

Three new species of anthericolous smut fungi on *Caryophyllaceae*

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Abstract. Based on molecular phylogenetic analyses and morphological studies, three new cryptic species of *Microbotryum*, *M. silenes-dioicae* on *Silene dioica*, *M. shykoffianum* on *Dianthus sylvestris*, and *M. carthusianorum* on *Dianthus carthusianorum*, are described and illustrated. For *Ustilago superba* on *Dianthus superbus*, a new combination in *Microbotryum*, *M. superbum*, is proposed.

Key words: *Caryophyllaceae*, *Dianthus*, *Microbotryum*, *Silene*, smut fungi, taxonomy

Introduction

Microbotryum violaceum s. lat. is a fungal basidiomycete species complex responsible for anther smut disease on many plant species in the *Caryophyllaceae* (Thrall *et al.* 1993). It has recently been shown to be composed of several sibling species highly specialized on their respective host plant and evolving independently without detectable gene flow despite their large range overlap (Lutz *et al.* 2005; Kemler *et al.* 2006; Le Gac *et al.* 2007a; Refrégier *et al.* 2008). Hybrid inviability and sterility have been detected when artificially crossing these species and inoculating them on plants (Le Gac *et al.* 2007b; de Vienne *et al.* 2009; Sloan *et al.* 2009). Several of these recently recognized sibling species have been formally named (Lutz *et al.* 2005, 2008), but others still await taxonomic description. Our aim here was to formally describe the *Microbotryum* species parasitizing respectively *Silene dioica*, *Dianthus sylvestris*, *D. superbus*, and *D. carthusianorum*. Previous studies using concordance between multiple gene phylogenies and experimental hybridizations showed that they indeed constitute separate species (Le Gac *et al.* 2007a, b; Refrégier *et al.* 2008; de Vienne *et al.* 2009; Sloan *et al.* 2009).

Microbotryum parasitizing *S. dioica* is referred to as *M. lychnidis-dioicae* in official taxonomy. However, while *M. lychnidis-dioicae* was initially described both on *S. latifolia* and *S. dioica* (Liro 1924), several studies showed that these

two host species are infected in nature by two different *Microbotryum* species (Van Putten *et al.* 2003, 2005; Le Gac *et al.* 2007a, b; Refrégier *et al.* 2008; de Vienne *et al.* 2009). A lectotype of *M. lychnidis-dioicae* was selected by Vánky (1985: 252) on *Melandrium album* (= *Silene latifolia* subsp. *alba*) (H.U.V. 9749; isoelectotypes in Sydow, Ustilagineen, no. 62; as *Ustilago violacea*). *Microbotryum* parasitizing *Dianthus* species are all called *M. dianthorum*, excepting the anthericolous smut fungus on *Dianthus superbus*, referred to *M. violaceum* s. lat., while multiple phylogenies revealed that several distinct species existed, in particular on *Dianthus sylvestris*, *D. superbus*, and *D. carthusianorum* (Le Gac *et al.* 2007a; Refrégier *et al.* 2008). *Microbotryum dianthorum* was described on *Dianthus deltoides* L. Microsatellite analyses showed that it is a separate species from those present in natural populations on *D. sylvestris*, *D. superbus*, and *D. carthusianorum* (M.E. Hood, unpubl. data).

Here, we therefore performed morphological studies in LM and SEM of specimens of anthericolous fungi on *Silene dioica*, *Dianthus sylvestris*, *D. superbus*, and *D. carthusianorum*. This yielded three new species of *Microbotryum*: *M. silenes-dioicae* on *Silene dioica*, *M. shykoffianum* on *Dianthus sylvestris*, and *M. carthusianorum* on *Dianthus carthusianorum*, which are described and illustrated in the present article. A new combination of *Ustilago superba* on *Dianthus superbus* in *Microbotryum* is also proposed.

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Materials and methods

Herbarium specimens were examined under light microscope (LM) and scanning electron microscope (SEM). For LM observations, the spores were mounted in lactophenol solution on glass slides, gently heated to boiling point and then cooled. The measurements of spores are given in the form: min–max (mean \pm 1 standard deviation). For SEM, the spores were attached to specimen holders by double-sided adhesive tape and coated with gold. The surface structure of spores was observed at 10 kV and photographed with a JEOL SM-6390 scanning electron microscope.

DNA was extracted from single-sporidial colonies using the Chelex (Biorad, Marne-la-coquette, France) protocol (Bucheli *et al.* 2001). PCR and sequencing was performed as in Le Gac *et al.* (2007a). The phylogenetic analyses are presented in Le Gac *et al.* (2007a) and Refrégier (2008). GenBank accession numbers of β -tubulin gene sequences of the *Microbotryum* examined specimens are provided in Table 1.

Results and discussion

We describe and illustrate below three new species of *Microbotryum*: *M. silenes-dioicae* on *Silene dioica*, *M. shykoffianum* on *Dianthus sylvestris*, and *M. carthusianorum* on *Dianthus carthusianorum*. These new species are morphologically identical with other specimens of anthericolous smut fungi referred to the *Microbotryum violaceum* complex and represent cryptic species differing in molecular phylogenetic characters (see Le Gac *et al.* 2007a; Refrégier *et al.* 2008) and showing hybrid inviability and sterility when artificially crossed with other *Microbotryum* species (Le Gac *et al.* 2007b; de Vienne *et al.* 2009; Sloan *et al.* 2009). In Le Gac *et al.* (2007a) these new species were discussed under the names MvSd for *M. silenes-dioicae*, MvDsp1 for *M. shykoffianum*, and MvDc for *M. carthusianorum*. *Microbotryum shykoffianum* was found to infect also *Dianthus neglectus* Loisel. and *D. carthusianorum*, and maybe other *Dianthus* species.

The anthericolous smut fungus on *Dianthus superbus* was discussed under the name MvDsp2 in Refrégier *et al.*

(2008) and was found to infect also *D. monspessulanus* and *D. gratianopolitanus*, and maybe other *Dianthus* species. We treat this fungus as a distinct species for which the name, *Ustilago superba*, needs to be transferred in *Microbotryum*.

Taxonomy

Microbotryum silenes-dioicae Giraud, Denchev & M.E. Hood, *sp. nov.* **Figs 1–2**

Mycobank # MB 515084

Sori antheras *Silenes dioicae* destruentes. *Massa sporarum pulverea, brunneo-vinosa*. *Sporae globosae, subglobosae vel late ellipsoideae*, 6.5–10.5 \times 5.5–9 (7.8 \pm 0.7 \times 7.1 \pm 0.6) μ m; *paries reticulatus*, 6–8 (–9) *maculis in diametro sporae, maculae irregulariter polyangulares, interstitiis laevis*. *Sequentia typi β -tubulini in collectione sequentiarum acidi nucleici NCBI (GenBank) numero DQ074513 deposita est.*

Holotypus in matrice Silene dioica (L.) Clairv. (Caryophyllaceae), Gallia, Brittany, 2004, Mickael le Gac, # Sdioica_b (SOMF 27 696).

Sori in anthers. **Spore mass** powdery, brown vinaceous (based on the *Colour identification chart* of Anonymous 1969, and Rayner's colour chart, Rayner 1970). **Spores** mainly globose, subglobose or broadly ellipsoidal, 6.5–10.5 \times 5.5–9 (7.8 \pm 0.7 \times 7.1 \pm 0.6) μ m ($n = 70$), pale coloured; spore wall reticulate, 6–8 (–9) meshes per spore diameter, meshes irregularly polygonal; in SEM interspaces smooth, rarely some interspaces with very low warts. The β -tubulin type sequence from the holotype (SOMF 27 696) is deposited in GenBank as DQ074513.

Etymology: the name refers to the host species.

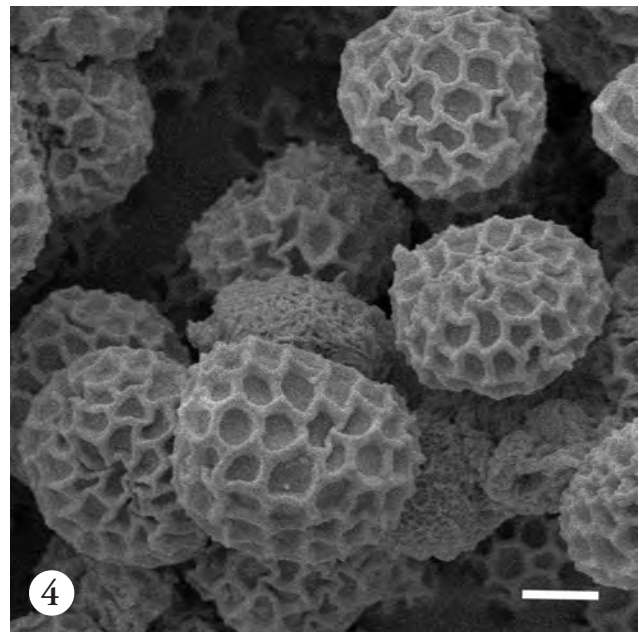
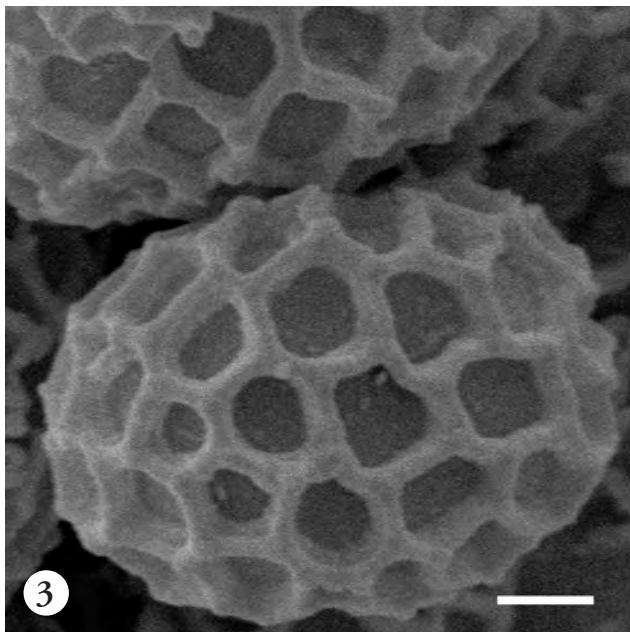
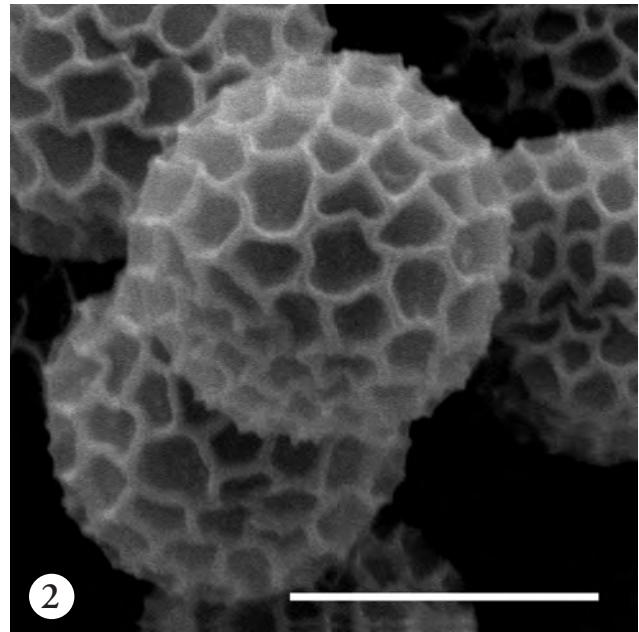
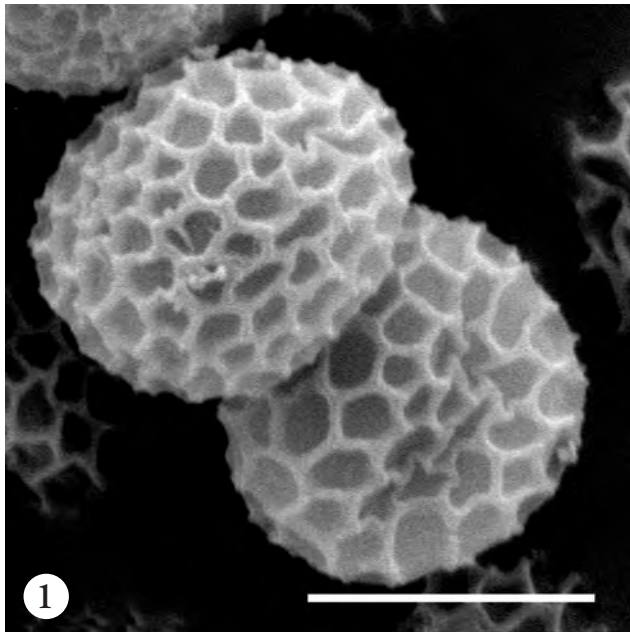
In Le Gac *et al.* (2007a), this species was discussed under the name MvSd.

Morphological species that most closely resembles *Microbotryum silenes-dioicae*: *M. lychnidis-dioicae* (Liro) G. Deml & Oberw.

Below we give a morphological description of an anthericolous smut fungus specimen on *Silene latifolia*, discussed in Le Gac *et al.* (2007a) under the name MvSl, and accepted by us as *Microbotryum lychnidis-dioicae*.

Table 1. Examined *Microbotryum* specimens with their host plants, locations of collection, and GenBank accession numbers for the type specimen

Species of <i>Microbotryum</i>	Host	Location	GenBank accession number for the sequence of the β -tubulin	Specimen number
<i>M. silenes-dioicae</i>	<i>Silene dioica</i>	France, Brittany	DQ074513	# Sdioica_b (Holotype)
<i>M. lychnidis-dioicae</i>	<i>Silene latifolia</i>	France, Paris region, Orsay	DQ074517	# 40.01 (SOMF 27 465)
<i>M. shykoffianum</i>	<i>Dianthus sylvestris</i>	Switzerland, the Alps, Zerne	DQ074496	# 91.11 (Holotype)
<i>M. carthusianorum</i>	<i>Dianthus carthusianorum</i>	France, the Pyrenees, val d'Esquierry	DQ074482	# 309.02 (Holotype)



Figs 1–2. Spores of *Microbotryum silenes-dioicae* Giraud, Denchev & M.E. Hood on *Silene dioica* (type) in SEM. Bars = 5 μm .
Figs 3–4. Spores of *Microbotryum lychnidis-dioicae* (Liro) G. Deml & Oberw. on *Silene latifolia* (SOMF 27 465) in SEM. Bars: 3 = 1 μm , 4 = 2 μm

Microbotryum lychnidis-dioicae (Liro) G. Deml & Oberw.

Figs 3–4

Sori in anthers. Spore mass powdery, brown vinaceous (based on the *Colour identification chart* of Anonymous 1969, and Rayner's colour chart, Rayner 1970). Spores mainly globose, subglobose or ovoid, 5.5–8 \times 5.5–7.5 (6.7 \pm 0.6 \times 6.1 \pm 0.4) μm ($n = 50$), pale coloured; spore wall reticulate, 6–7 (–8) meshes per spore diameter, meshes irregularly polygonal; in SEM interspaces smooth. The β -tubulin type sequence from SOMF 27 465 is deposited in GenBank as DQ074517.

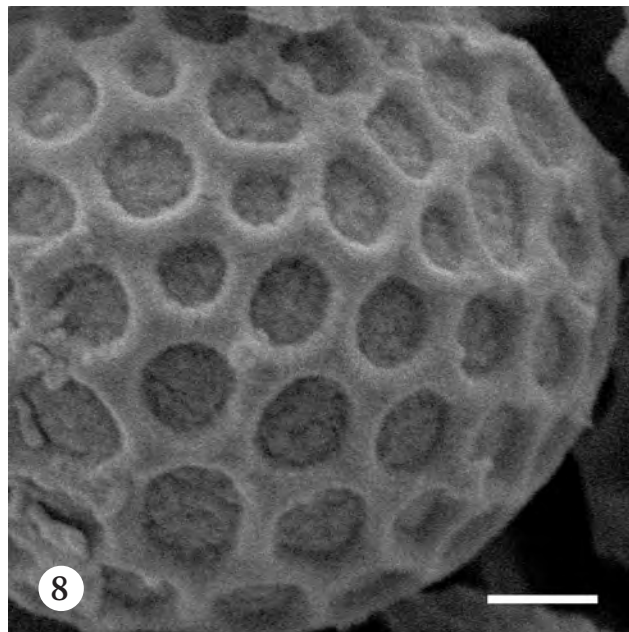
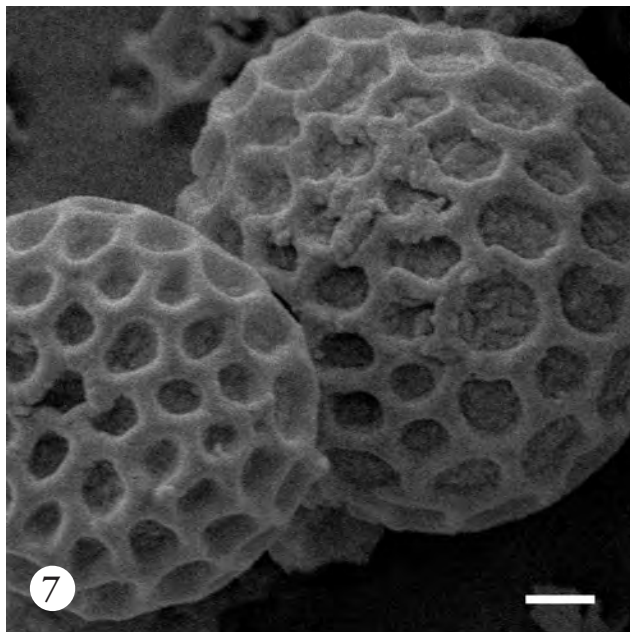
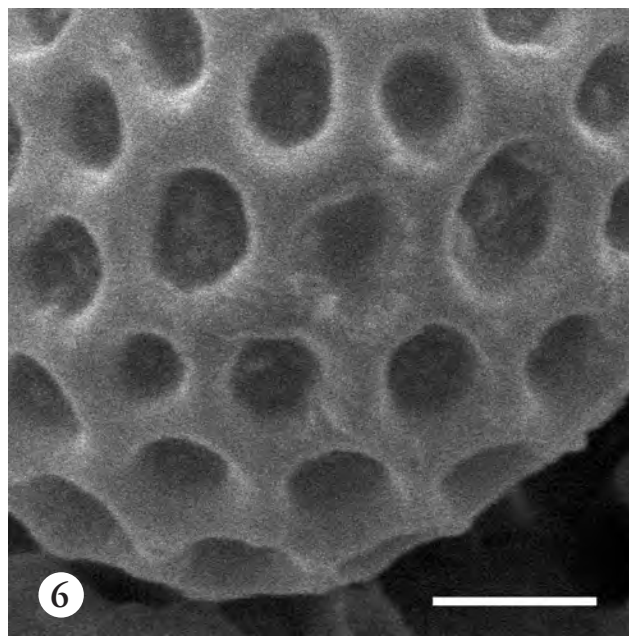
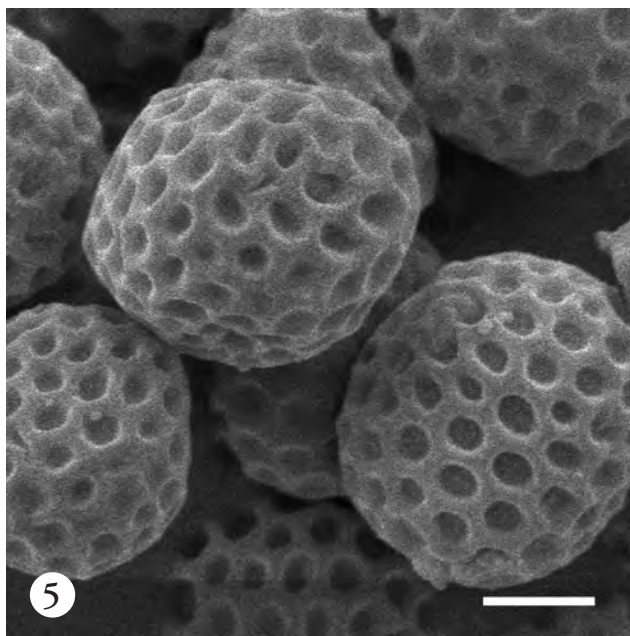
Specimen examined: on *Silene latifolia* Poir. (*Caryophyllaceae*), France, Paris region, Orsay, 2001, T. Giraud, # 40.01 (SOMF 27 465).

Microbotryum shykoffianum Giraud, Denchev & M.E. Hood, sp. nov.

Figs 5–6

Mycobank # MB 515085

Sori antheras *Dianthi sylvestris destruentes*. *Massa sporarum pulverea, brunneo-vinosa*. Spores globosae, subglobosae, late ellipsoideae vel ovoideae, 5.5–8 \times 5.5–6.5 (6.8 \pm 0.6 \times 6.2 \pm 0.4)



Figs 5–6. *Microbotryum shykoffianum* Giraud, Denchev & M.E. Hood on *Dianthus sylvestris* (type) in SEM. Bars: 5 = 2 μm , 6 = 1 μm . **Figs 7–8.** *Microbotryum carthusianorum* Denchev, Giraud & M.E. Hood on *Dianthus carthusianorum* (type) in SEM. Bars: = 1 μm

μm ; paries reticulatus, (6–) 7 (–8) maculis in diametro sporae, maculae rotundatae, interstitiis verruculosi. *Sequentia typi β -tubulini in collectione sequentiarum acidi nucleici NCBI (GenBank) numero DQ074496 deposita est.*

Holotypus in matrice *Dianthus sylvestris* Wulfen (*Caryophyllaceae*), Helvetia, Alpes, Zernezz, 2001, T. Giraud, # 91.11 (SOMF 27 466).

Sori in antheris. **Spore mass** powdery, brown vinaceous. **Spores** globose, subglobose, broadly ellipsoidal or ovoid, 5.5–8 \times 5.5–6.5 (6.8 \pm 0.6 \times 6.2 \pm 0.4) μm ($n = 50$), pale coloured; spore wall reticulate, (6–) 7 (–8) meshes per spore diameter,

meshes rounded with thick muri; in SEM interspaces verruculose. The β -tubulin type sequence from the holotype (SOMF 27 466) is deposited in GenBank as DQ074496.

Etymology: named in honour of the Canadian ecologist, Dr Jacqui Shykoff, who has contributed to the knowledge of population biology and ecology of the anthericolous smut fungi.

This smut fungus can be found also on *Dianthus neglectus* Loisel. and *D. carthusianorum*, and maybe on other *Dianthus* species. In Le Gac *et al.* (2007a), this species was discussed under the name MvDsp1.

Morphological species that most closely resembles *Microbotryum shykoffianum*: *M. dianthorum* (Liro) H. Scholz & I. Scholz.

Microbotryum carthusianorum Denchev, Giraud & M.E. Hood, **sp. nov.** Figs 7–8

Mycobank # MB 515086

Sori anthers *Dianthi carthusianori destruentes*. *Massa sporarum pulverea, brunneo-vinosa*. *Sporae praecipue globosae vel subglobosae, raro ovoideae vel leviter irregulares, 5.5–8 × 5.5–7.5 (6.7±0.4 × 6.5±0.4) µm; paries reticulatus, 7–8 maculis in diametro sporae, maculae rotundatae vel irregulariter polyangulares, interstitiis scabris vel verruculosis. Sequentia typi β-tubulini in collectione sequentiarum acidi nucleici NCBI (GenBank) numero DQ074482 deposita est.*

Holotypus in matrice Dianthus carthusianorum L. (Caryophyllaceae), Gallia, Pyrenaei Montes, val d'Esquierry, 2003, Mickael Le Gac, # 309.02 (SOMF 27 468).

Sori in anthers. **Spore mass** powdery, brown vinaceous (based on the *Colour identification chart* of Anonymous 1969, and Rayner's colour chart, Rayner 1970). **Spores** mainly globose or subglobose, rarely ovoid or slightly irregular, 5.5–8 × 5.5–7.5 (6.7±0.4 × 6.5±0.4) µm (*n* = 60), pale coloured; spore wall reticulate, 7–8 meshes per spore diameter, meshes rounded or irregularly polygonal; in SEM interspaces rough or verruculose. The β-tubulin type sequence from the holotype (SOMF 27 468) is deposited in GenBank as DQ074482.

Etymology: the name refers to the host species.

In Le Gac *et al.* (2007a), this species was discussed under the name MvDc.

Morphological species that most closely resembles *Microbotryum carthusianorum*: *M. dianthorum* (Liro) H. Scholz & I. Scholz.

Liro (1924) described *Ustilago superba* as a species, morphologically identical with *Ustilago dianthorum* Liro, but with a narrow specialization on *Dianthus superbus* — a plant host not attacked by *U. dianthorum*. Later, *U. superba* was reduced to a synonym of *Ustilago violacea* (Pers. : Pers.) Roussel and after its transfer in *Microbotryum*, to *M. violaceum* s. lat. (cfr Vánky 1994: 157). Denchev & Sharkova (1997) confirmed the lack of morphological differences between *Microbotryum dianthorum* and *M. violaceum* s. lat. Based on physiological (Liro op. cit.) and molecular phylogenetic (Le Gac *et al.* 2007a) characteristics, we propose here a transfer of *Ustilago superba* in *Microbotryum*, as a distinct species.

Microbotryum superbum (Liro) Denchev, Giraud & M.E. Hood, **comb. nov.**

Basionym: *Ustilago superba* Liro, Ann. Acad. Sci. Fenn., Ser. A, 17(1): 37, 1924.

Mycobank # MB 515087

Isolotypes: Sydow, Ustilagineen, no. 457 (as *Ustilago violacea*). For its illustration see Denchev & Sharkova (1997: Pl. I, Fig. 2, as *M. violaceum* var. *violaceum*).

In Refrégier *et al.* (2008), this species was discussed under the name MvDsp2 and was found to infect also *D. monspessulanus* and *D. gratianopolitanus*, and maybe other *Dianthus* species.

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