

## ***Tricholomopsis osiliensis* (Basidiomycota, Agaricales), recently described from Estonia, found in Slovakia**

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Holec J. (2012): *Tricholomopsis osiliensis* (Basidiomycota, Agaricales), recently described from Estonia, found in Slovakia. – Czech Mycol. 64(1): 93–100.

*Tricholomopsis osiliensis* is a rare, recently described species, known from two Estonian islands in the Baltic Sea. In 2009 it was also found in Dobročský prales virgin forest in Slovakia. Macro- and microcharacters of this collection are presented in detail. The Slovak collection has brighter colours, a more distinctly fibrillose pileus surface, larger and more elongated spores, larger basidia, and more abundant pleurocystidia than the holotype collection. However, a DNA study showed that the Estonian and Slovak collections form a well-supported, not very variable clade. In the light of this finding some differences in macro- and microcharacters are better understood. The darker colours and more pronounced fibrils on the pileus and stipe surface can be attributed to the earlier stage of drying up and ageing of the Slovak fruitbodies. The species has a broader range of spore sizes than was thought before. Its preferable habitats are natural mixed forests, where the fruitbodies appear on fallen trunks of conifers (*Picea*, *Abies*).

**Key words:** fungi, taxonomy, Central Europe, natural forests.

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*Tricholomopsis osiliensis* je vzácný, nedávno popsáný druh, známý jen ze dvou estonských ostrovů v Baltském moři. V roce 2009 byl nalezen v Dobročském pralesi na Slovensku. V článku jsou podrobně popsány znaky tohoto nálezu. Plodnice se od holotypu liší živější a tmavší barvou, výraznějšími vlákny na povrchu klobouku, většími a protáhlejšími výtrusy, většími bazidii a mnohem hojnějšími pleurocystidami. Studium DNA však ukázalo, že slovenský sběr tvoří málo variabilní klad s estonskými sběry. Ve světle tohoto zjištění lze lépe interpretovat některé rozdíly zmíněné výše. Tmavší barva a nápadnost vláken byla zřejmě způsobena částečným zaschnutím plodnic a jejich poškozením prvními nočními mrazíky. Druh má širší spektrum velikosti výtrusů než bylo zjištěno u dvou estonských sběrů. Preferuje zřejmě přirozené smíšené lesy, kde roste na padlých kmenech jehličnanů (smrku, jedle).

### INTRODUCTION

*Tricholomopsis osiliensis* Vauras is a recently described species (Vauras 2009) which differs from well-known *T. rutilans* (Schaeff.: Fr.) Singer by slenderer fruitbodies lacking any trace of violet and from *T. decora* (Fr.: Fr.) Singer by

a less bright colour, being pale brownish yellow with beige to ochre areas, and a smooth to tomentose-fibrillose pileus surface. Its type locality is the Estonian island of Saaremaa (Osilia in Latin) in the Baltic Sea, where it was found by J. Vauras in 2008. In 2010 it was collected by I. Saar on another Estonian island, Vormsi (Kalamees 2011). Testing of ITS sequences by Vauras (2009) and a more complex DNA study by Holec & Kolařík (2012) confirmed that *T. osiliensis* is a distinct species, forming a well-supported clade within the genus *Tricholomopsis*.

During an excursion to Dobročský prales, a virgin forest in central Slovakia, organised during the 10th field foray of the Slovak Mycological Society in Biele Vody in 2009, fruitbodies of an unknown *Tricholomopsis* species were found. Subsequent studies showed that they represent the first find of *T. osiliensis* outside Estonia. Results of a detailed morphological study are presented here. For the DNA study, see the paper by Holec & Kolařík (2012).

#### MATERIAL AND METHODS

Macrocharacters of the fresh fruitbodies were noted directly after the field excursion. A voucher specimen is kept in herbarium PRM (National Museum, Mycological Department, Prague, Czech Republic). For herbarium acronyms, see Thiers (2012). Microscopic mounts were made in a 5% KOH solution and studied under an Olympus BH-2 light microscope. Spore sizes of 40 spores measured (extremely large spores were omitted) are presented in the form of the main data range (c. 10–90 percentile values), complemented with extreme values in parentheses. The spores were measured directly under the microscope using an eyepiece micrometer with a basic scale division of 0.8  $\mu\text{m}$ , allowing for a max. precision of 0.4  $\mu\text{m}$ . Thus, the scale reading was performed using the following values (in  $\mu\text{m}$ ): 1.6, 2.0, 2.4, etc. Illustrations of microcharacters were drawn at a magnification of 1250 $\times$  using a drawing tube.

**A b b r e v i a t i o n s.** L = number of lamellae reaching up to the stipe, l = number of lamellulae between each pair of two lamellae, Q = quotient of length and width of the spores, Q<sub>av</sub> = mean value of Q of the 40 spores studied.

**Material studied.** Slovakia, Veporské vrchy Mts., ca. 5 km NNE of the village of Lom nad Rimavicou, Dobročský prales National Nature Reserve: central part (about 48°40'52.38" N, 19°40' 50.68" E; coordinates of the exact site of *T. osiliensis* are not known), NW slope, alt. 920 m, montane virgin forest (*Picea abies*, *Abies alba*, *Fagus sylvatica*), on fallen trunk of *Abies alba*, 29 Sep. 2009 leg. Štefan Malec and Pavol Žitňan, det. J. Holec (PRM 899184).

## RESULTS

*Tricholomopsis osiliensis* Vauras, Folia Cryptogamica Estonica 45: 87, 2009.

Figs. 1–3

**Collection.** Two fruitbodies were found and collected by Štefan Malec and Pavol Žitňan, and after the excursion transmitted to the author. The weather had been rather dry one week before the excursion (no rain), thus the fruitbodies were slightly dried-up. One of them was fully mature and relatively well-preserved (Fig. 1), the other one was old, partly decayed and damaged by the first night frosts, and, consequently, rather dark (Fig. 2). No young fruitbodies were seen.

**Macrocharacters.** Pileus 35–50 mm, plano-convex with a small umbo and deflexed margin, dry, not hygrophanous, with bright yellow to yellow-ochre ground, covered with coarse, adpressed, ochre-brown to orange-brown fibrils to cords of fibrils. Lamellae sparse, thick,  $L = 30\text{--}40$ ,  $l = 1\text{--}3$ , adnate with a short decurrent tooth, bright yellow when mature, then dull ochre-yellow, with rusty spots when old, edge uneven, eroded, paler than lamellae surface. Stipe 30–60 × 7–8 mm, cylindrical, hollow, with the same colour and surface structure as the pileus. Context pale yellow to yellow, paler than lamellae. Taste mild. Smell indistinct.

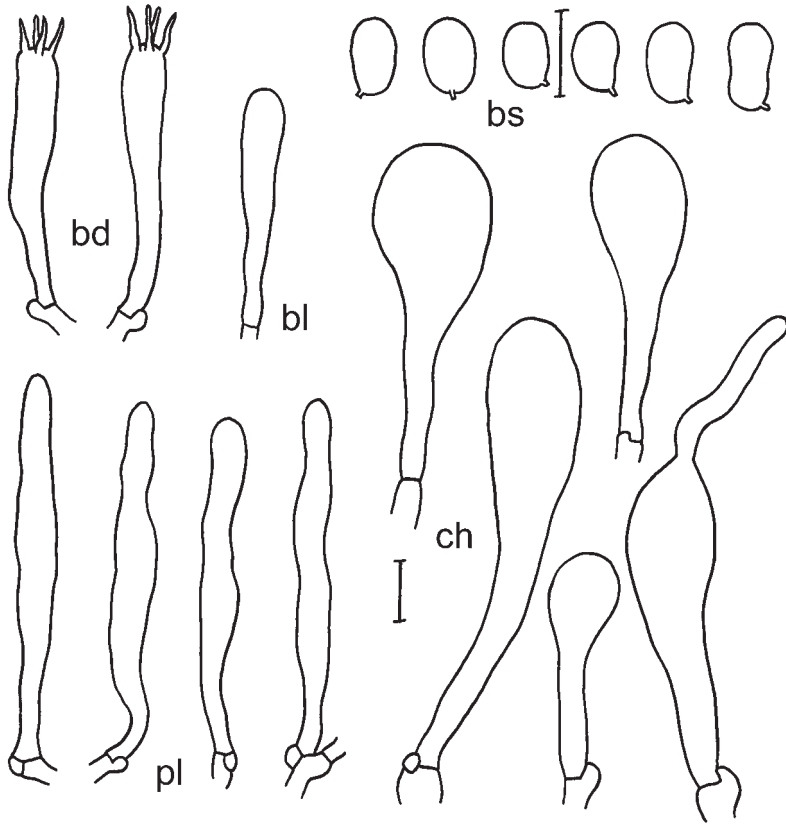
**Microcharacters.** Basidiospores  $8.0\text{--}9.6 \times (5.2)5.6\text{--}6.0(6.4) \mu\text{m}$ ,  $Q = 1.33\text{--}1.85$ ,  $Q_{av} = 1.52$ , ellipsoid to ovoid-ellipsoid, some of them slightly phaseoliform or with a slight median constriction in side view, thin-walled, hyaline or with a large oil droplet, hilar appendix distinct. A small percentage of aberrant, extremely long, ellipsoid-cylindrical spores was present in all mounts. Basidia  $40\text{--}46 \times 7\text{--}9 \mu\text{m}$ , 4-spored, cylindrical to narrowly clavate, sterigmata 5–7  $\mu\text{m}$  long. Basidiolae  $30\text{--}36 \times 6\text{--}8 \mu\text{m}$ , narrowly clavate. Cheilocystidia prominent, forming “nests” on the sterile lamellae edge,  $35\text{--}80 \times 11\text{--}20 \mu\text{m}$ , clavate, rarely with a cylindrical, up to 30  $\mu\text{m}$  long outgrowth, hyaline or filled with a refractive yellow-ochre content, at some places intermixed with pleurocystidia-like fusiform-cylindrical cystidia. Pleurocystidia numerous, narrow,  $56\text{--}76 \times 6\text{--}8 \mu\text{m}$ , fusiform-cylindrical, often slightly flexuous, with a pale yellow, homogeneous or finely granular content. Lamellar trama regular, made up of parallel, 3–12  $\mu\text{m}$  broad hyphae composed of cylindrical to slightly fusiformly inflated cells. Pileus cuticle a thin and very distinctive cutis of densely arranged cylindrical, 3–7(10)  $\mu\text{m}$  broad hyphae, with yellow-brown membranal pigmentation, covered with yellow-brown cords of densely arranged parallel, 4–22  $\mu\text{m}$  broad hyphae (forming dark fibrils on pileus surface), composed of cylindrical to slightly inflated cells, with coarse, yellow-brown, membranal pigmentation. Stipe cuticle a cutis of densely arranged parallel hyphae, 4–10  $\mu\text{m}$  broad, with yellow-brown membranal pigmentation, at places covered with nests of slightly upturned or flexuous hyphae, up to 16  $\mu\text{m}$  broad, with fusiform to narrowly clavate terminal cells, more



**Fig. 1.** *Tricholomopsis osiliensis*, Slovakia, Dobročský prales virgin forest, 29 Sep. 2009 (PRM 899184). Mature fruitbody, photographed on a wooden table after its collection. **a** – upper side. **b** – bottom side. Photo by J. Holec.



**Fig. 2.** *Tricholomopsis osiliensis*, Slovakia, Dobročský prales virgin forest, 29 Sep. 2009 (PRM 899184). Old fruitbodies, partly decayed and damaged by the first night frosts, photographed on a wooden table after its collection. Photo by J. Holec.



**Fig. 3.** *Tricholomopsis osiliensis*, Slovakia, Dobročský prales virgin forest, 29 Sep. 2009 (PRM 899184). Line drawings of microcharacters. **bd** – basidia, **bl** – basidiole, **bs** – basidiospores, **ch** – cheilocystidia, **pl** – pleurocystidia. Del. J. Holec.

strongly pigmented than the cutis hyphae. Pileo- and caulocystidia not observed. Clamp connections present in all tissues.

**Habitat and substrate.** The collectors only noticed its occurrence in the Dobročský prales National Nature Reserve and growth on a fallen trunk of *Abies alba*. The fruitbodies were certainly found inside the protected area, which is clearly marked in the field. This means that the find originates from a mixed montane forest composed of variously aged *Picea abies*, *Abies alba*, and *Fagus sylvatica* individuals and a plenty of fallen trunks in various stages of decay. The stand is a true virgin forest, never completely cut, influenced at most by selective removal of individual trees in the past.



## DISCUSSION

In 2009, I was not able to identify the collection according to its macro- and microcharacters. It was clear that the fungus differed from *T. rutilans* and *T. flammula* by the absence of any violet colours and from *T. decora* by its fibrillose cap surface without upraised scales. It was identified provisionally as "*T. ornata*" due to its resemblance to an illustration by Courtecuisse & Duhem (2000: fig. 419; yellow fungus with adpressed, fibrillose, vividly fulvous-brown scales). After publication of the new species *T. osiliensis* (Vauras 2009), the Slovak collection was compared with it, using the "classical" characters at first.

The comparison shows that the Slovak collection differs considerably from the holotype of *T. osiliensis* (Tab. 1). The Slovak fungus has brighter colours, a more distinctly fibrillose pileus surface, larger and more elongated spores, larger basidia, and more abundant pleurocystidia. Based on these facts, I thought that it represented an unknown taxon.

In a following stage, the collection was included into a detailed DNA study of European *Tricholomopsis* species (Holec & Kolařík 2012). Surprisingly, it proved to form a not very variable clade with the two hitherto known collections of *T. osiliensis* from Estonia (Saaremaa, Vormsi; Kalamees 2011). The minute infraspecific variability in ITS rDNA (less than 0.55%) and formation of a well-supported phylogenetic clade, suggest conspecificity of all three collections. There is only a difference from Saaremaa collection (PRM 899461: isotype of *T. osiliensis*) in 1 base pair (bp) and from the Vormsi collection (TU 101571) in 3 bp in the region containing complete ITS rDNA and partial LSU rDNA (550 bp).

In the light of this finding, some differences in macro- and microcharacters (Tab. 1) are better understood. The Slovak fruitbodies were collected after a period of dry weather and the first night frosts. They were slightly dried-up and, thus their water content was lower than in a fully fresh state. In such conditions, most fungi have darker fruitbodies due to the higher concentration of pigments in their (partly drained) cells. The strikingness of fibrils on pileus and stipe surface also seems to be caused by their drying up and ageing. The larger basidia and spores are harder to explain. We have to accept the fact that *T. osiliensis* has a rather broad range of spore sizes ( $6.0\text{--}9.5 \times 4.5\text{--}6.4 \mu\text{m}$ , based on the 3 collections known to date). Within *Tricholomopsis*, this phenomenon is known e.g. in *T. rutilans* (Boekhout & Noordeloos 1999:  $5.0\text{--}7.5 \times 4.0\text{--}6.5 \mu\text{m}$ , Vesterholt 2008:  $5\text{--}7.5 \times 4.5\text{--}6 \mu\text{m}$ , Holec & Kolařík 2012:  $5.0\text{--}8.5 \times 4.0\text{--}6.5 \mu\text{m}$ ).

**Tab. 1.** Comparison of characters of the Slovak collection (described here) and the holotype collection of *T. osiliensis* (Vauras 2009). Only deviating characters are listed.

Characters	Slovak collection (PRM 899184)	<i>T. osiliensis</i> (Vauras 2009)
age of the fruitbodies	mature, old (partly decayed)	young, mature
pileus colour: ground	bright yellow to yellow ochre	pale brownish yellow, with beige to ochre areas
pileus covering	ochre-brown to orange-brown fibrils to cords of fibrils	centre slightly tomentose, outwards fibrillose to almost smooth, margin with minute, concolorous squamules
lamellae	bright yellow, then dull ochre-yellow	pale yellow to yellow
stipe colour: ground	bright yellow to yellow ochre	pale yellow at apex, brownish yellow below
stipe covering	ochre-brown to orange-brown fibrils to cords of fibrils	brownish fibrils
spore size	8.0–9.6 × (5.2)5.6–6.0(6.4) μm	6.0–8.2 × 4.5–6.0 μm
spore size: Q value	1.33–1.85	1.2–1.6
spore shape	ellipsoid to ovoid-ellipsoid, some slightly phaseoliform or with a slight median constriction in side view	ellipsoid to broadly ellipsoid
basidia	40–46 × 7–9 μm	28–36 × 7–8 μm
pleurocystidia	numerous, fusiform-cylindrical	scarce, cylindrical to clavate

*Tricholomopsis ornata* (Fr.) Singer is thoroughly discussed by Holec & Kolařík (2012). It seems to be a dubious taxon, differently interpreted in the course of time. The original *Agaricus ornatus* Fries (1838: 130) is a medium-sized to large, fleshy fungus (pileus 5–12.5 cm broad, stipe about 1.2 cm thick) similar to *T. rutilans*, but having a pileus covered with brown flocculose scales. In my opinion, it represents an abnormal or old fruitbody of *T. rutilans*. The interpretations by Bon (1995) and Courtecuisse & Duhem (2000: fig. 419) certainly do not represent the Fries' fungus, but could be identical with *T. osiliensis*. A future revision of voucher specimens (if available) could help to confirm this.

Generally, more collections of *T. osiliensis* are necessary to understand its characters, variability and limits. It certainly is a rare fungus and therefore mycologists are asked to search for it and document all finds carefully. Based on the records discussed here, its preferred habitats are natural mixed forest, as two of the three finds originate from nature reserves. Moreover, the Slovak collection was found in one of the best-preserved old-growth forests of the Western Carpathians (for mycological characteristics of the Dobročský prales virgin forest, see e.g. Kotlaba & Pouzar 1962, Varjú 1994). In Estonia, the fruitbodies were found on decaying fallen trunks of *Picea abies* (Vauras 2009, Kalamees 2011), in Slovakia on *Abies alba*.

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