

## Additions to the smut fungi of the Iberian Peninsula

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**Abstract.** After examination of specimens, mainly from the herbarium (MA) and the mycological collection (MA-Fungi) of the Royal Botanic Garden of Madrid, we report several novelties on smut fungi within Europe. Two species of smut fungi, *Sporisorium egyptiacum* and *Tilletia viennotii*, are reported for the first time from Europe. A finding of *Sphacelotheca polygoni-serrulati* represents a second record for Europe. Six species of smut fungi, *Moreaua kochiana*, *Schizonella elyinae*, *Sporisorium egyptiacum*, *Thecaphora thlaspeos*, *Tilletia viennotii*, and *Ustanciosporium majus*, are recorded for the first time from the Iberian Peninsula. Five species of smut fungi, *Moreaua kochiana*, *Schizonella elyinae*, *Sporisorium egyptiacum*, *Thecaphora thlaspeos*, and *Ustanciosporium majus*, are newly recorded from Spain. Three species, *Moreaua kochiana*, *Sphacelotheca polygoni-serrulati*, and *Tilletia viennotii*, are new for Portugal. A specimen of *Moreaua kochiana* represents a new record for France. *Arabis serpillifolia* is reported as a new host of *Thecaphora thlaspeos*. New distribution records from the Iberian Peninsula are given for *Anthracoidea arenariae*, *Microbotryum minuartiae*, *M. silenens-saxifragae*, and *Tranzscheliella sparti*. We also include a phylogenetic analysis of DNA sequences of *Moreaua kochiana*, generated in this study, to understand this species' relationships within its genus.

**Keywords.** Iberian Peninsula, *Moreaua kochiana*, smut fungi, *Sporisorium egyptiacum*, *Tilletia viennotii*.

**Resumen.** Tras examinar especímenes principalmente del herbario (MA) y de la colección de hongos (MA-Fungi) del Real Jardín Botánico de Madrid reportamos varias novedades de carboneros dentro de Europa. Dos especies de carboneros, *Sporisorium egyptiacum* y *Tilletia viennotii*, se citan por primera vez para Europa. Reportamos también la segunda cita de *Sphacelotheca polygoni-serrulati* para Europa. Seis especies, *Moreaua kochiana*, *Schizonella elyinae*, *Sporisorium egyptiacum*, *Thecaphora thlaspeos*, *Tilletia viennotii*, y *Ustanciosporium majus*, se citan por primera vez para la Península Ibérica; cinco especies, *Moreaua kochiana*, *Schizonella elyinae*, *Sporisorium egyptiacum*, *Thecaphora thlaspeos*, y *Ustanciosporium majus*, son nuevas citas para España; tres especies, *Moreaua kochiana*, *Sphacelotheca polygoni-serrulati*, y *Tilletia viennotii*, son nuevas citas para Portugal. Una colección de *Moreaua kochiana* representa una nueva cita para Francia. *Arabis serpillifolia* se menciona por primera vez como un hospedante para *Thecaphora thlaspeos*. Se registran nuevas localidades en la Península Ibérica para *Anthracoidea arenariae*, *Microbotryum minuartiae*, *M. silenens-saxifragae* y *Tranzscheliella sparti*. Incluimos también un análisis filogenético de secuencias de ADN de dos especímenes de *Moreaua kochiana*, generadas en este estudio, para entender las relaciones filogenéticas de esta especie dentro su género.

**Palabras clave.** Península Ibérica, *Moreaua kochiana*, carboneros, *Sporisorium egyptiacum*, *Tilletia viennotii*.

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### INTRODUCTION

The Iberian Peninsula harbours an unusually high diversity of vascular plants, ranking among the highest in Europe and the Mediterranean area with 6276 species (Aedo & al. 2017). It is one of the most important centres of endemism with 1357 endemic species (22% of the total number of species) (Buirra & al. 2020). This level of plant diversity is expected to correlate with a high species richness of plant parasitic fungi. Although the smut fungi of the

Iberian Peninsula are a subject of long-term studies (e.g., González Fragoso 1914, 1917, 1919, 1923, 1924a, b, 1926; Unamuno 1928, 1930a, b, c, 1931, 1934a, b, 1942; Ciferri 1933; Maire 1933, 1943; Losa España 1942, 1944, 1949, 1954; Alcalde 1944; Cámara & Oliveira 1945; Cámara 1946; Guyot & al. 1955, 1958, 1960, 1969; Jørstad 1962; Durrieu 1966; Losa Quintana 1970; Llorens i Villagrasa 1985; Denchev 1995, 1997; Almaraz & Durrieu 1997; Al-

maraz 1998, 1999a, b, c, 2002; Almaraz & Medina 1998; Almaraz & Telleria 1998; Vánky 2011; Kemler & al. 2013, 2020; Denchev & Denchev 2017; Kruse & al. 2018), their inventory is incomplete and no regional monographic study has been published yet. Based on the modern taxonomic arrangement of the smut fungi, all known records from the Iberian Peninsula and Balearic Islands can be referred to 164 species.

In this article, we present new records of smut fungi from the Iberian Peninsula, as follows: two species for the first time from Europe, one species as a second record from Europe, six species for the first time from the Iberian Peninsula, five species as new for Spain, and three species as new for Portugal. We also generated DNA sequences (ITS and LSU rDNA regions) of *Moreaua kochiana* to provide a phylogenetic context for this species.

## MATERIAL AND METHODS

The collections, on which the records are based, were obtained during a visit of two of the co-authors (T.T.D. & C.M.D.) to the herbarium (MA) and mycological collection (MA-Fungi) at the Royal Botanic Garden, Madrid in April 2017. Dried specimens from the herbarium and mycological collection of the Royal Botanic Garden, Madrid and herbarium of the Botanic Garden and Botanical Museum Berlin were examined with a light microscope (LM) and scanning electron microscope (SEM). For LM observations and measurements, spores, spore balls, and sterile cells were mounted in lactoglycerol solution (w : la : gl = 1 : 1 : 2) on glass slides, gently heated to boiling point for rehydration, and then cooled. The measurements of spores and sterile cells are given in the form: min–max (extreme values) (mean  $\pm$  1 standard deviation). For SEM, spores were attached to specimen holders by double-sided adhesive tape and coated with gold in an ion sputter. The surface structure of spores was observed and photographed at 10 kV accelerating voltage using a Hitachi S-3000N scanning electron microscope. The shapes of spores, spore balls, and sterile cells are arranged in descending order of frequency. The width and height of the appendages of *Ustanciosporium majus* were measured in accordance with Denchev & Denchev (2016). The descriptions given below are based entirely on the specimens examined.

To elucidate the relationship of one of the species, *Moreaua kochiana* with other species of the genus and the *Anthracoideaceae*, the ITS and LSU rDNA regions of two specimens were analysed. Genomic DNA isolation was performed using DNeasyPlant Mini Qiagen (Qiagen, Valencia, California, US), following the manufacturer's instructions, except in three steps: the incubation with the RNase was done overnight at 65°C, a second drying at 20 000 $\times$ g was done for 2 min after cleaning with AW buffer, and elution

buffer was preheated to 60°C. Polymerase chain reactions (PCR) were performed using Ready-To-Go PCR beads (GE Healthcare, Buckinghamshire, UK) to amplify DNA from two regions with the following primer combinations: ITS1F (Gardens & Bruns 1993)/ITS4 (White & al. 1990), to obtain DNA amplifications of the nuclear ribosomal internal transcribed spacer regions ITS1 and ITS2, including 5.8S, ITS nrDNA barcode (Schoch & al. 2012); and LR0R/LR5r (White & al. 1990), for nrLSU region. Amplicons obtained were purified using the kit QIAquick Gel Extraction (Qiagen) following the protocol defined by the manufacturer. The purified PCR products were sent to Macrogen (Madrid) for sequencing both directions using the same primers used in the amplifications. The consensus sequences were obtained with the software Sequencher (Gene Codes Corporation Inc, Ann. Arbor, Michigan, USA).

Alignment of the newly generated DNA sequences and of selected ones from NCBI was performed using MAFFT v7.450 under the -einsi option (Katoh & al. 2002; Katoh & Standley 2013). Ambiguous sites, leading and trailing gaps were removed using GBlocks (Castresana 2000) as implemented in Seaview (Gouy & al. 2010), whereby smaller final blocks, gap positions within the final block, as well as less strict flanking positions were allowed. Alignment and GBlocks treatment were performed separately for the ITS and LSU datasets. Subsequently the two aligned matrices were concatenated using SequenceMatrix (Vaidya & al. 2011). Phylogenetic analyses were conducted using RAxML 7.3.5 (Stamatakis 2006) under the GTRGAMMA nucleotide substitution model and 1000 rapid bootstrap repetitions. The final ML tree was visualized using FigTree v1.4.3 (Rambaut 2016).

For the geographic distribution data provided to each taxon we follow the World Geographical Scheme for Recording Plant Distributions (Brummitt 2001).

## RESULTS AND DISCUSSION

### *Taxonomic treatment*

*Anthracoidea arenariae* (Syd.) Nannf., Bot. Not. 130: 365 (Nannfeldt 1977); *Cintractia arenariae* Syd., Ann. Mycol. 22: 289 (Sydow 1924). Type: on *Carex arenaria*, Poland, near Darłówko (as 'Pommern, Rügenwaldermünde'), Jul. 1893, P. Sydow s.n. (lectotype designated by Nannfeldt (1977: 365): S; isolectotypes: in Sydow, Ustilag., no. 5, as '*Ustilago caricis*').

*Specimen examined*.—On *Carex arenaria* L.: PORTUGAL. **Braga**: Esposende, Apúlia, 41°28'33.9"N, 8°46'23.7"W, 9 m, 6 Jun. 2010, A. Quintanar & al. AQ3864, "Iter Lusitanicus, VI-2010" (MA 824694).

*Distribution*.—On Cyperaceae: *Carex accrescens* Ohwi (*C. pallida* C.A.Mey), *C. arenaria*, *C. brizoides* L., *C. col-*

*chica* J.Gay (*C. ligerica* J.Gay), and *C. praecox* Schreb.; Europe, Asia, and North America (Vánky 2011).

*Comments.*—We report a new distribution record from Portugal.

***Microbotryum minuartiae*** M.Lutz, Piątek, Kemler & Chleb., Mycol. Res. 112: 1287 (Lutz & al. 2008). Type: on *Minuartia recurva* (All.) Schinz & Thell., Romania, Carpathian Mts, Bucegi Mts., Caraiman Peak, 2384 m, 26 Jul. 2004, A. Ronikier & M. Ronikier s.n. (holotype: KRAM-F 55483).

*Specimen examined.*—On *Minuartia villarii* (Balb.) Wilczek & Chenevard: SPAIN. **León:** Peña Ubiña, 1200 m, 10 Jul. 1994, S. Castroviejo s.n. (MA 247597).

*Distribution.*—On Caryophyllaceae: *Minuartia* spp.; Europe, Asia (Vánky 2011).

*Comments.*—In Spain, this species was known only from two localities: on *Minuartia villarii* from Province of Palencia, Velilla de Río Carrión, Espigüete; and on *M. recurva* subsp. *condensata* (C.Presl) Greuter & Burdet from Province of Soria, Castillo Vinuesa, Santa Inés (Almaraz 2002: 41, as '*Microbotryum stellariae*'). Molecular data are needed for clarification of the taxonomic status of the *Microbotryum* species on *Minuartia villarii*.

***Microbotryum silenes-saxifragae*** M.Lutz, Piątek & Kemler, IMA Fungus 4: 34 (Piątek & al. 2013). Type: on *Silene saxifraga*, Austria, Carinthia, Villach, Finkenstein, southern part of the Kanzianiberg, near the church, 630 m, 24 Jun. 2006, M. Lutz s.n. (holotype: KR-M-23890).

*Specimen examined.*—On *Silene saxifraga* L.: SPAIN. **Huesca:** Sobrarbe, Chisagüés, 1650 m, 20 Jun. 1996, M. Carrasco, C. Martín Blanco, and M. Velayos 8426 (MA 609550).

*Distribution.*—On Caryophyllaceae: *Silene saxifraga*; Europe (Piątek & al. 2013).

*Comments.*—A new distribution record from Spain.

***Moreaua kochiana*** (Gäum.) Vánky, Mycotaxon 74: 352 (Vánky 2000); *Tolyposporium kochianum* Gäum., Ber. Schweiz. Bot. Ges. 41: 179 (Gäumann 1932); *Thecaphora kochiana* (Gäum.) Thirum. & Neerg., Friesia 11: 186 (Thirumalachar & Neergaard 1978). Type: on *Schoenus* × *scheuchzeri* (as '*S. ferrugineus* × *S. nigricans*'), Switzerland, Kanton Zürich, at Greifensee Lake, Jun. 1932, W.Koch & L.Zobrist s.n. (holotype: ZT). Fig. 2a–d.

Infection systemic. Sori around filaments and gynoeium of all flowers of infected plant, concealed by adjacent glumes and outwardly inconspicuous; the mass of spore balls blackish brown, initially agglutinated, later powdery. Spore balls irregular, subglobose, broadly ellipsoidal or ovoid, composed of (2–)5–50 or more, firmly united spores,

occasionally single spores present, (17.5–)21–75(–85) × (14.5–)18–55(–68) µm, dark reddish brown to very dark reddish brown or medium reddish brown when composed of few spores, opaque when composed of tens of spores. Spores in surface view irregularly rounded, irregularly polygonal, subcuneate, subglobose, elliptical or broadly elliptical, measured from the free side (5.5–)6.5–15(–16) × (5–)6–11(–12) µm; radially (5–)6–15.5(–17) µm long; wall 1.2–3.2 µm thick at free surface, 0.5–1.0 µm thick at contact surfaces. In SEM, spore wall rugose to irregularly verrucose.

*Specimens examined.*—On *Schoenus nigricans* L.: PORTUGAL. **Aveiro** (as 'Beira Litoral'): Barrinha de Esmoriz, 12 Jul. 1977, Malato-Beliz 13586 and J.A. Guerra (MA 274860). SPAIN. **Pontevedra:** Cabo de Home, Playa de Melide, 9 Aug. 1985, E. Lago 566EL, S. Castroviejo, and X.R. Garcia (MA 875148). **Valencia:** Teresa de Cofrentes, Las Quebradas, 600 m, 3 Nov. 2003, M. Martínez Azorín s.n. (MA 836657).

*Additional collections examined.*—On *Schoenus nigricans*: AUSTRIA. **Lower Austria:** Gelber Berg near Purkersdorf, 0.9 km SE of Rudolfshöhe, 48°11'35"N, 16°12'01"E, 315 m, 29 Jul. 1999, B. Wallnöfer 13708 (MA 691763). FRANCE. **Occitanie:** Montpellier, Valmaillargues, 27 May 1934, L. Zobrist 14447 (MA 361735).

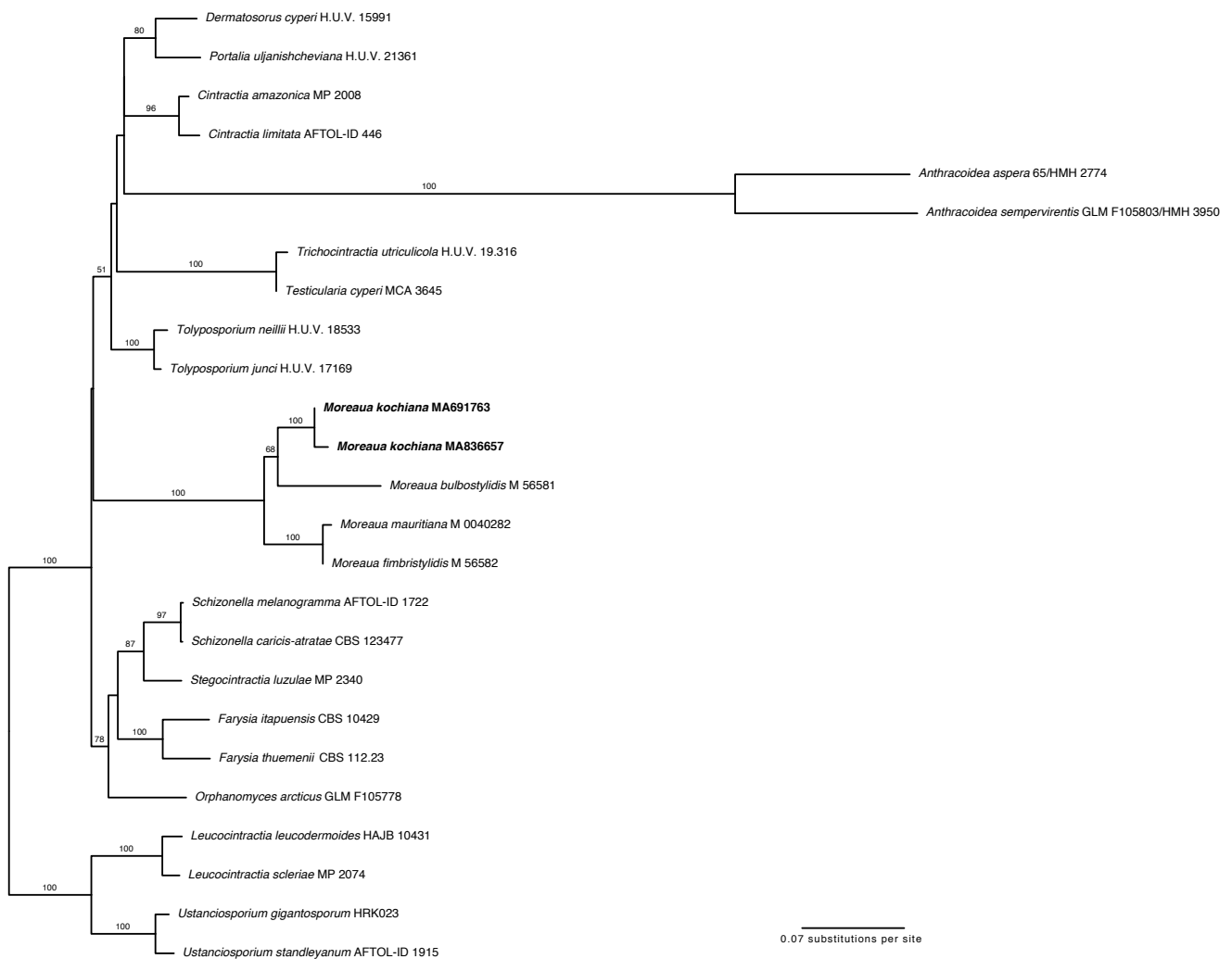
*Distribution.*—On Cyperaceae: *Schoenus carsei* Cheeseman, *S. nigricans*, and *Schoenus* × *scheuchzeri* Brügger (*S. ferrugineus* L. × *S. nigricans* L., *Schoenus* × *intermedius* Brügger); Europe (Austria, France, Germany, Italy, Netherlands, Portugal, Spain, Switzerland) and New Zealand.

*Comments.*—*Moreaua kochiana* is an infrequently collected smut fungus, considered by Vánky (1994: 273), in his monograph of the European smut fungi, as a rare species. Eighty-eight years after its description, it was known in Europe from only a few localities: on *Schoenus nigricans*, from the Netherlands (Ernst 2013) and Italy (Vánky, Ustilaginales Exsiccata, no. 861), and on *Schoenus* × *scheuchzeri*, from Switzerland (Gäumann 1932; Vánky 2000; Vánky, Ustilaginales Exsiccata, no. 189) and Germany (Kruse & al. 2014). Recently, it was recorded from Austria, on both host plants (Denchev & al. 2020b). *Moreaua kochiana* is reported herein for the first time from France, Spain, and Portugal, thus extending its geographic range to the Mediterranean region of France and the Iberian Peninsula. The significant increase in the knowledge about the distribution of this smut fungus suggests that its 'rarity' is rather due to its cryptic nature and that probably, this species has a larger geographic range.

*Moreaua kochiana* is also recorded on *Schoenus carsei* from New Zealand (McKenzie & Vánky 2001) but this association needs re-examination with molecular methods considering that the range of *S. carsei* is confined to SE

**Table 1.** List of sequences downloaded from GenBank and newly sequenced specimens used in the phylogenetic analyses with their respective GenBank accessions numbers for ITS and LSU.

Species	Host	Voucher	ITS	LSU
<i>Anthracoidea aspera</i> (Liro) Kukkonen	<i>Carex chordorrhiza</i> L.f.	65/HMH 2774	AJ586572	AY563607
<i>Anthracoidea sempervirentis</i> Vánky	<i>Carex sempervirens</i> Vill.	GLM-F105803/ HMH 3950	KY424498	AY563586
<i>Cintractia amazonica</i> Syd. & P.Syd.	<i>Rhynchospora barbata</i> (Vahl) Kunth	MP 2008	DQ875342	AJ236142
<i>Cintractia limitata</i> G.P.Clinton	<i>Cyperus</i> sp.	AFTOL-ID 446	DQ645508	DQ645506
<i>Dermatosorus cyperi</i> Vánky	<i>Cyperus celluloso-reticulatus</i> Boeckeler	H.U.V. 15991	DQ875343	AJ236157
<i>Farysia itapuensis</i> Landell & P.Valente ex Denchev & T.Denchev	n/a	CBS 10429	KY103405	KY107692
<i>Farysia thuemenii</i> (A.A.Fisch.Waldh.) Nannf.	n/a	CBS 112.23	MH854741	MH866248
<i>Leucocintractia leucodermoides</i> M.Piepenbr. & Begerow	<i>Rhynchospora holoschoenoides</i> (Rich.) Herter	HAJB 10431	DQ875346	DQ875363
<i>Leucocintractia scleriae</i> (DC.) M.Piepenbr. et al.	<i>Rhynchospora triflora</i> Vahl	MP 2074	AY740025	AJ236154
<i>Moreaua bulbostylidis</i> M.Piepenbr.	<i>Bulbostylis capillaris</i> C.B.Clarke	M 56581	DQ875349	DQ875366
<i>Moreaua fimbristylidis</i> Vánky & R.G.Shivas	<i>Fimbristylis dichotoma</i> (L.) Vahl	M 56582	DQ875350	DQ875367
<i>Moreaua kochiana</i> (Gäum.) Vánky	<i>Schoenus nigricans</i> L.	MA 836657	MW258623	MW258619
<i>Moreaua kochiana</i>	<i>Schoenus nigricans</i>	MA 691763	MW258622	MW258618
<i>Moreaua mauritiana</i> (Syd.) Vánky	<i>Fimbristylis ovata</i> (Burm.f.) J.Kern	M 0040282	KY424491	–
<i>Orphanomyces arcticus</i> (Rostr.) Savile	<i>Carex davalliana</i> Sm.	GLM-F105778	KY424454	–
<i>Portalia uljanishcheviana</i> (Schwarzman) V.González et al.	<i>Scirpoides holoschoenus</i> (L.) Soják (as ' <i>Holoschoenus vulgaris</i> ')	12 Jul. 1949, Schwarzman	–	EF118824
<i>Schizonella caricis-atratae</i> Prillinger et al. ex Denchev & T.Denchev	<i>Carex atrata</i> L.	CBS 123477	NR_158881	NG_064878
<i>Schizonella melanogramma</i> (DC.) J.Schröt.	<i>Carex</i> sp.	AFTOL-ID 1722	DQ832212	DQ832210
<i>Stegocintractia luzulae</i> (Sacc.) M.Piepenbr. et al.	<i>Luzula pilosa</i> (L.) Willd.	MP 2340	DQ875353	AJ236148
<i>Testicularia cyperi</i> Klotzsch	<i>Rhynchospora</i> sp.	MCA3645	KU147240	KU147242
<i>Tolyposporium junci</i> (J.Schröt.) Woronin	<i>Juncus bufonius</i> L.	H.U.V. 17169	AY344994	AF009876
<i>Tolyposporium neillii</i> (G.Cunn.) Vánky & McKenzie	<i>Isolepis nodosa</i> (Rottb.) R.Br.	H.U.V. 18533	EU246951	EU246952
<i>Trichocintractia utriculicola</i> (Henn.) M.Piepenbr.	<i>Rhynchospora corymbosa</i> (L.) Britton	H.U.V. 19316	KY424453	AF009877
<i>Ustanciosporium gigantosporum</i> (Liro) M.Piepenbr.	<i>Rhynchospora alba</i> (L.) Vahl	HRK023	JN367300	JN367325
<i>Ustanciosporium standleyanum</i> (Zundel) M.Piepenbr.	n/a	AFTOL-ID 1915	DQ846890	DQ846888



**Fig. 1.** Best-scoring ML phylogeny with bootstrap values > 50% written above branches. Specimens of *Moreaua kochiana* are shown in bold. The phylogeny was rooted with *Leucocintractia leucodermis*, *L. scleriae*, *Ustanciosporium gigantosporum*, and *U. standleyanum*.

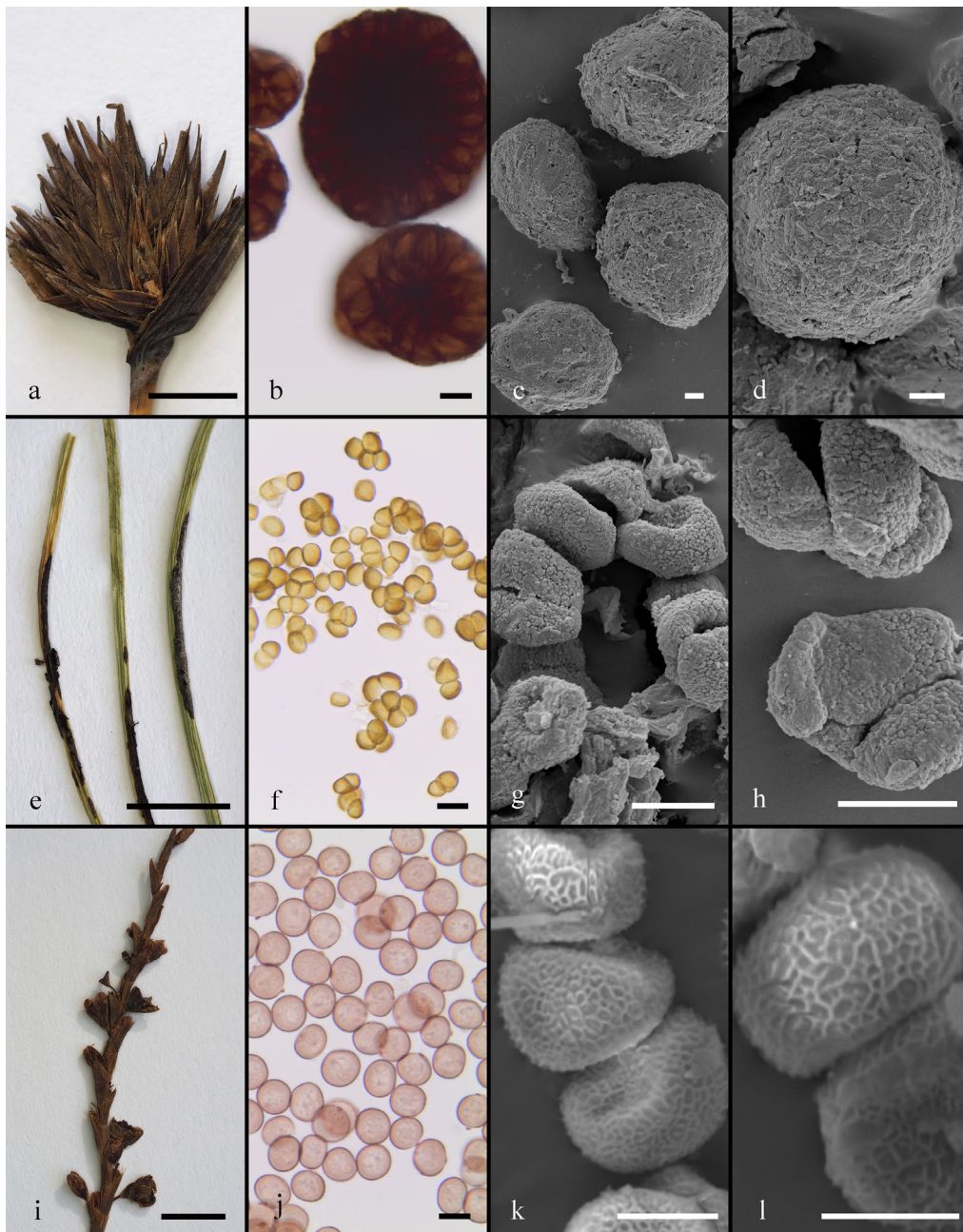
Australia and New Zealand (Govaerts 2020) and that the smut fungi usually have narrow host specialisation.

The new sequences of *Moreaua kochiana* generated in this study represent the first molecular data for this species (Table 1). The ITS sequences are identical, whereas the LSU sequences show minor sequence differences. The two accessions of *M. kochiana* included in the phylogenetic analysis form a statistically well-supported clade (Fig. 1). The species is closely related to *M. bulbostylidis* M. Piepenbr. and all species of *Moreaua* form a highly supported clade (Fig. 1).

*Schizonella elyanae* (A.Blytt) Liro, Ann. Acad. Sci. Fenn., Ser. A 42(1): 308 (Liro 1936); *Schizonella melanogramma* var. *elyanae* A.Blytt, Forh. Vidensk.-Selsk. Christiania 1896 (6): 33 (Blytt 1896, as ‘ $\beta$  *elyanae*’). Type: on *Carex myo-*

*suroides* (as ‘*Elyna spicata*’), Norway, Oppland, Dovre, Hjerkin, 8 Aug. 1889, A. Blytt s.n. (lectotype designated by Lindeberg (1959: 57): O). Fig. 2e–h.

Infection systemic. Sori in leaves as striae or irregular spots, initially covered by the silvery epidermis which later ruptures disclosing a semi-agglutinated, blackish brown mass of spores. Spores joined in pairs, sometimes in threes, often separating into single spores, depressed on the contact side, in plane view suborbicular, irregular, broadly elliptical or ovate in outline, in plane view (5–)5.5–9(–9.5)  $\times$  (4.5–)5–7.5(–8.5) (7.1  $\pm$  0.9  $\times$  6.1  $\pm$  0.6)  $\mu$ m (n = 100), in side view usually irregularly hemispherical; light yellowish brown to medium reddish brown; wall unevenly thickened, 0.6–1.3(–1.6)  $\mu$ m thick, thinner and lighter on the contact side, smooth. In SEM, spore wall densely, minutely verruculose,



**Fig. 2.** *Moreaua kochiana* (Gäum.) Vánky (MA 836657): **a**, habit; **b**, spore balls in LM; **c**, **d**, spore balls in SEM. *Schizonella elyngae* (A.Blytt) Liro (MA 342212): **e**, habit; **f**, spores in LM; **g**, **h**, spores in SEM. *Sphacelotheca polygoni-serrulati* Maire (B 10 0506861): **i**, habit; **j**, spores in LM; **k**, **l**, spores in SEM. Scale bars: a, e, i = 0.5 cm, b, f, j = 10  $\mu$ m, c, d, g, h, k, l = 5  $\mu$ m.

warts often confluent, forming small groups; ornaments up to 0.15  $\mu$ m in height; contact side with a concave area.

*Specimen examined.*—On *Carex myosuroides* Vill. (*Kobresia myosuroides* (Vill.) Fiori): SPAIN. **Cantabria**: Picos de Europa, Fuente Dé, upper station of the cable car to Horcados Rojos, 1900–2400 m, 14 Jul. 1985, M. Luceño and P. Vargas 419 (MA 342212).

*Distribution.*—On Cyperaceae: *Carex myosuroides*; Europe, Asia (East Siberia), and North America (Canada, Greenland).

*Comments.*—Both the smut fungus and its host plant are circumpolar–alpine species (Denchev & al. 2020a; Elven & al. 2020). In Europe, *Schizonella elyngae* is known from North Europe (Iceland, Norway, and Sweden) and the Alps

(Germany, Austria, and Italy) (Blytt 1896; Lindeberg 1959; Jørstad 1963; Helgi Hallgrímsson & Guðríður Gyða Eyjólfssdóttir 2004; Kruse & al. 2019; Denchev & al. 2020a). The present record extends the geographic range of this species to the Iberian Peninsula. *Schizonella elynae*, on *Carex pilulifera* L., was erroneously reported from Spain by Almaraz (1998: 123, 2002: 47), based on a wrongly revised specimen of González Frago, published by him (1924a: 121) as *S. melanogramma* (DC.) J.Schröt. on *C. praecox* Schreb.

***Sphacelotheca polygoni-serrulati*** Maire, Bull. Soc. Hist. Nat. Afrique N. 8: 74 (Maire 1917). Type: on *Persicaria decipiens* (as '*Polygonum serrulatum*'), Algeria, Algiers, Réghaïa, 15 Oct. 1915, R. Maire s.n. (lectotype designated by Vánky & Oberwinkler (1994: 28): MPU; isolectotypes: in Maire, Mycotheca Boreali-Africana, no. 229). Fig. 2i–l.

Sori in some ovaries of an inflorescence, ovoid, 2.5–4 mm long, covered by a thick, brownish and brittle peridium which later ruptures irregularly from its apex, exposing a semi-agglutinated, powdery on the surface, date brown (based on Colour identification chart of Anonymous 1969) mass of spores, surrounding a single columella. Spores subglobose, broadly ellipsoidal, slightly irregular, ovoid or globose, often slightly flattened, initially in chains, connected by disjunctors, later single, usually with two or sometimes three persistent appendages on the opposite sides of the spores (remnants of disjunctors), (9.5–)10–13(–14) × (8.5–)9.5–12(–13) (11.6 ± 0.8 × 10.4 ± 0.7) µm (n = 100), medium vinaceous; wall finely and irregularly reticulate, evenly thickened, 0.6–1.0 µm thick, spore profile not affected. In SEM, spore wall incompletely reticulate or labyrinthiform.

*Specimen examined*.—On *Persicaria decipiens* (R.Br.) K.L.Wilson (as '*Polygonum salicifolium* Brouss. ex Willd.'): PORTUGAL. **Setúbal**: Santiago do Cacem, Ribeira da Lezíria, 24 Oct. 1979, L.A. Grandvaux Barbosa 13330 (B 10 0506861).

*Distribution*.—On Polygonaceae: *Persicaria barbata* (L.) H.Hara (*Polygonum barbatum* L.), *P. decipiens* (*Polygonum salicifolium* Brouss. ex Willd., *P. serrulatum* Lag.), *P. maculosa* S.F.Gray, *P. pulchra* (Blume) Soják, and *P. setosula* (A.Rich.) K.L.Wilson (*Polygonum setosulum* A.Rich.); Europe (Portugal, Spain), Africa, Australasia (Australia, New Zealand).

*Comments*.—*Sphacelotheca polygoni-serrulati* is recorded here for the first time from Portugal. In Europe, this smut fungus has been previously reported only once, for Spain: on *Persicaria decipiens* (as '*Polygonum salicifolium*') in the Province of Barcelona, Gavá (Almaraz 2002). The present finding represents the second record for Europe.

In Africa, it is known from single localities in Algeria, Cameroon, the D.R. of the Congo, Madeira, Uganda,

Rwanda, and Zambia (Maire 1917; Liro 1924; Zundel 1944; Deml & al. 1985; Vánky & al. 2011; Piątek & al. 2012).

***Sporisorium egyptiacum*** (A.A.Fisch.Waldh.) Vánky (as '*aegypticum*'), Mycotaxon 33: 371 (Vánky 1988); *Ustilago egyptiaca* A.A.Fisch.Waldh. (as '*aegyptiaca*'), Hedwigia 18: 100 (Fischer von Waldheim 1879). Type: on *Schismus barbatus* (as '*S. calycinus*'), Egypt, near Cairo, 1820–1824, G. Ehrenberg s.n. (holotype: LE). Fig. 3a–d.

Infection systemic, in all spikelets of the inflorescence. Sori in the basal part of florets leaving intact the glumes and the distal part of the floret (in spikelets with mature sori, the basal part of the florets appears bullate, similar to sori of *Ustilago bullata* Berk.), 1.5–2.5 × 0.7–1.2 mm, ovoid or ellipsoidal, partially visible between the spreading glumes; initially covered by a thin, yellowish brown peridium that soon ruptures irregularly from its basal part, exposing a single, flattened, tapering columella as long as the sorus, surrounded by a powdery, blackish brown mass of spores and sterile cells. The infected plants are stunted. Sterile cells single, in irregular groups or in short chains, irregular, subglobose, broadly ellipsoidal or ellipsoidal, (6–)7–11.5(–12.5) × (5.5–)6.5–10(–11) (9.4 ± 1.6 × 8.0 ± 1.1) µm (n = 50), hyaline; wall 0.6–1.0 µm thick. *Spores* irregularly rounded, subglobose, broadly ellipsoidal, ellipsoidal or ovoid, (10.5–)11.5–14.5(–15.5) × (8.5–)9.5–12.5(–13.5) (13.0 ± 0.8 × 11.1 ± 0.9) µm (n = 100), medium reddish brown; wall 0.7–1.3 µm thick, minutely echinulate, ornaments up to 0.4(–0.5) µm high, spore profile slightly affected. In SEM, spore surface densely punctate between the spines.

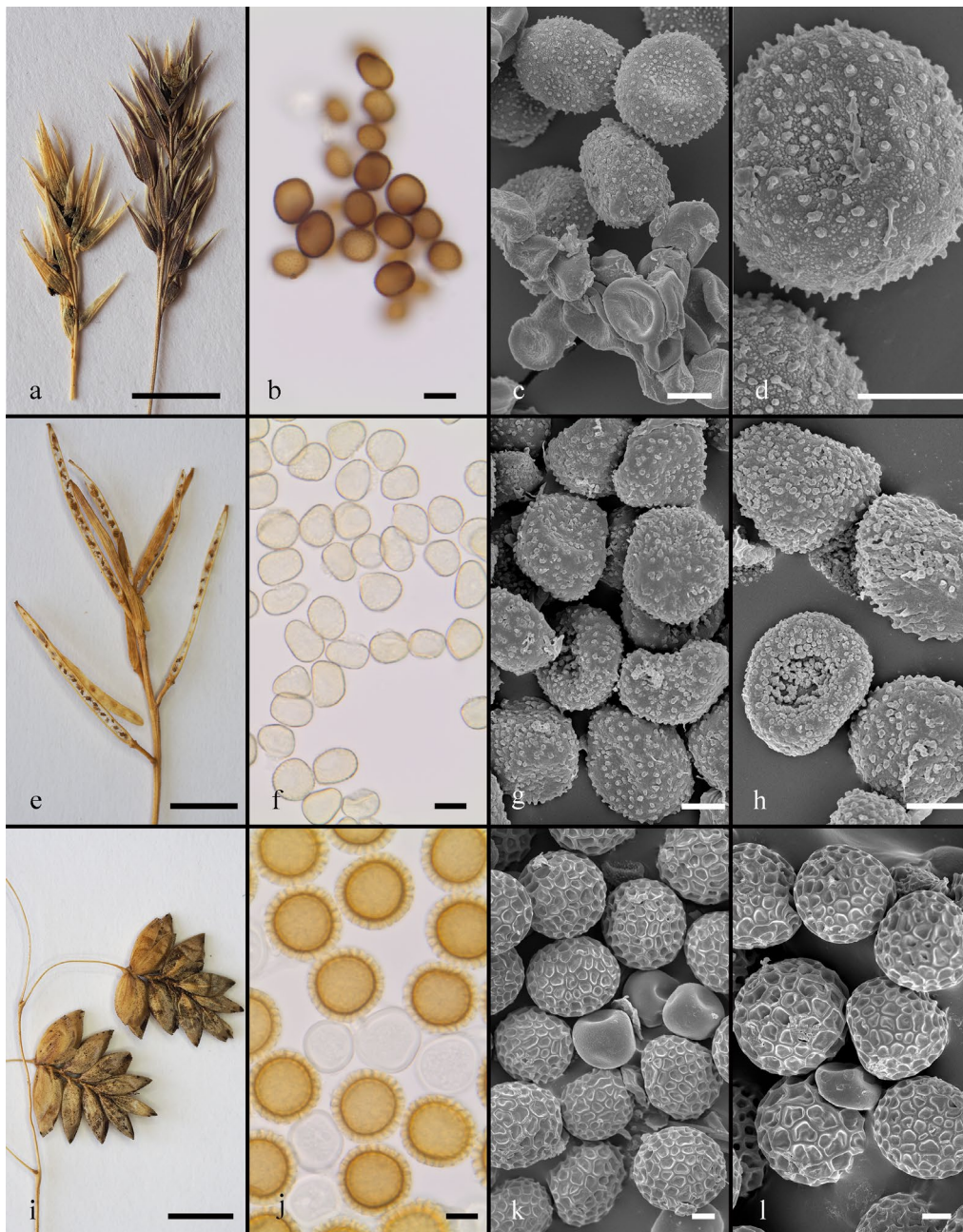
*Specimen examined*.—On *Schismus barbatus* (L.) Thell. (as '*S. calycinus* Cosson & Durieu'): SPAIN. **Almería**: Rioja near Gérgal, 11 Mar. 1970, J. Fernández Casas s.n. (MA 415522).

*Distribution*.—On Poaceae: *Schismus arabicus* Nees, *S. barbatus* (*S. calycinus* (Loefl.) K.Koch, *S. minutus* (Hoffm.) Roem. & Schult.); Europe (Spain), North Africa (Egypt, Libya), Asia, Australia.

*Comments*.—This finding of *Sporisorium egyptiacum* represents the first record for the Iberian Peninsula and Europe (cf. Vánky 1994, 2005).

***Thecaphora thlaspeos*** (Beck) Vánky, Mycotaxon 89: 111 (Vánky 2004); *Tilletia thlaspeos* Beck, Verh. K. K. Zool.-Bot. Ges. Wien 35: 362 (Beck 1886); *Ustilago thlaspeos* (Beck) Lagerh., in Sydow, Ustilaginales Exsiccata: no. 118 (1897). Type: on *Thlaspi alpestre*, Austria, Burgenland, near Redlschlag, V. Borbás s.n. (HUV 4776 in BRIP). Fig. 3e–h.

Infection systemic. Sori in siliquae, replacing the seeds. Spore mass powdery, yellowish brown, released when the siliquae open. Spores single, variable in shape, irregular,



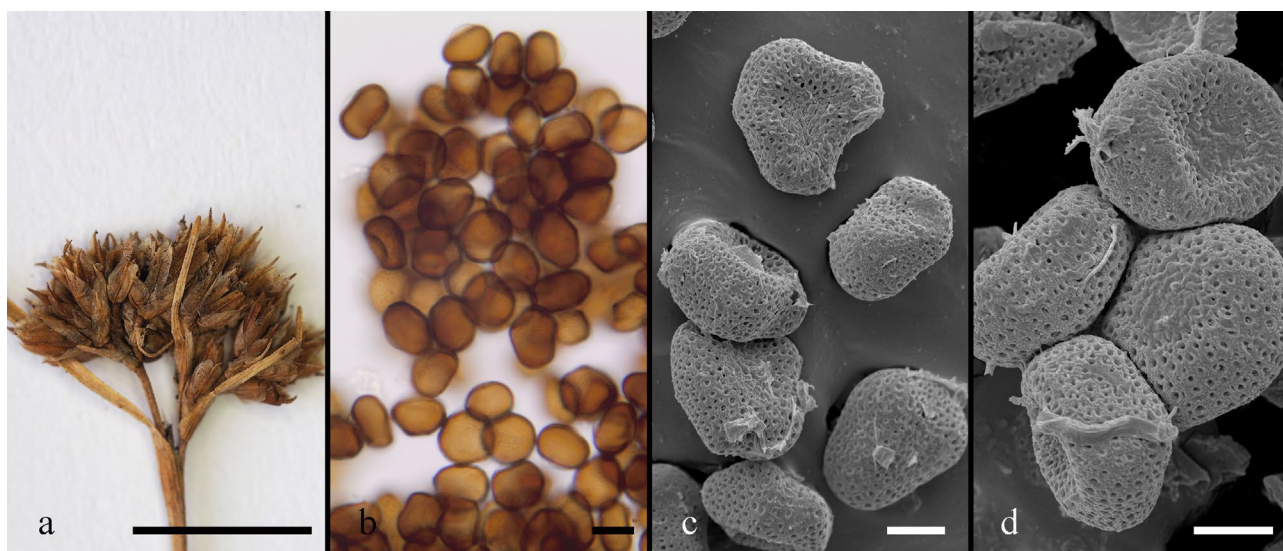
**Fig. 3.** *Sporisorium egyptiacum* (A.A.Fisch.Waldh.) Vánky (MA 415522): **a**, habit; **b**, spores in LM; **c**, **d**, spores and sterile cells in SEM. *Thecaphora thlaspeos* (Beck) Vánky (MA 331875): **e**, habit; **f**, spores in LM; **g**, **h**, spores in SEM. *Tilletia viennotii* Syd. (Álvarez & al. 1344 1A, MA-Fungi s.n.): **i**, habit; **j**, spores and sterile cells in LM; **k**, **l**, spores and sterile cells in SEM. Scale bars: **a**, **e**, **i** = 0.5 cm, **b**, **f**, **j** = 10  $\mu$ m, **c**, **d**, **g**, **h**, **k**, **l** = 5  $\mu$ m.

subglobose, broadly ellipsoidal, ellipsoidal, elongated, globose, ovoid or reniform, (9.5–)10.5–18(–20)  $\times$  (8–)9–12(–13.5) (13.4  $\pm$  1.8  $\times$  10.5  $\pm$  1.0)  $\mu$ m ( $n$  = 100), light to medium yellowish brown; wall 0.5–1.1  $\mu$ m thick, verrucose-echinate, ornaments up to 0.6  $\mu$ m high, on a restricted area of the wall ornaments coarser and higher, up to 2.0(–2.4)  $\mu$ m high. In SEM, spore wall verrucose-echinate, smooth to sparsely punctate between the ornaments.

*Specimen examined.*—On *Arabis serpillifolia* Vill.: SPAIN. **Huesca**: Valle de Ordesa, Faja Pelay, 1400 m, 29 Aug. 1969, J. Fernández Casas s.n. (MA 331875).

*Distribution.*—On Brassicaceae: *Alyssum reiseri* Velen., *Arabidopsis petraea* (L.) V.I.Dorof. (*Cardaminopsis petraea* (L.) Hiitonen), *Arabis alpina* L., *A. ciliata* Clairv. (*A. corymbiflora* Vest), *A. hirsuta* (L.) Scop., *A. pubescens* (Desf.) Poir., *A. sagittata* (Bertol.) DC., *A. serrata*





**Fig. 4.** *Ustanciosporium majus* (Desm.) M. Piepenbr.: **a**, habit (MA 798317); **b**, spores in LM (MA 483460); **c**, **d**, spores in SEM (MA 483460). Scale bars: a = 0.5 cm, b = 10  $\mu$ m, c, d = 5  $\mu$ m.

var. *hallaisanensis* (Nakai) Ohwi, *Cardamine bellidifolia* L., *Draba aizoides* L., *D. alpina* L., *D. incana* L., *Erysimum diffusum* Ehrh., *E. welcevii* Urum., *Noccaea alpestris* (Jacq.) Kerguelen (*Thlaspi alpinum* Crantz), *Noccaea brachypetala* (Jord.) F.K.Mey. (*Thlaspi brachypetalum* Jord.), and *N. caerulescens* (J. Presl & C. Presl) F.K.Mey. (*Thlaspi alpestre* L.); Europe, North Africa (Algeria), and Asia (South Korea).

**Comments.**—*Thecaphora thlaspeos* is a rather inconspicuous species, without obvious infection symptoms. This smut fungus can be seen once siliquae are opened and the spore mass becomes exposed (Denchev & Denchev 2019). *Thecaphora thlaspeos* is reported here for the first time from the Iberian Peninsula. *Arabis serpillifolia* is endemic to Europe, known from the Pyrenees, the Iberian System, Jura Mts, and the Alps (Jones & Akeroyd 1993; Talavera 1993). It is a new host plant record for *T. thlaspeos*.

***Tilletia viennotii*** Syd., Ann. Mycol. 35: 258 (Sydow 1937). Type: on *Briza maxima*, Madeira Island, Curral Grande, Aug. 1936, G. Viennot-Bourgin s.n. (holotype: PC). Fig. 3i–l.

Infection systemic. Sori in ovaries of all spikelets of an infected plant, hidden by the glumes, 2.5–3.5  $\times$  1.5–2 mm, covered by a thin, brown pericarp with parallel veins. Mass of spores and sterile cells powdery, umber (based on Rayner 1970) or snuff brown (based on Colour identification chart of Anonymous 1969), evident after rupturing of the pericarp. Sterile cells slightly irregular, subglobose or broadly ellipsoidal, (16.5–)17.5–23(–24)  $\times$  16–21(–22.5) (20.4  $\pm$  1.5  $\times$  18.7  $\pm$  1.3)  $\mu$ m (n = 50), hyaline; cell wall two-layered, (1.3–)1.5–2.3(–2.6)  $\mu$ m thick. In SEM, smooth to punctate. Spores subglobose, broadly ellipsoidal

or globose, (22.5–)23.5–28(–29)  $\times$  (21.5–)22.5–26(–27) (25.9  $\pm$  1.2  $\times$  24.2  $\pm$  1.0)  $\mu$ m (n = 100), medium yellowish brown to medium reddish brown, reticulate; spore wall (4.0–)4.3–5.5(–5.8)  $\mu$ m thick (including reticulum); meshes 6–8(–9) per spore diameter, polyhedral or irregular, (0.8–)1.2–5.0(–6.5)  $\mu$ m long; muri 22–31 on equatorial circumference, in optical median view subacute, acute or blunt, (2.3–)2.6–3.7(–4.2)  $\mu$ m high; often covered by thin, hyaline sheath. In SEM, interspaces smooth, sometimes with a very low, hemispherical protuberance.

**Specimen examined.**—On *Briza maxima* L.: PORTUGAL. **Beira Alta:** Guarda, between Vale de Estrela and Guarda, 950 m, 19 Jul. 1997, I. Álvarez 1344 IA, M.A. García, and L. Medina (MA-Fungi s.n.).

**Distribution.**—On Poaceae: *Briza maxima*, *B. minor* L.; Europe (Portugal), Africa, and Australia.

**Comments.**—*Briza maxima* and *B. minor* are native to the Mediterranean and Macaronesian regions (*B. minor* also in SW Asia to Iran) but they have been introduced to many countries throughout the world, as ornamental species, and widely naturalised there (Isabel & al. 2018; Clayton & al. 2020). Within its native range, *B. maxima* is reported as infected by *Tilletia viennotii* only from Madeira (Sydow 1937) while outside this range, there are records from South Africa (Zundel 1938; Vánky 1998; Vánky & al. 2011) and Australia (Vánky & Shivas 2008). *Briza minor* is known as a host plant only from Australia (Vánky & Shivas 2008). Thus, the finding reported here is of high interest, as it represents the first record of this smut fungus not only from the Iberian Peninsula but also from Europe.

*Tranzscheliella sparti* (Massenot) Vánky, Mycotaxon 85: 4 (Vánky 2003); *Ustilago sparti* Massenot, in Guyot & al., Rev. Pathol. Vég. Entomol. Agric. France 34: 216 (Guyot & al. 1955). Type: on *Lygeum spartum*, Tunisia, near Had-jeb-el-Aioun, 1 Oct. 1953, L. Guyot s.n. (lectotype designated by Vánky (1994: 376): PC; syntype: near Kasserine, 2 Oct. 1953, L. Guyot s.n.).

*Specimen examined*.—On *Lygeum spartum* L.: SPAIN. **Ciudad Real**: Alcázar de San Juan, 640 m, 25 Aug. 1998, L. Medina s.n. (MA-Fungi s.n.).

*Distribution*.—On Poaceae: *Lygeum spartum*; South Europe and North Africa (Vánky 2011).

*Comments*.—A new distribution record from Spain.

*Ustanciosporium majus* (Desm.) M. Piepenbr., Nova Hedwigia 70: 341 (Piepenbring 2000), **s. str.**; *Ustilago montagnei* var. *major* Desm., in Desmazières, Pl. Cryptog. N. France, ed. 1: no. 2126 (Desmazières 1851); *Cintractia major* (Desm.) Liro, Ann. Acad. Sci. Fenn., Ser. A 42(1): 46 (Liro 1935, in Liro 1938); *Ustilago intercedens* Lehtola, Ann. Bot. Soc. Zool.-Bot. Fenn. “Vanamo” 17(3): 23 (Lehtola 1942). Type: on *Rhynchospora alba*, France, *s.coll.* (isotype: in Desmazières, Pl. Cryptog. N. France, ed. 1: no. 2126, as ‘*Ustilago montagnei* var. *major*’). Fig. 4a–d.

Infection systemic. Sori in all spikelets of an infected inflorescence, hidden by the glumes, formed around more or less destroyed inner floral organs, naked; spore mass blackish brown, initially semi-agglutinated, later powdery. Spores single, flattened, in plane view usually irregularly rounded to subpolygonal or broadly elliptical, sometimes suborbicular or ovate, in plane view 11–16(–17) × (8.5–)9.5–13(–14) (13.6 ± 1.2 × 11.6 ± 1.0) µm (n = 100), in side view 6.5–9.5 µm thick, medium reddish brown; wall evenly to unevenly thickened, 0.5–1.0(–1.2) µm thick, finely, densely foveolate, sometimes with a hyaline or subhyaline appendage on one or both of the flattened sides, appendages wider than high 4–6.5 µm wide, 2–3.5 µm high; detached appendages occasionally present. In SEM, spore wall densely foveolate, punctate between the foveoles.

*Specimens examined*.—On *Rhynchospora alba* (L.) Vahl: SPAIN, **Ávila**: Navalguijo, Aug. 1986, M. Luceño and P. Vargas s.n. (MA 483460). **Zamora**: Rosinos de la Requejada, Doney de la Requejada, bank of Rio Negro River, 1100 m, 10 Oct. 2002, P. Bariego PB-1941 (MA 798317).

*Distribution*.—On Cyperaceae: *Rhynchospora alba*; Europe, Asia, and North America.

*Comments*.—This smut fungus is recorded here for the first time from the Iberian Peninsula.

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