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Review

Diversity in the *Botryosphaeriales*: Looking back, looking forward

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ABSTRACT

The *Botryosphaeriales* are amongst the most widespread, common and important fungal pathogens of woody plants. Many are also known to exist as endophytes in healthy plant tissues. This special issue highlights a number of key themes in the study of this group of fungi. In particular, there have been dramatic taxonomic changes over the past decade; from one family to nine (including two in this special issue) and from 10 to 33 genera known from culture. It is also clear from many studies that neither morphology nor single locus sequence data are sufficient to define taxa. This problem is exacerbated by the increasing recognition of cryptic species and hybrids (as highlighted for the first time in this special issue). It is futile that management strategies, including quarantine, continue to rely on outdated taxonomic definitions and identification tools. This is especially true in light of growing evidence that many species continue to be moved globally as endophytes in plants and plant products. A well defined natural classification and an extensive collection of tools to study the *Botryosphaeriaceae*, including a growing number of genomes, now provide a springboard for a much deeper exploration of their biology, biogeography and host associations.

Introduction

This special issue of Fungal Biology focuses on the *Botryosphaeriales*; the most common and widespread pathogens of woody hosts globally. These pathogens occur in virtually all woody plants that have been investigated for their presence, and they are commonly associated with branch, twig, leaf, fruit and seed diseases (Fig 1; Slippers & Wingfield 2007). When they occur, these diseases are closely associated with plant stress. This in turn raises the importance of the *Botryosphaeriales* under a scenario of global change, including climate change and other, mostly anthropogenic changes to the

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Fig 1 – (A). Botryosphaeriaceae die-back on *Vachellia karroo* (F. Jami). (B). *Neoscytalidium dematium* on *Citrus* (G. Polizzi). (C). *Botryosphaeria kuwatsukai* on *Malus* sp. (G. Sun). (D). *Dothiorella acacicola* on *Acacia heterophylla* (P.W. Crous). (E). *Botryosphaeria dothidea* on *Pistacia vera* (T. Michailides). (F). *Neofusicoccum ribis* on *Eucalyptus grandis* (C.A. Rodas). (G). *Lasiodiplodia* sp. on *Mangifera indica* (M.P.S. Câmara). (H). *Macrophomina phaseoli* on *Vigna nguiculata* (M.P. Melo). I. *Diplodia sapinea* on *Pinus patula* (B. Slippers). (J). *Alanphillipsia aloeicola* on *Aloe* sp. (P.W. Crous). (K). *Saccharata* sp. on *Banksia ashbyi* (P.W. Crous). (L). *Neofusicoccum* sp. on *Vitis vinifera* (P.W. Crous). (M). *Eutiarosporella* spp. infecting seed of *Triticum aestivum* (right) (S. Neate). (N). *Saccharata* sp. on *Banksia conferta* (P.W. Crous).

environment that place pressure on plant communities (Desprez-Loustau *et al.* 2007; Sturrock *et al.* 2011). There is also increasing evidence that these pathogens are being spread around the world with little constraint, and apparently with increasing frequency associated with globalization (Sakalidis *et al.* 2013; Burgess *et al.* 2017; Marsberg *et al.* 2017).

An increased and growing interest in the *Botryosphaeriales* globally is reflected in the rapid rise in publications on '*Botryosphaeria*' or '*Botryosphaeriaceae*'; the most commonly used historical and current terms referring to these fungi in recent years. This increased frequency represents just over 13 publications in the year 2000, to an average of more than 70 over the past Six years (ISI Web of Science core collection; Fig 2). While these data are likely affected by general factors such as overall increases in the numbers of scientific publications in most fields, this level of increase is unquestionably also linked to tools that have facilitated the study of the *Botryosphaeriales* and especially DNA sequence-based data. The growing interest in these fungi is clearly also linked to their growing importance as pathogens in natural and managed (e.g. agriculture and forestry) landscapes (Slippers & Wingfield 2007; Sarr *et al.* 2014; Chethana *et al.* 2016; Wyka & Broders 2016; Zlatković *et al.* 2016b; Mehl *et al.* 2017). This requires a clear understanding of the diversity of the group, as

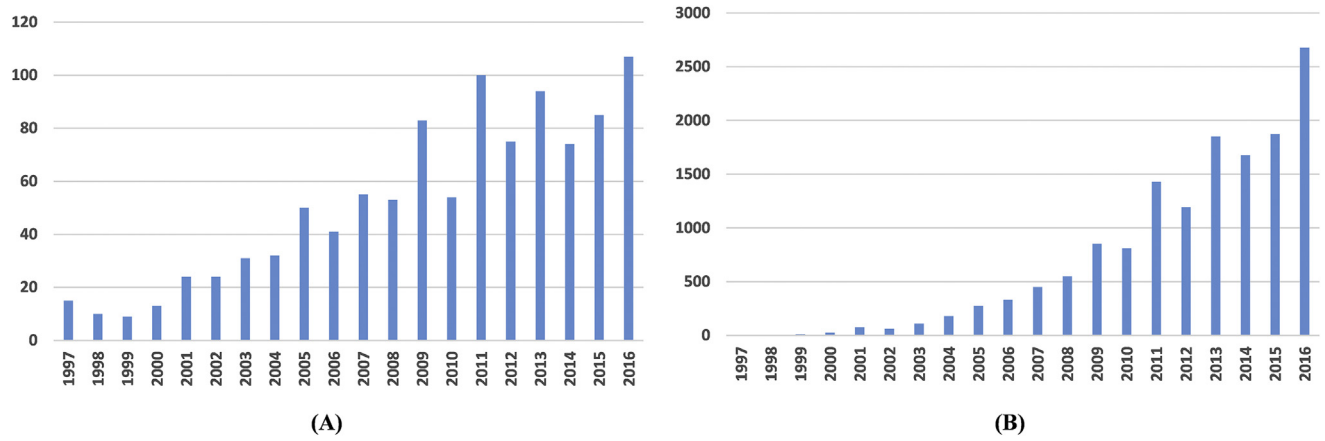

Fig 2 – The publications and citation history of papers citing *Botryosphaeria* or *Botryosphaeriaceae* in their titles over the past 20 years. (A). Publications per year. (B). Citations per year. Data was obtained from the *Web of Science*, www.webofknowledge.com.

well as of tools to accurately identify these fungi. This is necessary to ensure that they will be appropriately catered for in quarantine regulations, that we will better understand their invasion biology and that effective management strategies can be applied where disease problems are involved.

The first DNA sequence data were generated for the *Botryosphaeriales* fewer than 20 years ago (Jacobs & Rehner 1998). Since that time, there has been a massive increase in the number of sequences linked to this group. GenBank lists (November 2016) more than 10 000 gene sequences for the *Botryosphaeriales* (excluding genomes), covering more than 1160 sets linked to phylogenetic or population genetic studies. The phylogenetic analyses of these sequence data have significantly impacted all aspects of the systematics and taxonomy of the *Botryosphaeriales*. This has often led to significant and often confusing changes in their taxonomy. For example, in 1998 all taxa were known as species of *Botryosphaeria*, or at least they were thought to be *Botryosphaeria* where the asexual states had not been observed. This is in contrast to the current situation where only a small number of species reside in this genus and 33 other genera are now recognized (see below).

The papers in this issue of *Fungal Biology* represent a sampling of the dynamic changes that reflect our understanding of the diversity of the *Botryosphaeriales*. These include for example a redefinition of families and genera, identification of new species, cryptic species and (more recently) hybrids. These papers also illustrate the global distribution and importance of the *Botryosphaeriales*, as well as key aspects of their biology such as their mating biology, now being revealed through the availability of whole genome sequences.

In this review, we outline major historical developments in terms of species identification and taxonomy of the *Botryosphaeriales*. We also consider tools and techniques currently being used for identification and we propose best practices/standard suites of tools that can be utilized. We furthermore consider how a growing understanding of the diversity of the group is changing our understanding of global and regional patterns of diversity, host association and the epidemiology of diseases caused by a fascinating, important and yet relatively poorly understood group of fungi.

Understanding diversity and adapting the systematic framework

Our understanding of the diversity of the *Botryosphaeriales* has changed profoundly over the past 15 years. This includes the definition of the families, genera and species, as well as their relationships with each other. It was taxonomic work on the *Botryosphaeriales* that catalysed the 'one fungus one name' movement (Crous *et al.* 2006; Wingfield *et al.* 2012; Crous *et al.* 2015a). Arising from frustration at having to accommodate species in genera that were clearly very different to each other, Crous *et al.* (2006) took the dramatic step of defining all genera in the *Botryosphaeriales* based predominantly on phylogenetic inference and characteristics of their asexual morphs, and without morphological evidence of a sexual morph. In various cases, genera were thus established in the family based on asexual names. Under the new *International Code of Nomenclature for algae, fungi and plants* (McNeill *et al.* 2012), these asexual names are accepted for the holomorph and this is now true for many other fungi.

The most significant change to the systematics of the *Botryosphaeriales* in past two years has been at the family level. At the time when Schoch *et al.* (2006) described the *Botryosphaeriales*, all genera were accommodated in the *Botryosphaeriaceae*. Since then many authors (Minnis *et al.* 2012; Slippers *et al.* 2013; Wikee *et al.* 2013; Wyka & Broders 2016; Yang *et al.* 2017) have delineated an additional eight families, based on phylogenetic, morphological and ecological differences. With the description of *Septorioideaceae* (Wyka & Broders 2016), *Endomelanconiopsisaceae* and *Pseudofusicoccumaceae* (Yang *et al.* 2017) there are now nine families accommodated in the order (Table 1). Six of these nine *Botryosphaeriales* families are represented by only a single genus, while the *Botryosphaeriaceae* is represented by 23 genera, and *Aplosporellaceae* and *Planistromellaceae* by two genera each.

Table 1 – Families and genera described in the Botryosphaeriales.

Family	Genus	Reference
Aplosporellaceae Slippers et al. 2013	***Aplosporella*** Speg., Anal. Soc. cient. argent. 10(5–6): 157 (1880) Typus: ***A. chlorostroma*** Speg., Anal. Soc. cient. argent. 10(5–6): 158 [no. 117, reprint page 35] (1880)	Slippers et al. (2013)
	Bagnisiella Speg., Anal. Soc. cient. argent. 10(5–6): 146 (1880) Typus: ***B. australis*** Speg., Anal. Soc. cient. argent. 10(1): 22 (1880)	Slippers et al. (2013)
Botryosphaeriaceae Theiss. & Syd., 1918		Crous et al. (2006)
	Allanphillipsia Crous & M.J. Wingf. Persoonia 31: 197 (2013) Typus: ***A. aloes*** Crous & M.J. Wingf., Persoonia 31: 197 (2013)	Crous et al. (2013)
	Bahusutrabeeja Subram. & Bhat, Can. J. Bot. 55(16): 204 (1977) Typus: ***B. dwaya*** Subram. & Bhat, Can. J. Bot. 55(16): 2204 (1977)	Shenoy et al. (2010)
	Barriopsis A.J.L. Phillips et al., Persoonia 21: 39 (2008) Typus: ***B. fusca*** (N.E. Stevens) A.J.L. Phillips et al., Persoonia 21: 39 (2008)	Phillips et al. (2008)
	Botryobambusa Phook. et al., Fungal Diversity 57(1): 166 (2012) Typus: ***B. fusicoccum*** Phook. et al., Fungal Diversity 57(1): 166 (2012)	Liu et al. (2012)
	Botryosphaeria Ces. & De Not., Comm. Soc. crittog. Ital. 1(fasc. 4): 211 (1863) Typus: ***B. dothidea*** (Moug.) Ces. & De Not., Comm. Soc. crittog. Ital. 1(fasc. 4): 212 (1863)	Phillips et al. (2013)
	Cophinforma Doilom et al., Fungal Diversity 57(1): 174 (2012) Typus: ***C. eucalypti*** Doilom et al., Fungal Diversity 57(1): 174 (2012)	Liu et al. (2012)
	Diplodia Fr., in Montagne, Annls Sci. Nat., Bot., sér. 2 1: 302 (1834) Typus: ***D. mutila*** (Fr.) Mont., Annls Sci. Nat., Bot., sér. 2 1: 302 (1834)	Phillips et al. (2008)
	Dothiorella Sacc., Michelia 2(no. 6): 5 (1880) Typus: ***D. pyrenophora*** Berk. ex Sacc., Michelia 2(no. 6): 5 (1880)	Phillips et al. (2008), Yang et al. (2017)
	Eutiarosporella Crous, Phytotaxa 202(2): 85 (2015) Typus: ***E. tritici*** (B. Sutton & Marasas) Crous, Phytotaxa 202(2): 85 (2015)	Crous et al. (2015a, 2015b)
	Lasiodiplodia Ellis & Everh., Bot. Gaz. 21: 92 (1896) Typus: ***L. tubericola*** Ellis & Everh., Bot. Gaz. 21: 92 (1896)	Phillips et al. (2008)
	Macrophomina Petr., Annls mycol. 21(3/4): 314 (1923) Typus: ***M. philippinensis*** Petr., Annls mycol. 21(3/4): 314 (1923)	Phillips et al. (2013)
	Marasasiomyces Crous, Phytotaxa 202(2): 86 (2015) Typus: ***M. karoo*** (B. Sutton & Marasas) Crous, Phytotaxa 202(2): 86 (2015)	Crous et al. (2015a, 2015b)
	Mucoharknessia Crous et al., Phytotaxa 202(2): 86 (2015) Typus: ***M. cortaderiae*** Crous et al., Phytotaxa 202(2): 86 (2015)	Crous et al. (2015a, 2015b)
	Neodeightonia C. Booth, Mycol. Pap. 119: 17 (1970) Typus: ***N. subglobosa*** C. Booth, Mycol. Pap. 119: 19 (1970)	Phillips et al. (2008)
	Neofusicoccum Crous et al., Stud. Mycol. 55: 247 (2006) Typus: ***N. andinum*** (Mohali et al.) Mohali et al., Stud. Mycol. 55: 247 (2006)	Crous et al. (2006)
	Neoscytalidium Crous & Slippers, Stud. Mycol. 55: 244 (2006) Typus: ***N. dimidiatum*** (Penz.) Crous & Slippers, Stud. Mycol. 55: 244 (2006)	Crous et al. (2006)
	Oblongocollomyces Tao Yang & Crous, Fungal Biology 121: 322–346 (2017) Typus: ***O. variabilis*** (F.J.J. van der Walt, et al.) Tao Yang & Crous, Fungal Biology 2016	Yang et al. (2017)
	Otthia Nitschke ex Fuckel, Jb. nassau. Ver. Naturk. 23–24: 169 (1870) Typus: ***O. spiraeae*** (Fuckel) Fuckel, Jb. nassau. Ver. Naturk. 23–24: 170 (1870)	Schoch et al. (2009) [a]
	Phaeobotryon Theiss. & Syd., Annls mycol. 13(5/6): 664 (1915) Typus: ***P. cercidis*** (Cooke) Theiss. & Syd., Annls mycol. 13(5/6): 664 (1915)	Phillips et al. (2008)
	Sakireeta Subram. & K. Ramakr., J. Indian bot. Soc. 36: 83 (1957) Typus: ***S. madreeya*** Subram. & K. Ramakr., J. Indian bot. Soc. 36: 84 (1957)	Crous et al. (2015a, 2015b)
	Sardiniella Linaldeddu et al., Mycosphere 7 (7): 900 (2016) Typus: ***S. urbana*** Linaldeddu et al., Mycosphere 7 (7): 900 (2016)	Linaldeddu et al. (2016)
	Sphaeropsis Sacc., Michelia 2(no. 6): 105 (1880) Typus: ***S. visci*** (Alb. & Schwein.) Sacc., Michelia 2(no. 6): 105 (1880)	Phillips et al. (2013)
	Tiarosporella Höhn., in Weese, Ber. dt. bot. Ges. 37: 159 (1919) Typus: ***T. paludosa*** (Sacc. & Fiori) Höhn., in Weese, Ber. dt. bot. Ges. 37: 159 (1919)	Crous et al. (2015a, 2015b)
Endomelanopsisaceae Tao Yang & Crous, 2016	***Endomelanconiopsis*** E.I. Rojas & Samuels, Mycologia 100(5): 770 (2008) Typus: ***E. endophytica*** E.I. Rojas & Samuels, Mycologia 100(5): 770 (2008)	Yang et al. (2017)
Melanopsaceae Phillips et al. 2013	***Melanops*** Nitschke ex Fuckel, Jb. nassau. Ver. Naturk. 23–24: 225 (1870) Typus: ***M. tulasnei*** Fuckel, Jb. nassau. Ver. Naturk. 23–24: 225 (1870)	Slippers et al. (2013)
Phyllostictaceae Fr., 1849	***Phyllosticta*** Pers., Traité champ. (Paris). 55: 147 (1818) Typus: *P. convallariae* Pers., nom. inval. (= ***P. cruenta*** (Fr.) J. Kickx f.)	Wikee et al. (2013)
Planistromellaceae M.E. Barr, 1996	***Kellermania*** Ellis & Everh., J. Mycol. 1(12): 153 (1885) Typus: ***K. yuccigena*** Ellis & Everh., J. Mycol. 1(12): 154 (1885)	Minnis et al. (2012)

Table 1 – (*continued*)

Family	Genus	Reference
	Umthunziomyces Crous & M.J. Wingf. Persoonia 37: 315 (2016) Typus: ***U. hagahagensis*** Crous & M.J. Wingf. Persoonia 37: 315 (2016)	Crous *et al.* (2016a)
Pseudofusicoccumaceae Tao Yang & Crous, 2016	***Pseudofusicoccum*** Mohali *et al.*, Stud. Mycol. 55: 249 (2006) Typus: ***P. stromaticum*** (Mohali *et al.*) Mohali *et al.*, Stud. Mycol. 55: 249 (2006)	Yang *et al.* (2017)
Saccharataceae Slippers *et al.* 2013	***Saccharata*** Denman & Crous, CBS Diversity Ser. (Utrecht) 2: 104 (2004) Typus: ***S. proteae*** (Wakef.) Denman & Crous, CBS Diversity Ser. (Utrecht) 2: 104 (2004)	Slippers *et al.* (2013)
Septorioideaceae Wyka & Broders, 2016	***Septorioides*** Quaedvlieg *et al.*, Stud. Mycol. 75: 383 (2013) Typus: ***S. pini-thunbergii*** (S. Kaneko) Quaedvlieg *et al.*, Stud. Mycol. 75: 383 (2013)	Wyka & Broders (2016)

a The association of *Otthia* with *Botryosphaeriaceae* is uncertain. *Otthia spiraeae* was redescribed by Phillips *et al.* (2005) as the lectotype of the genus, but no isolates are available. Isolates that have been incorrectly assigned to *O. spiraeae* group with *Dothiorella*, but does not reflect relationships of *Otthia* as these isolates are not linked to the type (Phillips *et al.* 2005; Schoch *et al.* 2009). While *O. spiraeae* was separated from *Botryosphaeria* by Phillips *et al.* (2005), as defined at that stage, its relation to the rest of the *Botryosphaeriales* remain unresolved. An epitypification and designation of an ex-type isolate are needed to resolve this matter.

For most of these families, very few species are known from culture. As with genus and species recognition, it has been the phylogenetic perspective provided by the use of multiple genes, and especially LSU and (to a lesser extent) SSU rDNA sequence data that has given support for the backbone of the *Botryosphaeriales* phylogeny. This has provided confidence in the distinction of the different families as opposed to a system reliant primarily on morphological recognition. Analysis of the SSU rDNA data suggests that the split between these families ranges from 87 (40–163) – 38(16–73) million years ago (Slippers *et al.* 2013). The most diverse family in the *Botryosphaeriales* is the *Botryosphaeriaceae* that includes 23 genera. Considering the phylogenetic and morphological evidence that has been used to distinguish the nine families in the *Botryosphaeriales*, the main groups within the *Botryosphaeriaceae* should perhaps also be treated as distinct families (Slippers *et al.* 2013; Yang *et al.* 2017).

At the time when the first sequence-based phylogenies were produced for members of the *Botryosphaeriales*, most species were considered part of *Botryosphaeria*, and its dominant asexual morphs in *Fusicoccum*, *Diplodia* and *Lasiodiplodia* (Jacobs & Rehner 1998; Denman *et al.* 2000). As mentioned earlier, Crous *et al.* (2006) discarded the dual nomenclature for sexual and asexual morphs and recognized ten distinct lineages or genera within the *Botryosphaeriaceae*. Since then, 33 genera have been described in the order (Table 1), mostly based on characteristics of their asexual morphs. This increase in the identification of genera has, not surprisingly, been heavily influenced by sampling and the number of species known in each genus. While in most cases providing confidence for the distinction of new genera, better representation has also lead to recently described genera, such as *Dothiorella* (= *Spencermartinsia*) being reduced to synonymy (Yang *et al.* 2017). It is possible that similar blurring of distinctions between other closely related genera might occur as more species of recently described but still poorly sampled genera are discovered.

A large number of new species have been described in the *Botryosphaeriales* in recent years. These are mostly known from culture and DNA sequence-based differences, although morphology is still widely used in descriptions. In this special issue, eight new species are described, bringing the total number of species known from culture and sequence to 279 (Fig 3). Clearly the rate of discovery of new species over the past decade is not slowing. Recognition of further diversity is likely to be driven primarily by expanding the geographic sampling, and to a lesser extent by more intensive sampling on hosts in areas that have already been well studied (Slippers *et al.* 2014).

Our ability to recognize recently diverged yet morphologically similar cryptic species in the *Botryosphaeriaceae* has increased significantly by the application of Genealogical Concordance Phylogenetic Species Recognition (Taylor *et al.* 2000), or versions of it. Following this approach, concordance between individual gene trees is seen as evidence for a lack of recombination amongst groups and thus reflective of species barriers. The first application of this tool was for the distinction between *Neofusicoccum parvum* and *Neofusicoccum ribis*; two species that share very similar morphologies and ITS rDNA sequence data (Slippers *et al.* 2005). Currently, this species complex has been shown to include ten other cryptic species, many of which can be identified only through careful analyses of multiple gene sequences (Pavlic *et al.* 2009a; Begoude *et al.* 2010; Sakalidis *et al.* 2013). Some of these species are not consistently distinguishable using morphological data, and consequently Pavlic *et al.* (2009b) based the description on distinct Single Nucleotide Polymorphisms (SNP) that were fixed amongst the isolates of each species they studied. However, one limitation of this approach is that a sufficient number of isolates should be included per species for a robust identification of the truly fixed SNPs, which are not always available. It is now common to require sequence data for two or three gene regions to delineate species described from culture (see next section). Morphological characteristics have systematic and biological meaning, but they are no longer necessary for species distinction or routine identification. Thus, some of the commonly applied arguments relating to species descriptions, requiring for example examination of herbarium material, are not only superfluous but they are also meaningless (Slippers *et al.* 2014).

Prior to the publication of this special issue, hybrids were unknown amongst species of the *Botryosphaeriales*. Cruywagen *et al.* (2017) describe four hybrids and Rodríguez-Gálvez *et al.* (2017) describe one hybrid, amongst species of *Lasiodiplodia*. Some of these were previously described species for which phylogenetic data were inadequate and possibly incorrectly interpreted. Hybrids can be extremely difficult to identify and require sequences for multiple genes, representative collections, and the exclusion of other hypotheses to describe the variation. In this regard, Cruywagen *et al.* (2017) have provided a useful 'decision tree' to illustrate the approach taken in that study. These findings raise a number of critical questions. Why have hybrids been identified only in *Lasiodiplodia*? If hybrids are restricted to this genus, what drives hybridization within the group that is different from other genera? Does the global distribution of some species, well beyond their expected original ranges, contribute to the likelihood of hybridization in the group? What are the consequences of hybridization with respect to host range, pathogenicity and other characters that could have ecological and economic consequences? These and other questions have been studied for some other plant-associated fungi and Oomycetes, which will guide future studies in the *Botryosphaeriales* (Schardl & Craven 2003).

An increasingly urgent question needing consideration for the *Botryosphaeriales* (and numerous other important groups of fungi) is how best to deal with the hundreds of historical names known only from herbarium specimens. There are no cultures for the vast majority of these species and there is consequently little opportunity to verify their names based on sequence data. It is evident from changes in the taxonomy of the *Botryosphaeriales* described above, and especially the recognition of cryptic species and hybrids, that such DNA-based sequence data are not only important, but also essential for accurate routine identification of *Botryosphaeriales* in laboratories globally.

Epi- and neotypification of species is a valuable approach to address the problem of species known based only on herbarium specimens (e.g. see Slippers *et al.* 2004; Crous *et al.* 2015b; Dayarathne *et al.* 2016). However, while such typification actions can provide living cultures to be used in associated studies, it is expensive, time consuming and for most

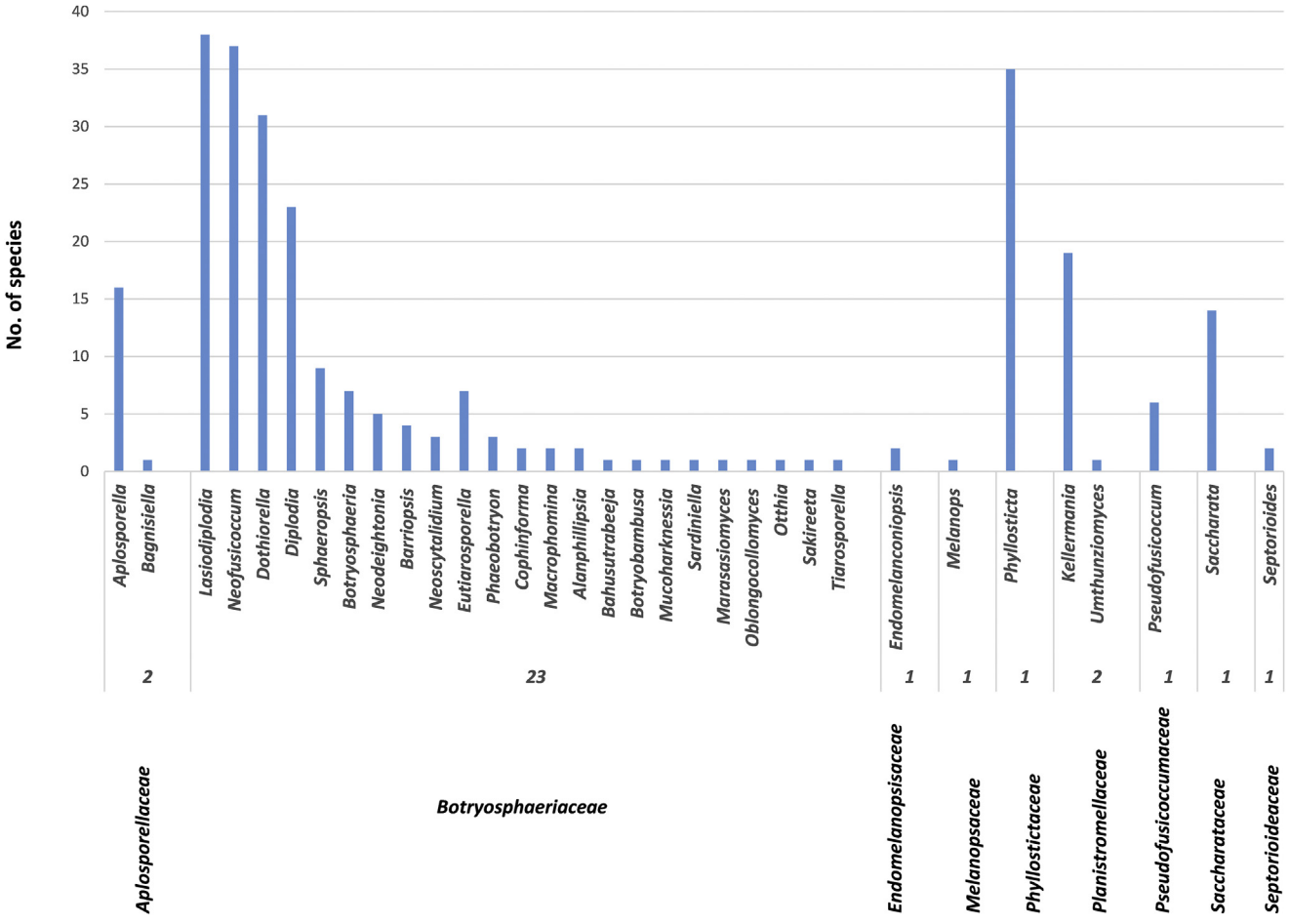

Fig 3 – The number of species known from culture in the 33 genera and nine families of the *Botryosphaeriales*. The bulk of the diversity is represented by the *Botryosphaeriaceae*, of which *Lasiodiplodia*, *Neofusicoccum*, *Dothiorella* and *Diplodia* contain 70 % of the species. There is no reason at present to believe that this skewed diversity is due to sampling or isolation bias, and rather represent genera that are more widely distributed geographically and on more hosts.

species this approach will not be practical or even possible. With advances in sequencing of very old and fragmented DNA, the sequencing of herbarium specimens provides an interesting alternative. A genomics project screening all type specimens in herbaria could be attempted (Weiss *et al.* 2016), although challenges of degraded DNA, mixed fungal infections, and depauperate specimens will need to be overcome. In addition, the probability is extremely low that sequences for all the commonly-used single-copy genes for species identification will be obtained from such DNA. Whatever the approach going forward, the use of older names without linked validated sequence data should be avoided, even for general identifications. In this regard, the list of names of species known from culture should be treated as the current acknowledged list of names.

Molecular tools for identification and taxonomic characterization

For phylogenetic relationships at the generic and family levels, sequence data from the Large- and Small-Subunit of the rDNA have been important, in addition to ITS and some other loci (Yang *et al.* 2017). The most complete phylogenies of representative groups in the order have been based on six loci (Slippers *et al.* 2013). Even from these, relationships between some groups are still only weakly supported or understood.

There are a variety of approaches used for DNA sequence-based description of taxa in the *Botryosphaeriales*. The internal transcribed spacer region of the ribosomal RNA (ITS) locus, amplified using the standard barcoding primers (ITS1 to ITS4 primers; White *et al.* 1990) is still the most widely used. This locus, however, does not distinguish many well-established cryptic species, or at least not with certainty (Slippers *et al.* 2004; Sakalidis *et al.* 2011). The translation elongation factor 1-α (*EF1-α*) is most often used in combination with the ITS locus, but it has limitations. For example, it has been problematic to amplify this region in some species, and lacks phylogenetic signal in some groups (e.g. *Lasiodiplodia*; see Cruywagen *et al.* 2017) (Fig 4). A portion of the β-tubulin (*TUB*) gene has been widely used, but it also lacks phylogenetic signal. The RNA polymerase II (*rpb2*) locus has shown much promise, providing phylogenetic signal between even closely related species (Pavlic *et al.* 2009b; Sakalidis *et al.* 2011; Yang *et al.* 2017). However, it has not been frequently used, sometimes due to failure to obtain amplicons for given genera or groups of species, and this implies that there are incomplete databases for this gene region. This is also true for numerous other loci that have been used in various studies (Table 2). Thus the ITS, *EF1-α*, *TUB*, and *rpb2* loci remain the most represented in databases, and they provide sufficient phylogenetic signal to distinguish most cryptic species. These latter loci should ideally all be sequenced for at least some representative isolates of any species that is being described as new.

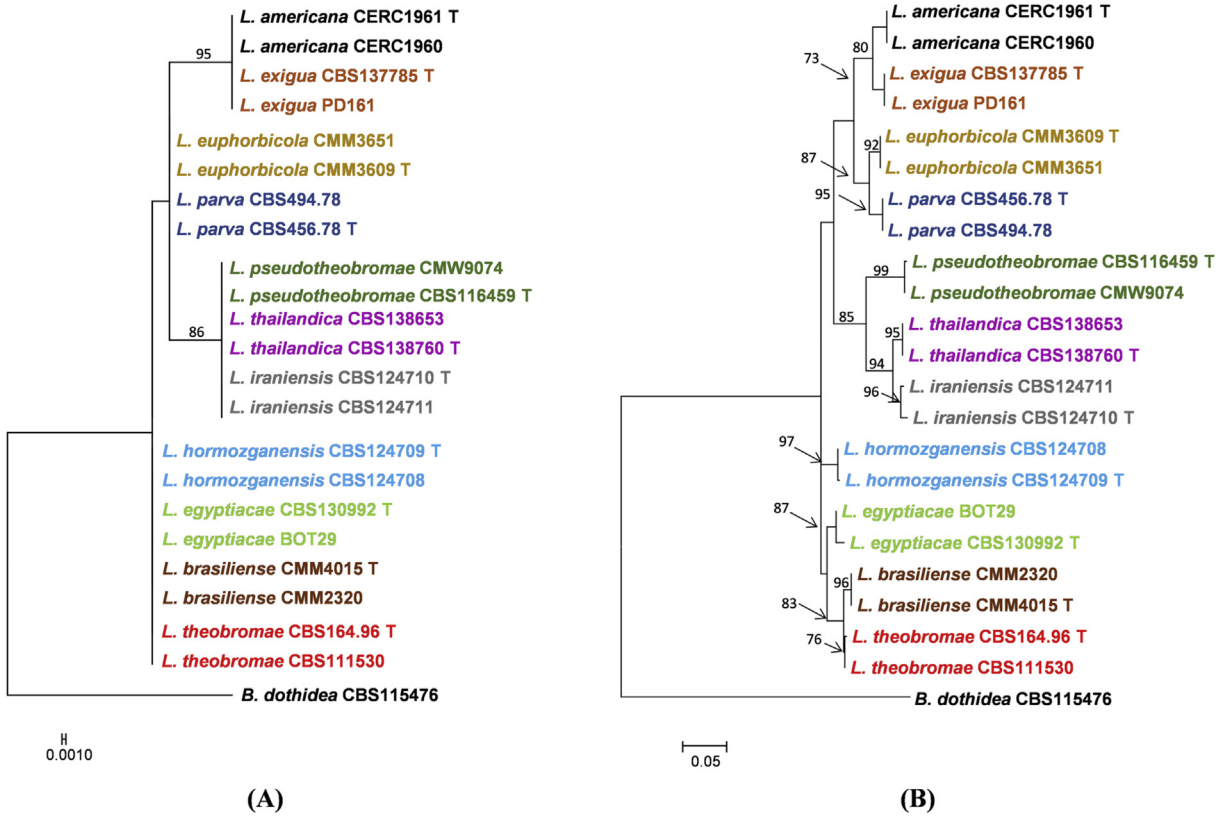

Fig 4 – Phylogenetic trees showing the difference between resolution of recognized species in *Lasiodiplodia* when using (A) ITS rDNA data compared to (B) a combined dataset of ITS rDNA, *EF1-α*, *TUB*, CAL and *rpb2* loci. Trees were drawn from GenBank data linked to the isolate number listed with each name. "T" represents ex-type cultures.

Table 2 – Primers commonly used to amplify loci for sequencing in phylogenetic studies.

Locus	Primer	Sequence 5′-3′	Reference
ITS rDNA	ITS1-F	CTTGGTCATTTAGAGGAAGTAA	Gardes & Bruns (1993)
	ITS1	TCCGTAGGTGAACCTGCGG	White et al. (1990)
	ITS4	TCCTCCGCTTATTGATATGC	White et al. (1990)
LSU rDNA	LR5	GGAAGTAAAAGTCGTAACAAGG	Vilgalys & Hester 1990
	LR0R	GTACCCGCTGAACTTAAGC	Rehner & Samuels (1994)
	LSU1 Fd	GRATCAGGTAGGRATACCCG	Crous et al. (2009)
SSU rDNA	NS1	GTAGTCATATGCTTGTCTC	White et al. (1990)
	NS4	CTTCCGTCAATTCCTTTAAG	White et al. (1990)
Translation Elongation factor-1α (*EF1-α*)	EF1-688F	CGGTCACTTGATCTACAAGTGC	Alves et al. (2008)
	EF1-1251R	CCTCGAACTCACCAGTACCG	Alves et al. (2008)
	EF-AF	CATCGAGAAGTTCGAGAAGG	Sakalidis et al. (2011)
	EF-BR	CRA TGG GTG ATA CCA RCG CTC	Sakalidis et al. (2011)
	EF1-728F	CATCGAGAAGTTCGAGAAGG	Carbone & Kohn (1999)
	EF1-986R	TACTTGAAGGAACCCTTACC	Carbone & Kohn (1999)
	EF2	GGARGTACCAGTSATCATGTT	O'Donnell et al. (1998)
	EF1-983F	GCYCCYGGHCAYCGTGAYTTYAT	Rehner (2001)
	EFgr	GCAATGTGGGCRGTRTGRCARTC	Rehner (2001)
β-tubulin (*TUB*)	Bt2a	GGTAACCAAATCGGTGCTGCTTTC	Glass & Donaldson (1995)
	Bt2b	ACCCTCAGTGTAGTGACCCTTGGC	Glass & Donaldson (1995)
	T1	AACATGCGTGAGATTGTAAGT	O'Donnell & Cigelnik (1997)
	T10	ACGATAGGTTCACCTCCAGAC	O'Donnell & Cigelnik (1997)
RNA polymerase II second largest subunit (*rpb2*)	*rpb2*-LasF	GGTAGCGACGTCACTCCT	Cruywagen et al. (2017)
	rpb2-LasR	GCGCAAATACCCAGAATCAT	Cruywagen et al. (2017)
	fRPB2-5F	GAYGAYMGWGATCAYTTYGG	Liu et al. (1999)
	fRPB2-414R	ACMANNCCCCARTGNGWRTTRTG	Quaedvlieg et al. (2011)
Calmodulin (*CAL*)	CAL-228F	GAGTTCAAGGAGGCCTTCTCCC	Carbone & Kohn (1999)
	CAL-737R	CATCTTTCTGGCCATCATGG	Carbone & Kohn (1999)
Actin (*ACT*)	ACT-512F	ATGTGCAAGGCCGGTTTCGC	Carbone & Kohn (1999)
	ACT-783R	TACGAGTCCTTCTGGCCCAT	Carbone & Kohn (1999)
Glyceraldehyde-3-phosphate dehydrogenase (*GPDH*)	Gpd1-LM	ATTGGCCGCATCGTCTTCCGCAA	Myllys et al. (2002)
	Gpd2-LM	CCCACTCGTTGTCGTACCA	Myllys et al. (2002)
	GDR1	GGGTGGAGTCGTACTTGAGCA TGT	Guerber et al. (2003)
	GDF1	GCCGTCAACGACCCCTTCATTGA	Guerber et al. (2003)
	GDPHR2	CTCRGMRGCRGCCTTGATGG	Glienke et al. (2011)
Mating type (*MAT1-1-1*)	Neo_MAT1_þ92F	TTGGCGCCACATACGCC	Lopes et al. (2017)
	Neo_MAT1_þ227F	GTCAATGCAGTATCCCCAGC	Lopes et al. (2017)
	Neo_MAT1_þ470F	CTTTGCTTCTGTGCTGTGCC	Lopes et al. (2017)
	Neo_MAT1_113Fb	CACTCTCAACTGCTTCGTCG	Lopes et al. (2017)
	Neo_MAT1_226F	CAGAAGGACAGGTCGGGC	Lopes et al. (2017)
	Neo_MAT1_240F	GACCTGTCCTTCTGTTGATGC	Lopes et al. (2017)
	Neo_MAT1_771F	TGCTGGGCATTCTGAGCAGC	Lopes et al. (2017)
	Neo_MAT1_1215Rb	CGAAGGTCCGAGTANTTG	Lopes et al. (2017)
	Neo_MAT1_1301R	CTTGATCGGACTGTCCAACC	Lopes et al. (2017)
	Neo_MAT1_1511R	CATTGTCAAAGTGGTCGGCG	Lopes et al. (2017)
	Neo_MAT1_-84R	GTGCAGTCCTACACGATTCC	Lopes et al. (2017)
	Neo_MAT1_-273R	GCATAAGTACTCGCCCAAGC	Lopes et al. (2017)
	Neo_MAT1_-283R	CGCTTGTGGCGCATAAGTACTCG	Lopes et al. (2017)
	Neo_MAT1_-1154R	GTTCATCTGCATCTGAGGATCG	Lopes et al. (2017)
	Neo_MAT1_-1892R	TACGATGTCGTGCATTGGGA	Lopes et al. (2017)
	Neo_MAT1_-2002R	TGGATTGGGTGGGGAATTGG	Lopes et al. (2017)
Mating type (*MAT1-2-1*)	Neo_MAT2_-359F	GGAAATACATACGCTCCTGTGG	Lopes et al. (2017)
	Neo_MAT2_-156Fb	TATCGTTCTTGGAGCGACTCAGC	Lopes et al. (2017)
	Neo_MAT2_-77F	TCACTGCTTTGGCTGCACACC	Lopes et al. (2017)
	Neo_MAT2_119F	CTACGAGCAACAAATGCCATTGC	Lopes et al. (2017)
	Neo_MAT2_268F	CTCAGCCTCTCATGAACCAG	Lopes et al. (2017)
	Neo_MAT2_1070Rb	GCATTGTCAGGATAGTCCGC	Lopes et al. (2017)
	Neo_MAT2_1405R	CAAGCGAAGTGAAGTCGAAGC	Lopes et al. (2017)
	Neo_MAT2_þ116R	AGGCAGTGGTCTTTCGTTCC	Lopes et al. (2017)
	Neo_MAT2_þ162R	CCTTGATCGAAAGACGCAGAGTG	Lopes et al. (2017)
	Neo_MAT2_þ975R	TGGGTGCGTCGTTGTTAGAGG	Lopes et al. (2017)

It is important to consider congruence amongst the phylogenies from the above-mentioned loci, and not only to lump these data in combined analyses (Taylor *et al.* 2000; Sakalidis *et al.* 2013). This is particularly evident in the use of the two most common loci in the *Botryosphaeriales* namely the ITS and *EF1-α*, which are often presented as combined analyses. In this regard, cryptic species can be erroneously distinguished in such combined trees when the distinction has support only from a single locus (e.g. *EF1-α*). Combined analyses inevitably strengthen the back-bones of the trees and often support interpretation of relationships amongst species and genera. But the distinction of cryptic species requires separate analyses of individual phylogenies, and typically for more than two informative loci. The identification of SNPs that are fixed within species and differ from other taxa, provide characters that can be used in the description of new species, as mentioned earlier (Pavlic *et al.* 2009b).

Microsatellite markers that are commonly used for population genetic studies have been useful to support the lack of gene flow between potential cryptic taxa that have been identified using gene sequences (Begoude *et al.* 2011; Sakalidis *et al.* 2011; Pavlic-Zupanc *et al.* 2015). While this approach should be applied with caution because distinction between populations and species can be confused, it is useful to test hypotheses of whether gene flow might occur between cryptic species.

Very few of the currently applied morphological characters for species descriptions of *Botryosphaeriales* have emerged as having taxonomic value (Slippers *et al.* 2014). Size of conidiomata, size, colour and septation in conidia and ascospores, presence and size of paraphyses, and various other typically described characters all overlap between species and vary greatly within species. Perhaps the most dramatic example of this problem is the occasional presence of highly variable synasexual morphs in many taxa (Barber *et al.* 2005; Crous *et al.* 2006; Phillips *et al.* 2013). While we do not suggest that the use of these characters should be blindly discarded in taxonomic descriptions, some consideration should be given to understanding which characters will most likely have value as metadata for biological, ecological or systematic studies in future. For example, accurate collection data (host, location, date), information regarding pathogenicity or disease symptoms, growth rate at certain temperatures and, in future, the genome and transcriptome data might have much greater value to future users of a description than dimensions of some morphological character. In this regard, a culture linked to a herbarium specimen is much more valuable than only a herbarium specimen; allowing researchers to characterize other features in future that are not considered important or that are not feasible at present.

The dramatic changes in the taxonomy of the *Botryosphaeriales* during the course of the past decade has led to high levels of uncertainty regarding previously described names in this family. As mentioned previously, this is true for names without associated sequence data, but is also a problem in the case of poorly characterized names for which sequence data are available. It is a problem that a very large proportion of the >10 000 gene sequences in GenBank have questionable taxonomic labels, and it is critical that this is addressed to support accurate future research (Bidartondo *et al.* 2008). The curated NCBI Reference Sequence Database (RefSeq) and associated curated databases such as MycoBank provide valuable resources to navigate through some of these data sets. It is ideal, however, to include reference sequences from ex-type cultures, as far as possible, in phylogenetic analyses and thus to avoid confusion.

Diversity, geographic distribution, host association, and disease

As an increasingly accurate view of the species diversity in the *Botryosphaeriales* has emerged, a fascinating but disturbing reality regarding the biogeography of the group is also emerging. For example, some species are clearly globally distributed and they have extremely wide host ranges. Examples include *Botryosphaeria dothidea* and *Lasiodiplodia theobromae* that interestingly were the most commonly used names in the order despite the taxonomic changes that have occurred. They also remain some of the most commonly occurring species globally. But there are many more species, especially in *Neofusicoccum*, *Diplodia*, and *Lasiodiplodia* that have a much wider global or near global distribution than was previously realized. These species are also those most often associated with disease in agriculture and forestry. These distributions can thus have far reaching consequences in terms of plant health in native ecosystems and commercial plant production alike. And this is especially important as plant communities come under pressure due to climate change (Desprez-Loustau *et al.* 2006). *B. dothidea*, for example, not only threatens apple and pistachio production in China and the USA, but it also causes disease on native *Ostrya carpinifolia* in Europe and *Acacia* in Southern Africa, amongst many other hosts around the world (Marsberg *et al.* 2017).

For some recently described species, the distribution appears to be limited to a single region or country. For example, the soil- and seed-borne polyphagous pathogen *Macrophomina pseudophaseolina*, which causes charcoal rot of *Abelmoschus esculentus, Arachis hypogaea, Hibiscus sabdarifa*, and *Vigna unguiculata*, is thus far only known to occur in Senegal (Sarr *et al.* 2014). Likewise, white grain disorder of wheat is caused by three different species of *Eutiarosporella*, but these have thus far been recorded only from Australia (Thynne *et al.* 2015), and not from other countries where wheat is cultivated.

Globally distributed species reflect a near total failure of quarantine systems to recognize or deal with the *Botryosphaeriales*. This is especially because the intercontinental spread of these fungi can be explained only through human movement of plant material. The *Botryosphaeriales* have sticky spores and are thought to be predominantly dispersed with wind and rain, and to a lesser extent via insects (Van Niekerk *et al.* 2010; Mehl *et al.* 2013; Moyo *et al.* 2014; Valencia *et al.* 2015). Consequently natural spread is expected to be relatively local. The *Botryosphaeriaceae* are, however, very common endophytes in various plant parts, including fruits, leaves, twigs and stems (Slippers & Wingfield 2007). The extensive movement of living plants and fresh plant products around the world is thus likely to have contributed substantially to the global distribution patterns of these fungi (Crous *et al.* 2016b; Burgess *et al.* 2017). Consider for example the trade in fruits such as mangoes or apples. Healthy fruits are very commonly

infected by a variety of *Botryosphaeriaceae* (Slippers *et al.* 2007). Once these start to rot, fruit are likely to be discarded. This would provide ample opportunity for these pathogens to spread to nearby woody plants and they would be able to successfully infect them due to their broad host ranges.

One of the best-studied cases of the global distribution of a *Botryosphaeriaceae* species is that of *Diplodia sapinea*. This pine-infecting species occurs in all areas of the world where *Pinus* spp. are planted as non-natives. In most of these populations the genetic diversity is very high (Burgess & Wingfield 2002), indicating extensive introduction from the native range. Bihon *et al.* (2011) have shown that this is unlikely to occur via seed, and has arisen more probably with the trade of living plants or plant tissues. This appears also to be a common pattern in other *Botryosphaeriaceae*.

One of the most dramatic surprises in terms of global distribution and impact of *Botryosphaeriaceae* species to emerge in the past decade is that concerning *Neofusicoccum parvum*. Studies have revealed an unexpectedly broad distribution and common occurrence of this pathogen on a wide range of woody plants. Interestingly, *N. parvum* was first described in the mid-1980's from New Zealand where it was found infecting kiwi fruit. But, since 2004 it has been shown to occur in many countries of the world and on at least 90 hosts (Sakalidis *et al.* 2013). Mehl *et al.* (2017) showed that *N. parvum* is one of the most common species infecting native and non-native *Anacardiaceae* in South Africa, namely Marula (*Sclerocarya birrea* subsp. *caffra*) and mango trees (*Mangifera indica*). It is also emerging as one of the most prominent pathogens in agriculture, including on *M. indica* (Trakunyingcharoen *et al.* 2014), *Prunus cerasoides* (Trakunyingcharoen *et al.* 2015), and *Vitis vinifera* (Van Niekerk *et al.* 2004; Úrbez-Torres & Gubler 2009; Massonnet *et al.* 2017).

The study by Mehl *et al.* (2017) in this issue highlights a pattern that is becoming increasingly obvious for some species of *Botryosphaeriaceae*. This is that, once introduced into a region, a *Botryosphaeriaceae* species will commonly spread between native and non-native plants and it can cause disease problems in both of these situations. This is particularly true with regards to broadly distributed and broad host range species (e.g. *N. parvum*) in disturbed environments (Pavlic-Zupanc *et al.* 2015; Zlatković *et al.* 2016a). Urban, forestry and agricultural landscapes appear to provide 'bridges' for these fungi to move to and from native ecosystems.

Not all species of the *Botryosphaeriales* have broad host and geographic distributions. Studies on a particular host across a wide geographic range, such as *Acacia* species across South Africa and Namibia (Slippers *et al.* 2014; Jami *et al.* 2015) and mangrove (Osorio *et al.* 2017) have shown that many species occur in rather limited areas. These findings, together with numerous other recent studies reporting new species from one or two locations, suggest that geography contributes significantly to species diversity in the order. This is not unexpected for fungi having a limited capacity to disperse over large areas.

Very few species of the *Botryosphaeriales* appear to be host specific. Where only one or two hosts are known, this commonly appears to be linked to small numbers of isolates (rare species) and possibly arises from limited sampling (Jami *et al.* 2014; Slippers *et al.* 2014). Most tree hosts appear to harbour many species of *Botryosphaeriales*. In fact, many intensive studies have revealed 16 or more species on a single host (Slippers *et al.* 2014; Jami *et al.* 2015; Osorio *et al.* 2017). These findings suggest that further geographic sampling, rather than a greater number of hosts, is likely to reveal more diversity in the group.

In recent years there have been a number of disease problems reported in plant communities across large areas linked to *Botryosphaeriales*. These have commonly also been linked to climate change events or cases of human disturbance. Notable examples are those for *Agonis flexuosa* in Western Australia (Dakin *et al.* 2010), *O. carpinifolia* in Slovenia and Italy (Piskur *et al.* 2011), *Acacia* species, Mangrove species (*Avicennia marina*, *Bruguiera gymnorrhiza*, *Ceriops tagal*, *Lumnitzera racemosa*, *Rhizophora mucronata*, and *Xylocarpus granatum*) and *M. indica* trees in Southern Africa (Jami *et al.* 2013; Slippers *et al.* 2014; Mehl *et al.* 2017; Osorio *et al.* 2017), *Schizolobium parahyba* in South America (Mehl *et al.* 2014), various ornamental trees across the Balkans (Zlatković *et al.* 2016b), *Anacardium occidentale* in Brazil (Netto *et al.* 2017), *Eriobotrya japonica* in Italy (Giambra *et al.* 2016), amongst others. Whether this trend is increasing is not clear. But it is certainly very prominent where such die-back and decline occurs and it will be necessary to monitor this situation carefully over time.

While patterns of host association and distribution are becoming increasingly better understood, the consequences of changes in these patterns, the spread of certain species and novel plant–fungal interactions are very poorly understood for the *Botryosphaeriales*. The pathogenicity of the vast majority of the species on most of the hosts on which they occur is unknown. How these communities change over time, and in response to various human-mediated environmental changes, is equally poorly understood (Jami *et al.* 2015). Given the broad distribution, common occurrence, and rapid global changes due to land use and climate change, these questions (amongst other raised here) are becoming increasingly urgent to be answered.

The future with (meta-)genomes and (meta-) transcriptomes

Genomics and transcriptomics are set to completely revolutionize our understanding of the diversity of the *Botryosphaeriales*. Even the initial analyses of the first *Botryosphaeriaceae* genomes have provided unprecedented insight into the biology of the group. To date genomes of 13 species residing in four families in the *Botryosphaeriales* have been sequenced. These are *Aplosporella prunicola*, *Botryosphaeria dothidea*, *Diplodia sapinea*, *Diplodia corticola*, *Diplodia scrobiculata*, *Diplodia seriata*, *Macrophomina phaseolina*, *Neofusicoccum parvum*, *Phyllosticta citriasiana, Phyllosticta citribraziliensis, Phyllosticta citricarpa, Phyllosticta capitalensis*, and *Saccharata proteae* (Table 3). Not all these genomes have been analysed, but where they have been studied, they illustrate a group of fungi uniquely interacting with their hosts (Marsberg *et al.* 2017). Their unique biology with prolonged endophytic life cycles and in which they apparently do not damage host cells and do not trigger a host response during infection is particularly intriguing. They can apparently deceive their hosts into allowing them

Table 3 – Available genomes of Botryosphaeriales.

Species	JGI website or GenBank ID	Associated publication	Size (Mb)	Gene count
Aplosporella prunicola	http://genome.jgi.doe.gov/Aplpr1/Aplpr1.home.html	Part of the 1000 Fungal Genomes Project.	32.8	12 579
Botryosphaeria dothidea	788841	Liu *et al.* (2016)	23.76	
Botryosphaeria dothidea	http://genome.jgi.doe.gov/Botdo1_1/Botdo1_1.home.html	Marsberg *et al.* (2017)	43.5	14 998
Diplodia corticola	50478	Fernandez *et al.* (2014)	34.98	10 839
Diplodia sapinea	31839	Bihon *et al.* (2014), Van der Nest *et al.* (2014)	35.6	
Diplodia scrobiculata	41697	Wingfield *et al.* (2015)	34.9	
Diplodia seriata	http://genome.jgi.doe.gov/Dipse1/Dipse1.home.html GenBank 38175	Morales-Cruz *et al.* (2015)	37.1	9343
Macrophomina phaseolina	12172	Islam *et al.* (2012)	48.8	13 806
Neofusicoccum parvum	16686	Blanco-Ulate *et al.* (2013)	42.6	10 366
Neofusicoccum parvum	UCD646So	Massonnet *et al.* (2017)	43.7	13 124
Phyllosticta citriasiana	http://genome.jgi.doe.gov/Phycit1/Phycit1.home.html	Part of the 1000 Fungal Genomes Project.	32.7	11 368
Phyllosticta citribraziliensis	http://genome.jgi.doe.gov/Phcit1/Phcit1.home.html	Part of the 1000 Fungal Genomes Project.	31.67	11 101
Phyllosticta citricarpa	713091	Wang *et al.* (2016)	31.13	5748
Phyllosticta capitalensis	http://genome.jgi.doe.gov/PhycapStandDraft_FD/PhycapStandDraft_FD.info.html	Part of the 1000 Fungal Genomes Project.	32.45	
Phyllosticta capitalensis strain Gm33	713081	Wang *et al.* (2016)		
Saccharata proteae	http://genome.jgi.doe.gov/Sacpr1/Sacpr1.home.html	Part of the 1000 Fungal Genomes Project.	31.4	9324

to infect, but later to become necrotrophic pathogens when the infected plants are subjected to stress, earning them the title of 'stealth pathogens' (Marsberg *et al.* 2017). Yet, the genomes of those species that have been analysed clearly show that they have a well developed arsenal of potential pathogenicity genes and virulence factors with which to overcome host defences and cause serious disease (Islam *et al.* 2012; Blanco-Ulate *et al.* 2013; Morales-Cruz *et al.* 2015; Marsberg *et al.* 2017; Massonnet *et al.* 2017). Studying the regulation of these genes during the infection and disease causing processes through transcriptomics and metatranscriptomics holds great potential, not only to shed light on this group of fungi, but also on the poorly understood endophytic fungal communities of plants in general.

Genomes will provide a rich source of markers for population and phylogenetic studies in the future. Earlier in this review we reflected on the rising recognition of cryptic species, hybrids and the movement of pathogens between hosts, environments and continents. Further studies on these topics are limited by available population markers. Every published genome has thousands of microsatellite markers from which to select. They also offer the opportunity to compare cryptic species and hybrids across whole genomes. Surprisingly, none of these approaches have been applied, but this situation is expected to change rapidly in coming years as more whole genomes of closely related cryptic species become available.

One of the first aspects of the biology of the *Botryosphaeriales* that genomes have revealed is their mating strategies. It is well known that fungi in this order have a sexual cycle, but this morph is seldom observed or artificially induced in the laboratory. Its influence on population diversity is largely unknown. Population genetic studies have occasionally suggested that this has a significant influence on populations, even in species such as *D. sapinea* where no sexual cycle is known (Bihon *et al.* 2012). And indeed the first genome analyses of this species, and subsequent population studies using markers from the genome analyses, revealed an apparently fully functional heterothallic reproductive system (Bihon *et al.* 2014; van der Nest *et al.* 2014). In contrast, *B. dothidea*, for which a sexual cycle is known, appears to be homothallic (Marsberg *et al.* 2017).

Lopes *et al.* (2017) illustrated the value of genomes for studying both phylogenetics and biology in the group. These authors characterized the mating type genes of a number of *Neofusicoccum* species, using markers designed from publicly available genomes of *N. parvum* and *D. sapinea*. They have shown that homothallism is the predominant mode of sexual reproduction in *Neofusicoccum*. Furthermore, these markers were shown to be useful to distinguish cryptic species in this genus. Similar studies on other genera, and using other markers from these genomes, are likely to proliferate in coming years.

Next generation sequencing is not only making it possible to sequence genomes, but also communities through metabarcoding approaches. Here too, our understanding of the *Botryosphaeriales* is set to change dramatically. For example, Kemler *et al.* (2013) used a metabarcoding approach to characterize the endophytic community of a single *Eucalyptus* tree, and showed that the *Botryosphaeriaceae* make up a dominant

portion; more than 30 % of the identified taxonomic units. While much work remains to be done to understand what this diversity represents, it is clear from the study of Kemler et al. (2013) that metabarcoding holds the key to our understanding of the phylogeography, host association and other ecologically important questions about the *Botryosphaeriales*.

Conclusions

Advances in DNA sequencing used for phylogenetics have helped determine a more natural classification of the *Botryosphaeriales* over the past decade. They have also raised serious doubts regarding past descriptions of taxa. Currently, decisions regarding new species are mostly made based on sequence data, and these can hardly be reliably linked to type material of 'old' species. The increasing recognition of cryptic species, hybrids and morphological variants of the same species make any reliance on other systems highly uncertain. Apart from requiring some molecular data to be linked to species descriptions, the use of multiple gene regions and preferably more than two genes become essential.

The time is overdue to move to a more sensible, sequence-based system to catalogue and describe new species in the *Botryosphaeriales* as well as in other fungi. Reliance on morphological descriptions alone that have little meaning in terms of identification or anchoring of a name in most cases reflect an unfortunate and unnecessary waste of time. This is particularly true in light of the potential threat that these fungi pose as invaders and pathogens of growing importance. This is also important against a background of the expected rise in taxon discovery as the power and utility of metabarcoding increases. Such a system will not only speed up discovery and description, but also make it accessible to a larger number of scientists and practitioners that can contribute to the understanding of distribution, host range and pathways of spread of these pathogens. It will also provide validated information allowing quarantine systems to consider management options for the apparently regular and continued spread of these fungi internationally.

One of the important remaining questions regarding the majority of *Botryosphaeriales* species concerns their biology. We are just beginning to understand fundamental issues regarding these fungi, such as whether species are homothallic or heterothallic, and whether they are undergoing sexual reproduction. Furthermore, virtually all species that have been studied in detail can exist as endophytes in healthy tissue for extended periods. Yet they have the ability to cause disease when inoculated and they are often associated with serious disease problems. How these species colonize their hosts, and in some cases hundreds of different and unrelated host species, without causing disease is unknown. It is also unknown what their roles are in broader ecosystems. Answers to these questions are important especially because they will impact strongly on issues relating to invasion biology and disease control it the future.

It is evident from a growing number of recent studies that species in the *Botryosphaeriales* are being moved around the world relatively freely. This spread has most likely been in healthy plant material, including fruits, and the pathogens would have been virtually impossible to detect in their endophytic state (Burgess et al. 2017). Even where symptoms are visible, quarantine can no longer rely on morphological identifications and old names for this group of fungi (Crous et al. 2016b). The only conceivable means to understand the magnitude and pathways of this spread will be through the use of direct sequencing, metabarcoding approaches. As our understanding of the biology of these organisms increases and as we come to understand the molecular mechanisms that underpin the many processes involved, we foresee that genomics and metagenomics (ie. considering the whole genome, and not only barcoding regions) will become the primary basis of quarantine and other management decisions (McTaggart et al. 2016). Clearly there is much work still to be done on this group before this will be a reality. Fortunately we now have a robust natural classification system for the *Botryosphaeriales* and many of the tools needed to understand and manage these fungi.

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