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Two new tricholomatoid dark-blue Entoloma species and epitypification of E. kansaiense

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Abstract

Tsuguo Hongo described numerous Japanese macrofungi, but many of his taxa remain poorly understood because usable DNA is often difficult to obtain from original material and information on fresh basidiomata is sometimes limited to monochrome line drawings. During ongoing surveys in the Kansai region, we investigated dark blue, tricholomatoid species of *Entoloma* and re-examined the rare *E. kansaiense*, originally described by Hongo from Otsu City, Shiga Prefecture. Morphological observations and maximum-likelihood phylogenetic analyses based on concatenated ITS, LSU, and *rpb2* sequences revealed three distinct taxonomic entities. Two species are described as new: *E. cyanovelutinum*, characterized by a smooth to subvelutinous dark navy-blue pileus and a compact palisadoderm pileipellis dominated by blue to indigo-blue hyphae; and *E. olidoatrocyaneum*, characterized by dull dark-blue basidiomata, an unpleasant beetle-like odor, and a bilayered pileipellis with a brown plagiotrichoderm suprapellis and bluish subpellis. Both species were placed within the Prunuloides clade, with *E. cyanovelutinum* nested in the Calliderma clade. Multiple collections of *E. kansaiense* revealed substantial variation in basidioma coloration and basidiospore dimensions. Its ITS sequence was identical to that of the holotype of the Chinese *E. caeruleoflavum*, and the LSU sequences differed by only one nucleotide. The morphological differences between the two taxa also fell within the observed variation of *E. kansaiense*. We therefore designate an epitype for *E. kansaiense* and treat *E. caeruleoflavum* as its synonym.

Introduction

Tsuguo Hongo (1923–2007) was one of the pioneers who established the foundation of mycology in Japan, having described numerous macrofungal species, mainly from the Kansai region, from the 1950s to the 2000s (Yokoyama 2007). Many of the species he described were subjected to fumigation for pest control prior to deposition in museums, resulting in extensive DNA fragmentation and hindering the recovery of sequence data (Sakuma 2019). Consequently, a considerable number of Hongo's taxa lack molecular data. In addition, many of Hongo's protologues were accompanied only by monochrome line drawings, providing limited information on the coloration of fresh basidiomata. Although illustrated field guides including color plates were later published (e.g., Imazeki and Hongo 1987; Imazeki and Hongo 1989), these are now out of print and not readily accessible. Due to these limitations in both morphological and molecular information, some of Hongo's taxa are not always adequately recognized or correctly interpreted in subsequent taxonomic studies, especially by researchers outside Japan. As a result, morphologically similar taxa may be described as new species without sufficient comparison with previously described taxa, potentially leading to an increase in synonyms. To stabilize the application of these names, epitypification based on recently collected, well-preserved material from near the type localities of Hongo's taxa, when appropriate, can be valuable when combined with detailed morphological observations and DNA sequence data.

The area around Otsu City, Shiga Prefecture, where Hongo conducted extensive fieldwork, includes numerous type localities of his taxa (e.g., *Hygrophorus otsuensis* Hongo, *Tylopilus otsuensis* Hongo, and

Russula omiensis Hongo). He also provided long-term taxonomic guidance and identification support to the Kansai Mycological Club (KMC), an amateur-based mycological society mainly in the Kansai region, which has played an important role in bridging professional researchers and citizen scientists (Kansai Mycological Club 2009). At present, continuous field surveys led by KMC members are conducted, including regular macrofungal surveys in the “Ryukoku-no-mori” area in Otsu City, Shiga Pref. (Tsuchiya et al. 2017) and at the Kamigamo Experimental Station in Kyoto City, Japan. These efforts have resulted in the discovery of previously undescribed taxa [e.g., *Russula ryukokuensis* (Shimono et al. 2021)]. Nevertheless, some of Hongo’s taxa are rare or difficult to rediscover, highlighting the importance of ongoing field investigations.

The first author has participated in these surveys with the aim of rediscovering *Entoloma cyanonigrum*, which was originally described by Hongo based on specimens from Otsu City. Although the species has not yet been rediscovered, several morphologically similar taxa have been identified. These taxa, including *E. quasicyanonigrum*, have been described as new taxa (Ohkubo et al. 2025). In the present study, two additional undescribed species of *Entoloma* with tricholomatoid basidiomata and dark blue to bluish black pilei were discovered and are described herein as new species. Furthermore, multiple specimens of the rare species *E. kansaiense*, previously lacking molecular data, were obtained, allowing the designation of an epitype. We herein report detailed morphological observations and molecular phylogenetic analyses of these taxa.

Materials and methods

Morphological analyses

Anatomical observations and measurements of newly collected materials were based on fresh material mounted in water, except as noted below. The dimensions of basidia and hyphal elements of the suprapellis of pileipellis were measured based on fresh material mounted in 3% (w/v) potassium hydroxide solution (KOH). For the dried specimen (OSA-MY-16519) of *E. kansaiense*, basidiospores were measured in 3% KOH and other tissues were measured in water. Congo red (CR) was also used to stain hyaline structures in 3% KOH (KOH/CR). Terminology and microscopic measurements followed Noordeloos et al. (2022). Slide preparations were examined and photographed using a BH2 microscope (Olympus, Tokyo, Japan) equipped with a WRAYCAM-EL510 digital camera (Wraymer, Osaka, Japan). All measurements were made using PhotoRuler version 1.1.3 (http://inocybe.info/). The lengths of curved structures, such as basidia, were measured along their longitudinal axes using the polyline tool in the program. The dimensions of basidiospores were measured by placing an imaginary rectangle around the spore, excluding the apiculi, as described by Noordeloos et al. (2022). The size range of basidiospores was described as (a–)b–c(–d), where b = mean – SD, c = mean + SD, and “a” and “d” represent the minimum and maximum values, respectively. The length/width ratio (Q) of each basidiospore was reported as a range of Q values. The number of basidiospores examined was expressed as “n.” The examined specimens were oven-dried and deposited in the Osaka Museum of Natural History (OSA; Osaka, Japan).

DNA isolation, amplification and sequencing

Genomic DNA was extracted from each specimen using a modified microwave method based on Izumitsu et al. (2012) and stored at − 20°C until PCR amplification. The nuclear ribosomal DNA region spanning the internal transcribed spacer (ITS) region to the D1/D2 domains of the large subunit (LSU) was amplified using the primer pair ITS1F (Gardes and Bruns 1993) and LR5 (Vilgalys and Hester 1990). The second-largest subunit of RNA polymerase II (*rpb2*) region was amplified using bRPB2-6F and bRPB2-7.1R (Matheny 2005). In addition, the translation elongation factor 1-alpha (*tef1*) region was amplified for selected specimens to provide supplementary sequence data, using EF1-983F and EF1-1567R (Rehner and Buckley 2005). PCR amplifications were performed in a total reaction volume of 20 µL containing 2 µL of template DNA, 0.4 µL of KOD FX Neo polymerase (TOYOBO Co., Ltd., Osaka, Japan), 10 µL of 2× Buffer for KOD FX Neo, 4 µL of 2 mM dNTPs, 0.3 µL of each primer (20 µM), and sterile distilled water to volume. PCR conditions for the ITS–LSU (D1/D2) region followed Ohkubo et al. (2025), whereas those for *rpb2* and *tef1* followed Matheny (2005) and Rehner and Buckley (2005), respectively. PCR products were purified by polyethylene glycol precipitation using a solution containing 13% (w/v) PEG 8000 and 1.6 M NaCl. Purified PCR products were subjected to Sanger sequencing by FASMAC Co., Ltd. (Kanagawa, Japan). The primers ITS1F and ITS4 (White et al. 1990) were used for sequencing the ITS region, LR0R (Rehner and Samuels 1994) and LR5 for the LSU region, and the same primer pairs for PCR amplification were used for *rpb2* and *tef1*. The nucleotide sequences generated in this study were deposited in the International Nucleotide Sequence Database Collaboration (INSDC) through the DNA Data Bank of Japan (DDBJ).

Phylogenetic analyses

To infer the phylogenetic placement of the collected specimens within the genus *Entoloma*, a concatenated phylogenetic analysis was conducted using sequences of the ITS, LSU, and *rpb2* regions. Reference sequences were retrieved from GenBank based on previous phylogenetic studies of *Entoloma* (e.g., Co-David et al. 2009; Morgado et al. 2013) and sequence similarity identified by BLAST searches (Supplementary Table S1). Sequence datasets for each locus were aligned separately using the MAFFT version 7 online service (Katoh et al. 2019). The resulting alignments were manually trimmed using SeaView version 5.1 (Gouy et al. 2021), and trimmed ITS, LSU, and *rpb2* alignments were subsequently concatenated in SeaView. The concatenated dataset was partitioned into ITS, LSU, and the first, second, and third codon positions of *rpb2*. The best-fitting nucleotide substitution model for each partition was selected using ModelTest-NG version 0.2.0 (Darriba et al. 2020) according to the Bayesian information criterion (BIC). The selected models were TPM2uf + I+G4 for ITS, TrN + I+G4 for LSU, TIM1 + G4 for the first codon position of *rpb2*, TPM3uf + I for the second codon position of *rpb2*, and K80 + I+G4 for the third codon position of *rpb2*. Maximum-likelihood (ML) phylogenetic analysis was performed using RAxML-NG version 1.2.2 (Kozlov et al. 2019), with the substitution model specified separately for each partition. Branch support was assessed using 1,000 bootstrap replicates. The resulting ML tree was visualized using FigTree version 1.4.4 and edited in Inkscape version 1.3.

Results

Morphological observations

Morphological observations revealed three distinct morphological patterns among the examined specimens. *Entoloma cyanovelutinum* differed in having a smooth to subvelutinous dark navy-blue pileus (Fig. 2b–d, f, g) and a compact palisadodermal pileipellis composed predominantly of blue to indigo-blue moniliform hyphae, with terminal cells sometimes brownish, particularly at the pileus center (Fig. 3a, b, d, e). The newly collected specimens of *E. kansaiense* exhibited considerable variation in basidioma coloration, ranging from predominantly yellow forms with grayish-violet pigmentation restricted to the pileus center (Fig. 6j–l) to forms in which most of the pileus and stipe were dark grayish violet (Fig. 6e, f, n). This color variation corresponded to differences in brown pigmentation of the terminal cells of the pileipellis and stipitipellis (Fig. 7). Some variation was also observed in basidiospore dimensions among specimens of *E. kansaiense*. *Entoloma olidoatrocyaneum* was characterized by dull dark-blue basidiomata (Fig. 9a, d, e, g, h) with an unpleasant odor and a bilayered pileipellis consisting of a brown plagiotrichodermal suprapellis and a bluish subpellis containing indigo-blue granular pigments (Fig. 10a–d). Detailed morphological descriptions and comparisons are provided in the Taxonomy section below.

Phylogenetic analysis

The concatenated ITS–LSU–*rpb2* phylogeny placed all three taxa examined in this study within the Prunuloides clade sensu Co-David et al. (2009) (Fig. 1). *Entoloma olidoatrocyaneum* formed an independent lineage outside Clades B1 and B2 recognized by Morgado et al. (2013), although its relationships with other members of the Prunuloides clade were not resolved. *Entoloma cyanovelutinum* was placed within Clade B2 and, more specifically, within the Calliderma clade recognized by Ohkubo et al. (2025). It formed a strongly supported clade with an unidentified Chinese specimen, HKAS 52713 (BS = 100). *Entoloma kansaiense* was also placed outside Clades B1 and B2 and formed a strongly supported clade with the Chinese taxon *E. caeruleoflavum*; this clade was in turn grouped with *E. luridum* and *E. manganaense* with strong support. The ITS sequences of the epitype of *E. kansaiense* and the holotype of *E. caeruleoflavum* were identical, and their LSU sequences differed by only one nucleotide (99.88% similarity, 863/864 bp), providing molecular evidence for their conspecificity. More detailed phylogenetic and sequence comparisons are presented in the notes for each taxon.

Taxonomy

Entoloma cyanovelutinum Ohkubo & H. Masumoto, sp. nov.

Figures 2, 3, 4a, 5a

Index Fungorum Identifier: 906425

Diagnosis

This species is characterized by a compact palisadoderm pileipellis composed of densely arranged, moniliform hyphae that are blue to pale bluish-violet or indigo blue and become darker toward the apex. The terminal cells are sometimes brownish, more frequently at the pileus center. The compact arrangement of the pileipellis hyphae gives the pileus surface a smooth to subvelutinous appearance.

Holotype: JAPAN. Kyoto Pref., Kyoto, Kita-ku, Kamigamo Experimental Station, solitary on an exposed slope beneath *Diplopterygium glaucum* (Houtt.) Nakai, below a broad-leaved forest, 21 Aug 2025, coll. Y. Ohkubo, OSA-MY-16518 (= ENT-CN_2501), GenBank accession no.: ITS (LC948578), nrLSU (LC948589), *rpb2* (LC948600).

Etymology

The epithet refers to the velvety, dark blue pileus.

Basidiomata tricholomatoid. **Pileus** 35–40 mm, plano-convex, not umbonate, dark navy-blue, becoming slightly paler at the margin, developing a brownish tinge at the center after collection; smooth to subvelutinous, not viscid, not hygrophanous; context white. **Lamellae** sinuate, close to subcrowded, up to 7 mm wide, nearly white when young, becoming dull flesh-colored, edge weakly serrulate to entire, with two tiers of lamellulae. **Stipe** 39–50 × 5–6 mm, equal to slightly thickened toward the base; surface bluish-violet, fibrillose with indistinct longitudinal striation, squamulose and pale bluish-violet beneath the pileus; context white, becoming pale brown with age, solid. **Odor** none. **Taste** mild. **Basidiospores** (8.7–)9.4–10.5(–11.4) × (7.6–)8.0–8.8(–9.2) µm (n = 50), Q = 1.11–1.25, subisodiametric to heterodiametric, 5–6-angled in side view. **Basidia** 51–61 × 14–17 µm (in KOH/CR), 4-spored, clavate, not basally clamped. **Lamella edge** fertile. **Cheilocystidia** absent. **Hymenophoral trama** subparallel, composed of cylindrical hyphae, 3.0–19 µm wide. **Pileipellis** a palisadoderm of densely arranged moniliform hyphae composed of chains of 4–6 cells, sometimes branched at the base, blue to pale bluish-violet to indigo blue, becoming darker toward the apex, sometimes with brownish terminal cells occurring throughout the pileus but more frequently at the center; terminal cells 15–43 × 6.0–9.4 µm (in KOH/CR) at the center, 11–40 × 6.4–11.5 µm (in KOH/CR) at the margin; subpellis composed of cylindrical hyphae, hyaline, 2.5–14.6 µm wide. **Pileitrama** composed of cylindrical hyphae, hyaline, 6.9–22.5 µm wide. **Stipitipellis** a cutis of cylindrical hyphae with dark indigo-blue to brownish granular pigment, 1.5–6.7 µm wide; in the middle part of the stipe, brownish, rod-shaped cystidioid hyphae projecting from the surface, and the outermost layer containing brownish hyphae. **Stipititrama** regular, composed of hyaline hyphae, 8.2–24.9 µm wide. **Clamp connections** present in hyphae of all tissues.

Habitat and distribution

Solitary on the ground at the margin of a broad-leaved forest. Currently confirmed from Kyoto Prefecture, Japan; sequence data suggest that the species also occurs in Shiga Prefecture, Japan, and Hunan Province, China.

Other specimens examined

JAPAN. Kyoto Pref., Kyoto, Kita-ku, Kamigamo Experimental Station, solitary on an exposed slope beneath *Diplopterygium glaucum* (Houtt.) Nakai, below a broad-leaved forest, 12 Sep 2024, coll. Y. Ohkubo, OSA-MY-16517 (= ENT-CN_2406).

Japanese name

Biroudo-koniro-gasa

Notes

This species is similar to *E. cyanonigrum* and *E. quasicyanonigrum*, but can be distinguished by its smooth to subvelutinous, rather than rugulose, pileus surface and by its compact palisadodermal pileipellis composed of densely arranged moniliform hyphae, with terminal cells sometimes brownish, especially at the pileus center.

Entoloma cyanovelutinum was placed within the Prunuloides clade sensu Co-David et al. (2009), specifically within Clade B2 sensu Morgado et al. (2013) and the Calliderma clade sensu Ohkubo et al. (2025) (Fig. 1). It formed a strongly supported clade with an unidentified *Entoloma* specimen from Hunan Province, China (HKAS 52713; BS = 100). The holotype of *E. cyanovelutinum* showed 99.39% ITS (491/494 bp) and 99.84% LSU (625/626 bp) sequence similarity to HKAS 52713. However, because no morphological information is available for this specimen, its taxonomic identity could not be further evaluated. High sequence similarity was also observed with a specimen from Tanakami, Shiga Prefecture, Japan (TNS-F-82989): 98.99% for the ITS1–partial 5.8S region (295/298 bp; LC786657) and 99.39% for the partial 5.8S–ITS2 region (327/329 bp; LC786708). The ITS sequence of the holotype also showed 99.10% similarity (612/619 bp) to that of a specimen from Hunan Province, China (MHHNU 31159), deposited as "*Entoloma kujuense*." Genuine *E. kujuense* (Hongo) Hongo, originally described from Japan by Hongo (1985), differs from *E. cyanovelutinum* in pileus and stipe surface characteristics and in basidiospore morphology; its basidiospores are more elongate and heterodiametric (8.5–13 × 6–8 μm), whereas those of *E. cyanovelutinum* are relatively broader and subisodiametric (9.4–10.5 × 8.0–8.8 μm). In contrast, the published morphology of MHHNU 31159 closely agrees with that of *E. cyanovelutinum*, and its basidiospores fall within the range observed in the latter species (Cheng and Zhang 2019). Together with the high ITS sequence similarity, these observations suggest that MHHNU 31159 was misidentified as *E. kujuense* and is likely conspecific with *E. cyanovelutinum*.

Entoloma kansaiense (Hongo) Noordel. & Co-David, in Co-David, Langeveld & Noordeloos, *Persoonia* 23: 170 (2009)

Figures 4b, 5b, 6–8

Index Fungorum Identifier: 513676

Basionym: *Rhodophyllus kansaiensis* Hongo, J. Jpn. Bot. 49: 299 (1974) [Index Fungorum Identifier: 322673]

= *Entoloma caeruleoflavum* T.H. Li & Xiao L. He, in He, Li, Jiang & Shen, *Mycol. Progr.* 11: 918 (2012) [Index Fungorum Identifier: 563507]

Type: JAPAN. Shiga Pref., Otsu, Ishiyama-Terabe, in *Pinus-Quercus* forest, 11 Sep 1973, K. Yokoyama, Hongo Herb. no. 4945 (TNS-F-237503 = holotype, not located); Shiga Pref., Ishiyama-Senjo, in *Pinus-Quercus* forest, 8 Sep 1968, Y. Shidei, Hongo Herb. no. 3728 (TNS-F-237504 = paratype, not located); Shiga Pref., Otsu, Gennai Pass, dispersed on the ground in a sparse forest of broad-leaved shrubs mixed with *Chamaecyparis obtusa* (Siebold et Zucc.) Endl., 9 Oct 2024, coll. Y. Ohkubo, OSA-MY-16520 (= ENT-KA2401-B; epitype, designated here; Index Fungorum Identifier: 906427, Figs. 6a, d, g–i, 7a–g, o, p), GenBank accession no.: ITS (LC948582), nrLSU (LC948593), *rpb2* (LC948603), and *tef1* (LC951350).

Basidiomata tricholomatoid. **Pileus** 32–67 mm, obtusely conical when young, convex with slight umbo at maturity, entirely dark grayish blue to violaceous when young, dark grayish violet at center and slightly faded at margin when matured, granular at center and squamulose at margin, dry, not hygrophanous; margin split, slightly decurved or deflexed at maturity, slightly undulate, occasionally revealing the context color; context white. **Lamellae** sinuate, subcrowded, 4.5–10.5 mm wide, slightly dull orange yellow, edge serrulate, with two tiers of lamellulae. **Stipe** 54–95 mm long, 6–8 mm wide just below the pileus, 7–12 mm at the middle part, and 8–10 mm near the base, tapered toward both ends, longitudinally fibrillose-striate, pale yellow beneath the pileus, becoming purplish brown downward, pale purplish brown to pale yellow near the base, the base white; context white, stuffed at the middle part and fistulose near the base, elastic and readily splitting longitudinally. **Odor** slightly fungoid, pleasant. **Taste** somewhat rancid, unpleasant. **Basidiospores** (6.5–)7.5–8.3(–8.6) × (5.8–)6.3–7.2(–7.7) µm (n = 50), Q = 1.05–1.27, isodiametric to subisodiametric, 6–7 relatively rounded angles, with relatively long apiculus. **Basidia** 36–45 × 9.7–14 µm (in KOH/CR), (1-)3- or 4-spored, clavate, basally clamped. **Lamella edge** fertile. **Cheilocystidia** absent. **Hymenophoral trama** parallel, composed of cylindrical hyphae, 9.2–28.5 µm wide, hyaline. **Pileipellis** bilayered; suprapellis a plagiotrichoderm composed of moniliform hyphae consisting of more than seven cells; terminal 2–3 cells dark grayish brown to brownish gray-violet; apical cells elongated, tapering toward the apex, 45–85 × 7.7–30 µm (in KOH/CR); subpellis arranged in a cutis-like layer of cylindrical hyphae, 6.0–20.9 µm wide, hyaline with grayish-blue pigment either as intracellular granules or diffused in the cytoplasm, some hyphae lacking pigment. **Pileitrama** composed of cylindrical hyphae, 12.2–23.7 µm wide, hyaline. **Stipitipellis** a cutis of cylindrical narrow hyphae, 3.0–8.9 µm wide, grayish red-violet to bluish, occasionally containing grayish-blue to indigo-blue granular pigment, or hyaline beneath the pileus. **Stipititrama** regular, composed of cylindrical 7–30 µm wide hyphae, hyaline. **Clamp connections** present in all tissues.

Habitat and distribution

Solitary or dispersed on the ground in *Pinus–Quercus* forests and in a sparse forest of broad-leaved shrubs mixed with *Chamaecyparis obtusa*. Currently known in Japan and China; Chinese records were

previously reported as *E. caeruleoflavum*.

Other specimens examined

JAPAN. Shiga Pref., Otsu, Gennai Pass, 9 Oct 2024, coll. Y. Ohkubo, OSA-MY-16519 (= ENT-KA2401-A); ibid., 9 Oct 2024, coll. Y. Ohkubo, OSA-MY-16521 (= ENT-KA2401-C); ibid., 9 Oct 2024, coll. Y. Ohkubo, OSA-MY-16522 (= ENT-KA2401-D); ibid., 9 Oct 2024, coll. Y. Ohkubo, OSA-MY-16523 (= ENT-KA2401-E); ibid., 24 Sep 2025, coll. Y. Ohkubo, OSA-MY-16524 (= ENT-KA2501); ibid., 28 Sep 2025, coll. Y. Ohkubo, OSA-MY-16525 (= ENT-KA2502).

Japanese name

Kihida-ippon-shimeji (Hongo 1974)

Notes

Entoloma kansaiense was originally described as *Rhodophyllus kansaiensis* Hongo based on specimens collected on the ground in forests of *Pinus densiflora* mixed with *Quercus serrata* in Otsu City, Shiga Prefecture (Hongo 1974). Although the original description contained no color illustrations, Hongo's original color drawings, some of which were subsequently published in Imazeki and Hongo (1987), are preserved in the Tsuguo Hongo Collection at the Osaka Museum of Natural History (OSA) (Fig. 8). The species has rarely been reported and has been regarded as endemic to Japan (Hosoya et al. 2016), with records mainly from temperate regions of the country (Imazeki and Hongo 1987; Hiroshima Jogakuin 1988; Ikeda 2014). It is currently listed as Vulnerable (VU) in Hiroshima Prefecture (Hiroshima Prefecture 2022) and Near Threatened (NT) in Shizuoka Prefecture (Shizuoka Prefecture 2020). Until the present study, no DNA sequence data were available for *E. kansaiense*, hindering its comparison with morphologically similar taxa. In the present study, multiple specimens were collected at Gennai Pass, Otsu City, approximately 6 km from the type locality at Ishiyama-Terabe. Because the original type specimens could not be located and the available original material is insufficient for unambiguous identification, we designate one of these newly collected specimen (OSA-MY-16520) as an epitype with DNA sequence data to clarify the application of the name *E. kansaiense*.

The newly collected specimens revealed considerable intraspecific variation in basidioma coloration. The typical form has a pileus that is dark grayish violet at the center and yellowish toward the margin (Fig. 6a, b, d, g–i, m). In some basidiomata, however, the grayish-violet coloration is restricted to the central portion of the pileus, whereas the pileus margin and the stipe are yellow (hereafter referred to as the “yellow form”; Fig. 6c, j–l). This difference is associated with pigmentation of the terminal cells of the pileipellis: these cells contain dark brown pigments in the typical form (Fig. 7a–e) but lack such pigmentation in the yellow form (Fig. 7h–l), resulting in predominantly yellow basidiomata that could superficially be mistaken for a distinct species. Conversely, basidiomata in which almost the entire pileus and stipe are dark grayish violet were also observed (Fig. 6e, f, n). These observations demonstrate substantial intraspecific variation in basidioma coloration in *E. kansaiense*. Basidiospore

dimensions also varied among specimens. For example, in the dried specimen (OSA-MY-16519), basidiospores measured (6.3–)6.9–7.9(–9.0) × (5.7–)6.2–6.9(–7.3) µm and were somewhat smaller than those observed in most of the other specimens examined.

Morphologically, *E. kansaiense* closely resembles *E. caeruleoflavum*, which was originally described from a single specimen from Yunnan Province, China (He et al. 2012) and has subsequently been reported from Hunan and Jiangxi Provinces (Cheng and Zhang 2019; Deng et al. 2020). Several differences can nevertheless be recognized from the published descriptions. Although both taxa have predominantly bluish pilei, *E. caeruleoflavum* was described as having marginal regions tinged not only with yellow or brown but also with green and pink. Given the substantial color variation observed among the newly collected specimens of *E. kansaiense*, however, these differences in pileus coloration are unlikely to warrant species-level separation.

Basidiospore size has also been regarded as a possible distinguishing character. Basidiospores of *E. caeruleoflavum* were reported as 6.5–7.5 × 6.3–7.3 µm, somewhat smaller than those given in the protologue of *E. kansaiense* (7.5–8.5 × 6.5–7.5 µm) and than the range observed in most specimens examined in the present study [(6.5–)7.5–8.3(–8.6) × (5.8–)6.3–7.2(–7.7) µm]. However, the relatively small basidiospores observed in the specimen OSA-MY-16519 substantially overlap with those reported for *E. caeruleoflavum*, indicating that spore size is variable within *E. kansaiense*. A similar pattern was observed in basidial dimensions. Basidia of *E. caeruleoflavum* were reported as 30–48 × 9–12 µm, whereas the protologue of *E. kansaiense* described shorter basidia, 23–31 × 9.5–12 µm. In the present material, however, basidia measured 36–45 × 9.7–14 µm and therefore broadly overlapped with those of *E. caeruleoflavum*. These differences in basidiospores and basidial dimensions are consequently best interpreted as intraspecific variation. One remaining morphological difference concerns the presence of oleiferous hyphae. *Entoloma caeruleoflavum* was described as having oleiferous hyphae scattered in the lamellar and pileal trama, whereas such structures were not observed in the specimens of *E. kansaiense* examined here. Nevertheless, this character alone appears insufficient to support recognition of the two taxa as separate species, particularly given the broad morphological variation observed in *E. kansaiense*.

Molecular data further support a close relationship between *E. kansaiense* from Japan and *E. caeruleoflavum* from China. The Japanese specimens of *E. kansaiense* examined in this study were placed within the Prunuloides clade sensu Co-David et al. (2009) but formed a distinct lineage outside Clades B1 and B2 recognized by Morgado et al. (2013) (Fig. 1). Within this lineage, *E. kansaiense* formed a strongly supported clade with *E. caeruleoflavum*. The ITS sequence of the epitype of *E. kansaiense* (LC948582) was identical to that of the holotype of *E. caeruleoflavum* (JQ320111), and the LSU sequences showed 99.88% similarity (863/864 bp). In addition, the ITS sequence of the epitype was also identical to that of specimen MHHNU30622 from Hunan Province, China, reported as *E. caeruleoflavum* (MK250920; 612/612 bp). These results revealed no substantial sequence divergence between the two taxa in the ITS and LSU regions examined.

Taken together, the absence of meaningful genetic divergence and the broad morphological variation observed in *E. kansaiense* provide no support for maintaining *E. caeruleoflavum* as a separate species. We therefore regard the two taxa as conspecific and place *E. caeruleoflavum* in synonymy with *E. kansaiense*, the earlier name.

Entoloma olidoatrocyaneum Ohkubo & H. Masumoto, sp. nov.

Figures 4c, 5c, 9, 10

Index Fungorum Identifier: 906426

Diagnosis

This species is characterized by a bilayered pileipellis consisting of a brown suprapellis and an indigo blue subpellis, giving the pileus a dark blue color, and an unpleasant beetle-like odor.

Holotype: JAPAN. Shiga Pref., Otsu, Ryukoku-no-Mori (Ryukoku Forest), on soil in broad-leaved forest mixed with *Chamaecyparis obtusa*, 9 Oct 2024, coll. Y. Ohkubo, OSA-MY-16513 (= ENT-CN_2402), GenBank accession no.: ITS (LC948575), nrLSU (LC948586), *rpb2* (LC948598), and *tef1* (LC951347).

Etymology

The epithet refers to the dark blue pileus (*atro-* = black, *cyaneum* = blue) and the unpleasant odor of basidiomata (*olidus* = emitting a smell).

Basidiomata tricholomatoid. **Pileus** 22–43 mm, conical when young, subumbonate when mature, dark blue with a nearly black center when fresh, darkening to almost black with a reddish purple to reddish brown tinge soon after collection, dry, scabrulose, indistinctly radially rugulose, not hygrophanous; margin slightly incurved when young, expanding to plane, upturned when old; context white. **Lamellae** sinuate, close to crowded, up to 6 mm wide, pale incarnate, edge wavy, with 2–3 tiers of lamellulae. **Stipe** 37–73 × 4–10 mm, thickened toward the base, grayish-blue and fibrillose-striate, rugulose, with white or pale yellow base; context white, solid. **Odor** beetle-like, unpleasant. **Taste** unpleasant. **Basidiospores** (7.2–)7.8–8.5(–8.9) × (6.5–)6.7–7.4(–7.6) µm (n = 25), Q = 1.10–1.20, subisodiametric, 5–6-angled in side view, with one to several guttules. **Basidia** 36–48 × 11–14 µm (in KOH/CR), 4-spored, clavate, occasionally basally clamped. **Lamella edge** fertile. **Cheilocystidia** absent. **Hymenophoral trama** subparallel, composed of cylindrical hyphae, 8.7–25 µm wide. **Pileipellis** bilayered; suprapellis a plagiotrichoderm of cylindrical to clavate hyphae, 27–81 × 8–23 µm (in KOH/CR), terminal elements consisting of a chain of 2–3 more or less inflated cells, constricted at septa, mainly dark brown or sometimes light blue, light reddish blue or hyaline, occasionally with indigo blue granules, basal cells hyaline or grayish with indigo blue granules; subpellis composed of cylindrical hyphae, 7.5–21 µm wide, hyaline, grayish, or pale bluish, with most hyphae containing accumulated indigo blue granular pigment. **Pileitrama** composed of cylindrical hyphae, hyaline, 11–33 µm wide. **Stipitipellis** a cutis of cylindrical hyphae; those of the outermost layer 2.2–6.3 µm wide, hyaline, containing indigo-blue granules, with

terminal cells often slightly inflated; underlying hyphae also cylindrical, grayish brown to reddish purple. **Stipititrama** regular, composed of cylindrical hyphae, 9–34 µm wide, hyaline. **Clamp connections** present in all tissues.

Habitat and distribution

Solitary or dispersed on the ground in a sparse forest of broad-leaved shrubs mixed with *Chamaecyparis obtusa*. Currently known only from Japan (Shiga).

Other specimens examined

JAPAN. Shiga Pref., Otsu, Ryukoku-no-Mori (Ryukoku Forest), on soil in broad-leaved forest mixed with *C. obtusa*, 22 Oct 2023, coll. Y. Ohkubo, OSA-MY-16510 (= ENT-CN_2301); ibid., 22 Oct 2023, coll. Y. Ohkubo, OSA-MY-16511 (= ENT-CN_2302); ibid., 9 Oct 2024, coll. Y. Ohkubo, OSA-MY-16514 (= ENT-CN_2403); ibid., 20 Oct 2024, coll. Y. Ohkubo, OSA-MY-16515 (= ENT-CN_2405); Shiga Pref., Otsu, Gennai Pass, on soil in a forest sparsely planted with *C. obtusa*, 9 Oct 2024, coll. Y. Ohkubo, OSA-MY-16512 (= ENT-CN_2401); ibid., 18 Sep 2025, coll. Y. Ohkubo, OSA-MY-16516 (= ENT-CN_2505).

Japanese name

Nioi-kon-iro-gasa

Notes

Entoloma olidoatrocyaneum produces tricholomatoid, dull dark-blue basidiomata and resembles *E. obscurocyaneum* and *E. purpureobrunneolum* in having a pileipellis composed of two layers of hyphae, one dark blue and the other brownish or purplish. However, the pileipellis of the latter two species is a hymeniderm- or palisadoderm type, with terminal hyphae not ascending obliquely, and they lack the characteristic unpleasant odor of *E. olidoatrocyaneum*.

Entoloma olidoatrocyaneum was supported as a member of the Prunuloides clade sensu Co-David et al. (2009) but formed a distinct lineage outside Clades B1 and B2 recognized by Morgado et al. (2013) (Fig. 1). Its phylogenetic relationships with other species of *Entoloma* remain unresolved. In addition, BLAST searches revealed no sequences showing high similarity to either the ITS or LSU sequence of the holotype of *E. olidoatrocyaneum*.

Discussion

Long-term, repeated macrofungal surveys in the Kansai region, particularly at the Kamigamo Experimental Station of Kyoto University, Ryukoku Forest of Ryukoku University, and the adjacent Gennai Pass, have led to the rediscovery of the rare *Entoloma kansaiense*, originally described by Hongo, enabling the designation of an epitype, as well as to the recognition of two previously undescribed dark-blue, tricholomatoid species, *E. cyanovelutinum* and *E. olidoatrocyaneum*.

Entoloma cyanovelutinum was placed in the Calliderma clade, whose members include several medium-sized, tricholomatoid species with dark bluish basidiomata and pileipelles composed of erect hyphal elements forming a hymeniderm or palisadoderm rather than a cutis (e.g., *E. callidermum*, *E. hymenidermum*, and *E. quasicyanonigrum*). Their relatively similar macromorphology emphasizes the taxonomic importance of microscopic features, particularly the organization and pigmentation of the pileipellis, for species recognition within this group. The close similarity between *E. cyanovelutinum* and a Chinese specimen (MHHNU 31159) reported as "*E. kujuense*" further illustrates the difficulty of applying names of poorly documented species in the absence of molecular data from authentic material. Because no sequence data are currently available from authentic Japanese collections of *E. kujuense*, the precise phylogenetic placement of *E. kujuense* and the identity of Chinese collections assigned to this name remain uncertain. The pileipellis of *E. kujuense* was described as a trichoderm (Hongo 1985), with hyphal elements arising approximately perpendicular to the pileus surface, a configuration that is not inconsistent with placement in the Calliderma clade. Recollection of *E. kujuense* from its type locality or nearby areas will therefore be important for determining whether sequences currently deposited under this name represent the species sensu Hongo.

The synonymization of *E. caeruleoflavum* with *E. kansaiense* substantially changes the known biogeographic interpretation of the latter. Rather than being restricted to Japan, *E. kansaiense* is now known to occur more broadly in East Asia, including records previously assigned to *E. caeruleoflavum* from China (He et al. 2012; Cheng and Zhang 2019; Deng et al. 2020). The phylogenetic placement of *E. kansaiense* close to *E. luridum* from North America and *E. manganaense* from Tasmania is also noteworthy (Fig. 1). These species share the presence of yellowish lamellae. The lamellae of *E. luridum* are initially described as mustard yellow and later become warm buff to pinkish, those of *E. manganaense* are cadmium yellow, and those of *E. kansaiense* are slightly dull orange-yellow. In contrast, pileus coloration differs markedly among them. The pileus of *E. luridum* is initially mustard yellow and later fades through cream to whitish (Hesler 1967), whereas that of *E. manganaense* is red-brown (Gates and Noordeloos 2007) and that of *E. kansaiense* varies from dark grayish violet to predominantly yellow. Clamp connections also differ: they are commonly present in *E. kansaiense* and *E. manganaense* but absent in *E. luridum*. This may indicate that yellow pigmentation of the lamellae is a relatively conserved morphological tendency within this lineage, whereas pileus pigmentation and clamp connections are more variable. Broader taxon sampling will be necessary to evaluate whether this character has phylogenetic significance within the Prunuloides clade.

Entoloma olidoatrocyaneum provides another example of the morphological similarity that complicates identification of dark-blue tricholomatoid *Entoloma*. Although it resembles *E. obscurocyaneum* and *E. purpureobrunneolum* in overall basidioma coloration and in having a bilayered pileipellis, its plagiotrichoderm-type pileipellis, its distinctive unpleasant beetle-like odor, and its distinct phylogenetic position (Fig. 1) support its recognition as a separate species. The coexistence of these superficially similar species in the Ryukoku Forest–Gennai Pass area is particularly interesting. *Entoloma obscurocyaneum* and *E. purpureobrunneolum* have been observed mainly from August to September (Ohkubo et al. 2025), whereas most collections of *E. olidoatrocyaneum* were obtained in October, with

one additional collection (OSA-MY-16516) in mid-September. Although additional observations are needed, this difference suggests that the three species may also differ in their seasonal fruiting phenology.

More broadly, the present study highlights the value of obtaining modern collections of historically described Japanese taxa from their type localities or nearby areas. For *E. kansaiense*, recollection provided sufficient morphological and molecular information to reassess its species limits, document previously underappreciated variation, and clarify its relationship to a subsequently described Chinese species. In contrast, the taxonomic status of *E. kujuense* remains difficult to evaluate because comparable molecular data from authentic Japanese material are lacking. These contrasting cases demonstrate that recollection and epitypification, when appropriate, can be critical for stabilizing the application of names based on historical collections. Continued integrative study of taxa described from the Kansai region may reveal additional overlooked synonymies and broader East Asian distributions, thereby improving both the taxonomic framework and the biogeographic interpretation of regional macrofungal diversity.

Declarations

Competing interests

The authors declare no competing interests.

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Author contribution

All authors contributed to the study conception and design. Conceptualization: YO, HM, DS; Material preparation, data collection and analysis: YO, HM; Writing—original draft preparation: YO, HM; Writing—review and editing: YO, HM; Resources: YO, HM, DS; Supervision: HM.

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Data availability

The sequences generated in this study are available in the International Nucleotide Sequence Database Collaboration (INSDC) under the accession numbers given in Supplementary Table S1.

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Figures

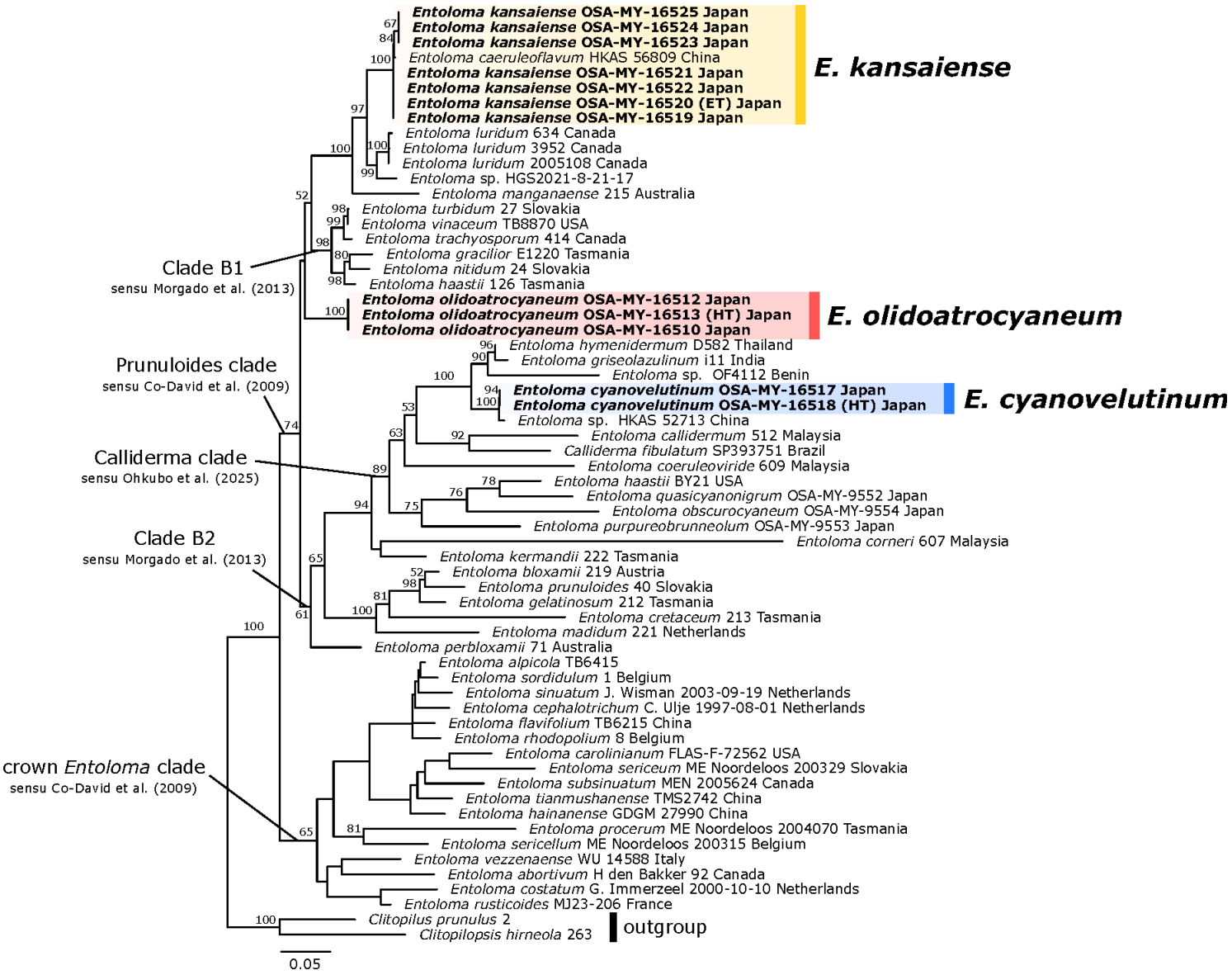

Figure 1

Maximum-likelihood (ML) phylogenetic tree of the genus *Entoloma* inferred from the concatenated ITS, LSU, and *rpb2*dataset. ML bootstrap support (BS) values ≥ 50% are indicated at the nodes. Taxon labels corresponding to sequences newly generated in this study are shown in bold. The scale bar indicates the number of substitutions per site. ET, epitype; HT, holotype.

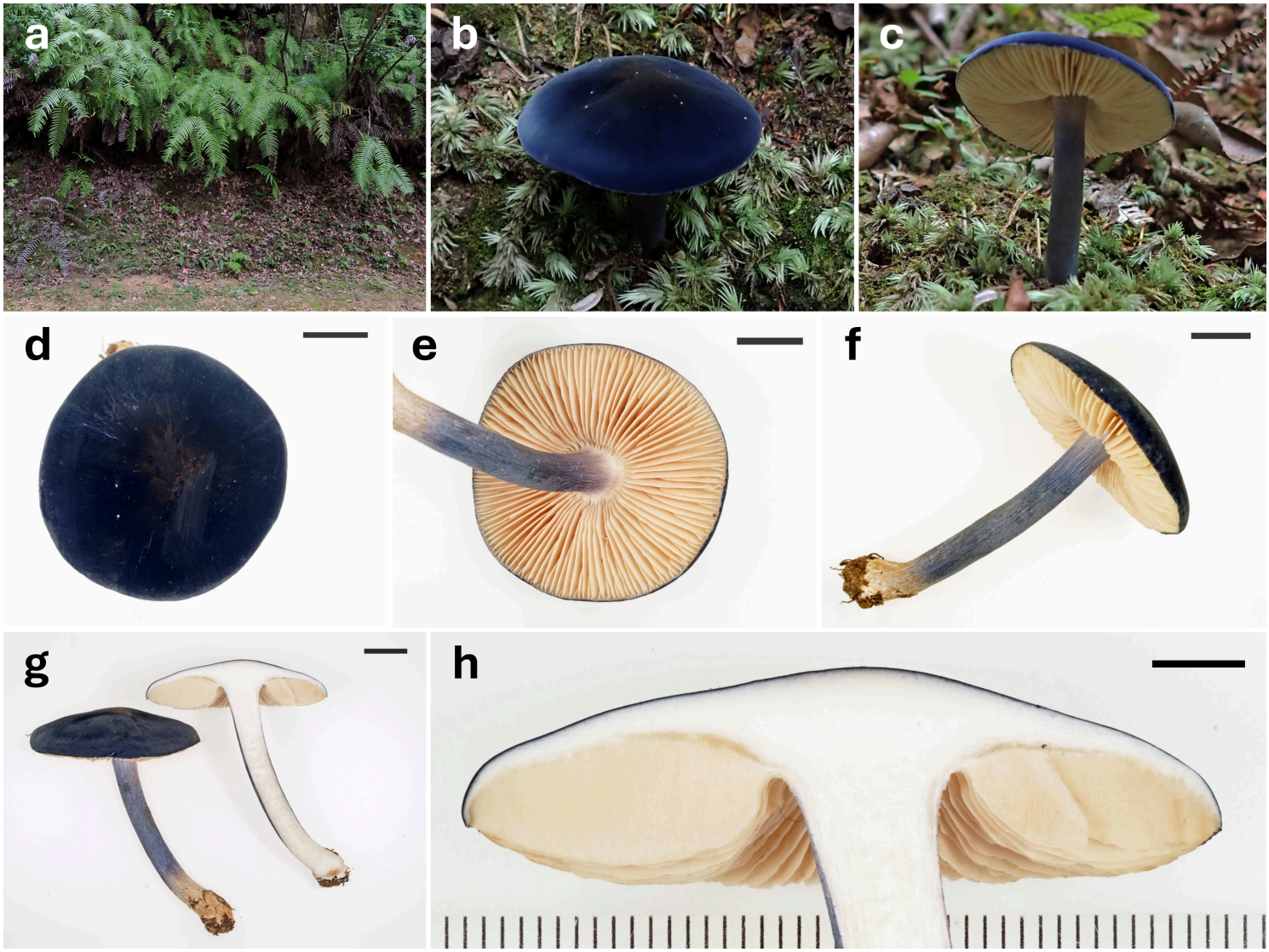

Figure 2

Macromorphology of *Entoloma cyanovelutinum*(OSA-MY-16518, holotype). **a** Habitat. **b, c** Basidiomata in the field. **d** Detail of the pileus surface. **e** Detail of the lamellae. **f** Detail of the stipe. **g** Sectioned basidioma. **h** Sectioned pileus. Bars: **d–g** 1 cm; **h** 5 mm.

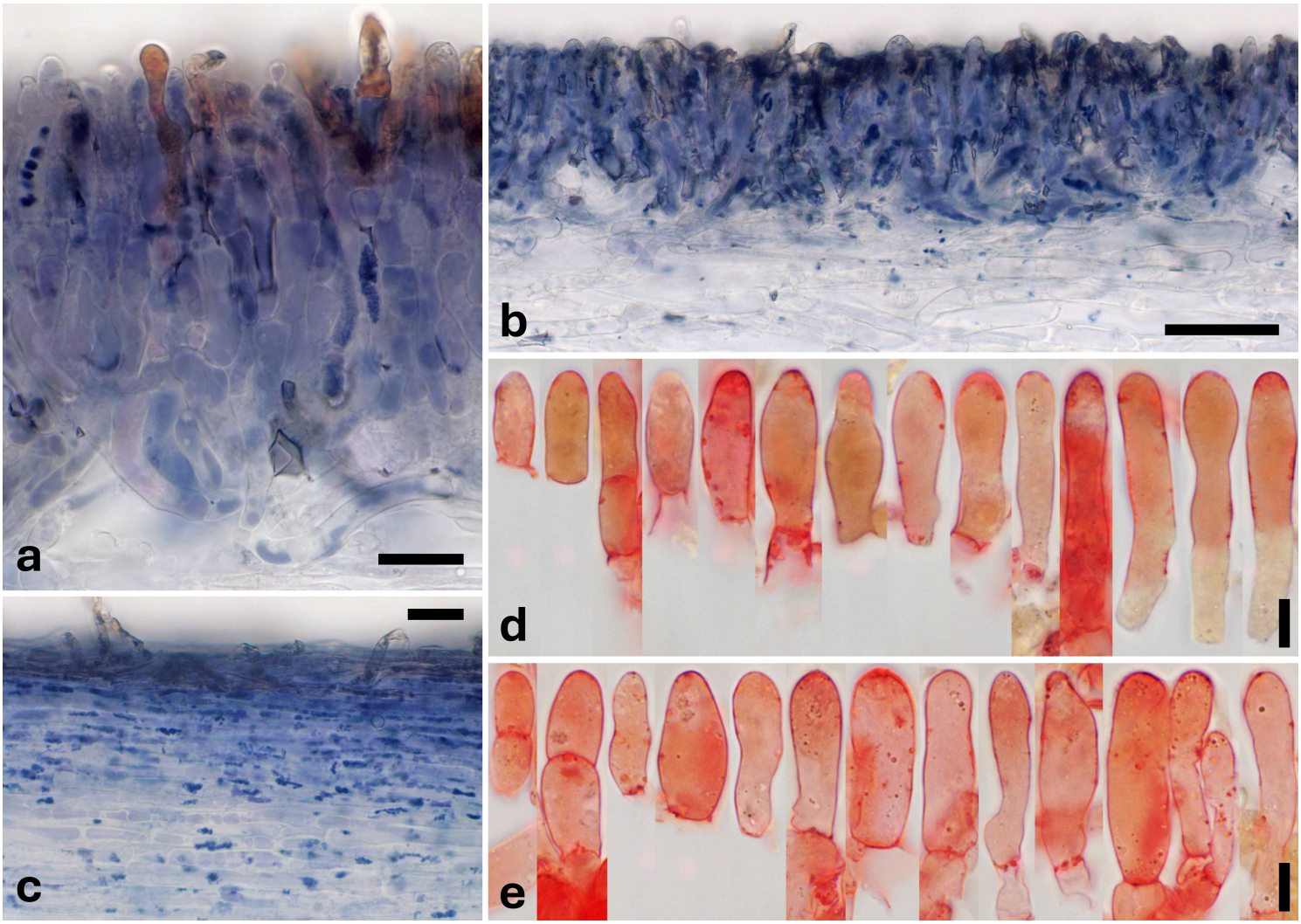

Figure 3

Pileipellis and stipitipellis of *Entoloma cyanovelutinum* (OSA-MY-16518, holotype). **a** Pileipellis at center of the pileus (in water). **b** Pileipellis at the pileus margin (in water). **c**Stipitipellis at the middle part of the stipe (in water). **d** Terminal cells of pileipellis at center of the pileus (in KOH with CR). **e** Terminal cells at the pileus margin (in KOH with CR). Bars: **b** 50 µm; **a, c** 20 µm; **d, e** 10 µm.

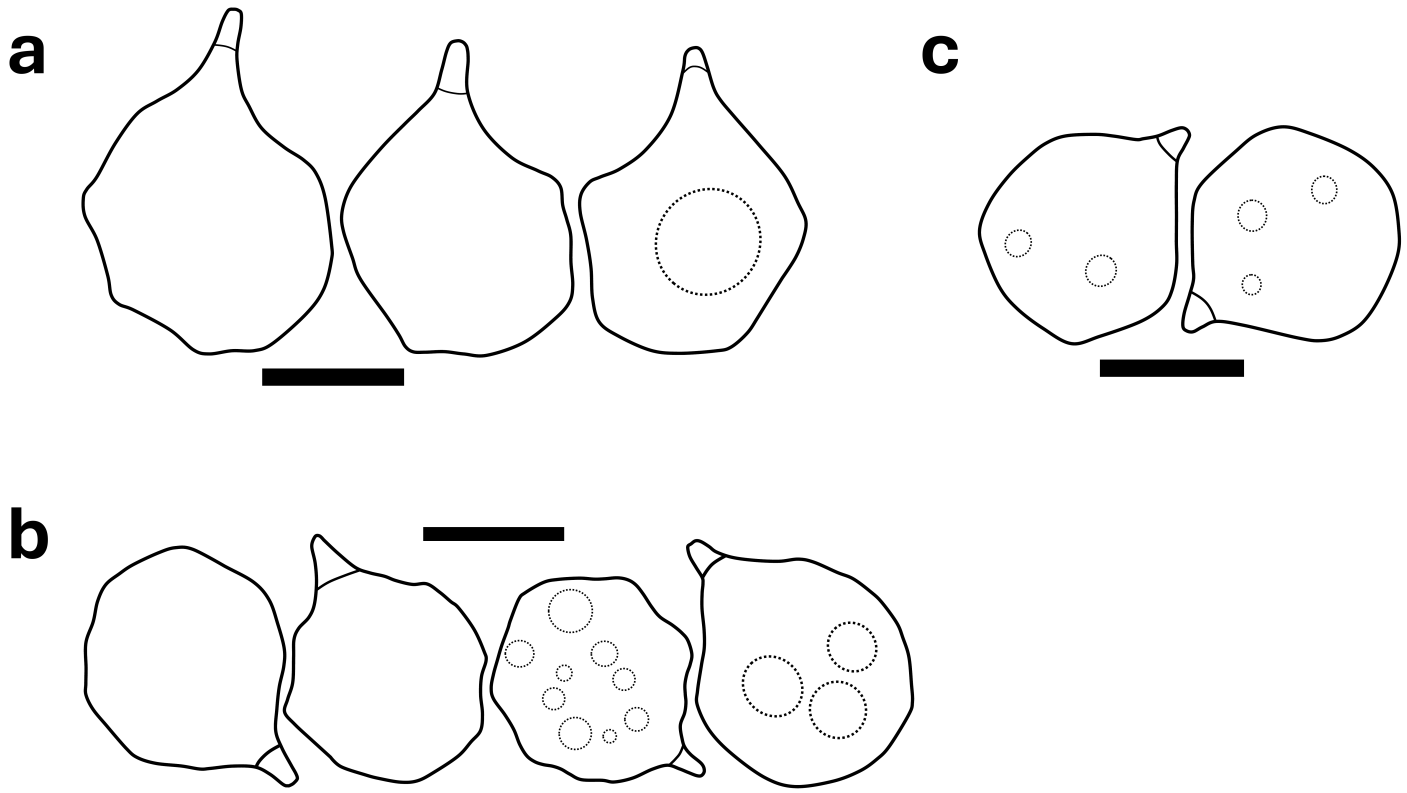

Figure 4

Basidiospores. **a** *Entoloma cyanovelutinum* (OSA-MY-16518, holotype). **b** *Entoloma kansaiense* (OSA-MY-16520, epitype). **c** *Entoloma olidoatrocyaneum* (OSA-MY-16513, holotype). Bars: 5 μm.

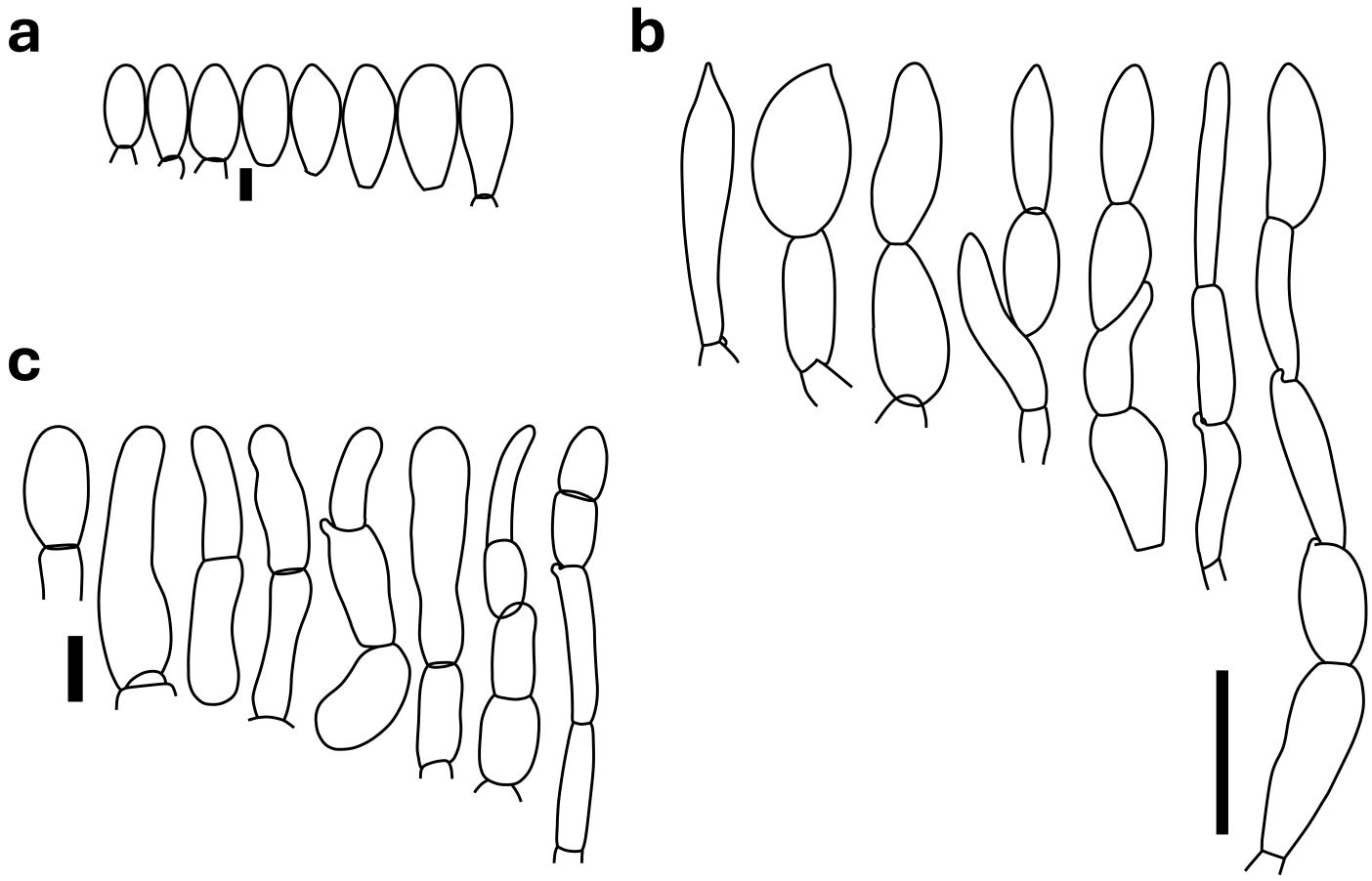

Figure 5

Terminal cells of pileipellis. **a** *Entoloma cyanovelutinum* (OSA-MY-16517). **b** *Entoloma kansaiense* (OSA-MY-16520, epitype). **c** *Entoloma olidoatrocyaneum* (OSA-MY-16513, holotype). Bars: **a** 10 µm; **b** 50 µm; **c** 20 µm.



Figure 6

Macromorphology of *Entoloma kansaiense*. **a–d** Basidiomata in the field. **e, f** Young basidiomata in the field. **g, j** Basidiomata. **h, k** Detail of the pileus surface. **i, l** Detail of the lamellae. **m** Sectioned basidiomata. **n**Sectioned young basidiomata. **o** Sectioned pileus. **a, d, g–i** OSA-MY-16520 (epitype); **b, m** OSA-MY-16519; **c, j–l, o** OSA-MY-16521; **e, f, n**OSA-MY-16523. Bars: 1 cm.

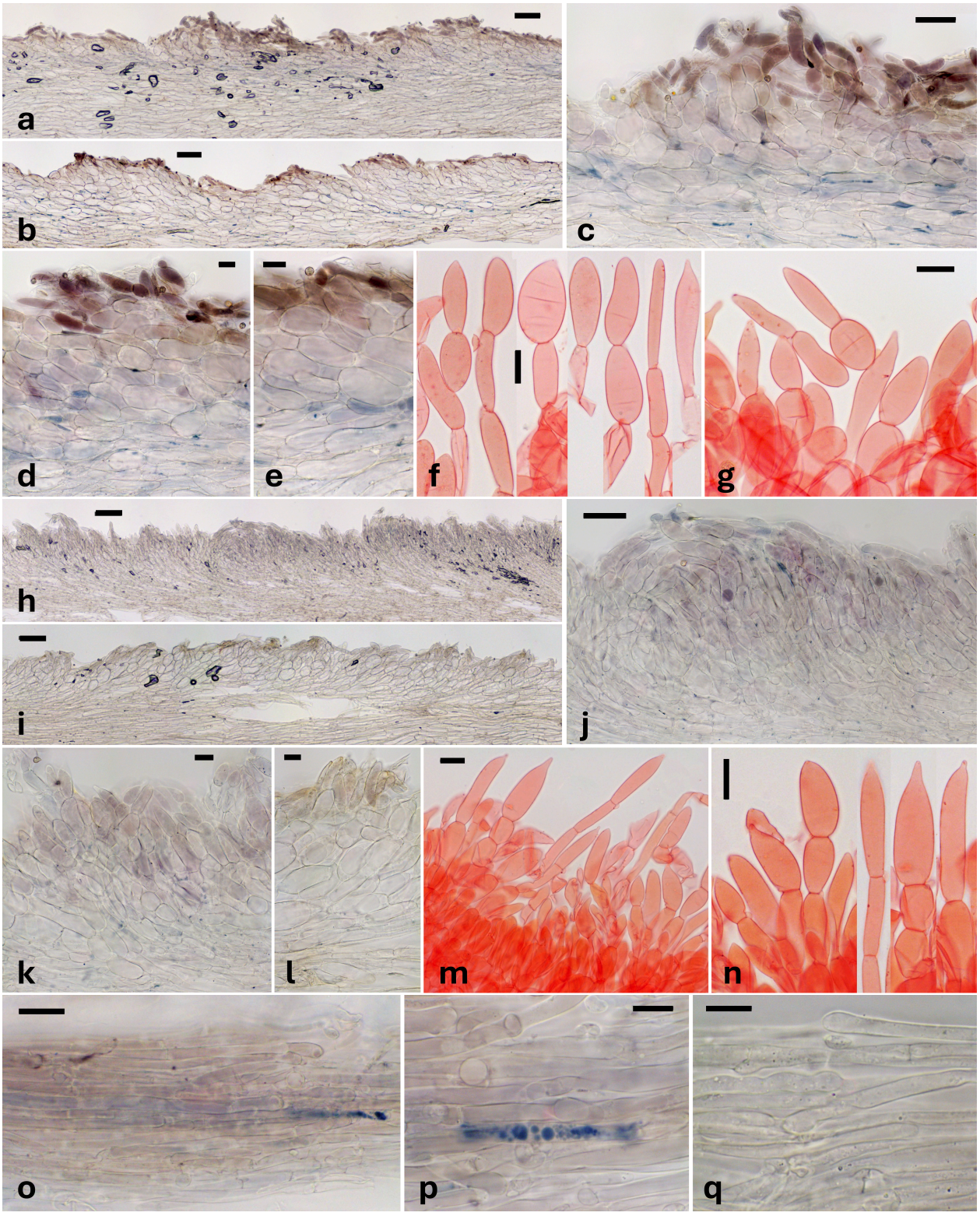

Figure 7

Pileipellis and stipitipellis of *E. kansaiense*. **a, c–e** Pileipellis at the center of pileus (in water). **b**Pileipellis at the pileus margin (in water). **f** Terminal cells of the pileipellis at the center of pileus (in KOH with CR). **g** Terminal cells of the pileipellis at the pileus margin (in KOH with CR). **h, j, k**Pileipellis at the center of pileus (in water). **i, l** Pileipellis at the pileus margin (in water). **m, n** Terminal cells of the pileipellis at the center of pileus (in KOH with CR). **o, p** Stipitipellis at the middle of the stipe (in water). **q** Stipitipellis

beneath the pileus (in water). **a–g, o, p** OSA-MY-16520 (epitype; typical form); **h–n, q** OSA-MY-16521 (yellowish form). Bars: **a, b, h, i** 100 µm; **c, j** 50 µm; **d–g, k–n, o** 20 µm; **p, q** 10 µm.

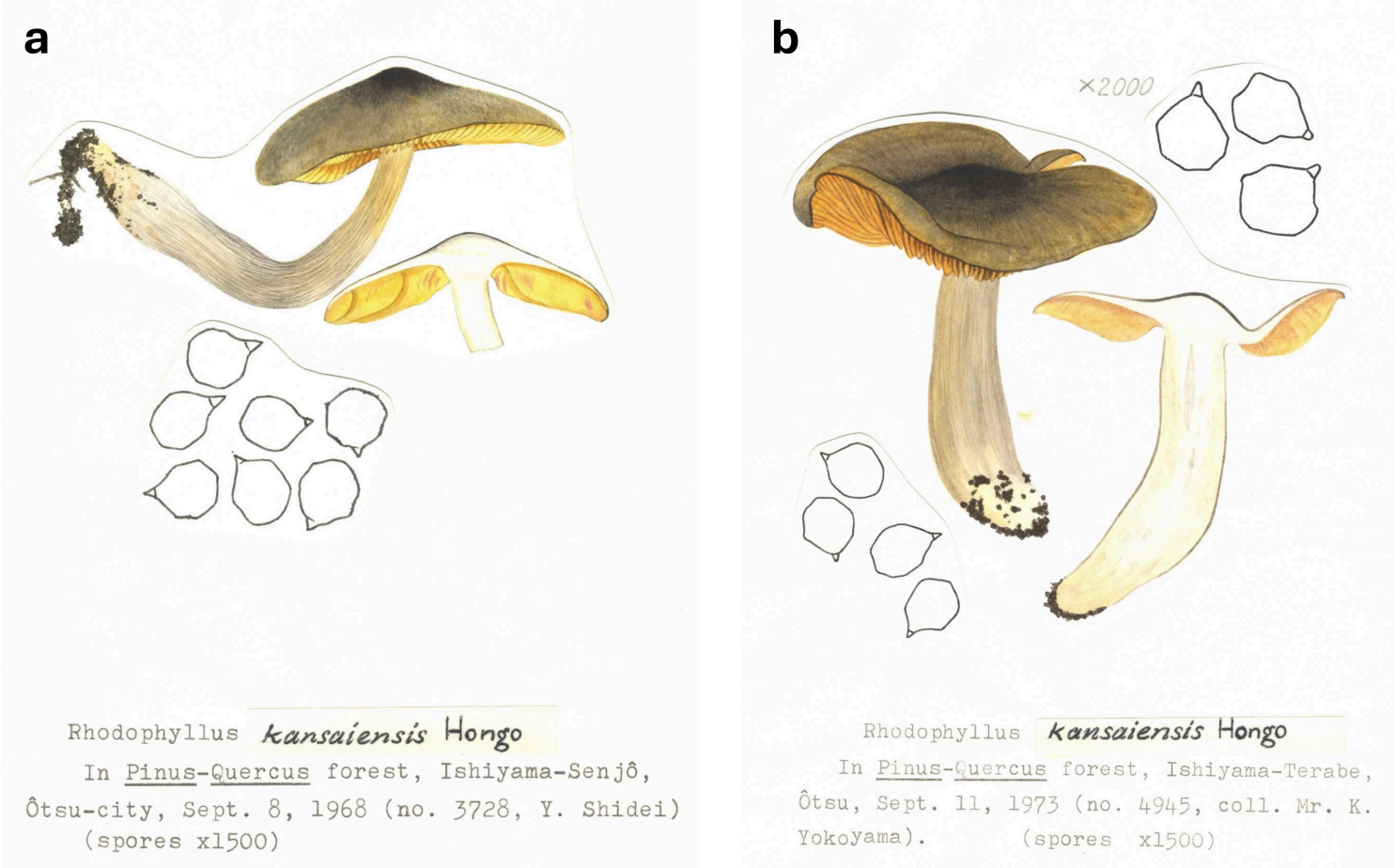

Figure 8

Color and line drawings of *Entoloma kansaiense* by Tsuguo Hongo. **a** Drawing of the paratype of *E. kansaiense* (Tsuguo Hongo no. 3728 (= TNS-F-237504); cited as *Rhodophyllus kansaiensis* Hongo). **b** Drawing of the holotype of *E. kansaiense* (Tsuguo Hongo no. 4945 (= TNS-F-237503); cited as *Rhodophyllus kansaiensis* Hongo). Source: Tsuguo Hongo Collection: Color drawings of Japanese Fungi by Osaka Museum of Natural History (OSA).

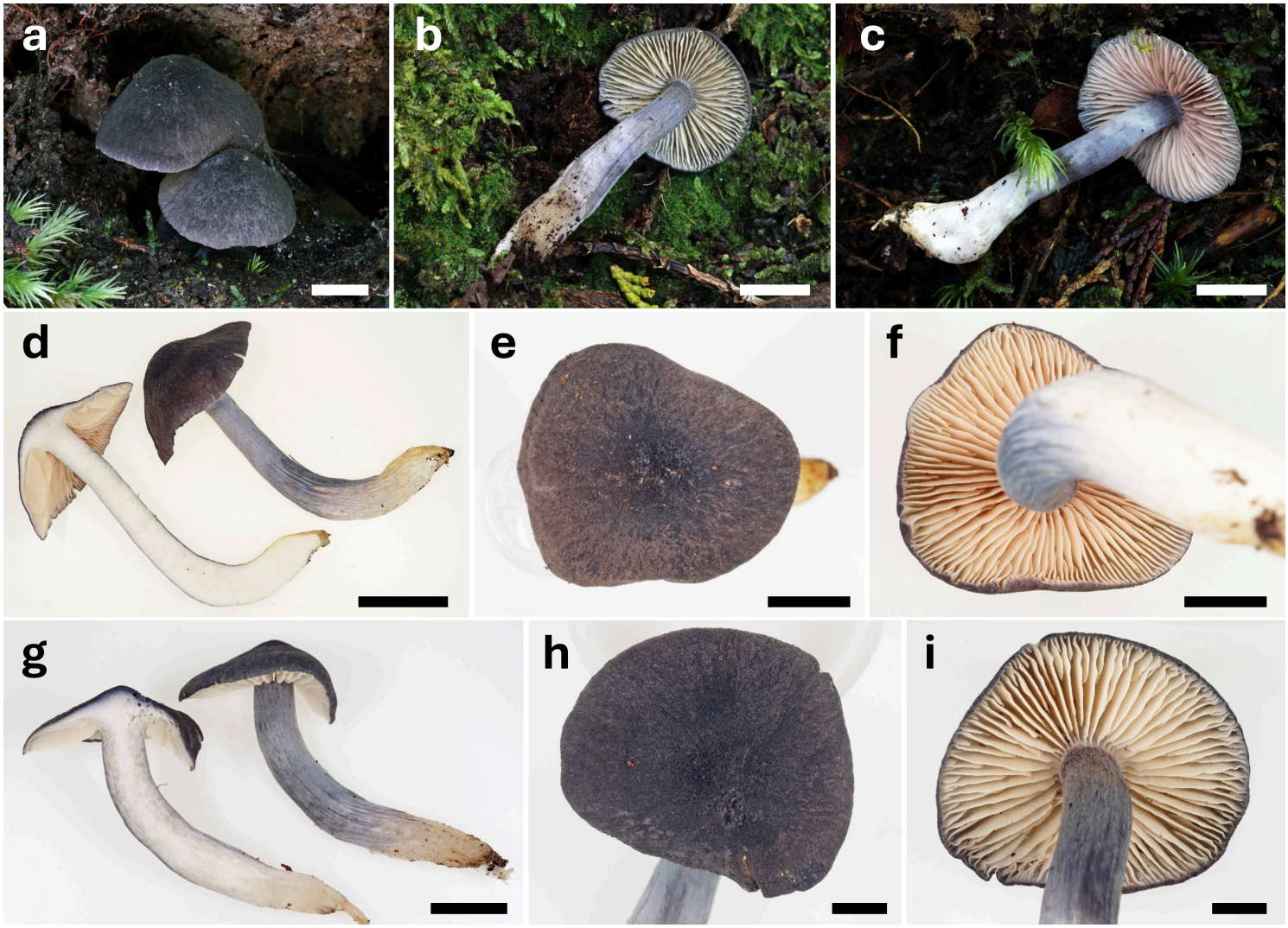

Figure 9

Macromorphology of *Entoloma olidoatrocyaneum*. **a–c** Basidiomata in the field. **d** Sectioned basidioma. **e**Detail of the pileus surface. **f** Detail of the lamellae. **g**Sectioned basidioma. **h** Detail of the pileus surface. **i** Detail of the lamellae. **a, d–f** OSA-MY-16513 (holotype); **b, g–i** OSA-MY-16510; **c** OSA-MY-16511. Bars: **a–c, e–g** 1 cm; **d** 2 cm; **h, i** 5 mm.

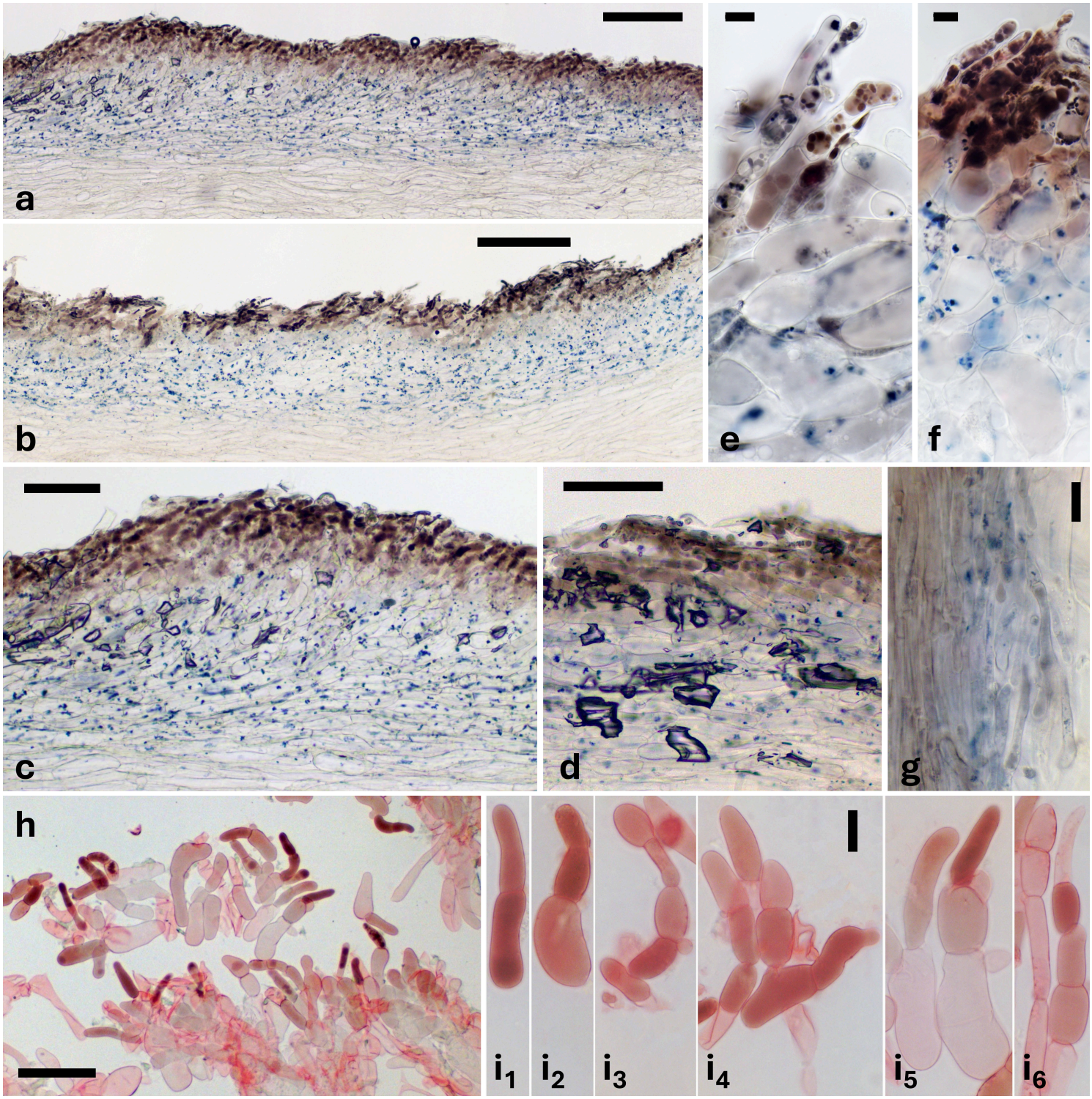

Figure 10

Pileipellis and stipitipellis of *Entoloma olidoatrocyaneum*. **a, b**Bicolored pileipelles (in water). **c, d** Detail of pileipelles (in water). **e, f** Higher-magnification views of the pileipelles (in water). **g**Stipitipellis just below the pileus (in water). **h** Squashed pileipellis (in KOH with CR). **i** Details of suprapellis hyphae (in KOH with CR). **a, c, g–i** OSA-MY-16510; **b, d** OSA-MY-16511; **e, f** OSA-MY-16515. Bars: **a, b** 200 µm; **c, d, h** 100 µm; **g, i** 20 µm; **e, f**10 µm.

Supplementary Files

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- SupplementaryTableS1.docx