Anther smuts of Caryophyllaceae: molecular characters indicate host-dependent species delimitation+

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Phylogenetic relationships of *Microbotryum* species (Urediniomycetes, Basidiomycota) inhabiting anthers of Caryophyllaceae were investigated by molecular analyses using internal transcribed spacer (ITS) sequences and collections from different host plants. The data show that the current taxonomy of *Microbotryum* on Caryophyllaceae is only partly satisfactory. *Microbotryum violaceum* is confirmed to be a paraphyletic grouping and is split up in monophyletic groups. *Microbotryum silenes-inflatae* and *M. violaceo-verrucosum* appear as polyphyletic. Host data are in good agreement with molecular results. Two new species, *Microbotryum chloranthae-verrucosum* and *M. saponariae*, are described based on morphological, ecological, and molecular characteristics. An emended circumscription of *Microbotryum dianthorum* is given. The name *Ustilago major* (= *Microbotryum major*) is lectotypified.

Taxonomic novelties: *Microbotryum chloranthae-verrucosum* M. Lutz, Göker, M. Piatek, Kemler, Begerow et Oberw.; *Microbotryum saponariae* M. Lutz, Göker, M. Piatek, Kemler, Begerow et Oberw.

Keywords: anther parasites, ITS, *Microbotryum*, molecular analysis, smut fungi, Urediniomycetes

In the current classification of *Microbotryum* (VÁNKY 1998), 15 species on Caryophyllaceae are accepted, eight of which occur in the host's anthers, more rarely also in other floral parts. These anther parasites exhibit a couple of outstanding features. Infected anthers become completely filled with masses of brownish-violet teliospores in mature disease stages (VÁNKY 1998). Amazingly, *Microbotryum* infection induces the production of anthers even in female individuals of dioecious host species, although the fungus probably is not the only cause for sex change in *Silene dioica* (HASSAN & MACDONALD 1971). As JENNERSTEN (1983) demonstrated, butterflies as regular pollinators of caryophyllaceous host plants may effectively carry out lateral transfer of the parasite's teliospores between host flowers. Moreover, *Microbotryum* may induce earlier flowering in infected plants, thus increasing the chance that teliospores are dispersed by pollinators (JENNERSTEN 1988). Hence, this interesting host-parasite system received attention of scientists interested in plant galls (cf. BUHR 1964, p. 737) or in the influence of host ecological factors (e.g., THRALL, BIERE & ANTONOVICS 1993) and numerous publications dealt with population studies (e.g., LEE 1981; MILLER ALEXANDER & ANTONOVICS 1995; MILLER ALEXANDER et al. 1996), including investigations in recent host shifts (ANTONOVICS, HOOD & PARTAIN 2002).

While there is consensus about the monophyly of the caryophyllacean anther smuts (e.g., DEML & OBERWINKLER 1982; ALMARAZ et al. 2002; KEMLER et al., in prep.) species delimitation within these parasites has long been discussed since, at least, LIRO (1924), who split *Ustilago violacea* (i.e., *Microbotryum violaceum*) into a couple of species, based on infection experiments and field observations. These were only in part accepted by later workers who failed to find morphological distinctions between most of LIRO'S species (NANNFELDT in LINDEBERG 1959, p. 142, p. 159; DURRIEU & ZAMBETTAKIS 1973; VÁNKY 1994). Some authors (e.g., PERLIN 1996; PERLIN et al. 1997; BUCHELI, GAUTSCHI & SHYKOFF 2000; FREEMAN et al. 2002) regard some or all of the anther infecting species as formae speciales of a single species, *Microbotryum violaceum* s.str. or s.l., respectively. On the other hand, BUCHELI, GAUTSCHI & SHYKOFF (2000) found *Microbotryum* specimens from different host plants to be genetically isolated. Thus, classification of *Microbotryum* species on Caryophyllaceae has been discussed controversially and molecular approaches should be applied in search for a natural arrangement of taxa (compare VÁNKY 2004).

The internal transcribed spacer (ITS) regions of the ribosomal RNA-coding nuclear DNA (rDNA) have been used

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successfully in numerous phylogenetic studies of fungi. With respect to smuts, ITS was mainly used below the genus level, i.e. for *Entyloma* (BEGEROW, LUTZ & OBERWINKLER 2002), *Tilletia* (LEVY et al. 2001), and *Ustilago/Sporisorium* (STOLL et al. 2003). SCORZETTI et al. (2002) found that ITS and large subunit (LSU) rDNA sequences are sufficient for species identification in basidiomycetous yeasts. ALMARAZ et al. (2002) used ITS sequences to infer phylogenetic trees of Microbotryaceae. FREEMAN et al. (2002) demonstrated that *Microbotryum* phylogenies derived from ITS were congruent with trees inferred from β- and γ-tubulin data, although the partition homogeneity test (FARRIS et al. 1995) conducted by these authors indicated significant conflict between the partitions. However, they discussed the possibility that the partition homogeneity test could be too conservative (for a general critique of the partition homogeneity test see, e.g., BARKER & LUTZONI 2002 and references therein). At least, FREEMAN et al. (2002) did not observe incongruent clades well supported by bootstrap analysis. These authors also showed that North American "isolates of *Microbotryum violaceum*" were genetically distinct from samples from European host plants.

The present study aims at clarifying taxonomical problems in anther-infecting *Microbotryum* species on Caryophyllaceae by ITS sequence analysis based on a larger sample of host species and, as far as possible, several specimens from the same host species. In addition, we intend to provide a framework for species delimitation in *Microbotryum* that is in agreement with the principles of phylogenetic systematics (HENNIG 1965) and useful for field studies. In our view, this implies that at least those obviously genetically isolated lineages that can be distinguished by other than molecular characters should be treated as different species.

Material and methods

Sample sources, and nomenclature

The *Microbotryum* specimens examined in this study are listed in Tab. 1. The nomenclature follows VÁNKY (1994; 1998). Assignment of *Microbotryum* specimens to species was based on location of sori, spore surface ornamentation, spore mass colour and host data as described by VÁNKY (1994). If specimens could not unequivocally be ascribed, the name "*Microbotryum violaceum* s.l." was used as in VÁNKY (1994).

Morphological examination

Teliospores of caryophyllacean anther smuts were mounted in Hoyer's Fluid (CUNNINGHAM 1972) and heated at 50 ºC for 10 min. A PZO BIOLAR stereomicroscope was used for bright field microscopy and spore measurements. For each specimen at least 25 spores were measured. Spore surface patterns were examined by light microscopy and could be assigned to the three character states verrucose, reticulate, and incompletely verrucose-reticulate, respectively. We did not attempt to describe size and form of the meshes on reticulate spores as these characters may be quite problematic (VÁNKY 2004).

Colour of spore masses was assessed by careful cross-examinations of infected anthers under a binocular lens. Three types of spore colours could be distinguished, using the *Microbotryum violaceo-irregulare* (TUB 011816) and *M. violaceo-verrucosum* (TUB 011815) specimens as standards for very dark-coloured and very light-coloured spore masses, respectively.

The spore surface of *Microbotryum major* on *Silene otites* (specimen: WRSL s.n.), *M. violaceo-verrucosum* on *Silene chlorantha* (specimen: B 700007571) and *M. violaceum* on *Saponaria officinalis* (specimen: M 0098773) was studied by scanning electron microscopy (SEM). In each case dry spores were mounted on clean glass and fixed to an aluminium stub with double-sided transparent tape. The stubs were sputter-coated with carbon using a Cressington sputter coater and viewed with a Hitachi S-4700 scanning electron microscope, with a working distance of ca. 12–13 mm.

DNA extraction, PCR, and sequencing

We isolated genomic DNA from 53 herbarium specimens and two cultures of the genus *Microbotryum* (Tab. 1). For methods of isolation and crushing of fungal material, DNA extraction, amplification, purification of PCR products, sequencing, and processing of the raw data see LUTZ et al. (2004). We amplified the ITS 1 and ITS 2 regions of the rDNA including the 5.8S rDNA (ITS, about 650 bp) using the primer pair ITS1 and ITS4 (WHITE et al. 1990) for PCR and cycle sequencing. For amplification of the ITS region we adjusted the annealing temperature to 45 °C. DNA sequences prepared in the course of this study were deposited in GenBank; accession numbers are given in Tab. 1.

Phylogenetic analyses

To elucidate the phylogenetic relations of the sequenced *Microbotryum* specimens, we analysed them together with the following *Microbotryum* sequences from GenBank: AF038830, AF038832, AF038833, AF038834, AF045872, AF045873, AF045874, AF045876, AF045877, AF045878, AF045879, AF045880, AF045881, AF444593, AY014213, AY014214, AY014215, AY014216, AY014217, AY014218, AY014219, AY014220, AY014221, AY014222, AY014223, AY014224, AY014225, AY014226, AY014227, AY014228, AY014229, AY014230, AY014231, AY014232, AY014235, AY014236, AY014238, AY014239, AY188368.

To align sequences we used MAFFT 3.85 (KATOH et al. 2002) using the FFT-NS-i option. The alignment produced by MAFFT (length: 724 bp, 313 variable sites) was used throughout its length.

To estimate phylogenetic relationships, we applied a Bayesian approach of phylogenetic inference using a Markov chain Monte Carlo (MCMC) technique as implemented in the computer program MrBayes 3.0B4 (HUELSENBECK & RON-

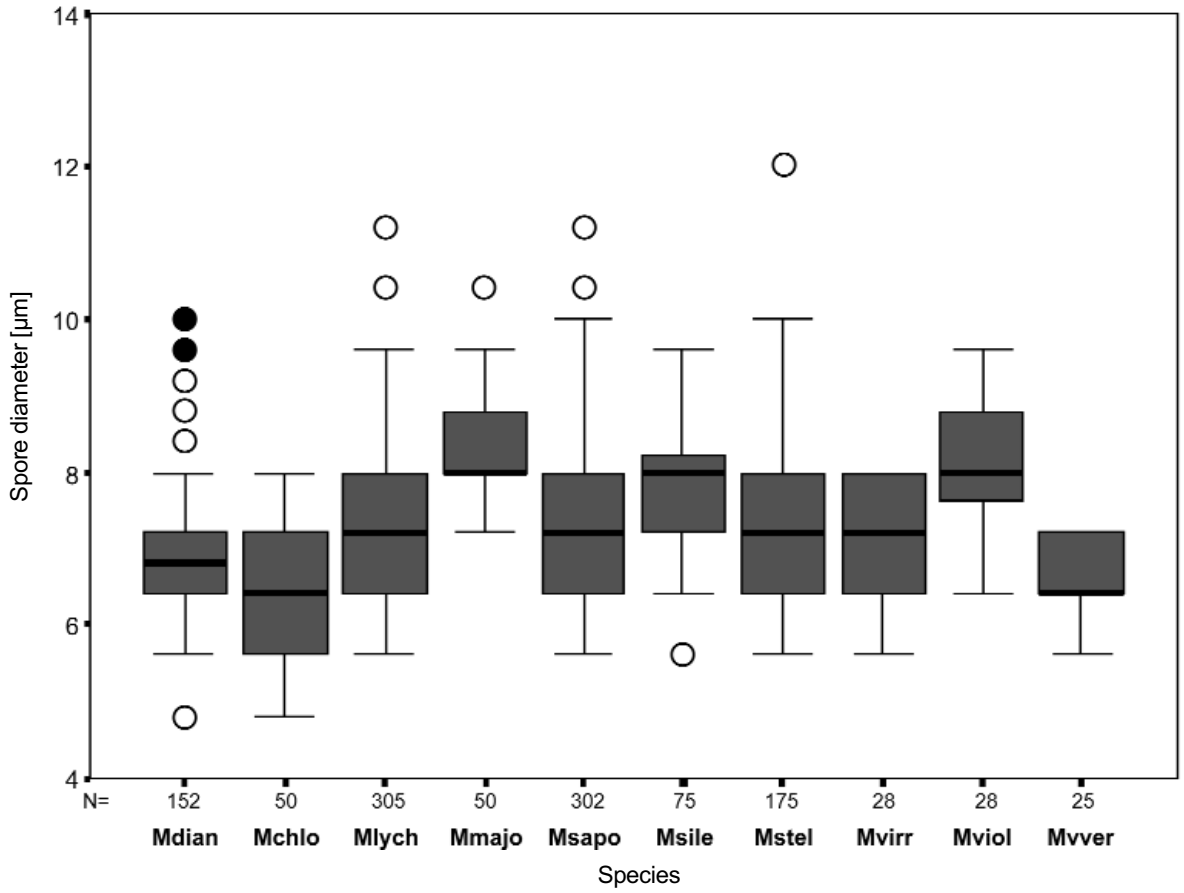


Fig. 1: Box plots of spore measurements. Specimens were grouped according to the suggested species boundaries. At least 25 spores per specimen were measured. Hollow circles represent outliers, closed circles represent extreme values, bold vertical lines represent the median values. Species abbreviations: Mchlo = *M. chloranthae-verrucosum*, Mdian = *M. dianthorum,* Mlych = *M. lychnidis-dioicae*, Mmajo = *M. major*, Msapo = *M. saponariae*, Msile = *M. silenes-inflatae*, Mstel = *M. stellariae*, Mviol = *M. violaceum* s.str., Mvirr = *M. violaceo-irregulare*, Mvver = *M. violaceo-verrucosum.*

QUIST 2001). For Bayesian analysis, the alignment was first analysed with MrModeltest 1.0b (POSADA & CRANDALL 1998) to find the most appropriate model of DNA substitution. The hierarchical likelihood ratio test proposed the DNA substitution model GTR+G (see SWOFFORD et al. 1996 for a survey of DNA substitution models). Thus, four incrementally heated simultaneous Markov chains were run over 2 000 000 generations using random starting trees and default starting parameters of the respective DNA substitution model (HUELSENBECK & RONQUIST 2001). Trees were sampled every 100th generation resulting in an overall sampling of 20 001. From these, the first 1 001 trees were discarded (burn-in = 1 001). The trees sampled after the process had reached stationarity (19 000 trees) were used to compute a 50 % majority rule consensus tree to obtain estimates for the a posteriori probabilities of groups of species. This Bayesian approach of phylogenetic analysis was repeated five times to test the independence of the results from topological priors (HUELSENBECK et al. 2002). Based on the classification in VÁNKY (1994; 1998) and the results of ALMARAZ et al. (2002), the trees were rooted with *Sphacelotheca polygoni-persicariae*.

Pair-wise relative base-pair differences were calculated from the MAFFT alignment with PAUP* version 4.0b10 using the PAIRDIFF command (SWOFFORD 2001). Note that gaps are not taken into account by this computation and the results are therefore not always fully compatible to branch lengths in a tree (Fig. 2), even if pair-wise differences are zero.

Results

Morphology

Mean and standard deviation of at least 25 spore diameter measurements of Caryophyllaceae-infecting *Microbotryum* specimens are included in Tab. 1. Fig. 1 shows box plots of spore diameter measurements of specimens included in the molecular analyses; species corresponding to the taxa proposed in Tab. 1 (see also Fig. 2). As examined by ANOVA and post-hoc-tests as implemented in SPSS 10.0.7 (which we do not show, as the main results may easily be inferred from Fig. 1), mean spore diameter showed statistically significant deviations in some cases after samples were aggregated to putative species. For instance, spore diameter in *Microbotryum major* was indeed

significantly larger than in most other specimens examined. However, even in that case we found no significant differences between the two *M. major* collections, the *M. violaceum* s.str. collection on its type host, *Silene nutans*, and the *M. silenes-inflatae* collections (Fig. 1). In the lectotype specimen of *Ustilago major* (= *Microbotryum major*), which is selected in this paper (see below), the dimensions of spores (mean 10.000 ± 0.870 µm) were larger than in the specimens used in the phylogenetic analyses (mean 8.256 ± 0.552 µm, 8.128 ± 0.822 µm, respectively), but matched well with variation of spores in different collections of *Microbotryum major* (VÁNKY 1994). On the other hand, the spores of specimens of *M. stellariae* examined in the course of this study, a species usually characterised by small spores, did not appear to be especially small-sized.

Spore surface ornamentation could only be used to separate *Microbotryum violaceo-irregulare* (incompletely verrucose-reticulate) and *M. violaceo-verrucosum* (on *Silene chlorantha* and *S. viscosa*; verrucose) from each other and all remaining *Microbotryum* species that showed reticulate spores.

Spore-mass colour is depicted in Fig. 2. It was mostly in accordance with literature (e.g., VÁNKY 1994, p. 154; VÁNKY 1998, p. 53f.).

Both the spores of *Microbotryum major* (Figs. 3a, b) and *M. violaceum* on *Saponaria officinalis* (Figs. 3c-e) appeared in the scanning electron microscope to be reticulate with more or less irregular meshes. The spores were often collapsed on the side view. The spores of *Microbotryum violaceo-verrucosum* on *Silene chlorantha* (Figs. 3f-h) were sparsely to densely verrucose with extremly finely verruculose spaces between the warts as is shown in Fig. 3h.

Phylogenetic analyses

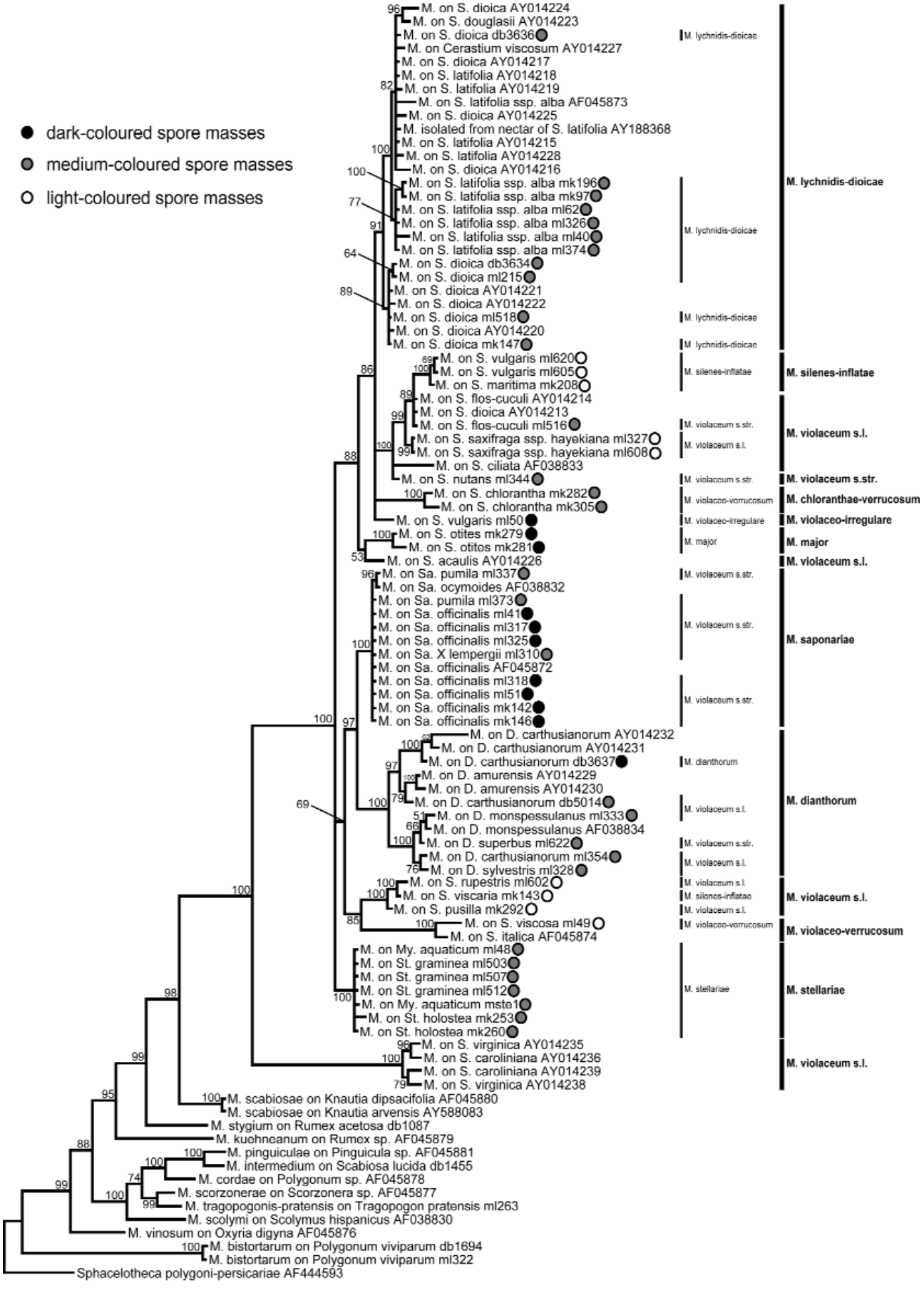
The different runs of Bayesian phylogenetic analyses that were performed yielded consistent topologies. We present the consensus tree of one run to illustrate the results (Fig. 2). All analysed *Microbotryum* specimens inhabiting anthers of Caryophyllaceae clustered together forming a monophyletic clade highly supported by an a posteriori probability of 100 %. In contrast, the *Microbotryum* species on Polygonaceae turned out to be a paraphyletic assemblage with *M. bistortarum* separating basally. The parasites of Dipsacaceae *(Microbotryum intermedium, M. scabiosae)* and Asteraceae *(M. scolymi, M. scorzonerae, M. tragopogonis-pratensis)* were revealed as polyphyletic, the first one of these clades with strong support.

The caryophyllacean anther smuts were subdivided into a cluster of specimens found exclusively on native North-American *Silene* species, i.e., *S. caroliniana* and *S. virginica* (1.5 ± 0.5 % base differences) and a large group containing all samples of European origin, four specimens collected in North America on naturalised hosts (*Microbotryum* on *Cerastium viscosum* and on *S. latifolia* AY014219/AY014215/AY188368), and two specimens from hosts native to North America (*Microbotryum* on *Silene acaulis* and on *S. douglasii*). Both clades were supported with an a posteriori probability of 100 %.

Within the latter group the *Silene*-inhabiting *Microbotryum* species were revealed as a paraphyletic grouping. In contrast, the anther smuts of the host genera *Stellaria*/*Myosoton*, *Saponaria*, and *Dianthus* clustered in three distinct groups, respectively, each of them supported by an a posteriori probability of 100 %. While the *Dianthus* parasites were relatively diverse (2.5 ± 1.2% base differences) and separated into several more or less strongly supported subgroups, the clusters of the parasites of *Stellaria/Myosoton* and *Saponaria*, respectively, showed low sequence diversity or none at all (0.3 ± 0.5 % or 0 % pair-wise nucleotide differences within, respectively). Thus, the relation within the *Stellaria/Myosoton* and *Saponaria* parasites, respectively, was not resolved. With an a posteriori probability of 97 % the specimens from *Dianthus* were revealed as sister group to the cluster of *Saponaria* parasites.

The *Silene*-inhabiting *Microbotryum* species formed two distinct groups. The first group contained several specimens from different *Silene* species and appeared as sister group of the specimens from *Dianthus* and *Saponaria*. This clade of *Silene* parasites was cut into two subgroups separated by a considerable genetic distance of which one could be assigned to *Microbotryum violaceo-verrucosum* (note that AF045874 was submitted to GenBank as *M. violaceo-verrucosum*; base-pair differences between these two sequences were 2.1 %). The other group contained specimens of *Microbotryum silenes-inflatae* and *M. violaceum* s.l. However, other specimens traditionally assigned to *Microbotryum silenes-inflatae* and *M. violaceo-verrucosum* clustered in the second cluster of *Silene* parasites. Thus, *Microbotryum silenes-inflatae* and *M. violaceo-verrucosum* were revealed as polyphyletic. As the

Fig. 2: Bayesian inference of phylogenetic relationships of the sampled *Microbotryum* specimens: Markov chain Monte Carlo analysis of an alignment of base sequences from the ITS1/2 region of the nuc-rDNA including the 5.8S rDNA using the GTR+G model of DNA substitution with gamma distributed substitution rates, random starting trees and default starting parameters of the DNA substitution model. A Majority-rule consensus tree computed from 19 000 trees that were sampled after the process had reached stationarity is shown. The topology was rooted with *Sphacelotheca polygoni-persicariae*. Numbers on branches are estimates for a posteriori probabilities. Branch lengths were averaged over the sampled trees. They are scaled in terms of expected numbers of nucleotide substitutions per site. Colour of spore masses, assignment to species after VÁNKY (1994) (left column, note that the taxonomical concept turned out to be artificial), and our suggestions for species boundaries (right column) are indicated. D. = *Dianthus*, M. = *Microbotryum*, My. = *Myosoton*, S. = *Silene*, Sa. = *Saponaria*, St. = *Stellaria*.


dark-coloured spore masses
medium-coloured spore masses
light-coloured spore masses
M. on S. dioica AY014224
M. on S. douglasii AY014223
M. on S. dioica db3636
M. on Cerastium viscosum AY014227
M. on S. dioica AY014217
M. on S. latifolia AY014218
M. on S. latifolia AY014219
M. on S. latifolia ssp. alba AF045873
M. on S. dioica AY014225
M. isolated from nectar of S. latifolia AY188368
M. on S. latifolia AY014215
M. on S. latifolia AY014228
M. on S. dioica AY014216
M. on S. latifolia ssp. alba mk196
M. on S. latifolia ssp. alba mk97
M. on S. latifolia ssp. alba ml62
M. on S. latifolia ssp. alba ml326
M. on S. latifolia ssp. alba ml40
M. on S. latifolia ssp. alba ml374
M. on S. dioica db3634
M. on S. dioica ml215
M. on S. dioica AY014221
M. on S. dioica AY014222
M. on S. dioica ml518
M. on S. dioica AY014220
M. on S. dioica mk147
M. on S. vulgaris ml620
M. on S. vulgaris ml605
M. on S. maritima mk208
M. on S. flos-cuculi AY014214
M. on S. dioica AY014213
M. on S. flos-cuculi ml516
M. on S. saxifraga ssp. hayekiana ml327
M. on S. saxifraga ssp. hayekiana ml608
M. on S. ciliata AF038833
M. on S. nutans ml344
M. on S. chlorantha mk282
M. on S. chlorantha mk305
M. on S. vulgaris ml50
M. on S. otites mk279
M. on S. otites mk281
M. on S. acaulis AY014226
M. on Sa. pumila ml337
M. on Sa. ocymoides AF038832
M. on Sa. pumila ml373
M. on Sa. officinalis ml41
M. on Sa. officinalis ml317
M. on Sa. officinalis ml325
M. on Sa. X lempergii ml310
M. on Sa. officinalis AF045872
M. on Sa. officinalis ml318
M. on Sa. officinalis ml51
M. on Sa. officinalis mk142
M. on Sa. officinalis mk146
M. on D. carthusianorum AY014232
M. on D. carthusianorum AY014231
M. on D. carthusianorum db3637
M. on D. amurensis AY014229
M. on D. amurensis AY014230
M. on D. carthusianorum db5014
M. on D. monspessulanus ml333
M. on D. monspessulanus AF038834
M. on D. superbus ml622
M. on D. carthusianorum ml354
M. on D. sylvestris ml328
M. on S. rupestris ml602
M. on S. viscaria mk143
M. on S. pusilla mk292
M. on S. viscosa ml49
M. on S. italica AF045874
M. on My. aquaticum ml48
M. on St. graminea ml503
M. on St. graminea ml507
M. on St. graminea ml512
M. on My. aquaticum mste1
M. on St. holostea mk253
M. on St. holostea mk260
M. on S. virginica AY014235
M. on S. caroliniana AY014236
M. on S. caroliniana AY014239
M. on S. virginica AY014238
M. scabiosae on Knautia dipsacifolia AF045880
M. scabiosae on Knautia arvensis AY588083
M. stygium on Rumex acetosa db1087
M. kuehneanum on Rumex sp. AF045879
M. pinguiculae on Pinguicula sp. AF045881
M. intermedium on Scabiosa lucida db1455
M. cordae on Polygonum sp. AF045878
M. scorzonerae on Scorzonera sp. AF045877
M. tragopogonis-pratensis on Tragopogon pratensis ml263
M. scolymi on Scolymus hispanicus AF038830
M. vinosum on Oxyria digyna AF045876
M. bistortarum on Polygonum viviparum db1694
M. bistortarum on Polygonum viviparum ml322
Sphacelotheca polygoni-persicariae AF444593
M. lychnidis-dioicae
M. silenes-inflatae
M. violaceum s.str.
M. violaceum s.l.
M. violacco-verrucosum
M. violaceo-irregulare
M. major
M. dianthorum
M. stellariae
M. lychnidis-dioicae
M. silenes-inflatae
M. violaceum s.l.
M. chloranthae-verrucosum
M. violaceo-irregulare
M. major
M. saponariae
M. dianthorum
M. stellariae
0.05 substitutions/site

other side of the same coin, *Microbotryum violaceum* inhabiting *Silene* species appeared as paraphyletic. Within the second group of *Silene*-inhabiting *Microbotryum* species the two *Microbotryum major* specimens (0.7 % base difference) and a specimen from *Silene acaulis*, which grouped together in a weakly supported sister relationship, separated basally. Furthermore, all specimens which were identified as *Microbotryum lychnidis-dioicae* formed a monophylum containing parasites of *Cerastium viscosum, Silene dioica, S. douglasii,* and *S. latifolia*. The clade was well supported by an a posteriori probability of 91 %; the pair-wise base differences within were 0.9 ± 0.6 %. The clade was subdivided into two main subgroups. One of them exclusively contained specimens from *Silene dioica*, the other clade consisted of specimens mostly from *S. latifolia* (incl. *S. latifolia* ssp. *alba*). Specimens from *Cerastium viscosum, Silene dioica* and *S. douglasii* were nested within the latter subgroup.

The sampled *Microbotryum violaceo-irregulare* specimen showed no apparent connection to other specimens sampled just as did the specimen of *M. violaceum* s.str. on the type host, *Silene nutans*, and the specimen from *S. ciliata.* In contrast, the specimens of *Microbotryum silenes-inflatae* (on *Silene vulgaris* and *S. maritima*), of *M. violaceo-verrucosum* (on *S. chlorantha*) and the specimens of *S. saxifraga* ssp. *hayekiana* formed well supported subgroups showing minimal (0–1.1 %) base differences, respectively.

Discussion

As ROUX, ALMARAZ & DURRIEU (1998), ALMARAZ et al. (2002) and FREEMAN et al. (2002) have shown, ITS sequences may successfully be used for phylogenetic studies in *Microbotryum*. The data presented here clearly corroborate the results of these authors since ITS phylogeny allows to draw conclusions with respect to the value of morphological and host characters for *Microbotryum* taxonomy as well as with respect to the different generic and species concepts applied in literature. These issues will be discussed in the following sections.

Morphological characters

Spore diameter turned out to be of quite limited value for species identification. For instance, spore diameter in *Microbotryum stellariae* is reported to range from 5 to 8 µm instead of 6 to 11 µm in most other *Microbotryum* species on Caryophyllaceae (VÁNKY 1994, p. 154; VÁNKY 1998, p. 53f.). In contrast, the *Microbotryum stellariae* specimens from *Stellaria* and *Myosoton* in our sampling did not show smaller spore sizes than specimens from other host genera, but ranged from 7.200 ± 0.516 to 7.872 ± 1.170 µm (Fig. 1). On the other hand, spore diameter in *Microbotryum major*, a species regarded to possess large spores and named accordingly (VÁNKY 1994), is indeed significantly larger than in most other specimens examined (Fig. 1). Thus, the value of spore diameter measurements in characterising *Microbotryum* species should not be overestimated, although it clearly is not zero. Note that Fig. 1 depicts spore measurements of collections that were aggregated into groups based on molecular and other data (see below). Naturally, it should be even more difficult to use spore size to ascribe single specimens to species reported in literature.

As depicted in Fig. 2, spore mass colour was mostly uniform within terminal monophyla revealed by ITS analysis (see below) and in accordance with literature (e.g., VÁNKY 1994, p. 154; VÁNKY 1998, p. 53f.). Hence, it proved to be taxonomically valuable. For instance, *Microbotryum silenes-inflatae* could easily be recognised by its light-coloured spore masses. On the other hand, spore masses of specimens from *Dianthus* and *Saponaria,* each of which turned out to be monophyletic groups, were not uniformly coloured. Instead, colouring could be host-dependent in some cases, as specimens from *Saponaria officinalis* were consistently coloured more darkly than specimens from other members of that host genus (Fig. 2).

Species delimitation

ITS sequence diversity (Fig. 2) and degree of specialization on different plant hosts are as large within *Microbotryum violaceum* s.l. as between *Microbotryum* species on non-caryophyllacean hosts. Hence, it would be taxonomically inconsistent to regard *Microbotryum* parasites of Caryophyllaceae as formae speciales of a single species, *M. violaceum* s.l.

Considering genetic distances our data do not support the view of PERLIN (1996), PERLIN et al. (1997), BUCHELI, GAUTSCHI & SHYKOFF (2000), and FREEMAN et al. (2002) to merge all *Microbotryum* specimens growing on members of the same host family into a single species. The present results confirm the opinion of LIRO (1924) that narrow species delimitations are appropriate in *Microbotryum* anther smuts. The lack of morphological differences between several *Microbotryum* specimens does not necessarily imply that they belong to the same species. As discussed below, stable morphological differences are apparent in many groups, but merging all morphologically uniform specimens into species would result in a number of non-monophyletic assemblages.

Our suggestions for species boundaries in accordance with this point of view are depicted in Fig. 2.

Apparently monophyletic lineages

With respect to taxa from Europe, a number of distinct lineages shown in the tree derived from our ITS sequence data can be ascribed to *Microbotryum* species mentioned in literature (Fig. 2) and appear to be in accordance with morphology. *Microbotryum violaceo-irregulare* is easily recognised by its incompletely reticulated spores and the darkly coloured spore masses (VÁNKY 1994; 1998) – it appeared as a valid taxon in ITS analysis, not nested within other species. Likewise, *Microbotryum major*, the spores of which are distinctly larger than in most other lineages (Fig. 1) and which is restricted to some

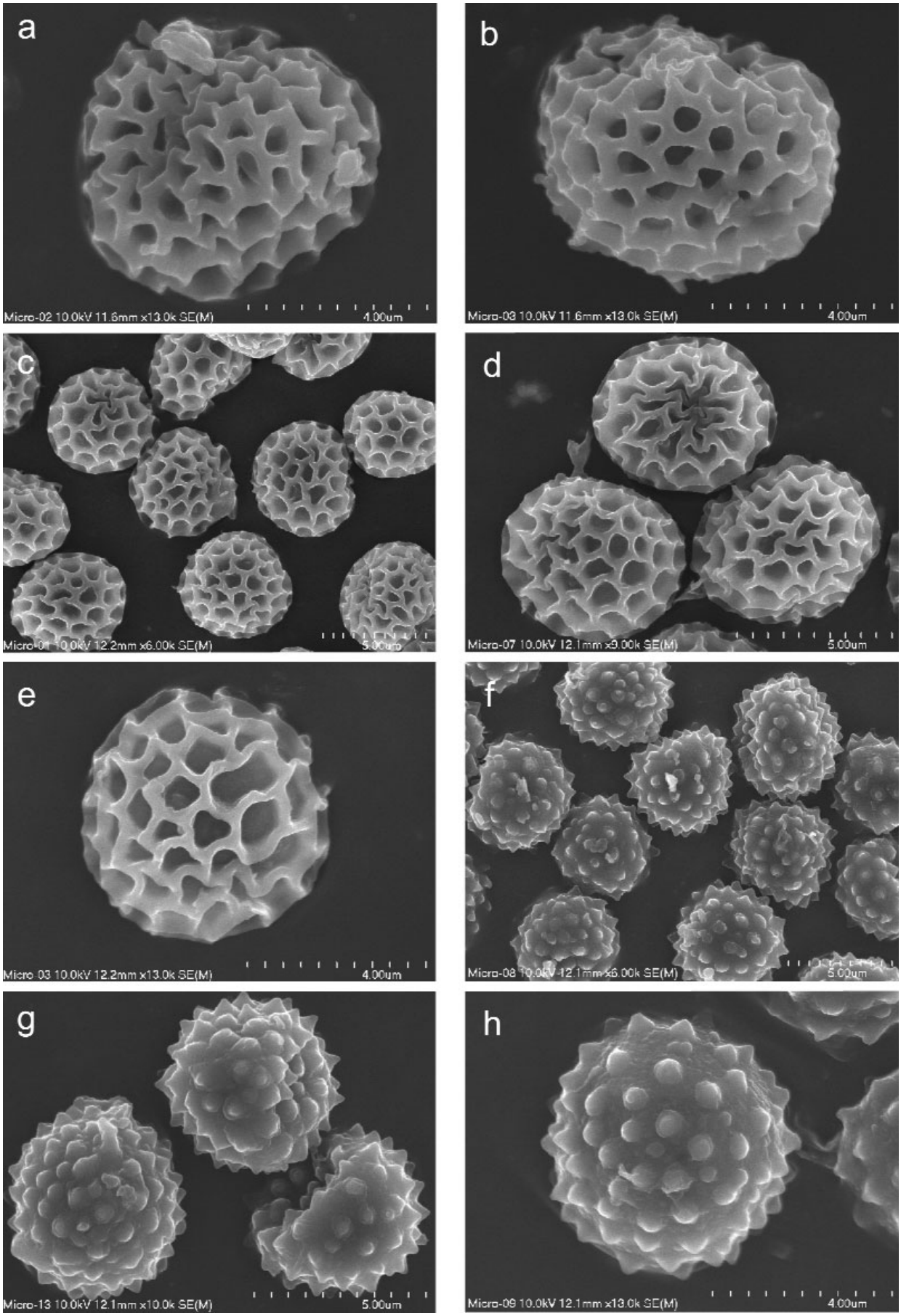


Fig. 3: SEM micrographs of teliospores. **a, b**. *Microbotryum major* (lectotype of *Ustilago major*, WRSL s.n.). **c-e**. *Microbotryum saponariae* (holotype, M 0098773). **f-h**. *Microbotryum chloranthae-verrucosum* (paratype, B 700007571).

species belonging to *Silene* sect. *Otites* (VÁNKY 1994), seems to be genetically different from other anther smut species.

Some other species could not be recognised by morphological features, but appeared as monophyletic groupings, too. Specimens to be included in *Microbotryum lychnidis-dioicae* could not be distinguished from *M. violaceum* by spore morphology and spore mass colour, but are characterised by their hosts being almost exclusively *Silene dioica* and *S. latifolia*.

As mentioned above, we failed to separate *Microbotryum stellariae* from remaining Caryophyllaceae-inhabiting *Microbotryum* species based on spore diameter. Nevertheless, parasites from *Stellaria* and *Myosoton* clustered tightly together, thus confirming the traditional species concept.

DENCHEV & SHARKOVA (1997) failed to find any morphological differences between *Microbotryum dianthorum* and *M. violaceum* and concluded that these species names should be regarded as synonyms. Our results confirm this view with respect to spore diameter and colour of spore masses. Specimens from *Dianthus* showed relatively high sequence divergence, but were strongly supported by ITS data as a monophyletic group. As they would partly belong to *Microbotryum violaceum* and partly to *M. dianthorum* according to the traditional concept, another species definition for *M. dianthorum* is needed. Further studies based on a larger sample of different hosts plants will probably allow to separate groups within *Microbotryum dianthorum*, confirming LIRO (1924) who showed by means of cross-infection experiments that parasites of *Dianthus superbus* and *D. deltoides* are ecologically distinct. However, the observed groups are not easily interpreted with respect to host distribution, as specimens growing on *Dianthus carthusianorum* cluster within all major lineages. Thus, currently a more conservative taxonomic arrangement should be applied.

Apparently non-monophyletic lineages

Microbotryum violaceo-verrucosum is characterised morphologically by its verrucose spores and the light-coloured spore masses, but it appeared as non-monophyletic in our studies. The specimens on *Silene chlorantha* were clearly separated from the specimens on *S. viscosa* and *S. italica*, respectively, and cluster in different phylogenetic lineages. As the type of *Microbotryum violaceo-verrucosum* was collected on *Silene italica*, we decided to describe a new species, *M. chloranthae-verrucosum*, to accommodate the anther smut of *S. chlorantha.*

Microbotryum silenes-inflatae, easily recognised by its light-coloured spore masses and a reticulate spore surface, appeared polyphyletic. Besides the group containing specimens from *Silene vulgaris*, the type host of *Microbotryum silenes-inflatae,* and *S. maritima,* there are two other quite remotely related lineages both containing hosts (*S. pusilla, S. rupestris, S. saxifraga* ssp. *hayekiana)* so far unknown for anther smuts with that combination of characters (therefore we decided to ascribe these specimens to *M. violaceum* s.l.). Against the background of the limitations of our dataset with respect to these lineages we decided not to draw taxonomical consequences but suggest to restrict *Microbotryum silenes-inflatae* to the group in which the specimens from the type host are nested in.

Microbotryum violaceum s.str. is characterised by medium-coloured spore masses and reticulate spores with rounded to regularly polygonal meshes (VÁNKY 1994). According to this definition and to the host range reported in literature, in addition to some of the *Dianthus* parasites mentioned earlier, at least the specimens from *Silene flos-cuculi, S. nutans* and *Saponaria* species should belong to *Microbotryum violaceum* s.str. As is evident from our molecular data (Fig. 2) and confirming the results of ALMARAZ et al. (2002) on a larger data basis, *Microbotryum violaceum* s.str. is a non-monophyletic assemblage. Furthermore, it did not appear as morphologically uniform, since dark spore masses were found in specimens from *Saponaria officinalis* and medium-coloured spore masses in collections from different hosts (Fig. 2).

While sequencing more specimens would be necessary to draw taxonomic conclusions with respect to *Silene*-inhabiting *Microbotryum violaceum*, our sampling included a number of collections from different *Saponaria* hosts from different European localities, which were nearly identical in ITS sequence. Genetic differences within *Saponaria* parasites were as low as in *Microbotryum stellariae* and lower than in *M. lychnidis-dioicae*. Consequently, *Microbotryum* on *Saponaria* can safely be regarded as a distinct species and should be proposed as such to achieve consistency in the species concept applied.

Based on field observations and infection experiments, LIRO (1924, p. 280) divided *Microbotryum violaceum* into a couple of species, including *M. silenes-nutantis* for the parasites of *Silene nutans*. According to his opinion, *Microbotryum violaceum* should be restricted to the fungi infecting *Saponaria*. As explained in the previous sections, our results strongly corroborate LIRO'S (1924) view of *Microbotryum violaceum* as a heterogeneous assemblage that should be split up to achieve a natural classification. However, VÁNKY (1998), confirming the view of NANNFELDT (in LINDEBERG 1959, p. 142), pointed out that LIRO (1924), as well as DEML & OBERWINKLER (1982), erroneously assumed *Saponaria officinalis* L. to be the type host of *Uredo violacea* Pers.: Pers. instead of the correct type host, *Silene nutans* L. Consequently, *Microbotryum violaceum* has to be accepted as to refer to the parasites of *Silene nutans* and a new epithet is to be proposed for *Microbotryum* infecting *Saponaria*. It should be pointed out that a smut fungus in the anthers of *Saponaria officinalis* has already been recognised by DE CANDOLLE (1815) as a variety *Uredo antherarum* γ *saponariae-officinalis* and by GIARD (1889) as a species *Ustilago saponariae*. However, both of these authors did not provide any diagnoses of their new taxa and, therefore, they actually are nomina nuda.

As distinguishing *Microbotryum* species by morphological features is apparently difficult, the question arises which characters to include in species definitions. The use of molecular markers not only for phylogenetic inference but also as

a taxonomic tool seems to be increasingly accepted in mycology. As TAYLOR et al. (2000) claimed, recognition of fungal species by molecular phylogenetic approaches can be superior to both morphological and biological species recognition. These authors listed examples in which morphologically identical fungi were genetically distinct or reproductively isolated. For instance, O'DONNELL, CIGELNIK & NIRENBERG (1998) proposed to split up the *Gibberella fujikuroi* complex into 23 new species, based on molecular analyses. This is in accordance with the result of BUCHELI, GAUTSCHI & SHYKOFF (2000) that gene flow between *Microbotryum* samples from different host plants is severely restricted or even absent. With respect to biological species recognition the problem occurs that strains may interbreed in vitro but do not exchange genes in nature (TAYLOR et al. 2000). SCORZETTI et al. (2002) pointed out that species identification in basidiomycetous yeasts may be achieved with molecular markers like ITS or LSU rDNA, but species definition and description "should not solely rely on nucleotide data".

As shown here, morphological data are mostly insufficient to separate *Microbotryum* species. However, basing species definitions on host data alone would be inappropriate, too, as different species may infect the same host plant. For example, *Silene vulgaris* is well-known to be parasitised by both *Microbotryum silenes-inflatae* and *M. violaceo-irregulare*. We also found cases that may represent occasional infections as exemplified by the specimen *Microbotryum lychnidis-dioicae* on *Cerastium viscosum* from GenBank. Consequently, we included an ITS type sequence in the following species definitions in addition to host specificity. A similar approach has been used by INÁCIO et al. (2004) who included LSU rDNA typification in their definitions of phylloplane yeasts in the genus *Lalaria*. However, we will not give any values for a percentage of nucleotide differences that must not be overcome for a sequence to be ascribed to the species defined below, as this would impose the assumption of a molecular clock (SCORZETTI et al. 2002). Hence, taxonomic conclusions listed below are only based on groupings which appeared as well-supported.

Phylogeny

As already revealed by the analyses of PERLIN et al. (1997) and FREEMAN et al. (2002), Caryophyllaceae-parasitising *Microbotryum* species are basally subdivided into specimens from native North-American *Silene* species and specimens which are collected mostly from European hosts. However, the European clade contains specimens from hosts naturalised in North America, specimens from hosts with circumpolar occurence (*Microbotryum* on *Silene acaulis)* and even from specimens from hosts native to North America (*Microbotryum* on *Silene douglasii*), too. The presence of the major European clade in North America suggests inter-continental migration of *Microbotryum* species via circumpolar hosts like *Silene acaulis*.

Our phylogenetic analyses reveal a high level of pathogen specialisation to particular host species. In a forthcoming article, we will discuss, whether this specialisation results in a strong correlation between host and parasite phylogenies in *Microbotryum* species infecting anthers of Caryophyllaceae (GÖKER et al., in prep.).

Taxonomy

Microbotryum chloranthae-verrucosum M. Lutz, Göker, M. Piatek, Kemler, Begerow et Oberw., **sp. nov.** Figs. 3f-h

Species characteribus generis. Sori in antheris *Silenes chloranthae* (Willd.) Ehrh. (Caryophyllaceae). Massa sporarum pallide brunneo-violacea. Teliosporae globosae, (4.8) 5.6–7.2 (8) µm diametro, parietibus verrucosis. Sequentia acidi nucleici ITS typi in collectione sequentiarum acidi nucleici NCBI (GenBank) numero AY877421 deposita est.

TYPUS [***hic designatus***] in matrice *Silene chlorantha* (Willd.) Ehrh.: Germany, Brandenburg, Barnim, Britz, leg. H. Scholz & I. Scholz, 29. 6. 2001 (HOLOTYPUS in B numero 700006053!, ISOTYPUS in HUV numero 21080 depositae sunt); Germany, Brandenburg, Eisenhüttenstadt, leg. S. Rätzel, 13. 6. 1999 (PARATYPUS in B numero 700007571! depositus).

With the characteristics of the genus. Sori in anthers. Infection systemic. Spore mass powdery, light brownish-violet. Teliospores pale brownish-violet, rounded, subglobose to irregular, (4.8) 5.6–7.2 (8) µm in diameter, mean 6.416 ± 0.783 µm, wall verrucose, warts sparsely to densely situated, pyramidal, interspaces between the warts extremely finely verruculose. On *Silene chlorantha* (Willd.) Ehrh. (Caryophyllaceae). The ITS type sequence from DNA isolation mk305 from the holotype (B 700006053) is deposited in GenBank as AY877421.

Etymology: named after the host species, *Silene chlorantha*, and the verrucose cell wall of the teliospores.

Microbotryum saponariae M. Lutz, Göker, M. Piatek, Kemler, Begerow et Oberw., **sp. nov.** **Figs. 3c-e**

= *Uredo antherarum* DC. γ *saponariae-officinalis* DC., Flore française III 6: 79 (1815), *nomen nudum*. – on *Saponaria officinalis* L., Central Europe.

= *Ustilago saponariae* Giard, Bulletin scientifique de la France et de la Belgique III 2: 157 (1889), *nomen nudum*. – on *Saponaria officinalis* L., France.

Species characteribus generis. Sori in antheris specierum generis *Saponariae* L. (Caryophyllaceae). Massa sporarum brunneo-violacea ad uligineo-violacea. Teliosporae globosae, (5.5) 6.5–8.5 (11) µm diametro, pariete reticulate decorato. Sequentia acidi nucleici ITS typi in collectione sequentiarum acidi nucleici NCBI (GenBank) numero AY588089 deposita est.

TYPUS [***hic designatus***] in matrice *Saponaria officinalis* L.: Germany, Bavaria, Günzburg, leg. M. Lutz, 18. 7. 2001 (HOLOTYPUS in M numero 0098773!, ISOTYPUS in TUB numero 011809 et HUV numero 21099 depositae sunt).

With the characteristics of the genus. Sori in anthers. Infection systemic. Spore mass powdery, brownish-violet to dark

brownish-violet. Teliospores rounded, subglobose, ovoid to irregular, (5.5) 6.5–8.5 (11) µm in diameter, mean 7.552 ± 0.940 µm, wall with reticulate ornamentation, 5-8 meshes per spore diameter. On Caryophyllaceae: *Saponaria* spp. The ITS type sequence from DNA isolation ml317 from the holotype (M 0098773) is deposited in GenBank as AY588089.

Etymology: named after the host genus, *Saponaria*.

Additionally, we propose to emend *Microbotryum dianthorum*:

Microbotryum dianthorum (Liro) H. & I. Scholz, Englera 8: 206 (1988), emend. M. Lutz, Göker, M. Piatek, Kemler, Begerow et Oberw.

= *Ustilago dianthorum* Liro, Annales Academiae Scientiarum Fennicae, Series A 17: 35 (1924). – Lectotype on *Dianthus deltoides* L., Finland, Tavastia borealis, Jyväskylä, Ristkivi, leg. J. L. Liro, 7. 7. 1912 (isolectotypes in Mycotheca fennica no. 350, HUV no. 7751).

With the characteristics of the genus. Sori in anthers. Infection systemic. Spore mass powdery, brownish-violet to dark brownish-violet. Teliospores rounded to subglobose, (5) 6–8 (10) µm in diameter, mean 6.953 ± 0.986 µm, wall with reticulate ornamentation. On Caryophyllaceae: *Dianthus* spp. Molecular characteristics: The ITS sequence from DNA isolation ml622 from the specimen M 0098771 is deposited in GenBank as AY588081.

Further, we lectotypify the name *Ustilago major* (= *Microbotryum major*):

Microbotryum major (J. Schröt.) G. Deml & Oberw., Phytopathol. Z. 104(4): 353 (1982). Figs. 3a, b

= *Ustilago major* J. Schröt., in Cohn, Kryptogamen-Flora von Schlesien 3(1): 273 (1887).

LECTOTYPUS [***hic designatus***]: on *Silene otites* (L.) Wibel, Poland, Breslau, Carlowitz, 1884, leg. ipse (WRSL s.n.!).

With the characteristics of the genus. Sori mostly in anthers, but sometimes also in the filaments and ovaries. Infection systemic. Spore mass powdery, dark blackish-violet. Teliospores light brownish-violet, rounded, subglobose, ovoid to irregular, 9–12 × 8–10 µm in diameter, mean 10.000 ± 0.870 µm, wall with reticulate ornamentation, 6–9 meshes per spore diameter. On Caryophyllaceae: *Silene* sect. *Otites*.

Commentary: When completing his monographs of Carpathian and European smut fungi, VÁNKY (1985, 1994) could not locate the original material of *Ustilago major*, described by SCHRÖTER (1887) from *Silene otites*. Thus, this smut fungus has not been typified. The original material was recently located in the herbarium of Museum of Natural History of Wroclaw University (WRSL) where the major part of collection of J. SCHRÖTER is preserved. In the protologue SCHRÖTER (1887) mentioned three localities in the Silesia (Grünberg: Lansitz; Wohlau: Leubus; Breslau: Karlowitz) where he found the fungus without indicating where the type was collected. In WRSL there are five specimens of *Ustilago major* collected in Karlowitz near Breslau (now Karlowice near Wroclaw). All of them are damaged by moulds and only one of them is relatively well maintained. This specimen is here selected as lectotype of *Ustilago major*. The description of this species given above is based on the lectotype specimen.

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References

ALMARAZ T, ROUX C, MAUMONT S, DURRIEU G (2002) Phylogenetic relationships among smut fungi parasitizing dicotyledons based on ITS sequence analysis. – Mycological Research **106**: 541-548.

ANTONOVICS J, HOOD M, PARTAIN J (2002) The ecology and genetics of a host shift: *Microbotryum* as a model system. – American Naturalist **160**: S40-S53.

BARKER FK, LUTZONI F (2002) The Utility of the Incongruence Length Difference Test. – Systematic Biology **51**: 625-637.

BEGEROW D, LUTZ M, OBERWINKLER F (2002) Implications of molecular characters for the phylogeny of the genus *Entyloma*. – Mycological Research **106**: 1392-1399.

BUCHELI E, GAUTSCHI B, SHYKOFF JA (2000) Host-specific differentiation in the anther smut fungus *Microbotryum violaceum* as revealed by microsatellites. – Journal of Evolutionary Biology **13**: 188-198.

BUHR H (1964) Bestimmungstabellen der Gallen (Zoo- und Phytocecidien) an Pflanzen Mittel- und Nordeuropas. Gustav Fischer Verlag, Jena.

CUNNINGHAM JL (1972) A miracle mounting fluid for permanent whole-mounts of microfungi. – Mycologia **64**: 906-911.

DE CANDOLLE AP (1815) Flore française. Ed. 3, vol. 6. Desray, Paris.

DEML G, OBERWINKLER F (1982) On *Ustilago violacea* (Pers.) Rouss. from *Saponaria officinalis* L. – Phytopathologische Zeitschrift **104**: 345-365.

DENCHEV C, SHARKOVA S (1997) Taxonomic revision of *Microbotryum* (Ustilaginales) on *Dianthus*. – Phytologia Balcanica **3**: 105-112.

DURRIEU G, ZAMBETTAKIS C (1973) Les *Ustilago* parasites des Caryophyllacées. Apports de la microscopie électronique. – Bulletin de la Société Mycologique de France **89**: 283-290.

FARRIS JS, KÄLLERSJÖ M, KLUGE AC, BULT C (1995) Testing significance of incongruence. – Cladistics **10**: 315-319.

FREEMAN AB, DOUNG KK, SHI T, HUGHES CF, PERLIN MH (2002) Isolates of *Microbotryum violaceum* from North American host species are phylogenetically distinct from their European host-derived counterparts. – Molecular Phylogenetics and Evolution **23**: 158-170.

GIARD A (1889) Sur la castration parasitaire du *Lychnis dioica* L. par *Ustilago antherarum* Fr. – Bulletin scientifique de la France et de la Belgique III, **2**: 153-158.

HASSAN A, MACDONALD JA (1971) *Ustilago violacea* on *Silene dioica*. – Transactions of the British Mycological Society **56**: 451-461.

HENNIG W (1965) Phylogenetic systematics. – Annual Review of Entomology **10**: 97-116.

HUELSENBECK JP, RONQUIST FR (2001) MRBAYES: Bayesian inference of phylogenetic trees. – Bioinformatics **17**: 754-755.

HUELSENBECK JP, LARGET B, MILLER RE, RONQUIST F (2002) Potential applications and pitfalls of Bayesian inference of phylogeny. – Systematic Biology **51**: 673-688.

INÁCIO J, RODRIGUES MG, SOBRAL P, FONSECA I (2004) Characterisation and classification of phylloplane yeasts from Portugal related to the genus *Taphrina* and description of five novel *Lalaria* species. – FEMS Yeast Research **4**: 541-555.

JENNERSTEN O (1983) Butterfly visitors as vectors of *Ustilago violacea* spores between Caryophyllaceous plants. – Oikos **40**: 125-130.

JENNERSTEN, O (1988) Insect dispersal of fungal disease: effects of *Ustilago* infection on pollinator attraction in *Viscaria vulgaris*. – Oikos **51**: 163-170.

KATOH K, MISAWA K, KUMA K, MIYATA T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. – Nucleic Acids Research **30**: 3059-3066.

LEE JA (1981) Variation in the infection of *Silene dioica* (L.) Clairv. by *Ustilago violacea* (Pers.) Fuckel in North West England. – New Phytologist **87**: 81-99.

LEVY LA, CASTLEBURY LM, CARRIS LJ, MEYER R, PIMENTEL G (2001) Internal transcribed spacer sequence-based phylogeny and polymerase chain reaction-restriction fragment length polymorphism differentiation of *Tilletia walkeri* and *T. indica*. – Phytopathology **91**: 935-940.

LINDEBERG B (1959) Ustilaginales of Sweden. – Symbolae Botanicae Upsalienses **16**: 1-175.

LIRO JI (1924) Die Ustilagineen Finnlands I. – Annales Academiae Scientiarum Fennicae, Series A **17**: 1-636.

LUTZ M, BAUER R, BEGEROW D, OBERWINKLER F, TRIEBEL D (2004) *Tuberculina*, rust relatives attack rusts. – Mycologia **96**: 614-626.

MILLER ALEXANDER H, ANTONOVICS J (1995) Spread of anther-smut disease (*Ustilago violacea*) and character correlations in a genetically variable experimental population of *Silene alba*. – Journal of Ecology **83**: 783-794.

MILLER ALEXANDER H, THRALL PH, ANTONOVICS J, JAROSZ AM, OUDEMANS PV (1996) Population dynamics and genetics of plant disease: A case study of anther-smut disease. – Ecology **77**: 990-996.

O'DONNELL K, CIGELNIK E, NIRENBERG HI (1998) Molecular systematics and phylogeography of the *Gibberella fujikuroi* complex. – Mycologia **90**: 165-493.

PERLIN MH (1996) Pathovars or formae speciales of *Microbotryum violaceum* differ in electrophoretic caryotype. – International Journal of Plant Sciences **157**: 447-452.

PERLIN MH, HUGHES C, WELCH J, AKKARAJU S, STEINECKER D, KUMAR A, SMITH B, GARR SS, BROWN SA, ANDOM T (1997) Molecular approaches to differentiate subpopulations or formae speciales of the fungal phytopathogen *Microbotryum violaceum*. – International Journal of Plant Sciences **158**: 568-574.

POSADA D, CRANDALL KA (1998) MODELTEST: testing the model of DNA substitution. – Bioinformatics **14**: 817-818.

ROUX C, ALMARAZ T, DURRIEU G (1998) Phylogénie de champignons responsables des charbons des végétaux à partir de l'-analyse des séquences ITS. – Comptes Rendus de l'Académie des Sciences. Série III-Vie. Académie des Sciences, Paris **321**: 603-609.

SCHRÖTER J (1887) Ustilaginei. In COHN F (ed) Kryptogamen-Flora von Schlesien **3**(1): 261-291. JU Kern's Verlag, Breslau.

SCORZETTI G, FELL JW, FONSECA A, STATZELL-TALLMAN A (2002) Systematics of basidiomycetous yeasts: a comparison of large subunit D1/D2 and internal transcribed spacer rDNA regions. – FEMS Yeast Research **2**: 495-517.

STOLL M, PIEPENBRING M, BEGEROW D, OBERWINKLER F (2003) Molecular phylogeny of *Ustilago* and *Sporisorium* species (Basidiomycota, Ustilaginales) based on internal transcribed spacer (ITS) sequences. – Canadian Journal of Botany **81**: 976-984.

SWOFFORD DL (2001) PAUP* Phylogenetic Analysis Using parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

SWOFFORD DL, OLSEN GJ, WADDELL PJ, HILLIS DM (1996) Phylogenetic Inference. In HILLIS DM, MORITZ C, MABLE BK (eds) Molecular Systematics, pp. 407-514. Sinauer Associates, Sunderland, Massachusetts.

TAYLOR JW, JACOBSON DJ, KROKEN S, KASUGA T, GEISER DM, HIBBETT DS, FISHER MC (2000) Phylogenetic species recognition and species concepts in fungi. – Fungal Genetics and Biology **31**: 21-32.

THRALL TY, BIERE A, ANTONOVICS J (1993) Plant life-history and disease susceptibility: the occurence of *Ustilago violacea* on different species within the Caryophyllaceae. – Journal of Ecology **81**: 489-498.

VÁNKY K (1985) Carpathian Ustilaginales. – Symbolae Botanicae Upsalienses **24**(2): 1-309.

VÁNKY K (1994) European smut fungi. Gustav Fischer Verlag, Stuttgart, Jena, New York.

VÁNKY K (1998) The genus *Microbotryum* (smut fungi). – Mycotaxon **67**: 33-60.

VÁNKY K (2004) Anther smuts of Caryophyllaceae. Taxonomy, nomenclature, problems in species delimitation. – Mycologia Balcanica **1**: 189-191.

WHITE TJ, BRUNS TD, LEE S, TAYLOR JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols, a guide to methods and applications, PP. 315-322. Academic Press, San Diego.

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Tab. 1: List of sequenced *Microbotryum* specimens with DNA isolation numbers, GenBank (**http://www.ncbi.nlm.nih.gov/**) accession numbers, spore sizes, proposed taxon names, and reference materials.

Microbotryum sp. on	DNA isolation no.	GenBank acc. no.	Spore size (µm)	Proposed taxon	Reference material[a]
Dianthus carthusianorum L.	db3637	AY588077	7.440 ± 0.683	*M. dianthorum* (Liro) H. & I. Scholz	Germany, Baden-Württemberg, Tübingen; leg. DB, 13.7.2000; D. Begerow 705
	db5014	AY588078	6.992 ± 0.826	*M. dianthorum* (Liro) H. & I. Scholz	Switzerland, Valais, Ulrichen; leg. UF & ML, 6.7.2002; TUB 011803
	ml354	AY588079	8.000 ± 1.120	*M. dianthorum* (Liro) H. & I. Scholz	Germany, Saxony-Anhalt, Halle; leg. ML, 24.9.2001; TUB 011801
D. monspessulanus L.	ml333	AY588080	6.784 ± 0.469	*M. dianthorum* (Liro) H. & I. Scholz	Slovenia, Bovec; leg. DB & ML, 10.8.2001; TUB 011802
D. superbus L.	ml622	AY588081	6.193 ± 0.848	*M. dianthorum* (Liro) H. & I. Scholz	Switzerland, Grisons, Sur; leg. ML & WM, 14.7.2003; M 0098771, TUB 011799
D. sylvestris Wulfen	ml328	AY588082	6.368 ± 0.541	*M. dianthorum* (Liro) H. & I. Scholz	Slovenia, Bovec; leg. DB & ML, 10.8.2001; TUB 011800
Myosoton aquaticum (L.) Moench	ml48	AY588084	7.744 ± 0.855	*M. stellariae* (Sowerby) G. Deml & Oberw.	Germany, Baden-Württemberg, Pfullendorf; leg. ML, 21.10.1998; TUB 011805
	mste1	AY588085	7.872 ± 1.170	*M. stellariae* (Sowerby) G. Deml & Oberw.	Germany, Baden-Württemberg, Kirchheim; leg. ML, 20.7.1998; TUB 011804
Polygonum viviparum L.	db1694	AY588113	–	*M. bistortarum* (DC.) Vánky	Switzerland, Grisons, Albulapaß; leg. MP, 3.8.1997; M. Piepenbring 2347
	ml322	AY588086	–	*M. bistortarum* (DC.) Vánky	Germany, Bavaria, Oberstdorf, leg. ML, 22.7.2001; TUB 011787
Rumex acetosa L.	db1087	AY588111	–	*M. stygium* (Liro) Vánky	Germany, Baden-Württemberg, Schloß Lichtenstein; leg. KV, 11.6.1988; K. Vánky 14028, TUB culture collection strain F566
Saponaria x *lempergii*	ml310	AY588087	7.664 ± 1.153	*M. saponariae* sp. nov.	Germany, Baden-Württemberg, Tübingen; leg. ML, 16.7.2001; TUB 011814
Sa. officinalis L.	mk142	AY877407	6.688 ± 0.606	*M. saponariae* sp. nov.	Germany, Baden-Württemberg, Gottmadingen; leg. MH, 23.6.2000; TUB 012111
	mk146	AY877406	6.784 ± 0.408	*M. saponariae* sp. nov.	Germany, Baden-Württemberg, Tübingen; leg. RB & MH, 17.8.2000; TUB 012110
	ml41	AY588088	8.832 ± 0.632	*M. saponariae* sp. nov.	Germany, Saxony, Königstein; leg. ML, 25.10.2000; TUB 011811
	ml51	AY877420	6.880 ± 0.730	*M. saponariae* sp. nov.	Germany, Baden-Württemberg, Tübingen; leg. ML, 4.7.2000; TUB 011781
	ml317	AY588089	7.289 ± 0.840	*M. saponariae* sp. nov.	Germany, Bavaria, Günzburg; leg. ML, 18.7.2001; M 0098773 - holotype, TUB 011809 - isotype
	ml318	AY877412	6.368 ± 0.489	*M. saponariae* sp. nov.	Germany, Bavaria, Günzburg; leg. ML, 18.7.2001; TUB 011780
	ml325	AY588090	7.264 ± 0.797	*M. saponariae* sp. nov.	Germany, Baden-Württemberg, Tübingen; leg. BG, 23.7.2001; TUB 011810
Sa. pumila Janchen	ml337	AY588091	7.600 ± 0.849	*M. saponariae* sp. nov.	Austria, Carinthia, Maltatal; leg. DB & ML, 31.7.2001; TUB 011812
	ml373	AY588092	7.312 ± 0.744	*M. saponariae* sp. nov.	Austria, Carinthia, Radenthein; leg. ML, 10.9.2001; TUB 011813
Scabiosa lucida Vill.	db1455	AY588112	–	*M. intermedium* (J. Schröt.) Vánky	Germany, Bavaria, Oberjoch; leg. KV, 14.9.1988; K. Vánky 13163, TUB culture collection strain F561
Silene chlorantha (Willd.) Ehrh.	mk282	AY877404	6.048 ± 0.656	*M. chloranthae-verrucosum* sp. nov.	Germany, Brandenburg, Eisenhüttenstadt; leg. SR, 13.6.1999; B 700007571 - paratype
	mk305	AY877421	6.784 ± 0.735	*M. chloranthae-verrucosum* sp. nov.	Germany, Brandenburg, Britz; leg. HS & IS, 29.6.2001; B 700006053 - holotype, Herbarium Ustilaginales Vánky 21080 - isotype
S. dioica (L.) Clairv.	db3634	AY588093	7.952 ± 0.835	*M. lychnidis-dioicae* (DC. ex Liro) G. Deml & Oberw.	France, Saône et Loire, Uchon; leg. DB, 2.6.2001; D. Begerow 700
	db3636	AY588114	7.184 ± 0.627	*M. lychnidis-dioicae* (DC. ex Liro) G. Deml & Oberw.	France, Maison du Parc Morvan, St. Brisson; leg. DB, 3.6.2001; TUB 011920
	mk147	AY877409	6.784 ± 0.697	*M. lychnidis-dioicae* (DC. ex Liro) G. Deml & Oberw.	Germany, Bavaria, Oberjoch; leg. MH & CAL, 15.6.2002; TUB 012109

Tab. 1: Continued

Microbotryum sp. on	DNA isolation no.	GenBank acc. no.	Spore size (µm)	Proposed taxon	Reference material[a]
S. dioica (L.) Clairv.	ml215	AY588094	7.686 ± 1.096	*M. lychnidis-dioicae* (DC. ex Liro) G. Deml & Oberw.	Germany, Baden-Württemberg, Reutlingen; leg. ML, 28.5.2001; TUB 011798
	ml518	AY877416	6.528 ± 0.680	*M. lychnidis-dioicae* (DC. ex Liro) G. Deml & Oberw.	Norway, Kristiansund, Farstad; leg. ML, 14.8.2002; TUB 012114
S. flos-cuculi (L.) Clairv.	ml516	AY877417	7.232 ± 0.588	*M. violaceum* s.l. (Pers.) G. Deml & Oberw.	Norway, Kristiansund, Farstad; leg. ML, 14.8.2002; TUB 012115
S. latifolia Poir. ssp. *alba* (Mill.) Greuter & Burdet	mk97	AY877405	6.464 ± 0.608	*M. lychnidis-dioicae* (DC. ex Liro) G. Deml & Oberw.	Russia, Yaroslavl; leg. KS, 21.6.1989; M 0066086
	mk196	AY877410	6.688 ± 0.688	*M. lychnidis-dioicae* (DC. ex Liro) G. Deml & Oberw.	Hungary, Nagykörös, Lajosmizse; leg. WM, 29.5.2003; TUB 012108
	ml40	AY588095	7.780 ± 0.927	*M. lychnidis-dioicae* (DC. ex Liro) G. Deml & Oberw.	Germany, Saxony, Königstein; leg. ML, 25.10.2000; TUB 011794
	ml62	AY588096	7.648 ± 1.060	*M. lychnidis-dioicae* (DC. ex Liro) G. Deml & Oberw.	Italy, South Tyrol, Sarntaler Alpen; leg. EB & ML, 9.8.2000; TUB 011795
	ml326	AY588097	7.877 ± 1.169	*M. lychnidis-dioicae* (DC. ex Liro) G. Deml & Oberw.	Slovenia, Bovec; leg. ML, 6.8.2001; TUB 011796
	ml374	AY588098	7.456 ± 0.799	*M. lychnidis-dioicae* (DC. ex Liro) G. Deml & Oberw.	Austria, Carinthia, Villach; leg. ML, 10.9.2001; TUB 011797
S. maritima With.	mk208	AY877408	7.200 ± 0.653	*M. silenes-inflatae* (DC. ex Liro) G. Deml & Oberw.	Norway, Vest-Agder, Lindesnes Fyr; leg. HS, 16.8.1978; B 700007632
S. nutans L.	ml344	AY588099	8.143 ± 0.845	*M. violaceum* s.l. (Pers.) G. Deml & Oberw.	Germany, Baden-Württemberg, Anhausen; leg. GMK, 9.6.2001; TUB 011818
S. otites (L.) Wibel	mk279	AY877419	8.256 ± 0.552	*M. major* (J. Schröt.) G. Deml & Oberw.	Germany, Brandenburg, Döberitz; leg. VK et al., 19.6.2002; B 700006042
	mk281	AY877418	8.128 ± 0.822	*M. major* (J. Schröt.) G. Deml & Oberw.	Germany, Brandenburg, Lebus; leg. SR, 26.6.1999; B 700007572
		–	10.000 ± 0.870	*M. major* (J. Schröt.) G. Deml & Oberw.	Poland, Breslau, Carlowitz; leg. ipse, 1884; WRSL s.n. - lectotype
S. pusilla W. & K.	mk292	AY877411	6.912 ± 0.726	*M. violaceum* s.l. (Pers.) G. Deml & Oberw.	Austria, Salzburgerland, Rauris; leg. VK, 8.7.2002; B 700006060
S. rupestris L.	ml602	AY588100	9.056 ± 0.765	*M. violaceum* s.l. (Pers.) G. Deml & Oberw.	Switzerland, canton Bern, Sustenpaß; leg. WM & ML, 12.6.2003; TUB 011817
S. saxifraga L. ssp. *hayekiana* (Hand.-Maz. & Janch.) Gr.	ml327	AY588101	6.943 ± 0.787	*M. violaceum* s.l. (Pers.) G. Deml & Oberw.	Slovenia, Bovec, Trenta; leg. DB & ML, 7.8.2001; TUB 011790
	ml608	AY588102	6.752 ± 0.686	*M. violaceum* s.l. (Pers.) G. Deml & Oberw.	Austria, Carinthia, Villach; leg. ML, 18.6.2003; TUB 011791
S. viscaria (L.) Jess.	mk143	AY877413	6.656 ± 0.642	*M. violaceum* s.l. (Pers.) G. Deml & Oberw.	Germany, Saxony, Tharandt; leg. MH, 25.5.2002; TUB 012112
S. viscosa (L.) Pers.	ml49	AY588103	6.720 ± 0.516	*M. violaceo-verrucosum (Brandenb. & Schwinn) Vánky*	France, Lozère, St. Chely du Tarn; leg. DB & ML, 19.5.1998; TUB 011815
S. vulgaris (Moench) Garcke	ml50	AY588104	7.114 ± 0.795	*M. violaceo-irregulare* (Brandenb. & Schwinn) G. Deml & Oberw.	Switzerland, Grisons, Maloja; leg. ML, 25.7.1998; TUB 011816
	ml605	AY588105	8.144 ± 0.738	*M. silenes-inflatae* (DC. ex Liro) G. Deml & Oberw.	Austria, Carinthia, Villach; leg. ML, 18.6.2003; TUB 011793
	ml620	AY588106	8.064 ± 0.709	*M. silenes-inflatae* (DC. ex Liro) G. Deml & Oberw.	Switzerland, Grisons, Sur; leg. ML & WM, 18.7.2003; TUB 011792

Tab. 1: Continued

Microbotryum sp. on	DNA isolation no.	GenBank acc. no.	Spore size (µm)	Proposed taxon	Reference material[a]
Stellaria graminea L.	ml503	AY588107	7.776 ± 0.880	*M. stellariae* (Sowerby) G. Deml & Oberw.	Sweden, Göteborg; leg. ML, 17.8.2002; TUB 011806
	ml507	AY588108	7.264 ± 1.100	*M. stellariae* (Sowerby) G. Deml & Oberw.	Norway, Oslo; leg. ML, 15.8.2002; TUB 011808
	ml512	AY588109	7.200 ± 0.516	*M. stellariae* (Sowerby) G. Deml & Oberw.	Norway, Molde; leg. ML, 14.8.2002; TUB 011807
St. holostea L.	mk253	AY877415	7.008 ± 0.740	*M. stellariae* (Sowerby) G. Deml & Oberw.	Germany, Mecklenburg-Vorpommern, Greifswald, leg. MS, 22.5.1999; B 700007591
	mk260	AY877414	6.560 ± 0.693	*M. stellariae* (Sowerby) G. Deml & Oberw.	Germany, Baden-Württemberg, Bodensee; leg. RBö, 9.5.1997; B 700007584
Tragopogon pratensis L.	ml263	AY588110	–	*M. tragopogonis-pratensis* (Pers.) R. Bauer & Oberw.	Switzerland, Grisons, Maloja; leg. ML, 25.7.1998; TUB 011788

[a] Acronyms: Collectors: BG, B. Grüninger; CAL, C. Adler-Lerner; DB, D. Begerow; EB, E. Bronner; GMK, G. M. Kovics; HS, H. Scholz; IS, I. Scholz; KS, K. Sutory; MH, M. Hendrichs; ML, M. Lutz; MS, M. Scholler; RB, R. Bauer; RBö, R. Böcker; SR, S. Rätzel; UF, U. Fischer; VK, V. Kummer; WM, W. Maier. Vouchers: M - Botanische Staatssammlung München, Germany; TUB - Herbarium of the Spezielle Botanik/Mykologie, Eberhard-Karls-Universität Tübingen, Germany.