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Entoloma mengsongense sp. nov. (Entolomataceae, Agaricales), a remarkable blue mushroom from Yunnan Province, China

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Abstract: A new agaric species, *Entoloma mengsongense*, is described and illustrated from samples collected in Mengsong, Yunnan Province, China. Microscopic observations were carried out to describe the micro- and macromorphological characteristics of specimens, and molecular identification was performed to verify the novelty of the species. The species is mainly recognized by its conical to plano-concave pileus, with yellowish brown fibril and 4–8 × 4–6 µm sized, cuboid–quadrate basidiospores. Molecular phylogenetic analysis using ITS sequence data also indicated that this is a new species closely related to *E. virescens* and *E. hochstetteri*. Morphological and molecular phylogenetic differences separate *E. mengsongense* from all other known species of *Entoloma*.

Key words: Basidiomycota, fungal taxonomy, Mengsong, molecular phylogeny, new species

1. Introduction

Species of the genus *Entoloma sensu lato* (*Entolomataceae*, Agaricales, Basidiomycota) are found throughout a wide geographic range, from the arctic to the tropical regions (Largent, 1994; Manimohan et al., 1995, 2006; Noordeloos, 2004; Gates and Noordeloos, 2007; Noordeloos and Hausknecht, 2007; Co-David et al., 2009; Noordeloos and Gates, 2009; Karstedt and Capelari, 2013). More than 1500 species from this genus have been described (Morozova and Noordeloos, 2010, He et al., 2012), most of which are saprobic, whilst some are mycorrhizal (Co-David et al., 2009). *Entoloma* species grow in disparate habitats including rainforests, temperate forests, woodlands, grasslands, moors, peat-bogs, and arctic or alpine forests (Co-David et al., 2009; Gates et al., 2009; Kasuya et al., 2010; Gargano et al., 2011). The genus is characterized by pinkish spore print and polyhedral basidiospores that are angular in all views. The attachment of the lamellae varies from almost free to deeply decurrent (Noordeloos, 1980, 1981; Gates and Noordeloos, 2007, 2009; Hausknecht and Noordeloos, 2007; Noordeloos and Gates, 2009; Noordeloos and Morozova, 2010).

The initial studies on *Entoloma* in China were conducted by Teng in 1932 (Horak, 1973) and over the past two decades researchers in China have continued to find and describe new species (Zhang and Li, 2001; Li et al., 2009; Li and Li, 2009). The first *Entoloma* species to be described was *E. quadratum*. Thereafter hundreds of *Entoloma* species have been described from China (Li et al., 2009; Li and Li, 2009; He et al., 2010). With the increased taxonomic studies carried out in China on the family *Entolomataceae*, several novel *Entoloma* species were described recently (He et al., 2012). Four new species—*Entoloma azureosquamulosum*, *E. caeruleoflavum*, *E. hainanense*, and *E.* subtenuicystidiatum—were recently discovered from southern China (He et al. 2012). Nearly 33 *Entoloma* species have been described from Hainan island (Li et al., 2009). In this study, we introduce a novel species of *Entoloma* from Mengsong, Yunnan Province, with the support of morphological characteristics and nrITS sequence data.

2. Materials and methods

2.1. Sample collection and macromorphological character examination

The *Entoloma* specimens were collected during field excursions in Mengsong, Yunnan Province, between May and September 2014. The basidiomata were photographed in the field using a digital camera. Information including habitat, ecotype, and attached substrate as well as

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macromorphological characteristics such as size, shape, and structure of the pileus and stipe were recorded. The color terminology used for macromorphological identification followed Kornerup and Wanscher (1978). The specimens were dried with a portable dryer at 40 °C for 24–48 h and sealed in zip lock plastic bags containing silica gel as a desiccant to control humidity. All the herbarium specimens were deposited in the Herbarium of Kunming Institute of Botany (HKAS), Kunming, China.

2.2. Micromorphological character examination

Microscopic observations on dry specimens were carried out using a dissecting microscope. Preparation of slides was implemented with 3%–5% KOH and congo red. Observations were carried out using a compound microscope (Nikon Model Eclipse Ci–s) for microcharacteristic evaluation at 200×, 400×, and 1000× magnifications. Photos were taken with a Canon 550D digital camera, attached to the microscope. For scanning electron microscopy of the basidiospores, fragments of lamellae were mounted on aluminum stubs with double-sided adhesive tape, coated with gold palladium alloy, and then observed under a SEM (Hitachi S4800) (Cook et al., 1997).

Q (the spore quotient) was used to represent the length/width ratio of a basidiospore in side view. The length and width of at least 25 basidiospores in side view were measured to find the range of basidiospore size. Dimensions were taken excluding the hilar appendix, using a compound light microscope (Nikon Model Eclipse Ci–s) at 1000× magnification.

2.3. DNA extraction, PCR, and sequencing process

Genomic DNA was extracted from dried specimens using a Biospin Fungus Genomic DNA Extraction Kit (Bioer Technology Co., Ltd., Hangzhou, P.R. China). The nuclear ribosomal internal transcribed spacer (nrITS) and large subunit (nrLSU) regions were amplified. The amplification process was carried out in 25-µL volumes carrying 1.0 µL of template DNA, 9.5 µL of double distilled water, 1.0 µL of each primer, and 12.5 µL of 2× Power Taq PCR Master Mix (a premixed, ready to use solution that includes 0.1 Units/µL Taq DNA polymerase, 500 µm of dNTP mixture each (dATP, dCTP, dGTP, dTTP), 20 mM of Tris–HCl pH 8.3, 100 mM KCl, 3 mM of $MgCl_2$, stabilizer, and enhancer)). During the reaction, each sample underwent 35 cycles according to the following settings: denaturation (95 °C, 30 s), annealing (52 °C, 30 s), extension (72 °C, 1 min), and final extension (72 °C, 10 min). The primers used for sequencing the whole ITS region were ITS5 (forward) and ITS 4 (reverse), while for LSU LROR (forward) and LR5 (reverse) primers were used. Amplified products were confirmed with 1% agarose gel electrophoresis stained with ethidium bromide. The amplified PCR fragments were sent to a commercial sequencing provider (Beijing Bai Mai Hui Kang Biological Engineering Technology Co., P.R. China). The nucleotide sequence data were deposited in GenBank.

2.4. Sequence alignment and phylogenetic analyses

The sequences obtained in this study were checked and assembled using BioEdit 7.0.9.0 (Hall, 1999) and compared to those available in the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) using the BLAST algorithm. Taxon information and GenBank accession numbers used in the molecular work are listed in Tables 1 and 2, including all sequences of the Chinese taxa. As shown in Table 1, the ITS dataset comprised 40 sequences, including 36 *Entoloma* sequences from GenBank, including the type species of the genus, *E. sinuatum*, and the new *Entoloma* collection from Mengsong, Yunnan province, China. As shown in Table 2, the LSU dataset comprised 60 sequences, including 59 *Entoloma* sequences from GenBank, and the new *Entoloma* collection from Mengsong, Yunnan province, China. *Lyophyllum decastes* and *Tricholoma vaccinum* were chosen as the outgroup taxa for both ITS and LSU phylogenetic trees. The ITS and the LSU sequence data were analyzed using maximum parsimony (MP), maximum likelihood (ML), and Bayesian analyses. MP analyses were performed in PAUP v. 4.0b10 (Swofford, 2002) using the heuristic search option with 1000 random taxa addition and tree bisection and reconnection (TBR) as the branch-swapping algorithm. The reconstruction of ML analysis was performed using raxmlGUI v.0.9b2 with the model GTRGAMMA. A Bayesian analysis was conducted with MrBayes v. 3.1.2 (GTR+I+G model) to valuate posterior probabilities (PP) by Markov chain Monte Carlo sampling (BMCMC). Sequences for each strain were aligned using Clustal X (Thompson et al., 1997). Ambiguously aligned regions were excluded from all analyses. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Maxtrees were unlimited, branches of zero length were collapsed, and all multiple parsimonious trees were saved. Clade stability of the trees resulting from the parsimony analyses was assessed by bootstrap analysis with 5000 replicates, each with 100 replicates of random stepwise addition of taxa (Felsenstein, 1985). Trees were displayed with TreeView ver. 1.6.6 (Page, 1996).

3. Results

3.1. Phylogenetic analyses

Trees of similar topologies were generated in MP, ML, and Bayesian analyses and the ML tree was selected to present the results. Our phylogenetic results show that the new species is supported by high bootstrap values in ITS phylogeny: 94% in ML, 90 in MP, and 0.99 in PP (Figure 1), and LSU phylogeny: 96% in ML, 91 in MP, and 0.99 in PP (Figure 2). It is closely related to two blue *Entoloma*

Table 1. Taxa information and GenBank accession numbers of ITS sequences of *Entoloma* specimens used in the molecular phylogenetic analyses.

Name of the species	Voucher	Locality	GenBank accession number (ITS, 5.8S)
Entoloma azureosquamulosum	HKAS53408	China	JQ410334
E. stylophorum	GDGM25736	China	JQ281480
E. subtenuicystidiatum	GDGM 29246	China	JQ320114
E. praegracile	GDGM 29256	China	JQ320107
E. caespitosum	GDGM24025	China	JQ281490
E. mastoideum	GDGM28820	China	JQ281476
E. coelestinum	HMLD1659	China	KC257434
E. holmvassdalenense (Type)	O–F75311	Norway	KM610321
E. caeruleopolitum	RBG Kew K M 102319	England	EU784210
E. chalybaeum var. lazulinum	RBG Kew K M 90810	England	EU784215
E. insidiosum	L376	Russia	KC898443
E. virescens	–	Japan	AB509863
E.hochstetteri	TL2573	New Zealand	KP191941
E. mengsongense (holotype)	HKAS90774	China	KU131556
E. petchii	HKAS56716	China	JQ281485
E.omiense	GDGM27563	China	JQ281487
E.inocephalum	LE262922	Vietnam	KC898449
E.griseocyaneum	LE254351	Russia	KC898444
E. turci	3882	USA	JF907993
E. incanum	HKAS54614	China	JQ281488
E. asprellum	RBG Kew K M 91347	England	EU784206
E. anatinum	RBG Kew K M 58618	England	EU784202
E. mougeotii	LE254352	Russia	KC898446
E. serrulatum	HKAS 52713	Iran	KT833862
Entoloma sp.	HKAS 52713	China	JQ410336
E. aff. kujuense	–	Japan	AB509866
E. nitidum	–	Canada	AY228340
E. bloxamii	–	Canada	EF530938
E. flavidum	GDGM24473	China	JQ281481
E. chalybaeum	LE254353	Russia	KC898445
E. abortivum	GDGM27313	China	JQ291565
E. shandongense	CUH AM109	India	KP241852
E. conferendum	HKAS48953	China	JQ281484
E. lampropus	LE9121	Russia	KC898378
E. subaraneosum	GDGM 28823	China	JQ320113
E. pallidocarpum	GDGM 28828	China	JQ320106
E. sinuatum	AFTOL–ID 524	USA	DQ486700
Lyophyllum decastes	Lc42 T5P	Switzerland	AF357060
L. decastes	901016	Sweden	HM572546
Tricholoma vaccinum	CBS55550	Germany	AF062628

Table 2. Taxa information and GenBank accession numbers of LSU sequences of *Entoloma* specimens used in the molecular phylogenetic analyses.

Name of the species	Voucher	Locality	GenBank accession number (LSU)
Entoloma hochstetteri	TL2570	New Zealand	KP191755
E. hochstetteri	TL2573	New Zealand	KP191758
E. hochstetteri	TL2572	New Zealand	KP191757
Entoloma sp.	LAM 0258	Malaysia	KY091023
E. virescens	Li 929	China	JQ993098
E. hochstetteri	TL2571	New Zealand	KP191756
Entoloma sp.	GDGM 26298	China	JQ993091
E. luteum	GDGM 27698	China	JQ320121
Entoloma sp.	GDGM 57161	China	KJ845724
E. murrayi	QI 1001	China	JQ993090
E. tectonicola	-	Netherlands	GQ289196
E. petchii	GDGM 27696	China	JX992853
E. petchii	HKAS 56716	China	JQ320120
E. flavovelutinum	LE_RUS_302078	Russia	KR052824
E. flavidum	GDGM 24473	China	JQ320122
E. flavovelutinum	LE_RUS_302075	Russia	KR052823
E. murrayi	QI 1002	China	JQ993089
E. tenuissimum	GDGM 28813	China	JQ993097
E. violaceovillosum	-	Netherlands	GQ289205
E. serrulatum	-	Netherlands	GQ289192
E. quadratum	EQ7695	-	AF261303
E. subaraneosum	KA12_1534	South Korea	KJ523137
E. albidoquadratum	-	-	GQ289151
E. porphyrescens	-	-	GQ289182
E. araneosum	-	-	GQ289153
E. luridum	-	-	KC710146
Entoloma sp.	-	Germany	KC261492
E. madidum	MEN 2004030	-	KC710158
E. transmutans	-	-	GQ289200
E. aff. luteum	GDGM 28991	China	JQ993093
E. bloxamii	TB6117	-	AF261289
E. gasteromycetoides	E2031	-	GQ289164
E. jubatum	-	Germany	KP965790
E. peralbidum	TLe1518	New Zealand	KP191742
E. peralbidum	TLe1520	New Zealand	KP191743
Entoloma sp.	2AK_2012 isolate_K479	-	AB692007
E. lividoalbum	MEN 200328	-	KC710152
E. changchunense	HMJAU 3886	China	JQ993095
E. procerum	-	-	GQ289183
E. cocles	-	-	GQ289159
Entoloma sp.	EM677	-	AB692015
E. hainanense	GDGM 27990	China	JQ320118
E. vinaceum	TB8870	-	GU384631
E. turbidum	-	-	GQ289201
E. costatum	-	-	GQ289161
E. manganaense	-	E369	KC710143
E. tenuissimum	GDGM 28814	China	JQ993096
E. tenuissimum	GDGM 28814	China	NG042670
E. subaraneosum	GDGM 28823	China	JQ410329
E. subaraneosum	GDGM 28823	China	JQ291568
E. subaraneosum	GDGM 28823	China	NG042606
E. omiense	GDGM 27563	China	JQ410330
E. omiense	GDGM 27229	China	JQ320124
E. furfuraceum	GDGM 28818	China	JQ993094
E. furfuraceum	GDGM 28818	China	NG042669
E. hypogaeum	K382	-	AB692009
E. hypogaeum	TNS F46869	-	NG042336
Lyophyllum decastes	GLM 45952	-	DQ071789
Tricholoma vaccinum	GLM 46037	Germany	DQ071796

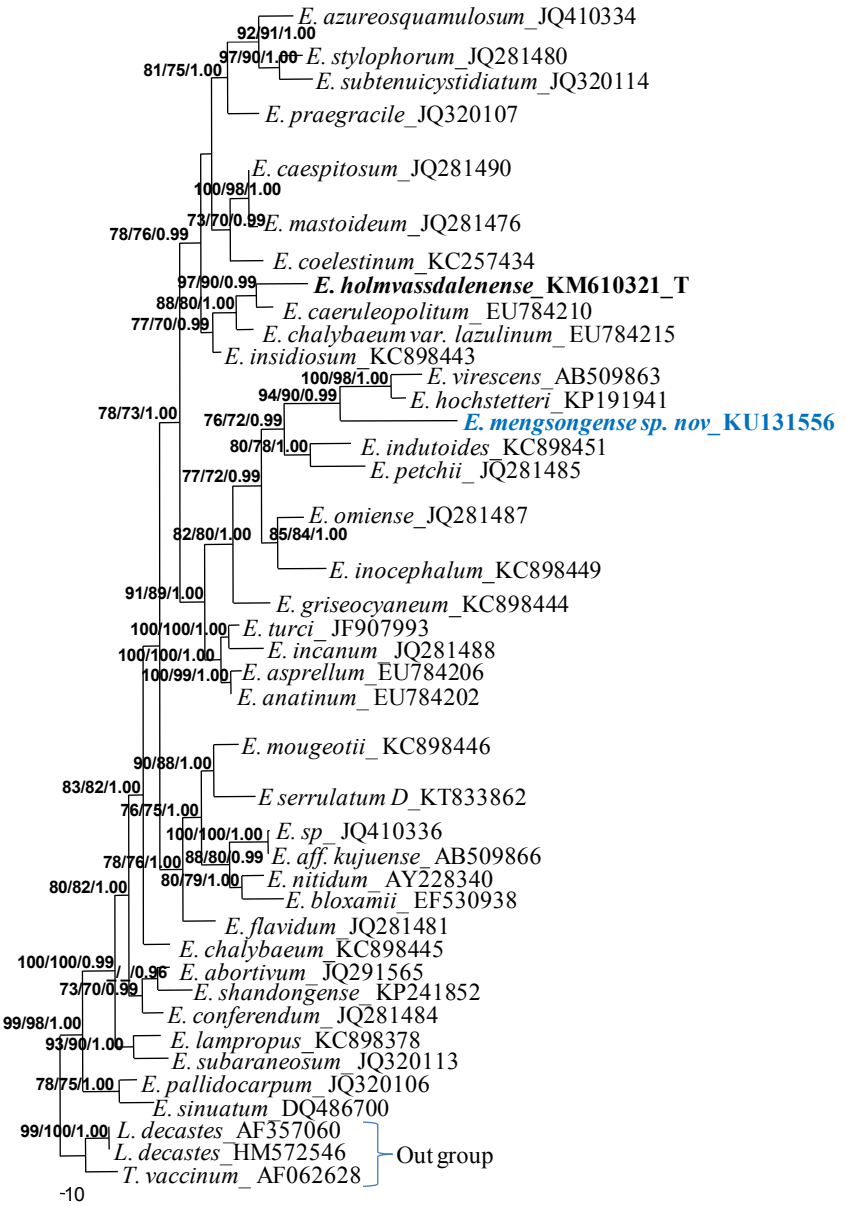

Figure 1. Phylogenetic tree obtained from RAxML analyses showing the phylogenetic position of *Entoloma mengsongense* based on the ITS sequence data. Bootstrap support values for maximum parsimony (MP, left), maximum likelihood (ML, middle) higher than 70%, and Bayesian posterior probabilities (BYPP, right) greater than 0.95 are provided at the nodes. The tree is rooted with *Lyophyllum decastes* and *Tricholoma vaccinum*. The new species is in blue bold and the type species are in black bold.

species, *E. virescens* and *E. hochstetteri*, but is distinct in having unique morphological characteristics (Table 3).

3.2. Taxonomy

Entoloma mengsongense A.N. Ediriweera, Karun., J.C. Xu, K.D. Hyde and P.E. Mortimer, sp. nov. **Figures 3–5**

MycoBank: MB 815072

Faces of fungi number: FoF 02080

Type: CHINA. Yunnan Province: Xishuangbanna, Mengsong Village, UTM N2379744.485281, UTM E653217. 753714, alt. 1600 m, on soil associated with mosses, 12 June 2012, Samantha C. Karunarathna (HKAS 90774, **holotype**).

Diagnosis: this species is characterized by a fibrillose, sky blue to greenish blue pileus; cuboid–quadrate, 4–8 × 4–6 µm sized basidiospores; clavate, 40–60 × 9–14 µm sized basidia and hyaline, clavate, 35–40 × 9–12 µm sized cheilocystidia.

Pileus 3.5–5 cm diam., conical when young, plano-concave when mature, with an umbo; surface dry, fibrillose all over the surface, more so at the center, yellowish brown

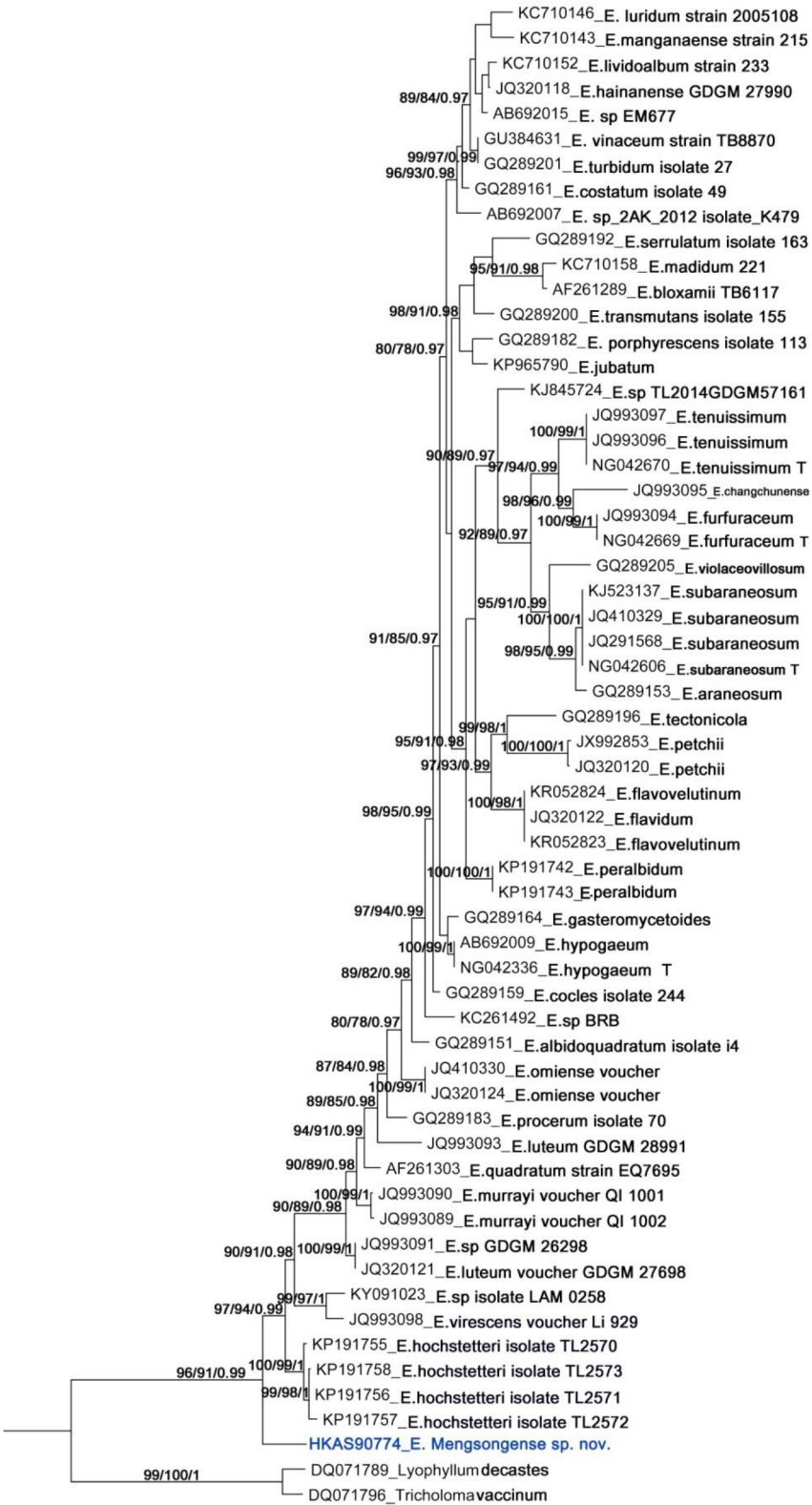

Figure 2. Phylogenetic tree obtained from RAxML analysis showing the phylogenetic position of *Entoloma mengsongense* based on the LSU sequence data. Bootstrap support values for maximum parsimony (MP, left), maximum likelihood (ML, middle) higher than 70%, and Bayesian posterior probabilities (BYPP, right) greater than 0.95 are provided at the nodes. The tree is rooted with *Lyophyllum decastes* and *Tricholoma vaccinum*. The new species is in blue bold and the type species are noted with a T after the species name.

Table 3. Comparison of *Entoloma mengsongense* with other morphologically and phylogenetically closely related *Entoloma* species.

Species	Basidiomata		Basidia	Basidiospores	Cheilocystidia
	Pileus	Stipe			
E. subaltissimum T.H. Li and C.H. Li	20–35 mm broad, conical to hemispherical, papillate or not when young and becomes convex with age, deep blue and sometimes pale blue near margin.	50–80 mm long. 2–3 mm thick at apex and slightly enlarged downwards, cylindrical and hollow, usually pale blue to light blue at apex and near base, glabrous.	27–50 × 10–15 µm and clavate. 2–4-spored, with sterigmata 2.5–5 µm long and clamped at base.	8–12.5 × 8–12 µm and subquadrate to quadrate, cuboid under scanning electron microscope with obvious apiculus, smooth and pinkish	50–76 × 15–29 µm, broadly clavate
E. virescens (Sacc.) E. Horak ex. Courtec.	10–35 mm broad and about 15 mm high, conical to acutely conical, often with slender, acute papilla, pileus surface radially wrinkled and often splitting from margin with age, deeply depressed at center.	45–70 × 1.5–3 mm and cylindrical, pastel blue, striate with loose, concolorous fibrils, fistulose.	30–52 × 10–17 µm. 4–spored and clamped, granular and brownish intracellular pigment present.	10.5–12.5 × 10.5–12 µm and cuboid	30–62 × 12–16 µm, cylindro-clavate
E. azureoviride E.Horak & Singer	24–38 mm diam., conical or conico-convex, with papilla, sometimes deep blue, surface completely covered with ocher-green or olive-brown fibrils.	49–96 × 3–9 mm, cylindrical, straight or with a bulbous base, deep blue.	40–54 × 10–12.5 µm, clavate, colorless, mostly with olive-yellow oil drops, thin-walled, tetrasporic.	7.5–10 × 7.5–10 µm, cuboid, with an evident apiculus, slightly pinkish yellow-brown, thin walled.	28–64 × 6.2–12.5 µm, clavate or rarely narrowly clavate, colorless or with yellow-brown intracellular pigment, sometimes with yellow-brown or olive oil drops, thin-walled.
E. altissimum (Massee) E. Horak	22 mm broad, conical to campanulate, light blue to pastel blue.	4–5 mm long, cylindrical, slightly broader towards the base, fistulose and longitudinally fibrillose, pastel blue.	40.2–54 × 11.3–14.1 µm and clavate. 4-spored and clamped.	6.5–9.1 × 6.4–8.3 µm, cuboid to subcuboid	56.5–100.9 × 9.9–13.6 µm, narrowly clavate
E. hochstetteri (Reich.) Stev.	40 mm broad and conical, indigo–blue with a green tint, fibrillose, the pileus margin is striate and rolled inwards	Stipe 50–100 × 3–5 mm, cylindrical and equal or attenuated upwards, concolorous with pileus, fading to brownish, whitish at the base, dry, fistulose and fragile	35.2–44.2 × 8.8–13.2 µm and club-shaped, hyaline, and has two or four sterigmata.	9.9–13.2 × 11.8–13.2 µm and tetrahedral, hyaline, smooth and thin-walled	40–60 ×8–14 µm, fusoid, with long tapering and apically rounded neck.
E. nitidum Quél.	20–40 mm broad and convex. Flattening, becoming broadly umbonate. Silky fibrillose surface; dark blue or grayish–blue.	30 – 60 mm long and 1–2 mm thick. Smooth, grayish–blue, white and felty at base. Longitudinally fibrillose and no stem ring	45–60 ×10–14 µm and club-shaped	7–9 × 6–8 µm. 5–8 angled in side view, thin walled.	Present
E. serrulatum (Fr.) Hesler	10–30 mm broad and convex. Radially fibrillose–silky or finely scaly. Black to bluish black, fading to gray.	20–40 mm long and 2–3 mm thick. Equal and silky at the apex and bald below. Hollow and black to bluish black.	38–45 × 9.5–12 µm	Spores 9–13 × 6.5–8 µm. 5–6 angled	40–65 × 10–13 µm. Cylindrical with subclavate, clavate, or subcapitate apices
E. bloxamii (Berk. & Broome) Sacc.	30–50 mm broad. Conical then conico- convex. Pale grayish blue.	20–45 mm long 2–3 mm thick. Clavate with broadest part in lower half. Almost white, with faint blue.	48–70 × 11–14.5 µm and clavate to oblong, clamped, 2–4-spored	7.4–9.4 × 6.7–9.7 µm. 7–10 angled in side view.	Absent
E. moongum Grgurinovic	19 mm broad with a surface that is finely fibrillose, and dark brown to purplish.	Stem 3.5 cm and equal.	34.4–48.8 × 9.6–12.8 µm and club-shaped.	9.6–13.6 × 5.6– 8.4 µm. 5–6 blunt angles.	Present
E. panniculus (Berkeley) Saccardo	20–30 mm broad. Conical to conico-convex. Uniformly deep blue.	40–70 × 2–4 mm. cylindrical, broadened at base or apex, deep blue.	20–40 × 6–9 µm, 4-spored, clamped.	9–12 × 6–8 µm, Q = 1.2–1.7 and 5–7 angled heterodiametric with pronounced angles. Irregular.	Absent
E. discrepans Noordeloos and G.M. Gates	10–40 mm broad. Hemispherical to convex and old specimens are dark blackish blue.	30–50 × 2–5 mm. Cylindrical or compressed with groove. Dark blue.	20–34 × 7–10 µm, 4-spored, clampless	8–10 × 6–7 µm, Q = 1.3–1.5, heterodiametric, 5–7 angled in side view with pronounced angles.	Absent
E. hymenidermum D.L.Largent	29–70 mm broad. Opaque. At first convex to broadly convex, sometimes campanulate– convex	27–79 × 4–10.5 mm. Broad at the apex, equal to clavate. Blackish blue, quickly becoming hollow with maturity.	38.9–48.9 × 9.7–14.5 µm .Narrowly clavate and tapered, relatively long and narrow.	6.8–10.0 × 6.2–8.8 µm, distinctly 5 to 6 angular, isodiametric to subisodiametric in profile and dorsiventral views. Q = 1.12 ± 0.07	Absent
E. shandongense T. Bau & J.R. Wang	13–22 mm broad. Convex with a slightly central depression when young. Surface entirely blue with somewhat purple tint.	23–28 × 3–7 mm. equal from the apex, become hollow with maturity	29–32 × 9–10 µm, 4–spored clavate to subclavate, thin-walled.	7.5 –8.4 × 5.4–6.0 µm. 5–8 angles in profile and hyaline, thick-walled.	Absent
E. mougeotii (Fr.) Hesler	10–20 mm broad. Planoconvex, becoming shallowly depressed, with or without a small umbo. Dark purple–gray.	25–50 × 2–4 mm and equal. Dry. Bald or finely silky. White at the apex.	27–30 × 8–10 µm, 4-spored, subclavate	9–11 × 5–6 µm, 5–9 shallow angles. Heterodiametric, smooth and hyaline	35–65 × 5–7.5 µm. Thin–walled and cylindrical–flexuous with rounded, subclavate, clavate, or subcapitate apices. Clearly differentiated
E. mengsongense	35–50 mm diameter. Conical when young, plano-convex when mature, umbo, surface dry. Yellowish brown fibrillose surface.	65 –90 mm × 6 –7 mm. Confluent with pileus central, brittle, cylindrical solid. Sky blue when mature with cottony white mycelium at base.	40–60 × 9–14 µm, mostly clavate, sometimes obclavate, 4-spored, occasionally 1-, 2-, or 3-spored.	4–8 × 4–6 µm, cuboid quadrate having 4 angles. Regular to irregular in profile. Have stramineous wall that is slightly thickened.	35–40 × 9–12 µm, clavate, thin walled, hyaline.

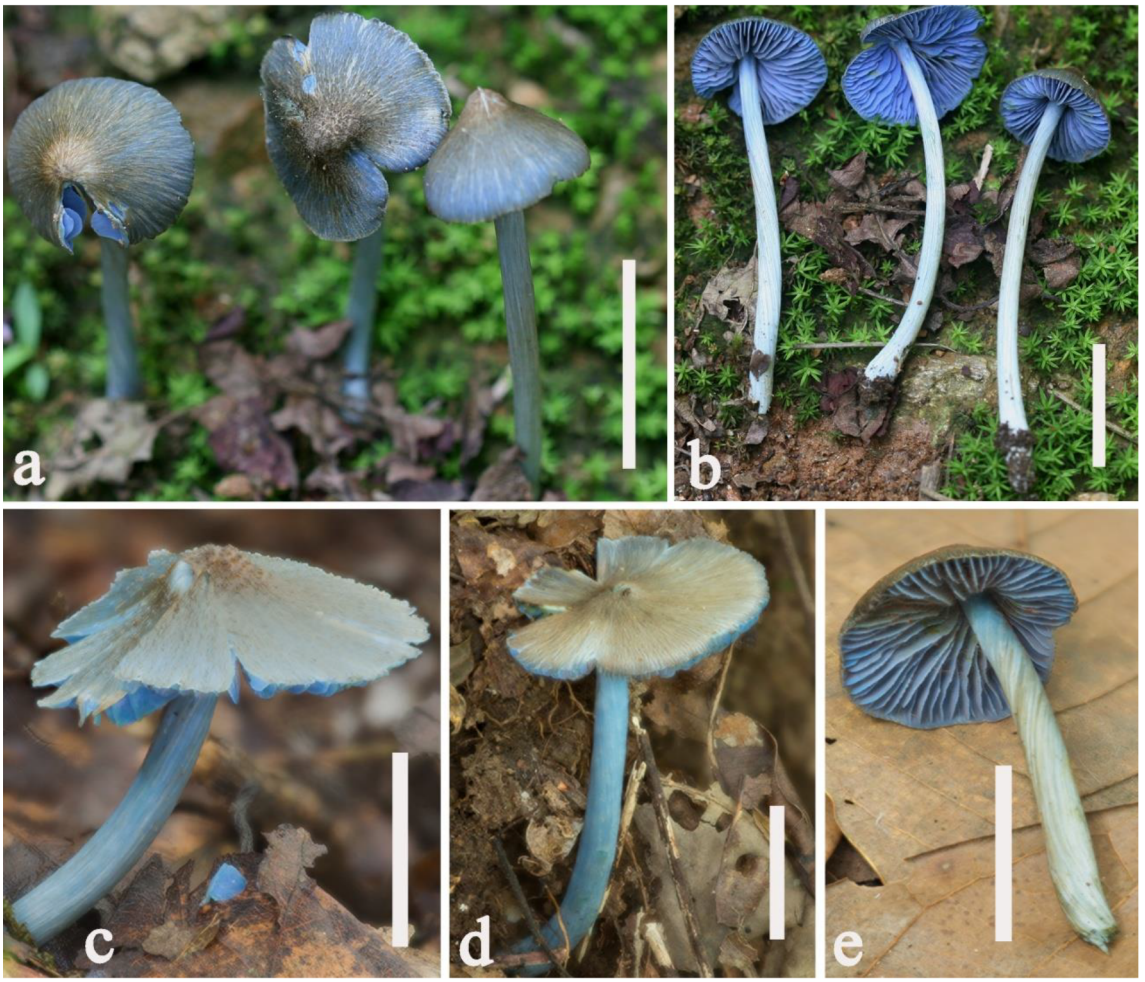

Figure 3. a–e Basidiomata of *E. mengsongense* in the field: a) HKAS 90774; b) HKAS 90774; c, d) HKAS 90775; and e) HKAS 90775. Scale bars: a–e = 5 cm.

(5E8); surface yellowish–brown (5E8) because of the fibrillose pileipellis, below the pileipellis sky blue becoming greenish blue (25E7) when drying or mature, and staining yellow–blue greenish (25A4) on bruising or cut. **Lamellae** adnexed, moderately crowded, with lamellulae of four lengths, ventricose, sky blue, turning yellowish green or greenish blue (25A4) on handling; margin smooth. **Stipe** 6.5–9 cm × 6–7 mm, confluent with the pileus, central, brittle, cylindrical, solid; surface, fibrillose as the pileus when young; concolorous with the pileus when young, becoming sky blue (21A6) when mature, with cottony white mycelium at the base. **Context** white and thin, turns yellowish green or greenish blue on bruising or drying. **Odor** peculiar and distinct. **Taste** was not tested.

Basidiospores 4–8 × 4–6 µm, μ_l (mean length of basidiospores) = 5.33 µm, μ_w (mean breadth of basidiospores) = 5.14 µm, σ_l (standard deviation of length) = 1.45, σ_w (standard deviation of breadth) = 0.6, Q = 0.68–1.49, Q_m = 1.01, cuboid–quadrate, having four angles that are regular to irregular in profile or side views, with a stramineous wall that is slightly thickened (Figure 5). Basidia 40–60 × 9–14 µm, mostly clavate, sometimes obclavate, 4-spored, occasionally 1-, 2-, or 3-spored; sterigmata up to 5 µm long. Cheilocystidia 35–40 × 9–12 µm, clavate, thin walled, hyaline. Hymenophoral trama subregular; hyphae 3–15 µm wide, hyaline and thin walled; subhymenium poorly developed. Pileal trama parallel–interwoven; hyphae similar to those of lamellar trama. Pileipellis a cutis, hyphae 2–10 µm diam., thin walled, pale yellowish. Stipitipellis a disrupted cutis; hyphae 1–8 µm broad, thin walled, hyaline, forming ascending or erect bundles of mostly clavate or occasionally cylindrical, 7–8.5 µm broad hyphal ends at the tip. Clamp connections present in all tissues.

3.3. Habitat/distribution

Gregarious on soil as small groups, so far only known from the type locality in China.

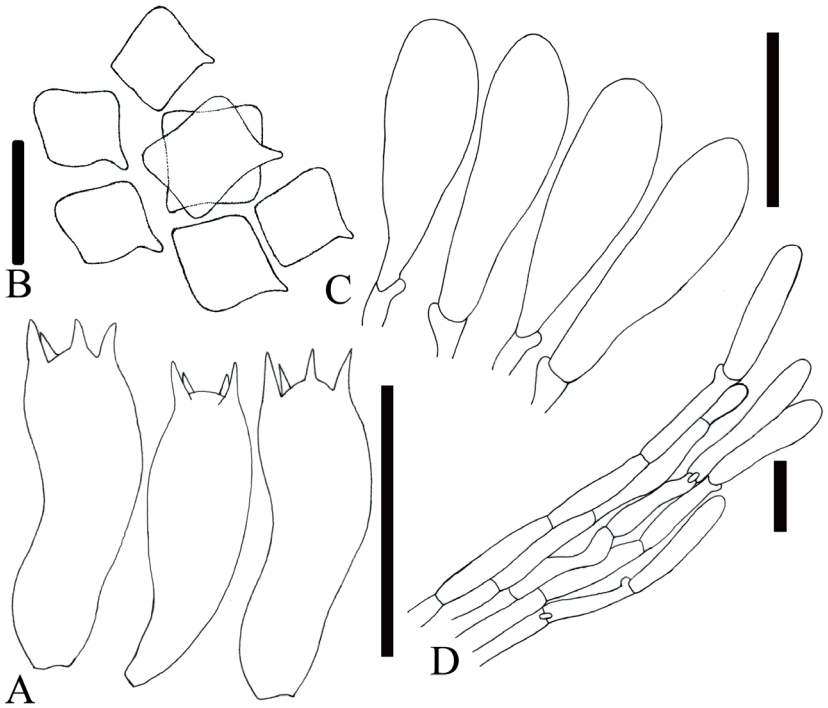

Figure 4. *Entoloma mengsongense* (from holotype HKAS 90774); A) Basidia; b) Basidiospores; c) Cheilocystidia; and d) Pileipellis. Scale bars: A = 50 μm; B = 10 μm; C, D = 20 μm.

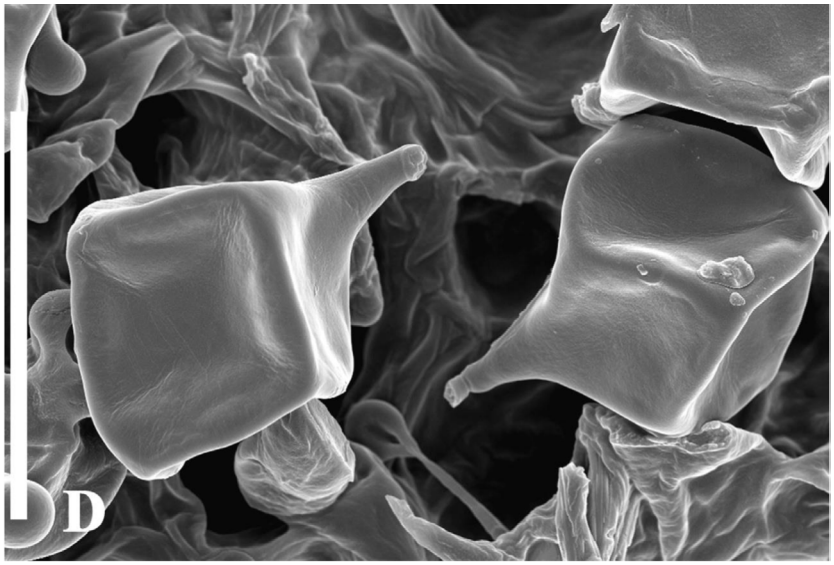

Figure 5. Scanning electron micrograph of basidiospores of *Entoloma mengsongense* (from holotype HKAS 90774); Scale bars: = 10 μm

3.4. Etymology

The species epithet "*mengsongense*" refers to the place where the type specimen was collected.

3.5. Other specimens examined

CHINA. Yunnan Province: Xishuangbanna, Mengsong Village, UTM N2379744.485281, UTM E653217. 753714, alt. 1600 m, on soil with decaying leaf litter, 15 July 2012, Samantha C. Karunarathna (HKAS 90775, **paratype**)

4. Discussion

Our new species is phylogenetically placed with maximum support in ITS phylogeny (94% in ML, 90 in MP, 0.99 in

PP in Figure 1) as well as LSU phylogeny (96% in ML, 91 in MP, 0.99 in PP in Figure 2) in a clade composed of two blue *Entoloma* species: *E. virescens* (Sacc.) E. Horak and *E. hochstetteri* (Reichardt) G. Stev. (Figures 1 and 2). Although *E. mengsongense* is phylogenetically closely related to *E. virescens* and *E. hochstetteri*, it is morphologically distinct. *Entoloma virescens* differs from *E. mengsongense* in having a wavy margin to the pileus, larger (10.5–12.5 × 10.5–12 µm) cuboid basidiospores, 30–52 × 10–17 µm size basidia, 30–62 × 12–16 µm size cylindro-clavate cheilocystidia (Alves and do Nascimento, 2012). *Entoloma hochstetteri* differs from our new species in having an indigo–blue pileus, 9.9–13.2 × 11.8–13.2 µm size basidiospores, 35–44 × 9–13 µm size basidia; and 40–60 × 8–14 µm, fusoid cheilocystidia. The cuboid basidiospores, long basidia, conic-campanulate pileus with appressed fibrils, and abundant clamp connections place our new species *E. mengsongense* in *Entoloma* subgenus *Inocephalus* (Noordel.) P.D. Orton. All the morphologically similar blue *Entoloma* species are compared with *Entoloma mengsongense* in Table 3.

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References

Alves MH, do Nascimento CC (2012). *Entoloma virescens* (Sacc.) E. Horak ex Courtec., 1986 (Agaricales: Entolomataceae): the first record for the Caatinga biome, Ceará, Brazil. Check List 8: 577-580.

Baroni T, Matheny PB (2011). A re-evaluation of Gasteroid and Cyphelloid species of Entolomataceae from Eastern North America. Harv Pap Bot 16: 293-310.

Burdsall HH, Czederpiltz DLL, Volk TJ (2001). Field observations and inoculation experiments to determine the nature of the carpophoroids associated with *Entoloma abortivum* and *Armillaria*. Mycologia 93: 841-851.

Co-David D, Langeveld D, Noordeloos ME (2009). Molecular phylogeny and spore evolution of Entolomataceae. Persoonia 23: 147-176.

Cook R, Inman A, Billings C (1997). Identification and classification of powdery mildew anamorphs using light and scanning electron microscopy and host range data. Mycol Res 101: 975-1002.

Felsenstein J (1985). Phylogenies and the comparative method. Am Nat 125: 1-15.

Gargano ML, Mandracchia G, Saitta A Venturella G (2011). Two rare Northern *Entoloma* species observed in Sicily under exceptionally cold weather conditions. The Scientific World 2012: 95722.

Gates GM, Noordeloos M (2007). Preliminary studies in the genus *Entoloma* in Tasmania-I. Persoonia 19: 157-226.

Gates GM, Hortan BM, Noordeloos M (2009). A new *Entoloma* (Basidiomycetes, Agaricales) from Tasmania. Mycotaxon 107: 175-179.

Gates GM, Noordeloos M (2009). Preliminary studies in the genus *Entoloma* in Tasmania-II. Cryptogamie Mycol 30: 107-140.

Hall TA (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, Nucleic Acids Symp Ser 41: 95-98.

He X, Jiang Z, Li T (2010). Three species of white *Entoloma* new to China. Mycosystema 29: 920-923.

He XL, Jiang ZD, Li TH, Shen YH (2012). Four new species of *Entoloma* s.l. (Agaricales) from southern China. Mycol Prog 11: 915-925.

Horak E (1973). On cuboid-spored species of Entoloma (Agaricales). Sydowia 28: 171-236.

Karstedt F, Capelari M (2013). *Inocephalus* (Entolomataceae, Agaricales) from São Paulo State, Brazil. Nova Hedwigia 96: 279-308.

Kasuya T, Takehashi S, Hoshino T, Noordeloos ME (2010). *Entoloma aprile* (Agaricales, Entolomataceae) new to Japan, with notes on its mycorrhiza associated with *Populus maximowiczii* in cool-temperate deciduous forests of Hokkaido. Sydowia 62: 205-223.

Kornerup A, Wanscher JH (1978). Methuen Handbook of Colour, London, UK: Methuen.

Largent DL (1994). Entolomatoid Fungi of the Pacific Northwest and Alaska. Eureka, CA, USA: Mad River Press.

Li CH, Li TH (2009). A new Entoloma species (Entolomataceae, Agaricales) from Hainan Island. Mycosystema 28: 641-643.

Manimohan P, Joseph AV, Leelavathy KM (1995). The genus *Entoloma* in Kerala State, India. Mycol Res 99: 1083-1097.

Manimohan P, Noordeloos ME, Dhanya, AM (2006). Studies on the genus *Entoloma* (Basidiomycetes, Agaricales) in Kerala state, India. Persoonia 19: 45-93.

Noordeloos ME (1980). *Entoloma* subgenus *Nolanea* in the Netherlands and adjacent regions with a reconnaissance of its remaining taxa in Europe. Persoonia 10: 427-534.

Noordeloos ME (1981). Introduction to the taxonomy of the genus *Entoloma* sensu lato (*Agaricales*). Persoonia 11: 121-151.

Noordeloos ME (2004). *Entoloma* s.l. Fungi Europaei, vol. 5a. Edizione Candusso, Italy.

Noordeloos ME, Hausknecht A (2007). The genus *Entoloma (Basidiomycetes, Agaricales)* of the Mascarenes and Seychelles. Fungal Divers 27: 111-144.

Noordeloos ME, Morozova OV (2010). New and noteworthy *Entoloma* species from the Primorsky Territory, Russian Far East. Mycotaxon 112: 231-255.

Page RDM (1996) Tree View: an application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences 12: 357-358.

Swofford DL (2002) PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0b10, Sinauer Associates, Sunderland.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25: 4876-4882.

Zhang W, Li TH (2001). A new species of *Entoloma* from Nanling National nature reserve. Mycosystema 21: 483-484.

Zhang W, Li TH (2001). A new subgenus and a new species of *Entoloma*. Mycosystema 21: 153-155.