



New records of *Melanogaster* species (Fungi, Boletales) in Bulgaria

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Abstract

Melanogaster ambiguus (Vittad.) Tul. & C. Tul. and *M. broomeanus* Berk., two of the rare and interesting hypogeous fungi in Bulgaria, were found in a new localities near hazelnut trees (*Corylus avellana* L.) in the Rhodopes. *Melanogaster broomeanus* are reported for the second time in the country. The morphological descriptions, habitat, ecology and geographical distribution of *M. broomeanus* in Bulgaria are reviewed. A key to the known Bulgarian species of *Melanogaster* Corda is given.

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Introduction

Most truffle-like fungi (including *Melanogaster* species) form ectomycorrhizae with plant hosts, particularly in the families Betulaceae, Cistaceae, Fagaceae, Pinaceae, and Saliaceae. The genus *Melanogaster* Corda includes hypogeous, psammophilous and thermophilous fungi found on almost all continents (Türkoğlu and Castellano, 2013).

The taxonomic status of the genus *Melanogaster* is formulated in various ways. For example, Hawksworth *et al.* (1995) place it in the order Melanogastrales and family Melanogastraceae, whereas Liu *et al.* (1989), Besl *et al.* (1996), and Cannon and Kirk (2007) include it in the order Boletales and family Boletaceae. According to Wang *et al.* (2005), Binder and Hibbett (2006), Kirk *et al.* (2008), and Index Fungorum (<http://www.indexfungorum.org/Names/Names.asp>), the genus *Melanogaster* belongs to the type Basidiomycota, class Basidiomycetes, order Boletales and family Paxillaceae.

Truffle fungi of Bulgaria are poorly known as only few mycologists have investigated them and especially is the development of its fruit bodies beneath the soil surface. In scientific literature only three species from the gasteroid fungi of genus *Melanogaster* have been published so far from the country: *Melanogaster ambiguus* (Vittad.) Tul. & C. Tul. (Stoichev and Gyosheva, 2005; Lacheva, 2011), *M. broomeianus* Berk. ex Tul. & Tul. (Lacheva, 2011), and *M. variegatus* (Vittad.) Tul. & C. Tul. (Hinkova, 1965; Hinkova and Fakirova, 1970; Gyosheva, 1991; Lacheva, 2011). The latter species appears on the Red List of fungi in Bulgaria as endangered (EN) (Gyosheva *et al.*, 2006).

In Bulgaria, *Melanogaster broomeianus* is a very rare fungus, known previously from only once localities (Lacheva, 2011, as *Melanogaster broomeianus* Berk. ex Tul. & Tul.), so the finding of a new locality in the Rhodopes is worth noting.

The aim of this work is to present the existing information about the species diversity and distribution of *Melanogaster* genus in Bulgaria. The paper also includes morphological descriptions of the species and keys to species identification in the genus *Melanogaster*.

Materials and methods

Collection and keeping of the samples

I collected the specimens under *Corylus avellana* L. during our field trips in the Western Rhodopi Mts, in June and October 2012. The specimens were examined in the laboratory and identified using the keys in Montecchi and Lazzari (1993), Pegler *et al.* (1993), and Montecchi and Sarasini (2000). Up to date nomenclature followed the Kirk *et al.* (2008) and online version of the Index Fungorum (<http://www.indexfungorum.org/Names/Names.asp>). The authors of fungal names are cited according to Kirk and Ansell (1992). Air-dried studied specimens of the species are deposited in the Mycological collection of the Agricultural University-Plovdiv (SOA).

Macro- and microscopic processing methods

Macromorphological description (size, fresh colour, bruising reactions, and odour) and data about the ecology of specimens were recorded, after which each specimen was photographed in its natural habitat. Micromorphological characteristics were recorded after tissue sections were rehydrated in 5% KOH or Melzer's reagent. Spores were photographed through a compound microscope. All the spore measurements were calculated from at least 20 individual measurements using Amplival ML light microscope with magnification of 1000.

A key to the species of *Melanogaster* reported from Bulgaria is also given. The key was prepared using character states derived from the literature.

Results

In this study, 2 truffle taxa belonging to 1 families were identified. These taxa are presented below with

their localities, habitats, collection dates, and accession numbers.

The specimens were identified according to Montecchi and Lazzari (1993), Pegler *et al.* (1993), and Montecchi and Sarasini (2000).

Melanogaster broomeanus Berk. (Fig. 1)



Fig. 1. Macro- and microscopic features of *Melanogaster broomeianus* from Bulgaria: a – basidiocarp at different stages of development *in situ*, b – basidiospores (photos and drawings by Maria Lacheva). Bar = 10 μm .

Macroscopic and microscopic features: Basidiocarp 2–4 cm in diameter, subglobose to irregularly lobed or slightly flattened, with irregular grooves with patches and pits, surface finely tomentose, at first yellow-brown, later purple-black to black, with or without rhizomorphs in the base. Gleba overall black but close examination reveals thin, pale yellow tramal plates with fairly regular locules that are filled with a near black gelatinous matrix containing brown spores. Peridium 300–400 μm thick, single-layered, the outer portion reddish-yellow with pigmented

cells, inner portion pale yellow to whitish, of generally parallel to the surface but also somewhat interwoven hyphae, 3.5–8.5 μm broad, clamp connections present. Trama 40–5.5 μm wide, of hyaline, gelatinised, interwoven hyphae, 3.5–8.5 μm broad, with clamp connections. Subhymenium irregular structure of hyaline, gelatinised, loosely interwoven hyphae, 3.8–8.5 μm hyphae. Basidia poorly reviving, clavate. Basidiospores 5.5–7.5 (–8) \times 4.5–7.5 μm , ellipsoid to cylindrical, base broadly truncate, apex obtuse, hyaline to pale dark-brown or olive brown, walls nearly black in cross-section.

Indicated: Rhodopi Mts (*Central*) – Bachishte locality near Dedevo village, Plovdiv distr., in a deciduous forest under *Corylus avellana* and *Pinus sylvestris*, 1–5 cm below the surface, 01.08.2005 (Lacheva, 2011).

Specimens examined: Bulgaria, Rhodopi Mts (*Western*) – near Byaga village, Pazardzhik distr., in soil among mosses and grasses, in an oak-hornbeam forest, under *Corylus avellana* L. (1 specimen), 25.10.2012, coll. and det. M. Lacheva (SOA 60 00372) – new locality to the country; Rhodopi Mts (*Central*) – Bachishte locality near Dedevo village, Plovdiv distr., in soil, in a deciduous forest under *Corylus avellana* L. and *Pinus sylvestris* L., (2 specimens), 01.08.2005, coll. G. Stoichev, det. G. Stoichev and M. Lacheva (SOA 60 0035) (Lacheva, 2011).

Habitat, ecology and phenology: In deciduous and mixed forests (*Fagus*, *Quercus*, *Carpinus*, *Pinus*) often under single hazelnut (*Corylus avellana* L.) trees and scotch pine (*Pinus sylvestris* L.) trees; fruiting bodies at or near the soil surface; grows solitary or gregarious; often form ectomycorrhizae with plant hosts, V–XI.

Melanogaster ambiguus (Vittad.) Tul. & C. Tul. (Fig. 2)

Specimens examined: Rhodopi Mts (*Western*) – near Topoli Dol village, Pazardzhik distr., in soil, in a community of *Pinus sylvestris* and *Betula pendula*,

under *Corylus avellana* (3 specimens), 12.06.2012, coll. and det. M. Lacheva (SOA 60 00371) – new locality to the country.

Relatively rare species, so far known from Vitosha Mt – Byalata Voda locality (Stoichev and Gyosheva, 2005), as well – near hut Selimitsa (Lacheva, 2011), and Central Rhodopi Mts – near Gulubovo village (Lacheva, 2011).



Fig. 2. Macro- and microscopic features of *Melanogaster ambiguus* from Bulgaria: a – basidiocarp at different stages of development *in situ*, b – basidiospores (photos and drawings by Maria Lacheva). Bar = 10 μm .

Discussion

Melanogaster broomeanus grows mostly in sandy, sandy-clay or calcareous soils, in an oak-hornbeam and coniferous forests. It is a widespread species found on almost all Mediterranean countries, where it is a relatively frequent fungus (Diamandis and Perlerou, 2008; Türkoğlu and Castellano, 2013).

It has been noted in Europe, including Denmark, Germany, Greece, Italy, Macedonia, Rumania, and United Kingdom (e.g., Vesterholt, 1998; Vesterholt *et al.*, 2003; Aulingera *et al.*, 2001; Diamandis and Perlerou, 2008; Montecchi and Lazzari, 1993; Montecchi and Sarasini, 2000; Chavdarova *et al.*, 2011; Tănase and Pop, 2005; Béres, 2012; Pegler *et al.*, 1993). It has been published from some regions of Azia; for example, it has not been noted in Turkey (Demirel, 1996, 1998, 1999; Demirel *et al.*, 2003, 2004; Sesli and Denchev, 2008; Türkoğlu and Castellano, 2013).

In Bulgaria, *M. broomeanus* was found for the first time in August 2005 from G. Stoichev and M. Lacheva, and published by Lacheva (2011) in the Bachishte locality near Dedevo village, Plovdiv distr., where it grew on a strongly insolated hill between mosses and grasses under *Corylus avellana* L. and *Pinus sylvestris* L.

A new locality of *Melanogaster broomeanus* was found in late autumn near Byaga village situated in Western Rhodopes. In October 2012, only one specimen was found on a steep slope with a southwest exposure, among mosses and grasses, in an oak-hornbeam forest with a substantial contribution of *Corylus avellana* L., developed in sandy soil. The locality of *Melanogaster broomeanus* in the Western Rhodopi Mts is the second locality of this species in Bulgaria.

Fruiting bodies of *Melanogaster broomeanus* may be mistaken with fruiting bodies of *M. variegatus* (Montecchi and Lazzari, 1993; Montecchi and Sarasini, 2000). According to Türkoğlu and Castellano (2013), the basidiocarps of *Melanogaster broomeanus* is 20-50 mm across and the fungus grows in mixed forests. I calculated the diameter of the specimens as 20-40 mm and collected the specimens under *Corylus avellana* L. and *Pinus sylvestris* L. among mosses and grasses. According to the same reference the spores are ellipsoid to subcylindrical and $4.5\text{--}5.5 \times 7.0\text{--}10.5 \mu\text{m}$. I measured

the spores as 5.5–7.5 (–8) × 4.5–7.5 µm, ellipsoid to cylindrical, which agree with the size presented of Montecchi and Lazzari (1993), namely 6.6–8.8 × 4.0 µm. *M. broomeianus* is the largest in size and the most common species of the genus *Melanogaster*. In United Kingdom it was common and in large numbers found in beech forests, usually on the surface or mixed with loose organic litter (Pegler *et al.* 1993). In Greece it was collected from the vicinity of hazelnut trees (*Corylus avellana*) in late autumn (Diamandis and Perlerou, 2008). In Macedonia it produced sporocarps under oaks at Osogovski Planini Mountain (Chavdarova *et al.*, 2011). In Turkey it was collected from mixed (*Pinus* spp. and *Quercus* spp.) forest (Demirel, 1999; Demirel *et al.*, 2003; Türkoğlu and Castellano, 2013) while in Romania (Béres, 2012) and Bulgaria it produced sporocarps under hazelnut trees (*Corylus avellana*) and scotch pine (*Pinus sylvestris*), as for example in the Rhodopi Mountain.

Melanogaster broomeanus is recorded here for the second time from Bulgaria. Contemporary knowledge of the diversity of Bulgarian fungi is based on over 100 years of investigations (Denchev and Bakalova, 2002). The known taxa of *Melanogaster* were recorded from Bulgaria between 1965 and 2012. They are as follows: *M. ambiguus*, *M. broomeanus* and *M. variegatus* (Denchev and Assyov, 2010; Lacheva, 2011). I hope that this paper will be helpful for creating a database of Bulgarian hypogeous fungi.

Identification Key to Species of Melanogaster Recorded in Bulgaria

1 Spores ellipsoid to cylindrical, basidiocarp with color other than yellow-brown, **2**

1* Spores 15.0–16.0 × 8.0–9.0 µm, ovate to fusiform, basidiocarp dark yellow-brown to olive-brown ***M. ambiguus***

2 Basidiocarp yellow-brown, purple-black to black, spores 5.5–8.0(–8.5) × 4.5–7.2 µm, mostly cylindrical.....***M. broomeanus***

2* Basidiocarp pale yellow-brown or red-brown, spores 6–11 × 3–5 µm, ellipsoid***M. variegatus***

Conclusion

Presently, the location in the Rhodopes is the only area of *Melanogaster broomeanus* Berk. that is currently known in Bulgaria. Due to its location, it is not threatened by any external factors. It is necessary to conduct further search for *Melanogaster broomeanus* in Bulgaria, as well as on other basidiomycete truffle species, as a rule.

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