Guignardia morindae frog eye–leaf spotting disease of *Morinda citrifolia* (Rubiaceae)

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Frog eye disease of leaves of *Morinda citrifolia* (Rubiaceae) was studied in Indonesia and Thailand. The causative species, *Guignardia morindae*, differs from species of *Guignardia* on other hosts by the distinct shape of its ascospores. The holotype for this taxon is missing, and therefore a neotype from Indonesia is designated. The species is illustrated from the neotype. New collections were also made from Thailand.

Key words – Disease record – Indonesia – New record – *Phyllosticta* – Taxonomy – Thailand

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Introduction

We are studying the important phytopathogen genus *Guignardia* and its anamorph *Phyllosticta* (Arx & Müller 1954, Reusser 1964, Aa 1973, Sivanesan 1984, Crous et al. 1993, Hyde 1995, Okane et al. 2001, Aa & Vanev 2002, Kobayashi 2003, Somritiphol & Hyde 2004, Motohashi et al. 2008a, b, Wulandari et al. 2009, Motohashi et al. 2010). A leaf spot caused by a species of *Guignardia* is very common on the plant *Morinda citrifolia* L. Frog eye leaf spot or shot-hole disease is caused by *G. morindae* (Koord.) Aa. This species was introduced as *Physalospora morindae* Koorders (1907) from Kedu Province, Central Java, Indonesia. This taxon was transferred as *Puiggarina morindae* (Koord.) Speg. by Spegazzini (1919) and later as *G. morindae* (Aa 1973). Petrak & Sydow (1927) introduced the anamorph species *Phyllostictina morindae* Petr. & Syd from *Morinda citrifolia*, while Aa (1973) transferred it as *Phyllosticta morindae* (Petr. & Syd.) Aa and linked it with the teleomorph *G. morindae*. Farr & Rossman (2010) list *G. morindae* from Australia, India, Indonesia, Japan and Samoa, but it is very common in Pacific island countries of American Samoa, Cook Islands, Federated States of Micronesia, Fiji, French Polynesia, Kiribati, Niue, Palau, Samoa, Tonga, Tuvalu, Vanuatu, Wallis and Futuna (Dingley et al. 1981, McKenzie 1989, 1990a,b, 1996, Shivas 1996).

Morinda citrifolia (Rubiaceae) is a highly prized medicinal plant. Noni juice extracted from the fermented fruit is marketed worldwide and has an estimated value of US$2 billion annually. Most of the raw product comes from Polynesia. Crude extracts of *M. citrifolia* and *M. elliptica* L. have been shown to have antiviral activity against foot and mouth disease virus (Chungsamarnyart et al. 2007).

Genotoxic and antigenotoxic effects of noni fruit juice produced in Thailand had genotoxic and antigenotoxi effects on human lymphocytes in the chromosome aberration assay and sister chromatid exchange (SCE) assay *in vitro*. (Ratanavalachai et al. 2008, Thani et al. 2010). Noni appears to restore the normal menstrual cycle problems and alleviate menstrual symptoms in mice (Chearskul et al. 2004, Thani et al. 2010) and inhibits murine tumor growth with a definite curative potential in mice (Furusawa 2002). Mathivanan et al. (2005) reviewed current research on *Morinda citrifolia* while Rethinam & Sivaraman (2007) discussed research developments in India and elsewhere and reviewed the literature. The objective of the research is two fold. This paper provides an updated description of *G. Morindae* and, since the type specimen is lost a neotype is designated in order to stabilize the application of this species name.

Results

Collections of *Guignardia morindae* from three different locations are compared. A description and illustration from the neotype specimen is made and a neotype is designated here.

Taxonomy

Guignardia morindae (Koord.) Aa, *Stud. Mycol*. 5: 69 (1973) Figs 1–33
MycoBank 314756

≡ *Physalospora morindae* Koord., *Verh. K. Akad. Vet. Amsterdam* 13(4): 190 (1907).

≡ *Puiggarina morindae* (Koord.) Speg., *Boletín de la Academia Nacional de Ciencias de Córdoba* 23: 486 (1919).

Anamorph *Phyllosticta morindae* (Petr. & Syd.) Aa, *Stud. Mycol*. 5: 69 (1973).

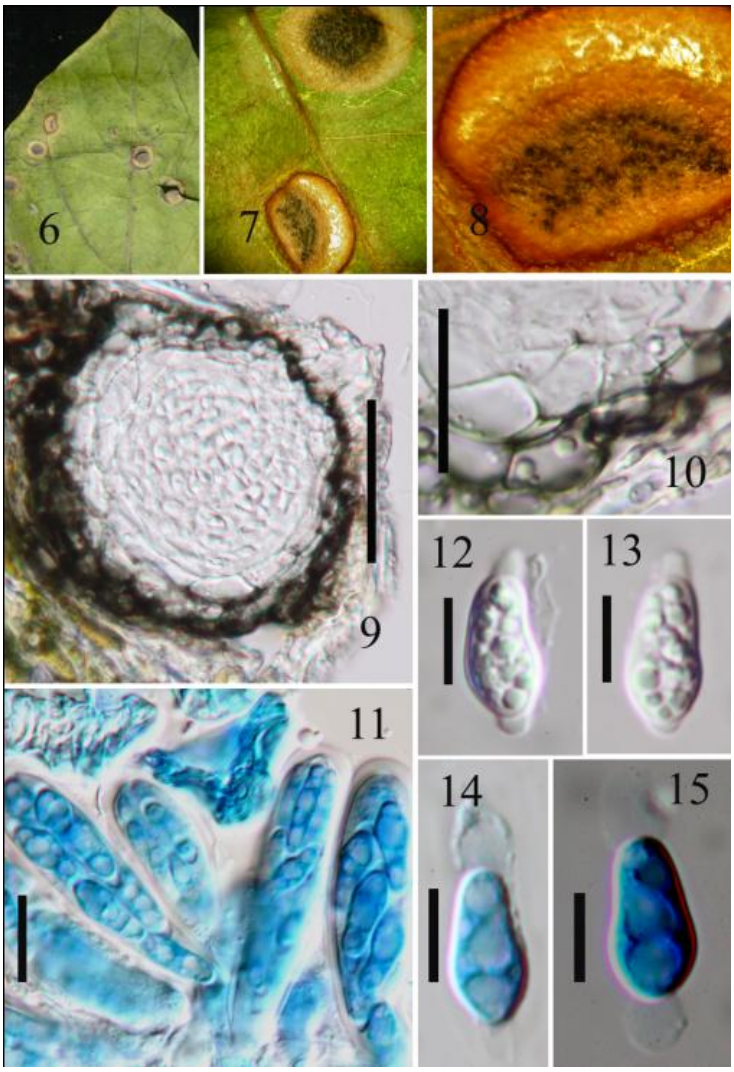
≡ *Phyllostictina morindae* Petr. & Syd., *Feddes Repert*., Beih. 42: 200 (1927).

Causing frog eye or shot-hole leaf spots, 0.3–0.8 × 0.5–1.2 cm, which are rounded to irregular with red to dark brown borders; the area where the fungi sporulates is transparent and often falls from the leaf (Figs 1, 2, 3). Ascomata 60–120 µm diameter, 90–100 µm high, on the upper and lower surfaces of the leaves, black, globose to subglobose, immersed in plant tissues, coriaceous, clustered, ostiolate,

Figs 1–5 – *Guignardia* frog eye disease on leaves. **1–3** Symptoms on leaves. **4–5** Appearance of ascoma on the host surface.

ostioles as black dots in the centre (Figs 4, 5, 6, 7, 8). Peridium 10–15 µm wide, composed of two to three layers of cells, of *textura angularis*, pigmented outwardly and around ostiole and paler inside (Figs 9, 10, 16). Pseudoparaphyses hypha-like, 2–3 µm in diam. Asci 39–65 × 11–14 µm ($\bar{x}$ = 50 × 12 µm, n = 20), 8-spored, bitunicate, fissitunicate, broadly cylindro-clavate, rounded at the apex, tapering gradually to a pedicel attached to the basal peridium (Figs 11, 17, 18). Ascospores 7–12 × 4–6 µm ($\bar{x}$ = 10 × 5 µm, n = 20) biseriate, obovoid, obtrullate, clavate, diamond shaped when viewed from above and inequilaterally ellipsoidal or ellipsoidal with one side flattened dorsally when viewed from side, hyaline or greenish, 1-celled, coarse-guttulate, smooth-walled, with rounded elongate ends and bipolar mucilaginous appendages (Figs 12–15, 19).

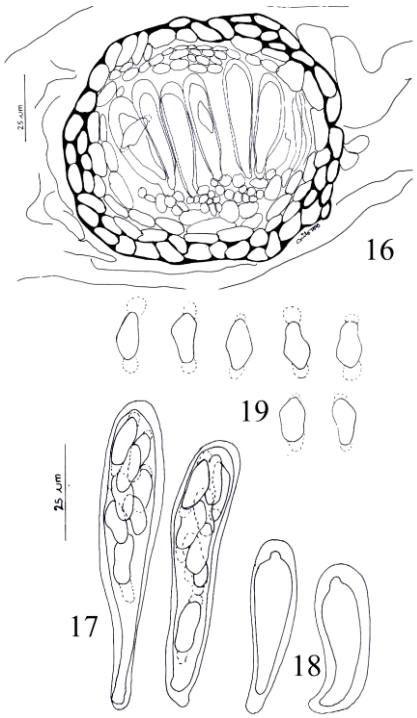
Pycnidia 85–95 µm diameter, 64–85 µm high, on the upper and lower leaf surface, black, globose to subglobose, immersed in plant tissues, coriaceous, solitary to clustered, ostiolate,



Figs 6–15 – *Guignardia morindae* (neotype). **6** Leaf spots. **7, 8** Appearance of ascomata on the host surface. **9** Section of ascoma. **10** Peridium comprising one strata of textura angularis comprising 2–3 layers of cells with an apex of thickened brown walls. **11** Asci. **12, 13, 14, 15** Ascospores with bipolar mucilaginous appendages with rounded elongate ends – Bars 12 = 50 µm, 13 = 20 µm, 14 = 10 µm, 15–18 = 5 µm.

ostioles as black dots in the centre, often growing together with ascomata. Peridium 11–15 µm wide, composed of two to three layers of cells, *textura angularis* and pigmented outwardly and around ostiole and paler inside (Figs 20, 21, 28). Conidiogenous cells 7–12 × 2–3 µm ($\bar{x}$ = 10 × 2 µm, n = 20), holoblastic, determinate, discrete, rarely integrated, hyaline cylindrical to doliiform, forming from cells lining the pycnidial locule (Figs 20, 29). Conidia 8–10 × 5–7 µm ($\bar{x}$ = 9 × 6 µm, n = 20), hyaline-greenish, 1-celled, coarse guttulate, smooth-walled, globose, ellipsoidal, clavate or obclavate, with an obtuse apex, sometimes truncate on the base, surrounded by 0.5–1 µm ($\bar{x}$ = 1 µm, n = 20) thick mucilaginous sheath which persists at maturity with a 2–7 µm ($\bar{x}$ = 4 µm, n = 20) single, hyaline, curved or straight appendage (Figs 23, 30).

Spermogonia 44–45 µm diameter, 42–47 µm high intermixed with pycnidia. Peridium 5–9 µm wide, composed of two to three layers of cells, *textura angularis* and pigmented outwardly and around ostiole and paler inside (Figs 24, 25, 31). Spermatiogenous cells 11–22 × 2–3 µm ($\bar{x}$ = 16 × 2 µm, n = 20), holoblastic, filamentous to cylindrical, simple or branched and easily discernible apical structure (Figs 26, 32). Spermatia 5–9 × 1–2 µm ($\bar{x}$ = 6 × 2 µm, n = 20) holoblastic, cylindrical to dumb-bel shaped, guttulate, straight or slightly curved forming singly in basipetal succession and

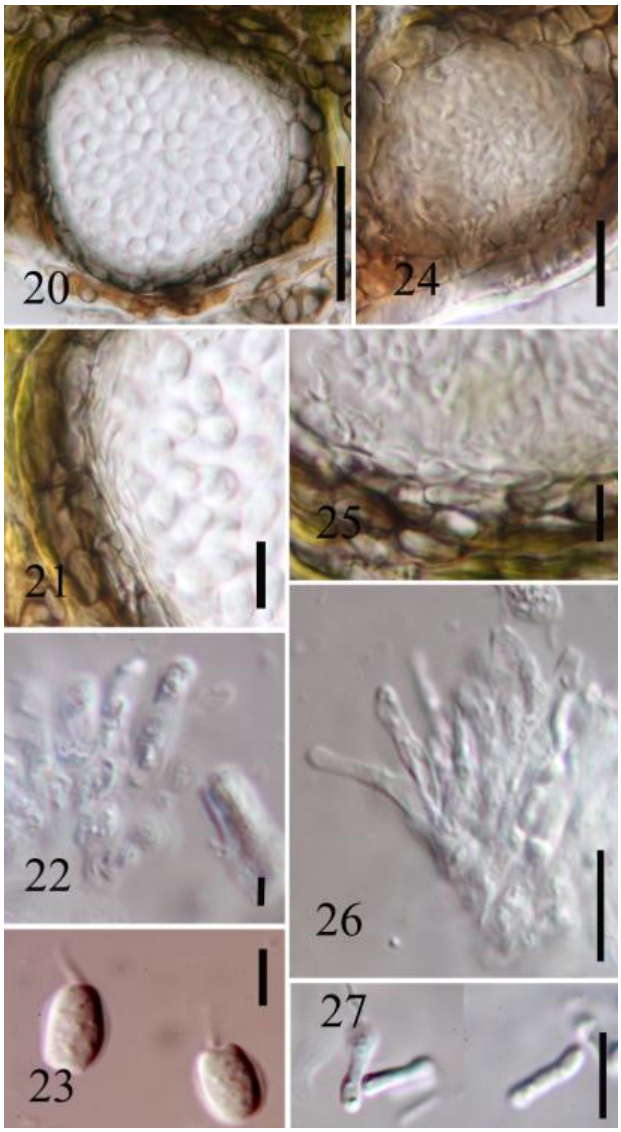


Figs 16–19 – *Guignardia morindae* (neotype) line drawing. **16** Section of ascoma. **17** Mature Asci. **18** Immature Asci. **19** Ascospores.

separating from the spermatiogenous cells by a septum (Figs 27, 33).

Material examined – INDONESIA, West Java Province, Bogor, Bogor Botanical Garden, on living leaf of *Morinda citrifolia*, 23 September 2010, N.F. Wulandari, NFW 361 (BO 22648 designated as neotype), spermatial stage, anamorph and teleomorph present; *ibid*., 23 September 2010, N.F. Wulandari, NFW 363 (BO 22650), teleomorph only present; *ibid*., 11 May 2006, N.F. Wulandari, NFW 169 (BO 22652), teleomorph and anamorph present; *ibid*., 27 June 2006, N.F. Wulandari, NFW 168 (BO 22651), anamorph only present; Central Java Province, Ngentak, Ngentak, Kedu, on living leaf of *Morinda citrifolia* 18 September 2010, N.F. Wulandari, NFW 362 (BO 22649), anamorph only present. THAILAND, Chiang Rai, Phahonyothin Road, on leaves of *Morinda citrifolia*, 20 January 2010, N.F. Wulandari, NFW 296 (MFLU 10 0453), teleomorph only present; *ibid*., 05 March 2010, N.F. Wulandari, NFW 313 (MFLU 10 0466), teleomorph only present.

Known distribution – American Samoa, Australia, Cook Islands, Federated States of Micronesia, Fiji, French Polynesia, Wallis and Futuna, India, Indonesia (Bogor, Kedu), Japan,

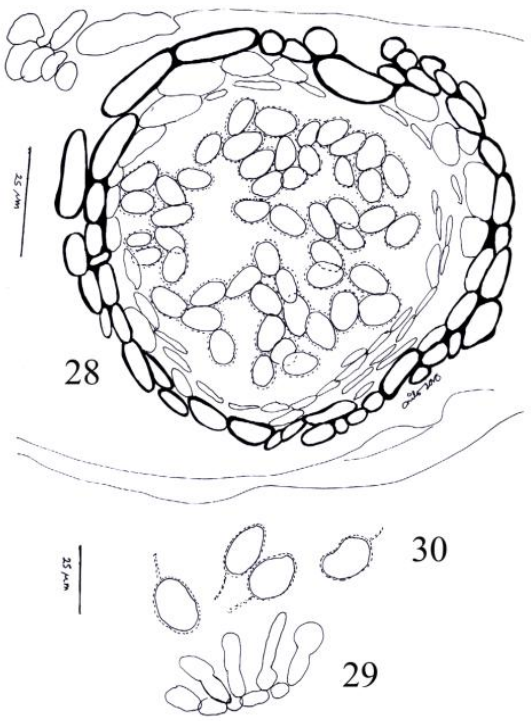


Figs 20–27 – *Phyllosticta* state of *G. morindae* (neotype). **20, 21**, **24, 25** Peridium comprising one strata of *textura angularis* comprising 2–3 layers of cells with an apex of thickened brown walls. **22** Conidiogenous cells. **23** Conidia. **24** Spermogonia. **26** Spermatiogenous cells **27** Spermatia – Bars 20 = 45 µm, 21 = 15 µm, 22 = 3 µm, 23 = 7 µm, 24 = 20 µm, 25, 27 = 9 µm, 26 = 22 µm.

Kiribati,Niue, Palau, Samao, Thailand (Chiang Mai, Chiang Rai), Tonga, Tuvalu, Vanuatu and Wallis.

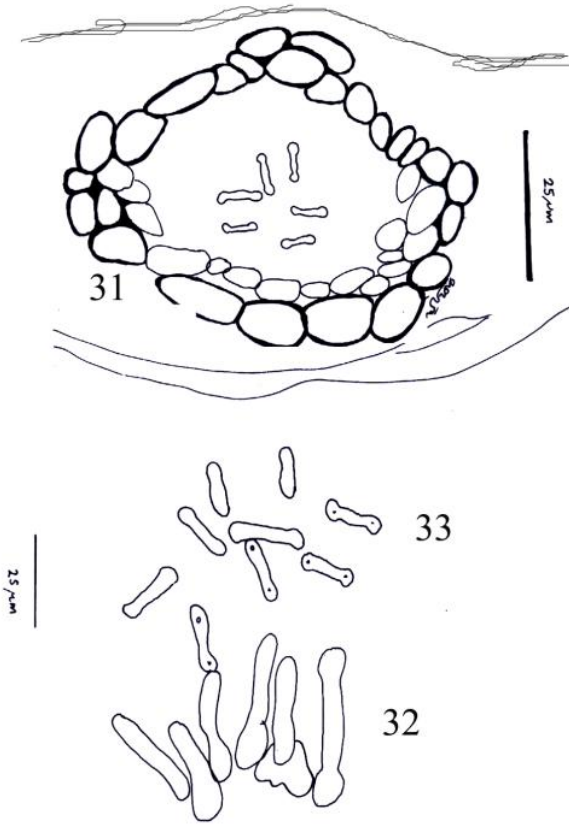
Discussion

The holotype of *Guignardia morindae* is not in BO; Koorders never deposited his specimens in the herbarium (Mien A. Rifai, pers. comm.) and there is no available ex-type culture. Since there is no type of *G. morindae* the species was recollected from Bogor at the



Figs 28–30 – *Phyllosticta* state of *G. morindae* (neotype) line drawing. **28** Section of pynidium. **29** Conidiogenous cells. **30** Conidia.

original place of collection. A neotype is a specimen or illustration selected to serve as nomenclatural type if no original material is extant, or is missing (Art 9.6) (McNeill et al. 2006). The need of neotypification is important in order to stabilize the application of the species name (McNeill et al. 2006). *Guignardia morindae* is recorded for the first time in Thailand. Molecular work is needed to discern the distinctness of this species. However, despite repeated attemps we could not isolate the fungus.



Figs 31–33 – *Leptodothiorella* state of *G. morindae* (neotype) line drawing. **31** Section of spermogonium. **32** Spermatiogenous cells. **33** Spermatia.

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