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Species of *Botryosphaeriaceae* associated with citrus branch diseases in China

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Key words

Botryosphaeria cankers
distribution
new taxa
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systematics

Abstract Citrus is an important and widely cultivated fruit crop in South China. Although the species of fungal diseases of leaves and fruits have been extensively studied, the causal organisms of branch diseases remain poorly known in China. Species of *Botryosphaeriaceae* are known as important fungal pathogens causing branch diseases on citrus in the USA and Europe. To determine the diversity of *Botryosphaeriaceae* species associated with citrus branch diseases in China, surveys were conducted in the major citrus-producing areas from 2017 to 2020. Diseased tissues were collected from twigs, branches and trunks with a range of symptoms including cankers, cracking, dieback and gummosis. Based on morphological characteristics and phylogenetic comparison of the DNA sequences of the internal transcribed spacer region (ITS), the translation elongation factor 1-alpha gene (*tef1*), the β-tubulin gene (*tub2*) and the DNA-directed RNA polymerase II second largest subunit (*rpb2*), 111 isolates from nine provinces were identified as 18 species of *Botryosphaeriaceae*, including *Botryosphaeria dothidea*, *B. fabicerciana*, *Diplodia seriata*, *Dothiorella alpina*, *Do. plurivora*, *Lasiodiplodia citricola*, *L. iraniensis*, *L. microconidia*, *L. pseudotheobromae*, *L. theobromae*, *Neodeightonia subglobosa*, *Neofusicoccum parvum*, and six previously undescribed species, namely *Do. citrimurcotticola*, *L. guilinensis*, *L. huangyanensis*, *L. linhaiensis*, *L. ponkanicola* and *Sphaeropsis linhaiensis* spp. nov. *Botryosphaeria dothidea* (28.8 %) was the most abundant species, followed by *L. pseudotheobromae* (23.4 %), which was the most widely distributed species on citrus, occurring in six of the nine provinces sampled. Pathogenicity tests indicated that all 18 species of *Botryosphaeriaceae* obtained from diseased citrus tissues in this study were pathogenic to the tested *Citrus reticulata* shoots *in vitro*, while not all species are pathogenic to the tested Cocktail grapefruit (*C. paradisi* × *C. reticulata*) shoots *in vivo*. In addition, *Lasiodiplodia* was the most aggressive genus both *in vitro* and *in vivo*. This is the first study to identify *Botryosphaeriaceae* species related to citrus branch diseases in China and the results provide a theoretical basis for the implementation of prevention and control measures.

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INTRODUCTION

The *Botryosphaeriaceae* was established by Theissen & Sydow (1918). The taxonomic status of *Botryosphaeriaceae* has been heavily debated and somewhat controversial until Schoch et al. (2006) proposed the *Botryosphaeriales* as a new order to accommodate the family (see Phillips et al. 2013). Presently, the *Botryosphaeriaceae* contains 23 genera and over 100 species that have been confirmed based on their DNA sequence data (Slippers et al. 2017, Yang et al. 2017, Zhang et al. 2021).

Species of *Botryosphaeriaceae* have a broad host range and cosmopolitan distribution (Slippers & Wingfield 2007, Phillips et al. 2013). Many species are important plant pathogens, especially for woody plant genera such as *Citrus*, causing bark rot, branch canker, gummosis, shoot blight, dieback and fruit rot, and even death of whole plants when conditions are conducive to disease development (Slippers & Wingfield 2007, Úrbez-Torres 2011). Citrus is one of the most important fruit crops globally. Citrus diseases caused by species in the *Botryosphaeriaceae* have been reported since the early 1900s when Fawcett & Burger (1911) isolated a *Diplodia* sp. from orange trees with gummosis, and from rotten grapefruits and oranges in Florida. The fungal agent was then considered to be *Diplodia natalensis*, which was regarded as the pathogen responsible for decay and gummosis in lemons and other citrus fruits in the USA and South Africa (Fawcett & Burger 1911, Adesemoye et al. 2014). Subsequent taxonomic revisions showed that *D. natalensis* represents as synonym of *Lasiodiplodia theobromae* (Alves et al. 2004). Further studies indicated that Diplodia stem-end rot caused by *L. theobromae* is one of the most important postharvest decays in warm, humid tropical and subtropical citrus-producing areas (Brown & Eckert 2000, Ismail & Zhang 2004, Zhang 2014). Several other species of *Botryosphaeriaceae* have subsequently been isolated from citrus with cankers, dieback, gummosis and fruit rot symptoms, including species of *Botryosphaeria* (Smith 1934, Adesemoye et al. 2011), *Diplodia* (Adesemoye et al. 2014, Berraf-Tebbal

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et al. 2020), *Dothiorella* (Adesemoye & Eskalen 2011, Abdollahzadeh et al. 2014, Berraf-Tebbal et al. 2020), *Lasiodiplodia* (Alves et al. 2008, Abdollahzadeh et al. 2010, Adesemoye et al. 2014, Linaldeddu et al. 2015, Coutinho et al. 2017, Guajardo et al. 2018, Bautista-Cruz et al. 2019, Berraf-Tebbal et al. 2020), *Macrophomina* (Azadeh et al. 2018), *Neofusicoccum* (Adesemoye & Eskalen 2011), *Neoscytalidium* (Polizzi et al. 2009, Adesemoye et al. 2014, Mayorquin et al. 2016) and *Sphaeropsis* (Phillips et al. 2013).

China has a history of more than 4 000 years of citrus cultivation (Deng et al. 2008, Shen 2019) and is the world's largest producer of citrus, with 37.92 M tons in 2018 (FAO 2018). Branch diseases including twig blight, branch dieback, bark rot, canker, crack and gummosis are commonly observed on citrus, especially in regions where stress factors such as frost and sunburn often occur. Resin (gummosis) caused by *Diaporthe citri* has been recorded as the most important fungal branch disease (Cai et al. 2011, Huang et al. 2013b), followed by Alternaria brown spot (dieback) caused by *Alternaria alternata* pathotype tangerine (Huang et al. 2012, Qin et al. 2012), anthracnose (twig blight and branch dieback) caused by *Colletotrichum gloeosporioides* (Cai et al. 2011, Huang et al. 2013a), and foot root caused by *Phytophthora* spp. (Cheng et al. 2004, Cai et al. 2011, Zhu et al. 2011). Species in other genera such as *Cytospora*, *Diplodia*, *Dothidea*, *Macrophoma*, *Phoma*, *Phyllosticta* and *Sphaeropsis*, have also been associated with citrus branch diseases (Chinese Academy of Agricultural Sciences 1960, Tai 1979). However, all fungal identifications were based on morphology or simply based on the symptoms before the 1990s and pathogenicity tests were lacking for most species (Tai 1979).

During 2017–2020, several surveys of citrus branch diseases were conducted in the major citrus production regions in China. The objectives of this study were to:

- identify the species of *Botryosphaeriaceae* associated with citrus branch diseases in China based on morphological traits and phylogenetic analysis;
- identify the dominant species associated with citrus branch diseases; and
- determine their pathogenicity.

MATERIALS AND METHODS

Disease symptoms, sample collection and fungal isolations

From 2017 to 2020, citrus branch disease samples with symptoms of canker, gummosis, twig blight and branch dieback (Fig. 1) were collected from the main citrus-producing regions in nine provinces of China, namely Chongqing, Fujian, Guangdong, Guangxi, Hunan, Jiangxi, Shaanxi, Shanghai and Zhejiang. The citrus species investigated and the number of samples collected would depend on the incidence of branch diseases in the orchard and region.

Fungal strains were isolated via two methods. Firstly, sporocarps visible on diseased tissue were transferred to a microtube containing sterile water to make a spore suspension. After dilution, 150 µL spore suspension was spread over the surface of water agar (WA) plates amended with 100 µg/mL ampicillin and 100 µg/mL streptomycin to suppress bacterial growth. After 24–36 h, germinating spores were retrieved and transferred onto potato dextrose agar plates (PDA, 200 g potatoes, 20 g glucose and 15 g agar/L water) with 100 µg/mL ampicillin and 100 µg/mL streptomycin (PDA-AS) and incubated at 25 °C. Axenic cultures were obtained by transferring a single colony onto PDA. Secondly, for samples lacking sporocarps, a tissue isolation method was used. A small section (about 3 × 3 mm) between the healthy and diseased tissue was aseptically cut and surface-sterilised in 70 % ethanol for 1 min, followed by 1 % NaClO solution for 1 min, and rinsed three times in sterile water. Tissue sections were dried on sterilised filter paper, placed on 1/2 PDA-AS plates and incubated at 25 °C. Axenic cultures were obtained by transferring single hyphal tips onto PDA. Specimens and isolates from this study were deposited in Zhejiang University, and ex-type cultures were deposited in the China General Microbiological Culture Collection Centre (CGMCC), Beijing, China.

DNA extraction, PCR amplification and sequencing

Isolates were grown on PDA plates and incubated at room temperature for 4–7 d. Surface mycelia were collected using

Fig. 1 Disease symptoms on citrus caused by *Botryosphaeriaceae*. a. Twig blight of *Citrus reticulata*; b. twig blight on Cocktail grapefruit; c. branch dieback of *C. reticulata*; d. death tree of *C. reticulata*; e. branch canker on *C. reticulata*; f. trunk canker of *C. unshiu*; g–h. gummosis on twig and trunk of Cocktail grapefruit; i. fungal fruitbody structures formed on dead branch of Cocktail grapefruit.

Table 1 Details of *Botryosphaeriaceae* isolates studied.

Species[a]	Isolate	Location	Collector	Host	Associated symptom	GenBank Accession no.[b]			
						ITS	*tef1*	*tub2*	*rpb2*
Botryosphaeria dothidea	BE1	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (*C. paradisi* × *C. reticulata*)	Twig gummosis	MT772261	MT775839	MT775849	MW884107
	BE2	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (*C. paradisi* × *C. reticulata*)	Twig gummosis	MT772262	MT775840	MT775850	MW884108
	BE60	Chenggu, Shaanxi, China	H.Y. Li & X.E. Xiao	*C. unshiu*	Trunk canker	MW862113	MW884017	MW884086	MW884109
	BE61	Changxing Island, Shanghai, China	X.E. Xiao	hybrid cv. Hongmeiren	Twig dieback	MW862116	MW884020	MW884087	MW884110
	BE62	Chunan, Zhejiang, China	H.Y. Li & X.E. Xiao	*C. unshiu*	Twig dieback	MW862110	MW884014	–	–
	BE63	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	*C. reticulata*	Twig gummosis	MW862111	MW884015	–	–
	BE64	Quzhou, Zhejiang, China	H.Y. Li & J.W. Lv	hybrid cv. Cocktail grapefruit (*C. paradisi* × *C. reticulata*)	Twig gummosis	MW862112	MW884016	–	–
	BE65	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (*C. paradisi* × *C. reticulata*)	Twig gummosis	MW862114	MW884018	–	–
	BE66	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (*C. paradisi* × *C. reticulata*)	Twig gummosis	MW862115	MW884019	–	–
	BE67	Xiangshan, Zhejiang, China	H.Y. Li	*C. unshiu*	Trunk canker	MW862117	MW884021	–	–
	BE68	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (*C. paradisi* × *C. reticulata*)	Twig gummosis	MW862118	MW884022	–	–
	BE69	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	*C. reticulata*	Twig gummosis	MW862119	MW884023	–	–
	BE72	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	*C. reticulata*	Twig dieback	MW881452	MW884024	–	–
	BE73	Chenggu, Shaanxi, China	H.Y. Li & X.E. Xiao	*C. unshiu*	Trunk gummosis	MW862120	MW884025	–	–
	BE75	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (*C. paradisi* × *C. reticulata*)	Twig gummosis	MW862121	MW884026	–	–
	BE76	Hangzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	*C. maxima*	Branch dieback	MW862122	MW884027	–	–
	BE77	Linhai, Zhejiang, China	H.Y. Li	*C. unshiu*	Branch canker	MW862123	MW884028	–	–
	BE79	Hangzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	*C. maxima*	Twig dieback	MW862124	MW884029	–	–
	BE81	Hangzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	*C. maxima*	Branch dieback	MW862125	MW884030	–	–
	BE90	Linhai, Zhejiang, China	H.Y. Li	*C. unshiu*	Twig dieback	MW862126	MW884031	–	–
	BE91	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	*C. reticulata*	Twig gummosis	MW862127	MW884032	–	–
	BE92	Shaoyang, Hunan, China	H.Y. Li & X.E. Xiao	*C. sinensis*	Twig dieback	MW862128	MW884033	–	–
	BE93	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (*C. paradisi* × *C. reticulata*)	Twig gummosis	MW862129	MW884034	–	–
	BE94	Linhai, Zhejiang, China	H.Y. Li	*C. unshiu*	Branch gummosis	MW862130	MW884035	–	–
	BE95	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	*C. reticulata*	Twig gummosis	MW862131	MW884036	–	–
	BE96	Chun'an, Zhejiang, China	H.Y. Li & X.E. Xiao	*C. unshiu*	Branch dieback	MW862132	MW884037	–	–
	BE97	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (*C. paradisi* × *C. reticulata*)	Twig gummosis	MW862133	MW884038	–	–
	BE98	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (*C. paradisi* × *C. reticulata*)	Twig gummosis	MW862134	MW884039	–	–
	BE101	Linhai, Zhejiang, China	H.Y. Li	*C. unshiu*	Twig dieback	MW862135	MW884040	–	–
	BE103	Linhai, Zhejiang, China	H.Y. Li	*C. unshiu*	Twig dieback	MW862136	MW884041	–	–
	BE105	Linhai, Zhejiang, China	H.Y. Li	*C. unshiu*	Twig dieback	MW862137	MW884042	–	–
	BE106	Linhai, Zhejiang, China	H.Y. Li	*C. unshiu*	Twig dieback	MW862138	MW884043	–	–
Botryosphaeria fabicerciana	BE3	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (*C. paradisi* × *C. reticulata*)	Twig gummosis	MT772263	MT775841	MT775851	MW884111
	BE78	Hangzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	*C. maxima*	Twig dieback	MW862139	MW884044	MW884088	MW884112

Table 1 (cont.)

Species[a]	Isolate	Location	Collector	Host	Associated symptom	GenBank Accession no.[b]			
						ITS	*tef1*	*tub2*	*rpb2*
Botryosphaeria fabicerciana (cont.)	BE85	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (*C. paradisi* × *C. reticulata*)	Twig gummosis	MW862140	MW884045	MW884089	MW884113
	BE86	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (*C. paradisi* × *C. reticulata*)	Twig gummosis	MW862141	MW884046	MW884090	MW884114
Diplodia seriata	BE4	Chenggu, Shaanxi, China	H.Y. Li & X.E. Xiao	*C. unshiu*	Branch canker	MW862142	MW884047	MW884091	MW884115
Dothiorella alpina	BE17	Shimen, Hunan, China	H.Y. Li & Y.T. Zeng	*C. unshiu*	Twig dieback	MW862143	MW884048	MW884092	MW884116
Do. citrimurcotticola	**BE5 = CGMCC3.20392**	**Linhai, Zhejiang, China**	**H.Y. Li**	***C. unshiu***	**Twig dieback**	**MW880663**	**MW884166**	**MW884195**	**MW884140**
	BE6	**Huangyan, Zhejiang, China**	**H.K. Wang & X.E. Xiao**	***C. maxima***	**Twig dieback**	**MW880664**	**MW884167**	**MW884196**	**MW884141**
	BE7 = CGMCC3.20393	**Quzhou, Zhejiang, China**	**H.Y. Li**	***C. maxima***	**Twig dieback**	**MW880665**	**MW884168**	**MW884197**	**MW884142**
	BE8 * = CGMCC3.20394	**Wanzhou, Chongqing, China**	**H.Y. Li & X.E. Xiao**	**hybrid cv. Murcott (*C. reticulata* × *C. sinensis*)**	**Twig dieback**	**MW880661**	**MW884164**	**MW884193**	**MW884138**
	BE9 = CGMCC3.20395	**Wanzhou, Chongqing, China**	**H.Y. Li & X.E. Xiao**	**hybrid cv. Murcott (*C. reticulata* × *C. sinensis*)**	**Twig dieback**	**MW880662**	**MW884165**	**MW884194**	**MW884139**
	BE71	**Linhai, Zhejiang, China**	**H.Y. Li**	***C. unshiu***	**Twig dieback**	**MW880666**	**MW884169**	**MW884198**	**MW884143**
Do. plurivora	BE16	Quzhou, Zhejiang, China	H.K. Wang & X.E. Xiao	*C. reticulata* cv. Ponkan	Twig dieback	MT772270	MT775848	MT775858	MW884117
	BE74	Linhai, Zhejiang, China	H.Y. Li	*C. unshiu*	Twig dieback	MW862144	MW884049	MW884093	MW884118
Lasiodiplodia citricola	BE13	Quzhou, Zhejiang, China	H.K. Wang & X.E. Xiao	hybrid cv. Cocktail grapefruit (*C. paradisi* × *C. reticulata*)	Branch gummosis	MT772267	MT775845	MT775855	MW884119
	BE38	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (*C. paradisi* × *C. reticulata*)	Twig gummosis	MW862145	MW884050	MW884094	MW884120
	BE45	Quzhou, Zhejiang, China	H.K. Wang & X.E. Xiao	*C. unshiu*	Twig dieback	MW862146	MW884051	MW884095	MW884121
	BE83	Wanzhou, Chongqing, China	H.Y. Li & X.E. Xiao	hybrid cv. Hongmeiren	Twig dieback	MW862148	MW884053	MW884096	MW884122
	BE89	Chenggu, Shaanxi, China	H.Y. Li & X.E. Xiao	*C. unshiu*	Trunk canker	MW862147	MW884052	–	–
	BE99	Lishui, Zhejiang, China	X.E. Xiao	*C. sinensis*	Twig dieback	MW862149	MW884054	–	–
	BE102	Xiangshan, Zhejiang, China	H.Y. Li & X.E. Xiao	*C. unshiu*	Branch canker	MW862150	MW884055	MW884097	MW884123
	BE104	Lishui, Zhejiang, China	X.E. Xiao	hybrid	Branch canker	MW862151	MW884056	MW884098	MW884124
L. guilinensis	**BE31 * = CGMCC3.20378**	**Guilin, Guangxi, China**	**H.Y. Li & X.E. Xiao**	***C. sinensis***	**Twig dieback**	**MW880672**	**MW884175**	**MW884204**	**MW884149**
	BE59 = CGMCC3.20379	**Linhai, Zhejiang, China**	**H.Y. Li**	***C. unshiu***	**Branch gummosis**	**MW880673**	**MW884176**	**MW884205**	**MW884150**
L. huangyanensis	**BE33 * = CGMCC3.20380**	**Huangyan, Zhejiang, China**	**X.E. Xiao & Q.B. Huang**	***C. reticulata***	**Twig dieback**	**MW880674**	**MW884177**	**MW884206**	**MW884151**
	BE50 = CGMCC3.20381	**Linhai, Zhejiang, China**	**W.L. Li**	***C. unshiu***	**Branch canker**	**MW880675**	**MW884178**	**MW884207**	**MW884152**
	BE111	**Huangyan, Zhejiang, China**	**H.Y. Li**	***C. unshiu***	**Twig dieback**	**MW880676**	**MW884179**	**MW884208**	**MW884153**
L. iranensis	BE27	Guilin, Guangxi, China	H.Y. Li & X.E. Xiao	*C. sinensis*	Twig dieback	MW880686	MW884189	MW884215	MW884160
	BE30	Guilin, Guangxi, China	H.Y. Li & X.E. Xiao	*C. sinensis*	Twig dieback	MW880687	MW884190	MW884216	MW884161
	BE36	Taizhou, Zhejiang, China	X.E. Xiao & Q.B. Huang	*C. reticulata*	Trunk canker	MW880688	MW884191	MW884217	MW884162
	BE41	Quzhou, Zhejiang, China	H.K. Wang & X.E. Xiao	*C. maxima*	Trunk canker	MW862152	MW884057	MW884099	MW884125
	BE42	Quzhou, Zhejiang, China	H.K. Wang & X.E. Xiao	*C. maxima*	Trunk canker	MW862153	MW884058	MW884100	MW884126
	BE100	Lishui, Zhejiang, China	X.E. Xiao	*C. maxima*	Trunk gummosis	MW880684	MW884187	MW884213	MW884158
L. linhaiensis	**BE51 * = CGMCC3.20386**	**Linhai, Zhejiang, China**	**W.L. Li**	***C. unshiu***	**Branch canker**	**MW880677**	**MW884180**	**MW884209**	**MW884154**
	BE28 = CGMCC3.20383	**Guilin, Guangxi, China**	**H.Y. Li & X.E. Xiao**	***C. sinensis***	**Twig dieback**	**MW880678**	**MW884181**	**MW884210**	**MW884155**
	BE34 = CGMCC3.20384	**Huangyan, Zhejiang, China**	**X.E. Xiao & Q.B. Huang**	***C. reticulata***	**Branch canker**	**MW880679**	**MW884182**	**MW884211**	**MW884156**
	BE40 = CGMCC3.20385	**Quzhou, Zhejiang, China**	**H.K. Wang & X.E. Xiao**	**hybrid cv. Cocktail grapefruit (*C. reticulata* × *C. sinensis*)**	**Twig dieback**	**MW880680**	**MW884183**	**MW884212**	**MW884157**
	BE43	**Quzhou, Zhejiang, China**	**H.K. Wang & X.E. Xiao**	***C. reticulata***	**Branch canker**	**MW880681**	**MW884184**	**–**	**–**
	BE49	**Linhai, Zhejiang, China**	**W.L. Li**	***C. unshiu***	**Branch canker**	**MW880682**	**MW884185**	**–**	**–**
	BE52	**Linhai, Zhejiang, China**	**W.L. Li**	***C. unshiu***	**Branch canker**	**MW880683**	**MW884186**	**–**	**–**

Table 1 (cont.)

Species[a]	Isolate	Location	Collector	Host	Associated symptom	GenBank Accession no.[b]			
						ITS	*tef1*	*tub2*	*rpb2*
L. microconidia	BE32	Huangyan, Zhejiang, China	H.K. Wang & X.E. Xiao	*C. reticulata*	Branch canker	MW880668	MW884171	MW884200	MW884145
	BE35	Huangyan, Zhejiang, China	X.E. Xiao & Q.B. Huang	*C. reticulata*	Branch canker	MW880669	MW884172	MW884201	MW884146
	BE80	Chun'an, Zhejiang, China	H.Y. Li & X.E. Xiao	*C. grandis*	Trunk canker	MW880670	MW884173	MW884202	MW884147
	BE87	Chun'an, Zhejiang, China	H.Y. Li & X.E. Xiao	*C. unshiu*	Trunk canker	MW880671	MW884174	MW884203	MW884148
	BE88	Chun'an, Zhejiang, China	H.Y. Li & X.E. Xiao	*C. grandis*	Twig dieback	MW880667	MW884170	MW884199	MW884144
L. ponkanicola	**BE44 * = CGMCC3.20388**	**Quzhou, Zhejiang, China**	**H.K. Wang & X.E. Xiao**	***C. reticulata***	**Trunk canker**	**MW880685**	**MW884188**	**MW884214**	**MW884159**
L. pseudotheobromae	BE10	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	*C. reticulata*	Twig gummosis	MT772264	MT775842	MT775852	MW884127
	BE11	Linhai, Zhejiang, China	H.Y. Li	*C. unshiu*	Twig dieback	MT772265	MT775843	MT775853	MW884128
	BE12	Fuzhou, Jiangxi, China	H.Y. Li & X.E. Xiao	*C. reticulata*	Twig dieback	MT772266	MT775844	MT775854	MW884129
	BE19	Guangzhou, Guangdong, China	H.Y. Li	*C. reticulata*	Twig dieback	MW862154	MW884059	MW884101	MW884130
	BE22	Guilin, Guangxi, China	H.Y. Li & X.E. Xiao	*C. sinensis*	Twig dieback	MW862158	MW884063	MW884102	MW884131
	BE23	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (*C. paradisi* × *C. reticulata*)	Twig gummosis	MW862160	MW884065	MW884103	MW884132
	BE24	Sihui, Guangdong, China	H.Y. Li	*C. reticulata*	Twig dieback	MW862155	MW884060	–	–
	BE26	Sihui, Guangdong, China	H.Y. Li	*C. reticulata*	Twig dieback	MW862156	MW884061	–	–
	BE29	Sihui, Guangdong, China	H.Y. Li	*C. reticulata*	Twig dieback	MW862157	MW884062	–	–
	BE37	Guilin, Guangxi, China	H.Y. Li & X.E. Xiao	*C. sinensis*	Twig dieback	MW862159	MW884064	–	–
	BE39	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (*C. paradisi* × *C. reticulata*)	Twig gummosis	MW862161	MW884066	–	–
	BE46	Linhai, Zhejiang, China	W.L. Li	*C. unshiu*	Trunk canker	MW862162	MW884067	–	–
	BE47	Linhai, Zhejiang, China	W.L. Li	*C. unshiu*	Trunk canker	MW862163	MW884068	–	–
	BE48	Linhai, Zhejiang, China	W.L. Li	*C. unshiu*	Trunk canker	MW862164	MW884069	–	–
	BE53	Linhai, Zhejiang, China	W.L. Li	*C. unshiu*	Trunk canker	MW862165	MW884070	–	–
	BE54	Linhai, Zhejiang, China	W.L. Li	*C. unshiu*	Trunk canker	MW862166	MW884071	–	–
	BE55	Linhai, Zhejiang, China	W.L. Li	*C. unshiu*	Trunk canker	MW862167	MW884072	–	–
	BE56	Linhai, Zhejiang, China	W.L. Li	*C. unshiu*	Trunk canker	MW862168	MW884073	–	–
	BE57	Linhai, Zhejiang, China	W.L. Li	*C. unshiu*	Trunk canker	MW862169	MW884074	–	–
	BE58	Linhai, Zhejiang, China	W.L. Li	*C. unshiu*	Trunk canker	MW862170	MW884075	–	–
	BE70	Wanzhou, Chongqing, China	H.Y. Li & X.E. Xiao	*C. limon*	Twig dieback	MW862171	MW884076	–	–
	BE82	Fuzhou, Jiangxi, China	H.Y. Li & X.E. Xiao	*C. reticulata*	Twig dieback	MW862172	MW884077	–	–
	BE84	Chun'an, Zhejiang, China	H.Y. Li & X.E. Xiao	*C. unshiu*	Trunk canker	MW862173	MW884078	–	–
	BE107	Yongchun, Fujian, China	X.E. Xiao	*C. reticulata*	Twig dieback	MW862174	MW884079	–	–
	BE109	Yongchun, Fujian, China	X.E. Xiao	*C. sinensis*	Twig dieback	MW862175	MW884080	–	–
	BE110	Yongchun, Fujian, China	X.E. Xiao	*C. reticulata*	Twig dieback	MW862176	MW884081	–	–
L. theobromae	BE20	Sihui, Guangdong, China	H.Y. Li	*C. reticulata*	Twig dieback	MW862177	MW884082	MW884104	MW884133
	BE21	Sihui, Guangdong, China	H.Y. Li	*C. reticulata*	Twig dieback	MW862178	MW884083	MW884105	MW884134
	BE25	Guilin, Guangxi, China	H.Y. Li & X.E. Xiao	*C. reticulata*	Twig dieback	MW862179	MW884084	MW884106	MW884135
	BE108	Yongchun, Fujian, China	X.E. Xiao	*C. sinensis*	Twig dieback	MW862180	MW884085	–	–
Neodeightonia subglobosa	BE14	Xiangshan, Zhejiang, China	H.Y. Li & B. Liu	*C. unshiu*	Trunk gummosis	MT772268	MT775846	MT775856	MW884136
Neofusicoccum parvum	BE15	Xiangshan, Zhejiang, China	X.E. Xiao	*C. unshiu*	Branch gummosis	MT772269	MT775847	MT775857	MW884137
Sphaeropsis linhaiensis	**BE18 * = CGMCC3.20382**	**Linhai, Zhejiang, China**	**H.Y. Li**	***C. unshiu***	**Twig dieback**	**MW880689**	**MW884192**	**MW884218**	**MW884163**

[a] Species names in **bold** represent new species described in this study.
[b] ITS, internal transcribed spacer region and intervening 5.8S nrRNA gene; *tef1*, translation elongation factor 1-alpha; *tub2*, β-tubulin; *rpb2*, DNA-directed RNA polymerase II second largest subunit.
* Isolates represent ex-type.

a sterile scalpel blade and genomic DNA was extracted by the CTAB (Cetyl trimethylammonium bromide) method (Van Burik et al. 1998). Partial regions of four loci were amplified. The internal transcribed spacer region (ITS) was amplified with primers ITS1 and ITS4 (White et al. 1990). Part of the translation elongation factor 1-alpha gene (*tef1*) was amplified with primers EF1-688F (Alves et al. 2008) or EF1-728F and EF1-986R (Carbone & Kohn 1999). Part of the β-tubulin gene (*tub2*) was amplified with Bt2a and Bt2b (Glass & Donaldson 1995). Part of the DNA directed RNA polymerase II second largest subunit (*rpb2*) was amplified with RPB2-6F and fRPB2-7cR (Liu et al. 1999) or *rpb2*-lasF and *rpb2*-lasR (Cruywagen et al. 2017). All amplification reactions were performed in a total volume of 25 µL mixture consisted of 12.5 µL of 2 × Taq Master Mix (Dye Plus) (Vazyme), 9.5 µL ddH_2O, 1 µL of each forward and reverse primer, and 1 µL DNA template. The amplification conditions consisted of an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 45 s, and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 5 min. The PCR products were separated by agarose gel electrophoresis and sent to Qingke Biotechnology (Hangzhou, China) for Sanger DNA sequencing. The nucleotide sequences were assembled and edited with MEGA v. 7.0.26 (Kumar et al. 2016). Sequences obtained in this study were deposited in GenBank nucleotide database (http://www.ncbi.nlm.nih.gov; Table 1).

Phylogenetic analyses

Sequences of the ITS and *tef1* locus for all the isolates obtained in this study were generated and blasted against the NCBIs GenBank nucleotide datasets (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to obtain an initial identification. Representative isolates were selected for sequencing of *tub2* and *rpb2* loci and further phylogenetic analyses. Sequences of ex-type strains closely related to the *Botryosphaeriaceae* isolates studied here were downloaded from NCBI and used for phylogenetic analyses (Table 2). Sequence alignments of each of the ITS, *tef1*, *tub2* and *rpb2* loci were initially aligned by using MAFFT v. 7 online service (https://mafft.cbrc.jp/alignment/server/index.html) (Katoh et al. 2019), with iterative refinement methods (FFT-NS-i), and then edited manually with MEGA v. 7.0.26 software. Aligned datasets and phylogenetic trees for the individual genes and combined alignments were deposited in TreeBASE (http://treebase.org; study number S28083).

The maximum parsimony (MP) analyses were conducted using PAUP v. 4.0b10 (Swofford 2003), with gaps treated as a fifth character. The characters were unordered and of equal weight with 1 000 random addition replicates. The equally most parsimonious trees were generated using the heuristic search option with the tree bisection-reconnection (TBR) branch swapping. MAXTREES were set to 5 000 and zero-length branches were collapsed. To assess clade stability, a bootstrap analysis was conducted with 1 000 replicates. Tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency index (RC) were recorded to evaluate the trees (Hillis & Bull 1993).

The maximum-likelihood (ML) analyses for each dataset were conducted using PhyML v. 3.0 (Guindon et al. 2010). The software package jModeltest v. 2.1.5 (Darriba et al. 2012) was used to determine the best nucleotide substitution model for each dataset. In PhyML, the retention of the maximum number of 1 000 trees was set and nodal support was determined by non-parametric bootstrapping with 1 000 replicates. For both the MP and ML analyses, the phylogenetic trees were viewed in MEGA v. 7.0.26 and FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree).

Morphology

Representative isolates of *Botryosphaeriaceae* that were identified as new species based on DNA sequence analysis were selected for morphological study. Sporulation was induced on pine needle agar (PNA) (Smith et al. 1996) by incubating cultures at 25 °C in 12/12 h fluorescent light/dark cycle for 4–6 wk. Sporocarps were embedded in a Leica Biosystem Tissue Freezing Medium (Leica Biosystems Nussloch GmbH, Nussloch, Germany) and sectioned (8 µm thick) using a freezing microtome (CryoStar NX50 HOP, Thermo Fisher Scientific, Walldorf, Germany) at -20 °C (Chen et al. 2018). Conidia and other microstructures were examined with a compound microscope (Eclipse 80i, Nikon, Japan) and images were recorded with a Nikon digital camera (NIS-Elements F3.0, Nikon, Japan). Measurements were made with Fiji-ImageJ software (Schindelin et al. 2012). One hundred conidia were measured per isolate, and 30 measurements were taken of other morphological structures. Results are presented as (minimum–) (mean – standard deviation) – (mean + standard deviation) (–maximum). The average length/average width ratio (L/W) of the conidial measurements were also calculated.

Colony characters on PDA were noted and colony colours were determined according to the colour charts of Rayner (1970). To determine growth rates in culture, agar plugs (5 mm diam) were taken from the edge of actively growing cultures of each representative isolate and transferred onto the centre of 90 mm diam Petri dishes containing PDA. Cultures were incubated at five temperature intervals from 5–40 °C in the dark. Five replicate plates of each representative isolate were incubated at each temperature. Perpendicular colony diameters were measured daily until the fastest growing cultures reached the edge of the Petri dish.

Pathogenicity tests

At least two representative isolates from each identified group, except for those with only one isolate, were selected for pathogenicity testing in this study. Inoculation tests were conducted both *in vitro* and *in vivo*. For *in vitro* inoculation, isolates were used to inoculate detached healthy green shoots (40 cm long, 0.6–1 cm diam) collected from *Citrus reticulata* trees and 10 shoots were inoculated with each isolate. One wound per shoot was made using a cork borer (5 mm diam) and a mycelial plug taken from the margins of colonies grown on PDA for 5 d in the dark was placed on the freshly wounded surface of each shoot, and the inoculated area was covered with Parafilm. The control treatment was inoculated with sterile PDA plugs. The inoculated shoots and controls were covered with liquid paraffin at their ends to prevent desiccation and incubated at 25 °C in moist chambers. Eight days after inoculation, the disease incidences were calculated and the internal lesions or wound lengths were measured. Data were analysed by one-way analysis of variance (ANOVA) using SPSS Statistics 20 software (SPSS 2011). To prove Koch's postulates, fungi were re-isolated by cutting small pieces of necrotic tissue from the edges of each lesion and plating them in PDA plates at 25 °C. The species were confirmed based on morphology.

For *in vivo* inoculation, the pathogenicity test was conducted on 6-yr-old healthy plants of Cocktail grapefruit (*C. paradisi* × *C. reticulata*). The plants were grown in vinyl house of the Xielong Family Farm in Kecheng District, Quzhou City, Zhejiang Province from 24 June to 9 July 2021. During this time, the environmental temperature ranged from 20–38 °C. Each representative isolate, as well as the control, was inoculated onto 10 shoots. After 15 d, the symptoms and disease incidences were assessed. Re-isolation was also conducted in the same way to fulfil Koch's postulates.

Table 2 Isolates from other studies used in the phylogenetic analyses.

Species	Isolate numbers[a]	Host	Location	Collector	GenBank accession numbers[b]			
					ITS	*tef1*	*tub2*	*rpb2*
Botryosphaeria agaves	CBS 133992 = MFLUCC11-0125 *	*Agave* sp.	Thailand	R. Phookamsak	JX646791	JX646856	JX646841	–
	MFLUCC 10-0051	*Agave* sp.	Thailand	P. Chomnunti	JX646790	JX646855	JX646840	–
Botryosphaeria corticis	CBS 119047 *	*Vaccinium corymbosum*	USA	P.V. Oudemans	DQ299245	EU017539	–	–
	ATCC 22927	*Vaccinium* sp.	USA	R.D. Millholland	DQ299247	EU673291	EU673108	–
Botryosphaeria dothidea	CBS 115476 = CMW 8000 *	*Prunus* sp.	Switzerland	B. Slippers	AY236949	AY236898	AY236927	EU339577
	CBS 110302	*Vitis vineifera*	Portugal	A.J.L. Phillips	AY259092	AY573218	EU673106	–
	CBS 145971 = CPC 29048	*Grevillea* sp.	Australia	P.W. Crous	MT587332	MT592034	MT592470	–
Botryosphaeria fabicerciana	CBS 127194 = CMW 27094 *	*Eucalyptus* sp.	China	M.J. Wingfield	HQ332197	HQ332213	KF779068	MF410137
	CERC 2948	*Eucalyptus* sp.	China	M.J. Wingfield	KX277983	KX278088	KX278193	MF410132
Botryosphaeria kuwatsukai	CBS 135219 = PG2 *	*Malus domestica*	China	C.S. Wang	KJ433388	KJ433410	–	–
	LSP5	*Pyrus* sp.	China	C.S. Wang	KJ433395	KJ433417	–	–
Botryosphaeria qingyuanensis	CERC 2946 = CGMCC 3.18742 *	*Eucalyptus* hybrid	China	S.F. Chen & G.Q. Li	KX278000	KX278105	KX278209	MF410151
	CERC 2947 = CGMCC 3.18744	*Eucalyptus* hybrid	China	S.F. Chen & G.Q. Li	KX278001	KX278106	KX278210	MF410152
Botryosphaeria ramosa	CBS 122069 = CMW 26167 *	*Eucalyptus camaldulensis*	Australia	T.I. Burgess	EU144055	EU144070	KF766132	–
	CGMCC 3.18006	*Myrtaceae*	China	–	KX197072	KX197092	KX197099	–
Botryosphaeria scharifii	CBS 124703 = IRAN 1529C *	*Mangifera indica*	Iran	J. Abdollahzadeh	JQ772020	JQ772057	–	–
	CBS 124702 = IRAN 1543C	*Mangifera indica*	Iran	J. Abdollahzadeh & A. Javadi	JQ772019	JQ772056	–	–
Diplodia africana	CBS 120835 = CPC 5908 *	*Prunus persica*	South Africa	U. Damm	KF766155	KF766397	KF766129	–
	STE-U 5946	*Prunus persica*	South Africa	U. Damm	EF445344	EF445383	–	–
Diplodia afrocarpi	CBS 131681 = CMW 35506	*Afrocarpus falcatus*, healthy twigs	South Africa	E.M. Cruywagen	MT587333	MT592035	MT592471	–
Diplodia agrifolia	CBS 124.30	*Ulmus* sp.	USA	–	KX464087	KX464557	KX464783	KX463953
Diplodia allocellula	CBS 130408 = CMW 36468 *	*Acacia karroo*	South Africa	F. Jami & M. Gryzenhout	JQ239397	JQ239384	JQ239378	–
	CMW 36470	*Acacia karroo*	South Africa	F. Jami & M. Gryzenhout	JQ239399	JQ239386	JQ239380	–
Diplodia arengae	MFLU 17-2769 = XTBG28 *	*Arenga hookeriana*	China	D.N. Wanasinghe	MG762771	MG762774	MG783039	–
Diplodia bulgarica	CBS 124254 *	*Malus sylvestris*	Bulgaria	S.G. Bobev	GQ923853	GQ923821	–	–
	CBS 124135	*Malus sylvestris*	Bulgaria	S.G. Bobev	GQ923852	GQ923820	–	–
Diplodia citricarpa	CBS 124715 = CJA 131 = IRAN 1578C *	*Citrus* sp., twigs	Iran	J. Abdollahzadeh & A. Javadi	KF890207	KF890189	KX464784	–
Diplodia corticola	CBS 112549 = CAP 134 *	*Quercus suber*	Portugal	A. Alves	AY259100	AY573227	DQ458853	–
	CBS 112546	Quercus ilex	Spain	–	AY259090	EU673310	EU673117	KX463954
Diplodia crataegicola	MFLU 15-1311 *	*Crataegus* sp.	Italy	–	KT290244	KT290248	KT290246	–
Diplodia cupressi	CBS 168.87 *	*Cupressus sempervirens*	Israel	Z. Solel	DQ458893	DQ458878	DQ458861	–
	CBS 261.85	*Cupressus sempervirens*	Israel	Z. Solel	DQ458894	DQ458879	DQ458862	–
Diplodia eriobotryicola	CBS 140851 = BN-21 *	*Eriobotrya japonica*	Spain	E. Gonzalez-Domınguez	KT240355	KT240193	MG015806	–
Diplodia estuarina	CMW 41363	*Rhizophora mucronata*	South Afric	J.A. Osorio & Jol. Roux.	KP860829	KP860674	KP860752	–
	CMW 41230	*Rhizophora mucronata*	South Afric	J.A. Osorio & Jol. Roux.	KP860830	KP860675	KP860753	–
Diplodia fraxini	CBS 136010 *	*Fraxinus angustifolia*	Portugal	A. Deidda	KF307700	KF318747	MG015807	–
	CBS 136011	*Fraxinus angustifolia*	Italy	B.T. Linaldeddu	KF307711	KF318748	MG015808	–
Diplodia galiicola	MFLU15-1310 *	*Galium* sp.	Italy	E. Camporesi	KT290245	KT290249	KT290247	–
Diplodia gallae	CBS 211.25	*Quercus* sp., fruit	–	–	KX464090	KX464564	KX464795	–
	CBS 212.25	*Quercus* sp., gall	–	–	KX464091	KX464565	KX464796	–
Diplodia malorum	CBS 124130 *	*Malus sylvestris*	Portugal	A.J.L. Phillips	GQ923865	GQ923833	–	–
	BN-37	*Eriobotrya japonica*	Spain	–	KT240360	KT240198	–	–
Diplodia mutila	CBS 112553 = CAP 062 *	*Vitis vinifera*	Portugal	A.J.L. Phillips	AY259093	AY573219	KY554743	–
Diplodia neojuniperi	CPC 22753 = B0031 *	*Juniperus chinensis*	Thailand	T. Trakunyingcharoen	KM006431	KM006462	–	–
	CPC 22754 = B0032	*Juniperus chinensis*	Thailand	T. Trakunyingcharoen	KM006432	KM006463	–	–

Table 2 (cont.)

Species	Isolate numbers[a]	Host	Location	Collector	GenBank accession numbers[b]			
					ITS	*tef1*	*tub2*	*rpb2*
Diplodia olivarum	CBS 121887 = CAP 254 *	*Olea europaea*	Italy	C. Lazzizera	EU392302	EU392279	HQ660079	–
	IMI 390972	*Carob* tree	Italy	–	HM028640	HQ660078	HQ660080	–
Diplodia pseudoseriata	CBS 124906 *	*Blepharocalyx salicifolius*	Uruguay	C. Pérez	EU080927	EU863181	MG015820	–
Diplodia quercivora	CBS 133852 *	*Quercus canariensis*	Tunisia	B. T. Linaldeddu	JX894205	JX894229	MG015821	–
	MEAN 1017	*Quercus suber*	Portugal	H. Braganca	KU311198	KU311201	–	–
Diplodia rosulata	CBS 116470 *	*Prunus africana*	Ethiopia	A. Gure	EU430265	EU430267	EU673132	–
	CBS 116472	*Prunus africana*	Ethiopia	A. Gure	EU430266	EU430268	EU673131	–
Diplodia sapinea	CBS 393.84 *	*Pinus nigra*	Netherlands	H.A. van der Aa	DQ458895	DQ458880	DQ458863	–
	CBS109726 = CMW 04880	*Pinus patula*	South Africa	M.J. Wingfield	KX464094	KX464568	KX464800	KX463956
Diplodia scrobiculata	CBS 118110 *	*Pinus resinosa*	USA	M.A. Palmer	AY253292	AY624253	AY624258	KX463959
Diplodia seriata	CBS 112555 = CAP 063 *	*Vitis vinifera*	Italy	A.J.L. Phillips	AY259094	AY573220	DQ458856	–
	CBS 117.82	*Rubus* sp., dead stem	Italy	H.A. van der Aa	KX464108	KX464598	KX464834	KX463964
Diplodia sp. 1	CBS 678.88	*Quercus suber*	Spain	J. Luque	AY259104	GU799459	GU799458	–
	UCD1275So	Grape vine	USA	–	GU799471	GU799468	GU799465	–
Diplodia subglobosa	CBS 124133 = JL 453 *	*Lonicera nigra*	Spain	J. Luque	GQ923856	GQ923824	MT592576	–
	CBS 124132 = JL 375	*Fraxinus excelsior*	Spain	J. Luque	DQ458887	DQ458871	DQ458852	–
Diplodia tsugae	CBS 418.64 = IMI 197143 *	*Tsuga heterophylla*	Canada	A. Funk	DQ458888	DQ458873	DQ458855	–
Dothiorella acacicola	CBS 141295 = CPC 26349 *	*Acacia mearnsii*	France	P.W. Crous & M.J. Wingfield	KX228269	KX228376	–	–
Dothiorella acericola	KUMCC 18-0137 *	*Acer palmatum*, dead hanging twigs	China	R. Phookamsak	MK359449	MK361182	–	–
	HNXX032	*Ziziphus jujuba*, branch	China	R. Zang	KY385661	KY393212	KY393178	
Dothiorella alpina	CGMCC 3.18001 *	*Platycladus orientalis*	China	W. He & J.R. Wu; det. Y. Zhang	KX499645	KX499651	–	–
Dothiorella brevicollis	CBS 130411 = CMW 36463 *	*Acacia karroo*	South Africa	F. Jami & M. Gryzenhout	JQ239403	JQ239390	JQ239371	–
	CMW 36464	*Acacia karroo*	South Africa	F. Jami & M. Gryzenhout	JQ239404	JQ239391	JQ239372	–
Dothiorella capri-amissi	CBS 121763 = CMW 25403 *	*Acacia erioloba*	South Africa	F.J.J. van der Walt & G.J. Marais	EU101323	EU101368	KX464850	–
	CMW 25404	*Acacia erioloba*	South Africa	F.J.J. van der Walt & G.J. Marais	EU101324	EU101369	–	–
Dothiorella casuarini	CBS 120688 = CMW 4855 *	*Casuarina* sp.	Australia	M.J. Wingfield	DQ846773	DQ875331	–	KX463970
	CBS 120690 = CMW 4857	*Casuarina* sp.	Australia	M.J. Wingfield	DQ846774	DQ875333	–	–
Dothiorella citricola	CBS 124729 = ICMP 16828 *	*Citrus sinensis*	New Zealand	S.R. Pennycook, P.R. Johnston & B.C. Paulus	EU673323	EU673290	KX464853	KX463971
	CBS 124728 = ICMP 16827	*Citrus sinensis*	New Zealand	S.R. Pennycook, P.R. Johnston & B.C. Paulus	EU673322	EU673289	KX464852	–
Dothiorella diospyricola	CBS 145972 = CPC 34653 *	*Diospyros mespiliformis*	South Africa	P.W. Crous	MT587398	MT592110	MT592581	
Dothiorella dulcispinae	CBS 130413 = CMW 36460 *	*Acacia karroo*	South Africa	F. Jami & M. Gryzenhout	JQ239400	JQ239387	JQ239373	–
	CMW 36462	*Acacia karroo*	South Africa	F. Jami & M. Gryzenhout	JQ239402	JQ239389	JQ239375	–
Dothiorella eriobotryae	CBS 140852 = CPC 29679 = BN 81 *	*Eriobotrya japonica*, branch canker	Spain	E. Gonzalez-Domınguez	KT240287	KT240262	MT592582	–
Dothiorella heterophyllae	CMW46458	*Acacia heterophylla*	La Réunion	M.J. Wingfield	MN103794	MH548348	MH548324	–
Dothiorella iranica	CBS 124722 = IRAN 1587C *	*Olea europea*	Iran	A. Javadi	KC898231	KC898214	KX464856	–
	MFLUCC 15-0656	*Paliurus*	Italy	E. Camporesi	KX765302	KX765303	–	–
Dothiorella koae	CMW48017	*Acacia heterophylla*	La Réunion	M.J. Wingfield	MH447652	MH548338	MH548327	–
Dothiorella lampangensis	MFLUCC 18-0232 *	*Rutaceae*, fallen fruit pericarp	Thailand	S.C. Jayasiri	MK347758	MK340869	MK412874	
Dothiorella longicollis	CBS 122068 = CMW 26166 *	*Lysiphyllum cunninghamii*	Australia	T.I. Burgess & M.J. Wingfield	EU144054	EU144069	KF766130	KX463972
	CBS 122066 = CMW 26166	*Terminalia* sp.	Australia	T.I. Burgess & M.J. Wingfield	EU144052	EU144067	KX464857	–
Dothiorella magnoliae	CFCC 51563 *	*Magnolia grandiflora*	China	C.J. You	KY111247	KY213686	–	
	CFCC 51564	*Magnolia grandiflora*	China	C.J. You	KY111248	KY213687	–	

Table 2 (cont.)

Species	Isolate numbers[a]	Host	Location	Collector	GenBank accession numbers[b]			
					ITS	*tef1*	*tub2*	*rpb2*
Dothiorella mangifericola	CBS 124727 = IRAN 1584C *	*Mangifera indica*	Iran	J. Abdollahzadeh & A. Javadi	KC898221	KC898204	–	KX463973
	IRAN 1545C	*Mangifera indica*	Iran	J. Abdollahzadeh & A. Javadi	KC898223	KC898206	–	–
Dothiorella moneti	MUCC 505 = WAC 13154 *	*Acacia rostellifera*	Australia	K.M. Taylor	EF591920	EF591971	EF591954	–
	MUCC 507	*Acacia rostellifera*	Australia	K.M. Taylor	EF591922	EF591973	EF591956	–
Dothiorella plurivora	CBS 124724 = IRAN 1557C *	*Citrus* sp.	Iran	J. Abdollahzadeh & A. Javadi	KC898225	KC898208	KX464874	–
	CBS 124725	*Prunus armeniaca*	Iran	J. Abdollahzadeh & A. Javadi	KC898225	KC898213	KX464875	–
Dothiorella pretoriensis	CBS 130404 = CMW 36480 *	*Acacia karroo*	South Africa	F. Jami & M. Gryzenhout	JQ239405	JQ239392	JQ239376	–
	CMW 36481	*Acacia karroo*	South Africa	F. Jami & M. Gryzenhout	JQ239406	JQ239393	JQ239377	–
Dothiorella prunicola	CBS 124723 = CAP 187 *	*Prunus dulcis*	Portugal	E. Diogo	EU673313	EU673280	–	–
Dothiorella reunionis	CMW46457 *	*Acacia heterophylla*	La Réunion	M.J. Wingfield	MH447649	MH548347	–	–
Dothiorella santali	MUCC 509 = WAC 13155 *	*Santalum acuminatum*	Australia	K.M. Taylor	EF591924	EF591975	EF591958	–
	MUCC 508	*Santalum acuminatum*	Australia	K.M. Taylor	EF591923	EF591974	EF591957	–
Dothiorella sarmentorum	IMI 63581b *	*Ulmus* sp.	England	E.A. Ellis	AY573212	AY573235	–	–
	CBS 115038	*Malus pumila*	Netherlands	A.J.L. Phillips	AY573206	AY573223	EU673101	–
Dothiorella sp. 1	CBS 121783 = CMW 25432 = CAMS 1187	*Acacia mearnsii*	South Africa	F.J.J. van der Walt & R.N. Heath	EU101333	EU101378	KX464859	–
	CBS 121784 = CMW 25430 = CAMS 1185	*Acacia mearnsii*	South Africa	F.J.J. van der Walt & R.N. Heath	EU101331	EU101376	KX464860	–
Dothiorella striata	CBS 124731= ICMP 16824 *	*Citrus sinensis*	New Zealand	S.R. Pennycook, P.R. Johnston & B.C. Paulus	EU673321	EU673288	EU673143	KX463976
	CBS 124730 = ICMP 16819	*Citrus sinensis*	New Zealand	S.R. Pennycook, P.R. Johnston & B.C. Paulus	EU673320	EU673287	EU673142	–
Dothiorella tectonae	MFLUCC 12-0382 = MD-2014 *	*Tectona grandis*	Thailand	M. Doilom	KM396899	KM409637	KM510357	–
Dothiorella thailandica	MFLUCC 11-0438 *	*Bamboo culm*	Thailand	D.Q. Dai	JX646796	JX646861	JX646844	–
Dothiorella thripsita	CBS 125445 = BRIP 51876 *	*Acacia harpophylla*	Australia	D.J. Tree & C.E.C. Tree	FJ824738	KJ573639	KJ577550	KX463977
Dothiorella ulmacea	CBS 138855 = CPC 24416 *	*Ulmus laevis*	Germany	R.K. Schumacher	KR611881	KR611910	KR611909	–
	CPC 24945	*Ulmus laevis*	Germany	R.K. Schumacher	KR611882	KR857697	–	–
Dothiorella uruguayensis	CBS 124908 = CMW 26763 =UY672 *	*Hexachlamis edulis*	Uruguay	C.A. Pérez	EU080923	EU863180	KX464886	–
Dothiorella vinea-gemmae	DAR 81012 = B116-3 *	*Vitis vinifera*	Australia	N. Wunderlich	KJ573644	KJ573641	–	–
Dothiorella viticola	CBS 117009 *	*Vitis vinifera* cv. Garnatxa Negra	Spain	J. Luque & S. Martos	AY905554	AY905559	EU673104	DQ677985
	GAR09	*Vitis* sp.	French	–	KT595694	KX098285	KT595695	–
Dothiorella yunnana	CGMCC 3.17999 *	*Camellia* sp.	China	W. He & J.R. Wu; det. Y. Zhang	KX499643	KX499649	–	–
	CGMCC 3.18000	*Camellia* sp.	China	W. He & J.R. Wu; det. Y. Zhang	KX499644	KX499650	–	–
Lasiodiplodia acaciae	CBS 136434 = CPC 20820 *	*Acacia* sp., leaf spot	Indonesia	M.J. Wingfield	MT587421	MT592133	MT592613	MT592307
Lasiodiplodia americana	CERC 1961 = CFCC 50065 *	*Pistachia vera*	USA	T.J. Michailides	KP217059	KP217067	KP217075	MF410161
	CERC 1960 = CFCC 50064	*Pistachia vera*	USA	T.J. Michailides	KP217058	KP217066	KP217074	MF410162
Lasiodiplodia aquilariae	CGMCC 3.18471 *	*Aquilaria crassna*	Laos	X. Sun	KY783442	KY848600	–	KY848562
Lasiodiplodia avicenniae	CMW 41467 *	*Avocennia marina*	South Africa	J.A. Osorio & J. Roux	KP860835	KP860680	KP860758	KU587878
	LAS 199	*Avocennia marina*	South Africa	J.A. Osorio & J. Roux	KU587957	KU587947	KU587868	KU587880
Lasiodiplodia brasiliense	CMM 4015 *	*Mangifera indica*	Brazil	M.W. Marques	JX464063	JX464049	–	–
	IBL 344	*Adansonia madagascariensis*	Madagascar	–	KT151808	KT151802	KT151805	–
Lasiodiplodia bruguierae	CMW 41470*	*Bruguiera gymnorrhiza*	South Africa	J.A. Osorio & J. Roux	KP860833	KP860678	KP860756	KU587875
	CMW 41614	*Bruguiera gymnorrhiza*	South Africa	J.A. Osorio & J. Roux	KP860834	KP860679	KP860757	KU587877
Lasiodiplodia cinnamomi	CFCC 51997 *	*Cinnamomum camphora*	China	N. Jiang	MG866028	MH236799	MH236797	MH236801
	CFCC 51998	*Cinnamomum camphora*	China	N. Jiang	MG866029	MH236800	MH236798	MH236802

Table 2 (cont.)

Species	Isolate numbers[a]	Host	Location	Collector	GenBank accession numbers[b]			
					ITS	*tef1*	*tub2*	*rpb2*
Lasiodiplodia citricola	CBS 124707 = IRAN 1522C *	*Citrus* sp.	Iran	J. Abdollahzadeh & A. Javadi	GU945354	GU945340	KP872405	KU696351
	CBS124706 = IRAN 1521C	*Citrus* sp.	Iran	A. Shekari	GU945353	GU945339	KP872406	KU696350
Lasiodiplodia crassispora	CBS 118741 = WAC12533 *	*Santalum album*	Australia	T.I. Burgess & B. Dell	DQ103550	EU673303	KU887506	KU696353
	CMM 4585	–	–	–	MG954354	MG979520	MG979552	MG979561
Lasiodiplodia euphorbicola	CMM 3609 *	*Jatropha curcas*	Brazil	A.R. Machado & O.L. Pereira	KF234543	KF226689	KF254926	–
	CMW 33350	*Adansonia digitata*	Botswana	–	KU887149	KU887026	KU887455	KU696346
Lasiodiplodia gilanensis	CBS 124704 = IRAN1523C= UCCE 940B *	*Citrus* sp., fallen twigs	Iran	J. Abdollahzadeh & A. Javadi	KX906851	KX906853	KX906849	KU696357
	CBS 124705 = IRAN 1501C	*Citrus* sp., fallen twigs	Iran	J. Abdollahzadeh & A. Javadi	GU945352	GU945341	KP872412	KU696356
Lasiodiplodia gonubiensis	CBS 115812 = CMW 14077 *	*Syzygium cordatum*	South Africa	D. Pavlic	AY639595	DQ103566	DQ458860	KU696359
	CMW 46621 = MTU 56	*Syzygium cordatum*	South Africa	D. Pavlic	KY052944	KY024623	KY000126	–
Lasiodiplodia gravistriata	CMM 4564 *	*Anacardium humile*	Brazil	M.S.B. Netto	KT250949	KT250950	–	–
	CMM 4565	*Anacardium humile*	Brazil	M.S.B. Netto	KT250947	KT266812	–	–
Lasiodiplodia hormozganensis	CBS 124709 = IRAN 1500C *	*Olea* sp.	Iran	J. Abdollahzadeh & A. Javadi	GU945355	GU945343	KP872413	KU696361
	CBS 124708 = IRAN 1498C	*Mangifera indica*	Iran	J. Abdollahzadeh & A. Javadi	GU945356	GU945344	KP872414	KU696360
Lasiodiplodia indica	IBP 1 *	*Angiospermous* tree	India	I.B. Prasher & G. Singh	KM376151	–	–	–
Lasiodiplodia iranensis	CBS 124710 = IRAN 1520C *	*Salvadora persica*	Iran	J. Abdollahzadeh & A. Javadi	GU945348	GU945336	KU887516	KU696363
	CBS 124711 = IRAN 1502C = CMM 4603	*Juglans* sp.	Iran	A. Javadi	GU945347	GU945335	MG979537	–
Lasiodiplodia laeliocattleyae	CBS 167.28 *	*Laeliocattleya*	Italy	C. Sibilia	KU507487	KU507454	–	–
	CMM 4724	*Vitis vinifera*	Brazil	–	MG954343	MG979508	MG979541	–
Lasiodiplodia lignicola	CBS 134112= MFLUCC 11-0435 *	Dead wood	Thailand	A.D. Ariyawansa	JX646797	KU887003	KT852958	KU696364
Lasiodiplodia macrospora	CMM 3833 *	*Jatropha curcas*	Brazil	A.R. Machado & O.L. Pereira	KF234557	KF226718	KF254941	–
Lasiodiplodia mahajangana	CBS 124925 = CMW 27801 *	*Terminalia catappa*	Madagascar	J. Roux	FJ900595	FJ900641	FJ900630	KU696365
	CMW 27818	*Terminalia catappa*	Madagascar	J. Roux	FJ900596	FJ900642	FJ900631	–
Lasiodiplodia margaritacea	CBS 122519 = CMW 26162 *	*Adansonia gibbosa*	Australia	T.I. Burgess & M.J. Wingfield	EU144050	EU144065	KX464903	KU696367
	CBS 122065	*Adansonia gibbosa*	Australia	T.I. Burgess & M.J. Wingfield	EU144051	EU144066	–	–
Lasiodiplodia mediterranea	CBS 137783 *	Holm oak	Italy	B.T. Linaldeddu	KJ638312	KJ638331	KU887521	KU696368
	CBS137784	Grapevine	Italy	S. Serra	KJ638311	KJ638330	KU887522	KU696369
Lasiodiplodia microconidia	CGMCC 3.18485 *	*Aquilaria crassna*	Laos	X. Sun	KY783441	KY848614	–	KY848561
Lasiodiplodia parva	CBS 456.78 *	Cassava-field soil	Colombia	O. Rangel	EF622083	EF622063	KU887523	KU696372
	CBS 494.78	Cassava-field soil	Colombia	O. Rangel	EF622084	EF622064	EU673114	KU696373
Lasiodiplodia plurivora	CBS 120832 = STE-U5803 *	*Prunus salicina*	South Africa	F. Halleen	EF445362	EF445395	KP872421	KU696374
	CBS 121103 = STE-U4583	*Vitis vinifera*	South Africa	F. Halleen	AY343482	EF445396	KP872422	KU696375
Lasiodiplodia pontae	CMW 1277 = IBL12 *	*Spondias purpurea*	Brazil	J.S. Lima & F.C.O. Freire	KT151794	KT151791	KT151797	–
Lasiodiplodia pseudotheobromae	CBS 116459 *	*Gmelina arborea*	Costa Rica	J. Carranza & Velásquez	EF622077	EF622057	EU673111	KU696376
	CMM 3887	*Jatropha curcas*	Brazil	A.R. Machado	KF234559	KF226722	KF254943	–
Lasiodiplodia rubropurpurea	CBS 118740 = CMW 14700 = WAC 12535 *	*Eucalyptus grandis*	Australia	T.I. Burgess & G. Pegg	DQ103553	DQ103571	KU887529	KU696380
	WAC 12536 = CMW 15207	*Eucalyptus grandis*	Australia	T.I. Burgess & G. Pegg	DQ103554	DQ103572	KP872425	KU696381
Lasiodiplodia subglobosa	CMM 3872 *	*Jatropha curcas*	Brazil	A.R. Machado & O.L. Pereira	KF234558	KF226721	KF254942	–
	CMM 4046	*Jatropha curcas*	Brazil	A.R. Machado & O.L. Pereira	KF234560	KF226723	KF254944	–
Lasiodiplodia syzygii	MFLUCC 19-0219.1 = GUCC 9719.1 *	*Syzygium samarangense*	Thailand	Q. Zhang	MT990531	MW016943	MW014331	–
	GUCC 9719.3	*Syzygium samarangense*	Thailand	Q. Zhang	MW081992	MW087102	MW087105	–
Lasiodiplodia thailandica	CPC 22795 *	*Mangifera indica*	Thailand	T. Trakunyingcharoen	KJ193637	KJ193681	–	–
	BJFU DZP160123-13	*Albizia chinensis*	China	Z.P. Dou & Z.C. Liu	KY676789	KY676798	KY751301	KY751298

Table 2 (cont.)

Species	Isolate numbers[a]	Host	Location	Collector	GenBank accession numbers[b]			
					ITS	*tef1*	*tub2*	*rpb2*
Lasiodiplodia theobromae	CBS 164.96 *	fruit along coral reef coast	Papua New Guinea	A. Aptroot	AY640255	AY640258	KU887532	KU696383
	CBS 111530	*Leucospermum* sp.	USA	J.E. Taylor	EF622074	EF622054	KU887531	KU69638
	CBS 124.13	–	USA	J.J. Taubenhaus	DQ458890	DQ458875	DQ458858	KY472887
Lasiodiplodia tropica	CGMCC 3.18477 *	*Aquilaria crassna*	Laos	X. Sun	KY783454	KY848616	KY848540	KY848574
Lasiodiplodia venezuelensis	CBS 118739 = CMW 13511 = WAC 12539 *	*Acacia mangium*	Venezuela	S. Mohali	DQ103547	EU673305	KU887533	KU696384
	CBS 129757	*Acacia mangium*	Venezuela	S. Mohali	JX545102	JX545122	JX545142	–
Lasiodiplodia viticola	CBS 128313 = UCD 2553AR *	*Vitis vinifera*	USA	R.D. Cartwright & W.D. Gubler	HQ288227	HQ288269	HQ288306	KU696385
	CBS 128315 = UCD 2604MO	*Vitis vinifera*	USA	K. Striegler & W.D. Gubler	HQ288228	HQ288270	HQ288307	KU696386
Lasiodiplodia vitis	CBS 124060 *	*Vitis vinifera*	Italy	S. Burruano	KX464148	KX464642	KX464917	–
Neodeightonia licuriensis	COAD 1780 *	*Syagrus coronata*	Brazil	O.L. Pereira	KP165429	KP165430	KP165431	–
Neodeightonia microspora	MFLUCC 11-0483	bamboo	Thailand	D.Q. Dai	KU940110	–	–	–
	MFLUCC 11-0504	bamboo	Thailand	D.Q. Dai	KU940111	–	–	–
Neodeightonia palmicola	MFLUCC10-0822 *	*Arenga westerhoutii*	Thailand	J.K. Liu	HQ199221	–	–	–
	FAFU 002	*Caryota mitis*	China	–	MK203813	–	MK208460	–
Neodeightonia phoenicum	CBS 122528 *	*Phoenix* sp.	Spain	F. Garcia	EU673340	EU673309	EU673116	KX463999
	CBS 169.34	*Phoenix dactylifera*	USA	H.S. Fawcett	EU673338	EU673307	EU673138	–
Neodeightonia planchoniae	MFLUCC 17-2427	*Planchonia* sp.	Thailand	S.C. Jayasiri	MK347755	–	–	–
Neodeightonia rattanica	MFLUCC 15-0712 *	*Calamus* sp.	Thailand	S. Konta	KX646357	KX646360	–	–
	MFLUCC 15-0313	*Calamus* sp.	Thailand	S. Konta	KX646358	KX646361	–	–
Neodeightonia rattanicola	MFLUCC 15-0319 *	*Calamus* sp.	Thailand	S. Konta	KX646359	KX646362	–	–
Neodeightonia subglobosa	CBS 448.91 *	*Bambusa arundinacea*	Sierra Leone	F.C. Deighton	EU673337	EU673306	EU673137	–
	MFLUCC 11-0607	bamboo	Thailand	D.Q. Dai	KU940113	–	–	–
Neofusicoccum algeriense	CBS 137504 = ALG1 *	*Vitis vinifera*	Algeria	A. Berraf-Tebbal	KJ657702	KJ657715	–	–
	ALG9	*Vitis vinifera*	Algeria	A. Berraf-Tebbal	KJ657704	KJ657721	–	–
Neofusicoccum andinum	CBS 117453 = CMW 13455 *	*Eucalyptus* sp.	Venezuela	S. Mohali	AY693976	AY693977	KX464923	KX464002
	CBS 117452 = CMW 13446	*Eucalyptus* sp.	Venezuela	S. Mohali	DQ306263	DQ306264	KX464922	KX464001
Neofusicoccum arbuti	CBS 116131 *	*Arbutus menziesii*	USA	A. Rossman	AY819720	–	KF531793	KX464003
	CBS 116575	*Arbutus menziesii*	USA	M. Elliott	KX464155	KX464650	KX464927	–
Neofusicoccum australe	CMW 6837 *	*Acacia* sp.	Australia	M.J. Wingfield	AY339262	AY339270	AY339254	EU339573
	C1.2	*Arctostphylos glauca*	USA	L. Drake-Schultheis	MH777002	MH754929	–	–
Neofusicoccum batangarum	CBS 124924 = CMW 28363 *	*Terminalia catappa*	Cameroon	D. Begoude & J. Roux	FJ900607	FJ900653	FJ900634	FJ900615
	OB45	*Opuntia ficus-indica*	Italy	–	MG609042	MG609076	MG609059	–
Neofusicoccum brasiliense	CMM 1338 *	*Mangifera indica*	Brazil	M.W. Marques	JX513630	JX513610	KC794031	–
	CMM 1269	*Mangifera indica*	Brazil	M.W. Marques	JX513629	JX513609	KC794032	–
Neofusicoccum cordaticola	CBS 123634 = CMW 13992 *	*Syzygium cordatum*	South Africa	D. Pavlic	EU821898	EU821868	EU821838	EU821928
	CBS123635 = CMW 14056	*Syzygium cordatum*	South Africa	D. Pavlic	EU821903	EU821873	EU821843	EU821933
Neofusicoccum cryptoaustrale	CBS 122813 = CMW 23785 *	*Eucalyptus* tree	South Africa	H.M. Maleme	FJ752742	FJ752713	FJ752756	KX464014
Neofusicoccum eucalypticola	CBS 115679 = CMW 6539 *	*Eucalyptus* sp.	Australia	M.J. Wingfield	AY615141	AY615133	AY615127	–
	CBS 6539 = CMW 6217	*Eucalyptus* sp.	Australia	M.J. Wingfield	AY615143	AY615135	AY615125	–
Neofusicoccum eucalyptorum	CBS 115791 = CMW 10125 *	*Eucalyptus grandis*	South Africa	H. Smith	AF283686	AY236891	AY236920	–
	CAA 518	*Eucalyptus globulus*	Portugal	–	KX871883	KX871839	KX871776	–
Neofusicoccum grevilleae	CBS 129518 = CPC 16999 *	*Grevillea aurea*	Australia	P.W. Crous & R.G. Shivas	JF951137	–	–	–
Neofusicoccum hellenicum	CERC 1947 = CFCC 50067 *	*Pistacia vera*	Greece	T.J. Michailides	KP217053	KP217061	KP217069	–
	CERC 1948 = CFCC 50068	*Pistacia vera*	Greece	T.J. Michailides	KP217054	KP217062	KP217070	–

Table 2 (cont.)

Species	Isolate numbers[a]	Host	Location	Collector	GenBank accession numbers[b]			
					ITS	*tef1*	*tub2*	*rpb2*
Neofusicoccum kwambonambiense	CBS 123639 = CMW 14023 *	*Syzygium cordatum*	South Africa	D. Pavlic	EU821900	EU821870	EU821840	EU821930
	CBS 123641 = CMW 14140	*Syzygium cordatum*	South Africa	D. Pavlic	EU821919	EU821889	EU821859	EU821949
Neofusicoccum lumnitzerae	CMW 41469 *	*Lumnitzera racemosa*	South Africa	J.A Osorio & Jol. Roux	KP860881	KP860724	KP860801	KU587925
	CMW 41228	*Lumnitzera racemosa*	South Africa	J.A Osorio & Jol. Roux	KP860882	KP860725	KP860803	KU587926
Neofusicoccum luteum	CBS 562.92 = ATCC 58193*	*Actinidia deliciosa*	New Zealand	S.R. Pennycook	KX464170	KX464690	KX464968	KX464020
	BRIP 5016	*Persea americana*	USA	–	MH057191	MH102254	–	–
Neofusicoccum macroclavatum	CBS 118223 = WAC 12444 *	*Eucalyptus globulus*	Australia	T.I. Burgess	DQ093196	DQ093217	DQ093206	KX464022
	WAC 12446	*Eucalyptus globulus*	Australia	T.I. Burgess	DQ093197	DQ093218	DQ093207	–
Neofusicoccum mangiferae	CBS 118531 = CMW 7024 *	*Mangifera indica*	Australia	G.I. Johnson	AY615185	DQ093221	AY615172	–
	CBS 118532 = CMW 7797	*Mangifera indica*	Australia	G.I. Johnson	AY615186	DQ093220	AY615173	KX464023
Neofusicoccum mangroviorum	CMW 41365 *	*Avicennia marina*	South Africa	J.A. Osorio	KP860859	KP860702	KP860779	KU587905
	CMW 42481	*Bruguiera gymnorrhiza*	South Afric	J.A. Osorio	KP860848	KP860692	KP860770	KU587895
Neofusicoccum mediterraneum	CBS 12718 = PD 312 *	*Eucalyptus* sp.	Greece	P.W. Crous, M.J. Wingfield & A.J.L. Phillips	GU251176	GU251308	GU251836	KX464024
Neofusicoccum microconidium	CGMCC 3.18750 = CERC 3497	*Eucalyptus urophylla* × *E. grandis*	China	S.F. Chen & G.Q. Li	KX278053	KX278158	KX278262	MF410203
Neofusicoccum nonquaesitum	CBS 126655 = PD 484 *	*Umbellularia californica*	USA	F.P. Trouillas	GU251163	GU251295	GU251823	KX464025
	PD301	*Vaccinium corymbosum*	Chile	E.X. Briceño, J.G. Espinoza & B. A.Latorre	GU251164	GU251296	GU251824	–
Neofusicoccum occulatum	CBS 128008 = MUCC 227 *	*Eucalyptus grandis*	Australia	T.I. Burgess	EU301030	EU339509	EU339472	EU339558
	MUCC 286 = WAC 12395	*Eucalyptus pellita*	Australia	T.I. Burgess	EU736947	EU339511	EU339474	EU339560
Neofusicoccum parvum	CMW 9081 = ATCC 58191*	*Populus nigra*	New Zealand	S.R. Pennycook	AY236943	AY236888	AY236917	EU821963
	CBS 110301	*Vitis vinifera*	Portugal	A.J.L. Phillips	AY259098	AY573221	EU673095	–
Neofusicoccum pennatisporum	MUCC 510 = WAC 13153 *	*Allocasuarina fraseriana*	Australia	K.M. Taylor	EF591976	EF591976	EF591959	–
Neofusicoccum pistaciae	CBS 595.76	*Pistacia vera*	Greece	D.G. Zachos	KX464163	KX464676	KX464953	KX464008
Neofusicoccum pistaciarum	CBS113083 = CPC 5263 *	*Pistacia vera*	USA	T.J. Michailides	KX464186	KX464712	KX464998	KX464027
	CBS113084 = CPC 5284	Redwood	USA	T.J. Michailides	KX464187	KX464713	KX464999	KX464028
Neofusicoccum protearum	CMW 39280	*Acacia karroo*	Africa	–	KF270041	KF270011	–	–
	CMW 39282	*Acacia karroo*	Africa	–	KF270043	KF270013	–	–
Neofusicoccum ribis	CBS 115475 = CMW 7772 *	*Ribes* sp.	USA	B. Slippers & G. Hudler	AY236935	AY236877	AY236906	EU821958
Neofusicoccum sinense	CGMCC 3.18315	Unknown wood plant	China	J.J. Gan	KY350148	KY817755	KY350154	–
Neofusicoccum stellenboschiana	CBS 110864	*Vitis vinifera*	South Africa	F. Halleen	AY343407	AY343348	KX465047	KX464042
Neofusicoccum terminaliae	CBS 125263 = CMW26679 *	*Terminalia sericea*	South Africa	D. Begoude & J. Roux	GQ471802	GQ471780	KX465052	KX464045
	CBS 125264 = CMW26683	*Terminalia sericea*	South Africa	D. Begoude & J. Roux	GQ471804	GQ471782	KX465053	KX464046
Neofusicoccum umdonicola	CBS 123645 = CMW 14058 *	*Syzygium cordatum*	South Africa	D. Pavlic	EU821904	EU821874	EU821844	EU821934
	CBS 123646 = CMW 14060	*Syzygium cordatum*	South Africa	D. Pavlic	EU821905	EU821875	EU821845	EU821935
Neofusicoccum ursorum	CBS 122811 = CMW 24480 *	*Eucalyptus* sp.	South Africa	H.M. Maleme	FJ752746	FJ752709	KX465056	KX464047
	CMW 23790	*Eucalyptus* sp.	South Africa	H.M. Maleme	FJ752745	FJ752708	KX465057	–
Neofusicoccum viticlavatum	CBS 112878 = STE-U 5044 *	*Vitis vinifera*	South Africa	F. Halleen	AY343381	AY343342	KX465058	KX464048
	CBS 112977 = STE-U 5041	*Vitis vinifera*	South Africa	F. Halleen	AY343380	AY343341	KX465059	–
Neofusicoccum vitifusiforme	CBS 110887 = STE-U 5252 *	*Vitis vinifera*	South Africa	J.M. van Niekerk	AY343383	AY343343	KX465061	KX464049
	CBS 110880 = STE-U 5050	*Vitis vinifera*	South Africa	J.M. van Niekerk	AY343382	AY343344	–	–
Sphaeropsis chromolaenicola	MFLUCC 17-1499 *	*Chromolaena odorata*	Thailand	A. Mapook	MT214366	–	–	–
Sphaeropsis citrigena	ICMP 16812 *	*Citrus sinensis*	New Zealand	S.R. Pennycook, P.R. Johnston & B.C.Paulus	EU673328	EU673294	EU673140	–

Table 2 (cont.)

Species	Isolate numbers[a]	Host	Location	Collector	GenBank accession numbers[b]			
					ITS	*tef1*	*tub2*	*rpb2*
Sphaeropsis citrigena (cont.)	ICMP 16818	*Citrus sinensis*	New Zealand	S.R. Pennycook, P.R. Johnston & B.C.Paulus	EU673329	EU673295	EU673141	–
Sphaeropsis eucalypticola	MFLUCC 11-0579 *	*Eucalyptus* sp.	Thailand	M. Doilom	JX646802	JX646867	JX646850	–
	MFLUCC 11-0654	*Eucalyptus* sp.	Thailand	M. Doilom	JX646803	JX646868	JX646851	–
Sphaeropsis porosa	CBS 110496 = STE-U 5132 *	*Vitis vinifera*	South Africa	J.M. van Niekerk	AY343379	AY343340	EU673130	KX464076
	CBS 110574 = STE-U 5046	*Vitis vinifera*	South Africa	J.M. van Niekerk	AY343378	AY343339	–	–
Sphaeropsis ulmicola	CBS 174.63	*Ulmus glabra*	Finland	–	MK134681	–	–	–
	PB-11f	*Ulmus glabra*	Poland	–	MK134682	–	–	–
Sphaeropsis visci	CBS 100163 *	*Vitis vinifera*	South Africa	J.M. van Niekerk	EU673324	EU673292	EU673127	KX464077
	CBS 186.97	*Viscum album*	Germany	T. Graefenhan	EU673325	EU673293	EU673128	KX464080

[a] ALG: Personal culture collection A. Berraf-Tebbal; ATCC: American Type Culture Collection, Virginia, USA; BL: Personal number of B.T. Linaldeddu; BRIP: Queensland Plant Pathology Herbarium, Brisbane, Australia; CAA: Personal culture collection Artur Alves, Universidade de Aveiro, Portugal; CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CERC: Culture collection of China Eucalypt Research Centre, Chinese Academy of Forestry, ZhanJiang, GuangDong, China; CFCC: China Forestry Culture Collection Center, Beijing, China; CGMCC: China General Microbiological Culture Collection Center, Beijing, China; CJA: Collection of J. Abdollahzadeh, Department of Plant Protection, Faculty of Agriculture, University of Kurdistan, Sanandaj, Iran; CMM: Culture Collection of Phytopathogenic Fungi 'Prof. Maria Menezes, Universidade Federal Rural de Pernambuco, Recife, Brazil; CMW: Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; CPC: Working collection of P.W. Crous, housed at CBS; GUCC: Guizhou University Culture Collection; GZCC: Guizhou Academy of Agricultural Sciences Culture Collection, GuiZhou, China; IBL: Personal culture collection, I.B.L. Coutinho; IBP: Personal culture collection, I.B. Prasher; ICMP: International Collection of Microorganisms from Plants, Auckland, New Zealand; IMI: Kew Royal Botanical Gardens, Kew, England; IRAN: Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Iran; JL: Personal culture collection of J. Luque, IRTA, Barcelona, Spain; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; MUCC: Culture collection of Murdoch University, Perth, Australia; PD: Culture Collection, University of California, Davis, USA; STE-U: Culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa; UCD: University of California, Davis, Plant Pathology Department Culture Collection; UCROK: Department of Plant Pathology and Microbiology, University of California, Riverside; UY: Department of Plant Pathology, University of Minnesota; WAC: Department of Agriculture, Western Australia Plant Pathogen Collection, South Perth, Western Australia; XTBG: Institutional Repository of Xishuangbanna Tropical Botanical Garden.

[b] ITS, internal transcribed spacer region and intervening 5.8S nrRNA gene; *tef1*, translation elongation factor 1-alpha; *tub2*, β-tubulin; *rpb2*, DNA-directed RNA polymerase II second largest subunit.

* Isolates represent ex-type.

RESULTS

Isolates

A total of 111 isolates from 88 collected citrus samples exhibited typical morphological characteristics of *Botryosphaeriaceae*. Eighty-one isolates were collected from Zhejiang, seven from Guangxi, six from Guangdong, four respectively from Chongqing, Fujian and Shaanxi, two respectively from Hunan and Jiangxi, and one from Shanghai. Among them, 52 isolates were obtained from twigs and branches with dieback, 31 were associated with branches and trunks with canker, and 28 from gummosis symptoms. In terms of *Citrus* species, 42 isolates were obtained from *C. unshiu*, 25 from *C. reticulata*, 10 from *C. sinensis*, nine from *C. maxima*, one from *C. limon*, and 24 from hybrids.

Phylogenetic analyses

The ITS and *tef1* sequences were amplified for all 111 isolates obtained in this study, and blast results indicated that these isolates resided in *Botryosphaeria*, *Diplodia*, *Dothiorella*, *Lasiodiplodia*, *Neodeightonia*, *Neofusicoccum* and *Sphaeropsis*. Fifty-seven representative isolates were subsequently selected to be sequenced for their *tub2* and *rpb2* loci (Table 1). Datasets for the seven genera, the parameters of the statistical values of the trees for the MP analyses and the best-fit substitution models for ML analyses are provided in Table 3. All sequences of *Botryosphaeriaceae* species obtained in this study were deposited in GenBank (Table 1).

Species of Botryosphaeria

Isolates clustered into two phylogenetic groups (Group A and B) for the individual genes (ITS, *tef1*, *tub2*, *rpb2*), as well as the combined gene dataset (Fig. 2, S1a–d). For the ITS sequences, isolates BE3, BE78, BE85 and BE86 (Group A) grouped with several species, while isolates BE1, BE2, BE63 and BE66 (Group B) formed a clade distinct from the other species (Fig. S1a). For the *tef1* and *rpb2* and combined ITS/*tef1*/*tub2*/*rpb2* datasets, isolates in Group A were closely related to *B. fabicerciana*, and isolates in Group B were most closely related to *B. dothidea* (Fig. 2, S1b, d). Therefore, isolates in Group A were identified as *B. fabicerciana*, and isolates in Group B were identified as *B. dothidea*.

Species of Diplodia

Isolate BE4 (Group C) clustered more closely to *D. seriata* and *D. galiicola* in the ITS datasets (Fig. S2a), while more closely to *D. seriata* and *D. sapinea* in the *tef1* datasets (Fig. S2b). For the *tub2* and *rpb2* sequences, isolate BE4 clustered with *D. seriata* (Fig. S2c–d). The analyses of the combined ITS, *tef1*, *tub2* and *rpb2* sequences demonstrated that isolate BE4 was most closely related to *D. seriata* (Fig. 3).

Species of Dothiorella

Eight isolates clustered in three clades (Group D–F). Isolate BE17 in Group D grouped with *Do. alpina* and *Do. magnoliae* based on the ITS sequences (Fig. S3a). For the *tef1* sequences, isolate BE17 formed an independent lineage close to *Do. alpina* and *Do. acericola* (Fig. S3b). For the *tub2* and *rpb2* sequences, isolate BE17 formed an independent lineage (Fig. S3c, d). For the combined ITS, *tef1* and *tub2* sequences, isolate BE17 clustered with *Do. alpina* (Fig. 4). For Group E, the sequence analyses of ITS, *tef1*, *tub2* and ITS/*tef1*/*tub2* sequences showed that isolates BE16 and BE74 clustered in the same clade (ITS, *tub2*) or close (*tef1*, ITS/*tef1*/*tub2*) to *Do. plurivora* (*rpb2* sequences are not available for *Do. plurivora*) (Fig. 4, S3a–c). Thus, isolates in Group D were identified as *Do. alpina*, while those in Group E were identified as *Do. plurivora*.

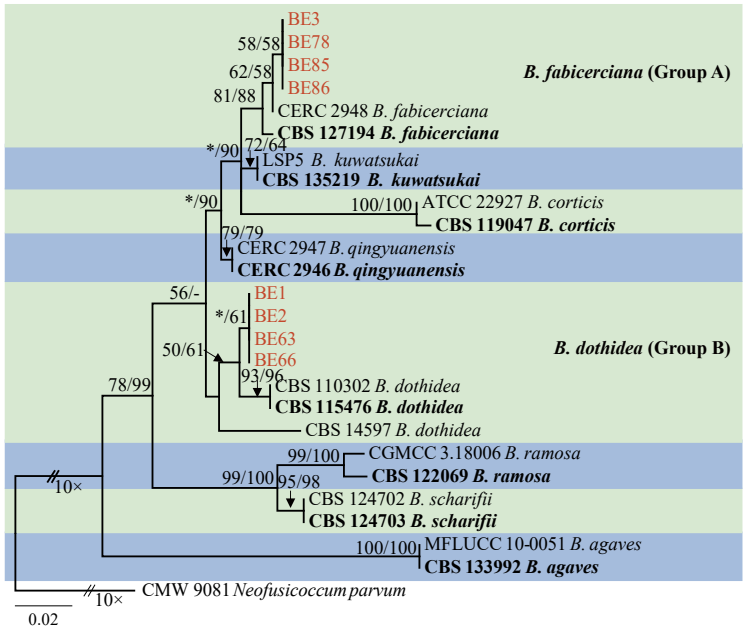


Fig. 2 Phylogenetic tree generated by maximum likelihood analyses based on the combined ITS, *tef1* and *tub2* sequence alignments of *Botryosphaeria*. Bootstrap support values ≥ 50 % for ML and MP are presented above branches as follows: ML/MP bootstrap values < 50 % are marked with *, and absent are marked with -. Newly generated sequences are in red and ex-type strains are in **bold**. The tree was rooted to *Neofusicoccum parvum* (CMW 9081).

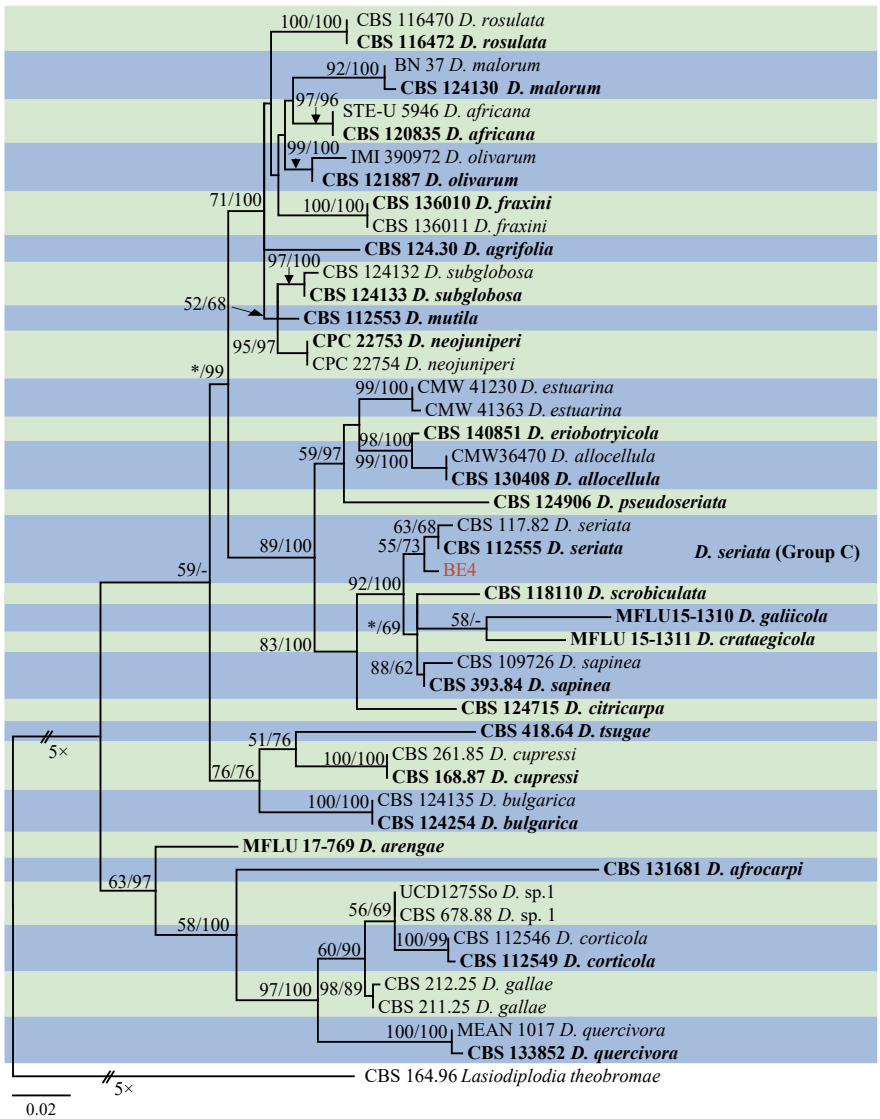


Fig. 3 Phylogenetic tree generated by maximum likelihood analyses based on the combined ITS, *tef1* and *tub2* sequence alignments of *Diplodia*. Bootstrap support values ≥ 50 % for ML and MP are presented above branches as follows: ML/MP bootstrap values < 50 % are marked with *, and absent are marked with -. Newly generated sequences are in red and ex-type strains are in **bold**. The tree was rooted to *Lasiodiplodia theobromae* (CBS 164. 96).

Table 3 Datasets used and statistics resulting from phylogenetic analyses in the current study.

Genus	Dataset	Maximum likelihood									
		Subst. model[1]	NST[2]	Rate matrix					Ti/Tv ratio[3]	p-inv	Gamma	Rates
Botryosphaeria	ITS	TrN+I	6	1.0000	1.4653	1.0000	1.0000	7.9294	–	0.8090	–	equal
	tef1	TPM3uf+I	6	0.3783	2.6378	1.0000	0.3783	2.6378	–	0.6000	–	equal
	tub2	TrN+I	6	1.0000	6.0784	1.0000	1.0000	14.3581	–	0.7170	–	equal
	rpb2	TIM3+G	6	9756.0724	15799.8965	1.0000	9756.0724	70110.9963	–	–	0.1480	gamma
	ITS/*tef1*/*tub2*	TrN+I	6	1.0000	3.4860	1.0000	1.0000	7.2459	–	0.761	–	equal
Diplodia	ITS	TVMef+I+G	6	6.1639	23.8085	4.6946	13.6614	23.8085	–	0.6580	0.6850	gamma
	tef1	TrN+G	6	1.0000	3.3131	1.0000	1.0000	5.5220	–	–	0.5530	gamma
	tub2	TrN+I	6	1.0000	3.1072	1.0000	1.0000	5.7192	–	0.676	–	equal
	rpb2	TrN+G	6	1.0000	5.1679	1.0000	1.0000	13.7446	–	–	0.228	gamma
	ITS/*tef1*/*tub2*	TrN+I+G	6	1.0000	3.6215	1.0000	1.0000	5.0395	–	0.5940	1.3320	gamma
Dothiorella	ITS	TrN+I+G	6	1.0000	1.4234	1.0000	1.0000	3.2819	–	0.4180	0.6840	gamma
	tef1	TPM2uf+G	6	2.0341	5.0971	2.0341	1.0000	5.0971	–	–	0.765	gamma
	tub2	HKY+I+G	2	–	–	–	–	–	1.7295	0.5600	0.9450	gamma
	rpb2	TrN+I	6	1.0000	3.8616	1.0000	1.0000	10.6645	–	0.6030	–	equal
	ITS/*tef1*/*tub2*	TIM2+G	6	1.2403	2.7176	1.2403	1.0000	4.1029	–	–	0.2160	gamma
Lasiodiplodia	ITS	K80+I	2	–	–	–	–	–	2.4444	0.7840	–	equal
	tef1	K80+G	2	–	–	–	–	–	1.7723	–	0.4130	gamma
	tub2	TrNef+I	6	1.0000	1.6752	1.0000	1.0000	6.7164	–	0.6540	–	equal
	rpb2	TrNef+G	6	1.0000	5.3397	1.0000	1.0000	12.4894	–	–	0.3110	gamma
	ITS/*tef1*/*tub2*/*rpb2*	TrN+I+G	6	1.0000	3.8560	1.0000	1.0000	7.6054	–	0.5410	0.6090	gamma
Neodeightonia	ITS	TPM3uf+I+G	6	3.2767	4.6927	1.0000	3.2767	4.6927	–	0.6640	0.5000	gamma
	tef1	TIM1	6	1.0000	1.0118	0.2049	0.2049	2.8685	–	–	–	equal
	tub2	TIM1+I	6	1.0000	1.2183	0.3558	0.3558	2.4674	–	0.6860	–	equal
	ITS/*tef1*/*tub2*	TIM1+G	6	1.0000	1.2491	0.5067	0.5067	3.6475	–	–	0.1940	gamma
Neofusicoccum	ITS	TIM1ef+I+G	6	1.0000	6.4363	2.1879	2.1879	17.3039	–	0.5780	0.6010	gamma
	tef1	HKY+G	2	–	–	–	–	–	2.5159	–	0.7750	gamma
	tub2	TrN+G	6	1.0000	3.1372	1.0000	1.0000	6.7052	–	–	0.2270	gamma
	rpb2	TrN+G	6	1.0000	5.5358	1.0000	1.0000	17.053	–	–	0.235	gamma
	ITS/*tef1*/*tub2*/*rpb2*	K80+G	2	–	–	–	–	–	2.265	–	–	gamma
Sphaeropsis	ITS	HKY+I+G	2	–	–	–	–	–	1.7294	0.5610	0.9480	gamma
	tef1	HKY+I+G	2	–	–	–	–	–	1.7294	0.5610	0.9480	gamma
	tub2	TIM2+G	6	1.2403	2.7176	1.2403	1.0000	4.1029	–	–	0.2160	gamma
	rpb2	TIM2+G	6	1.2403	2.7176	1.2403	1.0000	4.1029	–	–	0.2160	gamma
	ITS/*tef1*/*tub2*/*rpb2*	HKY+I+G	2	–	–	–	–	–	1.7294	–	0.5610	gamma

Table 3 (cont.)

Genus	Dataset	No. of taxa	No. of bp[4]	Maximum parsimony						
				PIC[5]	No. of trees	Tree length	CI[6]	RI[7]	RC[8]	HI[9]
Botryosphaeria	ITS	26	504	25	1	39	0.7949	0.8769	0.6970	0.2051
	tef1	26	347	103	42	129	0.8992	0.9378	0.8433	0.1008
	tub2	21	406	16	1	21	0.9048	0.9444	0.8545	0.0952
	rpb2	14	659	12	3	18	0.8889	0.9583	0.8519	0.1111
	ITS/*tef1*/*tub*	26	1257	144	3	193	0.8601	0.9129	0.7852	0.1399
Diplodia	ITS	47	535	91	1354	233	0.6652	0.8534	0.5677	0.3348
	tef1	47	287	117	5000	275	0.6291	0.9016	0.5672	0.3709
	tub	39	388	46	1103	95	0.6105	0.8571	0.5233	0.3895
	rpb2	7	594	41	2	61	0.8033	0.7857	0.6311	0.1967
	ITS/*tef1*/*tub2*	47	1210	42	42	658	0.5881	0.8536	0.5020	0.4119
Dothiorella	ITS	63	496	97	384	287	0.5714	0.8955	0.5117	0.4286
	tef1	63	324	217	400	791	0.5310	0.8594	0.4563	0.4690
	tub2	45	387	95	185	203	0.6946	0.8758	0.6083	0.3054
	rpb2	16	594	83	2	145	0.6621	0.8333	0.5517	0.3379
	ITS/*tef1*/*tub2*	63	1540	409	6	1336	0.5427	0.8584	0.4658	0.4573
Lasiodiplodia	ITS	99	486	49	5000	89	0.6517	0.8905	0.5803	0.3483
	tef1	98	291	132	802	284	0.6092	0.8911	0.5428	0.3908
	tub2	90	345	34	10	54	0.7222	0.9227	0.6664	0.2778
	rpb2	77	521	86	201	166	0.6024	0.8981	0.5411	0.3976
	ITS/*tef1*/*tub2*/*rpb2*	99	1643	313	670	956	0.485356	0.8422	0.4087	0.5146
Neodeightonia	ITS	15	502	51	1	111	0.7840	0.8490	0.6650	0.2160
	tef1	9	229	14	1	19	0.8420	0.8750	0.7370	0.1580
	tub2	7	418	6	1	9	0.8890	0.8570	0.7620	0.1110
	ITS/*tef1*/*tub2*	15	1150	78	3	162	0.7346	0.8114	0.5960	0.2654
Neofusicoccum	ITS	57	494	54	28	146	0.5548	0.8516	0.4725	0.4452
	tef1	55	280	115	5000	273	0.6923	0.9012	0.6239	0.3077
	tub2	51	422	67	5000	130	0.6231	0.8345	0.5199	0.3769
	rpb2	33	560	75	68	143	0.6364	0.8267	0.5261	0.3636
	ITS/*tef1*/*tub2*/*rpb2*	57	1756	334	24	817	0.5704	0.8208	0.4682	0.4296
Sphaeropsis	ITS	10	518	54	1	19	1.0000	1.0000	1.0000	1.0000
	tef1	10	297	77	1	117	0.8462	0.8583	0.7262	0.1538
	tub2	9	375	20	3	21	0.8095	0.8261	0.6687	0.1905
	rpb2	5	557	18	2	27	0.7410	0.6110	0.4530	0.2590
	ITS/*tef1*/*tub2*/*rpb2*	10	1747	130	2	188	0.8245	0.8245	0.6797	0.1755

[1] Subst. model = best fit substitution model.
[2] NST = number of substitution rate categories.
[3] Ti/Tv ratio = transition/transversion ratio.
[4] bp = base pairs.
[5] PIC = number of parsimony informative characters.
[6] CI = consistency index.
[7] RI = retention index.
[8] RC = rescaled consistency index.
[9] HI = homoplasy index.

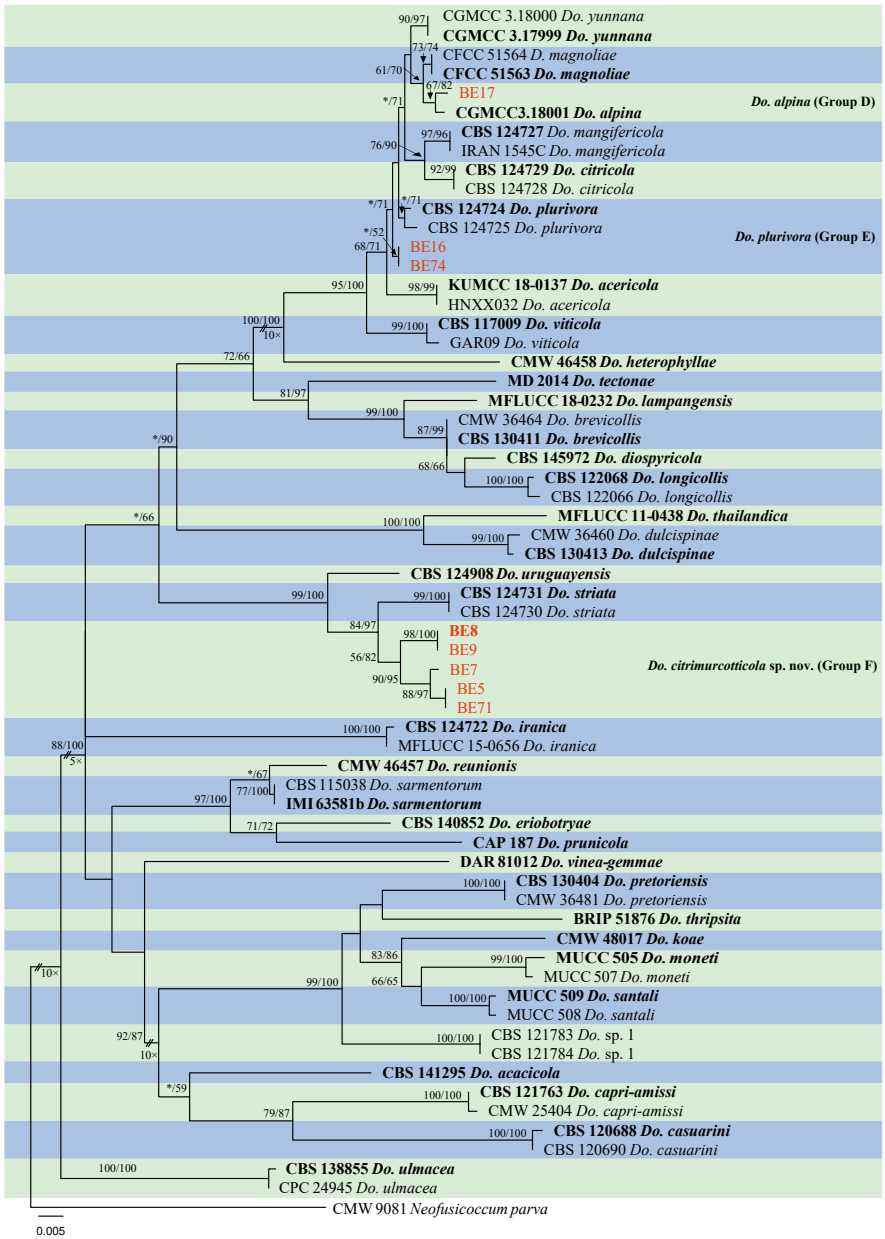


Fig. 4 Phylogenetic tree generated by maximum likelihood analyses based on the combined ITS, *tef1* and *tub2* sequence alignments of *Dothiorella*. Bootstrap support values ≥ 50 % for ML and MP are presented above branches as follows: ML/MP bootstrap values < 50 % are marked with *. Newly generated sequences are in red and ex-type strains are in **bold**. The tree was rooted to *Neofusicoccum parva* (CMW 9081).

Isolates in Group F (BE5, BE7, BE8, BE9, BE71) clustered close to *Do. striata* based on the ITS and *tef1* datasets (Fig. S3a–b), but formed independent clades that were separated from *Do. striata* with high bootstrap values in the *tub2*, *rpb2* and combined ITS/*tef1*/*tub2* datasets (*tub2*, ML/MP = 92 % / 94 %; *rpb2*, ML/MP = 100 % / 100 %; ITS/*tef1*/*tub2*, ML/MP = 84 % / 97 %) (Fig. 4, S3c–d). Thus, isolates in Group F were considered as an undescribed species in *Dothiorella*.

Species of Lasiodiplodia

Isolates resided in nine groups (Group G–O) based on the *tef1* and combined ITS/*tef1*/*tub2*/*rpb2* datasets (Fig. 5, S4b). Isolates in Group G (BE27, BE30, BE36, BE41, BE42, BE100) were closest to *L. iraniensis* and various other species based on the ITS, *tub2* and *rpb2* datasets (Fig. S4a, c–d). For the *tef1* and combined ITS/*tef1*/*tub2*/*rpb2* sequences, the six isolates were closest to *L. iraniensis* (Fig. 5, S4b). Thus, the six isolates in Group G were identified as *L. iraniensis*.

For isolates in Group H (BE20, BE21, BE25) and Group J (BE32, BE80, BE87, BE88), the analyses of the ITS, *tef1*, *tub2* and *rpb2* sequences indicated that isolates in Group H clustered into the same (ITS, *tub2*, *rpb2*) clade or close (*tef1*) to *L. theobromae*, while isolates in Group J clustered into the same (*rpb2*) clade or close (ITS, *tef1*) to *L. microconidia* (Fig. S4a–d). The combined ITS/*tef1*/*tub2*/*rpb2* datasets showed that isolates in Group H were more closely related to *L. theobromae*, while isolates in Group J clustered with *L. microconidia* (Fig. 5).

Isolates in Group I (BE28, BE34, BE40, BE51) and Group L (BE31, BE59) clustered with various *Lasiodiplodia* species based on ITS, *tub2* and *rpb2* datasets (Fig. S4a, c–d), but formed independent clades based on the *tef1* and combined ITS/*tef1*/*tub2*/*rpb2* trees, with high bootstrap values (Group I: *tef1*, ML/MP = 99 % / 100 %; ITS/*tef1*/*tub2*/*rpb2*, ML/MP = 99 % / 100 %; Group L: *tef1*, ML/MP = 98 % / 99 %; ITS/*tef1*/*tub2*/*rpb2*, ML/MP = 99 % / 100 %) (Fig. 5, S4b). Therefore, isolates in Group I and Group L were considered to represent two novel species.

Isolates in Group K (BE10, BE11, BE12, BE19, BE26, BE37) clustered with *L. pseudotheobromae* and various other species based on the ITS and *tub2* datasets (Fig. S4a, c). For

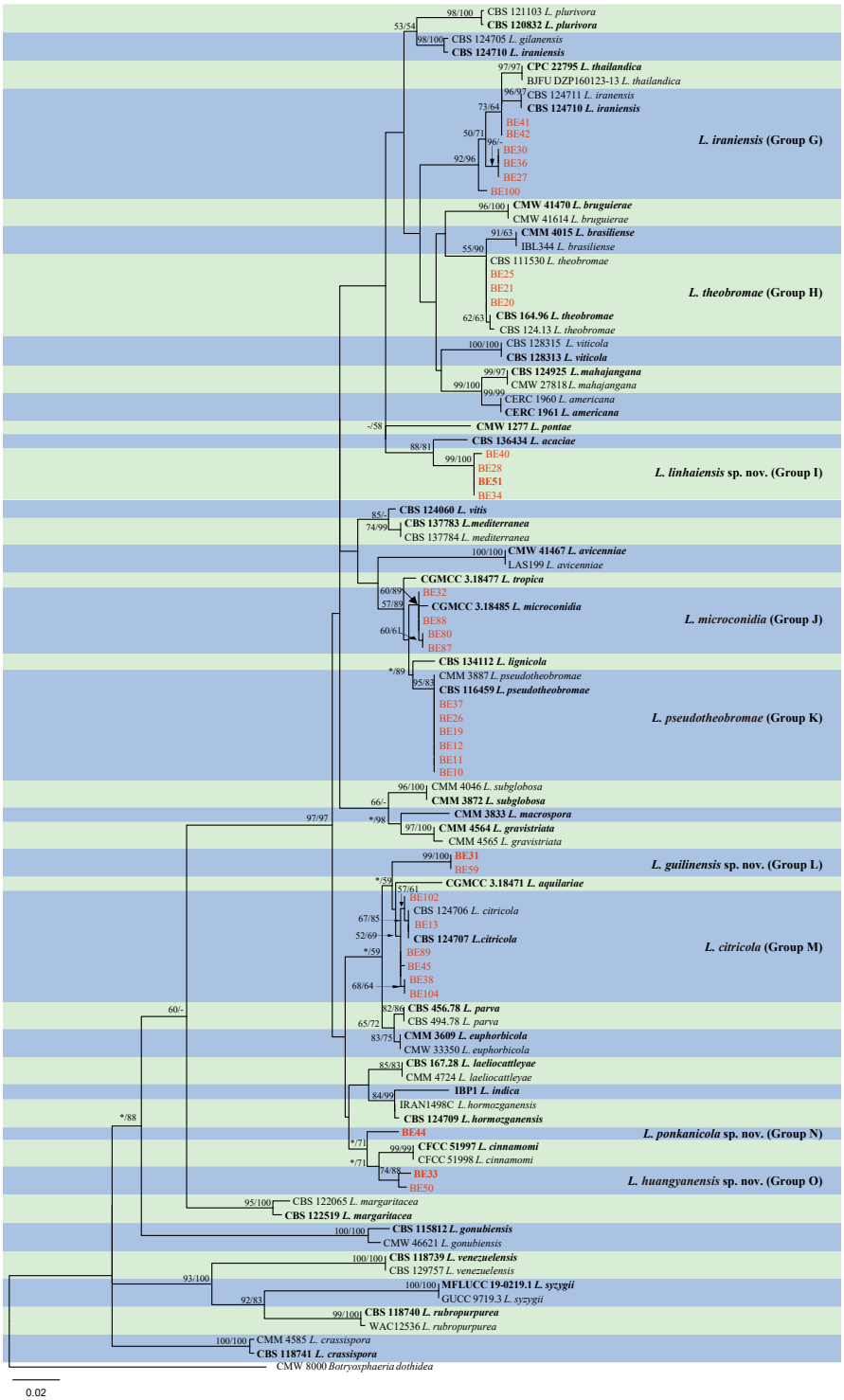


Fig. 5 Phylogenetic tree generated by maximum likelihood analyses based on the combined ITS, *tef1*, *tub2* and *rpb2* sequence alignments of *Lasiodiplodia*. Bootstrap support values ≥ 50 % for ML and MP are presented above branches as follows: ML/MP bootstrap values < 50 % are marked with *, and absent are marked with -. Newly generated sequences are in red and ex-type strains are in **bold**. The tree was rooted to *Botryosphaeria dothidea* (CMW 8000).

the analyses of *tef1*, *rpb2* and the combined ITS/*tef1*/*tub2*/*rpb2* datasets, the six isolates resided in the same clade with *L. pseudotheobromae* (Fig. 5, S4b, d). Therefore, the isolates in Group K were treated as *L. pseudotheobromae*. Isolates in Group M (BE13, BE38, BE45, BE89, BE102, BE104) clustered into the same (ITS, *rpb2*) clade or close (*tef1*, *rpb2*) to *L. citricola* (Fig. S4a–d). The combined ITS/*tef1*/*tub2*/*rpb2* datasets showed that isolates in Group M clustered with *L. citricola* (Fig. 5).

Isolate BE44 in Group N resided in a clade with *L. citricola* based on the analyses of the ITS and *tub2* datasets (Fig. S4a, c). For the *tef1* datasets, BE44 formed an independent lineage phylogenetically close to *L. aquilariae* (Fig. S4b). For the *rpb2* datasets, BE44 grouped with various other species (Fig. S4d). The analyses of the combined ITS/*tef1*/*tub2*/*rpb2* datasets indicated that isolate BE44 formed an independent lineage that was distinguished from other known phylogenetically related species (Fig. 5). Isolates in Group O (BE33, BE50) grouped together with various other *Lasiodiplodia* species in the ITS, *tub2* and *rpb2* trees (Fig. S4a, c–d). For the *tef1* datasets, the two isolates formed two independent clades but with low bootstrap support based on the *tef1* in the ML analyses, while they clustered together with Group L in the MP analyses (data not shown). The analyses of the combined ITS/*tef1*/*tub2*/*rpb2*

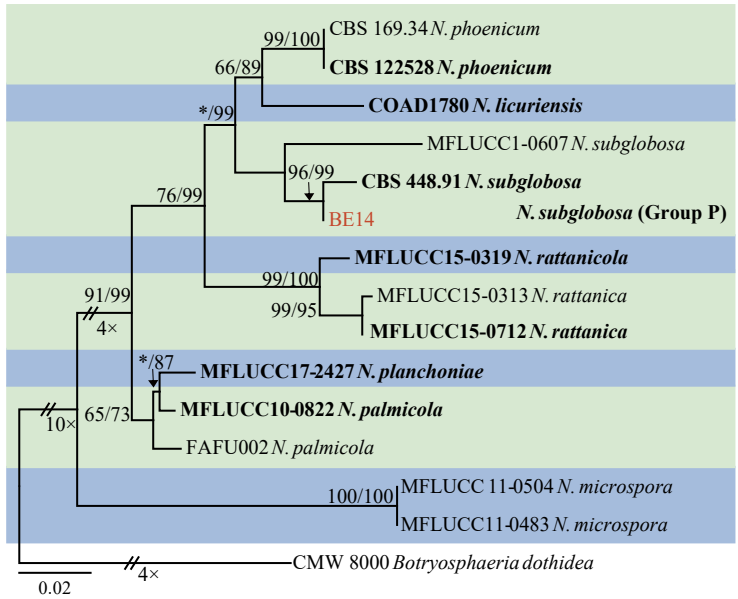


Fig. 6 Phylogenetic tree generated by maximum likelihood analyses based on the combined ITS, *tef1* and *tub2* sequence alignments of *Neodeightonia*. Bootstrap support values ≥ 50 % for ML and MP are presented above branches as follows: ML/MP bootstrap values < 50 % are marked with *. Newly generated sequences are in red and ex-type strains are in **bold**. The tree was rooted to *Botryosphaeria dothidea* (CMW 8000).

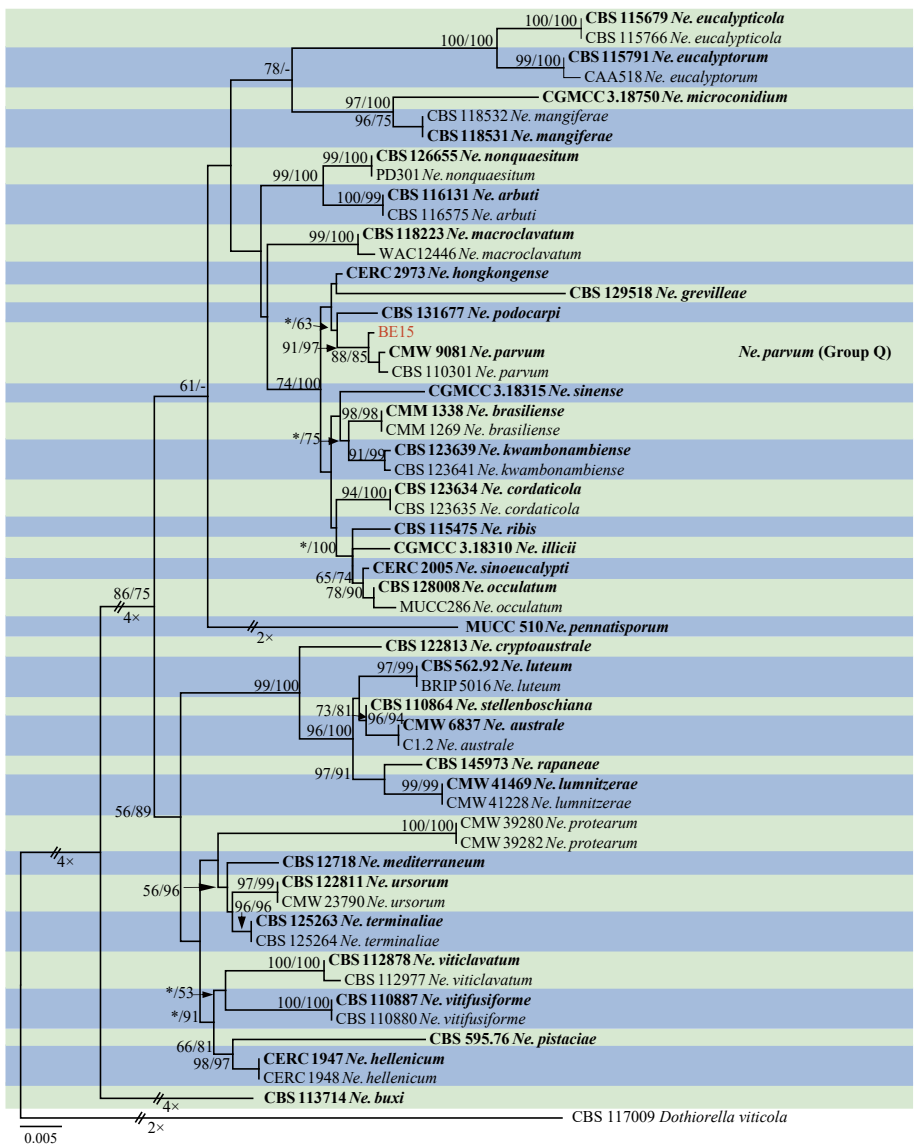


Fig. 7 Phylogenetic tree generated by maximum likelihood analyses based on the combined ITS, *tef1*, *tub2* and *rpb2* sequence alignments of *Neofusicoccum*. Bootstrap support values ≥ 50 % for ML and MP are presented above branches as follows: ML/MP bootstrap values < 50 % are marked with *, and absent are marked with -. Newly generated sequences are in red and ex-type strains are in **bold**. The tree was rooted to *Dothiorella viticola* (CBS 117009).

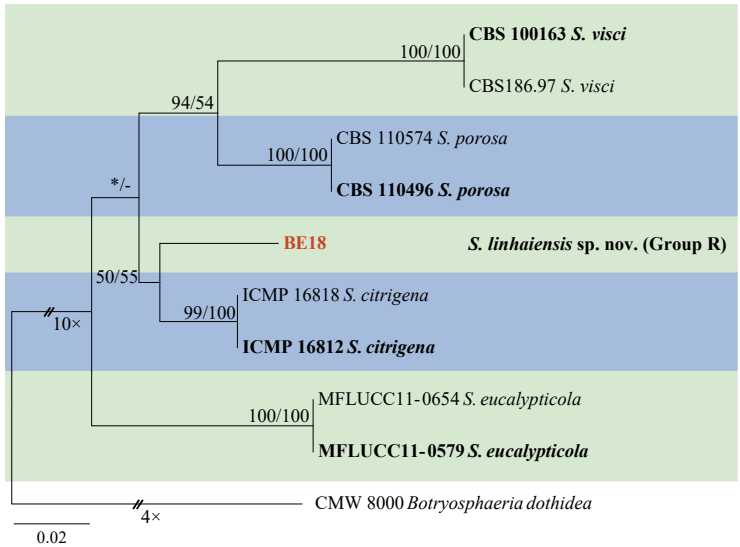


Fig. 8 Phylogenetic tree generated by maximum likelihood analyses based on the combined ITS, *tef1*, *tub2* and *rpb2* sequence alignments of *Sphaeropsis*. Bootstrap support values ≥ 50 % for ML and MP are presented above branches as follows: ML/MP bootstrap values < 50 % are marked with *, and absent are marked with -. Newly generated sequences are in red and ex-type strains are in **bold**. The tree was rooted to *Botryosphaeria dothidea* (CMW 8000).

datasets indicated that isolates in Group O formed an independent clade with high support bootstrap values (ML/MP = 74 % / 88 %) (Fig. 5). Consequently, isolates in Group N and Group O were identified as two new species of *Lasiodiplodia*.

Species of Neodeightonia

Isolate BE14 (Group P) grouped with *N. subglobosa* in one clade with high support value on the basis of the phylogenetic analyses for the ITS, *tef1*, *tub2* and ITS/*tef1*/*tub2* datasets (Fig. 6, S5a–d).

Species of Neofusicoccum

Phylogenetic analyses of ITS and *tef1* consistently indicated that isolate BE15 (Group Q) resided in one phylogenetic clade with *Ne. parvum* (Fig. S6a–b), and with *Ne. pennatisporum* based on *tub2* (Fig. S6c). Isolate BE15 clustered with *Ne. parvum*, *Ne. cryptoaustrale* and *Ne. mangiferae* based on *rpb2* (Fig. S6d). The phylogeny based on the combined ITS/*tef1*/*tub2*/*rpb2* sequences indicated that isolate BE15 was closely related to *Ne. parvum* (Fig. 7).

Species of Sphaeropsis

Isolate BE18 (Group R) formed an independent lineage that was distinct from any known species of *Sphaeropsis* based on the phylogenetic analyses for ITS, *tef1*, *tub2*, *rpb2* and the combined four gene datasets. The bootstrap values associated with the other species were higher than 50 % in ITS, *tef1*, *rpb2* and the combined datasets (ITS, ML/MP = 68 % / 64 %; *tef1*, MP = 93 %; *rpb2*, MP = 100 %; ITS/*tef1*/*tub2*/*rpb2*, ML/MP = 50 % / 55 %) (Fig. 8, S7a–b, d). Therefore, isolate BE18 was treated as a novel species of *Sphaeropsis*.

Morphology and taxonomy

The selected isolates for morphological studies produced pycnidia on PNA within 4–6 wk. No sexual structures were observed in this study. Based on DNA sequences and morphology, 18 species belonging to seven genera were identified. Of these, *Botryosphaeria dothidea*, *B. fabicerciana*, *Diplodia seriata*, *Dothiorella alpina*, *Do. plurivora*, *Lasiodiplodia citricola*, *L. iraniensis*, *L. microconidia*, *L. pseudotheobromae*, *L. theobromae*, *Neodeightonia subglobosa* and *Neofusicoccum parvum* are known species. The remaining six species are described below.

Dothiorella citrimurcotticola X.E. Xiao, P.W. Crous & H.Y. Li, *sp. nov.* — MycoBank MB 840681; Fig. 9

Etymology. Referring to the citrus host (Murcott) which it was isolated.

Typus. China, Chongqing Municipality, Wanzhou City, from a twig of Murcott (*C. reticulata* × *C. sinensis*), 23 Mar. 2019, *H.Y. Li & X.E. Xiao*, conidiomata induced on PNA (holotype ZJUE H-0008, culture ex-type CGMCC 3.20394 = BE8).

Sexual morph unknown. *Conidiomata* pycnidial, produced on PNA within 2–4 wk, dark brown to black, up to 823 µm diam, globose, superficial or semi-immersed, unilocular, thick-walled. *Conidiophores* absent. *Conidiogenous cells* cylindrical to fusiform or lageniform, hyaline, thin-walled, smooth, 4.5–10.5 × 2–5 µm. *Conidia* subcylindrical to ellipsoid or ovoid, initially hyaline, thin-walled, aseptate, becoming brown, thick-walled, 1-septate, externally smooth, internally verrucose, apex rounded, base truncate or rounded, (21.5–)23–25.5(–27) × (8.5–)9.5–11(–14) µm (av. = 24.4 × 10.3 µm, n = 100; L/W ratio = 2.4) (Table 4).

Culture characteristics — Colonies on PDA have abundant aerial mycelia, initially leaden grey in the centre, becoming pale mouse grey at the surface and greenish olivaceous to dull green at the reverse. Colonies cover the 90 mm plates after 3 d at the optimum temperature of 25 °C. No growth was observed at 40 °C. After 3 d, colonies at 5 °C, 10 °C, 15 °C, 20 °C, 30 °C and 35 °C reach 11 mm, 11 mm, 59 mm, 79 mm, 42 mm and 12 mm, respectively.

Additional materials examined. China, Zhejiang Province, Yongquan Town, from a twig of *C. unshiu*, Aug. 2018, *H.Y. Li*, conidiomata induced on PNA (holotype ZJUE H-0005, culture ex-type CGMCC 3.20392 = BE5); Chongqing Municipality, Wanzhou City, from a twig of Murcott (*C. reticulata* × *C. sinensis*), 23 Mar. 2019, *H.Y. Li & X.E. Xiao*, conidiomata induced on PNA (ZJUE H-0009, culture CGMCC 3.20395 = BE9); Zhejiang Province, Quzhou City, from a twig of *C. maxima*, 27 Apr. 2019, *H.Y. Li*, conidiomata induced on PNA (ZJUE H-0007, culture CGMCC 3.20393 = BE7).

Notes — Phylogenetically, *Do. citrimurcotticola* is closely related to *Do. striata* and *Do. uruguayensis*, but morphologically it can be distinguished based on their average conidial dimensions. Conidia of *Do. citrimurcotticola* (av. 24.4 × 10.3; L/W = 2.4) are larger than *Do. uruguayensis* (av. 22 × 9.25; L/W = 2.4) (Pérez et al. 2010) but smaller than *Do. striata* (av. 25.1 × 10.7; L/W = 2.4) (Abdollahzadeh et al. 2014) (Table 4). Moreover, *Do. citrimurcotticola* differs from these species based on nucleotide differences in ITS (*Do. striata*: 5 bp, *Do. urugua-*

yensis: 1 bp), *tef1* (*Do. striata*: 5 bp, *Do. uruguayensis*: 15 bp and including three gaps), *tub2* (*Do. striata*: 4 bp, *Do. uruguayensis*: 6 bp) and *rpb2* loci (*Do. striata*: 8 bp).

Lasiodiplodia guilinensis X.E. Xiao, P.W. Crous & H.Y. Li, *sp. nov.* — MycoBank MB 840682; Fig. 10

Etymology. Referring to the city, Guilin, where it was collected.

Typus. China, Guangxi Province, Guilin City, from a twig of *C. sinensis* cv. Valencia, 26 Mar. 2019, *H.Y. Li & X.E. Xiao*, conidiomata induced on PNA (holotype ZJUE H-0031, culture ex-type CGMCC 3.20378 = BE31).

Sexual morph unknown. *Conidiomata* stromatic, produced on PNA within 2–4 wk, superficial or semi-immersed, dark brown to black, up to 2 mm diam, solitary or aggregated, unilocular, covered by dense mycelium, globose, thick-walled, often releasing pale yellow to saffron yellow conidial tendrils or mass. *Paraphyses* hyaline, cylindrical, septate, unbranched, ends rounded, up to 75 µm long, 2–5 µm wide, formed among conidiogenous cells. *Conidiophores* absent. *Conidiogenous cells* holoblastic, hyaline, smooth, thin-walled, cylindrical, 8–54 × 3–9 µm. *Conidia* initially hyaline, aseptate, ellipsoid to ovoid, thin-walled with granular content, rounded at apex, base round or truncate, becoming dark brown, 1-septate with longitudinal striations, (23–)28–31(–33.5) × (13.5–)15–16.5(–17) µm (av. = 29.6 × 15.7 µm, n = 100; L/W ratio = 1.9) (Table 4).

Culture characteristics — Colonies on PDA with moderately dense aerial mycelium, initially white to smoke grey, turning grey olivaceous on the surface and greenish grey in reverse, becoming dark slate-blue with age. Colonies cover the 90 mm plates

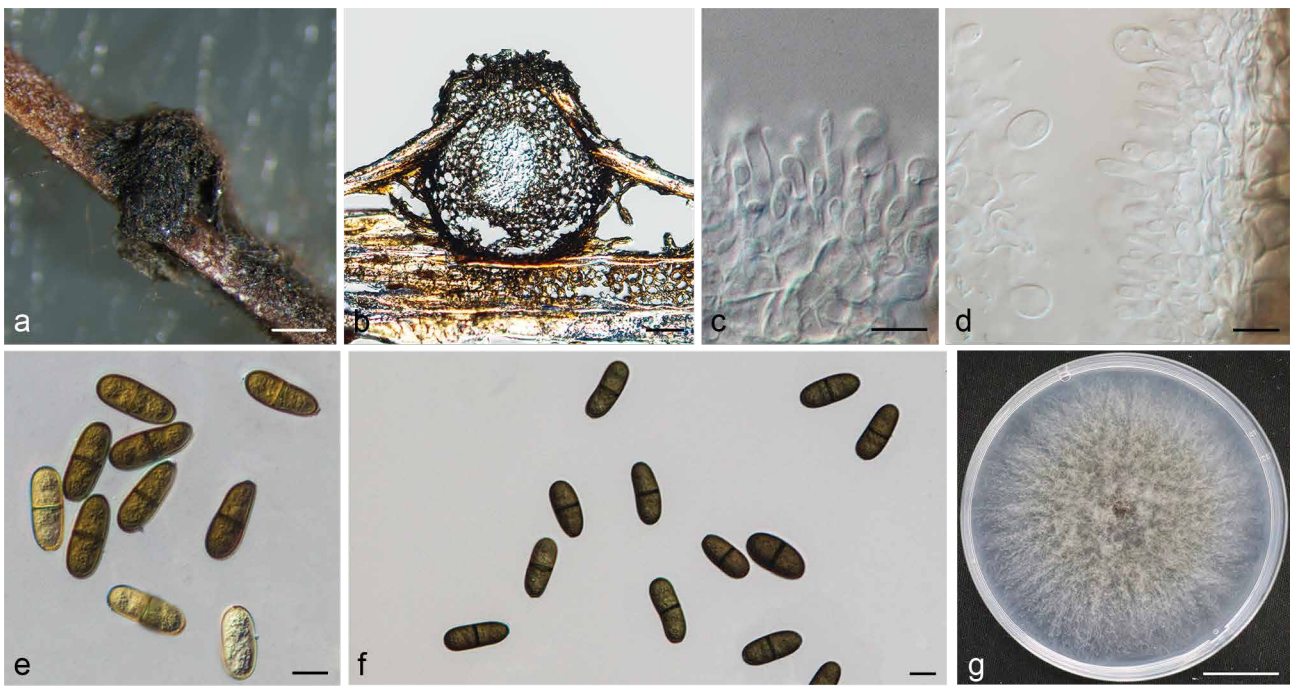


Fig. 9 *Dothiorella citrimurcotticola*. a. Conidioma formed on PNA; b. section view of conidioma; c–d. conidiogenous cells and developing conidia; e–f. conidia; g. colony growing on PDA after 3 d. — Scale bars: a = 100 µm; b = 50 µm; c–f = 10 µm; g = 2.1 cm.

Table 4 Conidial measurements of *Botryosphaeriaceae* species.

Species[1]	Conidia			Paraphyses		Reference
	Conidial size (µm) (L × W)[2]	Mean (µm) (L × W)[3]	L/W[4]	long (µm)[5]	wide (µm)[6]	
Do. citrimurcotticola	**(21.5–)23–25.5(–27) × (8.5–)9.5–11(–14)**	**24.4 × 10.3**	**2.4**	–	–	**This study**
Do. striata	(21–)23–26(–29.4) × (8.9–)9–12(–15.1)	25.1 × 10.7	2.4	–	–	Abdollahzadeh et al. (2014)
Do. uruguayensis	(17–)22–22.5(–26.5) × (7–)9–9.5(–12)	22 × 9.25	2.4	–	–	Pérez et al. (2010)
Lasiodiplodia acaciae	(21.5–)25–29.5(–31) × (11–)12–14(–15)	27.3 × 12.9	2.1	69	2–5	Zhang et al. (2021)
L. aquilariae	(23–)25–28(–29) × 12–16	26.9 × 14.1	1.8	100	3	Wang et al. (2019)
L. cinnamomi	(17.5–)18.7–21.1(–22.4) × (11.5–)12.7–14.1(–15.5)	19.9 × 13.4	1.5	106	3–4	Jiang et al. (2018)
L. citricola	(20–)22–27(–31) × (10.9–)12–17(–19)	24.5 × 15.4	1.6	125	3–4	Abdollahzadeh et al. (2010)
L. guilinensis	**(23–)28–31(–33.5) × (13.5–)15–16.5(–17)**	**29.6 × 15.7**	**1.9**	**75**	**2–5**	**This study**
L. huangyanensis	**(21–)28–32.5(–34) × (13–)14–16(–17)**	**30.1 × 15**	**2**	**82**	**3–4**	**This study**
L. linhaiensis	**(24.5–)27–30(–32) × (12.5–)13.5–15(–16)**	**28.5 × 14.2**	**2**	**80**	**2–6**	**This study**
L. microconidia	(18–)19–22(–23) × 10–15	20.8 × 13.2	1.5	90	3	Wang et al. (2019)
L. ponkanicola	**(16–)23.5–27.5(–28.5) × (11–)13–14.5(–15.5)**	**25.4 × 13.7**	**1.9**	**87**	**2–5**	**This study**
Sphaeropsis citrigena	(27–)28–33(–34) × (14.5–)15–18.5(–19)	30.5 × 16.8	1.8	25	3–5	Phillips et al. (2008)
S. linhaiensis	**(26.5–)28.5–35(–38) × (11.5–)14–18(–19.5)**	**31.6 × 15.9**	**2**	**27**	**1–5**	**This study**

[1] Isolates and measurements in **bold** were examined in this study.
[2] Minimum – (average – standard deviation) – (average + standard deviation) – maximum or minimum – maximum, L × W = length × width.
[3] L × W = average length × average width.
[4] L/W = average length/average width.
[5] Maximum.
[6] Maximum or minimum – maximum.

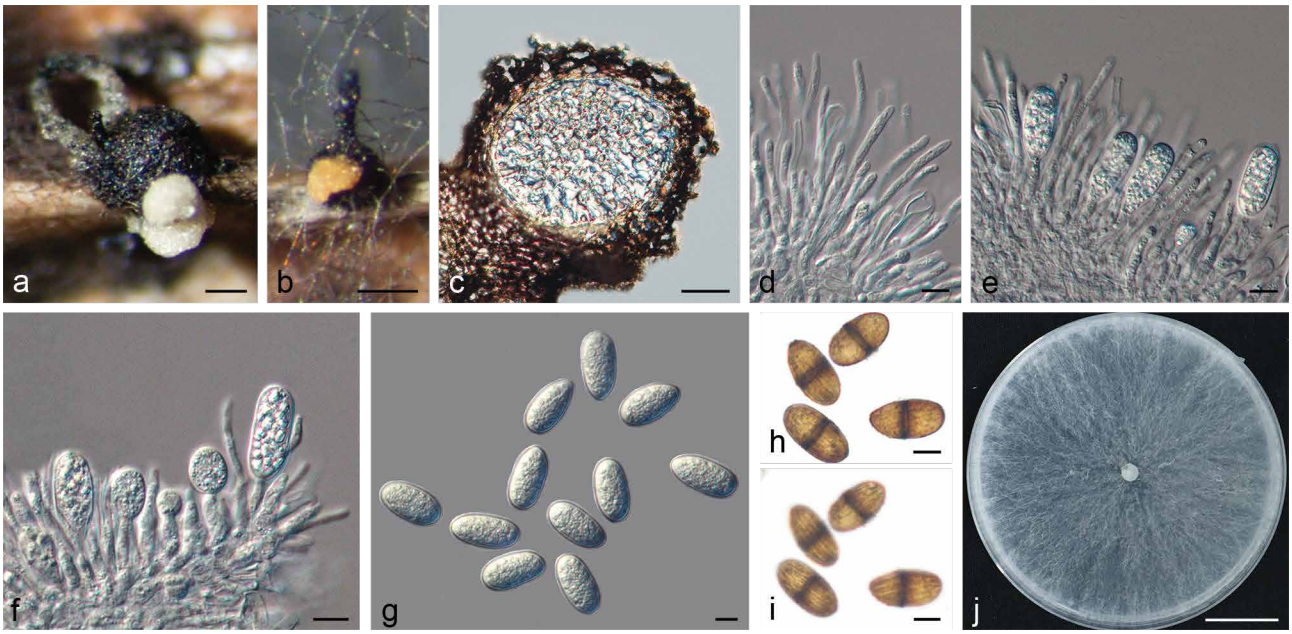


Fig. 10 *Lasiodiplodia guilinensis*. a–b. Pale yellow to saffron yellow conidial mass released from conidiomata formed on PNA; c. section view of conidioma; d. paraphyses; e–f. conidia developing on conidiogenous cells between paraphyses; g. hyaline, aseptate conidia; h–i. dark-brown, 1-septate conidia at two different focal planes to show the longitudinal striations; j. colony growing on PDA after 2 d. — Scale bars: a–b = 200 µm; c = 50 µm; d–i = 10 µm; j = 2 cm.

after 2 d in the dark at the optimum temperature of 25–30 °C. No growth was observed at 5 °C. After 2 d, colonies at 10 °C, 15 °C, 20 °C, 35 °C and 40 °C reach 11 mm, 32 mm, 64 mm, 72 mm and 12 mm, respectively.

Additional material examined. China, Zhejiang Province, Yongquan Town, from the branch of *C. unshiu*, 26 Sept. 2017, *H.Y. Li*, conidiomata induced on PNA (ZJUE H-0059, culture CGMCC 3.20379 = BE59).

Notes — Phylogenetically, *L. guilinensis* is closely related to *L. aquilariae* and *L. citricola*, but morphologically it can be separated from these species based on average conidial dimensions and length of its paraphyses. Conidia of *L. guilinensis* (av. 29.6 × 15.7; L/W = 1.9) are larger than those of *L. aquilariae* (av. 26.9 × 14.1; L/W = 1.8) (Wang et al. 2019) and *L. citricola* (av. 24.5 × 15.4; L/W = 1.6) (Abdollahzadeh et al. 2010). In terms of paraphyses, those of *L. guilinensis* (up to 75 µm long) are shorter than *L. aquilariae* (up to 100 µm long) (Wang et al. 2019) and *L. citricola* (up to 125 µm long) (Abdollahzadeh et al. 2010) (Table 4). Furthermore, *L. guilinensis* differs from these species by nucleotide differences in ITS (*L. aquilariae*: 6 bp, *L. citricola*: 3 bp), *tef1* (*L. aquilariae*: 16 bp, *L. citricola*: 14 bp), *tub2* (*L. citricola*: 3 bp) and *rpb2* loci (*L. aquilariae*: 3 bp).

Lasiodiplodia huangyanensis X.E. Xiao, P.W. Crous & H.Y. Li, *sp. nov.* — MycoBank MB 840683; Fig. 11

Etymology. Referring to the district, Huangyan, where it was collected.

Typus. China, Zhejiang Province, Huangyan District, from a twig of *C. reticulata* cv. Succosa, 22 Jan. 2019, *X.E. Xiao & Q.B. Huang*, conidiomata induced on PNA (holotype ZJUE H-0033, culture ex-type CGMCC 3.20380 = BE33).

Sexual morph unknown. *Conidiomata* stromatic, formed on PNA within 2–4 wk, superficial or semi-immersed, dark brown to black, up to 1.5 mm diam, solitary or aggregated, unilocular, covered by dense mycelium, globose, thick-walled, often releasing in pale yellow to saffron yellow conidial tendrils or mass. *Paraphyses* hyaline, cylindrical, septate, unbranched, ends rounded, up to 82 µm long, 3–4 µm wide, formed among conidiogenous cells. *Conidiophores* absent. *Conidiogenous cells* holoblastic, hyaline, smooth, thin-walled, cylindrical, 8–35 × 3.5–7 µm. *Conidia* initially hyaline, aseptate, ellipsoid to ovoid, thin-walled with granular content, rounded at apex, base round or truncate, becoming dark brown, 1-septate with longitudinal striations, (21–)28–32.5(–34) × (13–)14–16(–17) µm (av. = 30.1 × 15 µm, n = 100; L/W ratio = 2) (Table 4).

Culture characteristics — Colonies on PDA with moderately dense aerial mycelium, initially white to smoke grey, turning grey olivaceous on the surface and greenish grey in reverse, becoming dark slate-blue with age. Colonies cover the 90 mm plates after 2 d in the dark at the optimum temperature of 25–30 °C. No growth was observed at 5 °C. After 2 d, colonies at 10 °C, 15 °C, 20 °C, 35 °C and 40 °C reach 10 mm, 24 mm, 53 mm, 28 mm and 12 mm, respectively.

Additional material examined. China, Zhejiang Province, Linhai City, from the branch of *C. unshiu*, 13 Dec. 2018, *W.L. Li*, conidiomata induced on PNA (ZJUE H-0050, culture CGMCC 3.20381 = BE50).

Notes — Phylogenetically, *L. huangyanensis* is closely related to *L. cinnamomi* and *L. ponkanicola*. Morphologically, however, it can be distinguished based on the average conidial dimensions and length of its paraphyses. Conidia of *L. huangyanensis* (av. 30.1 × 15; L/W = 2) are larger than *L. cinnamomi* (av. 19.9 × 13.4; L/W = 1.5) (Jiang et al. 2018) and *L. ponkanicola* (av. 25.4 × 13.7; L/W = 1.9) (this study). Moreover, the paraphyses of *L. huangyanensis* (up to 82 µm long) are shorter than those of *L. cinnamomi* (up to 106 µm long) (Jiang et al. 2018) and *L. ponkanicola* (up to 87 µm long) (this study) (Table 4). Furthermore, *L. huangyanensis* differs from these species by nucleotide differences in ITS (*L. ponkanicola*: 3 bp), *tef1* (*L. cinnamomi*: 7 bp, *L. ponkanicola*: 10 bp), *tub2* (*L. ponkanicola*: 3 bp) and *rpb2* loci (*L. cinnamomi*: 9 bp).

Lasiodiplodia linhaiensis X.E. Xiao, P.W. Crous & H.Y. Li, *sp. nov.* — MycoBank MB 840684; Fig. 12

Etymology. Referring to the city, Linhai, where it was collected.

Typus. China, Zhejiang Province, Linhai City, from the branch of *C. unshiu*, 14 Dec. 2018, *W.L. Li*, conidiomata induced on PNA (holotype ZJUE H-0051, culture ex-type CGMCC 3.20386 = BE51).

Sexual morph unknown. *Conidiomata* stromatic, produced on PNA within 2–4 wk, superficial or semi-immersed, dark brown to black, up to 950 µm diam, solitary or aggregated, unilocular,

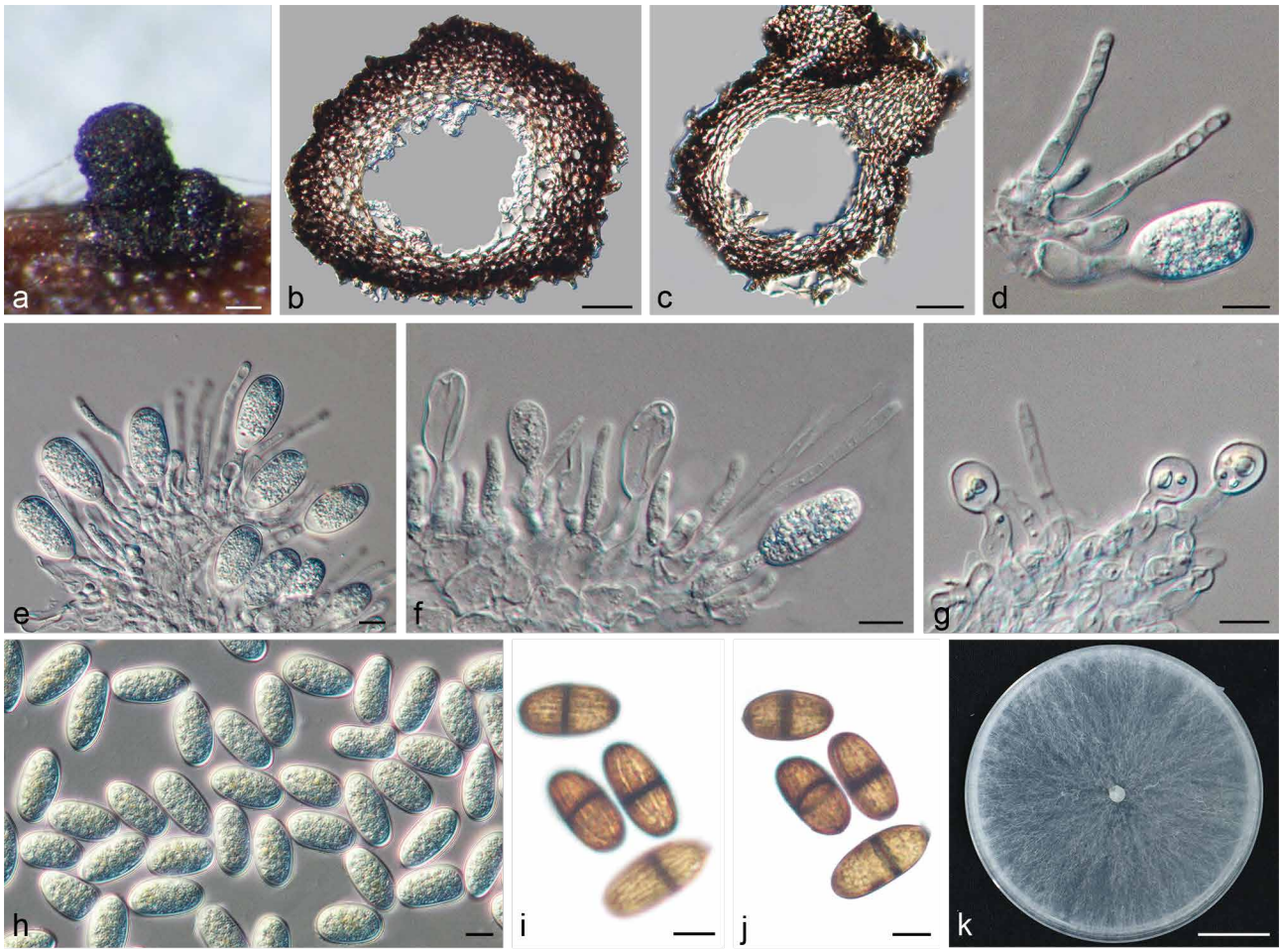


Fig. 11 *Lasiodiplodia huangyanensis*. a. Conidioma formed on PNA; b–c. section view of conidiomata; d–g. conidia developing on conidiogenous cells between paraphyses; h. hyaline, aseptate conidia; i–j. dark-brown, 1-septate conidia at two different focal planes to show the longitudinal striations; k. colony growing on PDA after 2 d. — Scale bars: a = 200 µm; b–c = 50 µm; d–j = 10 µm; k = 2.1 cm.

covered by dense mycelium, globose, thick-walled, often releasing in pale-yellow to saffron-yellow conidial tendrils or mass. *Paraphyses* hyaline, cylindrical, septate, unbranched, ends rounded, up to 80 µm long, 2–6 µm wide, formed among conidiogenous cells. *Conidiophores* absent. *Conidiogenous cells* holoblastic, hyaline, smooth, thin-walled, cylindrical, 7.5–22.5 × 3–5.5 µm. *Conidia* initially hyaline, aseptate, ellipsoid to ovoid, thin-walled with granular content, rounded at apex, base round or truncate, becoming dark brown, 1-septate with longitudinal striations, (24.5–)27–30(–32) × (12.5–)13.5–15(–16) µm (av. = 28.5 × 14.2 µm, n = 100; L/W ratio = 2) (Table 4).

Culture characteristics — Colonies on PDA with slightly dense aerial mycelium, initially white to smoke grey, turning grey olivaceous on the surface and greenish grey in reverse, becoming dark slate-blue with age. Colonies cover the 90 mm plates after 2 d in the dark at the optimum temperature of 25–30 °C. No growth was observed at 5 °C. After 2 d, colonies at 10 °C, 15 °C, 20 °C, 35 °C and 40 °C reach 12 mm, 18 mm, 42 mm, 22 mm and 11 mm, respectively. Isolates produced a pink pigment in PDA cultures at 35 °C.

Additional materials examined. China, Guangxi Province, Guilin City, from the dieback of *C. sinensis* cv. Valencia, 26 Mar. 2019, *H.Y. Li & X.E. Xiao*, conidiomata induced on PNA (ZJUE H-0028, culture CGMCC 3.20383 = BE 28); Zhejiang Province, Taizhou City, from the branch of *C. reticulata* cv. Succosa, 22 Jan. 2019, *X.E. Xiao & Q.B. Huang*, conidiomata induced on PNA (ZJUE H-0034, culture CGMCC 3.20384 = BE34); Zhejiang Province, Quzhou City, from the trunk of *C. reticulata* vs Ponkan, 23 Mar. 2018, *H.K. Wang & X.E. Xiao*, conidiomata induced on PNA (ZJUE H-0040, culture CGMCC3.20385 = BE40).

Notes — Phylogenetically, *L. linhaiensis* is closely related to *L. acaciae*, but can be separated from that species based on the length of its paraphyses. Paraphyses of *L. linhaiensis* (up to 80 µm long) are longer than those of *L. acaciae* (up to 69 µm long) (Zhang et al. 2021) (Table 4). Moreover, *L. linhaiensis* differs from *L. acaciae* by nucleotide differences in ITS (1 bp), *tef1* (5 bp) and *rpb2* loci (4 bp).

Lasiodiplodia ponkanicola X.E. Xiao, P.W. Crous & H.Y. Li, *sp. nov.* — MycoBank MB 840685; Fig. 13

Etymology. Referring to the host variety (Ponkan) from which the fungus was isolated.

Typus. China, Zhejiang Province, Quzhou City, from the trunk of *C. reticulata* cv. Ponkan, 23 Mar. 2018, *H.K. Wang & X.E. Xiao*, conidiomata induced on PNA (holotype ZJUE H-0044, culture ex-type CGMCC 3.20388 = BE44).

Sexual morph unknown. *Conidiomata* stromatic, produced on PNA within 2–4 wk, superficial or semi-immersed, dark brown to black, up to 1 mm diam, solitary or aggregated, unilocular, covered by mycelium, globose, thick-walled, often releasing pale yellow to saffron yellow conidial tendrils or mass. *Paraphyses* hyaline, cylindrical, septate, not branched, ends rounded, up to 87 µm long, 2–5 µm wide, formed among conidiogenous cells. *Conidiophores* absent. *Conidiogenous cells* holoblastic, hyaline, smooth, thin-walled, cylindrical, 8.5–40 × 2.5–9 µm. *Conidia* initially hyaline, aseptate, ellipsoid to ovoid, thin-walled with granular content, rounded at apex, base round or truncate, becoming pigmented, 1-septate with longitudinal striations, (16–)23.5–27.5(–28.5) × (11)–13–14.5(–15.5) µm (av. = 25.4 × 13.7 µm, n = 100; L/W ratio = 1.9) (Table 4).

Culture characteristics — Colonies on PDA with moderately dense aerial mycelium, initially white to smoke grey, turning grey

Fig. 12 *Lasiodiplodia linhaiensis*. a. Pale yellow conidial mass released from conidioma formed on PNA; b. section view of conidioma; c–e. conidia developing on conidiogenous; f. hyaline, aseptate conidia; g–h. dark-brown, 1-septate conidia at two different focal planes to show the longitudinal striations; i. colony growing on PDA after 2 d. — Scale bars: a = 200 µm; b = 50 µm; c–h = 10 µm; i = 1.8 cm.

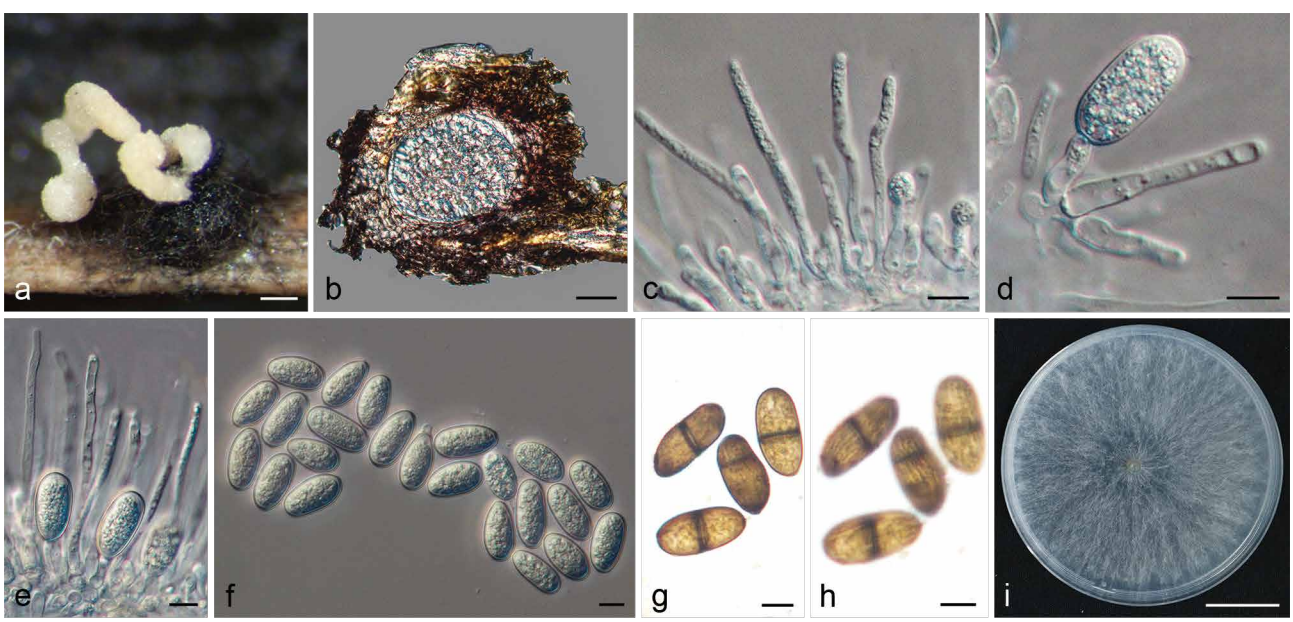


Fig. 13 *Lasiodiplodia ponkanicola*. a. Pale yellow conidial mass oozing from conidioma formed on PNA; b. section view of conidioma; c–e. conidia developing on conidiogenous; f. hyaline, aseptate conidia; g–h. dark-brown, 1-septate conidia at two different focal planes to show the longitudinal striations; i. colony growing on PDA after 7 d. — Scale bars: a = 200 µm; b = 50 µm; c–h = 10 µm; i = 2.3 cm.

olivaceous on the surface and greenish grey in reverse, becoming dark slate-blue with age. Colonies cover the 90 mm plates after 2 d in the dark at the optimum temperature of 25–30 °C. No growth was observed at 5 °C. After 2 d, colonies at 10 °C, 15 °C, 20 °C, 35 °C and 40 °C reach 9 mm, 19 mm, 43 mm, 71 mm and 26 mm, respectively.

Notes — Phylogenetically, *L. ponkanicola* is closely related to *L. aquilariae* (based on *tef1*), *L. citricola* (based on ITS and *tub2*), *L. cinnamomic* and *L. huangyanensis* (based on ITS/*tef1*/*tub2*/*rpb2*), but morphologically they can be separated on their average conidial dimensions and length of their paraphyses. Conidia of *L. ponkanicola* (av. 25.4 × 13.7; L/W = 1.9) are longer than *L. cinnamomi* (av. 19.9 × 13.4; L/W = 1.5) (Jiang et al. 2018) and *L. citricola* (av. 24.5 × 15.4; L/W = 1.6) (Abdollahzadeh et al. 2010), but shorter and narrower than *L. aquilariae* (av. 26.9 × 14.1; L/W = 1.8) (Wang et al. 2019) and *L. huangyanensis* (av. 30.1 × 15; L/W = 2) (this study). Moreover, the paraphyses of *L. ponkanicola* (up to 87 µm long) are longer than *L. huangyanensis* (up to 82 µm long) (this study)

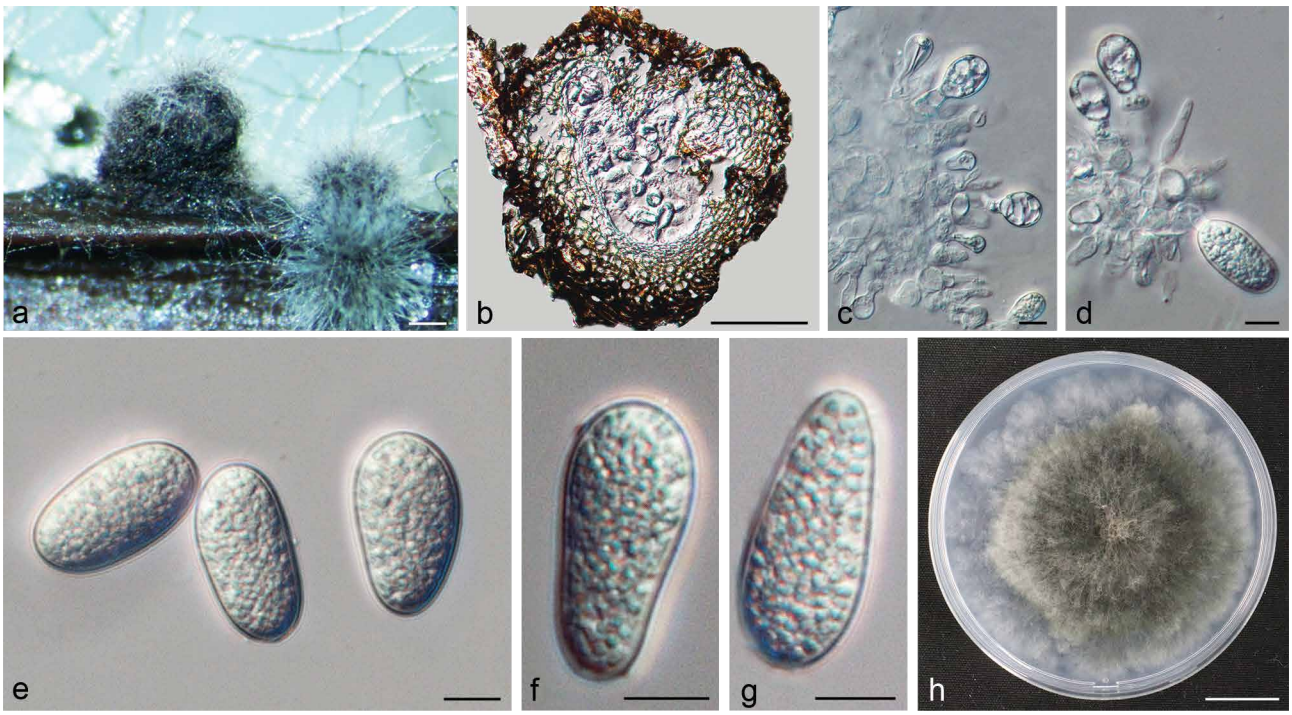


Fig. 14 *Sphaeropsis linhaiensis*. a. Conidiomata formed on PNA; b. section view of conidioma; c–d. conidia developing on conidiogenous cells; e–g. conidia; h. colony growing on PDA after 7 d. — Scale bars: a = 200 µm; b = 40 µm; c–g = 10 µm; h = 1.9 cm.

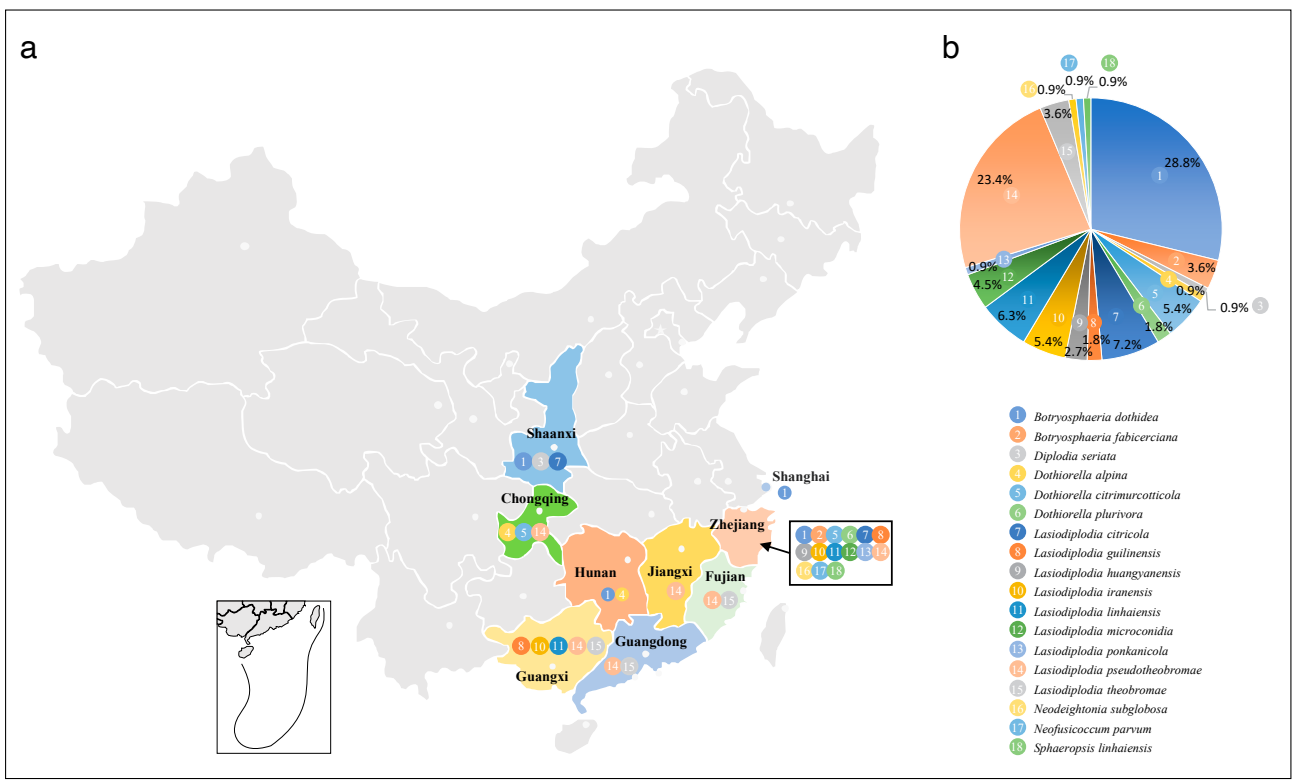


Fig. 15 The prevalence of *Botryosphaeriaceae* species isolated from citrus. a. Distribution of *Botryosphaeriaceae* species in China; b. overall isolation rate (%) of *Botryosphaeriaceae* species. Different species are represented by numbers with different colours.

but shorter than *L. aquilariae* (up to 100 µm long) (Wang et al. 2019), *L. cinnamomi* (up to 106 µm long) (Jiang et al. 2018) and *L. citricola* (up to 125 µm long) (Abdollahzadeh et al. 2010) (Table 4). Furthermore, *L. ponkanicola* differs from these species by nucleotide differences in ITS (*L. aquilariae*: 3 bp, *L. cinnamomi*: 3 bp, *L. huangyanensis*: 3 bp), *tef1* (*L. aquilariae*: 2 bp, *L. cinnamomi*: 6 bp, *L. citricola*: 7 bp, *L. huangyanensis*: 10 bp), *tub2* (*L. cinnamomi*: 3 bp, *L. huangyanensis*: 3 bp) and *rpb2* loci (*L. aquilariae*: 15 bp, *L. cinnamomi*: 9 bp, *L. citricola*: 15 bp). In view of the fact that isolate BE44 clustered with different species at different loci, it was considered that it may be a hybrid, which requires further research.

Sphaeropsis linhaiensis X.E. Xiao, P.W. Crous & H.Y. Li, *sp. nov.* — MycoBank MB 840686; Fig. 14

Etymology. Named after the Linhai City where it was isolated for the first time.

Typus. China, Zhejiang Province, Linhai City, from a twig of *C. unshiu*, 2 June 2018, *H.Y. Li*, conidiomata induced on PNA (holotype ZJUE H-0018, culture ex-type CGMCC 3.20382 = BE18).

Sexual morph unknown. *Conidiomata* pycnidial, produced on PNA within 2–4 wk, dark brown to black, unilocular, up to 880 µm diam, immersed in the needle tissue, globose to subglobose, ostiolate, wall composed of several layers of dark brown *textura angularis*. *Paraphyses* hyaline, aseptate, up to 27 µm long, 1–5 µm wide. *Conidiogenous cells* hyaline, discrete, proliferating internally to form periclinal thickenings, 4.5–11 × 3–8 µm. Poor sporulation, *conidia* hyaline, aseptate, guttulate, oval to broadly ellipsoid, apex obtuse, base obtuse or truncate, moderately thick-walled, (26.5–)28.5–35(–38) × (11.5–)14–18(–19.5) µm (av. = 31.6 × 15.9 µm, n = 30; L/W ratio = 2) (Table 4).

Culture characteristics — Colonies on PDA initially white, turning olivaceous grey gradually on the surface and leaden grey at the reverse. Colonies cover the 90 mm plates after 6 d at the optimum temperature of 25 °C. No growth was observed at 5 °C, 35 °C and 40 °C. After 6 d, colonies at 10 °C, 15 °C, 20 °C and 30 °C reach 31 mm, 64 mm, 80 mm and 19 mm, respectively.

Notes — Phylogenetically, *S. linhaiensis* is closely related to *S. citrigena*, but can be distinguished from *S. citrigena* based on its average conidial dimensions. Conidia of *S. linhaiensis* (av. 31.6 × 15.9; L/W = 2) are longer than those of *S. citrigena* (av. 30.5 × 16.8; L/W = 1.8) (Phillips et al. 2008). Moreover, *S. linhaiensis* differs from *S. citrigena* by nucleotide differences in ITS (6 bp), *tef1* (10 bp) and *tub2* (7 bp).

Prevalence of Botryosphaeriaceae species

In total, 18 species of *Botryosphaeriaceae* were identified from citrus branch diseases in the Chongqing, Fujian, Guangdong, Guangxi, Hunan, Jiangxi, Shaanxi, Shanghai and Zhejiang provinces of China (Fig. 15a). These species include *Botryosphaeria dothidea* (32 isolates, 28.8 %), *B. fabicerciana* (4 isolates, 3.6 %), *Diplodia seriata* (1 isolate, 0.9 %), *Dothiorella alpina* (1 isolate, 0.9 %), *Do. citrimurcotticola* (6 isolates, 5.4 %), *Do. plurivora* (2 isolates, 1.8 %), *Lasiodiplodia citricola* (8 isolates, 7.2 %), *L. guilinensis* (2 isolates, 1.8 %), *L. huangyanensis* (3 isolates, 2.7 %), *L. iraniensis* (6 isolates, 5.4 %), *L. linhaiensis* (7 isolates, 6.3 %), *L. microconidia* (5 isolates, 4.5 %), *L. ponkanicola* (1 isolate, 0.9 %), *L. pseudotheobromae* (26 isolates, 23.4 %), *L. theobromae* (4 isolates, 3.6 %), *Neodeightonia subglobosa* (1 isolate, 0.9 %), *Neofusicoccum parvum* (1 isolate, 0.9 %) and *Sphaeropsis linhaiensis* (1 isolate, 0.9 %) (Fig. 15b). Of these 18 species, *B. dothidea* (28.8 %) was most commonly isolated, followed by *L. pseudotheobromae* (23.4 %), *L. citricola* (8 isolates, 7.2 %) and *L. linhaiensis* (7 isolates, 6.3 %) (Fig. 15a). In terms of the source of isolates, Zhejiang Province has the most isolates and the most diversity in species, most likely because of the more intensive sampling in this province (Fig. S8). In addition, *L. pseudotheobromae* was the most widely distributed species found in six provinces, including Chongqing, Fujian, Guangdong, Guangxi, Shaanxi and Zhejiang (Fig. 15b, S8).

Pathogenicity tests

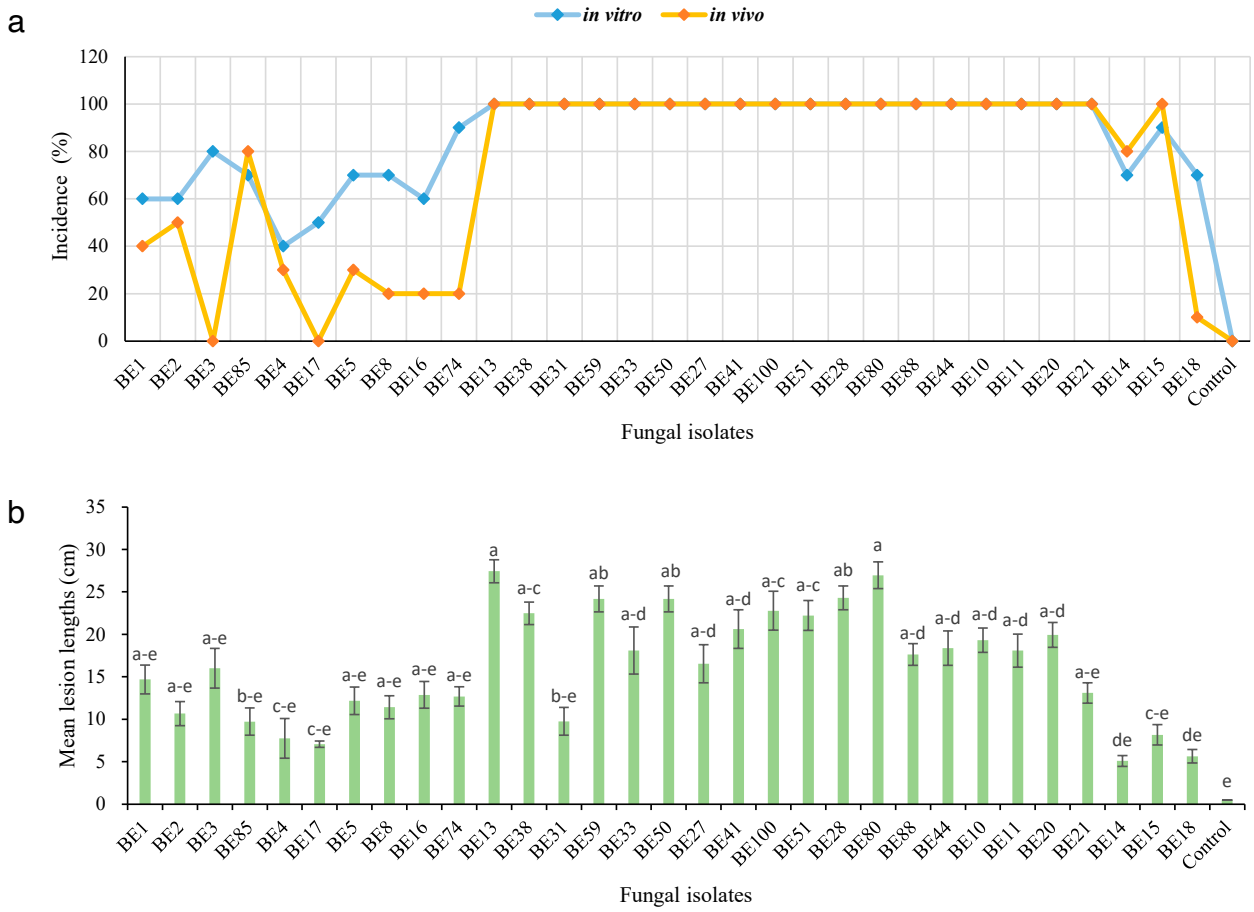
For *in vitro* inoculation, all 31 isolates inoculated were pathogenic to *C. reticulata* shoots with visible lesions. No lesions were produced on the shoots inoculated with PDA plugs (Fig. S9). Specifically, isolates of *Lasiodiplodia* spp. and *Ne. parvum* caused symptoms on all inoculated shoots (with incidence of 100 %), the isolates of *D. seriata* and *Do. alpina* caused symptoms on less than 60 % of the shoots, and the remaining isolates caused symptoms on shoots ranging from 60 to 100 % (Fig. 16a). Most isolates of *Lasiodiplodia* produced relatively longer lesions (mean lesion length > 15 cm) than the isolates from the other six genera. In contrast, isolates in *N. subglobosa* (5.09 cm), *S. linhaiensis* (5.64 cm), *Do. alpina* (7.06 cm) and *D. seriata* (7.75 cm) produced the shortest mean lesion lengths (Fig. 16b). In consideration of both disease incidence and lesion length, we concluded that species of *Lasiodiplodia* were more virulent to citrus than the other genera encountered.

For *in vivo* inoculation, all shoots of Cocktail grapefruit inoculated with the isolates listed in Fig. 16 produced lesions after 15 d inoculation, except for the isolates BE3 (*B. fabicerciana*) and BE17 (*Do. alpina*). Consistent with the results of *in vitro* inoculation, 100 % of the shoots inoculated with isolates of *Lasiodiplodia* spp. produced symptoms. The symptoms were similar to those observed in the field (Fig. S10a–g). However, the remaining isolates caused symptoms on less than 60 % of the shoots, except for isolates BE15 (100 %) and BE85 (80 %) (Fig. 16a). On the contrary, no necrosis symptoms were observed on the control shoots inspected 15 days after inoculation (Fig. 17h). All isolates inoculated *in vitro* and *in vivo* were re-isolated successfully from these lesions. As expected, no isolates of *Botryosphaeriaceae* were isolated from the control inoculations.

DISCUSSION

This study represents the first comprehensive characterisation of species in *Botryosphaeriaceae* isolated from citrus trees with twig and branch dieback, cankers and gummosis in China. Based on phylogenetic analyses and morphological characteristics, 18 species belonging to seven genera of *Botryosphaeriaceae* were identified. These species include *Botryosphaeria dothidea*, *B. fabicerciana*, *Diplodia seriata*, *Dothiorella alpina*, *Do. plurivora*, *Lasiodiplodia citricola*, *L. iraniensis*, *L. microconidia*, *L. pseudotheobromae*, *L. theobromae*, *Neodeightonia subglobosa*, *Neofusicoccum parvum*, and the new species described here, namely *Do. citrimurcotticola*, *L. guilinensis*, *L. huangyanensis*, *L. linhaiensis*, *L. ponkanicola* and *Sphaeropsis linhaiensis*. Of the 12 known species reported here, *B. fabicerciana*, *Do. alpina*, *L. microconidia* and *N. subglobosa* are reported on citrus in China for the first time.

Results of the pathogenicity tests indicate that all *Lasiodiplodia* species obtained in this study are pathogenic to the tested *C. reticulata* shoots *in vitro* and Cocktail grapefruit *in vivo*, with disease incidences of 100 %. Most of the remaining taxa were not as aggressive however, especially in the *in vivo* inoculation, which may be due to the higher ambient temperatures observed in the field (Zhang et al. 2016, 2021, this study). Moreover, significant differences in aggressiveness were observed among



Botryosphaeria dothidea: BE1, BE2
Botryosphaeria fabicerciana: BE3, BE85
Diplodia seriata: BE4
Dothiorella alpina: BE17
Dothiorella citrimurcotticola: BE5, BE8
Dothiorella plurivora: BE16, BE74
Lasiodiplodia citricola: BE13, BE38
Lasiodiplodia guilinensis: BE31, BE59
Lasiodiplodia huangyanensis: BE33, BE50
Lasiodiplodia iranensis: BE27, BE41, BE100
Lasiodiplodia linhaiensis: BE51, BE28
Lasiodiplodia microconidia: BE80, BE88
Lasiodiplodia ponkanicola: BE44
Lasiodiplodia pseudotheobromae: BE10, BE11
Lasiodiplodia theobromae: BE20, BE21
Neodeightonia subglobosa: BE14
Neofusicoccum parvum: BE15
Sphaeropsis linhaiensis: BE18

Fig. 16 Pathogenicity tests results of inoculated isolates in *Botryosphaeriaceae*. a. Incidences of shoots inoculated *in vitro* and *in vivo*, the blue diamond represents the incidence of shoots inoculated *in vitro*, while the orange diamond represents the incidence of shoots inoculated *in vivo*; b. mean lesion lengths on *C. reticulata* shoots inoculated *in vitro* after 8 d. Bars represent standard errors. Columns with different letters indicate significant differences according to LSD test with confidence level α = 0.05. Control: PDA plugs.

species. Most species of *Lasiodiplodia* were strongly aggressive. Conversely, *N. subglobosa*, *S. linhaiensis*, *Do. alpina* and *D. seriata* were weakly aggressive. In general, *Lasiodiplodia* was the most aggressive genus in the present study.

Botryosphaeria dothidea is the type species of *Botryosphaeria* and was considered as one of the most common and important pathogens of woody plants (Phillips et al. 2013, Marsberg et al. 2017). In this study, *B. dothidea* was the most commonly isolated species. Results of pathogenicity tests indicate that isolates of *B. dothidea* are moderately aggressive on *C. reticulata* shoots, which is similar to observations on *Pistacia vera* in California (Chen et al. 2014). *Botryosphaeria fabicerciana*, the other species of *Botryosphaeria* isolated in this study, was first reported on *Eucalyptus* and was weakly aggressive to this host (Chen et al. 2011). This is the first report of *B. fabicerciana* on citrus, and on *C. reticulata* it appeared to be mild to highly aggressive, while on Cocktail grapefruit it appeared to be weakly aggressive.

Diplodia seriata is regarded as a pathogen of citrus, causing branch canker and dieback in Algeria and the USA (Adesemoye et al. 2014, Berraf-Tebbal et al. 2020). Similarly, we isolated a single strain of *D. seriata* from *C. sinensis* with branch canker. This finding contrasts with that of Linaldeddu et al. (2015), who reported *D. seriata* to be the dominant species on *Vitis* in Italy. Pathogenicity tests indicate that *D. seriata* is less aggressive than most species of *Botryosphaeriaceae* obtained in this study.

Dothiorella gummosis refers to the occurrence of branch or trunk cankers on citrus caused by species of *Botryosphaeriaceae*. The pathogen was long believed to be *Do. gregaria*, the asexual morph of *Botryosphaeria ribis* (Adesemoye et al. 2011). However, other species that resided in the *Botryosphaeriaceae* were also found causing Dothiorella gummosis in citrus (Adesemoye et al. 2011). Therefore, the term 'Dothiorella gummosis' was no longer suitable for describing such symptoms, while 'Botryosphaeria gummosis' (Adesemoye et al. 2011) and 'Bot gummosis' (Adesemoye et al. 2014) were proposed as alternative. In this study, one undescribed *Dothiorella* species (*Do. citrimurcotticola*) and two previously reported species (*Do. alpina* and *Do. plurivora*) were isolated from branch dieback of *Citrus* spp. *Dothiorella alpina* was described from a dead tree of *Platycladus orientalis* in China (Zhang et al. 2016) and has not been reported from other hosts. Thus, this study represents the first report of this fungus on citrus. *Dothiorella plurivora* was first reported by Abdollahzadeh et al. (2014) in Iran and Spain, named for its broad host range, including twigs

of *Casuarina* sp., *Citrus* sp., *Cupressus sempervirens*, *Eucalyptus* sp., *Juglans regia*, *Malus domestica*, *Prunus armeniaca* and *Vitis vinifera*. In this study, the isolates of *Do. plurivora* were collected from *C. reticulata* and *C. unshiu* with twig dieback. To our knowledge, this is the first report of *Do. plurivora* occurring on citrus in China. Pathogenicity tests demonstrated that the three species of *Dothiorella* were pathogenic to *C. reticulata* shoots, and that *Do. alpina* was less aggressive than the other two species. However, isolates of *Do. citrimurcotticola* and *Do. plurivora* were weakly aggressive on Cocktail grapefruit shoots, with incidences lower than 50 %, while *Do. alpina* was non-pathogenic on Cocktail grapefruit shoots.

We obtained nine species of *Lasiodiplodia* from citrus diseased branches, accounting for 55.9 % of the total number of isolates, making *Lasiodiplodia* the most prevalent genus with the highest number of species encountered in this study. This finding is consistent with previous reports that *Lasiodiplodia* is common on citrus (Abdollahzadeh et al. 2010, Adesemoye et al. 2014, Coutinho et al. 2017, Guajardo et al. 2018, Bautista-Cruz et al. 2019, Berraf-Tebbal et al. 2020). Probable reasons why species of *Lasiodiplodia* are dominant on citrus include the following: Firstly, it was observed that species of *Lasiodiplodia* are fast growing, covering 90 mm plates in only 2 d, while species of other genera of *Botryosphaeriaceae* have slower growth rates. Species of *Lasiodiplodia* also proved to be more aggressive to citrus compared to other genera tested. Secondly, the optimum temperature of the other genera in this study is 25 °C, while the optimum growth temperature of *Lasiodiplodia* spp. ranges from 25 °C to 30 °C, thereby giving it an advantage over other species at higher temperatures, and this is probably the reason why *Lasiodiplodia* species are mostly found in tropical or subtropical regions, and rare in regions with temperate climates.

Lasiodiplodia pseudotheobromae can infect citrus, causing gummosis, trunk canker, and twig blight (Abdollahzadeh et al. 2010, Bautista-Cruz et al. 2019, Ahmed et al. 2020). In the present study, *L. pseudotheobromae* was the second most abundant species isolated from citrus with symptoms of gummosis, dieback and canker. Furthermore, the results of pathogenicity tests showed that *L. pseudotheobromae* is one of the most aggressive species on citrus shoots. Stem-end rot is a common and economically important postharvest disease of citrus fruits worldwide, and it is usually thought to be caused by *L. theobromae* (Zhang 2014). Sultana et al. (2018) found that *L. pseudotheobromae* was also associated with citrus stem-end rot in Bangladesh. Stem-end rot is also an important postharvest disease on citrus in China (Cai et al. 2011). Since *L. pseudotheobromae* is similar to *L. theobromae* and is common on citrus according to the isolation results obtained in this study, it is possible that several reports of *L. theobromae* could have in fact been *L. pseudotheobromae*. In summary, *L. pseudotheobromae* is widely distributed on citrus, highly aggressive and can cause stem-end rot and branch diseases. Therefore, we consider *L. pseudotheobromae* to be an important pathogen on citrus in China, urgently requiring further research to elucidate its impact on this crop.

Neodeightonia subglobosa was initially reported on dead culms of *Bambusa arundinacea* in Sierra Leone (Punithalingam 1969). Furthermore, *N. subglobosa* was also found to cause keratomycosis in human eyes (Phillips et al. 2008). There are few reports about *N. subglobosa*, and to our knowledge, this study is the first to report *N. subglobosa* associated with gummosis on citrus. However, pathogenicity tests indicated that *N. subglobosa* was only weakly aggressive on citrus.

Neofusicoccum parvum has a broad host range and distribution, and has been reported from 90 hosts across six continents and 29 countries (Sakalidis et al. 2013, Batista et al. 2021). The lack of host specificity, combined with both a sexual and an asexual cycle, and the ability to live as a latent pathogen are conducive to the infection and spread of *Ne. parvum* (Sakalidis et al. 2013). In China, *Ne. parvum* was found associated with canker and dieback on *Cupressus funebris* (Li et al. 2010), *Eucalyptus* (Chen et al. 2011), *Juglans regia* (Yu et al. 2015), *Prunus* (Li et al. 2019, Zhang et al. 2019), *Rhododendron* (Yang et al. 2015) and *Vitis heyneana* (Wu et al. 2015). In this study, we found that *Ne. parvum* also caused dieback and gummosis on *C. unshiu* in China. Based on the pathogenicity tests, *Ne. parvum* was less aggressive than most of the species in this study, but disease incidences *in vitro* and *in vivo* were high, second only to isolates in *Lasiodiplodia*.

Previous research revealed *Sphaeropsis citrigena* to occur on dead bark of citrus (Phillips et al. 2013). In the present study, another species of *Sphaeropsis*, namely *S. linhaiensis*, was found that was associated with twig dieback on *C. unshiu*. Pathogenicity tests, however, indicated that *S. linhaiensis* is weakly aggressiveness on citrus.

Branch diseases of citrus are a persistent and frequent problem in the main citrus production areas of China. Many fungal pathogens can induce branch diseases on citrus, including species within the *Botryosphaeriaceae*, *Diatrypaceae* and genera such as *Colletotrichum* and *Diaporthe* (Chinese Academy of Agricultural Sciences 1960, Tai 1979, Cai et al. 2011). Because branch diseases are complex, and symptoms may be induced by multiple pathogens at the same time, there are few effective measures to control such diseases. Previous research has shown that wounds caused by pruning, mechanical injury, frost and sunburn damage have become the entry point for *Botryosphaeriaceae* on woody hosts (Savocchia et al. 2007, Úrbez-Torres & Gubler 2009, Eskalen et al. 2013). Furthermore, fungal pathogens release the greatest number of spores during and after rainfall events (Eskalen & Gubler 2001, Amponsah et al. 2009, Eskalen et al. 2013). Therefore, to prevent and reduce the occurrence of citrus branch diseases, pruning should avoid rainy days, decaying or dead branches and twigs should be removed from orchards, wounds protected with sealant or fungicides, frost damage avoided where possible and trees protected from the sun in hot weather. Furthermore, because species of *Botryosphaeriaceae* are latent pathogens, they can become pathogenic when the trees are under stress or in weak vigour (Slippers & Wingfield 2007). Hence, the cultivation of good tree vigour is conducive to enhance disease resistance.

In conclusion, results of this study present the first detailed research of 18 species of *Botryosphaeriaceae* causing branch diseases on citrus in nine major citrus-producing provinces of China. Overall, *Lasiodiplodia* was found to be the most prevalent and aggressive genus in this study, which indicates that *Lasiodiplodia* is one of the most important genera causing citrus branch diseases. Besides, *L. pseudotheobromae* is one of the most abundant and most prevalent species, which together with its aggressiveness in branches and fruits, makes *L. pseudotheobromae* an economically important pathogen on citrus. To better prevent and control *L. pseudotheobromae*, further research is needed to study the relationship between *L. pseudotheobromae* in different regions and different citrus varieties, and its ability to cause stem-end rot. In the current study, several species were obtained as single isolates, but most of the isolates were from Zhejiang Province, so subsequent sampling needs to be expanded to investigate the prevalence of these species in other citrus-producing areas in China. Because species of *Botryosphaeriaceae* can be latent pathogens in woody host plants, and jump from hosts planted nearby (Damm et al. 2007, Slippers & Wingfield 2007, Begoude et al. 2012, James et al. 2017), collecting plant hosts adjacent to citrus is also useful for studying the diversity of the *Botryosphaeriaceae* species that could have an impact on citrus cultivation.

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Supplementary material

Fig. S1 Phylogenetic trees generated by maximum likelihood analyses based on the individual ITS, *tef1*, *tub2* and *rpb2* (a–d) sequence alignments of *Botryosphaeria*. Bootstrap support values ≥ 50 % for ML and MP are presented above branches as follows: ML/MP bootstrap values < 50 % are marked with *, and absent are marked with -. Newly generated sequences are in red and ex-type strains are in **bold**. The tree was rooted to *Neofusicoccum parvum* (CMW 9081).

Fig. S2 Phylogenetic trees generated by maximum likelihood analyses based on the individual ITS, *tef1*, *tub2* and *rpb2* (a–d) sequence alignments of *Diplodia*. Bootstrap support values ≥ 50 % for ML and MP are presented above branches as follows: ML/MP bootstrap values < 50 % are marked with *. Newly generated sequences are in red and ex-type strains are in **bold**. The tree was rooted to *Lasiodiplodia theobromae* (CBS 164. 96).

Fig. S3 Phylogenetic trees generated by maximum likelihood analyses based on the individual ITS, *tef1*, *tub2* and *rpb2* (a–d) sequence alignments of *Dothiorella*. Bootstrap support values ≥ 50 % for ML and MP are presented above branches as follows: ML/MP bootstrap values < 50 % are marked with *, and absent are marked with -. Newly generated sequences are in red and ex-type strains are in **bold**. The tree was rooted to *Neofusicoccum parva* (CMW 9081).

Fig. S4 Phylogenetic trees generated by maximum likelihood analyses based on the individual ITS, *tef1*, *tub2* and *rpb2* (a–d) sequence alignments of *Lasiodiplodia*. Bootstrap support values ≥ 50 % for ML and MP are presented above branches as follows: ML/MP bootstrap values < 50 % are marked with *, and absent are marked with -. Newly generated sequences are in red and type species are in red **bold**. The tree was rooted to *Botryosphaeria dothidea* (CMW 8000).

Fig. S5 Phylogenetic trees generated by maximum likelihood analyses based on the individual ITS, *tef1* and *tub2* (a–c) sequence alignments of *Neodeightonia*. Bootstrap support values ≥ 50 % for ML and MP are presented above branches as follows: ML/MP bootstrap values < 50 % are marked with *. Newly generated sequences are in red and ex-type strains are in **bold**. The tree was rooted to *Botryosphaeria dothidea* (CBS 115476).

Fig. S6 Phylogenetic trees generated by maximum likelihood analyses based on the individual ITS, *tef1*, *tub2* and *rpb2* (a–d) sequence alignments of *Neofusicoccum*. Bootstrap support values ≥ 50 % for ML and MP are presented above branches as follows: ML/MP bootstrap values < 50 % are marked with *. Newly generated sequences are in red and ex-type strains are in **bold**. The tree was rooted to *Dothiorella viticola* (CBS 117009).

Fig. S7 Phylogenetic trees generated by maximum likelihood analyses based on the individual ITS, *tef1*, *tub2* and *rpb2* (a–d) sequence alignments of *Sphaeropsis*. Bootstrap support values ≥ 50 % for ML and MP are presented above branches as follows: ML/MP bootstrap values < 50 % are marked with *, and absent are marked with -. Newly generated sequences are in red and ex-type strains are in **bold**. The tree was rooted to *Botryosphaeria dothidea* (CMW 8000).

Fig. S8 The distribution of *Botryosphaeriaceae* species obtained from nine provinces. Provinces are represented by different colours.

Fig. S9 Symptoms developed in the detached shoots of *C. reticulata* inoculated with isolates in *Botryosphaeriaceae* 8 d after inoculation. *B. dothidea*: BE1, BE2; *B. fabicerciana*: BE3, BE85; *D. seriata*: BE4; *Do. alpina*: BE17; *Do. citrimurcotticola*: BE5, BE8; *Do. plurivora*: BE16, BE74; *L. citricola*: BE13, BE38; *L. guilinensis*: BE31, BE59; *L. huangyanensis*: BE33, BE50; *L. iranensis*: BE27, BE41, BE100; *L. linhaiensis*: BE51, BE28; *L. microconidia*: BE80, BE88; *L. ponkanicola*: BE44; *L. pseudotheobromae*: BE10, BE11; *L. theobromae*: BE20, BE21; *N. subglobosa*: BE14; *Ne. parvum*: BE15; *S. linhaiensis*: BE18.

Fig. S10 Symptoms developed in shoots of Cocktail grapefruit plants inoculated with isolates of *Botryosphaeriaceae* 15 d after inoculation. a–b. Shoots inoculated with *B. fabicerciana* (BE85) producing gum exudate; c. gummosis caused by *Ne. parvum* (BE15); d. shoot showing symptoms of dieback and gummosis after inoculation with *L. guilinensis* (BE59); e. dieback with a large amount of gummosis caused by *L. pseudotheobromae* (BE10); f. dieback with gummosis caused by *L. huangyanensis* (BE50); g. conidiomata formed on the inoculated shoots with dieback; h. shoots inoculated with sterile PDA plugs. Red arrows indicate the inoculated positions; orange arrows indicate gum exudate; white arrow indicates a conidioma.