

# Addition and re-examination of Japanese species belonging to the genus *Cercospora* and allied genera. X: newly recorded species from Japan (5)

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**Abstract** *Cercospora yakushimensis* is proposed as a new combination, *Pseudocercospora yakushimensis* comb. nov., and the morphology of the species is characterized from herbarium specimens, including the type. Six additional species belonging to the genus *Cercospora* and its allied genera are newly added to the Japanese mycoflora, namely, *Cercospora dioscoreae-pyrifoliae* on *Dioscorea tokoro*, *Pseudocercospora catalpigena* on *Catalpa ovata*, *Pseudocercospora coriariae* on *Coriaria japonica*, *Pseudocercospora lythri* on two species of *Lythrum*, *Pseudocercospora pteroceltidis* on *Celtis boninensis*, and *Passalora puncta* on *Foeniculum vulgare*.

**Keywords** Fennel · Medicinal plant · New combination · Plant disease

## Introduction

This paper contributes to our increased understanding of cercosporoid fungi in Japan. Most of the specimens used in this study were collected from gardens dedicated to medicinal plants. According to the Fifteenth Edition of the Japanese Pharmacopoeia (2006, <http://jpd.b.nihs.go.jp/jp15e/>), 140 crude drugs in Japan are extracted from medicinal plants, which belong to 69 families and 130

plant genera. However, the diseases of these plants, including cercosporoid fungi, have been studied inadequately and the control of diseases is obstructed. In fact, in Japan, leaf blight of fennel (*Foeniculum vulgare* Mill.), which has been known as a globally important plant disease caused by a cercosporoid fungus, is newly recorded through this study, although it was introduced into the country as a medicinal plant more than 1,000 years ago.

As in our previous contributions, we report the addition of some new records to the Japanese mycoflora and describe a novel combination based on recent generic criteria for cercosporoid fungi, which were summarized by Crous and Braun (2003) previously.

## Materials and methods

### Collection and isolation of cercosporoid fungi

Samples of diseased leaves showing signs of leaf blight and/or spots with caespituli caused by cercosporoid fungi were collected. These samples were pressed and dried for 3–5 days between newspaper sheets, which were changed daily. Finally, the leaves were transferred into herbarium packets. These specimens were kept at the mycological herbarium of the Laboratory of Plant Pathology, Mie University (MUMH), Tsu, Japan, or in the herbarium of the Department of Botany, National Museum of Nature and Science (TNS), Tsukuba, Japan. Single conidium isolates were prepared on malt extract agar (MEA; Crous et al. 2009). Using a flame-sterilized micro-spatula, conidia were collected from caespituli or from affected spots of the leaves and suspended in sterilized water on a microscope slide. The conidial suspension was further diluted in sterilized water and pipetted onto a 2% aqueous agar in a Petri dish and

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spread over the surface using a flame-sterilized microspatula. After incubation at 20°C in the dark for 24 h, the germinating conidia were individually transferred onto MEA using a flame-sterilized micro-tube under a light microscope. Purified cultures are maintained in the culture collection of the Laboratory of Plant Pathology, Mie University (MUCC), Tsu, Japan, or the Genebank, National Institute of Agrobiological Sciences (MAFF), Tsukuba, Japan.

#### Morphological observation

Slides for microscopic examination were prepared by manual sectioning of freshly collected material and herbarium specimens using a razor blade. Specimens were mounted in Shear's medium (Chupp 1940) and examined under a light microscope.

#### Taxonomy

Epitypification and new combination for *Cercospora yakushimensis*

***Pseudocercospora yakushimensis*** (Togashi & Katsuki) C. Nakash. & Tak. Kobay., comb. nov. Figs. 1a, b

Basionym: *Cercospora yakushimensis* Togashi & Katsuki, Bot. Mag. (Tokyo) 65: 25, 1952.

≡ *Pseudocercospora yakushimensis* (Togashi & Katsuki) Tak. Kobay., Nishij. & C. Nakash., in Nakashima, Taxonomic study of *Cercospora* and allied genera in Japan: 168, 2001. *nom. inval.* [ICBN Art. 30.5.].

Leaf spots vein limited, indistinct to distinct at the border, angular, subcircular to irregular, pale brown, then turning dark brown with grayish white centers, 2–5 mm in size. Caespituli amphigenous. Stromata small to well developed, dark brown, 11–58 µm in diam. Conidiophores loosely to densely fasciculate, simple, rarely branched, straight to sinuous, rarely geniculate caused by sympodial proliferation, brown to pale olivaceous brown, paler towards apex, usually uniform in width, somewhat swelling at the apex, 20–110 × 2.5–5 µm, 1–8-septate; conidiogenous cells integrated, terminal or intercalary, with unthickened and truncate, or subconspicuous loci, proliferating sympodially or percurrently. Conidia pale to pale olivaceous, cylindrical to obclavate, with unthickened and obconically truncate basal end, tip acute, smooth, 25–90 × 2.5–4.5 µm, 2–10-septate.

Specimens examined: on *Hydrangea kawagoana* Koidz. var. *kawagoana*, Japan, Yaku Island, Kagoshima Prefecture, 19 October 1949, leg. S. Katsuki (TNS-F-243830, type specimen of *Cercospora yakushimensis*); Japan, Mt. Miy-anoura, Yaku Island, Kagoshima Prefecture, 5 August 1951, leg. K. Togashi & S. Katsuki; Japan, Mt. Inokawa-dake,

Tokunoshima Island, Kagoshima Prefecture, 9 November 1993, leg. T. Kobayashi & M. Muramoto (epitype specimen of *Cercospora yakushimensis* designated here: TNS-F-34701, ex-epitype culture deposited: MAFF237025); Japan, Okawa fall, Yaku, Kumage, Kagoshima Prefecture, 18 October 1997, by T. Kobayashi & C. Nakashima.

Notes: Togashi and Katsuki (1952) established that *C. yakushimensis* differs from *C. hydrangeae* Ellis & Everh. because it has non-geniculate conidiophores and is also distinct from *C. obtegens* Syd. & P. Syd. [= *Pseudocercospora obtegens* (Syd. & P. Syd.) U. Braun & Crous] and *C. arborescens* Tehon & E.Y. Daniels (= *C. hydrangeae*) by forming azonate and large lesions and with mostly hypophyllous conidiophores. However, there was no difference in the preference of the caespituli forming between the adaxial and the abaxial leaf surfaces on the type (TNS-F-243830). These species can be distinguished by their morphological characteristics, including the size of stromata, and characteristics of loci and/or hila, rather than the shape of conidiophores or type of symptoms. Further, because the caespituli on the type specimen of *C. yakushimensis* (TNS-F-243830) were almost broken, the epitype specimen (TNS-F-34701) with an ex-epitype culture obtained from a single conidium of a proposed epitype (MAFF237025) is designated as described above.

In this study, *C. yakushimensis* is transferred to the genus *Pseudocercospora* based on its morphological characteristics, especially the formation of unthickened loci on conidiogenous cells and a hilum on the basal end of the conidia. On the plant genus *Hydrangea*, three *Pseudocercospora* species were hitherto known—one is *P. obtegens* (Syd. & P. Syd.) U. Braun & Crous (2003), characterized by a lack of stromata, and the others are *P. hydrangeae-angustipetalae* Goh & W.H. Hsieh (Goh and Hsieh 1989) and *P. katongensis* (J.M. Yen) J.M. Yen (in Yen and Lim 1980), characterized by the well-developed stromata. In this study, it has not been possible to trace the type specimens of *P. hydrangeae-angustipetalae* and *P. katongensis*. Those morphological characteristics based on the original descriptions (Goh and Hsieh 1989; Yen 1966) are quite similar to *P. yakushimensis*. Specimens of *P. yakushimensis*, including the type specimen and freshly collected specimens from the type locality, show amphigenous caespituli, well-developed stromata, and branched conidiophores. From these results, *P. hydrangeae-angustipetalae* and *P. katongensis* may be synonymized as *P. yakushimensis*.

Newly added species to the Japanese mycoflora

***Cercospora dioscoreae-pyrifoliae*** J.M. Yen “*dioscoreae-pyrifoliae*”, Bull. Soc. Mycol. France 84: 5, 1968

Figs. 1c, d

Leaf spots circular to irregular, pale brown to blackish brown, indistinct at the border, often confluent. Caespituli amphigenous. Mycelium internal. Stromata brown, intraepidermal or substomatal, globose, small to well developed, 18–28  $\mu\text{m}$ . Conidiophores arising from stromata, solitary to densely fasciculate, pale brown, straight to curved, geniculate-sinuuous, uniform in width, often constricted at septa, simple, occasionally branched, 31–102  $\times$  3–5  $\mu\text{m}$ , 1–7-septate; conidiogenous cells integrated, terminal, or intercalary, proliferating sympodially; conidiogenous loci circumsperised or apical, multilocal, distinctly thickened, protuberant, darkened, 1.5–2.5  $\mu\text{m}$  in diam. Conidia hyaline, cylindrical to acicular, truncate, and thickened at the base, rarely obconically truncate at the slightly protuberant base, acute at the apex, straight or curved, 25–87  $\times$  2.5–3  $\mu\text{m}$ , 3–9-septate.

Specimen examined: on *Dioscorea tokoro* Makino (Dioscoreaceae), Japan, Koishikawa Botanical Garden, University of Tokyo, Bunkyo, Tokyo, 10 November 2007, leg. I. Araki & M. Harada (MUMH10951, deposited culture: MUCC849).

Notes: The morphological characteristics of the present species are similar to those described and illustrated by Yen (1968), except for the width of conidiophores and conidia, the sizes of which were described as 36–200  $\times$  6–8.5  $\mu\text{m}$  and 45.5–168  $\times$  3.5–6  $\mu\text{m}$ , respectively. The present species was treated as a synonym of *Cercospora apii* by Pons and Sutton (1988). Crous and Braun (2003) re-introduced it as an independent species, although it is morphologically similar to *C. apii* s.l. We believe that this species is distinguishable from *C. apii*, emended by Groenewald et al. (2005), by having circumsperised loci (Hennebert and Sutton 1994; Braun 1995) on conidiogenous cells. The characteristic location of the conidiogenous loci, which was discounted by Pons and Sutton (1988), should be treated as an important characteristic when the species within *C. apii* s.l. are classified.

*Mycosphaerella papuana*, the teleomorph of *Cercospora dioscoreae-pyrifoliae* reported by Sivanesan (1985), was not observed in our study.

***Pseudocercospora catalpigena*** U. Braun & Crous, in Braun et al. Mycol. Progr. 2: 198, 2003 Figs. 1e, f

Leaf spots circular to subcircular, distinct, 2–15 mm in diam., confluent, pale brown to reddish brown, dark brown to greenish brown at the border, with a yellowish halo. Caespituli amphigenous, visible as grayish black masses on lesions. Mycelium internal. Stromata well developed, 28–116  $\mu\text{m}$ , brown, immersed, intraepidermal, substomatal, without external hyphae. Conidiophores densely fasciculate, pale brown, simple, rarely branched, straight or geniculate-sinuuous, 10–25  $\times$  2–4.5  $\mu\text{m}$ , 0–2-septate; conidiogenous cells integrated, terminal, proliferating

sympodially; conidiogenous loci inconspicuous, unthickened. Conidia pale olivaceous, cylindrical to cylindro-obclavate, straight to mildly curved, smooth, truncate to long obconically truncate and unthickened at the base, obtuse at the apex, 24–44  $\times$  2–4  $\mu\text{m}$ , 1–5(–8)-septate indistinctly.

Specimen examined: on *Catalpa ovata* G. Don (Bignoniaceae), Japan, Hidakagawa, Wakayama Prefecture, 30 October 2007, leg. I. Araki & C. Nakashima (MUMH10868, culture deposited: MUCC743).

Notes: This is the second record for *P. catalpigena* following its original description from the USA. Conidiophores and conidia of the present specimen are somewhat narrower in width than those originally described for *P. catalpigena* (Braun et al. 2003; conidiophores 3–6  $\mu\text{m}$ , conidia 3–5  $\mu\text{m}$ ). However, the other characters are quite similar to those originally described.

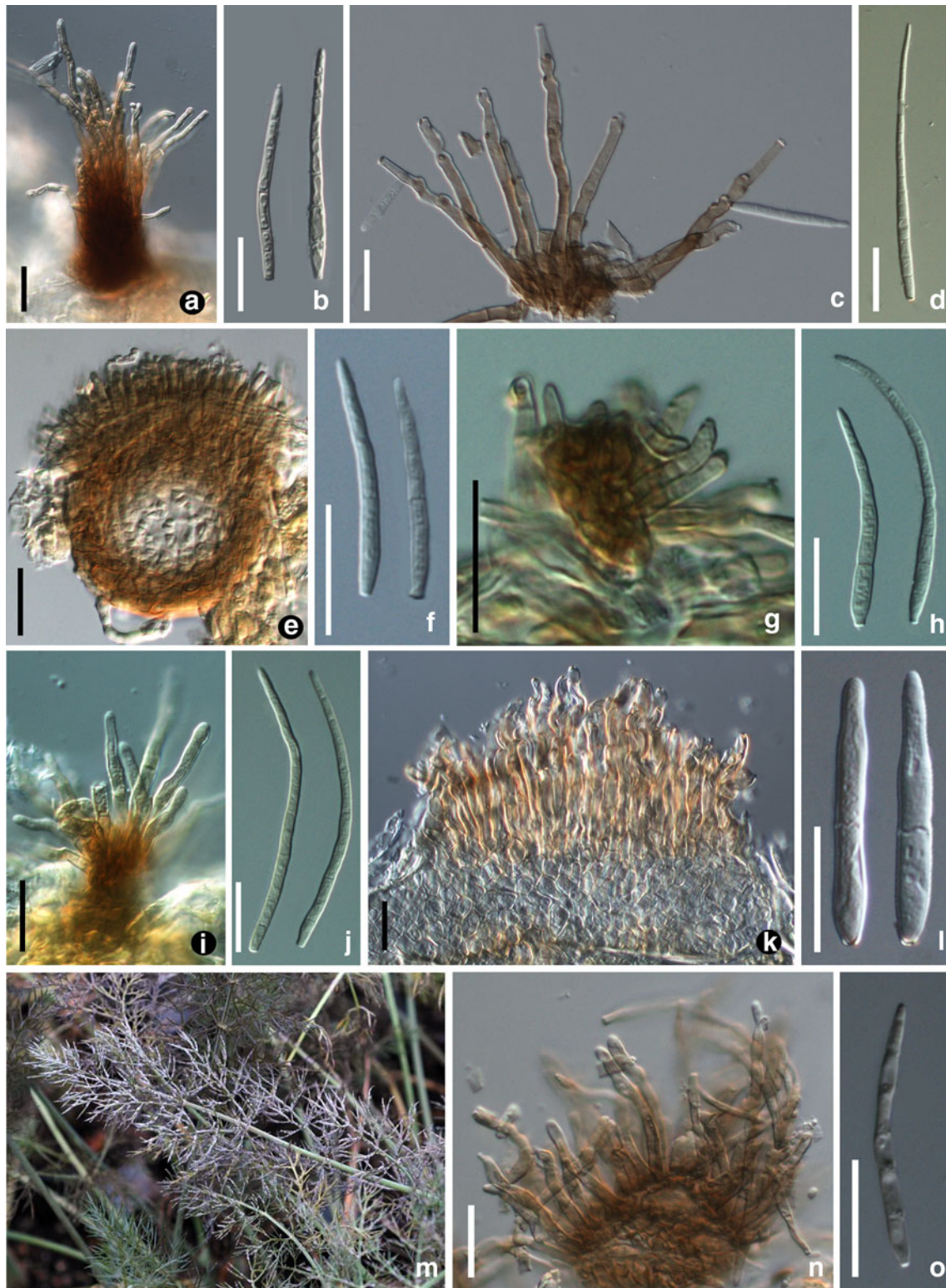
***Pseudocercospora coriariae*** (Chupp) X.J. Liu & Y.L. Guo, in Guo and Liu, Mycosystema 2: 232, 1989 Figs. 1g, h

Leaf spots grayish brown to sooty brown, indistinct in the early stage, definite later, angular to irregular, almost indistinct at the border, often confluent, up to 30 mm in size. Caespituli amphigenous, punctiform on the upper leaf surface, sooty on the lower leaf surface. Mycelium internal, external. Stromata mainly epiphyllous, brown, intraepidermal or substomatal, small to well developed, globose, 30–60  $\mu\text{m}$ . Conidiophores arising from stromata or external hyphae, cylindrical, solitary to densely fasciculate, pale brown, straight to geniculate-sinuuous, simple, occasionally branched, 2–3-septate, 10–75  $\times$  2–5  $\mu\text{m}$ ; conidiogenous cells integrated, terminal, proliferating sympodially or percurrently; conidiogenous loci inconspicuous or subconspicuous, unthickened. Conidia pale to pale brown, obclavate, smooth, truncate, and unthickened at the base, acute at the apex, 48–92  $\times$  3–4.5  $\mu\text{m}$ , 5–12-septate.

Specimen examined: on *Coriaria japonica* A. Gray (Coriariaceae), Japan, Koishikawa Botanical Garden, University of Tokyo, Bunkyo, Tokyo, 10 November 2007, leg. I. Araki & M. Harada (MUMH10942, deposited culture: MUCC840); Medicinal Plant Garden, Nihon University, Funabashi, Chiba Prefecture, 18 October 2008, leg. T. Kobayashi et al. [MUMH11323 (=TPPH:08-171), deposited culture: MUCC1117].

Notes: *Coriaria japonica* is newly added as a host plant for the present species.

The micromorphological characters on our specimens from Japan are variable. Especially, the dimensions of conidiophores and conidia in the early stages of infection of indistinct lesions (MUMH10942) were somewhat smaller than the descriptions given by other investigators (Chupp 1954; Guo and Hsieh 1995). However, the Japanese specimens were identified by the typical morphological



**Fig. 1** **a, b** *Pseudocercospora yakushimensis* (epitype, TNS-F-34701). **c, d** *Cercospora dioscoreae-pyrifoliae* on *Dioscorea tokoro*. **e, f** *Pseudocercospora catalpigena* on *Catalpa ovata*. **g, h** *Pseudocercospora coriariae* on *Coriaria japonica*. **i, j** *Pseudocercospora lythri* on *Lythrum anceps*. **k, l** *Passalora puncta* on *Foeniculum vulgare*. **m** Natural symptoms of the leaf blight disease of Fennel

caused by *P. puncta* (this photo was provided by Mr. Suzuki, who is a researcher for the Shizuoka Prefectural Research Institute of Agriculture and Forestry in the course of this study). **n, o** *Pseudocercospora pteroceltidis* on *Celtis boninensis*. **a, c, e, g, i, k, n** Stromata and conidiophores. **b, d, h, j, l, o** Conidia. Bars 20 μm

characteristics of *P. coriariae*, in which conidial masses emerge from well-developed external hyphae on well-defined lesions.

*Pseudocercospora lythri* H.D. Shin & U. Braun, Mycotaxon 74: 111, 2000 Figs. 1i, j

Leaf spots scattered, irregular, dark to reddish brown, later brown at the center, enlarged and confluent, 2–8 mm in size, almost indistinct at the border. Caespituli amphigenous. Stromata composed of a few large brown cells to well-developed, up to 46  $\mu\text{m}$  in diam., substomatal, immersed, subglobular, brown, with external mycelium rarely. Conidiophores solitary to densely fasciculate, geniculate-sinuous, simple, smooth, pale brown, paler towards the apex, irregular in width, 11–51  $\times$  2–4.5  $\mu\text{m}$ , 0–2-septate; conidiogenous cells integrated, apical, intercalary, proliferating sympodially, with truncate and unthickened conidiogenous loci. Conidia acicular to obclavate, straight to slightly curved, pale to pale olivaceous brown, unthickened and obconically truncate at the base, subacute at the apex, 42–100  $\times$  3–4  $\mu\text{m}$ , 5–9-septate.

Specimens examined: on *Lythrum salicaria* L. (Lythraceae), Japan, Medicinal Plant Garden, Nihon University, Funabashi, Chiba Prefecture, 10 July 2008, leg. I. Araki (MUMH11165); on *Lythrum anceps* (Koehne) Makino (Lythraceae), Japan, Medicinal Plant Garden, Nihon University, Funabashi, Chiba Prefecture, 10 July 2008, leg. I. Araki (MUMH11168); *ibid*, Koishikawa Botanical

Garden, University of Tokyo, Bunkyo, Tokyo, 10 November 2007, leg. I. Araki & M. Harada (MUMH11104, culture deposited: MUCC865).

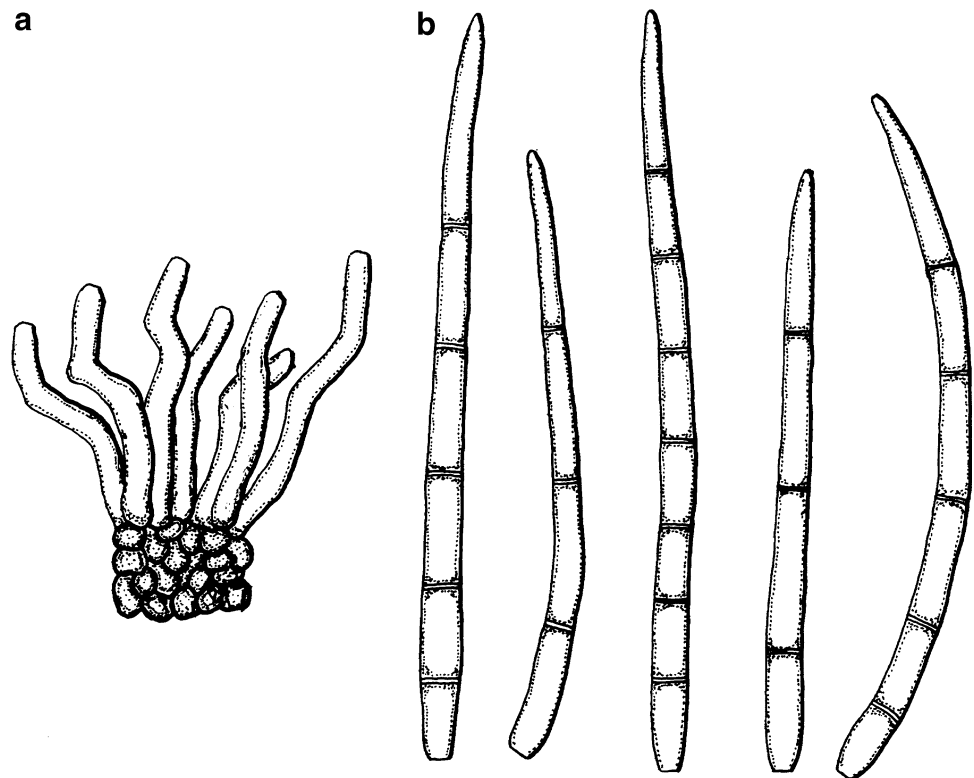
Notes: The present species was known only from the type locality in Korea, where it was described by Shin and Braun (2000).

*Pseudocercospora pteroceltidis* U. Braun & Y.L. Guo, in Braun & Melnik, Trudy Bot. Inst. im. V.L. Komarova 20: 95, 1997 Figs. 1n, o; 2a, b

Lesions visible as blight at leaf margin, dark brown at upper surface, brown at lower surface. Caespituli amphigenous. Stromata small to developed, substomatal, immersed, globular, olivaceous brown to dark brown, 30–58  $\mu\text{m}$  in diam., rarely with creeping external hyphae. Conidiophores emerging from the upper part of stromata or external hyphae, solitary to densely fasciculate, straight or geniculate-sinuous, pale brown, somewhat rough at the upper part, 26–46  $\times$  3–4.5  $\mu\text{m}$ ; conidiogenous cells integrated, terminal, proliferating sympodially, with unthickened and truncate conidiogenous loci. Conidia cylindrical to acicular, obclavate, hyaline to pale olivaceous brown, obconically truncate and unthickened at the base, acute at the apex, 31–79  $\times$  2.5–4.5  $\mu\text{m}$ , 2–8-septate.

Specimens examined: on *Celtis boninensis* Koidz. (Ulmaceae), Japan, China, Okinoerabu Island, Kagoshima Prefecture, 20 November 2001, leg. T. Kobayashi & Y. Ono (MUMH11330, culture deposited: MAFF239164).

**Fig. 2** *Pseudocercospora pteroceltidis*. **a** Stroma and conidiophores. **b** Conidia. Bars 20  $\mu\text{m}$



Notes: The morphology of the fungus on the Japanese specimen is similar to *Pseudocercospora pteroceltidis*, which is known as a species of cercosporoid fungi growing on *Pteroceltis*.

As for the cercosporoid fungi on *Celtis* and *Pteroceltis*, Guo and Liu (1993) re-described *Cercospora spegazzinii* as *Ps. spegazzinii* (Sacc.) Y.L. Guo & X.J. Liu, because the former should have been transferred to *Pseudocercospora* based on the study of Chinese specimens growing on *Celtis* and *Pteroceltis*. However, the morphology of the type specimen of *C. spegazzinii*, which has the characteristics of *Passalora*, is quite distinct from that of the Chinese specimens (Braun and Melnik 1997). Therefore, *Passalora spegazzinii* (Sacc.) U. Braun (in Braun and Melnik 1997) was re-described for the *Passalora* species on *Celtis*. Furthermore, *Ps. pteroceltidis* was established as the correct name for the *Pseudocercospora* species growing on *Pteroceltis tatarinowii* Maxim. in China. Braun and Melnik (1997) noted that the identities of the two Chinese specimens on *Celtis sinensis*, which were identified as *Ps. pteroceltidis* by Guo and Hsieh (1995), are uncertain, because secondary mycelia are absent or almost absent on these Chinese specimens, and the conidia are somewhat narrower and paler than those of *Ps. pteroceltidis* (Braun and Melnik 1997). These characteristics of the Chinese specimens on *Celtis* are similar to those of Japanese ones. It is generally difficult to delimit species based on whether they have secondary mycelia or not. In this study, we applied the epithet, *Ps. pteroceltidis*, to the Japanese *Pseudocercospora* species on *Celtis* and obtained an isolate for further studies using molecular data.

First record of *Passalora puncta* from Japan and its pathogenicity

***Passalora puncta*** (Delacr.) S. Petzoldt, in von Arx, Beih Nova Hedwigia 87: 288, 1987 Figs. 1k, l

Spots tiny yellow with caespituli on stems, peduncles, pedicels and leaves, pale brown, later enlarged, finally extended to apices. Caespituli amphigenous, mainly epiphyllous, visible as black dots with white conidial masses, often distributed over the whole of a pedicel. Stromata subglobose, dark brown, submerged, epidermal, 36.5–103  $\mu\text{m}$ . Conidiophores densely fasciculate, simple, straight to slightly curved, hyaline to pale colored, irregular in width, somewhat constricted at the shoulder following sympodial proliferation, rounded at apices, 0–1-septate, 12–31  $\times$  3–7  $\mu\text{m}$ ; conidiogenous cells integrated, terminal, proliferating sympodially, with darkened and thickened conidiogenous loci. Conidia cylindrical, obovoid to obclavate, smooth, hyaline, with thickened and protruded hilum at the base, rounded at the apex, 11.5–43  $\times$  4–7  $\mu\text{m}$ , 0–3-septate.

Specimens examined: on *Foeniculum vulgare* Mill. (Apiaceae), Japan, Medicinal Plant Garden, Nihon University, Funabashi, Chiba Prefecture, 10 July 2008, leg. I. Araki (MUMH11151); ibid, Hamamatsu, Shizuoka Prefecture, 21 February 2008, leg. M. Suzuki (MUMH11130, culture deposited: MUCC948); ibid, 13 June 2008, leg. M. Suzuki (MUMH11187).

Culture characteristics: Colonies on MEA irregular, rugged, with crenate to crispate margins, grayish black to black, reaching 10–20 mm in diameter after 1 month; aerial mycelium absent or sparse; fertile.

Notes: This is the first record of this species from Japan. *Foeniculum vulgare*, which belongs to the Apiaceae, is well known as a culinary or medicinal herb. It is believed that this plant originated from the Mediterranean shores, and it has been widely cultivated in many temperate areas (Chevallier 2000). The leaf blight disease pathogen *P. puncta* causes economically important losses in annual crops of fennel. Therefore, there have been attempts to establish corresponding resistant cultivars (Gabler 2002).

In Japan, *P. puncta* was found on *F. vulgare* in Shizuoka Prefecture in 2007 (Fig. 1m) with densely distributed caespituli on blighted plants. In our inoculation experiments, young seedlings of *F. vulgare* sprayed with conidial suspensions ( $2 \times 10^4$  conidia/ml), which were previously prepared by suspending the conidia collected in sterilized water from lesions (MUMH11187), showed leaf blight symptoms after 14 days and were completely covered with white conidial masses after 21 days. Symptoms of the inoculated plants and the morphology of the re-isolated fungi were identical to those found under natural conditions.

The taxonomic concept of the present species is based on Deighton (1967), who studied the lectotype specimen and transferred the species to *Cercosporidium* with several synonyms. Afterwards, von Arx (1987) re-transferred the fungus to *Passalora* as *P. puncta* (Delacr.) S. Petzoldt. However, Srivastava (1994) ignored this classification by von Arx (1987) and proposed a new combination, *P. puncta* (Delacr.) Poonam Srivast., without a full or direct reference to a basionym. Crous and Braun (2003) interpreted the epithet “*punctum*” (*Azosma* [neuter] *punctum*) as a noun “*punctum*” (=point) and, accordingly, altered the name *Passalora puncta* to *Passalora punctum*. This is, however, incorrect, since “*punctum/puncta*” is, in this case, undoubtedly the participle perfect passive of the verb “*pungere*” and means “punctate”, which undoubtedly refers to the characteristic punctiform sporodochial colonies of this species (U. Braun, in litt.).

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