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FULL PAPER

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Three species of *Exobasidium* causing Exobasidium leaf blight on subgenus *Hymenanthes*, *Rhododendron* spp., in Japan

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Abstract Three *Exobasidium* species causing Exobasidium leaf blight on *Rhododendron* of subgenus *Hymenanthes* in Japan are described and discussed. After examining the holotype and fresh materials of *E. shiraianum* on *R. degronianum*, its description is emended by the morphology of basidiospores that are ellipsoid to ovoid, or obovoid, 11–21 × 5–8 μm, and with 1–3 septa. Culture of this species showed yeast-like growth. *Exobasidium woronichinii* on *R. brachycarpum* observed from Hokkaido Prefecture to Nagano Prefecture is described as a new species characterized by its ellipsoid to ovoid, 11–19 × 3–4.5 μm, and 1–5(–6)-septated basidiospores. Culture of this species was gelatinous but obtrite, or thick and showed farinose appearance by conidiation. A fungus on *R. aureum* in Hokkaido and Nagano Prefectures is identified as *E. caucasicum*. This species is new to Japan and is characterized by its ellipsoid to ovoid, or obovoid, 11–19 × 3–6 μm, and 0–2-septated basidiospores. Culture of this species showed yeast-like growth.

Key words Basidiomycetes · Culture · *Exobasidium* · Germination · Japan · Taxonomy

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Introduction

Exobasidium shiraianum Henn. was described in 1903 as a new species to accommodate a fungus that caused Exobasidium leaf blight on *Rhododendron metternichii* Siebold et Zucc. collected by Prof. S. Kusano in Mt. Shirane, Japan (Hennings 1903). *Exobasidium hemisphaericum* Shirai was also reported to infect several Rhododendrons in the subgenus *Hymenanthes* and caused leaf gall (Anonymous 2000). This species was described in 1896 based on a specimen on *R. metternichii* var. *hondoense* Nakai (Shirai 1896). This leaf gall was well documented in Japanese textbooks of plant pathology (Ideta 1901, 1903, 1929), whereas *E. shiraianum* was merely noted in a textbook (Ideta 1929). Morphology and host range of *E. shiraianum* were described by Ito (1955). Even if a picture of symptoms of Exobasidium leaf blight on *Rhododendron* sp. (Fig. 38a in Ito 1955) and line drawings of basidia and basidiospores (Fig. 38b in Ito 1955) were given, the size of basidiospores was exactly the same as the original description. He listed *R. fauriae* Franch var. *rufescens* Nakai (Shirobana-syakunage in Japanese) and *R. chrysanthum* Pall. (Kibana-syakunage in Japanese) as host plants of this species. In the latest monographic work (Davidian 1992), legitimate names of *Rhododendron* species in the subgenus *Hymenanthes* were proposed. *Rhododendron metternichii* was synonymized in *R. degronianum* Carrière, and *R. fauriae* var. *rufescens* and *R. chrysanthum* were assigned to *R. brachycarpum* D. Don ex G. Don and to *R. aureum* Georgi, respectively. The host plant originally described in *E. shiraianum* was *R. degronianum*, called "Azuma-syakunage" in Japanese. Thus, there is discrepancy in the record of host plants: *Rhododendron brachycarpum* and *R. aureum* have been noted as hosts of *E. shiraianum* (Ito 1955; Ezuka 1998; Anonymous 2000). Host range noted in these reports is evidently different from that in the original description by Hennings (1903).

In 1999, Dr. A. Ezuka found Exobasidium leaf blight on *R. degronianum* in Nagano Prefecture. We also collected several samples of Exobasidium leaf blight on *R.*

brachycarpum to compare the morphology and cultural characteristics of these fungi. Based on these results, we discuss the taxonomy of *E. shiraianum* and propose a new species to accommodate an *Exobasidium* specimen on *R. brachycarpum*.

The symptom caused by *E. caucasicum* Woron. on *R. aureum* described in Russia (Woronichin 1921) was yellowing of developing leaves and formation of farinose hymenium on the lower side (Exobasidium leaf blight), quite similar to that caused by *E. shiraianum* (Woronichin 1926). The lack of records of *E. caucasicum* in the Japanese mycological literature (Anonymous 2000; Ezuka 1998; Ito 1955) gives us to believe that the occurrence of *E. caucasicum* has not been recognized in Japan. However, in our recent survey of the herbarium materials, there are several specimens collected in Japan that have been sorted as *E. caucasicum* in the Herbarium of the Hokkaido University Museum (SAPA). Examining these specimens and another fresh material collected in Nagano Prefecture in 2002, we here describe morphological features of *E. caucasicum* in Japan.

Materials and methods

Morphological observations

Fresh materials of *Exobasidium* species on *R. aureum, R. brachycarpum* and *R. degronianum* collected in the field were used for morphological observations. Specimens examined are listed in the description of the species (Table 1). Materials for morphological observations were prepared and conducted by light (LM) and scanning electron microscopy (SEM) as described previously (Nagao et al. 2003). Samples for SEM were prepared and observed as mentioned previously (Nagao et al. 2001). All materials were deposited in the Mycological Herbarium of Plant Parasitic Mycology, Institute of Agriculture and Forestry, University of Tsukuba (TSH) and the Herbarium of the National Institute of Agro-Environmental Sciences, Tsukuba, Ibaraki, Japan (NIAES).

Culture of basidiospore isolate

Fresh materials were kept in a plastic bag until newly sporulating lesions were observed. Colonies from a single basidiospore were obtained as described previously (Nagao et al. 2003). Cultures were kept in the Laboratory of Plant Parasitic Mycology, Institute of Agriculture and Forestry, University of Tsukuba, and some of the isolates of *Exobasidium* spp. obtained in this study were deposited in Genebank, National Institute of Agrobiological Sciences, Japan (MAFF).

Table 1. Morphological measurements of *Exobasidium* spp.

Species	Size of basidia (μm)	Size of sterigmata (μm)	Number of sterigmata	Size of basidiospores (μm)	Septal number of basidiospores
E. shiraianum					
Holotype S-F20843 (Hennings 1903)	nd	nd	4	7–11 × 2.5	nd
Holotype S-F20843	5–22 × 5–9	2–5 × 1–1.5	2–3	11–18 × 5–7	1–3
TSH-B 0023	nd	nd	nd	16–18 × 7–8	(1) 3
TSH-B 0025	nd	nd	nd	12–18 × 5.5–8	1–3
TSH-B 0026	nd	nd	nd	13–21 × 6–8	1–3
TSH-B 0027	15–30 × 5–7	2–6 × 1–2	2–3	14–18 × 6–8	1–3
E. woronichinii					
Holotype TSH-B 0081	22–40 × 4.5–8	4–5 (5.5) × 1.5–2	2–4	11–16 × 3–4.5	1–3 (5)
TSH-B 0085	11–50 × 6–8	4–5 × 1–2	2–3	11–16 × 3–4	(1) 2–5 (6)
TSH-B 0083	nd	nd		12–17 × 3–4	1–5
TSH-B 0114	13–60 × 6–7	2–6 × 1.5–2	2–3	11–17 × 3–4.5	1–5
TSH-B 0115	15–24 × 6–8	4–6 × 1–1.5	2	13–18 × 3–4.5	1–4
TSH-B 0116	20–30 × 6–12	3–5 × 1–1.5	2–3	12–19 × 3–4.5	2–4 (6)
TSH-B 0021	nd	nd		11–18 × 3–4	1–4
TSH-B 0022	10–40 × 5–8	2–6 × 1–2	2–4	12–17 × 3.5–4	1–3 (4)
NIAES20541	30 × 9–10	nd	3–4	12–19 (22) × 3–4	1–3
E. hemisphaericum					
(Shirai 1896)	nd	nd	4	15–19 × 3.5–4.5	nd
(Ito 1955)	nd	nd	4	15–19 × 3.5–4.5	3
TSH-B 0032	25–32 × 6–9	2–3 × 1–2	2–4	12–20 × 3–4	1–4
TSH-B 0033	22–35 × 7–8.5 (10)	2–3 × 1–1.5	2–3	20 × 7.5	nd
E. caucasicum					
Caucasus (Woronichin 1920)	39.6–42.9 × 6.6–8.8	nd	(2–3) 4	14–16.5 × 5	0–1
Kamchatka (Woronichin 1926)	42–52 × 7–10	nd	4	14–17.5 × 5–5.5	nd
NIAES20542	15–29 × 8–12	2–4 × 1–2	2–4	13–19 × 4–6	(0) 1–2
SAPA Aug. 5, 1925	nd	nd	nd	15 × 6	2
SAPA Aug. 4, 1903	20 × 6–7.5	nd	4	11–16 × 3–4.5	1–2
SAPA July 30, 1921	13–35 × 5–6	2–4 × 1.5–2	2–3	13–14 × 4	1
SAPA Aug. 1, 1895	nd	nd	nd	11–17 × 4–6	(0) 1

nd, not determined

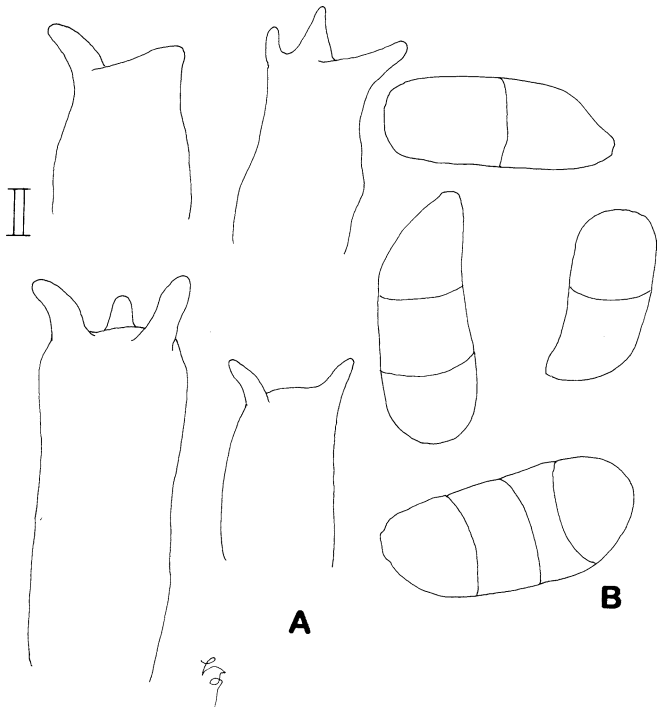

Fig. 1. Basidia (**A**) and basidiospores (**B**) of *Exobasidium shiraianum* holotype (S, F20843). *Bar* 3 μm

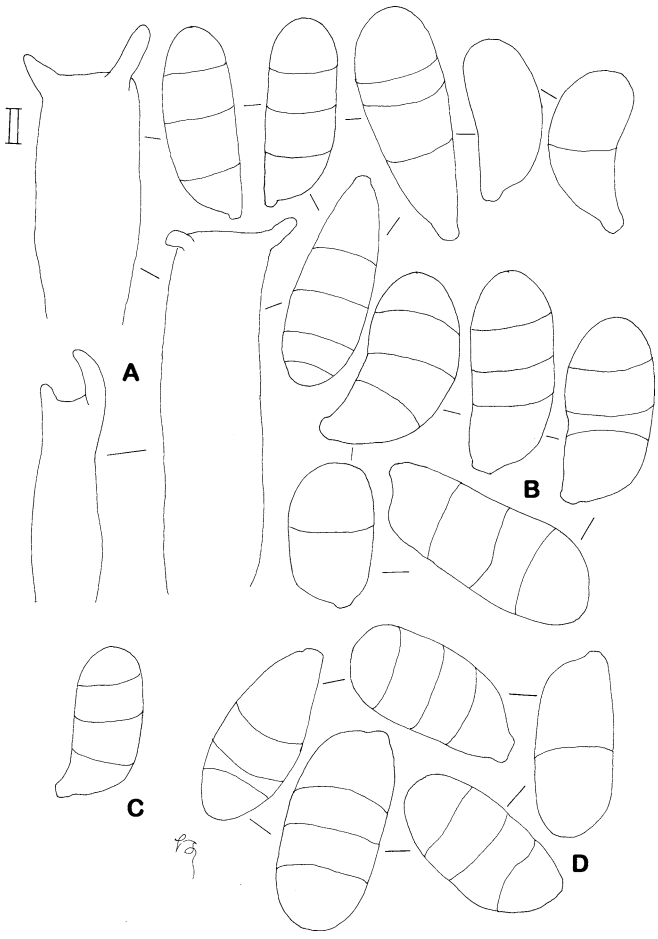

Fig. 2. Basidia and basidiospores of *E. shiraianum* TSH-B0027 (**A**), TSH-B0026 (**B**), TSH-B0025 (**C**), and TSH-B0023 (**D**). *Bar* 3 μm

Taxonomy

1. ***Exobasidium shiraianum*** Henn., Bot. Jahrb. 32: 38, 1903. emend Nagao Figs. 1, 2

Hymenium composed of basidia with 2 or 3 sterigmata and conidia. Hyphae not developing directly on the surface of epidermis. Basidia emerging directly from the host surface or through stomata, not fasciculate, clavate to cylindrical, 5–30 × 5–9 μm (Figs. 1A, 2A), obtuse at the apex. Sterigmata 1–2 μm in diameter at the base and 2–6 μm in height, tapering toward the tip (Fig. 11A–C). Basidiospores ellipsoid to oval, or obovoid, 11–21 × 5–8 μm, hyaline, smooth, with 1–3(–4) septa (Figs. 1B, 2, 11D, 12A). Septate basidiospores dropped on the agar surface germinating after 15 h (Fig. 3). Germ tubes or conidia emerging from the spore cells. Pseudohyphae not distinguishable from budded conidia. Conidia bacilliform or subfusiform (Fig. 9A–C), 2–10 × 1–2 μm, and budding polarly in culture (Figs. 3, 12A; Table 2) to produce daughter cells and also to develop pseudohyphae. Colonies on potato dextrose agar (PDA) growing gradually, reaching maximum 13 mm diameter in 21-day incubations at 22°C, and wrinkling irregularly at the periphery, with pale yellow and smooth surface not becoming farinose by conidial formation, glutinous and not fixed on the agar surface, composed of pseudohypha and conidia. The reverse of colonies dark yellow to pale pink. Dark pigmentation not exuded into PDA (Fig. 13A–C). Colonies from conidia showing the same morphological features as those from basidiospores.

Specimens examined: On *R. metternichii*, (syn. of *R. degranianum*) Prov. Shimozuke, Mt. Shirane, July 14, 1900, S. Kusano leg. (holotype S-F20843); on *R. degranianum,* Mt. Tengu-yama, Minamimaki-mura, Nagano Prefecture, July 26, 1999, A. Ezuka leg. (TSH-B0023, B0024, B0025, B0026)

Hennings (1903) briefly described the shapes of basidia and basidiospores, the number of sterigmata, and the size of basidiospores as 7–11 × 2.5 μm. He precisely described the symptom of infected leaves. As the hymenial appearance does not accompany gall formation, he again commented on this "atypical" symptom compared with the symptom caused by *E. pentasporium*. We examined the holotype and observed some basidiospores and basidia with sterigmata (Fig. 1). We noticed that the size of basidiospores (11–18 × 5–7 μm with 1–3 septa) was different from the description by Hennings (Fig. 1B). We complemented the morphological characteristics based upon the holotype and TSH-B 0027. This species is characterized by the width of basidiospores (5–8 μm) (Figs. 1B, 2, 11D, 12A). In our observations, size and shape of conidia of *E. shiraianum* (2–10 × 1–2 μm) were quite different from those of basidiospores of *E. shiraianum* (Tables 1, 2). We supposed that the size mentioned in the original description was that of conidia rather than that of basidiospores. Among 102 taxa of *Exobasidium* validly described so far, the following 8 taxa showed similarities to *E. shiraianum* in some morphological

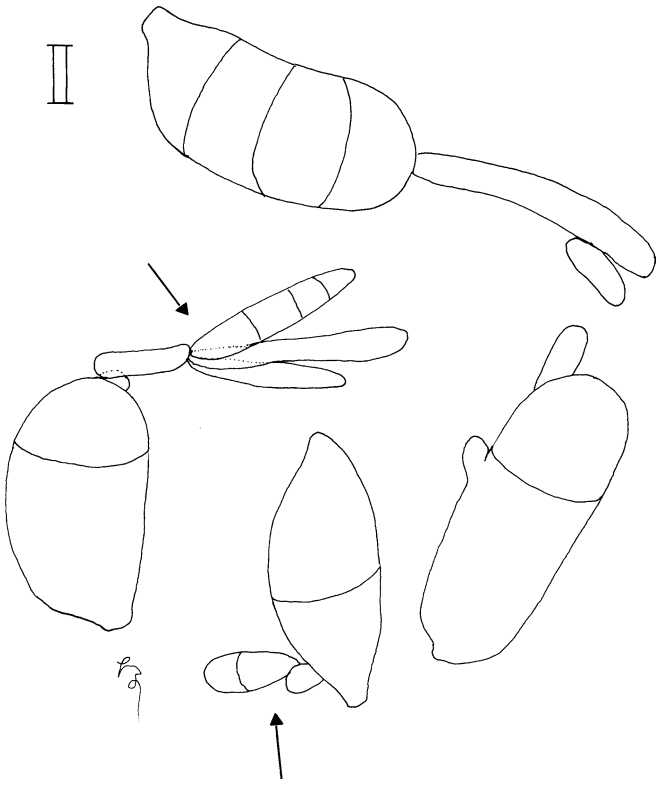

Fig. 3. Basidiospore germination of *E. shiraianum* (TSH-B 0025) on potato dextrose agar (PDA) after 12 h incubation. Some of the basidiospores produced conidia on the germ tube (*arrows*). *Bar* 3 μm

Table 2. Conidial morphology of *Exobasidium* spp.

Species	Size of conidia (μm)	Septal number of conidia
E. shiraianum		
MAFF238602	2–5 × 1–1.5	0
MAFF238603	3–10 × 1–2	0
MAFF238604	3–8 × 1–2	0
E. woronichinii		
MAFF238617	2–25 × 1–2	0
MAFF238618	3–19 × 1–2	0–1 (4)
MAFF238666	6–26 × 1–1.5	0
MAFF238667	2–17 × 1–2	0
MAFF238625	5–30 × 1–2	0–4
MAFF238622	4.5–18 × 1–2.5	0
MAFF238610	3–12 × 1–2	0
MAFF238825	5–21 × 1–2	0
E. hemisphaericum		
E-11[a]	5–24 × 1–1.5	0–2
E-13[a]	5–24 × 1–2	0
E. caucasicum		
Caucasus (Woronichin 1920)	5 × 1.5	nd
Kamchatka (Woronichin 1926)	7–10 × 1.75–3	nd
MAFF238830	3–7 × 1–2	0

nd, not determined
[a] Culture was deposited in the Laboratory of Plant Parasitic Mycology, Institute of Agriculture and Forestry, University of Tsukuba

measurements (Table 3). *Exobasidium shiraianum* differed from *E. aequale* Sacc., *E. dimorphosporum* Savile, *E. dracophylli* McNabb, *E. splendidum* Nannf., and *E. vaccinii-uliginosi* Boud. in the septal number of basidiospores, from *E. bisporum* Sawada ex Ezuka in the septal number and mode of germination of basidiospores, and from *E. camelliae* Shirai and *E. nudum* S. Ito et Y. Otani in the mode of germination of basidiospores. *Exobasidium shiraianum* is surely proved to be valid species. Exobasidium leaf blight on *R. degronianum* is characterized by the chlorosis and powdery appearance on the lower surface of newly developed leaves (Fig. 14A–C). The host plant and distribution of *E. shiraianum* may be restricted. When we surveyed the occurrence of this fungus on *R. degronianum* at the Hanazono-jinja shrine, Kitaibaraki-shi, Ibaraki Prefecture in June 2001, at Mt. Sanbon-yariga-take, Nasu, Tochigi Prefecture in June 2001, and at Yuno-ko Lake and Mt. Ohmanako-yama, Nikko-shi, Tochigi Prefecture in August 2000 and July 2001, we could not find it.

2. ***Exobasidium woronichinii*** Nagao, sp. nov. Fig. 4

Misapplied name: *Exobasidium shiraianum* sensu S. Ito, Mycological Flora of Japan 2(4):51, 1955–pro parte, non Henn. 1903.

Hymenium hypophyllum, effusum, saepe totum infrasuperficiem folii tegens. Folia infecta flava vel albolutescentia, infra concoloria dein albofarinosa, leviter carnosa. Basidia hyalina, clavato-cylindracea, 10–60 × 4.5–12 μm, terminaliter cum 2–4 sterigmatibus longiconoideis 2–6 × 1–2 μm praedita. Basidiosporae hyalinae, leaves, unciformes vel reclinatae, ad apicem muticae, ad basim curratae et angustatae, 11–19(22) × 3–4.5 μm, primo continuae dein 1–5(6)-septatae, per hyphas germinantes. Conidia hyalina, continua, laevia, linearia, 2–30 × 1–2.5 μm, 0–1(4) septata. Coloniae in PDA restricte crescentes, post 21 dies maxime 22 mm diameter attingens, corrugatae, gelatinosae, ad ambitum irregulares, ex hyphis circa 1 μm latis et conidiis constantes, pallide primulinae vel pallide aurantiacae, in agaro non pigmentiferae; reversum concolor.

Etymology: Referring to Russian mycologist, N. Woronichin, who described several new species of *Exobasidium*.

Holotypus in folliis vivis *Rhododendri brachycarpi* D. Don ex G. Don, Me-akan-dake spa, Hokkaido Pref. in Japonia, 14 VI 2002, H. Nagao leg., in Herbario Instituti Agriculturae et Silviculturae, Universitatis Tsukubensis, Tsukuba, Japonia (TSH-B0081) conservatus.

Specimens examined: TSH-B0083 (on *R. brachycarpum,* Me-akan-dake spa, Hokkaido Prefecture, June 14, 2001, H. Nagao leg.); TSH-B0018, TSH-B0021, TSH-B0022 (on *R. brachycarpum,* Mt. Tengu-yama, Minamimaki-mura, Nagano Prefecture, June 26, 1999, A. Ezuka leg.); TSH-B0114, TSH-B0115 (on *R. brachycarpum,* Kumami-sone, Mt. Nasu-dake, Nasu, Tochigi Prefecture, June 14, 2001, H. Nagao leg.); TSH-B0116 (on *R. brachycarpum,* Mt. Sanbon-yariga-dake, alt. 1917 m, Yumoto, Nasu-machi, Nasu-gun, Tochigi Prefecture, June 14, 2001, H. Nagao leg.); NIAES 20541 (on *R. brachycarpum,* Mt. Hakkoda Botanical

Table 3. Comparison of morphological measurement among *Exobasidium* spp.

Species	Sizes of basidia (μm)	Sizes of sterigmata (μm)	Number of sterigmata	Sizes of basidiospores (μm)	Number of septa of basidiospores	References
E. shiraianum	15–30 × 5–7	2–6 × 1–2	2–3	14–18 × 6–8	1–3	
E. aequale	6–8 wide	4–6 long	2	(14) 15–22 × (6) 7–9 (10)	0	Symb. Bot. Ups. 23(1981):1–72
E. bisporum	40–60 × 6–8	4–6 × 2–3	2 (3)	14–24 (27) × 4–7	1–7	Trans. Mycol. Soc. Jpn 32(1991):169–185
E. bisporum	60–80 × 5–7	4–7 × 1.5–2.5	2	15–22 × 5–8	1–4	Trans. Mycol. Soc. Jpn 32(1991):169–185
E. camelliae	130–160 × 6–12	4–6 × 2–3	2–3 (4)	15–25 × 5–7.5	(1) 3 (7)	Trans. Mycol. Soc. Jpn 31(1990):375–388
E. dimorphosporum	26–40 × 5–9.5	5–7 × 1.5–2.5	2–3	13–18.5 × 6.5–8.5; 18.5–28.5 (32) × 5–7 (8)	0 (1)	Can. J. Bot. 37(1959):641–656
E. dracophylii	25–45 × 5–6.5	6–8.5 long	2 (3)	20–27 × 5–8	0 (1)	Trans. N-Z. Bot. 1 (1962):259–268
E. nudum	100 × 5–8	4–5 × 2	(2) 4	10–20 × 4.5–8	1–3	Trans. Mycol. Soc. Jpn 31(1990):375–388
E. splendidum	6–8 wide	nd	2	(15) 20–27 × 6–11.5	0	Symb. Bot. Ups. 23(1981):1–72
E. vaccinii-uliginosi	9–10 wide	7 long	2	16–23 (28) × 6.5–9 (12)	0	Symb. Bot. Ups. 23(1981):1–72
E. woronichinii	22–40 × 4.5–8	4–5 (5.5) × 1.5–2	2–4	11–16 × 3–4.5	1–5 (6)	
E. canadense	24–40 × 5.5–8	2.5–4.5 × 1.5–2	2–4 (5)	14–20 × 3–4.7	1–3	Can. J. Bot. 37 (1959):641–656

nd, not determined

Fig. 4. Basidia and basidiospores of *E. woronichinii.* Holotype TSH-B0081 (**A**), NIAES 20541 (**B**), TSH-B0022 (**C**), and TSH-B0014 (**D**). *Bar* 3 μm

Garden, Tohoku Univ., Sukayu, Aomori Prefecture, June 9, 2002, Y. Harada leg.)

Hymenium composed of basidia with 2 to 4 sterigmata and conidia. Hyphae not developing directly on the surface of epidermis. Basidia clavate to cylindrical, 10–60 × 4.5–12 μm (Fig. 4A,C,D), with obtuse apex, emerging directly from leaf surface or through stomata, not fasciculate. Sterigmata 1–2 μm in diameter at the base and 2–6 μm in height, emerging outwardly and tapering toward the tip. Basidiospores ellipsoid to ovoid, 11–19(22) × 3–4.5 μm, hyaline, smooth, one-celled when formed, becoming septate with 1–5(6) septa (Figs. 4, 12B), slightly curved and tapering at the end. Septate basidiospores dropped on the agar surface germinated after 6 h (Fig. 5). Germ tubes of the basidiospores emerging from cells of both ends at first then from other cells and producing conidia at the tip or lateral of germ tubes 22 h after dropping (Fig. 5). Hyphae growing into pseudohyphae and branched. Conidia bacilliform, lacrimiform, subfusiform, and clavulate (Fig. 9D–G), 2–30 × 1–2.5 μm with 0–1(4) septa, and budding polarly (Table 2), to produce daughter cells polarly and also develop

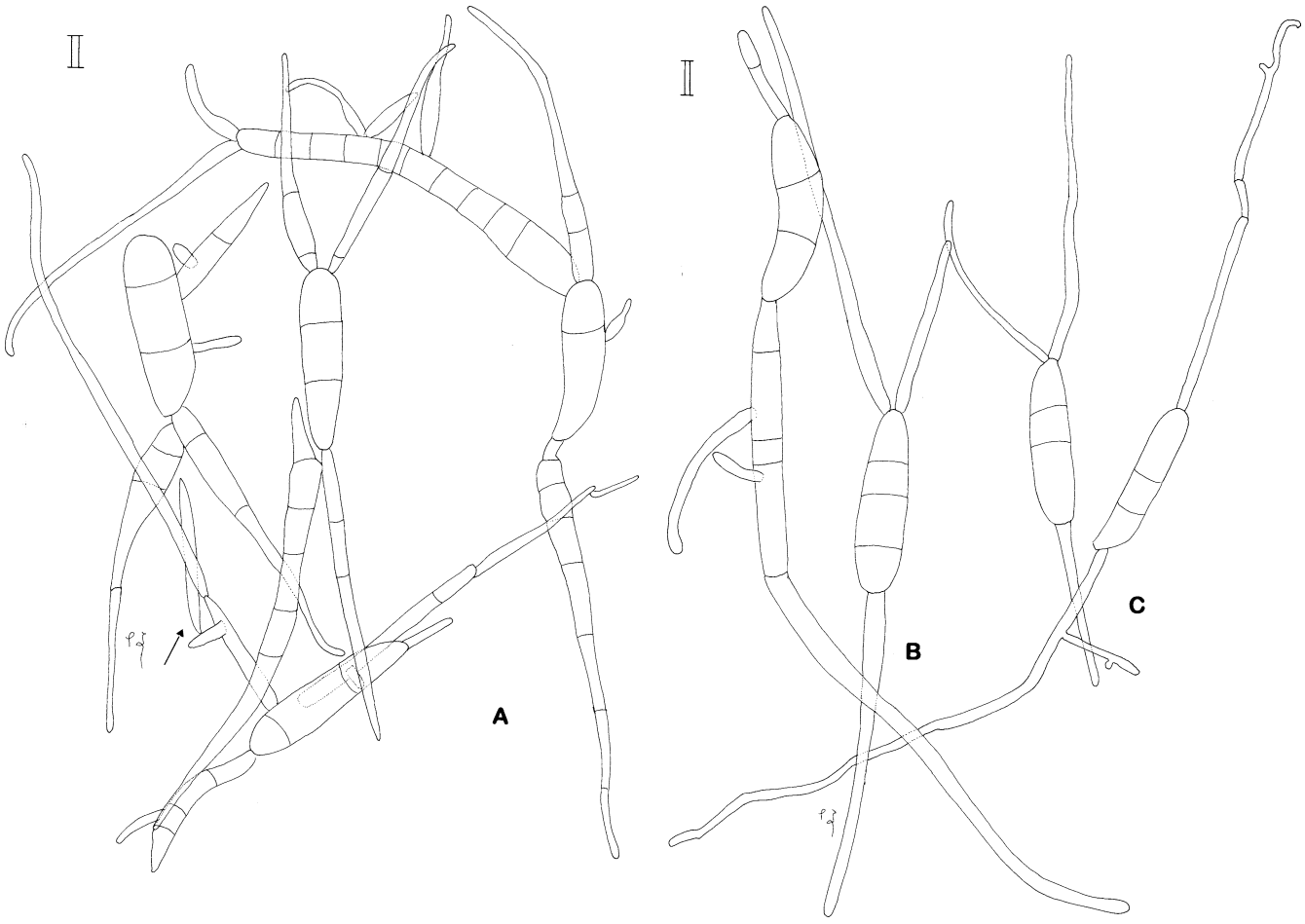

Fig. 5. Basidiospore germination of *E. woronichinii* (TSH-B 0040) on PDA after 22 h incubation. Some of the basidiospores produced conidia on the germ tube (*arrow*): TSH-B0116 (**A**), TSH-B0081 (**B**), and NIAES 14236 (**C**). *Bars* 3 μm

hyphae. Colonies on PDA growing gradually, reaching maximum 22 mm diameter in 21-day incubations at 22°C, and wrinkling irregularly at the periphery, with pale primrose-yellow to pale orange and corrugate surface, gelatinous but obtrite, or thick, often providing farinose appearance, composed of branching, intricate hypha and pseudohypha, and conidia. The reverse of colonies pale yellow to pale orange. Dark pigment not exuded on PDA (Fig. 13D,E). Colonies from conidia showing the same morphological features as those from basidiospores.

Among 102 taxa of *Exobasidium* that have been validly described, *E. canadense* Savile and *E. hemisphaericum* showed similarities in some morphological measurements (Tables 1, 3) to this new species, which, however, was distinguished from *E. canadense* in number of septa of basidiospores. The shape of basidiospores of *E. canadense* were musiform judged from the line drawing by Savile (1959) as well as that of *E. woronichinii*, whereas that of *E. hemisphaericum* was clavate to cylindrical. Especially, basidiospores of *E. woronichinii* showed strong curvature at the end of spores (Figs. 4A–D, 12B) and were distinguishable from *E. canadense* and *E. hemisphaericum* (see Fig. 10B; fig. 3 in Savile 1959). Exobasidium leaf blight on *R. brachycarpum* is characterized by the chlorosis and powdery appearance on the lower surface of newly developed leaves (Fig. 15A–F).

3. ***Exobasidium caucasicum*** Woron., Monit. Jard. Bot. Tiflis 51: 3, 1921. Figs. 6, 7

Hymenium composed of basidia with 2 to 4 sterigmata and conidia. Pseudohyphae not developing directly on the surface of epidermis. Basidia clavate to cylindrical, 13–35 × 5–12 μm, obtuse at the apex, not fasciculate. Sterigmata 1–2 μm in diameter at the base and 2–4 μm in height, emerging outwardly and tapering toward the tip (Figs. 6A,B, 7A). Basidiospores ellipsoid to oval, or obovoid, 11–19 × 3–6 μm, hyaline, smooth, one celled, or becoming septate with 1–2 septa (Figs. 6C, 7A–D). Septate basidiospores dropped on the agar surface germinating after 6 h (Fig. 8). Germ tubes or conidia emerging from the both end-cells and from the septal region of basidiospores or conidia produced at the tip of germ tubes 22 h after the dropping (Fig. 8). Conidia

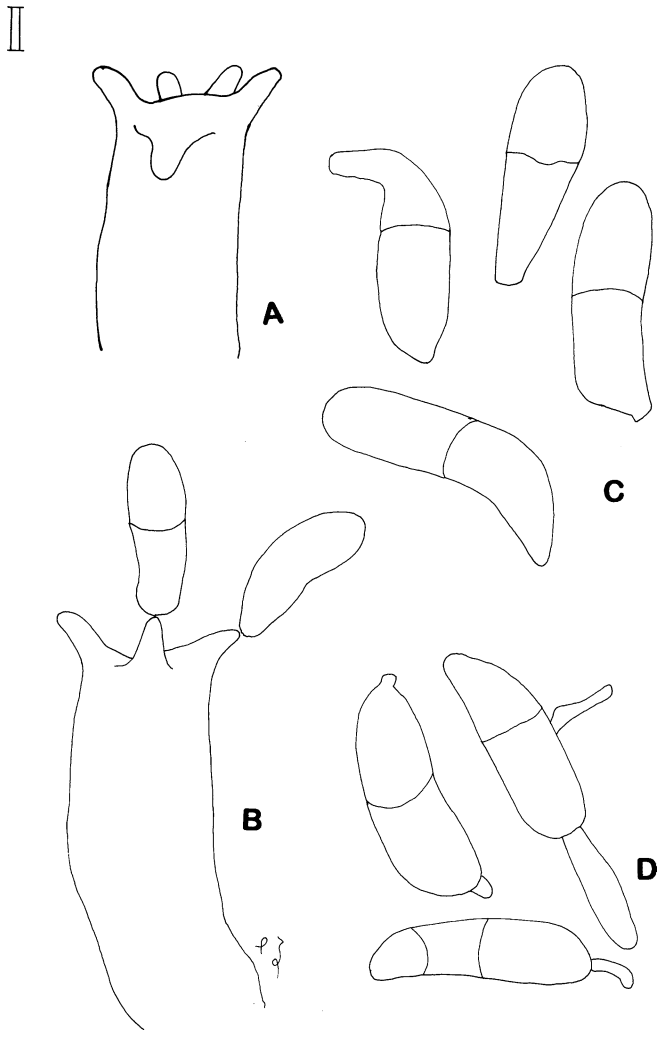

Fig. 6. Basidia and basidiospores of *E. caucasicum* NIAES 20542: basidium (**A**), basidium with basidiospores (**B**), basidiospores (**C**), and germlings in the hymenium (**D**). *Bar* 3 μm

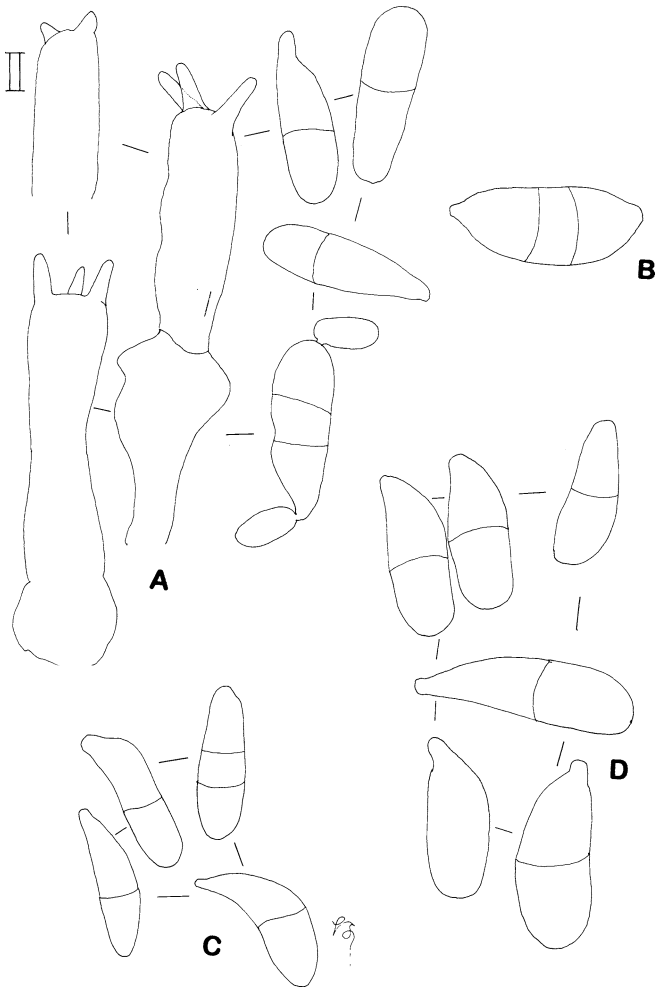

Fig. 7. Basidia and basidiospores of *E. caucasicum*: SAPA July 30, 1921 (**A**), SAPA Aug. 5, 1925 (**B**), SAPA Aug. 4, 1903 (**C**), and SAPA Aug. 1, 1895 (**D**). *Bar* 3 μm

bacilliform and lacrimiform (Fig. 9H), 3–7 × 1–2 μm, and budded polarly, to produce daughter cells and to develop pseudohyphae. Colonies on PDA growing gradually, reaching maximum 11 mm diameter in 21-day incubations, and wrinkling irregularly at the periphery. Colonies with pale orange and smooth surface not becoming farinose by conidial formation, glutinous and not fixed on the agar surface, composed of pseudohypha and conidia. The reverse of colonies pale yellow to pale pink. Dark pigment not exuded on PDA (Fig. 13G). Colonies from conidia showing the same morphological features as those from basidiospores.

Specimens examined: NIAES 20542 (on *R. aureum,* Shirakoma-ike, Yachiyo-mura, Minamisaku-gun, Nagano Prefecture, June 30, 2002, M. Kakishima et C.- m. Tian leg.), SAPA Aug. 1, 1895 (on *R. aureum,* Mt. Matsukarinupuri, Abuta-gun, Shiribeshi subprefecture, Hokkaido Prefecture, Aug. 1, 1895, T. Tozu leg.), SAPA Aug. 4, 1903 (on *R. aureum,* Mt. Yatsuga-take, Nagano Prefecture, Aug. 4, 1903, T. Miyake leg.), SAPA July 30, 1921 (on *R. aureum,* Mt. Matsukarinupuri, Abuta-gun, Shiribeshi subprefecture, Hokkaido Prefecture, July 30, 1921, Y. Homma leg.), SAPA Aug. 5, 1925 (on *R. aureum,* Mt. Hakuun-dake, Kamikawa-gun, Kamikawa subprefecture, Hokkaido Prefecture, Aug. 5, 1925, N. Hiratsuka leg.).

Exobasidium caucasicum was described based on the leaf blight of *R. aureum* collected in Caucasus, Russia (Woronichin 1921). Woronichin (1926) also reported its distribution in Kamchatka. An Exobasidium leaf blight of *R. aureum* (=*R. chrysanthum*) collected by Dr. N. Hiratsuka at Mt. Hakuun-dake, Hokkaido Prefecture in 1925 has been deposited as *E. caucasicum* in SAPA. The specimen mentioned above together with three others have been arranged in the same sheet in SAPA. As there was no record of *E. caucasicum* in the Japanese literature (Anonymous 2000; Ezuka 1998; Ito 1955), we reexamined these four specimens in SAPA comparing with the description of *E. caucasicum*.

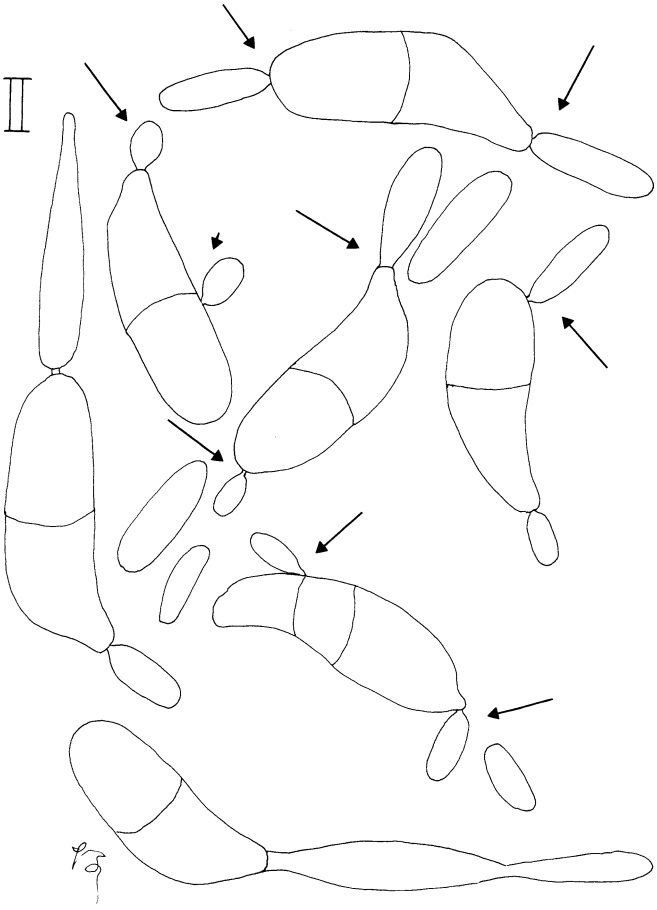

Fig. 8. Basidiospore germination of *E. caucasicum* (NIAES 20542) on PDA after 22 h incubation. Basidiospores budded conidia (*arrows*). *Bar* 3 μm

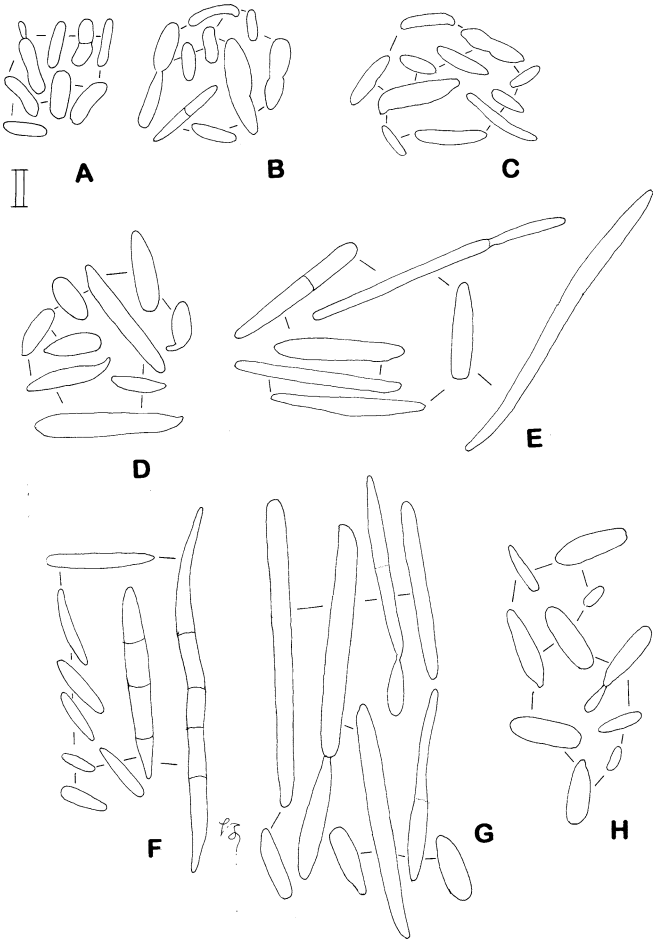

Fig. 9. Conidia of *E. shiraianum* MAFF 238602 (**A**), MAFF 238603 (**B**), MAFF 238604 (**C**), *E. woronichinii* MAFF 238610 (**D**), MAFF 238666 (**E**), MAFF 238617 (**F**), MAFF 238825 (**G**), and *E. caucasicum* MAFF 238830 (**H**) produced on PDA in 21-day incubations at 22°C. *Bar* 3 μm

Lengths of basidia were shorter than the original description, whereas widths of basidia and the number of sterigmata matched with the same ranges of the original description. The feature of basidiospores showed high similarity each other. The fresh material (NIAES 20542) collected in 2002 was identified as *E. caucasicum* after examination of its morphological characteristics. The difference of morphological features of three *Exobasidium* species treated here was in their basidiospores; i.e., *E. shiraianum* had wider and *E. woronichinii*. had more septated basidiospores than *E. caucasicum*. Colonies of *E. caucasicum* on PDA were glutinous and showed yeast like growth. In this cultural characteristic *E. caucasicum* was similar to *E. shiraianum* but not to *E. woronichinii*. Color of colonies of *E. caucasicum* was pale orange, and that of *E. shiraianum* was pale primrose-yellow to pale orange with farinose appearance. Anamorphic culture of *E. caucasicum* produced bacilliform and lacrimiform conidia (Fig. 9H) and pseudohyphae, whereas *E. woronichinii* produced variously shaped conidia (Fig. 9D–G) and pseudohyphae. Exobasidium leaf blight on *R. aureum* by *E. caucasicum* is characterized by chlorosis and powdery appearance on the lower surface of newly developed leaves (Fig. 16A–C). Woronichin (1926) discussed the distribution of *E. caucasicum* to be limited to high altitude or high latitude, and it might be due to adaptation to the climate in the glacial period. In Japan, *R. aureum* is distributed in the alpine area of Hokkaido and Nagano Prefectures, and *E. caucasicum* also followed or accompanied the distribution of *R. aureum*. Three *Exobasidium* species treated here may have host specificity on *Rhododendron* spp. (Figs. 14B, 15B,C,E, 16B), although inoculation tests are needed to demonstrate the host specificity.

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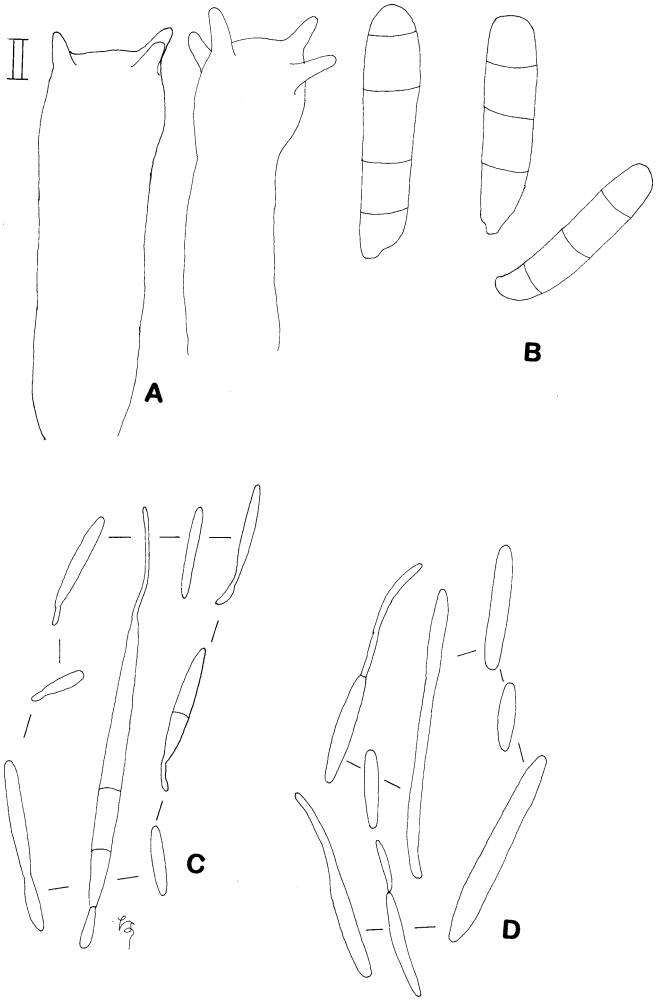

Fig. 10. Basidia and basidiospores of *E. hemisphaericum* TSH-B 0033. Basidium (**A**), basidium with basidiospores (**B**), and basidiospores (**C**) on *R. degronianum*. Conidia produced on PDA in 21-day incubations at 22°C (**D**, E11; **E**, E13). *Bar* 3 μm

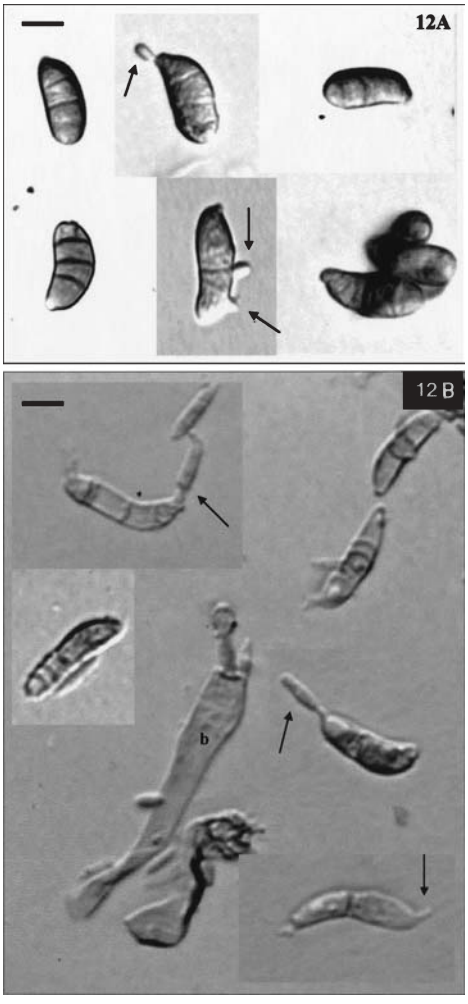

Fig. 12. Basidiospores of *E. shiraianum* and *E. woronichinii*: TSH-B0026 (**A**); TSH-B0114 (**B**). *b*, basidium; *arrows*, germination. *Bars* 3 μm

Fig. 11. Hymenium of *Exobasidium* spp. observed by scanning electron microscopy (SEM). **A** Basidia of *E. shiraianum* holotype (S, F20843) on infected leaf of *R. degronianum*. **B** Basidium and basidiospores of *E. shiraianum* holotype (S, F20843) on infected leaf of *R. degronianum*. **C** Basidium of *E. shiraianum* TSH-B0023 on infected leaf of *R. degronianum*. **D** Basidiospores of *E. shiraianum* TSH-B0023 on infected leaf of *R. degronianum*. There were 2–3 septa. *bs* and *s*, basidiospore and septum, respectively; *arrows*, sterigmata. *Bars* **A** 8.6 μm; **B** 5.0 μm; **C** 4.3 μm; **D** 6.0 μm

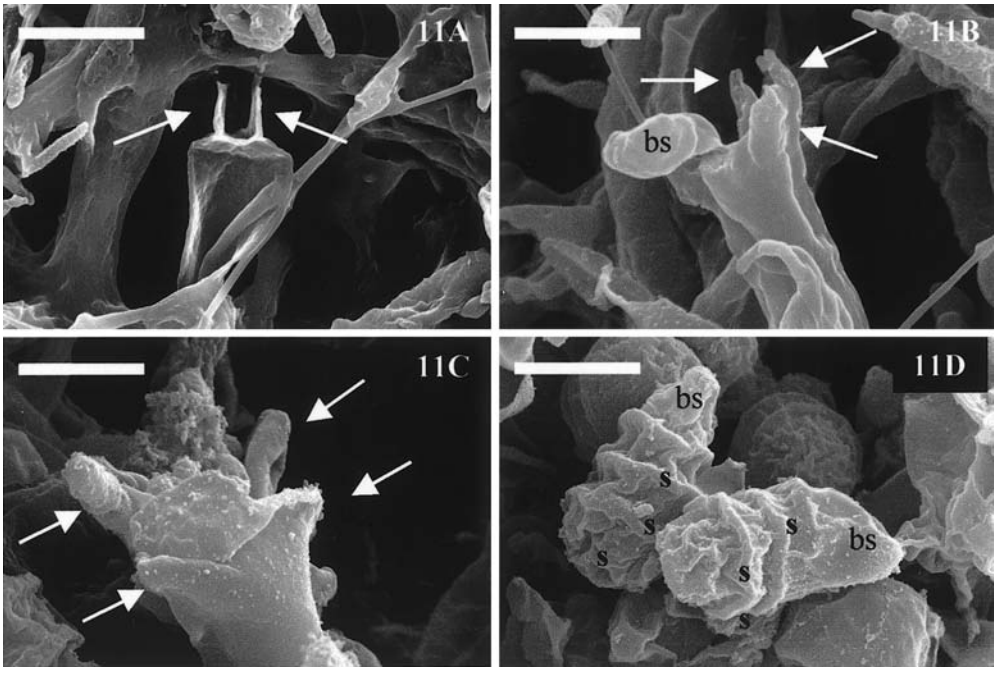

Fig. 13. Morphology and coloration of *Exobasidium* spp. on PDA. Surface of colonies of *E. shiraianum* MAFF 238602 (**A**), MAFF 238603 (**B**), 238604 (**C**); surface of colonies of *E. woronichinii* MAFF 238822 (**D**), MAFF 238825 (**E**); surface of colonies of *E. caucasicum* MAFF 238830 (**F**); surface of colonies of *E. hemisphaericum* E13 (**G**). *Bar* 5 mm

Fig. 14. Symptoms of Exobasidium leaf blight on *R. degronianum* by *E. shiraianum*. **A** Holotype collected on July 1900 in Gunma Prefecture. **B** Appearance of Exobasidium leaf blight on July 1999 in Nagano Prefecture. **C** Comparison of infected lower leaves (*right*) and healthy leaves (*left*). *Arrows*, chlorotic leaf

Fig. 15. Symptoms of Exobasidium leaf blight on *R. brachycarpum* by *E. woronichinii*. **A** Appearance of Exobasidium leaf blight on June 2001 in Hokkaido Prefecture. **B** Field appearance of Exobasidium leaf blight on June 2001 in Hokkaido Prefecture (*left*, healthy seedling; *right*, infected one). **C** Scratch lesions observed in some seedlings. **D** Comparison of infected lower leaf (*left*), partially infected lower leaf (*center*), and healthy leaf (*right*). **E** Appearance of Exobasidium leaf blight on June 1999 in Nagano Prefecture. **F** Comparison of infected lower leaf (*right*) and healthy leaf (*left*). Infected leaves were smaller than healthy ones. *Arrows*, chlorotic leaf

Fig. 16. Symptoms on Exobasidium leaf blight on *R. aureum* by *E. caucasicum*. **A** SAPA July 30, 1921, collected in Hokkaido Prefecture. **B** Appearance of Exobasidium leaf blight, June 2002, in Nagano Prefecture. **C** Comparison of infected lower leaf (*left*) and healthy leaf (*right*). *Arrows*, chlorotic leaf

brachycarpum; Dr. T. Yukawa, National Science Museum, Tokyo, for identifying the host plants including the host plant species, *R. degronianum*, of the holotype material of *E. shiraianum*; and Ms. H. Nakamura, for preparing the medium and helping with the experiments. This study was supported in part by a Grant-in-Aid for Scientific Research (B) (No.13460019), Japan Society for Promotion of Science (JSPS).

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