

Species of *Urocystis* (Ustilaginomycetes) on *Anemone* in Japan*

Cvetomir M. Denchev¹⁾, Makoto Kakishima^{2)**} and Yukio Harada³⁾

¹⁾ Institute of Botany, Bulgarian Academy of Sciences, 23 Acad. G. Bonchev Str., Sofia, BG-1113, Bulgaria

²⁾ Institute of Agriculture and Forestry, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

³⁾ Faculty of Agriculture and Life Sciences, Hirosaki University, Hirosaki, Aomori 036-8561, Japan

Accepted for publication 17 July 2000

Three species of *Urocystis* on *Anemone* (Ranunculaceae) are reported based on comparative morphology with specimens collected in Japan: *Urocystis anemones*, *U. japonica*, and *U. pseudoanemones* sp. nov. are separated by the number of ustilospores and sterile cells surrounding the ustilospores in the spore balls. Morphological characteristics, host plants and geographical distribution of these three species are also reported.

Key Words—*Anemone*; smut fungi; *Urocystis anemones*; *Urocystis japonica*; *Urocystis pseudoanemones*.

Two species of smut fungi, *Urocystis anemones* (Pers.: Pers.) G. Winter and *U. japonica* (Henn.) L. Ling have been reported on *Anemone* spp. (Ranunculaceae) in Japan (Ito, 1936; Kakishima, 1982). However, sori, spore balls, and ustilospores of these two species were morphologically variable among specimens. Therefore, we compared the morphology of the specimens identified as the above two species collected in Japan and revised *Urocystis* species on *Anemone* based on morphological differences. As a result, three species including a new species described here were recognized in Japan. Morphological characteristics, host plants, and geographic distribution of these three species are also reported.

Materials and Methods

Dry herbarium specimens were examined under light (LM) and scanning electron microscopy (SEM). These specimens were loaned by the following herbaria: the Mycological Herbarium of the Institute of Agriculture and Forestry, University of Tsukuba, Tsukuba, Japan (TSH); Faculty of Agriculture and Life Sciences, Hirosaki University, Hirosaki, Japan (Herb. Hirosaki Univ.); Faculty of Agriculture, Hokkaido University, Sapporo, Japan (SA-PA); and Department of Botany, National Science Museum, Tokyo (TNS). For LM observations, the spore balls were mounted in lactophenol solution on glass slides, gently heated to boiling point, then cooled. The measurements of ustilospores are given in the form: min-max (mean \pm standard deviation). In the description, the symbol 'n/x=' is used to indicate the total numbers of

measured specimens and spore balls or ustilospores, respectively. For SEM, the spore balls obtained from specimens were attached to specimen holders by double-sided adhesive tape and coated with platinum-palladium with a Hitachi E-1030 Ion Sputter. The surface structure of spore balls was observed with a Hitachi S-4200 SEM operating at 15 kV.

Results and Discussion

Urocystis anemones (Pers.: Pers.) G. Winter in Rabenh., Krypt.-Fl. Deutschl., 1(1): 123, 1881. Fig. 1

\equiv *Uredo anemones* Pers., Tent. Disp. Meth. Fung., p. 56, 1797.

\equiv *Tuburcinia anemones* (Pers.: Pers.) Liro, Ann. Univ. Fenn. Aboen. Ser. A1: 55, 1922.

Sori in the leaves, petioles, and stems, forming conspicuous swellings of varying shape and size, at first covered by the epidermis, becoming ruptured irregularly to expose the spore balls. Spore mass powdery, black-brown or black. Spore balls broadly ellipsoidal, ellipsoidal, ovoid, subglobose or irregular, 16–40 \times 14–39 μ m, composed of 1 (–3) central ustilospores [0=0.8%, 1=82.5%, 2=15.5%, 3=1.2%; n/8=1386], surrounded by a discontinuous layer of sterile cells, often of only few cells, or sterile cells absent. Sterile cells suborbicular, broadly elliptical, elliptical, ovate or angular in outline, or collapsed, 6–14 (–15.5) μ m long, light yellowish brown; wall 0.8–1 μ m thick, smooth or verruculose. Ustilospores subglobose, broadly ellipsoidal, ovoid or angular, 12.5–20.5 \times 10–17 (15.8 \pm 1.3 \times 13.5 \pm 1.1) μ m (n/7=240), dark or middle reddish brown; wall 0.9–1 μ m thick, verruculose.

Specimens examined: On *Anemone pseudoaltaica* H. Hara: Honshu, Aomori Pref., Hirosaki, Koguriyama, 3. V. 1970, Y. Harada (Y. H.) (Herb. Hirosaki Univ. 4369,

* Contribution No. 148, Laboratory of Plant Pathology and Mycology, Institute of Agriculture and Forestry, University of Tsukuba, Japan.

** Corresponding author.

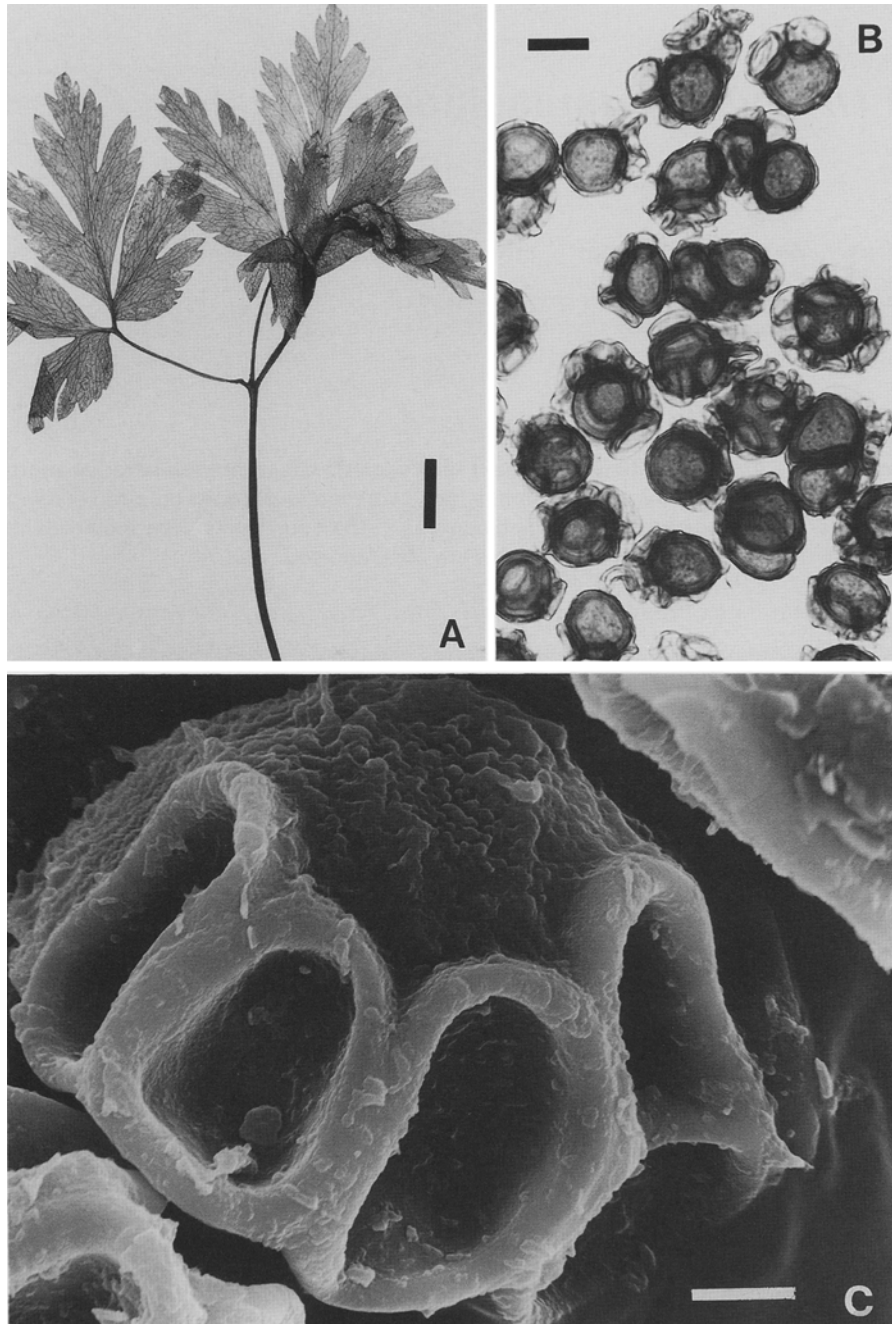


Fig. 1. *Urocystis anemones* on *Anemone pseudoaltaica*. A. Sori on *A. pseudoaltaica* (Herb. Hirosaki Univ. 13843). B. Spore balls (Herb. Hirosaki Univ. 13828). C. A spore ball observed under SEM (Herb. Hirosaki Univ. 15300). (Scale bars: A=8 mm, B=30 μ m, C=3 μ m)

4371); 17. V. 1970, Y. H. (Herb. Hirosaki Univ., 16925, 16926); 29. IV. 1972, Y. H. (Herb. Hirosaki Univ. 4380); 6. V. 1972, Y. H. (Herb. Hirosaki Univ. 4378, 4379); 11. V. 1973, Y. H. (Herb. Hirosaki Univ. 4376, 4377, 4382); Nishitsugaru-gun, Matsukami-mura, 9. IV. 1980, Y. H. (Herb. Hirosaki Univ. 10728); Kamikita-gun, 27. IV. 1983, Y. H. (Herb. Hirosaki Univ. 13816); Hirosaki, 30. IV. 1994, Y. H. (Herb. Hirosaki Univ. 22515); 10. V. 1983, Y. H. (Herb. Hirosaki Univ. 13827, 13828); 6. V.

1983, Y. H. (Herb. Hirosaki Univ. 13843); Towada Lake, 24. V. 1984, Y. H. (Herb. Hirosaki Univ. 15300); Oomagari, V. 1904, Tokubuchi (SAPA).

Host and distribution in Japan: On *Anemone pseudoaltaica* (*A. altaica* auct., non Fisch.) (Kikuzaki-ichige)-Honshu.

Distribution other than Japan: worldwide (Vánky, 1994; Wang, 1963; Zundel, 1953).

Urocystis anemones was first described from Germa-

ny (Vánky, 1994). The spore balls of the European specimens are composed predominantly of 1 ustilospore, rarely 2 or 3, which is surrounded by a discontinuous layer of sterile cells, often of only few cells, or sometimes sterile cells are absent (Vánky, 1994). Among Japanese speci-

mens of *Urocystis* on *Anemone* spp., only specimens on *A. pseudoaltaica* show morphological characteristics comparable of European specimens of *U. anemones*. *Urocystis anemones* seems to be restricted to *A. pseudoaltaica* in Japan.

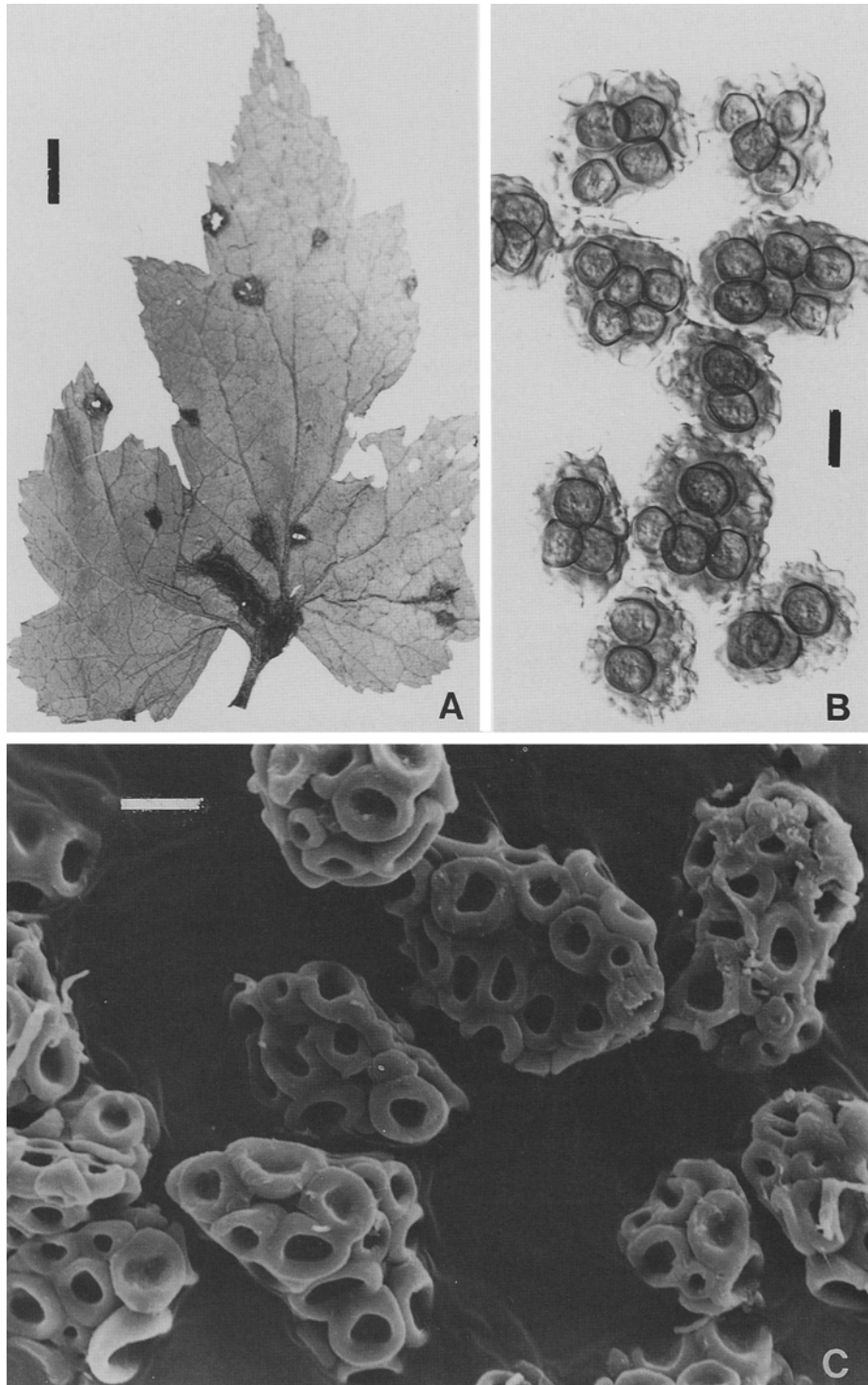


Fig. 2. *Urocystis japonica* on *Anemone hupehensis*. A. Sori on *A. hupehensis* (TSH-S397). B. Spore balls (TNS-F-229980). C. Spore balls observed under SEM (TNS-F-229980). (Scale bars: A=7 mm, B=30 μ m, C=10 μ m)

Urocystis japonica (Henn.) L. Ling, Mycol. Papers 11: 3, 1945. Fig. 2

≡ *Urocystis anemones* G. Winter var. *japonica* Henn., Hedwigia 43: 150, 1904.

≡ *Tubrcinia japonica* (Henn.) Liro, Ann. Univ. Fenn. Aboen. Ser. A1: 65, 1922.

Sori in petioles and stems, conspicuous, broadly elliptical to elliptical in outline, 2–9 mm long pustules,

often confluent and elongated up to 3.5 cm, or in leaves forming swellings of various shapes and sizes; at first covered by the epidermis then becoming ruptured exposing the spore balls. Spore mass powdery, black. Spore balls subglobose, broadly ellipsoidal, ellipsoidal, ovoid or slightly irregular, 22–56 × 19–55 μm, composed of (1–) 2–6 (–10) central ustilosporos [1=4%, 2=17%, 3=32%, 4=23%, 5=11%, 6=6%, 7=3.5%, 8=2%, 9=1.5%, 10=0.5%; n/6=824], surrounded by a contin-

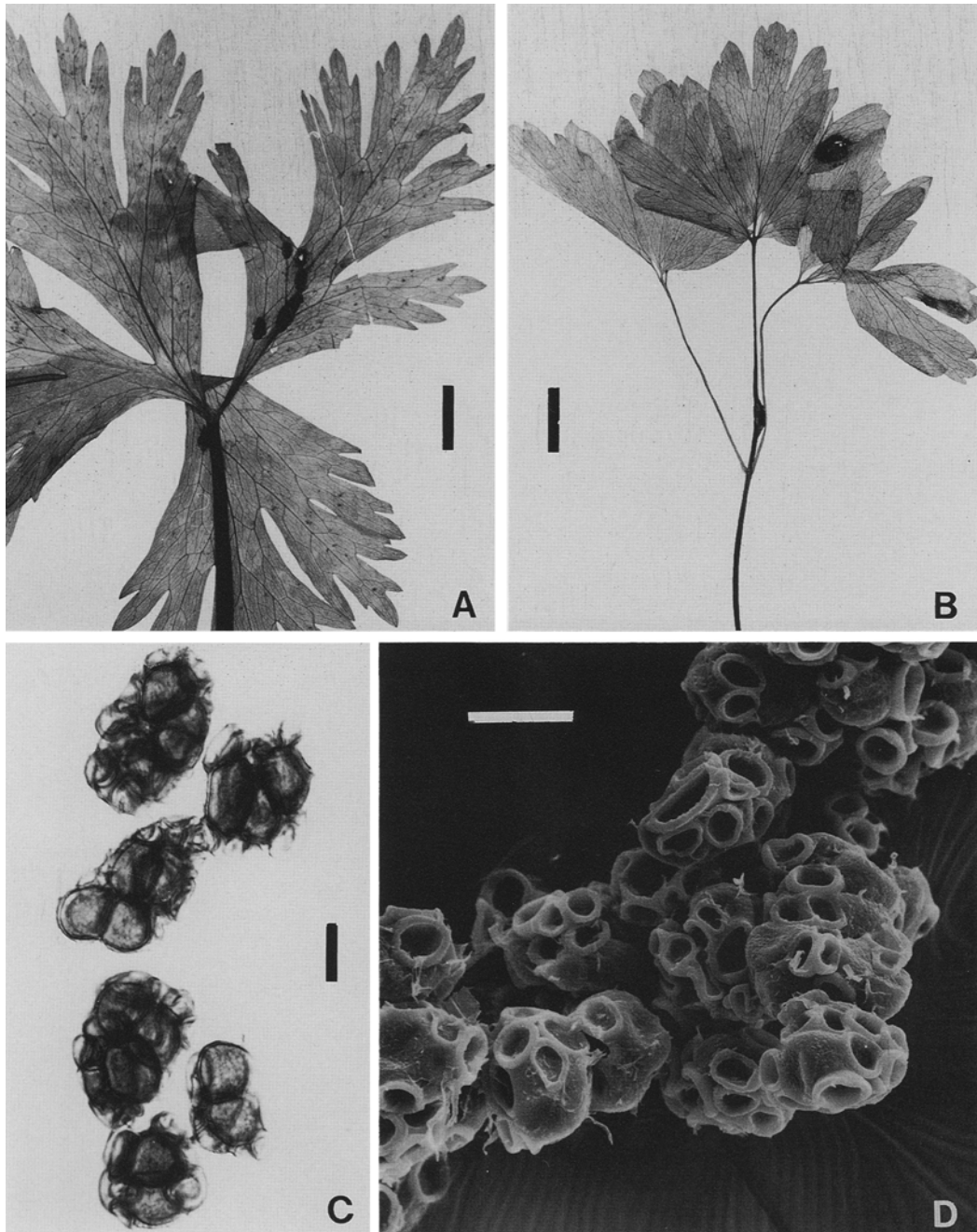


Fig. 3. *Urocystis pseudoanemones*. A. Sori on *Anemone flaccida* (Holotype). B. Sori on *A. raddeana* (Herb. Hirosaki Univ. 4372). C. Spore balls (Holotype, Herb. Hirosaki Univ. 15297). D. Spore balls observed under SEM (Holotype, Herb. Hirosaki Univ. 15297). (Scale bars: A, B=9 mm, C=30 μm, D=20 μm)

uous layer of sterile cells. Sterile cells broadly elliptical, elliptical, ovate or suborbicular in outline, or collapsed, 5–12 μm long, pale yellowish brown; wall 0.9–1.2 (–1.3) μm thick, smooth. Ustilosporia irregularly angular, subglobose, broadly ellipsoidal or ovoid, sometimes flattened on the contact sides, 10.5–18 \times 8.5–15 (13.8 \pm 1.2 \times 11.5 \pm 1.1) μm ($n/5=250$), dark reddish-brown; wall 1–1.2 (–1.35) μm thick, smooth.

Specimens examined: On *Anemone hupehensis* Lemoine var. *japonica* (Thunb. ex Murray) Bowles & Stearn: Honshu, Iwate Pref., Takada, 14. X. 1932, K. Chiba (SAPA); Shikoku, Kochi Pref., Kochi Park, VII. 1903, K. Nakano (TNS-F-229977, 229980); VI. 1903, K. Nakano (TNS-F-229976, SAPA); XII. 1904, K. Nakano (TNS-F-107963); VII. 1928, T. Yoshinaga (TNS F-228602, TSH-S396); Kochi Pref., Sakawa-machi, 9. VI. 1933, T. Yoshinaga (TSH-S397, SAPA); VII. 1933, T. Yoshinaga (TNS-F-230033); Kyushu, Kumamoto Pref., 8. X. 1905, K. Yoshino (TSH-S398, SAPA); Kyushu, Miyazaki Pref., 13. V. 1958, S. Hirata (TSH-S399).

Host and distribution in Japan: On *Anemone hupehensis* var. *japonica* [*A. japonica* (Thunb. ex Murray) Sieb. & Zucc., non Houtt.; *A. vitifolia* Buch.-Ham. var. *japonica* (Thunb. ex Murray) Finet & Gagnep; *A. sieboldii* Honda] (Shuumeigiku)-Honshu, Shikoku and Kyushu.

Distribution other than Japan: China (Wang, 1963), the Russian Far East (Azbukina et al., 1995).

Urocystis japonica differs from *U. anemones* in the spore number per spore ball and in the arrangement of sterile cells. Spore balls of *U. japonica* are completely surrounded by a layer of sterile cells, whereas those of *U. anemones* are surrounded by a discontinuous layer of sterile cells. Hennings (1904) originally described this smut fungus as possessing very long sori, sometimes more than 20 cm in length. Ciferri (1963) used this character to separate *U. japonica* from the *U. anemones* complex. However, the length of sori of this species is variable among the specimens observed. We believe that the length of sori is not a good taxonomic character.

Urocystis pseudoanemones Denchev, Kakish. & Y. Harada, sp. nov. Fig. 3

Sori in foliis, petiolis et caulibus tumores formantes, primo epidermide tecti. Massa sporarum nigricanto-brunnea vel nigra, pulverea. Glomeruli sporarum late ellipsoidei, ellipsoidei vel irregulares, 19–57 \times 14–40 μm , e ustilosporis 1–5 (–11) [numeri sporarum: 1=24%, 2=37%, 3=20%, 4=10%, 5=5%, 6=1.8%, 7=1.1%, 8=0.5%, 9=0.1%, 10=0.2%, 11=0.2%; $n/28=2978$] compositi, strato imperfecto cellularum sterili-um circumdantes, sed saepe cellulis sterilibus paucibus praesentes vel cum exceptione cellulis sterilibus absentes. Cellulae steriles suborbiculares, late ellipticae, ellipticae vel collapsae, 5.5–12 (–17) μm longae, pallide flavido-brunneae, pariete levi 1–1.4 μm crasso. Ustilosporae irregulariter angulares, subglobo- sae, late ellipsoideae vel lateribus contactis planis, 11–22 \times 9–17.5 (15.6 \pm 1.5 \times 12.6 \pm 1.3) μm ($n/26=948$), rufobrunneae vel atro-rufobrunneae; paries 0.7–1.3 (–1.7) μm crassus, verruculosus.

Holotypus: in foliis *Anemones flaccidae* F. Schmidt, Japonia, Honshu, Aomori Pref., ad lacum Towada, 24. V. 1984, Y. Harada (Herb. Hirosaki Univ. 15297).

Sori in the leaves, petioles and stems, forming conspicuous swellings of varying shape and size, at first covered by the epidermis then becoming ruptured irregularly to expose the spore balls. Spore mass powdery, black brown or black. Spore balls broadly ellipsoidal, ellipsoidal or irregular, 19–57 \times 14–40 μm , composed of 1–5 (–11) central ustilosporia [1=24%, 2=37%, 3=20%, 4=10%, 5=5%, 6=1.8%, 7=1.1%, 8=0.5%, 9=0.1%, 10=0.2%, 11=0.2%; $n/28=2978$], surrounded by a discontinuous layer of sterile cells, often of only few cells, sometimes sterile cells absent. Sterile cells suborbicular, broadly elliptical or elliptical in outline, or collapsed, 5.5–12 (–17) μm long, light yellowish brown; wall 1–1.4 μm thick, smooth. Ustilosporia irregularly angular, subglobose, broadly ellipsoidal or flattened on the contact sides, 11–22 \times 9–17.5 (15.6 \pm 1.5 \times 12.6 \pm 1.3) μm ($n/26=948$), medium to dark reddish brown; wall 0.7–1.3 (–1.7) μm thick, verruculose.

Other specimens examined: On *Anemone flaccida* F. Schmidt: Hokkaido, Shiribeshi-shicho, Zenibako, 27. V. 1926, N. Hiratsuka (N. H.) (SAPA); Shiribeshi-shicho, Asari, 27. V. 1926, N. H. (SAPA, TSH-S1117); Ishikari-shicho, Sapporo, Bot. Garden, 20. V. 1902, G. Yamada (SAPA); Sapporo, Mt. Moiwa, 3. VI. 1900, J. Hanzawa (SAPA); 28. V. 1925, N. H. (TSH-S380); 4. VI. 1926, S. Nagai (TNS-F-215333); 29. V. 1930, Y. Imai (SAPA); 16. V. 1953, S. Shimada (TSH-S381); Tobetsu, 26. V. 1906, K. Miura (SAPA); Nopporo, 31. V. 1932, Y. Imai (SAPA); Honshu, Aomori Pref., Nishitsugaru-gun, Iwasaki-mura, 15. IV. 1973, Y. Harada (Y. H.) (Herb. Hirosaki Univ. 4373, 4381); Misawa, 29. IV. 1985, Y. H. (Herb. Hirosaki Univ. 15290, 15378); Towada Lake, 24. V. 1985, Y. H. (Herb. Hirosaki Univ. 15325, 15326, 15348); 18. V. 1990, Y. H. (Herb. Hirosaki Univ. 19866); 18. V. 1991, Y. H. (Herb. Hirosaki Univ. 21098); Towada Lake, 19. V. 1991, Y. H. (Herb. Hirosaki Univ. 20656); Higashitsugaru-gun, Hiranai-machi, 12. V. 1987, Y. H. (Herb. Hirosaki Univ. 17149, 17153, 17157, 17206); Iwate Pref., near Morioka, Nanshozan, 8. V. 1904, G. Yamada (SAPA); Tochigi Pref., Kuroiso-machi, 5. V. 1939, K. Ide (TSH-S384); Ibaraki Pref., Gozenyama, 29. IV. 1978, M. Kakishima (TSH-S385); Tokyo, 2. V. 1899 (TSH-S1119); 30. IV. 1960, N. H. (TSH-S388); Tokyo, Shimura, 1. V. 1901, K. Miyabe (SAPA); 22. IV. 1907, Nambu (SAPA); Tokyo, Komaba, 18. IV. 1977, M. Kakishima (TSH-S1122); Tokyo, Mt. Takao, 5. V. 1949, T. Endo (TSH-S386); 5. V. 1949, S. Yoshida (TSH-S1121); 20. IV. 1957, N. H. & S. Sato (TSH-S387); 10. IV. 1959, T. Hiratsuka (TSH-S1120); 20. IV. 1963, N. H. & S. Sato (TSH-S389); 25. IV. 1965, S. Sato et al. (TSH-S390); 22. IV. 1967, S. Kaneko (TSH-S391); 29. IV. 1969, S. Sato (TSH-S1118); Nagano Pref., Sugadaira, 24. V. 1978, M. Kakishima (TSH-S383). On *Anemone raddeana* Regel: Hokkaido, Ishikari-shicho, Sapporo, University Farm, 12. V. 1925, I. Tanaka (SAPA); V. 1927, Y. Homma (TNS-F-217142); Sapporo, 16. V. 1925, N. H. (SAPA, TSH-

S1116); 5. VI. 1926, S. Ito (SAPA); 8. V. 1928, M. Terui (Herb. Hirosaki Univ. 4374); 27. V. 1929, Y. Tokunaga (SAPA); 6. V. 1930, Y. Imai (SAPA); Oshima-shicho, Tachimachi-saki, near Hakodate, 30. V. 1925, N. H. (TSH-S378, SAPA); Honshu, Aomori Pref., Hirosaki, Koguriyama, 17. V. 1970, Y. H. (Herb. Hirosaki Univ. 4370); 16. V. 1974, Y. H. (Herb. Hirosaki Univ. 4372); Hirosaki, 10. V. 1983, Y. H. (Herb. Hirosaki Univ. 13826); 6. V. 1983, Y. H. (Herb. Hirosaki Univ. 13847); Misawa, 29. IV. 1985, Y. H. (Herb. Hirosaki Univ. 15379); Nishistugaru-gun, Iwasaki-mura, 9. IV. 1986, Y. H. (Herb. Hirosaki Univ. 10730); Towada Lake, 26. V. 1984, Y. H. (Herb. Hirosaki Univ. 18284); Iwate Pref., Morioka, 11. V. 1904, G. Yamada (SAPA); 12. V. 1912, G. Yamada (SAPA); Mt. Himekami, 21. V. 1927, K. Togashi (SAPA); Ibaraki Pref., Gozenyama, 29. IV. 1978, M. Kakishima (TSH-S379). On *Anemone stolonifera* Maxim.: Hokkaido, Ishikari-shicho, Sapporo, Botanical Garden, 18. V. 1957, Y. H. (TSH-S382).

Hosts and distribution in Japan: On *Anemone flaccida* (Nirinsu)-Hokkaido and Honshu. On *A. raddeana* (Azumaichige)-Hokkaido and Honshu. On *A. stolonifera* (Sanrinso)-Hokkaido.

Distribution other than Japan: the Russian Far East (Sakhalin)

Urocystis pseudoanemones and *U. anemones* are distinct from *U. japonica* in the arrangement and number of sterile cells which surround the spore balls. The spore balls of the former two species are surrounded by a discontinuous layer of sterile cells, whereas those of *U. japonica* are completely surrounded by sterile cells. The spore balls of *U. pseudoanemones* are composed of 1–5 ustilospores, while those of *U. anemones* are predominantly composed of 1 ustilospore, rarely of 2 or 3. From the Mediterranean region (South Europe and North Africa), *U. antipolitana* Magnus on *Anemone* spp., whose spore balls are composed of 1–6 (–7) ustilospores, is reported (Vánky, 1994). However, its spore balls are completely covered by sterile cells, while those of *U. pseudoanemones* are covered by a discontinuous layer of sterile cells, often of only few cells. Recently, Guo (1999) reported this species based on a specimen collected in Qinling Mountains, China. However, we think that reevaluation of this specimen is required because its description is very similar to that of *U. pseudoanemones*.

Urocystis pseudoanemones is morphologically similar to *U. syncocca* (L. A. Kirchn.) B. Lindb. on *Hepatica* spp. *Urocystis syncocca* is mainly distributed in Europe and also reported from the Russian Far East (Azbukina et al., 1995). Markova (1927) reported that ustilospores of *U. syncocca* obtained from *H. triloba* Chaix did not infect *Trollius europaeus* L., *Anemone* spp., or *Ranunculus* spp. Therefore, we consider that *U. pseudoanemones* is different from *U. syncocca* in the host specificity and geographical distribution.

We assume that *U. pseudoanemones* is more widely

distributed in East Asia and Russia, especially in the Russian Far East and China. We found a specimen of *U. pseudoanemones* on *A. flaccida* which was collected in Sakhalin and was identified as *U. anemones* (SAPA). *Urocystis anemones* has been recorded from China on *A. raddeana* and *A. chinensis* Bge. (Ling, 1953; Wang, 1963). We suppose that specimens of *U. pseudoanemones* will be found among the specimens collected in China and identified as *U. anemones*.

Key to *Urocystis* species on *Anemone* in Japan

1. Ustilospores completely surrounded by a layer of sterile cells *U. japonica*
1. Ustilospores surrounded by a discontinuous layer of sterile cells 2
2. Spore balls composed of 1 (–3) ustilospores ... *U. anemones*
2. Spore balls composed of 1–5 (–11) ustilospores *U. pseudoanemones*

Acknowledgements—We wish to thank Prof. I. Uyeda, the Curator of SAPA (Herbarium of Hokkaido University, Sapporo, Japan), and Dr. Y. Doi, the Curator of TNS (the Department of Botany, National Science Museum, Tokyo, Japan), for the loan of the cited specimens. We are also thankful to the Japan Society for the Promotion of Science for financial support.

Literature cited

- Azbukina, Z. M., Karatygin, I. V. and Govorova, O. K. 1995. Ustomycetes (Ustilaginales). In: *Plantae non vasculares, Fungi et Bryopsida Orientis extremi Rossica*. Fungi, vol. 3, (ed. by Azbukina, Z. M.), pp. 9–205. Nauka, Petropoli. (In Russian.)
- Ciferri, R. 1963. Revisio Ustilaginearum. Pars I. Tilletiaceae. *Quaderno* 27: 1–143.
- Guo, L. 1999. *Urocystis antipolitana*, a smut species new to China. *J. Anhui Agric. Univ.* 26: 390.
- Hennings, P. 1904. Einige neue Pilze aus Japan. II. *Hedwigia* 43: 150–153.
- Ito, S. 1936. *Mycological Flora of Japan*, vol. 2, no. 1. Yokendo, Tokyo.
- Kakishima, M. 1982. A taxonomic study on the Ustilaginales in Japan. *Mem. Inst. Agr. For. Univ. Tsukuba (Agr. & For. Sci.)* 1: 1–124.
- Ling, L. 1953. The Ustilaginales of China. *Farlowia* 4: 305–351.
- Liro, J. I. 1938. Die Ustilagineen Finnlands. II. *Ann. Acad. Sci. Fenn., Ser. A*, 42: 1–720.
- Markova, M. F. 1927. Races biologiques de l'*Urocystis anemones* Wint. on *Ranunculaceae*. *Morbi Plant.* 16: 135–140. (In Russian.)
- Vánky, K. 1994. *European smut fungi*. G. Fischer Verlag, Stuttgart.
- Wang, Y. C. 1963. Ustilaginales of China. Science Press, Peking. (In Chinese.)
- Zundel, G. L. 1953. The Ustilaginales of the World. *Pennsylvania State Coll. School Agric. Dept. Bot., Contrib.* 176: 1–410.