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Review

A Review of *Botryosphaeriales* in Venezuela with Special Reference to Woody Plants

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Abstract: The *Botryosphaeriales* order are 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, *Botryosphaeriales* species have been identified in Venezuela using morphological descriptions in the 80's and 90's, and later, the mid-2000s using molecular techniques. The morphological descriptions of the asexual morphs were initially used for the identification of *Botryosphaeriales* genera and species. *Lasiodiplodia* spp., (as *L. theobromae*) was the most isolated fungus in Venezuela within the *Botryosphaeriales* and has been found in more than 50% of the hosts in native and non-native plants, followed by *Diplodia*, *Dothiorella*, *Fusicoccum*, *Lasiodiplodia*, *Microdiplodia*, *Macrophomina*, *Neofusicoccum*, *Sphaeropsis*, and *Botryosphaeria*, considered all of them cosmopolitan group. With molecular studies, that included DNA sequence data from multiple genes, such as the internal transcribed spacer of rDNA (ITS), translation elongation factor-1α (*tef1*), and β-tubulin (*btub*) used on the fungi isolated from woody plants, mainly trees or forest species, resulted in the presence of two families within the *Botryosphaeriales* order for Venezuela. *Botryosphaeriaceae* family with the genera: *Botryosphaeria*, *Cophinforma*, *Diplodia*, *Lasiodiplodia* and *Neofusicoccum*, and the *Pseudofusicoccumaceae* family that includes the genus *Pseudofusicoccum*. In *Botryosphaeriaceae* family was again the *Lasiodiplodia* genus the most predominant in most hosts, and the specie *L. theobromae* the most isolated in native and non-native plants; *Botryosphaeria dothidea*, *Cophinforma atrovirens*, *Diplodia scrobiculata* (syn. *Diplodia 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; while *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, 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*; Fungal Taxonomy

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1. 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 [1]. 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 [1,2]. Plantations in the tropics (planted forests of a single species) are usually of non-native species, such as the genera of *Pinus*, *Eucalyptus*, and *Acacia*, the main forest species planted in Venezuela.

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

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be introduced accidentally, or when they encounter novel pests to which they have no resistance, substantial damage or loss can ensue [3]. The longer non-native trees are planted in an area, the more threatened they become by native pests. Where the trees are of native species, they can be vulnerable to introduced 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 [4,5].

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 [6].

The *Botryosphaeriales* contains numerous fungal species that occur as saprophytes, parasites, or endophytes on a diverse range of plant hosts [7,8], as well as opportunistic pathogens of woody plants, especially when host plants are stressed [9]. Different species within of order *Botryosphaeriales* 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 [7,8]. An ecological and biological characteristic of the species in the *Botryosphaeriales* order is the lack of host specificity able to colonize and cause disease in diverse native and introduced plant hosts [7,10].

Different *Botryosphaeriales* genera can infect multiple hosts, increases 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 *Botryosphaeriales*, and that include those amongst and between native and non-native trees, we have *Botryosphaeriales* species have moved between trees in native stands of *Eucalyptus* (*Myrtaceae*) and adjacent plantations of these trees [11], between native waterberry trees (*Syzygium cordatum*; *Myrtaceae*) and related eucalypt plantations (*Myrtaceae*) [12], from *Pinus resinosa* windbreaks to pine nurseries [13], among various tree hosts in the *Casuarinaceae*, *Cupressaceae*, *Fabaceae*, *Myrtaceae*, *Proteaceae*, *Santalaceae* [14], and among native *Terminalia* spp. (*Combretaceae*) and between these trees and *Theobroma cacao* (*Malvaceae*) [15]. The epidemiology of *Botryosphaeriales* 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 [16]. In Venezuela, the *Botryosphaeriales* 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 *Botryosphaeriales* 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 [17,18]. Different species within the same genera of *Botryosphaeriales* frequently possess overlapping morphological features [19] 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 *Botryosphaeriales* genera [17,18,20–22]. The phylogenetic analyses of DNA sequence data have significantly impacted all aspects of the systematics and taxonomy of the *Botryosphaeriales*, including a redefinition of families and genera, identification of new species, cryptic species, and more recently hybrids [23]. Crous et al. [24] defined all genera in the *Botryosphaeriales* 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 *Botryosphaeriales* 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 *Botryosphaeriales* order, based on phylogenetic, morphological, and ecological differences [23]. These families are: *Aplosporellaceae* Slippers et al. 2013 [25], *Botryosphaeriaceae* Theiss. & Syd., 1918 [24], *Endomelanopsisaceae* TaoYang & Crous, 2016 [26], *Melanopsaceae* Phillips et al. 2013 [25], *Phyllostictaceae* Fr., 1849 [27], *Planistromellaceae* M.E. Barr, 1996 [28], *Pseudofusicoccumaceae* Tao Yang & Crous, 2016 [26], *Saccharataceae* Slippers et al. 2013 [25] and *Septorioideaceae* Wyka & Broders, 2016 [29], being the *Botryosphaeriaceae* family with the largest number of genera within it.

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

2. Genera and species of *Botryosphaeriales* identified with morphological descriptions in Venezuela

Few general morphological features of within *Botryosphaeriales* 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 [30–37].

Lasiodiplodia Ellis & Everh., species are well-known and widespread plant pathogens, occurring mostly in tropical and subtropical regions [38]. *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., has been widely reported and commonly occurs on different crops in Venezuela [39]. In a published list of plant diseases in Venezuela [39], *L. theobromae* was the common fungal pathogen. This list from Urtiaga [39] was updated using website records of fungi from 1998-2001 with specimens from the fungal collection of the Simon Bolivar University, Caracas-Venezuela [40], 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. [39,40]. 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 [41,42] (Table 1).

In Venezuela, at least eight genera of the *Botryosphaeriales* order within two families can be differentiated through the asexual morph. Seven genera belong to *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: *Cophinforma* 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
***Diplodia* Fr.**	*Ceiba pentandra* (L.) Gaertn-old leaves	Buena vista, Lara state	[39]
***Diplodia* sp.**	*Cassia* L.- **root**	-	[40]
***Diplodia ochromae* Pat.**	*Ochroma lagopus* Sw. -**trunk**	-	[40]
***Diplodia mutila* Fr. Apud Mont.**	*Pinus caribaea* morelet var. *hondurensis* (Barr. and Golf.)- **blue stain on wood**	Chaguaramas, Anzoátegui state	[36]
***Dothiorella* Sacc.**	*Delonix regia* (Bojer ex Hook) Raf. -**branches**	El Tocuyo, Lara state	[39]
***Dothiorella* sp.**	*Psidium guajava* L.-**fruit rot**	Merida and Zulia states	[33]
***Dothiorella dothidea* (= *Botryosphaeria dothidea*)**	*Prunus pérsica* (L.) Batsch- **brown rot of fruits**	El Arenal, Merida state	[31]

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***Fusicoccum* Corda**	*Echinodorus berteroi* (Spreng) Fassett-**leaves**	Guanare, Portuguesa state	[39]
***Lasiodiplodia theobromae* (Pat.) Griffon & Maubl.**	*Pachystachys lutea* Nees-**branches**	Barquisimeto, Lara state	[39]
L. theobromae	*Anacardium occidentale* L- **terminal branch death**	Barquisimeto, Lara state	[39]
L. theobromae	*Mangifera indica* L-**branches and seeds**	La Calzada de Páez, Barinas state	[39]
L. theobromae	*Annona reticulata* L.-**old leaves**	Barquisimeto, Lara state	[39]
L. theobromae	*Catharanthus roseus* (L.) G. Don-**Leaves**	Barquisimeto, Lara state	[39]
L. theobromae	*Crescentia cujete* L.- **branches and leaves**	Wide distribution in Venezuela	[39]
L. theobromae	*Citrullus lanatus* (Thunb.) Matsum. & Nakai-**fruits rot and branches**	La Miel, Lara state	[39]
L. theobromae	*Juniperus lucayana* Britton- **twigs**	Barquisimeto, Lara state	[39]
L. theobromae	*Curatella Americana* L.-**old leaves**	La Calzada de Páez, Barinas state	[39]
L. theobromae	*Codiaeum variegatum* (L.) Blume var. *pictum* (Lodd.) Muell	**-**	[39]
L. theobromae	*Hura crepitans* L.-**old leaves**	La Calzada de Páez, Barinas state	[39]
L. theobromae	*Manihot esculenta* Crantz-**branches**	Urachiche, Yaracuy state	[39]
Lasiodiplodia theobromae	*Duranta repens* L.-**branches**	Ureña, Táchira	[39]
L. theobromae	*Arachis hypogaea* L.-**root**	Buría Londres, Lara state	[39]
L. theobromae	*Phaseolus lunatus* L.-**branches**	Sabana de Parra, Yaracuy state	[39]
L. theobromae	*Sansevieria trifasciata* Prain.-**old leaves**	Barquisimeto, Lara state	[39]
L. theobromae	*Cedrela odorata* L.-**branches**	Chivacoa, Yaracuy state	[39]
L. theobromae	*Cecropia peltata* L.-**branches**	Chivacoa, Yaracuy	[39]
L. theobromae	*Ficus pumila* L.-**old leaves and galls on the leaves**	Barquisimeto, Lara state	[39]
L. theobromae	*Maxillaria* Ruiz & Pavon-**old leaves**	Duaca, Lara	[39]
L. theobromae	*Passiflora edulis* Sims. form *flavicarpa* Degener	El Eneal, Lara	[39]
L. theobromae	*Salix babylonica* L.- **black root rot**	Barquisimeto, Lara state	[39]
L. theobromae	*Pachystachys lutea* Nees-**branches**	Barquisimeto, Lara state	[39]
L. theobromae	*Cajanus indicus* Spreng.-**branches**	Lara state	[39]
L. theobromae	*Duranta repens* L.-**branches**	Tachira state	[39]
L. theobromae	*Theobroma cacao* L.	**-**	[40]
L. theobromae	*Vinca rosea* L.-**leaf and branch**	Lara state	[39]
L. theobromae	*Persea Americana* Mill.-**fruits**	Yaracuy state	[41]
L. theobromae	*Citrus latifolia* Tanaka-**fruits**	Yaracuy state	[41]
L. theobromae	*Citrus sinensis* (L.) Osbeck-**fruits**	Yaracuy state	[41]
Lasiodiplodia theobromae	*C. sinensis*-Lesion and Gummosis on the branches	Caño Amarillo, Tachira state	[30]
L. theobromae	*Citrus aurantiifolia*-**Lesion and Gummosis on the branches**	Caño Amarillo, Tachira state	[30]
L. theobromae	*Passiflora edulis* Sims f. *flavicarpa*-**Dieback on the branches**	South of Maracaibo Lake, Zulia and Merida states	[32]
L. theobromae	*Pinus caribaea* var. *hondurensis*-**blue stain on wood**	Uverito plantation and Uverito sawmill, Monagas state	[123]
L. theobromae	***Azadirachta indica* A. Juss-**blue stain on wood	Cojedes state	[37]

L. theobromae	*Pinus oocarpa* Schiede ex Schltdl	Merida state	[37]
L. theobromae	*Mangifera indica*-**branches dieback**	Maracay (INIA-CENIAP), Aragua state	[42]
***Microdiplodia buddleiae* Gucevicz**	*Opuntia caracasana* Salm.-**spot leaves**	Humocaro Bajo, Lara state	[39]
***Macrophomina phaseolina* (Tassi) Goidanich**	*Begonia* sp.- **spot on the leaf**	Barquisimeto, Lara state	[39]
Macrophomina phaseolina	*Calendula officinalis* L.-**stem and inflorescence**	Barinas state	[39]
M. phaseolina	*Ipomoea batata* (L.) Lam.-**stolons at the roots**	Siquisique, Lara state	[39]
M. phaseolina	*Phaseolus vulgaris* L.-**stem and basal rot**	Moroturo, Lara state	[39]
M. phaseolina	*Glycine* Willd.	-	[40]
M. phaseolina	*Gossypium* L.	-	[40]
M. phaseolina	*Ipomoea* L.	-	[40]
M. phaseolina	*Nicotiana* L.	-	[40]
M. phaseolina	*Phaseolus* L.	-	[40]
M. phaseolina	*Psidium guajava* L.-**Fruits**	-	[40]
M. phaseolina	*Solanum melongena* L.	-	[40]
M. phaseolina	*Vigna* Savi	-	[40]
***Neofusicoccum mangiferae* (Syd. & P. Syd.) Crous, Slippers & A.J.L. Phillips**	*Mangifera indica*- **death of branches**	Maracay (INIA-CENIAP), Aragua state	[42]
***Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips**	*Mangifera indica*- **death of branches**	Maracay (INIA-CENIAP), Aragua state	[42]
***Sphaeropsis* Sacc.**	*Cecropia peltata* L.-**branch and trunk knots**	Reserva Forestal de Ticoporo, Mirí, Barinas state	[39]
***Sphaeropsis* sp.**	*Phthirusa paniculata* (Kunth) J.F.Macbr.-**leaf**	Lara state	[39]
***Sphaeropsis palmarum* Cooke**	*Cocos nucifera* L.-**old leaves**	Cumanacoa, Sucre state	[39]
***Sphaeropsis sapinea* (Fr.) Dyko & B. Sutton**	*Pinus caribaea* Morelet- **Chlorosis in the needles and discoloration lesions on the stem**	Nirgua, Yaracuy state	[35]
S. sapinea	*Pinus oocarpa* Schiede-**blue stain on wood**	Andes region (1600 meters above sea level), Merida state	[37]
Sphaeropsis sapinea	*Pinus caribaea* var. *hondurensis*-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)	[34]
***Sphaeropsis tumefaciens* Hedges**	*Citrus* L.-**gall**	-	[40]
***Botryosphaeria festucae* (Lib.) Arx & E. Müll**	*Zea mays* L.-**bract, leaf, and seed**	-	[40]
***Botryosphaeria dothidea* (Moug. ex Fr.) Ces. & De Not**	Compositae-**stem**	Aragua state	[40]
***Botryosphaeria ribis* Grossenb. & Duggar**	*Rosa canina* L.-**branch**	Lara state	[39]

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

Genera	Conidia	Conidiomata	Conidiophores	Conidiogenesis cells	Paraphyses
***Cophinforma* 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
***Diplodia* Fr.**	Initially hyaline, aseptate, thick-walled, becoming 1–septate only rarely becoming 2-septate, pale transluscent 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
***Dothiorella* 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
***Lasiodiplodia* 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 conidiogenous cells, if present hyaline, simple, sometimes septate, rarely branched	Holoblastic, hyaline, smooth, cylindrical to subobpyriform, discrete, determinate, or indeterminate	Present
***Macrophomina* 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
***Neofusicoccum* 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 hyaline, cylindrical, branched at the base, smooth, 0–1 septate	Enteroblastic, integrated, hyaline, smooth, cylindrical	Absent
***Pseudofusicoccumaceae* Tao Yang & Crous, *Pseudofusicoccum* Mohali, Slippers & M.J. Wingf.**	Conidia are more cylindrical than in *Noefusicoccum* 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

Notes. ***Macrophomina*** has sclerotia black, smooth, hard, formed of dark brown, thick-walled cells [8]. ***Neofusicoccum*** was introduced by Crous et al. [24] 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

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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 [8]. Also, the absence of septa (aseptate) in mature conidia of ***Sphaeropsis*** separates it from the genus ***Diplodia*** which is characterized by conidia septate [8].

3. DNA Sequence-based identification of *Botryosphaeriales* in Venezuela

In the early 2000s, publications began appearing for identifying species within the *Botryosphaeriales* 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 [18,21]. However, single-gene genealogies were not always useful for resolving closely related or cryptic species of the *Botryosphaeriales*; moreover, comparisons of DNA sequence data from multiple genes or different gene regions were exceptionally useful for discriminating among several closely related species [19,43,44].

From mid-2000s through 2022, different species and genera within the *Botryosphaeriales* 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 *Botryosphaeriales* 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 *Botryosphaeriales* order identified with DNA sequence data in Venezuela.

Species	Accession number	Host	Locality	GenBank accession number			References
				ITS	**ITS**	**BTUB**	
Botryosphaeria dothidea	CMW8000 Ex-type	*Prunus* sp.	Switzerland	AY236949	AY236898	AY236927	[43]
B. dothidea	CMW13390= CBS117919	*Eucalyptus urophylla x E.grandis*	CR and WCR	EF118044	-	-	[53]
Cophinforma atrovirens	CMW13416= CBS117444	*E.urophylla x E.grandis*	CR and WCR	EF118050	GU134938	-	[53]
C. atrovirens	CMW13425= CBS117445	*Acacia mangium*	CR and WCR	EF118046	GU134939	-	[53]
C. atrovirens	CSM 72	*Theobroma cacao*	AR	MF436087	MF436099	MF436111	[54]
C. atrovirens	MFLUCC 11-0425 Ex-type	*Eucalyptus* sp	Thailand	JX646800	JX646865	JX646848	[56]
***Diplodia scrobiculata* (syn. *D. guayanensis*)**	CBS129749	*Acacia mangium*	NER	JX545106	JX545126	JX545146	[59]
***D. scrobiculata* (syn. *D. guayanensis*)**	CBS129750	*Acacia mangium*	NER	JX545108	JX545128	JX545148	[59]
D. scrobiculata	CMW189 = CBS 118110 Ex-type	*Pinus banksiana*	United States	KF766160	KF766399	AY624258	[60,127]
Lasiodiplodia brasiliense	CMM4015 Ex-type	*Mangifera indica*	Brazil	JX464063	JX464049	-	[69]
L. brasiliensis	CSM 11	*Theobroma cacao*	AR	MF436018	MF436006	MF435998	[54]
L. brasiliensis	CSM 15	*Theobroma cacao*	AR	MF436019	MF436007	MF435997	[54]

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L. crassispora	WAC 12533= CBS118741 Ex-type	*Santalum album*	Australia	DQ103550	EU673303	KU887506	[72,80]
L. crassispora	CMW 13488	*Eucalyptus urophylla*	CR and WCR	DQ103552	DQ103559	KU887507	[72,80]
L. pseudotheobromae	CBS 129752	*Acacia mangium*	NER	JX545091	JX545111	JX545131	[90]
L. pseudotheobromae	CBS116459 Ex-type	*Gmelina arborea*	Costa Rica	KF766193	EF622057	EU673111	[25,128]
Lasiodiplodia theobromae	CBS 164.96 Ex-neotype	From unidentified fruit along coral reef coast	Papua New Guinea, Madang	AY640255	AY640258	KU887532	[72,129]
L. theobromae	CSM 22	*Theobroma cacao*	AR	MF436023	MF436011	MF436005	[54]
L. theobromae	CBS129751	*Acacia mangium*	NER	JX545096	JX545116	JX545136	[59]
L. theobromae	CMW13487	*Europhylla urophylla x E. grandis*	CR and WCR	EF118053	-	-	[53]
L. theobromae	CBS129754	*Pinus caribaea* var. *hondurensis*	NER	JX545099	JX545119	JX545139	[59]
L. theobromae	CMW13489= CBS117922	*Eucalyptus urophylla x E. grandis*	CR and WCR	DQ103525	-	-	[53]
L. theobromae	CMW13510	*Acacia mangium*	CR and WCR	DQ103526	-	-	[43]
L. theobromae	CMW13520	*Pinus caribaea*	CR and WCR	DQ103527	-	-	[43]
L. theobromae	CAA006	*Ficus insipida*	GR	DQ458891	DQ458876	DQ458859	[106]
L. venezuelensis	CBS129755	*Pinus caribaea* var. *hondurensis*	NER	JX545104	JX545124	JX545144	[59]
L. venezuelensis	CBS129757	*Ficus insipida*	GR	JX545102	JX545122	-	[106]
L. venezuelensis	WAC12539= CBS118739 Ex-type	*Acacia mangium*	CR and WCR	DQ103547	DQ103568	KU887533	[72,80]
L. venezuelensis	CBS 129759	*Jacaranda copaia*	GR	JX545101	JX545121	JX545141	F. Castro-Medina/ S.R. Mohali-unpublished
Neofusicoccum arbuti	CBS 116131=AR 4014 Ex-type	*Arbutus menziesii*	USA	AY819720	KF531792	KF531793	[8,108]
***Neofusicoccum arbuti* (syn. *N. andinum*)**	CMW13455= CBS117453	*Eucalyptus* sp.	AR	AY693976	AY693977	KX464923	[26,105]

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***Neofusicoccum arbuti* (syn. *N. andinum*)**	CMW13446= CBS117452	*Eucalyptus* sp.	AR	DQ306263	DQ306264	KX464922	[26,105]
N. parvum	CMW9081 Ex-type	*Eucalyptus grandis*	South Africa	AY236943	AY236888		[43]
N. parvum	CMW13350= CBS117923	*Psidium guajava*	ZR	EF118036	-	-	[53]
N. parvum	CMW13355= CBS117915	*Eucalyptus urophylla*	CR and WCR	EF118035	-	-	[53]
N. ribis	CMW7772 Ex-type	*Ribes* sp.	New York, United States	AY236935	AY236877	-	[43]
N. ribis	CMW13360= CBS117916	*Eucalyptus urophylla*	CR and WCR	EF118037	-	-	[53]
N. ribis	CMW13410= CBS117443	*Eucalyptus urophylla*	CR and WCR	EF118038	-	-	[53]
Pseudofusicoccum stromaticum	CMW13434= CBS117448 Ex-type	*Eucalyptus urophylla x E. grandis*	CR and WCR	AY693974	AY693975	EU673094	[105,128]
P. stromaticum	CMW13426= PREM 58513	*Acacia mangium*	CR and WCR	EF118041	-	-	[53]

Acronyms of culture collections: CBS: Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; IBL: Independent Biological Laboratories Israel. KEFAR MALAL; **CMW**: Tree Patholgy Co-operative 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-Figure 3):** Central Region=**CR** (Cojedes state) and Western Central Region=**CR** and **WCR** (Falcon, Lara, and Portuguesa states). Los Andes Region (Mérida state) = **AR.** Northeastern Region (Anzoátegui and Monagas states) = **NER.** Guayana Region (Bolívar and Delta Amacuro states) = **GR.** Zulia Region (Zulia state) = **ZR. (-) = No sequences.**

3.1. 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 NCBI GenBank Database (http://www.ncbi.nlm.nih.gov). For this analysis, *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.

For the phylogenetic study were internal transcribed spacers 1 and 2 including the intervening 5.8S nrDNA gene (ITS) [45], the translation elongation factor 1-alpha gene (*tef1*) [46] and the beta-tubulin gene (*tub2*) [47]. The 3-loci concatenated alignment contained 1232 characters including gaps for *Lasiodiplodia* group (526 from ITS, 328 from *tef1* and 378 from *tub2*) and 1295 characters including gaps for *Botryosphaeriales* remaining (532 from ITS, 333 from *tef1* and 430 from *tub2*) (Table 3).

Phylogenetic analyses were performed for the combined datasets using two different methods: Maximum Likelihood (ML) and Bayesian Inference (BI). A partition homogeneity test (PHT) [48,49] 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, 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 [50], and BI phylogenies were performed with MRBAYES v3.2.1 [51]. All sequences from representative isolates were aligned using MUSCLE that along with BI phylogenies

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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 [52] using the Akaike Information Criterion (AIC) and for ML were determined with MEGA-X [50], with HKY+G substitution model used as the best model for both. Phylogenetic species were determined with ML ≥50%, and BI ≥0.6 for *Lasiodiplodia* group (Figure 1), and ML ≥ 90% and BI ≥ 0.90 for the remaining of *Botryosphaeriales* (Figure 2).

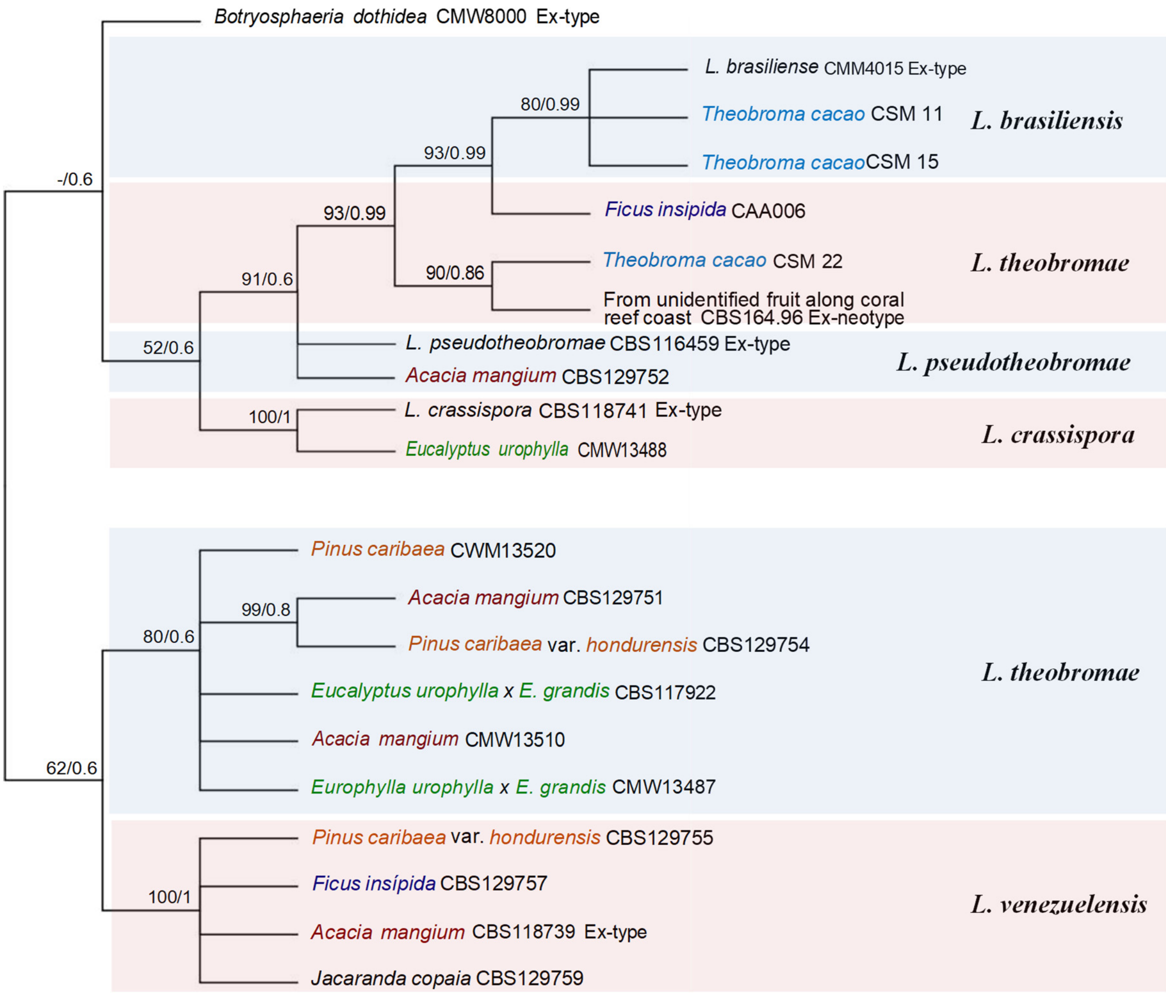

Figure 1. Phylogenetic tree of *Lasiodiplodia* genus in Venezuela results from Bayesian analysis (BI) of the combined ITS, *tef1*, and *tub2* sequence alignment. Maximum likelihood (ML) bootstrap support values (ML ≥50%) and Bayesian posterior probabilities (BI ≥0.6) are shown at the nodes (ML/BI). Ex-type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

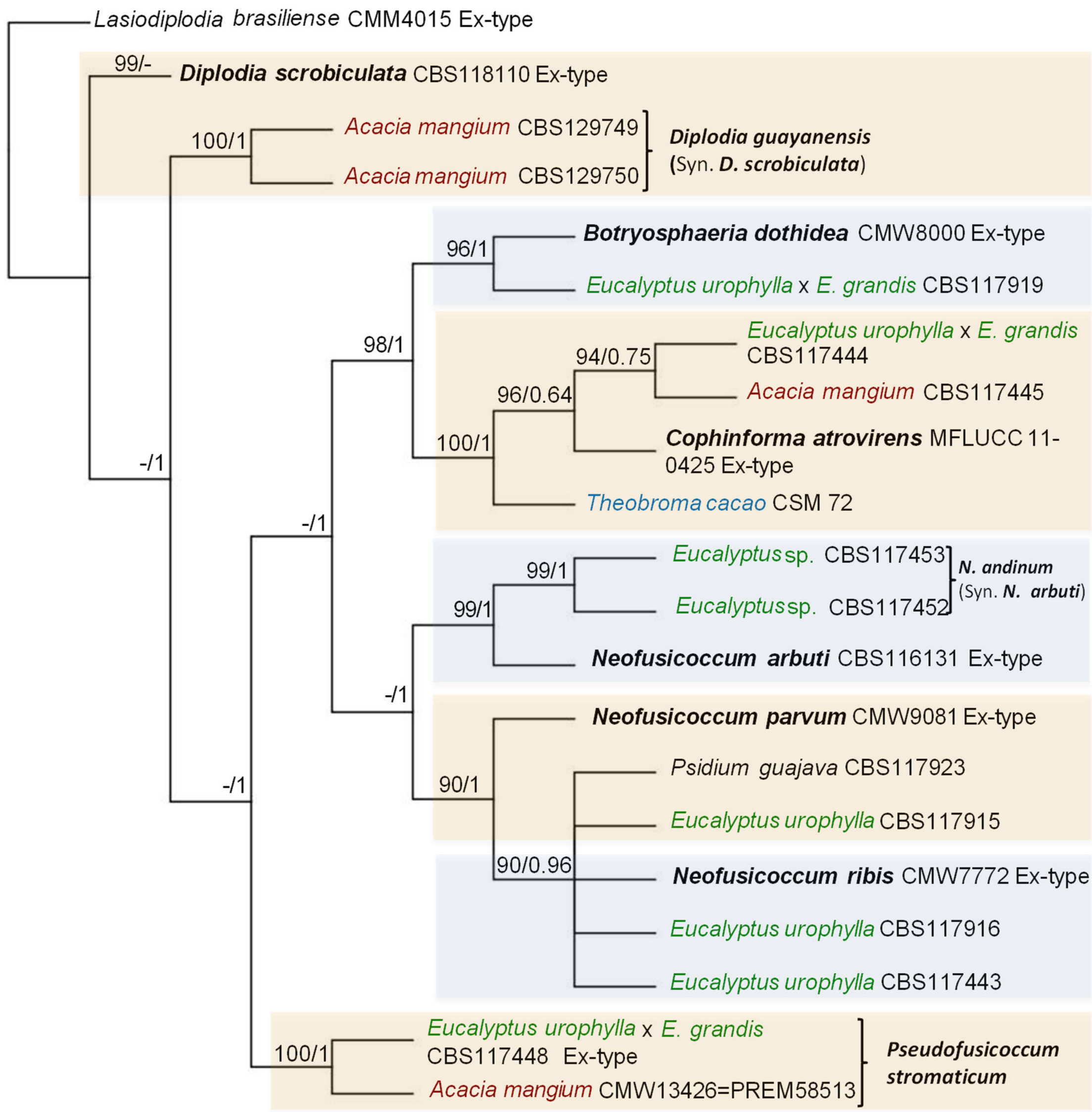

Figure 2. Phylogenetic tree of *Botryosphaeriales* remaining group in Venezuela results from Bayesian analysis (BI) of the combined ITS, *tef1*, and *tub2* sequence alignment. Maximum likelihood (ML) bootstrap support values (ML ≥ 90%) and Bayesian posterior probabilities (BI ≥ 0.90) are shown at the 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.

4. Taxonomy, diversity and distribution of a 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 1–3).

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

Cophinforma 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, [53], and from fruits and trees of *Theobroma cacao* L., in Merida state (AR) [54], Venezuela. Initially, Mohali et al. [53] reported this fungus as *Botryosphaeria mamane* D.E. Gardner (asexual morph *Cophinforma mamane* (D.E. Gardner) A.J.L. Phillips & A. Alves), but Phillips et al. [8] 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 [55]; dead branch of *Eucalyptus* sp., in Thailand [56] as *Cophinforma eucalypti* Doilom, J.K. Liu & K.D. Hyde; it was also isolated from *Dimocarpus longan* Lour., but producing lesions on inoculated seedlings of *Eucalyptus* sp., in China [57]; and stem rot and dieback on Cashew tree (*Anacardium occidentale*) in Brazil [58].

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 [59]. *Diplodia guayanensis* was distinguished from *D. scrobiculata* by its larger conidia [59]. Later, sequence alignment ITS, *tef1* and *btub* of *D. scrobiculata* and combining two (ITS and *tef1*) [60] and three loci (ITS, *tef1* and *btub*) [61] for phylogenetic analysis both concluded that *D. guayanensis* is indistinguishable from *D. scrobiculata* based on phylogenetic analyses, and considered it to be a synonym for *D. scrobiculata*, and this was further supported on the basis that Úrbez-Torres et al. [59] used older sequences for *D. scrobiculata* in their phylogenetic analyses [60], although Zhang et al. [61] used the old sequences and obtained the same results as Linaldeddu et al. [60]. Furthermore, morphological variability is common in these fungi [8,60]; 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 *Cfol* restriction fragments in *tef1* PCR products [59]. The PCR-

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RFLP fingerprinting profiles have been useful in this study to distinguish *Botryosphaeriales*, although overlapping RFLP patterns may be observed between some species using one, two, or more RE [62].

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) [63]; wilted twigs, branch dieback, necrosis and stem cankers on *Pinus halepensis* Mill., trees, in Tunisia [64]; symptomless and die-back on *Pinus patula* Schiede ex Schltdl. & Cham., in South Africa [65]; asymptomatic trees in *Pinus radiata* D. Don plantations but producing lesions on inoculated *P. radiata* seedlings in Spain [66]; *Pinus* sp., in Canada [67]; and dieback on Coast redwood (*Sequoia sempervirens* (Lamb. ex. D. Don) Endl.) in California, USA [68].

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 [54], although Zhang et al. [61], 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. [69] and other hosts in Brazil; saprobic on dead branch of teak (*Tectona grandis* L.f.), in Thailand [70]; Mango dieback, in Peru [71]; *Adansonia madagascariensis* Baill., in Madagascar [72]; *Eucalyptus* sp., in China [56]; as endophytic fungus isolated from healthy, brown, and ligaloes tissue of evergreen trees (*Aquilaria crassna* Pierre ex Lecomte), in Laos [73]; symptoms of gummosis, stem cankers, and dieback on Persian lime (*Citrus latifolia* Tan.), in Mexico [74]; *Gossypium hirsutum* L., in Australia [75]; leaf blight of *Sansevieria trifasciata* Prain (mother-in-law's tongue or snake plant), ornamental plant, in Malaysia [76]; dieback and corky bark on longan trees (*Dimocarpus longan* L.), in Puerto Rico [77]; branch dieback, *T. cacao*, Cameroon and *Psychotria tutcheri* Dunn fruits, in China [61].

Cruywagen et al. [72] and Farr & Rossman [78] 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* [79].

Lasiodiplodia crassispora Burgess, Barber sp. nov., was isolated for the first time from wood of living *E. urophylla* in Acarigua, Portuguesa State (WCR), Venezuela and canker of *Santalum album* L., (sandalwood) in Western Australia, Australia [80]. The sandalwood is native to southern India, eastern Indonesia, and northern Australia (https://en.wikipedia.org/wiki/Santalum_album), therefore, *L. crassispora* found in central-western Venezuela (WCR) could have been introduced through imported eucalyptus seeds used for the plantations in Venezuela.

Lasiodiplodia crassispora was associated the internal wood decay symptoms observed in the cordon samples on grapevine (*Vitis vinifera* L.), in South Africa [81]; *E. urophylla*, in Uruguay [82]; perennial cankers in the vascular tissue of grapevines, in California, USA [83]; endophytic in *Corymbia* sp. Hook, and minor lesions in inoculations on 4-month-old baobab seedlings (*Adansonia gregorii* F.Muell.), in Australia [84]; dieback and stem-end rot of mango, fresh fruit of table grape (*Vitis* spp.), and causing dieback on *Annonaceae* in Brazil [85–87]; dieback symptoms from trunks and branches on grapevines in Sonora and Baja California, Mexico [88]. *Lasiodiplodia crassispora* (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 [61,89].

Lasiodiplodia pseudotheobromae A.J.L. Phillips, A. Alves & Crous was reported for the first time in Uverito plantations, Monagas State (NER), Venezuela in *A. mangium* [90].

Lasiodiplodia pseudotheobromae was identified for the first time from *Gmelina arborea* Roxb., (Melina) and *A. mangium* in Costa Rica, *Rosa* sp., in Netherlands, *Coffea* sp., in Zaire and *Citrus aurantium* L., Suriname [91]; 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 [92]; dieback on blackthorn (*Acacia mellifera* (M.Vahl) Benth.), in Namibia [89]; *Adansonia digitata* L., in Mozambique and South Africa [71]; Cashew gummosis (*Anacardium humile* A.St.-Hil.), in Brazil [93]; *Annona muricata* L., in Australia [75]; *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 [94]; stem cankers, gummosis, and branches dieback *Citrus latifolia* Tan., in Mexico [74]; trunk cankers, *Citrus reticulata* Blanco, in Pakistan [95]; symptoms of branch dieback, cankers and fruit rot in *Citrus* sp., in Iran [96]; dieback, Mango, in Egypt, Peru and South Korea [71,97,98]; 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 [82]; dieback and fruit rot on Rambutan trees (*Nephelium lappaceum* L.), in Puerto Rico [77]; symptoms of branch dieback and cankers, and shoot and panicle blight in pistachio (*Pistacia* sp. and *Pistacia vera* L.), in Spain, [99]; shoot-dieback, gummosis, and sunken necrotic bark lesions in young nectarine (*Prunus persica*) trees, in Turkey [100]; *Rosa* sp., in Netherlands [91]; leaf blight of *Sansevieria trifasciata*, in Malaysia [76]; die-back disease on *Schizolobium parahyba* (Vell.) S. F. Blake var. *amazonicum* (Ducke) Barneby trees, in Ecuador [101]; trunk Diseases in *Vitis vinifera*, in Tunisia [102]; and post flowering stalk rot of maize (*Zea mays* L.), in India [103].

Lasiodiplodia theobromae (Pat.) Griffon & Maubl., is a cosmopolitan fungus occurring predominantly throughout tropical and subtropical regions [38,80]. It has also been known as a human pathogen causing keratomycosis and phaeohyphomycosis [103], 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 [38], and it also occurs as an endophyte [104].

Lasiodiplodia theobromae has been reported in Venezuela on *A. mangium*, and *E. urophylla*, in Portuguesa State (WCR) [105]; *P. caribaea* var. *hondurensis*, *E. urophylla* x *E. grandis*, and *A. mangium*, in Cojedes (CR), Falcon and Portuguesa States (WCR) [53]; *Pinus caribaea* and *A. mangium*, in Monagas State (NER) [59]; *Ficus insipida*, logs yard located within the natural forest of the Imataca Forest Reserve, between the Bolivar and Delta Amacuro States (GR) ([106]; *Theobroma cacao*, in Merida State (AR) [54].

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 [104].

Lasiodiplodia venezuelensis Burgess, Barber, Mohali, sp. nov., MB500237 was isolated and described for the first time from 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 Bolivar and Delta Amacuro States (GR) [59,80,106]. 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 for Venezuela as is the case of *F. insipida* and moving onto an exotic species as a pathogen in *A. mangium* plantations [59].

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.) [8,24,105], was isolated from asymptomatic branches of mature *Eucalyptus* sp., trees in Mucuchies (3140 m), Cordillera Los Andes mountains (AR), Venezuela [105].

Li et al. [57,107] using combination of ITS, *tef1*, *tub2*, and *rpb2* regions, with maximum parsimony (MP)/maximum likelihood (ML) tests analyses, they could separate cryptic species between *N. andinum* and *N. arbuti*. Later, Zhang et al. [61], 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. [105] did not include the *N. arbuti* sequences in the phylogenetic tree because these were not available at that time [61]. *Neofusicoccum arbuti* was isolated from cankers of *Arbutus menziesii* Pursh (Pacific madrone), in Washington and California, USA, and Canada [108], and stem canker and dieback of *Vaccinium* spp. (Blueberry), in Chile [109].

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., 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 *N. parvum* on *Psidium guajava* L., in Zulia State (ZR) [53].

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 [110].

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., [8,24,105], 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 [105].

Crous et al. [24] introduced to *Pseudofusicoccum* genus for species that are morphologically similar to *Fusicoccum* and *Neofusicoccum* but phylogenetically distinct from both of these genera. *Pseudofusicoccum* genus resembles species of *Fusicoccum* but distinct in having conidia encased in a persistent mucous sheath, and conidia are also more cylindrical than in *Fusicoccum* species [24]. Yang et al. [26] using robust backbone phylogeny for *Botryosphaeriales* (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 [110].

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 [111,112]; dieback, wilting of branches, discoloration of the vascular system, decline and subsequent death of Malay apple (*Syzygium malaccense* L.) trees [113]; associated with gummosis on native cashew (*Anacardium othonianum* Rizzinin) [93]; dieback and stem and branch cankers on cashew (*Anacardium occidentale* L.), guava (*Psidium guajava* L.) and caja-umbu (*Spondias mombin* L. x *S. tuberosa* Arruda) trees [114]; as endophyte in *Myracrodruon urundeuva* Fr. All. (*Anacardiaceae*) [115], and dieback of the *Annonaceae* [87]. In Uruguay, *P. stromaticum* was associated with cankers showing gummosis in peach shoots and showed moderate virulence on both inoculated apple and peach shoots [116].

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 podagrica* Hook, in India [117,118]; *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 [119]; *P. ardesiacum* Pavlic, T.I. Burgess, M.J. Wingf., on *A. gibbosa* and *Eucalyptus* sp., in Australia; *P. artocarpi* T. Trakunyingcharoen, L. Lombard & Crous, on twigs of *Artocarpus heterophyllus* Lam., in Chiang Mai Province, Thailand [94]; *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 [120]; *P. kimberleyense* Pavlic, T.I. Burgess, M.J. Wingf., on *Acacia synchronicia* Maslin, *Adansonia gibbosa*, *Eucalyptus* sp., and *Ficus opposite* Miq. in Australia [121] and *Persea americana* Mill., USA [61]; *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 [55,61]; *P. violaceum* Mehl & Slippers on asymptomatic branch of *P. angolensis* Mpumalanga Province, Mawewe Nature Reserve, in South Africa [55], and *Microcos paniculatus*, in Hong Kong, China [61].

This genus is known only as the asexual morph and thus far nine species have been reported [61]. 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 [61,117,118].

5. Symptoms associated with species from *Botryosphaeriales* in Venezuela

Botryosphaeriales 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 [7]. The Table 1, different genera within the *Botryosphaeriales* were found and isolated from different hosts and locations in Venezuela, associated with diverse symptoms, and identified through its asexual morph, and other were identified using DNA sequence data (Table 3, Figures 1 and 2).

Diplodia spp. and *Lasiodiplodia* spp., have 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 [122]. The blue color of the wood develops as an optical effect due to refraction of light [123], such as observed in the following examples: *Lasiodiplodia theobromae*, *L. venezuelensis* and *Diplodia mutila* (Fr.) Mont., on *Pinus caribaea* var. *hondurensis* (Figure 4a–i); *L. theobromae* and *L. venezuelensis* on *Ficus insípida* (Figure 4j,k). The discolorations in the wood of living trees/woody plants or dead logs are the result of diverse biotic and abiotic causes [124,125]. Wood discoloration and decay are often the result from wounding, such as those caused by animal chewing, branch breaking, pruning, mechanized wood harvest, construction injury, motor traffic, etc. [126], and insects. Further discolorations can result from tree-produced substances, such as deposition of heartwood substances developed by living tree cells, later microbial stains, and finally colored derivatives of wood decay processes [124], examples of tree/wood discolorations 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 *Cophinforma atrovirens* (Figure 4l,m); discolorations on *Acacia mangium* in Cojedes and Portuguesa States caused by *Lasiodiplodia theobromae*, *L. venezuelensis*, *Cophinforma atrovirens*, and *Pseudofusicoccum stromaticum* (Figure 4n,o).

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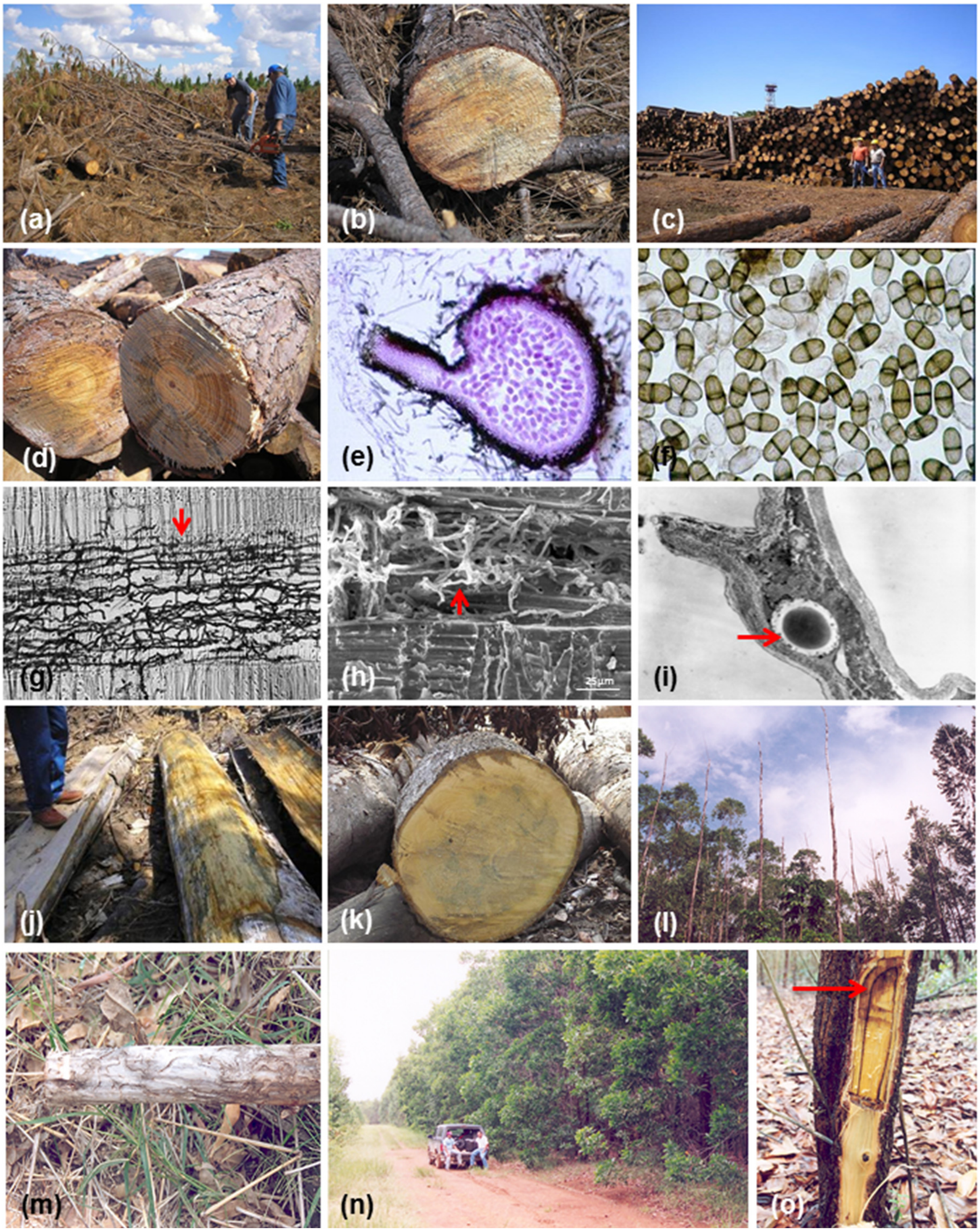

Figure 4. (**a**) *Pinus caribaea* var. *hondurensis* plantations (Maderas del Orinoco C.A) located in Uverito, Monagas state, Venezuela. (**b**) Blue stain of the wood, observed in fallen trees inside the plantations. (**c**) Log yard to be processed at the sawmill in Maderas del Orinoco company. (**d**) 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** [32]; **g** [123]; **h**, **i** [130]; **j**, **k** [106].

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Discolorations 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 (Figure 5a–d), as well decline symptoms observed in *A. mangium*, and *P. caribaea* var. *hondurensis*. Pathogenicity tests were carried out in commercial plantations at the company Maderas del Orinoco to investigate the status of *Botryosphaeriales* 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*. *A. mangium* showed bark swelling, vascular discoloration, necrosis, and cankering around the inoculation points (Figure 5e–g), while in *P. caribaea* var. *hondurensis* did not cause any lesions [59,90]. This study showed that *Lasiodiplodia* spp., and *Diplodia* sp., are highly virulent to *A. mangium*, showing. Other pathogenicity tests were carried out in the field, which gave us information about the susceptibility or tolerance to diseases, such as is the case of *Eucalyptus* spp., a forest species introduced in Venezuela to obtain fibers for cardboard production. These assessments of inoculations were made with different genera and species of *Botryosphaeriales* in commercial plantations of *Eucalyptus* at the company Smurfit Kappa Reforestadora Dos, Portuguesa State on different commercial clones of *Eucalyptus*-hybrids (*E. urophylla* x *E. grandis*), and where these clones were shown to be tolerant of *Botryosphaeriaceae* were observed [110]. *Cophinforma atrovirens* 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 [54] in Merida State (Figure 5h), producing discolorations in branches (Figure 5i) and stems (Figure 5j,k). These discolorations were mainly associated with wounds caused by bark beetles- *Scolytinae* (Figure 5l). 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 (Figure 5m,n) 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 [34]. Cedeño et al. [34] based their identification on the asexual morph, conidia 39,8 (37-45) x 12,7 (11-16) µm, one septum and rarely two or three septa (Figure 5o,p); measurements close to *Diplodia sapinea* (25.5-) 30.5-52.5 (-54) x (10-) 12.5-20 (-21) µm, *D. scrobiculata* (37.5-) 39.5 (-41.5) x (13-) 14 (-15.5) µm, and *D. scrobiculata* (syn. *D. guayanensis*) (33.5-) 40.6-42.4 (56) x (12-) 15.8-16.7 (-18.5) µm [59]. The absence of septa (aseptate) in mature conidia of *Sphaeropsis* separates it from the *Diplodia* genus, which is characterized by septate conidia [8] (Table 2), therefore, the diseases observed by Cedeño et al. [34] in the nurseries and plantations of *P. caribaea* var. *hondurensis* could have been caused by a fungal species in the *Diplodia* genus.

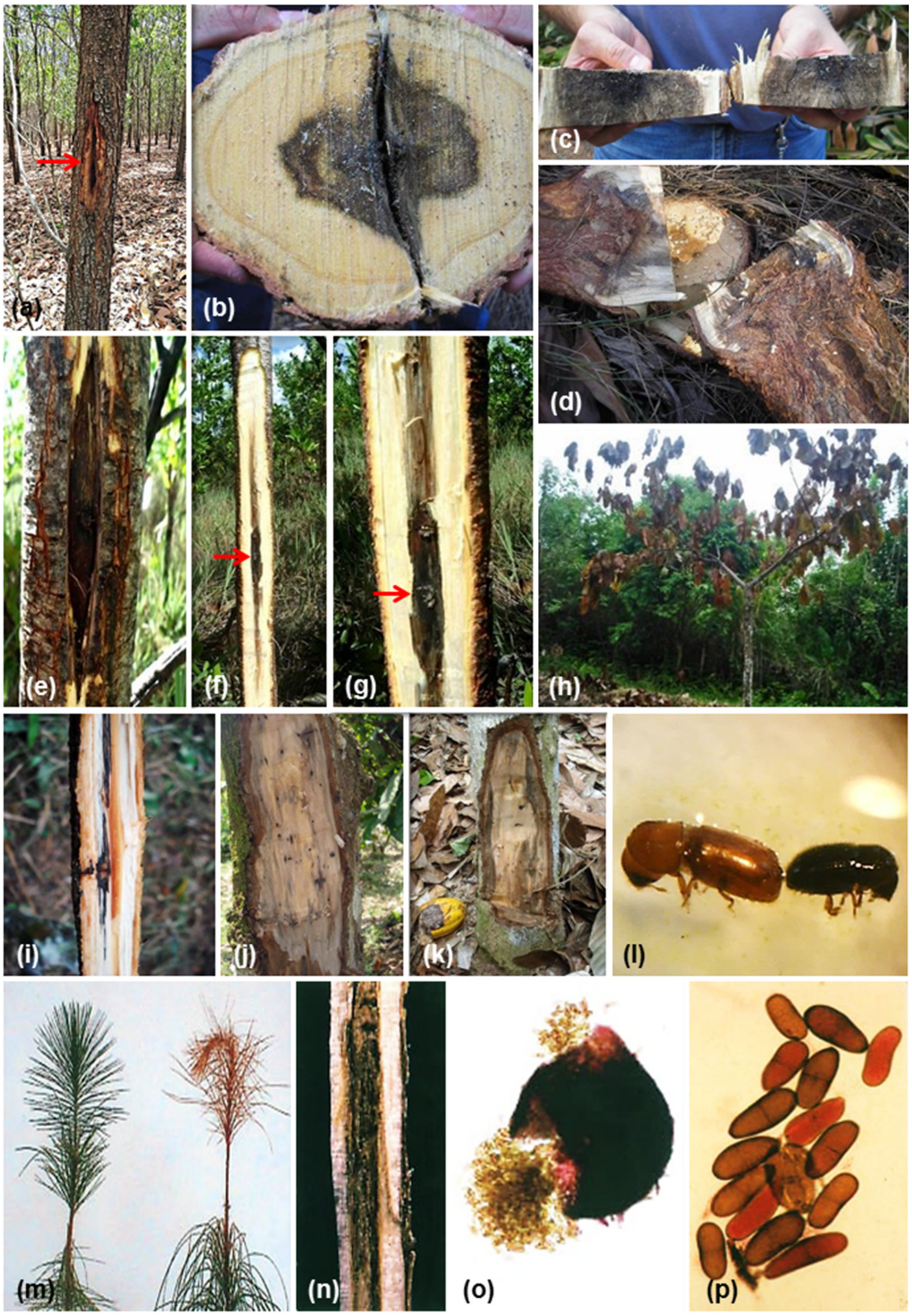

Figure 5. (**a**) *Acacia mangium* tree with canker in the stem (red arrow) in Maderas del Orinoco plantations. (**b**, **c**) Discoloration in the *A. mangium* stem. (**d**) Discoloration from *A. mangium* tree base. (**e**) Pathogenicity test with different *Botryosphaeria* species using standing trees of *A. mangium* in a commercial plantation, and where *A. mangium* tree showed bark swelling around the inoculation points and necrosis of the vascular system below the bark (canker) 12 weeks after inoculation. (**f**, **g**) Black exudation was observed when the outer bark was removed from inoculation points (red arrows). (**h**) Dieback or sudden death symptoms in *Theobroma cacao* tree. (**i**) Discoloration in branch; (**j**, **k**) Discolorations in stems of *T. cacao* with dieback or sudden death symptoms in Merida State. (**l**) Bark beetle (*Scolytinae*) collected from cacao tree stem with discoloration. (**m**) Control seedling (left) and, tip death symptom in a seedling inoculated with *Sphaeropsis sapinea* (right) in Caribbean pine three weeks after inoculation. (**n**) Seedling with longitudinal stem section inoculated with *S. sapinea* where the discoloration within the stem can be observed. (**o**) Pycnidium and, (**p**) *S. sapinea* Conidia (possible *Diplodia* sp.). Pictures **e-g** [59]; **m-p** [34].

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 (Figure 6a–d). Photographs of other *Botryosphaeriales* genera; *Diplodia scrobiculata* (syn. *Diplodia guayanensis*) (Figure 6e,f), *Pseudofusicoccum stromaticum* (Figure 6g–i); *Neofusicoccum ribis*/*Neofusicoccum parvum* (Figure 6j,k), *Cophinforma atrovirens* (Figure 6l, m), and *B. dothidea* (Figures 6n-p).

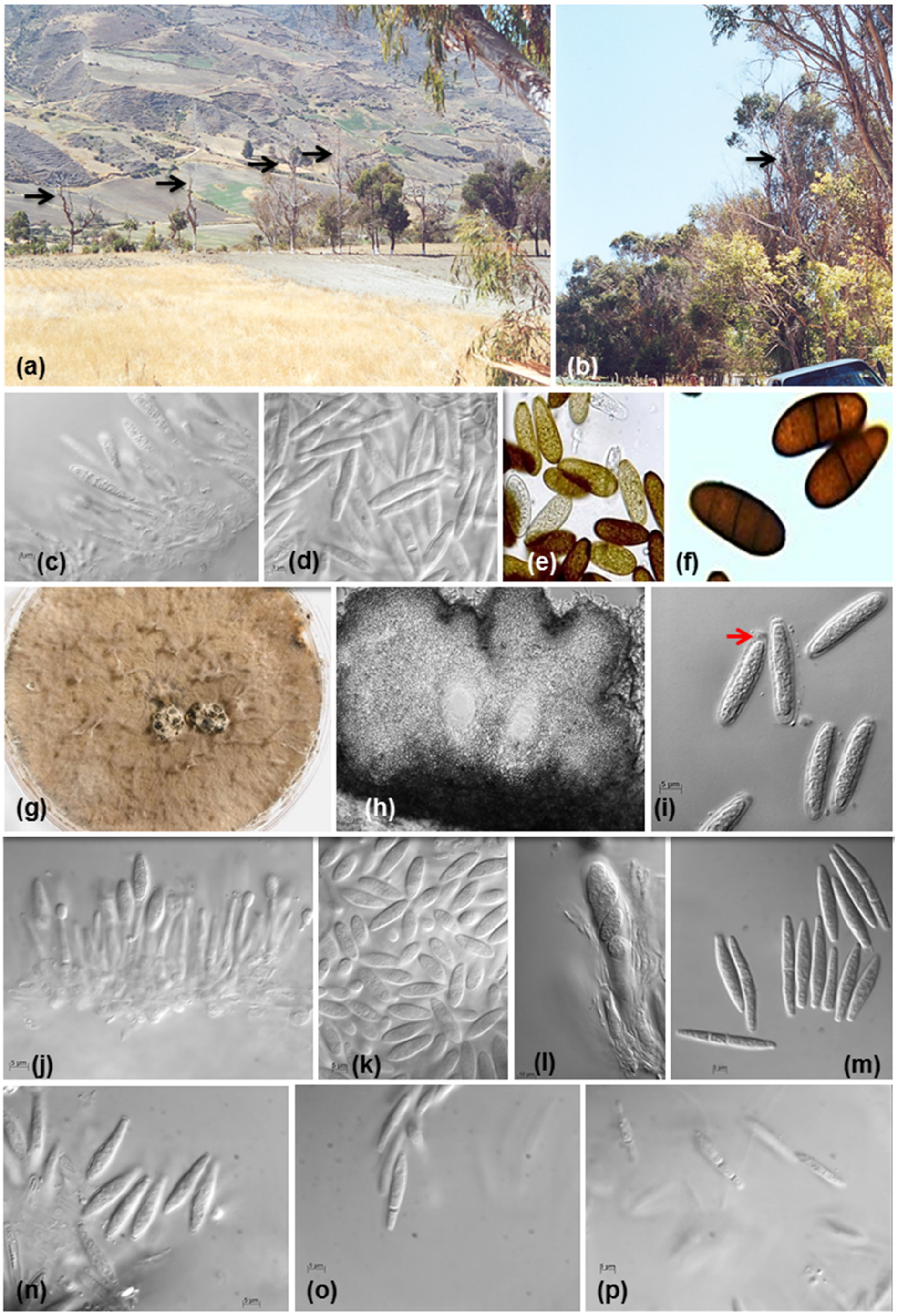

Figure 6. (**a**, **b**) *Eucalyptus* sp., at the Cordillera Los Andes Mountains, Merida state, Venezuela at an altitude of approx. 3140 meters above sea level. The black arrows show old *Eucalyptus* trees without apparent damage. (**c**) Conidiogenous cells and (**d**) Conidia of *Neofusicoccum arbuti* (syn. *Neofusicoccum andinum*) isolated from *Eucalyptus* sp., asymptomatic branches at the Cordillera Los Andes Mountains. (**e**) Conidia immature and mature of *Diplodia scrobiculata* (syn. *Diplodia guayanensis*). (**f**) Mature conidia of *D. guayanensis* with two and three septa. (**g**) *Pseudofusicoccum stromaticum* producing big conidioma on 2 % Malt Extract Agar. (**h**) Multilocular conidiomata of *P. stromaticum* without ostioles and embedded locule. (**i**) *Pseudofusicoccum stromaticum* conidia encased in a persistent

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mucous sheath (red arrow). (**j**) *Neofusicoccum ribis/parvum* complex conidiogenous cells. (**k**) *Neofusicoccum ribis/parvum* complex conidia. (**l**) *Cophinforma atrovirens*, asci bitunicate with ascospores aseptate, hyaline, with granular textured contents. (**m**) *Cophinforma atrovirens* conidia with one and two septa. (**n**-**p**) *Botryosphaeria dothidea* conidia with 0-2 septa. Pictures **e**, **f** [59]; **g-I** [105]; **j-p** [53].

6. Conclusions

This is a review and update of information that represents almost 40 years of research work with species pertaining to the order *Botryosphaeriales* that cause diseases, with special reference to woody plants. The nomenclature of the different species and genera found within the *Botryosphaeriales* order have been updated, including the identification of the news species of *Lasiodiplodia*, a new genus and specie of *Pseudofusiccocum*, and new reports for Venezuela using molecular tools.

At the morphological level, nine genera were isolated and identified within *Botryosphaeriales* order, where *Lasiodiplodia* spp is 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, and 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, showing 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; *B. dothidea* produced very small lesions, and the remainder of the fungi did not.

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, 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 Bolivar and Delta Amacuro States in Venezuela [106]. 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 specie exotics causing blue stain on pine wood, and canker in *A. mangium* plantations.

Batista et al. [2] have assumed that human movement and trade are the main routes of dispersal for all species within the order *Botryosphaeriales* with worldwide distribution across all continents, with the exception of Antarctica, with climatic variability being the main limitations for the appearance of new stable populations and additionally, they also highlight that the disease expression is mainly due to occasional climatic events that can affect the susceptibility of the host.

Botryosphaeriales 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 *Botryosphaeriales* order and their description at the morphological level in Venezuela is very

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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.

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