

Hypogeous ascomycetes in Bulgaria

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Abstract. The authors present summarized information about the species diversity and distribution of hypogeous ascomycetes in Bulgaria. A revision was carried out of all specimens of these fungi kept in the Mycological Collection of the Institute of Botany, Sofia (SOMF). Two truffle species – *Tuber brumale* and *T. excavatum* and new localities for four species are reported for the first time. Morphological descriptions of all investigated species are given.

Key words: Bulgaria, *Elaphomycetales*, hypogeous ascomycetes, new records, *Pezizales*, truffles

Introduction

There are few data concerning hypogeous ascomycetes in Bulgaria. In scientific literature only six species from the orders *Elaphomycetales* and *Pezizales* have been published so far from the country: *Choiromyces meandriiformis* Sacc. & Bizz. (Georgiev 1906; Barzakov 1931), *Elaphomyces granulatus* Fr. : Fr. (Kuthan & Kotlaba 1981; Stoichev 1981; Gyosheva 2000; Denchev & al. 2007), *Hydnotrya cerebriiformis* (Tul. & C. Tul.) Harkn. (Stoichev & Gyosheva 2005), *H. tulasnei* Berk. & Broome (Stoichev 1981), *Tuber aestivum* Vittad. (Hinkova 1965), *T. puberulum* Vittad. (Hinkova & Stoichev 1983).

In our opinion, the main reason for the very low level of knowledge about the interesting group of hypogeous ascomycetes in Bulgaria is the development of its fruit bodies beneath the soil surface, and especially the absence of any long tradition of truffle collecting and cultivation in Bulgaria.

The aim of this work is to present the existing information about the species diversity and distribution of hypogeous ascomycetes in Bulgaria. The paper also includes morphological descriptions of the fungi and keys to species identification in the genera *Hydnotrya* and *Tuber*.

Material and methods

The investigated material includes all specimens of hypogeous ascomycetes from the Mycological Collection of the Institute of Botany, Bulgarian Academy of Sciences (SOMF).

The specimens of the new truffle species to Bulgaria, *Tuber brumale* and *T. excavatum*, were collected in 2007.

The investigated dry specimens were rehydrated and squash mounts were prepared in Melzer's reagent. Asci and spores were observed and measured under a light microscope. Scanning electron microscopy (SEM) was carried out at the Institute of Physical Chemistry, Bulgarian Academy of Sciences. Specimens were examined on JSM-5300 scanning electron microscope, at 20 kV.

The nomenclature of hypogeous ascomycetes follows Kirk & al. (2001). The authors' abbreviations of fungi are according to Kirk & Ansell (2004). Hypogeous ascomycetes were determined following Moser (1963), Hennig (1971), Dennis (1978), Trappe (1979), Pegler & al. (1993), Breitenbach & Kränzlin (1984), and Dissing (2000). Distribution of the taxa is given according to the floristic regions adopted in the *Flora of the PR Bulgaria* (Jordanov 1966).

The new truffle species to Bulgaria in the list below are designated with asterisks. For the species with conservation value, the current IUCN threat categories are given, as applied in the *Red List of Fungi in Bulgaria* (Gyosheva & al. 2006).

Results

Eight species of hypogeous fungi have been known so far to occur in Bulgaria. They have been classified within two classes of the *Ascomycota* division: *Eurotiomycetes* (1) – *Elaphomyces granulatus*, and *Pezizomycetes* (7) – *Hydnotriza cerebriformis*, *H. tulasnei*, *Choiromyces meandriformis*, *Tuber aestivum*, *T. brumale*, *T. excavatum*, and *T. puberulum*.

Two truffle species, *Tuber brumale* and *T. excavatum*, are reported for the first time from Bulgaria. New localities for four species (*Elaphomyces granulatus*, *Hydnotriza tulasnei*, *Tuber aestivum*, and *T. puberulum*) have been established.

Five hypogeous species (*Elaphomyces granulatus*, *Hydnotriza tulasnei*, *Choiromyces meandriformis*, *Tuber aestivum*, and *T. puberulum*) are with high conservation value and are included in the *Red List of Fungi in Bulgaria* (Gyosheva & al. 2006).

Three *Tuber* species – *T. aestivum*, *T. brumale* and *T. excavatum* – are delicious edible fungi of commercial value in Europe. There are no official data about any gathered and sold amounts and of the trade value of truffles in Bulgaria.

List of Bulgarian species of hypogeous ascomycetes

Elaphomycetales

Elaphomycetaceae

Elaphomyces granulatus Fr. : Fr., *Syst. Mycol.*, 3, p. 58, 1829 (Fig. 1)

Ascomata hypogeous or subepigeous, subglobose to ellipsoid, up to 4 cm in diameter, with thick peridium composed of two layers: outer (rind) – thin-walled, pale-brown, divided into sections, covered by small polygonal warts, slightly pyramidal or blunt, and inner – homogeneous, white, pale to saturated brown, seldom black; gleba initially white, cotton-like, subsequently gray to brown, dusty-black at maturity. **Asci** 35–45 μm in diameter, subglobose or pyriform, 6–8-spored, thin-walled. **Ascospores** 24–32 μm in diameter, globose to pear-like, initial-

ly hyaline, subsequently black-brown, ornamented with short irregular spines.

Indicated: Black Sea Coast (*Southern*) – the Ropotamo Reserve, in oak forest, 18.06.1978, J. Kuthan (Kuthan & Kotlaba 1981, n.v.).

Specimens examined: Northeast Bulgaria – Razgrad Hills, under hornbeam, 07.09.2007, leg. I. Nedev, det. M. Gyosheva (MG) & E. Dimitrova (ED), SOMF 26657 – new locality for the country; Pirin Mts (*Southern*): above the town of Gotse Delchev, 10.09.1998, leg. M. Gyosheva (SOMF 22526), (Gyosheva 2000).

The species is included in the *Red List of Fungi in Bulgaria* (Gyosheva & al. 2006) under the category Critically Endangered (CR).

Pezizales

Discinaceae

Hydnotriza Berk. & Broome

Key to the Bulgarian species of *Hydnotriza*

1. Ascomata up to 1 cm in diameter, initially pale cream, subsequently darkened. Spores 17.5–25 μm in diameter, with a coarse reticulated coat and filiform appendages around *H. cerebriformis*
- 1*. Ascomata up to 5–7 cm in diameter, red-brown. Spores 27.5–35 (–40) μm in diameter with thick coarse warts on the coat *H. tulasnei*

Hydnotriza cerebriformis (Tul. & C. Tul.) Harkn., *Proc., Calif. Acad. Sci., ser. 3, Bot.*, 1, p. 266, 1889

Ascomata hypogeous, irregular globose, up to 0.5–1 cm in diameter with a smooth strong cerebriform convolute surface, initially pale cream, subsequently darkened; gleba of loosely woven hyphae, whitish. **Asci** 80–130 \times 62.5–80 μm , broadly ellipsoid or subglobose, short-stalked, thin-walled,

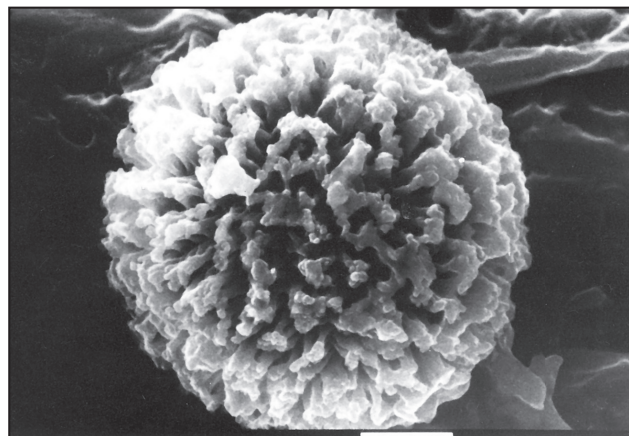


Fig. 1. *Elaphomyces granulatus*: ascospores in SEM. Scale bar = 5 μm .

8-spored, occasionally 4-7-spored. **Ascospores** globose, 17.5–25 μm in diameter (without appendages) with a large reticulate coat, initially hyaline, subsequently pale yellowish-brown with 1–2 big oil drops inside and filiform appendages around, long 2–3 μm .

Specimen examined: Rhodopi Mts (Central) – among mosses in *Pinus sylvestris* communities near Tsar Kalyan village, 10.11.2004, leg. G. Stoichev & M. Gyosheva (SOMF 25746), (Stoichev & Gyosheva 2005, sub *Hydnobolites cerebriformis* Tul. & C. Tul.).

Hydnotrya tulasnei Berk. & Broome, Ann. Mag. Nat. Hist., 18, p. 78, 1846

Ascomata hypogeous, irregularly globose, up to 5–7 cm in diameter, with wrinkled and pitted surface, reddish-brown, minutely downy or pruinose when fresh; gleba white or yellowish-gray, with pale orange twisted veins. **Asci** 175–210 \times 30–62.5 μm , broadly clavate or cylindrical, 4–8-spored. **Ascospores** 27.5–35 (–40) μm in diameter, excluding ornaments, globose, initially hyaline, subsequently reddish-brown at maturity, with a thick, coarse warty red-brown coat. Paraphyses cylindrical, obtuse, thin-walled, septate, 6–8 μm in diameter.

Indicated: Rhodopi Mts (Western) – the Beglika Reserve, in a conifer forest, 08.08.1976, leg. G. Stoichev (Stoichev 1981, n.v.).

Specimen examined: Rhodopi Mts (Central) – in a conifer forest at Bash Mandra locality, near Tsar Kalyan village, 28.08.1975, leg. G. Stoichev, det. G. Stoichev, M. Gyosheva & E. Dimitrova (SOMF 26654) – new locality for the country.

This species is included in the *Red List of Fungi in Bulgaria* (Gyosheva & al. 2006), under the category Critically Endangered (CR).

Tuberaceae

Choiromyces meandriformis Sacc. & Bizz., Monographia Tuberacearum (Milano), 2, p. 50, 1831 (Figs 2, 3)

Ascomata hypogeous or subhypogeous, irregular globose, up to 10 cm in diameter, sessile, lumpy, with smooth, folded yellow-brown outer surface; gleba soft, drying hard, initially white, at maturity cream to yellowish, marbled with numerous, irregular, wavy grayish to ochre veins. **Asci** 87.5–175 \times 57.5–62.5 μm , clavate, tapered below into a short stalk, 8-spored. **Ascospores** globose, 22.5–30 μm in diameter (without ornaments), yellowish-brown at maturity, with irreg-

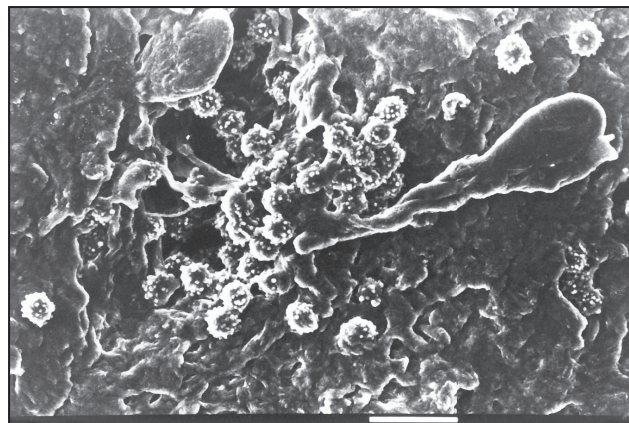


Fig. 2. *Choiromyces meandriformis*: ascus and ascospores in SEM. Scale bar = 5 μm .

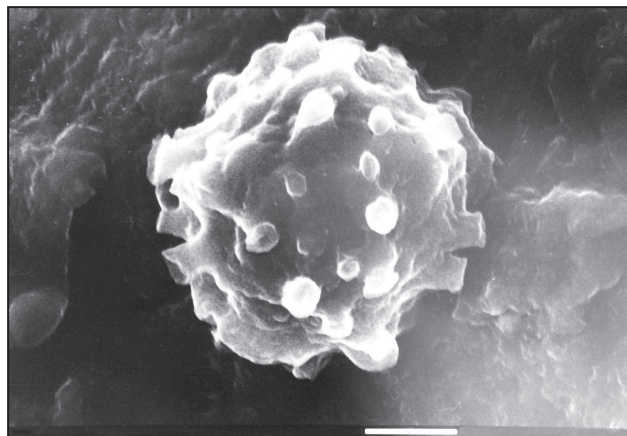


Fig. 3. *Choiromyces meandriformis*: ascospores in SEM. Scale bar = 50 μm .

ular acute or blunt pale brown spines on the surface, 3–4 μm long. Paraphyses cylindrical, obtuse, septate, 7.5–10.5 μm in diameter.

Indicated: Rila Mts – Borovets locality, July 1899 (Georgiev 1906, n.v.); the same locality, 10.08.1930, leg. I. Buresh (Barzakov 1931, 1932, n.v.).

Specimen examined: Vitosha region – Mt Vitosha, Zlatnite Mostove locality, 12.09.1959, leg. S. Dimitrov, det. Ts. Hinkova, M. Gyosheva & E. Dimitrova (SOMF 131) (Hinkova 1961).

The species is included in the *Red List of Fungi in Bulgaria* (Gyosheva & al. 2006) under the category Endangered (EN).

Tuber P. Micheli ex F.H. Wigg. : Fr.

Key to the Bulgarian species of *Tuber*

1. Spores with acute spines on the coat surface . . . 2

- 1*. Spores with large reticulate coat surface. 3
2. Asci 2-4-spored. Spores (30-) 32.5-37.5 (-40) × 25-27.5 μm; spines 3-7.5 × 1-1.5 μm in size.
 *T. excavatum*
- 2*. Asci 1-6-spored. Spores 22.5-30 (-32.5) × 15-20 μm; spines 3-5 × 1 μm in size. *T. brumale*
3. Ascumata 2-7 cm in diameter, black-brown, with coarse warts on the coat surface. Spores 25-50 × 17-37 μm, broadly ellipsoid. *T. aestivum*
- 3*. Ascumata up 2 cm in diameter, dark-brown, with acute hairs on the coat surface. Spores nearly globose, 22.5-40 μm in diameter *T. puberulum*

Tuber aestivum Vittad., Monographia Tubercarum (Milano), 2, p. 38, 1831 (Fig. 4)

Ascumata hypogeous, more or less globose, 2-7 cm in diameter, with thick black-brown coat covered with coarse, pyramidal warts; gleba initially whitish, subsequently yellowish or olive-brown, with a close network of whitish meandering veins. **Asci** 67.5-87.7 × 65-72.5 μm, clavate to nearly globose, short-stalked, 2-4 (6)-spored. **Ascospores** 25-50 × 17-37 μm (without ornaments), varying in size according to their number in the ascus, broadly ellipsoid, with a large reticulate coat, 2.5-3 μm high, initially hyaline, yellow to yellowish-brown at maturity.

Indicated: Valley of Struma River – near Kulata village, under *Astragalus* sp., 22.03.1960 (Hinkova 1965).

Specimens examined: Stara Planina Mts (Western) – near Ponor village in an oak-hornbeam forest, 05.11.2002, leg. S. Savev, det. V. Fakirova, M. Gyosheva & E. Dimitrova (SOMF 25 420) – new locality to the country; Valley of Struma River: near Kulata village, under *Astragalus* sp., 22.03.1960, leg. S. Kozuhrov, det. Ts. Hinkova, M. Gyosheva & E. Dimitrova (SOMF 4365) (Hinkova 1965).

The species is included in the *Red List of Fungi in Bulgaria* (Gyosheva & al. 2006) under the category Endangered (EN).

**Tuber brumale* Vittad., Monographia Tubercarum (Milano), 2, p. 37, 1831 (Fig. 5)

Ascumata hypogeous, subglobose, tuberiform, up to 1.6 cm in diameter and 0.8 cm high, with a strongly folded, initially reddish, at maturity black-brown coat, ornamented with coarse depressed warts, 2-3 mm wide; gleba initially watery, dirty

white-brown, at maturity fleshy, gray-brown, marbled with dendriform white veins. **Asci** 62.5-67.5 × 40-60 μm, broadly ellipsoid to nearly globose, short-stalked, 1-6-spored. **Ascospores** 22.5-30 (-32.5) ×

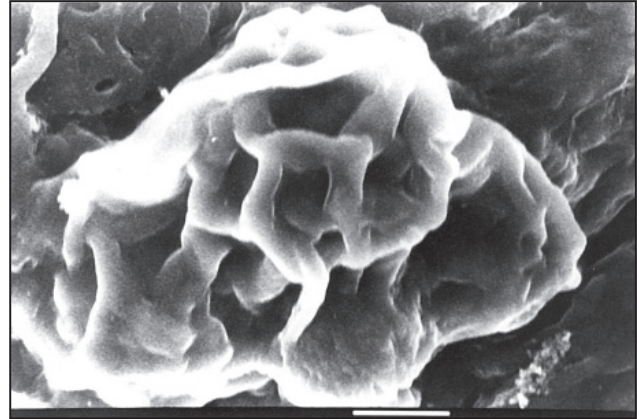


Fig. 4. *Tuber aestivum*: ascospores in SEM. Scale bar = 10 μm.

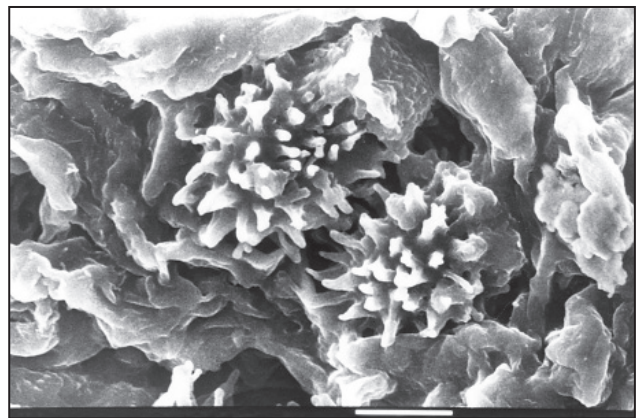


Fig. 5. *Tuber brumale*: ascospores in SEM. Scale bar = 10 μm.

15-20 μm, excluding ornaments, ellipsoid, initially hyaline, yellowish-brown at maturity, with acute cyanophilous spines on the surface, 3-5 μm long and 1 μm wide.

Specimen examined: Northeast Bulgaria – Lipnik Park near Nikolaevo village, near Ruse town, in a deciduous forest under *Quercus pubescens* Willd., November 2007, leg. I. Nedev, det. M. Gyosheva & E. Dimitrova (SOMF 26655).

**Tuber excavatum* Vittad., Monographia Tubercarum (Milano), 2, p. 49, 1831 (Fig. 6)

Ascumata hypogeous, subglobose, about 2 cm in diameter and 0.8 cm high, with a well-defined basal cavity, smooth outside, or with a slightly folded coat ornamented by small warts, initially yellow-brown, olive-brown at maturity; gleba initially white, subsequently yellowish to dark-brown finally, mar-

bled with radial cream coloured veins extending out of the basal cavity. **Asci** 92.5–125 × 65–80 µm, subglobose, short-stalked, 2-4 (-6)-spored. **Ascospores** (30-) 32.5–37.5 (-40) × 25–27.5 µm (without ornaments), ellipsoid to broadly ellipsoid, with bi-tunicate reticulate coat, yellowish-brown to brown at maturity, ornamented with coarse irregular spines, 3–7.5 × 1–1.5 µm in size.

Specimen examined: Northeast Bulgaria – Razgrad Hills, in an oak forest, November 2006, leg. I. Nedev, det. M. Gyosheva & E. Dimitrova (SOMF 26656).

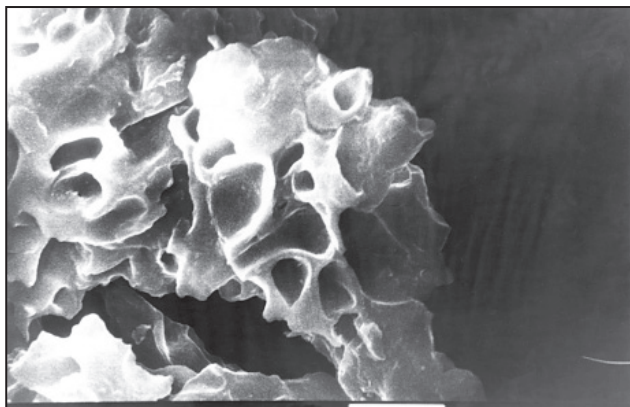


Fig. 6. *Tuber excavatum*: ascospores in SEM. Scale bar = 10 µm.

Tuber puberulum Berk. & Broome, Ann. Mag. Nat. Hist., 18, p. 81, 1846

Ascomata hypogeous or subepigeous at maturity, more or less globose, up to 2 cm in diameter, outside with thick surface, folded, initially pale yellowish-brown, at maturity dark-brown, covered with fine, white, pointed hairs, 100–160 × 5–10 µm in size; gleba initially whitish, subsequently pale-brown to orange-brown at maturity, with irregular, branching, whitish veins, radiating from the base. **Asci** 100–180 × 72.5–102.5 µm, oblong-globose to almost globose, some with stalks, 1-4-spored, seldom 5-spored. **Ascospores** nearly subglobose, 22.5–40 µm in diameter, excluding ornaments, varying in size according to their number in the ascus, initially hyaline, subsequently yellowish-brown at maturity, ornamented with a regular reticulum, 2.5–5 µm high.

Specimens examined: Rhodopi Mts (Central) – near Hrabrino village, Plovdiv district, 11.12.1979, leg. G. Stoichev, det. G. Stoichev, M. Gyosheva & E. Dimitrova (SOMF 15 144) – new locality for the country; around Zdravets chalet, 17.11.1979, leg. G. Stoichev, det. G. Stoichev, Ts. Hinkova, M. Gyoshe-

va & E. Dimitrova (SOMF 14 389) (Hinkova & Stoichev 1983).

This species is included in the *Red List of Fungi in Bulgaria* (Gyosheva & al. 2006) under the category Endangered (EN).

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