

# A review of *Botryosphaeraiales* in Venezuela with special reference to woody plants

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**Abstract** The *Botryosphaeraiales* order is best known for the diseases they cause in woody plants, as primary pathogens or latent pathogens residing in the woody tissue of asymptomatic hosts. In the first instance, *Botryosphaeraiales* species have been identified in Venezuela using morphological descriptions in the '80s and '90s, and later, the mid-2000s using molecular techniques. The morphological descriptions of the asexual morphs were initially used for the identification of *Botryosphaeraiales* genera and species. *Lasiodiplodia* spp. (as *L. theobromae*) was the most isolated fungus in Venezuela within the *Botryosphaeraiales* and has been found in more than 50% of the hosts in native and non-native plants, followed by *Diplodia*, *Dothiorella*, *Fusicoccum*, *Microdiplodia*, *Macrophomina*, *Neofusicoccum*, *Sphaeropsis*, and *Botryosphaeria*, considered all of them cosmopolitan group. Molecular studies, that included DNA sequence data from multiple genes, such as the internal transcribed spacer of rDNA (ITS), translation elongation factor-1 $\alpha$  (*tef1*), and  $\beta$ -tubulin (*btub*) used on the fungi isolated from woody plants, mainly trees or forest species, reveled the presence of two families within the *Botryosphaeraiales* order for Venezuela. *Botryosphaeriaceae* family includes the genera: *Botryosphaeria*, *Copriniforma*, *Diplodia*, *Lasiodiplodia* and *Neofusicoccum*, and the *Pseudofusicoccumaceae* family includes the genus *Pseudofusicoccum*. In the *Botryosphaeriaceae* the *Lasiodiplodia* genus was the most predominant in most hosts, and the species *L. theobromae* the most isolated in native and non-native plants. *Botryosphaeria dothidea*, *Copriniforma atrovirens*, *Diplodia scrobiculata* (syn. *D. guayanensis*), *Lasiodiplodia brasiliensis*, *L. crassispora*, *L. pseudotheobromae*, *Neofusicoccum arbuti* (syn. *N. andinum*), *N. parvum*, and *N. ribis* are cosmopolitan species, and they were isolated from native and non-native plants. *Pseudofusicoccum stromaticum* was found in plantations non-native of *Acacia mangium*, *E. urophylla x E. grandis*, *Eucalyptus urophylla*, and reported exclusively in South America. *Lasiodiplodia venezuelensis* has only been reported in Venezuela, and it was isolated from native and non-native plants. The presence, distribution, diversity, and symptoms of these fungi, mainly of the new genus, new species, and reports found in Venezuela and other parts of the world, were also reviewed.

**Keywords:** *Botryosphaeriaceae*, DNA sequence, forest, *Pseudofusicoccumaceae*, taxonomy.

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## Introduction

Forest ecosystems are a natural resource of great importance to humanity, since many people depend on them for their survival, in addition to other benefits such as human and environmental health, carbon sequestration, and genetic resources that underpin important wood and wood products-based industries (Wingfield et al. 2015). At present, the health of forests, both natural and managed, is more heavily threatened, and these threats arise from direct and indirect anthropogenic influences on fungal pathogens, and insect pests (Wingfield et al. 2015, Batista et al. 2021). Plantations in the tropics (monocultures) are usually of non-native species, such as the genera of *Pinus* spp., *Eucalyptus* spp., and *Acacia* spp., which are the main forest species planted in Venezuela, too.

Non-native trees in plantations are in part successful because they have been separated from their natural enemies, but when plantation trees are reunited with their coevolved pests, which may be introduced accidentally, or when they encounter novel pests to which they have no resistance, substantial damage or loss can ensue (Wingfield et al. 2008). The longer non-native trees are planted in an area, the more threatened they become by native pests. At the same time native species can be vulnerable to introduced (allochthonous, invasive) pests. But the relative species uniformity of monoculture stands in intensively managed native plantation forests can make them especially susceptible to the many native pests occurring in the surrounding natural forests (Wingfield 2003, Branco et al. 2015).

An example of an epidemic of native pathogens moving onto an exotic species is provided by the shoot pathogen *Gremmeniella abietina* (Lagerberg) Morelet, endemic and not particularly damaging on Scots pine in Sweden but causing widespread destruction of Swedish plantations of the extensively planted exotic lodgepole pine (Karlman et al. 1994).

The *Botryosphaerales* contains numerous fungal species that occur as saprophytes, parasites, or endophytes on a diverse range of plant hosts (Slippers & Wingfield 2007, Phillips et al. 2013), as well as opportunistic pathogens of woody plants, especially when host plants are stressed (Michailides & Morgan 1993). Different species within the order *Botryosphaerales* are well-known pathogens on forest trees and other woody plants associated with branch and trunk cankers, dieback, decline and mortality, and represent a growing threat to forest ecosystems worldwide (Slippers & Wingfield 2007, Phillips et al. 2013). An ecological and biological characteristic of the species in the *Botryosphaerales* order is the lack of host specificity thus being able to colonize and cause disease in diverse native and introduced plant hosts (Slippers & Wingfield 2007, Zlatković et al. 2018).

Different *Botryosphaerales* genera have the ability to infect multiple hosts, increasing the threat that they pose as potential economic and ecological important pathogens of native and cultivated trees around the world. Examples of inter-host exchanges of the *Botryosphaerales*, and that include those amongst and between native and non-native trees, we have *Botryosphaerales* species have moved between trees in native stands of *Eucalyptus* (*Myrtaceae*) and adjacent plantations of these trees (Burgess et al. 2006b), between native waterberry trees (*Syzygium cordatum*; *Myrtaceae*) and related eucalypt plantations (*Myrtaceae*) (Pavlic et al. 2007), from *Pinus resinosa* windbreaks to pine nurseries (Stanisz et al. 2007), among various tree hosts in the *Casuarinaceae*, *Cupressaceae*, *Fabaceae*, *Myrtaceae*, *Proteaceae*, *Santalaceae* (Sakalidis et al. 2011a), and among native *Terminalia* spp. (*Combretaceae*) and between these trees and *Theobroma cacao* (*Malvaceae*) (Begoude et al. 2012). The epidemiology of *Botryosphaerales* species is complex. These fungi can be monocyclic or oligocyclic

pathogens that cause polyetic epidemics. As monocyclic pathogens, they complete one disease cycle, or even part of one, in one season. Depending on the weather conditions, these species can be oligocyclic pathogens, i.e., polycyclic pathogens with a few (two or three) disease cycles per season (Moral et al. 2019). In Venezuela, the *Botryosphaerales* species are polycyclic since there are no marked seasons in the tropics as in the temperate regions. In the tropic, high temperatures and humidity are present almost all year round, therefore, these fungi will present several disease cycles and produce constant inoculum or spores throughout the year.

Species identification in *Botryosphaerales* has been largely based on the asexual morphs due to the lack of diversity among sexual morph features within this order and the difficulty of finding the sexual morphs in nature or obtaining them under laboratory conditions (Jacobs & Rehner 1998, Denman et al. 2000). Different species within the same genera of *Botryosphaerales* frequently possess overlapping morphological features (Pavlic et al. 2009) that can cause confusion in their accurate identification. In recent decades, several researchers began using identification techniques based on DNA sequencing and phylogenetic analyses to resolve the taxonomic problems associated with overlapping morphological characteristics among the species asexual morphs within *Botryosphaerales* genera (Jacobs & Rehner 1998, Denman et al. 2000, Smith & Stanosz 2001, Zhou & Stanosz 2001, Zhou et al. 2001). The phylogenetic analyses of DNA sequence data have significantly impacted all aspects of the systematics and taxonomy of the *Botryosphaerales*, including a redefinition of families and genera, identification of new species, cryptic species, and more recently hybrids (Slippers et al. 2017). Crous et al. (2006) defined all genera in the *Botryosphaerales* based predominantly on phylogenetic inference and characteristics of their asexual

morphs, and without morphological evidence of a sexual morph. In various cases, genera were thus established in the family based on asexual names.

The *Botryosphaerales* order has undergone changes in its systematics, mainly at the family level. A total of nine families have been included in the last 5 years within the *Botryosphaerales* order, based on phylogenetic, morphological, and ecological differences (Slippers et al. 2017). These families are: *Aplosporellaceae* Slippers et al. 2013 (Slippers et al. 2013), *Botryosphaeriaceae* Theiss. & Syd., 1918 (Crous et al. 2006), *Endomelanopsisaceae* TaoYang & Crous, 2016 (Yang et al. 2017), *Melanopsaceae* Phillips et al. 2013 (Slippers et al. 2013), *Phyllostictaceae* Fr., 1849 (Wikee et al. 2013), *Planistromellaceae* M.E. Barr, 1996 (Minnis et al. 2012), *Pseudofusicoccumaceae* Tao Yang & Crous, 2016 (Yang et al. 2017), *Saccharataceae* Slippers et al. 2013 (Slippers et al. 2013) and *Septorioideaceae* Wyka & Broders, 2016 (Wyka & Broders 2016), being the *Botryosphaeriaceae* family with the largest number of genera within it.

The purpose of this review was to update all relevant information on morphological descriptions and DNA sequencing data on the *Botryosphaerales* fungi that produce different diseases on cultivated and wild plants, as well as their distribution and diversity on woody plants in Venezuela.

### **Genera and species of *Botryosphaerales* identified with morphological descriptions in Venezuela**

Few general morphological features of within *Botryosphaerales* species have been reported in agricultural crops, forest plantations, and natural forests in Venezuela, where the taxonomic identification and associated reports have been initially based on morphological descriptions of the asexual morph. Such morphological descriptions are frequently based on 1) conidial features, such as septation, presence/absence of pigmentation,

and wall thickness, and 2) presence/absence of conidiophores, conidiogenous cells, and paraphyses in the conidiomata (Cedeño & Palacios-Prü 1992, Cedeño et al. 1994, 1995, 1998, 2001, Mohali 1997, Mohali & Encinas 2001, Mohali et al. 2002).

*Lasiodiplodia* Ellis & Everh., species are well-known and widespread plant pathogens, occurring mostly in tropical and subtropical regions (Punithalingam 1980). *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., has been widely reported and commonly occurs on different crops in Venezuela (Urtiaga 1986). In a published list of plant diseases in Venezuela, *L. theobromae* was the common fungal pathogen (Urtiaga 1986). This list from Urtiaga (1986) was updated using website records of fungi from 1998-2001 with specimens from the fungal collection of the Simon Bolívar University, Caracas-Venezuela (Iturriaga & Minter 2006), together with reports from Mohali and other authors during the 1990s through the mid-2000s (Table 1). In addition to *Lasiodiplodia*, other reported genera include *Diplodia* Fr., *Dothiorella* Sacc.,

*Botryosphaeria* Ces. & De Not., *Microdiplodia* Allesch., and *Macrophomina* Petr. (Urtiaga 1986, Iturriaga & Minter 2006). In addition to two *Neofusicoccum* Crous, Slippers & A.J.L. Phillips species isolated from *M. indica* in 2012 and 2016, where identified through their morphological descriptions (Table 1, Hernandez de Parra et al. 2012, Pacheco et al. 2016).

In Venezuela, at least eight genera of the *Botryosphaeriales* order within two families can be differentiated through the asexual morph. Seven genera belong to the *Botryosphaeriaceae* family, five with dark-conidia when mature age: *Diplodia* Fr., *Dothiorella* Sacc., *Lasiodiplodia* Ellis & Everh., *Macrophomina* Petr., *Sphaeropsis* Sacc., and two genera with hyaline conidia: *Copriniforma* Doilom, J.K. Liu & K.D. Hyde and *Neofusicoccum* Crous, Slippers & A.J.L. Phillips; and one genus in the *Pseudofusicoccumaceae* family, *Pseudofusicoccum* Mohali, Slippers & M.J. Wingf., with hyaline-conidia surrounded by a persistent mucous sheath (Table 2).

**Table 1** Different genera and species within *Botryosphaeriales* identified by their asexual morph in Venezuela.

Fungi	Host	Place	Reference
<i>Diplodia</i> Fr.	<i>Ceiba pentandra</i> (L.) Gaertn-old leaves	Buena vista, Lara state	Urtiaga 1986
<i>Diplodia</i> sp.	<i>Cassia</i> L.- root	-	Iturriaga & Minter 2006
<i>Diplodia ochracea</i> Pat.	<i>Ochroma lagopus</i> Sw.- trunk	-	Iturriaga & Minter 2006
<i>Diplodia mutila</i> Fr. Apud Mont.	<i>Pinus caribaea</i> morelet var. <i>hondurensis</i> (Barr. and Golf.) - blue stain on wood	Chaguanamas, Anzoátegui state	Mohali & Encinas 2001
<i>Dothiorella</i> Sacc.	<i>Delonix regia</i> (Bojer ex Hook) Raf. - branches	El Tocuyo, Lara state	Urtiaga 1986
<i>Dothiorella</i> sp.	<i>Psidium guajava</i> L.- fruit rot	Merida and Zulia states	Cedeño et al. 1998
<i>Dothiorella</i> <i>dothidea</i> (= <i>Botryosphaeria</i> <i>dothidea</i> )	<i>Prunus pérssica</i> (L.) Batsch - brown rot of fruits	El Arenal, Merida state	Cedeño et al. 1994
<i>Fusicoccum</i> Corda	<i>Echinodorus bertero</i> (Spreng) Fassett - leaves	Guanare, Portuguesa state	Urtiaga 1986
<i>Lasiodiplodia</i> <i>theobromae</i> (Pat.) Griffon & Maubl.	<i>Pachystachys lutea</i> Nees - branches	Barquisimeto, Lara state	Urtiaga 1986
<i>L. theobromae</i>	<i>Anacardium occidentale</i> L. - terminal branch death	Barquisimeto, Lara state	Urtiaga 1986
<i>L. theobromae</i>	<i>Mangifera indica</i> L. - branches and seeds	La Calzada de Páez, Barinas state	Urtiaga 1986
<i>L. theobromae</i>	<i>Annona reticulata</i> L. - old leaves	Barquisimeto, Lara state	Urtiaga 1986
<i>L. theobromae</i>	<i>Catharanthus roseus</i> (L.) G. Don - leaves	Barquisimeto, Lara state	Urtiaga 1986

Fungi	Host	Place	Reference
<i>L. theobromae</i>	<i>Crescentia cujete</i> L. - <b>branches and leaves</b>	Wide distribution in Venezuela	Urtiaga 1986
<i>L. theobromae</i>	<i>Citrus lanatus</i> (Thunb.) Matsum. & Nakai - <b>fruits rot and branches</b>	La Miel, Lara state	Urtiaga 1986
<i>L. theobromae</i>	<i>Juniperus lucayana</i> Britton - <b>twigs</b>	Barquisimeto, Lara state	Urtiaga 1986
<i>L. theobromae</i>	<i>Curatella Americana</i> L. - <b>old leaves</b>	La Calzada de Páez, Barinas state	Urtiaga 1986
<i>L. theobromae</i>	<i>Codiaeum variegatum</i> (L.) Blume var. <i>pictum</i> (Lodd.) Muell	-	Urtiaga 1986
<i>L. theobromae</i>	<i>Hura crepitans</i> L. - <b>old leaves</b>	La Calzada de Páez, Barinas state	Urtiaga 1986
<i>L. theobromae</i>	<i>Manihot esculenta</i> Crantz - <b>branches</b>	Uraciche, Yaracuy state	Urtiaga 1986
<i>L. theobromae</i>	<i>Arachis hypogaea</i> L. - <b>root</b>	Buría Londres, Lara state	Urtiaga 1986
<i>L. theobromae</i>	<i>Phaseolus lunatus</i> L. - <b>branches</b>	Sabana de Parra, Yaracuy state	Urtiaga 1986
<i>L. theobromae</i>	<i>Sansevieria trifasciata</i> Prain. - <b>old leaves</b>	Barquisimeto, Lara state	Urtiaga 1986
<i>L. theobromae</i>	<i>Cedrela odorata</i> L. - <b>branches</b>	Chivacoa, Yaracuy state	Urtiaga 1986
<i>L. theobromae</i>	<i>Cecropia peltata</i> L. - <b>branches</b>	Chivacoa, Yaracuy	Urtiaga 1986
<i>L. theobromae</i>	<i>Ficus pumila</i> L. - <b>old leaves and galls on the leaves</b>	Barquisimeto, Lara state	Urtiaga 1986
<i>L. theobromae</i>	<i>Maxillaria Ruiz &amp; Pavon</i> - <b>old leaves</b>	Duaca, Lara	Urtiaga 1986
<i>L. theobromae</i>	<i>Passiflora edulis</i> Sims. form <i>flavicarpa</i> Degener	El Eneal, Lara	Urtiaga 1986
<i>L. theobromae</i>	<i>Salix babylonica</i> L. - <b>black root rot</b>	Barquisimeto, Lara state	Urtiaga 1986
<i>L. theobromae</i>	<i>Duranta repens</i> L. - <b>branches</b>	Ureña, Táchira	Urtiaga 1986
<i>L. theobromae</i>	<i>Pachystachys lutea</i> Nees - <b>branches</b>	Barquisimeto, Lara state	Urtiaga 1986
<i>Lasiodiplodia theobromae</i>	<i>Cajanus indicus</i> Spreng. - <b>branches</b>	Lara state	Urtiaga 1986
<i>L. theobromae</i>	<i>Duranta repens</i> L. - <b>branches</b>	Tachira state	Urtiaga 1986
<i>L. theobromae</i>	<i>Theobroma cacao</i> L.	-	Iturriaga & Minter 2006
<i>L. theobromae</i>	<i>Vinca rosea</i> L. - <b>leaf and branch</b>	Lara state	Urtiaga
<i>L. theobromae</i>	<i>Persea Americana</i> Mill. - <b>fruits</b>	Yaracuy state	Hernandez de Parra et al. 2012
<i>L. theobromae</i>	<i>Citrus latifolia</i> Tanaka. - <b>fruits</b>	Yaracuy state	Hernandez de Parra et al. 2012
<i>L. theobromae</i>	<i>Citrus sinensis</i> (L.) Osbeck - <b>fruits</b>	Yaracuy state	Hernandez de Parra et al. 2012
<i>L. theobromae</i>	<i>C. sinensis</i> - <b>lesion and gummosis on the branches</b>	Caño Amarillo, Táchira state	Cedeño & Palacios-Prü 1992
<i>L. theobromae</i>	<i>Citrus aurantiifolia</i> - <b>lesion and gummosis on the branches</b>	Caño Amarillo, Táchira state	Cedeño &Palacios-Prü 1992
<i>L. theobromae</i>	<i>Passiflora edulis</i> Sims f. <i>flavicarpa</i> - <b>dieback on the branches</b>	South of Maracaibo Lake, Zulia and Mérida states	Cedeño et al. 1995
<i>L. theobromae</i>	<i>Pinus caribaea</i> var. <i>hondurensis</i> - <b>blue stain on wood</b>	Uverito plantation and Uverito sawmill, Monagas state	Mohali 1993
<i>L. theobromae</i>	<i>Azadirachta indica</i> A. juss - <b>blue stain on wood</b>	Cojedes state	Mohali et al. 2002
<i>L. theobromae</i>	<i>Pinus oocarpa</i> Schiede ex Schltdl	Merida state	Mohali et al. 2002
<i>L. theobromae</i>	<i>Mangifera indica</i> - <b>branches dieback</b>	Maracay (INIA-CENIAP), Aragua state	Pacheco et al. 2016
<i>Microdiplodia buddleiae</i> Guevitz	<i>Opuntia caracasana</i> Salm.- <b>spot leaves</b>	Humocaro Bajo, Lara state	Urtiaga 1986
<i>Macrophomina phaseolina</i> (Tassi) Goidanich	<i>Begonia</i> sp.- <b>spot on the leaf</b>	Barquisimeto, Lara state	Urtiaga 1986
<i>M. phaseolina</i>	<i>Calendula officinalis</i> L. - <b>stem and inflorescence</b>	Barinas state	Urtiaga 1986
<i>M. phaseolina</i>	<i>Ipomoea batata</i> (L.) Lam. - <b>stolons at the roots</b>	Siquisique, Lara state	Urtiaga 1986
<i>M. phaseolina</i>	<i>Phaseolus vulgaris</i> L. - <b>stem and basal rot</b>	Moroturo, Lara state	Urtiaga 1986
<i>M. phaseolina</i>	<i>Glycine</i> Willd.	-	Iturriaga & Minter (2006)
<i>Macrophomina phaseolina</i>	<i>Gossypium</i> L.	-	Iturriaga & Minter (2006)

Fungi	Host	Place	Reference
<i>M. phaseolina</i>	<i>Ipomoea</i> L.	-	Iturriaga & Minter (2006)
<i>M. phaseolina</i>	<i>Nicotiana</i> L.	-	Iturriaga & Minter (2006)
<i>M. phaseolina</i>	<i>Phaseolus</i> L.	-	Iturriaga & Minter (2006)
<i>M. phaseolina</i>	<i>Psidium guajava</i> L.- fruits	-	Iturriaga & Minter (2006)
<i>M. phaseolina</i>	<i>Solanum melongena</i> L.	-	Iturriaga & Minter (2006)
<i>M. phaseolina</i>	<i>Vigna</i> Savi	-	Iturriaga & Minter (2006)
<i>Neofusicoccum mangiferae</i> (Syd. & P. Syd.) Crous, Slippers & A.J.L. Phillips	<i>Mangifera indica</i> - death of branches	Maracay (INIA-CENIAP), Aragua state	Pacheco et al. 2016
<i>Neofusicoccum parvum</i> (Pennington & Samuels) Crous, Slippers & A.J.L. Phillips	<i>Mangifera indica</i> - death of branches	Maracay (INIA-CENIAP), Aragua state	Pacheco et al. 2016
<i>Sphaeropsis</i> Sacc.	<i>Cecropia peltata</i> L. - branch and trunk knots	Reserva Forestal de Ticoporo, Mirí, Barinas state	Urtiaga 1986
<i>Sphaeropsis</i> sp.	<i>Phthirusa paniculata</i> (Kunth) J.F.Macbr. - leaf	Lara state	Urtiaga 1986
<i>Sphaeropsis palmarum</i> Cooke	<i>Cocos nucifera</i> L. - old leaves	Cumanacoa, Sucre state	Urtiaga 1986
<i>Sphaeropsis sapinea</i> (Fr.) Dyko & B. Sutton	<i>Pinus caribaea</i> Morelet - chlorosis in the needles and discoloration lesions on the stem	Nirgua, Yaracuy state	Mohali 1997
<i>S. sapinea</i>	<i>Pinus caribaea</i> var. <i>hondurensis</i> - shoot blight, dieback and canker on trunks, branches and roots (plantations), and death at the tips of the needles (seedlings in nurseries)	Uverito (Monagas state), and Coloradito y Los Hachos (Anzoátegui state)	Cedeño et al. 2001
<i>S. sapinea</i>	<i>Pinus oocarpa</i> Schiede - blue stain on wood	Andes region (1600 meters above sea level), Merida state	Mohali et al. 2002
<i>Sphaeropsis tumefaciens</i> Hedges	<i>Citrus</i> L. - gall	-	Iturriaga & Minter (2006)
<i>Botryosphaeria festucae</i> (Lib.) Arx & E. Müll.	<i>Zea mays</i> L. - bract, leaf and seed	-	Iturriaga & Minter (2006)
<i>Botryosphaeria dothidea</i> (Moug. ex Fr.) Ces. & De Not	Compositae - stem	Aragua state	Iturriaga & Minter (2006)
<i>Botryosphaeria ribis</i> Grossenb. & Duggar	<i>Rosa canina</i> L. - branch	Lara state	Urtiaga 1986

**Table 2** Morphological differentiation between the *Botryosphaeriaceae* genera and a genus in *Pseudofusicoccumaceae* both belonging to the order *Botryosphaerales* found in Venezuela.

Genera	Conidia	Conidiomata	Conidiophores	Conidiogenesis cells	Paraphyses
<i>Copriniforma</i> Doilom, J.K. Liu & K.D. Hyde	Hyaline, thin walled, unicellular, aseptate, rarely becoming septate, mostly fusoid to ellipsoidal. Most conidia longer than 30 µm	Material pycnidial, superficial, multilocular, dark brown to black, eustromatic	Absent	Enteroblastic, hyaline, cylindrical	Absent

Genera	Conidia	Conidiomata	Conidiophores	Conidiogenesis cells	Paraphyses
<i>Diplodia</i> Fr.	Initially hyaline, aseptate, thick-walled, becoming 1-septate only rarely becoming 2-septate, pale translucent brown after discharge from the pycnidia. Some species the conidia become pigmented while still enclosed in the conidioma and these species the conidia rarely become septate.	Pycnidial, ostiolate, formed in uni- or multiloculate stromata	When present: hyaline, simple, occasionally septate, rarely branched, cylindrical,	Holoblastic, hyaline, cylindrical	Absent
<i>Dothiorella</i> Sacc.	Initially hyaline, becoming dark brown and one-euseptate within the pycnidial cavity, ellipsoid to ovoid, thick-walled, externally smooth or striate, internally verruculose	Stromatic, ostiolate, individual or in loose clusters of up to 10 conidiomata, immersed, breaking through the bark when mature.	Absent	Holoblastic, hyaline, smooth-walled, cylindrical	Absent
<i>Lasiodiplodia</i> Ellis & Everh.	Hyaline when young, later becoming medianly 1-euseptate, dark brown with longitudinal striations, thick-walled, oblong to ellipsoid, straight, broadly rounded at the apex, base truncate	Stromatic, immersed or superficial, separate or aggregated and confluent, globose, dark brown, uni- or multilocular	Often reduced to Holoblastic, conidiogenous cells, if present hyaline, simple, sometimes septate, rarely branched	hyaline, smooth, cylindrical to subobpyriform, discrete, determinate or indeterminate	Present
<i>Macrophomina</i> Petr.	Aseptate, obtuse at each end, straight, cylindrical to fusiform, thin-walled, smooth, guttulate, enclosed in mucoid sheath. Immature conidia hyaline, mature conidia becoming medium to dark brown.	Pycnidial, stromatic, dark brown to black, solitary or gregarious	Reduced to conidiogenous cells	Enteroblastic, phialidic, determinate, discrete, lageniform to doliiform, hyaline, smooth, with wide aperture and minute collarette, formed from the inner cells of the pycnidial wall, enclosed in mucoid sheath	Absent
<i>Neofusicoccum</i> Crous, Slippers & A.J.L. Phillips	Mostly fusoid to ellipsoidal, hyaline.	Stromatic, pycnidial, solitary or aggregated, often occurring within the same stroma as the ascomata, walls composed of dark brown	When present	Enteroblastic, integrated, hyaline, smooth, cylindrical	Absent

Genera	Conidia	Conidiomata	Conidiophores	Conidiogenesis cells	Paraphyses
<i>Pseudofusicoccumaceae</i> Tao Yang & Crous, <i>Pseudofusicoccum</i> Mohali, Slippers & M.J. Wingf.	Conidia are more cylindrical than in <i>Noefusicoccum</i> species and surrounded by a persistent mucous sheath, hyaline.	Large, superficial, unilocular or multilocular locule	Reduced to conidiogenous cells	Holoblastic, smooth, cylindrical to subcylindrical, hyaline	Present or absent
<i>Sphaeropsis</i> Sacc.	Oval, oblong or clavate, straight, aseptate, moderately thick-walled, initially hyaline, becoming brown.	Pycnidial, stromatic, immersed to erumpent, thick walled.	Reduced to conidiogenous cells	Hyaline, discrete, proliferating internally to form periclinal thickenings.	Present

**Notes:** *Macrophomina* has sclerotinia black, smooth, hard, formed of dark brown, thick-walled cells (Phillips et al. 2013). *Neofusicoccum* was introduced by Crous et al. (2006) for species that are morphologically similar to *Fusicoccum*, but phylogenetically distinct from them, and thus could no longer be accommodated in that genus. The presence of paraphyses in *Sphaeropsis* differentiates this genus from *Diplodia*, which does not have pycnidial paraphyses and striate conidia of *Lasiodiplodia* differentiate it from *Sphaeropsis*, which has smooth-walled conidia (Phillips et al. 2013). Also, the absence of septa (aseptate) in mature conidia of *Sphaeropsis* separates it from the genus *Diplodia* which is characterized by conidia septate (Phillips et al. 2013).

### DNA Sequence-based identification of *Botryosphaerales* in Venezuela

In the early 2000s, publications began appearing for identifying species within the *Botryosphaerales* using DNA sequence data. DNA-based approaches helped to solve the problem of identifying species with overlapping morphology, and the combination of morphological characteristics and DNA sequence data became a powerful tool to separate and identify new genera and species (Denman et al. 2000, Zhou & Stanosz 2001). However, single-gene genealogies were not always useful for resolving closely related or cryptic species of the *Botryosphaerales*;

moreover, comparisons of DNA sequence data from multiple genes or different gene regions were exceptionally useful for discriminating among several closely related species (Slippers et al. 2004a, 2004b, Pavlic et al. 2009).

From the mid-2000s through 2022, different species and genera within the *Botryosphaerales* in Venezuela were isolated. Analysis of the morphological characteristics and DNA sequences were used for identifying a new genus and four new species. Multiple DNA loci were used to identify these *Botryosphaerales* isolates from Venezuela including, the internal transcribed spacer of rDNA (ITS), translation elongation factor-1α (*tef1*), and β-tubulin (*btub*) (Table 3).

**Table 3.** Genera and species within *Botryosphaerales* order identified DNA sequence data in Venezuela.

Species	Accession number	Host	Locality	GenBank accession number			References
				ITS	TEFI	BTUB	
<i>Botryosphaeria dothidea</i>	CMW8000 Ex-type	<i>Prunus</i> sp.	Switzerland	AY236949	AY236898	AY236927	Slippers et al. 2004.
<i>B. dothidea</i>	CMW13390=CBS117919	<i>Eucalyptus urophylla</i> x <i>E. grandis</i>	CR and WCR	EF118044	-	-	Mohali et al. 2007
<i>Copriniforma atrovirens</i>	CMW13416=CBS 117444	<i>E. urophylla</i> x <i>E. grandis</i>	CR and WCR	EF118050	GU134938	-	Mohali et al. 2007
<i>C. atrovirens</i>	CMW13425=CBS117445	<i>Acacia mangium</i>	CR and WCR	EF118046	GU134939	-	Mohali et al. 2007
<i>C. atrovirens</i>	CSM 72	<i>Theobroma cacao</i>	AR	MF436087	MF436099	MF436111	Mohali et al. 2023

Species	Accession number	Host	Locality	GenBank accession number	References
				ITS TEFI BTUB	
<i>C. atrovirens</i>	MFLUCC 11-0425 Ex-type	<i>Eucalyptus</i> sp	Thailand	JX646800 JX646865 JX646848	Liu et al. 2012
<i>Diplodia scrobiculata</i> (Syn. <i>D. guayanensis</i> )	CBS129749	<i>Acacia mangium</i>	NER	JX545106 JX545126 JX545146	Úrbez-Torres et al. 2016
<i>D. scrobiculata</i> (Syn. <i>D. guayanensis</i> )	CBS129750	<i>Acacia mangium</i>	NER	JX545108 JX545128 JX545148	Úrbez-Torres et al. 2016
<i>D. scrobiculata</i>	CMW189 = CBS 118110 Ex-type	<i>Pinus banksiana</i>	United States	KF766160 KF766399 AY624258	Linaldeddu et al. 2016, de Wet et al. 2003
<i>Lasiodiplodia brasiliense</i>	CMM4015 Ex-type	<i>Mangifera indica</i>	Brazil	JX464063 JX464049 -	Netto et al. 2014
<i>L. brasiliensis</i>	CSM 11	<i>Theobroma cacao</i>	AR	MF436018 MF436006 MF435998	Mohali et al. 2023
<i>L. brasiliensis</i>	CSM 15	<i>Theobroma cacao</i>	AR	MF436019 MF436007 MF435997	Mohali et al. 2023
<i>L. crassispora</i>	WAC 12533= CBS118741 Ex-type	<i>Santalum album</i>	Australia	DQ103550 EU673303 KU887506	Burgess et al. 2006, Cruywagen et al. 2017
<i>L. crassispora</i>	CMW 13488	<i>Eucalyptus urophylla</i>	CR and WCR	DQ103552 DQ103559 KU887507	Burgess et al. 2006, Cruywagen et al. 2017
<i>L. pseudotheobromae</i>	CBS 129752	<i>Acacia mangium</i>	NER	JX545091 JX545111 JX545131	Castro-Medina et al. 2014
<i>L. pseudotheobromae</i>	CBS116459 Ex-type	<i>Gmelina arborea</i>	Costa Rica	KF766193 EF622057 EU673111	Phillips et al. 2008, Slippers et al. 2013
<i>Lasiodiplodia theobromae</i>	CBS 164.96 Ex-neotype	From unidentified fruit along coral reef coast	Papua New Guinea, Madang	AY640255 AY640258 KU887532	Phillips et al. 2005, Cruywagen et al. 2017
<i>L. theobromae</i>	CSM 22	<i>Theobroma cacao</i>	AR	MF436023 MF436011 MF436005	Mohali et al. 2023
<i>L. theobromae</i>	CBS129751	<i>Acacia mangium</i>	NER	JX545096 JX545116 JX545136	Úrbez-Torres et al. 2016
<i>L. theobromae</i>	CMW13487	<i>Europhylla urophylla</i> x <i>E. grandis</i>	CR and WCR	EF118053 - -	Mohali et al. 2007
<i>L. theobromae</i>	CBS129754	<i>Pinus caribaea</i> var. <i>hondurensis</i>	NER	JX545099 JX545119 JX545139	Úrbez-Torres et al. 2016
<i>L. theobromae</i>	CMW13489=CBS 117922	<i>Eucalyptus urophylla</i> x <i>E. grandis</i>	CR and WCR	DQ103525- -	Mohali et al. 2007
<i>L. theobromae</i>	CMW13510	<i>Acacia mangium</i>	CR and WCR	DQ103526- -	Burgess et al. 2006
<i>L. theobromae</i>	CMW13520	<i>Pinus caribaea</i>	CR and WCR	DQ103527- -	Burgess et al. 2006
<i>L. theobromae</i>	CAA006	<i>Ficus insipida</i> GR		DQ458891 DQ458876 DQ458859	Mohali et al. 2017
<i>L. venezuelensis</i>	CBS129755	<i>Pinus caribaea</i> var. <i>hondurensis</i>	NER	JX545104 JX545124 JX545144	Úrbez-Torres et al. 2016

Species	Accession number	Host	Locality	GenBank	accession	number	References
				ITS	TEFI	BTUB	
<i>L. venezuelensis</i>	CBS129757	<i>Ficus insipida</i>	GR	JX545102	JX545122	-	Mohali et al. 2017
<i>L. venezuelensis</i>	WAC12539=CBS 118739 Ex-type	<i>Acacia mangium</i>	CR and WCR	DQ103547	DQ103568	KU887533	Burgess et al. 2006, Cruywagen et al. 2017
<i>L. venezuelensis</i>	CBS 129759	<i>Jacaranda copaia</i>	GR	JX545101	JX545121	JX545141	F. Castro-Medina/S.R. Mohali-unpublished Farr et al. 2005, Phillips et al. 2013
<i>Neofusicoccum arbuti</i>	CBS 116131=AR 4014 Ex-type	<i>Arbutus menziesii</i>	USA	AY819720	KF531792	KF531793	Mohali et al. 2006, Yang et al. 2017
<i>Neofusicoccum arbuti</i> (Syn. <i>N. andinum</i> )	CMW13455=CBS 117453	<i>Eucalyptus</i> sp. AR		AY693976	AY693977	KX464923	Mohali et al. 2006, Yang et al. 2017
<i>Neofusicoccum arbuti</i> (Syn. <i>N. andinum</i> )	CMW13446=CBS 117452	<i>Eucalyptus</i> sp. AR		DQ306263	DQ306264	KX464922	Mohali et al. 2006, Yang et al. 2017
<i>N. parvum</i>	CMW9081 Ex-type	<i>Eucalyptus grandis</i>	South Africa	AY236943	AY236888		Slippers et al. 2004
<i>N. parvum</i>	CMW13350=CBS117923	<i>Psidium guajava</i>	ZR	EF118036	-	-	Mohali et al. 2007
<i>N. parvum</i>	CMW13355=CBS117915	<i>Eucalyptus urophylla</i>	CR and WCR	EF118035	-	-	Mohali et al. 2007
<i>N. ribis</i>	CMW7772 Ex-type	<i>Ribes</i> sp.	New York, United States	AY236935	AY236877	-	Slippers et al. 2004
<i>N. ribis</i>	CMW13360=CBS117916	<i>Eucalyptus urophylla</i>	CR and WCR	EF118037	-	-	Mohali et al. 2007
<i>N. ribis</i>	CMW13410=CBS117443	<i>Eucalyptus urophylla</i>	CR and WCR	EF118038	-	-	Mohali et al. 2007
<i>Pseudofusicoccum stromaticum</i>	CMW13434=CBS 117448 Ex-type	<i>Eucalyptus urophylla x E. grandis</i>	CR and WCR	AY693974	AY693975	EU673094	Mohali et al. 2006, Phillips et al. 2008
<i>P. stromaticum</i>	CMW13426=PREM58513	<i>Acacia mangium</i>	CR and WCR	EF118041	-	-	Mohali et al. 2007

Note: Acronyms of culture collections: CBS: Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; IBL: Independent Biological Laboratories Israel. KEFAR MALAL; CMW: Tree Pathology Cooperative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; WAC: Department of Agriculture, Western Australia Plant Pathogen Collection, South Perth, Western Australia; CSM: Personal culture collection deposited in the Department of Bioagricultural Sciences & Pest Management, Colorado State University, USA. MFLUCC: Mae Fah Luang University Culture Collection, ChiangRai, Thailand. CAA: A. Alves, Universidade de Aveiro, Portugal. **Locality in Venezuela** (see map-Fig. 3): Central Region (Cojedes state) and Western Central Region (Falcon, Lara and Portuguesa states) = CR and WCR; Los Andes Region (Mérida state) = AR; North Eastern Region (Anzoátegui and Monagas states) = NER; Guayana Region (Bolívar and Delta Amacuro states) = GR; Zulia Region (Zulia state) = ZR; (-)= No sequences.

## Phylogenetic analysis

For this review, a phylogenetic analysis was carried out for those genera and species of *Botryosphaeriales* in Venezuela that were identified by partial gene sequences available in the NCBI GenBank Database (<http://www.ncbi.nlm.nih.gov>). For this analysis, the *Lasiodiplodia* genus was analyzed separately from the remaining of the genera and species of *Botryosphaeriales*

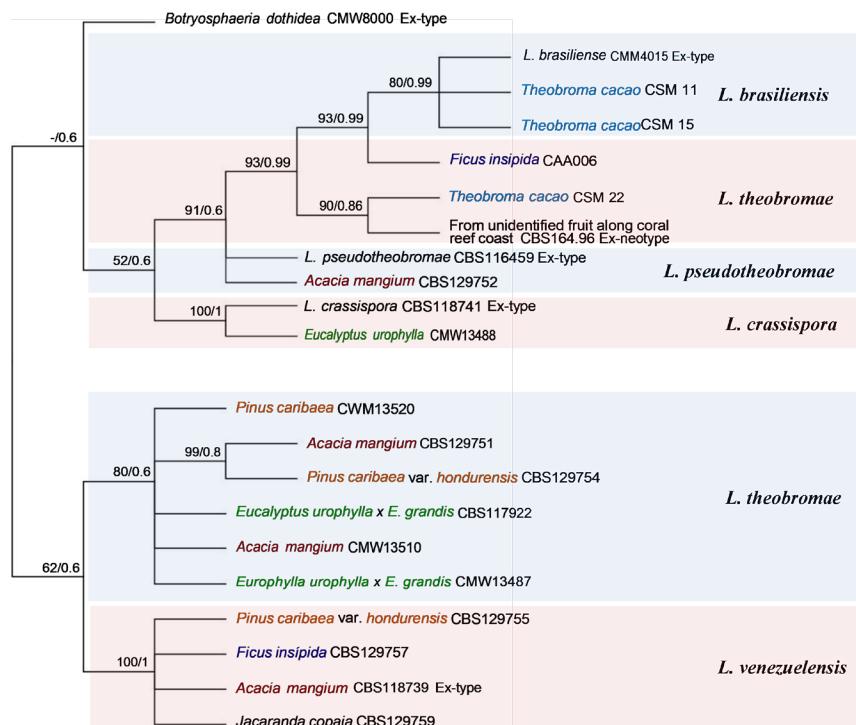
because it has the largest number of species reported for different hosts in Venezuela.

Three loci were used for the phylogenetic study: the internal transcribed spacers 1 and 2 including the intervening 5.8S nrDNA gene (ITS) (White et al. 1990), the translation elongation factor 1-alpha gene (*tef1*) (Carbone & Kohn 1999) and the beta-tubulin gene (*tub2*) (Glass & Donaldson 1995) (Table 3). The 3-loci concatenated alignment contained 1232

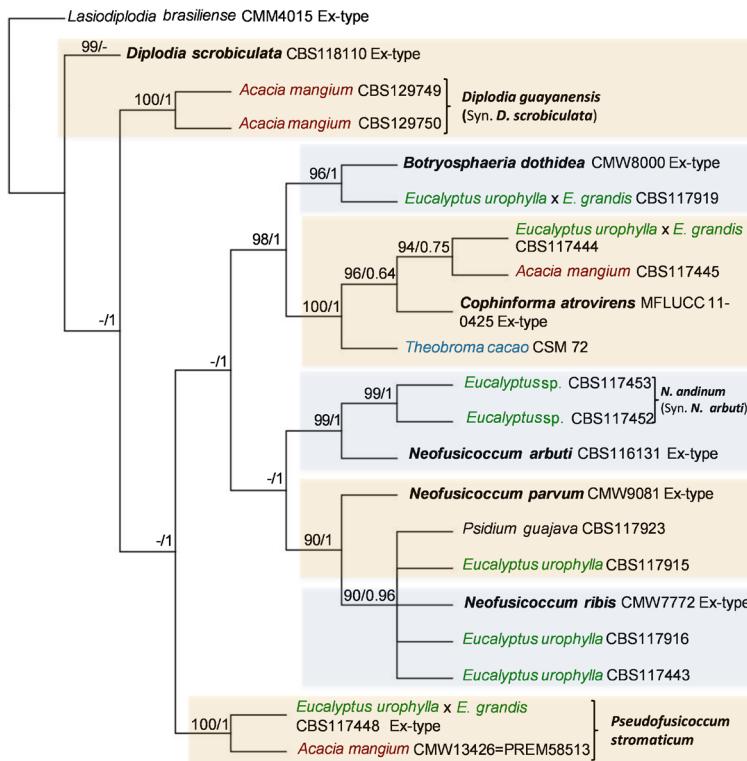
characters including gaps for the *Lasiodiplodia* group (526 from ITS, 328 from *tef1* and 378 from *tub2*) and 1295 characters including gaps for the remaining *Botryosphaerales* (532 from ITS, 333 from *tef1* and 430 from *tub2*).

Phylogenetic analyses were performed for the combined datasets using two different methods: Maximum Likelihood (ML) and Bayesian Inference (BI). A partition homogeneity test (PHT) (Farris et al. 1995, Swofford 2003) was conducted to determine whether the datasets for the three gene regions could be combined. The PHT performed on the concatenated dataset of three gene regions yielded a P-value = 0.01. The value P-value was significant, and datasets for multiple gene regions were combined for phylogenetic analysis. The ML phylogenies were evaluated with a bootstrapping (BS) method. ML phylogenies were performed with MEGA-X (Kumar et al. 2018), and BI

phylogenies were performed with MRBAYES v3.2.1 (Ronquist & Huelsenbeck 2003). All sequences from representative isolates were aligned using MUSCLE that along with BI phylogenies were used in association with the Geneious Prime software version 2020.1.2. The best-fit nucleotide substitution models for the combined datasets (ITS, *tef1*, and *btub*) were identified separately for ML and BI. For BI analyses, the best-fit nucleotide substitution models were determined with jModeltest 2.1.10 (Darriba et al. 2012) using the Akaike Information Criterion (AIC) and for ML were determined with MEGA-X (Kumar et al. 2018), with HKY+G substitution model used as the best model for both. Phylogenetic species were determined with ML  $\geq 50\%$ , and BI  $\geq 0.6$  for the *Lasiodiplodia* group (Figure 1), and ML  $\geq 90\%$  and BI  $\geq 0.90$  for the remaining of *Botryosphaerales* (Figure 2).



**Figure 1** Phylogenetic tree of *Lasiodiplodia* genus in Venezuela results from Bayesian analysis (BI) of the combined ITS, *tef1*, and *tub2* sequence alignment. Maximum likelihood (ML) bootstrap support values (ML  $\geq 50\%$ ) and Bayesian posterior probabilities (BI  $\geq 0.6$ ) are shown at the nodes (ML/BI). Ex-type strains are indicated and all hosts named in the tree belong to Venezuela. The tree was rooted to *Botryosphaeria dothidea* CMW8000 Ex-type.



**Figure 2** Phylogenetic tree of *Botryosphaeriales* remaining group in Venezuela results from Bayesian analysis (BI) of the combined ITS, *tef1*, and *tub2* sequence alignment. Maximum likelihood (ML) bootstrap support values (ML  $\geq 90\%$ ) and Bayesian posterior probabilities (BI  $\geq 0.90$ ) are shown at the nodes (ML/BI). Ex-type strains are indicated and all hosts named in the tree belong to Venezuela. The tree was rooted to *Lasiodiplodia brasiliense* CMM4015 Ex-type.

## Taxonomy, diversity, and distribution of new genus, new species and reports found in Venezuela and other regions of the world

The taxonomy of a new genus, new species, and reports of *Botryosphaeriales* identified by DNA sequences and their hosts in Venezuela are discussed below (Table 3; Figures 1, 2 and 3). *Copriniforma atrovirens* (Mehl & Slippers) A. Alves & A.J.L. Phillips (Basionym: *Fusicoccum atrovirens* Mehl & Slippers) was isolated from stems and branches of *A. mangium*, *Eucalyptus urophylla*-hybrids, *E. urophylla* x *E. grandis* and reported for the first time in Cojedes (CR) and Portuguesa (WCR) states, (Mohali et al. 2007), and from fruits and trees of *Theobroma cacao* L., in Merida state (AR) (Mohali et al. 2023), Venezuela. Initially,

Mohali et al. (2007) reported this fungus as *Botryosphaeria mamane* D.E. Gardner (asexual morph *Copriniforma mamane* (D.E. Gardner) A.J.L. Phillips & A. Alves), but Phillips et al. (2013) found that ITS sequences of the Venezuelan isolates of *C. mamane* are the same as the ITS sequence of *C. atrovirens*, therefore they consider the Venezuelan isolates to represent *C. atrovirens*.

In other regions of the world *C. atrovirens* was isolated from asymptomatic branches and twigs of *Pterocarpus angolensis*, in South Africa (Mehl et al. 2011); dead branch of *Eucalyptus* sp., in Thailand (Liu et al. 2012) as *Copriniforma eucalypti* Doilom, J.K. Liu & K.D. Hyde; it was also isolated from *Dimocarpus longan* Lour., but produced lesions on inoculated seedlings

## Political-administrative regions of Venezuela

### Regions (R) States

Los Andes	Mérida, Táchira, Trujillo, Barinas
Capital	Miranda, Vargas, Capital District
Central	Aragua, Carabobo, Cojedes
Western Central	Falcón, Lara, Portuguesa, Yaracuy
Guayana	Amazonas, Bolívar, Delta Amacuro
Island	Nueva Esparta
Los Llanos	Apure, Guárico
North Eastern	Anzoátegui, Monagas, Sucre
Zuliana	Zulia



**Figure 3** Localities or states in Venezuela where *Botryosphaerales* has been reported using sequences data (bold) and morphological methods (dark red).

of *Eucalyptus* sp., in China (Li et al. 2018); and stem rot and dieback on Cashew tree (*Anacardium occidentale*) in Brazil (Cardoso et al. 2019).

*Diplodia scrobiculata* J. de Wet, Slippers & M.J. Wingf., (syn. *Diplodia guayanensis* F. Castro-Medina, J.R. Úrbez-Torres, S.R. Mohali & W.D. Gubler sp. nov., MycoBank 812480) was isolated from the trunk of *A. mangium* in plantations of Monagas state, North Eastern Region (NER), Venezuela (Úrbez-Torres et al. 2016). *Diplodia guayanensis* was distinguished from *D. scrobiculata* by its larger conidia (Úrbez-Torres et al. 2016). Later, combining two (ITS and *tef1*) (Linaldeddu et al. 2016) and three loci (ITS, *tef1* and *btub*) (Zhang et al. 2021) of *D. scrobiculata* for phylogenetic analysis both concluded that *D. guayanensis* is indistinguishable from *D. scrobiculata*, and considered it to be a synonym for *D. scrobiculata*, and this was further supported on the basis that Úrbez-Torres et al. (2016) used older sequences for *D. scrobiculata* in their phylogenetic analyses (Linaldeddu et al. 2016). Zhang et al. (2021) used the old sequences and obtained the same results as Linaldeddu et al. (2016). Furthermore, morphological variability is common in these fungi (Phillips et al. 2013, Linaldeddu et al. 2016); however,

distinctive RFLP patterns were obtained for *D. guayanensis* compared against their closely related species *D. scrobiculata* and *D. sapinea* (Fr.) Fuckel (A and B) using *CfoI* restriction fragments in *tef1* PCR products (Úrbez-Torres et al. 2016). The PCR-RFLP fingerprinting profiles have been useful in this study to distinguish *Botryosphaerales*, although overlapping RFLP patterns may be observed between some species using one, two, or more RE (Slippers et al. 2004c).

*Diplodia scrobiculata* was isolated and identified for the first time from needles of *Pinus banksiana* Lamb., *P. resinosa* Aiton, *P. greggii* Engelm. ex Parl., in USA (Wisconsin, Minnesota, California), Mexico, and Europe (France, Italy) (de Wet et al. 2003); wilted twigs, branch dieback, necrosis and stem cankers on *Pinus halepensis* Mill., trees, in Tunisia (Hlaiem et al. 2019); symptomless and die-back on *Pinus patula* Schiede ex Schltdl. & Cham., in South Africa (Jamiet et al. 2017); asymptomatic trees in *Pinus radiata* D. Don plantations but producing lesions on inoculated *P. radiata* seedlings in Spain (Manzanos et al. 2017); *Pinus* sp., in Canada (Burgess et al. 2004); and dieback on Coast redwood (*Sequoia sempervirens* (Lamb. ex. D. Don) Endl.) in California, USA (Lee et al. 2022).

*Lasiodiplodia brasiliensis* M.S.B. Netto, M.W. Marques & A.J.L. Phillips was isolated for the first time in Venezuela from *T. cacao* plantations in the state of Merida (AR), Venezuela (Mohali et al. 2023), although Zhang et al. (2021), reported to *L. brasiliensis* on *P. caribaea* var. *hondurensis*, *F. insipida* and *J. copaia* wood in Venezuela, these authors taken

by mistake these sequences from GenBank that belong to *L. theobromae* from Venezuela (see Table S1 of these authors).

*Lasiodiplodia brasiliensis* was identified and reported for the first time in Brazil on stems of Mango (*Mangifera indica* L.) and fruits of *Carica papaya* L. (Netto et al. 2014) and other hosts in Brazil; saprobic on dead branch of teak (*Tectona grandis* L.f.), in Thailand (Doilom et al. 2015); Mango dieback, in Peru (Rodriguez-Galvez et al. 2017); *Adansonia madagascariensis* Baill., in Madagascar (Cruywagen et al. 2017); *Eucalyptus* sp., in China (Li et al. 2018); as endophytic fungus isolated from healthy, brown, and ligaloae tissue of evergreen trees (*Aquilaria crassna* Pierre ex Lecomte), in Laos (Wang et al. 2019); symptoms of gummosis, stem cankers, and dieback on Persian lime (*Citrus latifolia* Tan.), in Mexico (Bautista-Cruz et al. 2019); *Gossypium hirsutum* L., in Australia (Tan et al. 2019); leaf blight of *Sansevieria trifasciata* Prain (mother-in-law's tongue or snake plant), ornamental plant, in Malaysia (Kee et al. 2019); dieback and corky bark on longan trees (*Dimocarpus longan* L.), in Puerto Rico (Serrato-Diaz et al. 2020); branch dieback, *T. cacao*, Cameroon and *Psychotria tutcheri* Dunn fruits, in China (Zhang et al. 2021).

Cruywagen et al. (2017) and Farr & Rossman (Fungal Databases-June 13, 2023) mistakenly cited to *L. brasiliensis* as the causing of dieback in strawberries (*Fragaria x ananassa* Duchesne), in Turkey, but the pathogen reported was *L. theobromae* (Yildiz et al. 2014).

*Lasiodiplodia crassispore* Burgess, Barber sp. nov., was isolated for the first time from the wood of living *E. urophylla* in Acarigua, Portuguesa State (WCR), Venezuela and canker of *Santalum album* L., (sandalwood) in Western Australia, Australia (Burgess et al. 2006a). The sandalwood is native to southern India, eastern Indonesia, and northern Australia ([https://en.wikipedia.org/wiki/Santalum\\_album](https://en.wikipedia.org/wiki/Santalum_album)), therefore, *L. crassispore* found in

central-western Venezuela (WCR) could have been introduced through imported eucalyptus seeds used for the plantations in Venezuela.

*Lasiodiplodia crassispore* was associated with the internal wood decay symptoms observed in the cordon samples on the grapevine (*Vitis vinifera* L.), in South Africa (van Niekerk et al. 2010); *E. urophylla*, in Uruguay (Perez et al. 2010); perennial cankers in the vascular tissue of grapevines, in California, USA (Úrbez-Torres et al. 2010); endophytic in *Corymbia* sp. Hook, and minor lesions in inoculations on 4-month-old baobab seedlings (*Adansonia gregorii* F.Muell.), in Australia (Sakalidis et al. 2011b); dieback and stem-end rot of mango, fresh fruit of table grape (*Vitis* spp.), and causing dieback on *Annonaceae* in Brazil (Marques et al. 2013a, Correia et al. 2015, Machado et al. 2019); dieback symptoms from trunks and branches on grapevines in Sonora and Baja California, Mexico (Rangel-Montoya et al. 2021). *Lasiodiplodia crassispore* (syn. *Lasiodiplodia pyriformis* F.J.J. van der Walt, Slippers & G.J. Marais) isolated from the leading edges of lesions on branches of *Acacia mellifera* (M. Vahl) Benth., in Namibia (Slippers et al. 2014, Zhang et al. 2021).

*Lasiodiplodia pseudotheobromae* A.J.L. Phillips, A. Alves & Crous was reported for the first time in Uverito plantations, Monagas State (NER), Venezuela causing canker and wood stain symptoms in trunks and stems in *A. mangium* (Castro-Medina et al. 2014).

*Lasiodiplodia pseudotheobromae* was identified for the first time from *Gmelina arborea* Roxb., (Melina) and *A. mangium* in Costa Rica, *Rosa* sp., in the Netherlands, *Coffea* sp., in Zaire and *Citrus aurantium* L., Suriname (Alves et al. 2008); isolated from trees apparently healthy or showing canker and dieback symptoms of *Acacia confuse* Merr., *Albizia falcataria* (L.) Fosberg, *Eucalyptus* sp., *Mangifera sylvatica* Roxb., and *Paulownia fortunei* (Seem.) Hemsl., in China (Zhao et al. 2010); dieback on

blackthorn (*Acacia mellifera* (M.Vahl) Benth.), in Namibia (Slippers et al. 2014); *Adansonia digitata* L., in Mozambique and South Africa (Cruywagen et al. 2017); Cashew gummosis (*Anacardium humile* A.St.-Hil.), in Brazil (Netto et al. 2017); *Annona muricata* L., in Australia (Tan et al. 2019); *Bouea burmanica* Griff., *Hevea brasiliensis* (Willd. ex A. Juss.) Müll.Arg., *Persea americana* Mill., *Coffea arabica* L., *Mangifera indica*, *Ficus racemosa* L., *Syzygium samarangense* (Blume) Merr. & L.M.Perry, *Dimocarpus longan* Lour., in Thailand (Trakunyingcharoen et al. 2015); stem cankers, gummosis, and branches dieback *Citrus latifolia* Tan., in Mexico (Bautista-Cruz et al. 2019); trunk cankers, *Citrus reticulata* Blanco, in Pakistan (Ahmed et al. 2020); symptoms of branch dieback, cankers and fruit rot in *Citrus* sp., in Iran (Abdollahzadeh et al. 2010); dieback, Mango, in Egypt, Peru and South Korea (Ismail et al. 2012, Kwon et al. 2017, Rodriguez-Galvez et al. 2017); stem canker on the native Uruguayan tree, *Myrcianthes pungens* (O.Berg) D. Legrand and pathogenic in inoculated 4 month-old *Eucalyptus grandis* seedlings, in Uruguay (Perez et al. 2010); dieback and fruit rot on Rambutan trees (*Nephelium lappaceum* L.), in Puerto Rico (Serrato-Diaz et al. 2020); symptoms of branch dieback and cankers, and shoot and panicle blight in pistachio (*Pistacia* sp. and *Pistacia vera* L.), in Spain (Lopez-Moral et al. 2020); shoot-dieback, gummosis, and sunken necrotic bark lesions in young nectarine (*Prunus persica*) trees, in Turkey (Endes et al. 2016); *Rosa* sp., in Netherlands (Alves. et al. 2008); leaf blight of *Sansevieria trifasciata*, in Malaysia (Kee et al. 2019); die-back disease on *Schizolobium parahyba* (Vell.) S. F. Blake var. *amazonicum* (Ducke) Barneby trees, in Ecuador (Mehl et al. 2014); trunk Diseases in *Vitis vinifera*, in Tunisia (Rezgui et al. 2018); and post flowering stalk rot of maize (*Zea mays* L.), in India (Swamy et al. 2020).

***Lasiodiplodia theobromae*** (Pat.) Griffon & Maubl., is a cosmopolitan fungus occurring

predominantly throughout tropical and subtropical regions (Punithalingam 1980, Burgess et al. 2006a). It has also been known as a human pathogen causing keratomycosis and phaeohyphomycosis (Summerbell et al. 2004), and as a plant pathogen associated with about 500 plant hosts causing numerous diseases, including dieback, root rot, fruit rots, leaf spot and cankers of many others (Punithalingam 1980), and it also occurs as an endophyte (Punithalingam 1980).

***Lasiodiplodia theobromae*** has been reported in Venezuela on *A. mangium*, and *E. urophylla*, in Portuguesa State (WCR) (Mohali et al. 2006); *P. caribaea* var. *hondurensis*, *E. urophylla* x *E. grandis*, and *A. mangium*, in Cojedes (CR), Falcon and Portuguesa States (WCR) (Mohali et al. 2007); *Pinus caribaea* and *A. mangium*, in Monagas State (NER) (Úrbez-Torres et al. 2016); *Ficus insipida*, logs yard located within the natural forest of the Imataca Forest Reserve, between the Bolivar and Delta Amacuro States (GR) (Mohali et al. 2017); *Theobroma cacao*, in Merida State (AR) (Mohali et al. 2023).

In Venezuela, regarding the population structure of *L. theobromae* isolated from forest tree plantations was of a high gene flow between populations and a lack of population differentiation from the three host types considered, *A. mangium* and *Eucalyptus urophylla*, in Cojedes and Portuguesa State, and *P. caribaea* var. *hondurensis* in Falcon State, therefore the reproduction was predominantly clonal, and all three Venezuelan populations were pooled (Mohali et al. 2005).

***Lasiodiplodia venezuelensis*** Burgess, Barber, Mohali, sp. nov., MB500237 was isolated and described for the first time from the wood of living *Acacia mangium* Willd., in Acarigua, Portuguesa State (WCR), Venezuela. Later, was found causing blue stain on *Pinus caribaea* Morelet var. *hondurensis* (Sénécl.) W. H. Barrett & Golfari wood and light-brown cankers with a black exudate on *A. mangium* in Monagas State (NER), and blue stain on

*Ficus insipida* Willd., wood, Imataca Forest Reserve (natural forests), between the Bolívar and Delta Amacuro States (GR) (Burgess et al. 2006a, Úrbez-Torres et al. 2016, Mohali et al. 2017). To date, *L. venezuelensis* has only been reported in Venezuela, and found in the natural forest causing blue stain wood of *F. insipida*, and as a pathogen in *A. mangium* plantations. *L. venezuelensis* could be an endemic native fungus causing blue stain in light wood species native to Venezuela as is the case of *F. insipida* and moving onto an exotic species as a pathogen in *A. mangium* plantations.

***Neofusicoccum arbuti*** (D.F. Farr & M. Elliott) Crous, Slippers & A.J.L. Phillips (syn. ***Neofusicoccum andinum*** (Mohali, Slippers & M.J. Wingf.) Mohali, Slippers & M.J. Wingf. comb. nov. MycoBank MB500871. Basionym: *Fusicoccum andinum* Mohali, Slippers & M.J. Wingf.) (Crous et al. 2006, Mohali et al. 2006, Phillips et al. 2013), was isolated from asymptomatic branches of mature *Eucalyptus* sp., trees in Mucuchies (3140 m), Cordillera Los Andes mountains (AR), Venezuela (Mohali et al. 2006).

Li et al. (2018, 2020) using combination of ITS, *tef1*, *tub2*, and *rpb2* regions, with maximum parsimony (MP)/maximum likelihood (ML) tests analyses, they could separate cryptic species but between *N. andinum* and *N. arbuti* they obtained almost 100% similarity [2018 (100/99%) and (96/97%); 2020 (99/100%) and (99/97%)]. Later, Zhang et al. (2021), evaluated the species in *Botryosphaeriales*, and performed Bayesian analysis of the combined ITS, *tef1*, *tub2* and *rpb2* sequence alignment to obtain a new phylogenetic tree of *Neofusicoccum* spp. They found that the ex-type culture of *N. arbuti* had nucleotide similarities with the sequences of the ex-type of *N. andinum* [(ITS: 466/471 (98.94 %), *rpb2*: 536/537 (99.81 %), *tef1*: 240/241 (99.59 %)] and *tub2*: 376/376 (100 %), respectively], therefore *N. andinum* was reduced to synonymy with *N. arbuti*. Mohali et al. (2006) did not include the *N. arbuti* sequences in the phylogenetic tree because these were not available at that time (Zhang et al.

2021). *Neofusicoccum arbuti* was isolated from cankers of *Arbutus menziesii* Pursh (Pacific madrone), in Washington and California, USA, and Canada (Farr et al. 2005), and stem canker and dieback of *Vaccinium* spp. (Blueberry), in Chile (Espinoza et al. 2009).

***Neofusicoccum parvum*** (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips and ***Neofusicoccum ribis*** (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips both were isolated on *E. urophylla* S.T. Blake, and ***Botryosphaeria dothidea*** (Moug. ex Fr.) Ces. & De Not., was isolated on *E. urophylla* x *E. grandis* W. Hill ex Maiden hybrids all from asymptomatic plant tissue, as well as trees exhibiting blue stain and die-back and from entirely dead trees in Portuguesa State, and was isolated *N. parvum* on *Psidium guajava* L., in Zulia State (ZR) (Mohali et al. 2007).

An inoculation trial was conducted on *E. urophylla* x *E. grandis* hybrid stems in Portuguesa State with the fungi *B. dothidea*, *N. parvum* and *N. ribis*, and after 7 weeks lesions development was recorded. *Botryosphaeria dothidea* produced very small lesions in comparison to *N. ribis* and *N. parvum* which produced significantly larger lesions, bark swelling around the inoculation points and in some cases, the bark was cracked producing black kino exudation when the outer bark was removed from the points of inoculation (Mohali et al. 2009).

Information on the wide geographic distribution and host range of *L. theobromae*, *N. parvum*, *N. ribis* and *B. dothidea* can be found in Fungal Database (<https://nt.ars-grin.gov/fungaldatabases/>) and Mycobank Database (<https://www.mycobank.org/>).

***Pseudofusicoccum*** Mohali, Slippers & M.J. Wingf. gen. nov. MycoBank MB500884; ***Pseudofusicoccum stromaticum*** (Mohali, Slippers & M.J. Wingf.) Mohali, Slippers & M.J. Wingf., comb. nov. MycoBank MB500885, Basionym: *Fusicoccum stromaticum* Mohali, Slippers & M.J. Wingf., (Crous et al. 2006, Mohali et al. 2006, Phillips et al. 2013), was isolated from branches of *Eucalyptus urophylla*

S. T. Blake and *E. urophylla* x *E. grandis* W. Mill ex Maiden-hybrids, and from branches and stems of *Acacia mangium* Willd., in Western Central Region (WCR) of Venezuela (Mohali et al. 2006).

Crous et al. (2006) introduced to *Pseudofusicoccum* genus for species that are morphologically similar to *Fusicoccum* and *Neofusicoccum* but phylogenetically distinct from both of these genera. The *Pseudofusicoccum* genus resembles the species of *Fusicoccum* but is distinct in having conidia encased in a persistent mucous sheath, and conidia are also more cylindrical than in *Fusicoccum* species (Crous et al. 2006). Yang et al. (2017) using robust backbone phylogeny for *Botryosphaerales* (LSU and rpb2 genes) described and raised this genus as a new family, *Pseudofusicoccumaceae* Tao Yang & Crous where morphologically the family, is typified by *Pseudofusicoccum*.

In Venezuela, inoculations with *P. stromaticum* were made on 2-year-old trees in plantations of *E. urophylla* x *E. grandis* hybrid clones. Seven weeks after inoculation produced small lesions on the stems, but at the same time it was observed that the inoculation points had started to heal and produce callus by the end of the trial (Mohali et al. 2009).

*Pseudofusicoccum stromaticum* has been widely reported in Brazil causing diseases in different hosts such as: dieback on mango (*Mangifera indica* L.) stems, pathogenic on 5-month-old mango seedlings, and producing the small lesions on inoculated mango fruits (Marques et al. 2012, Marques et al. 2013b); dieback, wilting of branches, discolouration of the vascular system, decline and subsequent death of Malay apple (*Syzygium malaccense* L.) trees (Silveira et al. 2017); associated with gummosis on native cashew (*Anacardium othonianum* Rizzin) (Netto et al. 2017); dieback and stem and branch cankers the on cashew (*Anacardium occidentale* L.), guava (*Psidium guajava* L.) and caja-umbu (*Spondias mombin* L. x *S. tuberosa* Arruda) trees (Coutinho et al. 2018); as endophyte in *Myracrodruon*

*urundeuva* Fr. All. (*Anacardiaceae*) (Sobreira et al. 2018), and dieback of the *Annonaceae* (Machado et al. 2019). In Uruguay, *P. stromaticum* was associated with cankers showing gummosis in peach shoots and showed moderate virulence on both inoculated apple and peach shoots (Sessa et al. 2021).

In addition to *P. stromaticum*, eight species have subsequently been added to the genus, such as *Pseudofusicoccum adansoniae* Pavlic, T. I. Burgess, M. J. Wingf., on *Adansonia gibbosa* (A. Cunn.) Guymer ex D. A. Baum, *Acacia synchronicia* Maslin, *Eucalyptus* L'Hér., and *Ficus opposite* Miq., in Australia and, *Ficus krishnae* L. and *Jatropha podagraria* Hook, in India (Sharma et al. 2013, Prasher & Dhanda 2017); *P. africanum* Marinc., Jami & M.J. Wingf., on twigs of *Mimusops caffra* E. Mey. ex A. DC. (coastal red milkwood), in Eastern Cape Province, Haga Haga, South Africa (Jami et al. 2018); *P. ardesiacum* Pavlic, T.I. Burgess, M.J. Wingf., on *A. gibbosa* and *Eucalyptus* spp., in Australia; *P. artocarpi* T. Trakunyingcharoen, L. Lombard & Crous, on twigs of *Artocarpus heterophyllus* Lam., in Chiang Mai Province, Thailand (Trakunyingcharoen et al. 2015); *P. calophylli* Jayasiri, E.B.G. Jones & K.D. Hyde on decaying fruit pericarp of *Calophyllum inophyllum* L., in Krabi Province, Mueang Krabi District, Thailand (Jayasiri et al. 2019); *P. kimberleyense* Pavlic, T.I. Burgess, M.J. Wingf., on *Acacia synchronicia* Maslin, *Adansonia gibbosa*, *Eucalyptus* sp., and *Ficus opposite* Miq. in Australia (Pavlic et al. 2008) and *Persea americana* Mill., USA (Zhang et al. 2021); *P. olivaceum* Mehl & Slippers on asymptomatic branches and twigs of *Pterocarpus angolensis* (Kiaat), in Mpumalanga Province, Kruger National Park, Pretoriuskop, *Terminalia sericea* Burch. ex DC., and *Terminalia prunioides* M. A. Lawson, in South Africa (Mehl et al. 2011, Zhang et al. 2021); *P. violaceum* Mehl & Slippers on asymptomatic branch of *P. angolensis* Mpumalanga Province, Mawewe Nature Reserve, in South Africa (Mehl et al.

2011), and *Microcos paniculatus*, in Hong Kong, China (Zhang et al. 2021).

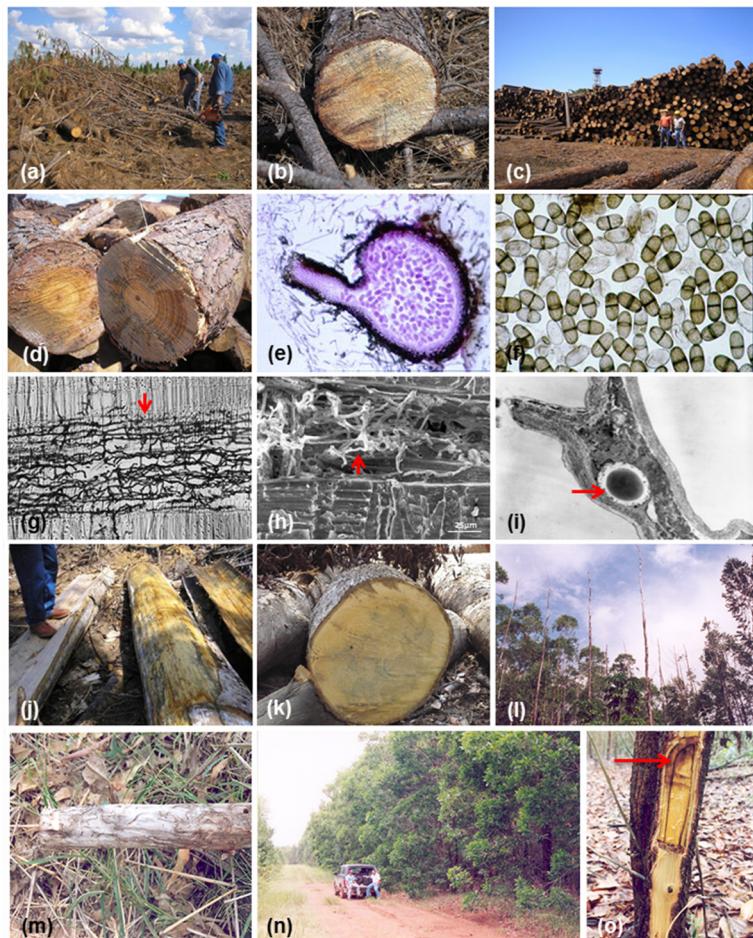
This genus is known only as the asexual morph and thus far nine species have been reported (Zhang et al. 2021). To date, *P. stromaticum* has been reported exclusively from South America while the remaining of the *Pseudofusicoccum* species have been reported from other regions, such as South Africa, Australia, Thailand, China, USA, and India (Sharma et al. 2013, Prasher & Dhanda 2017, Zhang et al. 2021).

### Symptoms associated with species from *Botryosphaerales* in Venezuela

*Botryosphaerales* species infect plants via wounds or through natural plant openings, such as buds, lenticels, and stomata, resulting in diverse symptoms, such as twig, branch, and main stem cankers; die-back of leaders, shoots, or whole branches; seed capsule abortion; collar rot; damping off or blight of seedlings; root cankers; blue-stain; decline; and death of whole trees in severe cases (Slippers & Wingfield 2007). The Table 1, different genera within the *Botryosphaerales* were

found and isolated from different hosts and locations in Venezuela, associated with diverse symptoms, and identified through its asexual morph, and others were identified using DNA sequence data (Figures 1 and 2; Table 3).

*Diplodia* spp. and *Lasiodiplodia* spp., have



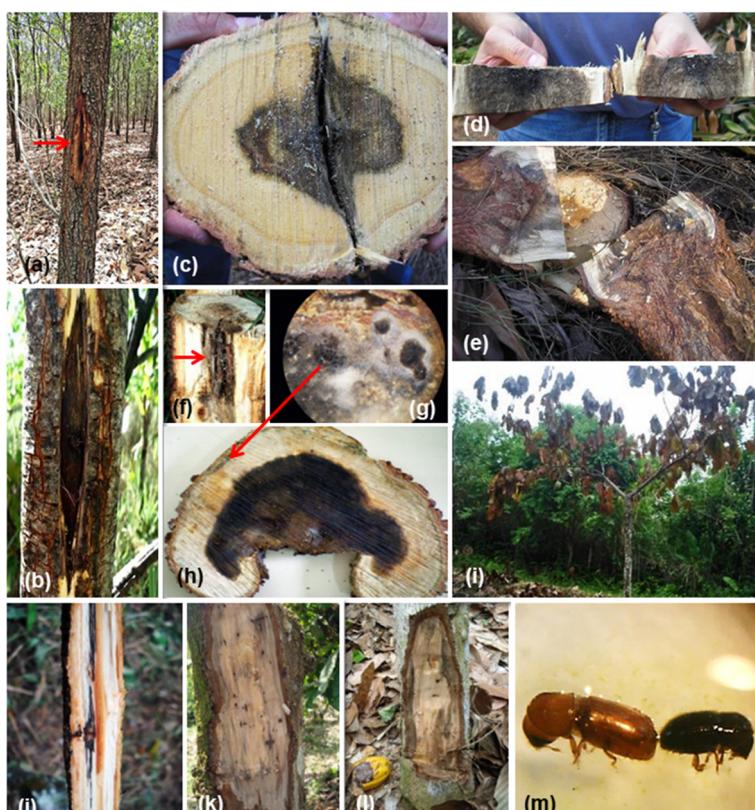
**Figure 4** (a) *Pinus caribaea* var. *hondurensis* plantations (Maderas del Orinoco C.A) located in Uverito, Monagas state, Venezuela. (b) Blue stain of the wood, observed in fallen trees inside the plantations. (c) Log yard to be processed at the sawmill in Maderas del Orinoco company. (d) Blue stain in the logs at the sawmill. (e) Pycnidium. (f) Conidia of *Lasiodiplodia* spp. (g, h) Hyphae of *Lasiodiplodia* spp. invading the medullary rays of *Pinus caribaea* var. *hondurensis* (red arrows), g=80X and h=Scanning Electron Microscopy. (i) Hypha found between tracheid or intercellular space of the wood of Caribbean pine (red arrow), Transmission Electron Microscope (19000X). (j) Blue stain in lumber of *Ficus insipida*; (k) Cross section *Ficus insipida* lumber with blue stain; (l) *Eucalyptus urophylla* trees exhibiting dieback or entirely dead trees (sudden death) in Portuguesa state. (m) *Eucalyptus urophylla* tree dead with blue stain. (n) *Acacia mangium* plantations in Portuguesa State. (o) Discoloration (red arrow) on *Acacia mangium* trees in Portuguesa State. Pictures e, f (Cedeño et al. 1995); g (Mohali 1993); h, i (Cedeño et al. 1996); j, k (Mohali et al. 2017).

been reported to cause different symptoms, such as blue stain (synonymous sap stain), which is a result of melanin, a pigment produced by the fungal pathogen (Zink & Fengel 1989). The blue colour of the wood develops as an optical effect due to refraction of light (Mohali 1993), such as observed in the following examples: *Lasiodiplodia theobromae*, *L. venezuelensis* and *Diplodia mutila* (Fr.) Mont. on *Pinus caribaea* var. *hondurensis* (Figures 4 a-i); *L. theobromae* and *L. venezuelensis* on *Ficus insípida* (Figures 4 j,k). The discolourations in the wood of living trees/woody plants or dead logs are the result of diverse biotic and abiotic causes (Bauch 1984, Kreber & Byrne 1994).

Wood discolouration and decay are often the result of wounding, such as those caused by animal chewing, branch breaking, pruning, mechanized wood harvest, construction injury, motor traffic, etc. (Tattar 1978), and insects. Further discolourations can result from tree-produced substances, such as deposition of heartwood substances developed by living tree cells, later microbial stains, and finally coloured derivatives of wood decay processes (Bauch et al. 1988), examples of tree/wood discolourations include the following: sudden death or die-back in *E. urophylla* and *Eucalyptus* hybrid of Portuguesa State caused by *Lasiodiplodia crassispora*, *L. theobromae*, *Neofusicoccum parvum*, *N. ribis*, *Botryosphaeria*

*dothidea*, *Pseudofusicoccum stromaticum*, and *Copriniforma atrovirens* (Figures 4 l, m); discolourations on *Acacia mangium* in Cojedes and Portuguesa States caused by *Lasiodiplodia theobromae*, *L. venezuelensis*, *Copriniforma atrovirens*, and *Pseudofusicoccum stromaticum* (Figures 4 n,o).

Discolourations and canker in the stem of *A. mangium* caused by *Lasiodiplodia pseudotheobromae*, *L. theobromae*, *L. venezuelensis*, and *Diplodia scrobiculata* (syn. *D. guayanensis*) in plantations of Maderas del Orinoco Company (Figures 5a-h). *Copriniforma atrovirens*

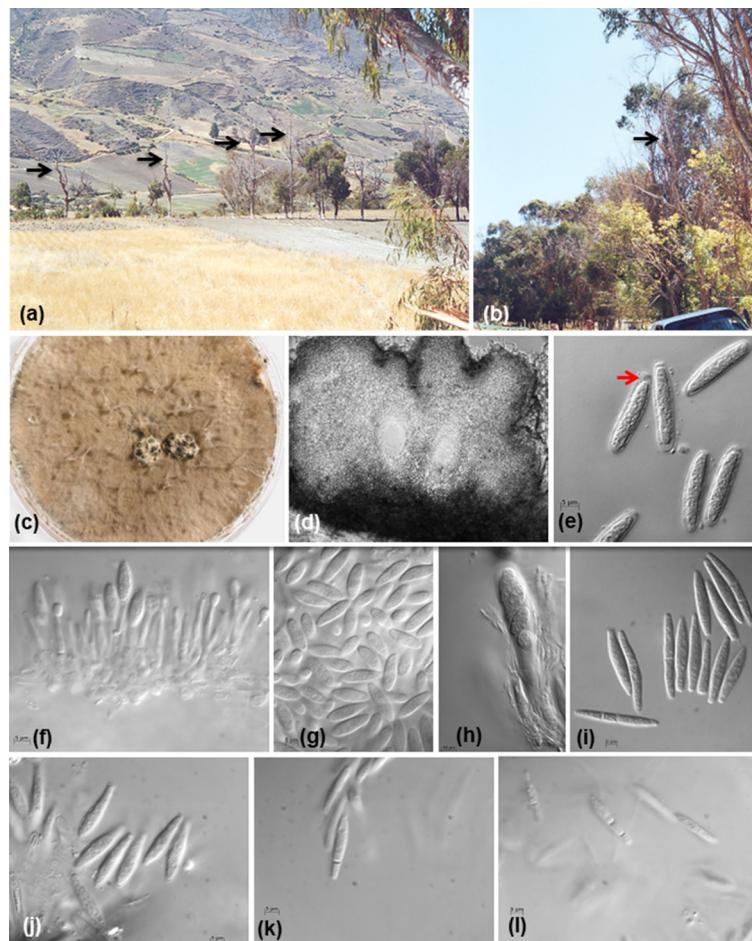


**Figure 5** (a, b) *Acacia mangium* tree with canker (red arrow) in Maderas del Orinoco plantations. (c, d) Discoloration in the *A. mangium* stem. (e) Discoloration from *A. mangium* tree base. (f) Termites attacking at the inoculation point and black exudation was observed when the outer bark was removed from inoculation points (red arrows). (g) *Lasiodiplodia* spp. pycnidia growing between the vascular cambium and the bark on wooden disc of *A. mangium* with canker (h) (red arrow). (i) Dieback or sudden death symptoms in *Theobroma cacao* tree. (j) Discoloration in branch; (k, l) Discolorations in stems of *T. cacao* with dieback or sudden death symptoms in Merida State. (m) Bark beetle (Scolytinae) collected from cacao tree stem with discolouration.

was isolated from *T. cacao* fruits with anthracnose and together with *Lasiodiplodia theobromae*, and *L. brasiliensis* were found in association with dieback or sudden death symptoms on *T. cacao* trees (Mohali et al. 2023) in Merida State (Figure 5i), producing discolourations in branches (Figure 5j) and stems (Figures 5k, l). These discolourations were mainly associated with wounds caused by bark beetles- *Scolytinae* (Figure 5 m).

Stems, branches, and roots with cankers and dieback on *P. caribaea* var. *hondurensis* trees in plantations from 4-to15-years old and in nurseries on 8-month-old seedlings in displaying completely browned needles were observed at the Maderas del Orinoco Company, and the main fungal pathogen reported as causing these diseases was *Sphaeropsis sapinea* (Fr.) Dyko & B. Sutton (Cedeño et al. 2001). Cedeño et al. (2001) based their identification on the asexual morph, conidia 39,8 (37-45) x 12,7 (11-16)  $\mu\text{m}$ , one septum and rarely two or three septa; measurements close to *Diplodia sapinea* (25.5-) 30.5-52.5 (-54) x (10-) 12.5-20 (-21)  $\mu\text{m}$ , *D. scrobiculata* (37.5-) 39.5 (-41.5) x (13-) 14 (-15.5)  $\mu\text{m}$ , and *D. scrobiculata* (syn. *D. guayanensis*) (33.5-) 40.6-42.4 (56) x (12-) 15.8-16.7 (-18.5)  $\mu\text{m}$  (Úrbez-Torres et

al. 2016). The absence of septa (aseptate) in mature conidia of *Sphaeropsis* separates it from the *Diplodia* genus, which is characterized by septate conidia (Table 2, Phillips et al. 2013) therefore, the diseases observed by Cedeño et al. (2001) in the nurseries and plantations of



**Figure 6** *Eucalyptus* sp., at the Cordillera Los Andes Mountains, Merida state, Venezuela at an altitude of approx. 3140 meters above sea level. The black arrows show old *Eucalyptus* trees without apparent damage. (c) *Pseudofusicoccum stromaticum* producing big conidioma on 2 % Malt Extract Agar. (d) Multilocular conidiomata of *P. stromaticum* without ostioles and embedded locule. (e) *Pseudofusicoccum stromaticum* conidia encased in a persistent mucous sheath (red arrow). (f) *Neofusicoccum ribis/parvum* complex conidiogenous cells. (g) *Neofusicoccum ribis/parvum* complex conidia. (h) *Copriniforma atrovirens*, ascii bitunicate with ascospores aseptate, hyaline, with granular textured contents. (i) *Copriniforma atrovirens* conidia with one and two septa. (j-l) *Botryosphaeria dothidea* conidia with 0-2 septa. Pictures c-e (Mohali et al. 2006); f, h, i (Mohali et al. 2007).

*P. caribaea* var. *hondurensis* could have been caused by a fungal species in the *Diplodia* genus.

*Neofusicoccum arbuti* (syn. *N. andinum*) was collected from asymptomatic branches of mature *Eucalyptus* sp. trees growing in the Cordillera Los Andes Mountains of Venezuela at an altitude of ca. 3000 m (Figures 6a, b). Photographs of other *Botryosphaerales* genera: *Pseudofusicoccum stromaticum* (Figures 6c-e); *Neofusicoccum ribis*/*Neofusicoccum parvum* (Figures 6f, g), *Copriniforma atrovirens* (Figures 6h, i), and *B. dothidea* (Figures 6j-l).

Pathogenicity tests were carried out in the field, which gave us information about the susceptibility or tolerance to diseases, such as in the case of *Eucalyptus* spp., a forest species introduced in Venezuela to obtain fibres for cardboard production. Inoculations with different genera and species of *Botryosphaerales* in commercial plantations of *Eucalyptus* at the company Smurfit Kappa Reforestadora Dos, Portuguesa State was done on different commercial clones of *Eucalyptus*-hybrids (*E. urophylla* x *E. grandis*). Clones tolerant to infection with all inoculated species of *Botryosphaeriaceae* were observed (Mohali et al. 2009). Also, other tests were carried out in commercial plantations at the company Maderas del Orinoco to investigate the status of *Botryosphaerales* associated with decline symptoms observed in *A. mangium* and *P. caribaea* var. *hondurensis*. Three *Lasiodiplodia* spp. and one *Diplodia* sp., were inoculated in *A. mangium*, and two *Lasiodiplodia* spp., on *P. caribaea* var. *hondurensis*, showing in this study that *Lasiodiplodia* spp., and *Diplodia* sp., are highly virulent to *A. mangium*, while the same pathogens in *P. caribaea* var. *hondurensis* did not cause any lesions (Castro-Medina et al. 2014, Úrbez-Torres et al. 2016).

## Conclusions

This is a review and update of information that represents more than 30 years of research work with species pertaining to the order

*Botryosphaerales* that cause diseases, with special reference to woody plants. The nomenclature of the different species and genera found within the *Botryosphaerales* order have been updated, including the identification of the new species of *Lasiodiplodia*, a new genus and species of *Pseudofusicoccum*, and new reports for Venezuela using molecular tools.

At the morphological level, nine genera were isolated and identified within *Botryosphaerales* order, where *Lasiodiplodia* spp. was the most abundant of all genera. This was isolated from fruit plantations such as citrus, mango, cacao, avocado, and forest tree plantations of exotic species such as pine, as well as from native forest species.

With molecular tools, it was possible to define exactly the name of the species that produce or are associated with forest diseases, especially in forest plantations of exotic species. *Lasiodiplodia theobromae* and *L. venezuelensis* both didn't cause lesions when they were inoculated in *Pinus caribaea* var. *hondurensis* trees, but they were routinely reisolated from asymptomatic wood which indicates the latent pathogen status of these species in this host, as well causing of blue stain on pine wood observed in fallen trees and in log yards at sawmills.

*Lasiodiplodia pseudotheobromae*, *L. theobromae*, *L. venezuelensis*, and *Diplodia scrobiculata* (=*D. guayanensis*) were isolated from trunks with symptoms light-brown cankers with a black exudate in *Acacia mangium* plantations. Inoculation tests carried out on this host showed bark swelling around the inoculation points and necrosis of the vascular system below the bark and black exudation, proving that these four species their high virulence on *A. mangium*.

The fungi *B. dothidea*, *C. atrovirens*, *L. theobromae*, *N. arbuti* (=*N. andinum*), *N. parvum*, *N. ribis* and *P. stromaticum* isolated from *Eucalyptus* spp., plantations, were inoculated on hybrid *Eucalyptus* trees, where *N. ribis* and *N. parvum* produced significantly

large lesions (canker) on the trunk, therefore these pathogens can be considered as new emerging diseases on these forest species introduced in the country. Moreover, *B. dothidea* produced very small lesions, while the remaining fungi did not produce any lesions when inoculated on the above-mentioned host.

Regarding natural tropical forests in Venezuela, the blue stain of the wood on *Ficus insipida* in lumber yards was caused by *L. theobromae* and *L. venezuelensis*.

Plantations of the non-native forest species, *Pinus caribaea* var. *hondurensis*, in the East of Venezuela (between the States of Anzoátegui and Monagas), began in 1961. This plantation had a planted area of approximately 600,000 ha, but currently there are 112,000 ha. Later, and on a smaller scale, a non-native species, *Acacia mangium*, was planted. These forest plantations border one of the largest natural forest reserves in South America, The Imataca Forest Reserve occupying approximately 3.7 million ha, and located between the Bolívar and Delta Amacuro States in Venezuela (Mohali et al 2017). The proximity between non-native and native species has allowed native pathogens, such as *Lasiodiplodia venezuelensis*, found so far only in Venezuela, and together with *L. theobromae*, *L. pseudotheobromae*, *D. arbuti* (=*D. guayanensis*) to be transferred to these exotic species causing blue stain on pine wood, and cankers in *A. mangium* plantations.

Batista et al. (2021) have assumed that human movement and trade were the main routes of dispersal for all species within the order *Botryosphaeraiales* with worldwide distribution across all continents, except for Antarctica, with climatic variability being the main limitations for the appearance of new stable populations. They also highlighted that the disease expression was mainly due to occasional climatic events that can affect the susceptibility of the host.

*Botryosphaeraiales* are reported as saprophytic, parasites, endophytic, and opportunistic pathogens in different crops,

natural forests, and plantations, causing significant losses to the Venezuelan economy, but these losses are not quantified. Information on diseases caused by fungi of the *Botryosphaeraiales* order and their description at the morphological level in Venezuela is very scarce, scattered, and with little information, in addition to future research in plant pathology, phylogenetic studies and fungal taxonomy, and the rest of the other areas of science that are developed in Venezuela is in great uncertainty due to current economic and political problems.

### Conflict of interest

The Author has no conflict of interest to declare.

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