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Phyllosticta capitalensis sporulating on ginkgo leaves in Taiwan

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Abstract

Formation of pycnidia by *Phyllosticta capitalensis* on living and dead leaves of *Ginkgo biloba* is recorded for the first time, based on morphology and the internal transcribed spacer sequence (ITS) of the ribosomal RNA gene of samples from Taiwan. Although the fungus is recorded as a widespread endophyte and weak pathogen from numerous plants, on ginkgo it has hitherto only been known as an endophyte in Japan. *Phyllosticta capitalensis* is the single verified *Phyllosticta* species on this host. Another fungus from ginkgo, the invalidly published *Pseudocercospora ginkgoana* is validated here.

Key words – Botryosphaeriales – *Diaporthe* – *Guignardia* – *Phoma* – plant pathogen

Introduction

Leaves of ginkgo (*Ginkgo biloba* L.) can be colonized by fungi from a wide range of systematic groups, such as Chytridiomycota (*Synchytrium macrosporum* Karling; Karling 1964), Ascomycota (several species; Kirschner & Okuda 2013), and Basidiomycota (*Bartheletia paradoxa* G. Arnaud ex Scheuer et al.; Scheuer et al. 2008, Kirschner & Okuda 2013, Koukol & Lotz-Winter 2016). Although *Bartheletia paradoxa* was claimed as a "living fossil" (Scheuer et al. 2008), the hitherto single known fungus on fossil ginkgo leaves is a microthyriaceous ascomycete without known extant counterpart (Sun et al. 2015). In our brief account of fungi on ginkgo leaves (Kirschner & Okuda 2013), we did not go into details about the records of *Phyllosticta* and overlooked a record of *Ph. capitalensis* from Japan (Motohashi et al. 2009). A recent finding of a *Phyllosticta* specimen on living and dead ginkgo leaves in Taiwan prompted complementing our previous notes about fungi on ginkgo. In addition, our invalid publication of *Pseudocercospora ginkgoana* is validated.

Materials & Methods

Attached and freshly fallen leaves of *Ginkgo biloba* with leaf spots were collected on the campus of National Central University in Taoyuan City, Taiwan, and investigated immediately with a dissecting microscope. Conidial masses visible on the top of pycnidia were picked up with a flamed acupuncture needle and transferred to corn meal agar (CMA, Fluka) with 0.2% chloramphenicol. Dried specimens were deposited at the fungal collection of the Museum of Natural Science, Taichung, Taiwan (TNM). For microscopic investigation, fresh specimens were mounted in 10% aqueous KOH and stained with 1% aqueous phloxine B. Measurements of conidia

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were made with 30 replicates and given as mean value ± standard deviation and extreme values in brackets. Other values are presented as extreme values. DNA was isolated from culture material derived from specimen R. Kirschner 4351 with the Genomic DNA Spin Kit (Plant; Bioman Scientific Co., Taiwan) according to the manufacturer's instructions. Amplification of the internal transcribed spacers (ITS) of the ribosomal RNA gene, sequencing of the amplicons, and editing of the sequences were done as in Kirschner & Okuda (2013). Sequence-based identification was made by searching with the BLAST algorithm in GenBank. The ITS sequence was deposited in GenBank under MG550984.

Results

Figs 1–4 – *Phyllosticta capitalensis* on *Ginkgo biloba*. 1, 2, 4 R. Kirschner 4351. 3 R. Kirschner 4513. 1 Infected leaf attached to a ginkgo tree. 2 Black pycnidia on the brown and green area (arrow) of the same leaf. 3 Conidiophores. 4 Conidia. – Bar 3, 4 = 10 µm.

Phyllosticta capitalensis Henn. (for synonyms see Wulandari et al. 2013) Figs 1–4

Pycnidia on green as well as brown discolored parts of the same leaf, particularly numerous on fallen brown leaves, amphigenous, developing supepidermally, rupturing epidermis during maturation, globose, black when seen with low magnification, brown in transmitted light in the light microscope, 70–120 µm high, 85–125 µm wide, wall 7–10 µm thick, composed of 2–3 layers of pale to dark brown textura epidermoidea, cells 6–20 × 3–13 µm, ostiole apical, not raised, circular, 10–15 µm diam. Internal hyphae intercellular, pale to dark brown, smooth, 2–6 µm wide. Conidiogenous cells sessile or formed on 1–2 subtending, swollen cells, lageniform or cylindrical, hyaline, 3–5 × 2–3 µm. Conidia ellipsoidal to obpyriform, hyaline, 1-celled, smooth-walled, 9.5–12(–13) × 6–7 µm (n = 30), surrounded by a 0.5–1 µm thick mucilaginous sheath, bearing a single

2–7 µm long apical appendage. Immature ascomata (sexual stage of the same species?) occurring on the same leaves.

When comparing ITS sequences exceeding 640 b in GenBank with BLAST, our sequence (657 b, GenBank MG550984) was 99–100% identical to those of *Ph. capitalensis/Guignardia mangiferae*, with 0–2 deviating positions. The single sequence (EU167584) not labelled under this name, but as *Ph. elongata* Weid. was corrected to *Ph. capitalensis* in Wikee et al. (2013). Searches excluding *Ph. capitalensis* as search term only revealed the same sequence of "*Ph. elongata*", while other sequences published with species names were shorter than 640 b. Similarities with *Phyllosticta* species more commonly recorded from gymnosperms, namely *Ph. abieticola* Wikee & Crous, *Ph. abietis* Bissett & M.E. Palm, *Ph. podocarpi* Crous, *Ph. podocarpicola* Wikee, Crous, K.D. Hyde & McKenzie, *Ph. pseudotsugae* L.E. Petrini, Petrini, Leuchtm. & G.C. Carroll, and *Ph. spinarum* (Died.) Nag Raj & M. Morelet, were considerably lower (< 92 %).

Known distribution – On many diverse hosts in tropical and subtropical regions (Wikee et al. 2013, Wulandari et al. 2013).

Material examined – On living attached and dead fallen leaves of *Ginkgo biloba* L., Taiwan, Taoyuan City, Zhongli District, National Central University, N 24 58.050 E 121 11.605, 130 m MSL, 28 Oct 2016, R. Kirschner 4351 (TNM); ibid., 20 Nov 2017, R. Kirschner 4513 (TNM).

Discussion

Three *Phyllosticta* species have been recorded in the literature as endophytic(?) isolates from living leaf of *G. biloba* in Japan (Motohashi et al. 2009, not mentioned in Kirschner & Okuda 2013), and from direct observation on leaves (Aa & Vanev 2002). The two species with pycnidia on leaves, however, do not belong to *Phyllosticta* (Aa & Vanev 2002):

Phyllosticta ginkgo Brunaud
On fallen leaves of *G. biloba*, France, conidia 3–8.5 × 2 µm (Berlese, A.N.; Voglino, P., Sylloge Fungorum. Additamenta ad Volumina I–IV: i–iv, 1–484: 434, 1886)
"Small spored *Phoma*" (Aa & Vanev 2002)

Phyllosticta salisburyae ('*salisburiae*') Tassi
On withering attached leaves of *G. biloba*, Italy, conidia 6–7 × 3 µm (Saccardo, P.A., Sylloge Fungorum XVI: 847, 1902)
"Probably a *Phoma* or *Phomopsis*" (Aa & Vanev 2002)

Since according to Aa & Vanev (2002), these two species described under *Phyllosticta* rather belong to *Phoma* or *Diaporthe*, they have to be considered when species of the latter two genera from ginkgo will be revised taxonomically. *Phyllosticta capitalensis* is presently the single verified *Phyllosticta* species known from ginkgo leaves. Its occurrence as an endophyte(?) reported in the literature (Motohashi et al. 2009) and sporulation on living and dead leaves of ginkgo prior and after leaf-fall in autumn observed in this study, indicate a role as both endophyte and saprobe or weak pathogen in senescent leaves of ginkgo as suggested by Wulandari et al. (2013) for other host plants. In Taiwan, *Ph. capitalensis* has been recorded on fruits of persimmon (*Dispyros kaki*; Duan et al. 2017). A record on *Citrus* sp. in Taiwan listed in Wikee et al. (2013) could not be traced in the given references and may be doubtful.

By courtesy of P. Kirk (Index Fungorum) and C. Bensch (MycoBank), we realized that we had presented the wrong repository identifier MB518046 for *Pseudocercospora ginkgoana* R. Kirschner in Kirschner & Okuda, Mycol. Progr. 12: 423 (2013), rendering the name invalid according to Art. 42.1 of the International Code of Nomenclature for algae, fungi and plants. By publishing a new repository accession, the name is validated here as ***Pseudocercospora ginkgoana*** R. Kirschner, sp. nov., under the new identifier MycoBank MB818606, with its holotype (Kirschner et al. 3561, TNM) and description as given in Kirschner & Okuda, Mycol. Progr. 12: 423 (2013). The TNM specimen accession has been designated as F0026006.

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References

Aa HA van der, Vanev S. 2002 – A revision of the species described in *Phyllosticta*. Centraalbureau voor Schimmelcultures, Utrecht.

Duan C-H, Chang C-M, Su C-C, Pan H-R, Wang C-C. 2017 – *Phyllosticta capitalensis* causes black spot of persimmon (*Diospyros kaki*) fruit in Taiwan. Australasian Plant Disease Notes 12:36, 1–4. Doi 10.1007/s13314-017-0263-4.

Karling JS. 1964 – *Synchytrium*. New York, Academic Press.

Kirschner R, Okuda T. 2013 – A new species of *Pseudocercospora* and new record of *Bartheletia paradoxa* on leaves of *Ginkgo biloba*. Mycological Progress 12, 421–426.

Koukol O, Lotz-Winter H. 2016 – Secondary conidia observed in *Bartheletia paradoxa*. Czech Mycology 68(1), 79–84.

Motohashi K, Inaba S, Anzai K, Takamatsu S, Nakashima C. 2009 – Phylogenetic analyses of Japanese species of *Phyllosticta* sensu stricto. Mycoscience 50, 291–302.

Scheuer C, Bauer R, Lutz M, Stabentheiner E, Melnik VA, Grube M. 2008 – *Bartheletia paradoxa* is a living fossil on ginkgo leaf litter with a unique septal structure in the Basidiomycota. Mycological Research 112, 1265–1279.

Sun C, Taylor TN, Na Y, Li T, Krings M. 2015 – Unusual preservation of a microthyriaceous fungus (Ascomycota) on *Sphenobaiera* (ginkgophyte foliage) from the Middle Jurassic of China. Review of Palaeobotany and Palynology 223, 21–30.

Wikee S, Lombard L, Crous PW, Nakashima C, Motohashi K, Chukeatirote E, Alias SA, McKenzie EHC, Hyde KD. 2013 – *Phyllosticta capitalensis*: a widespread endophyte of plants. Fungal Diversity 60, 91–105.

Wulandari NF, Bhat DJ, To-anun C. 2013 – A modern account of the genus *Phyllosticta*. Plant Pathology & Quarantine 3(2), 145–159. Doi 10.5943/ppq/3/2/4.