Life cycle of *Cerotelium asari* (Uredinales)

Yoshitaka Ono

Laboratory of Biological Sciences, Faculty of Education, Ibaraki University, 2-1-1 Bunkyo, Mito, Ibaraki 310, Japan

Ono, Y. (1995). Life cycle of *Cerotelium asari* (Uredinales). – Sydowia 47 (1): 54–64.

Cerotelium asari was known only from the type on *Asarum caulescens* in Shimane but it was recently rediscovered on the same host species in Ibaraki, Japan. Its heteroecious life cycle was proven by field observations and artificial inoculations, forming spermogonial and aecial stages on *Corydalis linearíloba* (Fumariaceae). The fungus was originally described in the genus *Cerotelium* because of the apparent formation of probasidia (teliospores) in chains in basipetal succession. A proximal cell of seemingly catenulate probasidia, however, has now been found to give rise to a new probasidium sympodially, a mode not found in *C. canavaliae*, the type of the genus *Cerotelium*. A taxonomic affinity of the *Corydalis-Asarum* alternating fungus to the genus *Aplopsora* is thus suggested.

Keywords: Rust fungi, *Cerotelium*, *Aplopsora*, *Corydalis*, *Asarum*.

Cerotelium asari Kaneko & al. had been known only from the type since it was collected on *Asarum caulescens* Maxim. in Shimane in 1972 and was described and named as a new species in 1983 (Kaneko & al., 1983). In October, 1992, this fungus was recorded on the same host species at Mt. Yamizosan, Ibaraki, Japan. Uredinial and probasidial (= telial) sori were present on the type and the newly collected specimens.

A heteroecious life cycle was expected for *C. asari* because it was considered to be taxonomically closely related to *C. dicentrae* Mains & Anders., the only *Cerotelium* species for which a complete heteroecious life cycle is known (Mains, 1921). Morphological similarities in spore ontogeny and mature spores may indicate close phylogenetic relationships among rust fungi, which are often also correlated with their host spectra (Ono & al., 1986; Ono & al., 1987). If this is the case, either aecial or telial host(s) previously known for one species would allow us to predict alternate host(s) of another morphologically related species. Accordingly, it was predicted that the aecial host(s) of *C. asari* is a member of the Fumariaceae as in *C. dicentrae*, the aecial host of which is *Dicentra cucularia* (L.) Bernh. (Fumariaceae) in North America (Mains, 1921).

This paper describes the heteroecious life cycle of *C. asari* and its hitherto not known, morphological characters.

Materials and methods

Specimens examined

Spermogonial and aecial stages on *Corydalis lineariloba* Sieb. & Zucc., Japan, Ibaraki, Kuji-gun, Daigo-machi, Mt. Yamizosan, Kusarezawa-rindo, 17.4.1993, Y.O. 2881 (IBA-6687); 26.4.1993, Y.O. 2884 (IBA-6690); 26.4.1993, Y.O. 2887 (IBA-6693); Mt. Yamizosan, Jaketsu-rindo, 8.5.1993, Y.O. 2891 (IBA-6697).

Uredinial and telial stages on *Asarum caulescens* Maxim., Japan, Shimane, Tsuwano-cho, Nayoshi, 8.10.1972, K. Katsumoto (HH-78461, holotype; YAM-21911, isotype); Ibaraki, Kuji-gun, Daigo-machi, Mt. Yamizosan, Kusarezawa-rindo, 24 .10.1992. Y.O. 2858 (IBA-6359); Mt. Yamizosan, Jaketsu-rindo, 25.6.1993. Y.O. 2913 (IBA-6720); 23.10.1993. Y.O. 2947 (IBA-7005); Mito, Bunkyo, Ibaraki Univ. Campus, 31.5.1993. Y.O. 2900 (IBA-6706; result of aeciospore inoculation); 18.6.1993. Y.O. 2911 (IBA-6718; result of urediniospore inoculation); 25.10.1993. Y.O. 2964 (IBA-7022; result of aeciospore inoculation).

Inocula and inoculations

Aeciospores from the *Aecidium*-infected *C. lineariloba* and *C. incisa* (Thunb.) Pers. were used as inoculum. The infected plants were collected at Mt. Yamizosan, Ibaraki, in April and May, 1993, replanted in a clay pot (15 cm diam.) with vermiculite, and maintained in a greenhouse at Ibaraki University Campus in Mito until used. Fresh aeciospores were scraped from the aecia and sprinkled on ca. 3 x 3 mm pieces of wet filter paper. The aeciospore-bearing pieces of filter paper were then placed on the abaxial leaf surface of apparently healthy plants of *A. caulescens* planted in a clay pot (15 cm diam.) containing vermiculite. The inoculated plants were sprayed with distilled water, incubated in a moist chamber under dark at ca. 20 C for 48 h. and subsequently transferred to a greenhouse for further observations. Five *Asarum* plants each were inoculated in two trials.

Preparations for light and scanning-electron microscopy

To examine sorus morphology and structure, freshly infected materials and dried herbarium specimens were free-hand sectioned under a dissecting microscope and thin sections were mounted in a drop of lactophenol on a glass slide. For morphological studies, spores were scraped from sori on herbarium specimens and mounted as

described above. The slide preparations were examined both by bright-field and by differential-interference-contrast microscopy (DIC).

For scanning electron microscopy (SEM), the *Aecidium*-infected leaves from the dried herbarium specimens were cut into ca. 3 x 3 mm pieces so that each piece bore a few sori, and each sorus-bearing piece was placed on double-adhesive tape on a specimen holder. The preparations were subsequently coated with gold by an Eiko IB-3 Ion Coater and examined with a Hitachi S-430 SEM at 20 kV.

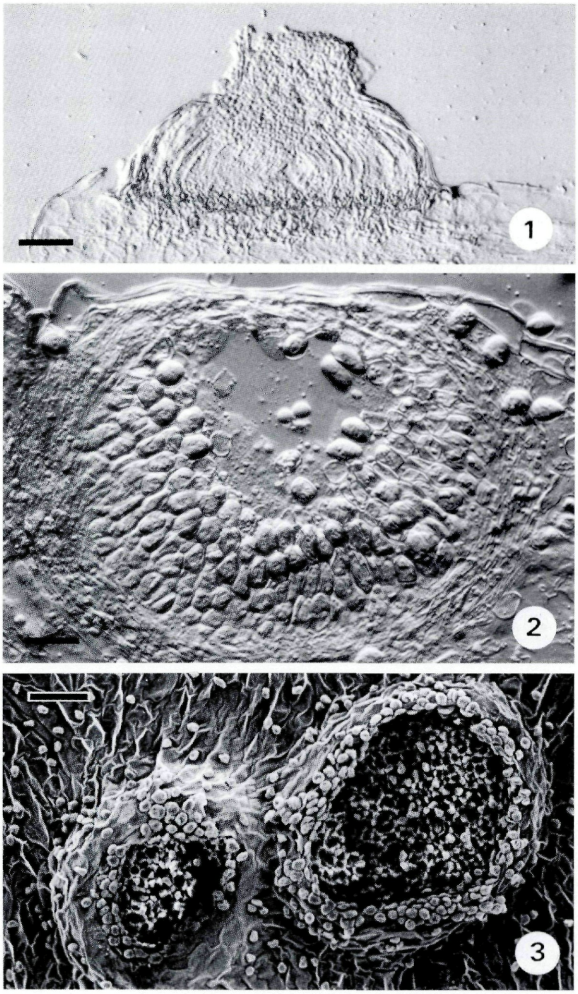
Results and discussion

Field observations

From late March through early May of 1993, the potential spermogonial-aecial hosts for the fungus were searched at several localities at Mt. Yamizosan where rust-infected *Asarum* plants were collected in the previous year. *Corydalis incisa* and *C. lineariloba* were the only plants on which spermogonia and aecia were formed during the survey period.

The fungus on *C. incisa*, *Aecidium corydalinum* Syd. & H. Syd., was proven, by reciprocal inoculations, to be the spermogonial-aecial state of *Ochropsora kraunhiae* (Diet.) Diet., the uredinial-telial stages of which occur on *Wisteria floribunda* (Willd.) DC. (Hiratsuka & Kaneko, 1978). Hiratsuka & Kaneko (1978) listed *C. ambigua* Cham. & Schlecht., *C. decumbens* (Thunb.) Pers., *C. incisa*, *C. pallida* (Thunb.) Pers., *C. pallida* var. *tenuis* Yatabe as the spermogonial-aecial hosts, and *W. brachybotrys* Sieb. & Zucc. f. *alba* (W. Mill.) Hurusawa and *W. floribunda* as the uredinial-telial hosts of *O. kraunhiae*. However, no rust fungus was so far reported for *C. lineariloba*.

Symptoms and signs produced on *C. incisa* were different from those on *C. lineariloba*. The former plant was systemically infected and the entire leaves were uniformly and densely covered by spermogonia and aecia. By contrast, in the latter species, only part of the incised, compound leaves or one of a few compound leaves were covered by spermogonia and aecia although the entire plant appeared to be systemically infected. The *Aecidium*-infected *C. lineariloba* was much lighter in color and erect with more or less hypertrophied leaflets even when no sori were produced on them, while uninfected plants were procumbent and had small, dark green leaflets. The different symptoms on *C. incisa* and *C. lineariloba* may or may not be caused by difference of the fungi involved in the infection. A possible



Figs. 1–3. – *Cerotelium asari* on *Corydalis lineariloba* (IBA-6697). – 1. Median cross section of spermogonium (DIC). – 2. Median cross section of unruptured aecium (DIC). – 3. Surface view of two mature aecia (SEM). – Scale bar = 40 μm in Figs. 1–2; 100 μm in Fig. 3.

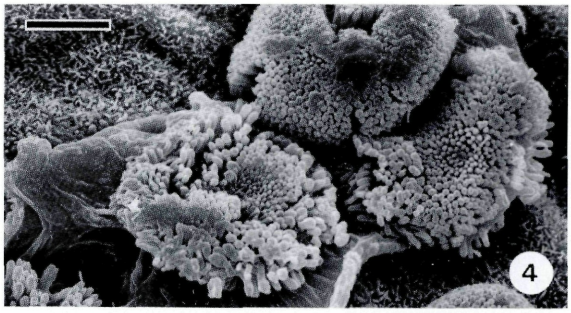
connection in the life-cycle of the *Aecidium* fungus on *C. lineariloba* with *C. asari* on *A. caulescens* was assumed.

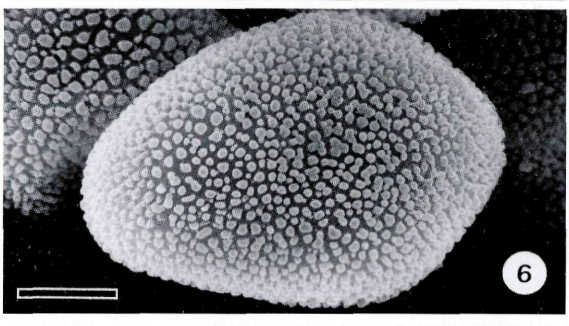
Life cycle of *Cerotelium asari*

Aeciospores from *C. lineariloba* always infected successfully and uredinia and telia were formed on the inoculated leaves of *A. caulescens*. Six or seven days after the inoculation, minute chlorotic spots appeared at the sites where the aeciospore-bearing pieces of filter paper were placed, and the production of mature uredinia with abundant masses of orange-yellow spores followed in the subsequent few days. Under greenhouse conditions, basidiosori started to form in early September. The basidiosori were minute, yellowish to reddish orange, waxy, and densely aggregated around uredinia that were formed by aeciospore inoculation. Contrary to the aeciospores on *C. linealiloba*, aeciospores from *C. incisa* failed to infect and produce urediniospores on the inoculated leaves of *A. caulescens*. Consequently, the *Aecidium* fungus on *C. lineariloba* was proven to be the spermogonial-aecial state of *C. asari* and to be different from *A. corydalinum* on *C. incisa* which is the spermogonial-aecial state of *O. kraunhiae*.

Corydalis lineariloba and *C. incisa* are different in growth habit. The former species produces sphaerical tubers, each of which forms a few compound leaves and a single stem that terminates in inflorescence. The latter species forms several stems in bunches from root stocks, but no tubers. *Corydalis lineariloba* is more closely related to *C. ambigua* and *C. decumbens* than to *C. incisa*, *C. pallida*, and *C. pallida* var. *tenuis*. This indicates that spermogonial and aecial stages formed on *C. ambigua* and *C. decumbens* may be *Cerotelium dicentrae* rather than *O. kraunhiae* as listed by Hiratsuka & Kaneko (1978).

The complete heteroecious life cycle of *C. asari* is now confirmed, but the question arises how this host alternation is assured in the field. Both rust-infected and non-infected *Corydalis* plants emerge early March and persist until early June at Mt. Yamizosan. By early April, when leaves of *Asarum* plants start to unfold, aecial sori on *Corydalis* plants mature and aeciospores are dispersed. Infection of *Asarum* plants with the aeciospores seems to take place from April through May. Uredinia begin to form on *Asarum* plants as early as mid May. Basidiosori, however, are not produced during summer when temperature is high and start to develop in early September both in the field and under greenhouse conditions in Mito. When probasidia, and successively metabasidia and basidiospores, are





Figs. 4–6. – *Cerotelium asari* on *Corydalis linearilobа* (IBA-6697). – 4. Disjointed peridial cells. Inner wall is densely covered with columnar projections (SEM). – 5. Aeciospores (DIC). – 6. Uniformly verrucose surface of aeciospore (SEM). – Scale bar = 10 μm in Figs. 4–5; 5 μm in Fig. 6.

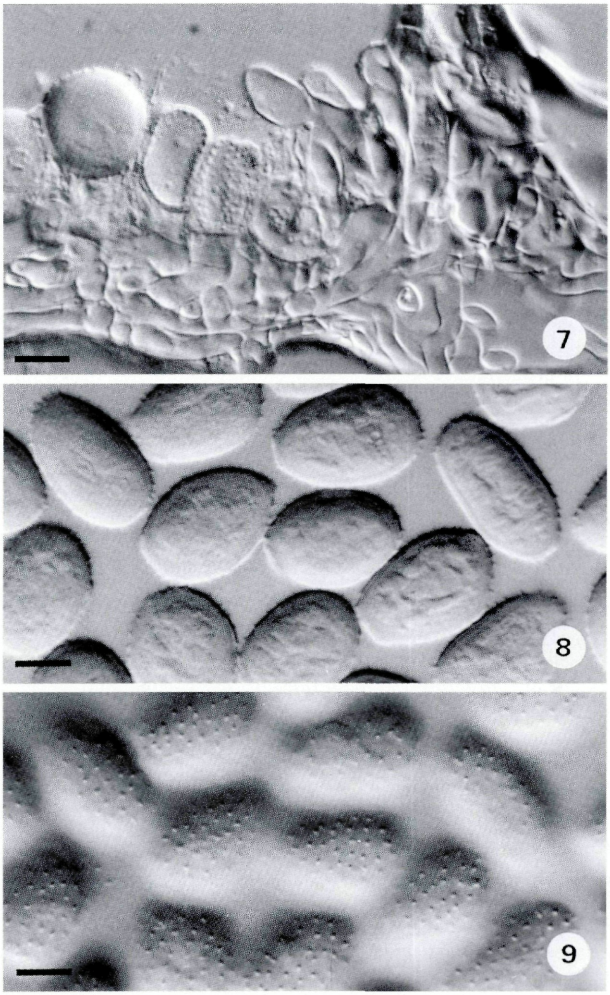
formed, no *Corydalis* plants are in actively growing from. They are resting in the form of tubers 5-10 cm deep in the ground.

The spermogonial and aecial stages on *Corydalis* plants are of systemic and perennial nature. Therefore, infection of *Asarum* plants by the aeciospores is possible every spring. It is not possible, however, to infect resting, underground tubers of *Corydalis* plants by basidiospores. Only tubers that are accidentally exposed to above ground in September to October may have the chance to be infected by the basidiospores. It is not known how often this kind of incident occurs in the field and whether or not this plays an important role in the host alternation in *C. asari*. The situation is exactly the same as observed in *C. dicentrae* for which this mechanism of host alternation was suggested (Mains, 1921).

Morphological characteristics

As mentioned above, the fungus apparently infects *Corydalis* plants systemically because the thin-walled probasidia on *Asarum* plants germinate upon maturity and collapse in the late growth season of a previous year, and because new leaves emerging from tubers in early spring in a succeeding year are already infected and bear sori. The fungus mycelia did apparently not spread equally to entire leaves: some leaflets of incised, compound leaves bore spermogonia and aecia while others of the same plant did not.

Spermogonia were formed almost always on abaxial surface of the leaves and are densely and uniformly distributed, subcuticular with a flat hymenium, hemispherical or discoid, 150–230 µm across and 90–130 µm high (Fig. 1). – Aecia occurred exclusively on the abaxial leaf surface. The sori were subepidermal in origin and became dome-shaped with central aparture (Figs. 2–3). As the sori developed and matured, the central aperture became larger. Although the sori were surrounded by peridium (Fig. 2), individual peridial cells were easily disjointed (Fig. 4). Therefore, the sori did not appear as typical, cup-shaped aecidia (Fig. 3). Aeciospores were subglobose, broadly ellipsoid or oblong-ellipsoid, and 22–32 x 18–24 µm in size (Fig. 5). The wall was evenly ca. 1 µm thick, colorless, and uniformly verrucose (Fig. 6). Thus, the aecial stage of this fungus differs from that of *O. kraunhiae* in spore size (14–24 x 11–16 µm, fide Sydow & Sydow, 1924) and in the degree of peridium integration (peridial cells being more or less firmly coherent, fide Sydow & Sydow, 1924). – Uredinia were formed in groups on abaxial leaf surface and were yellow and powdery due to mass of urediniospores. The sori were typical of the anamorph genus *Malupa* Ono & al. (Ono &



Figs. 7–9. – *Cerotelium asari* on *Asarum caulescens* (IBA-6706). – 7. Median cross section of uredinium (DIC). Thin-walled, cylindric paraphyses are present at periphery of the sorus. Urediniospores appear to be sessile. – 8. Urediniospores (DIC). – 9. Echinulation on the surface of urediniospores (DIC). – Scale bar = 10 μm.

al., 1992): they were marginally paraphysate and the urediniospores seemed sessile, being directly formed from the tip of hymenial mycelia in the sori (Fig. 7). The uredinial paraphyses were cylindrical or clavate, 32–40 μm long and 10–14 μm wide, and thin-walled (Fig. 7). The paraphyses merged at the base to become a pseudoparenchymatous peridial layer. Urediniospores were subglobose, obovoid-ellipsoid or oblong-ellipsoid, and 22–31 x 14–21 μm in size (Fig. 8). The wall was evenly 1–1.5 μm thick, colorless, and uniformly echinulate (Fig. 9). No germ pore was observed. – The basidiosori were minute, densely aggregate, reddish orange to orange-yellow, and waxy. The sori became pale yellowish and tomentose when most probasidia (teliospores) turned into basidiospore-bearing metabasidia. The metabasidium formation took place by continuous apical elongation of the probasidium. The probasidia were oblong or cylindric, seemingly catenulate in two (or rarely three) layers, and 34–54 X 8–14 μm in size (Fig. 10). The metabasidia were four-celled and a basidiospore (14–17 x 9–12 μm in size) was formed on a spiculum from each metabasidial cell (Fig. 11).

Comparison of uredinial and telial characteristics of the fungus found on *A. caulescens* at Mt. Yamizosan with those observed on the type specimen confirmed its taxonomic identity with *C. asari*. However, its generic placement in *Cerotelium* became questionable. As mentioned by Ono & al. (1992) for *C. tanakae*, species of *Cerotelium* that form thin-walled, cylindric probasidia that appear in two (or three) layers, i. e. *C. asari*, *C. dicentrae*, and *C. tanakae*, may have closer taxonomic affinity with *Aplopsora* than to *Cerotelium*. The probasidia of these three species appear to be two- (or rarely three-) layered, but may not be truly catenulate as in *C. canavaliae* (the type of *Cerotelium*), *C. bauhiniae*, *C. morobeana*, and *C. wagatae* (Ono & al., 1992).

Some cross-sections of the basidiosori of *C. asari* showed that a proximal cell of a seemingly two-layered probasidia buds give rise to another probasidium (Fig. 12), indicating that the proximal cell is not the probasidium but the basidiogenous cell. This mode is equivalent of sympodioconidia and comparable to the probasidium (teliospore) ontogeny observed in the Pucciniaceae (Hughes, 1970) and in the Chaconiaceae (Ono & Hennen, 1983). Because the first probasidium generated metabasidium and collapsed before the second probasidium attained its full maturity, it was not possible to determine the number of probasidia successively formed from the proximal, probably basidiogenous, cell.

For the rust genera with species forming thin-walled probasidia it is often difficult to determine the exact mode of probasidium ontogeny. However, probasidium ontogeny as well as mature morphology of probasidia are among the most important taxonomic

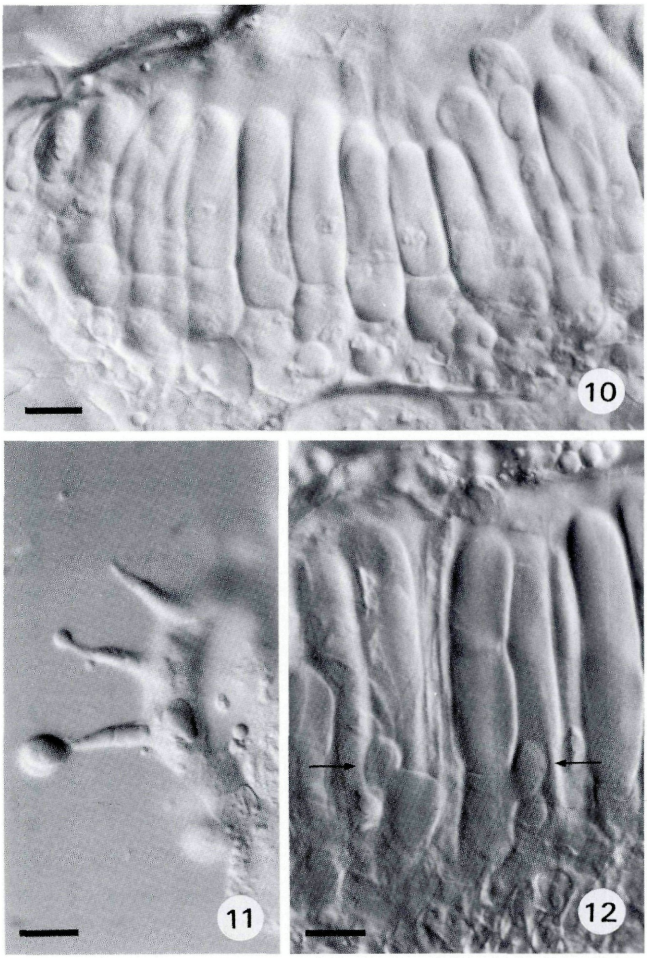


Fig. 10–12. – *Cerotelium asari* on *Asarum caulescens* (IBA-7022). – 10. Median cross section of telium (DIC). Cylindric probasidia are arranged in a pallisade layer and most are subtended by basal cell, thus appearing two-layered. – 11. Metabasidium with basidiospores on spicula (DIC). – 12. New probasidia (arrows) being formed from proximal cells of the seemingly two-layered probasidia (DIC). Scale bar = 10 μm.

characters to delimit rust genera. Therefore, the taxonomic identity or distinctness, and suggested relationships of the genera in the family Chaconiaceae are not conclusive (Ono & Hennen, 1983). It is not known whether probasidia of *Aplopsora* are successively formed from basal basidiogenous cell as in the genus *Chaconia* or whether distal cells of sorus-forming mycelia become probasidia as in the genus *Melampsora*. Perhaps species of *Aplopsora* form probasidia in a similar mode as was observed in *C. asari*. Until this issue is clarified, the *Corydalis-Asarum* alternating fungus is retained in *Cerotelium*.

Acknowledgments

SEM work was undertaken at the Institute of Agriculture and Forestry, University of Tsukuba; I thank Dr. M. Kakishima for technical assistance and for his invaluable comments on the study.

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(Manuscript accepted 7th September 1994)