Eduardo Vaz Lemos Pires Batista

Mapear, Detectar e Investigar espécies de Botryosphaeriaceae

Map, Detect and Research Botryosphaeriaceae species

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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Biologia Ecologia das Alterações Globais, realizada sob a orientação científica de Doutor Artur Jorge da Costa Peixoto Alves, Professor auxiliar com agregação do Departamento de Biologia da Universidade de Aveiro, e coorientação dos Doutores Pedro Manuel Alberto de Miranda, Professor catedrático da Universidade de Lisboa e Jorge Martín García, Professor auxiliar da Universidade de Valladolid.

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Palavras-chave Patologia florestal; "host-jumps"; nichos ecológicos; modelos de distribuição de espécies; análise de risco; alterações climáticas.

Resumo

A família de fungos Botryosphaeriaceae (Botryosphaeriales, Ascomycetes) é conhecida por incluir diversas espécies de patógenos oportunistas ou endófitos latentes que afectam várias espécies de angiospérmicas e gimnospérmicas em todo o mundo. Estes fungos, normalmente, atacam plantas expostas a stresses abióticos, como por exemplo seca ou plantas que já estão afectadas por outro tipo de patógenos ou pragas. Doenças causadas por estas espécies originam podridão de frutos, manchas foliares, tombamento apical, necroses, murchidão de rebentos e eventualmente pode originar a morte do hospedeiro. A quantidade de estudos com foco na distribuição, diversidade, ecologia e patogenicidade de espécies de Botryosphaeriaceae tem aumentado ao longo do tempo. Contudo, devido à falta de consistência na delimitação das espécies, no nome dos hospedeiros e na localização dos estudos, é praticamente impossível quantificar a presença destas espécies globalmente ou o número de diferentes relações fungo-hospedeiro que realmente ocorrem. Além disso, várias questões relacionadas com o potencial de patogenicidade e a capacidade destes organismos alternarem entre diferentes hospedeiros num cenário de alterações climáticas continuam por responder. A presente tese, no capítulo dois, apresenta uma perspectiva alargada sobre a diversidade global de espécies de Botryosphaeriaceae, sua dispersão, associações de hospedeiros, nichos ecológicos, patogenicidade e eficácia da comunicação de novas ocorrências e novas associações de fungos-hospedeiros, com recurso a uma base de dados curada a nível global. Esta base de dados, que contém mais de 2900 referência bibliográficas de 1692 diferentes hospedeiros em 149 países diferentes, foi transformada numa plataforma interactiva e aberta que permite ao utilizador final consultar e explorar toda a informação com facilidade. O capítulo três reflecte e avalia boas práticas para novas descrições de espécies de forma a garantir reprodutibilidade, transparência e consistência ao longo do tempo. Com base na nossa definição de boas práticas foi verificado que, num grupo representativo de 210 novas descrições: mais de 90% das descrições são acompanhadas por uma caracterização morfológica detalhada e com análises filogenéticas consistentes; nas caracterizações moleculares e nas interacções fungo-hospedeiro, 60% das descrições estão desactualizadas ou não preenchem os critérios mínimos para publicação e 50% dos autores não providenciam informações de acessibilidade e de reprodutibilidade suficientes. O capítulo quatro avalia como estas espécies podem adaptar o seu nicho ecológico em resposta às actuais e futuras alterações climáticas. Em geral, é esperado um aumento das áreas com condições adequadas para a ocorrência destes patógenos na grande maioria dos cenários climáticos e um consistente aumento do número de meses com condições óptimas para o desenvolvimento destes fungos, que eventualmente pode alterar a fenologia destes organismos e originar surtos mais frequentes e com maior intensidade. Adicionalmente, como caso de estudo, no capítulo cinco, foi realizada uma amostragem a nível nacional em Portugal para identificar espécies de Botryosphaeriaceae associadas aos principais hospedeiros florestais. Doze espécies diferentes foram identificadas e os testes de patogenicidade revelaram a capacidade de algumas espécies para alternarem hospedeiros demonstrando grande susceptibilidade de *Quercus suber* para com *Neofusicoccum parvum* e *N. eucalyptorum*, bem como de *Pinus pinaster* para com *Diplodia corticola*. Diferentes perspectivas foram exploradas de forma a melhorar o nosso conhecimento do desafio que as doenças relacionadas com espécies de Botryosphaeriaceae apresentam num cenário de alterações climáticas.

Keywords forest pathology; host-jumps; ecological niches; species distribution modelling; risk assessment; climate change.

Abstract

The family Botryosphaeriaceae (Botryosphaeriales, Ascomycetes) is known to include several species of opportunistic pathogens or latent endophytes that affect worldwide many angiosperm and gymnosperm hosts. These fungi usually attack plants exposed to environmental stress, like drought or plants that are already affected by other pathogens or pests. Diseases caused by these species result on fruit rots, leaf spots, seedlings damping-off and collar rot, cankers, blight of shoots and seedlings and eventually host death.
The number of studies targeting the distribution, diversity, ecology, and pathogenicity of Botryosphaeriaceae species is consistently increasing. However, with the lack of consistency in species delimitation, the name of hosts, and the locations of studies, it is almost impossible to quantify the presence of these species worldwide, or the number of different hosts–fungus interactions that occur. Also, several questions regarding pathogenicity potential and the capability of these organisms to jump among different hosts in a global change scenario is poorly understood.
The present thesis offers in chapter two, a broad perspective on Botryosphaeriaceae species global diversity, dispersion, host association, ecological niches, pathogenicity and communication efficiency of new occurrences and new host-fungus associations based on a worldwide cured dataset. This dataset, with more than 2900 literature references from 1692 different plant species in 149 countries was transformed in an interactive and open database that allows the end-user to easily consult and explore information. In chapter three, reflects and assess best practices for new fungal species descriptions to ensure reproducibility, transparency, and consistency over time. Based on our definition of best available practices, it was found that, from a representative group of 210 new fungal descriptions, over 90% of the descriptions are followed by a detailed morphological characterization and with consistent phylogenetic analyses, for molecular characterization and host-fungus interactions 60% of the descriptions are outdated or only meet the minimal requirements for publication and 50% of the authors do not provide enough accessible and reproducible information. Chapter four evaluates how these species may shift their ecological ranges in response to current and future climate changes. An overall increase of suitable areas for these pathogens was predicted in most of the future scenarios and a consistent increase of the optimal growth months, for fungi development, that eventually could impact the phenology of these organisms and originate more frequent and intensive outbreaks. Additionally, in chapter five, as a case study, a survey was conducted in Portugal to identify Botryosphaeriaceae species associated with the main forest tree species. Twelve different species were identified, and pathogenicity tests revealed the host-jump potential of some species, showing high susceptibility of *Quercus suber* to *Neofusicoccum parvum* and *N. eucalyptorum* and of *Pinus pinaster* to *Diplodia corticola*. Different perspectives were explored to contribute for a better understanding of the challenge that Botryosphaeriaceae-related diseases represent in a global change scenario.

Table of contents

List of figures

List of tables

List of abbreviations

RPB2	second largest subunit of RNA polymerase II
	translation elongation factor 1-alpha
TUB2	beta-tubulin
ISO	International Organization for Standardization
ITS	internal transcribed spacer region of rRNA region
MAT	mating-type
ML	Maximum Likelihood
MP	Maximum parsimony

Chapter 1

Thesis outline

The main purpose of this thesis was to map and assess the risk of Botryosphaeriaceae species occurrence worldwide. Additionally, as a study case, several forest hosts in Portugal were selected to detect the regions affected by these pathogens, to investigate the possibility of occurrence on new hosts and to model the dispersion of these plant pathogens under different future climate change scenarios. To achieve that, this thematic was explored from different perspectives using several methods: from field surveys to molecular and phylogenetic characterizations or from worldwide database analyses to species distribution modelling and risk assessment.

In Chapter 2 we collected and organized worldwide Botryosphaeriaceae occurrences in a single cured dataset, allowing for the first time a complete perspective on species global diversity, dispersion, host association, ecological niches, pathogenicity and communication efficiency of new occurrences and new host-fungus associations.

Chapter 3 we evaluated the quality of the standards used for publication of new Botryosphaeriaceae taxa. We selected a list of 210 Botryosphaeriaceae species, as representative of new fungal species descriptions, and each description was evaluated and scored according to a set of questions divided in five major topics: Morphological characterization; Molecular characterization; Phylogenetic analysis; Host-fungus interactions and Accessible information.

Chapter 4 we explored the role of global changes impacts, especially climate change, on Botryosphaeriaceae-related diseases by mapping suitable areas for five Botryosphaeriaceae species, according to three different future climate change scenarios.

Chapter 5 we analyzed Botryosphaeriaceae occurrence on the main forest hosts in Portugal. A survey was conducted on main forest tree species in Portugal, *Quercus suber*, *Eucalyptus globulus* and *Pinus pinaster*. Additionally, a meta-analysis was performed to compile all records known from Portugal, and several pathogenic trials were performed to explore host-fungus associations and possible new hosts jumps.

Finally, in chapter 6, a general discussion of the thesis is presented, and future challenges are discussed. In the end of the dissertation, supplementary data used is discriminated, allowing the reader to search detailed and complementary information.

The thesis is organized in article format. Some of the chapters have been published or are submitted for publication:

Chapter 2: Batista E, Lopes A, Alves A. What Do We Know about Botryosphaeriaceae? An Overview of a Worldwide Cured Dataset. Forests. 2021; 12(3):313. https://doi.org/10.3390/f12030313

Chapter 3: Batista E, Lopes A, Alves A. How good are we at describing a new fungal species? A case study based on the family Botryosphaeriaceae.

Chapter 4: Batista E, Lopes A, Miranda P, Alves A. Modelling current and future global distributions of five Botryosphaeriaceae species.

Chapter 5: Batista, E., Lopes, A., Alves, A. Botryosphaeriaceae species on forest trees in Portugal: diversity, distribution and pathogenicity. Eur J Plant Pathol 158, 693–720 (2020). https://doi.org/10.1007/s10658-020-02112-8

Chapter 2

What we know about Botryosphaeriaceae? – Overview of a worldwide cured dataset

The contents of this Chapter have been adapted from:

Batista E, Lopes A, Alves A. What Do We Know about Botryosphaeriaceae? An Overview of a Worldwide Cured Dataset. Forests. 2021; 12(3):313. https://doi.org/10.3390/f12030313

2.1 Abstract

Botryosphaeriaceae-related diseases occur worldwide in a wide variety of plant hosts. Studies targeting the distribution, diversity, ecology, and pathogenicity of Botryosphaeriaceae species are consistently increasing. However, with the lack of consistency on species delimitation, name of host or location among studies it is almost impossible to quantify the presence of these species worldwide, or the number of different host-fungus interactions. In this review we collected and organized Botryosphaeriaceae occurrences in a single cured dataset, allowing for the first time a complete perspective on species global diversity, dispersion, host association, ecological niches, pathogenicity and communication efficiency of new occurrences and new host-fungus associations. This dataset is freely available through an interactive and online application. The current release (version 1.0) contains 14405 cured isolates and 2989 literature references of 12121 different host-fungus interactions with 1692 different plant species from 149 countries.

2.2 Introduction

Species of Botryosphaeriaceae (Botryosphaeriales, Ascomycetes) are distributed worldwide and are known to have different ecological roles. These fungi can act as saprobic, endophytic, or latent pathogens (Slippers and Wingfield, 2007; Phillips *et al.*, 2013). Some members of this family are recognized as aggressive plant pathogens on different types of hosts. From agricultural crops to ornamental and forest hosts, these fungi have no boundaries (Trakunyingcharoen *et al.*, 2014; Linaldeddu *et al.*, 2015; Moricca *et al.*, 2016; Marsberg *et al.*, 2017; Mehl *et al.*, 2017b; Zlatković *et al.*, 2018). Their wide distribution, the ability to persist endophytically. Becoming pathogenic only when their hosts are under stress. Causing diseases that eventually may lead to host death, and the capability to adapt and colonize new hosts (Slippers and Wingfield, 2007; Batista *et al.*, 2020), turns these organisms into a big challenge for plant pathology in a changing world (Anderson *et al.*, 2004; La Porta *et al.*, 2008; Elad and Pertot, 2014).

Several species of Botryosphaeriaceae currently accepted have been described in the XIX century, as for example *Diplodia mutila* (1834), *Diplodia seriata* (1845), *Botryosphaeria dothidea* (1863), *Diplodia sapinea* (1870), among others. At that time, the description of new species, as well as the taxonomic position of the family Botryosphaeriaceae, was exclusively based on their micromorphological characteristics. Therefore, through years the taxonomic position of these organisms suffered multiple classifications (for a historical overview see (Phillips *et al.*, 2013)).

With the use of DNA sequencing and phylogenetic methods our understanding of the taxonomy and diversity of Botryosphaeriaceae species changed profoundly over time. Since 1996 several authors based on sequence data studied the phylogenetic relationships of this family and currently 20 genera and 280 species have been described (Crous *et al.*, 2006; Schoch *et al.*, 2006; Phillips *et al.*, 2008; Liu *et al.*, 2012; Phillips *et al.*, 2013; Slippers *et al.*, 2014; Dissanayake *et al.*, 2016; Phillips *et al.*, 2019).

Extensive surveys reporting diversity, distribution, and pathogenicity of Botryosphaeriaceae species have been performed in several countries providing valuable information in terms of frequency and diversity of hosts. As examples: Australia (Burgess *et al.*, 2019), Algeria (Mahamedi *et al.*, 2020), Brazil (Netto *et al.*, 2014; Rosado *et al.*, 2016), China (Xu *et al.*, 2015; Li *et al.*, 2018), Portugal (Batista *et al.*, 2020), United States of America (Inderbitzin *et al.*, 2010; Chen *et al.*, 2014), Serbia, Montenegro, Bosnia and Herzegovina (Zlatković *et al.*, 2016), South Africa (Mehl *et al.*, 2017b; Osorio *et al.*, 2017) and many others.

Information regarding these host-fungus interactions is rising (Slippers *et al.*, 2017). However, due to lack of consistency on the name of fungus, name of the host or even on the location, it is almost impossible to quantify the presence of these species worldwide or the number of different host-fungus interactions. Our review attempts to gather and standardize all in-formation found in the NCBI nucleotide database and all host-fungus interactions available in the U.S. National Fungus Collections. This information was cured

and organized to be easily available through a shiny interactive application.

2.3 Data analysis and extraction

2.3.1 Data extraction from Nucleotide – NCBI database

An initial query was performed on 12-05-2020 in Nucleotide – NCBI database[1] using the search term "Botryosphaeriaceae [Organism]" with the R package rentrez (Winter, 2017). On total, 49955 sequences were retrieved. Information such as organism, strain/culture collection, host, geographical coordinates, country, and title of publication were also extracted (Figure 2.1).

Screening was performed by removing duplicates and records without a strain or culture collection number. For each isolate, when available, sequences from the internal transcribed spacer region of rRNA region (ITS), translation elongation factor 1-alpha *TEF1* - α, *TUB2*, second largest subunit of RNA polymerase II (*RPB2*) and two alternate forms of the mating-type (MAT) locus (MAT1-1-1 and MAT1-2-1) were selected.

All sequences were grouped by the strain or culture collection number and all features were manually standardized. Special characters were removed from the strain/culture collection feature and organized by the main culture collections. Geographical coordinates were transformed to the decimal form of the WGS84 geodetic datum and countries names were organized according to the International Organization for Standardization (ISO) reference system. Host names were cured according to the Catalogue of Life: 2019 Annual Checklist from the CoL+ project (Bisby *et al.*, 2010). Climate variables were extracted from the CHELSEA project[2] only for records with geographical coordinates (Karger *et al.*, 2017). The organism name was verified and updated according to recent literature and a sequence quality screening was performed by running a pairwise blast analysis between the ITS of each isolate against the sequence of the type of each genus. Isolates with a similarity lower than 94.3% were removed (Vu *et al.*, 2019).

2.3.2 Data extraction from U.S. National Fungus Collections

A query was performed by genus *(Alanphillipsia, Barriopsis, Botryobambusa, Botryosphaeria, Cophinforma, Diplodia, Dothiorella, Endomelanconiopsis, Eutiarosporella, Lasiodiplodia, Macrophomina, Marasasiomyces, Mucoharknessia, Neodeightonia, Neofusicoccum, Neoscytalidium, Oblongocollomyces, Phaeobotryon, Sardiniella, Sphaeropsis)* on 12-05-2020. Data regarding Fungus – Hosts interactions was extracted and organized by country, year, and citation. In total, 22698 host-fungus interactions were extracted. Similarly, to the previous screening, duplicates were removed, and all features were standardized by the same rules (Figure 2.1).

[1] https://www.ncbi.nlm.nih.gov/
[2] https://chelsa-climate.org/

2.3.3 MDRBOT Database and Shiny Interface

A literature review was performed, and both datasets were analyzed to fill missing information. Several extra organization level features were built to allow different filter functions. A shiny interface was created to allow an easy access to both datasets.

2.3.4 The Site

The MDRBOT database[3] was built using R 3.6.0 with a web shiny interface. The website includes: a search engine to the cured Botryosphaeriaceae isolates from the Nucleotide – NCBI database and to the host-fungus interaction dataset where the user can perform multiple field search and download the output as an excel format file. A worldwide map generator tool for species occurrence is available, where the user can select an input species and generate a world occurrence map. A climate data analyses is included, where the user can select an input species and observe isolates with valid geographical information and to evaluate the main climate variables associated to these records (minimum, average and maximum annual temperature, and minimum, average, and maximum annual precipitation). The option to download the output maps as a portable network graphics file of both occurrence and climate data analyses is also available.

[3] https://mdr-bot-cesam-ua.shinyapps.io/bot_database/

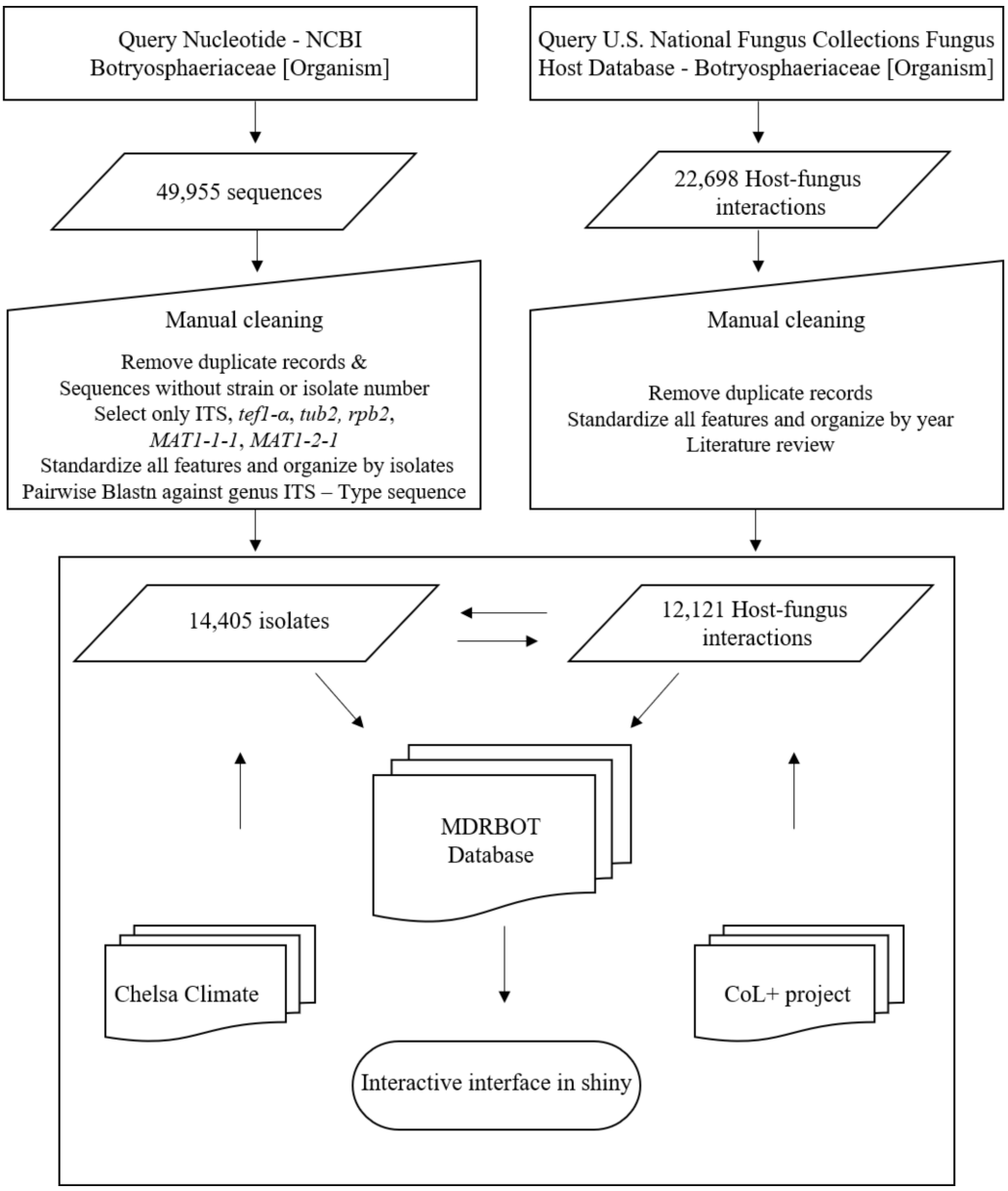


Figure 2.1: Workflow overview to cure and organize data extracted from the Nucleotide – NCBI database and the U.S. National Fungus Collections. Final output originated the Map Detect and Research BOTryosphaeriaceae database that can be access through an interactive shiny interface.

2.4 Diversity vs sampling effort. How much do we really know?

Despite all the efforts to characterize this fungal family it is impossible to evaluate global diversity of these organ-isms due to different levels of sampling effort across countries. For that reason, we compared diversity with countries sampling effort using the location of isolates from the Nucleotide - GenBank collection. In this analysis we consider that data in Nucleotide is representative for sampling effort. We found that 138 countries still have no records of Botryosphaeriaceae species deposited in GenBank (represented in white in figure 2.2) and 66% of the countries with records with less than 51 isolates. Concentrating 80% of all isolates in only 11 countries: China (1810 isolates), United States of America (1310), South Africa (1141), Brazil (1077), Australia (796) Italy (622) Iran (439), India (412), Spain (347), Malaysia (324) and Portugal (311). In a similar way, diversity tends to increase with sampling effort suggesting that we are still far away from reaching a plateau: China (72 species), United States of America (55), South Africa (62), Brazil (42), Australia (57) Italy (51) Iran (35), India (28), Spain (31), Malaysia (17) and Portugal (23). In figure 2 we produced a bi-variate world map to evaluate countries diversity vs sampling effort. It is clear the lack of data for several regions of the globe predominantly in Africa, Central and Western Europe, North, Central and Western Asia, Bolivia, and several countries in the Caribbean region.

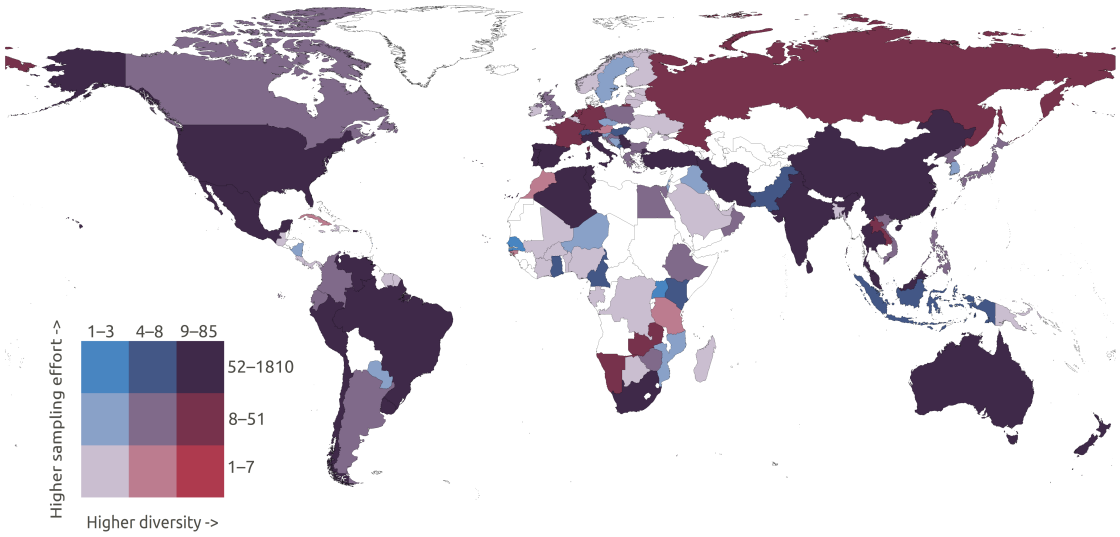


Figure 2.2: Bi-variate world map analyzing diversity vs sampling effort of Botryosphaeriaceae isolates. Data obtained from Nucleotide-GenBank (a total of 14405 isolates: 12593 with location and 1812 isolates without location). Countries in both variables were divided in quartiles with equal probability of occurrence (e.g., for species diversity the probability to randomly select a country is equal for the intervals with 1-3, 4-8 and 9-85 species) to facilitate visualization.

2.5 Worldwide occurrence – from where to where?

Evolutionary divergence studies show that Botryosphaeriaceae lineages emerged during the late Cretaceous period, over 66 million years ago, in a period dominated by expansion of angiosperms occupying environments previously dominated by conifers. It is hypothesised that evolution of modern Botryosphaeriaceae species was driven by the evolution and diversification during the Palaeocene epoch of their hosts, currently what we know as modern plants (Slippers *et al.*, 2013; Phillips *et al.*, 2019). Several authors investigated the origin of some species by performing population studies at the global scale among different hosts and did not identify an obvious phylogeographic origin (Burgess and Wingfield, 2002; Burgess *et al.*, 2004; Bihon *et al.*, 2012; Phillips *et al.*, 2013; Sakalidis *et al.*, 2013; Salahlou *et al.*, 2016; Mehl *et al.*, 2017a).

It is known that infection and colonization of hosts can occur through natural wounds on leaves, branches or stems and by other openings like lenticels and stomata. The distribution of these organism is favoured by the sticky spores dispersed by wind, rain, and insects (Slippers and Wingfield, 2007). At the intercontinental level, Human movement, and international trade of plants and derivates (timber and non-timber products) without appropriate quarantine measures leveraged the dispersion of these organism (Slippers *et al.*, 2017). Within this family several species are known to have limited distributions and a few species like *Botryosphaeria dothidea*, *Diplodia sapinea*, *D. seriata*, *Dothiorella sarmentorum*, *Neofusicoccum parvum* and *Lasiodiplodia theobromae* are recognized to be globally distributed (Phillips *et al.*, 2013; Dissanayake *et al.*, 2016; Mehl *et al.*, 2017a). To understand the distribution of the main Botryosphaeriaceae species we analysed the spatial distributions among continents and terrestrial ecoregions (Olson *et al.*, 2001; Dinerstein *et al.*, 2017). Within the studied species, so far, only *Diplodia corticola* and *Neofusicoccum mangiferae* are not reported in all continents. This observation confirms the ability of the remaining ones to spread globally. However, if we take in consideration the different terrestrial ecoregions, we can observe that some species are reported only in certain ecoregions. Based on occurrence data, we suggest a latitudinal shift among different types of climates where some species are clearly concentrated in some types of ecosystems. As example *Do. sarmentorum*, *D. corticola*, *D. mutila*, *N. australe*, *D. seriata* and *D. sapinea* found only in Temperate and Mediterranean ecosystems and others with a wider range of dispersion. *Neofusicoccum parvum* in our analysis appears to be the most adapted organism being detected from North to South with exception of boreal forests and montane grasslands. Boreal forests appear to be the most unlikely place to find Botryosphaeriaceae species, so far, only *D. sapinea* has been reported in this region (Figure 2.3).

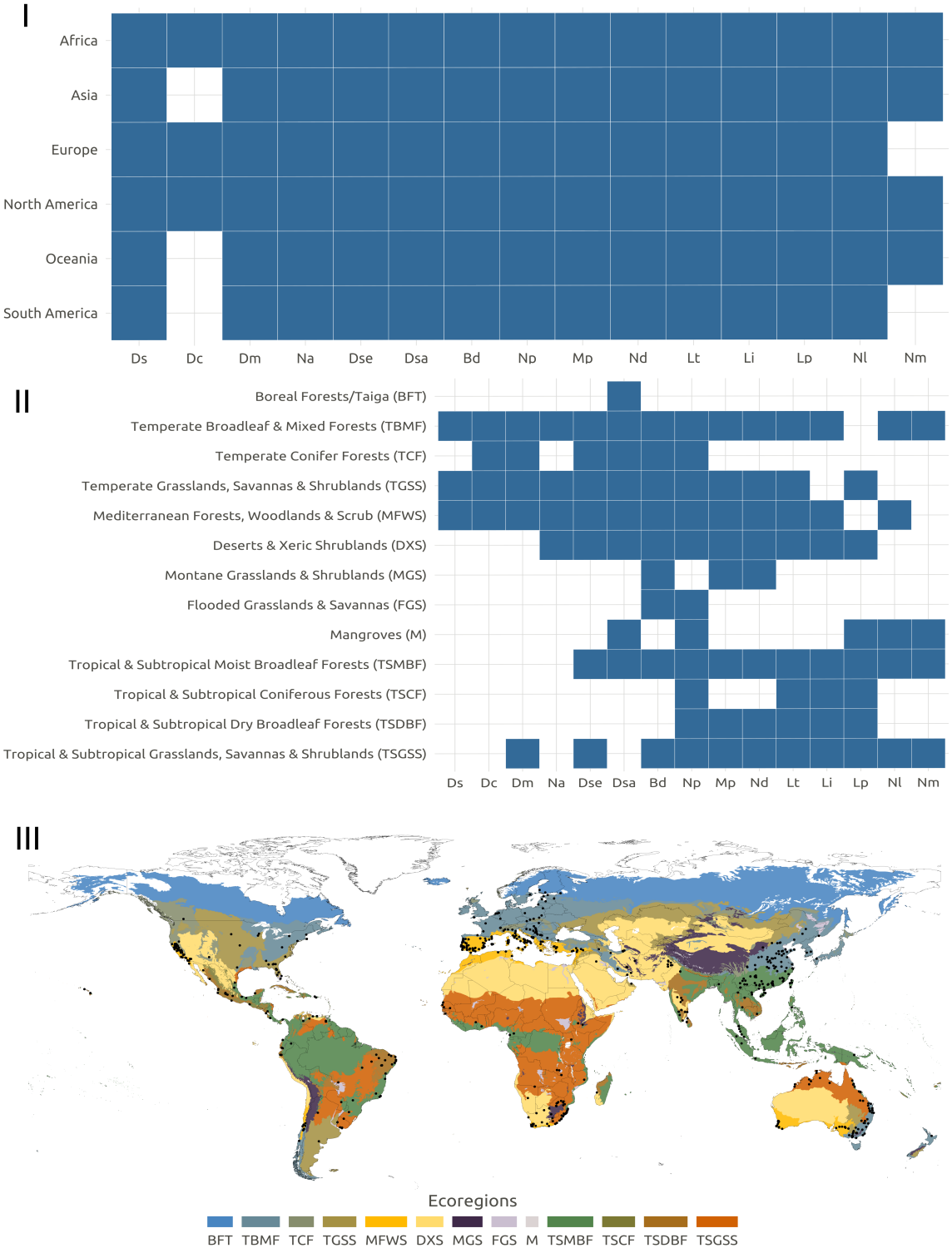


Figure 2.3: Worldwide occurrence of the main Botryosphaeriaceae species (*B. dothidea* (Bd), *D. corticola* (Dc), *D. mutila* (Dm), *D. sapinea* (Dsa), *D. seriata* (Dse), *Do. sarmentorum* (Ds), *L. iranensis* (Li), *L. pseudotheobromae* (Lp), *L. theobromae* (Lt), *M. phaseolina* (Mp), *N. australe* (Na), *Ne. dimidiatum* (Nd), *N. luteum* (Nl), *N. mangiferae* (Nm), *N. parvum* (Np)). Data for continental occurrence was obtained from literature. Points represent isolates with valid geographical coordinates from literature and GenBank. A total of 786 geographical references were used.

2.6 Understanding the process of host-jumps - can we spot host specificity?

Defining a host range and understand which factors favour future host-jumps is essential to study emerging and re-emerging fungal pathogens. Several drivers are often mentioned in the literature such as international trade, failure of quarantine and preventive measures, changes in land use or agricultural practices, pathogen evolution and plasticity, mechanisms of genome divergence (e.g., mutation, hybridization, sexual recombination and horizontal gene transfer and others), host-fungus genotype-by-genotype interactions, poor host health, climate change, among others (Burdon and Silk, 1997; Lambrechts, 2010; Brown and Tellier, 2011; Gange *et al.*, 2011; De Fine Licht, 2018; Corredor-Moreno and Saunders, 2020). Comparative genomics and omics studies are slowing unveiling host-fungus interaction mechanisms by dissecting the plant defence mechanism, the fungal pathogenic strategy and nutrient uptake pathways (Raffaele and Kamoun, 2012; Möller and Stukenbrock, 2017; Westermann *et al.*, 2017; Félix *et al.*, 2019; Han, 2019). To clarify a momentarily host range boundary and spot host specificity a complete overview of all mentioned areas is crucial.

Through time our knowledge regarding the biology and ecology of these pathogens is improving significantly. However, the mechanisms behind host-jumps and the worldwide extension of host association patterns across Botryosphaeriaceae genera are still poorly understood. In general, the most studied species lack host specificity and have proven capability to colonize and cause disease in diverse native and introduced plant hosts (Slippers and Wingfield, 2007; De Wet *et al.*, 2008; Jami *et al.*, 2017; Mehl *et al.*, 2017b; Pavlic-Zupanc *et al.*, 2017; Zlatković *et al.*, 2018; Liddle *et al.*, 2019; Batista *et al.*, 2020). Even species like *D. sapinea* and *D. corticola*, that have been consistently associated to a certain type of host, have been occasionally found to occur on other unrelated hosts in different regions of world (Lazzizera *et al.*, 2008; Barradas *et al.*, 2016; Zlatković *et al.*, 2017; Batista *et al.*, 2020).

To investigate possible host specificity, we compared the number of hosts against the total number of hosts of the genera, the number of isolates, the number of countries where these species were detected, the number of reports found in the literature and differences between the number of known associations with angiosperms and gymnosperms (Figure 2.4). Overall, *L. theobromae* is by far the organism with the largest number of known hosts (666 of 749 host reported for the genus *Lasiodiplodia*), with the largest number of isolates in GenBank (1944), the largest number of countries occurrence (97) and host-fungus reports (365). For the remaining species it is possible to observe, even with different research efforts in number of isolates or literature reports, that clearly some species have the capability to colonize an higher number of hosts (e.g. *N. parvum* with 223 hosts in 50 countries and *B. dothidea* with 403 hosts in 66 countries) and few have been consistently reported worldwide in a low number of hosts (e.g. *D. sapinea* was reported in 62 countries but only in 102 hosts, of which 83 are gymnosperms, or *D. seriata* with 121 hosts in 46

countries and *Lasiodiplodia pseudotheobromae* with 124 hosts in 44 countries).

However, a higher number of different hosts might be related only with the diversity of species among different plant functional groups and not with versatility to colonize different hosts from different plants groups. For example, the Pinopsida class also known as conifers have only 615 living species and the Liliopsida (monocotyledons) or the Magnoliopsida (dicotyledons) classes have an estimated 77267 and 246366 living species, respectively. To analyse host jumps among different functional groups we created a circular graph with shared hosts-fungus interactions where hosts were divided in different plants groups (Figure 2.5). Not surprisingly, *L. theobromae* shows capability to colonize hosts in all plant groups. However, *L. pseudotheobromae* and *N. parvum* with a considerably lower number of known hosts present a similar pattern of shared hosts-fungus interactions suggesting a clear capability to increase their host range in a similar way as *L. theobromae*. For other fungi, it is possible to explore range expansions: *N. mangiferae* and *L. iraniensis* have recently been described in several members of the Malvids group and occasionally found in other groups. *Diplodia sapinea* consistently described in Pinales species and *D. corticola* in *Quercus* species from the Fabids group but both are also starting to be found in different plant groups. These types of analyses can be useful for institutions and national authorities to guide their studies and to anticipate future hosts-jumps, especially for highly pathogenic organisms. Notably few studies have proven host specialization among different Botryosphaeriaceae species based on differences of pathogenicity-related genes (e.g., *B. dothidea* versus *Botryosphaeria kuwatsukai*) (Wang *et al.*, 2018).

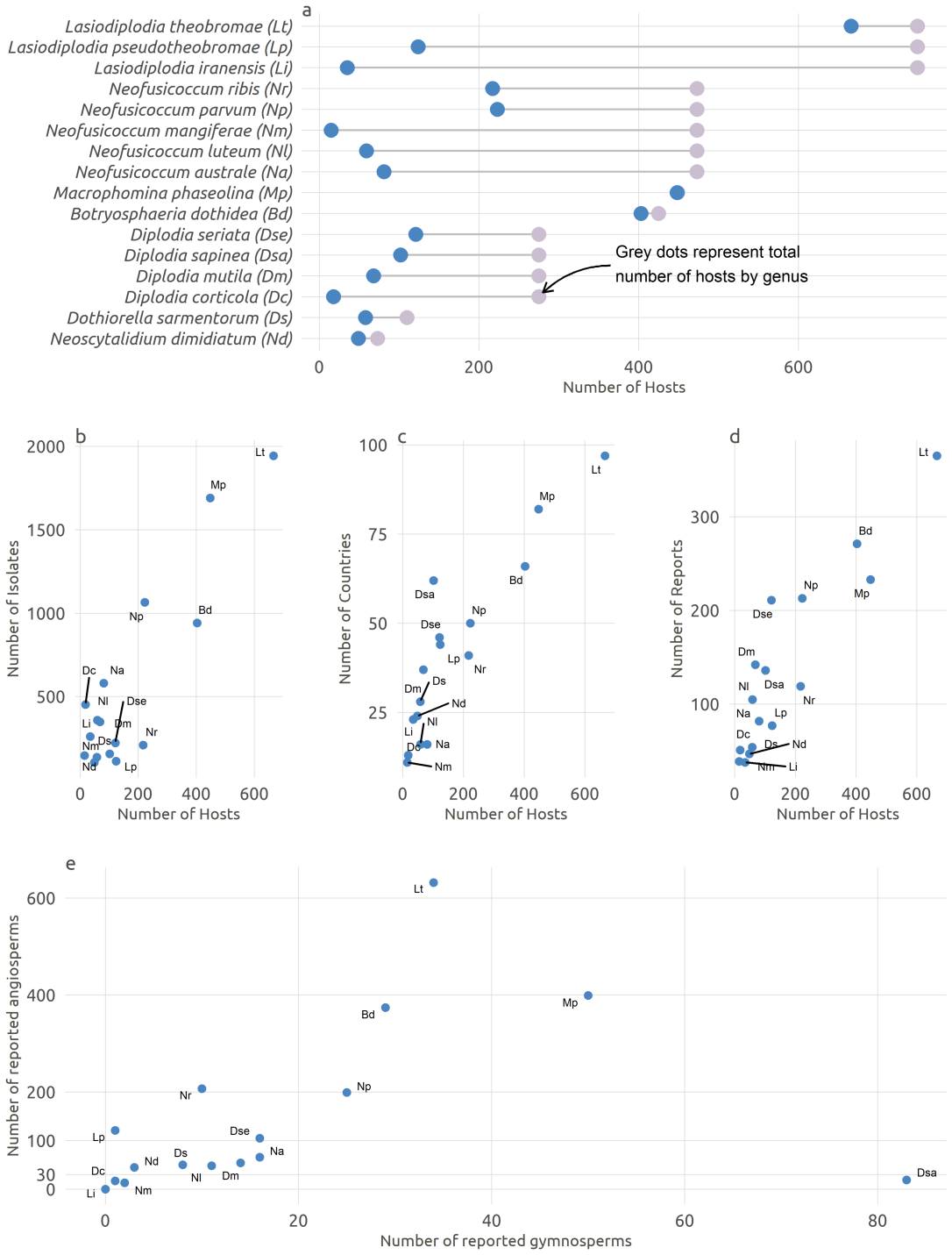


Figure 2.4: Host-fungus overview (I) Comparison of number of hosts by species within genera, (II) number of isolates versus number of hosts, (III) number of countries vs number of hosts, (IV) number of reports vs number of hosts and (V) number of known host associations with angiosperms vs gymnosperms. Data was collected from GenBank and literature review. Only host-fungus interactions identified to the species level were selected

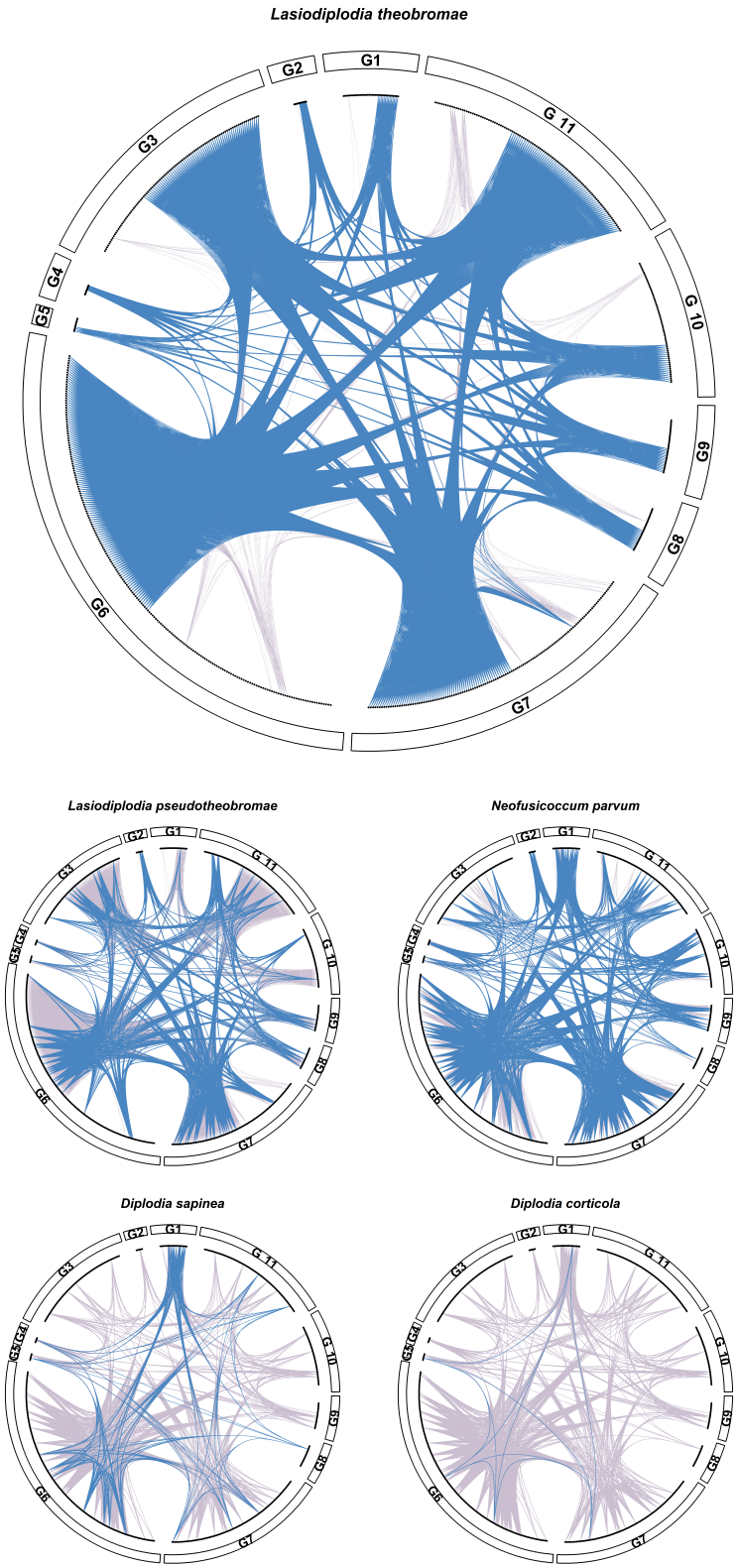


Figure 2.5: Shared hosts interactions worldwide based on GenBank and literature review. Nodes represent hosts genera and are grouped by taxonomic similarity. G1: Pinales and Ginkgoales, G2: Magnoliids, G3: Monocots, G4: Eudicots, G5: Superrosids and Rosids, G6: Fabids, G7: Malvids, G8: Superasterids, G9: Asterids, G10: Campanulids, G11: Lamiids. Lines represent host-fungus interactions, where background lines represent all known interactions of the respective Botryosphaeriaceae genus, blue lines represent known interactions of the respective Botryosphaeriaceae species.

2.7 How much do we know about pathogenicity and plant mortality?

Uncovering the complexity of a host-pathogen interaction is not a stationary science and depends on multiple variables from the environment and the interaction of both host-pathogen genomes artillery (Gururani *et al.*, 2012; Hossain *et al.*, 2019). Host-pathogenic interactions are similar to a chess game where for a specific outcome several actions are possible from each organism. Reproducibility of these interactions under controlled conditions might not always be representative of what occurs in nature and common pathogenicity trials do not fully expose pathogenic and resistance mechanisms (Manawasinghe *et al.*, 2016; Félix *et al.*, 2017). Nonetheless, sequence and annotation of both genomes opens the possibility for multi-omics analyses to provide a more complete overview of these interactions (Westermann *et al.*, 2016; Westermann *et al.*, 2017).

Pathogenicity of Botryosphaeriaceae isolates leading to plant mortality has been demonstrated under controlled conditions, mainly in seedlings, in well-watered conditions or under drought stress (Linaldeddu *et al.*, 2007; Pitt *et al.*, 2013a; Batista *et al.*, 2020). However, plant mortality in nature is often a combination of multiple biotic and abiotic stresses. On one hand, abiotic factors like drought or heat stress can disrupt plant physiological performance allowing the colonization of fungal pathogens and increasing disease susceptibility. On the other hand, colonization by fungal pathogens can reduce tolerance to biotic stress leading to higher mortality rates (Allen *et al.*, 2010; Wang *et al.*, 2012; Caldeira, 2019; Hossain *et al.*, 2019). The interaction effects of drought and fungal pathogen infection on plant mortality are resumed in Figure 2.6.

Several factors can induce mortality: carbon starvation when non-structural carbohydrates resources are depleted affecting the normal plant maintenance, growth, and defence mechanisms (Li *et al.*, 2019); toxic effect of fungal metabolites and, hydraulic failure of xylem water transport to the leaves due to embolism or phloem transport caused by an impaired xylem water potential (Oliva *et al.*, 2014). These factors can be directly or indirectly induced by both biotic and abiotic stresses. For example, drought and heat stress can induce stomata closure and simultaneously decrease carbon assimilation that is essential to maintain plant defence metabolism and functional sapwood maintenance. Pathogen infection can directly impact carbon assimilation by down-regulating genes involved in photosynthetic activities or trigger carbon starvation by inducing tree defences and/or by inhibiting the expression of genes involved in carbon metabolism and transport. Also, when these pathogens colonize the plant vascular tissues and vascular necrosis occur the whole-plant hydraulic conductance is reduced increasing the risk of hydraulic failure (Oliva *et al.*, 2014).

Genome and transcriptome analysis of some Botryosphaeriaceae species have shown a higher number of pathogenicity-related genes associated to cell wall degradation, nutrient uptake, secondary metabolism, and membrane transport functions, that are important for woody plant infection, when compared with other fungus with different life-styles (Wang *et*

al., 2018; Yan *et al.*, 2018). Also, several authors have demonstrated that during infection gene families related with carbohydrate catabolism, pectin, starch and sucrose metabolism, and pentose and glucuronate interconversion pathways were induced (Massonnet *et al.*, 2018; Gonçalves *et al.*, 2019; Ali *et al.*, 2020). Some of these genes' families were even induced by higher temperatures (Yan *et al.*, 2018; Félix *et al.*, 2019).

Furthermore, some species were proven to have the capability to exhibit cytotoxicity against mammalian cell lines and again temperature was suggested to modulate the expression of toxic compounds (Félix *et al.*, 2016; Félix *et al.*, 2019; Pour *et al.*, 2020).

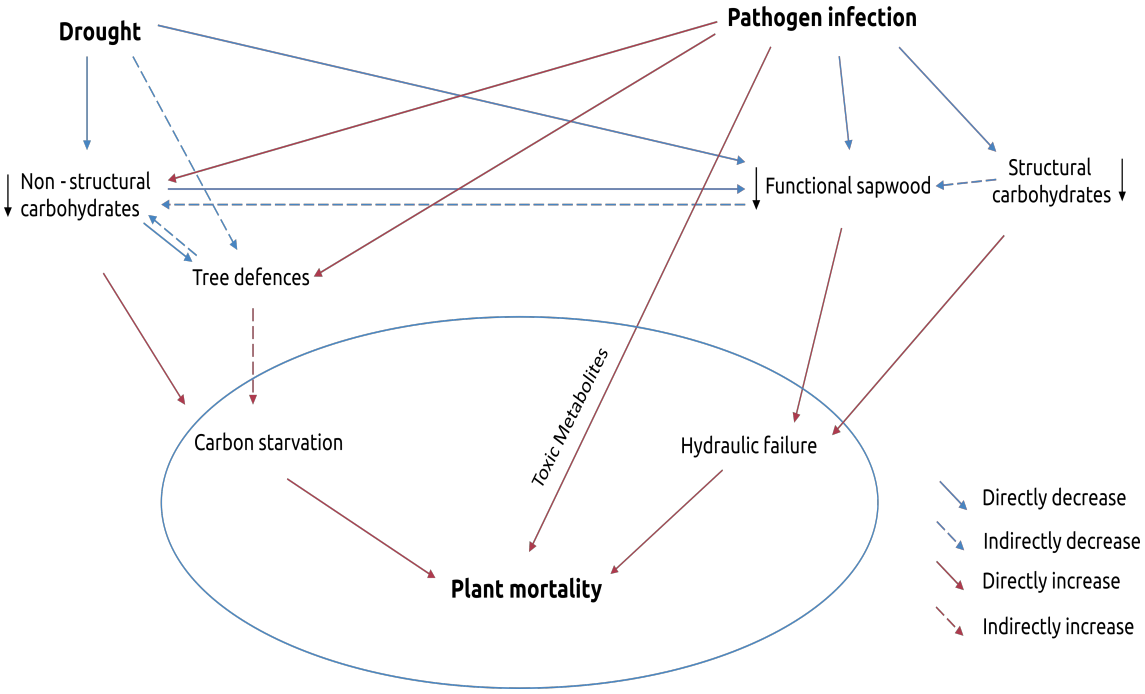


Figure 2.6: Combined effects of drought and pathogen infection on plant functioning, growth, and mortality. Adapted from (Oliva *et al.*, 2014; Caldeira, 2019).

2.8 Climate sensitivity, a hidden pattern?

Temperature growth studies suggest that in general Botryosphaeriaceae species present minimal growth rates at 5 °C or over 35 °C and optimal growth rates around 15 – 25 °C under controlled conditions (Phillips *et al.*, 2013; Dissanayake *et al.*, 2016). So far, no major studies were performed to characterise the natural bioclimatic envelopes of Botryosphaeriaceae species. Based on the geographical coordinates obtained during this review we analysed climatic variability of these records (Figure 2.7). In terms of annual mean temperature, it is clear that *B. dothidea*, *D. seriata* and *Do. iberica* are often collected in places with lower annual mean temperatures when compared with *L. theobromae*, *M. phaseolina*, *N. parvum*, and *Ne. dimidiatum*. For annual precipitation, this pattern is not so clear, with exception of *L. theobromae*, where the majority of records were collected in places with more than 1000 mm of annual precipitation (typical of tropical regions). Temperature and precipitation seasonality are calculated by using the standard deviation of the mean monthly. Regions with larger standard deviation have greater temperature and precipitation variability across the year. These metrics are important to understand the tolerance range for species in the future. For example, a species that is often present in tropical regions with tolerance to higher annual mean temperatures but without tolerance to strong temperature seasonality, may have problems to expand the distribution range in the future to a region with strong temperature variability across the year, even if the mean temperature increases (e.g., can *L. theobromae*, often collected in tropical regions, expand its range to Mediterranean and temperate regions with climate change?)

These results are consistent with our ecoregions analysis suggesting that probably the known host-fungus interactions and species distribution is strongly dependent on climate. We encourage authors to provide precise coordinates for occurrence data to improve the understanding of Botryosphaeriaceae species distribution.

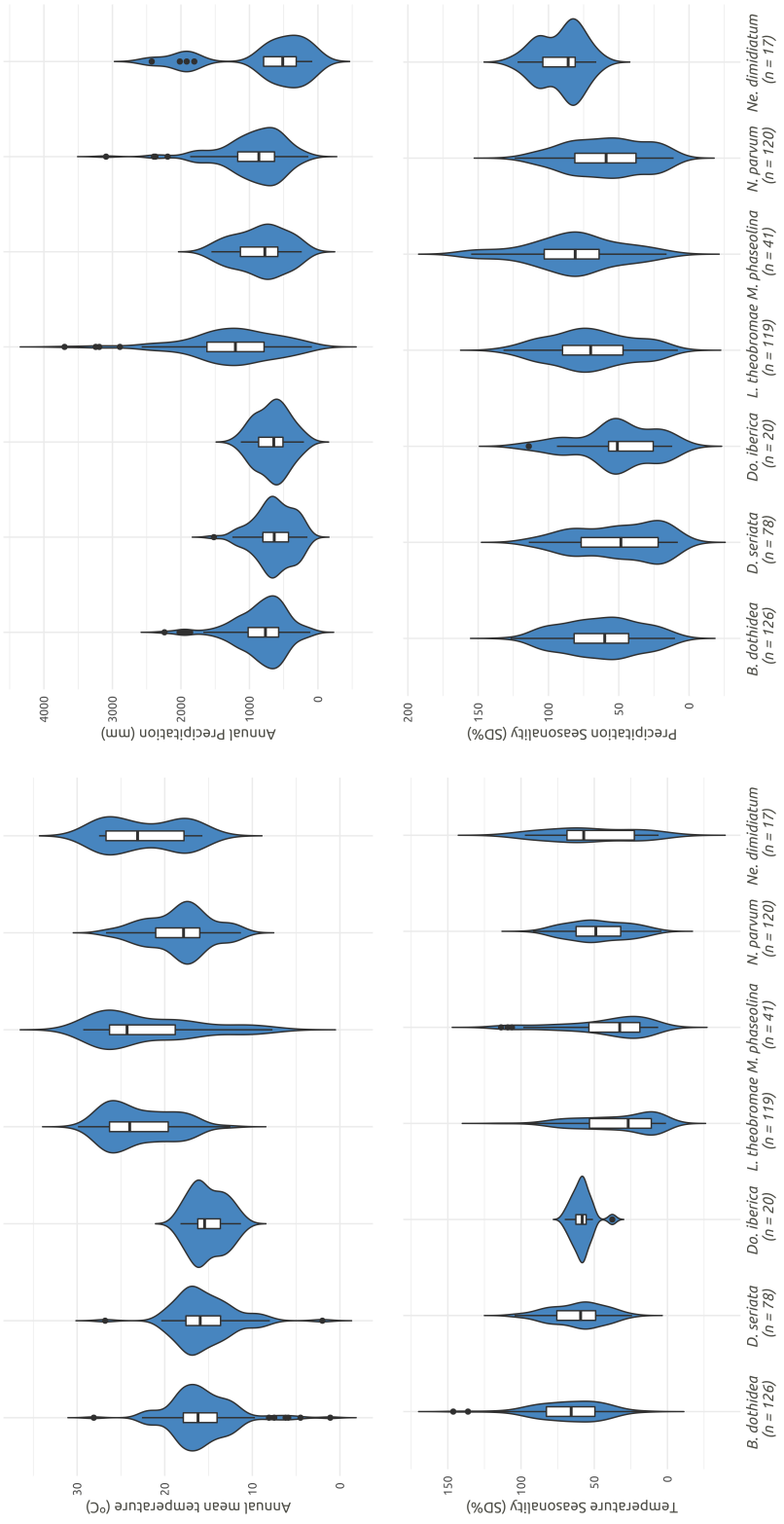


Figure 2.7: Annual mean temperature and precipitation seasonality for the main Botryosphaeriaceae species occurrence. Data was thin by 200km distance to avoid spatial autocorrelation using the R package ELSA (Naimi *et al.*, 2019). Climatic variables were extracted from the CHELSA project. Number of valid coordinates are indicated per species.

2.9 Global dispersion - How far can they go? - Framing ecological niche requirements for potential species distributions areas

As mentioned before, Botryosphaeriaceae species produce spores that are naturally dispersed through wind, rain, insects among other vectors. However, human induced activities are responsible for the majority of long-distance dispersion (Slippers and Wingfield, 2007; Slippers *et al.*, 2017). Taking in account the large number of potential hosts and the large quantity of plant material moving worldwide every day, it is virtually impossible to verify and detect efficiently latent pathogens living endophytically in symptomatic or asymptomatic material (Burgess *et al.*, 2016; Crous *et al.*, 2016; Slippers *et al.*, 2017). Thus, understanding the ecological niche requirements for potential species distributions areas might be a better solution to predict and prevent future outbreaks (Bosso *et al.*, 2017).

We propose an adaptation of the classical BAM diagram (Figure 2.8 I) to explain the influence of environmental conditions, biotic interactions, and dispersal in shaping species geographic distribution (Pulliam, 2000; Soberón and Peterson, 2005; Peterson *et al.*, 2011). Following the classical BAM diagram, B represents the geographic regions where the interaction factors with other species are favourable for species occurrence, A represents the geographical regions where the climatic conditions are favourable to maintain a viable long-term population and M correspond to the geographical region accessible to the species dispersion. However, for endophytes and latent pathogens this perspective does not fully represent the ecological dynamics of these organisms. We assume that: (1) Endophytic latent pathogens are mainly dispersed by human activities like movement and trade, (2) The introduction of a species in a new environment is likely to occur as human movement/trade exist and is favoured by lack of preventive and quarantine measures, therefore M is virtually unlimited (3), The establishment of these species is affected by climate. Nonetheless, unfavourable conditions might hide the presence of those species in asymptomatic hosts or by resistance structures. Seasonal effects might expand or decrease the growth of these organisms invalidating viable long term populations, (4) Optimal conditions for disease expression are mainly occasional climatic events that can affect the susceptibility of the host (i.e. Reduction of precipitation or/and temperature increments causing drought or heat stress to the host (Ragazzi *et al.*, 1999; Allen *et al.*, 2010; Barradas *et al.*, 2018)) or/and trigger the pathogenic behaviour of these organisms (i.e. variations in temperature, light intensity or atmospheric ozone inducing phytopathogenic mechanisms (Eastburn *et al.*, 2011; Herrera-Estrella and Horwitz, 2007; Félix *et al.*, 2016; Félix *et al.*, 2019; Pour *et al.*, 2020)), (5) Only when host-specificity is demonstrated, whether for a fungal species with limited ability to colonize and persist endophytically in certain hosts or/and for species with limited ability to infect and express disease symptoms in a certain type of hosts, is assumed that biotic interactions (B) can shape the geographical distribution (Figure 2.8

II).

As a result, for non-specific endophytes and latent pathogens, like many of the Botryosphaeriaceae species, assuming an imperfect quarantine system worldwide, climate is the main variable to constrain the geographical distribution of these organisms.

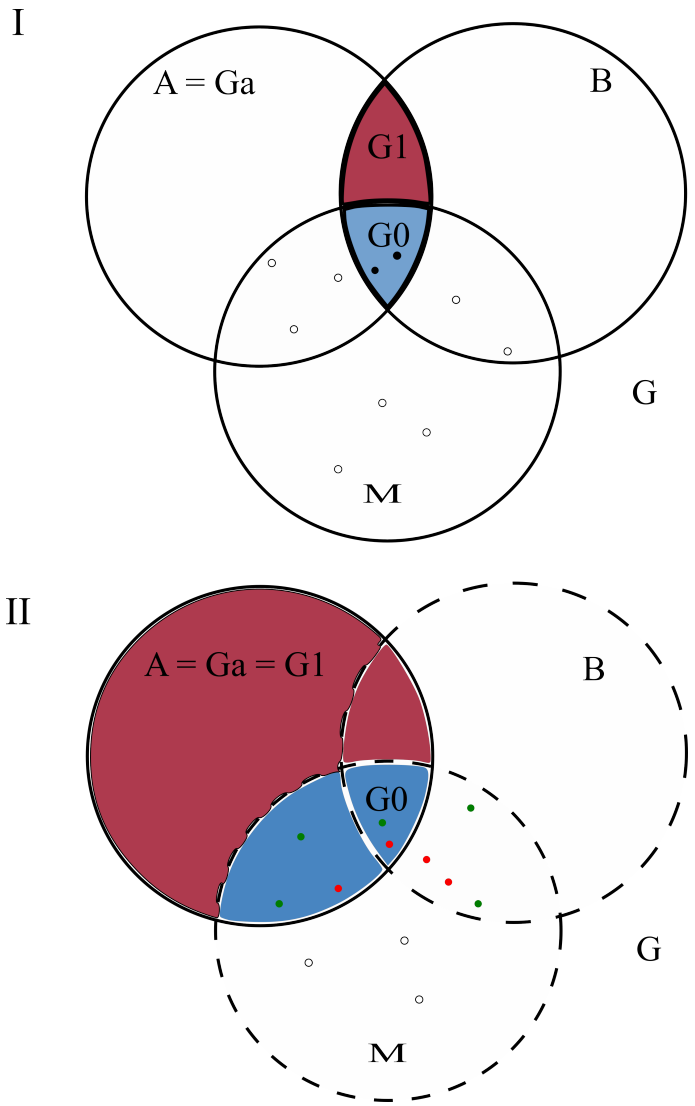


Figure 2.8: I) Representation of the classical BAM diagram adapted from (Peterson *et al.*, 2011). (II) Variation of the BAM diagram to represent endophytes and latent pathogens like Botryosphaeriaceae species. Three factors are suggested to define species geographic distribution biotic (B), abiotic (A), and movement (M). By interactions among these factors four areas can be defined: G the geographic space within which analyses are developed, Ga the abiotically suitable area, G0 the occupied distributional area, and G1 the invadable distributional area. Black solid circles indicate species occurrence, red solid circles occurrence in symptomatic hosts and green solid circles occurrence in asymptomatic hosts and open circles indicate absences.

2.10 How good are we at reporting new occurrences and host-fungus associations?

Despite the increase of new Botryosphaeriaceae-related studies worldwide, there is still a lack of standard databases that are consistently curated and maintained through time. The failure of efficiently report new occurrences and host-fungus associations increases the gap between science and society and dilutes the scientific effort to improve preventive and quarantine policy measures. Information regarding this fungal family is often reported in indexed scientific journals and should be followed by public sequence data that allow the scientific community to validate and confirm the taxonomic identification of these organisms. If information only present in literature might be outdated and difficult to verify, information only presented in GenBank or similar databases is also susceptible to be lost without a proper report. Ideally, a report of a new occurrence or a new host-fungus interaction should be documented in literature and supported with public genomic data. These data should be well-organized in public databases to allow national institutions like customs or agricultural/forestry authorities to be prepared for possible new threats.

In figure 2.9 we compared country occurrences and host-fungus interactions by country that are reported only in literature, in both literature and GenBank and only reported in GenBank. We also classified countries with the percentage of information that is not properly reported. We found that only 53.69% of the species occurrence by country is reported in both datasets and if we take in consideration host-fungus interactions by country only 23.07% of the current knowledge is well reported. Records prior to the massification of the DNA-based methodologies (older than the year 2000) are often poorly reported but there is also a large number of recent studies that fail to provide consistent information of host and location even when public genomic data is available.

2.11 Conclusions and future perspectives

Our database represents a single effort to clean and organize all Botryosphaeriaceae-related occurrences. This database will be continuously maintained and researchers working with these organisms are welcome to submit or update their information. Major updates on the source information are expected to happen every January and new features may be added over time as a result of users' feedback.

With the insights of a worldwide cured dataset, we verify a consistent growing interest of these plant-pathogenic fungi when taking in account the number of public records, publications, and the citation history of papers over the years (Slippers *et al.*, 2017). However, we demonstrate that 80% of all isolates with public sequence data is concentrated only in 11 countries, leading to a huge knowledge gap on Botryosphaeriaceae occurrence and diversity worldwide. Also, even the countries with a higher sampling effort are still far away from reaching a plateau on species diversity versus sampling effort, suggesting a high number of undescribed species. The problematic of estimating species numbers is

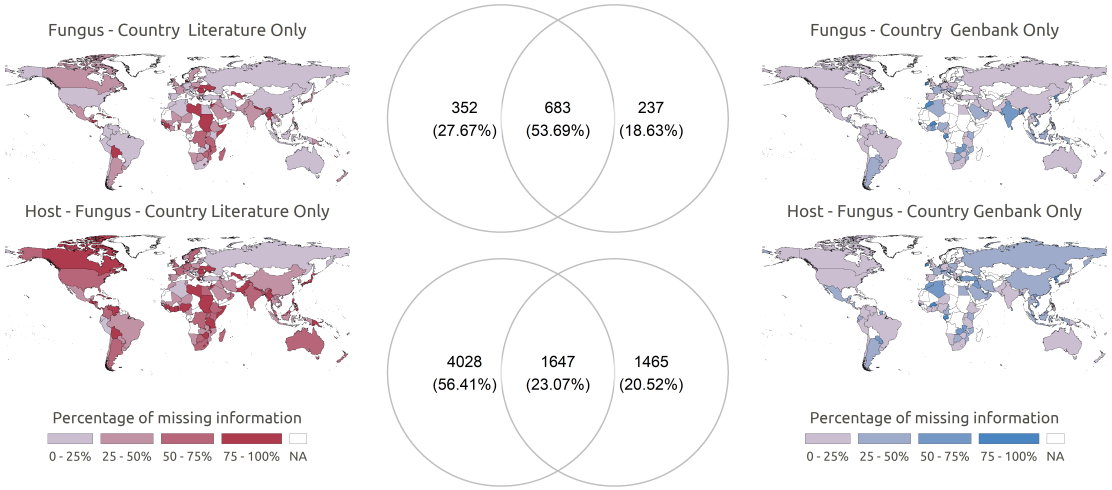


Figure 2.9: Worldwide percentage of missing information in both literature and GenBank datasets by country for occurrence and host-fungus interactions.

a common issue to other fungal families (Hyde *et al.*, 2020) raising the concern to fully understand the limit of a species boundary and to proper identify cryptic species on species complex as well the recognition of the hybridization phenomenon (Sakalidis *et al.*, 2013; Cruywagen *et al.*, 2017; Rodrıguez-Gálvez *et al.*, 2017).

In terms of ecology, we reflect about the climate influence on the distribution and dispersion of these organisms for the first time. Despite the worldwide distribution among all continents, with exception for Antarctica, was possible to observe a climatic preference for some species (e.g., *D. corticola*, *Do. sarmentorum* or *N. australe* by temperate and mediterranean regions) and other species with a wider tolerance from temperate to tropical regions (e.g., *B. dothidea*, *N. parvum* or *M. phaseolina*). In terms of dispersion, we propose a new framework to define the ecological niche requirements for most fungal latent endophytes. This framework is essential to improve our understanding of the current species distributions areas and to forecast future disease outbreaks (Bosso *et al.*, 2017). We assume that human movement and trade are the main dispersion routes of these organisms, being climate variability, the major constrain for the occurrence of new stable populations. Also, we highlight that disease expression is mainly due to occasional climatic events that can affect the susceptibility of the host. Raising the importance to sample asymptomatic hosts for an early detection of new species occurrence (e.g., *Diplodia insularis* was reported for the first time in Portugal in an asymptomatic host (Batista *et al.*, 2020)).

To finalize, we evaluate the consistency of known species occurrence and host associations reports in both indexed scientific journals and public sequence databases. We demon-

strate the incapability of our society to efficiently use and aggregate data of these emergent plant-pathogens. More than ever, we consider that consistent and open plant pathology databases are fundamental to address the challenge of Botryosphaeriaceae-related diseases in a changing world.

Chapter 3

How good are we at describing a new fungal species? A case study based on the family Botryosphaeriaceae

The contents of this Chapter have been adapted from: Batista, E., Lopes, A. Alves, A. How good are we at describing a new fungal species? A case study based on the family Botryosphaeriaceae (Dothideomycetes). Mycol Progress 21, 40 (2022). https://doi.org/10.1007/s11557-022-01796-y

3.1 Abstract

Best practices for describing a new fungal species is a topic often discussed by several authors. However, to our knowledge, no studies have evaluated the quality of standards used for publication of new taxa. We selected a list of 210 representatives of the family Botryosphaeriaceae, and their descriptions were evaluated and scored according to a set of questions divided in five major topics: morphological characterization; molecular characterization; phylogenetic analysis; host-fungus interactions and information accessibility. Based on our definition of best available practices, we found that over 90% of the descriptions are followed by a detailed morphological characterization and with consistent phylogenetic analyses, for molecular characterization and host-fungus interactions 60% of the descriptions are outdated or only meet the minimal requirements for publication, and 50% of the authors do not provide enough accessible and reproducible information. We verified that there is still room for improvement and the lack of formal standards over time do not follow a steady progress. Establishing well-defined best practices for new fungal species descriptions is crucial to ensure reproducibility, transparency, and consistency over time. Our goal is to raise awareness on what should be the minimal quality standards to describe a new fungal species within the Botryosphaeriaceae family.

3.2 Introduction

Fungi are the second most species-rich group of organisms after insects and cataloguing all this diversity before extinction is one of the biggest challenges for fungal taxonomists (Purvis and Hector, 2000). Over time, The International Commission on the Taxonomy of Fungi (ICTF) has provided publication requirements and best practices for describing a new fungal species (Sigler and Hawksworth, 1987; Seifert and Rossman, 2010; Aime *et al.*, 2021). These guidelines are essential for standardizing new taxon descriptions. However, the quality of these descriptions and the definition of minimal criteria for publication are often not well-defined and might vary among different fungal groups. Taxonomists working with fungi from different phyla have selected different criteria to describe a new species, making comparisons between groups difficult (Xu, 2020).

In plant pathology, fungal taxonomists are essential to define the language of communication about different organisms among scientists and society in general (Hibbett and Taylor, 2013). More than ever, to address the challenges of fungal diseases in plants in a changing world, an efficient communication is needed. In a recent study, we demonstrated that for Botryosphaeriaceae members only 23% of the known host–fungus interactions by country are reported simultaneously in peer review articles and with DNA sequences deposited in public databases like GenBank (Batista *et al.*, 2021).

Currently, the golden standard for fungal species delimitation is the genealogical concordance approach whereas concordance between multiple unlinked loci is used to assess species boundaries (Taylor *et al.*, 2000). The concept of fungal species delimitation is often reviewed and discussed (Steenkamp *et al.*, 2018; Matute and Sepúlveda, 2019; Xu, 2020; Chethana *et al.*, 2021; Jayawardena *et al.*, 2021; Maharachchikumbura *et al.*, 2021; Manawasinghe *et al.*, 2021). Fungal species boundaries can be misled by different factors such as hybridization phenomena(Cruywagen *et al.*, 2017), cryptic speciation and intra-specific variation (Alves *et al.*, 2008; Pavlic *et al.*, 2009; Bihon *et al.*, 2012; Lopes *et al.*, 2018) or convergent evolution (Shang *et al.*, 2016) which often lead to incongruencies within species (Taylor *et al.*, 2000). Also, confusion and conflicts with outdated scientific names perpetuate poor fungal identification (Crous *et al.*, 2015; Dayarathne, 2016).

As mentioned before, ICTF has defined several recommendations and requirements to standardize fungal descriptions. However, no formal quality standards have been defined for publication of new taxa and relevant information regarding morphological, molecular, and phylogenetic analyses or metadata information such as geographic distribution and hosts interactions are often not properly provided (Wu *et al.*, 2019; Durkin *et al.*, 2020).For example, the publication of DNA barcode sequences in a public repository is recommended but recommendations for minimal criteria to assess the quality of these sequences are lacking, allowing authors to describe new species based in DNA sequences with ambiguous nucleotide identification or with regions that were only partially sequenced.

Therefore, we decided to assess the consistency and quality over time of species descriptions in Botryosphaeriaceae, a family that is well known to contain several endophytic and

latent plant pathogens affecting agricultural crops as well as ornamental and forest hosts with a worldwide distribution (Batista *et al.*, 2021; Marsberg *et al.*, 2017; Slippers and Wingfield, 2007). The taxonomy of this family is often revisited and updated according to the newest molecular evidence that helps to clarify the phylogenetic relationships of these species.

However, the quality of the information behind each species descriptions by itself, to our knowledge, has never been assessed (Crous *et al.*, 2006; Pavlic *et al.*, 2009; Phillips *et al.*, 2008; Phillips *et al.*, 2013; Dissanayake *et al.*, 2016; Zhang *et al.*, 2021). In a recent study (Zhang *et al.*, 2021) 58 species, most of which from the family Botryosphaeriaceae, were reduced to synomymy. It is noteworthy that the vast majority of those species had been described quite recently (last 10 years), which could be an indication that standards for publication of novel species are not as high as they should be.

Best practices should be general, easily available for most of the research groups and should provide consistent information to allow accessibility to the ex-type culture and transparency and reproducibility to the supplementary information behind each new fungal report, such as culture techniques, DNA extraction and amplification protocols, isolation source and location, etc.

Our main goal is to raise awareness among authors, editors, and reviewers about what should be the minimal required information to describe a new fungal species within the Botryosphaeriaceae family, to ensure reproducibility, transparency, and consistency in future taxonomic, phylogenetic and plant pathology studies.

3.3 Material and methods

3.3.1 Data extraction

An initial query was performed on MycoBank[1] for species belonging to the genera *Botryosphaeria*, *Diplodia*, *Dothiorella*, *Lasiodiplodia* and *Neofusicoccum* as representatives of the Botryosphaeriaceae family (Figure 3.1). We selected all species described until the end of 2020. A list of 210 species was compiled (Supplementary data Table A), and a literature review was conducted to analyse the publication behind each species description. In the case of species that were described before 2013, besides the original publication of each description, our evaluation also considered the information collected by (Phillips *et al.*, 2013), to standardize and allow a better comparison with species that were described before the widespread use of DNA-based techniques.

[1] https://www.mycobank.org/

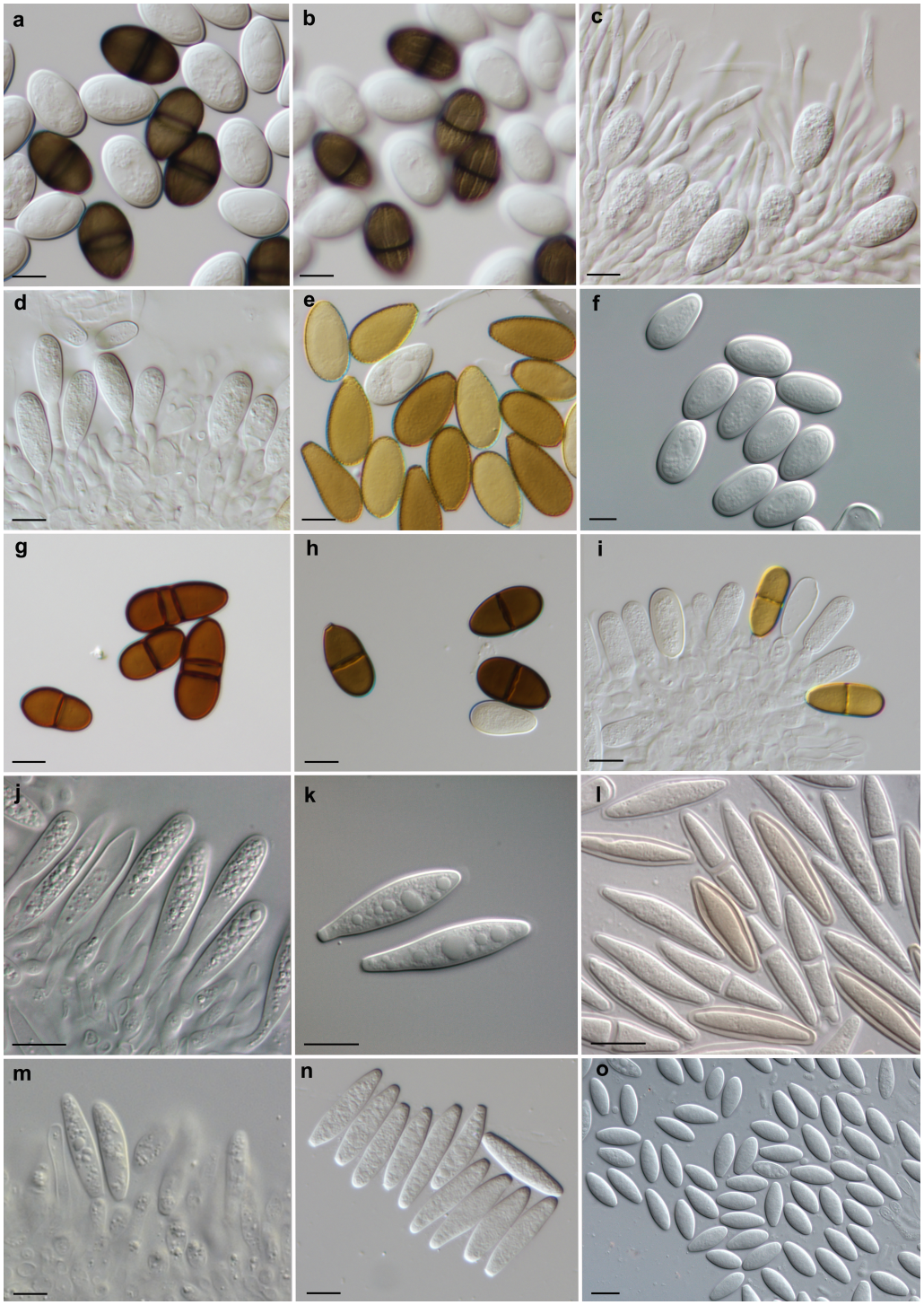


Figure 3.1: Typical asexual micromorphological characteristics of the genera *Lasiodiplodia* (a-b: immature hyaline and mature brown, 1-septate and striated conidia; c: conidiogenous layer with paraphyses), *Diplodia* (d: conidiogenous layer e-f: hyaline and brown coloured aseptate conidia. In some cases, conidia become 1-septate with aging), *Dothiorella* (g-h: brown coloured 1-septate conidia. Occasionally 2-septate conidia may be found; i: conidiogenous layer with coloured an septate conidia still attached to conidiogenous cells), *Botryosphaeria* (j: conidiogenous layer; k: typical fusiform, hyaline and aseptate conidia; l: although infrequently, some isolates produced coloured and septate conidia, and *Neofusicoccum* (m: conidiogenous layer; n-o: hyaline and aseptate conidia). Scale bar: 10 µm

3.3.2 Evaluation criteria and classification levels

Each species description was analysed and scored according to five major criteria: morphological characterization, molecular characterization, phylogenetic analysis, host-fungus interactions, and information accessibility. To evaluate the quality of each description, a set of 27 questions were prepared. For a consistent analysis, questions were constructed to allow only a binary response (Yes or No) and a list of minimal accepted criteria by question were defined (Table 3.1).

Subsequently, four different classification levels were defined with different requirements to evaluate the authors performance on each major criterium: level 1 - outdated/unacceptable practices corresponding to practices that no longer should be accepted by reviewers and editors; level 2 - minimum currently acceptable practices that should be required by reviewers and editors as essential information to describe a new fungal species; level 3 - best available practices that resemble the current cutting-edge approaches by providing consistent and transparent information; and level 4 - excellent and target future practices, that is defined by the ideal methods and should guide future developments to achieve an excellent level of fungal species descriptions (Table 3.2). These levels were defined taking into consideration our own perspective of the current practices to describe a new fungal species and we encourage the scientific community to discuss and improve the proposed standards levels.

Table 3.1: Set of questions used to evaluate each species description.

Topic	Sub-topic	Question	Criteria
Morphological characterization	Characterization of cultures	1	Authors provide a characterization of cultures
	Micromorphological characterization	2	Authors provide a micromorphological characterization - spores (e.g., size, colour, shape)
	Micromorphological characterization	3	Authors provide a micromorphological characterization - sporogenesis (e.g., ascostromata, asci, conidiomata, conidiogenous cells)
	Growth studies	4	Growth studies are performed with different temperature conditions
	Sporulation conditions	5	Growth media and optimal conditions to induce sporulation are provided
Molecular characterization	DNA extraction, PCR amplification, and sequencing	6	Protocols for DNA extraction, PCR amplification and sequencing are provided
	ITS region	7	ITS region is fully sequenced (e.g., recommend primers ITS1 (or ITS5) and ITS4 or similar)
	TEF1 - α region	8	*TEF1* - α region sequenced (e.g., minimal recommend primers EF-728F and EF-986R or similar)
	Other genes/regions	9	Species described using other sequenced regions (e.g., LSU, *TUB2*, *RPB2*)
	MAT Region	10	Species described with MAT genes

Table 3.1 continued from previous page

	Quality of sequence	11	Sequence is provided without dubious nucleotide identification or regions are fully sequenced using the minimal set of primers
Phylogenetic analysis	Single locus initial tree	12	Authors perform individual gene trees with all described species to select representatives and genealogical congruence between different loci is verified
	Multi-locus analysis	13	Authors perform multi-locus analysis with the selected representatives
	Phylogenetic methods	14	Authors perform more than one phylogenetic inference method (i.e., ML, MP, Bayesian) and evaluate congruence
	Relative comparison	15	Authors perform a morphological comparative analysis with the closest species
	Relative comparison	16	Authors perform a molecular comparative analysis with the closest species
Host-fungus interactions	Host description	17	Authors identify the host (genus level at least)
	Ecology	18	Authors suggest a type of ecology (e.g., pathogen, saprophyte)
	Pathogenicity trials	19	Authors perform pathogenicity trials
	Pathogenicity trials with stress	20	Authors perform pathogenicity trials on the host under one stress conditions (e.g., drought)
Accessible information	Sequences	21	Sequences are publicly available (e.g., GenBank)

Table 3.1 continued from previous page

type strain deposit	22	type strain is deposited in at least one international culture collection
type strain accessibility	23	type strain is deposited in at least two international (inter-continental) culture collections
Location	24	Authors provide geographical information (e.g., name of the city/region)
Location Lat - long	25	Authors provide geographical information (e.g., lat-long coordinates)
Number of strains	26	Authors support a new description with more than one strain collected independently (i.e., different hosts or locations)
Phylogenetic analysis	27	Authors provide raw data for phylogenetic analysis (e.g., TreeBase)

Table 3.2: Level of description by topic used to characterize the quality of new species reports.

Topic	Level	Description
Morphological characterization	1-Outdated/unacceptable practices	Species described without a micromorphological characterization
	2-Minimum currently acceptable practices	Species described with only cultures and spore's characterization
	3-Best available practices	Species described with a full characterization of cultures and micromorphological characteristics (spores and sporogenesis structures)
	4-Excellent and target future practices	Species described with a full characterization of cultures and micromorphological characteristics with temperature growth studies and well-defined sporulation conditions
Molecular characterization	1-Outdated/unacceptable practices	Species described without molecular characterization or with only one sequenced region
	2-Minimum currently acceptable practices	Protocols for DNA extraction, PCR amplification and sequencing are fully provided. Used regions/genes are partially sequenced
	3-Best available practices	Protocols for DNA extraction, PCR amplification and sequencing are fully provided. ITS or *TEF1* - α are fully sequenced without any dubious nucleotide identification
	4-Excellent and target future practices	Protocols for DNA extraction, PCR amplification and sequencing are fully provided. Sequenced regions (ITS or *TEF1* - α) are fully sequenced without any dubious nucleotide identification

Table 3.2 continued from previous page

Phylogenetic analysis	1-Outdated/unacceptable practices	Species described without a multi-locus phylogenetic analysis with the selected representatives.
	2-Minimum currently acceptable practices	Species described with molecular characterization with at least two sequenced regions (ITS + *TEF1* - α) Or initial phylogenetic analyses to select species representatives is not performed
	3-Best available practices	Species described with at least two sequenced regions (ITS+*TEF1* - α) and more than one phylogenetic inference method. Initial phylogenetic analyses are performed to select species representatives.
	4-Excellent and target future practices	Species described with more than two sequenced regions by more than one phylogenetic inference method and initial phylogenetic analyses are performed to select species representatives
Host-fungus interactions	1-Outdated/unacceptable practices	Host is not described
	2-Minimum currently acceptable practices	The host is well described
	3-Best available practices	Host is well described, and pathogenicity trials are performed under optimal conditions
	4-Excellent and target future practices	Host is well described, and pathogenicity trials are performed under optimal and at least one stress condition
Accessible information	1-Outdated/unacceptable practices	Sequences are not fully available on a public database and/or type strain is not deposited in one international culture collection

Table 3.2 continued from previous page

	Sequences are fully available in a public database and type strain is deposited in at least one international culture collection.
2-Minimum currently acceptable practices	Description is based on more than one strain. Geographical information is provided (e.g., name of the city/region) and raw data for phylogenetic analysis is available in a public database (e.g., TreeBase)
3-Best available practices	Sequences are fully available in a public database and Type strain is deposited in at least one international culture collection. Description is based in more than one strain. Geographical coordinates are provided and raw data for phylogenetic analysis is available in a public database (e.g., TreeBase)
4-Excellent and target future practices	Sequences are fully available in a public database and Type strain is deposited in at least two international culture collection. Description is based in more than one strain. Geographical coordinates are provided and raw data for phylogenetic analysis is available in a public database (e.g., TreeBase)

3.4 Results

Morphological characterization has been the essence of species descriptions since the beginning of fungal taxonomy. A detailed characterization of cultures and micromorphological elements such as spores or sporogenesis structures allows an initial verification process even before performing any DNA-based technique. Our evaluation analysis found that most of Botryosphaeriaceae descriptions are followed by a detailed morphological characterization (Q1, Q2 and Q3 in 3.2). In the future, even with a better molecular characterization, we should not underestimate or forget about the importance of a good morphological profile. Information regarding optimal conditions for growing and sporulation (Q4 and Q5) are often not reported in publications and might prevent the ability of different research groups to grow a species or to induce sporulation easily.

Molecular characterization is nowadays the main support for new species descriptions and therefore it is important that authors guarantee consistency and transparency of their work. In general, protocols for DNA extraction, PCR amplification and sequencing are fully provided (Q6) and most of the Botryosphaeriaceae species are described using the ITS and *TEF1* - α regions (Q7 and Q8). However, there is still no consensus in this family about which DNA regions should be considered essential for a new species description by genera (Q9). Moreover, despite several authors (Bihon *et al.*, 2014; Crous *et al.*, 2017; Lopes *et al.*, 2017; Lopes *et al.*, 2018) recognising the importance of the mating type (MAT) genes as an excellent phylogenetic marker, these genes have not been used yet to support any new description (Q10). Also, we found that 46% of the new species reports were done using sequences containing ambiguous nucleotide identification or regions that were only partially sequenced according to the minimal set of primers recommend for each region (Q11).

Phylogenetic analyses are important to compare information for genes, individuals, populations, or different species. The incorrect use of these tools might overvalue biological variations leading to an unjustified number of new species descriptions. We verified that 31% of the authors do not justify how representative species are selected or evaluate the genealogical congruence between different loci when performing multi-locus phylogenetic analyses (Q12). Overall, the use of multi-locus analyses with different phylogenetic inference methods is already a common practice among the studied species (Q13 and Q14). When describing new species, authors consistently do morphological comparisons with the closest relatives (Q15) although 70% failed to compare and specify molecular differences among the different sequenced regions (Q16).

In the case of fungal species often isolated from a plant host, it is important to mention the source and to provide some initial information about the host-fungus interaction. In that sense, we verify that authors consistently mention the host when describing a new species (Q17) but less than 50% suggested an ecological lifestyle (Q18). The use of pathogenicity assays to support a new fungal description was performed only in 28% of the cases under controlled conditions and so far, were never performed under stress conditions

(Q19 and Q20).

Accessibility to support information and type cultures is fundamental for the scientific community to verify the quality of each new fungal description. All the studied species were followed by public sequence data often deposited in GenBank database (Q21) and in 97% of the cases species were deposited in at least one international culture collection (Q22). To improve accessibility and security of type strains, we encourage authors to deposit cultures in at least two different culture collections preferably in different countries. We found out that only 20% of type species follow this practice (Q23). Information regarding the source location is often provided in a general way (e.g., name of the sampling region) (Q24) and only 20% of the authors provide precise geographic coordinates (Q25). A worldwide curated dataset of precise occurrence data can allow a wide variety of studies (e.g., species distribution models, risk analyses and others). In (Q26) we found that only 45% of the authors supported a new description with more than one isolate collected independently.

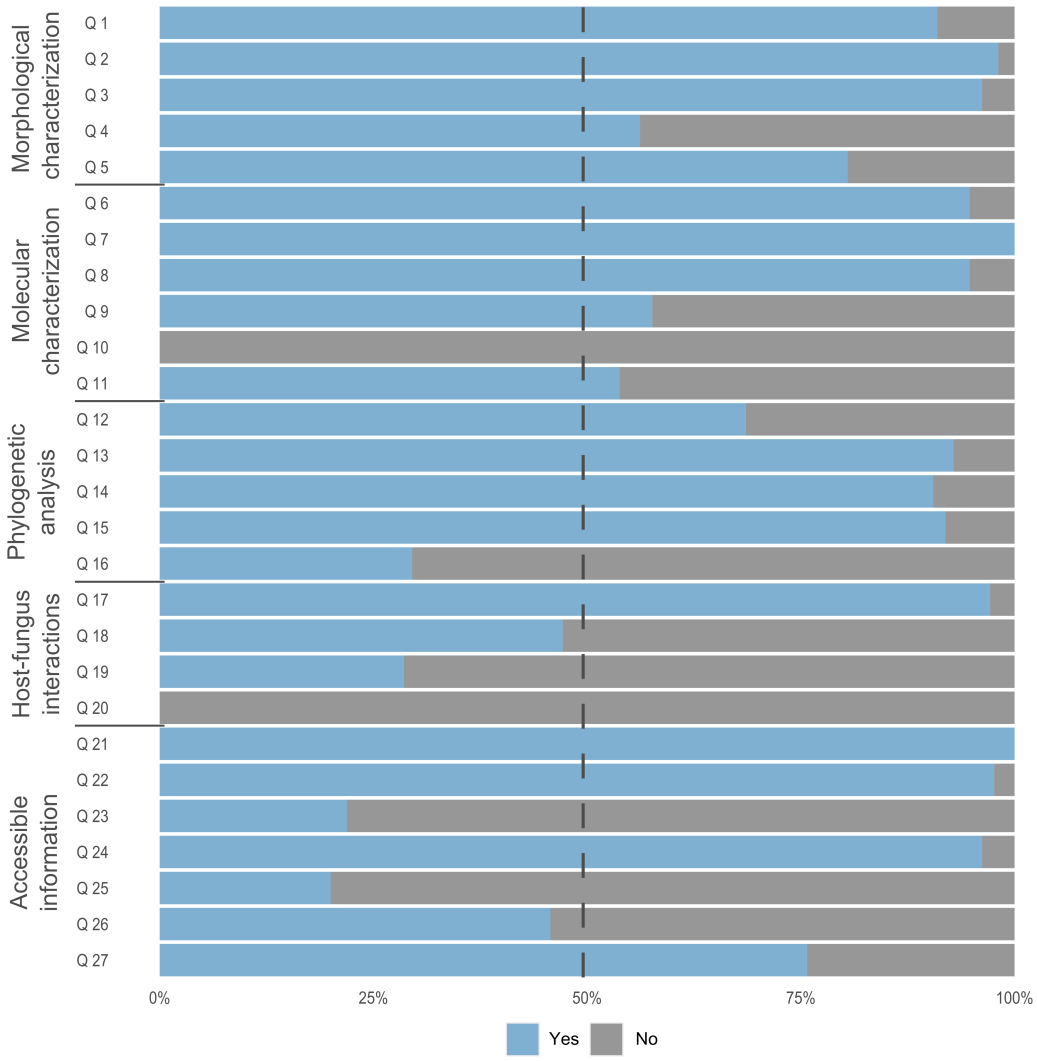


Figure 3.2: Evaluation of positive and negative results by question. A total of 210 new fungal descriptions of the Botryosphaeriaceae family were scored. Questions and answer criteria are defined in Table 3.1 and 3.2.

Describing a new fungal species should be the result of a consistent observation of a specimen with distinct morphological and molecular characteristics when compared with known similar species. For that reason, species descriptions based on a single isolate should be avoided. Finally, we found that 75% of the authors already provided the raw data used for phylogenetic analysis through databases such as TreeBase (Q27).

Based on the previous questions we scored each genus according to the different topics evaluated (Figure 3.3). We concluded that on average when describing new species of Botryosphaeriaceae, 90% of the authors had a satisfactory performance, according to our best available practices' standards, in the morphological characterization and phylogenetic analysis. However, in the remaining evaluated topics, we are still performing according to the minimum currently accepted practices, for molecular characterization and host-fungus interactions 60% of the descriptions are outdated or only meet the minimal requirements for publication and 50% of the authors do not provide enough accessible and reproducible information, leaving a lot of room for improvement (Table 3.3). It was also evident that species in some genera have been described with lower scores than the average in certain topics, specifically *Botryosphaeria* spp. in host-fungus interactions; *Diplodia* spp. in morphological characterization; *Dothiorella* spp. in molecular characterization, host-fungus interactions, and information accessibility; and *Lasiodiplodia* spp. in information accessibility. Only the *Neofusicoccum* spp. descriptions performed in line with or above the family average in all the analysed topics.

Figure 3.3: Comparison of species descriptions performance by genus with the family average.

As previously mentioned, species descriptions done before 2013 were complemented with information collected by (Phillips *et al.*, 2013) to allow a fair comparison with species described in the last decade. When we compared the improvement of quality by year (Figure 3.4) the morphological characterization criteria were constant over time in line with the best recommended practices. In the case of molecular characterization, phylogenetic analysis, and host-fungus interactions, the progress was irregular and always behind the level of best available practices but never below the minimum current practices. In the information accessibility group, we verified that most authors performed only slightly better than the minimum recommended practices and, in some years, even the quality of these criteria fell to the level of outdated and unacceptable practices. This analysis reinforces the need to define minimum criteria for publication to guarantee at least a constant progress over time. Therefore, we encourage authors, reviewers, and editors to discuss and suggest standard requirements for new fungal descriptions.

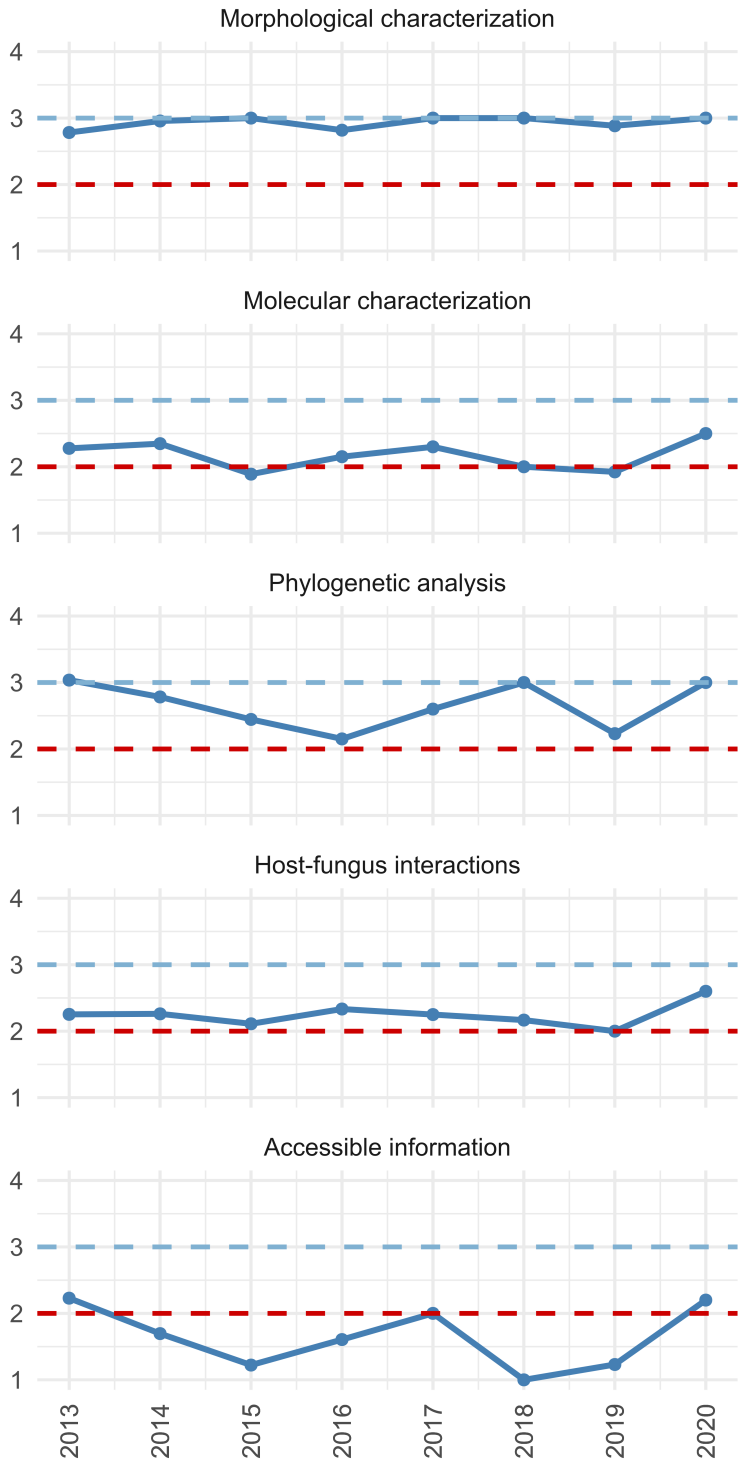


Figure 3.4: Temporal variation of species descriptions performance among the different evaluated groups. Classification levels were defined as 1-Outdated/unacceptable practices, 2-Minimum currently acceptable practices (represented in red), 3-Best available practices (represented in blue) and 4-Excellent and target future practices.

Table 3.3: Number of species descriptions scored in each level. Level 1 - outdated/unacceptable practices; level 2 - minimum currently acceptable; level 3 - best available practices; and level 4 - excellent and target future practices.

	Levels			
	1	2	3	4
Morphological characterization	4	20	79	107
Molecular characterization	16	134	60	-
Phylogenetic analysis	15	68	92	35
Host-fungus interactions	6	147	57	-
Accessible information	114	17	76	3

3.5 Discussion

Botryosphaeriaceae species are often isolated from symptomatic material. However, authors are encouraged to increase their sampling effort and consider collecting also asymptomatic material due to the latent endophytic lifestyle often common in this family (Slippers and Wingfield, 2007). The DNA regions most widely used to describe/identify a species in Botryosphaeriaceae are the ITS and *TEF1* - α followed by the *TUB2* gene (Phillips *et al.*, 2013). The ITS region has the highest number of sequences deposited in the GenBank. However, many of them are only partial sequences which can be restrictive for phylogenetic analyses and lead to erroneous description of novel species. (Linaldeddu *et al.*, 2016) clearly showed that the species *Diplodia guayanensis* (a synonym of *Diplodia scrobiculata*) and *Diplodia galiicola* (a synonym of *D. seriata*) were described based on shorter sequences and that contained sequencing errors. Also, (Berraf-Tebbal *et al.*, 2020) proved that *Lasiodiplodia vitis* was introduced as a novel species, distinct from *Lasiodiplodia mediterranea* based on sequences containing errors. Of the two nucleotide differences in the *TEF1* - α sequence distinguishing both species one was not real (sequencing error or lack of proper sequence edition) and the other was an artefact introduced by the sequence of primer EF-986R. To avoid this type of problem, we recommend the use for each region of the primer sets that allow to get the longest possible sequence. These primers are listed in Table 4. In the case of the genera *Neofusicoccum* and *Diplodia*, the MAT genes could also be amplified. Previous studies proved that these genes are better phylogenetic markers than the conventional ones, with a powerful capacity to identify and delimit even complexes of cryptic species (Lopes *et al.*, 2017; Lopes *et al.*, 2018). For this reason, we strongly suggest using these genes in the future for *Diplodia* and *Neofusicoccum* species descriptions.

Describing the complexity of a host-fungus interaction is not easy and depends on multiple variables from the environment and the interaction of both host-fungus genomes. An initial pathogenicity trial under controlled or stress conditions can provide important data to guide further studies, specially, in the case of latent fungal-related plant diseases.

Although we recognize that pathogenicity assays can be viewed only as an optional requirement to describe a new species, we consider that they can provide relevant information regarding the ecology and pathogenicity of a new species and can help to flag new emergent pathogens (Bhunjun *et al.*, 2021). Due to the economic and ecological relevance of Botryosphaeriaceae diseases, often associated to environmental stresses like drought and heat, we consider that initial pathogenic trials with well-watered and stress conditions can be important to be linked to the species description to improve communication. However, pathogenicity assays should be interpreted carefully. The timing of fungal inoculation when combined with different biotic stress (e.g., drought or heat stress) might affect the host in different ways and results should be interpreted wisely (Caldeira, 2019). Different pathogenicity assays make comparisons across studies difficult or even impossible, and standardized protocols must be defined for a better assessment of the pathogenicity potential among different host-fungus interactions.

Additionally, authors should not define levels of isolate aggressiveness exclusively based on the length of internal wood necrosis without taking into consideration the plant physiological and biochemical response, the variety of fungal pathogenesis mechanisms, the timing of the infection and the environment effect on the host-pathogen interaction (Manawasinghe *et al.*, 2016; Félix *et al.*, 2017; Batista *et al.*, 2021). Host-jump analyses should be taken more often into consideration. Testing emergent plant pathogens in relevant plant hosts can help to explore future expansion patterns for new host-jumps and guide further studies (Batista *et al.*, 2020). Selection of hosts for host-jump analyses should consider economically relevant plant species and species co-occurring in the same areas of current host(s). Also, species distribution models based in different climate change scenarios can help to identify potential emergent fungal diseases in new areas and guide host-jumps analyses in new important hosts.

To help improve future studies with Botryosphaeriaceae-related species, we propose a list of guidelines to improve taxonomic experiments based on the main protocols being used in our research group. It is not our intention to compare different protocols and we consider that authors applying different methods might achieve equal or better results. Therefore, this is just a proposal of a feasible and tested working solution for Botryosphaeriaceae species.

With this review we aimed to raise awareness on what should be the minimal criteria to publish a new fungal species besides the ones already defined by the International Code of Nomenclature for algae, fungi, and plants (Turland *et al.*, 2018). Moreover, we intended to understand which practices are often used and the ones frequently ignored by authors, when describing a new fungal species. Based on the family Botryosphaeriaceae, we verified that according to our best available practices authors had an adequate performance in the topics of morphological characterization and phylogenetic analysis. However, in molecular characterization, host-fungus interactions, and information accessibility, we are still performing according to the minimum currently accepted practices, leaving a lot of room for improvement. We also verified that the temporal variation of species descriptions

doesn't have a progressive performance and the lack of well-defined standards do not follow a constant progress. To help future descriptions, a new feature was added to the MDRBOT database[2] with the type sequences of species in this family and a survey to score putative new descriptions before submission. We hope to encourage authors, reviewers, and editors of peer-reviewed journals to reflect and discuss about these fungal description criteria and above all that authors follow them so that publication standards of new species are improved accordingly.

3.6 Guidelines to describe a new Botryosphaeriaceae species:

3.6.1 Species isolation

In general, members of Botryosphaeriaceae are isolated from woody plant material but they can be found also on leaves and fruits, as well as on soil and water samples. Symptomatic plants usually have one or several of the following symptoms: cankers, blight of shoots and seedlings and dieback. In the laboratory, wood material should be sterilised to remove any superficial fungal or bacterial contamination by placing the sample in 5 % sodium hypochlorite, followed by 96 % ethanol and sterile water for one min each. Isolations can be made by directly plating out small wood pieces of 5–10 mm on PDA (potato dextrose agar). Plates should be incubated at 20–25 °C and regularly checked for fungal growth. Sub-cultures can be established by subculturing hyphal tips every time that different mycelial observations are made during the initial seven days of growth. If possible single spore isolation should be done to obtain a final pure culture. Whenever fungal structures (ascomata or conidiomata) are found in the host single spore isolation should be attempted.

3.6.2 Morphological characterization [Q1 – Q5]

3.6.3 Macromorphological characterization:

Colony characteristics (color front and reverse) and pigment production should be recorded from cultures grown on full-strength PDA at room temperature (approximated 20–25 °C) and exposed to indirect sunlight. Growth at different temperatures should be determined on full-strength PDA at 5-degree intervals between 5 °C and 35 °C. Although not particularly relevant characteristics to discriminate species these macromorphological features should be an integral part of a species description.

3.6.4 Micromorphological characterization:

In general, isolates can be induced to sporulate by inoculation on ¼ strength PDA or WA (Water agar containing sterilised plant material e.g., pine needles, fennel stems,

[2] https://mdr-bot-cesam-ua.shinyapps.io/bot_database/

poplar twigs, oak twigs). Plates should be incubated at temperatures between 20 – 25 °C (room temperature) for 1–4 weeks under diffused daylight. When pycnidia are formed these should be mounted in a 100% lactic acid preparation or similar and observed with the support of a light microscope preferably equipped with differential interference contrast. Micromorphological characteristics of the conidia which include shape, size (length and width), colour, and septation septation) should be recorded. Also, mode of conidiogenesis and characteristics of conidiophores and conidiogenous cells should be registered. For a morphological reference of the main Botryosphaeriaceae species consult (Phillips *et al.*, 2013).

Micromorphological structures and cultures should be described with graphic element, photographs (preferably) or drawings, to facilitate each new description and allow visual comparisons with other species. As good examples we highlight the graphic element provided by (Alves *et al.*, 2004) and (Linaldeddu *et al.*, 2013). Several others can be found in (Phillips *et al.*, 2013).

3.6.5 Molecular characterization [Q6 – Q11]

Several protocols and commercial kits are available to perform fungal DNA extraction with high quality for sequencing. Cost and extraction times vary according to the method. In our laboratory we use an adaptation of the (Möller *et al.*, 1992) protocol, which works well for all species tested to date.

For PCR amplification we indicate a list of the best primer sets for the most used loci (ITS, *TEF1* - α and *TUB2*) and for each genus. The primer sets and PCR settings are only a reference to start with. Depending on the genus you are working with, some adjustments may be needed. (Table 3.4 and 3.5).

The set of primers used, and PCR amplification conditions must be clearly described so that these can be easily replicated. Amplicons should be sequenced in both strands. The nucleotide sequences need to be checked manually, and nucleotide arrangements at ambiguous positions clarified using both primer direction sequences.

Table 3.4: List of primers and respective PCR settings to get the largest sequences of ITS, *TEF1* - α and *TUB2* regions.

Region	Primers set	Initial denaturation	Denaturation	Annealing	Extension	Nr. of cycles	Final extension	References
ITS	ITS5/NL4	95C, 5'	94C, 30"	50C, 30"	72C, 1'30"	25	72C, 10'	(White *et al.*, 1990; Alves *et al.*, 2004; Rodrıguez-Gálvez *et al.*, 2020)
TEF1 - α	EF1-688F/EF1-1251R	95C, 5'	94C, 30"	52C, 30"	72C, 45"	30	72C, 10'	(Alves *et al.*, 2008)
TUB2	T1/Bt2b	95C, 3'	94C, 30"	50C, 30"	72C, 1'	35	72C, 10'	(Glass and Donaldson, 1995; O'Donnell and Cigelnik, 1997; Lopes *et al.*, 2016)

Table 3.5: List of primers and respective PCR settings to amplify MAT genes in the genera *Neofusicoccum* and *Diplodia*.

Genus	Region	Primers set	Initial denaturation	Denaturation	Annealing	Extension	Nr. of cycles	Final extension	References
Diplodia	MAT1-1-1	Dip_MAT1_-391F/Dip_MAT1_-1325R	95C, 3'	94C, 30"	50-56C, 30"	72C, 1'	35	72C, 10'	(Lopes *et al.*, 2018)
	MAT1-2-1	Dip_MAT2_-82F/Dip_MAT2_-1058R Dip_MAT2_-113F/Dip_MAT2_-1187R	95C, 3'	94C, 30"	50-52C, 30"	72C, 1'10"	35	72C, 10'	(Lopes *et al.*, 2018)
Neofusicoccum	MAT1-1-1	Neo_MAT1_-113F/Neo_MAT1_-1215R	95C, 3'	94C, 30"	48C, 30"	72C, 1'10"	35	72C, 10'	(Lopes *et al.*, 2017)
	MAT1-2-1	Neo_MAT2_-156F/Neo_MAT2_-1070R	95C, 3'	94C, 30"	52C, 30"	72C, 1'15"	35	72C, 10'	(Lopes *et al.*, 2017)

3.6.6 Phylogenetic analyses [Q12 – Q16]

Sequences can be aligned using different software freely available on the web. From our experience ClustalX v. 2.1 (Larkin *et al.*, 2007) works well using the following parameters: pairwise alignment parameters (gap opening = 10, gap extension = 0.1) and multiple alignment parameters (gap opening = 10, gap extension = 0.2, transition weight = 0.5, delay divergent sequences = 25%). The ideal situation is to work with full length sequences for each locus. However, sometimes this is impossible, and we must deal with missing data in the alignments as these may be problematic for phylogenetic analyses. The alignments can be truncated according to the length of the shortest sequence used. However, if the sequence is quite short, we may be excluding characters that would benefit our phylogenetic analyses. An alternative is to code the missing characters with a "?" and include them in the analyses. If manual adjustments are made to alignment these should be described.

Before concatenation of multiple loci, single locus analyses should be performed, and a phylogenetic analysis should be done to evaluate genealogical concordance between loci. Concatenation can be done using for example the software Sequence Matrix (Vaidya *et al.*, 2011). Maximum Likelihood (ML) phylogenetic trees should be built using the best model of DNA sequence evolution with 1000 bootstrap replicates. Additionally, Maximum parsimony (MP) and Bayesian inference (BI) analyses can also be performed to compare the robustness of tree branch support. Several different software is available to compute the previous analyses e.g., MEGA X (Kumar *et al.*, 2018), PAUP 4.0a (Swofford, Sullivan, *et al.*, 2003), MrBayes 3.0b4 (Ronquist and Huelsenbeck, 2003) *TUB2*.

In phylogenetic analyses it is important to use a balanced dataset, that is, include sequences that represent the whole known diversity within a group. Using a large number of identical sequences while excluding those more diverse ones may result in high but misleading support vales.

The new species being described should be compared with its closest phylogenetic relative(s), in terms of nucleotide differences between the sequenced loci. This comparison should include also differences (if any) in the micromorphological characters.

3.6.7 Host-fungus interactions [Q17 – Q20]

Pathogenicity assays can be used as an initial screening to test isolates for their pathogenicity. Plants should be exposed to one-month acclimatization period before inoculation and maintained at greenhouse temperature (e.g., 25/15 °C day/night) with a controlled photoperiod (e.g., 16/8 h day/night) depending on the host species used. Stress scenarios should be defined according to the host physiology and stress tolerance. Soil water content and host maximum stress tolerance should be calculated by species prior to inoculation for a better experimental design.

Fungal isolates can be grown on PDA, for 7 days at room temperature prior to inoculation. Inoculation should be performed at the base of the stem by placing a colonized agar plug in a wound and then wrapped with Parafilm. Control plants should be inoculated

plugs of sterile PDA. Symptoms such as cankers, blight of shoots or dieback should be daily observed and registered. Internal wood necroses should be recorded and measured at the end of the experiment. In case of seedling mortality during the experiment, the time and number of individuals should be recorded and reported. Koch's postulates should be fulfilled by transferring necrotic and surrounding healthy plant tissues to PDA medium for fungal isolation and identification.

For pathogenicity trials we recommend, among others, the examples provided by (Linaldeddu *et al.*, 2013; Batista *et al.*, 2020). We highlight the importance of expressing mortality numbers and the numbers of days when 100% mortality was observed, if observed.

3.6.8 Information accessibility [Q21 – Q27]

We encourage authors to provide as much information as possible through GenBank submissions as well as in manuscripts. For countries names please use ISO 3166 standards. Geographical coordinates should be provided, and geodetic datum should be mentioned (i.e., WGS84 geodetic datum). For plant host please confirm if your species name is accepted by the CoL+ Project (Bisby *et al.*, 2010). Clearly identify if the sequences concern ex-type strains. Preferably novel species should be described based on multiple strains, if possible, obtained from different samples and geographic locations. To deposit cultures in international collections please consult the World Federation for Culture Collections [3]. Full-length sequences of all loci analyzed must be deposited in GenBank. These sequences must be stripped out of the primer regions and not contain any ambiguous nucleotide positions. Additionally, they should properly annotated in order to identify introns (protein coding genes) and non-coding regions (Internal Transcribed Spacer). Alignments and outputs of phylogenetic analyses should be deposit in TreeBase.[4]

[3] http://www.wfcc.info/
[4] https://www.treebase.org/

Chapter 4

Modelling current and future global distributions of five Botryosphaeriaceae species

The contents of this Chapter have been submitted.

4.1 Abstract

Fungal species of the family Botryosphaeriaceae are distributed worldwide and are known to be important pathogens of a wide variety of forestry and agricultural plant hosts. The role of global changes impacts, especially climate change, on Botryosphaeriaceae-related diseases is still poorly understood. We mapped suitable areas for five Botryosphaeriaceae species, according to three different Shared Socio-economic Pathways (SSP) 126, 370 and 585 in different time slots: a historical climate series from 1970-2000 and two future projections 2021-2040 and 2081-2100. An overall increase of suitable areas for these pathogens is predicted in most of the studied scenarios and a possible range expansion in the northern hemisphere for *Botryosphaeria dothidea* and *Neofusicoccum parvum*. A consistent increase of the optimal growth months, for fungi development, was verified in most of the regions with predicted suitability of the north hemisphere that eventually could impact the phenology of these organisms and originate more frequent and intensive outbreaks. The ability to predict plant pathogens occurrence in space and time with species distribution models at local or global scale can help decision-makers to develop management strategies to prevent or minimize the impact of future disease outbreaks.

4.2 Introduction

The ability to predict species occurrence in space and time with species distribution models (SDMs) has been increasingly studied over the last decades. With a wide range of applications, these models have been commonly used to understand the impacts of biological invasions (e.g. (Thuiller *et al.*, 2005; Gallardo and Aldridge, 2013)), to support conservation and biodiversity studies (e.g. (Guisan *et al.*, 2013; Alagador *et al.*, 2014)) or to forecast climate change effects on species ecological niches (e.g. (Benito Garzón *et al.*, 2008; Fordham *et al.*, 2013)) among other examples. In a changing world, understanding how species shift their ecological ranges in response to on-going global changes is essential not only to prevent some species to face extinction but also to anticipate future impacts of biological invasions.

Throughout time, Human-induced activities have shaped the world landscape to answer the raising demand of natural resources. These changes are often associated to an increment of productivity in the agriculture and the forestry sectors where production process is optimized, and monocultures are usually favoured. Consequently, diversity in species communities and populations has decreased, rising our exposure to pathogenic organisms (Assessment *et al.*, 2005).

When compared to other taxa, the use of SDMs to identify potential suitability areas of fungal plant pathogens has been historically rare (Elith and Leathwick, 2009). However, several examples have been recently published increasing the attention among scholars and decision-makers to the different applicability of these tools (Hao *et al.*, 2020). Among these examples we can highlight several studies with Botryosphaeriaceae-related species (Desprez-Loustau *et al.*, 2007b; Fabre *et al.*, 2011; Qiu *et al.*, 2014; Iturritxa *et al.*, 2015; Bosso *et al.*, 2017) or with other well-known plant pathogens like *Fusarium* species (Backhouse, 2014; Shabani and Kumar, 2013; Shabani *et al.*, 2014; Serra-Varela *et al.*, 2017). In these studies, authors model, at local or global scale, known species occurrence in response to environmental predictor variables to identify current and future suitable areas under different climate scenarios to support management decisions concerning a wide diversity of plant hosts.

Species of the family Botryosphaeriaceae are distributed worldwide and are known to have different ecological roles ranging from saprobic to endophytic, or latent pathogens (Slippers and Wingfield, 2007; Phillips *et al.*, 2013). Taking into account the large number of potential hosts worldwide (Batista *et al.*, 2021), the ability of these organisms to persist endophytically becoming pathogenic only when their hosts are under stress (Slippers and Wingfield, 2007), and the large quantity of plant material moving worldwide every day due to Human activities (Desprez-Loustau *et al.*, 2007a; Hantula *et al.*, 2014), these species may turn into a potential biological threat. Understanding direct and indirect impacts of climate change on Botryosphaeriaceae-related diseases is complex and should be studied into detail for specific regions and for individual fungal-host interactions. If, in one hand, direct effects might favour pathogen multiplication and range expansion (Fabre *et al.*,

2011), in the other hand, climate change can indirectly affect the host resilience to these diseases (Wang *et al.*, 2012; Oliva *et al.*, 2014; Caldeira, 2019). Therefore, uncovering the ecological niche requirements to define potential suitable areas is essential to actively manage current and future outbreaks and guide future individual environment-host-fungus interactions.

Our study is focused on the distribution of five Botryosphaeriaceae species (*Botryosphaeria dothidea*, *Diplodia sapinea*, *D. seriata*, *Lasiodiplodia theobromae* and *Neofusicoccum parvum*) at the global level. We aim to map suitable areas for each species, according to three different Shared Socio-economic Pathways (SSP) 126, 370 and 585 in different time slots: a historical climate series from 1970-2000 (Near current time), and two future projections 2021-2040 and 2081-2100. We also explore the overlap distribution of these plant pathogen species in different land use areas and explore different risk priorities to help scientists and decision-makers flag potential outbreaks in new regions throughout time.

4.3 Material Methods

4.3.1 Species occurrence data

Geographical coordinates were obtained from the MDRBOT database[1] – a worldwide cured dataset of Botryosphaeriaceae species (Batista *et al.*, 2021). In this database each reference was verified, and occurrence data was confirmed by performing a pairwise BLAST analysis between the nuclear ribosomal internal transcribed spacer (ITS) region of each isolate against the ITS sequence of the type strain. Isolates with a similarity value lower than 99% were removed. Records for each species were screened for spatial autocorrelation using the R package ELSA (Naimi *et al.*, 2019) and thinned with the R package spThin (Aiello-Lammens *et al.*, 2015). Ten thousand pseudo-absences were randomly generated for each species model (Barbet-Massin *et al.*, 2012). The extension of background data to generate the pseudo-absences did not include Antarctica because there is no evidence of Botryosphaeriaceae occurrence in this region (Batista *et al.*, 2021).

All work related with the statistical modelling was performed in the High-Performance Computational System of Aveiro University (ARGUS) with the R software (version: 3.6.0; R Development Core Team, 2019).

4.3.2 Climate data

Environmental layers were downloaded from the Worldclim dataset version 2 (Fick and Hijmans, 2017). The average of the years 1970 – 2000 was used as historical data to build our near current time species distribution model prediction. For future data, eight global climate models (GCMs) were used: BCC-CSM2-MR, CNRM-CM6-1, CNRM-ESM2-1, CanESM5, IPSL-CM6A-LR, MIROC-ES2L and MIROC6 in scenarios following

[1] https://mdr-bot-cesam-ua.shinyapps.io/bot_database/

three different Shared Socio-economic Pathways (SSP) 126, 370 and 585. Future modelling predictions were performed for the period 2021 - 2040 and 2081 – 2100. For both datasets, the spatial resolution used was 5 arcminutes (approx. 10 km). The selection of bioclimatic variables was initially performed by a collinearity analysis using the Variance Inflation Factor (VIF) with the vifstep function of the usdm R package (Naimi *et al.*, 2014). Collinear variables were deleted for each species separately (VIF $>$ 10) (Chatterjee and Hadi, 2015). From the remaining variables, the final set of variables were chosen according to the potential biological meaning for this family according to different authors (Staden *et al.*, 2004; Fabre *et al.*, 2011; Iturritxa *et al.*, 2015; Bosso *et al.*, 2017; Batista *et al.*, 2021).

4.3.3 Statistical modelling

An ensemble forecasting approach using the sdm R package (Naimi and Araújo, 2016) was performed for each target species. An initial run was computed with different available algorithms: Generalized linear models (GLM) (McCullagh and Nelder, 1989), Generalized additive models (GAM) (Hastie and Tibshirani, 1990), Boosted regression trees (BRT) (Friedman, 2001), Support vector machine (SVM) (Vapnik, 2013), Classification and regression trees (CART) (Breiman *et al.*, 1984), Multivariate adaptive regression spline (MARS) (Friedman, 1991), Random forests (RF) (Breiman, 2001), and Maximum entropy (Maxent) (Phillips *et al.*, 2006) to evaluate model fitting and to optimize the computational resource consumption according to our processing capacity. Three algorithms, with the higher predictor capacity, were selected to model near current and future species distributions: GLMs, BRT and Maxent. To improve model evaluation three resampling methods were used for each algorithm: cross-validation (10-fold with 20 replicates), bootstrapping (n = 20) and subsampling (n = 20) where 70% of the occurrence data was used for training dataset and the remaining 30% to model evaluation. A total of 720 model runs gave a probability distribution for each cell and a consensus map was built based on the weighted of the True Skill Statistic value (TSS) (Naimi and Araújo, 2016). The final output was transformed in a suitable/unsuitable map where cells with a probability of occurrence higher than average TSS threshold were considered as suitable areas and cells with lower probability of occurrence were considered as unsuitable areas (Liu *et al.*, 2005). For future projections, the final output map took in consideration the result of each GCM and then a consensus map was built as previously described.

4.3.4 Land uses overlap analysis and risk assessment decision tree

The Corine Land Cover (CLC) 2012 (Version 2020 provided by European Union, Copernicus Land Monitoring Service, European Environment Agency (EEA)) was used to evaluate the suitability of the target species in different land use areas. All operations to extract suitable areas per different land use categories were performed using the raster R package (Hijmans *et al.*, 2014). A risk assessment for agriculture and forest areas was

performed for each tested species using the worst-case scenario of climate change (SPP585) to the time slot 2081-2100. Four categorical risk groups were defined according to the risk assessment decision tree (Figure 4.1).

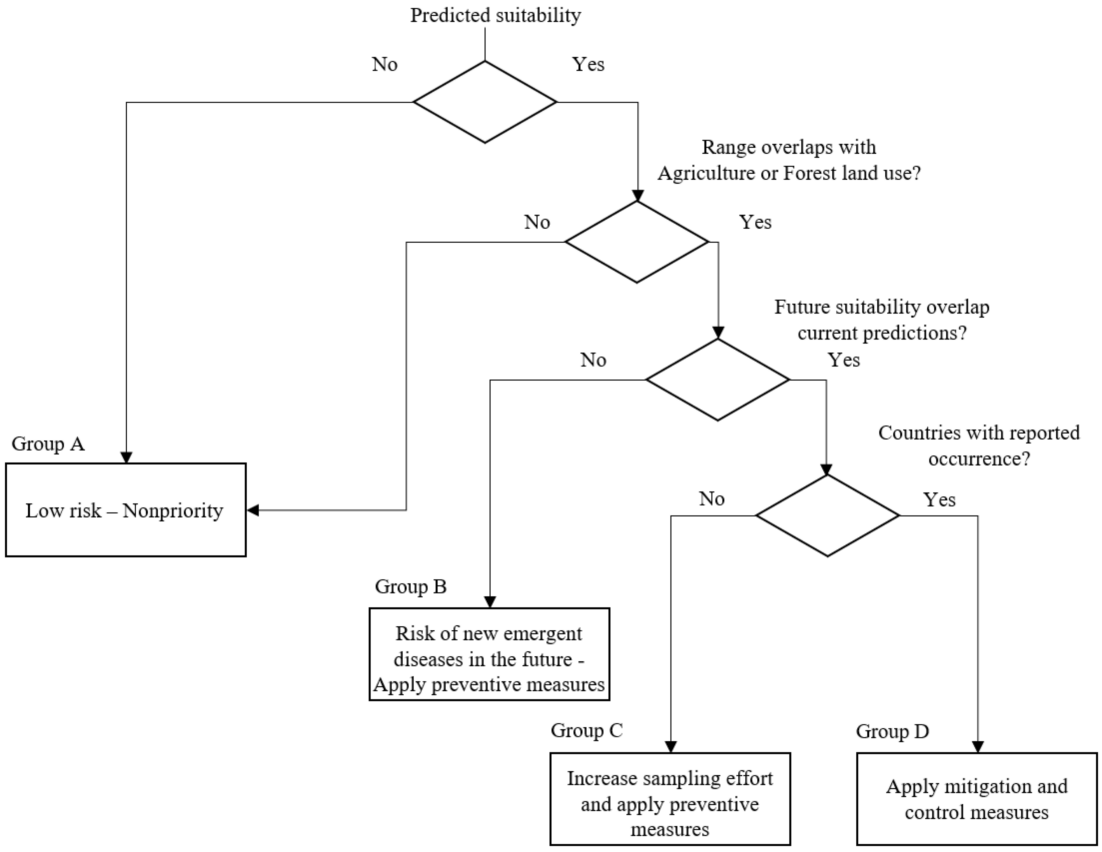


Figure 4.1: Risk assessment decision tree to prioritize sampling, preventive, and control measures.

4.3.5 Optimal growth months according to temperature

Optimal growth temperatures of the studied species were defined according to (Phillips *et al.*, 2013). An optimal range between 20 and 40 °C was selected to consider the overlap of the optimal range of all species. Minimal and maximum monthly temperature were used to calculated average monthly temperatures. This data was obtained from the Worldclim dataset version 2 for the historical climate 1970 – 2000 (Near current time). For the future climatic scenario SPP585 in the time slot 2081-2100, eight global climate models (GCMs) were used: BCC-CSM2-MR, CNRM-CM6-1, CNRM-ESM2-1, CanESM5, IPSL-CM6A-LR, MIROC-ES2L and MIROC6 (Fick and Hijmans, 2017). The final output corresponds to the average values of the mention GCMs. A consensus suitable area of all species was created taking in consideration the output models of the SPP585 2081-2100 final prediction. Changes in optimal growth months were calculated with reference to the near current time.

4.4 Results

4.4.1 Models' performance

After cleaning, the final dataset contained 126 occurrences of *B. dothidea*, 40 of *D. sapinea*, 59 of *D. seriata*, 107 of *L. theobromae* and 120 of *N. parvum*. In Figure 2, presence data used to train the model is represented by black dots. Overall, it was possible to collect information from several distinct locations (Africa, Asia, Europe, North and South America).

Environmental variables with collinearity problems (VIF $>$ 10) were removed. For each species, the final set of bioclimatic variables contained the following layers: bio4 - Temperature Seasonality, bio8 - Mean Temperature of Wettest Quarter, bio9 - Mean Temperature of Driest Quarter, bio18 - Precipitation of Warmest Quarter and bio19 - Precipitation of Coldest Quarter. Average variable importance was not consistent among species, our ensemble suggests a moderate to a strong contribution of the mean temperature of driest quarter for *B. dothidea*, *L. theobromae* and *N. parvum*. For *D. sapinea* and *D. seriata* the strongest contributor was precipitation of coldest quarter and precipitation of warmest quarter respectively (Figure 4.2 - I).

Taking in consideration the environmental variability of the occurrence locations used to train our models we found that *L. theobromae* is less susceptible to variations of temperature across the year (Figure 4.2 - II) and is adapted to high levels of precipitation in the warmest and the coldest quarter of the year and to the highest mean temperature of wettest and driest quarter. The remaining species showed a better adaptation to regions with strong temperature seasonality; however, average values in precipitation and temperatures were not consistent and varied independently by species.

The AUC values are in the range 0.67 – 0.97 indicating a reasonable to very good model performance (Table 4.1). BRT and Maxent algorithms performed better than GLMs. The

final suitability map was built based on the weighted of the True Skill Statistic value (TSS) where models with better performance received a higher weight in the final output.

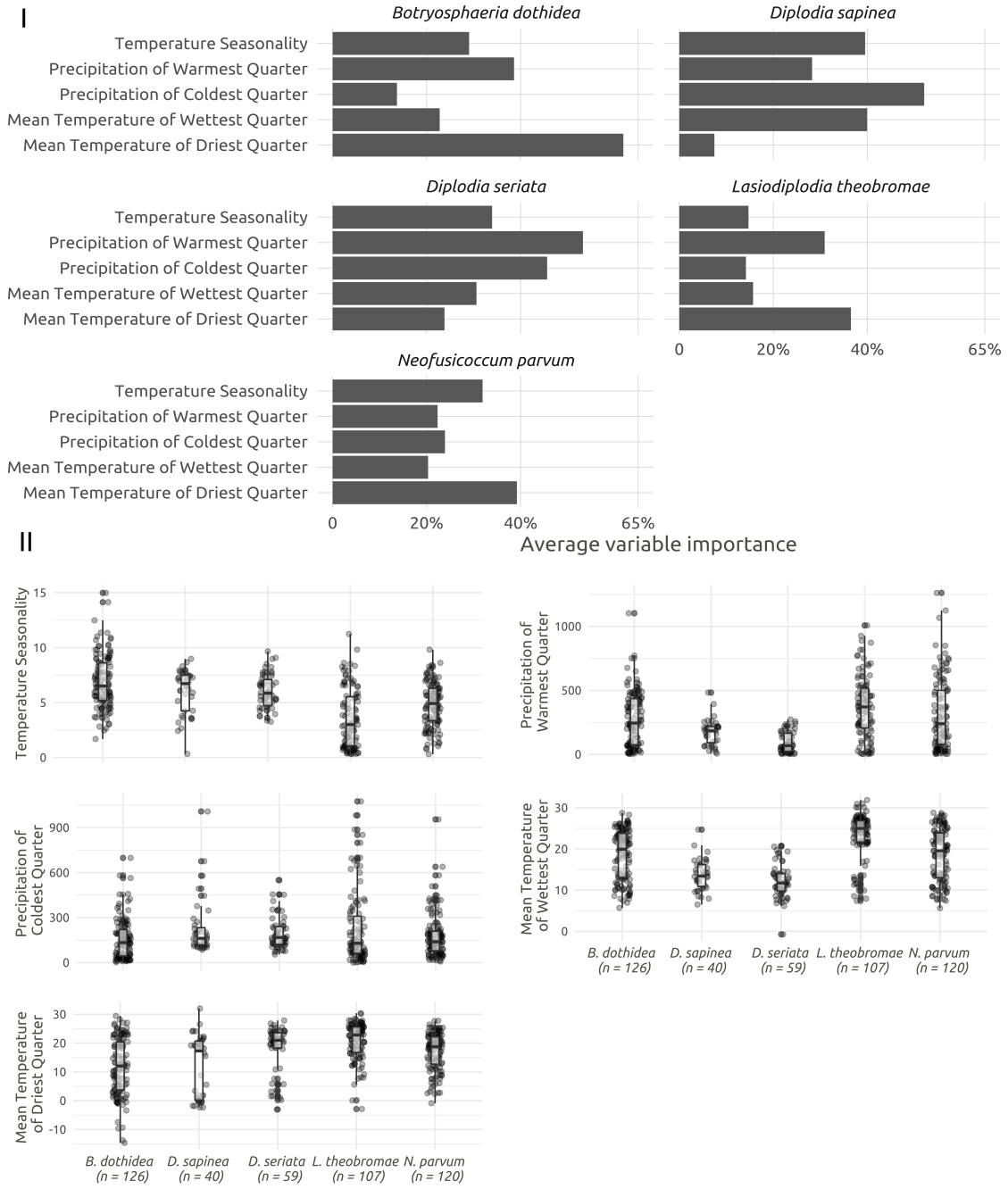


Figure 4.2: (I) Average variable importance of the climatic variables used to model habitat suitability. (II) environmental variability among regions where occurrence data were confirmed. Temperature seasonality was calculated using the standard deviation of the mean monthly values Regions with larger standard deviations have greater temperature variability across the year. Temperatures are represented in degrees Celsius and precipitation in millimeters. A quarter is a period of three months ($\frac{1}{4}$ of the year).

Table 4.1: Area under the receiver operating characteristics curve (AUC) and true skill statistic (TSS) by species for each of the algorithms.

Species	BRT			GLMs			Maxent		
	AUC	TSS	Deviance	AUC	TSS	Deviance	AUC	TSS	Deviance
Botryosphaeria dothidea	0.91	0.75	0.11	0.67	0.45	0.13	0.93	0.79	0.42
Diplodia sapinea	0.93	0.82	0.04	0.72	0.51	0.05	0.95	0.89	0.31
Diplodia seriata	0.92	0.78	0.05	0.85	0.65	0.06	0.97	0.91	0.2
Lasiodiplodia theobromae	0.85	0.66	0.11	0.8	0.59	0.1	0.87	0.68	0.7
Neofusicoccum parvum	0.91	0.74	0.11	0.72	0.51	0.12	0.92	0.79	0.47

4.4.2 Near current suitability

Suitability areas can be observed in grey in figure 3 for each tested species. *Botryosphaeria dothidea* and *N. parvum* were predicted consistently in all sampled regions and the remaining species showed some local restrictions, such as for example, *D. sapinea* and *D. seriata* in Europe, South of Australia, and western region of United States of America. *Lasiodiplodia theobromae* is mainly distributed in regions with a latitude range lower than 30ºN.

Suitable areas were compared with a list of countries with reported occurrence in literature (MDRBOT database[2]) and then divided in two categories: countries with literature reports and predicted suitability and countries with predicted suitability but without literature reports. Overall, our models were able to predict suitable areas in most of the countries with confirmed reports (represented in blue in figure 4.3). Countries delimited in red represent locations with predicted suitability but without any literature reports. We found that, among all studied species, approximately 43% of the countries with predicted suitability and without literature reports, so far, never reported any Botryosphaeriaceae species in literature suggesting that sampling probably never occurred on these regions. Our models, according to the literature, were not able to predict suitability in 6% of the countries with confirmed literature reports. A complementary table was created (Supplementary data B) with a complete country list divided by the mention categories.

[2] https://mdr-bot-cesam-ua.shinyapps.io/bot_database/

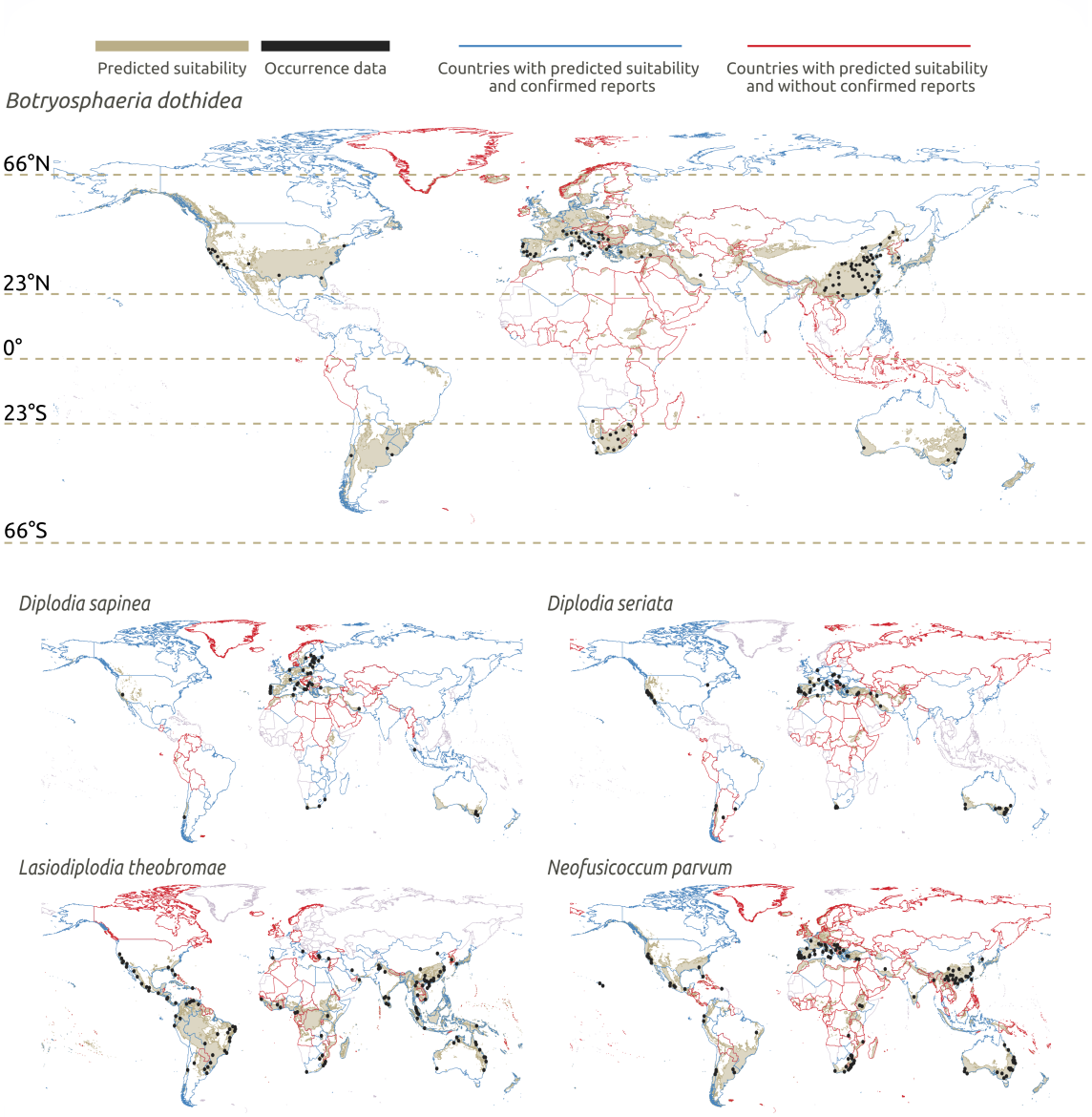
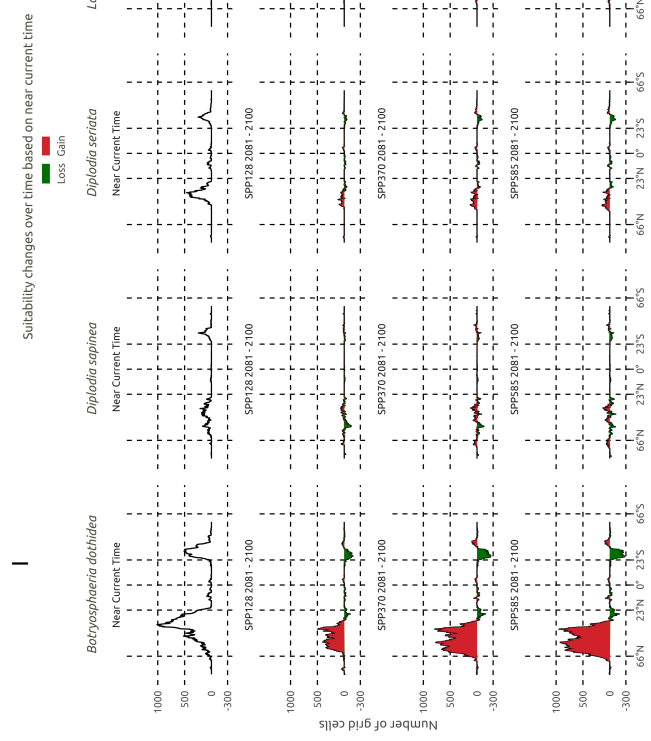


Figure 4.3: Predictions for near current suitability. Grey zones represent suitability areas predicted by the ensemble. Countries with border lines in blue stands for countries with species suitability predicted by the ensemble and with literature data verifying that occurrence. Countries with boarder lines in red stands for countries with species suitability predicted by the ensemble but without literature data. Black dots stand for occurrence data used to train the SDMs.

4.4.3 Future suitability

For future predictions our species models showed consistency within the two selected future time slots and among different climatic scenarios. Taking in consideration only the period 2081 – 2100 for our worst-case climatic scenario SPP585, *B. dothidea* and *L. theobromae* had the biggest percentual increment in suitable areas when compared with the near current prediction, +75% and +48% respectively (Figure 4 - II). These changes for *B. dothidea* were more significant above 23 ºN latitudinal degrees resulting in an expansion range in areas outside the natural range of this species. For *L. theobromae* we verified a consistent expansion within the normal latitudinal range observed in the near current time (Figure 4.4 - I). For *D. sapinea* and *D. seriata* changes were not constant in latitude and varied in several different regions. For example, it was predicted to expand in central Europe in areas that are exposed to the Atlantic Ocean such as United Kingdom, southwestern France, and North of Spain but to decrease in central Europe inland. For *N. parvum*, we found a consistent expansion in east European countries with latitudes above 47 ºN and a general loss of suitable areas below this latitudinal level. For detail world maps with suitability changes over time for all studied species, time slots and different climatic scenarios, please check Supplementary data C.

I



II

Species	SSP128		SSP370		SSP585	
	2021 - 2040	2081 - 2100	2021 - 2040	2081 - 2100	2021 - 2040	2081 - 2100
Botryosphaeria dothidea	+ 16.13%	+ 24.99%	+ 15.92%	+ 55.26%	+ 18.78%	+ 75.82%
Diplodia sapinea	- 4.82%	- 3.60%	- 3.76%	- 1.83%	- 4.17%	+ 1.82%
Diplodia seriata	+ 4.52%	+ 5.13%	+ 3.58%	+ 7.73%	+ 4.29%	+ 11.09%
Lasiodiplodia theobromae	+ 16.65%	+ 21.53%	+ 15.54%	+ 40.84%	+ 16.54%	+48.96%
Neofusicoccum parvum	- 1.56%	- 0.52%	- 2.13%	+ 1.32%	- 1.29%	+ 0.58%

Figure 4.4: (I) Approximated cumulative number of grid cells predicted by the ensemble over a latitude gradient. Areas under the curve in red represent gain of suitability and areas in green represent loss of suitability when compared with the near current time prediction. (II) Variation of total suitability areas by species. Values were obtained according to the percentual change of the respective climate scenario and time when compared with the predicted near current distribution. Percentual changes were calculated according to the number of grid cells with predicted suitability.

4.4.4 Land uses overlap analysis

Assuming a constant land use scenario over time we verify that, apart from *N. parvum*, all species increased their suitability range in all studied land use classes (Figure 4.5). For *N. parvum* we previously predicted a positive variation of 0.58% in total suitable areas when compared the SSP585 output scenario with the near current time. However, this small variation resulted in a reduction of suitable areas in most of the meaningful land use categories for Botryosphaeriaceae-related diseases.

Lasidiplodia theobromae presented the largest values in the total number of grid cells with predicted suitability. When compared with the remaining species, this species presented on average 2 times more suitable areas than *B. dothidea* and *N. parvum*, and 8 times more than *D. sapinea* and *D. seriata*.

Areas categorized as closed forest of evergreen and deciduous broadleaf tree species, closed forest of evergreen needleleaf species, herbaceous vegetation and cropland land uses were the ones consistently predicted as suitable among all the studied fungal species.

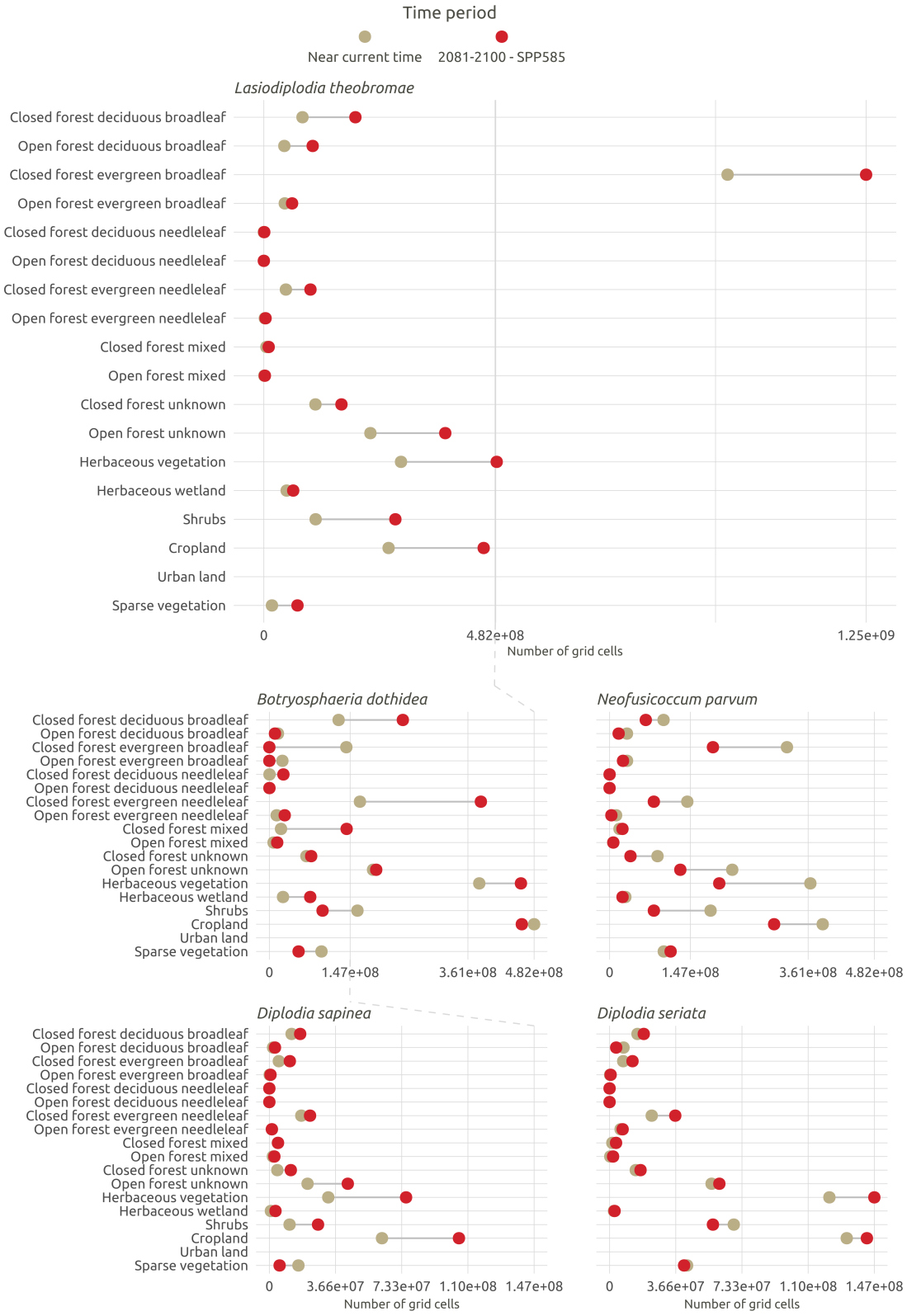


Figure 4.5: Approximated cumulative number of grid cells by different types of land use for the near current time and the tested climatic scenarios.

4.4.5 Risk scenarios

Figure 4.6 presents a map projection with the risk categories previously defined. We highlight for *B. dothidea* the possible risk of outbreaks in the future (Cat B) in the northern hemisphere in areas that are already connected with regions with current predicted suitability and with confirmed reports in literature (Cat D) and with areas without confirmed literature reports but with predicted suitability (Cat C). In *N. parvum*, *D. sapinea* and *D. seriata* we found a lower distribution range when compared with *B. dothidea* but with a similar distribution pattern in regions mostly located in the northern hemisphere. *Lasiodiplodia theobromae* was manly predicted in regions of the south hemisphere and areas with risk of future outbreaks are also often associated with regions where this species was already detected.

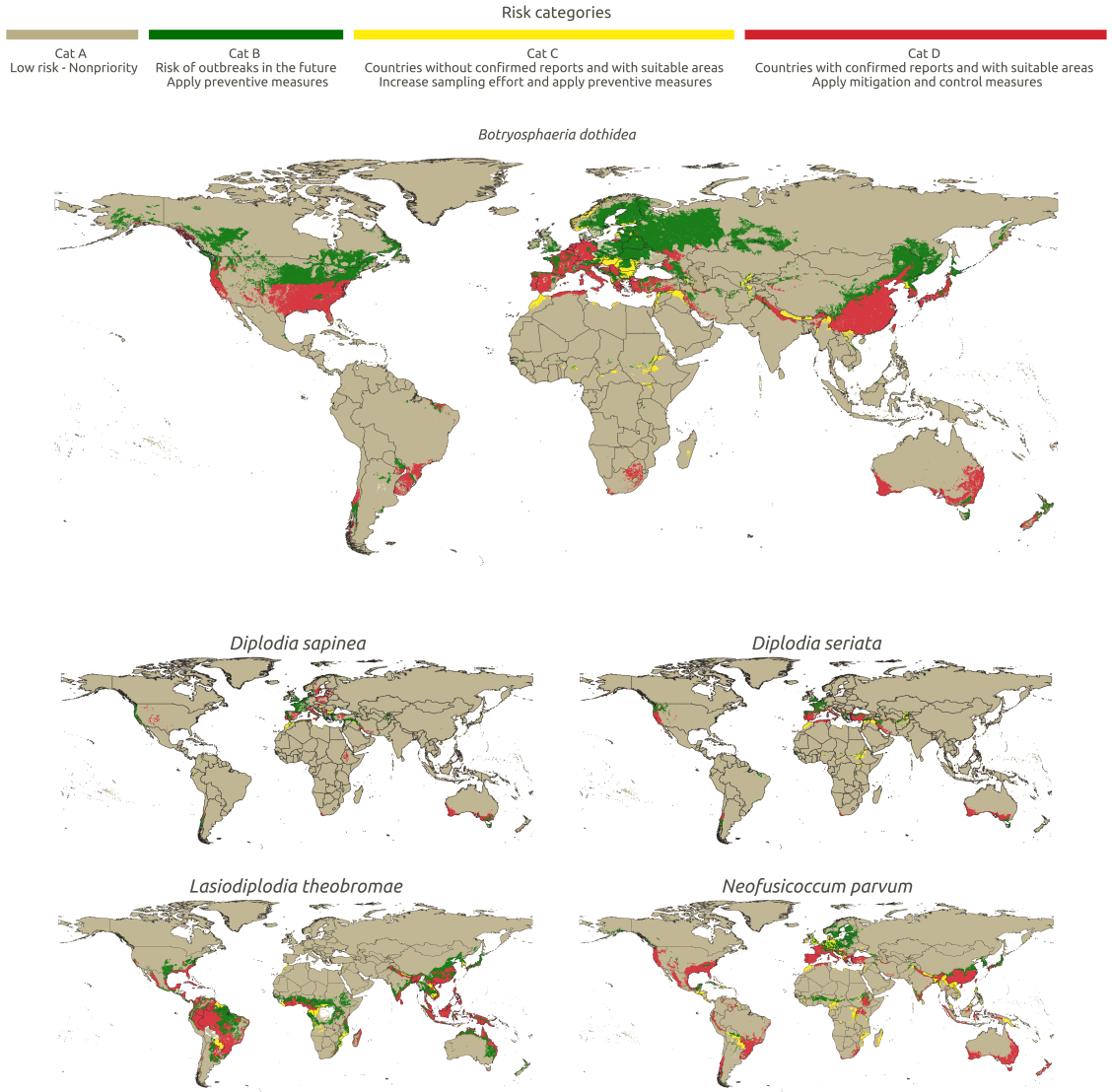


Figure 4.6: Risk assessment for the studied species.

4.4.6 Optimal growth months

Despite all the environmental conditions that characterize the ecological niche of each studied species we wanted to understand the possible impact of climate change in the number of optimal growth months per year based on the optimal growth temperature of these fungi (Figure 4.7). We verify a consistent increase of the optimal growth months in most of the regions with predicted suitability of the north hemisphere, Australia, South Africa, Argentina, and the South of Brazil, among others. Tropical regions showed almost no changes in the number of growth months with optimal temperatures. Future losses of optimal growth months were marginal and restricted to some regions in Persian Gulf countries and India.

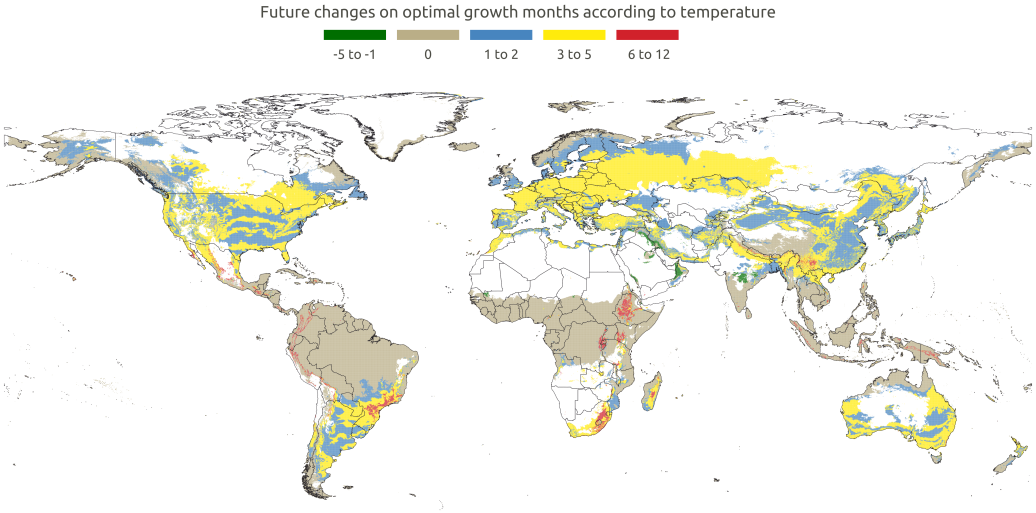


Figure 4.7: Future changes (2081 - 2100) on optimal growth months according to optimal temperature. To calculate the optimal growth months was consider the number of months with average temperature between 20 and 40ºC within the suitability range of all studied species.

4.4.7 Discussion

This study attempts for the first time to model worldwide suitability of five well-known and phytopathologically relevant Botryosphaeriaceae species in three different time slots. Modelling species distributions allows us to understand the ecological niche requirements of those species and to forecast possible future impacts. These models are always subjected to limitations, and in our case, we highlight the low number of occurrence data for *D. sapinea* and *D. seriata* and the low number of asymptomatic samples in all studied species. Also, we ignored evolution of these organisms within our timescale to simplify our modeling process. Several authors have discussed different mechanisms of pathogen evolution and coevolution with their hosts (Rausher, 2001; Brown and Tellier, 2011; Wingfield *et al.*, 2017; Ennos, 2015; Thines, 2019). For specific host-fungus-environment interactions could be interesting to consider evolutionary dynamics into the modeling process or as a risk factor in risk assessment analyses.

In a recent study we hypothesized that the establishment of these species is essentially affected by climate, and optimal conditions for disease expression are mainly due to occasional climatic events that can affect the susceptibility of the host or/and trigger the pathogenic behavior of these organisms (Ragazzi *et al.*, 1999; Allen *et al.*, 2010; Eastburn *et al.*, 2011; Félix *et al.*, 2016; Barradas *et al.*, 2018; Caldeira, 2019; Félix *et al.*, 2019; Pour *et al.*, 2020; Batista *et al.*, 2021) Therefore, we expect that unfavorable conditions might hide the occurrence of those organisms in asymptomatic hosts or through formation of resistance structures. The lack of records from asymptomatic hosts might underestimate distribution ranges in our models. We encourage authors to increase sampling in asymptomatic hosts to detect early species occurrence in new environments, geographic range expansion or new hosts-fungus associations and to improve the use of SDM's techniques with Botryosphaeriaceae-related species (Batista *et al.*, 2020).

Also, we hypothesized that seasonal effects might expand or decrease the growth of these fungal species, invalidating viable long-term populations, and that was verified by the environmental variables selected in our models (Batista *et al.*, 2021). The combination of temperature seasonality across the year, the variation of temperature and precipitation in the Wettest/ Driest and in the Warmest/ Coldest quarters of the year seem to be essential to guarantee long-term populations. These environmental set of variables are in line with other predictors used in similar studies (Staden *et al.*, 2004; Fabre *et al.*, 2011; Iturritxa *et al.*, 2015; Bosso *et al.*, 2017). Those environmental combinations were not consistent among species and is possible to identify different ecological requirements.

Botryosphaeria dothidea is commonly found in a wide variety of ecosystems from temperate and mediterranean regions to subtropical regions (Marsberg *et al.*, 2017; Batista *et al.*, 2021). This species tolerates a broad range of temperatures in the driest quarter however, our model suggests that high levels of precipitation in the warmest quarter can limit the distribution resulting in a lack of suitable areas in tropical regions. Our models suggest a future possible geographic range expansion in the northern hemisphere and our

land use overlap analysis show us that this fungal species will be a constant concern in all type of agricultural and forest land uses. *Diplodia sapinea* and *D. seriata* are often found in boreal, mediterranean and temperate regions (Burgess *et al.*, 2004; Phillips *et al.*, 2007; Slippers and Wingfield, 2007; Batista *et al.*, 2021). In our models, these species are often limited to regions with low levels of precipitation in the coldest quarter and to mean temperature in the wettest quarter below 20°C. The distribution of these organisms is marginal for the near current time and for the future scenarios when compared with the remaining studied species. However, both organisms have been described as aggressive pathogens to several plant hosts and these marginal suitability ranges should not be underestimated. Although with different map resolutions we found a similar pattern of *D. sapinea* in Italy when compared with the results obtained by (Bosso *et al.*, 2017) when using only environmental variables as a predictor for the near current time.

Lasiodiplodia theobromae, a well-known pathogen with a worldwide distribution, is often found associated to symptomatic hosts in tropical and sub-tropical habitats (Mehl *et al.*, 2017a). We found that this species is adapted to very high mean temperatures in the driest quarter and adapted to a very large range of precipitation in the warmest quarter of the year when compared with the other species. Our future scenarios do not predict an increase of ranges in terms of latitude in the northern hemisphere but rather an expansion within the normal suitability range in tropical and sub-tropical regions. The suitability area of *L. theobromae* is notorious in all studied land use cases when compared with the other fungal species; however, is not expectable that this species will be favored in future in the number of months with optimal growth conditions.

Neofusicoccum parvum, when compared with *L. theobromae*, presented a higher tolerance to a wide range of environmental conditions. However, the predicted suitability of this species is not similar to that of *L. theobromae*, being more present in the northern hemisphere and sub-tropical regions. This species has been described as one of the most aggressive pathogens within this family and we highlight the possibility in the future to a shift in its latitudinal range. It is expected that *N. parvum* will reduce the distribution range in areas with latitude lower than 47 °N but to increase in regions with higher latitude. This range shift can expose several agricultural and forest systems to this pathogen in regions that typically have low presence of Botryosphaeriaceae species due to climatic constraints.

Regarding our risk assessment analysis, it was considered as Low risk (Cat A): areas without predicted suitability or areas with predicted suitability but without any kind of agriculture or forest land use (Figure 4.6). This approach allows us to focus only on regions that are destined to produce agricultural crops or different forest products. However, this does not invalidate that target species might occur in natural hosts in those areas but without a relevant economic impact. Also, urban areas were not considered in our study due to the heterogeneous spatial structure of these regions and due to small geographic representation when compared with agricultural and forest systems. Although several authors highlight the importance of these pathogens in Urban areas or in ornamental hosts,

future studies should target specifically these host-fungus interactions in those ecosystems (Lopes *et al.*, 2016; Tiberi *et al.*, 2016; Pavlic-Zupanc *et al.*, 2017; Zlatković *et al.*, 2018). In Category B we highlight areas that currently do not have the most appropriate ecological niche conditions, but where future climatic conditions might favour the expansion of these ecological ranges. Therefore, the risk of potential outbreaks in the future should be considered. For these regions we recommend that preventive measures should be defined taking in consideration to current important plant hosts or future investments in new forest tree species or agricultural crops. If possible, host-jump trials should be considered to anticipate future impacts on new hosts and field surveys to monitor symptomatic and asymptomatic hosts should be frequently assessed. Countries without confirmed reports on literature but with predicted suitability are represented in yellow. We recommend the national authorities of these countries to increase the sampling effort and to create preventive measures to avoid species introductions on habitats with suitable conditions. Red represents countries with predicted suitability and with confirmed reports on the literature for which we recommend the development of active management solutions to control and mitigate the distribution and impact of these plant-pathogens. Also, the risk of commercial trade within these regions should be properly evaluated.

We also highlight the possible impact that the numbers of months with optimal growth conditions could have in the distribution, frequency, intensity, and severity of diseases impacts on plant hosts. Several authors have studied how temperature can affect the virulence and pathogenicity-related genes of several Botryosphaeriaceae species and we can expect that future temperature increments can lead to more frequent and higher infection rates (Qiu *et al.*, 2014; Félix *et al.*, 2016; Félix *et al.*, 2019; Corredor-Moreno and Saunders, 2020). We predicted from one to five more months per year with optimal growth conditions in most of the regions with predicted suitability of the north hemisphere, as well as in Australia, South Africa, Argentina, and the South of Brazil, among others.

Although the interaction of biotic variables with plant pathogenic organisms is commonly studied, the integration of these environmental stresses in modelling techniques to predict the frequency, intensity, and severity of diseases is poorly studied. As an example, several authors have examined the impacts of winter climate change, especially in boreal forests, in phenology of different bark beetles. Such changes influenced population abundance and originated more frequent and intensive outbreaks in large areas of pine stands (Berg *et al.*, 2006; Raffa *et al.*, 2008; Marshall *et al.*, 2020; Venäläinen *et al.*, 2020). To our knowledge, climate change impacts in the phenology of Botryosphaeriaceae-related species was never explored but we hypothesize that more months with optimal growth conditions could favor pathogen multiplication and increase population ranges and infection rates. The future extension of land areas with increased Botryosphaeriaceae fungal activity in northern hemisphere extratropical latitudes will be most certainly affected by the intensity of the process of Arctic Amplification of global warming (Screen and Simmonds, 2010) throughout the 21st century. Arctic Amplification has been a clear signal in recent climate change, leading to faster warming trends in northern land regions but

also to important changes in the extratropical circulation far away from the Arctic with impacts in extreme weather (Cohen *et al.*, 2014). However, a recent assessment of ensembles of CMIP6 models (Ye and Messori, 2021) identified a large spread in the intensity and spatial distribution of this process, suggesting that there is scope for larger impacts in local climate at some locations maybe leading to increased risks of intensified activity of pathogenic fungi, not captured by the ensemble mean of the models used in the present analysis. Future studies are needed to improve our understanding of how climatic events can trigger the pathogenic behavior of these organisms and how our model capacity could forecast future outbreaks. The study of global changes impacts, especially climate change, on Botryosphaeriaceae-related diseases is essential and should guide future studies and be implemented in management strategies to prevent or minimized the impact of future disease outbreaks.

Chapter 5

Botryosphaeriaceae species on forest trees in Portugal: diversity, distribution and pathogenicity

The contents of this Chapter have been adapted from:

Batista, E., Lopes, A. Alves, A. Botryosphaeriaceae species on forest trees in Portugal: diversity, distribution and pathogenicity. Eur J Plant Pathol 158, 693–720 (2020). https://doi.org/10.1007/s10658-020-02112-8

5.1 Abstract

Fungi in the family Botryosphaeriaceae are known as pathogens of diverse woody hosts, including forest tree species. Although several of these fungi have been described in different forest hosts in Portugal, their diversity and distribution is still poorly understood. A survey was conducted across the country to identify Botryosphaeriaceae species associated with the main forest tree species in Portugal, *Quercus suber*, *Eucalyptus globulus* and *Pinus pinaster*. Additionally, a meta-analysis was performed to compile and organize all records known from Portugal. From this meta-analysis, 22 different Botryosphaeriaceae species were reported and 40 different plant hosts were recorded in several studies from agricultural crops to ornamental and forest species. A total of 105 host-pathogen interactions were identified. In the national survey, 12 Botryosphaeriaceae species were identified, with *Diplodia corticola* being the most frequent. *Diplodia insularis*, *Diplodia pyri*, *Dothiorella plurivora* and *Dothiorella yunnana* are reported for the first time in Portugal. Of the 23 different host-fungus associations identified, 10 are also recognised as new hosts. Artificial inoculation tests confirmed the pathogenicity of all species, except *Dothiorella iberica*, *Dothiorella plurivora* and *Dothiorella yunnana*, which are regarded as weakly or non-pathogenic to the hosts tested. Pathogenicity tests revealed the host-jump potential of some species, showing high susceptibility of *Q. suber* to *Neofusicoccum parvum* and *N. eucalyptorum* and of *P. pinaster* to *D. corticola*. Our results show that Botryosphaeriaceae species have a widespread distribution across the country, however some species such as *D. corticola*, *D. sapinea* and *N. eucalyptorum* exhibit a distribution that overlaps the occurrence of the preferred hosts.

5.2 Introduction

The forest sector represents 39% of the land use in Portugal. Favoured by distinct seasons, typical of Mediterranean climates, the forest sector offers several timber and non-timber forest products along the year (IFN, 2013). According to the last National Forest Inventory, Portuguese forest is composed of *Eucalyptus* spp., mostly *Eucalytptus globulus* (811.943 ha), *Quercus suber* (736.775 ha), *Pinus pinaster* (714.445 ha), *Quercus rotundifolia* (331.179 ha), *Pinus pinea* (175.742 ha) and other woody plants (IFN, 2013). These ecosystems are an important source of income and represent approximately 2% of the national gross domestic product (Nunes *et al.*, 2019).

The fungal family Botryosphaeriaceae (Botryosphaeriales, Ascomycetes) includes several species of endophytes or latent pathogens that affect numerous angiosperm and gymnosperm plants (Crous *et al.*, 2006; Slippers and Wingfield, 2007). These fungi are essentially stress-related pathogens, expressing their pathogenicity towards plants exposed to environmental stress, like drought, or plants that are already affected by other pathogens or pests (Slippers and Wingfield, 2007; Phillips *et al.*, 2013).

Various species of Botryosphaeriaceae are well-known pathogens on forest trees, typically associated with branch and trunk cankers, dieback, decline and mortality, and represent a growing threat to forest ecosystems worldwide (Slippers and Wingfield, 2007; Phillips *et al.*, 2013; Chakusary *et al.*, 2019). Some good examples reside in the genus *Diplodia*, namely *D. sapinea* and *D. corticola*. *Diplodia sapinea* is one of the most important and disseminated pathogens of *Pinus* species, as well as other conifers, causing shoot blight, dieback, stem cankers, root diseases and even blue stain of wood diseases (Swart *et al.*, 1988; Phillips *et al.*, 2013). On its side, *D. corticola* has been reported as an important pathogen on oak trees (*Quercus* spp.). This fungus is common and widely distributed in the Mediterranean basin, where it is associated with dieback and canker of cork oak (*Q. suber*) and holm oak trees (*Q. rotundifolia*), being regarded as one of the main pathogens involved in the decline of these important Mediterranean forest ecosystems (Linaldeddu *et al.*, 2014; Smahi *et al.*, 2017). This fungal pathogen has also been implicated in the dieback, cankers, and mortality of native oak species (e.g. *Q. rubra*, *Q. virginiana*, *Q. chrysolepis*) in the United States (Dreaden *et al.*, 2014; Smith and Stanosz, 2018).

A notable aspect of the biology and ecology of Botryosphaeriaceae species is their lack of host specificity, which makes them able to colonize and cause disease in diverse native and introduced plant hosts (Slippers and Wingfield, 2007; Zlatković *et al.*, 2018). Even species such as *D. sapinea* and *D. corticola*, which clearly show a marked host preference, have been found to occur on other unrelated hosts (Lazzizera *et al.*, 2008; Barradas *et al.*, 2016; Zlatković *et al.*, 2017).

Species in the Botryosphaeriaceae thus appear to have the ability to jump to new hosts and some examples have been reported in the literature. The relevance of these host-jumps to the development of new pathogenic abilities and the potential damages they may cause

has not been widely addressed yet in these fungi (Barradas *et al.*, 2016; Lopes *et al.*, 2016; Zlatković *et al.*, 2017; Zlatković *et al.*, 2018).

Recent studies have identified the occurrence of four genera within the family Botryosphaeriaceae, namely *Botryosphaeria*, *Diplodia*, *Dothiorella* and *Neofusicoccum*, in association with forest trees in Portugal (Alves *et al.*, 2013; Barradas *et al.*, 2016; Lopes *et al.*, 2016). These fungi affect some important woody plant species like *Q. suber*, *Q. rotundifolia*, *P. pinaster*, *P. pinea*, *E. globulus* and many other plants of high economic, ecological and cultural value.

However, the current distribution of these pathogens and the possibility to infect new hosts is still poorly understood. In order to increase our knowledge on the occurrence, diversity and pathogenicity potential of these pathogens, the objectives of this study were: (1) to assess which species of Botryosphaeriaceae occur in Portugal through a national survey of forests ecosystems, complemented by a thorough literature review, (2) to map their distribution throughout the country and (3) to evaluate the pathogenic potential of the identified species towards the three main forest tree species found in Portuguese forests, *E. globulus*, *P. pinaster*, and *Q. suber*.

5.3 Material and methods

5.3.1 Sampling and fungal isolation

During the spring of 2018, surveys were carried out across Portugal to collect samples from the main forest tree species (*E. globulus*, *P. pinaster* and *Q. suber*) in this country. Occasionally, samples from other tree species were also collected. One hundred different sampling sites were randomly selected in Portugal. Samples were collected from branches showing symptoms of Botryosphaeriaceae-related diseases (cankers, blight of shoots and seedlings and dieback) and plants without symptoms.

Wood material was sterilised by placing it in 5% sodium hypochlorite, followed by 96% ethanol and sterile water for 1 min each. Cross sections were made and visually inspected for wood discoloration. Isolations were made directly by plating out 2 to 5 small wood pieces of 5–10 mm on PDA - potato dextrose agar (Merck, Germany). Plates were incubated at room temperature (20-25°C) and regularly checked for fungal growth. Pure cultures were established by subculturing hyphal tips every time that different mycelial observations were made during the initial seven days of growth.

5.3.2 Morphological identification

All isolates were morphological characterized according to (Phillips *et al.*, 2013), typical Botryosphaeriaceae cultures were induced to sporulate by inoculation on a ¼ strength PDA containing sterilised pine needles. Plates were incubated at room temperature for 2-3 weeks with diffused daylight. When pycnidia were formed, morphological characteristics

of the conidia (shape, size, colour, septation) and conidiogenous cells were recorded in a 100% lactic acid preparation with a Nikon ECLIPSE 80i microscope (Nikon, Japan).

5.3.3 Molecular characterization - DNA extraction, PCR fingerprinting, DNA sequencing

Isolates were grown on PDA for 7 days at room temperature and DNA extraction was done as described by (Alves *et al.*, 2004). All PCR reactions were carried out in 25 µL reaction mixtures with NZYTaq 2x Green Master Mix (2.5 mM MgCl2; 200 mM dNTPs; 0.2 U/mL DNA polymerase) (Nzytech, Lisbon, Portugal), in a Bio-Rad C-1000 Touch™ Thermal Cycler (Hercules, CA, USA). Negative controls with sterile water instead of template DNA were used in every PCR reaction.

Microsatellite-primed PCR (MSP-PCR) fingerprinting with the primer (GTG)5 was performed with the same conditions as defined previously (Alves *et al.*, 2007). The fingerprint profiles of all isolates were analysed with GelCompar II software (Applied Maths).

The ITS region of the ribosomal RNA cluster was amplified with the primers ITS1 and ITS4 (White *et al.*, 1990) using the same conditions previously described by (Alves *et al.*, 2004).

The translation elongation factor 1-alpha (*TEF1* - α) was amplified with the primers EF1- 688F and EF1-1251R (Alves *et al.*, 2008) and EF1-728F and EF1-986R (Carbone and Kohn, 1999) with the following thermal conditions: denaturation at 95°C for 8 min; 35 cycles at 94°C for 55 s, 50°C for 30 s, and 72°C for 1 min; final extension at 72°C for 10 min. Beta-tubulin (*TUB2*) loci were amplified with the primers Bt2a and Bt2b (Glass and Donaldson 1995) with the following conditions: denaturation at 95°C for 3 min; 40 cycles at 94°C for 30 s, 52°C for 30 s, and 72°C for 1 min; final extension at 72°C for 10 min. MAT1-1-1 gene was amplified with the primers `Neo_MAT1_113F` and `Neo_MAT1_1211R` as described previously by (Lopes *et al.*, 2017). PCR amplicons were purified with the DNA NZY Gelpure kit MB01102 (Nzytech, Lisbon, Portugal) before DNA sequencing and sequenced at GATC Biotech (Cologne, Germany). The nucleotide sequences were checked manually, and nucleotide arrangements at ambiguous positions were clarified using both primer direction sequences. All sequences were deposited in GenBank (Table 5.1).

Table 5.1: Identity of the isolates studied and GenBank accession numbers of the sequences used in phylogenetic analyses. Isolates in bold are ex-type cultures and isolates obtained in this study are in italic.

Species	Isolate	Origin	Host	ITS	*TEF1* - α	*TUB2*	MAT1-1-1
Botryosphaeria agaves	**CBS133992**	Thailand	*Agave sp.*	JX646791	JX646856	JX646841	
Botryosphaeria corticis	**CBS119047**	United States	*Vaccinium corymbosum*	DQ299245	EU017539	EU673107	
Botryosphaeria dothidea	**CBS115476**	Switzerland	*Prunus sp.*	AY236949	AY236898	AY236927	
Botryosphaeria dothidea	*CAA859*	Portugal	*Quercus ilex*	MK940302	MT309403	MT309378	
Botryosphaeria dothidea	*CAA938*	Portugal	*Quercus suber*	MT237173	MT309401	MT309379	
Botryosphaeria dothidea	*CAA860*	Portugal	*Quercus suber*	MK940295	MT309402	MT309380	
Botryosphaeria fabicerciana	**CBS127193**	China	*Eucalyptus sp.*	HQ332197	HQ332213	KF779068	
Botryosphaeria fusispora	**MFLUCC10 0098**	Thailand	*Entada sp.*	JX646789	JX646854	JX646839	
Botryosphaeria pseudoramosa	**CERC2001**	China	*Eucalyptus sp.*	KX277989	KX278094	KX278198	
Botryosphaeria qingyuanensis	**CERC2946**	China	*Eucalyptus sp.*	KX278000	KX278105	KX278209	
Botryosphaeria ramosa	**CBS122069**	Australia	*Eucalyptus camaldulensis*	EU144055	EU144070	KF766132	
Botryosphaeria rosaceae	**CGMCC3 18007**	China	-	KX197074	KX197094	KX197101	

Table 5.1 continued from previous page

Botryosphaeria wangensis	**CERC2298**	China	*Cedrus deodara*	KX278002	KX278107	KX278211
Diplodia africana	**CBS120835**	South Africa	*Prunus persica*	EF445343	EF445382	KF766129
Diplodia corticola	**CBS112546**	Spain	*Quercus ilex*	AY259090	EU673310	EU673117
Diplodia corticola	CBS112549	Portugal	*Quercus suber*	AY259100	AY573227	DQ458853
Diplodia corticola	*CAA862*	Portugal	*Eucalyptus globulus*	MK940298	MT309410	MT309381
Diplodia corticola	*CAA865*	Portugal	*Pinus pinaster*	MK940296	MT309411	MT309382
Diplodia corticola	*CAA870*	Portugal	*Quercus ilex*	MK940303	MT309408	MT309383
Diplodia corticola	*CAA875*	Portugal	*Quercus suber*	MK940297	MT309409	MT309384
Diplodia corticola	CAA499	Portugal	*Eucalyptus globulus*	MG015741	MG015723	MG015800
Diplodia corticola	CDFA519	United States	*Quercus sp.*	GU799472	GU799469	GU799466
Diplodia insularis	**CBS140350**	Italy	*Pistacia lentiscus*	KX833072	KX833073	MG015809
Diplodia insularis	*CAA890*	Portugal	*Eucalyptus globulus*	MK940299	MT309406	MT309385
Diplodia intermedia	CAA147	Portugal	*Malus pumila*	GQ923857	GQ923825	MG015811
Diplodia mutila	**CBS136014**	Portugal	*Populus alba*	KJ361837	KJ361829	MG015815
Diplodia mutila	CBS230.30	United States	*Phoenix dactylifera*	DQ458886	DQ458869	DQ458849
Diplodia mutila	CAA507	Portugal	*Fraxinus ornus*	MG015746	MG015728	MG015816
Diplodia pseudoseriata	**CBS124906**	Uruguay	*Blepharocalyx salicifolius*	EU080927	EU863181	MG015820
Diplodia pyri	**CBS121862**	Netherlands	*Pyrus communis*	KX464093	KX464567	KX464799
Diplodia pyri	*CAA891*	Portugal	*Eucalyptus globulus*	MK940300	MT309407	MT309386
Diplodia quercivora	**CBS133852**	Tunisia	*Quercus canariensis*	JX894205	JX894229	MG015821
Diplodia rosacearum	**CBS141915**	Italy	*Eriobotrya japonica*	KT956270	KU378605	MG015823
Diplodia sapinea	**CBS393.84**	Netherlands	*Pinus nigra*	DQ458895	DQ458880	DQ458863
Diplodia sapinea	*CAA892*	Portugal	*Pinus pinaster*	MK940292	MT309404	MT309387
Diplodia sapinea	*CAA903*	Portugal	*Quercus suber*	MK940312	MT309405	MT309388

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Diplodia seriata	**CBS112555**	Portugal	*Vitis vinifera*	AY259094	AY573220	DQ458856
Diplodia alatafructa	**CBS124931**	South Africa	*Pterocarpus angolensis*	FJ888460	FJ888444	MG015799
Diplodia scrobiculata	**CBS109944**	Mexico	*Pinus greggii*	DQ458899	DQ458884	DQ458867
Diplodia subglobosa	**CBS124132**	Spain	*Fraxinus excelsior*	DQ458887	DQ458871	DQ458852
Dothiorella acacicola	**CBS141295**	France	*Acacia mearnsii*	KX228269	KX228376	-
Dothiorella acericola	**KUMCC18-0137**	China	*Acer sp.*	MK359449	MK361182	-
Dothiorella alpina	**CGMCC3 18001**	China	*Platycladus orientalis*	KX499645	KX499651	-
Dothiorella americana	**CBS128309**	United States	*Vitis vinifera*	MH864851	HQ288262	HQ288297
Dothiorella californica	**CBS141587**	United States	*Umbellularia californica*	KX357188	KX357211	KX357165
Dothiorella citricola	**CBS124729**	New Zealand	*Citrus sinensis*	EU673323	EU673290	KX464853
Dothiorella iberica	**CBS115041**	Spain	*Quercus ilex*	AY573202	AY573222	EU673096
Dothiorella iberica	*CAA904*	Portugal	*Castanea sativa*	MK940306	MT309412	MT309389
Dothiorella iberica	*CAA905*	Portugal	*Eucalyptus globulus*	MK940310	MT309413	MT309390
Dothiorella iberica	*CAA906*	Portugal	*Quercus ilex*	MK940301	MT309414	MT309391
Dothiorella iberica	*CAA915*	Portugal	*Quercus suber*	MK940308	MT309415	MT309392
Dothiorella italica	**MFLUCC 170951**	Italy	*Rosa canina*	MF398891	MF398943	
Dothiorella magnoliae	**CFCC51563**	China	*Magnolia grandiflora*	KY111247	KY213686	
Dothiorella mangifericola	**CBS124727**	Iran	*Mangifera indica*	KC898221	KC898204	
Dothiorella parva	**CBS124720**	Iran	*Corylus sp.*	KC898234	KC898217	KX464866

Table 5.1 continued from previous page

Dothiorella plurivora	**CBS124724**	Iran	*Citrus sp.*	KC898225	KC898208		
Dothiorella plurivora	*CAA916*	Portugal	*Cupressus lusitanica*	MK940291	MT309417	MT309393	
Dothiorella prunicola	**CAP187**	Portugal	*Prunus dulcis*	EU673313	EU673280	EU673100	
Dothiorella rosulata	**CBS121760**	Namibia	*Vachellia karroo*	EU101290	EU101335	KX464877	
Dothiorella sarmentorum	**IMI63581b**	United Kingdom	*Ulmus sp.*	AY573212	AY573235		
Dothiorella sempervirentis	**CBS124718**	Iran	*Cupressus sempervirens*	KC898236	KC898219	KX464884	
Dothiorella symphoricarposicola	**MFLUCC13 0497**	Italy	*Symphoricarpos sp.*	KJ742378	KJ742381		
Dothiorella viticola	**CBS117009**	Spain	*Vitis vinifera*	KF766228	AY905559		
Dothiorella westralis	**CBS117007**	Spain	*Vitis vinifera*	AY905556	KX464623	KX464890	
Dothiorella yunnana	**CGMCC3 17999**	China	*Camellia sp.*	KX499643	KX499649		
Dothiorella yunnana	*CAA917*	Portugal	*Quercus ilex*	MK940307	MT309416	MT309394	
Neofusicoccum arbuti	CBS116131	United States	*Arbutus menziesii*	AY819720	KF531792	KF531793	KX505942
Neofusicoccum arbuti	CBS117090	United States	*Arbutus menziesii*	AY819724	KF531791	KF531794	KX505943
Neofusicoccum australe	**CMW6837**	Australia	*Acacia sp.*	AY339262	AY339270	AY339254	KY775140
Neofusicoccum australe	*CAA919*	Portugal	*Eucalyptus globulus*	MK940294	MT309423	MT309395	
Neofusicoccum australe	CAA434	Portugal	*Eucalyptus globulus*	KT440913	KT440973	KX505927	KX505951

Table 5.1 continued from previous page

Neofusicoccum australe	CAA455	Portugal	*Eucalyptus globulus*	KT440915	KT440975	KX505928	KX505952
Neofusicoccum batangarum	CBS124924	Cameroon	*Terminalia catappa*	FJ900607	FJ900653	FJ900634	
Neofusicoccum cordaticola	CMW14124	-	-	EU821925	EU821895	EU821865	KX766040
Neofusicoccum cordaticola	CBS123634	South Africa	*Syzygium cordatum*	EU821898	EU821868	EU821838	KY612503
Neofusicoccum cryptoaustrale	**CMW23785**	South Africa	*Eucalyptus sp.*	FJ752742	FJ752713	FJ752756	
Neofusicoccum cryptoaustrale	LM03	-	*Pistacia lentiscus*	KX505912	KX505903	KX505930	KX505955
Neofusicoccum cryptoaustrale	BL34	-	*Vitis vinifera*	KJ638328	KX505904	KX505931	KX505956
Neofusicoccum eucalypticola	**CBS115679**	Australia	*Eucalyptus grandis*	AY615141	AY615133	AY615125	
Neofusicoccum eucalyptorum	**CBS115791**	South Africa	*Eucalyptus grandis*	AF283686	AY236891	AY236920	
Neofusicoccum eucalyptorum	*CAA932*	Portugal	*Eucalyptus globulus*	MK940311	MT309422	MT309396	
Neofusicoccum eucalyptorum	CAA511	Portugal	*Eucalyptus globulus*	KX505907	KX505896	KX505919	KX505944
Neofusicoccum eucalyptorum	CAA709	Portugal	*Eucalyptus globulus*	KT440941	KT441001	KX505920	KX505945
Neofusicoccum eucalyptorum	CAA713	Portugal	*Eucalyptus globulus*	KT440943	KT441003	KX505921	KX505946
Neofusicoccum kwambonambiense	**CBS123639**	South Africa	*Syzygium cordatum*	EU821900	EU821870	EU821840	KY612505

Table 5.1 continued from previous page

Neofusicoccum kwambonambiense	CAA755	Portugal	*Eucalyptus globulus*	KT440946	KT441006	KX505917	KX505938
Neofusicoccum kwambonambiense	CMW14155	-	-	EU821923	EU821893	EU821863	KX766039
Neofusicoccum lumnitzerae	**CMW41469**	South Africa	*Barringtonia racemosa*	KP860881	KP860724	KP860801	
Neofusicoccum luteum	**CBS110299**	Portugal	*Vitis vinifera*	AY259091	KX464688	DQ458848	KX505953
Neofusicoccum luteum	*CAA935*	Portugal	*Eucalyptus globulus*	MK940305	MT309418	MT309397	
Neofusicoccum luteum	CAA628	Portugal	*Fraxinus excelsior*	KX505911	KX505902	KX505929	KX505954
Neofusicoccum luteum	CMW9076	-	-	AY236946	AY236893	AY236922	KY775141
Neofusicoccum mangiferae	**CBS118531**	Australia	*Mangifera indica*	AY615185	DQ093221	AY615172	
Neofusicoccum mangroviorum	**CMW41365**	South Africa	*Avicennia marina*	KP860859	KP860702	KP860779	
Neofusicoccum mediterraneum	**CBS121718**	Greece	*Eucalyptus sp.*	GU251176	GU251308	GU251836	MT339205
Neofusicoccum mediterraneum	CAA002	United States	*Pistacia vera*	EU017537	KX505900	KX505925	KX505949
Neofusicoccum mediterraneum	SPA9	-	*Pistacia lentiscus*	KX505910	KX505901	KX505926	KX505950
Neofusicoccum nonquaesitum	IMI500168	-	*Vaccinium corymbosum*	JX217819	KX505895	KX505918	KX505941
Neofusicoccum occulatum	**CBS128008**	Australia	*Eucalyptus grandis*	EU301030	EU339509	EU339472	

Table 5.1 continued from previous page

Neofusicoccum parvum	**CMW9081**	New Zealand	*Populus nigra*	AY236943	AY236888	AY236917	KX505932
Neofusicoccum parvum	*CAA940*	Portugal	*Eucalyptus globulus*	MK940304	MT309421	MT309399	
Neofusicoccum parvum	CMW9080	-	-	AY236942	AY236887	AY236916	KY612501
Neofusicoccum parvum	CAA322	Portugal	*Malus pumila*	KX505906	KX505894	KX505916	KX505937
Neofusicoccum pistaciarum	**CBS113083**	United States	*Pistacia vera*	KX464186	KX464712	KX464998	
Neofusicoccum pistaciarum	CBS113084	United States	-	KX464187	KX464713	KX464999	
Neofusicoccum pistaciicola	**CBS113089**	United States	*Pistacia vera*	KX464199	KX464727	KX465014	
Neofusicoccum ribis	**CBS115475**	United States	*Ribes sp.*	AY236935	AY236877	AY236906	KX505939
Neofusicoccum ribis	CBS121.26	-	*Ribes sp.*	AF241177	AY236879	AY236908	KX505940
Neofusicoccum sp1.	*CAA936*	Portugal	*Cupressus lusitanica*	MK940293	MT309419	MT309398	MT326193
Neofusicoccum sp2.	*CAA937*	Portugal	*Cupressus lusitanica*	MT237174	MT309420	MT309400	MT326195
Neofusicoccum umdonicola	CMW14106	-	-	EU821899	EU821869	EU821839	KX766037
Neofusicoccum umdonicola	CMW14058	-	-	EU821904	EU821874	EU821844	KY612502
Neofusicoccum vitifusiforme	B8	-	*Vitis vinifera*	KC469638	KC884948	KC884951	KX505947
Neofusicoccum vitifusiforme	B9	-	*Vitis vinifera*	KX505908	KX505898	KX505923	KX505948

Acronyms of culture collections: CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CAA: Personal culture collection Artur Alves, Universidade

de Aveiro, Portugal; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; CERC: CERC: Culture collection of China Eucalypt Research Centre, Chinese Academy of Forestry, ZhanJiang, GuangDong, China; CGMCC: China General Microbiological Culture Collection Center, Beijing, China; CDFA: California Department of Food and Agriculture, United States; KUMCC: Kunming Institute of Botany Culture Collection, Yunnan Province, China CFCC: China Forestry Culture Collection Centre, China; CAP: Personal culture collection Alan Phillips, Universidade de Lisboa, Portugal; IMI: International Mycological Institute, CBI-Bioscience, Egham, Bakeham Lane, UK; CMW: Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; BL: Linaldeddu, Università degli Studi di Sassari, Italy.

5.3.4 Phylogenetic analyses

Available ITS, *TEF1* - α, *TUB2* and MAT1-1-1 sequences from other isolates were retrieved using the R package rentrez (White *et al.*, 1990) from GenBank and included in the phylogenetic analyses (Table 1). Sequences were aligned with ClustalX v. 2.1 (Larkin *et al.*, 2007), using the following parameters: pairwise alignment parameters (gap opening = 10, gap extension = 0.1) and multiple alignment parameters (gap opening = 10, gap extension = 0.2, transition weight = 0.5, delay divergent sequences = 25%). The alignments were truncated according to the length of the smallest sequence used. Concatenation was done using the software Sequence Matrix (Vaidya *et al.*, 2011).

All (Maximum Likelihood (ML)) phylogenetic trees were built using the best model of DNA sequence evolution as selected by MEGA X, with 1000 bootstrap replicates to assess branch support (Kumar *et al.*, 2018). ML analysis was performed on a Neighbour-Joining starting tree automatically generated by the software. All alignment and phylogenetic trees were deposited in TreeBase[1]. Additional, (Maximum parsimony (MP)) analyses were performed in PAUP* 4.0a only to compare robustness of tree branch support in the multi-loci analyses.

Phylogenetic analyses were divided by genus. Before each analysis, single ML trees of ITS and *TEF1* - α with all currently described species were performed to select the closest representative species. Global single trees are available in supplementary data[2].

5.3.5 Pathogenicity trials

The experiment was conducted using one-year old seedlings of *Q. suber*, *E. globulus* and *P. pinaster*.

Before inoculation, plants were submitted to a one-month acclimatization period. During the whole experiment period, plants were maintained at greenhouse temperature (25/15°C day/night) with a controlled photoperiod (16/8h day/night) and watered every two days to maintain a non-stress scenario. For each host-fungal interaction 5 replicates were used. One isolate from different species were selected to conduct pathogenicity trials. Isolates were grown on PDA for 7 days at room temperature prior to inoculation. Inoculation was performed at the base of the stem by placing a colonized agar plug in a 5 mm wound and then wrapped with Parafilm. Control plants were inoculated with 5 mm pieces of sterile PDA. Symptoms such as cankers, blight of shoots or dieback were daily observed and registered.

Internal wood necroses in the cambium were recorded and measured after 40 days. In case of seedling mortality during the experiment the time and the number of individuals were recorded. Koch's postulates were fulfilled by transferring necrotic and surrounding plant tissues to PDA medium for fungal isolation. The identity of the isolates was confirmed by observation of typical micromorphological characteristics. The average relative

[1] https://www.treebase.org/
[2] https://link.springer.com/article/10.1007%2Fs10658-020-02112-8Sec18

necrosis was calculated by dividing the length of the necrosis by the length of the plant. This metric was selected instead of absolute values to allow a better comparison between different lesion lengths across the tested species.

5.3.6 Data sources for literature review

All Botryosphaeriaceae related sequences available on 12-05-2020 in Nucleotide - NCBI database were downloaded with the R package rentrez (Winter, 2017). Additionally, information such as strain/culture collection, host, geographical coordinates, country, and title of publication were also extracted. An initial screening was performed removing duplicates and records without a strain or culture collection number. Only records from Portugal were considered.

Simultaneously, a literature review was conducted with all reported cases of Botryosphaeriaceae related species in Portugal. All records without associated sequences were discarded. For missing fields, data was updated with literature information when available.

For each host-pathogen interaction reported in Portugal, a literature review was conducted to identify the existence or absence of pathogenicity trials.

5.3.7 Host jump analyses

A list of host-pathogen interaction was constructed based on the information collected during our survey, pathogenicity trials and the literature review. For this analysis, only reports from Portugal were used. Hosts were organized by taxonomic similarity in larger groups (G1: Pinales, G2: Proteales, G3: Vitales, G4 Asterids group: Apiales, Cornales, Ericales and Lamiales , G5 Rosids I group: Fabales, Fagales, Malpighiales and Rosales, G6 Rosids II group: Malvales, Myrtales and Sapindales). These groups were made based on the Angiosperm Phylogeny Group classification (Chase *et al.*, 2016). Gymnosperms hosts in Portugal so far are exclusively members of Pinales.

For this analysis, was considered that a fungal species that can colonize different plant hosts can "jump" among all hosts with a reciprocal effect. New hosts reports are made based on observed associations in nature during the field survey and possible new hosts jumps are based on tested associations during our pathogenicity trials.

5.4 Results

5.4.1 Sampling, fungal isolation and morphological characterization

A total of 429 trees were surveyed and wood samples were collected. The frequency of symptomatic and non-symptomatic trees per species is given in Table 5.2. From these trees, a total of 678 fungal isolates was obtained. Of these, 87 Botryosphaeriaceae isolates, were selected based on typical morphological characteristics and provisionally assigned to the genera based on conidial morphology *Botryosphaeria/Neofusicoccum* (hyaline, aseptate

and fusiform to ellipsoidal), *Diplodia* (ovoid to ellipsoid, brown and aseptate or hyaline and aseptate eventually becoming brown and 1-sepate after discharge from the pycnidium), and *Dothiorella* (ovoid to ellipsoid, brown and 1-septate while still attached to the conidiogenous cells). Other fungi commonly isolated from samples belonged to genera such as *Alternaria*, *Biscogniauxia*, *Cytospora*, *Diaporthe*, *Gnomoniopsis*, among others.

Table 5.2: Frequency of symptomatic and non-symptomatic trees sampled during the survey.

Species	Total	Symptomatic	Non-symptomatic
Quercus suber	151	75	76
Eucalyptus globulus	121	41	80
Pinus pinaster	120	49	71
Quercus ilex	17	10	7
Cupressus lusitanica	10	2	8
Pinus pinea	5	-	5
Castanea sativa	3	1	2
Quercus robur	2	-	2

5.4.2 Molecular characterization and phylogenetic analyses

According to MSP-PCR fingerprinting analyses, 23 representative isolates were selected for DNA sequence-based identification and phylogenetic analyses. An initial identification based on a BLASTn search of the ITS sequences against the nucleotide collection (nr/nt) database confirmed that the Botryosphaeriaceae isolates belonged to the genera *Botryosphaeria*, *Diplodia*, *Dothiorella* and *Neofusicoccum*.

For the phylogenetic analysis of the genus *Botryosphaeria* (Figure 5.1), the isolates obtained in this study were placed in the *B. dothidea* clade with a good bootstrap branch support (ML/MP = 88/64). Regarding the isolates belonging to the genus *Diplodia* these clustered into 4 distinct clades in the phylogenetic analysis (Figure 5.2), corresponding to the species *D. sapinea*, *D. insularis*, *D. pyri* and *D. corticola*. All these clades, apart from the *D. sapinea* one (ML/MP = 64/61), received high to very high bootstrap support. *Diplodia pyri* was grouped within the clade containing different isolates of *D. mutila*. However, bootstrap values between analyses were not consistent (ML/MP = 94/-). In a comparative analysis of the nucleotide sequences (Table 5.3), no differences were found between the ex-type cultures of *D. mutila* and *D. pyri* in the ITS region; whereas in the *TEF1* - α region the only difference is a deletion (GCTGCTGCT) in *D. pyri*.

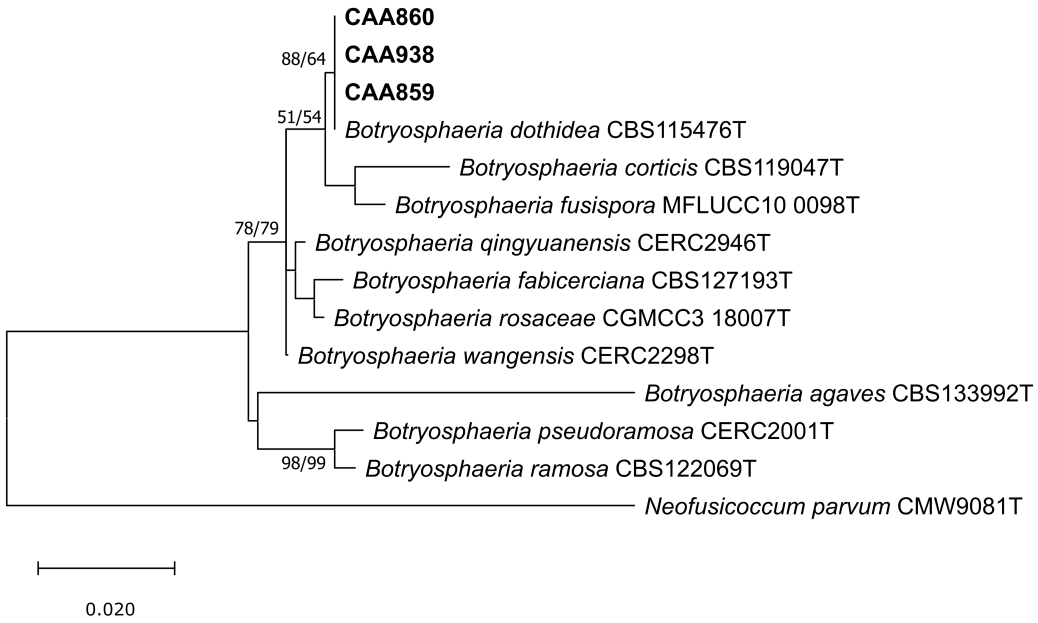

Figure 5.1: ML/MP Phylogenetic relationships of the *Botryosphaeria* isolates based on the combined ITS and *TEF1* - α sequence data. Bootstrap values (>50%) are given at the nodes. Isolates used in this study are given in bold. The tree is drawn to scale, with branch length measured in the number of substitutions per site. Ex-type strains are shown with a 'T' after the strain number

Table 5.3: Differences in the nucleotide sequences of the ITS and *TEF1* - α regions between isolates of *D. mutila* and *D. pyri*. The ex-type strains are indicated in bold and differences are highlighted in grey.

	ITS	*TEF1* - α
	22	12-20
D. mutila **CBS136014**	G	GCTGCTGCT
D. mutila CAA507	G	GCTGCTGCT
D. mutila CBS230.30	C	GCTGCTGCT
D. pyri **CBS121862**	G	-
D. pyri CAA891	G	-

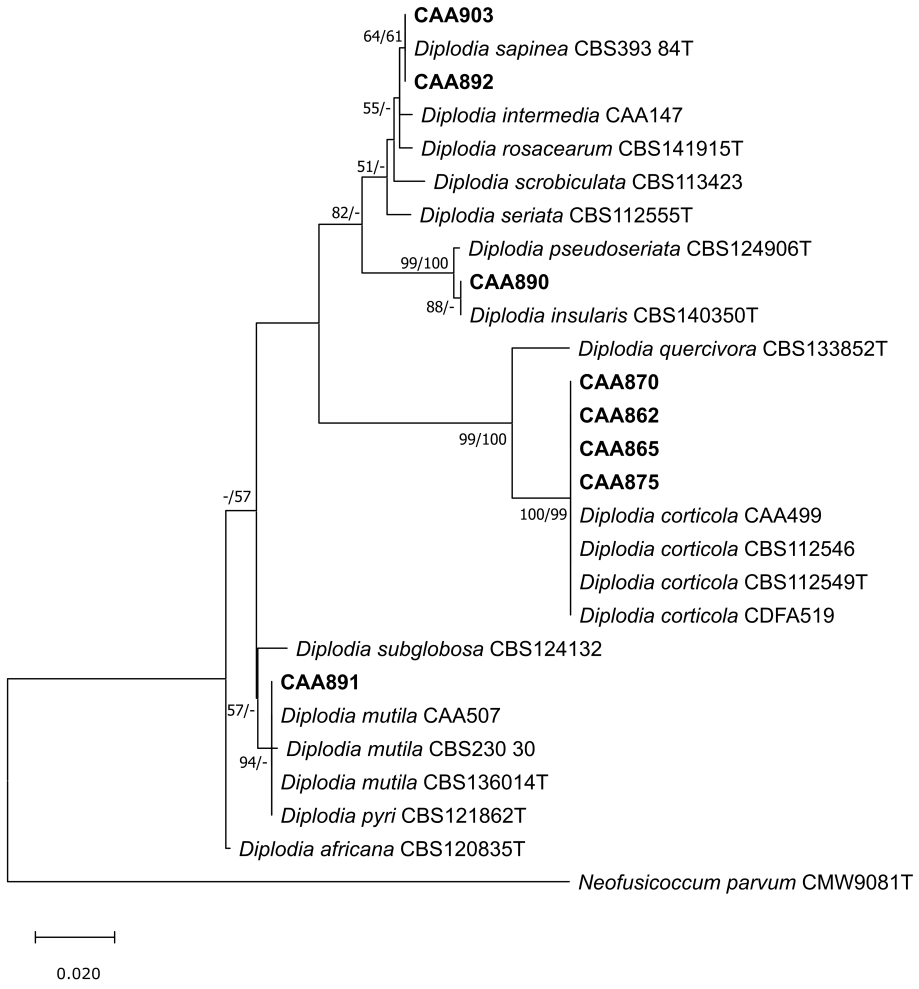

Figure 5.2: ML/MP Phylogenetic relationships of the *Diplodia* isolates based on the combined ITS and *TEF1* - α sequence data. Bootstrap values (>50 %) are given at the nodes. Isolates used in this study are given in bold. The tree is drawn to scale, with branch length measured in the number of substitutions per site. Ex-type strains are shown with a 'T' after the strain number

The *Dothiorella* isolates clustered into three distinct clades (Figure 5.3). Most of them grouped with *Dothiorella iberica* with a good bootstrap support (ML/MP = 88/70). However, isolates CAA916 and CAA917 clustered with *Dothiorella yunnana* and *Dothiorella plurivora*, respectively, but with bootstrap values lower than 50%.

For the genus *Neofusicoccum*, in the combined ITS, *TEF1* - α and *TUB2* phylogeny the isolates clustered into five separate clades receiving moderate to high bootstrap support (Figure 5.4 a). These included *Neofusicoccum australe*, *Neofusicoccum eucalyptorum*, *Neofusicoccum luteum* and *Neofusicoccum parvum*. One isolate (CAA936) was grouped

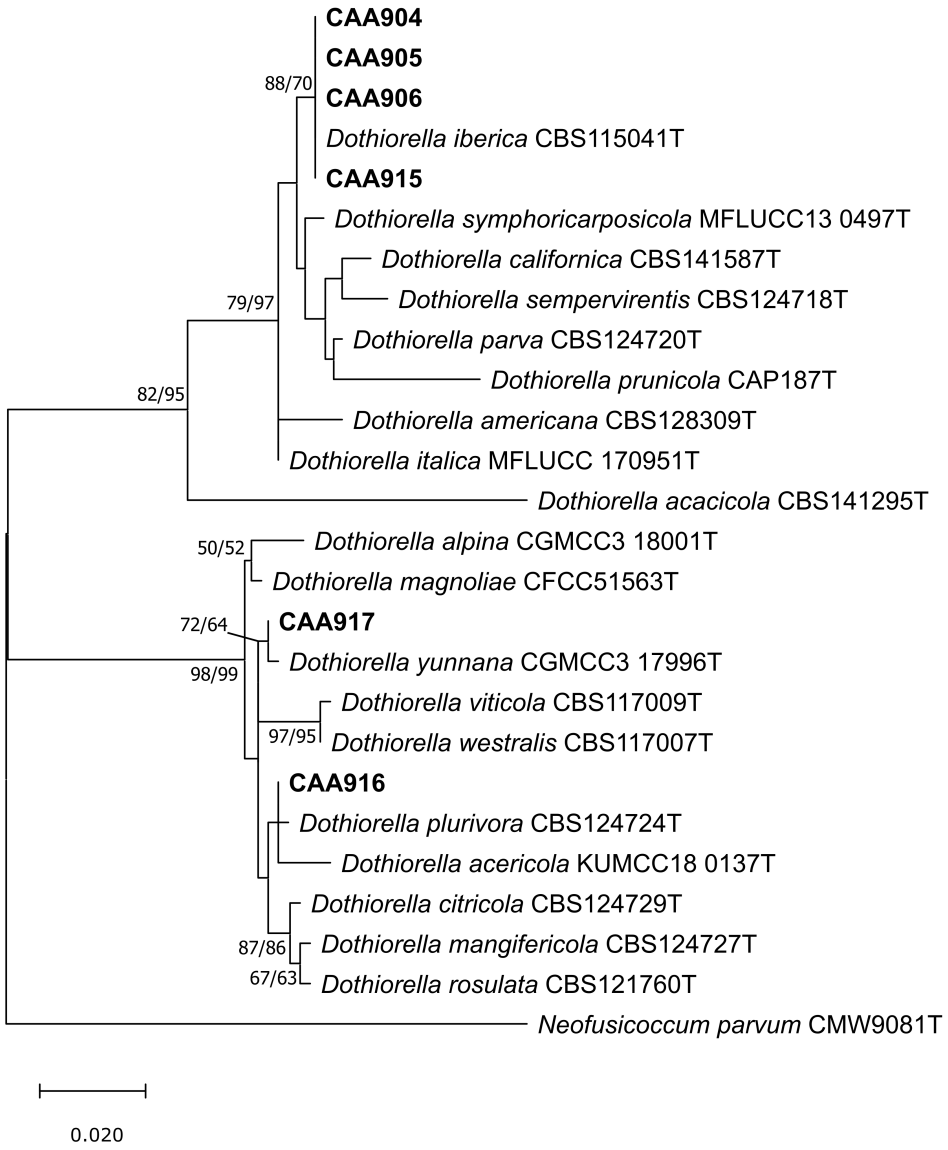

Figure 5.3: ML/MP Phylogenetic relationships of the *Dothiorella* isolates based on the combined ITS and *TEF1* - α sequence data. Bootstrap values (>50 %) are given at the nodes. Isolates used in this study are given in bold. The tree is drawn to scale, with branch length measured in the number of substitutions per site. Ex-type strains are shown with a 'T' after the strain number.

within the clade containing the ex-type strains of *Neofusicoccum mediterraneum*, *Neofusicoccum pistaciarum* and *Neofusicoccum pistaciicola*, along with other isolates identified as belonging to these three species. This clade received a very high bootstrap support (ML/MP = 98/96). In a comparative analysis of the nucleotide sequences (Table **??**) we

found only 3 differences in the ITS region between *N. pistaciarum*, *N. pistaciicola* and *N. mediterraneum*. No differences were found in the *TEF1* - α region among all sequences. One difference was found on *TUB2* region between *N. pistaciarum*, *N. pistaciicola* and *N. mediterraneum*. The isolate CAA936 compared to *N. mediterraneum* presented 2 differences on the ITS region and 2 differences on the *TUB2* region. Another isolate (CAA937) formed a sister clade to the previous one with moderate support (ML/MP = 73/94). When comparing sequences of this isolate, we found more differences in the *TUB2* region than in the ITS region. Again, no differences were found in the *TEF1* - α region. An additional phylogenetic analysis of the MAT1-1-1 gene was performed with isolates CAA936, CAA937 and *N. mediterraneum* CBS121718 (ex-type strain). MAT1-1-1 gene sequences of *N. pistaciarum* and *N. pistaciicola* were not available. In the MAT1-1-1 gene phylogeny these isolates formed a clade distinct from other *Neofusicocccum* species with (ML/MP = 100/100) bootstrap support. As can be seen in Figure 5.4 b isolates CAA936 and CAA937, are distinct from each other, and were separated from *N. mediterraneum* isolates which formed a sub-clade with very high support (ML/MP = 99/99). When comparing MAT1-1-1 gene sequences of CAA936, CAA937 and *N. mediterraneum* CBS121718 differences were obvious, with a minimum of 15 nucleotide substitutions and one sequence deletion (Table 5.5).

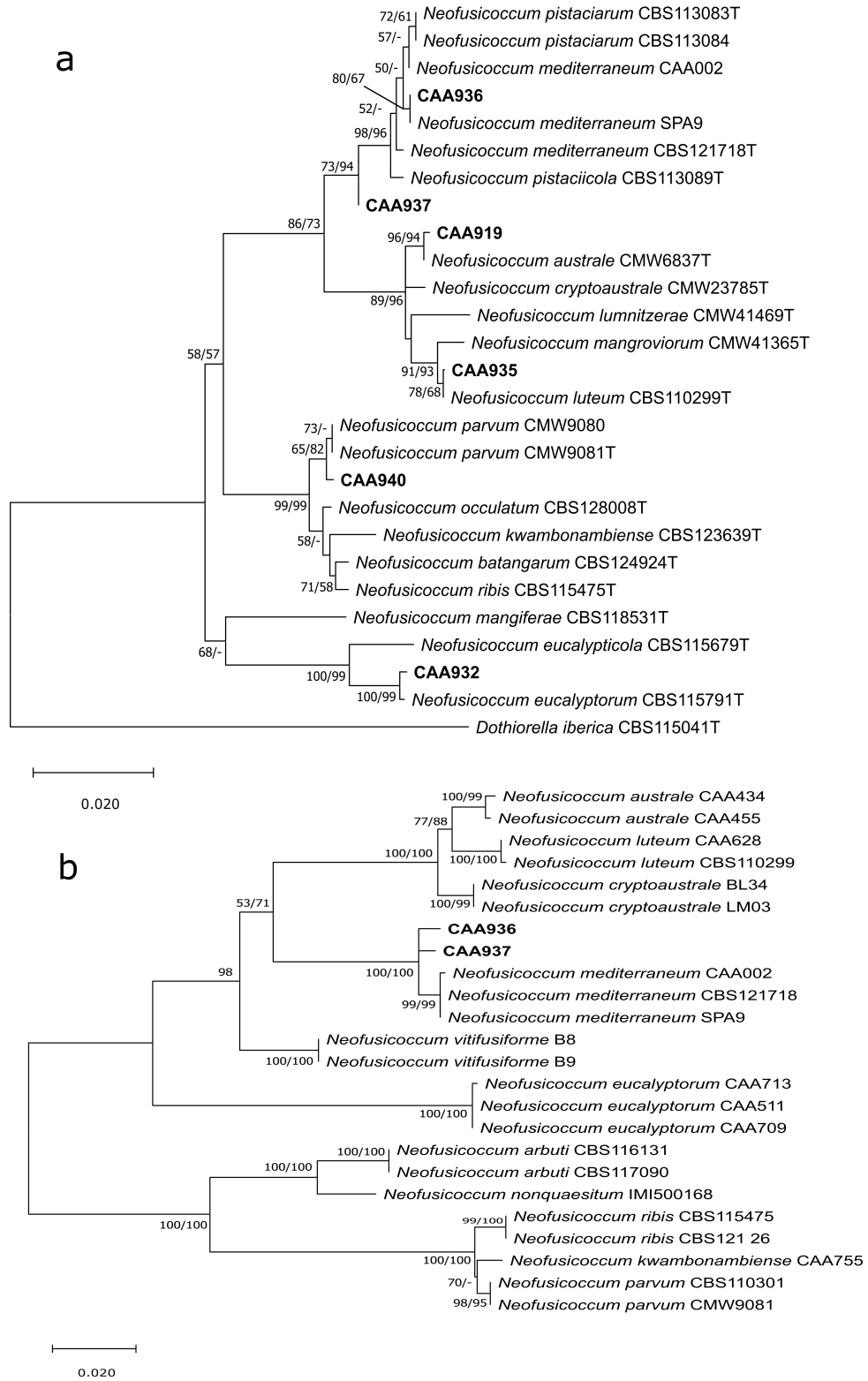

Figure 5.4: ML/MP Phylogenetic relationships of the *Neofusicoccum* isolates based on the combined ITS, *TEF1* - α and *TUB2* sequences data (a) and MAT1-1-1 gene (b). Isolates used in this study are given in bold. Bootstrap values (>50 %) are given at the nodes. Both trees are drawn to scale, with branch length measured in the number of substitutions per site. Ex-type strains are shown with a 'T' after the strain number

Table 5.4: Differences in the nucleotide sequences of the ITS, *TEF1* - α and *TUB2* regions between isolates of *N. pistaciarum*, *N. mediterraneum* and *N. pistaciicola*. The ex-type strain is highlighted in bold and differences are highlighted in grey.

	ITS					*TEF1* - α	*TUB2*								
	144	162	390	413	479		1	24	40	83	186	191	240	321	342
N. pistaciarum **CBS113083**	T	A	G	A	G	-	C	A	C	A	T	T	C	T	C
N. pistaciarum CBS113084	T	A	G	A	G	-	C	A	C	A	T	T	C	T	C
N. mediterraneum CAA002	T	A	G	G	G	-	C	A	C	A	T	T	C	T	C
CAA936	T	A	G	G	G	-	C	A	C	A	T	T	C	C	T
N. mediterraneum SPA9	T	A	G	G	G	-	C	A	C	A	T	T	C	C	T
N. mediterraneum **CBS121718**	C	A	G	G	A	-	C	A	C	A	T	T	C	C	C
N. pistaciicola **CBS113089**	T	G	A	G	A	-	C	A	C	A	T	T	C	T	C
CAA937	T	A	A	G	A	-	T	G	T	G	C	C	G	C	C

Table 5.5: Differences in the nucleotide sequences of the *MAT1-1-1* region between isolates of *N. mediterraneum*. The ex-type strain is indicated in bold and differences are highlighted in grey.

	MAT1-1-1													
	1	2	3	4	5	7	8	254	332	344	400	440	471	507
CAA936	A	G	A	C	C	T	A	T	C	G	G	T	C	A
CAA937	A	G	A	C	C	T	A	C	C	G	G	T	A	G
N. mediterraneum CAA002	T	A	G	A	G	C	G	C	T	A	A	A	C	G
N. mediterraneum **CBS121718**	A	G	A	C	C	T	A	C	T	G	A	A	C	G
N. mediterraneum SPA9	T	A	G	A	G	C	G	C	T	G	A	A	C	G

	516	578	592	640	685	698-718	725	765	806	827	999	1000	1001	1002
CAA936	G	C	T	A	T	-	G	C	C	T	A	T	C	A
CAA937	G	T	C	C	C	-	G	C	T	C	A	T	C	A
N. mediterraneum CAA002	A	C	C	C	T	TC-CTCAGGTTGCTCAGGCTGC	A	T	C	C	A	T	C	A
N. mediterraneum **CBS121718**	A	C	C	C	T	TC-CTCAGGTTGCTCAGGCTGC	A	T	C	C	T	C	A	T
N. mediterraneum SPA9	A	C	C	C	T	TC-CTCAGGTTGCTCAGGCTGC	A	T	C	C	A	T	C	A

5.4.3 Pathogenicity trials

The results from pathogenicity trials revealed 100% of mortality on *Q. suber* inoculated with *N. parvum* and *N. eucalyptorum* after 10 and 15 days respectively and on *P. pinaster* inoculated with *D. corticola* after 20 days (Table 5.6). All these plants started to show symptoms such as foliar chlorosis and dead leaves followed by a complete wood discoloration above the inoculation wound (Figure 5.5).

Mortality (40 – 60%) occurred in the interaction of *N. parvum* with *P. pinaster* and *E. globulus* and between *N. luteum* with *Q. suber* and *P. pinaster*. *Diplodia insularis* also showed aggressiveness against *P. pinaster*. These plants show similar disease symptoms to the previous group (foliar chlorosis and dead leaves), however after 40 days some plants shown only partial wood discoloration.

The remaining tested groups presented only small wood necrosis allowing the tree to maintain a functional vascular balance. In Table 5.6 the inoculated isolates are listed by the observed mortality and the remaining groups by the length of wood discoloration. Controls did not present symptoms although residual wood discoloration were observed due to damage caused by the inoculation process.

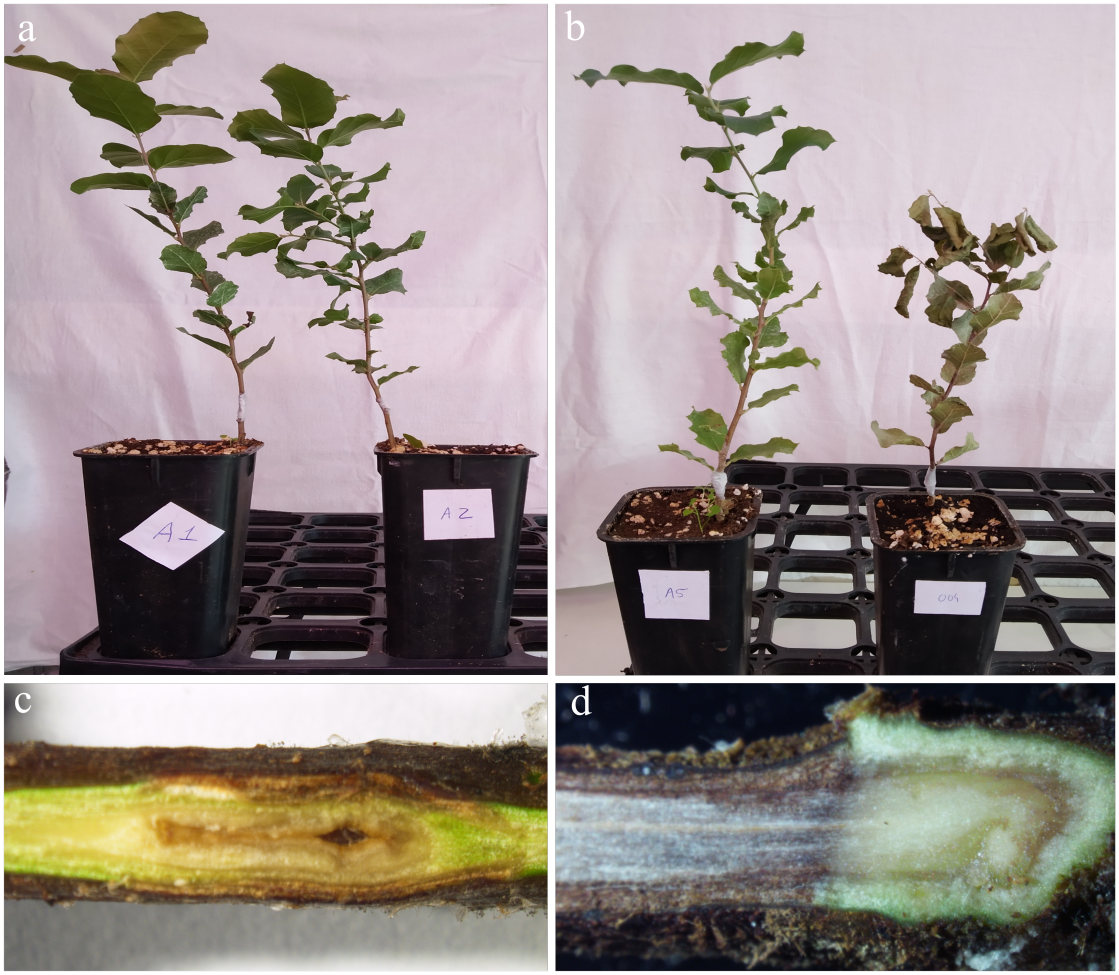

Figure 5.5: Symptoms on *Q. suber* caused by *N. parvum* (a) Control group at the inoculation day (day 0) (b) control group vs *Q. suber* inoculated with *N. parvum* at day 10 showing complete wood discoloration, (c) wood discoloration on the control group and (d) wood necrosis on the group inoculated with *N. parvum* at the end of the experiment.

Table 5.6: Average relative necrosis by the selected Botryosphaeriaceae species inoculated on *Q. suber*, *P. pinaster* and *E. globulus*. Mortality represent the number of plants deaths at the end of the experiment. Re-isolations represent the number of plants that fulfilled the Koch's postulates. 100% mortality shows the number of days after inoculation until 100% mortality was verified.

Species	Isolate	Host	Mortality (n)	Re-isolations (n)	100% Mortality (Days)	Average relative necrosis (%)	SD (%)
N. parvum	CAA940	*Q. suber*	5	5	10	100.00	0.00
N. eucalyptorum	CAA932	*Q. suber*	5	5	15	100.00	0.00
D. corticola	CAA865	*P. pinaster*	5	5	20	100.00	0.00
N. parvum	CAA940	*P. pinaster*	3	5	-	5.83	2.23
N. luteum	CAA935	*Q. suber*	3	5	-	5.28	1.19
N. parvum	CAA940	*E. globulus*	2	5	-	7.06	6.12
N. luteum	CAA935	*P. pinaster*	2	5	-	5.68	0.88
D. insularis	CAA890	*P. pinaster*	2	5	-	4.83	1.42
D. insularis	CAA890	*Q. suber*	1	5	-	3.87	1.10
N. eucalyptorum	CAA932	*P. pinaster*	0	5	-	5.04	2.48
Do. plurivora	CAA916	*Q. suber*	0	5	-	4.52	1.27
N. luteum	CAA935	*E. globulus*	0	5	-	4.50	0.83
D. pyri	CAA891	*Q. suber*	0	5	-	4.36	0.46
D. pyri	CAA891	*P. pinaster*	0	5	-	3.73	1.07
Do. plurivora	CAA916	*P. pinaster*	0	5	-	3.26	2.21
D. insularis	CAA890	*E. globulus*	0	5	-	3.08	0.44
Do. yunnana	CAA917	*Q. suber*	0	5	-	2.83	1.97
Do. plurivora	CAA916	*E. globulus*	0	5	-	2.80	1.12
Do. yunnana	CAA917	*P. pinaster*	0	5	-	2.80	2.63
D. sapinea	CAA903	*E. globulus*	0	4	-	2.55	0.59
D. pyri	CAA891	*E. globulus*	0	5	-	2.61	0.76
D. sapinea	CAA903	*Q. suber*	0	3	-	2.29	0.60
B. dothidea	CAA938	*Q. suber*	0	5	-	2.32	0.57
Do. yunnana	CAA917	*E. globulus*	0	5	-	2.15	1.01
Do. iberica	CAA905	*E. globulus*	0	5	-	2.10	0.41
Do. iberica	CAA905	*P. pinaster*	0	5	-	1.93	0.46
B. dothidea	CAA938	*P. pinaster*	0	2	-	1.54	0.87
Control	-	*P. pinaster*	0	0	-	1.19	1.09
Control	-	*Q. suber*	0	0	-	0.50	1.12
Control	-	*E. globulus*	0	0	-	0.28	0.39

5.4.4 Distribution and host association of Botryosphaeriaceae in Portugal

Isolates from the genera *Botryosphaeria*, *Diplodia*, *Dothiorella* and *Neofusicoccum* were sampled from both symptomatic and asymptomatic branches (Table 5.7). Co-occurrence of different Botryosphaeriaceae species was found only in two samples (a branch of *E. globulus* with *N. eucalyptorum* and *N. australe* and a branch of *Q. rotundifolia* with *D. corticola* and *Do. iberica*).

The frequency of Botryosphaeriaceae species on asymptomatic branches was higher in *E. globulus* when compared to other hosts. These species were mainly from the genus *Neofusicoccum*. In *Q. suber* and *P. pinaster*, Botryosphaeriaceae species were more abundant in symptomatic branches.

Combining data from the literature review with our present study, a total of 22 different Botryosphaeriaceae species have been isolated in Portugal and 41 different plant hosts. Several studies from agricultural crops to forest and ornamental species were considered. In supplementary data D is presented a compiled list of all isolates available in GenBank. It is possible to verify 105 host-pathogen interactions and a comprehensive list of pathogenicity trials for each interaction (Table 5.8).

Figure 5.6 illustrate isolates distribution by species in Portugal. Is possible to verify a wide range of distributions from north to south and from west to east. So far it is not possible to identify specific biological niches within species. However, current distributions for some species may reveal patterns regarding the preferential host distribution. As example, *D. corticola* is more abundant in the south of Portugal where the abundance of *Q. suber* is also higher; the higher prevalence of *D. sapinea* occurs in the west region, where the occurrence of *P. pinaster* is higher; the distribution of *N. eucalyptorum* commonly associated with *E. globulus*, also exhibits a wide distribution across the country.

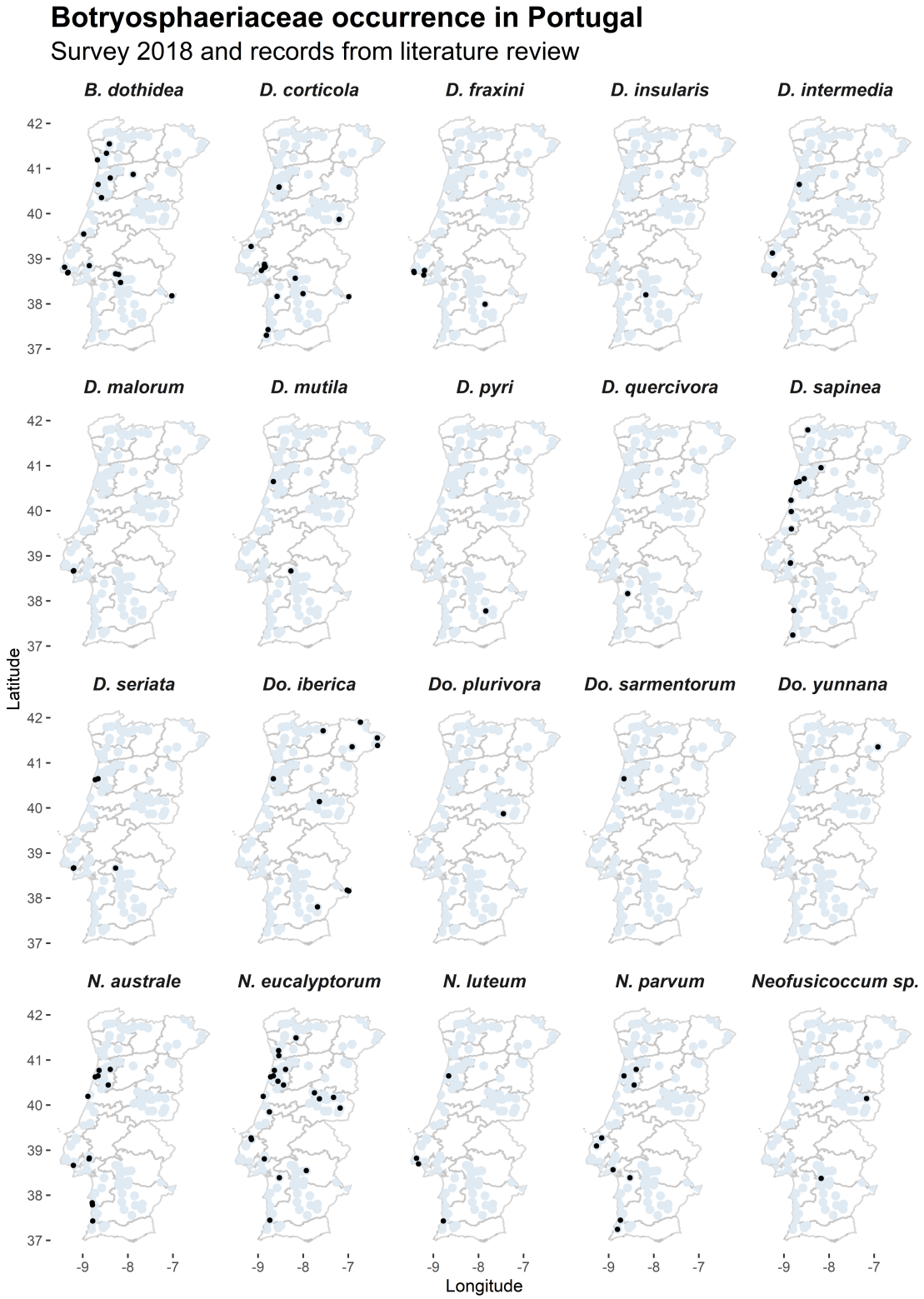

Figure 5.6: Botryosphaeriaceae occurrence in Portugal. Black dots stand for occurrence data and background blue dots stand for sampling areas.

Table 5.7: Frequency of Botryosphaeriaceae species isolated from asymptomatic or symptomatic trees.

Species	*E. globulus*		*P. pinaster*		*Q. suber*		*C. sativa*		*C. lusitanica*		*Q. ilex*	
	Asymp.	Sympt.	Asympt.	Sympt.	Asympt.	Sympt.	Asympt.	Sympt.	Asympt.	Sympt.	Asympt.	Sympt.
B. dothidea	-	-	-	-	1	2	-	-	-	-	-	1
D. corticola	2	-	-	3	5	15	-	-	-	-	3	1
D. insularis	1	-	-	-	-	-	-	-	-	-	-	-
D. pyri	1	-	-	-	-	-	-	-	-	-	-	-
D. sapinea	-	-	5	6	-	1	-	-	-	-	-	-
Do. iberica	-	1	-	-	2	1	-	1	-	-	-	7
Do. plurivora	-	-	-	-	-	-	-	-	1	-	-	-
Do. yunnana	-	-	-	-	-	-	-	-	-	-	-	1
N. australe	8	1	-	-	-	-	-	-	-	-	-	-
N. eucalyptorum	6	2	-	-	-	-	-	-	-	-	-	-
N. luteum	2	-	-	-	-	-	-	-	-	-	-	-
N. parvum	3	1	-	1	-	-	-	-	-	-	-	-
Neofusicoccum sp. CAA936	-	-	-	-	-	-	-	-	1	-	-	-
Neofusicoccum sp. CAA937	-	-	-	-	-	-		-	1	-	-	-
Total	23	5	5	10	8	19	0	1	3	0	3	10

Table 5.8: Literature compilation of all host-pathogen (Botryosphaeriaceae) interactions reported in Portugal.

Species	Host	Literature in Portugal	Pathogenicity trials
Botryosphaeria dothidea	*Eucalyptus globulus*	(Barradas *et al.*, 2016)	(Barradas *et al.*, 2016; Barradas *et al.*, 2019)
B. dothidea	*Vitis vinifera*	(Phillips and Lucas, 1997; Phillips *et al.*, 2002)	(Úrbez-Torres and Gubler, 2009; Pitt *et al.*, 2013b)
B. dothidea	*Fraxinus angustifolia*	(Phillips, 2002)	-
B. dothidea	*Styphnolobium japonicum*	(Phillips, 2002)	-
B. dothidea	*Populus nigra*	(Phillips *et al.*, 2005)	-
B. dothidea	*Juniperus communis*	(Alves *et al.*, 2013)	-
B. dothidea	*Quercus ilex*	This study	(Linaldeddu *et al.*, 2014)
B. dothidea	*Quercus suber*	This study	This study
B. dothidea	*Pinus pinaster*	-	This study
B. dothidea	*Vaccinium corymbosum*	(Hilário *et al.*, 2020)	(Hilário *et al.*, 2020)
Diplodia corticola	*Quercus suber*	(Alves *et al.*, 2004; Lopes *et al.*, 2018) and this study	(Linaldeddu *et al.*, 2009; Fernandes *et al.*, 2014; Smahi *et al.*, 2017)
D.corticola	*Eucalyptus globulus*	(Barradas *et al.*, 2016) and this study	(Barradas *et al.*, 2016; Barradas *et al.*, 2019)
D. corticola	*Pinus pinaster*	This study	This study
D. corticola	*Quercus ilex*	This study	(Linaldeddu *et al.*, 2009; Linaldeddu *et al.*, 2014)
Diplodia fraxini	*Fraxinus angustifolia*	(Alves *et al.*, 2014)	(Elena *et al.*, 2018)
Diplodia insularis	*Eucalyptus globulus*	This study	This study
D. insularis	*Quercus suber*	-	This study
D. insularis	*Pinus pinaster*	-	This study
Diplodia intermedia	*Malus sp.*	(Phillips *et al.*, 2012; Lopes *et al.*, 2018)	(Delgado-Cerrone *et al.*, 2016)
D. intermedia	*Cydonia oblonga*	(Phillips *et al.*, 2012)	-
D. intermedia	*Pyracantha coccinea*	(Phillips *et al.*, 2012)	-
D. intermedia	*Malus sp.*	(Phillips *et al.*, 2012)	-
Diplodia malorum	*Malus sp.*	(Alves *et al.*, 2006)	-
Diplodia mutila	*Vitis vinifera*	(Phillips, 2002)	(Úrbez-Torres and Gubler, 2009; Pitt *et al.*, 2013b)
D. mutila	*Taxus baccata*	(Alves *et al.*, 2013)	-
D. mutila	*Chamaecyparis lawsoniana*	(Alves *et al.*, 2014)	(Zlatković *et al.*, 2018)
D. mutila	*Populus alba*	(Alves *et al.*, 2014)	-
D. mutila	*Fraxinus ornus*	(Alves *et al.*, 2014)	-
Diplodia pyri	*Eucalyptus globulus*	This study	This study
D. pyri	*Quercus suber*	-	This study
D. pyri	*Pinus pinaster*	-	This study
Diplodia quercivora	*Quercus suber*	(Bragança *et al.*, 2016)	(Bragança *et al.*, 2016; Smahi *et al.*, 2017)
Diplodia sapinea	*Pinus nigra*	(Alves *et al.*, 2013)	(Iturritxa *et al.*, 2013; Zlatković *et al.*, 2017; Zlatković *et al.*, 2018)
D. sapinea	*Thuja plicata*	(Alves *et al.*, 2013)	-
D. sapinea	*Pinus pinaster*	(Alves *et al.*, 2013) and this study	(Swart *et al.*, 1988; Iturritxa *et al.*, 2013)
D. sapinea	*Quercus suber*	This study	(Smahi *et al.*, 2017) and this study
D. sapinea	*Eucalyptus globulus*	-	This study
Diplodia seriata	*Vitis vinifera*	(Phillips, 2002)	(Úrbez-Torres and Gubler, 2009; Pitt *et al.*, 2013b)
D. seriata	*Pyrus communis*	(Phillips *et al.*, 2012)	(Sessa *et al.*, 2016)

Table 5.8 continued from previous page

D. seriata	*Malus sp.*	(Phillips *et al.*, 2012)	(Delgado-Cerrone *et al.*, 2016; Sessa *et al.*, 2016)
D. seriata	*Thuja plicata*	(Alves *et al.*, 2013)	-
D. seriata	*Chamaecyparis lawsoniana*	(Alves *et al.*, 2013)	(Zlatković *et al.*, 2018)
D. seriata	*Fraxinus ornus*	(Lopes *et al.*, 2018)	-
D. seriata	*Eucalyptus globulus*	(Barradas *et al.*, 2016)	(Barradas *et al.*, 2016; Barradas *et al.*, 2019)
Dothiorella iberica	*Juniperus communis*	(Alves *et al.*, 2013)	-
Do. iberica	*Castanea sativa*	This study	-
Do. iberica	*Eucalyptus globulus*	This study	This study
Do. iberica	*Quercus ilex*	This study	-
Do. iberica	*Quercus suber*	This study	(Smahi *et al.*, 2017)
Do. iberica	*Pinus pinaster*	-	This study
Dothiorella sarmentorum	*Cupressus lusitanica*	(Alves *et al.*, 2013)	-
Dothiorella prunicola	*Prunus dulcis*	(Phillips *et al.*, 2008; Abdollahzadeh *et al.*, 2014)	-
Dothiorella plurivora	*Cupressus lusitanica*	This study	-
Do. plurivora	*Quercus suber*	-	This study
Do. plurivora	*Pinus pinaster*	-	This study
Do. plurivora	*Eucalyptus globulus*	-	This study
Dothiorella yunnana	*Quercus ilex*	This study	-
Do. yunnana	*Quercus suber*	-	This study
Do. yunnana	*Pinus pinaster*	-	This study
Do. yunnana	*Eucalyptus globulus*	-	This study
Neofusicoccum australe	*Acacia longifolia*	(Lopes *et al.*, 2016)	-
N. australe	*Chamaecyparis lawsoniana*	(Alves *et al.*, 2013)	-
N. australe	*Cupressus lusitanica*	(Alves *et al.*, 2013)	-
N. australe	*Eucalyptus globulus*	(Barradas *et al.*, 2016) and this study	(Barradas *et al.*, 2016; Barradas *et al.*, 2019)
N. australe	*Ferula communis*	(Lopes *et al.*, 2016)	-
N. australe	*Hydrangea macrophylla*	(Lopes *et al.*, 2016)	-
N. australe	*Melia azedarach*	(Lopes *et al.*, 2016)	-
N. australe	*Olea europaea*	(Lopes *et al.*, 2016)	-
N. australe	*Picea abies*	(Alves *et al.*, 2013)	-
N. australe	*Pinus pinaster*	(Alves *et al.*, 2013)	-
N. australe	*Pinus pinea*	(Alves *et al.*, 2013)	-
N. australe	*Pyracantha coccinea*	(Lopes *et al.*, 2016)	-
N. australe	*Quercus robur*	(Barradas *et al.*, 2013; Lopes *et al.*, 2016)	(Barradas *et al.*, 2013)
N. australe	*Sequoia sempervirens*	(Alves *et al.*, 2013)	-
N. australe	*Taxus baccata*	(Alves *et al.*, 2013)	-
N. australe	*Thuja plicata*	(Alves *et al.*, 2013)	-
N. australe	*Thujopsis dolabrata*	(Alves *et al.*, 2013)	-
N. australe	*Tilia platyphyllos*	(Lopes *et al.*, 2016)	-
N. australe	*Robinia pseudoacacia*	(Niekerk *et al.*, 2004)	-
N. australe	*Vaccinium corymbosum*	(Hilário *et al.*, 2020)	(Hilário *et al.*, 2020)
Neofusicoccum eucalyptorum	*Eucalyptus globulus*	(Barradas *et al.*, 2016) and this study	(Barradas *et al.*, 2016; Barradas *et al.*, 2019)
N. eucalyptorum	*Fraxinus excelsior*	(Lopes *et al.*, 2016)	-
N. eucalyptorum	*Pinus pinaster*	This study	This study
N. eucalyptorum	*Quercus suber*	-	This study
N. eucalyptorum	*Vaccinium corymbosum*	(Hilário *et al.*, 2020)	(Hilário *et al.*, 2020)

Table 5.8 continued from previous page

Neofusicoccum kwambonambiense	*Eucalyptus globulus*	(Barradas *et al.*, 2016)	(Barradas *et al.*, 2016; Barradas *et al.*, 2019)
Neofusicoccum luteum	*Styphnolobium japonicum*	(Niekerk *et al.*, 2004)	-
N. luteum	*Vitis vinifera*	(Phillips *et al.*, 2002)	(Úrbez-Torres and Gubler, 2009)
N. luteum	*Araucaria angustifolia*	(Alves *et al.*, 2013)	-
N. luteum	*Chamaecyparis lawsoniana*	(Alves *et al.*, 2013)	-
N. luteum	*Cupressus lusitanica*	(Alves *et al.*, 2013)	-
N. luteum	*Fraxinus excelsior*	(Lopes *et al.*, 2017)	-
N. luteum	*Fraxinus ornus*	(Lopes *et al.*, 2016)	-
N. luteum	*Melia azedarach*	(Lopes *et al.*, 2016)	-
N. luteum	*Pinus pinea*	(Alves *et al.*, 2013)	-
N. luteum	*Populus alba*	(Lopes *et al.*, 2016)	-
N. luteum	*Quercus robur*	(Barradas *et al.*, 2013; Barradas *et al.*, 2016)	(Barradas *et al.*, 2013)
N. luteum	*Sequoia sempervirens*	(Alves *et al.*, 2013)	
N. luteum	*Thuja plicata*	(Alves *et al.*, 2013)	-
N. luteum	*Thujopsis dolabrata*	(Alves *et al.*, 2013)	-
N. luteum	*Tilia platyphyllos*	(Lopes *et al.*, 2016)	-
N. luteum	*Eucalyptus globulus*	This study	This study
N. luteum	*Quercus suber*	-	This study
N. luteum	*Pinus pinaster*	-	This study
N. luteum	*Populus nigra*	(Yang *et al.*, 2017)	-
Neofusicoccum parvum	*Vitis vinifera*	(Phillips *et al.*, 2002)	(Úrbez-Torres and Gubler, 2009; Pitt *et al.*, 2013b)
N. parvum	*Aesculus hippocastanum*	(Lopes *et al.*, 2016)	(Zlatković *et al.*, 2018)
N. parvum	*Eucalyptus globulus*	(Lopes *et al.*, 2016) and this study	(Barradas *et al.*, 2016; Barradas *et al.*, 2019) and this study
N. parvum	*Quercus suber*	(Linaldeddu *et al.*, 2007)	(Linaldeddu *et al.*, 2007) and this study
N. parvum	*Pinus pinaster*	-	This study
N. parvum	*Ferula communis*	(Lopes *et al.*, 2016)	-
N. parvum	*Juniperus communis*	(Alves *et al.*, 2013)	-
N. parvum	*Malus sp.*	(Lopes *et al.*, 2017)	(Delgado-Cerrone *et al.*, 2016; Sessa *et al.*, 2016)
N. parvum	*Pinus pinea*	(Alves *et al.*, 2013)	-
N. parvum	*Rosa sp.*	(Lopes *et al.*, 2016)	-
N. parvum	*Thuja plicata*	(Alves *et al.*, 2013)	-
N. parvum	*Thujopsis dolabrata*	(Alves *et al.*, 2013)	-
N. parvum	*Protea cynaroides*	(Marincowitz *et al.*, 2008)	-
N. parvum	*Vaccinium corymbosum*	(Hilário *et al.*, 2020)	(Hilário *et al.*, 2020)
Neofusicoccum protearum	*Leucadendron sp.*	(Marincowitz *et al.*, 2008)	-
N. protearum	*Protea cynaroides*	(Marincowitz *et al.*, 2008)	-

5.4.5 Host jump analyses

Combining the information from our survey, pathogenicity trials and the literature review regarding the Botryosphaeriaceae species and known hosts interactions in Portugal a network of possible hosts jumps at the genus and species level was constructed (Figures 5.7 and 5.8). For the genus *Botryosphaeria*, only *B. dothidea* is known to occur in Portugal. We report for the first time in Portugal interactions with *Q. suber* and *Q. rotundifolia* (blue dots number 24 and 26 of Figure 5.7). Also, was confirmed the possibility of host-jump to *P. pinaster* during the pathogenicity trial (red dot number 6).

For the genus *Diplodia*, several interactions are described among different types of hosts (G1 Pinales, G3 Vitales, G4 Asterids group, G5 Rosids I group and G6 Rosids II group represented by blue background lines). With our survey, and concerning *D. corticola*, we report for the first time in Portugal an interaction with *Q. rotundifolia* and one new host, *P. pinaster*. Additional new interactions were described. For *D. pyri*, new host report, *E. globulus*, and first report in Portugal as well as possible host jump to *P. pinaster* and *Q. suber*. *Diplodia sapinea*, first report in Portugal on *Q. suber* and possible host jump to *E. globulus*. We report for the first time *D. insularis* in Portugal and in *E. globulus*. Pathogenicity trials confirmed the possibility of host-jump to *Q. suber* and *P. pinaster* (Figure 5.8).

Another example of expansion of host range is *N. eucalyptorum* (Figure 5.7). We report for the first time the occurrence of this species on *P. pinaster* and the first known record among the Pinales group (G1). During the pathogenicity trials the possibility to infect *Q. suber* was confirmed. *Neofusicoccum parvum* (Figure 5.7) follows the same range pattern of all combined *Neofusicoccum* species matching a huge variety of hosts in all hosts groups. In this study we report for the first time the interaction with *P. pinaster*. For *N. luteum* additional reports were made, new host- *E. globulus*, and possible hosts jumps - *Q. suber* and *P. pinaster* (Figure 5.8). For the genus *Dothiorella*, we report for *Do. iberica* as new hosts *E. globulus* and *Castanea sativa*. In Portugal we report for the first-time interactions with *Q. suber* and *Q. rotundifolia*. Also, we report for first time the occurrence in Portugal of *Do. plurivora* and *Do. yunnana* on *E. globulus* and *Q. rotundifolia*, respectively. Pathogenicity trials confirmed the possibility of host-jump to all tested hosts (Figure 5.8).

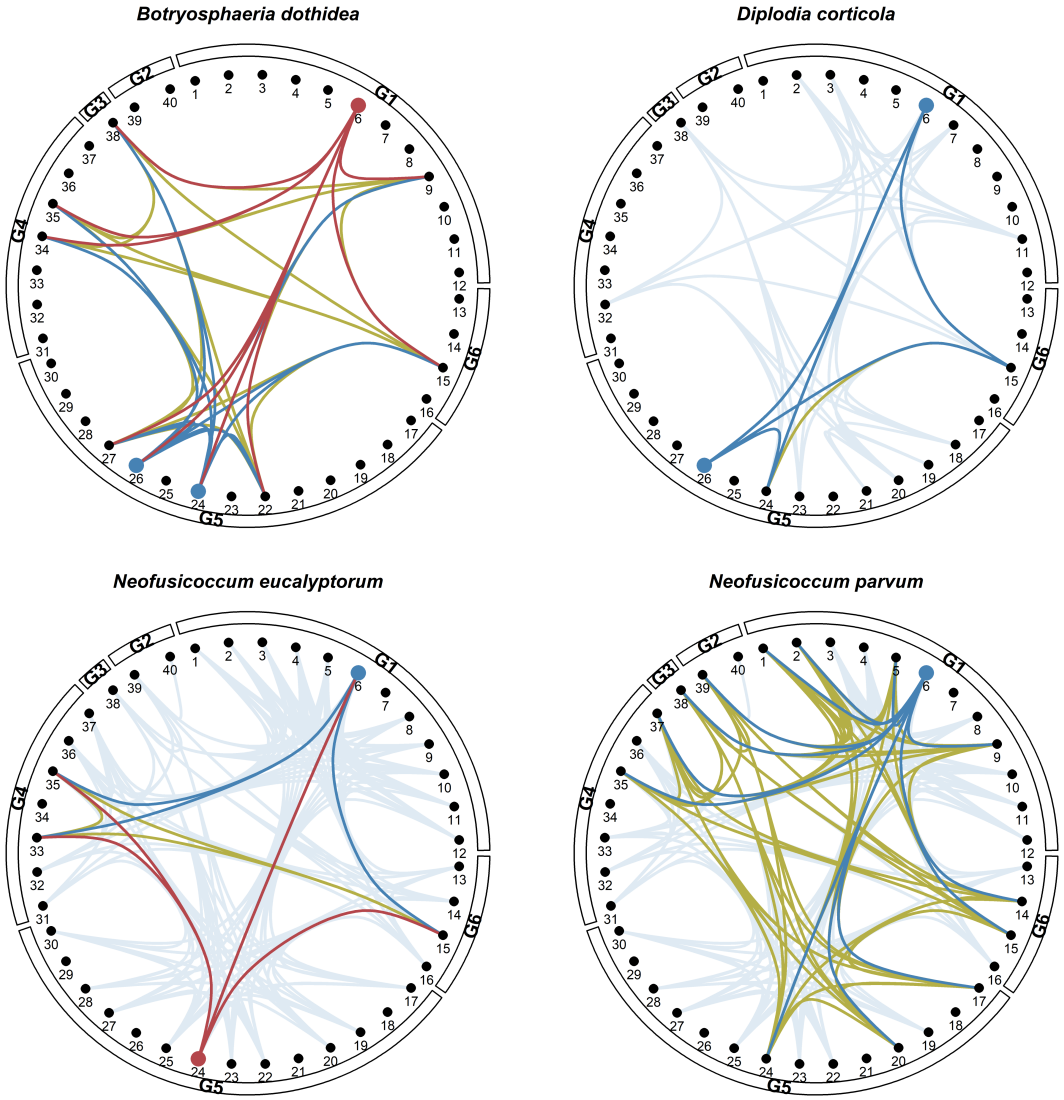

Figure 5.7: Shared hosts interactions in Portugal based on the survey 2018 and records from literature review. Nodes represent hosts and are grouped by taxonomic similarity. G1 Pinales group: 1 - *Thujopsis dolabrata*, 2 - *Thuja plicata*, 3 - *Taxus baccata*, 4 - *Sequoia sempervirens*, 5 - *Pinus pinea*, 6 - *Pinus pinaster*, 7 - *Pinus nigra*, 8 - *Picea abies*, 9 - *Juniperus communis*, 10 - *Cupressus lusitanica*, 11 - *Chamaecyparis lawsoniana*, 12 - *Araucaria angustifolia*. G6 Rosids II group: 13 - *Melia azedarach*, 14 - *Aesculus hippocastanum*, 15 - *Eucalyptus globulus*, 16 - *Tilia platyphyllos*. G5 Rosids I group: 17 - *Rosa* sp. ,18 - *Pyrus communis*, 19 - *Pyracantha coccinea*, 20 - *Malus* sp., 21- *Cydonia oblonga*, 22 - *Populus nigra*, 23 - *Populus alba*, 24 - *Quercus suber*, 25 - *Quercus robur*, 26 - *Quercus ilex*, 27 - *Styphnolobium japonicum*, 28 - *Robinia pseudoacacia*, 29 - *Castanea sativa*, 30 - *Acacia longifolia*. G4 Asterids group: 31 - *Olea europaea*, 32 - *Fraxinus ornus*, 33 - *Fraxinus excelsior*, 34 - *Fraxinus angustifolia*, 35 - *Vaccinium corymbosum*, 36 - *Hydrangea macrophylla*, 37 - *Ferula communis*. G3 Vitales group: 38 - *Vitis vinifera*. G2 Proteales group: 39 - *Protea cynaroides* and 40 - *Leucadendron* sp. Lines represent host-fungus interactions in Portugal, where background lines represent all known interactions of the respective Botryosphaeriaceae genus, green lines represent known interactions of the respective Botryosphaeriaceae species, blue lines represents new host-jumps observed in nature during this study and red lines represent new host-jumps observed during the pathogenicity trials.

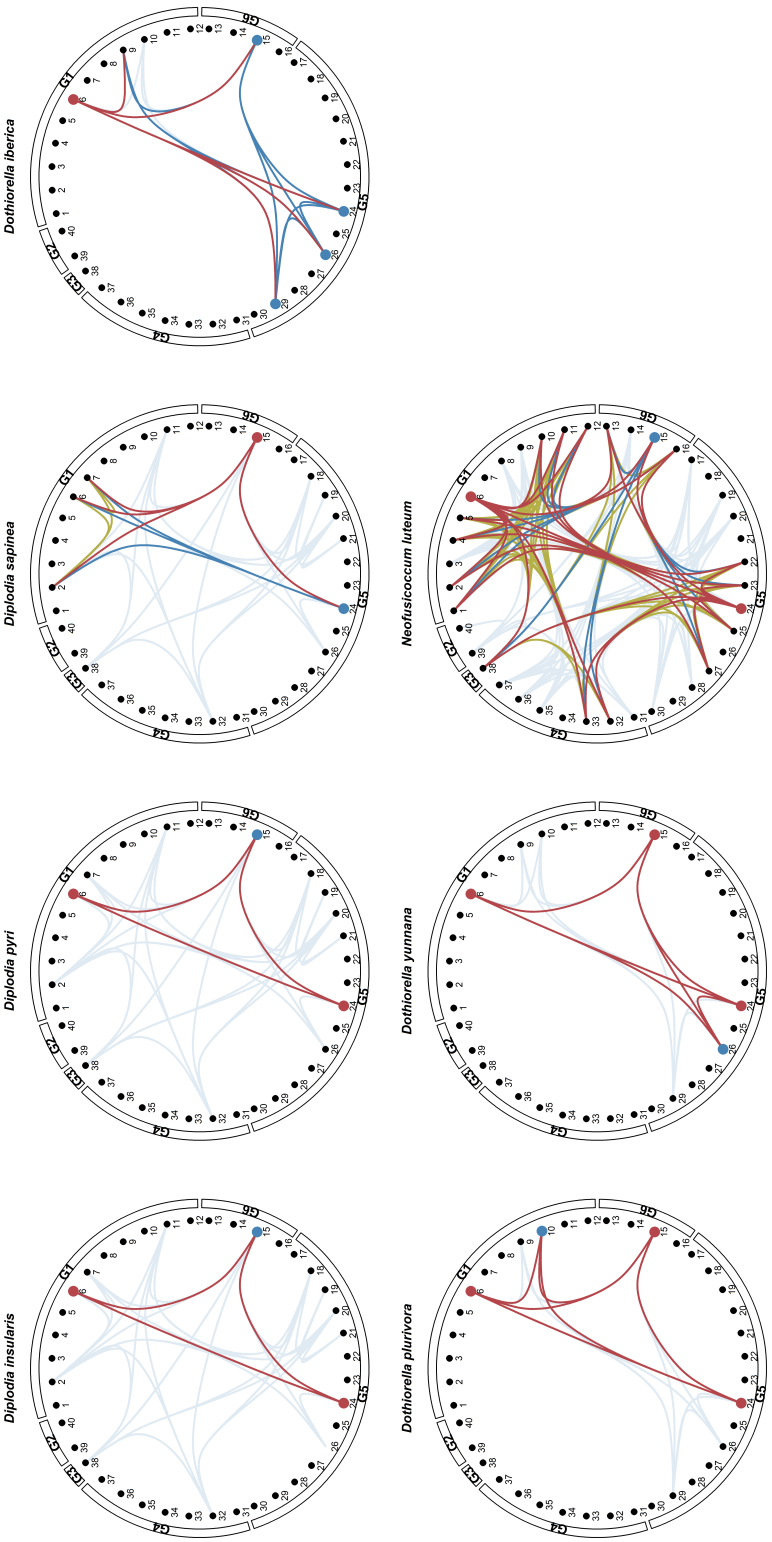

Figure 5.8: Shared hosts interactions in Portugal based on the survey 2018 and records from literature review. Nodes represent hosts and are grouped by taxonomic similarity. For nodes ID please check (Figure 5.7. Lines represent host-fungus interactions in Portugal, where background lines represent all known interactions of the respective Botryosphaeriaceae genus, green lines represent known interactions of the respective Botryosphaeriaceae species, blue lines represents new host-jumps observed in nature during this study and red lines represent new host-jumps observed during the pathogenicity trials.

5.5 Discussion

This study is a comprehensive overview of the Botryosphaeriaceae species occurring in Portugal with emphasis on their forest hosts. Over the last two decades efforts have been made to characterize the diversity of fungi of this family associated with different plant hosts (Table 5.8). However, these efforts often resulted from occasional sampling in restricted geographic locations. This national survey is our first attempt to characterize the Botryosphaeriaceae communities associated with the main forest tree species present throughout Portugal. Our extensive survey and sampling effort, focussed mostly on *E. globulus*, *P. pinaster* and *Q. suber*, resulted in the isolation and identification of 12 species belonging to four genera of Botryosphaeriaceae, specifically *Botryosphaeria* (*B. dothidea*), *Diplodia* (*D. corticola, D. insularis, D. pyri, D. sapinea*), *Dothiorella* (*Do. iberica, Do. plurivora, Do. yunnana*) and *Neofusicoccum* (*N. australe, N. eucalyptorum, N. luteum, N. parvum*).

In addition, two isolates (CAA936 and CAA937) obtained from *C. lusitanica* in two distinct locations, could not be undoubtedly affiliated to a species and were identified as *Neofusicoccum* sp.. In phylogenetic analyses these isolates grouped within a clade that included the species *N. mediterraneum*, *N. pistaciarum*, and *N. pistaciicola*. Few differences were found between these three species and the above-mentioned isolates in the analysis of ITS, *TEF1* - α and *TUB2* sequences, which suggests these may in fact represent a single species. Additional analysis using the MAT1-1-1 locus, which is known to help resolve closely related species (Lopes *et al.*, 2017) showed differences between isolates CAA936, CAA937 and *N. mediterraneum*. Nevertheless, in the MAT1-1-1 phylogeny this group of isolates formed a monophyletic and highly supported clade. Unfortunately, the ex-type cultures of *N. pistaciarum* and *N. pistaciicola* were not available for us to sequence their MAT1-1-1 gene, thus debilitating this analysis. Although our results strongly suggest that *N. pistaciarum* and *N. pistaciicola* are synonyms of *N. mediterraneum*, we refrain from formally introducing this synonymy until further studies are done.

In the genus *Diplodia* the separation between *D. mutila* and *D. pyri* is tenuous. They are distinguished by a single indel of a trinucleotide microsatellite repeat in the *TEF1* - α region, which could be interpreted as intraspecific variability. Future studies, including the analyses of additional markers such as the MAT locus (Lopes *et al.*, 2018) are needed to resolve the status of *D. pyri* as a distinct species.

From the 12 species identified *D. corticola* was the most frequent representing 35% of the total number of isolates. It is recognised as a pathogen of *Quercus* species, especially *Q. suber* and *Q. rotundifolia* in the Mediterranean region (Alves *et al.*, 2004; Linaldeddu *et al.*, 2017) but has been found on *Cercis canadensis*, *E. globulus* and *Vitis vinifera* (Slippers *et al.*, 2007; Úrbez-Torres *et al.*, 2010; Barradas *et al.*, 2013). Here we report it for the first time on *Q. rotundifolia* in Portugal, and for the first time on *P. pinaster* which represents a new host and reinforces the apparent host expansion tendency of this fungal species. Pathogenicity trials on *P. pinaster* showed a complete vascular discoloration after 20 days

in plants under well-watered conditions. This newly described host-jump should be taken in consideration for future studies to understand the prevalence of this new host-pathogen interaction. *Diplodia corticola* is still one of the most common Botryosphaeriaceae-related pathogens on cork oak with 70% of all public isolates being reported from this host (Supplementary data D).

Diplodia sapinea association with *P. pinaster* had been previously described (Alves *et al.*, 2013) and here we report it on *Q. suber* for the first time in Portugal. Our results confirm those of (Smahi *et al.*, 2017) who recently reported it as a pathogen of cork oak for the first time in Algeria. Two other *Diplodia* species, *D. insularis* and *D. pyri*, are new reports from Portugal, both associated with *E. globulus*. So far, *D. insularis* had been found on *Pistacia lentiscus* and *Fraxinus angustifolia* in Italy (Linaldeddu *et al.*, 2016), and *Eriobotrya japonica* in Spain (Giambra *et al.*, 2016) while *D. pyri* was known only from *Pyrus* sp. in The Netherlands (Yang *et al.*, 2017). In artificial inoculation trials both species proved to be pathogenic to *E. globulus*. Interestingly, both species were also more aggressive towards *P. pinaster* and *Q. suber*, suggesting that they hold potential to be pathogenic to these hosts.

Four species of *Neofusicoccum* (*N. australe, N. eucalyptorum, N. luteum* and *N. parvum*) were found on *E. globulus* during the survey. Additionally, *N. eucalyptorum* was also identified on *P. pinaster*. On a previous study of Botryosphaeriaceae associated with *Eucalyptus* spp. in Portugal (Barradas *et al.*, 2016) all these species have been reported, with the exception of *N. luteum*. In fact, *N. luteum* has only reported to occur on *Eucalyptus* spp.. once on *Eucalyptus camaldulensis* in Italy (Deidda *et al.*, 2016). Despite this, in pathogenicity trials it proved to be pathogenic and particularly aggressive to *E. globulus*, as well as to *P. pinaster* and *Q. suber*, two other hosts on which it has never been found. Following a similar trend, *N. eucalyptorum* and *N. parvum* were shown to be highly aggressive to these two hosts. Of special attention is the interaction of *N. parvum* with *Q. suber*, where only 10 days after inoculation were needed to achieve 100% of mortality. These results are consistent with those of (Linaldeddu *et al.*, 2007) and, to our knowledge, no other observations in nature have been made.

When compared to other species of the genus, *N. eucalyptorum* appears to be highly host specialized, often associated with *Eucalyptus* spp. and occasionally on other Myrtaceae (Slippers *et al.*, 2004; Barradas *et al.*, 2016). Only two other known host interactions outside of the family Myrtaceae are known, namely with *F. excelsior* (Lopes *et al.*, 2016) and *V. corymbosum* (Hilário *et al.*, 2020), both of them from Portugal. Here we report it for the first time in a coniferous host, *P. pinaster*. Usually described as weakly pathogenic (Barradas *et al.*, 2019; Hilário *et al.*, 2020), when tested on *Q. suber* a 100% mortality was registered only 15 days after inoculation. It thus has the potential to become a highly aggressive pathogen of cork oak. Similar to the case of *D. corticola*, *N. eucalyptorum* appears to hold potential to expand its host range.

In a context of climate change and knowing that this group of pathogens is often favoured by drought stress (Slippers and Wingfield, 2007) these results raise a big concern

for the future health status of the cork oak woodlands. Future studies should target these interactions in order to better understand them and mitigate possible damage.

Botryosphaeria dothidea is the only species of the genus *Botryosphaeria* known to occur in Portugal. We report it for the first time in Portugal in association with *Q. suber* and *Q. rotundifolia*, which have been previously established in other Mediterranean regions (Sánchez *et al.*, 2003; Linaldeddu *et al.*, 2014; Zlatković *et al.*, 2018). Also, it was confirmed the possibility of host-jump to *P. pinaster* during the pathogenicity trial, which is not completely unexpected as *B. dothidea* has been found on other *Pinus* species (Phillips *et al.*, 2013). What is striking is its low prevalence in our survey, which agrees with previous surveys on *Eucalyptus* spp. also in Portugal (Barradas *et al.*, 2016), but contrasts with other studies, in different geographic locations, where it was identified as the most abundant species on forest trees (e.g. (Chakusary *et al.*, 2019)).

We report for the first time the occurrence of *Do. plurivora* and *Do. yunnana* in Portugal, on *E. globulus* and *Q. rotundifolia*, respectively. Both species are known to occur on a diverse hosts (Zhang *et al.*, 2017) and these reports just reinforce their plurivorous nature. Pathogenicity trials of both *Dothiorella* species showed low or no effect on the health status of the tested hosts.

The analysis of distribution of the Botryosphaeriaceae species across the Portuguese territory showed that, in general, species of this family are widespread geographically. Some patterns of distribution were nevertheless identified for a few species, which could be somehow be explained by the host preference of the fungal species, namely *D. corticola* and *Q. suber* (mostly in the south), *D. sapinea* and *P. pinaster* (along the Atlantic coast), *N. eucalyptorum* and *E. globulus* (widely distributed but concentrated essentially along the Atlantic coast). Given the small number of reports for some species, further studies are needed in the future for a better definition of species distribution and the factors that affect it.

Overall, after compiling all the data with the literature review, we were able to identify 22 different Botryosphaeriaceae species and 105 hosts-pathogens interactions in Portugal. Also 16 possible host jumps were confirmed with our pathogenicity trials. Our knowledge regarding the pathogenicity effect of these organisms is still poor, since from the 105 known host-pathogen interactions in Portugal, only 42 were verified in pathogenicity trials under controlled, and most of them in a non-stress scenario. Pathogenicity effects under stress scenarios like drought are still not well understood for most of the host-pathogen interactions. Different set-ups of pathogenicity trials and assessment of effects makes comparisons across studies difficult or even impossible. Also, defining levels of aggressiveness exclusively on length of wood discoloration without taking in consideration: plant physiological and biochemical parameters, the variety of fungal pathogenesis mechanisms, the timing of the infection and the environment effect on the host-pathogen interaction might not correlate directly with strain aggressiveness and should not be extrapolated for other host-pathogen interactions (Manawasinghe *et al.*, 2016; Félix *et al.*, 2017; Caldeira, 2019; Wang *et al.*, 2018).

Several authors already identify that some phytotoxins, degradative and oxidative enzymes, cytotoxic proteins and a few secondary metabolites play a role in the infection mechanism (e.g for *D. corticola* on *Q. suber*, (Fernandes *et al.*, 2014; Masi *et al.*, 2016). However, to the extension of our work, that knowledge is still limited. For that reason, further studies should be conducted to understand the molecular mechanisms of pathogenesis among the host-fungus interactions were mortality was higher (e.g. *N. parvum* - *Q. suber*, *N. eucalyptorum* - *Q. suber* and *D. corticola* - *P. pinaster*).

Host-jumps analyses should be taken more often in consideration. Our approach of comparing known genera interactions against the known interactions of an individual species, allow us to explore possible expansion patterns for new host-jumps and guide further studies.

To finalize, our study summarizes the wide diversity and capability to infect new hosts of Botryosphaeriaceae species in Portugal. Our literature review and our hosts jump analyses identify several knowledge gaps that should be taken in consideration in the future for a better forest management.

Chapter 6

General discussion

Forests are complex and dynamic ecosystems, that should be observed and studied at different levels. This complexity underlies on a network of biotic and abiotic factors that have evolved through time in a slow and mature process that we often call, natural selection (with very few historical exceptions like rapid mass extinctions events). As a result of global changes, most of human-induced modifications have changed the dynamics in time, space, and use of this ecosystem affecting the evolution of these living entities as we know them.

Worldwide forests represent 31% of the total land use occupying about of 4.06 billion hectares. Since 1990, the world has lost 178 million hectares of forest mainly due to deforestation in South America and Africa. Decade after decade, countries mostly in Asia and Europe, have fight back this tendency by allowing ecosystems to regenerate naturally or by investing in planted forests (Food and Organization, 2021). Although the rate of new plantations has slowed in the last ten years, several countries have announced ambitious plans to plant billions of trees in the following years (Publications, 2021). This new era of "massive new forest plantations" raises several challenges to the plant physiology and plant pathology communities. One of many challenges is related with the large quantity of plant material moving worldwide every day, making almost virtually impossible to verify and detect efficiently latent pathogens living endophytically in symptomatic or asymptomatic material. Another challenge is related with our ability to create resilient ecosystems in a global change scenario taking into consideration several biotic and abiotic stresses. These stresses are often associated to rapid changes in climate or to the unexpected introduction of invasive and pathogenic organisms. Such changes are the opposite of what we initially described, as the normal and slow process of natural selection, and makes these ecosystems vulnerable. The emergence of stress-related fungal plant pathogens, like many Botryosphaeriaceae species, besides the negative economic impact on yield loss, can unbalance an entire ecosystem leading to biodiversity loss and land degradation. Therefore, more than ever it is urgent to understand the role of these organism in a global change scenario.

The present thesis aimed to Map, Detect and Research Botryosphaeriaceae species

occurrence worldwide in different plant hosts. This thematic was explored from different perspectives: from field surveys to molecular and phylogenetic characterizations or from worldwide database analyses to species distribution modelling and risk assessment. All these data allow us to have a better picture of the role of Botryosphaeriaceae-related diseases.

6.1 Data, data, and more data. What can plant pathology learn about data?

The empirical nature of the Human being to observe, measure, collect and record information to drive knowledge based on experience rather than beliefs has structured the foundation of scientific research along the history. However, the speed and capacity to collect and store information always over-passed our ability to analyze and process information. In plant pathology this is no exception. Humans have consistently observed and recorded information about plant diseases worldwide, although even nowadays we are not able to get the most out of these information's.

Initiatives like U.S. National Fungus Collections Specimen Database developed by U.S. Department of Agriculture or the EPPO standards and database developed by the European and Mediterranean Plant Protection Organization brought some consistency to monitor and diagnose plant-related diseases and invasive organisms by aggregating scientific information and developing international strategies against the introduction and spread of pest or by promoting safe and effective pest control methods. However, the scientific community still lacks standard measures to report and automatically aggregate data of new occurrences or new host-fungus associations. Making the process of maintaining constant updated databases costly and time-demanding.

During this project we aimed to build an aggregated and curated open-dataset of Botryosphaeriaceae-related diseases to offer a complete perspective on the species global diversity, dispersion, host association, ecological niches, pathogenicity and on the communication efficiency of new occurrences and new host-fungus associations. This dataset was transformed in an online and interactive database. Since the MDRBOT database release, this dataset has been consulted by several national and international researchers resulting in more than 15 hours per month of online visualizations. The raw data is freely available to be downloaded and to be aggregated to larger databases of plant related diseases.

6.2 How efficiently are countries monitoring and communicating the occurrence of these organisms?

In chapter 2, based on this dataset, we compared known diversity versus sampling effort around the world to understand how much we really know about these pathogens. We concluded that 138 countries still have no records of Botryosphaeriaceae species de-

posited in GenBank and 80% of all known isolates are concentrated in only 11 countries. This unbalanced sampling effort around the world suggests that we are still far from reaching a plateau in species diversity and the impact of these plant-pathogens may be underestimated. Also, these data allow us to dissect the idea that although most Botryosphaeriaceae species have a worldwide distribution, this distribution is probably mainly constrained by climate and not by host specificity. Also, in chapter two we analyzed the quality of the disease reports worldwide and we found only 23.07% of known host–fungus interactions by country are properly reported in both indexed scientific journals and public sequence databases. The incapability of our society to efficiently use and aggregate data of these emergent plant-pathogens will reflect in our future ability to prevent, react or mitigate impacts of new outbreaks. More than ever, we consider that consistent and open plant pathology databases are fundamental to address the challenge of Botryosphaeriaceae-related diseases in a changing world. In chapter 3, we evaluated the quality of standards used for publication of new Botryosphaeriaceae taxa based in a list of 210 representatives' species. We found that over 90% of the descriptions are followed by a detailed morphological characterization and with consistent phylogenetic analyses, for molecular characterization and host-fungus interactions 60% of the descriptions are outdated or only meet the minimal requirements for publication and 50% of the authors do not provide enough accessible and reproducible information. In plant pathology, fungal taxonomists are essential to define the language of communication about different organisms among scientists and society in general. These results are in line with the previous analyses of the quality of the diseases reports presented in chapter 2. Repeatedly, we stress that, to address the challenges of fungal diseases of plants in a changing world, an efficient communication is needed. With this chapter we aimed to raise the discussion between authors, editors, and reviewers to establishing well-defined best practices for new fungal species descriptions to ensure reproducibility, transparency, and consistency over time.

6.3 Global Dispersion - How Far Can They Go?

From a global perspective, we found the necessity to formulate five assumptions, that we believe, are essential to understand the ecological niche requirements and frame the role of these plant-pathogens organisms in a context of global change. To know: (1) endophytic latent pathogens are mainly dispersed by human activities such as movement and trade; (2) the introduction of a species in a new environment is likely to occur, as human movement/trade exists and is favored by a lack of preventive and quarantine measures; (3) the establishment of these species is affected by climate. Nonetheless, unfavorable conditions might hide the presence of those species in asymptomatic hosts or by resistance structures. Seasonal effects might expand or decrease the growth of these organisms, invalidating viable long-term populations; (4) optimal conditions for disease expression are mainly occasional climatic events that can affect the susceptibility of the host (i.e., a reduction in precipitation or/and temperature increments could cause drought or heat stress

to the host) or/and trigger the pathogenic behavior of these organisms (i.e., variations in temperature, light intensity, or atmospheric ozone could induce phytopathogenic mechanisms); (5) only when host specificity is demonstrated, whether for a fungal species with a limited ability to colonize and persist endophytically in certain hosts or/and for species with limited ability to infect and express disease symptoms in a certain type of hosts, is it assumed that biotic interactions can shape the geographical distribution. These assumptions were consistently used and tested along this thesis and therefore shaped our vision about the ecology of these organisms.

6.4 Understanding the role of these organisms in a global change scenario.

Based on these assumptions, in chapter 4 we mapped suitable areas for five Botryosphaeriaceae species, according to three different climate scenarios and three different time slots. We predicted an overall increase of suitable areas for these pathogens in most of the studied scenarios and a possible range expansion in the northern hemisphere for *Botryosphaeria dothidea* and *Neofusicoccum parvum*. A consistent increase of the optimal growth months for fungi development was verified in most of the regions with predicted suitability of the north hemisphere that eventually could impact the phenology of these organisms and originate more frequent and intensive outbreaks. Understanding direct and indirect impacts of climate change on Botryosphaeriaceae-related diseases is complex and should be studied in detail for specific regions and for individual fungal-host interactions. If, on one hand, direct effects might favor pathogen multiplication and range expansion, on the other hand, climate change can indirectly affect the host resilience to these diseases. The use of species distribution models proved to be an efficient tool to understand the ecological niche requirements of those species and to forecast possible future impacts. These models should be more often used and implemented in management strategies to prevent or minimize the impact of future disease outbreaks.

6.5 What do we know about Botryosphaeriaceae species occurrence and impacts in Portugal?

In chapter 5, we conducted a survey across the country to identify Botryosphaeriaceae species associated with the main forest tree species in Portugal, *Quercus suber*, *Eucalyptus globulus* and *Pinus pinaster*. Additionally, a meta-analysis was performed to compile and organize all records known from Portugal. In total, 22 different Botryosphaeriaceae species were reported, and 40 different plant hosts were recorded in several studies. A total of 105 host-pathogen interactions were identified. In the national survey, 12 Botryosphaeriaceae species were identified, with *Diplodia corticola* being the most frequent. *Diplodia insularis*, *Diplodia pyri*, *Dothiorella plurivora* and *Dothiorella yunnana* were reported for

the first time in Portugal. Of the 23 different host-fungus associations identified, 10 are also recognized as new hosts.

Based on the assumption that optimal conditions for disease expression are mainly occasional climatic events that can affect the susceptibility of the host or/and trigger the pathogenic behavior of these organisms we aimed to sample, consistently, both symptomatic and asymptomatic hosts during the survey. Sampling asymptomatic hosts was important for an early detection of new species occurrence (e.g., *Diplodia insularis* was reported for the first time in Portugal in an asymptomatic host). Therefore, we encourage researchers to increase their sampling effort and consider collecting also asymptomatic material due to the latent endophytic lifestyle often common in this family.

Artificial inoculation tests confirmed the pathogenicity of all species, except *Dothiorella iberica*, *Dothiorella plurivora* and *Dothiorella yunnana*, which are regarded as weakly or non-pathogenic to the hosts tested. Overall, we found that, from the 105 known host-pathogen interactions in Portugal, only 42 were verified in pathogenicity trials under controlled, and most of them in a non-stress scenario. This fact raises our concern of the need to obtain more information about the pathogenicity potential of these host-pathogen interactions.

Pathogenicity tests also revealed the host-jump potential of some species, showing high susceptibility of *Q. suber* to *Neofusicoccum parvum* and *N. eucalyptorum* and of *P. pinaster* to *D. corticola*. Of special attention is the interaction of *N. parvum* with *Q. suber*, where only 10 days after inoculation were needed to achieve 100% of mortality. These results are consistent with those of Linaldeddu et al. (2007) and Mahamedi et al. (2020) and, to our knowledge, no other observations in Portugal have been made. *Neofusicoccum parvum* is one of the most known aggressive pathogens of this family and might be triggered by environmental stress, like drought. Considering that fungi of this family are stress-related pathogens, in a scenario of climate change as the one predicted by the Intergovernmental Panel on Climate Change, the development of Botryosphaeriaceae-related diseases in Portugal would be clearly favored.

6.6 Can we define a host range and anticipate future host-jumps for fungal latent endophytic species?

The previous results raised our attention to understand which factors are essential to define a host range of a fungal species. Several drivers are often mentioned in the literature, such as international trade, the failure of quarantine and preventive measures, changes in land use or agricultural practices, pathogen evolution and plasticity, mechanisms of genome divergence (e.g., mutation, hybridization, sexual recombination, horizontal gene transfer, and others), host–fungus genotype-by-genotype interactions, poor host health, and climate change, among others (Burdon and Silk, 1997; Lambrechts, 2010; Brown and Tellier, 2011; Gange *et al.*, 2011; De Fine Licht, 2018; Corredor-Moreno and Saunders, 2020).

Comparative genomics and omics studies are slowing unveiling host–fungus interaction mechanisms by dissecting plant defence mechanisms, fungal pathogenic strategies, and nutrient uptake pathways. However, to clarify a momentary host range boundary and spot host specificity, a complete overview of all the mentioned areas is crucial and future studies are needed to understand better the process of a host-jump (Raffaele and Kamoun, 2012; Möller and Stukenbrock, 2017; Westermann *et al.*, 2017; Félix *et al.*, 2019; Han, 2019).

6.7 Final considerations, gaps, and future research opportunities

The present thesis offers a broad perspective on species global diversity, dispersion, host association, ecological niches, pathogenicity and communication efficiency of new occurrences and new host-fungus associations. It also discussed the importance of countries to ensure an efficient monitor, diagnose and communication strategy to prevent new emergent outbreaks and mitigate impacts of already known existing occurrences.

Several knowledge gaps are highlighted regarding: the genomic pathogenicity potential of these organisms; how the environment can promote/trigger the pathogenic behavior of these endophytic latent species in different host-fungus associations; which mechanisms are behind a new host-jump and in my opinion, the most important, what can be the role of global changes (international mobility and trade, land use changes, long term climatic changes and/or occasional extreme weather events) in the resilience of plant hosts across the world? This last question offers several future research opportunities, especially in northern hemisphere extratropical latitudes, where these host-fungus interactions will be most certainly affected by the intensity of the process of Arctic Amplification of global warming.

Portugal, although one of the most active countries in the scientific community regarding the study of Botryosphaeriaceae species, still fails to have consistent and standard strategies to monitor, diagnose and communicate new occurrences, new associations and evaluate the present ecological and economic impacts. Evaluating these impacts, not only for Botryosphaeriaceae species but also for other pests and invasive species, is probably one of the biggest challenges for the agriculture and forestry sectors in Portugal. An efficient assessment of the ecological and economic impacts of pathogenic and invasive organisms requires, among other things, a collaborative effort with different governmental, research, and private institutions not only to inventory different crops and forest land uses with an annual frequency but also to monitor, diagnose and publicly report relevant occurrences and impacts of different pathogenic and invasive organisms. It is impossible to create more resilient ecosystems for the future if we don't know the full current extension of our problems.

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Appendices

Appendix A

Supplementary data 1

Table A.1: Type strain and sequences considered in this study.

Species	Year	Strain	ITS	*TEF1* - α	*TUB2*
Botryosphaeria agaves	1911	CBS133992	JX646791	JX646856	JX646841
Botryosphaeria auasmontanum	2014	CMW25413	KF766167	EU101348	-
Botryosphaeria corticis	1954	CBS119047	DQ299245	EU017539	EU673107
Botryosphaeria dothidea	1863	CBS115476	AY236949	AY236898	AY236927
Botryosphaeria fabicerciana	2013	CBS127193	HQ332197	HQ332213	KF779068
Botryosphaeria fusispora	2012	MFLUCC10-0098	JX646789	JX646854	JX646839
Botryosphaeria guttulata	2020	CGMCC3.20094	MT327839	MT331606	-
Botryosphaeria kuwatsukai	2015	CBS135219	KJ433388	KJ433410	-
Botryosphaeria minutispermatia	2016	GAAS-01	KX447675	KX447678	-
Botryosphaeria pseudoramosa	2017	CERC2001	KX277989	KX278094	KX278198
Botryosphaeria puerensis	2020	CGMCC3.20081	MT028569	MT028735	MT028901
Botryosphaeria qingyuanensis	2017	CERC2946	KX278000	KX278105	KX278209
Botryosphaeria ramosa	2013	CBS122069	EU144055	EU144070	KF766132
Botryosphaeria rosaceae	2017	CGMCC3.18007	KX197074	KX197094	KX197101
Botryosphaeria scharifii	2013	CBS124703	JQ772020	JQ772057	-
Botryosphaeria sinensis	2016	CGMCC3.17722	KT343255	-	-
Botryosphaeria wangensis	2017	CERC2298	KX278002	KX278107	KX278211
Botryosphaeria qinlingensis	2019	CFCC52984	MK434301	MK425020	MK425022
Diplodia africana	2008	CBS120835	EF445343	EF445382	KF766129
Diplodia agrifoliae	2012	CBS132777	JN693507	JQ517317	-
Diplodia alatafructa	2011	CBS124931	FJ888460	FJ888444	MG015799
Diplodia allocellula	2012	CBS130408	JQ239397	JQ239385	JQ239379

Table A.1 continued from previous page

Diplodia arengae	2018	MFLU17-2769	MG762771	MG762774	MG783039
Diplodia bulgarica	2012	CBS124254	GQ923853	GQ923821	-
Diplodia citricarpa	2016	CBS124715	KF890207	KF890189	KX464784
Diplodia corticola	2004	CBS112549	AY259100	AY573227	DQ458853
Diplodia crataegicola	2015	MFLU15-1311	KT290244	KT290248	-
Diplodia cupressi	2006	CBS168.87	DQ458893	DQ458878	DQ458861
Diplodia eriobotryicola	2017	CBS140851	KT240355	KT240193	MG015806
Diplodia estuarina	2016	CMW41231	KP860831	KP860676	KP860754
Diplodia fraxini	1849	CBS136010	KF307700	KF318747	MG015807
Diplodia gallae	2016	CBS211.25	KX464090	KX464564	KX464795
Diplodia galiicola = D. seriata	2015	MFLU15-1310	KT290245	KT290249	KT290247
Diplodia guayanensis = D. scrobiculata	2016	CBS129750	JX545108	JX545128	JX545148
Diplodia huaxii	2016	GUCC0922-1	KU848201	MF421307	-
Diplodia insularis	2016	CBS140350	KX833072	KX833073	MG015809
Diplodia intermedia	2012	CBS124462	MH863374	GQ923826	-
Diplodia italica	2016	MFLUCC14-1007	KU848202	-	-
Diplodia magnoliigena	2019	MFLUCC18-1554	MK347807	-	MK412873
Diplodia malorum	1866	CBS124130	MH863354	GQ923833	-
Diplodia mutila	1834	CBS136014	KJ361837	KJ361829	MG015815
Diplodia neojuniperi	2014	CPC22753	KM006431	KM006462	-
Diplodia olivarum	2008	CBS121887	MH863159	EU392279	HQ660079
Diplodia porosum	2004	CBS110496	AY343379	AY343340	-
Diplodia pseudoplatani	2016	GUCCG603-1	KU848200	-	-
Diplodia pseudoseriata	2010	CBS124906	EU080927	EU863181	MG015820
Diplodia pyri	2016	CBS121862	KX464093	KX464567	KX464799
Diplodia quercicola	2019	CFCC53769	MN215831	MN205991	-
Diplodia quercivora	2013	CBS133852	JX894205	JX894229	MG015821
Diplodia rosacearum	2016	CBS141915	KT956270	KU378605	MG015823
Diplodia rosulata	2005	CBS116470	MH862997	EU430267	EU673132
Diplodia sapinea	1870	CBS393.84	DQ458895	DQ458880	DQ458863
Diplodia scrobiculata	2003	CBS118110	KF766160	KF766399	-
Diplodia seriata	1845	CBS112555	AY259094	AY573220	DQ458856
Diplodia subglobosa	2014	CBS124133	GQ923856	GQ923824	-
Diplodia tsugae	2012	CBS418.64	MH858473	DQ458873	DQ458855
Diplodia torilicola	2020	IT3612	MK625223	MK640502	-
Dothiorella acacicola	2016	CBS141295	KX228269	KX228376	-
Dothiorella acericola	2019	KUMCC18-0137	MK359449	MK361182	-
Dothiorella alpina	2019	CGMCC3.18001	KX499645	KX499651	-

Table A.1 continued from previous page

Dothiorella americana	2011	CBS128309	MH864851	HQ288262	HQ288297
Dothiorella brevicollis	2012	CBS130411	JQ239403	JQ239390	JQ239371
Dothiorella californica	2017	CBS141587	KX357188	KX357211	KX357165
Dothiorella capri-amissi	2014	CBS121878	EU101324	EU101369	KX464851
Dothiorella casuarinae	2009	CBS120688	MH863089	DQ875331	DQ875340
Dothiorella citricola	2016	CBS124729	EU673323	EU673290	KX464853
Dothiorella dulcispinae	2012	CBS130413	JQ239400	JQ239387	JQ239373
Dothiorella eriobotryae	2017	CBS140852	KT240287	KT240262	-
Dothiorella guttulata	2017	MFLUCC17-0242	KY797637	KY815020	-
Dothiorella heterophyllae	2019	CMW46458	MN103794	MH548348	MH548324
Dothiorella iberica	2005	CBS115041	AY573202	AY573222	EU673096
Dothiorella iranica	2014	CBS124722	KC898231	KC898214	KX464856
Dothiorella italica	2017	MFLUCC_-170951	MF398891	MF398943	-
Dothiorella koae	2019	CMW48017	MH447652	MH548338	MH548327
Dothiorella lampangensis	2019	MFLUCC18-0232	MK347758	MK340869	MK412874
Dothiorella longicollis	2008	CBS122068	MH863172	EU144069	KF766130
Dothiorella magnoliae	2017	CFCC51563	KY111247	KY213686	-
Dothiorella mangifericola	2016	CBS124727	KC898221	KC898204	-
Dothiorella moneti	2008	MUCC505	EF591920	EF591971	EF591954
Dothiorella neclivorem	2015	DAR80992	KJ573643	KJ573640	-
Dothiorella oblonga	2014	CBS121765	EU101300	EU101345	KX464862
Dothiorella omnivora	2016	CBS140349	KP205497	KP205470	-
Dothiorella parva	2014	CBS124720	KC898234	KC898217	KX464866
Dothiorella plurivora	2016	CBS124724	KC898225	KC898208	KX464874
Dothiorella pretoriensis	2013	CBS130404	JQ239405	JQ239392	JQ239376
Dothiorella prunicola	2014	CAP187	EU673313	EU673280	EU673100
Dothiorella reunionis	2019	CMW46457	MH447649	MH548347	-
Dothiorella rhamni	2016	MFLUCC_-150922	MF398893	MF398945	-
Dothiorella rosulata	2016	CBS121760	EU101290	EU101335	KX464877
Dothiorella santali	2008	WAC13155	EF591924	EF591975	EF591958
Dothiorella sarmentorum	2005	IMI63581b	AY573212	AY573235	EU673102
Dothiorella sempervirentis	2014	CBS124718	KC898236	KC898219	KX464884
Dothiorella striata	2014	CBS124731	EU673321	EU673288	EU673143
Dothiorella styphnolobii	2019	MFLU17-2256	MH880849	MK069594	-
Dothiorella symphoricarposicola	2014	MFLUCC13-0497	KJ742378	KJ742381	-
Dothiorella tectonae	2015	MFLUCC12-0381	KJ556515	KJ556516	KJ556517

Table A.1 continued from previous page

Dothiorella thailandica	2013	MFLUCC11-0438	JX646796	JX646861	JX646844
Dothiorella thripsita	2009	BRIP51876	KJ573642	KJ573639	KJ577550
Dothiorella ulmacea	2015	CBS140005	KR611882	KR857697	-
Dothiorella uruguayensis	2013	CBS124908	EU080923	EU863180	KX464886
Dothiorella vidmadera	2013	DAR78992	EU768874	EU768881	-
Dothiorella vinea-gemmae	2015	DAR81012	KJ573644	KJ573641	-
Dothiorella viticola	2005	CBS117009	KF766228	AY905559	EU673104
Dothiorella westralis	2016	CBS117007	AY905556	KX464623	KX464890
Dothiorella yunnana	2019	CGMCC3.17999	KX499643	KX499649	-
Lasiodiplodia americana = *L. exigua*	2015	CERC1961	KP217059	KP217067	KP217075
Lasiodiplodia aquilariae	2019	CGMCC318471	KY783442	KY848600	-
Lasiodiplodia avicenniae	2016	CMW41467	KP860835	KP860680	KP860758
Lasiodiplodia avicenniarum	2019	MFLUCC17-2591	MK347777	MK340867	-
Lasiodiplodia brasiliensis	2014	CMM4015	JX464063	JX464049	-
Lasiodiplodia bruguierae	2016	CMW41470	NR_147358	KP860678	KP860756
Lasiodiplodia caatinguensis	2016	IBL366	KT154760	KT008006	KT154767
Lasiodiplodia chinensis	2017	CGMCC3.18061	KX499889	KX499927	KX500002
Lasiodiplodia chonburiensis	2018	MFLUCC16-0376	MH275066	MH412773	MH412742
Lasiodiplodia cinnamomi	2018	CFCC51997	MG866028	MH236799	MH236797
Lasiodiplodia citricola	2010	IRAN1522C	GU945354	GU945340	KP872405
Lasiodiplodia crassispora	2006	CBS118741	DQ103550	EU673303	KU887506
Lasiodiplodia curvata	2019	GuoLD01906	KY783437	KY848596	KY848529
Lasiodiplodia egyptiacae = *L. laeliocattleyae*	2012	CBS130992	JN814397	JN814424	KU887508
Lasiodiplodia endophytica	2019	MFLUCC18-1121	MK501838	MK584572	MK550606
Lasiodiplodia euphorbiaceiola	2014	CMM3609	KF234543	KF226689	KF254926
Lasiodiplodia exigua	2014	CBS137785	KJ638317	KJ638336	KU887509
Lasiodiplodia gilanensis	2010	CBS124704	GU945351	GU945342	KP872411
Lasiodiplodia gonubiensis	2004	CBS115812	AY639595	DQ458877	DQ458860
Lasiodiplodia gravistriata	2016	CMM4564	KT250949	KT250950	-
Lasiodiplodia hormozganensis	2010	CBS124709	GU945355	GU945343	KP872413
Lasiodiplodia hyalina	2017	CGMCC3.17975	KX499879	KX499917	KX499992
Lasiodiplodia indica	2014	IBP1	NR_155317	-	-
Lasiodiplodia iranensis	2010	IRAN1520C	GU945346	GU945336	KP872415
Lasiodiplodia irregularis	2019	GuoLD01673	KY783472	KY848610	KY848553

Table A.1 continued from previous page

Lasiodiplodia jatrophicola = L. iranensis	2014	CMM3610	NR_147348	KF226690	KF254927
Lasiodiplodia krabiensis	2020	MFLU17_2617	MN047093	MN077070	-
Lasiodiplodia laeliocattleyae	2016	CBS167.28	MH866448	KU507454	-
Lasiodiplodia laosensis	2019	GuoLD01818	KY783471	KY848609	KY848552
Lasiodiplodia lignicola	2013	MFLUCC11-0435	JX646797	KU887003	JX646845
Lasiodiplodia macroconidia	2019	GuoLD01752	KY783438	KY848597	KY848530
Lasiodiplodia macrospora	2014	CMM3833	KF234557	KF226718	KF254941
Lasiodiplodia magnoliae	2019	MFLUCC18-0948	MK499387	MK568537	MK521587
Lasiodiplodia mahajangana	2009	CMW27820	FJ900597	FJ900643	-
Lasiodiplodia margaritacea	2008	CBS122519	KT852959	EU144065	KX464903
Lasiodiplodia marypalmiae = L. euphorbiaceiola	2014	CMM2275	KC484843	KC481567	-
Lasiodiplodia mediterranea	2014	CBS137783	KJ638312	KJ638331	KU887521
Lasiodiplodia microcondia	2019	GuoLD01889	KY783441	KY848614	-
Lasiodiplodia missouriana	2011	CBS128311	HQ288225	HQ288267	HQ288304
Lasiodiplodia mitidjana	2020	ALG111	MN104115	MN159114	-
Lasiodiplodia pandanicola	2018	MFLUCC16-0265	MH275068	MH412774	MH412744
Lasiodiplodia parva	2008	CBS456.78	MH861166	EF622063	KP872419
Lasiodiplodia plurivora	2008	STE-U5803	EF445362	EF445395	KP872421
Lasiodiplodia pontae	2016	IBL12	KT151794	KT151791	KT151797
Lasiodiplodia pseudotheobromae	2008	CBS116459	EF622077	EF622057	EU673111
Lasiodiplodia pyriformis	2014	CBS121770	EU101307	EU101352	KU887527
Lasiodiplodia rubropurpurea	2006	CBS118740	DQ103553	EU673304	KU887529
Lasiodiplodia sterculiae	2016	CBS342.78	KX464140	KX464634	KX464908
Lasiodiplodia subglobosa	2014	CMM3872	KF234558	KF226721	KF254942
Lasiodiplodia swieteniae	2019	MFLUCC18-0244	MK347789	MK340870	MK412877
Lasiodiplodia tenuiconidia	2019	CGMCC3.18449	KY783466	KY848619	-
Lasiodiplodia thailandica	2014	CPC22795	KM006433	KM006464	-
Lasiodiplodia theobromae	1909	CBS164.96	AY640255	AY640258	KU887532
Lasiodiplodia tropica	2019	CGMCC3.18477	KY783454	KY848616	KY848540
Lasiodiplodia vaccinii	2019	CGMCC3.19022	MH330318	MH330327	MH330324
Lasiodiplodia venezuelensis	2006	CBS118739	DQ103547	EU673305	KU887533
Lasiodiplodia viticola	2011	UCD2553AR	HQ288227	HQ288269	HQ288306
Lasiodiplodia vitis = L. mediterranea	2016	CBS124060	KX464148	KX464642	KX464917

Table A.1 continued from previous page

Neofusicoccum algeriense = N. parvum	2014	CBS137504	KJ657702	KX505893	KX505915
Neofusicoccum andinum	2006	CBS117453	AY693976	AY693977	KX464923
Neofusicoccum arbuti	2006	CBS116131	AY819720	KF531792	KF531793
Neofusicoccum australe	2006	CMW6837	AY339262	AY339270	AY339254
Neofusicoccum batangarum	2013	CBS124924	FJ900607	FJ900653	FJ900634
Neofusicoccum brasiliense	2013	CMM1338	JX513630	JX513610	KC794031
Neofusicoccum buxi	2016	CBS116.75	KX464165	KX464678	-
Neofusicoccum cordaticola	2009	CBS123634	EU821898	EU821868	EU821838
Neofusicoccum corticosae	2019	CBS120081	MN161920	KX464682	KX464958
Neofusicoccum cryptoaustrale	2013	CMW23785	FJ752742	FJ752713	FJ752756
Neofusicoccum dianense	2020	CGMCC3.20082	MT028605	MT028771	MT028937
Neofusicoccum eucalypticola	2006	CBS115679	AY615141	AY615133	AY615125
Neofusicoccum eucalyptorum	2006	CBS115791	AF283686	AY236891	AY236920
Neofusicoccum grevilleae	2011	CBS129518	JF951137	-	-
Neofusicoccum hellenicum	2015	CERC1947	KP217053	KP217061	KP217069
Neofusicoccum hongkongense	2017	CERC2973	KX278052	KX278157	KX278261
Neofusicoccum illicii	2017	BJFU2037	KY350149	-	KY350155
Neofusicoccum italicum	2017	MFLUCC15-0900	KY856755	KY856754	-
Neofusicoccum kwambonambiense	2009	CBS123639	EU821900	EU821870	EU821840
Neofusicoccum lumnitzerae	2016	CMW41469	KP860881	KP860724	KP860801
Neofusicoccum luteum	2006	CBS110299	AY259091	KX464688	DQ458848
Neofusicoccum macroclavatum	2006	CBS118223	DQ093196	DQ093217	DQ093206
Neofusicoccum magniconidium	2020	CGMCC3.20077	MT028612	MT028778	MT028944
Neofusicoccum mangiferae	2006	CBS118531	AY615185	DQ093221	AY615172
Neofusicoccum mangroviorum	2016	CMW41365	KP860859	KP860702	KP860779
Neofusicoccum mediterraneum	2007	CBS121718	GU251176	GU251308	GU251836
Neofusicoccum microconidium	2017	CERC3497	KX278053	KX278158	KX278262
Neofusicoccum ningerense	2020	CGMCC3.20078	MT028613	MT028779	MT028945
Neofusicoccum nonquaesitum	2010	CBS126655	KX357178	KX357201	KX357155
Neofusicoccum occulatum	2010	CBS128008	EU301030	EU339509	EU339472
Neofusicoccum pandanicola	2018	KUMCC17-0184	MH275072	MH412778	-

Table A.1 continued from previous page

Neofusicoccum parviconidium	2020	CGMCC3.20074	MT028615	MT028781	MT028947
Neofusicoccum parvum	2006	CMW9081	AY236943	AY236888	AY236917
Neofusicoccum pennatisporum	2009	MUCC510	EF591925	EF591976	EF591959
Neofusicoccum pistaciae	2016	CBS595.76	KX464163	KX464676	KX464953
Neofusicoccum pistaciarum	2016	CBS113083	KX464186	KX464712	KX464998
Neofusicoccum pistaciicola	2017	CBS113089	KX464199	KX464727	KX465014
Neofusicoccum protearum	2003	CBS114176	AF452539	KX464720	KX465006
Neofusicoccum pruni	2017	CBS121112	EF445349	EF445391	KX465016
Neofusicoccum ribis	2006	CBS115475	AY236935	AY236877	AY236906
Neofusicoccum sinense	2017	CGMCC3.18315	KY350148	KY817755	KY350154
Neofusicoccum sinoeucalypti	2017	CERC2265	KX278062	KX278167	KX278271
Neofusicoccum stellenboschiana	2016	CBS110864	AY343407	AY343348	KX465047
Neofusicoccum umdonicola	2009	CBS123645	MH863318	KF766427	KF766145
Neofusicoccum ursorum	2013	CBS122811	FJ752746	FJ752709	KX465056
Neofusicoccum variabile	2018	CMW37739	MH558608	-	MH569153
Neofusicoccum versiforme	2019	CBS118101	AY744376	GU251354	GU251882
Neofusicoccum viticlavatum	2006	CBS112878	AY343381	AY343342	KX465058
Neofusicoccum vitifusiforme	2006	CBS110887	MH862869	AY343343	KX465061
Neofusicoccum yunnanense	2020	CGMCC3.20083	MT028667	MT028833	MT028999

Appendix B

Supplementary data 2

Table B.1: Country list based on the near current time prediction for each studied species. Countries underlined represent regions without any literature report regarding other members of the Botryosphaeriaceae family. Countries abbreviations represent ISO3 codes and are listed in the footnote.

Species	Predicted and reported	Predicted but not reported	Not predicted but reported
Botryosphaeria dothidea	ARG, AUS, BEL, BIH, BOL, BRA, CAN, CHE, CHL, CHN, COL, CZE, DEU, DNK, DZA, ESP, FRA, GBR, GEO, GRC, HRV, IND, IRN, ITA, JPN, KEN, KOR, LTU, MEX, MNE, NAM, NLD, NZL, PAK, PAN, POL, PRT, PRY, RUS, SRB, SVN, SWE, TUN, TUR, TWN, UKR, URY, USA, ZAF, ZWE	AFG, ALB, AND, ARE, AUT, AZE, BEN, BFA, BGR, BLR, BTN, BWA, CAF, CMR, CYP, ECU, EGY, ERI, EST, ETH, FIN, FRO, GHA, GIN, GRL, HUN, IDN, IRL, IRQ, ISL, ISR, JEY, JOR, KAZ, KGZ, LAO, LBN, LBY, LIE, LKA, LSO, LUX, LVA, MAC, MAR, MDA, MDG, MKD, MLT, MMR, MOZ, NGA, NOR, NPL, OMN, PER, PNG, PRK, PSE, REU, ROU, RWA, SAU, SDN, SGS, SJM, SMR, SOM, SPM, SVK, SWZ, SYR, TCD, TJK, TKM, TZA, UGA, UZB, VNM, YEM	CUB, FJI, GTM, HKG, MWI, NCL, PHL, SLE, VEN

Table B.1 continued from previous page

Diplodia sapinea	ARG, AUS, AUT, BEL, BLR, CAN, CHL, CHN, COD, CYP, CZE, DEU, DZA, ESP, EST, ETH, FIN, FRA, GBR, GEO, GRC, IDN, IND, IRN, ISR, ITA, KEN, LTU, LVA, MEX, MKD, MNE, NLD, NZL, PAK, POL, PRT, ROU, RUS, SRB, SVK, SWE, TUN, TUR, TZA, UKR, URY, USA, ZAF	AFG, ALA, ALB, ARE, ARM, AZE, BDI, BGR, BIH, BOL, CMR, COL, DNK, ECU, EGY, FLK, GRL, GTM, HRV, HUN, IRQ, ISL, JOR, KAZ, LBN, LBY, MAR, MDA, MLT, MMR, NOR, NPL, OMN, PER, PSE, RWA, SAU, SDN, SJM, SMR, SVN, SYR, TJK, UGA, UZB, VEN	BRA, CHE, HND, JPN, LSO, MDG, MOZ, MUS, MWI, SGP, SWZ, THA, TWN, ZMB, ZWE
Diplodia seriata	AUS, BGR, BIH, BRA, CAN, CHL, CHN, DZA, ESP, FRA, GBR, GRC, HRV, IND, IRN, ITA, LBN, MEX, NZL, PAK, PRT, ROU, SRB, TUN, TUR, TZA, UKR, USA, ZAF	AFG, ALB, ARE, ARG, AZE, BDI, BEN, CAF, CMR, COD, CYP, ECU, EGY, ERI, ETH, FIN, GEO, GHA, GIN, IRQ, ISR, JOR, KAZ, KEN, KGZ, LBY, LKA, MAR, MKD, MLT, MNE, NGA, NPL, OMN, PAN, PER, PSE, RUS, RWA, SDN, SJM, SOM, SWE, SYR, TCD, TJK, TKM, UGA, UZB	AUT, BOL, CHE, CZE, DEU, JPN, NLD, POL, URY

Table B.1 continued from previous page

Lasiodiplodia theobromae	ARG, AUS, BEN, BGD, BOL, BRA, BRB, BRN, CHL, CHN, CIV, CMR, COD, COK, COL, CRI, CUB, CYP, DOM, ECU, EGY, ESP, ETH, FJI, GHA, GIN, GTM, HKG, HND, HTI, IDN, IND, IRN, ISR, JAM, JPN, KEN, LBY, LKA, MDG, MEX, MMR, MUS, MWI, MYS, NCL, NGA, NIC, NIU, NZL, OMN, PAK, PAN, PER, PHL, PNG, PRI, PRT, SAU, SDN, SGP, SLB, SLE, SLV, SOM, SYC, THA, TON, TTO, TUR, TWN, TZA, UGA, URY, USA, VEN, VIR, VNM, WSM, ZAF, ZMB, ZWE	ABW, AGO, AIA, ANT, ARE, ASM, ATG, BDI, BFA, BHS, BLM, BLZ, BTN, CAF, CAN, COG, COM, CXR, CYM, DEU, DJI, DMA, DZA, ERI, FRO, FSM, GAB, GBR, GEO, GLP, GNB, GNQ, GRC, GRD, GUF, GUM, GUY, IOT, IRL, ISL, JOR, KHM, KIR, KNA, KOR, LAO, LBN, LBR, LCA, MAC, MAR, MDV, MHL, MLI, MNP, MOZ, MSR, MTQ, MYT, NOR, NPL, PLW, PRK, PRY, PSE, PYF, REU, RWA, SEN, SHN, STP, SUR, SWZ, SYR, TCD, TGO, TLS, TUN, UMI, VCT, VGB, VUT, WLF	CHE, IRQ, ITA, MLT, NLD, UZB

Table B.1 continued from previous page

Neofusicoccum parvum	ARG, AUS, BGR, BRA, CAN, CHE, CHL, CHN, COL, DZA, ECU, ESP, ETH, FRA, GRC, HRV, IDN, IND, IRN, ITA, JPN, KEN, KOR, MEX, MLT, MNE, NLD, NZL, PER, PRI, PRT, SRB, SWZ, THA, TUN, TUR, TWN, UGA, URY, USA, VEN, ZAF, ZMB, ZWE	AFG, AGO, ALA, ALB, AND, ARE, ARM, ATF, AUT, AZE, BDI, BEL, BEN, BFA, BGD, BHR, BHS, BIH, BLZ, BOL, BTN, CAF, CIV, CMR, COD, COG, COM, CPV, CRI, CUB, CYP, CZE, DEU, DJI, DNK, DOM, EGY, ERI, EST, FIN, FJI, FLK, FRO, GAB, GBR, GEO, GGY, GHA, GIN, GNQ, GRL, GTM, GUY, HKG, HMD, HND, HTI, HUN, IMN, IRL, IRQ, ISL, ISR, JAM, JEY, JOR, KAZ, KHM, KIR, LAO, LBN, LBR, LBY, LIE, LKA, LSO, LUX, MAC, MAR, MDG, MKD, MMR, MOZ, MUS, MWI, MYS, NCL, NFK, NGA, NIC, NOR, NPL, OMN, PAK, PAN, PCN, PHL, PNG, POL, PRY, PSE, PYF, QAT, REU, ROU, RUS, RWA, SAU, SDN, SGS, SHN, SLB, SLE, SLV, SMR, SPM, STP, SVK, SVN, SWE, SYR, TCD, TGO, TJK, TLS, TZA, UKR, UMI, UZB, VNM, VUT, WSM, YEM	-

ABW - Aruba, AFG - Afghanistan, AGO - Angola, AIA - Anguilla, ALA - Åland Islands, ALB - Albania, AND - Andorra, ANT - Netherlands Antilles, ARE - United Arab Emirates (the), ARG - Argentina, ARM - Armenia, ASM - American Samoa, ATF - French Southern Territories (the), ATG - Antigua and Barbuda, AUS - Australia, AUT - Austria, AZE - Azerbaijan, BDI - Burundi, BEL - Belgium, BEN - Benin, BFA - Burkina Faso, BGD - Bangladesh, BGR - Bulgaria, BHR - Bahrain, BHS - Bahamas (the), BIH - Bosnia and Herzegovina, BLM - Saint Barthélemy, BLR - Belarus, BLZ - Belize, BOL - Bolivia (Plurinational State of), BRA - Brazil, BRB - Barbados, BRN - Brunei Darussalam, BTN - Bhutan, BWA - Botswana, CAF - Central African Republic (the), CAN - Canada, CHE - Switzerland, CHL - Chile, CHN - China, CIV - Côte d'Ivoire, CMR - Cameroon, COD - Congo (the Democratic Republic of the), COG - Congo (the), COK - Cook Islands (the), COL - Colombia, COM - Comoros (the), CPV - Cabo Verde, CRI - Costa Rica, CUB - Cuba, CXR - Christmas Island, CYM - Cayman Islands (the), CYP - Cyprus, CZE - Czechia, DEU - Germany, DJI - Djibouti, DMA - Dominica, DNK - Denmark, DOM - Dominican Republic (the), DZA - Algeria, ECU - Ecuador, EGY - Egypt, ERI - Eritrea, ESP - Spain, EST - Estonia, ETH - Ethiopia, FIN - Finland, FJI - Fiji, FLK - Falkland Islands (the) [Malvinas], FRA - France, FRO - Faroe Islands (the), FSM - Micronesia (Federated States of), GAB - Gabon, GBR - United Kingdom of Great Britain and Northern Ireland (the), GEO - Georgia, GGY - Guernsey, GHA - Ghana, GIN - Guinea, GLP - Guadeloupe, GNB - Guinea-Bissau, GNQ - Equatorial Guinea, GRC - Greece, GRD - Grenada, GRL - Greenland, GTM - Guatemala, GUF - French Guiana, GUM - Guam, GUY - Guyana, HKG - Hong Kong, HMD - Heard Island and McDonald Islands, HND - Honduras, HRV - Croatia, HTI - Haiti, HUN - Hungary, IDN - Indonesia, IMN - Isle of Man, IND - India, IOT - British Indian Ocean Territory (the), IRL - Ireland, IRN - Iran (Islamic Republic of), IRQ - Iraq, ISL - Iceland, ISR - Israel, ITA - Italy, JAM - Jamaica, JEY - Jersey, JOR - Jordan, JPN - Japan, KAZ - Kazakhstan, KEN - Kenya, KGZ - Kyrgyzstan, KHM - Cambodia, KIR - Kiribati, KNA - Saint Kitts and Nevis, KOR - Korea (the Republic of), LAO - Lao People's Democratic Republic (the), LBN - Lebanon, LBR - Liberia, LBY - Libya, LCA - Saint Lucia, LIE - Liechtenstein, LKA - Sri Lanka, LSO - Lesotho, LTU - Lithuania, LUX - Luxembourg, LVA - Latvia, MAC - Macao, MAR - Morocco, MDA - Moldova (the Republic of), MDG - Madagascar, MDV - Maldives, MEX - Mexico, MHL - Marshall Islands (the), MKD - Republic of North Macedonia, MLI - Mali, MLT - Malta, MMR - Myanmar, MNE - Montenegro, MNP - Northern Mariana Islands (the), MOZ - Mozambique, MSR - Montserrat, MTQ - Martinique, MUS - Mauritius, MWI - Malawi, MYS - Malaysia, MYT - Mayotte, NAM - Namibia, NCL - New Caledonia, NFK - Norfolk Island, NGA - Nigeria, NIC - Nicaragua, NIU - Niue, NLD - Netherlands (the), NOR - Norway, NPL - Nepal, NZL - New Zealand, OMN - Oman, PAK - Pakistan, PAN - Panama, PCN - Pitcairn, PER - Peru, PHL - Philippines (the), PLW - Palau, PNG - Papua New Guinea, POL - Poland, PRI - Puerto Rico, PRK - Korea (the Democratic People's Republic of), PRT - Portugal, PRY - Paraguay, PSE - Palestine, State of, PYF - French Polynesia, QAT - Qatar, REU - Réunion, ROU - Romania, RUS - Russian Federation

(the), RWA - Rwanda, SAU - Saudi Arabia, SDN - Sudan (the), SEN - Senegal, SGP - Singapore, SGS - South Georgia and the South Sandwich Islands, SHN - Saint Helena, Ascension and Tristan da Cunha, SJM - Svalbard and Jan Mayen, SLB - Solomon Islands, SLE - Sierra Leone, SLV - El Salvador, SMR - San Marino, SOM - Somalia, SPM - Saint Pierre and Miquelon, SRB - Serbia, STP - Sao Tome and Principe, SUR - Suriname, SVK - Slovakia, SVN - Slovenia, SWE - Sweden, SWZ - Eswatini, SYC - Seychelles, SYR - Syrian Arab Republic, TCD - Chad, TGO - Togo, THA - Thailand, TJK - Tajikistan, TKM - Turkmenistan, TLS - Timor-Leste, TON - Tonga, TTO - Trinidad and Tobago, TUN - Tunisia, TUR - Turkey, TWN - Taiwan (Province of China), TZA - Tanzania, United Republic of, UGA - Uganda, UKR - Ukraine, UMI - United States Minor Outlying Islands (the), URY - Uruguay, USA - United States of America (the), UZB - Uzbekistan, VCT - Saint Vincent and the Grenadines, VEN - Venezuela (Bolivarian Republic of), VGB - Virgin Islands (British), VIR - Virgin Islands (U.S.), VNM - Viet Nam, VUT - Vanuatu, WLF - Wallis and Futuna, WSM - Samoa, YEM - Yemen, ZAF - South Africa, ZMB - Zambia, ZWE – Zimbabwe.

Appendix C

Supplementary data 3

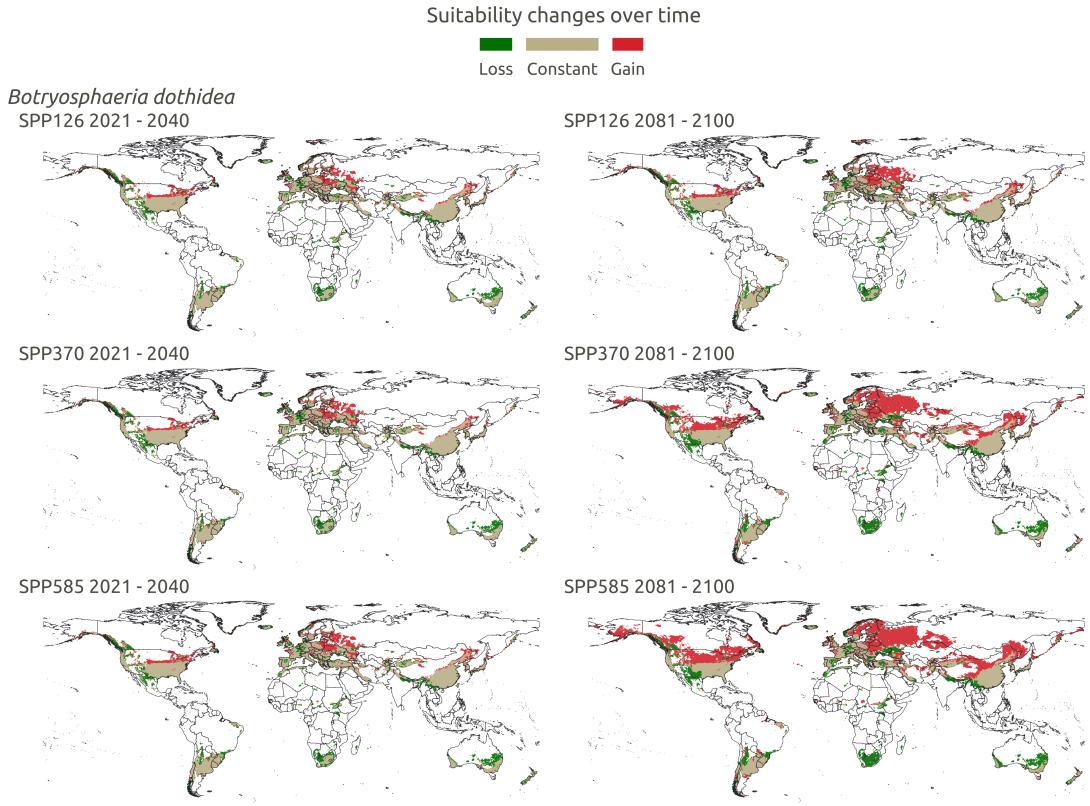

Figure C.1: Future suitability areas for *Botryosphaeria dothidea* according to three different climate scenarios (SSP128, SSP370 and SSP585) over two different time periods 2021 - 2040 and 2081 – 2100. Grey zones represent suitability areas predicted by the ensemble for the near current time. Green zones represent areas with loss of suitability over the time when compared with the near current predictions. Red zones represent areas with gain of suitability over the time when compared with the near current predictions.

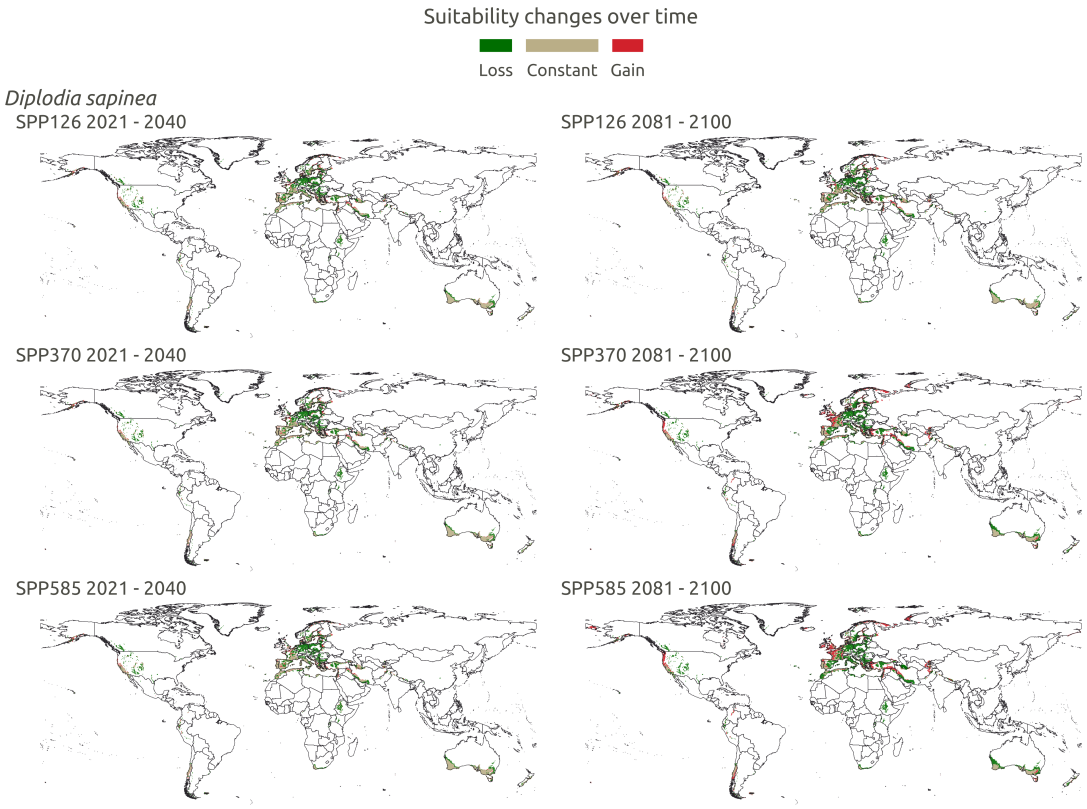

Figure C.2: Future suitability areas for *Diplodia sapinea* according to three different climate scenarios (SSP128, SSP370 and SSP585) over two different time periods 2021 - 2040 and 2081 – 2100. Grey zones represent suitability areas predicted by the ensemble for the near current time. Green zones represent areas with loss of suitability over the time when compared with the near current predictions. Red zones represent areas with gain of suitability over the time when compared with the near current predictions.

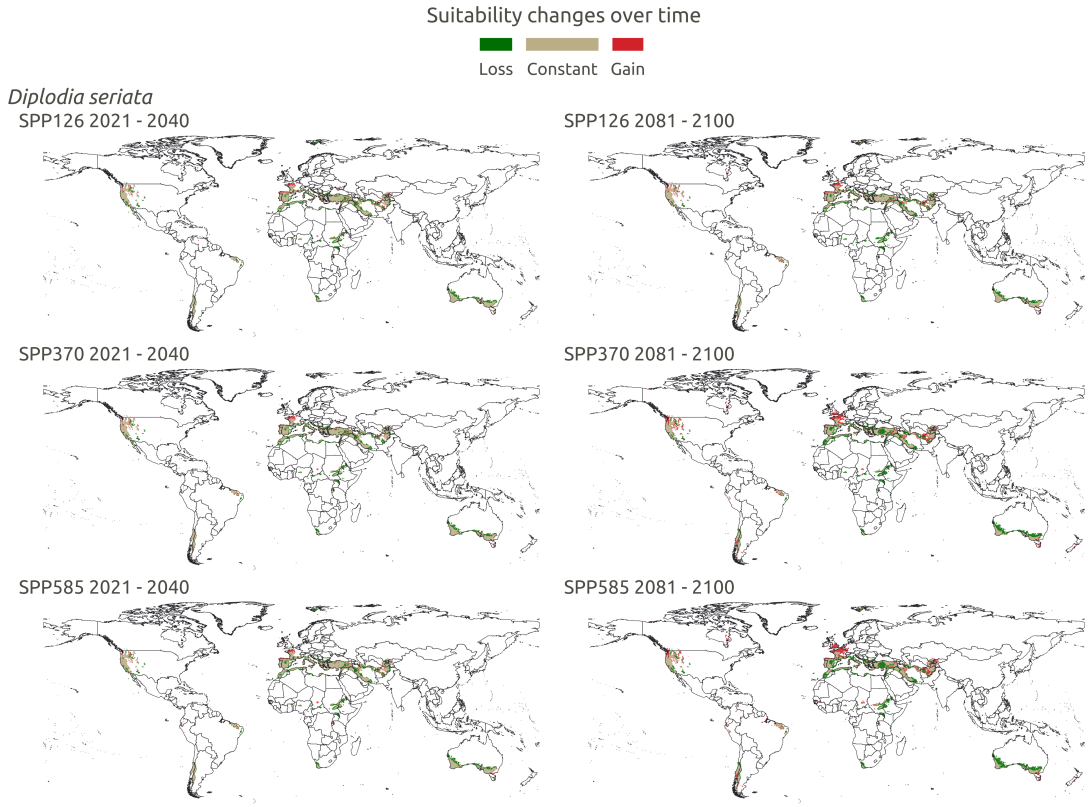

Figure C.3: Future suitability areas for *Diplodia seriata* according to three different climate scenarios (SSP128, SSP370 and SSP585) over two different time periods 2021 - 2040 and 2081 – 2100. Grey zones represent suitability areas predicted by the ensemble for the near current time. Green zones represent areas with loss of suitability over the time when compared with the near current predictions. Red zones represent areas with gain of suitability over the time when compared with the near current predictions.

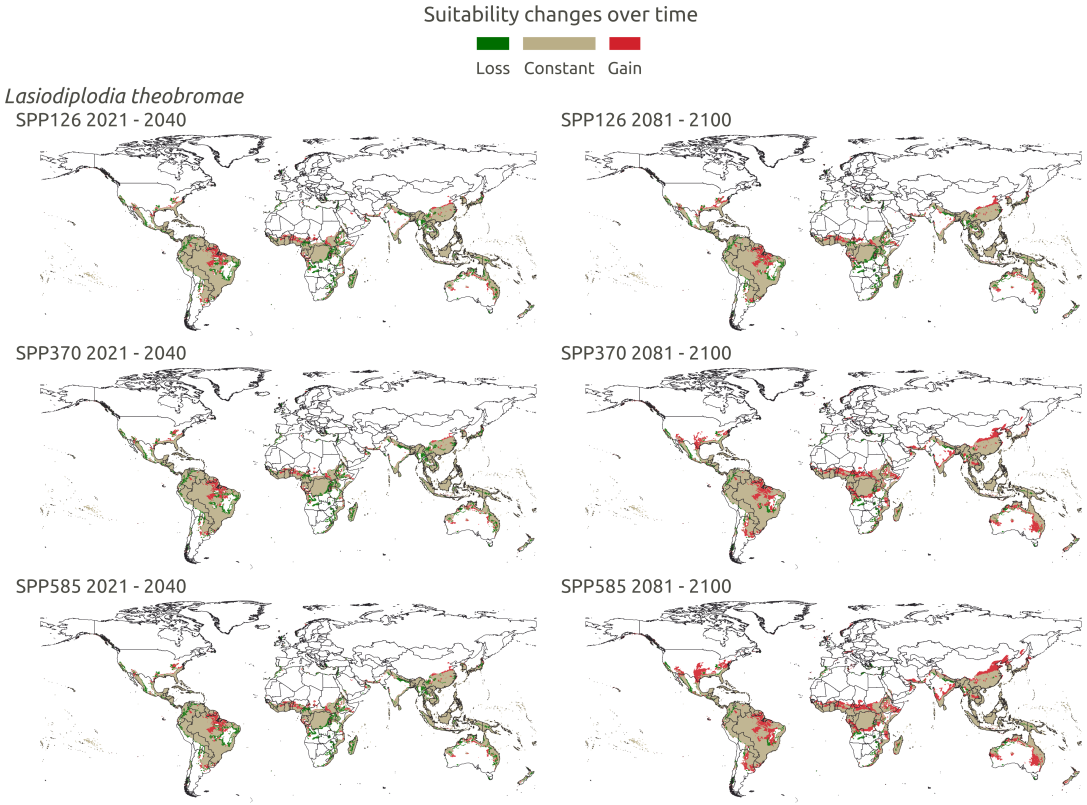

Figure C.4: Future suitability areas for *Lasiodiplodia theobromae* according to three different climate scenarios (SSP128, SSP370 and SSP585) over two different time periods 2021 - 2040 and 2081 – 2100. Grey zones represent suitability areas predicted by the ensemble for the near current time. Green zones represent areas with loss of suitability over the time when compared with the near current predictions. Red zones represent areas with gain of suitability over the time when compared with the near current predictions.

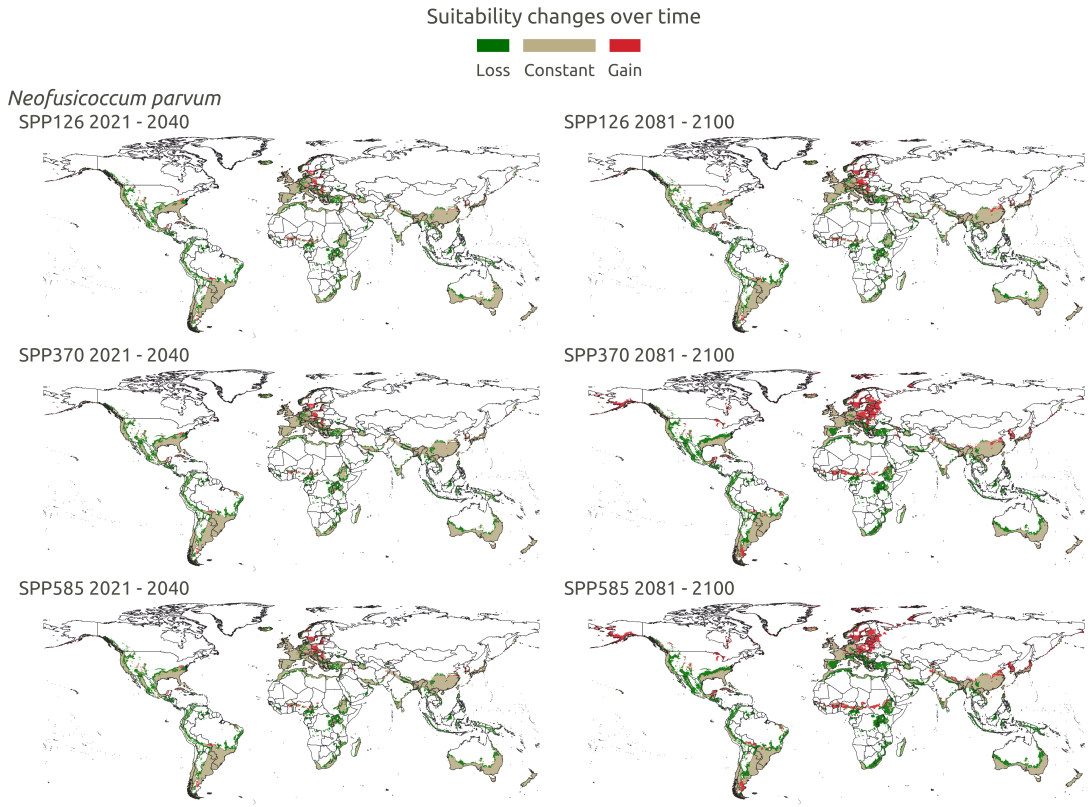

Figure C.5: Future suitability areas for *Neofusicoccum parvum* according to three different climate scenarios (SSP128, SSP370 and SSP585) over two different time periods 2021 - 2040 and 2081 – 2100. Grey zones represent suitability areas predicted by the ensemble for the near current time. Green zones represent areas with loss of suitability over the time when compared with the near current predictions. Red zones represent areas with gain of suitability over the time when compared with the near current predictions.

Appendix D

Supplementary data 4

Table D.1: Collection Botryosphaeriaceae isolates from Portugal

Species	Strain	ITS	*TEF1* - α	*TUB2*	*RPB2*	MAT1-1-1	MAT1-2-1	Species_Host
Botryosphaeria dothidea	CAA127	JX878554						*Juniperus communis*
Botryosphaeria dothidea	CAA642	KT440894	KT440953					*Eucalyptus globulus*
Botryosphaeria dothidea	CAA767	MK932747	MK932753					*Vaccinium corymbosum*
Botryosphaeria dothidea	CAA773	MK932748	MK932754					*Vaccinium corymbosum*
Botryosphaeria dothidea	CAA833	MK932724	MK932758					*Vaccinium corymbosum*
Botryosphaeria dothidea	CAA834	MK932727	MK932761					*Vaccinium corymbosum*
Botryosphaeria dothidea	CAA835	MK932729	MK932762					*Vaccinium corymbosum*
Botryosphaeria dothidea	CAA836	MK932733						*Vaccinium corymbosum*
Botryosphaeria dothidea	CAA837	MK932742	MK932765					*Vaccinium corymbosum*
Botryosphaeria dothidea	CAA859	MK940302						*Quercus ilex*
Botryosphaeria dothidea	CAA860	MK940295						*Quercus suber*
Botryosphaeria dothidea	CAA938	MT261004						*Quercus suber*
Botryosphaeria dothidea	CAP002	AF286255						*Vitis vinifera*
Botryosphaeria dothidea	CAP007	AF286256						*Vitis vinifera*
Botryosphaeria dothidea	CAP022	AF286259						*Vitis vinifera*
Botryosphaeria dothidea	CAP025	AF286260						*Vitis vinifera*
Botryosphaeria dothidea	CAP032	AF286261						*Vitis vinifera*
Botryosphaeria dothidea	CAP035	AF286262						*Vitis vinifera*
Botryosphaeria dothidea	CAP037	AF286263						*Vitis vinifera*
Botryosphaeria dothidea	CAP038	AF286264						*Vitis vinifera*
Botryosphaeria dothidea	CAP042	AF286265						*Vitis vinifera*
Botryosphaeria dothidea	CAP056	AF286266						*Fraxinus angustifolia*
Botryosphaeria dothidea	CAP058	AF286267						*Styphnolobium japonicum*
Botryosphaeria dothidea	CAP067	AF286268						*Vitis vinifera*
Botryosphaeria dothidea	CAP071	AF286269						*Vitis vinifera*
Botryosphaeria dothidea	CBS110300	AY640253	AY640256					*Populus nigra*
Botryosphaeria dothidea	CBS110302	AY259092	AY573218	EU673106				*Vitis vinifera*
Botryosphaeria dothidea	PE26	KT440893	KT440954					*Eucalyptus globulus*
Diplodia corticola	CAA007-2	AY259103						*Quercus suber*
Diplodia corticola	CAA009-2	JX894202	JX894226					*Quercus suber*
Diplodia corticola	CAA010	JX894203	JX894227					*Quercus suber*
Diplodia corticola	CAA499	MG015741	MG015723	MG015800			MG015776	*Eucalyptus globulus*
Diplodia corticola	CAA500	KT440895	KT440958	MG015801			MG015777	*Eucalyptus globulus*

Diplodia corticola	CAA691	KT440896	KT440959	MG015802	MG015754	*Eucalyptus globulus*
Diplodia corticola	CAA862	MK940298				*Eucalyptus globulus*
Diplodia corticola	CAA863	MT261002				*Eucalyptus globulus*
Diplodia corticola	CAA864	MT260992				*Pinus pinaster*
Diplodia corticola	CAA865	MK940296				*Pinus pinaster*
Diplodia corticola	CAA866	MT261001				*Pinus pinaster*
Diplodia corticola	CAA868	MT261005				*Quercus ilex*
Diplodia corticola	CAA869	MT261006				*Quercus ilex*
Diplodia corticola	CAA870	MK940303				*Quercus ilex*
Diplodia corticola	CAA871	MT260981				*Quercus suber*
Diplodia corticola	CAA872	MT260991				*Quercus suber*
Diplodia corticola	CAA873	MT260993				*Quercus suber*
Diplodia corticola	CAA874	MT260994				*Quercus suber*
Diplodia corticola	CAA875	MK940297				*Quercus suber*
Diplodia corticola	CAA876	MT260995				*Quercus suber*
Diplodia corticola	CAA877	MT260996				*Quercus suber*
Diplodia corticola	CAA881	MT260998				*Quercus suber*
Diplodia corticola	CAA882	MT260999				*Quercus suber*
Diplodia corticola	CAA884	MT261000				*Quercus suber*
Diplodia corticola	CAA886	MT261003				*Quercus suber*
Diplodia corticola	CAA888	MT261020				*Quercus suber*
Diplodia corticola	CAA889	MT261021				*Quercus suber*
Diplodia corticola	CBS112548	AY259099	KX464559	KX464789		*Quercus suber*
Diplodia corticola	CBS112549	AY259100	KF766398	DQ458853	MG015753	*Quercus suber*
Diplodia corticola	CBS112550	AY259097	KX464560	KX464790		*Quercus suber*
Diplodia corticola	CBS112551	AY259101	JX894225	KX464791		*Quercus suber*
Diplodia corticola	CBS112552	AY259102	KX464561	KX464792		*Quercus suber*
Diplodia corticola	MEAN_1020	KU891979	KU891980			*Quercus suber*
Diplodia fraxini	CAD002	KF307701	KF318748			*Fraxinus angustifolia*
Diplodia fraxini	CAD003	KF307702	KF318749			*Fraxinus angustifolia*
Diplodia fraxini	CAD004	KF307703	KF318750			*Fraxinus angustifolia*
Diplodia fraxini	CAD005	KF307704	KF318751			*Fraxinus angustifolia*
Diplodia fraxini	CAD006	KF307705	KF318752			*Fraxinus angustifolia*
Diplodia fraxini	CAD007	KF307706	KF318753			*Fraxinus angustifolia*
Diplodia fraxini	CAD008	KF307707	KF318754			*Fraxinus angustifolia*
Diplodia fraxini	CAD009	KF307708	KF318755			*Fraxinus angustifolia*

Diplodia fraxini	CAD010	KF307709	KF318756				*Fraxinus angustifolia*
Diplodia fraxini	CBS136010	KF307700	KF318747	MG015807	MG015759		*Fraxinus angustifolia*
Diplodia insularis	CAA890	MK940299					*Eucalyptus globulus*
Diplodia intermedia	CAA147	GQ923857	GQ923825	MG015811	MG015762		*Malus pumila*
Diplodia intermedia	CAA490	MG015744	MG015726	MG015812		MG015780	*Pyracantha coccinea*
Diplodia intermedia	CAA491	MG015745	MG015727	MG015813	MG015763		*Pyracantha coccinea*
Diplodia intermedia	CAP150	MG015743	MG015725	MG015814		MG015781	*Cydonia oblonga*
Diplodia intermedia	CAP273	GQ923858					*Malus pumila*
Diplodia intermedia	CBS124462	MH863374	GQ923826				*Malus pumila*
Diplodia malorum	CAP265	GQ923859	GQ923827				*Malus pumila*
Diplodia malorum	CAP266	GQ923860	GQ923828				*Malus pumila*
Diplodia malorum	CAP267	GQ923861	GQ923829				*Malus pumila*
Diplodia malorum	CAP268	GQ923862	GQ923830				*Malus pumila*
Diplodia malorum	CAP269	GQ923863	GQ923831				*Malus pumila*
Diplodia malorum	CAP270	GQ923864	GQ923832				*Malus pumila*
Diplodia malorum	CAP271	GQ923865					*Malus pumila*
Diplodia malorum	CAP272	GQ923866	GQ923834				*Malus pumila*
Diplodia malorum	CAP274	GQ923867					*Malus pumila*
Diplodia malorum	CAP275	GQ923868	GQ923836				*Malus pumila*
Diplodia malorum	CAP277	GQ923869	GQ923837				*Malus pumila*
Diplodia malorum	CAP278	GQ923870	GQ923838				*Malus pumila*
Diplodia malorum	CAP340	GQ923871	GQ923839				*Malus pumila*
Diplodia malorum	CAP341	GQ923872	GQ923840				*Malus pumila*
Diplodia malorum	CBS112554	AY259095	DQ458870	DQ458851	MG015764		*Malus sylvestris*
Diplodia malorum	CBS124130	MH863354	GQ923833				*Malus pumila*
Diplodia malorum	CBS124253		GQ923835				*Malus pumila*
Diplodia mutila	CAA096	JX878523	KJ361834				*Taxus baccata*
Diplodia mutila	CAA115	JX878524	KJ361835				*Chamaecyparis lawsoniana*
Diplodia mutila	CAA507	MG015746	MG015728	MG015816	MG015766		*Fraxinus ornus*
Diplodia mutila	CBS136014	KJ361837	KJ361829	MG015815	MG015765		*Populus alba*
Diplodia mutila	CBS136015	KJ361838	KJ361830				*Populus alba*
Diplodia mutila	CBS136016	KJ361839	KJ361831				*Fraxinus ornus*
Diplodia mutila	CBS136017	KJ361840	KJ361832				*Fraxinus ornus*
Diplodia mutila	STE-U5038	AY343484	AY343370				*Vitis vinifera*
Diplodia pyri	CAA891	MK940300					*Eucalyptus globulus*
Diplodia quercivora	MEAN_1016	KU311197	KU311200				*Quercus suber*

Diplodia quercivora	MEAN_1017	KU311198	KU311201				*Quercus suber*
Diplodia quercivora	MEAN_1018	KU311199					*Quercus suber*
Diplodia sapinea	CAA015	JX878559					*Pinus pinaster*
Diplodia sapinea	CAA025	JX878530					*Thuja plicata*
Diplodia sapinea	CAA068	JX878531					*Pinus nigra*
Diplodia sapinea	CAA070	JX878529					*Pinus nigra*
Diplodia sapinea	CAA892	MK940292					*Pinus pinaster*
Diplodia sapinea	CAA893	MT260983					*Pinus pinaster*
Diplodia sapinea	CAA894	MT260985					*Pinus pinaster*
Diplodia sapinea	CAA896	MT260988					*Pinus pinaster*
Diplodia sapinea	CAA897	MT260989					*Pinus pinaster*
Diplodia sapinea	CAA898	MT260990					*Pinus pinaster*
Diplodia sapinea	CAA902	MT261022					*Pinus pinaster*
Diplodia sapinea	CAA903	MK940312					*Quercus suber*
Diplodia seriata	CAA051	JX878532					*Thuja plicata*
Diplodia seriata	CAA108	JX878533					*Chamaecyparis lawsoniana*
Diplodia seriata	CAA317	KT440897	KT440955	MG015826		MG015794	*Eucalyptus globulus*
Diplodia seriata	CAA318	KT440898	KT440956				*Eucalyptus globulus*
Diplodia seriata	CAA339	KT440899	KT440957				*Eucalyptus globulus*
Diplodia seriata	CAA502	KJ361842	KJ361836				*Fraxinus ornus*
Diplodia seriata	CAA634	MG015749	MG015731	MG015827	MG015773		*Fraxinus ornus*
Diplodia seriata	CAA636	MG015750	MG015732	MG015828	MG015774		*Fraxinus ornus*
Diplodia seriata	CAP276	GQ923876	GQ923844				*Malus pumila*
Diplodia seriata	CBS112555	NR_-111151	AY573220	DQ458856		MG015793	*Vitis vinifera*
Diplodia seriata	CBS112556	AY259096	GQ923850				*Pyrus communis*
Diplodia seriata	STE-U5037	AY343446					*Vitis vinifera*
Dothiorella iberica	CAA129	JX878556					*Juniperus communis*
Dothiorella iberica	CAA131	JX878557					*Juniperus communis*
Dothiorella iberica	CAA904	MK940306					*Castanea sativa*
Dothiorella iberica	CAA905	MK940310					*Eucalyptus globulus*
Dothiorella iberica	CAA906	MK940301					*Quercus ilex*
Dothiorella iberica	CAA907	MT261007					*Quercus ilex*
Dothiorella iberica	CAA908	MT261008					*Quercus ilex*
Dothiorella iberica	CAA909	MT261009					*Quercus ilex*
Dothiorella iberica	CAA910	MT261010					*Quercus ilex*

Dothiorella iberica	CAA911	MT261024			*Quercus ilex*
Dothiorella iberica	CAA914	MT261023			*Quercus suber*
Dothiorella iberica	CAA915	MK940308			*Quercus suber*
Dothiorella iberica	P6_A4_1433	KU325273			*Olea sp.*
Dothiorella plurivora	CAA916	MK940291			*Cupressus lusitanica*
Dothiorella sarmentorum	CAA125	JX878555			*Cupressus lusitanica*
Dothiorella sp.	CAP187	EU673313	EU673280	EU673100	*Prunus dulcis*
Dothiorella yunnana	CAA917	MK940307			*Quercus ilex*
Macrophomina phaseolina	GA4R3P5	KX243300			*Olea europaea*
Macrophomina phaseolina	VA233RZ	KM519656			*Olea sp.*
Neofusicoccum australe	CAA018	JX878558			*Pinus pinaster*
Neofusicoccum australe	CAA031	JX878542			*Thuja plicata*
Neofusicoccum australe	CAA057	JX878549			*Cupressus lusitanica*
Neofusicoccum australe	CAA073	JX878543			*Pinus pinea*
Neofusicoccum australe	CAA083	JX878551			*Sequoia sempervirens*
Neofusicoccum australe	CAA090	JX878550			*Taxus baccata*
Neofusicoccum australe	CAA103	JX878525			*Thujopsis dolabrata*
Neofusicoccum australe	CAA112	JX878540			*Chamaecyparis lawsoniana*
Neofusicoccum australe	CAA118	JX878538			*Picea abies*
Neofusicoccum australe	CAA178	KX871844	KX871800	KX871709	*Ferula communis*
Neofusicoccum australe	CAA184	KX871845	KX871801	KX871710	*Ferula communis*
Neofusicoccum australe	CAA191	KX871846	KX871802	KX871711	*Ferula communis*
Neofusicoccum australe	CAA195	KX871847	KX871803	KX871712	*Ferula communis*
Neofusicoccum australe	CAA197	KX871848	KX871804	KX871713	*Ferula communis*
Neofusicoccum australe	CAA202	KX871849	KX871805	KX871714	*Melia azedarach*
Neofusicoccum australe	CAA231	KX871850	KX871806	KX871715	*Hydrangea macrophylla*
Neofusicoccum australe	CAA233	KX871851	KX871807	KX871716	*Hydrangea macrophylla*
Neofusicoccum australe	CAA242	KX871852	KX871808	KX871717	*Hydrangea macrophylla*
Neofusicoccum australe	CAA319	KT440900	KT440960	KX871718	*Eucalyptus globulus*
Neofusicoccum australe	CAA320	KT440901	KT440961	KX871719	*Eucalyptus globulus*
Neofusicoccum australe	CAA326	KX871853	KX871809	KX871720	*Pyracantha coccinea*
Neofusicoccum australe	CAA327	KX871854	KX871810	KX871721	*Pyracantha coccinea*
Neofusicoccum australe	CAA332	KT440902	KT440962	KX871722	*Eucalyptus globulus*
Neofusicoccum australe	CAA341	KT440903	KT440963	KX871723	*Eucalyptus globulus*
Neofusicoccum australe	CAA344	KT440904	KT440964	KX871724	*Eucalyptus globulus*
Neofusicoccum australe	CAA351	KT440905	KT440965	KX871725	*Eucalyptus globulus*

Neofusicoccum australe	CAA357	KT440906	KT440966	KX871726			*Eucalyptus globulus*
Neofusicoccum australe	CAA359	KT440907	KT440967	KX871727			*Eucalyptus globulus*
Neofusicoccum australe	CAA392	KX871855	KX871811	KX871728			*Quercus robur*
Neofusicoccum australe	CAA398	KX871856	KX871812	KX871729			*Eucalyptus globulus*
Neofusicoccum australe	CAA400	KT440908	KT440968	KX871730			*Eucalyptus globulus*
Neofusicoccum australe	CAA401	KT440909	KT440969	KX871731			*Eucalyptus globulus*
Neofusicoccum australe	CAA406	KT440910	KT440970	KX871732			*Eucalyptus globulus*
Neofusicoccum australe	CAA420	KT440911	KT440971	KX871733			*Eucalyptus globulus*
Neofusicoccum australe	CAA427	KT440912	KT440972	KX871734			*Eucalyptus globulus*
Neofusicoccum australe	CAA434	KT440913	KT440973	KX505927	KX505951	KX505885	*Eucalyptus globulus*
Neofusicoccum australe	CAA441	KT440914	KT440974	KX871735			*Eucalyptus globulus*
Neofusicoccum australe	CAA455	KT440915	KT440975	KX505928	KX505952	KX505886	*Eucalyptus globulus*
Neofusicoccum australe	CAA464	KT440916	KT440976	KX871736			*Eucalyptus globulus*
Neofusicoccum australe	CAA466	KT440917	KT440977	KX871737			*Eucalyptus globulus*
Neofusicoccum australe	CAA468	KX871857	KX871813	KX871738			*Olea europaea*
Neofusicoccum australe	CAA475	KX871858	KX871814	KX871739			*Olea europaea*
Neofusicoccum australe	CAA546	KT440918	KT440978	KX871740			*Eucalyptus globulus*
Neofusicoccum australe	CAA549	KT440919	KT440979	KX871741			*Eucalyptus globulus*
Neofusicoccum australe	CAA550	KX871859	KX871815	KX871742			*Eucalyptus globulus*
Neofusicoccum australe	CAA571	KX871860	KX871816	KX871743			*Eucalyptus globulus*
Neofusicoccum australe	CAA647	KT440920	KT440980	KX871744			*Eucalyptus globulus*
Neofusicoccum australe	CAA648	KT440921	KT440981	KX871745			*Eucalyptus globulus*
Neofusicoccum australe	CAA649	KX871861	KX871817	KX871746			*Eucalyptus globulus*
Neofusicoccum australe	CAA723	KX871862	KX871818	KX871747			*Tilia platyphyllos*
Neofusicoccum australe	CAA741	KX871863	KX871819	KX871748			*Acacia longifolia*
Neofusicoccum australe	CAA743	KX871864	KX871820	KX871749			*Acacia longifolia*
Neofusicoccum australe	CAA747	KX871865	KX871821	KX871750			*Acacia longifolia*
Neofusicoccum australe	CAA749	KX871866	KX871822	KX871751			*Acacia longifolia*
Neofusicoccum australe	CAA750	KX871867	KX871823	KX871752			*Acacia longifolia*
Neofusicoccum australe	CAA751	KX871868	KX871824	KX871753			*Acacia longifolia*
Neofusicoccum australe	CAA768	MK932752	MK932755				*Vaccinium corymbosum*
Neofusicoccum australe	CAA838	MK932725	MK932759				*Vaccinium corymbosum*
Neofusicoccum australe	CAA840	MK932732	MK932763				*Vaccinium corymbosum*
Neofusicoccum australe	CAA841	MK932744	MK932767				*Vaccinium corymbosum*
Neofusicoccum australe	CAA918	MT260986					*Eucalyptus globulus*
Neofusicoccum australe	CAA919	MK940294					*Eucalyptus globulus*

Neofusicoccum australe	CAA920	MT260997					*Eucalyptus globulus*
Neofusicoccum australe	CAA922	MT261011					*Eucalyptus globulus*
Neofusicoccum australe	CAA923	MT261012					*Eucalyptus globulus*
Neofusicoccum australe	CAA924	MT261013					*Eucalyptus globulus*
Neofusicoccum australe	CAA925	MT261017					*Eucalyptus globulus*
Neofusicoccum australe	CAA926	MT261018					*Eucalyptus globulus*
Neofusicoccum australe	CBS110490		KX464655	KX464931			*Robinia pseudoacacia*
Neofusicoccum australe	CBS119046	DQ299244	EU017541	KU198429			*Rubus sp.*
Neofusicoccum eucalyptorum	CAA369	KT440922	KT440982	KX871773			*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA450	KT440923	KT440983	KX871774			*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA511	KX505907	KX505896	KX505919	KX505944	KX505881	*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA517	KT440924	KT440984	KX871775			*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA518	KX871883	KX871839	KX871776			*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA520	KT440925	KT440985	KX871777			*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA522	KT440926	KT440986	KX871778			*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA528	KT440927	KT440987	KX871779			*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA532	KT440928	KT440988	KX871780			*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA535	KT440929	KT440989	KX871781			*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA536	KT440930	KT440990	KX871782			*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA539	KX871884	KX871840	KX871783			*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA542	KT440931	KT440991	KX871784			*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA558	KT440932	KT440992	KX871785			*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA561	KX871885	KX871841	KX871786			*Fraxinus excelsior*
Neofusicoccum eucalyptorum	CAA601	KT440933	KT440993	KX871787			*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA604	KT440934	KT440994	KX871788			*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA618	KT440935	KT440995	KX871789			*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA624	KT440936	KT440996	KX871790			*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA651	KT440937	KT440997	KX871791			*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA680	KT440938	KT440998	KX871792			*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA683	KT440939	KT440999	KX871793			*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA695	KT440940	KT441000	KX871794			*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA709	KT440941	KT441001	KX505920	KX505945	KX505882	*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA712	KT440942	KT441002	KX871795			*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA713	KT440943	KT441003	KX505921	KX505946	KX505883	*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA714	KX871886	KX871842	KX871796			*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA842	MK932723	MK932757				*Vaccinium corymbosum*

Neofusicoccum eucalyptorum	CAA845	MK932740	MK932764				*Vaccinium corymbosum*
Neofusicoccum eucalyptorum	CAA927	MT260979					*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA928	MT260980					*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA929	MT260982					*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA930	MT260984					*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA931	MT260987					*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA932	MK940311					*Eucalyptus globulus*
Neofusicoccum eucalyptorum	CAA933	MT261016					*Eucalyptus globulus*
Neofusicoccum eucalyptorum	PE20	KT440944	KT441004	KX871797			*Eucalyptus globulus*
Neofusicoccum eucalyptorum	PE21	KT440945	KT441005	KX871798			*Eucalyptus globulus*
Neofusicoccum eucalyptorum	PE23	KX871887	KX871843	KX871799			*Eucalyptus globulus*
Neofusicoccum kwambonambiense	CAA755	KT440946	KT441006	KX505917	KX505938	KX505878	*Eucalyptus globulus*
Neofusicoccum luteum	CAA046	JX878522					*Thuja plicata*
Neofusicoccum luteum	CAA047	JX878547					*Thuja plicata*
Neofusicoccum luteum	CAA049	JX878539					*Thuja plicata*
Neofusicoccum luteum	CAA061	JX878541					*Cupressus lusitanica*
Neofusicoccum luteum	CAA065	JX878545					*Cupressus lusitanica*
Neofusicoccum luteum	CAA072	JX878546					*Pinus pinea*
Neofusicoccum luteum	CAA086	JX878548					*Sequoia sempervirens*
Neofusicoccum luteum	CAA099	JX878553					*Thujopsis dolabrata*
Neofusicoccum luteum	CAA110	JX878544					*Chamaecyparis lawsoniana*
Neofusicoccum luteum	CAA124	JX878552					*Araucaria angustifolia*
Neofusicoccum luteum	CAA200	KX871869	KX871825	KX871754			*Melia azedarach*
Neofusicoccum luteum	CAA203	KX871870	KX871826	KX871755			*Melia azedarach*
Neofusicoccum luteum	CAA352	KX871871	KX871827	KX871756			*Quercus robur*
Neofusicoccum luteum	CAA360	KX871872	KX871828	KX871757			*Fraxinus ornus*
Neofusicoccum luteum	CAA362	KX871873	KX871829	KX871758			*Fraxinus ornus*
Neofusicoccum luteum	CAA365	KX871874	KX871830	KX871759			*Quercus robur*
Neofusicoccum luteum	CAA379	KX871875	KX871831	KX871760			*Melia azedarach*
Neofusicoccum luteum	CAA412	KX871876	KX871832	KX871761			*Populus alba*
Neofusicoccum luteum	CAA505	KX871877	KX871833	KX871762			*Fraxinus ornus*
Neofusicoccum luteum	CAA628	KX505911	KX505902	KX505929	KX505954	KX505888	*Fraxinus excelsior*
Neofusicoccum luteum	CAA720	KX871878	KX871834	KX871763			*Tilia platyphyllos*
Neofusicoccum luteum	CAA934	MT261019					*Eucalyptus globulus*
Neofusicoccum luteum	CAA935	MK940305					*Eucalyptus globulus*

Neofusicoccum luteum	CBS110299	AY259091	KX464688	DQ458848	KX464018	KX505953	KX505887	*Vitis vinifera*
Neofusicoccum luteum	CBS110487			KX464966	KX464019			*Populus nigra*
Neofusicoccum luteum	CBS110497	EU673311	EU673277	EU673092				*Vitis vinifera*
Neofusicoccum luteum	CMW10309	AY339258	AY339266	AY339250				*Vitis vinifera*
Neofusicoccum luteum	CMW10310	AY339259	AY339267	AY339251				*Vitis vinifera*
Neofusicoccum luteum	PD285	GU251221	GU251353	GU251881				*Vitis vinifera*
Neofusicoccum luteum	STE-U4592	AY343416	AY343351					*Styphnolobium japonicum*
Neofusicoccum luteum	STE-U4594	AY343418						*Vitis vinifera*
Neofusicoccum parvum	CAA022	JX878537						*Thuja plicata*
Neofusicoccum parvum	CAA074	JX878534						*Pinus pinea*
Neofusicoccum parvum	CAA107	JX878536						*Thujopsis dolabrata*
Neofusicoccum parvum	CAA126	JX878535						*Juniperus communis*
Neofusicoccum parvum	CAA189	KX871879	KX871835	KX871766				*Ferula communis*
Neofusicoccum parvum	CAA192	KX505905	KX505892	KX505913		KX505934	KX505874	*Ferula communis*
Neofusicoccum parvum	CAA322	KX505906	KX505894	KX505916		KX505937	KX505877	*Malus pumila*
Neofusicoccum parvum	CAA366	KT440951	KT441011	KX871764				*Eucalyptus globulus*
Neofusicoccum parvum	CAA384	KX871880	KX871836	KX871767				*Rosa sp.*
Neofusicoccum parvum	CAA386	KX871881	KX871837	KX871768				*Rosa sp.*
Neofusicoccum parvum	CAA608	KX871882	KX871838	KX871769				*Aesculus hippocastanum*
Neofusicoccum parvum	CAA692	KT440950	KT441010	KX871770				*Eucalyptus globulus*
Neofusicoccum parvum	CAA704	KT440947	KT441007	KX505914		KX505935	KX505875	*Eucalyptus globulus*
Neofusicoccum parvum	CAA846	MK932721	MK932756					*Vaccinium corymbosum*
Neofusicoccum parvum	CAA848	MK932726	MK932760					*Vaccinium corymbosum*
Neofusicoccum parvum	CAA856	MK932743	MK932766					*Vaccinium corymbosum*
Neofusicoccum parvum	CAA858	MK932746	MK932768					*Vaccinium corymbosum*
Neofusicoccum parvum	CAA939	MT261014						*Eucalyptus globulus*
Neofusicoccum parvum	CAA940	MK940304						*Eucalyptus globulus*
Neofusicoccum parvum	CAA941	MT261015						*Eucalyptus globulus*
Neofusicoccum parvum	CAA942	-						*Eucalyptus globulus*
Neofusicoccum parvum	CBS110301	AY259098	AY573221	EU673095		KX505933	KX505873	*Vitis vinifera*
Neofusicoccum parvum	CBS110882		KX464699	KX464978				*Vitis vinifera*
Neofusicoccum parvum	CBS110888			KX464979				*Vitis vinifera*
Neofusicoccum parvum	CBS115186	KX464179	KX464704	KX464989				*Protea cynaroides*
Neofusicoccum parvum	PE17	KT440948	KT441008	KX871771				*Eucalyptus globulus*
Neofusicoccum parvum	PE18	KT440949	KT441009	KX871772				*Eucalyptus globulus*
Neofusicoccum parvum	PE32	KT440952	KT441012	KX871765				*Eucalyptus globulus*

Neofusicoccum parvum	STE-U5035	AY343473		*Vitis vinifera*
Neofusicoccum parvum	STE-U5253	AY343477	AY343367	*Vitis vinifera*
Neofusicoccum protearum	CBS113071	FJ150700		*Protea cynaroides*
Neofusicoccum protearum	CBS113076	FJ150701		*Leucadendron sp.*
Neofusicoccum protearum	CBS115480			*Leucadendron sp.*
Neofusicoccum protearum	CBS115499	FJ150704		*Leucadendron sp.*
Neofusicoccum sp.	CAA936	MK940293		*Cupressus lusitanica*
Neofusicoccum sp.	CAA937	MT237174		*Cupressus lusitanica*

Appendix E

Supplementary data 5

Table E.1: Model used by phylogenetic analyses for each genera and alignments details.

				Number of characters				
Phylogenetic analyses	Model	Ingroup taxa	Outgroup taxa	Total length	ITS	*TEF1*	*TUB2*	MAT1-1-1
Botryosphaeria	T92+G	13	1	749 bp	487 bp	262 bp	-	-
Botryosphaeria	K2+G	13	1	487 bp	487 bp	-	-	-
Botryosphaeria	T92	13	1	262 bp	-	262 bp	-	-
Diplodia	T92+G	26	1	751 bp	500 bp	251 bp	-	-
Diplodia	K2+G	26	1	500 bp	500 bp	-	-	-
Diplodia	T92+I	26	1	251 bp	-	251 bp	-	-
Dothiorella	K2+G	25	1	654 bp	447 bp	207 bp	-	-
Dothiorella	K2+G+I	25	1	447 bp	447 bp	-	-	-
Dothiorella	K2+I	25	1	207 bp	-	207 bp	-	-
Neofusicoccum	T92+G	27	1	1031 bp	481 bp	234 bp	316 bp	-
Neofusicoccum	K2+G	27	1	481 bp	481 bp	-	-	-
Neofusicoccum	T92+I	27	1	234 bp	-	234 bp	-	-
Neofusicoccum	T92+G	27	1	316 bp	-	-	316 bp	-
Neofusicoccum	HKY+I	24	0	1059 bp	-	-	-	1059 bp

Abbreviations: HKY: Hasegawa-Kishino-Yano; T92: Tamura 3-parameter; K2: Kimura 2-parameter. Non-uniformity of evolutionary rates among sites may be modelled by using a discrete Gamma distribution (+G) or evolutionarily invariable (+I).