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FULL PAPER

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Taxonomy of *Exobasidium otanianum* causing Exobasidium leaf blight on *Rhododendron* species in Japan

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Abstract *Exobasidium otanianum* var. *otanianum* and var. *satsumense* have been reported as causal pathogens of Exobasidium leaf blight on *Rhododendron* species. Specimens of both varieties including types were morphologically examined. The type specimen of var. *otanianum* had 0–4-septated basidiospores although 1(–3)-septated basidiopores were reported in the original description. Observations of its herbarium specimens from different localities and its fresh materials from the type locality also confirmed the septal number of its basidiospores. *Exobasidium otanianum* var. *satsumense* was morphologically similar to var. *otanianum*, including the septal number of basidiospores. Germ tubes were consistently produced in these two varieties. From these observations, var. *satsumense* is treated as a synonym of var. *otanianum* and the description of *E. otanianum* is emended. *Rhododendron hyugaense* and *R. reticulatum* f. *glabrescens* are newly added to host plants of *E. otanianum*.

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

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Introduction

Exobasidium otanianum Ezuka var. *otanianum* on *R. reticulatum* D. Don has been reported to cause systemic infection of leaves and produces white hymenia on the lower side of leaves (Exobasidium leaf blight) (Ezuka 1991). *Exobasidium otanianum* var. *satsumense* X.Y. Zhang et K. Arai also causes Exobasidium leaf blight on *R. dilatatum* Miq. var. *satsumense* T. Yamaz. (Zhang et al. 1995). These two varieties are said to be distinguishable from each other in size and septal number of their basidiospores. In 2001, hitherto undescribed Exobasidium leaf blights on *Rhododendron hyugaense* (T. Yamaz.) T. Yamaz. and *R. reticulatum* f. *glabrescens* (Nakai et H. Hara) T. Yamaz. were observed in Miyazaki Pref., Japan. Therefore, we examined *Exobasdium* specimens on *R. hyugaense* and *R. reticulatum* f. *glabrescens* and compared them with two varieties of *E. otanianum* for identification. In these examinations, we noticed that there were no morphological differences between these two varieties, and the true morphology in the specimens was different from that in its original description. We report here the results of morphological observations including type studies with specimens of *E. otanianum* on *Rhododendron* species.

Materials and methods

Morphological observations

Specimens examined are listed in the description of the species. Fresh specimens on *R. hyugaense* and *R. reticulatum* f. *glabrescens* collected in the field were used for morphological observations. 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 Her-

barium of Laboratory 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 isolates

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 *E. otanianum* obtained in this study were deposited in Genebank, National Institute of Agrobiological Sciences, Japan (MAFF). Three isolates of *E. otanianum* var. *satsumense* were obtained from Laboratory of Plant Pathology, Faculty of Agriculture, Kagoshima University (EOS). An isolate of *E. otanianum* var. *otanianum* was also obtained from Institute of Fermentation, Japan (IFO).

Results and discussion

On reexamination of the type specimen of *E. otanianum* var. *otanianum*, we found a slight difference in the septal number of its basidiospores from those in the original description. The observed septal number in the type specimen was 0–4 instead of 1(–3) in the original description in Latin or 0–1(–3) in the explanation paragraph in Japanese (Ezuka 1991). We examined several herbarial specimens of *E. otanianum* collected from different localities and also fresh materials on *R. reticulatum* from the type locality to observe their morphological features, which were identical to those of the type (Table 1). These results of observations confirmed that the septal number in basidiospores of *E. otanianum* var. *otanianum* was 0–4.

E. otanianum var. *satsumense* was established because of the differences in the size of basidia and basidiospores and in septal number of basidiospores (Zhang et al. 1995). By examination of a specimen of *E. otanianum* var. *satsumense*, the morphological characteristics of var. *satsumense* such as the size of basidia and basidiospores, and septal number of basidiospores (see Table 1), are in the range of these of var. *otanianum*. Particularly, these observations confirmed that the septal number in basidiospores of *E. otanianum* var. *satsumense* was 1–3, same as that of var. *otanianum* (NIAES 10494). Therefore, we treat var. *satsumense* as a synonym of *E. otanianum* var. *otanianum*. Based on the morphological observations of two varieties and the resultant taxonomic treatment, the characteristics of *E. otanianum* are emended.

Exobasidium otanianum Ezuka, Trans. Mycol. Soc. Jpn. 32: 77, 1991, emend. Nagao Figs. 1,2 = *Exobasidium otanianum* var. *satsumense* X.Y. Zhang et K. Arai, Nippon Kingakkai Kaiho 36: 100.

Hymenium composed of basidia with 2–4 sterigmata and conidia (Fig. 3A). Hyphae not developing directly on the

Table 1. Morphological measurements of *Exobasidium otanianum*

Specimen	Size of basidia (µm)	Size of sterigmata (µm)	Number of sterigmata	Size of basidiospores (µm)	Number of septa of basidiospores
var. *otanianum* (Ezuka 1991a)	50–70 × 6–8	4.5–5.5 × 2	(1–)4–5(–8)	13–21(–23) × 3.5–6	0–1(–3)
var. *otanianum* NIAES 10494 (isotype)	14–34 × 5–9	2–6 × 1–2	2	11.5–21 × 3–6.5	1–3
var. *otanianum* NIAES 10495	9–27 × 5–9.5	2.5–5 × 1–3	2–4	10–16 × 3–5	0–4
var. *otanianum* NIAES 10496	12–25 × 5–8.5	2–3.5 × 1.5–2	2–4	12–17 × 3.5–5	1–3
var. *otanianum* NIAES 10539	18 × 5.5–7	3–3.5 × 1	3–4	12–16 × 3.5–6	1–3
var. *otanianum* NIAES 10540	10–22 × 6.5–10	3–5 × 1–2	2–4	11–17 × 3–5.5	1–4
(Ranges of above 5 specimens	9–34 × 5–10	2–6 × 1–3	2–4	10–21 × 3–6.5	0–4
var. *otanianum* NIAES62626	9–34 × 5–9.5	2–6 × 1–3	2–4	10–21 × 3–6.5	0–4
var. *satsumense* (Zhang et al. 1995)	52.5–72.5 × 6.3–8.8	3.8–6.3 × 2–2.5	2–3(–4)	10–15 × 3.8–6.3	0–3(–4)
var. *satsumense* KUP9505	15–29 × 7.5–11	4–7 × 2–3	2–3(–4)	12–17(–20) × 4–6	1–3
on *R. hyugaense* TSH-B0058	22–46 × 7–9	4–7 × 1.5–2.5	2–3	15–24 × 5–7	2–6
on *R. hyugaense* TSH-B0059	22–46 × 7–9	4–7 × 1.5–2.5	2–3	15–24 × 5–7	2–6
on *R. reticulatum* f. *glabrescens* TSH-B0061	nd	nd	nd	17–23 × 4–6	3–5

nd, not determined

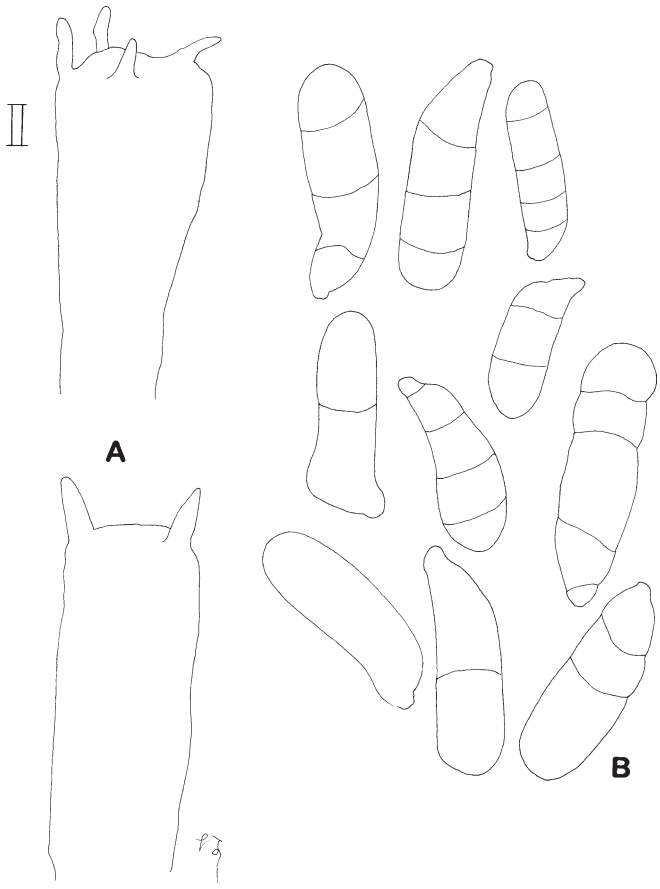

Fig. 1. Basidia (**A**) and basidiospores (**B**) of *Exobasidium otanianum* formed on the infected leaf on *Rhododendron reticulatum* NIAES10494 (Isotype). *Bar* 3 μm

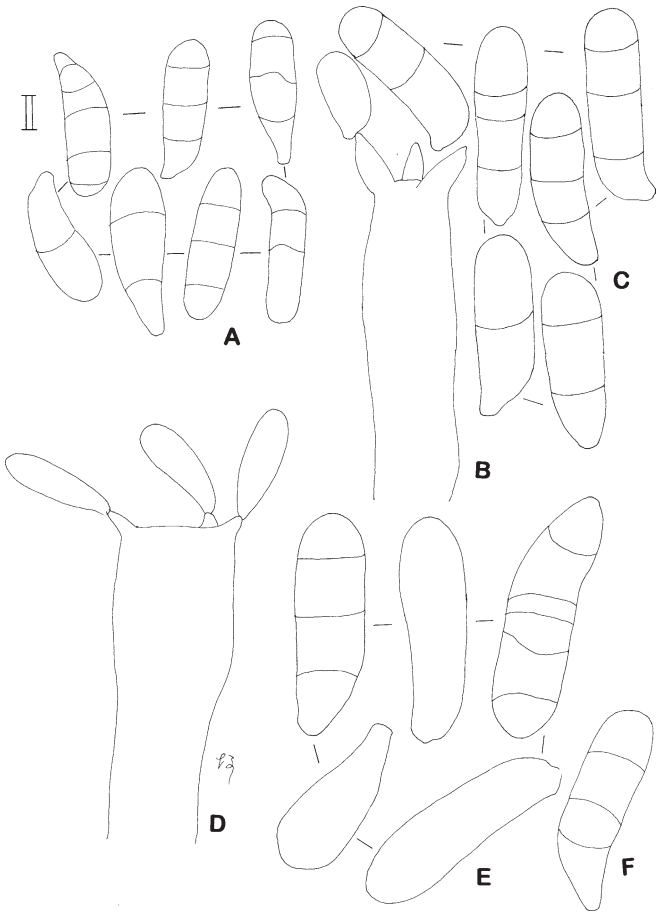

Fig. 2. Basidia and basidiospores of *E. otanianum* formed on the infected leaf on *Rhododendron* spp. Basidiospores on *R. reticulatum* NIAES303031 (**A**), basidium (**B**), and basidiospores (**C**), on *R. dilatatum* var. *satsumensis* KUP9505, basidium (**D**) and basidiospores (**E**) on *R. hyugaense* (TSH-B 0059), and basidiospores (**F**) on *R. reticulatum* f. *glabrescens* (TSH-B 0061). *Bar* 3 μm

surface of epidermis. Basidia clavate to cylindrical, 5–30 × 5–9 μm (Figs. 1A, 2C), obtuse at the apex, emerging directly from host surface or through stomata, not fasciculate. Sterigmata 1–3 μm in diameter at the base and 2–6 μm in height, tapering toward the tip (Fig. 3B). Basidiospores ellipsoid to ovoid, or obovoid, 10–21 × 3–6.5 μm, hyaline and smooth, with 0–4 septa (Figs. 1B, 2, 4), obtuse at the apex, slightly curving and tapering at the base. Septate basidiospores dropped on agar surface germinating after 15 h (Figs. 5, 6). Germ tubes emerged from the cells. Pseudohyphae indistinguishable from budded conidia. Conidia clavulate, bacilliform, or subfusiform (Fig. 7A–G), 5–20 × 1–2 μm, 0–1-septated, and budded polarly in culture (Table 2) to produce daughter cells polarly, and developing into pseudohyphae. Colonies on potato dextrose agar (PDA) growing gradually, reaching maximum 16 mm diameter in 21-day incubation and wrinkling irregularly at the periphery, gelatinous and not fixed on the agar surface, composed of branching, intricate hyphae and pseudohypha and conidia. Surface of colonies pale pink to pale orange and corrugate, not showing powdery appearance due to the conidial formation. Reverse of colonies pale pink. Dark pigmentation not exuded into PDA (Fig. 8). Colonies from conidia showed the same morphological features as those from basidiospores.

Specimens examined: on *R. reticulatum*, NIAES10494 (holotype of var. *otanianum*, Tenjin-yama, Zao-cho, Fukuyama-shi, Hiroshima Pref., April 22, 1971, A. Ezuka leg.), NIAES10495 (Kento-yama, Ishin-den, Tsu-shi, Mie Pref., April 25, 1971, A. Ezuka leg.), NIAES10496 (Kento-yama, Ishin-den, Tsu-shi, Mie Pref., May 2, 1971, A. Ezuka leg.), NIAES10539 (Mizuo, Ukyo-ku, Kyoto-shi, Kyoto Pref., April 29, 1990, A. Ezuka leg.), NIAES10540 (Mizuo, Ukyo-ku, Kyoto-shi, Kyoto Pref., May 1, 1990, A. Ezuka leg.), NIAES62626 (Tenjin-yama, Zao-cho, Fukuyama-shi, Hiroshima Pref., May 1, 2002, T. Kimura leg.); on *R. dilatatum* var. *satsumensis*, KUP-9505 (Yoshino-cho, Kagoshima-shi, Kagoshima Pref., May 5, 1995, X.-Y. Zhang and K. Arai leg.); on *R. hyugaense*, TSH-B0058, TSH-B0059 (Mt. Osuzu-yama, Tsuno-cho, Koyu-gun, Miyazaki Pref., March 28, 2001, H. Nagao and S. Kurogi leg.); on *R. reticulatum* f. *glabrescens*, TSH-B0061 (Mt. Morotsuka-yama, Takachiho-cho, Nishi-usuki-gun, Miyazaki Pref., May 8, 2001, S. Kurogi leg.).

Exobasidium species found on Exobasidium leaf blight on *R. hyugaense* and *R. reticulatum* f. *glabrescens* was iden-

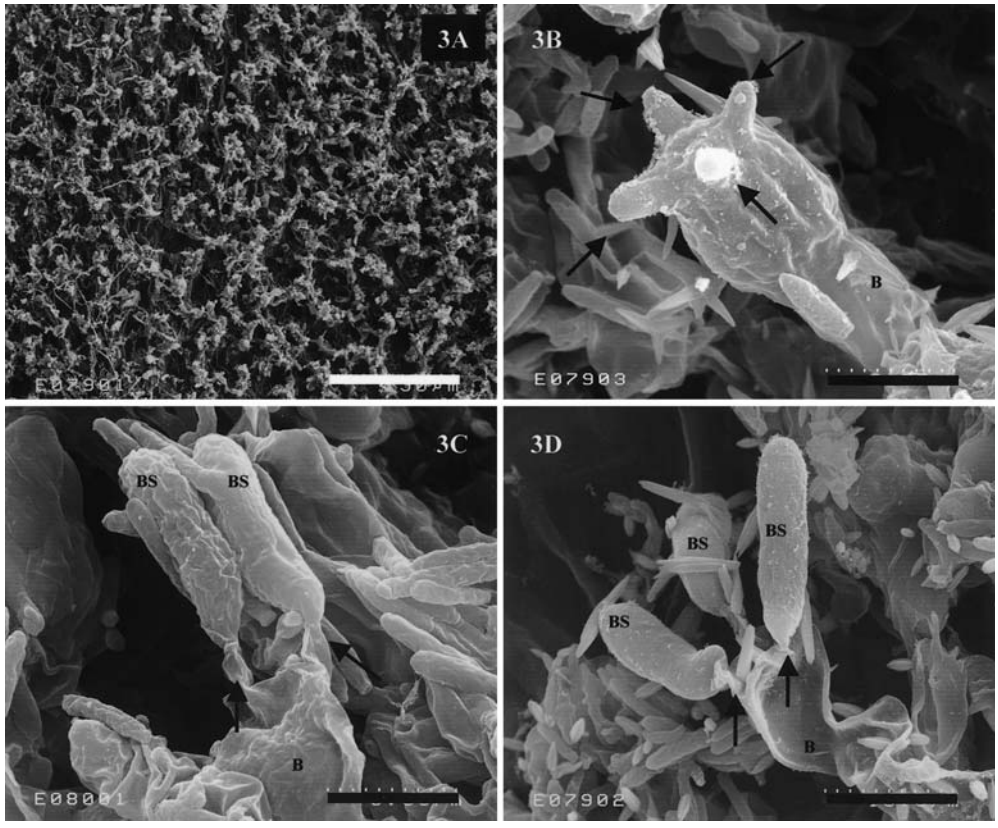

Fig. 3. Hymenium of *E. otanianum* observed by SEM. **A** Hymenium of *E. otanianum* on *R. hyugaense*; **B** basidium; **C,D** basidium with basidiospores. *Arrows* indicate sterigmata. *B*, basidium; *BS*, basidiospores. *Bars* **A** 150 μm; **B** 6.7 μm; **C** 6 μm; **D** 10 μm

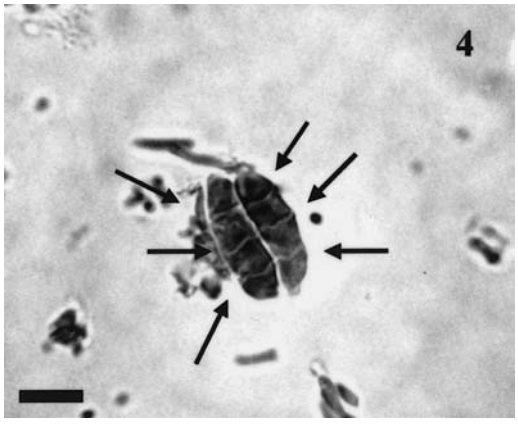

Fig. 4. Basidiospores of *E. otanianum* formed on the infected leaf on *R. reticulatum* NIAES10494 (isotype). Basidiospores were stained with Congo red. Septa were readily observed (*arrows*). *Bar* 10 μm

tified as *E. otanianum* (Fig. 2C–E, Table 1). Although basidiospores on the fungi on these hosts appeared to be somewhat longer, 15–24 × 4–7 μm, than those in the past record of *E. otanianum*, 13–21(–23) × 3.5–6 μm, the length and width of basidiospores were not discrete among these specimens. In the specimens obtained from *R. hyugaense* and *R. reticulatum* f. *glabrescens*, basidiospores consistently germi-

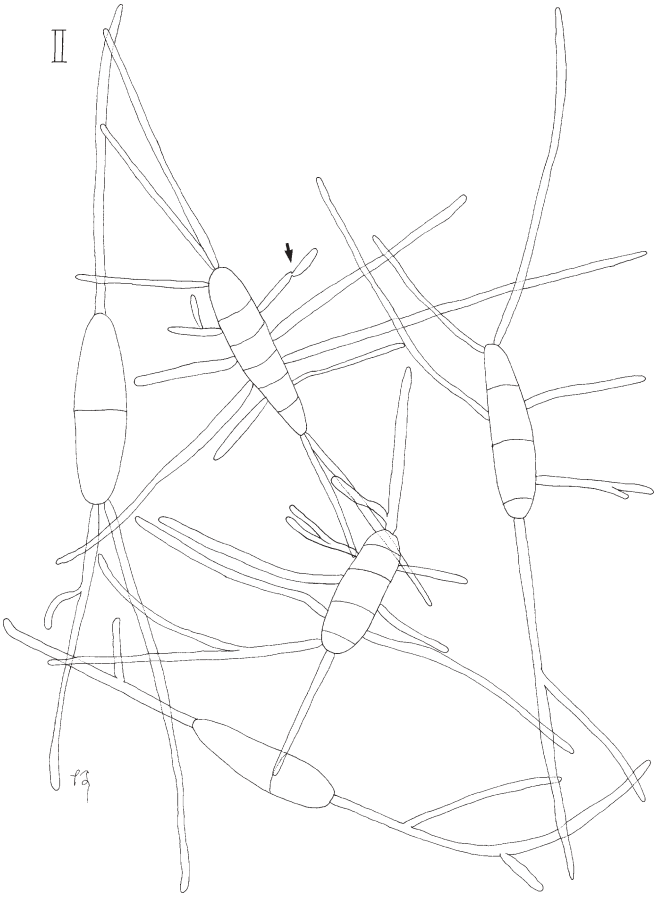

Fig. 5. Germination of the basidiospores of *E. otanianum* NIAES303031 on potato dextrose agar (PDA) after 15-h incubation. One of the basidiospores produced conidia on the germ tube (*arrow*). *Bar* 3 μm

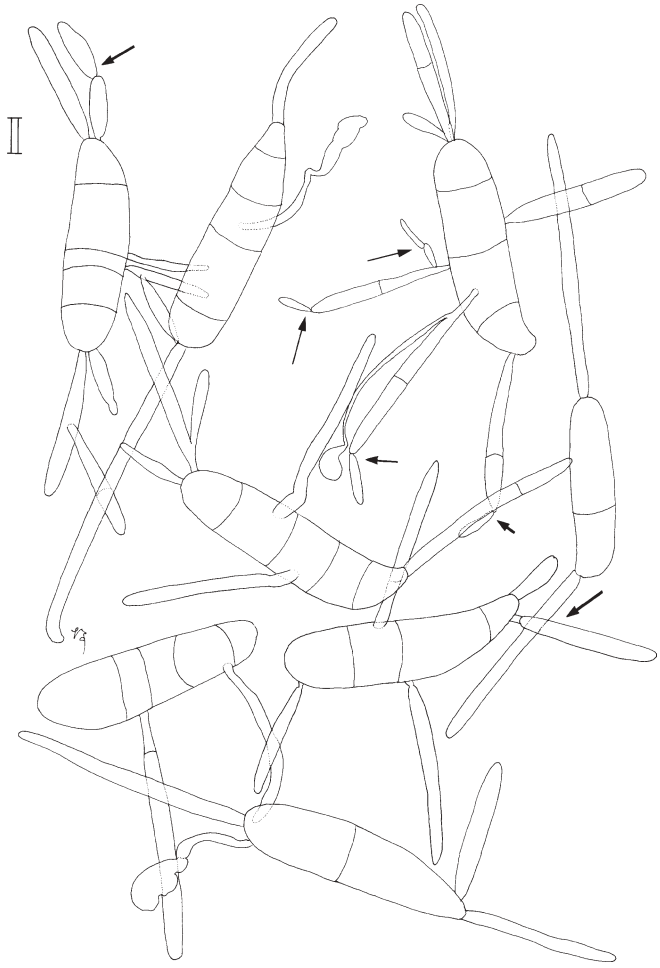

Fig. 6. Germination of the basidiospores of *E. otanianum* TSH-B 0059 on PDA after 15-h incubation. Some of the basidiospores produced conidia on the germ tube (*arrows*). *Bar* 3 μm

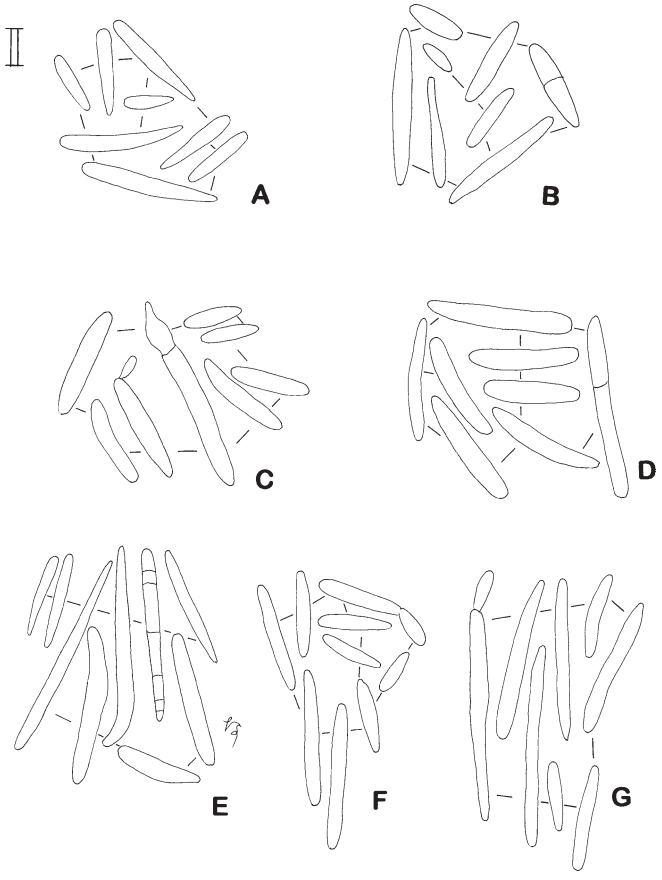

Fig. 7. Conidia produced on PDA in 21-day incubation at 22°C in isolates IFO9960 (**A**), MAFF238677 (**B**), EOS44 (**C**), EOS59 (**D**), MAFF 238611 (**E**), MAFF 238612 (**F**), and MAFF238613 (**G**). *Bar* 3 μm

Table 2. Conidial morphology of *Exobasidium otanianum*

Isolate	Size of conidia (μm)	Number of septa of conidia
var. *otanianum* (Ezuka, 1991a)	6–13 × 1–2(–2.5)	0
var. *otanianum* IFO9960	5–20 × 1–2	0–1
var. *otanianum* MAFF238677	3–10 × 1–1.5	0
var. *satsumense* (Zhang et al. 1995)	3.8–10 × 0.8–2	nd
var. *satsumense* EOS44	5–12 × 1–1.5	0–2
var. *satsumense* EOS59	7–15 × 1–2	0–1
on *R. hyugaense* MAFF238611	6–17 × 1–2	0 (–5)
on *R. hyugaense* MAFF238612	5–12 × 1–1.5	0–1
on *R. reticulatum* f. *glabrescens* MAFF238613	3–16 × 1–1.5	0

nd, not determined
EOS, Laboratory of Plant Pathology, Faculty of Agriculture, Kagoshima University; IFO, Institute of Fermentation, Japan; MAFF, Genebank, National Institute of Agrobiological Resources, Japan

nated with germ tubes, a symptom typical of Exobasidium leaf blight (Fig. 9A–C), infected leaves were larger and slightly thicker than healthy ones and pale green or whitish-green, white powdery hymenia were formed entirely on the lower side of these leaves (Fig. 9C), hymenium formation was not observed on twig and branch, and infected leaves then dried up rapidly, turned dark, and fell. *Rhododendron hyugaense* and *R. retuculatum* f. *glabrescens* were recognized as new hosts for *E. otanianum.*

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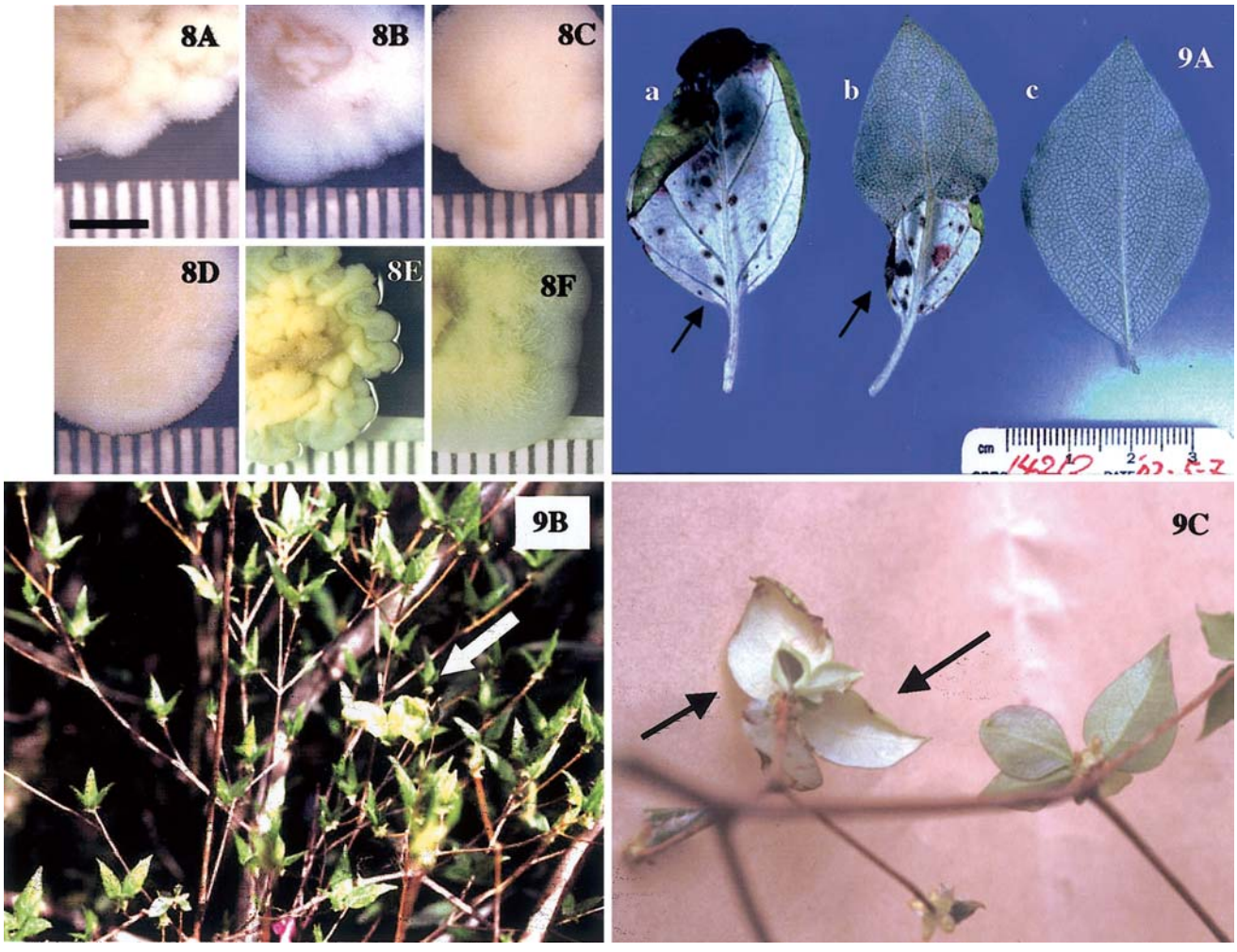

Fig. 8. Morphology and coloration of colonies formed by *E. otanianum* on PDA. **A** Surface of colonies of IFO9960 (**A**), MAFF238677 (**B**), EOS19 (**C**), EOS59 (**D**), MAFF 238611 (**E**), and MAFF238613 (**F**). Submerged hyphae in colonies were not pigmented. *Bar* 5 mm

Fig. 9. Symptoms of Exobasidium leaf blight. **A** Symptom on *R. reticulatum* observed on May 2002 in Hiroshima Prefecture: *a*, white hymenia produced all over the lower surface of a leaf; *b*, white hymenia produced on a part of the lower surface of a leaf; *c* a healthy leaf. **B** Symptom on *R. hyugaense* (*arrow*) observed on March 2001 in Miyazaki Prefecture. **C** White hymenia produced on the lower surface of leaves (*arrows*)

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