



Botryosphaeriaceae from *Eucalyptus* plantations and adjacent plants in China

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Key words

Botryosphaeria
Cophinforma
Lasiodiplodia
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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 *Cunninghamia 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- α (*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

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), *Chrysosporthe* (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 *Botryosphaeriaceae* 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 *Cunninghamia 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 cm²) 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 ddH₂O (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- α (*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 β -tubulin (*tub*) gene was amplified using the primers BT-2a/BT-2b (Glass & Donaldson 1995), a part of DNA-directed 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/RPB2bot7R 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™ Master Mix (contain 1.25 U TopTaq™ DNA Polymerase, 200 μM of each dNTP and 1.5 mM MgCl₂), 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

Table 1 Isolates sequenced and used for phylogenetic analyses, morphological studies and pathogenicity tests in this study.

Species ¹	Isolate No. ^{2,3}	Genotype ⁴	Host	Location	GPS information	Collector	ITS	<i>tefl</i>	<i>tub</i>	<i>rpb2</i>	<i>crnA</i>	LSU	SSU
<i>Botryosphaeria fusispora</i>	CERC1997	AAAA-AA	<i>Eucalyptus</i> hybrid	BeiHai Region, GuangXi Province, China	N21°35'41" E109°43'01"	S.F. Chen & G.Q. Li	KX277967	KX278072	KX278177	MF4-10116	N/A	MF4-10007	MF410205
	CERC2273	AAAA-AA	<i>Eucalyptus</i> hybrid	FuZhou Region, Fujian Province, China	N26°13'39" E119°10'51"	S.F. Chen & G.Q. Li	KX277968	KX278073	KX278178	MF4-10117	N/A	MF4-10008	MF410206
	CERC2274 ^{6,7}	AAAA-AA	<i>Eucalyptus</i> hybrid	FuZhou Region, Fujian Province, China	N26°13'39" E119°10'51"	S.F. Chen & G.Q. Li	KX277969	KX278074	KX278179	MF4-10118	N/A	MF4-10009	MF410207
	CERC2910	AAAA-AA	<i>Eucalyptus</i> hybrid	BeiHai Region, GuangXi Province, China	Unknown	S.F. Chen & G.Q. Li	KX277970	KX278075	KX278180	MF4-10119	N/A	MF4-10010	MF410208
	CERC2912	AAAA-AA	<i>Eucalyptus</i> hybrid	BeiHai Region, GuangXi Province, China	Unknown	S.F. Chen & G.Q. Li	KX277971	KX278076	KX278181	MF4-10120	N/A	MF4-10011	MF410209
	CERC2913	AAAA-AA	<i>Eucalyptus</i> hybrid	BeiHai Region, GuangXi Province, China	Unknown	S.F. Chen & G.Q. Li	KX277972	KX278077	KX278182	MF4-10121	N/A	MF4-10012	MF410210
	CERC3441 ⁶	AAAA-AA	<i>Eucalyptus</i> hybrid	ZhanJiang Region, Guangdong Province, China	N20°41'20" E110°01'17"	S.F. Chen & G.Q. Li	KX277974	KX278079	KX278184	MF4-10123	N/A	MF4-10014	MF410212
	CERC3442	AAAA-AA	<i>Eucalyptus</i> hybrid	ZhanJiang Region, Guangdong Province, China	N20°41'20" E110°01'17"	S.F. Chen & G.Q. Li	KX277975	KX278080	KX278185	MF4-10124	N/A	MF4-10015	MF410213
	CERC3474	AAAA-AA	<i>Eucalyptus</i> hybrid	ZhanJiang Region, Guangdong Province, China	N20°41'20" E110°01'17"	S.F. Chen & G.Q. Li	KX277976	KX278081	KX278186	MF4-10125	N/A	MF4-10016	MF410214
	CERC3426	AAAA-AB	<i>Eucalyptus</i> hybrid	BeiHai Region, GuangXi Province, China	N21°35'49" E109°43'49"	S.F. Chen & G.Q. Li	KX277973	KX278078	KX278187	MF4-10122	N/A	MF4-10013	MF410211
	CERC1998 ⁷	ABAA-AA	<i>Eucalyptus</i> hybrid	BeiHai Region, GuangXi Province, China	N21°35'41" E109°43'01"	S.F. Chen & G.Q. Li	KX277977	KX278082	KX278187	MF4-10126	N/A	MF4-10017	MF410215
	CERC2006	ABAA-AA	<i>Eucalyptus</i> hybrid	ZhanJiang Region, Guangdong Province, China	N21°15'26" E110°07'00"	S.F. Chen & G.Q. Li	KX277978	KX278083	KX278188	MF4-10127	N/A	MF4-10018	MF410216
	CERC2911 ⁶	ABAA-AA	<i>Eucalyptus</i> hybrid	BeiHai Region, GuangXi Province, China	Unknown	S.F. Chen & G.Q. Li	KX277979	KX278084	KX278189	MF4-10128	N/A	MF4-10019	MF410217
	CERC2918 ⁶	ABAA-AA	<i>Eucalyptus</i> hybrid	BeiHai Region, GuangXi Province, China	N21°35'41" E109°43'01"	S.F. Chen & G.Q. Li	KX277980	KX278085	KX278190	MF4-10129	N/A	MF4-10020	MF410218
	CERC2921	ABAA-AA	<i>Eucalyptus</i> hybrid	BeiHai Region, GuangXi Province, China	N21°35'41" E109°43'01"	S.F. Chen & G.Q. Li	KX277981	KX278086	KX278191	MF4-10130	N/A	MF4-10021	MF410219
	CERC2925	ABAA-AA	<i>Eucalyptus</i> hybrid	BeiHai Region, GuangXi Province, China	N21°35'41" E109°43'01"	S.F. Chen & G.Q. Li	KX277982	KX278087	KX278192	MF4-10131	N/A	MF4-10022	MF410220
	CERC2948	ABAA-AA	<i>Eucalyptus</i> hybrid	QingYuan Region, Guangdong Province, China	N23°51'44" E113°10'58"	S.F. Chen & G.Q. Li	KX277983	KX278088	KX278193	MF4-10132	N/A	MF4-10023	MF410221
	CERC2949	ABAA-AA	<i>Eucalyptus</i> hybrid	QingYuan Region, Guangdong Province, China	N23°51'44" E113°10'58"	S.F. Chen & G.Q. Li	KX277984	KX278089	KX278194	MF4-10133	N/A	MF4-10024	MF410222
	CERC2954	ABAA-AA	<i>Eucalyptus</i> hybrid	QingYuan Region, Guangdong Province, China	N23°51'44" E113°10'58"	S.F. Chen & G.Q. Li	KX277985	KX278090	KX278195	MF4-10134	N/A	MF4-10025	MF410223
	CERC3446 ⁷	ABAA-AA	<i>Eucalyptus</i> hybrid	ZhanJiang Region, Guangdong Province, China	N20°41'20" E110°01'17"	S.F. Chen & G.Q. Li	KX277986	KX278091	MF4-09964	MF4-10135	N/A	MF4-10026	MF410224
CERC2930 ⁷	ACAA-AA	<i>Eucalyptus</i> hybrid	BeiHai Region, GuangXi Province, China	N21°35'41" E109°43'01"	S.F. Chen & G.Q. Li	KX277987	KX278092	KX278196	MF4-10136	N/A	MF4-10027	MF410225	
CERC1999 ⁶	AAAA-AA	<i>Eucalyptus</i> hybrid	BeiHai Region, GuangXi Province, China	N21°35'41" E109°43'01"	S.F. Chen & G.Q. Li	KX277988	KX278093	KX278197	MF4-10139	N/A	MF4-10028	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	MF4-10140	N/A	MF4-10031	MF410229	
<i>B. pseudoramosa</i>	= CGMCC3.18739 ^{6,7,8,9}												
	CERC2004 ⁹	AAAA-AA	<i>Eucalyptus</i> hybrid	BeiHai Region, GuangXi Province, China	N21°35'41" E109°43'01"	S.F. Chen & G.Q. Li	KX277990	KX278095	KX278199	MF4-10141	N/A	MF4-10032	MF410230
	CERC2019	AAAA-AA	<i>Eucalyptus</i> hybrid	BeiHai Region, GuangXi Province, China	Unknown	S.F. Chen & G.Q. Li	KX277991	KX278096	KX278200	MF4-10142	N/A	MF4-10033	MF410231
	CERC2983	AAAA-AA	<i>Melastoma sanguineum</i>	ZhanJiang Region, Guangdong Province, China	N21°13'24" E110°24'04"	S.F. Chen	KX277992	KX278097	KX278201	MF4-10143	N/A	MF4-10034	MF410232
	= CGMCC3.18740 ⁶												
	CERC2985	AAAA-AA	<i>M. sanguineum</i>	ZhanJiang Region, Guangdong Province, China	N21°13'24" E110°24'04"	S.F. Chen	KX277993	KX278098	KX278202	MF4-10144	N/A	MF4-10035	MF410233
	CERC2987 ^{6,9}	AAAA-AA	<i>M. sanguineum</i>	ZhanJiang Region, Guangdong Province, China	N21°13'24" E110°24'04"	S.F. Chen	KX277994	KX278099	KX278203	MF4-10145	N/A	MF4-10036	MF410234
	CERC2988 ⁶	AAAA-AA	<i>M. sanguineum</i>	ZhanJiang Region, Guangdong Province, China	N21°13'24" E110°24'04"	S.F. Chen	KX277995	KX278100	KX278204	MF4-10146	N/A	MF4-10037	MF410235
	CERC3452 ⁷	AAAA-AA	<i>Eucalyptus</i> hybrid	ZhanJiang Region, Guangdong Province, China	N20°41'20" E110°01'17"	S.F. Chen & G.Q. Li	KX277996	KX278101	KX278205	MF4-10147	N/A	MF4-10038	MF410236
	CERC3455	AAAA-AA	<i>Eucalyptus</i> hybrid	ZhanJiang Region, Guangdong Province, China	N20°41'20" E110°01'17"	S.F. Chen & G.Q. Li	KX277997	KX278102	KX278206	MF4-10148	N/A	MF4-10039	MF410237
= CGMCC3.18741 ⁶													
CERC3462	AAAA-AA	<i>Eucalyptus</i> hybrid	ZhanJiang Region, Guangdong Province, China	N20°41'20" E110°01'17"	S.F. Chen & G.Q. Li	KX277998	KX278103	KX278207	MF4-10149	N/A	MF4-10040	MF410238	
CERC3472	AAAA-AA	<i>Eucalyptus</i> hybrid	ZhanJiang Region, Guangdong Province, China	N20°41'20" E110°01'17"	S.F. Chen & G.Q. Li	KX277999	KX278104	KX278208	MF4-10150	N/A	MF4-10041	MF410239	
CERC2946	AAAA-AA	<i>Eucalyptus</i> hybrid	QingYuan Region, Guangdong Province, China	N23°44'30" E112°48'49"	S.F. Chen & G.Q. Li	KX278000	KX278105	KX278209	MF4-10151	N/A	MF4-10042	MF410240	
= CGMCC3.18742 ^{6,7,8,9}													
CERC2947	AAAA-AA	<i>Eucalyptus</i> hybrid	QingYuan Region, Guangdong Province, China	N23°44'30" E112°48'49"	S.F. Chen & G.Q. Li	KX278001	KX278106	KX278210	MF4-10152	N/A	MF4-10043	MF410241	
= CGMCC3.18743 ^{7,9}													
CERC2298	AAAA-AA	<i>C. deodara</i>	XinZhuang, MangChuan, RuZhou Region, HeNan Province, China	N34°04'09.8" E112°49'00.7"	S.F. Chen	KX278002	KX278107	KX278211	MF4-10153	N/A	MF4-10044	MF410242	
= CGMCC3.18744 ^{6,7,8,9}													
CERC2299	AAAA-AA	<i>C. deodara</i>	XinZhuang, MangChuan, RuZhou Region, HeNan Province, China	N34°04'09.8" E112°49'00.7"	S.F. Chen	KX278003	KX278108	KX278212	MF4-10154	N/A	MF4-10045	MF410243	
= CGMCC3.18745 ^{6,7}													
CERC2300	AAAA-AA	<i>C. deodara</i>	XinZhuang, MangChuan, RuZhou Region, HeNan Province, China	N34°04'09.8" E112°49'00.7"	S.F. Chen	KX278004	KX278109	KX278213	MF4-10155	N/A	MF4-10046	MF410244	
= CGMCC3.18746 ^{6,9}													
CERC3481	AAAA-AA	<i>Dimocarpus longan</i>	ZhanJiang Region, Guangdong Province, China	Unknown	S.F. Chen	KX278005	KX278110	KX278214	MF4-10156	N/A	MF4-10047	MF410245	
CERC3482	AAAA-AA	<i>D. longan</i>	ZhanJiang Region, Guangdong Province, China	Unknown	S.F. Chen	KX278006	KX278111	KX278215	MF4-10157	N/A	MF4-10048	MF410246	
CERC3484 ⁷	AAAA-AA	<i>D. longan</i>	ZhanJiang Region, Guangdong Province, China	Unknown	S.F. Chen	KX278007	KX278112	KX278216	MF4-10158	N/A	MF4-10049	MF410247	
CERC3489 ⁷	BAAA-AA	<i>D. longan</i>	ZhanJiang Region, Guangdong Province, China	Unknown	S.F. Chen	KX278008	KX278113	KX278217	MF4-10159	N/A	MF4-10050	MF410248	
CERC3490	BAAA-AA	<i>D. longan</i>	ZhanJiang Region, Guangdong Province, China	Unknown	S.F. Chen	KX278009	KX278114	KX278218	MF4-10160	N/A	MF4-10051	MF410249	
CERC3499	BAAA-AA	<i>D. longan</i>	ZhanJiang Region, Guangdong Province, China	Unknown	S.F. Chen	KX278010	KX278115	KX278219	MF4-10163	MF4-09967	MF4-10054	MF410252	
CERC2284 ^{6,7}	AAAAAA	<i>Eucalyptus</i> hybrid	ZhangZhou Region, Fujian Province, China	N24°46'06" E117°51'02"	S.F. Chen & G.Q. Li	KX278011	KX278116	KX278220	MF4-10164	MF4-09968	MF4-10055	MF410253	
CERC2262	AAAAAA	<i>Eucalyptus</i> hybrid	Yulin Region, GuangXi Province, China	N22°09'12" E110°12'08"	S.F. Chen & G.Q. Li	KX278011	KX278116	KX278220	MF4-10164	MF4-09968	MF4-10055	MF410253	
CERC2280	AAAAAA	<i>Eucalyptus</i> hybrid	ZhangZhou Region, Fujian Province, China	N24°46'06" E117°51'02"	S.F. Chen & G.Q. Li	KX278012	KX278117	KX278221	MF4-10165	MF4-09969	MF4-10056	MF410254	

Table 1 (cont.)

Species ¹	Isolate No. ^{2,3}	Genotype ⁴	Host	Location	GPS information	Collector	GenBank accession No. ⁵						
							ITS	tef1	tub	rpb2	cmdA	LSU	SSU
<i>L. pseudotheobromae</i> (cont.)	CERC2281	AAAAAAA	<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
	CERC2282	AAAAAAA	<i>Eucalyptus</i> 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
	CERC2283	AAAAAAA	<i>Eucalyptus</i> hybrid	Zhangzhou Region, Fujian Province, China	N24°46'06" E117°51'02"	S.F. Chen & G.Q. Li	KX278015	KX278120	KX278224	MF410168	MF409972	MF410059	MF410257
	CERC2286 ^{6,7}	AAAAAAA	<i>Eucalyptus</i> hybrid	Zhangzhou Region, Fujian Province, China	N24°46'06" E117°51'02"	S.F. Chen & G.Q. Li	KX278016	KX278121	KX278225	MF410169	MF409973	MF410060	MF410258
	CERC2287 ⁶	AAAAAAA	<i>Eucalyptus</i> hybrid	Zhangzhou Region, Fujian Province, China	N24°46'06" E117°51'02"	S.F. Chen & G.Q. Li	KX278017	KX278122	KX278226	MF410170	MF409974	MF410061	MF410259
	CERC2288	AAAAAAA	<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	AAAAAAA	<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
	CERC2360	AAAAAAA	<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
	CERC2361	AAAAAAA	<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
	CERC3417	AAAAAAA	<i>Eucalyptus</i> hybrid	BeiHai Region, GuangXi Province, China	N21°35'49" E109°43'49"	S.F. Chen & G.Q. Li	KX278023	KX278128	KX278232	MF410175	MF409979	MF410066	MF410264
	CERC3432	AAAAAAA	<i>Eucalyptus</i> hybrid	BeiHai Region, GuangXi Province, China	N21°35'49" E109°43'49"	S.F. Chen & G.Q. Li	KX278024	KX278129	KX278233	MF410176	MF409981	MF410067	MF410265
	CERC3434	AAAAAAA	<i>Eucalyptus</i> hybrid	BeiHai Region, GuangXi Province, China	N21°35'49" E109°43'49"	S.F. Chen & G.Q. Li	KX278025	KX278130	KX278234	MF410177	MF409982	MF410068	MF410266
	CERC3438	AAAAAAA	<i>Eucalyptus</i> hybrid	BeiHai Region, GuangXi Province, China	N21°35'49" E109°43'49"	S.F. Chen & G.Q. Li	KX278026	KX278131	KX278235	MF410179	MF409983	MF410070	MF410268
	CERC3475	AAAAAAA	<i>Eucalyptus</i> hybrid	BeiHai Region, GuangXi Province, China	N21°35'49" E109°43'49"	S.F. Chen & G.Q. Li	KX278027	KX278132	KX278236	MF410180	MF409984	MF410071	MF410269
	CERC3495 ⁷	AAAAAAA	<i>E. urophylla</i> × <i>E. grandis</i>	Zhanjiang Region, Guangdong Province, China	N21°13'31" E110°23'47"	S.F. Chen & G.Q. Li	KX278028	KX278133	KX278237	MF410181	MF409985	MF410072	MF410270
	CERC3496	AAAAAAA	<i>E. urophylla</i> × <i>E. grandis</i>	Zhanjiang Region, Guangdong Province, China	N21°13'31" E110°23'47"	S.F. Chen & G.Q. Li	KX278029	KX278134	KX278238	MF410182	MF409986	MF410073	MF410271
	CERC2962	AAAAABA	<i>Eucalyptus</i> hybrid	YunFu Region, Guangdong Province, China	N23°15'12" E111°41'51"	S.F. Chen & G.Q. Li	KX278022	KX278127	KX278231	MF410175	MF409979	MF410066	MF410264
	CERC2024 ⁶	AAAAAAA	<i>Phoenix hanceana</i>	Zhanjiang Region, Guangdong Province, China	N21°15'26" E110°07'01"	S.F. Chen & G.Q. Li	KX278030	KX278135	KX278239	MF410183	MF409987	MF410074	MF410272
	CERC3420 ^{6,7}	AAAAAAA	<i>Eucalyptus</i> hybrid	BeiHai Region, GuangXi Province, China	N21°35'49" E109°43'49"	S.F. Chen & G.Q. Li	KX278031	KX278136	KX278240	MF410184	MF409988	MF410075	MF410273
	CERC3424 ⁶	AAAAAAA	<i>Eucalyptus</i> hybrid	BeiHai Region, GuangXi Province, China	N21°35'49" E109°43'49"	S.F. Chen & G.Q. Li	KX278032	KX278137	KX278241	MF410185	MF409989	MF410076	MF410274
CERC2025	ABAAAAA	<i>P. hanceana</i>	Zhanjiang Region, Guangdong Province, China	N21°15'26" E110°07'01"	S.F. Chen & G.Q. Li	KX278033	KX278138	KX278242	MF410186	MF409990	MF410077	MF410275	
CERC2284	ABAAAAA	<i>E. urophylla</i> × <i>E. grandis</i>	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	ABAAAAA	<i>E. urophylla</i> × <i>E. grandis</i>	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	ABAAAAA	<i>Eucalyptus</i> 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	ABAAAAA	<i>Cunninghamia lanceolata</i>	ShaoGuan Region, Guangdong Province, China	N24°31'32" E113°37'40"	S.F. Chen & G.Q. Li	KX278037	KX278142	KX278246	MF410190	MF409994	MF410081	MF410279	
CERC2958	ABAAAAA	<i>C. lanceolata</i>	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	ABAAAAA	<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	ABAAAAA	<i>Eucalyptus</i> 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	ABAAAAA	<i>Eucalyptus</i> 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	ABAAAAA	<i>D. longan</i>	Zhanjiang Region, Guangdong Province, China	Unknown	S.F. Chen	KX278042	KX278147	KX278251	MF410195	MF409999	MF410086	MF410284	
CERC3486	ABAAAAA	<i>D. longan</i>	Zhanjiang Region, Guangdong Province, China	Unknown	S.F. Chen	KX278043	KX278148	KX278252	MF410196	MF410000	MF410087	MF410285	
CERC3487	ABAAAAA	<i>D. longan</i>	Zhanjiang Region, Guangdong Province, China	Unknown	S.F. Chen	KX278044	KX278149	KX278253	MF410197	MF410001	MF410088	MF410286	
CERC3491	ABAAAAA	<i>D. longan</i>	Zhanjiang Region, Guangdong Province, China	Unknown	S.F. Chen	KX278045	KX278150	KX278254	MF410198	MF410002	MF410089	MF410287	
CERC3493	ABAAAAA	<i>D. longan</i>	Zhanjiang Region, Guangdong Province, China	Unknown	S.F. Chen	KX278046	KX278151	KX278255	MF410199	MF410003	MF410090	MF410288	
CERC3513 ^{6,7}	ABAAAAA	<i>E. urophylla</i> × <i>E. grandis</i>	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	ABAAAAA	<i>E. urophylla</i> × <i>E. grandis</i>	Zhanjiang Region, Guangdong Province, China	N21°13'31" E110°23'47"	S.F. Chen & G.Q. Li	KX278048	KX278153	KX278257	MF410201	MF410005	MF410092	MF410290	
CERC3516 ⁷	ABAAAAA	<i>E. urophylla</i> × <i>E. grandis</i>	Zhanjiang Region, Guangdong Province, China	N21°13'31" E110°23'47"	S.F. Chen & G.Q. Li	KX278049	KX278154	KX278258	MF410202	MF410006	MF410093	MF410291	
CERC2967	AAAA-AA	<i>Araucaria cunninghamii</i>	Hong Kong, China	Unknown	S.F. Chen	KX278050	KX278155	KX278259	KX278281	N/A	MF410094	MF410292	
		= CGMCC3.18747											
CERC2968	AABA-AA	<i>A. cunninghamii</i>	Hong Kong, China	Unknown	S.F. Chen	KX278051	KX278156	KX278260	KX278282	N/A	MF410095	MF410293	
		= CGMCC3.18748 ^{6,7,9}											
CERC2973	AABA-AA	<i>A. cunninghamii</i>	Hong Kong, China	Unknown	S.F. Chen	KX278052	KX278157	KX278261	KX278283	N/A	MF410096	MF410294	
		= CGMCC3.18749 ^{6,7,9,9}											
CERC3497	AAAA-AA	<i>E. urophylla</i> × <i>E. grandis</i>	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	
		= CGMCC3.18750 ^{6,7,9,9}											
CERC3498	AAAA-AA	<i>E. urophylla</i> × <i>E. grandis</i>	Zhanjiang Region, Guangdong Province, China	N21°13'31" E110°23'47"	S.F. Chen & G.Q. Li	KX278054	KX278159	KX278263	MF410204	N/A	MF410098	MF410296	
		= CGMCC3.18751 ^{6,7,9}											
CERC2951 ⁷	AAAA-AA	<i>E. urophylla</i> × <i>E. grandis</i>	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	
CERC3508	AAAA-AA	<i>E. urophylla</i> × <i>E. grandis</i>	Zhanjiang Region, Guangdong Province, China	N21°13'31" E110°23'47"	S.F. Chen & G.Q. Li	KX278056	KX278161	KX278265	KX278285	N/A	MF410100	MF410298	
CERC3509 ⁷	AAAA-AA	<i>E. urophylla</i> × <i>E. grandis</i>	Zhanjiang Region, Guangdong Province, China	N21°13'31" E110°23'47"	S.F. Chen & G.Q. Li	KX278057	KX278162	KX278266	KX278286	N/A	MF410101	MF410299	
CERC3502	ABAA-AA	<i>E. urophylla</i> × <i>E. grandis</i>	Zhanjiang Region, Guangdong Province, China	N21°13'31" E110°23'47"	S.F. Chen & G.Q. Li	KX278058	KX278163	KX278267	KX278287	N/A	MF410102	MF410300	
CERC3503 ⁶	ABAA-AA	<i>E. urophylla</i> × <i>E. grandis</i>	Zhanjiang Region, Guangdong Province, China	N21°13'31" E110°23'47"	S.F. Chen & G.Q. Li	KX278059	KX278164	KX278268	KX278288	N/A	MF410103	MF410301	
CERC3504 ^{6,7}	ABAA-AA	<i>E. urophylla</i> × <i>E. grandis</i>	Zhanjiang Region, Guangdong Province, China	N21°13'31" E110°23'47"	S.F. Chen & G.Q. Li	KX278060	KX278165	KX278269	KX278289	N/A	MF410104	MF410302	

Table 1 (cont.)

Species ¹	Isolate No. ^{2,3} Genotype ⁴ Host	Location	Host	GPS information	Collector	GenBank accession No. ⁵						
						ITS	tef1	tub	rpb2	cmdA	LSU	SSU
<i>N. sinoeucalypti</i>	CERC2005 AAAA-AA = CGMCC3.18752 ^{6,7,8,9}	Zhanjiang Region, Guangdong Province, China	<i>E. urophylla</i> × <i>E. grandis</i>	N21°15'26" E10°07'00"	S.F. Chen & G.Q. Li	KX278061	KX278166	KX278270	KX278290	N/A	MF410105	MF410303
	CERC3415 ⁶ AAAA-AA	BeiHai Region, GuangXi Province, China	<i>Eucalyptus</i> hybrid	N21°35'49" E109°43'49"	S.F. Chen & G.Q. Li	KX278063	KX278168	KX278272	KX278292	N/A	MF410107	MF410305
	CERC3416 AAAA-AA = CGMCC3.18754 ⁶	BeiHai Region, GuangXi Province, China	<i>Eucalyptus</i> hybrid	N21°35'49" E109°43'49"	S.F. Chen & G.Q. Li	KX278064	KX278169	KX278273	KX278293	N/A	MF410108	MF410306
	CERC3457 AAAA-AA	Zhanjiang Region, Guangdong Province, China	<i>E. urophylla</i> × <i>E. grandis</i>	N20°41'20" E110°01'17"	S.F. Chen & G.Q. Li	KX278066	KX278171	KX278275	KX278295	N/A	MF410110	MF410308
	CERC3458 AAAA-AA	Zhanjiang Region, Guangdong Province, China	<i>E. urophylla</i> × <i>E. grandis</i>	N20°41'20" E110°01'17"	S.F. Chen & G.Q. Li	KX278067	KX278172	KX278276	KX278296	N/A	MF410111	MF410309
	CERC3463 ⁷ AAAA-AA	Zhanjiang Region, Guangdong Province, China	<i>E. urophylla</i> × <i>E. grandis</i>	N20°41'20" E110°01'17"	S.F. Chen & G.Q. Li	KX278068	KX278173	KX278277	KX278297	N/A	MF410112	MF410310
	CERC3464 AAAA-AA	Zhanjiang Region, Guangdong Province, China	<i>E. urophylla</i> × <i>E. grandis</i>	N20°41'20" E110°01'17"	S.F. Chen & G.Q. Li	KX278069	KX278174	KX278278	KX278298	N/A	MF410113	MF410311
	CERC3467 AAAA-AA	Zhanjiang Region, Guangdong Province, China	<i>E. urophylla</i> × <i>E. grandis</i>	N20°41'20" E110°01'17"	S.F. Chen & G.Q. Li	KX278070	KX278175	KX278279	KX278299	N/A	MF410114	MF410312
	CERC3517 AAAA-AA	Zhanjiang Region, Guangdong Province, China	<i>E. urophylla</i> × <i>E. grandis</i>	N21°13'31" E110°23'47"	S.F. Chen & G.Q. Li	KX278071	KX278176	KX278280	KX278300	N/A	MF410115	MF410313
	CERC2265 AAAA-AB = CGMCC3.18753 ⁹	Yulin Region, GuangXi Province, China	<i>E. urophylla</i> × <i>E. grandis</i>	N22°08'55" E110°12'00"	S.F. Chen & G.Q. Li	KX278062	KX278167	KX278271	KX278291	N/A	MF410106	MF410304
CERC3451 AAAA-AB	Zhanjiang Region, Guangdong Province, China	<i>E. urophylla</i> × <i>E. grandis</i>	N20°41'20" E110°01'17"	S.F. Chen & G.Q. Li	KX278065	KX278170	KX278274	KX278294	N/A	MF410109	MF410307	

¹ Species names in **bold** are novel species described in this study.

² Isolates in **bold** are in the phylogenetic trees.

³ 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.

⁴ Genotype within each identified species, determined by ITS, *tef1*, *tub*, *rpb2*, *cmdA*, LSU and SSU regions; "-", means not available.

⁵ ITS, internal transcribed spacer region and intervening 5.8S rRNA gene; *tef1*, translation elongation factor 1- α ; *tub*, β -tubulin; *rpb2*, DNA-directed RNA polymerase II subunit; *cmdA*, calmodulin; LSU, nuclear ribosomal large subunit; SSU, nuclear ribosomal small subunit; N/A = not available.

⁶ Isolates used for morphological studies.

⁷ Isolates used for pathogenicity tests on three *Eucalyptus* clones.

⁸ Isolates represent ex-type.

⁹ 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 sequences. The sequences of the ex-type strains that were closely related to the *Botryosphaeriaceae* isolates sequenced in this 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/>) (Kato & 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>).

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 were combined for *Neofusicoccum* isolates.

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 1 000 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, 1 000 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).

Table 2 (cont.)

Species	Isolate numbers ¹	Host	Location	Collector	ITS	tef1	tub	rbp2	cmdA	LSU	SSU	Reference
<i>L. caatinguensis</i> (cont.)	IBL 40	<i>Spondias mombin</i>	Itarema, Ceará, Brazil	J.S. Lima & J.E. Cardoso	KT154762	KT154755	KT154769	N/A	N/A	N/A	N/A	Coutinho et al. (2017)
<i>L. chinensis</i>	CGMCC3 18061 ³ CGMCC3 18066	Unknown <i>Hevea brasiliensis</i>	China	W. He & Z.P. Dou	KX499889	KX499927	KX499965	N/A	N/A	N/A	N/A	Dou et al. (2017a)
<i>L. citricola</i>	CBS 124707 = IRAN 1522C ³ CBS 124706 = IRAN 1521C	<i>Citrus</i> sp. <i>Citrus</i> sp.	China Iran	Y. Zhang & Y.P. Zhou J. Abdollahzadeh & A. Javadi	GU945354	GU945340	KU887505	KU696351	KU886760	N/A	N/A	Abdollahzadeh et al. (2010), Cruywagen et al. (2017)
<i>L. crassispora</i>	CBS 118741 = WAC12533 ³ CBS 110492	<i>Santalum album</i>	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)
<i>L. euphorbicola</i>	CMM 3609 ³ CMM 33350	Unknown <i>Jatropha curcas</i>	Unknown Brazil	Unknown A.R. Machado & O.L. Pereira	EF622086 KF234543	EF622066 KF254926	EU673134 N/A	N/A	N/A	EU673251	N/A	Alves et al. (2008), Phillips et al. (2008), Machado et al. (2014)
<i>L. exigua</i>	CBS 137785 ³ BL 184	<i>Adansonia digitata</i> <i>Rehmannia raietam</i>	Botswana Tunisia	B.T. Linaiddu B.T. Linaiddu	KU887149 KJ638317	KU887026 KJ638336	KU696346 KU887509	N/A	KU886754	N/A	N/A	Cruywagen et al. (2017)
<i>L. glanensis</i>	CBS 124704 = IRAN 1523C ³ CBS 124705 = IRAN 1501C	Unknown <i>Rehmannia raietam</i>	Tunisia	B.T. Linaiddu J. Abdollahzadeh & A. Javadi	KJ638318 GU945351	KJ638337 GU945342	N/A KU887511	N/A	KU886765	N/A	N/A	Linaiddu et al. (2015), Cruywagen et al. (2017)
<i>L. gonubiensis</i>	CBS 115812 = CMW 14077 ³ CBS 116355 = CMW 14078	Unknown <i>Syzygium cordatum</i>	Iran	J. Abdollahzadeh & A. Javadi D. Pavlic	GU945352 AY639595	GU945341 DQ103566	KU887510 DQ458860	KU696356	KU886766	N/A	N/A	Abdollahzadeh et al. (2010), Cruywagen et al. (2017)
<i>L. gravistriata</i>	CMM 4564 ³ CMM 4565	<i>Anacardium humile</i> <i>Anacardium humile</i>	Brazil	M.S.B. Netto M.S.B. Netto	KT250949 KT250947	N/A	N/A	N/A	N/A	N/A	N/A	Netto et al. (2017)
<i>L. hormozganensis</i>	CBS 124709 = IRAN 1500C ³ CBS 124708 = IRAN 1498C	<i>Olea</i> sp. <i>Mangifera indica</i>	Iran	J. Abdollahzadeh & A. Javadi J. Abdollahzadeh & A. Javadi	GU945355 GU945356	GU945343 GU945344	KU887515 KU887514	KU696361	KU886770	N/A	N/A	Abdollahzadeh et al. (2010), Cruywagen et al. (2017)
<i>L. hyalina</i>	CGMCC3.17975 ³ CGMCC3.18383 = B 6180	<i>Acacia confusa</i> Unknown tree	China	Y. Zhang & Y.P. Zhou Z.P. Dou & Z.C. Liu	KX499879 KY767661	KX499917 KY751302	KX499955 KY751296	N/A	N/A	N/A	N/A	Dou et al. (2017b)
<i>L. indica</i>	IBP 01 ³	<i>Argiospermous</i> tree	India	I.B. Prasher & G. Singh	KM376151	N/A	N/A	N/A	N/A	N/A	N/A	Prasher & Singh (2014)
<i>L. iraniensis</i>	IRAN 1520C ³ IRAN 1502C	<i>Salvadora persica</i> <i>Juglans</i> sp.	Iran	J. Abdollahzadeh & A. Javadi A. Javadi	GU945348 GU945347	GU945336 GU945335	KU887516 KU887517	KU696363 KU696362	KU886771	N/A	N/A	Abdollahzadeh et al. (2010), Cruywagen et al. (2017)
<i>L. laelocattleyae</i>	CBS 167.28 ³ LAREP1	<i>Laelocattleya</i>	Italy	C. Sibilia	KU507487	N/A	N/A	N/A	N/A	DQ377892	N/A	Crous et al. (2006), Rodriguez-Gálvez et al. (2017)
<i>L. lignicola</i>	MFLUCC 11-0435 = CBS134112 ³	Unknown <i>Mangifera indica</i>	Reparitidor, Peru Thailand	P. Guerrero A.D. Ariyawansa	KU507484 JX646797	KU507451	N/A	N/A	N/A	JX646814	JX646830	Rodriguez-Gálvez et al. (2017)
<i>L. macrospora</i>	CMM 3833 ³	<i>Jatropha curcas</i>	Brazil	A.R. Machado & O.L. Pereira	KF234557	KF226718	KF254941	N/A	N/A	N/A	N/A	Machado et al. (2014)
<i>L. mahajangana</i>	CBS 124925 = CMW 27801 ³ CBS 124926 = CMW 27820	<i>Terminalia catappa</i>	Madagascar	J. Roux	FJ900595	FJ900641	FJ900630	KU696365	KU886773	N/A	N/A	Begoude et al. (2010), Cruywagen et al. (2017)
<i>L. margaritacea</i>	CBS 122519 = CMW 26162 ³	<i>Terminalia catappa</i>	Madagascar	J. Roux	FJ900596	FJ900642	KU887519	KU696366	KU886774	N/A	N/A	Begoude et al. (2010), Cruywagen et al. (2017)
<i>L. mediterranea</i>	CBS 137783 ³ CBS 137784	<i>Quercus ilex</i> <i>Vitis vinifera</i>	Italy Italy	B.T. Linaiddu S. Serra	KJ638312 KJ638311	KJ638330	KU887521	KU696368	KU886776	N/A	N/A	Linaiddu et al. (2015)
<i>L. missouriiana</i>	CBS 128311 = UC02193MO ³ CBS 128312 = UC02199MO	<i>Vitis</i> sp. x <i>Vitis labruscana</i>	Missouri, USA Missouri, USA	K. Stieglar & G.M. Leavitt K. Stieglar & G.M. Leavitt	HQ288225 HQ288226	HQ288267	HQ288304	KU696370	KU886778	N/A	N/A	Úrbez-Torres et al. (2012), Cruywagen et al. (2017)
<i>L. parva</i>	CBS 456.78 ³	Cassava-field soil	Colombia	O. Rangel	EF622083	EF622063	KU887523	KU696372	KU886780	KF766362	N/A	Alves et al. (2008), Cruywagen et al. (2017)

Table 2 (cont.)

Species	Isolate numbers ¹	Host	Location	Collector	ITS	<i>tef1</i>	<i>tub</i>	<i>rbp2</i>	<i>cmdA</i>	LSU	SSU	Reference
<i>N. batangarum</i> (cont.)	CBS 124923 = CMW 28320	<i>Terminalia catappa</i>	Cameroon	D. Begoude & J. Roux	FJ900608	FJ900654	FJ900635	FJ900616	N/A	KX464400	N/A	Begoude et al. (2010), Yang et al. (2017)
<i>N. brasiliense</i>	CMM 1338 ³ CMM 1285	<i>Mangifera indica</i>	Brazil	M.W. Marques	JX513630 JX513628	KC794031 KC794030	N/A	N/A	N/A	N/A	N/A	Marques et al. (2013)
<i>N. buxi</i>	CBS 11675 ³ CBS 113714	<i>Buxus sempervirens</i> <i>Buxus sempervirens</i>	France Sweden	H.A. van der Aa O. Constantinescu	KX464165 KX464164	N/A KX464677	KX464010 KX464009	N/A	N/A	KX464406 KX464405	N/A	Yang et al. (2017) Yang et al. (2017)
<i>N. cordaticola</i>	CBS 123634 = CMW 13982 ³ = CBS 123635 = CMW 14056	<i>Syzgium cordatum</i> <i>Syzgium cordatum</i>	South Africa South Africa	D. Pavlic D. Pavlic	EU821898 EU821903	EU821868 EU821873	EU821928 EU821843	N/A	N/A	KX464409 KX464410	N/A	Pavlic et al. (2009b), Yang et al. (2017) Pavlic et al. (2009b), Yang et al. (2017)
<i>N. cryptobaustale</i>	CMM 23785 = CBS 122813 ³	<i>Eucalyptus trees</i>	South Africa	H.M. Maleme	FJ752742	FJ752713	FJ752756	KX464014	N/A	KX464416	N/A	Crous et al. (2013), Yang et al. (2017)
<i>N. eucalypticola</i>	CBS 115679 = CMW 6539 ³ CBS 115766 = CMW 6217	<i>Eucalyptus grandis</i> <i>Eucalyptus grandis</i> <i>Eucalyptus rossii</i>	Orbost, Victoria, Australia Tidbinbilla, NSW, Australia	M.J. Wingfield M.J. Wingfield	AY615141 AY615143	AY615133 AY615135	AY615125 AY615127	N/A	N/A	KF766368 N/A	N/A	Slippers et al. (2004c, 2013) Slippers et al. (2004c, 2013)
<i>N. eucalyptorum</i>	CBS 115791 = CMW 10125 ³ CMM 10126 CBS 129518 = CPC 16999 ³	<i>Eucalyptus grandis</i> <i>Eucalyptus grandis</i> <i>Grevillea aurea</i>	South Africa South Africa Australia	H. Smith H. Smith P.W. Crous & R.G. Shivas	AF283686 AF283687 JF951137	AY236891 AY236892 N/A	N/A N/A N/A	N/A	N/A	N/A N/A JF951157	N/A	Smith et al. (2001), Slippers et al. (2004b) Smith et al. (2001), Slippers et al. (2004b) Crous et al. (2011)
<i>N. hellenicum</i>	CERC1947 = FCCC50067 ³ CERC1948 = CFCC50068	<i>Pistachia vera</i> <i>Pistachia vera</i>	Thessaloniki, Greece Aghios Mamas, Chaikidiki, Greece	T.J. Michailides T.J. Michailides	KP217053 KP217054	KP217061 KP217062	N/A	N/A	N/A	N/A	N/A	Chen et al. (2015) Chen et al. (2015)
<i>N. lilicii</i>	CGMCC3.18310 ³ CGMCC3.18311	<i>Illicium verum</i> <i>Illicium verum</i>	Guangxi, China Guangxi, China	L. Wang L. Wang	KY350149 KY350150	N/A KY1817756	N/A KY350155 KY350156	N/A	N/A	N/A N/A	N/A	Zhang et al. (2017) Zhang et al. (2017)
<i>N. kwambonambiense</i>	CBS 123639 = CMW 14023 ³ CBS 123641 = CMW 14140 CMM 41469 ³ CMM 41228	<i>Syzgium cordatum</i> <i>Syzgium cordatum</i> <i>Lumnitzera racemosa</i> <i>Lumnitzera racemosa</i>	South Africa South Africa South Africa South Africa	D. Pavlic D. Pavlic J.A. Osorio & J. Roux J.A. Osorio & J. Roux	EU821900 EU821919 KP860881 KP860882	EU821870 EU821889 KP860724 KP860725	EU821840 EU821859 KP860801 KP860803	EU821930 N/A N/A N/A	N/A	KX464422 KX464424 N/A N/A	N/A	Pavlic et al. (2009b), Yang et al. (2017) Pavlic et al. (2009b), Yang et al. (2017) Pavlic et al. (2009b), Yang et al. (2017) Osorio et al. (2017) Osorio et al. (2017)
<i>N. luteum</i>	CBS 562.92 = ATCC 58193 ³ CBS 118223 = WAC 12444 ³	<i>Actinidia deliciosa</i> lesion on ripe fruit <i>Eucalyptus globulus</i>	New Zealand Western Australia	S.R. Pennycook T. Burgess	KX464170 DQ093196	KX464690 DQ093217	KX464020 KX464022	N/A	N/A	KX464430 KX464436	N/A	Yang et al. (2017) Burgess et al. (2005), Yang et al. (2017)
<i>N. mangiferae</i>	CBS 118531 = CMW 7024 ³ CBS 118532 = CMW 7797	<i>Mangifera indica</i> <i>Mangifera indica</i>	Australia Australia	G.I. Johnson G.I. Johnson	AY615185 AY615186	DQ093221 DQ093220	AY615172 AY615173	N/A	N/A	DQ377920 DQ377921	EU673153 EU673154	Slippers et al. (2005), Phillips et al. (2008) Slippers et al. (2005), Phillips et al. (2008), Yang et al. (2017)
<i>N. mangroviatum</i>	CMM 41365 ³ CMM 42481	<i>Avicennia marina</i> <i>Bruguiera gymnorrhiza</i>	South Africa South Africa	J.A. Osorio & J. Roux J.A. Osorio & J. Roux	KP860859 KP860848	KP860702 KP860692	KU587905 KU587895	N/A	N/A	N/A N/A	N/A	Osorio et al. (2017) Osorio et al. (2017)
<i>N. mediterraneum</i>	CBS 121718 = CPC 13137 ³	<i>Eucalyptus</i> sp.	Greece	P.W. Crous, M.J. Wingfield & A.J.L. Phillips	GU251176	GU251308	KX464024	N/A	N/A	N/A	N/A	Crous et al. (2007), Yang et al. (2017)
<i>N. nonquaeasium</i>	CBS 126655 = PD 484 ³ PD 301	<i>Umbellularia californica</i>	USA	F.P. Trouillas	GU251163	GU251295	KX464025	N/A	N/A	KX464437	N/A	Inderbitzin et al. (2010), Yang et al. (2017)
<i>N. occulatum</i>	CBS 128008 = MUCC 227 ³	<i>Vaccinium corymbosum</i> cv. Elliot <i>Eucalyptus grandis</i> hybrid	Chile Australia	E.X. Briceno, J.G. Espinoza, B.A. Latorre & J.G. Espinoza T.I. Burgess	GU251164 EU301030	GU251296 EU339509	N/A EU339472	N/A	N/A	N/A KX464438	N/A	Inderbitzin et al. (2010) Sakalidis et al. (2011), Yang et al. (2017)

Table 2 (cont.)

Species	Isolate numbers ¹	Host	Location	Collector	ITS	<i>tef1</i>	<i>tub</i>	<i>rpb2</i>	<i>cmdA</i>	LSU	SSU	Reference
<i>N. occulatum</i> (cont.)	MUCC 286 = WAC 12395	<i>Eucalyptus pellita</i>	Australia	T.I. Burgess	EU736947	EU339511	EU339474	EU339560	N/A	N/A	N/A	Sakalidis et al. (2011)
<i>N. pernum</i>	ATCC 58191 = CMW 9081 ³ CMW 9080 = ICMP 8002	<i>Populus nigra</i>	New Zealand	G.J. Samuels	AY236943	AY236888	AY236917	EU821963	N/A	AY928045	EU873151	Slippers et al. (2004a), Alves et al. (2005), Phillips et al. (2008), Pavlic et al. (2009b)
<i>N. pennatisporum</i>	WAC 13153 = MUCC 510 ³	<i>Allocausarima fraseriana</i>	Western Australia	K.M. Taylor	EF591925	EF591976	EF591959	N/A	N/A	EF591942	N/A	Taylor et al. (2009)
<i>N. pistaciae</i>	CBS 595.76 ³	<i>Pistacia vera</i>	Greece	D.G. Zachos	KX464163	KX464676	KX464953	KX464008	N/A	KX464404	N/A	Yang et al. (2017)
<i>N. pistaciarum</i>	CBS 113083 = CPC 5263 ³ CBS 113084 = CPC 5284	<i>Pistacia vera</i>	USA	T.J. Michalides	KX464186	KX464712	KX464998	KX464027	N/A	KX464465	N/A	Yang et al. (2017)
<i>N. protearum</i>	CBS 114176 = STE-U 1775 ³ CBS 111200 = CPC 1357	<i>Leucadendron salignum</i>	South Africa	S. Denman	AF452539	KX464720	KX465006	KX464029	N/A	JX556245	N/A	Denman et al. (2003), Yang et al. (2017)
<i>N. ribis</i>	CBS 115475 = CMW 7772 ³ CBS 121.26 = CMW 7054	<i>Leucadendron</i> sp.	South Africa	P.W. Crous	KX464193	KX464719	KX465005	N/A	N/A	KX464472	N/A	Yang et al. (2017)
<i>N. sinense</i>	CGMCC3.18315 ³	Unknown woody plant	Guizhou, China	B. Slippers & G. Hudler	AY236935	AY236877	AY236906	EU821958	N/A	AY928044	KF766292	Slippers et al. (2004a, 2013), Alves et al. (2005), Pavlic et al. (2009b)
<i>N. stellerboschiana</i>	CBS 110864 = CPC 4598 ³	<i>Vitis vinifera</i>	South Africa	F. Halleen	AY343407	AY343348	KX465047	KX464042	N/A	KX464513	N/A	Slippers et al. (2004a), Pavlic et al. (2009b), Yang et al. (2017)
<i>N. terminaliae</i>	CBS 125263 = CMW 26679 ³ CBS 125264 = CMW 26683	<i>Terminalia sericea</i>	South Africa	D. Begoude & J. Roux	GQ471802	GQ471780	KX465052	KX464045	N/A	KX464518	N/A	Begoude (2010), Yang et al. (2017)
<i>N. urundicola</i>	CBS 123645 = CMW 14058 ³ CBS 123646 = CMW 14060	<i>Syzgium cordatum</i>	South Africa	D. Begoude & J. Roux	GQ471804	GQ471782	KX465053	KX464046	N/A	KX464519	N/A	Begoude (2010), Yang et al. (2017)
<i>N. ursorum</i>	CBS 122811 ³ CMW 23790	<i>Eucalyptus trees</i>	South Africa	H.M. Maleme	FJ752746	FJ752709	KX465056	KX464047	N/A	N/A	N/A	Crous et al. (2013), Yang et al. (2017)
<i>N. vitiflavatum</i>	CBS 112878 = STE-U 5044 ³ CBS 112977 = STE-U 5041	<i>Vitis vinifera</i>	South Africa	F. Halleen	FJ752745	FJ752708	KX465057	N/A	N/A	N/A	N/A	Crous et al. (2013), Yang et al. (2017)
<i>N. vitifusiforme</i>	CBS 110887 = STE-U 5252 ³ CBS 110880 = STE-U 5050	<i>Vitis vinifera</i>	South Africa	J.M. van Niekerk	AY343381	AY343342	KX465058	KX464048	N/A	KX464527	N/A	Phillips et al. (2013), Yang et al. (2017)
		<i>Vitis vinifera</i>	South Africa	J.M. van Niekerk	AY343380	AY343341	KX465059	N/A	N/A	KX464528	N/A	Phillips et al. (2013), Yang et al. (2017)
		<i>Vitis vinifera</i>	South Africa	J.M. van Niekerk	AY343383	AY343343	KX465061	KX464049	N/A	KX464530	N/A	Van Niekerk et al. (2004), Yang et al. (2017)
		<i>Vitis vinifera</i>	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)

¹ ALG: Personal culture collection A.; Berraf-Tebbati; ATCC: American Type Culture Collection, Virginia, USA; BL: Personal number of B. T. Linaldeddu; CAA: Personal culture collection Artur Alves, Universidade de Aveiro, Portugal; CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CERCC: Culture collection of China Eucalypt Research Centre, Chinese Academy of Forestry, Zhanjiang, Guangdong, China; CFCC: China Forestry Culture Collection Center, Beijing, China; CGMCC: China General Microbiological Culture Collection Center, Beijing, China; CMM: Culture Collection of Phytopathogenic Fungi 'Prof. Maria Menezes', Universidade Federal Rural de Pernambuco, Recife, Brazil; CMW: Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; CPC: Working collection of P.W. Crous, housed at CBS; GZCC: Guizhou Academy of Agricultural Sciences Culture Collection, GuiZhou, China; IBL: Personal culture collection, I.B.L. Coutinho; IBP: Personal culture collection, I.B. Prasher; ICMP: International Collection of Microorganisms from Plants, Auckland, New Zealand; IRAN: Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Iran; MFLUCC: Mae Fah Luang University Culture 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; UCDC: University of California, Davis, Plant Pathology Department Culture Collection; WAC: Department of Agriculture, Western Australia Plant Pathogen Collection, South Perth, Western Australia.

² ITS: internal transcribed spacer region and intervening 5.8S nrRNA gene; *tef1*: translation elongation factor 1- α ; *tub*: β -tubulin; *rpb2*: DNA-directed RNA polymerase II subunit; *cmdA*: calmodulin; LSU: nuclear ribosomal large subunit; SSU: nuclear ribosomal small subunit; N/A = not available.

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

Morphology

Representative isolates for each genotype of *Botryosphaeria*-*ceae* 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-mm-diam 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 *Botryosphaeria*-*ceae* 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 *Botryosphaeria*-*ceae* 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 *Botryosphaeria*-*ceae* 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 *Botryosphaeria*-*ceae* 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*

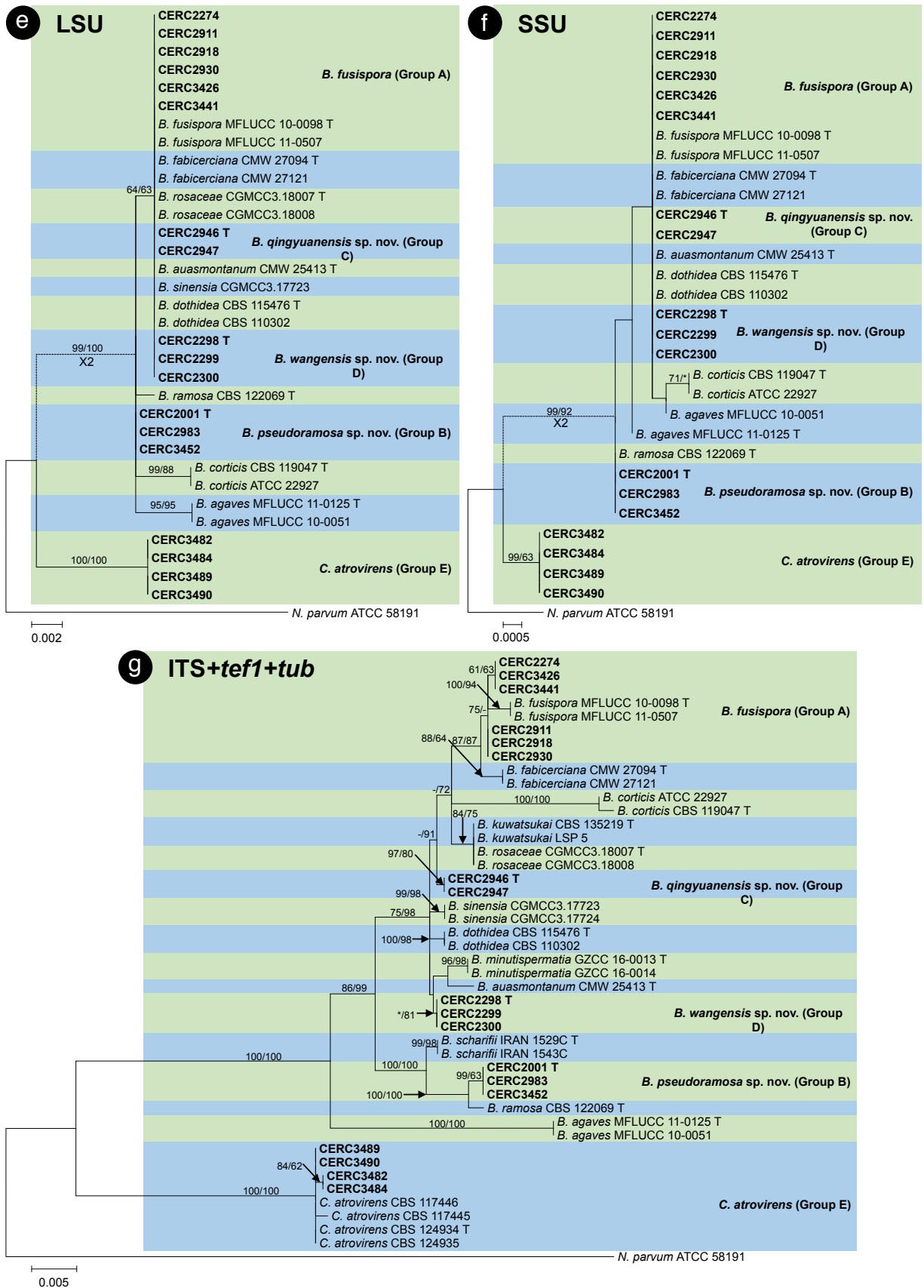
For the isolates grouping in the genus *Botryosphaeria*, isolates clustered into four phylogenetic groups (Groups A–D) for each of the ITS, *tef1*, *tub*, *rpb2* and ITS/*tef1*/*tub* datasets (Fig. 2a–d, g). For each of the LSU and SSU datasets, Groups A, C and D clustered together (Fig. 2e–f). The ITS sequences of *Botryosphaeria fabricerciana*, *B. fusicarpa*, *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. fabricerciana* and *B. fusicarpa* (Fig. 2b). For the *tub* sequences, the isolates in Group A resided in the same phylogenetic clade with *B. fusicarpa* (Fig. 2c). For the *rpb2*, LSU and SSU sequences, the isolates in Group A clustered to the same clade with *B. fabricerciana* and *B. fusicarpa* (*rpb2* is not available to *B. fusicarpa*) (Fig. 2d–f). The phylogenetic analyses for ITS, *tef1*, *tub*, *rpb2*, LSU and SSU sequences

Table 3 Datasets used and statistics resulting from phylogenetic analyses.

Genus	Dataset	No. of taxa	No. of bp ¹	Maximum parsimony					RC ⁵	HI ⁶
				PIC ²	No. of trees	Tree length	CI ³	RI ⁴		
<i>Botryosphaeria/Cophiniforma</i>	ITS	45	543	42	18	67	0.8209	0.9506	0.7804	0.1791
	<i>tef1</i>	45	356	147	875	206	0.8932	0.9677	0.8643	0.1068
	<i>tub</i>	34	415	43	8	58	0.9138	0.9688	0.8852	0.0862
	<i>rbp2</i>	22	718	92	2	116	0.9397	0.9809	0.9217	0.0603
	LSU	34	847	21	3	24	0.9583	0.9865	0.9454	0.0417
	SSU	31	1024	9	342	9	1.0000	1.0000	1.0000	1.0000
	ITS/ <i>tef1/tub</i>	45	1314	232	5000	337	0.8665	0.9585	0.8305	0.1335
	ITS	76	526	48	5000	87	0.6897	0.8945	0.6169	0.3103
	<i>tef1</i>	75	332	142	852	402	0.6219	0.9067	0.5639	0.3781
	<i>tub</i>	67	409	41	5000	60	0.7667	0.9352	0.7170	0.2333
<i>Lasiodiplodia</i>	<i>rbp2</i>	57	532	104	861	190	0.6421	0.8761	0.5626	0.3579
	<i>cmdA</i>	45	521	74	496	91	0.9121	0.9776	0.8916	0.0879
	LSU	28	835	20	3	27	0.7407	0.9247	0.6850	0.2593
	SSU	19	1020	17	2	23	0.8261	0.9048	0.7474	0.1739
	ITS/ <i>tef1/tub/rbp2/cmdA</i>	76	2320	409	5000	948	0.5918	0.8713	0.5156	0.4082
	ITS	77	532	81	1404	187	0.5615	0.8707	0.4889	0.4385
	<i>tef1</i>	75	307	147	5000	303	0.7426	0.9321	0.6922	0.2574
	<i>tub</i>	75	424	71	1430	141	0.6170	0.8784	0.5420	0.3830
	<i>rbp2</i>	55	607	114	652	191	0.7173	0.9069	0.6505	0.2827
	LSU	55	841	33	3260	70	0.5714	0.8324	0.4757	0.4286
<i>Neofusicoccum</i>	SSU	21	1027	3	1	3	1.0000	1.0000	1.0000	1.0000
	ITS/ <i>tef1/tub/rbp2</i>	77	1870	413	336	884	0.6267	0.8824	0.5530	0.3733
	Dataset									
	Subst. model ⁷		NST ⁸		Rate matrix		Maximum likelihood			
	ITS	TrN+I	6	1.0000	1.4207	1.0000	1.0000	8.3891	0.8010	Equal
	<i>tef1</i>	TPM2uf+G	6	1.7386	4.6965	1.7386	1.0000	4.6965	—	Gamma
	<i>tub</i>	HKY+G	2	—	—	—	—	—	4.1414	Gamma
	<i>rbp2</i>	TrN+G	6	1.0000	3.1864	1.0000	1.0000	10.4238	—	Gamma
	LSU	TrN+I	6	1.0000	5.1213	1.0000	1.0000	14.2608	—	Gamma
	SSU	TIM2	6	0.2353	0.4397	0.2353	1.0000	3.3084	—	Equal
ITS/ <i>tef1/tub</i>	TrN+G	6	1.0000	3.2977	1.0000	1.0000	5.9498	—	Equal	
ITS	TPM1uf+H+G	6	1.0000	8.3075	3.1185	3.1185	8.3075	—	Gamma	
<i>tef1</i>	TrN+G	6	1.0000	3.6014	1.0000	1.0000	5.5149	—	Gamma	
<i>tub</i>	TIM3+G	6	2.6761	3.8909	1.0000	2.6761	10.7362	—	Gamma	
<i>rbp2</i>	TrN+G	6	1.0000	4.8566	1.0000	1.0000	13.8753	—	Gamma	
<i>cmdA</i>	HKY+I	2	—	—	—	—	—	2.7918	Gamma	
LSU	TrN+I	6	1.0000	7.7385	1.0000	1.0000	16.1138	—	Equal	
SSU	TIM2+I	6	3.1234	3.8646	3.1234	1.0000	15.5731	—	Equal	
ITS/ <i>tef1/tub/rbp2/cmdA</i>	TIM3+H+G	6	0.6986	3.2898	1.0000	0.6986	5.4586	—	Gamma	
ITS	TIM1+H+G	6	1.0000	11.6895	3.1944	3.1944	22.131	—	Gamma	
<i>tef1</i>	HKY+G	2	—	—	—	—	—	2.8135	Gamma	
<i>tub</i>	TrN+G	6	1.0000	4.0352	1.0000	1.0000	7.8348	—	Gamma	
<i>rbp2</i>	TIM3+G	6	1.9463	7.0524	1.0000	1.9463	19.4804	—	Gamma	
LSU	TrN+I	6	1.0000	6.0800	1.0000	1.0000	25.6908	—	Equal	
SSU	TrN	6	1.0000	0.9096	1.0000	1.0000	7.9272	—	Equal	
ITS/ <i>tef1/tub/rbp2</i>	TrN+H+G	6	1.0000	4.8874	1.0000	1.0000	9.1711	—	Gamma	

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

Fig. 2 (cont.)



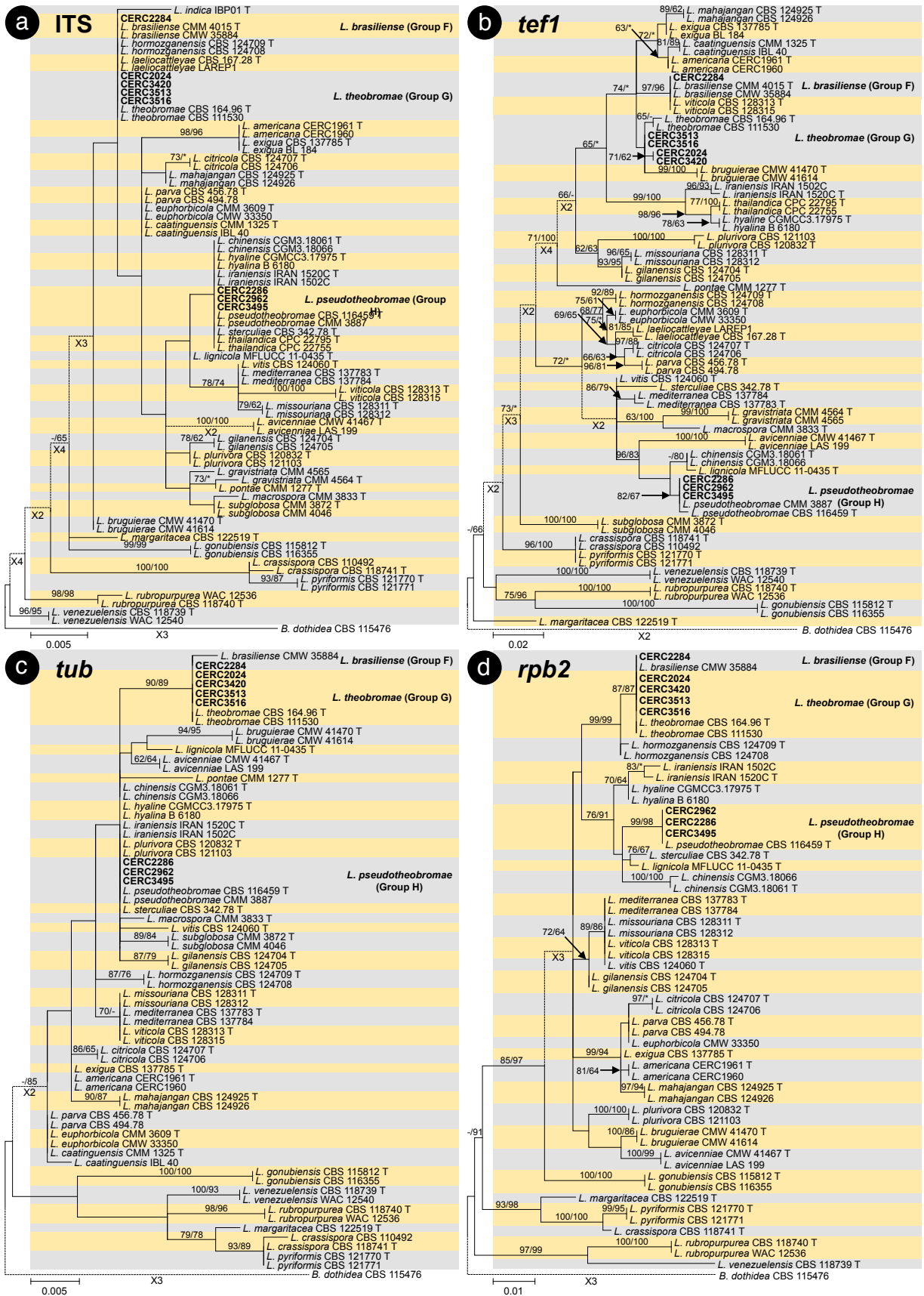
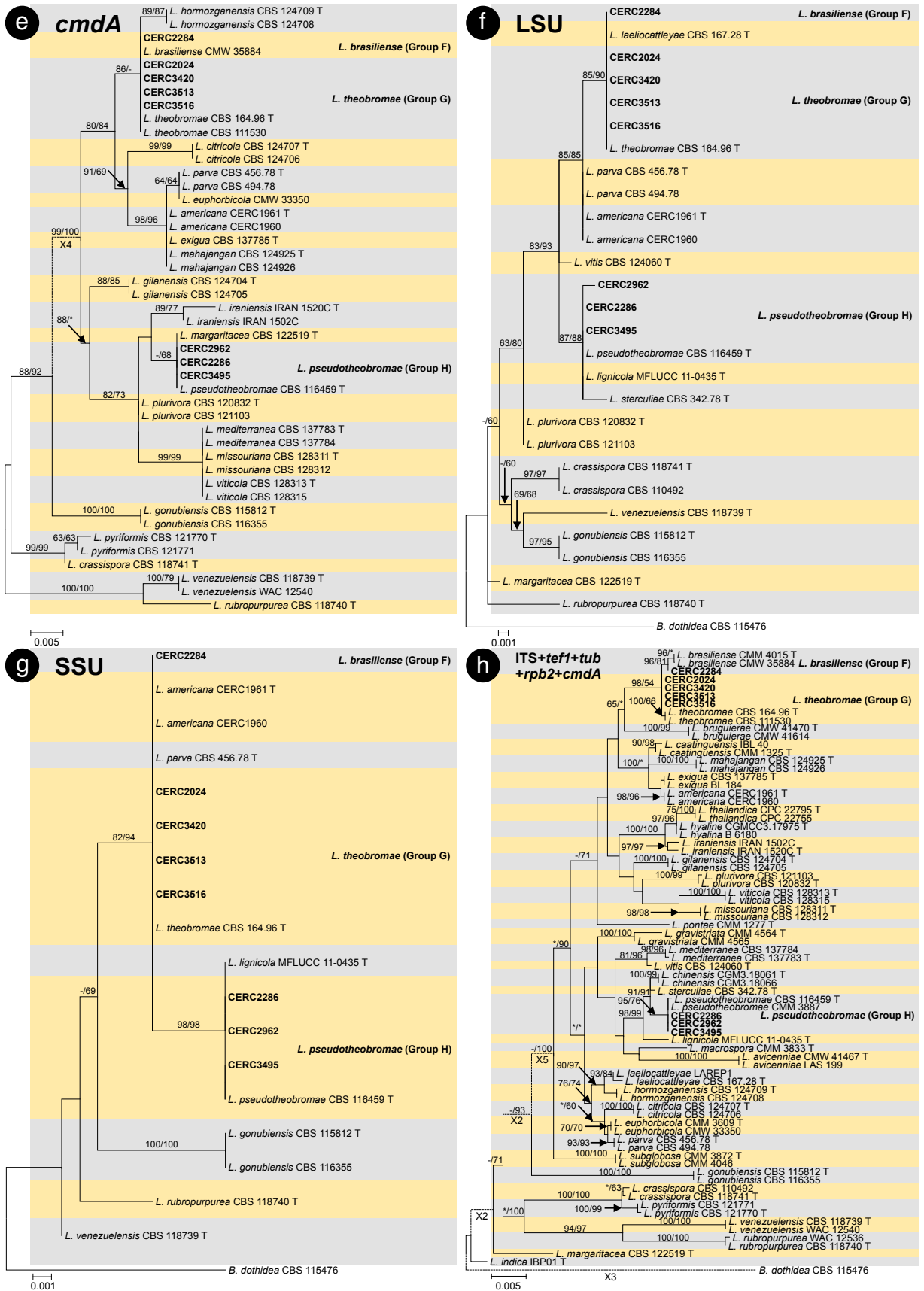


Fig. 3 Phylogenetic trees based on maximum likelihood (ML) analyses for species in *Lasiodiplodia*. a. ITS region; b. *tef1* gene region; c. *tub* gene region; d. *rpb2* gene region; e. *cmdA* gene region; f. LSU region; g. SSU region; h. combination of ITS, *tef1*, *tub*, *rpb2* and *cmdA* regions. 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. 3 (cont.)



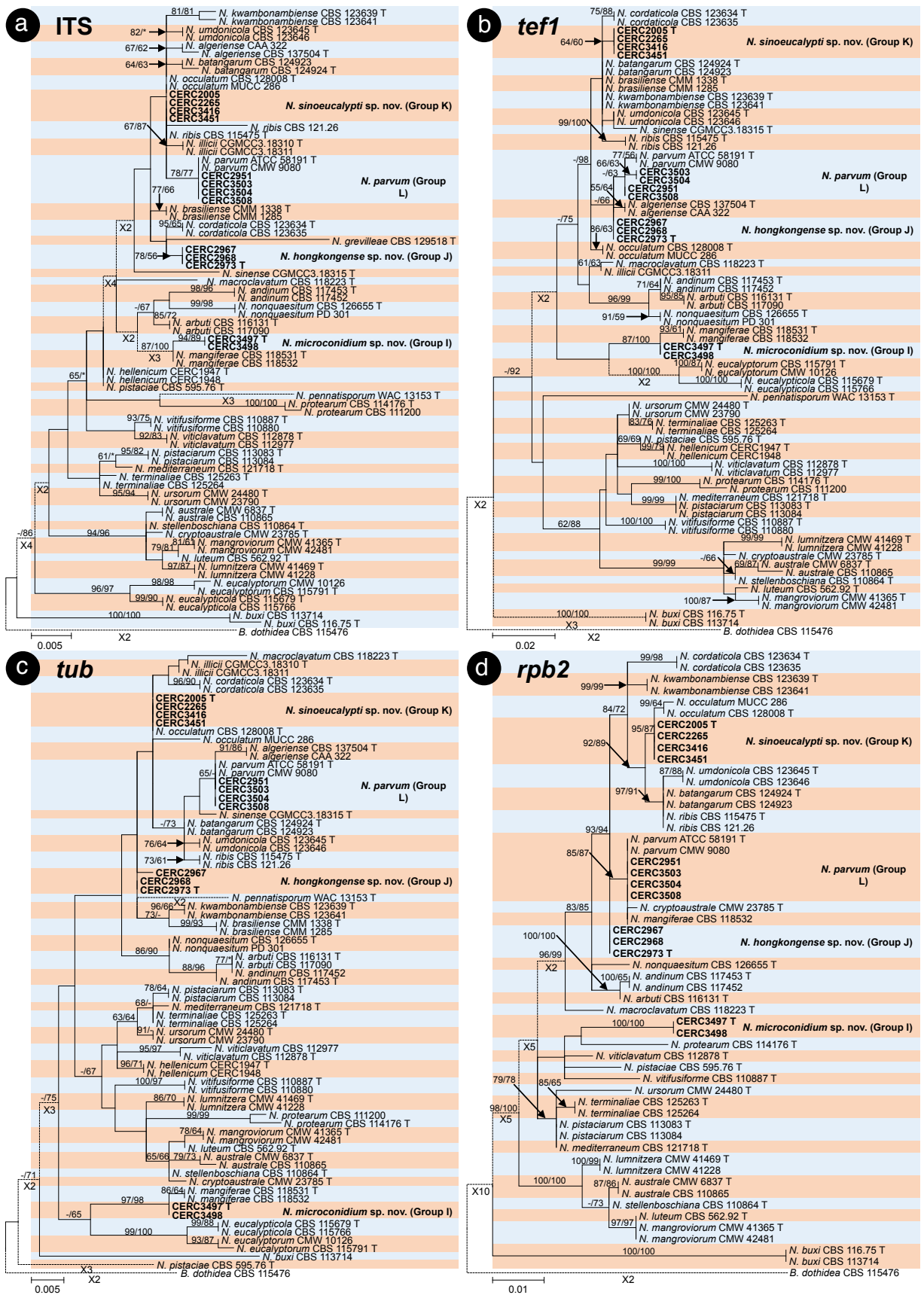
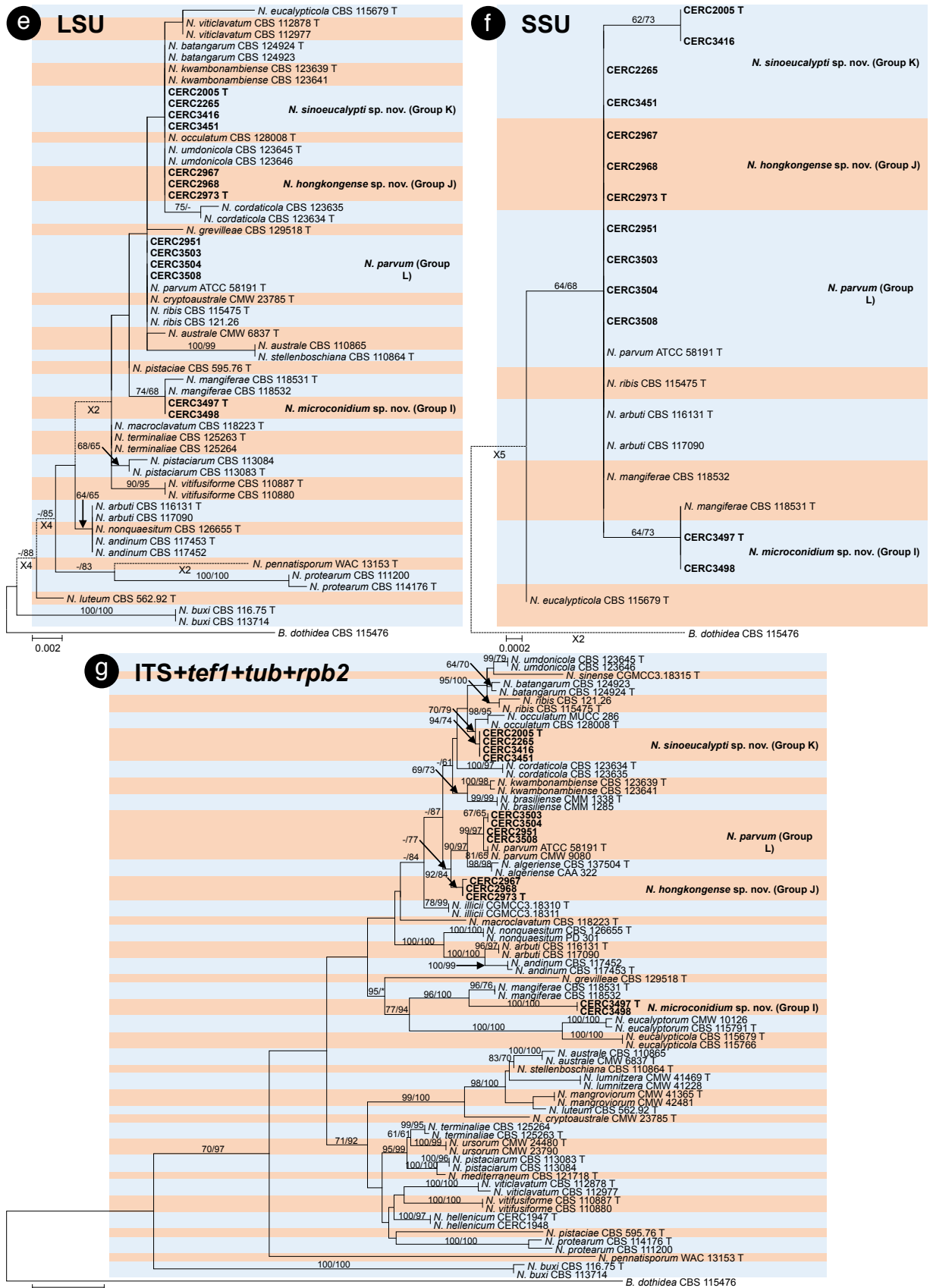


Fig. 4 Phylogenetic trees based on maximum likelihood (ML) analyses for species in *Neofusicoccum*. a. ITS region; b. *tef1* gene region; c. *tub* gene region; d. *rpb2* gene region; e. LSU region; f. SSU region; g. combination of ITS, *tef1*, *tub* and *rpb2* regions. Isolates sequenced in this study 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.

Fig. 4 (cont.)



showed that the six Chinese isolates in Group A are most closely related to *B. fabicerciana* and *B. fuisispora* (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. fuisispora* 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. fuisispora*.

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. minutispermata* and *B. sinensis* (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. fuisispora*, *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.

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

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

¹ Isolates and measurements in **bold** were examined in this study.

² Minimum–(average – standard deviation)–(average + standard deviation)–maximum or minimum–maximum, L × W = length × width.

³ L × W = average length × average width.

⁴ L/W = average length/average width.

⁵ 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) × (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) × (4–)4.5–5(–6) µm (av. = 11.5 × 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^{mm}f) to pale mouse grey (15^{mm}d) at the surface and olivaceous (21^{mm}k) to iron grey (23^{mm}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) × (2–)2.5–3.5 µm. *Paraphyses* not seen. *Conidia* hyaline, thin-walled, smooth with

granular contents, unicellular, aseptate narrowly fusiform, base subtruncate to bluntly rounded, $(15\text{--}19.5\text{--}24.5\text{--}28.5) \times (5\text{--}6\text{--}6.5\text{--}7.5) \mu\text{m}$ (av. = $22 \times 6.2 \mu\text{m}$, $n = 100$; $L/W = 3.5$) (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 $(25\text{--}30)^\circ\text{C}$, 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. fabriciana*, *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. 22×6.2 ; $L/W = 3.5$) are wider than these of *B. fabriciana* (av. 22×5.8 ; $L/W = 3.8$) and the optimal growth temperature of *B. qingyuanensis* ($(25\text{--}30)^\circ\text{C}$) is different from that of *B. fabriciana* ($(25\text{--}30)^\circ\text{C}$) (Chen et al. 2011c). Conidia of *B. qingyuanensis* are longer and wider than *B. fusispora* (av. 20×5 ; $L/W = 4$) (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. 26.2×6.7 ; $L/W = 3.9$) (Zhou et al. 2017). Conidia of *B. qingyuanensis* are slightly shorter than *B. kuwatsukai* (av. 22.3×6.2 ; $L/W = 3.6$) (Xu et al. 2015a) and no conidia or microconidia are observed for *B. qingyuanensis*, and no conidia with 1–3 septa before germination and microconidia ($3\text{--}8 \times 1\text{--}2 \mu\text{m}$) 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

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 $698 \mu\text{m}$ wide, $484 \mu\text{m}$ high, embedded in needle tissue, semi-immersed to superficial, unilocular, with a central ostiole, exuding conidia in a yellow mucoid mass. **Conidiophores** absent. **Conidiogenous cells** holoblastic, discrete, hyaline, cylindrical to lageniform, phialidic with periclinal thickening, $(6\text{--})8.5\text{--}13.5\text{--}15) \times 2\text{--}3\text{--}3.5) \mu\text{m}$. **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, $(20.5\text{--})22\text{--}26\text{--}29) \times (4.5\text{--})5.5\text{--}6.5\text{--}7.5) \mu\text{m}$ (av. = $23.8 \times 6 \mu\text{m}$, $n = 100$; $L/W = 3.9$) (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, $6.5\text{--}16 \times 1.5\text{--}2.5 \mu\text{m}$. **Spermatia** unicellular, aseptate, hyaline, thin-walled, allantoid to rod-shaped, $3.5\text{--}4.5 \times 1\text{--}1.5 \mu\text{m}$, $L/W = 2.9$.

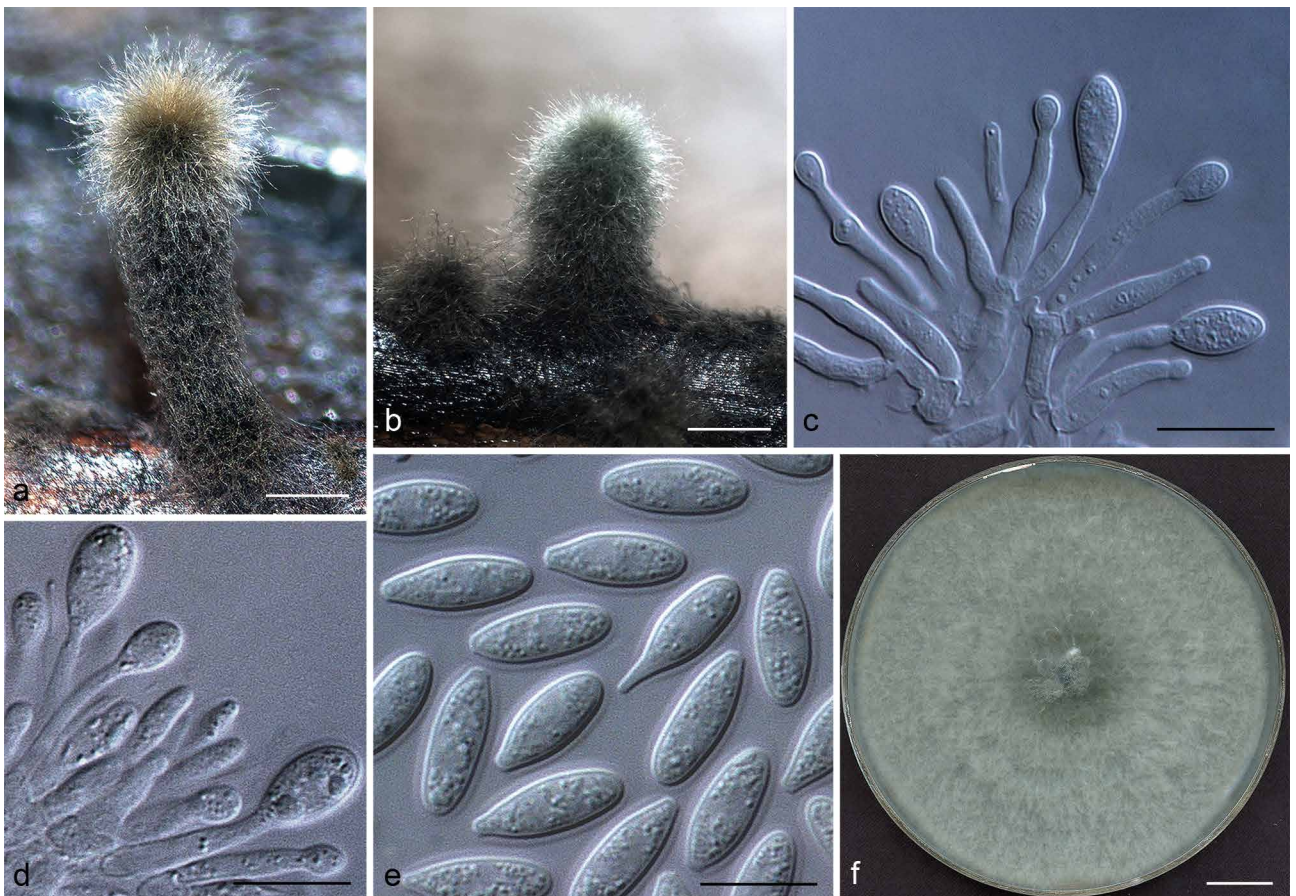


Fig. 5 *Botryosphaeria pseudoramosa*. a–b. Conidiomata formed on pine needle culture; c–d. conidiogenous cells and developing conidia; e. conidia; f. living culture after 10 d on 2% MEA (front). — Scale bars: a–b = $500 \mu\text{m}$; c–e = $10 \mu\text{m}$; f = 1cm .

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. minutispermata* 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 × 3.4; L/W = 3) (Slippers et al. 2014) and *B. minutispermata* (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 µm; b–d = 10 µm; e = 1 cm.

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

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) \times (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 \times 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*

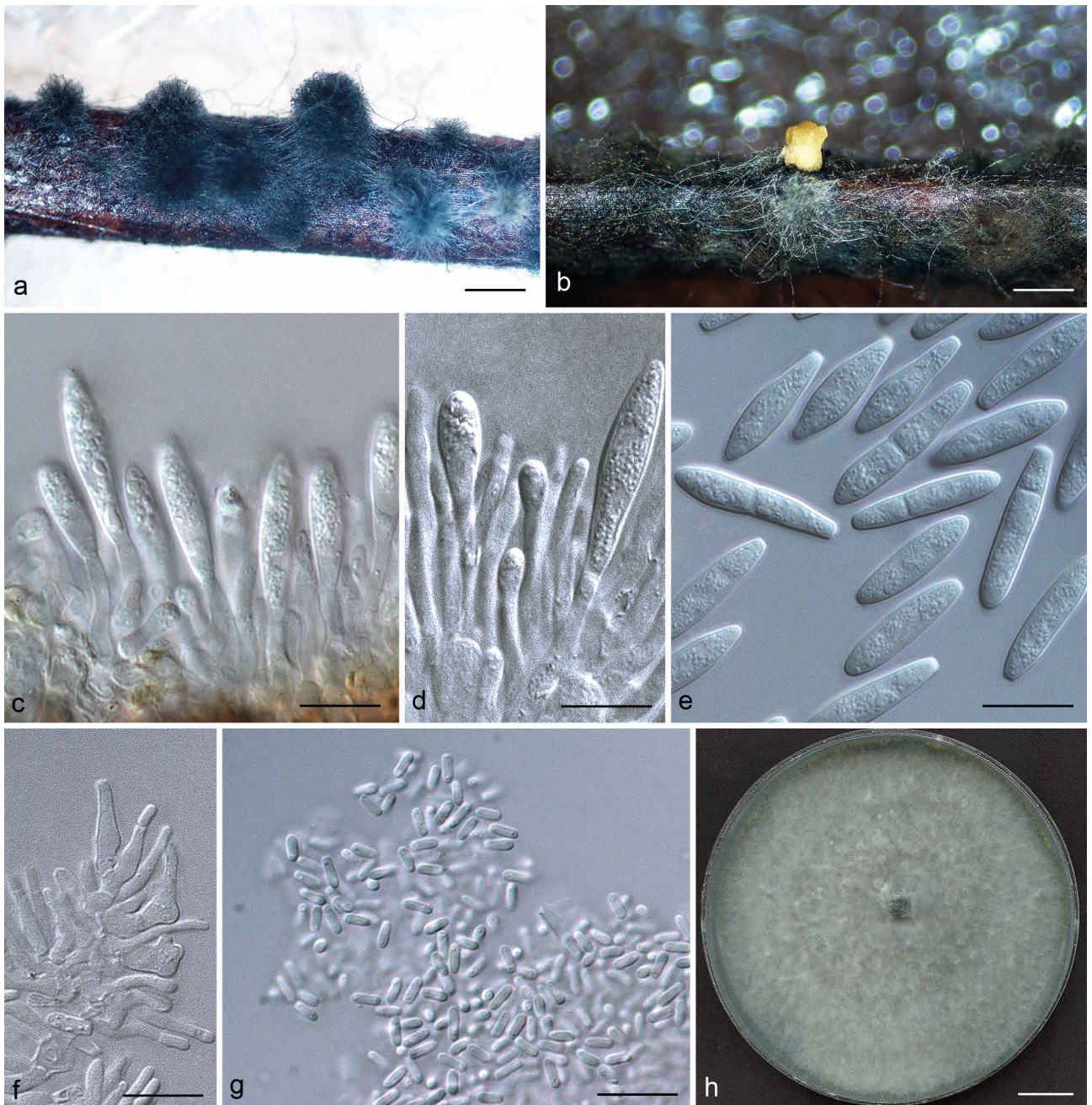


Fig. 7 *Botryosphaeria wangensis*. a–b. Conidiomata on pine needle culture; c–d. conidiogenous cells and developing conidia; e. conidia with 1 septum; f. spermatogenous cells; g. spermatia; h. living culture after 10 d on 2% MEA (front). — Scale bars: a–b = 500 μm ; c–g = 10 μm ; h = 1 cm.

(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. occultatum* (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. sinoeucalypti* (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 $895 \mu\text{m}$ wide, $1729 \mu\text{m}$ 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, $(10.5\text{--}12.5\text{--}18\text{--}20.5) \times (2\text{--}2.5\text{--}3\text{--}3.5) \mu\text{m}$. *Paraphyses* not seen. *Conidia* hyaline, thin-walled, smooth with granular contents, unicellular, aseptate narrowly fusiform, base subtruncate to bluntly rounded, $(10\text{--}11.5\text{--}13\text{--}14.5) \times (4\text{--}4.5\text{--}5.5\text{--}6) \mu\text{m}$ (av. = $12.3 \times 5 \mu\text{m}$, $n = 100$; L/W = 2.5) (Table 4).

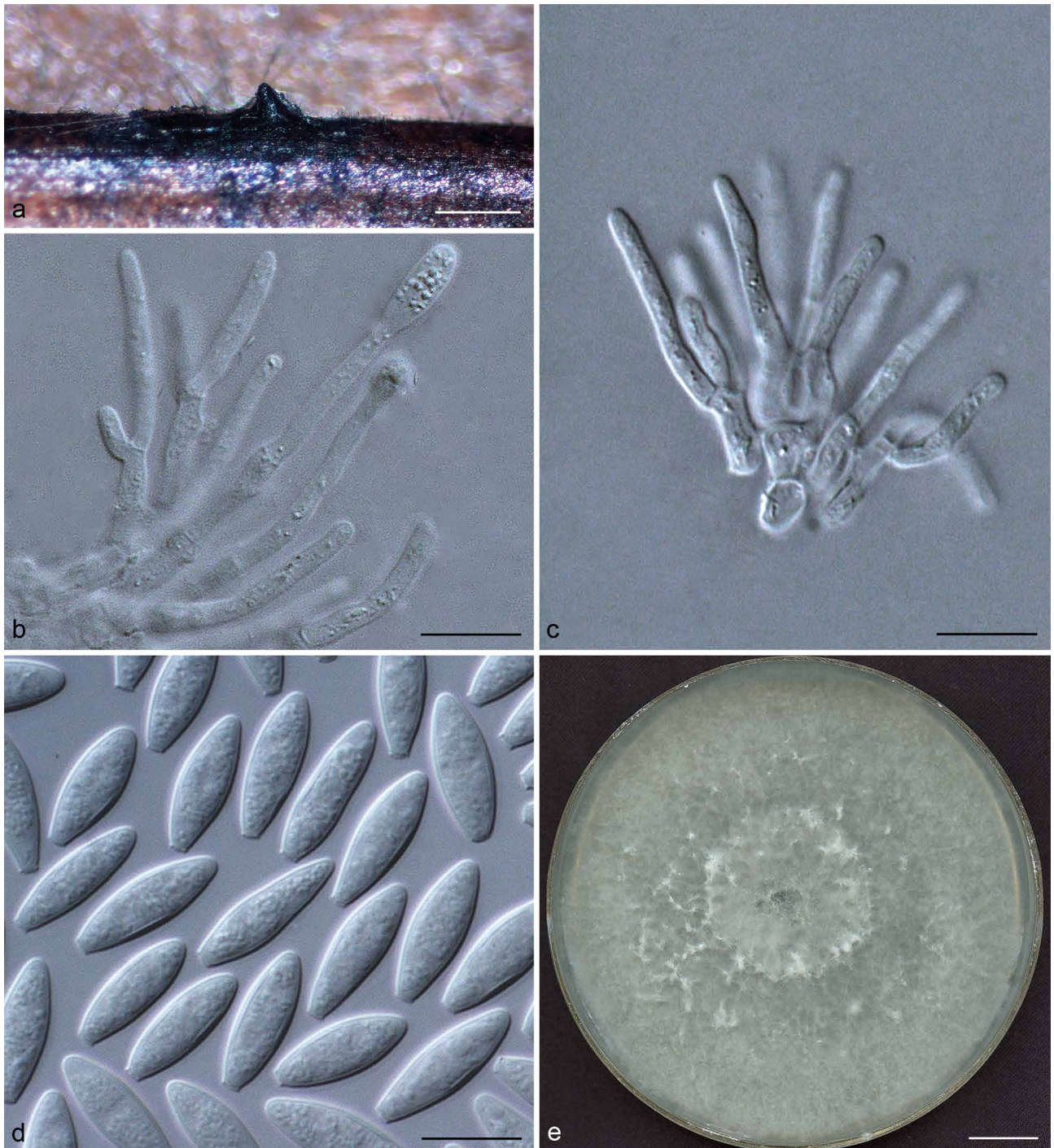


Fig. 8 *Neofusicoccum hongkongense*. a. Conidiomata formed on pine needle culture; b. conidiogenous cells and developing conidia; c. conidiogenous cells; d. conidia; e. living culture after 10 d on 2% MEA (front). — Scale bars: a = $500 \mu\text{m}$; b–d = $10 \mu\text{m}$; e = 1cm .

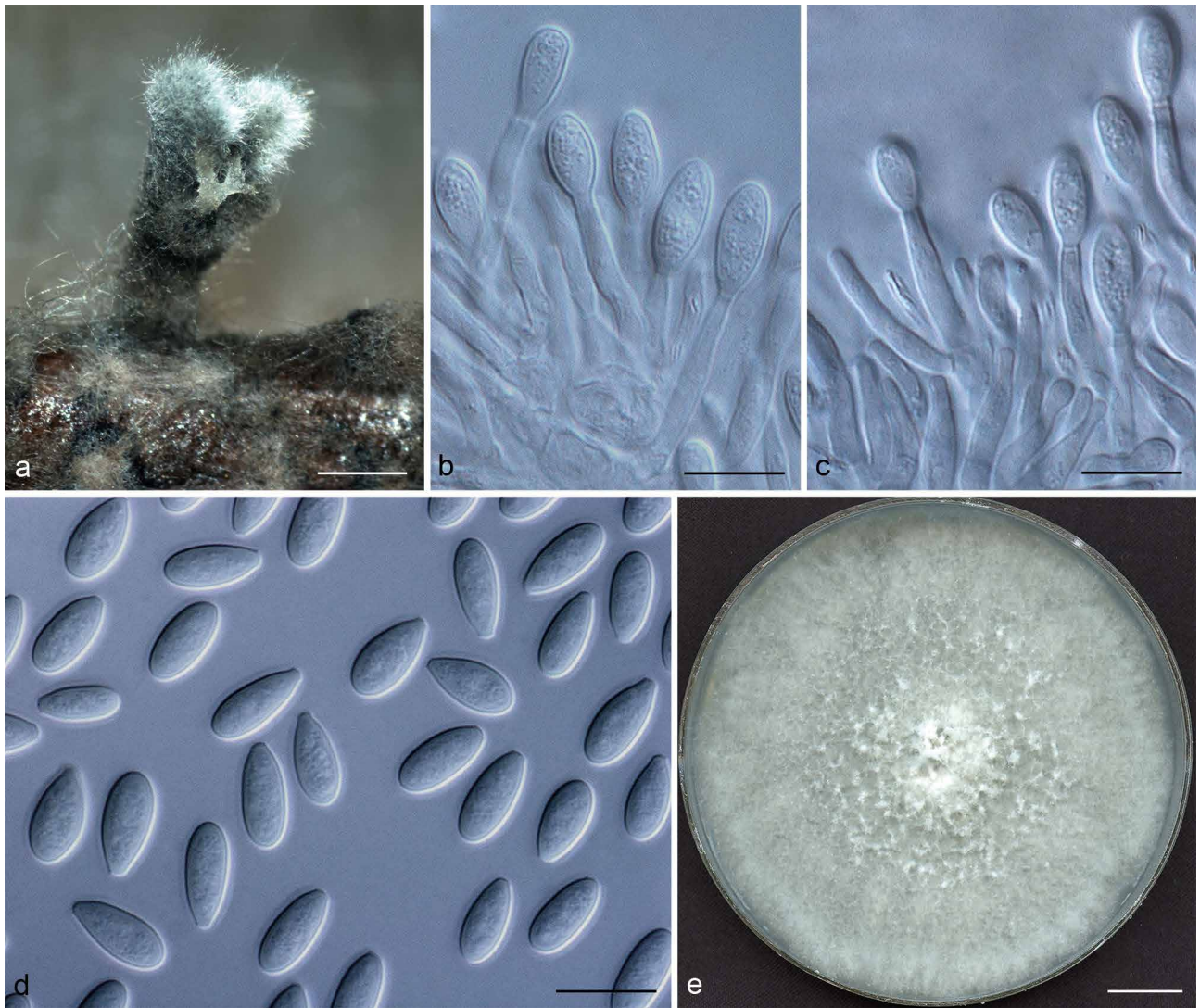


Fig. 9 *Neofusicoccum microconidium*. a. Conidiomata formed on pine needle culture; b–c. conidiogenous cells and developing conidia; d. conidia; e. living culture after 10 d on 2% MEA (front). — Scale bars: a = 500 μ m; b–d = 10 μ m; e = 1 cm.

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* \times *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* \times *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 μ m wide, 685 μ 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 μ 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) μ m (av. = 17.7 \times 5.2 μ m, 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 μ m. **Spermatia** unicellular, aseptate, hyaline, thin-walled, allantoid to rod-shaped, 2.5–4.5 \times 1.5 μ 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

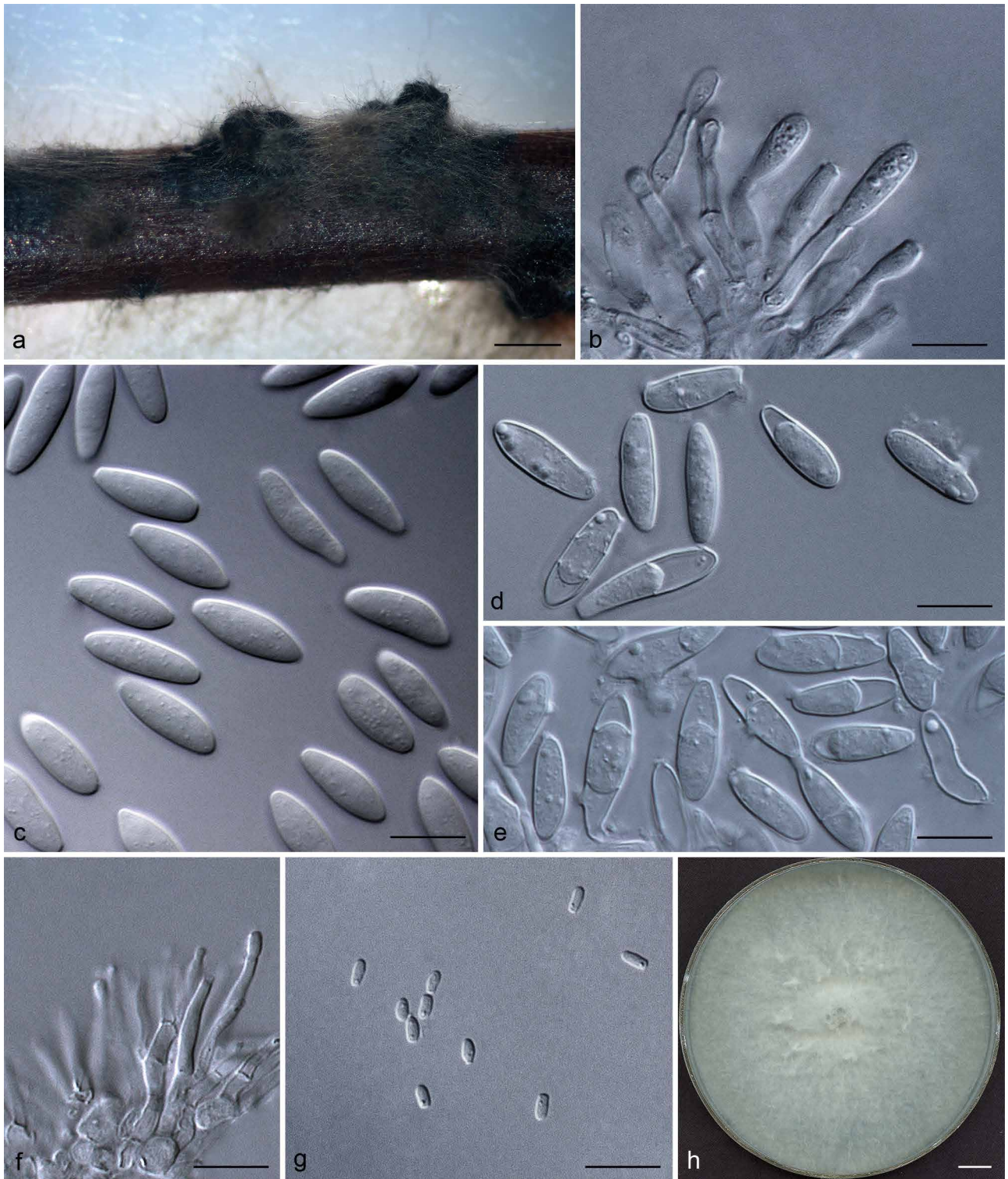


Fig. 10 *Neofusicoccum sinoeucalypti*. a. Conidiomata formed on pine needle culture; b. conidiogenous cells and developing conidia; c. immature conidia; d–e. mature conidia with 1–2 septa; f. spermatogenous cells; g. spermatia; h. living culture after 10 d on 2 % MEA (front). — Scale bars: a = 500 μ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

white, becoming pale mouse grey (15th d) to mouse grey (13th d) at the surface and olivaceous buff (21st d) to iron grey (23rd d) 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* \times *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 ex-type CERC2005 = CGMCC3.18752); GuangXi, from twigs of *E. urophylla* \times

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. occultatum*, *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. 15.5×5.5 ; 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).

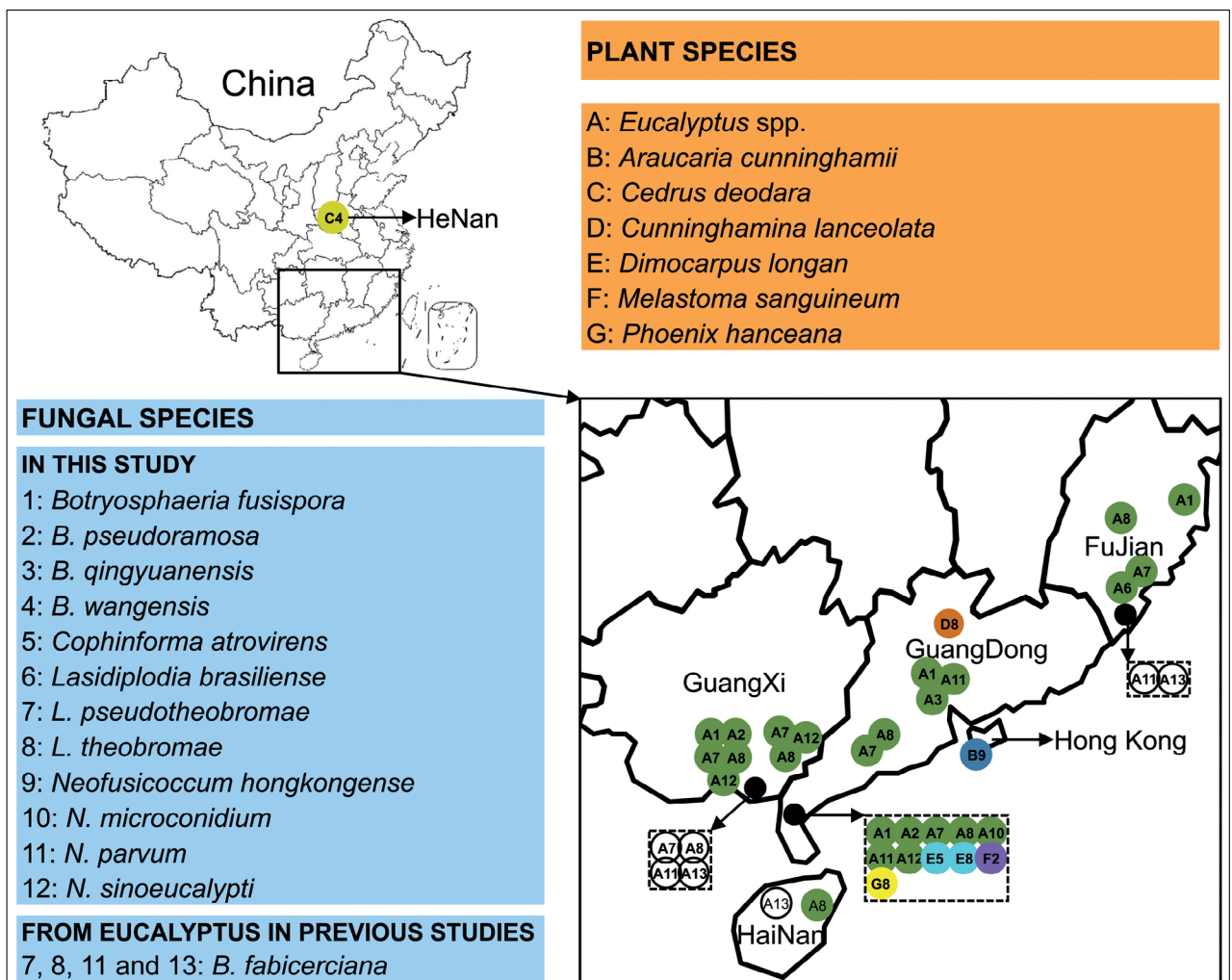


Fig. 11 Map showing the 12 species of *Botryosphaeriaceae* detected from different regions and plant hosts. The different *Botryosphaeriaceae* species are indicated as numbers 1 to 12; the plant hosts are shown as letters A to G. For example, A8 indicates *L. theobromae* (number 8 of fungal species) 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	<i>Eucalyptus</i> clones		
		CEPT-11	CEPT-12	CEPT-13
<i>Botryosphaeria fusispora</i>	CERC1998	17.6 ± 1.8 m-p ¹	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
<i>B. pseudoramosa</i>	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
<i>B. qingyuanensis</i>	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
<i>B. wangensis</i>	CERC2298	10.6 ± 0.5 op	12.3 ± 0.8 op	10.0 ± 0.2 op
	CERC2299	9.3 ± 1.1 op	11.1 ± 0.3 op	9.8 ± 0.1 op
<i>Cophinforma atrovirens</i>	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
<i>Lasiodiplodia brasiliense</i>	CERC2284	41.7 ± 5.6 j-m	139.7 ± 27.3 cd	95.8 ± 15.7 f
<i>L. pseudotheobromae</i>	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
<i>L. theobromae</i>	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
<i>Neofusicoccum hongkongense</i>	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
<i>N. microconidium</i>	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
	CERC2951	10.3 ± 0.5 op	11.9 ± 0.9 op	10.3 ± 0.3 op
<i>N. parvum</i>	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
	CERC2005	27.5 ± 4.7 k-p	68.0 ± 9.0 g-i	62.3 ± 8.7 h-j
<i>N. sinoeucalypti</i>	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-o
Control		10.5 ± 0.6 op	10.0 ± 0.2 op	9.4 ± 0.3 op

¹ 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 *Eucalyptus* 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 ± 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 brasiliense*, *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. qingyuanensis*, 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. sanguineum*, 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 *Botryosphaeriaceae* 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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