

Article



http://dx.doi.org/10.11646/phytotaxa.255.1.2

Chaetothyrina mangiferae sp. nov., a new species of Chaetothyrina

CHONTICHA SINGTRIPOP^{1,2,3}, SINANG HONGSANAN^{1,2,3}, JUNFU LI ^{1,2,3}, NIMALI INDEEWARI DE SILVA^{1,2,3}, ALAN J.L. PHILLIPS⁵, E.B. GARETH JONES⁴, ALI H. BAHKALI⁴ & KEVIN D. HYDE^{1,2,3}

¹Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

Correspondence to*: kdhyde3@gmail.com

Abstract

Chaetothyrina musarum and C. mangiferae sp. nov. (Micropeltidaceae, Dothideomycetes) are reported from fruits of Musa sp. (Musa acuminata × Musa balbisiana) and Mangifera indica, respectively. Introduction of a new species, Chaetothyrina mangiferae, and provision of a reference specimen for C. musarum with sequence data, supported by morphology will help to stabilize the taxonomy of the genus and family. Maximum likelihood and Bayesian analyses of LSU and SSU sequence data show that C. musarum forms a distinct clade with other genera in Micropeltidaceae within Capnodiales and C. mangiferae clusters in a poorly supported clade in Dothideomycetes, Illustrated accounts of Chaetothyrina musarum (reference specimen) and C. mangiferae are provided and compared with similar taxa. This is the first report of C. musarum causing sooty blotch and flyspeck disease on Musa sp. (Musaceae) in Thailand and C. mangiferae causing sooty blotch and flyspeck disease of Mangifera indica (Anacardiaceae) worldwide.

Key words: Dothideomycetes, *Mangifera indica*, Micropeltidaceae, Microthyriales, *Musa*, new species

Introduction

The order Microthyriales was introduced by Arnaud (1918). This order presently comprises two families, Micropeltidaceae and Microthyriaceae. The order Microthyriales has been poorly studied and there are few representative cultures in culture collections and sequence data in GenBank is minimal. This order comprises saprobes, pathogens and epiphytes found on dead or living leaves or stems (Kirk *et al.* 2008, Schoch *et al.* 2009, Wu *et al.* 2010, 2011a, b, Hongsanan *et al.* 2014, 2015). Species of Microthyriales are characterized by small and inconspicuous black spots on host tissues, which are the flattened, usually poorly developed at the base, ostiolate thyriothecia. Asci are bitunicate with fissitunicate dehiscence and pseudoparaphyses may be present or lacking. Ascospores are hyaline to brown and uni- to multi-septate (Arnaud 1918, von Arx & Müller 1975, Barr 1987, Kirk *et al.* 2008, Wu *et al.* 2011, Hyde *et al.* 2013, Hongsanan *et al.* 2014, 2015).

The family Micropeltidaceae was introduced by Clements & Shear (1931). The number of accepted genera has varied from eight (von Arx & Müller 1975) to 27 (Kirk *et al.* 2008). Barr (1987) accepted five genera, Lumbsch & Huhndorf (2010) accepted 24 genera, Hyde *et al.* (2013) included ten genera and Wijayawardene *et al.* (2014) included 13 genera. This family is distinguished by superficial, flattened and scutate thyriothecia, with an upper wall comprising non-radiate, often meandrous interwoven cells, and a poorly developed base. The hamathecium comprises narrow cellular pseudoparaphyses. Asci are bitunicate, and clavate to cylindrical and ascospores are hyaline with long-clavate ascospores and usually with more than two septa (Clements & Shear 1931, Batista 1959, von Arx & Müller 1975, Barr 1987, Wu *et al.* 2011a, Hyde *et al.* 2013, Hongsanan *et al.* 2014). The family has been poorly studied (Wu *et al.* 2011, Hyde *et al.* 2013, Hongsanan *et al.* 2015).

The genus *Chaetothyrina* was introduced by Theissen (1913) and is typified by *Chaetothyrina musarum* (Speg.) Theiss. *Chaetothyrina* is characterized by ascomata with setae, superficial hyphae which are absent or inconspicuous

²World Agroforestry Centre, East and Central Asia, 132 Lanhei Road, Kunming 650201, China

³Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand

⁴Botany and Microbiology Department, College of Science, King Saud University, Riyadh 11442, Saudi Arabia

⁵Centro de Recursos Microbiológicos, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516, Caparica Lisbon, Portugal

and hyaline, 1-septate ascospores (Reynolds & Gilbert 2005, Hyde *et al.* 2013). This genus consists of 18 species records in MycoBank and 17 species records in Index Fungorum (2015). The genus *Chaetothyrina* causes flyspeck disease, e.g., *C. musarum* is found on banana (*Musa* sp.) (Muller & von Arx 1962) and *C. panamensis* on *Oncoba laurina* (Muller & von Arx 1962, Hofmann & Piepenbring 2006).

Sooty blotch flyspeck disease (SBFS) is a disease complex caused by a diverse array of epiphytic fungi that colonize the epicuticular wax layer of fruits, stems and leaves of many plants in humid areas, worldwide (Batzer et al. 2005, 2010, Yang et al. 2010, Cooley et al. 2011, Gleason et al. 2011, Miñarro et al. 2012, Hao et al. 2013, Mayfield et al. 2013, Zhang et al. 2015). The causal fungi are characterized by colonies produced on host tissues, from superficial, spreading, dark, irregular blotches of mycelium, with or without sclerotium-like structures or fruiting bodies and are referred to as "sooty blotch". "Flyspeck" on the other hand designates clusters of shiny, small black sclerotium-like structures or fruiting bodies, lacking visible intercalary mycelium (Batzer et al. 2005, Frank et al. 2010, Yang et al. 2010, Gleason et al. 2011, Mayfield et al. 2013). A typical example of flyspeck is on apple and is caused by Schizothyrium pomi (Mont. & Fr.) Arx (Farr 1961, Batzer et al. 2002, Gleason et al. 2011). Infected fruits are generally rejected by the market and consumers, resulting in considerable economic loss to growers (Batzer et al. 2010, Miñarro et al. 2012, Zhang et al. 2015). The SBFS complex is estimated to comprise 60–80 species (Batzer et al. 2005, Gleason et al. 2011, Díaz Arias et al. 2010), most are either taxonomically undetermined or undescribed (Batzer et al. 2008, Sun et al. 2008, Schoch et al. 2009, Yang et al. 2010).

The aim of this paper is to introduce and describe a new species, *Chaetothyrina mangiferae* and provide a reference specimen for *C. musarum* with molecular data. Both species cause sooty blotch and flyspeck disease; *C. musarum* on fruits of *Musa* sp. and *C. mangiferae* on fruits of *Mangifera indica* in northern Thailand. The type material of *C. musarum* is also illustrated along with the reference specimen. *Chaetothyrina mangiferae* is introduced based on morphological and phylogenetic evidence.

Material and methods

Sample collection and morphological studies

Fruits of *Musa* sp. (*M. acuminata* × *M. balbisiana*) and *Mangifera indica* were collected in Chiang Rai Province, Thailand and brought to the laboratory in paper bags where they were examined using a Motic SMZ 168 series dissecting stereo-microscope. Free-hand sections of ascomata were mounted in 10% lactoglycerol for photomicrography and microscopic studies. The taxa were examined using a Nikon ECLIPSE 80i compound microscope and photographed by a Canon 550D and 600D digital camera fitted to the microscope. Tarosoft (R) Image Frame Work program was used for measuring, and images used for figures were made with Adobe Photoshop CS5 Extended version 12.0 software (Adobe Systems, USA). Isolation was carried out from single ascospores following the method described by Chomnunti *et al.* (2014) and germinating single spores were transferred directly to malt extract agar (MEA) plates and grown at 15–18°C. Characters of colonies were observed and measured after 1–2 weeks.

The type materials are deposited in the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand and Kunming Herbarium Collection (KUN), Kunming, China. Cultures are deposited at the Mae Fah Luang University Culture Collection (MFLUCC), Chiang Rai, Thailand and Mycothèque de l'Université Catholique de Louvain (MULC), Belgium. Faces of Fungi and Index Fungorum numbers were obtained as explained in Jayasiri *et al.* (2015) and Index Fungorum (2015).

DNA extraction and PCR reaction

Genomic DNA was extracted from mycelium grown on MEA for 1–2 weeks at 25–28°C using a Biospin Fungus Genomic DNA Extraction Kit (BioFlux®) following the manufacturers' protocol. The amplification of rDNA regions of the small subunit rDNA (SSU) and large subunit (LSU) was carried out using NS1 and NS4 (White *et al.* 1990) and LROR and LR5 (Vilgalys & Hester 1990). The amplification procedure was performed in a 25 μl reaction volume containing 1 μl DNA, 1 μl of primer 1 and primer 2, 12.5 μl of 2xMaster Mix and 9.5 μl of ddH₂O. Amplification conditions were setup for initial denaturation of 3 min at 94°C, followed by 35 cycles of 30 s at 94°C, 50 s at 55°C and 1 min at 72°C, and a final extension period of 10 min at 72°C. The PCR products were checked on 1% agarose electrophoresis gels stained with ethidium bromide. Purification and sequencing of PCR products were done by Shangkai Majorbio Biopharm Technology Co., China.

Sequence alignment and phylogenetic analyses

The two generated sequences were compared with other sequences in GenBank by nucleotide megablast searches. Multiple sequence alignment was carried out in BioEdit (Hall 1999) and Clustal X (Thompson *et al.* 1997). Maximum likelihood analysis was performed by using raxmlGUIv.0.9b2 (Silvestro & Michalak 2012). The search strategy was set to rapid bootstrapping and the analysis carried out using the GTRGAMMAI model of nucleotide substitution. The number of replicates was inferred using the stopping criterion (Pattengale *et al.* 2009). Maximum likelihood (ML) bootstrap values equal or greater than 60% are given above the nodes (Fig. 1). The model of evolution was carried out using MrModeltest 2.2 (Nylander *et al.* 2008). Posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (BMCMC) using MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001). Bayesian posterior probabilities (BYPP) equal or greater than 0.90 are given below the nodes (Fig. 1). A single species of Arthoniomycetes, the sister class to Dothideomycetes, *Schismatomma decolorans* (Turner & Borrer ex Sm.) Clauzade & Vězda (DUKE 0047570) was chosen as the out group taxon (Wu *et al.* 2011, 2014, Hongsanan *et al.* 2014).

Results

DNA sequencing—SSU and LSU

The combined gene analysis of the LSU and SSU dataset comprised 53 taxa including our new strains of *Chaetothyrina* musarum and C. mangiferae sp. nov. Sequence data from taxa in Capnodiaceae, Dissoconiaceae, Micropeltidaceae, Microthyriaceae, Mycosphaerellaceae, Natipusillaceae, Phaeotrichaceae, Schizothyriaceae, Symphoventuriaceae, Venturiaceae and Zeloasperisporiaceae were downloaded from GenBank to supplement the dataset (Table 1). Phylogenetic analyses used combined LSU and SSU sequence data (Fig. 1). The Micropeltidaceae I clade comprises six available strains from GenBank and formed within Capnodiales (68% ML, 0.9 PP support). Chaetothyrina musarum groups with strains of Stomiopeltis and Houjia in the clade Micropeltidaceae I, but is separated from other strains with relatively strong bootstrap support (90% ML, 0.95 PP support, Fig. 1) and forms a robust clade sister to Stomiopeltis versicolor (Desm.) Arx (GA3 23C2b). The Micropeltidaceae II clade comprises five available strains from GenBank, and three of the five strains belong to Micropeltis which is the generic type of Micropeltidaceae. The Micropeltidaceae II clade is clustered as a sister group to the Microthyriaceae clade. Chaetothyrina mangiferae clusters with Paramicrothyrium chinensis H.X. Wu & K.D. Hyde (IFRDCC 2258) with high support (100% ML, 1.0 PP support, Fig. 1). However, Chaetothyrina mangiferae is not morphologically similar to Paramicrothyrium chinensis (IFRDCC 2258) but similar to Chaetothyrina musarum, thus introduced as a new species. Although support for the different genera of *Micropeltidaceae* is generally weak, we suspect this is because the family is poorly populated with sequence data and some genus / species names are putative (e.g., Stomiopeltis versicolor).

Phylogeny

TABLE 1. Taxa used in the phylogenetic analysis and their corresponding GenBank accession numbers. All ex-type sequences are indicated in bold, the new sequences are in red bold and reference specimen in blue bold.

Species	Culture Accession No.	GenBank Accession No.	
		LSU	SSU
Apiosporina collinsii	CBS 118973	GU301798	GU296135
Capnodium coartatum	MFLUCC10-0069	JN832614	JN832599
Capnodium coffeae	CBS 147.52	GU214400	DQ247808
Chaetothyrina mangiferae	MFLUCC14-0201	KU710172	KU710173
Chaetothyrina musarum	MFLUCC15-0383	KU710171	KU710174
Chaetothyriothecium elegans	CPC 21375	KF268420	-
Dissoconium aciculare	CBS 204.89	GU214419	GU214523
Dothistroma septosporum	CBS:112498	GQ852597	JX901744

...Continued on next page

TABLE 1. (Continued)

Species	Culture Accession No.	GenBank Accession No.	
		LSU	SSU
Fusarium ramoconidii	CBS 463.82	EU035439	-
Fusicladium pini	CBS 462.82	EU035436	-
Houjia yanglingensis	YHLB20	GQ433630	-
Houjia yanglingensis	YHJN13	GQ433631	-
Leptoxyphium cacuminum	MFLUCC10-0049	JN832602	JN832587
Leptoxyphium kurandae	CPC:17274	KF982311	KF982312
Micropeltis dendrophthoes	MFLUCC15-0599	KT588595	KT588597
Micropeltis dendrophthoes	MFLUCC15-0600	KT588596	KT588598
Micropeltis zingiberacicola	IFRDCC 2264	JQ036227	JQ036222
Microthyrium buxicola	MFLUCC15-0212	KT306551	KT306549
Microthyrium buxicola	MFLUCC15-0213	KT306552	KT306550
Microthyrium microscopicum	CBS 115976	GU301846	GU296175
Mycosphaerella ellipsoidea	CBS:110843	GQ852602	AY725545
Mycosphaerella keniensis	CBS:111001	GQ852610	-
Mycosphaerella marksii	CBS:110942	GQ852612	-
Mycosphaerella punctiformis	CBS 113265	NG_027571	AY490775
Natipusilla decorospora	L-A236-1A	HM196369	HM196376
Natipusilla limonensis	L-AF286-1A	HM196370	HM196377
Natipusilla naponensis	L-AF217-1A	HM196371	HM196378
Paramicrothyrium chinensis	IFRDCC 2258	JQ036229	JQ036224
Phaeothecoidiella illinoisensis	CBS:125223	GU117901	-
Phaeothecoidiella missouriensis	CBS:118959	GU117903	-
Phaeotrichum benjaminii	CBS 541.72	GU357788	AY538349
Phloeospora maculans	CBS 115123	GU214670	GU214670
Phragmocapnias betle	MFLUCC10-0053	JN832606	JN832591
Pseudoveronaea ellipsoidea	MI3 34F1a	JQ622103	-
Ramichloridium apiculatum	CBS 400.76	EU041851	EU041794
Schizothyrium pomi	CUA1a	AY598895	-
Schizothyrium pomi	Flyspeck1924-Zj001	AY598894	-
Schizothyrium pomi	VA1_7A1d	FJ147155	-
Stomiopeltis betulae	CBS 114420	GU214701	GU214701
Stomiopeltis versicolor	GA3 23C2b	FJ147163	-
Sympoventuria capensis	CPC 120136	KF156104	KF156094
Trichodelitschia bisporula	CBS 262.69	GU348996	GU296202
Trichodelitschia munkii	Kruys 201 (UPS)	DQ384096	-
Trypethelium eluteriae	111	GU327726	GU327704
Tumidispora shoreae	MFLUCC 12-0409	KT314073	KT314075

...Continued on next page

TABLE 1. (Continued)

Species	Culture Accession No.	GenBank Accession No.	
		LSU	SSU
Tumidispora shoreae	MFLUCC 14-0574	KT314074	KT314076
Venturia inaequalis	CBS 594.70	GU301879	KF156093
Zeloasperisporium eucalyptorum	CBS:14603	GQ303329	-
Zeloasperisporium hyphopodioides	CBS218.95	EU035442	-
Zeloasperisporium siamense	IFRDCC 2194	JQ036227	JQ036223
Zeloasperisporium wrightiae	MFLUCC15-0225	KT387737	KT387738
Zygophiala cryptogama	KY1_1.2A1c	EF164902	-
Zygophiala wisconsinensis	MSTA8a	AY598897	-

Taxonomy

The present study resulted in the discovery of a new species, *Chaetothyrina mangiferae*, and a reference specimen of *C. musarum* is provided. These species were associated with SBFS on mango (*Mangifera indica*) and banana (*Musa acuminata* × *Musa balbisiana*), respectively. The genus and species are treated below.

Chaetothyrina Theiss., Annls mycol. 11(6): 495 (1913)

Epiphytic on stems, leaves or fruit surfaces, as very small, black, shiny dots, with long setae. Sexual morph: Thyriothecium superficial, easily removed from host, circular, brown to dark brown, rounded and darkened at the margin, base poorly developed, with long setae, lacking superficial mycelium. Setae straight, rounded at the apex, unbranched, with dark septa, brown to dark brown. Upper wall comprising brown to dark brown cells of textura epidermoidea. Hamathecium comprising septate, hyaline, pseudoparaphyses. Asci 8-spored, bitunicate, broadly obovoid to pyriform, apically rounded, with an ocular chamber, smooth-walled. Ascospores 2–3-seriate, broadly ellipsoidal to fusoid, 1-septate, slightly constricted at the septum, hyaline, smooth-walled, upper cell mostly larger than lower cell, base with rounded ends.

Type species:—Chaetothyrina musarum (Speg.) Theiss.

Notes:—Chaetothyrina was introduced by Theissen (1913) as a monotypic genus and typified by Chaetothyrina musarum. (Speg.) Theiss. Müller & von Arx (1962) provided an illustrated account. The genus is characterized by ascomata with setae, lack of superficial hyphae and hyaline, 1-septate ascospores (Reynolds & Gilbert 2005, Hyde et al. 2013).

Chaetothyrina musarum (Speg.) Theiss., Annls mycol. 11(6): 495 (1913)

≡ Chaetothyrium musarum Speg., Anales del Museo Nacional de Historia Natural Buenos Aires 6: 297 (1898).

Description from BPI 646466 (Fig 2.)

Epiphytic on the surface of fruit of Musa sapientum L. Sexual morph: Thyriothecia 110–155 × 105–150 μm (\overline{x} = 134 × 123 μm, n = 5), superficial on host tissues, solitary, circular, brown to dark brown, rounded and darkened at the margin, easily removed, base poorly developed, with setae, ostiole central, lacking superficial mycelium. Setae 90–120 μm long, arising from the surface of thyriothecia, straight, rounded at the apex, unbranched, septate, darkened at the septa, brown to dark brown. Upper wall comprising 2–3 layers of textura epidermoidea, brown to dark brown. Hamathecium comprising septate, hyaline, pseudoparaphyses. Asci 24–38 × 10–14 μm (\overline{x} = 32 × 12 μm, n = 8), 8-spored, bitunicate, broadly obovoid to pyriform, apex apically rounded, with an ocular chamber, smooth-walled. Ascospores 10–13 × 3–4 μm (\overline{x} = 12 × 4 μm, n = 20), 2–3-seriate, hyaline, broadly ellipsoidal to fusoid, 1-septate, slightly constricted at the septum, upper cell mostly larger than lower cell, basal cell with rounded ends, smooth-walled.

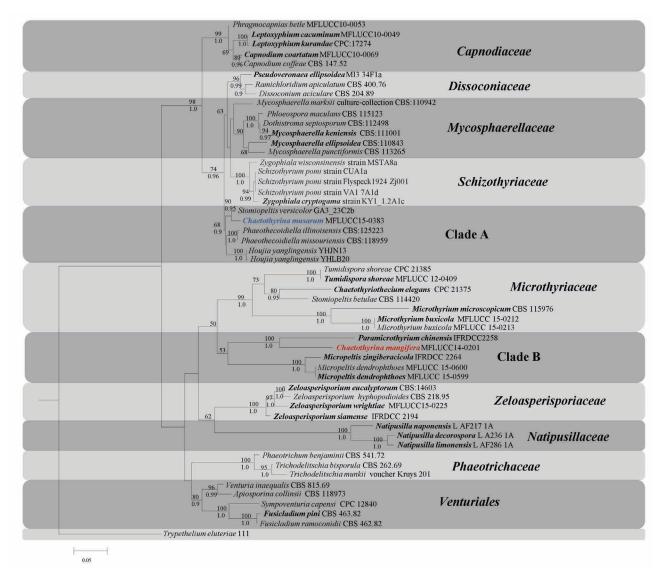


FIGURE 1. RAXML maximum likelihood tree obtained from a representative dataset of 53 taxa (LSU and SSU sequence data) of Capnodiaceae, Dissoconiaceae, Micropeltidaceae, Microthyriaceae, Mycosphaerellaceae, Natipusillaceae, Phaeotrichaceae, Schizothyriaceae, Symphoventuriaceae, Venturiaceae and Zeloasperisporiaceae and rooted with Trypethelium eluteriae as the out group taxon. Bootstrap support values greater than 50% for maximum likelihood (ML), expressed from 1,000 repetitions, are shown above the nodes. The values below the nodes are Bayesian posterior probabilities above 0.90. The new species is in red bold and the reference specimen in blue bold. All other type strains are in black bold and the original isolate numbers are noted after the species names.

Material examined:—BRAZIL. Districto Federal: on fruit of *Musa sapientum* L., 30 December 1931, H. Grillo (BPI 646466).

Notes:—Chaetothyrina musarum was introduced by Theissen (1913) as a monotypic genus, and Chaetothyrium musarum Speg. (1899) and Plochmopeltidella smilasina Mendoza (1925) are synonyms. The fungus is reported from Musa sp., Musa paradisiaca L. (Musaceae), Smilax spp. (Liliaceae) and other plants in tropical America, Africa and South Asia (Müller & von Arx 1962).

Description from reference collection (Fig. 3)

Faceoffungi Number: FoF02009

Epiphytic associated with sooty blotch and flyspeck complex disease on the surface of fruit of *Musa* sp., appearing as very small, black, shiny dots, rounded to ovoid, with setae. Sexual morph: Thyriothecia 125–160 \times 125–150 μ m ($\overline{x} = 140 \times 138 \ \mu$ m, n = 5), superficial or partly immersed in host tissues, solitary, circular, brown to dark brown, rounded and darkened at the margin, easily removed, base poorly developed, with long setae, ostiole central, lacking

superficial mycelium. Setae $105-135\mu m$ long, arising from the surface of thyriothecium, straight, rounded at the apex, unbranched, septate, darkened at the septa, brown to dark brown, smooth-walled. Upper wall comprising 2–3 layers of brown to dark brown cells of textura epidermoidea. Hamathecium comprising $1-1.8 \mu m$ wide, septate, hyaline pseudoparaphyses. Asci $30-41 \times 10-13 \mu m$ ($\overline{x} = 35 \times 12 \mu m$, n = 10), 8-spored, bitunicate, broadly obovoid to pyriform, apically rounded, with an ocular chamber. Ascospores $11-14 \times 4-5 \mu m$ ($\overline{x} = 12 \times 4 \mu m$, n = 20), 2–3-seriate, hyaline, broadly ellipsoidal to fusoid, somewhat curved, 1-septate, slightly constricted at the septum, smooth-walled, upper cell mostly larger than lower cell, base with rounded end. Asexual morph: Undetermined.



FIGURE 2. Chaetothyrina musarum (BPI 646466). A. Specimen label. B. Herbarium specimens. C. Appearance of thyriothecia on host. D. Thyriothecia when viewed in squash mount. E. Section through thyriothecium. F. Seta arising from the surface of thyriothecium. G. Upper wall of thyriothecium when viewed in squash mount. h, i. Immature asci. j, k. Ascospores. Scale bars: $d=50 \mu m$, $e=100 \mu m$, $f-i=10 \mu m$, j, $k=5 \mu m$.

Culture characters:—Ascospores germinating on MEA at 25–28°C after 24–36 hours, germ tubes appearing from each end of the ascospore, colonies reaching 1.8–2 cm diam. after 15 days on MEA at 25–28°C, raised, comprising raised, dark grey mycelium, white to greyish at the margin, surface of colonies velvety.

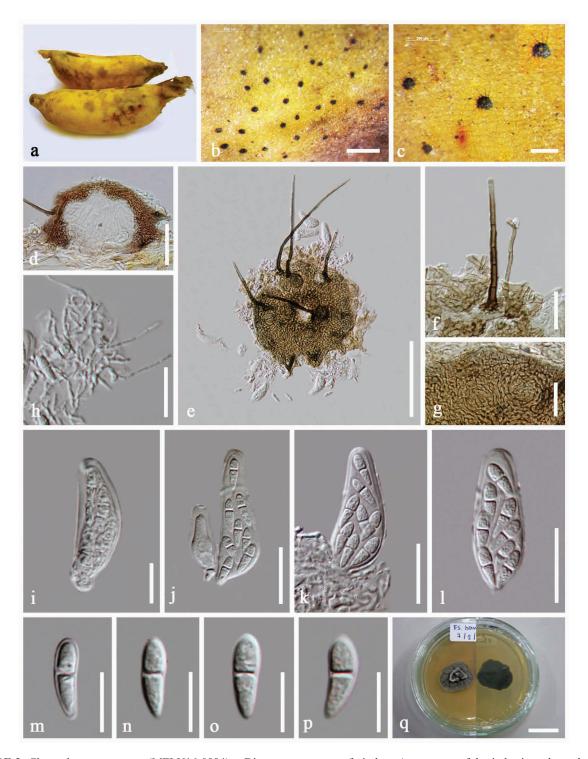


FIGURE 3. Chaetothyrina musarum (MFLU16-0884) a. Disease symptom on fruit. b, c. Appearance of thyriothecia on host. d. Section through thyriothecium. e. Thyriothecium when viewed in squash mount. f. Seta arising from the surface of thyriothecium. g. Upper wall of thyriothecium when viewed in squash mount. h. Pseudoparaphyses. i. Immature ascus. j–l. Mature asci. m–p. Ascospores. q. Colonies on MEA from above and below. Scale bars: $b=500 \mu m$, $c=200 \mu m$, $d=50 \mu m$, $e=100 \mu m$, $f=l=20 \mu m$, $m-p=10 \mu m$, q=2 cm.

Material examined:—THAILAND. Chiang Rai Province: Tar Sud District, on fruit of *Musa* sp. (*M. acuminata* × *M. balbisiana*, Musaceae), 12 January 2014, Chonticha Singtripop, FS02, (MFLU16-0884, HKAS90976, **reference specimen designated here**); Chiang Rai Province: Tar Sud District, on fruit of *Musa* sp. (*M. acuminata* × *M. balbisiana*) 22 February 2015, Chonticha Singtripop, (MFLU16-0885), living culture, MFLUCC 15-0383, MUCL55904.

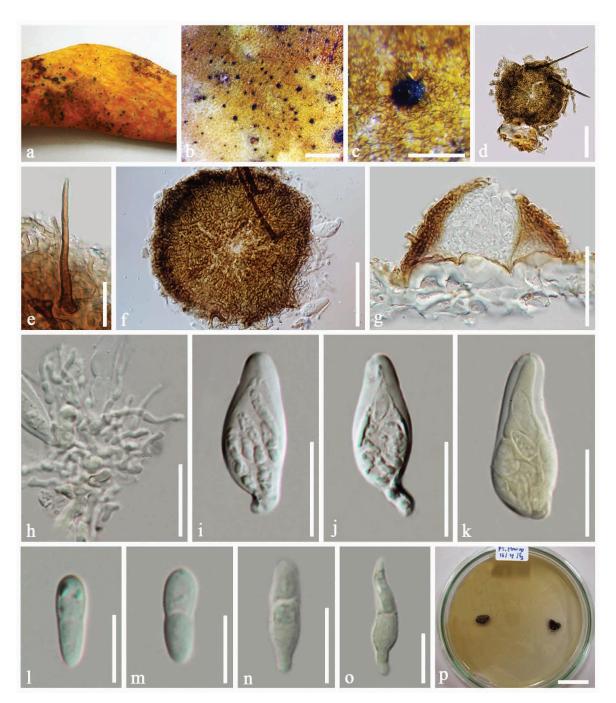


FIGURE 4. Chaetothyrina mangiferae (holotype). a, b. Disease symptoms on fruit. c. Appearance of thyriothecium on host. d. Thyriothecium when viewed in squash mount. e. Seta arising from the surface of thyriothecium. f. Upper wall of thyriothecium when viewed in squash mount. g. Section of thyriothecium. h. Pseudoparaphyses. i, j. Asci. k. Ascus in Melzer's reagent. l, m. Ascospores. n, o. Ascospores germinating in Melzer's reagent. p. Colonies on MEA plate viewed from above. Scale bars: $b=1,000 \mu m$, $c=200 \mu m$, $d=100 \mu m$, $f=100 \mu m$,

Chaetothyrina mangiferae Singtripop, Hongsanan & K.D. Hyde, sp. nov.

Facesoffungi number: FoF02010 (Fig. 4)

Etymology:—mangiferae refers to host, Mangifera indica

Holotype:—MFLU 14-0191.

Epiphytes associated with the sooty blotch and flyspeck complex disease of fruit of Mangifera indica, appearing as very small, black, shiny dots, round to ovoid, with long setae. **Sexual morph**: Thyriothecium 90–130 × 80–120 μ m (\overline{x} =110 × 104 μ m, n=5), superficial or partly immersed in host tissues, solitary, circular, brown to dark brown, rounded and darkened at the margin, easily removed, base poorly developed, with setae, ostiole central, lacking superficial

mycelium. Setae 50–120 µm long, arising from the surface of thyriothecium, straight, rounded at the apex, unbranched, septate, darkened at the septa, brown to dark brown, smooth-walled. Upper wall comprising 2–3 layers of brown to dark brown cells of textura epidermoidea. Hamathecium of 3–5 µm wide, septate, hyaline pseudoparaphyses. Asci $26-36 \times 11-12 \text{ µm}$ ($\overline{x}=32 \times 12 \text{ µm}$, n=5), 8-spored, bitunicate, broadly oblong to pyriform, ends apically rounded, with an ocular chamber. Ascospores $11-23 \times 3-6 \text{ µm}$ ($\overline{x}=16 \times 5 \text{ µm}$, n=10), 2–3-seriate, hyaline, broadly ellipsoidal to fusoid, 1-septate, slightly constricted at the septa, upper cell mostly larger than lower cell, rounded ends, smoothwalled. Asexual morph: Undetermined.

Culture characters:—Ascospores germinating on MEA at 25–28°C after 24–36 hours in the dark, germ tubes appearing from each end of the ascospores. Colonies reaching 0.8–1 cm diam. after 15 days on MEA at 25–28°C, raised, comprising raised dark grey mycelium, white to greyish at the margin.

Material examined:—THAILAND. Chiang Rai Province: Tar Sud District, on living fruit of *Mangifera indica* (*Anacardiaceae*), 7 December 2013, Chonticha Singtripop FS01 (MFLU 14-0191, **holotype**, HKAS 90977, **isotype**); Tar Sud District, on living fruit of *M. indica*, 18 January 2015, Chonticha Singtripop (MFLU16-0886), ex-type living culture, MFLUCC 14-0201, MUCL55902.

Notes:—Chaetothyrina mangiferae is most similar to C. applanata (Ellis & G. Martin) M.E. Barr, in having superficial scutate thyriothecia with dark brown, septate setae, an upper wall comprising cells of textura epidermoidea, bitunicate, oblong to pyriform asci and 1-septate, hyaline ascospores (Barr 1993), However, it differs in having smaller thyriothecia, larger, ellipsoidal to fusoid ascospores and a different host.

Discussion

The present study treats a new species, *Chaetothyrina mangiferae* and provides a reference specimen (*sensu* Ariywansa *et al.* 2014) for *C. musarum* with molecular data. The species were associated with SBFS of mango (*Mangifera indica*) and banana (*Musa acuminata* × *M. balbisiana*), respectively, and were collected in northern Thailand. Although *C. musarum* and *C. mangiferae* are morphologically similar, they have differences in several base pairs in SSU and LSU sequence data. Ismali *et al.* (2016) showed the placement of Micropeltidaceae in Capnodiales in their phylogenetic tree; however, they did not include the strains of *Micropeltis*, which is the type genus of this family. This study uses LSU and SSU sequence data to show the placement of Micropeltidaceae species in two separate clades (Fig. 1). The first group clustered as a distinct clade in Capnodiales (similar to Ismail *et al.* 2016), and the other clustered as family *incertae sedis* in Dothideomycetes. More collections and sequence data are needed to clarify and provide a reasonable placement of Micropeltidaceae in the phylogenetic tree. *Chaetothyrina musarum* clustered with other species of Micropeltidaceae within Capnodiales, while *C. mangiferae* is a distinct species in the clade that comprises three strains of *Micropeltis*, but outside Capnodiales (Fig. 1). These results probably reflect the fact that the trees are poorly populated with taxa from Micropeltidaceae.

Based on the morphological study *Chaetothyrina mangiferae* (MFLUCC 14-0201) is introduced as a new species even though the phylogenetic tree shows it groups with *Paramicrothyrium chinensis* (100% ML, 1.0 PP support, Fig. 1). The phylogenetic tree branch length of *Chaetothyrina mangiferae* is longer than *Paramicrothyrium chinensis* (IFRDCC 2258). *Chaetothyrina musarum* and *C. mangiferae* are often found on post-harvest fruits in markets in Thailand during the cold season (November to February).

Acknowledgements

This work was supported by the International Research Group Program (IRG-14-27), Deanship of Scientific Research, King Saud University, Saudi Arabia. We would like to thank Mae Fah Luang University grant "Taxonomy and Phylogeny of selected families of Dothideomycetes (Grant number: 56101020032)" for supporting this study. Thanks to Humidtropics, a CGIAR Research Program that aims to develop new opportunities for improved livelihoods in a sustainable environment, for partially funding this work, Chinese Academy of Sciences, project number 2013T2S0030, for the award of Visiting Professorship for Senior International Scientists at Kunming Institute of Botany, Plant Germplasm and Genomics Center in Germplasm Bank of Wild Species, Kunming Institute of Botany for the help with molecular work. Chonticha Singtripop thanks Saranyaphat Boonmee, Mingkhuan Doilom, Ausana Mapook, Sirinapa Konta and Chada Norphanphoun for their valuable suggestions and help.

References

- Ariyawansa, H.A., Hawksworth, D.L. & Hyde, K.D. (2014) Epitypification and neotypification: guidelines with appropriate and inappropriate examples. *Fungal Diversity* 69 (1): 57–91.
- Arnaud, G. (1918) Les Astérinées. Annals d'École National d'Agriculture de Montpellier série 16: 1–288.
- Arx, J.A. von & Müller, E. (1975) A re-evaluation of the bitunicate ascomycetes with key to families and genera. *Studies in Mycology* 9: 1–159
- Barr, M.E. (1987) New taxa and combinations in the Loculoascomycetes. Mycotaxon 29: 501-505.
- Barr, M.E. (1993) Redisposition of some taxa described by J.B. Ellis. *Mycotaxon* 46: 45–76.
- Batista, A.C. (1959) Monografia dos fungos Micropeltaceae. Publicações Instituto de Micologia da Universidade do Recife 56: 1-519.
- Batzer, J.C., Gleason, M.L., Weldon, B., Dixon, P.M. & Nutter, F.W. (2002) Evaluation of postharvest removal of sooty blotch and flyspeck on apples using sodium hypochlorite, hydrogen peroxide with peroxyacetic acid, and soap. *Plant Disease* 86: 1325–1332. http://dx.doi.org/10.1094/PDIS.2002.86.12.1325
- Batzer, J.C., Gleason, M.L., Harrington, T.C. & Tiffany, L.H. (2005) Expansion of the sooty blotch and flyspeck complex on apples based on analysis of ribosomal DNA gene sequences and morphology. *Mycologia* 97: 1268–1286. http://dx.doi.org/10.3852/mycologia.97.6.1268
- Batzer, J.C., Díaz-Arias, M.M., Harrington, T.C., Gleason, M.L., Groenewald, J.Z. & Crous, P.W. (2008) Four species of *Zygophiala* (Schizothyriaceae, Capnodiales) are associated with the sooty blotch and flyspeck complex on apple. *Mycologia* 100: 246–258. http://dx.doi.org/10.3852/mycologia.100.2.246
- Batzer, J.C., Hernandez Rincon, S., Mueller, D.S., Petersen, B.J., Le Corronc, F., McManus, P.S., Dixon, P.M. & Gleason, M.L. (2010) Effect of temperature and nutrient concentration on the growth of six species of sooty blotch and flyspeck fungi. *Phytopathologia Mediterranea* 49: 3–10.
 - http://dx.doi.org/10.1007/s13225-014-0278-5
- Brown, E.M. & Sutton, T.B. (1993) Time of infection of *Gloeodes pomigena* and *Schizothyrium pomi* on apple in North Carolina and potential control by an eradicant spray program. *Plant Disease* 77: 451–455. http://dx.doi.org/10.1094/PD-77-0451
- Chomnunti, P., Hongsanan, S., Hudson, B.A., Tian, Q., Peršoh, D., Dhami, M.K., Alias, A.S., Xu, J., Liu, X., Stadler, M. & Hyde, K.D. (2014) The sooty moulds. *Fungal Diversity* 66: 1–36. http://dx.doi.org/10.1007/s13225-014-0278-5
- Clements, F.E. & Shear, C.L. (1931) Genera of fungi. 2 Vols. H.W. Wilson, USA, New York, 496 pp.
- Cooley, D.R., Rosenberger, D.A., Gleason, M.L., Koehler, G., Cox, K., Clements, J.M., Sutton, T.B., Madeiras, A. & Hartman, J.R. (2011) Variability among forecast models for the apple sooty blotch/flyspeck disease complex. *Plant Disease* 95: 1179–1186. http://dx.doi.org/10.1094/PDIS-03-11-0248
- Díaz Arias, M.M., Batzer, J.C., Wang Wong, A., Bost, S.C., Cooley, D.R., Ellis, M.A., Hartman, J.R., Rosenberger, D.A., Sundin, G.W., Sutton, T.B., Travis, J.W., Wheeler, M.J., Yoder, K.S. & Gleason, M.L. (2010) Diversity and biogeography of sooty blotch and flyspeck fungi on apple in the eastern and midwestern United States. *Phytopathology* 100: 345–355. http://dx.doi.org/10.1094/PHYTO-100-4-0345
- Farr, M.L. (1961) Mycological notes 11. New taxa, synonyms, and records. *The American Midland Naturalist* 66: 355–362. http://dx.doi.org/10.2307/2423035
- Frank, J., Crous, P.W., Groenewald, J.Z., Oertel, B., Hyde, K.D., Phengsintham, P. & Schroers, H.J. (2010) *Microcyclospora* and *Microcyclosporella*: novel genera accommodating epiphytic fungi causing sooty blotch on apple. *Persoonia* 24: 93–105. http://dx.doi.org/10.3767/003158510X510560
- Gleason, M.L., Batzer, J.C., Guangyu, S., Zhang, R., Díaz Arias, M.M., Sutton, T.B., Crous, P.W., Lvanovlć, M., McManus, P.S., Cooley, D.R., Mayr, U., Weber, R.W.S., Yoder, K.S., Ponte, E.M.D., Biggs, A.R. & Oertel, B. (2011) A new view of sooty blotch and flyspeck. *Plant Disease* 95: 368–383. http://dx.doi.org/10.1094/PDIS-08-10-0590
- Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium* 41: 95–98.
- Hao, L., Chen, C., Zhang, R., Zhu, M., Sun, G. & Gleason, M.L. (2013) A new species of *Scolecobasidium* associated with the sooty blotch and flyspeck complex on banana from China. *Mycological Progress* 12: 489–495. http://dx.doi.org/10.1007/s11557-012-0855-5
- Hofmann, T.A. & Piepenbring, M. (2006) New records and host plants of fly-speck fungi from Panama. Fungal Diversity 22: 55-70.
- Hongsanan, S., Chomnunti, P., Crous, P.W., Chukeatirote, E. & Hyde, K.D. (2014) Introducing Chaetothyriothecium, a new genus of

- Microthyriales. *Phytotaxa* 161 (2): 157–164. http://dx.doi.org/10.11646/phytotaxa.161.2.7
- Hongsanan, S., Tian, Q., Bahkali, A.H., Jun-Bo Yang, J.B., McKenzie, E.H.C. & Hyde, K.D. (2015) Zeloasperisporiales order nov., and two new species of *Zeloasperisporium*. *Cryptogamie Mycologie* 36 (3): 301–317. http://dx.doi.org/10.7872/cryb/v36.iss3.2015.301
- Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755. http://dx.doi.org/10.1093/bioinformatics/17.8.754
- Hyde, K.D., Jones, E.B.G., Liu, J.K., Ariyawansha, H., Boehm, E., Boonmee, S., Braun, U., Chomnunti, P., Crous, P., Dai, D.Q., Diederich, P., Dissanayake, A., Doilom, M., Doveri, F., Hongsanan, S., Jayawardena, R., Lawrey, J.D., Li, Y.M., Liu. Y.X., Lücking, R., Monkai, J., Nelsen, M.P., Phookamsak, R., Muggia, L., Pang, K.L., Senanayake, I., Shearer, C.A., Wijayawardene, N., Wu, H.X., Thambugala, M., Suetrong, S., Tanaka, K., Wikee, S., Zhang, Y., Hudson, B.A., Alias, S.A., Aptroot, A., Bahkali, A.H., Bezerra, L.J., Bhat, J.D., Camporesi, E., Chukeatirote, E., Hoog, S.D., Gueidan, C., Hawksworth, D.L., Hirayama, K., Kang, J.C., Knudsen, K., Li, W.J., Liu, Z.Y., McKenzie, E.H.C., Miller, A.N., Nadeeshan, D., Phillip, A.J.L., Mapook, A., Raja, H.A., Tian, Q., Zhang, M., Scheuer, C., Schumm, F., Taylor, J., Yacharoen, S., Tibpromma, S., Wang, Y., Yan, J. & Li, X. (2013) Families of Dothideomycetes. Fungal Diversity 63: 1–313.
 - http://dx.doi.org/10.1007/s13225-013-0263-4
- Ismail, S.I., Batzer, J.C., Harrington, T.C., Crous, P.W., Lavrov, D.V., Li, H. & Gleason, M.L. (in press) Ancestral state reconstruction infers phytopathogenic origins of sooty blotch and flyspeck fungi on apple. *Mycologia*.
- Jayasiri, S.C., Hyde, K.D., Ariyawansa, H.A., Bhat, J., Buyck, B., Cai, L., Dai, Y.C., Abd-Elsalam, K.A., Ertz, D., Hidayat, I., Jeewon, R., Jones, E.B.G., Bahkali, A.H., Karunarathna, S.C., Liu, J.K., Luangsa-ard, J.J., Lumbsch, H.T., Maharachchikumbura, S.S.N., McKenzie, E.H.C., Moncalvo, J.M., Ghobad-Nejhad, M., Nilsson, H., Pang, K.A., Pereira, O.L., Phillips, A.J.L., Raspé, O., Rollins, A.W., Romero, A.I., Etayo, J., Selçuk, F., Stephenson, S.L., Suetrong, S., Taylor, J.E., Tsui, C.K.M., Vizzini, A., Abdel-Wahab, M.A., Wen, T.C., Boonmee, S., Dai, D.Q., Daranagama, D.A., Dissanayake, A.J., Ekanayaka, A.H., Fryar, S.C., Hongsanan, S., Jayawardena, R.S., Li, W.J., Perera, R.H., Phookamsak, R., de Silva, N.I., Thambugala, K.M., Tian, Q., Wijayawardene, N.N., Zhao, R.L., Zhao, Q., Kang, J.C. & Promputtha, I. (2015) The Faces of Fungi database: Fungal names linked with morphology, molecular and human attributes. Fungal Diversity 74: 3–18.
 - http://dx.doi.org/10.1007/s13225-015-0351-8
- Kirk, P.M., Cannon, P.F., Minter, D.W. & Stalpers, J.A. (2008) *Ainsworth & Bisby's dictionary of the fungi, 10th edition.* CAB International, Wallingford, UK. 428 pp.
 - http://dx.doi.org/10.1007/s10658-012-0123-1
- Lumbsch, H.T. & Huhndorf, S.M. (2010) Myconet volume 14, part one. Outline of ascomycota—2009, part two. Notes on ascomycete systematics. Nos. 4751–5113. *Fieldiana, Life and Earth Sciences* 1: 1–64. http://dx.doi.org/10.3158/1557.1
- Mayfield, D.A., Karakaya, A., Batzer, J.C., Blase, J.M. & Gleason, M.L. (2013) Diversity of sooty blotch and flyspeck fungi from apples in northeastern Turkey. *European Journal of Plant Pathology* 135: 805–815. http://dx.doi.org/10.1007/s10658-012-0123-1
- Miñarro, M. & Dapena, E. (2012) *Proceedings of the 15th International Conference on Organic Fruit Growing*. February 20th–22nd Hohenheim, Germany, pp. 47–53.
- Müller, E. & Arx, J.A. von (1962) Die Gattungen der didymosporen Pyrenomyceten. *Beiträge zur Kryptogamenflora der Schweiz* 11 (2): 1–922.
- Nylander, J.A.A., Wilgenbusch, J.C., Warren, D.L. & Swofford, D.L. (2008) AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics* 24: 581–583. http://dx.doi.org/10.1093/bioinformatics/btm388
- Pattengale N.D., Alipour, M., Bininda-emonds, O.R.P., Moret, B.M.E. & Stamatakis, A. (2009) How many bootstrap replicates are necessary? *Lecture Notes in Computer Science* 5541: 184–200. http://dx.doi.org/10.1071/SB04030
- Rannala, B. & Yang, Z. (1996) Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular Evolution* 43: 304–311. http://dx.doi.org/10.1007/BF02338839
- Reynolds, D.R. & Gilbert, G.S. (2005) Epifoliar fungi from Queensland, Australia. *Australian Systematic Botany* 18: 265–289. http://dx.doi.org/10.1071/SB04030
- Schoch, C.L., Crous, P.W., Groenewald, J.Z., Boehm, E.W., Burgess, T.I., de Gruyter, J., de Hoog, G.S., Dixon, L.J., Grube, M., Gueidan, C., Harada, Y., Hatakeyama, S., Hirayama, K., Hosoya, T., Huhndorf, S.M., Hyde, K.D., Jones, E.B., Kohlmeyer, J., Kruys, A., Li, YM., Lucking, R., Lumbsch, H.T., Marvanova, L., Mbatchou, J.S., McVay, A.H., Miller, A.N., Mugambi, G.K., Muggia, L.,

- Nelsen, M.P., Nelson, P., Owensby, C.A., Phillips, A.J., Phongpaichit, S., Pointing, S.B., Pujade-Renaud, V., Raja, H.A., Plata, E.R., Robbertse, B., Ruibal, C., Sakayaroj, J., Sano, T., Selbmann, L., Shearer, C.A., Shirouzu, T., Slippers, B., Suetrong, S., Tanaka, K., Volkmann-Kohlmeyer, B., Wingfield, M.J., Wood, A.R., Woudenberg, J.H., Yonezawa, H., Zhang, Y. & Spatafora, J.W. (2009) A class-wide phylogenetic assessment of Dothideomycetes. *Studies in Mycology* 64: 1–15. http://dx.doi.org/10.3114/sim.2009.64.01
- Silvestro, D. & Michalak, I. (2012) RaxmlGUI: a graphical front-end for RAxML. *Organisms Diversity and Evolution* 12: 335–337. http://dx.doi.org/10.1007/s13127-011-0056-0
- Spegazzini, C. (1899) Fungi Argentini novi vel critici. Anales del Museo Nacional de Historia Natural Buenos Aires 6: 81–365.
- Sun, G.Y., Zhang, R., Li, H. & Gleason, M.L. (2008) Diversity of fungi causing fly-speck like signs on apple in China. *Phytopathology* 98: S153.
- Theissen, F. (1913) Über einige Mikrothyriaceen. Annales Mycologici 11: 493–511.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25: 4876. http://dx.doi.org/10.1093/nar/25.24.4876
- Vilgalys, R. & Hester, M. (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172: 4238–4246.
- White, T.J., Bruns, T.D., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics In: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) PCR Protocols: a guide to methods and applications. Academic, New York, pp. 315–322. http://dx.doi.org/10.1016/b978-0-12-372180-8.50042-1
- Wijayawardene, N.N., Crous, P.W., Kirk, P.M., Hawksworth, D.L., Boonmee, S., Braun, U., Dai, D.Q., D'souza, M.J., Diederich, P., Dissanayake, A., Doilom, M., Hongsanan, S., Jones, E.B.G., Groenewald, J.Z., Jayawardena, R., Lawrey, J.D., Liu, J.K., Lücking, R., Madrid, H., Manamgoda, D.S., Muggia, L., Nelsen, M,P., Phookamsak, R., Suetrong, S., Tanaka, K., Thambugala, K.M., Wanasinghe, D.N., Wikee,, S., Zhang Y., Aptroot, A., Ariyawansa, H.A., Bahkali, A.H., Bhat, D.J., Gueidan, C., Chomnunti, P., Hoog, G.S.D., Knudsen, K., Li, W.J., McKenzie, E.H.C., Miller, A.N., Phillips, A.J.L., Piątek, M., Raja, H.A., Shivas, R.S., Slippers, B., Taylor, J.E., Tian, Q., Wang, Y., Woudenberg, J.H.C., Cai, L., Jaklitsch, W.M. & Hyde, K.D. (2014) Naming and outline of Dothideomycetes 2014 including proposals for the protection or suppression of generic names. *Fungal Diversity* 69: 1–55. http://dx.doi.org/10.1007/s13225-014-0309-2
- Wu, H.X., Li, Y.M., Chen, H. & Hyde, K.D. (2010) Studies on Microthyriaceae: some excluded genera. *Mycotaxon* 113: 147–156. http://dx.doi.org/10.5248/113.147
- Wu, H.X., Schoch, C.L., Boonmee, S., Bahkali, A.H., Chomnunti, P. & Hyde, K.D. (2011) A reappraisal of Microthyriaceae. Fungal Diversity 51: 189–248. http://dx.doi.org/10.1007/s13225-011-0143-8
- Wu, H.X., Li, Y.M, Ariyawansa, H.A., Li, W.J., Yang, H. & Hyde, K.D. (2014) A new species of *Microthyrium* from Yunnan, China. *Phytotaxa* 176 (1): 213–218. http://dx.doi.org/10.11646/phytotaxa.176.1.21
- Yang, H.L., Sun, G.Y., Batzer, J.C., Crous, P.W., Groenewald, J.Z & Gleason, M.L. (2010) Novel fungal genera and species associated with the sooty blotch and flyspeck complex on apple in China and the United States. *Persoonia* 24: 29–37. http://dx.doi.org/10.3767/003158510X492101
- Zhang, M., Gao Shang, S., Han, X., Zhang, R., Latinović, J., Latinović, N., Batzer, J.C., Gleason, M.L. & Sun, G. (2015) New species and record of *Zygophiala* (Capnodiales, Mycosphaerellaceae) on apple from Montenegro. *Phytotaxa* 195 (3): 227–235. http://dx.doi.org/10.11646/phytotaxa.195.3.2
- Zhaxybayeva, O. & Gogarten, J.P. (2002) Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses. *BMC Genomics* 3: 4. http://dx.doi.org/10.1186/1471-2164-3-4