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Life cycle and morphology of *Melampsora yezoensis* on *Salix serissaefolia**

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Uredinial and telial states of a *Melampsora* species occurring on the leaves of *Salix serissaefolia* were for the first time recorded. Field observations and inoculation experiments showed that the spermogonial and aecial states of the fungus were formed on *Chelidonium majus* var. *asiaticum* and *Corydalis incisa*. The fungus was identified as *Melampsora yezoensis* based on the morphological observation of all the spore states. Urediniospores were able to infect *S. eriocarpa* and *S. pierotii* as well as *S. serissaefolia* and *S. jessoensis*. *Salix serissaefolia*, *S. eriocarpa*, and *S. pierotii* are new uredinial and telial hosts of *M. yezoensis*, and *C. majus* var. *asiaticum* and *C. incisa* are new spermogonial and aecial hosts of the fungus.

Key Words——life cycle; *Melampsora yezoensis*; rust fungus; *Salix serissaefolia*; Uredinales.

In August 1989, we found abundant uredinia of a rust fungus on leaves of *Salix serissaefolia* Kimura on the exposed riverbed and riverbank of the Azusa River, Azusakawa-mura, Minamiazumi-gun, Nagano Prefecture. The fungus was suspected to be a species of *Melampsora*, based on the morphological characteristics of the uredinia and urediniospores. Hiratsuka and Kaneko (1982) and Hiratsuka et al. (1992) reported 12 species of *Melampsora* parasitic on *Salix* species in Japan and listed 23 *Salix* species as their host plants. However, *S. serissaefolia* was not included in this list. Field observation at the Azusa River in the spring of 1990 showed that spermogonia and aecia occurred on leaves of *Chelidonium majus* L. var. *asiaticum* (Hara) Ohwi near trees of *S. serissaefolia*. Furthermore, spermogonia and aecia occurring on leaves of *Corydalis incisa* (Thunb.) Pers. as well as on leaves of *C. majus* var. *asiaticum* were collected at the Azusa River in the spring of 1993. We therefore suspected host alternation of the fungus between *S. serissaefolia* and *C. majus* var. *asiaticum* and/or *C. incisa*.

The purpose of the present study was to clarify the life cycle of the rust fungus found on *S. serissaefolia* and to identify the fungus.

Materials and Methods

Basidiospore inoculation Leaves of *S. serissaefolia* with abundant telia of *Melampsora* sp. were collected on the exposed riverbed and riverbank of the Azusa River, Azusakawa-mura, Minamiazumi-gun, Nagano Pref. on four occasions, in March and November 1990, April 1992, and March 1993. Samples were kept in a refrigerator at ca. 5°C until use, except that those collected in November 1990 were put in a gauze bag and left outside for about 1.5 mo before transfer to the refrigerator.

The leaves were immersed in running tap water (ca. 20°C) for several days to induce germination of teliospores, then placed on water-soaked filter paper in Petri dishes and incubated at 20°C in the dark. The teliospores germinated and produced abundant basidiospores after 24–72 h. Small pieces of the leaves with germinating teliospores were placed on young, healthy leaves of *C. majus* var. *asiaticum*, *Corydalis ambigua* Cham. et Schlecht., *C. incisa*, *Larix leptolepis* (Sieb. et Zucc.) Gordon, *Pinus densiflora* Sieb. et Zucc., *P. thunbergii* Parl., and *Tsuga sieboldii* Carr. The inoculated plants were sprayed with distilled water and placed in a dark, moist chamber at ca. 20°C for 2 d, then transferred to a growth cabinet controlled at approximately 20°C and with a 16 h L: 8 h D photoperiod. The inoculated plants were observed for 3 wk after inoculation. The inoculation experiments with basidiospores were conducted in total 17 times from 1990 to 1993. *Chelidonium majus* var. *asiaticum* was used in all the experiments.

Aeciospore inoculation Aeciospores formed on *C. majus* var. *asiaticum* and *C. incisa* by the basidiospore inoculations in 1990 and 1993 were used as inocula. Aeciospores formed on the plant were dusted with a scalpel onto wet filter papers (ca. 3×3 mm), which were then placed on the lower surface of young, healthy leaves of *S. serissaefolia* planted in clay pots. The inoculated

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plants were placed in a dark, moist chamber at ca. 20°C for 2 d, then transferred to a growth cabinet controlled at approximately 20°C and with a 16 h L: 8 h D photoperiod.

Aeciospores on *C. majus* var. *asiaticum* collected at the Azusa River in May of 1990, 1991, and 1993 and those on *C. incisa* collected on the same place in May of 1993 were also used as inocula. *Salix serissaefolia* and the other *Salix* species found at the Azusa River and other suspected host plants (Table 1) were inoculated by the same methods as described above.

Urediniospore inoculation Two uredinial cultures obtained from the aeciospore inoculation in 1990 and 1993 were used for the urediniospore inoculation to examine the host range of the fungus. They were maintained on *S. serissaefolia* by periodically inoculating the plants with urediniospores. *Salix* species found at the Azusa River and other suspected host plants (Table 1) were inoculated by the same methods as used in the aeciospore inoculation.

Morphological observation Uredinia and telia of *Melampsora* sp. on *S. serissaefolia*, and spermogonia and aecia on *C. majus* var. *asiaticum* and *C. incisa* collected at the Azusa River and those obtained from the inoculation experiments in this study were used for morphological observation. The samples used were deposited as dry herbarium specimens at the Herbarium (TSH), Institute of Agriculture and Forestry, University of Tsukuba.

The specimens used were as follows: II, III on *S. serissaefolia* collected on the exposed riverbed and riverbank of the Azusa River (TSH-R1504, TSH-R1507, TSH-R1508, TSH-R1509); II, III on *S. serissaefolia* obtained from the inoculation experiments (TSH-R1510,TSH-R1512); 0, I on *C. majus* var. *asiaticum* collected on the exposed riverbed and riverbank of the Azusa River (TSH-R1505, TSH-R1506); 0, I on *C. majus* var. *asiaticum* (TSH-R1511, TSH-R1514) and 0, I on *C. incisa* obtained from the inoculation experiments (TSH-R1513, TSH-R1515).

For morphological comparison, dry herbarium specimens of *M. yezoensis* (0, I on *C. ambigua*, HH-53171; II, III on *S. jessoensis*, HH-53165) were examined. Hand sections of spermogonia, aecia, uredinia and telia were mounted in a drop of lactophenol solution on glass slides for light microscopic observation. Aeciospores and urediniospores were also mounted with a drop of lactophenol solution on glass slides and observed under a light microscope. Thirty spores of each sample were measured by use of an image analyzer (Olympus CIA-102). To observe the germ pores, urediniospores were mixed with a drop of lactophenol-cotton blue solution on a glass slide, heated until the solution began to "smoke," then covered with a coverslip while still warm. The coverslip was pressed hard on to the slide until the spore contents were expelled and the spore walls came into one plane of focus. The squashed spores were observed under a phase-contrast microscope (Kaneko and Hiratsuka, 1982; Jennings et al., 1989).

For scanning electron microscopy, spores from dry herbarium specimens were dusted on double-sided adhesive tape on specimen-holders, and coated with gold by use of an Eiko IB-3 Ion Coater. They were examined with a Hitachi S-430 SEM operating at 20 kV.

Results

Basidiospore inoculation Six to eight days after inoculation with basidiospores, spermogonia, mostly grouped in yellowish green lesions, appeared on the upper surface of the inoculated leaves and/or petioles of *C. majus* var. *asiaticum* (on 16 out of 17 plants inoculated) (Fig. 1) and *C. incisa* (on 4 out of 6 plants inoculated) (Fig. 2). Two to three days later, pale orange aecia occurred on both surfaces of the plants (Figs. 1, 2). Spermogonia also

Table 1. *Salix* species used in the inoculation experiments with aeciospores and urediniospores.

Species	Clone No.	Origin
Salix chaenomeloides	SC-1	Tsukuba Botanical Garden, National Science Museum, Tsukuba, Ibaraki Pref.
S. eriocarpa	HB426	Botanical Garden, Tohoku University, Sendai, Miyagi Pref.
S. gilgiana	SGI-1	Exposed riverbed and riverbank of Azusa River, Nagano Pref.
S. gracilistyla	SGR-1	Exposed riverbed and riverbank of Azusa River, Nagano Pref.
S. integra	SI-2	Exposed riverbed and riverbank of Azusa River, Nagano Pref.
S. integra	SI-3	Minamimaki-mura, Minamisaku-gun, Nagano Pref.
S. jessoensis	SJ-1	The Tokyo University Forest in Hokkaido, Furano, Hokkaido
S. jessoensis	SJ-2	Botanical Garden, Tohoku University, Sendai, Miyagi Pref.
S. matsudana f. *tortuosa*	SMA-2	Exposed riverbed and riverbank of Azusa River, Nagano Pref.
S. pierotii	Green44	Botanical Garden, Tohoku University, Sendai, Miyagi Pref.
S. sachalinensis	SSA-2	Botanical Garden, Tohoku University, Sendai, Miyagi Pref.
S. sachalinensis	SSA-4	Exposed riverbed and riverbank of Azusa River, Nagano Pref.
S. serissaefolia	HB419	Botanical Garden, Tohoku University, Sendai, Miyagi Pref.
S. serissaefolia	SSE-1	Botanical Garden, Tohoku University, Sendai, Miyagi Pref.
S. serissaefolia	SSE-2	Exposed riverbed and riverbank of Azusa River, Nagano Pref.
S. subfragilis	SSU-2	Exposed riverbed and riverbank of Azusa River, Nagano Pref.

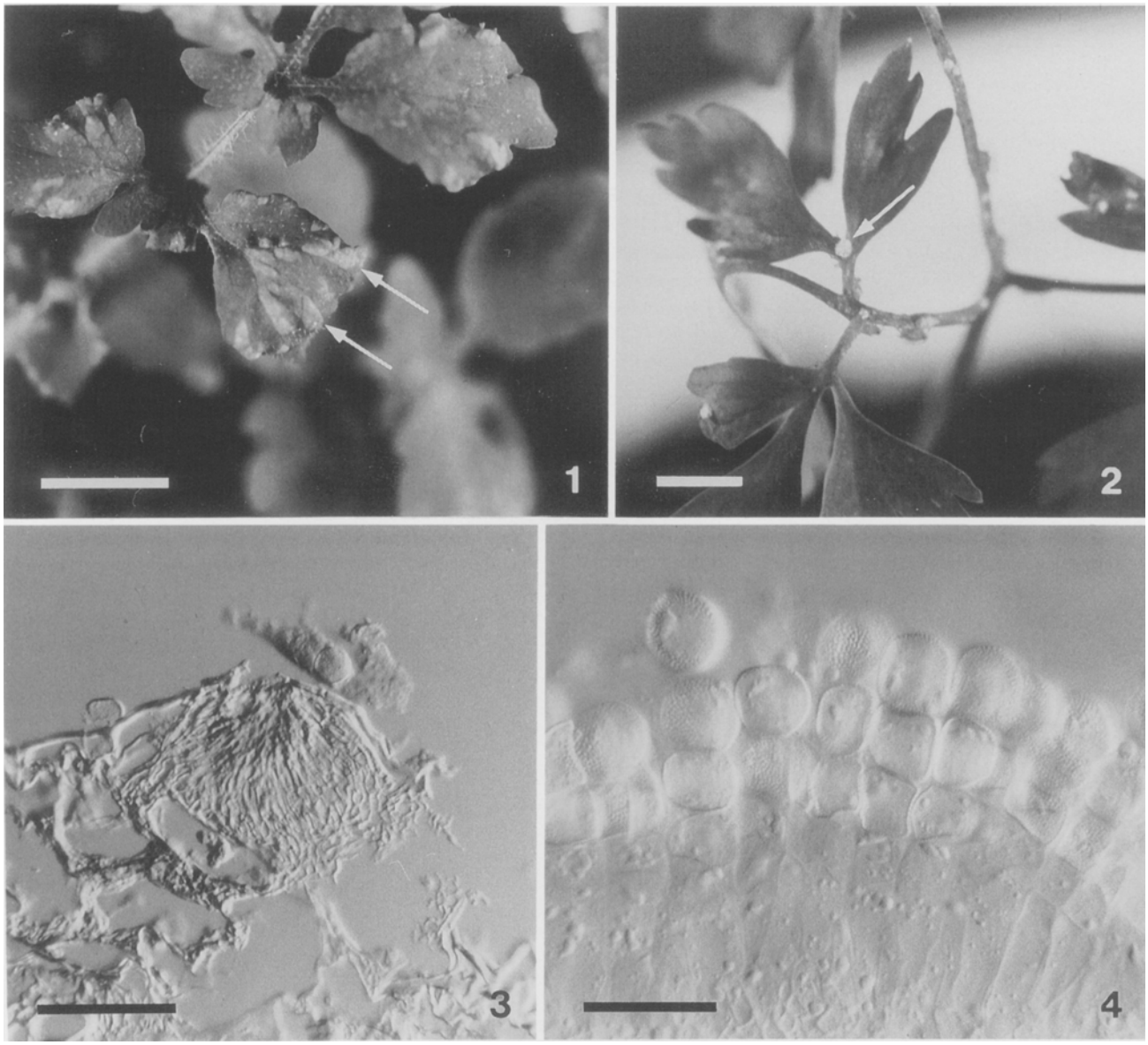

Fig. 1. Spermogonia and aecia on *Chelidonium majus* var. *asiaticum* obtained from an inoculation experiment.
Fig. 2. Spermogonia and aecia on *Corydalis incisa* obtained from an inoculation experiment.
Fig. 3. A cross section of a spermogonium on *Chelidonium majus* var. *asiaticum*.
Fig. 4. A cross section of an aecium on *Chelidonium majus* var. *asiaticum*.
Scale bars: 1, 2=2 cm, 3=50 μm, 4=20 μm.

appeared on the petioles of *C. ambigua* 8 d after inoculation, but the inoculated plants died before aecia were produced there. No sign of infection was found on *L. leptolepis*, *P. densiflora*, *P. thunbergii*, *T. sieboldii* and *S. serissaefolia*.

Aeciospore inoculation Aeciospores produced on *C. majus* var. *asiaticum* and *C. incisa* as a results of basidiospore inoculation were used as inocula. Yellow to yellowish orange uredinia were produced on the lower surface of the inoculated leaves of *S. serissaefolia* 6 to 10 d after inoculation.

Salix species collected at the Azusa River were inoculated with aeciospores produced on *C. majus* var. *asiaticum* collected in the spring of 1991 and 1993 and those on *C. incisa* collected in the spring 1993 at the same place. Uredinia were produced on the leaves of *S. serissaefolia* and *S. jessoensis* Seem. but not on the leaves of the other five *Salix* species (Table 2).

Urediniospore inoculation Two uredinial cultures derived from aeciospore inoculation were used for the urediniospore inoculation to examine host range of the fungus. Both cultures showed the same results. Uredinia were produced on *S. jessoensis*, *S. serissaefolia*, *S. eriocarpa* Franch. et Savat., and *S. pierotii* Miq. (Table 3). Uredinia produced on *S. pierotii* were smaller than those on the other three *Salix* species and located on the

Table 2. Results of inoculation experiments with aeciospores collected in the field.

Aeciospores on	Plant inoculated	No. of plants	
		inoculated	producing uredinia
Chelidonium majus var. *asiaticum*	*S. serissaefolia* (SSE-2)	4	4
	S. gilgiana (SGI-1)	4	0
	S. gracilistyla (SGR-1)	1	0
	S. integra (SI-2)	1	0
	S. matsudana f. *tortuosa* (SMA-2)	1	0
	S. subfragilis (SSU-2)	2	0
Corydalis incisa	*S. serissaefolia* (SSE-2)	4	4
	S. gilgiana (SGI-1)	2	0
	S. integra (SI-2)	1	0
	S. jessoensis (SJ-2)	2	2
	S. matsudana f. *tortuosa* (SMA-2)	1	0

Table 3. Results of inoculation experiments with urediniospores that resulted from aeciospore inoculation.

Plant inoculated	No. of plants	
	inoculated	producing uredinia
S. serissaefolia (SSE-2)	12	12
S. serissaefolia (SSE-1)	3	3
S. serissaefolia (HB-419)	1	1
S. chaenomeloides (SC-1)	1	0
S. eriocarpa (HB426)	5	4
S. gilgiana (SGI-1)	3	0
S. gracilistyla (SGR-1)	2	0
S. integra (SI-3)	1	0
S. jessoensis (SJ-1)	2	2
S. jessoensis (SJ-2)	6	6
S. matsudana f. *tortuosa* (SMA-2)	1	0
S. pierotii (Green44)	10	7[a]
S. sachalinensis (SSA-2)	1	0
S. sachalinensis (SSA-4)	1	0
S. subfragilis (SSU-2)	2	0

a) Uredinia were produced on necrotic lesions.

necrotic lesions.

Morphological observations The spermogonia on *C. majus* var. *asiaticum* collected in the field and obtained in the inoculation experiments were epiphyllous, densely grouped, subepidermal, and ellipsoid (type 2 of Hiratsuka and Cummins, 1963) (Fig. 3). Aecia were mostly hypophyllous and orange in colour (caeoma I of Sato and Sato, 1984). The aeciospores were globoid or broadly ellipsoid and 10.2–25.0 × 8.2–24.8 μm (Fig. 4). Their walls were 0.3–1.4 μm thick and verrucose (Fig. 9). The spermogonia and the aecia produced on *C. incisa* collected in the field and obtained in the inoculation experiments were morphologically the same as those on *C. majus* var. *asiaticum*. The aeciospores on *C. incisa* were globoid or broadly ellipsoid and 11.5–22.9 × 8.7–18.4 μm. Their walls were 0.4–1.8 μm thick and verrucose. The uredinia on *S. serissaefolia* were mostly hypophyllous, scattered and yellow in colour (Fig. 5). The urediniospores were obovoid or ellipsoid and 10.5–28.0 × 8.8–20.2 μm (Fig. 6). Their walls were 0.4–2.1 μm thick and echinulate (Fig. 10), but rarely with a smooth spot (Fig. 11). Germpores were 5–8 in the scattered pattern. Many capitate to clavate paraphyses were intermixed in the uredinia. Telia were hypophyllous, subcuticular, in groups or scattered, and dark brown in colour (Fig. 7). The teliospores were 16.0–35.4 × 6.0–12.0 μm (Fig.8). Their walls were uniform and 0.6–1.5 μm thick.

Discussion

The field observation and the inoculation experiments in the present study demonstrated that the fungus formed

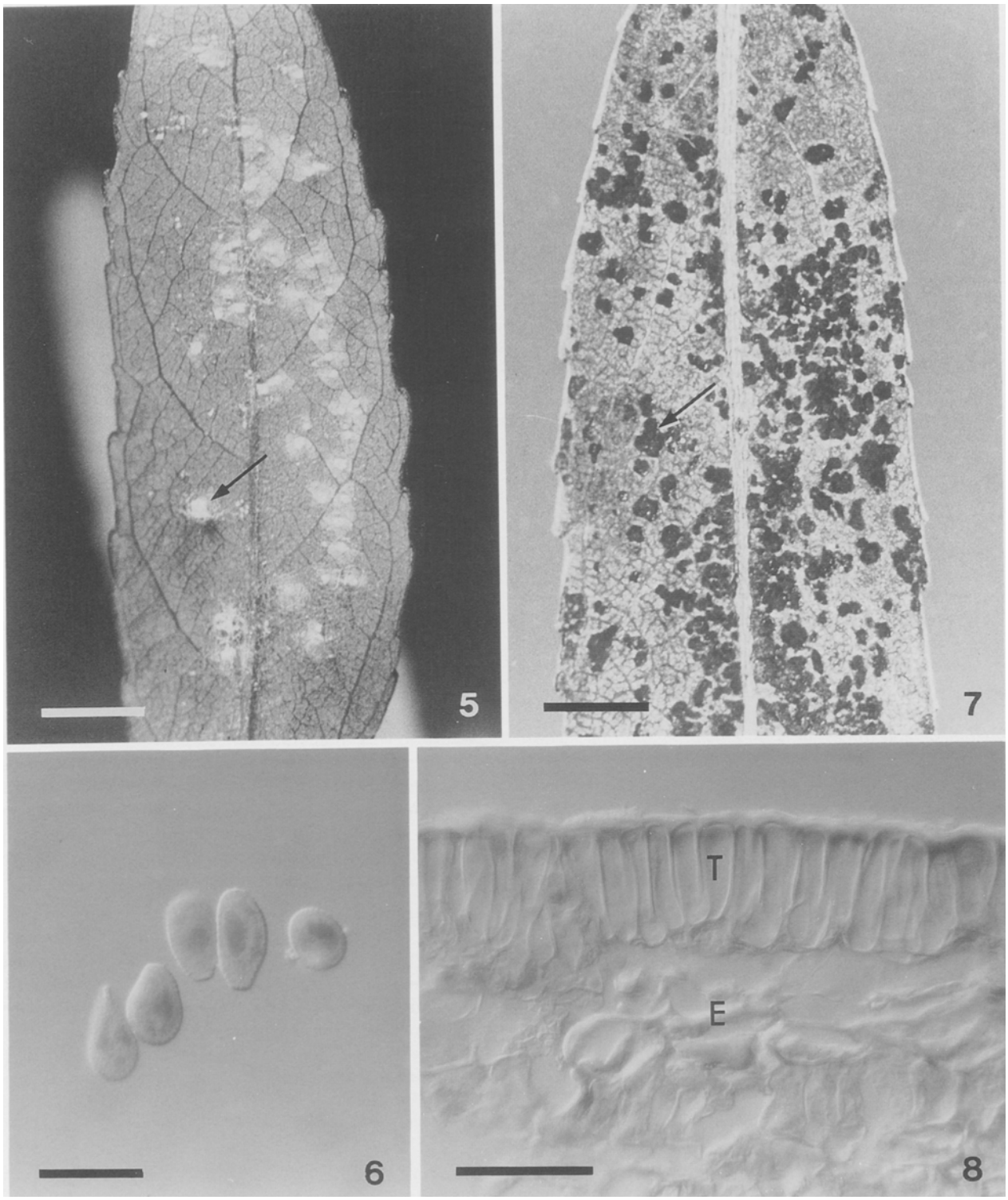

Fig. 5. Uredinia on *Salix serissaefolia*.
Fig. 6. Urediniospores.
Fig. 7. Telia on *Salix serissaefolia*.
Fig. 8. A cross section of a telium on *Salix serissaefolia* showing teliospores (T) and epidermis (E).
Scale bars: 5=5 mm, 6=20 μm, 7=5 mm and 8=30 μm.

the uredinial and telial states on *S. serissaefolia* and the spermogonial and aecial states on *C. majus* var. *asiaticum* and *C. incisa* on the exposed riverbed and riverbank of the Azusa River.

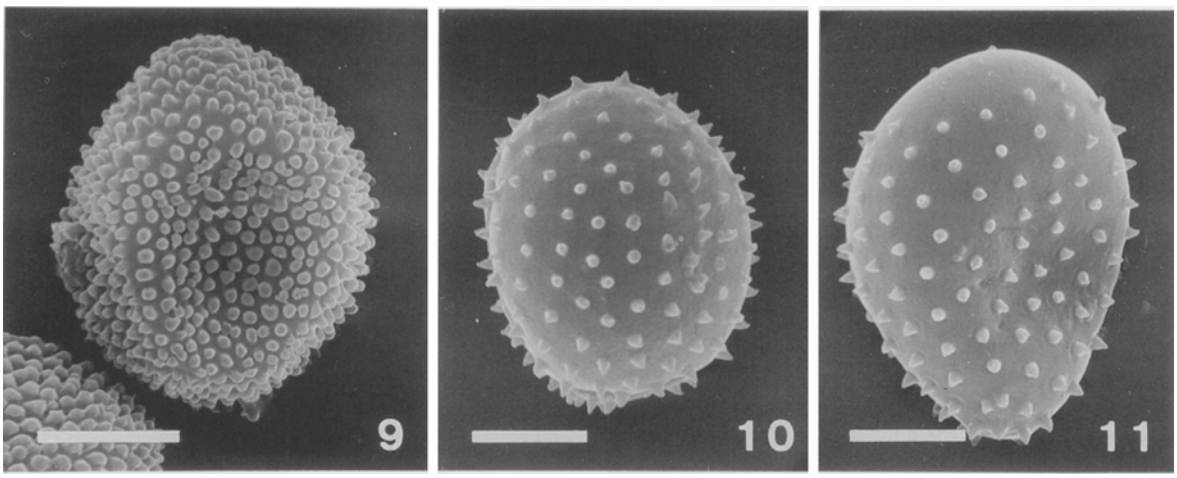

Fig. 9. An aeciospore produced on *Chelidonium majus* var. *asiaticum* observed under SEM.
Fig. 10. A urediniospore produced on *Salix serissaefolia* observed under SEM.
Fig. 11. A urediniospore produced on *Salix serissaefolia* with a smooth spot observed under SEM.
Scale bars: 9, 10 and 11 = 5 μm.

This is the first report of this rust on *S. serissaefolia*. Morphological characteristics and host range of the fungus were compared with the description of all the willow rusts found in Arthur (1934), Kuprevich and Transhel (1957), Wilson and Henderson (1966), Ziller (1974), Hiratsuka and Kaneko (1982), Azbukina (1984), and Hiratsuka et al. (1992). Two species of *Melampsora* parasitic on willows produced spermogonia and aecia on the plants belonging to Papaveraceae. One of these is *M. yezoensis* and the other is *M. chelidonii-pierotii*. The former forms the uredinial and telial states on *S. jessoensis* and the spermogonial and aecial states on *C. ambigua* (Matsumoto, 1915; Hiratsuka, 1932, 1944, 1960; Ito, 1938; Kuprevich and Transhel, 1957; Hiratsuka and Kaneko, 1982). The latter forms the uredinial and telial states on *S. pierotii* and *S. chaenomeloides* Kimura and the spermogonial and aecial states on *C. majus* var. *asiaticum* and *C. incisa* (Matsumoto, 1926; Hiratsuka and Kaneko, 1982). The present fungus covered the host ranges of both *M. yezoensis* and *M. chelidonii-pierotii*. *Melampsora yezoensis* and *M. chelidonii-pierotii* were morphologically distinguishable based on the position of telia produced on the host leaves and the length of teliospores. Telia of the former were mostly hypophyllous, while those of the latter mostly epiphyllous. The former has shorter teliospores than the latter. The morphological characters of the present fungus fitted well with those of *M. yezoensis*. Therefore, we identified the rust fungus on *S. serissaefolia* as *M. yezoensis*. *Salix serissaefolia* was added as a new uredinial and telial host of *M. yezoensis*, and *C. majus* var. *asiaticum* and *C. incisa* were added as new spermogonial and aecial hosts.

The morphological characteristics of the spermogonia of *M. yezoensis* are described for the first time in the present study. The spermogonia of the fungus was of type 2 of Hiratsuka and Cummins (1963). *Melampsora* species that are known to have spermogonia have type 2 or type 3 spermogonia (Hiratsuka and Cummins, 1963).

The inoculation experiments with urediniospores showed that four *Salix* species, *S. jessoensis*, *S. serissaefolia*, *S. eriocarpa*, and *S. pierotii*, can be considered to be host plants of *M. yezoensis*. The former two species were natural host plants, since the fungus was found on them in nature. On the other hand, *M. yezoensis* was not found on the other two *Salix* species in nature. In the present inoculation experiments, necrotic lesions surrounding uredinia were observed on the leaves of *S. pierotii*. This indicated that *S. pierotii*, or at least the clone of the plant used, was not susceptible to the strains of *M. yezoensis* used. *Salix eriocarpa* and *S. pierotii* are sometimes treated as the same species (Kitamura and Murata, 1980). In the present study, we followed the taxonomic system proposed by Kimura (1989), and thus they were treated as separate species.

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