

Article

New Contributions to *Entoloma* sect. *Madida* (*Entolomataceae*, *Agaricales*) from Subtropical Montane Forests of China: Two New Taxa

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Abstract

Entoloma is a species-rich and taxonomically complex genus in *Entolomataceae*, characterised by pink to pinkish-brown spore prints and angular basidiospores. Within this genus, *Entoloma* sect. *Madida*, corresponding to the *Prunuloides* lineage in recent infrageneric treatments, includes several morphologically similar taxa related to the *E. bloxamii*–*prunuloides* complex. However, this lineage remains poorly documented from subtropical eastern China. In this study, we examined *Entoloma* collections from the Huangshan region of Anhui Province, China, using macromorphological observations, micromorphological characters, scanning electron microscopy (SEM) of basidiospores, and multilocus phylogenetic analyses based on ITS, LSU and *rpb2*. We recognised two independent and well-supported species-level lineages, described here as *Entoloma theobrunneum* and *E. ochrorubrum*. *Entoloma theobrunneum* is phylogenetically close to *E. corneri* but differs in its smoky grey to tea-brown pileus, snakeskin-like stipe pattern, higher basidiospore Q values, larger basidia, and the presence of pleurocystidia and caulocystidia. *Entoloma ochrorubrum* is associated with taxa of the *E. bloxamii* complex but is distinguished by its darker iron-red to ochre-brown pileus, blackish-brown central papilla, cracking pileus margin, longitudinally fibrillose stipe, higher basidiospore Q values, and basidia with granular contents. SEM observations are provided only as supplementary documentation of the three-dimensional architecture of basidiospores. These findings add two phylogenetically supported Chinese species to the current framework of *E. sect. Madida*, help fill a regional documentation gap in subtropical eastern China, and provide additional East Asian data relevant to future evaluation of the current infrageneric framework of *Entoloma*.

Keywords: *Agaricales*; *Entolomataceae*; taxonomy; new taxa



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1. Introduction

The genus *Entoloma* (Fr.) P. Kumm. (*Entolomataceae*, *Agaricales*) is typically characterised by a pink to pinkish-brown spore print and basidiospores that appear polygonal in outline from all angles [1–3]. Species of this genus are widely distributed, occurring from temperate to tropical regions and from high-altitude to lowland habitats; most grow on forest soil, among mosses, or on humus-rich substrates [4–6]. They constitute an important component of terrestrial fungal diversity and may also play significant roles in forest

ecosystems. Owing to the marked variation in basidiomata size, colour, pileus surface structure, and basidiospore morphology, substantial morphological overlap is common among species of *Entoloma*. As a result, species delimitation and infrageneric classification have long remained central and challenging issues in the systematics of macrofungi. The traditional morphology-based system established by Noordeloos [5,6] and others provided an important foundation for studies of the genus. However, in taxonomic groups containing numerous closely related species, similar combinations of basidiomata size, pileus colouration, surface ornamentation, and basidiospore morphology may be shared by phylogenetically distinct taxa.

With the widespread application of molecular systematics, the taxonomic framework of *Entoloma* has been further refined. Based on multigene data, Co-David et al. [3] showed that *Entoloma* in the broad sense is monophyletic, whereas the higher-level groups circumscribed on the basis of traditional morphology do not fully correspond to natural phylogenetic lineages. Subsequently, Morgado et al. [7] conducted multilocus phylogenetic analyses of the *E. bloxamii*–*prunuloides* complex and further demonstrated that some putatively widespread species treated under broad morphological concepts actually comprise multiple geographically separated and phylogenetically independent sister lineages. Building on these phylogenetic insights, Noordeloos et al. [8] treated the *Prunuloides* clade as *Entoloma* sect. *Madida*, a circumscription broadly corresponding to Clade B2 in Morgado et al., namely the lineage related to the *E. bloxamii*–*prunuloides* complex. This section includes medium- to large-sized taxa such as *E. madidum*, *E. bloxamii*, *E. prunuloides*, and allied species, many of which are difficult to delimit using morphology alone. Within sect. *Madida*, species are most effectively distinguished by combinations of pileus colour and surface structure, lamellar attachment, stipe ornamentation, basidiospore dimensions and Q values, basidial size, and the presence or absence of cystidia, rather than by any single morphological character.

In China, recent taxonomic studies of *Entoloma* have revealed considerable species diversity in several infrageneric groups, including *Cyanula*, *Cubospora*, and *Claudopus* [9–12]. However, these advances have not been evenly distributed among lineages. *Entoloma* sect. *Madida*, which includes taxa allied to the *E. bloxamii*–*prunuloides* complex, remains poorly documented from China, especially from subtropical montane forests of eastern China. At present, most morphological concepts and available comparative sequences for this section are based mainly on material from Europe, North America, Australasia, and Southeast Asia. Although several Chinese species of *Entoloma* have been documented, no morphology-based Chinese records can currently be assigned confidently to sect. *Madida* without further molecular verification. As a result, Chinese collections resembling members of sect. *Madida* cannot be confidently assigned to known taxa without detailed morphological comparison and multilocus phylogenetic assessment.

The Huangshan Mountains in Anhui Province are part of the subtropical montane forest system of eastern China and provide heterogeneous habitats suitable for diverse macrofungal communities [13–15]. Nevertheless, molecularly verified records of *Entoloma* from this region remain limited, and sect. *Madida* has not been adequately evaluated using an integrative taxonomic framework. This creates a specific regional knowledge gap: it is unclear whether Chinese collections morphologically similar to the *E. bloxamii*–*prunuloides* complex represent geographical variants of known Eurasian or Southeast Asian taxa, or independent species-level lineages within sect. *Madida*.

To address this gap, we examined *Entoloma* collections from Anhui Province obtained during 2023–2025. Particular attention was paid to collections showing morphological affinity to sect. *Madida* but differing from described taxa in pileus colouration, stipe surface structure, basidiospore morphology, and other micromorphological characters.

We combined macromorphological observations, micromorphological analyses, scanning electron microscopy of basidiospores, and phylogenetic analyses based on ITS, LSU, and *rpb2*. The aims of this study were to determine the phylogenetic placement of these collections, assess their morphological differentiation from closely allied taxa, and formally describe those supported by integrative evidence as new species. This study therefore provides a focused, molecularly supported assessment of *Entoloma* sect. *Madida* from subtropical eastern China and helps evaluate the applicability of current species concepts to East Asian material.

2. Materials and Methods

2.1. Specimen Collection

During a broader survey of *Entoloma* diversity in Anhui Province, China (114°54'–119°37' E, 29°41'–34°38' N), conducted from May to October in 2023–2025, a total of 107 *Entoloma* collections were obtained from subtropical montane forests. These collections were photographed in their natural habitats, and macroscopic characters of fresh basidiomata were recorded. After preliminary morphological screening, only three collections, representing two taxa, were assigned to *Entoloma* sect. *Madida*. Preliminary assignment to sect. *Madida* was based on the overall combination of medium- to large-sized, robust basidiomata, a fleshy pileus, a central fibrous stipe, and morphological affinity to taxa of the *E. bloxamii*–*prunuloides* complex. Collections showing clearly different basidioma stature, pileus structure, stipe morphology, or basidiospore characters were excluded from further analysis in the present study. These collections are the focus of the present study because they showed morphological affinity to the *E. bloxamii*–*prunuloides* complex but could not be assigned to any described species. The remaining *Entoloma* collections belonged to other lineages or did not show diagnostic characters of sect. *Madida*. The three collections assigned to sect. *Madida* were used for detailed morphological examination and molecular analyses in the present study. All specimens examined in this study were dried at 40–45 °C and deposited in the Mycological Herbarium of Jilin Agricultural University (HMJAU).

2.2. Morphological Studies

Macromorphological descriptions were based on fresh basidiomata, whereas micromorphological descriptions were based on dried specimens. All measurements of basidiomata were taken from mature specimens. Colours of fresh basidiomata were recorded under natural light and followed the Methuen Handbook of Colour [16]. Microscopic structures were examined using a ZEISS Axioscope 5 light microscope (Carl Zeiss Microscopy GmbH, Jena, Germany), and all sections were prepared manually. For each taxon, 50 basidiospores and 50 basidia were measured. When cystidia were present, 50 cystidia were measured, and 50 measurements were also obtained for each available pileipellis, stipitipellis, and contextual element. Immature, collapsed, or obviously abnormal structures were excluded from measurements. Spore dimensions are presented as (minimum) main range (maximum), and Q refers to the length/width ratio of individual basidiospores. Tissues were rehydrated in 5–10% KOH. Sections of the pileipellis and stipitipellis were cut longitudinally and perpendicular to the surface, whereas sections of the hymenium were taken from the lamellar edge towards the base. Congo red was applied when necessary, and Melzer's reagent was used to test for amyloid or dextrinoid reactions. For ultrastructural observations of basidiospores [17,18], scanning electron microscopy (SEM) was performed on basidiospores from the holotype collections of the two new species. Fifty basidiospores were examined for each holotype collection, and representative spores were photographed from different orientations. SEM observations were restricted to the holotype collections because these specimens represent the nomenclatural reference material

for the two new species and contained sufficient mature basidiospores for examination. This sampling documents representative three-dimensional spore architecture but does not allow variation among collections or within species to be fully assessed. SEM was performed using a JEOL JSM-IT800 (JEOL Ltd., Akishima, Tokyo, Japan) at an accelerating voltage of 8.0 kV. Specimens were first dried on silica gel and then sputter-coated with gold (chamber pressure $< 1.0 \times 10^{-3}$ Pa; sputtering time, 45 s). SEM observations were used only to provide supplementary information on the three-dimensional architecture of basidiospores.

2.3. DNA Extraction, PCR, and Sequencing

Genomic DNA was extracted from dried basidiomata using the DNasecure Plant Genomic DNA Extraction Kit (Tiangen Biotechnology Co., Ltd., Beijing, China) according to the manufacturer's instructions. The extracted DNA was stored at -20°C , and DNA concentration was measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Three loci commonly used in molecular phylogenetic studies of macrofungi were selected in this study: the internal transcribed spacer (ITS) region, including ITS1, 5.8S rDNA, and ITS2; the nuclear large subunit ribosomal DNA (LSU); and a partial sequence of the second largest subunit of RNA polymerase II (*rpb2*). The primer pairs ITS1/ITS4 [19], LR0R/LR5 [20], and RPB2-5F/fRPB2-7cR [21] were used to amplify ITS, LSU, and *rpb2*, respectively.

PCR amplifications were carried out in 25 μL reaction mixtures containing 12.5 μL of Yuanye $2 \times$ HiFi PCR Master Mix, 1 μL of each primer (5 μM ; Sangon Biotech, Shanghai, China), 0.8–1.0 μL of DNA template depending on DNA concentration, and ddH₂O to a final volume of 25 μL . The PCR programme consisted of an initial denaturation at 94°C for 5 min, followed by 32 cycles of denaturation at 94°C for 30 s, annealing at 52, 53, and 56°C for ITS, LSU, and *rpb2*, respectively, for 40 s, and extension at 72°C for 1.5 min, with a final extension at 72°C for 7 min. The amplification products were then held at 4°C . PCR products were examined by electrophoresis on 1.0% agarose gels and visualised under UV light. Successful amplicons were purified using a DNA purification and recovery kit (Beijing Quanshijin Biotechnology Co., Ltd., Beijing, China) according to the manufacturer's instructions, and the purified products were sent to Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China) for Sanger sequencing. Newly generated sequences were assembled in SeqMan v.7.1 (DNASTAR, Madison, WI, USA), and the consensus sequences were deposited in GenBank.

2.4. Phylogenetic Analyses

Taxon sampling focused on representatives of the *Prunuloides* lineage, corresponding to *Entoloma* sect. *Madida*, and morphologically similar taxa, especially species related to the *E. bloxamii*–*prunuloides* complex. Species from related lineages, including *E.* sect. *Turfosa* and other allied taxa, were also included to test the phylogenetic placement of the new collections. Newly generated sequences were then combined with relevant sequences retrieved from GenBank, including taxa selected on the basis of morphological similarity and preliminary BLAST searches [7–9,22], to construct a phylogenetic tree for the multigene locus (ITS-LSU-*rpb2*). Before phylogenetic analyses, forward and reverse chromatograms of the newly generated sequences were manually inspected in SeqMan v.7.1. Low-quality terminal regions were trimmed, and ambiguous base calls were checked against both strands. Positions that could not be reliably resolved were treated as ambiguous bases. The newly generated sequences were also checked for consistency with preliminary BLAST results before inclusion in the final dataset. The resulting gap-free sequence lengths ranged from 623 to 648 bp for ITS and from 734 to 932 bp for LSU, whereas the *rpb2* sequence was 615 bp

long. Voucher-specific sequence lengths and GenBank accession numbers are provided in Table A2. The final combined dataset included 115 taxa and 202 sequences representing ITS, LSU, and *rpb2*. Because many available GenBank accessions of *Entoloma* sect. *Madida* and allied taxa are incomplete, some taxa lacked one or more loci. These taxa were retained in the analyses to maximise taxonomic coverage and to include morphologically or phylogenetically important comparative species. Missing loci were coded as missing data in the concatenated matrix. Entirely unavailable loci accounted for 36.6% of the total matrix cells, based on 14 taxa lacking ITS, 45 lacking LSU, and 84 lacking *rpb2*; ordinary alignment gaps and ambiguous nucleotide codes were not included in this calculation. Because missing data were unevenly distributed among loci and were particularly concentrated in the *rpb2* partition, they may reduce the resolution and support of some internal branches, especially for taxa represented by only one or two loci. The phylogenetic placement of the new taxa was therefore interpreted together with the available molecular evidence and diagnostic morphological characters.

The ITS, LSU, and *rpb2* sequences were aligned separately using the L-INS-i algorithm in MAFFT v.7 [23]. The resulting alignments were manually adjusted in BioEdit v.7.1.3 and then concatenated in PhyloSuite v.1.2.2 [24]. The best-fit substitution models for each partition were selected using ModelFinder [25] under the Bayesian information criterion (BIC). Maximum likelihood (ML) analyses were conducted in IQ-TREE v.1.6.8 [26], and Bayesian inference (BI) was performed in MrBayes v.3.2.6 with two parallel runs for 2,000,000 generations. The first 25% of sampled trees were discarded as burn-in. Convergence of the Bayesian analysis was considered to have been reached when the average standard deviation of split frequencies fell below 0.01. Branch support for the ML analysis was assessed using 1000 bootstrap replicates. All of the above programs were implemented in PhyloSuite v.1.2.2. The resulting trees were visualised in FigTree v.1.4 and further edited in Adobe Illustrator v.30.0.

To ensure transparency and reproducibility of the phylogenetic analyses, the full list of specimens, vouchers, GenBank accession numbers, countries of origin, and references is provided in Table 1.

Table 1. Specimens and sequences used in this study.

Species	Specimen Voucher	GenBank No.			Country	References
		ITS	LSU	<i>rpb2</i>		
<i>Clitopilus hirneolus</i>	MEN 199956	KC710132	GQ289211	GQ289278	Italy	[7]
<i>C. fallax</i>	MEN 200367	-	GQ289210	GQ289276	Slovakia	[7]
<i>C. nitellinus</i>	MEN 200435	-	GQ289215	GQ289282	Austria	[7]
<i>Entoloma albidum</i>	Y.Lamoureux 3218	KC710102	KC710151	-	Canada	[7]
<i>E. albomagnum</i>	G Gates E2030	KC710065	KC710137	-	Australia	[7]
<i>E. alcedicolor</i>	E Arnolds 02 760276	KC710123	GQ289152	GQ289224	Netherlands	[7]
<i>E. araneosum</i>	MEN 200314	KC710056	GQ289153	GQ289225	Belgium	[7]
<i>E. baronii</i>	G Gates E2292	KC710093	-	-	Australia	[7]
<i>E. bloxamii</i>	MEN 200442	KC710087	GQ289154	GQ289226	Austria	[7]
<i>E. bloxamii</i>	MEN 02165	KC710083	-	-	Italy	[7]

Table 1. Cont.

Species	Specimen Voucher	GenBank No.			Country	References
		ITS	LSU	<i>rpb2</i>		
<i>E. bloxamii</i>	LNM 171211	KC710077	-	-	Portugal	[7]
<i>E. bloxamii</i>	S.Baireuther 1-10-2010	KC710098	-	-	Germany	[7]
<i>E. bloxamii</i> v. <i>rubellum</i>	JCVegeau 01-10-2000	KC710082	-	-	France	[7]
<i>E. bloxamii</i> v. <i>rubellum</i>	MMaletii 28-10-1998	KC710068	-	-	Italy	[7]
<i>E. bloxamii</i> v. <i>rubellum</i>	G.Wölfel E20-04	KC710066	-	-	Germany	[7]
<i>E. bloxamii</i> v. <i>rubellum</i>	S.Baireuther 1-10-2001	KC710070	-	-	Germany	[7]
<i>E. aff. prunuloides</i>	HDThiers 53901	KC710071	KC710139	-	United States	[7]
<i>E. madidum</i>	OSC1064185	EU526002	-	-	United States	[7]
<i>E. aff. prunuloides</i>	TJB7876	KC710081	-	-	United States	[7]
<i>E. bloxamii</i>	UBCF16248	EF530938	-	-	Canada	[7]
<i>E. caccabus</i>	MEN 200324	KC710063	GQ289155	GQ289227	Belgium	[7]
<i>E. caesiolamellatum</i>	Wölfel 2022000	KC710126	KC710157	-	Spain	[7]
<i>E. callidermum</i>	Stubbe 06252	KC710115	KC710153	-	Malaysia	[7]
<i>E. clypeatum</i>	MEN 198302	KC710059	KC710136	-	Netherlands	[7]
<i>E. coeruleogracilis</i>	MEN 2004055	KC710107	GQ289167	GQ289238	Australia	[7]
<i>E. coeruleogracilis</i>	G-Gates E1777	KC710069	GQ289168	GQ289239	Australia	[7]
<i>E. coeruleoviride</i>	Stubbe 06236	KC710057	KC710134	-	Malaysia	[7]
<i>E. conferendum</i>	MEN 200313	-	GQ289160	GQ289231	Belgium	[7]
<i>E. conferendum</i>	MEN 200330	KC710055	KC710133	KC710191	Slovakia	[7]
<i>E. corneri</i>	D.Stubbe 06241	KC710058	KC710135	-	Malaysia	[7]
<i>E. cretaceum</i>	G.Gates E1181	KC710064	GQ289162	GQ289233	Australia	[7]
<i>E. cretaceum</i>	MEN 2010039	KC710090	-	-	Canada	[7]
<i>E. cretaceum</i>	MEN 2011022	KC710074	-	-	Australia	[7]
<i>E. flavifolium</i>	Y.Lamoureux 2846	KC710097	KC710150	-	Canada	[7]
<i>E. aff. flavifolium</i>	TB 6215	-	AF261301	GU384644	United States	[7]
<i>E. fumosobrunneum</i>	MEN2005113	KC710124	KC710155	-	Canada	[7]
<i>E. fumosobrunneum</i>	MEN2005120	KC710125	KC710156	-	Canada	[7]
<i>E. gelatinosum</i>	G-Gates E792	KC710103	GQ289165	GQ289236	Australia	[7]
<i>E. gracilior</i>	MEN 2011043	KC710079	-	-	Australia	[7]
<i>E. gracilior</i>	G-Gates E1220	KC710112	GQ289169	GQ289240	Australia	[7]
<i>E. aff. griseopruinatum</i>	LNM 221111	KC710118	-	-	Portugal	[7]
<i>E. haastii</i>	MEN 2004055 53	KC710086	KC710144	-	Australia	[7]

Table 1. Cont.

Species	Specimen Voucher	GenBank No.			Country	References
		ITS	LSU	<i>rpb2</i>		
<i>E. haastii</i>	MEN 2006617	KC710089	KC710145	-	New Zealand	[7]
<i>E. haastii</i>	MEN 2010034	KC710113	-	-	Australia	[7]
<i>E. haastii</i>	MEN 2011045	KC710101	-	-	Australia	[7]
<i>E. indigoticoum-brinum</i>	MEN 200406	-	GQ289171	GQ289242	Australia	[7]
<i>E. kermantii</i>	G.Gates E227	-	GQ289173	GQ289244	Australia	[7]
<i>E. kermantii</i>	G.Gates E227	KC710075	-	-	Australia	[7]
<i>E. lividoalbum</i>	MEN 200328	KC710114	KC710152	-	Belgium	[7]
<i>E. luridum</i>	MEN 2005634	KC710080	KC710141	-	Canada	[7]
<i>E. luridum</i>	Y.Lamoureux 3952	KC710094	KC710148	-	Canada	[7]
<i>E. luridum</i>	MEN 2005108	KC710091	KC710146	KC710192	Canada	[7]
<i>E. madidum</i>	MEN 2004030	KC710127	KC710158	-	Netherlands	[7]
<i>E. madidum</i>	985	JF907990	-	-	Italy	[7]
<i>E. madidum</i>	EC-Vellinga	KC710131	-	-	Netherlands	[7]
<i>E. madidum</i>	MEN 2165	KC710129	-	-	Italy	[7]
<i>E. madidum</i>	67195	KC710130	-	-	Norway	[7]
<i>E. manganaense</i>	G.Gates E369	KC710085	KC710143	-	Australia	[7]
<i>E. myrmecophilum</i>	G.Tjallingii Beukers 1981-10-30	KC710120	GQ289174	GQ289245	Netherlands	[7]
<i>E. nitidum</i>	MEN 200324	KC710122	GQ289175	GQ289246	Slovakia	[7]
<i>E. nitidum</i>	287	JF907989	-	-	Italy	[7]
<i>E. nitidum</i>	MEN 8376	KC710076	-	-	Scotland	[7]
<i>E. nitidum</i>	Hausknecht 2006201	KC710100	-	-	Austria	[7]
<i>E. nitidum</i>	F14054	AF335449	-	-	Canada	[7]
<i>E. nitidum</i>	F14288	AY228340	-	-	Canada	[7]
<i>E. nitidum</i>	TB 7526	-	GU384626	GU384655	United States	[7]
<i>E. ochreoprunuloides</i>	D.Harries 3-10-2010	KC710106	-	-	United Kingdom	[7]
<i>E. ochreoprunuloides</i>	E.Arnolds 01-142	KC710092	KC710147	-	Germany	[7]
<i>E. ochreoprunuloides</i>	RA Maas Geesteranus 15721	KC710111	-	-	France	[7]
<i>E. ochreoprunuloides</i> f. <i>hyacinthinum</i>	D.Harries 25 9 2010	KC710105	-	-	United Kingdom	[7]
<i>E. perbloxamii</i>	MEN 2004071	KC710117	GQ289178	GQ289249	Australia	[7]
<i>E. perbloxamii</i>	MEN 2010037	KC710095	-	-	Australia	[7]

Table 1. Cont.

Species	Specimen Voucher	GenBank No.			Country	References
		ITS	LSU	<i>rpb2</i>		
<i>E. prunuloides</i>	R.Kristiansen 27-7-1980	KC710099	-	-	Norway	[7]
<i>E. prunuloides</i>	MEN 200340	KC710073	GQ289184	GQ289255	Slovakia	[7]
<i>E. prunuloides</i>	MEN 99202	KC710072	-	-	United Kingdom	[7]
<i>E. pseudo-prunuloides</i>	MEN 2005115	KC710078	KC710140	-	Canada	[7]
<i>E. sinuatum</i>	MEN 09-10-2001	KC710110	-	-	Austria	[7]
<i>E. sinuatum</i>	AReijnders 45	KC710108	-	-	Netherlands	[7]
<i>E. sinuatum</i>	JWisman 2003-09-19	KC710109	GQ289193	GQ289264	Netherlands	[7]
<i>E. sinuatum</i>	JVauras 8181F	KC710116	KC710154	-	Finland	[7]
<i>E. sinuatum</i>	H 6003960	GU373512	-	-	Finland	[7]
<i>E. sordidulum</i>	Co-David 2003	KC710062	GQ289194	GQ289265	Belgium	[7]
<i>E. sordidulum</i>	LNМ 191211	KC710119	-	-	Portugal	[7]
<i>E. bloxamii</i>	RBG Kew.K(M)128736	EU784208	-	-	United Kingdom	[7]
<i>E. aff. subsinuatum</i>	TB 5349	DQ486700	AY691891	-	United States	[7]
<i>E. sinuatum</i>	BHS2009-07	GU289652	-	-	United States	[7]
<i>E. haastii</i>	BY21	-	AF261309	-	United States	[7]
<i>E. sinuatum</i>	MLS007	GQ397994	-	-	United States	[7]
<i>E. aff. subsinuatum</i>	TB 5034	-	AF261294	-	United States	[7]
<i>E. caesiolamellatum</i>	TB 6117	KC710128	AF261289	-	United States	[7]
<i>E. aff. subsinuatum</i>	TB 6807	-	AF261295	-	United States	[7]
<i>E. aff. whiteae</i>	TJB4765	DQ206983	-	DQ385883	United States	[7]
<i>E. sinuatum</i>	TM 02-134	-	EU522735	-	Canada	[7]
<i>E. sinuatum</i>	TM 02-335	-	EU522771	-	Canada	[7]
<i>E. sinuatum</i>	TM 03-429	-	EU522811	-	Canada	[7]
<i>E. aff. myrmecophilum</i>	TRTC156542	JN021020	-	-	Canada	[7]
<i>E. aff. myrmecophilum</i>	TRTC156546	JN021019	-	-	Canada	[7]
<i>E. sphagneti</i>	C.Bas 686	KC710061	GQ289195	-	Netherlands	[7]
<i>E. subsinuatum</i>	MEN 2005624	KC710067	KC710138	-	Canada	[7]
<i>E. subsinuatum</i>	Y.Lamoureux 2269	KC710096	KC710149	-	Canada	[7]
<i>E. subsinuatum</i>	MEN 2005100	KC710104	-	-	Canada	[7]
<i>E. theobrunneum</i> HT	HMJAU81631	PZ236460	PZ232092	-	China	This study
<i>E. theobrunneum</i>	HMJAU81633	PZ236461	-	-	China	This study

Table 1. Cont.

Species	Specimen Voucher	GenBank No.			Country	References
		ITS	LSU	<i>rpb2</i>		
<i>E. trachyosporum</i>	H.den Bakker 1153	KC710088	GQ289198	-	Canada	[7]
<i>E. trachyosporum</i>	H.den Bakker 1901	KC710121	GQ289199	-	Canada	[7]
<i>E. trachyosporum</i>	DAVFP 28111	JF899553	-	-	Canada	[7]
<i>E. trachyosporum</i>	TB 5856	-	GU384629	GU384658	United States	[7]
<i>E. turbidum</i>	MEN 200351	KC710060	GQ289201	GQ289269	Slovakia	[7]
<i>E. whiteae</i>	Y.Lamoureux 1430	KC710084	KC710142	-	Canada	[7]
<i>E. zuccherellii</i>	AZuccherelli 1996-01-25	-	GQ289206	-	Italy	[7]
<i>Lyophyllum leucophaeatum</i>	Hae 25197	AF357032	AF223202	DQ367434	Switzerland	[27,28]
<i>Rugosomyces carneus</i>	CBS 55250	AF357028	AF223175	DQ825407	Switzerland	GenBank
<i>E. ochrorubrum</i> HT	HMJAU81632	PZ236462	PZ232093	PZ235941	China	This study
<i>E. omiense</i>	B23052606	OR905725	OR905734	-	China	GenBank
<i>E. omiense</i>	B22052303	OR905724	OR905733	-	China	GenBank
<i>E. omiense</i>	GDGM27563	JQ281487	JQ410330	-	China	[29]

3. Results

3.1. Molecular Phylogeny

The ITS dataset comprised 101 sequences and 1022 characters, including gaps. The LSU dataset comprised 70 sequences and 1009 characters, including gaps, whereas the *rpb2* dataset comprised 31 sequences and 615 characters, including gaps. The concatenated three-gene matrix included 115 taxa and had a total length of 2646 bp, including gaps. Entirely missing loci accounted for 36.6% of the combined matrix cells, with the greatest proportion of missing data occurring in the *rpb2* partition. The best-fit substitution models selected by ModelFinder were TIM2 + F + I + G4 for both ITS and LSU, and TIM3e + I + G4 for *rpb2*. The phylogenetic tree inferred from maximum likelihood (ML) analysis showed a topology largely congruent with that obtained from Bayesian inference (BI). The complete ITS–LSU–*rpb2* combined tree is provided in Appendix A, Figure A1. To improve readability and to focus on the phylogenetic placement of the two new taxa, the main text presents an enlarged view of *Entoloma* sect. *Madida* (Figure 1). Because several comparative taxa lacked one or more loci, support values for some internal branches should be interpreted cautiously. Nevertheless, the two newly described taxa were recovered as independent lineages in the combined analysis, and their recognition is further supported by diagnostic morphological differences.

The phylogeny recovered a clade corresponding to *Entoloma* sect. *Madida*, including taxa related to the *E. bloxamii*–*prunuloides* complex. The newly generated sequences from Anhui formed two distinct species-level lineages within this framework. For *E. theobrunneum*, the holotype collection HMJAU81631 was represented by ITS and LSU sequences, whereas the additional collection HMJAU81633 was represented by ITS only. Therefore, the additional collection provides ITS-level support for its placement with the

holotype, rather than independent multilocus confirmation. *Entoloma theobrunneum* was recovered as the sister lineage of *E. corneri* with strong support (BPP = 0.98, MLB = 96), whereas *E. ochrorubrum* formed an independent lineage near taxa of the *E. bloxamii* complex (BPP = 0.99, MLB = 100). These results support the placement of both taxa within the broader *E. sect. Madida* framework and indicate that they represent independent species-level lineages rather than variants of previously sampled taxa.

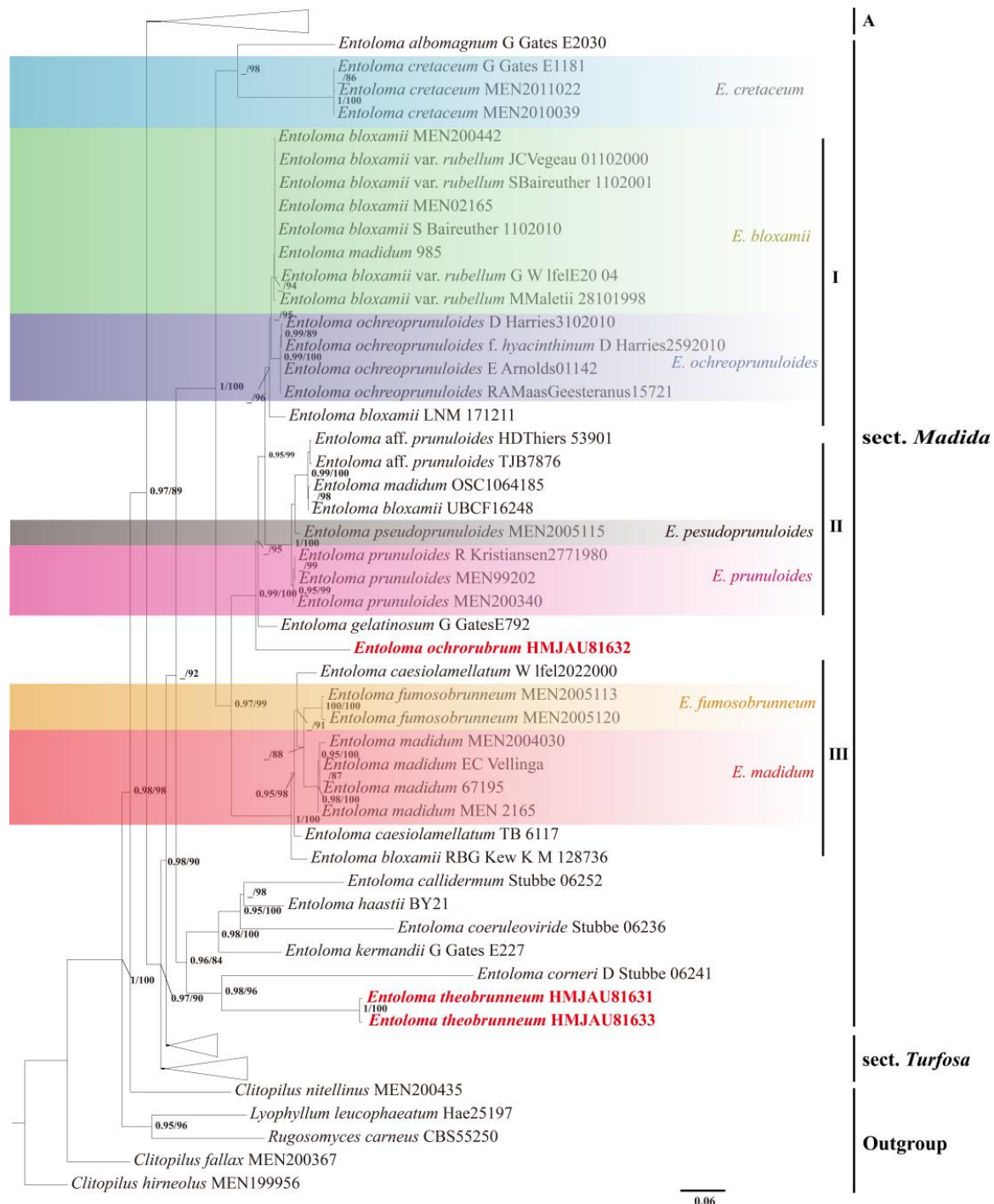


Figure 1. Enlarged phylogenetic tree of *Entoloma* sect. *Madida* inferred from the combined ITS, LSU and *rpb2* dataset. Values on branches indicate maximum likelihood bootstrap support and Bayesian posterior probabilities, shown as MLB/BPP. Only MLB $\geq$ 70% and BPP $\geq$ 0.95 are displayed. Newly described taxa are highlighted in bold red. The complete combined tree is shown in Appendix A, Figure A1.

To facilitate comparison among the relevant taxa, the principal morphological, ecological, and distributional differences between the two new species and their closest phylogenetic and morphologically allied taxa are summarised in Appendix B, Table A1.

3.2. Taxonomy

Entoloma theobrunneum H. Zhang, Q. Wang & Yu Li, sp. nov.; Figures 2 and 3.

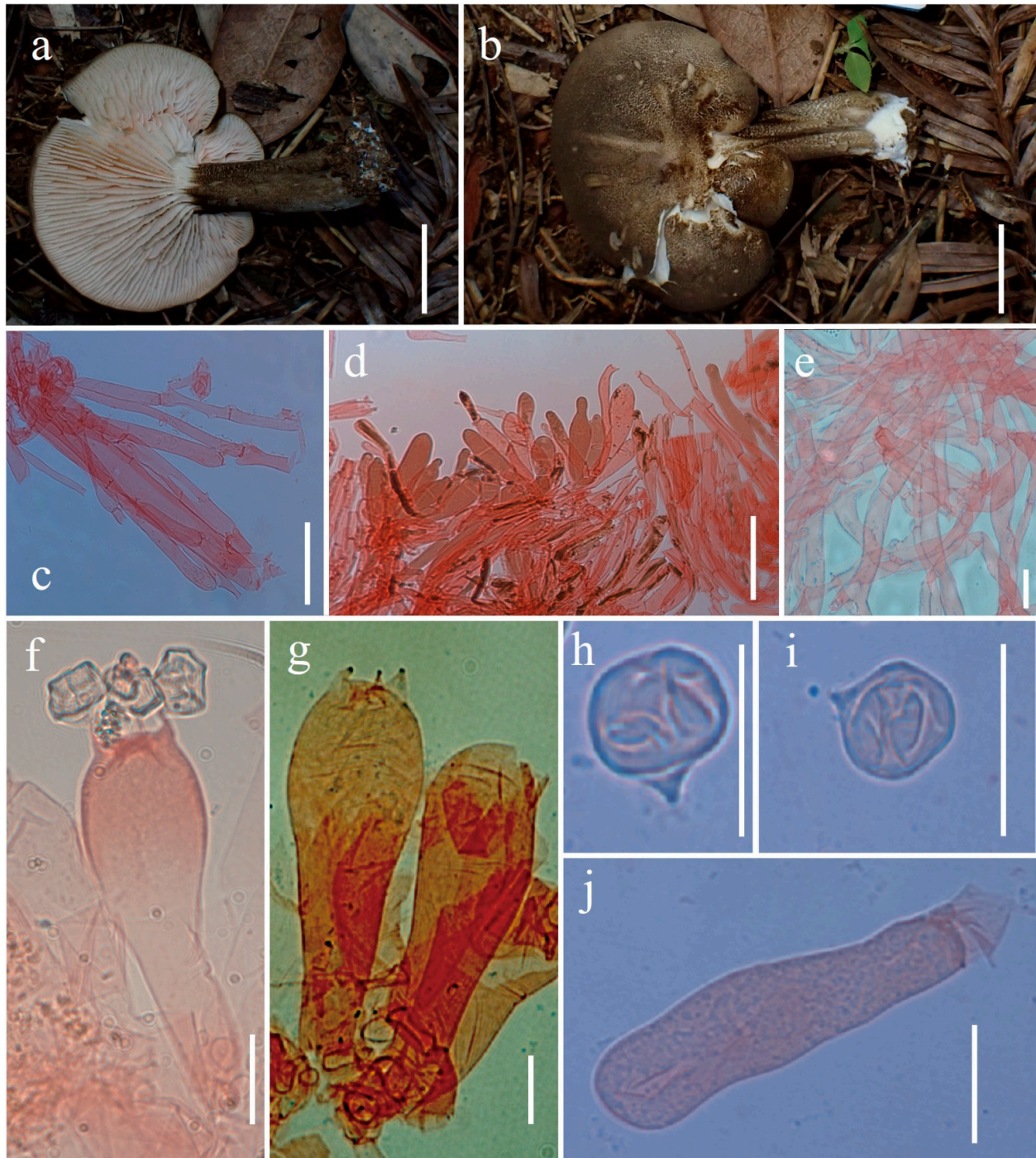


Figure 2. Macroscopic and microscopic features of *E. theobrunneum* (HMJAU81631, holotype). (a,b) Basidiomata; (c) Stipitipellis; (d) Caulocystidia; (e) Pileipellis; (f,g) Basidia; (h,i) Basidiospores; (j) Pleurocystidia. Scale bar: (a,b) = 2 cm; (c,d) = 20 μ m; (e) = 2 μ m; (f–j) = 10 μ m. Line drawing of *E. theobrunneum* is provided in Appendix C, Figure A2.

Fungal Names No.: FN573597.

Etymology: The specific epithet “*theobrunneum*” is derived from the Latin words “*thea*” (tea) and “*brunneum*” (brown), referring to the species’ overall tea-brown coloration.

Holotype: CHINA. Anhui Province, Huangshan City, Huangshan Wild Monkey Valley, 30°08′52.43″ N, 118°14′31.47″ E. Elevation 637.8 m. In the soil of a mixed coniferous and broad-leaved forest. 8 September 2025. Zhang Hui (HMJAU81631). GenBank accession numbers: ITS = PZ236460 and LSU = PZ232092.

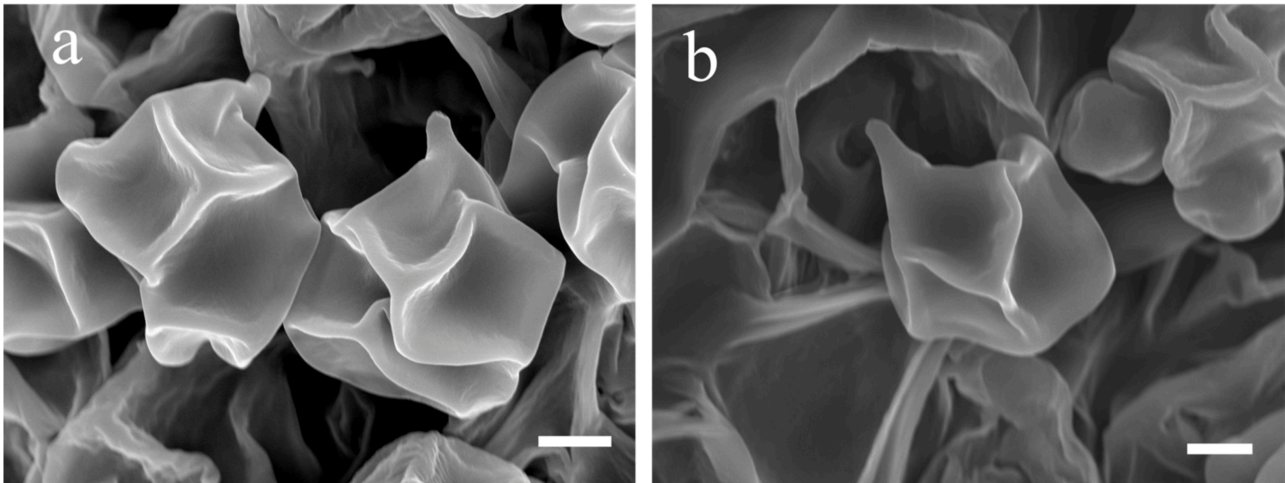


Figure 3. Scanning electron microscope (SEM) images of basidiospores. Scale bar: (a,b) = 2 μ m.

Diagnosis: Based on the available collections, *Entoloma theobrunneum* differs from morphologically similar species by its smoky grey to tea-brown pileus with a dense patchy pattern, a stipe concolorous or nearly concolorous with the pileus and bearing a distinct snakeskin-like pattern, relatively large basidiospores, larger basidia, and the presence of pleurocystidia and caulocystidia.

Description: As shown in Figure 2, basidiomata medium-sized to large. Pileus 6.5–8.0 cm in diam., distinctly depressed at the centre, surface rough, with a dense patchy pattern; margin usually entire, occasionally with irregular notches; non-striate; smoky grey to tea-brown; pileus nearly plane when young, becoming upturned at maturity; not viscid when moist. Context thin, white to off-white. Lamellae fairly crowded, unequal in length, adnate with a decurrent tooth, white to pale pink; lamellar edge near the stipe dark brown; unchanged when bruised. Stipe 4.0–6.0 $\times$ 1.5–1.8 cm, central, cylindrical, slightly swollen at the base; surface dark brown to greyish brown, with a distinct snakeskin-like pattern, fibrous; solid when young, becoming hollow at maturity; base covered with white mycelium. Odour indistinct.

Basidiospores (7.6)8.6–9.0(10.0) $\times$ (6.7)7.3–7.5(7.9) μ m, $Q = 1.15$ –1.27, $Q_{av} = 1.21$ ($n = 50$), polygonal in outline, with 4–6 angles and indistinct edges, thin-walled. Basidia 56.2–60.1 $\times$ 18.2–22.8 μ m ($n = 50$), clavate, gradually tapering toward the base, 3–4-spored. Pleurocystidia scattered on the lamellar sides, 31.8–39.2 $\times$ 9.1–10.5 μ m ($n = 50$), mostly cylindrical. Caulocystidia abundant and fasciculate on the stipe surface, 37.8–46.1 $\times$ 7.4–9.1 μ m ($n = 50$), variable in shape, bowling-pin-shaped to cylindrical, containing pale brown intracellular pigment. Stipitipellis composed of narrow cylindrical hyphae 3.8–4.2 μ m in diameter. Pileipellis composed of hyphae 2.9–3.5 μ m in diameter. Clamp connections present in all tissues.

Scanning electron micrographs of the basidiospores are shown in Figure 3. Under SEM, the basidiospores appear distinctly polyhedral in three dimensions, with the spa-

tial arrangement of the facets not fully reflected by conventional two-dimensional light-microscopic observation.

Habitat and distribution: Solitary to scattered on the ground in a mixed coniferous–broadleaf forest composed of *Pinus massoniana* and *Quercus chenii*.

World distribution: China.

China distribution: Anhui Province.

Additional specimens examined: China. Anhui Province, Huangshan City, Huangshan Wild Monkey Valley. 30°05′05.18″ N, 118°08′37.81″ E. Elevation 633.4 m. On soil in a bamboo forest (*Phyllostachys edulis*). 8 September 2025. H. Zhang (HMJAU81633). GenBank accession number: ITS = PZ236461.

Notes: Morphologically, *Entoloma theobrunneum* is most similar to *E. corneri* from Malaysia. Both species produce medium- to large-sized basidiomata with an overall brownish appearance. In the available collections, *E. theobrunneum* is characterized by a smoky grey to tea-brown pileus with a densely patchy surface, a stipe concolorous or nearly concolorous with the pileus and ornamented with a conspicuous snakeskin-like pattern, and adnate lamellae with a decurrent tooth and tea-brown edges. These macromorphological characters were observed in both available collections, although minor variation was present in pileus size and the degree of central depression, probably reflecting developmental stage or habitat conditions.

Compared with *E. corneri*, which has an olive-brown to cigar-brown pileus with a smooth surface and silky sheen and a stipe usually showing an olive tint, *E. theobrunneum* differs by the combined characters of pileus colouration, pileus surface pattern, stipe ornamentation, and lamellar attachment. Micromorphologically, the new species is further distinguished by higher basidiospore Q values, markedly larger basidia, and the presence of pleurocystidia and caulocystidia containing pale brown intracellular pigment. Pleurocystidia were observed on the lamellar sides, and caulocystidia were present on the stipe surface, locally occurring in clusters.

Phylogenetic analyses showed that *E. theobrunneum* formed a distinct lineage sister to *E. corneri*. It should be noted that multilocus evidence for this species is mainly based on the holotype collection HMJAU81631, whereas the additional collection HMJAU81633 is represented by ITS only. In combination with the diagnostic morphological differences outlined above, these phylogenetic results support the recognition of *E. theobrunneum* as a distinct new species.

Entoloma ochrorubrum H. Zhang, Q. Wang & Yu Li, sp. nov.; Figures 4 and 5.

Fungal Names No.: FN573601.

Etymology: The epithet “*ochrorubrum*” is derived from the Latin words *ochro-* (ochre-yellow or earth-yellow in tone) and *rubrum* (red), referring to the reddish pileus tinged with ochre tones in this species.

Holotype: China. Anhui Province, Huangshan City, Huangshan Wild Monkey Valley. 30°05′24.84″ N, 118°08′49.97″ E. Elevation 694.1 m. On soil in a mixed coniferous–broadleaf forest. 7 July 2025. H. Zhang (HMJAU81632). GenBank accession numbers: ITS = PZ236462; LSU = PZ232093; *rpb2* = PZ235941.

Diagnosis: *Entoloma ochrorubrum* is currently known only from the holotype collection. Based on the available material, it differs from *E. bloxamii* var. *rubellum* and *E. ochreoprunuloides* by its darker pileus, which is iron-red to ochre-brown with a blackish-brown central papilla, an irregular pileus margin that cracks at maturity, and relatively large basidiospores (6.8–7.4 × 5.3–6.5 µm). The range of intraspecific variation remains unknown.

Description: Based on the holotype collection. As shown in Figure 4, basidiomata medium-sized to large, 5.5–6.5 cm high. Pileus 7.5–8.5 cm in diam., nearly plane to shallowly infundibuliform, centrally depressed, with a papillate projection in the centre; margin

irregular, locally cracked, exposing the white context; surface nearly smooth to finely fibrillose, iron-red to ochre-brown; central papilla blackish brown, gradually paler towards the margin. Context thin, white. Lamellae relatively distant, 0.8–1.2 cm broad, unequal in length, decurrent, pale pink to light brown, with irregular edges; unchanged when bruised. Stipe 4.5–5.5 × 1.5–2.0 cm, central, cylindrical, slightly curved, hollow, fibrous, pale yellow to yellowish brown, with a distinct longitudinally fibrillose pattern on the surface. Odour indistinct.

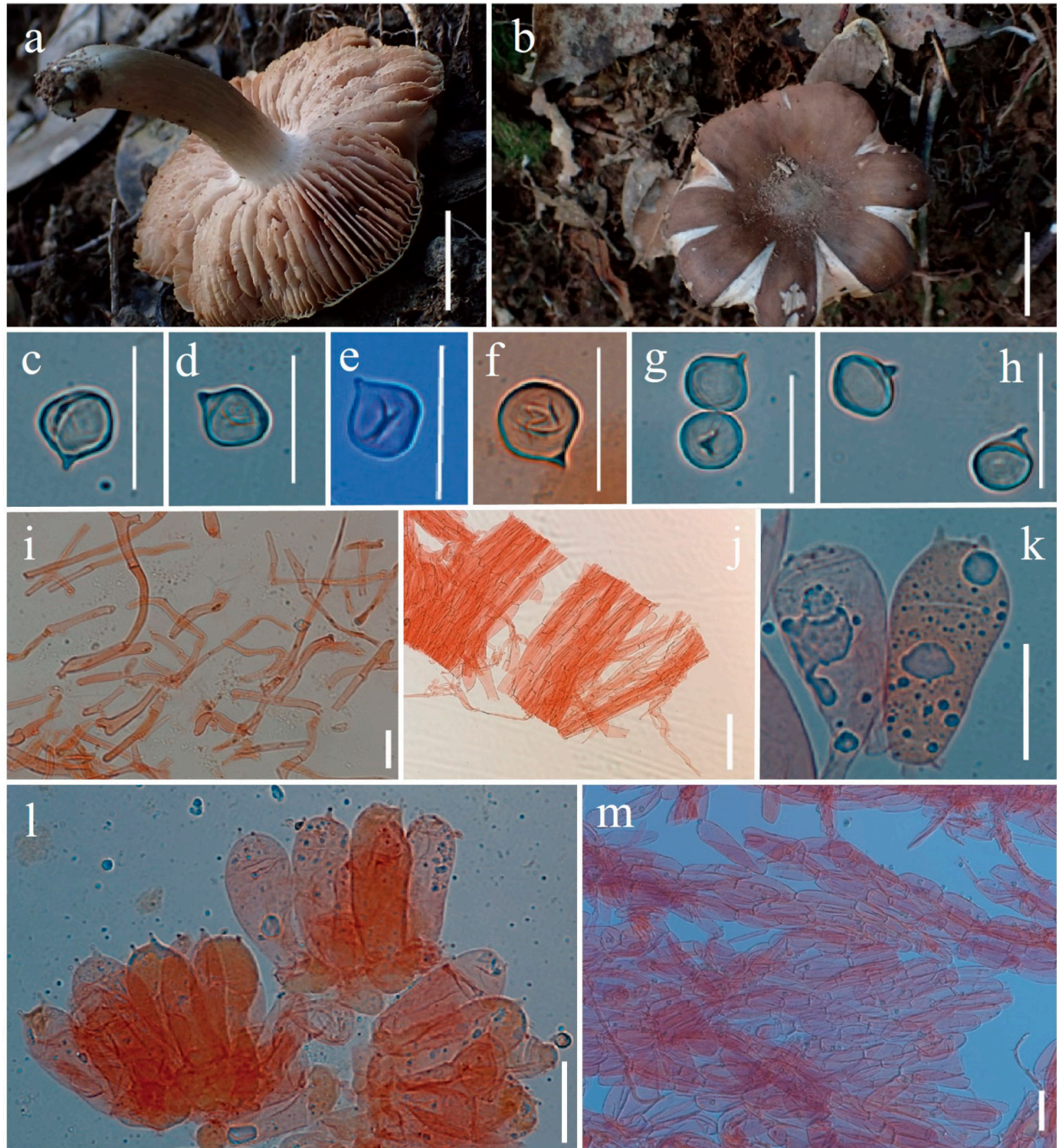


Figure 4. Macroscopic and microscopic features of *E. ochrorubrum* (HMJAU81632, holotype). (a,b) Basidiomata; (c–h) Basidiospores; (i) Pileipellis; (j) Stipitipellis; (k,l) Basidia; (m) Contextual hyphae. Scale bar: (a,b) = 2 cm; (c–h) = 10 µm; (i,j) = 20 µm; (k,l) = 10 µm; (m) = 20 µm. Line drawing of *E. ochrorubrum* is provided in Appendix C, Figure A3.

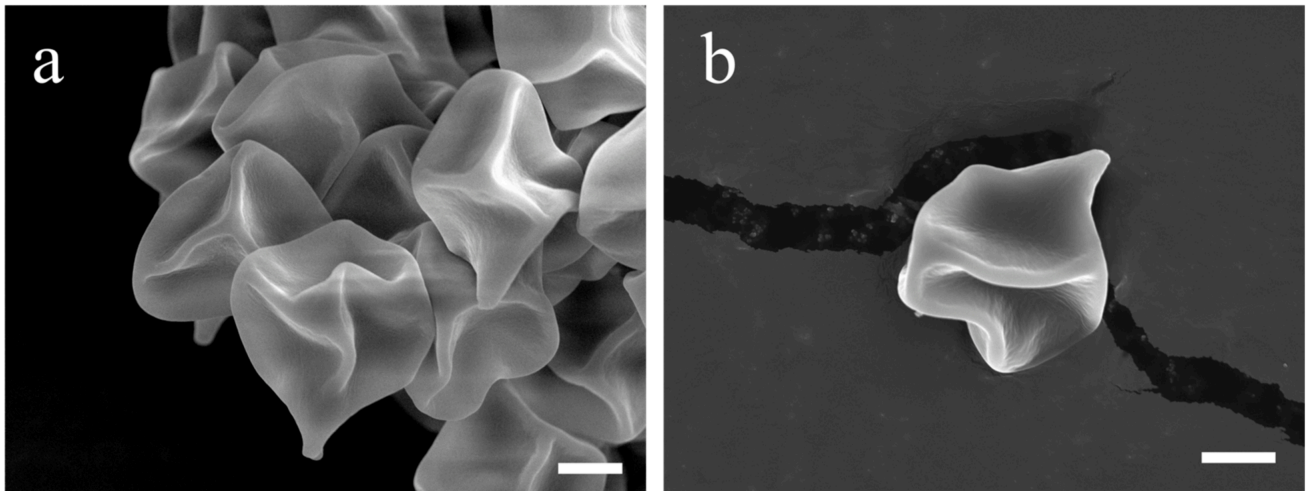


Figure 5. Scanning electron microscope (SEM) images of basidiospores. Scale bars: (a,b) = 2 μm .

Basidiospores $(5.8)6.8\text{--}7.4(7.9) \times (4.8)5.3\text{--}6.5(6.6) \mu\text{m}$, $Q = 1.14\text{--}1.39(1.42)$, $Q_{\text{av}} = 1.26$ ($n = 50$), polygonal in outline, with 5–6 angles, angles indistinct, walls moderately thick. Basidia $24.6\text{--}26.8 \times 11.5\text{--}12.2 \mu\text{m}$ ($n = 50$), clavate, usually with granular contents, 2–4-spored. Stipitipellis composed of hyphae $4.3\text{--}5.0 \mu\text{m}$ wide. Pileipellis composed of hyphae $2.9\text{--}3.2 \mu\text{m}$ wide. Clamp connections present in all tissues. Context composed of sub-parallel, slightly inflated cylindrical to ellipsoid cells, $29.5\text{--}32.3 \times 8.9\text{--}10.1 \mu\text{m}$ ($n = 50$).

Scanning electron micrographs of the basidiospores are shown in Figure 5. Under SEM, the basidiospores are distinctly polyhedral, with multiple facets and ridges visible on the spore surface, indicating that their three-dimensional structure cannot be fully represented by a two-dimensional outline alone.

Habitat and distribution: Solitary on the ground in a mixed coniferous–broadleaf forest dominated by *Pinus massoniana* and *Quercus chenii*.

World distribution: China.

China distribution: Anhui Province.

Notes: Morphologically, *Entoloma ochrorubrum* is similar to the European species *E. bloxamii* var. *rubellum* and *E. ochreoprunuloides*; all three have reddish-brown to pinkish-ochre pileus colours and yellowish to yellowish-brown stipes. However, *E. ochrorubrum* differs by its darker iron-red to ochre-brown pileus, blackish-brown central papilla, irregular pileus margin that cracks at maturity, and distinctly longitudinally fibrillose stipe. This combination of characters supports the separation of *E. ochrorubrum* from these morphologically similar taxa, although additional collections are needed to evaluate the stability of these diagnostic characters.

Micromorphologically, *E. ochrorubrum* differs from *E. bloxamii* var. *rubellum* and *E. ochreoprunuloides* by its higher basidiospore Q values and relatively shorter but broader basidia. Compared with *E. bloxamii* var. *rubellum*, which has basidiospores with $Q = 1.0\text{--}1.2$ and basidia $22\text{--}38 \times 8\text{--}11 \mu\text{m}$, *E. ochrorubrum* has basidiospores with $Q = 1.14\text{--}1.39$ and basidia $24.6\text{--}26.8 \times 11.5\text{--}12.2 \mu\text{m}$. Compared with *E. ochreoprunuloides*, which has basidiospores with $Q = 1.02\text{--}1.04$ and basidia $27\text{--}40 \times 7\text{--}11 \mu\text{m}$, *E. ochrorubrum* has more elongate basidiospores and shorter but broader basidia.

Phylogenetic analysis recovered *E. ochrorubrum* near taxa related to the *E. bloxamii* complex, although it did not cluster with any previously described species represented in the dataset. It is also separated from other related taxa included in the phylogeny, such as *E. bloxamii*, *E. prunuloides*, *E. madidum*, *E. perbloxamii*, and *E. omiense*, by the combination of a dark iron-red to ochre-brown pileus, blackish-brown central papilla, cracking pileus margin, longitudinally fibrillose stipe, relatively high basidiospore Q values, and basidia

with conspicuous granular contents. This phylogenetic position is consistent with the available macroscopic and microscopic evidence. However, because *E. ochrorubrum* is represented by a single collection, its intraspecific variation, ecological amplitude, and distribution range remain insufficiently known. Additional collections will be necessary to further evaluate the stability of its diagnostic characters.

4. Discussion

The present study provides an integrative taxonomic assessment of three collections of *Entoloma* sect. *Madida* from subtropical montane forests of Anhui Province, eastern China. The two taxa described here, *E. theobrunneum* and *E. ochrorubrum*, were recovered as independent species-level lineages in the combined ITS–LSU–*rpb2* phylogeny and can be distinguished from closely related taxa by different combinations of macromorphological and micromorphological characters. Therefore, their recognition is based on the congruence between phylogenetic placement and diagnostic morphology, rather than on a single divergent character.

The phylogenetic results place both new species within the framework of *E.* sect. *Madida*, a lineage that remains insufficiently documented from subtropical eastern China. However, the main taxonomic significance of the present material lies in the specific differences between the two Chinese taxa and their closest relatives, rather than in the general diversity of *Entoloma* in China [28,29].

Entoloma theobrunneum formed a distinct and strongly supported lineage in the combined phylogeny (BPP = 0.98, MLB = 96) and was recovered as the sister lineage of *E. corneri*. Previous studies have shown that, in *Entoloma*, morphologically similar but geographically separated materials often represent closely related yet independent lineages in multilocus analyses, rather than a single genuinely widespread species [7,30]. Morphologically, both taxa are terrestrial and share an overall brownish habit [7,31]. However, *E. theobrunneum* differs in having a smoky grey to tea-brown pileus with a densely patchy surface and a distinctly depressed centre, a stipe concolorous or nearly concolorous with the pileus and bearing a conspicuous snakeskin-like pattern, and adnate lamellae with a decurrent tooth and a dark brown lamellar edge near the stipe. By contrast, *E. corneri* has an olive-brown to olive-green pileus with a smooth, silky surface and a pale yellow to olivaceous stipe. Micromorphological characters further support the distinction between the two taxa. *Entoloma theobrunneum* has larger basidiospores with higher Q values, markedly larger basidia, and possesses pleurocystidia and caulocystidia containing pale brown intracellular pigment, whereas *E. corneri* has basidiospores that are more nearly isodiametric, smaller basidia, and lacks cystidia. Thus, *E. theobrunneum* is not separated from *E. corneri* only by pileus colour, but by a combination of phylogenetic position, pileus surface pattern, stipe ornamentation, lamellar characters, larger basidia, higher basidiospore Q values, and the presence of pleurocystidia and fasciculate caulocystidia.

Similar to the case of *E. theobrunneum*, *E. ochrorubrum* also formed a distinct and strongly supported lineage in the combined phylogeny (BPP = 0.99, MLB = 100) and was recovered near taxa related to the *E. bloxamii* complex. Previous studies have shown that the traditional broad concept surrounding *E. bloxamii* in fact encompasses multiple independent phylogenetic species. Accordingly, the phylogenetic position of *E. ochrorubrum* in the present study should not be interpreted simply as a colour variant within this complex, but more likely represents a previously undocumented Chinese lineage [7]. Morphologically, *E. ochrorubrum* is most similar to *E. bloxamii* var. *rubellum* and *E. ochreoprunuloides*. In addition, we also considered phylogenetically related taxa included in the present tree, including *E. bloxamii*, *E. prunuloides*, *E. madidum*, *E. perbloxamii*, and *E. omiense*, so that the comparison was not limited to two morphologically similar European

taxa. *Entoloma ochrorubrum* can be distinguished by its darker pileus, blackish-brown central papilla, pileus margin that cracks at maturity, and stipe with a conspicuous longitudinally fibrillose pattern. Micromorphologically, it further differs in having higher basidiospore Q values, relatively shorter and broader basidia, and conspicuous granular contents in the basidia [32–35]. The combination of these morphological characters and its independent phylogenetic position indicates that *E. ochrorubrum* represents a distinct Chinese species-level lineage rather than a colour variant of previously known allied taxa.

Although the available molecular and morphological evidence supports the recognition of *E. ochrorubrum* as an independent species-level lineage, this taxon is currently represented only by the holotype collection. Repeated field surveys conducted in the same area did not yield additional material, but the limited sampling prevents a full assessment of its intraspecific morphological variation, ecological amplitude, and geographical distribution. Therefore, the diagnostic differences reported here should be interpreted with caution until additional collections become available. Broader sampling from Huangshan and other subtropical montane forests will be necessary to test the stability of these characters and to clarify the distribution of this species in eastern China.

The SEM observations incorporated in this study were included primarily to supplement light-microscopic examination and to provide a clearer visualization of basidiospore three-dimensional architecture. Under SEM, the basidiospores of both *Entoloma theobrunneum* and *E. ochrorubrum* appear distinctly polyhedral, with multiple facets and angles that are only partially represented in two-dimensional light-microscopic views, consistent with the observations reported by Reschke et al. [36]. However, these SEM observations are intended only as supplementary morphological documentation and were not used as primary evidence for species delimitation, particularly because comparable SEM data are unavailable for many species of *Entoloma* sect. *Madida* and related lineages. Accordingly, the recognition of *E. theobrunneum* and *E. ochrorubrum* is based mainly on the combined evidence from macromorphological characters, micromorphological measurements, and multilocus phylogenetic analyses, whereas the SEM images serve only to illustrate basidiospore three-dimensional structure and complement the morphological descriptions.

The occurrence of two distinct lineages within a restricted area may partly reflect the environmental heterogeneity of the Huangshan montane landscape, where mixed coniferous–broadleaf forests and bamboo forests provide contrasting substrates and microclimatic conditions. Geographic isolation among montane habitats may also contribute to the persistence of regionally differentiated lineages. However, because the present study is based on only three collections, these explanations remain preliminary hypotheses that require broader geographic and population-level sampling across East Asia.

Overall, the present results expand the documented diversity of *Entoloma* sect. *Madida* in subtropical eastern China and demonstrate the value of integrating multilocus phylogenetic analyses with detailed morphological comparisons. The closest allied taxa used for comparison with the two new species are represented mainly by collections from Europe, Malaysia, North America and other regions, whereas confirmed Chinese molecular records of this lineage remain limited. In this context, the discovery of *E. theobrunneum* and *E. ochrorubrum* helps fill a regional documentation gap and supports the need for further integrative studies of *E.* sect. *Madida* in China and East Asia.

5. Conclusions

This study examined *Entoloma* collections from subtropical montane forests of Anhui Province, eastern China, using an integrative approach combining macromorphology and micromorphology, SEM observations of basidiospores, and multilocus phylogenetic analyses based on ITS, LSU and *rpb2*. Two independent and well-supported species-level lineages were recognised and described as *Entoloma theobrunneum* and *E. ochrorubrum*. Their phylogenetic placement and diagnostic morphological characters support their recognition as distinct species within the *E. sect. Madida* framework.

The discovery of these two taxa adds new Chinese material to a lineage that remains poorly documented from subtropical eastern China and further demonstrates the value of combining detailed morphological comparisons with multilocus phylogenetic evidence when assessing collections allied to the *E. bloxamii–prunuloides* complex. SEM observations provide supplementary documentation of basidiospore architecture but were not used as primary evidence for species delimitation. However, because the present conclusions are based on only three collections from the Huangshan region of Anhui Province, their broader applicability should be interpreted cautiously. Additional collections from other regions of China, particularly of *E. ochrorubrum*, are needed to assess intraspecific variation, ecological amplitude, geographical distribution, and the stability of the inferred species boundaries.

Author Contributions: H.Z.: Investigation, Conceptualization, Data curation, Software, Visualization, Writing—original draft preparation, Writing—review and editing. S.W.: Methodology, Writing—review and editing. Y.D.: Data curation, Software. W.D.: Software. S.L.: Supervision. Q.W.: Conceptualization, Project administration, Funding acquisition, Resources, Writing—review and editing. Y.L.: Funding acquisition, Project administration. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The species registration names and gene accession numbers referenced in this study are publicly available in the following online databases: Fungal Names, <https://nmdc.cn/fungalnames/> (accessed on 5 April 2026) and NCBI GenBank, <https://www.ncbi.nlm.nih.gov/genbank/> (accessed on 13 April 2026).

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Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix B. Morphological Comparison of the Two New Species and Closely Related Taxa in *Entoloma* sect. *Madida*

Table A1. Morphological comparison of *Entoloma theobrunneum*, *E. ochrorubrum*, and their closest phylogenetic and morphologically allied taxa in *Entoloma* sect. *Madida*.

Species	Pileus		Stipe	Basidia		Basidiospores			Habitat	References	Distribution
	Colour	Size (cm)	Size (cm)	Size (µm)	Size (µm)	av.L × W (µm)	Q	Angles			
<i>E. bloxamii</i> var. <i>rubellum</i>	pink to pale pink	3–5	2–4.5 × 1–1.8	33–45 × 10–12	7.4–9.4 × 6.7–9.7	8.2 × 8.3	1.0–1.2	7–8	Calcareous meadows	[7]	Europe
<i>E. bloxamii</i>	greyish blue to greyish brown	3–5	2–4.5 × 1–1.8	33–45 × 10–12	7.4–9.4 × 6.7–9.7	8.2 × 8.3	1.0–1.2	7–10	Calcareous subalpine meadows	[7]	Europe
<i>E. corneri</i>	olive green to greyish brown	3.7–9.2	2.3–7.3 × 0.6–1.5	18–40 × 6–11	7.0–7.5 × 6.0–7.5	7.2 × 7.3	1.0–1.15	5–8	Tropical <i>Shorea</i> forests	[7]	Malaysia
<i>ochreoprunuloides</i>	dark brown to greyish brown	2–5	3–5 × 0.5–0.8	30–35 × 8–11.4	5.9–7.1 × 5.7–7.2	6.6 × 6.4	1.0–1.16	5–6	Calcareous grasslands; broad-leaved forests	[7,34]	Europe
<i>E.theobrunneum</i>	smoky brown to tea-brown	6.5–8	4.0–6.0 × 1.5–1.8	56.2–60.1 × 18.2–22.8	8.6–9.0 × 7.3–7.5	8.8 × 7.2	1.15–1.27	4–6	Mixed coniferous–broadleaf forests; bamboo forests	This study	China
<i>E.ochrorubrum</i>	iron red to ochraceous brown	7.5–8.5	4.5–5.5 × 1.5–2.0	24.6–26.8 × 11.5–12.2	6.8–7.4 × 5.3–6.5	7.1 × 6.0	1.14–1.39	5–6	Mixed coniferous–broadleaf forests	This study	China
<i>E. flavifolium</i>	olive-brown to yellowish brown	2–6	3.5–9 × 0.4–1	37–48 × 9–11	7.0–9.0 × 6.5–7.5	8.5 × 7.0	1.1–1.3	5–7	<i>Quercus</i> forests	[7]	North America
<i>E. whiteae</i>	greyish brown to beige-brown	7–20	5–15 × 1–3.5	22–40 × 7–12	7–9.6 × 6.5–8.5	8.5 × 7.4	1.1–1.4	5–6	Mixed deciduous forests	[7]	North America
<i>E. luridum</i>	ivory white to very pale brown	4–12	3–7.5 × 1–1.6	35–43 × 8.5–14	6.5–8.5 × 6.0–8.5	7.5 × 7.2	1.0–1.1	5–6	<i>Picea</i> forest margins; mixed forests	[7]	North America

Table A1. Cont.

Species	Pileus		Stipe	Basidia		Basidiospores			Habitat	References	Distribution
	Colour	Size (cm)	Size (cm)	Size (µm)	Size (µm)	av.L × W (µm)	Q	Angles			
<i>E. pseudo-prunuloides</i>	pale to medium yellowish brown	4–6	2–5 × 0.8–1.3	34–47 × 8–11	6.5–8.1 × 6.5–7.8	7.5 × 7.1	1.0–1.17	5–7	Grasslands with scattered <i>Abies</i>	[7]	Canada
<i>E. subsinuatum</i>	off-white to pale yellow	10–13	7.5–10 × 1–3	33–41 × 8.5–10.6	6–8.1 × 6.3–8	7.1 × 7.1	1.0–1.13	5–6	Mixed coniferous–broadleaf forests	[7]	North America
<i>E. madidum</i>	deep blue with a purplish tint	3–8	4–9 × 1–2	33–48 × 8.5–10.5	5.9–7.6 × 5.8–7.5	6.8 × 6.7	1.0–1.2	6–8	Mixed deciduous forests	[7]	Europe

Appendix C. Line Drawings of *Entoloma theobrunneum* and *E. ochrorubrum*

Appendix C.1. *Entoloma theobrunneum*

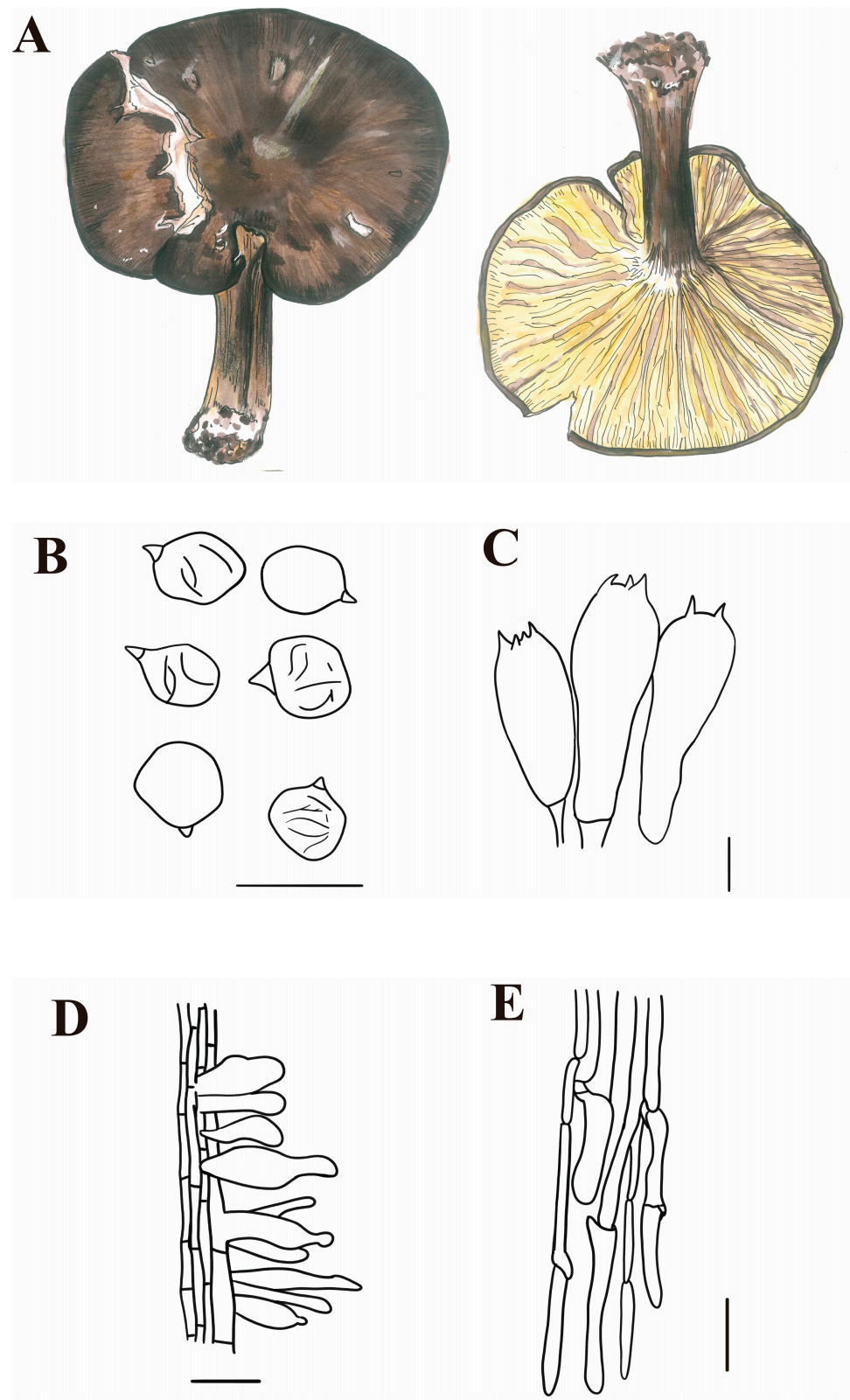


Figure A2. Line drawing of *Entoloma theobrunneum*. (A) Basidiomata; (B) Basidiospores; (C) Basidia; (D) Caulocystidia; (E) Stipitipellis. Scale bars: (A) = 2 cm; (B,C) = 10 µm; (D,E) = 20 µm.

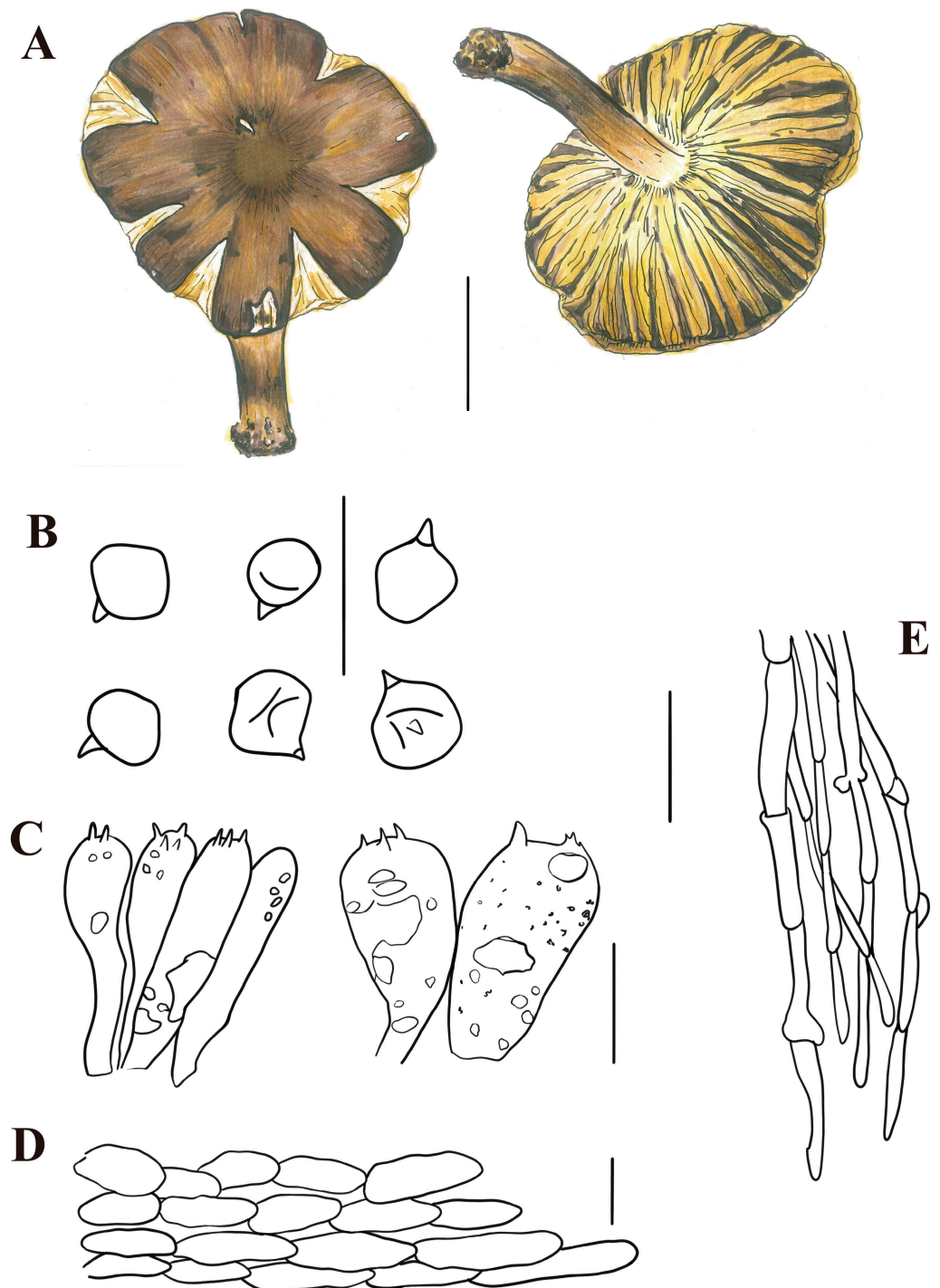
Appendix C.2. *Entoloma ochrorubrum*

Figure A3. Line drawing of *Entoloma ochrorubrum*. (A) Basidiomata; (B) Basidiospores; (C) Basidia; (D) Contextual hyphae; (E) Pileipellis. Scale bars: (A) = 2 cm; (B,C) = 10 μm; (D,E) = 20 μm.

Appendix D. Newly Generated Sequence Data

Table A2. Gap-free lengths of the newly generated ITS, LSU, and *rpb2* sequences included in the final trimmed alignments.

Taxon	Voucher	Locus	GenBank Accession	Final Edited Sequence Length (bp)
<i>Entoloma theobrunneum</i> HT	HMJAU81631	ITS	PZ236460	623
<i>Entoloma theobrunneum</i>	HMJAU81631	LSU	PZ232092	932
<i>Entoloma theobrunneum</i>	HMJAU81633	ITS	PZ236461	639
<i>Entoloma ochrorubrum</i> HT	HMJAU81632	ITS	PZ236462	648
<i>Entoloma ochrorubrum</i>	HMJAU81632	LSU	PZ232093	734
<i>Entoloma ochrorubrum</i>	HMJAU81632	<i>rpb2</i>	PZ235941	615

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