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; isolecotypes 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 ± 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-sporedial 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 Réfrégier (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-dioica on Silene dioica, M. shykoffianum on Dianthus sylvestris, and M. carthusianorum on Dianthus carthusianorum. These new species are morphologically identical with other species 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; Réfrégier 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-dioica, 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 Réfrégier et al. (2008) and was found to infect also D. mospessulanus and D. grattianopolitanus, 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-dioica 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 × 5.5–9 (7.8±0.7 × 7.1±0.6) μm; partes reticulatus, 6–8 (–9) maculis in diametro sporae, maculae irregulariter polygonales, intersititiae laevae. Sequentia typi β-tubulini in collectione sequentiarum acidic nuclei 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, subglobe or broadly ellipsoid, 6.5–10.5 × 5.5–9 (7.8±0.7 × 7.1±0.6) μm (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

<table>
<thead>
<tr>
<th>Species of Microbotryum</th>
<th>Host</th>
<th>Location</th>
<th>GenBank accession number for the sequence of the β-tubulin</th>
<th>Specimen number</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. silenes-dioica</td>
<td>Silene dioica</td>
<td>France, Brittany</td>
<td>DQ074513</td>
<td># Sdioica_b (Holotype)</td>
</tr>
<tr>
<td>M. lychnidis-dioica</td>
<td>Silene latifolia</td>
<td>France, Paris region, Ficheux</td>
<td>DQ074517</td>
<td># 40.01 (SOMF 27 465)</td>
</tr>
<tr>
<td>M. shykoffianum</td>
<td>Dianthus sylvestris</td>
<td>Switzerland, the Alps, Zernez</td>
<td>DQ074496</td>
<td># 91.11 (Holotype)</td>
</tr>
<tr>
<td>M. carthusianorum</td>
<td>Dianthus carthusianorum</td>
<td>France, the Pyrenees, val d’Esquierry</td>
<td>DQ074482</td>
<td># 309.02 (Holotype)</td>
</tr>
</tbody>
</table>
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 × 5.5–7.5 (6.7±0.6 × 6.1±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, Ficheux, 2007, 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. Sporae globosae, subgloboseae, late ellipsoideae vel ovoideae, 5.5–8 × 5.5–6.5 (6.8±0.6 × 6.2±0.4)
µm; paries reticulatus, (6–) 7 (–8) maculis in diametro sporae, maculae rotundatae, interstitiis verruculosis. Sequenta typi β-tubulini in collectione sequentarum acidi nucleici NCBI (GenBank) numero DQ074496 deposita est.

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

Sori in anthers. Spore mass powdery, brown vinaceous. Spores globose, subglobose, broadly ellipsoidal or ovoid, 5.5–8 × 5.5–6.5 (6.8±0.6 × 6.2±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* (*Liro*) H. Scholz & I. Scholz.

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

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

*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. (cf 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.


MycoBank # MB 515087

Isolectotypes: 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. monspesulanus* and *D. gretianopolitanus*, and maybe other *Dianthus* species.

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**References**


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Microbotryum violaceum from the host plants Silene latifolia and S. dioica.