

***Phyllosticta* species from banana (*Musa* sp.) in Chongqing and Guizhou Provinces, China**

SHI-PING WU^{1,2,3}, YONG-XIANG LIU³, JIE YUAN², YONG WANG¹, KEVIN D. HYDE⁴ & ZUO-YI LIU^{3,5*}

¹ Agricultural College of Guizhou University, Guiyang, 550006, P.R. China

² Guizhou Institute of Plant Protections, Guiyang, 550006 P.R. China

³ Key Laboratory for Agricultural Biotechnology of Guizhou, Guiyang, 550006 P.R. China

⁴ Institute of Excellence in Fungal Research, and School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand

⁵ Guizhou Academy of Agricultural Science, 550006 P.R. China

*Corresponding author: Zuo-Yi Liu: gziuzuoyi@163.com

Abstract

Six *Phyllosticta* strains were isolated from diseased leaves of *Musa* species in Chongqing and Guizhou provinces, China. Morphological and molecular analysis of LSU and combined ITS, ACT, TEF-1, and GPDH gene sequences, identified these strains as *P. capitalensis* (3 strains), *P. musarum* (1 strain) and two isolates were distinct from known *Phyllosticta* species. The latter is herein introduced as *Phyllosticta musaechinensis* sp. nov. A description and illustrations are provided, and the new species is compared with other species from *Musa* in this paper.

Key words: *Musa*, Phylogeny, New species, Taxonomy

Introduction

The genus *Phyllosticta* is an important causal agent of banana (*Musa* sp.) leaf and post-harvest diseases (Meredith 1968, Jones & Alcorn 1982, Wulandari *et al.* 2010). Seven species of *Phyllosticta* have been recorded from banana although their identification is confusing (Meredith 1968, Chuang 1981, Brown *et al.* 1998, Photita *et al.* 2001, van der Aa & Vanev 2002, Pu *et al.* 2008, Wong *et al.* 2013, Wulandari *et al.* 2010). Wulandari *et al.* (2010) investigated the *Guignardia/Phyllosticta* species associated with freckle disease on banana leaves, re-examined the holotype of each epithet, and reported that the agents of banana freckle are *Guignardia musae* Racib. and *G. musicola* Wulandari, L. Cai & K.D. Hyde. Wong *et al.* (2012) used the name *Phyllosticta* rather than *Guignardia*, found five species on *Musa* in Australia, designated the epitypes for *Phyllosticta maculata* M.H. Wong & Crous and *P. musarum* (Cooke) Aa, and described *P. cavendishii* M.H. Wong & Crous as a new species. The history of *Phyllosticta* on banana was also discussed by Wulandari *et al.* (2010) and Wong *et al.* (2012). *Phyllosticta capitalensis* Henn., *P. cocoicola* (Bat.) Sivan., *Phyllosticta musae* (as *Guignardia musae*), *Phyllosticta musicola* and *Guignardia sydowniana* Trotter have been also recorded as endophytes on banana (Brown *et al.* 1998, Photita *et al.* 2001, 2002).

Wikee *et al.* (2013) provided a multilocus backbone tree for *Phyllosticta* species based on combined ITS, TEF-1, ACT, LSU and GPDH region genes, however, they did not include pathogens from banana (except the ubiquitous endophyte, *P. capitalensis*). In the present study six, *Phyllosticta* strains were isolated from diseased leaves of *Musa* spp. from Chongqing and Guizhou provinces in China. Among them, one taxon differed from known *Phyllosticta* species from banana and other hosts. The aim of this paper is to describe the new species based on morphological and molecular data and investigate the relationship of *P. musaechinensis* with other species.

Materials and methods

Isolates

Symptomatic banana leaves with small to expanding lesions were selected for isolation. The leaves were cut into pieces

TABLE 2. Conidia morphology of *Phyllosticta* / *Guignardia* spp. described from *Musa*.

Taxa	Pycnidia size (µm)	Conidia size (µm)	Mucilaginous sheath thickness (µm)	Appendage length (µm)
<i>P. cavendishii</i>	78–137	12–17 × 8–10	1–3	8–20
<i>P. maculata</i>	84–137	15–21 × 9–13	2–6	12–37
<i>P. musarum</i>	69–118	12–20 × 7–11	1–3	14–20
<i>G. stevensii</i> (no asexual state)	–	–	–	–
<i>G. sydowiana</i> (no asexual state)	–	–	–	–
<i>G. musicola</i>	90–125	12–17 × 8–11	2–4	10–15
<i>P. capitalensis</i>	300	10–14 × 5–7	2–4	6–8
<i>P. musaechinensis</i>	45–145	15.5–22.5 × 8.5–13	0.5–3.5	4–18.5

References

- Brown, K.B., Hyde, K.D. & Guest, D.I. (1998) Preliminary studies on endophytic fungal communities of *Musa acuminata* species complex in Hong Kong and Australia. *Fungal Diversity* 1:27–51.
- Chomnunti, P., Hongsanan, S., Aguirre-Hudson, B., Tian, Q., Persoh, D., Dhami, M.K., Alias, A.S., Xu, J., Liu, X.Z. & Stadler, M. (2014) The sooty moulds. *Fungal Diversity* 66: 1–36.
<http://dx.doi.org/10.1007/s13225-014-0278-5>
- Chuang, T.Y. (1981) Isolation of *Phyllosticta musarum*, causal organism of banana freckle. *Transactions of the British Mycological Society* 77(3):670–671.
[http://dx.doi.org/10.1016/s0007-1536\(81\)80127-1](http://dx.doi.org/10.1016/s0007-1536(81)80127-1)
- Cubero, O.F., Crespo, A., Fatehi, J. & Bridge, P.D. (1999) DNA extraction and PCR amplification method suitable for fresh, herbarium-stored, lichenized, and other fungi. *Plant Systematics and Evolution* 216(3–4):243–249.
<http://dx.doi.org/10.1007/bf01084401>
- Glez-Peña, D., Gómez-Blanco, D., Reboiro-Jato, M., Fdez-Riverola, F. & Posada, D. (2010) ALTER: program-oriented conversion of DNA and protein alignments. *Nucleic acids research* 38(suppl. 2):W14–W18.
<http://dx.doi.org/10.1093/nar/gkq321>
- Hoog, G.S. & Ende, A. (1998) Molecular diagnostics of clinical strains of filamentous Basidiomycetes. *Mycoses* 41(5–6):183–189.
<http://dx.doi.org/10.1111/j.1439-0507.1998.tb00321.x>
- Jones, D. & Alcorn, J. (1982) Freckle and black Sigatoka diseases of banana in far north Queensland. *Australasian Plant Pathology* 11(1):7–9.
- Katoh, K., Kuma, K.-I., Toh, H. & Miyata, T. (2005) MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Research* 33(2):511–518.
<http://dx.doi.org/10.1093/nar/gki198>
- Liu, J.-K., Phookamsak, R., Doilom, M., Wikee, S., Li, Y.-M., Ariyawansa, H., Boonmee, S., Chomnunti, P., Dai, D.-Q. & Bhat, J.D. (2012) Towards a natural classification of Botryosphaerales. *Fungal Diversity* 57(1):149–210.
<http://dx.doi.org/10.1007/s13225-012-0207-4>
- Meredith, D. (1968) Freckle disease of banana in Hawaii caused by *Phyllostictina musarum* (Cke.) Petr. *Annals of Applied Biology* 62(2):329–340.
<http://dx.doi.org/10.1111/j.1744-7348.1968.tb02828.x>
- Nylander, J. (2004) *MrModeltest* v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Photita, W., Lumyong, S., Lumyong, P. & Hyde, K.D. (2001) Endophytic fungi of wild banana *Musa acuminata* at Doi Suthep Pui National Park, Thailand. *Mycological Research* 105(12):1508–1513.
<http://dx.doi.org/10.1017/S0953756201004968>
- Photita, W., Lumyong, S., Lumyong, P. & Hyde, K.D. McKenzie, E.H.C. (2002) Index of fungi described from the Musaceae. *Mycotaxon* 81(9): 491–503.
- Pu, J., Xie, Y., Zhang, X., Qi, Y., Zhang, C. & Liu, X. (2008) Preinfection behaviour of *Phyllosticta musarum* on banana leaves. *Australasian Plant Pathology* 37(1): 60–64.
<http://dx.doi.org/10.1071/ap07079>

- Rehner, S.A. & Samuels, G.J. (1994) Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. *Mycological Research* 98(6):625–634.
[http://dx.doi.org/10.1016/s0953-7562\(09\)80409-7](http://dx.doi.org/10.1016/s0953-7562(09)80409-7)
- Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19(12):1572–1574.
<http://dx.doi.org/10.1093/bioinformatics/btg180>
- Silvestro, D. & Michalak, I. (2012) raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity & Evolution* 12(4):335–337.
<http://dx.doi.org/10.1007/s13127-011-0056-0>
- Swofford, D. (2003) *PAUP*: phylogenetic analysis using parsimony, version 4.0 b10*.
- van der Aa, H.A. & Vanev, S. (2002) A revision of the species described in *Phyllosticta*. In: Aptroot, A., Summerbell, R.C. & Verkley, G.J. (Eds.) *A revision of the species described in Phyllosticta*. CBS.
- Vilgalys, R. & Hester, M. (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172(8):4238–4246.
- White, T.J., Bruns, T., Lee, S. & Taylor, J. (Eds.) (1990) *PCR protocols: a guide to methods and applications*.
- Wikee, S., Lombard, L., Nakashima, C., Motohashi, K., Chukeatirote, E., Cheewangkoon, R., McKenzie, E.H.C., Hyde, K.D. & Crous, P.W. (2013) A phylogenetic re-evaluation of *Phyllosticta* (Botryosphaerales). *Studies in Mycology* 76: 1–29.
<http://dx.doi.org/10.3114/sim0019>
- Wong, M.-H., Crous, P.W., Henderson, J., Groenewald, J.Z. & Drenth, A. (2012) *Phyllosticta* species associated with freckle disease of banana. *Fungal Diversity* 56: 173–187.
<http://dx.doi.org/10.1007/s13225-012-0182-9>
- Wong, M.-H., Henderson, J., & Drenth, A. (2013) Identification and differentiation of *Phyllosticta* species causing freckle disease of banana using high resolution melting (HRM) analysis. *Plant Pathology* 62: 1285–1293.
<http://dx.doi.org/10.1111/ppa.12056>
- Wulandari, N.F., To-Anun, C., Lei, C., Abd-Elsalam, K.A. & Hyde, K.D. (2010) *Guignardia/Phyllosticta* species on banana. *Cryptogamie, Mycologie* 31(4):403–418.
- Xia, X., Xie, Z., Salemi, M., Chen, L. & Wang, Y. (2003) An index of substitution saturation and its application. *Molecular Phylogenetics and Evolution* 26(1):1–7.
[http://dx.doi.org/10.1016/S1055-7903\(02\)00326-3](http://dx.doi.org/10.1016/S1055-7903(02)00326-3)