Contribution to the smut fungi of Greece

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Abstract: After examination of specimens in the herbarium of the Botanic Garden and Botanical Museum Berlin, eight species of smut fungi are reported for the first time from Greece: Microbotryum dianthorum on Dianthus viscidus, Sporisorium pulverulentum on Saccharum strictum, Tilletia fusca on Vulpia ciliata, T. lolii on Lolium temulentum, Urocystis dactylidina on Dactylis glomerata subsp. hackelii, U. johansonii on Juncus bufonius, U. ornithogali on Ornithogalum sp. and U. ulei on Festuca jeanpertii. Four species of smut fungi are recorded on new host plants making new fungus-host combinations: Microbotryum dianthorum on Dianthus viscidus, Tranzscheliella williamsii on Stipa isoldeae, Urocystis dactylidina on Dactylis glomerata subsp. hackelii and U. ulei on Festuca jeanpertii. Two plant species are reported as new hosts of smut fungi already known from Greece: Stipa isoldeae for Tranzscheliella williamsii, and Lygeum spartum for T. hypodytes. All ten species of smut fungi are illustrated. A description is also provided for Urocystis agropyri, recorded in Greece on Thinopyrum junceum.

Key words: Dactylis glomerata subsp. hackelii, Dianthus viscidus, Festuca jeanpertii, Greece, Microbotryum, smut fungi, Sporisorium, taxonomy, Stipa isoldeae, Tilletia, Tranzscheliella, Urocystis

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Introduction

Greece is home to an unusually high diversity of vascular plants, ranking among the highest in Europe and the Mediterranean area with 5752 species, and one of the most important centres of endemism with 1278 endemic species (22.2 % of the total number of species) (Dinopoulos & al. 2013). This is a prerequisite for a high species richness of parasitic fungi on plants. However, the diversity of the smut fungi in Greece (Ustilaginomycotina and Microbotryales) is not intensively studied. No regional monographic study has been carried out yet. To date, only 66 species on 77 vascular plant species have been reported, making 86 smut-host combinations (Hohenbühl 1868; Bornmüller 1894, 1928; Magnus 1894; Maire 1905, 1917; Sarejanni 1935, 1939; Sydow 1935; Săvulescu 1937; Konstantinia-Sulidu 1939; Maire & Politis 1940; Petrak 1943, 1944, 1956; Apostolidis 1952; Crittenpoles 1953; Demetriades & Zachos 1962; Durrieu 1968; Brandenburg 1969; Pyrowolakis & Weltzman 1970; Vánky 1980, 1985b, 1986, 1989, 1990a, b, 1991, 1992, 1996, 1998, 2003a, b, 2008, 2011; Scholz & Scholz 1988; Scheuer 1992, 2010; Vánky & Oberwinkler 1994; Denchev 1997; Triebel 1998, 1999; Vánky & Scholz 2001; Vánky & Berner 2003; Kashefi & Vánky 2004; Vánky & al. 2005; Denchev & Minter 2008, 2011a, b; Braun 2013; Denchev & al. 2013; Savchenko & al. 2014).

In the present article, eight species of smut fungi (indicated by * in the text) are reported for the first time from Greece: Microbotryum dianthorum, Sporisorium pulverulentum, Tilletia fusca, T. lolii, Urocystis dactylidina, U. johansonii, U. ornithogali and U. ulei. Four fungus-host combinations are recorded for the first time: Microbotryum dianthorum on Dianthus viscidus, Tranzscheliella williamsii on Stipa isoldeae, Urocystis dactylidina on Dactylis glomerata subsp. hackelii and Urocystis ulei on Festuca jeanpertii. Two plant species are reported as new hosts of smut fungi already known from Greece: Stipa

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isoldeae for Tranzscheliella williamsii and Lygeum spartum for T. hypodrys.

The smut fungi reported here were found during examination of specimens in the herbarium of the Botanic Garden and Botanical Museum Berlin.

Material and methods

Dried specimens from the herbarium of B (herbarium code according to Thiers 2016+) were examined under a light microscope (LM) and a scanning electron microscope (SEM). For LM observations and measurements, spores were mounted in lactoglycerol solution (w : l : a : 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 (mean ± 1 standard deviation). For SEM, spores were attached to specimen holders by double-sided adhesive tape and coated with platinum-palladium in an ion sputter. The surface structure of the spores was observed and photographed at 5 kV accelerating voltage using a JEOL JSM-7600F scanning electron microscope (Naturalis Biodiversity Center, Leiden, The Netherlands). The descriptions below are based entirely on the specimens examined. The description of spore ornamentation is in accordance with Denchev & al. (2013). The height of the wall ornamentation (warts and spines) was measured with SEM. Lists of shapes of sterile cells or spores are arranged in descending order of frequency.

New records of smut fungi for Greece

*Microbotryum dianthorum* (Liro) H. Scholz & I. Scholz in Englera 8: 206. 1988, s. lat. – Fig. 1A–C.

Sori in anthers (some flowers may be unaffacted). Spore mass dark reddish brown. Spores globose, subglobose, broadly ellipsoidal, ellipsoidal or ovoid, (5.5–6–8.5–9.5) × (5–5.5–7.5–8) (7.2 ± 0.6 × 6.4 ± 0.5) μm (n = 100), pale vinaceous; spore wall reticulate, 1–1.5 μm thick; meshes (5–6–9) per spore diameter, polygonal or rounded, 0.4–1.1–1.8 μm wide; muri 0.5–0.9 μm high; interspaces in SEM smooth or rugulate.


Note — *Dianthus viscidus* is a new host for *Microbotryum dianthorum.*

*Sporisorium pulverulentum* (Cooke & Massée) Vánky in Symb. Bot. Upsal. 24(2): 120. 1985. – Fig. 1D–F.

Sori in all spikelets of inflorescence, elongate to cylindric, c. 1.5 × 0.7 mm, partially concealed by glumes, covered by a thick yellow-brown to rusty brown peridium that ruptures irregularly (usually at apex) exposing a single, stout, tapering, sometimes slightly branched columella with shallow, longitudinal furrows. Columella to 5 mm long, surrounded by powdery, blackish brown mass of spores and sterile cells. *Sterile cells* in irregular groups (single sterile cells not seen), subglobose, broadly ellipsoidal or irregular, often collapsed, (7–8.5–16.5–18.5) μm long, hyaline; cell wall 0.6–1.2 μm thick, smooth. *Spores* subglobose, broadly ellipsoidal, slightly irregular or ovoid, sometimes ellipsoidal, (9.5–10–13.5–15) × (8.5–9.5–12–13) (12.0 ± 0.8 × 10.8 ± 0.8) μm (n = 100), medium yellow-brown; spore wall ± evenly thickened, 0.6–0.8(–1) μm thick, often with one or two paler, rounded areas of 2.5–4 μm in diameter, minutely verruculose, spore profile not affected or slightly affected, in SEM minutely echinulate; spines to 0.2 μm high, spore surface densely punctate between spines.

Specimen examined — On *Saccharum strictum* (Host) Sprenq. – GREECE: Rodos island, c. 1 km E of Archangelos, 23 Oct 2003, M. Ristow (B 70 0015526).

Note — *Sporisorium pulverulentum* is a rare species known only from S Europe and S and SE Asia (India, Malaysia and Indonesia) (Vánky 2011; Chalkley 2015). In Europe, it has previously been recorded only from Serbia (Mt Fruska Gora near Novi Sad; Vánky 1985a, b).

*Tilletia fusca* Ellis & Everh. in J. Mycol. 3: 55. 1887. – Fig. 2A–C.

Sori in ovaries of most spikelets of infected plant, ovoid, 1.5–2.2 mm long, partially concealed by floral envelopes, initially covered by thin, dark brown pericarp that later ruptures exposing powdery, dark reddish brown mass of spores and sterile cells. *Sterile cells* subglobose or broadly ellipsoidal, sometimes slightly irregular, (9.5–10.5–14.5–15.5) × (9–10–13.5–14.5) μm, hyaline; cell wall 0.9–1.6 μm thick, in SEM smooth. *Spores* globose, subglobose or broadly ellipsoidal, (16.5–17.5–20.5–21.5) × (16–17–19.5–20.5) (19.1 ± 1.0 × 18.2 ± 1.0) μm (n = 100), light to medium yellow-brown, completely reticulate; spore wall 3–3.8 μm thick (including reticulum and hardly visible, 0.5–0.7 μm-thick inner layer); meshes (5–6–8–9) per spore diameter, usually polygonal, sometimes rounded, (0.9–1.2–3.5–4) μm wide; muri 21–29 on equatorial circumference, in optical median view subacute or blunt, (1.2–1.4–1.8–2.2) μm high, in SEM some meshes with a low hemispherical protuberance on bottom.

Specimen examined — On *Vulpia ciliata* Dumort. – GREECE: Crete, Agia Triada, 27 Mar 1979, R. Böcker (B 70 0015527).
Fig. 1. A–C: Microbotryum dianthorum on Dianthus viscidus (B 70 0007639); A: habit; B, C: spores in LM and SEM, respectively. – D–F: Sporisorium pulverulentum on Saccharum strictum (B 70 0015526); D: habit; E, F: spores in LM and SEM, respectively. – Scale bars: A = 0.5 cm, B = 10 µm, C = 1 µm, D = 0.2 cm, E = 10 µm, F = 5 µm.
Fig. 2. A–C: *Tilletia fusca* on *Vulpia ciliata* (B 70 0015527); A: habit; B: spores in LM; C: spores and a sterile cell in SEM. – D–F: *Tilletia lolii* on *Lolium temulentum* (B 70 0015546); D: habit; E: spores in LM; F: spores and a sterile cell in SEM. – Scale bars: A = 0.2 cm, B = 10 µm, C = 5 µm, D = 0.2 cm, E = 10 µm, F = 5 µm.
*Tilletia lolii* Auersw. ex G. Winter, Rabenh. Krypt.-Fl., ed. 2, 1(1): 109. 1881. – Fig. 2D–F.

Sori in all ovaries of infected plant, ovoid, 3–4 × 1.2–1.8 mm, partially concealed by floral envelopes, initially covered by thin, purplish brown pericarp that later ruptures exposing powdery, cinnamon-brown mass of spores and sterile cells. Sterile cells subglobose, broadly ellipsoidal or slightly irregular, (11.5–12.5–15.5(–18) × (10.5–)11.5–14.5(–16.5) μm, subhyaline; cell wall (0.9–)1.2–1.8(–2.3) μm thick, smooth. Spores globose, broadly ellipsoidal or subglobose, (18–)18.5–21.5(–23) × (17.5–)18–20(–21) (19.9 ± 0.7 × 19.0 ± 0.6) μm (n = 100), light yellow-brown, completely reticulate; spore wall 2.9–4 μm thick (including reticulum and 0.5–0.8 μm-thick inner layer), covered by a hyaline sheath 0.3–0.6 μm thick; meshes (5–)6–7(–8) per spore diameter, usually polyhedral, sometimes rounded, 0.7–3.8(–4.3) μm wide; muri 25–33 on equatorial circumference, in optical median view acute or subacute, 1.3–2.2(–2.5) μm high, in SEM single meshes with a low hemispherical or conical protuberance on bottom.

Specimen examined — On Lolium temulentum L. – GREECE: Crete, Prov. Rethymno, between the mountain peaks TsiliVidas and Xilos Korifi near Kali Sikia village, 720 m, 30 May 1983, Greuter & Matthäus (B 70 0015546).

*Tranzscheliella hypodytes* (Schltdl.) Vánky & McKenzie, Smut Fungi New Zealand: 156. 2002, s. lat. – Fig. 3A–C.

Sori around upper internode or around branches of aborted inflorescence, initially covered by upper leaf sheaths or spatheole, respectively, later exposed; peridium absent. Spore mass powdery, blackish brown. Infection systemic. Spores slightly flattened, in plane view suborbicular, orbicular or broadly elliptic, sometimes slightly irregular, in plane view (5.5–)6–9(–9.5) × (5–)5.5–7.5(–8) (7.4 ± 0.8 × 6.8 ± 0.5) μm (n = 100), medium olivaceous brown; spore wall 0.6–1 μm thick, smooth; exospore often cracked, bearing 2 persistent appendages on flattened sides, in SEM with low, flattened, densely packed ornaments connected in small groups and short rows or forming labyrinthiform pattern.


Notes — From Greece, *Tranzscheliella williamsii* has been previously recorded only once, as *Ustilago athenae* (Maire). This relevant specimen is kept at the Mycological Collection of the Natural History Museum, Vienna (W): Epír, Distr. Ioannina, Montes Pindus, in monte Tsuka Rossa ditionis pagi Vovousa (Viosa), 1600–1980 m, in pinetis, substr. serpent., 1–2 Aug 1956, K. H. Rechinger 18586 (K. H. Rechinger, Iter Graecum VIII, 1956; W 1976-04903) (Fig. 3D). This specimen was re-examined by us and its identification was confirmed.

*Stipa isoleae* is endemic to Greece. This grass is reported here as a new host of *Tranzscheliella williamsii*.

*Urocystis agropyri* (Preuss) A. A. Fisch. Waldh. in Bull. Soc. Imp. Naturalistes Moscou 15: 477. 1977. – Fig. 3D–F; Fig. 4A.

Sori around upper internodes or aborted inflorescence branches, initially covered by upper leaf sheaths, later exposed. Spore mass powdery, blackish brown. Infection systemic. Spores slightly flattened, in plane view suborbicular, orbicular or broadly elliptic, sometimes slightly irregular, in plane view (5.5–)6–9(–9.5) × (5–)5.5–7.5(–8) (7.4 ± 0.8 × 6.8 ± 0.5) μm (n = 100), medium olivaceous brown; spore wall 0.6–1 μm thick, smooth; exospore often cracked, bearing 2 persistent appendages on flattened sides, in SEM with low, flattened, densely packed ornaments connected in small groups and short rows or forming labyrinthiform pattern.

Specimen examined — On Ustilago sp. (Petrak 1956). The relevant specimen is kept at the Mycological Collection of the Natural History Museum, Vienna (W): Epír, Distr. Ioannina, Montes Pindus, in monte Tsuka Rossa ditionis pagi Vovousa (Viosa), 1600–1980 m, in pinetis, substr. serpent., 1–2 Aug 1956, K. H. Rechinger 18586 (K. H. Rechinger, Iter Graecum VIII, 1956; W 1976-04903) (Fig. 3D). This specimen was re-examined by us and its identification was confirmed.

*Urocystis agropyri* is reported here as a new host of *Tranzscheliella williamsii*.
Fig. 3. A–C: *Tranzscheliella hypodytes* on *Lygeum spartum* (B); A: habit; B, C: spores in LM and SEM, respectively. – D: Habit of *Tranzscheliella williamsii* on *Stipa* sp. (W). – E, F: *Tranzscheliella williamsii* on *Stipa isoldea* (B); E: habit; F: spores in LM. – Scale bars: A = 1 cm, B = 10 µm, C = 1 µm, D = 1 cm, E = 0.2 cm, F = 10 µm.
Fig. 4. A: Spores of Tranzscheliella williamsii on Stipa isoldeae (B) in SEM. – B, C: Urocystis dactylidina on Dactylis glomerata subsp. hackelii (B); B: spore balls in LM; C: spore ball in SEM. – D–F: Urocystis johansonii on Juncus bufonius (B); D: habit; E, F: spore balls in LM and SEM, respectively. – Scale bars: A = 1 µm, B = 10 µm, C = 5 µm, D = 0.5 cm, E = 10 µm, F = 5 µm.
on side proximal to spores thicker, smooth, in SEM punctate; projections irregularly arranged, often fused. *Spores* broadly ellipsoidal, subpolyhedral, subglobose, ellipsoidal or ovoid, sometimes cuneate or elongate, often slightly flattened on a few places, (11.5–)12.5–17.5–(19.5)×(10–)10.5–14.5–(16) (15.8 ± 1.4 × 12.8 ± 1.2) µm (n = 100), medium reddish brown; spore wall slightly uneven, 0.7–1.1 µm thick.


*Note* — In some websites, *Urocystis agropyri* is reported from Greece as collected on wheat. In fact, the correct name of the smut fungus in leaves and stems of *Triticum* is *U. tritici* Körn. The only other known record of *U. agropyri* from Greece is on *Thinopyrum junceum* (as *Elymus farctus*) from Kos island (collected on 21 Apr 1990, *H. Scholz* & *I. Scholz*, Vánky, *Ustilaginales exsiccata*, no. 769, Vánky 1990b). *Urocystis agropyri* in its broad sense represents a species complex (Vánky 2011) that probably contains a few species. For clarification of their number and specialization and whether these species are morphologically recognizable, this complex needs a combined molecular and morphological study. For this reason, we considered that it would be helpful to include here a description of the Greek specimen examined by us on *Thinopyrum junceum*.

*Urocystis dactylidina* (Lavrov) Zundel in Contr. Dept. Bot. School Agric. Pennsylvania State Coll. 176: 314. 1953. – Fig. 4B, C.

*Sori* in leaves and sheaths as long striae, initially covered by epidermis that later ruptures disclosing powdery, blackish brown mass of spore balls. *Spore balls* irregular, broadly ellipsoidal, subglobulo or ovoid, composed of 1–3(or 4) central spores (1 = 42.5 %, 2 = 45.3 %, 3 = 9.7 %, 4 = 2.5 %; n = 746), surrounded by a continuous or almost continuous layer of sterile cells, (18–)20–29–(32) × (16–)17–24–(28) µm [with 1 spore], (24–)26–34–(37) × (18–)20–28–(32) µm [with 2 spores], (29–)31–38–(42) × (23.5–)25–33–(37) µm [with 3 spores]. *Sterile cells* suborbicular, broadly elliptical, irregular, elliptic or ovate in outline, often collapsed, (4.5–)5.5–14–(17) µm long, medium yellow-brown; cell wall irregularly thickened, on side distal to spores 0.7–2.3(–2.6) µm thick, on side proximal to spores thicker, smooth, in SEM punctate; projections sometimes connected, forming fine, irregular pattern. *Sporidesmium* subpolyhedral, broadly ellipsoidal, subglobose or ovoid, sometimes ellipsoidal or cuneate, sometimes slightly flattened on a few places, (11.5–)12.5–18.5–(20) × (10–)11–15.5–(16.5) (15.3 ± 1.5 × 13.1 ± 1.2) µm (n = 100), medium reddish brown; spore wall slightly uneven, 0.8–1.2(–1.4) µm thick.


*Note* — *Urocystis dactylidina* is a rare species collected only a few times in C Europe (Czech Republic, Switzerland, ?Poland) and Asia (W and E Siberia) (Vánky 1985a, 1994, 2011; Azbukina & Karatygin 1995; Karatygin 2012). Vánky (2011) noted that the host plant identity of all collections, seen by him, was suspected to be wrongly identified. For this reason, *Dactylis glomerata* subsp. *hackelli*, reported here, is a new host of *U. dactylidina*.

*Urocystis johnsonii* (Lagerh.) Magnus in Verh. Bot. Vereins Prov. Brandenburg 37: 94. 1896. – Fig. 4D–F.

*Sori* at basal part of leaves as bulb-like swellings, initially covered by epidermis that later ruptures disclosing powdery, blackish brown mass of spore balls. *Spore balls* subglobose, irregular, broadly ellipsoidal, ovoid or ellipsoidal, composed of 1–5(–9) central spores (1 = 6 %, 2 = 29.6 %, 3 = 35.2 %, 4 = 15.6 %, 5 = 8.8 %, 6 = 2.8 %, 7 = 1 %, 8 = 0.7 %, 9 = 0.3 %; n = 609), surrounded by a continuous layer of sterile cells, (13.5–)14.5–20–(21) × (12.5–)13.5–17.5–(18.5) µm [with 1 spore], (18–)20–26–(29) × (14–)16–20–(22) µm [with 2 spores], (21–)22–30–(31.5) × (18–)19–23–(24.5) µm [with 3 spores], (23.5–)25–33–(35) × (19.5–)21–26–(28.5) µm [with 4 spores], (27–)29–41–(45) × (20–)22–31–(33) µm [with 5 spores]. *Sterile cells* irregular, suborbicular, broadly elliptical or ovate in outline, collapsed, 3.5–13(–15) µm long, light or medium yellow-brown; cell wall on side distal to spores 0.5–0.8 µm thick, on side proximal to spores thicker, smooth, in SEM smooth. *Spores* subglobose, broadly ellipsoidal, slightly irregular, ellipsoidal or ovoid, sometimes slightly flattened on a few places, 10.5–15–(16) × (8–)9–12–(13) (13.0 ± 1.0 × 10.7 ± 0.8) µm (n = 100), dark reddish brown; spore wall slightly uneven, 0.9–1.4(–1.6) µm thick.


*Urocystis ornithogali* Körn. ex A. A. Fisch. Waldh., Aperçu Syst. Ustilag.: 41. 1877. – Fig. 5A–C.
Fig. 5. A–C: Urocystis ornithogali on Ornithogalum sp. (B); A: habit; B, C: spore balls in LM and SEM, respectively. – D–F: Urocystis ulei on Festuca jeanpertii (B); D: habit; E, F: spore balls in LM and SEM, respectively. – Scale bars: A = 0.5 cm, B, C = 10 µm, D = 0.5 cm, E, F = 10 µm.
Sori in leaves and sheaths as small to large pustules, initially covered by epidermis that later ruptures disclosing powdery, blackish brown mass of spore balls. Spore balls irregular, broadly ellipsoidal, subglobose or ovoid, composed of 1–3(–5) central spores (1 = 31 %, 2 = 41.7 %, 3 = 21.1 %, 4 = 5.3 %, 5 = 0.9 %; n = 546), surrounded by a continuous layer of sterile cells, (18–)20–26(–28) × (16.5–)18–23(–25.5) µm [with 1 spore], (22–)25–33(–35) × (19.5–)21–26(–28) µm [with 2 spores], (24.5–)27–40(–44) × (22.5–)25–32(–35) µm [with 3 spores]. Sterile cells irregular, suborbicular, orbicular or ovate in outline, collapsed, 5–13(–15) µm long, light or medium yellow-brown; cell wall on side distal to spores 0.4–0.8 µm thick, on side proximal to spores thicker, smooth, in SEM smooth to sparsely punc- tate. Spores subhyphal, broadly ellipsoidal, subglobose or cuneate, sometimes slightly flattened on a few places, (13–)14.5–20.5(–22.5) × (10.5–)11.5–16(–17) (17.3 ± 1.4 × 14.2 ± 1.0) µm (n = 100), medium reddish brown; spore wall slightly uneven, 0.7–1 µm thick.


*Urocytis ulei* Magnus in Rabenhorst, Fungi Europ. ex- sicc. 17: no. 2390. 1878. – Fig. 5D–F.

Sori in leaves and sheaths (infected plants usually ster- ile) as long, slightly swollen striae, initially covered by epidermis that later ruptures disclosing powdery, blackish brown mass of spore balls. Spore balls subglobose, broadly ellipsoidal, irregular, ellipsoidal or ovoid, composed of 1 or 2(–4) central spores (1 = 78.4 %, 2 = 16.6 %, 3 = 4.2 %, 4 = 0.8 %; n = 529), surrounded by a continuous or almost continuous layer of sterile cells, (14.5–)16–27(–29) × (12–)13.5–22(–23.5) µm [with 1 spore], (17.5–)20–34(–38) × (14.5–)16–25(–27) µm [with 2 spores], (27–)29–37(–40) × (22–)24–32(–35) µm [with 3 spores]. Sterile cells suborbicular, orbicular, irregular, broadly elliptic, elliptic or ovate in outline, collapsed, (5–)6–13(–15) µm long, light or medium yellow-brown; cell wall on side distal to spores 0.5–0.7 µm thick, on side proximal to spores thicker, smooth, in SEM punctate. Spores subglobose, broadly ellipsoidal, subhyphal, slightly irregular, ellipsoidal or ovoid, sometimes elongate, sometimes slightly flattened on a few places, (11.5–)13–17(–19.5) × (10–)11–14.5(–15.5) (14.8 ± 1.4 × 12.7 ± 1.2) µm (n = 100), medium reddish brown; spore wall slightly uneven, 0.8–1.5 µm thick.


Note — *Festuca jeanpertii* is a new host for *Urocytis ulei*.

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