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Original Scientific Paper

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*

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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 '*heliospermae*'). (Fig. 1a–c)

Infection systemic. Sori in the considerably swollen anthers, filling the pollen sacs with a pulverulent, sepia (based on Rayner 1970) or date brown (based on the colour identification chart of Anonymous 1969) spore mass. Spores subglobose, 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, Romania, 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 pulverulent, umber or sepia (based on Rayner 1970) or umber (based on the colour identification chart of Anonymous 1969) spore mass. Spores variable in shape, subglobose, globose, ovoid, irregularly rounded, broadly ellipsoidal, ellipsoidal, elongated or occasionally pyriform or lacrymiform, (5–)6–9(–10) × (5–)5.5–7.5(–8) (7.4 ± 0.7 × 6.6 ± 0.6) µm ($n/_1$ = 100), light vinaceous; wall 0.6–1.0 µm thick, finely striate; striae up to 0.25 µm high. In SEM, spore wall striate, striae parallel or irregularly arranged, often ramifying or anastomosing.

Specimen examined. On *Koenigia alpina* (All.) T.M. Schust. & Reveal (*Polygonum alpinum* All., *Aconogonon alpinum* (All.) Schur): Bulgaria, Mt. Vitosha, below Golyam Rezen Peak, N 42°33'54.8", E 23°17'46.6", alt. 1943 m, 6 July 2016, T.T. Denchev & C.M. Denchev, no. 1637 (SOMF 30247) (Fig. 2).

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; Sultan *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 reticulatum (Liro) R. Bauer & Oberw., in Bauer *et al.*, Can. J. Bot. 75: 1311, 1997. (Fig. 1g–i)

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 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).

It is a cosmopolitan species (Vánky 2011), recorded here for the first time from Greece.

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

Infection systemic. Sori in the considerably swollen anthers, filling the pollen sacs with a pulverulent, clay buff (based on Rayner 1970) or hazel (based on the colour identification chart of Anonymous 1969) spore mass. Spores subglobose, broadly ellipsoidal, ovoid, slightly irregular, globose or ellipsoidal, (6.5–)7–10.5(–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; Lindeberg 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 & Fraiture 2014). It is reported here for the first time from Bulgaria.

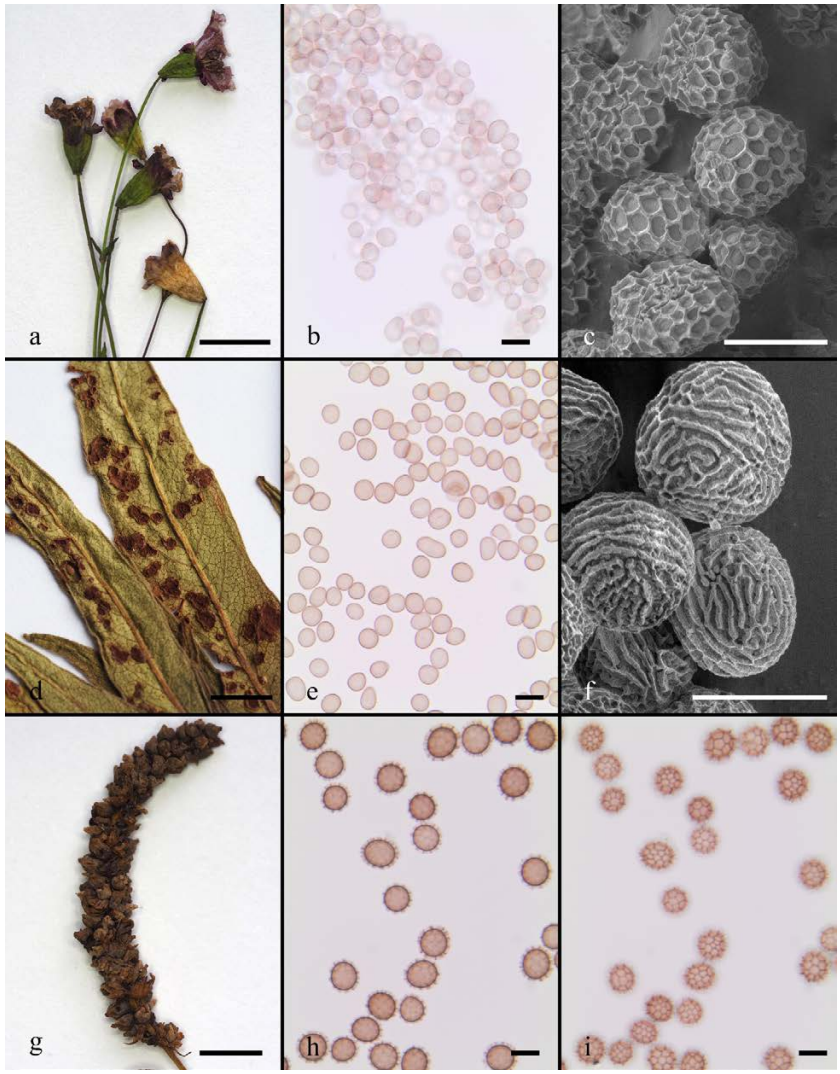

Fig. 1. *Microbotryum* from the Balkan Peninsula. **a–c** *M. heliospermatis* (Bulgaria, SOMF 30382): a – habit, b – spores in LM, c – spores in SEM; **d–f** *M. piperi* (Bulgaria, SOMF 30247): d – habit, e – spores in LM, f – spores in SEM; **g–i** *M. reticulatum* (Greece, SOMF 30294): g – habit, h, i – spores in LM, in median and surface view, respectively. Scale bars: a, d, g = 0.5 cm; b, e, h, i = 10 µm, c, f = 5 µm.

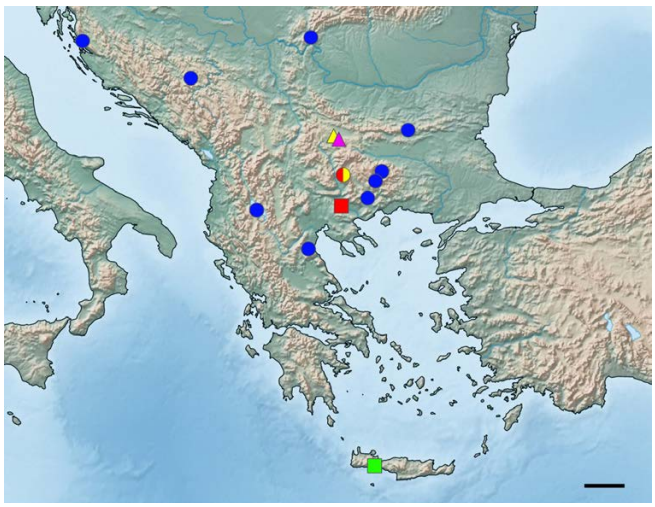

Fig. 2. Map of the specimen localities: *M. heliospermatis* (red circle; the gathering from the Central Stara Planina Mts. cannot be located), *M. piperi* (rose triangle), *M. reticulatum* (red square), *M. scabiosae* (yellow circle), *M. silenes-dioicae* (yellow triangle), *M. silenes-saxifragae* (blue circles), and *M. stygium* (light green square). Scale bar = 100 km.

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

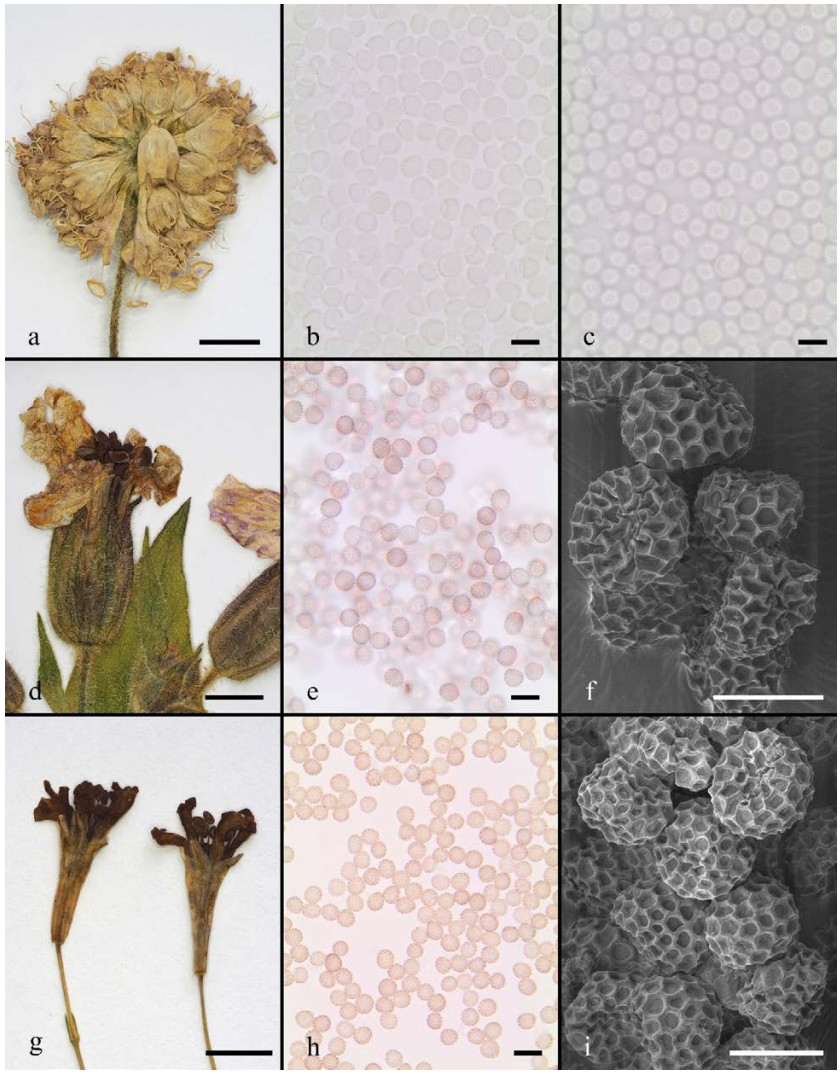

Fig. 3. *Microbotryum* from the Balkan Peninsula. **a–c** *M. scabiosae* (Bulgaria, SOMF 30299): a – habit, b, c – spores in LM, in median and surface view, respectively; **d–f** *M. silenes-dioicae* (Bulgaria, SOMF 30295): d – habit, e – spores in LM, f – spores in SEM; **g–i** *M. silenes-saxifragae* (Bulgaria, SOMF 30220): g – habit, h – spores in LM, i – spores in SEM. Scale bars: a, d, g = 0.5 cm; b, c, e, h = 10 µm, f, i = 5 µm.

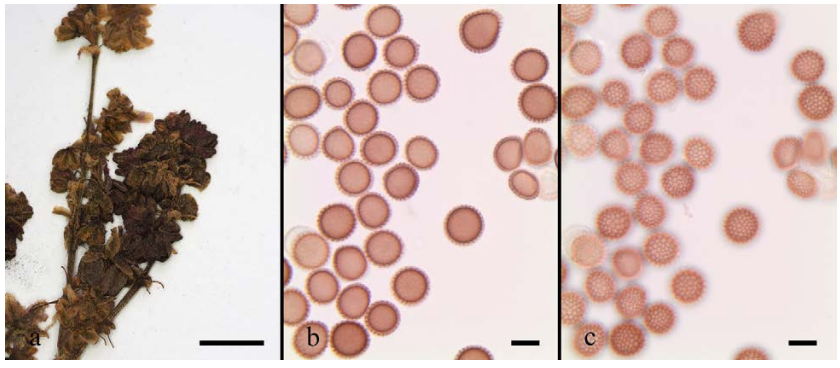

Fig. 4. *Microbotryum stygium* (Crete, H 7 035 350): a – habit, b, c – spores in LM, in median and surface view, respectively. Scale bars: a = 0.5 cm; b, c = 10 µm.

1959; Kochman 1960; Zogg 1986; Scholz & Scholz 1988, 2001, 2004, 2013; Karatygin & Azbukina 1989; Zwetko & Blanz 2004; Le Gac *et al.* 2007; van Putten *et al.* 2007; Denchev *et al.* 2009; Lutz & Vánky 2009; Jage *et al.* 2010; Vercken *et al.* 2010; Vanderweyen & Fraiture 2014). It is reported here for the first time from Bulgaria.

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) × (5–)5.5–6.5(–7) (6.5 ± 0.4 × 5.9 ± 0.3) µm ($n/_1$ = 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, Băile Herculane, Prolaz, alt. 300 m, 18 May 1920, leg. A. Borza, Fl. Roman. exsicc., no. 42 (as '*Silene petraea*') (SOMF 30296). — Croatia, Velebit Mts., near Oštarije, June 1886, leg. Th. Pichler, s.n. (SO 21410). — Bosnia and Herzegovina, Mt. Romanija, June 1888., leg. G. de Beck, Plantae Bosniae et Hercegovinae, no. 162 (W 1961-0021813). — North Macedonia, Mt. Galičica, above the pass between Trpejca and Oteševo, 3–8 July 1982, leg. A. Touw, no. 21882 (L 3745283). — Bulgaria, Stara Planina Mts., Kameshnitsa Reserve, above the village of Enina, alt. ca 1000 m, 7 July 2002, leg. D. Stoyanov, s.n. (SOMF 30297); Rhodopes Mts., near Choudnite Mostove Hut, July 1996, C.M. Denchev, s.n. (SOMF 30220); Rhodopes Mts., above Trigradski Skali Hut, ca 1200 m, 15 August 1991, C.M. Denchev, no. 9158 (SOMF 30298). — Greece, Eastern Macedonia and Thrace region, near Drama, 25 July 1970, leg. E. Stamatiadou, no. 10155 (W 1971-0000458); Thessaly region, Mt. Olympus, alt. 2000–2500 m, 21–22 July 1937, leg. N. Stoyanoff & D. Jordanoff, s.n. (SO 21232) (Fig. 2).

Microbotryum silenes-saxifragae has been previously reported from Austria, France, Germany, Italy, Montenegro, North Macedonia, Romania, Slovenia, Spain, and Switzerland (Maire 1908; Lindtner 1950; Zogg 1986; Scholz & Scholz 1988; Negrean 1993; Almaraz 2002; Piątek *et al.* 2013). This smut fungus is reported here for the first time from Bosnia and Herzegovina, Bulgaria, Croatia, and Greece. A further two localities are added to its distribution in North Macedonia and Romania.

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) × (10–)11–14(–15) (13.6 ± 1.0 × 12.4 ± 0.9) µm ($n/_1$ = 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 7 035 350, 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; Săvulescu 1957; Lindeberg 1959; Jørstad 1963; Vánky 1985; Zogg 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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REZIME

Botanica SERBICA

Novi nalazi *Microbotryum* (Microbotryaceae) sa Balkanskog poluostrva

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Sedam gljiva iz roda *Microbotryum* je zabeleženo po prvi put na Balkanskom poluostrvu: *M. heliospermatis*, *M. piperi*, *M. scabiosae*, *M. silenes-dioicae* i *M. silenes-saxifragae* u Bugarskoj, *M. reticulatum*, *M. silenes-saxifragae* i *M. stygium* u Grčkoj, i *M. silenes-saxifragae* u Bosni i Hercegovini i Hrvatskoj. Pronalazak *M. piperi* predstavlja prvi nalaz ove vrste na Balkanu, dok je na području Evrope ova vrsta do sada bila zabeležena samo na Pirinejima u Francuskoj i Alpima u Italiji. Pronalazak vrste *M. stygium* na Kritu značajno širi njen areal na području Evrope, a vrsta *Rumex tuberosus* subsp. *creticus* je zabeležena kao njen novi domaćin.

Ključne reči: Bosna i Hercegovina, Bugarska, Krit, Hrvatska, Grčka, gljive, *Microbotryaceae*