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Puccinia carici-adenocauli, a new rust fungus on *Carex*, and its anamorph, *Aecidium adenocauli**

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Field observations and inoculation experiments showed that *Aecidium adenocauli* on *Adenocaulon himalaicum* was an anamorph of a *Puccinia* on *Carex hakonensis* and *C. uda*. From comparative morphology with caricicolous puccinias, the rust on these *Carex* species was considered as a new species and was named as *Puccinia carici-adenocauli*.

Key Words——*Aecidium adenocauli*; *Adenocaulon*; *Carex*; *Puccinia carici-adenocauli*; rust fungus.

Aecidium adenocauli H. Sydow et Sydow, an anamorphic rust fungus (Uredinales), was first described in 1907 based on spermogonial and aecial stages on *Adenocaulon bicolor* Hook. (=*A. himalaicum* Edgew.) collected in Iwate Pref., Japan (Sydow and Sydow, 1913). It is widely distributed in Honshu and Hokkaido, Japan (Ito, 1950; Hiratsuka, 1960, Hiratsuka et al., 1992), Korea (Hiratsuka, 1963) and the Russian Far East (Azbukina, 1974). Kakishima and Sato (1984) carried out inoculation experiments with materials collected in Yamanakakomura, Yamanashi Pref., Japan and reported that the *Aecidium* was the anamorph of a *Puccinia* species on *C. hakonensis* Franch. et Savat. However, they could not identify the rust species.

In June 1996, we found *A. adenocauli* on leaves of *A. himalaicum* at Tomakomai Experiment Forest of Hokkaido University, near Sapporo, Hokkaido. Simultaneously, we found *C. hakonensis* and *C. uda* Maxim. bearing telia of a *Puccinia* species on their leaves and stems in the same location. Because of the report by Kakishima and Sato (1984), we suspected that host alternation of the same rust fungus occurred between *A. himalaicum* and *C. hakonensis* and/or *C. uda*.

Here, we report inoculation experiments conducted to confirm the life cycle connection between the *Aecidium* on *A. himalaicum* and the *Puccinia* on two *Carex* species. We also carried out morphological comparisons of the specimens obtained by inoculation experiments and those collected in Yamanashi Pref. and Hokkaido to identify the rusts concerned. As a result, we propose the rust fungus on *Carex* as a new *Puccinia* species.

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Materials and Methods

Basidiospore inoculation Leaves of *C. hakonensis* with telia of *Puccinia* sp. were collected at Tomakomai Experiment Forest on October 30, 1996. The leaves were placed on the ground and overwintered under snow at Hirosaki Univ. Campus. In early April, they were collected from the field and kept in a refrigerator at 0°C until use. The leaves were placed on wet filter paper in Petri dishes for about 2 wk at room temperature (15–20°C) to induce the germination of the teliospores. The teliospores germinated to produce numerous basidiospores within several days. For inoculation, the leaves with germinating teliospores were placed on wire net set on healthy leaves of *A. himalaicum* to allow basidiospores to fall on to the leaves (wire net method). As a second method of inoculation, leaves with germinating teliospores were crushed into small pieces, suspended in distilled water, and the suspensions were sprayed on healthy leaves of *A. himalaicum* with an atomizer (spray method). The inoculated plants were kept in a moist chamber with diffused sunlight at about 20°C. Seven days later, the inoculated plants were transferred to a shaded place in the field.

Aeciospore inoculation Aeciospores formed on *A. himalaicum* by basidiospore inoculations were used as inocula. The aeciospores collected from aecia on the leaves were suspended in distilled water and inoculated on young leaves of *C. hakonensis* and *C. uda* with a small hair brush. The inoculated plants were kept in a moist chamber with diffused sunlight at about 20°C for 1 wk, then transferred to the shaded place in the field.

Morphological observations Dry specimens on *A. himalaicum*, *C. hakonensis*, and *C. uda* obtained from the inoculations and/or collected in the fields were used for morphological observations. Specimens examined are listed in the description of the species. Morphological observations were made by light (LM) and scanning elec-

tron microscopy (SEM). The spores or thin sections of sori were mounted in lactophenol solution on glass slides for LM observations. For SEM, sori or spores obtained from dry specimens were attached to specimen holders by double-sided adhesive tape and coated with gold in a high vacuum with an Eiko IB-3 Ion Coater. They were examined with a Hitachi S-430 SEM operating at 20 kV.

Results and Discussion

Life cycle About 10–12 d after inoculation with teliospores and/or basidiospores, yellowish green spermogonia appeared on the upper surface of the inoculated leaves of *A. himalaicum* and 7–14 d later, aecia were produced on the lower surface of the leaves. Spermogonia and aecia on the leaves appeared earlier and were more abundant on leaves inoculated by the wire net method than on those inoculated by the spray method.

About 1 wk after aeciospore inoculations, white to pale yellow spots appeared on inoculated leaves and stems of *C. hakonensis* and *C. uda*, and 3–7 d later, these spots turned yellowish brown and produced uredinia. After about 10 wk, telia were also produced on the leaves and stems of both *Carex* species.

These inoculation experiments confirmed the results of inoculations carried out by Kakishima and Sato (1984)

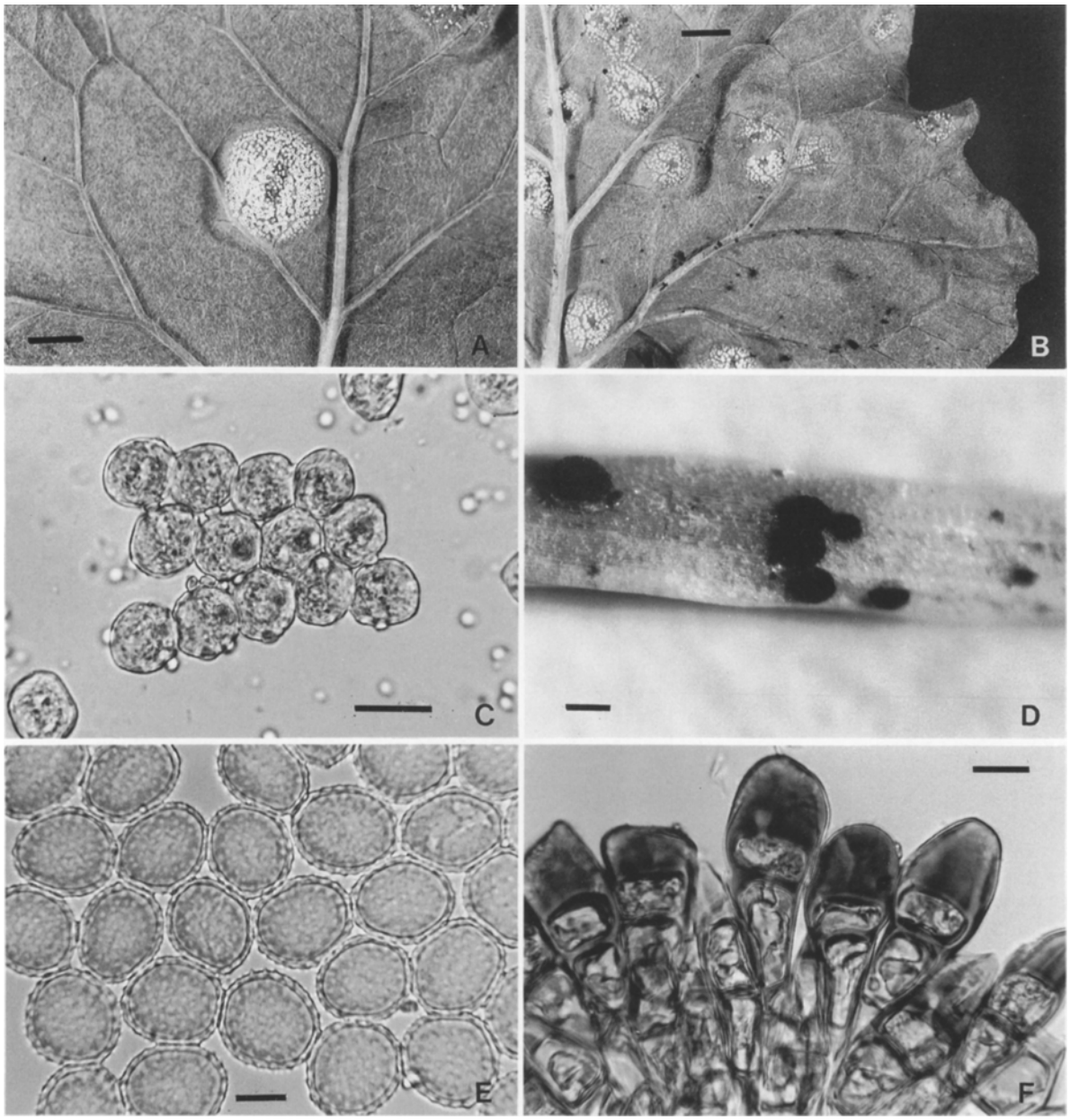

Fig. 1. *Puccinia carici-adenocauli*. A, B. Aecia on *Adenocaulon himalaicum*. C. Aeciospores with large granules. D. Telia on *Carex hakonensis*. E. Urediniospores. F. Teliospores. (Scale bars: A=5 mm, B=1 cm, C=20 μm, D=0.5 mm, E=10 μm, F=10 μm)

and showed that *A.adenocauli* on *A. himalaicum* was the anamorph of a *Puccinia* species on *Carex hakonensis* and *C. uda*.

Morphology The spermogonia on *A. himalaicum* were epiphyllous, surrounded by orange-yellow lesions, densely grouped, yellow to brown, subepidermal, and flask-shaped (type 4 of Hiratsuka and Cummins, 1963). Aecia were hypophyllous, densely grouped, cupulate with peridia, and pale yellow (Figs. 1A, B; 2A). The aeciospores were globose to subglobose, often angular, and 15–21 × 12–17 μm (Figs. 1C; 2B). Their walls were 1–1.8 μm thick, hyaline, and verrucose with large granules

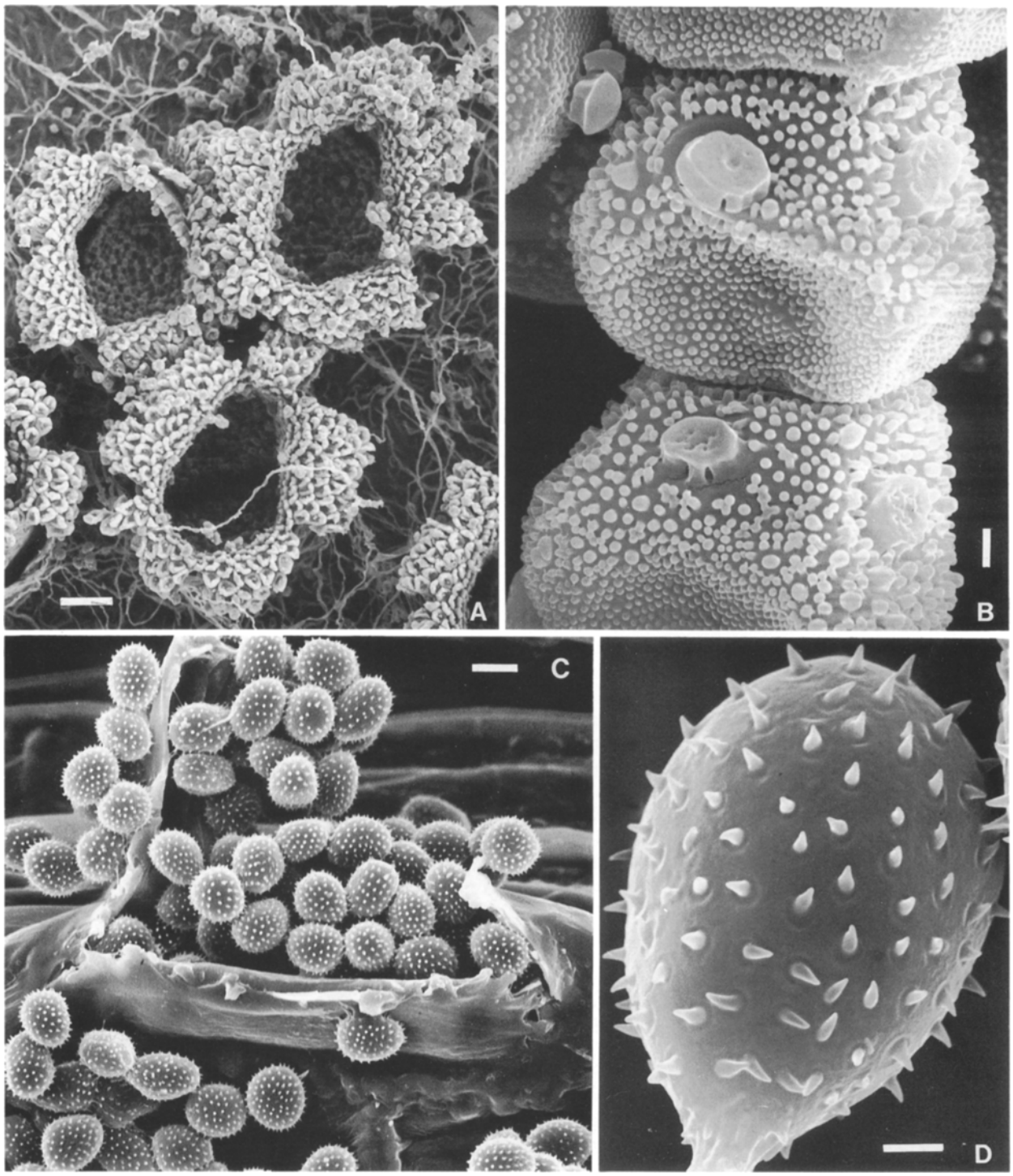

Fig. 2. *Puccinia carici-adenocauli* observed under SEM. A. Aecia on *Adenocaulon himalaicum*. B. Aeciospores with large granules. C. A uredinium on *Carex hakonensis*. D. A urediniospore. (Scale bars: A = 50 μm, B = 1 μm, C = 10 μm, D = 2 μm)

(2–6 μm in diam) (Fig. 2B). These morphological characteristics were identical with the descriptions of *A. adenocauli* by Sydow and Sydow (1913), Ito (1950), and Hiratsuka et al. (1992). Therefore, we confirmed the anamorphic stage on *A. himalaicum* as *A. adenocauli*.

The uredinia on two *Carex* species were hypophyllous, scattered, erumpent, and brown to pale brown without paraphyses. Urediniospores were obovoid to ellipsoid, and 16–25 × 12–20 μm (Figs. 1E; 2C). Their walls were 1.5–2.5 μm thick, hyaline, and echinulate (Figs. 1E; 2C, D). The germ pores were obscure. Telia were hypohyllous, scattered, rounded to ellipsoid, erumpent, and dark brown without paraphyses (Fig. 1D). Teliospores were clavate to oblong with round to obutuse apices and attenuate toward bases, constricted at the septa, and 30–58 × 10–23 μm (Fig. 1F). Walls were dark brown with apical thickning of 7–20 μm, and smooth. Pedicels were 15–65 μm long, and persistent. These morphological characteristics were different from descriptions of *P. limosae* Magnus on *C. hakonensis* (Ito, 1950; Arthur, 1934; Gäumann, 1959).

Taxonomy From field observations and inoculation experiments it was clarified that the teleomorph of *A. adenocauli* on *A. himalaicum* was produced on *C. hakonensis* and *C. uda* and that this rust fungus had heteroecious macrocyclic life cycle. There is no record of a rust fungus with a life cycle alternating between *Adenocaulon* and *Carex*.

From morphological observations, the main teleomorphic characteristics of this rust fungus are as follows: (1) urediniospores are relatively small, (2) walls of urediniospores are hyaline and echinulate, (3) germ pores of urediniospores are obscure, (4) walls of teliospores are dark brown and their apical walls are thick, (5) teliospores germinate after a period of dormancy, and (6) pedicels are long and persistent.

Puccinia limosae is the only rust fungus that is known to produce uredinial and telial stages on *C. hakonensis* (Ito, 1950; Hiratsuka, 1960; Hiratsuka et al., 1992). Teliospores of the present rust fungus are similar to those of *P. limosae*. However, urediniospores of *P. limosae* have three distinct germ pores at the equatorial part and thier walls are pale brown (Arthur, 1934; Ito, 1950; Gäumann, 1959; Hiratsuka et al., 1992). The anamorph of *P. limosae* is reported to be produced on *Lysimachia* species (Arthur, 1934; Ito, 1950; Gäumann, 1959; Hiratsuka et al., 1992). Therefore, the present fungus is different from *P. limosae*.

Morphological comparison of the *Puccinia* species on *Carex* with other rust fungi revealed no other fungi with the same characteristics. Therefore, we concluded that the present rust fungus was a new species. Its description is as follows:

Puccinia carici-adenocauli Kakishima, M. Yokoi et Y. Harada, sp. nov. Figs. 1–2

Anamorph: *Aecidium adenocauli* H. Sydow et Sydow, Ann. Mycol. 11: 111, 1913.

Uredinia hypophylla, sparsa, mox nuda, brunnea, pallide fusca; urediniosporae obovatae vel ellipsoideae, 16–25 × 12–20 μm, membrana 1.5–2.5 μm crassa, hyalina, echinulata, pori germinationis obscuri; paraphysis nulla. Telia hypophylla, sparsa, rotundata vel ellipsoidea, mox nuda, atro-brunnea; teliosporae clavatae vel oblongae, apice rotundatae vel obtusae, basi attenuatae, medio leviter constrictae, 30–58 × 10–23 μm, membrana laevi atro-brunnea apice 7–20 μm incrassatae pedicello persistenti, 15–65 μm longo praeditae; paraphysis nulla.

Holotype: On *Carex hakonensis* Franch. et Savat., Yamanakako-mura, Yamanashi Pref., Japan, May 8, 1983, II, III, M.K., TSH-R1693.

Specimens examined: On *A. himalaicum* (=*A. adhaerescens*, *A. bicolor*), Yamanakako-mura, Yamanashi Pref., Japan, 25 May 1983, 0, I, M.K., TSH-R1689; Ussuri Reserve, Primorye Prov., 60 km North of Vladivostok, Far East of Russia, 31 July 1992, 0, I, Z. M. Azbukina et al., TSH-R9030; cultured with TSH-R1693 at Tsukuba, 30 May 1983, 0, I, M.K., TSH-R1690; cultured with TSH-R1693 at Tsukuba, 7 June 1983, 0, I, M.K., TSH-R1691; cultured with TSH-R1693 at Tsukuba, 18 June 1983, 0, I, M.K., TSH-R1692; cultured at Hirosaki, 15 July 1997, 0, I, M.Y., no. 24148. On *C. hakonensis*, Yamanakako-mura, Yamanashi Pref., Japan, 8 May 1983, II, III, M.K., TSH-R1693, Holotype; cultured with TSH-R1690 at Tsukuba, 6 June 1983, II, M.K., TSH-R1694; cultured with TSH-R1690 at Tsukuba, 16 June 1983, II, M.K., TSH-R1695; cultured with TSH-R1690 at Tsukuba, June 25, 1983, II, M.K., TSH-R1696; cultured with TSH-R1690 at Tsukuba, 7 Dec. 1983, III, M.K., TSH-R1697; cultured at Hirosaki, 7 Aug. 1997, II, M.Y., no. 24282; cultured at Hirosaki, 12 Nov. 1997, III, M.Y., no. 24283. On *C. uda*, cultured at Hirosaki, 1 Sept. 1997, II, M.Y., no. 24280; cultured at Hirosaki, 12 Nov. 1997, III, M.Y., no. 24284.

Hosts and Distribution: 0, I On *A. himalaicum* (=*A. adhaerescens*, *A. bicolor*) (Nobuki) – Japan (Honshu, Hokkaido) (Ito, 1950; Hiratsuka, 1960, Hiratsuka et al., 1992), Korea (Hiratsuka, 1963), Russia (Far East) (Azbukina, 1974). II, III On *C. hakonensis* (Koharisuge) – Japan (Honshu, Hokkaido). II, III On *C. uda* (Ezoharisuge) – Japan (Hokkaido).

Specimens used in this study were deposited in the Mycological Herbarium, Institute of Agriculture and Forestry, University of Tsukuba (TSH) or in the Herbarium of the Faculty of Agiculture and Life Science, Hirosaki University for those cultured at Hirosaki.

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