First Bulgarian collections of *Mattirolomyces terfezioides* (*Pezizaceae*), a potentially valuable hypogeous fungus

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Abstract. The paper discusses occurrence of *Mattirolomyces terfezioides* in Bulgaria, presenting the easternmost locality in Europe known so far. Description and illustrations of the first Bulgarian collections are included.

Key words: Bulgarian mycota, *Pezizales,* subterranean ascomycetes, *Terfeziaceae,* truffle-like fungi

Introduction

Truffles (i.e. the members of genus *Tuber* Micheli ex F.H. Wigg.) are well-known and prized delicacy mushrooms which are extensively traded. Some truffle-like species of other genera are also subject of commercial interest, notably members of the genera *Terfezia* (Tul. & C.Tul.) Tul. & C.Tul. and *Tirmania* Chatin. Another related genus, *Mattirolomyces* E. Fisch., and its only European species, *M. terfezioides* (Mattir.) E. Fisch., have received little attention so far, irrespective of some peculiar properties, not found in other hypogeous fungi (Gógán Csorbainé 2009). In 2015, the authors received from correspondents two specimens of hypogeous fungus, which turned out to be the first Bulgarian collections of *M. terfezioides.* Those findings are presented and discussed herein.

Materials and methods

One of the specimens was received in fresh state shortly after collecting. Ascomata were photographed *ex situ* and important macroscopic and organoleptic characters were noted down. The second collection was presented to us in dried state, supplied with photographs taken *in situ* and *ex situ.* Microscopic examination was held on fresh and dried materials with the aid of Amscope T360B light microscope, equipped with Amscope MU900 digital camera. Microscopic slides were mostly prepared in tap water, but Congo red in ammonia, aqueous Floxine and aqueous cotton blue were also used arbitrarily to visualize better some of the microscopic structures. Amiloidity was tested with Melzer's reagent and IKI. Measurements were taken on pre-calibrated digital photographs with Piximetre ver. 5.9. Ascospore measurements reported below refer to 50 spores, measured without the ornamentation on slides in water. In the quotation of ascospore size, the figures in parenthesis represent the average value and the respective standard deviation, while the minimum and maximum values are not included in parenthesis. For the remaining microscopic structures only the minimum and maximum values are recorded; the figures are based on 20 measurements for asci and 15 for cells of peridium and gleba.

Description of species

***Mattirolomyces terfezioides* (Mattir.) E. Fisch.,** in Engler & Harms, Nat. Pflanzenfam., Edn 2, 5bVIII: 39 (1938); *Choiromyces terfezioides* Mattir., Mém. R. Accad. Sci. Torino, Ser. 2, 38: 384 (1888); *Terfezia terfezioides* (Mattir.) Trappe, Trans. Brit. Mycol. Soc. 57(1): 91 (1971). (Fig. 1a-i)

Ascomata hypogeous, later semihypogeous, tuberiform, irregularly wrinkled to lobate, up to 11 cm across. Peridium smooth or with pubescent appearance, occasionally disappearing in places, initially white, subsequently darkening to somewhat yellowish- ochraceous or ochraceous, changing to yellow when bruised. Gleba initially compact and brittle, with age becoming softer, at first whitish, then cream to pale-ochraceous, with numerous whitish sterile veins, branching and anastomosing and separating different in size fertile areas. Odour agreeable or somewhat spermatic in young and mature specimens, unpleasant in overmature ascomata. Taste characteristic, very sweet, reminding of sweeteners. Asci ovoid to ovoid elongate, pedicellate, 122.5–199.5 × 45.5–71.9 µm, mostly with irregularly disposed ascospores, seldom loosely biseriate, 8-spored, inamyloid. Ascospores globose, 14.8–(16.6±1.0)–18.6 µm across, hyaline, with up to 0.6 µm high, in places incomplete, areolate-reticulate ornamentation; meshes regular, polygonal, up to 6 across the spore diameter, at the corners with projecting aculei up to 1.8 µm high. Peridium composed of loosely connected inflated elements, 17–69.1 × 18.7–74.9 µm. Gleba composed of randomly disposed asci and chains of variable in shape inflated cells up to 146.5 × 40.9 µm, tissues of the veins more or less similar to those of the fertile areas.

Specimens examined. Bulgaria: in the vicinity of Karantsi village, close to *Robinia pseudoacacia* L., 13.11.2015, D. Dimitrov (SOMF 28802); in the vicinity of Svishtov town, 16.11.2015, C. Geneva (SOMF 28803); idem, 20.12.2015, C. Geneva (obs.).

Discussion

The studied specimens in their macromorphology and microscopic features agree perfectly with the contemporary descriptions of *M. terfezioides* (Astier 1998; Montecchi & Sarasini 2000; Gori 2005). Konstantinidis & Kaounas (2014) describing their collection of the species have found a slightly different size range of the ascospores. Nevertheless, the values from our material fall within this range. Gori (2005) also cited a wider range, but his measurements apparently include the spore ornamentation.

The species was originally described by Mattirolo (1888) as *Choiromyces terfezioides* and provided with ample description and detailed illustration. Fischer (1938) erected a new genus, *Mattirolomyces.* Later on, Trappe (1971) proposed placement in the genus *Terfezia,* but retained *Mattirolomyces* at subgeneric level. Further molecular research did not confirm the accommodation of the fungus in this genus, but rather supported the resurrection of *Mattirolomyces* (Percudani & al. 1999; Díez & al. 2002). The genus *Mattirolomyces* includes four more species, all extra-European. *Mattirolomyces spinosus* (Harkn.) Kovacs, Trappe & Alsheikh and *M. mexicanus* Kovacs, Trappe & Alsheikh are established as distinct by ITS and LSU phylogenetic analysis. They are described and discussed in detail in Kovács & al. (2011b). Both species share a lot of similarities and are difficult to distinguish from *M. terfezioides,* although *M. spinosus* is said to have indistinctive flavour (Kovács & al. 2011b). *Mattirolomyces mulpu* Kovacs, Trappe & Claridge is described in Trappe & al. (2010a) and *M. austroafricanus* (Trappe & Marasas) Kovacs, Trappe & Claridge is presented in Trappe & al. (2010a, b). Those two species have larger spores (exceeding 19 µm) with higher ornamentation.

Mattirolomyces terfezioides is well-distinguished from the related hypogeous fungi of the genera *Terfezia* and *Tirmania* by a combination of characters, including morphology of ascomata, peridium of inflate elements, inamyloid asci and ornamentation of the ascospores. The species of *Tirmania* have amyloid asci and smooth ascospores and thus are separated easily from *Mattirolomyces* (Díez & al. 2002). The genus *Terfezia* includes species with non-amyloid asci and variable ornamentation: spinose or reticulate. The members of this genus, however, feature variable in structure, but compact peridium, different from the peridium of *M. terfezioides,* which is composed of loosely connected elements and therefore is often fleeting. Healy & Kovács (2010) provided ultrastructural evidence for the distinction of *Mattirolomyces* from *Terfezia,* namely heterogeneously staining spines of the spore wall and hexagonal Woronin bodies. Several species of *Terfezia* possess reticulate ornamentation. In Europe and adjacent areas, these are

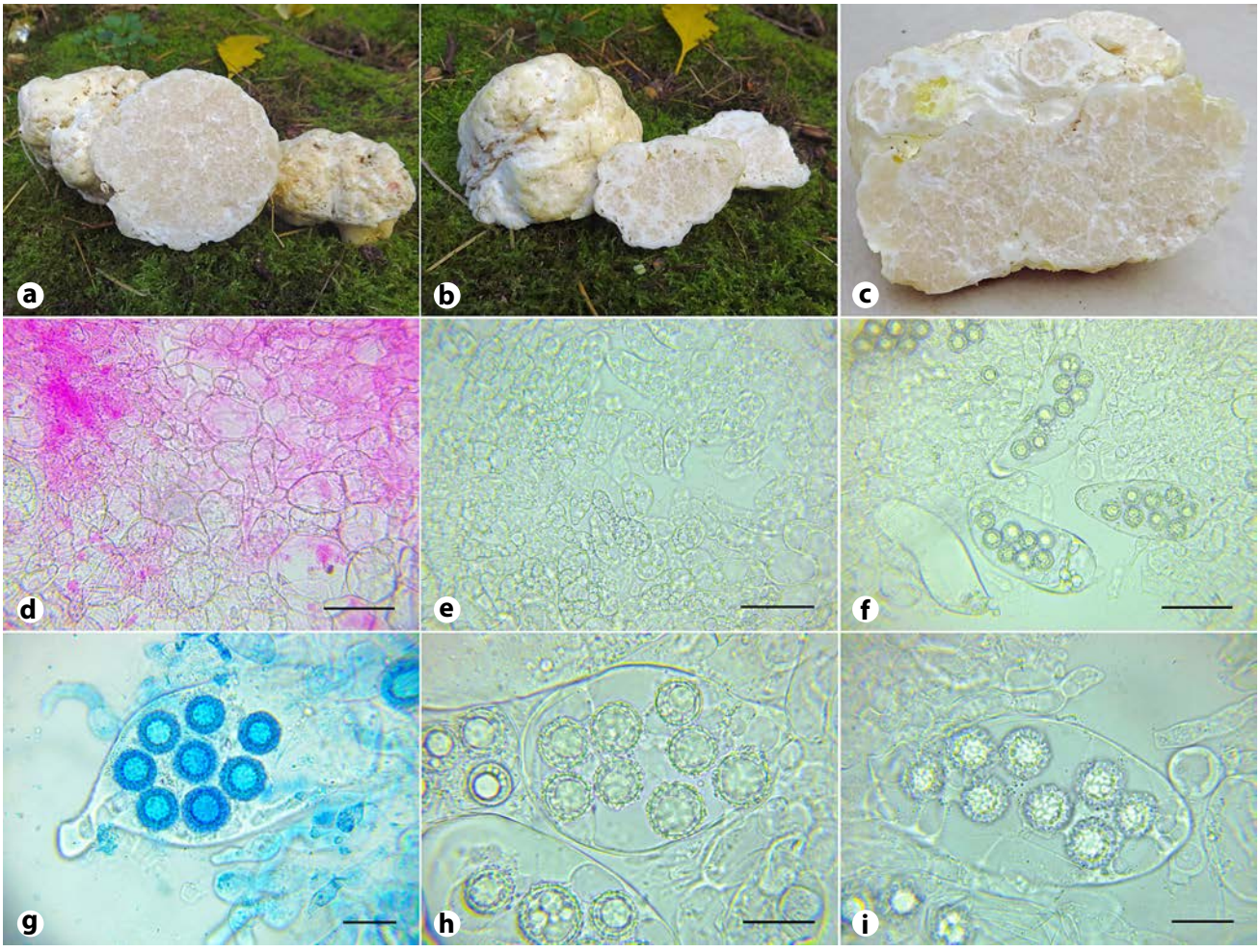

Fig. 1. Macromorphological and microscopic features: **a-c** – ascomata ex situ; **d** – cross section of peridium; **e** – gleba (sterile veins); **f** – randomly disposed asci with ascospores; **g-i** – asci with ascospores, with emphasis on spore ornamentation (**d** – aqueous floxine, **g** – aqueous cotton blue, **e,f,h,i** – tap water). Scale bars = 50 µm (d-f) & 20 µm (g-i).

T. alsheikhii Kovács, M.P. Martín & Calonge (reticulate ascospores), *T. boudieri* Chatin (warty-reticulate), *T. canariens* Bordallo & Ant. Rodr. (reticulate), and *T. claveryi* Chatin (reticulate). All four species have either pseudoparenchymatic or hyphal peridium (Kovács & al. 2011a, Bordallo & al. 2012). From ecological point of view, the species of this genus are known to form mycorrhizae with representatives of the plant family *Cistaceae* Juss. On the other hand, *Mattirolomyces terfezioides* has been documented to enter into relationship with a broader spectrum of vascular plants (see comments below).

The distribution of *M. terfezioides* is apparently yet to be clarified, judging from the Bulgarian findings, which expand further its known geographic range and appear to be its easternmost part so far known. So far it seems that the fungus is most common in Hungary (Szemere 1970; Ławrynowicz 1990; Király & Bratek 1992), where it is collected for alimentary purposes. Scattered records exist also from France (Ławrynowicz 1990; Ławrynowicz & al. 1997), Italy (Montecchi & Sarasini 2000; Bizio 2002), Slovakia (Glejdura & Kunca 2012), and Spain (Kovács & al. 2009). In the Balkan countries, previous records are known only from Serbia (Ławrynowicz & al. 1997) and Greece (Konstantinidis & Kaounas 2014), in both countries rather localized. Data for the occurrence of the species outside Europe is included in a single report from India (Khare 1975) and another one from China (Zhang 1992). In relation to the collection of Khare (1975), the discovery of the very similar *M. spinosus* in Pakistan should be noted (Kovács & al. 2011b). Mention deserves the fact that the ascospores of the Indian collection were reported to be larger, 18.5–24 µm. Extra-European records of *M. terfezioides* are of special interest, as it is yet unclear whether it is a native species in Europe,

a question raised by its common putative association with some non-native trees. Various host plants were reported in Europe, including representatives of the genera *Ficus* L., *Robinia* L., *Prunus* L., and *Asparagus* L. (Astier 1998; Montecchi & Sarasini 2000). Studies by Kovács & al. (2003, 2007) have found evidence for the relation of *M. terfezioides* with both woody and herbaceous plants, namely *Celtis occidentalis* L., *Crataegus monogyna* Jacq., *Helianthemum ovatum* (Viv.) Dunal, *Ligustrum vulgare* L., *Muscari racemosum* (L.) Mill., *Robinia pseudoacacia*, *Salvia glutinosa* L., and *Viola cyanea* Čelak.

Mattirolomyces terfezioides presents certain interest for practical purposes as it has peculiar flavour and taste, being the only European hypogeous ascomycete with strong sweet taste, very similar to that of sweeteners. Although not very popular, which is probably due to the fact that it is not found in most European countries, it has interesting potential applications. In Hungary, where it seems more common, it is collected and used for preparation of various deserts (Gógán Csorbainè 2009). Due to this, it has been considered as a species with potential in truffle gastronomy (Bratek & al. 2013). The authors are not aware if the fungus is collected for practical purposes in Bulgaria.

Another important peculiarity of *M. terfezioides* is that it associates with the roots of different plants, but very often with *R. pseudoacacia.* The association might be achieved also under laboratory conditions (Bratek & al. 1996; Kovács & al. 2002, 2003), a fact which could encourage further attempts for cultivation. *Robinia pseudoacacia* is a non-native tree, which is extensively planted in various parts of Bulgaria and has become one of the most important invasive species in this country (Petrova & al. 2012). A recent account shows that plantations of this species cover approximately 150 000 ha in Bulgaria (Dimitrova 2012). Although there is no published evidence so far, our field experience has shown that *Robinia* stands in this country are generally poor in terms of edible fungi. The presence of species of potential commercial importance could thus elevate the value of such artificial plantations. So far the authors have been aware of the existence of two localities of *M. terfezioides* in Bulgaria, although it could be much more widespread. Further research should reveal the actual extent of its occurrence and provide precise characterization of its habitats.

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