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Rossbeevera subalbida (Boletaceae, Boletales), a new, rare truffle-like species from Japan

国内に稀産する新種トリュフ型菌、ウノハナツチダマタケ *Rossbeevera subalbida*（イグチ目イグチ科）

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Abstract

The genus *Rossbeevera* comprises truffle-like species characterized by rubbery basidiomata with bluish discoloration and fusoid to citriform basidiospores with 3–5 prominent longitudinal ridges. In this study, *Rossbeevera subalbida* is described as a new species from Japan based on morphological characteristics and phylogenetic analyses of the internal transcribed spacer region and the large subunit (28S) of the nuclear ribosomal RNA gene. To date, this species has been collected only sporadically in association with evergreen *Quercus* spp. and *Castanopsis sieboldii* across Honshu, Kyushu and Hachijo Island. Morphologically, this species is characterized by its persistent, white to whitish peridium and basidiomata with very weak bluish discoloration. The phylogenetic analyses revealed that *R. subalbida* is sister to *R. griseovelutina*, a more common, widely distributed species throughout Japan. Key diagnostic characters separating *R. subalbida* from other morphologically similar *Rossbeevera* species are provided, and the rarity of this species and its potential distribution are also discussed.

要旨

トリュフ型地下生菌からなるツチダマタケ属 *Rossbeevera* は、青変性のある子実体と紡錘形～レモン型で 3–5 つの大きな畝をもつ担子胞子を特徴とする一群である。本研究では、本州、九州および八丈島のカシ類及びスダジイ樹下の計 3 地点から採集された本属菌標本の形態学的検討および分子系統解析結果に基づき、新種 *Rossbeevera subalbida* を記載した。本種は子実体の青変性が弱く、白色～帯白色で残存性の外皮を有する点により形態的に特徴づけられる。核 rDNA の ITS 領域および大サブユニット（LSU）を用いた分子系統解析の結果、本種は日本国内に広く分布する *R. griseovelutina* と系統的に姉妹関係にあることが示された。また、本稿では種の記載、類似種との識別点を詳述したほか、本種の希少性および潜在的分布についても考察を加えた。なお、本種の和名は、子実体の特徴的な色合いを日本の伝統色の一つである卯の花色に見立て、ウノハナツチダマタケ（新称）とする。

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Introduction

The genus *Rossbeevera* T. Lebel & Orihara (Boletaceae, Boletales) is established in 2011 to accommodate truffule-like species that have rubbery basidiomata with bluish discoloration and fusoid to citriform basidiospores with 3–5 large, longitudinal ridges (Lebel et al., 2012). Currently, a total of 11 *Rossbeevera* species has been known from East and Southeast Asia and Australasia (Hosen et al., 2019; Lebel et al., 2012; Orihara, 2018; Orihara et al., 2012a, 2016). The genus belongs to the Leccinoideae and is phylogenetically close to the epigeous bolete genera *Leccinellum* Bresinsky & Manfr. Binder, *Garcileccinum* Ayala-Vásquez & Pérez-Moreno and *Leccinum* Grey, together with its sister truffle-like genus *Turmalinea* Orihara & N. Maek. (Ayala-Vásquez et al., 2023; Orihara et al., 2016, 2021; Wu et al., 2016; note that *Garcileccinum* is currently *nom. inval.* according to the Shenzhen Code Art. 40.1; the genus typifies an invalid species, whose identifier refers to a different taxon).

In Japan, five *Rossbeevera* species have been reported from Honshu, Shikoku, Kyusyu, and Ryukyu Islands (Lebel et al., 2012; Orihara, 2018; Orihara et al., 2016). Since 2014, another unknown *Rossbeevera* species has been sporadically collected from Hachijo Island in the Izu Islands, Honshu and Kyushu. Based on its morphology and phylogeny, I describe it as a new species, *Rossbeevera subalbida*, and clarify its morphological and phylogenetical characteristics in the genus.

Materials and methods

Taxon sampling and morphological observation

Basidiomata of *Rossbeevera* sp. were collected during a field survey in Izu Islands in 2014, in a foray of the Japanese Association for Truffle Science (JATS) in Miyazaki Pref. in 2017, and by a personal contribution to the author. After DNA extraction, the basidiomata were freeze-dried or air-dried at 46°C for later examination. The specimens are deposited in Kanagawa Prefectural Museum of Natural History, Japan (KPM). For light microscopy, hand-cut sections of dried specimens were presoaked in 70% ethanol and 3% KOH. After briefly rinsing out those sections with diluted water, they were mounted in lacto-glycerol or 1% phloxine B aqueous solution. Basidiospore dimensions (e.g., range of spore length spore × width, length of hilar appendages) and their standard deviations (SD) were determined based on 50 measurements. Measurements of basidiospores include the hilar appendage but not spore ornamentation or the pedicel. Both endpoints of the spore dimensions are shown without parentheses. Two additional spore features are shown; the length to width ratio (*Q*; mean *Q*m) and the hilar appendage to spore length ratio (*HA*/*S*; mean *HA*/*S*m) proposed for a key diagnostic value in *Rossbeevera* by Orihara et al. (2012a).

DNA extraction, PCR amplification and sequencing

DNA was extracted from internal tissue of a fresh basidiomata using the Indicating FTA Cards (Whatman International Ltd, Maidstone, England) or the protocol of Izumitsu et al. (2012). Specimens of other taxa newly included for phylogenetic analyses were collected from various localities in Japan (Table 1). PCR amplification of the internal transcribed spacer region (ITS) and the large subunit (LSU [or 28S]) of nuclear ribosomal RNA gene (rDNA) followed Orihara et al. (2012a). PCR primers were ITS1F (Gardes & Bruns, 1993) and ITS4 (White et al., 1990) for the ITS region, and LR0R and LR5 (Vilgalys & Hester, 1990) for LSU rDNA. Cycle sequencing of the PCR products in forward and reverse directions were completed according to Orihara et al. (2012b). Sequences were edited and assembled with Chromas v. 2.6 (https://technelysium.com.au/wp/chromas/) and SeaView v. 5 (Gouy et al., 2021). A total of 32 newly obtained sequences were deposited in the International Nucleotide Sequence Database (INSD) (Table 1).

Phylogenetic analyses

In addition to these newly generated rDNA sequences for this study, I retrieved 44 ITS and LSU rDNA sequences from 23 specimens that cover overall phylogenetic relationships among *Rossbeevera* and *Turmalinea* from INSD (Table 1). *Leccinellum griseum* (Quél.) Bresinsky & Manfr. Binder, *Leccinellum albellum* (Peck) Bresinsky & Manfr. Binder, and *Leccinum* aff. *intusrubens* (Corner) Høil were selected as outgroups for the analyses. ITS and LSU datasets were independently aligned with the online version of MAFFT version 7 (Katoh & Standley, 2013) under default settings (i.e., the alignment algorithm is automatically selected from FFT-NS-1, FFT-NS-2, FFT-NS-i or L-INS-i). Subsequently, the sites with obvious alignment errors were manually adjusted in SeaView. The ITS and LSU datasets were then concatenated using the "concatenate" option in SeaView. The results of the GBlocks option in SeaView (Castresana, 2000) was referred to exclude ambiguously aligned sites. Accordingly, the long nucleotide insertion at the 3' end of ITS2 found in *Rossbeevera* and *Turmalinea* spp. (Orihara et al., 2016) was partially included in the dataset. The concatenated dataset was partitioned into ITS1-5.8S-ITS2 and LSU. The best substitution model for each partition was estimated by the Akaike Information Criterion (AIC) under jModelTest2 (Darriba et al., 2012).

Maximum likelihood (ML) analyses were performed with RAxML 8.2.10 (Stamatakis, 2014). RAxML analysis was conducted under the GTR+G+I model with rapid bootstrap replicates set to 1000.

Table 1. Specimens and nucleotide sequences used for phylogenetic analyses in this study. Newly generated sequences for this study are shown in bold
表 1. 分子系統解析に用いた標本の情報およびヌクレオチド配列． 本研究において新たに INSD に登録された配列は太字で示した

Taxon	Locality	Voucher no.	GenBank acc. no.	
			ITS	LSU
Leccinellum albellum	USA, NC, Buncombe Co.	FLAS-F-61741	MH488723	MH620337
Leccinellum griseum	Japan, Tottori Pref., Tottori-shi	KPM-NC 17832	**PZ191805**	JN378509
Leccinum aff. *intusrubens*	Japan, Mie Pref., Matsusaka-shi	KPM-NC 27950	**PZ191806**	**PZ191450**
Rossbeevera paracyanea	Japan, Nara Pref., Nara-shi	KPM-NC 18087	KJ001086	KJ001100
Rossbeevera paracyanea	Japan, Kagoshima Pref., Yakushima Isl.	KPM-NC 27944	**PZ191807**	**PZ191451**
Rossbeevera paracyanea	Japan, Nara Pref., Nara-shi	KPM-NC 18086	**PZ191808**	**PZ191452**
Rossbeevera paracyanea	China, Guangdong Prov., Boluo Co., Xiangtoushan Natl. Nat. Reserve	GDGM 45913	MH532534	MH537793
Rossbeevera paracyanea	China, Guangdong Prov., Dinghu District	KPM-NC 28186	MT934784	MT812704
Rossbeevera paracyanea	China, Guangdong Prov., Baiyun District	GDGM 46631	MK035705	MK036348
Rossbeevera aff. *vittatispora*	Australia, New South Wales,	TO-AUS-46	KC551978	JN378507
Rossbeevera vittatispora	Australia, New South Wales, Southern Tablelands	MEL2128491	MT934781	KX685725
Rossbeevera aff. *westraliensis*	Australia, Victoria, East Gippsland	TO-AUS-72	KC551977	KC552025
Rossbeevera pachydermis	New Zealand, South Isl., Fiordland National Park	MEL 2079341	KC551974	KC552021
Rossbeevera pachydermis	New Zealand, North Isl., Te Urewera National Park	KPM-NC 23336	KJ001088	KJ001095
Rossbeevera griseovelutina	Japan, Tokyo Met., Hachijo Isl.	KPM-NC 25968	**PZ191809**	**PZ191453**
Rossbeevera griseovelutina	Japan, Tokyo Met., Hachijo Isl.	KPM-NC 25980	**PZ191810**	**PZ191454**
Rossbeevera griseovelutina	Japan, Tokyo Met., Hachijo Isl.	KPM-NC 25974	**PZ191811**	**PZ191455**
Rossbeevera griseovelutina	Japan, Okinawa Pref., Kumejima Isl.	KPM-NC 26881	**PZ191812**	**PZ191456**
Rossbeevera griseovelutina	Japan, Chiba Pref., Kamogawa-shi	KPM-NC 25347	**PZ191813**	**PZ191457**
Rossbeevera griseovelutina	Japan, Nagano Pref., Iida-shi	KPM-NC 18094	KJ001091	KJ001101
Rossbeevera griseovelutina	Japan, Kanagawa Pref., Odawara-shi	KPM-NC 18044	KC551986	KC552033
"*Rossbeevera subalbida*"	Japan, Tokyo Met., Hachijo Isl.	KPM-NC 25954	**PZ191814**	**PZ191458**
"*Rossbeevera subalbida*"	Japan, Miyazaki Pref., Aya-cho	KPM-NC 25692	**PZ191815**	**PZ191459**
"*Rossbeevera subalbida*"	Japan, Osaka Pref., Yao-shi	KPM-NC 25099	**PZ191816**	**PZ191460**
Rossbeevera cryptocyanea	Japan, Kagoshima Pref., Amami-oshima Isl., Yamato-son	KPM-NC 17843	**PZ191817**	KC552030
Rossbeevera cryptocyanea	Japan, Miyazaki Pref., Aya-cho	KPM-NC 25699	**PZ191818**	**PZ191461**
Rossbeevera cryptocyanea	Japan, Okinawa Pref., Ishigaki Isl.	KPM-NC 23387	KP222893	KP222899
Rossbeevera eucyanea	Japan, Tottori Pref., Tottori-shi	TNS-F-36986	HQ693875	HQ693880
Rossbeevera eucyanea	Japan, Kagoshima Pref., Amami-oshima Isl., Yamato-son	KPM-NC 17844	KC551984	KT581439
Rossbeevera yunnanensis	China, Yunnan Prov., Chu Xang Pref.	KPM-NC 17850	KC551990	JN979437
Rossbeevera yunnanensis	Japan, Hiroshima Pref., Hatsukaichi-machi	KPM-NC 23352	MF357925	MF354015
Turmalinea persicina	Japan, Kyoto Pref., Kyoto-shi Sakyo-ku	KPM-NC 18001	KC551991	KC552038
Turmalinea persicina	Japan, Aichi Pref., Okazaki-shi	KPM-NC 26137	**PZ191819**	**PZ191462**
Turmalinea persicina	Japan, Nagasaki Pref., Tsushima Isl.., Tsushima-shi	KPM-NC 31939	**PZ191820**	**PZ191463**
Turmalinea yuwanensis	Japan, Okinawa Pref., Tokashiki Isl.	KPM-NC 23377	KJ001092	KJ001098
Turmalinea mesomorpha subsp. *mesomorpha*	Japan, Hokkaido, Kaminokuni-cho	KPM-NC 27158	**PZ191821**	**PZ191464**
Turmalinea mesomorpha subsp. *mesomorpha*	Japan, Ehime Pref., Matsuyama-shi	KPM-NC 17743	KC552002	KC552050
Turmalinea chrysocarpa	China, Yunnan Prov., Honghe Hani and Yi Autonomous Pref., Gejiu City	KPM-NC 18068	KC552003	KC552051

Bayesian analyses were conducted with MrBayes 3.2 (Ronquist & Huelsenbeck, 2003). The GTR+I+G was selected as the best-fit model for the both partitions. Bayesian posterior probabilities (PPs) were estimated by the Metropolis-coupled Markov chain Monte Carlo method (Geyer, 1991). The two parallel runs were conducted with one cold and seven heated chains each for 3M generations. Temperature parameter of the seven heated chains in both runs was set to 0.20 (as a default). Trees were saved to a file every 1000th generation. I determined that the two runs reached convergence when the average standard deviation of split frequencies (ASDSF) was continuously lower than 0.01. The ASDSF was monitored every 5000 generations. Trees obtained before reaching convergence were discarded as the burn-in, and the remaining trees were used to calculate a 50% majority consensus topology and to determine PP values for individual branches.

Results

Phylogenetic analyses

The ITS-LSU combined dataset was composed of 1851 aligned nucleotide positions. In the RAxML analysis, the final ML optimization of log likelihood (lnL) was -2662.341248. In the Bayesian inference, the two parallel runs reached convergence after *ca*. 535,000 generations. Accordingly, the first 540 trees in each run were discarded, and the remaining 2461 trees in each run were summarized to approximate Bayesian PPs. The total arithmetic and harmonic mean of estimated marginal lnL for runs were -7644.44 and -7680.43, respectively.

Because the resulting overall ML and Bayesian topologies were congruent, only the Bayesian tree is shown (Fig. 1). The tree mostly recovered overall relationships within *Rossbeevera* and *Turmalinea*. The three *Rossbeevera* sp. samples shown as "*R. subalbida*" in Fig. 1 formed a strongly supported clade sister to *R. griseovelutina* Orihara, a common species widely distributed across Japan excluding Hokkaido. Genetic divergence between the two lineages was comparable to other sister species within the genus. Within "*R. subalbida*," the specimen collected from Hachijo Island, Tokyo, was slightly divergent from those collected from Miyazaki and Osaka Prefectures (nucleotide identity in ITS: 841 bp/851 bp [98.82%]; query coverage: 100%).

Taxonomy

The unidentified *Rossbeevera* specimens can be morphologically distinguished from other *Rossbeevera* species as described below. The phylogenetic analyses based on ITS-LSU rDNA dataset robustly support its distinctness within the genus *Rossbeevera* (Fig. 1). Accordingly, I herein propose *R. subalbida* as a new species.

Rossbeevera subalbida Orihara, **sp. nov.**

MycoBank # 862521

Fig. 2.

Etymology: The Latin, *subalbida* means "rather whitish," referring to the distinctive color of the basidiomata.

Holotype: JAPAN, Miyazaki Pref., Higashimorokata-gun Aya-cho, Kawanaka Natural Park, under *Quercus gilva*, 22 July 2017, *T. Orihara*, KPM-NC 25692.

Description: Basidiomata solitary or sparse, subglobose to depressed-globose, 10–22 mm in diam. at maturity, soft-rubbery, surface smooth to somewhat felty, white, in some cases tinged with pale brown or greyish in patches, finally becoming light brown to beige, often gradually turning light blue-green when rubbed, bruised or injured, but the reaction is weak, with a short stipe up to 3.5 mm high at the base. Peridium thin, persistent. Gleba whitish when immture, becoming dark reddish brown to blackish brown at maturity, of dense, minute locules. Stipe tissue subgelatinous, sometimes gradually turning sky blue when cut. Rhizomorph present at the bottom of the stipe, white. Odor sweetish but not pleasant.

Peridium 50–350 µm thick, of densely interwoven, thin-walled, pale ochraceous to colorless filamentous hyphae 2.5–10 µm broad, partially inflated up to 18 µm broad. Peridiopellis composed of loosely interwoven, branched, thin-walled (<0.6 µm), colorless filamentous hyphae 2.5–7 µm broad. Sterile base of colorless pseudomarenchymatous cells 20–55 µm in diam. Tramal hyphae running parallel to hymenia, 2–6 µm broad, oleiferous hyphae sparsely present within the tramal hyphae. Basidia 16–22 × 7–11 µm, 3- or 4-spored, clavate to clavulate, evanescent, colorless. Basidioles abundant on hymenium, 11–17 µm in diam., inflated, clavulate or balloon-like, colorless. Subhymenium absent. Basidiospores fusiform to citriform, 13.6–17.4 × 6.5–8.4 µm, mean 15.2 × 7.4 µm, SD: 0.89 × 0.46 µm, Q = 1.8–2.4, Qm = 2.1, smooth, inamyloid, ochraceous brown to reddish brown at maturity, with 3–5 longitudinal ridges 1.2–2 µm high, with a developed hilar appendage 1.6–3.6 µm (mean µm), *HA*/*S* (length of a hilar appendage divided by spore length) = 0.11–0.23, *HA*/*S*m = 0.17, spore walls 0.5–0.8 µm thick.

Habitat, distribution and fruiting seasons: Sub-hypogeous under evergreen *Quercus* spp. and *Castanopsis sieboldii*, presumably ectomycorrhizal with these trees; Japan (Honshu, Kyushu and Izu Islands); summer (July to August).

Japanese name: *Unohana-tsuchidamatake* (*Unohana* refers to a traditional Japanese color inspired by *Deutzia crenata* flowers, characterized by a white hue with a pale yellow or bluish tint; *tsuchidamatake* is a Japanese name of the genus *Rossbeevera*).

Other specimens examined: JAPAN, Tokyo Met., Hachijo Island, Hachijo-machi, Nakanogoh, under *C. sieboldii*, 15 July 2015, *T. Orihara*, KPM-NC 25954; Osaka Pref., Yao-shi, Nishi-kyuhoji, Kyuhoji Ryokuchi Park, under *Q. glauca*, 10 July 2021, *N. Mori*, KPM-NC 25098; *ibid*, 11 July 2021, *N. Mori*, KPM-NC 25099; Miyazaki Pref., Higashimorokata-gun Aya-cho, Kawanaka Natural Park, under *Quercus gilva*, 22 July 2017, *Y. Sunada*, KPM-NC 25693.

Remarks: A sister species, *Rossbeevera griseovelutina*, resembles

R. subalbida in the relatively weak discoloration of basidiomata. Although *R. griseovelutina* occasionally has a whitish peridium similar to that of *R. subalbida*, it can be distinguished by more or less developed peridiopellis (i.e., pileipellis) on the peridial surface, and the larger, fusoid to fusiform basidiospores 14.4–31.9 × 6.7–10.4 µm with fewer ridges (mostly 3, more rarely 4). Two Australian species, *R. vittatispora* (G.W. Beaton, Pegler & T.W.K. Young) T. Lebel and *R. westraliensis* T. Lebel also resemble *R. subalbida*, but differ in their significantly smaller basidiospores and their host association with *Eucalyptus* spp. (Lebel et al., 2012). Another rare species, *R. yunnanensis* Orihara & M.E. Sm., which is reported from China and Japan, is macromorphologically similar to *R. subalbida*, but the former has a thinner peridium with more distinct discoloration and longer, fusoid to fusiform basidiospores (Q = 2–3.8, Qm = 3.1 [isoptype]; Orihara, 2018; Orihara et al., 2012a). The holotype of *R. subalbida* was collected during the second truffle foray held by the Japanese Association for Truffle Science in 2017.

Fig. 1. Bayesian tree of *Rossbeevara* and its allied taxa based on the nuclear rDNA ITS-LSU dataset. Values of Bayesian PPs and RAxML rapid bootstrapping (MLBS) are designated above or below branches (PP/MLBS). Three species of *Leccinellum* and *Leccinum* are selected as outgroups. Branches supported by both PP ≥ 0.98 and BS ≥ 75% are depicted as thickened black lines. Branches supported by either PP ≥ 0.98 or BS ≥ 75% are shown as thickened grey lines.

図 1. 核 rDNA ITS 領域および大サブユニットのデータセットに基づく，*Rossbeevera* およびその近縁属のベイズ系統樹．ベイズ事後確率（PP）および RAxML 高速ブートストラップ法（MLBS）の値を各分岐に示す（PP/MLBS）．*Rossbeevera* および *Turmalinea* に近縁である *Leccinellum*（クロヤマイグチ属）および *Leccinum*（ヤマイグチ属）の 3 種をアウトグループに用いた．PP ≥ 0.98 かつ BS ≥ 75% で指示された分岐は黒い太線で，PP ≥ 0.98 もしくは BS ≥ 75% のいずれかで指示された分岐は灰色の太線で表した．

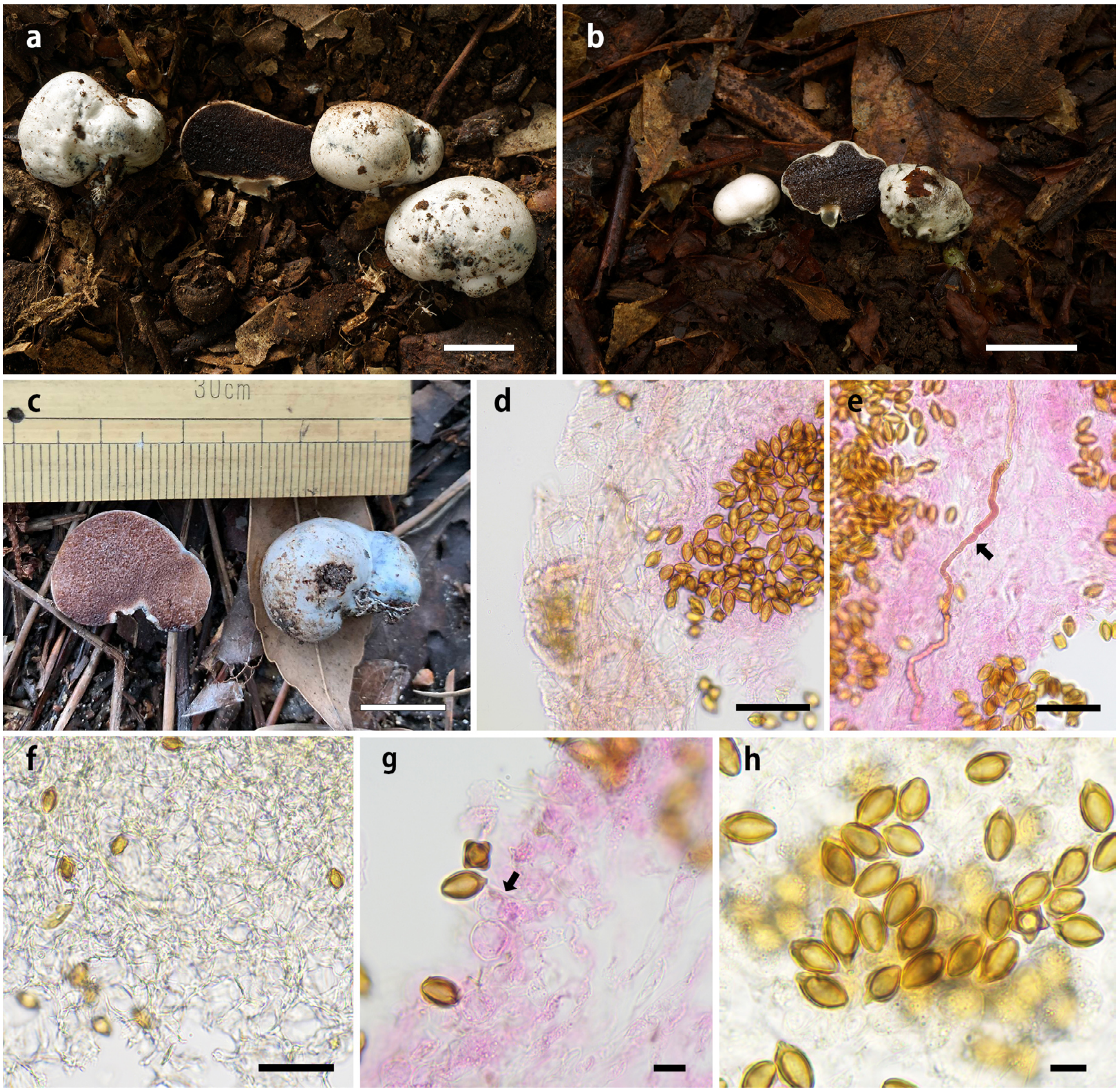

Fig. 2. *Rossbeevera subalbida* Orihara: (a–c) Basidiomata (a: KPM-NC 25692, holotype; b: KPM-NC 25954; c: KPM-NC 25098 [photo by Norito Mori). (d) Peridial tissue. (e) Oleiferous hyphae within tramal tissue (arrow). (f) Stipe tissue. (g) Basidiospores and basidia (arrow). (h) Basidiospores. (d, e, g) stained with 1% phloxine B aqueous solution. Micrographs (d–h) are of the holotype (KPM-NC 25692). Bars: (a–c) 1 cm; (d–f) 50 µm; (g, h) 10 µm.

図 2. *Rossbeevera subalbida* Orihara（ウノハナツチダマタケ）：（a–c）子実体（a: KPM-NC 25692, holotype; b: KPM-NC 25954; c: KPM-NC 25098［撮影：森紀翔］）．（d）外皮を構成する菌糸．（e）基層板の組織中に存在する乳管菌糸（矢印）．（f）短柄を構成する細胞．（g）担子器（矢印）と担子胞子．（h）担子胞子．（d, e, g）1% フロキシン水溶液で染色．顕微鏡写真（d–h）はホロタイプ（KPM-NC 25692）を撮影したもの．スケール：(a–c) 1 cm; (d–f) 50 µm; (g, h) 10 µm.

Discussion

The new species, *R. subalbida*, is characterized by its persistent, white to whitish peridium with very weak bluish discoloration. Despite extensive sampling effort across Japan by many truffle researchers and collectors, this species has been recorded from only three localities to date. This suggests that *R. subalbida* is a rare species, highlighting the need for future conservation of its habitats. The habitats of *R. subalbida* vary from well-preserved evergreen forests (i.e., Aya-cho, Miyazaki Pref.) to a small plantation of *Quercus* trees within an urban park (i.e., Yao-shi, Osaka Pref.). Its sister species, *R. griseovelutina*, is more common in these habitats although it also inhabits cool temperate broadleaved forest. Based on current data, it remains difficult to explain why *R. subalbida* is much rarer than *R. griseovelutina*. Additionally, it is noteworthy that three *R. griseovelutina* specimens collected from Hachijo Island are provided for molecular phylogenetic analyses for the first time in this study.

The phylogenetic analyses based on ITS-LSU rDNA dataset clarified most of the interspecific relationships within *Rossbeevera* with high statistical supports. The relatively weak, bluish discoloration observed in *R. subalbida* and *R. griseovelutina*, compared to other

Rossbeevera spp., may be a synapomorphy of these two sister species. At the intraspecific level, the *R. subalbida* specimen collected from Hachijo Island was genetically divergent from those from Honshu and Kyushu. This divergence may indicate high genetic diversity of *R. subalbida* despite its rarity, suggesting a potentially broader distribution of the species. Although this species is currently considered endemic to Japan, it may also distribute in East Asia since another rare species, *R. yunnanensis*, which had been recorded only once in Yunnan Province, China, was discovered in Hiroshima Prefecture, Japan in 2013, approximately 3,150 km distant from the type locality (Orihara, 2018). Future fieldworks across East Asia may reveal the true distribution of *R. subalbida*.

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