Research note

First Report of *Hydnotrya tulasnei* in Central Taiwan

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【Summary】

In August 2016, 2 *Hydnotrya* specimens were collected from Taichung City of central Taiwan. One was found under *Pseudotsuga wilsoniana*, in a mixed forest of *Picea morrisonicola*, *Pinus armandii* and *Tsuga formosana* in the Piluchi-Lishan region, while the other was found in a plantation of *P. morrisonicola* along the Dasyueshan Trail. Both were at an elevation of 2500 m. Morphological analyses revealed that both specimens matched the most widely distributed truffle *Hydnotrya tulasnei*. This is the first report of *Hydnotrya* species in Taiwan.

Key words: Ascomycota, ectomycorrhiza, hypogeous fungi, truffles.

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研究簡報

台灣地下真菌新紀錄種—涂氏腔塊菌

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摘 要

2016年8月，於臺灣中部山區發現兩顆腔塊菌屬的子囊果，分別於台中市畢祿溪—梨山地區之臺灣雲杉、華山松及臺灣鐵杉混合林內的臺灣黃杉樹圈範圍內；以及台中市大雪山林道的臺灣雲杉人工林下發現挖出，兩處海拔皆為2,500 m。以形態鑑定兩顆子囊果同為涂氏腔塊菌(*Hydnotrya tulasnei*)，為臺灣的新紀錄屬種。

關鍵詞：子囊菌、外生菌根菌、地下真菌、松露。

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Hydnotrya Berk. & Br. is a genus under Discinaceae of Pezizales of Ascomycota, which was described in 1846 by Berkeley and Broome. They are hypogeous fungi and also commonly known as truffles. The genus distribute across the Northern Hemisphere including Europe (Bulgaria, Czech Republic, Denmark, Germany, the Netherlands and the UK), North America (the USA and Canada), Australia and Asia (China) (Gilkey 1954, Pegler et al. 1993, Abbott and Currah 1997, Dimitrova and Gyosheva 2008, Kirk et al. 2008, Stielow et al. 2010, Li et al. 2013). Five species have been reported in China since 1989: *H. mechaelis* and *H. tulasnei* in Jilin Province, *H. cerebriformis* in Shanxi, Xinjiang and Xizang Provinces (Tao and Liu 1989), *H. cubispora* in Xizang Province (Xu 2000), and *H. laojunshanensis* as a unique new species from Yunnan Province (Li et al. 2013).

In this study, we reported *Hydnotrya tulasnei* (Berk.) Berk. & Br. as a newly recorded hypogeous fungus in Taiwan. Two specimens were collected from Taichung City of central Taiwan, one found under *Pseudotsuga wilsoniana* Hayata, in a mixed forest of *Picea morrisonicola* Hayata, *Pinus armandii* Franch and *Tsuga formosana* Hayata, along No. 8 Taiwan Provincial Highway in the Piluchi-Lishan region; while another was found on a plantation of *Picea morrisonicola* Hayata along the Dasyueshan Trail, both at an elevation of 2500 m.

Specimens were cleaned with a dry toothbrush, and the fresh size, surface texture and color of each specimen were noted. Specimens were then cut into half, allowing observation of gleba color or any color change upon air-exposure and the orientation of the glebal cavities. Slides of specimens were then made with a razor-blade by hand, stained and mounted on slides with lactophenol solution or cottonblue-lactophenol solution. Microscopic descriptions and measurement of the peridium, asci and ascospores (n = 160) were done with an ocular micrometer under an LEICA DMLB light microscope.

For scanning electron microscopy (SEM), ascospores from air-dried ascocarps were mounted on conventional SEM-stubs with carbon double-sided tapes (Nisshin EM

Co. Ltd., Tokyo, Japan), coated with gold-palladium for 2 min, then examined and photographed with a HITACHI TM3000 tabletop SEM. Stubs and dried specimens were stored in Room 610, Forestry Research Building, Taiwan Forestry Research Institute (Taipei, Taiwan).

Gleba tissues from fresh ascocarps were ground in a plastic pestle with 800 µL of lysis buffer in a 1.5 mL centrifuge tube for DNA extraction. DNA was then extracted using the TANBead® fungal Nucleic Acid Extraction Kit and TANBead® Nucleic Acid Extractor following the protocol of the manufacturer (Taiwan Advanced Nanotech Inc., Taoyuan, Taiwan). Forward primer ITS5 (5' GGAAGTAAAAGTCGTAACAAGG 3') was used in combination with reverse primer ITS4 (5' TCCTCCGCTTATTGATATGC 3') for amplifying the internal transcribed spacer (ITS) regions of ribosomal DNA (White et al., 1990). Polymerase chain reaction (PCR) were performed with initial denaturation at 94℃ for 3 min, then at 94℃ for 30 s, annealing at 56℃ for 30 s, extension at 72℃ for 30 s for 35 cycles and a final extension at 72℃ for 5 min on a multigene thermal cycler (Labnet International Inc.). PCR products were checked on agarose gel containing 1.4% agarose and 0.5×Tris-acetate-EDTA (TAE) buffer and stained with Healthview™ nucleic acid stain under UV light by multilmage™ light cabinet (Alphalmager 2200). The PCR products were sent to Seeing Bioscience Co. Ltd. (Taipei, Taiwan) for purification and sequencing.

Taxonomy

Hydnotrya tulasnei (Berk.) Berk. & Br., Ann. Mag. Nat. Hist. 18:78 (1846) (Fig.1)
= *Hydnobolites tulasnei* Berk., Ann. Mag. Nat. Hist. 13:357 (1844)
= *Hydnotrya carnea* Zobel, Icones Fungorum 6:61 (1854)
= *Hydnotrya jurana* Quél., Enchiridion Fungorum 262 (1886)

Ascocarp hypogeous, solid and firm texture, irregularly globose, ca. 2 cm in diam., with reddish-brown and pitted surface. **Odor** musty, unpleasant when old. **Peridium** 100~160 µm thick, composed of brown interwoven hyphae, more reddish-brown towards the surface. **Gleba** pink with white veins when immature, light-brown with orange veins when mature, later dark-brown when old, infolding into small irregular cavities. **Asci** 92.5~162.5×50~70 µm, broadly cylindrical or clavate (especially when immature), with (4-)7~8-spored, numerous asci in subhymenium. **Ascospores** (20-)25~37.5(-40) µm in diameter, irregularly arranged in asci, initially hyaline or light- orange, turn reddish-brown when mature, generally globose, ornamented with large, coarse warty surface of thickness 2.5~10 µm. **Paraphyses** 5~7.5 µm in diameter, cylindrical, hyaline, thin-walled, septate.

Specimen examined: TAIWAN. Taichung City, Piluchi-Lishan region, along No. 8 Taiwan Provincial Highway, 2500 m elev., under *Pseudotsuga wilsoniana* in mixed forest of Pinaceae, 29 July 2016, *Lin*, *C.-L.* Dasyueshan Trail, 2500 m elev., beneath piled leaves under plantation of *Picea morrisonicola*, 8 Aug 2016, *Lin, W.-W.*, dried-specimen stored in Room 610, Forestry Research Building, Taiwan Forestry Research Institute, Taipei, Taiwan, with accession number D31.

Distribution: Bulgaria, China, Czech Republic, Denmark, the Netherlands, Taiwan, UK and the USA.

Notes: Characteristics of the 2 specimens matched the description of *Hydnotrya tulasnei* holotype in Berkeley and Broome (1846): with rusty-vermilion ascocarps, ca. 2 cm in diam., asci oblong-elliptic, containing 8 globose warty ascospores. H. *bailii* Soehner

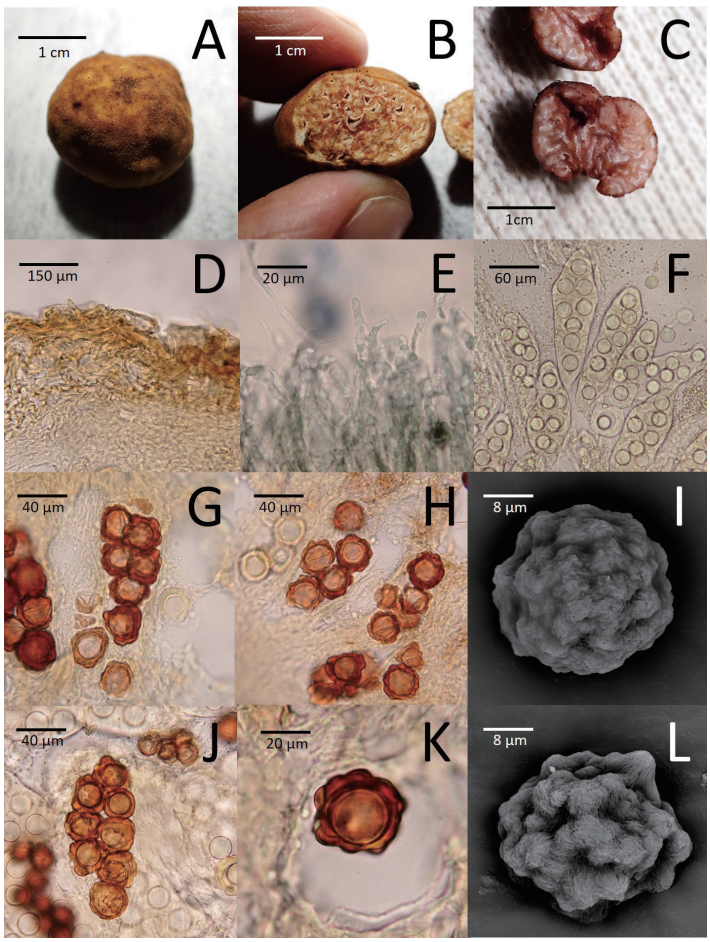

Fig. 1. Morphologocal characteristics of *Hydnotrya tulasnei* (Berk.) Berk. & Broome. A, B, Ascocarp from the Piluchi-Lishan region; C, ascocarp from Dasyueshan; D, peridium; E, paraphyses; F, immature asci; G, H, J, K, ascospores under a light microscope; I, L, ascospores under SEM.

has very similar warty spores but can be distinguished from *H. tulasnei* by the strictly uniseriate spores (Soehner 1959, Stielow et al. 2010). *H. tulasnei* is ectomycorrhiza but apparently has not been associated with any particular host. *H. tulasnei* was reported to be associated with a broad host range of both deciduous and coniferous trees, such as *Pinus* (e.g., *P. contorta* Dougl. and *P. sylvestris* L.), *Abies*, *Pseudotsuga* (e.g., *P. menziesii* (Mirb.) Franco), *Tsuga* (e.g., *T. heterophylla* (Raf.) Sarg.), *Picea* (e.g., *P. abies* (L.) Karst.), *Fagus* (e.g., *F. sylvatica* L.), *Quercus* (e.g., *Q. mongolica* Fisch.), *Acer* (e.g., *A. psedoplatanus* L.) and *Corylus* (e.g., *C. avellana* L.) (Tao and Liu 1989, Pegler et al. 1993, Abbott and Currah 1997, Trappe et al. 2007, Stielow et al. 2010). In this study, specimens were found under *Pseudotsuga wilsoniana* and *Picea morrisonicola*, which are both endemic species of Taiwan. The discoveries of this study verify the possibility of the association of *H. tulasnei* with coniferous spruce and Douglas-fir in montane regions of central Taiwan,

although Stielow et al. (2010) distinguished *H. bailii* from *H. tulasnei* by its preference of association with spruce in montane to boreal habitats, in addition to the difference in their morphological and phylogenetic characters.

H. tulasnei is reported to be found in North America in April-November, mostly from northeast and northwest coastal forests and occasionally in mountains. There are also reports of fruiting begining from late June (Abbott and Currah 1997, Trappe et al. 2007). On the other hand, *H. tulasnei* is widespread throughout Europe and widely distributed in the British Isles while more frequently found in the south. They are recorded in July-October and became mature from late summer (Pegler et al. 1993). In this study, half-mature specimens were found in early August at an elevation of 2500 m in the montane region of central Taiwan, with an extreme mountain climate and rainy typhoon season from July to October.

ITS sequences of ascocarps from Piluchi-Lishan and Dasyueshan were deposited at GenBank with respective accession numbers of LC360403 and LC360404. Both sequences consist of 751 bp and shared the highest sequence similarity with *Hydnotrya* sp. (KU878593) in GenBank. Specimens are recognized as *H. tulasnei* from morphological taxonomy, while a larger sample size is preferred for further reliable molecular analysis and phylogenetic study.

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