Research Note

Ten Records of Cercosporoid Fungi from the Lienhuachih Forest in Taiwan

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【Summary】

Ten cercosporoid fungi were found in the Lienhuachih forest, Yuchi, Nantou County, west-central Taiwan. They are *Pseudocercospora borreriae*, *P. eupatorii-formosani*, *P. brachypus*, *P. wedeliae*, *P. puerariicola*, *Cercospora bidentis*, *C. stachytarphetae*, *C. ageraticola*, *C. camptothecae*, and *Pseudocercosporella oxalidis*. Among them, *P. borreriae* and *P. brachypus* are first reported herein in Taiwan. Descriptions and illustrations are provided for these 2 species.

Key words: cercosporoid fungi, new record, Lienhuachih.

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研究簡報

臺灣蓮華池森林十種尾孢菌類真菌之紀錄

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摘 要

於臺灣南投縣魚池鄉蓮華池地區採集到10種尾孢菌類真菌，分別為：豐花草假尾孢菌(*Pseudocercospora borreriae*)、香澤蘭假尾孢菌(*P. eupatorii–formosani*)、細窄假尾孢菌(*P. brachypus*)、蟛蜞菊假尾孢菌(*P. wedeliae*)、葛藤假尾孢菌(*P. puerariicola*)、鬼針草尾孢菌(*Cercospora bidentis*)、長穗木尾孢菌(*C. stachytarphetae*)、藿香薊尾孢菌(*C. ageraticola*)、喜樹尾孢菌(*C. camptothecae*) 和酢漿草假小尾孢菌(*Pseudocercosporella oxalidis*)。其中，豐花草假尾孢菌和細窄假尾孢菌為臺灣新紀錄種。本文亦將對此新紀錄種進行詳細描述。

關鍵詞：尾孢菌類真菌、新紀錄、蓮華池。

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INTRODUCTION

Cercosporoid fungi are hyphomycetes resembling *Cercospora* that taxonomically belong to the family Mycosphaerellaceae (Ascomycota) (Crous and Braun 2003). Cercosporoid fungi are comprised of the genera *Cercospora*, *Pseudocercospora*, *Ramularia*, *Passalora*, *Stenella*, *Cercosporella*, *Pseudocercosporella*, etc., with the former 4 genera being the most prevalent. The major characters used for identification are the shape and pigmentation of the conidiophores and conidia, the texture of the conidial wall, and the presence or absence of conidial scars. Most cercosporoid fungi are plant pathogens causing leaf spots on ferns, gymnosperms, monocots, and dicots, and have high host specificity. Some of them are economically important pathogens of crops and trees, such as angular leaf spot of beans (*Pseudocercospora griseola*), leaf spot of sugar beets (*Cercospora beticola*), angular leaf spot of camptotheca trees (*Cercospora camptothecae*), *mycosphaerella* leaf *disease* of eucalypt trees (Crous, 1998), etc.

The earliest studies of cercosporoid fungi in Taiwan were conducted by Kaneyoshi Sawada (Sawada 1919, 1922, 1928, 1931, 1933, 1942, 1943a, 1943b, 1944, 1959), followed by Drs. Hsieh and Goh, who included nearly 240 species of cercosporoid fungi in their valuable book *Cercospora and similar fungi from Taiwan* (Hsieh and Goh 1990). In recent years, some new species and newly recorded species of cercosporoid fungi have continuously been published (Kirschner et al. 2004, Kirschner and Chen 2007, Kirschner and Okuda 2013, Kirschner 2014, Kirschner and Liu 2014, Kirschner and Wang 2015, Chen et al. 2020). To the present, approximately 410 species of cercosporoid fungi have been recorded in Taiwan. The Lienhuachih forest, known for its great biodiversity, is located at Yuchi Township, Nantou County, west-central Taiwan, at an elevation of 576~925 m. The annual average temperature is about 21℃, and annual average rain-

fall is about 2200 mm in the area. In a survey of cercosporoid fungi in the Lienhuachih forest, 10 species were obtained. Identification of these species was mainly based on host specificity, disease symptoms, and microscopic fungal characters. Two species are new records to Taiwan. Fresh cultures of these 2 species were obtained for subsequent internal transcribed spacer (ITS) sequencing. Thereafter, the identification was further justified.

MATERIALS AND METHODS

All fresh samples with disease lesions were collected by H. H. Shih at Lienhuachih, Nantou County, Taiwan. Samples were examined for the presence of cercosporoid fungi under a Leica MZ125 stereo microscope (Germany). To identify the fungi, detailed fungal microscopic characters were determined using a Leica DM2500 light microscope with an oil-immersion lens (1000×), and about 20~30 conidia and conidiophores were measured. Single spores were isolated for fungi first reported in Taiwan. Potato dextrose agar (PDA) medium was used to incubate the fungi at room temperature. Purified cultures were deposited at the Bioresource Collection and Research Center (BCRC; Hsinchu, Taiwan), and dry specimens were deposited at the Lienhuachih Research Center, Taiwan Forestry Research Institute (TFRILHC).

DNA was extracted as described by the protocol of Sambrook and Russell (2001). For DNA barcoding, primer pairs of ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3')/ ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990) and V9G (5'-TTACGTCCCTGCCCTTTGTA-3') (Gerrits van den Ende and de Hoog 1999)/LR1 (5'-GGTTGGTTTCTTTTCCT-3') (Vilgalys and Hester 1990) were used to amplify the internal transcribed spacer (ITS). A polymerase chain reaction (PCR) was performed with initial denaturation at 94℃ for 5 min, followed by 35 cycles of 94℃ for 30 s, 30 s at 55℃, and 60 s at 72℃, with a final extension of 10 min at 72℃. ITS sequences were sequenced using the same primers for the PCR amplification which was performed on an ABI PRISM 377 DNA sequencer at the Biotechnology Center of National Chung Hsing Univ (Taichung, Taiwan). Sequences were submitted through the submission system of the DNA Data Bank of Japan (DDBJ) (Mashima et al. 2016).

RESULTS

Ten cercosporoid fungi including 4 *Cercospora* species, 5 *Pseudocercospora* species, and 1 *Pseudocercosporella* species were collected and studied during 2020~2022 in the Lienhuachih forest. They are *Cercospora ageraticola* on *Ageratum houstonianum* (Asteraceae), *C. bidentis* on *Bidens pilosa* (Asteraceae), *C. camptothecae* on *Camtoptheca acuminate* (Nyssaceae), *C. stachytarphetae* on *Stachytarpheta jamaicensis* (Verbenaceae), *Pseudocercospora brachypus* on *Ampelopsis cantoniensis* (Vitaceae), *P. eupatorii–formosani* on *Chromolaena odoratum* (Asteraceae), *P. puerariicola* on *Pueraria montana* var. *lobata* (Fabaceae), *P. borreriae* on *Spermacoce latifolia* (Rubiaceae), *P. wedeliae* on *Wedelia triloba* (Asteraceae), and *Pseudocercosporella oxalidis* on *Oxalis debilis* var. *corymbosa* (Oxalidaceae). Among them, *P. borreriae* and *P. brachypus* were newly recorded in Taiwan. The collection information with morphological descriptions for the 2 new Taiwan records are as follows.

Cercospora ageraticola Goh & Hsieh, Trans Mycol Soc Rep China 4(2-3):40, 1989 (藿香薊尾孢菌)

Specimens examined: On leaves of *Ag-*

eratum houstonianum (Asteraceae), 30 Nov. 2021 (TFRILHC-1101130-7); 11 Feb. 2022 (TFRILHC-1110211-3).

Early report in Taiwan: Hsieh and Goh (1990).

Cercospora bidentis Tharp, Mycologia 9(2):108, 1917 (鬼針草尾孢菌) = *Cercospora bidentis* Marchal & Steyaert, Bull Soc Roy Bot Belgique 61:167, 1929. = *Cercospora bidentis-pilosae* Sawada, Taiwan Agric Res Inst Rep 85:98, 1943.

Specimens examined: On leaves of *Bidens pilosa* (Asteraceae), 5 Nov. 2021 (TFRILHC-1101105-9); 25 Nov. 2021 (TFRILHC-1101125-1).

Early report in Taiwan: Hsieh and Goh (1990).

Cercospora camptothecae Tai, Lloydia 11:39, 1948 (喜樹尾孢菌)

Specimen examined: On leaves of *Camptotheca acuminate* (Nyssaceae), 2 Dec. 2021 (TFRILHC-1101202-4).

Early report in Taiwan: Chen (1968).

Cercospora stachytarphetae Ellis & Everh., Missouri Bot Gard Ann Rep 9:120, 1898 (長穗木尾孢菌)

Specimens examined: On leaves of *Stachytarpheta jamaicensis* (Verbenaceae), 15 Jan. 2021 (TFRILHC-1100115-1); 17 Nov. 2021 (TFRILHC-1101117-1).

Early report in Taiwan: Hsieh and Goh (1987).

Pseudocercospora borreriae (Ellis & Everh.) Deighton, Mycol Pap 140:140, 1976. Fig. 1. (豐花草假尾孢菌)

≡ *Cercospora borreriae* Ellis & Everh. Proc Acad Nat Sci Phil 46(3):379, 1894

Leaf spots amphigenous, irregular to circular, 5~15 mm in diam., pale-brown to grayish-brown in center, with dark-brown border, sometimes many small leaf spots fusing to appear like leaf blight. Stromata amphigenous, well-developed, immersed. Conidiophores densely fasciculate, cylindrical, straight to slightly curved, pale-brown, unbranched or rarely branched, conically truncate at apex, 76~165 × (2.5~)3~5 μm with 3~7 septa, conidial scars not thickened. Conidia solitary, straight or slightly curved, subhyaline to pale-olivaceous, cylindrical to obclavate or cylindric-obclavate, 45~105 × 3.7~5.5 μm with 3~7(~8) septa, with round to obtuse apex and conically truncated base, 2~3 μm wide, hila neither thickened nor darkened. Colony on PDA slow-growing, dome-shaped, lobate, white, gray, to dark-gray.

Specimens examined: On leaves of *Spermacoce latifolia* (Rubiaceae), 23 Oct. 2020 (TFRILHC-1091023-1; living culture: BCRC FU31708); 23 Aug. 2021 (TFRILHC-1100823-1); 23 Sept. 2021 (TFRILHC-1100923-2); 19 Oct. 2021 (TFRILHC-1101019-3); 5 Nov. 2021 (TFRILHC-1101105-5).

Note: *Pseudocercospora borreriae*, based on *Cercospora borreriae*, was originally reported on *Borreria micrantha* (= *Spermacoce remota*) (Ellis and Everhart 1894), and has now been widely reported on many other plant genera in *Borreria*, *Mitracarpus*, and *Spermacoce* (Farr and Rossman 2022), all belonging to the family Rubiaceae. The original description (Ellis and Everhart 1894) of this species was too simple to tell characteristic differences from other species. Nevertheless, the justification for this species was largely based on host identity, i.e., *Spermacoce* affinity. The fungus isolated from *Spermacoce latifolia* in Taiwan conforms to the description of *Pseudocercospora borreriae* by Dennis (1970) on *Spermacoce latifolia*, and Guo (2012) on *Borreria stricta*. The ITS sequence

(accession no. LC700328) of 496 base pairs was successfully obtained in this study. Unfortunately, there was no sequence named under *Pseudocercospora borreriae* in GenBank for comparison. In a blast search, the highest hit was NR147312.1, namely *Pseudocercospora richardsoniicola*, with 99.17% similarity. *Pseudocercospora richardsoniicola* was reported on *Richardsonia* sp. and *Richardia brasiliensis* (Rubiaceae) (Silva et al. 2016), which are phylogenetically closely related to *Spermacoce* (Groeninckx et al. 2009). *Pseudocercospora richardsoniicola* is morphologically indistinguishable from *P. borreriae* based on the available description. They are probably the same fungus, but type sequences of both species must be compared. However, according to the host identity, *Pseudocercospora borreriae* was applied to name the Taiwan material.

Pseudocercospora coremioides was reported on *Diodia*, a genus phylogenetically closely related to *Spermacoce* (Groeninckx et al. 2009). However, *P. coremioides* can be distinguished by having longer and solitary conidiophores (40~300 μm) bearing wider conidia (4~6.5 μm) with more septa (3~12) (Braun and Urtiaga 2013).

Pseudocercospora brachypus (Ellis & Everh.) Liu & Guo, Acta Mycol Sin 11:128, 1922; The Genus *Pseudocercospora* in China, p 352, 1995. Fig. 2. (細窄假尾孢菌)

≡ *Cercospora brachypus* Ellis & Everh., J Mycol 8:71, 1902.

Leaf spots angular to irregular, 4~10 mm in diam., brown, dark-reddish-brown to dark-brown with a blackish-brown margin. Secondary mycelium well-developed, hyphae subhyaline to pale-olivaceous, branched, septate, arcuate, 1.5~2.5 μm wide, bearing secondary conidiophores. Stromata nearly globular, dark-brown, 20~45 μm wide. Conidiophores densely fasciculate, pale-brown, cylindric, irregular in width, mostly straight and erect, unbranched, 0(~1) septate, obtuse at apex, 10~25 × 2.3~3.6 μm, conidial scars not thickened. Conidia subhyaline to pale-olivaceous, cylindric, straight to slightly curved, (2~)3~10 septate, subobtuse to rounded at apex, obconically truncate at base, 23~68(~85) × 1.9~3.7 μm, hila neither thickened nor darkened, 1~1.5 μm wide. Colony on PDA slow-growing, dome-shaped, lobate, gray at periphery, dark-gray to dark-olivaceous-gray in center.

Specimens examined: On leaves of *Ampelopsis cantoniensis* (Vitaceae), 24 Nov. 2021 (TFRILHC-1101124-1; living culture: BCRC FU31709); 30 Nov. 2021 (TFRILHC-1101130-2); 20 Jan. 2022 (TFRILHC-1110120-2).

Note: Two *Pseudocercospora* species, *P. brachypus* and *P. ampelopsis*, have been reported on species of the host genus *Ampelopsis* (Guo and Hsieh 1995, Crous et al. 2013). In addition to *Ampelopsis brevipedunculata* (Vitaceae), *P. brachypus* was also reported on *Parthenocissus tricuspidata* (Vitaceae) and *Vitis balanserma* (Vitaceae), while *P. ampelopsis* was only reported on *Ampelopsis glandulosa* var. *heterophylla* (Vitaceae). The fungus of the Taiwan material conforms to the description of *P. brachypus* by Guo and Hsieh (1995) and significantly differs from *P. ampelopsis* in the morphology of the conidiophores and conidia. *Pseudocercospora ampelopsis* is distinct in having larger conidiophores (20~80 × (2.5~)3~5(~6) μm) with more septa (3~6) bearing larger conidia ((35~)40~90(~110) × 3~5(~6) μm) (Crous et al. 2013). Unfortunately, there was no sequence named under *Pseudocercospora brachypus* in GenBank for comparison. In a blast search using the ITS sequence (accession no. LC700327) of the Taiwan material, the high-

est hits were sequences under the names of *P. ampelopsis* (DQ303088) (Crous et al. 2013) and *P. flavomarginata* (MH863876/GU269799) reported on *Eucalyptus* spp. (Myrtaceae), *P. struthanthi* (NR147304/KT290141) reported on *Struthanthus flexicaulis* (Loranthaceae), and *P. schizolobii* (GQ852765/GQ885903) reported on *Eucalyptus camaldulensis* and *Schixolobium parahyba* (Fabaceae). *Pseudocercospora flavomarginata* is distinct in having larger stromata (56 × 47 µm), larger conidiophores ((18~)32~36(~53) × (2~)3~4(~5) µm), larger conidia ((28~)46~54(~90) × (2~)3(~4) µm), and different numbers of septa of conidiophores and conidia (Hunter et al. 2006); *P. struthanthi* in having wider conidiophores (7.5~31 × 3~5.5 µm), larger conidia (41~83.5 × 3~4 µm), and more septa (0~3) of the conidiophores (Silva et al. 2016); *P. schizolobii* in having larger conidiophores (30~70 × 3.5~4 µm) and conidia ((22~) 40~60 (~90) × (3~) 3.5 (~4) µm) and more septa (1~4) of the conidiophores (Crous et al. 2009). *Pseudocercospora brachypus* is the name best accommodating the Taiwan material.

Pseudocercospora eupatorii–formosani Yen ex Guo & Hsieh, Mycosystema Monographicum 2:67, 1995 (香澤蘭假尾孢菌)

≡ *Cercospora eupatori-formosani* Sawada, Taiwan Agric Res Inst Rep 86:169, 1943.

Specimen examined: On leaves of *Chromolaena odoratum* (Asteraceae), 5 Nov. 2021 (TFRILHC-1101105-3).

Early report in Taiwan: Hsieh and Goh (1990).

Pseudocercospora puerariicola (Yamam.) Deighton, Mycol Pap 140:151, 1976 (葛藤假尾孢菌)

≡ *Cercospora puerariicola* Yamamoto, Trans Sapporo Nat Hist Soc 13:142, 1934.

Specimens examined: On leaves of *Pueraria montana var. lobata* (Fabaceae), 24 Sept. 2021 (TFRILHC-1100924-1); 18 Oct. 2021 (TFRILHC-1101018-2); 17 Feb. 2022 (TFRILHC-1110217-3).

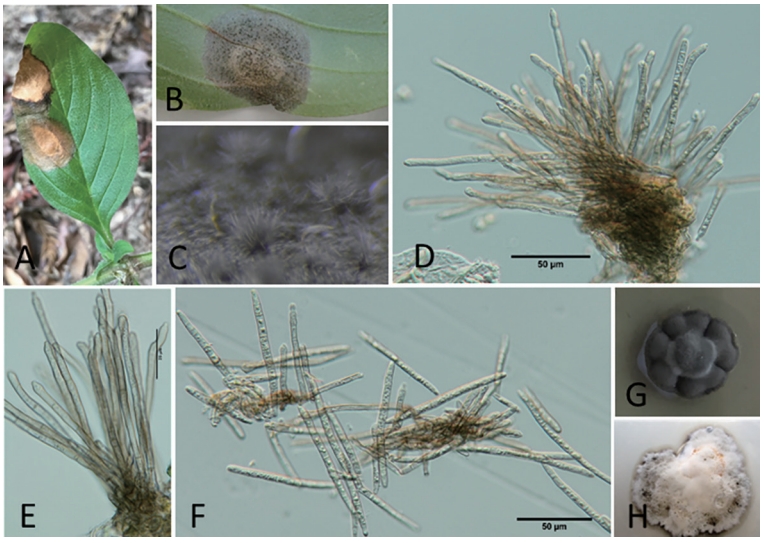

Fig. 1. *Pseudocercospora borreriae*. A, Leaf spots on *Spermacoce latifolia*; B, close-up of lesion; C, close-up of leaf spot with superficial sporulation structures; D and E, fasciculate conidiophores; F, conidia; G and H, colony on PDA.

Early report in Taiwan: Hsieh and Goh (1990).

Pseudocercospora wedeliae (Kar & Mandal) Deighton, Mycol Pap 140:155, 1976 (蟛蜞菊假尾孢菌)

≡ *Cercospora wedeliae* A.K. Kar & M. Mandal, Trans Br Mycol Soc 54(3):428, 1970.

Specimens examined: On leaves of *Wedelia triloba* (Asteraceae), 24 Nov. 2021 (TFRILHC-1111124-2); 25 Jan. 2022 (TFRILHC-1111124-2-1).

Early report in Taiwan: Kirschner (2014).

Pseudocercosporella oxalidis (Goh & Hsieh) U. Braun, Nova Hedwigia 55(1-2):218, 1992 (酢漿草假小尾孢菌)

Specimens examined: On leaves of *Oxalis debilis* var. *corymbosa* (Oxalidaceae), 30 Nov. 2021 (TFRILHC-1101130-4); 8 Dec. 2021 (TFRILHC-1101208-1); 3 Mar. 2022 (TFRILHC-1110303-1).

Early report in Taiwan: Goh and Hsieh (1989).

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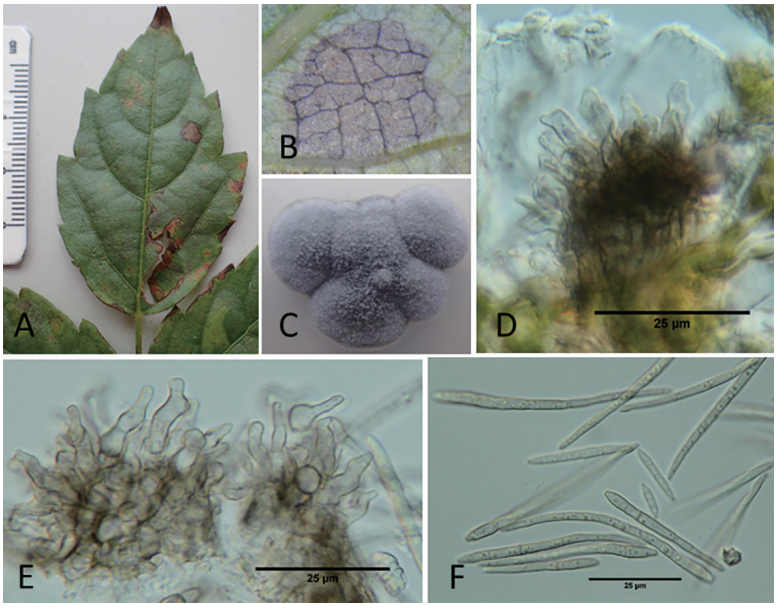

Fig. 2. *Pseudocercospora brachypus*. A, Leaf spots on *Ampelopsis cantoniensis*; B, close-up of lesion; C, colony on PDA; D and E, fasciculate conidiophores; F, conidia.

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