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FULL PAPER

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Two new species of *Exobasidium* causing Exobasidium diseases on *Vaccinium* spp. in Japan

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Abstract Two new *Exobasidium* species on *Vaccinium* spp. in Japan are described and discussed. *Exobasidium kishianum*, which causes Exobasidium leaf blight on *V. hirtum* var. *pubescens* and *V. smallii*, is characterized by its ellipsoid to ovoid basidiospores with (0–)1–3 septa. Its systemic infection is also observed. *Exobasidium inconspicuum*, causing Exobasidium leaf blister on *V. hirtum* var. *pubescens*, is characterized by its obovoid or ellipsoid to oval basidiospores with 0–4 septa. Mode of germination of the basidiospores is by germ tube in both species.

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

Introduction

Twenty-seven species of *Vaccinium* have been recorded as host plants of *Exobasidium* spp. in Europe and North America. The type species of *Exobasidium* was erected from the material of systemic infection on *V. vitis-idaea* L. and named *E. vaccinii* (Fuckel) Woronin. *Exobasidium vaccinii* merely has basidia with 4 sterigmata and aseptate basidiospores. This simple morphology gathers most species with 0–1-septate basidiospores into varieties of *E. vaccinii* irrespective of cultural characteristics, pathogenicity, and host plant (Savile 1959). Sundström (1964) found the stability of mode of germination to characterize each host race of *E. vaccinii*. His physiological, morphological, and serological studies constructed a clear taxonomic structure of *Exobasidium*. Then, Nannfeldt (1981) followed his work and considered the host range and symptom as a valuable key for species. According to his species concept, *Exobasidium vaccinii* sensu lato was systematically emended into *E. vaccinii* sensu stricto and 18 species. In Japan, 2 *Exobasidium* species have been recorded on *V. hirtum* Thumb. ex Murray var. *pubescens* (Koidz.) T. Yamaz. but have not been described (Anonymous 2000; Ezuka 1991). We recently collected fresh samples of these 2 *Exobasidium* species and could examine the morphology and cultural characteristics of these fungi compared with the known species. Following Nannfeldt's concept (1981), we propose 2 new species to accommodate these *Exobasidium* specimens on *Vaccinium* spp.

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Materials and methods

Morphological observations

Fresh materials of *Exobasidium* species on *V. hirtum* var. *pubescens* and *V. smallii* A. Gray var. *smallii* 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 reported 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).

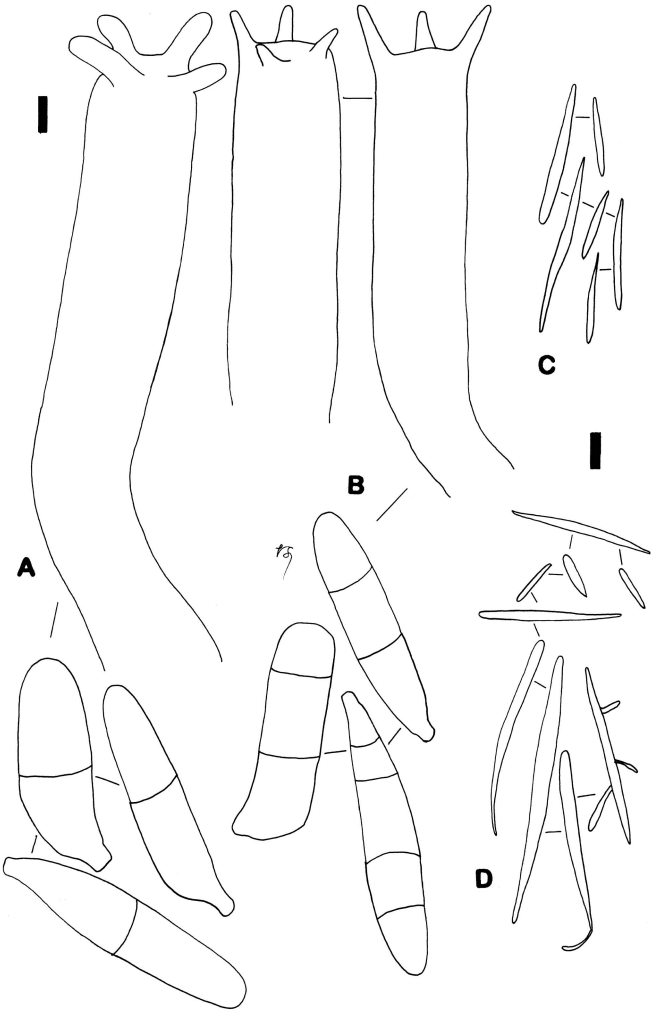

Fig. 1. Upper part of basidia and basidiospores of *E. kishianum* TSH-B0070 (**A**) and TSH-B0071 (**B**). Conidia of *E. kishianum* MAFF 238623 (**C**) and MAFF 238624 (**D**) produced on potato dextrose agar (PDA) in 21-day incubation at 22°C. *Bars* 2 μm

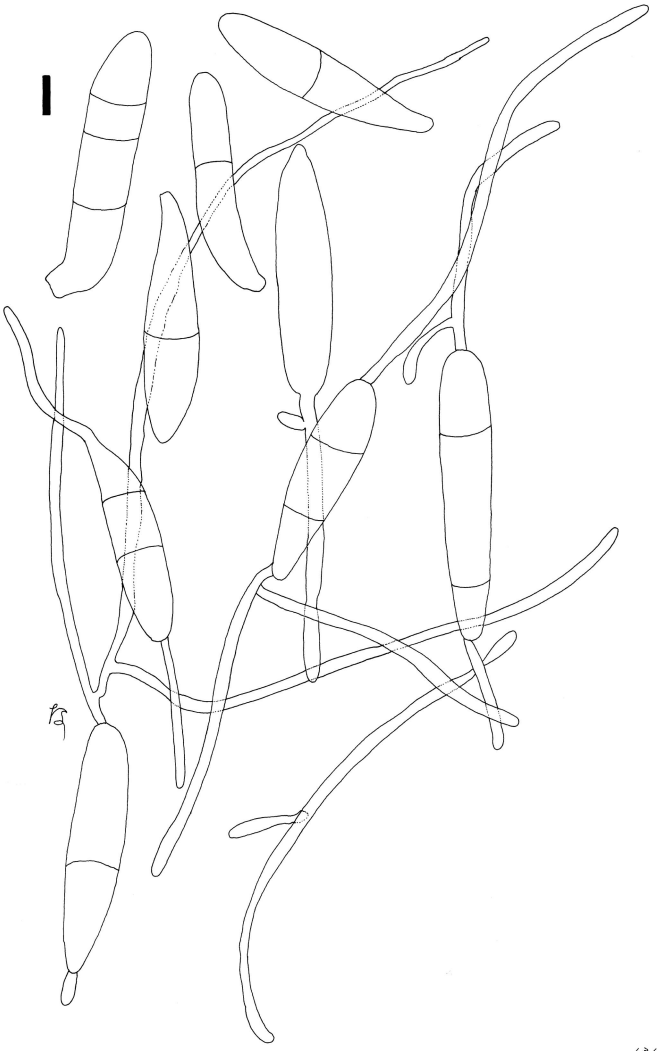

Fig. 2. Basidiospore germination of *E. kishianum* (TSH-B 0070) on PDA after 12 h incubation. *Bar* 2.5 μm

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 were deposited in Genebank, National Institute of Agrobiological Sciences, Japan (MAFF).

Taxonomy

1. ***Exobasidium kishianum*** Nagao et Ezuka, sp. nov. Fig. 1

Exobasidium sp. A. Ezuka, Ann. Phytopathol Soc Jpn 41:100, 1975

Exobasidium sp. ④ A. Ezuka, Trans Mycol Soc Jpn 32:174–176, 1991

Hymenium hypophyllum, effusum, saepe totum infrasuperficiem folii tegens. Folia infecta supra viridiflava vel viridescentia, infra albofarinosa, leviter carnosa. Interdum, ramuli caespitosi in ramis formantes. Basidia hyalina, clavato-cylindracea, 50–70 × 4–6.3 μm, terminaliter cum 4–5(–6) sterigmatibus longiconoideis 2–4 × 1–2 μm praedita. Basidiosporae hyalinae, laeves, cylindricae, saepe unciformes vel reclinatae, ad apicem semiorbiculares, ad basim angustatae, 11–18(–21.3) × 2.5–3.8(–5) μm, primo continuae dein (0–)1–3-septatae, per hyphas germinantes. Conidia hyalina, continua, laevia, linearia, 2–19 × 0.5–1 μm. Coloniae in PDA restricte crescentes, post 21 dies maxime 9–15 mm diameter attingens, corrugatae, fragiles, ad ambitum irregulares, ex hyphis circa 1 μm latis et conidiis constantes, pallide persicinae vel pallide aurantiacae, in agaro non pigmentiferae; reversum pallide primulinum.

Etymology: Referring to Japanese plant pathologist, K. Kishi, who first collected this new *Exobasidium* species.

Table 1. Comparison of morphological measurements among *Exobasidium* spp.

Species	Size of basidia	Size of sterigmata	Number of sterigmata	Size of basidiospores	Number of septa of basidiospores
E. kishianum	50–70 × 4–6.3	2–4 × 1–2	4–5(6)	11–18(21.3) × 2.5–3.8(5)	(0)1–3
E. asebi	60–80 × 4–7	4–6 × 1.5–2.5	3–4	16–23 × 3–5.5	1–3
E. gaultheriae	70 × 5–7.8	4–6.5 long	3–6	12–15.3 × 3.5–5	1–3
E. vaccinii	13–27(30) × 3–6(7)	2–4.5 × 1–1.7	(2)3–6(8)	8–14.5 × 2–3.2	(0)1(3)
E. vaccinii-uliginosi	9–10 wide	7 long	2	16–23(28) × 6.5–9(12)	0
E. inconspicuum	80–100 × 4–8	2–6 × 1.5–2	2–4(5)	9–19 × 3.5–6	0–4
E. cylindrosporum	50–60 × 5–7	5–6 × 2	(4)5(6)	12–22 × 2.8–4.4	(1)3
E. sakishimaense	37.5–50 × 5–7.5	5–7 × 3	(2)3(4)	15–24 × 5–6	1–2

All measurements are in micrometers (μm)

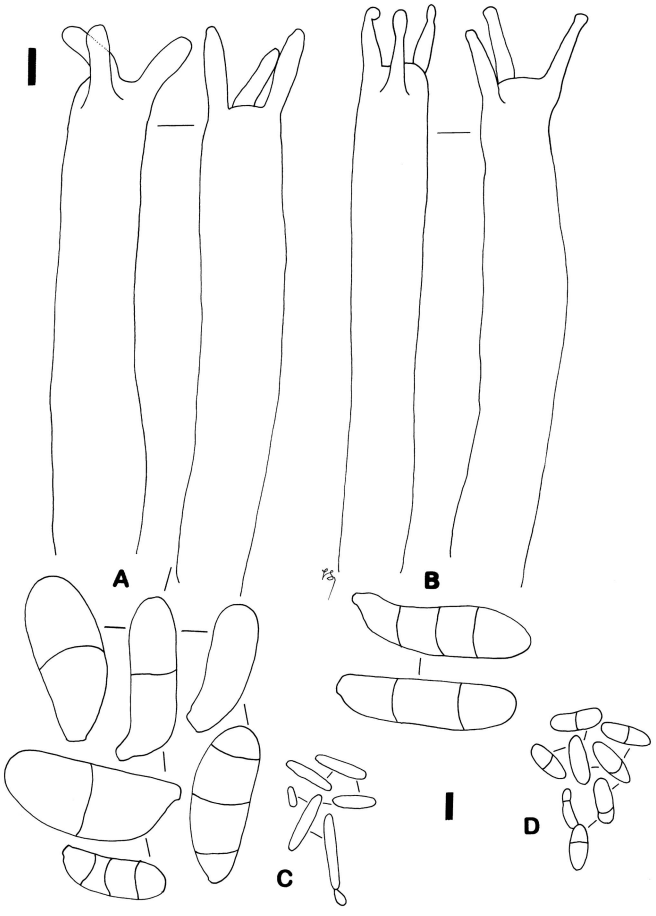

Fig. 3. Upper part of basidia and basidiospores of *E. inconspicuum*: TSH-B0080 (**A**) and TSH-B0086 (**B**). Conidia of *E. inconspicuum* MAFF 238616 (**C**) and MAFF 238619 (**D**) produced on PDA in 21-day incubation at 22°C. *Bars* **A**, **B** 3 μm; **C**, **D** 2 μm

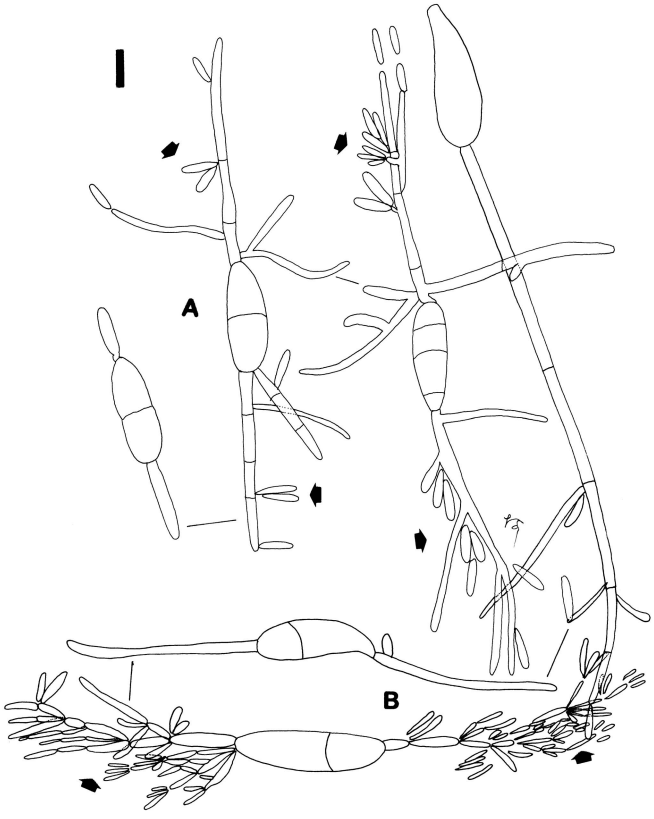

Fig. 4. Basidiospore germination of *E. inconspicuum* on PDA after 12 h incubation. Some of the basidiospores produced conidia on the germ tubes (*arrows*). **A** TSH-B0080; **B** TSH-B0086. *Bar* 5 μm

Holotypus in folliis vivis *Vaccinii hirtum* Thumb. ex Murray var. *pubescens* (Koidz.) T. Yamaz., Ishinden, Tsu, Mie Prefecture in Japonia, 1 IV 1974, A. Ezuka leg., in Herbario Instituti Nationalis Scientiae Agroenvironmentalis, Tsukuba, Japonia (NIAES 10519) conservatus.

Specimens examined: TSH-B0070 and TSH-B0071 (on *V. smallii* var. *smallii*, Zatoh-ishi, Hirosaki, Aomori Prefecture, June 12, 2001, Y. Harada leg.); NIAES 10517 (on *V. hirtum* var. *pubescens*, Ishinden, Tsu, Mie Prefecture, May 25, 1974, K. Kishi leg.); NIAES 10518 (on *V. hirtum* var. *pubescens*, Ishinden, Tsu, Mie Prefecture, June 1, 1974, H. Ukaji leg.).

Hymenium composed of basidia with 4–5(–6) sterigmata and conidia (Fig. 5A). Hyphae not developing directly on the surface of epidermis. Basidia clavate to cylindrical, 50–70 × 4–6.3 μm (Figs. 1A,B, 5C,D), with obtuse apex, emerging directly from leaf surface or through stomata, not fasciculate. Sterigmata 1–2 μm in width at the base and 2–4 μm in height, emerging outwardly and tapering toward the tip. Basidiospores ellipsoid to ovoid, 11–18(–21.3) × 2.5–3.8(–5) μm, hyaline, smooth, one-celled when formed, becoming septate with (0–)1–3 septa (Figs. 1A,B, 5B, 6A–C), slightly curved and tapering at the basal end. Septate basidiospores dropped on the agar surface germinated after 6 h,

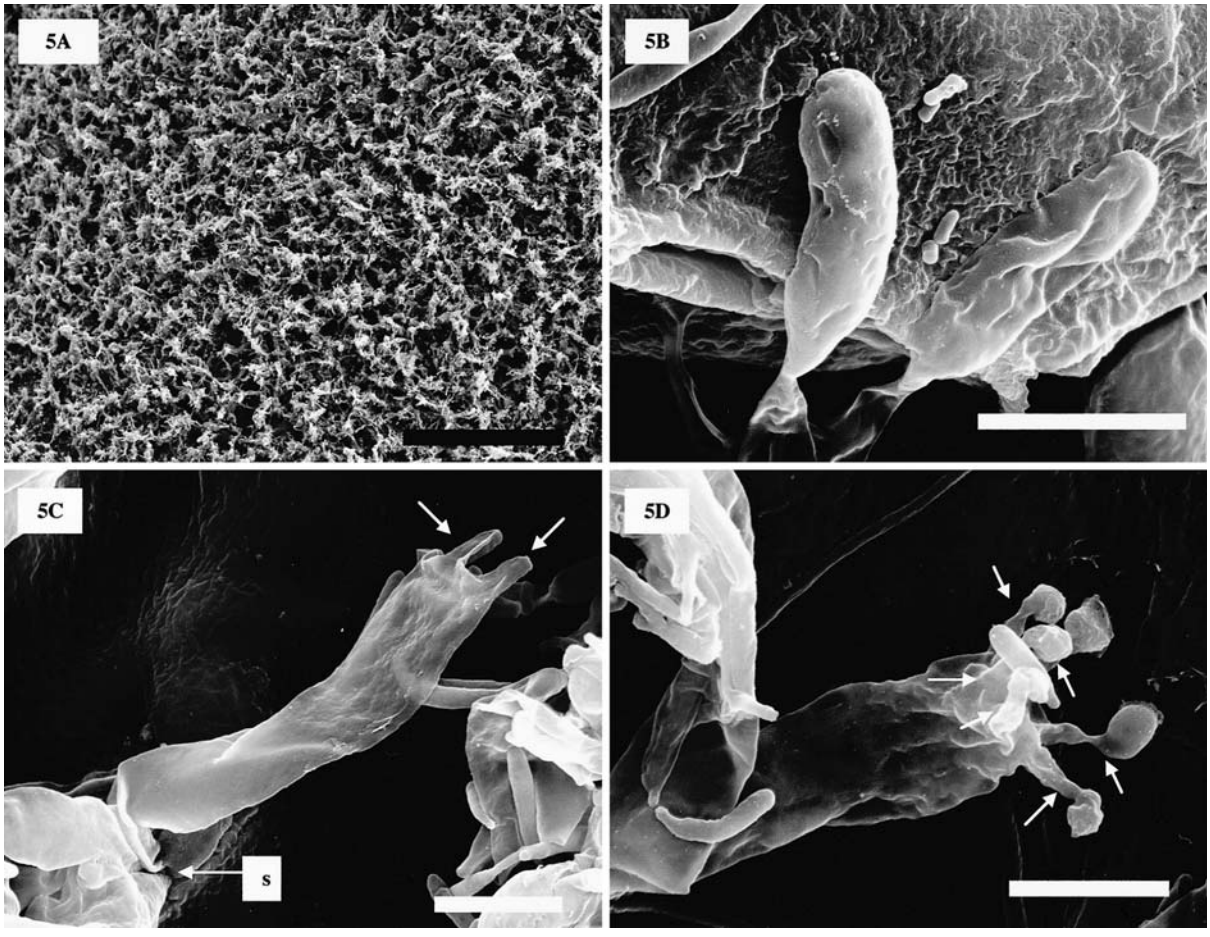

Fig. 5. Hymenium of *Exobasidium kishianum* on infected leaf of *V. smallii* TSH-B0071 observed by scanning electron microscopy (SEM). **A** Hymenium emerged on leaf surface without rupture. **B** Mature basidiospores with smooth surface. **C** Basidium from a stoma (*s*). *Arrows*, sterigmata. **D** Basidium bearing immature basidiospores. *Arrows*, sterigmata. *Bars* **A** 150 µm; **B–D** 5.0 µm

forming germ tubes 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 the dropping (Fig. 2). Hyphae growing into pseudohyphae and branched. Conidia subfusiform and clavulate (Fig. 1C,D), 2–19 × 0.5–1 µm aseptate, and budding polarly to produce daughter cells and also develop hyphae. Colonies on potato dextrose agar (PDA) growing gradually, reaching maximum 15 mm diameter in 21-day incubations at 22°C, and wrinkling irregularly at the periphery, with pale peach-colored to pale orange and corrugate surface, gelatinous but obtrite and fixed on agar surface, often providing farinose appearance, composed of branching, intricate hyphae and pseudohyphae, and conidia (Fig. 7A,B). The reverse of colonies pale peach-colored. Dark pigment not exuded on PDA. Colonies from conidia showing the same morphological features as those from basidiospores.

Among 103 taxa of *Exobasidium* that have been validly described, *E. asebi* Hara et Ezuka, *E. gaultheriae* Sawada, and *E. vaccinii* showed similarities in some morphological measurements (Table 1) to this new species. However, this species was distinguished from *E. asebi* and *E. gaultheriae* in length of the basidiospores and sterigmata. In addition to these differences, the mode of germination of the basidiospore was budding in *E. gaultheriae*. Morphological measurements and its shape were in the range of *E. vaccinii*, whereas the mode of germination of *E. vaccinii* was budding. *Exobasidium vaccini-ulginosi* Boud. was reported on *V. smallii* var. *smallii* (Ito 1955). Width of basidia and basidiospores and size of sterigmata of *E. vaccini-ulginosi* were markedly larger than those of *E. kishianum*. The number of sterigmata was also different from *E. kishianum*. The symptom of *E. kishianum* was Exobasidium leaf blight, whereas that of *E. asebi* was Exobasidium leaf blister, and *E. gaultheriae* formed a gall on leaf and bud.

Exobasidium leaf blight on *V. hirtum* var. *pubescens* and *V. smallii* var. *smallii* was characterized by chlorosis and a powdery appearance on the lower surface of newly developed leaves (Fig. 8A–C). In some materials, cladomaniac symptom was also observed.

2. *Exobasidium inconspicuum* Nagao et Ezuka, sp. nov. Fig. 3

Exobasidium sp. A. Ezuka, Ann. Phytopathol Soc Jpn 41:100, 1975

Exobasidium sp. ③ A. Ezuka, Trans Mycol Soc Jpn 32:172–173, 1991

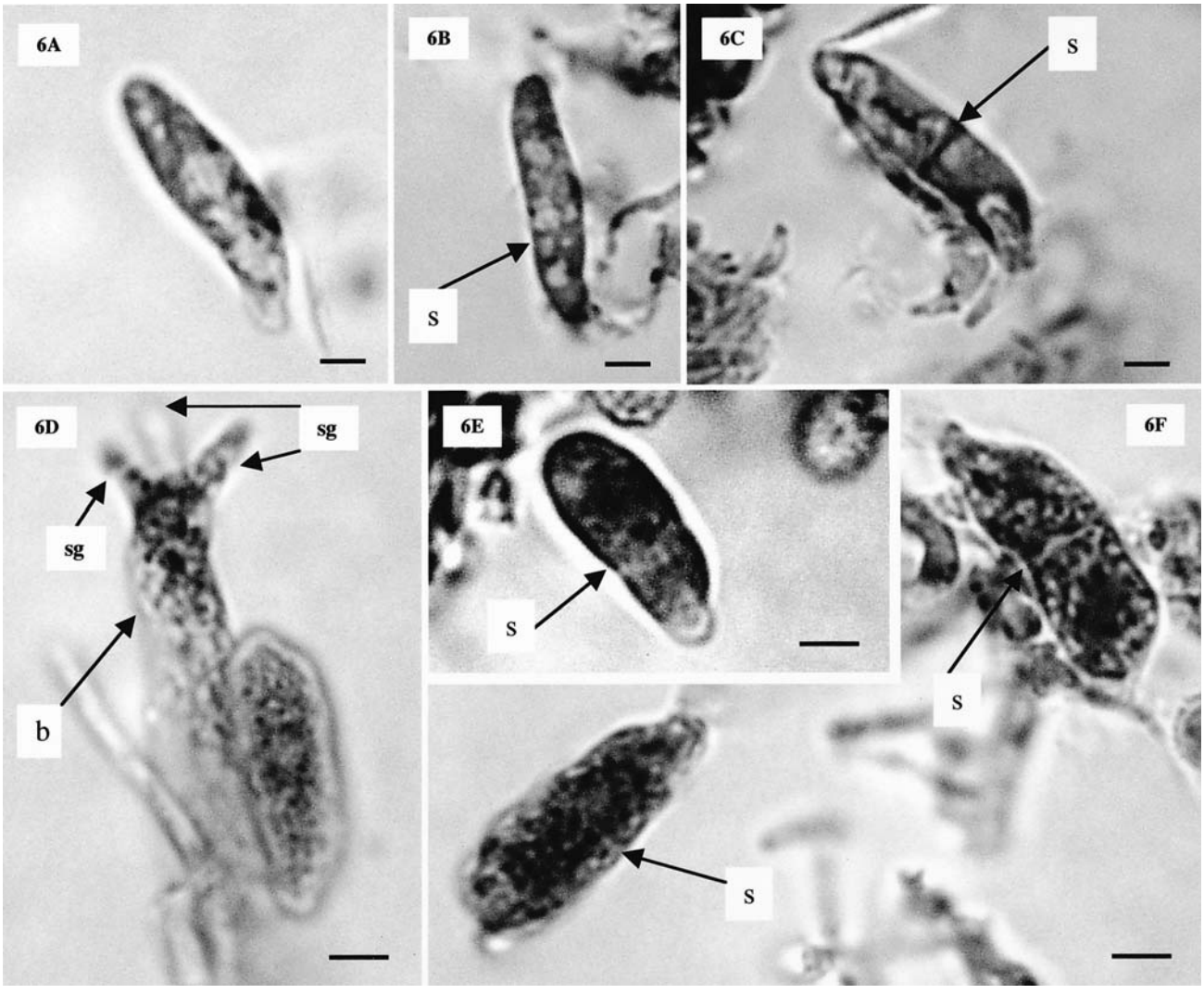

Fig. 6. Basidiospores of *E. kishianum* NIAES 10518 (**A–C**) and *E. inconspicuum* NIAES 10513 (**D–F**). *b*, *s*, and *sg* indicate basidium, septum, and sterigma, respectively. *Bars* 3 µm

Maculae in foliis rotundae, 2–8 mm diametro, planae et leviter vel non incrassatae, supra flavae vel viridiflavae et infra albo-farinosae. Hymenium hypophyllum, determinatum, infra maculas. Basidia hyalina, clavato-cylindracea, 80–100 × 4–8 µm, ad apicem obtusata vel deplanata, terminaliter cum 2–4(–5) sterigmatibus longiconicis 2–6 × 1.5–2 µm praedita. Basidiosporae hyalinae, laeves, obovatae vel cylindricae, ad apicem rotundatae, ad basim curvatae et angustatae, 9–19 × 3.5–6 µm, primo continuae dein 0–4 septatae, per hyphas germinantes. Conidia hyalina, laevia, linearia vel reniformai, 3–6 × 1–1.5 µm, primo continua dein 0–1 septata. Coloniae in PDA restricte crescentes, post 21 dies maxime 15 mm diametro attingens, ad ambitum irregulariter rugosae, ex hyphis circa 1 µm lais et conidiis constantes, cremeae vel pallide aurantiacae, in agaro no pigmentiferae; reversum coloniis concoloria.

Holotypus in foliis *Vaccinii hirtum* var. *pubescens*, Hatsukura, Haibara, Shizuoka Prefecture in Japonia, 8 V 1956, A. Ezuka leg., in Herbario Instituti Nationalis Scientiae Agroenvironmentalis, Tsukuba, Japonia (NIAES 10512) conservatus.

Etymology: Referring to obscure symptom.

Specimens examined: NIAES 10511 (on *V. hirtum* var. *pubescens*, Hatsukura, Haibara, Shizuoka Prefecture, April 26, 1956, A. Ezuka leg.), NIAES 10513 (on *V. hirtum* var. *pubescens*, Shimonagao, Nakakawane, Haibara, Shizuoka Prefecture, May 10, 1956, A. Ezuka leg.), NIAES 10514 (on *V. hirtum* var. *pubescens*, Kawaue, Ena, Gifu Prefecture, May 29, 1956, K. Hara leg.), NIAES 10515 (on *V. hirtum* var. *pubescens*, Hiromineyama, Himeji, Hyogo Prefecture, May 24, 1959, A. Ezuka leg.), NIAES 10516 (on *V. hirtum* var. *pubescens*, Shimonagao, Nakakawane, Haibara, Shizuoka Prefecture, May 12, 1963, A. Ezuka leg.), NIAES 10545 (on *V. hirtum* var. *pubescens*, Toriimoto, Ukyo-ku, Kyoto, Kyoto Prefecture, May 1, 1990, A. Ezuka leg.), TSH-B0080 and TSH-B0086 (on *V. hirtum* var. *pubescens*, Meakan Spa, Kamirawan, Ashoro, Hokkaido Prefecture, June 21, 2001, H. Nagao leg.).

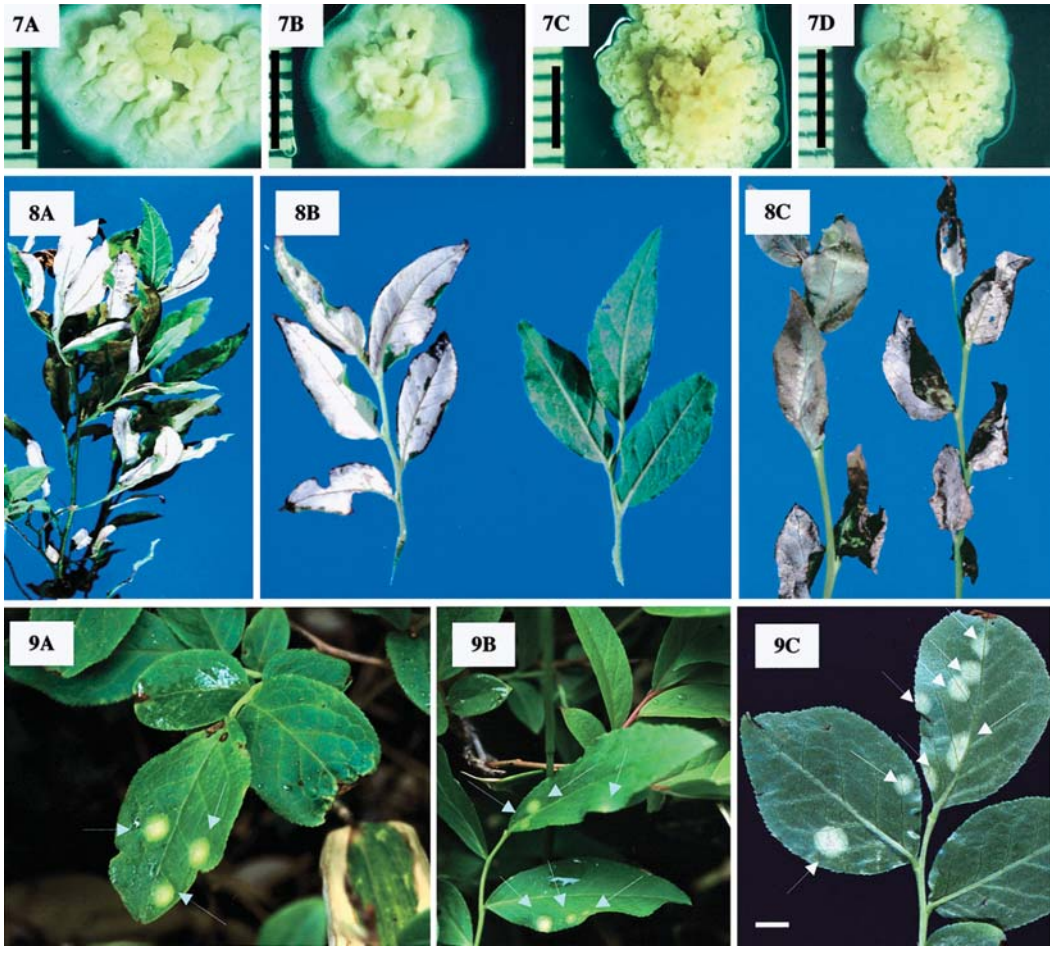

Fig. 7. Morphology and coloration of *Exobasidium* spp. on PDA. Surface of colonies of *E. kishianum* MAFF 238623 (**A**), MAFF 238624 (**B**); surface of colonies of *E. inconspicuum* MAFF 238616 (**C**), MAFF 238619 (**D**). *Bars* 5 mm

Fig. 8. Symptoms of Exobasidium leaf blight on *Vaccinium* spp. by *E. kishianum*. **A** Appearance of Exobasidium leaf blight on *V. smallii* TSH-B0071 in June 2000 in Aomori Prefecture. **B** Comparison of infected lower leaves (*left*) and healthy leaves (*right*). **C** Appearance of Exobasidium leaf blight on *V. hirtum* var. *pubescens* TSH-B0070, showing infected chlorotic upper leaves (*left*) and farinose lower leaves (*right*)

Fig. 9. Symptoms of Exobasidium leaf blister on *V. hirtum* var. *pubescens* by *E. inconspicuum*. Field appearance of Exobasidium leaf blister TSH-B0080 (**A**) and TSH-B0086 (**B**) in June 2001 in Hokkaido Prefecture. *Arrows*, chlorotic spots. **C** Sign on lower leaves of Exobasidium leaf blight TSH-B0080 (*arrows*). *Bar* 5 mm

Hymenium composed of basidia with 2–4(–5) sterigmata and conidia. Pseudohyphae not developing directly on the surface of epidermis. Basidia clavate to cylindrical, 80–100 × 4–8 µm, obtuse at the apex, not fasciculate. Sterigmata 1.5–2 µm in diameter at the base and 2–6 µm in height, emerging outwardly and tapering toward the tip (Fig. 3A,B). Basidiospores obovoid, or ellipsoid to oval, 9–19 × 3.5–6 µm, hyaline, smooth, one-celled or becoming septate with 0–4 septa (Figs. 3A,B, 6D–F). Septate basidiospores dropped on the agar surface germinating after 6 h forming germ tubes or conidia emerging from both their end cells and septal region or also forming conidia at the tip of germ tubes 22 h after the dropping (Fig. 4A,B). Conidia bacilliform (Fig. 3C,D), 3–6 × 1–1.5 µm, and budded polarly to produce daughter cells and to develop pseudohyphae. Colonies on PDA growing gradually, reaching maximum 15 mm diameter in 21-day incubations, and wrinkling irregularly at the periphery, with pale orange to peach-colored and corrugate surface not becoming farinose by conidial formation, obtrite and not fixed on the agar surface, composed of pseudohyphae and conidia (Fig. 7C,D). The reverse of colonies showing pale yellow to pale pink. Dark pigment not exuded on PDA. Colonies from conidia showing the same morphological features as those from basidiospores.

Among 103 taxa of *Exobasidium* that have been validly described, *E. asebi*, *E. cylindrosporum* Ezuka, *E. gaultheriae*, and *E. sakishimaense* Y. Otani showed similarities in some morphological measurements, especially in size of basidia and basidiospores, to this new species (Table 1). However, the mode of germination of basidiospore was budding in *E. gaultheriae*. This species was distinguished in the length of sterigmata. The basidiospores of this new species showed an obovoid shape predominantly, which was a critical difference from these species even though morphological measurements were in their ranges. The basidiospores of *E. asebi*, *E. cylindrosporum*, and *E.*

sakishimaense were ellipsoidal in shape. The symptom of *E. inconspicuum* was very obscure, although it was a round leaf spot of 2–8 mm diameter, chlorosis appeared faintly, a circumscribed rind did not occur, and no farinose hymenium appeared on the leaves (Fig. 9A–C). The symptom of *E. inconspicuum* and *E. asebi* was Exobasidium leaf blister, whereas that of *E. cylindrosporum* and *E. sakishimaense* was leaf gall.

Symptom of Exobasidium disease on a host plant resulted from a seasonal succession of two to three fungal generations (Richards 1896) or from two or three different *Exobasidium* species with localized or systemic mycelia (Nannfeldt 1981). In the case of *E. inconspicuum* and *E. kishianum*, two morphologically distinguishable species attacked *V. hirtum* var. *pubescens* in the same season. *Exobasidium inconspicuum* is characterized by its obovoid, or ellipsoid to oval, basidiospores and *E. kishianum* by its ellipsoid to ovoid basidiospores (Fig. 6, Table 1).

Acknowledgments We profoundly appreciate the cooperation of Dr. M. Akimoto, Donan Branch, Hokkaido Forestry Research Institute, and Dr. W. Abe, Graduate School of Science, University of Hokkaido, for their kind help with the sampling of *V. hirtum* var. *pubescens* and *V. smallii*, and thank Ms. H. Nakamura for preparing the medium and helping with the experiments.

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