

# Botryosphaeriaceae from Eucalyptus plantations and adjacent plants in China

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#### Key words

Botryosphaeria Cophinforma Lasiodiplodia Neofusicoccum pathogenicity plant pathogen

Abstract The Botryosphaeriaceae is a species-rich family that includes pathogens of a wide variety of plants, including species of Eucalyptus. Recently, during disease surveys in China, diseased samples associated with species of Botryosphaeriaceae were collected from plantation Eucalyptus and other plants, including Cunninghamina lanceolata, Dimocarpus longan, Melastoma sanguineum and Phoenix hanceana, which were growing adjacent to Eucalyptus. In addition, few samples from Araucaria cunninghamii and Cedrus deodara in two gardens were also included in this study. Disease symptoms observed mainly included stem canker, shoot and twig blight. In this study, 105 isolates of Botryosphaeriaceae were collected from six provinces, of which 81 isolates were from Eucalyptus trees. These isolates were identified based on comparisons of the DNA sequences of the internal transcribed spacer regions and intervening 5.8S nrRNA gene (ITS), and partial translation elongation factor 1-alpha (tef1), β-tubulin (tub), DNA-directed RNA polymerase II subunit (rpb2) and calmodulin (cmdA) genes, the nuclear ribosomal large subunit (LSU) and the nuclear ribosomal small subunit (SSU), and combined with their morphological characteristics. Results showed that these isolates represent 12 species of Botryosphaeriaceae, including Botryosphaeria fusispora, Cophinforma atrovirens, Lasiodiplodia brasiliense, L. pseudotheobromae, L. theobromae and Neofusicoccum parvum, and six previously undescribed species of Botryosphaeria and Neofusicoccum, namely B. pseudoramosa sp. nov., B. qingyuanensis sp. nov., B. wangensis sp. nov., N. hongkongense sp. nov., N. microconidium sp. nov. and N. sinoeucalypti sp. nov. Aside from B. wangensis, C. atrovirens and N. hongkongense, the other nine Botryosphaeriaceae species were isolated from Eucalyptus trees in South China. Botryosphaeria fusispora (26 % of the isolates from Eucalyptus) is the dominant species, followed by L. pseudotheobromae (23 % of the isolates from Eucalyptus). In addition to species found on Eucalyptus trees, we also found B. pseudoramosa on M. sanguineum; B. wangensis on C. deodara; C. atrovirens on D. longan; L. theobromae on C. lanceolata, D. longan and P. hanceana; and N. hongkongense on A. cunninghamii. Pathogenicity tests showed that the 12 species of Botryosphaeriaceae are pathogenic to three Eucalyptus clones and that Lasiodiplodia species are the most aggressive. The results of our study suggest that many more species of the Botryosphaeriaceae remain to be discovered in China. This study also provides confirmation for the wide host range of Botryosphaeriaceae species on different plants.

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#### INTRODUCTION

The Botryosphaeriaceae includes a range of phylogenetically and morphologically diverse fungi with a broad host range and geographic distribution globally (Punithalingam 1980, Slippers & Wingfield 2007, Liu et al. 2012, Phillips et al. 2013). These fungi occur primarily on woody plants including both economically important crops and native trees (Slippers & Wingfield 2007). Many species of Botryosphaeriaceae are well-known pathogens that can cause stem canker, shoot blight and dieback on woody plants; however, some species of Botryosphaeriaceae have been described as latent pathogens or endophytes that cause disease when the plant is under stress conditions (Slippers & Wingfield 2007).

Species of Eucalyptus are widely planted in more than 100 countries, and because of the rapid growth of some Eucalyptus trees, they represent one of the most widely planted genera for commercial forestry worldwide, with approximately 20 million hectares (Mha) established in plantations (Iglesias-Trabad et al. 2009). In China, Eucalyptus plantations have expanded substantially during the past 30 years, with more than 4.5 Mha

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of Eucalyptus established in South China by the end of 2013 (Chen & Chen 2013). Industrial Eucalyptus plantations in China are typically single species or hybrid plantings, often from a few clones that share a common parentage (Wei 2005, Turnbull 2007, Zhou & Wingfield 2011). The model of large-scale plantations with few clones greatly increases the threat from pests and diseases (Wingfield 2003, Wingfield et al. 2008). In recent years, the sustainable development of Eucalyptus plantations in China has been increasingly threatened by pathogens and pests (Zhou & Wingfield 2011). The important diseases in Chinese Eucalyptus plantations include stem canker/wilt caused by species of Botryosphaeriaceae (Chen et al. 2011c), Ceratocystis (Chen et al. 2013, Liu et al. 2015), Chrysoporthe (Chen et al. 2010) and Teratosphaeria (Chen et al. 2011a); leaf blight/ spot caused by species of Teratosphaeriaceae (Burgess et al. 2006), Mycosphaerellaceae (Burgess et al. 2007), Calonectria (Lombard et al. 2010, Chen et al. 2011b) and Quambalaria (Zhou et al. 2007); and bacterial wilt associated with Ralstonia solanacearum (Cao 1982, Old et al. 2003).

Relatively little research has been conducted on diseases caused by Botryosphaeriaceae on Eucalyptus trees in China (Chen et al. 2011c, Li et al. 2015a). Based on DNA sequence comparisons and morphological features, five species of Botryosphaeriaceae have been identified from Eucalyptus in China to date, including Botryosphaeria fabicerciana from FuJian,

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Fig. 1 Disease symptoms on *Eucalyptus* trees caused by *Botryosphaeriaceae*. a. Typical dieback of a *Eucalyptus grandis* clone in FunJian Province; b. dieback of *Eucalyptus globulus*; c-e. stem cankers and lesions on main stems of different *Eucalyptus* clones/genotypes; f. branch and twig blight of a *Eucalyptus grandis* clone; g. fruiting structures with abundant mature dark conidia on a *Eucalyptus* branch; h. new branches germinated after main stem infection.

GuangXi and HaiNan Provinces, *Lasiodiplodia pseudotheobromae* from GuangXi Province, *L. theobromae* from GuangDong and GuangXi Provinces, *Neofusicoccum parvum* from FuJian and GuangXi Provinces and *N. ribis* s.lat. from FuJian Province (Chen et al. 2011c, Li et al. 2015a). These species were collected from cankered stems and blighted branches or twigs, and pathogenicity tests showed that all five species could produce lesions on *Eucalyptus* seedlings or trees (Chen et al. 2011c, Li et al. 2015a).

In China, species of *Botryosphaeriaceae* also have been isolated from a number of other woody and horticultural plants, including *Acacia confusa* (Zhao et al. 2010), *Actinidia chinensis* (Zhou et al. 2015), *Bougainvillea spectabilis*, *Polyscias balfouriana* (Li et al. 2015a), *Juglans regia* (Li et al. 2015b, Yu et al. 2015), *Malus domestica* (Tang et al. 2012, Xu et al. 2015a), *Rosa rugosa* (Chen et al. 2016), *Vitis vinifera* (Yan et al. 2012, 2013) and *Vaccinium corymbosum* (Xu et al. 2015b). *Botryosphaeriaceae* species identified from these plants resided in *Botryosphaeria, Lasiodiplodia* and *Neofusicoccum*. These *Botryosphaeriaceae* were all isolated from diseased tissue of the respective plant hosts.

From 2013–2014, surveys were conducted on *Eucalyptus* in plantations and some plants adjacent to *Eucalyptus*, and diseases with symptoms typical of those caused by *Botryosphaeriaceae* were observed. Diseased samples were collected and the putative *Botryosphaeriaceae* fungi (based on microscopic morphology) were isolated. In addition, few samples previously collected from *Araucaria cunninghamii* and *Cedrus deodara* were also included in this study. The aims of this study are to:

- identify these species of *Botryosphaeriaceae* based on phylogenetic analyses and morphological characteristics;
- clarify the geographic distribution of these *Botryosphae*riaceae species; and
- evaluate pathogenicity of the identified Botryosphaeriaceae species on different Eucalyptus clones.

## MATERIALS AND METHODS

#### Disease symptoms, sample collection and fungal isolation

Disease surveys were mainly conducted on species of Eucalyptus in plantations distributed in FuJian, GuangDong, GuangXi and HaiNan Provinces. Disease symptoms typically caused by Botryosphaeriaceae include tree dieback, stem canker, branch canker and twig blight (Fig. 1). Other plants, including Cunninghamina lanceolata, Dimocarpus longan, Melastoma sanguineum and Phoenix hanceana, which were growing in close proximity to Eucalyptus trees, were also randomly surveyed in this study. These surveys were conducted during 2013-2014. Samples of diseased materials, including stems, branches and twigs that showed typical symptoms of Botryosphaeriaceae infection, were collected and taken to the laboratory for fungal isolation. Diseased branches of C. deodara in HeNan Province and A. cunninghamii in Hong Kong Region with similar symptoms typical of Botryosphaeriaceae collected previously, were also added in this study (Fig. 1).

Fungi were isolated from diseased stems, branches and twigs, as well as from pycnidia produced on diseased tissues of *Eucalyptus* and other plants. When pycnidia formed on the surface of diseased tissue, the pycnidia were scratched lightly with a sterile scalpel and transferred with a sterile steel needle to 2 % malt extract agar (MEA) media containing 20 g of malt extract powder (Beijing Shuangxuan Microbial Culture Medium Products Factory, Beijing, China) and 20 g of agar per litre of water (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) under a stereomicroscope (Carl Zeiss Ltd., Munchen, Germany). For diseased tissues that did not produce pycnidia,

small tissue pieces (approximately 0.25 cm<sup>2</sup>) were cut from inner wood and transferred to 2 % MEA. Pieces of pycnidia and wood were incubated at room temperature for 2–5 d until colonies formed. Colonies with morphological characteristics typical of *Botryosphaeriaceae* were transferred to fresh 2 % MEA plates. Pure cultures were obtained by transferring single hyphal tips from colonies to 2 % MEA. Cultures were deposited in the culture collection of the China Eucalypt Research Centre (CERC), Chinese Academy of Forestry (CAF), ZhanJiang, GuangDong Province, China. Isolates linked to type specimens of the fungal species were deposited in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China. The specimens were deposited in the Collection of Central South Forestry Fungi of China (CSFF), GuangDong Province, China.

## DNA extraction, PCR amplification and sequencing

DNA extractions and sequence comparisons were conducted on selected isolates collected from different trees and different regions (Table 1). For the selected isolates, mycelia were scraped from 7-d-old cultures using sterile scalpels and transferred to 2 mL Eppendorf tubes. A CTAB-based protocol, 'Method 5' described by Van Burik et al. (1998), was used to extract the DNA samples. The resulting DNA was checked for purity and concentration using a NanoDrop 2000 Spectrometer (Thermo Fisher Scientific Inc. Waltham, MA, USA). Prior to PCR amplification, each DNA sample was diluted to approximately 100 ng/µL with DNase/RNase-free ddH<sub>2</sub>O (Sangon Biotech Co., Ltd., Shanghai, China). The internal transcribed spacer (ITS) region was amplified using the primers ITS1/ITS4 (White et al. 1990), a part of the translation elongation factor 1-alpha (tef1) gene was amplified using the primers EF1-728F/EF1-986R (Carbone & Kohn 1999) or EF1F/EF2R (Jacobs et al. 2004), a part of the  $\beta$ -tubulin (*tub*) gene was amplified using the primers BT-2a/BT-2b (Glass & Donaldson 1995), a part of DNAdirected RNA polymerase II subunit (rpb2) gene was amplified using the primers fRPB2-5F/fRPB2-7cR for Botryosphaeria and Cophinforma (Liu et al. 1999), rpb2-LasF/rpb2-LasR for Lasiodiplodia (Cruywagen et al. 2017) and RPB2bot6F/RPB-2bot7R for Neofusicoccum (Pavlic et al. 2009a, Sakalidis et al. 2011), the nuclear ribosomal large subunit (LSU) region was amplified using the primers LR0R/LR5 (Vilgalys & Hester 1990, Cubeta et al. 1991), the nuclear ribosomal small subunit (SSU) region was amplified using the primers NS1/NS4 (White et al. 1990). For the isolates of Lasiodiplodia, a portion of the calmodulin (cmdA) gene was amplified using the primers CAL-228F/CAL-737R (Carbone & Kohn 1999). All primers were synthesised by Life Technologies (Thermo Fisher Scientific Inc., Shanghai, China). The PCR mixtures to amplify the ITS, tef1, tub, rpb2, cmdA, LSU, SSU regions used the TopTaq™ Master Mix Kit (Qiagen Inc., Hilden, Germany). All amplification reactions consisted of 25 µL TopTaq<sup>™</sup> Master Mix (contain 1.25 U TopTaq<sup>™</sup> DNA Polymerase, 200 µM of each dNTP and 1.5 mM MgCl<sub>2</sub>), 0.2 mM of each primer and 50 ng template DNA (made up to a total volume of 50 µL with RNase-free water). The amplification conditions consisted of an initial denaturation step at 94 °C for 3 min, 35 cycles of 94 °C for 1 min, 55 °C (except 45 °C for SSU) for 1 min, and 72 °C for 1 min, followed by a final elongation step at 72 °C for 10 min.

PCR amplifications were carried out in a thermocycler (Bio-Rad Laboratories, Inc., Berkeley, California, USA). The PCR products were separated by electrophoresis in 1.5 % agarose gels with SYBR Safe DNA Gel Stain (Thermo Fisher Scientific Inc., USA) in 1× Tris-acetate-EDTA (TAE) buffer at a constant voltage (80 V) for 30 min. All PCR products were sequenced in both directions using the primers specified above by Beijing Genomics Institution, Guangzhou, GuangDong Province, China. The

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Species <sup>1</sup>	Isolate No. <sup>2,3</sup>	Genotype⁴	Host	Location	GPS information	Collector			GenBank	accession N	lo. <sup>5</sup>		
							ITS	tef1	tub	rpb2	cmdA	LSU	SSU
Botryosphaeria fusispor	a CERC1997	AAAA-AA	Eucalyptus hybrid	BeiHai Region, GuangXi Province, China	N21°35'41" E109°43'01"	S.F. Chen & G.Q. Li	KX277967	KX278072	KX278177	MF410116	N/A	MF410007	MF410205
	CERC2273	AAAA-AA	Eucalyptus hybrid	FuZhou Region, FuJian Province, China	N26°13'39" E119°10'51"	S.F. Chen & G.Q. Li	KX277968	KX278073	KX278178	MF410117	N/A	MF410008	MF410206
	CERC227467	AAAA-AA	Eucalyptus hybrid	FuZhou Region, FuJian Province, China	N26°13'39" E119°10'51"	S.F. Chen & G.Q. Li	KX277969	KX278074	KX278179	MF410118	N/A	MF410009	MF410207
	CERC2910	AAAA-AA	Eucalyptus hybrid	BeiHai Region, GuangXi Province, China	Unknown	S.F. Chen & G.Q. Li	KX277970	KX278075	KX278180	MF410119	N/A	MF410010	MF410208
	CERC2912	AAAA-AA	Eucalyptus hybrid	BeiHai Region, GuangXi Province, China Daittai Dacian, CuranzVi Dravinco, China	Unknown	S.F. Chen & G.Q. Li	KX277971	KX278076	KX278181	MF410120	N/A	MF410011	MF410209
	CERC2813		Eucaryptus riyorid Eucalvatus hybrid	Deinal Region, GuangAl Flovince, Clillia Zhan liang Bagion GuangDong Province, China	UIIKIIUWII NO0°44'30" E440°04'47"		KY27707A	KX278070	KY778184	ME410121		ME410012	ME410210
	CERC3469		Eucaryptus riybrid Fucalvotus hvhrid	Zhan Jiang Region, Guangoong Frovince, China Zhan Jiang Region Guanghong Province China	N20°41'20" E110'01'17" N20°41'20" E110°01'17"		KX277975	KX278080	KX278185	MF410123		ME410015	ME410213
	CERC3474	AAAA-AA	Eucalvotus hybrid	ZhanJiang Region, GuangDong Province, China	N20°41'20" E110°01'17"	S.F. Chen & G.Q. Li	KX277976	KX278081	KX278186	MF410125	N/A	MF410016	MF410214
	<b>CERC3426</b>	AAAA-AB	Eucalyptus hybrid	BeiHai Region, GuangXi Province, China	N21°35'49" E109°43'49"	S.F. Chen & G.Q. Li	KX277973	KX278078	KX278183	MF410122	N/A	MF410013	MF410211
	CERC19987	ABAA-AA	Eucalyptus hybrid	BeiHai Region, GuangXi Province, China	N21°35'41" E109°43'01"	S.F. Chen & G.Q. Li	KX277977	KX278082	KX278187	MF410126	N/A	MF410017	MF410215
	CERC2006	ABAA-AA	Eucalyptus hybrid	ZhanJiang Region, GuangDong Province, China	N21°15'26" E110°07'00"	S.F. Chen & G.Q. Li	KX277978	KX278083	KX278188	MF410127	N/A	MF410018	MF410216
	CERC2911 <sup>6</sup>	ABAA-AA	Eucalyptus hybrid	BeiHai Region, GuangXi Province, China	Unknown	S.F. Chen & G.Q. Li	KX277979	KX278084	KX278189	MF410128	N/A	MF410019	MF410217
	CERC2918°	ABAA-AA	Eucalyptus hybrid	BeiHai Region, GuangXi Province, China	N21°35'41" E109°43'01"	S.F. Chen & G.Q. Li	KX277980	KX278085	KX278190	MF410129	N/A	MF410020	MF410218
	CERC2921	ABAA-AA	Eucalyptus hybrid	BeiHai Region, GuangXi Province, China	N21°35'41" E109°43'01"	S.F. Chen & G.Q. Li	KX277981	KX278086	KX278191	MF410130	N/A	MF410021	MF410219
	CERC2925	ABAA-AA	Eucalyptus hybrid	BeiHai Region, GuangXi Province, China	N21°35'41" E109°43'01"	S.F. Chen & G.Q. Li	KX277982	KX278087	KX278192	MF410131	N/A	MF410022	MF410220
	CERC2948	ABAA-AA	Eucalyptus hybrid	QingYuan Region, GuangDong Province, China	N23°51'44" E113°10'58"	S.F. Chen & G.Q. Li	KX277983	KX278088	KX278193	MF410132	N/A	MF410023	MF410221
	CERC2949	ABAA-AA	Eucalyptus hybrid	QingYuan Region, GuangDong Province, China	N23°51'44" E113°10'58"	S.F. Chen & G.Q. Li	KX277984	KX278089	KX278194	MF410133	N/A	MF410024	MF410222
	CERC2954	ABAA-AA	Eucalyptus hybrid	QingYuan Region, GuangDong Province, China	N23°51'44" E113°10'58"	S.F. Chen & G.Q. Li	KX277985	KX278090	KX278195	MF410134	N/A	MF410025	MF410223
	CERC3446	ABAA-AA	Eucalyptus hybrid	ZhanJiang Region, GuangDong Province, China	N20°41'20" E110°01'17"	S.F. Chen & G.Q. Li	KX277986	KX278091	MF409964	MF410135	A/A	MF410026	MF410224
	CERC2930	ACAA-AA	Eucalyptus hybrid	BeiHai Region, GuangXi Province, China	N21°35'41" E109°43'01"	S.F. Chen & G.Q. Li	KX277987	KX278092	KX278196	MF410136	A/N	MF410027	MF410225
B. pseudoramosa	CERC1999°	AAAA-AA	Eucalyptus hybrid	BeiHai Region, GuangXi Province, China	N21°35'41" E109°43'01"	S.F. Chen & G.Q. Li	KX277988	KX278093	KX278197	MF410139	A/A	MF410030	MF410228
	CERC2001	AAAA-AA	<i>Eucalyptus</i> hybrid	BeiHai Region, GuangXi Province, China	N21°35'41" E109°43'01"	S.F. Chen & G.Q. Li	KX277989	KX278094	KX278198	MF410140	N/A	MF410031	MF410229
		18739°'''''''	Eucolynatics highrid	Doithei Booice, Guerra Vi Brovince, Chine	NO4026141" E400042104"		00027077	10002CV	00197077	ME410141	N/N	ME410030	ME410230
	CERC:2019	AAAA-AA	Eucalyptus nybrid	Beillai Region, GuandXi Frovince, China Beillai Region GuandXi Province China		S.F.Chen & G.O. Li	KX277991	KX278096	KX278200	MF410142	A/N	MF410033	MF410231
	CERC2983	AAAA-AA	Melastoma sanguineum	ZhanJiang Region, Guangong Province, China ZhanJiang Region, GuangDong Province, China	N21°13'24" E110°24'04"	S.F. Chen	KX277992	KX278097	KX278201	MF410143	A/N	MF410034	MF410232
	= CGMCC3	.18740 <sup>6</sup>											
	CERC2985	AAAA-AA	M. sanguineum	ZhanJiang Region, GuangDong Province, China	N21°13'24" E110°24'04"	S.F. Chen	KX277993	KX278098	KX278202	MF410144	N/A	MF410035	MF410233
	CERC298769	AAAA-AA	M. sanguineum	ZhanJiang Region, GuangDong Province, China	N21°13'24" E110°24'04"	S.F. Chen	KX277994	KX278099	KX278203	MF410145	N/A	MF410036	MF410234
	CERC2988°	AAAA-AA	M. sanguineum	ZhanJiang Region, GuangDong Province, China	N21°13'24" E110°24'04"	S.F. Chen	KX277995	KX278100	KX278204	MF410146	N/A	MF410037	MF410235
	CERC34527	AAAA-AA	Eucalyptus hybrid	ZhanJiang Region, GuangDong Province, China	N20°41'20" E110°01'17"	S.F. Chen & G.Q. Li	KX277996	KX278101	KX278205	MF410147	N/A	MF410038	MF410236
	CERC3455	AAAA-AA	Eucalyptus hybrid	ZhanJiang Region, GuangDong Province, China	N20°41'20" E110°01'17"	S.F. Chen & G.Q. Li	KX277997	KX278102	KX278206	MF410148	N/A	MF410039	MF410237
	= CGMCC:	5.18/41°											
	CERC3462	AAAA-AA	Eucalyptus hybrid	ZhanJiang Region, GuangDong Province, China Zhan liang Province, CuangDong Province, China	N20°41'20" E110°01'17" N20°41'20" E110°01'17"	S.F. Chen & G.Q. Li	KX277998	KX278103	KX278207	MF410149	N/A	MF410040	MF410238
B. ainavuanensis	CERC2946	AA-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	Eucaryptus nybrid Fucalvotus hvbrid	Zitariotarig Region, Guariguorig Province, Citita DingYuan Region, GuangDong Province, China	N23°44'30" F112°48'49"	S.F. Chen & G.Q. Li	KX278000	KX278105	KX278209	MF410150 MF410151	A/N	MF410041 MF410042	MF410239
	= CGMCC3	.187426.7,8.9				5							
	<b>CERC2947</b>	AAAA-AA	Eucalyptus hybrid	QingYuan Region, GuangDong Province, China	N23°44'30" E112°48'49"	S.F. Chen & G.Q. Li	KX278001	KX278106	KX278210	MF410152	N/A	MF410043	MF410241
	= CGMCC3	18743 <sup>7,9</sup>											
B. wangensis	CERC2298	AAAA-AA	C. deodara	XinZhuang, MangChuan, RuZhou Region,	N34°04'09.8"	S.F. Chen	KX278002	KX278107	KX278211	MF410153	N/A	MF410044	MF410242
	= CGMCCS	A A A A - A A	caepoep J	YinZhuana Province, China YinZhuana ManaChuan BuiZhou Beaton	E112°49'00.7" Ni34°04'00 8"	S F Chan	KX778003	K X778108	KX778717	ME410154	N/A	MEATODAR	ME410243
		.187456.7	0. 00000	Henan Province, China	E112°49'00.7"	1910 . 10			7170/7001				
	<b>CERC2300</b>	AAAA-AA	C. deodara	XinZhuang, MangChuan, RuZhou Region,	N34°04'09.8"	S.F. Chen	KX278004	KX278109	KX278213	MF410155	N/A	MF410046	MF410244
	= CGMCC3	18746 <sup>6,9</sup>		HeNan Province, China	E112°49'00.7"								
Cophinforma atrovirens	CERC3481	AAAA-AA	Dimocarpus longan	ZhanJiang Region, GuangDong Province, China	Unknown	S.F. Chen	KX278005	KX278110	KX278214	MF410156	N/A	MF410047	MF410245
	CERC3482	AAAA-AA	D. longan	ZhanJiang Region, GuangDong Province, China Zhan liang Bogina, CuangDong Province, China	Unknown	S.F. Chen	KX2/8006	KX2/8111	612872XX	MF41015/	N/A	MF410048	MF410246
	CERC3404	RAAA-AA	D. Iongan	ZhanJang Region, GuangDong Frovince, Cilina Zhan liang Region, GuangDong Province, China	l lnknown	S.F. Chen	KX278008	KX278113	KX278217	MF410150		ME410050	MF410248
	CERC3490	BAAA-AA	D. longan	Zhan Jiang Region, GuangDong Province, China	Unknown	S.F. Chen	KX278009	KX278114	KX278218	MF410160	N/A	MF410051	MF410249
Lasiodiplodia brasiliense	• CERC228467	AAAAAA	Eucalyptus hybrid	ZhangZhou Region, FuJian Province, China	N24°46'06" E117°51'02"	S.F. Chen & G.Q. Li	KX278010	KX278115	KX278219	MF410163	MF409967	MF410054	MF410252
L. pseudotheobromae	CERC2262	AAAAAA	Eucalyptus hybrid	YuLin Region, GuangXi Province, China	N22°09'12" E110°12'08"	S.F. Chen & G.Q. Li	KX278011	KX278116	KX278220	MF410164	MF409968	MF410055	MF410253
	CERC2280	AAAAAA	Eucalyptus hybrid	ZhangZhou Region, FuJian Province, China	N24°46'06" E117°51'02"	S.F. Chen & G.Q. Li	KX278012	KX278117	KX278221	MF410165	MF409969	MF410056	MF410254

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Species <sup>1</sup>	Isolate No. <sup>2/5</sup>	<sup>5</sup> Genotype⁴	Host	Location	GPS information	Collector			GenBank	accession No	0. <sup>5</sup>		
						I	ITS	tef1	tub	rpb2	cmdA	LSU	SSU
L. pseudotheobromae	CERC2281	AAAAAA	<i>Eucalyptus</i> hybrid	ZhangZhou Region, FuJian Province, China	N24°46'06" E117°51'02"	S.F. Chen & G.Q. Li	KX278013	KX278118	KX278222	MF410166	MF409970	MF410057	MF410255
(cont.)	CERC2282	AAAAAA	Eucalyptus hybrid	ZhangZhou Region, FuJian Province, China	N24°46'06" E117°51'02"	S.F. Chen & G.Q. Li	KX278014	KX278119	KX278223	MF410167	MF409971	MF410058	MF410256
	CFRC2283	AAAAAA	Eucalvotus hybrid	ZhandZhou Redion Fullian Province China	N24°46'06" E 117°51'02"	S F Chen & G O Li	KX278015	KX278120	KX278224	MF410168	MF409972	MF410059	MF410257
	CEDC000667	~~~~~	Eucolimatics by brid	ZhanaZhou Dogion Eu lian Drovince China	NO4°46'06" E447°64'00"		NV77016	10107077	K V770775	MEALOLED	ME400073	ME410060	ME410260
				Zhangzhou region, ruoian riovinec, Onna Zhanzzhan Dazian Fritian Daniman Ohina					077017020			AF 4 0004	
								7710/774					
	CERC2288	AAAAAA	<i>Eucalyptus</i> hybrid	ZhangZhou Region, FuJian Province, China	N24*46'06" E117*51'02"	S.F. Chen & G.Q. LI	KX278018	KX278123	KX278227	MF410171	MF409975	MF410062	MF410260
	CERC2289	AAAAAA	<i>Eucalyptus</i> hybrid	ZhangZhou Region, FuJian Province, China	N24°46'06" E117°51'02"	S.F. Chen & G.Q. Li	KX278019	KX278124	KX278228	MF410172	MF409976	MF410063	MF410261
	CERC2960	AAAAAA	<i>Eucalyptus</i> hybrid	YunFu Region, GuangDong Province, China	N23°15'12" E111°41'51"	S.F. Chen & G.Q. Li	KX278020	KX278125	KX278229	MF410173	MF409977	MF410064	MF410262
	CERC2961	AAAAAA	<i>Eucalyptus</i> hybrid	YunFu Region, GuangDong Province, China	N23°15'12" E111°41'51"	S.F. Chen & G.Q. Li	KX278021	KX278126	KX278230	MF410174	MF409978	MF410065	MF410263
	CERC34177	AAAAAA	<i>Eucalyptus</i> hybrid	BeiHai Region, GuangXi Province, China	N21°35'49" E109°43'49"	S.F. Chen & G.Q. Li	KX278023	KX278128	KX278232	MF410176	MF409980	MF410067	MF410265
	CERC3432 <sup>6</sup>	AAAAAA	Eucalvotus hybrid	BeiHai Region, GuangXi Province, China	N21°35'49" E109°43'49"	S.F. Chen & G.Q. Li	KX278024	KX278129	KX278233	MF410177	MF409981	MF410068	MF410266
	CFRC3434	AAAAAA	Eucalvotus hybrid	BeiHai Region GuandXi Province, China	N21°35'49" F109°43'49"	S.F. Chen & G.O. Li	KX278025	KX278130	KX278234	MF410178	MF409982	MF410069	MF410267
	CERC3438	AAAAAA	Eucalvatus hvhrid	BeiHai Region GuangXi Province China	N21°35'49" F109°43'49"	S F Chen & G O Li	KX278026	KX278131	KX778235	MF410179	MF409983	MF410070	MF410268
	CERC3475	AAAAAA	Eucalvotus hybrid	BeiHai Region, GuandXi Province, China	N21°35'49" E109°43'49"	S.F. Chen & G.O. Li	KX278027	KX278132	KX778236	MF410180	MF409984	MF410071	MF410269
	CERC34957	AAAAAA	E uronhvlla × F orandis	Zhan Iiang Region Guandhong Province China	N21°13'31" E110°23'47"	S.F. Chen & G.O. Li	KX278028	KX278133	KX778237	MF410181	MF409985	MF410072	MF410270
	CEDC3406		E. archina × E. grandis E. urchinla × E. grandis	Zhan Jiang Degion, Guangbong Frovince, Omia Zhan Jiang Degion, Guangbong Drovince, China	ND1010121 E110 20 47		KY778070	KX278134	KY778738	ME410182	MEADOORG	ME410073	ME410271
			E. uropriyira × E. grarius Ericohistischischich	Zitatiotalig Regioni, Guanguorig Flovince, Crinia Vineri Dogion Guond Drovinco Phino	ND20161401 E110 2047		670012XX	701012VV	122020727	ME410102		ME410073	ME410271
1 40000000	CERC2302		Eucarypius Iriyoriu	Tuliru Regioni, Gualiguolig Province, Cilila Zhan liana Bazian CuanaDana Davinca China	1014 1113 7101 0701		72001277	121012V	10201277	ME410173	ME409979	ME410054	ME410204
			Fridelik handearia	Zitariotarig Neglori, Guariguorig Frovince, Crima Beitlei Bozien Cuenzy: Bravince, Chine	ND4005140" E100 01 01			96192077				ME410075	ME410272
	CERC3420		Eucalyptus Itybrid	Beithai Regioti, GuanigAt Frovince, Crinta Deithai Deaton, CueneXi Brevince, China	N21026140" E100 4349"		1002200	75197577	VV270241	ME410104			ME410273
									1 4 20 1 2001	MF410103	MT 400000	MI 410070	MI 410275
	CERCZUZS	ABAAAAA	P. nanceana	ZhanJiang Region, GuangDong Province, China	NZ1-15.26 E110-07.01		KX2/8033	KXZ/8138	KX2/8242	MIF410186		MF410077	G/Z01.4-1M
	CERC2264	ABAAAA	E. urophylla × E. grandis	YuLin Region, GuangXi Province, China	N22°09'12" E110°12'08"	S.F. Chen & G.Q. Li	KX278034	KX278139	KX278243	MF410187	MF409991	MF410078	MF410276
	CERC2275	ABAAAA	E. urophylla × E. grandis	YongAn Region, FuJian Province, China	N26°01'40" E117°27'11"	S.F. Chen & G.Q. Li	KX278035	KX278140	KX278244	MF410188	MF409992	MF410079	MF410277
	CERC2934	ABAAAA	Eucalyptus hybrid	DingAn County, HaiNan Province, China	N19°36'41" E110°17'16"	S.F. Chen & G.Q. Li	KX278036	KX278141	KX278245	MF410189	MF409993	MF410080	MF410278
	CERC2957	ABAAAA	Cunninghamina	ShaoGuan Region, GuangDong Province, China	N24°31'32" E113°37'40"	S.F. Chen & G.Q. Li	KX278037	KX278142	KX278246	MF410190	MF409994	MF410081	MF410279
			lanceolata										
	CERC2958	ABAAAA	C. lanceolata	ShaoGuan Region, GuangDong Province, China	N24°31'32" E113°37'40"	S.F. Chen & G.Q. Li	KX278038	KX278143	KX278247	MF410191	MF409995	MF410082	MF410280
	CERC2963	ABAAAA	<i>Eucalyptus</i> hybrid	YunFu Region, GuangDong Province, China	N23°15'12" E111°41'51"	S.F. Chen & G.Q. Li	KX278039	KX278144	KX278248	MF410192	MF409996	MF410083	MF410281
	CERC3418	ABAAAA	Eucalyptus hybrid	BeiHai Region, GuangXi Province, China	N21°35'49" E109°43'49"	S.F. Chen & G.Q. Li	KX278040	KX278145	KX278249	MF410193	MF409997	MF410084	MF410282
	CERC3422	ABAAAA	Eucalyptus hybrid	BeiHai Region, GuangXi Province, China	N21°35'49" E109°43'49"	S.F. Chen & G.Q. Li	KX278041	KX278146	KX278250	MF410194	MF409998	MF410085	MF410283
	CERC3485	ABAAAA	D. longan	ZhanJiang Region, GuangDong Province, China	Unknown	S.F. Chen	KX278042	KX278147	KX278251	MF410195	MF409999	MF410086	MF410284
	CERC3486	ABAAAA	D. longan	ZhanJiang Region, GuangDong Province, China	Unknown	S.F. Chen	KX278043	KX278148	KX278252	MF410196	MF410000	MF410087	MF410285
	CERC3487	ABAAAA	D. longan	ZhanJiang Region, GuangDong Province, China	Unknown	S.F. Chen	KX278044	KX278149	KX278253	MF410197	MF410001	MF410088	MF410286
	CERC3491	ABAAAA	D. longan	ZhanJiang Region, GuangDong Province, China	Unknown	S.F. Chen	KX278045	KX278150	KX278254	MF410198	MF410002	MF410089	MF410287
	CERC3493	ABAAAA	D. longan	ZhanJiang Region, GuangDong Province, China	Unknown	S.F. Chen	KX278046	KX278151	KX278255	MF410199	MF410003	MF410090	MF410288
	CERC3513 <sup>60</sup>	7 ABAAAAA	E. urophylla × E. grandis	ZhanJiang Region, GuangDong Province, China	N21°13'31" E110°23'47"	S.F. Chen & G.Q. Li	KX278047	KX278152	KX278256	MF410200	MF410004	MF410091	MF410289
	CERC3514	ABAAAA	E. urophylla × E. grandis	ZhanJiang Region, GuangDong Province, China	N21°13'31" E110°23'47"	S.F. Chen & G.Q. Li	KX278048	KX278153	KX278257	MF410201	MF410005	MF410092	MF410290
	CERC35167	ABAAAA	E. urophylla × E. grandis	ZhanJiang Region, GuangDong Province, China	N21°13'31" E110°23'47"	S.F. Chen & G.Q. Li	KX278049	KX278154	KX278258	MF410202	MF410006	MF410093	MF410291
Neofusicoccum	CERC2967	AAAA-AA	Araucaria cunninghamii	Hong Kong, China	Unknown	S.F. Chen	KX278050	KX278155	KX278259	KX278281	N/A	MF410094	MF410292
hongkongense	= CGMCCS	8.18747											
	CERC2968	AABA-AA	A. cunninghamii	Hong Kong, China	Unknown	S.F. Chen	KX278051	KX278156	KX278260	KX278282	N/A	MF410095	MF410293
		01-01-00-00-00-00-00-00-00-00-00-00-00-0		Lana Kana China		2040 L 0		2102020	10000000			940.440006	100011
	= CGMCC3	18749 <sup>6,7,8,9</sup>	A. curringnami	пону кону, Сшна		O.F. OIEI	7000/774	1010/700	1070/774	C020/2VV	Y N	MIF4 10090	MF410234
N. microconidium	CERC3497	AAAA-AA	E. urophylla × E. grandis	ZhanJiang Region, GuangDong Province, China	N21°13'31" E110°23'47"	S.F. Chen & G.Q. Li	KX278053	KX278158	KX278262	MF410203	N/A	MF410097	MF410295
	= CGMCC:	3.18750 <sup>6.7.8,9</sup> ΔΔΔΔ-ΔΔ	E uronhvilla < E arandis	Zhan Ijang Begion, Guanghong Province, China	ND1°13'31" E110°03'47"	S E Chan & G O Li	K X 278054	KX778150	КХЭТВЭЕЗ	ME410204	N/A	ME410098	ME410296
		.18751 <sup>67,9</sup>											
N. parvum	CERC29517	AAAA-AA	E. urophylla × E. grandis	QingYuan Region, GuangDong Province, China	N23°51'44" E113°10'58"	S.F. Chen & G.Q. Li	KX278055	KX278160	KX278264	KX278284	N/A	MF410099	MF410297
		AAAA-AA	E. urophylia × E. grandis E. urophylia × E. crandis	ZhanJiang Region, GuangDong Province, China Zhan liang Bedion, GuandDong Brovince, China	NZT 13.31 E110 Z34/ N21°13'31" E110°23'17"			KX2/8101 KY778167	2028/2XA	00201277	N/A	ME410100	ME410298
	CERC3502	ABAA-AA	E. urophyla × E. grandis E. urophyla × E. grandis	Zhanshang region, Guangbong Frovince, China Zhan liang Region, Guangbong Province, China	N21°13'31" E110°23'47"	S.F. Chen & G.O. Li	KX278058	KX278163	KX278267	KX278287	N/A	MF410102	MF410300
	CERC3503 <sup>6</sup>	ABAA-AA	E. urophvlla × E. grandis	ZhanJiang Region, GuangDong Province, China	N21°13'31" E110°23'47"	S.F. Chen & G.O. Li	KX278059	KX278164	KX278268	KX278288	N/A	MF410103	MF410301
	CERC3504	ABAA-AA	E urophvlla × E. grandis	Zhan Ilang Region. GuangDong Province, China	N21°13'31" E110°23'47"	S.F. Chen & G.Q. Li	KX278060	KX278165	KX278269	KX278289	N/A	MF410104	MF410302

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Species <sup>1</sup>	Isolate No. <sup>25</sup>	³ Genotype⁴	Host	Location	GPS information	Collector			GenBank	accession N	0. <sup>5</sup>		
							ПS	tef1	tub	rpb2	cmdA	LSU	SSU
N. sinoeucalypti	CERC2005 = CGMCC3	AAAA-AA .187526.7.8.9	E. urophylla × E. grandis	ZhanJiang Region, GuangDong Province, China	N21°15'26" E110°07'00"	S.F. Chen & G.Q. Li	KX278061	KX278166	KX278270	KX278290	N/A	MF410105	MF410303
	CERC34156	AAAA-AA	Eucalyptus hybrid	BeiHai Region, GuangXi Province, China	N21°35'49" E109°43'49"	S.F. Chen & G.Q. Li	KX278063	KX278168	KX278272	KX278292	N/A	MF410107	MF410305
	CERC3416 = CGMCC3	AAAA-AA 18754°	Eucalyptus hybrid	BeiHai Region, GuangXi Province, China	N21°35'49" E109°43'49"	S.F. Chen & G.Q. Li	KX278064	KX278169	KX278273	KX278293	N/A	MF410108	MF410306
	CERC3457	AAAA-AA	E. urophylla × E. grandis	ZhanJiang Region, GuangDong Province, China	N20°41'20" E110°01'17"	S.F. Chen & G.Q. Li	KX278066	KX278171	KX278275	KX278295	N/A	MF410110	MF410308
	CERC3458	AAAA-AA	E. urophylla × E. grandis	ZhanJiang Region, GuangDong Province, China	N20°41'20" E110°01'17"	S.F. Chen & G.Q. Li	KX278067	KX278172	KX278276	KX278296	N/A	MF410111	MF410309
	CERC3463 7	AAAA-AA	E. urophylla × E. grandis	ZhanJiang Region, GuangDong Province, China	N20°41'20" E110°01'17"	S.F. Chen & G.Q. Li	KX278068	KX278173	KX278277	KX278297	N/A	MF410112	MF410310
	CERC3464	AAAA-AA	E. urophylla × E. grandis	ZhanJiang Region, GuangDong Province, China	N20°41'20" E110°01'17"	S.F. Chen & G.Q. Li	KX278069	KX278174	KX278278	KX278298	N/A	MF410113	MF410311
	CERC3467	AAAA-AA	E. urophylla × E. grandis	ZhanJiang Region, GuangDong Province, China	N20°41'20" E110°01'17"	S.F. Chen & G.Q. Li	KX278070	KX278175	KX278279	KX278299	N/A	MF410114	MF410312
	CERC3517	AAAA-AA	E. urophylla × E. grandis	ZhanJiang Region, GuangDong Province, China	N21°13'31" E110°23'47"	S.F. Chen & G.Q. Li	KX278071	KX278176	KX278280	KX278300	N/A	MF410115	MF410313
	<b>CERC2265</b>	AAAA-AB	E. urophylla × E. grandis	YuLin Region, GuangXi Province, China	N22°08'55" E110°12'00"	S.F. Chen & G.Q. Li	KX278062	KX278167	KX278271	KX278291	N/A	MF410106	MF410304
	= CGMCC3	1.18753 <sup>6,9</sup>											
	CERC3451	AAA-AB	E. urophylla × E. grandis	ZhanJiang Region, GuangDong Province, China	N20°41'20" E110°01'17"	S.F. Chen & G.Q. Li	KX278065	KX278170	KX278274	KX278294	N/A	MF410109	MF410307
<sup>1</sup> Species names in <b>bc</b>	ld are novel spe	cies describe	ed in this study.										

Isolates in **bold** are in the phylogenetic trees. CERC: Culture Collection of China Eucalypt Research Centre, Chinese Academy of Forestry, ZhanJiang, GuangDong Province, China; CGMCC: China General Microbiological Culture Collection Center, Beijing, China

calmodulin; LSU, nuclear ribosomal large subunit; SSU, nuclear ribosomal small subunit; N/A Genotype within each identified species, determined by ITS, *terf., tub, rpb2, cmd*A, LSU and SSU regions; --- means not available. ITS, internal transcribed spacer region and intervening 5.8S mrRNA gene; *terf.*, translation elongation factor 1-alpha; *tub*, *f*-tubulin; *rpb2*, DNA-directed RNA polymerase II subunit; *cmdA*,

Isolates used for morphological = not available.

Eucalyptus clones studies

Isolates used for pathogenicity tests on three Isolates represent ex-type. Isolates used for culture growth studies.

nucleotide sequences were edited with MEGA v. 6.0.5 software (Tamura et al. 2013). Sequences obtained in this study were all deposited in GenBank (http://www.ncbi.nlm.nih.gov) (Table 1).

## Phylogenetic analyses

The preliminary identities of the isolates sequenced in this study were obtained by conducting a standard nucleotide BLAST search using the ITS, tef1, tub, rpb2, cmdA, LSU, SSU seguences. The sequences of the ex-type strains that were closely related to the Botryosphaeriaceae isolates sequenced in this

The BLAST results showed that the isolates collected in this study were grouped in the genera Botryosphaeria, Cophinforma, Lasiodiplodia and Neofusicoccum. Phylogenetic analyses were conducted for each of the ITS, tef1, tub, rpb2, cmdA, LSU and SSU datasets for genera Botryosphaeria/Cophinforma, Lasiodiplodia and Neofusicoccum, respectively. As the cmdA sequences are only available for Lasiodiplodia, and not for Botryosphaeria, Cophinforma and Neofusicoccum, the analyses for cmdA sequences were only conducted for the genus Lasiodiplodia.

Phylogenetic analyses were also conducted for combined datasets, as the LSU and SSU sequences are not available for some of the previously described species of Botryosphaeria, Cophinforma, Lasiodiplodia and Neofusicoccum, and the rpb2 sequences are not available for some species of Botryosphaeria. The ITS, tef1 and tub sequences were combined for phylogenetic analyses of Botryosphaeria/Cophinforma isolates, the ITS, tef1, tub, rpb2 and cmdA sequences were combined for Lasiodiplodia isolates, and ITS, tef1, tub and rpb2 sequences

Two phylogenetic analysis methods were used: PAUP v. 4.0b10 (Swofford 2003) for the maximum parsimony (MP) analyses and PhyML v. 3.0 (Guindon et al. 2010) for maximum likelihood (ML) tests. For MP analyses, gaps are treated as a fifth character and the characters are unordered and of equal weight with 1000 random addition replicates. The equally most parsimonious trees were obtained using the heuristic search function and tree bisection and reconstruction (TBR) as the branch swapping algorithms. MAXTREES were limited to 5 000, and branch lengths of zero were collapsed. A bootstrap analysis (50 % majority rule, 1000 replicates) was performed to determine the confidence levels of the tree-branching points (Felsenstein

1985). Tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) were used to evaluate the trees (Hillis & Huelsenbeck 1992). For ML analyses of each dataset, the best models of nucleotide

substitution were determined using jModelTest v. 2.1.5 (Darriba et al. 2012). Additional ML parameters in PhyML include the

retention of the maximum number of 1 000 trees and the determination of nodal support by non-parametric bootstrapping

with 1 000 replicates. All phylogenetic trees were viewed using MEGA v. 6.0.5 (Tamura et al. 2013). Neofusicoccum parvum (ATCC 58191) was used as the outgroup taxon for analyses of Botryosphaeria and Cophinforma; Botryosphaeria dothidea

(CBS 115476) was used as the outgroup taxon for analyses of

Lasiodiplodia and Neofusicoccum (Table 2).

were combined for Neofusicoccum isolates.

study were downloaded from NCBI (http://www.ncbi.nlm.nih. gov/) and used for polygenetic analyses (Table 2). Sequences were aligned using MAFFT online v. 7 (http://mafft.cbrc.jp/ alignment/server/) (Katoh & Standley 2013), with the iterative refinement method (FFT-NS-i setting). The alignments were further edited manually with MEGA v. 6.0.5 software (Tamura et al. 2013). Resulting alignments and phylogenetic trees for all the datasets were deposited in TreeBASE (http://treebase.org).

Species	Isolate numbers <sup>1</sup>	Host	Location	Collector		)	GenBank acc	ession numbe	ers <sup>2</sup>			Reference
					ITS	tef1	tub	rpb2	cmdA	LSU	SSU	
Botryosphaeria agaves	MFLUCC 11-0125 = CBS 133992 <sup>3</sup>	Agave sp.	Thailand	R. Phookamsak	JX646791	JX646856	JX646841	N/A	N/A	JX646808	JX646825	Liu et al. (2012)
	MFLUCC 10-0051	Agave sp.	Thailand	P. Chomnunti	JX646790	JX646855	JX646840	N/A	N/A	JX646807	JX646824	Liu et al. (2012)
B. auasmontanum	CMW 25413 = CBS 121769 <sup>3</sup>	Acacia mellifera	Namibia	F.J.J. van der Walt & J. Roux	EU101303	EU101348	N/A	N/A	N/A	KF766332	KF766252	Slippers et al. (2013, 2014)
B. corticis	CBS 119047 <sup>3</sup> ATCC 22927	Vaccinium corymbosum Vaccinium sp.	USA USA	P.V. Oudemans R.D. Millholland	DQ299245 DQ299247	EU017539 EU673291	EU673107 EU673108	N/A N/A	N/A N/A	EU673244 EU673245	EU673175 EU673176	Phillips et al. (2006, 2008), Lazzizera et al. (2008) Phillips et al. (2006, 2008)
B. dothidea	CBS 115476 = CMW 8000 <sup>3</sup>	Prunus sp.	Switzerland	B. Slippers	AY236949	AY236898	AY236927	EU339577	N/A	AY928047	EU673173	Slippers et al. (2004a), Phillips et al. (2008)
	CBS 110302	Vitis vinifera	Portugal	A.J.L. Phillips	AY 259092	AY573218	EU673106	N/A	N/A	EU673243	EU673174	Alves et al. (2004), Phillips et al. (2008)
B. fabicerciana	CMW 27094 = CBS 127193 <sup>3</sup>	Eucalyptus sp.	China	M.J. Wingfield	HQ332197	HQ332213	KF779068	MF410137	N/A	MF410028	MF410226	Chen et al. (2011c), This study
	CMW 27121 = CBS 127194	Eucalyptus sp.	China	M.J. Wingfield	HQ332198	HQ332214	KF779069	MF410138	N/A	MF410029	MF410227	Chen et al. (2011c), This study
B. fusispora	MFLUCC 10-0098 <sup>3</sup> MFLUCC 11-0507	Entada sp. Entada sp.	Thailand Thailand	S. Boonmee R. Cheewangkoon	JX646789 JX646788	JX646854 JX646853	JX646839 JX646838	N/A N/A	N/A N/A	JX646806 JX646805	JX646823 JX646822	Liu et al. (2012) Liu et al. (2012)
B. kuwatsukai	CBS 135219 = PG 2 <sup>3</sup> LSP 5	Malus domestica Pyrus sp.	China China	C.S. Wang C.S. Wang	KJ433388 KJ433395	KJ433410 KJ433417	N/A N/A	N/A N/A	N/A N/A	N/A N/A	N/A N/A	Xu et al. (2015a) Xu et al. (2015a)
B. minutispermatia	GZCC 16-0013 <sup>3</sup> GZCC 16-0014	Dead wood Dead wood	Guizhou, China Guizhou, China	H.A. Ariyawansa H.A. Ariyawansa	KX447675 KX447676	KX447678 KX447679	N/A N/A	N/A N/A	N/A N/A	N/A N/A	N/A N/A	Ariyawansa et al. (2016) Ariyawansa et al. (2016)
B. ramosa	CBS 122069 = CMW 26167 <sup>3</sup>	Eucalyptus camaldulensis	Australia	T.I. Burgess	EU144055	EU144070	KF766132	N/A	N/A	KF766333	KF766253	Pavlic et al. (2008), Slippers et al. (2013)
B. rosaceae	CGMCC3.18007 <sup>3</sup> CGMCC3.18008	Malus sp. Amygdalus sp.	Shandong, China Shandong, China	Y. Zhang & J.Q. Zhang Y. Zhang, J.Q. Zhang & Z.P. Dou	KX197074 KX197075	KX197094 KX197095	KX197101 KX197102	N/A N/A	N/A N/A	KX197083 KX197084	N/A N/A	Zhou et al. (2017) Zhou et al. (2017)
B. scharifii	IRAN 1529C = CBS 124703 <sup>3</sup>	Mangifera indica	Iran	J. Abdollahzadeh	JQ772020	JQ772057	N/A	N/A	N/A	N/A	N/A	Abdollahzadeh et al. (2013)
	IRAN 1543C = CBS 124702	Mangifera indica	Iran	J. Abdollahzadeh & A. Javadi	JQ772019	JQ772056	N/A	N/A	N/A	N/A	N/A	Abdollahzadeh et al. (2013)
B. sinensia	CGMCC3.17723 CGMCC3.17724	Morus sp. Juglans regia	Henan, China Henan, China	Z.P. Dou Z.P. Dou	KT343254 KT343256	KU221233 KU221234	KX197107 KX197108	N/A N/A	N/A N/A	KX197090 N/A	N/A N/A	Zhou et al. (2016, 2017) Zhou et al. (2016, 2017)
Cophinforma atrovirens	<ul> <li>CBS 124934</li> <li>= CMW 22674<sup>3</sup></li> </ul>	Pterocarpus angolensis	South Africa	J. Mehl & J. Roux	FJ888473	FJ888456	N/A	N/A	N/A	N/A	N/A	Mehl et al. (2011)
	CBS 124935 = CMW 22682	Pterocarpus angolensis	South Africa	J. Mehl & J. Roux	FJ888476	FJ888457	N/A	N/A	N/A	N/A	N/A	Mehl et al. (2011)
	CBS 117445 = CMW 13425	Acacia mangium	Venezuela	S. Mohali	EF118046	GU134939	N/A	N/A	N/A	N/A	N/A	Mohali et al. (2007)
	CBS 117446 = CMW 13429	Eucalyptus hybrid	Venezuela	S. Mohali	EF118048	GU134940	N/A	N/A	N/A	N/A	N/A	Mohali et al. (2007)
Lasiodiplodia avicenniae	CMW 41467 <sup>3</sup> LAS 199 (DNA)	Avicennia marina Avicennia marina	South Africa South Africa	J.A. Osorio & J. Roux J.A. Osorio & J. Roux	KP860835 KU587957	KP860680 KU587947	KP860758 KU587868	KU587878 KU587880	N/A N/A	N/A N/A	N/A N/A	Osorio et al. (2017) Osorio et al. (2017)
L. americana	CERC1961 = CFCC50065 <sup>3</sup>	Pistachia vera	Arizona, USA	T.J. Michailides	KP217059	KP217067	KP217075	MF410161	MF409965	MF410052	MF410250	Chen et al. (2015), This study
	CERC1960 = CFCC50064	Pistachia vera	Arizona, USA	T.J. Michailides	KP217058	KP217066	KP217074	MF410162	MF409966	MF410053	MF410251	Chen et al. (2015), This study
L. brasiliense	CMM 4015 <sup>3</sup> CMW 35884	Mangifera indica Adansonia madagascariensis	Brazil Madagascar	M.W. Marques	JX464063 KU887094	JX464049 KU886972	N/A KU887466	N/A KU696345	N/A KU886755	N/A N/A	N/A N/A	Netto et al. (2014) Cruywagen et al. (2017)
L. bruguierae	CMW 41470 <sup>3</sup> CMW 41614	Bruguiera gymnorrhiza Bruguiera gymnorrhiza	South Africa South Africa	J.A. Osorio & J. Roux J.A. Osorio & J. Roux	KP860833 KP860834	KP860678 KP860679	KP860756 KP860757	KU587875 KU587877	N/A N/A	N/A N/A	N/A N/A	Osorio et al. (2017) Osorio et al. (2017)
L. caatinguensis	CMM 1325 <sup>3</sup>	Citrus sinensis	ltarema, Ceará, Brazil	I.B.L. Coutinho & J.S. Lima	KT154760	KT008006	KT154767	N/A	N/A	N/A	N/A	Coutinho et al. (2017)

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Species	Isolate numbers <sup>1</sup>	Host	Location	Collector		U	GenBank acc	ession numb	ers <sup>2</sup>			Reference
					ITS	tef1	tub	rpb2	cmdA	LSU	SSU	
L. caatinguensis (cont.	.) IBL 40	Spondias mombin	ltarema, Ceará, Brazil	J.S. Lima & J.E. Cardoso	KT154762	KT154755	KT154769	N/A	N/A	N/A	N/A	Coutinho et al. (2017)
L. chinensis	CGMCC3.18061 <sup>3</sup> CGMCC3.18066	Unknown Hevea brasiliensis	China China	W. He & Z.P. Dou Y. Zhang & Y.P. Zhou	KX499889 KX499899	KX499927 KX499937	KX500002 KX500012	KX499965 KX499974	N/A N/A	N/A N/A	N/A N/A	Dou et al. (2017a) Dou et al. (2017a)
L. citricola	CBS 124707 = IRAN 1522C <sup>3</sup>	Citrus sp.	Iran	J. Abdollahzadeh & A. Javadi	GU945354	GU945340	KU887505	KU696351	KU886760	N/A	N/A	Abdollahzadeh et al. (2010), Cruywagen et al. (2017)
	CBS 124706 = IRAN 1521C	<i>Citrus</i> sp.	Iran	A. Shekari	GU945353	GU945339	KU887504	KU696350	KU886759	N/A	N/A	Abdollahzadeh etal. (2010), Cruywagen etal. (2017)
L. crassispora	CBS 118741 = WAC12533 <sup>3</sup>	Santalum album	Kununurra, Australia	T.I. Burgess & B. Dell	DQ103550	EU673303	KU887506	KU696353	KU886761	DQ377901	N/A	Burgess et al. (2006), Phillips et al. (2008), Cruywagen et al. (2017)
	CBS 110492	Unknown	Unknown	Unknown	EF622086	EF622066	EU673134	A/A	N/A	EU673251	N/A	Alves et al. (2008), Phillips et al. (2008)
L. euphorbicola	CMM 36095 CMW 33350	Jatropha curcas Adansonia digitata	Brazil Botswana	A.K. Machado & O.L. Pereira	KF-234543 KU887149	KF226689 KU887026	KF254926 KU887455	N/A KU696346	N/A KU886754	A/A N/A	N/A	Machado et al. (2014) Cruywagen et al. (2017)
L. exigua	CBS 137785 <sup>3</sup> BL 184	Retama raetam Retama raetam	Tunisia Tunisia	B.T. Linaldeddu B.T. Linaldeddu	KJ638317 KJ638318	KJ638336 KJ638337	KU887509 N/A	KU696355 N/A	KU886764 N/A	N/A N/A	N/A N/A	Linaldeddu et al. (2015), Cruywagen et al. (2017) Linaldeddu et al. (2015)
L. gilanensis	CBS 124704 = IRAN 1523C <sup>3</sup>	Unknown	Iran	J. Abdollahzadeh & A. Javadi	GU945351	GU945342	KU887511	KU696357	KU886765	N/A	N/A	Abdollahzadeh etal. (2010), Cruywagen etal. (2017)
	CBS 124705 = IRAN 1501C	Unknown	Iran	J. Abdollahzadeh & A. Javadi	GU945352	GU945341	KU887510	KU696356	KU886766	N/A	N/A	Abdollahzadeh etal. (2010), Cruywagen etal. (2017)
L. gonubiensis	CBS 115812 = CMW 14077 <sup>3</sup>	Syzygium cordatum	South Africa	D. Pavlic	AY639595	DQ103566	DQ458860	KU696359	KU886768	DQ377902	EU673193	Pavlic et al. (2004), Burgess et al. (2006), Phillips et al. (2008). Cruvwagen et al. (2017)
	CBS 116355 = CMW 14078	Syzygium cordatum	South Africa	D. Pavlic	AY639594	DQ103567	EU673126	KU696358	KU886767	EU673252	EU673194	Pavlic et al. (2004), Burgess et al. (2006), Phillips et al. (2008), Cruywagen et al. (2017)
L. gravistriata	CMM 4564 <sup>3</sup> CMM 4565	Anacardium humile Anacardium humile	Brazil Brazil	M.S.B. Netto M.S.B. Netto	KT250949 KT250947	KT250950 KT266812	N/A N/A	N/A N/A	N/A N/A	N/A N/A	N/A N/A	Netto et al. (2017) Netto et al. (2017)
L. hormozganensis	CBS 124709 = IRAN 1500C <sup>3</sup>	Olea sp.	Iran	J. Abdollahzadeh & A. Javadi	GU945355	GU945343	KU887515	KU696361	KU886770	N/A	N/A	Abdollahzadeh etal. (2010), Cruywagen etal. (2017)
	CBS 124708 = IRAN 1498C	Mangifera indica	Iran	J. Abdollahzadeh & A. Javadi	GU945356	GU945344	KU887514	KU696360	KU886769	N/A	N/A	Abdollahzadeh etal. (2010), Cruywagen etal. (2017)
L. hyalina	CGMCC3.17975 <sup>3</sup> CGMCC3.18383 = B 6180	Acacia confusa Unknown tree	China China	Y. Zhang & Y.P. Zhou Z.P. Dou & Z.C. Liu	KX499879 KY767661	KX499917 KY751302	КХ499992 КҮ751299	KX499955 KY751296	N/A N/A	N/A N/A	N/A N/A	Dou et al. (2017b) Dou et al. (2017b)
L. indica	IBP 01 <sup>3</sup>	Angiospermous tree	India	I.B. Prasher & G. Singh	KM376151	N/A	N/A	N/A	N/A	N/A	N/A	Prasher & Singh (2014)
L. iraniensis	IRAN 1520C <sup>3</sup> IRAN 1502C	Salvadora persica Juglans sp.	lran Iran	J. Abdollahzadeh & A. Javadi A. Javadi	GU945348 GU945347	GU945336 GU945335	KU887516 KU887517	KU696363 KU696362	KU886771 KU886772	N/A N/A	N/A N/A	Abdollahzadeh et al. (2010), Cruywagen et al. (2017) Abdollahzadeh et al. (2010), Cruywagen et al. (2017)
L. laeliocattleyae	CBS 167.28 <sup>3</sup> LAREP1	Laeliocattleya Mangifera indica	Italy Repartidor, Peru	C. Sibilia P. Guerrero	KU507487 KU507484	KU507454 KU507451	N/A N/A	N/A N/A	N/A N/A	DQ377892 N/A	N/A N/A	Crous et al. (2006), Rodríguez-Gálvez et al. (2017) Rodríguez-Gálvez et al. (2017)
L. lignicola	MFLUCC 11-0435 = CBS134112 <sup>3</sup>	Unknown	Thailand	A.D. Ariyawansa	JX646797	KU887003	JX646845	KU696364	N/A	JX646814	JX646830	Liu et al. (2012), Cruywagen et al. (2017)
L. macrospora	CMM 3833 <sup>3</sup>	Jatropha curcas	Brazil	A.R. Machado & O.L. Pereira	KF234557	KF226718	KF254941	N/A	N/A	N/A	N/A	Machado et al. (2014)
L. mahajangana	CBS 124925 = CMW 27801 <sup>3</sup>	Terminalia catappa	Madagascar	J. Roux	FJ900595	FJ900641	FJ900630	KU696365	KU886773	N/A	N/A	Begoude et al. (2010), Cruywagen et al. (2017)
	CBS 124926 = CMW 27820	Terminalia catappa	Madagascar	J. Roux	FJ900596	FJ900642	KU887519	KU696366	KU886774	N/A	N/A	Begoude et al. (2010), Cruywagen et al. (2017)
L. margaritacea	CBS 122519 = CMW 26162 <sup>3</sup>	Adansonia gibbosa	WA, Tunnel Creek Gorge	T.I. Burgess	EU144050	EU144065	KU887520	KU696367	KU886775	KX464354	N/A	Pavlic et al. (2008), Cruywagen et al. (2017)
L. mediterranea	CBS 137783 <sup>3</sup> CBS 137784	Quercus ilex Vitis vinifera	Italy Italy	B.T. Linaldeddu S. Serra	KJ638312 KJ638311	KJ638331 KJ638330	KU887521 KU887522	KU696368 KU696369	KU886776 KU886777	N/A N/A	N/A N/A	Linaldeddu et al. (2015) Linaldeddu et al. (2015)
L. missouriana	CBS 128311 = UCD2193MO <sup>3</sup>	Vitis sp. × Vitis labruscana	Missouri, USA	K. Striegler & G.M. Leavitt	HQ288225	HQ288267	HQ288304	KU696370	KU886778	N/A	N/A	Úrbez-Torres et al. (2012), Cruywagen et al. (2017)
	CBS 128312 = UCD2199MO	Vitis sp. × Vitis labruscana	Missouri, USA	K. Striegler & G.M. Leavitt	HQ288226	HQ288268	HQ288305	KU696371	KU886779	N/A	N/A	Úrbez-Torres et al. (2012), Cruywagen et al. (2017)
ennen l	CBS 456 783	Cassava-field soil	Colombia	lenned O	EF622083	FF622063	KI 1887523	K11696372	KI IRRE7RO	KE766362	N/A	Alviae at al 72008) Critiviviadian et al (2017)

Species	Isolate numbers <sup>1</sup>	Host	Location	Collector			GenBank aco	ession numbe	∋rS²			Reference
					ITS	tef1	tub	rpb2	cmdA	LSU	SSU	
L. parva (cont.)	CBS 494.78	Cassava-field soil	Colombia	O. Rangel	EF622084	EF622064	EU673114	KU696373	KU886781	EU673258	EU673201	Alves et al. (2008), Phillips et al. (2008), Cruywagen et al. (2017)
L. plurivora	CBS 120832 <sup>3</sup>	Prunus salicina	Stellenbosch, Western Cape, Sou	U. Damm uth Africa	EF445362	EF445395	KU887524	KU696374	KU886782	KX464356	N/A	Damm et al. (2007), Cruywagen et al. (2017)
	CBS 121103	Vitis vinifera	South Africa	F. Halleen	AY 343482	EF445396	KU887525	KU696375	KU886783	KX464357	N/A	Damm et al. (2007), Cruywagen et al. (2017)
L. pontae	CMM 1277 <sup>3</sup>	Spondias purpurea	Pio-IX/Piauí/Brazil	J.S. Lima & F.C.O. Freire	KT151794	KT151791	KT151797	N/A	N/A	N/A	N/A	Coutinho et al. (2017)
L. pseudotheobromae	CBS 116459 <sup>3</sup>	Gmelina arborea	Costa Rica	J. Carranza & Velásquez	EF622077	EF622057	EU673111	KU696376	KU886784	EU673256	EU673199	Alves et al. (2008), Phillips et al. (2008), Crimmonan et al. (2017)
	CMM 3887	Jatropha curcas	Brazil	A. R. Machado	KF234559	KF226722	KF254943	N/A	N/A	N/A	N/A	Machado et al. (2014)
L. pyriformis	CBS 121770 - CMM 264143	Acacia mellifera	Dordabis, Namibia	F.J.J. van der Walt & J. Roux	EU101307	EU101352	KU887527	KU696378	KU886786	N/A	N/A	Slippers et al. (2014), Cruywagen et al. (2017)
	- CMW 23414 CBS 121771 = CMW 25415	Acacia mellifera	Dordabis, Namibia	F.J.J. van der Walt & J. Roux	EU101308	EU101353	KU887528	KU696379	KU886787	N/A	N/A	Slippers et al. (2014), Cruywagen et al. (2017)
L. rubropurpurea	CBS 118740 = CMW 14700 = WAC 12535 <sup>3</sup>	Eucalyptus grandis	Tully, Queensland	T.I. Burgess & G. Pegg	DQ103553	DQ103571	EU673136	KU696380	KU886788	DQ377903	EU673191	Burgess et al. (2006), Phillips et al. (2008), Cruywagen et al. (2017)
	WAC 12536 = CMW 15207	Eucalyptus grandis	Tully, Queensland	T.I. Burgess & G. Pegg	DQ103554	DQ103572	KU887530	KU696381	N/A	N/A	N/A	Burgess et al. (2006), Cruywagen et al. (2017)
L. sterculiae	CBS 342.78 <sup>3</sup>	Sterculia oblonga	Germany	S. Bruhn	KX464140	KX464634	KX464908	KX463989	N/A	JX681073	N/A	Yang et al. (2017)
L. subglobosa	CMM 3872 <sup>3</sup> CMM 4046	Jatropha curcas Jatropha curcas	Brazil Brazil	A.R. Machado & O.L. Pereira A.R. Machado & O.L. Pereira	KF234558 KF234560	KF226721 KF226723	KF254942 KF254944	N/A N/A	N/A N/A	N/A N/A	N/A N/A	Machado et al. (2014) Machado et al. (2014)
L. thailandica	CPC 22795 <sup>3</sup> CPC 22755	Mangifera indica Phyllanthus acidus	Thailand Thailand	T. Trakunyingcharoen T. Trakunyingcharoen	KJ193637 KM006433	KJ193681 KM006464	N/A N/A	N/A N/A	N/A N/A	N/A N/A	N/A N/A	Trakunyingcharoen et al. (2015) Trakunyingcharoen et al. (2015)
L. theobromae	CBS 164.96 <sup>3</sup>	Fruit along coral	New Guinea	A. Aptroot	AY640255	AY640258	KU887532	KU696383	KU886789	EU673253	EU673196	Alves et al. (2008), Phillips et al. (2008),
	CBS 111530	reef coast Unknown	Unknown	Unknown	EF622074	EF622054	KU887531	KU696382	KU886790	N/A	N/A	Cruywagen et al. (2017) Alves et al. (2008), Cruywagen et al. (2017)
L. venezuelensis	CBS 118739 = CMW 13511 = WAC 12539 <sup>3</sup>	Acacia mangium	Acarigua, Venezuela	S. Mohali	DQ103547	DQ103568	KU887533	KU696384	KU886791	DQ377904	EU673192	Burgess et al. (2006), Phillips et al. (2008), Cruywagen et al. (2017)
	CMW 13512 = WAC 12540	Acacia mangium	Acarigua, Venezuela	S. Mohali	DQ103548	DQ103569	KU887534	N/A	KU886792	N/A	N/A	Burgess et al. (2006), Cruywagen et al. (2017)
L. viticola	CBS 128313 = UCD 2553AR <sup>3</sup>	Vitis vinifera	USA	K. Striegler & G.M. Leavitt	HQ288227	HQ288269	HQ288306	KU696385	KU886793	N/A	N/A	Úrbez-Torres et al. (2012), Cruywagen et al. (2017)
	CBS 128315 = UCD 2604MO	Vitis vinifera	USA	K. Striegler & G.M. Leavitt	HQ288228	HQ288270	HQ288307	KU696386	KU886794	N/A	N/A	Úrbez-Torres et al. (2012), Cruywagen et al. (2017)
L. vitis	CBS 124060 <sup>3</sup>	Vitis vinifera	Italy	S. Burruano	KX464148	KX464642	KX464917	KX463994	N/A	KX464367	N/A	Yang et al. (2017)
Neofusicoccum algeriense	CBS 137504 = ALG 1 <sup>3</sup>	Vitis vinifera	Algeria	A. Berraf-Tebbal	KJ657702	KJ657715	KX505915	N/A	N/A	N/A	N/A	Berraf-Tebbal et al. (2014), Lopes et al. (2017)
	CAA 322	Malus domestica	Portugal		KX505906	KX505894	KX505916	N/A	N/A	N/A	N/A	Lopes et al. (2017)
N. andinum	CBS 117453 = CMW 134553	Eucalyptus sp.	Me' rida state, Venezuela	S. Mohali	AY693976	AY693977	KX464923	KX464002	N/A	KX464373	N/A	Mohali et al. (2006), Yang et al. (2017)
	<ul><li>CBS 117452</li><li>= CMW 13446</li></ul>	Eucalyptus sp.	Venezuela	S. Mohali	DQ306263	DQ306264	KX464922	KX464001	N/A	DQ377914	N/A	Mohali et al. (2006), Yang et al. (2017)
N. arbuti	CBS 116131 <sup>3</sup>	Arbutus menziesii	Washington, USA	M. Elliott	AY819720	KF531792	KF531793	KX464003	N/A	DQ377915	KF531814	Farr et al. (2005), Crous et al. (2006), Phillins et al. (2013), Vann et al. (2017)
	CBS 117090	Arbutus menziesii	California, USA	M. Elliott	AY819724	KF531791	KF531794	N/A	N/A	DQ377919	KF531813	Farr et al. (2005), Crous et al. (2006), Phillips et al. (2013)
N. australe	CMW 6837 <sup>3</sup>	<i>Acacia</i> sp.	Batemans Bay, Australia	M.J. Wingfield	AY339262	AY 339270	AY339254	EU339573	N/A	KF766367	N/A	Slippers et al. (2004b, 2013), Burgess et al. (2007)
	CBS 110865 = CPC 4599	Vitis vinifera	South Africa	F. Halleen	AY343408	KX464661	KX464937	KX464005	N/A	KX464385	N/A	Van Niekerk et al. (2004), Yang et al. (2017)
N. batangarum	CBS 124924 = CMW 28363 <sup>3</sup>	Terminalia catappa	Cameroon	D. Begoude & J. Roux	FJ900607	FJ900653	FJ900634	FJ900615	N/A	KX464401	N/A	Begoude et al. (2010), Yang et al. (2017)

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Species	Isolate numbers <sup>1</sup>	Host	Location	Collector		U	enBank acc	ession numb	ers <sup>2</sup>			Reference
					ПS	tef1	tub	rpb2	cmdA	LSU	SSU	
N. batangarum (cont.)	CBS 124923 = CMW 28320	Terminalia catappa	Cameroon	D. Begoude & J. Roux	FJ900608	FJ900654	FJ900635	FJ900616	N/A	KX464400	N/A	Begoude et al. (2010), Yang et al. (2017)
N. brasiliense	CMM 1338 <sup>3</sup> CMM 1285	Mangifera indica Mangifera indica	Brazil Brazil	M.W. Marques M.W. Marques	JX513630 JX513628	JX513610 JX513608	KC794031 KC794030	N/A N/A	N/A N/A	N/A N/A	N/A N/A	Marques et al. (2013) Marques et al. (2013)
N. buxi	CBS 116.75 <sup>3</sup> CBS 113714	Buxus sempervirens Buxus sempervirens	France Sweden	H.A. van der Aa O. Constantinescu	KX464165 KX464164	KX464678 KX464677	N/A KX464954	KX464010 KX464009	N/A N/A	KX464406 KX464405	N/A N/A	Yang et al. (2017) Yang et al. (2017)
N. cordaticola	CBS 123634 = CMW 13992 <sup>3</sup>	Syzigium cordatum	South Africa	D. Pavlic	EU821898	EU821868	EU821838	EU821928	N/A	KX464409	N/A	Pavlic et al. (2009b), Yang et al. (2017)
	CBS 123635 = CMW 14056	Syzigium cordatum	South Africa	D. Pavlic	EU821903	EU821873	EU821843	EU821933	N/A	KX464410	N/A	Pavlic et al. (2009b), Yang et al. (2017)
N. cryptoaustrale	CMW 23785 = CBS 122813 <sup>3</sup>	Eucalyptus trees	South Africa	H.M. Maleme	FJ752742	FJ752713	FJ752756	KX464014	N/A	KX464416	N/A	Crous et al. (2013), Yang et al. (2017)
N. eucalypticola	CBS 115679 = CMW 6539 <sup>3</sup>	Eucalyptus grandis	Orbost, Victoria, Australia	M.J. Wingfield	AY615141	AY615133	AY615125	N/A	N/A	KF766368	KF766288	Slippers et al. (2004c, 2013)
	CBS 115766 = CMW 6217	Eucalyptus rossii	Tidbinbilla, NSW, Australia	M.J. Wingfield	AY615143	AY615135	AY615127	N/A	N/A	N/A	N/A	Slippers et al. (2004c, 2013)
N. eucalyptorum	CBS 115791 = CMW 10125 <sup>3</sup>	Eucalyptus grandis	South Africa	H. Smith	AF283686	AY236891	AY236920	N/A	N/A	N/A	N/A	Smith et al. (2001), Slippers et al. (2004b)
N. grevilleae	CMW 10126 CBS 129518 - CDC 160003	Eucalyptus grandis Grevillea aurea	South Africa Australia	H. Smith P.W. Crous & R.G. Shivas	AF283687 JF951137	AY 236892 N/A	AY236921 N/A	N/A N/A	A/N N/A	N/A JF951157	N/A N/A	Smith et al. (2001), Slippers et al. (2004b) Crous et al. (2011)
N. hellenicum	CERC1947	Pistachia vera	Thessaloniki, Greece	T.J. Michailides	KP217053	KP217061	KP217069	N/A	N/A	N/A	N/A	Chen et al. (2015)
	= CFCC50067 CERC1948 = CFCC50068	Pistachia vera	Aghios Mamas, Chalkidiki, Greece	T.J. Michailides	KP217054	KP217062	KP217070	N/A	N/A	N/A	N/A	Chen et al. (2015)
N. illicii	CGMCC3.18310 <sup>3</sup> CGMCC3.18311	Illicium verum Illicium verum	Guangxi, China Guangxi, China	L. Wang L. Wang	KY350149 KY350150	N/A KY817756	KY350155 KY350156	N/A N/A	N/A N/A	N/A N/A	N/A N/A	Zhang et al. (2017) Zhang et al. (2017)
N. kwambonambiense	CBS 123639 = CMW 14023 <sup>3</sup> CBS 123641	Syzigium cordatum	South Africa	D. Pavlic	EU821900	EU821870	EU821840	EU821930	N/A	KX464422	N/A	Pavlic et al. (2009b), Yang et al. (2017)
	= CMW 14140	Syzigium cordatum	South Africa	D. Pavlic	EU821919	EU821889	EU821859	EU821949	N/A	KX464424	N/A	Pavlic et al. (2009b), Yang et al. (2017)
N. lumnitzerae	CMW 41469 <sup>3</sup> CMW 41228	Lumnitzera racemosa Lumnitzera racemosa	South Africa South Africa	J.A. Osorio & J. Roux J.A. Osorio & J. Roux	KP860881 KP860882	KP860724 KP860725	KP860801 KP860803	KU587925 KU587926	N/A N/A	N/A N/A	N/A N/A	Osorio et al. (2017) Osorio et al. (2017)
N. Iuteum	CBS 562.92 = ATCC 58193 <sup>3</sup>	Actinidia deliciosa, lesion on ripe fruit	New Zealand	S.R. Pennycook	KX464170	KX464690	KX464968	KX464020	N/A	KX464430	N/A	Yang et al. (2017)
N. macroclavatum	CBS 118223 = WAC 12444 <sup>3</sup>	Eucalyptus globulus	Western Australia	T. Burgess	DQ093196	DQ093217	DQ093206	KX464022	N/A	KX464436	N/A	Burgess et al. (2005), Yang et al. (2017)
N. mangiferae	CBS 118531 = CMW 7024 <sup>3</sup>	Mangifera indica	Australia	G.I. Johnson	AY615185	DQ093221	AY615172	N/A	N/A	DQ377920	EU673153	Slippers et al. (2005), Phillips et al. (2008)
	CBS 118532 = CMW 7797	Mangifera indica	Australia	G.I. Johnson	AY615186	DQ093220	AY615173	KX464023	N/A	DQ377921	EU673154	Slippers et al. (2005), Phillips et al. (2008), Yang et al. (2017)
N. mangroviorum	CMW 41365 <sup>3</sup> CMW 42481	Avicennia marina Bruguiera gymnorrhiza	South Africa South Africa	J.A. Osorio & J. Roux J.A. Osorio & J. Roux	KP860859 KP860848	KP860702 KP860692	KP860779 KP860770	KU587905 KU587895	N/A N/A	N/A N/A	N/A N/A	Osorio et al. (2017) Osorio et al. (2017)
N. mediterraneum	CBS 121718 = CPC 13137 <sup>3</sup>	Eucalyptus sp.	Greece	P.W. Crous, M.J. Wingfield & A.J.L. Phillips	GU251176	GU251308	GU251836	KX464024	N/A	N/A	N/A	Crous et al. (2007), Yang et al. (2017)
N. nonquaesitum	CBS 126655 = PD 484 <sup>3</sup>	Umbellularia californica	NSA	F.P. Trouillas	GU251163	GU251295	GU251823	KX464025	N/A	KX464437	N/A	Inderbitzin et al. (2010), Yang et al. (2017)
	PD 301	Vaccinum corymbosum cv. Elliot	Chile	E.X. Bricenő, J.G. Espinoza, B.A. Latorre & J.G. Espinoza	GU251164	GU251296	GU251824	N/A	N/A	N/A	N/A	Inderbitzin et al. (2010)
N. occulatum	CBS 128008 = MUCC 227 <sup>3</sup>	<i>Eucalyptus grandis</i> hybrid	Australia	T.I. Burgess	EU301030	EU339509	EU339472	EU339558	N/A	KX464438	N/A	Sakalidis et al. (2011), Yang et al. (2017)

Species	Isolate numbers <sup>1</sup>	Host	Location	Collector		0	3enBank acc	ession numb	ers <sup>2</sup>			Reference
					ITS	tef1	tub	rpb2	cmdA	LSU	SSU	
N. occulatum (cont.)	MUCC 286 = WAC 12395	Eucalyptus pellita	Australia	T.I. Burgess	EU736947	EU339511	EU339474	EU339560	N/A	N/A	N/A	Sakalidis et al. (2011)
N. parvum	ATCC 58191 = CMW 9081 <sup>3</sup>	Populus nigra	New Zealand	G.J. Samuels	AY236943	AY236888	AY236917	EU821963	N/A	AY928045	EU673151	Slippers et al. (2004a), Alves et al. (2005), Phillips et al. (2008), Pavlic et al. (2009b)
	CMW 9080 = ICMP 8002	Populus nigra	New Zealand	G.J. Samuels	AY236942	AY236887	AY236916	EU821962	N/A	N/A	N/A	Slippers et al. (2004a), Pavlic et al. (2009b)
N. pennatisporum	WAC 13153 = MUCC 510 <sup>3</sup>	Allocasuarina fraserianá	Western Australia	K.M. Taylor	EF591925	EF591976	EF591959	N/A	N/A	EF591942	N/A	Taylor et al. (2009)
N. pistaciae	CBS 595.76 <sup>3</sup>	Pistacia vera	Greece	D.G. Zachos	KX464163	KX464676	KX464953	KX464008	N/A	KX464404	N/A	Yang et al. (2017)
N. pistaciarum	CBS 113083 = CPC 5263 <sup>3</sup>	Pistacia vera	NSA	T.J. Michailides	KX464186	KX464712	KX464998	KX464027	N/A	KX464465	N/A	Yang et al. (2017)
	CBS 113084 = CPC 5284	Redwood	USA	T.J. Michailides	KX464187	KX464713	KX464999	KX464028	N/A	KX464466	N/A	Yang et al. (2017)
N. protearum	CBS 114176 = STF-I1 1775 <sup>3</sup>	Leucadendron salignum	South Africa	S. Denman	AF452539	KX464720	KX465006	KX464029	N/A	JX556245	N/A	Denman et al. (2003), Yang et al. (2017)
	CBS 111200 = CPC 1357	Leucadendron sp.	South Africa	P.W. Crous	KX464193	KX464719	KX465005	N/A	N/A	KX464472	N/A	Yang et al. (2017)
N. ribis	CBS 115475 = CMW 7772 <sup>3</sup>	<i>Ribes</i> sp.	USA	B. Slippers & G. Hudler	AY236935	AY236877	AY 236906	EU821958	N/A	AY928044	KF766292	Slippers et al. (2004a, 2013), Alves et al. (2005), Pavlic et al. (2009b)
	CBS 121.26 = CMW 7054	Ribes rubrum	USA	N.E. Stevens	AF241177	AY236879	AY236908	EU821960	N/A	KX464473	N/A	Slippers et al. (2004a), Pavlic et al. (2009b), Yang et al. (2017)
N. sinense	CGMCC3.183153	Unknown woody plant	Guizhou, China	J.J. Gan	KY350148	KY817755	KY350154	N/A	N/A	N/A	N/A	Zhang et al. (2017)
N. stellenboschiana	CBS 110864 = CPC 4598 <sup>3</sup>	Vitis vinifera	South Africa	F. Halleen	AY343407	AY343348	KX465047	KX464042	N/A	KX464513	N/A	Van Niekerk et al. (2004), Yang et al. (2017)
N. terminaliae	CBS 125263 = CMW 26679 <sup>3</sup>	Terminalia sericea	South Africa	D. Begoude & J. Roux	GQ471802	GQ471780	KX465052	KX464045	N/A	KX464518	N/A	Begoude (2010), Yang et al. (2017)
	CBS 125264 = CMW 26683	Terminalia sericea	South Africa	D. Begoude & J. Roux	GQ471804	GQ471782	KX465053	KX464046	N/A	KX464519	N/A	Begoude (2010), Yang et al. (2017)
N. umdonicola	CBS 123645 = CMW 14058 <sup>3</sup>	Syzigium cordatum	South Africa	D. Pavlic	EU821904	EU821874	EU821844	EU821934	N/A	KX464522	N/A	Pavlic et al. (2009b), Yang et al. (2017)
	CBS 123646 = CMW 14060	Syzigium cordatum	South Africa	D. Pavlic	EU821905	EU821875	EU821845	EU821935	N/A	KX464523	N/A	Pavlic et al. (2009b), Yang et al. (2017)
N. ursorum	CMW 24480 = CBS 122811 <sup>3</sup>	Eucalyptus trees	South Africa	H.M. Maleme	FJ752746	FJ752709	KX465056	KX464047	N/A	N/A	N/A	Crous et al. (2013), Yang et al. (2017)
	CMW 23790	Eucalyptus trees	South Africa	H.M. Maleme	FJ752745	FJ752708	KX465057	N/A	N/A	N/A	N/A	Crous et al. (2013), Yang et al. (2017)
N. viticlavatum	CBS 112878 = STF-U 5044 <sup>3</sup>	Vitis vinifera	South Africa	F. Halleen	AY343381	AY343342	KX465058	KX464048	N/A	KX464527	N/A	Phillips et al. (2013), Yang et al. (2017)
	CBS 112977 = STE-U 5041	Vitis vinifera	South Africa	F. Halleen	AY343380	AY343341	KX465059	N/A	N/A	KX464528	N/A	Phillips et al. (2013), Yang et al. (2017)
N. vitifusiforme	CBS 110887 = STE-U 5252 <sup>3</sup>	Vitis vinifera	South Africa	J.M. van Niekerk	AY343383	AY343343	KX465061	KX464049	N/A	KX464530	N/A	Van Niekerk et al. (2004), Yang et al. (2017)
	CBS 110880 = STE_I1 5050	Vitis vinifera	South Africa	J.M. van Niekerk	AY343382	AY343344	KX465008	N/A	N/A	KX464475	N/A	Van Niekerk et al. (2004), Yang et al. (2017)

Africa; CPC: Working collection of P.W. Crous, housed at CBS; GZCC; Guizhou Academy of Agricultural Sciences Culture Collection, GuiZhou, China; IBL: Personal culture collection, I.B. Prasher; ICMP: International Collection of Africa; CPC; Working collection of P.W. Crous, housed at CBS; GZCC; Guizhou Academy of Agricultural Sciences Culture Collection, GuiZhou, China; IBL: Personal culture collection, I.B. Prasher; ICMP: International Collection of P.W. Crous, housed at CBS; GZCC; Guizhou Academy of Agricultural Sciences Culture Collection, GuiZhou, China; IBL: Personal culture collection, I.B. Prasher; ICMP: International Collection of P.W. Crous, housed at CBS; GZCC; Guizhou Academy of Agricultural Sciences Culture Collection, GuiZhou, China; IBL: Personal culture collection, I.B. Prasher; ICMP: International Collection of P.W. Crous, housed at CBS; GZCC; Guizhou Academy of Agricultural Sciences Culture Collection, GuiZhou, China; IBL: Personal culture collection, I.B. Prasher; ICMP: International Collection of P.W. Crous, housed at CBS; GZCC; Guizhou Academy of Agricultural Sciences Culture Collection, GuiZhou, China; IBL: Personal culture collection, I.B. Prasher; ICMP: International Collection, GuiZhou, China; IBL: Personal culture collection, I.B. Prasher; ICMP: International Collection, GuiZhou, China; IBL: Personal culture collection, I.B. Prasher; ICMP: International Collection, GuiZhou, China; IBL: Personal culture collection, I.B. Prasher; ICMP: International Collection, GuiZhou, China; IBL: Personal culture collection, I.B. Prasher; ICMP: International Collection, GuiZhou, China; IBL: Personal culture collection, GuiZhou, China; IBL: Personal culture collection, I.B. Prasher; ICMP: International Collection, I.B. Prasher; ICMP: International Collection, I.B. Prasher; I Utrecht, The Netherlands; CERC: Culture collection of China Eucalypt Research Centre, Chinese Academy of Foresty, ZhanJiang, GuangDong, China, CFCC; China Foresty Culture Collection Center, Beijing, China; CBMCC: China General Microbiological Culture Collection Center, Beijing, China; CMM: Cuture Collection of Phytopathogenic Fungi 'Prof. Maria Menezes', Universidade Federal Rural de Pernambuco, Recife, Brazil; CMW: Tree Pathology Co-operative Program, Foresty and Agricultural Biotechnology Institute, Universidade Federal Rural de Pernambuco, Recife, Brazil; CMW: Tree Pathology Co-operative Program, Foresty and Agricultural Biotechnology Institute, Universidade Federal Rural de Pernambuco, Recife, Brazil; CMW: Tree Pathology Co-operative Program, Foresty and Agricultural Biotechnology Institute, Universidade Federal Rural de Pernambuco, Recife, Brazil; CMM: Cuture Collection of Phytopathogenic Fungi Prof. Maria Menezes', Universidade Federal Rural de Pernambuco, Recife, Brazil; CMM: Tree Pathology Co-operative Program, Foresty and Agricultural Biotechnology Institute, Universidade Federal Rural de Pernambuco, Recife, Brazil; CMM: Tree Pathology Co-operative Program, Foresty and Agricultural Biotechnology Institute, Universidade Federal Rural de Pernambuco, Recife, Brazil; CMM: Tree Pathology Co-operative Program, Foresty and Agricultural Biotechnology Institute, Universidade Federal Rural de Pernambuco, Recife, Brazil; CMM: Tree Pathology Co-operative Program, Foresty and Agricultural Biotechnology Institute, Universidade Federal Rural de Pernambuco, Recife, Brazil; CMM: Tree Pathology Co-operative Program, Foresty and Agricultura Biotechnology Institute, Inst Microorganisms from Plants, Auckland, New Zealand; IRAN: Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Iran; MFLUCC: Mae Fah Luang University Collection, Chiang Rai, Thailand; MUCC: Culture collection of Murdoch University, Perth, Australia, 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, Chiang Rai, Thailand; MUCC: Culture collection of the Department of Agriculture, Western Australia Plant Pathogen Collection. South Perth, Western Australia.

ALG: Personal culture collection A. Berraf-Tebbal; ATCC: American Type Culture Collection, Virginia, USA; BL: Personal number of B.T. Linaldedu; CAA: Personal culture collection Artur Alves, Universidade de Aveiro, Portugal; CBS: CBS-KNAW Fungal Biodiversity Centre,

ITS, internal transcribed spacer region and intervening 5.8S nrRNA gene; *tef1*, translation elongation factor 1-alpha; *tub*, B-tubulin; *rp*2, DNA-directed RNA polymerase II subunit; *cmd4*, calmodulin; LSU, nuclear ribosomal large subunit; SSU, nuclear ribosomal small subunit; NA = not available.

<sup>3</sup> Isolates represent ex-type or are from samples that have been linked morphologically to type materials of the species.

Table 2 (cont.)

## Morphology

Representative isolates for each genotype of Botryosphaeriaceae species identified by DNA sequence comparisons were selected for morphological study. To induce sporulation, selected isolates were transferred to 2 % water agar (WA) media (20 g of agar per litre of water) with double-sterilised pine needles placed on the surface of the media (Smith et al. 1996). These cultures were incubated at 25 °C under near-ultraviolet light for 4-6 wk. Conidia in the pycnidia were mounted in one drop of 80 % lactic acid on glass slides and examined under a stereomicroscope (Carl Zeiss Ltd., Munchen, Germany). Conidia and other structures were examined and recorded using a Zeiss Axio Imager A1 microscope and a Zeiss AxioCam MRc digital camera with Zeiss Axio Vision v. 4.8 software (Carl Zeiss Ltd.). Measurements of conidiomata, conidiophores and conidiogenous cells were made to determine the smallest and the largest values. For the isolates selected as a holotype, the lengths and widths of 100 conidia per isolate were measured, as well as 25 measurements of the remaining isolates of each taxon. Average (mean), standard deviation (SD), minimum (min) and maximum (max) measurements are presented as (min-) (mean-SD)-(mean+SD)(-max). The average length/average width ratio (L/W) of the conidial measurements was calculated.

Colony morphology was characterised by cultures grown on 2 % MEA for 7 d and colony colour was determined using the colour charts of Rayner (1970). For growth studies, a 5-mmdiam plug from the growing margin of 7-d-old colonies of each representative isolate was placed in the centre of 90-mm-diam Petri plates containing 2 % MEA. These cultures were incubated in the dark at 5 °C intervals from 5–40 °C. Five replicate plates of each isolate at each temperature were conducted. Two diameter measurements, orthogonally, were recorded daily until the fastest growing culture reached the edge of the Petri plate. The experiment was repeated once and the average for each of the eight temperatures was calculated.

## Pathogenicity tests

To determine the pathogenicity of the identified species on Eucalyptus seedlings, representative isolates of all Botryosphaeriaceae species identified in this study were selected to inoculate on Eucalyptus seedlings. Three Eucalyptus clones, CEPT-11 (Eucalyptus urophylla × E. grandis), CEPT-12 (E. urophylla) and CEPT-13 (E. urophylla × E. tereticornis), were used for inoculations. The Eucalyptus seedlings were 1-yr-old, approximately 1.7 m in height, and had a 2.0 cm diam at the root collar. For each clone, 10 seedlings were inoculated with each isolate. On each inoculated seedling, a 5-mm-diam wound was made on the tree stem using a cork borer to remove the bark and expose the xylem. The wounds are located approximately 30 cm above the root collar. For inoculation, 5-mm-diam plugs of mycelia from the margins of colonies grown on 2 % MEA for 7 d in the dark were taken and placed into the wounds with the mycelia facing the cambium. Inoculated wounds were encased with masking tape to prevent contamination and desiccation. Ten seedlings of each Eucalyptus clone were inoculated with sterile MEA plugs to serve as negative controls. One month after inoculation, the bark of inoculated seedlings was removed and the internal lesion/wound length on the cambium was measured. The inoculated fungi were re-isolated by cutting small pieces of wood from the edges of the lesions and cultivating them in 2 % MEA at 25 °C. Re-isolations were made from the seedlings inoculated by mycelium plugs and MEA plugs. The data were analysed by one-way analyses of variance (ANOVA) using SAS v. 9.3 (SAS Institute Inc. 2011).

## RESULTS

#### Fungal isolation

In this study, 105 isolates from *Eucalyptus* and other plants that show typical morphology of *Botryosphaeriaceae* were isolated. Eighty-one isolates were collected from *Eucalyptus* trees: 12 from FuJian Province, 39 from GuangDong Province, 29 from GuangXi Province and one from HaiNan Province. Eighteen isolates with typical characteristics of *Botryosphaeriaceae* were collected from other plants which were growing in close proximity to *Eucalyptus*: two from *C. lanceolata*, 10 from *D. longan*, four from *M. sanguineum*, and two from *P. hanceana*. In addition, three isolates were collected from *A. cunninghamii* and *C. deodara*, respectively (Table 1).

#### Phylogenetic analyses

For all the 105 isolates in this study, ITS, tef1, tub, rpb2, cmdA, LSU and SSU sequence data were generated and deposited in GenBank (Table 1). The PCR fragments are approximately 520 bps for the ITS region, 280 bps for the tef1 region, 430 bps for the tub region, 610 bps for the rpb2 region, 850 bps for the LSU region and 1040 bps for the SSU region. The genotype for each isolate was determined by the ITS, tef1, tub, rpb2, LSU, SSU sequences for isolates in the genera Botryosphaeria, Cophinforma and Neofusicoccum, and by ITS, tef1, tub, rpb2, cmdA, LSU, SSU sequences for isolates in the genus Lasiodiplodia (Table 1). The preliminary identities of the isolates were determined from conducting a standard nucleotide BLAST with the sequences of ITS, tef1, tub, rpb2, cmdA, LSU and SSU, the results consistently showed that the isolates sequenced in this study resided in Botryosphaeria, Cophinforma, Lasiodiplodia or Neofusicoccum. One to two isolates of each genotype were selected and used for phylogenetic analyses, depending on the number of isolates of each genotype (Table 1). Based on the comparisons for six to seven region sequences generated in this study and published sequences from ex-type strains of Botryosphaeriaceae downloaded from NCBI, sequences of Botryosphaeria, Cophinforma, Lasiodiplodia or Neofusicoccum related to species emerging from this study were used for analyses (Table 2). The aligned sequences of each region of ITS, tef1, tub, rpb2, cmdA, LSU, SSU, as well as the combined sequences of three to five (Botryosphaeria/Cophinforma: three; Lasiodiplodia: five; Neofusicoccum: four) regions were deposited in TreeBASE (No. 21430). These datasets for genera Botryosphaeria/Cophinforma, Lasiodiplodia and Neofusicoccum, as well as statistical values for the trees for the MP analyses and parameters for the best-fit substitution models of ML analyses, are provided in Table 3.

#### Species residing in Botryosphaeria

For the isolates grouping in the genus Botryosphaeria, isolates clustered into four phylogenetic groups (Groups A-D) for each of the ITS, tef1, tub, rpb2 and ITS/tef1/tub datasets (Fig. 2a-d, g). For each of the LSU and SSU datasets, Groups A, C and D clustered together (Fig. 2e-f). The ITS sequences of Botryosphaeria fabicerciana, B. fusispora, B. kuwatsukai, B. rosaceae and the six Chinese isolates (CERC2274, CERC2911, CERC2918, CERC2930, CERC3426 and CERC3441) in Group A are consistent, and all of them grouped into one phylogenetic clade (Fig. 2a). For the tef1 sequence analyses, the isolates in Group A clustered closely to B. fabicerciana and B. fusispora (Fig. 2b). For the tub sequences, the isolates in Group A resided in the same phylogenetic clade with B. fusispora (Fig. 2c). For the rpb2, LSU and SSU sequences, the isolates in Group A clustered to the same clade with B. fabicerciana and B. fusispora (rpb2 is not available to *B. fusispora*) (Fig. 2d-f). The phylogenetic analyses for ITS, tef1, tub, rpb2, LSU and SSU sequences

Genus	Dataset	No. of taxa	No. of $bp^{1}$			Maxin	num parsimon	y				
				PIC <sup>2</sup>	No. of trees	Tree length	CI <sup>3</sup>	RI <sup>4</sup>	RC <sup>5</sup>	HI <sup>6</sup>		
Botryosphaeria/Cophinforma Lasiodiplodia Neofusicoccum	ITS teff tub rpb2 LSU SSU ITS/tef1/tub TS/tef1/tub/rpb2/cmdA ITS/tef1/tub/rpb2/cmdA TTS/tef1/tub/rpb2/cmdA TTS/tef1/tub/rpb2/cmdA TTS/tef1/tub/rpb2/cmdA TTS/tef1/tub/rpb2/cmdA TTS/tef1/tub/rpb2/cmdA	4 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	543 356 415 415 718 718 718 7124 1024 1024 1024 1025 532 532 532 532 532 532 532 532 532 5	4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	18 875 875 875 861 861 861 861 861 861 861 861 861 861	206 206 24 24 24 24 24 23 29 190 190 23 23 23 23 23 23 23 23 23 23 23 23 23	0.8209 0.8332 0.9138 0.93397 0.9387 0.9583 1.0000 1.0000 0.8665 0.8665 0.8665 0.8665 0.6219 0.6219 0.7426 0.7426 0.7426 0.7426 0.7426 0.7426 0.7426 0.7426 0.7426 0.7426 0.7713 0.5714 1.0000 0.7713 0.5714 0.5714 0.5714 0.5716 0.5717 0.5717 0.5717 0.5716 0.5716 0.5716 0.5716 0.5716 0.57170 0.57170 0.57170000000000000000000000000000000000	0.9506 0.9688 0.9869 0.9865 1.0000 0.9865 0.9865 0.9865 0.9865 0.976 0.9776 0.9776 0.9776 0.9776 0.9778 0.9777 0.9776 0.9777 0.9776 0.9777 0.9776 0.9777 0.9776 0.97770 0.977700 0.977700 0.977700 0.977700 0.9770000000000	0.7804 0.8643 0.8852 0.9454 1.0000 0.9454 1.0000 0.8305 0.6169 0.5626 0.8916 0.5639 0.7170 0.5639 0.5639 0.5626 0.8916 0.5639 0.7474 0.5630 0.5422 0.5420 0.5420 0.5420 0.5420 0.5530	0.1791 0.0668 0.0862 0.0417 1.0000 0.1335 0.3181 0.3781 0.3781 0.3781 0.3783 0.3781 0.3783 0.3783 0.2593 0.4789 0.2574 0.2574 0.2574 0.2574 0.2574 0.2574 0.2574 0.2573 0.1733 0.1733 0.1733 0.2574 0.2574 0.2574 0.2573 0.27730 0.27730 0.27730 0.27730 0.27730 0.27730 0.2773000000000000000000		
Genus	Dataset	Subst. model <sup>7</sup>	NST <sup>®</sup>			Maximum Rate matrix	likelihood		Ti/Tv ratio <sup>9</sup>	p-inv	Gamma	Rates
Botryosphaeria/Cophinforma Lasiodiplodia Neofusicoccum	ITS teff tub rpb2 LSU SSU TTS/tef1/tub TTS/tef1/tub/pb2/cmdA LSU SSU TTS/tef1/tub/pb2/cmdA TTS/tef1/tub/pb2/cmdA TTS/tef1/tub/pb2/cmdA TTS/tef1/tub/pb2	TrN+I TPM2uf+G HKY+G TrN+G TrN+G TrN+G TrN+G TrN+G TrN+G TrN+G TrN+I TrN+I TrN+I TrN+I TrN+I TrN+I TrN+I TrN+I TrN+I TrN+I TrN+I TrN+I TrN+I TrN+I TrN+I TrN+I TrN+G	а о и а о о о о о о о о о о о о о о о о	1.0000 1.7386 1.7386 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000	1.4207 4.6965 5.1213 5.1213 0.4397 3.2875 8.3075 3.2014 4.8566 4.8566 3.8009 4.8566 3.8646 3.2898 11.6895 3.2898 11.6895 3.2898 11.6895 4.0352 7.0524 6.0800 0.9096	1,0000 1,7386 1,7386 1,0000 0,2353 1,0000 1,0000 1,0000 1,0000 3,1234 3,1234 1,0000 1,0000 1,0000 1,0000 1,0000 1,0000	1.0000 1.0000 1.0000 1.0000 1.0000 3.1185 1.0000 1.0000 1.0000 1.0000 1.9463 1.0000 1.9463 1.0000 1.9463 1.0000 1.0000 1.0000 1.0000	8.3891 4.6965 10.4238 14.2608 3.3084 5.9498 8.3075 5.5149 10.7362 13.8753 16.1138 15.5731 16.1138 15.5731 5.4586 22.131 5.4586 22.131 5.4586 22.131 5.4586 22.131 5.4586 25.6908 17.9272 9.1711	4.14 4.14 1414 2.8 135 2.8 135	0.8010 - 0.8440 0.8440 0.6760 0.6760 0.6760 0.5770 0.5710 0.7220 0.5510 0.5510 0.5510 0.0740 0.0740 0.0740 0.0740 0.0740 0.0740 0.0740 0.0740 0.0740 0.0740 0.0740 0.0740 0.0740 0.0740 0.0740 0.0740 0.07700 0.0770 0.077000 0.077000 0.0770000000000	- 4470 0.4470 0.2610 - 2610 0.7400 0.7400 0.4190 0.4190 0.4190 0.4190 0.3530 0.4190 0.3530 0.6330 0.6330 0.66330 0.66330 0.6810 0.2840 0.2840 0.2840 0.2840 0.7150	Equal Gamma Gamma Equal Equal Gamma Gamma Equal Equal Gamma Gamma Gamma Gamma Gamma
<sup>1</sup> bp         =         base pairs.           2         PIC         =         number of parsimony infi           3         Cl         =         consistency index.           4         Rl         =         retention index.           6         RC         =         retention index.           6         RC         =         restelled consistency index.	ormative characters. lex.	<ul> <li>HI</li> <li>Subst. model</li> <li>NST</li> <li>TI/Tv ratio</li> </ul>	homoplasy index. best fit substitution mo number of substitution transition/transversion	odel. r rate categories. r ratio.								

 Table 3
 Datasets used and statistics resulting from phylogenetic analyses.



**Fig. 2** Phylogenetic trees based on maximum likelihood (ML) analyses for species in *Botryosphaeria* and *Cophinforma*. a. ITS region; b. *tef1* gene region; c. *tub* gene region; d. *rpb2* gene region; e. LSU region; f. SSU region; g. combination of ITS, *tef1* and *tub* regions. Isolates sequenced in this study are in **bold**. Bootstrap support values  $\geq$  60 % for ML and MP are presented above branches as follows: ML/MP, bootstrap support values < 60 % are marked with '-', and absent (< 50 %) are marked with '\*'. Isolates representing ex-type sequences are marked with 'T'. *Neofusicoccum parvum* (ATCC 58191) was used as the outgroup taxon.





**Fig. 3** Phylogenetic trees based on maximum likelihood (ML) analyses for species in *Lasiodiplodia*. a. ITS region; b. *tef1* gene region; c. *tub* gene region; d. *rpb2* gene region; e. *cmdA* gene region; f. LSU region; g. SSU region; h. combination of ITS, *tef1*, *tub*, *rpb2* and *cmdA* regions. Isolates sequenced in this study are in **bold**. Bootstrap support values  $\geq$  60 % for ML and MP are presented above branches as follows: ML/MP, bootstrap values < 60 % are marked with '-', and absent (< 50 %) are marked with '\*'. Isolates representing ex-type sequences are marked with 'T'. *Botryosphaeria dothidea* (CBS 115476) was used as the outgroup taxon.





**Fig. 4** Phylogenetic trees based on maximum likelihood (ML) analyses for species in *Neofusicoccum*. a. ITS region; b. *tef1* gene region; c. *tub* gene region; d. *rpb2* gene region; e. LSU region; f. SSU region; g. combination of ITS, *tef1*, *tub* and *rpb2* regions. Isolates sequenced in this study are in **bold**. Bootstrap support values  $\geq$  60 % for ML and MP are presented above branches as follows: ML/MP, bootstrap support values < 60 % are marked with '-', and absent (< 50 %) are marked with '\*'. Isolates representing ex-type sequences are marked with 'T'. *Botryosphaeria dothidea* (CBS 115476) was used as the outgroup taxon.



showed that the six Chinese isolates in Group A are most closely related to *B. fabicerciana* and *B. fusispora* (Fig. 2a–f). The analyses of the combination of ITS, *tef1* and *tub* sequences indicated that the six isolates are not forming a well-resolved clade, but are phylogenetically more closely related to *B. fusispora* than to *B. fabicerciana* (Fig. 2g). Based on the phylogenetic analyses for ITS, *tef1*, *tub*, *rpb2*, LSU, SSU and the combination of the ITS, *tef1* and *tub* sequences, the six isolates were identified as *B. fusispora*.

Isolates in Group B (CERC2001, CERC2983 and CERC3452) and Group C (CERC2946 and CERC2947) were found to be consistently distinct from other known phylogenetically related species of *Botryosphaeria* by congruent distinction in the multiple datasets (Group B: ITS, *tef1*, *rpb2* and LSU datasets; Group C: ITS, *tef1* and *rpb2* datasets) (Fig. 2a–f). The analyses of the combination of ITS, *tef1* and *tub* sequences indicated that the isolates in Group B and Group C form two well-resolved clades supported by relatively high bootstrap values (Fig. 2g). Isolates in Groups B and C represent two previously undescribed species of *Botryosphaeria*.

The phylogenetic analyses based on ITS, *tef1*, *tub*, *rpb2*, LSU and SSU sequences consistently showed that three isolates (CERC2298, CERC2299 and CERC2300) in Group D were phylogenetically most closely related to *B. auasmontanum*, *B. dothidea*, *B. minutispermatia* and *B. sinensia* (Fig. 2a–f). The analyses of combined ITS, *tef1* and *tub* sequences showed that isolates in Group D form one well-resolved clade (Fig. 2g). Isolates in Group D were identified as a new species of *Botryosphaeria*.

#### Species residing in Cophinforma

The BLAST results for ITS sequences show that isolates CERC3482, CERC3484, CERC3489 and CERC3490 (Group E) are related to the genus Cophinforma. Only two species of Cophinforma have previously been described, Cophinforma atrovirens (Mehl et al. 2011, Phillips et al. 2013) and C. mamane (Gardner 1997, Phillips et al. 2013). The two species of Cophinforma are morphologically very similar, but can be distinguished based on ITS sequence data. BLAST results of the ITS sequences indicate that the four Chinese isolates are more closely related to C. atrovirens than to C. mamane. A BLAST search of the tef1 sequences show that the Chinese isolates and the ex-type isolate of C. atrovirens (CBS 124934) are identical. Cophinforma mamane does not have a tef1 sequence and cultures are not available (Phillips et al. 2013). Based on the sequence comparisons of the ITS and tef1 regions (tub gene sequences are not available for species of Cophinforma), isolates in Group E were identified as C. atrovirens (Fig. 2a-b, g).

## Species residing in Lasiodiplodia

The isolates in our study that clustered in the genus Lasiodiplodia grouped into three phylogenetic groups for the tef1 dataset (Group F: CERC2284; Group G: CERC2024, CERC3420, CERC3513, CERC3516; Group H: CERC2286, CERC2962, CERC3495) (Fig. 3b), and two clades (Group F = Group G; Group H) for the ITS, tub, rpb2, cmdA, LSU and SSU datasets (Fig. 3a, c-g). For Group F, the sequence analyses of the ITS, tef1, tub, rpb2, cmdA datasets showed that the Chinese isolates clustered into the same (ITS, tef1, rpb2 and cmdA) clade or close (tub) to L. brasiliense (LSU and SSU sequences are not available to *L. brasiliense*) (Fig. 3a-g). The analyses indicated that isolates in Group G and Group H clustered into the same (ITS, tub, rpb2, cmdA, LSU and SSU) clade or close (tef1) to L. theobromae and L. pseudotheobromae, respectively (Fig. 3a-g). The analyses of the combination of the ITS, tef1, tub, rpb2 and cmdA sequences

indicated that the isolates in Groups F, G and H are phylogenetically most closely related to *L. brasiliense*, *L. theobromae* and *L. pseudotheobromae*, respectively (Fig. 3h). Altogether, the results of these phylogenetic analyses identified isolates in Groups F, G and H as *L. brasiliense*, *L. theobromae* and *L. pseudotheobromae*, respectively.

#### Species residing in Neofusicoccum

For the Chinese isolates that grouped in the genus *Neofusicoccum*, the isolates in this study clustered into four phylogenetic groups for the ITS, *tef1*, *tub* and *rpb2* datasets (Group I: CERC3497, CERC3498; Group J: CERC2967, CERC2968, CERC2973; Group K: CERC2005, CERC2265, CERC3416, CERC3451; Group L: CERC2951, CERC3503, CERC3504, CERC3508) (Fig. 4a–d). The Chinese *Neofusicoccum* isolates clustered into three groups (Group I, Group J = Group K, Group L) for the LSU dataset, and two groups (Group I, Group J = Group K = Group L) for the SSU dataset (Fig. 4e–f).

Previous studies have shown that phylogenetic analyses of the ITS, tef1, tub and rpb2 sequences, especially a combination of the four gene sequences, is an efficient method for species identification in Neofusicoccum (Pavlic et al. 2009a, Sakalidis et al. 2011, Osorio et al. 2017). The isolates in each of Group I and J were found to be consistently distinct from other known phylogenetically closely related species of *Neofusicoccum* by congruent distinction in all the ITS, tef1, tub and rpb2 datasets (Fig. 4a–d). Isolates in Group K formed a single independent clade that was distinct from any known Neofusicoccum species in the tef1 and rpb2 datasets (Fig. 4b, d). The analyses of the combination of the ITS, tef1, tub and rpb2 sequences indicated that isolates in each of Groups I, J and K formed a well-resolved clade that was distinct from any described Neofusicoccum species which are supported by high bootstrap values (Fig. 4g). Therefore, we considered isolates in Groups I, J and K to represent three undescribed species of Neofusicoccum.

The Chinese isolates in Group L grouped in the same clade as *N. parvum* based on the ITS, *tub*, *rpb2*, LSU and SSU sequence analyses (Fig. 4a, c-f), and close to *N. parvum* on the *tef1* sequence analysis (Fig. 4b). In the phylogenetic analyses combining four gene regions, isolates in Group L were identified as *N. parvum* (Fig. 4g).

#### Morphology and taxonomy

Representative isolates (Table 1, 4) selected for morphological studies produced asexual fruiting structures on pine needles on WA media within 4–6 wk. No sexual structures were observed during the same period of time. For the 12 phylogenetic groups of Botryosphaeriaceae which were distinguished by DNA sequences, morphological studies, including culture and conidia characteristics, show that isolates in each of Group A, E, F, G, H and L were morphologically similar to the type specimens linked to it via sequence data, especially in the morphological characterisation of conidia (Table 4), namely B. fusispora, C. atrovirens, L. brasiliense, L. theobromae, L. pseudotheobromae and N. parvum, respectively. For phylogenetic groups B, C, D, I, J and K, morphological differences were observed compared to the phylogenetically most closely related species based on sequence data, and consequently each of the six groups were considered as new species. Based on the phylogenetic analyses and the morphological characteristics, the fungi collected from *Eucalyptus* and other plants in this study represent 12 species of Botryosphaeriaceae, including six previously undescribed species. These new species are described as follows.

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Table 4 Conidial measurements of Botryosphaeriaceae species examined in this study and comparison with measurements described in previous studies.

Species <sup>1</sup>	Conidial size (µm) (L × W) <sup>2</sup>	Mean (µm) (L × W) <sup>3</sup>	L/W <sup>4</sup>	Reference
Botryosphaeria auasmontanum	(8.1–)8.8–11.3(–13) × (2.5–)2.9–3.9(–5)	10.1 × 3.4	3.0	Slippers et al. (2014)
B. corticis	(20.5–)23.5–32.5(–34.5) × (5.0–)5.5–7(–7.5)	28.9 × 6.4	4.5	Phillips et al. (2006)
B. dothidea	$(20-)23-27(-30) \times 4-5(-6)$	26.2 × 5.4	4.9	Slippers et al. (2004a)
B. fabicerciana	$(16.5-)19.5-24.5(-26) \times (4.5-)5-6.5(-7.5)$	22.0 × 5.8	3.8	Chen et al. (2011c)
B. fusispora	(16.5-)19-23.5(-28.5) × 5-6(-8)	21.2 × 5.6	3.8	This study
B. fusispora	$16-22 \times 4-5.5$	20.0 × 5.0	4.0	Liu et al. (2012)
B. kuwatsukai	(18.5–)20–24.5(–26) × 5–7(–8)	22.3 × 6.2	3.6	Xu et al. (2015a)
B. minutispermatia	8–14 × 3–4	13.0 × 3.5	3.7	Ariyawansa et al. (2016)
B. pseudoramosa⁵	(8–)10–13(–16) × (4–)4.5–5(–6)	11.5 × 4.6	2.5	This study
B. qingyuanensis⁵	(15–)19.5–24.5(–28.5) × (5–)6–6.5(–7.5)	22.0 × 6.2	3.5	This study
B. ramosa	(11–)12–15(–16) × (4.7–)5–6(–7)	13.5 × 5.5	2.3	Pavlic et al. (2008)
B. rosaceae	20-31 × 6-8	26.2 × 6.7	3.9	Zhou et al. (2017)
B. scharifii	(11.5–)13–17(–19) × 4–6.5	15.4 × 5.2	2.7	Abdollahzadeh et al. (2013)
B. sinensia	(15–)19–29 × 5–7	24.3 × 5.9	4.1	Zhou et al. (2016)
B. wangensis⁵	(20.5–)22–26(–29) × (4.5–)5.5–6.5(–7.5)	$\textbf{23.8} \times \textbf{6.0}$	3.9	This study
Lasiodiplodia brasiliense	22.7–29.2 × 11.7–17	26.0 × 14.6	1.8	Netto et al. (2014)
L. brasiliense	(22–)25–27(–28) × (12–)13.5–15(–15.5)	26.0 × 14.4	1.8	This study
L. pseudotheobromae	(22.5-)23.5-32(-33) × (13.5-)14-18(-20)	28.0 × 16.0	1.7	Alves et al. (2008)
L. pseudotheobromae	(22.5–)24.5–28.5(–31.5) × (12–)13–15(–16)	26.5 × 13.8	1.9	This study
L. theobromae	(19–)21–31(–32.5) × (12–)13–15.5(–18.5)	26.2 × 14.2	1.9	Alves et al. (2008)
L. theobromae	(21–)24–26.5(–29.5) × (11–)12.5–14(–16)	25.3 × 13.1	1.9	This study
Neofusicoccum algeriense	(14.5–)17–18(–21) × (4.5–)5.5–5.7(–6.5)	17.6 × 5.6	3.1	Berraf-Tebbal et al. (2014)
N. batangarum	$(12-)14-17.5(-20) \times (4-)4.5-6(-6.5)$	15.5 × 5.5	2.9	Begoude et al. (2010)
N. cordaticola	18-28 × 4.5-7	23.3 × 5.3	4.3	Pavlic et al. (2009b)
N. hongkongense <sup>5</sup>	(11.5–)13–15.5(–17.5) × (4–)4.5–5(–5.5)	14.1 × 4.7	3.0	This study
N. kwambonambiense	$16-28 \times 5-8$	22.3 × 6.3	3.6	Pavlic et al. (2009b)
N. microconidium <sup>5</sup>	(10–)11.5–13(–14.5) × (4–)4.5–5.5(–6)	12.3 × 5.0	2.5	This study
N. mangiferae	(11–)12–15(–17.5) × 5–6.6	13.6 × 5.4	2.0-2.5	Slippers et al. (2005)
N. occulatum	14-22 × 3.5-7.5	18.3 × 5.2	3.5	Sakalidis et al. (2011)
N. parvum	(12–)13.5–21(–24) × 4–6(–10)	17.1 × 5.5	3.2	Phillips et al. (2013)
N. parvum	(15.5–)16.5–19(–21) × (4.5–)5–6(–6.5)	17.9 × 5.5	3.3	This study
N. ribis	(16–)19–23(–24) × 5–6(–7)	20.8 × 5.5	3.8	Slippers et al. (2004a)
N. sinense	(15.2–)17.6–20.4(–23) × (6.9–)7.4–8(–9)	18.7 × 7.7	2.4	Zhang et al. (2017)
N. sinoeucalypti⁵	(13–)15–20.5(–25.5) × (4–)5–5.5(–6.5)	17.7 × 5.2	3.4	This study
N. umdonicola	15-23.5 × 4.5-6.5	19.4 × 5.5	3.5	Pavlic et al. (2009b)

<sup>1</sup> Isolates and measurements in **bold** were examined in this study.

<sup>2</sup> Minimum-(average - standard deviation)-(average + standard deviation)-maximum or minimum-maximum, L × W = length × width.

 $^{3}$  L × W = average length × average width.

<sup>4</sup> L/W = average length/average width.

5 Novel species described in this study.

## TAXONOMY

Botryosphaeria pseudoramosa G.Q. Li & S.F. Chen, sp. nov. — MycoBank MB822323; Fig. 5

Etymology. Named for its phylogenetic resemblance to B. ramosa.

Sexual morph unknown. Conidiomata pycnidial, produced on pine needles on WA within 2–4 wk, globose to ovoid, dark brown to black, up to 698 µm wide, sometimes with a neck up to 1660 µm long, arising from the substrate, covered by hyphal hairs, embedded in needle tissue, semi-immersed to superficial, unilocular, with a central ostiole. Conidiophores reduced to conidiogenous cells. Conidiogenous cells holoblastic, discrete, hyaline, cylindrical to lageniform, phialidic with periclinal thickening,  $(10-)11-16(-22.5) \times (1-)2-3.5(-4)$  µm. Paraphyses not seen. Conidia hyaline, thin-walled, smooth with granular contents, unicellular, aseptate ellipsoid to fusoid, base subtruncate to bluntly rounded,  $(8-)10-13(-16) \times (4-)4.5-5(-6)$  µm (av. =  $11.5 \times 4.6$  µm, n = 100; L/W = 2.5) (Table 4).

Culture characteristics — Colonies on MEA have fluffy mycelia with an uneven margin and a few cottony aerial mycelia reaching to the lid of the Petri plate, with an appressed mycelial mat that is sparse to moderately dense. Colony mycelia initially white, becoming smoke grey (21""f) to pale mouse grey (15""'d) at the surface and olivaceous (21"k) to iron grey (23""'k) at the reverse within 10–14 d. Optimal growth temperature is 30 °C, covering the 90 mm plates after 5 d. No growth at 5 °C. After 5 d, colonies at 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, 35 °C and 40 °C reached 17 mm, 20 mm, 64 mm, 80 mm, 87 mm, 33 mm and 8 mm, respectively.

Specimens examined. CHINA, GuangXi, from twigs of one *Eucalyptus* tree, fruiting structures induced on needles of *Pinus* sp. on water agar, 24 May 2014, *S.F. Chen & G.Q. Li* (holotype CSFF2025, culture ex-type CERC2001 = CGMCC3.18739); GuangDong, from twigs of one *Eucalyptus* tree, 24 May 2014, *S.F. Chen & G.Q. Li* (CSFF2026, culture CERC3455 = CGMCC3.18741); GuangDong, from twigs of one *Melastoma sanguineum* plant, 17 Mar. 2014, *S.F. Chen* (CSFF2027, culture CERC2983 = CGMCC3.18740).

Notes — Botryosphaeria pseudoramosa is phylogenetically closely related to *B. ramosa* and *B. scharifii. Botryosphaeria pseudoramosa* can be distinguished from *B. ramosa* and *B. scharifii* based on the morphology of their conidia. Conidia of *B. pseudoramosa* (av. 11.5 × 4.6; L/W = 2.5) are smaller than *B. ramosa* (av. 13.5 × 5.5; L/W = 2.3) (Pavlic et al. 2008) and *B. scharifii* (av. 15.4 × 5.2; L/W = 2.7) (Abdollahzadeh et al. 2013) (Table 4).

#### Botryosphaeria qingyuanensis G.Q. Li & S.F. Chen, sp. nov. — MycoBank MB822324; Fig. 6

*Etymology*. Named for the QingYuan Region where the fungus was isolated for the first time.

Sexual morph unknown. Conidiomata pycnidial, produced on pine needles on WA within 2–4 wk, solitary, globose to ovoid, dark brown to black, up to 317 µm wide, 229 µm high, embedded in needle tissue, semi-immersed to superficial, unilocular, with a central ostiole. Conidiophores absent. Conidiogenous cells holoblastic, discrete, hyaline, cylindrical to lageniform, phialidic with periclinal thickening,  $(7-)7.5-12(-14.5) \times (2-)2.5-3.5 \ \mum. Paraphyses$  not seen. Conidia hyaline, thin-walled, smooth with

granular contents, unicellular, aseptate narrowly fusiform, base subtruncate to bluntly rounded,  $(15-)19.5-24.5(-28.5) \times (5-)6-6.5(-7.5) \mu m$  (av. = 22 × 6.2  $\mu m$ , n = 100; L/W = 3.5) (Table 4).

Culture characteristics — Colonies on MEA have fluffy mycelia with an uneven margin and few cottony aerial mycelia reaching to the lid of the Petri plate, with an appressed mycelial mat that is sparse to moderately dense. Colony mycelia initially white, becoming smoke grey (21""f) to pale mouse grey (15""d) at the surface and smoke grey (21""f) to iron grey (23""k) at the reverse within 10–14 d. Optimal growth temperature is (25–)30 °C, reaching the edge of the 90 mm plates after 5 d. No growth is observed at 5 °C and 40 °C. After 5 d, colonies at 10 °C, 15 °C, 20 °C, 25 °C, 30 °C and 35 °C reach 14 mm, 22 mm, 52 mm, 73 mm, 74 mm and 12 mm, respectively.

Specimens examined. CHINA, GuangDong, from twigs of one *Eucalyptus* tree, fruiting structures induced on needles of *Pinus* sp. on water agar, 4 Dec. 2013, *S.F. Chen & G.Q. Li* (holotype CSFF2028, culture ex-type CERC2946 = CGMCC3.18742); GuangDong, from twigs of one *Eucalyptus* hybrid tree, fruiting structures induced on needles of *Pinus* sp. on water agar, 4 Dec. 2013, *S.F. Chen & G.Q. Li* (CSFF2029, culture CERC2947 = CGMCC3.18743).

Notes — Botryosphaeria qingyuanensis is phylogenetically closely related to *B. corticis*, *B. fabicerciana*, *B. fusispora*, *B. kuwatsukai* and *B. rosaceae*, but can be distinguished from these species based on morphological or growth characteristics. Conidia of *B. qingyuanensis* (av.  $22 \times 6.2$ ; L/W = 3.5) are wider than these of *B. fabicerciana* (av.  $22 \times 5.8$ ; L/W = 3.8) and the optimal growth temperature of *B. qingyuanensis* ((25–)30 °C) is different from that of *B. fabicerciana* (25(–30) °C) (Chen et al. 2011c). Conidia of *B. qingyuanensis* are longer and wider than *B. fusispora* (av.  $20 \times 5$ ; L/W = 4) (Liu et al. 2012). Conidia of *B. qingyuanensis* are smaller than *B. corticis* (av. 28.9 × 6.4;

L/W = 4.5) (Phillips et al. 2006) and *B. rosaceae* (av.  $26.2 \times 6.7$ ; L/W = 3.9) (Zhou et al. 2017). Conidia of *B. qingyuanensis* are slightly shorter than *B. kuwatsukai* (av.  $22.3 \times 6.2$ ; L/W = 3.6) (Xu et al. 2015a) and no conidia or microconidia are observed for *B. qingyuanensis*, but conidia with 1–3 septa before germination and microconidia ( $3-8 \times 1-2 \mu m$ ) have been found for *B. kuwatsukai* (Xu et al. 2015a) (Table 4).

#### Botryosphaeria wangensis G.Q. Li & S.F. Chen, sp. nov. — MycoBank MB822325; Fig. 7

 $\ensuremath{\textit{Etymology}}$  . Named after the Wang village where the fungus was isolated for the first time.

Sexual morph unknown. Conidiomata pycnidial, produced on pine needles on WA within 2-4 wk, solitary, globose to ovoid, dark brown to black, up to 698 µm wide, 484 µm high, embedded in needle tissue, semi-immersed to superficial, unilocular, with a central ostiole, exuding conidia in a vellow mucoid mass. Conidiophores absent. Conidiogenous cells holoblastic, discrete, hyaline, cylindrical to lageniform, phialidic with periclinal thickening, (6-)8.5-13.5(-15) × 2-3(-3.5) µm. Paraphyses not seen. Conidia hyaline, thin-walled, smooth with granular contents, unicellular, aseptate, becoming 1-septate before germination, narrowly fusiform, base subtruncate to bluntly rounded,  $(20.5-)22-26(-29) \times (4.5-)5.5-6.5(-7.5) \mu m$  (av. =  $23.8 \times 6 \mu m$ , n = 100; L/W = 3.9) (Table 4). Spermatophores hyaline, smooth, branched, cylindrical to subcylindrical (Fig. 7f). Spermatogenous cells discrete or integrated, hyaline, smooth, cylindrical, producing spermatia on their tips, holoblastic or proliferating via phialides with periclinal thickenings, 6.5-16 × 1.5-2.5 µm. Spermatia unicellular, aseptate, hyaline, thinwalled, allantoid to rod-shaped,  $3.5-4.5 \times 1-1.5 \mu m$ , L/W = 2.9.



**Fig. 5** Botryosphaeria pseudoramosa. a-b. Conidiomata formed on pine needle culture; c-d. conidiogenous cells and developing conidia; e. conidia; f. living culture after 10 d on 2 % MEA (front). — Scale bars:  $a-b = 500 \mu m$ ;  $c-e = 10 \mu m$ ; f = 1 cm.

Culture characteristics — Colonies on MEA have fluffy mycelia with an uneven margin and a few cottony aerial mycelia reaching to the lid of the Petri plate, with an appressed mycelial mat that is sparse to moderately dense. Colony mycelia initially white, becoming smoke grey (21""f) to mouse grey (13""i) at the surface and olivaceous grey (21""i) to iron grey (23""k) at the reverse within 10–14 d. Optimal growth temperature is 30 °C, covering the 90 mm plates after 5 d. No growth at 5 °C and 40 °C. After 5 d, colonies at 10 °C, 15 °C, 20 °C, 25 °C, 30 °C and 35 °C reach 15 mm, 21 mm, 50 mm, 69 mm, 89 mm and 24 mm, respectively.

Specimens examined. CHINA, HeNan, from twigs of one *Cedrus deodara* tree, fruiting structures induced on needles of *Pinus* sp. on water agar, 26 Nov. 2013, *S.F. Chen* (holotype CSFF2030, culture ex-type CERC2298 =

CGMCC3.18744); HeNan, from twigs of one *Cedrus deodara* tree, 26 Nov. 2013, *S.F. Chen* (CSFF2031, culture CERC2300 = CGMCC3.18746).

Notes — *Botryosphaeria wangensis* is phylogenetically closely related to *B. auasmontanum*, *B. dothidea*, *B. minutispermatia* and *B. sinensia*. *Botryosphaeria wangensis* can be distinguished from its phylogenetically closely related species by the size of their conidia. Conidia of *B. wangensis* (av. 23.8 × 6; L/W = 3.9) are longer and wider than those of *B. auasmontanum* (av.  $10.1 \times 3.4$ ; L/W = 3) (Slippers et al. 2014) and *B. minutispermatia* (av. 13 × 3.5; L/W = 3.7) (Ariyawansa et al. 2016) and shorter and wider than those of *B. dothidea* (av. 26.2 × 5.4; L/W = 4.9) (Slippers et al. 2004a) and *B. sinensia* (av. 24.3 × 5.9; L/W = 4.1) (Zhou et al. 2016) (Table 4).



**Fig. 6** Botryosphaeria qingyuanensis. a. Conidiomata formed on pine needle culture; b-c. conidiogenous cells and developing conidia; d. conidia; e. living culture after 10 d on 2 % MEA (front). — Scale bars:  $a = 500 \mu m$ ;  $b-d = 10 \mu m$ ; e = 1 cm.

#### Neofusicoccum hongkongense G.Q. Li & S.F. Chen, sp. nov. — MycoBank MB822328; Fig. 8

 $\ensuremath{\textit{Etymology}}$  . Named after the Hong Kong Region where it was isolated for the first time.

Sexual morph unknown. Conidiomata pycnidial, produced on pine needles on WA within 2–4 wk, solitary, globose to ovoid, dark brown to black, up to 694 µm wide, up to 776 µm high, embedded in needle tissue, semi-immersed to superficial, unilocular, with a central ostiole. Conidiophores reduced to conidiogenous cells. Conidiogenous cells holoblastic, discrete, hyaline, cylindrical, phialidic with periclinal thickening, (9.5-) 12–18.5(–22) × (1.5-)2-2.5(-3) µm. Paraphyses not seen. Conidia hyaline, thin-walled, smooth with granular contents, unicellular, aseptate narrowly fusiform, base subtruncate to bluntly rounded,  $(11.5-)13-15.5(-17.5) \times (4-)4.5-5(-5.5)$  µm (av. = 14.1 × 4.7 µm, n = 100; L/W = 3) (Table 4).

Culture characteristics — Colonies on MEA have fluffy mycelia with an uneven margin and a few cottony aerial mycelia

reaching to the lid of the Petri plate, with an appressed mycelial mat that is sparse to moderately dense. Colony mycelia initially white, becoming smoke grey (21""f) to grey olivaceous (21""b) at the surface and grey olivaceous (21""b) to olivaceous grey (21""i) at the reverse within 10–14 d. Optimal growth temperature is 25 °C, covering the 90 mm plates after 5 d. No growth at 5 °C or 40 °C. After 5 d, colonies grown at 10 °C, 15 °C, 20 °C, 25 °C, 30 °C and 35 °C reach 25 mm, 41 mm, 66 mm, 90 mm, 84 mm and 9 mm, respectively.

Specimens examined. CHINA, Hong Kong, from twigs of *Araucaria cunninghamii*, fruiting structures induced on needles of *Pinus* sp. on water agar, 11 Mar. 2014, *S.F. Chen* (holotype CSFF2034, culture ex-type CERC2973 = CGMCC3.18749); Hong Kong, from twigs of *Araucaria cunninghamii*, 11 Mar. 2014, *S.F. Chen* (CSFF2035, culture CERC2968 = CGMCC3.18748).

Notes — Based on phylogenetic analyses, *N. hongkongense* phylogenetically clustered in the *N. parvum/N. ribis* species complex. *Neofusicoccum hongkongense* can be distinguished from other species in the *N. parvum/N. ribis* complex by the size and shape of their conidia. The conidia of *N. hongkongense* 



**Fig. 7** *Botryosphaeria wangensis.* a-b. Conidiomata on pine needle culture; c-d. conidiogenous cells and developing conidia; e. conidia with 1 septum; f. spermatogenous cells; g. spermatia; h. living culture after 10 d on 2 % MEA (front). — Scale bars:  $a-b = 500 \mu$ m;  $c-g = 10 \mu$ m; h = 1 cm.

(av. 14.1 × 4.7; L/W = 3) are shorter and narrower than those of *N. algeriense* (av. 17.6 × 5.6; L/W = 3.1) (Berraf-Tebbal et al. 2014), *N. batangarum* (av. 15.5 × 5.5; L/W = 2.9) (Begoude et al. 2010), *N. cordaticola* (av. 23.3 × 5.3; L/W = 4.3) (Pavlic et al. 2009b), *N. kwambonambiense* (av. 22.3 × 6.3; L/W = 3.6) (Pavlic et al. 2009b), *N. occulatum* (av. 18.3 × 5.2; L/W = 3.5) (Sakalidis et al. 2011), *N. parvum* (av. 17.1 × 5.5; L/W = 3.2) (Phillips et al. 2013), *N. ribis* (av. 20.8 × 5.5; L/W = 3.8) (Slippers et al. 2004a), *N. sinense* (av. 18.7 × 7.7; L/W = 2.4) (Zhang et al. 2017), *N. sineucalypti* (av. 17.7 × 5.2; L/W = 3.4) (this study) and *N. umdonicola* (av. 19.4 × 5.5; L/W = 3.5) (Pavlic et al. 2009b). The conidial size of *N. brasiliense* remains unknown (Marques et al. 2013) (Table 4).

## Neofusicoccum microconidium G.Q. Li & S.F. Chen, sp. nov. — MycoBank MB822326; Fig. 9

Etymology. Named for the small conidia of this fungus.

Sexual morph unknown. Conidiomata pycnidial, produced on pine needles on WA within 2–4 wk, solitary, globose to ovoid, dark brown to black, up to 895 µm wide, 1729 µm high, embedded in needle tissue, semi-immersed to superficial, unilocular, with a central ostiole, exuding conidia in a white mucoid mass. Conidiophores reduced to conidiogenous cells. Conidiogenous cells holoblastic, discrete, hyaline, cylindrical, phialidic with periclinal thickening,  $(10.5-)12.5-18(-20.5) \times (2-)2.5-3(-3.5)$  µm. Paraphyses not seen. Conidia hyaline, thin-walled, smooth with granular contents, unicellular, aseptate narrowly fusiform, base subtruncate to bluntly rounded,  $(10-)11.5-13(-14.5) \times (4-)4.5-5.5(-6)$  µm (av. =  $12.3 \times 5$  µm, n = 100; L/W = 2.5) (Table 4).



**Fig. 8** Neofusicoccum hongkongense. a. Conidiomata formed on pine needle culture; b. conidiogenous cells and developing conidia; c. conidiogenous cells; d. conidia; e. living culture after 10 d on 2 % MEA (front). — Scale bars:  $a = 500 \mu m$ ;  $b-d = 10 \mu m$ ; e = 1 cm.



**Fig. 9** Neofusicoccum microconidium. a. Conidiomata formed on pine needle culture; b-c. conidiogenous cells and developing conidia; d. conidia; e. living culture after 10 d on 2 % MEA (front). — Scale bars:  $a = 500 \mu m$ ;  $b-d = 10 \mu m$ ; e = 1 cm.

Culture characteristics — Colonies on MEA have fluffy mycelia with an uneven margin and a few cottony aerial mycelia reaching to the lid of the Petri plate, with an appressed mycelial mat that is sparse to moderately dense. Colony mycelia initially white, becoming pale mouse grey (15""'d) to olivaceous grey (21""'i) at the surface and olivaceous grey (21""'i) to iron grey (23""'k) at the reverse within 10–14 d. Optimal growth temperature is 30 °C, reaching the edge of the 90 mm plates after 5 d. No growth at 5 °C. After 5 d, colonies at 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, 35 °C and 40 °C reach 24 mm, 34 mm, 66 mm, 74 mm, 86 mm, 36 mm and 8 mm, respectively.

Specimens examined. CHINA, GuangDong, from twigs of *E. urophylla* × *E. grandis*, fruiting structures induced on needles of *Pinus* sp. on water agar, 22 July 2014, *S.F. Chen* & *G.Q. Li* (holotype CSFF2032, culture ex-type CERC3497 = CGMCC3.18750); GuangDong, from twigs of *E. urophylla* × *E. grandis*, 22 July 2014, *S.F. Chen* & *G.Q. Li* (CSFF2033, culture CERC3498 = CGMCC3.18751).

Notes — *Neofusicoccum microconidium* is phylogenetically closely related to *N. mangiferae*. The two species can be distinguished from each other based on conidial morphology. Conidia of *N. microconidium* (av.  $12.3 \times 5$ ; L/W = 2.5) are smaller than those of *N. mangiferae* (av.  $13.6 \times 5.4$ ; L/W = 2–2.5) (Slippers et al. 2005) (Table 4).

## Neofusicoccum sinoeucalypti G.Q. Li & S.F. Chen, sp. nov. — MycoBank MB822327; Fig. 10

*Etymology*. Named after the host genus *Eucalyptus* from which it was isolated for the first time.

Sexual morph solitary, globose to ovoid, dark brown to black, up to 1 007  $\mu$ m wide, 685  $\mu$ m high, embedded in needle tissue, semi-immersed to superficial, unilocular, with a central ostiole. Conidiophores absent. Conidiogenous cells holoblastic, discrete, hyaline, cylindrical to lageniform, phialidic with periclinal thickening,  $(10-)10.5-11 \times 2-3 \mu m$ . Paraphyses not seen. Conidia hyaline, thin-walled, smooth with granular contents, unicellular, aseptate, narrowly fusiform, base subtruncate to bluntly rounded,  $(13-)15-20.5(-25.5) \times (4-)5-5.5(-6.5) \mu m$ (av. = 17.7 × 5.2 µm, n = 100; L/W = 3.4). Spermatophores hyaline, smooth, cylindrical to subcylindrical. Spermatogenous cells discrete or integrated, hyaline, smooth, cylindrical, producing spermatia on their tips, holoblastic or proliferating via phialides with periclinal thickenings,  $8.5-15.5 \times 1.5-2 \mu m$ . Spermatia unicellular, aseptate, hyaline, thin-walled, allantoid to rod-shaped,  $2.5-4.5 \times 1.5 \ \mu m$ , L/W = 2.1.

Culture characteristics — Colonies on MEA have fluffy mycelia with an uneven margin and a few cottony aerial mycelia that reach to the lid of the Petri plate, with an appressed mycelial mat that is sparse to moderately dense. Colony mycelia are initially



**Fig. 10** Neofusicoccum sinoeucalypti. a. Conidiomata formed on pine needle culture; b. conidiogenous cells and developing conidia; c. immature conidia; d–e. mature conidia with 1–2 septa; f. spermatogenous cells; g. spermatia; h. living culture after 10 d on 2 % MEA (front). — Scale bars:  $a = 500 \mu m$ ;  $b-g = 10 \mu m$ ; h = 1 cm.

white, becoming pale mouse grey  $(15^{\text{min}}d)$  to mouse grey  $(13^{\text{min}})$  at the surface and olivaceous buff  $(21^{\text{min}}d)$  to iron grey  $(23^{\text{min}}k)$  at the reverse within 10–14 d. Optimal growth temperature is 30 °C, reaching the edge of the 90 mm plates after 5 d. No growth at 5 °C or 40 °C. After 5 d, colonies at 10 °C, 15 °C, 20 °C, 25 °C, 30 °C and 35 °C reach 25 mm, 31 mm, 53 mm, 78 mm, 90 mm and 11 mm, respectively.

Specimens examined. CHINA, GuangDong, from twigs of *E. urophylla* × *E. grandis*, fruiting structures induced on needles of *Pinus* sp. on water agar, 30 July 2013, *S.F. Chen & G.Q. Li* (holotype CSFF2036, culture extype CERC2005 = CGMCC3.18752); GuangXi, from twigs of *E. urophylla* ×

*E. grandis*, 25 Oct. 2013, *S.F. Chen & G.Q. Li* (CSFF2037, culture CERC2265 = CGMCC3.18753); GuangXi, from twigs of *Eucalyptus* hybrid, 22 May 2014, *S.F. Chen & G.Q. Li* (CSFF2038, culture CERC3416 = CGMCC3.18754).

Notes — Neofusicoccum sinoeucalypti clustered in the N. parvum/N. ribis species complex. Other species in this complex include N. algeriense, N. batangarum, N. brasiliense, N. cordaticola, N. hongkongense (this study), N. kwambonambiense, N. occulatum, N. parvum, N. ribis and N. umdonicola. For these species, except N. brasiliense (morphological data not available) (Marques et al. 2013), spermatia have been reported only in N. sinoeucalypti and are allantoid to rod-shaped. Conidia of N. sinoeucalypti (av. 17.7 × 5.2; L/W = 3.4) are longer and wider than those of *N. hongkongense* (av. 14.1 × 4.7; L/W = 3), longer and narrower than those of N. batangarum (av.  $15.5 \times$ 5.5; L/W = 2.9) (Begoude et al. 2010) and N. parvum (av. 17.1 × 5.5; L/W = 3.2) (Phillips et al. 2013), shorter and narrower than those of N. cordaticola (av. 23.3 × 5.3; L/W = 4.3) (Pavlic et al. 2009b), N. kwambonambiense (av. 22.3 × 6.3; L/W = 3.6) (Pavlic et al. 2009b), N. ribis (av. 20.8 × 5.5; L/W = 3.8) (Slippers et al. 2004a), N. sinense (av. 18.7 × 7.7; L/W = 2.4) (Zhang et al. 2017) and N. umdonicola (av. 19.4 × 5.5; L/W = 3.5) (Pavlic et al. 2009b), shorter than those of N. occulatum (av. 18.3 × 5.2; L/W = 3.5) (Sakalidis et al. 2011), and narrower than those of N. algeriense (av. 17.6 × 5.6; L/W = 3.1) (Berraf-Tebbal et al. 2014). The optimal growth temperature of N. sinoeucalypti (30 °C) is different compared to N. algeriense (25 °C) (Berraf-Tebbal et al. 2014), N. batangarum (25 °C) (Begoude et al. 2010), N. brasiliense (27.7 °C) (Marques et al. 2013), N. hongkongense (25 °C) (this study), N. occulatum (25 °C) (Sakalidis et al. 2011) and N. ribis (25 °C) (Slippers et al. 2004a) (Table 4).

## Distribution of Botryosphaeriaceae

According to the phylogenetic and morphological analyses of the 105 isolates collected in this study, twelve species of *Botryosphaeriaceae* were identified from seven hosts in the FuJian, GuangDong, GuangXi, HaiNan and HeNan Provinces and the Hong Kong Region of China (Fig. 11). These species include B. fusispora (21 isolates: all from Eucalyptus hybrids), B. pseudoramosa (12 isolates: 8 from Eucalyptus hybrids, 4 from M. sanguineum), B. qingyuanensis (2 isolates: both from one Eucalyptus hybrid), B. wangensis (3 isolates: all from C. deodara), C. atrovirens (5 isolates: all from D. longan), L. brasiliense (1 isolate: from a Eucalyptus hybrid), L. pseudotheobromae (19 isolates: 17 from unknown Eucalyptus hybrids, two from *E. urophylla* × *E. grandis*), *L. theobromae* (20 isolates: six from unknown Eucalyptus hybrids, five from E. urophylla × E. grandis, 2 from C. lanceolata, 5 from D. longan, 2 from P. hanceana), N. hongkongense (3 isolates: all from A. cunninghamii), N. microconidium (2 isolates: both from E. urophylla × E. grandis), N. parvum (6 isolates: all from E. urophylla × E. grandis) and N. sinoeucalypti (11 isolates: nine from E. urophylla × E. grandis, two from Eucalyptus hybrids) (Table 1, Fig. 11). The 81 isolates collected from Eucalyptus trees include nine species (except for B. wangensis, C. atrovirens and N. hongkongense) of Botryosphaeriaceae. Of these nine species from Eucalyptus, B. fusispora (26 % of the isolates), L. pseudotheobromae (23 % of the isolates) and L. theobromae (14 % of the isolates) are dominant and are distributed throughout the surveyed Provinces of South China. Of the 12 species of Botryosphaeriaceae, L. theobromae (isolated from C. lanceolata, D. longan, a Eucalyptus hybrid and P. hanceana) and B. pseudoramosa (isolated from a Eucalyptus hybrid and M. sanguineum) were collected from more than one plant host (Fig. 11).



Fig. 11 Map showing the 12 species of *Botryosphaeriaceae* detected from different regions and plant hosts. The different *Botryosphaeriaceae* species are indicated as numbers 1 to 12; the plant hosts are shown as letters A to G. For example, A8 indicates *L. theobromae* (number 8 of fungal species) isolated from *Eucalyptus* spp. (letter A of plant species) in HaiNan Province. The pies in colours indicate *Botryosphaeriaceae* isolated from different plant hosts in this study, the pies without colour indicate *Botryosphaeriaceae* species reported from *Eucalyptus* in previous studies (Chen et al. 2011c, Li et al. 2015a).

#### Table 5 Average lesion length (mm) on seedlings of three Eucalyptus clones inoculated with Botryosphaeriaceae.

Species	Isolates		Eucalyptus clones	
		CEPT-11	CEPT-12	CEPT-13
Botryosphaeria fusispora	CERC1998	17.6 ± 1.8 m-p <sup>1</sup>	27.1 ± 9.3 k-p	10.6 ± 0.7 op
	CERC2274	10.3 ± 0.4 op	10.7 ± 0.4 op	9.2 ± 0.2 op
	CERC2930	12.3 ± 1.2 op	13.9 ± 2.4 m-p	14.5 ± 1.6 m-p
	CERC3446	12.0 ± 0.6 op	17.5 ± 4.5 m-p	15.5 ± 2.8 m-p
B. pseudoramosa	CERC2001	13.5 ± 0.8 n-p	15.5 ± 4.0 m-p	11.1 ± 0.5 op
·	CERC3452	16.8 ± 2.0 m-p	26.9 ± 7.3 k-p	13.9 ± 1.9 m-p
B. qingyuanensis	CERC2946	10.4 ± 0.5 op	16.2 ± 5.6 m-p	11.1 ± 0.8 op
	CERC2947	11.0 ± 0.5 op	18.2 ± 5.2 l-p	10.4 ± 0.5 op
B. wangensis	CERC2298	10.6 ± 0.5 op	12.3 ± 0.8 op	10.0 ± 0.2 op
C C	CERC2299	9.3 ± 1.1 op	11.1 ± 0.3 op	9.8 ± 0.1 op
Cophinforma atrovirens	CERC3484	11.0 ± 1.5 op	10.0 ± 1.5 op	8.8 ± 1.1 op
,	CERC3489	12.2 ± 0.7 op	9.2 ± 0.2 op	9.4 ± 0.3 op
Lasiodiplodia brasiliense	CERC2284	41.7 ± 5.6 j-m	139.7 ± 27.3 cd	95.8 ± 15.7 f
L. pseudotheobromae	CERC2286	90.7 ± 16.9 fg	84.5 ± 12.6 f-h	100.1 ± 21.8 ef
	CERC3417	78.4 ± 9.7 fg	121.0 ± 12.6 de	128.4 ± 12.2 d
	CERC3495	120.9 ± 11.5 de	138.1 ± 12.8 cd	85.9 ± 9.5 fg
L. theobromae	CERC3420	26.7 ± 2.3 k-p	46.6 ± 7.5 i-l	26.8 ± 5.6 k-p
	CERC3513	123.9 ± 16.0 d	150.7 ± 21.9 b	219.5 ± 19.8 a
	CERC3516	126.3 ± 13.1 d	142.0 ± 21.6 bc	173.5 ± 18.7 b
Neofusicoccum hongkongense	CERC2968	17.7 ± 1.2 m-p	12.9 ± 2.2 op	14.6 ± 1.2 m-p
	CERC2973	21.6 ± 1.2 k-p	31.8 ± 5.6 k-p	18.7 ± 1.3 m-p
N. microconidium	CERC3497	32.7 ± 2.2 k-p	47.7 ± 7.5 i-k	40.8 ± 6.8 j-n
	CERC3498	16.6 ± 1.2 m-p	17.2 ± 1.7 m-p	20.8 ± 4.3 l-p
N. parvum	CERC2951	10.3 ± 0.5 op	11.9 ± 0.9 op	10.3 ± 0.3 op
	CERC3504	17.3 ± 0.7 m-p	30.1 ± 5.2 k-p	22.1 ± 3.2 k-p
	CERC3509	16.0 ± 1.5 m-p	17.5 ± 4.0 k-p	15.3 ± 2.4 m-p
N. sinoeucalypti	CERC2005	27.5 ± 4.7 k-p	68.0 ± 9.0 g-i	62.3 ± 8.7 h-j
	CERC3463	30.9 ± 4.8 k-p	24.0 ± 4.3 k-p	39.3 ± 7.7 j-0
Control		10.5 ± 0.6 op	10.0 ± 0.2 op	9.4 ± 0.3 op

<sup>1</sup> Mean ± SE followed by different lowercase letters indicates treatments that are significantly different (P < 0.05); Mean = average lesion length; SE = standard error of mean.

#### Pathogenicity tests

Twenty-eight isolates representing the 12 species of Botryosphaeriaceae identified in this study were used for inoculations on three different Eucalyptus clones (different parents) (Table 1, 5). Pathogenicity tests indicate that all of the Botryosphaeriaceae isolates tested produce lesions on stems of the three Eucalyptus clones, while MEA unclonised plugs produced only wounds. Overall, isolates in species of Lasiodiplodia produce relatively longer lesions than that of Botryosphaeria, Cophinforma and Neofusicoccum. For all three tested Eucalyptus clones, the lesions produced by Lasiodiplodia isolates are all significantly longer than the wounds caused by negative controls, except isolate CERC3420 (L. theobromae) on CEPT-11 and CEPT-13 (P < 0.05) (Table 5). For isolates in the genera of Botryosphaeria, Cophinforma and Neofusicoccum, isolates CERC3497 (N. microconidium) and CERC2005 (N. sinoeucalypti) also produce significantly longer lesions on CEPT-11 and CEPT-13 (P < 0.05) (Table 5). Analysis of variance shows significant differences in the susceptibility of the three Euca*lyptus* clones to some of the isolates we tested. For example, the lesions produced by isolate CERC2284 (L. brasiliense) on three Eucalyptus clones are significantly different from each other (P < 0.05) (Table 5). Analysis of results also show that not all the isolates of the same species of Botryosphaeriaceae react in the same manner to the Eucalyptus clones. For example, lesions produced by isolate CERC3420 (L. theobromae) on clone CEPT-12 are significantly longer than those on CEPT-13, whereas lesions produced by isolate CERC3513 (L. theobromae) on CEPT-12 are significantly shorter than those on CEPT-13 (P < 0.05) (Table 5). In addition, based on the lesions caused by all Botryosphaeriaceae isolates in this study, CEPT-11 (average lesion length: 33.0 ± 2.4 mm) is more tolerant than CEPT-12 (average lesion length:  $44.2 \pm 3.2$  mm) and CEPT-13 (average lesion length: 42.0 ± 3.4 mm). All 12 species of Botryosphaeriaceae were re-isolated successfully from the lesions, and no *Botryosphaeriaceae* were isolated from the negative controls, thus fulfilling Koch's postulates.

#### DISCUSSION

In this study, disease samples from symptomatic trees with stem cankers, shoot and twig blight were collected mainly from Eucalyptus and six other plant hosts in China. Botryosphaeriaceae was isolated from these diseased samples. Based on phylogenetic analyses and morphological characteristics, 12 species of Botryosphaeriaceae were isolated from these samples and the genera Botryosphaeria, Cophinforma, Lasiodiplodia and Neofusicoccum were identified from among a relatively large collection of isolates. These species include Botryosphaeria fusispora, Cophinforma atrovirens, Lasiodiplodia brasilience, L. pseudotheobromae, L. theobromae, Neofusicoccum parvum and each of three previously undescribed species of Botryosphaeria and Neofusicoccum, namely B. pseudoramosa sp. nov., B. qingyuanensis sp. nov., B. wangensis sp. nov., N. hongkongense sp. nov., N. microconidium sp. nov. and N. sinoeucalypti sp. nov.

In this study, ITS, *tef1*, *tub*, *rpb2*, *cmdA*, LSU and SSU sequences were generated to distinguish and describe new species of *Botryosphaeria*, *Cophinforma*, *Lasiodiplodia* and *Neofusicoccum*. For the six to seven regions used for analyses of *Botryosphaeria*, *Lasiodiplodia* and *Neofusicoccum*, phylogenetic analyses based on sequence comparisons show that polymorphic nucleotides exist between some isolates collected in this study and other closely related species. Sequences of the ITS, *tef1* and *tub* regions are widely used to distinguish and describe new species of *Botryosphaeria*, *Lasiodiplodia* and *Neofusicoccum* of *Botryosphaeriaceae* (Phillips et al. 2013, Chen et al. 2015, Linaldeddu et al. 2015, Coutinho et al. 2017), except ITS, *tef1* and *tub*, *rpb2* genes are also used for the species identification of *Neofusicoccum* (Pavlic et al. 2009a, Sakalidis et al. 2011, Osorio et al. 2017, Yang et al. 2017) and rpb2 and cmdA are also used for Lasiodiplodia (Cruywagen et al. 2017, Dou et al. 2017a, b, Osorio et al. 2017). The phylogenetic analyses based on a combination of the three to five regions (Botryosphaeria: ITS, tef1 and tub; Lasiodiplodia: ITS, tef1, tub, rpb2 and cmdA; Neofusicoccum: ITS, tef1, tub and rpb2) indicated that these isolates form independent phylogenetic clades supported by high bootstrap values, which are identified and described as six new species. In the other Chinese isolates, the differences we did find occurred only in one of the two (Cophinforma), six (Botryosphaeria and Neofusicoccum) or seven (Lasiodiplodia) regions, these isolates reside in the same clade to previously identified species or form independent phylogenetic clades but not supported by high bootstrap values, and they were identified as B. fusispora, C. atrovirens, L. brasiliense, L. pseudotheobromae, L. theobroma and N. parvum.

The identification of 12 Botryosphaeriaceae species is also supported by morphological and/or biological characteristics. For each of the six species that have been described previously, their culture morphology and conidial characteristics are very similar to that of the type specimens. For the six newly described species in this study, morphological differences exist among them and other phylogenetically closely related species, especially in terms of the size and shape of conidia, as well as conidium septum characteristics. We also observed biological differences, for example optimal growth temperatures, among some of the species. For the six new species, B. pseudoramosa, B. wangensis, N. hongkongense and N. microconidium are easily distinguished from other phylogenetically close species based on conidial morphology. Although some overlap in conidial shape and size is observed among some species, such as B. fabicerciana, B. kuwatsukai and B. gingyuanensis, these species can be distinguished from each other by the presence of a conidial septum (older conidia) and microconidia, as well as the optimal growth temperature. The newly described species N. sinoeucalypti can be distinguished from other species with similar conidia in the N. parvum/N. ribis complex by conidial morphology and the optimal growth temperature.

Except for B. wangensis, C. atrovirens and N. hongkongense, the other nine species were isolated from *Eucalyptus* trees in South China. Of the Botryosphaeriaceae species isolated from Eucalyptus, B. fusispora, L. pseudotheobromae and L. theobromae are dominant and distributed in the GuangDong, GuangXi and HaiNan Provinces; L. pseudotheobromae and L. theobromae have also been found in previous studies (Chen et al. 2011c, Li et al. 2015a), suggesting that they may be widely distributed on Eucalyptus trees in other areas in South China. Four new species, B. pseudoramosa, B. qingyuanensis, N. microconidium and N. sinoeucalypti, were isolated from Eucalyptus in China. This study also presents the first report of *L. brasiliense* on Eucalyptus in the world. Species of Botryosphaeriaceae are distributed in all the areas surveyed where Eucalyptus is planted. The results of our study suggest that the species diversity of Botryosphaeriaceae on Eucalyptus in China may be higher than what was previously expected (Chen et al. 2011c).

In addition to *Botryosphaeriaceae* species identified on *Eucalyptus*, we also identified *B. pseudoramosa* from *Melastoma* sanguineum, *B. wangensis* from *C. deodara*, *C. atrovirens* from *D. longan*, *L. theobromae* from *C. lanceolata*, *D. longan* and *P. hanceana*, and *N. hongkongense* from *A. cunninghamii*. Aside from *L. theobromae* from *P. hanceana* (Lu et al. 2000), which has been reported previously, these *Botryosphaeriaceae* species are reported from their respective plant hosts for the first time. Disease materials were collected randomly from limited areas, including the areas which were adjacent to *Eucalyptus* plantations, and further work is needed to better understand

the biodiversity and distribution of *Botryosphaeriaceae* on their hosts.

Based on sequence comparisons of the seven gene regions, the same genotype of *L. theobromae* was shared by species of *Eucalyptus* in all the surveyed provinces in South China, and *C. lanceolata*, *D. longan* and *P. hanceana* planted in GuangDong Province (Table 1). We isolated the newly described species *B. pseudoramosa* from both *Eucalyptus* trees and *M. sanguine-um*, and isolates from different hosts in geographically close areas do share the same genotype (Table 1). These results provide confirmation for the wide host range of *L. theobromae* and *B. pseudoramosa* on different plants. Previous studies used genetic diversity and geographic distribution comparisons to show the wide host range of *N. mediterraneum* on different crop trees in California (Chen et al. 2014a, b). The results of our current study further show that some *Botryosphaeriaceae* have wide geographic and host ranges.

Inoculation experiments revealed that all species of Botryosphaeriaceae identified in this study are pathogenic to the tested Eucalyptus clones, which is consistent with previous work showing that Botryosphaeriaceae species include important pathogens of Eucalyptus (Pavlic et al. 2007, Mohali et al. 2009, Rodas et al. 2009, Chen et al. 2011c). Pathogenicity tests in this study showed that species of Lasiodiplodia are more aggressive than Botryosphaeria and Neofusicoccum on three Eucalyptus clones, including one clone of E. urophylla × E. grandis, which is consistent with results in previous studies (Chen et al. 2011c). Results in Mohali et al. (2009) showed that some species of *Neofusicoccum* were more aggressive than Lasiodiplodia on clones of E. urophylla × E. grandis, which indicated that resistance of different genotypes of E. urophylla × E. grandis can be significantly different. Therefore, the identification of commercially available Eucalyptus genotypes resistant to Botryosphaeriaceae will promote the selection of resistant materials for wide-scale planting.

Of the fungal species we found, *L. theobromae* and *L. pseudotheobromae* are the most aggressive and are also widely distributed on *Eucalyptus* trees in different regions; it is essential that these pathogens be monitored carefully to help make decisions regarding disease management. Except for species of *Lasiodiplodia*, other fungi of the genera *Botryosphaeria*, *Cophinforma* and *Neofusicoccum* also produce lesions on inoculated seedlings; although these species are not highly virulent to *Eucalyptus* and are not widespread, these fungi still need to be monitored carefully because some of them may be highly aggressive to their original hosts or may spread and act as important pathogens in a suitable environment.

Our results in this study indicate that some species of *Botryo-sphaeriaceae* are widely distributed in different geographic regions on different hosts. These fungal species have significant potential to cause diseases of *Eucalyptus*. Management of the diseases on *Eucalyptus* reported in this study will need to rely on sound breeding programs to select *Eucalyptus* genotypes to match climatic and edaphic factors and silvicultural practices (spacing and thinning) as part of an integrated management strategy (Old et al. 2003). Further study is needed to better understand the genetic diversity of the species at the population level and to understand the biological and epidemiological characteristics of these species to help with long-term disease management.

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