

Heteroecious life cycle of two graminicolous *Puccinia* (Uredinales)

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Puccinia erythropus, whose uredinial-telial stage occurs on *Miscanthus* and *Eularia* spp. (Gramineae), was found to have a heteroecious macrocyclic life cycle with the spermogonial-aecial stage on *Cynanchum sublanceolatum* var. *obtusum* (Asclepiadaceae). *Puccinia miyoshiana*, which forms the uredinial-telial stage on *Bothriochloa*, *Capillipedium*, *Eccoilopus*, and *Spodiopogon* (Gramineae), is known to form its spermogonial-aecial stage on *Bupleurum* spp. (Umbelliferae). By field observations and artificial inoculations, *Bupleurum komarovianum* was proved to serve as an additional spermogonial-aecial host of this fungus.

Key Words—Asclepiadaceae; Gramineae; *Puccinia*; Umbelliferae; Uredinales.

Puccinia erythropus Dietel, Bot. Jahrb. 37: 101. 1905.

Taxonomic identity of a fungus with *Puccinia erythropus*

In 1992–1995, heavy infections of *Miscanthus sinensis* Anderss. with a *Puccinia* fungus were noticed in every autumn around Mt. Nantaisan at Daigo-machi, Kuji-gun, Ibaraki Pref. The fungus was uredinial-telial in the life cycle stage. Both uredinia and telia were formed on abaxial surface of the leaves. Individual sori were minute but often coalesced to become elongated along the parallel veins of the leaves. The infection often resulted in reddish brown or purplish brown flecks on the leaves. However, sometimes no discoloration was caused on the leaves; thus the occurrence of the fungus was not appreciable on the adaxial surface in spite of dense production of rust sori that appeared dark brown or almost black on the abaxial surface.

The teliospores were two-celled, mostly broadly ellipsoid, obovoid-ellipsoid or oblong-ellipsoid, rounded at both ends, moderately constricted at the septum, and 28–43 × 16–24 μm in size (Fig. 1). The wall was chestnut-brown, smooth, and 1.5–2.5 μm thick at sides and up to 8 μm thick at the apex. One germ pore was located in each cell: apical in the upper and septal in the lower cell. The pedicel was 80–140 μm long and persistent. The urediniospores were broadly ellipsoid or obovoid-ellipsoid and 24–35 × 18–23 μm in size (Fig. 2). The wall was cinnamon-brown, 2–3 μm thick at sides and up to 7 μm at the apex (Fig. 2), and uniformly echinulate (Fig. 3). Three or four germ pores were distributed in the equatorial zone of the spores.

Five species of *Puccinia* have been recognized on *Miscanthus* spp. in the world: *Puccinia daisenensis* Hiratsuka, f., *P. erythropus* Dietel, *P. miscanthi* Miura, *P. miscanthidii* Doidge, and *P. miscanthicola* Tai & Cheo (Cum-

mins, 1971). The former three species have been recorded in Japan, where *P. daisenensis* is known to form its spermogonial-aecial stage on *Veronicastrum sibiricum* (L.) Pennel var. *japonicum* (Nakai) Hara (Scrophulariaceae) (Kaneko and Hiratsuka, 1983) and *P. miscanthi* is known to form its spermogonial-aecial stage on *Lysimachia clethroides* Duby (Primulaceae) (Sato and Kakishima, 1982), *Plantago asiatica* L. (Plantaginaceae) (Hiratsuka, 1933), and *Plantago camtschatica* Cham. (Hiratsuka and Sato, 1984).

Comparison of the observed characteristics of the fungus under discussion with the described morphological characteristics of the above five species (Cummins, 1971; Doidge, 1939; Hiratsuka, 1932; Miura, 1928) led to the conclusion that the fungus was taxonomically identical with *P. erythropus*. Except for the presence of multicellular teliospores, the fungus was also similar to *P. miscanthicola*, for which only telial characteristics were described in the original report (Tai and Cheo, 1937) and urediniospore characteristics were reported later (Wang, 1983). Cummins (1953, 1971) suggested that *P. miscanthicola* might be a morphological variant of and thus synonymous with *P. erythropus*. However, Wang (1983) argued the taxonomic distinctness of the former rust because of the presence of 3- or 4-celled teliospores in the type specimen, while no multicelled teliospores were found on *P. erythropus* specimens collected in China. No multicellular teliospores were observed in the specimens examined in this study.

Ito (1950) mentioned that *P. erythropus* formed abundant paraphyses, that were capitate, apically thick-walled, and 40 × 16 μm in size, in the uredinia, although no uredinial paraphysis was mentioned in the original description (Dietel, 1905). No uredinial paraphyses were found in the specimens cited in this study. Hira-

tsuka and Kaneko (1983) stated that two forms, i.e., one with and another without uredinial paraphyses, were present among the specimens they examined, which were otherwise morphologically identifiable as *P. erythropus*. Consistency or flexibility of paraphysis formation in the uredinium of the fungal populations that are identifiable as *P. erythropus* must be carefully studied in relation to their taxonomic identity.

Heteroecious life cycle of *Puccinia erythropus* In the places where *P. erythropus* was found in autumn, several spermogonial-aecial fungi were found, in the previous or following spring, on *Berchemia racemosa* Sieb. & Zucc., *Cimicifuga acerina* (Sieb. & Zucc.) C. Tanaka, *Clematis terniflora* DC., *Cynanchum sublancoelatum* (Miq.) Matsum. var. *obtusum* (Fr. & Sav.) Matsum., *Deutzia crenata* Sieb. & Zucc., *Disporum sessile* Don., *Hamamelis japonica* Sieb. & Zucc., *Hydrangea hirta* Sieb. & Zucc., *Pertya scandens* (Thunb.) Sch.-Bip., *Ligustrum obtusifolium* Sieb. & Zucc., and *Plantago asiatica*. Among these spermogonial-aecial fungi, only those on *C. sublancoelatum* var. *obtusum* and *L. obtusifolium* had not been connected to any teleomorphic fungi. One on *L. obtusifolium* was named as *Aecidium klugkistianum* Dietel and no rust fungus had been known on *C. sublancoelatum* var. *obtusum*.

Close association of the unconnected spermogonial-aecial fungi with *P. erythropus* infecting *M. sinensis* indicated that either one on the *Cynanchum* plant or another on the *Ligustrum* plant was a spermogonial-aecial stage of *P. erythropus*. Consequently, artificial inoculations were carried out to confirm this possible connection of the *Miscanthus* fungus to either one of two *Aecidium* fungi.

In April of 1995, telium-bearing *Miscanthus* leaves that had overwintered in the field were collected at Saigane, Daigo-machi, Ibaraki Pref. The leaves were preserved in a plastic bag at room temperature. The telium-bearing leaves were soaked in running tap water for 7–14 d to induce germination, then cut into small pieces (ca. 2 × 5 mm), placed on water-saturated filter paper in a Petri dish and incubated in the dark at ca. 18°C. The teliospores germinated well within 2 d to form metabasidia and basidiospores. The basidiospore-bearing pieces of leaves were placed on adaxial surfaces of apparently healthy leaves of the *Cynanchum* and *Ligustrum* plants, which had been planted in loam soil in clay pots (18 cm in diam). *Clematis terniflora* was also inoculated, because this plant is thought to serve as a spermogonial-aecial host for a variety of graminicolous *Puccinia* species. The inoculated plants were sprayed with distilled water and placed in a moist chamber at room temperature (18–22°C) for 48 h. The plants were subsequently transferred to a glasshouse for further observations. The inoculation experiments were repeated three times with three plants being inoculated each time. The basidiospore inoculation was repeated in the same manner in April, 1996.

Five to 7 d after the inoculation, pale yellowish flecks appeared on the adaxial surface of the inoculated leaves of *Cynanchum* plants and spermogonium formation fol-

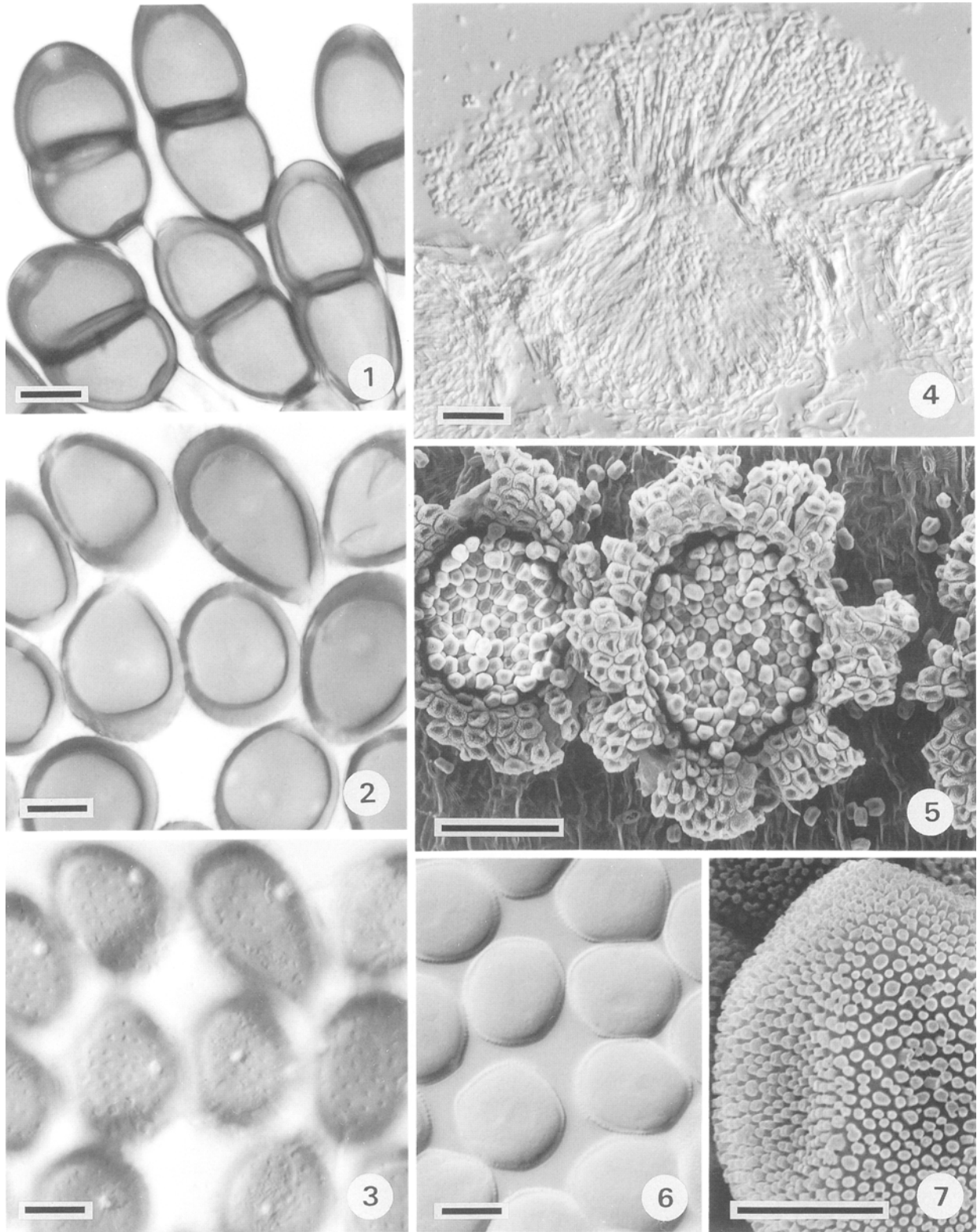
lowed in the subsequent 2 or 3 d. Ten to 12 d after the inoculation, dense aggregates of aecia were formed on the abaxial surface of the inoculated leaves, which had become distorted due to heavy infection. On the other hand, no sign of infection was observed on the inoculated *Ligustrum* and *Clematis* plants. Consequently, *P. erythropus* was proved to form its spermogonial-aecial stage on *C. sublancoelatum* var. *obtusum*.

Taxonomic identity of *Aecidium* on *C. sublancoelatum* var. *obtusum* Infection habits and morphology of spermogonia and aecia observed on the *Cynanchum* plant in the inoculation experiments were not significantly different from those observed in nature. The spermogonia were subepidermal, mostly subglobose, and 80–110 µm high and 85–120 µm across (Fig. 4). The aecia were aecidioid, surrounded by a well-developed peridium with its margin being segmented in 8–10 lobes (Fig. 5). The aeciospores were formed in chains, mostly subglobose or broadly ellipsoid, and 17–25 × 15–22 µm in size (Fig. 6). The wall was evenly ca. 1 µm thick, colorless, and uniformly verrucose (Fig. 7). No germ pore was observed on the wall.

No rust fungus was previously recorded on *C. sublancoelatum* var. *obtusum*. The spermogonial-aecial fungus on the *Cynanchum* plants was compared with unconnected *Aecidium* species on the Asclepiadaceae. *Aecidium musashiense* H. Sydow & Sydow and *A. vincetoxici* Hennings & Shirai are known to be distributed in Japan, the former occurring on an unidentified species of *Tylophora* (Sydow and Sydow, 1917) and the latter on *Cynanchum caudata* (Miq.) Maxim., *C. nipponicum* Matsum. var. *glabrum* (Nakai) Maxim., *C. sublancoelatum* (Miq.) Matsum. var. *macranthum* (Maxim.) Matsum., and *Tylophora japonicum* Miq. (Hennings, 1900; Hiratsuka et al., 1992). *Aecidium musashiense* forms aeciospores whose wall is apically thickened (2–6 µm thick) (Sydow and Sydow, 1917; Hiratsuka et al., 1992) and thus morphologically different from the *Aecidium* fungus under discussion. Contrarily, morphological characteristics of the *Aecidium* on *C. sublancoelatum* var. *obtusum* were not significantly different from those described for *A. vincetoxici*, and the host plant was indicative of the close taxonomic affinity of the *Aecidium* fungus to *A. vincetoxici*: accordingly, the taxonomic identity of the *Aecidium* fungus with *A. vincetoxici* was concluded.

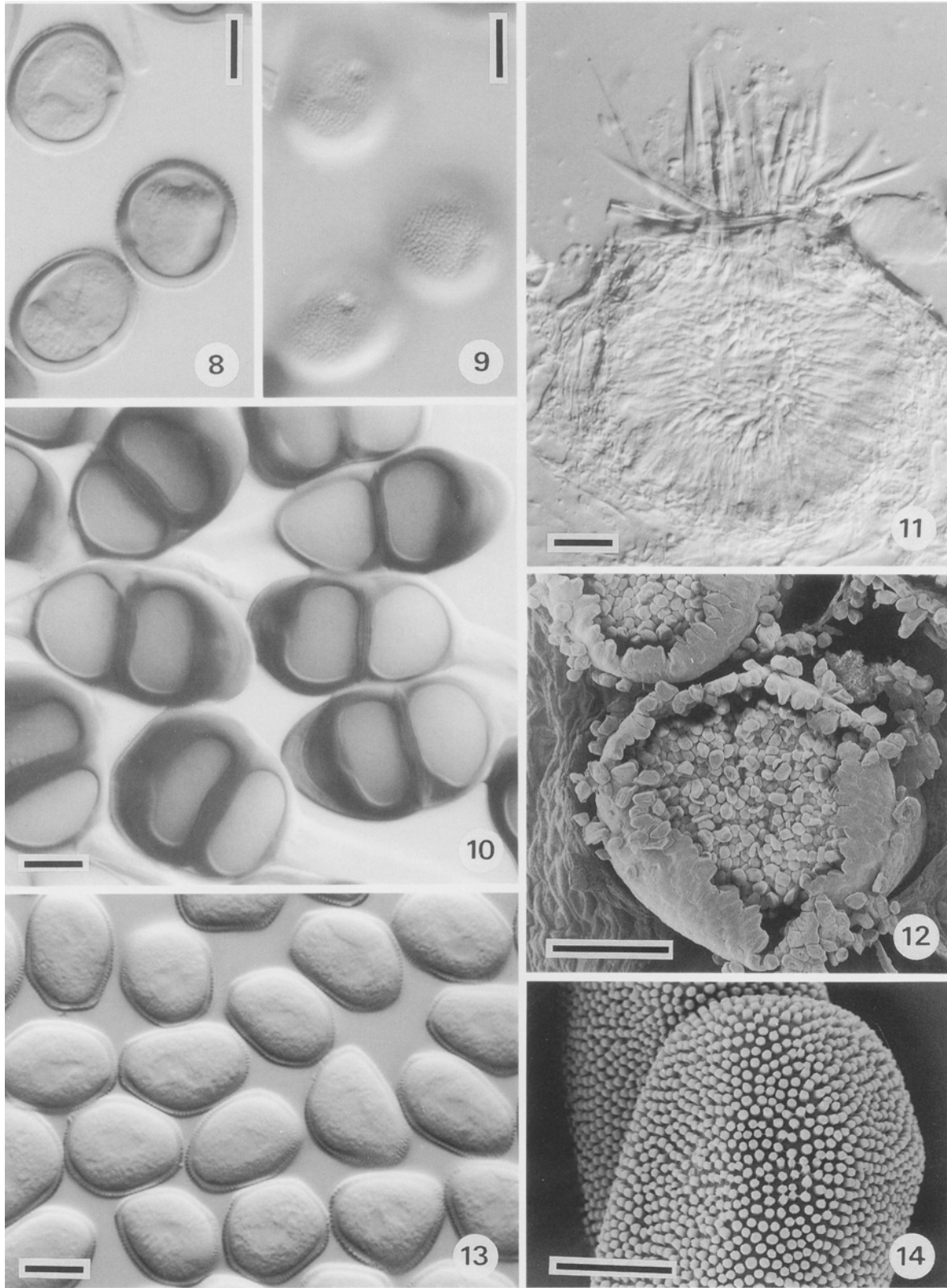
Puccinia erythropus is now concluded to have *A. vincetoxici* as the spermogonial-aecial anamorph. Two other graminicolous *Puccinia*, i.e., *Puccinia chloridis* Spegazzini and *P. seymouriana* Arthur, are known to have their spermogonial-aecial anamorph on the Asclepiadaceae in the Americas (Cummins, 1971). Morphological comparisons of *P. erythropus* with the latter two species suggest no phylogenetic relationship among them.

Specimens examined On *C. sublancoelatum* var. *obtusum*, Saigane, Daigo-machi, Kuji-gun, Ibaraki, 27 May 1990, Y.O. (IBA-4734); 31 May 1994, Y.O. (IBA-7163); 2 June 1995, Y.O. (IBA-7586); Result of basidiospore inoculation, 12 May 1995, Y.O. (IBA-7337); 20 May 1995, Y. Ono (IBA-7339); on *M. sinensis*, Saigane,



Figs. 1–7. *Puccinia erythropus*.

1. Teliospores (IBA-7615). 2. Urediniospores focused in a median plane (IBA-7615). The wall is thickened apically. Mostly 4 germ pores are distributed in an equatorial zone. 3. Urediniospores, same as Fig. 2 but focused in a tangential plane. Echinulae are uniformly distributed over the wall. 4. Spermogonium (IBA-7339). 5. Aecium surrounded by the well-developed peridium with several-lobes at the margin (IBA-4734). 6. Aeciospores (IBA-7339). 7. Surface view of an aeciospore (IBA-4734). Scales: 10 μm in Figs. 1–3, 6; 20 μm in Fig. 4; 100 μm in Fig. 5; 5 μm in Fig. 7.



Figs. 8–14. *Puccinia miyoshiana*.

8. Urediniospores focused in a median plane (IBA-7554). 9. Urediniospores, same as Fig. 8 but focused in a tangential plane. The wall is minutely verrucose-echinulate. 10. Teliospores (IBA-7554). 11. Spermogonium (IBA-7370). 12. Aecia surrounded by a well-developed peridium (IBA-7370). 13. Aeciospores (IBA-7370). 14. Surface view of an aeciospore (IBA-7370). Scales: 10 μm in Figs. 8–10, 13; 20 μm in Fig. 11; 100 μm in Fig. 12; 5 μm in Fig. 14.

Daigo-machi, Kuji-gun, Ibaraki, 11 April 1995, Y.O. (IBA-7331); 31 Sept. 1995, Y.O. (IBA-7615); 15 Sept. 1996, Y.O. (IBA-7761).

Puccinia miyoshiana Dietel, Bot. Jahrb. 27: 569. 1899.

Taxonomic identity of the *Puccinia* fungus Telial-ureidial stage of *P. miyoshiana* occurs on *Bothriochloa parviflora* Ohwi, *Capillipedium parviflorum* (R. Br.) Stapf., *Eccoilopus cotulifer* (Thunb.) A. Camus, *Spodiopogon sibiricus* Trin. var. *sibiricus*, and *S. sibiricus* var. *tomentosus* Koidz. in East and Northeast Asia (Dietel, 1899; Cummins, 1971; Hiratsuka et al., 1992; Wang, 1983).

In August of 1995, plants of *S. sibiricus* were found to be heavily infected by a *Puccinia* fungus with abundant telia formed on the leaves at Blagodatonoe in Sikhote-Alin Reserve, Primorsky Territory, Russia. Urediniospores of the fungus were intermixed in the telia, subglobose, broadly ellipsoid or oblong-ellipsoid, and $23\text{--}29 \times 18\text{--}23 \mu\text{m}$ in size (Fig. 8). The wall was uniformly $2.5\text{--}3.5 \mu\text{m}$ thick, golden brown to yellowish brown, and minutely verrucose-echinulate (Fig. 9). Three (sometimes 2 or 4) germ pores were distributed in the equatorial zone of the spores. Teliospores were obovoid, obovoid-ellipsoid or oblong-ellipsoid, rounded at the apex, rounded or attenuate at the base, not or slightly constricted at the septum, and $34\text{--}50 \times 18\text{--}29 \mu\text{m}$ in size (Fig. 10). The wall was chestnut-brown, smooth, $2\text{--}4 \mu\text{m}$ thick at sides and $8\text{--}13 \mu\text{m}$ thick apically. These morphological characteristics showed the taxonomic identity of the fungus with *P. miyoshiana*.

Anamorph of *P. miyoshiana* Tranzschel (1938) proved for the first time by inoculation experiments that the spermogonial-aecial stage of the fungus occurred on *Bupleurum falcatum* L. (= *B. scorzoneraefolium* Willd.) and *B. longiradiatum* Turcz. in Russia. *Aecidium bupleuri-sachalinensis* Miyake described on *B. longiradiatum* var. *breviradiatum* Fr. Schm. (= *B. sachalinense* Fr. Schm.) in Hokkaido, Japan, was said to be the spermogonial-aecial anamorph (Cummins, 1971; Hiratsuka et al., 1992), although Ito (1950) and Ito and Murayama (1950) only suggested this possible life cycle connection, for which there has been no direct proof. The spermogonial-aecial fungus on various *Bupleurum* species in East Asia has been reported as the anamorph of *P. miyoshiana*: on *B. longiradiatum* var. *breviradiatum* in Japan, on *Bupleurum chinense* DC., *B. longicaule* Wall., and an unidentified *Bupleurum* species in China (Wang, 1983). Azbukina (1984) indicated the occurrence of the spermogonial-aecial stage of the fungus on *Bupleurum* spp. in Russian Far East but did not specify the species and locality. Again, there has been no experimental proof for the life cycle connection between the spermogonial-aecial anamorph on these *Bupleurum* species and *P. miyoshiana*.

In June, 1995, *Aecidium*-infected plants of *Bupleurum komarovianum* Lincz. were found at Blagodatonoe in Sikhote-Alin Reserve, Primorsky Territory, Russia. The same locality was revisited in August of the same year, when the *Bupleurum* plants were found to bear neither

uredinia nor telia. Instead, *Spodiopogon sibiricus* plants growing around the *Bupleurum* plants were heavily infected with *P. miyoshiana* as described above.

It was then reasonable to conjecture a life cycle connection between *P. miyoshiana* on *S. sibiricus* and the *Aecidium* fungus on *B. komarovianum*. In order to prove this possible connection, inoculation experiments were conducted in spring of 1996. The *Spodiopogon* plants that bore abundant telia on the leaves were collected at Blagodatonoe in August, 1995, and preserved in a refrigerator at ca. 5°C until March and April, 1996. The basidiospore inoculations onto *B. komarovianum* were conducted in the same manner as described above for *P. erythropus*. The inoculation experiments were repeated twice with three plants being inoculated each time.

Nine to 10 d after the inoculation, spermogonia appeared on the adaxial surface of the inoculated leaves and aecium formation followed in subsequent 3 or 4 d. Infection habits and morphology of spermogonia and aecia observed in the inoculation experiments were similar to those observed in nature. The spermogonia were subepidermal, mostly subglobose, and $105\text{--}126 \mu\text{m}$ high and $100\text{--}129 \mu\text{m}$ across (Fig. 11). The aecia were aecidioid, surrounded by a well-developed peridium (Fig. 12). The aeciospores were formed in chain, mostly subglobose or broadly ellipsoid, often angular, and $19\text{--}28 \times 12\text{--}18 \mu\text{m}$ in size (Fig. 13). The wall was evenly ca. $1 \mu\text{m}$ thick, colorless, and uniformly verrucose (Fig. 14). No germ pore was observed on the wall.

Bupleurum komarovianum is now proved to be an additional spermogonial-aecial host of *P. miyoshiana*. The above-described morphology of the *Aecidium* fungus is, however, different from that described for *A. bupleuri-sachalinensis*: according to Miyake (1906), the aeciospores are "globose or subglobose, thicker walled, verrucose, darker colored, and $22\text{--}25 \mu\text{m}$ in diam." Because the type specimen was not available for study, we were unable to determine the taxonomic identity of the spermogonial-aecial fungus reported herein with *A. bupleuri-sachalinensis*.

Specimens examined On *B. komarovianum*, Blagodatonoe, Sikhote-Alin Reserve, Primorsky Territory, Russia, 22 June 1995, Y.O. (IBA-7370); Result of basidiospore inoculation, 7 May 1996, Y.O. (IBA-7753); on *S. sibiricus*, Blagodatonoe, Sikhote-Alin Reserve, Primorsky Territory, Russia, 22 June 1995, Y.O. (IBA-7367); 29 Aug. 1995, Y.O., S. Kaneko and Z. M. Azbukina (IBA-7554).

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