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Seifertia shangrilaensis sp. *nov.* (Melanommataceae), a new species from Southwest China

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Abstract

A new *Seifertia* species was isolated from hanging rachides of *Rhododendron decorum* in Yunnan Province, Southwest China. The new taxon was compared with the type species, *S. azalea* and differs in having wider conidiophores, with hyaline to subhyaline and smaller conidia, while *S. azalea* has olive-brown to brown, rarely branched conidiophores, and pale brown or olive-brown, very rarely septate conidia. Phylogenetic analyses of combined LSU, SSU and TEF1-α sequence data show that *S. shangrilaensis* forms a robust clade with *S. azalea* nested among the species of Melanommataceae in the order Pleosporales. A new species, *S. shangrilaensis* is introduced in this study, and *Seifertia* should be placed in Melanommataceae (Pleosporales, Dothideomycetes) based on phylogenetic analysis. Description and illustration of *Seifertia shangrilaensis* are provided with notes and its introduction is supported by molecular data.

Keywords: Dothideomycetes, hyphomycetous fungi, Melanommataceae, phylogeny, taxonomy

Introduction

Seifertia was introduced as a monotypic genus by Partridge and Morgan-Jones (2002) and is typified by *S. azaleae* (Peck) Partridge & Morgan-Jones which was originally described as *Periconia azaleae* Peck. with the names *Briosia azaleae* (Peck) Dearn, *Cephalotrichum azaleae* (Peck) Kuntze, *Pycnostysanus azalea* (Peck) E.W. and *Sporocybe azaleae* (Peck) Sacc. treated as synonyms (Partridge & Morgan-Jones 2002, Glawe & Hummel 2006, Index Fungorum 2016).

Seifertia azalea has been reported as a cosmopolitan taxon causing bud blast and twig blight of azaleas and rhododendrons in Japan, Europe and North America (Mason 1941, Ellis 1976, Farr *et al.* 1996, Partridge & Morgan-Jones 2002, Glawe & Hummel 2006). However, *Seifertia* species are relatively poorly studied worldwide, and have not been studied in the southwest of China. Based on phylogenetic analysis of LSU sequence data, Seifert *et al.* (2007) treated *Seifertia azalea* in Dothideomycetes, as closely related to *Mycosphaerella mycopappi* A. Funk & Dorworth, but unrelated to *Mycosphaerella sensu stricto*. Crous *et al.* (2009, 2013) placed *Seifertia* in Pleosporales which was shown to be allied to *Xenostigmina*, a synanamorph of *Mycopappus*, based on combined ITS and LSU phylogenetic analyses. There is little molecular data to resolve the natural placement of *Seifertia* in Dothideomycetes.

The aim of this study is to introduce a new species, *Seifertia shangrilaensis*, collected from Yunnan Province, Southwest China. The species is described and illustrated with phylogenetic support. *Seifertia shangrilaensis* is the first record of *Seifertia* for China.

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Materials and methods

Isolation and morphology

The specimen was collected from a living branch of *Rhododendron decorum* Franch. (Ericaceae) in Yunnan Province, southwest China and brought to the laboratory for examination and description of fungal morphology. The specimens were observed under a Motic SMZ 168 series dissecting stereo-microscope. The conidial structures were picked up using a surgical needle and a squash mount was prepared in 10% lacto-glycerol for examination under a Nikon Eclipse 80i compound microscope. Photographs were made with a Canon 600D digital camera using DIC microscopy. The macro-morphological structures were photographed with a Discovery V.8 stereo-microscope fitted with a CARL ZEISS Axio Cam ERc5S microscope camera. Tarosoft (R) Image Frame Work program and a Adobe Photoshop version CS5 (Adobe Systems Inc., The United States) were used for measurements and photographic plates.

Single spore isolation was carried out to obtain a pure culture as described in Chomnunti *et al.* (2014). Type specimen is deposited in the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand and duplicated in the Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (KUN-HKAS), Yunnan, China. Ex-type living cultures are deposited in Mae Fah Luang University Culture Collection (MFLUCC) and Kunming Institute of Botany Culture Collection (KUMCC). Faces of Fungi and Index Fungorum numbers were provided as in Jayasiri *et al.* (2015) and Index Fungorum (2016).

DNA extraction, PCR amplification and sequencing

The fungal mycelia were scraped by using a sterilized lancet to reduce the contamination and kept in a 1.5 ml microcentrifuge tube for DNA extraction. The genomic DNA was extracted by using the Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux®, P.R. China) following the protocols in the manufacturer's instructions. The DNA product was maintained at 4 °C for the DNA amplification and duplicated at -20 °C for long term storage.

The DNA amplification was performed by polymerase chain reaction (PCR) using the respective genes (LSU, SSU and TEF1-α). The primers LROR and LR5 (Vilgalys & Hester 1990) were used to amplify the partial ribosomal RNA for the nuclear large subunit (LSU), NS1 and NS4 (White *et al.* 1990) were used to amplify the partial ribosomal RNA for the nuclear small subunit (SSU) and EF1-983F and EF1-2218R (Rehner 2001) were used to amplify the translation elongation factor 1-alpha gene (TEF1-α). The final volume of the PCR reaction were 25 µl, containing 1 µl of DNA template, 1 µl of each forward and reward primer, 12.5 µl of 2x Master mixture and 9.5 µl of ddH_2O. The PCR thermal cycling conditions were processed by initialization at 94 °C for 3 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 50 seconds, elongation at 72 °C for 1 minute, and a final extension at 72 °C for 10 minutes, and final kept at 4°C for keeping DNA. The PCR products were checked on 1% agarose gel electrophoresis stained with ethidium bromide. Purification and DNA sequencing were carried out at Shanghai Biological Engineering Technology Co., Ltd, P.R. China.

Sequence alignment and phylogenetic analyses

Phylogenetic analyses were performed by combined LSU, SSU and TEF1-α sequence data. Sequences generated from this study were analyzed with other similar sequences, obtained from the GenBank and those derived from Crous *et al.* (2013) and Tian *et al.* (2015) (Table 1). A single gene alignment was performed by using MAFFT v. 7 (Katoh & Standley, 2013: http://mafft.cbrc.jp/alignment/server/) and manual aligned where necessary in MEGA version 6.0 (Tamura *et al.* 2013). Further analyses were performed by using RAxML GUI v.0.9b2 (Stamatakis 2006, 2014, Stamatakis *et al.* 2008, Silvestro & Michalak 2010) and Bayesian analysis (BI) (Huelsenbeck & Ronquist 2001, Huelsenbeck *et al.* 2001) following the methodology as described in Phookamsak *et al.* (2015).

The phylograms are represented in Treeview (Page 1996) and drawn in Microsoft power point and converted to jpeg file in Adobe Photoshop version CS5 (Adobe Systems Inc., The United States). The new sequences were submitted in GenBank (Table 1).

Results

Phylogenetic analyses

The combined LSU, SSU and TEF1-α sequence data comprised 36 taxa with *Hysterium angustatum* (CBS 123334, CBS 236.34) selected as the outgroup taxon. The best scoring tree from RAxML analysis is chosen to represent

the phylogenetic relationships among a newly generated taxon with other genera in Melanommataceae and Pleomassariaceae in Dothideomycetes (Fig. 1). The phylogenetic analyses obtained from maximum likelihood and Bayesian analyses gave similar topologies of the relationships in Melanommataceae and Pleomassariaceae and were not significantly different. Phylogenetically shows that *Seifertia shangrilaensis* (MFLUCC 16-0238) belongs to the family Melanommataceae (Pleosporales, Dothideomycetes) which forms a robust clade to *S. azalea* (97% ML, 0.99 PP) and formed a sister group with *Xenostigmina zilleri* (A. Funk) Crous (Fig. 1). Therefore, a new species, *Seifertia shangrilaensis* is established.

TABLE 1. Taxa used in the phylogenetic analyses and their corresponding GenBank numbers. The newly generated sequences are indicated in red bold and the type strains are in bold.

Species	Culture collection	GenBank accession numbers		
		LSU	SSU	TEF1-α
Aposphaeria populina	**CBS 543.70**	**EU754130**	**EU754031**	-
Aposphaeria populina	CBS 350.82	JF740265	-	-
Byssosphaeria jamaicana	**SMH 1403**	**GU385152**	-	**GU327746**
Byssosphaeria jamaicana	SMH 3085	GU385154	-	-
Byssosphaeria rhodomphala	SMH 3086	GU385155	-	-
Byssosphaeria rhodomphala	**GKM L153N**	**GU385157**	-	**GU327747**
Byssosphaeria salebrosa	**SMH 2387**	**GU385162**	-	**GU327748**
Byssosphaeria schiedermayeriana	SMH 3157	GU385163	-	GU327745
Byssosphaeria schiedermayeriana	**GKM 152N**	**GU385168**	-	**GU327749**
Byssosphaeria villosa	GKM 204N	GU385151	-	GU327751
Byssosphaeria musae	**MFLUCC 11-0146**	**KP744477**	**KP753947**	-
Byssosphaeria siamensis	**MFLUCC 10-0099**	**KT289895**	**KT289897**	**KT962059**
Byssosphaeria schiedermayeriana	**MFLUCC 10-0100**	**KT289894**	**KT289896**	**KT962060**
Herpotrichia diffusa	**CBS 250.62**	**DQ678071**	**DQ678019**	**DQ677915**
Herpotrichia juniper	**CBS 200.31**	**DQ678080**	**DQ678029**	**DQ677925**
Herpotrichia juniperi	CBS 468.64	DQ384093	DQ384077	-
Herpotrichia macrotricha	GKM 196N	GU385176	-	GU327755
Herpotrichia macrotricha	SMH 269	GU385177	-	GU327756
Hysterium angustatum	CBS 123334	FJ161207	FJ161167	FJ161111
Hysterium angustatum	CBS 236.34	FJ161180.	GU397359	FJ161096
Melanomma pulvis-pyrius	**CBS 124080**	**GU456323**	**GU456302**	**GU456265**
Melanomma pulvis-pyrius	CBS 109.77	FJ201986	FJ201987	GU456274
Melanomma pulvis-pyrius	CBS 371.75	FJ201988	FJ201989	GU349019
Melanomma rhododendri	ANM 73	GU385198	-	-
Monotosporella tuberculata	CBS 256.84	GU301851	-	GU349006
Mycopappus aceris	CBS 124109	FJ839660	-	-
Pleomassaria siparia	**CBS 279.74**	**DQ678078**	**DQ678027**	**DQ677923**
Prosthemium betulinum	**CBS 127468**	**AB553754**	**AB553644**	**-**
Prosthemium canba	JCM 16966	AB553760	AB553646	-
Prosthemium orientale	JCM 12841	AB553748	AB553641	-
Prosthemium stellar	**CBS 126964**	**AB553781**	**AB553650**	**-**
Seifertia azalea	**DAOM 239136**	**EU030276**	**-**	**-**
Seifertia shangrilaensis	**MFLUCC 16-0238**	**KU954100**	**KU954102**	**KU954101**
Xenostigmina zilleri	CBS 115686	GU253858	-	-
Xenostigmina zilleri	**CBS 115685**	**GU253857**	-	-
Xenostigmina zilleri	CBS 124108	FJ839675	-	-

Abbreviations: ANM: A.N. Miller; CBS Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; **DAOM**: Canadian Collection of Fungal Cultures, Ottawa, Canada; **GKM**: G.K. Mugambi; **IL**: I. Lopez; **JK**: J. Kohlmeyer; **KT**: K. Tanaka; **MAFF**: Ministry of Agriculture, Forestry and Fisheries, Japan; **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **SMH**: S.M. Huhndorf.

Taxonomy

Seifertia shangrilaensis J.F. Li, Phookamsak & K.D. Hyde**,** *sp. nov*. Fig. 2

Index Fungorum number: IF552010; Facesoffungi number: FoF 01885

Etymology:—Refers to the location, where the holotype was collected.

Diagnosis:—Differs from *Seifertia azalea* in its shorter and hyaline to subhyaline conidia.

Holotype: MFLU 16-0267

Epiphytic on living rachides of *Rhododendron decorum* Franch. ***Sexual morph***: Undetermined. ***Asexual morph***: Synnemata 1000–2300 µm high, 120–200 µm wide, erect, simple, unbranched, dark brown to black, with bubble-like, tightly interwoven, branched hyphae, compacted into an elongate bundle. *Mycelium* superficial, partly immersed on the substrate, composed of septate, branched, smooth, thin-walled, pale white to white hyphae. *Conidiophores* (1000–) 1300–2100 (–2150) × (9.5–) 10–11 (–13) µm, ($\bar{x}$ = 1804.7 × 10.3 µm, n = 40), synnematous, closely packed into a bundle, hyaline to pale brown, thin-walled, smooth, septate, unbranched, straight or flexuous, cylindrical. *Conidiogenous cells* (5–) 7–11 (–12.5) × (4.5–) 5.5–6.5 (–7) µm, ($\bar{x}$ = 7.9 × 5.8 µm, n = 100), holoblastic, phialidic, integrated, terminal, determinate, subhyaline, cylindrical to subclavate, smooth. *Conidia* (2.5–) 3.5–5 (–6) × 2.5–3.5 µm ($\bar{x}$ = 4.2 × 3.1 µm, n = 100), phialoconidia, hyaline to subhyaline, fusiform to subglobose, or ellipsoidal, aseptate, borne in basipetally, developing pseudo-chains, clavate, smooth, thin-walled, sometimes aggregated into slimy masses at the apex of the synnema.

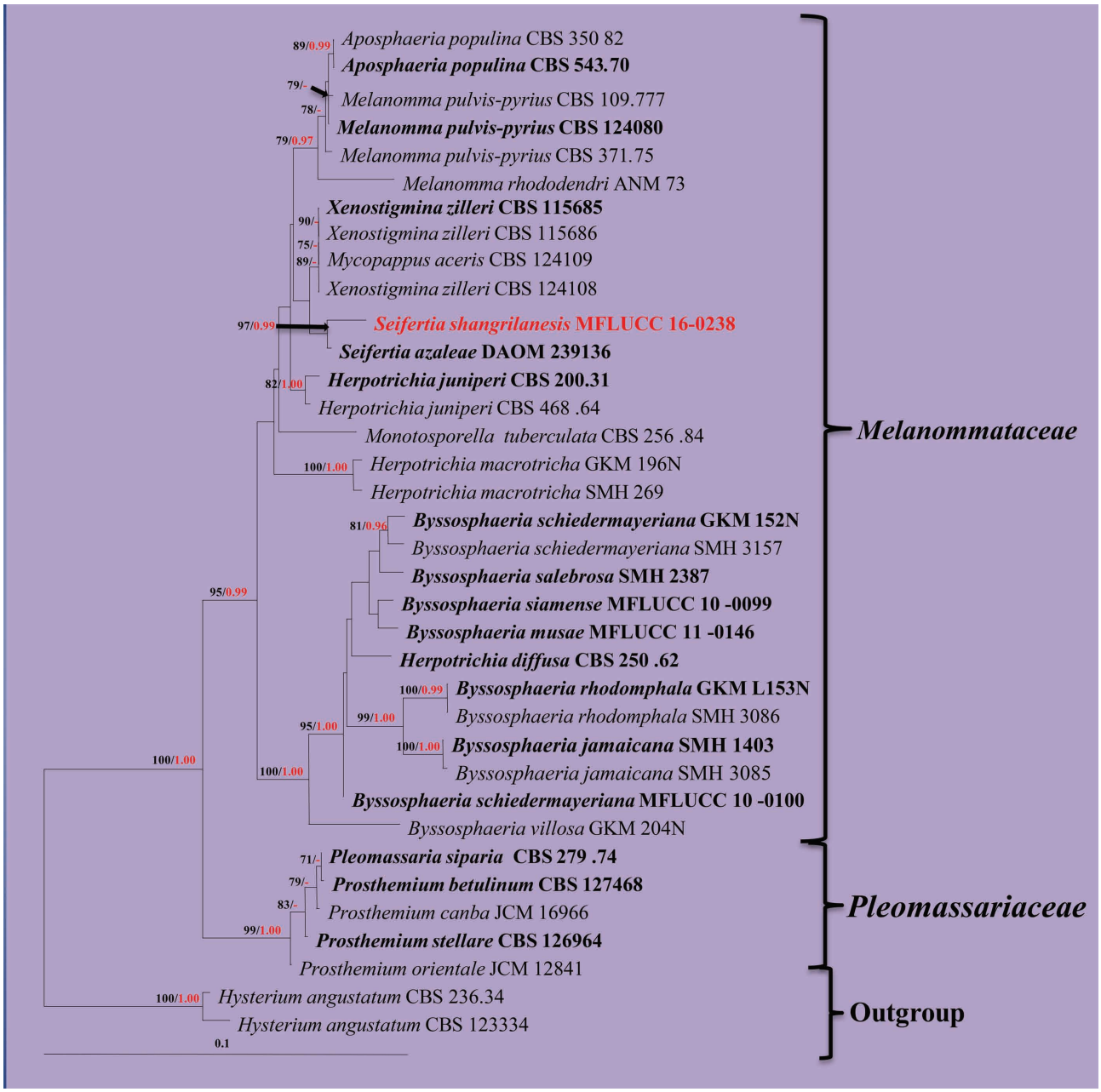

FIGURE 1. Phylogenetic construction using RAxML tree based analysis of a combined dataset of LSU, SSU and TEF1-α. Bootstrap support values for maximum likelihood (ML, black), equal or greater than 70% and Bayesian posterior probabilities (PP, red) equal to or greater than 0.95 are shown above the nodes. The tree is rooted to *Hysterium angustatum* (CBS 236.34 and CBS 123334). The type strains are in black bold and the newly generated sequences are indicated in red bold.

Cultural characteristics:—Conidia germinating on PDA within 14 hours at 15 ºC, germ tubes produced from apex. Colonies growing on PDA, reaching 5 mm diam. after 4 weeks at 25 ºC, mycelium semi-immersed to superficial, irregular in shape, flat, slightly raised, with undulate edge, slightly rough on surface, cottony to fairy fluffy, colony from above, initially write to cream at the margin, yellowish-brown in the centre, becoming white at the margin, dark brown at the centre after 4 weeks; from below, initially, cream at the margin, orangish-brown to reddish-brown at the centre, becoming dark brown to black after 4 weeks, producing brown pigmentation in agar.

Material examined:—CHINA, Yunnan Province, Shangrila, on living rachides of *Rhododendron decorum* (Ericaceae), 20 October 2014, R. Phookamsak, SgL027 (MFLU 16-0267, **holotype!**; KUN-HKAS 93733, **isotype!**), ex-type living culture, MFLUCC 16-0238!, KUMCC 16-0002.

Known distribution:—widespread in temperate and boreal regions.

Notes:—*Seifertia shangrilaensis* is similar to the type species, *Seifertia azalea* in having narrow and pale conidia, with one or two conidiogenous loci of conidiogenous cells, dark conidiophores and erect, synnemata (Partridge & Morgan-Jones 2002, Glawe *et al.* 2006, Seifert *et al.* 2007). However, *S. shangrilaensis* differs from *S. azalea* in having hyaline to subhyaline, and wider conidiophores (9.5–12.6 µm vs. 4–7 µm), and smaller conidia (2.5–6 × 2.5–3.6 µm vs. 4–12 × 4–8 µm).

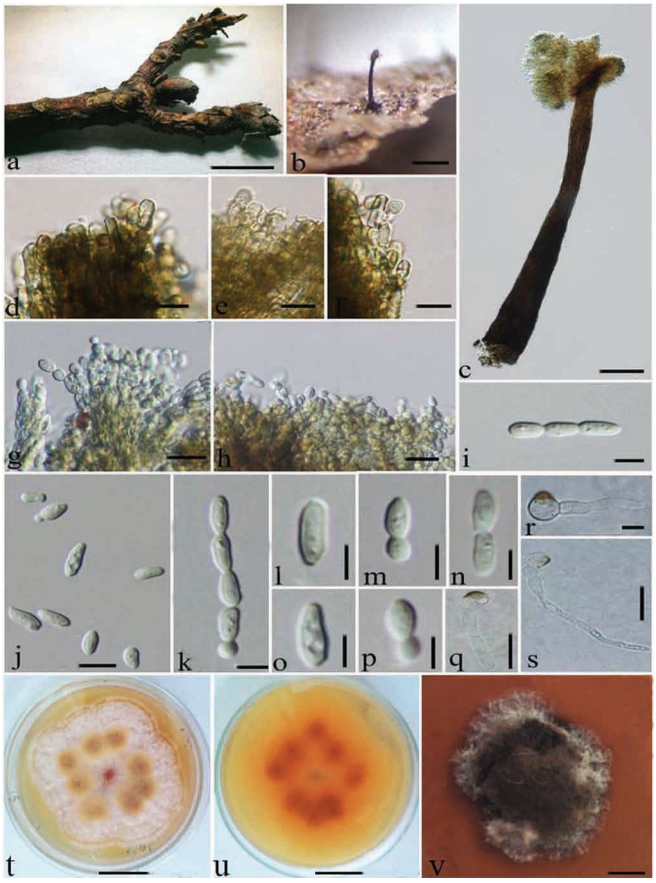

FIGURE. 2. ***Seifertia shangrilaensis*** (MFLU 16-0267, **holotype**). **a**. Living rachides of *Rhododendron decorum*. **b**. Appearance of synnema on rachides. **c**. A synnema visualized under the compound microscope. **d–h**. Conidiogenous cells. **i–p**. Conidia. **q–s**. Germinating conidia. **t**. Multiple culture colonies from above. **u**. Multiple culture colonies. **v**. Single colony on PDA after 4 weeks. Scale bars: t–u = 2 cm, v = 1 cm, a = 0.5 cm, b = 500 µm, c = 200 µm, g–h, j–k, q–s = 10 µm, d–f, i, l–n = 5 µm.

Discussion

The genus *Seifertia* is relatively poorly studied and was introduced to accommodate a pathogen species occurring on *Rhododendron* in the UK. Based on the few sequence data available in GenBank, the placement of *Seifertia* is uncertain. According to its morphological characters, *Seifertia* was previously treated in various genera such as *Periconia* (Peck 1873), *Pycnostysanus* (Mason 1941) and also considered a taxonomic synonymy of *Sorocybe* (Ellis 1976, Carmichael *et al.* 1980). The type species, *Seifertia azalea* is characterized by erect, simple, and dark synnemata, unicellular or

very rarely septate, narrow, paler conidia laterally thick-walled, macronematous and synnematous conidiophores and holoblastic, integrated, terminal and determinate conidiogenous cells (Partridge & Morgan-Jones 2002).

Seifertia is known to occur on azaleas and rhododendrons and is a cosmopolitan species causing bud blight or bud blast disease in Japan, Europe and North America (Mason 1941, Ellis 1976, Farr *et al.* 1996, Partridge & Morgan-Jones 2002, Glawe & Hummel 2006). Insects were thought to play a role of pathogenic distribution (Howell *et al.* 1962, Pirone 1978, Viennot-Bourgin 1981). Disease symptoms were as initially, infecting dead flower buds, and later becoming blackened bearing numerous synnemata (Glawe & Hummel 2006).

Pirone (1978) reported that 90% death of *Rhododendron* flowers were caused by *S. azalea*, with a perennial infection (Chant & Gbaja 1984, Glawe & Hummel 2006). Farr *et al.* (2007) listed eight *Rhododendron* species infected by *Seifertia azalea* in the USA viz. *R. arborescens* (Pursh) Torr., *R. catawbiense* Michx., *R. macrophyllum* D. Don ex G. Don, *R. maximum* L., *R. minus* Michx., *R. nudiflorum* (L.) Torr., *R. vaseyi* A. Gray and *R. viscosum* (L.) Torr. Recently, Farr and Rossman (2016) listed only two *Rhododendron* species, *R. hemsleyanum* and *R. ponticum* caused by *S. azalea* in the USA. However, further occurrence, incidence, host and geographical range were relatively poorly known (Glawe & Hummel 2006). Glawe & Hummel (2006) reported a new host, *R. hemsleyanum* infected by *S. azalea* in North America. They mentioned that the disease incidence was limited by a low number of vulnerable genotypes in collection, critical environmental conditions and some other factors.

Seifertia species has so far not been reported to occur on *Rhododendron decorum*. *Seifertia shangrilaensis* was collected from living rachides of *R. decorum* in South-west China. The species occurs on both living rachides and dead hanging rachides of *R. decorum*. *Seifertia shangrilaensis* appears to be a pathogen causing death of flower buds of *R. decorum*. The development of disease infection might be initially limited where the synnemata occurred, and later, infected widely in other host cells. However, pathogenicity test is not done in this study.

Seifert *et al.* (2007) treated *Seifertia* in Dothideomycetes based on the large subunit LSU gene sequence data and it clustered with *Mycosphaerella mycopappi*, but was unrelated to *Mycosphaerella sensu stricto.* Crous *et al.* (2009) re-examined *Xenostigmina* and confirmed that *Xenostigmina* is a synanamorph of *Mycopappus* and showed to be allied with *Seifertia* in Pleosporales. Furthermore, Crous *et al.* (2013) revealed that *Xenostigmina* and *Mycopappus* clustered in the Phaeosphaeriaceae. Phookamsak *et al.* (2014), however, they excluded *Mycopappus* and *Xenostigmina* from Phaeosphaeriaceae due to these genera forming a single clade close to Melanommataceae. Tian *et al.* (2015) accepted *Xenostigmina* and *Mycopappus* in Melanommataceae based on multiple gene phylogenetic analyses. Hyde *et al* (2013) and Wijayawardene *et al.* (2014) did not treat *Seifertia*, while Tian *et al.* (2015) discussed its relationship with *Xenostigmina* and *Mycopappus*, but did not place it in Melanommataceae.

Several asexual morphs of melanommataceous genera have been reported. *Pyrenochaeta* is the asexual morph of *Herpotrichia* (Sivanesan 1984). *Aposphaeria* and *Phoma*-like asexual morphs have been reported for *Melanomma* species (Sivanesan 1984, Tian *et al.* 2015). These links however, need to be confirmed in the light of modern taxonomic treatments and molecular data (Tian *et al.* 2015). In this study, phylogenetic analyses of combined LSU, SSU and TEF1-α sequence data provide a support that *Sefertia* belongs in the family Melanommataceae, Dothideomycetes (*sensu* Hyde *et al.* 2013, Wijayawardene *et al.* 2014) and is distinct from *Mycopappus* and *Xenostigmina*.

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