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# New records of *Microbotryum* species parasitizing *Caryophyllaceae* from Ukraine

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Four records of smut fungi belonging to the genus *Microbotryum* Lév. are reported. Two species were found on new hosts, namely *M. dianthorum* on *Dianthus borbasii* and *D. pseudoserotinus* and *M. superbum* on *D. stenocalyx. Microbotryum lagerheimii* on *Lychnis viscaria* is a new species for Ukraine.

Key words: smut fungi, Microbotryum, Dianthus, Lychnis

#### INTRODUCTION

The comprehensive information regarding European smut fungi has been given in the monograph by Vánky (1994), where the author listed 400 species known for the continent and 70 provisory species. However, some new species have been described later and species known in other regions have been found here. Thus, in the new check-list of smut fungi (Vánky 2005a) 14 additional species were included. Currently, there are 38 species which were described or collected after 1994 in Europe (Almaraz, Telleria 1998; Bauer et al. 2008; Denchev 2007; Denchev, Giraud and Hood 2009; Lutz et al. 2005, 2008; Lutz, Vánky 2009; Piątek 2006; Prillinger et al. 2009; Vánky 1998, 2003, 2005a,b, 2007, 2008a,b, 2009; Vánky and Berner 2003; Vánky, Horita and Jage 2005a; Vánky, Jage and Scholz 2005b; Vánky et al. 2008; Vánky, Lutz 2007; Vánky, Scholz 2001). Among them there are also representatives of the genus *Microbotryum* Lév., mainly described as new species, e.g., *M. adenopetalae* M. Lutz, Kemler et Chleb., *M. cartusianorum* Denchev, Giraud et M.E. Hood, *M. chloranthae-verrucosum* M. Lutz, Göker, Piątek, Kemler, Begerow et Oberw., *M. lagerheimii* Denchev, *M. minuartiae* M. Lutz, Piątek, Kemler et Chleb., *M. saponariae* M. Lutz, Göker, Piątek, Kemler,

Begerow et Oberw., *M. schykoffianum* Giraud, Denchev et M.E. Hood, *M. silenesacaulis* M. Lutz, Piątek, Kemler et Chleb., *M. silenes-dioicae* Giraud, Denchev et M.E. Hood, and *M. silybum* Vánky et Berner (Denchev 2007; Denchev et al. 2009; Lutz et al. 2005, 2008; Vánky, Berner 2003). *Microbotryum jehudanum* (Zundel) Vánky was found for the first time in the territory of Europe (Vánky et al. 2005b). Another species well known as *Ustilago superba* Liro was transferred into the genus *Microbotryum* with creation of a new combination *M. superbum* (Liro) Denchev, Giraud et M.E. Hood (Denchev et al. 2009).

It is known that many species of the genus *Microbotryum* develop in the anthers of plants belonging to the *Caryophyllaceae*. They are the so-called "anthericolous smut fungi". These species easily produce hybrid forms which can subsequently become new species (Chlebicki, Suková 2005). Therefore, for each region the establishment of the host-plants ranges of these species thorough morphological and, when it is possible, molecular investigations are all important.

Ukraine is a region with a considerable diversity of vascular plants, including the *Caryophyllaceae* with the level of endemism at about 24% (Fedoronchuk 2009). So, during the studies on smut fungi both rare species belonging to the genus *Microbotryum* and new species of their host-plants may be found. This paper deals with such fungi which we have found on the *Lychnis* L. and *Dianthus* L. species in 2009.

### MATERIALS AND METHODS

Sori and spores were studied using dried herbarium specimens. For light microscopy (LM), spores were dispersed in a droplet of lactophenol on a microscope slide, covered with a cover glass, gently heated to boiling point to rehydrate the spores and eliminate air bubbles, and examined at  $400 \times$  and  $1000 \times$  magnification. For scanning electron microscopy (SEM), spores were placed on double-sided adhesive tape, mounted on a specimen stub, sputter-coated with gold, ca. 20 nm, and examined in SEM at 30 kV. The studied materials are stored in the National Herbarium of Ukraine of M.G. Kholodny Institute of Botany (KW).

## RESULTS AND DISCUSSION

Smut fungi on *Dianthus* species. For a long time, the smut fungi which develop their sori in the anthers of different *Dianthus* species were included in *Ustilago violacea* Pers.: Pers., a combined cosmopolitan parasite of hosts belonging to the *Caryophyllaceae*. However, in 1924 Liro described two separate species, *U. dianthorum* Liro in the anthers of *D. deltoides* L. and *U. superba* Liro on *D. superbus* L. Much later, a new genus *Microbotryum* was offered for some anthericolous smut fungi, and *U. violacea* s. lat. was included in this genus as *M. violaceum* (Pers.) G. Deml et Oberw. (Deml, Oberwinkler 1982). Scholz and Scholz (1988) transferred smut fungi that

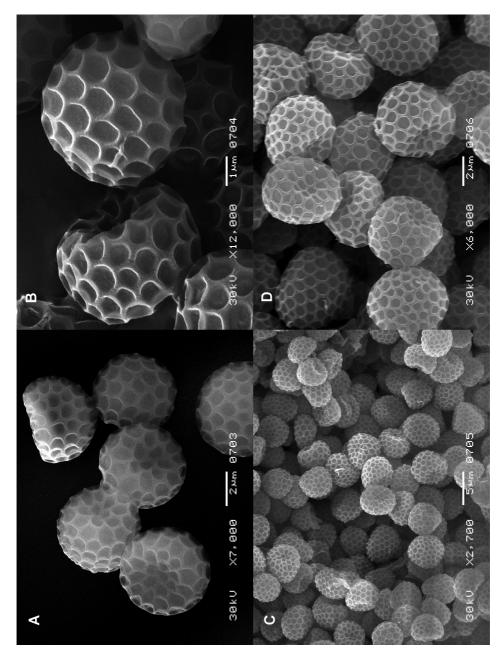


Fig. 1. Spores of *Microbotryum dianthorum*: A, B – on *Dianthus borbasii*; C, D – on *Dianthus pseudoserotinus*. All photos in SEM. Scale bars for A, D =  $2 \mu m$ ; B =  $1 \mu m$ ; C =  $5 \mu m$ .



Fig. 2. Microbotryum superbum on Dianthus stenocalyx: A – infected flower, B, C – spores. Microbotryum lagerhemii Denchev on Lychnis viscaria: D – infected flowers, E, F – spores; B, C, E, F – in SEM. Scale bars for A, D = 5 mm; B = 5  $\mu$ m; C = 2  $\mu$ m; E, F = 1  $\mu$ m.

developed in anthers of many Dianthus species into M. dianthorum (Liro) H. Scholz et I. Scholz, but Liro's *U. superba* was left in *M. violaceum*. Denchev and Scharkova (1997), having investigated 229 specimens of smut fungi on 22 Dianthus species, concluded that all of them belong to the same species, namely M. violaceum s. lat. However, Vánky (2004) supposed that this species can be divided into smaller ones through the use of analyses of as many of type specimens as possible with employment of both traditional morphological and molecular-phylogenetic methods. Such studies have been made by Lutz et al. (2005, 2008), and their results confirmed the separation of M. dianthorum. However, within this species a great variability in the spore dimensions was found. In addition, the number of meshes per spore diameter also strongly varied. In spite of this, all specimens were clustered together by molecular data. Similar investigations regarding other fungi from M. violcaeum-complex parasitizing D. sylvestris Wulfen and D. cartusianorum L. showed insignificant morphological differences between both of these samples and from other specimens of this complex. However, they differed by molecular-biological data and features of microsatellite analyses. Thus, they were cryptic species (Denchev et al. 2009). The results of molecular-phylogenetic investigations also made it possible to restore a species status of smut fungus on D. superbus and regard it not any more as U. superba but as M. superbum (Denchev et al. 2009).

Only two species of smut fungi parasitizing *Dianthus* species were known in Ukraine, e.g., *M. dianthorum* (reported as *U. dianthorum*) on *D. pseudoarmeria* M. Bieb. from the forest-steppe zone and *M. superbum* (reported as *U. superba*) on *D. superbus* from Polissia region (Zerova et al. 1971). No specimens of these fungi were found in the National Herbarium of Ukraine of the M.G. Kholodny Institute of Botany (Fungi of Ukraine 1996). So far, the smut fungi on *Dianthus* have been considered to be rare in Ukraine. However, in summer 2009 we collected three specimens of the anthericolous smut fungi on *D. borbasii* Vandas, *D. pseudoserotinus* Błocki (*D. arenarius* p.p.) and *D. stenocalyx* Juz. (*D. superbus* p.p.). The first and the second of them were identified by us as *M. dianthorum* and the last as *M. superbum*. The characteristics of these fungi on new hosts are given below.

## Microbotryum dianthorum (Liro) H. Scholz et I. Scholz

Fig. 1

Sori develop in the anthers of host plants. Spore mass powdery, from medium violet to brownish violet. Spores globose or subglobose, sometimes ovoid to slightly elongated, 5-8  $\times$  5-7  $\mu m$ . Spore wall reticulate, on *Dianthus borbasii* 5-7 meshes per spore diameter, meshes irregularly polygonal to rounded, 0.6-0.9  $\times$  0.8-1  $\mu m$ ; on *D. pseudoserotinus* (6) 7-9 meshes per spore diameter, meshes rounded to slightly irregularly polygonal, 0.6  $\times$  0.7  $\mu m$ .

DISTRIBUTION IN UKRAINE. On *Dianthus borbasii* Vandas: Cherkasy region, Kaniv distr., Trakhtemyriv Regional Landscape Park, 49°59'10" N, 31°22'20" E, 03.06.2009, leg. K. Savchenko (KW 36849F). On *D. pseudoarmeria* M. Bieb.: Right-Bank Forest-Steppe zone (Zerova et al. 1971). On *D. pseudoserotinus* Błocki: Volhynian region, Lubeshiv distr., Pripyat-Stokhid National Park, 51°53'16" N, 24°57'26" E, 09.08.2009, leg. K. Savchenko and V. Heluta (KW 36851F).

Microbotryum superbum (Liro) Denchev, Giraud et M.E. Hood

Figs 2 A-C

Sori develop in the anthers of host plants. Spore mass dark violet brown, powdery. Spores subglobose, ovoid to slightly elongated, sometimes rather irregular,

 $5-6~(6.5) \times 4-6~\mu m$  (at the mean  $5.6 \times 4.7~\mu m$ ). Spore wall reticulate, 5-7 meshes per spore diameter, meshes slightly irregular polygonal,  $0.7 \times 0.8~\mu m$ .

DISTRIBUTION IN UKRAINE. On *Dianthus stenocalyx* Juz.: Volhynian region, Lubeshiv distr., Pripyat-Stokhid National Park, mixed forest, 51°53'40" N, 24°57'35" E, 08.08.2009, leg. V. Heluta (KW 36852F). On *D. superbus* L. s. lat.: Right-Bank and Left-Bank Polissia (Zerova et al. 1971). *Microbotryum superbum* differs from *M. dianthorum* in having smaller spores and by the color of spore mass.

Anthericolous smut fungus on Lychnis. During field research in Cherkasy region (Ukraine) intense development of smut fungus in the anthers of *Lychnis viscaria* L. was recorded. Thus, about 20% of plants in the studied population were affected. The fungus was identified as *Microbotryum lagerheimii*. This species has been recently described (Denchev 2007) in the anthers of *L. alpina* L. and *L. viscaria*. It is closely related to *M. silenes-inflatae* (DC. ex Liro) G. Deml et Oberw. known on *Lychnis alpina*, *Silene vulgaris* subsp. *glareosa* (Jord.) Marsden-Jones et Turrill, *S. vulgaris* subsp. *vulgaris* (Moench) Garcke (*Oberna behen* (L.) Ikonn.), *S. cucubalus* Wibel, *S. inflata* Sm. and *S. venosa* Asch.) but differs in having far less (small or medium) intensity of spore mass coloration (Denchev, Minter 2008). *Microbotryum lagerhemii* is also known from Czech, Finland, Germany, Italy, Latvia, Norway, Poland, Russia, and Sweden (Denchev 2007). Thus, the fungus is a European endemic within a boreal-temperate area. In Ukraine it was found for the first time.

# Microbotryum lagerheimii Denchev

Figs 2 D-F

Sori develop in the anthers of host plant. Spore mass pale to medium violet, powdery. Spores globose, subglobose or slightly ellipsoidal to irregular,  $4.5-8\times4.5-7$  µm, length/width ratio 1.06–1.18, practically hyaline to weakly coloured. Spore wall reticulate, 5-8 meshes per spore diameter, meshes globose to ellipsoidal and irregularly elongated, 0.3-1.1 µm.

DISTRIBUTION IN UKRAINE. Cherkasy region, Kaniv distr., Trakhtemyriv Regional Landscape Park, 49°59'06" N, 31°20'53" E, 04.06.2009, leg. K. Savchenko (KW 36366F).

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