# 黒穂菌(Ustilaginomycotina)5種の日本新産記録


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# 黒穂菌（Ustilaginomycotina）5種の日本新産記録

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# Five new records of smut fungi (Ustilaginomycotina) in Japan

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Five smut fungi collected in Japan are described here: *Pilocintractia fimbristylidicola* on *Fimbristylis miliacea*, *Sporisorium manilense* on *Sacciolepis indica*, *Tilletia arundinellae* on *Arundinella hirta*, *Tilletia vittata* on *Oplismenus undulatifolius*, and *Ustilago phragmitis* on *Phragmites australis*. These species are reported in Japan for the first time. Besides, *Neovossia moliniae* on *P. australis* is described. This is the second record of this smut fungus in Japan.

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Key Words——*Neovossia*, *Pilocintractia*, *Sporisorium*, *Tilletia*, *Ustilago*

Many smut fungi (Basidiomycota, Ustilaginomycotina) form sori on the flowers of grasses or sedges. Currently recognized species of smut fungi in Japan have been summarized by Kakishima (2016). During a number of surveys of phytopathogenic fungi on grasses and sedges, *Pilocintractia fimbristylidicola* (Ustilaginales, Anthracoideaceae), *Sporisorium manilense* (Ustilaginales, Ustilaginaceae), *Tilletia arundinellae* (Tilletiales, Tilletiaceae), *Tilletia vittata* (Tilletiaceae), and *Ustilago phragmitis* (Ustilaginaceae) were identified as newly recorded species Japan; *Neovossia moliniae* (Tilletiaceae) was identified as the second recorded species in Japan based on the hosts, morphological characteristics, and, when possible, DNA sequence data.

Samples of smut fungi were collected from different areas of Japan. These specimens were dried at room temperature and maintained in our laboratory until deposit in the mycological herbarium of the National Museum of Nature and Science (Tsukuba, Japan). For light microscopic (LM) study, the spores were mounted in 20% glycerol and observed using differential interference contrast microscopy (E-800 or Ni, Nikon, Tokyo, Japan). For scanning electron microscopic (SEM) study, the spores were fixed with vapor from 1% $OsO_4$ in 0.05 M cacodylate buffer at pH7.2 for 2 h then coated with 8 nm thick platinum using an ion sputter (E-1010, Hitachi), and observed using field emission scanning electron microscopy (S-4700, Hitachi High-Technologies Corp., Tokyo, Japan) as shown in a previous study (Tanaka & Honda, 2017). Spores were suspended in distilled water and spread on water agar (1.5% w/v) in plastic Petri dishes for cultures. The Petri dishes were incubated at 18℃ or 4℃ under dark conditions. The dishes incubated at 4℃ were transferred to 18℃ after three mo or six mo. The dishes incubated at 18℃ were observed under a microscope to monitor spore germination. After confirming spore germination, images were acquired using an inverted microscope (CKX 41, Olympus, Tokyo, Japan). A few colonies derived from a single spore were separately cultured. Some cultures were deposited in NITE Biological Resource Center (NBRC, Japan). Morphological identifications were confirmed by comparing DNA sequences of

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the internal transcribed spacer (ITS) region and D1/D2 region of the large subunit (LSU) ribosomal DNA gene as described in a previous study (Tanaka et al., 2019). Taxonomic classification in this manuscript was based on Vánky (2012).

***Pilocintractia fimbristylidicola*** (Pavgi & Mundk.) Vánky, Mycologia Balcanica 1: 173 (2004) [MB 510113] Fig. 1
Holotype: INDIA, Orissa, Ganjam, Chatrapur, Aug. 30, 1904 (HCIO 1438; isotype BPI 171548, HUV 15462)
Basionym: *Cintractia fimbristylidicola* Pavgi & Mundk., Indian Phytopathology 1: 108 (1949). ≡ *Cintractia fimbristylicola* Pavgi & Mundk., Indian Phytopathology 1 : 108 (1949).

**Sori** in and around ovaries, scattered in the inflorescence, black, hard, not powdery. Peridium absent. **Spores** flattened, in side view elliptic, (5−)6−8 µm wide, in plane-view elliptic, ovate or subpolygonally irregular, 8−11.5(−13) × 6−10 µm, yellowish brown; in LM punctate or finely granular-verrucose, in SEM warts partly confluent forming a rough, irregular reticulum. **Spore germination** result in phragmobasidia on which lateral and terminal ellipsoidal basidiospores are produced.

Habitat: On Cyperaceae, *Fimbristylis* spp. Distribution: Japan, Australia, India, Thai, Central & S America

Specimen examined: JAPAN, Ishikawa, Hakusan, Anyoji, 36° 29′ 42″N 136° 35′ 36″E, on *Fimbristylis miliacea*, Oct. 23, 2015, E. Tanaka, ITS-LSU sequence LC603326, TNS-F-91241 (culture NBRC 115020).

Notes: This Japanese sample was identified as *P. fimbristylidicola* despite the spore surface reticulation of this Japanese sample appearing thinner than that of the type specimen (Vánky, 2004a). Smut fungi on *Fimbristylis* have been summarized by Piatek & Vánky (2005). The hard sori, spore size, and spore surface ornamentation differentiate this species from other smut fungi on *Fimbristylis*. The genus *Pilocintractia* was recorded here in Japan for the first time. When the D1/D2 sequences (605 bp) were compared, there was 99.3% identity, with four different base-pairs between this Japanese sample (LC603326) and MP 2213 assigned to *Cintractia fimbristylicola* in the GenBank (AJ 236143). The MP 2213 was morphologically identified by M. Piepenbring and phylogenetically examined at the molecular level (Piepenbring et al., 1999). Kakishima (1982) reported two smut fungi on *F. miliacea* in Japan: *Cintractia fimbristylis-miliaceae* Hennings and *Cintractia pulchra* S. Ito. However, their spore surfaces are verrucose-echinulate, unlike that of *P. fimbristylidicola*.

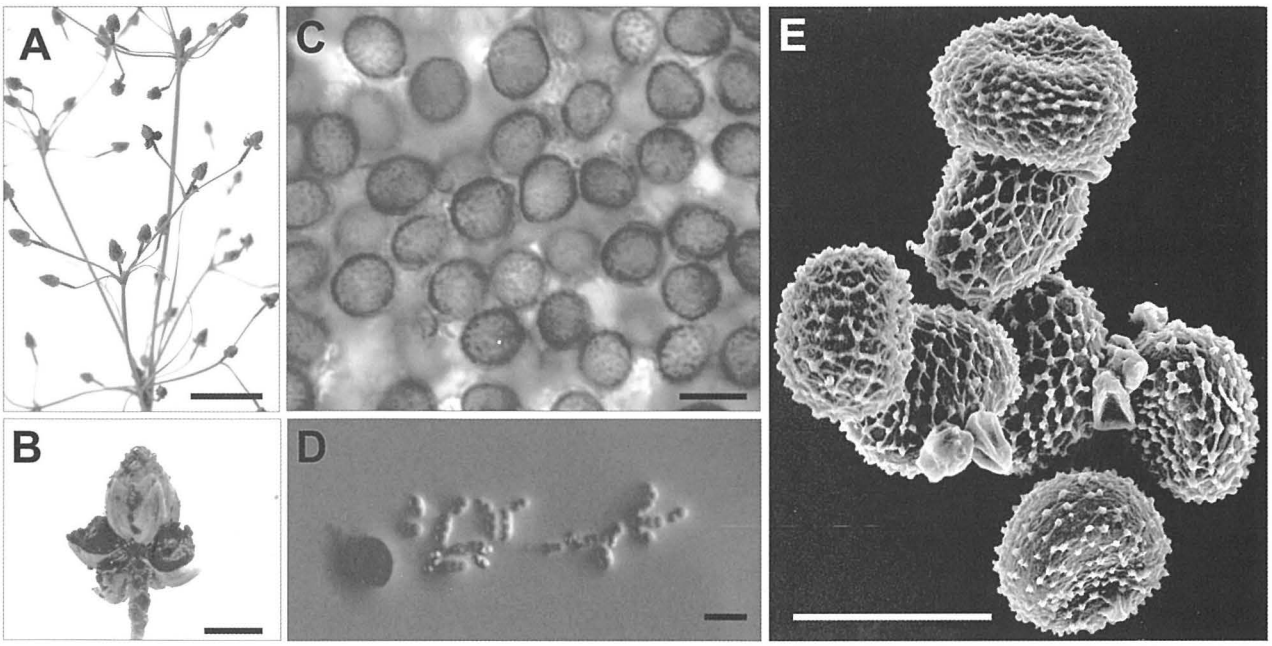


Fig. 1. *Pilocintractia fimbristylidicola* on *Fimbristylis millacea* (TNS-F-91241). A, B: Sori. C: Spores in LM. D: Spore germination. E: Spores in SEM. *Bars*: A 1 cm; B 1 mm; C–E 10 µm.

***Sporisorium manilense*** (Syd. & P. Syd.) Vánky, Mycotaxon 59: 110 (1996) [MB 415732] Fig. 2

Holotype: INDIA, Mysore, Bhadravati, on *Sacciolepis indica*, Aug. 18, 1947 (HCIO 18808; isotype IMI 38397)

Basionym: *Ustilago manilensis* Syd. & P. Syd., Annales Mycologici 10 (1): 77 (1912). ≡ *Sphacelotheca manilensis* (H. & P. Sydow) L. Ling, Sydowia 4: 78 (1950).

= *Sphacelotheca sacciolepidis* Thirumalachar (as *saccolepidis*), Lloydia 13 : 173 (1950). ≡ *Sporisorium sacciolepidis* (Thirum.) Vánky, Mycotaxon 48: 41 (1993).

**Sori** in all spikelets of an inflorescence comprising the ovaries and innermost floral organs. **Spores** globose, subglobose to ovoid, 10 − 13 (− 15) × 8 − 12 µm, brown; surface in LM densely echinulate, in SEM echinulate between the spins verrucose. **Spore germination** result in mycelium on water agar.

Habitat: On *Sacciolepis indica*. Distribution: Japan, Africa, SE Asia.

Specimen examined: JAPAN, Ishikawa, Hakusan, Ozo, 36° 16′ 04″ N 136° 42′ 53″ E, on *S. indica*, Oct. 06, 2016, E. Tanaka, ITS-LSU sequence LC603327, TNS-F-91242 (culture NBRC 115021).

Notes: This smut fungus on *S. indica* was identified as *S. manilense*. The other smut fungi on *Sacciolepis* are *Ustilago trichophora* and an unknown *Tilletia* species, distinguished from *S. manilense* based on the size and surface ornamentation of the spores. There were 100% ITS and D1/D2 sequence identities between the Japanese sample (LC603327) and Ust.Exs.854 (M), labeled as *S. manilense* in GenBank (AY740059 and AY740112). The Ust.Exs.854 (M) was morphologically examined by K. Vánky and phylogenetically examined at the molecular level (Stoll et al., 2005).

***Tilletia arundinellae*** L. Ling, Mycological Papers 11 : 1 (1945) [MB 291406] Fig. 3

Holotype: CHINA, Sichuan Prov. Chengdu, on *Arundinella anomala* (= *A. hirta*), Leg. L. Ling (BPI 196372).

**Sori** in ovaries, scattered in the inflorescence, ovoid, 3 − 4 mm long, covered by the thin, green pericarp, which later turned brown to black, later ruptures, exposing powdery, dark spores. **Spores** globose or subglobose, 25 − 33 × 23 − 30 µm, brown or blackish brown; surface in LM irregularly verrucurose, in SEM the warts confluent forming parallel or spirally arranged rows. **Spore germination** result in aseptate basidium, apically producing numerous short acicular basidiospores.

Habitat: On *Arundinella hirta*. Distribution: Japan, China.

Specimen examined: JAPAN, Ishikawa, Nomi, Tate, 36° 25′ 18″ N, 136° 33′ 17″ E, on *A. hirta*, Oct. 25, 2016, E.

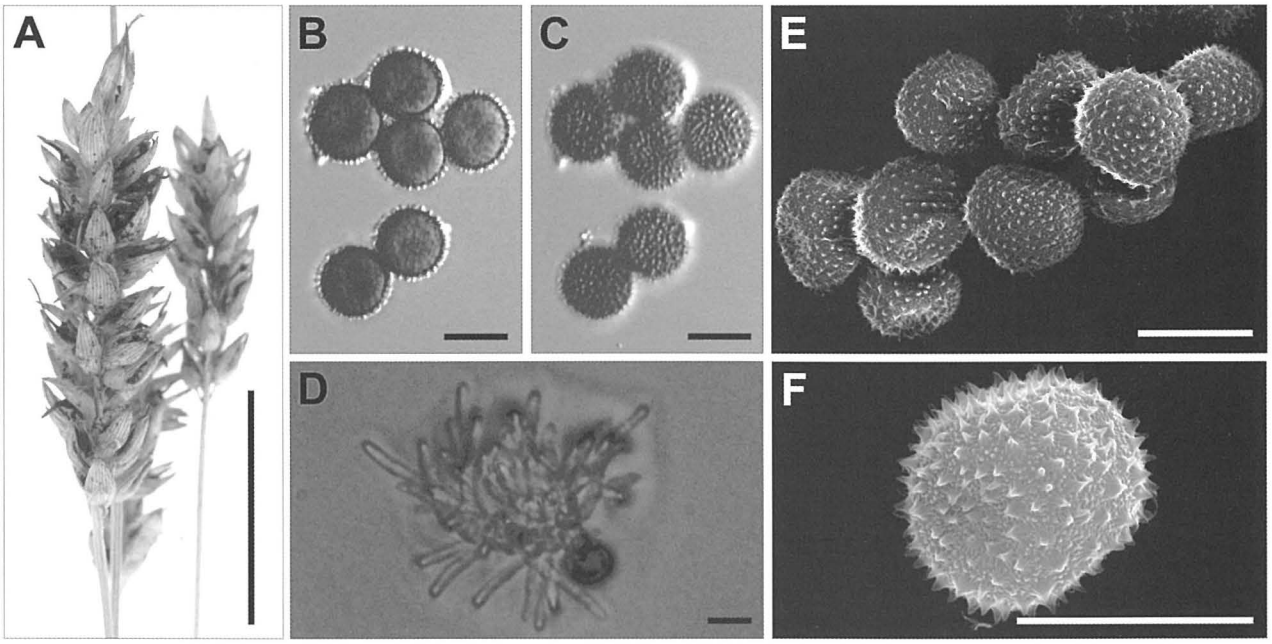


Fig. 2. *Sorosporium manilense* on *Sacciolepis indica* (TNS-F-91242). A: Sori. B, C: Spores in LM. B: In median view. C: In surface view. E, F: Spores in SEM. *Bars*: A 1 cm; B–E 10 µm.

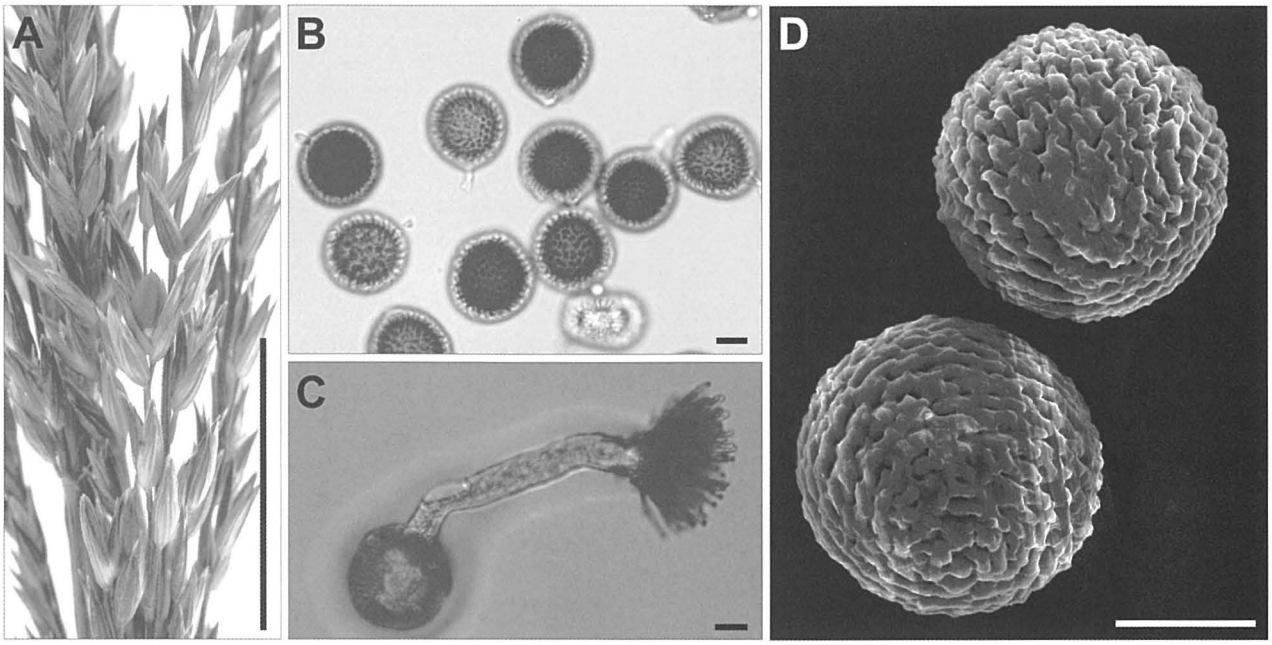


Fig. 3. *Tilletia arundinellae* on *Arundinella hirta* (TNS-F-91243). A: Sori. B: Spores in LM. C: Spore germination. D: Spores in SEM. *Bars*: A 1 cm; B–D 10 μm.

Tanaka, ITS-LSU sequence LC603328, TNS-F-91243 (culture NBRC 115022); JAPAN, Ishikawa, Kanazawa, Azukizawa, Mt. Kigo, 36°31′34″N, 136°44′51″E, on *A. hirta*, Nov. 13, 2016, E. Tanaka, ITS-LSU sequence LC603330, TNS-F-91245 (culture NBRC 115024); JAPAN, Miyazaki, Miyakonojo, Yasuhisa, 31°40′58″N, 131°05′21″E, on *A. hirta*, Nov. 19, 2020, E. Tanaka, ITS-LSU sequence LC603329, TNS-F-91244.

Notes: A smut fungus on *A. hirta* was collected from three locations in Japan and identified as *T. arundinellae*. Smut fungi on *Arundinella* spp. have been summarized by Shivas & Vánky (2005). The spore size and spore surface ornamentation differentiate this species from other smut fungi on *Arundinella*. This is the second report of this fungus; *T. arundinellae* has been only known as a type specimen from China (Lee, 1945). There were no DNA data of *T. arundinellae* in GenBank for comparison. The ITS and D1/D2 sequences of this sample were closely related to some *Tilletia* spp. *Sorosporium arundinellae* Sydow on *A. hirta* was reported from Japan (Ito, 1936; Kakishima, 1982). However, its spores (5–8 μm) were small, but its sori were formed into ovaries like *T. arundinellae*. Furthermore, *S. arundinellae* may belong to Ascomycota (Vánky, 2004b). *Ustilago arundinellae-hirtae* S. Ito (= *Sporisorium kusanoi*) on *A. hirta* was also reported from Japan (Ito, 1936). However, the sori of *U. arundinellae-hirtae* occupy the entire inflorescence, unlike those of *T. arundinellae*.

***Tilletia vittata*** (Berk.) Mundk., Trans. Brit. Mycol. Soc. 24: 312 (1940) [1941] [MB 291424] Fig. 4

Basionym: *Ustilago vittata* Berk., Gardeners' Chronicle 1853: 148 (1853). ≡*Neovossia vittata* (Berk.) H.S. Shetty & Safeeulla, Indian Phytopathology 33: 399 (1981).

Holotype on Oplismenoid grass (*O. compositus* (L.) P. Beauv., det. C.E. Hubbard, INDIA, Bihar, near the summit of Paras Nath, alt. ca 1200 m, before 1854, leg. W.J. Hooker, s. n. (K 84475).

**Sori** in ovaries, some spikelets in the inflorescence, exceeding cylindrical, 2.5–20×0.8–2 mm, covered by a thin, green to purple, hispid peridium of host origin with parallel veins. When the peridia rupture, exposing black powdery spores. **Spores** globose to subglobose, 14–19×13–18 μm, dark brown; in LM verrucose, in SEM warts densely, frequently confluent. **Spore germination** result in septate basidium, apically producing numerous long acicular basidiospores.

Habitat: On *Oplismenus* spp. Distribution: Japan, S & SE Asia, N & Central America, Africa and Australia

Specimen examined: JAPAN, Ishikawa, Kanazawa, Nu-

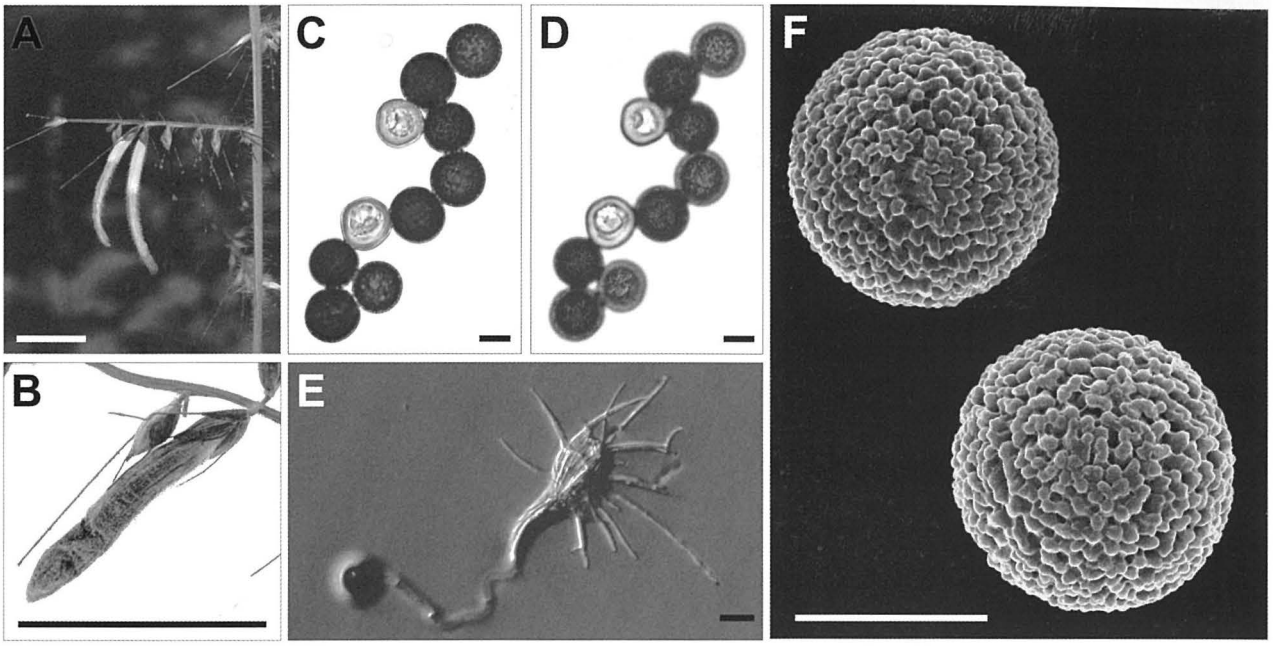


Fig. 4. *Tilletia vittata* on *Oplismenus undulatifolius* (TNS-F-91246). A, B: Sori. C, D: Spores and sterile cells in LM. C: In median view. D: In surface view. E: Spore germination. F: Spores in SEM. *Bars*: A, B 1 cm; C–F 10 μm.

kadani, 36° 30′ 25″ N, 136° 37′ 56″ E, on *O. undulatifolius*, Oct. 27, 2015, E. Tanaka, ITS-LSU sequence LC 603331, TNS-F-91246 (culture NBRC 115023); JAPAN, Ishikawa, Hakusan, Chugu, 36° 15′ 47″ N, 136° 45′ 39″ E, on *O. undulatifolius*, Oct. 6, 2016, E. Tanaka, ITS-LSU sequence LC603332, TNS-F-91247; JAPAN, Ishikawa, Kanazawa, Azukizawa, Mt. Kigo, 36° 31′ 20″ N, 136° 44′ 43″ E, on *O. undulatifolius*, Nov. 13, 2016, E. Tanaka, TNS-F-91248; JAPAN, Niigata, Murakami, Takane, 38°20′ N, 139°37′ E, on *O. undulatifolius*, Nov. 16, 2016, Kazuhito Tanada, ITS-LSU sequence LC603333, TNS-F-91249 (culture NBRC 115025).

Notes: A smut fungus on *O. undulatifolius* was collected from four locations in Japan and identified as *T. vittata*. Smut fungi on *Oplismenus* have been summarized by Vánky (2004). The hypertrophy of the infected ovary, spore size, and spore surface ornamentation differentiate this species from other smut fungi on *Oplismenus*. When the D1/D2 sequences (598 bp) were compared, there was 100% identity between TNS-F-91246 (LC603331) and BRIP 54207 (MH231814)/HUV 19.160 (AY818985), labeled as *T. vittata* in GenBank; there were 99.7% identities with a two base-pair difference between TNS-F-91247 (LC603332)/TNS-F-91249 (LC603333) and BRIP 54207/HUV 19.160. The HUV 19.160 was morphologically identified by K. Vánky and phylogenetically examined at the molecular level (Castlebury et al., 2005).

***Ustilago phragmitis*** L. Ling, Sydowia 4 : 76 (1950) [MB 307643] Fig. 5

Holotype: MALAYSIA, North Borneo (Sabah), Elopera near Sandakan, on *Phragmites karka*, 15 Nov. 1947 (isotype HUV 15448).

**Sori** in some ovaries of groups of spikelets, ovoid, 1 – 2.5 mm long, covered by a green to brown peridium. Spores globose to irregular, subangular, 8 – 12.5 × 6.5 – 10 μm, yellowish brown; in LM punctate, in SEM finely, punctate-verruculose. **Spores** germinate and develop septate basidia forming basidiospores. **Spore germination** results in 4-celled basidia on which lateral and terminal ellipsoidal basidiospores are produced.

Habitat: *Phragmites* spp. Distribution: Japan, E & SE Asia.

Specimen examined: JAPAN, Tokyo, Ome, Sawai, on *P. australis*, Nov. 3, 2018, Saho Shibata, ITS-LSU sequence LC603337, TNS-F-91254 (culture NBRC 115016).

Notes: This Japanese sample was identified as *U. phragmitis*, although the Japanese sample spores are somewhat smaller than those typical of the type. With the exception of *Neovossia moliniae*, which is described below, the other smut fungus that forms sori in the ovaries of *Phragmitis*

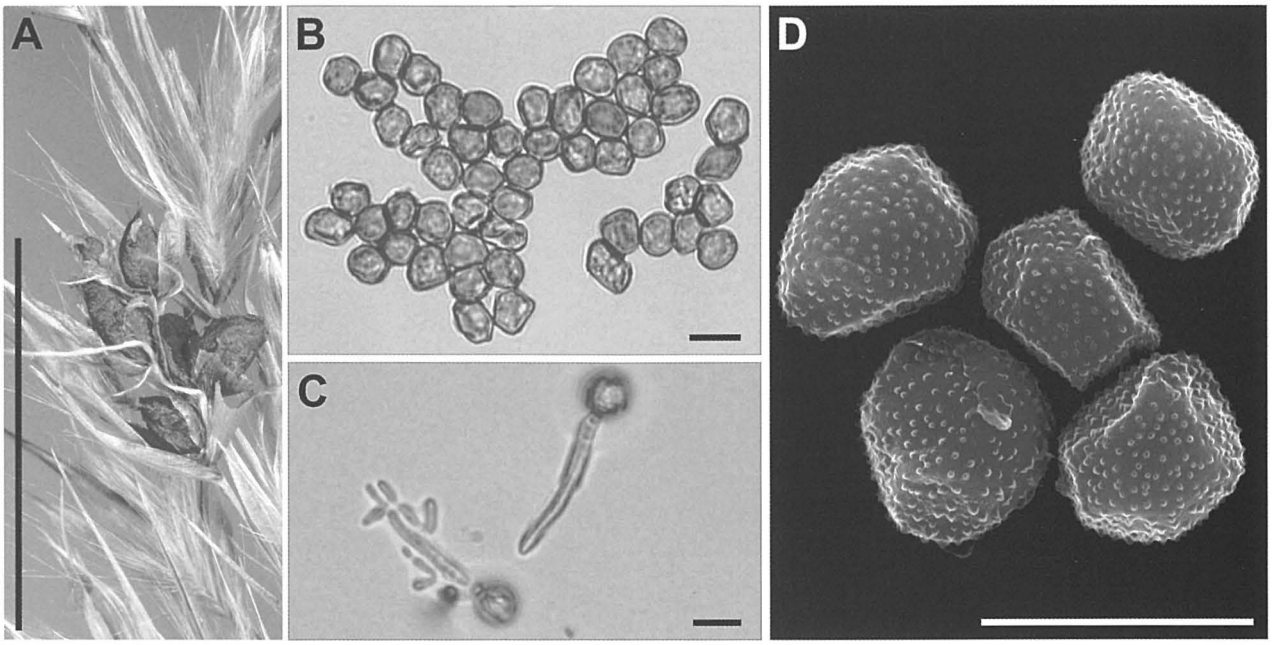


Fig. 5. *Ustilago phragmitis* on *Phragmites australis* (TNS-F-91254). A: Sori. B: Spores in LM. C: Spores germination. D: Spores in SEM. *Bars*: A 1 cm; B–D 10 μm.

spp. is *Ustilago mauritiana* K. Vánky. However, the sori of *U. mauritiana* are pubescent, unlike that of *U. phragmitis*. There were no DNA sequence data for *U. phragmitis* in GenBank for comparison. The D1/D2 sequence of this sample was closely related (99.3%) to CBS 13471 (MH877397), labeled as *Ustilago sparsa* in GenBank. *Ustilago grandis* E. Fries on *P. australis* has been reported in Japan. However, its sori are formed in culms.

***Neovossia moliniae*** (Thüm.) Körn., Österreichische Botanische Zeitschrift 29: 217 (1879) [MB 181885] Fig. 6

Holotype: SLOVENIA, Ljubjhanaon, *Molinia caerulea*, Oct. 1878, Q. Voss, (HUV 9092)

Basionym: *Vossia moliniae* Thüm., Österreichische Botanische Zeitschrift 29: 19 (1879). ≡*Tilletia moliniae* (Thüm.) G. Winter, Rabenhorst's Kryptogamen-Flora, Edn. 2, 1(1): 109 (1881) [1884].

=*Neovossia iowensis* H.H. Hume & Hodson, Botanical Gazette 30 : 274 (1900). ≡*Tilletia iowensis* (H.H. Hume & Hodson) Cif., Quad. Ist. Bot. Univ. Pavia 27: 229 (1963).

=*Neovossia danubialis* Săvul., Commun. Acad. Repub. pop. rom.: 71 (1955).

**Sori** in some ovaries of an inflorescence, ovoid, 4 – 5 mm long, covered by a green smooth peridium which later turn to brown to black. **Spores** ellipsoid to oblong, 21 – 32 × 14 – 18 μm, brown to dark brown enveloped in a hyaline sheath with long hyaline appendage; in LM and SEM foveolate. **Spore germination** result in aseptate basidia, apically forming a lot of acicular basidiospores.

Specimen examined: JAPAN, Ishikawa, Komatsu, Imae, 36° 22′ 35″ N 136° 26′ 56″ E, on *P. australis*, Oct. 25, 2016, E. Tanaka, ITS-LSU sequence LC603334, TNS-F-91250 (culture NBRC 115018); JAPAN, Fukui, Awara, Hamasaka, 36° 16′ 34″ N 136° 14′ 46″ E, on *P. australis*, Nov. 10, 2016, E. Tanaka, TNS-F-91251; JAPAN, Fukui, Wakasa, Kiyama, 35° 34′ 13″ N 135° 54′ 22″ E, on *P. australis*, Nov. 30, 2016, E. Tanaka, ITS-LSU sequence LC603335, TNS-F-91252 (culture NBRC 115019); JAPAN, Chiba, Sakura, Usuida, 35° 44′ 48″ N 140° 10′ 43″ E, on *P. australis*, Jan. 01, 2018, E. Tanaka; JAPAN, Ibaraki, Tsukuba, Hanamuro, 36° 05′ 06″ N 140° 07′ 39″ E, on *P. australis*, Mar. 21, 2019, E. Tanaka.

Culture examined: JAPAN, Aomori, Hirosaki, Koguriyama, on *P. communis* (=*P. australis*), 1972, Y. Harada, NBRC 9940 (as *T. danubialis*).

Habitat: *Phragmites* spp., *Molinia caerulea*. Distribution: Japan, Asia, Europe, N America.

Notes: A smut fungus on *P. australis* was collected from five locations in Japan and identified as *N. moliniae*. The long hyaline appendage of the spores is a characteristic morphology that distinguishes it from other smut fungi.

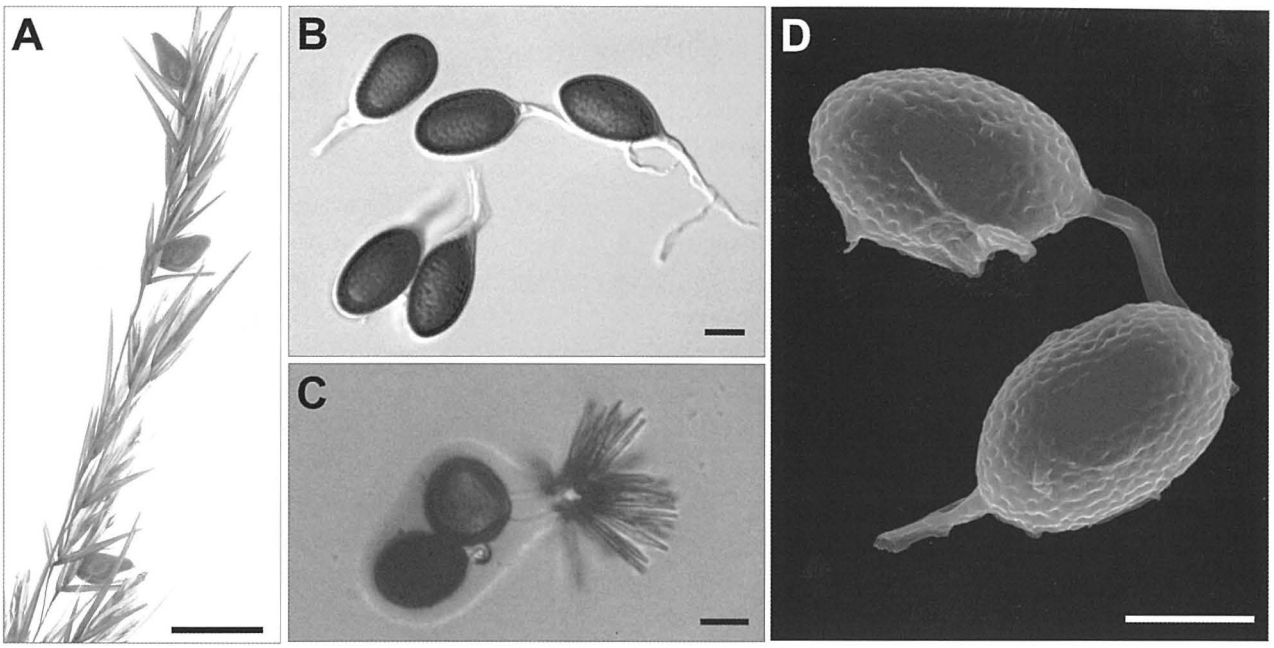


Fig. 6. *Neovossia moliniae* on *Phragmites australis* (TNS-F-91250). A: Sori. B: Spores in LM. C: Spore germination (upper right spore). D: Spores in SEM. *Bars*: A 1 cm; B–D 10 µm.

There was about 99% identity between the ITS and D1/D2 sequences of the Japanese specimens (TNS-F-91250 and TNS-F-91252) and eight published specimens labeled as *T. moliniae* or *T. iowensis* in GenBank. Some of the specimens were morphologically identified by K. Vánky. This fungus has been reported only in Aomori Prefecture in Japan as *Neovossia danubialis* Savulescu (Terui & Harada, 1974). There were 100% ITS and D1/D2 sequence identities between TNS-F-91250 and NBRC 9940 from the Aomori Prefecture.


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## 摘　要

カヤツリグサ科のヒデリコを宿主とする *Pilocintractia fimbristylidicola*，イネ科のハイヌメリを宿主とする *Sporisorium manilense*，イネ科のトダシバを宿主とする *Tilletia arundinellae*，イネ科のチヂミザサを宿主とする *Tilletia vittata*，イネ科のヨシを宿主とする *Ustilago phragmitis* の5種を黒穂菌類（Ustilaginomycotina）の日本新産記録として記載した．また，イネ科のヨシを宿主とする黒穂菌 *Neovossia moliniae* について日本で2例目の記録として記載した．


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