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Studies of botryosphaerialean fungi associated with canker and dieback of tree hosts in Dongling Mountain of China

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Abstract

Botryosphaeriales is an order comprising of latent fungal pathogens with a wide range of woody hosts. These pathogens represent interesting and diverse fungi with a confusing taxonomy due to their similar morphological characters. Many genera or families of this order have not been robustly sampled or systematically studied in separate hosts and regions, although recent studies have made enormous progress. In this study, five species of *Aplosporellaceae* and *Botryosphaeriaceae* were isolated from *Juglans regia* (Juglandaceae), *Rhus typhina* (Anacardiaceae) and *Ziziphus jujuba* (Rhamnaceae) in Dongling Mountain of China. These species include *Aplosporella ginkgonis*, *Aplosporella javeedii*, *Botryosphaeria dothidea*, *Phaeobotryon rhoinum* sp. nov. and *Phaeobotryon rhois*. Of which, *Aplosporella javeedii* and *A. ginkgonis* were identified as the first records from *Ziziphus jujube* and *Rhus typhina*, respectively. *Phaeobotryon rhoinum* is characterised by its globose, scattered to gregarious pycnidia with ellipsoid to oblong, brown, 1-septate conidia. It can be distinguished from the similar species *P. cercidis*, *P. cupressi*, *P. mamane*, *P. quercicola* and *P. rhois* based on host association and conidial size and colour. The results represent the first attempts to study *Aplosporella*, *Botryosphaeria* and *Phaeobotryon* with descriptions and multilocus phylogenies (ITS, LSU and TEF-1α) in Dongling Mountain of China.

Key words: *Aplosporella*, *Botryosphaeria*, *Phaeobotryon*, Phylogeny, Taxonomy

Introduction

The Botryosphaeriales C.L. Schoch, Crous & Shoemaker was established by Schoch *et al.* (2006) and encompass one family, Botryosphaeriaceae Theiss. & Syd., which confused a large number of mycologists and taxonomists. In the past, this family continuously varied until Liu *et al.* (2012) listed all of the various primary treatments and redefined 29 genera using multilocus phylogenetic techniques based on examinations of the types of genera. However, the interrelations of several genera remained disordered, and it is likely that Botryosphaeriales consists of more than one or two families (Crous *et al.* 2006; Liu *et al.* 2012). Slippers *et al.* (2013) investigated the systematics and evolution of the phylogenetic lineages of the Botryosphaeriales, including Aplosporellaceae Slippers, Boissin & Crous, Botryosphaeriaceae, Melanopsaceae A.J.L. Phillips, Slippers, Boissin & Crous, Phyllostictaceae Fr., Planistromellaceae M.E. Barr and Saccharataceae Slippers, Boissin & Crous. With the introductions of Septorioideaceae Wyka & Broders (Wyka & Broders 2016), Endomelanconiopsisaceae Tao Yang & Crous and Pseudofusicoccumaceae Tao Yang & Crous (Yang *et al.* 2017), there are now nine families accommodated in the order listed by Slippers *et al.* (2017). Six of these nine Botryosphaeriales families contain a single genus, while Botryosphaeriaceae consists 23 genera, representing the largest family in this order (Dissanayake *et al.* 2016; Slippers *et al.* 2017). Aplosporellaceae and Planistromellaceae are represented by three and two genera, respectively (Sharma *et al.* 2017; Slippers *et al.* 2017).

Aplosporella Speg. (Aplosporellaceae) was introduced by Spegazzini (1880) to accommodate six species with *A. chlorostroma* Speg. as the generic type, and subsequently fell into a long period of confusion with many synonyms, particularly *Haplosporella* Speg. (Tilak & Rao 1964; Tai 1979). This genus is characterized by multilocular conidiomata with a single ostiole and brown, aseptate conidia. The identifications and descriptions of most *Aplosporella* species were based on the host association, whereas current studies suggested that these species lack host specificity (Damm *et al.* 2007). Thus there have been more than 330 epithets in Index Fungorum (2018) with an estimated 66 epithets in Kirk *et al.* (2008). Recent studies have confirmed that *Aplosporella* is better to be positioned in Botryosphaeriaceae

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(Damm *et al.* 2007; Liu *et al.* 2012). Slippers *et al.* (2013) recognized that this genus should be separated from Botryosphaeriaceae under the distinct family name of Aplosporellaceae using six loci phylogeny. Slippers *et al.* (2013) proposed consistent connections between *Aplosporella* and another similar genus *Bagnisiella* Speg. and believed that *Bagnisiella* should be reduced to synonymy with *Aplosporella*. Sharma *et al.* (2017) proposed genus *Alanomyces* Roh. Sharma in Aplosporellaceae, which consist of saprobes on soil attached to the base of macrofungus stipes in mixed forest.

Botryosphaeria Ces. & De Not. (Botryosphaeriaceae) was proposed by de Cesati & de Notaris (1863) with 12 species. Barr (1972) designated *B. dothidea* (Moug.) Ces. & De Not. as the lectotype species of the genus. However, no ex-type cultures were available for *B. dothidea*. Slippers *et al.* (2004) designated a neotype for *B. dothidea* and designated it as an epitype to stabilize the type species *B. dothidea* with molecular data. Crous *et al.* (2006) suggested that *Botryosphaeria sensu lato* is composed of 10 phylogenetic lineages. Phillips *et al*. (2013) separated them and recognised seven species in *Botryosphaeria*. The genus is characterized by clavate asci with hyaline (sometimes becoming pale brown with age), aseptate (sometimes becoming 1–2 septate with age), fusoid to ellipsoid or ovoid ascospores; with hyaline (sometimes becoming olivaceous or darker with age), thin-walled, smooth, aseptate (occasionally forming 1–2 septate with age or before germination), elliptical to fusiform or clavate conidia (Phillips *et al*. 2013). Several recent studies increased the species to 11 in *Botryosphaeria* (Slippers *et al.* 2014, Ariyawansa *et al.* 2016, Zhou *et al.* 2016, 2017). Of which *Botryosphaeria dothidea* was regarded as one of the most frequent species as well as latent pathogen associated with canker disease of woody plants (Marsberg *et al.* 2017).

Phaeobotryon Theiss. & Syd. (Botryosphaeriaceae) was established by Theissen & Sydow (1915) to accommodate *Dothidea cercidis* Cooke and subsequently involved in the broad concept of the *Botryosphaeria* species. However, recent studies showed that *Phaeobotryon* represents an individual genus and is distinct from all other genera in Botryosphaeriaceae (Phillips *et al.* 2008, 2013). This genus is characterized by clavate to cylindrical-clavate asci with 2-septate, brown ascospores with conical apiculi at each end; and ellipsoidal to oblong or obovoid, hyaline or brown conidia, that are mostly 2-septate at maturity (Phillips *et al.* 2013, Fan *et al.* 2015a). *Phaeobotryon* consists of nine species in Index Fungorum (2018) with an estimated four epithets in Kirk *et al.* (2008), of which only four species (*P. cupressi* Abdollahz., Zare & A.J.L. Phillips, *P. mamane* Crous & A.J.L. Phillips, *P. negundinis* Daranag., Bulgakov & K.D. Hyde and *P. rhois* C.M. Tian, X.L. Fan & K.D. Hyde) have been studied with living culture (Liu *et al.* 2012; Phillips *et al.* 2013; Slippers *et al.* 2013; Fan *et al.* 2015a).

During the course of cognitive practice to investigate forest pathogenic fungi in Dongling Mountain, isolates of *Aplosporella*, *Botryosphaeria* and *Phaeobotryon* were obtained from three unrelated hosts, i.e., *Juglans regia* L. (Juglandaceae DC. ex Perleb), *Rhus typhina* L. (Anacardiaceae R. Br.) and *Ziziphus jujuba* Miller (Rhamnaceae Juss.). The current study aims to clarify the systematics and taxonomy of these Botryosphaeriales fungi with detailed descriptions.

Materials and methods

Sampling and isolation

Seventeen isolates were isolated from symptomatic branches and stems of *Juglans regia* (Juglandaceae), *Rhus typhina* (Anacardiaceae) and *Ziziphus jujuba* (Rhamnaceae) during the course of cognitive practice supporting by Beijing Forestry University (BJFU) in Dongling Mountain of Beijing, China (Table 1). The suspension of conidia was established by removing a mucoid spore mass from conidiomata or ascomata, and spread the suspension on the surface of 1.8 % potato dextrose agar (PDA) in a petri-dish, and incubated at 25 °C for up to 24 h. Single germinating conidia were transferred onto fresh PDA plates. Specimens and isolates were deposited in the Key Laboratory for Silviculture and Conservation of the Ministry of Education in BJFU, and the working Collection of X.L. Fan (CF) housed at the BJFU. Axenic cultures are maintained in the China Forestry Culture Collection Centre (CFCC).

Morphology

Species identification was based on the morphological characteristics of the conidiomata from infected host materials. The macro-morphological photographs were captured using a Leica stereomicroscope M205 FA (Leica Microsystems, Wetzlar, Germany), including structure and size of stromata; number, structure and size of ectostromatic disc and ostioles. Micro-morphological observations include shape and size of conidiophores and conidia determined under a Nikon Eclipse 80i microscope (Nikon Corporation, Tokyo, Japan) equipped with a Nikon digital sight DS-Ri2 high

definition colour camera (Nikon Corporation, Tokyo, Japan), using differential interference contrast (DIC) illumination and the Nikon software NIS-Elements D Package v. 3.00. Adobe Bridge CS v. 6 and Adobe Photoshop CS v. 5 were used for the manual editing. A total of 20 conidiomata and 50 conidia were measured to calculate the mean size and standard deviation (SD). Nomenclatural novelties and descriptions were deposited in MycoBank (Crous *et al.* 2004). Colony diameters were measured, and the colony colours described after 3 wk according to the colour charts of Rayner (1970).

DNA isolation, amplification and sequencing

Genomic DNA was extracted using a modified CTAB method, with fungal mycelium harvested from PDA plates with cellophane (Doyle & Doyle 1990). The PCR amplifications were performed in a DNA Engine (PTC-200) Peltier Thermal Cycler (Biorad Laboratories, CA, USA). The internal transcribed spacer (ITS) region was amplified using the primers ITS1 and ITS4 (White *et al.* 1990). The nuclear ribosomal RNA large subunit (LSU) region was amplified using the primers LR0R and LR7 (Vilgalys & Hester 1990). The translation elongation factor 1-alpha (TEF-1α) region was amplified using the primers TEF1-688F and TEF1-1251R (Alves *et al.* 2008). The PCR mixture for the all regions consisted of 1 µL genomic DNA, 3 mM $MgCl_2$, 20 µM of each dNTP, 0.2 µM of each primer and 0.25 U BIOTAQ DNA polymerase (Bioline Reagents, London, UK). Conditions for PCR cycle of ITS and LSU genes constituted 35 cycles of 30 s at 95 °C, 30 s at 48 °C and 1 min at 72 °C, while the TEF-1α gene was performed using 35 cycles of 30 s at 95 °C, 45 s at 56 °C and 1 min at 72 °C. The PCR amplification products were visually estimated by electrophoresis in 2 % agarose gels. The PCR products were sequenced in two directions using the PCR primers and the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), and performed with an ABI Prism 3730XL Sequencer (Applied Biosystems) according to the instructions of the manufacturer.

Phylogenetic analyses

DNA sequences generated by each primer combination were used to obtain consensus sequences using Seqman v. 7.1.0 in the DNASTAR lasergene core suite software (DNASTAR Inc., Madison, WI, USA). Reference sequences were selected based on sequence availability from relevant published literature (Phillips *et al.* 2013; Slippers *et al.* 2013, 2017; Fan *et al.* 2015a, b; Dou *et al.* 2017; Du *et al.* 2017; Sharma *et al.* 2017) (Table 1). Sequences were aligned using MAFFT v. 6 (Katoh & Standley 2013) and edited manually using MEGA v. 6.0 (Tamura *et al.* 2013). A partition homogeneity test (PHT) test with heuristic search and 1000 homogeneities was performed using PAUP v.4.0b10 to test the discrepancy between the ITS-LSU and EF-1α in reconstructing phylogenetic trees. A maximum parsimony (MP) analysis was performed using PAUP v. 4.0b10 with a heuristic search option of 1000 random-addition sequences using a tree bisection and reconnection (TBR) branch swapping algorithm (Swofford *et al.* 2003). The branches of zero length were collapsed and all equally parsimonious trees were saved. Other parsimony scores such as tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency (RC) were calculated (Swofford *et al*. 2003). A maximum likelihood (ML) analysis was performed with GTR+G+I model of site substitution including estimation of Gamma-distributed rate heterogeneity and a proportion of invariant sites using RAxMLv.7.2.8 (Stamatakis 2006).

MrModeltest v. 2.3 was performed to estimate the best nucleotide substitution model settings for each gene (Posada & Crandall 1998). A Bayesian inference (BI) employing a Markov Chain Monte Carlo (MCMC) algorithm was performed in MrBayes v. 3.1.2 based on the individual DNA dataset from the results of the MrModeltest (Ronquist & Huelsenbeck 2003). Two MCMC chains were run from random trees for 1000000 generations and trees were sampled by each 100th generations. The first 25 % of trees were discarded as the burn-in phase of each analysis, and the posterior probabilities (BPP) were calculated to assess the remaining trees (Rannala & Yang 1996). The branch support from MP and ML analysis was evaluated with a bootstrapping (BS) method of 1000 replicates (Hillis & Bull 1993). *Fusicladium convolvularum* (CBS 122706), *F. effusum* (STE-U 4525) and *F. oleagineum* (CBS 113427) were selected as outgroup taxa in all analyses (Slippers *et al.* 2013). Phylograms are shown using Figtree v. 1.3.1 (Rambaut & Drummond 2010). Novel sequence data were deposited in GenBank (Table 1) and the multilocus sequence alignment file and ITS sequence-alignment file were maintained in TreeBASE (www.treebase.org; accession number: S22512).

Results

The alignment included 137 *Botryosphaeriales* ingroup strains with a total of 2003 characters including gaps from three gene portions (694 for the ITS, 852 for the LSU and 457 for the TEF-1-α). In the alignment 1041 characters

are constant, 172 variable characters are parsimony-uninformative and 790 characters are variable and parsimony-informative. The results from the PHT test were not significant and supported a decision to combine the three gene datasets. MP analyses generated 200 parsimonious trees, one of which is presented in Fig. 1 (TL = 4280, CI = 0.411, RI = 0.856, RC = 0.352). Topologies of ML (final likelihood value of -23106.782503) and Bayesian analyses were similar to the MP tree. The MP bootstrap supports (BS) equal to or above 50 were shown in branches in Fig. 1. The branches with significant Bayesian posterior probabilities (BPP) equal to or above 0.95 are shown in the phylogram (Table 1). The phylogram included nine known lineages: Aplosporellaceae, Botryosphaeriaceae, Endomelanconiopsisaceae, Melanopsaceae, Phyllostictaceae, Planistromellaceae, Pseudofusicoccumaceae, Saccharataceae and Septorioideaceae, representing nine families in Botryosphaeriales. The current sequences from our 17 Chinese collections clustered into five clades within *Aplosporella* (Aplosporellaceae), *Botryosphaeria* and *Phaeobotryon* (Botryosphaeriaceae), representing *Aplosporella ginkgonis* C.M. Tian, Z. Du & K.D. Hyde, *Aplosporella javeedii* Jami, Gryzenh., Slippers & M.J. Wingf., *Botryosphaeria dothidea*, *Phaeobotryon rhoinum* and *Phaeobotryon rhois* (Fig. 1). The three isolates of *Phaeobotryon rhoinum* from *Rhus typhina* clustered in the subclade of *Phaeobotryon* and are distinct from other species of *Phaeobotryon*. The three strains clustered in an individual clade representing a novel species with high support values (MP/ML/BI = 99/100/1); this is also supported by morphology.

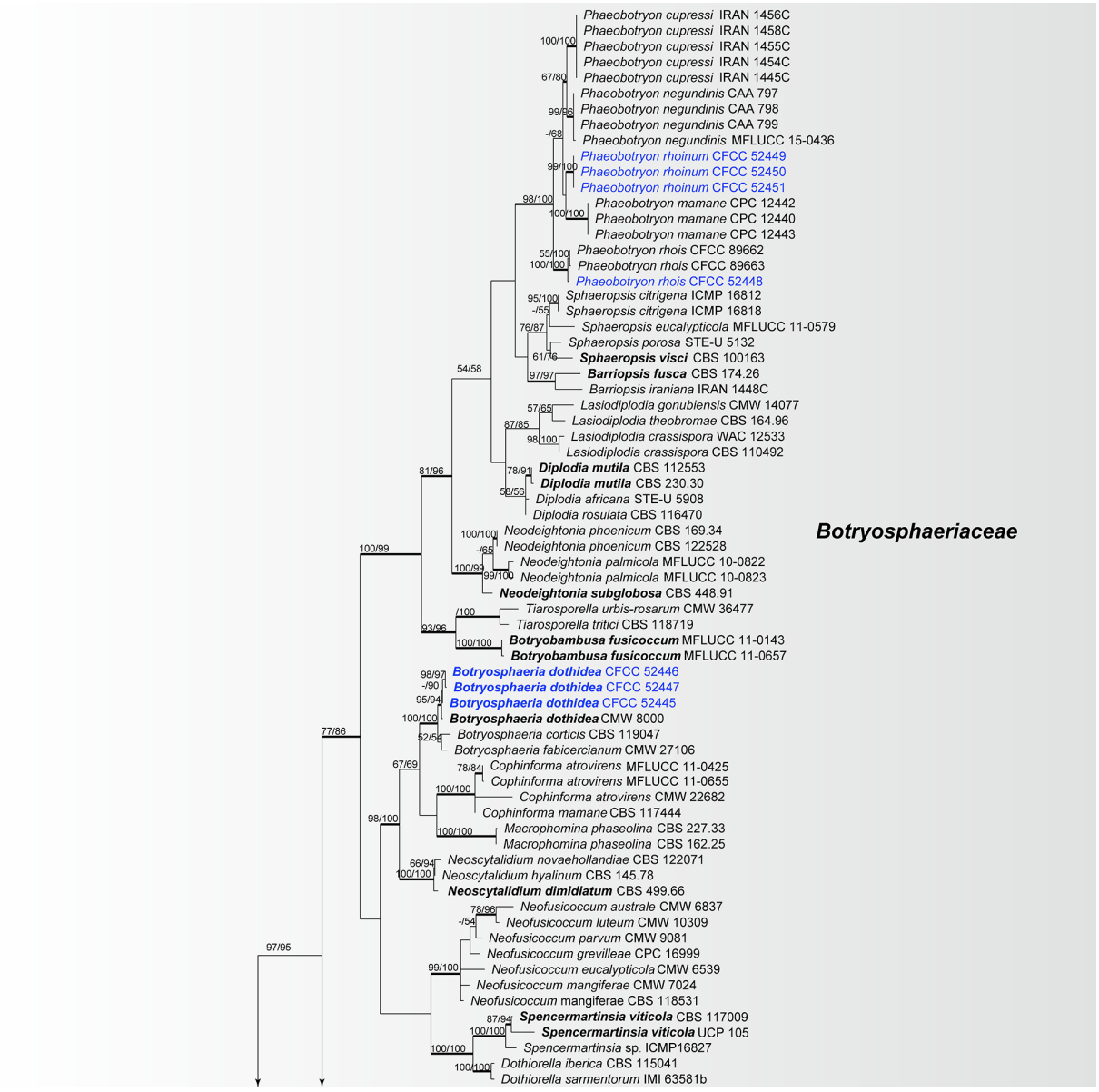

FIGURE 1. Phylogram of *Botryosphaeriales* based on combined ITS, LSU, and TEF-1α genes. MP and ML bootstrap support values above 50 % are shown at the first and second position respectively. Thickened branches represent posterior probabilities above 0.95 from BI. Type species are in bold. Strains in the current study are in blue.

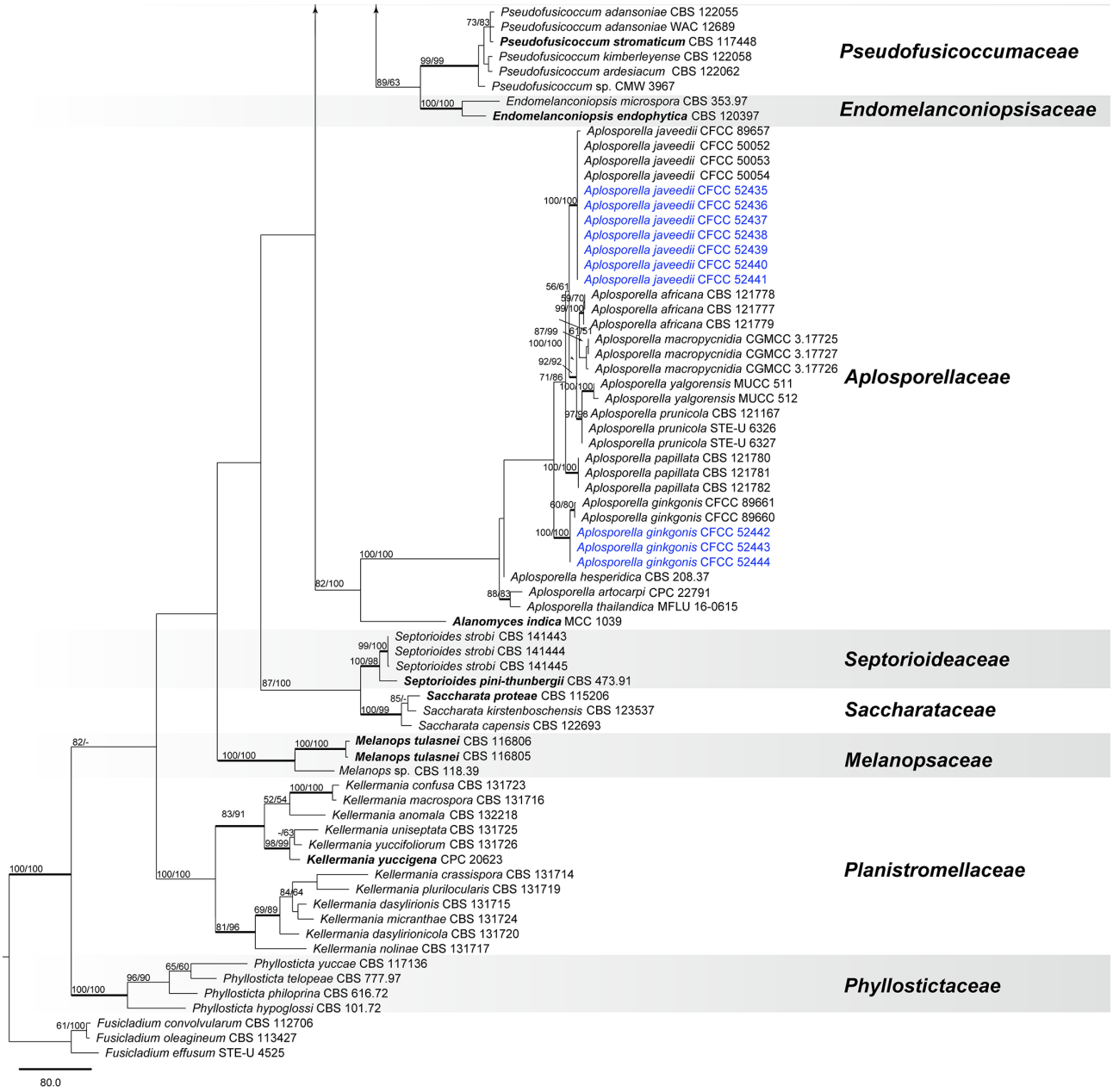

FIGURE 1 (cont.).

Taxonomy

Aplosporella ginkgonis C.M. Tian, Z. Du & K.D. Hyde, in Du, Fan, Yang, Hyde & Tian, Mycosphere 8(2): 1249 (2017)

Materials examined: **China, Beijing City**, **Mentougou District**, Dongling Mountain, Xiaolongmen Forestry Centre, from branches of *Rhus typhina*, 21 Aug. 2017, H.Y. Zhu & X.L. Fan, deposited by X.L. Fan, CF 2017821, living culture CFCC 52442; *ibid.* CF 2017824, living culture CFCC 52443; *ibid.* CF 2017805, living culture CFCC 52444.
Notes: *Aplosporella ginkgonis* was known to cause canker and dieback disease of *Ginkgo biloba* L. and *Morus alba* L. in China (Du *et al.* 2017). This fungus is illustrated and characterised by its multilocular conidiomata with one to four ostioles, and aseptate, brown, ellipsoid to oblong conidia (16–20.5 × 6.0–7.5 µm) (Du *et al.* 2017). Both morphology and the sequence data confirmed that our three isolates belong to this species. Therefore, this represents a new host record (*Rhus typhina*) for *Aplosporella ginkgonis.*

Aplosporella javeedii Jami, Gryzenh., Slippers & M.J. Wingf., Fungal Biology 118(2): 174 (2013)

Materials examined: **China, Beijing City**, **Mentougou District**, Dongling Mountain, Xiaolongmen Forestry Centre, from branches of *Ziziphus jujube*, 22 Aug. 2017, H.Y. Zhu & X.L. Fan, deposited by X.L. Fan, CF 2017816, living culture CFCC 52435; *ibid.* CF 2017817, living culture CFCC 52436; *ibid.* CF 2017819, living culture CFCC 52437; **Beijing City**, **Mentougou District**, Dongling Mountain, Xiaolongmen Forestry Centre, from branches of *Rhus typhina*, 21 Aug. 2017, H.Y. Zhu & X.L. Fan, deposited by X.L. Fan, CF 2017822, living culture CFCC 52438; *ibid.* CF 2017823, living culture CFCC 52439; *ibid.* CF 2017827, living culture CFCC 52440.; *ibid.* CF 2017881, living culture CFCC 52441.

Notes: *Aplosporella javeedii* was known as an endophyte from healthy trees of two dicotyledonous host species [*Celtis Africana* Burm. f. (Cannabaceae Martinov) and *Searsia lancea* (L. f.) F.A. Barkley (Anacardiaceae)] in South Africa (Jami *et al.* 2014). Fan *et al.* (2015) firstly reported and illustrated this fungus in China, associating with canker or dieback disease of five hosts, i.e. *Albizia julibrissin* Durazz. (Fabaceae Lindl.), *Broussonetia papyrifera* (L.) Vent. (Moraceae Gaudich.), *Gleditsia sinensis* Lam. (Fabaceae), *Juniperus chinensis* L. (Cupressaceae Gray), and *Styphnolobium japonicum* (L.) Schott (Fabaceae) (Fan *et al.* 2015b). The current study extends the host range to *Rhus typhina* (Anacardiaceae) and *Ziziphus jujube* (Rhamnaceae).

Botryosphaeria dothidea (Moug.) Ces. & De Not., Comm. Soc. Crittog. Ital. 1: 212 (1863)
Synonyms: *Sphaeria dothidea* Moug. *Syst. mycol.* 2(2): 423 (1823)

Materials examined: **China, Beijing City**, **Mentougou District**, Dongling Mountain, Xiaolongmen Forestry Centre, from branches of *Juglans regia*, 22 Aug. 2017, H.Y. Zhu & X.L. Fan, deposited by X.L. Fan, CF 2017840, living culture CFCC 52445; *ibid.* CF 2017875, living culture CFCC 52446; *ibid.* CF 2017873, living culture CFCC 52447;
Notes: *Botryosphaeria dothidea* is the type species of *Botryosphaeria* (Botryosphaeriaceae, Botryosphaeriales), which was regarded as a latent pathogen of global importance to woody plant health (over 24 genera plants) (Marsberg *et al.* 2017). This fungus was reported to be the most commonly species causing canker disease with a wide host range in China (Deng 1963; Tai 1979; Wei 1979; Zhuang 2005). The current study suggests *Botryosphaeria dothidea* is the causal agent of walnut canker in Dongling Mountain.

Phaeobotryon rhoinum Fan ***sp. nov.*** Fig. 2
MycoBank MB 824808
Holotype:—**China, Beijing City**, **Mentougou District**, Dongling Mountain, Xiaolongmen Forestry Centre, from branches of *Rhus typhina*, 17 Aug. 2017, H.Y. Zhu & X.L. Fan, deposited by X.L. Fan, holotype CF 2017820, ex-type living culture CFCC 52449.

Etymology:—Named after the host genus on which it was collected, *Rhus*.

Descriptions:—Asexual morph: *Pycnidial stromata* immersed in the bark, globose, scattered to gregarious, erumpent slightly through the surface of bark, unilocular. *Ectostromatic disc* honey to hazel, inconspicuous, circular. *Ostioles* black, inconspicuous, at the same level as the disc surface, surrounded below disc by lighter entostroma. *Locule* single, globose, (230–)250–420(–450) µm in diam. *Conidiogenous cells* formed from the cells lining the inner walls of the locules, hyaline, smooth, inconspicuous. *Conidia* ellipsoid to oblong, smooth to verruculose, moderately thick-walled, guttulate, ends rounded, initial hyaline, aseptate, becoming brown, 1-septate when mature, (18.5–)19–21(–21.5) × (7–)7.5–9 ($\bar{x}$ = 20.1 ± 0.8 × 8.2 ± 0.5 µm, n = 50) µm. Sexual morph: not observed.

Culture characteristics: Culture on PDA is initially white, becoming olivaceous to fuscous black after 7–10 days. The colony is felt-like, thick and fluffy with abundant aerial mycelium. Pycnidia distributed irregularly on the medium surface.

Materials examined:—**China, Beijing City**, **Mentougou District**, Dongling Mountain, Xiaolongmen Forestry Centre, from branches of *Rhus typhina*, 18 Aug. 2017, H.Y. Zhu & X.L. Fan, deposited by X.L. Fan, CF 2017825, living culture CFCC 52450; *ibid.* CF 2017828, living culture CFCC 52451.

Notes:—*Phaeobotryon rhoinum* is associated with canker disease of *Rhus typhina*, which has similar characteristics with *P. rhois*. Morphologically, *P. rhoinum* has smaller conidia (19–21 ×7.5–9 µm) as compared with the conidia (20–25 × 10–12 µm) of *P. rhois* (Fig. 2) (Fan *et al.* 2015a). Phylogenetically, it clusters in a separate lineage (MP/ML/BI = 99/100/1) compared to all other strains included in this study, and therefore we describe this species as a new.

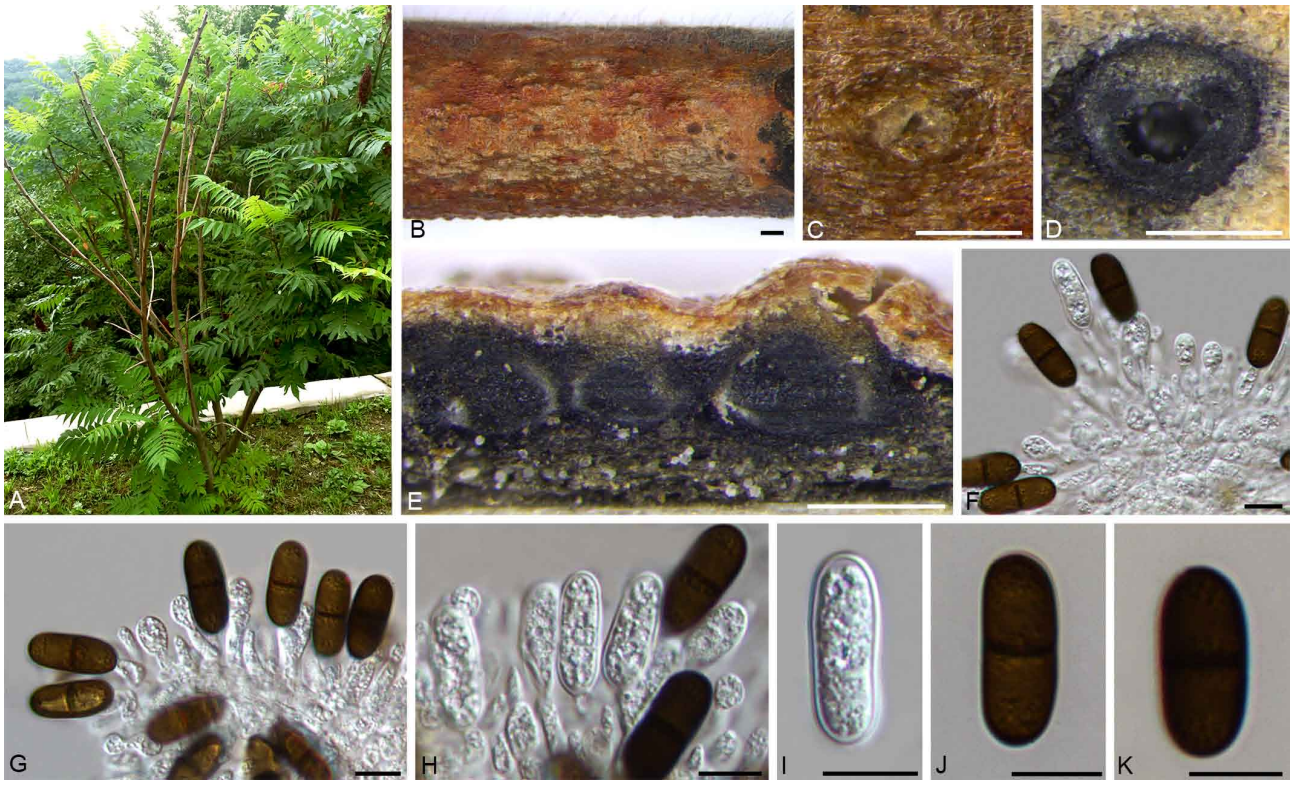

FIGURE 2. Morphology of *Phaeobotryon rhoinum* from *Rhus typhina* (CF 201782). A: Symptoms on the host. B, C: Habit of pycnidia on a twig. D: Transverse section of pycnidia. E: Longitudinal section through pycnidia. F–H: Conidiogenous cells and conidia. I: immature conidia. J–K: mature conidia. Scale bars: B = 1 mm; C–E = 500 μm; F–K = 10 μm.

Phaeobotryon rhois C.M. Tian, X.L. Fan & K.D. Hyde, Phytotaxa 205(2): 95 (2015)

Material examined: **China, Beijing City**, **Mentougou District**, Dongling Mountain, Xiaolongmen Forestry Centre, from branches of *Rhus typhina*, 21 Aug. 2017, H.Y. Zhu & X.L. Fan, deposited by X.L. Fan, CF 2017826, living culture CFCC 52448

Notes: *Phaeobotryon rhois* was known to cause canker and dieback disease of *Rhus typhina* in China (Fan *et al.* 2015a). This fungus is illustrated and characterised by its globose, unilocular fruiting bodies and small, brown, 1-septate conidia (20–25 × 10–12 μm) (Fan *et al.* 2015a). Both morphology and the sequence data confirmed that our three isolates belong to this species.

Discussion

In this study, five species of *Aplosporellaceae* and *Botryosphaeriaceae* (*Botryosphaeriales*) were isolated from *Juglans regia*, *Rhus typhina* and *Ziziphus jujuba* in Dongling Mountain of China. These species include *Aplosporella ginkgonis*, *Aplosporella javeedii*, *Botryosphaeria dothidea*, *Phaeobotryon rhoinum* and *Phaeobotryon rhois*. Among them, *Aplosporella javeedii* and *A. ginkgonis* were identified as the first records from *Ziziphus jujube* and *Rhus typhina*, respectively. *Phaeobotryon rhoinum* is introduced here as new. The results represent the first attempts to study *Botryosphaeriales* fungi with morphology and multi-locus phylogenies (ITS, LSU and TEF-1α) in Dongling Mountain of China, which is considered as a practice base of biodiversity with a high diversity for forest species in Beijing Forestry University. In the current study, 17 specimens were collected from symptomatic branches and twigs associated with canker or dieback disease. Four species were isolated from 11 specimens of *Rhus typhina* with various symptoms, suggesting that many additional undiscovered species of botryosphaeriaceous fungi exist in China.

Botryosphaeriales species are typically regarded as latent or opportunistic pathogens and seem to have a wide range of hosts and unclear patterns of host association (Schoch *et al.* 2006; Slippers *et al.* 2013). Recent studies

have suggested that these species prefer hosts that are suffering from environmental stress, particularly drought stress (Slippers & Wingfield 2007; Marsberg *et al.* 2017). Additionally, de Wet *et al.* (2008) and Alves *et al.* (2013) observed and analysed the patterns of host association of some genera of botryosphaeriaceous fungi, e.g., *Botryosphaeria*, *Dothiorella*, *Diplodia*, *Lasiodiplodia* and *Neofusicoccum*, which suggested that both host generalists and species specialists were present in all lineages in this order, and proposed some assumptions such as site-specific factors or host-associated co-evolution. In the present study, most fungi infected only one single species (apart from *Aplosporella javeedii,* which were proved to infect several host species) (Fan *et al.* 2015b). These results also suggest that host selectivity is a universal characteristic in some taxa.

In the future studies of Botryosphaeriales fungi, extensive fresh materials should be collected to help clarify the confused species concepts. Most taxa in this order still lacked type materials linking to multigene DNA data. The fungal diversity of Botryosphaeriales associated with canker or dieback disease seems to be an attractive region of discovery.

TABLE 1. Strains of Botryosphaeriales taxa used in the molecular analyses in this study.

Species	Isolate No.	Host	Location	GenBank Accession No.		
				ITS	LSU	TEF-1α
Alanomyces indica	MCC 1039	Soil	India	HF563622	HF563623	AB872219
Aplosporella africana	CBS 121777 = CMW 25424	*Acacia mellifera*	Namibia	KF766196	EU101380	EU101360
Aplosporella africana	CBS 121778 = CMW 25425	*Acacia mellifera*	Namibia	EU101316	EU101381	EU101361
Aplosporella africana	CBS 121779 = CMW 25426	*Acacia mellifera*	Namibia	EU101317	EU101382	EU101362
Aplosporella artocarpi	CPC 22791	*Artocarpus heterophyllus*	Thailand	KM006450	NA	KM006481
Aplosporella ginkgonis	CFCC 89660	*Morus alba*	China	KM030582	KM030589	KM030596
Aplosporella ginkgonis	CFCC 89661	*Ginkgo biloba*	China	KM030583	KM030590	KM030597
Aplosporella ginkgonis	CFCC 52442*	*Rhus typhina*	China	MH133916	MH133933	MH133950
Aplosporella ginkgonis	CFCC 52443*	*Rhus typhina*	China	MH133917	MH133934	MH133951
Aplosporella ginkgonis	CFCC 52444*	*Rhus typhina*	China	MH133918	MH133935	MH133952
Aplosporella hesperidica	CBS 208.37	*Citrus sinensis*	Zimbabwe	JX681069	NA	NA
Aplosporella javeedii	CFCC 89657	*Albizia julibrissin*	China	KM030579	KM030586	KM030593
Aplosporella javeedii	CFCC 50052	*Gleditsia sinensis*	China	KP208838	KP208841	KP208844
Aplosporella javeedii	CFCC 50053	*Styphnolobium japonicum*	China	KP208839	KP208842	KP208845
Aplosporella javeedii	CFCC 50054	*Juniperus chinensis*	China	KP208840	KP208843	KP208846
Aplosporella javeedii	CFCC 52435*	*Ziziphus jujube*	China	MH133909	MH133926	MH133943
Aplosporella javeedii	CFCC 52436*	*Ziziphus jujube*	China	MH133910	MH133927	MH133944
Aplosporella javeedii	CFCC 52437*	*Ziziphus jujube*	China	MH133911	MH133928	MH133945
Aplosporella javeedii	CFCC 52438*	*Rhus typhina*	China	MH133912	MH133929	MH133946
Aplosporella javeedii	CFCC 52439*	*Rhus typhina*	China	MH133913	MH133930	MH133947
Aplosporella javeedii	CFCC 52440*	*Rhus typhina*	China	MH133914	MH133931	MH133948
Aplosporella javeedii	CFCC 52441*	*Rhus typhina*	China	MH133915	MH133932	MH133949
Aplosporella macropycnidia	CGMCC 3.17725	*Cerasus yedoensis*	China	KT343648	NA	KX011176
Aplosporella macropycnidia	CGMCC 3.17726	*Cerasus yedoensis*	China	KT343649	NA	KX011177
Aplosporella macropycnidia	CGMCC 3.17727	*Cerasus yedoensis*	China	KT343647	NA	KX011175
Aplosporella papillata	CBS 121780	*Acacia tortillas*	South Africa	EU101328	EU101383	EU101373
Aplosporella papillata	CBS 121781	*Acacia tortillas*	South Africa	EU101329	EU101384	EU101374
Aplosporella papillata	CBS 121782	*Acacia tortillas*	South Africa	EU101330	EU101385	EU101375
Aplosporella prunicola	CBS 121167	*Prunus persica* var. *nucipersica*	South Africa	KF766147	KF766315	NA
Aplosporella prunicola	STE-U 6326	*Prunus persica* var. *nucipersica*	South Africa	EF564375	EF564377	NA
Aplosporella prunicola	STE-U 6327	*Prunus persica* var. *nucipersica*	South Africa	EF564376	EF564378	NA
Aplosporella thailandica	MFLU 16-0615	Dead stems	Thailand	KX423536	NA	KX423537
Aplosporella yalgorensis	MUCC 511	*Acacia cochlearis*	Australia	EF591926	EF591943	EF591977
Aplosporella yalgorensis	MUCC 512	*Eucalyptus gomphocephala*	Australia	EF591927	EF591944	EF591978

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TABLE 1. (Continued)

Species	Isolate No.	Host	Location	GenBank Accession No.		
				ITS	LSU	TEF-1α
Barriopsis iraniana	IRAN 1448C	*Mangifera indica*	Iran	NR137030	KF766318	FJ919652
Botryobambusa fusicoccum	MFLUCC 11-0143	*Bambusa* sp.	Thailand	JX646792	JX646809	JX646857
Botryobambusa fusicoccum	MFLUCC 11-0657	*Bambusa* sp.	Thailand	JX646793	JX646810	JX646858
Botryosphaeria corticis	CBS 119047	*Vaccinium corymbosum*	USA	DQ299245	EU673244	EU017539
Botryosphaeria dothidea	CFCC 52445*	*Juglans regia*	China	MH133919	MH133936	MH133953
Botryosphaeria dothidea	CFCC 52446*	*Juglans regia*	China	MH133920	MH133937	MH133954
Botryosphaeria dothidea	CFCC 52447*	*Juglans regia*	China	MH133921	MH133938	MH133955
Botryosphaeria dothidea	CMW 8000	*Prunus* sp.	Portugal	AY236949	AY928047	AY236898
Botryosphaeria fabicercianum	CMW 27106	*Eucalyptus* sp.	China	HQ332199	NA	HQ332215
Cophinforma atrovirens	MFLUCC 110425	*Eucalyptus* sp.	Thailand	JX646800	JX646817	JX646865
Cophinforma atrovirens	MFLUCC 110655	*Eucalyptus* sp.	Thailand	JX646801	JX646818	JX646866
Cophinforma atrovirens	CMW 22682	*Pterocarpus angolensis*	South Africa	FJ888476	NA	NA
Cophinforma mamane	CBS 117444	*Eucalyptus urophylla*	Venezuela	KF531822	DQ377855	KF531801
Diplodia africana	STE-U 5908	*Prunus persica*	South Africa	EF445343	NA	EF445382
Diplodia mutila	CBS 112553	*Vitis vinifera*	Portugal	AY259093	AY928049	AY573219
Diplodia mutila	CBS 230.30	*Phoenix dactylifera*	USA	DQ458886	EU673265	DQ458869
Diplodia rosulata	CBS 116470	*Prunus africana*	Ethiopia	EU430265	NA	EU430267
Dothiorella iberica	CBS 115041	*Quercus ilex*	Spain	AY573202	AY928053	AY573222
Dothiorella sarmentorum	IMI 63581b	*Ulmus* sp.	UK	AY573212	AY928052	AY573235
Endomelanconiopsis endophytica	CBS 120397	*Theobroma cacao*	Panama	EU683656	EU683629	EU683637
Endomelanconiopsis microspora	CBS 353.97	Soil	Papua New Guinea	EU683655	EU683628	EU683636
Fusicladium convolvularum	CBS 112706	*Convolvulus arvensis*	New Zealand	NA	EU035428	NA
Fusicladium effusum	STE-U 4525 = CPC 4525	*Carya illinoinensis*	USA	AY251085	EU035430	KF766428
Fusicladium oleagineum	CBS 113427	*Olea europaea*	New Zealand	KF766166	NA	NA
Kellermania anomala	CBS 132218 = AR 3471	*Yucca brevifolia*	USA	KF766173	NG042700	KF766404
Kellermania confusa	CBS 131723 = AR 3469	*Yucca thornberi*	USA	KF766174	NG042701	KF766405
Kellermania crassispora	CBS 131714 = AR 3463	*Nolina micrantha*	USA	KF766175	NG042702	KF766406
Kellermania dasylirionicola	CBS 131720 = AR 3465	*Dasylirion leiophyllum*	USA	KF766176	NG042703.	KF766407
Kellermania dasylirionis	CBS 131715 = AR 3464	*Dasylirion leiophyllum*	USA	KF766177	NG042704	KF766408
Kellermania macrospora	CBS 131716 = AR 3468	*Agave* sp.	USA	KF766178	NG042705	KF766409
Kellermania micranthae	CBS 131724 = AR 3474	*Nolina micrantha*	USA	KF766179	NG042706	KF766410
Kellermania nolinae	CBS 131717 = AR 3475	*Nolina erumpens*	USA	KF766180	NG042707	KF766411
Kellermania plurilocularis	CBS 131719 = AR 3467	*Yucca baccata*	USA	KF766181	NG042709	KF766412
Kellermania uniseptata	CBS 131725 = AR 3476	*Yucca rupicola*	USA	KF766184	NG042712	KF766415
Kellermania yuccifoliorum	CBS 131726 = AR 3472	*Yucca brevifolia*	USA	KF766185	NG042713	KF766416
Kellermania yuccigena	CPC 20623	*Yucca rostrata*	USA	KF766189	KJ710448	KF766420
Lasiodiplodia crassispora	WAC 12533	*Santalum album*	Australia	DQ103550	NA	DQ103557
Lasiodiplodia crassispora	CBS 110492	NA	NA	EF622086	EU673251	EF622066
Lasiodiplodia gonubiensis	CMW 14077	*Syzygium cordatum*	South Africa	AY639595	NA	DQ103566

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TABLE 1. (Continued)

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				ITS	LSU	TEF-1α
Lasiodiplodia theobromae	CBS 164.96	*Papua New Guinea*	Fruit	NR111174	EU673253	AY640258
Macrophomina phaseolina	CBS 227.33	*Zea mays*	Israel	KF531825	DQ377906	KF531804
Macrophomina phaseolina	CBS 162.25	*Eucalyptus sp.*	Uganda	KF531826	DQ377905	KF531803
Melanops sp.	CBS 118.39	*Quercus borealis*	USA	FJ824771	DQ377856	FJ824776
Melanops tulasnei	CBS 116805	*Quercus robur*	Germany	FJ824769	FJ824764	FJ824774
Melanops tulasnei	CBS 116806	*Quercus robur*	Germany	FJ824770	FJ824765	FJ824775
Neodeightonia palmicola	MFLUCC 10-0822	*Arenga westerhoutii*	Thailand	HQ199221	HQ199222	NA
Neodeightonia palmicola	MFLUCC 10-0823	*Caryota urens*	Thailand	HQ199224	HQ199225	NA
Neodeightonia phoenicum	CBS 169.34	*Phoenix dactylifera*	USA	EU673338	EU673259	EU673307
Neodeightonia phoenicum	CBS 122528	*Phoenix dactylifera*	Spain	EU673340	EU673261	EU673309
Neodeightonia subglobosa	CBS 448.91	*Homo sapiens*	UK	EU673337	DQ377866	EU673306
Neofusicoccum australe	CMW 6837	*Acacia* sp.	Australia	AY339262	NA	AY339270
Neofusicoccum eucalypticola	CMW 6539	*Eucalyptus grandis*	Australia	KF766201	KF766368	AY615133
Neofusicoccum grevilleae	CPC 16999	*Grevillea aurea*	Australia	JF951137	JF951157	NA
Neofusicoccum luteum	CMW 10309	*Vitis vinifera*	Portugal	KF766369	KF766202	KF766424
Neofusicoccum mangiferae	CMW 7024	*Mangifera indica*	Australia	AY615185	NA	DQ093221
Neofusicoccum mangiferae	CBS 118531	*Mangifera indica*	Australia	NA	DQ377920	NA
Neofusicoccum parvum	CMW 9081	*Populus nigra*	New Zealand	KF766204	AY928045	KF766426
Neoscytalidium dimidiatum	CBS 499.66	*Mangifera indica*	Mali	KF531820	DQ377925	KF531798
Neoscytalidium hyalinum	CBS 145.78	*Homo sapiens*	UK	KF531816	DQ377922	KF531795
Neoscytalidium novaehollandiae	CBS 122071	*Crotalaria medicaginea*	Australia	EF585540	NA	EF585580
Phaeobotryon cupressi	IRAN 1456C	*Cupressus sempervirens*	Iran	FJ919670	NA	FJ919659
Phaeobotryon cupressi	IRAN 1458C	*Cupressus sempervirens*	Iran	FJ919671	NA	FJ919660
Phaeobotryon cupressi	IRAN 1455C	*Cupressus sempervirens*	Iran	FJ919672	NA	FJ919661
Phaeobotryon cupressi	IRAN 1454C	*Cupressus sempervirens*	Iran	FJ919673	NA	FJ919662
Phaeobotryon cupressi	IRAN 1445C	*Cupressus sempervirens*	Iran	KF766208	NA	KF766428
Phaeobotryon mamane	CPC 12442	*Sophora chrysophylla*	USA	EU673333	DQ377899	EU673299
Phaeobotryon mamane	CPC 12440	*Sophora chrysophylla*	USA	KF766209	EU673248	EU673298
Phaeobotryon mamane	CPC 12443	*Sophora chrysophylla*	USA	EU673334	EU673249	EU673300
Phaeobotryon negundinis	CAA 797	*Acer negundo*	Russia	KX061513	NA	KX061507
Phaeobotryon negundinis	CAA 798	*Ligustrum vulgare*	Russia	KX061514	NA	KX061508
Phaeobotryon negundinis	CAA 799	*Forsythia* x *intermedia*	Russia	KX061515	NA	KX061509
Phaeobotryon negundinis	MFLUCC 15-0436	*Acer negundo*	Russia	KU820970	KU820971	KU853997
Phaeobotryon rhoinum	CFCC 52449*	*Rhus typhina*	China	MH133923	MH133940	MH133957
Phaeobotryon rhoinum	CFCC 52450*	*Rhus typhina*	China	MH133924	MH133941	MH133958
Phaeobotryon rhoinum	CFCC 52451*	*Rhus typhina*	China	MH133925	MH133942	MH133959
Phaeobotryon rhois	CFCC 89662	*Rhus typhina*	China	KM030584	KM030591	KM030598
Phaeobotryon rhois	CFCC 89663	*Rhus typhina*	China	KM030585	KM030592	KM030599
Phaeobotryon rhois	CFCC 52448*	*Rhus typhina*	China	MH133922	MH133939	MH133956
Phyllosticta hypoglossi	CBS 101.72	*Ruscus aculeatus*	Italy	FJ538365	KF206326	FJ538423
Phyllosticta philoprina	CBS 616.72	*Ilex aquifolium*	Netherlands	KF289205	KF206296	KF154279
Phyllosticta telopeae	CBS 777.97	*Telopea speciosissima*	Australia	KF206205	KF206285	KF289210
Phyllosticta yuccae	CBS 117136	*Yucca elephantipes*	New Zealand	KF766219	KF766385	KF766436
Pseudofusicoccum adansoniae	CBS 122055	*Adansonia gibbosa*	Australia	EF585523	NA	EF585571

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TABLE 1. (Continued)

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Pseudofusicoccum ardesiacum	CBS 122062	*Adansonia gibbosa*	Australia	EU144060	NA	EU144075
Pseudofusicoccum kimberleyense	CBS 122058	*Acacia synchronicia*	Australia	EU144057	NA	EU144072
Pseudofusicoccum sp.	CMW 3967	*Mangifera indica*	Brazil	JX464106	NA	JX464113
Pseudofusicoccum stromaticum	CBS 117448	*Eucalyptus hibrido*	Venezuela	AY693974	DQ377931	AY693975
Saccharata capensis	CBS 122693	*Mimetes cucullata*	South Africa	KF766224	KF766390	EU552095
Saccharata kirstenboschensis	CBS 123537	*Encephalartos princeps*	South Africa	FJ372392	FJ372409	KX464770
Saccharata proteae	CBS 115206	*Protea* sp.	Australia	KF766226	DQ377882	KF766438
Septorioides pini-thunbergii	CBS 473.91	*Pinus thunbergii*	Japan	KF251243	KF251746	NA
Septorioides strobi	CBS 141443	*Pinus strobus*	USA	KT884699	KT884685	KT884713
Septorioides strobi	CBS 141444	*Pinus strobus*	USA	KT884700	KT884686	KT884714
Septorioides strobi	CBS 141445	*Pinus strobus*	USA	KT884701	KT884687	KT884715
Spencermartinsia sp.	ICMP16827	*Citrus sinensis*	New Zealand	EU673322	EU673241	EU673289
Spencermartinsia viticola	CBS 117009	*Vitis vinifera*	Spain	AY905554	DQ377873	AY905559
Spencermartinsia viticola	UCP 105	*Citrus* sp.	USA	JF271748	NA	JF271784
Sphaeropsis citrigena	ICMP 16812	*Citrus sinensis*	Luxembourg	EU673328	EU673246	EU673294
Sphaeropsis citrigena	ICMP 16818	*Citrus sinensis*	New Zealand	EU673329	EU673247	EU673295
Sphaeropsis eucalypticola	MFLUCC 11-0579	*Eucalyptus* sp.	Thailand	JX646802	JX646819	JX646867
Sphaeropsis porosa	STE-U 5132	*Vitis vinifera*	South Africa	AY343379	NA	AY343340
Sphaeropsis visci	CBS 100163	*Viscum album*	Luxembourg	EU673324	DQ377870	EU673292
Tiarosporella tritic	CBS 118719	*Triticum* sp.	South Africa	KF531830	DQ377941	KF531809
Tiarosporella urbis-rosarum	CMW 36477	*Acacia karroo*	South Africa	JQ239407	JQ239420	JQ239394

Notes: CBS: Westerdijk Fungal Biodiversity Institute (CBS-KNAW Fungal Biodiversity Centre), Utrecht, The Netherlands; CFCC: China Forestry Culture Collection Centre, Beijing, China; CGMCC: China General Microbiological Culture Collection Centre; CMW: Culture collection of Michael Wingfield, University of Pretoria, South Africa; CPC: Culture collection of Pedro Crous, The Netherlands; ICMP: International Collection of Microorganisms from Plants; IMI: CABI Bioscience, Egham, UK; MFLUCC: Mae Fah Luang University Culture Collection, Thailand; STE-U: Department of Plant Pathology, University of Stellenbosch, South Africa; WAC: Department of Agriculture Western Australia Plant Pathogen Collection; UCP: University of California, Riverside Citrus Project; NA: not applicable. All the new isolates used in this study are marked by an asterisk (*) and the strains from generic type species are in bold.

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