



A case for re-inventory of Australia's plant pathogens

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Key words

disease associated fungi
disease threats
molecular phylogeny
quarantine
taxonomy

Abstract Australia has efficient and visible plant quarantine measures, which through various border controls and survey activities attempt to prevent the entry of unwanted pests and diseases. The ability to successfully perform this task relies heavily on determining what pathogens are present and established in Australia as well as those pathogens that are exotic and threatening. There are detailed checklists and databases of fungal plant pathogens in Australia, compiled, in part, from surveys over many years sponsored by Federal and State programmes. These checklists and databases are mostly specimen-based, which enables validation of records with reference herbarium specimens and sometimes associated cultures. Most of the identifications have been based on morphological examination. The use of molecular methods, particularly the analysis of DNA sequence data, has recently shown that several well-known and important plant pathogenic species are actually complexes of cryptic species. We provide examples of this in the important plant pathogenic genera *Botryosphaeria* and its anamorphs, *Colletotrichum*, *Fusarium*, *Phomopsis* / *Diaporthe* and *Mycosphaerella* and its anamorphs. The discovery of these cryptic species indicates that many of the fungal names in checklists need scrutiny. It is difficult, and often impossible, to extract DNA for sequence analysis from herbarium specimens in order to validate identifications that may now be considered suspect. This validation can only be done if specimens are recollected, re-isolated and subjected to DNA analysis. Where possible, herbarium specimens as well as living cultures are needed to support records. Accurate knowledge of the plant pathogens within Australia's borders is an essential prerequisite for the effective discharge of plant quarantine activities that will prevent or delay the arrival of unwanted plant pathogens.

Article info Received: 1 November 2010; Accepted: 14 November 2010; Published: 1 December 2010.

INTRODUCTION

Most records of plant pathogenic fungi in Australia are derived from, and substantiated by, dried herbarium specimens and relatively few are based on living cultures. There are several reasons for this including, i) many groups of pathogens are obligate and cannot be cultured; ii) many pathogens can be identified with confidence *in situ* based on morphology and thus cultures are not necessary for diagnosis; iii) living cultures are often difficult to preserve, especially over time and may lose their ability to sporulate or retain pathogenicity or other physiological properties; iv) maintaining living cultures is relatively costly; and v) isolation of a presumptive causal organism does not demonstrate pathogenicity unless Koch's postulates are fulfilled. As an example, consider that most plant disease surveys in northern Australia (Hyde & Alcorn 1993, Shivas 1995, Shivas & Alcorn 1996), and neighbouring countries of Papua New Guinea (Hyde & Philemon 1994) and Irian Jaya (Shivas et al. 1996), often as part of the Northern Australian

Quarantine Strategy (NAQS), were based almost entirely on herbarium specimens. The NAQS surveys often focused on remote parts of these regions, looking for early signs of pest and disease incursions. The remoteness of many locations meant that facilities were not available to obtain and look after cultures. Furthermore, the scientists themselves were often not allowed to move specimens that might harbour living and exotic pathogens, particularly if the specimens were collected offshore.

The importance of maintaining accurate records of pathogens in Australia is their value in determining those pathogens that are not present (exotic) and potentially a threat if introduced, through a process known as pest risk assessment (Plant Health Australia 2010). The aim of quarantine in Australia is to prevent entry of exotic pests, diseases and weeds that could have serious environmental and economic consequences if introduced. For example, the cost of introducing some exotic plant diseases, e.g. Karnal bunt of wheat, into Australia could cost billions of dollars (<http://www.dfat.gov.au/facts/quarantine.html>). Several island countries, including Australia, New Zealand, and the Philippines, through their isolation have avoided the introduction of many important exotic threats.

Specimen-based records of most of the plant pathogens that occur in Australia can be accessed through the Australian Plant Disease Database and the Australian Plant Pest Database (Shivas et al. 2006). Both of these important databases are username and password protected which limits their availability. These databases, together with the three large Australian herbaria of plant pathogens that underpin them, are important resources for resolving quarantine issues and facilitating the pest risk assessment process.

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Prior to the availability of DNA sequence data, the identification of plant pathogenic fungi was primarily based on morphology, with herbarium specimens serving as proof of identity for future reference. The relatively recent application of molecular phylogenetic analysis to species identification has revealed that many traditionally accepted species actually represent complexes of species. This is especially true for many important plant pathogenic genera such as anamorphic *Mycosphaerellaceae* (including *Cladosporium*), *Botryosphaeria* and its anamorphs, *Colletotrichum*, *Fusarium*, *Guignardia* with its *Phyllosticta* anamorphs, and *Diaporthe* with its *Phomopsis* anamorphs (Crous et al. 2006, 2009a–c, Damm et al. 2007, Alves et al. 2008, Cai et al. 2009, Hyde et al. 2009a, b, Kvas et al. 2009, Phillips et al. 2008, Shivas & Tan 2009, Wulandari et al. 2009, Zhang et al. 2009, Schoch et al. 2009, Phoulivong et al. 2010, Summerell et al. 2010, Walsh et al. 2010). As a result of these recent advances many of the taxa listed in the aforementioned Australian checklists and databases of fungi associated with plant diseases are now outdated. There is a pressing need to address this problem. The purpose of this paper is to provide selected examples where there is a need to carry out a re-inventory of the fungal pathogens of plants in Australia so that the checklist and databases are both accurate and up to date.

MATERIALS AND METHODS

Selected sequences from five plant pathogenic fungal genera were downloaded from GenBank and aligned using either Clustal X or the online MAFFT server (<http://mafft.cbrc.jp/alignment/server/index.html>). The alignments were optimised manually to allow maximum alignment and maximum sequence similarity. Gaps were treated as missing data or fifth character states (*Mycosphaerella* and *Phomopsis* alignments). Phylogenetic analyses were carried out based on the aligned dataset using PAUP v4.0b10 (Swofford 2003). Ambiguously aligned regions were excluded from all analyses, where present. Trees were inferred using the heuristic search option with TBR branch swapping and 100–1 000 random sequence additions. Max-trees were unlimited, branches of zero length were collapsed, and all multiple parsimonious trees were saved. Trees were drawn in TreeView (Page 1996).

RESULTS AND DISCUSSION

Botryosphaeriaceae and its anamorphs

Several *Botryosphaeria* species and their anamorphs, such as *Lasiodiplodia theobromae*, were identified during NAQS quarantine surveys of plant disease associated fungi of northern Australia (Hyde & Alcorn 1993, Shivas 1995, Shivas & Alcorn 1996). However, several recent studies have shown that species of *Botryosphaeria* such as *B. dothidea* and anamorph taxa such as *Lasiodiplodia theobromae* are species complexes (Alves et al. 2008, Phillips et al. 2008, Abdollahzadeh et al. 2010). *Botryosphaeria dothidea* was epitypified by Slippers et al. (2004) with a specimen from *Prunus* sp. collected on the Italy-Switzerland border. The taxon has proved to be a complex comprising several species (Smith et al. 2001, Denman et al. 2003, Slippers et al. 2004). Several other *Botryosphaeria* species and related anamorphs have also been epitypified (see Fig. 1 in black bold) and this has led to advances in understanding the genus (Crous et al. 2006, Alves et al. 2008, Phillips et al. 2008). By epitypifying these taxa with living cultures it is now possible to compare recent collections with that of the type to establish whether they are the same species. This is necessary for all diseases for which *Botryosphaeria* and its anamorphs are linked to establish accurate disease records.

In Fig. 1 we present a phylogramme comprising 59 ITS sequences downloaded from GenBank including 17 named as *Botryosphaeria dothidea* with its epitype sequence highlighted in red. Sequences from ex-type strains of *Neofusicoccum australe* (= *B. australis*; Crous et al. 2006), *N. luteum* (= *B. lutea*), *Diplodia seriata* (= *B. obtusa*; Phillips et al. 2007), *N. parvum* (= *B. parva*), *N. ribis* (= *B. ribis*), *D. mutila* (= *B. stevensii*), '*Botryosphaeria*' *tsugae*, *Lasiodiplodia crassispora*, *L. gonubensis* and *Phaeobotryosphaeria porosa* (= *Diplodia porosa*; Phillips et al. 2008) are also included. Although the majority of *Botryosphaeria dothidea* strains cluster around the type sequence in the upper part of the tree, there are five disparate strains scattered in the lower part of the tree. This suggests that there are likely to be many sequences for *B. dothidea* in GenBank with wrongly applied names. We do not show the data here but there are similar situations for *Botryosphaeria rhodina* and *Lasiodiplodia theobromae*. These taxa have not been epitypified and at present we do not know which strain in GenBank (if any) represents *B. rhodina* or *L. theobromae*, although this situation is likely to be solved in a future publication (A.J.L. Phillips pers. comm.). Besides the problem with wrongly applied names in GenBank, very few of the GenBank sequences are based on Australian specimens and therefore they need to be recollected, sequenced and checked against verified or typified names. Specific examples of erroneous names concern the species of *Botryosphaeria* that cause disease of palms. Recent studies in Thailand have shown some species belong to *Neodeightonia*, a genus that is quite similar to *Botryosphaeria* (Phillips et al. 2008, Liu et al. in press). Therefore it is essential that every *Botryosphaeria*-like species associated with disease of palms should be re-assessed and this should also be the case with other hosts.

Colletotrichum

The anamorphic ascomycete genus *Colletotrichum* contains many well-known plant pathogens that cause a range of diseases worldwide (Crouch & Beirn 2009, Crouch et al. 2009, Damm et al. 2009, Hyde et al. 2009a, b). MycoBank currently contains 676 records of *Colletotrichum* (www.mycobank.org), while only 66 species names are in current use (Hyde et al. 2009a). Cai et al. (2009) outlined a polyphasic approach for studying *Colletotrichum* and provided a backbone tree comprising 42 ex-type ITS sequences. Many ubiquitous *Colletotrichum* species have now been shown to be species complexes containing numerous cryptic species. For example, *Colletotrichum acutatum* s.lat. has been shown to contain discrete morphological and molecular groups that represent discrete species as found in some Australian isolates (Shivas & Tan 2009).

Perhaps the most commonly known species in the genus is *Colletotrichum gloeosporioides* (teleomorph *Glomerella cingulata*), which is represented in Australian collections by more than 5 000 specimens from several hundred host plant species in about 100 different plant families. Very little is known about whether these fungal records represent saprobes, weak or opportunistic pathogens, or genuine pathogens. It is possible that many of these records are misidentified as the morphological characteristics that define *C. gloeosporioides* are unreliable or even misleading, i.e. having cylindrical conidia with rounded ends, and less than 4.5 µm wide according to the widely used key by Sutton (1980). Cannon et al. (2008) epitypified *C. gloeosporioides*; conidia of the ex-epitype strain are on average wider, measuring 14.4 × 5.6 µm. The species had been synonymised by von Arx (1957) with about 600 names, some of which might represent discrete species.

Because of the morphological similarities between *C. gloeosporioides* and other *Colletotrichum* species, the close relationship between species within the *C. gloeosporioides* species complex

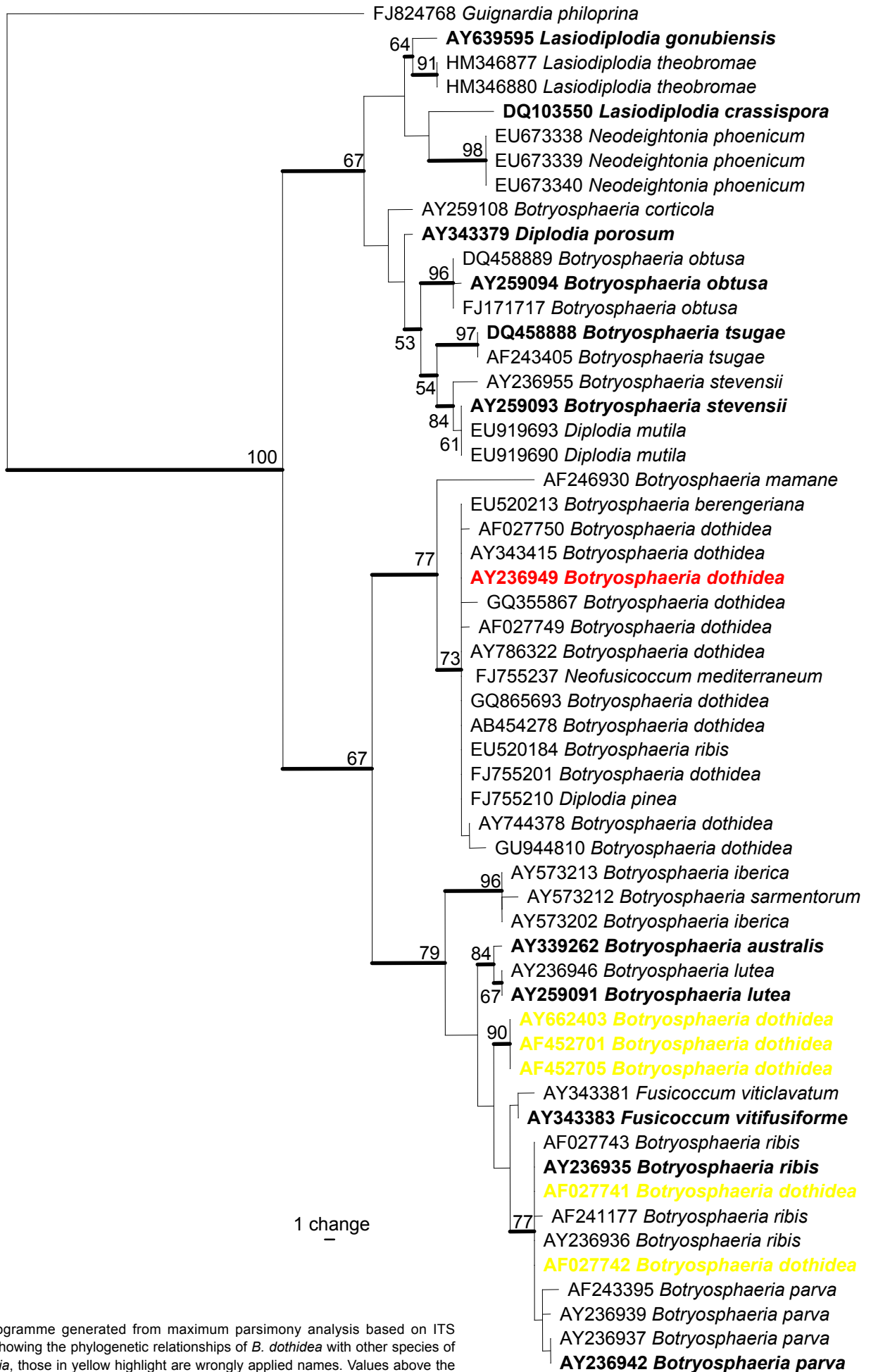


Fig. 1 Phylogramme generated from maximum parsimony analysis based on ITS sequences, showing the phylogenetic relationships of *B. dothidea* with other species of *Botryosphaeria*, those in yellow highlight are wrongly applied names. Values above the branches are parsimony bootstrap (> 50 %). Ex-type strains are shown in bold.

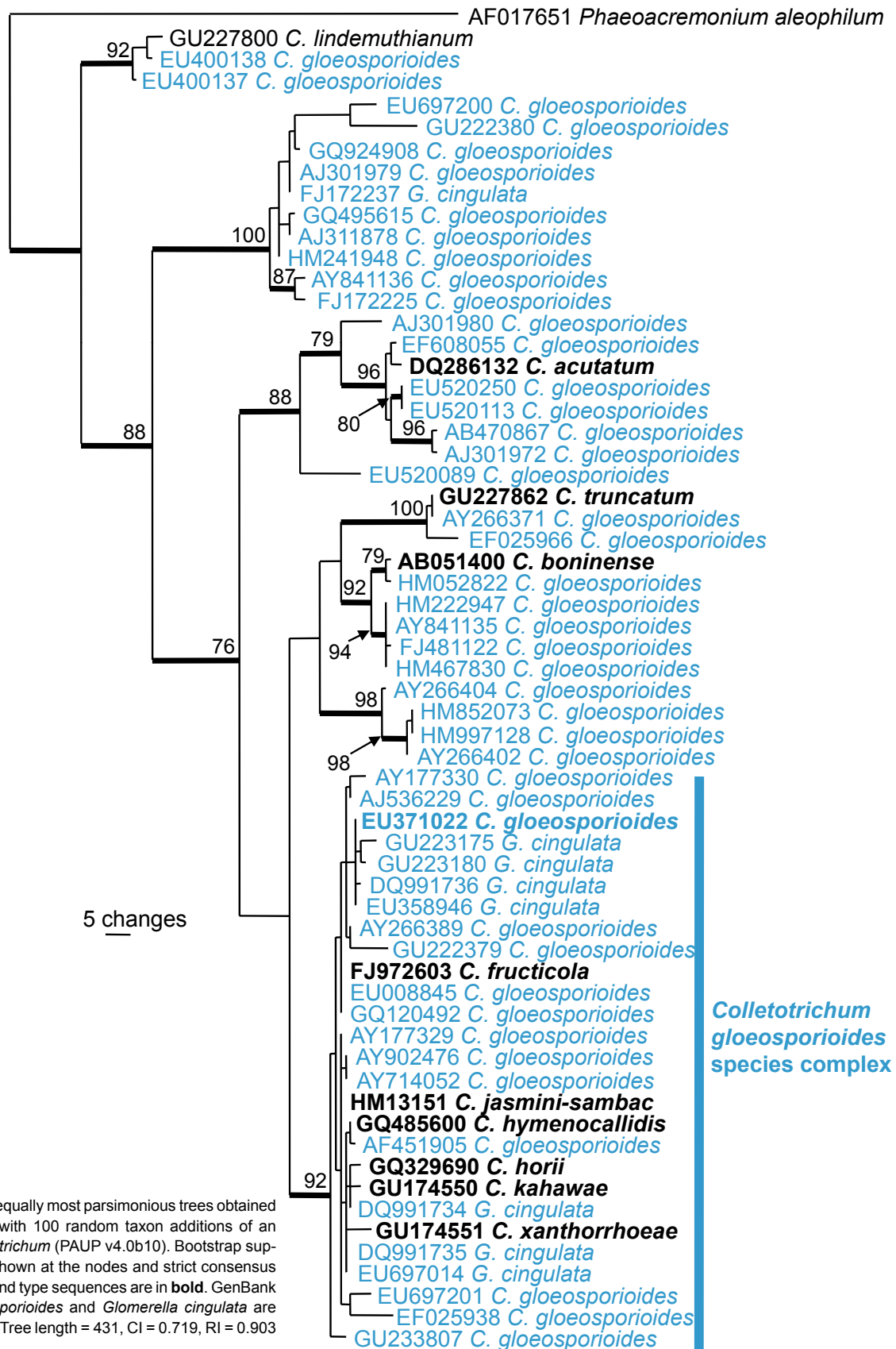


Fig. 2 The first of 8 900 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of an ITS alignment for *Colletotrichum* (PAUP v4.0b10). Bootstrap support values > 69 % are shown at the nodes and strict consensus branches are thickened and type sequences are in bold. GenBank accessions of *C. gloeosporioides* and *Glomerella cingulata* are indicated in blue colours. Tree length = 431, CI = 0.719, RI = 0.903 and RC = 0.649.

and the only recent epitypification, most of the sequences lodged in GenBank as *C. gloeosporioides* are doubtful and belong to many different species. As demonstrated in Fig. 2, ITS sequences lodged in GenBank as *C. gloeosporioides* and *G. cingulata* are found throughout the *C. gloeosporioides* species complex and outside the *C. gloeosporioides* complex, the latter applies to more than 100 of the about 750 ITS sequences

of *C. gloeosporioides* in GenBank. Furthermore, Fig. 2 shows the difficulty of species recognition within the *C. gloeosporioides* species complex using ITS sequences only. Only a few of these GenBank sequences were derived from specimens from Australia.

The *C. gloeosporioides* aggregate is currently the subject of intensive phylogenetic analysis and Hyde et al. (2009a) noted

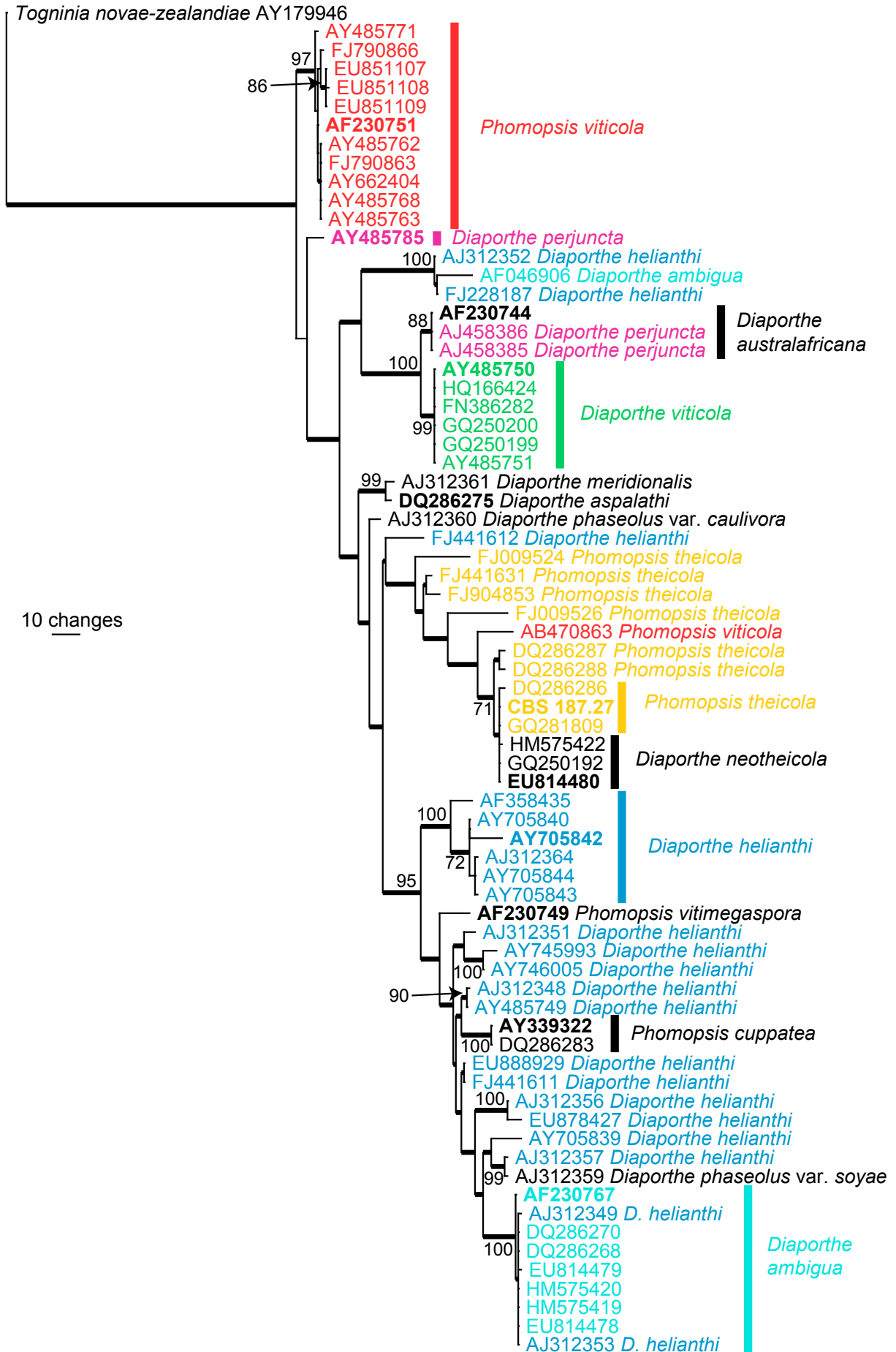


Fig. 3 The first of 319 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of an ITS alignment for *Phomopsis* (PAUP v4.0b10). Bootstrap support values > 69 % are shown at the nodes and strict consensus branches are thickened and type sequences are in **bold**. Names of paraphyletic species are indicated in different colours and clades containing the type sequences of these paraphyletic species are indicated with a coloured bar corresponding to the colour of the species name. Tree length = 568, CI = 0.583, RI = 0.876 and RC = 0.511.

that it was likely a series of well-supported monophyletic (though not host-specific) clades may be identified. This research has resulted in several publications revealing, describing or typifying species within the *C. gloeosporioides* species complex (Shivas & Tan 2009, Yang et al. 2009, Phoulivong et al. 2010, Rojas et al. 2010, Weir & Johnston 2010). The taxon was previously considered as a pathogen of many tropical fruits, causing anthracnose (Holliday 1980). Phoulivong et al. (2010) isolated *Colletotrichum* species from anthracnose symptoms of eight tropical fruits in Laos and Thailand and none of these isolates was *C. gloeosporioides*. This illustrates the need to re-investigate the *Colletotrichum* species in Australia using molecular data to establish which species occur in this country.

Diaporthe/Phomopsis

The anamorphic ascomycete genus *Phomopsis* contains about 1 000 species names (Uecker 1988) with teleomorphic connections in *Diaporthe* for about 180 species (van der Aa & Vanev 2002). *Phomopsis* spp. are widespread and occur on a diversity of host plants as pathogens, endophytes and saprobes (Uecker 1988). Plant pathogenic species cause serious diseases of many cultivated plants worldwide, including grapevines (van Niekerk et al. 2005), sunflower (Gulya et al. 1997), strawberry (Maas 1998), and soybean (Li et al. 2010). Interest in *Phomopsis* has also focused on the secondary metabolites produced by some endophytic and saprobic forms. Two examples are given. Firstly, *Diaporthe toxica* is known to produce toxic metabolites, phomopsins, on infected *Lupinus* stubble or seed, which can result in the death of grazing animals (Peterson et al. 1987, Cowley et al. 2010). Secondly, strains of endophytic *Phomopsis* from healthy plants have been shown to produce taxol, which has strong cytotoxicity towards human cancer cells (Kumaran & Hur 2009).

Species delimitation in *Phomopsis* has been traditionally based on host association as morphological characters are few and not reliable, for example, most species do not produce β -conidia or the teleomorph in culture (Rehner & Uecker 1994). Increasingly molecular phylogenies, especially those derived from the sequences of the internal transcribed spacer (ITS) region of the ribosomal DNA have been used to identify species (Mostert et al. 2001, van Niekerk et al. 2005, van Rensburg et al. 2006, Santos & Phillips 2009, Ash et al. 2010). Fig. 3 shows an ITS phylogeny consisting of 72 sequences (including the outgroup sequence) obtained from NCBI's GenBank nucleotide database. A total of 489 characters were used in the analysis, of which 134 characters were parsimony informative, 267 were constant and 88 variable characters were parsimony uninformative. The tree clearly illustrates the confusion around the application of species names such as *Diaporthe ambigua* and *D. helianthi*.

In Australia, specimens of *Phomopsis* deposited in the major plant pathology herbaria are mostly not identified to species level. The reason for this is that the species concept in *Phomopsis* needs modernisation, particularly in light of additional biological, biochemical and molecular data (van der Aa & Vanev 2002). Discarding the host-based species concept is the first step in the development of a useful and reliable classification for *Phomopsis*. This needs to be followed by a major international collaborative effort, as is happening in *Botryosphaeria*, *Colletotrichum* and *Fusarium*, to develop a reliable taxonomy.

Fusarium

Fusarium contains some of the most damaging plant pathogenic fungi as well as species that are important toxin producers and human pathogens (Desjardin 2006, Leslie & Summerell 2006). It is also one of the most actively researched groups of fungi and consequently the taxonomic concepts of many of the key

plant pathogens have changed dramatically over the past two decades (Leslie & Summerell 2006). Added to this there have been many new species of *Fusarium* described from a diversity of environments and host substrates, including a number from Australia, e.g. *F. aywerte* and *F. nurragi* (Benyon et al. 2000), *F. babinda* (Summerell et al. 1995), *F. beomiforme* (Nelson et al. 1987), *F. gaditjirri* (Phan et al. 2004), *F. lyarnte* and *F. werrikimbe* (Walsh et al. 2010). Like the other genera described above, *Fusarium* has been shown to be rich in cryptic species, and while some may argue that cryptic speciation is of interest only to taxonomists and evolutionary biologists in *Fusarium* these species have clearly been demonstrated to have critical importance to plant pathologists, plant breeders and quarantine officials.

Several species provide good examples of the problems in *Fusarium*. Stalk rot of maize is caused by *F. verticillioides* but much of the literature and most of the specimens in Australian collections will use the name *F. moniliforme*. This latter name we now know refers to at least three species currently described, namely *F. verticillioides*, *F. thapsinum* and *F. andiyazi* (Seifert et al. 2003, Leslie et al. 2005) with a number of undescribed taxa known; all are morphologically identical but differ biologically, ecologically and phylogenetically. We now know that *F. verticillioides* causes stalk rot of maize and produces the mycotoxin fumonisin, *F. thapsinum* causes stalk rot of sorghum and does not produce fumonisin, but does produce other less important toxins and *F. andiyazi* causes some disease in sorghum (Leslie et al. 2005). Clearly in this case accurate identification is critical because of the implications to crop and human health. An even more complex situation occurs within a large group of species previously described as *F. subglutinans*. Fig. 4 shows a phylogenetic tree comprised of 72 translation elongation factor sequences sourced from GenBank of species either named as *F. subglutinans* or previously called *F. subglutinans* and those species closely related in the *Gibberella fujikuroi* clade. This tree highlights the diversity that exists within this species concept and how names are wrongly applied to data present in GenBank, and also demonstrates the confusion that surrounds a number of the species complexes within *Fusarium*, especially when using data sourced from databases such as GenBank. Detailed phylogeny-based investigations of the previously broad species concept that applied to *F. subglutinans* have shown it to include more than 20 species (Steenkamp et al. 2002, Leslie & Summerell 2006) of some extremely important plant pathogens including *F. sacchari*, *F. circinatum* and *F. mangiferae*; the latter two being of high importance to Australia as pathogens of quarantine importance. Several other species such as *F. oxysporum* (Wang et al. 2004), *F. solani* (O'Donnell 2000), *F. dimerum* (Schroers et al. 2009) and *F. graminearum* (O'Donnell et al. 2004) have been shown to be quite diverse species complexes and the impact of these studies on the identity of cultures in the collections still awaits to be explored.

Unfortunately, none of the major plant pathogen reference collections or major research collections of *Fusarium* has completed a full analysis of their holdings and as such urgently need review to determine the status of the isolates held. The preliminary analyses that have been done using DNA based techniques have shown that there is considerable diversity held in these collections that are not reflected in the lists of species present in Australia and as such it is difficult to determine the quarantine status of many species of *Fusarium*. A checklist including plant pathogenic and quarantine status of *Fusarium* in Australia has been prepared (Summerell et al. in press) and this will provide a basis on which to analyse the species found in collections in Australia.

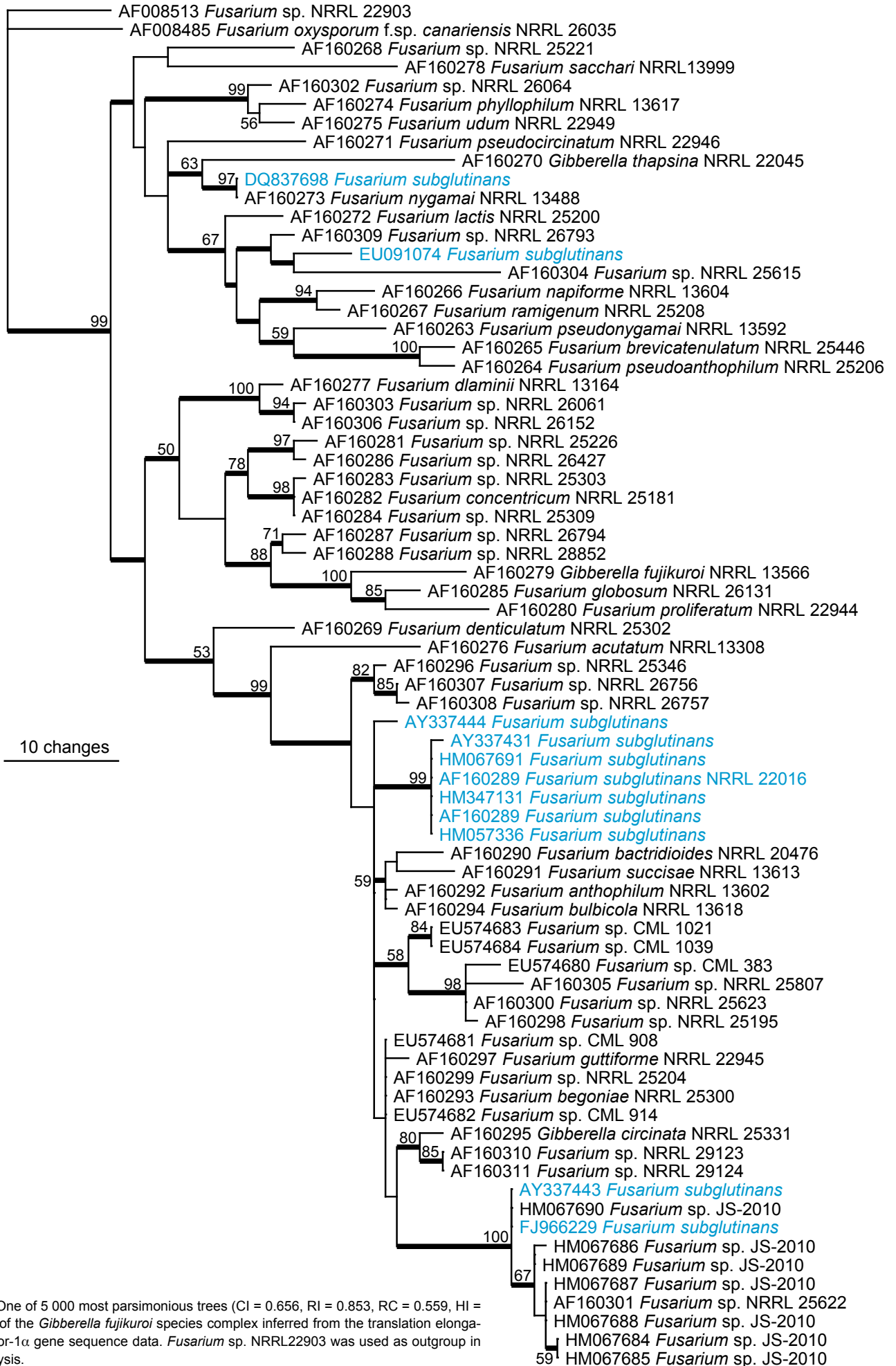


Fig. 4 One of 5 000 most parsimonious trees (CI = 0.656, RI = 0.853, RC = 0.559, HI = 0.4463) of the *Gibberella fujikuroi* species complex inferred from the translation elongation factor-1 α gene sequence data. *Fusarium* sp. NRRL22903 was used as outgroup in the analysis.

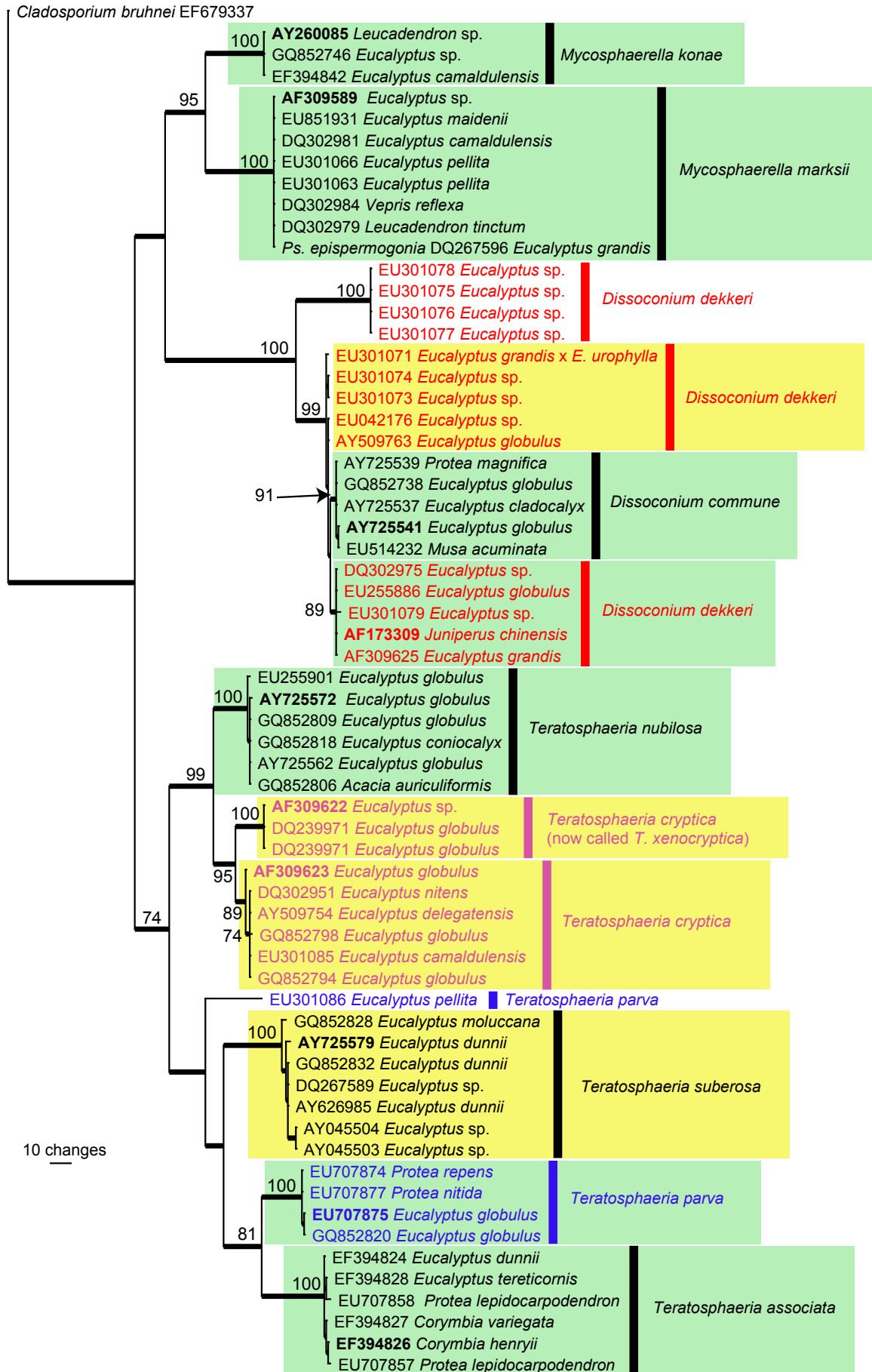


Fig. 5 The first of four equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions (PAUP v4.0b10). Bootstrap support values > 69 % are shown at the nodes and strict consensus branches are thickened and type sequences are in **bold**. Names of paraphyletic species are indicated in different colours. Green blocks represent species with a wide host range or host jumping between different host **genera** whereas the yellow blocks represent those with a wide host range or host jumping across host **species** (i.e. same genus but different species of the genus). Tree length = 555, CI = 0.674, RI = 0.952 and RC = 0.641.

Mycosphaerella and its anamorphs

The genus *Mycosphaerella* s.lat. is commonly accepted as the largest genus of *Ascomycetes*, containing over 10 000 taxa if anamorph states are included. Recent studies have shown, however, that *Mycosphaerella* is para- and polyphyletic (Hunter et al. 2006, Schoch et al. 2006, Crous et al. 2007a, b, 2009a–c, Arzanlou et al. 2007, Batzer et al. 2008), and in fact contains numerous genera, most of which can only be distinguished based on their unique anamorphs. In most cases anamorph genera are now used as holomorph names for these different clades, and new teleomorph names have not been introduced in an effort to stop the proliferation of dual nomenclature in this complex.

Most species in *Mycosphaerella* s.lat. (incl. *Teratosphaeria*) have been described on the assumption that they are host-specific (Chupp 1954, Corlett 1991, Braun 1995, 1998, Crous & Braun 2003, Aptroot 2006). Although this assumption holds true for many species, such as *M. fijiensis*, *M. musicola* and *M. eumusae* on banana (Arzanlou et al. 2008) and *M. graminicola* on wheat (Stukenbrock et al. 2007), some *Mycosphaerella* species are able to colonise different and even unrelated hosts (Crous et al. 2004b, Crous & Groenewald 2005). Furthermore, examples are also known of host-specific necrotrophic pathogenic species of *Mycosphaerella* and *Teratosphaeria* that appeared to also exhibit a facultative saprobic behaviour (Crous et al. 2009a, b). It is imperative, therefore, that to identify all species occurring in a specific lesion, DNA techniques are also employed.

For the purpose of this paper, we chose to focus on the host genus *Eucalyptus*, which is indigenous to Australia, but also cultivated as exotics in commercial plantations in many countries of the world. Specific examples of the *Mycosphaerella* complex that occur on eucalypts and have a wider host range include the following: *Dissoconium commune* (on *Eucalyptus* in South Africa, Spain, New Zealand, *Musa* in Trinidad, *Protea magnifica* in Australia) (Crous et al. 2009c), *M. konae* (*Leucospermum* in Hawaii, *Eucalyptus* in Thailand) (Crous et al. 2007c), *M. marksii* (*Eucalyptus*, Australia, Bolivia, China, Ecuador, Ethiopia, Papua New Guinea, New Zealand, South Africa, Spain, Tanzania, Uruguay, *Leucadendron* on the Madeira Islands, and *Musa* in Mozambique) (Arzanlou et al. 2008), *Teratosphaeria associata* (*Eucalyptus* and *Protea* in Australia) (Summerell et al. 2006, Crous et al. 2007c), *T. parva* (*Eucalyptus* in Australia, Chile, Ethiopia, Portugal, South Africa, Spain, and *Protea* in South Africa), *T. nubilosa* (*Eucalyptus* in Australia, New Zealand, Europe, South America, and *Acacia* in Thailand (Crous & Groenewald 2005, Hunter et al. 2009), and *Mycosphaerella citri* (*Musa* in Florida, *Acacia* in Thailand, and *Eucalyptus* in Vietnam, and *Aeglopsis*, *Citrus*, *Fortunella*, *Murraya*, and *Poncirus* in North and South America, as well as Asia (Pretorius et al. 2003, Crous et al. 2004a, b, Crous & Groenewald 2005, Burgess et al. 2007) (Fig. 5).

Furthermore, to illustrate the complexity of the problem, several species occurring on eucalypts were initially described from exotic plantations outside of their native range. These include *M. heimii* from Madagascar (Crous & Swart 1995), *M. fori* and *M. ellipsoidea* from South Africa (Crous & Wingfield 1996, Hunter et al. 2006), *Teratosphaeria tasmaniensis* from Tasmania (Crous et al. 1998), *T. molleriana* from Portugal and California (Crous & Wingfield 1997), *Dissoconium dekkeri* from Europe and Africa (de Hoog et al. 1983, Crous & Wingfield 1996), and *T. mexicana* from Mexico (Crous 1998), which were only later reported from Australia (Maxwell et al. 2003, Whyte et al. 2005, Jackson et al. 2008). Fig. 5 shows an ITS phylogeny consisting of 119 sequences (including the outgroup sequence) obtained from GenBank. A total of 447 characters were used in the analysis, of which 203 characters were parsimony informative,

230 were constant and 14 variable characters were parsimony uninformative. Although the taxonomy of *Mycosphaerella* / *Teratosphaeria* has been subjected to a concerted effort by several research groups to clarify species boundaries, the tree clearly illustrates the confusion around the application of some species names such as *Dissoconium dekkeri* and *Teratosphaeria parva*. Furthermore, species with wider host ranges or involved in host jumping are also indicated on the tree.

CONCLUSIONS

In this paper we have looked at five fungal groups and have shown that in each case the present knowledge of plant disease associated fungi in these genera is often based on names that have now been shown to be species complexes. There are numerous other plant pathogenic genera where recent publications have revealed that what we thought were species now comprise species complexes and the species present in Australia need reassessing. These include *Cladosporium* (Crous et al. 2007b, Schubert et al. 2007, Bensch et al. 2010), *Phoma* (Aveskamp et al. 2008, 2010, de Gruyter et al. 2009), *Phyllosticta* (Wulanderi et al. 2009), and *Mycosphaerella* and its anamorphs (Crous 2009). We predict the situation to be the same in many other plant pathogenic genera such as *Alternaria*, *Ascochyta*, the helminthosporioid genera (including *Bipolaris*, *Drechslera*, *Exserohilum*, *Curvularia* and their teleomorphs) and *Pestalotiopsis*.

It is evident based on newly emerging molecular data that checklists of many plant pathogenic genera in Australia are now outdated and in need of revision. There is an urgent need for re-assessment of these plant-associated pathogens in order to preserve the effectiveness of Australia's biosecurity measures. Unfortunately, it is often difficult or impossible to extract DNA from herbarium specimens in order to validate identifications, and morphology alone cannot always differentiate taxa in species complexes. Mycologists and plant pathologists need to go back to the field and recollect specimens from which fungal pathogens can be isolated and their DNA extracted and sequenced for the purpose of validating identifications. Revised checklists and databases must be supported by herbarium material, living cultures and DNA libraries. In Australia the Biosecurity Bank provides a reference collection of DNA from a range of agriculturally important plant pathogens and pests for molecular analyses, linking DNA specimens to voucher specimens for taxonomic verification (www.biosecuritybank.com). It is only through the combination of molecular and morphological approaches that the plant pathogens within Australia's borders will be reliably identified. This in turn will preserve the effective role that quarantine plays in keeping unwanted plant pathogens out of Australia.

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