# Four *Tuber* species accompanying *T. mesentericum* in natural sites in Poland

by

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#### **Abstract**

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Tuber rufum Pico: Fr., T. ferrugineum Vittad., T. maculatum Vittad. and T. fulgens Quél. have been identified among hypogeous fungi occurring together with T. mesentericum in natural sites in the Częstochowa region (S Poland). All these species have been recorded in this locality for the first time; T. ferrugineum and T. fulgens are new to Poland. Descriptions of the materials collected, scanning electron micrographs of spores, as well as comments on chorology of the species are provided in the paper.

**Keywords:** hypogeous fungi, chorology, ecology, taxonomy, Poland.

#### Introduction

The area of Poland lies in the temperate climate zone. The records of edible truffles in the country represent the *Tuber aestivum* group (Ławrynowicz & al., 2008). T. mesentericum seems to be the most frequent among them. It was found in 1981 in the Zielona Góra forest nature reserve close to the border of the Częstochowa town (Ławrynowicz, 1988) in oak-beech forest occupying calcareous hill (300 m a.s.l.). A vicinity of the town inhabited by 200 thousand people, thousands of pilgrims coming to this place every year and intense tourist activity due to great landscape result in countless trails converging on the town borders. Tuber mesentericum has been found to produce great numbers of fruitbodies almost each year in the most attended places. In 1997, the species was found in another locality - on the south-western slope of a calcareous hill of the Warta river gorge. The site was

#### Resumen

Ławrynowicz, M. 2009. Cuatro especies de Tuber que acompañan a T. mesentericum en localidades naturales en Polonia. *Anales Jard. Bot. Madrid* 66S1: 145-149 (en inglés).

Tuber rufum Pico: Fr., T. ferrugineum Vittad., T. maculatum Vittad. y T. fulgens Quél. han sido identificados entre los hongos hipogeos presentes junto con T. mesentericum en localidades naturales en la región de Częstochowa (S de Polonia). Todas estas especies se constatan por primera vez en esta localidad; T. ferrugineum y T. fulgens son nuevos en Polonia. En este estudio se ofrecen descripciones de todo el material recolectado, el escaneado de micrografías electrónicas de esporas, además de comentarios sobre la corología de las especies.

**Palabras clave:** hongos hipogeos, corología, ecología, taxonomía, Polonia.

occupied with 30 years old *Quercus robur* forest (Ławrynowicz, 1999).

Hypogeous fungi are a subject of thorough and systematic field searching in Poland. It is easy to observe that these fungi produce their fruit bodies only in some specific places called oases or nests. In such sites usually several species representing different systematic groups can be found but not every year. The climatic conditions of temperate zone are suitable for production of hypogeous carpophores only exceptionally. The year 2007 was favourable for hypogeous fungi including edible truffles from *Tuber aestivum* group – they were collected in great quantities in some regions in southern Poland (Ławrynowicz & al., 2008).

The sites of the first collections of *Tuber mesente-ricum* have been carefully monitored every year for more than twenty years. Special attention has been paid to other *Tuber* species accompanying *T. mesentericum*. Four of them are presented in the paper: *T. rufum*,

T. ferrugineum, T. maculatum and T. fulgens. T. ferrugineum and T. fulgens are the species reported in Poland for the first time.

#### Material and methods

The material was collected by the author in course of study of hypogeous fungi in the Częstochowa Upland. Repeated searching in two places in 2007 yielded several species of hypogeous fungi, among them four species of *Tuber* accompanying *T. mesentericum*. All the fruit bodies were analysed taxonomically using classical methods. The micromorphological and anatomical study was carried out. Peridium, asci and ascospores were examined under the Nikon Eclypse E-600 microscope (200×, 400× and 600×) and in scanning electron microscope. Comments on taxonomy, ecology and chorology of the presented species are given. In case of the species new for Poland, detailed descriptions are also provided. Dried materials have been preserved in Herbarium Universitatis Lodziensis (LOD).

#### Results and discussion

**Tuber rufum** Pico: Fr., Meleth. inaug. fung. gen. prop. p. 80. 1788.-Fries, Syst. Mycol. 2, p. 292. 1823

This species is well represented in the Polish collections, morphological description and chorology is given by Ławrynowicz (1988, 1990).

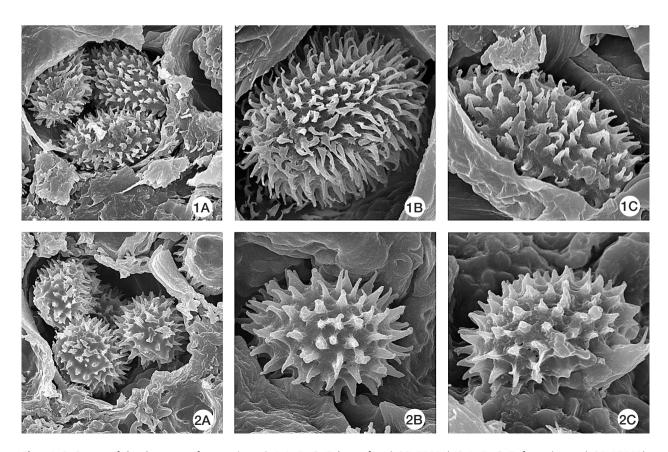
Taxonomical delimitation of this species is subject to different interpretations (e.g. Pegler & al., 1993; Calonge & al., 1994a, b, c; Montecchi & Sarasini, 2000; Riousset & al., 2001). The analysis of the Polish material contributes to better understanding of the differentiation between *T. rufum* and *T. ferrugineum*.

The illustrations of spores and asci in SEM demonstrate the differences in comparison with respective images of *T. ferrugineum* (Figs. 1A, B, C; 2A, B, C).

T. rufum was found under Quercus, Tilia, Corylus avellana, Fagus sylvatica, in humus layer of light soils, often together with other hypogeous species. It prefers places with bare soil, on roads and footpaths with removed litter layer, in woods and town areas, as well. T. rufum is rather common in large part of Europe in regions with a temperate or Mediterranean climate (Ławrynowicz, 1992).

Examined specimens: Częstochowa Upland, Wancerzów, under Quercus robur, VII-2007, leg. M. Ławrynowicz, LOD 22021.

**Tuber ferrugineum** Vittad. Mon. Tub. p. 46, pl. 3. f. 10. 1831



Figs. 1, 2. Stages of development of spores in asci. 1 A, B, C, Tuber rufum (LOD 22021); 2 A, B, C, T. ferrugineum (LOD 22022).

Fruit body 0,5-2 cm in diam., surface reddish brown, similar to *Balsamia platyspora*. Ripe specimens covered with small warts. Gleba whitish gray becoming ochre brown, finally rust or chocolate brown with white veins. No distinct odour has been noted.

Asci 60-180 µm with stalk 6-20 µm. Spores ellipsoid or subglobose 18-40 × 15-30 µm, yellow, becoming red brown with age, covered with rather firm spines connected with each other at their bases, forming ± regular reticulum visible in light microscope. These net-like connections have been the reason of confusion in the species discriptions. Some authors (e.g. Fischer, 1897) write about reticulated spores, other mention spores as spiny and come to the conclusion that *T. ferrugineum* is closely related to *T. rufum* (Knapp, 1951; Szemere, 1965; Pegler & al., 1993; Montecchi & Sarasini, 2000; Riousset & al., 2001).

The study of the Polish material supports the identity of *T. ferrugineum*. The differences between spores ornamentations of *T. rufum* (Fig. 1A, B, C) and *T. ferrugineum* (Fig. 2A, B, C) are very distinct. Such point of view was also expressed by Calonge & al. (1977).

Tuber sp. described and illustrated by Calonge & al. (1994b) seems to have spore structure similar to *T. ferrugineum*, but sometimes also nodular, determined as "... Esporas elipsoides de dos tipos: unas espinosas y otras nodulosas...". The similar phenomenon has been observed in the Polish material and is partly visible in the Fig. 2C. Nodular spores or nodular parts of spores seem to be covered with a kind of delicate *velum* during the process of their maturation. This phenomenon is even more distinct in case of *T. maculatum* (Fig. 3A, B).

*T. ferrugineum* seems to be rare because it was not included in many publications as a result of various taxonomical interpretations and not separating it from *T. rufum*.

*T. ferrugineum* was collected in Poland for the first time. It was found as three small groups of 4-8 fruit bodies and was collected only once in the site, where *T. mesentericum* had been observed every year for more than twenty years.

Examined specimens: Częstochowa Upland, Wancerzów, under Quercus robur, VII-2007, leg. M. Ławrynowicz, LOD 22022.

# **Tuber maculatum** Vittad. Mon. Tub. p. 45, pl. III. fig. 16. 1831

The species is rare, known in Poland only from two localities up till now. Morphology and chorology of the species were described by Ławrynowicz (1988, 1990).

Fruit bodies globose, with white surface changing

colour into yellow with reddish brown or brown patches. Peridium thin, without pseudoparenchymatous layer – the feature distinguishing the species from similar ones. Spores ellipsoid or ovoid, covered with regular reticulum, mashes usually not exceed 10  $\mu$ m in diam. Fig. 3A, B, C demonstrate three successive stages of development of spores.

T. maculatum is widespread in Europe between 45° N and 60° N. It is known from Sweden, Norway, Finland and the Saint Petersburg region in Russia. It occurs in nemoral and boreal zones of temperate climate in Europe. In the southern part of its range it occurs in mountain forests (Ławrynowicz, 1992).

The specimens presented in the paper come from a new locality, where they accompanied *T. mesente-ricum*. It is thus the third locality of this species in Poland. A group of 7 fruit bodies were collected, but only two of them were ripe. The species is known only from calcareous area in the south of Poland (Ławrynowicz, 1990).

Examined specimens: Częstochowa Upland, Wancerzów, under Quercus robur, VII-2007, leg. M. Ławrynowicz, LOD 22023.

## Tuber fulgens Quél., Grevillea 8: 115-117. 1879

Fruit bodies globose or subglobose, 1-4 cm in diam., at first white, then yellow, becoming orange reddish and finally rust brown. Surface covered with small warts. Gleba of apricot colour becoming chocolate brown with white veins.

Asci 120-130×90-100  $\mu$ m, mostly 1-4 spored. Spores globose or subglobose, 34-50  $\times$  30-40  $\mu$ m, covered with reticulum of wide alveolae with high walls (Fig. 4A, B, C).

According to some authors, *T. fulgens* is the subspecies of *T. excavatum* (Fischer, 1897) or its form (Ceruti, 1960). According to Knapp (1952), Szemere (1965), Montecchi & Sarasini (2000), Riousset & al. (2001) it is a separate species.

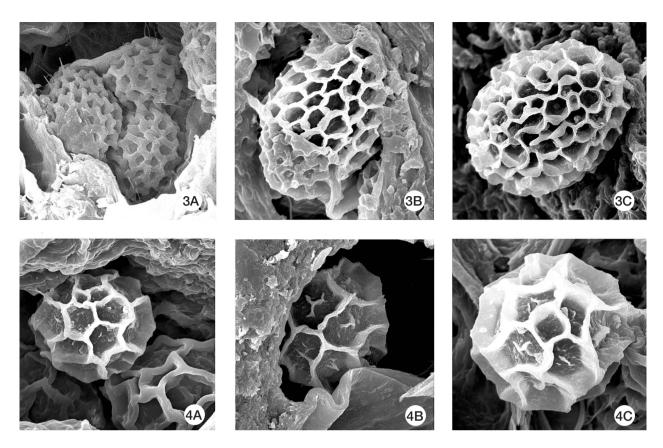
The species is new to Poland. The examination of morphology and SEM pictures of specimens from the Polish collection support the concept of *T. fulgens* as a distinct species.

It was found under *Quercus robur, Carpinus betulus* in calcareous area, in the same site as *T. mesentericum*.

Examined specimens: Częstochowa Upland, Zielona Góra reserve, VII-2007, leg. M. Ławrynowicz, LOD 22024.

#### Conclusions

Tuber mesentericum is the most known species among the black truffles in Poland, recorded in five localities within three geographical regions in the south of the country (Ławrynowicz & al., 2008).



Figs. 3, 4. Stages of development of spores in asci. 3 A, B, C, Tuber maculatum (LOD 22023); 4 A, B, C, T. fulgens (LOD 22024).

The two localities near Częstochowa town, discovered in 1981 and 1997 respectively, are subject to every year monitoring. The examination yielded also some other hypogeous species, among them four collections representing the genus *Tuber* found together with *T. mesentericum*, which are presented in the paper. Micromorphology of the spores has been illustrated by scanning electron micrographs supporting their identity and species concept. Comments on the distribution in Poland and Europe are given.

Tuber ferrugineum and T. fulgens are published from Poland for the first time. T. maculatum seems to be rare, T. rufum is rather common; both species are widespread in nemoral and boreal zones.

The results of examination of ecological conditions suitable for hypogeous fungi growing together with edible truffles enable to better understand their environmental requirements. An interest in truffles cultivation has been significantly increasing in the last years and attempts towards introduction of truffle orchards have been recently made in the whole Europe, including Poland (Chevalier, 2008; Hilszczańska & al., 2008).

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