Guignardia/Phyllosticta species on banana

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Abstract – Guignardia musae is the reported causal agent of freckle disease of banana. The epithet has, however, been introduced on three separate occasions and only one name is valid. We therefore investigated this problem. We examined the types of G. musae Racib., G. musae F. Stevens and G. musae Syd. & P. Syd. and also made fresh collections from banana in northern Thailand. Guignardia musae Racib. is the earliest name and takes precedence over the other two names which are homonyms. G. musae F. Stevens is a different species and therefore a new name G. stevensii Wulandari & K.D. Hyde is introduced to accommodate it. The name G. sydowiana Trotter has previously been introduced to accommodate G. musae Syd. & P Syd.; type material is, however, depauperate. Guignardia musicola Wulandari, L. Cai & K.D. Hyde sp. nov. is introduced as a new species from Thailand. The three species from banana are compared and their differences described.

Banana freckle disease / New species / Taxonomy
INTRODUCTION

Freckle disease occurs on several species and varieties in Musaceae (Jones & Alcorn, 1982; Jones, 1984, 1993, 1994a, b, 1999; Pitakpaivan, 1985; Shivas et al., 1996). The causal agent induces freckling on the leaves and fruits, causing a series of black, raised spots with a sand paper-like texture; this is due to the protruding pycnidia and/or ascomata. Leaves turn yellow with time and eventually scensce. The causal agent of banana freckle is reported to be Guignardia musae (Aa, 1973; Aa & Vanev, 2002; CABI, 1990, 2005; Chuang, 1981; Dingley et al., 1981; Hwang et al., 1984; Jones & Alcorn, 1982; Jones, 1984, 1993, 1994a, b, 1999; Meredith, 1968; Pitakpaivan, 1985; Ploetz et al., 2003; Sivanesan, 1984; Shivas et al., 1996; Tsai et al., 1993; Zhou & Xie 1992) and its anamorph is reported to be Phyllosticta musarum (Aa, 1973; Aa & Vanev, 2002; Sivanesan, 1984).

The name Guignardia musae has been introduced on three occasions. It was first introduced by Raciborski (1909) for a fungus on Musa paradisiaca from Indonesia. This was followed by G. musae F. Stevens from Musa sp. in Hawaii (Stevens, 1925) and G. musae Syd. & P. Syd from Musa sp. in the Democratic Republic of Congo (Sydow & Sydow, 1912). The latter two names are homonyms and thus invalid. In the literature and generally on the world-wide web, the cause of freckle is listed as Guignardia-Phyllosticta sp. (http://www.pestnet.org/Summaries/Crops/Plantationcrops/Banana/Fungi/Frecklediseaseofbanana/tabid/1350/Default.aspx; http://www2.dpi.qld.gov.au/horticulture/7926.html and http://www.indexfungorum.org) and the exact name of the species is not often listed.

Banana freckle occurs worldwide (Table 1). It is common in Asia where the causative agent is usually listed as Guignardia musae Racib. (anamorph Phyllosticta musarum (Cook) Aa.). In Thailand freckle has been recorded on various Musa species (Sontirat et al., 1994). Photita et al. (2002) recorded G. musae Racib., G. musae Syd. & P. Syd and G. sydowiana Trotter from Musaceae, while Photita et al. (2001) reported G. cocicola Punith. as a common endophyte from wild banana in northern Thailand. Brown et al. (1998) reported P.usicola F. Stevens nom. inval. as a common endophyte from Musa acuminata in Hong Kong. There is obviously confusion surrounding the species of these genera occurring on banana.

The purpose of this paper is to investigate the Guignardia/Phyllosticta spp. associated with freckle disease on leaves. We re-examined the holotype of each epithet and also made fresh collections from banana in Asia.

MATERIAL AND METHODS

Specimens examined

Holotype specimens were loaned from S (Sweden), KRA (Poland) and BISH (Hawaii), while fresh specimens of freckle disease on banana were collected from Thailand. Herbarium acronyms follow Index Herbariorum (Holmgren & Holmgren, 1998).
Table 1. Countries in which banana freckle disease has been recorded

<table>
<thead>
<tr>
<th>Region/country</th>
<th>Reference</th>
<th>Species name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiji</td>
<td>Dingley et al. (1981), CABI (1990)</td>
<td>G. musae</td>
</tr>
<tr>
<td>New Caledonia</td>
<td>CABI (1990)</td>
<td>G. musae</td>
</tr>
<tr>
<td>Niue</td>
<td>Dingley et al. (1981)</td>
<td>G. musae</td>
</tr>
<tr>
<td>Hawaii (USA)</td>
<td>Steven (1925)</td>
<td>G. musae</td>
</tr>
<tr>
<td>Papua New Guinea</td>
<td>CABI (1990)</td>
<td>G. musae</td>
</tr>
<tr>
<td>Samoa (USA)</td>
<td>CABI (1990)</td>
<td>G. musae</td>
</tr>
<tr>
<td>Tonga</td>
<td>Dingley et al. (1981), CABI (1990)</td>
<td>G. musae</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>CABI (1990)</td>
<td>G. musae</td>
</tr>
<tr>
<td>Bhutan</td>
<td>CABI (1990)</td>
<td>G. musae</td>
</tr>
<tr>
<td>Brunei Darussalam</td>
<td>CABI (1990), Farr &amp; Rossman (2010)</td>
<td>G. musae, P. musarum</td>
</tr>
<tr>
<td>China (Fujian, Guangdong, Guangxi, Yunnan)</td>
<td>Zhou &amp; Xie (1992), Farr &amp; Rossman (2010)</td>
<td>G. musae, P. musarum</td>
</tr>
<tr>
<td>Hong Kong, Taiwan</td>
<td>CABI (1990)</td>
<td>G. musae</td>
</tr>
<tr>
<td>Christmas Island</td>
<td>Shivas &amp; Hilton (1990)</td>
<td>G. musae</td>
</tr>
<tr>
<td>India (Karnataka, Uttar, Pradesh)</td>
<td>CABI (1990), Farr &amp; Rossman (2010)</td>
<td>G. musae, P. musarum</td>
</tr>
<tr>
<td>Indonesia (Java, Irian Jaya)</td>
<td>Raciborski (1908), Shivas et al. (1996)</td>
<td>G. musae</td>
</tr>
<tr>
<td>Malaysia (Peninsular Sabah, Sarawak)</td>
<td>Jones (1993), CABI (1990)</td>
<td>G. musae</td>
</tr>
<tr>
<td>Myanmar</td>
<td>CABI (1990), Farr &amp; Rossman (2010)</td>
<td>G. musae</td>
</tr>
<tr>
<td>Nepal</td>
<td>CABI (1990)</td>
<td>G. musae, P. musarum</td>
</tr>
<tr>
<td>Pakistan</td>
<td>CABI (1990)</td>
<td>G. musae</td>
</tr>
<tr>
<td>Philippines</td>
<td>CABI (1990)</td>
<td>G. musae</td>
</tr>
<tr>
<td>Sri Lanka</td>
<td>CABI (1991)</td>
<td>G. musae</td>
</tr>
<tr>
<td>Thailand</td>
<td>Sontirat &amp; Jones (1994)</td>
<td>G. musae</td>
</tr>
<tr>
<td>Cook Islands</td>
<td>Dingley et al. (1981)</td>
<td>G. musae</td>
</tr>
<tr>
<td>Samoa</td>
<td>Dingley et al. (1981)</td>
<td>G. musae</td>
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<tr>
<td>Solomon Islands</td>
<td>McKenzie &amp; Jackson (1986)</td>
<td>G. musae</td>
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<tr>
<td>Vanuatu</td>
<td>McKenzie (1989)</td>
<td>G. musae</td>
</tr>
<tr>
<td>Palau</td>
<td>McKenzie (1990a)</td>
<td>G. musae</td>
</tr>
<tr>
<td>Federated States of Micronesia</td>
<td>McKenzie (1990b)</td>
<td>G. musae</td>
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</tbody>
</table>
Morphology

Specimens were studied using a Nikon eclipse 80i with EOS 450 D Nikon camera (× 1000 magnification) and an Olympus CX-41 research microscope fitted with a drawing tube and Olympus SMZ 168. Hand sections were made for microscopic examination. Preparations and measurements were made in lactoglycerol (lactic acid: water: glycerol = 1:2:1) for semi-permanent slide and lactophenol cotton blue. The 95% confidence intervals were derived from 30 observations of spores formed on water agar plates, with extremes in parentheses.

RESULTS

Taxonomy

MycoBank: MB 271864
(Figs. 2-9, 17-19)

Ascomata 100-125 µm high, 75-150 µm diam, on upper and lower surface of leaves and on banana fruit skin, globose to subglobose, black, semi-immersed in plant tissues, coriaceous, solitary to clustered, ostiolate, ostioles as black central dots (Fig. 2). Peridium 12.5-20 µm wide, upper part composed of compressed, brownish, thin-walled cells, 1-4 cells thick, lower part hyaline, composed of flattened, dark brown cells, darkest around the ostiole (Figs. 3-5, 17). Pseudoparaphyses not observed. Asci 49-105 × 16-28 µm (̄ = 74 × 21 µm, n = 20), 8-spored, bitunicate, broadly cylindro-clavate, rounded at the apex, where the diameter is 8-21 µm, tapering gradually to a 5-10 µm diam. × 5-10 µm long pedicel attached to the basal peridium, ocular chamber 3-8 µm high (Figs. 6-7, 18). Ascospores 20-25 × 8-13 µm (̄ = 22 × 10 µm, n = 20), uniseriate or occasionally overlapping biseriate, clavate to oblong, not laterally compressed, having the same shape when viewed from above or from the side, hyaline to greenish, 1-celled, guttulate, smooth-walled, lacking a mucilaginous sheath or appendages at the ends (possibly due to nature of old specimens) (Figs. 8-9, 19).

Material examined. INDONESIA, Bogor, on leaves of Musa acuminata, no date, Raciborski, (KRA 063561, holotype of Guignardia musae Racib.), only teleomorph present.

Notes: This is the earliest species of Guignardia or Phyllosticta described from Musa species and therefore takes precedence over G. musae F. Stevens and G. musae Syd. & P. Syd. The ascospores in this species are distinct because of their size (20-25 × 8-13 µm) and shape (clavate to oblong, not laterally compressed having the same shape when viewed from above or the side) (Table 2).

Guignardia stevensii Wulandari & K.D. Hyde, nom. nov.
MycoBank: MB 519089
(Figs. 10-13, 20-22)

Etymology: Named after its collector, F.L. Stevens.
Figs. 1a-d. Freckle disease on *Musa* spp. in Thailand caused by *Guignardia musicola*.
Ascomata 50-125 µm high, 60-95 µm diam, on upper surface of leaves, globose to subglobose, black, semi-immersed in plant tissues, coriaceous, solitary to clustered, ostiolate, ostioles as black central dots. Peridium 20-25 µm wide, composed of compressed, brownish, thin-walled cells, in the upper part 1-4 cells thick, composed of flattened, dark brown cells, darkest around the ostiole, hyaline towards the lower region (Fig. 20). Pseudoparaphyses not observed. Asci 40-59 × 11-15 µm (\(\bar{x} = 50 \times 13 \mu m, n = 20\)), 8-spored, bitunicate, cylindro-clavate, rounded at the apex, where the diameter is 10-12 µm, tapering gradually to a 2-7 µm diam. × 3-7 µm long pedicel attached to the basal peridium, ocular chamber 3-8 µm high (Figs. 10-11, 21). Ascospores 14-17 × 5-6 µm (\(\bar{x} = 15 \times 5 \mu m, n = 20\)), uniseriate or occasionally overlapping biseriate, ellipsoidal, widest 2/5th from the apex (obtrullate) in one plane, inequilaterally ellipsoidal, or ellipsoidal with one side flattened when viewed from the side, hyaline to greenish, 1-celled, guttulate, smooth-walled, with a mucilaginous appendage at each end (Figs. 12-13, 22).

Material examined. HAWAII. Oahu, Hakipu, on leaves of Musa sp., 12 June 1921, F.L. Stevens, No. 565 (BISH 596860, holotype; BISH 499904 isotype of Guignardia musae F. Stevens), teleomorph only present.

Notes: The ascospores of Guignardia musae F. Stevens differ markedly from those of G. musae Racib. being 14-17 × 5-6 µm, obtrullate from above, inequilaterally ellipsoidal, or ellipsoidal and flattened on one when viewed from

Table 2. Synopsis of ascospores and conidia of Guignardia species on Musa spp.

<table>
<thead>
<tr>
<th></th>
<th>G. musae Racib.</th>
<th>G. stevensii</th>
<th>G. musicola</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascus (µm)</td>
<td>49-105 × 16-28, broadly clavate</td>
<td>40-59 × 11-15, cylindro-clavate</td>
<td>133-150 × 19-20, cylindrical to cylindro-clavate</td>
</tr>
<tr>
<td>Ascospores (µm)</td>
<td>20-25 × 8-13, clavate to oblong symmetrical, without appendages</td>
<td>14-17 × 5-6, widest 2/5 near the apex (obtrullate), inequilateral, or ellipsoidal with one side flattened, and with appendages</td>
<td>12-21 × 7-10, obclavate to oblong, symmetrical, with appendages</td>
</tr>
<tr>
<td>Phyllosticta state (µm)</td>
<td>Not present</td>
<td>Not present</td>
<td>Conidia 12-17 × 8-11, with appendage 10-15 long, sheath 2-4 wide</td>
</tr>
</tbody>
</table>
the side (Table 2). Since G. Musae F. Stevens is a homonym of G. Musae Racib.
we provide a new name. Fresh living collections from Hawaii are needed to fully
circumscribe this taxon from *Musa* sp. with DNA sequence comparison.

**Guignardia sydowiana** Trotter, in Saccardo, Syll. Fung. (Abellini) 24(2): 788
(1928)

[name is invalid as homonym of *G. musae* Racib.].

Material examined. Democratic Republic of Congo, on dead leaf of *Musa* sp., Vanderyst, ex
Herb. Sydow (S, 10753, holotype of *Guignardia musae* Syd. & P. Syd).

Notes: The name *Guignardia sydowiana* Trotter was introduced to
replace *G. musae* Syd. & P. Syd., which is a homonym of *G. musae* Racib., and
thus invalid. The type material examined is not in a good condition as ascomata
were dry and depauperate.

**Guignardia musicola** N.F. Wulandari, L. Cai & K.D. Hyde, sp. nov.
Mycobank no.: MB 519088

(Figs. 14-16, 23-31)

Etymology: Named after its host plant, *Musa* sp. and -cola meaning dwelling on.

*Guignardiae musae* Racib. similis, sed ascosporae 12-21 × 7-10 µm.

Leaf spot occupying marginal areas of the leaf and pinna, bleached, the
leaf breaking at the edge to the middle of lamina, ascomata visible to the unaided
eye on surface of the leaves, surface rough indicating protruding ascomata and
pycnidia (Fig. 1). *Ascomata* 100-125 µm diam, 100-125 µm high, on upper surface
of leaves, globose to subglobose, black, semi-immersed in plant tissues,
coriaceous, solitary to clustered, ostiolate, ostioles as black central dots. *Peridium*
22.5-25 µm wide, comprising 2 layers of *textura angularis* cells with thickened
brown walls around ostiole (Fig. 23). *Pseudoparaphyses* not observed. *Asci*
133-150 × 19-20 µm (x = 137 × 20 µm, n = 20), 8-spored, bitunicate, fissitunicate,
cylindrical to cyindo-clavate, rounded at the apex, where the diameter is
12-13 µm, tapering gradually to a 10-20 µm diam. × 5-6 µm long pedicel attached
to the basal peridium, ocular chamber 2-5 µm high (Figs. 14, 24). *Ascosperes*
12-21 × 7-10 µm (x = 19 × 9 µm, n = 20), uniseriate or occasionally overlapping
biseriate, ellipsoidal to clavate, not laterally compressed, having the same shape
when viewed from above or the side, hyaline to greenish, 1-celled, guttulate,
smooth-walled, with a mucilaginous appendage at each end, not (Figs. 15-16, 25).

*Pycnidia* 95-125 µm diam, 75-125 µm high, epiphyllous, black, globose to pyriform,
immersed in plant tissues, coriaceous, solitary to clustered, ostiolate, ostioles as white
dots in the centre. *Peridium* 22-25 µm wide, one stratum of *textura angularis*
comprising 2 layers of cells with thickened brown walls around ostiole (Fig. 26).
*Conidiogenous cells* 10-12 × 8-9 µm (x = 11 × 8 µm, n = 5), holoblastic,
determinate, discrete, sometimes rarely integrated, hyaline, cylindrical to
doliiform cells lining the pycnidial locule (Fig. 27). *Conidia* 12-17 × 8-11 µm
(x = 14 × 10 µm, n = 20), hyaline to greenish, 1-celled, guttulate, smooth-walled,
globose, ellipsoidal, clavate or obclavate, with an obtuse apex, sometimes truncate
at the base, surrounded by 2-4 µm thick mucilaginous sheath which persists at
maturity and in some specimens with a single, hyaline, curved or straight,
10-15 µm long appendage (Fig. 28). *Spermatogonia* 95-125 µm in diameter,
75-125 µm high, epiphyllous, black, globose to subglobose, immersed in plant
tissues, coriaceous, solitary to clustered, ostiolate, ostioles as white dots in the
centre, similar to pycnidia. *Peridium* 22-25 µm wide, one stratum of *textura*
angularis comprising 2 layers of cells with thickened brown walls around ostiole (Fig. 29). Spermatiogenous cells 7-10 × 1 µm (̄x = 9.8 × 1 µm, n = 20), holoblastic, filamentous to cylindrical, simple or branched as distinct phialides with a very characteristic and easily discernible apical structure (Fig. 30). Spermatia 5-8 × 1-2 µm (̄x = 7 × 1 µm, n = 20), cylindrical to dumb-bell shaped, guttulate, straight or slightly curved forming singly in basipetal succession and separating from the spermatiogenous cells by a septum (Fig. 31).

Material examined. THAILAND, Chiang Mai Province, Chiang Mai, Tung Jaow Village, on leaves of Musa acuminata, 18 July 2007, N.F. Wulandari, NFW 154 (MFLU 10 0235, holotype) teleomorph and anamorph present; extype cultures CBS 123405; ibid., Srilanna, on leaves of Musa paradisiaca, 12 July 2007, N.F. Wulandari, NFW 140 (MFLU 10 0233) teleomorph and anamorph present; Bahn Pa Deng, T. Pa Pae, Mae Taeng, Mushroom Research Centre, on leaves of M. paradisiaca, 24 August 2006, N.F. Wulandari, NFW 084 (MFLU 10 0222), teleomorph only present; ibid., 3 June 2007, N.F. Wulandari NFW 128 (MFLU 10 0231), teleomorph only present; ibid., 20 July 2007, N.F. Wulandari, NFW 161 (MFLU 10 0236), teleomorph only present; ibid., 13 August 2007, N.F. Wulandari, NFW 176 (MFLU 10 0237), teleomorph and anamorph present; ibid., 21 August 2007, N.F. Wulandari, NFW 182 (MFLU 10 0238), teleomorph and anamorph present; ibid., 12 September 2007, N.F. Wulandari, NFW 219 (MFLU 10 0244), teleomorph only present. Tumbon, Chiangdoaw, on leaves of M. paradisiaca, 5 September 2007, N.F. Wulandari, NFW 184 (MFLU 10 0239), teleomorph, anamorph and spermatial stage present; ibid., 5 September 2007, N.F. Wulandari, NFW 185 (MFLU 10 0240), teleomorph only present; ibid., 5 September 2007, N.F. Wulandari, NFW 188 (MFLU 10 0242), teleomorph only present. Bahn Pha Deng, Mae Lod, Royal Project, on leaves of M. paradisiaca, 11 September 2007, N.F. Wulandari, NFW 210 (MFLU 10 0243), teleomorph only present; ibid., Chiang Mai University on leaves of M. paradisiaca, 16 June 2006, N.F. Wulandari, NFW 114 (MFLU 10 0225), teleomorph only present; ibid., 19 June 2007, N.F. Wulandari, NFW 117 (MFLU 10 0228), teleomorph only present; ibid., 19 June 2006, N.F. Wulandari, NFW 118 (MFLU 10 0229), teleomorph only present; Chiang Mai University Shop garden, on leaves of M. paradisiaca, 15 September 2007, W. Tajeena & N.F. Wulandari, NFW 221 (MFLU 10 0245), teleomorph and anamorph present; Medicinal Plant Garden on leaves of Musa paradisiaca, 15 September 2007, N.F. Wulandari, NFW 230 (MFLU 10 0246), teleomorph only present. Bahn Pha Deng, Pathummikaram Temple, on leaves of M. paradisiaca, 1 July 2007, N.F. Wulandari, NFW 123 (MFLU 10 0230), teleomorph only present; Bahn Pha Deng Mushroom Research Centre, on leaves of M. paradisiaca, 24 August 2006, N.F. Wulandari, NFW 079 (MFLU 10 0220), teleomorph only present; ibid., 22 August 2006, N.F. Wulandari NFW 080 (MFLU 10 0221), teleomorph only present; ibid., 18 June 2007, N.F. Wulandari, NFW 115 (MFLU 10 0226), teleomorph only present; ibid., 18 June 2007, N.F. Wulandari, NFW 116 (MFLU 10 0227), teleomorph only present; ibid., N.F. Wulandari, NFW 131 (MFLU 10 0232), teleomorph only present; ibid., 17 July 2007, N.F. Wulandari NFW 151 (MFLU 10 0234), teleomorph only present. Chiang Rai, Nam Tok Huey Mesak Forest Park, on leaves of M. paradisiaca, 6 February 2010, N.F. Wulandari & P. Syshophanthong, NFW 306 (MFLU 10 0281), teleomorph only present.

Notes: Guignardia musicola is distinct from G. musae Racib. as ascospores in G. musicola are smaller 12-21 × 7-10 µm (̄x = 19 × 9 µm), compared with those of Guignardia musae Racib. (20-25 × 8-13 µm, ̄x = 22 × 10 µm) (Table 2).

**DISCUSSION**

This study redescribes G. musae Racib. and shows it to be a morphologically distinct species. Fresh collections are needed from Indonesia, however, to epitypify this species for molecular study. Guignardia musae
F. Stevens and *G. musae* Syd. & Syd. are homonyms of *G. musae* Racib. and thus invalid. *Guignardia musae* F. Stevens is, however, a distinct species and a new name *G. stephensii* is introduced for this taxon. One new species of *Guignardia* isolated from leaves of banana with freckle symptoms in Thailand (Fig. 1) is also introduced. The study shows that more than one species is responsible for freckle symptoms of banana and a worldwide study is justified. Several other species, e.g. *Macrophoma musae* (Sacc.) Berl. & Voglino, *Phoma musae* Sacc., *Phoma musae* C.W. Carp., *Phyllosticta musarum* (Cooke) Aa, *Sphaeropsis musarum* Cooke and *Phyllostictina musarum* (Cooke) Petr. have at one time or another been considered to be synonyms of *G. musae* Racib. (Aa, 1973; Carpenter, 1919; Petrak and Ciferri, 1931; Raciborski, 1909; Sivanesan, 1984). The synonymies, however, were based on morphological data and the taxa need recollecting and subjecting to molecular analysis. Further collections and sequence analysis are needed from different continents and various musaceous hosts to establish which species induce freckle disease of banana.

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