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RESEARCH ARTICLE

New records of a rare fungus *Tricholoma focale* (*Agaricales*) in Ukraine

Maryna M. SUKHOMLYN¹ , Mariia V. PASAILIUK^{2*} , Nina M. FONTANA³ 

¹ Institute for Evolutionary Ecology, National Academy of Sciences of Ukraine,
37 Lebedeva Str., Kyiv 03143, Ukraine

² Hutsulshchyna National Nature Park,
84 Druzhby Str., Kosiv 78600, Ukraine

³ Department of Native American Studies, University of California,
Davis, California, Shields Ave 1, 95616, USA

* Author for correspondence: mariia.pasailiuk@gmail.com

Abstract. New records of *Tricholoma focale*, a species listed in the *Red Data Book of Ukraine* as Vulnerable, are reported. During 2018–2021, we observed over 15 fruit bodies of the fungus near Kozyntsi village (Bucha District, Kyiv Region). Including our data, in total 15 localities of the fungus are known in Ukraine; ten of them are outside the territories designated as protected areas. Due to the ongoing military activities, four previously reported records are located within the temporarily occupied areas and thus are inaccessible for further observations. Conservation measures both *in situ* and *ex situ* to preserve the genetic diversity of *Tricholoma focale* are required.

Keywords: conservation, distribution, morphological characters, new localities, *Red Data Book of Ukraine*

Introduction

Tricholoma focale (Fr.) Ricken (*Tricholomataceae*, *Basidiomycota*) is a boreal species with a disjunct distribution, recorded in Europe, Asia, and North America (Herink, 1965; Brooks, 2009; Reschke et al., 2018). The fungus grows in sandy pine and pine-mixed forests, particularly in oligotrophic boreal heath and oligo-mesotrophic boreal forests. It was also found in wooded oligotrophic boreal heath grasslands and wooded coastal dunes, typically under *Pinus sylvestris* L., in September and October (Kalamees, 2010).

The main macroscopic morphological features for distinguishing the species are as follows. Cap 50–150 mm, at first hemispherical to obtusely conical with involute margin, later convex to plane, with a broad umbo, coarsely radially fibrillose, becoming finely scaly in marginal zone, viscid when moist, ochraceous orange, yellowish brown, orange brown or dark brick red with margin paler, warm buff, ochraceous or dark pinkish buff, often with honey to pale olivaceous streaks and spots, in young fruit bodies often with whitish, cottony remains of velum near margin. Stipe up to 40–140 mm long and 8–30 mm wide, cylindrical, often tapering towards

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the root base, with a well-defined, felt-like fibrous ring. Above the ring, the stipe surface smooth, white or pink, while under the ring, the surface covered with soft felt reddish-brown scales, forming stripes. Flesh solid, white or pale pink; taste farinaceous, sometimes with a cucumber-like or rancid odor (Christensen, Heilmann-Clausen, 2013).

Throughout its extensive taxonomic history, *Tricholoma focale* has had numerous alternative (synonymic) names (<https://www.mycobank.org/>, <https://www.speciesfungorum.org/>). The fungus was first described in 1838 by Elias Magnus Fries as *Agaricus focalis* Fr. In 1879, the German mycologist Adalbert Ricken transferred the species to the genus *Tricholoma* (Ricken, 1915), and thus the currently accepted name *Tricholoma focale* has been established.

Tricholoma focale and some other species of the genus *Tricholoma* (Fr.) Staude, such as *T. bakamatsutake* Hongo, *T. caligatum* (Viv.) Ricken, *T. duciolens* Kytöv., *T. fulvocastaneum* Hongo, *T. magnivelare* (Peck) Redhead, *T. quercicola* M. Zang, *T. robustum* (Alb. & Schwein.) Ricken, and *T. zelleri* (D.E. Stuntz. & A.H. Sm.) Ovrebo & Tylutki are closely related to *T. matsutake* (S. Ito & S. Imai) Singer. However, *T. focale* lacks the characteristic aroma and taste of *T. matsutake* (Breitenbach, Kränzlin, 1991). None of the species listed above has been found in the Southern Hemisphere (Yun et al., 1997).

Some authors consider *Tricholoma focale* found in Europe to be highly likely identical (synonymous) to *T. robustum* that occurs in Japan. The species often grows alongside *T. matsutake*, so it is called matsutakemotoke (a matsutake indicator). Like the species mentioned above, it also lacks the distinctive odor of *T. matsutake* (Breitenbach, Kränzlin, 1991). Some authors consider *T. focale* and *T. robustum* to be the same species (Cuesta, Rodríguez, 2009). However, others believe that *T. robustum* may be a distinct species, with larger spores (6–7 × 4.5–5.5 µm) and larger fruiting bodies (Christensen, Heilmann-Clausen, 2013). The ITS data show that *Tricholoma focale* is quite distantly related to *T. matsutake* and allies, but rather closely related to *T. batschii* Gulden ex Mort. Chr. & Noordel. (Christensen, Heilmann-Clausen, 2013).

Tricholoma focale is a rare fungus, regarded as a threatened species in Europe (Ing, 1993). Currently, it is listed in the Red Lists of the Czech Republic (Holec, Beran, 2006), Bulgaria (Gyosheva, 2006), Hungary (Siller, Vasas, 1995), Spain (Cuesta,

Rodríguez, 2009), and the United Kingdom (Evans et al., 2006). The fungus is considered a rare species in Estonia (Kalamees, 2010). *Tricholoma focale* has a conservation status of Vulnerable species in the *Red Data Book of Ukraine* (Prydiuk, 2009; <https://zakon.rada.gov.ua/laws/show/z0370-21?lang=en#Text>). Negative changes in fungus populations resulted from various factors, such as traditional gathering practice, deforestation leading to the destruction of forest ecosystems, forest fires, recreational activity in their habitats, etc. (Prydiuk, 2009).

Unlike *Tricholoma matsutake*, *T. focale* lacks commercial value primarily due to its rare status and poor taste (Zambonelli, Bonito, 2012). It is a mycorrhizal fungus classified as an edible mushroom of the fourth category, although some authors state that it may irritate the gastrointestinal tract (Patrick et al., 2009).

Nevertheless, scientists are keenly interested in studying this species because of its pharmacological and medicinal properties. *Tricholoma focale* shows promise in treating cancer, as researchers have extracted and isolated a new type of steroids with three biologically active antitumor components from its fruit bodies (Singeç et al., 2017). The species has been found to possess antioxidant properties. Additionally, the fatty acid profile of the fruit bodies is notable, with oleic acid being the primary acid (35.0%), along with palmitic acid (5.12%) and high content of linoleic acid (31.0%) (Kaplaner et al., 2017).

In this article, the first reports of *Tricholoma focale* in Kyiv Region are provided, as well as details of the macroscopic and microscopic features of the fungus, along with the analysis of its previously known occurrences in Ukraine.

Materials and Methods

Field observations took place in Kyiv Region in 2018–2021. The macromorphological characteristics of fresh fruit bodies were described based on fresh material and documented with field notes and photographs. For species identification, various literature sources were used (Noordeloos, Christensen, 1999; Riva, 2003; Galli, 2005; Kibby, 2010; Heilmann-Clausen et al., 2017; Şen, Alli, 2019). Microscopy of spores and hymenium was conducted using a Primo Star light microscope (Carl Zeiss, Germany) equipped with Canon A 300 camera, using AxioVision 4.7. Thirty spores per sample were measured,



Fig. 1. *Tricholoma focale*, fruit bodies *in situ*, Kozyntsi village, Kyiv Region

where present. The total number of measured spores is 300. For scanning electron microscope (SEM) analysis, small pieces of hymenial lamellae were placed on metal stubs, then sputter-coated with gold and observed under SEM (Jeol 6060LA, Japan).

Results

New occurrences of *Tricholoma focale* were observed by M.M. Sukhomlyn on 13 October 2018, 19 October 2019, and 21 October 2021 in a pine forest near Kozyntsi village (Irpin territorial community, Bucha District, Kyiv Region, Ukraine, N50°30'06", E30°03'30"). More than 15 fruit bodies of *T. focale* were found in one location, within about 50 meters

from each other, on sandy soil in *Pinus sylvestris* plantations (Fig. 1). Some fruit bodies of *T. focale* were observed already collected and left on the ground, being confused by mushroom gatherers with other edible mushrooms. It is worth noting that these are the first records of the fungus reported for the central part of Ukraine.

Macroscopic characters

Caps of the observed fruit bodies ranged from 30 to 135 mm in diam. (Fig. 1) and varied in color from orange-brown to reddish-brown. In young non-sporulating fruit bodies, the caps were convex or convex-flattened in shape (Fig. 2A) and sticky to the touch. At the sporulation stage (in mature basidiomata), the caps were flat-spread with a noticeable radial elevation at the center, not sticky and relatively dry (Fig. 2B). In terms of consistency, the caps are moderately fleshy, predominantly brick-red, with remnants of the cover. With age, the caps become cracked. The hymenophore is lamellar (Fig. 2C). In mature fruit bodies, the gills are thin, dense, pale-yellowish in color, firmly attached to the stem by a notch. When pressed, the mature gills exhibit reddish-brown coloration and are firmly attached to the mushroom stem. The stems range from 45–100 mm in length and are 10–20 mm thick. Positioned centrally on the caps, the stems taper slightly towards a root-like base. The ring, two-thirds up above stem base, appears leathery with reddish-brown hues. The ring divides the stem visually and chromatically: the upper portion under the cap is predominately white, while the area below the ring shows reddish-brown tones (Fig. 2D). The fungus flesh is elastic, white in color, with a scent and taste that is mealy, and with a slightly bitter taste. KOH on the cap surface changes color from dark to bright red.

Microscopic characters

Basidiospores smooth, ellipsoid, colorless, $3.5\text{--}5.5 \times 2.5\text{--}3.5 \mu\text{m}$ (Figs. 3, 4A–D).

Discussion

Having analyzed the literature data, we found information about 15 reports of the species from eight regions of Ukraine (Table 1). Of them, seven records are located in Poltava Region, two in Luhansk Region, and one location in each of Mykolaiv, Dnipropetrovsk, Kherson, Volyn, Ivano-Frankivsk, and Kyiv Regions (including our report).



Fig. 2. Fruit bodies of *Tricholoma focale*. A: young fruit body; B: mature fruit body; C: mature fruit body showing hymenophore; D: mature fruit body showing hymenophore and ring on the stem

The first occurrences of the fungus as separate fruit bodies were documented in 1960 from seven localities in Zavorsklyanskyi mixed (subboreal) forests, along the pine terrace of the Vorskla River, within modern Poltava District in Poltava Region (Hanzha, 1960) (Table 1). After that, there were no more findings in this area, and all reports of this fungus in Poltava Region derive from these original records (Besedina, Stetsiuk, 2010).

Subsequently, the fruit bodies were discovered in the Pervomaisk forestry of Mykolaiv Region, within pine plantations (Sheliah-Sosonko et al., 1996). The reports of the fungus from Poltava and Mykolaiv regions were included in the first edition of the *Red Data Book of Ukraine* (Sheliah-Sosonko et al., 1996). In the second edition of the *Red Data Book of Ukraine* (2009), the reports from Poltava, Dnipropetrovsk, Luhansk, and Kherson regions were added, one from each region (Taran et al., 1989; Prydiuk, 2005; Prydiuk, 2009). As a result, in that edition seven records of the species in Poltava Region are presented as one locality, while the record from Mykolaiv Region is omitted.

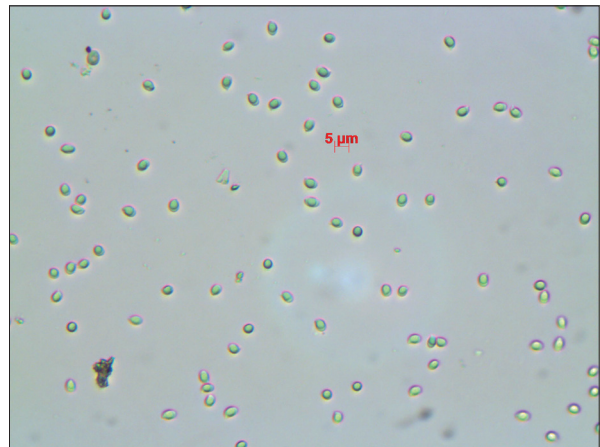


Fig. 3. Spores of *Tricholoma focale* (LM)

The additional localities of the fungus in the second edition of the *Red Data Book of Ukraine* are as follows: one in Dnipropetrovsk Region, in Prysamarskyi forest (Taran et al., 1989), two in Luhansk Region (one near Provallya village, Dovzhansk District, within the Provalskyi Step unit of Luhansk Nature Reserve: Dudka et al., 2009, one

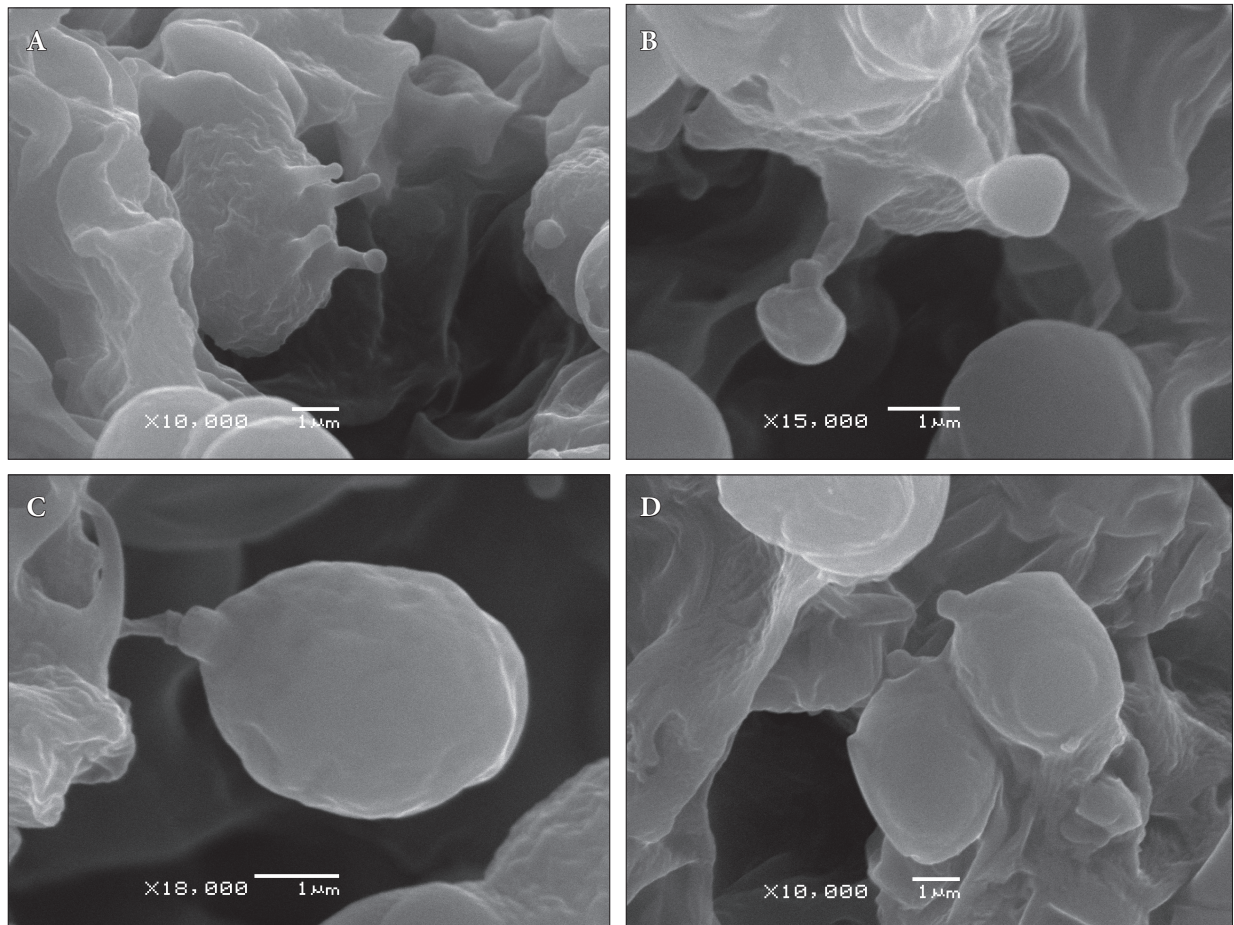


Fig. 4. *Tricholoma focale*, hymenium elements (SEM). A: basidia with sterigmata; B: young basidiospores on sterigmata; C: basidiospore on sterigma; D: basidiospores with apiculi. Scale: 1 µm

more in the Prydintsivska floodplain, Stanychno-Luhansk section of Luhansk Nature Reserve, in a pine forest, 3 km east of Khrystove village: Prydiuk, 2005), and another one in Kherson Region (near Olhivka village, Beryslav District: Prydiuk, 2009). These locations correspond to the Left Bank Forest-Steppe, Left Bank Grass Meadow Steppe, Starobilsk Grass Meadow Steppe, and Right Bank Grass Steppe, respectively (Prydiuk, 2009).

Tricholoma focale was also found by V.P. Heluta and O.P. Vysotska on 25 September 2008 in a pine forest, 2 kilometers north of the village of Zarudchi (Kamin-Kashyrskiy District, Volyn Region) (Heluta et al., 2010). This area is located within the Prypiat-Stokhid National Nature Park (NNP). This location is not reported in the *Red Data Book of Ukraine* (2009). In 2009, the fungus was also listed as an

endangered species in the inventory of rare species of the eastern Ukraine (Leshan, Pakhomov, 2009).

The species was also recorded in Hutsulshchyna National Nature Park (Kosiv District, Ivano-Frankivsk Region) as early as 2005 (Bazuk-Dubei, 2010). For some time, Hutsulshchyna NNP was the only protected area in western of Ukraine with a single location of this fungus (Dudka et al., 2009; Dudka et al., 2019). However, in 2020 the fungus was rediscovered in the NNP by S.I. Fokshei and O.O. Pohribnyi (2021).

Many records of this rare species in Ukraine are reported from the protected areas, as a trend attributed to the increased research efforts by mycologists within these regions. Additionally, reserve territories provide better protection for rare species (see Table 1). However, *Tricholoma focale* faces a different scenario. The temporarily occupation

Table 1. A list of known localities of *Tricholoma focale* in Ukraine

No.	Locality	Nature protection status of the area	Accessibility of the site *	Year of the report
1	Poltava Region, Poltava District (former Kyshenky District), Zavorsklyanskyi mixed forests on the pine terrace of the Vorskla River	–	Available to visit	1960
2	Poltava Region, Poltava District (former Kobelyaky District), Zavorsklyanskyi mixed forests on the pine terrace of the Vorskla River	–	Available to visit	1960
3	Poltava Region, Poltava District (former Novi Sanzhary District), Zavorsklyanskyi mixed forests on the pine terrace of the Vorskla River	–	Available to visit	1960
4	Poltava Region, Poltava District (former Poltava District) Zavorsklyanskyi mixed forests on the pine terrace of the Vorskla River	–	Available to visit	1960
5	Poltava Region, Poltava District (former Dykanka District), Zavorsklyanskyi mixed forests on the pine terrace of the Vorskla River	–	Available to visit	1960
6	Poltava Region, Poltava District (former Opishnya District) Zavorsklyanskyi mixed forests on the pine terrace of the Vorskla River	–	Available to visit	1960
7	Poltava Region, Poltava District (former Kotelva District) Zavorsklyanskyi mixed forests on the pine terrace of the Vorskla River	–	Available to visit	1960
8	Mykolaiv Region, Pervomaysk District, Pervomaysk forestry	–	Available to visit	1996
9	Dnipropetrovsk Region, Pavlohrad District, Prysamarskyi forest	–	Available to visit	1989
10	Luhansk Region, Luhansk District, 3 km east of Khristove village, Prydintsivska floodplain, Stanychno-Luhanske branch of Luhansk Nature Reserve, pine forest	Luhansk Nature Reserve	Temporarily occupied	2005
11	Luhansk Region, Dovzhansk District, Provallia village, Provalsky Steppe branch of Luhansk Nature Reserve	Luhansk Nature Reserve	Temporarily occupied	2009
12	Kherson Region, Beryslav District, vicinity of Olhivka village	Nyzhnodniprovskyi National Nature Park	Temporarily closed to the public	2009
13	Volyn Region, Kamin-Kashyrskyi District, Zarudchi village	Prypiat-Stokhid National Nature Park	Partially restricted to visit	2008
14	Ivano-Frankivsk Region, Kosiv District	Hutsulshchyna National Nature Park	Available to visit	2010, 2020
15	Kyiv Region, Bucha District, near Kozyntsi village	–	Available to visit	This study

* The restricted access due to Russian aggression is provided here according to the website of the Ministry of Environmental Protection and Natural Resources of Ukraine: <https://wownature.in.ua/karta/>.

of Ukraine by the aggressor state, forest clearance, trenching, timber harvesting, forest fires, and active combat operations are detrimental to the preservation of natural habitats and pose a significant threat to the survival of flora and fauna.

Thus, out of 15 known records of the species, ten are located outside protected areas. Four of them are not currently accessible for further observation

due to ongoing military activities nearby. For the rare species, such access is essential for updating information on their distribution, particularly in those regions where occurrence data has been lacking for an extended period.

It is noteworthy that in all previously documented sites, solitary fruit bodies were observed, typically no more than two or three fruit bodies. In

contrast, the currently reported site yielded over 15 fruit bodies, making it the most abundant location recorded in Ukraine. Given that this species was found in an area without any protected status, it is crucial to implement conservation measures such as establishing conservation zones, disseminating relevant information, and enforcing appropriate environmental regulations within the area.

Conclusion

In Ukraine, new records of *Tricholoma focale* were observed in Kyiv Region in 2018, 2019, and 2021, with over 15 fruit bodies. The occurrence of this fungus in Ukraine is quite rare and sporadic. Currently, 15 locations of the species in eight administrative regions of Ukraine are known: seven locations in Poltava Region, two in Luhansk Region, and one in each of Mykolaiv, Dnipropetrovsk, Kherson, Volyn, Ivano-Frankivsk, and Kyiv regions (including our discovery).

Of the 15 known records of the species, ten are located in the areas that do not have protected status (not included into the nature reserve fund of the country). For four of these locations, due to military actions and temporarily occupation of Ukraine's territory by the aggressor state, there is no possibility to conduct further research in the area, where the information on the species occurrence has not been

updated already for quite a long time. Thus, there are no guarantees for preservation of the species in these areas.

Therefore, in our opinion, thorough observations of the areas, where *T. focale* was previously found, should be conducted after the end of the war and subsequent demining efforts to develop conservation measures both *in situ* and *ex situ*. Although we have been unable to isolate the fungus in pure culture, due to reported successful results in cultivating *T. focale* on artificial media (Sánchez et al., 2001), it can be suggested that attempts to obtain pure cultures for gene pool preservation of the fungus remain relevant.

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ETHICS DECLARATION

The authors declare no conflict of interest.

ORCID

M.M. Sukhomlyn:  <https://orcid.org/0000-0001-6573-9699>

M.V. Pasailiuk:  <https://orcid.org/0000-0001-6103-1247>

N.M. Fontana:  <https://orcid.org/0000-0002-9622-103X>

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Нові знахідки рідкісного гриба *Tricholoma focale* (Agaricales) в Україні

М.М. СУХОМЛИН¹, М.В. ПАСАЙЛЮК², Н.М. ФОНТАНА³

¹ Інститут еволюційної екології НАН України, вул. Лебедева 37, Київ 03143, Україна

² Національний природний парк "Гуцульщина", вул. Дружби 84, Косів 78600, Україна

³ Каліфорнійський університет, Дейвіс, Каліфорнія, США

Реферат. Вид *Tricholoma focale* включений до Червоної книги України зі статусом "вразливий". У 2018–2021 рр. ми виявили новий локалітет гриба поблизу села Козинці (Ірпінська територіальна громада, Бучанський район, Київська область), де спостерігали його масове плодоношення — понад 15 екземплярів. Ми встановили, що разом із нашими даними в Україні загалом відомо 15 місцезнаходжень гриба; з них 10 локалітетів знаходяться поза межами територій, які мають статус природно-заповідних. Встановлено також, що повторні дослідження чотирьох попередніх місцезнаходжень є неможливими через недоступність цих територій внаслідок російського вторгнення в Україну та воєнної дії. Необхідно розробити заходи охорони *T. focale in situ* та виділити цей вид в чисту культуру для збереження його генетичного різноманіття *ex situ*.

Ключові слова: збереження, морфологічні ознаки, нові локалітети, поширення, Червона книга України