www.biodicon.com Biological Diversity and Conservation

ISSN 1308-8084 Online; ISSN 1308-5301 Print 8/3 (2015) 01-05

Research article/Araştırma makalesi

Distribution in Ukraine and cultural features of a rare fungus *Leucoagaricus barssii* (Agaricales, Basidiomycota)

Vasyl P. HELUTA[1], Maryna M. SUKHOMLYN [* 2]

[1] M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine, Department of Mycology, Kyiv, Ukraine

[2] Taras Shevchenko Kyiv National University, Institute of Biology, Department of Botany, Kyiv, Ukraine

Abstract

Two new localities of *Leucoagaricus barssii* (Zeller) Vellinga, a rare fungus in Ukraine, were characterized. A pure culture of the fungus was obtained, cultural-morphological features of this species were studied, and a possibility to obtain fertile fruit bodies in vitro was demonstrated. These results open up the prospect for recovery of populations of this rare fungus in nature.

Key words: *Leucoagaricus macrorhizus*, Red Data Book of Ukraine, pure culture, fruiting, in vitro

1. Introduction

There are thousands of species of fungi in Ukraine, which are known from a few or several habitats in the country. Only a tiny part of them (57 species) is included in the latest edition of the Red Data Book of Ukraine (Chervona knyha…, 2009). *Leucoagaricus barssii* (Zeller) Vellinga found at the time of publication of the Red Data Book only in two localities in Ukraine (Sumy and Khmelnitsky regions) (Wasser, 1976, 1980; Karpenko, 2004) also belongs to such rare fungi. In the mentioned works, and in the Red Data Book, this species is presented as *L. macrorhizus* Locq. ex Horak. However, Vellinga (2000) has shown that at least 12 different names belonging to three groups are known for the fungus. This author conducted a comparative study of type specimens and descriptions of the three valid species of these groups, viz. *Lepiota barssii* Zeller, *Leucoagaricus pinguipes* (A. Pearson) Bon, and *L. macrorhizus* Locq. ex E. Horak. It was found that all of them belong to the same species of the genus *Leucoagaricus*. As the oldest legitimate name is *Lepiota barssii*, based on this name, a new combination *Leucoagaricus barssii* was proposed. Thus, only this name is correct for this species in the genus *Leucoagaricus*. That is why the fungus should be listed in next editions of the Red Data Book of Ukraine as *L. barssii* (Zeller) Vellinga.

Leucoagaricus barssii has a very wide distribution. The fungus is known in Europe (Austria, Bulgaria, France, Germany, Great Britain, Italy, Netherlands, Russia, Slovenia, Ukraine), Asia (Armenia, India, Thailand, Turkey), Africa (Canary Islands, Morocco), Northern America (Canada, USA), and Australia (Wasser, 1980; Yilmaz and Işiloğlu, 2002; Houben and Kelderman, 2008; Chandrasrikul et al., 2011; Babenko and Popova, 2013; Kumari and Atri, 2013; López Quintanilla et al., 2013; Akata et al., 2014; Lacheva, 2014; Outcoumit et al., 2014). Among neighboring countries, it was reported only from Russia (Rostov region) (Vyshchepan, 1990). However, in all the above countries *L. barssii* is regarded as a rare fungus.

The authors of this paper have recorded *L. barssii* in two new localities in Ukraine (Fig. 1). In addition, from the fruit body collected in one of the localities (Fig. 2a) a pure culture was isolated (Sukhomlyn, 2010; Makarenko et al., 2013) and even fruit bodies were obtained under sterile conditions (Fig. 2b). Therefore, taking into account the considerable rarity of this fungus, we believe that it is necessary to discuss briefly its distribution in Ukraine, ecological characteristics of the habitats as well as some morphological and cultural characteristics of the fungus.

2. Materials and methods

Pure culture of *L. barssii* was isolated from the fruit body. The corresponding strain (LA 1) is maintained in the pure culture collection of the Department of Botany of Taras Shevchenko Kyiv National University (FCKU). The

[*] *Corresponding author* / Haberleşmeden sorumlu yazar: Tel.: +380506234905; Fax.: +380506234905; E-mail: marsuh@i.ua

 BioDiCon. 501-0615

growth of fungus colonies and their morphological characteristics in long-term storage were investigated in Petri dishes on nutrient media of different composition (grams per liter): *Doksy-Chapek agar* (saccharose – 30.0, sodium nitrate ($NaNO_3$) – 3.0, magnesium sulfate ($MgSO_4 \cdot 7H_2O$) – 0.5, potassium chloride (KCl) – 0.5, ferrous sulfate ($FeSO_4$) – 0.01, dibasic potassium phosphate (KH_2PO_4) – 1.0, agar-agar – 13.0); *potato-glucose agar* (glucose – 10.0, potatoes – 200.0, agar-agar – 10.0); *carrot agar* (glucose – 10.0, carrots – 200.0, agar-agar – 10.0); *cereal agar* (glucose – 10.0, wheat – 200.0, agar-agar – 10.0); *Saburo agar* (glucose – 40.0, peptone – 10.0, agar-agar – 10.0); *soil agar* (glucose – 10.0, soil – 200.0, agar-agar – 10.0); *agar with yeast extract* (glucose – 5.0, peptone – 2,5, yeast extract – 0.5, agar – 10.0) (Dudka et al., 1982, Molitoris, 2000). Inoculation was performed by mycelial disc 4 mm diam. at the center of the culture medium in Petri dishes and incubated for 5–10 days at 24 °C until nutrition medium surface was overgrown by mycelium.

In culture, when *L. barssii* was grown on agar media, primordia of the fruit bodies were not formed. Only on sterile organic substrate (sunflower husk), the fungus formed fertile basidiomata. Experiments on fruit bodies formation were conducted during the winter (December to March). Overgrowth of the substrate occurred for 25 days, then mycelium was covered with sterile soil and flasks were exposed to light. Additional wetting of the soil was necessary during incubation. Sterile water was added into the flask after two months since inoculation of the substrate.

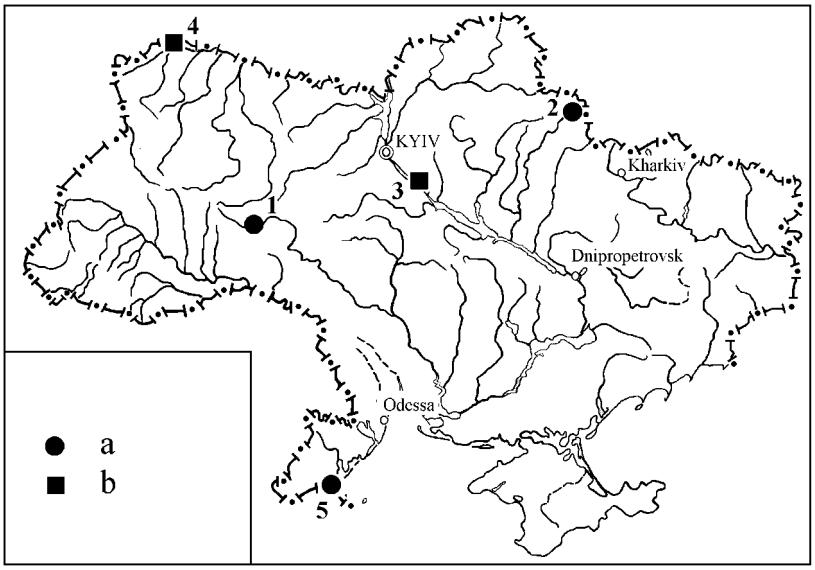

Figure 1. Distribution of *Leucoagaricus barssii* in Ukraine: literature data (**a**), new records (**b**). Numbering of localities is in accordance with text

Mycelium was studied using living culture. Hyphae examined and photographed under a light microscope Primo Star (Carl Zeiss, Germany) using the Camera Canon A 300 and software AxioVision 4.7. For scanning electron microscope (SEM) investigation, small pieces of dried mycelium and hymenial lamellae were placed on metal stubs, then coated with gold and observed under the SEM (Jeol 6060LA, Japan).

3. Results

As noted above, the latest edition of the Red Data Book of Ukraine (2009) contains information on two localities of *L. barssii*. Subsequently, we found the fungus in Cherkasy and Volhynian regions. In addition, it was reported from Odessa region (Babenko and Popova, 2013). Thus, to date this fungus is registered in the West Polissia, the Right and the Left Bank Forest-Steppes and the Right Bank Grass Steppes of Ukraine, i.e. altogether in five localities only (Fig. 1). Two of them are reported for the first time. Ecological information on these habitats is given below.

1. Khmelnytskyi region, Letychiv district, settlement Medzhybizh, 14.06.1974, leg. S. Wasser. The fungus was collected on a lawn near the castle (Wasser, 1976, 1980). It is possible that this habitat could be destroyed during archaeological excavations and because of the large recreational pressure caused by the restoration of the castle as a tourist site.

2. Sumy region, Krasnopillia district, surroundings of the village Taratutyne, 28.09.2000, leg. K. Karpenko. According to the literature data (Karpenko, 2004), we only know that the fungus was collected in meadow steppe.

3. Cherkasy region, Kaniv district, Regional Landscape Park Trakhtemyriv, area of the former village Trakhtemyriv, 02.11.2008, leg. V. Heluta and M. Sukhomlyn. The fungus (only two fruit bodies) were found on an artificial slope covered by grass eight years ago. Soil contains a large admixture of sand.

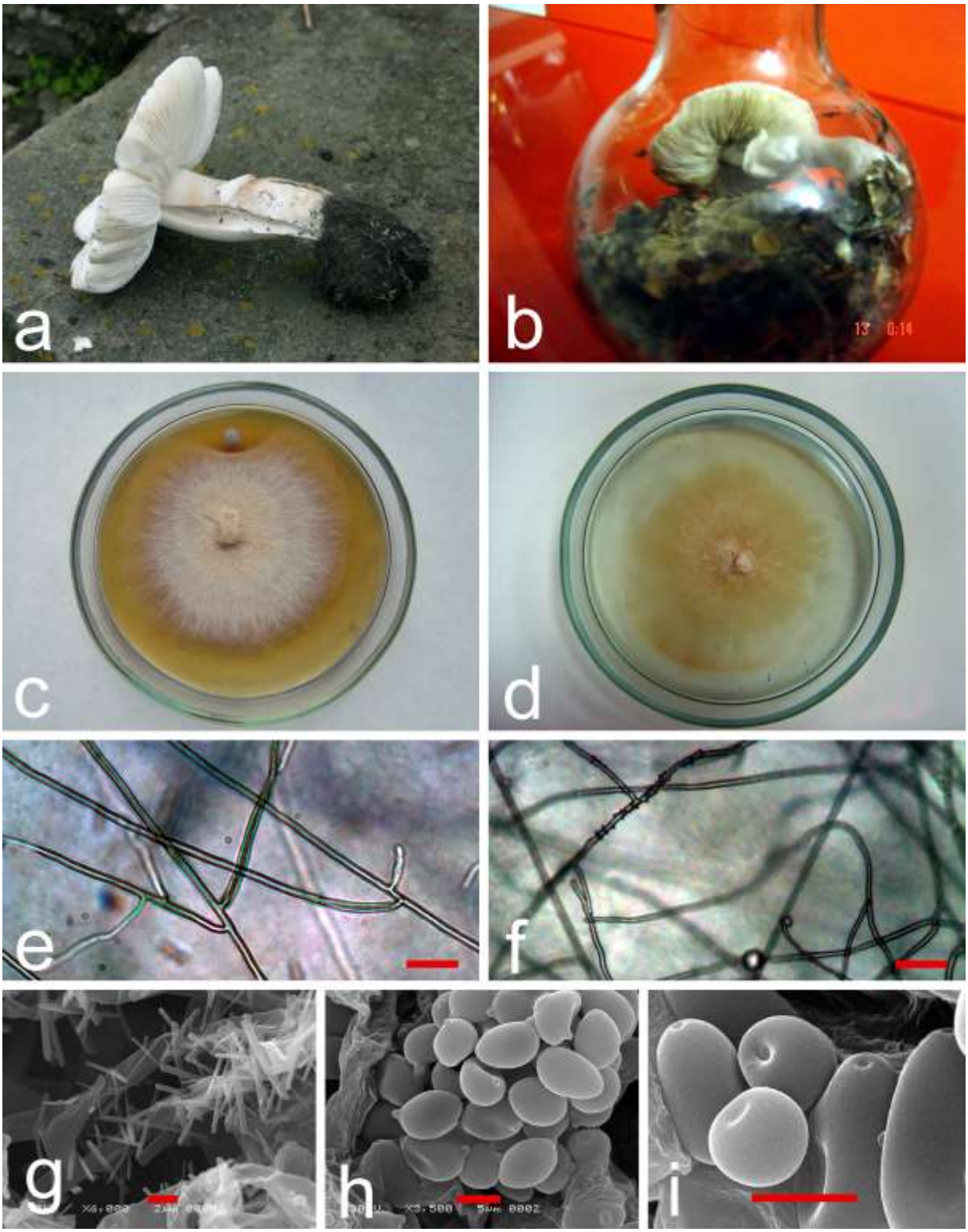

Figure 2. *Leucoagaricus barssii* in nature and in pure culture: fruit body collected from natural environment (**a**); fruit body obtained in culture (**b**); colony on PGA (potato-glucose agar) (**c**); colony on the medium with peptone (**d**); hyphae of the fungus in pure culture on PGA (**e**–**g**; **f**–**g** – with incrustation); basidiospores formed by hymenium of the fruit body obtained in the laboratory (**h**–**i**; **i** – with conspicuous apical germ pores) (**e**–**f** – LEM, **g**–**i** – SEM). Scale bars: 20 μm (**e**–**f**), 2 μm (**g**), 5 μm (**h**–**i**).

From the one fruit body a pure culture was isolated, which is stored in Taras Shevchenko Kyiv National University. There are no threats of destruction in this locality.

4. Volhynian region, Ratne district, north-eastern surroundings of the village Zalukhiv, 22.09.2009, leg. V. Heluta.

Only two fruit bodies were found in a sandy meadow with very sparse and trampled vegetation. Soil is represented by almost pure sand and a site is located next to a water drainage channel. Consequently, the fungus can develop only after prolonged rains. The source of nutrients consisted of the remains of plant (grasses) roots and possibly cow dung. There are no threats of destruction.

5. Odessa region, Kiliya district, surroundings of the village Lisky, Danube Biosphere Reserve, 21.10.2009, leg. O. Babenko. The fungus was found in sandy soil in artificial forest plantations formed by deciduous plants, with *Phragmites australis* (Cav.) Trin. ex Steud. in grass cover (Babenko and Popova, 2013).

As we can see, almost all known habitats of *L. barssii* are associated with anthropogenically transformed ecosystems. There is a significant restriction to open areas with sparse vegetation formed on sand soils. Such habitats are strongly heated by sunlight that is in accordance with the needs of this thermophilic species.

In conditions of Ukraine, *L. barssii* forms fruit bodies in autumn, in September and November. The fungus was among the species studied by Turkish mycologists with respect to the content of heavy metals in fruit bodies. It was found, as compared to other fungi, a high copper content (93 mg/kg), zinc (190 mg/kg) and iron (460 mg/kg) in fruit bodies collected near the road (Işiloğlu et al., 2001).

Under natural conditions, protection of fungal species that are threatened obviously is best achieved by preserving habitats where they grow. However, these habitats do not always fall within the nature reserve areas. Unfortunately, rare species of fungi are frequently recorded in the sites that are not protected. Therefore, for them the threat of habitat destruction persists. However, another important way to protect fungi is *ex situ* conservation, i.e. outside their habitats, in vitro, in pure culture collections (Hawksworth, 1992; Badalyan, 2002).

In view of the above, we have isolated a pure culture of *L. barssii*. In different media, the fungus formed whitish colonies consisting of depth (substrate) and surface hyphae. Reversum of the substrate mycelium caused ochre tint of the colonies; surface mycelium formed white, threadlike, thin, uneven bands (Figs. 2c and 2d). In media where the fungus showed better growth, aerial mycelium grew stronger and colonies looked more fluffy. Advancing zone of colony was appressed, fringed, reversum was ochre. No mycelial clamp connexions were formed (Figs. 2e and 2f). With age, crystalline incrustation on hyphae was observed (Figs. 2f and 2g).

We have studied the growth rate of the mycelium on various nutrient media. The following results were obtained: in potato-glucose agar 1.63 mm/day, Saburo agar 1.38 mm/day, cereal agar 1.20 mm/day, agar with yeast extract 1.13 mm/day, Doksy-Chapek agar 0.91 mm/day, carrot agar 0.82 mm/day, and soil agar 0.76 mm/day. We can conclude that the best for cultivation were two media, viz. potato-glucose agar and Saburo agar. The least suitable for the growth of the fungus were media with soil and carrot agar.

Fruiting occurred only when the substrate was covered by a layer of sterile soil. Fruit bodies obtained in vitro (Fig. 2b) hardly differed from those described from nature conditions (Fig. 2a), but were somewhat smaller in size. Caps were hemispherical, thick fleshy in the center, white-creamy, silky-fiber, in the center with a wide tubercle, 4–5 cm in diam. Stipe central, cylindrical, curved, extended to the base to form a small tuber, smooth, finely pubescent, with a ring at the top, length reached 5 cm.

Fruit bodies obtained in vitro have formed normal hymenophore on which basidiospores appeared (Figs. 2h and 2i). The basidiospores are oval to elliptical, with a smooth surface and germ pores exclusively located on top of them, 9.4 × 6.0 μm (Fig. 2i). The spores germinate well on the medium with potato-glucose agar. Thus, the fruit bodies of the fungus obtained in vitro are fertile.

It should be also noted that the studied mushroom can provide valuable biological material for application purposes. Several Ukrainian researchers (Dovhyi et al., 2013; Makarenko et al., 2013) found the impact of culture broth and mycelium extract of *L. barssii* on cytotoxic effect, namely oxygen-dependent metabolism, of peritoneal macrophages of mice, as well as on characters of immune system such as metabolic activity of macrophages and mononuclear leukocytes of mouse spleen, and relative weight and cellularity of lymphoid organs.

To sum up, we found two new localities of a rare fungus *L. barssii*, isolated pure culture of this species, studied its cultural-morphological characteristics and obtained fruit bodies in vitro for possible future recovery of this fungus in nature.

Acknowledgements

Authors are grateful to Prof. S.P. Wasser for kindly providing advices and some reprints of articles on *L. barssii.* We gratefully acknowledge Mr. V.M. Novichenko, for his help with scanning electron microscopy.

References

Akata, I., Uzun, Y., Kaya, A. 2014. Macromycetes determined in Yomra (Trabzon) district. Turk J Bot. 38. 999-1012.

Babenko, O.A., Popova, O.M. 2013. Novi mistseznakhodzhennia dvokh ridkisnykh vydiv makromitsetiv u Pivnichno-Zakhidnomu Prychornomorji (Ukraina). Ukr Bot Z. 70/4. 547-551.

Badalyan, S.M. 2002. Kollekciya kultur makromitsetov laboratorii eksperimentalnoi mikologii Yerevanskogo gosudarstvennogo universiteta. Mikol Fitopatol. 26/1. 55-57.

Chandrasrikul, A., Suwanarit, P., Sangwanit, U., Lumyong, S., Payapanon, A., Sanoamuang, N. et al. 2011.Checklist of Mushrooms (Basidiomycetes) in Thailand. Office of Natural Resources and Environmental Policy and Planning, Bangkok.

Chervona knyha Ukrainy. 2009. Roslynnyi svit. Globalkonsalting, Kyiv.

Dovhyi, R.S., Pozur, V.V., Svyatetska, V.M., Sukhomlyn, M.M., Livinska, O.P., Makarenko, O.M. 2013. Imunobiolohichna diya ekstraktiv ta zasobiv, otrymanykh iz vyshchykh hrybiv *Ganoderma lucidum*, *Cordyceps sinensis* ta *Leucoagaricus macrorhizus*. Aktualni problemy suchasnoi medytsyny: Visnyk Ukrains'koi medychnoi stomatolohichnoi akademii. 13 [4/44)]. 92-97.

Dudka, I.A., Wasser, S.P., Elanskaya, I.A., Koval, E.Z., Gorbik, L.T., Nikolskaya, Ye.A. et al. 1982. Metody eksperimentalnoi mikologii. Naukova dumka, Kiev.

Hawksworth, D.L. 1992. Obshchee kolichestvo gribov, ikh znachenie v funktsionirovanii ekosistem, sokhranenie i znachenie dlia cheloveka. Mikol Fitopatol. 26/2. 152-166.

Houben, M., Kelderman, P. 2008. *Leucoagaricus barssii* de zeldzame Wortelende champignonparasol. PSL-Nieuws. 15/2. 11-12.

Işiloğlu, M., Merdivan, M., Yilmaz, F. 2001. Heavy metal contents in some macrofungi collected in the Northwestern Part of Turkey. Arch Environ Contam Toxicol. 41. 1-7.

Karpenko, K.K. 2004. Novi ta ridkisni dlia Ukrainy vydy macromitsetiv iz pivnichno-skhidnoi chastyny Livoberezhnoho Lisostepu Ukrainy. Ukr Bot Z. 61/2. 34-40.

Kumari, B., Atri, N.S. 2013. New additions of basidiomycetous fungi in Indian mycoflora. Mycosphere. 4/1. 53-59.

Lacheva, M. 2014. New data for *Leucoagaricus* and *Leucocoprinus* (*Agaricaceae*) in Bulgaria. J Agric Sci Technol. 6/1. 76-79.

López Quintanilla, J.F., Velaz Vergara, J.I., Luque Víboras, M., Escobio García, V.J. 2013. Contribución al conocimiento de los hongos del Jardín Botánico Viera y Clavijo (Gran Canaria, Islas Canarias). (I). Bot Macaronésica. 28. 51-62.

Makarenko, O.M., Rudyk, M.P., Pozur, V.V., Sukhomlyn, M.M., Sviatetska, V.M., Dovhyi, R.S. 2013. Vplyv kulturalnoi ridyny ta ekstraktu mitseliyu hryba *Leucoagaricus macrorhizus* na kysnezalezhnyi metabolizm perytonealnykh makrofahiv myshei. Bukovynskyi medychnyi visnyk. 17 [1/65)]. 64-67.

Molitoris, H.P. 2000. Methods for determination of enzymatic activities of marine fungi. Czech Mycol. 52/2. 97.

Outcoumit, A., El Kholfy, S., Ouazzani Touham,i A., Douira, A. 2014. Bibliographic inventory of Tangier fungi: catalogue of the Basidiomycetes fungal flora. Int J Plant Anim Environ Sci. 4/1. 205-256.

Sukhomlyn, M.M. 2010. Kolektsiya kultur hrybiv-makromitsetiv yak osnova stratehii zberezhennia henofondu bazydialnykh i askomitsetnykh hrybiv. Pryroda Zakhidnoho Polissia ta prylehlykh terytoriy. 7. 102-107.

Vellinga, E.C. 2000. Notes on *Lepiota* and *Leucoagaricus.* Type studies on *Lepiota magnispora*, *Lepiota barssii*, and *Agaricus americanus.* Mycotaxon. 76. 429-438.

Vyshchepan, S.L. 1990. Konspekt mikromitsetov nizoviy Dona i Severnogo Priazovia (v predelakh Rostovskoi oblasti). Dissertatsia kand. biol. nauk. Kiev.

Wasser, S.P. 1976. Do vyvchennia Basidiomycetes Ukrains'koi RSR. V. Novi ta ridkisni vydy z rodyny *Agaricaceae* Cohn (poriadok Agaricales Clem.). Ukr Bot Z. 33/5. 493-497.

Wasser, S.P. 1980. Flora gribov Ukrainy. Agarikovye griby. Naukova dumka, Kiev.

Yilmaz ,F., Işiloğlu, M. 2002. Macrofungi of Değirmenboğazı (Balıkesir). Turk J Bot. 26.161-164.

(Received for publication 29 June 2015; The date of publication 15 December 2015)