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FULL PAPER

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Addition and reexamination of Japanese species belonging to the genus *Cercospora* and allied genera. VIII. Newly recorded species from Japan (3)

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Abstract Four species belonging to the genus *Cercospora* and allied genera were newly added to the Japanese mycoflora. They are *Cercospora plantaginis* on *Plantago asiatica*, *Pseudocercospora asystasiae* on *Asystasia gengetica*, *P. cassiae-fistulae* on *Cassia fistula*, and *Pseudocercosporella ranunculacearum* on *Clematis grata* var. *ryukyuensis*.

Key words *Cercospora* · New to Japan · *Pseudocercospora* · *Pseudocercosporella*

Introduction

In recent years, taxonomic reexamination of *Cercospora* and allied genera has been carried out throughout the world based on new generic concepts (Braun 1995, 1998; Crous and Braun 2003). In Japan, Katsuki (1965) published a monograph of Japanese Cercosporae including 226 species. However, his monograph should be revised according to new generic concepts. Moreover, many species of this group are being added to Japanese mycoflora (Kobayashi et al. 1998, 2002; Nakashima 2004; Nakashima et al. 2002). In this article, four species of *Cercospora* and related genera with no previous record in Japan were identified, described, and discussed. These taxa consist of one species of the genus *Cercospora*, two of *Pseudocercospora*, and one of *Pseudocercosporella*.

In this study, slides for the light microscope were prepared by hand sectioning of fresh materials. Mounting medium used was Shear's fluid. The dried specimens are maintained in the Laboratory of Forest Pathology Herbarium, Forestry and Forest Products Research Institute, Japan (TFM: FPH).

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Descriptions

Cercospora plantaginis Saccardo, Michelia 1:267, 1879 (Saccardo, Sylloge Fungorum 4:219, 1886); Chupp, A monograph of the fungus genus *Cercospora* 443, 1954; Braun and Castaneda, Crypt Bot 2/3:291, 1991; Braun and Melnik, Cercosporoid fungi from Russia and adjacent countries 80, 1997; Shin and Kim, *Cercospora* and allied genera from Korea 93–95, 2001.

[*Cercospora apii* s. lat. (Crous and Braun 2003)]

Synonym: *Cercosporella plantaginella* Tehon, Mycologia 16:139, 1924.

Leaf spots circular to subcircular, 1–3 mm in diameter, pale brown with dark brown concentric rings, scattered. Fruit bodies amphigenous. Stromata small, composed of a few dark brown cells. Conidiophores loosely fasciculate, erumpent, simple, septate, brown, straight or geniculate, with thickened conidial scars, 25–75 × 3–3.8 μm. External hyphae not observed. Conidia acicular, straight, smooth, hyaline, with thickened and truncate hilum, tip acute, pluriseptate, 20–95 × 2.5 μm.

Host: *Plantago asiatica* L. (Japanese name: Ohbako).

Specimen examined: Morioka, Iwate Pref., by Kaneyoshi Sawada (TFM: FPH-6236), as *Cercospora* sp.

Note: On the host plant genus *Plantago*, two species of *Cercospora* have hitherto been known (Crous and Braun 2003). *Cercospora pantoleuca* Saccardo differs from the present species by having hyaline, short (5–15 μm) conidiophores and verruculose conidia. The other species, *C. plantaginis* Saccardo, has been well known as having conidiophores of variable shape (Shin and Kim 2001). According to Chupp (1954) and Braun and Castaneda (1991), conidiophores were measured from 20 to 300 μm in length, and Shin and Kim (2001) observed them extending to 150 μm. In the case of the Japanese material, variability of form and length of the conidiophores seemed to be less than that described in these previous records. However, as the other morphological characteristics and symptoms of the present fungus are identical with those of *C. plantaginis* Saccardo, it was identified as *C. plantaginis*.

Crous and Braun (2003) proposed a "compound species" named *C. apii* s. lat. comprising all cercosporoid hyphomycetes indistinguishable from *Cercospora* on *Apium graveolens* L. Then, [*Cercospora apii* s. lat.] was added onto the species description to indicate the taxonomy currently applied. *C. plantaginis* is also included in *Cercospora apii* s. lat. based on its morphological characteristics. On the other hand, as they have pointed out, it is necessary to do the inoculation test to define the host range and to do the phylogenetic studies. However, these experiments for *C. plantaginis* have not yet been conducted. Therefore, *C. plantaginis* should be treated as a independent species until this research is done and [*Cercospora apii* s. lat.] is added onto its description in this study.

The present fungus has been recorded from Armenia, Azerbaijan, Brazil, Bulgaria, Cuba, Cyprus, Egypt, Germany, Great Britain, Hawaii, Italy, Kazakhstan, Korea, Mauritius, Panama, Portugal, Romania, Russia (European part), South Africa, Ukraine, United States, and Uzbekistan, Venezuela (Braun and Castaneda 1991; Braun and Melnik 1997; Crous and Braun 2003; Chupp 1954; Shin and Kim 2001). According to Shin and Kim (2001), the present species in Korea has been commonly known as the causal agent of severe leaf spot on *Plantago lanceolata* L. On the other hand, this species has not been reported from Japan up to the present. However, it was found in Sawada's specimens labeled as *Cercospora* sp. deposited in TFM: FPH of FFPRI.

Pseudocercospora asystasiae (Yen) Yen, in Yen & Lim, Gardens' Bull., Singapore 33:169, 1980. Figs. 1, 4, 5

Synonym: *Cercospora asystasiae* Yen, Rev. Mycol. (Paris) 32:178, 1967; Gardens' Bull., Singapore 33:169, 1980.

Leaf spots circular, brown, pale brown in the center. Fruit bodies amphigenous. Stromata brown, amphigenous, mostly hypophyllous, 15–55 μm in diameter. Conidiophores arising from stromata, hyaline to brown, straight or sinuous, densely fasciculate, 20–57.5 × 2.5–5 μm, with unthickened conidial scars. External hyphae not observed. Conidia pale olivaceous, obclavate to cylindrical, 17–77 × 2.5–3.75 μm, 0–4-septate, with truncate and thin basal end.

Host: *Asystasia gengetica* (L.) Anderson (Japanese name: Sekido-sakuraso).

Specimen examined: Okinawa Memorial Park, Motobu (Okinawa Is.), Okinawa Pref., June 6, 1998, by Takao Kobayashi (TK) and Chiharu Nakashima (CN) (TFM: FPH-7644).

Note: On the host plant genus *Asystasia*, two species of *Cercospora* and allied genera have been known, namely *Cercospora asystasiana* Yen (1967) and *C. asystasiae* Yen (1967). Thereafter, the latter species was transferred to the genus *Pseudocercospora* based on its morphological characteristics (Yen and Lim 1980).

In this time, the present material was identified as *P. asystasiae*, although it had slightly larger stromata (14.4–30 μm in diameter), shorter conidiophores (24–126 in length), and smaller conidia (37.2–126 × 3.6–5 μm in size) compared with the original description of *C. asystasiae* by Yen (1967). The present species was reported from the Ivory Coast and Singapore (Crous and Braun 2003; Yen 1967).

The host plant was introduced from a foreign country to botanical gardens in Japan as a ground cover plant.

Pseudocercospora cassiae-fistulae Goh & W.H. Hsieh, in Hsieh and Goh, *Cercospora* and similar fungi from Taiwan 180, 1990 Figs. 2, 6, 7, 8

Leaf spots distinct, angular, blackish-brown, vein limited, 1–3 mm in size, often confluent. Fruit bodies strictly hypophyllous, observed on lower leaf surface as blackish mycelial mat composed of external hyphae, conidiophores, and conidia. Stromata brown to blackish-brown, 25–75 μm in diameter, with external hyphae. Conidiophores densely arising from upper part of stromata or singly from external

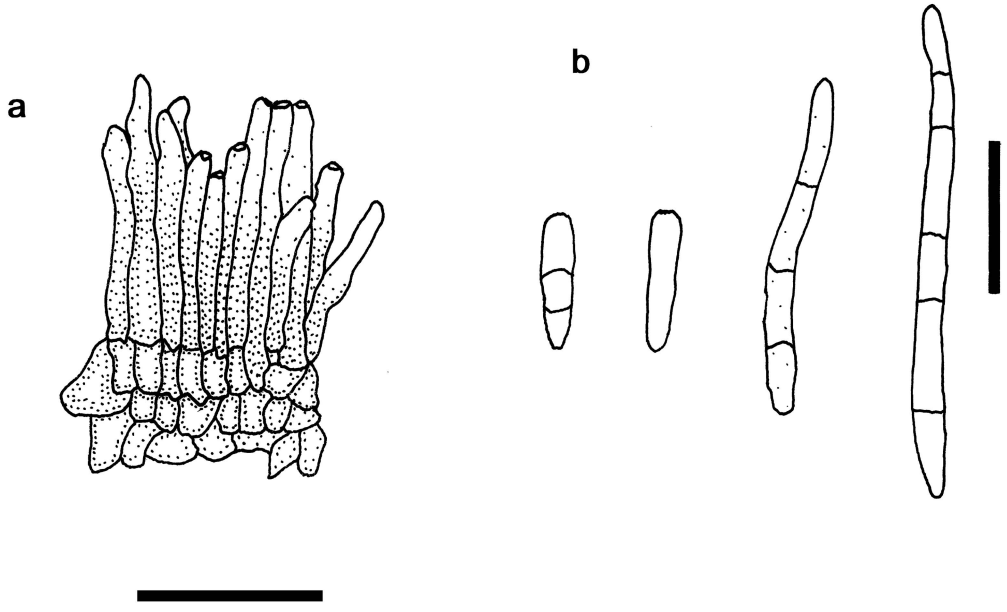

Fig. 1. *Pseudocercospora asystasiae.* **a** Stroma and conidiophores. **b** Conidia. *Bars* 20 μm

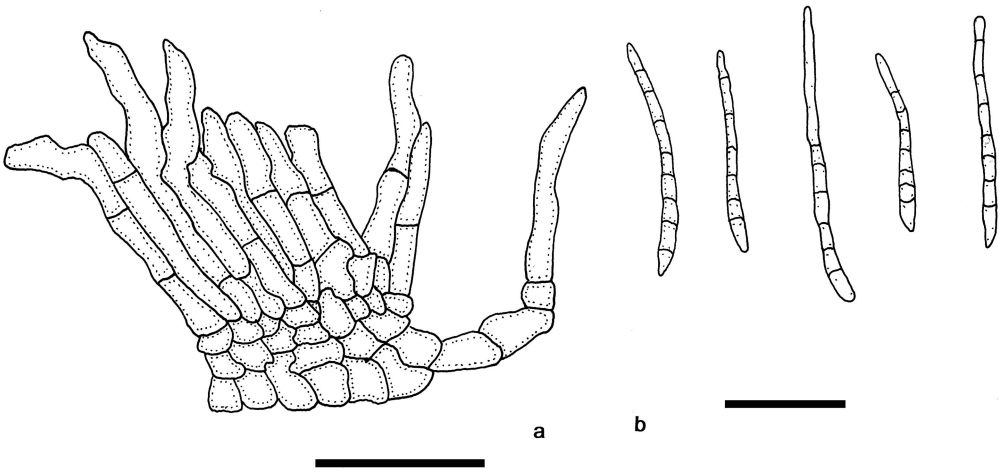

Fig. 2. *Pseudocercospora cassiae-fistulae.* **a** Stroma and conidiophores. **b** Conidia. *Bars* 20 μm

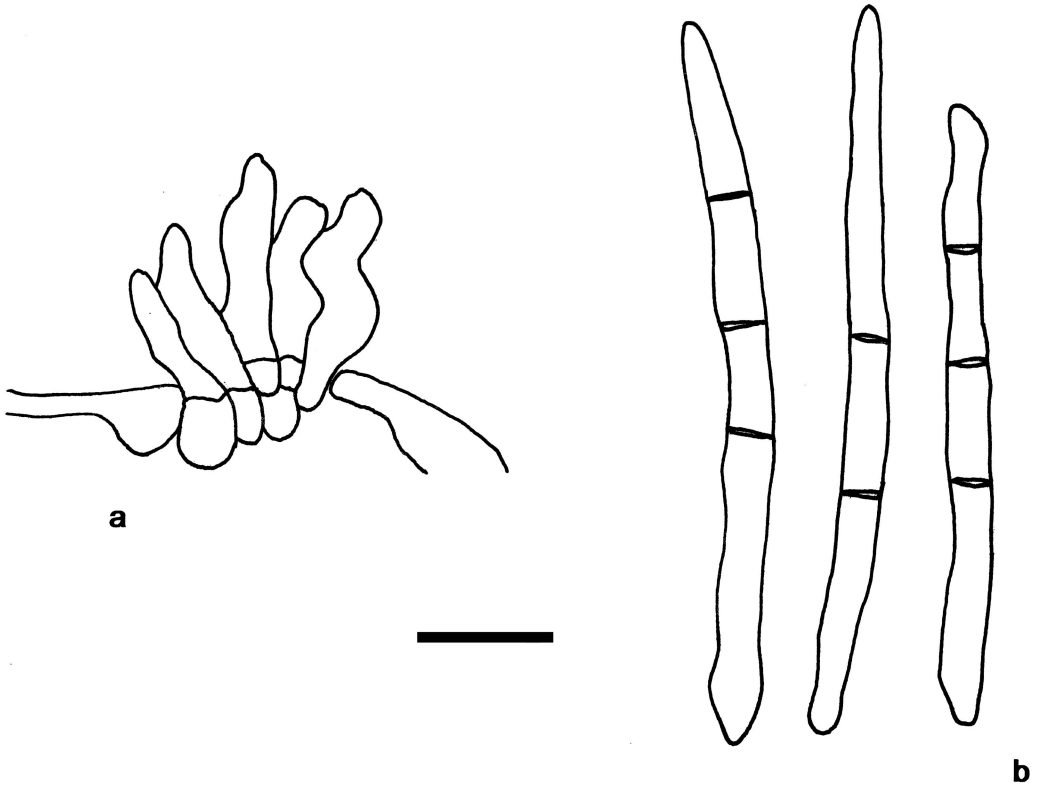

Fig. 3. *Pseudocercosporella ranunculacearum.* **a** Conidiophores. **b** Conidia. *Bars* 10 μm

hyphae, straight or flexuous, simple or branched, pale olivaceous-brown to olivaceous-brown, with thin conidia scars, 17–43 × 2.5–3 μm. Conidia pale olive, straight, obclavate, with thin and obconically truncate basal end, tip rounded, 17–80 × 2.5–3.8 μm, 1–9-septated.

Host: *Cassia fistula* L. (Japanese name: Nanban-saikachi).

Specimen examined: Tonan Botanical Garden, Chibana, Okinawa (Okinawa Is.), Okinawa Pref., March 7, 1998, by TK and CN (TFM: FPH-7645).

Note: On the host plant genus *Cassia*, many species of *Cercospora* and allied genera, mainly *Pseudocercospora*, have been known. The differences among these species on *Cassia* were published by Braun and Morgan-Jones (1977) and Braun (1989) with a dichotomous key. The dichotomous key for *Pseudocercospora* species on *Cassia*, which has alterations from Braun's (1989), is shown below.

1. Conidial scars unthickened, often inconspicuous, flat, broad, sometimes protuberant 2
1′. Conidial scars conspicuous, thickened, often also dark and bulging
..... *Cercospora* and allied genera except *Pseudocercospora*; *Cercospora canescens* [*Cercospora apii* s. lat.], *Cercospora cassiae-montanae*, *Cercospora cassiae-nodosae*, *Cercospora cassiatorae*, *Cercospora*

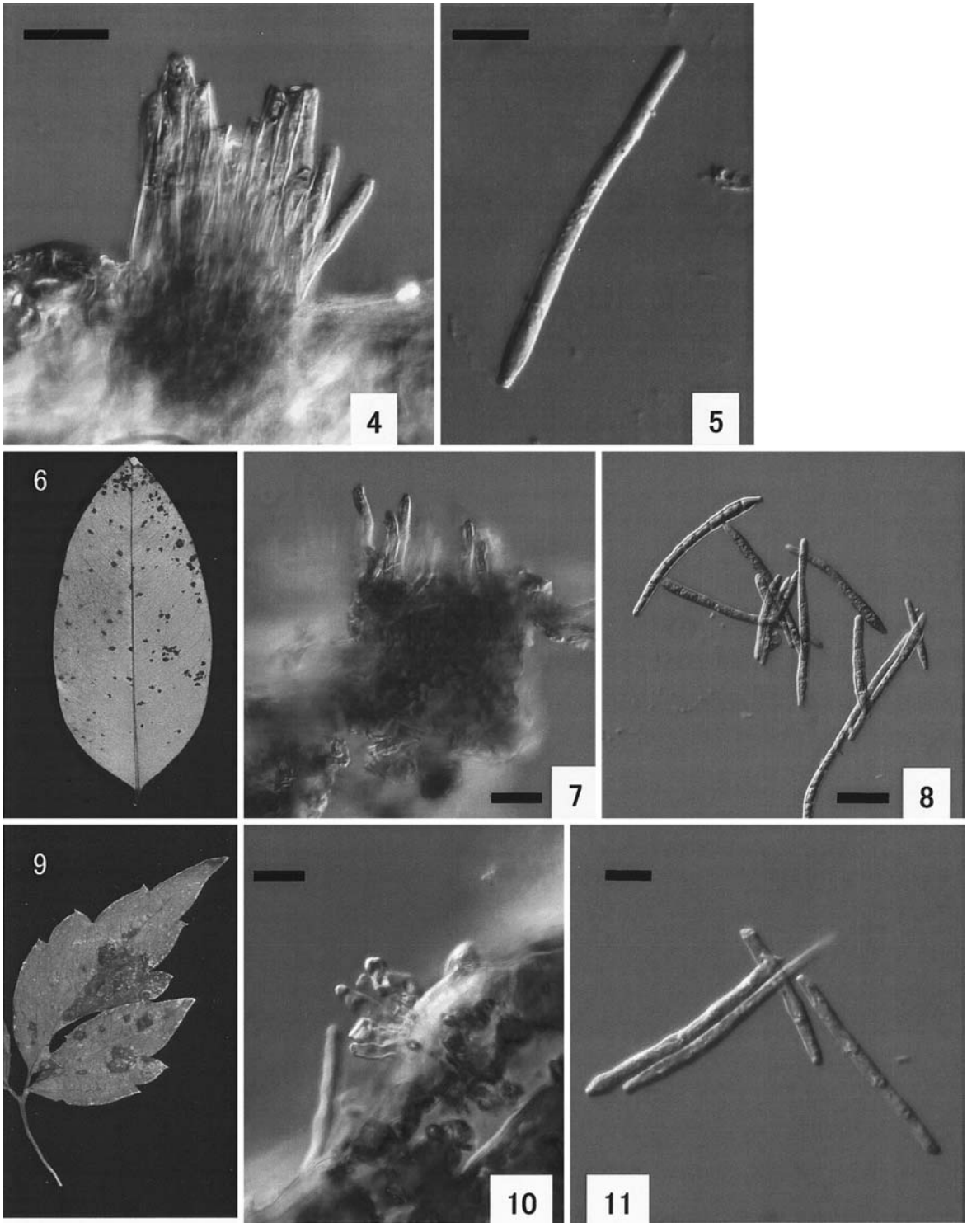

Fig. 4. Stroma of *Pseudocercospora asystasiae. Bar* 10 μm
Fig. 5. Conidium of *Pseudocercospora asystasiae. Bar* 10 μm
Fig. 6. Symptom associated with *Pseudocercospora cassiae-fistulae* on *Cassia fistula*
Fig. 7. Stroma of *Pseudocercospora cassiae-fistulae. Bar* 10 μm
Fig. 8. Conidia of *Pseudocercospora cassiae-fistulae. Bar* 10 μm
Fig. 9. Symptom associated with *Pseudocercosporella ranunculacearum* on *Clematis grata* var. *ryukyuensis*
Fig. 10. Conidiophores of *Pseudocercosporella ranunculacearum. Bar* 10 μm
Fig. 11. Conidia of *Pseudocercosporella ranunculacearum. Bar* 10 μm

cassiicola [*Cercospora apii* s. lat.], *Cercospora cassiocarpa* [*Cercospora apii* s. lat.], *Cercospora kikuchii* [*Cercospora apii* s. lat.], *Cercospora lambareneesis*, *Cercospora pinnulaecola*, *Passalora aenea*, *Passalora chamaecristae*, *Passalora greciana*, *Passalora occidentalis*

2. Mycelium internal as well as external, superficial, creeping (external) hyphae developed 3
2′. Mycelium internal, no external hyphae 5

3. Stromata well developed, fruit bodies strictly hypophyllous, conidiophores arising from stromata and external hyphae, dense fascicle, simple or branched, 10–43 × 2.5–5 μm, conidia 17–80 × 2.5–4 μm. On *Cassia fistula*, Taiwan and Japan ..... *Pseudocercospora cassiae-fistulae* Goh & W.H. Hsieh (Hsieh and Goh 1990)

3′. Stromata small or lacking 4

4. Conidiophores in small fascicles, emerging through stomata, and also solitary conidiophores, arising from external hyphae, 5–70 × 2.5–7 μm, conidia 1–5, mostly 1–3-septate, 12–55 × 3–6 μm. On *Cassia diphylla*, Brazil ..... *Pseudocercospora cassiae-diphyllae* Braun (Braun 1989)
4′. Conidiophores nonfasciculate, only solitary, arising from external hyphae, 3–45 × 2.5–3 μm, conidia 1–10-septate, 15–90 × 1.5–2 μm. On *Cassia alata*, Singapore ..... *Pseudocercospora cassiae-alatae* (Yen & Lim) Yen (Yen and Lim 1980)

5. Stromata well developed, conidiophores in dense fascicle, up to 25 μm in length, conidia small, up to 55 μm in length and 3 μm in width 6
5′. Conidiophores longer, conidia mostly larger 7

6. Stromata 25–40 μm in diameter, conidiophores very short, 6–11 × 2–3 μm, conidia small, 22–36 × 2–2.5 μm, mostly 3-septate. On *Cassia alata*, Singapore ..... *Pseudocercospora cassiigena* (Yen & Lim) Yen (Yen and Lim 1980)
6′. Stromata 30–100 μm in diameter, conidiophores 10–25 × 1–3 μm, conidia 20–55 × 1.5–3 μm, 1–6 indistinctly septate. On *Cassia fistulae*, Taiwan ..... *Pseudocercospora taichungensis* Goh & W.H. Hsieh (Hsieh and Goh 1990)

7. Stromata absent or only few brown cells, conidiophores in loose fascicle, 3–10, very long, 50–300 × 3–5 μm, fairly rigid, stiff, brown, paler upward, relatively thick walled and obviously septated, upper part characteristically undulate-geniculate and denticulate, conidia 20–55 × 3–6 μm, 1–5, mostly 1–3-septate, obclavate (subcylindrical). On *Cassia alata*, *C. hirsta*, and *C. marylandica*, North America, West Indies, South America ..... *Pseudocercospora simulata* (Ellis & Everh.) Braun & Castaneda (Castaneda and Braun 1989)
7′. Conidiophores shorter, about 20–100 μm in length, and/or conidia longer, or stromata well developed, large 8

8. Conidiophores apically obviously denticulate, up to 37.5 × 2–2.5 μm, simple, rarely branched, conidia pale olivaceous, obclavate-cylindrical, filiform, 1–8-septate, 27.5–72.5 × 2.5–3 μm, stromata developed. On *Cassia sieberiana*, India, Africa, Sierra Leone ..... *Pseudocercospora sieberiana* Raghu Ram & Mallaiah (Raghu Ram and Mallaiah 1993) (Teleomorph, *Mycosphaerella sieberiana*)
8′. Conidiophores not obviously denticulate 9

9. Stromata developed, large, 15–40 μm in diameter, conidiophores densely fasciculate, fairly short, about 10–50 μm in length 10
9′. Stromata absent or only very small, a few swollen cells, stromata up to 20 μm in diameter, conidiophores longer, or stromata present, but conidiophores much longer 11

10. Conidiophores about 15–30 × 3–4 μm, brown, conidia about 30–95 × 3.5–4 μm, light brown, 3–10-septate, narrowly cylindrical-obclavate. On *Cassia siamea*, India ..... *Pseudocercospora cassiae-siameae* (Chidd.) Deighton (Deighton 1976)
10′. Conidiophores 10–50 × 2–3.5 μm, pale olivaceous-brown, conidia 15–75 × 2–4 μm, 3–7-septate, subhyaline to pale olivaceous-brown. On *Cassia hirsuta*, South America, Columbia [according to Chupp (1954), stromata are small] ..... *Pseudocercospora angustata* (Chupp & Solheim) Deighton (Deighton 1976)

11. Leaf spot at first indefinite, later grayish-brown, suborbicular to irregular, stromata nearly absent to small, composed of a few brown cells, about 10–20 μm in diameter, conidiophores loosely fasciculate, about 2–15, straight to flexuous or slightly geniculate, (15–)20–100(–125) × 2.5–6 μm, olivaceous-brown, paler toward the apex, not denticulate, simple, occasionally irregularly branched (in some collections frequently branched), septate, conidia obclavate, subcylindrical, faintly colored, olivaceous, (15–)20–65(–80) × 3.5–6 μm, 1–6-septate. On *Cassia* species, widespread, North and South America, West Indies, Africa, Asia ..... *Pseudocercospora nigricans* (Cooke) Deighton (Deighton 1976)
11′. Leaf spot distinct, subcircular, yellowish-gray to brownish-gray, vein limited on the upper side, scattered, 0.5–4 mm in diameter, stromata lacking, conidiophores poor to medium fasciculate, 2–10, simple or branched, 31–77 × 4.5–5.5 μm, conidia cylindrical or obclavato-cylindrical, pale olivaceous, 31–77 × 4.5–5.5 μm, generally 3-septate (rarely 1- or 4-septate). On *Cassia occidentalis*, Singapore ..... *Pseudocercospora singaporensis* (Yen) Yen (Yen and Lim 1980)

In Japan, only one species of the genus *Pseudocercospora*, *P. nigricans* (Cooke) Deighton, has been reported on *Cassia occidentalis* L. (Japanese name: Habuso; Yamamoto and Maeda 1960; Katsuki 1965). The

present sample on *Cassia* collected in Okinawa Prefecture differs from *P. nigricans* in that symptom and small or lacking stromata. From those morphological characteristics and symptoms, it was completely equated with the description of *P. cassiae-fistulae*. According to the original description of the present species by Hsieh and Goh (1990), it also differs from the other *Pseudocercospora* on *Cassia* by its strictly hypophyllous fruiting and the simultaneous presence of the external hyphae and the densely fasciculate conidiophores on a developed stroma. This is the first record of this species outside Taiwan.

The present host plants are cultivated as a medicinal crop and also as an ornamental tree. Leaf spot caused by the present species is so serious that a control measure for the disease is urgently needed.

Pseudocercosporella ranunculacearum Braun, Mycotaxon 51:50, 1994; A monograph of *Cercosporella*, *Ramularia* and allied genera 1:182, 1995. Figs. 3, 9, 10, 11

Teleomorph: *Mycosphaerella ranunculi* (Karst.) Lind, Meddel. Gronland. 71:167, 1926 (Braun 1995).

Leaf spots black, angular to irregular with halo, confluent, 2–5 mm in size. Stromata absent or composed of a few small and hyaline cells, amphigenous, mainly hypophyllous, hyaline or slightly colored. Conidiophores hyaline or slightly colored, simple, straight or sinuous, 7–18 × 2–4 μm, with inconspicuously conidial scars. External hypha not observed. Conidia noncatenulate, scolecosporous or long-cylindrical to narrowly obclavate, narrowed toward apex, hyaline, unthickened at basal end, 0–6-septate, mainly 1–3-septate, 25–73 × 2.5 μm.

Host: *Clematis grata* Wall. var. *ryukyuensis* Tamura (Japanese name: Ryukyu-Botandzuru).

Specimens examined: Tagami, Tomigususku (Okinawa Is.), Okinawa Pref., November 16, 1999, by TK and Erica Imaizumi (EI) (TFM: FPH-7647); Tohashina, Tomigusuku (Okinawa Is.), Okinawa Pref., November 16, 1999, by TK and EI (TFM: FPH-7648); Higashi-nakasone, Hirara, Miyako (Miyako Is.), Okinawa Pref., November 20, 1999, by TK and EI (TFM: FPH-7646).

Note: The host plant, *Clematis grata* var. *ryukyuensis*, is a wild plant used also for a traditional event on Ryukyu Islands, including the Amami Islands of the southern part of Japan. Habitats are Ryukyu Islands and Taiwan.

The present fungus was identified as *P. ranunculacearum* on the same plant family Ranunculaceae because its morphological characteristics were equated with the original description by Braun (1994) except for the slightly wide conidiophores (2–3 μm in width; Braun 1994) and more septate conidia (1–4-septate; Braun 1994). The plant genus *Clematis* is newly added to host plants of the present species.

According to Braun (1994), conidiophores of the present species are associated with pseudothecia of *Mycosphaerella ranunculi* on *Ranunculus.* In the case of Japanese specimens, the telemorph had not been observed on leaf lesions. The habitat of present species was recorded from Canada (Braun 1995).

On the same host plant genus, *Clematis*, some species of *Cercospora*, and allied genera have been known. Especially, *Pseudocercospora clematidis* Goh & W.H. Hsieh (Goh and Hsieh 1989; Hsieh and Goh 1990) has been recorded from Taiwan. The description of the fungus and symptoms were quite similar to the Japanese descriptions except for slightly colored conidia. On the other hand, *P. clematidis* recorded from China (Guo and Hsieh 1995), Taiwan (Guo and Hsieh 1995), and Korea (Shin and Kim 2001) have distinctly colored stromata and developed external hyphae.

Japanese specimens must be identified as the genus *Pseudocercosporella* based on its morphological characteristics (hyaline conidiophores and conidia, inconspicuous conidial scars and scolecospore). However, the genus *Pseudocercosporella* has been known to have faintly colored conidiophores and conidia (Braun 1990). For these reasons, *Pseudocercospora clematidis* sensu Goh and Hsieh (1989) and Hsieh and Goh (1990) will probably be transferred to the genus *Pseudocercosporella.* Then, it should be treated as a synonym of *P. ranunculacearum*. More detailed comparative study for distinction of both species will be required after examining the type specimen.

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