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A new species of *Exobasidium* causing witches' broom on *Rhododendron wadanum*

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Witches' broom on *Rhododendron wadanum* has been observed since 1994 in Nagano Pref. and recently, in 2000, it was found at Mt. Tsukuba, Ibaraki Pref., Japan. In comparison with the morphology, the mode of germination of basidiospores and the cultural characteristics of the 99 validly described taxa, this fungus was considered as a new species and named *Exobasidium nobeyamense*.

Key Words——Basidiomycetes; Japan; *Rhododendron wadanum*; taxonomy; yeast-like growth.

Witches' broom on *Rhododendron wadanum* Makino has been observed since 1994 in Nagano Pref. and was found in 2000 at Mt. Tsukuba in Ibaraki Pref., Japan. *Rhododendron wadanum*, endemic in Japan, belongs to Subgen. *Sciadorhodion*, Sect. *Brachycalyx*. Most plants in this section are distributed in China and Japan. Two *Exobasidium* species have been reported on Sect. *Brachycalyx* (Ezuka, 1991a). Hymenia of *E. yoshinagae* Henn. appear on *R. wadanum*, *R. dilatatum* Miq., *R. kiyosumense* Makino, *R. reticulatum* D. Don, *R. viscistylum* Nakai var. *viscistylum*, *R. viscistylum* var. *glaucum* Sugim. and *R. weyrichii* Maxim. This fungus causes small leaf spot (*Exobasidium* leaf blister) (Ezuka, 1991a). On the other hand, *E. otanianum* Ezuka var. *otanianum* on *R. reticulatum* causes systemic infection of leaves and produces white hymenia on the lower side of leaves (*Exobasidium* leaf blight) (Ezuka, 1991a). Eventually, the infected leaves dry up and fall. *Exobasidium otanianum* var. *satsumense* X. Y. Zhang et K. Arai on *R. dilatatum* var. *satsumense* T. Yamaz. causes systemic infection of leaves and produces white hymenia on the lower side of leaves (*Exobasidium* leaf blight) (Zhang et al., 1995). These fungi cause symptoms on newly developing leaves but do not affect branches and twigs; accordingly witches' broom does not occur. Furthermore, *E. pentasporium* Shirai, described in 1896, causes witches' broom of *R. obtusum* (Lindl.) Planch. var. *kaempferi* (Planch.) E. H. Wilson and *R. macrosepalum* Maxim., which belong to other Subgen. *Tsutsusi* (Ezuka, 1990). However, no species was reported to cause witches' broom in Sect. *Brachycalyx*.

Therefore, we carried out morphological comparisons of the specimens collected in Ibaraki and Nagano Pref. with other *Exobasidium* spp. on *Rhododendron* spp. We propose the fungus producing witches' broom on *R. wadanum* as a new *Exobasidium* species.

Materials and Methods

Morphological observations Fresh specimens on *R. wadanum* collected in the field were used for morphological observations. Specimens examined are listed in the description of the species. Morphological observations were conducted by light and scanning electron microscopy. The basidiospores and conidia or thin sections of hymenia were mounted in Shear's solution on glass slides for LM observations. For SEM, the infected leaves with hymenia stored in FAA were used. Samples for SEM were prepared by modifying a standard procedure (Tanaka, 1992), using a glutaraldehyde (2% v/v) fixation procedure, dehydration, then t-butyl alcohol freeze-drying and coating with platinum-palladium in a high vacuum with a Hitachi E-1030 ion sputter. They were examined with a Hitachi S-4200 SEM operating at 15 kV.

Taxonomy

Exobasidium nobeyamense Nagao et Ezuka, sp. nov.
Figs. 1–6

Ramuli caespitosi in ramis cum nodis agangulioneis formantes. Hymenuim hypophyllum, effusum, saepe

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totam infrasuperficiem folii tegens. Folia infecta supra flava vel viridiflava, infra viridescentia dein albofarinosa, leviter carnosa. Basidia hyalina, clavato-cylindracea, 35–40 × 7–10 μm, terminaliter cum 3–6 sterigmatibus longiconicis 3.5–5.5 × 2–2.5 μm praedita. Basidiosporae hyalinae, laeves, cylindricae vel falcatae, ad apicem semiorbiculares, ad basim curvatae et angustatae, 12–21 × 2–5.5 μm, primo continuae dein 2–4 septatae, per hyphas germinantes. Conidia hyalina, continua, laevia, linearia, 7–12 × 1–2 μm. Coloniae in PDA restricte crescentes, ad ambitum irregulariter rugosae, ex hyphis circa 1 μm latis et conidiis constantes, pallide persicinae vel pallide aurantiacae, in agaro non pigmentiferae; reversum coloniis concolor.

Holotypus in foliis *Rhododendri wadani* Makino, Nobeyama, Minami-maki-mura, Minami-saku-gun, Nagano Pref. in Japonia, 25 V 1994. A. Ezuka leg., in Herbario Instituti Nationalis Scientiae Agro-Environmentalis, Tsukuba, Japania conservatus (NIAES 10569).

Etymology: Nobeyama, referring to the type locality.

Specimens examined: TSH-B0001, TSH-B0002 (Nagao 99775, 99776, Minami-maki-mura, Nagano Pref., May 26, 1999), TSH-B0003 (Nagao 12935, Mt. Tsukuba, Ibaraki Pref., May 25, 2000). All materials were deposited in the Herbarium of Laboratory of Plant Parasitic Mycology, Institute of Agriculture and Forestry, University of Tsukuba (TSH).

Hymenium was composed of basidia with 3 to 6 sterigmata and conidia (Fig. 2A). Hyphae did not develop directly on the surface of the epidermis. Basidia were clavate to cylindrical, 35–40 × 7–10 μm. Sterigmata were 2–2.5 μm in diam at the base and 3.5–5.5 μm in height, emerging outwardly and tapering toward the tip (Fig. 2B). Basidiospores were ellipsoid to ovoid, 12–21 × 2–5.5 μm, hyaline, smooth, one-celled when formed, becoming 2–4-septated (Fig. 1A, C). Septate basidiospores germinated after 6 h when dropped on the agar surface (Fig. 3). Germ tubes were 3–5 × 2–3 μm, emerging from each cell and producing conidia at the tip 12 h after of the dropping. Hyphae grew into pseudohyphae and branched. Conidia were long fusiform, 7–12 × 1–2 μm and budded polarly (Fig. 1B, D). Conidia budded to produce daughter cells polarly but did not develop germ tubes or hyphae. Colonies on PDA grew tightly, heaping up to form a mound, and wrinkled irregularly around the periphery. Surface of colonies was pale pink to pale orange and rather dry in appearance. Colonies composed of branching, intricate hyphae and conidia. The reverse of colonies was also pale pink to pale orange. Dark pigment was not produced on PDA (Fig. 4). Colonies from conidia showed the same morphological features as those from basidiospores.

The taxonomy of *Exobasidium* has been subjected to controversy because of the simple morphology and the highly variable symptoms (Burt, 1915; Ezuka, 1991b; Nannfeldt, 1981; McNabb, 1962; Savile, 1959; Sundström, 1964). Savile (1959) examined the morphology of basidia, basidiospores and sterigmata and synonymized several species described on *Rhododendron* from various parts of the world into *Exobasidium vaccinii*. In his judgment, the shape and size of basidiospores, the width of basidia, the size and number of sterigmata are more valuable taxonomic characters. Ninety-nine taxa of *Exobasidium* have been validly described at present. Of these, 16 taxa show similarities in some morphological measurements. However, *E. burtii* Zeller differs in the number of septa. Six taxa, *E. asebi* Hara et Ezuka, *E. canadense* Savile, *E. caucasicum* Woron., *E. japonicum* Shirai var. *japonicum*, *E. japonicum* Shirai var. *hypophyllum* Ezuka and *E. unedonis* Mre, differ in the sizes of basidia and numbers of septa of basidiospores. Three taxa, *E. cylindricum* Ezuka, *E. otanianum* Ezuka var. *otanianum* and *E. gracile* (Shirai) Syd. et P. Syd., differ in the sizes of basidia but agree in the number of septa of basidiospores. *Exobasidium karstenii* Sacc. et Trott. differ in the sizes of basidia and the mode of germination of basidiospores (budding). Five taxa, *E. affine* Racib., *E. dimorphosporum* Savile, *E. euryae* Syd. et P. Syd., *E. hachijoense* Y. Otani et al. and *E. sakishimense* Y. Otani, differ in both sizes of basidiospores and number of septa of basidiospores. The new species on *R. wadanum* differs morphologically from *E. yoshinagae*, causing leaf blister on *R. wadanum* and *E. pentasporium*, and witches' broom on *R. obtusum* var. *kaempferi*. Basidiospores of the new species are narrower than those of *E. yoshinagae* at their middle part. Basidiospores of the new species also differ from those of *E. pentasporium* in the number of septa. Sterigmata of the new species emerge outwardly, while those of *E. pentasporium*

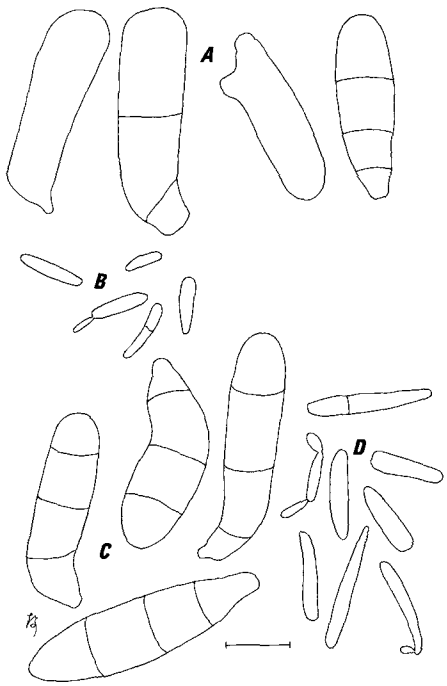


Fig. 1. Basidiospores and conidia of *Exobasidium nobeyamense* formed on the infected leaf of witches' broom on *Rhododendron wadanum*. Basidiospores (A) and conidia (B) collected in Nagano Pref. (TSH-B 0001). Basidiospores (C) and conidia (D) collected in Ibaraki Pref. (TSH-B 0003). Scale bar = 10 μm.

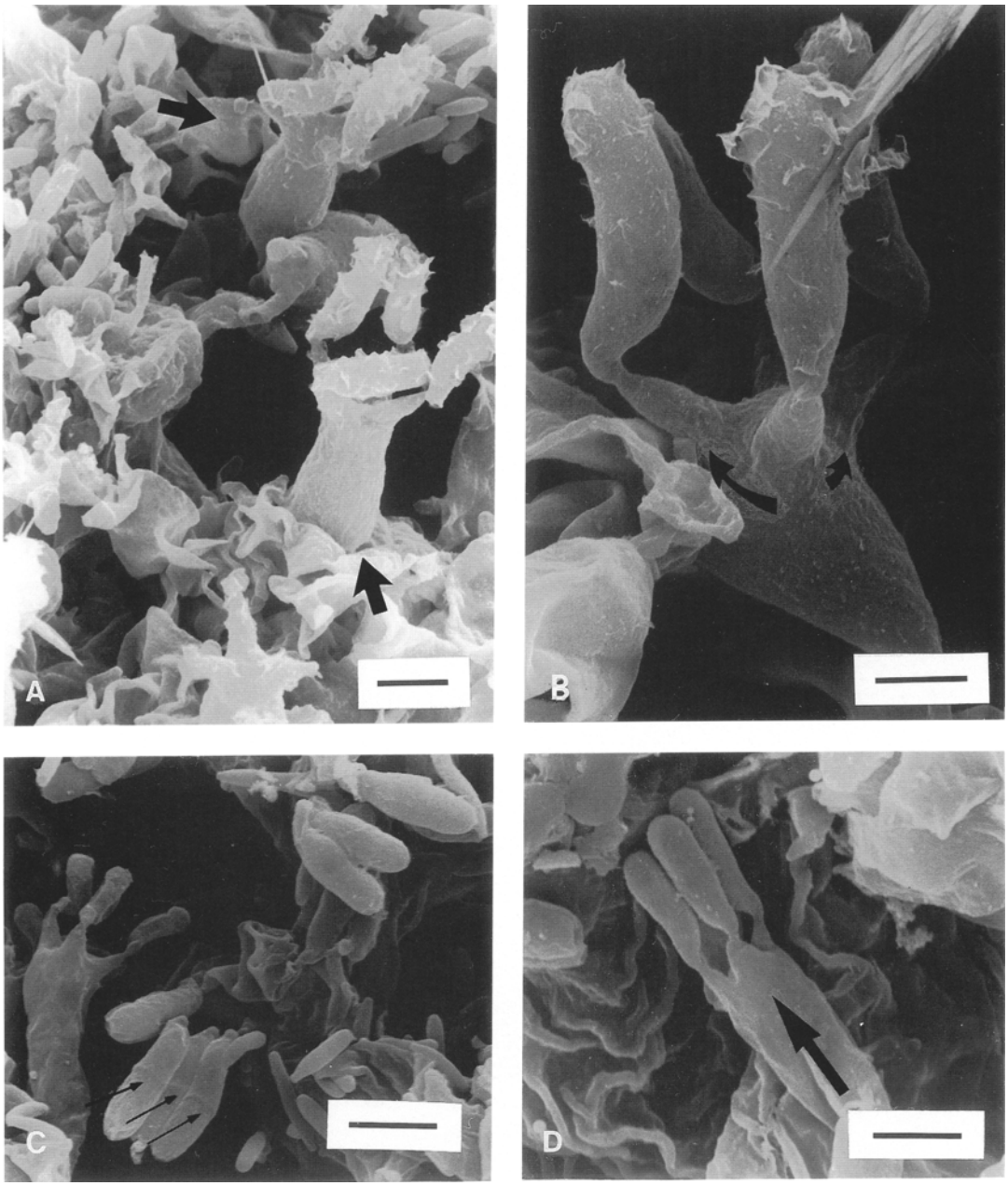
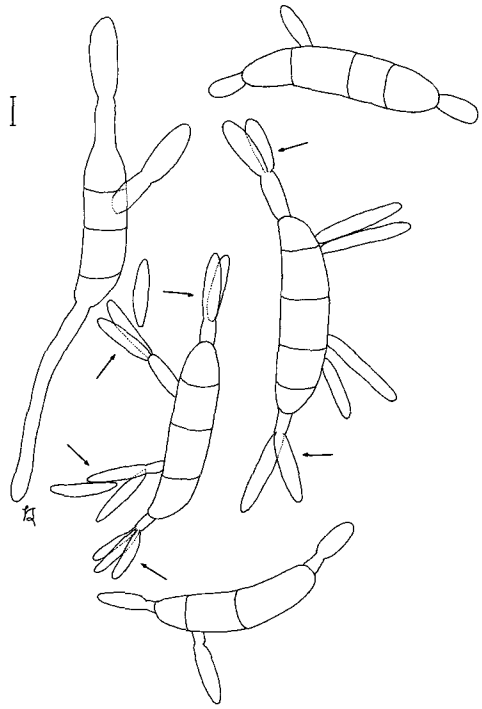


Fig. 2. Comparison of basidia of *E. nobeyamense* and *E. pentasporium*. *Exobasidium nobeyamense* observed by SEM. A. Hymenium of *E. nobeyamense* on *R. wadanum*. Arrows indicate two basidia bearing basidiospores. B. Basidium with immature basidiospores. Sterigmata were obviously emerging outwardly (arrowed). C. Hymenium of *E. pentasporium* on *R. obtusum* var. *kaempferi*. Septated basidiospores were observed (arrowed). D. Basidium with immature basidiospores. Sterigmata were emerging vertically (arrowed). Scale bar = 5 μm.

emerge vertically (Fig. 2C, D). Basidiospores germinate into a germ tube in all these species, but the new species differs from *E. pentasporium* in the number of germ tubes. Colonies of *E. pentasporium* on PDA produce dark pigment, but cultures of *E. nobeyamense* and *E. yoshinagae* do not show dark pigmentation on PDA (Fig. 4).

Graafland (1960) performed cross-inoculations between *E. vaccinii* and *E. japonicum* var. *japonicum*, which were assigned to one species because of the morphological similarities by Savile (1959). These cross-inoculations demonstrated a difference in pathogenicity between these fungi. Graafland (1960) considered that the physiological specialization of the fungus and the difference in aspect of the pure cultures were sufficient to distinguish the species. Although not all descriptions of *Exobasidium* species indicate the aspect of the pure cultures, this aspect would be as useful as the mode of germination to distinguish the species. In our observations, the germination mode and absence of dark pigment



in culture of the new species were distinctive characteristics.

Numerous twigs characterize witches' broom on *R. wadanum* and smaller young leaves, the abaxial surface of which is covered by white hymenia. Leaves from infected branches were smaller and slightly thicker than healthy ones, and white powdery hymenia were formed entirely or partially on the lower side of these leaves (Fig. 6). Hymenia were not observed on twigs and branches. Infected leaves then dried up rapidly, turned dark and fell. In witches' broom on *R. wadanum*, the branch was slightly thickened at the beginning of the infection. The number of twigs subsequently increased and the basis of the infected branch thickened year by year. The witches' broom grew to around 40 × 50 cm (Figs. 5ABC). Twigs on the infected branches did not thicken. Witches' broom on *R. obtusum* var. *kaempferi* infected by *E. pentasporium* formed a gall at the basis of infected branches (Fig. 5D). However, gall formation on *R.*

Fig. 3. Germination of the basidiospore of *E. nobeyamense* of witches' broom on *R. wadanum* after 8 h of incubation. Some of the basidiospores produced conidia at the tip of germ tubes (arrowed). Scale bar = 3 μm.

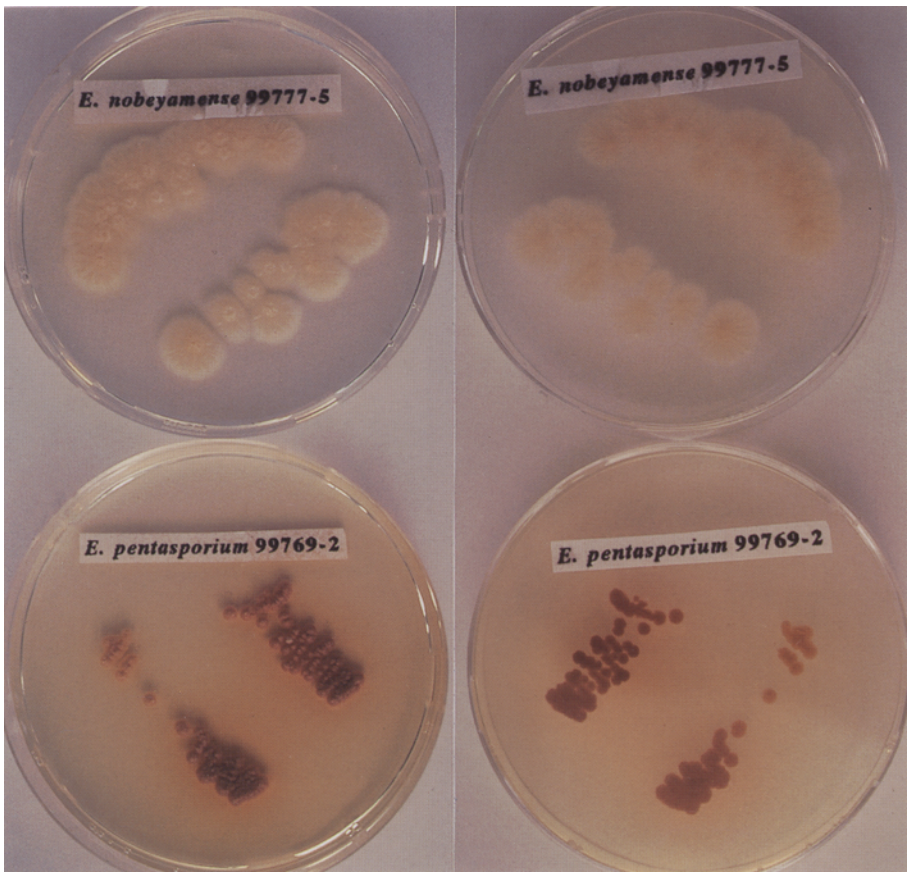


Fig. 4. Morphology and coloration of colonies formed by *E. nobeyamense* and *E. pentasporium* on PDA. Left. Surface of colonies of *E. nobeyamense* (upper) and *E. pentasporium* (lower). Unlike *E. pentasporium*, colonies of *E. nobeyamense* did not produce pigment on this medium. Right. Reverse of colonies of *E. nobeyamense* (upper) and *E. pentasporium* (lower). Submerged hypha in colonies of *E. nobeyamense* were not pigmented.

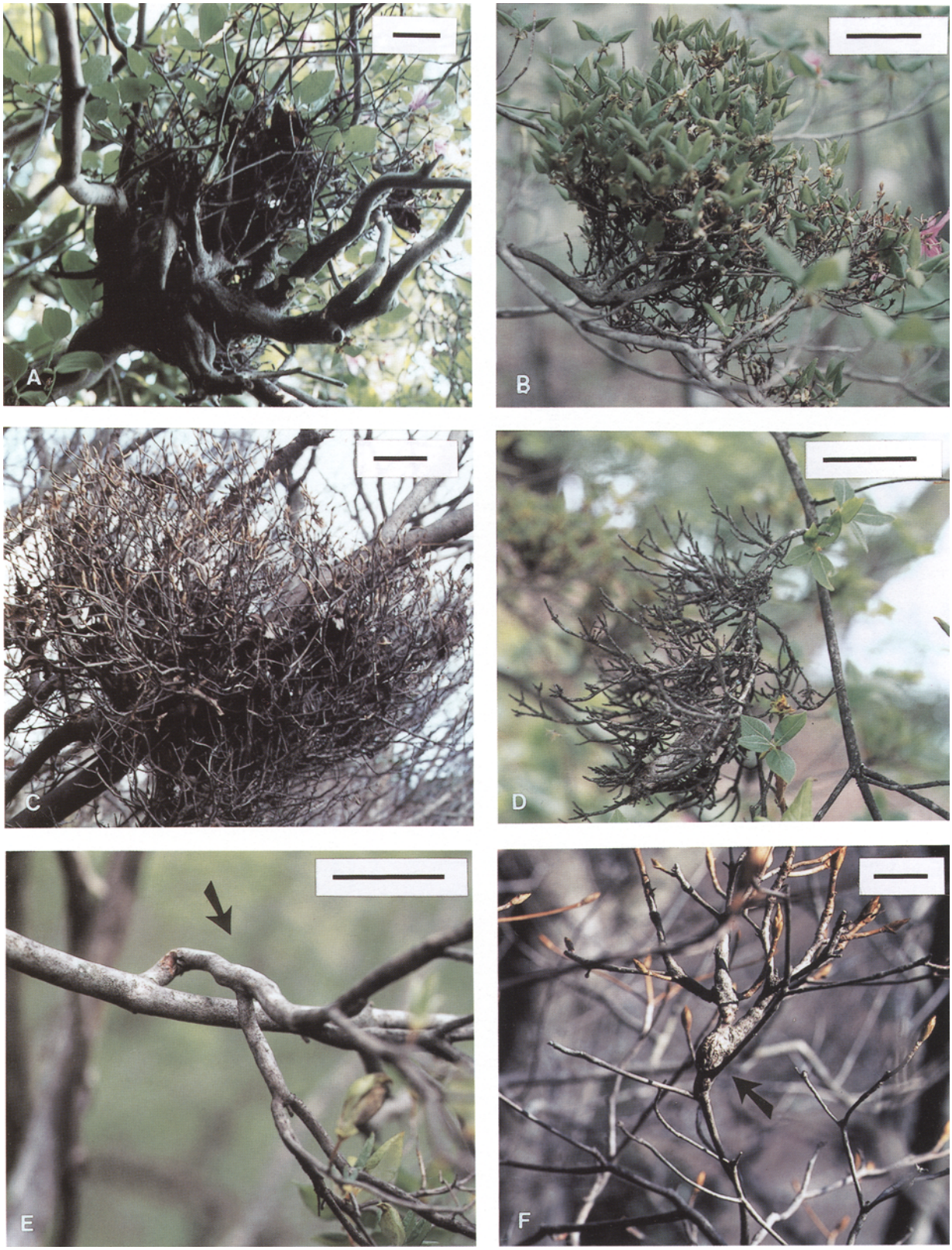


Fig. 5. Symptom of witches' broom on *Rhododendron* spp. by *Exobasidium* spp. A. Symptom of witches' broom observed on May, 2000 in Ibaraki Pref. B. Symptom of witches' broom observed on May, 1994 in Nagano Pref. C. Symptom of witches' broom before blooming observed on April, 2000 in Ibaraki Pref. D. Symptom of witches' broom on *R. obtusum* var. *kaempferi* by *E. pentasporium*. E. Typical symptom on branch of witches' broom on *R. wadanum* by *E. nobeyamense*. Infected branch was thickened but did not form gall (arrow). F. Symptom on branch of witches' broom on *R. wadanum* by *E. nobeyamense*. Infected branch formed round gall (arrow). Scale bars: A, B, C, D=50 mm; E=25 mm; F=20 mm.

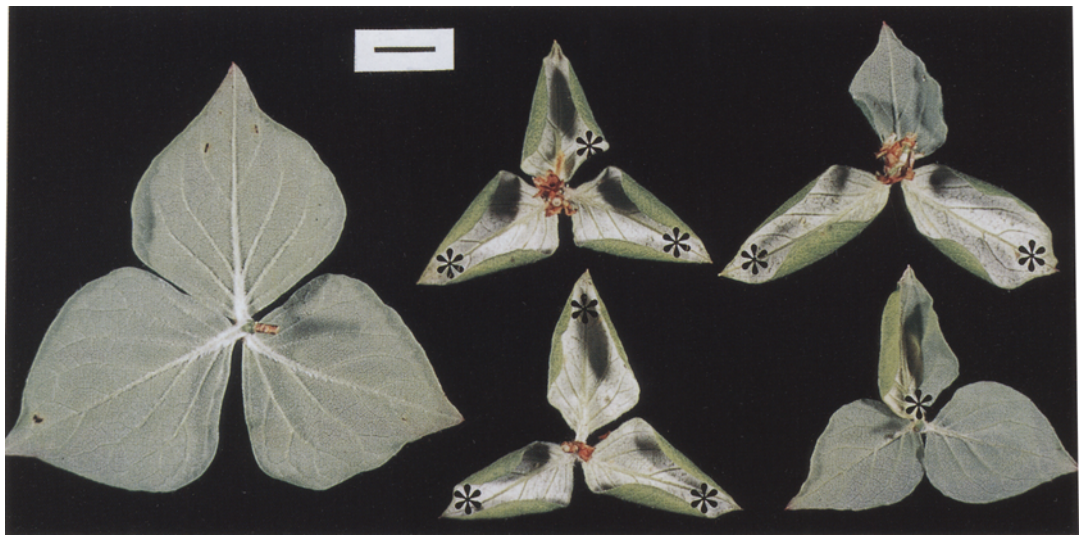


Fig. 6. Symptoms on leaf of witches' broom on *R. wadanum* by *E. nobeyamense*. White hymenia occurred on the lower surface of leaves (middle and right) and the infected leaves were smaller than healthy leaves (left). White hymenia formed partially on the lower surface of leaves (right). Asterisks indicate the regions of hymenia. Scale bar = 10 mm.

wadanum by *E. nobeyamense* was rarely observed at the basis of infected branches of witches' broom (Fig. 5E). No gall was observed on *R. wadanum* in Nagano Pref., but galls were found on the specimens in Ibaraki Pref. (Fig. 5F). *Rhododendron wadanum* infected by *E. yoshinagae* exhibited *Exobasidium* leaf blister but not witches' broom (Ezuka, 1991a). In most cases, blooming decreased on infected branches.

In contrast, the symptoms on *R. obtusum* var. *kaempferi* caused by *E. pentasporium* were characterized by increased number of twigs, smaller infected leaves, no blooming and formation of a round gall at the base of infected branches. Thickening of infected branches is a typical symptom of witches' broom on *R. wadanum* except a case observed at Mt. Tsukuba, Ibaraki Pref. Most of witches' brooms at Mt. Tsukuba were larger than those in Minami-maki-mura, Nagano Pref. Thickening of branches and the increase in the number of twigs of *R. wadanum* are considered to be the results of physiological reactions to *Exobasidium* infection, and therefore the mechanism of gall formation, and the effect of the age of infected plants and growth conditions should be studied *in situ* and/or with inoculation tests.

The difference in host plant and morphological characters of the species producing witches' broom on *R. wadanum* suggest that it is a new species.

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