Mycoscience (2004) 45:200–205
DOI 10.1007/s10267-003-0178-0

FULL PAPER

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Puccinia calystegiae-soldanellae, a new rust species on *Calystegia soldanella* from Japan

Received: November 13, 2003 / Accepted: December 22, 2003

Abstract A rust species on *Calystegia soldanella* in Japan has been treated as *Puccinia convolvuli* to date. However, morphological characteristics of specimens on *C. soldanella* collected from Japan are significantly different from those of specimens on other *Calystegia* and *Convolvulus* species from different areas of the world. It is proved by inoculation experiment that the rust on *C. soldanella* is specific to *C. soldanella*. Based on these results, *Puccinia* rust on *C. soldanella* from Japan is described as a new species, *Puccinia calystegiae-soldanellae.*

Key words *Calystegia* · Life cycle · *Puccinia* · Rust fungi · Taxonomy

Introduction

Since *Puccinia convolvuli* Castagne was described by Castagne (1843), it has been found on *Calystegia* R. Br., *Convolvulus* L., and *Ipomoea* L. of Convolvulaceae from America, Europe, Japan, and northern Africa (Sydow and Sydow 1904; Ito 1950; Gäumann 1959; Arthur 1934; Wilson and Henderson 1966; Hiratsuka et al. 1992). However, recently, when rust fungi from Aomori Pref., Japan were surveyed by the authors, it was found that the urediniospore characteristics of specimens on *Calystegia soldanella* (L.) Roem. & Schult. were different from those of *P. convolvuli* described previously. Therefore, comparative morphological studies of specimens on *C. soldanella* collected from Japan as well as specimens on other *Calystegia* and *Convolvulus* species from different areas of the world were carried out. Meanwhile, urediniospores on *C. soldanella* and *Calystegia japonica* Choisy were inoculated to *C. soldanella* and *C. japonica* to examine the suspected host specificity of rust fungi on *C. soldanella.*

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Materials and methods

Specimens examined

A total of 85 rust specimens from various herbaria were used for morphological study: the collection data were described as follows.

On *Calystegia hederacea* Wall., China: Yunnan Prov. (HMAS35289, 50202: U; HMAS51894: U, T); Sichuan Prov. (HMAS44415, 65825: U; HMAS65824: T); Shaanxi Prov. (HMAS55999: U; HMAS56349: S, A). On *Calystegia sepium* (L.) R. Br. (=*Convolvulus sepium* L.), Japan: Iwate Pref. (BPI-0058466: T); Hokkaido (HHUF27952, 27953: S, A); U.S.A.: Kansas (BPI-0058457: U; E00159115: U, T); location unknown (BPI-0058459: S, A); New York (BPI-0058464: S, A; PUR51632: U, T; BPI-0058460: T); Illinois (BPI-0058461, 0058462: U, T; BPI-0058463, 0058465, 0058468–0058470: U; BPI-0058467, 0058472: T); Indiana (PURN1970–1972: U; PURN4618, 4619: U, T); Ohio (PUR32448, 66171: U; PUR42970, 66004: U, T; PUR66168, 66169: T); Montana (E00159114: T); Romania: Prahova (E00159112: T); Bulgaria: Distr. Ivailovgrad. (E00159111, BPI-0058471: S, A); Italy: Riva on Gardasee (BPI-0058473: U). On *Calystegia japonica* Choisy, Japan: Shimane Pref. (BPI-0058474: T); Aomori Pref. (HHUF12105: U, T; HHUF12108, 12109, 12119, 12120: T); Korea: Ryuen-men (Yoshin-gun) (BPI-0058456: U). On *Convolvulus arvensis* L., U.S.A.: Indiana (PUR89268: U); Turkey: Rize Prov. (E00159113: S, A). On *Calystegia spithamaea* (L.) Pursh, U.S.A.: Minnesota (BPI-0058483, 0058484: U, T). On *Calystegia* spp., China: Anhwei Prov. (BPI-0058454: T); Kweichow Prov. (BPI-0058455: U); U.S.A.: Indiana (PUR65501: S, A); Swiss: Distr. Lugano (BPI-0058453: U). On *Calystegia soldanella* (L.) Roem. & Schult., Japan:

Okinawa Is. (BPI-0058475, 0058476: U); Okayama Pref. (BPI-0058477: T); Tottori Pref. (BPI-0058479: U); Oita Pref. (BPI-0058480: U); Kagoshima Pref. (BPI-0058481: U); Hokkaido (BPI-0058482: U; BPI-0058478: T); Aomori Pref. (HHUF28049–28055: S, A; HHUF13122: A, U; HHUF15602, 18804, 18808, 18827, 28042–28045: U; HHUF14264: U, T; HHUF28046–28048: T).

Capital letters S, A, U, and T following the herbarium accession number denote spermogonial, aecial, uredinial, and telial states, respectively. All the above-cited specimens were obtained from the following herbaria: Arthur Herbarium, Purdue University, USA (PUR); The National Fungus Collections, U.S. Department of Agriculture, USA (BPI); Herbarium of Mycology and Lichenology, the Institute of Microbiology, Chinese Academy of Sciences, China (HMAS); the Herbarium, Royal Botanic Garden, Scotland, Great Britain (E); and Mycological Herbarium of the Faculty of Agriculture and Life Science, Hirosaki University, Japan (HHUF). The names of the host plants were according to Brako et al. (1995), Hutchinson (1972), Ohwi and Kitagawa (1992), and Satake et al. (1982). The system of terminology proposed by Cummins and Hiratsuka (1983) was used to describe life cycle states of rust fungi throughout the study.

Microscopic observation

For light microscopy, spores were scraped from sori on herbarium specimens and mounted in a drop of lactophenol on glass slides for observation. Morphology and size of 50 or 100 randomly selected spores were examined for each specimen.

For scanning electron microscopy (SEM), rust-infected leaves from dried herbarium specimens were cut into pieces about 3 × 3 mm containing a few sori, and each piece was placed on double-adhesive tape on a specimen holder. The preparations were subsequently coated with platinum-paladium using a JEOL JFC–1100 Ion Sputter and examined with a JEOL JSM–5300 scanning electron microscope (SEM) at 15 kV.

Basidiospore inoculation

Teliospores on *C. soldanella* were used for the basidiospore inoculation to confirm a autoecious nature of life cycle of the fungus. Basidiospore inoculations were undertaken by the method described by Ono (1994) and Ono and Azbukina (1997). Telium-bearing leaves of *C. soldanella* were collected at Toriizaki, Aomori Pref. in November 2002. The leaves were preserved in a refrigerator at 5°C until use. They were then soaked in running tap water for 7–14 days to induce germination of teliospores. Leaves were cut into small pieces (about 2 × 2 mm), placed on wetted filter paper in a Petri dish, and incubated in the dark at about 20°C. The leaf pieces with germinated teliospores were placed on adaxial surfaces of apparently healthy leaves of *C. soldanella* plants that had been planted with loam soil in clay pots (18 cm diameter). The inoculated plants were sprayed with distilled water and placed in a moist chamber at 18°–22°C for 48 h. The plants were subsequently transferred to a glasshouse (~20°C) for further observations. The inoculation experiments were repeated four times in February and March 2003. In each experiments, two to five leaves of one or two plants were inoculated.

Urediniospore inoculation

Urediniospores on *C. soldanella* and *C. japonica* were used as inocula. Uredinium-bearing leaves of *C. soldanella* were collected at Toriizaki, Aomori Pref. in July 2002, and those of *C. japonica,* which were obtained from inoculations with the aeciospores on *C. sepium* collected from Sapporo, Hokkaido in July 2002, were temporarily preserved in a refrigerator at 5°C until use. The urediniospores, scraped from uredinia on the leaves of both host plants and suspended in distilled water, were inoculated on surfaces of apparently healthy leaves of *C. japonica* and *C. soldanella* with a small hair brush. The inoculated plants were sprayed with distilled water and placed in a dark, moist chamber at about 20°C for 48 h. The plants were subsequently transferred to a glasshouse for further observations. The inoculation experiments were repeated five times for urediniospores on *C. soldanella* and four times for urediniospores on *C. japonica* in July to August 2002. In each experiment, three to five leaves of one or two plants were inoculated, respectively.

Results

Basidiospore inoculation

In inoculation of *C. soldanella* with basidiospores from teliospores on *C. soldanella*, spermogonia appeared on the upper surface of the inoculated leaves and/or petioles 12–15 days after inoculation and aecia followed in 10–15 days more. Then, uredinia and telia were produced on the leaves of *C. soldanella* 2 months and 6 months after inoculation, respectively. Spermogonia were seen on the adaxial leaf surface or on the petiole; they were densely grouped, subepidermal, globose or subglobose, yellowish, 90–150 μm in diameter (Fig. 1A). Spermatia were ellipsoid to oblong-ellipsoid, hyaline, 4–7 × 2–3 μm in size. Aecia were mostly on abaxial leaf surfaces or on petioles, densely grouped, on brownish or purplish spots, cupulate with a well-developed peridium reflexing upon maturity. Aeciospores were globose to subglobose, often angular, 18–28 × 15–21 μm, the walls about 1 μm thick, colorless or pale yellow, verrucose (Fig. 1B). Uredinia were mostly amphigenous, scattered, brown to dark brown, powdery. Urediniospores were subglobose, obovoid, or broadly ellipsoidal, 27–38 × 23–31 μm, walls 4–6 μm thick, brown, completely echinulate, germ pores 2–3, mostly equatorial (Fig. 1D). Telia were amphigenous, rounded, scattered, or often confluent into rounded clusters, long covered by the epidermis, pulvinate,

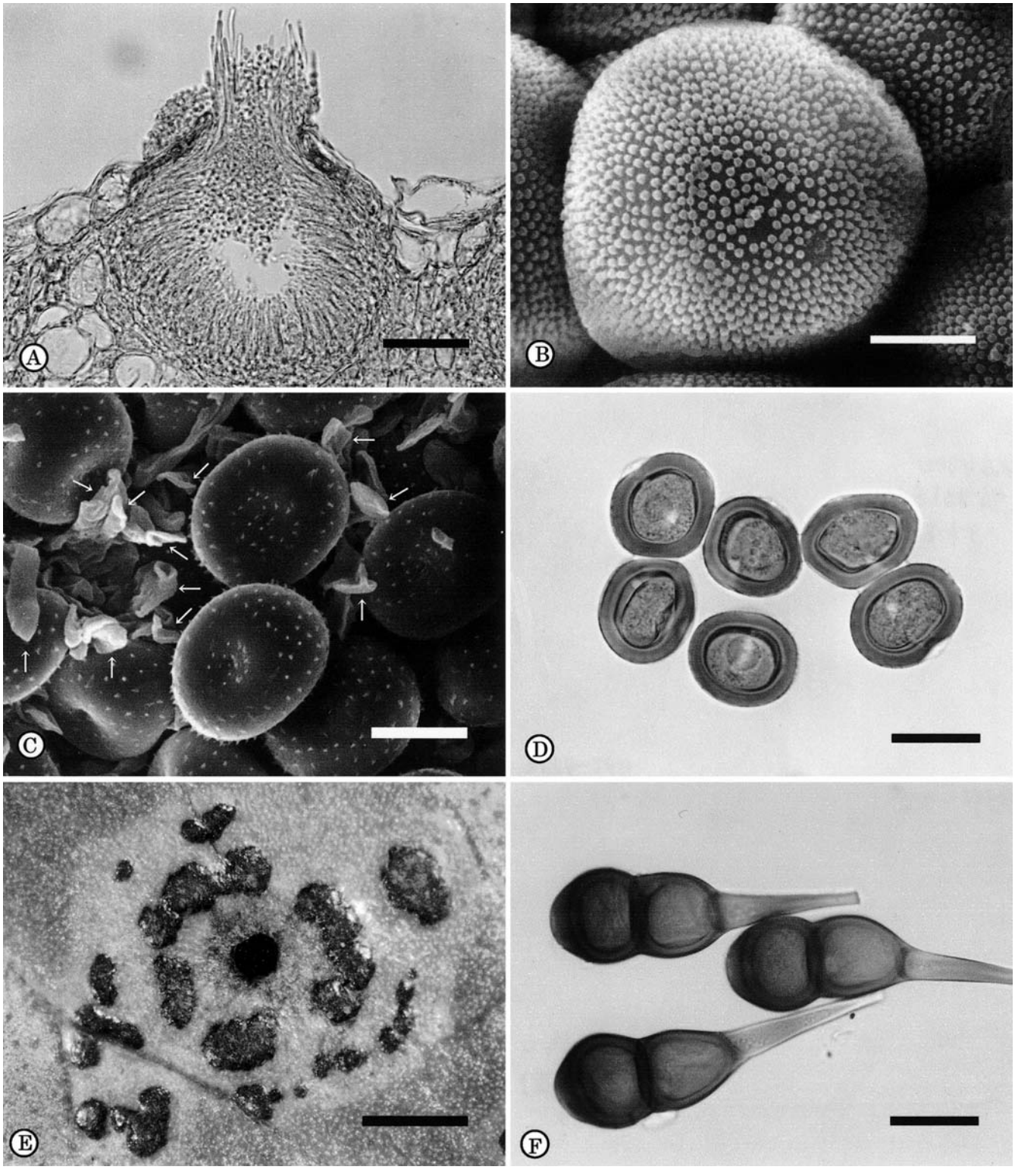

Fig. 1. *Puccinia calystegiae-soldanellae.* **A** Spermogonium (HHUF28050); **B** surface structure of aeciospores (HHUF28050) (SEM); **C** uredinium (abundant and developed paraphyses among urediniospores indicated by *arrows*) (SEM) (HHUF28042); **D** urediniospores (HHUF28042); **E** telia (HHUF28046); **F** teliospores (HHUF28046). *Bars* **A** 50 μm; **B** 5 μm; **C** 15 μm; **D,F** 25 μm; **E** 2 mm

and black (Fig. 1E). Teliospores were broadly ellipsoidal, rounded at the apex, 35–65 × 22–35 μm. The wall was smooth, brown to chestnut-brown, 5–10 μm thick at the apex, pedicels brown to pale brownish, persistent, up to 60 μm long (Fig. 1F).

Urediniospore inoculation

When *C. soldanella* and *C. japonica* were inoculated with urediniospores on *C. soldanella*, uredinia were produced on leaves of *C. soldanella* in 8–12 days after inoculation, but not on leaves of *C. japonica*. Uredinia were produced on leaves of *C. japonica* in 8–12 days after inoculation, but not on leaves of *C. soldanella* in inoculations with urediniospores on *C. japonica*.

Morphology

No significant differences were found in morphology among the aeciospores of all specimens. The size of aeciospores was (17–)19–28(–30) × (14–)15–21(–23) μm, wall thickness was 1 μm, and surface structure type was verrucose.

However, the specimens were separated into two groups based on size and wall thickness of urediniospores. In the first group, the size was (25–)27–38(–40) × (20–)23–31(–33) μm, and thickness was 3–6 μm (Fig. 1D). In the second group, it was smaller, (20–)22–31(–35) × (16–)18–25(–27) μm in size, and thinner than the first group in wall thickness, 1–3 μm (Fig. 2B). All urediniospores of specimens on *C. soldanella* belonged to the first group and the others belonged to the second group.

In addition, abundantly developed paraphyses were often observed with uredinia of specimens on *C. soldanella* (Fig. 1C), but they were scarcely or only poorly developed with uredinia of the specimens on other hosts (Fig. 2A).

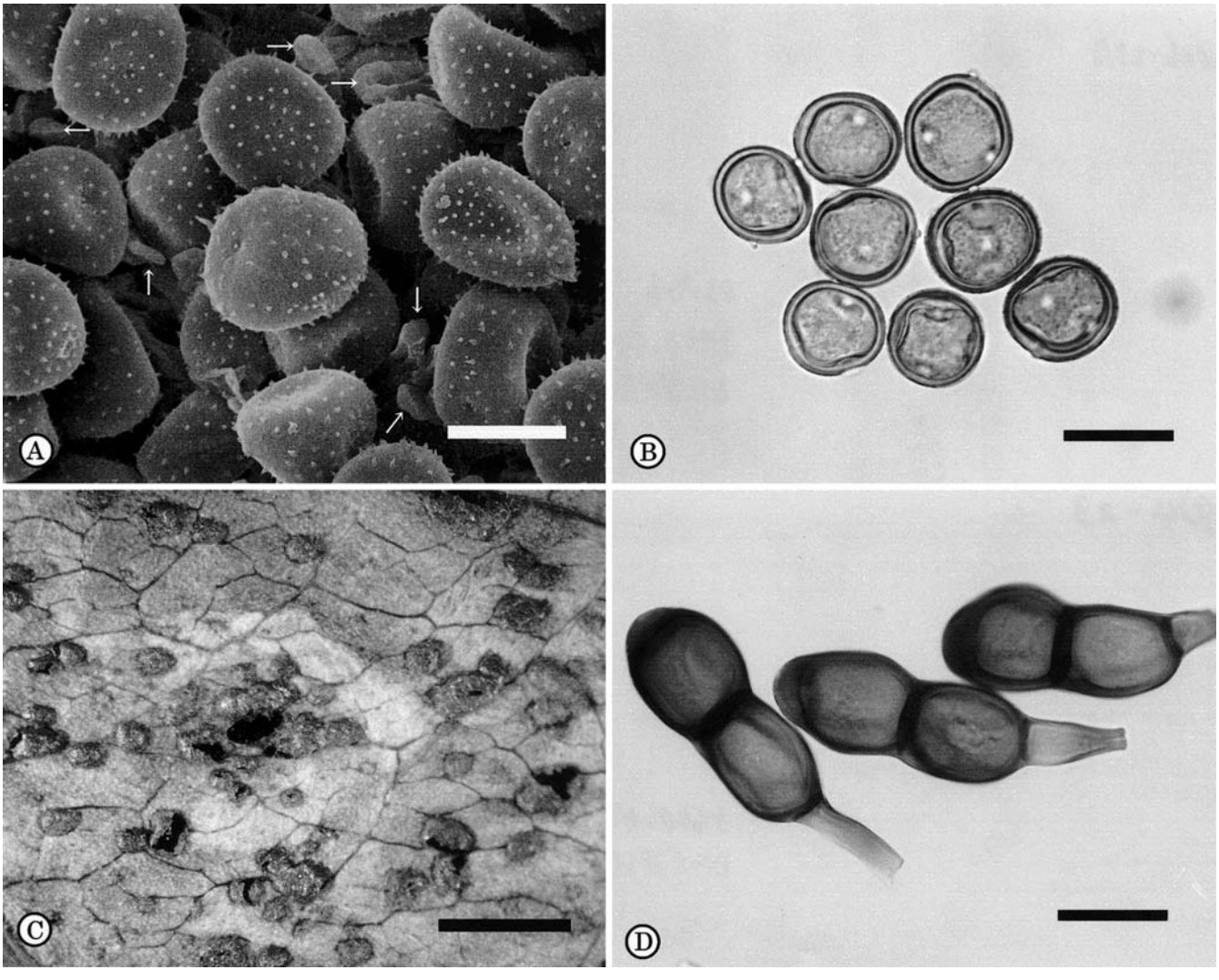

Fig. 2. *Puccinia convolvuli*. **A** Uredinium (scarce and undeveloped paraphyses among urediniospores indicated by *arrows*) (SEM) (HHUF12105); **B** urediniospores (HHUF12105); **C** telia (HHUF12108); **D** teliospores (HHUF12108). *Bars* **A** 15 μm; **B,D** 25 μm; **C** 2 mm

In specimens with telial state, the specimens were separated into two types based on morphological characteristics of telia and teliospores. The first type was found in all specimens on *C. soldanella.* Telia in this type were scattered and often confluent into rounded clusters (Fig. 1E). Teliospores were broadly ellipsoidal, rounded at the apex, and pedicels were persistent, up to 60 μm (Fig. 1F). The second type of telia was found in the specimens on other hosts. Telia in this type were scattered (Fig. 2C). Teliospores were cuneiform to clavate, obtuse at the apex; pedicels were fragile and often deciduous, up to 50 μm (Fig. 2D).

In the analysis of teliospore size, the average width range of teliospores of specimens on *C. soldanella* was greater than that of specimens on other hosts, although no significant difference was observed in range of teliospore size [(35–)38–65(–70) × (20–)22–33(–35) μm] among all specimens in this study (Fig. 3). Correspondingly, the shape of teliospores in specimens on *C. soldanella* was broadly ellipsoid versus cuneiform to clavate in specimens on other hosts. Meanwhile, the walls of teliospores of specimens on *C. soldanella* were generally thicker [(2.5–)3–5(–6) μm] than those of teliospores on other specimens [(1.5–)2–4(–4.5) μm].

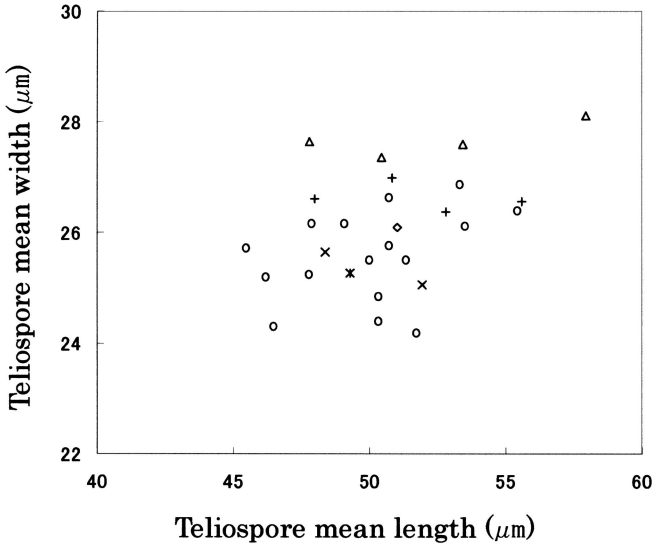

Fig. 3. Mean length versus mean width of teliospores: ×, specimens on *Calystegia hederacea*; +, specimens on *C. japonica*; ◇, specimens on *C. spithamaea*; ∗, specimens on *Calystegia* sp.; ○, specimens on *C. sepium*; △, specimens on *C. soldanella*

Discussion

Observation of specimens showed that there were significant differences in morphological characteristics of uredinial and telial states between the specimens on *C. soldanella* from Japan and specimens on other hosts, except *C. soldanella*, from different areas of the world.

In the uredinial state, urediniospore specimens on *C. soldanella* were bigger, the wall of spores was thicker and, if present, paraphyses developed more abundantly in uredinium comparing specimens on hosts other than *C. soldanella*.

In telial state, the telia of specimens on *C. soldanella* were often confluent into rounded clusters, the teliospore was broadly ellipsoidal, rounded at the apex, the average width was greater, the wall of the spore was thicker, and the pedicel was persistent, and longer, up to 60 μm, comparing with specimens on other hosts.

Development of such structural differences was probably correlated with the different ecological niches between seashores and inland; i.e., the thicker urediniospore wall, well-developed paraphyses, and thicker teliospore wall of the rust on *C. soldanella* might reflect the severe ecological environment of the seashore where *C. soldanella* has its habitat.

So far, only *P. convolvuli* has been known among *Puccinia* species on *Calystegia* and *Convolvulus* species. The measurements of *P. convolvuli* reported previously by different authors were compared (Table 1). It was shown that measurements of *P. convolvuli* reported previously were much closer to those of specimens on other hosts except *C. soldanella* in this study, but were different from specimens on *C. soldanella*. Therefore, specimens on *C. soldanella* from Japan in this study were not the same as *P. convolvuli.*

In addition, it was proved by inoculation experiment that the rust fungi on *C. soldanella* were specific *C. soldanella* and did not infect *C. japonica.* This result was not in agree-

Table 1. Measurements of *Puccinia convolvuli* reported previously

Authors	Aeciospores (μm)	Urediniospores (μm) (wall thickness)	Teliospores (μm)
Sydow and Sydow (1904)	17–28 in diameter	22–30 × 18–26 (not described)	38–66 × 18–30
Gäumann (1959)	21–35 (diameter) × 18–22	25–32 × 18–25 (2–2.5)	43–73 × 25–32
Arthur (1934)	18–25 × 15–20	23–33 × 18–26 (2–2.5)	42–55 × 23–31
Wilson and Henderson (1966)	17–28 in diameter	22–30 × 18–26 (not described)	38–66 × 18–30
Hiratsuka et al. (1992)	18–25 × 15–21	22–30 × 18–26 (not described)	38–66 × 18–30

ment with the previous report that rust fungi on *C. soldanella* and *C. japonica* were the same rust fungi, *P. convolvuli*. Rust fungi on *C. soldanella* could not infect *C. japonica* by inoculation experiment. This finding strongly suggested that rust fungi on *C. soldanella* and *C. japonica* were not the same species.

The urediniospore morphology of specimens on *C. soldanella* in this study was similar to that of *Uredo calystegiae* Sawada, 28–36 × 23–28 μm in size, walls 3–5 μm thick, which was reported as a new species based on the specimen on *C. soldanella* collected from Taiwan by Sawada (1944). Thus, *U. calystegiae* was considered as a uredinial state of rust fungi on *C. soldanella* in this study.

Based upon the preceding results of the experiment, we conclude that the rust on *C. soldanella* from Japan, formerly treated as *P. convolvuli*, belongs to a distinct species, which is here named *Puccinia calystegiae-soldanellae*, as follows.

Puccinia calystegiae-soldanellae Z. Li, F. Nakai & Y. Harada, sp. nov. Fig. 1

Misapplied name: *Puccinia convolvuli* auct. jap. pro parte, non Castagne, Obs. 1: 16, 1843. Anamorph: *Uredo calystegiae* Sawada, Descr. Catal. Form. Fungi 9: 135, 1944 (nom. inval.).

Spermogoniis saepe epiphyllis vel petiolaribus, aggregatis, minutis, subepidermalibus, globosis vel subglobosis, flavis, 90–150 μm; spermatiis ellipsoideis vel oblongo-ellipsoideis, hyalinis, 4–7 × 2–3 μm; aeciis saepe hypophyllis vel petiolaribus, aggregatis, maculis brunneis vel purpureis insidentibus, peridiatis, cupularibus, albis; aeciosporis globosis vel subglobosis, saepe angularibus, 18–28 × 15–21 μm, episporiis 1 μm crassis, pallidis vel pallido-flavis, verrucosis; urediniis amphigenis, sparsis, brunneis; urediniosporis subglobosis, obovatis vel late ellipsoideis, 27–38 × 23–31 μm, episporiis 4–6 μm crassis, brunneis, omnino echinulatis, poris germinationis 2–3 saepe aequatoriis praeditis; teliis amphigenis, rotundatis, sparsis vel saepe confluentibus, diu epidermidibus obtectis, pulvinatis, nigris; teliosporis ellipsoideis vel late ellipsoideis, apice obtusatis rotundatis, ad basim rotundatis vel attenuatis, 35–65 × 22–35 μm, episporiis laevis brunneis vel castaneo-brunneis apice 5–10 μm crassis; pedicellis brunneis vel pallide brunneis, persistentibus, usque ad 60 μm longis; teliosporis interdum 1- et rarius 3-cellulosis.

Hab. in foliis *Calystegiae soldanellae* (L.) Roem. et Schult. (Convolvulaceae).

Spermogonia mostly epiphyllous or petiolicolous, in groups, minute, subepidermal, globose or subglobose, yellowish, 90–150 μm in diameter. Spermatia ellipsoid to oblong-ellipsoid, hyaline, 4–7 × 2–3 μm in size. Aecia mostly hypophyllous or petiolicolous, in groups, on brownish or purplish spots, peridiate, cupulate, white and recurved and torn at the margin. Aeciospores globose to subglobose, often angular, 18–28 × 15–21 μm, the walls about 1 μm thick, colorless or pale yellow, verrucose with refractive granules at the upper side. Uredinia amphigenous, scattered, brown to dark brown, powdery. Urediniospores subglobose, obovoid or broadly ellipsoidal, 27–38 × 23–31 μm, walls 4–6 μm thick, brown, completely echinulate, germ pores 2–3, mostly equatorial. Telia amphigenous, rounded, scattered or often confluent into rounded clusters, long covered by the epidermis, pulvinate, and black. Teliospores ellipsoid to broadly ellipsoidal, obtuse or rounded at the apex, rounded or attenuate at base, 35–65 × 22–35 μm. The walls smooth, brown to chestnut-brown, 5–10 μm thick at the apex, pedicels brown to pale brownish, persistent, up to 60 μm long, sometimes one-celled teliospores intermixed, rarely three-celled spores also occur.

Holotype: On *Calystegia soldanella* (L.) Roem. & Schult. (Convolvulaceae), Toriizaki, Fukaura-machi, Nishitsugaru-gun, Aomori Pref., Japan, 140°00.1′ E, 40°44.0′ N, Nov. 12, 2002, Z. Li (HHUF28046), kept in Mycological Herbarium of the Faculty of Agriculture and Life Science, Hirosaki University, Japan.

Host and distribution: On *Calystegia soldanella*, Japan and Taiwan.

Acknowledgments We express our thanks to the curators of the following herbaria for the loan of specimens: Arthur Herbarium, Purdue University, USA (PUR); Herbarium, Royal Botanic Garden, Scotland, Great Britain (E); The National Fungus Collections, United States Department of Agriculture, USA (BPI); and Herbarium of Mycology and Lichenology, Institute of Microbiology, Chinese Academy of Sciences, China (HMAS).

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