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RESEARCH ARTICLE

The second record of a rare fungus *Flammulina ononidis* (*Physalacriaceae*) in Ukraine

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Abstract. The second record in Ukraine of a rare fungus *Flammulina ononidis* (*Physalacriaceae*, *Agaricales*) is reported. This species is known in 16 European countries but is rare in most of them. In Ukraine, it has not been found for more than 50 years. The article provides a complete description of the macro- and microscopic details of the collected fruit bodies, as well as distribution data. The differences from some related species are described and original illustrations of the fungus are provided.

Keywords: *Agaricales*, *Flammulina*, new record, *Ononis spinosa*

Introduction

Recently an interesting collection of a fungus apparently belonging to the genus *Flammulina* P. Karst. (*Physalacriaceae*, *Agaricales*) was recorded in Ukraine. The fruiting bodies were found in a pasture among various herbaceous plants, including dry remnants of *Ononis spinosa* L. The fungus was preliminary identified as *Flammulina ononidis* Arnolds. Afterwards, the study of microscopic features of the collected fruiting bodies has confirmed this identification. This is a very remarkable find, because *F. ononidis* is a relatively rare species in Europe. It grows saprotrophically on dry roots of *Ononis spinosa* (Arnolds, 1977; Ripková et al., 2008) and is

currently known from 16 European countries. In most of them it is rather rare (reported from less than 10 locations): Austria — 7, Italy — 7, Czech Republic — 6, France — 4, Spain — 3, Croatia — 2, Switzerland — 2, Estonia — 1, Hungary — 1, Poland — 1, and Slovakia — 1 (Ripková et al., 2008; Mešić, 2019; GBIF..., 2022). An exception is Germany with about 40 reported localities (Mešić, 2019), though in the GBIF online database (GBIF..., 2022) only 17 localities are recorded for this country. The species is also included in *The IUCN Red List of Threatened Species* as Vulnerable (VU) (Mešić, 2019) and in the national fungal Red Lists of four European countries: Austria — Endangered (EN) (Dämon, Krisai-Greilhuber, 2016), Croatia — Vulnerable

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Fig. 1. Fruit bodies of *Flammulina ononidis* (KW-M71531). Bar — 1 cm

(VU) (Tkalčec et al., 2008), the Czech Republic — Critically Endangered (CR) (Holec, Beran, 2006), and Germany — Endangered (EN) (Dämmrich et al., 2016). As to Ukraine, *F. ononidis* was previously recorded only once, more than 50 years ago (Wasser, 1971). Unfortunately, its specimens have not been deposited in the herbarium and so cannot be investigated. Our collection represents the second locality of this rare fungus in our country and is worthwhile to be described more in details.

Materials and Methods

All macroscopic and microscopic features of the fungus are based on one collection. The microscopic structures were observed in dried material. Microscopic sections of lamellae were made at about $\frac{1}{2}$ radius of the pileus, those of pileipellis were

made near the margin of the pileus. Preparations were mounted and examined in 3% KOH. Spore sizes are based on at least 20 spore measurements per fruit body. For basidia, cystidia, and terminal cells of ixohyphidia, the statistics are based on 10 measurements per fruit body.

The following abbreviations are used in the description of spores: av. W = average width of the spores in frontal view; av. L = average length of the spores; Q = length/width ratio; av. Q = average Q. The following abbreviations are used in the description of fruit bodies: L = number of lamellae reaching stipe; l = number of short lamellae (not reaching stipe) between two long ones.

The specimen reported in the article is deposited in the Herbarium of the M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, Kyiv, Ukraine (KW-M).

Results and Discussion

Flammulina ononidis Arnolds, Westfälische Pilzbriefe 11(3-4): 33. 1977. — Figs. 1, 2.

Flammulina velutipes var. *pratensis* Schief., Z. Pilzk. 21: 21. 1949. — *Collybia velutipes* subsp. *pratensis* (Schief.) Schief., Z. Pilzk. 29: 109. 1963.

Fruit bodies collybioid. Pileus 0.5–3.0 cm in diameter, at first hemispheric, later convex to expanded, sometimes with low umbo in centre, viscid, honey yellow, pale orange to pale orange brown, in centre darker, up to orange brown, translucently striate at margin. Lamellae rather distant ($L = 20\text{--}26$; $l = 1\text{--}3$), adnate, yellowish white to pale ochraceous yellow. Stipe 1.5–4.5 × 0.1–0.3 cm, cylindrical or slightly tapering downwards, sometimes with root-like basis, velvety, pale yellowish at top, towards base darkening, orange brown, reddish brown to dark brown. Flesh in pileus whitish, up to 0.1 cm thick, in stipe pale yellowish at the top, downwards darkening up to dark brown at base. Taste mild, smell insignificant.

Spores (7.0–)8.0–11.5 × 4.0–4.5 μm ($n = 60$), av. $L = 9.7 \pm 1.24$ μm, av. $W = 4.7 \pm 0.50$ μm, $Q = 1.70\text{--}2.30$, av. $Q = 2.08 \pm 0.16$, ellipsoid, narrowly-ellipsoid, cylindrical-ellipsoid, smooth, thin-walled, hyaline, inamyloid. Basidia 25.0–27.0 × 6.0–7.0 μm, narrowly-clavate, 4-spored. Cheilocystidia 40.0–70.0 × 9.5–12.0 μm ($n = 30$), narrowly-utriform to lageniform, apex 3.5–7.0 μm broad, thin-walled, hyaline, abundant. Pleurocystidia 40.0–60.0 × 8.0–12.0 μm ($n = 30$), similar to cheilocystidia, apex 5.0–8.5 μm broad, very scattered. Pileipellis an ixotrichoderm, consisting of ixohyphidia and rather numerous pileocystidia. Pileocystidia 45.0–110.0 × 8.0–10.5 μm ($n = 30$), narrowly-fusiform to narrowly-lageniform, apex 4.0–5.0 μm broad, rather thick-walled (up to 0.5 μm), brownish yellow. Ixohyphidia 1.0–1.5(–2.0) μm wide, thin-walled, hyaline; terminal cells of ixohyphidia 30.0–60.0 × 3.6–6.0 μm ($n = 30$), narrowly fusiform with mucronate tip, sometimes with slightly inflated medium part, usually branched, with 1–3 lateral branches (with mucronate tips too), as a rule with many constrictions along all length, thin-walled but in basal half with slightly thickened walls, hyaline. Stipitipellis consisting of parallel hyphae with numerous erect terminal cells, those 25.0–100.0 × 5.0–6.0 μm, with slightly narrowed tips, thick-walled, yellow-brown. Caulocystidia 50.0–85.0 × 9.5–11.5 μm ($n = 30$), rather numerous, fusiform, narrowly-fusiform to

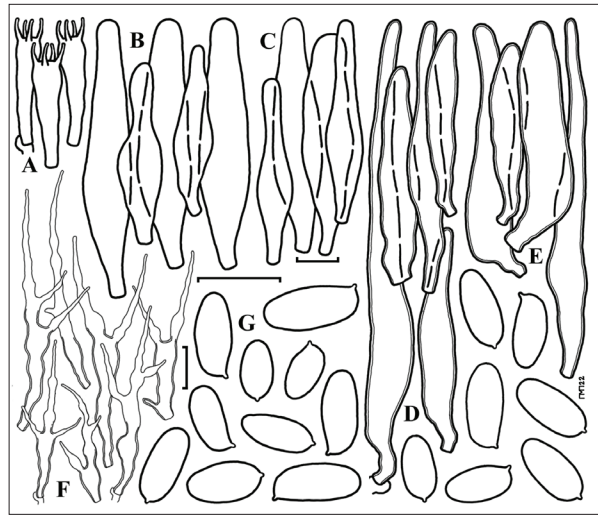


Fig. 2. Microscopical features of *Flammulina ononidis* (KW-M71531). A: basidia; B: cheilocystidia; C: pleurocystidia; D: pileocystidia; E: caulocystidia; F: terminal cells of ixohyphidia; G: spores. Bars — 10 μm

narrowly lageniform, apex 3.0–6.0 μm broad, rather thick-walled (up to 0.5 μm), brownish yellow. Hyphae in all tissues with clamp connections.

Fruit bodies grow solitary or in small groups (1–3 specimens), on dead roots and stem bases of *Ononis spinosa*, in pastures and meadows. According to literature data, it prefers calcareous soils (Arnolds, 1977; Klán, 1978; Vesterholt, 2012; Mešić, 2019). A rare species, up to date only two localities have been found in Ukraine. May–November.

Specimen examined. Zakarpatska (Transcarpathian) Region, Rakhiv District, village of Chorna Tysa, pasture, N 48°17'26.7", E 24°22'14.6", 08.11.2022 (KW-M71531), coll. K.I. Fedorova.

Other known localities: Kherson Region, Kakhovka District, Askania-Nova Biosphere Reserve, arboretum, May–November 1968, coll. S.P. Wasser (Wasser, 1971, as *Flammulina velutipes* var. *pratensis*).

General distribution. Europe: Austria, Belgium, Croatia, Czech Republic, Denmark, Estonia, France, Germany, Hungary, Italy, Poland, Russian Federation, Slovakia, Spain, Switzerland, Ukraine. Asia: Turkey (Ripková et al., 2008; Mešić, 2019; GBIF..., 2022).

One of main distinctive macromorphological features of *Flammulina ononidis* is its pseudorhiza, solitary (or in small groups) growth (not in large clusters), and connection to remnants (mainly roots) of herbaceous plant *Ononis spinosa*. Another European

species with similar peculiarities is *F. cephalariae* Pérez-Butrón & Fern.-Vic. which grows on roots of *Cephalaria leucantha* (L.) Roem. & Schult. in Spain (Pérez-Butrón, Fernández-Vicente, 2007). A rather important distinctive sign of both species is also their habitat, namely seminatural grasslands, while other representatives of the genus prefer wood habitats, both natural and anthropogenic.

Microscopically *Flammulina ononidis* differs from most representatives of the genus by rather large spores (over 8.0 µm long and over 4.0 µm wide). *Flammulina cephalariae* and *F. rossica* Redhead & R. H. Petersen have also large spores, particularly *F. cephalariae* possessing spores 12.0–17.0 µm long (Ripková et al., 2010), while spores of the second species are rather similar to those of *F. ononidis*. However, *F. rossica* can be separated by the form of the prevailing type of terminal cells of ixohyphidia, which have distinct central or terminal globose inflation up to 9.0 µm wide and 1–3 lateral nodules or short branches (not mucronate as ones of *F. ononidis*) (Adamčík, Ripková, 2008). Besides, *Flammulina rossica* is a lignicolous species (Adamčík, Ripková, 2008; Ripková et al., 2010). Spores of similar length are also reported for *F. elastica* (Sacc.) Redhead & R.H. Petersen, but they are distinctly narrower (as a rule up to 4.0 µm wide) (Ripková et al., 2008, 2010).

It is worth mentioning that various authors provide rather different information about the size of spores of *Flammulina ononidis*. Their data vary from (7.5–)8.1–10.1(–10.7) × 4.1–5.0(–5.2) µm (Ripková et al., 2010) to (7.5–)8.5–13.0(–14.0) × (4.0–)4.5–6.0 µm (Pérez-Butrón, Fernández-Vicente, 2007). The spores of our specimens of *Flammulina ononidis* were most similar to the data of Arnolds (1977): (7.5–)8.5–11.0 × (4.0–)4.5–5.5(–6.0) µm.

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- Flammulina ononidis* is probably a very rare fungus both in Ukraine and in Europe. Its suitable habitats, semi-natural grasslands, still occupy rather large territories in Ukraine but their areas gradually decrease (National..., 2018). They are very dependent on traditional agricultural practices, namely grazing by large herbivorous animals (cattle, horses, sheep, etc.) as well as regular mowing. During later decades these traditional agricultural practices are constantly declining in our country, and that is also a general European trend. In absence of grazing and mowing, grasslands become gradually overgrown by shrubs and trees and thus become unsuitable for many native fungi, including *F. ononidis*. It is one of the main threats for the fungus both in Europe and in Ukraine. Another large peril is intensification of agriculture (increasing in Ukraine) which also destroys suitable habitats by replacing them with monoculture fields. In Ukraine, still an additional factor exists. The previous Ukrainian record of *F. ononidis* is located within a territory currently occupied by the Russian Federation and can potentially be destroyed at the scene of war. The consequences of this destruction for natural habitats are very difficult to predict. Taking into account the fact that up to now *F. ononidis* has been recorded in Ukraine only twice and the above mentioned threats for it, the fungus has to be considered as a candidate for inclusion in the *Red Data Book of Ukraine*.

Ethics Declaration

The authors declare no conflict of interest.

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**Друга в Україні знахідка рідкісного гриба
Flammulina ononidis (Physalacriaceae)**

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Реферат. Повідомляється про другу в Україні знахідку рідкісного гриба *Flammulina ononidis* (Physalacriaceae, Agaricales). Цей вид трапляється в 16 європейських країнах, але є рідкісним у більшості з них. В Україні його не знаходили понад 50 років. У статті наведений детальний опис макро- та мікроскопічних деталей будови зібраних плодових тіл та надана інформація про розповсюдження цього гриба. Описані його відмінності від споріднених видів і подані оригінальні ілюстрації цього гриба.

Ключові слова: нова знахідка, Agaricales, *Flammulina*, *Ononis spinosa*