Molecular and morphological evidence reveals a new smut fungus, Microbotryum arcticum (Microbotryaceae), on Silene uralensis (Caryophyllaceae) from Greenland and Canada

Authors: Denchev, Teodor T., Kemler, Martin, Begerow, Dominik, and Denchev, Cvetomir M.

Source: Willdenowia, 49(2) : 241-255

Published By: Botanic Garden and Botanical Museum Berlin (BGBM)

URL: https://doi.org/10.3372/wi.49.49212

Willdenowia
Annals of the Botanic Garden and Botanical Museum Berlin

TEODOR T. DENCHEV[1*], MARTIN KEMLER[2], DOMINIK BEGEROW[2] & CVETOMIR M. DENCHEV[1]

Molecular and morphological evidence reveals a new smut fungus, *Microbotryum arcticum* (*Microbotryaceae*), on *Silene uralensis* (*Caryophyllaceae*) from Greenland and Canada

Version of record first published online on 29 August 2019 ahead of inclusion in August 2019 issue.

Abstract: The group of the anthericolous *Microbotryum* species on *Silene* consists of narrowly host-specialized fungi. Despite intensive taxonomic and phylogenetic studies in this group over the past two decades, the actual species richness has not yet been fully uncovered. Thirteen of these species cause typical anther infection, with soral development restricted to the anthers. Three other species cause atypical infection, with soral development resulting in swollen and deformed flowers (completely filled with spores) and affecting both the anthers and the filaments. A comparative morphological study and molecular phylogenetic analyses, using ITS and LSU rDNA sequences, revealed a new species, *Microbotryum arcticum*, causing anther infection of *Silene uralensis* subsp. *arctica*. *Microbotryum arcticum* is described and illustrated on the basis of material from Greenland and the eastern Canadian Arctic. An emended description of *M. savilei*, which causes atypical infection of the same host plant in the eastern Canadian Arctic, is also given. Morphological characters of healthy flowers of *S. uralensis* subsp. *arctica* are compared with those of flowers with anther infection and with those of flowers with atypical infection.

Key words: Arctic, Canada, *Caryophyllaceae*, Greenland, *Microbotryaceae*, *Microbotryum*, phylogeny, *Silene uralensis*, smut fungi, taxonomy

Article history: Received 29 March 2019; peer-review completed 5 June 2019; received in revised form 9 June 2019; accepted for publication 20 June 2019.

Citation: Denchev T. T., Kemler M., Begerow D. & Denchev C. M. 2019: Molecular and morphological evidence reveals a new smut fungus, *Microbotryum arcticum* (*Microbotryaceae*), on *Silene uralensis* (*Caryophyllaceae*) from Greenland and Canada. – Willdenowia 49: 241–255. doi: https://doi.org/10.3372/wi.49.49212

Introduction

The anthericolous smut fungi on hosts in the *Caryophyllaceae* are among the longest-studied species of smut fungi. The name *Uredo violacea* Pers., the basionym of *Microbotryum violaceum* (Pers.) G. Deml & Oberw., s. str., was described by Persoon (1797), making this species one of the oldest known smut fungi (Vánky 2004). On the basis of inoculation tests, Liro (1924) recognized nine species in this complex of anthericolous fungi. Subsequent lumping and splitting treated this complex as one to many species (cf. Fischer 1953; Savile 1953; Lindeberg 1959; Nannfeldt 1959; Deml & Oberwinkler 1982, 1983; Scholz & Scholz 1988; Vánky 1985, 1994). For a long period, these species were considered as members of *Ustilago* (Pers.) Roussel and placed in the *Ustilaginaceae*, until Deml & Oberwinkler (1982) reinstated *Microbotryum* Lév. (with *M. violaceum* as the type species) for seven anthericolous species on caryophyllaceous hosts. Thereafter, the concept for this genus was altered several times. Vánky (1998), in his monographic treatment of *Microbotryum*, extended the definition of this genus and considered it in

1 Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin St., 1113 Sofia, Bulgaria; *e-mail: ttdenchev@gmail.com (author for correspondence); cmdenchev@yahoo.co.uk
2 AG Geobotanik, Ruhr-Universität Bochum, ND 03, Universitätsstr. 150, 44801 Bochum, Germany; e-mail: martin.kemler@rub.de; dominik.begerow@rub.de

a broad sense, including 76 species, eight of which occur in the anthers of *Caryophyllaceae*. Later, on the basis of ultrastructural characteristics and molecular analyses, it was demonstrated that the species of *Microbotryum* were more closely related to the rust fungi (*Pucciniomycotina*) than to the true smut fungi (*Ustilaginomycotina*) (Bauer & al. 1997, 2006; Begerow & al. 1998). Over the past two decades, phylogenetic and genomic approaches have considered the anthericolous species on hosts in the *Caryophyllaceae* as a model system for studying host-pathogen interactions in natural populations (e.g. Antonovics & al. 2002, 2003; Garber & Ruddat 2002; Uchida & al. 2003; Kazama & al. 2005; Lutz & al. 2005, 2008; Le Gac & al. 2007; Giraud & al. 2008; Refrégier & al. 2008; Sloan & al. 2008; Bernasconi & al. 2009; Denchev & al. 2009; Hood & al. 2010; Piątek & al. 2012, 2013; Perlin & al. 2015; Petit & al. 2017; Branco & al. 2018).

The anther-smut fungi of *Microbotryum* on hosts in the *Caryophyllaceae* cause formation of teliospores instead of pollen in the anthers of bisexual flowers. When female flowers of dioecious species (e.g. *Silene latifolia* Poir. and *S. dioica* (L.) Clairv.) are infected, suppression of stamen development does not occur, and development of spore-bearing anthers is induced (Kazama & al. 2005). These smut fungi infect mainly perennial plants (Thrall & al. 1993; Hood & al. 2010). The teliospores are transmitted from diseased to healthy plants mostly by insects that normally serve as pollinators (Fontaine & al. 2013).

The most widely studied anthericolous smuts are those in the anthers of *Silene* L. It is a group of highly host-specific fungi. The intensive taxonomic and phylogenetic studies of this group yielded resurrection of several species from the catch-all species *Ustilago violacea* and additional description of new species (Deml & Oberwinkler 1982; Chlebicki & Suková 2005; Lutz & al. 2005, 2008; Denchev 2007a, b; Le Gac & al. 2007; Refrégier & al. 2008; Denchev & al. 2009; Denchev & Denchev 2011; Piątek & al. 2012, 2013).

Sorus morphology divides the recognized 16 species of *Microbotryum* on *Silene* into two groups. The first group comprises 13 species that cause typical anther infection: *M. bardanense* Chleb. & Suková, *M. chloranthae-verrucosum* M. Lutz & al., *M. coronariae* (Liro) Denchev & T. Denchev, *M. heliospermae* Piątek & M. Lutz, *M. lagerheimii* Denchev, *M. lychnidis-dioicae* (DC.) G. Deml & Oberw., *M. silenes-acaulis* M. Lutz & al., *M. silenes-dioicae* T. Giraud & al., *M. silenes-inflatae* (DC. ex Liro) G. Deml & Oberw., *M. silenes-saxifragae* M. Lutz & al., *M. violaceoirregulare* (Brandenb. & Schwinn) G. Deml & Oberw., *M. violaceoverrucosum* (Brandenb. & Schwinn) Vánky and *M. violaceum* (Pers.) G. Deml & Oberw. The development of their sori is restricted to the anthers. The second group has three species: *M. adenopetalae* M. Lutz & al., *M. majus* (J. Schröt.) G. Deml & Oberw. and *M. savilei* Denchev. The development of their sori results in formation of swollen and deformed flowers, completely filled with spore mass. The sori of these species are usually formed not only in the anthers but also in the filaments, which at a more advanced stage of soral development leads to destruction of the stamens.

In the course of a study of the smut fungi of Greenland, carried out by two of the authors (TTD & CMD), the caryophyllaceous collection in the Greenland Herbarium, which is part of the herbarium of the Natural History Museum of Denmark, University of Copenhagen (C; herbarium code according to Thiers 2019+), was examined for presence of smut fungus infection. Four specimens of *Silene uralensis* subsp. *arctica* (Th. Fr.) Bocquet were found infected by a *Microbotryum* species that caused typical anther infection. On the same host, another species of *Microbotryum*, *M. savilei*, has already been described from Canada (Denchev 2007b), but it causes atypical anther infection. For the first time, presence of two kinds of infection of the flowers of *S. uralensis* subsp. *arctica* was observed by D. B. O. Savile during his field trips to the Canadian High Arctic islands in 1958–1959 and the early 1960s. Savile never published this observation but the herbarium packets of the specimens, collected during these expeditions, are thoroughly annotated inside. However, he published information about another, strange type of infection of the same host plant, a stigma infection, and supplied a table with comparative data about the morphology of the healthy plants, those with stigma infection, and those with typical anther infection, based on examination of a Somerset Island collection *no. 3628* (Savile 1959: 983–984). Our revision of this specimen did not confirm presence of a peculiar infection and we consider the smutted plants in this specimen (DAOM 02-01000869745) as infected by the typical anther infection.

It was found that the above-mentioned specimens on *Silene uralensis* subsp. *arctica* from Greenland belong to a species morphologically distinct from *Microbotryum savilei*. Additionally, ITS and LSU rDNA sequences of the *Microbotryum* species from Greenland were obtained and compared with sequences of *Microbotryum* species on caryophyllaceous hosts, available in the NCBI nucleotide database. The morphological differences and molecular phylogenetic analyses revealed the existence of a new smut fungus on *S. uralensis* subsp. *arctica*, which is described and illustrated here.

Material and methods

Morphological examination

Dried specimens from the Canadian National Mycological Herbarium, Ottawa (DAOM) and the herbarium of the Natural History Museum of Denmark, University of Copenhagen (C) were examined under light microscope (LM) and scanning electron microscope (SEM). For LM observations and measurements, spores were mounted in lactoglycerol solution (w : la : gl = 1 : 1 : 2) on glass slides, gently heated to boiling point to rehydrate the spores, and then cooled. The measurements of spores are

given as min–max (extreme values) (mean ± 1 standard deviation). For SEM, spores were attached to specimen holders by double-sided adhesive tape and coated with platinum or gold in an ion sputter. The surface structure of spores was observed and photographed at 10 kV accelerating voltage using a JEOL JSM 6610-LV scanning electron microscope (Natural History Museum, Vienna) and Hitachi SU3500 (National Museum of Natural History, Paris). The descriptions below are based on the specimens examined. The spore mass colour treatment is based on Rayner (1970). The shapes of spores are arranged in descending order of frequency.

DNA extraction, PCR amplification, and sequencing

DNA was isolated from two or three anthers from infected flowers of *Silene uralensis* subsp. *arctica* using the MyBudget Plant DNA Kit (Bio-Budget Technologies GmbH, Krefeld), according to the manufacturer's protocol (protocol 1: "Isolation of DNA from plant material using lysis buffer SLS"). PCR was used to amplify the ITS and parts of the LSU using the primer pair ITS1F/ITS4 (White & al. 1990; Gardes & Bruns 1993) and NL1/NL4 (O'Donnell 1992, 1993), respectively. Amplicons were sequenced in both directions by GATC (Eurofins Genomics, Konstanz), and subsequently quality controlled and forward and reverse read were merged in Sequencher 5.1 (Gene Codes Corporation, Ann Arbor). Sequences were deposited in the NCBI nucleotide database (see Table 1 for accession numbers).

Phylogenetic analyses

For phylogenetic analysis, sequences of selected *Microbotryum* species were downloaded from the NCBI nucleotide database (Table 1) and alignments were constructed separately for ITS and LSU in MAFFT v7.310 (Katoh & al. 2002; Katoh & Standley 2013) using the local alignment option (linsi). Ambiguous regions, as well as leading and trailing sequences, were removed using GBLOCK (Castresana 2000) implemented in SeaView (Gouy & al. 2010), whereby smaller final blocks, gap positions and less strict flanking positions were allowed. Subsequently, the alignments of ITS and LSU were concatenated in SequenceMatrix 1.7.8 (Vaidya & al. 2011). A Maximum Likelihood phylogeny was inferred with RAxML 8.2.11 (Stamatakis 2014) under the GTRGAMMA option and 1000 rapid bootstrap replicates. The resulting phylogeny was visualized in FigTree v1.4.3 (Rambaut 2012).

Results

Phylogenetic analyses

The phylogenetic analysis of the ITS and LSU data confirms previous studies on the evolutionary relationships within the caryophyllaceous anther smuts (Fig. 1). Species on *Dianthus* L., *Saponaria* L. and *Stellaria* L. are monophyletic, whereas the species on *Silene* are inferred as paraphyletic. However, in our analysis, there is no strong statistical support for this paraphyly. Most recognized species are inferred as monophyletic with strong statistical support. An exception is *Microbotryum heliospermae*, which is paraphyletic in our analysis. The new species *M. arcticum*, described below, forms a highly supported sister group to *M. majus*. Together they form a sister clade to a group of anthericolous *Microbotryum* species that mainly parasitize other *Silene* species and also *M. minuartiae* on *Minuartia*. However, this sister-group relationship has no statistical support.

Morphology

The observation that the anthericolous species on *Silene uralensis* subsp. *arctica* from Greenland is distinct from all phylogenetically studied *Microbotryum* species on *Silene* required revision of the Canadian specimens of *M. violaceum* s. lat. causing typical anther infection of *S. uralensis* subsp. *arctica*, kept at DAOM, and re-examination of the type specimen of *M. savilei*, which infects the same host plant.

All *Microbotryum* species are characterized by a limited number of microscopic diagnostic features. In Table 2, data are presented from a comparative morphological study of spores of four Greenlandic and nine Canadian *Microbotryum* specimens, causing typical anther infection of flowers of *Silene uralensis* subsp. *arctica*, and *M. savilei*. With respect to the spore sizes, the Greenlandic and Canadian specimens of the smut fungus causing typical anther infection are similar, while the comparison of this species with *M. savilei* shows that the latter possesses spores with higher maximum and mean values for spore length and width. Significantly, the sorus development of *M. savilei* causes formation of swollen and deformed flowers, completely filled with a spore mass. The differences in the localization and soral morphology of both species are discussed below.

Taxonomy

Re-examination of the type of *Microbotryum savilei* requires an emended description of its soral morphology.

Microbotryum savilei Denchev in Mycol. Balcan. 4: 72. 2007. – Holotype: on *Silene uralensis* subsp. *arctica*, Canada, Nunavut, Kivalliq Region, Southampton Island, Coral Harbour, 16 Aug 1959, *D. B. O. Savile 3952, J. A. Calder & I. Kukkonen* (DAOM 02-01000571594/DAOM 66879!). – Paratype: on same host, Canada, Nunavut, Kivalliq Region, Southampton Island, Coral Harbour, 16 Aug 1959, *D. B. O. Savile 3953, J. A. Calder & I. Kukkonen* (DAOM 02-01000868580/DAOM 66880! [white-flowered plants]). – Fig. 2, 3.

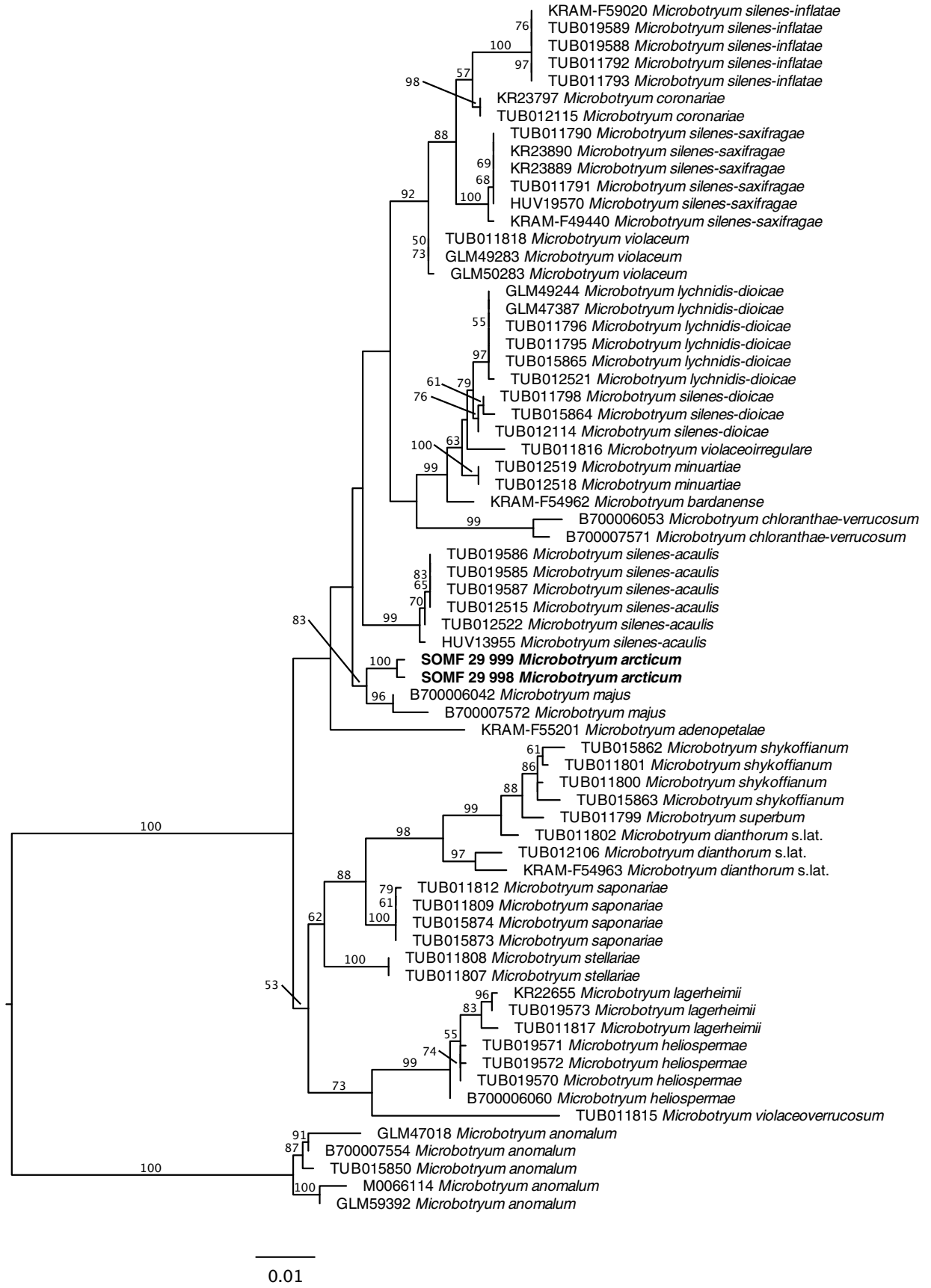

Fig. 1. Best tree of the Maximum Likelihood analysis of caryophyllaceous anther smuts in the genus *Microbotryum* based on a concatenated ITS and LSU dataset. The tree was rooted with *M. anomalum* (J. Kunze ex G. Winter) Vánky (based on Kemler & al. 2006). Bootstrap values ≥ 50 are indicated above branches.

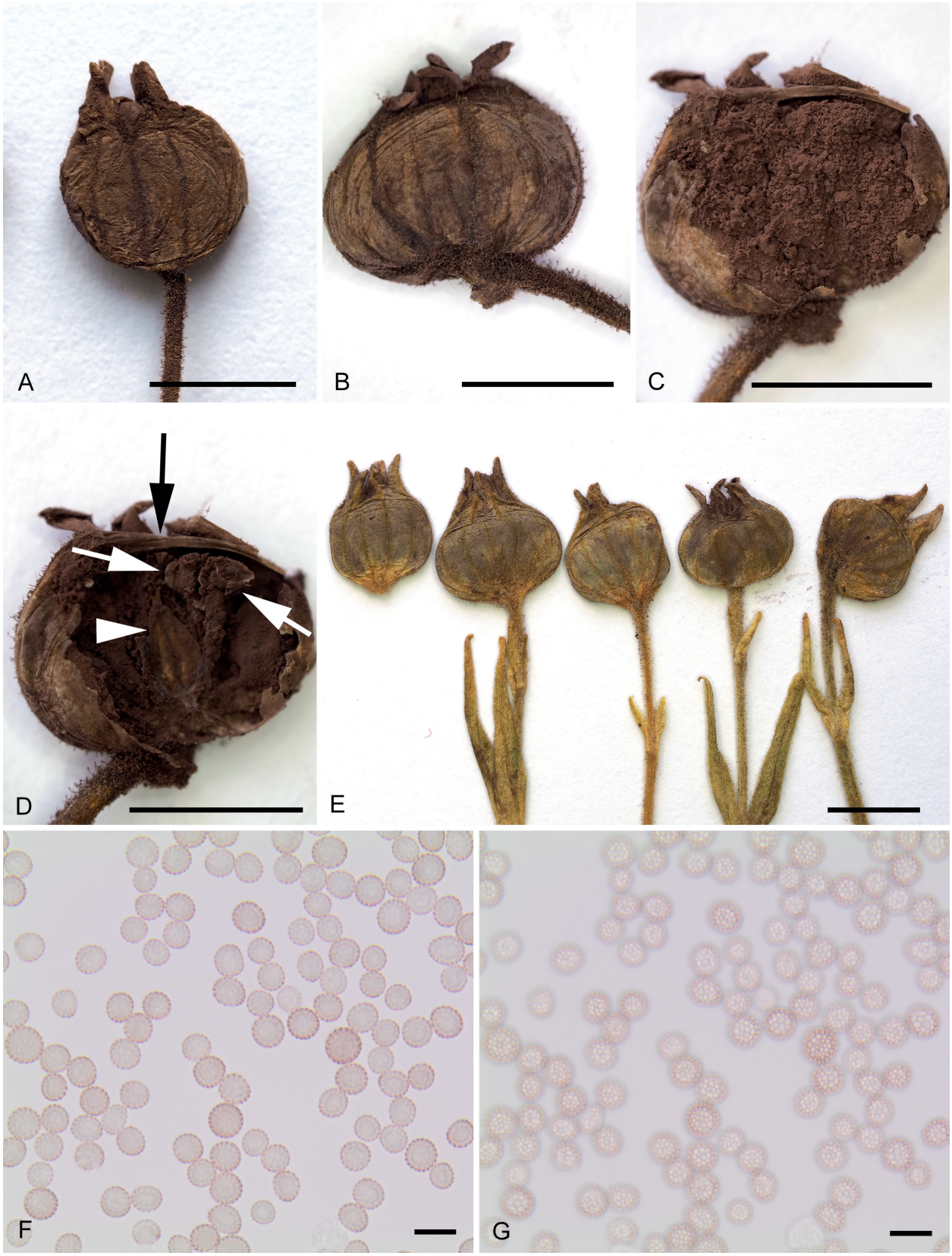

Fig. 2. *Microbotryum savilei* on *Silene uralensis* subsp. *arctica*. – A–D: habit (holotype); note deformed and swollen flower (enclosed by corolla; black arrow in D shows petals that remain within closed calyx), destroyed stamens (white arrows), and aborted ovary and styles (arrowhead); E: habit (paratype, on white-flowered plants); F, G: spores in LM, in median and surface view, respectively (holotype). – Scale bars: A–E = 0.5 cm; F, G = 10 µm.

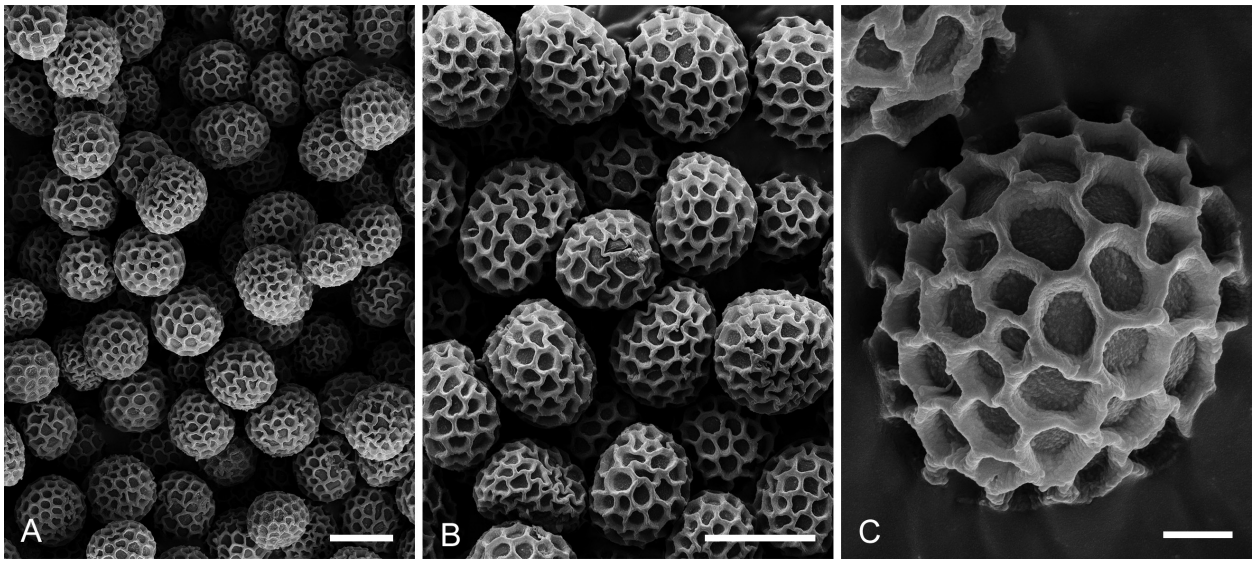

Fig. 3. *Microbotryum savilei* on *Silene uralensis* subsp. *arctica*. – A–C: spores in SEM. – Scale bars: A, B = 5 µm; C = 1 µm.

Description — Infection systemic. *Sori* in deformed and swollen flowers (Fig. 2A, B, E), enclosed by corolla (petals remain within closed calyx) (Fig. 2C, D), affecting anthers and filaments; stamens may or may not be completely destroyed; ovary and styles aborted (Fig. 2D). Calyx of infected flowers erect and closed at throat, broadly ovoid or very broadly ovoid (with approximate height/width ratio 0.8–1.2) (Fig. 2A, B, E). Spore mass filling affected flower completely, enclosed by corolla; pulverulent, dark livid or dark purple (Fig. 2C). *Spores* subglobose, globose or broadly ellipsoidal, sometimes ellipsoidal, (5.5–)6–9(–10) × (5–)5.5–8(–9) (7.4 ± 0.7 × 6.7 ± 0.6) µm ($n/_1$ = 300), length/width ratio 1.08–1.10, light vinaceous; wall reticulate, 1–1.7(–2) µm thick (including reticulum); meshes (4 or)5–8(or 9) per spore diameter, polyhedral or irregular, 0.4–1.8(–2.5) µm long; muri (15 or)16–20(–22) on equatorial circumference, to 0.4(–0.5) µm high; meshes rugulose on bottom in SEM (Fig. 3A–C). *Spore germination* unknown.

Host plant and distribution — On *Caryophyllaceae*: *Silene* sect. *Physolychnis* (Benth.) Bocquet: *S. uralensis* subsp. *arctica*, North America (Canadian Arctic). Known only from the type locality (Fig. 5).

Remarks — The paratype was collected by the same collectors and at the same site as the holotype, but, according to Savile's note (placed inside the herbarium packet), the infected plants of the paratype are white-flowered.

Based on our phylogenetic and morphological studies, we propose a new species of *Microbotryum* on *Silene uralensis* subsp. *arctica*.

Microbotryum arcticum T. Denchev, Denchev, Kemler & Begerow, **sp. nov.** – Fig. 4.
Index Fungorum number: IF 555874.

Holotype: on *Silene uralensis* subsp. *arctica*, Greenland, Peary Land, 10 km NW of Mudderbugt, just S of Ndr. Ladegårdså, 82°29–30'N, 21°30–35'W, 7 Aug 1991, *B. Fredskild 91-433* (SOMF 29 999).

Diagnosis — Microbotryum arcticum differs morphologically from *M. savilei* in soral development, restricted to the anthers, with presence of intact filaments (vs formation of swollen and deformed flowers, completely filled with a spore mass, and soral development not only in the anthers but also in the filaments, which are morphologically affected; Table 3), and by having spores with lower maximum and mean values of the spore length and width, to 7.5(–8.5) µm long (6.4 ± 0.5) and to 6.5(–7.5) µm wide (5.9 ± 0.4) vs to 9(–10) µm long (7.4 ± 0.7) and to 8(–9) µm wide (6.7 ± 0.6) for *M. savilei* (Table 2). *Microbotryum arcticum* is distinguished from the other anther-infecting *Microbotryum* species by molecular data, based on ITS sequence (deposited in the NCBI nucleotide database with accession no. MK474659) and LSU sequence (accession no. MK474658) derived from the holotype, and ITS sequence (accession no. MK474660) derived from a paratype (SOMF 29 998).

Description — Infection systemic. *Sori* in considerably swollen anthers, filling pollen sacs with a pulverulent, dark livid or livid vinaceous spore mass. *Spores* subglobose, globose, broadly ellipsoidal or ovoid, sometimes ellipsoidal or slightly irregular, (5–)5.5–7.5(–8.5) × (4.5–)5–6.5(–7.5) (6.4 ± 0.5 × 5.9 ± 0.4) µm ($n/_{14}$ = 1600), length/width ratio 1.06–1.12, pale vinaceous; wall reticulate, 0.8–1.3(–1.5) µm thick (including reticulum); meshes 5–8(or 9) per spore diameter, polyhedral or irregular, 0.3–1(–1.5) µm long; muri (15 or)16–21(–23) on equatorial circumference, to 0.4 µm high; meshes smooth or rugulose on bottom in SEM (Fig. 4H–J). *Spore germination* (Parmelee, unpublished notes and photo-

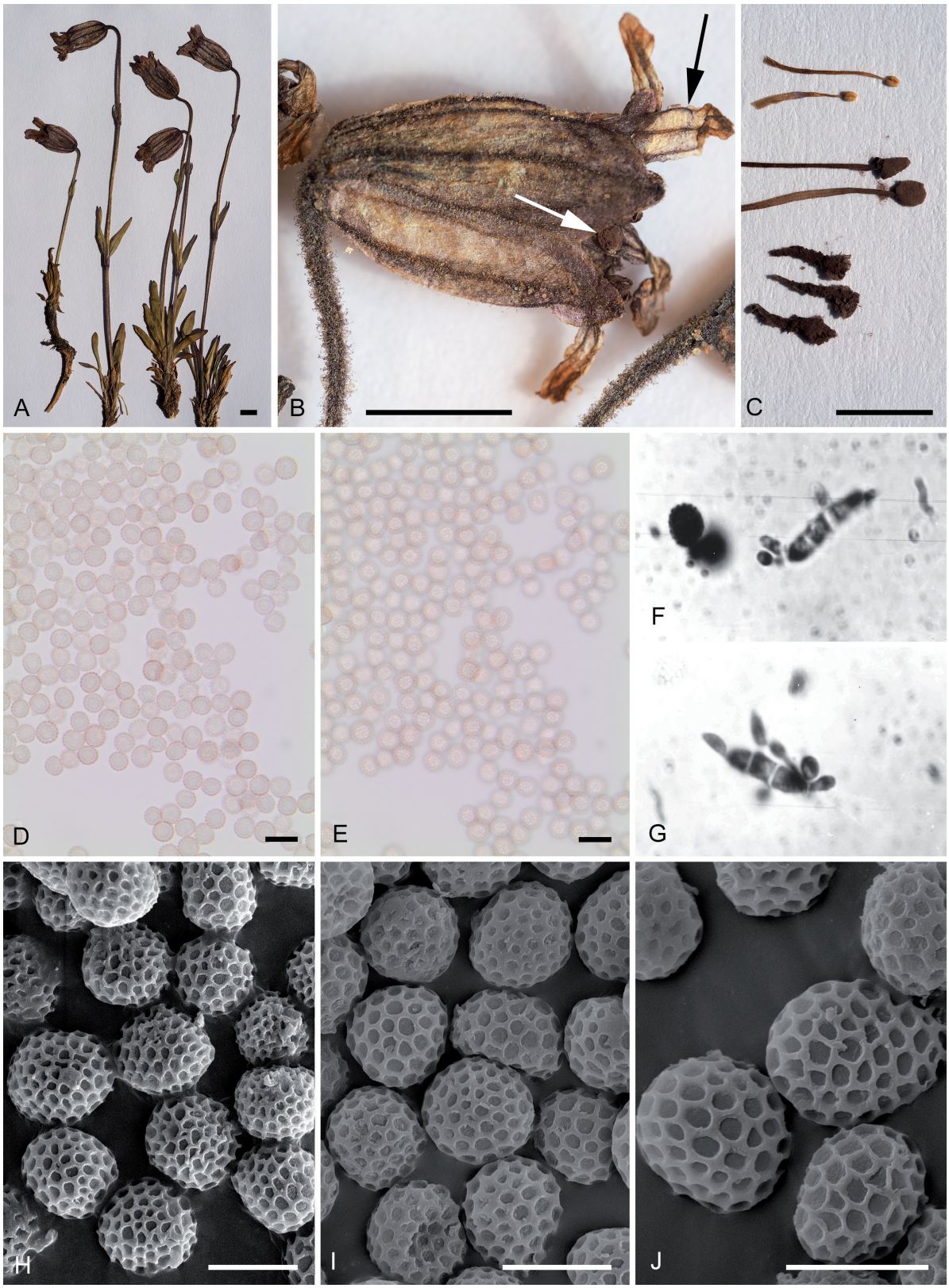

Fig. 4. *Microbotryum arcticum* on *Silene uralensis* subsp. *arctica*. – A: habit (DAOM 66883); note nodding, open and not deformed flowers; B: habit (holotype); note petals emerging from calyx (black arrow) and affected anther (white arrow); C: stamens of healthy plants of *S. uralensis* subsp. *arctica* (top, DAOM 93735), plants infected by *M. arcticum* (middle, DAOM 66883) and plants infected by *M. savilei* (bottom, holotype); D, E: spores in LM, in median and surface view, respectively (holotype); F, G: germination of spores (unpublished photographs by J. A. Parmelee in herbarium packet of DAOM 93735; used by permission of J. Parmelee [17 Jan 2019] and S. Redhead, Curator of DAOM); H–J: spores in SEM (H: Inglefield Land; I, J: Washington Land). – Scale bars: A–C = 0.5 cm; D, E = 10 µm; H–J = 5 µm.

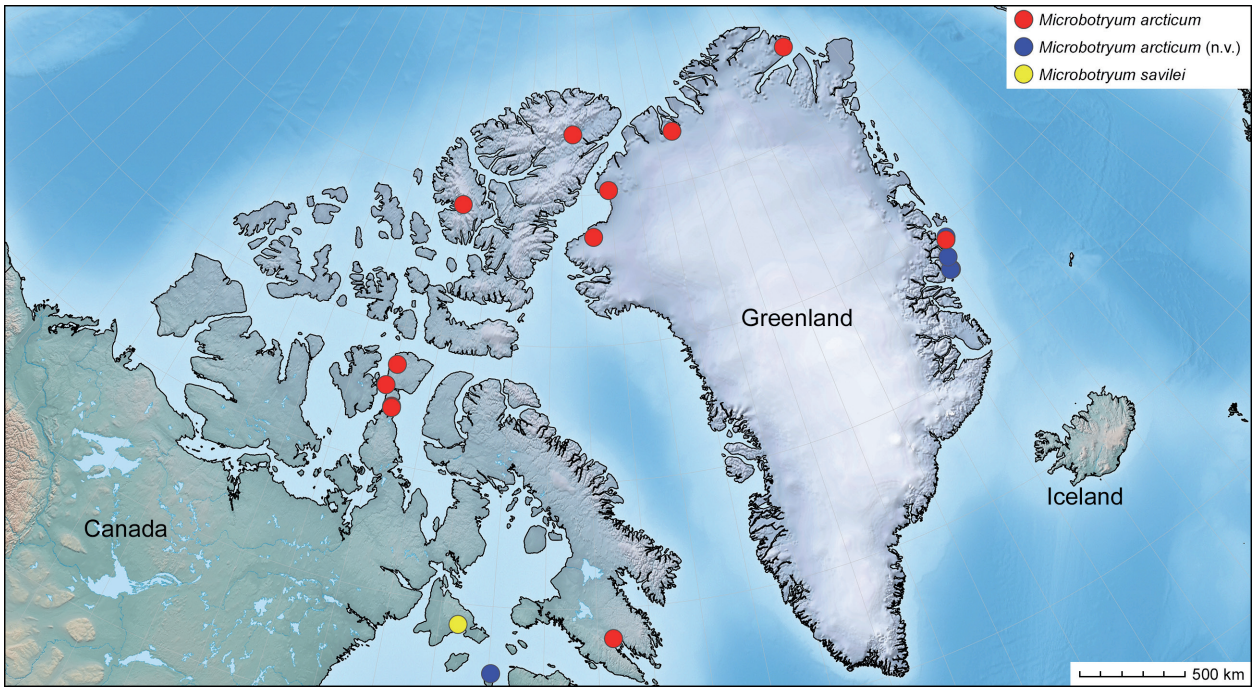

Fig. 5. Geographic distribution of *Microbotryum arcticum* and *M. savilei* (generated with SimpleMappr, Shorthouse 2010).

graphs in herbarium packet of DAOM 93735) results in a 4-celled basidium, separating during basidiospore formation as a 3-celled basidium (1 cell remaining attached to teliospore) and producing basidiospores laterally and terminally (Fig. 4F, G).

Host plant and distribution — On *Caryophyllaceae*: *Silene* sect. *Physolychnis*: *S. uralensis* subsp. *arctica*, North America (Canadian Arctic and Greenland, with localities at 82°30'N–61°35'N) (Fig. 5).

Additional specimens examined (paratypes) — On *Silene uralensis* subsp. *arctica*. — Canada: Nunavut, Qikiqtaaluk Region, Ellesmere Island, 2 mi NNW of Hazen Camp, 81°49'N, 71°21'W, alt. 1400 ft, 17 Jul 1962, *D. B. O. Savile 4656* (DAOM 02-01000869753/DAOM 92911; in Savile & Parmelee 1964: 711 as "*U[stilago]. violacea* var. *violacea*"); Ellesmere Island, 6 mi WSW of Hazen Camp, 81°49'N, 71°21'W, alt. 1500 ft, 10 Jul 1962, *D. B. O. Savile 4601* (DAOM 02-01000869752/DAOM 92910; in Savile & Parmelee 1964: 711 as "*U. violacea* var. *violacea*"); Axel Heiberg Island, Jacobsen-McGill Base Camp, on margins of Colour Lake, 79°25'N, 90°30'W, 7 Aug 1961, *J. A. Parmelee 2215* (DAOM 02-01000869751/DAOM 93735; in Savile & Parmelee 1964: 711 as "*U. violacea* var. *violacea*"); Somerset Island, Aston Bay, 73°39'N, 94°45'W, 8 Aug 1958, *D. B. O. Savile 3729* (DAOM 02-01000869750/DAOM 60120; in Savile 1959: 983 as "*U. violacea* var. *violacea*"); Somerset Island, Four Rivers Bay, 72°50'N, 95°30'W, 31 Jul 1958, *D. B. O. Savile 3717* (DAOM 02-01000869749/DAOM 60135; in Savile 1959: 983 as "*U. violacea* var. *violacea*"); Somerset Island, 2 mi NNW of Base Camp, 72°07'N, 94°12'W, 27 Jul 1958, *D. B. O. Savile 3628* (DAOM 02-01000869745/DAOM 60165; in Savile 1959: 983 as "*U. violacea* var. *violacea*"); Somerset Island, N slope of Hazard Inlet, 72°04'N, 94°10'W, 22 Jul 1958, *D. B. O. Savile 3583* (DAOM 02-01000869746/DAOM 59984; in Savile 1959: 983 as "*U. violacea* var. *violacea*"); Baffin Island, Frobisher Bay, 63°45'N, 68°32'W, 10 Jul 1948, *H. A. Senn 3868 & J. A. Calder* (DAOM 02-01000869747/DAOM 60136); ibid., Frobisher Bay, 10 Aug 1959, *D. B. O. Savile 3925, J. A. Calder & I. Kukkonen* (DAOM 02-01000869748/DAOM 66883, as *U. violacea* var. *stellariae*). — Greenland: Warming Land, GGU (Grønlands Geologiske Undersøgelse) Base Camp, 81°32'N, 51°31'W, 13 Aug 1985, *C. Bay 85-434* (C-Greenland herb. s.n.); Washington Land, Cass Fjord, Nygaard Bugt, 80°06'N, 65°10'W, alt. 10 m, 5 Aug 1976, *P. Frykman & B. Fredskild s.n.* (C-Greenland herb. s.n.); Inglefield Land, central inland, plain plateau, 78°40'N, 68°18'W, alt. 450 m, 16 Aug 1999, *J. Feilberg 534* (SOMF 29 998 ex C-Greenland herb. s.n.); Wollaston Forland, Landingsdalen, 74°27.5'N, 19°03.1'W, 28 Jul 1929, *J. Vaage s.n.* (O-V-688113).

Literature record (specimen not seen) — On *Silene uralensis* subsp. *arctica.* – Canada: Nunavut, Qikiqtaaluk Region, Hudson Bay, Mansel Island (located at c. 61°35'–62°25'N), 30 Aug 1884, *R. Bell 2621* (CAN 54784, n.v., host as "*Lychnis nesophila* Holm"; in Savile 1959: 994 as "*U[stilago]. violacea*") (Fig. 5).

Etymology — The epithet refers to the distribution of the species in the High Arctic of Greenland and in the Canadian Arctic Archipelago.

Table 1. *Microbotryum* herbarium specimens and NCBI nucleotide database accession numbers used for phylogenetic analysis (newly generated sequences indicated in boldface).

Species	Host	Specimen	ITS accession no.	LSU accession no.
M. adenopetalae	*Silene adenopetala*	KRAM F-55201	DQ366848	DQ366876
M. anomalum	*Fallopia baldschuanica*	M-0066114	DQ238721	EF621956
M. anomalum	*Fallopia baldschuanica*	TUB 015850	EF621919	EF621958
M. anomalum	*Fallopia convolvulus*	B 70 0007554	EF621918	EF621957
M. anomalum	*Fallopia convolvulus*	GLM 59392	EF621921	EF621960
M. anomalum	*Fallopia dumetorum*	GLM 47018	EF621920	EF621959
***M. arcticum* sp. nov.**	*Silene uralensis* subsp. *arctica*	SOMF 29 999	**MK474659**	**MK474658**
***M. arcticum* sp. nov.**	*Silene uralensis* subsp. *arctica*	SOMF 29 998	**MK474660**	–
M. bardanense	*Silene moorcroftiana*	KRAM F-54962	DQ366856	DQ366877
M. chloranthae-verrucosum	*Silene chlorantha*	B 70 0006053	AY877421	DQ366883
M. chloranthae-verrucosum	*Silene chlorantha*	B 70 0007571	AY877404	DQ366878
M. coronariae	*Silene flos-cuculi*	KR 23797	KC684887	KC684886
M. coronariae	*Silene flos-cuculi*	TUB 012115	AY877417[1]	KC684885
M. dianthorum s. lat.	*Dianthus jacquemontii*	KRAM F-54963	DQ366844	DQ366869
M. dianthorum s. lat.	*Dianthus monspessulanus*	TUB 011802	AY588080	DQ366871
M. dianthorum s. lat.	*Petrorhagia saxifraga*	TUB 012106	DQ366845	DQ366866
M. heliospermae	*Silene pusilla*	B 70 0006060	AY877411	DQ366881
M. heliospermae	*Silene pusilla*	TUB 019570	HQ832086	HQ832087
M. heliospermae	*Silene pusilla*	TUB 019571	HQ832084	HQ832085
M. heliospermae	*Silene pusilla*	TUB 019572	HQ832082	HQ832083
M. lagerheimii	*Silene rupestris*	KR 22655	HQ832090	HQ832091
M. lagerheimii	*Silene rupestris*	TUB 011817	AY588100	DQ366874
M. lagerheimii	*Silene rupestris*	TUB 019573	HQ832089	HQ832088
M. lychnidis-dioicae	*Silene baccifera*	GLM 47387	DQ640064	DQ640067
M. lychnidis-dioicae	*Silene baccifera*	GLM 49244	DQ640066	DQ640068
M. lychnidis-dioicae	*Silene latifolia*	TUB 011795	AY588096	DQ366886
M. lychnidis-dioicae	*Silene latifolia*	TUB 011796	AY588097	DQ366865
M. lychnidis-dioicae	*Silene latifolia*	TUB 015865	EF621936	EF621984
M. lychnidis-dioicae	*Silene zawadzkii*	TUB 012521	DQ366847	DQ366860
M. majus	*Silene otites*	B 70 0007572	AY877418	EF621986
M. majus	*Silene otites*	B 70 0006042	AY877419	DQ366858
M. minuartiae	*Minuartia recurva*	TUB 012518	DQ366852	DQ366863
M. minuartiae	*Minuartia recurva*	TUB 012519	DQ366853	DQ366862
M. saponariae	*Saponaria officinalis*	TUB 011809	AY588089	DQ366887
M. saponariae	*Saponaria officinalis*	TUB 015873	EF621948	EF622001
M. saponariae	*Saponaria officinalis*	TUB 015874	EF621949	EF622002
M. saponariae	*Saponaria pumila*	TUB 011812	AY588091	DQ366864
M. shykoffianum	*Dianthus carthusianorum*	TUB 011801	AY588079	DQ366889
M. shykoffianum	*Dianthus sylvestris*	TUB 015863	EF621935[2]	EF621979[2]
M. shykoffianum	*Dianthus sylvestris*	TUB 015862	EF621934[2]	EF621980[2]
M. shykoffianum	*Dianthus sylvestris*	TUB 011800	AY588082	DQ366857
M. silenes-acaulis	*Silene acaulis*	HUV 13955	DQ366854	DQ366888
M. silenes-acaulis	*Silene acaulis*	TUB 012515	DQ366849	DQ366885
M. silenes-acaulis	*Silene acaulis*	TUB 012522	DQ366846	DQ366870
M. silenes-acaulis	*Silene acaulis*	TUB 019585	JN223408	JN223413
M. silenes-acaulis	*Silene acaulis*	TUB 019586	JN223406	JN223411
M. silenes-acaulis	*Silene acaulis*	TUB 019587	JN223407	JN223412

[1] as *M.* aff. *violaceum* in NCBI

[2] as *M. dianthorum* in NCBI

continued on next page

continued from previous page

Species	Host	Specimen	ITS accession no.	LSU accession no.
M. silenes-dioicae	*Silene dioica*	TUB 011798	AY588094	DQ366859
M. silenes-dioicae	*Silene dioica*	TUB 012114	AY877416	DQ366868
M. silenes-dioicae	*Silene dioica*	TUB 015864	EF621937[3]	EF621985[3]
M. silenes-inflatae	*Silene uniflora*	KRAM F-59020	KY321304	KY321305
M. silenes-inflatae	*Silene uniflora*	TUB 019588	JN223405	JN223410
M. silenes-inflatae	*Silene uniflora*	TUB 019589	JN223404	JN223409
M. silenes-inflatae	*Silene vulgaris*	TUB 011793	AY588105	DQ366884
M. silenes-inflatae	*Silene vulgaris*	TUB 011792	AY588106	DQ366879
M. silenes-saxifragae	*Silene saxifraga*	HUV 19570	JN000074	JN000078
M. silenes-saxifragae	*Silene saxifraga*	KR 23889	JN000073	JN000079
M. silenes-saxifragae	*Silene saxifraga*	KR 23890	JN000071	JN000075
M. silenes-saxifragae	*Silene saxifraga*	KRAM F-49440	JN000072	JN000080
M. silenes-saxifragae	*Silene saxifraga*	TUB 011790	AY588101	JN000076
M. silenes-saxifragae	*Silene saxifraga*	TUB 011791	AY588102	JN000077
M. stellariae	*Stellaria graminea*	TUB 011807	AY588109	DQ366872
M. stellariae	*Stellaria graminea*	TUB 011808	AY588108	DQ366873
M. superbum	*Dianthus superbus*	TUB 011799	AY588081	DQ366867
M. violaceoirregulare	*Silene vulgaris*	TUB 011816	AY588104	DQ366875
M. violaceoverrucosum	*Silene viscosa*	TUB 011815	AY588103	DQ366882
M. violaceum	*Silene nutans*	GLM 49283	DQ640071	DQ640069
M. violaceum	*Silene nutans*	GLM 50283	DQ640065	DQ640070
M. violaceum	*Silene nutans*	TUB 011818	AY588099	DQ366880

[3] as *M. lychnidis-dioicae* in NCBI

Discussion

For the first time from Greenland, anther-smut fungal infection of *Silene uralensis* subsp. *arctica* was reported by Hagen (1947, as *Ustilago violacea* on *Melandrium apetalum* (L.) Fenzl), based on the following collections:

Greenland: Sabine Island, near Germania Havn (on the south side of the island, c. 74°32.2'N, 18°49.9'W), 22 Jul 1932 & 16 Aug 1932, *S. Aandstad s.n.* (n.v.), and 21 Jul 1933, *A. Hagen, the Norwegian Expedition to NE Greenland 1933 s.n.* (n.v.); Gael Hamke Bugt, Jackson Island, c. 73°55'N, 11 Aug 1933, *A. Hagen, the Norwegian Expedition to NE Greenland 1933 s.n.* (n.v.); Hold with Hope, Stormdalen, c. 73°29.5'N, 20°46.9'W, 9 Aug 1933, *A. Hagen, the Norwegian Expedition to NE Greenland 1933 s.n.* (n.v.); Troldsøen (as "Trollvatnet"), c. 73°29'N, 20°39'W, 9 Aug 1933, *A. Hagen, the Norwegian Expedition to NE Greenland 1933 s.n.* (n.v.) (Fig. 5); as well as on the last specimen in the above-mentioned list of paratypes of *Microbotryum arcticum*.

For six of these collections, no voucher specimens were found in the herbarium in Oslo (O) (K.-H. Larsson, pers. comm.), although we assume that they represent *Microbotryum arcticum*, as Hagen's spore measurements (5–8 µm in diam.) correspond to our data.

The host plant of the Canadian specimens of *Microbotryum arcticum* listed here was originally identified by D. B. O. Savile and J. A. Parmelee (coll. no. 2215) as "*Lychnis apetala* var. *arctica* (Th. Fr.) Cody". The infected plant specimens listed from North Greenland (deposited in C) were originally identified as "*Melandrium apetalum* subsp. *arcticum* (Th. Fr.) Hultén" or "*Silene uralensis* subsp. *apetala* (L.) Bocquet" (coll. no. 534). All of these are considered here as *S. uralensis* subsp. *arctica*, in accordance with Elven & al. (2011).

The taxonomic treatments of this plant vary considerably. *Silene uralensis* (Rupr.) Bocquet (*S.* sect. *Physolychnis*) is a very variable species complex (Morton 2005) with not completely clarified specific and infraspecific delimitation. In Bocquet's treatment of *S.* sect. *Physolychnis* (1967), four subspecies were recognized within *S. uralensis*, namely subsp. *uralensis*, subsp. *apetala*, subsp. *arctica* and subsp. *porsildii* Bocquet (the last one a tetraploid taxon). The populations in Svalbard were treated as an endemic subsp. *arctica*, while those in Scandinavia and the islands of the Bering Sea were recognized as subsp. *apetala* (*S. wahlbergella* Chowdhuri). The remaining populations were referred to subsp. *uralensis* (with a northern circumpolar distribution). Hultén (1968) accepted two subspecies: *Melandrium apetalum* subsp. *arcticum*, mapped by him with a circumpolar distribution, and subsp. *apetalum* from Scandinavia. In *Flora nordica* (Jonsell 2001), however, the Fennoscandian plants were treated as a distinct species, *S. wahlbergella*, and, ac-

Table 2. Comparative morphological data for *Microbotryum arcticum* and *M. savilei*, causing anther infection and atypical infection, respectively, on *Silene uralensis* subsp. *arctica*.

Species/specimens	Spore length (L) (µm)	Spore width (W) (µm)	M ± 1σ L	M ± 1σ W
Microbotryum arcticum				
Peary Land (holotype, SOMF 29 999)	(5–)5.5–7.5(–8)	(4.5–)5–6.5(–7)	6.4 ± 0.5	5.9 ± 0.4
Warming Land (C-Greenland herb.)	5.5–7.5(–8.5)	(4.5–)5–7(–7.5)	6.6 ± 0.5	6.2 ± 0.5
Washington Land (C-Greenland herb.)	5.5–7.5(–8)	(4.5–)5–6.5(–7)	6.4 ± 0.4	6.0 ± 0.3
Inglefield Land (C-Greenland herb.)	(5–)5.5–7.5(–8.5)	(4.5–)5–7(–7.5)	6.6 ± 0.5	6.2 ± 0.4
Wollaston Forland (O-V-688113)	(5–)5.5–7.5(–8)	(4.5–)5–7(–7.5)	6.5 ± 0.7	6.1 ± 0.6
Ellesmere Is. (DAOM 02-01000869753)	5.5–7.5(–8.5)	5–6.5(–7.5)	6.4 ± 0.5	5.8 ± 0.4
Ellesmere Is. (DAOM 02-01000869752)	(5–)5.5–7.5(–8.5)	(4.5–)5–6.5(–7)	6.2 ± 0.6	5.6 ± 0.4
Axel Heiberg Is. (DAOM 02-01000869751)	(5–)5.5–7.5(–8.5)	(4.5–)5–6.5(–7)	6.3 ± 0.5	5.7 ± 0.4
Somerset Is. (DAOM 02-01000869750)	(5–)5.5–7.5(–8.5)	(4.5–)5–6.5(–7.5)	6.4 ± 0.8	5.8 ± 0.6
Somerset Is. (DAOM 02-01000869749)	(5–)5.5–7(–7.5)	(4.5–)5–6.5(–7)	6.1 ± 0.4	5.6 ± 0.4
Somerset Is. (DAOM 02-01000869745)	(5.5–)6–7.5(–8.5)	(4.5–)5–6.5(–7.5)	6.4 ± 0.5	5.8 ± 0.4
Somerset Is. (DAOM 02-01000869746)	(5.5–)6–7.5(–8)	5–6.5(–7)	6.4 ± 0.5	5.8 ± 0.4
Baffin Is. (DAOM 02-01000869747)	(5–)5.5–7.5(–8)	(4.5–)5–6.5(–7)	6.3 ± 0.5	5.8 ± 0.4
Baffin Is. (DAOM 02-01000869748)	(5–)5.5–7.5(–8.5)	(4.5–)5–6.5(–7)	6.5 ± 0.6	5.8 ± 0.5
Microbotryum savilei				
Southampton Is. (holotype, DAOM 02-01000571594)	(5.5–)6–9(–10)	(5–)5.5–8(–9)	7.4 ± 0.7	6.7 ± 0.6

Table 3. Effect of *Microbotryum arcticum* and *M. savilei* on flower morphology of *Silene uralensis* subsp. *arctica.*

Flower condition	Healthy flowers	Anther infection (by *M. arcticum*)	Atypical infection (by *M. savilei*)
Habit	nodding in flowering stage	nodding throughout summer, not deformed	erect, deformed
Pigmentation	light	abnormally deeply pigmented	n/a
Filling with a spore mass	spore mass absent	spore mass restricted to anthers	flowers completely filled with a spore mass
Calyx orientation and shape	nodding, inflated, in flowering stage with height/width ratio c. 1.5	nodding, more elongated than in healthy flowers	erect, swollen, broadly ovoid or very broadly ovoid, with height/width ratio 0.8–1.2
Calyx at throat	open	more open at throat than in healthy flowers	closed at throat
Corolla	petals emerging from calyx	petals emerging more from calyx than in healthy flowers	petals remaining within closed calyx and never emergent
Stamens	developed	anthers somewhat misshapen, filled with teliospores; filaments intact; stamens not completely destroyed	anthers misshapen, filled with teliospores; filaments affected; stamens may or may not be completely destroyed
Ovary	developed	remaining rudimentary	remaining rudimentary
Styles	developed	remaining rudimentary	remaining rudimentary

cordingly, only the arctic plants from North America and Asia were considered related to *S. uralensis*. In the *Silene* treatment for *Flora of North America* (Morton 2005), three subspecies were recognized within the *S. uralensis* complex: subsp. *uralensis*, widespread with an arctic circumpolar distribution; subsp. *porsildii*, in Yukon, Alaska and Arctic Asia; and subsp. *ogilviensis* (A. E. Porsild) D. F. Brunt. from the Canadian Low Arctic. Of these, only subsp. *uralensis* was given as present in Greenland and the eastern Canadian Arctic Archipelago. Elven & al.

(2011) disagreed with Bocquet's view that subsp. *arctica* was restricted to Svalbard, and recognized three subspecies of *S. uralensis*: subsp. *uralensis*, with a circumpolar distribution (NE Europe, Arctic Asia, Bering Sea islands, Alaska, Canada and W and S Greenland); subsp. *arctica*, also with a circumpolar distribution (Arctic Far East of Russia, northernmost Alaska and Canada, Greenland and Svalbard); and subsp. *ogilviensis*.

Two subspecies of *Silene uralensis* are present in Greenland (according to the taxonomic scheme of Elven & al. 2011). *Silene uralensis* subsp. *uralensis* is characterized by a calyx that is not strongly inflated and usually longer than broad and by petals slightly emerging from the calyx, less so than in subsp. *arctica* (Elven & al. 2011). The calyx of *S. uralensis* subsp. *arctica* is inflated, in the flowering stage c. 1.5 times as long as broad (Alsos & al. 2018). In Greenland, there is an overlap in the ranges of the northern subsp. *arctica* and more southern subsp. *uralensis* at 70–71°N, but there are no obvious transitional plants (Elven & al. 2011). In the first half of the last century, the High Arctic entity in Greenland, subsp. *arctica*, was referred to as "*Melandrium apetalum*" (e.g. Kruuse 1905; Ostenfeld & Lundager 1910; Hartz & Kruuse 1911; Ostenfeld 1926; Porsild 1926). The southernmost localities of subsp. *arctica* are at 69°42'N in West Greenland (Porsild 1926) and 69°30'N in East Greenland (Kruuse 1905), while northward it reaches 83°06'N (Maguire 1950). Considering that subsp. *uralensis* is distributed only on the western and southern coasts of Greenland, the host plant of the *Microbotryum* specimens recorded by Hagen (1947) on "*Melandrium apetalum*" from East Greenland (at 73°29'–74°32'N) should be accepted as belonging to subsp. *arctica*.

The above-mentioned specimen from Mansel Island was examined by Savile (1959). The plant specimen is a type of *Lychnis nesophila* Holm (Holm 1907; see Aiken & al. 2007a). As was noted by Savile (1959: 983), this type is based on material of *Silene uralensis* subsp. *arctica* infected with a smut fungus causing typical anther infection. This smut fungus was not revised in the course of this study, but, based on the symptoms described by Savile, it may be presumed that it belongs to *Microbotryum arcticum*. This locality is the southernmost for *M. arcticum*.

Other Canadian specimens of "*Ustilago violacea*" on "*Melandrium apetalum* f. *arctica*" [sic] were recorded by Lind (1910), from Nunavut, King William Island (in the vicinity of Gjoa Haven, as "Gjöa Harbour", 68°37'38"N, 96°23'40"W, 2 Aug 1905, *C. H. Ostenfeld s.n.*), and by Liro (1924) and Savile (1957), from Nunavut, Ellesmere Island (Goose Fjord, c. 76°30'N, 88°35'W, 15 Aug 1901, *H. G. Simmons s.n.*, H). These records were not taken into account in this treatment, but probably they also belong to *Microbotryum arcticum*.

Microbotryum arcticum seems to be a common species on *Silene uralensis* subsp. *arctica* in the Canadian Arctic Archipelago. Under the name *Ustilago violacea* var. *violacea*, it was reported as a widespread smut fungus on this host at Queen Elizabeth Islands (Savile & Parmelee 1964) and throughout Somerset Island (Savile 1959).

On the basis of available information in Savile (1959), Kevan & Parmelee (1972), Aiken & al. (2007b), Elven & al. (2011), and Alsos & al. (2018), as well as our observations on the cited *Microbotryum* specimens from DAOM, contrastive morphological characters of the healthy flowers of *Silene uralensis* subsp. *arctica* are compared with those of flowers of the same plant with anther infection, and with flowers with atypical infection (Table 3).

Microbotryum arcticum causes typical anther infection, leading to the development of smutted anthers (Fig. 4B, C), which are filled with teliospores instead of fertile pollen grains.

Because the soral development of *Microbotryum savilei* has not yet been studied, the infection caused by this species is provisionally referred to here as atypical infection. A suite of characters distinguishes both species. *Microbotryum savilei* can be easily differentiated from the newly described species by the soral morphology and changes in the affected flowers (Table 3). The healthy plants of *S. uralensis* subsp. *arctica* (in the flowering stage) and the plants infected by *M. arcticum* have nodding flowers, whereas the plants infected by *M. savilei* have erect flowers. The flowers infected by *M. arcticum* are not deformed; they are open and not completely filled with a spore mass. The flowers infected by *M. savilei* are closed, deformed and swollen (more so than in the healthy flowers), with a broadly ovoid or very broadly ovoid calyx (with height/width ratio 0.8–1.2), and are completely filled with a spore mass. In the infected flowers, the petals remain within the closed calyx and are never emergent; the calyx is erect and closed at the throat; not only the anthers but also the filaments are affected (Fig. 2D, 4C); the stamens may or may not be completely destroyed; the ovary and styles remain rudimentary and no seeds are produced.

As transmission of the fungus is mediated by insects that are both vectors of the disease and pollinators of the plant (Kevan & Parmelee 1972; Biere & Honders 1996; Fontaine & al. 2013), the flower modifications due to *Microbotryum arcticum* (specifically, the deeply pigmented flower, dark-coloured spore mass in the smutted anthers, and presence of a more elongated calyx that is more open at the throat, and more exserted petals) could result in more insect visits, which is extremely important under the High Arctic conditions.

Acknowledgements

This study was funded by the Program for Support of Young Researchers and PhD Students at the Bulgarian Academy of Sciences (Grant no. DFNP-17-93/28.07.2017), and received support from the SYN-

THESYS Project http://www.synthesys.info/, which is financed by the European Community Research Infrastructure Action under the FP7 "Capacities" Program at the Natural History Museum of Denmark, University of Copenhagen (Grant no. DK-TAF-5927), the Natural History Museum, Vienna (Grant no. AT-TAF-6810) and the National Museum of Natural History, Paris (Grant no. FR-TAF-6628). The assistance of Dr Scott A. Redhead (Curator of DAOM), Dr Henning Knudsen (Curator of Fungi in the herbarium of the Natural History Museum of Denmark, C) and Prof. Karl-Henrik Larsson and Assoc. Prof. Charlotte S. Bjorå (curators in the herbarium of the Botanical Museum, University of Oslo, O), for sending specimens or providing access to the examined specimens, is kindly acknowledged. The authors are very grateful to Dr Eric H. C. McKenzie (Manaaki Whenua Landcare Research, Auckland), Prof. Roger G. Shivas (University of Southern Queensland, Toowoomba, Queensland) and Nicholas Turland (Botanic Garden and Botanical Museum Berlin) for critically reading the manuscript and helpful corrections and suggestions.

References

Aiken S. G., Dallwitz M. J., Consaul L. L., McJannet C. L., Boles R. L., Argus G. W., Gillett J. M., Scott P. J., Elven R., LeBlanc M. C., Gillespie L. J., Brysting A. K., Solstad H. & Harris J. G. 2007a: Type specimen of *Lychnis nesophila*. – In: Flora of the Canadian Arctic Archipelago. Ottawa: NRC Research Press, National Research Council of Canada. – Published at https://nature.ca/aaflora/images/casiurt1.jpg [accessed 27 Oct 2018].

Aiken S. G., Dallwitz M. J., Consaul L. L., McJannet C. L., Boles R. L., Argus G. W., Gillett J. M., Scott P. J., Elven R., LeBlanc M. C., Gillespie L. J., Brysting A. K., Solstad H. & Harris J. G. 2007b: *Silene uralensis* subsp. *arctica* (Th. Fr.) Bocquet. – In: Flora of the Canadian Arctic Archipelago. Ottawa: NRC Research Press, National Research Council of Canada. – Published at https://nature.ca/aaflora/data/www/casiur.htm [accessed 16 Oct 2018].

Alsos I. G., Arnesen G. & Elven R. 2018: The flora of Svalbard. – Published at http://www.svalbardflora.no [accessed 16 Oct 2018].

Antonovics J., Hood M. E. & Partain J. 2002: The ecology and genetics of a host shift: *Microbotryum* as a model system. – Amer. Naturalist **160:** S40–S53.

Antonovics J., Hood M. E., Thrall P. H., Abrams J. Y. & Duthie G. M. 2003: Herbarium studies on the distribution of anther-smut fungus (*Microbotryum violaceum*) and *Silene* species (*Caryophyllaceae*) in the eastern United States. – Amer. J. Bot. **90:** 1522–1531.

Bauer R., Begerow D., Sampaio J. P., Weiß M. & Oberwinkler F. 2006: The simple-septate basidiomycetes: a synopsis. – Mycol. Progr. **5:** 41–66.

Bauer R., Oberwinkler F. & Vánky K. 1997: Ultrastructural markers and systematics in smut fungi and allied taxa. – Canad. J. Bot. **75:** 1273–1314.

Begerow D., Bauer R. & Oberwinkler F. 1998 ["1997"]: Phylogenetic studies on nuclear large subunit ribosomal DNA sequences of smut fungi and related taxa. – Canad. J. Bot. **75:** 2045–2056.

Bernasconi G., Antonovics J., Biere A., Charlesworth D., Delph L. F., Filatov D., Giraud T., Hood M. E., Marais G. A. B., McCauley D., Pannell J. R., Shykoff J. A., Vyskot B., Wolfe L. M. & Widmer A. 2009: *Silene* as a model system in ecology and evolution. – Heredity **103:** 5–14.

Biere A. & Honders S. 1996: Host adaptation in the anther smut fungus *Ustilago violacea* (*Microbotryum violaceum*): infection success, spore production and alteration of floral traits on two host species and their F1-hybrid. – Oecologia **107:** 307–320.

Bocquet G. 1967: Physolychnidium olim Gastrolychnidium nomenclaturae fundamentum includens combinationes taxaque nova nonnulla Silenes generis. – Candollea **22:** 1–38.

Branco S., Carpentier F., Rodríguez de la Vega R. C., Badouin H., Snirc A., Le Prieur S., Coelho M. A., de Vienne D. M., Hartmann F. E., Begerow D., Hood M. E. & Giraud T. 2018: Multiple convergent supergene evolution events in mating-type chromosomes. – Nat. Commun. **9:** 2000 [1–13].

Castresana J. 2000: Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. – Molec. Biol. Evol. **17:** 540–552.

Chlebicki A. & Suková M. 2005: Two *Microbotryum* species from the Himalayas. – Mycotaxon **93:** 149–154.

Deml G. & Oberwinkler F. 1982: Studies in *Heterobasidiomycetes*. Part 24. On *Ustilago violacea* (Pers.) Rouss. from *Saponaria officinalis* L. – Phytopathol. Z. **104:** 345–356.

Deml G. & Oberwinkler F. 1983: Studies in *Heterobasidiomycetes*. Part 31. On the anther smuts of *Silene vulgaris* (Moench) Garcke. – Phytopathol. Z. **108:** 61–70.

Denchev C. M. 2007a: *Microbotryum lagerheimii* sp. nov. (*Microbotryaceae*). – Mycol. Balcan. **4:** 62–67.

Denchev C. M. 2007b: *Microbotryum savilei* sp. nov. (*Microbotryaceae*). – Mycol. Balcan. **4:** 69–73.

Denchev C. M. & Denchev T. T. 2011: New records of smut fungi. 4. *Microbotryum coronariae* comb. nov. – Mycotaxon **118:** 53–56.

Denchev C. M., Giraud T. & Hood M. E. 2009: Three new species of anthericolous smut fungi on *Caryophyllaceae*. – Mycol. Balcan. **6:** 79–84.

Elven R., Murray D. F. & Petrovsky V. V. 2011: *Silene uralensis*. – In: Elven R. (ed.), Annotated checklist of the Panarctic Flora (PAF). Vascular plants, version 1.0. – Published at https://nhm2.uio.no/paf/ [accessed 16 Oct 2018].

Fischer G. W. 1953: Manual of the North American smut fungi. – New York: Ronald Press Co.

Fontaine M. C., Gladieux P., Hood M. E. & Giraud T. 2013: History of the invasion of the anther smut pathogen on *Silene latifolia* in North America. – New Phytol. **198:** 946–956.

Garber E. D. & Ruddat M. 2002: Transmission genetics of *Microbotryum violaceum* (*Ustilago violacea*): a case study. – Advances Appl. Microbiol. **51:** 107–127.

Gardes M. & Bruns T. D. 1993: ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. – Molec. Ecol. **2:** 113–118.

Giraud T., Yockteng R., López-Villavicencio M., Refrégier G. & Hood M. E. 2008: Mating system of the anther smut fungus *Microbotryum violaceum*: selfing under heterothallism. – Eukaryot. Cell **7:** 765–775.

Gouy M., Guindon S. & Gascuel O. 2010: SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. – Molec. Biol. Evol. **27:** 221–224.

Hagen A. 1947: *Ustilagineae* from East Greenland. – Sydowia **1:** 283–288.

Hartz N. & Kruuse C. 1911: The vegetation of Northeast Greenland 69°25' lat. n. – 75° lat. n. of East Greenland. – Meddel. Grønland **30:** 333–431.

Holm C. T. 1907: New plants from Arctic North America. – Repert. Spec. Nov. Regni Veg. **3:** 337–338.

Hood M. E., Mena-Alí J. I., Gibson A. K., Oxelman B., Giraud T., Yockteng R., Arroyo M. T. K., Conti F., Pedersen A. B., Gladieux P. & Antonovics J. 2010: Distribution of the anther-smut pathogen *Microbotryum* on species of the *Caryophyllaceae*. – New Phytol. **187:** 217–229.

Hultén E. 1968: Flora of Alaska and neighboring territories. – Stanford: Stanford University Press.

Jonsell B. (ed.) 2001: Flora nordica **2.** – Stockholm: The Bergius Foundation.

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 Res. **30:** 3059–3066.

Katoh K. & Standley D. M. 2013: MAFFT multiple sequence alignment software version 7: improvements in performance and usability. – Molec. Biol. Evol. **30:** 772–780.

Kazama Y., Koizumi A., Uchida W., Ageez A. & Kawano S. 2005: Expression of the floral B-function gene *SLM2* in female flowers of *Silene latifolia* infected with the smut fungus *Microbotryum violaceum*. – Pl. Cell Physiol. **46:** 806–811.

Kemler M., Göker M., Oberwinkler F. & Begerow D. 2006: Implications of molecular characters for the phylogeny of the *Microbotryaceae* (*Basidiomycota*: *Urediniomycetes*). – B. M. C. Evol. Biol. **6:** 35.

Kevan P. G. & Parmelee J. A. 1972: Insect-flower-fungus relationships for the transmission of the smut, *Ustilago violacea* by flower-visiting insects in the High Arctic. – Greenhouse-Garden-Grass **11(1):** 6–13.

Kruuse C. 1905: List of the phanerogams and vascular cryptogams found on the coast 75°–66°20' Iat. N of East Greenland. – Meddel. Grønland **30:** 143–208.

Le Gac M., Hood M. E., Fournier E. & Giraud T. 2007: Phylogenetic evidence of host-specific cryptic species in the anther smut fungus. – Evolution **61:** 15–26.

Lind J. 1910: Fungi (micromycetes), collected in Arctic North America (King William Land, King Point and Herschel Isl.) by the Gjöa Expedition under Captain Roald Amundsen, 1904–1906. – Kongel. Danske Vidensk. Selsk. Skr., I. Math.-Nat. Klasse **1909(9):** 1–25 + pl.

Lindeberg B. 1959: *Ustilaginales* of Sweden (exclusive of the Cintractias on *Caricoideae*). – Symb. Bot. Upsal. **16(2):** 1–175.

Liro J. I. 1924: Die Ustilagineen Finnlands I. – Ann. Acad. Sci. Fenn., Ser. A, **17(1):** 1–636.

Lutz M., Göker M., Piątek M., Kemler M., Begerow D. & Oberwinkler F. 2005: Anther smuts of *Caryophyllaceae*: molecular characters indicate host-dependent species delimitation. – Mycol. Progr. **4:** 225–238.

Lutz M., Piątek M., Kemler M., Chlebicki A. & Oberwinkler F. 2008: Anther smuts of *Caryophyllaceae*: molecular analyses reveal further new species. – Mycol. Res. **112:** 1280–1296.

Maguire B. 1950: Studies in the *Caryophyllaceae* IV: a synopsis of the North American species of the subfamily *Silenoideae*. – Rhodora **52:** 233–245.

Morton J. K. 2005: *Silene* L. – Pp. 166–214 in: Flora of North America Editorial Committee (ed.), Flora of North America **5.** – New York: Oxford University Press.

Nannfeldt J. A. 1959: On the anther smuts on the *Caryophyllaceae*. – In: Lindeberg B., *Ustilaginales* of Sweden (exclusive of the Cintractias on *Caricoideae*). – Symb. Bot. Upsal. **16(2):** 159–160.

O'Donnell K. 1992: Ribosomal DNA internal transcribed spacers are highly divergent in the phytopathogenic ascomycete *Fusarium sambucinum* (*Gibberella pulicaris*). – Curr. Genet. **22:** 213–220.

O'Donnell K. L. 1993: *Fusarium* and its relatives. – Pp. 225–233 in: Taylor J. W. & Reynolds D. R. (ed.), The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics. – Wallingford: CAB International.

Ostenfeld C. H. 1926: The flora of Greenland and its origin. – Kongel. Danske Vidensk. Selsk., Biolog. Meddel. **6(3):** 1–71.

Ostenfeld C. H. & Lundager A. 1910: List of vascular plants from North-East Greenland (N. of 76°N lat.) collected by the Danmark-Expedition 1906–1908. – Meddel. Grønland **43:** 4–32 + pl. I–VI.

Perlin M. H., Amselem J., Fontanillas E., Toh S. S., Chen Z., Goldberg J., Duplessis S., Henrissat B., Young S., Zeng Q., Aguileta G., Petit E., Badouin H., Andrews J., Razeeq D., Gabaldón T., Quesneville H., Giraud T., Hood M. E., Schultz D. J. & Cuomo C. A. 2015:

Sex and parasites: genomic and transcriptomic analysis of *Microbotryum lychnidis-dioicae*, the biotrophic and plant-castrating anther smut fungus. – B. M. C. Genomics **16:** 461.

Persoon C. H. 1797: Tentamen dispositionis methodicae fungorum […]. – Lipsiae: apud Petrum Philippum Wolf.

Petit E., Silver C., Cornille A., Gladieux P., Rosenthal L., Bruns E., Yee S., Antonovics J., Giraud T. & Hood M. E. 2017: Co-occurrence and hybridization of anther-smut pathogens specialized on *Dianthus* hosts. – Molec. Ecol. **26:** 1877–1890.

Piątek M., Lutz M. & Kemler M. 2013: *Microbotryum silenes-saxifragae* sp. nov. sporulating in the anthers of *Silene saxifraga* in southern European mountains. – IMA Fungus **4:** 29–40.

Piątek M., Lutz M., Ronikier A., Kemler M. & Swiderska-Burek U. 2012: *Microbotryum heliospermae*, a new anther smut fungus parasitic on *Heliosperma pusillum* in the mountains of the European Alpine System. – Fungal Biol. **116:** 185–195.

Porsild M. P. 1926: The flora of Disco Island and the adjacent coast of West Greenland. – Meddel. Grønland **58:** 1–156.

Rambaut A. 2012: FigTree v1.4. – Published at http://tree.bio.ed.ac.uk/software/figtree/

Rayner R. W. 1970: A mycological colour chart. – Surrey: CMI; Kew: British Mycological Society.

Refrégier G., Le Gac M., Jabbour F., Widmer A., Hood M. E., Yockteng R., Shykoff J. A., Giraud T. 2008: Cophylogeny of the anther smut fungi and their caryophyllaceous hosts: prevalence of host shifts and importance of delimiting parasite species. – B. M. C. Evol. Biol. **8:** 100.

Scholz H. & Scholz I. 1988: Die Brandpilze Deutschlands (*Ustilaginales*). – Englera **8:** 1–691.

Savile D. B. O. 1953: Notes on boreal *Ustilaginales*. – Canad. J. Bot. **31:** 663–674.

Savile D. B. O. 1957: Notes on boreal *Ustilaginales*. II. – Canad. J. Bot. **35:** 279–286.

Savile D. B. O. 1959: The botany of Somerset Island, District of Franklin. – Canad. J. Bot. **37:** 959–1002.

Savile D. B. O. & Parmelee J. A. 1964: Parasitic fungi of the Queen Elisabeth Islands. – Canad. J. Bot. **42:** 699–722.

Shorthouse D. P. 2010: SimpleMappr, an online tool to produce publication-quality point maps. – Published at http://www.simplemappr.net [accessed 17 Jan 2019].

Sloan D., Giraud T. & Hood M. E. 2008: Maximized virulence in a sterilizing pathogen: the anther-smut fungus and its co-evolved hosts. – J. Evol. Biol. **21:** 1544–1554.

Stamatakis A. 2014: RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. – Bioinformatics **30:** 1312–1313.

Thiers B. 2019+ [continuously updated]: Index herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's virtual herbarium. – Published at http://sweetgum.nybg.org/science/ih/ [last accessed 16 Jan 2019].

Uchida W., Matsunaga S., Sugiyama R., Kazama Y. & Shigeyuki K. 2003: Morphological development of anthers induced by the dimorphic smut fungus *Microbotryum violaceum* in female flowers of the dioecious plant *Silene latifolia*. – Planta **218:** 240–248.

Vaidya G., Lohman D. J. & Meier R. 2011: SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. – Cladistics **27:** 171–180.

Vánky K. 1985: Carpathian *Ustilaginales*. – Symb. Bot. Upsal. **24(2):** 1–309.

Vánky K. 1994: European smut fungi. – Stuttgart: Gustav Fischer Verlag.

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. – Mycol. Balcan. **1:** 189–191.

White T. J., Bruns T., Lee S. & Taylor J. 1990: Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. – Pp. 315–322 in: Innis M., Gelfand D., Shinsky J. & White T. (ed.), PCR protocols: a guide to methods and applications. – San Diego: Academic Press.

Willdenowia
Open-access online edition bioone.org/journals/willdenowia
Online ISSN 1868-6397 · Print ISSN 0511-9618 · Impact factor 1.156
Published by the Botanic Garden and Botanical Museum Berlin, Freie Universität Berlin