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

Wulandari NF\(^1\), To-Anun C\(^1\) and Hyde KD\(^3\)

\(^1\)Department of Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai, 51200, Thailand.
\(^2\)Microbiology Division, Research Centre for Biology (RCB), Indonesian Institute of Sciences (LIPI), Cibinong Science Centre, Jl. Raya Jakarta Bogor KM. 46, Cibinong 16911, Indonesia.
\(^3\)School of Mae Fah Luang University, 333 M. 1. T. Tasud Muang District, Chiang Rai 57100, Thailand.


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

**Article Information**
Received 1 November 2010
Accepted 25 November 2010
Published online 12 December 2010

*Corresponding author: Nilam Wulandari – e-mail – nilamwulandari@gmail.com

**Introduction**


*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 antigenotoxic 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


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, 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 (\(\bar{x} = 50 \times 12\) 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 (\(\bar{x} = 10 \times 5\) µm, n = 20) biseriate, obovoid, ob-trullate, clavate, diamond shaped when viewed from above and inequilaterally ellipsoidal or ellipsoidial 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 (\(\bar{x} = 10 \times 2 \mu m, n = 20\)), 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 (\(\bar{x} = 9 \times 6 \mu m, n = 20\)), 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 (\(\bar{x} = 1 \mu m, n = 20\)) thick mucilaginous sheath which persists at maturity with a 2–7 µm (\(\bar{x} = 4 \mu m, n = 20\)) single, hyaline, curved or straight appendage (Figs 23, 30).

Spermodonia 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 (\(\bar{x} = 16 \times 2 \mu m, n = 20\)), holoblastic, filamentous to cylindrical, simple or branched and easily discernible apical structure (Figs 26, 32). Spermatia 5–9 × 1–2 µm (\(\bar{x} = 6 \times 2 \mu m, n = 20\)) holoblastic, cylindrical to dumb-bell 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 Ascii. 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, Kiribati, Niue, Palau, Samoa, 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 attempts we could not isolate the fungus.

Acknowledgements

Nilam Wulandari is grateful to the Graduate School, Chiang Mai University, Thailand for financial support and the School of Science, Mae Fah Luang University for laboratory facilities. Josse Rizal is thanked for financial support for the collecting trip in Bogor, West Java and Kedu, Central Java, Indonesia. Uben Syarifuddin, Rismita Sari and Ira wati, Bogor Botanical Garden, Bogor, Indonesia and Sri Ngatini, Ngentak, Kedu, Central Java, Indonesia are thanked for Morinda citrifolia leaf samples. Herbarium Bogoriense (BO), Indonesia through Eko Baroto Walujo, Kartini Kramadibrata are thanked for specimens certificate permit No. 1184/IPH.1.02/K.S.01.04/2010. Mien A. Rifai and Dewi Susan are thanked for valuable information concerning the holotype of Physalospora morindae in BO. Mae Fah Luang University is thanked for the award of a grant no 53101020017 (17/2553) to study Phyllosticta in northern Thailand. BRT, Thailand is acknowledged for the award of a grant (BRT No R251181) to study Dothideomycetes in northern Thailand. The Mushroom Research Foundation is thanked for a scholarship to carry our studies towards a PhD. Eric McKenzie is thanked for improving the draft manuscript.

References

Aa HA Van der, Vanov S. 2002 – A Revision of the species described in Phyllosticta.
Centraalbureau voor Schimmelcultures, Utrech, The Netherlands. 1–49.
Ratanavalachai T, Thitiorul S, Nandhasri P. 2008 – *In vitro* genotoxic and antigenotoxic studies of Thai noni fruit juice by chromosomal aberration and sister chromatid exchange assays in