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Aecidium dispori is the aecial anamorph of *Puccinia albispora*, sp. nov. (Uredinales)

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Aecidium dispori **forms spermogonium and aecium on** ***Disporum sessile*** **and** ***D. smilacinum*****, which are distributed in East Asia. The** ***Aecidium*** **species is found to be an aecial anamorph of a** ***Puccinia*** **fungus, with its uredinial-telial stage being formed on** ***Carex conica, C. dolichostachya*** **subsp.** ***multifolia, C. pisiformis*** **subsp.** ***alterniflora*** **and** ***C. rugata*****. Urediniospores of this fungus are large, colorless, thick-walled with 4–5 equatorial germ pores. The morphological characteristics of urediniospores and the spermogonial-aecial host do not fit to any set of circumscribing characters of previously described species. We consider the fungus to be a new species and propose a new name,** ***Puccinia albispora*****, for the fungus.**

Key Words——*Carex*; Cyperaceae; *Disporum*; Liliaceae; rust fungus.

Aecidium dispori Dietel was first described for a fungus found on *Disporum sessile* Don at Togakushi, Nagano Pref., Japan (Dietel, 1899). It has since been found to occur also on *D. smilacinum* A. Gray and to be widely distributed in Japan, China and Taiwan (Hiratsuka et al., 1992).

It is empirically known that *A. dispori* does not repeat the spermogonial-aecial stage on the same host species. No uredinial-telial fungus has been discovered on *Disporum* species in Japan, although *Puccinia dispori* Sydow is know to occur on *Disporum* species in the Philippines (Sydow and Petrak, 1931), China (Zhuang et al., 1998) and Taiwan (Hiratsuka and Hashioka, 1937). Thus, *A. dispori* is believed to be an aecial anamorph of a fungus that may belong either to *Puccinia* or to *Uromyces*.

This paper reports the life-cycle connection between *A. dispori* on *Disporum* and a uredinial-telial *Puccinia* on *Carex*, which was proven by field observations and repeated inoculations, and proposes a name for the new holomorphic fungus based on morphological examinations of closely related species.

Materials and Methods

Specimens examined Specimens deposited in the mycological herbaria of the Faculty of Education, Ibaraki University (IBA), the Institute of Agriculture and Forestry, University of Tsukuba (TSH), the Faculty of Agriculture, Hokkaido University (SAPA) and the Swedish Museum of Natural History, Sweden (S) were examined and are listed under each species in the description section below.

Basidiospore inoculation Basidiospore inoculations were undertaken by the method described by Ono (1995) and Ono and Azubukina (1997). Telium-bearing leaves of *Carex* species were collected at various locations in different years as follows: *C. pisiformis* subsp. *alterniflora* at Asakawa, Tokyo, in April, 1976; *C. conica* at Mt. Tsukubasan, Ibaraki, in March, 1979; *C. dolichostachya* subsp. *multifolia* at Takefu, Ibaraki, in 1979, at Mt. Gozenyama, Ibaraki, in March 1980 and at Tokuda, Ibaraki, in March 1999; and *C. rugata* at Okami, Ibaraki, in March, 1999 and February 2000. In each year, the collected telium-bearing leaves were preserved in a refrigerator at ca. 5°C until the time when they were soaked in running tap water at room temperature for 7–14 d to induce germination. Then, the leaves were cut into small pieces (ca. 3–5 mm long), placed on water-saturated filter paper in a Petri dish and incubated in the dark at ca. 18°C. The leaf pieces with germinated teliospores were placed on the adaxial surface of apparently healthy leaves of the following plants, which had been planted with loam soil in clay pots (18 cm diam): *Disporum smilacinum, Cardiocrinum cordatum* Makino, *Aster scaber* Thunb. and *A. ageratoides* Turzc. subsp. *ovatus* (Fr. & Sav.) Nakai were inoculated with the fungus on *C. pisiformis* subsp. *alterniflora* in 1976; *D. sessile, D. smilacinum, C. cordatum* and *Polygonatum lasiantum* Maxim. with the fungus on *C. dolichostachya* subsp. *multifolia* from Takefu in 1979; *D. sessile* and *D. smilacinum* with the fungus on *C. dolichostachya* subsp. *multifolia* from Mt. Gozenyama in 1980; *D. sessile, D. smilacinum* and *C. cordatum* with the fungus on *C. conica* in 1979; *D. smilacinum, C. cordatum, Majanthemum dilatatum* Nels. & Macbr., *Tricyrtis macropoda* Miq., *A. ageratoides* subsp. *ovatus* and *Circaea mollis* Sieb. & Zucc. with the fungus on *C. dolichostachya* subsp. *multifolia* in 1999; and *D. sessile* with the fungus on *C. rugata* in 2000. The inoculated plants were chosen because

they were often found infected with an *Aecidium* and located near the *Puccinia*-infected *Carex* species, except for *M. dilatatum* and *T. macropoda,* which were not aecidium-bearing or located near the rusted sedges, but which were shown by previous records to be possible aecial hosts of the *Puccinia* fungus. The inoculated plants were sprayed with distilled water and placed in a moist chamber at room temperature (18–22°C) for 48 h, then transferred to a glasshouse for further observation.

Aeciospore inoculation Aeciospores formed a plant inoculated with basidiospores of the fungus on *C. conica* or *C. dolichostachya* subsp. *multifolia* were inoculated onto apparently healthy leaves of *C. conica, C. dolichostachya* subsp. *multifolia* and *C. pisiformis* subsp. *alterniflora*. Aeciospores were scraped from the sori and dusted on small pieces (ca. 3×3 mm) of water-saturated filter paper, which were then placed on the abaxial surfaces of leaves. The inoculated plants were sprayed with distilled water and placed in a moist chamber at room temperature (18–22°C) for 48 h, then transferred to a glasshouse for further observation.

Microscopic observation To examine morphology and structure of spermogonia and aecia, fresh infected materials and dried herbarium specimens were freehand-sectioned under a binocular dissecting microscope. Thin sections were mounted in a drop of lactophenol solution without staining. To examine morphology and measure size, the spores were scraped from sori on herbarium specimens and mounted by the same method as described above.

To observe germ pores in urediniospores, the spores were placed in a drop of lactic acid on a microscopic slide, heated to boiling for a few seconds and mounted with an additional drop of lactophenol solution with aniline blue. The spores on the slide were smashed by applying gentle pressure over a cover slip on the preparation.

For scanning electron microscopy (SEM), rust-infected leaves from dried herbarium specimens were cut into ca. 3×3 mm pieces containing a few sori, and each piece was placed on double-adhesive tape on a specimen holder. The preparations were coated with platinum-palladium using a Hitachi E-1030 Ion Sputter and examined with a Hitachi S-4200 SEM at 15kV.

Results and Discussion

Life cycle Basidiospores were abundantly formed under the conditions described above; however, the time lag before initiation of basidiospore production varied from a few hours to a few days depending on the conditions of teliospores used for inoculation experiments and the year of collection. The basidiospores of the fungus on *C. pisiformis* subsp. *alterniflora* infected *D. smilacinum,* resulting in spermogonium production 7–10 d after the inoculation and aecium production 5–7 d later. No sign of infection was detected on *C. cordatum, A. scaber* and *A. ageratoides* subsp. *ovatus*. Similarly, basidiospores of the fungus on *C. conica* were successfully inoculated only on *D. smilacinum*, with spermogonium and aecium production. No sign of infection was observed on *D. sessile* and *C. cordatum*. Basidiospores of the fungus on *C. rugata* also infected only *D. smilacinum*, resulting in spermogonium and aecium production. *Cardiocrinum cordatum, M. dilatatum, T. macropoda, A. ageratoides* subsp. *ovatus* and *C. mollis* were not infected.

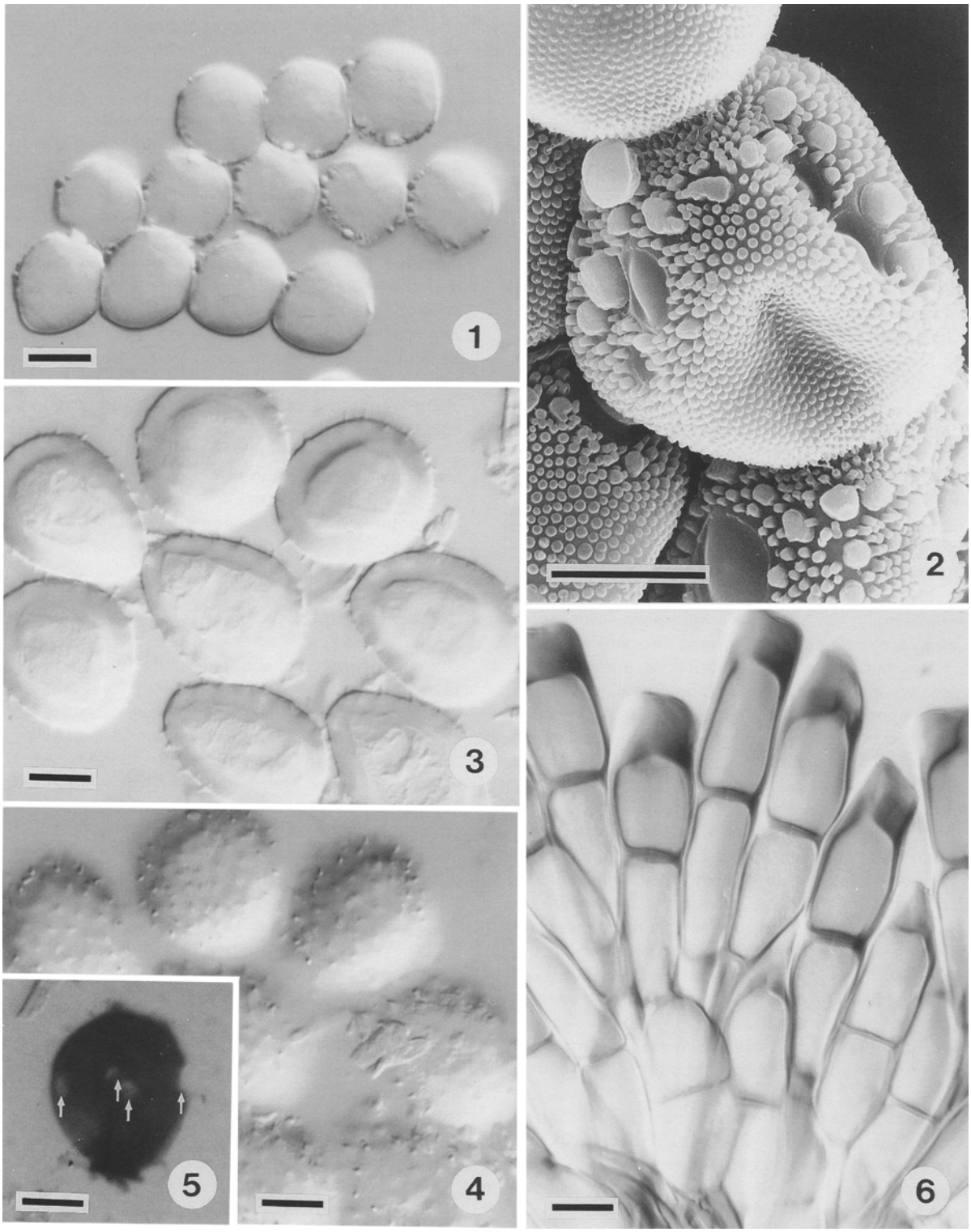
On the other hand, basidiospores of the fungus on *C. dolichostachya* subsp. *multifolia* from Takefu were successfully inoculated both on *D. smilacinum* and on *D. sessile* resulting in spermogonium and aecium production. No infection took place on *C. cordatum* and *P. lasianthum*. Basidiospores of the fungus on *C. dolichostachya* subsp. *multifolia* from Mt. Gozenyama infected only *D. sessile,* resulting in spermogonium and aecium production. No sign of infection was detected on *D. smilacinum*.

Aeciospores formed by the basidiospore inoculation of the fungus on *C. dolichostachya* subsp. *multifolia* from Takefu were successfully inoculated only on the same host species, resulting in uredinium production 10–14 d after the inoculation. No infection occurred on *C. conica* and *C. pisiformis* subsp. *alterniflora*. Similarly, aeciospores formed by the basidiospore inoculation of the fungus on *C. conica* successfully infected only the same host species, resulting in uredinium production. Naturally formed aeciospores on *D. smilacinum* collected at Okami was also successfully inoculated on *C. rugata*, resulting in uredinium and telium production.

The successful basidiospore inoculations proved the life-cycle connection between the *Aecidium* fungus on *D. smilacinum* and the *Puccinia* fungus on *C. conica, C. dolichostachya* subsp. *multifolia, C. pisiformis* subsp. *alterniflora* and *C. rugata* and between the *Aecidium* fungus on *D. sessile* and the *Puccinia* fungus on *C. dolichostachya* subsp. *multifolia*.

Both basidiospore and aeciospore inoculations indicated some degree of host specificity among the fungal populations studied. Because the inoculation experiment was not comprehensive, however, the degree of host specificity of both the spermogonial-aecial hosts and the uredinial-telial hosts and its taxonomic implications were not determined. Nevertheless, the fact that the fungus on *C. dolichostachya* subsp. *multifolia* from Takefu infected both *D. smilacinum* and *D. sessile* indicates that the *Aecidium* on both *D. smilacinum* and *D. sessile* is an aecial anamorph of the *Puccinia* on the four *Carex* species.

Morphology No significant difference was observed in aecia and aeciospores formed, either naturally or by the basidiospore inoculation, on *D. smilacinum* and *D. sessile*. Spermogonia occurred on the adaxial leaf surface or on petiole and were subepidermal, subglobose or ovoid, 110–150 μm high and 90–140 μm wide. Aecia were formed mostly on abaxial leaf surfaces or on petioles and were subepidermal, cupulate with a well-developed peridium reflexing upon maturity. Aeciospores were catenulate, subglobose or broadly ellipsoid, often angular, 16–21×15–18 μm (Fig. 1). The aeciospore wall was ca. 1 μm thick, colorless and verrucose with refractive granules at the upper side (type 5 of Savile



Figs. 1–6. *Puccinia albispora*. 1. Aeciospores (IBA-8521). Bar=10 μm. 2. Aeciospores surface structure, SEM (IBA-8521). Bar=5 μm. 3. Urediniospores focused on a median plane (Holotype, IBA-8492). Bar=10 μm. 4. Urediniospores focused on a tangential plane (Holotype, IBA-8492). Bar=10 μm. 5. Urediniospore germ pores (arrows) (Holotype, IBA-8492). Bar=10 μm. 6. Teliospores (Holotype, IBA-8492). Bar=10 μm.

1973; Fig. 2).

The morphology of the *Aecidium* fungus on the *Disporum* plants agrees with that of *A. dispori* Dietel originally described for a fungus on *D. sessile* from Nagano

Pref. (Dietel, 1899). Although Dietel (1899) did not mention refractive granules on the wall, other similar morphological characteristics and the host relationship are sufficient to conclude the taxonomic identity of the *Aecidium* fungus with *A. dispori*.

Uredinial and telial characteristics of the fungi on the four *Carex* species were also identical. Uredinia occurred on the abaxial leaf surface and were small, subepidermal, long-covered by the epidermis, rupturing at maturity and pale brownish or whitish. Urediniospores were subglobose, broadly obovoid or ellipsoid and 21–34 × 20–31 μm (Fig. 3). The urediniospore wall was evenly (2.5–) 3.0–5.0(–6.0) μm thick, pale yellowish or almost colorless and completely echinulate (Fig. 4). Four or five germ pores were distributed in the equatorial zone (Fig. 5). Telia occurred also on abaxial leaf surface and were subepidermal, soon rupturing at maturity, pulvinate and black. Teliospores were clavate or oblong-ellipsoid, rounded, truncate or conical at the apex, not to weakly constricted at the septum and 32–55 × 12–23 μm (Fig. 6). The teliospore wall was brown, smooth and 7–15 μm thick at the apex. The pedicel was persistent and 25–50 μm long.

Uredinial characteristics of the *Disporum-Carex* alternating fungus under discussion are similar to *P. breviculmis* Dietel (Dietel, 1907), *Uredo breviculmis* Hennings (Hennings, 1901), *P. iriensis* Y. Morimoto (Morimoto, 1962) and *P. caricis-conicae* Homma (Ito and Homma, 1938). The number and distribution of urediniospore germ pores were not mentioned in the original description of the three species (Morimoto, 1962; Hennings, 1901; Ito and Homma, 1938). Therefore, holo- and/or isotype specimens of *U. breviculmis* (Herb. SAPA), *P. iriensis* (Herb. TSH) and *P. caricis-conicae* (Herb. S & SAPA) were examined for the comparison.

Although the urediniospores of *U. breviculmis* were described as subglobose, ovoid or ellipsoid and 22–32 × 20–25 μm, with a thick, colorless wall (Hennings, 1901), our measurement of the spores was much larger (25–36 × 21–34 μm) than that of the original description and significantly different from that of the fungus under discussion. The wall was colorless and (2.5–)3.0–6.0 μm thick. Four or five (infrequently 3) germ pores were distributed on an equatorial zone. In the original description, Dietel (1907) cited *U. breviculmis* as the uredinial state of *P. breviculmis* without mentioning the uredinial morphology.

In contrast, the urediniospore size of *P. iriensis* (24.2–29.6 × 20.1–25.5 μm; 20–34 × 17–19 μm in the original description, Morimoto 1962) was similar to those of *P. breviculmis* and the fungus under discussion, but the wall thickness was thinner [2.5–5 μm; 2–3.5(–5) μm in the original description]. The germ pore seemed not to be differentiated in *P. iriensis*.

In addition to the difference in the urediniospore morphology, *P. iriensis* was proven to host-alternate on *Smilax china* L. (Morimoto, 1962). No cross-inoculation was undertaken between *P. iriensis* and the fungus under discussion. However, a distant taxonomic relationship between *Smilax* (Smilacaceae in Dioscoreales) and *Disporum* (Uvulariaceae in Liliales) (Dahlgren et al., 1985) suggests that the two fungi do not share both plant species as common spermogonial-aecial hosts and, thus, the two fungi are presumed to be reproductively isolated.

According to Ito and Homma (1938), *P. caricis-conicae* forms globose or ovate urediniospores of 31–39 × 23–37 μm with thick (4–5 μm), colorless and echinulate urediniospores and clavate teliospores of 52–84 × 17–28 μm (Ito and Homma, 1938). The number and distribution of urediniospore germ pores was stated to be obscure in the original description. Examination of the holotype (Herb. S) and isotype (Herb. SAPA) revealed that the urediniospores were subglobose, broadly obovoid or broadly ellipsoid and 38–45 × 31–39 μm in size. The wall was 3.4–4.8(–5.7) μm thick, colorless and echinulate. Five or six germ pores were distributed in the equatorial zone. Thus, the fungus under discussion is apparently different from *P. caricis-conicae* in the uredinial morphology.

Because the fungus under discussion possesses morphological features and life cycle distinct from those of any previously described species, the fungus is concluded to be a new species and the following name is proposed:

Puccinia albispora Ono & Kakishima, sp. nov. Figs. 1–6
Aecial anamorph: *Aecidium dispori* Dietel, Bot. Jahrb. **27**: 571. 1899.
Uredinia hypophylla, subepidermalia, diu epidermide tecta, pulverulenta, pallide brunnea vel alba. Urediniosporae subglobosae, late obovoideae vel late ellipsoideae, 21–34 × 20–31 μm, episporio aequaliter (2.5–) 3.0–5.0(–6.0) μm crasso pallide flavido vel hyalino echinulato, poris germinationis 4–5 aequalitorialibus. Telia subepidermalia, mox nuda, pulvinata, atra. Teliosporae clavatae, ad apicem roundatae, truncatae vel conicae, basin versus attenuatae, non vel leniter constrictae ad septum, 32–55 × 12–23 μm, episporio brunneo laevi ad apicem 7–15 μm crasso, pedicello persistenti 25–50 μm longo.
HOLOTYPE: On *Carex rugata* Ohwi, JAPAN: Ibaraki, Mito (obtained from aeciospore inoculation), 22 Nov. 1999, *Y. Ono 4533* (IBA-8492).
Spermogonia epiphyllous or petiolicolous, subepidermal, subglobose or ovoid, 110–150 μm high and 90–140 μm wide. Aecia mostly hypophyllous or petiolicolous, subepidermal, cupulate with a well-developed peridium reflexing upon maturity. Aeciospores catenulate, subglobose or broadly ellipsoid, often angular, 16–25 × 15–21 μm in size. The aeciospore wall ca. 1 μm thick, colorless and verrucose with refractive granules at the upper side. Uredinia hypophyllous, small, subepidermal, long-covered by the epidermis, rupturing at maturity and pale brownish or whitish. Urediniospores subglobose, broadly obovoid or ellipsoid, 21–34 × 20–31 μm in size. The wall evenly (2.5–)3.0–5.0(–6.0) μm thick, pale yellowish or colorless and completely echinulate. Germ pores 4–5, equatorial. Telia hypophyllous, subepidermal, soon rupturing at maturity, pulvinate and black. Teliospores clavate or oblong-ellipsoid, rounded, truncate or conical

at the apex, attenuate toward the base, not to weakly constricted at the septum and 32–55 × 12–23 μm in size. The wall brown, smooth and 7–15 μm thick at the apex. The pedicel persistent and 25–50 μm long.

Host and distribution: On *Disporum sessile, D. smilacinum, Carex conica, C. dolichostachya* subsp. *multifolia, Carex pisiformis* subsp. *alterniflora* and *C. rugata*—widely distributed in Japan and perhaps also in China and Taiwan

Specimens examined: On *Disporum sessile*, **JAPAN: Gumma**, Tone-gun, Niiharu-mura, Mikuni-toge, 18 Jul. 1973, *Y. Ono* (*Y. O.*) *67* (IBA-1608); **Ibaraki**, Kuji-gun, Suifu-mura, Takefu, 16 Aug. 1978, *Makoto Kakishima* (*M. K.*) (TSH-R1733); Mito, Watari, 10 May 1980, *Y. O. 363* (IBA-1899); Tsukuba (obtained from basidiospore inoculation), 14 May 1979, *M. K.* (TSH-R1723); 7 May 1979, *M. K.* (TSH-R1724); 23 May 1979, *M. K.* (TSH-R1726); 14 May 1980, *M. K.* (TSH-R1727); 28 May 1980, *M. K.* (TSH-R1729); 1 Jun. 1979, *M. K.* (TSH-R1730). On *D. smilacinum*, **JAPAN: Hokkaido**, Nemuro, Mt. Rausudake, 6 Jul. 1998, *Y. O. 4181* (IBA-8124); **Ibaraki**, Higashiibaraki-gun, Katsura-mura, Mt. Gozenyama, 10 Jul. 1980. *Y. O. 423* (IBA-1960); 3 Jul. 1999, *Y. O. & Kaori Ishimiya* (*K. I.*) *4366* (IBA-8316); Kuji-gun, Daigo-machi, Mt. Nantaisan, 14 Jun. 1999, *Y. O. & K. I. 4353* (IBA-8306); 9 May 1998, *Y. O. & K. I. 4110* (IBA-8053); 27 May 1990, *Y. O. 2085* (IBA-4777); 27 May 1990, *Y. O. 2088* (IBA-4780); 31 May 1994, *Y. O. 3001* (IBA-7168); 27 May 1990, *Y. O. 2096* (IBA-4788); 31 May 1989, *Y. O. 1695* (IBA-3242); 31 May 1989, *Y. O. 1697* (IBA-3244); 31 May 1989, *Y. O. 1702* (IBA-3249); Kuji-gun, Satomi-mura, Nanatan, 8 June 1990, *Y. O. 2112* (IBA-4804); Okami, 14 Jun. 1999, *Y. O. 4349* (IBA-8299); Kuji-gun, Suifu-mura, between Okubo and Kamegafuchi, 9 Jun. 1991, *Y. O. et al. 2367* (IBA-5723); Mito, Watari, 4 Jun. 1980, *Y. O. 380* (IBA-1918); 9 Apr. 1999, *Y. O. & K. I. 4298b* (IBA-8248); Mito (obtained from basidiospore inoculation), 18 Jun. 1999, *Y. O. & K. I. 4357* (IBA-8307); 18 Jun. 1999, *Y. O. & K. I. 4358* (IBA-8308); 2 May 1999, *Y. O. & K. I. 4350* (IBA-8300); 25 May 2000, *Y. O. & K. I. 4572* (IBA-8520); 31 May 2000. *Y. O. & K. I. 4573a* (IBA-8521); 31 May 2000. *Y. O. & K. I. 4573b* (IBA-8522); Mito, Narusawa, Mito Forest Park, 28 Jun. 1980, *Y. O. 407* (IBA-1945); Tsukuba (obtained from basidiospore inoculation), 25 May 1977, *M. K.* (TSH-R1712); 2 June 1977, *M. K.* (TSH-R1713); 17 Jun. 1977, *M. K.* (TSH-R1714); 14 Jun. 1977, *M. K.* (TSH-R1715); 27 May 1979, *M. K.* (TSH-R1717); 31 May 1979, *M. K.* (TSH-R1718); 17 May 1979, *M. K.* (TSH-R1719); 5 Jun. 1979. *M. K.* (TSH-R1721); Tsukuba-gun, Tsukuba-machi, Mt. Tsukubasan, 17 Jun. 1978, *M. K.* (TSH-R1732); **Nagano**, Minamisaku-gun, Minamimaki-mura, Nobeyama, 25 Jul. 1978, *M. K.* (TSH-R1731); **Tochigi**, Nikko, between Kotoku and Karenuma, alt. 1650 m, 18 Jun. 1990. *Y. O. 2126* (IBA-4818). On *Carex conica*, **Ibaraki**, Tsukuba-gun, Tsukuba-machi, Mt. Tsukubasan, 6 Mar. 1979, *M. K.* (TSH-R1716); 12 Mar. 1980, *M. K.* (TSH-R1720). On *C. dolichostachya* subsp. *multifolia*, **Japan: Ibaraki**, Kuji-gun, Suifu-mura, Takefu, 23 Mar. 1979, *M. K.* (TSH-R1732); Satomi-mura, Tokuda, 2 Mar. 1999, *Y. O. & K. I. 4266* (IBA-8217); Tsukuba (obtained from aeciospore inoculation), 17 Aug. 1979, *M. K.* (TSH-R1728); Kuji-gun, Katsura-mura, Mt. Gozenyama, 14 Mar. 1979, *M. K.* (TSH-R1725). On *C. pisiformis* subsp. *alterniflora*, **JAPAN: Tokyo**, Hachioji, Asakawa, 24 April 1977, *M. K.* (TSH-R1711). On *C. rugata* Ohwi, **JAPAN: Ibaraki**, Kuji-gun, Satomi-mura, Okami, 11 Sep. 1999, *Y. O. & K. I. 4381* (IBA-8331; 15 Feb. 2000, *Y. O. & K. I. 4535* (IBA-8538); 15 Feb. 2000, *Y. O. & K. I. 4536* (IBA-8539); 2 Mar. 1999, *Y. O. & K. I. 4270* (IBA-8221); 2 March 1999, *Y. O. & K. I. 4271* (IBA-8222); 9 Aug. 1999, *Y. O. 4369* (IBA-8319); Mito (obtained from aeciospore inoculation), 22 Nov. 1999, *Y. O. 4533* (IBA-8492); 30 Nov. 1999, *Y. O. 4534* (IBA-8493).

Additional specimens examined: *Puccinia breviculmis* Dietel on *Carex breviculmis* R. Br., **JAPAN: Kochi**, Sakawa, Jun. 1901, *T. Yoshinaga* (Isotype of *Uredo breviculmis* Hennings in Herb. SAPA). *Puccinia caricis-conicae* Homma on *C. conica*, **JAPAN: Hokkaido**, Makomanai, 16 Oct. 1910, *M. Miura* (Holotype in Herb. S and Isotype in Herb. SAPA). *Puccinia iriensis* Y. Morimoto on *C. maximowzii* Miq., **JAPAN: Hiroshima**, Kaiji, 23 Sept. 1960, *Y. Morimoto* (Isotype in Herb. TSH).

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