



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

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Key words

Botryosphaeria cankers
distribution
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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- α 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*, *Dothiella 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* $\times$ *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 the 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 fruiting 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
<i>Botryosphaeria dothidea</i>	BE1	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (<i>C. paradisi</i> × <i>C. reticulata</i>)	Twig gummosis	MT772261	MT775839	MT775849	MW884107
	BE2	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (<i>C. paradisi</i> × <i>C. reticulata</i>)	Twig gummosis	MT772262	MT775840	MT775850	MW884108
	BE60	Chenggu, Shaanxi, China	H.Y. Li & X.E. Xiao	<i>C. unshiu</i>	Trunk canker	MW862113	MW884017	MW894086	MW884109
	BE61	Changxing Island, Shanghai, China	X.E. Xiao	hybrid cv. Hongmeiren	Twig dieback	MW862116	MW884020	MW894087	MW884110
	BE62	Chunan, Zhejiang, China	H.Y. Li & X.E. Xiao	<i>C. unshiu</i>	Twig dieback	MW862110	MW884014	–	–
	BE63	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	<i>C. reticulata</i>	Twig gummosis	MW862111	MW884015	–	–
	BE64	Quzhou, Zhejiang, China	H.Y. Li & J.W. Lv	hybrid cv. Cocktail grapefruit (<i>C. paradisi</i> × <i>C. reticulata</i>)	Twig gummosis	MW862112	MW884016	–	–
	BE65	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (<i>C. paradisi</i> × <i>C. reticulata</i>)	Twig gummosis	MW862114	MW884018	–	–
	BE66	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (<i>C. paradisi</i> × <i>C. reticulata</i>)	Twig gummosis	MW862115	MW884019	–	–
	BE67	Xiangshan, Zhejiang, China	H.Y. Li	<i>C. unshiu</i>	Trunk canker	MW862117	MW884021	–	–
	BE68	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (<i>C. paradisi</i> × <i>C. reticulata</i>)	Twig gummosis	MW862118	MW884022	–	–
	BE69	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	<i>C. reticulata</i>	Twig gummosis	MW862119	MW884023	–	–
	BE72	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	<i>C. reticulata</i>	Twig dieback	MW881452	MW884024	–	–
	BE73	Chenggu, Shaanxi, China	H.Y. Li & X.E. Xiao	<i>C. unshiu</i>	Trunk gummosis	MW862120	MW884025	–	–
	BE75	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (<i>C. paradisi</i> × <i>C. reticulata</i>)	Twig gummosis	MW862121	MW884026	–	–
	BE76	Hangzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	<i>C. maxima</i>	Branch dieback	MW862122	MW884027	–	–
	BE77	Linhai, Zhejiang, China	H.Y. Li	<i>C. unshiu</i>	Branch canker	MW862123	MW884028	–	–
	BE79	Hangzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	<i>C. maxima</i>	Twig dieback	MW862124	MW884029	–	–
	BE81	Hangzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	<i>C. maxima</i>	Branch dieback	MW862125	MW884030	–	–
	BE90	Linhai, Zhejiang, China	H.Y. Li	<i>C. unshiu</i>	Twig dieback	MW862126	MW884031	–	–
	BE91	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	<i>C. reticulata</i>	Twig gummosis	MW862127	MW884032	–	–
	BE92	Shaoyang, Hunan, China	H.Y. Li & X.E. Xiao	<i>C. sinensis</i>	Twig dieback	MW862128	MW884033	–	–
	BE93	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (<i>C. paradisi</i> × <i>C. reticulata</i>)	Twig gummosis	MW862129	MW884034	–	–
	BE94	Linhai, Zhejiang, China	H.Y. Li	<i>C. unshiu</i>	Branch gummosis	MW862130	MW884035	–	–
	BE95	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	<i>C. reticulata</i>	Twig gummosis	MW862131	MW884036	–	–
	BE96	Chun'an, Zhejiang, China	H.Y. Li & X.E. Xiao	<i>C. unshiu</i>	Branch dieback	MW862132	MW884037	–	–
	BE97	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (<i>C. paradisi</i> × <i>C. reticulata</i>)	Twig gummosis	MW862133	MW884038	–	–
	BE98	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (<i>C. paradisi</i> × <i>C. reticulata</i>)	Twig gummosis	MW862134	MW884039	–	–
	BE101	Linhai, Zhejiang, China	H.Y. Li	<i>C. unshiu</i>	Twig dieback	MW862135	MW884040	–	–
	BE103	Linhai, Zhejiang, China	H.Y. Li	<i>C. unshiu</i>	Twig dieback	MW862136	MW884041	–	–
	BE105	Linhai, Zhejiang, China	H.Y. Li	<i>C. unshiu</i>	Twig dieback	MW862137	MW884042	–	–
	BE106	Linhai, Zhejiang, China	H.Y. Li	<i>C. unshiu</i>	Twig dieback	MW862138	MW884043	–	–
<i>Botryosphaeria fabierciana</i>	BE3	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (<i>C. paradisi</i> × <i>C. reticulata</i>)	Twig gummosis	MT772263	MT775841	MT775851	MW884111
	BE78	Hangzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	<i>C. maxima</i>	Twig dieback	MW862139	MW884044	MW894088	MW884112

Table 1 (cont.)

Species ^a	Isolate	Location	Collector	Host	Associated symptom	GenBank Accession no. ^b			
						ITS	tef1	tub2	rpb2
<i>Botryosphaeria fabierciana</i> (cont.)	BE5	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (C. paradisi × C. reticulata)	Twig gummosis	MW862140	MW884045	MW884089	MW884113
	BE6	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (C. paradisi × C. reticulata)	Twig gummosis	MW862141	MW884046	MW884090	MW884114
<i>Diplodia seriata</i>	BE4	Chenggu, Shaanxi, China	H.Y. Li & X.E. Xiao	C. unshiu	Branch canker	MW862142	MW884047	MW884091	MW884115
<i>Dothiorella alpina</i>	BE17	Shimen, Hunan, China	H.Y. Li & Y.T. Zeng	C. unshiu	Twig dieback	MW862143	MW884048	MW884092	MW884116
	BE5 = CGMCC3.20392	Linhai, Zhejiang, China	H.Y. Li	C. unshiu	Twig dieback	MW880663	MW884166	MW884195	MW884140
<i>Do. citrimurcoticola</i>	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
<i>Do. plurivora</i>	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
<i>Lasiodiplodia citricola</i>	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
<i>L. guilinensis</i>	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
	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
	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
<i>L. iranensis</i>	BE111	Huangyan, Zhejiang, China	H.Y. Li	C. unshiu	Twig dieback	MW880676	MW884179	MW884208	MW884153
	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
	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
<i>L. linhaiensis</i>	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
<i>L. linhaiensis</i>	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
<i>L. microconidia</i>	BE32	Huangyan, Zhejiang, China	H.K. Wang & X.E. Xiao	<i>C. reticulata</i>	Branch canker	MW880668	MW884171	MW884200	MW884145
	BE35	Huangyan, Zhejiang, China	X.E. Xiao & Q.B. Huang	<i>C. reticulata</i>	Branch canker	MW880669	MW884172	MW884201	MW884146
	BE80	Chun'an, Zhejiang, China	H.Y. Li & X.E. Xiao	<i>C. grandis</i>	Trunk canker	MW880670	MW884173	MW884202	MW884147
	BE87	Chun'an, Zhejiang, China	H.Y. Li & X.E. Xiao	<i>C. unshiu</i>	Trunk canker	MW880671	MW884174	MW884203	MW884148
	BE88	Chun'an, Zhejiang, China	H.Y. Li & X.E. Xiao	<i>C. grandis</i>	Twig dieback	MW880667	MW884170	MW884199	MW884144
	BE44 * = CGMCC3.20388	Quzhou, Zhejiang, China	H.K. Wang & X.E. Xiao	<i>C. reticulata</i>	Trunk canker	MW880685	MW884188	MW884214	MW884159
	BE10	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	<i>C. reticulata</i>	Twig gummosis	MT772264	MT775842	MT775852	MW884127
	BE11	Linhai, Zhejiang, China	H.Y. Li	<i>C. unshiu</i>	Twig dieback	MT772265	MT775843	MT775853	MW884128
<i>L. pseudotheobromae</i>	BE12	Fuzhou, Jiangxi, China	H.Y. Li & X.E. Xiao	<i>C. reticulata</i>	Twig dieback	MT772266	MT775844	MT775854	MW884129
	BE19	Guangzhou, Guangdong, China	H.Y. Li	<i>C. reticulata</i>	Twig dieback	MW862154	MW884059	MW884101	MW884130
	BE22	Gullin, Guangxi, China	H.Y. Li & X.E. Xiao	<i>C. sinensis</i>	Twig dieback	MW862158	MW884063	MW884102	MW884131
	BE23	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (<i>C. paradisi</i> × <i>C. reticulata</i>)	Twig gummosis	MW862160	MW884065	MW884103	MW884132
	BE24	Sihui, Guangdong, China	H.Y. Li	<i>C. reticulata</i>	Twig dieback	MW862155	MW884060	–	–
	BE26	Sihui, Guangdong, China	H.Y. Li	<i>C. reticulata</i>	Twig dieback	MW862156	MW884061	–	–
	BE29	Sihui, Guangdong, China	H.Y. Li	<i>C. reticulata</i>	Twig dieback	MW862157	MW884062	–	–
	BE37	Gullin, Guangxi, China	H.Y. Li & X.E. Xiao	<i>C. sinensis</i>	Twig dieback	MW862159	MW884064	–	–
	BE39	Quzhou, Zhejiang, China	H.Y. Li & X.E. Xiao	hybrid cv. Cocktail grapefruit (<i>C. paradisi</i> × <i>C. reticulata</i>)	Twig gummosis	MW862161	MW884066	–	–
	BE46	Linhai, Zhejiang, China	W.L. Li	<i>C. unshiu</i>	Trunk canker	MW862162	MW884067	–	–
	BE47	Linhai, Zhejiang, China	W.L. Li	<i>C. unshiu</i>	Trunk canker	MW862163	MW884068	–	–
	BE48	Linhai, Zhejiang, China	W.L. Li	<i>C. unshiu</i>	Trunk canker	MW862164	MW884069	–	–
	BE53	Linhai, Zhejiang, China	W.L. Li	<i>C. unshiu</i>	Trunk canker	MW862165	MW884070	–	–
	BE54	Linhai, Zhejiang, China	W.L. Li	<i>C. unshiu</i>	Trunk canker	MW862166	MW884071	–	–
	BE55	Linhai, Zhejiang, China	W.L. Li	<i>C. unshiu</i>	Trunk canker	MW862167	MW884072	–	–
	BE56	Linhai, Zhejiang, China	W.L. Li	<i>C. unshiu</i>	Trunk canker	MW862168	MW884073	–	–
	BE57	Linhai, Zhejiang, China	W.L. Li	<i>C. unshiu</i>	Trunk canker	MW862169	MW884074	–	–
	BE58	Linhai, Zhejiang, China	W.L. Li	<i>C. unshiu</i>	Trunk canker	MW862170	MW884075	–	–
	BE70	Wanzhou, Chongqing, China	H.Y. Li & X.E. Xiao	<i>C. limon</i>	Twig dieback	MW862171	MW884076	–	–
<i>L. theobromae</i>	BE82	Fuzhou, Jiangxi, China	H.Y. Li & X.E. Xiao	<i>C. reticulata</i>	Twig dieback	MW862172	MW884077	–	–
	BE84	Chun'an, Zhejiang, China	H.Y. Li & X.E. Xiao	<i>C. unshiu</i>	Trunk canker	MW862173	MW884078	–	–
	BE107	Yongchun, Fujian, China	X.E. Xiao	<i>C. reticulata</i>	Twig dieback	MW862174	MW884079	–	–
	BE109	Yongchun, Fujian, China	X.E. Xiao	<i>C. sinensis</i>	Twig dieback	MW862175	MW884080	–	–
	BE110	Yongchun, Fujian, China	X.E. Xiao	<i>C. reticulata</i>	Twig dieback	MW862176	MW884081	–	–
	BE20	Sihui, Guangdong, China	H.Y. Li	<i>C. reticulata</i>	Twig dieback	MW862177	MW884082	MW884104	MW884133
	BE21	Sihui, Guangdong, China	H.Y. Li	<i>C. reticulata</i>	Twig dieback	MW862178	MW884083	MW884105	MW884134
	BE25	Gullin, Guangxi, China	H.Y. Li & X.E. Xiao	<i>C. reticulata</i>	Twig dieback	MW862179	MW884084	MW884106	MW884135
	BE108	Yongchun, Fujian, China	X.E. Xiao	<i>C. sinensis</i>	Twig dieback	MW862180	MW884085	–	–
	BE14	Xiangshan, Zhejiang, China	H.Y. Li & B. Liu	<i>C. unshiu</i>	Trunk gummosis	MT772268	MT775846	MT775856	MW884136
<i>Neodeightonia subglobosa</i>	BE15	Xiangshan, Zhejiang, China	X.E. Xiao	<i>C. unshiu</i>	Branch gummosis	MT772269	MT775847	MT775857	MW884137
<i>Neofusicoccum parvum</i>	BE18 * = CGMCC3.20382	Linhai, Zhejiang, China	H.Y. Li	<i>C. unshiu</i>	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- α ; *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- α 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 rRPB2-7cR (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 μ L mixture consisted of 12.5 μ L of 2 $\times$ Taq Master Mix (Dye Plus) (Vazyme), 9.5 μ L ddH₂O, 1 μ L of each forward and reverse primer, and 1 μ L 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 NCBI 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>) (Kato 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 μ m 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* $\times$ *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
<i>Botryosphaeria agaves</i>	CBS 133992 = MFLUCC11-0125 * MFLUCC 10-0051	Agave sp. Agave sp.	Thailand Thailand	R. Phookamsak P. Chormunti	JX646791 JX646790	JX646856 JX646855	JX646841 JX646840	– –
<i>Botryosphaeria corticis</i>	CBS 119047 * ATCC 29297	<i>Vaccinium corymbosum</i> <i>Vaccinium</i> sp.	USA USA	P.V. Oudemans R.D. Millholland	DQ299245 DQ299247	EU017539 EU673291	– EU673108	– –
<i>Botryosphaeria dothidea</i>	CBS 115476 = CMW 8000 * CBS 110302 CBS 145971 = CPC 29048	<i>Prunus</i> sp. <i>Vitis vinifera</i> <i>Grevillea</i> sp.	Switzerland Portugal Australia	B. Slippers A.J.L. Phillips P.W. Crous	AY236949 AY573218 MT592034	AY236898 AY573218 MT592034	EY236927 EU673106 MT592470	EU339577 – –
<i>Botryosphaeria fabierciana</i>	CBS 127194 = CMW 27094 * CERC 2948	<i>Eucalyptus</i> sp. <i>Eucalyptus</i> sp.	China China	M.J. Wingfield M.J. Wingfield	HQ332197 KX277983	HQ332213 KX278088	KF779068 KX278193	MF410137 MF410132
<i>Botryosphaeria kuwatsukai</i>	CBS 135219 = PG2 * LSP5	<i>Malus domestica</i> <i>Pyrus</i> sp.	China China	C.S. Wang C.S. Wang	KJ433388 KJ433395	KJ433410 KJ433417	– –	– –
<i>Botryosphaeria qingyuanensis</i>	CERC 2946 = CGMCC 3.18742 * CERC 2947 = CGMCC 3.18744	<i>Eucalyptus</i> hybrid <i>Eucalyptus</i> hybrid	China China	S.F. Chen & G.Q. Li S.F. Chen & G.Q. Li	KX278000 KX278001	KX278105 KX278106	KX278209 KX278210	MF410151 MF410152
<i>Botryosphaeria ramosa</i>	CBS 122069 = CMW 26167 * CGMCC 3.18006	<i>Eucalyptus camaldulensis</i> <i>Myrtaceae</i>	Australia China	T.I. Burgess –	EU144055 KX197072	EU144070 KX197092	KF766132 KX197099	– –
<i>Botryosphaeria scharifii</i>	CBS 124703 = IRAN 1529C * CBS 124702 = IRAN 1543C	<i>Mangifera indica</i> <i>Mangifera indica</i>	Iran Iran	J. Abdollahzadeh J. Abdollahzadeh & A. Javadi	JQ772020 JQ772019	JQ772057 JQ772056	– –	– –
<i>Diplodia africana</i>	CBS 120835 = CPC 5908 * STE-U 5946	<i>Prunus persica</i> <i>Prunus persica</i>	South Africa South Africa	U. Damm U. Damm	KF766155 EF445344	KF766397 EF445383	KF766129 –	– –
<i>Diplodia afrocarpi</i>	CBS 131681 = CMW 35506	<i>Afrocarpus falcatus</i> , healthy twigs	South Africa	E.M. Cruywagen	MT587333	MT592035	MT592471	–
<i>Diplodia agrifolia</i>	CBS 124.30	<i>Ulmus</i> sp.	USA	–	KX464087	KX464557	KX464783	KX463953
<i>Diplodia allocellula</i>	CBS 130408 = CMW 36468 * CMW 36470	<i>Acacia karroo</i> <i>Acacia karroo</i>	South Africa South Africa	F. Jami & M. Gryzenhout F. Jami & M. Gryzenhout	JQ239397 JQ239399	JQ239384 JQ239386	JQ239378 JQ239380	– –
<i>Diplodia arengae</i>	MFLU 17-2769 = XTBG28 *	<i>Arenga hookeriana</i>	China	D.N. Wanasinghe	MG762771	MG762774	MG783039	–
<i>Diplodia bulgarica</i>	CBS 124254 * CBS 124135	<i>Malus sylvestris</i> <i>Malus sylvestris</i>	Bulgaria Bulgaria	S.G. Bobev S.G. Bobev	GQ923853 GQ923852	GQ923821 GQ923820	– –	– –
<i>Diplodia citricarpa</i>	CBS 124715 = CJA 131 = IRAN 1578C *	<i>Citrus</i> sp., twigs	Iran	J. Abdollahzadeh & A. Javadi	KF890207	KF890189	KX464784	–
<i>Diplodia corticola</i>	CBS 112549 = CAP 134 * CBS 112546	<i>Quercus suber</i> <i>Quercus ilex</i>	Portugal Spain	A. Alves –	AY259100 AY259090	AY573227 EU673310	DQ458853 EU673117	– KX463954
<i>Diplodia crataegicola</i>	MFLU 15-1311 *	<i>Crataegus</i> sp.	Italy	–	KT290244	KT290248	KT290246	–
<i>Diplodia cupressi</i>	CBS 168.87 * CBS 261.85	<i>Cupressus sempervirens</i> <i>Cupressus sempervirens</i>	Israel Israel	Z. Solel Z. Solel	DQ458893 DQ458894	DQ458878 DQ458879	DQ458861 DQ458862	– –
<i>Diplodia eriobotryicola</i>	CBS 140851 = BN-21 *	<i>Eriobotrya japonica</i>	Spain	E. Gonzalez-Dominguez	KT240355	KT240193	MG015806	–
<i>Diplodia estuarina</i>	CMW 41363 CMW 41230	<i>Rhizophora mucronata</i> <i>Rhizophora mucronata</i>	South Afric South Afric	J.A. Osorio & Jol. Roux. J.A. Osorio & Jol. Roux.	KP860829 KP860830	KP860674 KP860675	KP860752 KP860753	– –
<i>Diplodia fraxini</i>	CBS 136010 * CBS 136011	<i>Fraxinus angustifolia</i> <i>Fraxinus angustifolia</i>	Portugal Italy	A. Deidda B.T. Linaleddu	KF307700 KF307711	KF318747 KF318748	MG015807 MG015808	– –
<i>Diplodia galicola</i>	MFLU15-1310 *	<i>Galium</i> sp.	Italy	E. Camporesi	KT290245	KT290249	KT290247	–
<i>Diplodia gallae</i>	CBS 211.25 CBS 212.25	<i>Quercus</i> sp., fruit <i>Quercus</i> sp., gall	– –	– –	KX464090 KX464091	KX464564 KX464565	KX464795 KX464796	– –
<i>Diplodia malorum</i>	CBS 124130 * BN-37	<i>Malus sylvestris</i> <i>Eriobotrya japonica</i>	Portugal Spain	A.J.L. Phillips –	GQ923865 KT240360	GQ923833 KT240198	– –	– –
<i>Diplodia mutilla</i>	CBS 112553 = CAP 062 *	<i>Vitis vinifera</i>	Portugal	A.J.L. Phillips	AY259093	AY573219	KY554743	–
<i>Diplodia neojuniperi</i>	CPC 22753 = B0031 * CPC 22754 = B0032	<i>Juniperus chinensis</i> <i>Juniperus chinensis</i>	Thailand Thailand	T. Trakunyingcharoen T. Trakunyingcharoen	KM006431 KM006432	KM006462 KM006463	– –	– –

Table 2 (cont.)

Species	Isolate numbers ^a	Host	Location	Collector	GenBank accession numbers ^b			
					ITS	tef1	tub2	rpb2
<i>Diplodia olivarum</i>	CBS 121887 = CAP 254 * IMI 390972	<i>Olea europaea</i> Carob tree	Italy	C. Lazzizera	EU392302	EU392279	HQ660079	–
			Italy	–	HM028640	HQ660078	HQ660080	–
<i>Diplodia pseudoseriata</i>	CBS 124906 *	<i>Blepharocalyx salicifolius</i>	Uruguay	C. Pérez	EU080927	EU863181	MG015820	–
<i>Diplodia quercivora</i>	CBS 133852 * MEAN 1017	<i>Quercus canariensis</i> <i>Quercus suber</i>	Tunisia Portugal	B. T. Linaldeddu H. Braganca	JX894205 KU311198	JX894229 KU311201	MG015821	–
<i>Diplodia rosulata</i>	CBS 116470 * CBS 116472	<i>Prunus africana</i> <i>Prunus africana</i>	Ethiopia Ethiopia	A. Gure A. Gure	EU430265 EU430266	EU430267 EU430268	EU673132 EU673131	–
<i>Diplodia sapinea</i>	CBS 393.84 * CBS109726 = CMW 04880	<i>Pinus nigra</i> <i>Pinus patula</i>	Netherlands South Africa	H.A. van der Aa M.J. Wingfield	DQ458895 KX464094	DQ458880 KX464568	DQ458863 KX464800	– KX463956
<i>Diplodia scrobiculata</i>	CBS 118110 *	<i>Pinus resinosa</i>	USA	M.A. Palmer	AY253292	AY624253	AY624258	KX463959
<i>Diplodia seriata</i>	CBS 112555 = CAP 063 * CBS 117.82	<i>Vitis vinifera</i> <i>Rubus</i> sp., dead stem	Italy Italy	A.J.L. Phillips H.A. van der Aa	AY259094 KX464108	AY573220 KX464598	DQ458856 KX464834	– KX463964
<i>Diplodia</i> sp. 1	CBS 678.88 UCD1275So	<i>Quercus suber</i> Grape vine	Spain USA	J. Luque –	AY259104 GU799471	GU799459 GU799468	GU799458 GU799465	– –
<i>Diplodia subglobosa</i>	CBS 124133 = JL 453 * CBS 124132 = JL 375	<i>Lonicera nigra</i> <i>Fraxinus excelsior</i>	Spain Spain	J. Luque J. Luque	GQ923856 DQ458887	GQ923824 DQ458871	MT592576 DQ458852	– –
<i>Diplodia tsugae</i>	CBS 418.64 = IMI 197143 *	<i>Tsuga heterophylla</i>	Canada	A. Funk	DQ458888	DQ458873	DQ458855	–
<i>Dothiorella acacicola</i>	CBS 141295 = CPC 26349 *	<i>Acacia mearnsii</i>	France	P.W. Crous & M.J. Wingfield	KX228269	KX228376	–	–
<i>Dothiorella acericola</i>	KUMCC 18-0137 * HNXX032	<i>Acer palmatum</i> , dead hanging twigs <i>Ziziphus jujuba</i> , branch	China China	R. Phookamsak R. Zang	MK359449 KY385661	MK361182 KY393212	– KY393178	– –
<i>Dothiorella alpina</i>	CGMCC 3.18001 *	<i>Platyclusus orientalis</i>	China	W. He & J.R. Wu; det. Y. Zhang	KX499645	KX499651	–	–
<i>Dothiorella brevicollis</i>	CBS 130411 = CMW 36463 * CMW 36464	<i>Acacia karroo</i> <i>Acacia karroo</i>	South Africa South Africa	F. Jami & M. Gryzenhout F. Jami & M. Gryzenhout	JO239403 JO239404	JO239390 JO239391	JO239371 JO239372	– –
<i>Dothiorella capri-amissi</i>	CBS 121763 = CMW 25403 * CMW 25404	<i>Acacia erioloba</i> <i>Acacia erioloba</i>	South Africa South Africa	F.J.J. van der Walt & G.J. Marais F.J.J. van der Walt & G.J. Marais	EU101323 EU101324	EU101368 EU101369	KX464850	–
<i>Dothiorella casuarini</i>	CBS 120688 = CMW 4855 * CBS 120690 = CMW 4857	<i>Casuarina</i> sp. <i>Casuarina</i> sp.	Australia Australia	M.J. Wingfield M.J. Wingfield	DQ846773 DQ846774	DQ875331 DQ875333	– –	KX463970
<i>Dothiorella citricola</i>	CBS 124729 = ICMP 16828 *	<i>Citrus sinensis</i>	New Zealand	S.R. Pennycook, P.R. Johnston & B.C. Paulus	EU673323	EU673290	KX464853	KX463971
	CBS 124728 = ICMP 16827	<i>Citrus sinensis</i>	New Zealand	S.R. Pennycook, P.R. Johnston & B.C. Paulus	EU673322	EU673289	KX464852	–
<i>Dothiorella diospyricola</i>	CBS 145972 = CPC 34653 *	<i>Diospyros mespiliformis</i>	South Africa	P.W. Crous	MT587398	MT592110	MT592581	–
<i>Dothiorella dulcispinae</i>	CBS 130413 = CMW 36460 * CMW 36462	<i>Acacia karroo</i> <i>Acacia karroo</i>	South Africa South Africa	F. Jami & M. Gryzenhout F. Jami & M. Gryzenhout	JO239400 JO239389	JO239387 JO239389	JO239373 JO239375	– –
<i>Dothiorella eriobotryae</i>	CBS 140852 = CPC 29679 = BN 81 * CMW46458	<i>Eriobotrya japonica</i> , branch canker <i>Acacia heterophylla</i>	Spain La Réunion	E. Gonzalez-Dominguez M.J. Wingfield	KT240287 MN103794	KT240262 MH548348	MT592582 MH548324	– –
<i>Dothiorella iranica</i>	CBS 124722 = IRAN 1587C * MFLUCC 15-0656	<i>Olea europea</i> <i>Paliurus</i>	Iran Italy	A. Javadi E. Camporesi	KC898231 KX765302	KC898214 KX765303	KX464856	–
<i>Dothiorella koae</i>	CMW48017	<i>Acacia heterophylla</i>	La Réunion	M.J. Wingfield	MH447652	MH548338	MH548327	–
<i>Dothiorella lampangensis</i>	MFLUCC 18-0232 *	<i>Rutaceae</i> , fallen fruit pericarp	Thailand	S.C. Jayasiri	MK347758	MK340869	MK412874	–
<i>Dothiorella longicollis</i>	CBS 122068 = CMW 26166 * CBS 122066 = CMW 26166	<i>Lysiphyllum cunninghamii</i> <i>Terminalia</i> sp.	Australia Australia	T.I. Burgess & M.J. Wingfield T.I. Burgess & M.J. Wingfield	EU144054 EU144052	EU144069 EU144067	KF766130 KX464857	KX463972
<i>Dothiorella magnoliae</i>	CFCC 51563 * CFCC 51564	<i>Magnolia grandiflora</i> <i>Magnolia grandiflora</i>	China China	C.J. You C.J. You	KY111247 KY111248	KY213686 KY213687	– –	– –

Table 2 (cont.)

Species	Isolate numbers ^a	Host	Location	Collector	GenBank accession numbers ^b			
					ITS	tef1	tub2	rpb2
<i>Dothiorella mangifera</i>	CBS 124727 = IRAN 1584C * IRAN 1545C	<i>Mangifera indica</i> <i>Mangifera indica</i>	Iran	J. Abdollahzadeh & A. Javadi	KC898221	KC898204	–	KX463973
<i>Dothiorella moneti</i>	MUCC 505 = WAC 13154 * MUCC 507	<i>Acacia rostellifera</i> <i>Acacia rostellifera</i>	Australia	K.M. Taylor	KC898223	KC898206	–	–
<i>Dothiorella plurivora</i>	CBS 124724 = IRAN 1557C * CBS 124725	<i>Citrus</i> sp. <i>Prunus armeniaca</i>	Australia	K.M. Taylor	EF591920	EF591971	EF591954	–
<i>Dothiorella pretoriensis</i>	CBS 130404 = CMW 36480 * CMW 36481	<i>Acacia karroo</i> <i>Acacia karroo</i>	Iran	J. Abdollahzadeh & A. Javadi	EF591922	EF591973	EF591956	–
<i>Dothiorella prunicola</i>	CBS 124723 = CAP 187 * CMW46457 *	<i>Prunus dulcis</i>	South Africa	F. Jami & M. Gryzenhout	KC898225	KC898208	KX464874	–
<i>Dothiorella reunionis</i>	MUCC 509 = WAC 13155 * MUCC 508	<i>Acacia heterophylla</i> <i>Santalum acuminatum</i>	South Africa	J. Abdollahzadeh & A. Javadi	KC898225	KC898213	KX464875	–
<i>Dothiorella sarmentorum</i>	IMI 63581b * CBS 115038	<i>Ulmus</i> sp. <i>Malus pumila</i>	Portugal	F. Jami & M. Gryzenhout	QJ239405	QJ239392	QJ239376	–
<i>Dothiorella</i> sp. 1	CBS 121783 = CMW 25432 = CAMS 1187 CBS 121784 = CMW 25430 = CAMS 1185	<i>Acacia mearnsii</i> <i>Acacia mearnsii</i>	La Réunion	E. Diogo	QJ239406	QJ239393	QJ239377	–
<i>Dothiorella striata</i>	CBS 124731 = ICMP 16824 * CBS 124730 = ICMP 16819	<i>Citrus sinensis</i> <i>Citrus sinensis</i>	Australia	M.J. Wingfield	EU673313	EU673280	–	–
<i>Dothiorella tectonae</i>	MFLUCC 12-0382 = MD-2014 *	<i>Tectona grandis</i>	England	K.M. Taylor	MH447649	MH548347	–	–
<i>Dothiorella thailandica</i>	MFLUCC 11-0438 *	<i>Bamboos culm</i>	Netherlands	K.M. Taylor	EF591924	EF591975	EF591958	–
<i>Dothiorella thripita</i>	CBS 125445 = BRIP 51876 *	<i>Acacia harpophylla</i>	South Africa	E.A. Ellis	EF591923	EF591974	EF591957	–
<i>Dothiorella ulmacea</i>	CBS 138855 = CPC 24416 * CPC 24945	<i>Ulmus laevis</i> <i>Ulmus laevis</i>	South Africa	A.J.L. Phillips	AY573212	AY573235	–	–
<i>Dothiorella uruguayensis</i>	CBS 124908 = CMW 26763 = UY672 *	<i>Hexachlamis edulis</i>	New Zealand	F.J.J. van der Walt & R.N. Heath	AY573206	AY573223	EU673101	–
<i>Dothiorella vinea-germae</i>	DAR 81012 = B116-3 *	<i>Vitis vinifera</i>	New Zealand	F.J.J. van der Walt & R.N. Heath	EU101333	EU101378	KX464859	–
<i>Dothiorella viticola</i>	CBS 117009 * GAR09	<i>Vitis vinifera</i> cv. Gamatxa Negra <i>Vitis</i> sp.	New Zealand	S.R. Pennycook, P.R. Johnston & B.C. Paulus	EU101331	EU101376	KX464860	–
<i>Dothiorella yunnana</i>	CGMCC 3.17999 * CGMCC 3.18000	<i>Camellia</i> sp. <i>Camellia</i> sp.	Thailand	S.R. Pennycook, P.R. Johnston & B.C. Paulus	EU673321	EU673288	EU673143	KX463976
<i>Lasiodiplodia acaciae</i>	CERC 1961 = CFCC 50065 * CERC 1960 = CFCC 50064	<i>Acacia</i> sp., leaf spot <i>Pistachia vera</i>	Thailand	M. Dolom	EU673320	EU673287	EU673142	–
<i>Lasiodiplodia americana</i>	CGMCC 3.18471 *	<i>Aquilaria crassna</i>	Thailand	D.Q. Dai	KM396899	KM409637	KM510357	–
<i>Lasiodiplodia aquilariae</i>	CMW 41467 *	<i>Avocennia marina</i>	Australia	D.J. Tree & C.E.C. Tree	JX646796	JX646861	JX646844	–
<i>Lasiodiplodia avicenniae</i>	LAS 199	<i>Avocennia marina</i>	Germany	R.K. Schumacher	FJ824738	KJ573639	KJ577550	KX463977
<i>Lasiodiplodia brasiliense</i>	CMW 4015 * IBL 344	<i>Mangifera indica</i> <i>Adansonia madagascariensis</i>	Germany	R.K. Schumacher	KR611881	KR611910	KR611909	–
<i>Lasiodiplodia bruguierae</i>	CMW 41470 * CMW 41614	<i>Bruguiera gymnorrhiza</i> <i>Bruguiera gymnorrhiza</i>	Uruguay	C.A. Pérez	KR611882	KR857697	–	–
<i>Lasiodiplodia cinnamomi</i>	CFCC 51997 * CFCC 51998	<i>Cinnamomum camphora</i> <i>Cinnamomum camphora</i>	Australia	N. Wunderlich	EU080923	EU863180	KX464886	–

Table 2 (cont.)

Species	Isolate numbers ^a	Host	Location	Collector	GenBank accession numbers ^b			
					ITS	tef1	tub2	rpb2
<i>Lasiodiplodia citricola</i>	CBS 124707 = IRAN 1522C * CBS124706 = IRAN 1521C	Citrus sp. Citrus sp.	Iran Iran	J. Abdollahzadeh & A. Javadi A. Shekari	GU945354 GU945353	GU945340 GU945339	KP872405 KP872406	KU696351 KU696350
<i>Lasiodiplodia crassipora</i>	CBS 118741 = WAC12533 * CMM 4585	<i>Sanitalum album</i>	Australia	T.I. Burgess & B. Dell	DQ103550 MG954354	EU673303 MG979520	KU887506 MG979552	KU696353 MG979561
<i>Lasiodiplodia euphorbicola</i>	CMM 3609 * CMM 33350	<i>Jatropha curcas</i> <i>Adansonia digitata</i>	Brazil Botswana	A.R. Machado & O.L. Pereira —	KF234543 KU887149	KF226689 KU887026	KF254926 KU887455	— KU696346
<i>Lasiodiplodia gilanensis</i>	CBS 124704 = IRAN1523C= UCCE 940B * CBS 124705 = IRAN 1501C	Citrus sp., fallen twigs Citrus sp., fallen twigs	Iran Iran	J. Abdollahzadeh & A. Javadi J. Abdollahzadeh & A. Javadi	KX906851 GU945352	KX906853 GU945341	KX906849 KP872412	KU696357 KU696356
<i>Lasiodiplodia gonubiensis</i>	CBS 115812 = CMW 14077 * CMW 46621 = MTU 56	<i>Syzygium cordatum</i> <i>Syzygium cordatum</i>	South Africa South Africa	D. Pavlic D. Pavlic	AY639595 KY052944	DQ103566 KY024623	DQ458860 KY000126	KU696359 —
<i>Lasiodiplodia gravistriata</i>	CMM 4564 * CMM 4565	<i>Anacardium humile</i> <i>Anacardium humile</i>	Brazil Brazil	M.S.B. Netto M.S.B. Netto	KT250949 KT250947	KT250950 KT266812	— —	— —
<i>Lasiodiplodia hormozganensis</i>	CBS 124709 = IRAN 1500C * CBS 124708 = IRAN 1498C	<i>Olea</i> sp. <i>Mangifera indica</i>	Iran Iran	J. Abdollahzadeh & A. Javadi J. Abdollahzadeh & A. Javadi	GU945355 GU945356	GU945343 GU945344	KP872413 KP872414	KU696361 KU696360
<i>Lasiodiplodia indica</i>	IBP 1 *	<i>Angiospermous tree</i>	India	I.B. Prasher & G. Singh	KM376151	—	—	—
<i>Lasiodiplodia iranensis</i>	CBS 124710 = IRAN 1520C * CBS 124711 = IRAN 1502C = CMM 4603	<i>Salvadora persica</i> <i>Juglans</i> sp.	Iran Iran	J. Abdollahzadeh & A. Javadi A. Javadi	GU945348 GU945347	GU945336 GU945335	KU887516 MG979537	KU696363 —
<i>Lasiodiplodia laeliocattleyae</i>	CBS 167 28 * CMM 4724	<i>Laeliocattleya</i> <i>Vitis vinifera</i>	Italy Brazil	C. Sibilia —	KU507487 MG954343	KU507454 MG979508	— MG979541	— —
<i>Lasiodiplodia lignicola</i>	CBS 134112= MFLUCC 11-0435 *	Dead wood	Thailand	A.D. Ariyawansa	JX646797	KU887003	KT852958	KU696364
<i>Lasiodiplodia macrospora</i>	CMM 3833 *	<i>Jatropha curcas</i>	Brazil	A.R. Machado & O.L. Pereira	KF234557	KF226718	KF254941	—
<i>Lasiodiplodia mahajangana</i>	CBS 124925 = CMW 27801 * CMW 27818	<i>Terminalia catappa</i> <i>Terminalia catappa</i>	Madagascar Madagascar	J. Roux J. Roux	FJ900595 FJ900596	FJ900641 FJ900642	FJ900630 FJ900631	KU696365 —
<i>Lasiodiplodia margaritacea</i>	CBS 122519 = CMW 26162 * CBS 122065	<i>Adansonia gibbosa</i> <i>Adansonia gibbosa</i>	Australia Australia	T.I. Burgess & M.J. Wingfield T.I. Burgess & M.J. Wingfield	EU144050 EU144051	EU144065 EU144066	KX464903 —	KU696367 —
<i>Lasiodiplodia mediterranea</i>	CBS 137783 * CBS137784	Holm oak Grapevine	Italy Italy	B.T. Linaldeddu S. Serra	KJ638312 KJ638311	KJ638331 KJ638330	KU887521 KU887522	KU696368 KU696369
<i>Lasiodiplodia microconidia</i>	CGMCC 3.18485 *	<i>Aquilaria crassna</i>	Laos	X. Sun	KY783441	KY848614	—	KY848561
<i>Lasiodiplodia parva</i>	CBS 456 78 * CBS 494 78	Cassava-field soil Cassava-field soil	Colombia Colombia	O. Rangel O. Rangel	EF622083 EF622084	EF622063 EF622064	KU887523 EU673114	KU696372 KU696373
<i>Lasiodiplodia plurivora</i>	CBS 120832 = STE-U5803 * CBS 121103 = STE-U4583	<i>Prunus salicina</i> <i>Vitis vinifera</i>	South Africa South Africa	F. Halleen F. Halleen	EF445362 AY343482	EF445395 EF445396	KP872421 KP872422	KU696374 KU696375
<i>Lasiodiplodia pontae</i>	CMW 1277 = IBL12 *	<i>Spondias purpurea</i>	Brazil	J.S. Lima & F.C.O. Freire	KT151794	KT151791	KT151797	—
<i>Lasiodiplodia pseudotheobromae</i>	CBS 116459 * CMM 3887	<i>Gmelina arborea</i> <i>Jatropha curcas</i>	Costa Rica Brazil	J. Carranza & Velásquez A.R. Machado	EF622077 KF234559	EF622057 KF226722	EU673111 KF254943	KU696376 —
<i>Lasiodiplodia rubropurpurea</i>	CBS 118740 = CMW 14700 = WAC 12535 * WAC 12536 = CMW 15207	<i>Eucalyptus grandis</i> <i>Eucalyptus grandis</i>	Australia Australia	T.I. Burgess & G. Pegg T.I. Burgess & G. Pegg	DQ103553 DQ103554	DQ103571 DQ103572	KU887529 KP872425	KU696380 KU696381
<i>Lasiodiplodia subglobosa</i>	CMM 3872 * CMM 4046	<i>Jatropha curcas</i> <i>Jatropha curcas</i>	Brazil Brazil	A.R. Machado & O.L. Pereira A.R. Machado & O.L. Pereira	KF234558 KF234560	KF226721 KF226723	KF254942 KF254944	— —
<i>Lasiodiplodia syzygii</i>	MFLUCC 19-0219.1 = GUCC 9719.1 * GUCC 9719.3	<i>Syzygium samarangense</i> <i>Syzygium samarangense</i>	Thailand Thailand	Q. Zhang Q. Zhang	MT990531 MW081992	MW016943 MW087102	MW014331 MW087105	— —
<i>Lasiodiplodia thailandica</i>	CPC 22795 * BUFU DZP160123-13	<i>Mangifera indica</i> <i>Albizia chinensis</i>	Thailand China	T. Trakunyingcharoen Z.P. Dou & Z.C. Liu	KJ193637 KY676789	KJ193681 KY676798	— KY751301	— KY751298

Table 2 (cont.)

Species	Isolate numbers ^a	Host	Location	Collector	GenBank accession numbers ^b			
					ITS	tef1	tub2	rpb2
<i>Lasiodiplodia theobromae</i>	CBS 164.96 * CBS 111530 CBS 124.13	fruit along coral reef coast <i>Leucospermum</i> sp.	Papua New Guinea USA	A. Aptroot J.E. Taylor	AY640255 EF622074 DQ458890	AY640258 EF622054 DQ458875	KU887532 KU887531 DQ458858	KU696383 KU69638 KY472887
<i>Lasiodiplodia tropica</i>	CGMCC 3.18477 *	<i>Aquilaria crassna</i>	Laos	X. Sun	KY783454	KY848616	KY848540	KY848574
<i>Lasiodiplodia venezuelensis</i>	CBS 118739 = CMW 13511 = WAC 12539 * CBS 129757	<i>Acacia mangium</i> <i>Acacia mangium</i>	Venezuela Venezuela	S. Mohali S. Mohali	DQ103547 JX545102	EU673305 JX545122	KU887533 JX545142	KU696384 –
<i>Lasiodiplodia viticola</i>	CBS 128313 = UCD 2553AR * CBS 128315 = UCD 2604MO	<i>Vitis vinifera</i> <i>Vitis vinifera</i>	USA USA	R.D. Cartwright & W.D. Gubler K. Stiegler & W.D. Gubler	HQ288227 HQ288228	HQ288269 HQ288270	HQ288306 HQ288307	KU696385 KU696386
<i>Lasiodiplodia vitis</i>	CBS 124060 *	<i>Vitis vinifera</i>	Italy	S. Burruano	KX464148	KX464642	KX464917	–
<i>Neodeightonia licuriensis</i>	COAD 1780 *	<i>Syagrus coronata</i>	Brazil	O.L. Pereira	KP165429	KP165430	KP165431	–
<i>Neodeightonia microspora</i>	MFLUCC 11-0483 MFLUCC 11-0504	bamboo	Thailand Thailand	D.Q. Dai D.Q. Dai	KU940110 KU940111	–	–	–
<i>Neodeightonia palmicola</i>	MFLUCC10-0822 * FAFU 002	<i>Arenga westerhoutii</i> <i>Caryota mitis</i>	Thailand China	J.K. Liu –	HQ199221 MK203813	–	–	–
<i>Neodeightonia phoenicum</i>	CBS 122528 * CBS 169.34	<i>Phoenix</i> sp. <i>Phoenix dactylifera</i>	Spain USA	F. Garcia H.S. Fawcett	EU673340 EU673338	EU673309 EU673307	EU673116 EU673138	KX463999 –
<i>Neodeightonia planchoniae</i>	MFLUCC 17-2427	<i>Planchonia</i> sp.	Thailand	S.C. Jayasiri	MK347755	–	–	–
<i>Neodeightonia rattanica</i>	MFLUCC 15-0712 * MFLUCC 15-0313	<i>Calamus</i> sp. <i>Calamus</i> sp.	Thailand Thailand	S. Konta S. Konta	KX646357 KX646361	KX646360 KX646361	–	–
<i>Neodeightonia rattanicola</i>	MFLUCC 15-0319 *	<i>Calamus</i> sp.	Thailand	S. Konta	KX646359	KX646362	–	–
<i>Neodeightonia subglobosa</i>	CBS 448.91 * MFLUCC 11-0607	<i>Bambusa arundinacea</i> bamboo	Sierra Leone Thailand	F.C. Deighton D.Q. Dai	EU673337 KU940113	EU673306	EU673137	–
<i>Neofusicoccum algeriense</i>	CBS 137504 = ALG1 * ALG9	<i>Vitis vinifera</i> <i>Vitis vinifera</i>	Algeria Algeria	A. Berraf-Tebbal A. Berraf-Tebbal	KJ657702 KJ657704	KJ657715 KJ657721	–	–
<i>Neofusicoccum andinum</i>	CBS 117453 = CMW 13455 * CBS 117452 = CMW 13446	<i>Eucalyptus</i> sp. <i>Eucalyptus</i> sp.	Venezuela Venezuela	S. Mohali S. Mohali	AY693976 DQ306263	AY693977 DQ306264	KX464923 KX464922	KX464002 KX464001
<i>Neofusicoccum arbuti</i>	CBS 116131 * CBS 116575	<i>Arbutus menziesii</i> <i>Arbutus menziesii</i>	USA USA	A. Rossman M. Elliott	XY819720 KX464155	–	KF531793 KX464927	KX464003 –
<i>Neofusicoccum australe</i>	CMW 6837 * C1.2	<i>Acacia</i> sp. <i>Arctostaphylos glauca</i>	Australia USA	M.J. Wingfield L. Drake-Schultheis	AY339262 MH77002	AY339270 MH754929	AY339254	EU339573
<i>Neofusicoccum batangarum</i>	CBS 124924 = CMW 28363 * OB45	<i>Terminalia catappa</i> <i>Opuntia ficus-indica</i>	Cameroon Italy	D. Begoude & J. Roux –	FJ900607 MG609042	FJ900653 MG609076	FJ900634 MG609059	FJ900615 –
<i>Neofusicoccum brasiliense</i>	CMW 1338 * CMW 1269	<i>Mangifera indica</i> <i>Mangifera indica</i>	Brazil Brazil	M.W. Marques M.W. Marques	JX513630 JX513629	JX513610 JX513609	KC794031 KC794032	–
<i>Neofusicoccum cordaticola</i>	CBS 123634 = CMW 13992 * CBS 123635 = CMW 14056	<i>Syzygium cordatum</i> <i>Syzygium cordatum</i>	South Africa South Africa	D. Pavlic D. Pavlic	EU821898 EU821903	EU821868 EU821873	EU821838 EU821843	EU821928 EU821933
<i>Neofusicoccum cryptoaustrale</i>	CBS 122813 = CMW 23785 *	<i>Eucalyptus</i> tree	South Africa	H.M. Maleme	FJ752742	FJ752713	FJ752756	KX464014
<i>Neofusicoccum eucalypticola</i>	CBS 115679 = CMW 6539 * CBS 6539 = CMW 6217	<i>Eucalyptus</i> sp. <i>Eucalyptus</i> sp.	Australia Australia	M.J. Wingfield M.J. Wingfield	AY615141 AY615143	AY615133 AY615135	AY615127 AY615125	–
<i>Neofusicoccum eucalyptorum</i>	CBS 115791 = CMW 10125 * CAA 518	<i>Eucalyptus grandis</i> <i>Eucalyptus globulus</i>	South Africa Portugal	H. Smith –	AF283686 KX871883	AY236891 KX871839	AY236920 KX871776	–
<i>Neofusicoccum grevilleae</i>	CBS 129518 = CPC 16999 *	<i>Grevillea aurea</i>	Australia	P.W. Crous & R.G. Shivas	JF951137	–	–	–
<i>Neofusicoccum hellenicum</i>	CERC 1947 = CFCC 50067 * CERC 1948 = CFCC 50068	<i>Pistacia vera</i> <i>Pistacia vera</i>	Greece Greece	T.J. Michalides T.J. Michalides	KP217053 KP217054	KP217061 KP217062	KP217069 KP217070	–

Table 2 (cont.)

Species	Isolate numbers ^a	Host	Location	Collector	GenBank accession numbers ^b			
					ITS	tef1	tub2	rpb2
<i>Neofusicoccum kwambambiense</i>	CBS 123639 = CMW 14023 * CBS 123641 = CMW 14140	<i>Syzygium cordatum</i> <i>Syzygium cordatum</i>	South Africa South Africa	D. Pavlic D. Pavlic	EU821900 EU821919	EU821870 EU821889	EU821840 EU821859	EU821930 EU821949
<i>Neofusicoccum luminizerae</i>	CMW 41469 * CMW 41228	<i>Lumnitzera racemosa</i> <i>Lumnitzera racemosa</i>	South Africa South Africa	J.A Osorio & Jol. Roux J.A Osorio & Jol. Roux	KP860881 KP860882	KP860724 KP860725	KP860801 KP860803	KU587925 KU587926
<i>Neofusicoccum luteum</i>	CBS 562.92 = ATCC 58193* BRIP 5016	<i>Actinidia deliciosa</i> <i>Persea americana</i>	New Zealand USA	S.R. Pennycook –	KX464170 MH057191	KX464690 MH102254	KX464968 –	KX464020 –
<i>Neofusicoccum macroclavatum</i>	CBS 118223 = WAC 12444 * WAC 12446	<i>Eucalyptus globulus</i> <i>Eucalyptus globulus</i>	Australia Australia	T.I. Burgess T.I. Burgess	DQ093196 DQ093197	DQ093217 DQ093218	DQ093206 DQ093207	KX464022 –
<i>Neofusicoccum mangiferae</i>	CBS 118531 = CMW 7024 * CBS 118532 = CMW 7797	<i>Mangifera indica</i> <i>Mangifera indica</i>	Australia Australia	G.I. Johnson G.I. Johnson	AY615185 AY615186	DQ093221 DQ093220	AY615172 AY615173	– KX464023
<i>Neofusicoccum mangroviorium</i>	CMW 41365 * CMW 42481	<i>Avicennia marina</i> <i>Bruguiera gymnorhiza</i>	South Africa South Afric	J.A. Osorio J.A. Osorio	KP860859 KP860848	KP860702 KP860692	KP860779 KP860770	KU587905 KU587895
<i>Neofusicoccum mediterraneum</i>	CBS 12718 = PD 312 *	<i>Eucalyptus</i> sp.	Greece	P.W. Crous, M.J. Wingfield & A.J.L. Phillips	GU251176	GU251308	GU251836	KX464024
<i>Neofusicoccum microconidium</i>	CGMCC 3.18750 = CERC 3497	<i>Eucalyptus urophylla</i> × <i>E. grandis</i>	China	S.F. Chen & G.Q. Li	KX278053	KX278158	KX278262	MF410203
<i>Neofusicoccum nonquaesitum</i>	CBS 126655 = PD 484 * PD301	<i>Umbellularia californica</i> <i>Vaccinium corymbosum</i>	USA Chile	F.P. Trouillas E.X. Briceño, J.G. Espinoza & B. A. Latorre	GU251163 GU251164	GU251295 GU251296	GU251823 GU251824	KX464025 –
<i>Neofusicoccum occultatum</i>	CBS 128008 = MUCC 227 * MUCC 286 = WAC 12395	<i>Eucalyptus grandis</i> <i>Eucalyptus pellita</i>	Australia Australia	T.I. Burgess T.I. Burgess	EU301030 EU736947	EU339509 EU339511	EU339472 EU339474	EU339558 EU339560
<i>Neofusicoccum parvum</i>	CMW 9081 = ATCC 58191* CBS 110301	<i>Populus nigra</i> <i>Vitis vinifera</i>	New Zealand Portugal	S.R. Pennycook A.J.L. Phillips	AY236943 AY259098	AY236888 AY573221	AY236917 EU673095	EU821963 –
<i>Neofusicoccum pennatisporum</i>	MUCC 510 = WAC 13153 *	<i>Allocasuarina fraseriana</i>	Australia	K.M. Taylor	EF591976	EF591976	EF591959	–
<i>Neofusicoccum pistaciae</i>	CBS 595.76	<i>Pistacia vera</i>	Greece	D.G. Zachos	KX464163	KX464676	KX464953	KX464008
<i>Neofusicoccum pistaciarium</i>	CBS113083 = CPC 5263 * CBS113084 = CPC 5284	<i>Pistacia vera</i> Redwood	USA USA	T.J. Michalides T.J. Michalides	KX464186 KX464187	KX464712 KX464713	KX464998 KX464999	KX464027 KX464028
<i>Neofusicoccum protearum</i>	CMW 39280 CMW 39282	<i>Acacia karroo</i> <i>Acacia karroo</i>	Africa Africa	– –	KF270041 KF270043	KF270011 KF270013	– –	– –
<i>Neofusicoccum ribis</i>	CBS 115475 = CMW 7772 *	<i>Ribes</i> sp.	USA	B. Slippers & G. Hudler	AY236935	AY236877	AY236906	EU821958
<i>Neofusicoccum sinense</i>	CGMCC 3.18315	Unknown wood plant	China	J.J. Gan	KY350148	KY817755	KY350154	–
<i>Neofusicoccum stellenboschiana</i>	CBS 110864	<i>Vitis vinifera</i>	South Africa	F. Halleen	AY343407	AY343348	KX465047	KX464042
<i>Neofusicoccum terminaliae</i>	CBS 125263 = CMW26679 * CBS 125264 = CMW26683	<i>Terminalia sericea</i> <i>Terminalia sericea</i>	South Africa South Africa	D. Begoude & J. Roux D. Begoude & J. Roux	GQ471802 GQ471804	GQ471780 GQ471782	KX465052 KX465053	KX464045 KX464046
<i>Neofusicoccum umdonicola</i>	CBS 123645 = CMW 14058 * CBS 123646 = CMW 14060	<i>Syzygium cordatum</i> <i>Syzygium cordatum</i>	South Africa South Africa	D. Pavlic D. Pavlic	EU821904 EU821905	EU821874 EU821875	EU821844 EU821845	EU821934 EU821935
<i>Neofusicoccum ursorum</i>	CBS 122811 = CMW 24480 * CMW 23790	<i>Eucalyptus</i> sp. <i>Eucalyptus</i> sp.	South Africa South Africa	H.M. Maleme H.M. Maleme	FJ752745 FJ752746	FJ752709 FJ752708	KX465056 KX465057	KX464047 –
<i>Neofusicoccum viticlavatum</i>	CBS 112878 = STE-U 5044 * CBS 112977 = STE-U 5041	<i>Vitis vinifera</i> <i>Vitis vinifera</i>	South Africa South Africa	F. Halleen F. Halleen	AY343381 AY343380	AY343342 AY343341	KX465058 KX465059	KX464048 –
<i>Neofusicoccum vitiforme</i>	CBS 110887 = STE-U 5252 * CBS 110880 = STE-U 5050	<i>Vitis vinifera</i> <i>Vitis vinifera</i>	South Africa South Africa	J.M. van Niekerk J.M. van Niekerk	AY343383 AY343382	AY343343 AY343344	KX465061 –	KX464049 –
<i>Sphaeropsis chromolaenicola</i>	MFLUCC 17-1499 *	<i>Chromolaena odorata</i>	Thailand	A. Mapook	MT214366	–	–	–
<i>Sphaeropsis citrigena</i>	ICMP 16812 *	<i>Citrus sinensis</i>	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
<i>Sphaeropsis citrigena</i> (cont.)	ICMP 16818	<i>Citrus sinensis</i>	New Zealand	S.R. Pennycook, P.R. Johnston & B.C. Paulus	EU673329	EU673295	EU673141	–
<i>Sphaeropsis eucalypticola</i>	MFLUCC 11-0579 *	<i>Eucalyptus</i> sp.	Thailand	M. Dolom	JX646802	JX646867	JX646850	–
	MFLUCC 11-0654	<i>Eucalyptus</i> sp.	Thailand	M. Dolom	JX646803	JX646868	JX646851	–
<i>Sphaeropsis porosa</i>	CBS 110496 = STE-U 5132 *	<i>Vitis vinifera</i>	South Africa	J.M. van Niekerk	AY343379	AY343340	EU673130	KX464076
	CBS 110574 = STE-U 5046	<i>Vitis vinifera</i>	South Africa	J.M. van Niekerk	AY343378	AY343339	–	–
<i>Sphaeropsis ulmicola</i>	CBS 174.63	<i>Ulmus glabra</i>	Finland	–	MK134681	–	–	–
	PB-11f	<i>Ulmus glabra</i>	Poland	–	MK134682	–	–	–
<i>Sphaeropsis visci</i>	CBS 100163 *	<i>Vitis vinifera</i>	South Africa	J.M. van Niekerk	EU673324	EU673292	EU673127	KX464077
	CBS 186.97	<i>Viscum album</i>	Germany	T. Graefenhan	EU673325	EU673293	EU673128	KX464080

^a ALG: Personal culture collection A. Berraf-Tebbat; 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; GCMCC: China General Microbiological Culture Collection Center, Beijing, China; CJA: Collection of J. Abdollahzadeh, Department of Agriculture, University of Kurdistan, Sanandaj, Iran; CMV: 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 Botanic 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; VAC: 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 rRNA 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*.

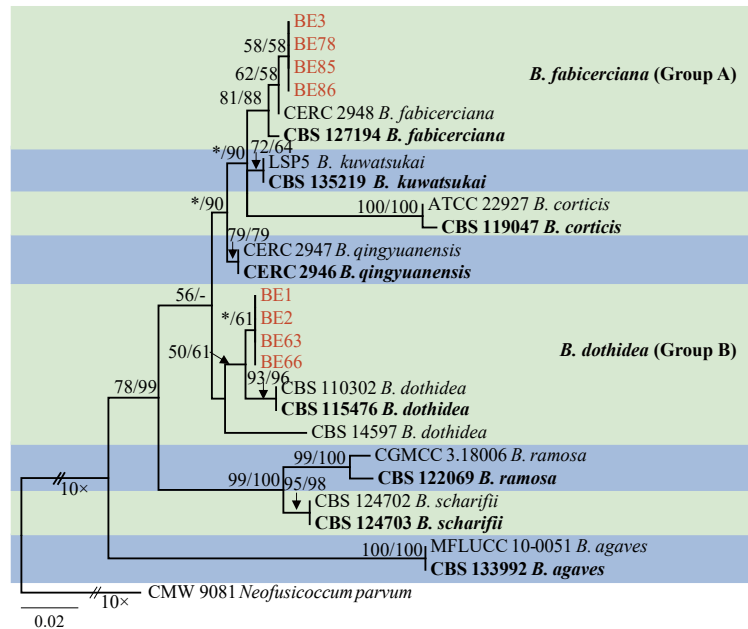


Fig. 2 Phylogenetic tree generated by maximum likelihood analyses based on the combined ITS, *tef1* and *tub2* sequence alignments of *Botryosphaeria*. Bootstrap support values $\geq 50\%$ for ML and MP are presented above branches as follows: ML/MP bootstrap values $< 50\%$ are marked with *, and absent 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).

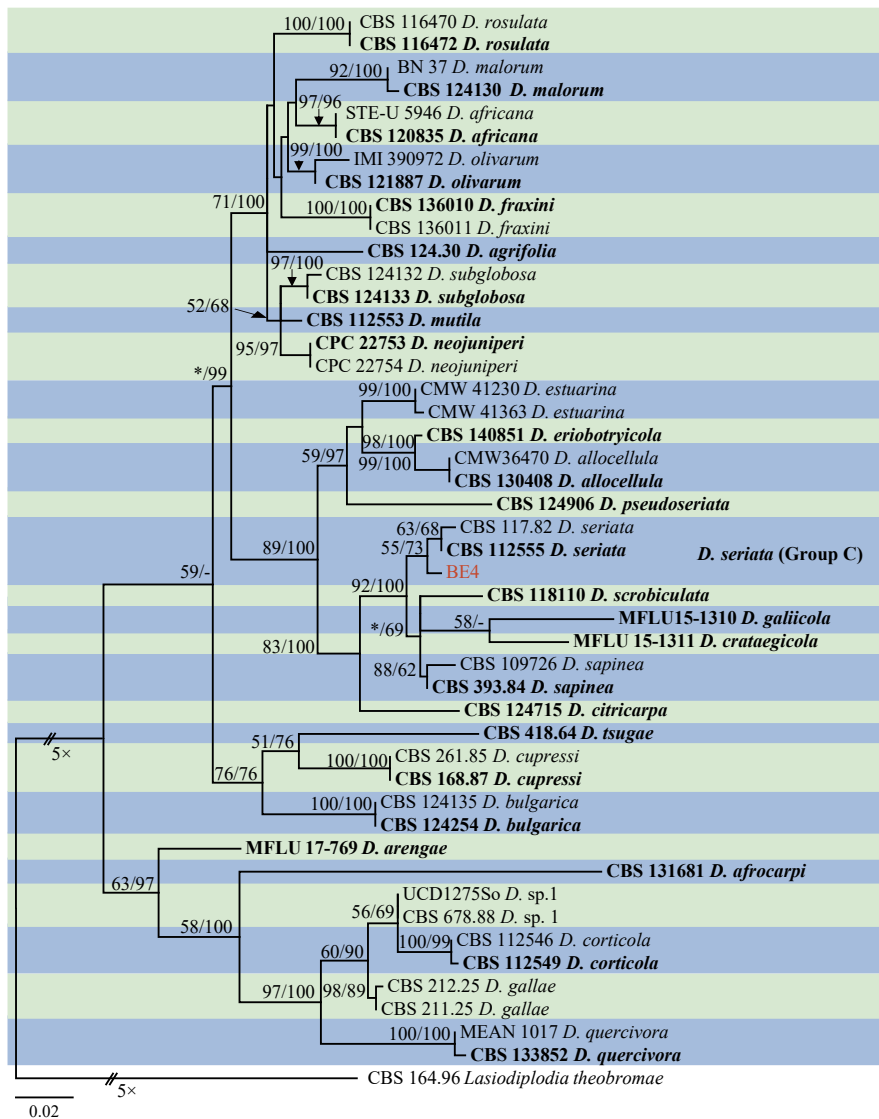


Fig. 3 Phylogenetic tree generated by maximum likelihood analyses based on the combined ITS, *tef1* and *tub2* sequence alignments of *Diplodia*. Bootstrap support values $\geq 50\%$ for ML and MP are presented above branches as follows: ML/MP bootstrap values $< 50\%$ are marked with *, and absent are 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
<i>Botryosphaeria</i>	ITS	TrN+I	6	1.0000	1.4653	1.0000	1.0000	7.9294	0.8090	–	equal
	<i>tef1</i>	TPM3uf+I	6	0.3783	2.6378	1.0000	0.3783	2.6378	0.6000	–	equal
	<i>tub2</i>	TrN+I	6	1.0000	6.0784	1.0000	1.0000	14.3581	0.7170	–	equal
	<i>rpb2</i>	TIM3+G	6	9756.0724	15799.8965	1.0000	9756.0724	70110.9963	–	0.1480	gamma
<i>Diplodia</i>	ITS/ <i>tef1</i> / <i>tub2</i>	TrN+I	6	1.0000	3.4860	1.0000	1.0000	7.2459	0.761	–	equal
	ITS	TVMeft+I+G	6	6.1639	23.8085	4.6946	13.6614	23.8085	0.6580	0.6850	gamma
	<i>tef1</i>	TrN+G	6	1.0000	3.3131	1.0000	1.0000	5.5220	–	0.5530	gamma
	<i>tub2</i>	TrN+I	6	1.0000	3.1072	1.0000	1.0000	5.7192	0.676	–	equal
<i>Dothiorella</i>	<i>rpb2</i>	TrN+G	6	1.0000	5.1679	1.0000	1.0000	13.7446	–	0.228	gamma
	ITS/ <i>tef1</i> / <i>tub2</i>	TrN+I+G	6	1.0000	3.6215	1.0000	1.0000	5.0395	0.5940	1.3320	gamma
	ITS	TrN+I+G	6	1.0000	1.4234	1.0000	1.0000	3.2819	0.4180	0.6840	gamma
	<i>tef1</i>	TPM2uf+G	6	2.0341	5.0971	2.0341	1.0000	5.0971	–	0.765	gamma
<i>Lasiodiplodia</i>	<i>tub2</i>	HKY+I+G	2	–	–	–	–	–	1.7295	0.9450	gamma
	<i>rpb2</i>	TrN+I	6	1.0000	3.8616	1.0000	1.0000	10.6645	0.6030	–	equal
	ITS/ <i>tef1</i> / <i>tub2</i>	TIM2+G	6	1.2403	2.7176	1.2403	1.0000	4.1029	–	0.2160	gamma
	ITS	K80+I	2	–	–	–	–	–	2.4444	–	equal
<i>Neodeightonia</i>	<i>tef1</i>	K80+G	2	–	–	–	–	–	1.7723	0.4130	gamma
	<i>tub2</i>	TrNef+I	6	1.0000	1.6752	1.0000	1.0000	6.7164	0.6540	–	equal
	<i>rpb2</i>	TrNef+G	6	1.0000	5.3397	1.0000	1.0000	12.4894	–	0.3110	gamma
	ITS/ <i>tef1</i> / <i>tub2</i> / <i>rpb2</i>	TrN+I+G	6	1.0000	3.8560	1.0000	1.0000	7.6054	0.5410	0.6090	gamma
<i>Neofusicoccum</i>	ITS	TPM3uf+I+G	6	3.2767	4.6927	1.0000	3.2767	4.6927	0.6640	0.5000	gamma
	<i>tef1</i>	TIM1	6	1.0000	1.0118	0.2049	0.2049	2.8685	–	–	equal
	<i>tub2</i>	TIM1+I	6	1.0000	1.2183	0.3558	0.3558	2.4674	0.6860	–	equal
	ITS/ <i>tef1</i> / <i>tub2</i>	TIM1+G	6	1.0000	1.2491	0.5067	0.5067	3.6475	–	0.1940	gamma
<i>Sphaeropsis</i>	ITS	TIM1ef+I+G	6	1.0000	6.4363	2.1879	2.1879	17.3039	0.5780	0.6010	gamma
	<i>tef1</i>	HKY+G	2	–	–	–	–	–	2.5159	0.7750	gamma
	<i>tub2</i>	TrN+G	6	1.0000	3.1372	1.0000	1.0000	6.7052	–	0.2270	gamma
	<i>rpb2</i>	TrN+G	6	1.0000	5.5358	1.0000	1.0000	17.053	–	0.235	gamma
<i>Sphaeropsis</i>	ITS/ <i>tef1</i> / <i>tub2</i> / <i>rpb2</i>	K80+G	2	–	–	–	–	–	2.265	–	gamma
	ITS	HKY+I+G	2	–	–	–	–	–	1.7294	0.9480	gamma
	<i>tef1</i>	HKY+I+G	2	–	–	–	–	–	1.7294	0.9480	gamma
	<i>tub2</i>	TIM2+G	6	1.2403	2.7176	1.2403	1.0000	4.1029	–	0.2160	gamma
<i>Sphaeropsis</i>	<i>rpb2</i>	TIM2+G	6	1.2403	2.7176	1.2403	1.0000	4.1029	–	0.2160	gamma
	ITS/ <i>tef1</i> / <i>tub2</i> / <i>rpb2</i>	HKY+I+G	2	–	–	–	–	–	1.7294	0.5610	gamma
	ITS	HKY+I+G	2	–	–	–	–	–	1.7294	0.5610	gamma
	<i>tef1</i>	HKY+I+G	2	–	–	–	–	–	1.7294	0.5610	gamma

Table 3 (cont.)

Genus	Dataset	No. of taxa	No. of bp ⁴	Maximum parsimony				RC ⁸	HI ⁹
				PIC ⁵	No. of trees	Tree length	CI ⁶		
Botryosphaeria	ITS	26	504	25	1	39	0.7949	0.8769	0.6970
	tef1	26	347	103	42	129	0.8992	0.9378	0.8433
	tub2	21	406	16	1	21	0.9048	0.9444	0.8545
	rbp2	14	659	12	3	18	0.8889	0.9583	0.8519
	ITSitef1/tub	26	1257	144	3	193	0.8601	0.9129	0.7852
Diplodia	ITS	47	535	91	1354	233	0.6652	0.8534	0.5677
	tef1	47	287	117	5000	275	0.6291	0.9016	0.5672
	tub	39	388	46	1103	95	0.6105	0.8571	0.5233
	rbp2	7	594	41	2	61	0.8033	0.7857	0.6311
	ITSitef1/tub2	47	1210	42	42	658	0.5881	0.8536	0.5020
Dothiorella	ITS	63	496	97	384	287	0.5714	0.8955	0.5117
	tef1	63	324	217	400	791	0.5310	0.8594	0.4563
	tub2	45	387	95	185	203	0.6946	0.8758	0.6083
	rbp2	16	594	83	2	145	0.6621	0.8333	0.5517
	ITSitef1/tub2	63	1540	409	6	1336	0.5427	0.8584	0.4658
Lasiodiplodia	ITS	99	486	49	5000	89	0.6517	0.8905	0.5803
	tef1	98	291	132	802	284	0.6092	0.8911	0.5428
	tub2	90	345	34	10	54	0.7222	0.9227	0.6664
	rbp2	77	521	86	201	166	0.6024	0.8981	0.5411
	ITSitef1/tub2/rbp2	99	1643	313	670	956	0.485356	0.8422	0.4087
Neodeightonia	ITS	15	502	51	1	111	0.7840	0.8490	0.6650
	tef1	9	229	14	1	19	0.8420	0.8750	0.7370
	tub2	7	418	6	1	9	0.8890	0.8570	0.7620
	ITSitef1/tub2	15	1150	78	3	162	0.7346	0.8114	0.5960
Neofusicoccum	ITS	57	494	54	28	146	0.5548	0.8516	0.4725
	tef1	55	280	115	5000	273	0.6923	0.9012	0.6239
	tub2	51	422	67	5000	130	0.6231	0.8345	0.5199
	rbp2	33	560	75	68	143	0.6364	0.8267	0.5261
	ITSitef1/tub2/rbp2	57	1756	334	24	817	0.5704	0.8208	0.4682
Sphaeropsis	ITS	10	518	54	1	19	1.0000	1.0000	1.0000
	tef1	10	297	77	1	117	0.8462	0.8583	0.7262
	tub2	9	375	20	3	21	0.8095	0.8261	0.6687
	rbp2	5	557	18	2	27	0.7410	0.6110	0.4530
	ITSitef1/tub2/rbp2	10	1747	130	2	188	0.8245	0.8245	0.6797

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.

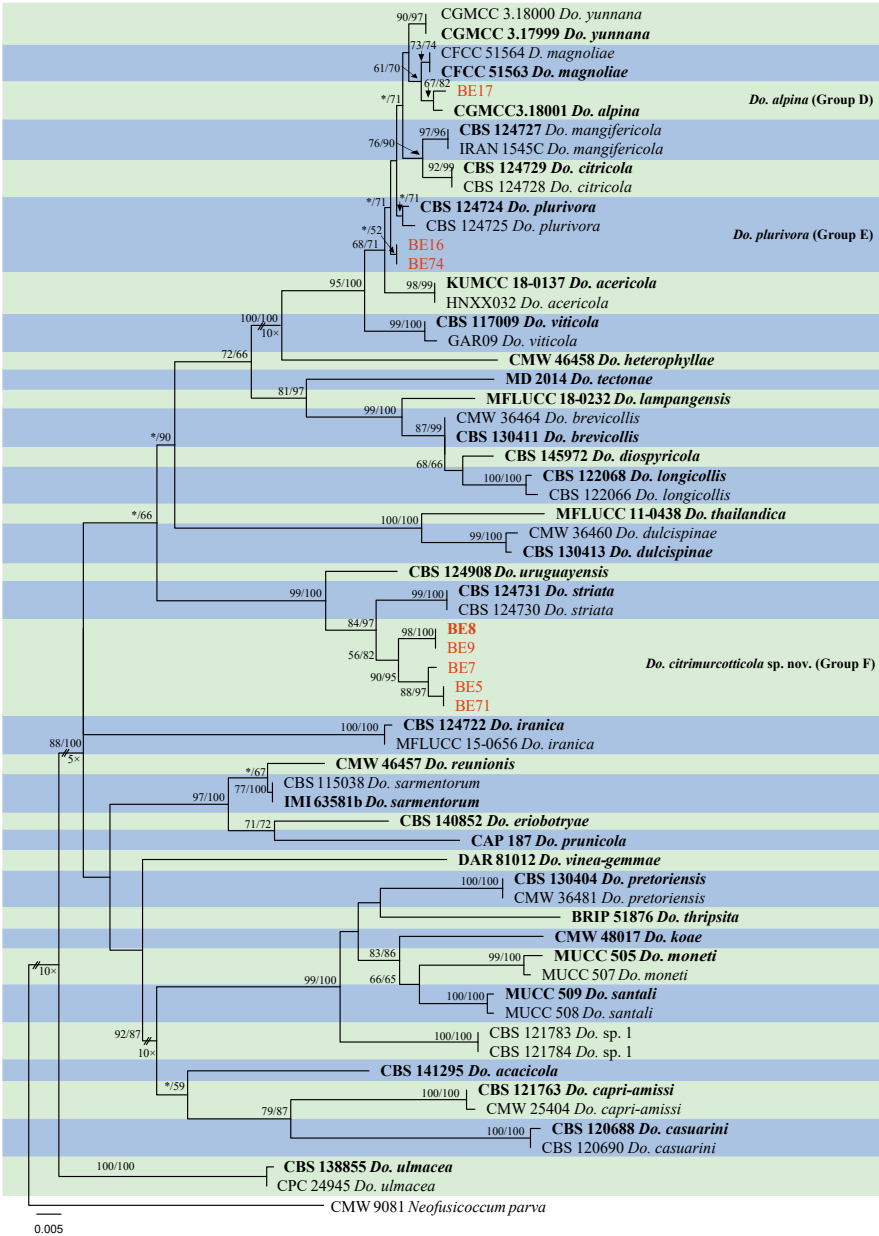


Fig. 4 Phylogenetic tree generated by maximum likelihood analyses based on the combined ITS, *tef1* and *tub2* sequence alignments of *Dothiorella*. Bootstrap support values ≥ 50 % for ML and MP are presented above branches as follows: ML/MP bootstrap values < 50 % are marked with *. Newly generated 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

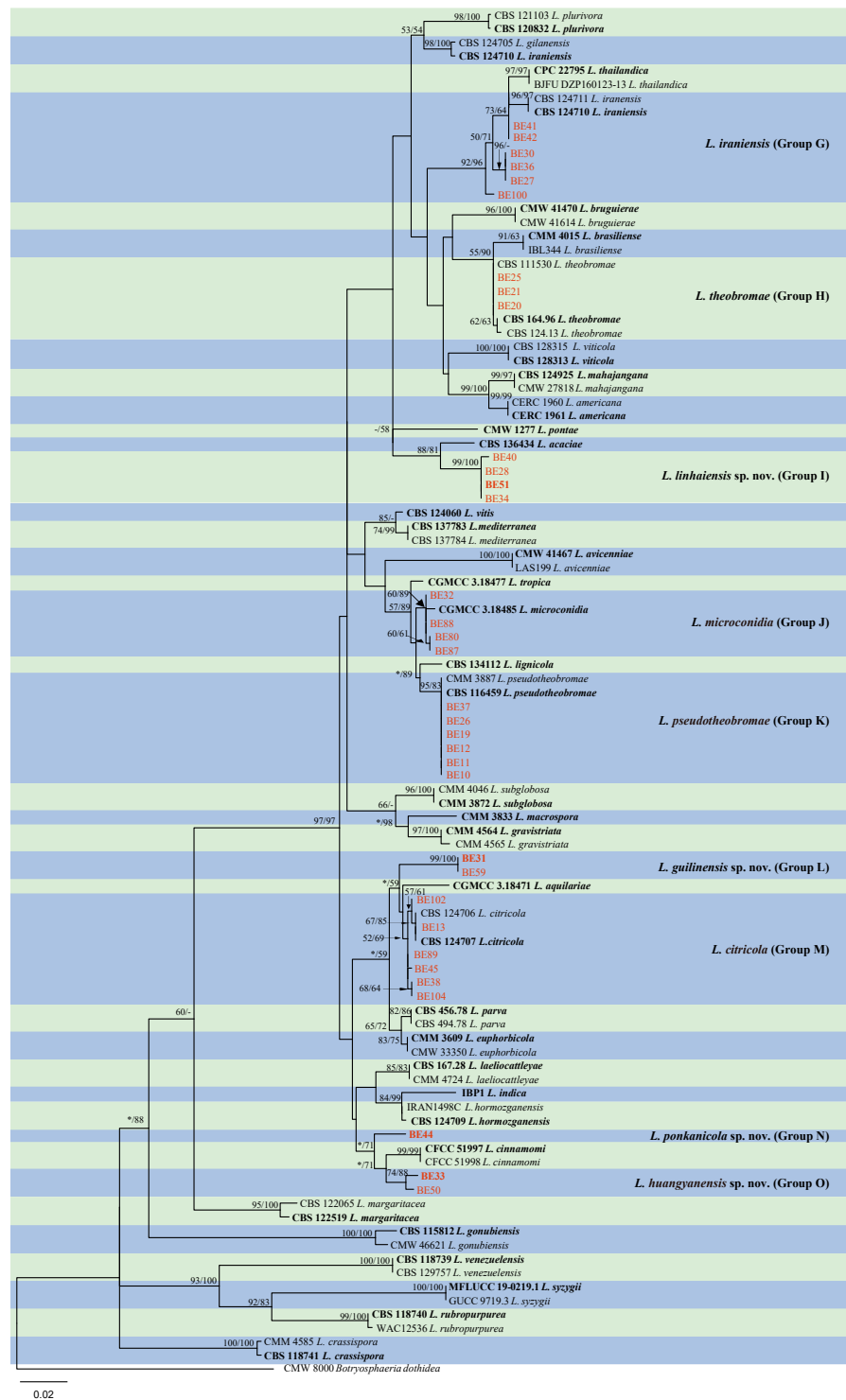


Fig. 5 Phylogenetic tree generated by maximum likelihood analyses based on the combined ITS, *tef1*, *tub2* and *rpb2* sequence alignments of *Lasiodiplodia*. Bootstrap support values $\geq 50\%$ for ML and MP are presented above branches as follows: ML/MP bootstrap values $< 50\%$ are marked with *, 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*

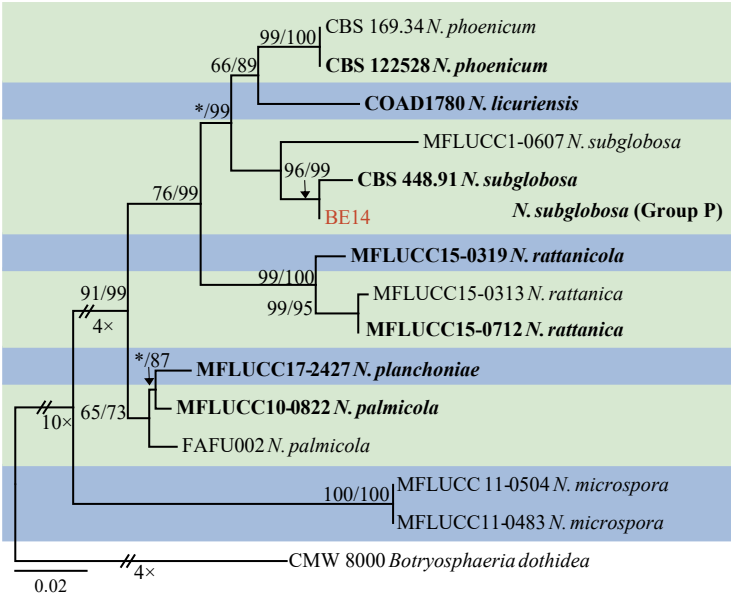


Fig. 6 Phylogenetic tree generated by maximum likelihood analyses based on the combined ITS, *tef1* and *tub2* sequence alignments of *Neodeighonia*. Bootstrap support values $\geq 50\%$ for ML and MP are presented above branches as follows: ML/MP bootstrap values $< 50\%$ are marked with *. Newly generated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

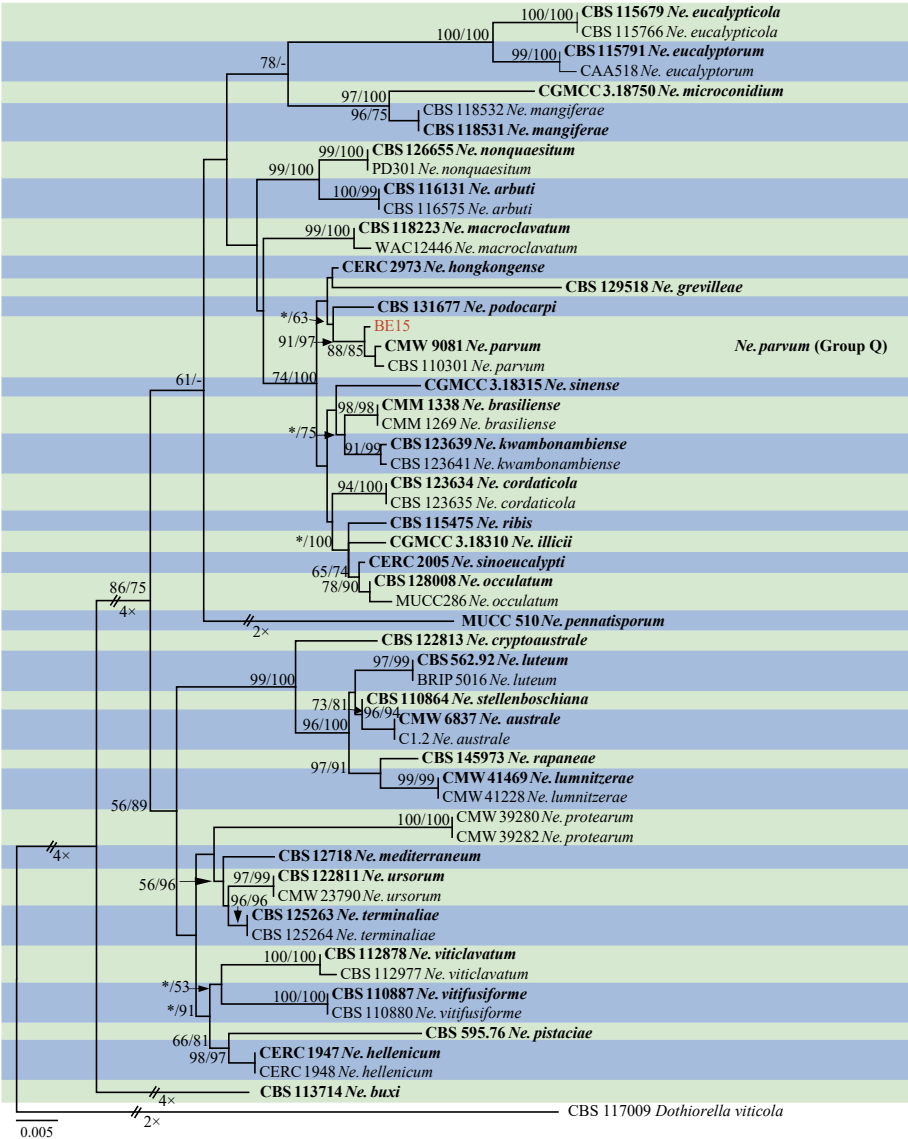


Fig. 7 Phylogenetic tree generated by maximum likelihood analyses based on the combined ITS, *tef1*, *tub2* and *rpb2* sequence alignments of *Neofusicoccum*. Bootstrap support values $\geq 50\%$ for ML and MP are presented above branches as follows: ML/MP bootstrap values $< 50\%$ are marked with *, 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).

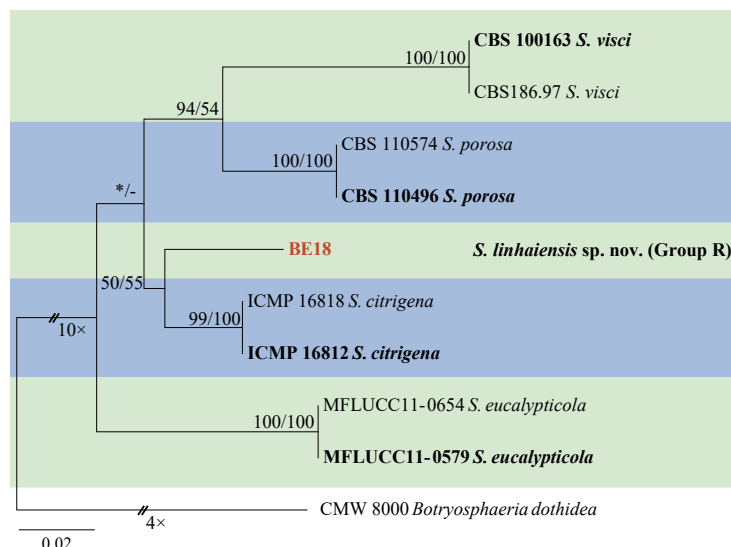


Fig. 8 Phylogenetic tree generated by maximum likelihood analyses based on the combined ITS, *tef1*, *tub2* and *rpb2* sequence alignments of *Sphaeropsis*. Bootstrap support values $\geq 50\%$ for ML and MP are presented above branches as follows: ML/MP bootstrap values $< 50\%$ are marked with *, and 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. urguay-*

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

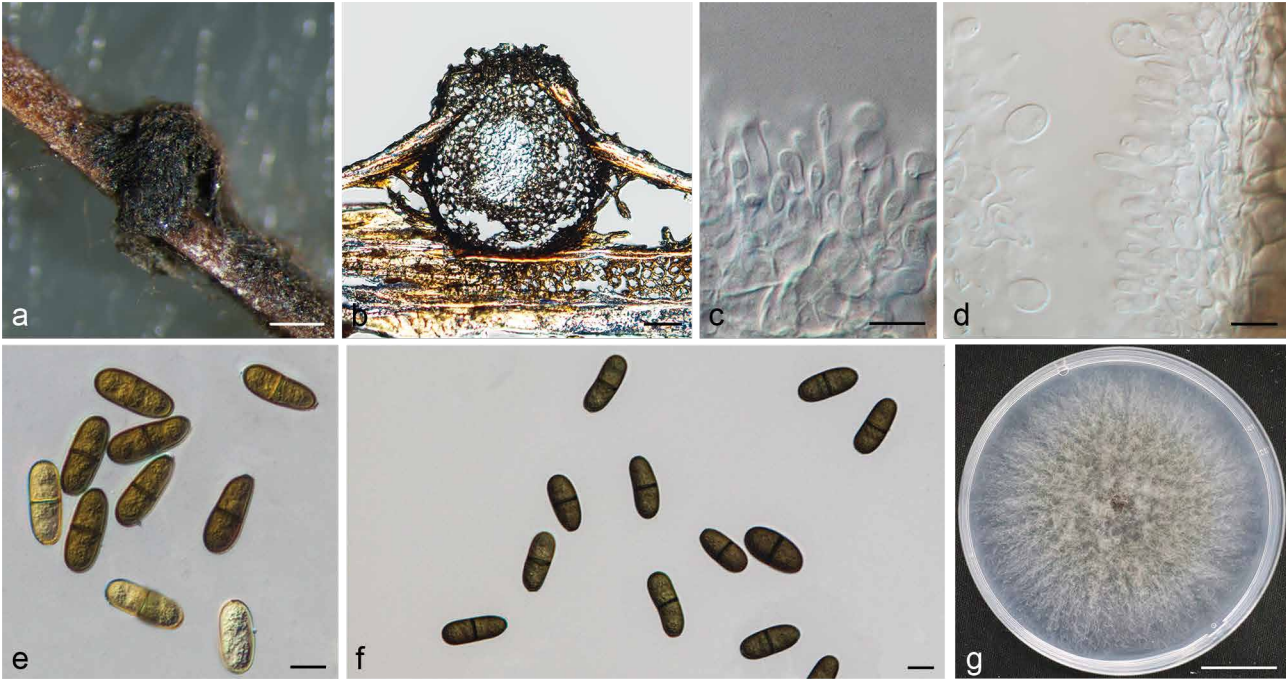


Fig. 9 *Dothiorella citrimurcoticola*. a. Conidioma formed on PNA; b. section view of conidioma; c–d. conidiogenous cells and developing conidia; e–f. conidia; g. colony growing on PDA after 3 d. — Scale bars: a = 100 µm; b = 50 µm; c–f = 10 µm; g = 2.1 cm.

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	
<i>Do. citrimurcoticola</i>	(21.5–)23–25.5(–27) × (8.5–)9.5–11(–14)	24.4 × 10.3	2.4	–	–	This study
<i>Do. striata</i>	(21–)23–26(–29.4) × (8.9–)9–12(–15.1)	25.1 × 10.7	2.4	–	–	Abdollahzadeh et al. (2014)
<i>Do. uruguayensis</i>	(17–)22–22.5(–26.5) × (7–)9–9.5(–12)	22 × 9.25	2.4	–	–	Pérez et al. (2010)
<i>Lasiodiplodia acaciae</i>	(21.5–)25–29.5(–31) × (11–)12–14(–15)	27.3 × 12.9	2.1	69	2–5	Zhang et al. (2021)
<i>L. aquilariae</i>	(23–)25–28(–29) × 12–16	26.9 × 14.1	1.8	100	3	Wang et al. (2019)
<i>L. cinnamomi</i>	(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)
<i>L. citricola</i>	(20–)22–27(–31) × (10.9–)12–17(–19)	24.5 × 15.4	1.6	125	3–4	Abdollahzadeh et al. (2010)
<i>L. guilinensis</i>	(23–)28–31(–33.5) × (13.5–)15–16.5(–17)	29.6 × 15.7	1.9	75	2–5	This study
<i>L. huangyanensis</i>	(21–)28–32.5(–34) × (13–)14–16(–17)	30.1 × 15	2	82	3–4	This study
<i>L. linhaiensis</i>	(24.5–)27–30(–32) × (12.5–)13.5–15(–16)	28.5 × 14.2	2	80	2–6	This study
<i>L. microconidia</i>	(18–)19–22(–23) × 10–15	20.8 × 13.2	1.5	90	3	Wang et al. (2019)
<i>L. ponkanicola</i>	(16–)23.5–27.5(–28.5) × (11–)13–14.5(–15.5)	25.4 × 13.7	1.9	87	2–5	This study
<i>Sphaeropsis citrigena</i>	(27–)28–33(–34) × (14.5–)15–18.5(–19)	30.5 × 16.8	1.8	25	3–5	Phillips et al. (2008)
<i>S. linhaiensis</i>	(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.

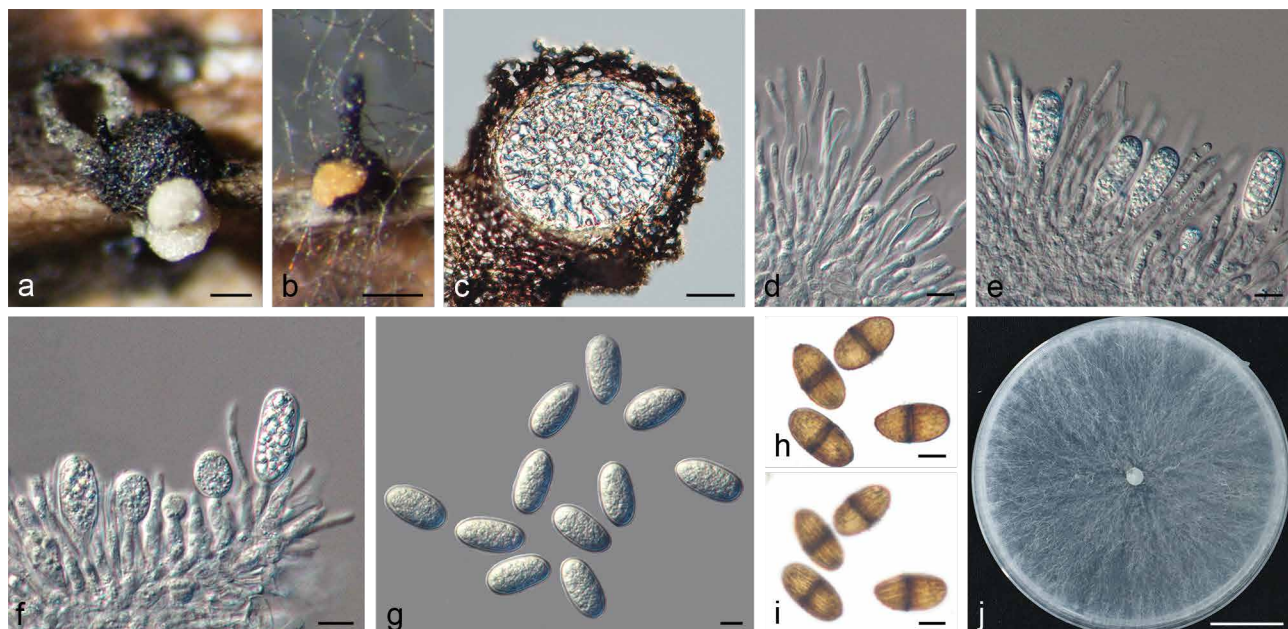


Fig. 10 *Lasiodiplodia guilinensis*. a–b. Pale yellow to saffron yellow conidial mass released from conidiomata formed on PNA; c. section view of conidioma; d. paraphyses; e–f. conidia developing on conidiogenous cells between paraphyses; g. hyaline, aseptate conidia; h–i. dark-brown, 1-septate conidia at two different focal planes to show the longitudinal striations; j. colony growing on PDA after 2 d. — Scale bars: a–b = 200 μ m; c = 50 μ m; d–i = 10 μ m; 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 $\times$ 15.7; L/W = 1.9) are larger than those of *L. aquilariae* (av. 26.9 $\times$ 14.1; L/W = 1.8) (Wang et al. 2019) and *L. citricola* (av. 24.5 $\times$ 15.4; L/W = 1.6) (Abdollahzadeh et al. 2010). In terms of paraphyses, those of *L. guilinensis* (up to 75 μ m long) are shorter than *L. aquilariae* (up to 100 μ m long) (Wang et al. 2019) and *L. citricola* (up to 125 μ m 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 μ m long, 3–4 μ m wide, formed among conidiogenous cells. **Conidiophores** absent. **Conidiogenous cells** holoblastic, hyaline, smooth, thin-walled, cylindrical, 8–35 $\times$ 3.5–7 μ m. **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) $\times$ (13–)14–16(–17) μ m (av. = 30.1 $\times$ 15 μ m, 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 $\times$ 15; L/W = 2) are larger than *L. cinnamomi* (av. 19.9 $\times$ 13.4; L/W = 1.5) (Jiang et al. 2018) and *L. ponkanicola* (av. 25.4 $\times$ 13.7; L/W = 1.9) (this study). Moreover, the paraphyses of *L. huangyanensis* (up to 82 μ m long) are shorter than those of *L. cinnamomi* (up to 106 μ m long) (Jiang et al. 2018) and *L. ponkanicola* (up to 87 μ m 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 μ m diam, solitary or aggregated, unilocular,

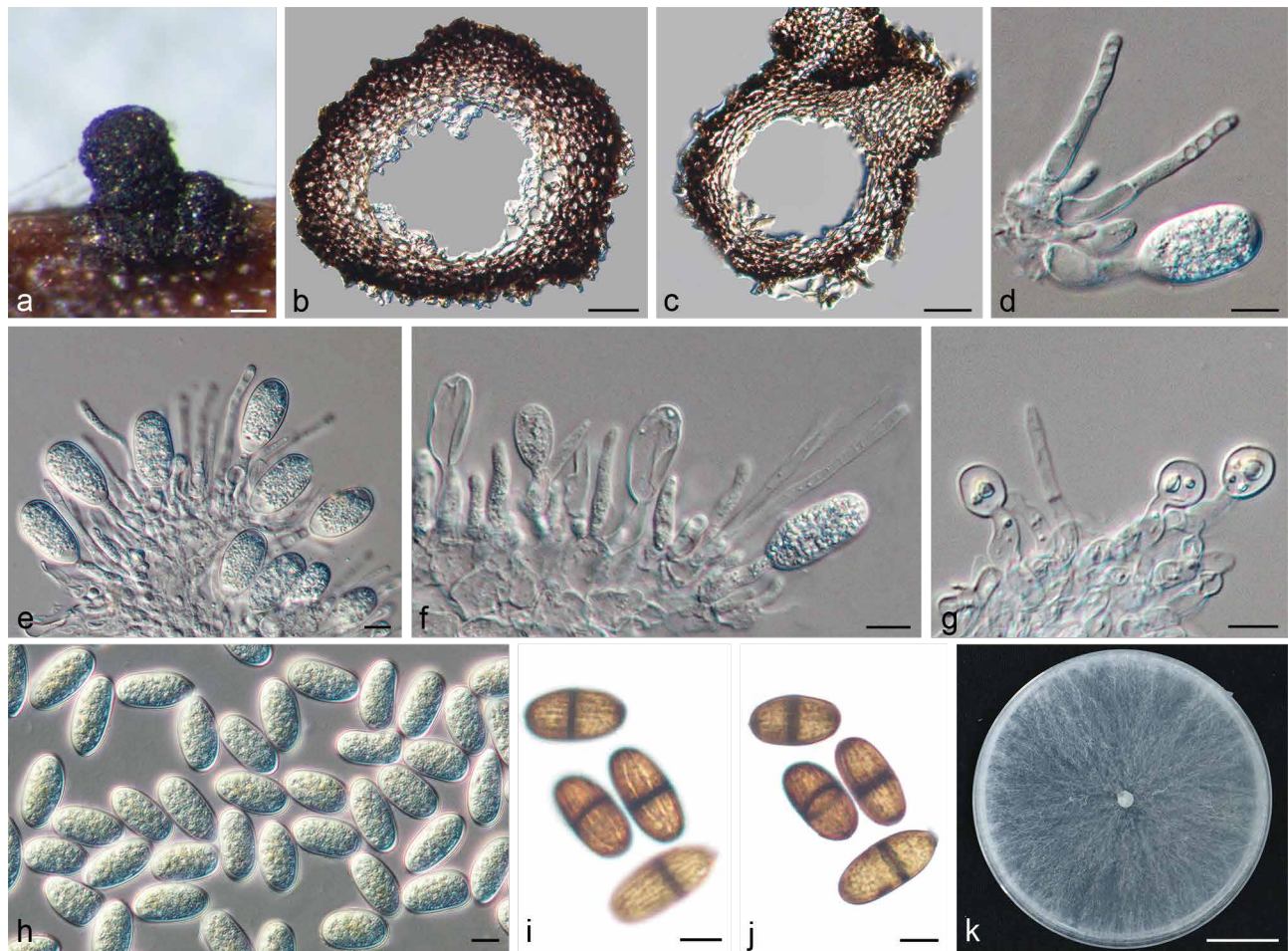


Fig. 11 *Lasiodiplodia huangyanensis*. a. Conidioma formed on PNA; b–c. section view of conidiomata; d–g. conidia developing on conidiogenous cells between paraphyses; h. hyaline, aseptate conidia; i–j. dark-brown, 1-septate conidia at two different focal planes to show the longitudinal striations; k. colony growing on PDA after 2 d. — Scale bars: a = 200 μ m; b–c = 50 μ m; d–j = 10 μ m; 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 μ m long, 2–6 μ m wide, formed among conidiogenous cells. *Conidiophores* absent. *Conidiogenous cells* holoblastic, hyaline, smooth, thin-walled, cylindrical, 7.5–22.5 $\times$ 3–5.5 μ m. *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) $\times$ (12.5–)13.5–15(–16) μ m (av. = 28.5 $\times$ 14.2 μ m, 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 $^{\circ}$ C. No growth was observed at 5 $^{\circ}$ C. After 2 d, colonies at 10 $^{\circ}$ C, 15 $^{\circ}$ C, 20 $^{\circ}$ C, 35 $^{\circ}$ C and 40 $^{\circ}$ C reach 12 mm, 18 mm, 42 mm, 22 mm and 11 mm, respectively. Isolates produced a pink pigment in PDA cultures at 35 $^{\circ}$ 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 μ m long) are longer than those of *L. acaciae* (up to 69 μ m 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 μ m long, 2–5 μ m wide, formed among conidiogenous cells. *Conidiophores* absent. *Conidiogenous cells* holoblastic, hyaline, smooth, thin-walled, cylindrical, 8.5–40 $\times$ 2.5–9 μ m. *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) $\times$ (11)–13–14.5(–15.5) μ m (av. = 25.4 $\times$ 13.7 μ m, 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 μ m; b = 50 μ m; c–h = 10 μ m; 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 μ m; b = 50 μ m; c–h = 10 μ m; 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)

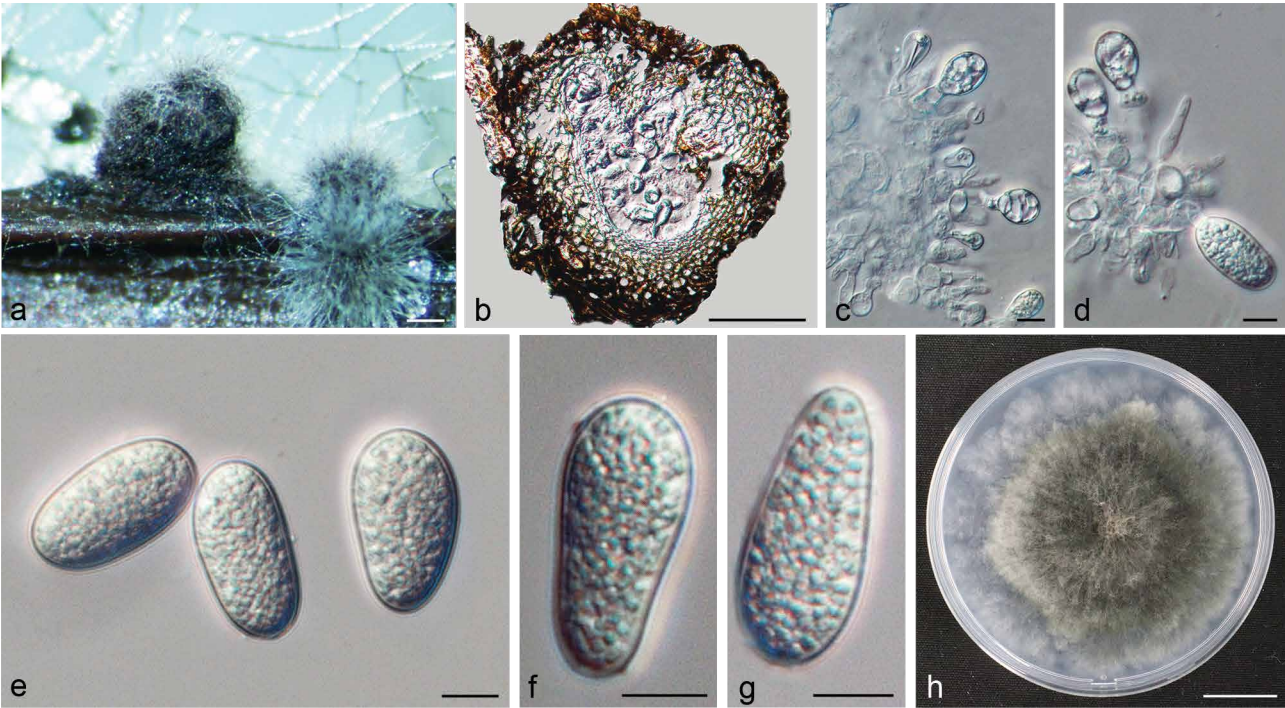


Fig. 14 *Sphaeropsis linhaiensis*. a. Conidiomata formed on PNA; b. section view of conidioma; c–d. conidia developing on conidiogenous cells; e–g. conidia; h. colony growing on PDA after 7 d. — Scale bars: a = 200 µm; b = 40 µm; c–g = 10 µm; h = 1.9 cm.

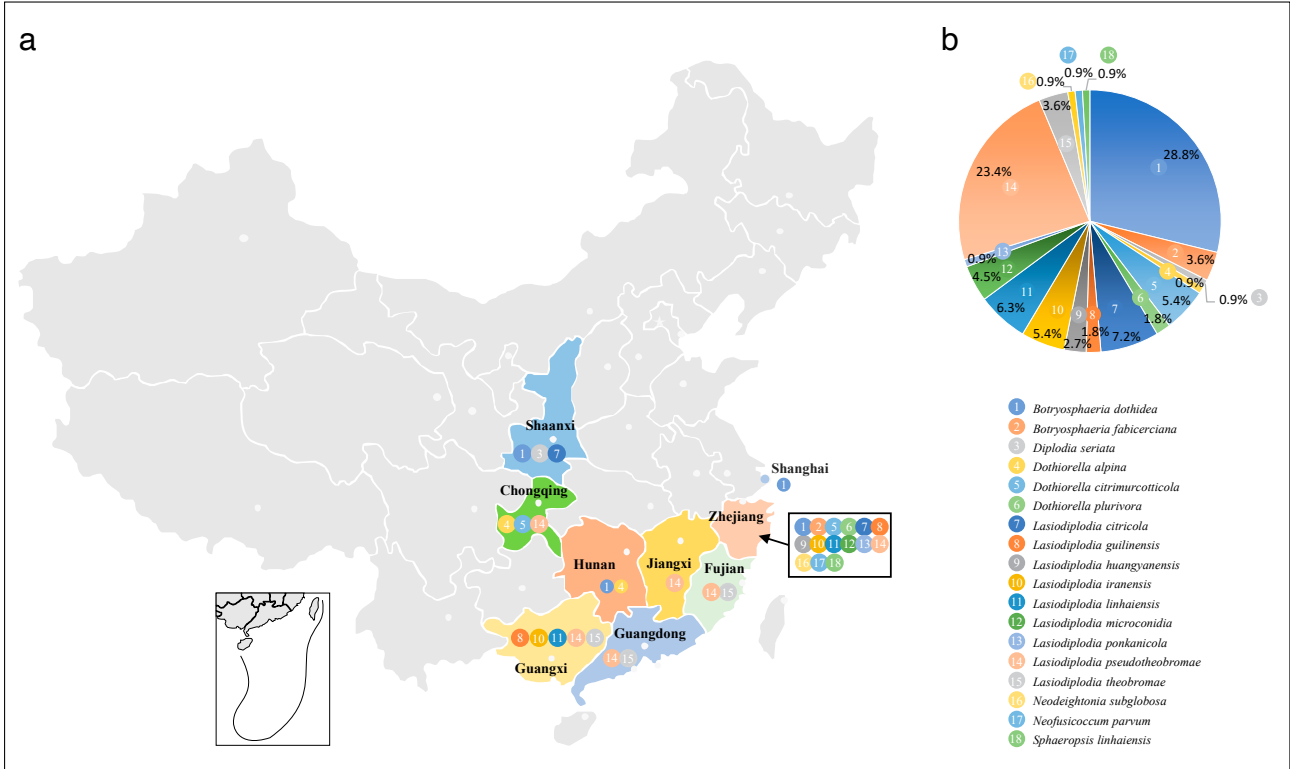


Fig. 15 The prevalence of *Botryosphaeriaceae* species isolated from citrus. a. Distribution of *Botryosphaeriaceae* species in China; b. overall isolation rate (%) of *Botryosphaeriaceae* species. Different species are represented by numbers with different colours.

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. citrimurcoticola* (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. citrimurcoticola*, *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

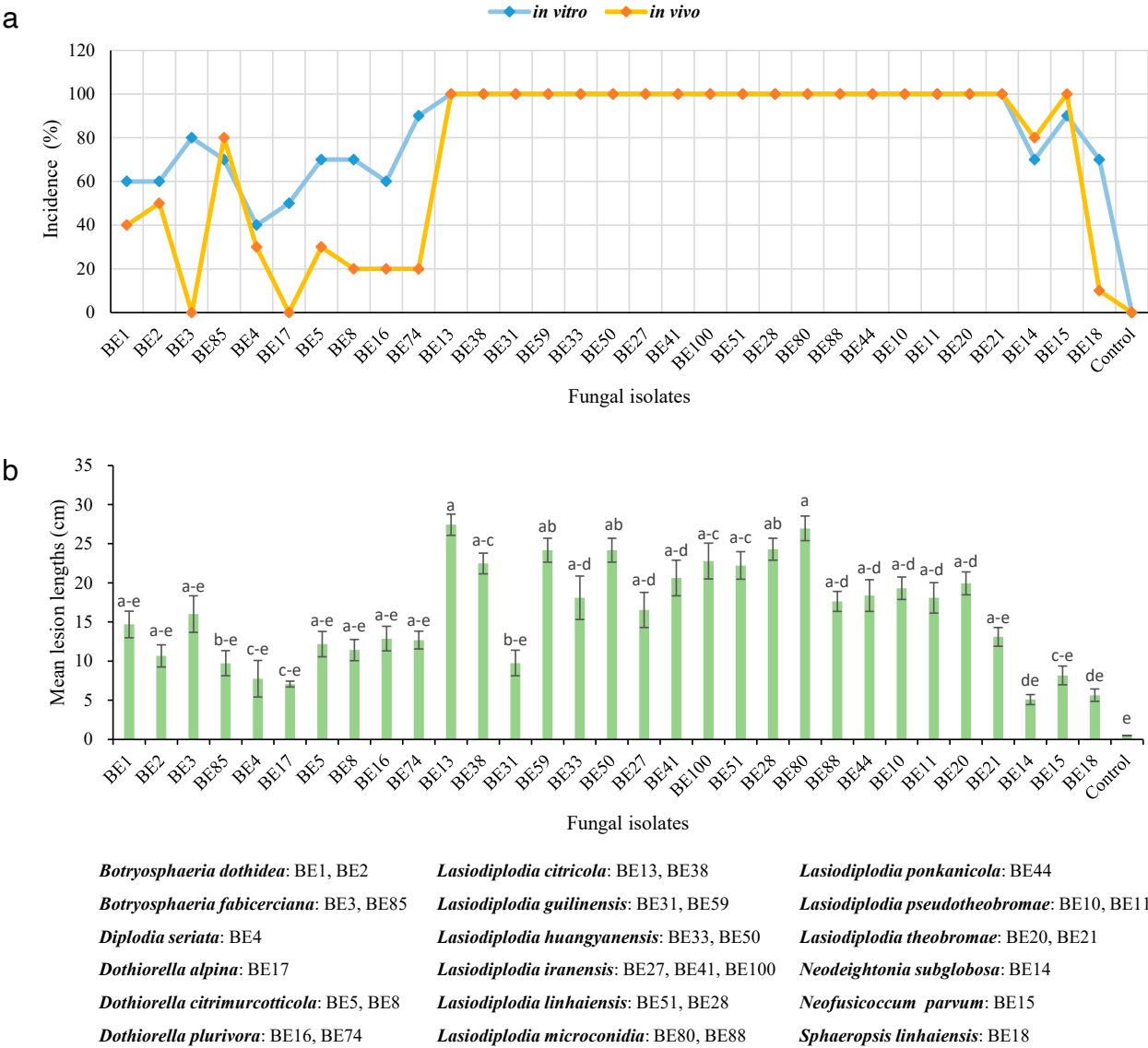


Fig. 16 Pathogenicity tests results of inoculated isolates in *Botryosphaeriaceae*. a. Incidences of shoots inoculated *in vitro* and *in vivo*, the blue diamond represents the incidence of shoots inoculated *in vitro*, while the orange diamond represents the incidence of shoots inoculated *in vivo*; 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 $\alpha = 0.05$. 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 $\geq 50\%$ 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 $\geq 50\%$ 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 $\geq 50\%$ 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 $\geq 50\%$ 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 **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 $\geq 50\%$ 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 $\geq 50\%$ 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 $\geq 50\%$ 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. fabierciana*: 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. fabierciana* (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.