeled with Maximum Likelihood bootstrap values equal to or above 70%, and Bayesian posterior probabilities equal to or above 0.95. The new species are in bold.

Taxonomy

Melanogaster sinensis Xin Yang & C.L. Zhao, *sp. nov.* Figs. 2, 3
MycoBank No.: MB 862143

Etymology: *sinensis* (Lat.): refers to the species being found in China.

Holotype: China. Jilin Province, Changchun City, Chaoyang County, Jilin University, 43°49'20"N, 125°16'44"E, elev. 212.5 m, on the ground, 1 October 2023, CLZhao 48944 (SWFC 00048944).

Description: *Basidiomata* 1.6–2.5 × 1.1–2.4 cm, hypogeous to semi-hypogeous, subglobose to ellipsoidal, and occasionally folded inside, grayish brown to dark brown, surface smooth, with honey-yellow rhizomorphs. *Peridium* single-layered, 34–112 µm thick, composed of interwoven hyphae; thin-walled, branched, interwoven, (3.0–)4.5–9.0(–10.5) µm diam., smooth, with abundant clamp connections; textura; honey-yellow to grayish brown walls toward the outside surface. *Gleba* black, solid, gelatinous, filled with small chambers separated by sterile white veins, thin-walled to slightly thick-walled, branched, interwoven, 3.0–4.5 µm diam., with abundant clamp connections, textura. *Basidia* 10.5–16.0 (–19.5) × 6.0–9.0 µm, clavate-capitate, hyaline, 8 spores, randomly distributed, gelatinized at maturity. *Basidiospores* fusiform, (4.1–)4.8–6.2 × 3.0–3.8(–4.1) µm (Lm × Wm = 5.4 ± 0.5 × 3.4 ± 0.3, Q = 1.5–1.6, Qm = 1.5

± 0.1 , $n = 60/2$), dark brown at maturity, thick-walled, smooth, with broadly truncate-cupped base and short remnants of sterigmata.

Habit, habitat and distribution: Currently only known from the type locality in Jilin Province, Northeast China, growing in moss under *Tilia* sp.

Additional specimen examined (paratype): China. Jilin Province, Changchun, Chaoyang County City, Jilin University, 43°49'20"N, 125°16'44"E, elev. 212.5 m, on the ground, 1 October 2023, CLZhao 48945 (SWFC 00048945).

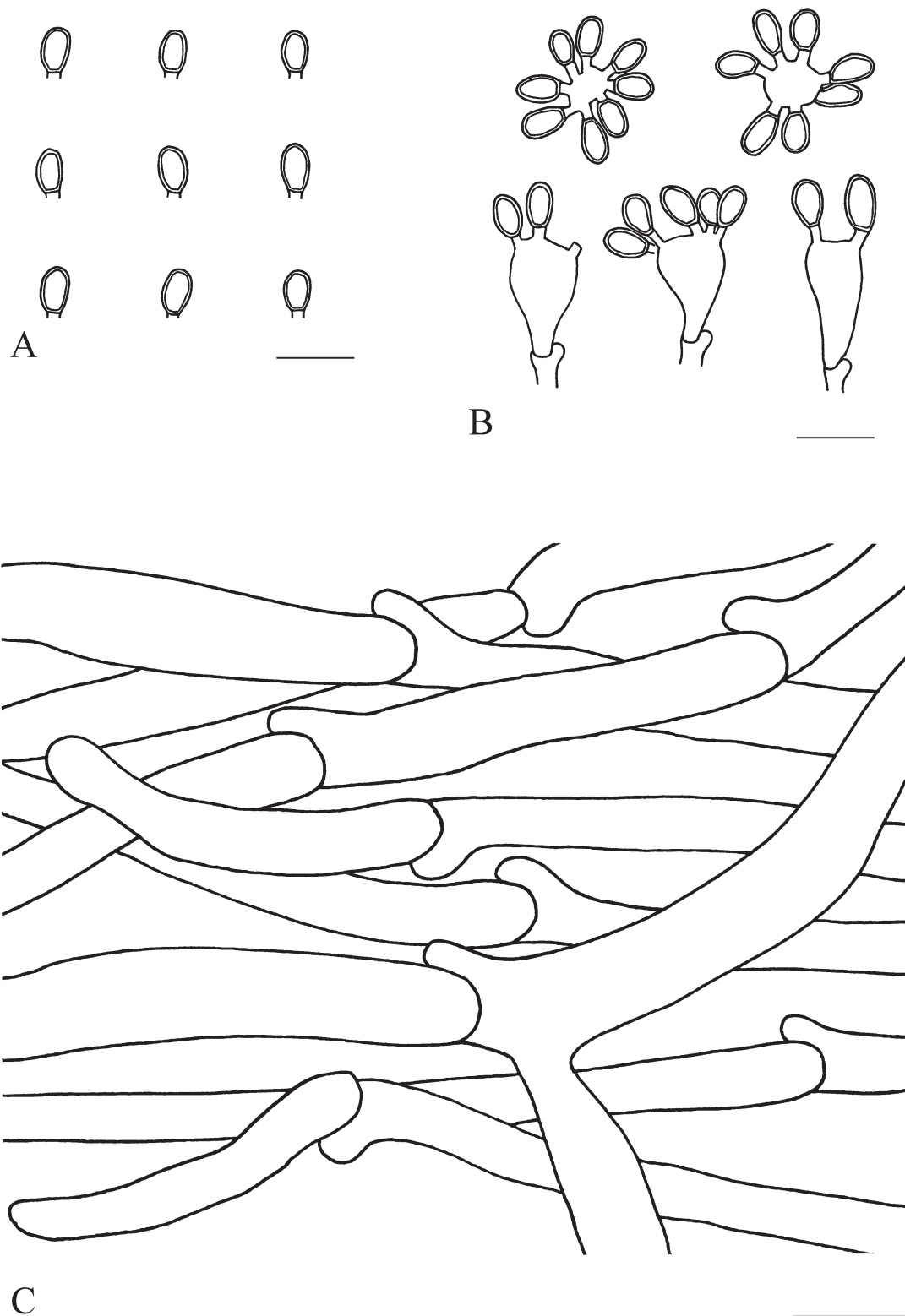


FIGURE 2. *Melanogaster sinensis* (CLZhao 48944, holotype). (A) Basidiospores; (B) Basidia; (C) Peridium hyphae. Scale bars: A–C = 10 μ m.

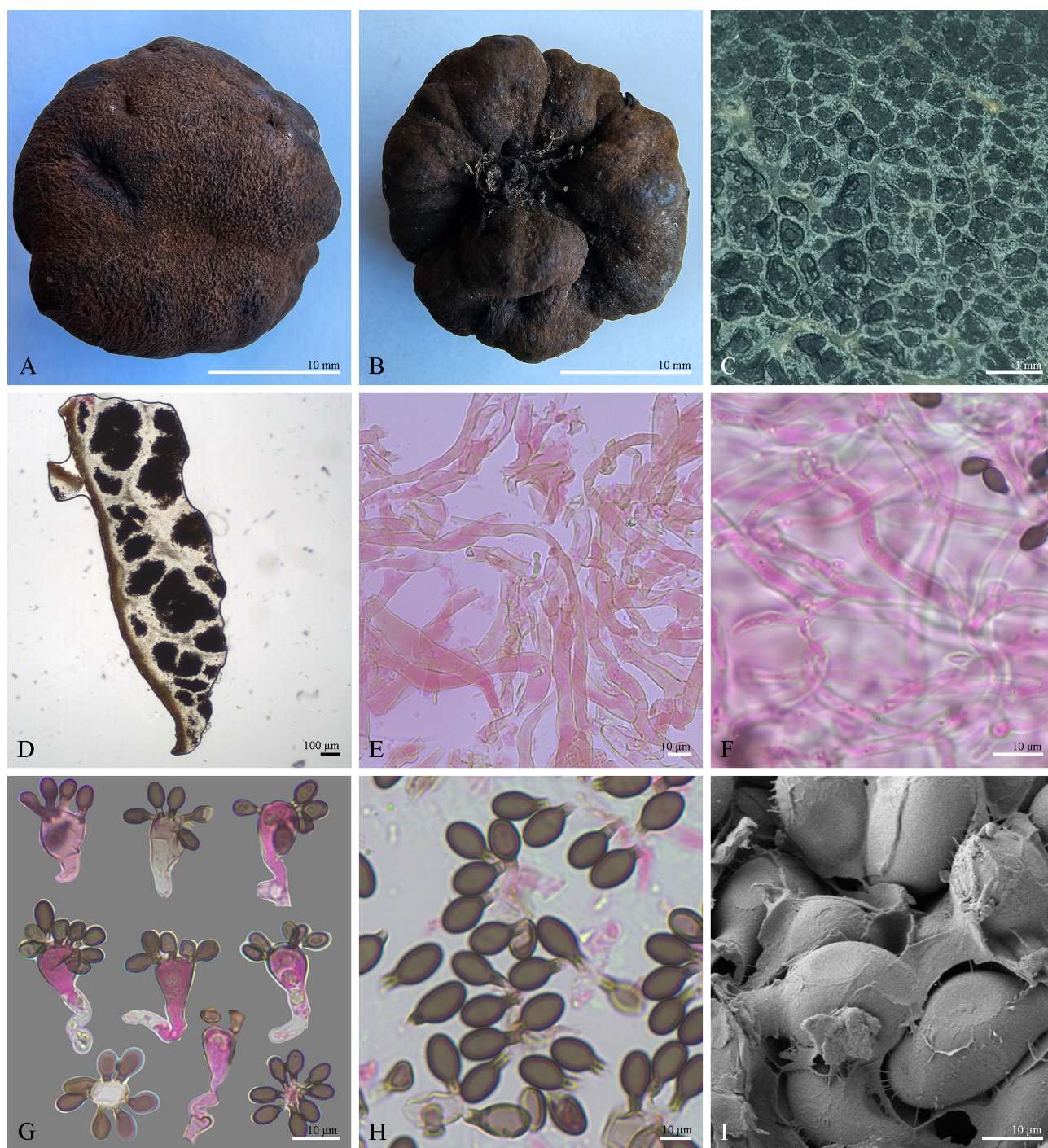


FIGURE 3. *Melanogaster sinensis* (CLZhao 48944, holotype) (A–C) Basidiomata; (D) Peridium; (E) Peridium hyphae; (F) Gleba hyphae; (G) Basidia; (H) Basidiospores; (I) Basidiospores by SEM.

Discussion

This study describes one new species, *Melanogaster sinensis*, based on ITS-based phylogenetic analyses and morphological observations. Phylogenetic analysis of the ITS dataset (Fig. 1) showed that *Melanogaster sinensis* was assigned within *Melanogaster* and formed a monophyletic lineage.

Morphologically, the new species resembles six morphologically similar species within the genus *Melanogaster*, namely *M. anatolicus*, *M. luteus*, *M. minobovatus*, *M. panzhihuaensis*, *M. rivularis*, and *M. truncatisporus*. However, *Melanogaster sinensis* can be distinguished from these related species by several diagnostic features. Notably, *M. sinensis* differs from the 4-spored *M. truncatisporus* by having 8-spored basidia (Yuan *et al.* 2024). Furthermore, the

new species differs from *M. panzhihuaensis* in having smaller basidiospores ($4.8\text{--}6.2 \times 3.0\text{--}3.8 \mu\text{m}$ vs. $8.7\text{--}12.4 \times 5.2\text{--}7.6 \mu\text{m}$; Xu *et al.* 2022), and from *M. anatolicus* by possessing a single-layered peridium rather than a two-layered peridium (Crous *et al.* 2023). Table 2 presents a comprehensive morphological comparison between the new species and these taxa.

The discovery and description of *Melanogaster sinensis* contribute to a better understanding of the diversity of this genus in China. However, we acknowledge several limitations, including reliance on a single ITS locus for phylogenetic inference, the limited number of available specimens, and restricted geographic sampling. Future studies should incorporate more extensive field sampling across regions and use multi-locus molecular analyses to clarify phylogenetic relationships and species diversity within *Melanogaster*. Such integrative approaches may also facilitate the discovery of additional undescribed *Melanogaster* taxa in China.

TABLE 2. A morphological comparison among our new species and six similar species of *Melanogaster*. Newly introduced taxa are indicated in bold.

Species name	Basidiomata	Peridium	Gleba	Basidiospores	Basidia	References
<i>Melanogaster anatolicus</i>	hypogeous or semi-hypogeous, subglobose or irregularly tuberous, 35–100 mm diam.	two-layered, 150–360 μm thick	shiny gelatinous	mostly oblong to broadly fusiform, sometimes ellipsoid or subcylindrical, smooth, thick-walled, $6.7\text{--}11.5 \times 4.4\text{--}6.0 \mu\text{m}$	narrowly clavate to clavate, with a long and sinuous stipe, 2–4 spored, $25.0\text{--}35.0 \times 5.9\text{--}8.0 \mu\text{m}$	Crous <i>et al.</i> 2023
<i>Melanogaster luteus</i>	hypogeous, subglobose to ellipsoidal, somewhat flattened, $0.7\text{--}1.2 \times 0.4\text{--}1.0 \text{ mm}$	two-layered, 280–430 μm thick	gelatinous, early deliquescent when mature	asymmetric in profile view, somewhat inflated to club-shaped, cylindro-elliptical to cylindrical, thick-walled, $5.3\text{--}6.8 \times 2.4\text{--}3.0 \mu\text{m}$	clavate-capitate when young, 2–8 spored, $18\text{--}24 \times 5\text{--}6.5 \mu\text{m}$	Moreau <i>et al.</i> 2011
<i>Melanogaster minobovatus</i>	hypogeous, subglobose, $35 \times 45 \text{ mm}$	two-layered, 320–500 μm thick	usually liquefying at over-maturity	obovoid, $5.1\text{--}6.9 \times 4.2\text{--}5.1 \mu\text{m}$	not observed	Xu <i>et al.</i> 2022
<i>Melanogaster panzhihuaensis</i>	hypogeous, subglobose, $15 \times 17 \text{ mm}$	single-layered, 150–220 μm thick	firm, with sterile white veins scattered throughout	oval, apex blunt and narrow, $8.7\text{--}12.4 \times 5.2\text{--}7.6 \mu\text{m}$	not observed	Xu <i>et al.</i> 2022
<i>Melanogaster rivularis</i>	hypogeous to half-emergent, globose to subglobose, $6\text{--}25 \times 4\text{--}10 \text{ mm}$	two-layered, 90–135 μm thick	gelatinous-firm	cylindro-elliptical to cylindrical, with an amygdaloid tendency in profile view, $5.1\text{--}6.2 \times 2.7\text{--}3.1 \mu\text{m}$	shortly clavate, 6–8 spored, $18\text{--}26 \times 5.5\text{--}6.5 \mu\text{m}$	Moreau <i>et al.</i> 2011
<i>Melanogaster sinensis</i>	hypogeous or semi-hypogeous, subglobose to ellipsoidal	single-layered, 34–112 μm thick	solid, gelatinous,	fusiform, $4.8\text{--}6.2 \times 3.0\text{--}3.8 \mu\text{m}$	clavate-capitate, 8 spored, $10.5\text{--}16.0 \times 6.0\text{--}9.0 \mu\text{m}$	This study
<i>Melanogaster truncatisporus</i>	hypogeous or semi-hypogeous, subglobose to oval, occasionally irregular-elongated, $15\text{--}30 \times 4\text{--}23 \text{ mm}$	two-layered, 200–450 μm thick	solid, hard when dried	subglobose to globose or irregularly elongate-pyriform, $3.5\text{--}9.5 \times 3.0\text{--}7.0 \mu\text{m}$	4 spored	Yuan <i>et al.</i> 2024

Acknowledgements

The research was supported by the Yunnan Province Graduate Supervisor Team Project—Taxonomy and Phylogeny of Forest Edible and Medicinal Fungi in Yunnan Province (Changlin Zhao team: 503260614), Science and Technology Project of Yunnan Province—Major Science and Technology Special Project Program (202602AE090021), Modern Industry School of Edible-fungi, Southwest Forestry University (Project No. SYJ2601), and the Forestry and Grass Science and Technology Innovation Joint Project of Yunnan Province (Project No. 202404CB090008), and Yunnan Province College Students Innovation and Entrepreneurship Training Program (Project Nos. 202510677105, S202510677109).

References

- Alvarado, P., Cabero, J., Moreno-Mateos, D., Vizzini, A., Alonso, J., Lebeuf, R., Siquier, J.L. & Vidal, J.M. (2021) Phylogenetic relationships among false truffle genera of Paxillaceae—*Alpova*, *Melanogaster*, *Nealpova*, and *Paralpova*, gen. nov. *Mycologia* 113 (4): 828–841.
<https://doi.org/10.1080/00275514.2021.1911552>
- Arraiano-Castilho, R., Bidartondo, M.I., Niskanen, T., Clarkson, J.J., Brunner, I., Zimmermann, S., Senn-Irlet, B., Frey, B., Peintner, U., Mrak, T. & Suz, L.M. (2021) Habitat specialisation controls ectomycorrhizal fungi above the treeline in the European Alps. *The New Phytologist* 229 (5): 2901–2916.
<https://doi.org/10.1111/nph.17033>
- Binder, M. & Hibbett, D.S. (2006) Molecular systematics and biological diversification of Boletales. *Mycologia* 98 (6): 971–981.
<https://doi.org/10.1080/15572536.2006.11832626>
- Brock, P.M., Döring, H. & Bidartondo, M.I. (2009) How to know unknown fungi: The role of a herbarium. *The New Phytologist* 181 (3): 719–724.
<https://doi.org/10.1111/j.1469-8137.2008.02703.x>
- Castellano, M.A., Trappe, J.M., Maser, Z. & Maser, S. (1986) *Key to spores of the genera of hypogeous fungi of North America, with reference to animal mycophagy*. Mad River Press, Eureka, USA, pp. 1–185.
- Comandini, O., Contu, M. & Rinaldi, A.C. (2006) An overview of Cistus ectomycorrhizal fungi. *Mycorrhiza* 16 (6): 381–395.
<https://doi.org/10.1007/s00572-006-0047-8>
- Corda, A.C.J. (1831) Deutschlands Flora, Abtheilung III. *Die Pilze Deutschlands* 3 (11): 1–32.
- Crous, P.W., Osieck, E.R., Shivas, R.G., Tan, Y.P., Bishop-Hurley, S.L., EsteveRaventós, F., Larsson, E., Luangsa-ard, J.J., Pancorbo, F., Balashov, S., Baseia, I.G., Boekhout, T., Chandranayaka, S., Cowan, D.A., Cruz, R.H.S.F., Czachura, P., De la Peña-Lastra, S., Dovana, F., Drury, B., Fell, J., Flakus, A., Fotedar, R., Jurjević, Ž., Kolečka, A., Mack, J., Maggs-Köling, G., Mahadevakumar, S., Mateos, A., Mongkolsamrit, S., Noisripoom, W., Plaza, M., Overy, D.P., Piątek, M., Sandoval-Denis, M., Vauras, J., Wingfield, M.J., Abell, S.E., Ahmadpour, A., Akulov, A., Alavi, F., Alavi, Z., Altés, A., Alvarado, P., Anand, G., Ashtekar, N., Assyov, B., Banc-Prandi, G., Barbosa, K.D., Barreto, G.G., Bellanger, J.M., Bezerra, J.L., Bhat, D.J., Bilański, P., Bose, T., Bozok, F., Chaves, J., Costa-Rezende, D.H., Danteswari, C., Darmostuk, V., Delgado, G., Denman, S., Eichmeier, A., Etayo, J., Eyssartier, G., Faulwetter, S., Ganga, K.G.G., Ghosta, Y., Goh, J., Góis, J.S., Gramaje, D., Granit, L., Groenewald, M., Gulden, G., Gusmão, L.F.P., Hammerbacher, A., Heidarian, Z., Hywel-Jones, N., Jankowiak, R., Kaliyaperumal, M., Kaygusuz, O., Kezo, K., Khonsanit, A., Kumar, S., Kuo, C.H., Læssøe, T., Latha, K.P.D., Loizides, M., Luo, S.M., Maciá-Vicente, J.G., Manimohan, P., Marbach, P.A.S., Marinho, P., Marney, T.S., Marques, G., Martín, M.P., Miller, A.N., Mondello, F., Moreno, G., Mufeeda, K.T., Mun, H.Y., Nau, T., Nkomo, T., Okraśińska, A., Oliveira, J.P.A.F., Oliveira, R.L., Ortiz, D.A., Pawłowska, J., Pérez-De-Gregorio, M.À., Podile, A.R., Portugal, A., Privitera, N., Rajeshkumar, K.C., Rauf, I., Rian, B., Rigueiro-Rodríguez, A., Rivas-Torres, G.F., Rodríguez-Flakus, P., Romero-Gordillo, M., Saar, I., Saba, M., Santos, C.D., Sarma, P.V.S.R.N., Siquier, J.L., Sleiman, S., Spetik, M., Sridhar, K.R., Stryjak-Bogacka, M., Szczepańska, K., Taşkın, H., Tennakoon, D.S., Thanakitpipattana, D., Trovão, J., Türkeul, İ., van Iperen, A.L., van 't Hof, P., Vasquez, G., Visagie, C.M., Wingfield, B.D., Wong, P.T.W., Yang, W.X., Yasar, M., Yarden, O., Yilmaz, N., Zhang, N., Zhu, Y.N. & Groenewald, J.Z. (2023) Fungal Planet description sheets: 1478–1549. *Persoonia* 50: 158–310.
<https://doi.org/10.3767/persoonia.2023.50.05>
- de la Fuente, J.I., Martínez-González, C.R., Oros-Ortega, I., Guevara, G., Bandala, V.M., Córdova-Lara, I., Vela-Hernández, R.Y., López, C.Y. & García Jiménez, J. (2021) *Melanogaster coccolobae* sp. nov. (Paxillaceae, Boletales), a tropical hypogeous fungus from the urban areas of Quintana Roo, Mexico. *Acta Botánica Mexicana* 128 (128): e1896.
<https://doi.org/10.21829/abm128.2021.1896>

- Gu, Z.R., Zhou, C.Q., Deng, Q.H., Zhang, J.L., Li, M. & Zhou, H.M. (2025) Morphological and phylogenetic evidence reveal a new species of *Protomerulius* (Basidiomycota) from China. *Phytotaxa* 716 (1): 36–44.
<https://doi.org/10.11646/phytotaxa.716.1.4>
- Jargeat, P., Chaumeton, J.P., Navaud, O., Vizzini, A. & Gryta, H. (2014) The *Paxillus involutus* (Boletales, Paxillaceae) complex in Europe: Genetic diversity and morphological description of the new species *Paxillus cuprinus*, typification of *P. involutus* s.s., and synthesis of species boundaries. *Fungal Biology* 118 (1): 12–31.
<https://doi.org/10.1016/j.funbio.2013.10.008>
- Katoh, K., Rozewicki, J. & Yamada, K.D. (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20: 1160–1166.
<https://doi.org/10.1093/bib/bbx108>
- Knapp, A. (1954) Die Europäischen Hypogaeen-Gattungen und ihre Gattungstypen. II. Teil (Fortsetzung). Die Gattungstypen der Melanogastraceae und weitere spezie. *Schweizerisches Zeitschrift für Pilzkunde* 32 (8): 117–130.
- Lacheva, M. (2015) New records of *Melanogaster* species (Fungi, Boletales) in Bulgaria. *Journal of Biodiversity and Environmental Sciences* 6 (1): 492–498.
- Larsson, A. (2014) AliView: a fast and lightweight alignment viewer and editor for large data sets. *Bioinformatics* 30: 3276–3278.
<https://doi.org/10.1093/bioinformatics/btu531>
- Liu, B., Tao, K. & Cang, M.C. (1989) Two new species of *Melanogaster* from China. *Acta Mycologica Sinica* 8 (3): 210–213.
- Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings of the Gateway Computing Environments Workshop*, New Orleans, pp. 1–8.
<https://doi.org/10.1109/GCE.2010.567612>
- Moreau, P.A., Rochet, J., Richard, F., Chassagne, F., Manzi, S. & Gardes, M. (2011) Taxonomy of *Alnus*-associated hypogeous species of *Alpova* and *Melanogaster* (Basidiomycota, Paxillaceae) in Europe. *Cryptogamie Mycologie* 32 (1): 33–62.
<https://doi.org/10.7872/crym.v32.iss1.2012.033>
- Moreau, P.A., Stéphane, W., Branislav, P., Jargeat, P., Manzi, S. & Vizzini, A. (2013) *Alpova komoviana* (Boletales, Paxillaceae), a new sequestrate fungus from montenegro, with a revised phylogeny of the genus in Europe. *Mycological Progress* 12 (1): 109–119.
<https://doi.org/10.1007/s11557-012-0818-x>
- Nguyen, L., Schmidt, H.A., Haeseler, A. & Minh, B.Q. (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* 32 (1): 268–274.
<https://doi.org/10.1093/molbev/msu300>
- Pegler, D.N., Spooner, B.M. & Young, T.W.K. (1993) *British truffles: a revision of British hypogeous fungi*. Royal Botanical Gardens, Kew, London, UK, pp. 1–216.
- Petersen, J.H. (1996) *Farvekort. The danish Mycological Society's Colour-Chart*. Foreningen til Svampekundskabens Fremme, Greve, Germany, pp. 1–6.
- Rathnayaka, A.R., Tennakoon, D.S., Jones, G.E., Wanasinghe, D.N., Bhat, D.J., Priyashantha, A.H., Stephenson, S.L., Tibpromma, S. & Karunarathna, S.C. (2025) Significance of precise documentation of hosts and geospatial data of fungal collections, with an emphasis on plant-associated fungi. *New Zealand Journal of Botany* 63 (2–3): 462–489.
<https://doi.org/10.1080/0028825X.2024.2381734>
- Svrček, M. (1958) VIII. řad Melanogastrales-Černouškotvaré. *Flora ČSR B (1). Gasteromycetes*. Czechoslovakian Academy, Praha, pp. 527–556, 795–802.
- Teng, S.Q. (1963) *Fungi of China*. Science Press, Beijing, pp. 1–808.
- Trappe, J.M., Molina, R., Luoma, D.L., Cázares, E., Pilz, D., Smith, J.E., Castellano, M.A., Miller, S.L. & Trappe, M.J. (2009) *Diversity, ecology, and conservation of truffle fungi in forests of the Pacific northwest*. USDA general technical report, Forest Service, Pacific northwest Research Station, Portland, Oregon, pp. 1–196.
<https://doi.org/10.2737/PNW-GTR-772>
- Türkoğlu, A. & Castellano, M.A. (2013) New records of truffle fungi (Basidiomycetes) from Turkey. *Turkish Journal of Botany* 37: 970–976.
<https://doi.org/10.3906/bot-1212-54>
- Vizzini, A., Zotti, M., Ryman, S. & Ghignone, S. (2010) Typification of *Octaviania rubescens* (Paxillineae, Boletales) and phylogenetic hypotheses for genus *Alpova*. *Mycologia* 102 (4): 967–975.
<https://doi.org/10.3852/09-256>
- Wang, Y., Chang, M.C., Tao, K. & Liu, B. (1995) New species and new varieties in the genus *Melanogaster* from China. *Journal of Shanxi University (Natural Science Edition)* 18 (4): 449–453.
- White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR protocols: A guide to methods and applications*. Academic Press, San Diego, pp. 315–322.

<https://doi.org/10.1016/B978-0-12-372180-8.50042-1>

- Xiang, C.Y., Gao, F.L., Jakovlić, I., Lei, H.P., Hu, Y., Zhang, H., Zou, H., Wang, G.T. & Zhang, D. (2023) Using PhyloSuite for molecular phylogeny and tree-based analyses. *iMeta* 2 (1): e87.
<https://doi.org/10.1002/imt.2.87>
- Xu, Y.Y., Yan, X.Y., Li, T., Zhao, T.Y., Lv, J.C. & Fan, L. (2022) The taxonomic revision of *Melanogaster* (Paxillaceae, Boletales) in China based on molecular and morphological evidence. *Mycological Progress* 21 (12): e98.
<https://doi.org/10.1007/s11557-022-01842-9>
- Yang, X., Su, J.Q., Zhou, H.M. & Zhao, C.L. (2024) Morphological characteristics and phylogenetic analyses revealed *Geastrum yunnanense* sp. nov. (Gastrales, Basidiomycota) from Southwest China. *Phytotaxa* 665 (3): 179–192.
<https://doi.org/10.11646/phytotaxa.665.3.1>
- Yang, Y., Xu, Y., Wang, L., Jiang, Q.Q., Su, J.Q., Li, R., Zhou, H.M. & Zhao, C.L. (2025b) Multigene phylogeny of seven wood-inhabiting fungal orders in Basidiomycota, and proposal of a new genus and thirteen new species. *Mycosphere* 16: 245–295.
<https://doi.org/10.5943/mycosphere/16/1/4>
- Yuan, Q., You, S.C., Zhou, S.L., Zhu, Y.G., Li, W., Zhao, C.L. & Xu, Y.Y. (2025) Morphological characteristics and phylogenetic analyses reveal *Purpureocorticium niveum* sp. nov. (Agaricomycetes, Basidiomycota) from southern China. *Phytotaxa* 726 (3): 143–154.
<https://doi.org/10.11646/phytotaxa.726.3.2>
- Yuan, T.J., Luo, H.M., Su, K.M., Li, S.H. & Raspé, O. (2024) Three new *Melanogaster* species (Boletales, Paxillaceae) from southwestern China based on morphological and molecular evidence. *MycKeys* 107: 141–160.
<https://doi.org/10.3897/mycokeys.107.123565>
- Zeller, S.M. (1949) Keys to the orders, families, and genera of the Gasteromycetes. *Mycologia* 41: 36–58.
<https://doi.org/10.1080/00275514.1949.12017751>
- Zhang, D., Gao, F., Jakovlić, I., Zou, H., Zhang, J., Li, W.X. & Wang, G.T. (2020) PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Molecular Ecology Resource* 20 (1): 348–55.
<https://doi.org/10.1111/1755-0998.13096>
- Zhao, C.L. & Wu, Z.Q. (2017) *Ceriporiopsis kunmingensis* sp. nov. (Polyporales, Basidiomycota) evidenced by morphological characters and phylogenetic analysis. *Mycological Progress* 16: 93–100.
<https://doi.org/10.1007/s11557-016-1259-8>
- Zhao, C.L., Qu, M.H., Huang, R.X. & Karunarathna, S.C. (2023a) Multi-Gene Phylogeny and taxonomy of the wood-rotting fungal genus *Phlebia* sensu lato (Polyporales, Basidiomycota). *Journal of Fungi* 9: 320.
<https://doi.org/10.3390/jof9030320>