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Pseudocercosporella stellariicola, a new hyphomycete occurring on *Stellaria aquatica*

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Abstract — A new cercosporoid fungus, *Pseudocercosporella stellariicola*, was collected on leaves of *Stellaria aquatica* (*Caryophyllaceae*). This fungus is described, discussed, and compared with other similar hyphomycetes occurring on *Stellaria* and related genera: *Cercosporella echinulata*, *Phacellium episphaerium*, *Pseudocercosporella cerastii*, and *Ps. woronovii*. The phylogenetic relationship of *Ps. stellariicola* and other *Pseudocercosporella* species within *Mycosphaerellaceae* is also inferred from the ITS rDNA sequence data.

Key words — anamorphic *Mycosphaerella*, imperfect fungi, plant pathogen

Introduction

As a result of extensive studies conducted on the mycobiotas of cercosporoid fungi in Korea from 1990 to 2000, Shin & Kim (2001) published a monograph comprising 127 species in 14 genera on 151 species of host plants. Although *Stellaria aquatica*, one of the most common wild plants in Korea, was frequently found infected with *Phacellium episphaerium*, a previously undescribed leaf-spotting hyphomycete has also been found on this plant since 2004. This fungus differed from other similar hyphomycetes known to occur on *Stellaria* and related genera. It was well characterized by colorless conidiophores in dense fascicles, inconspicuous, unthickened conidial scars and solitary, hyaline conidia. These features agree with the concept of *Pseudocercosporella* proposed by Braun (1990), and this hyphomycete is identified and characterized as a novel species in that genus.

Materials & methods

For microscopy, free-hand sections of fresh materials were mounted in water. Morphological characteristics were measured using a model BX51 microscope (Olympus, Tokyo, Japan), and photographed using an Axio imager microscope (Carl

Zeiss, Göttingen, Germany). The voucher specimens were deposited in the Korea University herbarium (KUS).

Monoconidial isolates were deposited at the Korean Agricultural Culture Collection (KACC) of the National Academy of Agricultural Science in Korea and the Centraalbureau voor Schimmelcultures (CBS) in the Netherlands. Genomic DNA was extracted using mycelia harvested from colonies following a previously described method (Lee & Taylor 1990). The ITS rDNA region was amplified using universal primers ITS1/ITS4 (White et al. 1990). The PCR amplicons were purified using a MultiScreen HTS™ PCR filter plates (Millipore, Carrigtwohill, Cork, Ireland) and sequenced using an ABI Prism™ 377 automatic DNA sequencer (Applied Biosystems, Foster City, CA, USA) with a BigDye™ cycle sequencing kit v. 3.1 (Applied Biosystems). The raw sequences were edited using the DNASTAR computer package v. 5.05 (Lasergene, Madison, WI, USA). The ITS rDNA sequences obtained from three isolates (KACC42363, KACC42395, and CPC11297) have been deposited in GenBank with accession numbers EF600945, EF600956, and GU214693, respectively. For phylogenetic analysis, available ITS rDNA sequences of *Pseudocercosporella* spp. and closely related taxa belonging to *Mycosphaerellaceae* were retrieved from GenBank. The sequences were aligned with ClustalX v. 2.1 (Larkin et al. 2007). The Bayesian inference analysis was performed with MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001). The GTR + G as nucleotide substitution model was selected using jModeltest v. 0.1.1 (Posada 2008). One million generations with four chains were run and a tree every 100th generation was saved. The first 1000 of the 10,000 saved trees were discarded as burn-in. The resulting 50% majority-rule consensus tree was visualized with TreeViewX (Page 1996). Branch support was calculated as posterior probability (PP).

Taxonomy

Pseudocercosporella stellariicola M.J. Park, J.H. Park & H.D. Shin, **sp. nov.** Fig. 1

MycoBank MB 563164

Similar to *Pseudocercosporella cerastii,* but with wider (2–3 µm) conidia and distinct leaf lesions. Similar to *P. woronovii,* but with shorter (40–80 µm), straight to slightly curved, 1–3(–4)-euseptate conidia.

Type: On living leaves of *Stellaria aquatica* (L.) Scop. (*Caryophyllaceae*), KOREA, Namyangju, Experimental Farm of Korea University, 37°35′01″N 127°14′13″E, 3 May 2006, H.D. Shin & M.J. Park (**Holotype**, KUS-F21740; culture ex-type, KACC42363).

Etymology: The epithet is derived from the host plant genus.

Leaf spots amphigenous, circular to subcircular, 2–7 mm diam., whitish to pale brown. Caespituli amphigenous, grayish-brown, punctiform, evenly and more or less densely distributed over the spot. Mycelium internal; hyphae septate, branched, hyaline, 3–5 µm wide; superficial mycelium absent. Stromata well-developed, erumpent, subcuticular or intraepidermal, sometimes substomatal, consisting of medium brown, pseudoparenchymatal cells, ca. 30–100 µm wide and 25–65 µm high. Conidiophores numerous in a dense divergent fascicle, hyaline throughout, aseptate, straight, conic to

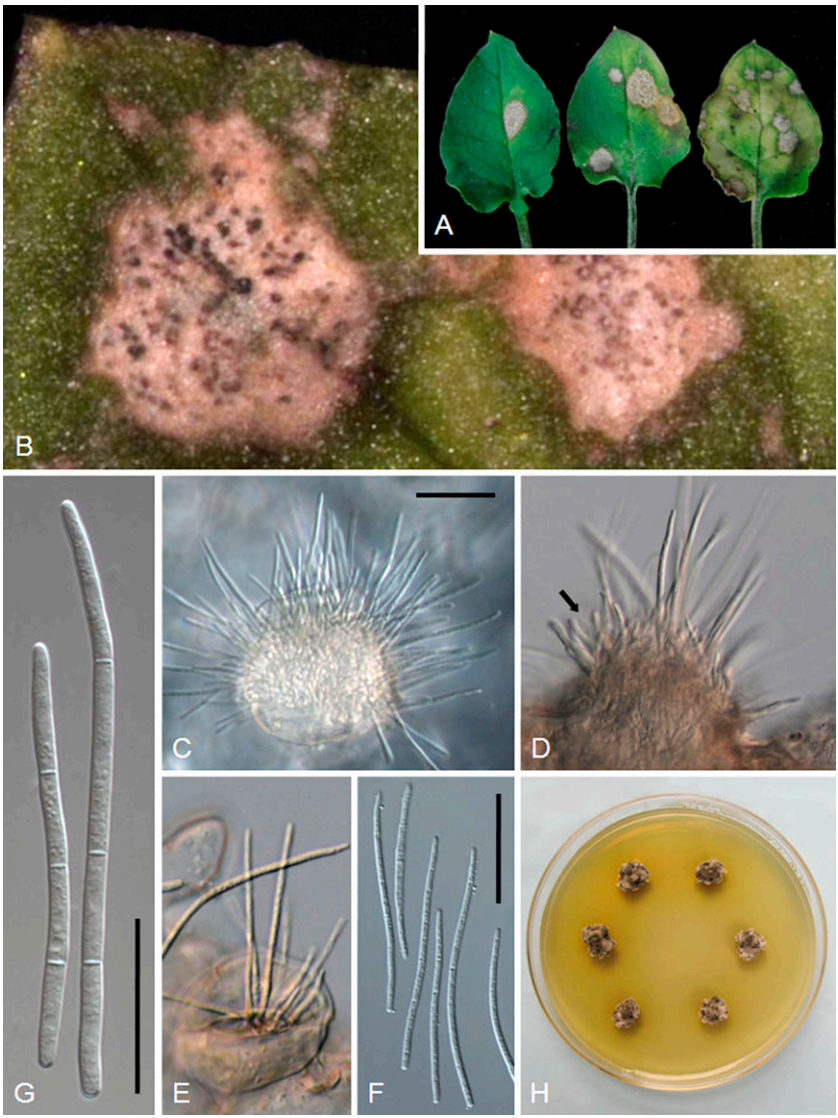

Fig. 1. *Pseudocercosporella stellariicola*. A: Symptoms on leaves of *Stellaria aquatica*. B: Close-up of the leaf lesions showing many stromata on the infected tissue. C: Upper view of a stroma emerging from a stoma of the host plant. D: Vertical view of a stroma protruding from the cuticle of the host plant. E: Small stroma with a few conidiophores emerging through the substomatal cavity. F: Conidia. G: Close-up of conidia. H: Six-week-old colonies formed on potato dextrose agar. Scale bar: C–D = 50 µm, E–F = 30 µm, G = 20 µm.

mildly geniculate-sinuous, 8–15(–20) µm in length, 2–3 µm in width; conidial scars unthickened, not darkened. Conidia solitary, filiform, straight to slightly curved, hyaline, 1–3(–4)-euseptate, non-constricted at the septa, irregularly guttulate, obtuse at the apex, subtruncate to subobtuse at the base, 40–80 × 2–3 µm; hilum unthickened, not darkened. Colonies on PDA attaining ca. 10 mm diam after 6 weeks at 25°C under 12-h fluorescent light, dark brownish to blackish, somewhat crumpled, consisting of a slimy mass due to abundant sporulation, producing sparse aerial mycelia. Teleomorph unknown.

Additional specimens examined – **KOREA**, Yangpyeong, Experimental Forest of Korea University, 37°30′12″N 127°41′55″E, 5 May 2004, H.D. Shin, KUS-F20158; 26 May 2004, H.D. Shin, KUS-F20239 (culture: CPC11297); 20 May 2005, H.D. Shin, KUS-F21102; 25 May 2006, H.D. Shin & M.J. Park, KUS-F21808 (culture: KACC42395); Cheongun-myeon, 37°32′04″N 127°44′48″E, 20 May 2007, H.D. Shin & M.J. Park, KUS-F22591 (culture: KACC42830); Namyangju, Experimental Farm of Korea University, 37°35′01″N 127°14′13″E, 17 May 2004, H.D. Shin, KUS-F20216; 19 April 2008, H.D. Shin & M.J. Park, KUS-F23281 (culture: KACC43763); 25 April 2011, H.D. Shin & J.H. Park, KUS-F25669; Taean, Nam-myeon, 36°41′11″N 126°17′05″E, 17 April 2007, H.D. Shin & M.J. Park, KUS-F22539; Hongcheon, Sangoan-ri, 37°39′25″N 127°49′18″E, 28 April 2007, H.D. Shin & M.J. Park, KUS-F22565 (culture: KACC42827); Seongdong-ri, 37°44′41″N 127°52′01″E, 29 April 2007, H.D. Shin & M.J. Park, KUS-F22578; Hoengseong, Eupha-ri, 37°29′47″N 127°58′52″E, 29 April 2011, H.D. Shin & J.H. Park, KUS-F25676; Seowon-myeon, 37°31′33″N 127°52′29″E, 6 May 2011, H.D. Shin, KUS-F25679.

Discussion

Two cercosporoid hyphomycetes are presently known to occur on *Stellaria* species. *Cercosporella echinulata* Garb., described on *Myosoton aquaticum* (L.) Moench (≡ *Stellaria aquatica*), differs from the new species by conidiophores with an echinulate, denticulate apical part. Additionally, *C. echinulata* possesses much longer (35–110 µm) conidiophores and narrower (1–2 µm) conidia. Although the identity of *C. echinulata* is unclear (according to Braun 1995), it is easily differentiated from *Ps. stellariicola*. *Phacellium episphaerium* (Desm.) U. Braun is also readily differentiated from the present species by its synnematous fascicles of conidiophores and aseptate conidia.

Two *Pseudocercosporella* species are known on other caryophyllaceous host plants. *Pseudocercosporella cerastii* (H.C. Greene) U. Braun (Braun 1995) known on *Cerastium* spp. from Central Asia and North America is distinguished from the new species by much narrower (0.75–2 µm) conidia. The leaf lesions associated with *Ps. cerastii* are almost absent or diffuse, whereas those of *Ps. stellariicola* are quite distinct by being circular to subcircular and delimited by a dark border.

Pseudocercosporella woronovii (Siemaszko) U. Braun recorded on *Silene* spp. and *Lychnis* spp. in Europe (see Braun 1995) also produces (sub-)circular lesions and amphigenous colonies on the leaves. However, *Ps. woronovii* differs

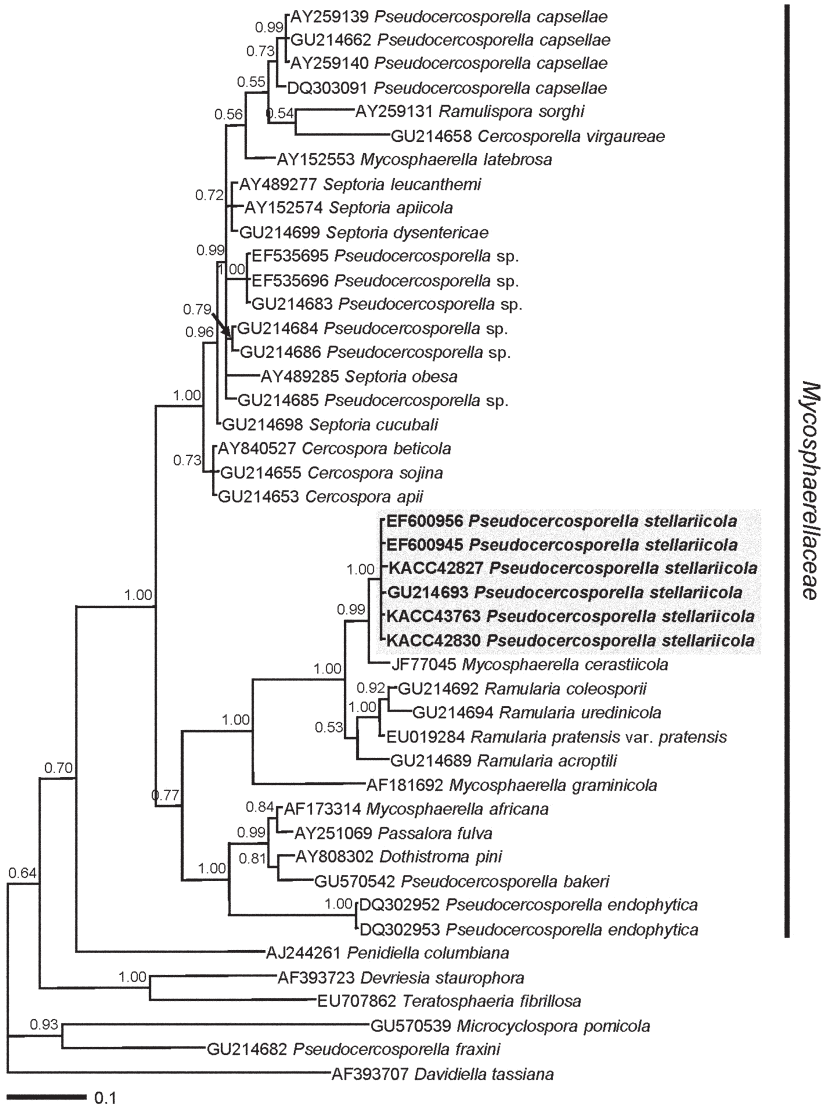

Fig. 2. A 50% majority-rule consensus tree from Bayesian analysis of the ITS rDNA sequences of *Pseudocercosporella* spp. and closely related taxa belonging to *Mycosphaerellaceae*. Numbers above the branches indicate the posterior probability values. The number of nucleotide changes between taxa is represented by branch length and the scale bar corresponds to the number of substitution per site. The sequences of *Ps. stellariicola* are shown in bold and a well-supported clade accommodating them is highlighted in gray box.

from *Ps. stellariicola* by longer (50–100 µm), straight to flexuous, obscurely 1–5-septate conidia.

The complete ITS rDNA sequence data of the six *Ps. stellariicola* isolates obtained were nearly identical, with only one base pair difference between KACC42827 and the other isolates. They formed a separate clade distinct from the other species of *Mycosphaerellaceae* (1.00 PP value) in the Bayesian tree (Fig. 2). Our phylogenetic analysis indicated that *Mycosphaerella cerastiicola* Crous is sister to the *Ps. stellariicola* clade (0.99 PP value); *Ramularia* spp. are also phylogenetically close to the new species. In recent studies, based on 18S, 28S, and ITS-5.8S rDNA sequence analyses (Crous et al. 2009 & 2011), isolate CPC11297 (representing *Ps. stellariicola*) was tentatively identified as a *Ramularia* sp. based on its placement in the *Ramularia* clade. Nevertheless, based on the morphological characteristics described in this study, the isolate is better accommodated in *Pseudocercosporella*. In addition, the fact that *Ps. stellariicola* is closely related with *Ramularia* spp. is not surprising, as *Pseudocercosporella* is polyphyletic within *Mycosphaerellaceae* and *Capnodiales*, as shown in previous phylogenetic studies (Crous et al. 2009, Frank et al. 2010) and the present analysis. In conclusion, both morphological and molecular data support *Ps. stellariicola* as a novel species.

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