Saudi Journal of Biological Sciences (2012) **19**, 13–16

King Saud University

Saudi Journal of Biological Sciences

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Phyllosticta ophiopogonis sp. nov. from *Ophiopogon japonicus* (Liliaceae)

S. Wikee [a], N.F. Wulandari [a,b,c], E.H.C. McKenzie [d], K.D. Hyde [a,*]

[a] *School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[b] *Department of Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai 51200, Thailand*
[c] *Microbiology Division, Research Centre for Biology, Indonesian Institute of Sciences, Cibinong Science Centre, Jl. Raya Jakarta Bogor KM. 46, Cibinong 16911, Indonesia*
[d] *Manaaki Whenua Landcare Research, Private Bag 92170, Auckland, New Zealand*

Received 9 July 2011; revised 8 October 2011; accepted 8 October 2011
Available online 19 October 2011

KEYWORDS

Coelomycetes;
Botryosphaeriaceae;
Dothideales;
Leaf spot;
Pathogen;
Taxonomy

Abstract A leaf spotting disease of an ornamental variety of *Ophiopogon japonicus* was discovered at several locations in northern Thailand. In all cases a species of *Phyllosticta* was associated with the lesions. *Phyllosticta ophiopogonis* sp. nov. is distinguished from *Phyllosticta* species from Liliaceae in conidia size, mucilaginous sheath and appendage thus the species is introduced as new in this paper. The new species which causes unsightly lesions on this ornamental plant is described, illustrated and compared with other similar *Phyllosticta* species.

1. Introduction

The genus *Phyllosticta* had been relatively well-studied worldwide and a monograph has been published with details of the excepted species (van der Aa and Vanev, 2002). Five species have been introduced since the publication of van der Aa and Vanev (2002), mainly based on morphology and host occurrence. Four new species were introduced from Japan by Motohashi et al. (2008), while *Phyllosticta citriasiana* Wulandari, Crous and Gruyter, from the peel of the fruit *Citrus maxima* was introduced from China and Thailand (Wulandari et al., 2009). Three new species of the sexual state of *Phyllosticta* have also recently been introduced and include *Guignardia musicola*, Wulandari, Cai and Hyde, *Guignardia bispora*, Wulandari and Hyde, and *Guignardia ellipsoidea*, Wulandari and Hyde from the palms in northern Thailand (Wulandari et al., 2010a,b, 2011). Glienke et al. (2011) also introduced *Phyllosticta bifrenariae* Pereira, Glienke and Crous on orchids, *Phyllosticta citribraziliensis* Glienke and Crous on *Citrus* and *Phyllosticta brazilianiae*, Stringri, Glienke and Crous on *Mangifera indica* and epitypified *Phyllosticta capitalensis* Henn. and *Phyllosticta citricarpa* (McAlpine) Aa.

Ophiopogon japonicus (L.f.) Ker Gawl is an ornamental plant grown in gardens and parks throughout northern Thailand. During field surveys we repeatedly came across severely diseased plants, with symptoms ranging from numerous

* Corresponding author. Tel.: +66 871791761.
E-mail address: kdhyde3@gmail.com (K.D. Hyde).

1319-562X

Peer review under responsibility of King Saud University.
doi:10.1016/j.sjbs.2011.10.003

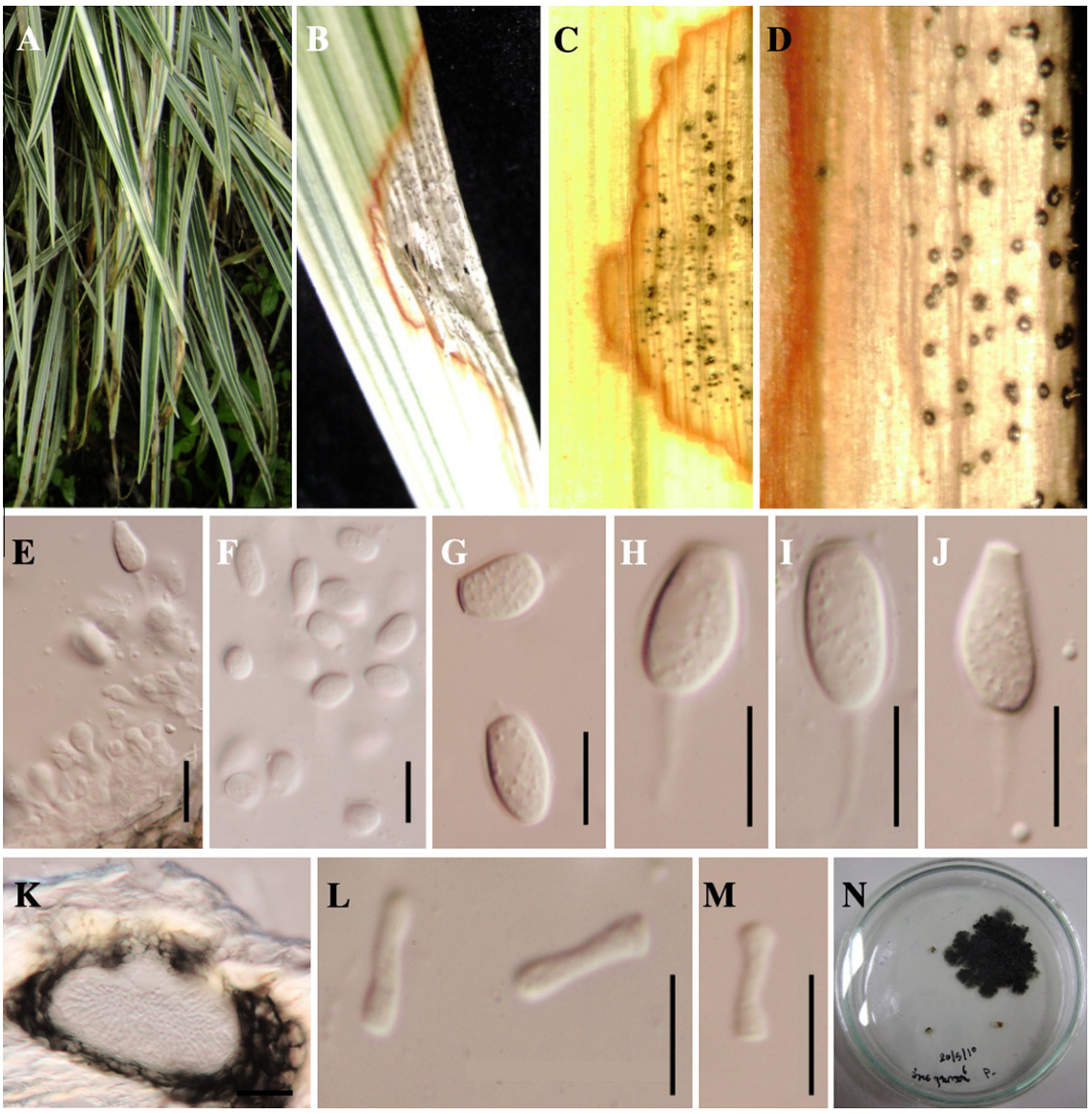

Figure 1 (A–N) *Phyllosticta ophiopogonis* (MFLU11 0027, holotype). (A) Symptom of disease. (B–D) Pycnidia growing on infected leaf of Liliaceae. (E) Conidiogenous cell. (F–J) Conidia Cross section through (K) *Leptodothiorella* state; scale bar = 50 µm. (L and M) Spermatia; scale bar = 10 µm. (N) Upper of cultures after 5 weeks.

leaf spots to severe blighting (Fig. 1). The separation of *Phyllosticta* species is presently mainly based on morphological characters, although molecular data has recently helped to differentiate some taxa, e.g. *P. bifrenariae*, *P. brazilianiae*, *P. citriasiana*, *P. citribraziliensis* (Wulandari et al., 2009; Glienke et al., 2011). However, despite the use of molecular data, we still need to rely on morphological data (Hyde et al., 2010; Udayanga et al., 2011).

There is no report of *Phyllosticta* species on *Ophiopogon* sp. in northern Thailand or worldwide. We therefore compare the species from *O. japonicus* with the species from other members from Liliaceae. *Phyllosticta ophiopogonis* is however distinct. The aim of this paper is therefore to introduce the new species from *O. japonicus* based on morphology as it causes unsightly disease of this ornamental. The new species is compared with other *Phyllosticta* species described from Liliaceae in Table 1.

2. Material and Methods

Diseased leaves of *O. japonicus* were collected from various sites in Chiang Rai Province, Thailand. Morphological characters were recorded using the methods described by Wulandari et al. (2010a,b). Single spore isolates were prepared using the method of Choi et al. (1999).

3. Results

Phyllosticta ophiopogonis Wulandari, Wikee and Hyde, sp. nov. MycoBank: MB 519321 (Figs. 1A–N and 2M–O).

Etymology named after its host plant, *O. japonicus*.

Leaf spots on lamina and apex of the leaf, at first minute, later becoming large spots with a dark reddish border, eventually coalescing to form blights on the leaves, centre of lesions pale

Table 1 *Phyllosticta* spp. described from Liliaceae.

Phyllosticta species	Host plant and family*	Pycnidia size (μm)	Peridium thickness (μm)	Conidiogenous cells (μm)	Conidia (μm)	Sheath size (μm)	Appendage size (μm)	Reference
P. aspidistricola	*Aspidistra elatior* var. *elatior* Liliaceae	61–118 × 86–110	–	7–12.5 × 1.2–2.5	9.5–12.5 × 8.5–10	–	17–24.5	Motohashi et al. (2008)
P. cruenta var. *discincta*	*Polygonatum latifolium*, Liliaceae	100–195 × 145	9–21	4–14 × 3–6	12–23 × 6.4–10	0.2	4–17	Bissett (1979a) present study
P. crypta	*Smilax* sp., Liliaceae	70–130 × 45–95	4–14	5–12 × 2–3.5	5.4–8.9 × 3.8–6.2	0.3–1.0	3–8	Bissett (1979b)
P. cumminsii	*Smilax* sp., Liliaceae	75–140 × 110	4–19	3.5–14 × 3–6	6.7–10.5	1–2	5–20	Bissett (1979b)
P. discincta	*Uvularia grandiflora*, Liliaceae	65–120	5–12	4–13 × 2.5–6	5–8.6 × 3.9–6.6	Less than 0.8	4–14	van der Aa and Vanev (2002)
P. hemerocallidis	*Hemerocallis fulva*, Liliaceae	84–139	–	–	8–13 × 3–5	–	3–10	van der Aa and Vanev (2002)
P. hypoglossi	*Ruscus hypoglossum*, Liliaceae	120–250	12–30	4–10 × 2–3.5	8–15 (−18) × 6–10	–	10 up to 35	Van der Aa (1973)
P. ophiopogonis	*Ophiopogon japonicus*, Liliaceae	96–100 × 75–80	9–15	7–12 × 2–4	10–14 × 7–8	0.8–1	5–16	Present study
P. subeffusa	*Smilax herbacca*, Liliaceae	90–150 × 120–140	5–16	5–8 × 3–4.5	7–13 × 7–10	–	5–7 up to 15	Van der Aa (1973)
P. yuccae	*Yucca elephantipes*, Liliaceae	90–150	14–38	5.4–9.8 × 2.7–6	7.5–15.4 × 6–9.5	1	4–15	Bissett (1986)

* They may now belong to other families.

brown with numerous pycnidia (Fig. 1A–C). *Pycnidia* 96–99 μm diameter, 75–81 μm high, on the surface of leaves, black, globose to pyriform, immersed in plant tissues, coriaceous, solitary to clustered, ostiolate, ostioles as black dots in the centre (Fig. 1D). *Conidiogenous cells* 7–12 × 2–4 μm ($\bar{x} = 9 \times 3$ μm, $n = 20$), holoblastic, determinate, discrete, hyaline, sometimes rarely integrated, with cylindrical to doliiform cells lining the pycnidial locule (Fig. 1E). *Conidia* 10–14 × 7–8 μm ($\bar{x} = 12 \times 7$ μm, $n = 20$), hyaline, 1-celled, coarse-guttulate, smooth-walled, globose, ellipsoidal, clavate or obclavate, with an obtuse apex, sometimes truncate at the base, surrounded by 0.8–1 μm thick mucilaginous sheath which persists at maturity and with 5–16 μm a single, hyaline, curved or straight appendage (Fig. 1H–J).

Leptodothiorella state, 60–80 μm in length, 40–50 μm in wide and thick 22 μm. *Spermatia* are produced from spermatiogenous cells, cylindrical and globose at two ends 6–8 μm long, 6.7–8.3 × 1.3–1.6 μm.

Colonies black, fimbriate, black in reverse, reaching 3–5 cm in diameter after 21 days incubation at 28 °C of on half strength PDA.

Habitat: On living leaves causing leaf spots.
Host: O. japonicus (Lilliaceae).
Known distribution: Thailand (Chiang Rai).
Material examined: Thailand, Chiang Rai Province, Khun.

Korn Waterfall, on the leaves of *O. japonicus*, 10 November 2010, Wikee, WK 10 (MFLU11-0027, *holotype*); culture ex-type MFLUCC11-0057; Wieng Chiang Rung, Houi Mae Sak Waterfall, on the leaves of *O. japonicus*, 10 September 2010, S. Wikee, WK 12 (MFLU11-0028), culture MFLUCC11-0059; Nang Lae, Pasangwiwat, on the leaves of *O. japonicus*, 10 December 2010, Wikee, WK 17 (MFLU11-0029), culture MFLUCC11-0063; *ibid.* 11 November 2010, Wikee, WK 23 (MFLU11-0030), culture MFLUCC11-0069; Weing Kan, on the leaves of *O. japonicus*, 6 January 2011, Wikee, WK 26 (MFLU11-0031), culture MFLUCC10-0132. Mae Fah Luang University, on the leaves of *O. japonicus*, 30 June 2010, Wulandari, NFW 330 (MFLU10-0480); *ibid.*, 06 August 2010, Wulandari, NFW 332 (MFLU10-0482); *ibid.*, Hue Pui Temple, on the leaves of *O. japonicus*, 28 October 2010, Wulandari, NFW 341 (MFLU10-0980); *ibid.*, Hue Pui Temple, on the leaves of *O. japonicus*, 17 August 2010, Wulandari, NFW 343 (MFLU10-0982).

Notes: Van der Aa (1973) and van der Aa and Vanev (2002) distinguished nine species of *Phyllosticta* on Liliaceae (Table 1).

Of the *Phyllosticta* spp. that occurs on Liliaceae, *P. cruenta* var. *discincta* is the most similar. The conidia of *P. ophiopogonis* differ as they are smaller than *P. cruenta* var. *discincta* (10–14 × 7–8 μm ($\bar{x} = 12 \times 7$ μm, $n = 20$) versus 12–23 × 6.4–10 μm) and the pycnidia are also smaller in *P. ophiopogonis* (96–99 μm diameter, 75–81 μm high versus 100–195 μm diameter 145 μm high). *P. ophiopogonis* also differs from *Phyllosticta hypoglossi* in having shorter appendages, 5–16 μm versus 10 up to 35 μm long (van der Aa, 1973).

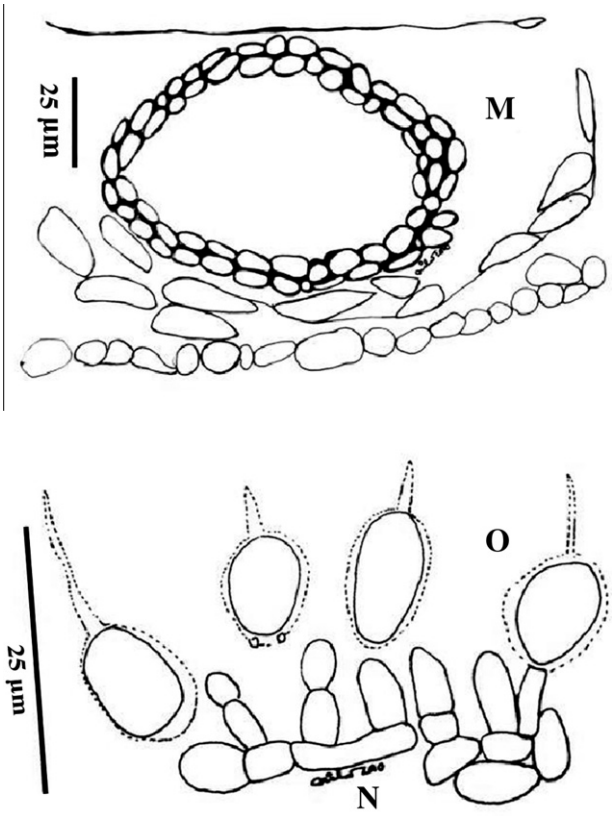

Figure 2 (M–O) *Phyllosticta ophiopogonis* (MFLU 10-0480) line drawing. (M) Section of pycnidia in the leaf (darkened area are plant cells – arrowed). (N) Conidiogenous cells. (O) Conidia with appendage and sheath.

4. Discussion

There is a move towards use of one name for a single biological species instead of different names for different morphs (Hyde et al., 2011). In this study, the sexual *Guignardia* state did not form in any of the collections made or in culture. We choose to use the oldest name *Phyllosticta* as compared to *Guignardia* for this taxon as *Phyllosticta* species usually cause serious disease. If the teleomorph is found later it can be described under *P. ophiopogonis*. This disease is important as it causes unsightly spots and blights on this commonly used ornamental (Fig. 1A).

Acknowledgements

The Global Research Network for Fungal Biology and King Saud University are thanked for supporting this research. Nilam Wulandari is grateful to the Mushroom Research Foundation for a scholarship to carry our studies towards a Ph.D. and the Graduate School, Chiang Mai University, Thailand for the financial support and the School of Science, Mae Fah Luang University for the laboratory facilities. MFLU awarded Grant No. 53101020017 to study the genus *Phyllosticta* in northern Thailand and the National Research Council of Thailand awarded Grant No. 54201020004 to study the genus *Phyllosticta* in Thailand. The Thailand Research Fund in the Royal Golden Ph.D. Jubilee Program agreement No. Ph.D. / 0198/2552 in 2.B.M.F./52/A.1.N.XX to study the taxonomy and phylogeny of *Phyllosticta* is acknowledged. Crous, CBS, the Netherlands is thanked for partially funding this research. Wara Asfiya (LIPI) and Samantha Karunarathna (MFLU) are thanked for valuable references on *Phyllosticta* species.

References

Bissett, J., 1986. Discochora yuccae sp. nov. with Phyllosticta and *Leptodothiorella synanamorphs*. Canadian Journal of Botany 64, 1720–1726.

Bissett, J., 1979a. Coelomycetes on Liliales: the Genus *Phyllosticta*. Canadian Journal of Botany 57, 2082–2095.

Bissett, J., 1979b. Coelomycetes on Liliales: *Dothiorella smilacinae* and *Stagonospora smilacis*. Canadian Journal of Botany 57, 2071–2081.

Choi, Y.W., Hyde, K.D., Ho, W.H., 1999. Single spore isolation of fungi. Fungal Diversity 3, 29–38.

Glienke, C., Pereira, O.L., Stringri, D., Fabris, J., Kava-Cordeiro, V., galli-terasawa, L., Cunnington, J., Shivas, R.G., Groenewald, J.Z., Crous, P.W., 2011. Endophytic and pathogenic *Phyllosticta* species, with reference to those associated with Citrus Black Spot. Persoonia 26, 47–56.

Hyde, K.D., Abd-Elsalam, K., Cai, L., 2010. Morphology: still essential in a molecular world. Mycotaxon 114, 439–451.

Hyde, K.D., KoKo, T.W., McKenzie, E.H.C., 2011. Towards incorporating anamorphic fungi in a natural classification – Checklist and notes for 2010. Mycosphere 2 (1), 1–88.

Motohashi, K., Araki, I., Nakashima, C., 2008. Four new species of *Phyllosticta*, one new species of *Pseudocercospora*, and one new combination in Passalora from Japan. Mycoscience 49 (2), 138–146.

Udayanga, D., Liu, X.Z., McKenzie, E.H.C., Chukeatorate, E., Bahkali, H.A., Hyde, K.D., 2011. The genus *Phomopsis*: biology, species concepts, future and names of important phytopathogens. Fungal Diversity 50, 189–225.

Van Der Aa, H.A., 1973. Studies in *Phyllosticta* 1. Studies in Mycology 5, 1–110.

Van der Aa, H.A., Vanev, S., 2002. A Revision of the Species Described in *Phyllosticta*. Centraalbureau voor Schimmelcultures, Utrech, The Netherlands, pp. 1–49.

Wulandari, N.F., To-anun, C., Hyde, K.D., Duong, L.M., De Gruyter, J., Meffert, J.P., Groenewald, J.Z., Crous, P.W., 2009. Phyllosticta citri-asiana sp. nov, the cause of Citrus tan spot of *Citrus maxima* in Asia. Fungal Diversity 34, 23–39.

Wulandari, N.F., To-anun, C., Cai, L., Abd-Elsalam, K., Hyde, K.D., 2010a. *Guignardia*/*Phyllosticta* species on banana. Cryptogamie Mycologie 31 (4), 403–418.

Wulandari, N.F., To-anun, C., Hyde, K.D., 2010b. *Guignardia morindae* frog eye-leaf spotting disease of *Morinda citrifolia* (Rubiaceae). Mycosphere 1 (4), 325–331.

Wulandari, N.F., To-anun, C., McKenzie, E.H.C., Hyde, K.D., 2011. *Guignardia bispora* and *G. ellipsoidea* spp. nov. and other *Guignardia* species from palms (Arecaceae). Mycosphere 2 (2), 115–118.