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FULL PAPER

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A new species of *Discostroma* and its anamorph *Seimatosporium* with two morphological types of conidia, isolated from the stems of *Paeonia suffruticosa*

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Abstract Conidia of two morphologically different types, one with a basal appendage only and the other with appendage at both ends, were isolated from the stems of *Paeonia suffruticosa*. Single conidial isolates of both types of conidia yield identical colonies, which then produced both types of conidia on agar media depending on temperature, thus showing that both types of conidia belong to the same fungus. *Seimatosporium botan* is described based on its morphological characteristics. The teleomorph of the fungus was first found on sterilized *P. suffruticosa* stems placed on water agar, when grown at 5°C for 2 months in 12-h photoperiod. *Discostroma botan* is described for this fungus. The teleomorph is also found on the same host in the field.

Key words Amphisphaeriaceae · *Discostroma botan* · *Paeonia suffruticosa* · *Seimatosporium botan*

Introduction

In February 2002, we collected stems of *Paeonia suffruticosa* Andr. with acervuli of an interesting fungus. Its acervuli contained conidia with a basal appendage only, which belonged to *Seimatosporium* Corda. Single conidial isolates, however, yielded only conidia with appendage at both ends when grown on potato sucrose agar under room temperature conditions. The second collection from the same site in May 2002 was found to have both types of conidia.

To determine the identity of the producer of both types of conidia, a series of cultural experiments were conducted, with an attempt to find the teleomorph of the fungus.

Materials and methods

Fungus collections and isolates

Collected plant materials were brought into the laboratory; portions of the material were examined immediately, while the remainders were pressed and dried at room temperature and kept as dried herbarium specimens.

To obtain isolates, conidia from the materials were suspended in a drop of sterilized distilled water on sterilized glass slides, and then a loop of the suspension was streaked on the surface of glucose agar – GA; 20 g Wako glucose (Wako, Osaka, Japan), 20 g Wako agar, and 1000 ml distilled water – plates and kept at room temperature. In 1 day, individual germinating conidia were transferred onto potato sucrose agar (PSA; 200 g potato, 20 g Wako sucrose, 20 g Wako agar, and 1000 ml distilled water) slants. At the same time, the position of cells developing into the germ tube was recorded. Single conidial isolates were used throughout the present study, and stock cultures were grown on PSA slants and kept at 5°C. Single ascospore isolates were obtained as described above. Herbarium specimens as well as isolates were all deposited in the Herbarium of Hirosaki University, Fungi (HHUF). In collector name, S.H. stands for Satoshi Hatakeyama.

Morphological observation

Fungal morphology was observed under Olympus BH2 and CK40 light microscopes (Olympus, Tokyo, Japan). The fruiting body was crushed in a drop of distilled water on a glass slide and then covered with a coverslip for observation. An oil immersion lens at 1000× was used for measurement. Melzer's reagent was used to test amyloid reactions of unitunicate ascal apices. Fruiting body sections were made

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using a freezing microtome (HM 400R; Microm, Walldorf, Germany) and mounted in distilled water for observation and photomicrography.

Cultural experiments

To determine the identity of the producer of both types of conidia, single conidial isolates were grown on malt extract agar – MA; 20 g Difco malt extract (Difco, Detroit, MI, USA), 20 g Wako agar, and 1000 ml distilled water – and potato dextrose agar (PDA; 39 g Difco potato dextrose agar, 1000 ml distilled water) at temperatures ranging from 5°C to 25°C at 5°C intervals, each with a 12 h-photoperiod, and the conidia newly formed were observed.

Growth rate and colony characteristics were recorded from cultures grown on MA and PDA plates at 20°C in the dark. Measurements were performed after 2 weeks of incubation. As a color standard, the *Methuen Handbook of Colour* (Kornerup and Wanscher 1978) was used.

Production of teleomorph in culture

To find the teleomorph of the fungus, *P. suffruticosa* stems (about 5 cm long) were washed clean and were autoclaved for 20 min at 121°C in Petri dishes. The sterilized stems were immersed halfway into the water agar (WA; 20 g Wako agar, 1000 ml distilled water) plate. A small piece of mycelial culture was placed as inoculum on the stems and incubated at the temperatures ranging from 5°C to 25°C at 5°C intervals, each with a 12 h-photoperiod, for 2 months.

Results

Identification of the producer of both types of conidia (conidia with a basal appendage only and conidia with appendage at both ends)

Germ tubes were observed to develop from pigmented cells only. No conidia were seen to germinate from hyaline cells or appendages (see Figs. 10, 11).

On MA, the fungus produced blackish conidial mass or a pionnote, 100–150 μm diameter. The type of conidia changed with the temperature condition.

At 5° or 10°C, conidia with a basal appendage only (type 1, as named here) were only formed (see Fig. 14), while at 20°C, conidia with appendage at both ends (type 2, as named here) were only formed (see Fig. 15). At 15°C, both types of conidia, type 1 and type 2, were formed in the same plate, but were not present together in the same pionnote. At 25°C, no conidia were formed.

The size, shape, and color of conidia produced on MA plates were identical with those on the host plant.

On PDA, the fungus produced an orange conidial mass or pionnote, 100–150 μm diameter, which only contained type 2 conidia at all temperatures except 25°C, although the conidial shape was variable under different temperatures (see Fig. 16). At 25°C, no conidia were formed.

Single conidial isolates of both types of conidia yielded identical colonies, which then produced both types of conidia on the MA plate, depending on temperature condition, thus showing that both types of conidia belonged to the same fungus.

Production of teleomorph in culture

At 15° or 20°C, anamorph or conidiomata was produced within 1 month of inoculation, but no teleomorph or ascomata was produced even in the following 2 months. At 5° or 10°C, the anamorph was produced within 1 month of incubation and, in 1 more month, the teleomorph was produced. At 25°C, neither anamorph nor teleomorph was produced.

The shape, number of septa, and color of ascospores produced under cultural condition were similar to those on the host plant, although numerous hyphal hairs developed around the ostiole absent in the ascomata in nature (see Figs. 5, 7).

In ascospore of the fungus, germ tubes usually developed from both end cells, but sometimes from other cells (see Fig. 2). Single ascospore isolates and single conidial isolates of both types of conidia yielded identical colonies on the same medium; thus, the teleomorph–anamorph relationship of the fungus was confirmed.

Single ascospore isolates of the fungus produced both *Discostroma* Clem. teleomorph and *Seimatosporium* anamorph under cultural condition, and therefore this fungus is considered homothallic.

Taxonomy

Teleomorph

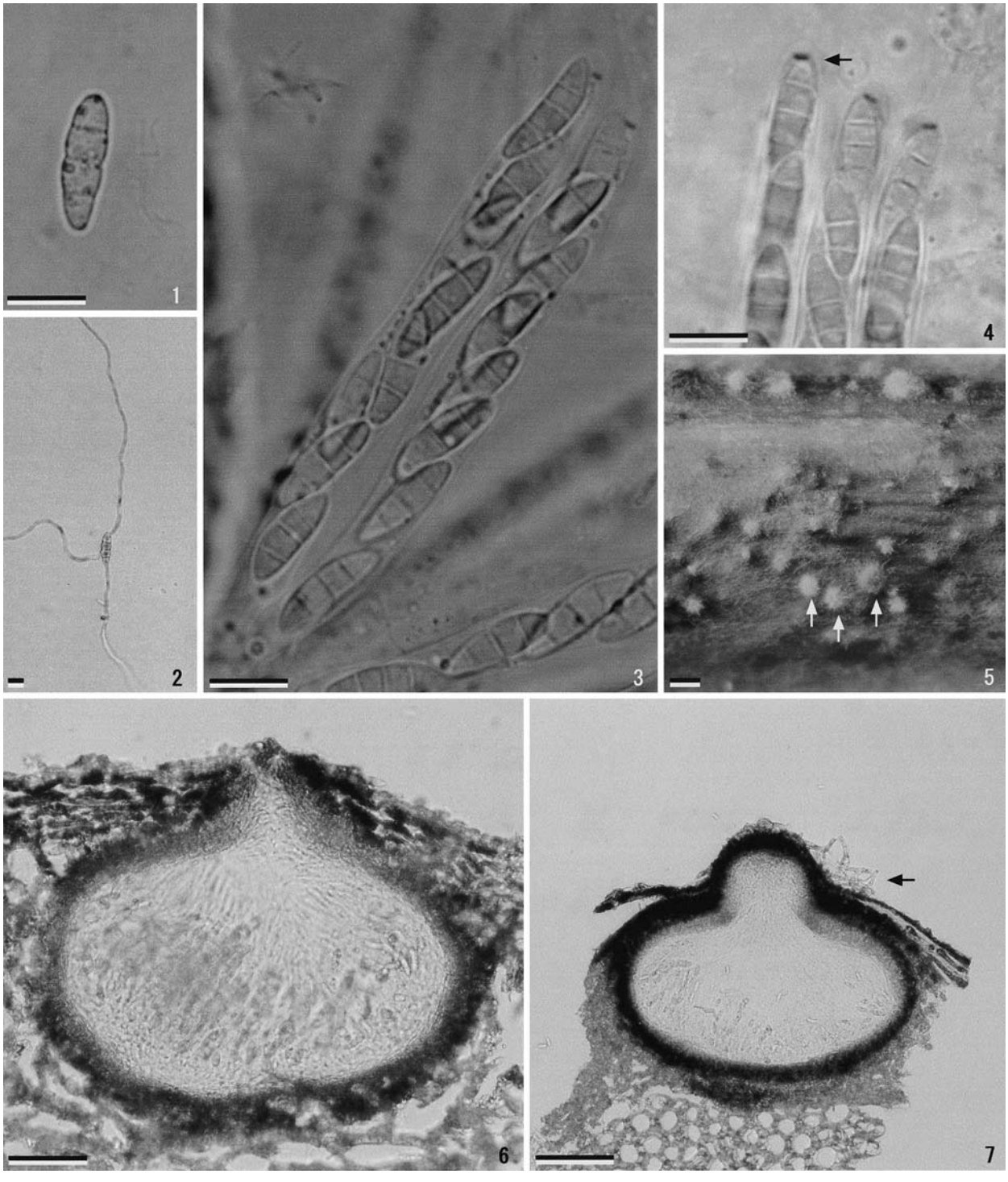
Discostroma botan Sat. Hatakeyama & Y. Harada, sp. nov. Figs. 1–7

Etymology: Botan = Japanese name of the host plant.

Ascomata perithecialia, solitalia, substrato immersa vel partim immersa, globosa vel subglobosa, brunnea vel atro-brunnea, 200–250 μm alta, 300–360 μm diametro, papillata, cellulis in stratis externis brunneis, in stratis internis hyalinis. Paraphyses filiformes, hyalinae, simplices, septatae. Asci unitunicati, cylindrici, apice rotundati, annulo apicali jodo cyanescenti praediti, breviter stipitati, octospori, (62–) 70–111 × 6–10 μm. Ascosporae uniseriales, ellipticae vel subfusiformes, hyalinae, transverse 3-septatae, ad septa non vel vix constrictae, laeves, 12–18 × 4–6 μm.

Holotypus HHUF 25865. On stems of *Paeonia suffruticosa*: Kirida, Towada, Aomori, Apr. 3, 2003, S.H. culture 4642, kept in the Herbarium (Fungi) of the Faculty of Agriculture and Life Science, Hirosaki University.

Ascomata perithecial, slightly papillate with periphysate ostiole, solitary, partly to completely immersed in the host substrate, globose to subglobose, brown to dark brown, 200–250 μm high, 300–360 μm diameter. Ascomatal walls composed of several layers, cells brown in outer layers, becoming hyaline in inner layers. Paraphyses filiform,



Figs. 1–7. *Discostroma botan*. **1** Ascospore. **2** Germinating ascospore. **3** Asci. **4** Ascal apex with an apical apparatus (*arrow*). **5** Ascomata formed under cultural condition, with hyphal hairs developed around the ostiole (*arrows*). **6** Cross section of an ascoma (in nature). **7** Cross section of an ascoma (formed under cultural condition), with hyphal hairs (*arrow*). (**1–4, 6** HHUF 25865; **5, 7** HHUF 27924.) *Bars* **1–4** 10 μm; **5** 500 μm; **6**, **7** 50 μm

hyaline, unbranched, septate. Asci unitunicate, cylindrical, apically rounded with an amyloid apical apparatus, short stalked, containing 8 uniseriate ascospores, (62–) 70–111 × 6–10 μm (mean, 92.8 × 7.7 μm; $n = 50$). Ascospores elliptic to subfusiform, hyaline, transversely 3-septate, nonconstricted or slightly constricted at the middle septum, smooth, 12–18 × 4–6 μm (mean, 15.3 × 4.9 μm; $n = 50$), length/width ratio (L/W) = 2.3–4.3 (mean, 3.1).

Additional specimens examined: On stems of *Paeonia suffruticosa*: Kirida, Towada, Aomori, Feb. 16, 2003, S.H. (HHUF 27925, culture 4604); Yagami, Towada, Aomori; Apr. 3, 2003, S.H. (HHUF 25866); Eainishikicho, Furukawa,

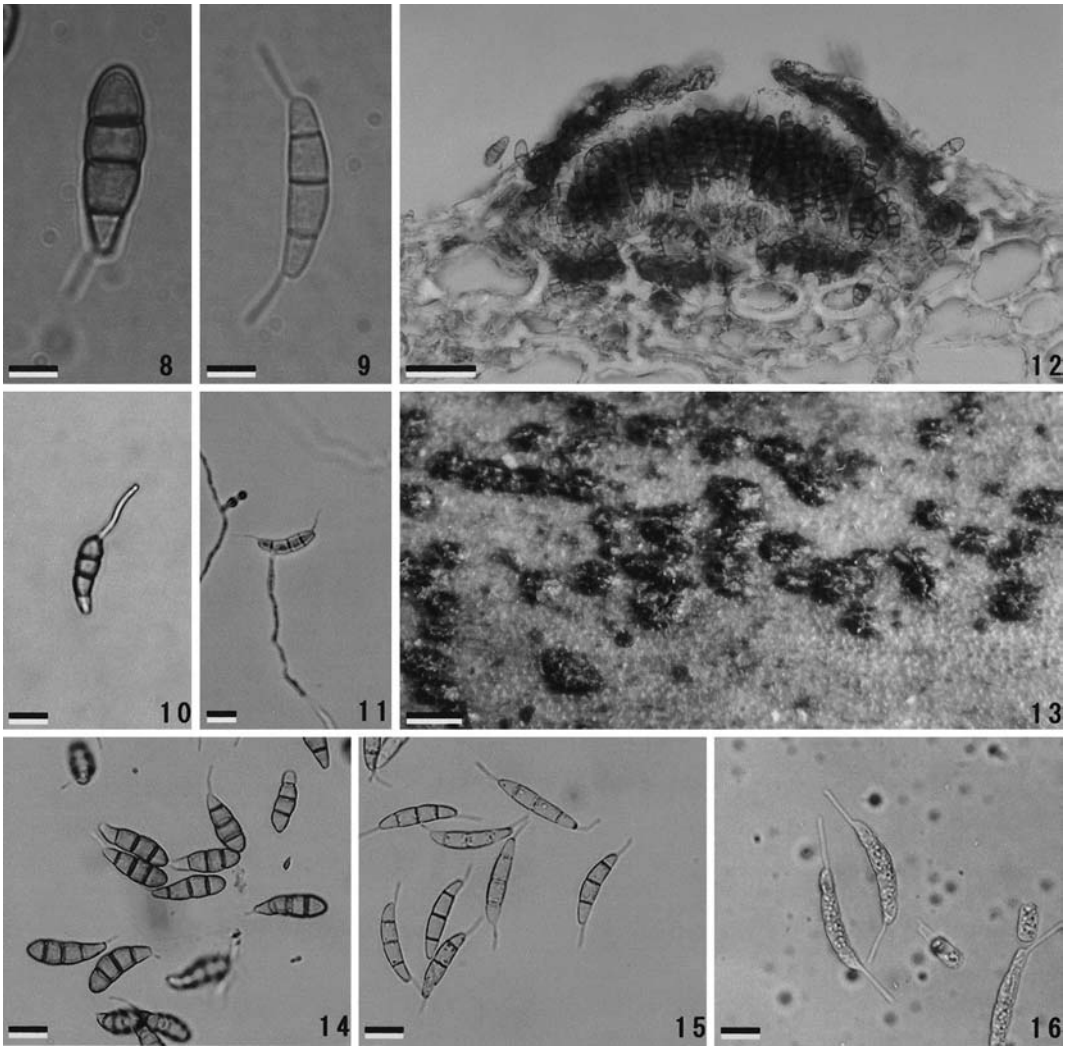
Miyagi, Apr. 6, 2003, Y. Takahashi (HHUF 28094); June 15, 2003, Y. Takahashi (HHUF 28095). Dried culture specimen: culture with stems of *P. suffruticosa* that was inoculated with culture 4618 (HHUF 27924, culture 4603).

Anamorph

Seimatosporium botan Sat. Hatakeyama & Y. Harada, sp. nov. Figs. 8–16

Etymology: Botan = Japanese name of the host plant.

Conidiomata acervulata, sparsa vel gregaria, applanata, semiimmersa vel erumpentia, ovalia vel orbicularia, brunnea vel fusca, 150–200 μm alta, 250–300 μm diametro, glabra, unilocularia, dehiscentia e textura obducenti; tela basalis et lateralis "textura angularis," cellulis in stratis externis brunneis, in stratis internis hyalinis. Conidiophora e basi conidiomatis enascentia, simplicia vel irregulatim ramosa, hyalina, septata, laevia, usque ad 20 μm. Cellulae conidiogenae cylindricae, hyalinae, holoblasticae, annellidicae. Conidia biformia: conidia cum appendice basali ellipsoidea vel fusiformia, transverse 3-septata, 16–20 × 5–7 μm, laevia, cellula basali obconica basi truncata subhyalina 3–5 μm longa, 2 cellulis centralibus subcylindricis vel doliiformibus brunneis vel arto-brunneis 8–10 μm longis, cellula apicali subconica apice rotundata concolori ut in cellulis centralibus 4–5 μm longa, appendice basali filiformi simplici excentrica 4–8 μm longa, appendice appicali nulla; conidia utrinque cum appendice fusiformia vel subcylindrica, transverse 3-septata, 16–20 × 4–5 μm, laevia, apice utrinque appendiculata, cellula basali obconica basi truncata subhyalina 4–5 μm longa, 2 cellulis centralibus subcylindrica vel doliiformibus pallide brunneis 8–11 μm longis, cellula apicali subacuta hyalina vel subhyalina 4–5 μm longa, appendice basali filiformi simplici



Figs. 8–16. *Seimatosporium botan*. **8** Conidium with a basal appendage only (type 1). **9** Conidium with appendage at both ends (type 2). **10** Germinating conidium (type 1). **11** Germinating conidium (type 2). **12** Cross section of a conidioma. **13** Conidiomata on host surface. **14** Type 1 conidia produced on malt extract agar (MA) plate at 5°C. **15** Type 2 conidia produced on MA plate at 20°C. **16** Type 2 conidia of irregular shape produced on potato dextrose agar (PDA) plate at 20°C. (**8–11** HHUF 27946; **12–13** HHUF 27945; **14–16** culture 4618.) *Bars* **8**, **9** 5 μm; **10**, **11** 10 μm; **12** 25 μm; **13** 250 μm; **14–16** 10 μm

excentrica 4–8 μm longa, appendice apicali filiformi simplici 4–8 μm longa.

Holotypus HHUF 27946. On stems of *Paeonia suffruticosa*: Kirida, Towada, Aomori, May 27, 2002, S.H. culture 4619, kept in the Herbarium (Fungi) of the Faculty of Agriculture and Life Science, Hirosaki University.

Conidiomata acervular, scattered to gregarious, applanate, subimmersed or erumpent, oval to orbicular, brown to blackish-brown, 150–200 μm high, 250–300 μm diameter, glabrous, unilocular, dehiscing by a split in the overlaying host tissue, basal and lateral tissue of textura angularis, cells brown in outer layers, hyaline in inner layers. Conidiophores arising at the base of the cavity of the conidioma, unbranched or irregularly branched, hyaline, septate, smooth, up to 20 μm long. Conidiogenous cells cylindrical, hyaline, holoblastic, annellidic. Conidia of two types: conidia with a basal appendage only, ellipsoid to fusiform, transversely 3-septate, 16–20 × 5–7 μm (mean, 18.0 × 6.0 μm; $n = 50$), L/W = 2.6–3.8 (mean, 3.0), smooth; basal cell obconic with a truncate base, subhyaline, 3–5 μm long (mean, 4.0 μm long); two central cells subcylindrical to doliiform, brown to dark brown, 8–10 μm long (mean, 9.1 μm long) [the second cell from the base 4–5 μm long (mean, 4.6 μm long), the third cell 4–5 μm long (mean, 4.5 μm long)]; apical cell conical with rounded apex, concolous with the central cells, 4–5 μm long (mean, 4.8 μm long); basal appendage filiform, unbranched, excentric, 4–8 μm long (mean, 6.0 μm long); apical appendage absent. Conidia with appendage at both ends fusiform to subcylindrical, transversely 3-septate, 16–20 × 4–5 μm (mean, 18.0 × 4.0 μm; $n = 50$), L/W = 4.0–5.0 (mean, 4.6), smooth, bearing appendage at both ends; basal cell obconic with a truncate base, subhyaline, 4–5 μm long (mean, 4.3 μm long); two central cells subcylindrical to doliiform, pale brown, 8–11 μm long (mean, 9.8 μm long) [each cell 4–6 μm long (mean, 4.9 μm long)]; apical cell conical with an acute apex, hyaline to subhyaline, 4–5 μm long (mean, 4.0 μm long); basal appendage filiform, unbranched, excentric, 4–8 μm long (mean, 5.4 μm long); apical appendage filiform, unbranched, 4–8 μm long (mean, 5.8 μm long).

Additional specimens examined: On stems of *Paeonia suffruticosa*: Kirida, Towada, Aomori, Feb. 24, 2002, S.H. (HHUF 27945, culture 4618); Aug. 14, 2002, S.H. (HHUF 27947); Nov. 30, 2002, S.H. (HHUF 27948); Yagami, Towada, Aomori; Nov. 9, 2002, S.H. (HHUF 27949).

Cultural characteristics

Colonies on PDA attaining a diameter of 5.7–5.9 cm within 14 days at 20°C, slimy in appearance, Salmon (6A4) to Pale Orange (6A3) with white, velvety, irregular margin; reverse similar; no pigment produced.

Colonies on MA attaining a diameter of 7.4–7.8 cm within 14 days at 20°C, velvety in appearance, white and Nutria (5F3) with radiating lines from the center, margin entire; reverse similar; no pigment produced.

Discussion

Seimatosporium botan is characterized by its fusiform to subcylindrical conidia having 3-septate, two median colored cells, an excentric basal appendage, and with or without an apical appendage. *Seimatosporium botan* is morphologically differentiated from three other most similar *Seimatosporium* species, having 3-septate conidia, in apparently longer conidia (Table 1). *Seimatosporium hebeiense* W.P. Wu was isolated from the same host in China (Wu 1992). Conidia of *S. hebeiense* are shorter than those of *S. botan*, and lack an apical appendage.

Discostroma botan is characterized by its unitunicate asci with an amyloid apical apparatus, hyaline, 3-septate ascospores, and the *Seimatosporium* anamorph. *Discostroma botan* is morphologically differentiated from the known species of *Discostroma*, having transversely 3-septate ascospores, in the size of the asci and ascospores (Table 2). *Discostroma polymorphum* Brockmann is closest to *D. botan*, but differs from the latter in brown ascospores and an different anamorph of *Sprocadus* Corda.

Seimatosporium botan produced type 1 conidia only on MA plates at 5°–10°C; at 20°C, it produced type 2 conidia only. These results showed the lower temperatures affected formation of type 1 conidia. Both types of conidia were produced on MA plates at 15°C, which seemed a boundary for temperature reaction. It is likely that a similar phenomenon might occur in nature. For instance, only type 1 conidia were observed with specimens collected from November through March (HHUF 25865, 25866, 27925, 27945, 27948, 27949, 28094) whereas only type 2 conidia were observed with specimens collected from June through August (HHUF 27947, 28095), and both types of conidia were present together on the same stem that was collected in late May (HHUF 27946). *Discostroma botan* was observed with specimens collected from November through May (HHUF 25865, 25866, 27925, 28094, 28095).

There are some reports referring to alteration in spore morphology by temperature and other factors. For example, in *Ulocladium chartarum* (Preuss) E.G. Simmons and *Stemphylium floridanum* C.I. Hannon & G.F. Weber,

Table 1. Morphological comparison between *Seimatosporium botan* and *Seimatosporium* species having 3-septate conidia

Species	Conidia size (μm)	Appendages length (μm)	Literature
S. grammitum	12–18.5 × 3.5–5.5	2–8	Nag Raj (1993)
S. hebeiense	13.5–17.6 × 4.5–6.5	3–5.7	Wu (1992)
S. hysterioides	12–18 × 5.5–6.5	1–14	Nag Raj (1993)
S. botan	16–20 × 4–7	4–8	Present paper

Table 2. Morphological comparison between *Discostroma botan* and *Discostroma* species having transversely 3-septate ascospores

Species	Asci (μm)	Amyloid apical ring	Ascospores		Literature
			Size (μm)	Color	
D. fruticosum	90–130 × 9–11	+	18–26 × 6–8	Hyaline	Yuan and Barr (1994)
D. fuscellum	115–132 × 7–12	+	15–21 × 5–9.5	Hyaline	Huhndorf (1992)
D. muricatum	100–110 × 12–17	−	18–20 × 8–10	Brown	Barr (1983)
D. osyridis	75–80 × 5–6	−	11.5–13.8 × 4–4.5	Brown	Sivanesan (1983)
D. polymorphum	85–110 × 7–10	+	11–16.7 × 5.6–7.3	Brown	Brockmann (1976)
D. propendulum	110–140 × 18–20	+	19–22 × 10–13	Hyaline	Eriksson (1974)
D. rubicola	100–160 × 9–13	+	15.5–24 × 6.5–9	Hyaline	Barr (1993)
D. saccardoanum	117–150 × 8–10	+	15–18 × 5.5–7.5	Pale brown	Brockmann (1976)
D. sanguineae	105–130 × 8.5–13.3	+	16.5–20 × 7.5–9	Hyaline	Brockmann (1976)
D. strobiligenum	55–70 × 5–7	+	7–11 × 3–4	Hyaline	Müller and Loeffler (1957)
D. botan	70–111 × 6–10	+	12–18 × 4–6	Hyaline	Present paper

conidial morphology was altered from "*Alternaria*-like" to "*Stemphylium*-like" according to a temperature rise (Leach and Aragaki 1970). The size of conidia of *Alternaria cichorii* Nattrass changed with different temperature and light intensity (Vakalounakis and Christias 1985). Some other factors, such as age of culture, have been reported to alter conidial morphology (Kaneko 1980; Yokoyama 1975).

This is the first report about temperature effect on conidial types of *Seimatosporium*. Although shape and size as well as color and ornamentation of conidia have been used as important criteria in delimitation of fungal species, there have been no reports for *Seimatosporium*. It is necessary to perform a cultural examination to see if there is such effect for other species of *Seimatosporium* for which only either type 1 or type 2 conidia were known.

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