

Original peer-reviewed article (原著論文; 査読有)

First report of a rare sequestrate fungus, *Rossbeevera yunnanensis* (Boletaceae, Boletales) from Japan

希少シクエストレート菌、ウナンツチダマタケ *Rossbeevera yunnanensis* (イグチ目イグチ科) の日本における初報告

Takamichi Orihara

折原 貴道

Kanagawa Prefectural Museum of Natural History, 499 Iryuda, Odawara, Kanagawa 250-0031, Japan

神奈川県立生命の星・地球博物館, 〒250-0031 神奈川県小田原市入生田 499

E-mail: t_orihara@nh.kanagawa-museum.jp

Abstract

A sequestrate, truffle-like bolete species, *Rossbeevera yunnanensis* was described in 2012 based on a single specimen collected from Chuxiong Yi Autonomous Prefecture, Yunnan Province, China. I collected a second specimen from Hiroshima Prefecture, Japan. This is the first report of this truffle in Japan. The overall morphology of the Japanese specimen was identical to the holotype from China, and the internal transcribed spacer region and 28S of the nuclear ribosomal RNA gene between the Japanese and Chinese specimens showed 99.6% and 99.9% similarity, respectively. I discuss the possibility of long-distance spore dispersal of the species.

要旨

トリュフ型(シクエストレート性)イグチ類からなるツチダマタケ属(*Rossbeevera*)の一種、*Rossbeevera yunnanensis*は、2012年に中華人民共和国(中国)雲南省産の標本1点を基に記載された稀産種である。この度、広島県廿日市市の落葉広葉樹林コナラ樹下において、日本新産となる本種子実体を採集したので報告する。本種は極めて薄い白色~類白色の外皮と、紡錘形の大型の担子胞子(平均径 $20.1 \times 7.8 \mu\text{m}$)が特徴で、外皮は手で触れると淡青色に変色する。日本産標本は形態の特徴が中国産基準標本とほぼ一致し、核リボソームRNA遺伝子のITS領域および28S(大サブユニット)の配列についても、両標本はそれぞれ99.6%および99.9%の相同性を示した。このことは、中国南部と日本(本州)という離れた地域間で、比較的短期間に本種の分散が生じたことを示唆している。なお、本種の和名は「ウナンツチダマタケ」とする。

Article Info: Submitted: 24 June 2017 Accepted: 30 November 2017 Published: 31 March 2018

Introduction

The genus *Rossbeevera* T. Lebel & Orihara was proposed in 2011 to accommodate sequestrate (truffle-like) species phylogenetically related to epigeous bolete genera *Leccinum* Gray and *Leccinellum* Bresinsky & Manfr. Binder *s. l.* in the Boletaceae (Lebel et al., 2012). The genus currently includes 10 species from East Asia to Australasia, and is characterized by ellipsoid basidiospores with 3–5 longitudinally ridges and bluish discoloration of the fruitbody surface (Orihara et al., 2016).

Species of the genus presumably form ectomycorrhizas with trees of Fagaceae, Casuarinaceae, Myrtaceae and Nothofagaceae. Orihara et al. (2016) proposed a sister, sequestrate genus *Turmalinea* Orihara & N. Maek. that accommodates four species and one subspecies from Japan and China. This genus is easily distinguished from *Rossbeevera* by the basidiospores with 6–9 longitudinal ridges and a cushion-shaped, colorless sterile base.

Shortly after the proposal of the genus *Rossbeevera* by Lebel

et al. (2012), Orihara et al. (2012a) described an additional species, *R. yunnanensis* Orihara & M. E. Sm. based on a single specimen collected from Mt. Zixi, Chuxiong Yi Autonomous Prefecture, Yunnan Province, China. *Rossbeevera yunnanensis* forms the earliest diverging lineage of *Rossbeevera* (Orihara et al., 2012a, 2016), and is considered an important species to clarify the phylogeography of *Rossbeevera* and *Turmalinea*. In the course of field surveys in Japan, I unexpectedly collected fruitbodies of *R. yunnanensis* in Hiroshima Prefecture, Japan. Here I give a short description and accompanying information on the specimen.

Materials and methods

Taxon sampling and morphological observation

Fruitbodies were collected during the fungal foray in Hiroshima Prefecture held by Mycological Society of Japan during 27–28 Sep. 2013. After DNA extraction, the fruitbodies were air-dried for later examination. The specimen is deposited in Kanagawa Prefectural Museum of Natural History (KPM), Japan. For microscopy, hand-cut sections were mounted in water, 3% KOH, lacto-glycerol, or 1% phloxine B aqueous solution. Basidiospore dimensions (e.g., range of spore length \times spore width, length of hilar appendages) and their standard deviations (SD) were determined based on 30 measurements. The 95% prediction intervals of basidiospore diameter are shown without parentheses in taxonomic descriptions. Both endpoints of the spore dimensions are shown in parentheses. Two additional spore features are shown; the length to width ratio (Q) and the hilar appendage to spore length ratio (HA/S ; Orihara et al., 2012a). Measurements include the hilar appendage but not spore ornamentation or the pedicel.

DNA extraction, PCR amplification and sequencing

DNA was extracted using the FTA Classic Card or Indicating FTA Cards (Whatman International Ltd, Maidstone, England) based on the manufacturer's protocol for plants (www.whatman.com/References/WGI_1397_PlantPoster_V6.pdf). PCR amplification of the internal transcribed spacer region (ITS) and 28S (large subunit; LSU) of the nuclear rRNA gene (rDNA) followed the protocol in Orihara et al. (2012b). 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 28S 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 Sequence Scanner v. 1.0 (Applied Biosystems, Foster City, California, USA), BioEdit v. 7.0.9 (Hall, 1999) and SeaView v. 4 (Galtier et al., 1996). The ITS and 28S rDNA sequences were deposited in the International Nucleotide Sequence Databases (INSD) under the accession numbers MF357925 and MF354015, respectively. Nucleotide sequence similarity was examined using NCBI BLAST searches (<https://blast.ncbi.nlm.nih.gov/BlastAlign.cgi>).

Results

Rossbeevera yunnanensis Orihara & M.E. Sm., Mycotaxon 120: 141.

Fig. 1.

Specimen examined: JAPAN, Hiroshima Prefecture, Hatsukaichi City, Mominoki Forest Park, subhypogeous under *Quercus serrata* Murray, 28 Sep. 2013, T. Orihara, KPM-NC 23352.

INSD (GenBank) ID of the nucleotide sequences: ITS region of rDNA: MF357925; 28S rDNA: MF354015.

Japanese name: Unnan-tsuchidama-take (“Unnan” = Yunnan; “tsuchidama-take” = the Japanese name of *Rossbeevera*).

Morphological and molecular comparison with the isotype specimen

Two fruitbodies were collected in deciduous *Quercus* forest in a temperate, low mountain area. The specimen was morphologically identical to the original description of *Rossbeevera yunnanensis* provided by Orihara et al. (2012a). The fruitbodies were 8 mm and 9.5 mm in diam., and the surface quickly turned light blue or blue-green when touched. The peridium was smooth, and extremely thin or almost absent in some parts of the fruitbodies. While the holotype specimen was only partially mature and the gleba was mostly off-white to beige, one of the fruitbodies of the Japanese specimen was fully mature, and the gleba was blackish brown. The basidiospores were 17.1–(17.8–) 22.9 (–25) \times (7.4–) 7.5–(9.1) –9.2 μm (SD: 1.41 (length), 0.41 (width)), mean $20 \times 8.3 \mu\text{m}$ in diam., $Q = 2.1$ – 2.9 (mean $Q = 2.4$), fusoid to fusiform, brown at maturity, with 3–5 longitudinal ridges up to 1.5 μm high and a basal hilar appendage 2–4.8 μm long ($HA/S = 0.1$ – 0.2 , mean $HA/S = 0.15$).

The ITS and 28S rDNA sequences of the Japanese specimen (KPM-NC 23352) obtained for the study were 791 bp and 923 bp, respectively. The nucleotide similarity of ITS sequence between the Japanese specimen and the holotype was 99.6% (738 bp /741 bp). The similarity of 28S between the two specimens was 99.9% (813 bp /814 bp).

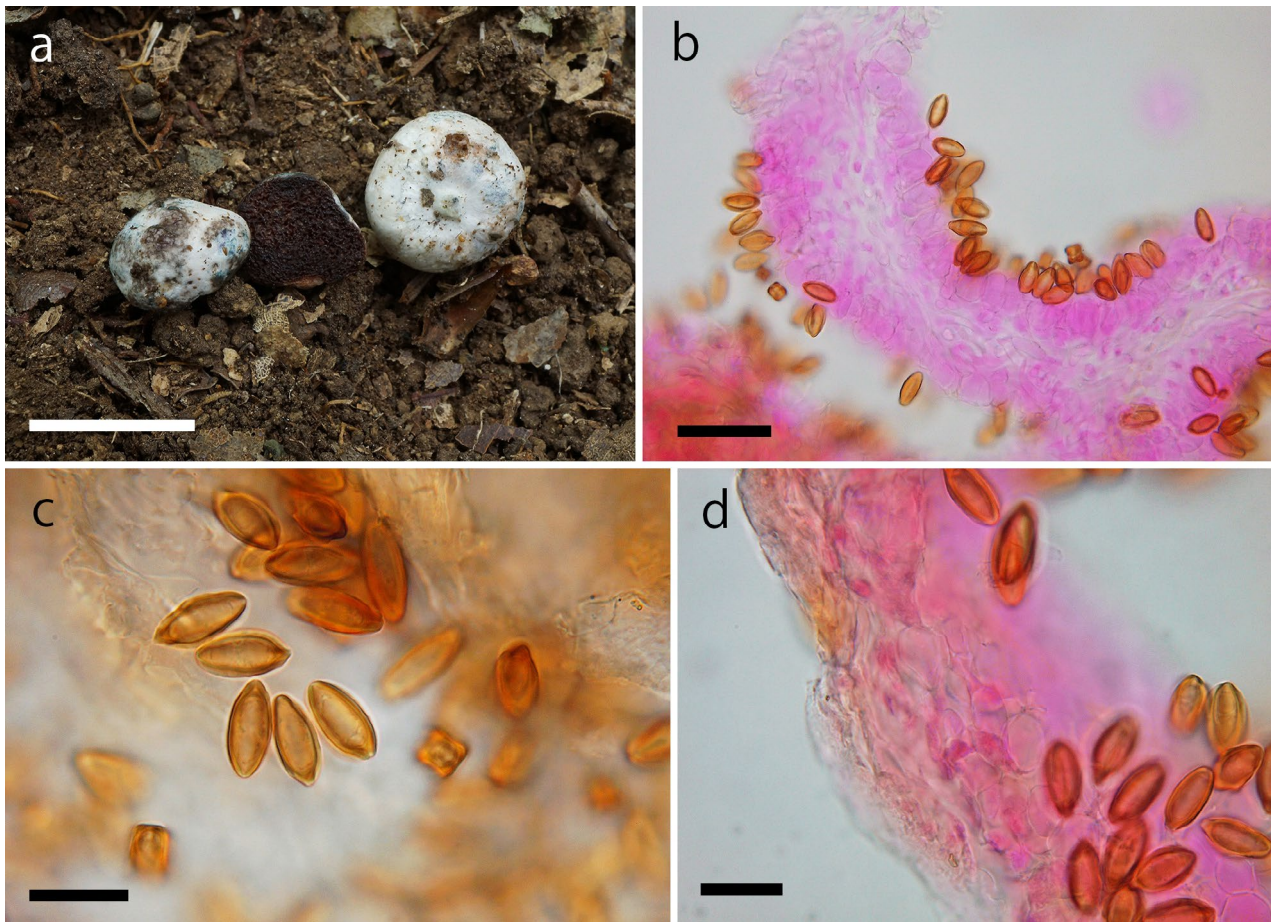


Fig. 1. *Rossbeevera yunnanensis* collected from Hiroshima Prefecture, Japan (KPM-NC 23352): a. Basidiomata (fruitbodies); b. Trama, hymenia and basidiospores (stained with 1% phloxine aqueous solution); c. Basidiospores mounted in lactoglycerol; d. Peridium and trama (stained with 1% phloxine aqueous solution). Bars: a = 1 cm; b = 50 μ m; c, d = 20 μ m.

図 1. ウンナンツチダマタケ *Rossbeevera yunnanensis* (広島県産標本; KPM-NC 23352) : a. 子実体; b. グレバ基層、子実層および担子胞子 (1% フロキシシン水溶液により染色); c. 担子胞子 (ラクトグリセロールにより封入); d. 外皮およびグレバ基層 (1% フロキシシン水溶液により染色). スケール : a = 1 cm; b = 50 μ m; c, d = 20 μ m.

Discussion

This is the first report of *R. yunnanensis* from Japan and only the second known specimen of the species in the world (Orihara et al., 2012a). The species is characteristic in the genus *Rossbeevera* in the remarkably thin peridium that turns light blue or blue green, and the large, fusoid to fusiform basidiospores (average dimension: 20.1 \times 7.8 μ m; Orihara et al., 2012a). The overall morphology of the Japanese specimen is almost identical to the Chinese holotype specimen. This confirms the accuracy of the description by Orihara et al. (2012a) despite the fact that it was based only on a single specimen. The basidiospore dimension of the Japanese specimen was slightly wider than that of the holotype. This is probably due to the difference of the degree of maturity noted above.

Nucleotide comparison using BLAST searches showed that there were only three nucleotide substitutions in the ITS region between the Chinese and Japanese specimens, and only one

substitution in the 28S rDNA. These findings suggest that they spread across Asia relatively recently, despite the fact that those two collection localities are far apart from each other (i.e., Chuxiong, Yunnan Province, China vs. Hiroshima Prefecture, Japan; ca. 3,150 km in distance). Although it is generally believed that sequestrate, truffle-like basidiomycetes rely on mycophagy by insects or mammals for spore dispersal (Claridge & May, 1994; Fogel & Peck, 1975; Maser et al., 1978), the results suggest that *R. yunnanensis* may be able to disperse its spores over long distances.

Acknowledgements

I am grateful to Dr. Matthew E. Smith for critically reading the manuscript and for giving me valuable suggestions. I thank Ms. Suyama-Watanabe Mai for supporting the molecular experiments for this study. This study was supported partially by JSPS KAKENHI Grant-in-Aid for Young Scientists (B)

(no. 17K15184) and the 27th Pro Natura Fund from Pro Natura Foundation Japan (PNFJ).

References

- Claridge A.W., May T.W. (1994) Mycophagy among Australian mammals. *Australian Journal of Ecology* 19: 251–275.
- Fogel R., Peck S.B. (1975) Ecological studies of hypogeous fungi. I. Coleoptera associated with sporocarps. *Mycologia* 67: 741–747.
- Hall T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 96/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Galtier N., Gouy M., Gautier C. (1996) SEAVIEW and PHYLO_WIN: Two graphic tools for sequence alignment and molecular phylogeny. *Computer Applications in the Biosciences* 12: 543–548.
- Gardes M., Bruns T.D. (1993) ITS primers with enhanced specificity for basidiomycetes: application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113–118.
- Lebel T., Orihara T., Maekawa N. (2012) The sequestrate genus *Rosbeeva* T. Lebel & Orihara gen. nov. (Boletaceae) from Australasia and Japan: new species and new combinations. *Fungal Diversity* 52: 49–71+73.
- Maser C., Trappe J.M., Nussbaum R.A. (1978) Fungal-small mammal interrelationships with emphasis on Oregon coniferous forests. *Ecology* 59: 799–809.
- Orihara T., Smith M.E., Ge Z.-W., Maekawa N. (2012a) *Rossbeevera yunnanensis* (Boletaceae, Boletales), a new sequestrate species from southern China. *Mycotaxon* 120: 139–147.
- Orihara T., Smith M.E., Shimomura N., Iwase K., Maekawa N. (2012b) Diversity and systematics of the sequestrate genus *Octaviania* in Japan: two new subgenera and eleven new species. *Persoonia* 28: 85–112.
- Orihara T., Lebel T., Ge Z.-W., Smith M.E., Maekawa N. (2016) Evolutionary history of the sequestrate genus *Rossbeevera* (Boletaceae) reveals a new genus *Turmalinea* and highlights the utility of ITS minisatellite-like insertions for molecular identification. *Persoonia* 37: 173–198.
- Vilgalys R., Hester M. (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172: 4238–4246.
- White T.J., Bruns T., Lee S., Taylor J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M.A., Gelfand D.H., Sninsky J.J., White T.J. (eds.) *PCR protocols: a guide to methods and applications*. Academic Press, USA, pp. 315–322.