Anther smuts of Caryophyllaceae. Taxonomy, nomenclature, problems in species delimitation *

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Abstract. After a short historical review, taxonomy and nomenclature of the genus *Microbotryum* in general, and those of the anther smuts of Caryophyllaceae in special, are presented. Problems in species delimitation of these smut fungi are discussed, which is still not solved satisfactorily. Until a better classification of the anther smuts of Caryophyllaceae will be elaborated, the use of the name of *M. violaceum* s. lat. is proposed for *M. dianthorum, M. lychnidis-dioicae, M. silenes-inflatae, Ustilago coronariae, U. silenes-nutantis,* and *U. superba.*

Key words: anther smuts, Caryophyllaceae, *Microbotryum violaceum,* smut fungi, species delimitation, taxonomy

Motto: Recognise what you are working with

The anther smuts of Caryophyllaceae are among the oldest described smut fungi. In 1797, the fungus in the anthers of *Silene nutans* was named by Persoon *Uredo violacea.* Since that, its name and place in the classificatory system was changed several times. It was called *Ustilago violacea* (Pers. : Pers.) Roussel (1806: 47), *Caeoma violaceum* (Pers. : Pers.) Nees (1817: 14), and *Microbotryum violaceum* (Pers. : Pers.) G. Deml & Oberw. (Deml & Oberwinkler 1982: 353). Until a few years ago, it was placed in the Ustilaginaceae family of the order Ustilaginales. Using chemical, ultrastructural, and especially molecular biological methods, it was shown that the species of *Microbotryum* are more closely related to the rust fungi than to the true smut fungi, despite of their identical spore morphology and biology. It would take too long to show all the steps on the way towards the placement of *Microbotryum* into the class Urediniomycetes Swann & J.W. Taylor, subclass Microbotryomycetidae Swann, order Microbotryales R. Bauer & Oberw., family Microbotryaceae R.T. Moore. An important role in this process played mycologists in Tübingen, Germany, during the last two decades. A detailed description of history, taxonomic and nomenclatural problems of *Microbotryum* Lév. (Léveillé 1847: 372), emend. Vánky (1998: 39) was published by me (Vánky 1998) in a monograph of the genus. It would also take too long to show how the number of species of *Microbotryum* increased to 76 at present, on 7 host plant families. Of the 76 species, 14 occur on Caryophyllaceae, of which 8 are in the anthers of Caryophyllaceae. However, it is interesting to notice that this increase of species was fertilised by the ingenious vision of Savile (1953: 667) and Nannfeldt (in Lindeberg 1959: 150) who considered the purple-spored *Ustilago* species as forming a natural group. *Microbotryum* also belongs to this group. Many years ago, I formulated a working hypothesis, that species of *Ustilago* are restricted to host plants in the Poaceae. This hypothesis proved to be fruitful. The revision of "*Ustilago*" species on host plants other than Poaceae lead, on the one hand, to the description of several new genera [*Bauerago* Vánky (1999) for *Ustilago* species on Cyperaceae and Juncaceae, *Tothiella* Vánky (1999) on Cruciferae, or *Vankya* Ershad (2000) on Liliaceae], on the other hand, it led to the transfer of further 55 *Ustilago* species into the genus *Microbotryum* (Vánky 1998), in addition to the 17 species, placed before in this genus with help of chemical, ultrastructural or molecular biological methods. In this process, I selected all 66 known "*Ustilago*" species on dicotyledonous host plants. Of these, 55 species, possessing spores with a

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violet tint besides the brown colour, were transferred into the genus *Microbotryum.* A big difficulty was that in many species, the violet tint was so weak that it was difficult to see its presence, which was decisive. To overcome this difficulty, I invented the so called "colour contrast method". Spores of the suspected *Microbotryum* species and those of *Ustilago hordei* (Pers. : Pers.) Lagerh., which are typically brown and smooth, were mixed in a droplet of lactophenol and studied under a light microscope at high (1000×) magnification. A comparison of the colours of the two kinds of spores side by side easily revealed even a pale violet tint.

Returning to the *Microbotryum* species of Caryophyllaceae, of which the great majority has teliospores possessing a reticulate spore wall, we can state that the applied criteria for species delimitation and naming these fungi changed considerably during the times, and this problem is still not solved satisfactorily. According to one extreme, represented by the Finnish mycologist Liro (1924), all physiological forms, infecting a certain host plant species or a group of species, but not others, were considered a good species. Contrary to this extreme, only morphologically well characterised species are recognised. Recently, this species concept is generally accepted. This, maybe until pure molecular taxonomy will take over the morphological one or perhaps a combination of these two methods will succeed. Species delimitation and naming is not only a theoretically important thing. In all fields of biology, including mycology, it is very important to know exactly which organism, which species we are working with, choosing as objects for our gen experiments or for showing sexual polarity or other experiments.

Regarding the identification of anther smuts of Caryophyllaceae, one species, *Microbotryum majus* (J. Schröt.) G. Deml & Oberw., on different *Silene* species of the sect. *Otites,* is easy to recognise and differentiate from all other anthericolous smut fungi of Caryophyllaceae because the sori may comprise besides the anthers also the filaments, ovaries, and the basal part of the petals. Using powerful, modern light microscopes (LM), and especially Scanning Electron Microscopes (SEM), earlier unknown morphological differences of teliospores became evident. Brandenburger & Schwinn (1974) showed that not all anther smuts of Caryophyllaceae possess reticulate spores. *Ustilago violaceo-verrucosa* and *U. violaceo-irregularis* have verrucose or verrucose-reticulate spores, respectively. Denchev (1993, 1994, 1995a, b, 1997, 2003) and Denchev *et al.* (1997) checking hundreds of specimens, showed, that these two species are actually not rare and are present in numerous countries of the World. Morphological differences were also demonstrated for reticulate-spored *Microbotryum* species, but only to a certain level. E.g., the spores of *M. stellariae* (Liro) G. Deml & Oberw., occurring on members of the Alsinoideae subfamily (*Arenaria, Cerastium, Minuartia, Myosoton, Stellaria*), are usually small, measuring 5-8 µm in diameter. Further four species, occurring in the anthers of host plants in the Silenoideae subfamily, have spores of 6-11 µm in diameter and are difficult to differentiate by spore morphology. These four species are *M. dianthorum* (Liro) H. Scholz & I. Scholz, *M. lychnidis-dioicae* (DC. ex Liro) G. Deml & Oberw., *M. silenes-inflatae* (DC. ex Liro) G. Deml & Oberw., and *M. violaceum* (Pers. : Pers.) G. Deml & Oberw. s. str. A key constructed for these four species, based on the descriptions by Scholz & Scholz (1988), reveals how small (and unfortunately also variable) the differences between them are.

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1 Meshes rounded to polygonal **2**

2 Meshes rounded, 0.5-0.8 µm in diam. Spore mass dark violet. On *Dianthus* . . . . . ***M. dianthorum***

2* Meshes rounded to regularly polygonal, up to 1 µm in diam. Spore mass brownish violet. On *Cucubalus, Dianthus, Gypsophila, Lychnis, Petrorhagia, Saponaria, Silene* . . . . . ***M. violaceum***

1* Meshes irregularly polygonal **3**

3 Meshes up to 1.4 µm in diam. Spore mass dark brownish violet. On *Silene* . . . ***M. lychnidis-dioicae***

3* Meshes up to 1.2 µm in diam. Spore mass pale greyish violet. On *Silene, Lychnis* . . . . . ***M. silenes-inflatae***

In addition to these four species, there are several names of anther smuts, proposed by Liro (1924), based on host specificity. These cannot be differentiated morphologically from each other and from the above mentioned 4 species. These are: *Ustilago coronariae* Liro, on *Lychnis flos-cuculi, U. silenes-nutantis* DC. ex Liro, on *Silene nutans* L, or *U. superba* Liro, on *Dianthus superbus* L., all on members of the Silenoideae subfamily. Therefore, the tendency among classical mycologists is only natural to consider all these 7 "species" as one species, *M. violaceum* s. lat.

As we can see, there are big difficulties in species delimitation within this group of anther smuts. The problem is complicated by the fact that these smuts are not strictly host specific. The same fungus may infect several host plant species within the same genus or even of different Caryophyllaceae genera. At the same time, the same host plant species or genus can be infected by several anther smuts. e.g., on *Silene* there are four anther smuts, as we can see above.

From this impasse, maybe molecular biology will help. On the 7th IMC in Oslo, Begerow & Bauer (2002) presented the results of molecular biological investigations of numerous *Microbotryum* species and also of their host plants under the title "Evolution in smuts". They found that there is a correlation between the parasites and their hosts, confirming Liro's results based on field observations and numerous infection experiments. The speakers concluded that there has been a co-evolution between the parasites and their host plants. The big problem with the presented work is that most of the studied smut fungi have no names only sp. numbers, and do not help in solving the problem of species delimitation of this group. If their experiments

could have been performed with the type specimens, in addition to showing the nice correlation between host plants and parasites, they could have contributed also to solving, at least partly, the problem of species delimitation of anther smuts of Caryophyllaceae. Therefore, I would like to recommend for molecular biologists that similar studies should be done with as many as possible type specimens to see which are identical and which are different in their molecular characters. The obtained results, correlated to spore morphology, could maybe solve the difficult problem of species delimitation in this group.

In conclusion, we can say that it is still valid what Liro (1924: 280) wrote 80 years ago, namely, that "We still cannot identify most of the anther smuts of Caryophyllaceae with certainty and, consequently, cannot name them with certainty". Therefore, until a better classification of the anther smuts of Caryophyllaceae will be elaborated, I would like to propose the use of the name of ***Microbotryum violaceum*** (Pers. : Pers.) G. Deml & Oberw. **s. lat.** for the names of *M. dianthorum, M. lychnidis-dioicae, M. silenes-inflatae, Ustilago coronariae, U. silenes-nutantis,* and *U. superba.*

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