

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

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Summary: Description of the first findings of *Tuber macrosporum* in Bulgaria and Greece, illustrated by photographs of macroscopic and microscopic features, including SEM-microphotographs.

Keywords: Ascomycetes, Balkan mycota, *Pezizales*, truffle, *Tuberaceae*, *Tuberales*

Résumé : description des premières récoltes de *Tuber macrosporum* de Bulgarie et de Grèce, illustrée par des photographies des caractères macroscopiques et microscopiques, y compris microphotographies au MEB.

Mots-clés : ascomycètes, mycota des Balkans, *Pezizales*, truffe, *Tuberaceae*, *Tuberales*.

Introduction

The hypogeous mycota of the western parts of the Balkan Peninsula is still little known (GLAMOČLIJA *et al.*, 1997; ZERVAKIS *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 photo-

graphed 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), MONTECCHI & 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

Tuber macrosporum Vittad., *Monogr. Tuberc.*, p. 35 (1831).

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 ×



Fig. 1. – *Tuber macrosporum*

On left, collection GK 5899; photo: G. Konstantinidis. On right, collection SOMF 29373; photo: D. Stoykov.

73 μm , inamyloid. **Ascospores** (33–) 53.5 ± 7.5 (–79) \times (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

BULGARIA: Pleven distr., near Somovit town, by the riverside of Danube River, under *Populus nigra* L., 10.12.2011, leg. P. Borisova (SOMF 29373). GREECE: Kastoria, under broad-leaf trees, 04.11.2011, leg. G. Setkos (GK 5877); idem, 11.11.2011, leg. G. Setkos (GK 5899).

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; ŁAWRYNOWICZ, 1988; PEGLER *et al.*, 1993; GLAMOČLIJA *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; GLAMOČLIJA *et al.*, 1997; RIOUSSET *et al.*, 2001; ŞANDRU, 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 $37 \times 25 \mu\text{m}$) 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 $20 \times 16 \mu\text{m}$) 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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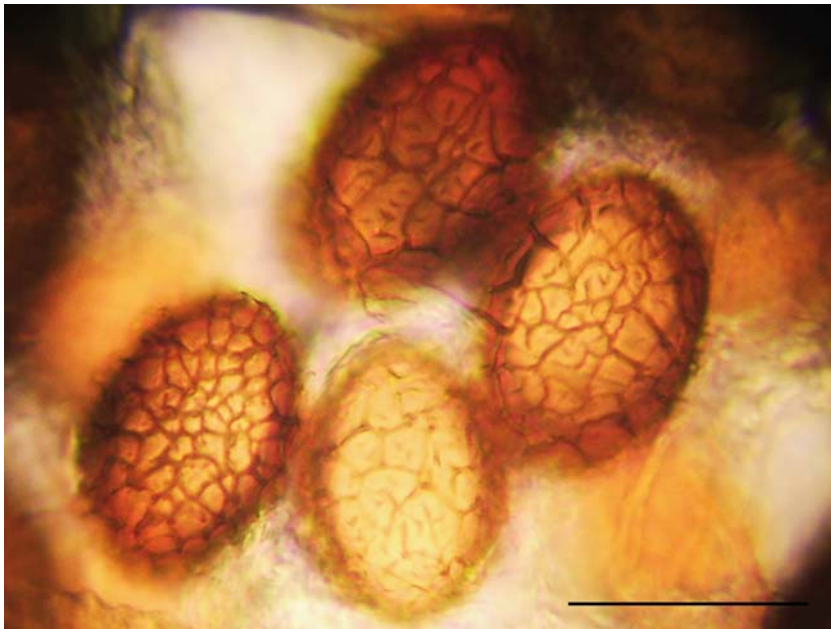


Fig. 2 – *Tuber macrosporium*. Hymenial elements (asci, ascospores) in LM. Scale bar = 50 μ m
Photo: G. Konstantinidis

Fig. 3 – *Tuber macrosporium*. Hymenial elements (asci, ascospores) in LM. Scale bar = 50 μ m
Photo: D. Stoykov

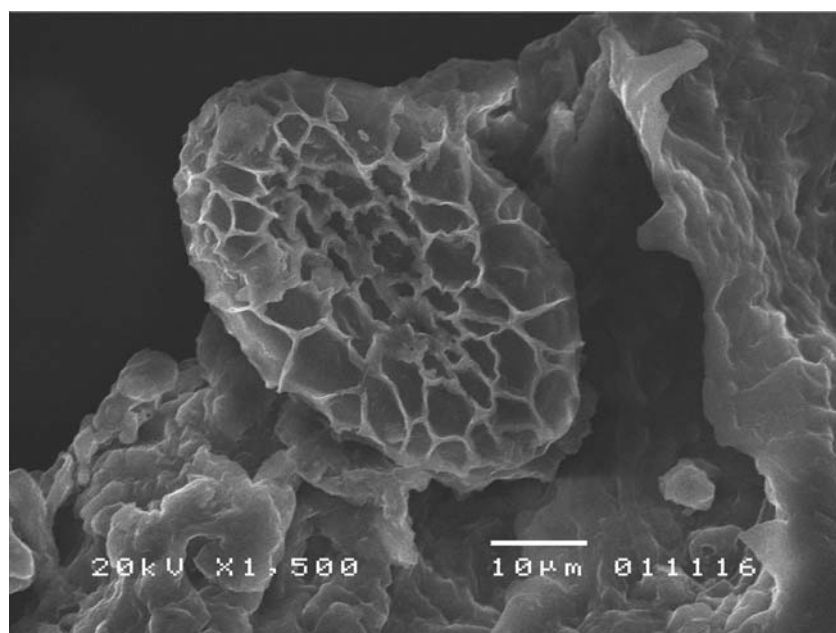


Fig. 4 – *Tuber macrosporium*. Hymenial elements (ascus, ascospore) in SEM. Collection SOMF 29373.
Photo: M. Gyosheva

Authors/studied specimens	Spore length (µm)	Spore width (µm)
HAWKER (1954)	55–70–80	39–49–60
SZEMERE (1970)	38–82	28–45–60
ŁAWRYNOWICZ (1988)	(38–) 55–80	(28–) 39–60
PEGLER <i>et al.</i> (1993)	45–75	30–50
ASTIER (1998)	40–80	30–55
MONTECCHI & SARASINI (2000)	36–53	23–32
RIOUSSET <i>et al.</i> (2001)	(30–) 40–80 (–92)	(25–) 30–55 (–62)
GORI (2005)	38–75	25–45
SOMF 29373 (n=30)	(45–) 60.3 ± 5.5 (–75)	(30–) 42 ± 2.7 (–50)
GK 5877 (n=28)	(39–) 52.9 ± 9.4 (–79)	(21.5–) 29.3 ± 6.6 (–47.5)
GK 5899 (n=22)	(33–) 47.3 ± 7.5 (–62)	(21.5–) 29.9 ± 4.4 (–44.5)

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

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