

Hydnotrya michaelis – an Uncommon Fungus from Unexpected Habitat

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Abstract. The paper introduces the first records in Southeastern Europe of *Hydnotrya michaelis*, rare ascomycete, collected in artificial plantation with Macedonian pine (*Pinus peuce*) and compared with specimens found with other Pinaceae. The species was studied for the first time on living materials and description and illustrations of the Bulgarian specimens are provided. The first nLSU sequence of this rare and little-known species is also released.

Key words: Bulgarian mycota, Discinaceae, hypogeous fungi, Pezizales, truffle-like fungi.

Introduction

Despite the vast array of diverse habitats, the knowledge on hypogeous fungi was until very recently surprisingly limited in the Balkan Peninsula (Chavdarova et al., 2011; Kaounas et al., 2011; Polemis et al., 2019). While the studies have become more intensive in the last decade due to the interest in species of commercial potential, the number of species has notably increased, including with some previously undescribed (see e. g. Kaounas et al., 2015, 2016; Milenković et al., 2016; Polemis et al., 2019; Vidal et al., 2019). *Hydnotrya* Berk. & Broome is a genus of hypogeous ascomycetes, with eleven species so far described from across Europe, some of them pending taxonomic reassessment as suggested by recent molecular studies (Stielow et al., 2010). Of these only two have been recorded so far in some Balkan countries: *H. cerebriformis* Harkn. and *H. tulasnei* (Berk.)

Berk. & Broome from Bulgaria (Dimitrova & Gyosheva, 2008), and *H. tulasnei* in Greece (Konstantinidis & Kaounas, 2014). Our collecting efforts yielded one particularly interesting *Hydnotrya* specimen from habitats with the Balkan endemic pine *Pinus peuce* Griseb., followed by another collection from boreal coniferous forests. Their study revealed that these are the first collections of the rare and less-known *H. michaelis* (E. Fisch.) Trappe in Southeastern Europe. As far as it is a species seldomly described in the mycological literature, detailed morphological characterization is provided herein.

Material and Methods

The fungus was retrieved with the aid of trained dogs. The specimens were photographed and documented in the field, as well as *ex-situ*. The microscopic study was held

on fresh specimens on slides in tap water (Baral, 1992). Melzer's reagent was used for additional observations. All measurements of microscopic structures in the description are reported from slides in water. The colours of the different parts of ascomata as closely as possible refer to the "Flora of British Fungi Colour Identification Chart" (Anonymous, 1969). Air-dried voucher specimens are preserved in the Mycological Collection of the Institute of Biodiversity and Ecosystem Research (SOMF).

DNA extraction, amplification, and sequencing were handled by ALVALAB (Spain) by the following protocol. Total DNA was extracted from dry specimens employing a modified protocol based on Murray & Thompson (1980). PCR reactions (Mullis & Faloona, 1987) included 35 cycles with an annealing temperature of 54°C. The primers LR0R and LR3-Asc (Cubeta et al., 1991, Tedersoo et al., 2008) were used to amplify the 28S rDNA region (LSU). The PCR product was checked in a 1% agarose gel, and the amplicon was sequenced with primer LR3-Asc.

Results and Discussion

Hydnotrya michaelis (E. Fisch.) Trappe, Mycotaxon 2(1): 113 (1975).

Macroscopic features. Ascomata hypogeous, up to 6 cm across, initially subspherical, with rounded opening, then with maturation more or less irregular, wrinkled, lobulate, with numerous invaginations. Peridium irregularly coloured in buff, clay pink to chestnut brown, with pronounced vinaceous or purple tinges, finely pubescent to smooth. Gleba labyrinthoid, with large, sinuous cavities, separated by folded inwards portions of ascomatal wall; trama clay pink to pale vinaceous, with distinct, narrow, livid vinaceous subhymenial line; hymenial layer continuous, in surface view off-white to vinaceous pink or clay buff, in section whitish. Odour very strong, somewhat pungent, rather persistent.

Microscopic features. Peridium following the ascomatal surface and the respective side of the invaginated parts of

the wall, 80–150 µm thick, composed of 5–8 layers of almost spherical, ovoid or polygonal elements and sometimes slightly elongate and narrow apical elements, 17.5–40.0 × 12–35 µm; with numerous spherical lipid bodies, yellowish to brownish in water and in places with extracellular granules of brown pigment; walls not thickened or by exception thickened up to 3 µm, encrusting particles not seen. Trama composed of strongly interwoven, thin-walled, branched, septate, hyaline hyphae 2–7 µm wide, with numerous lipid bodies; some inflated up to 17 µm hyphae present, occasionally with walls up to 2 µm thick and with somewhat granular appearance. Hymenium lining the cavities, composed of regularly arranged asci and paraphyses. Asci cylindrical, 200–220 × 30–35 µm, including up to 30 µm narrowed basal part, 8-spored, thin-walled, inamyloid. Paraphyses considerably longer than asci (by some 100–150 µm), narrowly cylindrical, 5–6 µm wide, sometimes slightly widened (up to 8 µm) at the apex, thin-walled, septate, hyaline, with numerous lipid bodies. Ascospores (21.2–)24.9–29.6(–32.2) × (18.8–)19.8–22.4 (–24.9) µm; Q = (1.1–)1.2–1.4 (–1.5), on average 27.5 × 21.3 µm; Q_{av} = 1.3 (n=60, ornamentation excluded), broadly ellipsoid, honey-yellow, thick-walled, with one large central guttule; ornamentation 2–6 µm, consisting of large, dense, mostly irregular to ameboid warts with usually rounded or truncate apices, forming somewhat incomplete reticulate pattern.

Specimens examined. Bulgaria: Western Stara Planina Mts: Petrohan Pass (Sofia distr.), 43°06'53.6"N 23°07'34.6"E, artificial plantation, under *Pinus peuce* Griseb., 04.07.2016, B. Assyov (SOMF 30345, GenBank MW879528); Western Rodopi Mts, Atoluka resort (Pazardzhik distr.), forest with *Picea abies* (L.) H. Karst. and *Abies alba* Mill., 17.06.2018, M. Slavova (SOMF 30346).

The Bulgarian collections of *H. michaelis* (Fig. 1a–g) correspond well, both macro- and microscopically to the existing descriptions of the species in the mycological literature (Fischer, 1878; Pegler et al., 1993; Vidal, 1994; Montecchi &

Sarasini, 2000; Gori, 2005; Kříž et al., 2017). The specimen from *P. peuce* stand agrees in all aspects to the second studied collection, associated with other Pinaceae. As far as possible to judge from the few descriptions in literature, the Bulgarian specimens are the first to be studied microscopically in living state. Among the European species of the genus, *H. michaelis* is recognized by ascomata with large, irregular

cavities, combined with ellipsoid spores with ornamentation of broad, irregularly-shaped warts. An attempt was made to obtain barcoding sequences of one of our specimens. An nLSU sequence was successfully acquired and appears to be the first publicly available of this species (GenBank MW879528). Sequence of the nrITS region could not be obtained for the moment.



Fig. 1. Morphological features of *Hydnotryia michaelis*: A,B – ascomata, C – section of ascoma, D – parts of asci and paraphyses, E,F – ascospores, G – peridium. Scale bars: A,B = 1 cm, C = 100 μ m, D,E,F,G = 30 μ m.

Hydnotryia michaelis was described by Fischer (1878) and for a long time after its description remained relatively little-known, with more records starting to emerge only in the late XXth century. In Europe until now it has been found in Austria, Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, Netherlands, Norway, Poland, Spain, Sweden and the United Kingdom (Kers, 1989; Ławrynowicz, 1990; Vidal, 1994; Montecchi & Sarasini, 2000; Spooner, 2003; Gori, 2005; Dieker, 2010; Huntinen, 2010). The closest known localities are apparently those in the Italian Alps (Montecchi & Sarasini, 2000). The Bulgarian findings are among the southernmost so far known and represent an outpost in the disjunct range of the species, which is mostly confined to Central and Northern Europe. *Hydnotryia*

michaelis is known to be mycorrhizal species, linked to mountain coniferous trees, primarily *Picea abies* (L.) H. Karst., but also to *Abies alba* Mill., *Pinus* spp. and *Larix decidua* L. (Vidal, 1994; Ławrynowicz, 1990; Montecchi & Sarasini, 2000). The first Bulgarian locality is an old artificial plantation of mixed conifers with scattered trees of *Fagus sylvatica* L. *Pinus peuce* is the dominant coniferous species on the spot and *Picea abies*, *A. alba* and *Pinus sylvestris* L. are also present in the stand. Ascomata of *H. michaelis* were however only recovered under trees of *P. peuce* and far from other conifers, making the mycorrhizal relation to this tree a plausible assumption. The species was consequently collected also in another distant locality, where it seemed associated with *A. alba* or *P. abies*. In both above places

the ascomata were found at shallow depth, slightly buried in the top soil layer and only covered by 1–5 cm thick layer of leaf-litter. *Hydnotrya michaelis* appears to be the first record of a hypogeous fungus allegedly

associated with *P. peuce*, unequivocally showing that the subterranean mycobiota of this peculiar and restricted to the Balkan Peninsula mycorrhizal host is so far underexplored and merits further attention.

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