New records of Microbotryum (Microbotryaceae) from the Balkan Peninsula

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ABSTRACT:
Seven smut fungi belonging to the genus Microbotryum are reported for the first time from the following Balkan countries: M. heliospermatis, M. piperi, M. scabiosae, M. silenes-dioicae, and M. silenes-saxifragae from Bulgaria, M. reticulatum, M. silenes-saxifragae, and M. stygium from Greece, and M. silenes-saxifragae from Bosnia and Herzegovina and Croatia. The finding of M. piperi represents the first Balkan record of this smut fungus which, elsewhere in Europe, is only known from the French Pyrenees and the Italian Alps. The finding of M. stygium in Crete considerably extends its distribution in Europe. Rumex tuberosus subsp. creticus is reported as a new host for M. stygium.

Keywords:
Bosnia and Herzegovina, Bulgaria, Crete, Croatia, Greece, smut fungi, Microbotryaceae

INTRODUCTION
Microbotryum Lév. (Microbotryaceae) is a genus of smut fungi that comprises 99 species on host plants belonging to ten asterid families (listed alphabetically): Asteraceae, Caprifoliaceae, Caryophyllaceae, Gentianaceae, Lamiaceae, Lentibulariaceae, Montiaceae, Onagraceae, Polygonaceae, and Primulaceae (Kemler et al. 2020). The members of this genus form sori in various organs of the infected plants (flowers, anthers, ovules, filaments of stamens, pedicels, inflorescence branches, capitulum, stems, and leaves), containing powdery, pale yellowish brown to dark reddish brown or dark purple spore mass. The sori are without sterile cells. The spores are single, subhyaline to dark reddish brown or dark purple, variously ornamented (reticulate, verruculose, verruculose-reticulate, echinate or striate). Spore germination results in a phragmobasidium, on which sessile basidiospores are produced (Vánky 2011, 2013). The mature septa is without pores; the host-parasite interaction is achieved by intercellular hyphae, without deposits of specific fungal vesicles (Bauer et al. 1997; Vánky 2013).

Although Microbotryum has a nearly world-wide distribution, many of the species are known from one or a limited number of localities (Vánky 2011), and some species are under-recorded. The present article is aimed at increasing knowledge about the distribution of this genus in the Balkan Peninsula. Sixteen new records of seven Microbotryum species are reported from Bosnia and Herzegovina, Bulgaria, Croatia, Greece, North Macedonia, and Romania.

MATERIALS AND METHODS
Dried specimens from H, L, SO, SOMF, and W (herbarium codes according to Thiers 2021) were examined by both light microscopy (LM) and scanning electron microscopy (SEM). For the LM observations and measurements, the 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 the spores are given as min–max (mean ± 1 standard deviation). For SEM, the spores were attached to specimen holders by double-sided adhesive tape and coated with gold in an ion sputter. The surface structure of the spores was observed and photographed at 10 kV accelerating voltage using a JEOL SM-6390 scanning electron microscope. The descriptions of the smut fungi given...
below are based entirely on the specimens examined. The shapes of the spores are arranged in descending order of frequency.

RESULTS AND DISCUSSION

*Microbotryum heliospermatis* Piątek & M. Lutz, in Piątek et al., Fungal Biology 116: 192, 2012 (as ‘heliospermæ’). (Fig. 1a–c)

Infection systemic. Sori in the considerably swollen anthers, filling the pollen sacs with a pulvérulent, sepia (based on Rayner 1970) or date brown (based on the colour identification chart of Anonymous 1969) spore mass. Spores subglobule, globose, broadly ellipsoidal, ovoid, slightly irregular or ellipsoidal, (5.5–)6–8.5(–9.5) × (5–)5.5–7.5(–8.5) (6.9 ± 0.7 × 6.2 ± 0.6) μm (n/1 = 100), light livid vinaceous; wall reticulate, 0.8–1.3(–1.5) μm thick (including reticulum), meshes 6–9(–11) per spore diameter, polyhedral or irregular, 0.3–1.0(–1.3) μm wide, muri 0.15–0.35 μm high. In SEM, meshes smooth on the bottom.

**Specimens examined.** On *Silene pusilla* Waldst. & Kit.: Bulgaria, Central Stara Planina Mts., alt. 1700–1900 m, 18 July 1928, leg. A. Drenovski, s.n. (SOMF 30246); Blagoevgrad Province, Pirin Mts., above Vihren Hut, N 41°45’32.2”, E 23°24’43.0”, alt. 2121 m, 22 August 2019, T.T. Denchev & C.M. Denchev, no. 1979 (SOMF 30382) (Fig. 2).

*Microbotryum heliospermatis* is known from Austria, France, Germany, Montenegro, Poland, Slovakia, and Spain (Vánky 1985; Negrean 1993; Scholz & Scholz 2001; Almaraz 2002; Zwetko & Blanz 2004; Piątek et al. 2012). It is reported here for the first time from Bulgaria.

*Microbotryum piperi* (G.P. Clinton) Vánky, Mycotaxon 67: 48, 1998 (as ‘piperii’). (Fig. 1d–f)

Infection systemic. Sori as pustules on the abaxial side of leaves, often confluent, at maturity rupturing the epidermis exposing a pulvérulent, umber or sepia (based on Rayner 1970) or umber or sepia (based on the colour identification chart of Anonymous 1969) spore mass. Spores subglobe, broadly ellipsoidal or globose, sometimes ovoid, (9.5–)10.5–13.5(–14.5) × (8.5–)9.5–12(–13) (12.1 ± 0.9 × 11.0 ± 0.8) μm (n/1 = 100), vinaceous; wall reticulate, (1.3–)1.5–2.2(–2.4) μm thick (including reticulum), meshes 3–5 per spore diameter, polyhedral or irregular, 0.8–4.3 μm wide, muri 0.5–1.3(–1.6) μm high.

**Specimen examined.** On *Persicaria lapathifolia* (L.) Delarbre: Greece, Macedonia, Serres, near Provatas, N 41°03’44.9”, E 23°21’01.1”, alt. 19 m, 23 September 2011, T.T. Denchev & C.M. Denchev, no. 1101 (SOMF 30294) (Fig. 2).

*Microbotryum scabiosae* (Sowerby) G. Deml & Prillinger, in Prillinger et al., Bot. Acta 104: 10, 1991. (Fig. 3a–c)

Infection systemic. Sori in flowers, spore mass sepia (based on Rayner 1970) or date brown (based on the colour identification chart of Anonymous 1969). Spores subglobe, broadly ellipsoidal or globose, sometimes ovoid, (6.5–)7–10(–11.5) × (6–)6.5–8.5(–9.5) (8.3 ± 1.0 × 7.3 ± 0.7) μm (n/1 = 100), hyaline; wall reticulate, 1.4–2.0 μm thick (including reticulum), meshes 5–9(–10) per spore diameter, polyhedral or irregular, 0.4–1.5 μm wide, muri 0.6–0.9(–1.1) μm high.

**Specimen examined.** On *Knautia arvensis* (L.) DC. s. lat.: Bulgaria, Blagoevgrad Province, Pirin Mts., near Bunderitsa Hut, N 41°46’04.9”, E 23°25’23.2”, alt. 1909 m, 15 July 2020, T.T. Denchev & C.M. Denchev, no. 2030 (SOMF 30299) (Fig. 2).

*Microbotryum scabiosae* is distributed in Europe (Austria, Belgium, Czechia, Denmark, Finland, France, Hungary, Italy, Latvia, Lithuania, Norway, the Netherlands, Poland, Romania, Russia, Serbia, Slovenia, Spain, Sweden, the UK, and Ukraine) (Lindtner 1950; Săvulescu 1957; Lindberg 1959; Jørstad 1963; Vánky 1985; Zogg 1986; Scholz & Scholz 1988; Karatygin & Azbukina 1989; Almaraz 2002; Zwetko & Blanz 2004; Lutz & Vánky 2009; Savchenko & Heluta 2012; Vanderweyen & Fraitrage 2014). It is reported here for the first time from Bulgaria.

*Microbotryum piperi* is known from a few localities in Europe (France and Italy), Asia (China, Kazakhstan, Nepal, Pakistan, Russian Far East, and Uzbekistan), and North America (U.S.A.) (Clinton 1904; Balfour-Browne 1968; Karatygin & Azbukina 1989; Vánky & Oberwinkler 1994; Azbukina et al. 1995; Guo 2000; Chlebicki 2006; Sultán et al. 2007). This is the first Balkan record of this smut fungus which, elsewhere in Europe, is only known from the French Pyrenees and the Italian Alps (Vánky 2011; Dueñas 2020).
Microbotryum silenes-dioicae T. Giraud, Denchev & M.E. Hood, in Denchev et al., Mycol. Balcan. 6: 80, 2009. (Fig. 3d–f)

Infection systemic. Sori in the considerably swollen anthers, filling the pollen sacs with a pulverulent, sepia (based on Rayner 1970) or purplish date (based on the colour identification chart of Anonymous 1969) spore mass. Spores subglobose, globose, broadly ellipsoidal, ovoid, slightly irregular or ellipsoidal, (5.5–)6–9.5(–10) × (5–)5.5–8(–8.5) (7.2 ± 0.8 × 6.5 ± 0.6) μm (n/1 = 100), light livid vinaceous; wall reticulate, 1.0–1.4(–1.6) μm thick (including reticulum), meshes (5–)6–8(–9) per spore diameter, polyhedral or irregular, 0.4–1.6(–1.9) μm wide, muri 0.25–0.45 μm high. In SEM, meshes smooth on the bottom.

Specimen examined. On Silene dioica (L.) Clairv.: Bulgaria, Mt. Lyulin, May 2010, leg. D. Stoyanov, s.n. (SOMF 30295) (Fig. 2).

Microbotryum silenes-dioicae is known from Austria, Belgium, Denmark, Finland, France, Germany, Italy, Lithuania, the Netherlands, Poland, Slovenia, Sweden, Switzerland, and the UK (LIND 1913; LIRO 1924; LINDEBERG...

**Microbotryum silenes-saxifragae** M. Lutz, Piątek & Kemler, IMA Fungus 4: 34, 2013. (Fig. 3g–i)

Infection systemic. Sori in the considerably swollen anthers, filling the pollen sacs with a pulverulent, umber (based on Rayner 1970) or purplish date (based on the colour identification chart of Anonymous 1969) spore mass. Spores subglobose, globose, broadly ellipsoidal or
ovoid, 5.5–7.5(-8.5) x (5–)5.5–6.5(-7) (6.5 ± 0.4 x 5.9 ± 0.3) μm (n //= 100), light vinaceous; wall reticulate, 0.9–1.3 μm thick (including reticulum), meshes (5–)6–7–8 per spore diameter, polyhedral or irregular, 0.3–1.3(-1.6) μm wide, muri 0.25–0.40 μm high. In SEM meshes smooth or rugulose on the bottom.

Specimens examined. On Silene saxifraga L.: Romania, Banat, Caraș-Severin, Bâile Herculane, Prolaz, alt. 300 m, 18 May 1920, leg. A. Borza, Fl. Roman. exsicc., no. 42 (as ‘Silene petraea’) (SOMF 30297). — Germany, Alemania, Schleswig-Holstein, Gifhorn, alt. 20 m, 20 May 1920, leg. E. Kuchar, no. 11099 (H 7035 350, SO 21232) (Fig. 2).

Microbotryum silenes-saxifragae has been previously reported from Austria, France, Germany, Italy, Monte negro, North Macedonia, Romania, Slovenia, Spain, and Switzerland (MAIRE 1908; LINDTNER 1950; ZOOG 1986; SCHOLZ & SCHOLZ 1988, 1993; ALMARAZ 2002; KEMLER M, DENCHEV TT, DENCHEV CM, BEGEROW D, PIĄTEK M & LUTZ M. 2020. Host preference and sorus location correlate with parasite phylogeny in the smut fungal genus Microbotryum (Basidiomycota, Microbotryales). Mycological Progress 19: 481–493.

Microbotryum stygium (Liro) Vánky, Mycotaxon 67: 50, 1998. (Fig. 4a–c)

Infection systemic. Sori in flowers, spore mass sepia (based on RAYNER 1970) or date brown (based on the colour identification chart of ANONYMOUS 1969). Spores subglobose, broadly ellipsoidal, ovoid, slightly irregular, globose or sometimes ellipsoidal, (11–)12–15.5(-16.5) x (10–)11–14–15 (13.6 ± 1.0 x 12.4 ± 0.9) μm (n //= 100), reddish brown; wall reticulate, (1.6–)1.8–2.3(-2.7) μm thick (including reticulum), meshes (7–)8–11–12 per spore diameter, polyhedral or irregular, 0.5–2.7 μm wide, muri (0.6–)0.8–1.6–(-1.9) μm high.

Specimen examined. On Rumex tuberosus subsp. creticus (Boiss.) Rech. f.: Greece, Crete, Imbros, Imbros Valley, alongside the path near the village of Imbros, alt. 780 m, 29 April 2002, leg. P. Alanko, no. 113545 (H 7035 350, as ‘Ustilago sp.’) (Fig. 2).

Microbotryum stygium is known on members of Rumex, reported from Europe (Austria, the Czech Republic, Finland, France, Germany, Norway, Romania, Russia, Sweden, Switzerland, and the UK – LIRO 1924; SAVULESCU 1957; LINDEBERG 1959; JØRSTAD 1963; VÁNKY 1985; ZOOG 1986; SCHOLZ & SCHOLZ 1988, 2001, 2004, 2013; KARATYGIN & AZBUKINA 1989; ZWETKO & BLANZ 2004) and Asia (VÁNKY 2011). The citation here constitutes the first record of M. stygium from Greece, extending its distribution in Europe to Crete. Rumex tuberosus subsp. creticus is a new host for this smut fungus.

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