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Two new smut fungi on *Ventenata* (*Poaceae*): *Tilletia elizabethae* from Slovakia and *T. ventenatae* from Turkey

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Abstract: Two new smut fungi on *Ventenata* (*Poaceae*), *Tilletia elizabethae* (*Tilletiaceae*) on *V. dubia* from Slovakia, and *T. ventenatae* on *V. subenervis* from Turkey, are described and illustrated. They differ from all other *Tilletia* species by specialization on *Ventenata*. *Tilletia elizabethae* is distinguished from *T. ventenatae* by having larger spores and a dark cinnamon spore mass, while the spore mass of *T. ventenatae* is very dark reddish brown.

Key words: *Apera spica-venti*, biocontrol, invasive plants, *Poaceae*, Slovakia, smut fungi, taxonomy, *Tilletia*, *Tilletiaceae*, Turkey, *Ventenata*

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Introduction

Ventenata Koeler (*Poaceae*) is a small genus in the tribe *Poeae*, subtribe *Ventenatinae*, which contains 21 species in six genera, specifically, *Apera* Adans., *Bellardiochloa* Chiov., *Gaudinopsis* (Boiss.) Eig, *Nephelochloa* Boiss., *Parvotrisetum* Chrtek and *Ventenata* (Soreng & al. 2017). *Ventenata* comprises four species, with a geographic range extending from C and S Europe and Algeria to Crimea, the Caucasus, Turkey, Syria, Kazakhstan and Iran.

During an examination of grass specimens in the herbarium of the Naturalis Biodiversity Center, National Herbarium of the Netherlands, Leiden (L; herbarium code according to Thiers 2018+), two ovariicolous smut fungi belonging to the genus *Tilletia* Tul. & C. Tul. were found on specimens of *Ventenata*. These fungi were considered to represent unknown species of *Tilletia*. Currently, 185 species are recognized in *Tilletia* (Bao & al. 2010; Vánky 2011, 2013; Denchev & Denchev 2013; Li & al.

2014). Most commonly, their sori are produced in the ovaries, which fill with a semi-agglutinated or powdery spore mass intermixed with sterile cells. In some species, the sori are formed on leaves and culms, as streaks. Exceptionally, the sori appear as swellings on the culms or cover the surface of the leaves, or form witches' brooms (Vánky 2013). On grasses in the subtribe *Ventenatinae*, species of *Tilletia* have been previously recorded only on species of *Apera*. None of the currently recognized species of smut fungi is known to infect *Ventenata* species.

The only record of a smut fungus (*Ustilaginomyco-tina*) on *Ventenata* was reported by Vánky in his monograph of the Carpathian *Ustilaginales* (1985: 132, as *Tilletia bromi* (Brockm.) Brockm. on *V. dubia* (Leers) Coss. & Durieu from Slovakia, Trenčín Co., Zemanske-Podhrad, collected by F. Hazslinszky, BP, Herbarium *Ustilaginales* Vánky). In this monograph, *T. bromi* was treated in a very broad sense, as a species complex of smut fungi that produce reticulate spores, occurring on

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host plants in *Aira* L., *Bromus* L., *Festuca* L., *Poa* L., *Ventenata* and *Vulpia* C. C. Gmel. Vánky (1994) listed the host genera of *T. bromi* as reduced to *Bromus*, *Festuca*, *Ventenata* and *Vulpia*. Later, it was shown that *T. fusca* Ellis & Everh. on *Vulpia* species and *T. goloskokovii* Schwarzman on *Apera interrupta* (L.) P. Beauv. were distinct species (Boyd & Carris 1997, 1998; Boyd & al. 1998). *Tilletia bromi* was considered by Vánky (2011) as restricted to *Bromus* species, and the record of “*T. bromi*” on *Ventenata* was given as *Tilletia* sp. (Vánky 2011: 856).

In the present article, two new species of *Tilletia* on *Ventenata* are described and illustrated. A key to the species of *Tilletia* that infect *Ventenata* and *Apera* is also provided.

Material and methods

Dried specimens from the herbarium of the Naturalis Biodiversity Center, Leiden (L) were examined under a light microscope (LM) and a scanning electron microscope (SEM). For LM observations and measurements, spores and sterile cells were mounted in lactoglycerol solution (w : la : gl = 1 : 1 : 2) on glass slides, gently heated to boiling point to rehydrate the spores and sterile cells, and then cooled. The measurements of spores are given as min–max (extreme values) (mean \pm 1 standard deviation). For SEM, spores and sterile cells were attached to specimen holders by double-sided adhesive tape and coated with platinum in an ion sputter. The surface structure of spores and sterile cells was observed and photographed at 10 kV accelerating voltage using a JEOL JSM 6610-LV scanning electron microscope (Natural History Museum Vienna, Austria). The descriptions below are based entirely on the specimens examined. The shapes of sterile cells and spores are arranged in descending order of frequency.

Taxonomy

***Tilletia elizabethae* T. Denchev & Denchev, sp. nov.** – Fig. 1A–E.

Index Fungorum IF 554244.

Holotype: On *Ventenata dubia* (Leers) Coss. & Durieu. – Slovakia, Trenčín Region, Nové Mesto nad Váhom District, Zemianske Podhradie village (as “Ungarn, Ns. Podhrad”), sine datum, *J. L. Holuby s.n.* (SOMF 29 800; isotype: L 1351477).

Diagnosis — Differs from other *Tilletia* species by specialization on *Ventenata*. Differs from *T. ventenatae* (described herein) by (1) longer spores (21–)22–26(–27) μ m long compared to (15.5–)16–19.5(–21) μ m long in *T. ventenatae*, and (2) spore mass dark cinnamon compared to very dark reddish brown in *T. ventenatae* (Fig. 1F). Differs from *T. separata* by (1) shorter sterile cells (8–)9.5–16(–17.5) μ m long compared to

(15–)17–26(–27) μ m long in *T. separata*, and (2) sterile cells with thinner walls (0.6–)0.8–1.5(–1.8) μ m thick compared to (1.3–)1.5–2.0(–2.3) μ m thick in *T. separata*.

Description — **Infection** systemic, all spikelets of a panicle affected. **Sori** in all ovaries of an infected plant, broadly fusiform or ellipsoid, 2–4.5 mm long, with a short, acute tip, bearing a rudimentary style and stigmas, visible between spreading floral bracts, covered by a thin, purplish brown or yellow-brown pericarp that later ruptures to expose a powdery, dark cinnamon mass of spores and sterile cells. **Sterile cells** irregular, sometimes subglobose, broadly ellipsoidal, reniform or ellipsoidal, (8–)9.5–16(–17.5) \times (7.5–)8.5–14(–15.5) μ m, hyaline or subhyaline; cell wall (0.6–)0.8–1.5(–1.8) μ m thick, rarely with a short papilla, in SEM smooth. **Spores** subglobose, broadly ellipsoidal, globose or ovoid, (21–)22–26(–27) \times (19–)20–23.5(–24.5) (23.9 \pm 1.1 \times 21.8 \pm 1.0) μ m (n = 200), medium reddish brown or medium yellow-brown, completely or sometimes incompletely reticulate; spore wall (3.2–)3.4–3.9(–4.2) μ m thick (including reticulum and an inconspicuous inner layer 0.4–0.6 μ m thick); meshes 4–6(or 7) per spore diameter, polyhedral or irregular, (1.4–)1.6–7.0(–9.5) μ m long; muri (20–)22–27(–29) on equatorial circumference, in optical median view subacute or acute, (1.1–)1.3–2.2(–2.5) μ m high, in SEM interspaces smooth or rugulose, often with a very low, hemispherical protuberance.

Host plant and distribution — On *Poaceae*: *Ventenata dubia*, Europe (Slovakia). Known only from the type locality (Fig. 1G).

Eponymy — Named in loving memory of Elizabeth Dencheva (1958–2015), wife of Cvetomir and mother of Teodor.

Remarks — Further to the distinctions mentioned in the diagnosis, *Tilletia elizabethae* differs from *T. goloskokovii* on *Apera interrupta* by (1) shorter sterile cells (8–)9.5–16(–17.5) μ m long compared to *T. goloskokovii* 12–30 μ m long (after Vánky 2011) or 15–28 μ m long (after Boyd & al. 1998), and (2) by sterile cells with thinner walls (0.6–)0.8–1.5(–1.8) μ m thick compared to *T. goloskokovii* 1–5 μ m thick (after Vánky 2011) or 1–2.5 μ m thick (after Boyd & al. 1998).

It is difficult to determine the exact date that the type specimen of *Tilletia elizabethae* was collected, but it can be assumed that this happened during the period 1861–1909 when J. Holuby served as a Lutheran priest in Zemianske Podhradie and collected plants and fungi in that area.

Ventenata dubia (syn. *Avena tenuis* Moench, *V. avenacea* Koeler) is distributed from C and S Europe and Algeria to Crimea, the Caucasus, Turkey, Kazakhstan and Iran (Hamzeh’ee & al. 2008; Contu 2013; Clayton & al. 2018). As mentioned, a smut fungus on *V. dubia* has been



Fig. 1. A–E: *Tilletia elizabethae* on *Ventenata dubia* (holotype); A: habit; B, C: spores and a sterile cell in LM (in median and surface view, respectively); black arrow shows a sterile cell; D, E: spores and sterile cells in SEM. – F: spore mass colour of *Tilletia elizabethae* (above) and *T. ventenatae* (below). – G: type localities of *T. elizabethae* (red circle) and *T. ventenatae* (blue circle) (generated with SimpleMapp; Shorthouse 2010). – Scale bars: A = 2 mm; B–E = 10 μ m; G = 200 km.

previously reported only once (Vánky 1985), despite the relatively extensive geographic range of the plant and the fact that the smut fungi of C Europe are among the best studied in the world. The specimen of Hazslinszky, cited by Vánky (as *Tilletia bromi*, see Introduction), was collected in the same locality from which *T. elizabethae* is described here, if not a part of the same gathering, due to the fact that at the time when J. Holuby studied the flora of Zemianske Podhradie, he had close professional relationships with F. Hazslinszky (see Holuby 1874: 310). It seems that the smut fungus on *V. dubia* is a rare species, not simply a rarely recorded one.

Ventenata dubia is an alien species in Canada (British Columbia, Alberta, and the E provinces; Contu 2013; Fryer 2017), U.S.A. (CA, ID, MT, OH, OR, NY, ME, UT, WA, WI, and WY; Contu 2013; Fryer 2017), and Japan (Koba & al. 2005). In the U.S.A., it appears to be most prevalent in the intermountain Pacific Northwest, growing in a variety of dry, open, and often disturbed habitats (Draft Washington State Noxious Weed Control Board 2016). In some states, it has become a highly invasive species, replacing perennial grasses and herbs along roadsides and in pastures, rangelands and CRP (Conservation Reserve Program) fields (Scheinost & al. 2009; Contu 2013). Additionally, there are concerns that the shallow root system of *V. dubia* may cause the soil to be more prone to erosion (Scheinost & al. 2009). It is worth noting that along with *Bromus tectorum* L. (cheatgrass), *V. dubia* is a serious threat to the critically endangered Palouse Prairie plant communities in the Pacific Northwest of the U.S.A. (Nyamai & al. 2011). Biological control with a fungal species has not been considered (Scheinost & al. 2009; Draft Washington State Noxious Weed Control Board 2016). *Ventenata dubia* is an annual plant that reproduces only by seeds. Since *Tilletia elizabethae* has a high level of host specificity and causes systemic infection that prevents seed production, this smut fungus has potential as a biological control agent for *V. dubia* in the U.S.A.

***Tilletia ventenatae* T. Denchev & Denchev, sp. nov.** – Fig. 2A–E.

Index Fungorum IF 554245.

Holotype: On *Ventenata subenervis* Boiss. & Balansa. – Turkey, near Izmir, N of the tomb of Tantalus, 21 May 1865, B. Balansa, *Plantes d'Orient*, s.n. (SOMF 29 801; isotype: L 1351399).

Diagnosis — Differs from other *Tilletia* species by specialization on *Ventenata*. Differs from *T. elizabethae* (described herein) by (1) shorter spores (15.5–)16–19.5(–21) µm long compared to (21–)22–26(–27) µm long in *T. elizabethae*, and (2) spore mass very dark reddish brown compared to dark cinnamon in *T. elizabethae* (Fig. 1F). Differs from *T. separata* by (1) shorter spores (15.5–)16–19.5(–21) µm long compared to (20–)21–26.5(–28.5) µm long in *T. separata*, (2) shorter

sterile cells (10.5–)11.5–19.5(–21) µm long compared to (15–)17–26(–27) µm long in *T. separata*, and (3) sterile cells with thinner walls (0.6–)0.7–1.1(–1.3) µm thick compared to (1.3–)1.5–2.0(–2.3) µm thick in *T. separata*.

Description — *Infection* systemic, all spikelets of a panicle affected. *Sori* in all ovaries of an infected plant, ellipsoid, 1–2 mm long, with a short, acute tip, bearing a rudimentary style and stigmas, partially visible between spreading floral bracts, covered by a thin, blackish brown pericarp that later ruptures exposing a powdery, very dark reddish brown mass of spores and sterile cells. *Sterile cells* irregular, broadly ellipsoidal, subglobose or reniform, (10.5–)11.5–19.5(–21) × (9.5–)10.5–17.5(–18.5) µm, hyaline; cell wall (0.6–)0.7–1.1(–1.3) µm thick, in SEM smooth. *Spores* subglobose, broadly ellipsoidal or globose, sometimes ovoid, (15.5–)16–19.5(–21) × (14.5–)15–18(–19) (18.0 ± 1.0 × 16.6 ± 0.8) µm (*n* = 200), medium reddish brown, completely or partially reticulate; spore wall (2.4–)2.7–3.4(–3.7) µm thick (including reticulum and an inner layer 0.4–0.6 µm thick); meshes (3 or)4–6(or 7) per spore diameter, polyhedral or irregular, (0.7–)1.0–5.0(–7.5) µm long; muri (17–)19–23(–25) on equatorial circumference, in optical median view subacute or acute, (0.7–)1.0–1.7(–2.0) µm high, in SEM interspaces often with a hemispherical or irregular protuberance.

Host plant and distribution — On *Poaceae*: *Ventenata subenervis*, Asia (Turkey). Known only from the type locality (Fig. 1G).

Eponymy — The epithet refers to the host genus.

Remarks — Further to the distinctions mentioned in the diagnosis, *Tilletia ventenatae* differs from *T. goloskokovii* on *Apera interrupta* by (1) shorter sterile cells (10.5–)11.5–19.5(–21) µm long compared to *T. goloskokovii* 12–30 µm long (after Vánky 2011) or 15–28 µm long (after Boyd & al. 1998), and (2) sterile cells with thinner walls (0.6–)0.7–1.1(–1.3) µm thick compared to *T. goloskokovii* 1–5 µm thick (after Vánky 2011) or 1–2.5 µm thick (after Boyd & al. 1998).

The host, *Ventenata subenervis*, is an E Mediterranean species, distributed in E Crete (Greuter & al. 1985), the East Aegean Island of Lesvos (Candargy 1897) and W Turkey (from the Aegean region to inner Anatolia, Doğan 1985).

The newly described *Tilletia* species were compared with a German specimen of *T. separata*, a smut fungus originally described on a specimen from Germany.

***Tilletia separata* J. Kunze ex G. Winter, Rabenh. Krypt.-Fl., ed. 2, 1(1): 111. 1881.** – Fig. 2F–H.

Description — *Sori* in all ovaries of an infected plant, ellipsoid or ovoid, 1–2.5 mm long, with rudimentary style

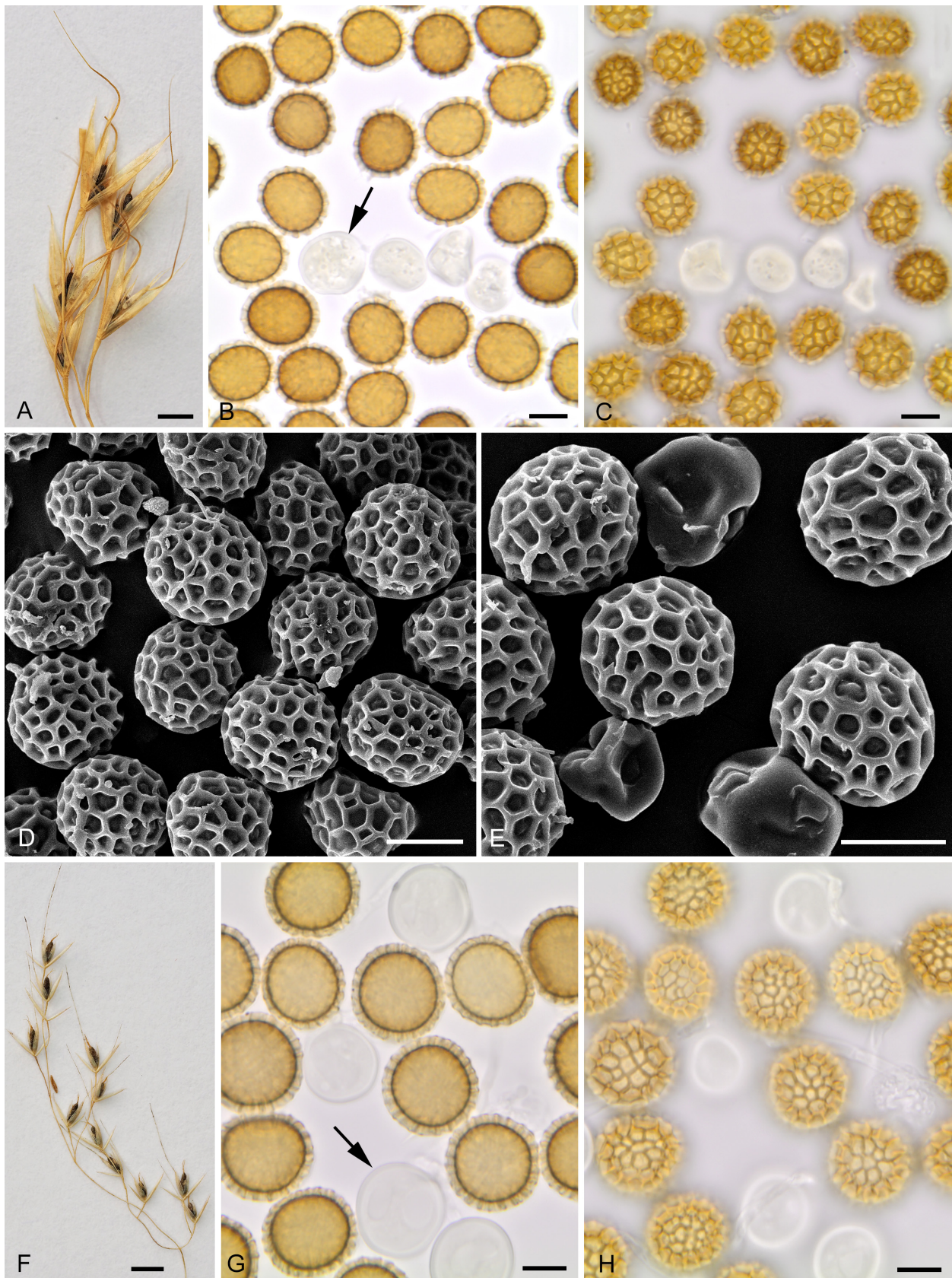


Fig. 2. A–E: *Tilletia ventenatae* on *Ventenata subenervis* (holotype); A: habit; B, C: spores and sterile cells in LM (in median and surface view, respectively); black arrow shows a sterile cell; D, E: spores and sterile cells in SEM. – F–H: *Tilletia separata* on *Apera spica-venti* (K. Vánky, Ustilaginales exsiccata, no. 136); F: habit; G, H: spores and sterile cells in LM (in median and surface view, respectively); black arrow shows a sterile cell. – Scale bars: A, F = 2 mm; B–E, G, H = 10 μ m.

and stigmas, visible between spreading floral bracts, initially covered by a thin, purplish brown, dark brown or blackish brown pericarp that later ruptures exposing a powdery, dark reddish brown mass of spores and sterile cells. *Sterile cells* subglobose, broadly ellipsoidal or globose, sometimes ovoid or slightly irregularly rounded, (15–)17–26(–27) × (13.5–)16–23.5(–25) µm, hyaline; cell wall 2-layered, (1.3–)1.5–2.0(–2.3) µm thick. *Spores* subglobose, globose or broadly ellipsoidal, sometimes ovoid, (20–)21–26.5(–28.5) × (19–)20–23.5(–25) (24.0 ± 1.1 × 22.2 ± 0.9) µm ($n = 100$), medium reddish brown, completely reticulate, less often incompletely reticulate; spore wall 3.3–4.0(–4.5) µm thick (including reticulum and hardly visible, 0.4–0.8 µm thick inner layer); meshes 5–8(or 9) per spore diameter, polyhedral or irregular, (1.1–)1.4–7.0(–8.5) µm long; muri (21–)23–29(–31) on equatorial circumference, in optical median view subacute or acute, (0.9–)1.2–2.3(–2.7) µm high.

Specimen examined — On *Apera spica-venti* (L.) P. Beauv. — Germany, Bayern, prope pagum Rothenburg ob der Taube, 25 Jul 1971, K. Vánky 829 (K. Vánky, Ustilaginales exsiccata, no. 136).

Host plant and distribution — On *Poaceae*: *Apera spica-venti*, Europe.

Remarks — *Tilletia madeirensis* Syd. on *Aira praecox* L. was treated by Vánky (2011) as a synonym of *T. separata*, but, considering the phylogenetic position of *Aira*, these smut fungi should be recognized as different species, which will be discussed further in another article.

The second *Tilletia* species on *Apera*, *T. goloskokovii* Schwarzman, is known on *A. interrupta* from Kazakhstan and the U.S.A. (Schwarzman 1960; Boyd & al. 1998; Vánky 2011).

The newly described *Tilletia* species and that on *Apera* may be distinguished by the following key.

Key to the *Tilletia* species on hosts from subtribe *Ventenatinae*

1. Sterile cells maximum length less than 19.5 µm. [On *Ventenata*] **2**
– Sterile cells maximum length equal to or more than 26 µm. [On *Apera*] **3**
2. Spore mass dark cinnamon; spores longer than 21 µm. [Currently known on *V. dubia*] ***T. elizabethae***
– Spore mass very dark reddish brown; spores up to 21 µm long. [Currently known on *V. subenervis*] ***T. ventenatae***
3. Wall of sterile cells (1.3–)1.5–2.0(–2.3) µm thick. [Currently known on *A. spica-venti*] ***T. separata***
– Wall of sterile cells 1–5 µm thick. [Currently known on *A. interrupta*] ***T. goloskokovii***

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