# Three New Species of Cercosporoid Fungi from Northern Thailand

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**Abstract** A collection of Cercosporoid fungi occurring in leaf spots from Northern Thailand during 2013–2014 is described and illustrated. These fungi include three new species, *Cercospora posoneae*, *Pseudocercospora combretina* and *Pseudocercospora glycinicola*. Eleven species are new records of host plants for Thailand. Six species of *Cercospora* morphologically similar to *Cercospora apii* sensu lato were also found in this study.

Keywords: Cercosporoid, Cercospora, Pseudocercospora

#### Introduction

Cercosporoid fungi are well known as one of the largest hyphomycete genera, and numerous species of this fungus have been linked to leaf spots symptoms. Many hyphomycetes genera are linked to fungus *Mycosphaerella* and many host plant species are damaged by these fungi (Crous, 2009).

Among the Cercosporoid fungi, *Cercospora* and *Pseudocercospora* genera are considered to be important fungi because of numerous plant disease reports from Northern Thailand (Gavin *et al.*, 2006; Nakachima *et al.*, 2007; Meeboon *et al.*, 2007; To–anun *et al.*, 2011). They cause lesion symptoms, including necrotic leaf spots and leaf blight on many host plants including as agronomic crops, ornamental plants and forest trees (Crous *et al.*, 2004, 2006, 2007; Arzanlou *et al.*, 2008).

Ecologically these fungi are also known to be saprobes, parasites of other fungi (Jackson *et al.*, 2004) as well important plant pathogens, and are mostly found in tropical and sub–tropical areas. Although, most Cercospora

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species are host specific (Arzanlou *et al.*, 2007), but some species still produce lesions on various different plants (Groenewold *et al.*, 2006) through co–infection with an unrelated fungus in the same lesion.

The present study on Cercosporoid fungi is similar to those conducted in many other countries which have sought to discover new *Cercospora* species. Moreover, there have been numerous reports of new species, new hosts and new records of Cercosporoid fungi in South–East Asia (Burgess *et al.*, 2007). In Thailand and neighboring countries there have been many reports of new Cercosporoid fungi, such as *Cercospora*, *Passalora*, *Pseudocercospora* and other fungi. (Nakachima *et al.*, 2007; Meeboon *et al.*, 2007; To–anun *et al.*, 2011; Phengsintham *et al.*, 2013). These reports were mainly based on the morphological taxonomic concepts introduced by Chupp (1954) and further developed and modified by Crous & Braun (2003). The taxonomic identification key of Cercosporoid fungi used in the current study was based on these reports.

#### Materials and methods

The specimens were collected in Chiang Mai, Chiang Rai and Lampang provinces in Northern Thailand. Leaf lesions were examined under a stereo microscope for the presence the Cercosporoid fungi. The slides for microscopic examination were prepared by hand sections from plant materials and mounted in lactic acid.

Detailed observations of morphological characteristics were carried out by means of an Olympus CX 31 light microscope (100×). Thirty conidia, conidiophores and stromata were measured for each specimen. Dried specimens were deposited at Plant Pathology Herbarium, Department of Agriculture, Chiang Mai University.

#### Results

Cercospora pisoneae Wongsopa and Cheewangkoon, sp. nov.

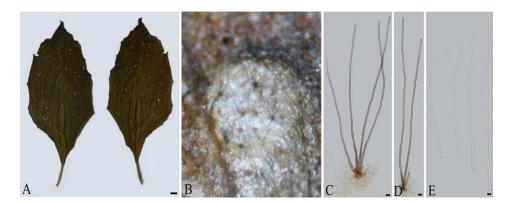
Etymology: pisoneae, derived from the genus name of the host plant. Maculae amphigenae, circulares vel subcirculares, centro griseo-brunnea, margine indefinitis, 5 mm dium. Caespituli amphigeni. Stromata nulla vel parva, evolutis, substomatalia, 10–30 μm diam., atro-brunnea. Conidiophora 3–5 laxe fasciculate, cylindrical, erecta vel decumbentia, recta vel leviter curvata, 1–3-geniculata, non ramosa, (45–) 275–370 (–400) ×4–7

 $\mu$ m, 8–10 (–13) –septata, medio brunnea, laevia. Cellulae conidiogenae terminals, sympodieles, Loci conidiogeni conspicui, incrassati, fuscati. Conidia solitaria, fusiformia vel obclavata, recta vel leviter curvata, (200–) 215–340 (–380)  $\times$  (3–) 4 (–5)  $\mu$ m, (16–) 17–28 (–32) -septata, hyaline, non ramosa, laevia, apice subacuto vel abtusa et basi obconice truncate, hila incrassate et fuscata.

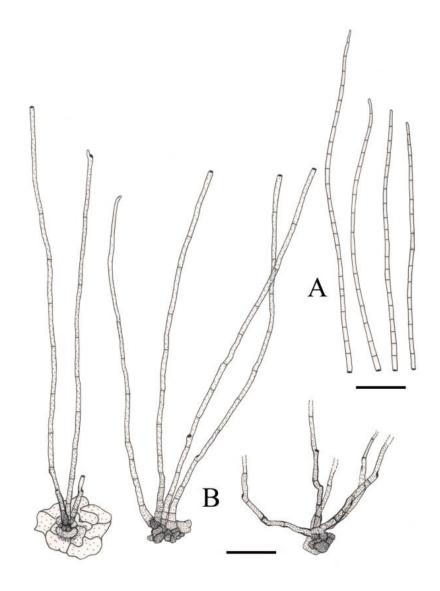
Leaf spots circular to subcircular, centre grayish to pale brown and dark brown indefinite margins, 5 mm in diameter. Caespituli amphigenous. Stromata lacking to small–developed, subepidermal, arising from stomata cells, 10–30 μm in diameter, dark brown. Conidiophores loosely fasciculate, arising from stromata (3–5 per fascicle), cylindrical, erect to decumbent, straight or slightly curved, sometimes 1–3-geniculate at base, unbranched, (45–) 275–370 (–400) × 4–7 μm, 8–10 (–13) -septate, medium brown, smooth. Conidiogenous cells terminal, conidiogenous loci conspicuous, thickened and darkened. Conidia solitary, filiform to narrowly obclavate, straight to slightly curved, (200–) 215–340 (–380) × (3–) 4 (–5) μm, (16–) 17–28 (–32) -septate, hyaline, unbranched, smooth, apex obtuse and base obconically truncated, Hila thicken and darkened. (Fig. 1–2)

Habitat: On leaves of *Pisonia grandis* R. Br. (Nyctaginaceae) *Material examined*: Thailand, Chiang Mai Province, Mae Rim district, Nang Hoi, 26 July 2013, Khelang Wongsopa and Ratchadawan Cheewangkoon (Plant Pathology Herbarium HY32).

*Notes*: Numerous reports can be found describing *Cercospora* spp. on Nyctaginaceae, but this is the first report of *Cercospora pisoneae* on Genus *Pisonea* (Nyctaginaceae) from Thailand.



**Figure 1**. *Cercospora pisoneae* **A.** Leaf spots on *Pisonia grandis* R. Br. (Scale bars = 1 cm) **B.** Caespituli, **C–D**. Stromata and conidiophores, E. Conidia (C–E: Scale bars = 10 μm).



**Figure 2**. *Cercospora pisoneae* on *Pisonia grandis* R. Br. (From holotype). A. Conidia B. Stromata and conidiophores. Scale bars  $= 50 \mu m$ .

# Pseudocercospora combretina Wongsopa and Cheewangkoon, sp. nov.

*Etymology*: combretina, derived from the genus name of the host plant. Maculae amphigenae subcirculares vel irregulares, centro griseo—albidis vel pallide brunnea, medio brunneo vel atro—brunnea margine indefinitis, 5–15 mm dium., Caespituli epiphyllis. Stromata bene—evolutis, substomatalia vel

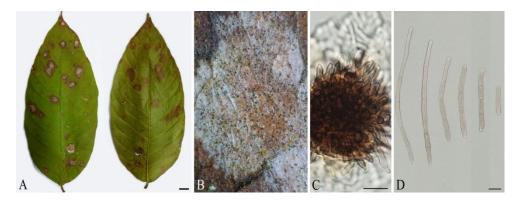
intraepidermalia, usque 50  $\mu$ m dium., subglobosis, atro-brunnea vel cinereo. Conidiophora 15–25 laxe vel dense fasciculate, acicuribus vel subcylindrica, erecta vel decumbentia, recta vel lenviter curvata,geniculate et apice, non ramusa, (6–) 10–20  $\times$  2.5–4  $\mu$ m, 0–1 (–2) -septata, pallide vel medio brunneo. Laevia, rotumdato vel subconico et apice. Cellulae conidiogenae terminals. Loci conidiogeni inconspicui. Conidia solitaria, obclavata vel subcylindrica, recta vel lenviter curvata, sinuosa, (12–) 20–82 (–88)  $\times$  3–4  $\mu$ m, 1–5-septata, conspicui, brunneo ad apicem versus pallidiora, non ramosa, laevia, apice obtuse, et basi obconice truncata.

Leaf spots subcircular to irregular, centre grayish, white to pale brown and medium brown to dark brown indefinite margins, 5–15 mm in diameter. Caespituli mainly epiphyllous. Stromata well–developed, subepidermal to intraepidermal, up to 50 μm in diameter, subglobular, dark brown to blackish. Conidiophores loosely to moderate fasciculate, arising from stromata (15–25 per fascicle), acicular to subcylindrical, erect to decumbent, straight or slightly curved, sometimes geniculate at apex, unbranched, (6–) 10–20 × 2.5–4 μm, 0–1 (–2) -septate, pale to medium brown, smooth, small rounded to subconic apex. Conidiogenous cells terminal, conidiogenous loci inconspicuous, unthickened and not darkened. Conidia solitary, narrowly obclavate to subcylindrical, straight to slightly curved, sinuous, (12–) 20–82 (–88) × 3–4 μm, 1–5-septate conspicuous, brown, and paler toward the apex, unbranched, smooth, apex obtuse and base obconically truncated. (Fig. 3–4)

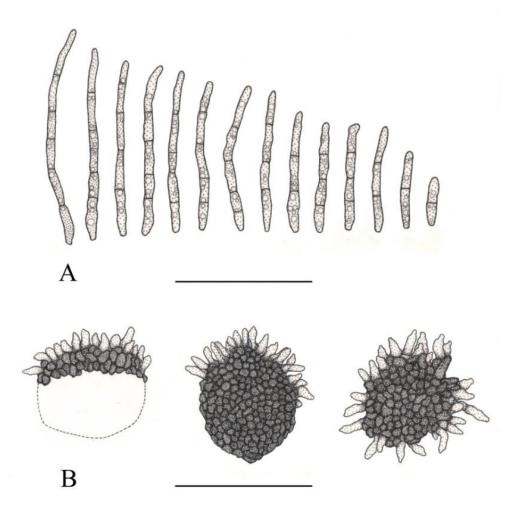
*Habitat*: On leaves of *Combretum procursum* Craib (Combretaceae)

*Material examined*: Thailand, Lampang Province, Chaeson National Park, 8 January 2014, Khelang Wongsopa and Ratchadawan Cheewangkoon (Plant Herbarium HY41).

*Notes*: This is a new record for this species from Thailand. Previously two species of *Pseudocercospora* spp. were reported on *Combretum* spp. The current specimen is distinguished by having *stromata* dark brown to blackish, *Conidiophores* are shorter (6–)  $10-20 \times 2.5-4 \mu m$  and *Conidia* are brown to dark brown.



**Figure 3.** Pseudocercospora combretina A. Leaf spots on Combretum procursum Craib(Scale bars = 1 cm), B. Caespituli, C. Stromata and conidiophores, D. Conidia (C–D: Scale bars =  $10 \mu m$ ).



**Figure 4**. *Pseudocercospora combretina* on *Combretum procursum* Craib (From holotype). A. Conidia, B. Stromata and conidiophores. (Scale bars: = 50 μm.)

## *Pseudocercospora glycinicola* Wongsopa & Cheewangkoon, sp. nov.

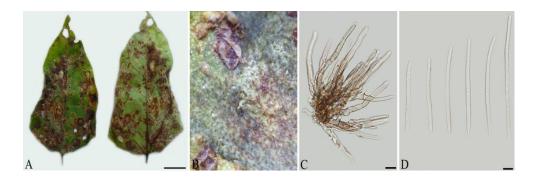
Etymology: glycinicola, derived from the genus name of the host plant. Maculae amphigenae, subcirculares vel irregulares, griseo vel brunneo, 1–5 mm dium., Caespituli amphigenae. Stromata nulla vel parva–evolutis, substomatalia, 20–25 (–50) μm diam., brunneo vel atro–brunnea, Conidiophora 15–21 fasciculata, subcylindrica, erecta vel decumbentia, recta, leviter curvata vel sinuosa, ramosa, (30–) 40–70 (–75) × 5–6 (–7) μm, 4–6-septata, laevia, pallide brunnea vel brunneo, ad apicum versus pallidiora, apicem subacuto vel rotundato ad subtruncata. Cellulae conidiogenae terminals, sympodie, Loci conidiogeni inconspicui. Conidia solitaria, subcylindrica vel cylindrical, obclavata, recta vel leviter curvata, (40–) 65–95 (–100) μm, (3–) 4–7-septata, gutulata, subhyalina vel pallide brunnea, laevia, apice obtuse vel rotundato, et basi obconice truncate.

Leaf spots subcircular to irregular, grayish to brown, 1–5 mm in diameter, Caespituli amphigenous. Stromata lacking to small–developed, substomatal, 20–25 (–50) μm in diameter, brown to dark brown. Conidiophores fasciculate (15–21 per fascicle), erumpent through the cuticle, subcylindrical and narrower towards at apex, erect to decumbent, straight, slightly curved to sinuous, branched, (30–) 40–70 (–75) × 5–6 (–7) μm, 4–6-septate, smooth, brown to pale brown, and paler toward at apex, subacute to round and subtruncate at apex. Conidiogenous cells sympodial, terminal, conidiogenous loci inconspicuous, unthickened, not darkened. Conidia solitary, subcylindrical to cylindrical, sometimes narrowly obclavate, mostly straight to slightly curved, (40–) 65–95 (–100) μm, (3–) 4–7-septate, gutulate, subhyaline to pale brown, smooth, apex obtuse, base obconically truncated. (Fig. 5–6)

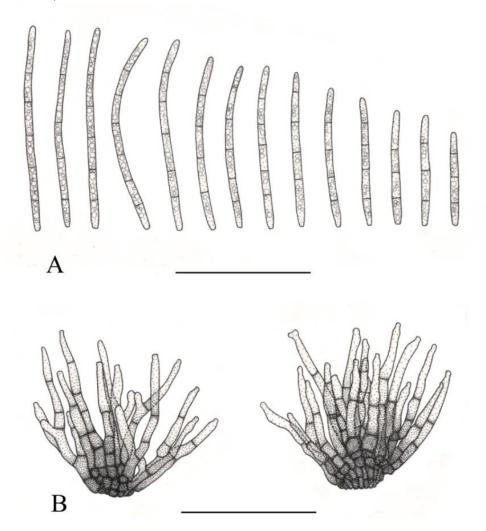
*Habitat*: On leaves of *Glycine max* (L.) Merr. (Fabaceae)

*Material examined*: Thailand, Chiang Mai Province, Mae Wang district, 26 July 2013, Khelang Wongsopa (Plant Pathology Herbarium HY25).

Notes: Only *Pseudocercospora glycines* species found on Genus *Glycine* spp. by Hsieh and Goh (1990), *P. glycines* was previously reported only from Australia and Taiwan. *P. glycinicola* is distinguished by having *conidiophores* branched and longer than *P. glycines* (30–) 40–70 (–75)  $\times$  5–6 (–7) µm Vs. 10–25  $\times$  1.5–3 µm.



**Figure 5**. *Pseudocercospora glycinicola* A. Leaf spots on *Glycine max* (L.) Merr (Scale bars = 1 cm), B. Caespituli, C. Stromata and conidiophores, D. Conidia (C–D: Scale bars = 10 μm).



**Figure 6**. *Pseudocercospora glycinicola* on *Glycine max* (L.) Merr. (From holotype). A. Conidia B. Stromata and conidiophores. Scale bars:  $= 50 \mu m$ .

Cercospora apii Fresen. sensu lato, emend. Crous and Braun, Mycosphaerella and its anamorphs: 1. Names published in Cercospora and Passalora: 35, 2003.

Crous and Braun (2003) organized the several phylogenetic species and their morphologically similar species of the *Cercospora apii* complex from many host species. However, their scheme lacks the taxonomic identification of the genus *Cercospora* from Northern Thailand.

## Cercospora apii (Crous and Braun, 2003)

*Caespituli* amphigenous. *Stromata* substomatal to intraepidermal, 25–40  $\mu$ m in diam., dark brown. *Conidiophores* cylindrical, 125–240 (–250)  $\times$  5–6  $\mu$ m, (2–) 3–8 (–9) -septate, brown. *Conidia* filiform to narrowly obclavate, (35–) 70–300 (–380)  $\times$  (3–) 4–5  $\mu$ m, (2–) 6–22 (–29) -septate, hyaline to subhyaline.

Habitat: On leaves of Brassica rapa L. (Cruciferae)

*Material examined*: Thailand, Chiang Mai Province, Multiple Cropping Center, Faculty of Agriculture Chiang Mai University, 15 August 2013, Khelang Wongsopa and Ratchadawan Cheewangkoon (Plant Pathology Herbarium HY33).

*Notes*: This is the first report of a new host from Thailand.

## Cercospora ipomoeae (Hsieh and Goh, 1990)

(=Cercospora apii Crous and Braun, 2003)

*Caespituli* amphigenous, *Stromata* substomatal, 20–60 µm in diam., dark brown to blackish. *Conidiophores* cylindrical, (45–) 50–120  $\times$ 5–6 µm, 2–3-septate, unbranch, pale brown to mediam brown. *Conidia* narrowly obclavate, (75–) 100–210 (–310)  $\times$  4–5 µm, (7–) 10–15 (–17) -septate, gutulate, hyaline to subhyline.

*Habitat*: On leaves of *Merremia vitifolia* (Burm. f.) Hallier f. (Convolvulaceae)

*Material examined*: Thailand, Chiang Mai Province, Mueang District, Mae Hia, 10 June 2013, Khelang Wongsopa and Ratchadawan Cheewangkoon (Plant Pathology Herbarium HY20).

Notes: This is the first report of this species on a new host from Thailand.

## Cercospora apii (Crous and Braun, 2003)

Caespituli amphigenous, Stromata substomatal, 25–80  $\mu$ m in diam., dark brown. Conidiophores cylindrical, (80–) 85–180 (–190)  $\times$  (4–) 5–6 (–7)

 $\mu$ m, (2–) 3–4 (–5)-septate, unbranch, pale brown. *Conidia* narrowly obclavate–subcylindrical, (70–) 90–275 (–300)  $\times$  4–5  $\mu$ m, (8–) 13–21 (–24) -septate, hyaline.

Habitat: On leaves of Abelmoschus esculentus (L.) Moench (Malvaceae)

*Material examined*: Thailand, Chiang Mai Province, Mae Wang District, 28 June 2013, Khelang Wongsopa and Ratchadawan Cheewangkoon (Plant Pathology Herbarium HY22).

*Notes*: This is the first report on this host from Thailand.

## Cercospora apii (Crous and Braun, 2003)

Caespituli amphigenous, Stromata substomatal, 50–65 μm in diam., dark brown. Conidiophores cylindrical, (130–) 140–190 (–210)  $\times$  5–6 μm, 4–6 (–8) -septate, unbranch, pale brown. Conidia filiform–narrowly obclavate, 70–280  $\times$  4 μm, 10–28-septate, gutulate, hyaline.

Habitat: On leaves of Thunbergia laurifolia Lindl. (Acanthaceae)

*Material examined*: Thailand, Chiang Mai Province, Muang District, Mae Hia, 14 February 2013, Khelang Wongsopa and Ratchadawan Cheewangkoon (Plant Pathology Herbarium HY15).

*Notes*: This is the first report of a new host from Thailand.

## Cercospora paederiicola (Guo and Liu, 1991)

(=Cercospora apii Crous and Braun, 2003)

*Caespituli* amphigenous, *Stromata* substomatal, 100–120 μm in diam., dark brown to blackish. *Conidiophores* cylindrical, (110–) 115–130 (–150)  $\times$  4–6 μm, 5–6-septate, unbranch, pale brown. *Conidia* narrowly obclavate, (75–) 100–210 (–310)  $\times$  4–5 μm, (11–) 13–25 (38) -septate, gutulate, hyaline.

*Habitat*: On leaves of *Paederia pallida* Craib (Rubiaceae)

*Material examined*: Thailand, Chiang Mai Province, Muang District, Mae Hia, 10 June 2013, Khelang Wongsopa and Ratchadawan Cheewangkoon (Plant Pathology Herbarium HY18).

*Notes*: This is the first report of a new host from Thailand.

## Pseudocercospora liquidam Scalebarsicola (J.M. Yen) Braun, (2000)

*Caespituli* amphigenous, *stromata* substomatal, up to 65 in diam., dark brown. *Conidiophores* 9–10 (–20)  $\times$  3–4  $\mu$ m, 0–1-septate, unbranched, pale brown. *Conidia* acicular–narrowly obclavate, subcylindrical (30–) 35–52 (–65)  $\times$  (2.5–) 3  $\mu$ m, (2–) 3–5 (–7) -septate, gutalate, greyish to brown.

Habitat: On leaves of Liquidambar formosana Hance. (Hamamelidaceae)

*Material examined*: Thailand, Chiang Mai Province, Chom Thong District, Ban Mae Tao, 13 November 2012, KhelangWongsopa and RatchadawanCheewangkoon (Plant Pathology Herbarium HY1).

Notes: This is the first report of this species from Thailand.

# *Pseudocercospora fici* Heald & F.A. Wolf (Liu and Guo, 1991)

(=Cercospora fici Heald & Wolf, 1911)

*Caespituli* amphigenous. *Stromata* substomatal, 25–35 μm in diam, brown to dark brown. *Conidiophores* (9–) 10–20 (–25)  $\times$  (2.5–) 3–3.5 (–4) μm, 0–1-septate, unbranched, subhyaline to pale brown. *Conidia* subcylindrical, (60–) 65–80  $\times$  (2.5–) 3 (–4) μm, (4–) 5–8 (–10) -septate, gutulate, subhyaline to pale brown.

Habitat: On leaves of Ficus pumila L. (Moraceae)

*Material examined*: Thailand, Chiang Rai Province, Mae Fah Luang District, Doi Tung, 3 February 2013, Khelang Wongsopa and Ratchadawan Cheewangkoon (Plant Pathology Herbarium HY9).

*Notes*: This is the first report of this species on a new host from Thailand.

## Pseudocercospora mori (Hara) Deighton, (1976)

*Caespituli* hypophyllous, *Stromata* substomatal, 35–55 µm in diam., dark brown. *Conidiophores* cylindrical, (5–) 6–9 (–10)  $\times$  2–5 µm, aseptate, unbranched, pale brown. *Conidia* narrowly obclavate–subcylindrical, (20–) 25–55 (–65)  $\times$  2–3 µm, (1–) 2–6 (–7) -septate, gutulate, *subhyaline to greyish*.

Habitat: On leaves of Morus alba L. (Moraceae)

Material examined: Thailand, Chiang Mai Province, Muang District, Falcuty of Agriculture, Chiang Mai University, 15 August 2013, Khelang Wongsopa and Ratchadawan Cheewangkoon (Plant Pathology Herbarium HY34).

*Notes*: This is the first report of this species on a new host from Thailand.

## Pseudocercospora rubi (Sacc.) Deighton, (1976)

Caespituli epiphyllous, Stromata substomatal-intraepidermatal, 40–80 µm in diam., dark brown. Conidiophores cylindrical, geniculate-sinuous,

(10–) 15–20 (–25)  $\times$  3–4  $\mu$ m, 0–1-septate, unbranched, pale brown to hyaline. *Conidia* mostly curved, subcylindrical–cylindrical, (40–) 50–80 (–105)  $\times$  3–4  $\mu$ m, (1–) 3–8 (–9) -septate, gutulate, grayish to subhyaline.

Habitat: On leaves of Rubus blepharoneurus Card. (Rosaceae)

*Material examined*: Thailand, Chiang Rai Province, Thoeng District, Phu Chi Fa, 10 December 2013, Khelang Wongsopa and Ratchadawan Cheewangkoon (Plant Pathology Herbarium HY38).

*Notes*: This is the first report of this species on a new host from Thailand.

## Pseudocercospora egenula (Syd.) Braun and Crous, (2003)

Caespituli amphigenous, Stromata substomatal, 25–45  $\mu$ m in diam., brown to dark brown. Conidiophores subcylindrical, (17–) 20–35 (–45)  $\times$  (3.5–) 4–5 (–6)  $\mu$ m, (0–) 1–2 (–4) -septate, unbranched, pale brown to brown. Conidia narrowly obclavate–subcylindrical, (16–) 35–75 (–95)  $\times$  3–4 (–5)  $\mu$ m, 1–7 (–9) -septate, greyish to subhyaline.

Habitat: On Leaves of Brugmansia × candida Pers. (Solanaceae)

*Material examined*: Thailand, Chiang Mai Province, Chom Thong District, Doi Intanon, 27 January 2013, Khelang Wongsopa and Ratchadawan Cheewangkoon (Plant Pathology Herbarium HY4).

*Notes*: This is the first report of this species on a new host from Thailand.

## Pseudocercospora daturina (J. M. Yen) Deighton, (1976)

*Caespituli* amphigenous, *Stromata* substomatal, 35–65 μm in diam., brown to dark brown. *Conidiophores* subcylindrical, (10–) 15–40 (–45)  $\times$ 4–5(–6) μm, 0–2 (–3) -septate, branched to unbranched, greyish to pale brown. *Conidia* narrowly obclavate to subcylindrical, (22–) 42–87 (–110)  $\times$  (3–) 4–5 μm, (1–) 4–8 (–11) -septate, gutulate, grayish to pale brown.

*Habitat*: On Leaves of *Brugmansia* × *candida* Pers. (Solanaceae)

*Material examined*: Thailand, Chiang Rai Province, Mae Fah Luang District, Doi Tung, 3 Febuary 2013, Khelang Wongsopa and Ratchadawan Cheewangkoon (Plant Pathology Herbarium HY10).

*Notes*: This is the first report of this species on a new host from Thailand.

#### **Discussion**

The Cercosporoid fungi are well known to be associated with *Mycosphaerella* teleomorphic fungi, and they have been linked to leaf spots symptoms on many hosts. Numerous reports can indicate that they include some of most important living tissue pathogens in tropical regions (Crous, 2009).

A specific focus of this study was to survey for Cercosporoid fungi in forests and plantations in Northern Thailand, because a number of previous reports on these fungi identified new species, new hosts and new records (Meeboon *et al.*, 2007; Nakachima *et al.*, 2007; To–anun *et al.*, 2011; Phengsintham *et al.*, 2011).

Our research obtained 14 isolates of *Cercospora* spp. and *Pseudocercospora* spp. from lesions on host plants, including new species from Thailand: *Cercospora pisoneae* on *Pisonia grandis* (Nyctagina), *Pseudocercospora combretina* on *Combretum procursum* Craib (Combretaceae) and *Pseudocercospora glycinicola* on *Glycine max* (L.) Merr (Fabaceae). These isolates differed taxonomically from all known Cercosporoid fungi and can be considered new species.

In addition, the lists of *Cercospora apii* sensu lato on many host plants in this report was consistent reporting in "*Mycosphaerella* and its anamorph: 1. Names published in *Cercospora* and *Passalora*" reported by Crous and Braun (2003).

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