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Phyllosticta Capitalensis, P. helicteres, P. sterculiae and other *Phyllosticta* Species from Sterculiaceae

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Abstract Phyllosticta as an important plant pathogenic genus associated with plants as endophytes, pathogens or saprobes. *Phyllosticta sterculiae* occurs on *Sterculia foetida* (Sterculiaceae) in the Philippines, where it causes a leaf blight. The holotype of *P. sterculiae* is redescribed and compared with a Phyllosticta species collected on *Sterculia monosperma* in Thailand. *P. sterculiae* has oblong to obovoid ascospores whereas the fungus from Thailand has ellipsoidal ascospores that are swollen in the middle and possess polar appendages. The comparison between other Phyllosticta species on Sterculiaceae revealed Phyllosticta species from Thailand collection is different in ascospores dimention. Furthermore, the anamorph in culture compare to the Thailand collections with *Phyllosticta melochiae* from Indonesia on host subtrate posseses similar character. The BLAST result showed that the Thailand species is belonging *Phyllosticta capitalensis*. The need of epitypification for all the *Phyllosticta spp*. occurs in Sterculiaceae is needed here to clarify the species name. The Thailand fungus is described, illustrated and discussed.

Keywords: Blast, Dothideomycetes, Indonesia, leaf blight, morphology, plant disease, taxonomy, Thailand

Introduction

Phyllosticta is an important plant pathogenic genus with Guignardia sexual state as previously recorded (Sivanesan, 1984; Bussaban *et al*., 2004; Farr and Rossman, 2012; Hawksworth, 2012; Stevens, 1917; Van der Aa, 1973; Van der Aa and Vanev, 2002; Rehm, 1914; Yates, 1918; Von Arx and Müller, 1954; Hyde, 1995; Benjapalakorn, 2006; Wong *et al*., 2012; Wulandari *et al*., 2009; Wulandari *et al*., 2010a,b,c; Wulandari *et al*., 2011; McMakin, 2000; Hennings, 1980; Sontirat *et al*., 1994; Crous *et al*., 2004; Raciborski, 1990; Rayner, 1994; Gardner *et al*., 2000; Somrithipol and Hyde, 2004; Thongkontha

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et al., 2008; Wikee *et al*., 2011, 2012; The Royal Institute (1995), Photita *et al*., 2001; Choi *et al*., 1999). Species occur as endophytes, pathogens and saprobes on a wide range of plant families such as, Agavaceae (Hawksworth, 2012; Van der Aa and Vanev, 2002; Rehm, 1914; Wulandari *et al*., 2010b; Crous *et al*., 2004; Wikee *et al*., 2011) Arecaceae (palms) (Hawksworth, 2012; Van der Aa and Vanev, 2002; Rehm, 1914; Sivanesan, 1984; McMakin, 2000; Crous *et al*., 2004). Musaceae (Sivanesan, 1984; Stevens, 1917; Yates, 1918; 1994; Crous *et al*., 2004; Raciborski, 1990). Orchidaceae (Sivanesan, 1984; Hawksworth, 2012; an der Aa and Vanev, 2002; Sontirat *et al*., 1994), Pandanaceae Rehm (1914), Zingiberaceae (Bussaban *et al*., 2004; Van der Aa, 1973; Rehm, 1914) (monocotyledons), and dicotyledons: Acanthaceae Rehm (1914), Caesalpiniaceae Rehm (1914), Custutaceae Rehm (1914), Dioscoreaceae (Sivanesan, 1984; Hawksworth, 2012; Van der Aa and Vanev, 2002; Rehm 1914; Thongkontha *et al*., 2008), Dipterocarpaceae Rehm (1914), Fabaceae (Hawksworth, 2012; Van der Aa and Vanev, 2002; Rehm, 1914; Wulandari *et al*., 2010b; Crous *et al*., 2004), Rubiaceae (Sivanesan, 1984; Hawksworth, 2012 Van der Aa and Vanev, 2002; Rehm, 1914; Wulandari *et al*., 2010a,b, Wulandari *et al*., 2011) and Rutaceae (Sivanesan, 1984; Hawksworth, 2012 Van der Aa and Vanev, 2002; Rehm, 1914; Wulandari *et al*., 2010a,b, Wulandari *et al*., 2011). We are studying the genus Phyllosticta in Northern Thailand and in this paper report Phyllosticta species on Sterculiaceae.

The Sterculiaceae compise large trees native to China and Thailand (http://www.quisqualis.com/tvPEFC2P063.html) and widely introduce into Southern China, Sumatra (Indonesia) and Taiwan. Theobroma cacao is an important genus in this family and is used for cocoa production. Sterculia monosperma Vent. is known as Chinese cheshnut, noble battle tree, or as pheng pok in Thai (Somrithipol and Hyde, 2004) and its starch granules may be used as thickening in chili sauce (Wong *et al*., 2012). Melochia umbellata is known as Melochia (Somrithipol and Hyde, 2004), the wood is used for handycraft and tool (Somrithipol and Hyde, 2004). Sterculia foetida known as Sumrong [Thai] and it has unpleasent aroma of the flower (Hennings, 1980).

Phyllosticta melochiae, P. helicteres and P. sterculiae are Phyllosticta species known from Sterculiaceae. Phyllosticta melochia was found in Delhi, Borneo, Indonesia (Rehm, 1914) with specific zona leaf spot, P. helicteres was found on Helicteres jamaicensis in Puerto Rico with target spot (Van der Aa, 1973) and Phyllosticta sterculiae was reported on Sterculia foetida in the Philippines, where it causes leaf blight (Yates, 1918). In this study we made collections of Phyllosticta species from Sterculia monosperma in Thailand. The species found was distinct from P. sterculiae and P. helicteres and it is described, illustrated and discussed. For comparative purposes we also

examined the type material of P. sterculiae and P. helicteres. BLAST result were also used to support the finding. By using BLAST, it can compare nucleotide sequence to a whole sequence library and will gave the high sequence similarity (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Materials and methods

Type specimens of sexual state of Phyllosticta sterculiae were borrowed from ILL (USA) and S (Sweden), P. helicteres from BPI (USA) and NY (USA) while fresh collections on Sterculia monosperma were made in Chiang Rai Province, Northern Thailand. Fungi were isolated by single spore method as described in (Choi *et al*., 1999). Morphological character were recorded using a Nikon 80i microscope with Tarosof program for measuring spores and camera Lucida attachment for line drawing. Strains were isolated onto Difco malt extract agar (MEA) and growth rate was measured at 24^{o} C in triplicate; colour nomenclature and pigmentation follow Rayner (Gardner *et al*., 2000). Holotype specimens Myco Bank number were provided by MycoBank website (Raciborski, 1990).

Results

Hawksworth (2012) reported that can be only one fungus represent one name can be applied to all the state of certain fungi. This paper will treated all Guignardia name into Phyllosticta name. The isotype of P. sterculiae from Philippines and P. helicteres from Puerto Rico were reexamined and it is redescribed and illustrated here. Collections of Phyllosticta species on Sterculia monosperma from Chiang Rai Province in Thailand differed from P. sterculiae and P. helicteres, from BLAST (Basic Local Alignment Search Tool, http://blast.ncbi.nlm.nih.gov/Blast.cgi) the fungus is recognize as Phyllosticta capitalensis. All Phyllosticta species occurs on Sterculiaceae will be described and illustrated below.

Phyllosticta capitalensis (Hennings, 1980) epitypify (Farr and Rossman, 2012) MycoBank: MB 168326

Leaf blight, necrotic blotches 6–10 cm, rounded, with a brown border and pale brown centre, ascomata visible on the surface as numerous black dots. Ascomata 135–160 µm, on upper surface, black, globose to subglobose, immersed in plant tissues, coriaceous, solitary to clustered, ostiolate, ostioles as black dots in the center. Peridium 15–25 µm wide, comprising one strata of textura angularis comprising 2–3 layers of cells with thickened brown walls.

Pseudoparaphyses not observed. Asci 50–91 × 9–14 µm (= 69 × 12 µm, n = 20), 8–spored, bitunicate, fissitunicate, broadly cylindrical to cylindro–clavate, rounded at the apex, where the diameter is 7–9 µm, ocular chamber 3–6 µm high, tapering gradually to a 6–8 × 5–7 µm long pedicel attached to the basal peridium. Ascospores 14–19 × 5–7 µm (= 17 × 6 µm, n = 20), biseriate or occasionally overlapping uniseriate, ellipsoidal, swollen in the centre, flattened on one side when viewed from above, hyaline–greenish, 1–celled, coarse–guttulate, smooth–walled, with a 6–8 × 5–7 µm long mucilaginous appendage at each end.

Cultural characteristics: Colonies on MEA colonies reaching 4.1 mm in 1 week. Flat, slightly raised, irregular to lobate edge, black–olivaceous to black above and black–olivaceous reverse.

Pycnidia 39–68 µm diameter, 90–103 um high, singly, black, globose to elongate, immersed in media. Peridium 15–17 µm in diameter. Conidiogenous cells 7–15 × 2–4 µm (= 12 × 3 µm, n = 20), holoblastic, determinate, discrete, rarely integrated, hyaline, cylindrical to doliiform cells lining the pycnidial locule. Conidia 7–11 × 5–7 µm (= 10 × 6 µm, n = 20), hyaline–greenish, 1–celled, coarse–guttulate, smooth–walled, globose, ellipsoidal, clavate or obclavate, with an obtuse apex, sometimes truncate at the base, surrounded by 1–2 µm thick mucilaginous sheath which persists at maturity and in some spores with a single, hyaline, curved or straight, 1–5 µm long appendage.

Habitat: On living leaves of Sterculia monosperma Vent. (Sterculiaceae) causing leaf blotch/leaf blight.

Known Distribution: Thailand.

Material examined: Thailand, Chiang Rai, on leaves of Sterculia monosperma, 20 November 2009, N. F. Wulandari, NFW 249 (MFLU10 0292; MFLUCC 0340, living culture) teleomorph only present; ibid., 17 December 2009, NFW 266 (MFLU10 0293) teleomorph only present; ibid., 21 December 2009, N. F. Wulandari, NFW 308 (MFLU10 0294) teleomorph only present.

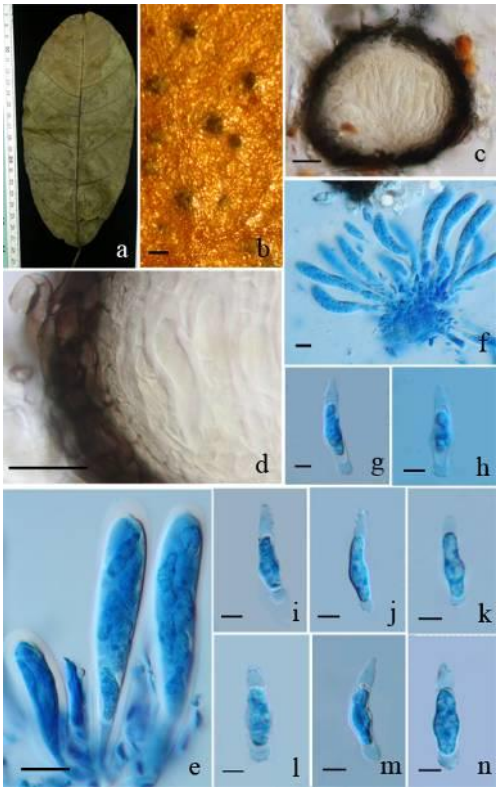

Fig. 1a. *Phyllosticta capitalensis* (MFLU10 0292) a. Leaf blight (arrowed) on leaf. b. Appearance of ascomata on the host surface. c. Section of ascoma on the leaf. d. Peridium of textura angularis comprising 2–3 layers of cells with thickened angular brown walls. e–f. Asci with ocular chamber. g–n. Ascospores with bipolar mucilaginous appendages, rounded at the base and pointed at the apex. Scale bars: b = 100 µm, c–d = 20 µm, e–f = 25 µm, g–n = 10 µm.

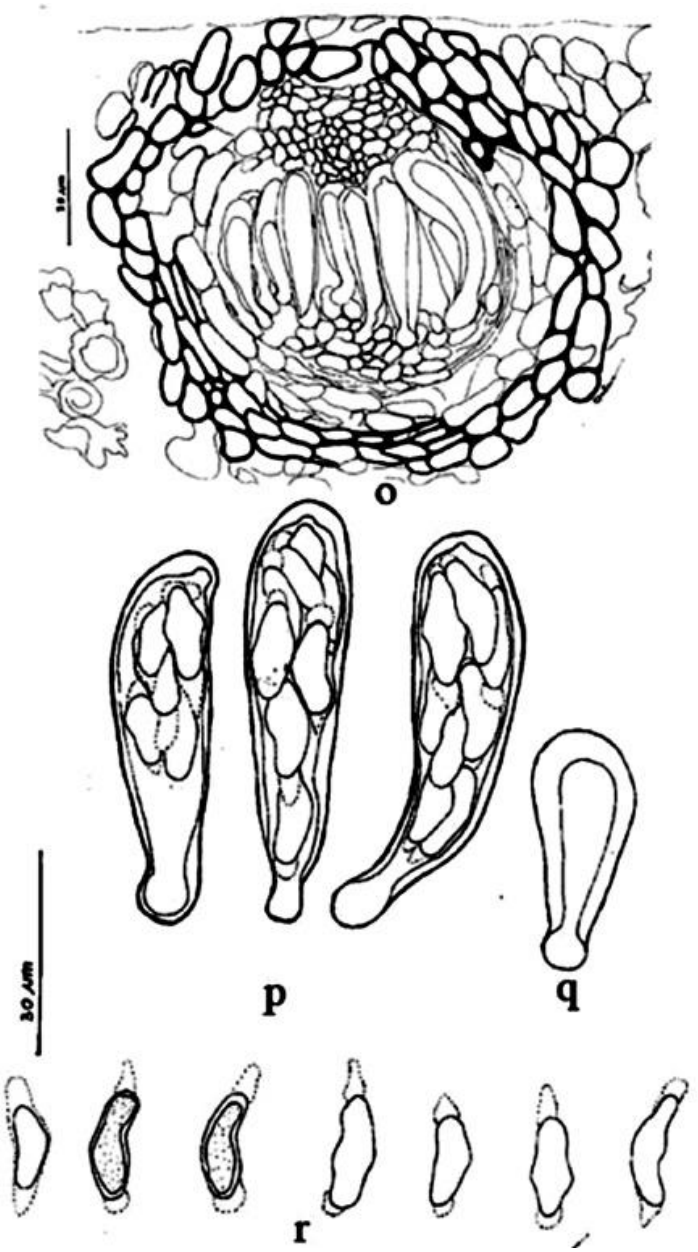

Fig. 1b. *Phyllosticta capitalensis* (MFLU10 0292) line drawing. o. Section of ascoma in the leaf (darkened area is fungal cells, arrowed) p. Asci. q. Immature ascus. r. Ascospores

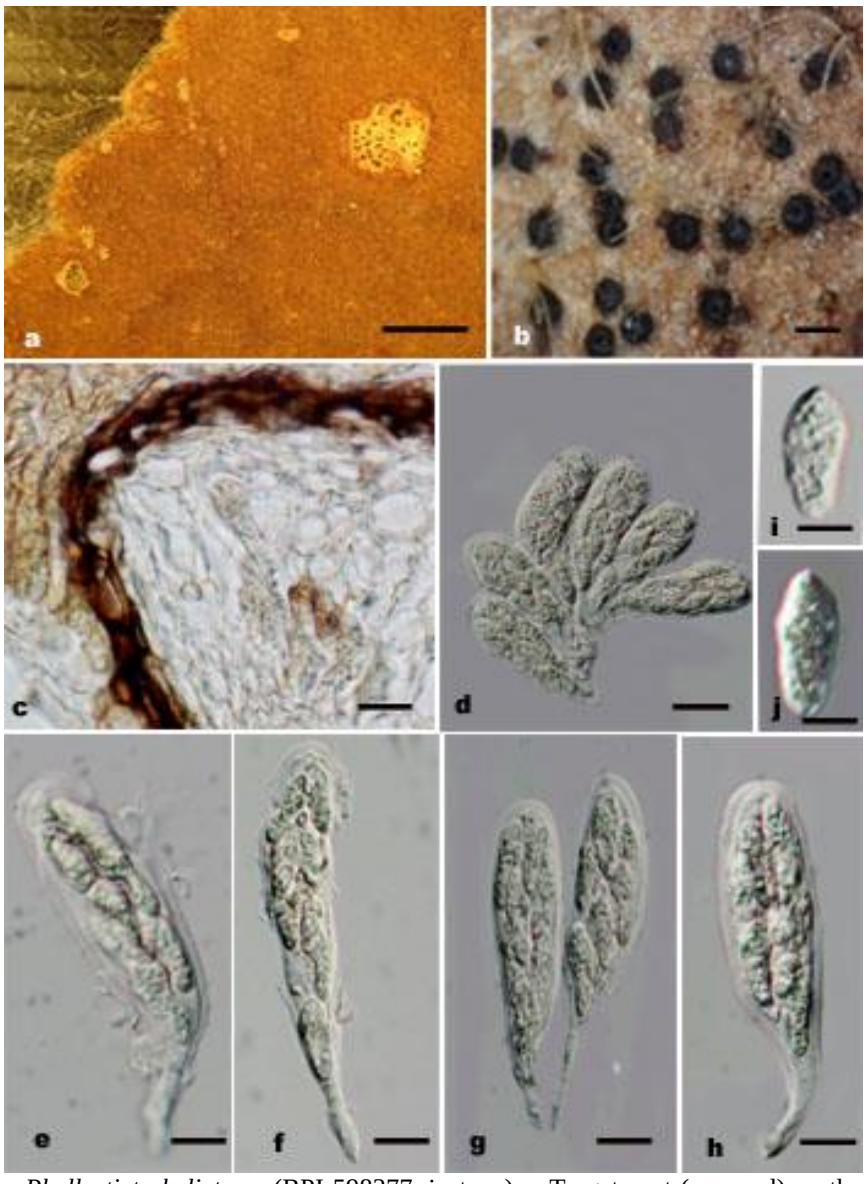

Fig. 2a. *Phyllosticta helicteres* (BPI 598377, isotype) a. Target spot (arrowed) on the leaf. b. Appearance of ascomata on the host surface. c. Peridium comprising one strata of textura angularis comprising 2–3 layers of cells with thickened brown walls. d–h. Asci. i–j. Ascospores. Scale bars: a = 3 mm, b = 100 μm, c–h = 10 μm.

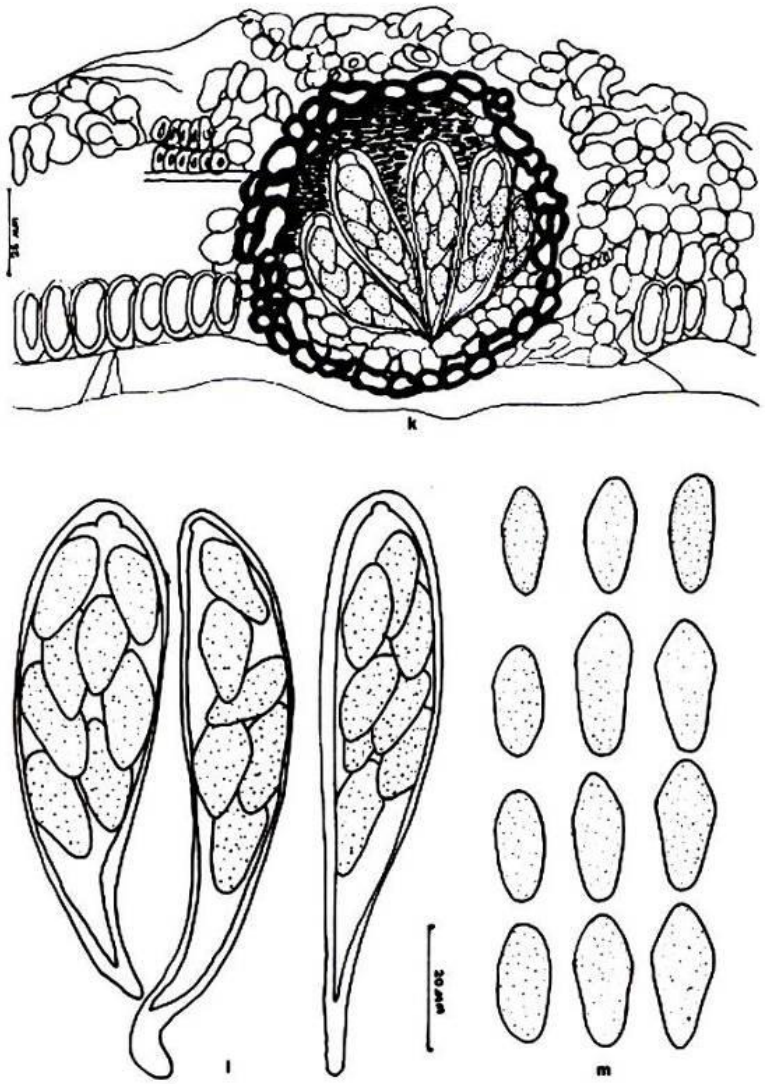

Fig. 2b. *Phyllosticta helicteres* (BPI 598377, isotype) line drawing. k. Section of ascoma in the leaf (darkened area are fungal cells in arrowed) l. Asci. m. Ascospores without mucilaginous sheath.

Phyllosticta sterculiae (Rehm) Wulandari & K. D. Hyde, comb. nov. = Guignardia sterculiae (Rehm, 1914). MycoBank: MB 528760

Ascomata 110–165 µm diameter, 105–155 µm high, on upper and lower leaf surface, black, globose to subglobose, immersed in plant tissues. Peridium

13–25 µm wide, one strata of textura angularis comprising 1–2 layers of cells with thickened brown angular walls. Pseudopharaphyses not observed. Asci 55–105 × 19–25 µm (= 78 × 21 µm, n = 10), 8–spored, bitunicate, cylindrical to cylindro–clavate, rounded at the apex, where the diameter is 14–16 µm, tapering gradually to a 6–19 × 6–8 µm pedicel attached to the basal peridium. Ascospores 15–21 × 8–13 µm (= 18 × 9 um, n = 20), uniseriate to biseriate, ellipsoidal, oblong, or obovoid when viewed in any plane, hyaline–greenish, 1–celled, coarse–guttulate, smooth–walled, without mucilaginous appendages.

Habitat: On dead leaves of Sterculia foetidae (Sterculiaceae) causing leaf blight.

Known Distribution: Philippines.

Material examined: Philippines, Luzon, Laguna, Los Baños on leaves of Sterculia foetidae, September 1914, C.F Baker, Fungi Malayana No. 31, Philippines (ILL 9762, isotype; F 10723, holotype) teleomorph only present.

Notes: This species differs from Phyllosticta helicteres in having bigger ascospores and longer asci, 15–21 × 8–13 µm; 55–105 × 19–25 µm for P. sterculiae and 14–18 × 5–9 µm; 50–94 × 8–20 µm for P. helicteres. Furthermore, the ascopsores shape is also differ respectively, ellipsoidal, oblong to obovoid for P. sterculiae and ellipsoidal widest 2/5 near the apex for P. helicteres.

Phyllosticta melochiae (Van der Aa, 1973; Van der Aa and Vanev, 2002; Yates, 1918) MycoBank: MB 519218From the original reference:

Pycnidia 45–90 µm diameter, singly, black, globose to elongate, immersed in media. Peridium 15–17 µm in diameter. Conidiogenous cells 7–12 × 5–7 µm, some of the cell sometimes reduced. Conidia 7–12 × 5–7 µm, hyaline–greenish, 1–celled, coarse–guttulate, smooth–walled, obovoidal, ovoidal, slightly globose, with an truncate base when young, broadly rounded apically, surrounded by thick mucilaginous sheath containing a large number of coarse guttulate, with an apical appendage.

Habitat: On living leaves of Melochia umbellata (Sterculiaceae/Buettneriaceae)

causing leaf spot.

Known Distribution: Indonesia

Note: The examination of two Phyllosticta spp. on the same genus of Melochia found that those two species differ. Phyllosticta melochiae from Melochia umbellata possesses smaller conidia than Phyllosticta sp. from Melochia sp. (Van der Aa and Vanev, 2002). This result showed there might be

two different species of Phyllosticta occur on Melochia spp. (Sterculiaceae). This Phyllosticta species cause zona spot on the leaf.

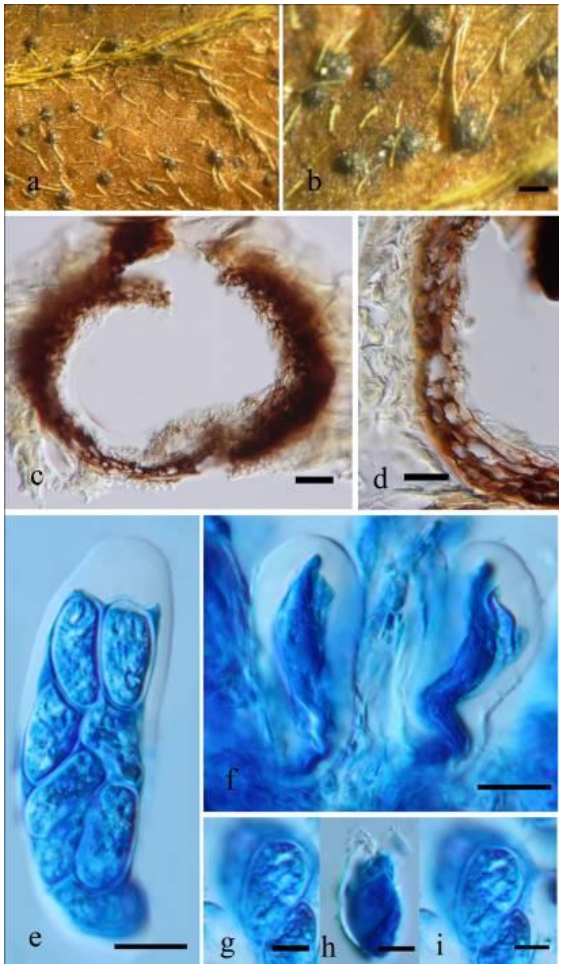

Fig. 3a. *Phyllosticta sterculiae* (ILL 9762, isotype). a–b. Appearance of ascomata on host surface. c–d. Peridium of textura angularis comprising 1–2 layers of cells with thickened angular brown walls. e–f. Asci. g–i. Ascospores obovoid when viewed in any plane. Scale bars. b = 100 µm, c–d = 10 µm, e–f = 18 µm, g–i = 10 µm.

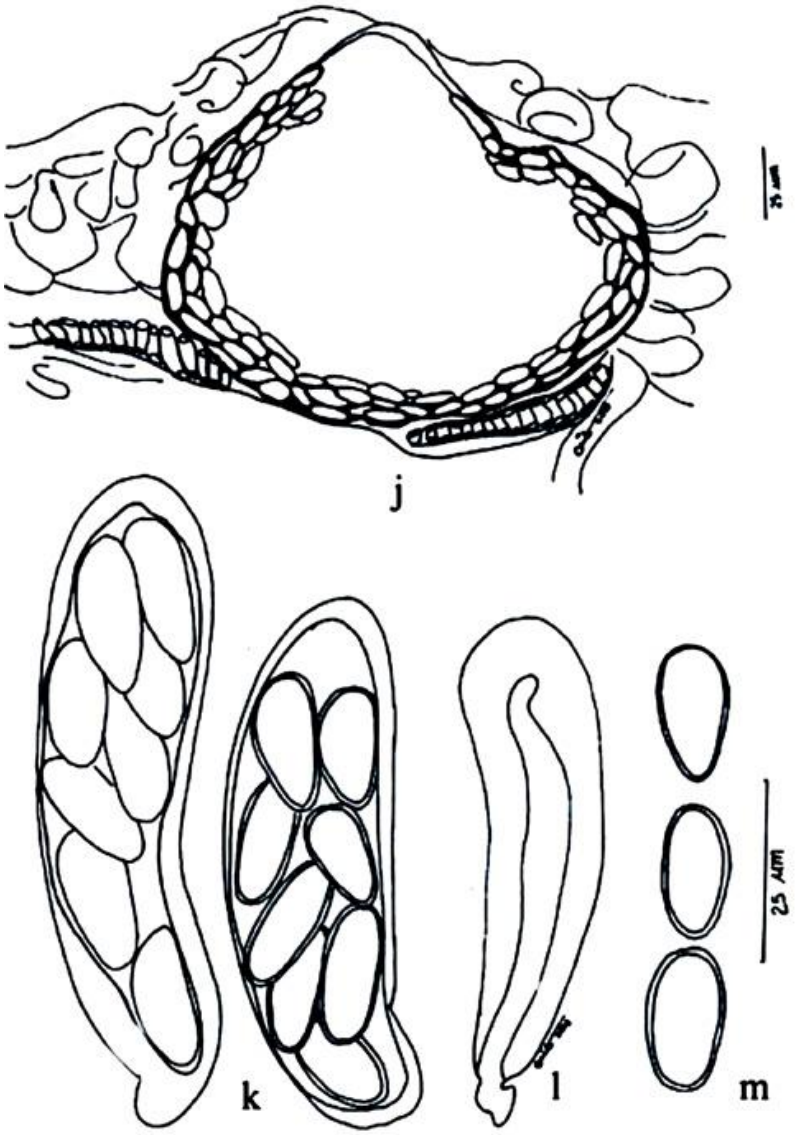

Fig. 3b. *Phyllosticta sterculiae* (ILL 9762, isotype) line drawing. j. Section of ascoma in the leaf (darkened area is fungal cells, arrowed) k. Asci. l. Immature ascus. m. Ascospores.

Table 1. Asci and ascospores shape, size and sheath/appendage of Phyllosticta species on Sterculiaceae

	P. sterculiae	*P. helicteres*	*P. capitalensis*
Asci size (µm)	55–105 × 19–25	50–94 × 8–20	50–91 × 9–14
Pedicel (µm)	6–19 × 6–8	8–26 × 3–6	6–8 × 5–7
Ascospores shape/sheath/ Appendage	Ellipsoidal to oblong, no mucilaginous appendage	Ellipsoidal widest 2/5 near the apex (obtrullate), no mucilaginous appendage	Ellipsoidal swolen in the centre, polar mucilaginous appendages present
Ascospores size (µm)	15–21 × 8–13	14–18 × 5–9	14–19 × 5–7
Reference	Rehm (1914)	Stevens (1917)	Present study

Discussions

The collection of Phyllosticta from Sterculiaceae in Thailand differs from the type of P. sterculiae in ascospore shape (ellipsoidal, swollen in the centre in P. capitalensis vs ellipsoidal to obovoid in P. sterculiae), size of ascospores (14–19 × 5–7 µm in P. capitalensis vs 15–21 × 8–13 µm in P. sterculiae) and mucilaginous appendages (present in P. capitalensis vs absent in P. sterculiae). P. capitalensis also similar to P. helicteres (Stevens, 1917), but they posses shorter asci (50–91 × 9–14 µm in P. capitalensis vs 50–94 × 8–20 µm in P. helicteres) and mucilaginous appendage on ascospores present in P. capitalensis vs absent in P. helicteres. The anamorph in culture of P. capitalensis as introduced in this paper has similar anamorph state to Phyllosticta melochiae from Melochia umbellata (Sterculiaceae) in conidia dimention, only slighly different in pycnidia diameter, the pycnidia in P. melochia is bigger. P. capitalensis leaf blight disease made the tree severe with brownish tip of leaf that finaly falling down. This fungus also recorded distributed at many place of Chiang Rai Province, Northern Thailand (pers. observ.). P. capitalensis in which typically the species has ellipsoidal, swollen in the middle with polar mucilaginous appendages ascospores (Farr and Rossman, 2012). This research findings showed that more than one species of Phyllosticta might be occur in Sterculiaceae. Pathogenicity test, epitypification were needed for a final taxonomic decision.

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