Phytotaxa 176 (1): 260–269
www.mapress.com/phytotaxa/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

http://dx.doi.org/10.11646/phytotaxa.176.1.25

The sexual state of *Setophoma*

RUNGTIWA PHOOKAMSAK[1,2,3,4,5], JIAN-KUI LIU[3,4], DIMUTHU S. MANAMGODA[3,4], EKACHAI CHUKEATIROTE[3,4], PETER E. MORTIMER[1,2], ERIC H.C. MCKENZIE[6] & KEVIN D. HYDE[1,2,3,4,5]

[1] *World Agroforestry Centre, East and Central Asia, Kunming 650201, China*

[2] *Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China*

[3] *Institute of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand*

[4] *School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand*

[5] *International Fungal Research & Development Centre, Research Institute of Resource Insects, Chinese Academy of Forestry, Kunming, Yunnan, 650224, PR China*

[6] *Landcare Research, Private Bag 92170, Auckland, New Zealand*

Abstract

A sexual state of *Setophoma*, a coelomycete genus of *Phaeosphaeriaceae*, was found causing leaf spots of sugarcane (*Saccharum officinarum*). Pure cultures from single ascospores produced the asexual morph on rice straw and bamboo pieces on water agar. Multiple gene phylogenetic analysis using ITS, LSU and *RPB2* showed that our strains belong to the family *Phaeosphaeriaceae.* The strains clustered with *Setophoma sacchari* with strong support (100% ML, 100% MP and 1.00 PP) and formed a well-supported clade with other *Setophoma* species. Therefore our strains are identified as *S. sacchari.* In this paper descriptions and photographs of the sexual and asexual morphs of *S. sacchari* are provided. The sexual state of *S. sacchari* is compared with *Leptosphaeria sacchari*, *L. saccharicola, Phaeosphaeria nodorum* and *Sphaerulina sacchari* which have similar morphological characters; however they differ in size and colour of ascospores and in the characters of the asexual state. *Setophoma* is clearly separated from other *Phaeosphaeria* species based on the phylogenetic analysis.

Key words: asexual morph, Phaeosphaeriaceae, phylogeny, *Setophoma sacchari*, sugarcane, taxonomy

Introduction

Phaeosphaeriaceae is a large and important family of *Pleosporales*, in the suborder *Pleosporineae*, Dothideomycetes (Câmara *et al.* 2002, Kodsueb *et al.* 2006, Schoch *et al.* 2006, 2009, Suetrong *et al.* 2009, Zhang *et al.* 2009, 2012, Gruyter *et al.* 2010, Hyde *et al.* 2013). Members of *Phaeosphaeriaceae* can be endophytic, epiphytic, saprobic, or pathogenic on plants with many species and their asexual morphs causing serious diseases of crops worldwide (Shoemaker & Babcock 1989, Schoch *et al.* 2006, 2009, Zhang *et al.* 2009, 2012, Hyde *et al.* 2013). Barr (1979) introduced *Phaeosphaeriaceae* with *Phaeosphaeria* as the type genus and described the family as saprobic, pathogenic or hyperparasitic. She reported ascomata as immersed, erumpent or superficial, globose or conical, sometimes multiloculate, short papillate or rostrate, small to medium sized, while the asci as bitunicate and the ascospores as hyaline, yellowish or brown, narrowly or widely obovoid or acerose, aseptate or septate.

Barr (1979) included 15 genera in the family, although some have been subsequently placed in other families. Zhang *et al.* (2012) re-examined the *Phaeosphaeriaceae* and accepted 18 genera. Based on multigene phylogenetic analysis, she gave a bolder familial concept with members of the *Phaeosphaeriaceae* having small to medium sized ascomata, and septate, ellipsoidal to fusiform or filiform ascospores. Hyde *et al.* (2013) re-circumscribed the families of Dothideomycetes and accepted 27 genera in *Phaeosphaeriaceae* based on morphology and phylogeny of both sexual and asexual morphs. *Phaeosphaeriaceae* are often associated with monocotyledonous plants, have a peridium of pseudoparenchymatous cells and asexual morphs are mostly classified in *Ampelomyces*,

 Accepted by Gareth Jones: 3 Mar. 2014; published: 20 Aug. 2014

Chaetosphaeronema, Neosetophoma, *Neostagonospora Parahendersonia, Paraphoma*, *Parastagonospora*, *Phaeoseptoria, Sclerostagonospora*, *Setophoma, Tiarospora, Vrystaatia* , *Wojnowicia*, *Xenoseptoria* (Câmara *et al.* 2002, Schoch *et al.* 2009, Zhang *et al.* 2009, 2012, Gruyter *et al.* 2010, Wijayawardene *et al.* 2012, Hyde *et al.* 2013, Quaedvlieg *et al.* 2013). However, many species of *Phaeosphaeriaceae* have recently been reported on dicotyledonous plants and their phylogeny needs to be investigated using fresh collections (Zhang *et al.* 2012, Hyde *et al.* 2013).

Setophoma is an asexual genus in *Phaeosphaeriaceae*, which was described by Gruyter *et al.* (2010). Three species, *Setophoma sacchari* (Bitanc.) Gruyter *et al., Setophoma terrestris* (H.N. Hansen) Gruyter *et al.* and *Setophoma chromolaenae* Quaedvlieg *et al.* are accommodated in this genus based on phylogenetic investigation which form a clade related to *Phaeosphaeriaceae* with low bootstrap support (Gruyter *et al.* 2010, Quaedvlieg *et al.* 2013). On agar the genus produces superficial or submerged pycnidia covered by setae, phialidic conidiogenous cells, and hyaline, ellipsoidal to subcylindrical, aseptate, guttulate conidia (Gruyter *et al.* 2010, Quaedvlieg *et al.* 2013). No sexual state is known for *Setophoma*. *Setophoma* formed a weakly supported clade with *Neosetophoma* and was related to the sexual morph genera *Ophiosphaerella*, *Phaeosphaeria* and *Phaeosphaeriopsis* in Gruyter *et al.* (2010).

In the present study, we collected the sexual state of *Setophoma sacchari.* Both states are described and illustrated. Phylogenetic analysis also shows *Setophoma* to belong in *Phaeosphaeriaceae*.

Material and methods

Isolation and identification

Sugarcane leaves with diseased spots were collected from Chiang Mai Province, Thailand and returned to the laboratory for examination following the methods described by Taylor & Hyde (2003) and Phookamsak *et al*. (2013). Pure cultures were derived from single ascospores following methods described in Chomnunti *et al.* (2011) and Phookamsak *et al.* (2013). Corn meal agar (CMA, 17 g/l sterile distilled water, Difco corn meal agar), malt extract agar (MEA; 33.6 g/l sterile distilled water, Difco malt extract) and potato dextrose agar (PDA; 39 g/l sterile distilled water, Difco potato dextrose) were utilized for cultivation of fungal colonies and recording their growth rate. Sterile bamboo pieces on water agar (WA; 15 g/l sterile distilled water) were used to encourage sporulation. Fungi isolated in our study are deposited in Mae Fah Luang University Culture Collection (MFLUCC) with duplicates in the International Collection of Microorganisms from Plants (ICMP), Landcare Research, New Zealand. Dried specimens are deposited in the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand.

DNA extraction, PCR amplification and sequencing

Fungal genomic DNA was extracted from fresh fungal mycelium grown on PDA media at 25–27 °C for 4 weeks. A Biospin Fungus Genomic DNA Extraction Kit (BioFlux®, China) was used to extract DNA according to the manufacturer's instructions (Hangzhou, P.R. China) (Phookamsak *et al.* 2013).

Polymerase chain reaction (PCR) was performed for DNA amplification using the primers LROR and LR5 (Vilgalys & Hester 1990) for the partial large subunit nuclear rDNA (28S, LSU); NS1 and NS4 (White *et al.* 1990) for the small subunit nuclear rDNA (18S, SSU); ITS4 and ITS5 (White *et al.* 1990) for the internal transcribed spacers (5.8S, ITS); EF1-983F and EF1-2218R (Rehner 2001) for the translation elongation factor 1-alpha gene (*TEF1α*); and fRPB2-5F and fRPB2-7cR (Liu *et al.* 1999) for the partial RNA polymerase second largest subunit (*RPB2*). The procedure of DNA amplification followed Phookamsak *et al.* (2013).

Phylogenetic analysis

Phylogenetic analysis was performed by combining partial LSU, ITS and *RPB2* genes. The generated sequences were analyzed with other closest match sequences obtained from GenBank (Table 1). In addition, members of *Cucurbitariaceae, Didymellaceae, Dothidotthiaceae, Leptosphaeriaceae,* and *Pleosporaceae* were included in the analysis. *Melanomma pulvis-pyrius* was designated as an outgroup taxon. The fungal sequence strains were combined and aligned using MAFFT v. 7.036 (Katoh & Standley 2013) and improved manually where necessary in BioEdit v. 7.2 (Hall 1999). The phylogenetic analysis was performed using maximum-parsimony (MP) in PAUP v. 4.0b10 (Swofford 2002), Bayesian analyses in MrBayes v. 3.0b4 (Ronquist & Huelsenbeck 2003) and RAxML v.7.2.8 as part of the "RAxML-HPC2 on TG" tool (Stamatakis 2006, Stamatakis *et al.* 2008) at the CIPRES webportal (Miller *et al.* 2010) for maximum likelihood analysis (ML). Maximum-parsimony was

conducted using the heuristic search option with 1,000 random sequences addition. Maxtrees were setup to 5,000 with tree-bisection reconnection (TBR) of branch-swapping algorithm and a zero of maximum branch length was collapsed. Gaps were treated as missing data. The setup detail of the following phylogenetic analysis programs are described in Liu *et al.* (2012) and Phookamsak *et al.* (2013). The phylogram was visualized in Treeview (Page 1996) with bootstrap values above and below the branches (Fig. 1). The sequences generated in this study are deposited in GenBank.

Results

Phylogenetic analysis

The molecular phylogeny based on combined LSU, ITS, and *RPB2* gene data comprised 40 taxa, with the dataset consisting of 2,692 aligned nucleotide characters, of which 1,613 characters are constant, 236 variable and 780 parsimony informative. Six equally parsimonious trees were generated and the first of the most parsimonious tree is selected. The Kishino-Hasegawa test shows length = 3577 steps with CI = 0.479, RI = 0.531, RC = 0.254 and HI = 0.521. The phylogenetic trees obtained from MP, ML and Bayesian analysis are similar in topology and not significantly different. The best scoring RAxML tree is chosen to represent the relationships among each taxon (Fig. 1). The values of the Bayesian posterior probabilities (PP) from MCMC analyses provided significant (equal or higher than 95%) support for most of the clades as defined below the nodes. Bootstrap support (BS) values of MP and ML are shown above the nodes (equal or greater than 60%).

TABLE 1. Isolates used in this study and their GenBank accession numbers. The newly generated sequences are indicated in bold.

Taxon	Culture/voucher	GenBank Accession		
		ITS	LSU	RPB2
Cochliobolus heterostrophus	CBS 134.39	DQ491489	AY544645	DQ247790
Cucurbitaria berberidis	CBS 394.84	–	GQ387605	–
Cucurbitaria berberidis	CBS 363.93	JF740191	GQ387606	–
Dothidotthia aspera	CPC 12933	–	EU673276	–
Dothidotthia symphoricarpi	CPC 12929 / CBS 119687^{T}	–	EU673273	–
Entodesmium rude	CBS 650.86	–	GU301812	–
Leptosphaeria doliolum	CBS 505.75^{T}	JF740205	GU301827	–
Leptosphaeria maculans	DAOM 229267	–	DQ470946	DQ470894
Leptosphaerulina australis	CBS 311.51^{T}	–	FJ795500	GU456357
Loratospora aestuarii	JK 5535B	–	GU301838	GU371760
Melanomma pulvis-pyrius	CBS 124080^{T}	–	GU456323	GU456350
Neosetophoma samarorum	CBS 138.96^{T}	KF251160	KF251664	KF252168
Ophiosphaerella herpotricha	CBS 620.86	KF498728	DQ678062	DQ677958
Paraphoma radicina	CBS 111.79^{T}	KF251172	KF251676	KF252180
Parastagonospora nodorum	CBS 110109	KF251177	KF251681	KF252185
Phaeosphaeria alpina	CBS 456.84^{T}	KF251181	KF251684	KF252188
Phaeosphaeria ammophilae	CBS 114595	–	GU301859	GU371724
Phaeosphaeria eustoma	CBS 573.86	AF439479	DQ678063	DQ677959
Phaeosphaeria juncicola	CBS 110108	KF251183	KF251686	KF252190
Phaeosphaeria nigrans	CBS 307.79	KF251184	KF251687	KF252191
Phaeosphaeria oryzae	CBS 110110^{T}	KF251186	KF251689	KF252193
Phaeosphaeria papayae	CBS 135416/S528^{T}	KF251187	KF251690	KF252194

.....continued on the next page

TABLE 1. (Continued)

Taxon	Culture/voucher	GenBank Accession		
		ITS	LSU	RPB2
Phaeosphaeria phragmiticola	CBS 459.84	KF251188	KF251691	KF252195
Phaeosphaeria typharum	CBS 296.54	KF251192	KF251695	KF252199
Phaeosphaeria vagans	CBS 604.86	KF251193	KF251696	KF252200
Phaeosphaeriopsis musae	CBS 120026^{T}	DQ885894	GU301862	–
Phoma herbarum	CBS 276.37^{T}	JF810524	DQ678066	DQ677962
Plenodomus biglobosus	CBS 119951	JF740198	JF740274	–
Pleospora herbarum	CBS 191.86^{T}	DQ491516	DQ247804	DQ247794
Pyrenochaeta nobilis	CBS 407.76^{T}	EU930011	DQ678096	DQ677991
Pyrenochaeta nobilis	CBS 566.75	–	GQ387616	–
Pyrenophora phaeocomes	DAOM 222769	DQ491507	DQ499596	DQ497614
Setomelanomma holmii	CBS 110217	–	–	GU371800
Setophoma chromolaena	CBS 135105^{T}	KF251244	KF251747	KF252249
Setophoma sacchari	CBS 333.39^{T}	KF251245	KF251748	KF252250
Setophoma sacchari	**MFLUCC11-0154**	**KJ476144**	**KJ476146**	**KJ461317**
Setophoma sacchari	**MFLUCC 12-0241**	**KJ476145**	**KJ476147**	**–**
Setophoma terrestris	CBS 335.87^{T}	KF251247	KF251750	KF252252
Xenoseptoria neosaccardoi	CBS 128665^{T}	KF251281	KF251784	KF252286

Abbreviations: CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; DAOM: Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; Culture and specimen abbreviations: CPC**:** Collection of Pedro Crous housed at CBS**;** JK: J. Kohlmeyer; S: Working collection of William Quaedvlieg; T ex-type/ex-epitype isolates.

The phylogenetic analysis obtained from maximum likelihood, maximum parsimony and Bayesian analysis gave similar results for related families. Five strains of *Setophoma* formed a single clade in the family *Phaeosphaeriaceae*. Our strains (MFLUCC11-0154 and MFLUCC12-0241) from Thailand are closely related to *S. sacchari* (CBS 333.39) from Brazil with strong support (100% ML, 100% MP and 1.00 PP) and are regarded as the same species. A comparison of the ITS gene region between the two strains from Thailand and the strain from Brazil show 11 base pair differences, which are not significantly different. Therefore, we name our strains as *S. sacchari*.

The various *Phaeosphaeria* species are divided into several subclades. *Phaeosphaeria ammophilae* (Lasch) Kohlm. & E. Kohlm, *Phaeosphaeria phragmiticola* Leuchtm and *Phaeosphaeria vagans* (Niessl) O.E. Eriksss. form a well-supported clade with *Ophiosphaerella herpotricha* (Fr.) J. Walker and *Neosetophoma samarorum* (Desm.) Gruyter *et al.*, while *Phaeosphaeria alpina* Leuchtm. and *Phaeosphaeria typharum* (Desm.) L. Holm form a sister clade with *Phaeosphaeria eustoma* (Fuckel) L. Holm, *Phaeosphaeria nigrans* (Roberge ex Desm.) L. Holm and *Phaeosphaeria nodorum* (E. Müll.) Hedjar. *Phaeosphaeriopsis musae* Arzanlou & Crous often groups with *Phaeosphaeria oryzae* I. Miyake and *Phaeosphaeria papaya* (Speg.) Quaedvlieg *et al.* in our phylogenetic analysis. The morphological characters of *Ph. musae* are more typical of *Phaeosphaeria* than *Phaeosphaeriopsis* species. *Setomelanomma holmii* M. Morelet forms a well-supported clade with two asexual morphs, *Xenoseptoria neosaccardoi* Quaedvlieg *et al.* and *Paraphoma radicina* (McAlpine) Morgan-Jones & J.F. White, while *Phaeosphaeria juncicola* (Rehm ex G. Winter) L. Holm forms a sister group with *Loratospora aestuarii* Kohlm. & Volkm.-Kohlm and *Entodesmium rude* Riess.

Based on morphology and phylogeny, we propose to accommodate our strains under the name *Setophoma sacchari* and provide the first report of the sexual state. Detailed descriptions and illustrations are provided below.

Taxonomy

The sexual state of *Setophoma sacchari* was found on leaf spots on sugarcane and produced an asexual state on bamboo pieces on WA after 4 weeks. The morphology and illustration of both sexual and asexual state are provided.

Setophoma sacchari (Bitanc.) Gruyter, Aveskamp & Verkley, Mycologia 102(5): 1077 (2010) Figs 2, 3

≡ *Pyrenochaeta sacchari* Bitanc., Archos Inst. biol., S. Paulo 9(27): 301 (1938) MycoBank: MB 514660

Pathogenic or *saprobic* on sugarcane causing a ring spot disease, lesions 0.7–2 cm long, ovoid to elongate or irregular, initially reddish brown or reddish purple to brown, becoming white to pale brown in the center, separated from the healthy tissue by a reddish brown to purple-brown or black margin. Sexual state: *Ascomata* 120–180 µm high, 140–190 µm diam, immersed or semi-immersed, visible as minute black dots on host surface, uniloculate, globose to subglobose, brown to dark brown, solitary or gregarious; centrally ostiolate, circular, papillate. *Peridium* 8.5–15 µm wide, with 3–5 layers, the outer layers composed of brown, thick-walled cells of *textura angularis*, the inner layers composed of hyaline to pale brown, thin-walled cells of *textura prismatica*. *Hamathecium* composed of numerous, hyaline, frequently septate, broadly cellular 1.5–3 µm wide pseudoparaphyses, often constricted at the septa, branching at the apex, embedded in mucilage. *Asci* 60–75(–85) × 12–15(–17) µm ($\overline{x}$ = 67.3 × 14.1 µm, n = 25), 8-spored, bitunicate, fissitunicate, cylindrical to cylindric-clavate, short pedicellate, apically rounded with well-developed narrowly ocular chamber (1–2.5 µm wide), smooth-walled, arising from the base of ascoma. *Ascospores* 20–23(–25) × 5–6 µm ($\overline{x}$ = 22.5 × 5.6 µm, n = 30), cylindrical to cylindric-clavate, hyaline, overlapping or irregularly biseriate, 3-septate, usually widest at the second cell from apex, smooth-walled with large guttules. *Ascospores germinate* within 12 hours on water agar, geminating from end cells. Asexual state: produced on rice straw and bamboo stem on water agar after 4 weeks. *Conidiomata* superficial, initially light brown, becoming brown to dark brown, hairy, globose to subglobose, scattered to clustered, thin-walled, composed of pseudoparenchymatous cells of *textura angularis*, light brown to brown. *Conidiogenous cells* 5–11 × 2–3.5 µm ($\overline{x}$ = 7.7 × 2.7 µm, n = 10), single, phailidic, unbranched, oblong to cylindrical, cylindric-clavate, or ampulliform, hyaline, slightly curved, aseptate, lining the inner cavity. *Conidia* 8–11.5 × 3–5 µm ($\overline{x}$ = 8.9 × 3.5 µm, n = 30), oblong to ellipsoidal, rarely irregular, with rounded to obtuse ends, hyaline, aseptate, smooth-walled with large guttules when immature, becoming rough-walled at maturity.

Culture characters:—Colonies on corn meal agar (CMA, 17 g/l sterile distilled water, Difco corn meal agar) slow growing, 10–11 mm diam after 2 weeks at 25–30°C, circular, white at the edge, yellowish to orangish in the centre; reverse white at the edge, yellowish to orangish in the centre, dense, convex or dome-shaped to umbonate, with entire edge, glabrous with tiny granular on surface, no pigment produced.

Material examined:—THAILAND. Chiang Mai Province: Mae Wang District, Khun Wang Royal Project, on living leaves of *Saccharum officinarum* L. (Poaceae), 5 October 2010, *R. Phookamsak* RP0070 (MFLU11-0190!); living culture = MFLUCC11-0154!; *ibid.,* San Sai District, Maejo University, on dead leaves of *Saccharum officinarum*, 5 February 2012, *R. Phookamsak* RP0127 (MFLU12-2470!); living culture = MFLUCC12-0241! = ICMP 20048.

Gene sequence data:—MFLUCC11-0154 = ITS (KJ476144), LSU (KJ476146), SSU (KJ476148), *RPB2* (KJ461317), and *TEF1α* (KJ461319); MFLUCC12-0241 = ITS (KJ476145), LSU (KJ476147), SSU (KJ476149), and *TEF1α* (KJ461318).

Notes:—*Setophoma sacchari* was introduced by Gruyter *et al.* (2010) to accommodate *Pyrenochaeta sacchari* Bitanc. The species was described as causing a leaf spot disease on sugarcane in Brazil. Bitancourt (1938) stated that typically disease occurs on young leaves with the first symptoms visible as small pure white spots, well-delimited on lower surface, with smaller and much less conspicuous spots on the upper surface. The individual spots are usually surrounded by a thin purple to vinaceous-buff coloured zone, which can often be confused with *Leptosphaeria sacchari* Breda de Haan. Our strains are morphologically similar to the original description of the symptoms on leaves and conidia of *S. sacchari*. Other *Setophoma* species form thick-walled pycnidia covered by setae (Gruyter *et al.* 2010, Quaedvlieg *et al*. 2013). Our strains formed thin-walled pycnidia which were initially covered by hairs, but became glabrous at maturity.

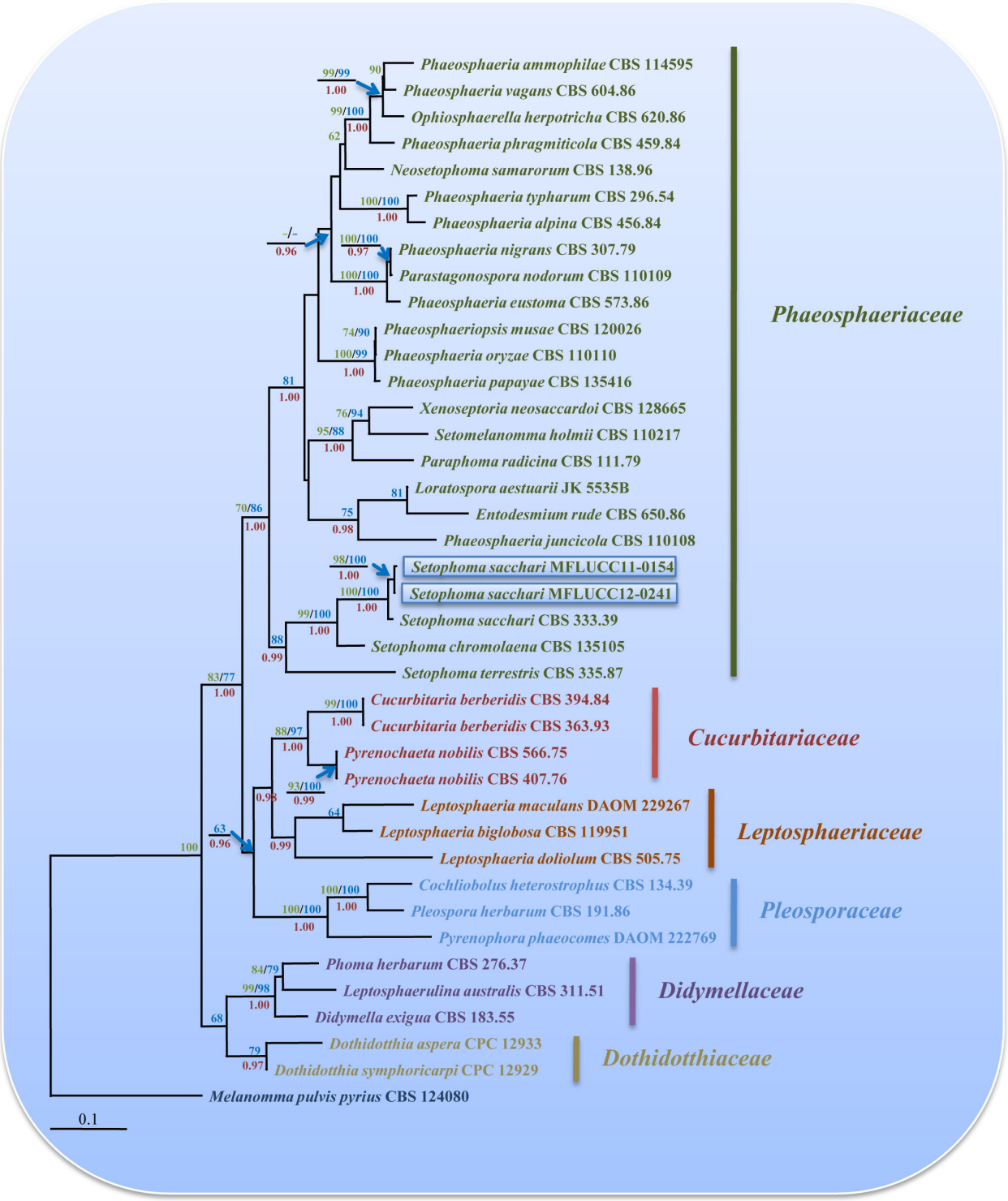

FIGURE 1. RAxML tree based on a combined dataset of LSU, ITS and RPB2 gene sequences. Bootstrap support values for maximum parsimony (MP, green) and maximum likelihood (ML, blue) higher than 60% are given above the nodes. Bayesian posterior probabilities (BYPP, red) greater than 0.95 are provided below the nodes. The tree is rooted to *Melanomma pulvis-pyrius* (CBS 124080).

Earlier names for *Setophoma sacchari* might exist in *Leptosphaeria sacchari* Breda de Haan, *Leptosphaeria sacchari* Speg., *Leptosphaeria saccharicola* Henn., *Sphaerulina sacchari* Henn. and some *Phaeosphaeria* species, such as *Phaeosphaeria nodorum.* According to Hudson (1960), Breda de Haan (1892) described *Leptosphaeria sacchari* as the cause of ring spot disease of sugarcane. Symptoms included irregularly oval, initially dark green, later brown and colourless 0.5–1 cm lesions, which are dry in the centre and, separated from the healthy tissue by

red-brown margin. Ascomata are 140 µm diam, globose, with a thin brown ascomal wall, and ascospores are 20–24 µm long, 4-celled, with one main cell thicker than cells on either side, the thinner cells are mostly 3 µm diam, becoming dark in colour when mature or outside the asci. Our stains are similar to *L. sacchari*, however, they cause larger leaf lesions, the ascomata are larger, and the ascospores do not become dark when mature or discharged from the asci. However, it is possible that *L. sacchari* is an earlier name for *S. sacchari.*

FIGURE 2. Sexual state of *Setophoma sacchari* on living leaves of *Saccharum officinarum*. a. Symptoms on host. b. Ascomata immersed in host tissue. c. Section through ascoma. d. Section through peridium. e. Pseudoparaphyses. f–i. Asci. j–n. Ascospores. o. Spore germination. p–q. Colony on CMA (p from above, q from below). Scale bars: c = 50 µm, d–i, o = 20 µm, j–n = 10 µm.

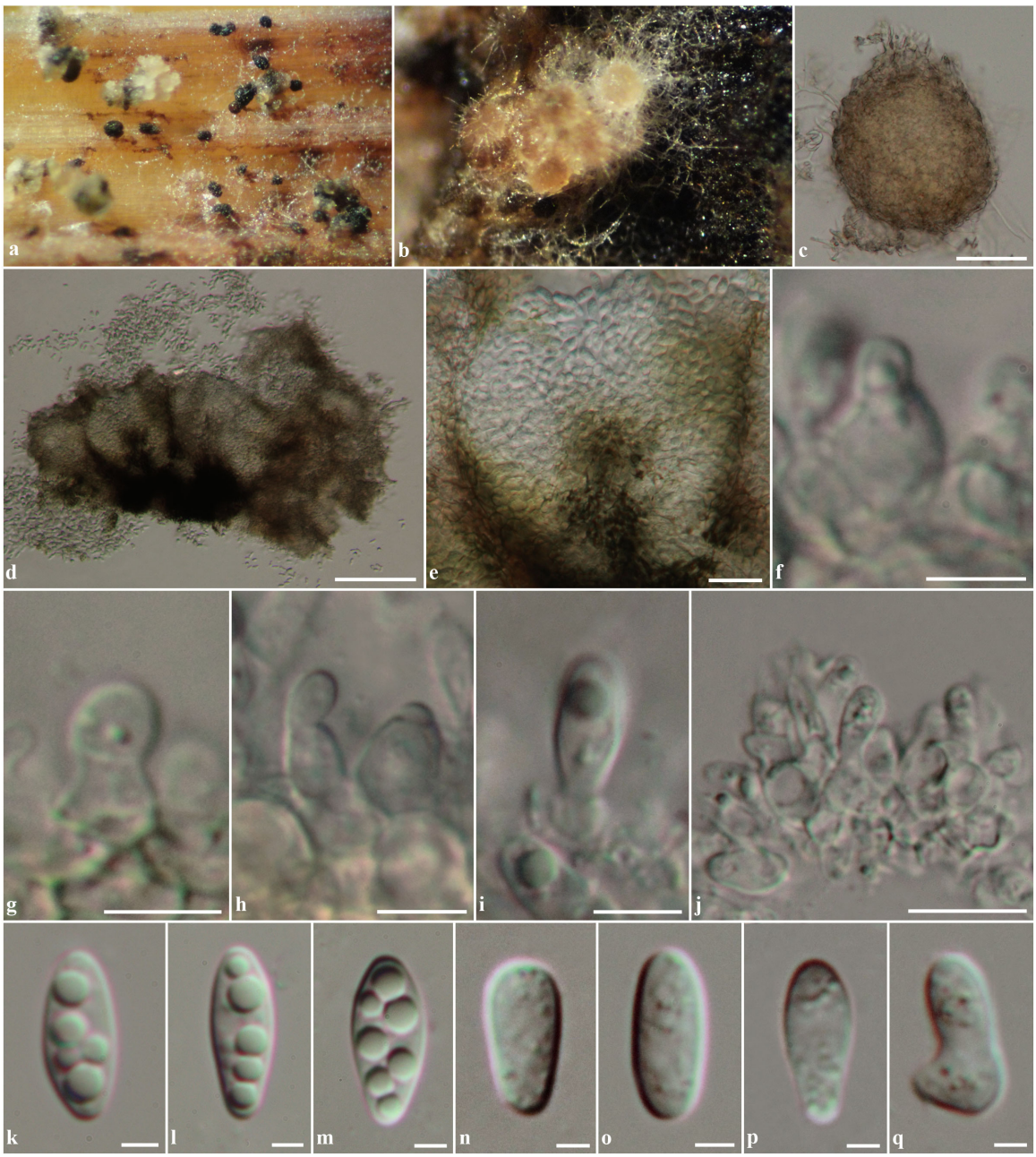

FIGURE 3. Asexual state of *Setophoma sacchari*. a. Conidiomata on rice straw on WA after 4 weeks (MFLUCC11-0154). b. Immature conidiomata on living culture (MFLUCC11-0154). c. Conidiomata on WA (MFLUCC12-0241). d. Section through conidiomata (MFLUCC12-0241). e. Section through pycnidial wall (MFLUCC12-0241). f–j. Conidiogenous cells (MFLUCC12-0241). k–m. Conidia (MFLUCC11-0154). n–q. Conidia (MFLUCC12-0241). Scale bars: c, d = 100 µm, e = 20 µm, j = 10 µm, f–i = 5 µm, k–q = 2 µm.

Leptosphaeria sacchari Speg., which has similar-sized (25 × 5–6 µm), 3-septate ascospores is a homonym of *L. sacchari* Breda de Haan and it was thus renamed *L. spegazzinii* Sacc. & P. Syd. (Saccardo & Sydow 1899). *Leptosphaeria spegazzinii* has smaller ascomata than *Setophoma sacchari* and its ascospores are pale olivaceous (Saccardo & Sydow 1899). *Leptosphaeria saccharicola* forms pale leaf spots on *Saccharum* and has 3–4-septate ascospores (15–19 × 4 µm), which become brown when mature (Hennings 1900, Saccardo 1902). *Sphaerulina sacchari* also forms leaf spots on sugarcane and has 3-septate ascospores (Hennings 1905), which are, however, smaller (15–20 × 3.5–4 µm). The sexual state of *Setophoma sacchari* is similar to *Phaeosphaeria nodorum* in size and septation of ascospores, asci and ascomata, but ascospores and asexual characters differ in colour (Bitancourt 1938, Hedjaroude 1968). *Setophoma sacchari* has hyaline ascospores and forms a *Phoma*-like state, while *Phaeosphaeria nodorum* has subhyaline to pale yellowish ascospores and forms a *Stagonospora*-like asexual state

(Bitancourt 1938, Câmara *et al.* 2002, Hedjaroude 1968). Most *Phaeosphaeria* species have yellowish to brown ascospores and grow fast in agar media (Shoemaker & Babcock 1989), while *Setophoma sacchari* has hyaline ascospores and grows very slowly in culture. Multigene phylogenetic analysis shows that *S. sacchari* is in a related clade to *Phaeosphaeriaceae* but separate from other *Phaeosphaeria* species. It is clear that more than one ascomycete species with somewhat similar ascospores, with three septa and the second cells being swollen, causes ring spot disease of sugarcane as indicated by Hudson (1960). One of these earlier names may represent *Setophoma sacchari,* but as it is unlikely that sequences could be obtained from the old herbarium specimens and, in some cases, it is not possible to examine type material (e.g. PAD and LPS). Therefore, we utilize the name *Setophoma sacchari* for the disease of sugarcane as cultures and herbarium materials are readily available.

Acknowledgements

The Royal Golden Jubilee Ph. D. Program (PHD/0090/2551) under Thailand Research Fund and Dothideomycetes grant under Mae Fah Luang University (56 1 01 02 00 32), and Humidtropics, a CGIAR Research Program that aims to develop new opportunities for improved livelihoods in a sustainable environment are gratefully acknowledged for financial and laboratory support. The Chinese Academy of Sciences, project number 2013T2S0030 is gratefully thanked by KD Hyde, for the award of Visiting Professorship for Senior International Scientists at Kunming Institute of Botany. Wen Jing Li and International Fungal Research & Development Centre, Research Institute of Resource Insects, Chinese Academy of Forestry are thanked for molecular data.

References

Barr, M.E. (1979) A classification of Loculoascomycetes. *Mycologia* 71: 935–957. http://dx.doi.org/10.2307/3759283

Bitancourt, A.A. (1938) *Pyrenochaeta sacchari* n.sp. and a leaf spot of sugar-cane. *Archivos do Instituto Biologico, Sao Paulo* 9(27): 299–302.

Câmara, M.P.S., Palm, M.E., van Berkum, P. & O'Neill, N.R. (2002) Molecular phylogeny of *Leptosphaeria* and *Phaeosphaeria*. *Mycologia* 94: 630–640.

Chomnunti, P., Schoch, C.L., Aguirre-Hudson, B., KoKo, T.W., Hongsanan, S., Jones, E.B.G., Kodsub, R., Chukeatirote, E., Bahkali, A.H. & Hyde, K.D. (2011) *Capnodiaceae*. *Fungal Diversity* 51: 103–134. http://dx.doi.org/10.1007/s13225-011-0145-6

Gruyter, J.D., Woudenberg, J.H.C., Aveskamp, M.M., Verkley, G.J.M., Groenewald, J.Z. & Crous, P.W. (2010) Systematic reappraisal of species in *Phoma* section *Paraphoma*, *Pyrenochaeta* and *Pleurophoma*. *Mycologia* 102: 1066–1081. http://dx.doi.org/doi:10.3852/09-240

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.

Hedjaroude, A. (1968) Études taxonomiques sur les *Phaeosphaeria* Miyake et leurs formes voisines (ascomycètes). *Sydowia* 22(1-4): 57–107.

Hennings, P.C. (1900) Fungi Paráenses I. *Hedwigia Beiblatt* 39: 79.

Hennings, P.C. (1905) Fungi Amazonici IV a cl. Ernesto Ule collecti (Appendix). *Hedwigia* 44: 62.

Hudson, H.J. (1960) Pyrenomycetes of sugarcane and others grasses in Jamaica; I. Conidia of *Apiospora camptospora* and *Leptosphaeria sacchari*. *Transactions of the British Mycological Society* 43(4): 607–616. http://dx.doi.org/10.1016/S0007-1536(60)80051-4

Hyde, K.D., Jones, E.B.G., Liu, J.K., Ariyawansa, H., Boehm, E., Boonmee, S., Braun, U., Chomnunti, P., Crous, P.W., 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., Monka, J., Muggia, L., Nelsen, M.P., Pang, K.L., Phookamsak, R., Senanayake, I.C., Shearer, C.A., Seutrong, S., Tanaka, K., Thambugala, K.M., Wijayawardene, N.N., Wikee, S., Wu, H.X., Zhang, Y., Aguirre-Hudson, B., Alias, S.A., Aptroot A., Bahkali, A.H., Bezerra, J.L., Bhat, D.J., Camporesi, E., Chukeatirote, E., Gueidan, C., Hawksworth, D.L., Hirayama, K., De Hoog, S., Kang, J.C., Knudsen, K., Li, W.J., Li, X., Liu, Z.Y., Mapook, A., McKenzie, E.H.C., Miller, A.N., Mortimer, P.E., Phillips, A.J.L., Raja, H.A., Scheuer, C., Schumm, F., Taylor, J.E., Tian, Q., Tibpromma, S., Wanasinghe D.N., Wang, Y., Xu J., Yacharoen, S. Yan, J. & Zhang, M. (2013) Families of Dothideomycetes. *Fungal Diversity* 63: 1–313. http://dx.doi.org/10.1007/s13225-013-0263-4

Katoh, K. & Standley, K. (2013) MAFFT Multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* http://mbe.oxfordjournals.org/ (accessed June 22, 2013).

Kodsueb, R., Dhanasekaran, V., Aptroot, A., Lumyong, S, McKenzie, E.H.C., Hyde, K.D. & Jeewon, R. (2006) The family *Pleosporaceae*: intergeneric relationships and phylogenetic perspectives based on sequence analyses of partial 28S rDNA.

Mycologia 98(4): 571–583.
http://dx.doi.org/10.3852/mycologia.98.4.571

Liu, J.K., Phookamsak, R., Doilom, M., Wikee, S., Li, Y.M., Ariyawansha, H., Boonmee, S., Chomnunti, P., Dai, D.Q., Bhat, D.J., Romero, A.I., Zhuang, W.Y., Monkai, J., Jones, E.B.G., Chukeatirote, E., Ko Ko, T.W., Zhao, Y.C., Wang, Y. & Hyde, K.D. (2012) Towards a natural classification of *Botryosphaeriales*. *Fungal Diversity* 57: 149–210.
http://dx.doi.org/10.1007/s13225-012-0207-4

Liu, Y.J., Whelen, S. & Hall, B.D. (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. *Molecular Biology and Evolution* 16: 1799–1808.

Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) *Creating the CIPRES Science Gateway for inference of large phylogenetic trees*. Gateway Computing Environments Workshop 2010 (GCE), pp. 1–8.

Page, R.D.M. (1996) TreeView: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* 12(4): 357–358.

Phookamsak, R., Liu, J.K., Chukeatirote, E., McKenzie, E.H.C. & Hyde, K.D. (2013) Phylogeny and morphology of *Leptosphaerulina saccharicola* sp. nov. and *Pleosphaerulina oryzae* and relationships with *Pithomyces*. *Cryptogamie Mycologie* 34(4): 303–319.
http://dx.doi.org/10.7872/crym.v34.iss4.2013.303

Quaedvlieg, W., Verkley, G.J.M., Shin, H.-D., Barreto, R.W., Alfenas, A.C., Swart, W.J., Groenewald, J.Z. & Crous, P.W. (2013) Sizing up *Septoria*. *Studies in Mycology* 75: 307–390.
http://dx.doi.org/10.3114/sim0017

Rehner, S. (2001) *Primers for Elongation Factor 1-α (EF1- α)*. http://ocid.NACSE.ORG/research/deephyphae/EF1primer.pdf.

Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19(12): 1572.
http://dx.doi.org/10.1093/bioinformatics/btg180

Saccardo, P.A. (1902) *Sylloge Fungorum* 16: 156–157.

Saccardo, P.A. & Sydow, P. (1899). *Sylloge Fungorum* 14(1): 1–724.

Schoch, C.L., Shoemaker, R.A., Seifert, K.A., Hambleton, S., Spatafora, J.W. & Crous, P.W. (2006) A multigene phylogeny of the Dothideomycetes using four nuclear loci. *Mycologia* 98: 1041–1052.
http://dx.doi.org/10.3852/mycologia.98.6.1041

Schoch, C.L., Crous, P.W., Groenewald, J.Z., Boehm, E.W.A., 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.G., Kohlmeyer, J., Kruys, A., Li, Y.M., 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

Shoemaker, R.A. & Babcock, C.E. (1989) *Phaeosphaeria*. *Canadian Journal of Botany* 67: 1500–1599.
http://dx.doi.org/10.1139/b89-199

Stamatakis, A. (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690.
http://dx.doi.org/10.1093/bioinformatics/btl446

Stamatakis, A., Hoover, P. & Rougemont, J. (2008) A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* 57: 758–771.

Suetrong, S., Schoch, C.L., Spatafora, J.W., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Sakayaroj, J., Phongpaichit, S., Tanaka, K., Hirayama, K. & Jones, E.B.G. (2009) Molecular systematics of the marine Dothideomycetes. *Studies in Mycology* 64: 155–173.
http://dx.doi.org/10.3114/sim.2009.64.09

Swofford, D.L. (2002) *PAUP: Phylogenetic analysis using parsimony.* Version 4.0 b10. Sinauer Associates, Sunderland, MA.

Taylor, J.E. & Hyde KD (2003) *Microfungi of tropical and temperate palms*. Fungal Diversity Research Series 12: 1–459.

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., Bruns, T., 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* 18: 315–322.

Wijayawardene, D.N.N., McKenzie, E.H.C. & Hyde, K.D. (2012) Towards incorporating anamorphic fungi in a natural classification checklist and notes for 2011. *Mycosphere* 3: 157–228.

Zhang, Y., Schoch, C.L., Fournier, J., Crous, P.W., De Gruyter, J., Woudenberg, J.H.C., Hirayama, K., Tanaka, K., Pointing, S.B., Spatafora, J.W. & Hyde, K.D. (2009) Multi-locus phylogeny of *Pleosporales*: a taxonomic, ecological and evolutionary reevaluation. *Studies in Mycology* 64: 85–102.
http://dx.doi.org/10.3114/sim.2009.64.04

Zhang, Y., Crous, P.W., Schoch, C.L. & Hyde, K.D. (2012). *Pleosporales*. *Fungal Diversity* 53: 1–221.
http://dx.doi.org/10.1007/s13225-011-0117-x