Collections of *Tuber macrosporum* from the Balkan Peninsula (Bulgaria and Greece)

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Introduction

The hypogeous mycota of the western parts of the Balkan Peninsula is still little known (GLAMOČLIJA et al., 1997; ZERVA-KIS et al., 1998, 1999; DIMITROVA & GYOSHEVA, 2008; SESLI & DENCHEV, 2008; DENCHEV & ASSYOV, 2010). The ongoing research in the last few years however, has produced a number of intriguing records, thus contributing to the taxonomy and the systematic position of several uncommon species (DIAMANDIS & PERLEROU, 2008; KONSTANTINIDIS, 2009; AGNELLO & KAOUNAS, 2010; ALVARADO et al., 2011; KAOUNAS et al., 2011; GYOSHEVA et al., 2012). Field surveys in 2011 led to almost simultaneous discovery of *Tuber macrosporum* in Bulgaria and Greece, being the first record for both countries. Those collections are presented herein.

Materials and methods

The collections were found with the aid of trained dogs. The fungi are documented with colour photographs and preserved in air-dried state. The Bulgarian specimen is kept in the Mycological Collection of the Institute of Biodiversity and Ecosystem Research (SOMF) and the Greek ones are conserved in the private collection of G. Konstantinidis, noted in the text as GK.

The microscopic study was held on dried specimens. Microscopic features were observed in tap water, KOH 5% and Melzer’s solution and measured in water under Olympus BX-41 and Nikon Eclipse E100 light microscopes. The measurements in statistically relevant numbers are presented below in the form (min–) mean±standard deviation (–max); ‘n=’ denotes the number of measurements taken. The surface structures of the ascospores were studied and photographed with JEOL JSM-5300 scanning electron microscope at 20 kV. Spores for the SEM-preparation were obtained from pieces of glebal tissues mounted on metal stubs with double-sided adhesive tape and sputter-coated with gold.

The identification was confirmed mostly by the use of the works of PEGLER et al. (1993), ASTIER (1998), MONTECHI & SARASINI (2000), RIOUSSET et al. (2001), CERUTI et al. (2003), and GORI (2005), but further useful works are cited throughout the text. For extensive bibliography on this species the reader might consult PEGLER et al. (1993) and CERUTI et al. (2003). For the phylogenetic position of *T. macrosporum*, see BONITO et al. (2010a, 2010b).

Description


Macroscopic features

Ascomata hypogeous, globose or subglobose to irregular, up to 4 cm across, firm, grayish brown, purple brown to blackish brown, sometimes to almost rusty in places; outer surface with small, angular, densely crowded flattened warts, up to 2 mm across and up to 1 mm high. Gleba somewhat whitish at first, later grayish brown to blackish, marbled; veins numerous, whitish; glebal tissue under lens stippled by asci with large dark spores; smell strong, of onion or garlic-like; taste agreeable.

Microscopic features

Peridium thin, up to 300 μm thick, agglutinated throughout; the outermost layer composed of reddish brown, thick-walled elements, the inner layer of interwoven, yellowish, thick-walled hyphae, forming small, irregular cells. Asci 1- to 4-spored, subglobose to ellipsoidal, short-stalked, up to 140 ×...
73 μm, inamyloid. **Ascospores** (33–) 53.5 ± 7.5 (–79) × (21.5–) 33.7 ± 4.6 (–50) μm (n=80), length/width ratio (1.1–) 1.6 ± 0.2 (–2.3), ellipsoid, yellowish brown to reddish brown in water, ornamented with a small-meshed reticulum up to 4 μm high; meshes 6–9 across the width and 7–12 across the length of the ascospore, up to 24 μm wide (n=30); the size of the spores in general related to the number of spores in asci (largest spores usually seen in 1-spored asci).

**Specimens examined**

**Comments**
The Bulgarian and the Greek specimens match well macro- and microscopically both the original (Vittadini, 1831) and the later descriptions (Fischer, 1897; Hawker, 1954; Henning, 1971; Lawrynowicz, 1988; Pegler et al., 1993; Glamoclia et al., 1997; Astier, 1998; Montecchi & Sarasini, 2000; Coste & Rey, 2001; Riousset et al., 2001; Ceruti et al., 2003; Gori, 2005). Comparison of the values for the size of the ascospores obtained from our collections with data from the literature is presented in table 1. In the literature, available data about the spore quotient are reported only by Montecchi & Sarasini (2000), who quote it as 1.6–1.7, which corresponds to the mean value found in the Greek and the Bulgarian specimens.

**Tuber macrosporum** is considered to be a widespread but mostly uncommon species (Riousset et al., 2001; Ceruti et al., 2003). Records are known so far from Hungary, Italy, France, Germany, Romania, Spain, Serbia, Switzerland and the United Kingdom (see e.g. Fischer, 1897; Szemere, 1970; Pegler et al., 1993; Glamoclia et al., 1997; Riousset et al., 2001; Sandru, 2009; Flammer, 2011) and now also from Bulgaria and Greece. It is yet uncertain whether it is truly rare or under-recorded.

**Tuber macrosporum** superficially resembles *T. malenconii* Donadini, Riousset, G. Riousset & G. Chev. and *T. regianum* Montecchi & Lazzari. All three species share the finely warty peridium surface, which easily sets them apart from other species of *Tuber* with reticulate spores and peridium with large angular or pyramidal warts, e.g. *T. aestivum* Vittad., *T. mesentericum* Vittad. and *T. uncinatum* Chatin. Among the first mentioned species *T. macrosporum* is recognized by its 1- to 4-spored asci with gigantic spores with irregular reticulum of meshes of different shape and size. *Tuber malenconii* is distinguished by its smaller (up to 37 × 25 μm) ascospores with rather regular reticulum with meshes up to 4 μm wide, as well as by the asci, which are up to 8-spored. *Tuber regianum* is separated by the blackish brown to reddish black peridium, the rusty to brick or reddish brown gleba, the asci which bear up to 8 spores, as well as by the distinctly smaller (up to 20 × 16 μm) ascospores with rather regular reticulum composed by meshes up to 5 μm wide and number up to 5-6 across the length of the spore.

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**Fig. 1. – Tuber macrosporum**
On left, collection GK 5899; photo: G. Konstantinidis. On right, collection SOMF 29373; photo: D. Stoykov.
Fig. 2 — *Tuber macrosporum*. Hymenial elements (asci, ascospores) in LM. Scale bar = 50 µm
Photo: G. Konstantinidis

Fig. 3 — *Tuber macrosporum*. Hymenial elements (asci, ascospores) in LM. Scale bar = 50 µm
Photo: D. Stoykov

Fig. 4 — *Tuber macrosporum*. Hymenial elements (ascus, ascospore) in SEM. Collection SOMF 29373.
Photo: M. Gyosheva
<table>
<thead>
<tr>
<th>Authors/studied specimens</th>
<th>Spore length (μm)</th>
<th>Spore width (μm)</th>
</tr>
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<tbody>
<tr>
<td>HAWKER (1954)</td>
<td>55–70–80</td>
<td>39–49–60</td>
</tr>
<tr>
<td>SZEMERE (1970)</td>
<td>38–82</td>
<td>28–45–60</td>
</tr>
<tr>
<td>PEGLER et al. (1993)</td>
<td>45–75</td>
<td>30–50</td>
</tr>
<tr>
<td>GORI (2005)</td>
<td>38–75</td>
<td>25–45</td>
</tr>
<tr>
<td>SOMF 29373 (n=30)</td>
<td>(45–) 60.3 ± 5.5 (–75)</td>
<td>(30–) 42 ± 2.7 (–44.5)</td>
</tr>
<tr>
<td>GK 5877 (n=28)</td>
<td>(39–) 52.9 ± 9.4 (–79)</td>
<td>(21.5–) 29.3 ± 6.6 (–47.5)</td>
</tr>
<tr>
<td>GK 5899 (n=22)</td>
<td>(33–) 47.3 ± 7.5 (–62)</td>
<td>(21.5–) 29.9 ± 4.4 (–44.5)</td>
</tr>
</tbody>
</table>

**Tab. 1.** – Ascospores of *T. macrosporum* – comparative data.

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**References**


