

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

## *Balsamia oblonga* (Helvellaceae), a new species from a subalpine forest in Japan

### 日本の亜高山帯において採集された新種ナガミノツチレイシタケ *Balsamia oblonga* (ノボリリュウ科)

Kohei Yamamoto<sup>1\*</sup>, Naoki Endo<sup>2</sup>, Muneyuki Ohmae<sup>3</sup>, Takamichi Orihara<sup>4</sup>山本 航平<sup>1\*</sup>, 遠藤 直樹<sup>2</sup>, 大前 宗之<sup>3</sup>, 折原 貴道<sup>4</sup>

<sup>1</sup> Department of Botany, National Museum of Nature and Science, 4-1-1 Amakubo, Tsukuba-shi, Ibaraki 305-0005, Japan  
国立科学博物館植物研究部, 〒 305-0005 茨城県つくば市天久保 4-1-1

<sup>2</sup> Fungus/Mushroom Resource and Research Center, Faculty of Agriculture, Tottori University, 4-101 Koyama, Tottori 680-8553, Japan  
鳥取大学農学部附属菌類きのこ遺伝資源研究センター, 〒 680-8553 鳥取県鳥取市湖山町南 4 丁目 101

<sup>3</sup> Hokken Co. Ltd., 7-3 Ekihigashimachi, Mibu-machi, Shimotsuga-gun, Tochigi 321-0222, Japan  
株式会社北研, 〒 321-0222 栃木県下都賀郡壬生町駅東町 7-3

<sup>4</sup> Kanagawa Prefectural Museum of Natural History, 499 Iryuda, Odawara-shi, 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

\* Corresponding author (主著者)

E-mail: kohei081@yahoo.co.jp

## Abstract

*Balsamia* is a member of the family Helvellaceae, and all but one of the 25 species form subterranean truffle-like ascomata. This genus is broadly distributed across the Northern Hemisphere, i.e., Europe, North America, and Asia (China). However, this genus had not been found in Japan. In September 2013, an unidentified truffle-like fungus was collected in a subalpine forest in Gunma Prefecture, Japan. Morphological observations and molecular phylogenetic analyses revealed that this fungus belongs to the genus *Balsamia*. Here, we describe the new species *B. oblonga*. This species is characterized by large, oblong ascospores (up to 33.5 μm) with a large length-to-width ratio (up to 3.3). Phylogenetic analyses resolved this species as a distinct species-level branch.

## 要旨

*Balsamia* はノボリリュウ科に属する子囊菌類で、全 25 種の内 1 種を除くすべてが地中性のトリュフ型の子実体を形成する。これまでにヨーロッパ、北米および中国における分布が報じられてきたが、日本からは未発見であった。2013 年 9 月に群馬県の亜高山帯において採集された未同定菌について、形態観察と分子系統解析を行った結果、本邦初となる *Balsamia* 属種であることが明らかになった。本属既知種と比較した結果、子嚢胞子が大型の長楕円形（長径最大 33.5 μm）で、縦横比（最大 3.3）が大きい点でいずれにも合致せず、系統学的にも独立種であることが支持された。以上の結果に基づき、本種を *B. oblonga* として記載した。本属の和名は、褐色で突起に覆われる外皮と白色の基本体から構成される子実体が、植物のレイシの果実（ライチ）に類似することから、ツチレイシタケ属とする。また、*B. oblonga* の和名は、上記の子嚢胞子の特徴に基づきナガミノツチレイシタケとする。

Article Info: Submitted: 15 December 2020 Accepted: 1 March 2021 Published: 31 March 2021

## Introduction

The genus *Balsamia* (Helvellaceae) is characterized by brown to black sequestrate ascomata covered by warts, ovoid, eight-spored asci, and distinct ellipsoid to cylindrical ascospores (Southworth et al., 2018). This genus is considered ectomycorrhizal (EM) (Tedersoo & Smith, 2013) based on the observation of EM roots formed between *Bal. platyspora* Berk. and *Tilia* (Ceruti & Bussetti, 1962) and between *Bal. alba* Harkn. and *Pinus* (Agerer & Rambold, 2004–2020), and isotopic measurements of the ascoma of *Bal. magnata* Harkn. (Hobbie et al., 2001).

*Balsamia* was described based on a truffle-like species, *Bal. vulgaris* Vittad. (Vittadini, 1831), and currently includes 25 species. By the 1900s, *Bal. ambigua* Petib., *Bal. fusispora* Schulzer, *Bal. platyspora* (= *Bal. fragiformis* Tul. & C. Tul.), and *Bal. polysperma* Vittad. from Europe and *Bal. alba*, *Bal. filamentosa* Harkn., *Bal. magnata*, and *Bal. nigrans* Harkn. from western North America had been described (Tulasne & Tulasne, 1843; Berkeley, 1844; Schulzer, 1870; Harkness, 1899; Petitberghien, 1966). More recently, Southworth et al. (2018) described *Bal. cascadenensis* D. Southw., J.L. Frank & Castellano, *Bal. latisporea* D. Southw., J.L. Frank & Castellano, *Bal. lazyana* Trappe, D. Southw. & Amaranthus, *Bal. limuwensis* D. Southw., J.L. Frank & Castellano, *Bal. pallida* D. Southw., J.L. Frank & Castellano, *Bal. quiericola* D. Southw., M.E. Sm. & J.L. Frank, and *Bal. trappei* D. Southw., J.L. Frank & Castellano from western North America, and *Bal. alba*, *Bal. filamentosa*, *Bal. magnata*, *Bal. nigrans*, and *Bal. setchellii* (E. Fisch.) Trappe, D. Southw. & Castellano (= *Pseudobalsamia setchellii* E. Fisch.) were emended. Hansen et al. (2019) expanded the definition of *Balsamia* based on morphological and phylogenetic studies, transferring a cup-shaped epigeous fungus, *Acetabula aestivalis* R. Heim & L. Rémy, to *Balsamia* and synonymizing the genus *Barssia*. They also transferred four Mediterranean *Barssia* species (i.e., *Bar. gunerii* H.H. Doğan, Bozok & Taşkın, *Bar. hellenica* Kaounas, Agnello, P. Alvarado & Slavova, and *Bar. maroccana* G. Moreno, Manjón, Carlavilla & P. Alvarado) and North America (*Bar. oregonensis* Gilkey) to *Balsamia*.

By contrast, in Asia, *Bal. platyspora* was reported from China only once (Liu & Tao, 1990), and this genus had not been found in Japan. In recent years, three new species of *Balsamia* (*Bal. guozigouensis* (L. Fan & Y.Y. Xu) L. Fan & Y.Y. Xu, *Bal. lishanensis* L. Fan & Y.Y. Xu, and *Bal. hyashanensis* (L. Fan & Y.Y. Xu) L. Fan & Y.Y. Xu) were described from China (Xu et al., 2018, 2020), and the diversity of this genus in East Asia is becoming more clearly known. During our taxonomic study of sequestrate fungi from subalpine forest in Japan, an unidentified ascomycete was examined. Based on morphological

observations and molecular phylogenetic analyses, this fungus was identified as a new species of *Balsamia*. Here, we describe this species as *Bal. oblonga*.

## Materials and methods

### Morphological observations

Specimen was collected in Katashina-mura, Gunma Prefecture in September 2013. Specimen was photographed and oven-dried at 60°C. For light microscopy, hand-cut sections of the dried specimen were reconstituted in water for 24 h and then mounted in water or 5% KOH. Melzer's reagent was used to test the amyloid reaction of the asci and ascospores. All measurements were made using PhotoRuler version 1.1.3 (<http://inocybe.info/>). *Q* value was obtained from the length-to-width ratio of mature ascospores. The specimen is deposited in the herbarium of the Kanagawa Prefectural Museum of Natural History (KPM) and National Museum of Nature and Science, Tokyo (TNS) in Japan.

### DNA sequencing and phylogenetic analyses

DNA was extracted as described by Izumitsu et al. (2012) with minor modifications (Yamamoto et al., 2019). PCR amplification of the internal transcribed spacer (ITS) region and the large subunit (LSU) of nuclear ribosomal DNA was performed using the primer pair ITS1F (Gardes & Bruns, 1993) and LR5 (Vilgalys & Hester, 1990). PCR was performed in a total volume of 25 µL containing 5 µL dNTPs (0.4 mM each), 0.75 µL each primer (0.3 µM each), 12.5 µL 2× PCR buffer for KOD FX Neo (Toyobo, Osaka, Japan), 0.5 µL 1.0 U µL<sup>-1</sup> KOD FX Neo (Toyobo), 1.25 µL template DNA, and 4.25 µL sterile distilled water. The PCR conditions were 94 °C for 2 min and 35 cycles of 98 °C for 10 s, 58 °C for 30 s, and 68 °C for 45 s. The PCR products were purified using illustra™ ExoProStar™ (GE Healthcare, Buckinghamshire, UK) and sent to Macrogen Japan (Tokyo, Japan) for sequencing using four primers: ITS1F, ITS4 (White et al., 1990), LR0R (Vilgalys & Hester, 1990), and LR5. The resulting bidirectional sequences were edited using 4Peaks 1.8 (<http://nucleobytes.com/4peaks>) and assembled using MEGA X (Kumar et al., 2018). Newly generated sequences were deposited in the DNA Data Bank of Japan (<http://www.ddbj.nig.ac.jp>) under accession numbers LC600234 (ITS) and LC600235 (LSU).

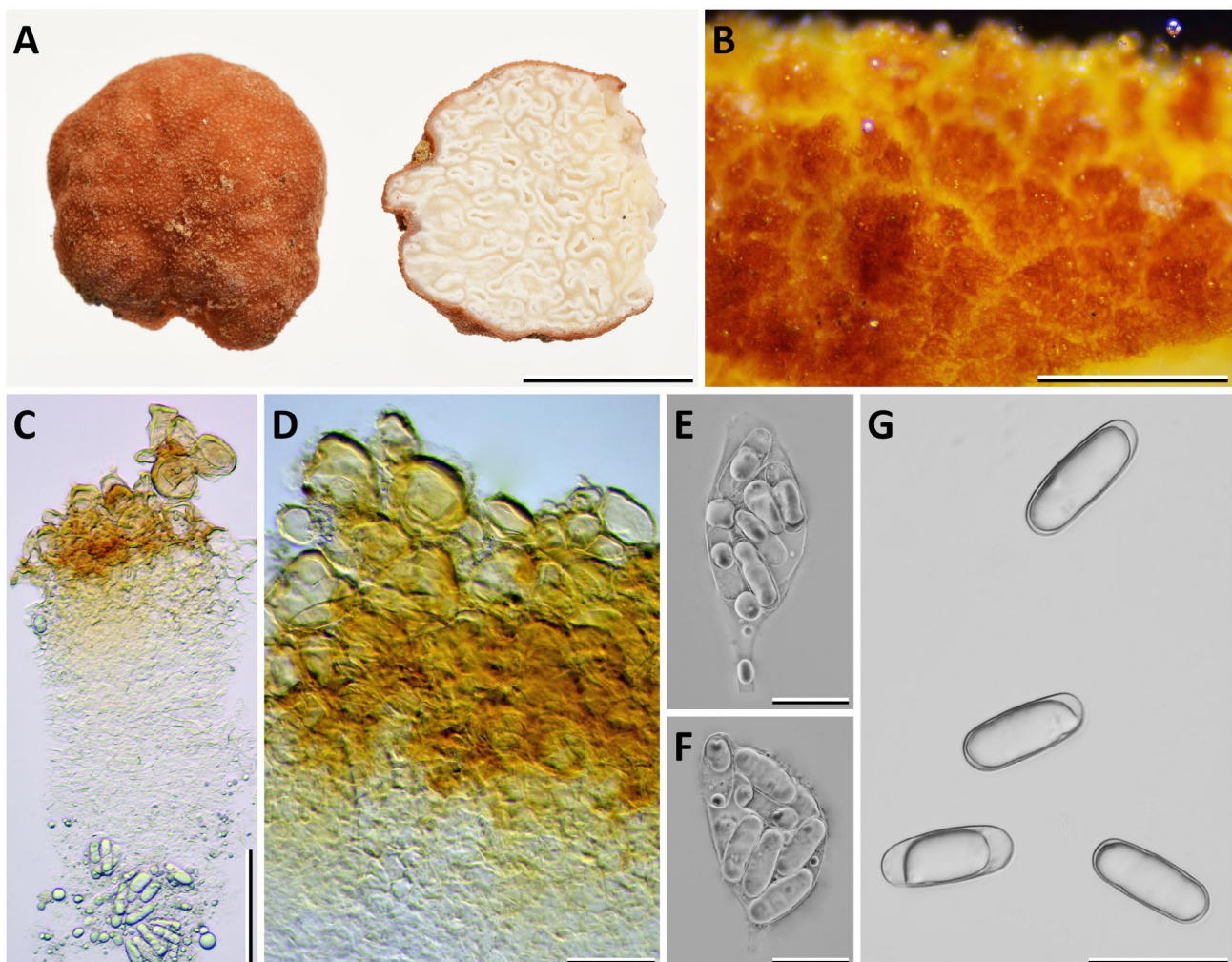
For phylogenetic analyses, 102 ITS sequences and 34 LSU sequences from *Balsamia* spp. were retrieved from NCBI (<https://www.ncbi.nlm.nih.gov/nucleotide>) and included in the dataset. *Wynnella subalpina* Q. Zhao, Zhu L. Yang & K.D. Hyde and *Helvella dryophila* Vellinga & N.H. Nguyen (Helvellaceae) were selected as outgroups. The sequences were aligned following Yamamoto et al.

(2020). Ambiguously aligned sites were removed using the Gblocks server ([http://molevol.cmima.csic.es/castresana/Gblocks\\_server.html](http://molevol.cmima.csic.es/castresana/Gblocks_server.html)) under the least stringent setting. Topological conflicts between the two trees (ITS, 724 bp; LSU, 839 bp) were checked directly by topological comparison of the preliminary maximum likelihood (ML) trees. Next, the two datasets were combined into a single 1563 bp dataset and deposited in TreeBASE (accession URL: <http://purl.org/phylo/treebase/phyloids/study/TB2:S27402>). ML analysis of the combined dataset was conducted based on Yamamoto et al. (2020) using raxmlGUI 1.31 (Silvestro & Michalak, 2012) under a general time reversible model of nucleotide substitution, with a discrete gamma distribution (+G) and invariant sites (+I), selected by MEGA X. A maximum parsimony (MP) analysis was conducted based on Yamamoto et al. (2020).

## Results

An ascoma was collected from a subalpine forest dominated by *Abies* and *Betula* which were potential host trees. The macroscopic characters, i.e., a sequestrate, reddish-brown ascoma without folding and apical depression, and opened glebal chambers (Fig. 1), were similar to those of *Bal. ambigua*, *Bal. lazyana*, *Bal. lishanensis*, *Bal. platyspora*, *Bal. polysperma*, *Bal. trappei*, and *Bal. vulgaris* (Hawker, 1954; Montecchi & Sarasini, 2000; Southworth et al., 2018; Xu et al., 2020). However, the ascospores were narrower than those of *Bal. vulgaris* and longer than those of the remaining species (Fig. 2).

ITS (1093 bp) and LSU (869 bp) sequences were obtained from the specimen. A NCBI BLAST ([https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE\\_TYPE=BlastSearch](https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch)) search showed that ITS sequence from the Japanese specimen was most similar to that of *Balsamia* spp. and had the highest homology (88% [640 / 726]) with that of



**Fig. 1.** *Balsamia oblonga* (KPM-NC 28613) collected from Gunma, Japan. (A) Ascoma showing surface (left) and gleba (right). (B) Surface view of the peridium. (C) Peridium (upper) and gleba (lower) mounted in 5% KOH. (D) Peridium mounted in 5% KOH. (E–F) Asci mounted in water. (G) Ascospores mounted in water. Bars: A = 1 cm; B = 500  $\mu$ m; C = 100  $\mu$ m; D = 50  $\mu$ m; E–G = 30  $\mu$ m.

**図 1.** 群馬県産 *Balsamia oblonga* (KPM-NC 28613)。A: 子実体。右は断面。B: 外皮表面。C: 外皮(上部)および子実層(下部)の断面(5% KOH 封入)。D: 外皮(5% KOH 封入)。E–F: 子嚢(水封)。G: 子嚢胞子(水封)。スケール: A = 1 cm; B = 500  $\mu$ m; C = 100  $\mu$ m; D = 50  $\mu$ m; E–G = 30  $\mu$ m。



*Bal. lishanensis* (MT232904). Fig. 3 shows the ML phylogeny of the combined ITS and LSU dataset (ln L = -10449.792713). The MP statistics were as follows: tree length = 1723, consistency index = 0.564, retention index = 0.896, and composite index = 0.552. The genus *Balsamia* formed a well-supported clade, and monophyly of each species in the genus was well supported, except for *Bal. platyspora* and *Bal. vulgaris*. The sequence from the Japanese specimen belonged to the clade composed of the six abovementioned species (except for *Bal. ambigua*) and environmental sequences from uncultured EM (Bootstrap support: 96 / - ), and the specimen was formed a sister lineage to *Bal. lishanensis*.

**Taxonomy**

***Balsamia oblonga* Koh. Yamam., N. Endo, Ohmae & Orihara, sp. nov.**

[Mycobank ID: MB 838245]

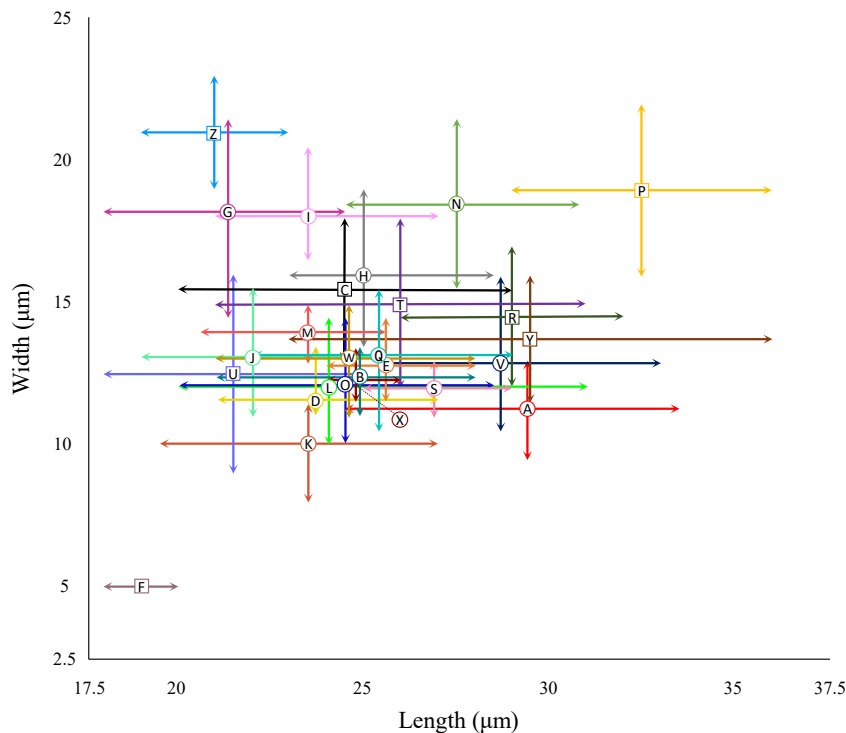
**Diagnosis:** This species is distinguishable from other *Balsamia* spp. by its long, slender ascospores.

**Etymology:** From the Latin *oblongus* = oblong, referring to the oblong ascospore of this species.

**Type:** JAPAN, Gunma Prefecture, Katashina-mura, a mountain trail on Mt. Nikko-Shirane, semi-hypogeous under a subalpine forest dominated by *Abies veitchii* Lindl., *A. mariesii* Mast., and *Betula ermanii* Cham., 27 Sep. 2013, N. Endo (holotype, KPM-NC 28613; isotype, TNS-F-82005).

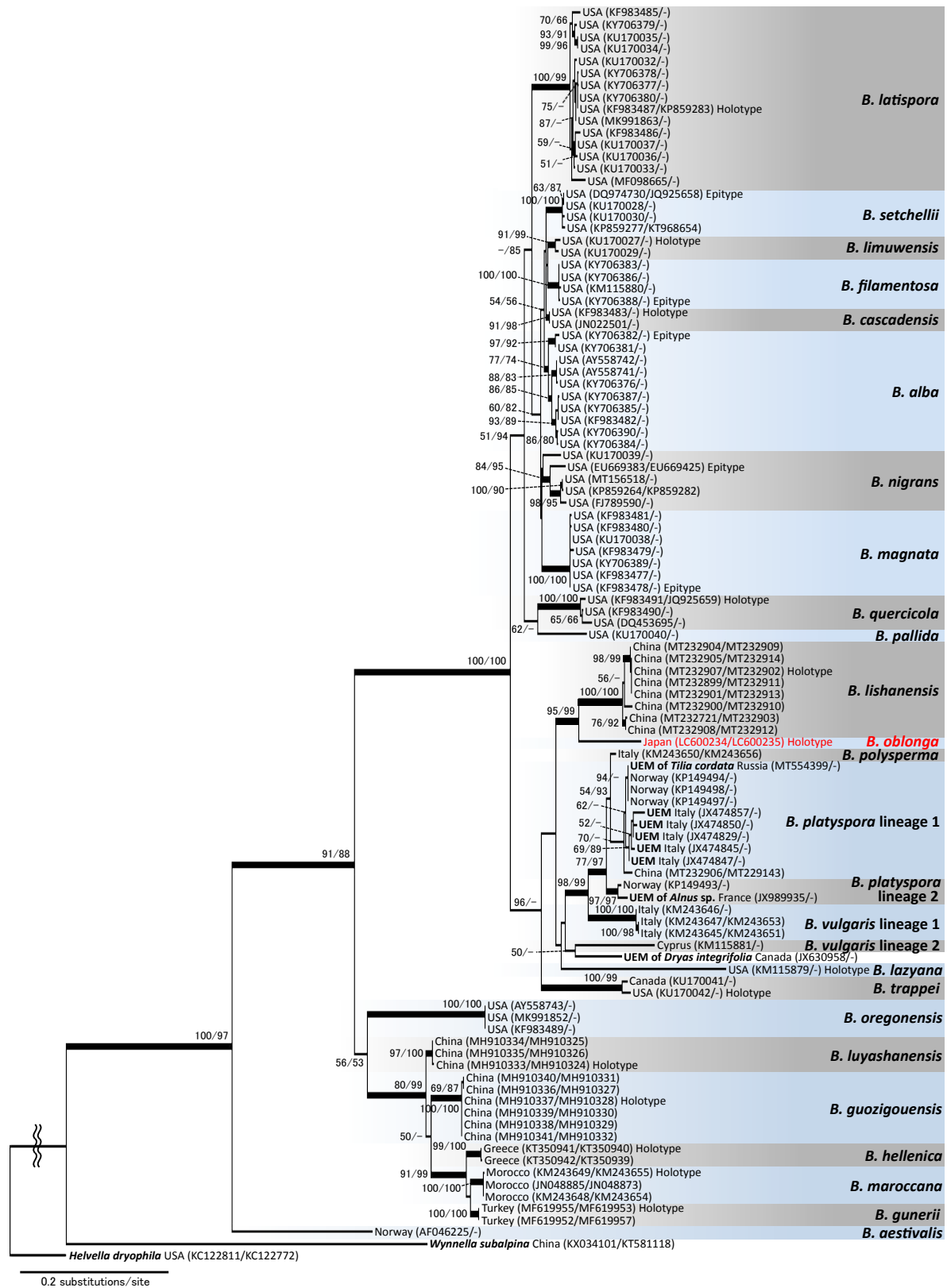
**Description:** Ascoma pychothecium, closed, spheroid, 18 × 17 mm; surface brick-red, warty. Gleba chambered, white; chambers open, irregular, up to 0.8 mm wide. Odor not recorded. Peridium brown to pale brown in 5% KOH, 100–240 μm thick; outer layer strongly pigmented, textura globulosa, 50–165 μm thick, composed of 4 to 5 layers of cells 10–42 μm wide, wall up to 8 μm thick; inner layer textura angularis, 62–88 μm thick, cells 9–21 μm wide, wall up to 2 μm thick. Glebal tissue of textura intricata; composed of hyphae

Fig. 1.



**Fig. 2.** Measurements of the ascospores of sequestrate species in *Balsamia* and *Barssia yezo-montana*. A = *Bal. oblonga*; B = *Bal. alba*; C = *Bal. ambigua*; D = *Bal. cascadenensis*; E = *Bal. filamentosa*; F = *Bal. fusispora*; G = *Bal. gunerii*; H = *Bal. guozigouensis*; I = *Bal. hellenica*; J = *Bal. latispora*; K = *Bal. lazyana*; L = *Bal. limuwensis*; M = *Bal. lishanensis*; N = *Bal. luyashanensis*; O = *Bal. magnata*; P = *Bal. maroccana*; Q = *Bal. nigrans*; R = *Bal. oregonensis*; S = *Bal. pallida*; T = *Bal. platyspora*; U = *Bal. polysperma*; V = *Bal. quercicola*; W = *Bal. setchellii*; X = *Bal. trappei*; Y = *Bal. vulgaris*; Z = *Bar. yezo-montana*. Sources of ascospore size: Southworth et al. (2018) for B, D, E, J–L, O, Q, S, V–X; Petitberghien (1966) for C; Schulzer (1870) for F; Doğan et al. (2018) for G; Xu et al. (2018) for H and N; Kaounas et al. (2015) for I; Xu et al. (2020) for M; Crous et al. (2014) for P; Gilkey (1939) for R; Pegler et al. (1993) for T and Y; Montecchi & Sarasini (2000) for U; Kobayasi (1937) for Z. Circles, mean values; squares, median values.

**図 2.** *Balsamia* に属す地下生菌およびツチクレタケ (*Barssia yezo-montana*) の子嚢胞子サイズの比較。縦軸と横軸はそれぞれ幅と長さを示す。円および四角の位置はそれぞれ、幅と長さの平均値および中間値を示す。各アルファベットに対応する種名およびサイズの出典は英文キャプションを参照。



**Fig. 3.** Maximum likelihood (ML) phylogenetic tree of the combined dataset of internal transcribed spacer (ITS) and large subunit (LSU) sequences of *Balsamia*. *Wynnella subalpina* and *Helvella dryophila* were used as outgroups. GenBank accession numbers for ITS (left) and LSU (right) are shown in parentheses. Bootstrap (BS) values (1,000 replicates) > 50% from ML (left) and maximum parsimony (MP) (right) analyses are shown near the nodes. Branches supported by BS values ≥ 70% from both ML and MP analyses are depicted as thick lines. UEM = uncultured ectomycorrhiza.

**図 3.** *Balsamia oblonga* および *Balsamia* に属すその他の種の系統関係を示した。ITS および LSU の結合データセットに基づく最尤法 (ML) 系統樹。外群には *Wynnella subalpina* ならびに *Helvella dryophila* を用いた。ITS (左) および LSU (右) の GenBank アクセッション番号を括弧内に示した。ノード付近に、最尤法 (左) ならびに最節約法 (MP) (右) のブートストラップ値 (BS) を“MLBS/MPBS”として表示した。MLBS ≥ 70% かつ MPBS ≥ 70% で支持された分岐を太線で示した。UEM = 外生菌根由来の環境配列。

hyaline, septate, 3.5–10.5 µm broad, thin-walled. Paraphyses obscure possibly due to maturation. Asci irregularly distributed, with 8 spores, hyaline, ellipsoid to citriform, 64–90.5 × 32.5–45 µm (excluding stipe) in water, thin-walled; stipe arising from crozier, up to 23.5 µm long, 6.5 µm broad; not reacting in Melzer's reagent with pretreatment in 5% KOH. Ascospores oblong, hyaline, smooth, 24.5–33.5 × 9.5–13 µm (mean, 29.4 × 11.3 µm; n = 81), Q = 2.3–3.3 (mean, 2.6; n = 81) in water, thin-walled; containing 1–3 (mostly single) large central oil droplets; not reacting in Melzer's reagent with pretreatment in 5% KOH.

## Discussion

The genus *Balsamia*, excluding the earliest diverging apothecial species *Bal. aestivaris* K. Hansen, Skrede & T. Schumacher and species formerly classified as *Barssia*, is largely divided into two major clades (Fig. 3). One comprises species distributed in Eurasia (*Bal. lishanensis*, *Bal. oblonga*, *Bal. platyspora*, *Bal. polysperma*, and *Bal. vulgaris*) and North America (*Bal. lazyana* and *Bal. trappei*). The species in this clade are characterized by reddish ascomata and opened glebal chambers. The other clade is composed of 10 North American species, which implies that significant speciation of *Balsamia* occurred within western North America.

An enigmatic Japanese species, *Barssia yezo-montana* (Kobayasi) Trappe (= *Phymatomyces yezo-montanus*) is known only from the type specimen collected in Hokkaido, Japan (Kobayasi, 1937). Furthermore, the holotype of this species seemed to have been lost (Gilkey, 1961). Recently, Hansen et al. (2019) synonymized *Barssia* with *Balsamia*, but they retained the classification of *Bar. yezo-montana* as *Barssia* due to a lack of enough specimens to evaluate. In August 2017, the first and last authors attempted a field survey in the type locality of *Bar. yezo-montana*, which was unsuccessful. *Barssia yezo-montana* forms cylindrical asci and globose ascospores (Fig. 2), and thus, this species is suggested to belong to another genus.

## Acknowledgements

We thank Mr. Teruhisa Masaki for supporting DNA sequencing. This study was financially supported by JSPS KAKENHI Grant-in-Aid for Young Scientists (B) (nos. 17K15184) for the last author.

## References

Agerer R., Rambold G. (2004–2020) DEEMY – An information system for characterization and determination of ectomycorrhizae. Available at [www.deemy.de](http://www.deemy.de) (accessed on 8 Dec. 2020).  
Berkeley M.J. (1844) Notices of British fungi. *Annals and Magazine of Natural History* 13:340–360.

Ceruti A., Bussetti L. (1962) Sulla simbiosi micorrizica tra tigli e *Boletus subtomentosus*, *Russula grisea*, *Balsamia platyspora* e *Hysterangium clathroides*. *Allionia* 8: 55–66.  
Crous P.W., Wingfield M.J., Schumacher R.K., Summerell B.A., Giraldo A., Gené J., Guarro J., Wanasinghe D.N., Hyde K.D., Camporesi E., Gareth Jones E.B., Thambugala K.M., Malysheva E.F., Malysheva V.F., Acharya K., Álvarez J., Alvarado P., Assefa A., Barnes C.W., Bartlett J.S., Blanchette R.A., Burgess T.I., Carlavilla J.R., Coetzee M.P., Damm U., Decock C.A., den Breeÿen A., de Vries B., Dutta A.K., Holdom D.G., Rooney-Latham S., Manjón J.L., Marinowitz S., Mirabolfathy M., Moreno G., Nakashima C., Papizadeh M., Shahzadeh Fazeli S. A., Amoozegar M.A., Romberg M.K., Shivas R.G., Stalpers J.A., Stielow B., Stukely M.J., Swart W.J., Tan Y.P., van der Bank M., Wood A.R., Zhang Y., Groenewald J.Z. (2014) Fungal Planet description sheets: 281–319. *Persoonia* 33: 212–289.  
Doğan H.H., Bozok F., Taşkın H. (2018) A new species of *Barssia* (Ascomycota, Helvellaceae) from Turkey. *Turkish Journal of Botany* 42: 636–643.  
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.  
Gilkey H.M. (1939) Tuberales of North America. Oregon State Monographs: Studies in Botany 1: 1–63.  
Gilkey H.M. (1961) New species and revisions in the order Tuberales. *Mycologia* 53: 215–220.  
Hansen K., Schumacher T., Skrede I., Huhtinen S., Wang X.-H. (2019) *Pindara* revisited – evolution and generic limits in Helvellaceae. *Persoonia* 42: 186–204.  
Harkness H.W. (1899) Californian hypogæous fungi. *Proceedings of the California Academy of Science, Third series, Botany* 1: 241–286.  
Hawker L.E. (1954) British hypogeous fungi. *Philosophical Transactions of the Royal Society B* 237: 429–546.  
Hobbie E.A., Weber N.S., Trappe J.M. (2001) Mycorrhizal vs saprotrophic status of fungi: the isotopic evidence. *New Phytologist* 150: 601–610.  
Izumitsu K., Hatoh K., Sumita T., Kitade Y., Morita A., Gafur A., Ohta A., Kawai M., Yamanaka T., Neda H., Ota Y., Tanaka C. (2012) Rapid and simple preparation of mushroom DNA directly from colonies and fruiting bodies for PCR. *Mycoscience* 53: 396–401.  
Kaounas V., Agnello C., Alvarado P., Slavova M. (2015) *Barssia hellenica* sp. nov. (Ascomycota, Pezizales), a new hypogeous species from Greece. *Ascomycete.org* 7: 213–219.  
Kobayasi Y. (1937) *Phymatomyces*, a new genus of the Tuberales. *Journal of Japanese Botany* 13: 912–914.  
Kumar S., Stecher G., Li M., Knyaz C., Tamura K. (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35: 1547–1549.  
Liu B., Tao K. (1990) New species and new records of hypogeous fungi from China III. *Acta Mycologica Sinica* 9: 25–30.

- Montecchi A., Sarasini M. (2000) *Funghi ipogei d'Europa*. Fondazione Centro Studi Micologici, Trento.
- Pegler D.N., Spooner B.M., Young T.W.K. (1993) *British truffles: A revision of British hypogeous fungi*. Royal Botanic Gardens, Kew.
- Petitberghien A. (1966) Note sur deux champignons hypogés. *Bulletin Trimestriel de la Société Mycologique de France* 82: 460–466.
- Schulzer S. (1870) *Mykologische beobachtungen aus Nord-Ungarn im Herbst 1869*. *Verhandlungen der Zoologisch-Botanischen Gesellschaft in Österreich* 20: 169–210.
- Silvestro D., Michalak I. (2012) raxmlGUI: a graphical front-end for RAxML. *Organisms, Diversity and Evolution* 12: 335–337.
- Southworth D., Frank J.L., Castellano M.A., Smith M.E., Trappe J.M. (2018) *Balsamia* (sequestrate Helvellaceae, Ascomycota) in western North America. *Fungal Systematics and Evolution* 2: 11–36.
- Tedersoo L., Smith M.E. (2013) Lineages of ectomycorrhizal fungi revisited: Foraging strategies and novel lineages revealed by sequences from belowground. *Fungal Biology Reviews* 27: 83–99.
- Tulasne L.R., Tulasne C. (1843) Champignons hypogés de la famille des Lycoperdaceés, observés dans les environs de Paris et les départements de la Vienne et d'Indre-et-Loire. *Annales des Sciences Naturelles Série 2* 19: 373–381.
- 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.
- Vittadini C. (1831) *Monographia Tuberacearum*. Mediolani.
- 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.
- Xu Y.-Y., Guo L.-J., Li T., Fan L. (2018) Two new species of *Barssia* from China. *Phytotaxa* 374: 129–138.
- Xu Y.-Y., Yan X.-Y., Li T., Fan L. (2020) A taxonomic reassessment of the genus *Balsamia* from China. *MycKeys* 67: 81–94.
- Yamamoto K., Ohmae M., Orihara T. (2019) First report of a hypogeous fungus, *Pachyphlodes nemoralis* (Pezizaceae) from subalpine forest in Japan. *Truffology* 2:1–5.
- Yamamoto K., Ohmae M., Orihara T. (2020) *Metarhizium brachyspermum* sp. nov. (Clavicipitaceae), a new species parasitic on Elateridae from Japan. *Mycoscience* 61: 37–42.