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# *Tuber decipiens*, a new black *Tuber* species from Croatia

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## Abstract

***Background and Purpose:*** *The species diversity within the genus* Tuber *is a highly mysterious. The aims of this study are to give detailed taxonomical, morphological and ecological characteristics of the new species* Tuber decipiens.

***Material and Method:*** *Based on fungarium studies, field observations and historical review, morphological studies have been conducted on the species. The recording morphological details were carried out using a microscope.*

***Results and Conclusions:*** Tuber decipiens *is a black colored truffle and a new species belonging to order* Pezizales *and family* Tuberaceae. *It was found in autumn (November-December 2010) near the Adriatic Sea (Rovinj, Croatia) and grows in pure pine forest (*Pinus halepensis*) in the calcareous-gravel soil (pH 7.5–7.8).* Tuber decipiens *is different in the morphological and other characteristics from all the other known black. The new species is most similar to the winter truffle* Tuber brumale *var.* moscatum *which grows in autumn and winter in symbiosis with deciduous trees. Unlike the winter truffle, a new species (*Tuber decipiens*) grows in symbiosis with* Pinus halepensis *and permanently has a basal depression with one or more cavities, clearly visible in the cross section of fruitbodies. Very important characteristics are the color of gleba, which is never blackish or black and in mature fruitbodies remains gray-brownish, the asci are spherical or very rarely ovoid-ellipsoid, containing 1–6 spores. The odor of the new species is not pleasant and reminiscent of the soil or mould while the odor of Tuber brumale var. moscatum is delicate and persistent, and reminiscent of the musk.* Tuber decipiens*, a new species from Croatia is described.*

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## INTRODUCTION

The study of the underground fungi began in Italy (Vittadini 1800–1865), then in France (Tulasne 1815–1885, Quelet 1832–1899), again in Italy (Saccardo 1845–1920, Mattirolo 1856–1947, Ceruti 1911–2000) and then by many authors in other countries. On the territory of Croatia truffles were not researched and there are no scientific papers about the underground fungi. *Tuber decipiens* is a new species and grows in pure pine forest (*Pinus halepensis* Mill.) near the Adriatic Sea (Rovinj, Croatia). Truffles were found for the first time, November 15, 2010 in the calcareous-gravelly red soil (pH 7.5–7.8) at the depth of 15 cm. Some black colored truffles produce allelochemicals substances *(1)* that inhibit the growth and the reproduction of grass.

This group includes *Tuber melanosporum* Vittad., *Tuber aestivum* Vittad., *Tuber aestivum var. uncinatum (*Chatin) Montecchi and Borelli. A new species of black truffle grows in the high green grass without traces (disappearance of grass) around mycorrhizal pine.

After a scientific review of the European species of truffles Ceruti et al. *(2),* only nine species and forms of black colored truffles are recognized. Many other species of black and other truffles described by the mycologists over the centuries (from the 18th to the 20th) have become only synonymous. The two most important morphological characteristics of black truffles are certainly the form and the ornament of the spores. Within the recognized species only three species (*Tuber brumale* Vittad., *T. melanosporum* Vittad. and *T. brumale var. moscatum* (Ferry) Montecchi and Lazzari) have spores with single spines as *Tuber decipiens*. The spores of other known black truffles (six) have a reticulate-alveolate ornament with polygonal meshes.

Accordingly, the new species can only be compared with black truffles whose spores are densely ornamented with single spines, well spaced, rigid and sharp. The color of gleba and the density of sterile veins are other important characteristics of each species, which are significantly different when compared with similar black truffles. The odor is also an important characteristic of truffles and it depends on the composition of different volatile chemicals substances *(3)*. *Tuber melanosporum* Vittad. contains fourteen volatile substances, six alcohols, four aldehydes, two ketones, dimethy sulfide and anisol *(3)*. In the study of volatile compounds of white truffle (*Tuber magnatum* Pico) it was published that the bis (methylthio) methane is the only component responsible for the odor *(4)*. The odor of *Tuber decipiens* is not pleasant and is reminiscent of the soil or mold and it would be very useful to determine the volatile substances.

## MATERIAL AND METHODS

The fruitbodies of *Tuber decipiens* were found November 15, and December 12, 2010, near the Adriatic Sea (Rovinj, Croatia). Based on field observations, fungarium studies and historical review of the all other known black truffles, numerical and morphological studies have been conducted on the species. The microscopy of peridium, asci, spores, hyphae and their measurement was performed with a microscope Carl-Zeiss Jena, Germany. The recording of the morphological details was carried out using a microscope Carl-Zeiss Jenamed 2, Germany and camera Olympus, Camedo C-5050 Zoom. The acidity of the soil in aqueous solution (pH) was measured with pH-meter IQ150 (IQ Scientific Instruments, USA).

## RESULTS

### Description of the new species

*Tuber decipiens* Božac, Širić et Kos, sp. nov.
MycoBank MB 563741

Etymology: from latin decipiens = deceiving

Additional specimens examined. CROATIA. ISTRIAN PENINSULA: Rovinj, 35 km NE of Pula, 45°04'17.19" N, 13°38'15.5' E, atl. 9 m s. m., in symbiosis with *Pinus halepensis* Mill., leg. R. Božac, November 15, and December 12, 2010.

The form of fruitbodies is more or less irregular, can be rounded, tuberiform, in some places lobed, but also regular and subglobose, not very hard, very soon become soft and decaying (Figure 1A). The average diameter is 4–7 cm, and the surface is blackish-brown or nearly black and covered with warts. The fruitbodies may have a basal depression or cavity or more holes clearly evident in the appropriate section. It is almost always eaten by thick white worms.

The peridium is composed of very irregular blackish--brown or nearly black warts which are irregularly polygonal with 3–7 angles, flattened or depressed at the center, very different in size, in some places are larger, and in some places smaller (Figure 1B). The warts are irregularly radially cracked by 3–6 grooves, and with the help of a lens small transversal lines are clearly visible, the base is never reddish brown as visible in the *Tuber brumale* Vittad. and *Tuber melanosporum* Vittad. The peridium is 200–400 μm thick, the structure consists of very compacted hyphae with short and inflated particles (pseudoparenchymatous), the polygonal cells are 5–17 μm large.

The gleba, medium hard and juicy, initially white, when fully matured spores become grayish-brownish--blackish but never become gray black, on the air does not change the color, marbled with very rare and thin white and unchangeable meandriform veins, at places where veins are branched wide and irregular fields are visible (Figure 1C). The odor is not particularly pleasant because it reminds of the soil or mold. Asci are round or rarely broadly ovate-elliptic, round have a diameter 52–65–85 μm, ovate-elliptic have dimensions 75–85 × 70–80 μm, the pedunculum is very short (4–5 μm) or is not visible, contains 1–6 spores, mostly 3–5 spores.

The spores are yellow-brownish to light brown, ovate--globose, densely ornamented with single spines, well spaced, rigid and sharp, the dimensions are 38–45 × 23–32 μm measured without the ornamentation (spines), spines are 3.5–5 μm long, straight and quite dense, the length/width of spores (Q) is 1.5–1.8 (Figure 1D).

It grows in autumn (November, December) near the Adriatic Sea (Rovinj, Croatia) in symbiosis (mycorrhiza) with pine *Pinus halepensis* Mill. The fruitbodies were found at the depth of 15 cm in gravelly red soil, pH in aqueous solution was 7.5–7.8. Dense grass grows at that place, which suggests that *Tuber decipiens* does not produce allelochemicals substances that inhibit the growth and the reproduction of grass.

### Taxonomic notes

The most important differences of this species in relation to similar black truffles (*Tuber brumale* Vittad., *T. brumale* var. *moscatum*(Ferry) Montecchi and Lazzari

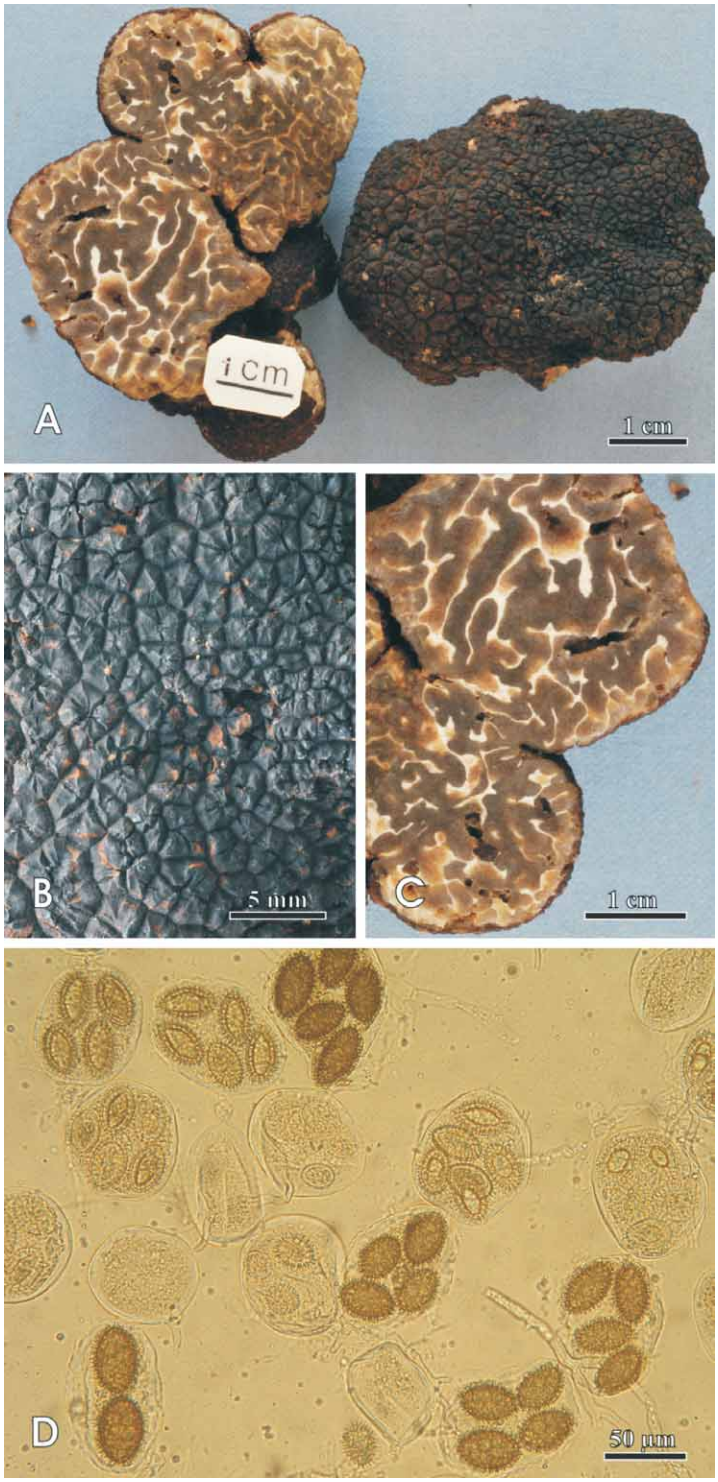


**Figure 1.** Tuber decipiens *(holotype). A. Fruitbodies, B. Warts of peridium, C. Cross section of fruitbodies, D. Asci and ascospores.*

and *T. melanosporum* Vittad.) which grow at the same time (autumn-winter) and similar habitats are the color, the structure and the consistency of the gleba, form and size of spores and the odor that is reminiscent of the soil or mould. In addition, very significant traits are visible transversal striate on the warts of peridium and the form and size of warts whose base is never reddish brown. All other European species of black truffle (*Tuber aestivum* Vittad., *T. bellonae* Quelet, *T. aestivum* var.*uncinatum* (Chatin) Montecchi and Borelli, *T. macrosporum* Vittad., *T. malenconi* Donadini,Rousset and Chevalier, *T. mesentericum* Vittad., *T. regianum* Montecchi and Lazzari, and

*T. donnagotto* Božac, Širić and Kos) have spores with irregular polygonal meshes.

## DISCUSSION

The new species belongs to a group of black colored truffles in which there are 11 European species, varieties and forms *(2, 5, 6)*. However, due to the significantly different forms and color of fruitbodies and reticulate--alveolate ornament of spores, which is composed of irregular polygonal meshes, the possibility of confusion with 7 European species of black truffle is excluded. Only three known species have elliptical spores with an ornament composed of dense single spines, well spaced, rigid and sharp (*Tuber brumale* Vittad., *T. brumale* var. *moscatum* (Ferry) Montecchi and Lazzari, *T. melanosporum* Vittad.), as well as a new species *T. decipiens*. *Tuber melanosporum* Vittad. is essentially different due to the almost black gleba with very densely interwoven sterile veins, the size and color of spores, the size and color of warts on the peridium and due to the very pleasant and strong odor *(2, 5)*. A typical *Tuber brumale* Vittad. which grows in symbiosis with the deciduous trees is also very different due to the smaller and more regular form of fruitbodies, brown-black warts which have reddish-brown base, gray-black gleba, more rounded spores (Q = 1.4–1.7), and above all, the strong and persistent sharp odor. According to the morphological characteristics of the new species, *Tuber brumale* var. *moscatum* (Ferry) Montecchi and Lazzari, which grows in symbiosis with *Quercus sp*. and other deciduous trees *(2, 5, 7)*, is most similar, while *Tuber decipiens* grows in mycorrhiza with pine (*Pinus halepensis* Mill.*)*. Roux *et al*. *(8)* have studied phylogenetic relationships between *Tuber brumale* Vittad. and *Tuber brumale* var. *moscatum* (Ferry) Montecchi and Lazzari. It is interesting that there is no difference between the interval transcribe spacers (ITS sequence). Gandeboeut *et al*. *(9)* have investigated rapid profiles (RAPD) between *Tuber brumale* Vittad. and *Tuber brumale* var. *moscatum* (Ferry) Montecchi and Lazzari and the results show homogeneity between these two species, which also confirms their taxonomic equality. However, despite this fact Ceruti *et al*. *(2)* recognized the validity of *Tuber brumale* var. *moscatum* (Ferry) Montecchi and Lazzari. French researchers *(8, 9, 10)* analyzed the biochemical and molecular differences between *Tuber aestivum* Vittad. and *Tuber aestivum* var. *uncinatum* (Chatin) Montecchi and Borelli. In this study no significant differences in data between these kinds of truffles were identified. In this case, Ceruti *et al*. *(2)* accepted the results of French researchers and did not recognize the validity of *Tuber aestivum* var. *uncinatum* (Chatin) Montecchi and Borelli and indicated it only as a synonym. Chevalier *et al*. *(11)* and Chevalier and Frochot *(12)* have carefully studied the fruitbodies that belonged to *Tuber uncinatum* (Chatin) Montecchi and Borelli and *T. aestivum* Vittad. and concluded that *T. uncinatum* (Chatin) Montecchi and Borelli is simply a variety of summer truffle *T. aestivum* Vittad. In our opinion the French mycologist Chatin *(13)* described perfectly the differences between *Tuber aestivum* Vittad. and *T. aestivum* var. *uncinatum* (Chatin) Montecchi and Borelli, which grows in autumn. This is confirmed by numerous commercial truffle gatherers who say that they will never accept a scientific analysis that ignores the macroscopic characteristics of truffles. There are numerous contrasts and contradictions about individual species, varieties and forms in the literature. The reasons for this are different procedures, materials and methods and errors in determination of the mushrooms. For example, the forms of asci and the ornament on the spores quckly change (dry) during microscopy, which leads to the wrong data. Based on numerous studies and analysis of all other known similar black truffles, Tuber decipiens is a new species that grows in autumn in mycorrhiza with *Pinus halepensis* Mill.

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