Taxonomy and biodiversity of plant pathogenic fungi from Australia

Timor Sea

NORTHERN TERRITORY

QUEENSLAND

Australia

WESTERN AUSTRALIA

SOUTH AUSTRALIA

Brisbane

Perth

NEW SOUTH WALES

Newcastle

Sydney

Adelaide

ACT

Great Australian Bight

VICTORIA

Melbourne

TASMANIA

Bipolaris
Botryosphaeriaceae
Curvularia
Diaporthe

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Front and back cover: Spatial records of *Bipolaris*, *Curvularia*, *Diaporthe* and *Botryosphaeriaceae* across the continent of Australia, sourced from the Atlas of Living Australia (http://www.ala.org.au). Accessed 12 March 2019.

Taxonomy and biodiversity of plant pathogenic fungi from Australia

Taxonomie en biodiversiteit van plantpathogene schimmels van Australië
(met een samenvatting in het Nederlands)

Proefschrift

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CHAPTER 1

General introduction

Plant biosecurity in Australia

Australia has remained free of many serious plant pests and diseases of agriculture and forestry that are found in many other parts of the world. This has been due in a large part to Australia's geographical isolation as well as more than a century of effective plant quarantine measures (Plant Health Australia 2017). The Australian plant biosecurity system is one of the most effective and visible in the world, supporting the access of Australian crop and horticultural products to overseas markets, and further protecting the Australian economy and environment from the impacts of unwanted pests and diseases.

A sophisticated diagnostic and surveillance system, which relies on close collaboration between plant pathologists and entomologists across Australia, underpins the national plant biosecurity system (Craik et al. 2017). The Australian biosecurity system is multilayered, with protective and complementary measures applied offshore, at the border and onshore. The biosecurity system is enforced by a broad range of participants, covering all Australian governments (national, State, territory and local), industry bodies, exporters and importers, farmers, miners, tourists, researchers and the broader community. This strong and effective biosecurity system is possible because of contributions from, and cooperation between, all participants across a wide range of biosecurity activities (Craik et al. 2017).

Underpinning the Australian biosecurity system is the National Plant Biosecurity Diagnostic Network, which is a nationally integrated network for plant diagnosticians in Australia (http://plantbiosecuritydiagnostics.net.au/). The activities supported by this network enable the efficient and effective diagnosis of plant pests by Australian plant diagnosticians. However, the ability to successfully diagnose unwanted and threatening plant diseases relies heavily on sound knowledge about the pathogens that are already present and established in Australia. Much of this information is currently sourced from outdated published host-pathogen checklists that were compiled, in part, from surveys conducted over many years by Federal and State government programs (Noble 1936, Talbot 1964, Simmonds 1966, Shivas 1989, Hyde and Alcorn 1993, Shivas 1995, Shivas and Alcorn 1996). These checklists have become outdated as new records of plant diseases are discovered and reported. Online databases have replaced these checklists, with the advantage that these can be regularly updated and made accessible, either freely (e.g. Atlas of Living Australia https://www.ala.org.au/, U.S. National Fungus Collections https://nt.ars-grin.gov/fungaldatabases/) or restricted (e.g. Australian Plant Pest Database https://appd.ala.org.au/appd-hub/index).

Host-pathogen checklists and online databases are only reliable if the records are specimen-based, which enables validation of the pathogenic microorganism and plant host against reference material that may be either herbarium specimens, living cultures or DNA accessions. This standard of reliability is evidenced by the requirement that most scientific journals will only publish papers, including first records, that reference specimens lodged in internationally recognised culture collections or herbaria, such as those listed in *Index Herbariorum* (Thiers 2018, http://sweetgum.nybg.org/science/ih/) and the *World Federation for Culture Collections* (http://www.wfcc.info/).

Prior to the availability of DNA sequence data, the identification of plant pathogenic fungi was primarily based on morphology, with herbarium specimens and their associated cultures

serving as proof of identity for future reference. The application of molecular-based methods, in particular the phylogenetic analysis of DNA sequence data, to species classification and identification has revealed that many traditionally accepted species are actually complexes of multiple species (Crous and Groenewald 2005), and has led to a subsequent taxonomic revolution that has accelerated fungal species discovery (Hawksworth and Lücking 2017). Nowadays, molecular methods using DNA sequences are routinely used by plant pathologists and mycologists to identify and classify fungal plant pathogens. Over the past decade, several well-known and important plant pathogenic species have been shown to be complexes of cryptic species, i.e. morphologically similar yet phylogenetically distinct. For example, *Colletotrichum acutatum* and *C. gloeosporioides* were each considered pathogens of a wide range of host plants for the better part of the last 50 years, until taxonomic reassessment by multilocus molecular phylogenetic analyses divided the two taxa into 31 and 22 species, respectively (Shivas and Tan 2009, Damm et al. 2012, Weir et al. 2012), each with comparatively narrow host ranges and geographic distribution. It is difficult, and sometimes impossible, to extract DNA of sufficient quality for multilocus sequence analysis (MLSA) from herbarium specimens, especially old specimens or ones that have been maintained in suboptimal conditions (Telle and Thines 2008). As MLSA is often necessary to verify many of the records of plant pathogens in the plant pathology literature, this has rendered many of those records unreliable. Verification of many plant disease records will require specimens to be recollected, re-isolated and subjected to DNA sequence analysis (Hyde et al. 2010).

The molecular-based method of fungal identification has short-term consequences and long-term benefits for Australian plant biosecurity. It is apparent that many, if not most, of the names applied to specimens in Australian reference collections, as well as names used in the plant pathology literature, are either incorrect or applied to *sensu lato* concepts (literally broadly defined).

Most specimens in the majority of reference collections were collected over a decade ago, when pathogen identifications were based almost entirely on morphology and/or with host identity. Identifications based on morphology were often difficult as most fungi are pleomorphic, and may occur as either sexual, asexual or synasexual morphs. Throughout the 20th century, different names were applied to different morphs, a practice known as dual nomenclature (Briquet 1905), which meant that the same fungus could legitimately have different generic and sometimes different species names for each morph. Sometimes these names were based on the same type specimens (nomenclatural synonyms), and sometimes on different type specimens (taxonomic synonyms). The dual nomenclature system was abolished at the 18th International Botanical Congress (Hawksworth et al. 2011, McNeil et al. 2012), and the application of single names for phytopathogenic fungi has become entrenched in the *International Code of Nomenclature for algae, fungi and plants* (Turland et al. 2018). This change has had a positive and progressive effect on plant pathology and plant biosecurity as DNA sequence comparisons have made it possible to reliably connect the different morphological morphs, and to place a fungus in its appropriate phylogenetic position, even if sterile (Wingfield et al. 2012, Crous et al. 2015, 2016).

Records of plant pathogens in Australia

Currently records of plant pathogenic fungi in Australia, whether published or available online, were mostly derived from herbarium specimens, which are pressed, dried and non-viable

(Shivas et al. 2006). Relatively few records of plant pathogens have been substantiated by living cultures of microorganisms, whether fungi, oomycetes or bacteria. There are several reasons for this, notably, (1) many large groups of plant pathogens are obligate and cannot be cultured, such as downy mildews (*Peronosporaceae*), powdery mildews (*Erysiphales*), tar spots (*Phyllachoraceae*), rusts (*Pucciniales*), smuts (*Ustilaginomycetes*); (2) many identifications of pathogens were based on morphology and cultures were not necessary for diagnosis; (3) living cultures are often difficult to preserve, especially over time and may lose their ability to sporulate or retain pathogenicity or other physiological properties; (4) long-term maintenance of living cultures is relatively costly; and (5) isolation of a presumptive causal organism does not demonstrate pathogenicity unless Koch's postulates are fulfilled. For example, consider that records of plant pathogenic fungi in remote parts of northern Australia (Hyde and Alcorn 1993, Shivas 1995, Shivas and Alcorn 1996), and in the neighbouring countries of Papua New Guinea (Hyde & Philemon 1994) and Indonesia (Shivas et al. 1996), were mostly based on microscopic examination of herbarium specimens. The remoteness of many locations in northern Australia has meant that facilities were not immediately available to isolate and preserve living cultures of plant pathogenic fungi. Furthermore, the Australian biosecurity system prohibits movement of specimens that might harbour living and exotic pathogens, unless accompanied by a biological import permit issued by the Australian Department of Agriculture and Water Resources. These permits invariably stipulate that the herbarium specimens must not be used for *in vitro* studies.

The importance of maintaining accurate records of pathogens in Australia is essential for a process known as pest risk analysis (Plant Health Australia 2013). Pest risk analysis is a form of risk analysis conducted by regulatory plant health authorities to identify the appropriate phytosanitary measures needed to protect plant or plant products from new or emerging pests and diseases. The process of pest risk analysis also determines whether exotic pathogens are a potential threat to agriculture or the environment. The aim of the plant biosecurity system in Australia is to prevent the entry of exotic pests, diseases and weeds that could have serious environmental and economic consequences if introduced and allowed to establish (Craik et al. 2016).

Specimen-based records of most of the plant pathogens in Australia can be accessed through the Australian Plant Pest Database (APPD) (Shivas et al. 2006, https://appd.ala.org.au/appd-hub/index). Access to this database is currently restricted by username and password, which limits its accessibility. The APPD includes records of specimens from the three largest herbaria of plant pathogens in Australia, namely the Queensland Plant Pathology Herbarium (BRIP, http://collections.daff.qld.gov.au/), the New South Wales Plant Pathology and Mycology Herbarium (DAR, https://www.dpi.nsw.gov.au/about-us/services/collections/herbarium), the and the Victorian Plant Pathology Herbarium (VPRI). For several of these records, the herbaria maintain associated living cultures. With over 18 000 isolates, BRIP holds Australia's largest collection of cultures of plant pathogenic fungi. These records are critical resources for the resolution of biosecurity issues and underpin the pest risk analysis process in Australia.

Molecular phylogenetic methods are now routinely used in most diagnostics laboratories in Australia. Many commonly encountered groups of plant pathogenic fungi have been shown to include complexes of cryptic species, such as *Mycosphaerellaceae* (including *Cercospora*, *Cladosporium*, *Pseudocercospora*), *Botryosphaeriaceae* (including *Lasiodiplodia*, *Neofusicoccum*), as well as the genera *Calonectria*, *Colletotrichum*, *Diaporthe*, *Fusarium*, and

Phyllosticta (Crous et al. 2006a–e, Alves et al. 2008, Cai et al. 2009, Hyde et al. 2009a, Lombard et al. 2010, Bensch et al. 2012, Cannon et al. 2012, Damm et al. 2012a, 2012b, Gomes et al. 2012, Udayanga et al. 2012, Weir et al. 2012, Groenewald et al. 2013, Wikee et al. 2013, Aoki et al. 2014, Damm et al. 2014, Laurence et al. 2014, Liu et al. 2014, Damm et al. 2019). Many of these studies have included some Australian isolates, but for the most part, the extent of fungal diversity in Australia is poorly known. This knowledge gap, as far as it pertains to fungal plant pathogens, poses a risk to the Australian biosecurity system as checklists and databases of fungi associated with plant diseases in Australia are outdated.

The purpose of the studies described in this thesis was to demonstrate that there is hidden diversity in the Australian plant pathogen culture collections. A re-inventory of these culture collections will allow checklists and databases to be updated. Accurate and updated lists of Australian plant pathogens are essential to ensure that the Australian plant biosecurity system remains strong and effective.

Important plant pathogenic fungal genera that are poorly resolved in Australia

Botryosphaeriaceae

The *Botryosphaeriaceae*, with over 2000 names, represents a large, well-studied group of fungi that cause canker diseases on woody plant hosts around the world (Yang et al. 2017, http://www.indexfungorum.org/). Until recently, there was very little reliable baseline data about the species of *Botryosphaeriaceae* that were present in Australia (Tan et al. 2018, Burgess et al. 2018). There have been many taxonomic changes in this fungal family over the past decade, with the establishment of several new genera and species, as well as the reclassification of many species (Dissanayake et al. 2016, Slippers et al. 2017, Yang et al. 2017).

There are currently 24 genera containing 222 species in the *Botryosphaeriaceae* worldwide; with 9 genera and 62 species represented in Australia (Tan et al. 2018, Burgess et al. 2018). Some species have a wide host range with many records worldwide, such as *Botryosphaeria dothidea*, *Lasiodiplodia theobromae* and *Neofusicoccum parvum* (Marsberg et al. 2017, Slippers et al. 2017), while for some recently described species, such as *Macrophomina pseudophaseolina*, and *Eutiarosporella* spp., the distribution appears to be limited (Sarr et al. 2014, Thynne et al. 2015).

Systematic collection of species in the *Botryosphaeriaceae* is needed to support biosecurity decision making and provide a foundation for future taxonomic and diagnostic studies into this group of important pathogens. Australian plant pathology herbaria and culture collections contain many specimen-based records of *Botryosphaeriaceae* (Plant Health Australia 2001). However, most of these records need revision as they predate the application of molecular phylogenetic methods. Morphological characters alone are inadequate to define genera or identify species of *Botryosphaeriaceae*. Phillips et al. (2013) suggested that many taxa linked to the *Botryosphaeriaceae*, for which cultures or DNA sequence data were not available, will have to be disregarded unless they were epitypified. Molecular studies have shown that species of *Botryosphaeria* such as *B. dothidea* and *L. theobromae* are species complexes (Alves et al. 2008, Phillips et al. 2008, Abdollahzadeh et al. 2010). *Botryosphaeria dothidea* was shown to be a complex comprising several species (Smith et al. 2001, Denman et al. 2003), necessitating its epitypification with a specimen from *Prunus* sp., collected near the Italian-Swiss border

(Slippers et al. 2004). Subsequently, several other species of *Botryosphaeria* have been epitypified, which has advanced the taxonomic resolution of this genus (Crous et al. 2006d, Alves et al. 2008, Phillips et al. 2008). The epitypification of these taxa has stabilised many names by removing uncertainty about their application.

Diaporthe

The genus *Diaporthe* includes over 800 species, with approximately 900 species names available in the synonymous genus *Phomopsis* (http://www.indexfungorum.org/). However, only 213 of these species are supported by DNA sequences from ex-type cultures (Dissanayake et al. 2017, Marin-Felix et al. 2019). *Diaporthe* includes species reported as pathogens, endophytes and saprobes, which are widespread in both temperate and tropical regions (Santos and Phillips 2009, Santos et al. 2011, Udayanga et al. 2011, 2012, Gomes et al. 2013, Hyde et al. 2014, Dissanayake et al. 2017, Marin-Felix et al. 2019).

Plant pathogenic species of *Diaporthe* cause serious diseases of many cultivated plants worldwide, including grapevines (van Niekerk et al. 2005), soybean (Li et al. 2010), strawberry (Maas 1998), and sunflower (Muntanola-Cvetkovic et al. 1981, Thompson et al. 2011, 2015). In Australia, several exotic species of *Diaporthe* have been recognised by the regulatory agencies or by the agricultural or horticultural industry as potential biosecurity threats, including *D. amygdali* on almonds; *D. eres* and *D. tanakae* on apples and pears; *D. melonis* on cucurbits; *D. helianthi* on sunflowers; *D. vaccini* on blueberries and small cranberries; and *D. viticola* on grapes (Plant Health Australia 2006, 2007, 2010, 2018, DPIRD 2018)

In Australia, there are over 1 300 specimens of *Diaporthe* (including *Phomopsis*) deposited in the major plant pathology herbaria (Plant Health Australia 2001). Most of the specimens have not been identified to species level, or require revision as they predate the application of molecular phylogenetic methods. Cryptic diversification, phenotypic plasticity and extensive host associations have long complicated accurate identifications of *Diaporthe* species (Udayanga et al. 2012). Species recognition in *Diaporthe* was based on morphological characters and host associations (Wehmeyer 1933). Molecular phylogenetic studies have demonstrated that morphological characters were often unreliable for species identification due to variability in environmental conditions (Gomes et al. 2013). Host associations are also unreliable as a single species can be found on diverse hosts, or more than one *Diaporthe* species may be reported as causative agents of the same disease (Thompson et al. 2011, Guarnaccia et al. 2016, 2018). Identification and description of *Diaporthe* species are now based on multilocus phylogenetic inferences (Santos et al. 2011, Thompson et al. 2011, Udayanga et al. 2012, Gomes et al. 2013; Tan et al. 2013; Udayanga et al. 2014, Lombard et al. 2014, Thompson et al. 2015, Udayanga et al. 2014a, 2014b, 2015, Du et al. 2016, Gao et al. 2015, Senanayake et al. 2017). However, the creation of a stable taxonomy for *Diaporthe* has been hindered by the lack of ex-type cultures for many species.

Bipolaris* and *Curvularia

The genus *Bipolaris* (Shoemaker 1959) includes over 100 species, and *Curvularia* (Boefijn 1933) includes over 170 species. In addition, there are approximately 50 species names available in *Cochliobolus* (Drechslera 1934) that have mostly been synonymised into *Bipolaris*

or *Curvularia*. Species of *Bipolaris* and *Curvularia* include phytopathogens, particularly of grasses (*Poaceae*), but are also associated with human, and non-living substrates such as air, fresh water, and soil (Sivanesan 1987, Manamgoda et al. 2011, Rangaswamy et al. 2013, Verma et al. 2013, Manamgoda et al. 2014, 2015, Marin-Felix et al. 2018).

In Australia, there are over 1 200 specimens of *Bipolaris* and over 800 specimens of *Curvularia* deposited in the major plant pathology herbaria (Plant Health Australia 2001). Most of the specimens have not been identified to species level, or require revisions as they predate the application of molecular phylogenetic methods. In the past, identification and classification of *Bipolaris* and *Curvularia* species were entirely based on morphological characters. In particular, *Curvularia* species were considered to have curved conidia (or disproportionate swelling of at least one of the conidial cells) with true septa, whereas *Bipolaris* species had straight conidia with pseudosepta (Sivanesan 1987). *Bipolaris* and *Curvularia* species share many similar and overlapping asexual morphological characteristics, and share similar sexual morphs (previously classified as *Cochliobolus*), which led to debates as to whether the two genera should be synonymised (von Arx and Luttrell 1979, Sivanesan 1987).

Molecular phylogenetic analyses have demonstrated that *Bipolaris* and *Curvularia* were broadly monophyletic and separated into two well resolved clades (Berbee et al. 1999, Manamgoda et al. 2012). To stabilise the taxonomy, there have been attempts to source the ex-type cultures of existing species and apply molecular phylogenetic methods to resolve or confirm the taxonomy of 41 *Bipolaris* and 73 *Curvularia* species (Manamgoda et al. 2012, 2014, Tan et al. 2014, Manamgoda et al. 2015). This has resulted in the reassignment of 26 *Bipolaris* species to *Curvularia*, and the introduction of the monotypic genus, *Johnalcornia*, to accommodate an aberrant *Bipolaris* species (Manamgoda et al. 2012, 2014, Tan et al. 2014).

Outline of the thesis

The research presented in this thesis is mainly related to the taxonomy and biodiversity of cultures from the Queensland Plant Pathology Herbarium (BRIP), Australia's largest collection of phytopathogenic fungi. The new insights gained from this thesis will influence future research into the taxonomy and biodiversity of other underrepresented microfungi in Australia, and provide a firm foundation for plant biosecurity in Australia.

Thesis chapters

Chapter 1: An overview of plant biosecurity in Australia.

Chapter 2: Six new species of *Diaporthe*, *D. beilharziae* on *Indigofera australis*, *D. fraxini-angustifoliae* on *Fraxinus angustifolia* subsp. *oxycarpa*, *D. litchicola* on *Litchi chinensis*, *D. nothofagi* on *Nothofagus cunninghamii*, *D. pascoei* on *Persea americana* and *D. salicicola* on *Salix purpurea* from Australia are described and illustrated based on morphological characteristics and molecular analyses. Three of the new species no longer produced sporulating structures in culture and two of these were morphologically described from voucher specimens. Phylogenetic relationships of the new species with other *Diaporthe* species are revealed by DNA sequence analyses based on the internal transcribed spacer (ITS) region, and partial regions of the β-tubulin (*tub2*) and translation elongation factor 1-alpha (*tef1α*).

Chapter 3: An examination of ex-type and authentic cultures of 34 species of *Bipolaris* and *Curvularia* by phylogenetic analysis of four loci (*gapdh*, ITS, LSU and *tef1α*) resulted in nine new combinations in *Curvularia*, as well as new synonymies for some species of *Bipolaris* and *Curvularia*. Lectotypes are designated for *Bipolaris secalis* and *Curvularia richardiae*, and an epitype is designated for *Curvularia crustacea*. A new monotypic genus, *Johnalcornia*, is introduced to accommodate *Bipolaris aberrans*, which clusters sister to the newly described *Porocercospora*. *Johnalcornia* differs morphologically from this taxon by producing distinctive conidia-like chlamydospores as well as comparatively thick-walled, geniculate conidiophores, with conidiogenous cells that have conspicuous scars. *Johnalcornia* further differs from related genera by the second conidial septum forming distally to delimit the apical cell.

Chapter 4: Several unidentified specimens of *Bipolaris* deposited in the Queensland Plant Pathology Herbarium (BRIP) from 1976–1988 and recognised by Dr. John L. Alcorn as taxonomically interesting were re-examined. The morphology of conidia and conidiophores, as well as phylogenetic inference from the analyses of three loci (ITS, *gapdh* and *tef1α*) supported the classification of eight novel *Bipolaris* species, which were originally isolated from leaf spots on grasses (*Poaceae*).

Chapter 5: Several unidentified specimens of *Curvularia* deposited in the Queensland Plant Pathology Herbarium were re-examined. Phylogenetic analyses based on sequence data of the internal transcribed spacer region, partial fragments of the glyceraldehyde-3-phosphate dehydrogenase and the translation elongation factor 1-α genes, supported the introduction of 13 novel *Curvularia* species. Eight of the species described, namely, *C. beasleyi*, *C. beerburrumensis*, *C. eragrosticola*, *C. kenpeggii*, *C. mebaldsii*, *C. petersonii*, *C. platzii* and *C. warraberensis*, were isolated from grasses (*Poaceae*) exotic to Australia. Only two species, *C. lamingtonensis* and *C. sporobolicola*, were described from native Australian grasses. Two species were described from hosts in other families, namely, *C. coatsiae* from *Litchi chinensis* (*Sapindaceae*) and *C. colbranii* from *Crinum zeylanicum* (*Amaryllidaceae*). *Curvularia reesii* was described from an isolate obtained from an air sample. Furthermore, DNA sequences from ex-type cultures supported the generic placement of *C. neoindica* and the transfer of *Drechslera boeremae* to *Curvularia*.

Chapter 6: The *Botryosphaeriaceae* is one of the most widespread and cosmopolitan endophytic groups of fungi. However, the species of this family can cause severe disease when the hosts are exposed to stressful conditions. The aim of this study was to identify living cultures from the Botryosphaeriaceae preserved in the Queensland and Victorian Plant Pathology Herbaria using DNA sequence analyses. The 51 isolates examined were collected between 1971 and 2017 from 35 different host genera, with the dominant host genera being *Mangifera* (11 isolates), *Acacia* (10), and *Persea* (5). Multilocus sequence analyses resulted in the re-identification of 41 isolates to the genera *Botryosphaeria* (2 isolates), *Diplodia* (4), *Dothiorella* (1), *Lasiodiplodia* (19), and *Neofusicoccum* (15), as well as some that belonged to genera outside of the *Botryosphaeriaceae* (10). New records for Australia were *Botryosphaeria sinensis*, *Diplodia alatafructa*, *Lasiodiplodia gonubiensis*, *Neofusicoccum cryptoaustrale*, and *N. mangroviorum*. These taxa were identified as a result of a workshop organised by the Subcommittee on Plant Health Diagnostics. The results of this study provide fundamental information regarding the diversity of *Botryosphaeriaceae* species present in Australia.

Chapter 7: The taxonomic decisions presented in this thesis and their implications for plant biosecurity in Australia are discussed. The impact of identification and resolution of species complexes has important benefits for the pest risk analysis process and the exclusion of exotic plant pathogens from Australia. The Queensland Plant Pathology Herbarium holds the largest culture collection of plant pathogenic fungi in Australia, with over 23 000 strains. This collection is an essential asset for plant biosecurity in Australia. An English and Dutch summary of the results and conclusions of this thesis are provided in the Appendix.

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CHAPTER 2

Molecular phylogenetic analysis reveals six new species of *Diaporthe* from Australia

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Key words: *Diaporthe beilharziae*, *Diaporthe fraxiniangustifoliae*, *Diaporthe litchicola*, *Diaporthe nothofagi*, *Diaporthe pascoei*, *Diaporthe salicicola*, *tub2*, ITS, Phylogeny *Phomopsis*, *tef1α*

Abstract

Six new species of *Diaporthe*, *D. beilharziae* on *Indigofera australis*, *D. fraxini-angustifoliae* on *Fraxinus angustifolia* subsp. *oxycarpa*, *D. litchicola* on *Litchi chinensis*, *D. nothofagi* on *Nothofagus cunninghamii*, *D. pascoei* on *Persea americana* and *D. salicicola* on *Salix purpurea* from Australia are described and illustrated based on morphological characteristics and molecular analyses. Three of the new species no longer produced sporulating structures in culture and two of these were morphologically described from voucher specimens. Phylogenetic relationships of the new species with other *Diaporthe* species are revealed by DNA sequence analyses based on the internal transcribed spacer (ITS) region, and partial regions of the β-tubulin (*tub2*) and translation elongation factor 1-alpha (*tef1α*).

INTRODUCTION

Diaporthe species (including their asexual *Phomopsis* states) are found worldwide on a diverse range of host plants as endophytes, pathogens and saprobes (Uecker 1988). Previously, host association was often the basis for species classification and identification in *Diaporthe* and *Phomopsis*, as morphological and cultural characteristics were often inadequate or unreliable (van der Aa et al. 1990, van Rensburg et al. 2006). In recent studies, species of *Diaporthe* were distinguished mainly by their molecular phylogenies, especially those derived from analyses of the internal transcribed spacer (ITS) region of the nuclear ribosomal RNA (Mostert et al. 2001, van Niekerk et al. 2005, van Rensburg et al. 2006, Santos and Phillips 2009, Ash et al. 2010, Crous et al. 2011). Some authors have also used other gene regions as markers for phylogenetic analysis in *Diaporthe* and *Phomopsis*, such as actin, β-tubulin (*tub2*), calmodulin, histone, mating type genes, and translation elongation factor 1-alpha (*tef1α*) (van Rensburg et al. 2006, Santos et al. 2010, Udayanga et al. 2011, 2012a, 2012b, Gomes et al. 2013).

DNA sequencing has enabled the link between anamorphic (*Phomopsis*) and teleomorphic (*Diaporthe*) states irrespective of whether the taxon under study produces asexual or sexual structures (Udayanga et al. 2012b). Recent changes to the rules that govern fungal nomenclature require that only one name for a single species should be used instead of different names for different sexual stages (Hawksworth 2011). The earlier generic name *Diaporthe* (Nitschke 1870) has priority over *Phomopsis* (Saccardo and Roumeguère 1884) and has been adopted as the generic name for these taxa in recent studies in Australia (Thompson et al. 2011) and overseas (Santos et al. 2010, 2011, Crous et al. 2011, 2012, Udayanga et al. 2012a, 2012b, Gomes et al. 2013). In this study, six new species of *Diaporthe* from Australia are described based on morphological characters and phylogenies derived from ITS, *tef1α* and *tub2* gene sequences.

MATERIALS AND METHODS

Isolates

Unidentified isolates of *Phomopsis* were sourced from the culture collections at the Queensland Plant Pathology Herbarium (BRIP, Dutton Park, Queensland, Australia) and the Victorian Plant Pathology Herbarium (VPRI, Bundoora, Victoria, Australia). All isolates were deposited in BRIP as both living and dried cultures (Table 1).

Morphology

For fungal morphological studies, isolates were grown on potato dextrose agar (PDA) (Oxoid), oatmeal agar (OMA) (Oxoid), and water agar (WA) with sterile pieces of wheat stems, and incubated under 12 h near ultraviolet light/12 h dark (Smith 2002) at room temperature (approx. 23–25 °C). Fungal structures were mounted on glass slides in 100 % lactic acid for microscopic examination after 28 days of incubation. Ranges were expressed as either min.–max. or as (min.–) mean-SD–mean+SD (−max.) with values rounded to 0.5 µm. Means and standard deviations (SD) were made from at least 20 measurements. Images were captured with a Leica DFC 500 camera attached to a Leica DM5500B compound microscope with Nomarski differential interference contrast.

For colony morphology, 3-d-old cultures on 9 cm diam. plates of PDA and OMA that had been grown in the dark at room temperature were grown for a further 7 days under 12 h near ultraviolet light/12 h dark. Colony colours (surface and reverse) were matched and described according to the colour charts of Rayner (1970).

DNA isolation, amplification and analyses

Mycelia were scraped off PDA cultures and macerated with 0.5 mm glass beads (Daintree Scientific) in a Tissue Lyser (QIAGEN). Genomic DNA was then extracted with the Gentra Puregene DNA Extraction kit (QIAGEN) according to the manufacturer's instructions.

The primers V9G (de Hoog and Gerrits van den Ende 1998) and ITS4 (White et al. 1990) were used to amplify the ITS region of the ribosome genes. The primers EF1-728 F (Carbone and Kohn 1999) and EF2 (O'Donnell et al. 1998) were used to amplify part of the *tef1α* gene, and the primers T1 (O'Donnell and Cigelnik 1997) and Bt2b (Glass and Donaldson 1995) were used to amplify part of the *tub2* gene. All gene regions were amplified with the Phusion High-Fidelity PCR Master Mix (Finnzymes). The PCR products were purified with the QIAquick PCR Purification Kit (QIAGEN), and sequenced by Macrogen Incorporated (Seoul, Korea) on the 3730xl DNA Analyzer (Applied Biosystems) using the amplifying primers.

All sequences generated in this study were assembled using Vector NTi Advance 11.0 (Invitrogen). The ITS sequences were initially aligned with representative *Diaporthe* spp. using ClustalW in MEGA 5.2 (Tamura et al. 2011). *Diaporthella corylina* was selected as the outgroup taxon. A Neighbour-Joining (NJ) analysis using the Kimura-2 parameter with Gamma distribution was applied (data not shown; TreeBASE study S13424), and the closest phylogenetic neighbours were selected for a combined analyses using ITS, *tef1α* and *tub2* genes. The sequences of each gene were aligned separately and manually adjusted where needed. Alignment gaps were treated as missing character states, and all characters were unordered and of equal weight. Suitable Maximum Likelihood (ML) nucleotide substitution model for each gene was determined using the model test function in MEGA 5.2, and then used for the phylogenetic analysis of the ITS, *tef1α* and *tub2* sequences individually, as well as for the combined dataset. A ML tree using the combined dataset was generated in MEGA 5.2 using the Tamura-Nei substitution model with Gamma distribution. Bootstrap support values with 1000 replications were calculated for tree branches. The newly generated sequences were deposited into GenBank (Table 1) and the concatenated alignment in TreeBASE (Study S13424). Nomenclatural novelties were deposited

Table 1. *Diaporthe* species analysed in this study.

Species	Isolate no.[a]	Host	Locality	GenBank accession numbers[b]		
				ITS	*tef1α*	*tub2*
D. arengae	CBS 114979[T]	*Arenga engleri*	Hong Kong	KC343034	KC343760	KC344002
D. australafricana	CBS 111886[T]	*Vitis Vinifera*	Australia	KC343038	KC343764	KC344006
D. beckhausii	CBS 138.27	*Viburnum* sp.		KC343041	KC343767	KC344009
D. beilharziae sp. nov.	BRIP 54792[T] VPRI 16602	*Indigofera australis*	Australia	**JX862529**	**JX862535**	-
D. cynaroidis	CBS 122676[T]	*Protea cynaroides*	South Africa	KC343058	KC343784	KC344026
D. eugeniae	CBS 444.82	*Eugenia aromatica*	Indonesia	KC343098	KC343824	KC344066
D. fraxini-angustifoliae sp. nov.	BRIP 54781[T] VPRI 10911	*Fraxinus angustifolia* subsp. *oxycarpa*	Australia	**JX862528**	**JX862534**	-
D. ganjae	CBS 180.91[T]	*Cannabis sativa*	USA	KC343112	KC343838	KC344080
D. infecunda	CBS 133812[T]	*Schinus terebinthifolius*	Brazil	KC343126	KC343852	KC344094
D. litchicola sp. nov.	BRIP 54900[T]	*Litchi chinensis*	Australia	**JX862533**	**JX862539**	-
D. manihotia	CBS 505.76	*Manihot utilissima*	Rwanda	KC343138	KC343864	KC344106
D. musigena	CBS 129519[T] CPC 17025	*Musa* sp.	Australia	KC343143	KC343869	KC344111
D. nothofagi sp. nov.	BRIP 54801[T] VPRI 22429b	*Nothofagus cunninghamii*	Australia	**JX862530**	**JX862536**	-
D. oncostoma	CBS 589.78	*Robinia pseudoacacia*	Germany	KC343160	KC343886	KC344128
D. pascoei sp. nov.	BRIP 54847[T] VPRI 16058	*Persea americana*	Australia	**JX862532**	**JX862538**	-
D. perseae	CBS 151.73	*Persea gratissima*	Netherlands	KC343173	KC343899	KC344141
D. pseudomangiferae	CBS 101339[T]	*Mangifera indica*	Dominican Republic	KC343181	KC343907	KC344149
D. pustulata	CBS 109742	*Acer pseudoplatanus*	Austria	KC343185	KC343911	KC344153
D. salicicola sp. nov.	BRIP 54825[T] VPRI 32789	*Salix purpurea*	Australia	**JX862531**	**JX862537**	-
D. schini	CBS 133181[T]	*Schinus terebinthifolius*	Brazil	KC343191	KC343917	KC344159

Table 1. (Ctd).

Species	Isolate no.[a]	Host	Locality	GenBank accession numbers[b]		
				ITS	*tef1α*	*tub2*
D. tecomae	CBS 100547	*Tabebuia* sp.	Brazil	KC343215	KC343941	KC344183
D. toxica	CBS 534.93[T]	*Lupinus angustifolius*	Australia	KC343220	KC343946	KC344188
D. viticola	CBS 113201[T]	*Vitis Vinifera*	Portugal	KC343234	KC343960	KC344202
Diaporthe sp. 6	CBS 115584	*Maesa perlarius*	Hong Kong	KC343208	KC343934	KC344176
Diaporthella corylina	CBS 121124	*Corylus* sp.	China	KC343004	KC343730	KC343972

[a] BRIP: Plant Pathology Herbarium, Dutton Park, Queensland, Australia; CBS: Westerdijke Fungal Biodiversity Institute, Utrecht, The Netherlands; CPC: Collection Pedro Crous, housed at CBS; VPRI: National Collection of Fungi, Knoxfield, Victoria, Australia.

[b] ITS: internal transcribed spacer; *tef1α*: translation elongation factor 1-alpha; *tub2*: β-tubulin

Newly deposited sequences are in **bold**. All other sequences were downloaded from GenBank and published in Gomes et al. (2013).

[T] Ex-type culture.

in MycoBank (www.MycoBank.org) (Crous et al. 2004).

Unique fixed nucleotides positions are used to characterise and describe three sterile species. For each sterile species that was described, the closest phylogenetic neighbour was selected and this focused dataset was subjected to single nucleotide polymorphisms (SNPs) analyses. These SNPs were determined for each aligned data partition using DnaSP 5.10.01 (Librado and Rozas 2009).

RESULTS

Phylogenetic analysis

Approximately 600 bases of the ITS region were sequenced from the isolates in this study and added to the alignment (TreeBASE study S13424). The alignment included 118 sequences from 51 *Diaporthe* (including *Phomopsis*) spp., most of which were from ex-type cultures. The evolutionary relationships of these sequences were analysed using NJ method based on a Kimura-2 parameter model with Gamma distribution (data not shown; TreeBASE study S13424). From this NJ phylogenetic tree, 18 taxa closest to the isolates in this study were select for a combined analyses using the ITS, *tef1α* and *tub2* genes.

The combined (ITS, *tef1α* and *tub2*) alignment for the ML analysis contained 25 isolates (including the outgroup) and 1956 characters were used in the phylogenetic analysis.

The combined phylogenetic tree showed that three of the newly described species in this study clustered closely with each other as well as to *Diaporthe musigena* (Fig. 1). The phylogenetic tree also showed that one of the new species clustered close to *Diaporthe cynaroidis* (Fig. 1). Comparison of the ITS, *tef1α* and *tub2* sequences between *D. cynaroidis* and the new taxon identified fixed nucleotide differences which accurately delineate between the two.

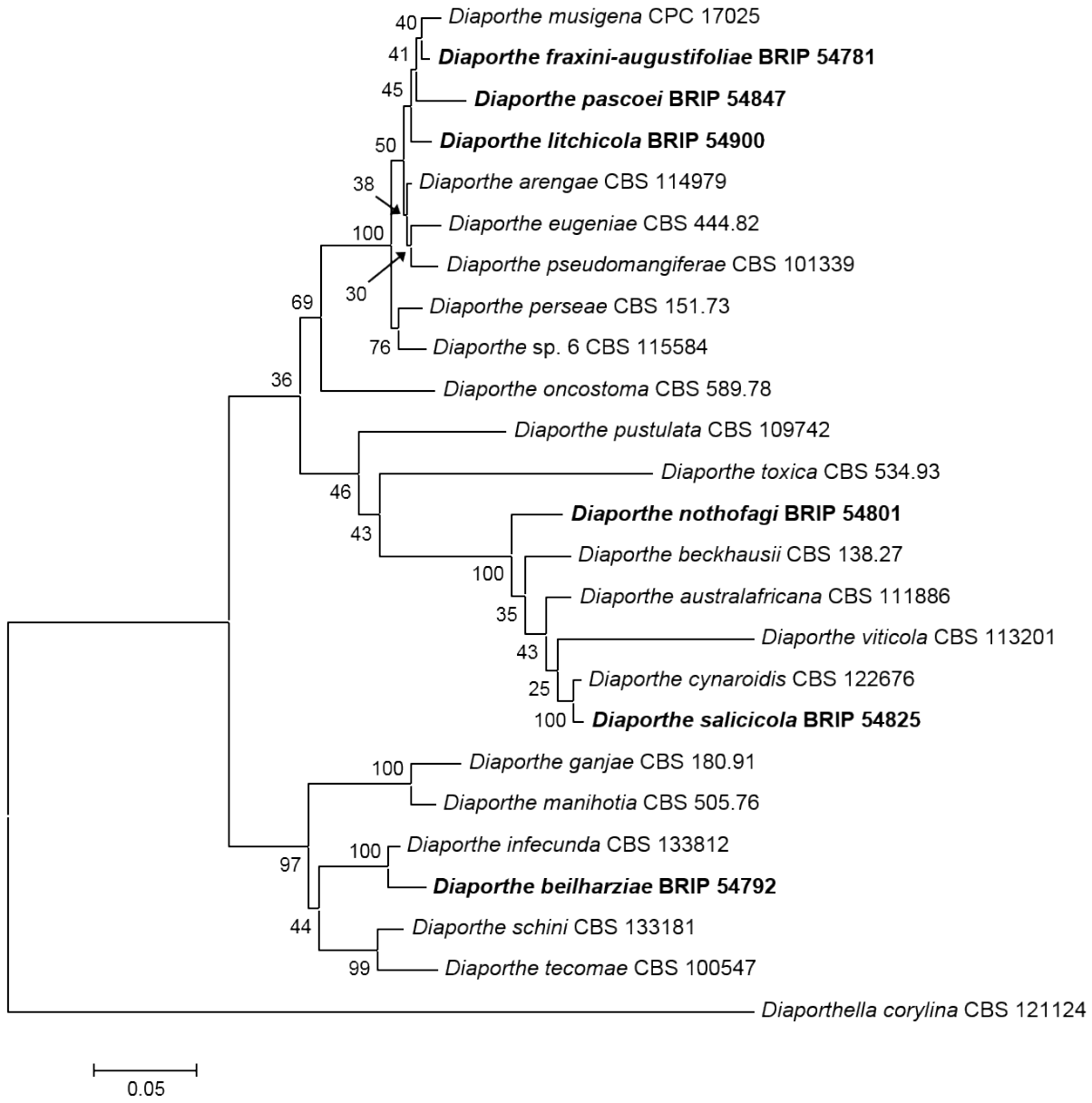

Fig. 1. Maximum likelihood tree inferred from analysis of three combined three genes (ITS, *tef1α* and *tub2*). The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree was rooted to *Diaporthella corylina*. The alignment and tree are deposited in TreeBASE (S13424). Species described in this study are in **bold**.

Taxonomy

Six undescribed species of *Diaporthe* were recognised based on DNA sequence analysis, together with cultural morphology, and sometimes a description of anamorphic structures. Although none of the new fungi produced a teleomorphic stage in culture, all have been described in *Diaporthe* according to rules in the *International Code of Nomenclature for algae, fungi and plants* (Hawksworth 2011) on the basis that *Diaporthe* was established 14 years before *Phomopsis* in accordance with previous studies (Santos and Phillips 2009, Santos et al. 2010, 2011, Thompson et al. 2011, Udayanga et al. 2012a, 2012b).

Diaporthe beilharziae R.G. Shivas, J. Edwards & Y.P. Tan, **sp. nov. –** MycoBank MB802383. Fig. 2a–e

Etymology: In recognition of Dr. Vyrna Beilharz, a highly respected Australian mycologist who first collected and isolated this fungus.

Conidiomata pycnidial, solitary, scattered or aggregated in small groups, abundant on PDA, OMA, and wheat straw pieces on WA after 4 weeks, solitary and immersed in WA after 4 weeks, subglobose, up to 250 µm diam., ostiolate, beaks absent or less than 300 µm, abundant pale yellow to salmon conidial droplets exuded from ostioles; walls thin, composed of an inner layer of yellowish brown textura angularis and an outer layer of darker yellowish brown textura epidermoidea. *Conidiophores* formed from the inner layer of the locular wall, reduced to conidiogenous cells or 1-septate, hyaline to pale yellowish brown, ampulliform to cylindrical, 5–15 × 1.5–3.5 µm, *Conidiogenous cells* cylindrical to flexuous, tapered towards the apex, hyaline, 5–20 × 1.5–3.0 µm. *Alpha conidia* abundant, oval to cylindrical, rounded at the apex, obconically truncate at base, mostly biguttulate, hyaline, (5.5–) 6.5–9 (−10) × 2–2.5 (−3) µm. *Beta conidia* scarce amongst the alpha conidia, flexuous, hyaline, 15–25 × 1.0–1.5 µm. *Perithecia* not seen.

Culture characteristics: Colonies on PDA covering entire plate after 10 days, adpressed to slightly ropey with pycnidia visible as hundreds of small black dots, transparent becoming pale greyish sepia towards the centre; reverse similar to the surface. On OMA covering the entire plate after 10 days, adpressed, transparent to pale mouse grey with pycnidia apparent as small black dots or irregular patches less than 200 µm diam.; reverse similar to the surface.

Specimen examined: AUSTRALIA, New South Wales, Mittagong, on *Indigofera australis*, 30 Apr. 1991, *V.C. Beilharz* (VPRI 16602 holotype, includes ex-type culture), (BRIP 54792 isotype, includes ex-type culture).

Notes: *Diaporthe beilharziae* was isolated from a leaf spot on *Indigofera australis*. Two other species, *Diaporthe indigoferae* on dead branches of *I. gerardiana* in Pakistan (Müller and Ahmad 1958) and *Phomopsis indigoferae* on stems of *I. dosua* and *I. dalea* from Europe (Uecker 1988), have been reported on *Indigofera*. Conidia were not described for *D. indigoferae*. *Phomopsis indigoferae* has larger alpha conidia (8 × 3–4 µm) than *D. beilharziae* (5.5–10 × 2–3 µm). The role of all of these fungi as pathogens is not known.

The phylogenetic inference from the combined sequence data showed *D. beilharziae* clustered close to *D. infecunda* (Gomes et al. 2013) (Fig. 1). In culture, *D. beilharzae* produced abundant pycnidia on PDA and OMA, compared to *D. infecunda*, which was sterile.

Diaporthe fraxini-angustifoliae R.G. Shivas, J. Edwards & Y.P. Tan, **sp. nov. –** MycoBank MB802384. Fig. 2f–j

Etymology: Named after the host genus and species from which it was collected, *Fraxinus angustifolius*.

Conidiomata pycnidial, solitary or aggregated in groups up to 2 mm diam. and abundant on PDA, OMA and wheat straw pieces on WA after 4 weeks, solitary and scarce on WA after 4 weeks,

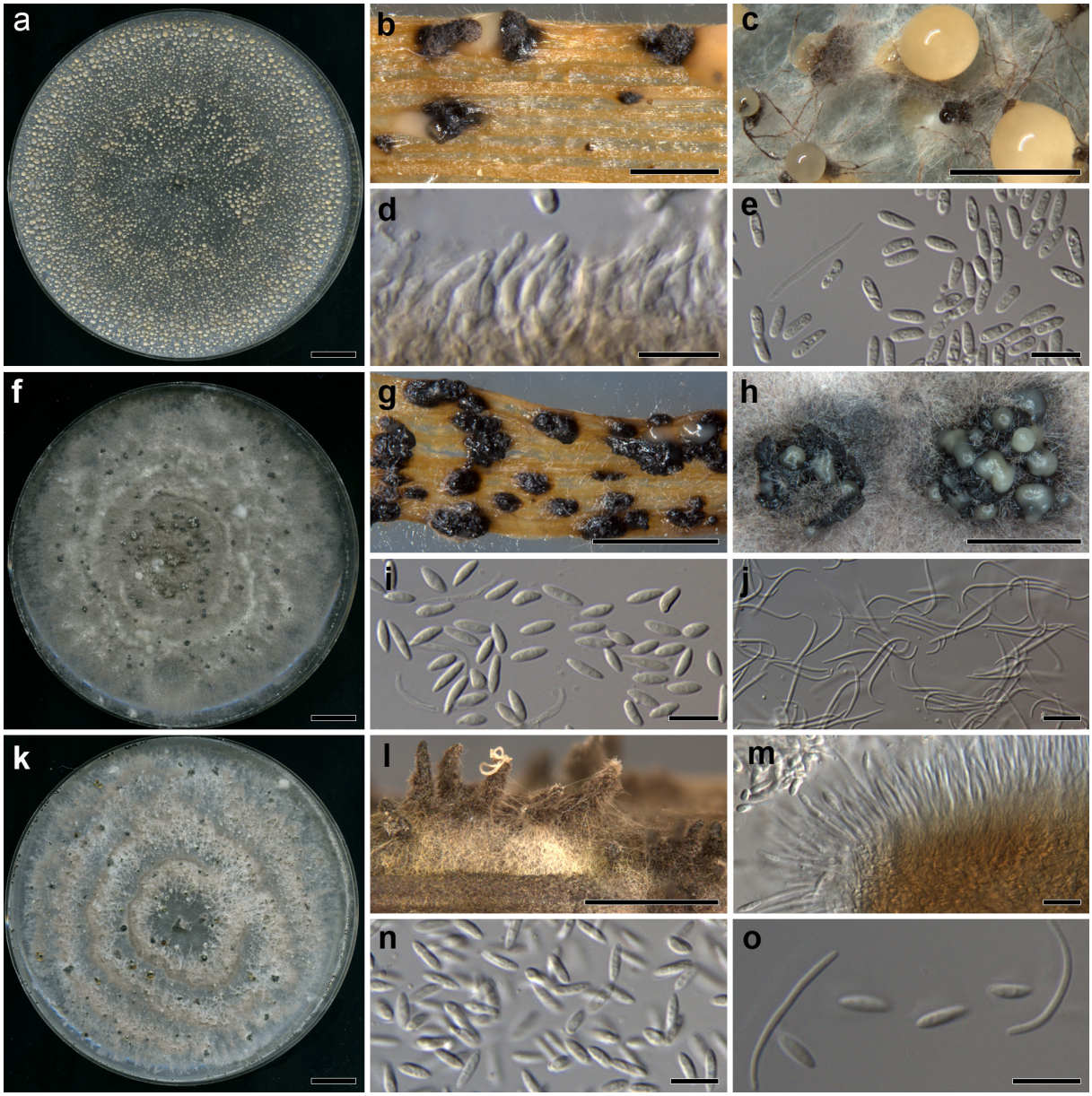

Fig. 2. ***Diaporthe beilharziae*** (ex-type BRIP 54792) after 4 weeks, **a** culture on PDA, **b** pycnidia on sterilised wheat straw, **c** conidial ooze, **d** conidiophores, **e** alpha conidia and a solitary beta conidium. ***Diaporthe fraxini-angustifoliae*** (ex-type BRIP 54781) after 4 weeks, **f** culture on PDA, **g** pycnidia on sterilised wheat straw, **h** conidial ooze, **i** alpha conidia, **j** beta conidia. ***Diaporthe litchicola*** (ex-type BRIP 54900) after 4 weeks, **k** culture on PDA, **l** pycnidia on sterilised wheat straw, **m** conidiophores, **n** alpha conidia, **o** alpha and beta conidia. Scale bars: a, f, k = 1 cm; b–c, g–h, l = 1 mm; d–e, i–j, m–o = 10 µm.

subglobose, with tan to white conidial droplets exuded from ostioles, ostiolar beaks mostly absent or rarely up to 100 µm high; walls thick, composed of inner layers of olivaceous brown textura angularis and an outer layer of reddish brown textura epidermoidea. Conidiophores formed from the inner layer of the locular wall, reduced to conidiogenous cell or 1-septate, hyaline to pale brown, cylindrical to lageniform, straight to sinuous, 5–30 × 1.5–4.0 µm. *Conidiogenous cells* phialidic, terminal, cylindrical, 5–15 × 1–2 µm, tapered towards the apex,

hyaline. *Alpha conidia* scarce, cylindrical to oval, attenuated at the ends, hyaline to subhyaline, (4–) 5–8.5 (−10) × 2–3 µm. *Beta conidia* abundant, flexuous to lunate, hyaline, (16–) 17–21 (−22) × 1.0 µm, truncate at the base, narrowed towards the acute apex, mostly curved through 45°-180° in the apical third. *Perithecia* not seen.

Culture characteristics: Colonies on PDA covering entire plate after 10 days, mouse grey, adpressed with scant aerial mycelium; reverse fuscous black. On OMA covering the entire plate after 10 days, with numerous confluent scattered tufts of mouse grey mycelium, adpressed in the centre with a 2 cm diam.; reverse mouse grey becoming fuscous black after 4 weeks.

Specimen examined: AUSTRALIA, Victoria, on *Fraxinus angustifolia* subsp. *oxycarpa* cv. Claret Ash, 31 Oct. 1979, *L. Smith* (VPRI 10911 holotype, includes ex-type culture), (BRIP 54781 isotype, includes ex-type culture).

Notes: Eight species of *Diaporthe* (Wehmeyer 1933) and five of *Phomopsis* (Uecker 1988) have been reported on *Fraxinus*. Wehmeyer (1933) placed all of these names in synonymy with *D. eres*, which he considered a large species complex that could not be separated by morphology. Some of these taxa have been linked as anamorph-teleomorph connections but these should be considered tentative as most connections in *Diaporthe* and *Phomopsis* are unproven (Uecker 1988). The taxa and presumed connections (in brackets) that have been reported on *Fraxinus* are *D. ciliaris*, *D. controversa* (*P. controversa*), *D. fraxini*, *D. obscurans*, *D. priva*, *D. samaricola* (*P. pterophila*, *P. samarorum*), *D. scobina* (*P. scobina*), *D. scobinoides* and *P. scobinella* (Wehmeyer 1933, Uecker 1988). Each of these species was reported from Europe, where stem necrosis on European ash (*Fraxinus excelsior*) is widely distributed in some countries (Przybł 2002). The role of these *Diaporthe* species in dieback of European ash is unclear (MacDonald and Russell 1937).

Diaporthe fraxini-angustifoliae was isolated from stems of *Fraxinus* sp. exhibiting dieback. Its role as a pathogen is not proven and uncertain. A massive occurrence of dieback of European ash in Austria in 2007 was attributed to *Hymenoscyphus pseudoalbidus* (syn. *Chalara fraxinea*) (Keβler et al. 2012). *Diaporthe fraxini-angustifoliae* produces copious amounts of beta conidia that measure 15–25 µm, a character that separates it from all other species except *P. scobinella*. *Diaporthe fraxini-angustifoliae* has much shorter alpha conidia (4–10 µm) than *P. scobinella* (8–12 µm).

The phylogenetic inference from the combined sequence data showed *D. fraxini-angustifoliae* clustered closely with *D. litchicola* and *D. pascoei*, which are newly described below, as well as with *D. musigena* (Fig. 1). *Diaporthe fraxini-angustifoliae* differs from *D. pascoei* in three loci: ITS positions 422 (G) and 424 (G); *tef1α* 93 % match (Identities 533/574 (93 %), Gaps 6/574 (1 %)); *tub2* 97 % match (Identities 649/666 (97 %), Gaps 1/666 (0 %)). *Diaporthe fraxini-angustifoliae* has longer and wider alpha conidia (4–10 × 2–3 µm) than *D. pascoei* (3.5–5×1–2 µm); and shorter beta conida (16–22 µm) *than D. litchicola* (17–37 µm). *Diaporthe fraxini-angustifoliae* cannot be differentiated from *D. musigena* (7–12 × 2–3 µm) by conidial size.

Diaporthe litchicola R.G. Shivas, K.R.E. Grice & Y.P. Tan, **sp. nov. –** MycoBank MB802385. Fig. 2k–o

Etymology: Named after the host genus from which it was collected, *Litchi*.

Pycnidia formed abundantly on OMA, PDA and wheat stems on WA after 4 weeks, solitary or in groups of up to 20 on a dark stroma with a sharp slightly raised and blackened margin, with black cylindrical ostiolate necks up to 1.5 mm. subglobose, up to 400 µm diam., *Conidiophores* reduced to *conidiogenous cells*, formed from the inner layer of the locular wall, hyaline, smooth, cylindrical, straight to sinuous, tapered towards the apex, 20–45 × 1.5–2.0 µm. *Alpha conidia* hyaline, smooth, guttulate, fusiform to oval, tapered at both ends, cylindrical to ellipsoidal, (5–) 6.5–9.5 (−10) × 1.5–2 (−2.5) µm. *Beta conidia* scattered amongst the alpha conidia, flexuous to lunate, (17–) 20–32 (−37) × 1.0–1.5 µm.

Culture characteristics: Colonies on PDA covering the entire plate after 10 days, ropey with abundant tufted white aerial mycelium, buff, numerous black conidiomata less than 0.5 mm diam. form in the mycelium mostly towards the edge of the colony; reverse buff with darker zonate and irregular lines corresponding to embedded conidiomata. On OMA covering the entire plate after 10 days, adpressed with scant white aerial mycelium and numerous scattered black conidiomata less than 1.0 mm diam.; reverse buff with numerous black conidiomata less than 1.0 mm diam. and ochreous irregular patches up to 6 mm diam., becoming rosy buff after 4 weeks. On WA covering the entire plate after 4 weeks, transparent, agar tinted rosy vinaceous.

Specimen examined: AUSTRALIA, Queensland, Mareeba, on *Litchi chinensis*, 22 Nov. 2011, *K.R.E. Grice* (BRIP 54900 holotype, includes ex-type culture).

Notes: *Diaporthe litchicola* was isolated from dieback of lychee (*Litchi chinensis*) in northern Queensland. One other species has been reported from lychee, namely *P. litchichinensis*, which was described from dark brown leaf spots in India (Prameela and Chowdhry 2004). *Diaporthe litchicola* has shorter and narrower alpha conidia (5–10×1.5–2.5 µm) than *P. litchi-chinensis* (10–12×3–5 µm) for which there is no DNA sequence data available. A culture of was *P. litchi-chinensis* was deposited in the Indian Type Culture Collection (ITCC 5420), which will facilitate future comparative study.

The phylogenetic inference from the combined sequence data showed *D. litchicola* cluster closely with *D. fraxini-angustifoliae* and *D. pascoei* (Fig. 1). Unique nucleotide differences are relied upon to differentiate *D. litchicola* from *D. pascoei*. *Diaporthe litchicola* differs from *D. pascoei* in three loci: ITS positions 92 (C), 421 (G) and 424 (G); *tef1α* 92 % match (Identities 558/609 (92 %), Gaps 7/609 (1 %)); *tub2* 96 % match (Identities 641/667(96 %), Gaps 2/667 (0 %)). Morphologically *D. litchicola* has narrower alpha conidia that *D. fraxini-angustifoliae* (2–3 µm) and longer alpha conidia than *D. pascoei* (3.5–5 µm).

Diaporthe nothofagi R.G. Shivas, J. Edwards & Y.P. Tan, **sp. nov. –** MycoBank MB802386. Fig. 3h–i

Etymology: Named after the host genus from which it was collected, *Nothofagus*.

Hyphae on PDA after 4 weeks septate, smooth, mostly hyaline 1–3 µm wide, scarcely brown 3–8 µm wide. Perithecia and pycnidia not produced on PDA, OMA or wheat straw pieces on WA after 4 weeks.

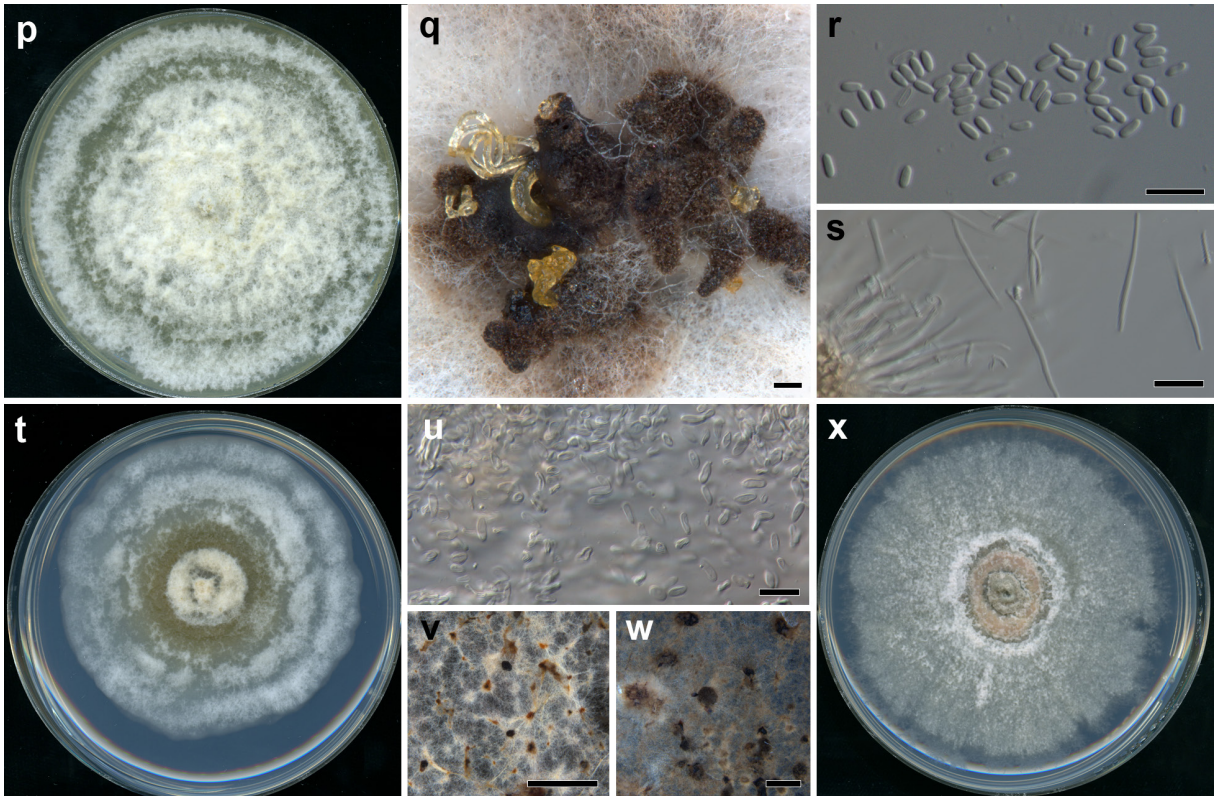

Fig. 3. ***Diaporthe pascoei*** (ex-type VPRI 16058) after 4 weeks, **p** on PDA, **q** pycnidia on dried plate of PDA, **r** alpha conidia, **s** conidiophores and beta conidia. ***Diaporthe salicicola*** (ex-type VPRI 32789) after 4 weeks, **t** on PDA, **u** alpha conidia from dried voucher specimen, **v** dried culture on PDA; ***Diaporthe nothofagi*** (ex-type VPRI 22429) after 4 weeks **w** dried culture on PDA, **x** on PDA. Scale bars: a, e, i = 1 cm; b = 100 µm; c–d, f = 10 µm; g–h = 1 mm.

Culture characteristics: Colonies on PDA reaching 6 cm diam. after 10 days, adpressed, white to pale grey becoming amber in the centre, lighter towards the margin, after 4 weeks reaching 7 cm diam., becoming flesh coloured in the centre; reverse amber, darker towards the centre. On OMA covering the entire plate after 10 days, adpressed, transparent with scant white aerial mycelium, rosy buff towards the central 2 cm diam. with scant grey aerial mycelium, after 4 weeks covering entire plate, salmon towards the margins and flesh coloured in the centre; reverse salmon in the central part becoming lighter and transparent towards the margin.

Specimen examined: AUSTRALIA, Victoria, Carlton, on *Nothofagus cunninghamii*, 31 Oct. 2000, *C. Brenchley* (VPRI 22429b holotype, includes ex-type culture), (BRIP 54801 isotype, includes ex-type culture).

Notes: *Diaporthe nothofagi* was isolated from brown streaks at the base of the trunk of *Nothofagus cunninghamii*. There are no apparent literature records of *Diaporthe* or *Phomopsis* species on *Nothofagus*. It is not known whether *D. nothofagi* is a pathogen, saprobe or endophyte.

The Victorian voucher specimen of *D. nothofagi* comprised a dried culture on PDA in a 9 cm Petri dish. The specimen had numerous scattered immature pycnidia without beaks and with empty locules. The associated living culture was sterile. The phylogenetic inference from

combined dataset show a very strong bootstrap value at the node (100 %), thus supporting the introduction of *D. nothofagi* as a new taxon.

Diaporthe pascoei R.G. Shivas, J. Edwards & Y.P. Tan, **sp. nov. –** MycoBank MB802387. Fig. 3a–d

Etymology: In recognition of Ian Pascoe, an excellent mycologist and plant pathologist, mentor and friend who collected and isolated the fungus.

Conidiomata pycnidial, scattered, solitary or aggregated in groups up to 6 mm diam.on PDA, with conidial droplets exuded from ostioles, ostiolar beaksmostly up to 1.5mm high. *Conidiophores* formed from the inner layer of the locular wall, 1–2-septate near the base, hyaline, cylindrical, straight, unbranched, 5–40 × 2–3 µm. *Conidiogenous cells* phialidic, terminal, cylindrical, 5–30 × 2–3 µm, tapered towards the apex, hyaline. *Alpha conidia* scarce, cylindrical, rounded at the apex, slightly attenuated at the base, hyaline, (3.5–) 4–5 × 1–2 µm. *Beta conidia* abundant, flexuous to lunate, hyaline, (15–) 19–31 (−39) × 1.0–1.5 µm, truncate at the base, narrowed towards the apex, often curved up to 90° in the apical part. *Perithecia* and pycnidia not produced on PDA, OMA or wheat straw pieces on WA after 4 weeks.

Culture characteristics: Colonies on PDA covering the entire plate after 10 days, pale luteous with abundant white compact aerial mycelium; reverse pale luteous becoming umber towards the centre. OnOMA covering the entire plate after 10 days, adpressed, honey with abundant white aerial mycelium towards the margin; reverse honey with three zonate isabelline rings.

Specimen examined: AUSTRALIA, Victoria, on *Persea americana*, 29 Nov. 1988, *I.G. Pascoe* (VPRI 16058 holotype, includes ex-type culture), (BRIP 54847 isotype, includes ex-type culture).

Notes: The ex-type culture of *D. pascoei* had lost its ability to sporulate. The morphological description given above is based on the holotype, which comprised three dried cultures grown on PDA supplemented with aureomycin in 9 cm diam. Petri dishes. *Diaporthe pascoei* was isolated from fruit rot of avocado (*Persea americana*). A note with the holotype specimen states that the fungus was isolated from pocket rot of the stem end with severe discoloration of vascular throughout the fruit. Uecker (1988) lists one species, *P. perseae*, on dying branches of avocado. *Phomopsis perseae* was part of the complex of fungi that caused stem-end rot of avocado in Australia (Peterson 1978) and South Africa (Darvas and Kotzé 1987). *Diaporthe pascoei* has much smaller alpha conidia (3.5–5 × 1–2 µm), than those of *P. perseae*, which Uecker (1988) listed as 7–10.2 × 2.3–2.5 µm.

There are no DNA sequences available for any ex-type culture of *P. perseae*, although Gomes et al. (2013) provided sequence data for a strain (CBS 151.73) they considered authentic when transferring *P. perseae* to *Diaporthe*. The MegaBLAST comparison of the ITS sequence of *D. pascoei* against the three available sequences of *P. perseae* showed a 92 % match to an isolate from South Africa (GU967697) (Identities=489/534 (92 %), Gaps=9/534 (9 %)), a 87 % match to the ITS1 sequence of an Australian isolate BRIP 5513 (AY705859) (Identities 155/179 (87 %), Gaps 7/179 (4 %)), and a 91 % match to the ITS2 sequence of BRIP 5513 (AY705860) (Identities 147/161 (91 %), Gaps 2/161 (1 %)) (data not shown).

The phylogenetic inference from the combined sequence data showed *D. pascoei* clustered close to *D. fraxini-angustifoliae*, *D. litchicola* and *D. musigena* (Fig. 1). *Diaporthe pascoei* differs from *D. musigena* in three loci: ITS positions 92 (T) and 541 (T); *tef1α* 92 % match (Identities 280/303 (92 %), Gaps 4/303(1 %)); *tub2* 97 % match (Identities 645/666 (97 %), Gaps 1/666 (0 %)). Refer to the discussion under the earlier description of *D. fraxini-angustifoliae* for morphological differentiation of these three species.

Diaporthe salicicola R.G. Shivas, J. Edwards & Y.P. Tan, **sp. nov. –** MycoBank MB803338; Fig. 3e–g

Etymology: Named after the host genus from which it was collected, *Salix*.

Mycelium on PDA after 4 weeks adpressed, forming a pellicle on the surface. *Conidiomata* pycnidial, solitary, scattered, with ostiolar beaks mostly up to 400 µm high on PDA. *Conidiophores* formed from the inner layer of the locular wall, hyaline, cylindrical, straight, 1–3-septate, unbranched, 10–25 × 2–3 µm. *Conidiogenous cells* phialidic, terminal, cylindrical, 10–20 × 2–3 µm, tapered towards the apex, hyaline. *Alpha conidia* abundant, cylindrical to oval, rounded at the apex, slightly attenuated at the base, hyaline, (4–) 5–7 (−8) × 1.5–2.5 µm. *Beta conidia* not seen. *Perithecia* and pycnidia not produced on PDA, OMA or wheat straw pieces on WA after 4 weeks.

Culture characteristics: Colonies on PDA reaching 7 cm diam. after 10 days, adpressed towards the centre with abundant white aerial mycelium towards the margin, ochreous towards the centre, zonate; reverse umber at the centre becoming ochreous and then transparent towards the margin, zonate. On OMA reaching 6 cm after 10 days, adpressed, transparent to buff with scant white aerial mycelium; reverse transparent to faintly buff.

Specimens examined: AUSTRALIA, Tasmania, Blackfish Creek, on *Salix purpurea*, 31 Jul. 2007, *K. Finlay & R. Adair* (VPRI 32789 holotype, includes ex-type culture), (BRIP 54825 isotype, includes ex-type culture).

Notes: *Diaporthe salicicola* has conidia that are similar in size to *Phomopsis salicina*, which was originally described as *Phoma salicina* from branches and bark of *Salix* in France and Germany (Saccardo 1884, Diedicke 1911). The morphological description of *D. salicicola* was based on two dried cultures grown on PDA in 9 cm diam. Petri dishes that formed part of the voucher specimen. *Diaporthe salicicola* was associated with a leaf spot of *Salix purpurea*, which is native to Europe and an invasive weedy shrub or small tree in southern Australia (Parsons and Cuthbertson 2001). We have chosen to describe the Australian isolate as a new species of *Diaporthe* rather than identify it as *Phomopsis salicina*, which occurs in Europe. This approach creates a more stable taxonomy, especially if it was later learnt that there is a complex of small-spored cryptic *Diaporthe* species associated with *Salix*.

At least five species of *Diaporthe* (Wehmeyer 1933) and four species of *Phomopsis* (Uecker 1988) have been reported on *Salix*. Some of these taxa have been linked as anamorph-teleomorph connections but these should be considered tentative as most connections in *Diaporthe* and *Phomopsis* are unproven (Uecker 1988). The taxa and presumed connections (in brackets) that have been reported on *Salix* are *D. glyptica*, *D. mucronata*, *D. spina* (*P. leucostoma* as

'*leucostemum*'), *D. tessella* (*P. systema-solare*), *D. tessellata*, *P. pallida*, *P. salicina* and *P. salicina* f. *longipes* (Wehmeyer 1933, Uecker 1988). The role of these *Diaporthe* species in diseases of Salix is unclear. *Phomopsis salicina* was listed as a potential biological control agent for *Salix* spp. in Australia based on its association with leaf and stem spots in Lithuania (Adair et al. 2006).

The phylogenetic inference from combined sequence data show that *D. salicicola* clustered close to *D. cynaroidis* (Fig. 1). *Diaporthe salicicola* differs from *D. cynaroidis* in three loci: ITS positions 124 (G), 459 (T), 512 (C) and 533 (T); *tef1α* positions 1 (G) and 548 (C); *tub2* positions 7 (G), 112 (C), 113 (A), 129 (C), 143 (A), 534 (C), 637 (T) 673 (G) and 719 (T).

DISCUSSION

In this study, six new species have been described in *Diaporthe*, on the basis of morphological and molecular characteristics. Three of the species, *D. nothofagi*, *D. pascoei*, and *D. salicicola*, were sterile under the conditions that they were grown and did not produce any sporocarps. Voucher specimens of *D. pascoei* and *D. salicicola* from the original collections dating back to 1988 and 2007, respectively, had pycnidia and conidia that allowed morphological descriptions to be completed.

A phylogenetic tree derived from an alignment of ITS sequences is useful as a guide for identification of isolates of *Diaporthe* species (Udayanga et al. 2012b). ITS sequences provide persuasive evidence for species delineation where a few taxa are analysed, such as species associated with the same host (Santos and Phillips 2009; Santos et al. 2011; Thompson et al. 2011), although confusion arises when a large number of species from a wide range of host species are analysed. Santos et al. (2010) suggested that *tef1α* is a better phylogenetic marker in *Diaporthe* than ITS, and has been widely used as a secondary locus for phylogenetic studies (Santos et al. 2011, Udayanga et al. 2012a, 2012b). Gomes et al. (2013) examined five loci from 95 species. They found that *tef1α* poorly discriminated species, and suggested that histone and BT were the better candidates as secondary phylogenetic markers to accompany the official fungi barcode, ITS. In this study, a combined three gene analysis of ITS, *tef1α* and *tub2* was used to support the introduction of six new *Diaporthe* species.

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CHAPTER 3

Johnalcornia gen. et. comb. nov., and nine new combinations in *Curvularia* based on molecular phylogenetic analysis

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Abstract

An examination of ex-type and authentic cultures of 34 species of *Bipolaris* and *Curvularia* by phylogenetic analysis of four loci (*gapdh*, ITS, LSU and *tef1α*) resulted in nine new combinations in *Curvularia*, as well as new synonymies for some species of *Bipolaris* and *Curvularia*. Lectotypes are designated for *Bipolaris secalis* and *Curvularia richardiae*, and an epitype is designated for *Curvularia crustacea*. A new monotypic genus, *Johnalcornia*, is introduced to accommodate *Bipolaris aberrans*, which clusters sister to the newly described *Porocercospora*. *Johnalcornia* differs morphologically from this taxon by producing distinctive conidia-like chlamydospores as well as comparatively thick-walled, geniculate conidiophores, with conidiogenous cells that have conspicuous scars. *Johnalcornia* further differs from related genera by forming the second conidial septum in the apical cell.

INTRODUCTION

The helminthosporioid genera *Bipolaris* (Shoemaker 1959) and *Curvularia* (Boedijn 1933) include many important plant pathogens, particularly of grasses (*Poaceae*). These fungi are associated with diseases on more than 60 host plant genera (Sivanesan 1987, Manamgoda et al. 2011). In the past, the classification of *Bipolaris* and *Curvularia* spp. was entirely based on morphological characteristics. Index Fungorum lists 116 species of *Bipolaris* and 120 of *Curvularia*, of which 96 and 85 %, respectively, were originally described solely from morphology. As *Bipolaris* and *Curvularia* share many similar morphological characteristics, this approach has been unreliable for the allocation of new taxa to either genus. In the last decade, molecular phylogenetic approaches have provided additional reliable criteria that have allowed the development of a more stable taxonomy for these genera (Manamgoda et al. 2012b).

Bipolaris and *Curvularia* have morphologically similar sexual morphs, which were often classified in *Cochliobolus* (Drechsler 1934), although sexual morphs are not known for all species of *Bipolaris* and *Curvularia* (Sivanesan 1987). Berbee et al. (1999) investigated the phylogenetic relationships between *Bipolaris*, *Cochliobolus* and *Curvularia* by comparison of DNA sequences from the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (nrDNA), and a fragment of the glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) gene. They observed that species of *Bipolaris* and *Curvularia*, with and without known sexual morphs, were monophyletic in the broad sense, yet clustered into two well resolved groups. Berbee et al. (1999), were reluctant to separate these two groups into formal taxonomic ranks, i.e. genera, as this required the transfer of *Cochliobolus hawaiiensis* (asexual morph: *B. hawaiiensis*) and *Cochliobolus ravenelii* (asexual morph: *B. ravenelii*), which have straight conidia, into *Curvularia* that typically has curved conidia. Such a taxonomic decision would have been in contrast to the original morphological concepts accepted for these genera.

Manamgoda et al. (2012b) also showed that species of *Bipolaris* and *Curvularia* separated into two well-defined groups based on phylogenetic analyses of either two genetic loci (ITS and *gapdh*) or four loci (ITS, 28S large subunit nrDNA (LSU), *gapdh*, and translation elongation factor-1α (*tef1α*)). They subsequently transferred *B. hawaiiensis*, *B. ravenellii* and four other *Bipolaris* species to *Curvularia* on the basis of phylogenetic analysis that included DNA sequences from a designated neotype of *Curvularia lunata*, which is the type species of *Curvularia*.

Manamgoda et al. (2012b) noted that there remained 95 *Bipolaris* spp., 30 *Cochliobolus* spp., and 101 *Curvularia* spp. whose ex-type cultures, if available, had not been sequenced. Consequently, these species could not be assigned to either of the newly circumscribed genera *Bipolaris* or *Curvularia*. In this paper, we applied molecular phylogenetic methods to 50 taxa (including the outgroup) to resolve or confirm the taxonomy of 34 species of *Bipolaris* and *Curvularia*, by examining selected regions of genomic DNA extracted from ex-type and reference cultures.

MATERIALS AND METHODS

Isolates and morphology

All isolates examined, including 45 isolates located at the Queensland Plant Pathology Herbarium (BRIP, Dutton Park, Australia), are listed in Table 1. Isolates from both BRIP and the Westerdijk Fungal Biodiversity Centre (CBS) are retained as living cultures in a metabolically inactive state (deep-frozen), which renders them acceptable as types according to Art. 8.4 of the current edition of the International Code of Nomenclature for Algae, Fungi, and Plants (Melbourne Code). The images presented in Figs. 2–4 were taken from dried specimens mounted on glass slides in lactic acid (100 % v/v), and captured with a Leica DFC 500 camera attached to a Leica DM5500B compound microscope with Nomarski differential interference contrast illumination.

DNA isolation, amplification, and phylogenetic analyses

The isolates were grown on potato dextrose agar (PDA) (Becton Dickinson) for 7 days at room temperature (approx. 23–25 °C). Mycelia were scraped off the PDA cultures and macerated with 0.5 mm glass beads (Daintree Scientific) in a Tissue Lyser (QIAGEN). Genomic DNA was extracted with the Gentra Puregene DNA Extraction Kit (QIAGEN) according to the manufacturer's instructions.

The primers V9G (de Hoog and Gerits van den Ende 1998) and ITS4 (White et al. 1990) were used to amplify the ITS region of the nrDNA. The primers gpd1 and gpd2 (Berbee et al. 1999) were used to amplify part of the *gapdh* gene. Partial regions of the LSU and *tef1α* loci were amplified using the primers LR0R/LR5 and EF1983/EF12218R, respectively (Schoch et al. 2009). All loci were amplified with the Phusion High-Fidelity PCR Master Mix (New England Biolabs). The PCR products were purified and sequenced by Macrogen Incorporated (Seoul, Korea) on the 3730xl DNA Analyzer (Applied Biosystems) using the amplifying primers.

All sequences generated were assembled using Vector NTi Advance v. 11.0 (Invitrogen), and deposited in GenBank (Table 1, in bold). These sequences were aligned with sequences of *Bipolaris* and *Curvularia* spp. obtained from GenBank (Table 1) using the MAFFT alignment algorithm (Katoh et al. 2009) in the software Geneious (Biomatters Ltd). *Alternaria alternata* (CBS 916.96) was included as the outgroup (Table 1). The sequences of each locus were aligned separately and manually adjusted as necessary. Alignment gaps were treated as missing character states, and all characters were unordered and of equal weight. The alignments were uploaded to Gblocks Server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html), and curated to remove poorly aligned positions and divergent regions (Talavera and Castresana 2007). The alignments were trimmed as follows: ITS from 423 nucleotides, including gaps, to

Table 1. *Bipolaris* and *Curvularia* isolates examined.

Species	Isolate no.[a]	Host/Substrate	Locality	GenBank accession numbers[b]			
				ITS	*gapdh*	LSU	*tef1α*
Alternaria alternata	CBS 916.96[T]	*Arachis hypogaea*	India	AF071346	AF081400	DQ678082	DQ677927
Bipolaris chloridis	BRIP 10965	*Chloris gayana*	Australia	**KJ415523**	**KJ415423**	**KJ415476**	**KJ415472**
Bipolaris clavata	BRIP 12530[T]	*Dactyloctenium radulans*	Australia	**KJ415524**	**KJ415422**	**KJ415477**	**KJ415471**
Bipolaris coffeana	BRIP 14845[T]	*Coffea arabica*	Kenya	**KJ415525**	**KJ415421**	**KJ415478**	**KJ415470**
Bipolaris crotonis	BRIP 14838[T]	*Croton* sp.	Samoa	**KJ415526**	**KJ415420**	**KJ415479**	**KJ415469**
	BRIP 15875	*Eleusine indica*	Australia	**KJ415527**	**KJ415419**	**KJ415480**	**KJ415468**
Bipolaris gossypina	BRIP 14840[T]	*Gossypium* sp.	Kenya	**KJ415528**	**KJ415418**	**KJ415481**	**KJ415467**
Bipolaris heliconiae	BRIP 17189	*Heliconia chartacea*	Australia	**KJ415529**	**KJ415416**	**KJ415482**	**KJ415466**
	BRIP 17186[T]	*Heliconia psittacorum*	Australia	**KJ415530**	**KJ415417**	**KJ415483**	**KJ415465**
Bipolaris luttrellii	BRIP 14643[T]	*Dactyloctenium aegyptium*	Australia	AF071350	AF081402	**KJ415484**	**KJ415464**
Bipolaris maydis	CBS 136.29[T]	*Zea mays*	Japan	HF934926	HG779086	HF934879	KJ415463
Bipolaris microlaenae	CBS 280.91[T]	*Microlaena stipoides*	Australia	JN600974	JN600974	JN600995	JN601017
Bipolaris panici-miliacei	BRIP 12282[T]	*Panicum miliaceum*	Japan	**KJ415531**	**KJ415415**	**KJ415485**	**KJ415462**
Bipolaris peregianensis	BRIP 12790[T]	*Cynodon dactylon*	Australia	JN601034	JN600977	JN601000	JN601022
Bipolaris pluriseptata	BRIP 14839[T]	*Eleusine coracana*	Zambia	**KJ415532**	**KJ415414**	**KJ415486**	**KJ415461**
Bipolaris salviniae	BRIP 12312	*Melinis minutiflora*	Australia	**KJ415533**	**KJ415413**	**KJ415487**	**KJ415460**
	BRIP 12898			JN601035	JN600972	**KJ415488**	**KJ415459**
	BRIP 15895			**KJ415534**	**KJ415412**	**KJ415489**	**KJ415458**
	BRIP 16571[T]	*Salvinia auriculata*	Brazil	**KJ415535**	**KJ415411**	**KJ415490**	**KJ415457**
	BRIP 13795	*Triticum aestivum*	Paraguay	**KJ415536**	**KJ415410**	**KJ415491**	**KJ415456**

Table 1. (Ctd).

Species	Isolate no.[a]	Host/Substrate	Locality	GenBank accession numbers[b]			
				ITS	*gapdh*	LSU	*tef1α*
Bipolaris zeae	BRIP 11512^{T}	*Zea mays*	Australia	**KJ415538**	**KJ415408**	**KJ415493**	**KJ415454**
Curvularia akaiiensis	BRIP 16080^{T}	unknown	India	**KJ415539**	**KJ415407**	**KJ415494**	**KJ415453**
Curvularia alcornii	MFLUCC 10-0703^{T}	*Zea mays*	Thailand	JX256420	JX276433	JX256387	JX266589
Curvularia asianensis	MFLUCC 10-0711^{T}	*Panicum* sp.	Thailand	JX256424	JX276436	JX256391	JX266593
Curvularia australiensis	CBS 172.57	*Oryza sativa*	Vietnam	JN601026	JN601036	JN600981	JN601003
	BRIP 12044^{T}		Australia	**KJ415540**	**KJ415406**	**KJ415495**	**KJ415452**
Curvularia australis comb. nov.	BRIP 12521^{T}	*Sporobolus caroli*	Australia	**KJ415541**	**KJ415405**	**KJ415496**	**KJ415451**
Curvularia bannonii	BRIP 16732^{T}	*Jacquemontia tamnifolia*	USA	**KJ415542**	**KJ415404**	**KJ415497**	**KJ415450**
Curvularia bothriochloae	BRIP 12522^{T}	*Bothriochloa bladhii*	Australia	**KJ415543**	**KJ415403**	**KJ415498**	**KJ415449**
Curvularia coicis	CBS 192.29^{T}	*Coix lacryma-jobi*	Japan	AF081447	AF081410	JN600984	JN601006
Curvularia crustacea comb. nov.	BRIP 13524^{T}	*Sporobolus* sp.	Indonesia	**KJ415544**	**KJ415402**	**KJ415499**	**KJ415448**
Curvularia dactyloctenii comb. nov.	BRIP 12846^{T}	*Dactyloctenium radulans*	Australia	**KJ415545**	**KJ415401**	**KJ415500**	**KJ415447**
Curvularia ellisii	CBS 193.62	air	Pakistan	JN192375	JN600963	JN600963	JN601007
Curvularia graminicola	BRIP 23186	*Aristida ingrata*	Australia	JN192376	JN600964	JN600986	JN601008
Curvularia harveyi	BRIP 57412^{T}	*Triticum aestivum*	Australia	**KJ415546**	**KJ415400**	**KJ415501**	**KJ415446**
Curvularia hawaiiensis	BRIP 11987^{T}	*Oryza sativa*	USA	**KJ415547**	**KJ415399**	**KJ415502**	**KJ415445**
Curvularia heteropogonicola	BRIP 14579^{T}	*Heteropogon contortus*	India	**KJ415548**	**KJ415398**	**KJ415503**	**KJ415444**

Table 1. (Ctd).

Species	Isolate no.[a]	Host/Substrate	Locality	GenBank accession numbers[b]			
				ITS	*gapdh*	LSU	*tef1α*
Curvularia heteropogonis	CBS 284.91	*Heteropogon contortus*	Australia	KJ415549	JN600969	JN600990	JN601013
Curvularia homomorpha comb. nov.	BRIP 59391^{T}	air	USA	JN192380	JN600970	JN600991	JN601014
Curvularia lunata	CBS 730.96^{T}	Human lung biopsy	USA	JX256429	JX276441	JX256396	JX266596
Curvularia neergaardii comb. nov.	BRIP 12919^{T}	*Oryza sativa*	Ghana	**KJ415550**	**KJ415397**	**KJ415504**	**KJ415443**
Curvularia nicotiae comb. nov.	BRIP 11983^{T}	soil	Algeria	**KJ415551**	**KJ415396**	**KJ415505**	**KJ415442**
Curvularia ovariicola	CBS 470.90^{T}	*Eragrostis interrupta*	Australia	JN192384	JN600976	JN600998	JN601020
Curvularia papendorfii	BRIP 57608^{T}	*Acacia karroo*	South Africa	**KJ415552**	**KJ415395**	**KJ415506**	**KJ415441**
Curvularia perotidis	BRIP 13466^{T}	*Perotis rara*	Australia	JN192385	KJ415394	JN600999	JN601021
Curvularia portulacae comb. nov.	BRIP 14541^{T}	*Portulaca oleracea*	USA	**KJ415553**	**KJ415393**	**KJ415507**	**KJ415440**
	BRIP 14837^{T}	soil	New Zealand	**KJ415554**	**KJ415392**	**KJ415508**	**KJ415439**
Curvularia ravenelii	BRIP 13165^{T}	*Sporobolus fertilis*	Australia	JN192386	JN600978	JN601001	JN601024
Curvularia richardiae	BRIP 4371^{T}	*Richardia brasiliensis*	Australia	**KJ415555**	**KJ415391**	**KJ415509**	**KJ415438**
Curvularia ryleyi comb. et nom. nov.	BRIP 12554^{T}	*Sporobolus creber*	Australia	**KJ415556**	**KJ415390**	**KJ415510**	**KJ415437**
	BRIP 12637	*Sporobolus elongatus*	Australia	**KJ415557**	**KJ415389**	**KJ415511**	**KJ415436**
Curvularia sorghina	BRIP 15900^{T}	*Sorghum bicolor*	Australia	**KJ415558**	**KJ415388**	**KJ415512**	**KJ415435**

Table 1. (Ctd).

Species	Isolate no.[a]	Host/Substrate	Locality	GenBank accession numbers[b]			
				ITS	*gapdh*	LSU	*tef1α*
Curvularia spicifera	CBS 274.52[T]	soil	Spain	JN192387	JN600979	JX256400	JN601023
Curvularia tripogonis	BRIP 12375[T]	*Tripogon loliiformis*	Australia	JN192388	JN600980	JN601002	JN601025
Curvularia tropicalis comb. nov.	BRIP 14834[T]	*Coffea arabica*	India	**KJ415559**	**KJ415387**	**KJ415513**	**KJ415434**
Curvularia tuberculata	CBS 146.63	*Zea mays*	India	JX256433	JX276445	JX256401	JX266599
Johnalcornia aberrans gen. et. comb. nov.	BRIP 16281[T]	*Eragrostis parviflora*	Australia	**KJ415522**	**KJ415424**	**KJ415475**	**KJ415473**
Porocercospora seminalis	CBS 134906[T]	*Bouteloua dactyloides*	USA	HF934941	KJ415379	HF934862	KJ415426

[a] BRIP: Plant Pathology Herbarium, Dutton Park, Queensland, Australia; CBS: Westerdijke Fungal Biodiversity Institute, Utrecht, The Netherlands; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; VPRI: Victorian Plant Pathology Herbarium, Bundoora, Victoria, Australia.

[b] ITS: internal transcribed spacer; *gapdh*: glyceraldehyde-3-phosphate dehydrogenase; LSU: large subunit; *tef1α*: translation elongation factor 1-alpha.

Newly deposited sequences are in **bold**.

[T] Ex-type culture.

317 with no gaps; and *gapdh* from 513, including gaps, to 386 with no gaps. The alignments of LSU and *tef1α* did not require trimming. Bayesian analysis was performed with MrBayes v. 3.2.1 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003) in Geneious. The Markov Chain Monte Carlo (MCMC) analysis used four chains and started from a random tree topology. The sample frequency was set at 200 and the temperature of the heated chain was 0.3. Burn-in was set at 25 % after which the likelihood values were stationary. Maximum Likelihood (ML) analysis including 1 000 bootstrap replicates was run using RAxML v. 7.2.8 (Stamatakis and Alchiotis 2010) in Geneious. The nucleotide substitution model used was General Time Reversible (GTR) with a gamma-distributed rate variation. The concatenated alignment and resulting tree were deposited in TreeBASE (Study S15335), and nomenclatural novelties were deposited in MycoBank (www.MycoBank.org, Crous et al. 2004).

RESULTS

Phylogenetic analysis

The combined (ITS, *gapdh*, LSU and *tef1α*) alignment is composed of 59 isolates (including the outgroup) and 2 303 characters. The inferred phylogenetic tree validated the generic placement of 12 species of *Bipolaris* and eight species of *Curvularia* (Fig. 1). A further nine species of *Bipolaris* appeared in the clade that include the type species of *Curvularia* (Fig. 1). Consequently, these species of *Bipolaris* are transferred to *Curvularia* and listed below as novel combinations, alongside species of *Curvularia* that have not been previously validated by DNA sequences.

The phylogenetic tree indicates that *B. aberrans* does not belong to either *Bipolaris* or *Curvularia* (Fig. 1). It is sister to the recently described monotypic genus, *Porocercospora* (Amaradasa et al. 2014). *Bipolaris aberrans* differs morphologically from *P. seminalis*, which has comparatively thin-walled, non-geniculate conidiophores, with conidiogenous cells that have unthickened and inconspicuous scars. Based on multilocus sequence analysis (Fig. 1) and morphology (Fig. 2), *B. aberrans* is assigned to a new genus.

Taxonomy

Johnalcornia Y.P. Tan & R.G. Shivas, **gen. nov.** – MycoBank MB807731. Fig. 2

Etymology: Named after the Australian mycologist, John Leonard Alcorn, in recognition of his substantial contributions towards the taxonomy of helminthosporioid fungi, including the original description of *Bipolaris aberrans*.

Conidiophores solitary or in fascicles, simple or branched, straight to flexuous, apically geniculate, cylindrical, smooth. *Conidiogenous cells* integrated, cylindrical, proliferating sympodially, smooth to verruculose, with thickened and conspicuous scars. *Conidia* solitary, straight to curved, smooth, hilum inconspicuous, distoseptate, germinating from both polar cells, first conidial septum median or submedian, second conidial septum delimiting the apical cell, third septum basal. *Ascomata* globose with a short neck. *Asci* cylindrical to fusoid, straight or curved. *Ascospores* hyaline, filiform, tightly coiled.

Type species: *Johnalcornia aberrans* (Alcorn) Y.P. Tan & R.G. Shivas

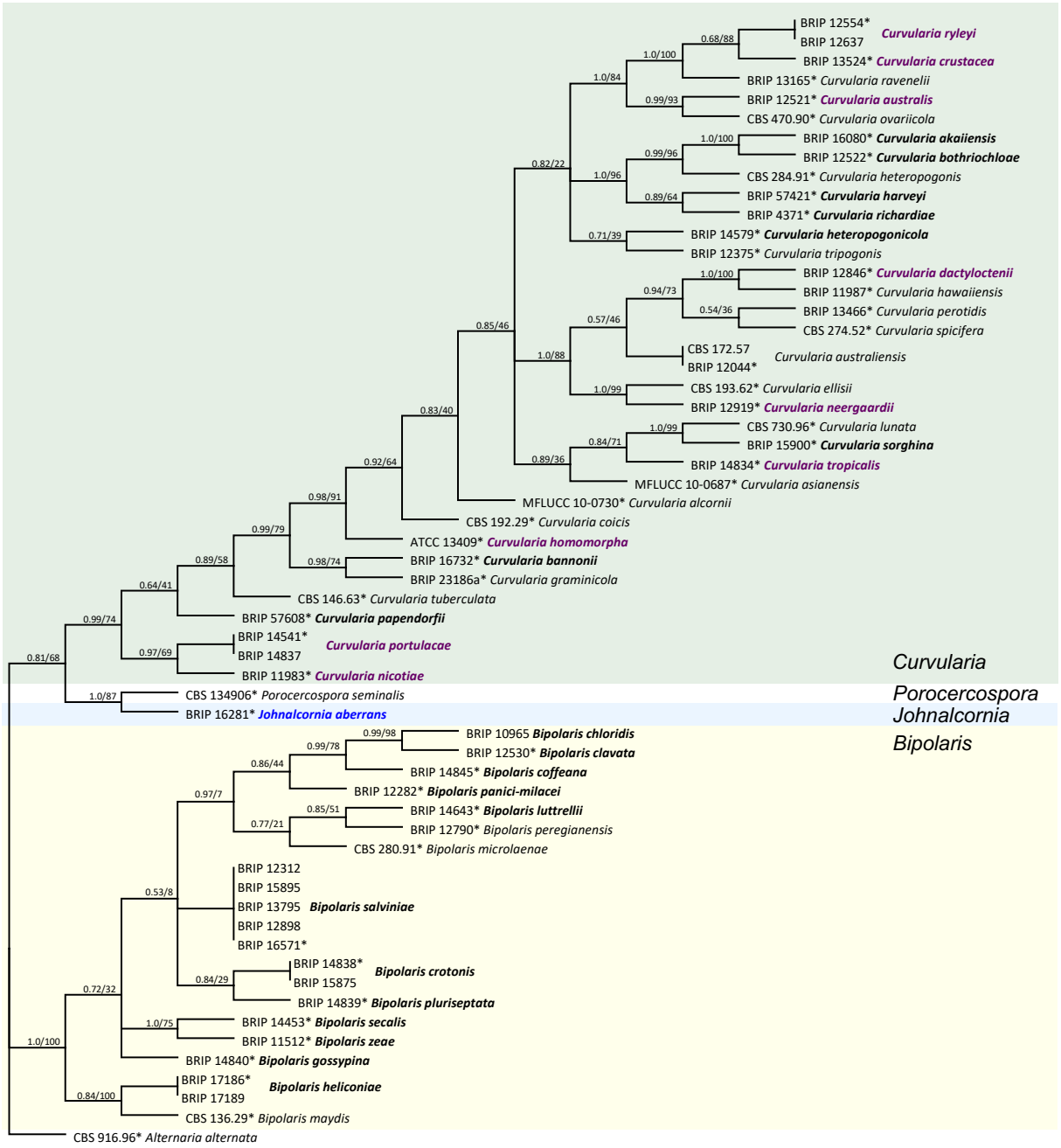

Fig. 1. Phylogenetic tree based on the combined multilocus alignment. Bayesian posterior probabilities (pp) and RAxML bootstrap values (bs) are given at the nodes (pp/bs). Species transferred from *Bipolaris* to *Curvularia* are shown in bold violet, and *Johnalcornia aberrans* gen. et. comb. nov. is shown in bold blue. The *Bipolaris*, *Curvularia* and *Johnalcornia* clades are highlighted by coloured boxes. Species that are validated in this study are in bold. Ex-type isolates of the currently accepted names are marked with an asterisk (*). The tree is rooted to *Alternaria alternata* (see Table 1).

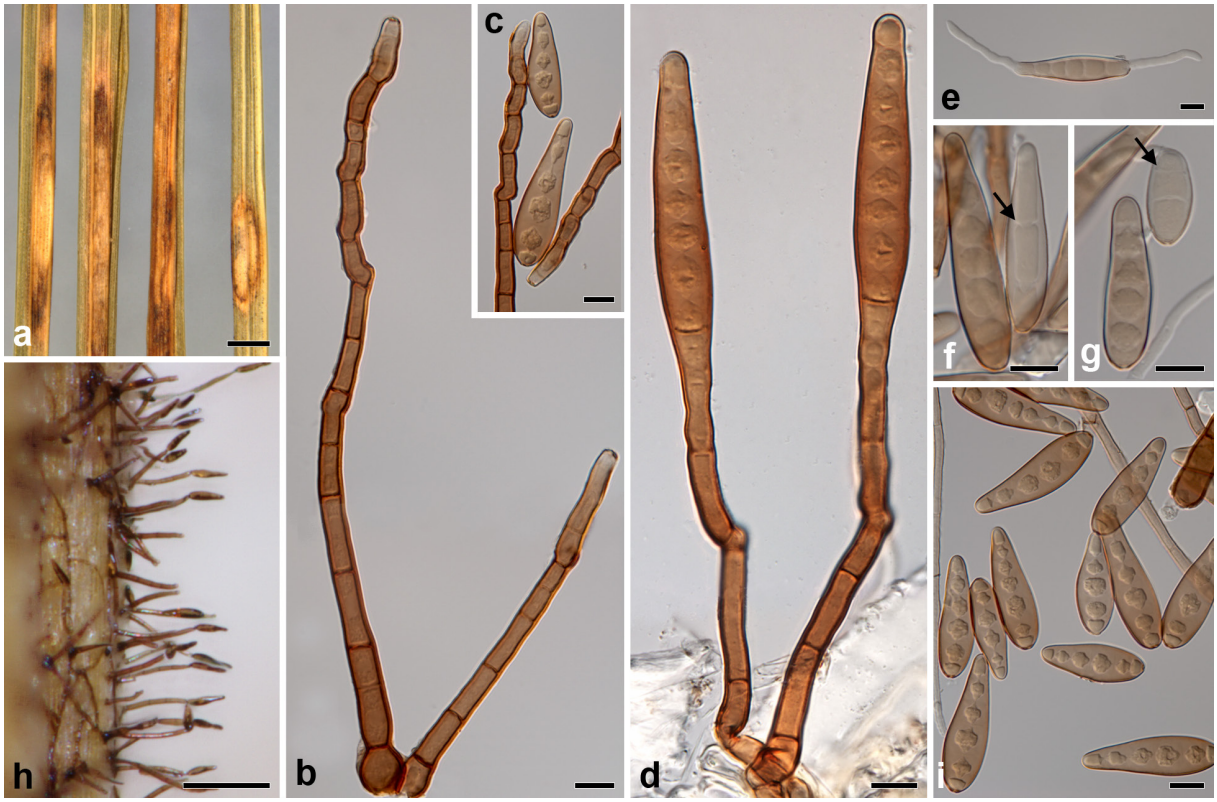

Fig. 2. *Johnalcornia aberrans* (holotype, BRIP 16281). **a** Leaf spots on *Eragrostis parviflora*, **b** conidiophores, **c** conidia, **d** chlamydospores, **e** bipolar conidial germination, **f–g** second formed conidial septum (arrowed) delimiting the apical cell, **h** conidiophores and conidia on wheat straw, **i** conidia. Scale bars: a = 1 mm; b–g, i = 10 µm, h = 100 µm.

Johnalcornia aberrans (Alcorn) Y.P. Tan & R.G. Shivas, **comb. nov.** – MycoBank MB807732. Fig. 2

Basionym: *Bipolaris aberrans* Alcorn, Mycotaxon 39: 364. 1990.

= *Cochliobolus aberrans* Alcorn, Mycotaxon 39: 362. 1990

Leaf spots elongated, about 0.5 mm wide and up to 1 cm long, with pale brown to grey centres and darker brown margins (Fig. 2a). *Conidiophores* single or in loose fascicles, arising from a swollen basal cell, simple or rarely once branched apically, olivaceous brown below, paler at apex, straight and smooth in the sterile part, becoming geniculate and smooth to finely verruculose towards the apex, sympodial, thick-walled, multiseptate, 75–270 µm long, swollen to 9–15 µm wide at base, 6–7.5 µm wide just above base and 3.5–5 µm at apex. *Conidiogenous* cells enteroblastic, cylindrical, integrated, terminal, proliferating sympodially, smooth to finely verruculose, (Figs. 2b–c, h). *Conidia* pale to mid olivaceous brown, slightly paler apically, obclavate, mostly straight, smooth, base rounded to slightly truncate, 3–7 septate, 25–60 × 9–12.5 µm; *hilum* inconspicuous, 2–3 µm wide (Figs. 2e–i). *Chlamydospores* (holoblastic conidia sensu Alcorn 1990) resemble conidiophores with conidia-like apices, terminal, olivaceous brown, straight, fusiform, 55–95 × 7–14 µm (Fig. 2d); sometimes producing at the apex either a secondary chlamydospore or a conidiophore that bears conidia normally through pores. Conidial germination bipolar, with apical germ tube axial and the basal germ tube semi-axial and often displacing the hilum. *Ascomata* black, up to 620 µm high, with a globose body

205–410 µm diam., ostiolar neck 50–155 µm high, the body bearing hyphal hairs and short blunt setae, beak glabrous. *Asci* bitunicate, cylindrical to fusoid, straight or curved, tapered, sometimes sinuate at the base, occasionally pedicellate, 98–188×12.5–19 µm. *Ascospores* hyaline, filiform, (105–) 135–260 × 3.5–4.5 µm, 5–13-septate, tightly coiled over much of ascus length, slightly less so apically, generally tapered slightly to apex and base, widest in median or upper part, base narrower (1.5–2.5 µm) than apex (2–3 µm).

Specimen examined: AUSTRALIA, Queensland, Wivenhoe Dam, from leaf spot of *Eragrostis parviflora*, 22 Mar. 1988, *J.L. Alcorn 8868* (BRIP 16281, *B. aberrans* holotype, includes the ex-type culture), (IMI 335210 isotype), (ex-isotype culture CBS 510.91).

Notes: This description is based on Alcorn (1990) and examination of the holotype specimen of *B. aberrans*, together with microscope slides of this specimen prepared by John Alcorn in 1989. This specimen only contains an asexual morph, which Alcorn (1990) described as *B. aberrans*. The sexual morph of this fungus was described by Alcorn (1990) as *Cochliobolus aberrans*. *Bipolaris aberrans* was described by Alcorn (1990) as producing holoblastic conidia that did not secede from the conidiophores. We prefer to use the term chlamydospores to describe these structures (Seifert et al. 2011), which are illustrated here for the first time (Fig. 2).

Johnalcornia is introduced as a monotypic genus for *Bipolaris aberrans*. It is phylogenetically close to *Bipolaris*, *Curvularia*, and *Porocercospora* (Fig. 1). All of these genera produce tretic (poroblastic) conidiogenous cells and distoseptate conidia. In *Johnalcornia*, the second conidial septum forms distally delimiting the apical cell (Figs. 2f, g), which differs from species of *Bipolaris* and *Curvularia* that form the second conidial septum proximally to delimit the basal cell (Alcorn 1990). Alcorn (1990) reported that paired cultures of single-spored isolates from the ex-type culture of *B. aberrans* produced a sexual morph with filiform ascospores. This differentiates *Johnalcornia* from the sexual state of *Exserohilum*, which has fusoid ascospores (Sivanesan 1987). The ascospores of *Cochliobolus abberans* were originally described as often bearing hyaline, obovoid to dumbbell-shaped or irregularly moniliform protrusions, which Alcorn (1990) considered as cellular appendages. Such structures have not been reported in sexual stages of *Bipolaris* or *Curvularia* (Sivanesan 1987). We suspect that these protrusions are malformed germ tubes, rather than appendages.

Bipolaris Shoemaker, Can. J. Bot. 37(5): 882. 1959.

Type species: *Bipolaris maydis* (Y. Nisik. & C. Miyake) Shoemaker, Can. J. Bot. 37: 882. 1959.

Descriptions: Shoemaker (1959), Alcorn (1983a), Sivanesan (1987), Manamgoda et al. (2012b).

Conidiophores solitary or in loose fascicles, simple or branched, straight to flexuous, often geniculate, sometimes nodose, cylindrical, smooth. *Conidiogenous cells* integrated, terminal and intercalary, cylindrical, proliferating sympodially, smooth to verruculose, with thickened scars. *Conidia* solitary, straight to curved, variable in shape from fusiform to oval, brown, mostly smooth or rarely echinulate to rough-walled, two or more distoseptate, septa sometimes thickened and dark brown, germinating from one or both polar cells, first conidial septum median or sub-median, second conidial septum delimiting the basal cell, third septum distal. *Ascomata* up to 700 µm wide, brown or black, immersed, erumpent, partially embedded or

superficial, free or on flat stroma, mostly globose to ellipsoidal, sometimes ampulliform or flattened, smooth or covered with vegetative hyphae, ostiole central, often papillate or with a sub-conical, conical, paraboloid or cylindrical neck. Peridium of pseudoparenchymatous cells of equal thickness or slightly thickened at the apex. Hamathecium of septate, filiform, and branched pseudoparaphyses. *Asci* 2–8-spored, clavate, cylindrical-clavate or broadly fusoid, straight or slightly curved, thin-walled, bitunicate, fissitunicate, often distended prior to dehiscence, short pedicellate, rounded at the apex. *Ascospores* filiform, hyaline to pale brown at maturity, transversely multiseptate, loosely to strongly coiled in a helix within the ascus, often with a thin mucilaginous sheath.

Note: Twelve species of *Bipolaris* are validated by multilocus analysis of ex-type or reference isolates (Fig. 1).

Bipolaris chloridis (Alcorn) Alcorn, Mycotaxon 16: 373. 1983.
Basionym: *Drechslera chloridis* Alcorn, Trans. Br. mycol. Soc. 67: 148. 1976.
= *Cochliobolus chloridis* Alcorn, Trans Br. mycol. Soc. 70: 61. 1978.

Specimen examined: AUSTRALIA, Queensland, Booie, from leaf lesion on *Chloris gayana*, 12 Dec. 1972, *J.L. Alcorn 20338* (BRIP 10965, holotype of *D. chloridis*, includes the ex-type culture), (IMI 181067 isotype).

Notes: *Bipolaris chloridis* is sister *to B. clavata* (Fig. 1), which differs by the ITS and *tef1α* loci (Table 1). *Cochliobolus chloridis* was described from paired cultures of isolates collected from Kalbar, Queensland, Australia. Alcorn (1983b) transferred *D. chloridis* to *B. chloridis* as it had a sexual morph that had been described in *Cochliobolus*. *Bipolaris chloridis* causes leaf spot or leaf blight on *C. gayana* worldwide (Sivanesan 1987).

Bipolaris clavata Alcorn, Mycotaxon 15: 15. 1982.

Specimen examined: AUSTRALIA, Queensland, Goondiwindi, from leaf spot on *Dactyloctenium radulans*, 12 May 1977, *J.L. Alcorn 77144c* (BRIP 12530 holotype, includes the ex-type culture), (IMI 264352 isotype, includes the ex-type culture).

Note: *Bipolaris clavata* is known only from the type specimen on *D. radulans* in Queensland, Australia.

Bipolaris coffeana Sivan., Trans. Br. mycol. Soc. 84: 404. 1985.

Specimen examined: KENYA, from leaf of *Coffea arabica*, 31 Oct. 1969, *I. Furtado 23* (IMI 144159 holotype), (ex-isotype culture BRIP 14845).

Note: *Bipolaris coffeana* is known only from the type specimen on *Coffea arabica*.

Bipolaris crotonis Sivan., Trans. Br. mycol. Soc. 84: 404. 1985.
= *Bipolaris eleusines* Alcorn & R.G. Shivas, in Alcorn, Mycotaxon 39: 369. 1990 (nom. inval. Art. 53.1), non *Bipolaris eleusines* J.H. Peng & J.Y. Lu [as '*eleusinea*'], Journal of Nanjing Agricultural University 12(4): 47. 1989.

= *Cochliobolus eleusines* Alcorn, Mycotaxon 39: 367. 1990.

Specimens examined: AUSTRALIA, Queensland, Goldsborough, from leaf spot of *Eleusine indica*, 1 May 1987, *J.L. Alcorn 8786a* (BRIP 15875, holotype of *B. eleusines*, includes the ex-type culture), (IMI 335212 isotype), (ex-isotype culture CBS 274.91). SAMOA, from leaf of *Croton* sp., 21 Nov. 1977, *G.F. Laundon LEV12488* (IMI 223682, holotype of *B. crotonis*), (ex-isotype culture BRIP 14838).

Notes: The phylogenetic tree inferred from the combined multilocus alignment shows that the ex-isotype isolate of *B. crotonis* (BRIP 14838) and the ex-type isolate of *B. eleusines* (BRIP 15875) (Alcorn 1990) are identical (Fig. 1). The morphology of these two taxa as given in the original descriptions are also very similar as both have slightly protruding and truncated hila, and similar conidial measurements, 80–110 × 18–29 µm for *B. crotonis* compared with 75–170 × 15–26 µm for *B. eleusines*. However the name *B. eleusines* Alcorn & R.G. Shivas in Alcorn (1990) is illegitimate as the epithet is a homonym of *B. eleusines* J.H. Peng & J.Y. Lu (as 'eleusinea'), which was isolated from a leaf of *Eleusine indica* collected Jiangsu Province, China, and described one year earlier (Peng and Lu 1989). *Bipolaris crotonis* is sister to *B. pluriseptata* (Fig. 1), which differs by the ITS, *gapdh*, LSU and *tef1α* loci (Table 1).

Bipolaris gossypina Sivan., Trans. Br. mycol. Soc. 84: 404. 1985.

Specimen examined: KENYA, from seed of *Gossypium* sp., M.H. White (IMI 123377 holotype), (ex-isotype culture BRIP 14840).

Note: *Bipolaris gossypina* is known only from the type specimen on *Gossypium* sp.

Bipolaris heliconiae Alcorn, Aust. Syst. Bot. 9: 814. 1996.
= *Cochliobolus heliconiae* Alcorn, Aust. Syst. Bot. 9: 813. 1996.

Specimens examined: AUSTRALIA, Northern Territory, Batchelor, from inflorescence of *Heliconia psittacorum* cv. 'Parakeet', July 1990, *J.D. Duff NT17605* (BRIP 17186, holotype of *B. heliconiae*, includes the ex-type culture); same location, from leaf of *Heliconia chartacea*, July 1990, *J.D. Duff NT17610* (ex-paratype culture BRIP 17189.

Notes: *Bipolaris heliconiae* is sister to *B. maydis* (Fig. 1), which differs by the ITS, *gapdh* and *tef1α* loci (Table 1). *Bipolaris heliconiae* has only been reported on species of *Heliconia* in Australia (Alcorn 1996). Single-spored isolates in paired cultures from the ex-holotype culture of *B. heliconiae* produced a sexual morph described as *Cochliobolus heliconiae* (Alcorn 1996).

Bipolaris luttrellii Alcorn, Mycotaxon 39: 378. 1990.
= *Cochliobolus luttrellii* Alcorn, Mycotaxon 39: 377. 1990.

Specimen examined: AUSTRALIA, Northern Territory, Manbulloo, from leaf of *Dactyloctenium aegyptium*, 31 Mar. 1985, *R.A. Peterson* (BRIP 14643, holotype of *B. luttrelii*, includes the ex-type culture), (IMI 335216 isotype).

Notes: *Bipolaris luttrellii* is sister to *B. peregianensis* (Fig. 1), which differs by the ITS, *gapdh*, LSU and *tef1α* loci (Table 1). *Bipolaris luttrelii* is only known from the type specimen on *D. aegyptium*. A single-spored isolate from the ex-holotype culture of *B. luttrellii* was used to produce a sexual morph described as *Cochliobolus luttrellii* (Alcorn 1996).

Bipolaris maydis (Y. Nisik. & C. Miyake) Shoemaker, Can. J. Bot. 37: 882. 1959.
Basionym: *Helminthosporium maydis* Y. Nisik & C. Miyake, Sci. Res. Alumni Assoc. Mirioka agric. Col. Japan 3: 46. 1926.
≡ *Drechslera maydis* (Y. Nisik. & C. Miyake) Subram. & Jain, Curr. Sci. 35: 354. 1966.
= *Ophiobolus heterostrophus* Drechsler, J. agric. Res. 31: 723. 1925.
= *Cochliobolus heterostrophus* (Drechsler) Drechsler, Phytopathology 24: 973. 1934.

Specimen examined: JAPAN, from *Zea mays*, Nov. 1929, *Y. Nisikado* (*Helminthosporium maydis* ex-paratype culture CBS 136.29).

Notes: *Bipolaris maydis* is an economically important fungal pathogen that causes southern leaf blight of maize around the world (Sivanesan 1987). *Bipolaris maydis* and *Cochliobolus heterostrophus* are the asexual and sexual morphs, as well as the generic types, respectively, of the same biological species (Manamgoda et al. 2012b).

Bipolaris panici-miliacei (Y. Nisik.) Shoemaker, Can. J. Bot. 37: 884. 1959.
Basionym: *Helminthosporium panici-miliacei* Y. Nisik., Ber. Ohara Inst. Landwirt. Fursch. 4: 120. 1929.
≡ *Drechslera panici-miliacei* (Y. Nisik.) Subram. & B.L. Jain, Curr. Sci. 35: 354. 1966.

Specimen examined: JAPAN, Kurashiki, from *Panicum miliaceum*, Nov. 1929, *Y. Nisikado* (ex-syntype culture CBS 199.29), (ex-isosyntype culture BRIP 12282).

Notes: *Bipolaris panici-miliacei* is a leaf pathogen on *P. miliaceum*. It has also been recorded on other grasses in Australia, India and Papua New Guinea (Sivanesan 1987).

Bipolaris pluriseptata (Khetarpal, R. Nath & S.P. Lal) Alcorn, Mycotaxon 41: 329. 1991.
Basionym: *Drechslera pluriseptata* Khetarpal, R. Nath & S.P. Lal, Indian Phytopath. 37: 320. 1984.

Specimen examined: ZAMBIA, from seeds of *Eleusine coracana*, Feb. 1981, *R.K. Khetarpal, S.P. Lal and R. Nath* (ITCC 3131 holotype), (IMI 259810 isotype, includes the extype culture), (ex-isotype culture BRIP 14839).

Notes: Sivanesan (1987) considered that B. *curvispora* and *B. melinidis* were synonyms of *B. pluriseptata*. Alcorn (1991) maintained *B. pluriseptata* as a distinct species by comparison of the cultures of each species grown under identical conditions. The phylogenetic tree inferred from the combined multilocus alignment supports *B. pluriseptata* as a distinct taxon (Fig. 1).

Bipolaris salviniae (J.J. Muchovej) Alcorn, Mycotaxon 41: 331. 1991.
Basionym: *Drechslera salviniae* J.J. Muchovej, Trans. Br. mycol. Soc. 72: 331. 1979.
= *Drechslera curvispora* El Shafie, Trans. Br. mycol. Soc. 78: 545. 1982.

≡ *Bipolaris curvispora* (El Shafie) Sivan., Mycol. Pap. 158: 47. 1987.
= *Cochliobolus melinidis* Alcorn, Mycotaxon 15: 5. 1982.
= *Bipolaris melinidis* Alcorn, Mycotaxon 15: 7. 1982.

Specimens examined: AUSTRALIA, Queensland, Kuranda, from leaf spot of *Melinis minutiflora*, 04 Jul. 1977, *K.G. Pegg* (culture BRIP 12312); Maleny (near Mary Cairncross Park), from leaf spot of *Melinis minutiflora*, 24 May 1979, *J.L. Alcorn 7965*, (BRIP 12898, holotype of *B. melinidis*, includes the ex-type culture), (IMI 264354 isotype, includes the ex-isotype culture); Julatten, 20 Jul. 1987, *J.L. Alcorn 8795* (BRIP 15895, holotype of *C. melinidis*, includes the ex-type culture). BRAZIL, Minas Gerais, Vicosa, from *Salvinia auriculata*, 16 May 1978, *J.J. Muchovej*, (IMI 228224, lectotype of *D. salviniae*, includes the ex-type culture), (ex-isolectotype culture BRIP 16571). PARAGUAY, from seed of *Triticum aestivum*, Aug. 1982, *A.E. El Shafie* (IMI 253986, isotype of *D. curvispora*), (ex-isotype culture BRIP 13795).

Notes: Alcorn (1991) showed that the ex-type cultures of *D. curvispora* (BRIP 13795) and *D. salviniae* (BRIP 16571) formed fertile ascomata when paired with a reference isolate of *B. melinidi*s (BRIP 12312) as well as with single-ascospore isolates of *C. melinidis* (BRIP 15931). Alcorn (1991) proposed a new combination based on the earliest epithet, *D. salviniae*. This combination was not recognised in the recent taxonomic re-evaluation of *Bipolaris* and *Curvularia* (Manamgoda et al. 2012b). The multilocus phylogenetic analysis of the ex-type cultures of *B. melinidis, C. melinidis, D. curvispora*, and *D. salviniae* confirm the synonymies proposed by Alcorn (1991) (Fig. 1).

Bipolaris secalis Sisterna, Pl. Path. 38:98. 1989.

Specimen examined: ARGENTINA, Buenos Aires, Los Hornos, from seed of *Secale cereale*, Aug. 1984, *M.N. Sisterna*, (IMI 286591, lectotype designated here, MBT177161), (BRIP 14453 isolectotype, includes the ex-type culture).

Notes: Sisterna (1989) designated the holotype as "IMI 286591 = BRIP 14453." As the specimens are in different herbaria, the name may be considered nom. inval. by virtue of Article 40.2, Melbourne Code, which states that a type specimen must refer to a single specimen. Although, both specimens are part of the same collection, for taxonomic clarity, IMI 286591 is designated as the lectotype for *B. secalis*, and BRIP 14453 becomes the isolectotype. *Bipolaris secalis* is sister *to B. zeae* (Fig. 1), which differs by the *gapdh* and *tef1α* loci (Table 1).

Bipolaris zeae Sivan., Trans. Br. mycol. Soc. 84: 418. 1985.

Specimen examined: AUSTRALIA, Queensland, Kingaroy, from leaf spot of Zea mays, 18 Jan. 1973, P.E. Mayers 20424, IMI 202084 (paratype), BRIP 11512 (ex-isoparatype).

Notes: *Bipolaris zeae* was first isolated from *Zea mays* in Australia, but has since been recorded on multiple grasses worldwide (Sivanesan 1987). A sexual morph was later described as *Cochliobolus zeae*, from paired cultures isolated from *Pennisetum clandestinum* in central Taiwan (Chang 1992). The synonymy of *B. zeae* and *C. zeae* cannot be assumed at this point as the mating studies were not undertaken with the ex-type culture or a reference isolate of *B. zeae*. The type culture of *C. zeae* needs to be examined to determine whether it represents the same species.

Curvularia Boedijn, Bull. Jard. bot. Buitenz, 3 Sér. 13(1): 123. 1933.
= *Curvusporium* Corbetta [as '*Curvosporium*'], Riso 12(3): 28, 30. 1963.
= *Malustela* Bat. & J.A. Lima, Publicações Inst. Micol. Recife 263: 5. 1960.

Type species: *Curvularia lunata* (Wakker) Boedijn, Bull. Jard. bot. Buitenz, 3 Sér. 13(1): 127. 1933.

Descriptions: Ellis (1971), Alcorn (1983a), Sivanesan (1987), Manamgoda et al. (2012b).

Notes: Although several species have recently been transferred from *Bipolaris* to *Curvularia* (Manamgoda et al. 2012b), both genera can still be recognised morphologically. As observed by Madrid et al. (2014), *Bipolaris* the conidia are fusiform, straight to slightly curved, smooth and typically long, sometimes up to 225 μm, whereas in *Curvularia* the conidia tend to be shorter (usually less than 130 μm) and more variable in shape and ornamentation, appearing clavate, ellipsoid, subcylindrical or more or less curved at a swollen intermediate cell, smooth to strongly verrucose. A strongly protruding hilum is produced by some species of *Curvularia* (e.g. *C. harveyi* and *C. heteropogonicola*) but has not been reported in *Bipolaris*. Nine species of *Bipolaris* are transferred to *Curvularia* on the basis of a combined multilocus phylogenetic analysis, and are listed below as novel combinations. A further eight species of *Curvularia* are herein validated by analysis of ex-type or reference isolates (Fig. 1)

Curvularia akaiiensis Sivan., Mycol. Pap. 158: 110. 1987.
≡ *Cochliobolus a*kaiiensis Sivan., Mycol. Pap. 158: 110. 1987.

Specimen examined. INDIA, Maharashtra, Dhulia, 10 Dec. 1972, *M.S. Rane J90* (IMI 172167 holotype), (ex-isotype culture BRIP 16080).

Notes: *Curvularia akaiiensis* is sister to *C. bothriochloae* (Fig. 1), which differs by the ITS, *gapdh* and *tef1α* loci (Table 1). Both the asexual and sexual morphs of this species were described from the same specimen.

Curvularia australis (Alcorn) Y.P. Tan & R.G. Shivas, **comb. nov.** – MycoBank MB807749. Fig. 3a
Basionym: *Bipolaris australis* Alcorn, Mycotaxon 15: 38. 1982.

Specimen examined: AUSTRALIA, Queensland, Goondiwindi, from inflorescence of *Sporobolus caroli*, 12 May 1977, *J.L. Alcorn 77134* (BRIP 12521 holotype, includes the ex-type culture), (IMI 261917 isotype, includes the ex-type culture), (ex-isotype culture CBS 309.90).

Notes: This species is transferred from *Bipolaris* to *Curvularia* as a result of the phylogenetic analysis (Fig. 1). *Curvularia australis* is mostly found as a pathogen on the inflorescences of *Sporobolus* spp., and has only been recorded in Australia (Sivanesan 1987). *Curvularia australis* is sister to *C. ovariicola* (Fig. 1), which differs by the ITS, *gapdh* and *tef1α* loci (Table 1). *Curvularia ovariicola* infects inflorescences of *Eragrostis* spp., and is morphologically similar to *C. australis*. However, *C. australis* has narrower conidia (8.5–15 μm) than *C. ovariicola* (12.5–20 μm) (Alcorn 1982, Sivanesan 1987).

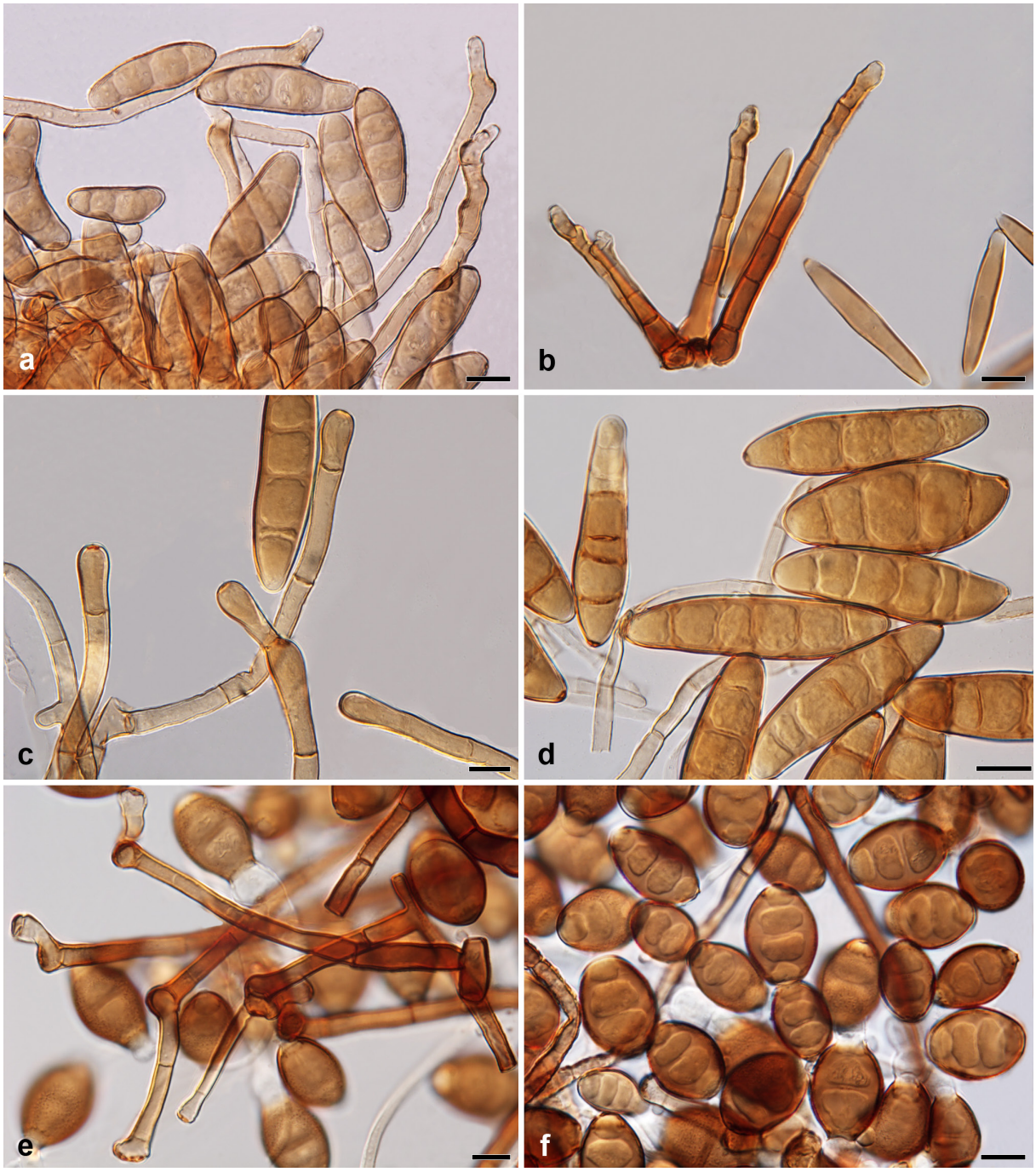

Fig. 3. Conidia and conidiophores of *Curvularia* species transferred from *Bipolaris* in this paper. **a** *Curvularia australis* (holotype BRIP 12521), **b** *Curvularia dactylotenii* (holotype, BRIP 12846), **c–d** *Curvularia crustacea* (epitype, BRIP 13524), **e–f** *Curvularia neergaardii* (isotype, BRIP 12919). Scale bars = 10 µm.

Curvularia bannonii Morgan-Jones, Mycotaxon 33: 407. 1988.

Specimen examined: USA, Louisiana, Ruston, from leaf of *Jacquemontia tamnifolia*, Jun. 1986, *J.S. Bannon* (AUAM 2602 holotype), (ex-isotype culture BRIP 16732).

Notes: *Curvularia bannonii* is sister to C. graminicola (Fig. 1), which differs by the ITS, *gapdh*, LSU and *tef1α* loci (Table 1). *Curvularia bannonii* is known only from the type specimen on *J. tamnifolia*.

Curvularia bothriochloae Sivan., Alcorn & R.G. Shivas, Aust. Syst. Bot. 16: 275. 2003.

Specimen examined: AUSTRALIA, Queensland, Goondiwindi, from leaf of *Bothriochloa baldhii*, 12 May 1977, *J.L. Alcorn 77135* (BRIP 12522 holotype, includes the ex-type culture).

Note: *Curvularia bothriochloae* is known only from the type specimen on *B. baldhii*.

Curvularia crustacea (Henn.) Y.P. Tan & R.G. Shivas, **comb. nov.** – MycoBank MB807750. Figs. 3c–d
Basionym: *Helminthosporium crustaceum* Henn., Hedwigia 41: 147. 1902.
≡ *Bipolaris crustacea* (Henn.) Alcorn, Mycotaxon 15: 27. 1982.

Type specimen: INDONESIA, Java, Bogor (formerly Buitenzorg), from *Sporobolus* sp., 1901, Zimmermann, (BRIP 12435 isotype).

Specimen examined: INDONESIA, Java, Yogyakarta, from inflorescence of *Sporobolus* sp., 25 Feb. 1982, J.*K. Somodiryo 8207*, (BRIP 13524, epitype designated here, MBT177162, includes the ex-type culture).

Notes: An ex-type culture of *B. crustaceae* does not exist. For this reason it is necessary to designate an epitype, with a living culture and DNA sequence data, to stabilise its taxonomy and aid in future phylogenetic studies. Ideally, an epitype should be derived from the same locality and host as the holotype (Cannon et al. 2012). A search of culture collection databases (ATCC, BRIP, CBS, IMI) found isolate BRIP 13524 as the most suitable candidate for epitypification of *B. crustaceae*, as it shared a similar host (*Sporobolus* sp.) and locality (central Java, Indonesia) to those of the holotype. The ex-epitype culture was sequenced and the phylogenetic analysis supported the transfer of *B. crustaceae* to *Curvularia* (Fig. 1).

Curvularia dactyloctenii (Alcorn) Y.P. Tan & R.G. Shivas, **comb. nov.** – MycoBank MB807751. Fig. 3b
Basionym: *Bipolaris dactyloctenii* Alcorn, Mycotaxon 15: 3. 1982.
= *Cochliobolus dactyloctenii* Alcorn, Mycotaxon 15: 3. 1982.

Specimen examined: AUSTRALIA, Queensland, Goondiwindi, from leaf of *Dactyloctenium radulans*, 15 Mar. 1979, *J.L. Alcorn 7909* (BRIP 12846, holotype of *B. dactyloctenii*, includes the ex-type culture), (isotype IMI 264353).

Notes: A single-spored isolate from the ex-holotype culture of *B. dactyloctenii* (BRIP 12846) was used in paired cultures to produce the sexual morph that was named *Cochiobolus dactyloctenii* (Alcorn 1996). This species is transferred from *Bipolaris* to *Curvularia* based on the phylogenetic analysis (Fig. 1). *Curvularia dactyloctenii* infects the leaves of *D. radulans* and the seeds of *Melinis* sp. (Sivanesan 1987). *Curvularia dactyloctenii* is sister to *C. hawaiiensis* (Fig. 1), which differs by the *gapdh* and *tef1α* loci (Table 1).

Curvularia harveyi Shipton, Trans. Br. mycol. Soc. 49: 523. 1966.

Specimen examined: AUSTRALIA, Western Australia, Goomalling, from seed of *Triticum* sp., 16 Jul. 1965 (IMI 114257 holotype, includes the ex-type culture), (ex-isotype culture BRIP 57412).

Notes: *Curvularia harveyi* is sister to *C. richardiae* (Fig. 1), which differs by the ITS, *gapdh* and *tef1α* loci (Table 1). *Curvularia harveyi* has only been reported on *Triticum* in Australia (Shipton 1966), and on *Zea mays* in Bangladesh (Shamsi and Yasmin 2007).

Curvularia hawaiiensis (Bugnic.) Manamgoda, L. Cai & K.D. Hyde, Fungal Diversity 56: 141. 2012.
Basionym: *Drechslera hawaiiensis* Bugnic. ex M.B. Ellis, Dematiaceous Hyphomycetes (Kew): 415. 1971.
≡ *Helminthosporium hawaiiensis* Bugnic., [as '*hawaiiense*'] Rev. gén. Bot. 62: 238. 1955 (nom. inval., Art. 39.1 Melbourne Code).
≡ *Drechslera hawaiiensis* Bugnic. ex Subram. & B.L. Jain [as '*hawaiiense*'], Curr. Sci. 35: 354. 1966 (nom. inval., Art. 39.1).
≡ *Bipolaris hawaiiensis* (M.B. Ellis) J.Y. Uchida & Aragaki, Phytopathology 69: 1115. 1979
≡ *Bipolaris hawaiiensis* (M.B. Ellis) Tsuda & Ueyama, Mycologia 73: 89. 1981 (nom. inval. Art. 53).
= *Cochliobolus hawaiiensis* Alcorn, Trans. Br. mycol. Soc. 70: 64. 1978.
≡ *Pseudocochliobolus hawaiiensis* (Alcorn) Tsuda & Ueyama, Mycologia 73: 92. 1981.

Specimen examined: USA, Hawaii, Honolulu, from *Oryza sativa*, Apr. 1950, *F. Bugnicourt* (IMI 53993, lectotype of *D. hawaiiensis*), (ex-isolectotype culture BRIP 11987).

Notes: This taxon was transferred into *Curvularia* on the basis of a combined phylogenetic analysis of ITS and *gapdh* sequences of an ex-syntype of *H. hawaiiensis* (CBS 173.57) collected by F. Bugnicourt from *Oryza sativa* in Vietnam (Manamgoda et al. 2012b). Ellis (1971) designated a lectotype (IMI 53993) when adding a Latin diagnosis *for H. hawaiiensis*. For our multilocus phylogenetic analysis, we elected to use the ex-isolectotype culture (BRIP 11987). *Curvularia hawaiiensis* has been found worldwide on many *Poaceae* hosts, but also on a wide variety of other plant hosts and non-plant substrates, e.g. air, soil, (Sivanesan 1987). It is also a known agent of opportunistic infections in humans (McGinnis et al. 1986).

Curvularia heteropogonicola (Sivan.) Alcorn, Mycotaxon 41: 332. 1991.
Basionym: *Exserohilum heteropogonicola* Sivan., Trans. Br. mycol. Soc. 83: 321. 1984.

Specimen examined: INDIA, West Himalaya, Uttaranchal, Pithoragarh, from leaf of *Heteropogon contortus*, 27 Jun. 1982, *R.S. Adhikari* (IMI 268958 holotype, includes the ex-type culture), (ex-isotype culture BRIP 14579).

Notes: Goh et al. (1998) used restriction analysis of the ITS/28S regions derived from the ex-type culture (IMI 268958) to support Alcorn's (1991) transfer of this species from *Exserohilum* into *Curvularia*. In the phylogenetic analysis, *C. heteropogonicola* is sister to *C. tripogonis* (Fig. 1), which differs by the ITS, *gapdh*, LSU and *tef1α* loci (Table 1). Additionally, *C.*

heteropogonicola has a distinctly protuberant hilum compared to *C. tripogonis*, which has a flush hilum.

Curvularia homomorpha (Luttr. & Rogerson) Y.P. Tan & R.G. Shivas, **comb. nov.** – MycoBank MB807752.
Basionym: *Helminthosporium homomorphus* Luttr. & Rogerson [as 'homomorphus'], Mycologia 51: 195. 1959.
≡ *Cochliobolus homomorphus* Luttr. & Rogerson, Mycologia 51: 195. 1959.
≡ *Drechslera homomorpha* (Luttr. & Rogerson) Sivan., Bitunicate Ascomycetes and their Anamorphs (Vaduz): 375. 1984.
≡ *Bipolaris homomorpha* (Luttr. & Rogerson) Subram. ex Alcorn [as '*homomorphus*'], Mycotaxon 16: 374. 1983.

Specimen examined: USA, Kansas, Kansas State College, from air, *C.T. Rogerson B227-14*, 11 Jun. 1957, (ex-holotype culture CBS 156.60), (ex-isotype culture BRIP 59391).

Notes: In a combined ITS and *gapdh* phylogeny by Berbee et al. (1999), the ex-type culture of *Cochliobolus homomorphus* did not cluster either with *Bipolaris* or *Curvularia*. Manamgoda et al. (2012b) excluded this taxon from their analyses but remarked that its conidial morphology is similar to *Curvularia*. The phylogenetic tree inferred from our combined multilocus alignment showed that *B. homomorpha* resided in the *Curvularia* clade (Fig. 1). Both the asexual and sexual morphs of *Curvularia homomopha* were described from the same specimen (Luttrell and Rogerson 1959).

Curvularia neergaardii (Danquah) Y.P. Tan & R.G. Shivas, **comb. nov.** – MycoBank MB807753. Figs. 3e–f
Basionym: *Drechslera neergaardii* Danquah, Trans. Br. mycol. Soc. 64: 545. 1975.
≡ *Bipolaris neergaardii* (Danquah) Alcorn, Mycotaxon 17: 68. 1983.
= *Cochliobolus neergaardii* Alcorn, Mycotaxon 39: 385. 1990.

Specimen examined: GHANA, from seed of *Oryza sativa*, 1973, *O.A. Danquah* (IMI 174949 holotype), (*D. neergaardii* ex-holotype culture DAOM 15428), (ex-isotype culture BRIP 12919).

Notes: Alcorn (1990) showed that single-spored isolates paired in cultures from the ex-holotype culture of *D. neergaardii* (DAOM 154282) produced a sexual morph, which he described as *Cochliobolus neergaardii* (Alcorn 1990). This species is transferred from *Bipolaris* to *Curvularia* based on the phylogenetic analysis (Fig. 1). *Curvularia neergaardii* is sister to *C. ellisii* (Fig. 1), which differs by the *gapdh* and *tef1α* loci (Table 1).

Curvularia nicotiae (Mouch.) Y.P. Tan & R.G. Shivas, **comb. nov.** – MycoBank MB807754. Fig. 4e
Basionym: *Drechslera nicotiae* Mouch., Revue Mycol., Paris 38: 108. 1973.
≡ *Bipolaris nicotiae* (Mouch.) Alcorn, Mycotaxon 17: 68. 1983.

Specimen examined: ALGERIA, Tamanrasset, Tassili Plateau, from soil, 1974, *J. Nicot PC2230*, (ex-isotype cultures CBS 655.74, BRIP 11983).

Fig. 4. Conidia and conidiophores of *Curvularia* species transferred from *Bipolaris* in this paper. **a–b** *Curvularia ryleyi* (holotype, BRIP 12554), **c** *Curvularia portulacea* (isotype, BRIP 14541), **d, f** *Curvularia tropicalis* (isotype, BRIP 14834), **e** *Curvularia nicotiae* (isotype, BRIP 11983). Scale bars = 10 µm.

Notes: This species is transferred from *Bipolaris* to *Curvularia* based on the phylogenetic analysis (Fig. 1). *Curvularia nicotiae* is sister to *C. portulacae* (Fig. 1), which differs by the ITS, *gapdh*, LSU and *tef1α* loci (Table 1). These two species differ in that *C. nicotiae* has broadly ellipsoidal conidia up to 50 µm long (Fig. 4e), compared to *C. portulaceae*, that has cylindrical conidia up to 180 µm long (Fig. 4c).

Curvularia papendorfii Aa, Persoonia 5(1): 45. 1967.
≡ *Drechslera papendorfii* (Aa) M.B. Ellis, Dematiaceous Hyphomycetes (Kew): 413. 1971.
≡ *Bipolaris papendorfii* (Aa) Alcorn, Mycotaxon 17: 68. 1983.
= *Curvularia siddiquii* S.I. Ahmed & M. Qureshi [as '*siddiqui*'], Pakist. J. scient. ind. Res. 3: 177. 1960 (nom. inval., Art. 39.1 Melbourne Code).

Specimen examined: SOUTH AFRICA, Transvaal, Potchefstroom, from leaf litter composed of *Acacia karroo*, 1967, *M.C. Papendorf Aa1021* (*D. papendorfii* ex-holotype culture CBS 308.67), (ex-isotype culture BRIP 57608).

Note: *Curvularia papendorfii* is recognised as a distinct species on the basis of the phylogenetic analysis (Fig. 1), despite having been transferred to *Drechslera* and *Bipolaris*.

Curvularia portulacae (Rader) Y.P. Tan & R.G. Shivas, **comb. nov.** – MycoBank MB807755. Fig. 4c
Basionym: *Helminthosporium portulacae* Rader, Mycologia 40: 344. 1948.
≡ *Drechslera portulacae* (Rader) de Hoog & Oorschot, Proc. K. Ned. Akad. Wet., Ser. C, Biol. Med. Sci. 86: 59. 1983.
≡ *Bipolaris portulacae* (Rader) Strider & Chi, Plant Diseases 68: 826. 1984 (nom. inval. Art. 35.2 Melbourne Code).
≡ *Bipolaris portulacae* (Rader) Alcorn, Mycotaxon 41: 330. 1991.
= *Drechslera helianthi* Hulea, Proc. 6th Int. Sunflower Conf. (Bucharest): 665. 1974 1975 (nom. inval., Art. 39.1 Melbourne Code).
≡ *Drechslera helianthi* Iliescu, Hulea & Bunescu, Proc. 6th Internat. Sunflower Conf. (1974) Bucharest, p. 665. 1975 (nom. inval., Art. 39.1 Melbourne Code).
= *Bipolaris novae-zelandiae* Sivan., Trans. Br. mycol. Soc. 84: 406. 1985.

Specimens examined: *New Zealand*, Nelson, Mouteka, from soil, 25 Oct. 1977, *K.N. Brunette 12347* (IMI 222864, holotype of *B. novae-zelandiae*, includes the ex-type culture), (BRIP 14837 isotype, includes ex-type culture). USA, New York, Watkins Glen, from *Portulaca oleracea*, 1947, *W.E. Rader* (*H. portulacae* ex-isotype cultures CBS 239.48, BRIP 14541).

Notes: Alcorn (1991) synonymised *B. novae-zelandiae* with *B. portulacae* based on conidial morphology. The phylogenetic tree inferred from the combined multilocus sequence alignment showed that the two ex-isotype isolates of *H. portulacae* (BRIP 14541) and *B. novae-zealandiae* (BRIP 14837) are identical, thereby supporting Alcorn's synonymy (Fig. 1). This species is transferred from *Bipolaris* to *Curvularia* based on multilocus phylogenetic analysis (Fig. 1).

Curvularia richardiae Alcorn, Trans. Br. mycol. Soc. 56: 155. 1971.

Specimen examined: AUSTRALIA, Queensland, Cleveland, Redlands Horticulture Research Station, from leaf of *Richardia brasiliensis*, 02 May 1969, *J.L. Alcorn 18390–2* (DAR 19772, lectotype designated here, MBT177193, includes the ex-type culture), (IMI 150233 isolectotype), (ex-isolectotype culture BRIP 4371).

Notes: Alcorn (1971) referred to both DAR 19772 and IMI 150233 as the type specimens, but did not specify which one was the holotype. As the specimens are in different herbaria, the name may be considered nom. inval. by virtue of Article 40.3, Melbourne Code, which states a type specimen must refer to a single specimen. Although, both specimens are part of the same collection, for taxonomic clarity we designate DAR 19772 as the lectotype for *C. richardiae*, and IMI 150233 and BRIP 4371 as the isolectotypes.

Curvularia ryleyi (Alcorn) Y.P. Tan & R.G. Shivas, **comb. et nom. nov.** – MycoBank MB807756. Figs. 4a–b
Replaced synonym: *Bipolaris cylindrica* Alcorn, Mycotaxon 15: 42. 1982.
≠ *Curvularia cylindrica* Meng Zhang & T.Y. Zhang, Mycosystema 24(4): [473] 2005.

Etymology: Named after Dr. Malcolm J. Ryley, an eminent Australian plant pathologist and mycologist.

Specimens examined: AUSTRALIA, New South Wales, Yetman, from inflorescence of *Sporobolus creber*, 12 May 1977, *J.L. Alcorn 77154* (BRIP 12554 holotype, includes the ex-type culture), (IMI 261918 isotype, includes the ex-type culture); Queensland, Warrill View, from inflorescence of *Sporobolus elongatus*, 11 May 1978, *M.J. Ryley 7824* (culture BRIP 12637).

Notes: *Bipolaris cylindrica* is transferred from *Bipolaris* to *Curvularia* based on phylogenetic analysis (Fig. 1). A nom. nov. is introduced to avoid creating a homonym with *C. cylindrica* Meng Zhang & T.Y. Zhang, which was isolated from the leaves of *Allium fistulosum*, in Xinjiang, China (Zhang and Zhang 2005). *Curvularia ryleyi* has longer conidiophores (up to 350 µm) and larger conidia (on the host, 45–100 µm × 10–17.5 µm) than *C. cylindrica* (conidiophores less than 120 µm long, conidia 30–49 µm × 7.5−12 µm). *Curvularia ryleyi* is sister to *C. crustacea* (Fig. 1), which differs by the ITS, LSU and *tef1α* loci (Table 1).

Curvularia sorghina R.G. Shivas & Sivan., Trans. Br. mycol. Soc. 88: 269. 1987.

Specimen examined: AUSTRALIA, Western Australia, Kununurra, from leaf of *Sorghum bicolor*, Apr. 1984, *R.G. Shivas WA 2279* (IMI 289262 holotype, includes the ex-type culture), (ex-isotype culture BRIP 15900).

Notes: In the phylogenetic analysis, *C. sorghina* is sister to *C. lunata* (Fig. 1), which differs by the *gapdh* and *tef1α* loci (Table 1). *Curvularia sorghina* has also been identified by morphology on sorghum in Canada (Funnell-Harris et al. 2013).

Curvularia tropicalis (Sivan.) Y.P. Tan & R.G. Shivas, **comb. nov.** – MycoBank MB807757. Figs. 4d, f
Basionym: *Bipolaris tropicalis* Sivan., Trans. Br. mycol. Soc. 84: 411. 1985.

Specimen examined: INDIA, Karnataka, Central Coffee Research Station, from leaf of *Coffea arabica*, 6 Feb. 1979, *B.N. Muthappa 39* (IMI 235542 holotype, includes the ex-type culture), (ex-isotype culture BRIP 14834).

Notes: This species is transferred from *Bipolaris* to *Curvularia* based on the phylogenetic analysis (Fig. 1). *Curvularia tropicalis* is known only from the type specimen on *Coffea arabica* in India (Sivanesan 1985).

DISCUSSION

It has long been recognised that species of *Bipolaris* and *Curvularia* are morphologically similar (Ellis 1971, Luttrell 1979, Sivanesan 1987, Goh et al. 1998, Manamgoda et al. 2012b). The first to suggest that *Bipolaris* and *Curvularia* should be synonymised were von Arx and Luttrell (1979). Differentiation between *Bipolaris* and *Curvularia* has been predominantly based on conidial morphology (Ellis 1971, Sivanesan 1987, Manamgoda et al. 2012b), particularly their size, number of septa, degree of curvature, presence of a flush or protuberant conidial hilum, and the presence or absence of a disproportionately swollen intermediate cell. Generic assignment based on these characters is often difficult (if not impossible) as the conidia of many *Bipolaris* and *Curvularia* species share similar characteristics. Analysis of *Bipolaris* and *Curvularia* species using DNA gene sequences (Berbee et al. 1999, Manamgoda et al. 2011, 2012b) and restriction fragment length polymorphism (Goh et al. 1998) has also raised doubts as to the utility of conidial morphology in differentiating the two genera.

Previous studies have shown that phylogenetic analyses based on ITS and *gapdh* sequences, either individually or in combination, provide sufficient resolution for delimiting taxa within *Bipolaris* and *Curvularia* (Berbee et al. 1999, Manamgoda et al. 2012b). However, a four-loci dataset, including ITS, *gapdh*, LSU and *tef1α*, provided a better resolution at the terminal clades, and are able to provide stronger support for the description of new species (Manamgoda et al. 2012a, b). We used a similar combined multilocus dataset and phylogenetic analyses to resolve the taxonomic status of 34 species of *Bipolaris* and *Curvularia*, resulting in the transfer of nine species from *Bipolaris* to *Curvularia*, the synonymy of four species, and fixing the generic application of a further 20 species names.

The generic placement of *B. homomorpha* has been considered problematic based on morphology (Berbee et al. 1999). It has been excluded from previous phylogenetic analyses because of this ambiguity (Berbee et al. 1999, Manamgoda et al. 2012b). The combined multilocus analyses in our study showed *B. homomorpha* clustered in the *Curvularia* clade, and taxonomic uncertainty is resolved with its transfer.

A new monotypic genus, *Johnalcornia*, has been introduced to accommodate *B. aberrans*, which is characterised by forming the second conidial septum in the apical cell. The combined multilocus phylogenetic analysis showed that *J. aberrans* did not belong to either the *Bipolaris* or *Curvularia*, as currently circumscribed (Manamgoda et al. 2012b), although all three genera are morphologically similar in that they have conidiophores that are often thick-walled and geniculate, with conidiogenous cells that have thickened scars, and distoseptate conidia. *Johnalcornia aberrans* is phylogenetically close to the recently described monotypic genus, *Porocercospora* (Amaradasa et al. 2014) that was established to accommodate *Cercospora seminalis* (on seeds of *Buchloë dactyloides*, USA).

Further studies are required to phylogenetically resolve remaining taxa into either *Bipolaris* or *Curvularia*. This work should be based upon ex-type or epitype cultures and the application of multilocus analyses. We recommend that future descriptions of new taxon be accompanied by at least sequence data for the ITS region, which is the official fungal barcode (Schoch et al. 2012). Additional sequences from other loci (e.g. *gapdh*, LSU and *tef1α*) are valuable for in silico identification and analyses. We are currently using this approach to study the phylogenetic relationships of 100 unidentified isolates of *Bipolaris* and *Curvularia* maintained in the BRIP collection (unpubl. data).

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CHAPTER 4

Eight novel *Bipolaris* species identified from John L. Alcorn's collections at the Queensland Plant Pathology Herbarium (BRIP)

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Key words: Helminthosporioid fungi, Novel species,Taxonomy

Abstract

Several unidentified specimens of *Bipolaris* deposited in the Queensland Plant Pathology Herbarium (BRIP) that were previously recognised by Dr. John L. Alcorn as taxonomically interesting were re-examined. The morphology of conidia and conidiophores, as well as phylogenetic inference from the analyses of three loci (ITS, *gapdh* and *tef1α*) supported the classification of eight novel *Bipolaris* species, which were originally isolated from leaf spots on grasses (*Poaceae*).

INTRODUCTION

The genus *Bipolaris* (Shoemaker 1959) has traditionally been treated as part of the helminthosporioid complex, so-called because the conidia and conidiophores morphologically resemble species of Helminthosporium (Link 1809). *Bipolaris* was originally established to accommodate species that formed fusoid conidia with two or more septa that exhibited bipolar germination, but also included some species with curved conidia and hyaline apical cells (Shoemaker 1959).

Until the late 1990s, the classification and identification of *Bipolaris* species was based entirely on morphological characteristics (Sivanesan 1987). This proved problematic, as conidia and conidiophores are highly variable within species. In recent years, the generic limits for the helminthosporioid fungi (including *Curvularia*, *Drechslera*, *Exserohilum*, *Johnalcornia* and *Porocercospora*) have been more clearly defined with the aid of molecular sequence data (Ahmadpour et al. 2012, da Cunha et al. 2012, Madrid et al. 2014, Manamgoda et al. 2012, 2014, Tan et al. 2014). Subsequent analyses of DNA sequence data have established the synonymy between *Bipolaris* (typified by *B. maydis*) and its sexual morph, *Cochliobolus* Drechsler (1934) (typified by *C. heterostrophus*) (Manamgoda et al. 2012, Rossman et al. 2013). The rules of nomenclature for fungi only allow one name for each genus, instead of different names for different morphs in the fungal life cycle (McNeill et al. 2012). Although *Cochliobolus* is the older name, *Bipolaris* is more frequently used by plant pathologists in disease reports and widely applied in the taxonomic literature. The name *Bipolaris* was subsequently proposed for conservation against the earlier name *Cochliobolus* (Rossman et al. 2013).

Species of *Bipolaris* are commonly associated with leaf spots, leaf blights and root rots on hosts in the *Poaceae* (Ellis 1971, Sivanesan 1987, Manamgoda et al. 2011, 2014). Some species that are considered serious pathogens are those on high-value commodity cereal crops, such as *B. maydis* on maize, *B. oryzae* on rice (Sunder et al. 2014) and *B. sorokiniana* on wheat (Acharya et al. 2011). Several species have multiple grass hosts, including other cereals and weeds, which presents additional problems related to crop rotation and disease management (Strange and Scott 2005, Iftikhar et al. 2009, Sunder et al. 2014). Furthermore, many *Bipolaris* species are saprobes or pathogens on hosts in the families Anacardiaceae, Araceae, Euphorbiaceae, Fabaceae, Malvaceae, Rutaceae and Zingiberaceae (Ellis 1971, Manamgoda et al. 2011, 2014). There are approximately 47 species of *Bipolaris* (Manamgoda et al. 2014), of which 29 occur in Australia (Alcorn 1982, Sivanesan 1985, 1987, Alcorn 1990, 1996, DAF Biological Collections 2016). Most of these species were associated with hosts in the *Poaceae*, with the exceptions of *B. cactivora* and *B. incurvata*, which were only recorded on hosts in the families Cactaceae and Arecaceae, respectively (Forsberg 1985, Shivas 1995, Fröhlich et al. 1997, DAF Biological Collections 2016).

Accurate identification of *Bipolaris* species based on DNA sequences is dependent on the availability of ex-type cultures. In recent years, many DNA sequences from ex-type or reference cultures of *Bipolaris* species have been made available in GenBank (Manamgoda et al. 2012, 2014, Tan et al. 2014). In this study, 13 unidentified isolates of *Bipolaris* held in the Queensland Plant Pathology Herbarium (BRIP) were examined by molecular and morphological methods, and compared with ex-type and reference isolates. Most of the fungi were collected and isolated by Dr. John L. Alcorn as curator of the BRIP from the early 1960s through to the late 1990s. Ten new species of *Bipolaris* were revealed from the combined data analyses and morphological studies, and are herein introduced and described.

MATERIALS AND METHODS

Isolates and morphology

All isolates examined are listed in Table 1. The unidentified isolates of *Bipolaris* were obtained from BRIP, which retains cultures in a metabolically inactive state at -80 °C in a sterile solution of 15 % v/v glycerol. In order to observe conidia and conidiophores, living cultures were grown on sterilised leaf pieces of *Zea mays* placed on modified Sachs agar or sterilised wheat straws on water agar, incubated at room temperature (approx. 23–25 °C) for 4 weeks, and exposed to near ultraviolet light source on a 12-h light / dark diurnal cycle (Sivanesan 1987). Conidia and conidiophores were mounted on glass slides in lactic acid (100 % v/v) and images captured with a Leica DFC500 camera attached to a Leica DM5500 B compound microscope with Nomarski differential interference contrast illumination. The images presented in Fig. 3d–e were taken from dried cultures, and Figs. 2e, i and 3a were taken from dried herbarium specimens. Conidial widths were measured at the widest part of each conidium. Means and standard deviations (SDs) were calculated from at least 20 measurements. Ranges were expressed as (min.–) mean-SD–mean+SD (–max.), with values rounded to 0.5 µm. Images of the herbarium specimens were captured by an Epson Perfection V700 scanner at 300 dpi resolution.

DNA isolation, amplification, and phylogenetic analyses

The isolates were grown on potato dextrose agar (PDA) (Oxoid) for 7 days at room temperature. Mycelia were scraped off the PDA cultures and macerated with 0.5 mm glass beads (Daintree Scientific) in a TissueLyser (QIAGEN). Genomic DNA was extracted with the Gentra Puregene DNA Extraction Kit (QIAGEN), according to the manufacturer's instructions.

The primers V9G (de Hoog and Gerits van den Ende 1998) and ITS4 (White et al. 1990) were used to amplify the internal transcribed spacer (ITS) region of the nrDNA. The primers gpd1 and gpd2 (Berbee et al. 1999) were used to amplify part of the glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) gene. A partial region of the translation elongation factor 1-α (*tef1α*) locus was amplified using the primers EF1983/EF12218R (Schoch et al. 2009). All loci were amplified with the Phusion High-Fidelity PCR Master Mix (New England Biolabs). The PCR products were purified and sequenced by Macrogen Incorporated (Seoul, Korea).

All sequences generated were assembled using Geneious v. 9.1.5 (Biomatters Ltd.) and deposited in GenBank (Table 1, in bold). These sequences were aligned with selected sequences of *Bipolaris* species obtained from GenBank (Table 1) using the MAFFT alignment algorithm (Katoh et al.

Table 1. *Bipolaris* isolates examined in this study.

Species	Isolate no.[a]	*Host*	Location	GenBank accession numbers[b]			
				ITS	*gapdh*	*tef1α*	LSU
Bipolaris austrostipae sp. nov.	BRIP 12490^{T}	*Austrostipa verticillata*	Australia	**KX452442**	**KX452408**	**KX452459**	**KX452425**
Bipolaris axonopicola sp. nov.	BRIP 11740^{T}	*Axonopus fissifolius*	Australia	**KX452443**	**KX452409**	**KX452460**	**KX452426**
Bipolaris bamagaensis sp. nov.	BRIP 13577^{T}	*Brachiaria subquadripara*	Australia	**KX452445**	**KX452411**	**KX452462**	**KX452428**
	BRIP 10711	*Dactyloctenium aegyptium*	Australia	**KX452444**	**KX452410**	**KX452461**	**KX452427**
	BRIP 14847	*Dactyloctenium aegyptium*	Australia	**KX452446**	**KX452412**	**KX452463**	**KX452429**
	BRIP 15934	*Dactyloctenium aegyptium*	Australia	**KX452447**	**KX452413**	**KX452464**	**KX452430**
Bipolaris bicolor	CBS 690.96	unknown	Cuba	KJ909762	KM042893	KM243287	-
Bipolaris chloridis	BRIP 10965^{T}	*Chloris gayana*	Australia	KJ415523	KJ415423	KJ415472	-
Bipolaris clavata	BRIP 12530^{T}	*Dactyloctenium radulans*	Australia	KJ415524	KJ415422	KJ415471	-
Bipolaris coffeana	BRIP 14845^{T}	*Coffea arabica*	Kenya	KJ415525	KJ415421	KJ415470	-
Bipolaris cookei	MAFF 51191	*Sorghum bicolor*	Japan	KJ922392	KM034834	KM093777	-
Bipolaris crotonis	BRIP 14838^{T}	*Croton* sp.	Samoa	KJ415526	KJ415420	KJ415469	-
Bipolaris cynodontis	CBS 109894^{T}	*Cynodon dactylon*	Hungary	KJ909767	KM034838	KM243288	-
Bipolaris drechsleri	CBS 136207^{T}	*Microstegium vimineum*	USA	KF500530	KF500533	KM093760	-
Bipolaris gossypina	BRIP 14840^{T}	*Gossypium* sp.	Kenya	KJ415528	KJ415418	KJ415467	-
Bipolaris heliconiae	BRIP 17186^{T}	*Heliconia psittacorum*	Australia	KJ415530	KJ415417	KJ415465	-
Bipolaris heveae	CBS 241.92	*Hevea* sp.	Nigeria	KJ909763	KM034843	KM243294	-
Bipolaris luttrellii	BRIP 14643^{T}	*Dactyloctenium aegyptium*	Australia	AF071350	AF081402	KJ415464	-
Bipolaris maydis	CBS 136.29^{T}	*Zea mays*	Japan	HF934926	HG779086	KJ415463	-

Table 1. (Ctd).

Species	Isolate no.[a]	*Host*	Location	GenBank accession numbers[b]			
				ITS	*gapdh*	*tef1α*	LSU
Bipolaris microlaenae	CBS 280.91[T]	*Microlaena stipoides*	Australia	JN600974	JN600974	JN601017	-
Bipolaris microstegii	CBS 132550[T]	*Microstegium vimineum*	USA	JX089579	JX089575	JX100808	-
Bipolaris oryzae	MFLUCC 10-0715[T]	*Oryza sativa*	Thailand	JX256416	JX276430	JX256384	-
Bipolaris panici-miliacei	BRIP 12282[T]	*Panicum miliaceum*	Japan	KJ415531	KJ415415	KJ415462	-
Bipolaris peregianensis	BRIP 12790[T]	*Cynodon dactylon*	Australia	JN601034	JN600977	JN601022	-
Bipolaris pluriseptata	BRIP 14839[T]	*Eleusine coracana*	Zambia	KJ415532	KJ415414	KJ415461	-
Bipolaris sacchari	ICMP 6227	*Oplismenus imbecilis*	New Zealand	KJ922386	KM034842	KM093785	-
Bipolaris salviniae	BRIP 16571[T]	*Salvinia auriculata*	Brazil	KJ415535	KJ415411	KJ415457	-
Bipolaris secalis	BRIP 14453[T]	*Secale cereale*	Argentina	KJ415537	KJ415409	KJ415455	-
Bipolaris shoemakeri sp. nov.	BRIP 15806	*Ischaemum rugosum* var. *segetum*	Australia	**KX452452**	**KX452418**	**KX452469**	**KX452435**
	BRIP 15929[T]		Australia	**KX452453**	**KX452419**	**KX452470**	**KX452436**
Bipolaris simmondsii sp. nov.	BRIP 12030[T]	*Zoysia macrantha*	Australia	**KX452454**	**KX452420**	**KX452471**	**KX452437**
Bipolaris sivanesaniana sp. nov.	BRIP 15847[T]	*Paspalidium distans*	Australia	**KX452455**	**KX452421**	**KX452472**	**KX452438**
	BRIP 15822	*Setaria sphaecelata*	Australia	**KX452456**	**KX452422**	**KX452473**	**KX452439**
Bipolaris sorokiniaia (=B. multiformis)	CBS 480.74[T]	*Tribulus terrestris*	South Africa	KJ909771	KM034827	KM243282	-
Bipolaris subramanianii sp. nov.	BRIP 16226[T]	*Setaria sphacelata*	Australia	**KX452457**	**KX452423**	**KX452474**	**KX452440**
Bipolaris urochloae	ATCC 58317[T]	*Urochloa panicoides*	Australia	KJ922389	KM230396	KM093770	-
Bipolaris victoriae	CBS 327.64[T]	*Avena sativa*	USA	KJ909778	KM034811	KM093748	-

Table 1. (Ctd).

Species	Isolate no.[a]	*Host*	Location	GenBank accession numbers[b]			
				ITS	*gapdh*	*tef1α*	LSU
Bipolaris woodii sp. nov.	BRIP 12239[T]	*Paspalidium caespitosum*	Australia	**KX452458**	**KX452424**	**KX452475**	**KX452441**
Bipolaris yamadae	CBS 202.29	*Panicum milaceum*	Japan	KJ909779	KM034830	KM243275	-
Bipolaris zeae	BRIP 11512[T]	*Zea mays*	Australia	KJ415538	KJ415408	KJ415454	-
Bipolaris zeicola	FIP532[T]	*Zea mays*	USA	KM230398	KM034815	KM093752	-
Curvularia lunata	CBS 730.96[T]	Human lung biopsy	USA	JX256429	JX276441	JX266596	-

[a] ATCC: American Type Culture Collection; BRIP Queensland Plant Pathology Herbarium, Brisbane, Queensland, Australia; CBS: Westerdijke Fungal Biodiversity Institute, Utrecht, The Netherlands; ICMP: International Collection of Microorganisms from Plants, Auckland, New Zealand; MAFF: MAFF Genebank Project, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand.

[b] ITS: internal transcribed spacer; *gapdh*: glyceraldehyde-3-phosphate dehydrogenase; LSU: large subunit; *tef1α*: translation elongation factor 1-alpha.

GenBank accessions derived from this study are shown in **bold**.

[T] Indicates ex-type culture

2009) in Geneious. *Curvularia lunata* CBS 730.96 was included as the outgroup (Table 1). The sequences of each locus were aligned separately and manually adjusted as necessary. Alignment gaps were treated as missing character states, and all characters were unordered and of equal weight. The Markov chain Monte Carlo (MCMC) algorithm was used to create a phylogenetic tree based on Bayesian probabilities using MrBayes v. 3.2.1 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003) in Geneious. To remove the need for a priori model testing, the MCMC analysis was set to sample across the entire general time-reversible (GTR) model space with a gamma-distributed rate variation across the sites. Ten million random trees were generated using the MCMC procedure with four chains. The sample frequency was set at 100 and the temperature of the heated chain was 0.1. Burn-in was set at 25 %, after which the likelihood values were stationary. Maximum likelihood (ML) analysis was run using RAxML v. 7.2.8 (Stamatakis and Alachiotis 2010) in Geneious and started from a random tree topology. The nucleotide substitution model used was GTR with a gamma-distributed rate variation. The concatenated alignment was deposited in TreeBASE (Study 19483). All novel sequences were deposited in GenBank (Table 1).

In order to determine the species limits, the criterion of genealogical concordance phylogenetic species recognition (GCPSR) was applied to the molecular data (Taylor et al. 2000). A combined analysis of three genes was used to determine the final species boundaries with the support of all single gene trees inferred. Unique fixed nucleotides are used to characterise genetic differences in the new species. For each species description, the closest phylogenetic neighbour was selected and these alignments were subject to single nucleotide polymorphism (SNP) analyses. These SNPs were determined for each aligned locus using the Find Variation/SNPs feature in Geneious. SNPs were determined based on a minimum variant frequency of 0.2. Taxonomic novelties were registered in MycoBank (http://www.mycobank.org, Crous et al. 2004).

RESULTS

Phylogenetic analysis

On average, 860 bp of the ITS region, 551 bp of the *gapdh* gene and 876 bp of the *tef1α* gene were sequenced from the BRIP isolates. For the phylogenetic analyses, the ITS and *gapdh* were trimmed to 474 and 445 bp, respectively. The combined alignment deposited in TreeBASE is composed of 1733 characters from 46 isolates, of which 96 bp (20.3 %), 156 bp (35.1 %) and 99 bp (11.3 %) were variable for ITS, *gapdh* and *tef1α*, respectively. The ITS alignment was able to resolve 19 out of 38 *Bipolaris* species, including four of the new species (data not shown). Individually, both the *gapdh* and *tef1α* alignments were able to resolve 36 out of 38 *Bipolaris* species, including the ten new species described here (data not shown). None of the ITS, *gapdh* or the *tef1α* alignments were able to differentiate between the ex-holotype strain of *B. coffeana* and the recently designated ex-epitype strain of *B. cynodontis* (Manamgoda et al. 2014). A pairwise comparison of the unannotated sequences of *B. coffeana* and *B. cynodontis* showed 100 % identity in the ITS and *tef1α* loci, and one SNP in the *gapdh* locus, indicating a potential synonymy. Morphologically, *B. coffeana* can have conidiophores longer than *B. cynodontis* (up to 260 µm versus 170 µm), although the conidial dimensions of *B. coffeana* (32–75 × 11–14 µm) falls within the range described for *B. cynodontis* (30–75 × 10–16 µm). To avoid duplication, the novel taxa described below are, therefore, compared to *B. cynodontis*. The

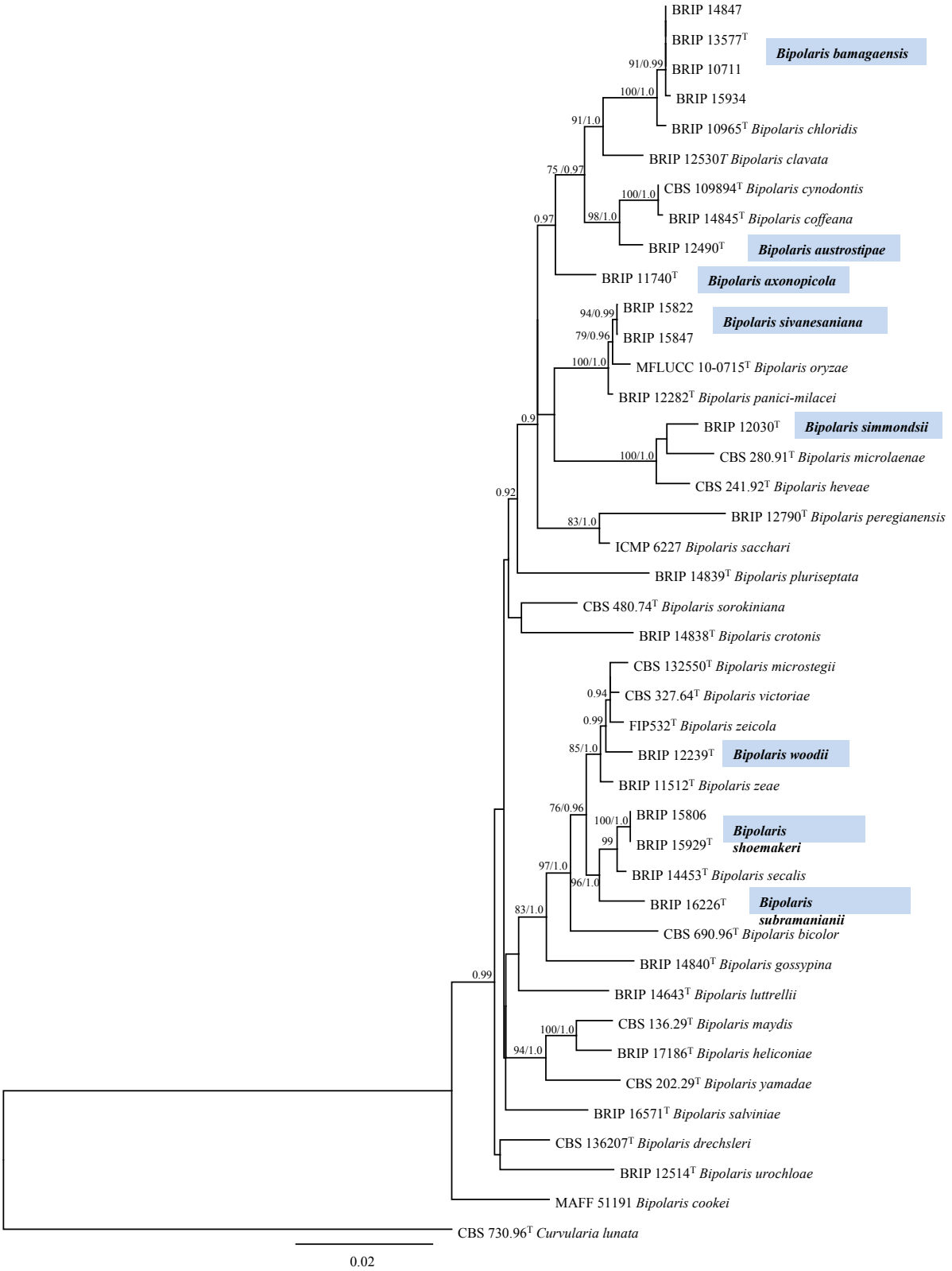


Fig. 1. Phylogenetic tree based on maximum likelihood analysis of the combined multilocus alignment. RAxML bootstrap values (bs) greater than 70 % and Bayesian posterior probabilities (pp) greater than 0.9 are given at the nodes (bs/pp). Novel species are in bold and highlighted in blue. Ex-type isolates are marked with a superscript T. The outgroup is *Curvularia lunata*.

inferred phylogenetic tree based on the concatenated alignment resolved the 17 BRIP isolates into ten well-supported and unique clades, which are accepted in this study as novel species (Fig. 1).

Taxonomy

Bipolaris austrostipae Y.P. Tan & R.G. Shivas, **sp. nov.** – MycoBank MB817461. Fig. 2a–b

Etymology: Named after *Austrostipa*, the grass genus from which it was isolated.

Conidiophores mononematous, erect, straight to flexuous, rarely branched, geniculate towards the apex, uniformly brown to dark brown, smooth, septate, up to 260 µm × 5–6 µm; basal cell swollen and darker than the other cells, up to 10 µm diam. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, mono- or polytretic. *Conidiogenous nodes* darkening and becoming verruculose. *Conidia* fusiform, straight to slightly curved, (55–) 70–77 (–86) × (11–) 14–15.5 (–20) µm, brown to dark brown, 6–9-distoseptate. *Hilum* thick and darkened.

Specimen examined: AUSTRALIA, Queensland, Leyburn, from *Austrostipa verticillata* (Nees ex Spreng.) S.W.L. Jacobs & J. Everett, 11 May 1977, *J.L. Alcorn* (BRIP 12490 holotype, includes ex-type culture).

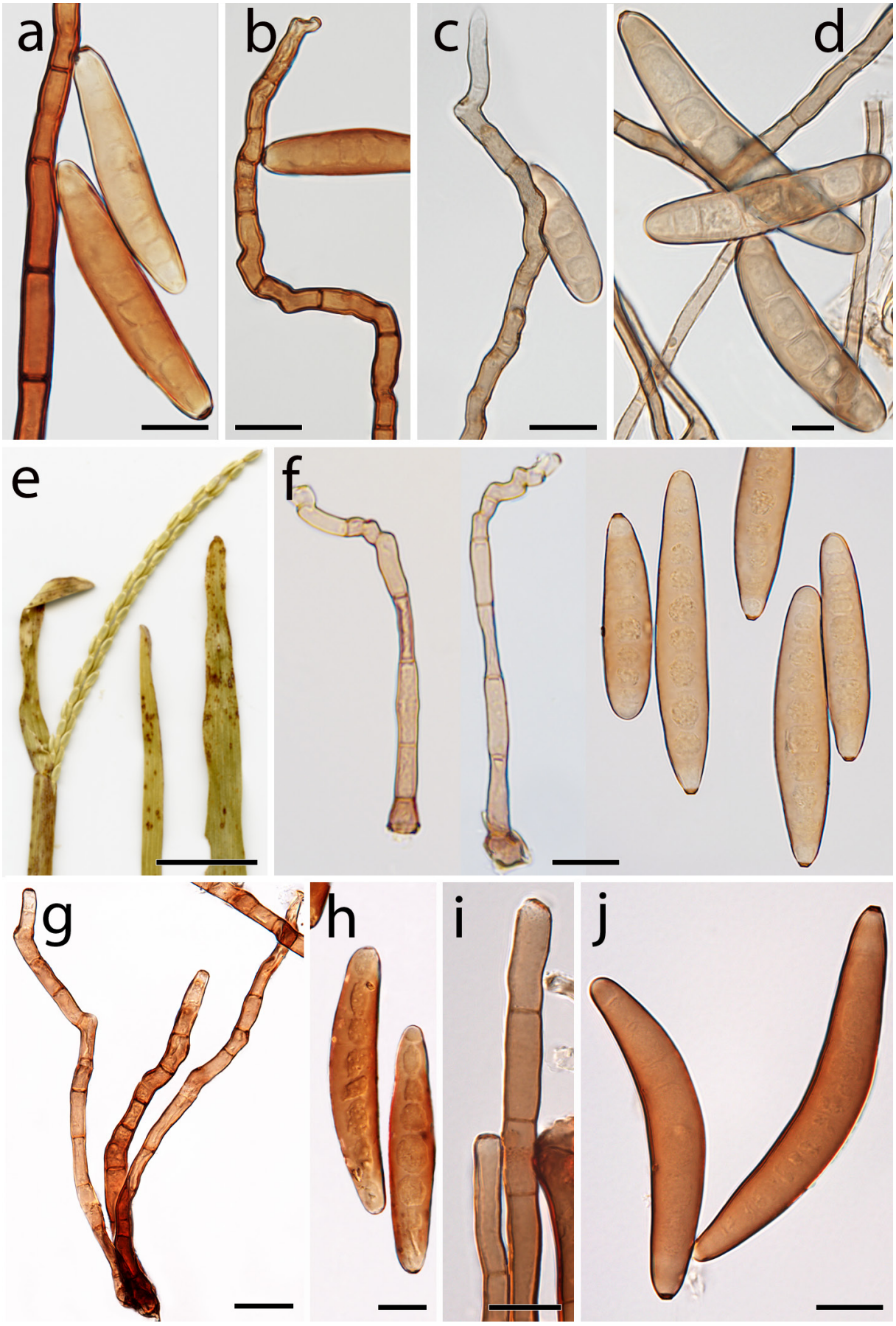
Notes: Bipolaris austrostipae is only known from the type specimen on *Austrostipa verticillata*, which is an Australian perennial grass found predominantly in Queensland and New South Wales (Simon and Alfonso 2011). *Bipolaris austrostipae* is phylogenetically close to *B. cynodontis* (Fig. 1), and its conidial size falls within the range given for *B. cynodontis* (30–75 × 10–16 µm) (Sivanesan 1987). *Bipolaris austrostipae* differs from the ex-type culture of *B. cynodontis* in two loci: *gapdh* 98 % match (Identities 432/443, Gaps 0/443); *tef1α* positions 225 (C), 266 (G) and 717 (T).

Bipolaris axonopicola Y.P. Tan & R.G. Shivas, **sp. nov.** – MycoBank MB817462. Fig. 2e–f

Etymology: Named after *Axonopus*, the grass genus from which it was isolated.

Leaf spots on Axonopus fissifolius, narrowly ellipsoidal, up to 1 × 0.5 mm, reddish brown, larger spots with grey centres. *Conidiophores* mononematous, erect, straight to flexuous, rarely branched, geniculate towards the apex, uniformly pale brown to brown, smooth, septate, up to 250 µm × 5–9 µm; basal cell swollen and darker than the other cells, up to 18 µm diam. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, mono- or polytretic with undarkened circular scars. Co*nidiogenous nodes* distinct, slightly verruculose below the node. *Conidia* fusiform to subcylindrical or obclavate, (40–) 55–60 (–71) × (10–) 11.5–12.5 (–14) µm, pale brown with the end cells slightly paler than the central cells, smooth, 5–10-distoseptate, apex rounded, base obconically truncate or rounded. *Hilum* darkened and sometimes thickened. Germination bipolar.

Culture characteristics: Colonies on PDA cover the entire plate; surface grey olivaceous with smoky grey patches, velutinous with abundant aerial mycelium.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Specimen examined: AUSTRALIA, Queensland, Peregian Beach, from leaf spot on *Axonopus fissifolius*, 6 June 1976, *J.L. Alcorn* (BRIP 11740 holotype, includes ex-type culture).

Notes: *Bipolaris axonopicola* is only known from a single specimen on *Axonopus fissifolius* in south-east Queensland. *Axonopus fissifolius* is native to the Americas and was introduced to Australia as a pasture grass (Simon and Alfonso 2011). The conidial dimensions of B. *axonopicola* overlap with those of *B. cynodontis* (30–75 × 10–16 µm). Marignoni (1909) described *Helminthosporium cynodontis* (synonym of *B. cynodontis*) as having conidia 60–75 µm long and also illustrated them as slightly curved. Subsequently, many morphologically similar isolates with slightly curved conidia have been assigned to *B. cynodontis* from a wide range of hosts (Manamgoda et al. 2014), including *A. fissifolius* (Sivanesan 1987). *Bipolaris axonopicola* has straight conidia, which distinguishes it from *B. cynodontis*.

Bipolaris axonopicola is phylogenetically close to *B. cynodontis* and *B. austrostipae* (Fig. 1). *Bipolaris axonopicola* differs from *B. cynodontis* in three loci: ITS 99 % match (Identities 451/457, Gaps 2/457); *gapdh* 97 % match (Identities 427/441, Gaps 0/441); and *tef1α* 99 % match (Identities 865/873, Gaps 0/873). The straight conidia of *B. axonopicola* distinguishes it from the slightly curved conidia of *B. austrostipae*, in addition to differences in three loci: ITS 98 % match (Identities 450/457, Gaps 2/457); *gapdh* 98 % match (Identities 431/441, Gaps 0/441); and *tef1α* 99 % match (Identities 866/875, Gaps 0/875).

Bipolaris bamagaensis Y.P. Tan & R.G. Shivas, **sp. nov.** – MycoBank MB817463. Fig. 3a–c

Etymology: Named after the locality, Bamaga, from where it was collected.

Conidiophores mononematous, erect, straight to flexuous, rarely branched, geniculate towards the apex, pale brown to brown to subhyaline at the apex, smooth, septate, up to 370 µm × 4 µm, base sometimes swollen (7–9 µm). *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, mono- or polytretic with undarkened circular scars. *Conidiogenous nodes* dark, distinct and slightly verruculose. *Conidia* ellipsoidal, fusiform, straight to slightly curved, (40–) 50–55 (–70) × (10–) 12–13 (–17) µm, uniformly pale brown to brown, smooth, 3–7 (usually 5)-distoseptate. *Hilum* darkened and sometimes thickened.

Specimens examined: AUSTRALIA, Queensland, Bamaga, from necrotic leaf on Urochloa subquadripara, 28 May 1981, *J.L. Alcorn* (BRIP 13577 holotype, includes ex-type culture); from leaf on *Dactyloctenium aegyptium*, 29 May 1981, *J.L. Alcorn* (culture BRIP 10711); culture formed in vitro by crossing isolates BRIP 13577 and BRIP 10711, 26 June 1985, *J.L. Alcorn* (culture BRIP 14847); on Yarrabah Road, Mackey Creek (near Gordonvale), from leaf

Fig. 2. *Bipolaris austrostipae* (ex-holotype BRIP 12490) **a** conidiophore with conidia, **b** conidiophore with conidium. *Bipolaris shoemakeri* (ex-holotype BRIP 15929) **c** conidiophore with a conidium, **d** conidia. *Bipolaris axonopicola* (ex-holotype BRIP 11740) **e** leaf spots on *A. fissifolius,* **f** conidiophores and conidia. *Bipolaris subramanianii* (ex-holotype BRIP 16226) **g** conidiophore, **h** conidia, **i** leaf spots on *S. sphacelata*. *Bipolaris woodii* (ex-holotype 12239) **j** conidiophore and conidia. Scale bars: e, i = 1 cm; a–d, f, h, j = 20 µm; g = 10 µm.

blight on *D. aegyptium*, 1 May 1987, *J.L. Alcorn* (culture BRIP 15879); culture formed in vitro by single-spored isolates of BRIP 15897, June 1987, *J.L. Alcorn* (culture BRIP 15934).

Notes: *Bipolaris bamagaensis* is known from specimens on *Dactyloctenium aegyptium* and *Urochloa subquadripara* with leaf necrosis. Although both grass hosts are found across Australia, *B. bamagaensis* has only been found in northern Queensland. Many *Bipolaris* species have been associated with *Dactyloctenium*, including *B. clavata, B. cynodontis, B. luttrellii* and *B. maydis* (Sivanesan 1987, Manamgoda et al. 2014), while only one species, *B. urochloae*, has been recorded on *Urochloa* (Sivanesan 1987, Manamgoda et al. 2014). There may be other records in the literature of *Bipolaris* species on *Urochloa*, as many *Brachiaria* species were transferred to *Urochloa* (Webster 1987).

Bipolaris bamagaensis formed its sexual morph in culture (BRIP 14847) when single-spored isolates from different cultures (ex-holotype BRIP 13577 and BRIP 10711), as well as from the same culture (BRIP 15879), were crossed (J.L. Alcorn herbarium notes). The sexual morph was not observed during this study, and, therefore, a description could not be provided. Morphologically, the conidiophores of *B. bamagaensis* in culture are much shorter than that observed for *B. chloridis* (up to 1.2 mm long), and the dimensions of the typically straight to slightly curved conidia fall within the range described for the mostly curved conidia of *B. chloridis* (30–100 × 10–20 µm). *Bipolaris bamagaensis* differs from *B. chloridis* in two loci: *gapdh* positions 20 (C) and 62 (T); *tef1α* positions 307 (A) and 312 (G).

Bipolaris shoemakeri Y.P. Tan & R.G. Shivas, **sp. nov.** – MycoBank MB817466. Fig. 2c–d

Etymology: Named after Professor Robert Alan Shoemaker, an internationally respected mycologist and plant pathologist, who established *Bipolaris* for helminthosporioid species with fusoid conidia and bipolar germination, thereby differentiating it from *Drechslera* and *Helminthosporium* (Shoemaker 1959).

Conidiophores mononematous, erect, straight to flexuous, rarely branched, uniformly pale brown to brown, smooth, septate, up to 1.8 mm× 6 µm. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale to subhyaline, smooth, mono- or polytretic with undarkened circular scars. *Conidiogenous nodes* distinct and slightly verruculose. *Conidia* fusiform, straight to slightly curved, (60–) 70–80 (–100) × (10–) 13.5–15 (–19) µm, pale brown to brown, smooth, 4–10 (usually 8)-distoseptate. *Hilum* darkened.

Specimens examined: AUSTRALIA, Queensland, Mount Molloy, from leaf spot on *Ischaemum rugosum* var. *segetum*, culture formed in vitro by crossing single-spored isolates, June 1987, *J.L. Alcorn* (BRIP 15929 holotype, includes ex-type culture); Mount Molloy, from leaf spot on *Ischaemum rugosum* var. *segetum*, 30 Apr. 1987, *J.L. Alcorn* (culture BRIP 15806).

Notes: *Bipolaris shoemakeri* was isolated from *Ischaemum rugosum* var. *segetum*, which is found mainly in the northern coastal region of Australia, and extends from India to Taiwan (Simon and Alfonso 2011). The ex-holotype culture (BRIP 15929) produced ascospores and was derived in vitro from self-crossed single-spored isolates of BRIP 15806 (J.L. Alcorn herbarium notes). The sexual morph was not observed during this study, and, therefore, a description could not be provided. Other species recorded on *I. rugosum* are B. *cynodontis,*

B. oryzae and *B. setariae* (Sivanesan 1987, Manamgoda et al. 2014, Farr and Rossman 2016, Herbarium Catalogue 2016). *Bipolaris shoemakeri* has longer conidiophores (up to 1.8 mm) than *B. cynodontis* (up to 170 µm), *B. oryzae* (up to 600 µm) and *B. setariae* (200 µm). *Bipolaris shoemakeri* is phylogenetically close to *B. secalis* (Fig. 1). Morphologically, the very long, straight to flexuous conidiophores of *B. shoemakeri* differ from the shorter (up to 300 µm) and apically geniculate conidiophores of B. *secalis*. *Bipolaris shoemakeri* differs from B. secalis in three loci: ITS positions 103 (G) and 339 (indel); *gapdh* positions 209 (T) and 446 (C); *tef1α* positions 453 (C) and 816 (T).

Bipolaris simmondsii Y.P. Tan & R.G. Shivas, **sp. nov.** – MycoBank MB817467. Fig. 3d–e

Etymology: Named after the Australian plant pathologist Dr. John Howard (Jack) Simmonds MBE, who listed the first helminthosporioid fungi found in Queensland (Simmonds 1966).

Conidiophores mononematous, erect, straight to flexuous, rarely branched, sometimes geniculate towards the apex, uniformly yellowish brown, paler at the apex, smooth, septate, up to 240 µm × 8 µm, basal cell swollen, up to 18 µm diam. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, monoor polytretic with circular scars. *Conidiogenous nodes* distinct and darkened. *Conidia* fusiform, straight or slightly curved, (70–) 78–116 (–130) × (12–) 13–17 (–18) µm, widest at the middle, yellowish brown to pale yellowish brown, paler at the ends, 7–10-distoseptate. *Hilum* darkened.

Specimen examined: AUSTRALIA, Queensland, Peregian Beach, on leaf spot on *Zoysia macrantha*, 14 Nov. 1976, *J.L. Alcorn* (BRIP 12030 holotype, includes ex-type culture).

Notes: *Bipolaris simmondsii* is only known from the type specimen on *Zoysia macrantha*, an endemic temperate Australian grass. The ex-type isolate was sterile under the conditions it was grown. Fortunately, dried culture specimens from the original collection in 1976 had conidiophores and conidia that allowed morphological descriptions to be made. *Bipolaris simmondsii* is phylogenetically close to *B. heveae*, which has been associated with leaf spots on Zoysia japonica in Japan (Tsukiboshi et al. 2005). *Bipolaris heveae* has conidia that sometimes have a slightly protuberant hilum (3–4 µm), while *B. simmondsii* has an inconspicuous hilum. *Bipolaris simmondsii* differs from *B. heveae* in three loci: ITS positions
452 (indel), 453 (C) and 456 (T); *gapdh* 98 % match (Identities 435/443, Gaps 0/443); *tef1α* positions 9 (T), 102 (C), 307 (G), 453 (C), 655 (G), 735 (C) and 771 (C).

Bipolaris sivanesaniana Y.P. Tan & R.G. Shivas, **sp. nov.** – MycoBank MB817468. Fig. 3f–g

Etymology: Named after Dr. Asaipillai Sivanesan, in recognition of his contributions to mycology and plant pathology, especially his seminal monograph on graminicolous helminthosporioid fungi (Sivanesan 1987).

Conidiophores mononematous, erect, straight to flexuous, rarely branched, uniformly pale brown to brown, smooth, septate, up to 600 µm × 4–6 µm; basal cell swollen and darker than the other cells, up to 18 µm diam. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to subhyaline, smooth, mono- or polytretic with undarkened circular scars. *Conidiogenous nodes* distinct and swollen. *Conidia* fusiform, straight to slightly

Fig. 3. *Bipolaris bamagaensis* (ex-holotype BRIP 16634) **a** necrotic leaves from *U. subquadripara*, **b** conidiophore, **c** conidia. *Bipolaris simmondsii* (ex-holotype BRIP 12030) **d** conidiophore, **e** conidia. *Bipolaris sivanesaniana* (ex-holotype BRIP 15847) **f** conidiophores, **g** conidia. Scale bars: a = 1 cm; b–g = 10 µm.

curved, (60–) 70–77 (–86) × (11–) 14–15.5 (–20) µm, pale brown to brown, 5–8-distoseptate. *Hilum* darkened and sometimes thickened.

Specimens examined: AUSTRALIA, Queensland, Atherton, from *Paspalidium distans*, 1 May 1987, *J.L. Alcorn* (BRIP 15847 holotype, includes ex-type culture); Julatten, from *Setaria sphacelata*, 30 Apr. 1987, *J.L. Alcorn* (culture BRIP 15822).

Notes: *Bipolaris sivanesaniana* is known from *Paspalidium distans* and *Setaria sphacelata* in Queensland. This hints at a co-evolutionary relationship as the grass hosts, *Setaria* and *Paspalidium*, are closely related (Kellogg et al. 2009, Morrone et al. 2012). *Bipolaris*

sivanesaniana is the only species described on *P. distans*, a native Australian perennial grass found in temperate and tropical regions of Asia and the Pacific. One other species, *B. setariae*, has been recorded on *P. flavidum* (Farr and Rossman 2016). *Bipolaris sivanesaniana* has longer conidiophores (up to 600 µm) than *B. setariae* (up to 200 µm long). Molecular phylogenetic comparison with *B. setariae* cannot be reliably made, as there are no available sequences for a type or authentic strain. Other *Bipolaris* species recorded on *Setaria* are *B. bicolor*, *B. cynodontis, B. leersiae, B. maydis, B. oryzae, B. panici-milacei, B. sacchari, B. salviniae, B. setariae, B. sorokiniana, B. victoriae, B. yamadae* and *B. zeicola* (Sivanesan 1987, Manamgoda et al. 2014, Farr and Rossman 2016, Herbarium Catalogue 2016), although some of these identifications have not been verified by DNA sequencing analyses.

Bipolaris sivanesaniana is phylogenetically close to B. *oryzae* and *B. panici-milacei* (Fig. 1). Morphologically, *B. sivanesaniana* has shorter conidia (60–86 µm) than *B. oryzae* (63–153 µm), and fewer septa (up to 8 versus 14). *Bipolaris sivanesaniana* has longer conidiophores than *B. panici-milacei* (up to 255 µm long). *Bipolaris sivanesaniana* differs from *B. oryzae* in two loci: ITS position 97 (C); *tef1α* position 381 (C). *Bipolaris sivanesaniana* differs from *B. panici-milacei* in three loci: ITS position 97 (C); *gapdh* position 182 (A); *tef1α* position 342 (C).

Bipolaris subramanianii Y.P. Tan & R.G. Shivas, **sp. nov.** – MycoBank MB817469. Fig. 2g–i

Etymology: Named after Professor C.V. Subramanian, in recognition of his contributions to mycology and plant pathology, especially his widely referenced monograph on hyphomycetes (Subramanian 1983).

Leaf spots on Setaria sphacelata, narrowly ellipsoidal, grey spots with brown margins, at first 1 × 0.5 mm, then expanding up to 5 cm in length with water-soaked appearance. *Conidiophores* mononematous, erect, straight to flexuous, never branched, uniformly brown to pale brown at the apex, smooth, septate, up to 830 µm × 5 µm; basal cell swollen and darker than the other cells, up to 13 µm diam. *Conidiogenous nodes* distinct and slightly swollen. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, mono- or polytretic with undarkened circular scars. *Conidia* straight to fusiform to subcylindrical, (70–) 90–99 (–130) × (9–) 11–12.5 (–15) µm, uniformly pale brown to subhyaline, smooth, 5–8-distoseptate, apex rounded, base obconically truncate. *Hilum* distinct and protuberant.

Specimen examined: AUSTRALIA, Queensland, Maclean Bridge, from leaf spot on *Setaria sphacelata*, 17 Mar. 1988, *J.L. Alcorn* (BRIP 16226 holotype, includes ex-type culture).

Notes: *Bipolaris subramanianii* is only known from the type specimen on *Setaria sphacelata*, which is a perennial African grass that has a worldwide distribution (Simon and Alfonso 2011). Other species recorded on *S. sphaecelata* are *B. cynodontis, B. maydis* and *B. zeicola* (DAF Biological Collections 2016; Farr and Rossman 2016; Herbarium Catalogue 2016); however, some of these records require verification by molecular methods. *Bipolaris subramanianii* has longer conidiophores (up to 830 µm) than *B. cynodontis* (up to 170 µm) and *B. zeicola* (up to 250 µm). *Bipolaris subramanianii* has longer conidia (70–130 µm) than *B. cynodontis* (30–75 µm). The conidia of *B. subramanianii* are typically straight to subcylindrical, whereas *B.*

cynodontis and *B. zeicola* have slightly curved conidia that are broadest in the middle and taper towards the rounded ends. *Bipolaris maydis* has conidia that are distinctly curved.

Bipolaris subramanianii is phylogenetically close to *B. shoemakeri* and *B. secalis* (Fig. 1). The conidiophores of *B. subramanianii* are shorter than *B. shoemakeri* (up to 1.8 mm), but longer than *B. secalis* (up to 300 µm). The typically straight conidia of B. subramanianii are slightly longer and thinner than the slightly curved conidia of *B. shoemakeri* (70–80 × 13.5–15 µm). The conidia of *B. subramanianii* are uniformly paler in colour and have fewer septa than the conidia of *B. secalis*, which are mostly 10-distoseptate. *Bipolaris subramanianii* differs from *B. shoemakeri* by three loci: ITS 98 % match (Identities 452/461, Gaps 3/461); *gapdh* positions 26 (T), 55 (T), 77 (G) and 209 (C); TEF1α positions 30 (G), 255 (C), 266 (G), 450 (A), 816 (C) and 843 (C). Bipolaris subramanianii differs from B. secalis by three loci: ITS 98 % match (Identities 453/460, Gaps 2/460); *gapdh* positions 26 (T), 55 (T), 77 (G) and 446 (T); *tef1α* positions 30 (G), 255 (C), 266 (G), 450 (A), 453 (C) and 843 (C).

Bipolaris woodii Y.P. Tan & R.G. Shivas, **sp. nov.** – MycoBank MB817470. Fig. 2j

Etymology: Named after Dr. Peter Wood, in recognition of his mentorship of microbiologists at the Queensland University of Technology, including the lead author.

Conidiophores mononematous, erect, straight to flexuous, rarely branched, geniculate towards the apex, uniformly pale brown to brown, smooth, septate, up to 250 µm × 5–10 µm. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, monoor polytretic with darkened circular scars. *Conidiogenous nodes* distinct, darkened and verruculose. *Conidia* fusiform, straight to slightly curved, (60–) 69–76 (–86) × (10–) 12.5–13.5 (–15) µm, brown, smooth, 7–10-distoseptate. *Hilum* darkened and sometimes thickened.

Specimen examined: AUSTRALIA, Queensland, Goondiwindi, from *Paspalidium caespitosum*, 25 Apr. 1977, *J. Brouwer* (BRIP 12239 holotype, includes ex-type culture).

Notes: *Bipolaris woodii* is only known from a single specimen on *Paspalidium caespitosum*. This grass is a native species widely distributed across inland regions of eastern Australia (Simon and Alfonso 2011). Two other species recorded on *Paspalidium* are *B. setariae* on *P. flavidum* (Farr and Rossman 2016) and *B. sivanesaniana* described in this study from *P. distans*. *Bipolaris woodii* has shorter conidiophores (up to 250 µm) than *B. sivanesaniana* (up to 600 µm). Molecular phylogenetic comparison with *B. setariae* cannot be reliably made at this point in time as there are no available sequences for an ex-type or authentic strain of *B. setariae*.

Bipolaris woodii is phylogenetically close to *B. microstegii, B. victoriae* and *B. zeicola* (Fig. 1). *Bipolaris woodii* differs from *B. microstegii* in three loci: ITS 98 % match (Identities 452/461, Gap 4/461); *gapdh* positions 83 (T), 111 (T) and 383 (T); *tef1α* positions 138 (T), 265 (T) and 572 (C). *Bipolaris woodii* also differs in morphology, with shorter conidiophores than *B. microstegii* (up to 750 µm). *Bipolaris woodii* differs from *B. victoriae* in three loci: ITS 98 % match (Identities 454/461, Gaps 4/461); *gapdh* positions 83 (T), 98 (T), 111 (T) and 383 (T); *tef1α* positions 333 (C) and 573 (C). *Bipolaris woodii* has slightly smaller and darker conidia than *B. victoriae* (40–120 × 12–19 µm) (Sivanesan 1987). *Bipolaris woodii*

differs from *B. zeicola* in three loci: ITS 98 % match (Identities 452/462, Gaps 5/462); *gapdh* positions 83 (T), 111 (T), 383 (T) and 425 (C); *tef1α* position 573 (C). *Bipolaris woodii* has a darkened and conspicuous hilum, and, thereby, differs from *B. zeicola*, which has an inconspicuous hilum.

DISCUSSION

Phylogenetic analyses based on ITS and *gapdh* sequences, either individually or concatenated, provided sufficient resolution for delimiting taxa within *Bipolaris* (Berbee et al. 1999, Manamgoda et al. 2012, 2014, Tan et al. 2014). Further, a four-locus dataset (ITS, *gapdh*, LSU and *tef1α*) provided stronger support for the description of new helminthosporioid species (Manamgoda et al. 2012, Tan et al. 2014). In this study, 13 isolates from the BRIP collection, recognised by Dr. John L. Alcorn as taxonomically interesting and potentially distinct, were analysed against reference sequences of cultures available from currently accepted *Bipolaris* species based on three loci, ITS, *gapdh* and *tef1α*. Analyses with LSU were omitted in the dataset as they provided little information to warrant inclusion. Nonetheless, LSU sequences have been deposited in GenBank to facilitate future studies (Table 1). The phylogenetic analyses of the combined three locus dataset resolved the 13 BRIP isolates into eight novel *Bipolaris* species. It is not known whether the species are pathogens, endophytes or saprobes. The description of these species provides a foundation upon which additional sampling and accumulation of molecular data will improve knowledge of their host ranges and ecological roles.

The ITS locus is the universal barcode marker for fungi (Schoch et al. 2012). The ITS alignment used in this study was able to resolve 19 out of 36 *Bipolaris* species, including four of the new species. However, some studies have used only ITS to identify and describe *Bipolaris* species (Ahmadpour et al. 2012, da Cunha et al. 2012). Most recently, taxonomists have accepted that a secondary locus is essential for the accurate identification of many taxa (Madrid et al. 2014, Manamgoda et al. 2012, Tan et al. 2014, Manamgoda et al. 2015, Stielow et al. 2015). The protein-coding loci of *gapdh*, *tef1α* and RNA polymerase II second largest subunit (*rpb2*) have been reported to be phylogenetically informative in the analyses of helminthosporioid species, and complement species identification and classification studies (Crous et al. 2012, 2013, Manamgoda et al. 2012, 2014, 2015, Madrid et al. 2014, Tan et al. 2014). The *gapdh* and *tef1α* alignments used in this study were able to resolve 34 out of 36 *Bipolaris* species, including the eight new species described here. None of the ITS, *gapdh* or the *tef1α* alignments were able to differentiate between the ex-holotype strain of *B. coffeana* and the recently designated ex-epitype strain of *B. cynodontis* (Manamgoda et al. 2014). A comparison of the sequences of *B. coffeana* and *B. cynodontis* indicates a potential synonymy, which is supported by shared conidial characteristics. The loci *gapdh* and *tef1α* were determined to be the most suitable single locus marker for species-level identification within *Bipolaris*. Madrid et al. (2014) found *rpb2*, followed by *gapdh*, to be the most informative loci for helminthosporioid phylogeny. Analyses with *rpb2*could not be included in this study as sequences were only available for ex-type isolates of three *Bipolaris* species. It is strongly suggested that the classification of new taxa in *Bipolaris* be accompanied by the official fungal barcode, ITS and a secondary locus, *gapdh*, *tef1α* or *rpb2*.

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CHAPTER 5

Cryptic species of *Curvularia* in the culture collection of the Queensland Plant Pathology Herbarium

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Key words: *Dothideomycetes*, Multigene phylogeny, Taxonomy, 13 new species.

Abstract

Several unidentified specimens of *Curvularia* deposited in the Queensland Plant Pathology Herbarium were re-examined. Phylogenetic analyses based on sequence data of the internal transcribed spacer region, partial fragments of the glyceraldehyde-3-phosphate dehydrogenase and the translation elongation factor 1-α genes, supported the introduction of 13 novel *Curvularia* species. Eight of the species described, namely, *C. beasleyi* sp. nov., *C. beerburrumensis* sp. nov., *C. eragrosticola* sp. nov., *C. kenpeggii* sp. nov., *C. mebaldsii* sp. nov., *C. petersonii* sp. nov., *C. platzii* sp. nov. and *C. warraberensis* sp. nov., were isolated from grasses (*Poaceae*) exotic to Australia. Only two species, *C. lamingtonensis* sp. nov. and *C. sporobolicola* sp. nov., were described from native Australian grasses. Two species were described from hosts in other families, namely, *C. coatsiae* sp. nov. from *Litchi chinensis* (*Sapindaceae*) and *C. colbranii* sp. nov. from *Crinum zeylanicum* (*Amaryllidaceae*). *Curvularia reesii* sp. nov. was described from an isolate obtained from an air sample. Furthermore, DNA sequences from ex-type cultures supported the generic placement of *C. neoindica* and the transfer of *Drechslera boeremae* to *Curvularia*.

INTRODUCTION

Curvularia is a species-rich genus of pathogens and saprobes associated with plant, human and animals worldwide (Sivanesan 1987, Hyde et al. 2014, Madrid et al. 2014, Manamgoda et al. 2015, Marin-Felix et al. 2017a, 2017b). *Curvularia* species have also been reported from substrates such as air (Almaguer et al. 2012, Hargreaves et al. 2013), aquatic environments (Verma et al. 2013, Su et al. 2015, Sharma et al. 2016) and soil (Manamgoda et al. 2011, Marin-Felix et al. 2017a).

Species delimitation within *Curvularia* based solely on morphology is difficult as many species share similar characters and have overlapping conidial dimensions. Currently, there are 131 species of *Curvularia* (excluding varieties) listed in *Index Fungorum* (accessed on 4 January 2018). Phylogenetic studies based on multilocus sequence analyses of ex-type or reference cultures have recently delimited many cryptic species (Deng et al. 2014, Manamgoda et al. 2014, Tan et al. 2014, Manamgoda et al. 2015, Marin-Felix et al. 2017a, 2017b). Presently, there are 81 accepted species for which taxonomic placement has been established by DNA barcodes to allow accurate identification and comparison (Marin-Felix et al. 2017a, 2017b).

In Australia, 64 species of *Curvularia* have been reported (DAF Biological Collections 2018, Farr and Rossman 2018). Of these, 17 species were described from Australia, namely *C. australiensis, C. australis, C. bothriochloae, C. crustacea, C. dactyloctenii, C. graminicola, C. harveyi, C. heteropogonis, C. micrairae, C. ovariicola, C. perotidis, C. queenslandica, C. ravenelii, C. richardiae, C. ryleyi, C. sorghina* and *C. tripogonis*. Eight of the Australian *Curvularia* species were originally placed in the closely related genus, *Bipolaris*, before transfer to *Curvularia* based on molecular studies (Manamgoda et al. 2012, 2014, Tan et al. 2014).

In this study, 17 unidentified isolates of *Curvularia* maintained in the culture collection held in the Queensland Plant Pathology Herbarium (BRIP) were compared with ex-type and reference isolates. Thirteen new species of *Curvularia* were revealed based on multilocus phylogenetic analyses and are formally described here. In addition, phylogenetic analyses of ex-type cultures

have confirmed the placement of a *Curvularia* species, as well as the introduction of a new combination.

MATERIALS AND METHODS

Isolates and morphology

Unidentified isolates of *Curvularia* were obtained from BRIP (Table 1), which retains cultures in a metabolically inactive state at -80 °C in a sterile solution of 15 % v/v glycerol. In order to observe conidia and conidiophores, living cultures were grown on sterilised leaf pieces of *Zea mays* on modified Sachs agar and on sterilised wheat straws on water agar, incubated at room temperature (approx. 25 °C) for seven days and exposed to near ultraviolet light on a 12 h light/dark diurnal cycle (Sivanesan 1987). Conidia and conidiophores were mounted on glass slides in lactic acid (100 % v/v). Images were captured with a Leica DFC 500 camera attached to a Leica DM5500B compound microscope with Nomarski differential interference contrast illumination. Conidial widths were measured at the widest part of each conidium. Means and standard deviations (SD) were calculated from at least 20 measurements. Ranges were expressed as (minimum value–) mean-SD–mean+SD (−maximum value) with values rounded to 0.5 µm.

Colonies were described from 7-d-old cultures grown on potato dextrose agar (PDA) (Becton Dickinson), incubated at room temperature (approx. 25°C) and exposed to near-ultraviolet light on a diurnal cycle. Images of the colonies and herbarium specimens were captured by an Epson Perfection V700 scanner at a 300 dpi resolution. Colour of the colonies was rated according to Rayner (1970). Taxonomic novelties were deposited in MycoBank (www.MycoBank.org; Crous et al. 2004).

DNA isolation, amplification, and phylogenetic analyses

Isolates were grown on PDA for 7 d at room temperature (approx. 25 °C). Mycelium was scraped off the PDA cultures and macerated with 0.5 mm glass beads (Daintree Scientific) in a Tissue Lyser (Qiagen). Genomic DNA was extracted with the Gentra Puregene DNA Extraction Kit (Qiagen) according to the manufacturer's instructions. Amplification and sequencing of the internal transcribed spacer (ITS) region, glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) and the translation elongation factor 1-alpha (*tef1α*) loci followed the methods by Tan et al. (2014). All sequences generated were assembled using Geneious v. 9.1.8 (Biomatters Ltd) and deposited in GenBank (Table 1, in bold). Sequences were aligned with selected sequences of *Curvularia* species obtained from GenBank (Table 1) using the MAFFT alignment algorithm (Katoh et al. 2009) in Geneious. *Bipolaris maydis* (CBS 136.29) was included as the outgroup. The sequences of each locus were aligned separately and manually adjusted where necessary. The alignment included sequences from ex-type cultures of 63 species of *Curvularia* and from the reference cultures of 16 species. The Maximum-Likelihood (ML) and Bayesian Inference (BI) methods were used in phylogenetic analyses as described by Tan et al. (2016). Briefly, the ML analysis was run using RAxML v. 7.2.8 (Stamatakis and Alachiotis 2010) in Geneious and started from a random tree topology. The nucleotide substitution model used was GTR with a gamma-distributed rate variation. The Markov chain Monte Carlo (MCMC) algorithm was used to create a phylogenetic tree based on Bayesian probabilities using MrBayes v. 3.2.1

Table 1. *Curvularia* isolates examined.

				GenBank accession numbers[b]		
Species	**Isolate no.[a]**	***Host***	**Location**	**ITS**	***gapdh***	***tef1a***
Bipolaris maydis	CBS 136.29^T	*Zea mays*	USA	AF071325	KM034846	KM093794
Curvularia aeria	CBS 294.61^T	air	Brazil	HF934910	HG779148	-
Curvularia affinis	CBS 154.34^T	unknown	Indonesia	KJ909780	KM230401	KM196566
Curvularia akaii	CBS 317.86	unknown	Japan	KJ909782	KM230402	KM196569
Curvularia akaiiensis	BRIP 16080^T	unknown	India	KJ415539	KJ415407	KJ415453
Curvularia alcornii	MFLUCC 10-0703^T	*Zea mays*	Thailand	JX256420	JX276433	JX266589
Curvularia americana	UTHSC 08-3414^T	*Homo sapiens*	USA	HE861833	HF565488	-
Curvularia asiatica	MFLUCC 10-0711^T	*Panicum* sp.	Thailand	JX256424	JX276436	JX266593
Curvularia australiensis	BRIP 12044^T	*Oryza sativa*	Australia	KJ415540	KJ415406	KJ415452
Curvularia australis	BRIP 12521^T	*Sporobolus caroli*	Australia	KJ415541	KJ415405	KJ415451
Curvularia bannonii	BRIP 16732^T	*Jacquemontia tamnifolia*	USA	KJ415542	KJ415404	KJ415450
Curvularia beasleyi sp. nov.	BRIP 10972^T	*Chloris gayana*	Australia	**MH414892**	**MH433638**	**MH433654**
	BRIP 15854	*Leersia hexandra*	Australia	**MH414893**	**MH433639**	**MH433655**
Curvularia beerburrumensis sp. nov.	BRIP 12942^T	*Eragrostis bahiensis*	Australia	**MH414894**	**MH433634**	**MH433657**
	BRIP 12555	*Eragrostis sororia*	Australia	**MH414895**	**MH433640**	**MH433656**
Curvularia boeremae comb. nov.	IMI 164633^T	*Portulaca oleracea*	India	**MH414911**	**MH433641**	**-**
Curvularia borreriae	MFLUCC 11-0422	unknown Poaceae	Thailand	KP400638	KP419987	KM196571
Curvularia bothriochloae	BRIP 12522^T	*Bothriochloa bladhii*	Australia	KJ415543	KJ415403	KJ415449
Curvularia brachyspora	CBS 186.50	Soil	India	KJ922372	KM061784	KM230405

Table 1. (Ctd).

Species	Isolate no.[a]	*Host*	Location	GenBank accession numbers[b]		
				ITS	*gapdh*	*tef1a*
Curvularia buchloës	CBS 246.49[T]	*Buchloë dactyloides*	USA	KJ909765	KM061789	KM196588
Curvularia carica-papayae	CBS 135941[T]	*Carica papaya*	India	HG778984	HG779146	-
Curvularia chiangmaiensis	CPC 28829[T]	*Zea mays*	Thailand	MF490814	MF490836	MF490857
Curvularia chlamydospora	UTHSC 07-2764[T]	*Homo sapiens*	USA	HG779021	HG779151	-
Curvularia coatsiae sp. nov.	BRIP 24170	air	Australia	**MH414896**	**MH433635**	**MH433658**
	BRIP 24261[T]	*Litchi chinensis*	Australia	**MH414897**	**MH433636**	**MH433659**
Curvularia clavata	BRIP 61680b	*Oryza rufipogon*	Australia	KU552205	KU552167	KU552159
Curvularia coicis	CBS 192.29[T]	*Coix lacryma-jobi*	Japan	AF081447	AF081410	JN601006
Curvularia colbranii sp. nov.	BRIP 13066[T]	*Crinum zeylanicum*	Australia	**MH414898**	**MH433642**	**MH433660**
Curvularia crustacea	BRIP 13524[T]	*Sporobolus* sp.	Indonesia	KJ415544	KJ415402	KJ415448
Curvularia cymbopogonis	CBS 419.78	*Yucca* sp.	Netherlands	HG778985	HG779129	-
Curvularia dactyloctenicola	CPC 28810[T]	*Dactyloctenium aegyptium*	Thailand	MF490815	MF490837	MF490858
Curvularia dactyloctenii	BRIP 12846[T]	*Dactyloctenium radulans*	Australia	KJ415545	KJ415401	KJ415447
Curvularia ellisii	CBS 193.62[T]	air	Pakistan	JN192375	JN600963	JN601007
Curvularia eragrostidis	CBS 189.48	*Sorghum* sp.	Indonesia	HG778986	HG779154	-
Curvularia eragrosticola sp. nov.	BRIP 12538[T]	*Eragrostis pilosa*	Australia	**MH414899**	**MH433643**	**MH433661**
Curvularia geniculata	CBS 187.50	*Andropogon sorghum*	Indonesia	KJ909781	KM083609	KM230410
Curvularia gladioli	CBS 210.79	*Gladiolus* sp.	Romania	HG778987	HG779123	-
Curvularia graminicola	BRIP 23186[T]	*Aristida ingrata*	Australia	JN192376	JN600964	JN601008

Table 1. (Ctd).

Species	Isolate no.[a]	*Host*	Location	GenBank accession numbers[b]		
				ITS	*gapdh*	*tef1a*
Curvularia harveyi	BRIP 57412^T	*Triticum aestivum*	Australia	KJ415546	KJ415400	KJ415446
Curvularia hawaiiensis	BRIP 11987^T	*Oryza sativa*	USA	KJ415547	KJ415399	KJ415445
Curvularia heteropogonicola	BRIP 14579^T	*Heteropogon contortus*	India	KJ415548	KJ415398	KJ415444
Curvularia heteropogonis	CBS 284.91^T	*Heteropogon contortus*	Australia	KJ415549	JN600969	JN601013
Curvularia hominis	CBS 136985^T	*Homo sapiens*	USA	HG779011	HG779106	-
Curvularia homomorpha	CBS 156.60^T	air	USA	JN192380	JN600970	JN601014
Curvularia inequalis	CBS 102.42^T	soil	France	KJ922375	KM061787	KM196574
Curvularia intermedia	CBS 334.64	*Avena versicolor*	USA	HG778991	HG779155	-
Curvularia ischaemi	CBS 630.82^T	*Ischaemum indicum*	Solomon Islands	JX256428	JX276440	-
Curvularia kenpeggii sp. nov.	BRIP 14530^T	*Triticum aestivum*	Australia	**MH414900**	**MH433644**	**MH433662**
Curvularia kusanoi	CBS 137.29	*Eragrostis major*	Japan	JN192381	-	JN601016
Curvularia lamingtonensis sp. nov.	BRIP 12259^T	*Microlaena stipoides*	Australia	**MH414901**	**MH433645**	**MH433663**
Curvularia lunata	CBS 730.96^T	*Homo sapiens*	USA	JX256429	JX276441	JX266596
Curvularia malina	CBS 131274^T	*Zoysia matrella*	USA	JF812154	KP153179	KR493095
Curvularia mebaldsii sp. nov.	BRIP 12900^T	*Cynodon transvaalensis*	Australia	**MH414902**	**MH433647**	**MH433664**
	BRIP 13983	*Cynodon dactylon x transvaalensis*	Australia	**MH414903**	**MH433646**	**MH433665**
Curvularia miyakei	CBS 197.29^T	*Eragrostis pilosa*	Japan	KJ909770	KM083611	KM196568
Curvularia muehlenbeckiae	CBS 144.63^T	*Sorghum* sp.	USA	KP400647	KP419996	KM196578

Table 1. (Ctd).

Species	Isolate no.[a]	*Host*	Location	GenBank accession numbers[b]		
				ITS	*gapdh*	*tef1a*
Curvularia neergaardii	BRIP 12919^{T}	*Oryza sativa*	Ghana	KJ415550	KJ415397	KJ415443
Curvularia neoindica	IMI 129790^{T}	*Brassica nigra*	India	**MH414910**	**MH433649**	**MH433667**
Curvularia nicotiae	BRIP 11983^{T}	soil	Algeria	KJ415551	KJ415396	KJ415442
Curvularia nodosa	CPC 28800^{T}	*Digitaria ciliaris*	Thailand	MF490816	MF490838	MF490859
Curvularia nodulosa	CBS 160.58	*Eleusine indica*	USA	JN601033	JN600975	JN601019
Curvularia oryzae	CBS 169.53^{T}	*Oryza sativa*	Vietnam	KP400650	KP645344	KM196590
Curvularia ovariicola	CBS 470.90^{T}	*Eragrostis interrupta*	Australia	JN192384	JN600976	JN601020
Curvularia pallescens	CBS 156.35^{T}	air	Indonesia	KJ922380	KM083606	KM196570
Curvularia papendorfii	CBS 308.67^{T}	*Acacia karroo*	South Africa	KJ415552	KJ415395	KJ415441
Curvularia petersonii sp. nov.	BRIP 14642^{T}	*Dactyloctenium aegyptium*	Australia	**MH414905**	**MH433650**	**MH433668**
Curvularia perotidis	CBS 350.90^{T}	*Perotis rara*	Australia	JN192385	KJ415394	JN601021
Curvularia pisi	CBS 190.48^{T}	*Pisum sativum*	Canada	KY905678	KY905690	KY905697
Curvularia platzii sp. nov.	BRIP 27703b^{T}	*Cenchrus clandestinum*	Australia	**MH414906**	**MH433651**	**MH433669**
Curvularia portulacae	BRIP 14541^{T}	*Portulaca oleracea*	USA	KJ415553	KJ415393	KJ415440
Curvularia prasadii	CBS 143.64^{T}	*Jasminum sambac*	India	KJ922373	KM061785	KM230408
Curvularia protuberata	CBS 376.65^{T}	*Deschampsia flexuosa*	UK	KJ922376	KM083605	KM196576
Curvularia pseudobrachyspora	CPC 28808^{T}	*Eleusine indica*	Thailand	MF490819	MF490841	MF490862
Curvularia pseudolunata	UTHSC 09-2092^{T}	*Homo sapiens*	USA	HE861842	HE861842	-

Table 1. (Ctd).

Species	Isolate no.[a]	*Host*	Location	GenBank accession numbers[b]		
				ITS	*gapdh*	*tef1a*
Curvularia pseudorobusta	UTHSC 08-3458	*Homo sapiens*	USA	HE861838	HF565476	-
Curvularia ravenelii	BRIP 13165^{T}	*Sporobolus fertilis*	Australia	JN192386	JN600978	JN601024
Curvularia reesii sp. nov.	BRIP 4358^{T}	air	Australia	**MH414907**	**MH433637**	**MH433670**
Curvularia richardiae	BRIP 4371^{T}	*Richardia brasiliensis*	Australia	KJ415555	KJ415391	KJ415438
Curvularia robusta	CBS 624.68^{T}	*Dichanthium annulatum*	USA	KJ909783	KM083613	KM196577
Curvularia ryleyi	BRIP 12554^{T}	*Sporobolus creber*	Australia	KJ415556	KJ415390	KJ415437
Curvularia senegalensis	*Curvularia* S 149.71	unknown	Nigeria	HG779001	HG779128	-
Curvularia soli	CBS 222.96^{T}	soil	Papua New Guinea	KY905679	KY905691	KY905698
Curvularia sorghina	BRIP 15900^{T}	*Sorghum bicolor*	Australia	KJ415558	KJ415388	KJ415435
Curvularia spicifera	CBS 274.52	soil	Spain	JN192387	JN600979	JN601023
Curvularia sporobolicola sp. nov.	BRIP 23040b^{T}	*Sporobolus australasicus*	Australia	**MH414908**	**MH433652**	**MH433671**
Curvularia subpapendorfii	CBS 656.74^{T}	soil	Egypt	KJ909777	KM061791	KM196585
Curvularia trifolii	CBS 173.55	*Trifolium repens*	USA	HG779023	HG779124	-
Curvularia tripogonis	BRIP 12375^{T}	*Tripogon loliiformis*	Australia	JN192388	JN600980	JN601025
Curvularia tropicalis	BRIP 14834^{T}	*Coffea arabica*	India	KJ415559	KJ415387	KJ415434
Curvularia tsudae	ATCC 44764^{T}	*Chloris gayana*	Japan	KC424596	KC747745	KC503940
Curvularia tuberculata	CBS 146.63^{T}	*Zea mays*	India	JX256433	JX276445	JX266599
Curvularia uncinata	CBS 221.52^{T}	*Oryza sativa*	Vietnam	HG779024	HG779134	-
Curvularia variabilis	CPC 28815^{T}	*Chloris barbata*	Thailand	MF490822	MF490844	MF490865

Table 1. (Ctd).

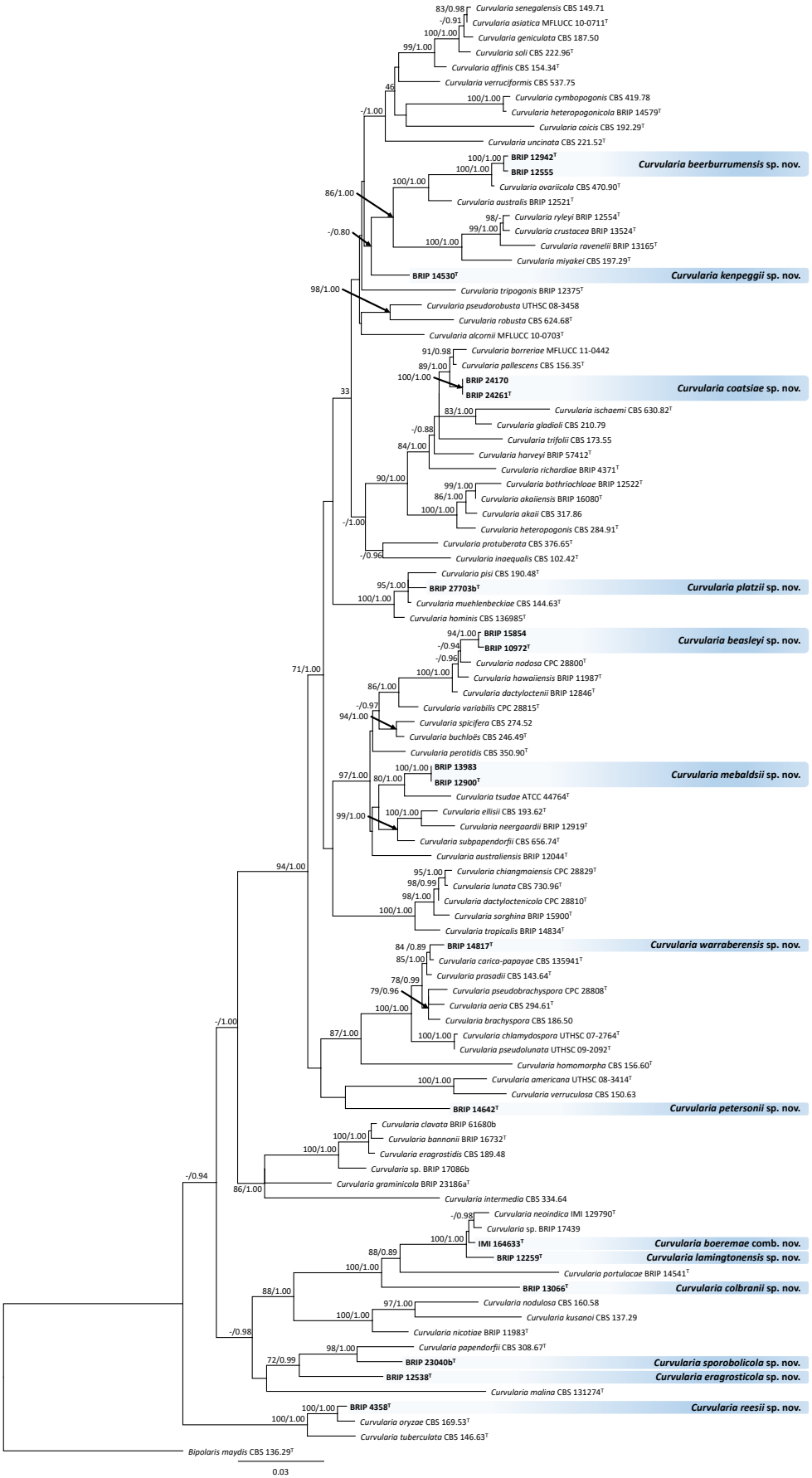
Species	Isolate no.[a]	*Host*	Location	GenBank accession numbers[b]		
				ITS	*gapdh*	*tef1a*
Curvularia verruciformis	CBS 537.75	*Vanellus miles*	New Zealand	HG779026	HG779133	-
Curvularia verruculosa	CBS 150.63	*Punica granatum*	India	KP400652	KP645346	KP735695
Curvularia warraberensis sp. nov.	BRIP 14817[T]	*Dactyloctenium aegyptium*	Australia	**MH414909**	**MH433653**	**MH433672**
Curvularia sp.	BRIP 17068b	*Micraira subulifolia*	Australia	**MH414904**	**MH433648**	**MH433666**
	BRIP 17439	*Trianthema portulacastrum*	Australia	AF081449	AF081406	**MH445455**

[a] ATCC: American Type Culture Collection, Manassas, Virginia, USA; BRIP: Queensland Plant Pathology Herbarium, Brisbane, Australia; CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CPC: cultures of Pedro Crous, housed at Westerdijk Fungal Biodiversity Institute; ICMP: International Collection of Microorganisms for Plants, Auckland, New Zealand; IMI: International Mycological Institute, CABI-Bioscience, Egham, United Kingdom; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; UTHSC: Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio, Texas, USA.

[b] ITS: internal transcribed spacer; *gapdh*: glyceraldehyde-3-phosphate dehydrogenase; *tef1α*: translation elongation factor 1-alpha.

GenBank accessions derived from this study are shown in **bold**.

[T] Indicates ex-type culture


Curvularia senegalensis CBS 149.71
Curvularia asiatica MFLUCC 10-0711T
Curvularia geniculata CBS 187.50
Curvularia soli CBS 222.96T
Curvularia affinis CBS 154.34T
Curvularia verruciformis CBS 537.75
Curvularia cymbopogonis CBS 419.78
Curvularia heteropogonicola BRIP 14579T
Curvularia coicis CBS 192.29T
Curvularia uncinata CBS 221.52T
BRIP 12942T
BRIP 12555
Curvularia beerburrumensis sp. nov.
Curvularia ovariicola CBS 470.90T
Curvularia australis BRIP 12521T
Curvularia ryleyi BRIP 12554T
Curvularia crustacea BRIP 13524T
Curvularia ravenelii BRIP 13165T
Curvularia miyakei CBS 197.29T
BRIP 14530T
Curvularia kenpeggii sp. nov.
Curvularia tripogonis BRIP 12375T
Curvularia pseudorobusta UTHSC 08-3458
Curvularia robusta CBS 624.68T
Curvularia alcornii MFLUCC 10-0703T
Curvularia borreriae MFLUCC 11-0442
Curvularia pallescens CBS 156.35T
BRIP 24170
BRIP 24261T
Curvularia coatsiae sp. nov.
Curvularia ischaemi CBS 630.82T
Curvularia gladioli CBS 210.79
Curvularia trifolii CBS 173.55
Curvularia harveyi BRIP 57412T
Curvularia richardiae BRIP 4371T
Curvularia bothriochloae BRIP 12522T
Curvularia akaiiensis BRIP 16080T
Curvularia akaii CBS 317.86
Curvularia heteropogonis CBS 284.91T
Curvularia protuberata CBS 376.65T
Curvularia inaequalis CBS 102.42T
Curvularia pisi CBS 190.48T
BRIP 27703bT
Curvularia platzii sp. nov.
Curvularia muehlenbeckiae CBS 144.63T
Curvularia hominis CBS 136985T
BRIP 15854
BRIP 10972T
Curvularia beasleyi sp. nov.
Curvularia nodosa CPC 28800T
Curvularia hawaiiensis BRIP 11987T
Curvularia dactyloctenii BRIP 12846T
Curvularia variabilis CPC 28815T
Curvularia spicifera CBS 274.52
Curvularia buchloës CBS 246.49T
Curvularia perotidis CBS 350.90T
BRIP 13983
BRIP 12900T
Curvularia mebaldsii sp. nov.
Curvularia tsudae ATCC 44764T
Curvularia ellisii CBS 193.62T
Curvularia neergaardii BRIP 12919T
Curvularia subpapendorfii CBS 656.74T
Curvularia australiensis BRIP 12044T
Curvularia chiangmaiensis CPC 28829T
Curvularia lunata CBS 730.96T
Curvularia dactyloctenicola CPC 28810T
Curvularia sorghina BRIP 15900T
Curvularia tropicalis BRIP 14834T
BRIP 14817T
Curvularia warraberensis sp. nov.
Curvularia carica-papayae CBS 135941T
Curvularia prasadii CBS 143.64T
Curvularia pseudobrachyspora CPC 28808T
Curvularia aeria CBS 294.61T
Curvularia brachyspora CBS 186.50
Curvularia chlamydospora UTHSC 07-2764T
Curvularia pseudolunata UTHSC 09-2092T
Curvularia homomorpha CBS 156.60T
Curvularia americana UTHSC 08-3414T
Curvularia verruculosa CBS 150.63
BRIP 14642T
Curvularia petersonii sp. nov.
Curvularia clavata BRIP 61680b
Curvularia bannonii BRIP 16732T
Curvularia eragrostidis CBS 189.48
Curvularia sp. BRIP 17086b
Curvularia graminicola BRIP 23186aT
Curvularia intermedia CBS 334.64
Curvularia neoindica IMI 129790T
Curvularia sp. BRIP 17439
IMI 164633T
Curvularia boeremae comb. nov.
BRIP 12259T
Curvularia lamingtonensis sp. nov.
Curvularia portulacae BRIP 14541T
BRIP 13066T
Curvularia colbranii sp. nov.
Curvularia nodulosa CBS 160.58
Curvularia kusanoi CBS 137.29
Curvularia nicotiae BRIP 11983T
Curvularia papendorfii CBS 308.67T
BRIP 23040bT
Curvularia sporobolicola sp. nov.
BRIP 12538T
Curvularia eragrosticola sp. nov.
Curvularia malina CBS 131274T
BRIP 4358T
Curvularia reesii sp. nov.
Curvularia oryzae CBS 169.53T
Curvularia tuberculata CBS 146.63T
Bipolaris maydis CBS 136.29T
0.03

(Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003) in Geneious. To remove the need for *a priori* model testing, the MCMC analysis was set to sample across the entire general time-reversible (GTR) model space with a gamma-distributed rate variation across the sites. Ten million random trees were generated using the MCMC procedure with four chains. The sample frequency was set at 100 and the temperature of the heated chain was 0.1. Burn-in was set at 25 %, after which the likelihood values were stationary. The concatenated alignment was deposited in TreeBASE (S22563).

Unique fixed nucleotide positions were used to characterise and describe two cryptic species (see applicable species notes). For each of the cryptic species that was described, the closest phylogenetic neighbour was selected (Fig. 1) and this focused dataset was subjected to single nucleotide polymorphism (SNP) analysis. These SNPs were determined for each aligned locus using the Find Variation / SNPs feature in Geneious. The SNPs were determined based on a minimum variant frequency of 0.2.

RESULTS

Molecular phylogeny

Approximately 800 bp of the ITS region, 598 bp of the partial region of the *gapdh* gene and 969 bp of the partial region of the *tef1α* gene were sequenced from the BRIP isolates. After removing ambiguously aligned regions, the ITS, *gapdh* and *tef1α* alignments were trimmed to 474 bp, 544 bp and 867 bp, respectively. The ITS phylogeny was able to resolve 53 of 79 *Curvularia* species, including 10 of the new species (data not shown). The *gapdh* phylogeny inferred 12 new species and the *tef1α* phylogeny resolved all 13 of the new species (data not shown). As the topologies of the single locus phylogenies for the tree datasets did not show any conflicts, they were analysed in a concatenated alignment. The phylogenetic tree based on the concatenated alignment resolved the 17 BRIP isolates into 13 well-supported and unique clades (Fig. 1), which are described in this study as novel species.

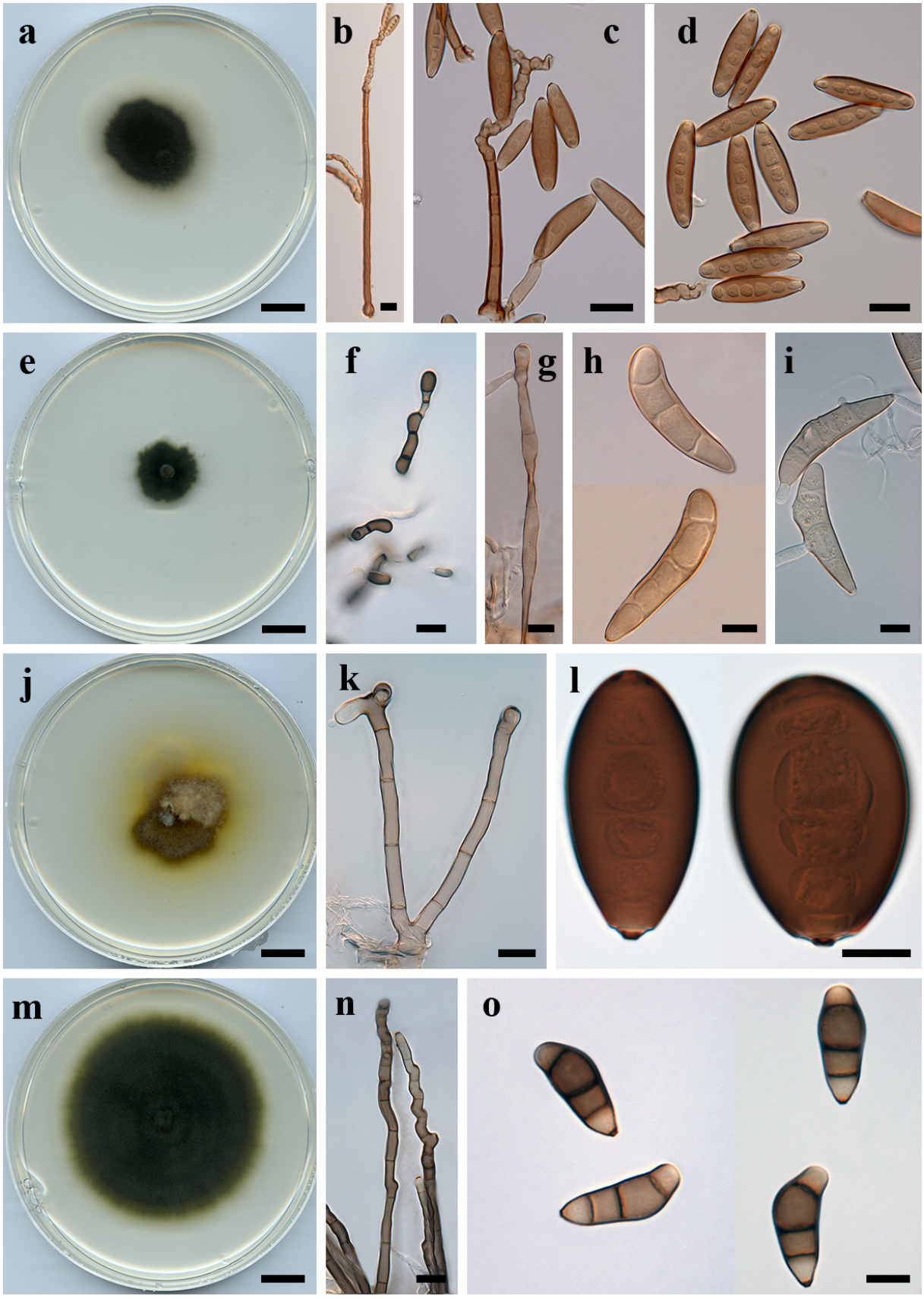
Taxonomy

Curvularia beasleyi Y.P. Tan & R.G. Shivas, **sp. nov. –** MycoBank MB825449. Fig. 2a–d

Etymology: In recognition of Dr. Dean R. Beasley, an Australian plant pathologist, for his dedication and numerous innovative contributions to the curation and promotion of the Queensland Plant Pathology Herbarium (BRIP).

Colonies on PDA approx. 4 cm diam. after 7 d at 25 °C, surface funiculose, margin fimbriate, olivaceous black. *Hyphae* subhyaline, smooth to branched, septate, up to 3 µm in width.

Fig. 1. Phylogenetic tree based on maximum likelihood analysis of the combined multilocus alignment. RAxML bootstrap values (bs) greater than 70 % and Bayesian posterior probabilities (pp) greater than 0.7 are given at the nodes (bs/pp). Novel species names are highlighted in blue. Ex-type isolates are marked with a T. The outgroup is *Bipolaris maydis* ex-type strain CBS 136.29.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Conidiophores branched, erect, straight to flexuous, geniculate towards apex, brown, paler towards apex, smooth, septate, up to 110 µm long, 4 µm wide; basal cell swollen and darker than the other cells, up to 6 µm diam. *Conidiogenous cells* integrated, terminal or intercalary, sympodial, pale brown, smooth, with darkened scars. *Conidia* fusiform, straight to slightly curved, rounded at the apex, (14–) 26–29 (–34) × (5–) 6.5–7.5 (–9) µm, brown to dark brown, 3–7 (mostly 5)-distoseptate. *Hila* conspicuous, slightly protuberant, thickened and darkened, 1−1.5 µm wide.

Specimens examined: AUSTRALIA, Queensland, Beaudesert, from leaf spot on *Chloris gayana*, 9 Jan. 1974, *J.L. Alcorn* (BRIP 10972 holotype, includes ex-type culture); Atherton, from leaf spot on *Leersia hexandra*, 1 May 1987, *J.L. Alcorn* (BRIP 15854, includes culture).

Notes: *Curvularia beasleyi* is placed in the same clade as *C. dactyloctenii*, *C. hawaiiensis* and *C. nodosa* (Fig. 1). *Curvularia dactyloctenii* and *C. hawaiiensis* have been recorded in Australia (Sivanesan 1987, Tan et al. 2014), but the recently described *C. nodosa* has only been reported from Thailand (Marin-Felix et al. 2017b). *Curvularia beasleyi* is distinguished in two loci from the ex-type cultures of *C. dactyloctenii* (99 % in *gapdh* and 99 % in *tef1α*), *C. hawaiiensis* (98 % in *gapdh* and 99 % in *tef1α*) and *C. nodosa* (99 % in *gapdh* and 99 % in *tef1α*). The conidia of *C. beasleyi* are longer than those of *C. nodosa* (12–25 µm, Marin-Felix et al. 2017b) and shorter than those of *C. dactyloctenii* (32–55 µm, Sivanesan 1987). *Curvularia beasleyi* is morphologically similar to *C. hawaiiensis*, however the later species has never been recorded on *Leersia* (Farr and Rossman 2018).

Curvularia beasleyi is only known from Queensland on two unrelated grasses, the introduced host *Chloris gayana* and the native *Leersia hexandra*. There are many *Curvularia* species reported as associated with *Chloris* spp. (*C. australiensis*, *C. australis, C. hawaiiensis*, *C. lunata*, *C. nodosa*, *C. pallescens*, *C. tsudae*, *C. variabilis*, C. *verruculosa*) (Sivanesan 1987, Deng et al. 2014, Manamgoda et al. 2014, Marin-Felix et al. 2017b) and *Leersia* spp. (*C. australiensis*, *C. geniculata*, and *C. heteropogonicola*) (DAF Biological Collections 2018, Farr and Rossman 2018, Herbarium Catalogue 2018), although not all of the reports have been verified by molecular phylogenetic analyses.

Curvularia beerburrumensis Y.P. Tan & R.G. Shivas, **sp. nov. –** MycoBank MB825450. Fig. 2e–i

Etymology: Named after the town Beerburrum, where the holotype was collected.

Colonies on PDA approx. 2 cm diam. after 7 d at 25 °C, surface funiculose, margin fimbrillate, olivaceous black. *Hyphae* subhyaline, smooth to asperulate, branched, septate, 3−4 µm in width; chlamydospores intercalary in chains, 4–9 µm, smooth, thick-walled. *Conidiophores*

Fig. 2. *Curvularia beasleyi* (ex-holotype BRIP 10972): **a** colony on PDA **b–c** conidiophores and conidia **d** conidia. *Curvularia beerburrumensis* (ex-holotype BRIP 12942) **e** colony on PDA **f** chlamydospores **g** conidiophore **h–i** conidia. *Curvularia boeremae* (ex-holotype IMI 164633) **j** colony on PDA **k c**onidiophores **l** conidia. *Curvularia coatesiae* (ex-holotype BRIP 24261) **m** colony on PDA **n** conidiophores **o** conidia. Scale bars: 1 cm (**a, e, j, m**); all others – 10 µm.

erect, straight to flexuous, geniculate towards apex, subhyaline to pale brown, smooth, septate, up to 500 µm long, 5−6 µm wide. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, mono- or polytretic, with darkened scars. *Conidia* fusiform to subcylindrical or clavate, straight to slightly curved, rounded at the apex, (40–) 51–56 (–71) × (10–) 12–13 (–14) µm, subhyaline to pale yellowish-brown, 2–4 (mostly 3)-distoseptate. *Hila* mostly inconspicuous or minutely thickened and darkened.

Specimens examined: AUSTRALIA, Queensland, Beerburrum, from blackened inflorescence of *Eragrostis bahiensis*, 24 May 1979, *J.L. Alcorn* (BRIP 12942 holotype, includes ex-type culture); New South Wales, Yetman, blackened inflorescence of *Eragrostis sororia*, 12 May 1977, *J.L. Alcorn* (BRIP 12555, includes culture).

Notes: *Curvularia beerburrumensis* is phylogenetically sister to *C. australis* and *C. ovariicola* (Fig. 1), which have both been recorded in Australia on *Eragrostis* (Sivanesan 1987, Tan et al. 2014). *Curvularia beerburrumensis* is distinguished from the ex-type culture of *C. australis* in three loci (98 % in ITS, 96 % in *gapdh* and 98 % in *tef1α*). Furthermore, *C. beerburrumensis* has larger conidia than *C. australis* (25−48 × 9.0−12.5 µm, Sivanesan 1987). *Curvularia beerburrumensis* differs from the ex-type culture of *C. ovariicola* in three loci (99 % in ITS, 99 % in *gapdh* and 99 % in *tef1α*). *Curvularia beerburrumensis* has longer conidiophores than *C. ovariicola* (up to 325 µm, Sivanesan 1987). *Curvularia beerburrumensis* also produced chlamydospores in culture, which are not known for *C. australis* and *C. ovariicola*.

Curvularia beerburrumensis is only known from inflorescences of the invasive South American grass *Eragrostis bahiensis*, as well as the Australian native *E. sororia* (Simon and Alfonso 2011). Other *Curvularia* associated with *Eragrostis* include *C. australis*, *C. clavata*, *C. crustacea*, *C. ellisii, C. eragrostidis*, *C. geniculata*, *C. kusanoi*, *C. lunata*, *C. miyakei*, *C. nodulosa*, *C. ovariicola*, *C. perotidis*, *C. protuberata*, *C. ravenelii* and *C. verrucosa*, (Sivanesan 1987, Farr and Rossman 2018, Herbarium Catalogue 2018), although many of these reports are yet to be verified by molecular phylogenetic analyses.

Curvularia boeremae (A.S. Patil & V.G. Rao) Y.P. Tan & R.G. Shivas, **comb. nov. –** MycoBank MB825451. Fig. 2j–l
Basionym: *Drechslera boeremae* A.S. Patil & V.G. Rao, *Antonie van Leeuwenhoek* 42: 129. 1976.

Colonies on PDA approx. 3 cm diam. after 7 d at 25 °C, surface funiculose, margin fimbriate, olivaceous green to citrine, velutinous with aerial mycelium. *Hyphae* subhyaline, smooth to asperulate, branched, septate, 2–3 µm in width. *Conidiophores* straight to flexuous, slightly geniculate towards apex, uniformly subhyaline to pale brown, smooth, septate, up to 110 µm long, 4 µm wide. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, mono- or polytretic, with darkened scars. *Conidia* broadly ellipsoidal to oval, brown to dark brown, smooth, (42–) 46–52 (–55) × (17–) 20–23 (–25) µm, brown to dark brown, 4–6-distoseptate. *Hila* protuberant, thickened and darkened, 2–3 µm wide.

Specimen examined: INDIA, Poona, from leaves of *Portulaca oleracea*, 28 Apr. 1970, *A.S. Patil* (IMI 164633 holotype, includes ex-type culture), (BRIP 13934 isotype, includes ex-type culture).

Notes: Multilocus phylogenetic analyses placed the ex-type culture of *D. boeremae* within the clade that includes *C. lunata*, the type species of the genus (Fig. 1). *Curvularia boeremae* differs from *C. neoindica* in one locus (98 % identities in *gapdh*). Furthermore, *C. boeremae* has shorter conidia than *C. neoindica* (27–65 µm, Manamgoda et al. 2014). Sivanesan's (1987) synonymy of *Dreschlera boeremae* with *Bipolaris indica* was based on similar conidial morphology and is not supported by the phylogenetic analyses in this study.

Curvularia boeremae is only known from the type specimen on *P. oleraceae* and has not been recorded in Australia. *Curvularia portulacae* is the only other species recorded on *P. oleraceae* (Farr and Rossman 2018). *Curvularia boeremae* is morphologically distinct from *C. portulacae*, which has comparatively long, cylindrical conidia (average 110 × 13 µm, Rader 1948).

Curvularia coatesiae Y.P. Tan & R.G. Shivas, **sp. nov. –** MycoBank MB825452. Fig. 2m–o

Etymology: Named after Dr. Lindel (Lindy) M. Coates, an Australian plant pathologist in recognition of her contributions to the study of post-harvest fruit pathology.

Colonies on PDA 6–7 cm diam. after 7 d at 25 °C, surface funiculose, floccose, olivaceous black at the centre, olivaceous to grey olivaceous towards the edge, margin fimbriate. *Hyphae* subhyaline, smooth to asperulate, septate, up to 3 µm in width. *Conidiophores* erect, flexuous, geniculate in the top half, uniformly brown, sometimes pale towards apex, septate, up to 190 µm long, 4 µm wide; basal cell sometimes swollen, up to 8 µm diam. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown, mono- or polytretic, with darkened nodes. *Conidia* ellipsoidal to obovoid, asymmetrical, sometimes the third cell from base is unequally enlarged, intermediate cells dark brown and usually verruculose, end cells paler and less ornamented than central cells, (20–) 23–26 (–30) × (7–) 8–9 (–10) µm, 3-distoseptate. *Hila* protuberant, thickened and darkened, 1–2 µm wide.

Specimens examined: AUSTRALIA, Queensland, Eudlo, from rotted fruit of *Litchi chinensis*, 28 Jan. 1992, *L.M. Coates* (BRIP 24261 holotype, includes ex-type culture); New South Wales, Alstonville, isolated from the air in a mango orchard, 11 Mar. 1991, *G.I. Johnson* (BRIP 24170, includes culture).

Notes: *Curvularia coatesiae* is morphologically similar and phylogenetically related to a reference culture of *C. borreriae* and the ex-type culture of *C. pallescens* (Fig. 1). *Curvularia coatesiae* differs from the ex-type culture of *C. pallescens* in three loci: ITS position 439 (T); *gapdh* positions 219 (C), 287 (C); *tef1α* positions 43 (C), 257 (C), 259 (C). Although *C. borreriae* and *C. pallescens* have been recorded in Australia, these have not been verified by molecular phylogenetic analyses and there have been no additional records beyond the 1980s (Sivanesan 1987, Shivas 1989). Other species recorded from *L. chinensis* are *C. geniculata*, *C. hawaiiensis*, *C. lunata* and *C. pallescens* (DAF Biological Collections 2018, Herbarium Catalogue 2018), although not all the reports have been verified by molecular phylogenetic analyses.

Curvularia colbranii Y.P. Tan & R.G. Shivas, **sp. nov. –** MycoBank MB825453. Fig. 3a–d

Etymology: Named after Dr. Robert (Bob) Chester Colbran (1926–2010), an Australian nematologist and Director of the Plant Pathology Branch, Queensland Department of Primary Industries, in recognition of his significant contributions to Australian plant pathology.

Colonies on PDA approx. 5 cm diam. after 7 d at 25 °C, surface funiculose, margin fimbriate, olivaceous black, aerial mycelium white. *Hyphae* subhyaline, smooth, septate, up to 3 µm in width. *Conidiophores* erect, flexuous, geniculate, uniformly pale brown to brown, smooth, septate, up to 145 µm long, 4–6 µm wide, basal cell sometimes swollen, up to 8 µm diam. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, mono- or polytretic, with darkened scars. *Conidia* fusiform to subcylindrical with rounded apex and obconical at the base, brown, end cells pale, (54–) 83–92 (–110) × (13–) 14–16 (–17) µm, brown to dark brown, 6–9-distoseptate. *Hila* slightly protuberant, thickened and darkened, 1–2 µm wide.

Specimen examined: AUSTRALIA, Queensland, Brisbane, from leaf spot on *Crinum zeylanicum*, 11 Oct. 1976, *R.C. Colbran* (BRIP 13066 holotype, includes ex-type culture).

Notes: *Curvularia colbranii* is sister to *C. boeremae*, *C. lamingtonensis* (see this paper), *C. neoindica* and *C. portulacae*, although separated by a considerable genetic distance (Fig. 1). *Curvularia colbranii* has fusiform to subcylindrical conidia that are distinct from the ellipsoidal to oval conidia of *C. boeremae* (42–55 × 17–25 µm, this study) and *C. neoindica* (27–65 × 17–27 µm, Manamgoda et al. 2014) and longer than those of *C. lamingtonensis* (45–76 × 11–14 µm, this study). *Curvularia colbranii* has conidia that are 6–9-distoseptate, while *C. portulacae* has conidia reported as 3–15 celled (Rader 1948).

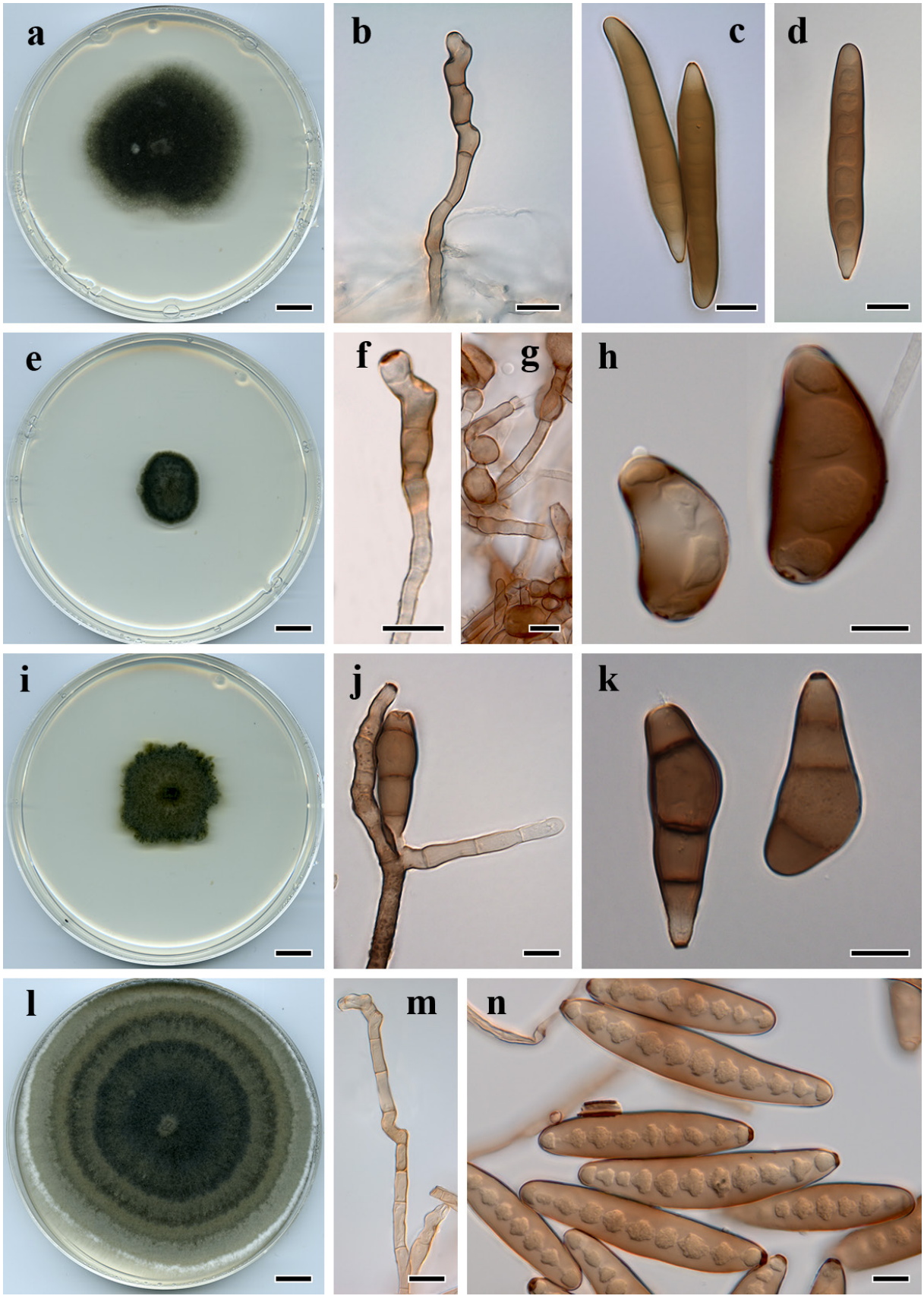
Only one other species, *C. trifolii*, has been reported on *Crinum* sp. (Shaw 1984), but this record has not been verified by phylogenetic analyses. *Curvularia colbranii* is morphologically distinct from *C. trifolii*, which has curved conidia.

Curvularia eragrosticola Y.P. Tan & R.G. Shivas, **sp. nov. –** MycoBank MB825454. Fig. 3e–h

Etymology: Named after *Eragrostis*, the grass genus from which this fungus was isolated.

Colonies on PDA approx. 2 cm diam. after 7 d at 25 °C, surface funiculose, margin fimbriate, dark olive with white patches, velutinous with some aerial mycelium. *Hyphae* subhyaline, smooth, branched, septate, 4−5 µm wide; chlamydospores abundant, subglobose to ellipsoidal or irregular, terminal and intercalary, 5−20 µm diam. *Conidiophores* erect, straight to flexuous, slightly geniculate, pale brown to brown, paler towards apex smooth, septate, up to 145 µm long, 4−5 µm wide. *Conidiogenous cells* integrated, terminal or intercalary, sympodial, pale brown to

Fig. 3. *Curvularia colbranii* (ex-holotype BRIP 13066): **a** colony on PDA **b** conidiophore **c–d** conidia. *Curvularia eragrosticola* (ex-holotype BRIP 12538) **e** colony on PDA **f** conidiophore **g** chlamydosphores **h** conidia. *Curvularia kenpeggii* (ex-holotype BRIP 14530) **i** colony on PDA **j** conidiophores and conidium **k** conidia. *Curvularia lamingtonensis* (ex-holotype BRIP 12259) **l** colony on PDA **n** conidiophore **n** conidia. Scale bars: 1 cm (**a, e, i, l**); all others – 10 µm.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brown, smooth, with darkened scars. *Conidia* hemi-ellipsoidal, curved, asymmetrical, brown to dark brown, end cells slightly paler, (25–) 26–30 (–34) × (9–) 13–15 (–19) µm, 3-distoseptate with a faint narrow median septum. *Hila* non-protuberant, minutely thickened and darkened.

Specimen examined: AUSTRALIA, New South Wales, Yetman, from inflorescence on *Eragrostis pilosa*, 12 May 1977, *J.L. Alcorn* (holotype BRIP 12538, includes ex-type culture).

Notes: *Curvularia eragrosticola* is phylogenetically close to *C. papendorfii* and *C. sporobolicola* (see this paper) (Fig. 1). *Curvularia eragrosticola* is distinguished in three loci from the ex-type culture of *C. papendorfii* (97 % in ITS, 92 % in *gapdh* and 98 % in *tef1α*) and *C. sporobolicola* (98 % in ITS, 92 % in *gapdh* and 98 % in *tef1α*). *Curvularia eragrosticola* has conidia that are smaller than *C. papendorfii* (30–50 × 17–30 µm, Sivanesan 1987) and *C. sporobolicola* (34–45 × 14–23 µm, this study).

Curvularia eragrosticola is only known from the type specimen on *Eragrostis pilosa*, which is native to Eurasia and Africa and is considered a troublesome weed in Australia (Simon & Alfonso 2011). Neither *C. papendorfii* nor *C. sporobolicola* have been reported on *Eragrostis*. Other *Curvularia* spp. associated with *Eragrostis* are listed in the notes for *C. beerburrumensis*.

Curvularia kenpeggii Y.P. Tan & R.G. Shivas, **sp. nov. –** MycoBank MB825455. Fig. 3i–k

Etymology: Named after Dr. Kenneth G. Pegg AM (member of the Order of Australia), in celebration of his 60 years of dedication to plant pathology in Australia and to thank him for his generous mentorship.

Colonies on PDA 3–4 cm diam. after 7 d at 25 °C, surface funiculose, margin fimbriate, floccose and olivaceous black at the centre with white patches, velutinous with some aerial mycelium. *Hyphae* hyaline, asperulate, branched, septate, 4−5 µm in width. *Conidiophores* erect, straight to flexuous, slightly geniculate in the upper part, pale brown to brown, sometimes paler towards the apex, verrucose, septate, up to 360 µm long, 4−5 µm wide, basal cell sometimes swollen, up to 8 µm. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, mono- or polytretic, with darkened scars. *Conidia* ellipsoidal to clavate to obovoid, asymmetrical, third cell from the base is unequally enlarged, brown, end cells paler, verruculose, (31–) 35–39 (–42) × (10–) 13–14 (–15) µm, 3-distoseptate. *Hila* protuberant, thickened and darkened, 1–2 µm wide.

Specimen examined: AUSTRALIA, Queensland, from mouldy grain of *Triticum aestivum*, 26 Oct. 1984, *J.L. Alcorn* (BRIP 14530 holotype, includes ex-type culture), (IMI 290719 isotype).

Notes: *Curvularia kenpeggii* is only known from the holotype specimen and is genetically distinct from all other *Curvularia* species (Fig. 1). *Curvularia kenpeggii* is basal to a clade comprised of *C. australis, C. beerburrumensis, C. crustaceae, C. miyakei, C. ovariicola, C. ravenellii* and *C. ryleyi*. These species are mostly reported as pathogens of *Eragrostis* and *Sporobolus* spp. and not known to be associated with wheat (*Triticum aestivum*). *Curvularia* species associated with *T. aestivum* in Australia are *C. brachyspora, C. harveyi, C. hawaiiensis, C. lunata, C. perotidis, C. ramosa* and *C. spicifera*, (Shivas 1989, Farr and Rossman 2018), although not all the reports have been verified by molecular phylogenetic analyses.

Curvularia lamingtonensis Y.P. Tan & R.G. Shivas, **sp. nov. –** MycoBank MB825456. Fig. 3l–n

Etymology: Named after the locality, Lamington National Park, where the holotype was collected.

Colonies on PDA cover the whole plate after 7 d at 25 °C, surface funiculose, margin fimbriate, olivaceous green, velutinous with some aerial mycelium. *Hyphae* hyaline, branched, septate, 4 µm in width. *Conidiophores* erect, straight to flexuous, geniculate towards apex, pale brown to dark brown on wheat straw agar, septate, up to 160 µm long, 3−4 µm wide. *Conidiogenous cells* integrated, terminal or intercalary, sympodial, pale brown to brown, smooth, with darkened scars. *Conidia* ellipsoidal to fusiform, straight, pale brown, (45–) 59–66 (–76) × (11–) 11.5–13 (–14) µm, 4–11-distoseptate with inconspicuous transverse septa. *Hila* protuberant, thickened and darkened, 1–2 µm wide.

Specimen examined: AUSTRALIA, Queensland, Lamington National Park, from *Microlaena stipoides*, 9 May 1977, *J.L. Alcorn* (BRIP 12259 holotype, includes ex-type culture).

Notes: *Curvularia lamingtonensis* is phylogenetically closely related to C. *boeremae* and *C. neoindica*. *Curvularia lamingtonensis* is distinguished from the ex-type culture of *C. boeremae* in two loci (96 % in ITS and 98 % in *gapdh*) and from the ex-type culture of *C. neoindica* in three loci (95 % in ITS, 98 % in *gapdh* and 99 % in *tef1α*). *Curvularia lamingtonensis* has longer and straighter conidia than *C. boeremae* and *C. neoindica*, both of which have broad, ellipsoidal conidia (42–55 × 20–23 µm, and 27–65 × 17–27 µm, respectively). *Curvularia lamingtonensis* is only known from the type specimen on *Microlaena stipoides*. This is the first record of a *Curvularia* species associated with *Microlaena*.

Curvularia mebaldsii Y.P. Tan & R.G. Shivas, **sp. nov. –** MycoBank MB825457. Fig. 4a–c

Etymology: Named after the collector, Martin Mebalds, in recognition of his contributions to Australian plant pathology and biosecurity.

Colonies on PDA approx. 5 cm diam. after 7 d at 25 °C, surface funiculose, margin fimbriate, olivaceous black with white patches, velutinous with some aerial mycelium. *Hyphae* hyaline to subhyaline, smooth to asperulate, septate, 3–4 µm wide. *Conidiophores* erect, straight to flexuous, sometimes slightly geniculate towards apex, branched, uniformly brown, paler at apex, smooth to asperulate, septate, up to 180 µm long, 4–5 µm wide. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, subhyaline to pale brown, smooth, mono- or polytretic, with darkened scars. *Conidia* ellipsoidal to obovoid, sometimes straight to slightly curved, rounded at the apex, (22–) 25–28 (–30) × (7–) 8–9 (–10) µm, pale brown to brown, 3-distoseptate. *Hila* protuberant, thickened and darkened, 1–2 µm wide.

Specimens examined: AUSTRALIA, Victoria, Hopetoun, from *Cynodon transvaalensis*, Apr. 1979, *M. Mebalds* (BRIP 12900 holotype, includes ex-type culture); New South Wales, Tweed Heads, from necrotic leaf on *Cynodon dactylon* × *transvaalensis*, 10 Jun. 1983, *G. Thomas* (BRIP 13983, includes culture).

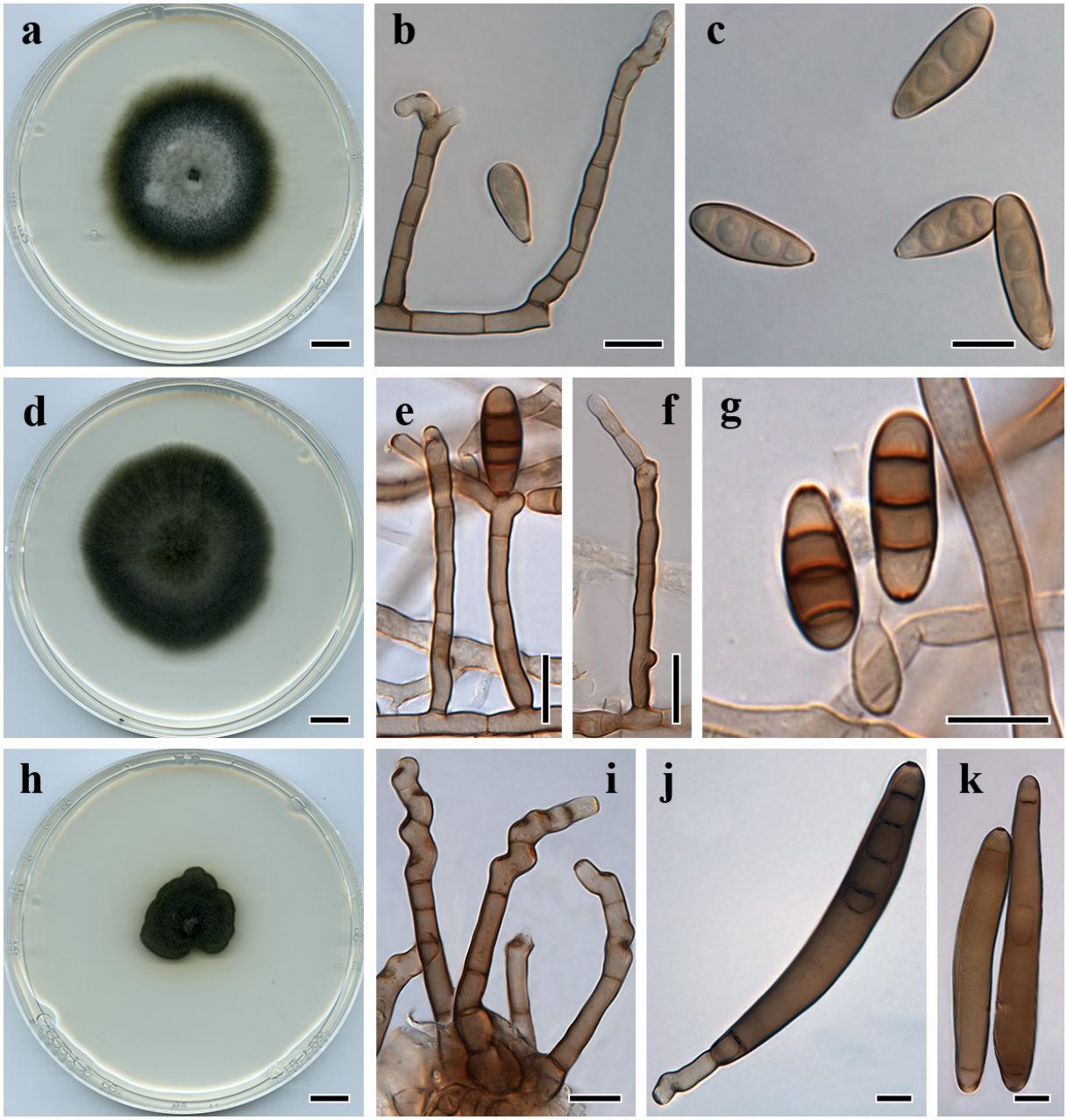

Fig. 4. *Curvularia mebaldsii* (ex-holotype BRIP 12900): **a** colony on PDA **b** conidiophores and conidium **c** conidia. *Curvularia petersonii* (ex-holotype BRIP 14642) **d** colony on PDA **e–f** conidiophores and conidium **g** conidia. *Curvularia platzii* (ex-holotype BRIP 27703b) **h** colony on PDA **i** conidiophores **j–k** conidia. Scale bars: 1 cm (**a, d, h**); all others – 10 µm.

Notes: The multilocus phylogenetic analyses showed that *C. mebaldsii* was sister to *C. tsudae*, although separated by a considerable genetic distance (Fig. 1). *Curvularia mebaldsii* is distinguished from the ex-type culture of *C. tsudae* in three loci (98 % in ITS, 97 % in *gapdh* and 99 % in *tef1α*). Morphologically, *C. mebaldsii* cannot be reliably separated from *C. tsudae*. *Curvularia mebaldsii* is known from two specimens on *Cynodon* spp. Several *Curvularia* species have been associated with *Cynodon*, including *C. aeria*, *C. australiensis*, *C. brachyspora*, *C. clavata*, *C. fallax*, *C. geniculata*, *C. hawaiiensis*, *C. inaequalis*, *C. lunata*, *C. pallescens*,

C. ramosa, *C. senegalensis*, *C. spicata*, *C. spicifera* and *C. verruculosa* (DAF Biological Collections 2018, Farr and Rossman 2018, Herbarium Catalogue 2018), although these records have not been verified by phylogenetic analyses.

Curvularia petersonii Y.P. Tan & R.G. Shivas, **sp. nov. –** MycoBank MB825458. Fig. 4d–g

Etymology: Named after Ron A. Peterson, an Australian plant pathologist, in recognition of his contributions to tropical plant pathology.

Colonies on PDA approx. 5 cm diam. after 7 d at 25 °C, surface funiculose, olivaceous black, velutinous with some aerial mycelium, margin fimbriate. *Hyphae* subhyaline, smooth to asperulate, septate, up to 3 µm in width. *Conidiophores* erect, straight to flexuous, rarely branched, slightly geniculate, uniformly brown, sometimes pale brown at apex, smooth, septate, up to 110 µm long, 4 µm wide. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, mono- or polytretic, with darkened scars. *Conidia* obovoid to ellipsoidal, straight to slightly curved, (15–) 17–19 (–21) × (5–) 5.5–6 (–7) µm, brown, end cells pale, 3-distoseptate. *Hila* non-protuberant, thickened and darkened.

Specimen examined: AUSTRALIA, Northern Territory, Daly Waters, from leaf spot on *Dactyloctenium aegyptium*, 20 Mar. 1985, *R.A. Peterson* (BRIP 14642 holotype, includes ex-type culture).

Notes: The multilocus phylogenetic analyses placed *C. petersonii* sister to *C. americana* and *C. verruculosa*, although separated by a considerable genetic distance (Fig. 1). Both *C. americana* and *C. verruculosa* have been found in Australia (DAF Biological Collections 2018, Herbarium Catalogue 2018). *Curvularia petersonii* is distinguished from the ex-type culture of *C. americana* in two loci (94 % in ITS and 92 % in *gapdh*) and from a reference culture of *C. verruculosa* in three loci (92 % in ITS, 92 % in *gapdh* and 98 % in *tef1α*). *Curvularia petersonii* has smaller conidia than *C. americana* (13–28 × 7–15 µm, Madrid et al. 2014) and *C. verruculosa* (20–40 × 12–17 µm, Sivanesan 1987).

Curvularia petersonii is only known from a single specimen on *Dactyloctenium aegyptium* in the Northern Territory. Many *Curvularia* species have been associated with *Dactyloctenium*, including *C. clavata*, *C. dactyloctenicola*, *C. dactyloctenii*, *C. eragrostidis*, *C. lunata*, *C. neergaardii*, *C. pallenscens* and *C. verruculosa* (Sivanesan 1987, Manamgoda et al. 2014, Farr and Rossman 2018, Herbarium Catalogue 2018, Marin-Felix et al. 2017b), although these records have not been verified by phylogenetic analyses.

Curvularia platzii Y.P. Tan & R.G. Shivas, **sp. nov. –** MycoBank MB825459. Fig. 4h–k

Etymology: Named after Gregory (Greg) J. Platz, in recognition of his contributions to Australian cereal plant pathology for the past 30 years, as well as his prowess as an international and Queensland rugby league footballer.

Colonies on PDA approx. 2 cm diam. after 7 d at 25 °C, surface dark olivaceous green. *Hyphae* subhyaline, smooth, septate, up to 3 µm wide. *Conidiophores* erect, straight to flexuous, geniculate towards apex, uniformly brown, sometimes pale brown towards apex, septate, up to

75 µm long, 5–6 µm wide, swollen at base, 8–10 µm. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, mono- or polytretic, with darkened scars. *Conidia* fusiform to narrowly clavate, brown, end cells sometimes paler, (65–) 94–105 (–115) × (11–) 12.5–13.5 (–14) µm, 9–13-distoseptate. *Hila* non-protuberant, thickened and darkened.

Specimen examined: AUSTRALIA, Queensland, Warwick, from leaf spot on *Cenchrus clandestinus*, 24 Jan. 2001, *G.J. Platz* (BRIP 27703b holotype, includes ex-type culture).

Notes: The multilocus phylogenetic analyses indicated *C. platzii* was sister to *C. hominis*, *C. meuhlenbeckiae* and *C. pisi* (Fig. 1). *Curvularia platzii* is distinguished in one locus from the ex-type culture of *C. hominis* (97 % in *tef1α*) and in two loci from the reference culture of *C. meuhlenbeckiae* (99 % in *gapdh* and 99 % in *tef1α*) and the ex-type culture of *C. pisi* (98 % in *gapdh* and 99 % in *tef1α*). *Curvularia platzii* differs from *C. hominis*, *C. meuhlenbeckiae* and *C. pisi*, which have much shorter asymmetrical conidia with fewer septa (Madrid et al. 2014, Marin-Felix et al. 2017a).

Curvularia platzii is only known from the holotype. The host, *Cenchrus clandestinus* (syn. *Pennisetum clandestinum*), is a perennial grass with a worldwide distribution (Simon & Alfonso 2011). Other *Curvularia* species associated with *C. clandestinus* are *C. lunata*, *C. nodulosa* and *C. trifolii* (Farr and Rossman 2018, Herbarium Catalogue 2017), although these records have not been verified by phylogenetic analyses.

Curvularia reesii Y.P. Tan & R.G. Shivas, **sp. nov. –** MycoBank MB825460. Fig. 5a–c

Etymology: Named after Dr. Robert (Bob) G. Rees, an Australian plant pathologist, in recognition of his extensive contributions to cereal pathology.

Colonies on PDA approx. 6−7 cm diam. after 7 d at 25 °C, surface funiculose, greenish-grey, velutinous with some aerial mycelium, margin fimbriate. *Hyphae* hyaline, branched, septate, 3−4 µm in width. *Conidiophores* erect, straight to flexuous, slightly geniculate towards apex, pale brown to brown, sometimes paler towards the apex, septate, up to 200 µm long, 4−5 µm wide. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, mono- or polytretic, with darkened scars. *Conidia* ellipsoidal to obclavate, straight, third cell from pore swollen, brown, end cells paler, smooth, (28–) 31–35 (–39) × (10–) 12–13 (–14) µm, mostly 3 septate. *Hila* inconspicuous, sometimes darkened.

Specimen examined: AUSTRALIA, Queensland, Brisbane, isolated from air, 22 Jun. 1963, *R.G. Rees* (BRIP 4358 holotype, includes ex-type culture).

Notes: The multilocus phylogenetic analyses indicated *C. reesii* was sister to *C. oryzae* and *C. tuberculata*. *Curvularia reesii* is distinguished in two loci from the ex-type cultures of *C. oryzae* (98 % in *gapdh* and 99 % in *tef1α*) and *C. tuberculata* (96 % in *gapdh* and 99 % in *tef1α*). Morphologically, *C. reesii* has conidia similar in size to *C. oryzae* (24–40 × 12–22 µm, Sivanesan 1987) and *C. tuberculata* (23–52 × 13–20 µm, Sivanesan 1987). The isolate of *C. reesii* examined in this study had become sterile.

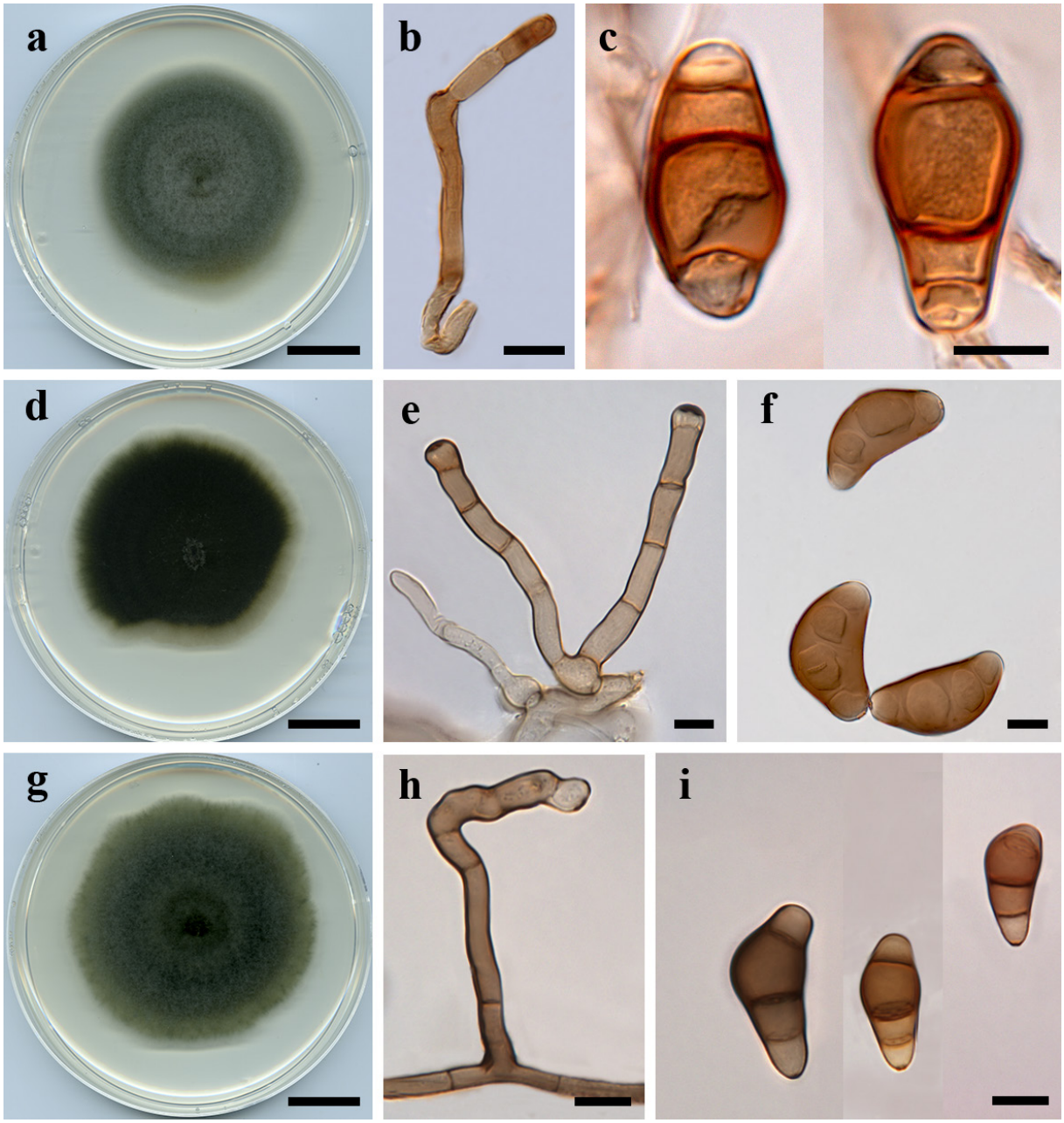

Fig. 5. *Curvularia reesii* (holotype BRIP 4358): **a** colony on PDA **b** conidiophore **c** conidia. *Curvularia sporobolicola* (ex-holotype BRIP 23040b) **d** colony on PDA **e** conidiophores **f** conidia. *Curvularia warraberensis* (ex-holotype BRIP 14817) **g** colony on PDA **h** conidiophore **i** conidia. Scale bars: 1 cm (**a, d, g**); all others – 10 µm.

Curvularia sporobolicola Y.P. Tan & R.G. Shivas, **sp. nov. –** MycoBank MB825461. Fig. 5d–f

Etymology: Named after *Sporobolus*, the grass genus from which it was isolated.

Colonies on PDA approx. 6 cm diam. after 7 d at 25 °C, surface funiculose, margin fimbriate, olivaceous black, velutinous. *Hyphae* subhyaline, smooth, branched, septate, 3 µm wide. *Conidiophores* erect, straight to flexuous, geniculate, pale yellowish-brown, septate, up to 55

µm long, 4−5 µm wide, basal cell swollen, 6−10 µm diam. *Conidiogenous cells* cylindrical, slightly flared at the apex, integrated, sympodial, pale brown, smooth, with darkened and thickened scars. *Conidia* hemi-ellipsoidal, curved, 4-distoseptate with a faint narrow median septum, (34–) 37–41 (–45) × (14–) 17–20 (–23) µm, brown to dark brown, end cells rounded and paler. *Hila* non-protuberant, sometimes darkened.

Specimen examined: AUSTRALIA, Queensland, Musselbrook Reserve, leaf of *Sporobolus australasicus*, 2 May 1995, *J.L. Alcorn* (BRIP 23040b holotype, includes ex-type culture).

Notes: Based on multilocus phylogenetic analyses, *C. sporobolicola* clustered sister to *C. papendorfii*, which are both sister to *C. eragrosticola* (Fig. 1). *Curvularia sporobolicola* is distinguished in three loci from the ex-type cultures of *C. papendorfii* (99 % in ITS, 96 % in *gapdh* and 98 % in *tef1α*) and *C. eragrosticola* (98 % in ITS, 92 % in *gapdh* and 98 % in *tef1α*). These three species are similar in having dark brown, hemi-ellipsoidal, curved, conidia, which makes identification by morphology difficult. The conidia of *C. sporobolicola* tend to be wider than those of C. *eragrosticola* (25–35 × 9–19 µm, this study) and C. *papendorfii* (30–50 × 9–19 µm, Sivanesan 1987).

Curvularia sporobolicola is only known from the type specimen on *S. australasicus*, which is a native Australian grass with a broad distribution in the tropics and subtropics (Simon & Alfonso 2011). Other *Curvularia* species associated with *Sporobolus* include *C. australis, C. crustacea, C. eragrostis, C. geniculata, C. lunata, C. ovariicola, C. pallescens, C. ravenelli* and *C. ryleyi* (Sivanesan 1987, Farr and Rossman 2018), although this is the first *Curvularia* species associated with *S. australasicus*.

Curvularia warraberensis Y.P. Tan & R.G. Shivas, **sp. nov. –** MycoBank MB825462. Fig. 5g–i

Etymology: Named after the locality, Warraber Island in the Torres Straits, where the specimen was collected.

Colonies on PDA 6–7 mm diam. after 7 d at 25 °C, surface funiculose, margin fimbriate, olivaceous green, velutinous with some aerial mycelium. *Hyphae* subhyaline, smooth, septate, up to 3 µm wide. *Conidiophores* erect, flexuous, geniculate towards apex, uniformly pale brown to brown, septate, up to 360 µm long, 4–5 µm wide, basal cell sometimes swollen, 6–8 µm diam. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, mono- or polytretic, with darkened scars. *Conidia* ellipsoidal, curved, the third cell from base swollen, end cells paler, smooth, (20–) 23–26 (–28) × (8–) 9.5–11 µm, pale brown to brown, 3-distoseptate. *Hila* conspicuous, sometimes slightly protuberant, thickened and darkened.

Specimen examined: AUSTRALIA, Queensland, Torres Strait, Warraber Island, from leaf spot on *Dactyloctenium aegyptium*, 2 Jun. 1985, *R.A. Peterson* (BRIP 14817 holotype, includes ex-type culture).

Notes: Multilocus phylogenetic analyses placed *C. warraberensis* sister to *C. caricae-papayae* and *C. prasadii* (Fig. 1). *Curvularia warraberensis* differs from the ex-type culture of *C. caricae-papayae* in *gapdh* positions 40 (C), 102 (C), 230 (A), 233 (C) and 321 (A) and from the ex-type

culture of *C. prasadii* in two loci, *gapdh* positions 102 (C), 131 (C), 230 (A), 233 (C), 321 (A) and *tef1α* positions 214 (C), 337 (C), 542 (A), 543 (C), 685 (C). These three species belong to the *lunata*-clade sensu Madrid et al. (2014), which also includes *C. aeria, C. brachyspora, C. chlamydospora, C. lunata* and *C. pseudolunata*. All the species in the *lunata*-clade sensu Madrid et al. (2014) have 4-celled conidia in which the third cell from the base is often swollen (unequally sided and larger) and darker than the other cells. *Curvularia warraberensis* has longer conidiophores than *C. caricae-papayae* (up to 100 µm long, Srivastava and Bilgrami 1963) and longer conidia than *C. caricae-papayae* (12.8–18.0 × 6–8 µm) and *C. prasadii* (12.8–18.0 × 6–8 µm, Mathur and Mathur 1959).

Curvularia warraberensis is only known from the holotype. *Curvularia* species associated with *Dactyloctenium* are listed in the notes for *C. petersonii*.

DISCUSSION

Although the ITS locus is the universal barcode marker for fungi (Schoch et al. 2012), secondary loci are often essential for the accurate identification of many helminthosporioid species (Manamgoda et al. 2012, 2015, Madrid et al. 2014, Tan et al. 2014, 2016, Stielow et al. 2015. Hernández-Restrepo et al. 2018). The protein-coding loci of *gapdh*, *tef1α* and RNA polymerase II second largest subunit (*rpb2*) have been reported as phylogenetically informative in the phylogenetic analyses of sequence data from species of *Curvularia* (Hernández-Restrepo et al. 2018, Manamgoda et al. 2014, Marin-Felix et al. 2017a, 2017b). In this study, sequences of three loci (ITS, *gapdh* and *tef1α*) from 17 cultures in BRIP were compared with those from ex-type cultures as well as published reference cultures for species of *Bipolaris* and *Curvularia*. The phylogenetic analyses of the concatenated three-locus dataset resolved the 17 BRIP isolates into 13 novel *Curvularia* species.

Eight *Curvularia* species are described here from grasses (*Poaceae*) exotic to Australia, namely, *C. beasleyi* on *Chloris gayana*, *C. beerburrumensis* on *Eragrostis bahiensis*, *C. eragrosticola* on *E. pilosa*, *C. kenpeggii* on *Triticum aestivum*, *C. mebaldsii* on *Cynodon dactylon* × *transvaalensis*, *C. petersonii* and *C. warraberensis* on *Dactyloctenium aegyptium* and *C. platzii* on *Cenchrus clandestinus*. Only two species were described from native Australian grasses, *C. lamingtonensis* on *Microlaena stipoides* and *C. sporobolicola* on *Sporobolus australasicus*. Two species were described from other hosts, *C. coatesiae* from *Litchi chinensis* (Sapindaceae) and *C. colbranii* from *Crinum zeylanicum* (Amaryllidaceae). One species, *C. reesii*, was described from an isolate obtained from an air sample. Furthermore, DNA sequences derived from ex-type cultures have supported the generic placement of *C. neoindica* and the transfer of *Drechslera boeremae* to *Curvularia*.

It is not known whether the species described here are pathogens, endophytes or saprobes. It is also unclear as to whether these species are native or introduced. *Curvularia beasleyi* and *C. beerburrumensis* were both isolated from a native Australian grass species, as well as an exotic host. Some grass species have been reported to be associated with multiple *Curvularia* species, such as *Chloris* and *Cynodon*, with nine and 15 species, respectively. Many of the published records on *Chloris* and *Cynodon* have not been verified by molecular analyses. The number of new species described from non-Australian grasses indicates a need for a molecular-based reassessment of previous host-species records. The description of these species provides

a foundation upon which additional sampling and accumulation of molecular data will improve knowledge of the host ranges and ecological roles of helminthosporioid fungi in Australia and overseas.

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CHAPTER 6

Australian cultures of *Botryosphaeriaceae* held in Queensland and Victoria plant pathology herbaria revisited

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Abstract

The *Botryosphaeriaceae* is one of the most widespread and cosmopolitan endophytic group of fungi. However, the species of this group can cause severe disease when the hosts are under stressful conditions. The aim of this study was to identify living cultures from the *Botryosphaeriaceae* family preserved in the Queensland and Victorian Plant Pathology Herbaria using DNA sequence analyses. The 51 isolates were collected between 1971 and 2017, from 35 different host genera, with the dominant host genera being *Mangifera* (11 isolates), *Acacia* (10), and *Persea* (5). Multilocus sequence analyses resulted in the re-identification of 41 isolates to the genera *Botryosphaeria* (2 isolates), *Diplodia* (4), *Dothiorella* (1), *Lasiodiplodia* (19), and *Neofusicoccum* (15), as well as some that belonged to genera outside of the *Botryosphaeriaceae* (10). New records for Australia were *Botryosphaeria sinensis*, *Diplodia alatafructa*, *Lasiodiplodia gonubiensis*, *Neofusicoccum cryptoaustrale*, and *N. mangroviorum*. These were identified as a result of a workshop organised by the Subcommittee on Plant Health Diagnostics. The results of this study provide the fundamental information regarding the diversity of *Botryosphaeriaceae* species present in Australian.

INTRODUCTION

The *Botryosphaeriaceae* (*Dothideomycetes*: *Botryosphaeriales*) includes 24 genera of ecologically diverse fungi that occur as saprobes, endophytes or plant pathogens (Slippers et al. 2017, Yang et al. 2017). Some of these fungi are important pathogens of woody plant species, causing dieback and stem cankers, especially in the tropics and subtropics. Several species of *Botryosphaeriaceae* can remain as latent pathogens in localised infections for many years, facilitating their global spread through trade in agricultural and forestry products (Burgess et al. 2016, Crous et al. 2016).

The accurate identification of *Botryosphaeriaceae* by DNA sequence data rather than relying on morphological descriptions, provides the best means to halt their spread and reduce the threat of these fungi. Recent taxonomic changes and the recognition of cryptic species have made the identification of species in the *Botryosphaeriaceae* challenging. Phillips et al. (2013) recommended that at least two loci, the internal transcribed spacer (ITS) region, and the translation elongation factor 1-alpha (*tef1α*), be used for species separation within *Botryosphaeriaceae*. However, Slippers et al. (2013) recommended the use of four loci, including the ITS region, *tef1α*, beta-tubulin (*tub*), and the RNA polymerase II (*rpb2*), as these loci will provide sufficient resolution to distinguish cryptic species. The amplification of *rpb2* is challenging and subsequently there is lack of data for comparisons (Slippers et al. 2013).

Recent research into grapevine trunk diseases has identified at least 14 *Botryosphaeriaceae* species that impact Australian viticulture (Pitt et al. 2010, Wunderlich et al. 2011, Pitt et al. 2013, 2015,). Similarly, in Western Australia, many fungi that belong to *Botryosphaeriaceae* have been associated with dieback of mango and forest trees (Sakalidis et al. 2011a, 2011b, 2013). Further information about the species of *Botryosphaeriaceae* elsewhere in Australia must be treated with caution as it predates the recent molecular focussed taxonomic revisions.

Australian plant biosecurity is underpinned by the ability to accurately determine what pathogens are present and established in Australia, in order to recognise pathogens that are exotic. National

plant pest reference collections, such as the Queensland and Victorian Plant Pathology Herbaria (BRIP and VPRI, respectively), play a crucial role in diagnostics by providing specimen-based records of Australia's plant pathogens. This information can be rapidly accessed by Australian biosecurity practitioners through the Australian Plant Pathogen Database (Plant Health Australia 2001). In light of ongoing taxonomic revisions, there is a need for specimens in Australian reference collections to be verified, as well as for the continued professional development of Australian plant biosecurity diagnosticians. To this end, a workshop was held at the University of Southern Queensland (26–30 June, 2017) to provide training for 23 professional plant pathologists on the latest developments in morphological and molecular methods for the identification and classification of fungi in the *Botryosphaeriaceae*.

Materials and methods

Specimens and species identification

Living cultures of 51 specimens were sourced from the Queensland Plant Pathology Herbarium (BRIP) and Victorian Plant Pathology Herbarium (VPRI) (Tables 1 and 2). Identification of the specimens to species level required unambiguous DNA sequence reads that matched data from the ex-type reference specimens on GenBank (Table 3).

DNA extraction, PCR amplification and phylogenetic analyses

Mycelia were collected from cultures grown on potato dextrose agar (Difco™, Becton, Dickinson and Company) and macerated with 0.5 mm glass beads (Daintree Scientific) in a Tissue Lyser (QIAGEN). Genomic DNA was extracted with the DNeasy Plant Mini Kit (QIAGEN) according to the manufacturer's instructions.

The primers V9G (de Hoog and Gerits van den Ende 1998) and ITS4 (White et al. 1990) were used to amplify the ITS region of the nrDNA, and the amplification of the partial region of the *tef1α* locus was achieved by either the primer sets EF1-728F (Carbone & Kohn 1999) and EF2 (O'Donnell et al. 1998) or EF1-688F and EF1-1251R (Alves et al. 2008). All loci were amplified with the Phusion High-Fidelity PCR Master Mix with HF Buffer (New England Biolabs). The PCR mix included: 12.5 µL of Phusion Master Mix, 0.5 µL of 10 mM of each primer, and 1 µL of DNA template. Sterile water was used as no-template control. The amplification conditions were as follows: initial denaturation of 98 °C for 30 secs, followed by 30 cycles of 98 °C for 10 secs, 55 °C for 30 secs, and 72 °C for 30 secs, and a final extension at 72 °C for 5 mins. The amplified products were purified and sequenced by Macrogen Incorporated (Seoul, Korea).

All sequences generated were assembled using Geneious v. 9.1.8 (Biomatters Ltd.) and deposited in GenBank (Table 2). These sequences were aligned with selected sequences of ex-type or authentic representative *Botryosphaeriaceae* genera (Table 3) using the MAFFT alignment algorithm (Katoh et al. 2009) in Geneious. *Pseudofusicoccum stromaticum* strain CBS 117448 was included as the outgroup (Table 3). The sequences of each locus were aligned separately and manually adjusted as necessary. Alignment gaps were treated as missing character states and all characters were unordered and of equal weight. The Markov chain Monte Carlo (MCMC) algorithm was used to create a phylogenetic tree based on Bayesian probabilities using MrBayes v. 3.2.1 (Huelensbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003)

Table 1. Non-Botryosphaeriaceae re-identified based on DNA analyses.

Taxon	Strain [a]	Former identification	Host	State, city/town/region
Cladosporium sp.	BRIP 52463	*Fusicoccum* sp.	*Cycas* sp.	Qld, Townsville
Coniothyrum sp.	VPRI 41605 (=BRIP 65675)	*Diplodia* sp.	*Acacia pycnantha*	Vic, Grampians National Park
	VPRI 41618 (=BRIP 65676)		*Acacia pycnantha*	Vic, Grampians National Park
	VPRI 41620 (=BRIP 65677)		*Acacia pycnantha*	Vic, Grampians National Park
	VPRI 41626 (=BRIP 65678)		*Acacia pycnantha*	Vic, Grampians National Park
Diaporthe sp.	BRIP 52819b	*Fusicoccum* sp.	*Acacia* sp.	Qld, Brisbane
	BRIP 52820		*Acacia* sp.	Qld, Brisbane
	BRIP 52999b		Acacia sp.	Qld, Brisbane
Fusarium sp.	BRIP 52819d	*Botryosphaeria* sp.	Acacia sp.	Qld, Brisbane
Huntiella sp.	BRIP 28467	*Fusicoccum luteum*	*Mangifera indica*	Qld, Ayr

[a] BRIP, Queensland Plant Pathology Herbarium, Brisbane, Queensland; VPRI, Victorian Plant Pathology Herbarium, Agribio, Bundoora, Victoria.

[b] Qld, Queensland; Vic, Victoria.

Table 2. List of isolates identified or re-identified by DNA sequencing in this study. New Australian fungal or host records are in **bold**.

Taxon	Strain [a]	Former identification	Host	State[b], city/ town/region	GenBank Accessions	
					ITS	*tef1α*
Botryosphaeria sinensis	BRIP 19781	*Fusicoccum* sp.	***Mangifera indica***	Qld, Ayr	MH057165	MH102228
***Botryosphaeria* sp.**	BRIP 24140	*Neofusicoccum parvum*	*Mangifera indica*	Qld, Rita Island	MH057166	MH102229
Diplodia africana	VPRI 41783 (=BRIP 53702)	undetermined	***Pinus muricata***	Vic, Melbourne	MH057169	MH102232

Table 2. (Ctd).

Taxon	Strain [a]	Former identification	Host	State[b], city/ town/region	GenBank Accessions	
					ITS	*tef1α*
	BRIP 53072	*Zasmidium scaevolicola*	***Scaevola taccada***	Qld, Cape Tribulation	MH057168	MH102231
Diplodia alatafructa	BRIP 52819a	*Botryosphaeria* sp.	***Acacia* sp.**	Qld, Brisbane	MH057167	MH102230
Diplodia seriata	VPRI 42125 (=BRIP 65679)	undetermined	***Araucaria heterophylla***	Vic, Melbourne	MH057170	MH102233
***Dothiorella* sp.**	VPRI 13932 (=BRIP 64673)	*Botryosphaeria sarmentorum*	*Alyxia buxifolia*	Vic, Melbourne	MH057171	MH102234
Lasiodiplodia brasiliensis	BRIP 60182e	undetermined	***Gossypium hirsutum***	Qld, Emerald	MH057184	MH102247
Lasiodiplodia gonubiensis	BRIP 58865	*Lasiodiplodia* sp.	***Acmena smithii***	Qld, Brisbane	MH057180	MH102243
	BRIP 64897c	*Lasiodiplodia* sp.	***Camellia sinensis***	Qld, Topaz	MH057176	MH102239
	BRIP 58861	*Lasiodiplodia* sp.	***Lenwebbia lasioclada***	Qld, Brisbane	MH057179	MH102242
Lasiodiplodia iraniensis	BRIP 63318	undetermined	***Vaccinium* sp.**	Qld, Brisbane	MH057172	MH102235
Lasiodiplodia mahajangana	BRIP 63052	undetermined	***Annona reticulata***	Qld, Alloway	MH057187	MH102250
	BRIP 63346	*Lasiodiplodia* sp.	***Musa* sp.**	Qld, Upper Daradgee	MH057188	MH102251
	BRIP 55402	*Lasiodiplodia theobromae*	***Persea americana***	NSW, Duranbah	MH057177	MH102240
Lasiodiplodia pseudotheobromae	BRIP 64096b	*Lasiodiplodia* sp.	*Annona muricata*	Qld, Tully	MH057189	MH102252
	BRIP 53572	undetermined	*Dimocarpus longan*	Qld, Mareeba	MH057174	MH102237
	BRIP 53606	undetermined	*Macadamia* sp.	Qld, Tolga	MH057175	MH102238

Table 2. (Ctd).

Taxon	Strain [a]	Former identification	Host	State[b], city/ town/region	GenBank Accessions	
					ITS	*tef1α*
	BRIP 51631	*Lasiodiplodia theobromae*	*Mangifera indica*	Qld, Gumlu	MH057173	MH102236
	BRIP 62846	*Lasiodiplodia theobromae*	*Rosa* sp.	Qld, Tolga	MH057185	MH102248
Lasiodiplodia theobromae	BRIP 58919	*Botryosphaeria* sp.	*Syzygium nervosum*	Qld, Brisbane	MH057182	MH102245
	BRIP 58866	*Botryosphaeria* sp.	*Syzygium wilsonii*	Qld, Brisbane	MH057181	MH102244
	BRIP 62872	*Lasiodiplodia* sp.	*Pinus caribaea*	Qld, Kalpower	MH057186	MH102249
	BRIP 64718	undetermined	*Passiflora edulis*	Qld, Cooktown	MH057190	MH102253
Lasiodiplodia sp.	BRIP 58969	undetermined	*Acacia mangium*	Qld, Mareeba	MH057183	MH102246
	BRIP 58042b	*Lasiodiplodia* sp.	*Vitis vinifera*	Qld, Dimbulah	MH057178	MH102241
Neofusicoccum australe	VPRI 42853	*Botryosphaeria* sp.	*Banksia* sp.	Vic, Mornington	MH057204	MH102267
	(=BRIP 65680)					
	VPRI 42863 (=BRIP 65681)	undetermined	*Juglans* sp.	NSW, Leeton	MH057205	MH102268
	BRIP 59728	undetermined	*Persea americana*	WA, Kalamunda	MH057198	MH102261
Neofusicoccum cryptoaustrale	BRIP 63679	*Neofusicoccum* sp.	***Mangifera indica***	WA, Northampton	MH057200	MH102263
Neofusicoccum luteum	BRIP 5016	*Dothiorella aromatic*	*Persea americana*	Qld, Brisbane	MH057191	MH102254
	BRIP 54746	*Neofusicoccum parvum*	*Mangifera indica*	Qld, Mundubbera	MH057194	MH102257
Neofusicoccum mangroviorum	BRIP 57901	*Neofusicoccum luteum*	*Helianthus annuus*	Qld, Gatton	MH057196	MH102259

Table 2. (Ctd).

Taxon	Strain [a]	Former identification	Host	State[b], city/ town/region	GenBank Accessions	
					ITS	*tef1α*
Neofusicoccum occulatum	BRIP 64094	*Lasiodiplodia theobromae*	*Vaccinium* sp.	Qld, Tolga	MH057202	MH102265
Neofusicoccum parvum	BRIP 19486	*Dothiorella dominicana*	*Persea americana*	Qld, Maleny	MH057192	MH102255
	BRIP 55401	*Dothiorella* sp.	*Persea americana*	Qld, Gingin	MH057195	MH102258
	BRIP 62250a	*Dothiorella* sp.	*Persea americana*	WA, Busselton	MH057199	MH102262
	BRIP 65440	*Dothiorella* sp.	*Mangifera indica*	Qld, Spring Creek	MH057195	MH102266
	BRIP 24083	*Fusicoccum mangiferae*	*Mangifera indica*	Qld, Bowen	MH057193	MH102256
	BRIP 58868	*Botryosphaeria* sp.	*Xanthostemon* sp.	Qld, Beerburrum	MH057197	MH102260
Neofusicoccum vitifusiforme	BRIP 64010	*Neofusicoccum* sp.	*Geijera salicifolia*	Qld, Kingsthorpe	MH057201	MH102264

[a] BRIP, Queensland Plant Pathology Herbarium, Brisbane, Qld; VPRI, Victorian Plant Pathology Herbarium, Agribio, Bundoora, Vic

[b] NSW, New South Wales; Qld, Queensland; Vic, Victoria; WA, Western Australia

Table 3. List of reference sequences included in the phylogenetic analyses.

Taxon	Strain [a]	Host	Country	GenBank Accessions	
				ITS	*tef1α*
Botryosphaeria dothidea	CBS 115476[ET]	*Prunus* sp.	Switzerland	AY236949	AY236898
Botryosphaeria fabicerciana	CBS 127193[HT]	*Eucalyptus* sp.	China	HQ332197	HQ332213
Botryosphaeria fusispora	MFLUCC 10-0098[HT]	*Entada* sp.	Thailand	JX646789	JX646854
Botryosphaeria ramosa	CBS 122069[HT]	*Eucalyptus camaldulensis*	Australia	EU144055	EU144070

Table 3. List of reference sequences included in the phylogenetic analyses.

Taxon	Strain [a]	Host	Country	GenBank Accessions	
				ITS	*tef1α*
Botryosphaeria scharifii	CBS 124703[IS]	*Mangifera indica*	Iran	JQ772020	JQ772057
Botryosphaeria sinensis	CGMCC 3.17723[PT]	*Populus* sp.	China	KT343254	KU221233
Diplodia africana	CBS 120835[HT]	*Prunus persica*	South Africa	EF445343	EF445382
Diplodia alatafructa	CBS 124931[HT]	*Pterocarpus angolensis*	South Africa	FJ888460	FJ888444
Diplodia allocellula	CBS 130408[HT]	*Acacia karroo*	South Africa	JQ239399	JQ239386
Diplodia crataegicola	MFLU 15-1311[HT]	*Crataegus* sp.	Italy	KT290244	KT290248
Diplodia estuarine	CMW 41230[PT]	*Avicennia marina*	South Africa	KP860831	KP860676
Diplodia fraxini	CBS 136010[NT]	*Fraxinus angustifolia*	Portugal	KF307700	KF318747
Diplodia galiicola	MFLU 15-1310[HT]	*Galium* sp.	Italy	KT290245	KT290249
Diplodia seriata	CBS 112555[ET]	*Vitis vinifera*	Portugal	AY259094	AY573220
Dothiorella americana	CBS 128309[HT]	*Vitis vinifera*	USA	HQ288218	HQ288262
Dothiorella californica	CBS 141587[HT]	*Umbellularia californica*	USA	KX357188	KX357211
Dothiorella iberica	CBS 115041[HT]	*Quercus ilex*	Spain	AY573202	AY573222
Dothiorella omnivore	CBS 140349[HT]	*Corylus avellana*	Italy	KP205497	KP205470
Dothiorella parva	IRAN1579C[HT] (=CBS 24720[IS])	*Corylus avellana*	Iran	KC898234	KC898217
Dothiorella sarmentorum	IMI 63581b[HT]	*Ulmus* sp.	England	AY573212	AY573235
Dothiorella sempervirentis	IRAN1583C[HT] (=CBS 124718[IS])	*Cupressus sempervirens*	Iran	KC898236	KC898219
Dothiorella symphoricarposicola	MFLUCC 13-0497[IS]	*Symphoricarpos* sp.	Italy	KJ742378	KJ742381
Dothiorella vidmadera	DAR 78992[HT]	*Vitis vinifera*	Australia	EU768874	EU768881

Table 3. List of reference sequences included in the phylogenetic analyses.

Taxon	Strain [a]	Host	Country	GenBank Accessions	
				ITS	*tef1α*
Lasiodiplodia brasiliensis	CMM 4015[HT]	*Mangifera indica*	Brazil	JX464063	JX464049
Lasiodiplodia bruguierae	CMW 41470[HT]	*Bruguiera gymnorrhiza*	South Africa	KP860832	KP860677
Lasiodiplodia caatinguensis	CMM 1325[HT]	*Citrus sinensis*	Brazil	KT154760	KT008006
Lasiodiplodia exigua	CBS 137785[HT]	*Quercus ilex*	Tunisia	KJ638317	KJ638336
Lasiodiplodia gonubiensis	CBS 115812[HT]	*Syzygium cordatum*	South Africa	AY639595	DQ103566
Lasiodiplodia gravistriata	CMM 4564	*Anacardium humile*	Brazil	KT250949	KT250950
Lasiodiplodia iraniensis	CBS 124710[HT]	*Salvadora persica*	Iran	GU945346	GU945334
Lasiodiplodia jatrophicola	CMM 3610[HT]	*Jatropha curcas*	Brazil	KF234544	KF226690
Lasiodiplodia macrospora	CMM 3833[HT]	*Jatropha curcas*	Brazil	KF234557	KF226718
Lasiodiplodia mahajangana	CBS 124927[IS]	*Terminalia catappa*	Madagascar	FJ900597	FJ900643
Lasiodiplodia pseudotheobromae	CBS 116459[HT]	*Gmelina arborea*	Costa Rica	EF622077	EF622057
Lasiodiplodia subglobosa	CMM 3872[HT]	*Jatropha curcas*	Brazil	KF234558	KF226721
Lasiodiplodia thailandica	CBS 138760[HT] (=CPC 22795)	*Mangifera indica*	Thailand	KJ193637	KJ193681
Lasiodiplodia theobromae	CBS 164.96[NT]	unknown fruit on coral reef coast	Papua New Guinea	AY640255	AY640258
Neofusicoccum australe	CMW 6837[HT]	*Acacia* sp.	Australia	AY339262	AY339270
Neofusicoccum cryptoaustrale	CBS 122813[HT]	*Eucalyptus* sp.	South Africa	FJ752742	FJ752713
Neofusicoccum eucalypticola	CBS 115766[IS]	*Eucalyptus grandis*	Australia	AY615143	AY615135
Neofusicoccum eucalyptorum	CBS 115791	*Eucalyptus grandis*	South Africa	AF283686	AY236891
Neofusicoccum luteum	CBS 110299[HT]	*Vitis vinifera*	Portugal	AY259091	AY573217

Table 3. List of reference sequences included in the phylogenetic analyses.

Taxon	Strain [a]	Host	Country	GenBank Accessions	
				ITS	*tef1α*
Neofusicoccum mangiferae	CBS 118531	*Mangifera indica*	Australia	AY615185	DQ093221
Neofusicoccum mangroviorum	CMW 41365[HT]	*Avicennia marina*	South Africa	KP860859	KP860702
Neofusicoccum mediterraneum	CBS 121718[HT]	*Eucalyptus* sp.	Greece	GU251176	GU251308
Neofusicoccum occulatum	CBS 128008[HT]	*Eucalyptus grandis*	Australia	EU301030	EU339509
Neofusicoccum parvum	CMW 9081[ET]	*Populus nigra*	New Zealand	AY236943	AY236888
Neofusicoccum ursorum	CMW 24480[HT] (=CBS 122811[IS])	*Eucalyptus* sp.	South Africa	FJ752746	FJ752709
Neofusicoccum vitisiforme	CBS 110887[HT]	*Vitis vinifera*	South Africa	AY343383	AY343343
Pseudofusicoccum stromaticum	CBS 117448[HT]	*Eucalyptus urophylla*	Venezuela	AY693974	AY693975

[a] CBS, Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; CERC, Culture Collection of China Eucalypt Research Centre, Chinese Academy of Forestry, Zhan Jiang, Guang Dong, China; CGMCC, China General Microbiological Culture Collection Center, Institute of Microbiology, Chinese Academy of Sciences, China; CMM, Culture Collection of Phytopathogenic Fungi Prof. Maria Menezes, Federal Rural University of Pernambuco, Brazil; CMW, Collection of the Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; DAR, New South Wales Plant Pathology Herbarium, Orange, NSW; GZCC, Guizhou Academy of Agricultural Sciences, Guizhou, China; IMI, CABI Genetic Resource Collection, Surrey, UK; MUCC, Mie University Culture Collection, Tsu City, Mie Prefecture, Japan.

Ex-type isolates: [ET], ex-epitype; [HT], ex-holotype; [IS], ex-isotype; [NT], ex-neotype; [PT], ex-paratype

in Geneious. To remove the need for *a priori* model testing, the MCMC analysis was set to sample across the entire general time-reversible (GTR) model space with a gamma-distributed rate variation across the sites. Five million random trees were generated using the MCMC procedure with four chains. The sample frequency was set at 1000 and the temperature of the heated chain was 0.1. Burn-in was set at 25 %, after which the likelihood values were stationary. Maximum likelihood (ML) analysis was run using RAxML v. 7.2.8 (Stamatakis and Alachiotis 2010) in Geneious and started from a random tree topology. The nucleotide substitution model used was GTR with a gamma-distributed rate variation.

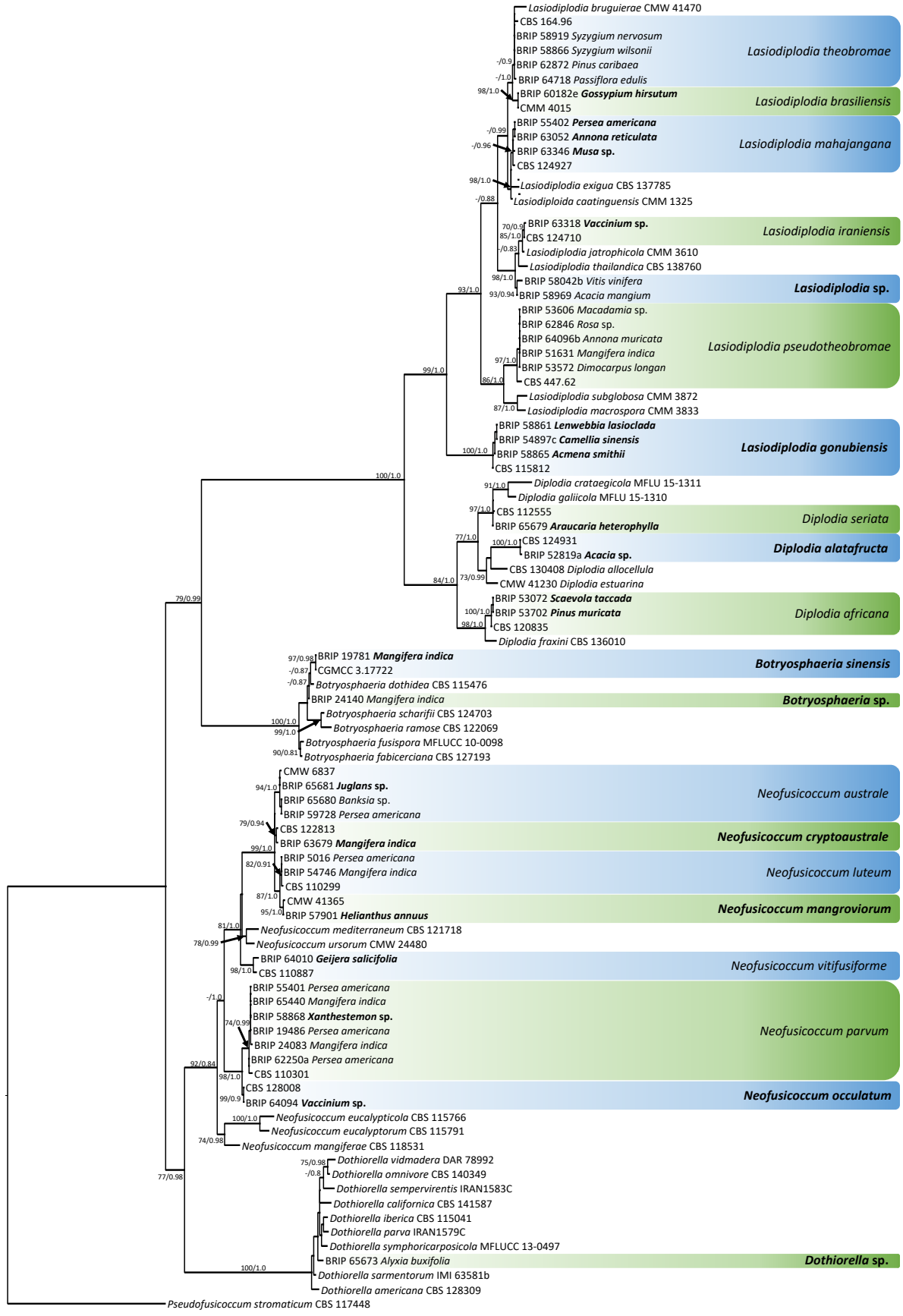
RESULTS

All 51 isolates were successfully amplified for both ITS and *tef1α* and their sequence datasets were analysed individually and in combination. The dataset contained 650 bp for the ITS region and 420 bp for the *tef1α* locus. The ITS and *tef1α* alignments were trimmed to 525 and 333 bp, respectively, and combined for phylogenetic analyses. The combined alignment was composed of 859 characters from 144 isolates, of which 99 bp (18.9 %), and 99 bp (29.4 %) were variable for ITS and *tef1α*, respectively. Species identification was confirmed through careful analyses of the combined ITS and *tef1α* sequence data.

Ten isolates that had been deposited as *Botryosphaeria* (1 isolate), *Diplodia* (4), and *Fusicoccum* (5), were identified as non-*Botryosphaeriaceae* based on BLASTn search results of the ITS sequences against the GenBank database (Table 1). The remaining 41 isolates that had been deposited as *Botryosphaeria* (5), *Dothiorella* (7), *Fusicoccum* (2), *Lasiodiplodia* (8), *Neofusicoccum* (5), and undetermined (9) were re-identified based on analyses of the combined ITS and *tef1α* sequences (Table 2, Fig. 1).

Seven of these re-identified isolates represent five new species records for Australia. One isolate (BRIP 19781) obtained from *Mangifera indica* (Anacardiaceae) in Ayr, Queensland (Qld), was identified as *Botryosphaeria sinensis* based on 100 % identity in the ITS and in the *tef1α* to the ex-paratype strain CGMCC 3.17723. One isolate (BRIP 52819a) obtained from *Acacia* sp. (Fabaceae) in Brisbane, Qld, was identified as *Diplodia alatafructa* based on 100 % identity in the ITS, and 99 % (1 single nucleotide polymorphism) identity in the *tef1α* to the ex-holotype strain CBS 124931 (Fig. 1). Three isolates (BRIP 54897c, 58861, and 54897) obtained from dead branches of *Acmena smithii* (Myrtaceae) and *Lenwebbia lasioclada* (Myrtaceae) in Brisbane, as well as from *Camellia sinensis* (Theaceae) in northern Qld were identified as *Lasiodiplodia gonubiensis* (Fig. 1). All three BRIP isolates differed from the ex-holotype strain CBS 115812 by 1 single nucleotide polymorphism (SNP) in the ITS region, while the isolates from *C. sinensis* (BRIP 54897c) and *L. lasioclada* (BRIP 58861) differed by 1 SNP in the *tef1α* sequence. An isolate (BRIP 63679) from a leaf of *M. indica* in Western Australia was identified as *Neofusicoccum cryptoaustrale* based on 99 % (1 SNP) identity in the ITS region, and 99 % (1 SNP) identity in the *tef1α* to the ex-type strain CBS 122813 (Fig. 1). An isolate (BRIP 57901) obtained from *Helianthus annuus* (Asteraceae) in a sunflower screening trial at Gatton, Qld, most likely as an endophyte, was identified as *N. mangroviorum* based on 99 % identity (1 SNP) in the ITS, and 99 % (2 SNP) identity in the *tef1α* to the ex-type strain CMW 41365 (Fig. 1).

Furthermore, four isolates were clustered in three distinct taxa in the current phylogenetic tree (Fig. 1). These isolates will remain as undescribed species as they require more loci sequences


Lasiodiplodia bruguierae CMW 41470
CBS 164.96
BRIP 58919 Syzygium nervosum
BRIP 58866 Syzygium wilsonii
BRIP 62872 Pinus caribaea
BRIP 64718 Passiflora edulis
BRIP 60182e Gossypium hirsutum
CMM 4015
Lasiodiplodia theobromae
Lasiodiplodia brasiliensis
BRIP 55402 Persea americana
BRIP 63052 Annona reticulata
BRIP 63346 Musa sp.
CBS 124927
Lasiodiplodia mahajangana
Lasiodiplodia exigua CBS 137785
Lasiodiploida caatinguensis CMM 1325
BRIP 63318 Vaccinium sp.
CBS 124710
Lasiodiplodia jatrophicola CMM 3610
Lasiodiplodia thailandica CBS 138760
Lasiodiplodia iraniensis
BRIP 58042b Vitis vinifera
BRIP 58969 Acacia mangium
Lasiodiplodia sp.
BRIP 53606 Macadamia sp.
BRIP 62846 Rosa sp.
BRIP 64096b Annona muricata
BRIP 51631 Mangifera indica
BRIP 53572 Dimocarpus longan
CBS 447.62
Lasiodiplodia pseudotheobromae
Lasiodiplodia subglobosa CMM 3872
Lasiodiplodia macrospora CMM 3833
BRIP 58861 Lenwebbia lasioclada
BRIP 54897c Camellia sinensis
BRIP 58865 Acmena smithii
CBS 115812
Lasiodiplodia gonubiensis
Diplodia crataegicola MFLU 15-1311
Diplodia galiicola MFLU 15-1310
CBS 112555
BRIP 65679 Araucaria heterophylla
Diplodia seriata
CBS 124931
BRIP 52819a Acacia sp.
Diplodia alatafructa
CBS 130408 Diplodia allocellula
CMW 41230 Diplodia estuarina
BRIP 53072 Scaevola taccada
BRIP 53702 Pinus muricata
CBS 120835
Diplodia africana
Diplodia fraxini CBS 136010
BRIP 19781 Mangifera indica
CGMCC 3.17722
Botryosphaeria dothidea CBS 115476
Botryosphaeria sinensis
BRIP 24140 Mangifera indica
Botryosphaeria sp.
Botryosphaeria scharifii CBS 124703
Botryosphaeria ramose CBS 122069
Botryosphaeria fusispora MFLUCC 10-0098
Botryosphaeria fabicerciana CBS 127193
CMW 6837
BRIP 65681 Juglans sp.
BRIP 65680 Banksia sp.
BRIP 59728 Persea americana
Neofusicoccum australe
CBS 122813
BRIP 63679 Mangifera indica
Neofusicoccum cryptoaustrale
BRIP 5016 Persea americana
BRIP 54746 Mangifera indica
CBS 110299
Neofusicoccum luteum
CMW 41365
BRIP 57901 Helianthus annuus
Neofusicoccum mangroviorum
Neofusicoccum mediterraneum CBS 121718
Neofusicoccum ursorum CMW 24480
BRIP 64010 Geijera salicifolia
CBS 110887
Neofusicoccum vitifusiforme
BRIP 55401 Persea americana
BRIP 65440 Mangifera indica
BRIP 58868 Xanthestemon sp.
BRIP 19486 Persea americana
BRIP 24083 Mangifera indica
BRIP 62250a Persea americana
CBS 110301
Neofusicoccum parvum
CBS 128008
BRIP 64094 Vaccinium sp.
Neofusicoccum occulatum
Neofusicoccum eucalypticola CBS 115766
Neofusicoccum eucalyptorum CBS 115791
Neofusicoccum mangiferae CBS 118531
Dothiorella vidmadera DAR 78992
Dothiorella omnivore CBS 140349
Dothiorella sempervirentis IRAN1583C
Dothiorella californica CBS 141587
Dothiorella iberica CBS 115041
Dothiorella parva IRAN1579C
Dothiorella symphoricarposicola MFLUCC 13-0497
BRIP 65673 Alyxia buxifolia
Dothiorella sp.
Dothiorella sarmentorum IMI 63581b
Dothiorella americana CBS 128309
Pseudofusicoccum stromaticum CBS 117448

to support their introduction as novel species. One isolate (BRIP 24140) is a sister clade to *B. dothidea* and *B. sinensis*, and differs from both species by 4 bp in *tef1α*. Two other isolates (BRIP 58042b and 58969), *Lasiodiplodia* sp., is a sister clade to *L. iraniensis*, *L. jatrophicola*, and *L. thailandica*. *Lasiodiplodia* sp. differs from *L. iraniensis* by 2 bp in ITS and 7 bp in *tef1α*, from *L. jatrophicola* by 3 bp in ITS and 5 bp in *tef1α*, and from *L. thailandica* by an 8 bp deletion in *tef1α*. The isolate, VPRI 13932, represents a distinct taxon in *Dothiorella*, and differs from the other species by a 26 bp deletion in *tef1α*.

DISCUSSION

Multilocus sequence analyses re-identified 41 isolates from the two herbaria into five genera and 20 species, including 18 known species and three unknown species in *Botryosphaeriaceae*. Five of these species, *Botryosphaeria sinensis*, *Diplodia alatafructa*, *Lasiodiplodia gonubiensis*, *Neofusicoccum cryoptoaustrale,* and *N. mangroviorum*, are reported for the first time in Australia. New hosts are reported for 14 species, namely *B. sinensis*, *D. africana*, *D. alatafructa*, *D. seriata*, *L. brasiliensis*, *L. gonubiensis*, *L. iraniensis, L. mahajangana*, *N. australe*, *N. cryptoaustrale*, *N. mangroviorum, N. occulatum, N. parvum*, and *N. vitifusiforme*.

Two *Botryosphaeria* species were identified in this study, *B. sinensis* and an undescribed *Botryosphaeria* sp. *Botryosphaeria sinensis* was recently described from *Juglans regia* (Juglandaceae), *Morus alba* (Moraceae), and *Populus* sp. (Salicaceae) in China (Zhou et al. 2016), as a sister taxon to *B. dothidea*. The isolate, BRIP 19781, from *M. indica* represents a new species record for Australia, and a new host association.

Three *Diplodia* species, including *D. africana*, *D. alatafructa* and *D. seriata*, were identified in this study. *Diplodia africana* was first described as a potential pathogen on *Prunus* spp. in South Africa (Damm et al. 2007), and has since been found on *Juniperus phoenicea* (Cupressaceae) in Italy (Alves et al. 2014). In this study, *D. africana* was identified on *Pinus muricata* (Pinaceae) and *Scaevola taccada* (Goodeniaceae). *Diplodia alatafructa* was first described from a stem wound on *Pterocarpus angolensis* (Fabaceae) in South Africa (Mehl et al. 2011), and has been shown to cause stem lesions and vascular discolouration on *Eriobotrya japonica* (Rosaceae) in Spain (González-Domínguez et al. 2017). The isolate of *D. alatafructa* (BRIP 52819a) from *Acacia* sp. represents a new species record for Australia. *Diplodia seriata* has over 300 host associations and is found worldwide (Farr and Rossman 2017). Despite its plurivorous nature, the identification of *D. seriata* on *Araucaria heterophylla* (Araucariaceae) in Australia represents an extension of its host family. Results of this study not only expand the host associations for these three species, but also a new geographical location for *D. alatafructa*.

Seven *Lasiodiploda* species were identified in this study, *L. brasiliensis, L. gonubiensis, L. iraniensis, L. mahajangana, L. pseudotheobromae, L. theobromae,* and an undescribed *Lasiodiplodia* sp. *Lasiodiplodia brasiliensis* was originally described as a minor pathogen

Fig. 1. Phylogenetic tree based on maximum likelihood analysis of the combined ITS and *tef1α* alignment. RAxML bootstrap values (bs) greater than 70 % and Bayesian posterior probabilities (pp) greater than 0.8 are given at the nodes (bs/pp). The outgroup is *Pseudofusicoccum stromaticum* ex-type strain CBS 117448. New species reported in Australia and new host records are in **bold**.

associated with stem-end rot of *Carica papaya* (Caricaceae) and of *M. indica* in Brazil (Marques et al. 201, Netto et al. 2014). Since then, it has been isolated from other hosts in Brazil, including *Anacardium occidentale* (Anacardiaceae), *Annona squamosa* (Annonaceae), *Cocos nucifera* (Arecaceae), *Spondias purpurea* (Anacardiaceae), and *Vitis vinifera* (Vitaceae) (Cardoso et al. 2017, Correia et al. 2016, Coutinho et al. 2017, Netto et al. 2017, Rosado et al. 2015). It has also been reported from other countries, including in Madagascar from *Adansonia madagascariensis* (Malvaceae), in Thailand from *Tectona grandis* (Lamiaceae) and in Turkey from *Fragaria* × *ananassa* (Rosaceae) (Cruywagen et al. 2017, Doilom et al. 2015). The isolate, BRIP 60182e, from *Gossypium hirsutum* (Malvaceae) represents an extension of its host range. *Lasiodiplodia gonubiensis* was originally described as an endophyte from *Syzygium cordatum* (Myrtaceae) in South Africa (Pavlic et al. 2004), where it has subsequently been isolated from healthy and/or diseased *Bruguiera gymnorrhiza* (Rhizophoraceae), *Ceriops tagal* (Rhizophoraceae), *Sclerocarya birrea* subsp. *caffra* (Anacardiaceae), and *Vachellia karroo* (Fabaceae) in South Africa (Jami et al. 2015, 2017, Osorio et al. 2017, Mehl et al. 2017). *Lasiodiplodia gonubiensis* has also been reported from *Adansonia digitata* (Malvaceae) in Mozambique, *Anacardium humile* (Anacardiaceae) in Brazil, and *Phyllanthus emblica* (Phyllanthaceae) in Thailand (Cruywagen et al. 2017, Netto et al. 2017, Trakunyingcharoen et al. 2015). The isolates in this study represent the first record of *L. gonubiensis* in Australia, as well as new host associations for this species. *Lasiodiplodia iraniensis* has been isolated from various hosts in Iran, namely *Citrus* sp. (Rutaceae), *Eucalyptus* sp. (Myrtaceae), *Juglans* sp. (Jualandaceae), *M. indica, Salvadora persica* (Salvadoraceae), and *Terminalia catappa* (Combretaceae) (Abdollahzadeh et al. 2010, Mohammadi et al. 2013). It has also been reported from *A. digitata* throughout central and southern Africa (Cruywagen et al. 2017), *A. occidentale* in Brazil (Netto et al. 2017), *M. indica* in Australia, Brazil and Peru (Netto et al. 2017, Rodriguez-Galvez et al. 2017, Sakalidis et al. 2011b), *S. persica* in Colombia (Úrbez-Torres et al. 2012b), and *Sclerocarya birrea* subsp. *caffra* (Anacardiaceae) in South Africa (Mehl et al. 2017). The isolate, BRIP 63318, from *Vaccinium* sp. (Ericaceae) represents a new host association for *L. iraniensis*. *Lasiodiplodia mahajangana* is predominantly associated with woody hosts in the southern Africa continent (Begoude et al. 2010, Jami et al. 2017, Mehl et al. 2017, Phillips et al. 2013). The isolates in this study represents expansion of its host range to include *Annona reticulata* (Annonaceae) and *Persea americana* (Lauraceae), and an herbaceous host, *Musa* sp. (Musaceae).

Seven *Neofusicoccum* species were identified in this study, including *N. australe, N. cryptoaustrale, N. luteum, N. mangroviorum, N. occulatu, N. parvum* and *N. vitifusiforme*. *Neofusicoccum australe* has been reported from 73 different hosts mainly from countries located in the southern hemisphere (Farr and Rossman 2017). Despite its plurivorous nature, the identification of *N. australe* on *Juglans* sp. in this study represents an extension of its host range. *Neofusicoccum cryptoaustrale* was first described as an endophyte from branches and leaves of *Eucalyptus* trees in South Africa (Pavlic-Zupanc et al. 2013), where it has subsequently been isolated from healthy and/or diseased *Avicennia marina* (Acanthaceae), *Barringtonia racemosa* (Lecythidaceae), *Bruguiera gymnorrhiza* (Rhizophoraceae), *Ceriops tagal* (Rhizophoraceae), *Eucalyptus* spp., *Lumnitzera racemose* (Combretaceae), *Podocarpus henkelii* (Podocarpaceae), *P. latifolius* (Podocarpaceae), and *Rhizophora mucronata* (Rhizophoraceae) (Osorio et al. 2017, Pavlic-Zupanc et al. 2017). The isolate in this study represents the first record of *N. cryptoaustrale* in Australia, as well as a new host association. *Neofusicoccum mangroviorum* was isolated from symptomless branches of four genera of mangrove (*Avicennia*, *Bruguiera*, *Lumnitzera*, and *Rhizophora*) and *Mimusops caffra* (Sapotaceae) in South Africa (Osorio et

al. 2017, Jami et al. unpublished). The identification of this species on *H. annuus* represents a new species record for Australia, and a new host association. *Neofusicoccum occulatum* was first described from *Eucalyptus* spp. (Myrtaceae) and *Wollemia nobilis* (Araucariaceae), as pathogens on stems of *E. globulus* (Sakalidis et al. 2011a). *Neofusicoccum occulatum* has since been isolated from other woody hosts, such as *Blepharocalyx salicifolius* (Myrtaceae) in Uruguay, *Grevillea* sp. (Proteaceae) in Uganda, *Eucalyptus* spp. in Hawaii, and *V. vinifera* in Australia (Sakalidis et al. 2013). The identification of *N. occulatum* on *Vaccinium* sp. represents a host new host association. *Neofusicoccum parvum* has been reported globally from over 150 different hosts (Farr and Rossman 2017). Despite its plurivorous nature, the identification of *N. parvum* on *Xanthostemon* sp., a tree endemic only to north eastern Qld, represents a new host association. *Neofusicoccum vitifusiforme* has a wide host range having been found to cause, or be associated with, grapevine dieback in South Africa (van Niekerk 2004, who first described and named this species *Fusicoccum vitifusiforme*), Spain (Luque et al. 2009), Mexico (Candolfi-Arballo et al. 2010), USA (Úrbez-Torres 2011) and Italy (Mondello et al. 2013); olive (*Olea europaea*) drupe rot in Italy (Lazzizera et al. 2008, Úrbez-Torres et al. 2012a); dieback of stone-fruit trees (*Prunus* spp.) (Damm et al. 2007) and pome fruit trees (*Malus* and *Pyrus* spp.) in South Africa (Cloete et al. 2011), and blight of blueberry (*Vaccinium corymbosum*) in China (Kong et al. 2010). In this study, *N. vitifusiforme* was identified on leaves of *Geijera salicifolia* (Rutaceae), which is native to dry rainforests in eastern Australia, and represents a new host association.

Species in the *Botryosphaeriaceae* are spreading around the world, likely facilitated by movement of plant material, including fruits. These fungi are virtually impossible to detect in their endophytic state (Burgess et al. 2016). Even where symptoms are visible, biosecurity measures, including quarantining plant material, must no longer rely on morphological identifications and outdated taxonomy for this group of fungi (Crous et al. 2016). The re-identification of 41 isolates in this study based on phylogenetic analyses of the ITS and *tef1α* loci demonstrates the inadequacy of morphological characters for species level identifications. Ten isolates were identified as not belong to *Botryosphaeriaceae*, which also illustrates the difficulties faced by plant pathologists and plant diagnosticians even at the generic level. This has also shown to be the case for *Colletotrichum* (Shivas and Tan 2009, Shivas et al. 2016), *Fusarium* (Summerell et al. 2011), *Phytophthora* (Burgess et al. 2009), downy mildew (Shivas et al. 2012), and powdery mildew (Cunnington et al. 2003). Thus, laboratory capability to identify these fungi must be maintained and extensive reference collections supported if effective surveillance and monitoring of the family is to continue.

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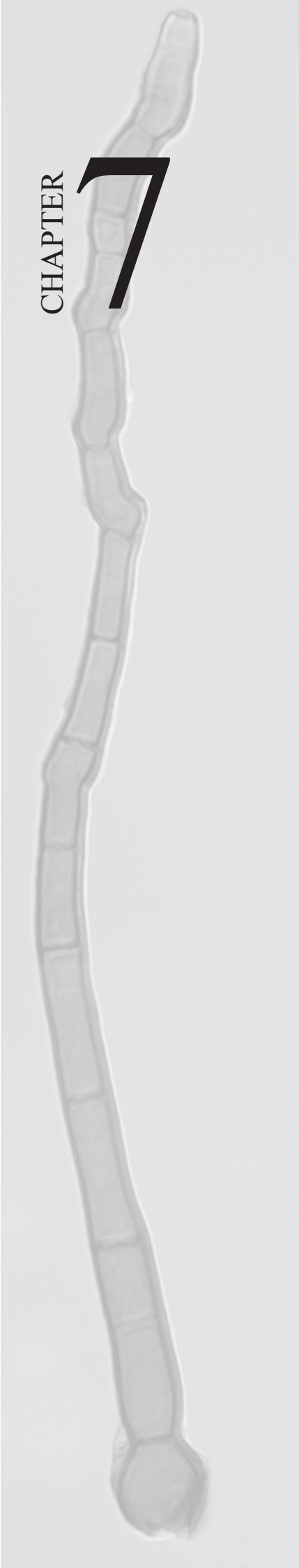
CHAPTER 7

General discussion



In the past decade, there has been an upsurge in the discovery of cryptic species and species complexes in many taxonomic groups of fungal plant pathogens from around the world. This has led to major taxonomic revisions for many important groups of plant pathogenic fungi. Some examples are *Alternaria* (Woudenberg et al. 2013, 2014, 2015), *Botryosphaeriaceae* (Phillips et al. 2013, Slippers et al. 2013, 2014, Dissanayake et al. 2016), *Cercospora* (Groenewald et al. 2013), *Cladosporium* (Bensch et al. 2010, 2012, 2015, 2018), *Colleotrichum* (Damm et al. 2012a, 2012b, Weir et al. 2012, Damm et al. 2013, Crouch 2014, Damm et al. 2014, Liu et al. 2014, Damm et al. 2019), *Diaporthe* (Thompson et al. 2011, Udayanga et al. 2011, Gomes et al. 2012, Udayanga et al. 2014a, 2014b, 2015, Dissanayake et al. 2017), *Elsinoë* (Miles et al. 2015, Fan et al. 2017), *Endoraecium* (McTaggart et al. 2015), *Erysiphe* (Takamatsu et al. 2013, 2015, Pastirčáková et al. 2016, Takamatsu et al. 2016), *Exserohilum* (Hernández-Restrepo et al. 2018), *Fusarium* (Aoki et al. 2014, O'Donnell et al. 2015, Maryani et al. 2017), *Macalpinomyces* (McTaggart et al. 2012), *Microdochium* and *Monographella* (Hernández-Restrepo et al. 2016), Nectriaceae (Lombard et al. 2010, Lombard and Crous 2012, Lombard et al. 2012, 2014a, 2015), *Nigrospora* (Wang et al. 2017), *Phoma* (Aveskamp et al. 2010, de Gruyter er al. 2010, Chen et al. 2015), *Phyllosticta* (Wikee et al. 2013), *Pseudocercospora* (Crous et al. 2013a), and *Ustilago* (McTaggart et al. 2012).

The upsurge in the discovery and classification of cryptic species has been directly attributed to a taxonomic revolution that was a consequence of the adoption of DNA sequence-based techniques and phylogenetic analyses in most taxonomic and diagnostic plant pathology laboratories worldwide. Symbolically, the revolution began with the *Amsterdam Declaration on Fungal Nomenclature* agreed at an international symposium convened in Amsterdam, the Netherlands, 19–20 April 2011 under the auspices of the International Commission on the Taxonomy of Fungi (ICTF; Hawksworth et al. 2011). The purpose of the symposium was to address the issue of whether the current system of naming pleomorphic fungi should be maintained or changed since molecular data were increasingly available. The issue was considered urgent as mycologists were following different practices, and there was a lack of consensus from the Special Committee appointed in 2005 by the International Botanical Congress to provide advice on the problem. The *Amsterdam Declaration on Fungal Nomenclature* recognised the need for an orderly transition to a single-name nomenclatural system for all fungi, and to provide mechanisms to protect names that would otherwise become endangered. The revolution was won on 1 January 2012, when the changes to the set of rules that cover fungal nomenclature, i.e. the *International Code of Nomenclature for algae, fungi, and plants* (ICN, McNeill et al. 2012), which had been approved by the 18th International Botanical Congress in Melbourne, Australia in July 2011, came into effect (Hawskworth 2011). These rule changes included the abolishment of the dual nomenclatural system for fungi and its replacement by a system whereby one fungus can only have one name, regardless of its biological (morphological) state. Furthermore, online publications of new fungal and plant species were allowed.

Since 2012, a significant decrease in costs and improved molecular methods have accelerated the incorporation of DNA sequence-based phylogenetic methods and analyses in taxonomic studies. The changes to the ICN together with the increased understanding of systematic relationships among fungi based on molecular phylogenetic methods have resulted in a large number of taxonomic revisions in recent years. Although these name changes might have caused confusion for plant pathologists and biosecurity agencies in the short term, the more accurate application of names based on DNA sequence-based data will ensure long-term stability in the taxonomy of all biological organisms.

As the cost of next generation sequencing technologies has decreased over the last decade, the number of fungal genomes that have been sequenced and made available has increased significantly (Spatafora 2011, Aylward et al. 2017). The availability and analysis of fungal genomes has enabled *in silico* predictions that provide an insight into the pathogenicity and life style of the organism (as reviewed by Aylward et al. 2017). The information gathered from analyses of fungal genomes is likely to affect the way biosecurity agencies assess the ability of plant pathogens to infect host plants, establish in new locations, and cause detrimental economic and environmental consequences.

In traditional taxon-based approaches to biosecurity, the focus has been on known and named pathogens to the exclusion of those that were unknown or undescribed. It has been suggested that this traditional approach may complement or even be replaced by the genes-based approach to biosecurity, where the risk is assessed based on regions of the genome related to the pathogenicity and/or life style, i.e. mating genes, pathogenicity genes and transposons (Crous et al. 2016, McTaggart et al. 2016). An example of a genes-based approach to biosecurity is illustrated by species formerly classified as *Fusarium oxysporum* f.sp. *cubense* (*Foc*), the causal agent of Panama disease on bananas (Stover 1962, Maryani et al. 2019). *Foc* was divided into 24 genotypes called Vegetative Compatibility Groups (VCGs) (Kistler et al. 1998). Some diverse VCGs that were able to infect cultivars of Gros Michel (Musa AAA) and Lady Finger (Musa AAB) were labelled as Race 1 (Ploetz 2006, Ordonez et al. 2016). However, the main strain of economic concern was VCG 01213/16, also known as Tropical Race 4 (TR4), which infects Cavendish cultivars (*Musa* AAA) and all cultivars suspectible to Race 1. In Australia, TR4 was detected in the Northern Territory in 1997 (Conde and Pikethley 2001), and in northern Queensland in 2015 (O'Neill et al. 2016). A recent study into the genetic diversity of *Foc* in Indonesia identified nine independent lineages and formally described these as novel species (Maryani et al. 2019). TR4 was classified as *F. odoratissimum* based on pathogenicity on Cavendish cultivars, while Race 1 was separated into six species (*F. grosmichelii*, *F. duoseptatum*, *F. hexaseptatum*, *F. purprascens*, *F. phialophorum*, and *F. tardichlamydosporum*) based on pathogenicity to cultivars of Gros Michel only (Maryani et al. 2019).

The re-classification of *Foc* into nine species has implications for biosecurity and diagnostics in all banana-producing countries, especially Australia. Identification of the fungus is fundamental to prevention and management of this soil-borne pathogen. The most widely used molecular-based test was designed around the intergenic spacer region of a poorly-defined species complex (Dita et al. 2010). This test was recently abandoned by Australian diagnostic and biosecurity agencies as being unreliable because it was susceptible to false positives (McKillop 2016, Zonca 2016). The diagnostic test adopted by most biosecurity agencies for *Foc* has traditionally been the the time-consuming VCG test, which can take up to four weeks for a result. Other methods are currently in the pipeline to facilitate rapid detection of TR4 strains, such as a molecular test based on pathogenicity genes (Fraser-Smith et al. 2014) or high-throughput genome analyses (Ordonez et al. 2016). However, these tests need re-evaluation considering the taxonomic revision of *Foc* (Maryani et al. 2019).

The seven *Fusarium* species from the *Foc* complex classified by Maryani et al. (2019) were isolated from *Musa* hosts. It is now imperative that the identity of all *F. oxysporum* isolates in the Australian plant pathology herbaria are re-evaluated to determine if there are alternate host plants species. It is not known which of the six *Fusarium* species that now represent Race 1 are present in Australia, although Race 1 strains have been in Australia since 1876 (Bancroft

1876, Pegg et al. 1996). The Australian reference culture collections of *Foc* will help determine the distribution of these *Fusarium* species in Australia. This information may have important implications for biosecurity regulations around the movement of plant material and agricultural equipment across the Australian continent. The genes-based approach to biosecurity for exotic or novel fungal plant pathogens of concern is only in its infancy, and uptake by biosecurity agencies is some years away. Until then, an accurate identification system that underpins the taxon-based approach is still needed in Australia.

In many countries, particularly in Australia, the accurate identification of plant pathogenic fungi is important to national biosecurity agencies, which have a mandate to prevent, detect or delay the introduction of exotic pests and pathogens. Hyde et al. (2010) highlighted the urgent need for a re-assessment of fungal plant pathogens in Australia in order to preserve the effectiveness of biosecurity measures. Lists of plant pathogens recorded in Australia have become outdated as molecular methods uncover many new cryptic species and species complexes. Future revision of Australian plant pathogens checklists and databases must be supported by herbarium material, living cultures and DNA libraries (Hyde et al. 2010). Recent studies have started to reassess plant pathogens in the Australian context, such as *Botryosphaeriaceae* (Billones-Baaijens et al. 2018, Burgess et al. 2018, Tan et al. 2018), *Colletotrichum* (Shivas et al. 2016), *Fusarium* (Summerell et al. 2011, Liew et al. 2016), and *Phytophthora* (Burgess et al. 2017).

In Australia, the three largest culture collections of plant pathogenic fungi collectively contain over 30 000 strains. The largest of these collections is housed at the Queensland Plant Pathology Herbarium (BRIP), Dutton Park, Queensland, and contains about 18 000 living fungal cultures permanently preserved in a metabolically inactive state at ultra low temperature (-70 °C) in a sterile solution of 15 % v/v glycerol. These strains are extremely valuable to the Australian biosecurity system that relies on them as reference isolates when making decisions about potential exotic incursions. The accurate identification of these reference isolates is paramount to the protection of Australian agriculture and the environment from new plant diseases. Most of these reference isolates have been collected over the past 50 years and were identified based on morphology. However, many of the names of these isolates are unverified or inaccurate because of DNA-based phylogenetic methods.

The research aim of this thesis was to establish whether cultures of plant pathogens held in the major Australian culture collections were accurately and reliably identified (Chapter 1). The BRIP holds particulary rich collections of *Diaporthe*, helminthosporioid fungi and *Botryosphaeriaceae*. These taxonomic groups contain both endemic and exotic species that were assembled mostly by plant pathologists working for the Queensland Government in the past 50 years or even longer. The helminthosporioid collection was assembled by the mycologist Dr. John L. Alcorn (former Curator of BRIP) who had become, at the turn of the twentieth century, one of the foremost experts with these fungi by discovering and describing many new taxa. However, Alcorn worked mostly in the pre-molecular era and he did not have the tools by which species complexes could be resolved. The collections of *Diaporthe*, helminthosporioid fungi and *Botryosphaeriaceae* held in the Queensland Plant Pathology Herbarium were chosen for molecular taxonomic reassessment because of both the diversity and agricultural importance (Chapters 2, 3, 4, 5 and 6). Molecular, morphological and phylogenetic methods were used to determine and taxonomically resolve cryptic species and species complexes in these fungal groups. The implication of these studies for Australia plant biosecurity are discussed below.

Diaporthe

Diaporthe species (*Sordariomycetes*, *Diaporthales*, *Diaporthaceae*) are known as pathogens, endophytes or saprobes on a wide range of host plants (Udayanga et al. 2011, Gomes et al. 2013, Udayanga et al. 2015, Dissanyake et al. 2017, Marin-Felix et al. 2019). More than one *Diaporthe* species may be causal agents of the same disease (Thompson et al. 2011, Guarnaccia et al. 2017, 2018), and a single *Diaporthe* species can be found on diverse hosts (Dissanayake et al. 2017). Consequently, the identification of *Diaporthe* species based on host association and morphology is not reliable. Recent studies have provided a multilocus backbone tree for species delimination and inference (Dissanayake et al. 2017, Marin-Felix et al. 2019).

In Chapter 2, isolates that had been identified as belonging to *Diaporthe* sp. (syn. *Phomopsis*) in the BRIP and the Victorian Plant Pathology Herbarium (Bundaroo, Vic, Australia) were examined. Multilocus phylogenetic analyses revealed that the Australian isolates represented six unique taxa (Tan et al. 2013). *Diaporthe beilharziae*, *D. fraxini-angustifoliae* and *D. litchicola*, were described based on morphological and molecular characteristics. The other three species, *D. nothofagi*, *D. pascoei*, and *D. salicicola*, were sterile under the conditions that they were grown and did not produce any fruiting structures. Voucher specimens of *D. pascoei* and *D. salicicola* from the original collections dating back to 1988 and 2007, respectively, had pycnidia and conidia that allowed the completion of morphological descriptions. Strong phylogenetic evidence based on multilocus sequence analyses supported the establishment of *D. nothofagi* as a unique taxon, despite the absence of morphological characteristics.

The genus *Diaporthe* now includes 213 species that are supported by ex-type cultures and DNA barcodes (Marin-Felix et al. 2019). Only 50 species of *Diaporthe* have been reported in Australia, of which 26 were described from Australian specimens (Fig. 1) (Williamson et al. 1994, van Niekerk et al. 2005, Crous et al. 2011, Thompson et al. 2011, Crous et al. 2012, Gomes et al. 2013, Tan et al. 2013, Crous et al. 2015, Thompson et al. 2015). Two of the species described in Chapter 2 have been subsequently reported outside of Australia, namely *Diaporthe fraxini-angustifoliae* from *Persea americana* in Florida, USA (Shetty et al. 2016), and *D. litchicola* from *Cinchona calisaya* in Indonesia (Radiastuti et al. 2016).

The data from Chapter 2 provided an insight into the hidden diversity of *Diaporthe* species in Australian plant pathology herbaria. This apparent diversity is neither suprising nor unique to Australia. The application of a multilocus phylogenetic approach has resulted in a rapid increase in the description of novel species. Recent studies have uncovered multiple species of *Diaporthe* from the same host plant species (Thompson et al. 2011, 2015, Santos et al. 2011, Huang et al. 2013, Lombard et al. 2014b, Gao et al. 2015, 2016, Udayanga et al. 2015, Guarnaccia et al. 2016, 2017, 2018). In each of those studies, established species were identified as well as novel species described. Some species appeared to be opportunistic pathogens found on multiple hosts, such as *D. foeniculina* and *D. novem* (Santos et al. 2011, Gomes et al. 2013, Udayanga et al. 2014b, Guarnaccia et al. 2017, Thompson et al. 2018).

The association of multiple species of *Diaporthe* with the same plant disease symptoms highlights the importance of accurate, molecular-based identification of species of *Diaporthe* for plant biosecurity. For example, stem canker on sunflower (*Helianthus annuus*) (Fig. 2a−b) caused by *D. helianthi* has never been recorded in Australia. *Diaporthe helianthi* was first reported in the

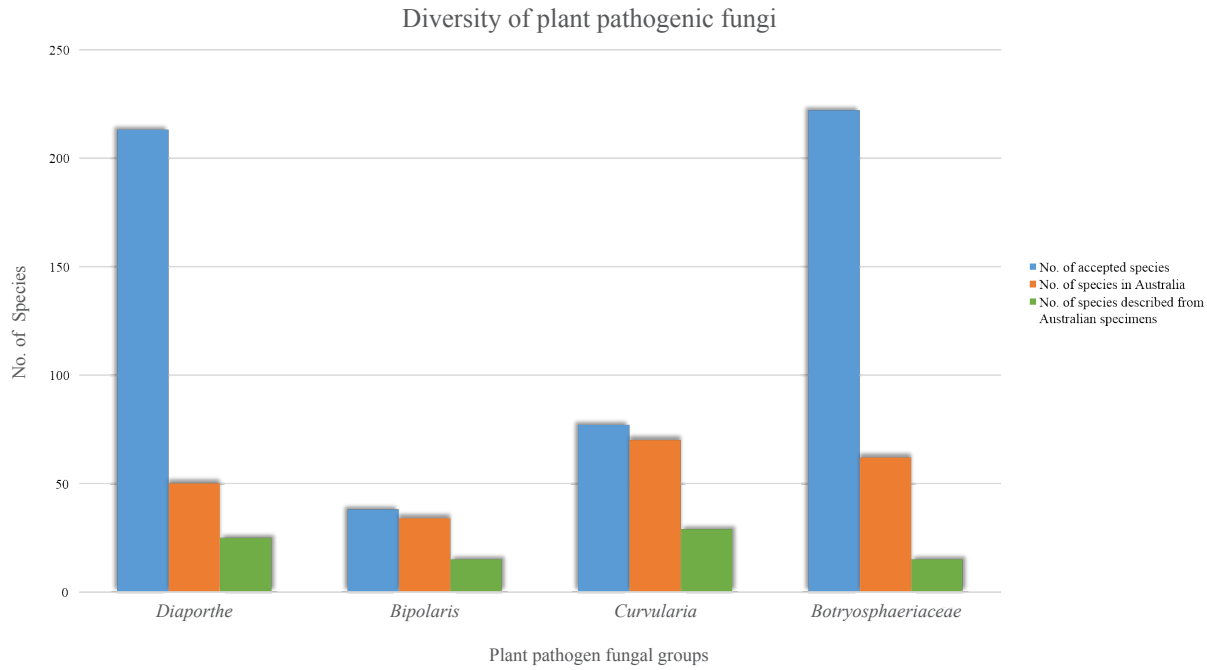


Fig. 1. Species diversity of *Bipolaris*, *Curvularia*, *Diaporthe* and *Botryosphaeriaceae*. This graph shows the total number of accepted species of each plant pathogenic fungal groups examined in this thesis (blue column). Of this total number, the number of species reported in Australia (orange), and the number of species described from Australian specimens (green).

former Yugoslavia in the early 1980s (Muntañola-Cvetkovic´ et al. 1981). A study of *Diaporthe* isolates from sunflower stem canker in Australia revealed three novel species, including one, *D. gulyae*, that caused sysmptoms of equal severity as the exotic *D. helianthi* (Thompson et al. 2011). *Diaporthe gulyae* has since been reported in North America from sunflower (Mathew et al. 2015a, 2015b) and *Glycine max* (Mathew et al. 2018a). The existence of two species of *Diaporthe*, one exotic and one established in Australia, that cause a severe sunflower stem canker disease has implications for biosecurity and sunflower disease management (Mathew et al. 2018b). The biosecurity implications are that an outbreak of sunflower stem canker disease in Australia does not necessarily indicate an exotic incursion, and that molecular-based methods must be used to determine the causal fungus. An implication for the management of sunflower stem canker in Australia is that breeding programs that select resistant sunflower varieties must ensure that plants are challenged with accurately identified *Diaporthe* species. It is not yet known whether *D. gulyae* and *D. helianthi* have similar virulence profiles towards differential sets of sunflower varieties. More recently, a third species, *D. novem*, has been reported to cause sunflower stem canker in Australia (Thompson et al. 2018). This is significant as *D. novem* has a broad host range and overwinters on plant debris (Gomes et al. 2013, Thompson et al. 2015, Dissanayake et al. 2017).

Recent studies on the diversity of *Diaporthe* isolated from economically significant plants have recognised several species including taxonomic novelties (Huang et al. 2013, Lombard et al. 2014b, Gao et al. 2015, 2016, Udayanga et al. 2015, Guarnaccia et al. 2016, 2017, 2018). For example, *D. citri* was described as the cause of citrus melanose and stem-end rot (Fawcett 1912). The species concept of *D. citri* was resolved based on multilocus phylogenetic analysis

Fig. 2. Disease symptoms. Stem canker on *Helianthus annuus*: **a** *Diaporthe* canker lesion on sunflower stalk, **b** lodging of mature plants due to severe infection. Helminthosporioid infection on *Poaceae*: **c** leaf spot on *Arundinella* sp., near Toowoomba, Queensland, **d** mouldy inflorescence on *Sporobolus* sp., south-east Queensland, **e** leaf spot on *Panicum* sp., near Mareeba, Queensland. Botryospheriaceae dieback of an Australian native plant: **f** *Acacia harpophylla*, near Marburg, Queensland.
Images supplied by S.M. Thompson (a−b), R.G. Shivas (c−e), and D.J. Tree (f).

(Udayanga et al. 2014b) that enabled subsequent studies to determine the diversity of *Diaporthe* on *Citrus*. A study from China identified *D. citri* and two novel species, *D. citriasiana* and *D. citrichinensis* (Huang et al. 2013). A similar study in Europe identified three novel species (*D. infertilis*, *D. limonicola* and *D. melitensis*), three known species (*D. baccae*, *D. foeniculina* and *D. novem*), but did not find *D. citri* (Guarnaccia et al. 2017). *Diaporthe citri* is reported in Australia (Simmonds 1966, Hyde and Alcorn 1993), although this does not appear to have been verified by molecular methods. The *Diaporthe* species that are present on *Citrus* in Australia needs to be determined and verified in order to determine the exotic species. Once this is done, the biosecurity risks that exotic species of *Diaporthe* pose to the Australian *Citrus*-based industries can be assessed.

Helminthosporioid genera

In Chapters 3, 4 and 5, the helminthosporioid genera *Bipolaris* and *Curvularia* (*Dothideomycetes*, *Pleosporales*, *Pleosporaceae*) were taxonomically revised through the examination of cultures held in the Queensland Plant Pathology Herbarium. Species of *Bipolaris* and *Curvularia* have been associated with diseases on more than 60 host plant genera, mostly grasses (*Poaceae*) (Fig. 2c−e) (Sivanesan 1987, Manamgoda et al. 2011, 2014, 2015, Marin-Felix et al. 2017a). In the past, the identification of *Bipolaris* and *Curvularia* in Australia was based on the extensive morphological studies by the Australian taxonomic mycologist Dr. John L. Alcorn, who was the curator of the Queensland Plant Pathology Herbarium from 1960–1999. Most of the helminthosporioid fungal isolates examined in these chapters had been collected and studied by Alcorn (see Alcorn 1971, 1982a, 1982b, 1983, 1990, 1996, 1998, Sivanesan et al. 2003). As *Bipolaris* and *Curvularia* share overlapping morphological characters, this approach has been unreliable, and consequently many of Alcorn's specimens remained undescribed.

In the past decade, molecular phylogenetic approaches have provided additional criteria that have allowed the development of a stable taxonomy for the helminthosporioid genera (Manamgoda

et al. 2012b). In Chapter 3, a molecular phylogenetic approach was applied to ex-type cultures of 34 species *Bipolaris* and *Curvularia* to resolve or confirm their taxonomic status (Tan et al. 2014). The phylogenetic analyses supported the transfer of nine species of *Bipolaris* to *Curvularia*; the synonymy of four species; and linked the names of a further 20 species to DNA barcodes. A new monotypic genus, *Johnalcornia*, was also introduced to accommodate *Bipolaris aberrans*, as it did not belong to either *Bipolaris* or *Curvularia* as circumscribed by Manamgoda et al. (2012b). The results from Chapter 3 stabilised the genera *Bipolaris* and *Curvularia* by providing a multilocus backbone tree based on ex-type cultures. This was the basis for the analyses of unidentified *Bipolaris* and *Curvularia* isolates in Chapters 4 and 5, respectively.

In Chapter 4, 13 *Bipolaris* isolates from Australia were examined from BRIP. These had been recognised by Alcorn as taxonomically interesting and potentially distinct according to herbarium label annotations. The multilocus phylogenetic analyses supported the introduction of eight new species of *Bipolaris* (Tan et al. 2016). Species of *Bipolaris* are commonly associated with leaf spots, leaf blights and root rots on hosts in the *Poaceae* (grasses), and can also be pathogens or saprobes on hosts from *Anacardiaceae*, *Araceae*, *Euphorbiaceae*, *Fabaceae*, *Malvaceae*, *Rutaceae* and *Zingiberaceae* (Ellis 1971, Manamgoda et al. 2011, 2014). The eight species of *Bipolaris* described by Tan et al. (2016) were all associated with grasses, although it is unclear whether the fungal species were pathogens, endophytes or saprobes. Four of the species, *B. austrostipae, B. simmondsii, B. sivanesaniana*, and *B. woodii*, were described from native Australian grasses, while the other four, B. *axonopicola, B. bamagaensis, B. shoemakeri,* and *B. subramanianii*, were described from introduced grass hosts.

In Chapter 5, 17 *Curvularia* isolates were examined from BRIP. The multilocus phylogenetic analyses supported the introduction of 13 new species of *Curvularia* (Tan et al. 2018). *Curvularia* is a species-rich genus of pathogens and saprobes associated with plants and animals worldwide (Sivanesan 1987, Madrid et al. 2014, Manamgoda et al. 2015, Marin-Felix et al. 2017a, b). *Curvularia* species have also been reported from substrates including air (Almaguer et al. 2012, Hargreaves et al. 2013), aquatic environment (Verma et al. 2013, Sharma et al. 2016) and soil (Manamgoda et al. 2011, Marin-Felix et al. 2017a). Eight of the species, *C. beasleyi, C. beerburrumensis, C. eragrosticola, C. kenpeggii, C. mebaldsii, C. petersonii, C. platzii* and *C. warraberensis*, were isolated from grass hosts exotic to Australia. Only two species, *C. lamingtonensis* and *C. sporobolicola*, were described from native Australian grasses. Two species were described from hosts in other families, *C. coatesiae* from *Litchi chinensis* (Sapindaceae) and *C. colbranii* from *Crinum zeylanicum* (Amaryllidaceae). *Curvularia reesii* was described from an isolate obtained from an air sample.

The genera *Bipolaris* and *Curvularia* now comprise of 38 and 77 accepted species, respectively, which are supported by ex-type cultures and DNA barcodes (Hyde et al. 2017, Marin-Felix et al. 2017a, b, Heidari et al. 2018, Hernández-Restrepo et al. 2018, Liang et al. 2018, Tan et al. 2018). Thirty-four species of *Bipolaris* and 70 species of *Curvularia* have been reported in Australia (Fig. 1) (Sivanesan 1987, Manamgoda et al. 2014). Of these, 17 species of *Bipolaris* and 28 species of *Curvularia* were described from Australian specimens (Fig. 1, Table 1). This represents the greatest number of *Bipolaris* and *Curvularia* species described from a single country, followed by the USA (Table 1). The number of Australian taxa described may be attributed to the intense taxonomic treatment by Alcorn (Alcorn 1982a, 1982b, 1983a, 1983b,

Table 1. Accepted species of *Bipolaris* and *Curvularia,* summary of host and country of type specimens.

Taxon	Host genus (type)	*Poaceae* host subfamily	Country
Bipolaris austrostipae	*Austrostipa*	Pooideae	Australia
B. axonopicola	*Axonopus*	Panicoideae	Australia
B. bamagaensis	*Dactyloctenium*	Chloridoideae	Australia
B. bicolor	*Triticum*	Pooideae	India
B. chloridis	*Chloris*	Chloridoideae	Australia
B. clavata	*Dactyloctenium*	Chloridoideae	Australia
B. coffeana	*Coffea*		Kenya
B. cookei	*Sorghum*	Panicoideae	USA
B. crotonis	*Eleusine*	Chloridoideae	Australia
B. cynodontis	*Cynodon*	Chloridoideae	Italy
B. drechsleri	*Microstegium*	Panicoideae	USA
B. gossypina	*Gossypium*		Kenyta
B. heliconiae	*Heliconia*		Australia
B. heaveae	*Hevea*		Sri Lanka
B. leersiae	*Leersia*	Oryzoidea	USA
B. luttrellii	*Dactyloctenium*	Chloridoideae	Australia
B. microlaena	*Microlaena*	Oryzoideae	Australia
B. microstegii	*Microstegium*		USA
B. oryzae	*Oryza*	Oryzoidea	Japan
B. panici-miliacei	*Panicum*	Panicoideae	Japan
B. peregianensis	*Cynodon*	Chloridoideae	Australia
B. pluriseptata	*Eleusine*	Chloridoideae	Zambia
B. sacchari	*Saccharum*	Panicoideae	India
B. saccharicola	*Saccharum*	Panicoideae	unknown
B. salkadehensis	*Sparganium*	Orthoteliinae	Iran
B. salviniae	*Melinis*	Panicoideae	Australia
B. secalis	*Secale*	Pooideae	Argentina
B. setariae	*Setaria*	Panicoideae	Denmakr
B. shoemakeri	*Ischaemum*	Panicoideae	Australia
B. simmondsii	*Zoysia*	Chloridoideae	Australia
B. sivanesaniana	*Paspalidium*	Panicoideae	Australia
B. sorokiniana (=*B. multiformis*)	*Tribulus*		South Africa
B. stenospila	*Saccharum*	Panicoideae	USA
B. subramanianii	*Setaria*	Panicoideae	Australia
B. urochloae	*Urochloa*	Panicoideae	UK

Table 1. (Ctd).

Taxon	Host genus (type)	*Poaceae* host subfamily	Country
B. variabilis	*Pennisetum*	Panicoideae	Argentina
B. victoriae	*Avena*	Pooideae	USA
B. woodii	*Paspalidium*	Panicoideae	Australia
B. zeae	*Zea*	Panicoideae	Australia
B. zeicola	*Zea*	Panicoideae	USA
Curvularia aeria	air		Brazil
C. affinis	unknown		Indonesia
C. akaii	unknown		Japan
C. akaiiensis	unknown		India
C. alcornii	*Zea*	Panicoideae	Thailand
C. americana	*Homo*		USA
C. asiatica	*Panicum*	Panicoideae	Thailand
C. australiensis	*Oryza*	Oryzoidea	Australia
C. australis	*Sporobolus*	Chloridoideae	Australia
C. bannonii	*Jacquemontia*		USA
C. beasleyi	*Chloris*	Chloridoideae	Australia
C. beerburrumensis	*Eragrostis*	Chloridoideae	Australia
C. borreriae	unknown Poaceae		Thailand
C. bothriochloae	*Bothriochloa*	Panicoideae	Australia
C. brachyspora	soil		India
C. buchloes	*Bouteloua*	Chloridoideae	USA
C. carica-papayae	*Carica*		India
C. chiangmaiensis	*Zea*	Panicoideae	Thailand
C. chlamydospora	*Homo*		USA
C. clavata	*Tripogonis*	Chloridoideae	India
C. coatesiae	*Litchi*		Australia
C. coicis	*Coix*	Panicoideae	Japan
C. colbranii	*Crinum*		Australia
C. crustacea	*Sporobolus*	Chloridoideae	
C. cymbopogonis	*Cymbopogon*	Panicoideae	Guatemala
C. dactyloctenicola	*Dactyloctenium*	Chloridoideae	Thailand
C. dactyloctenii	*Dactyloctenium*	Chloridoideae	Australia
C. ellisii	air		Pakistan
C. eragrosticola	*Eragrostis*	Chloridoideae	Australia
C. eragrostidis	*Eragrostis*	Chloridoideae	Congo

Table 1. (Ctd).

Taxon	Host genus (type)	*Poaceae* host subfamily	Country
C. geniculata	*Eragrostis*	Chloridoideae	USA
C. gladioli	*Gladiolus*		Canada
C. graminicola	*Aristida*	Aristidoideae	Australia
C. gudauskasii	*Zea*	Panicoideae	USA
C. harveyi	*Triticum*	Pooideae	Australia
C. hawaiiensis	*Oryza*	Oryzoidea	USA
C. heteropogonicola	*Heteropogon*	Panicoideae	India
C. heteropogonis	*Heteropogon*	Panicoideae	Australia
C. hominis	*Homo*		USA
C. homomorpha	*Hordeum*	Pooideae	USA
C. inequalis	air		France
C. intermedia	*Avena*	Pooideae	USA
C. ischaemi	*Ischaemum*	Panicoideae	Solomon Islands
C. kenpeggii	*Triticum*	Pooideae	Australia
C. kusanoi	*Eragrostis*	Chloridoideae	Japan
C. lamingtonensis	*Microlaena*	Ehrhartoideae	Australia
C. lunata	*Saccharum*	Panicoideae	Indonesia
C. malina	*Zoysia*	Chloridoideae	USA
C. mebaldsii	*Cynodon*	Chloridoideae	Australia
C. micrairae	*Micraira*	Micrairoideae	Australia
C. miyakei	*Eragrostis*	Chloridoideae	Japan
C. muehlenbeckiae	*Sorghum*	Panicoideae	USA
C. neergaardii	*Oryza*	Oryzoidea	Ghana
C. neoindica	*Brassica*		India
C. nicotiae	soil		Algeria
C. nodosa	*Digitaria*	Panicoideae	Thailand
C. nodulosa	*Eleusine*	Chloridoideae	USA
C. oryzae	*Oryza*	Oryzoidea	Vietnam
C. ovariicola	*Eragrostis*	Chloridoideae	Australia
C. pallescens	air		Indonesia
C. papendorfii	*Acacia*		South Africa
C. perotidis	*Perotis*	Chloridoideae	Australia
C. petersonii	*Dactyloctenium*	Chloridoideae	Australia
C. pisi	*Pisum*	Canada	

Table 1. (Ctd).

Taxon	Host genus (type)	*Poaceae* host subfamily	Country
C. platzii	*Cenchrus*	Panicoideae	Australia
C. portulacae	*Portulaca*		USA
C. prasadii	*Jasminum*		India
C. protuberata	*Deschampsia*	Pooideae	UK
C.pseudobrachyspora	*Eleusine*		Thailand
C. pseudolunata	*Homo*		USA
C. pseudorobusta	*Homo*		USA
C. ravenelii	*Sporobolus*	Chloridoideae	Australia
C. reesii	air		Australia
C. richardiae	*Richardia*		Australia
C. robusta	*Dichanthium*	Panicoideae	USA
C. ryleyi	*Sporobolus*	Chloridoideae	Australia
C. sengalensis	unknown		Nigera
C. soli	soil		Papua New Guinea
C. sorghina	*Sorghum*	Panicoideae	Australia
C. spicifera	unknown		unknown
C. sporobolicola	*Sporobolus*	Chloridoideae	Australia
C. subpapendorfii	soil		Egypt
C. trifolii	*Trifolium*		USA
C. tripogonis	*Tripogon*	Chloridoideae	Australia
C. tropicalis	*Coffea*		India
C. tsudae	*Chloris*	Chloridoideae	Japan
C. tuberculata	*Zea*		India
C. uncinata	*Oryza*		Vietnam
C. variabilis	*Chloris*	Chloridoideae	Thailand
C. verruciformis	*Triticum*	Pooideae	India
C. verruculosa	*Cycas*		India
C. warraberensis	*Dactyloctenium*	Chloridoideae	Australia

Table compiled from Manamgoda et al. 2012, 2014, Marin-Felix et al. (2017a, 2017b), and Tan et al. 2018.

1988, 1990, 1991) and Sivanesan (Sivanesan 1985, 1987, 1992, Sivanesan et al. 2003) over decades. However, recent studies from the USA and Thailand have not uncovered as many taxonomic novelties (Manamgoda et al. 2012a, 2014, Marin-Felix et al. 2017b). Most of the *Bipolaris* and *Curvularia* species described from Australian specimens were associated with native and exotic grasses from *Chloridoideae* and *Panicoidea* (Table 1), which contains most of the grasses found in Australia (Simons and Alfonso 2011, Grass Phylogeny Working Group II 2012).

There is limited information about the host range and distribution of Australian *Bipolaris* and *Curvularia* species. Some appear to be restricted to specific hosts, such as *B. heliconiae* on *Heliconia* spp. (Alcorn 1996), and *C. micrairae* on *Micraira subulifolia* (Sivanesan et al. 2003). The former species, *B. heliconiae*, is likely introduced and established as is its host plant species, whilst the latter, *C. micrairae*, is certainly endemic. Other species found in Australia appear to be more cosmopolitan, such as *B. zeae* and *C. australiensis* (Farr and Rossman 2018). It may be that species of helminthosporioid fungi with restricted host range and distribution co-evolved with their hosts. Alternatively, these species may have been simply overlooked or misidentified because their morphological characters are indistinct. Given the unreliability of morphology as a means to identify species of *Bipolaris* and *Curvularia*, DNA barcodes linked to ex-type cultures are needed for accurate and reliable identification. Most information about the host range and distribution of *Bipolaris* and *Curvularia* species from the literature and databases should be considered doubtful unless they have been verified by molecular phylogenetic analysis. Chapters 4 and 5 uncovered novel taxa from historic collections, and it is likely that more taxonomic novelties await discovery amongst the remaining 700 unexamined specimens of *Bipolaris* and 500 of *Curvularia* in Australian plant pathology herbaria and culture collections.

The descriptions of the newly recognised helminthosporioid species from Chapters 4 and 5 provide a foundation upon which to base additional specimen sampling and accumulation of molecular data. This will improve knowledge about host ranges and the ecological roles of helminthosporioid fungi in Australia and overseas. This information will allow Australian biosecurity agencies to better assess the risk that these fungi pose to agriculture and natural ecosystems.

Botryosphaeriaceae

The *Botryosphaeriaceae* (*Dothideomycetes: Botryosphaeriales*) has received little attention from a plant biosecurity perspective in Australia and globally. Despite the on-going calls for better regulation of the international trade in live plants to prevent the inadvertent introduction of pests and pathogens (Hantula et al. 2014, Wingfield et al. 2015, Rouget et al. 2016), microorganisms hidden within plants, seeds and soil are largely ignored (Burgess et al. 2016). Recent taxonomic changes and the recognition of cryptic species have made the identification of species in the *Botryosphaeriaceae* challenging (Phillips et al. 2013, Dou et al. 2017, Slippers et al. 2017). The *Botryosphaeriaceae* comprises 24 genera of ecologically diverse fungi that occur as saprobes, endophytes or plant pathogens (Slippers et al. 2017, Yang et al. 2017). Several of these fungi are important pathogens of woody plant species, causing dieback and stem cankers, especially in the tropics and subtropics (Fig. 2f). The accurate identification of *Botryosphaeriaceae* by DNA sequence data rather than relying on morphological descriptions provides the best means to identify these fungi and thereby halt their spread into new agricultural and natural ecosystems (Burgess et al. 2016, Crous et al. 2016).

In Chapter 6, 51 isolates from *Botryosphaeriaceae* preserved in BRIP and the Victorian Plant Pathology Herbarium were examined. These isolates were collected from 1971–2017 from 35 different host genera. Prior to 2017, all of these isolates had been identified only by morphological characters. Of these isolates, 41 isolates were re-identified based on multilocus phylogenetic analyses, which demonstrated the inadequacy of morphological characters for species level identifications. Ten isolates were identified as not belonging to *Botryosphaeriaceae*, which also illustrates the difficulties faced by plant pathologists and plant diagnosticians even at the generic level. Five of the species identified represented new records for Australia, namely *Botryosphaeria sinensis*, *Diplodia alatafructa*, *Lasiodiplodia gonubiensis*, *Neofusicoccum cryptoaustrale* and *N. mangroviorum*.

Only nine genera and 64 species of *Botryosphaeriaceae* have been recorded in Australia, of which eight genera and 27 species were described from Australian specimens (Fig 1) (Dissanayake et al. 2016, Burgess et al. 2018). This leaves 24 genera that contain 195 known exotic species of cryptic *Botryosphaeriaceae* that pose a major challenge for biosecurity agencies. Several species of *Botryosphaeriaceae* can remain within host plant tissues as latent pathogens. This makes their detection particularly difficult as they can reside as benign endophytes or in localised infections for many years, until casuing serious diseases when the plants are stressed (Slippers and Wingfield 2007).

The spread of *Botryosphaeriaceae* has been facilitated in part by (i) the global demand in the trade of live plants (Liebhold et al. 2012), (ii) the introduction of infected non-native plants in novel environments (Burgess et al. 2016), and (iii) an ability to spread across a wide host range (Slippers and Wingfield 2007, Slippers et al. 2017). *Botryosphaeriaceae* are not typically transmitted by seed, but are mostly acquired from the environment as seedlings emerge (Burgess and Wingfield 2002, Bihon et al. 2011). Current biosecurity measures in Australia for the *Botryosphaeriaceae*, including the quarantine of plant material, rely on visual inspection for disease symptoms, morphological identifications and outdated taxonomy. The application of molecular-based methods for their detection will lead to better biosecurity decisions and improved border protection. Current molecular-based and data aggregation technologies already exist but are not widely used for biosecurity purposes, due to the lack of human capacity development within the biosecurity framework (Gao and Zhang 2013, Crous et al. 2016, Billones-Baaijens et al. 2018).

Conclusions and future directions

In this thesis, living cultures of isolates from four fungal groups, *Bipolaris, Curvularia, Diaporthe* and *Botryosphaeriaceae*, were examined. In each case, it was shown that many of the collection names used for these fungi were incongruent with modern taxonomy. In the case of *Bipolaris, Curvularia* and *Diaporthe*, 21 novel species were uncovered and formally described.

There are other genera of fungi that have recently been shown to contain species complexes as well as containing species of biosecurity importance to Australia. These genera include *Alternaria* (Woudenberg et al. 2013, 2014, 2015), *Cladosporium* (Bensch et al. 2010, 2012), *Pestalotiopsis* (Maharachchikumbura et al. 2014), *Phoma* (Aveskamp et al. 2010, de Gruyter et al. 2010), *Phyllosticta* (Wikee et al. 2013, Wong et al. 2013), and *Mycosphaerellaceae* (Videira

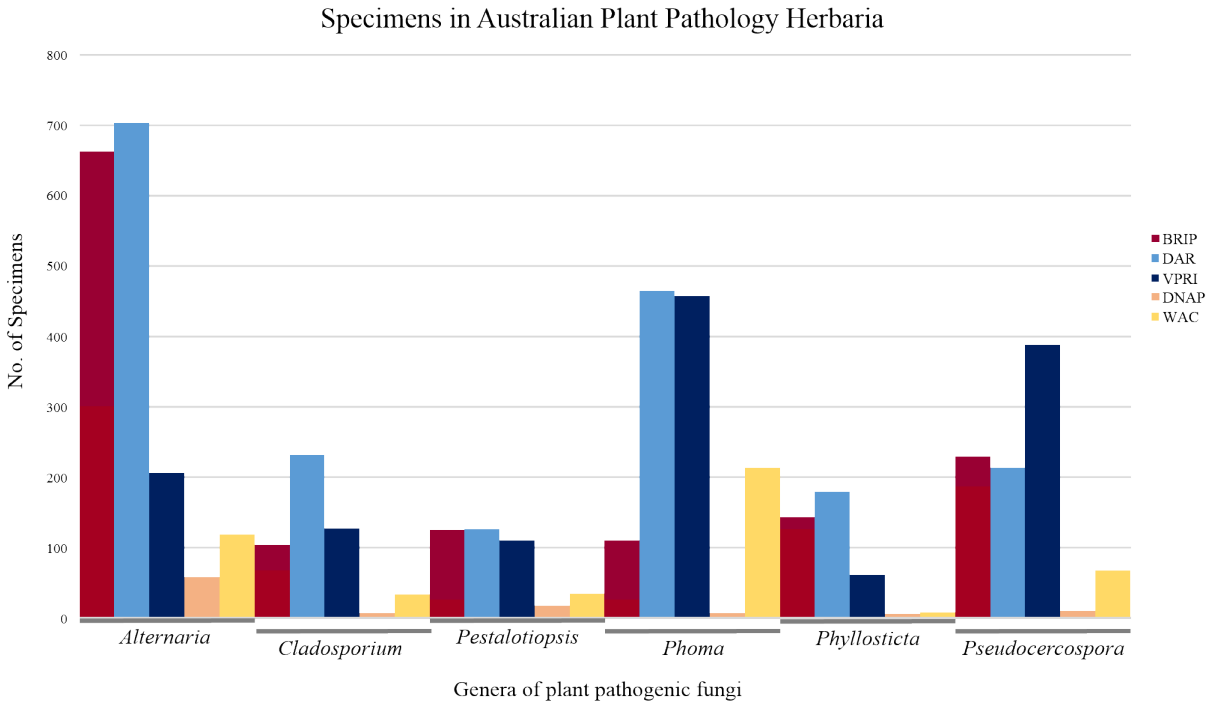


Fig. 3. Specimens held in Australian plant pathogy herbaria. This graph shows the number of specimens from six plant pathogenic genera held in five plant pathology herbaria across Australia. BRIP: Queensland Plant Pathology Herbarium, Ecosciences Precinct, Queensland; DAR: New South Wales Plant Pathology Herbarium, Orange, New South Wales; VPRI: Victorian Plant Pathology Herbarium, Bundoora, Victoria; DNAP: Northern Territoriy Plant Pathology Herbarium, Berrimah, Northern Territory; WAC: Western Australia Plant Pathology Reference Culture Collection, South Perth, Western Australia.

et al. 2017). Some of the agriculturally and environmentally important fungal species of biosecurity concern in Australia include *Alternatria humicola* on field peas, *Cladosporium allii* on onion, *Pestalotiopsis palmarum* on palms, *Phoma tracheiphila* on *Citrus* spp., *Phyllosticta cavendishii* on *Musa* spp., *Phyllosticta citrichinanensis* on *Citrus* spp., and *Pseudocercospora fijiensis* on *Musa* spp. These genera also require taxonomic reassessment in Australia, similar to the studies reported in this thesis. Most of the specimens from these genera held in the major plant pathology herbaria in Australia are unreliably identified or undetermined at the species level (Figs. 3 and 4) (Plant Health Australia 2001). It is likely that all these specimens will require re-assessment considering the recent taxonomic studies within these genera.

This thesis has provided a glimpse into the unique biodiversity of microfungi in Australia. It has demonstrated that the collections of plant pathogenic fungi held in the major Australian herbaria hold a high diversity of unique taxa at both the generic and species level. There is still a need to re-identify and taxonomically re-assess the specimens held in these collections, which are mostly unidentified, or unreliably identified, at the species level (Figs. 3 and 4). The reference specimens in these collections underpin the Australian biosecurity system, which currently relies on accurate and rapid identification of new incursions. It is only through the combination of molecular and morphological approaches that plant pathogens in Australia will be reliably identified. This in turn will preserve the effective role that biosecurity plays in keeping unwanted plant pathogens out of Australia.

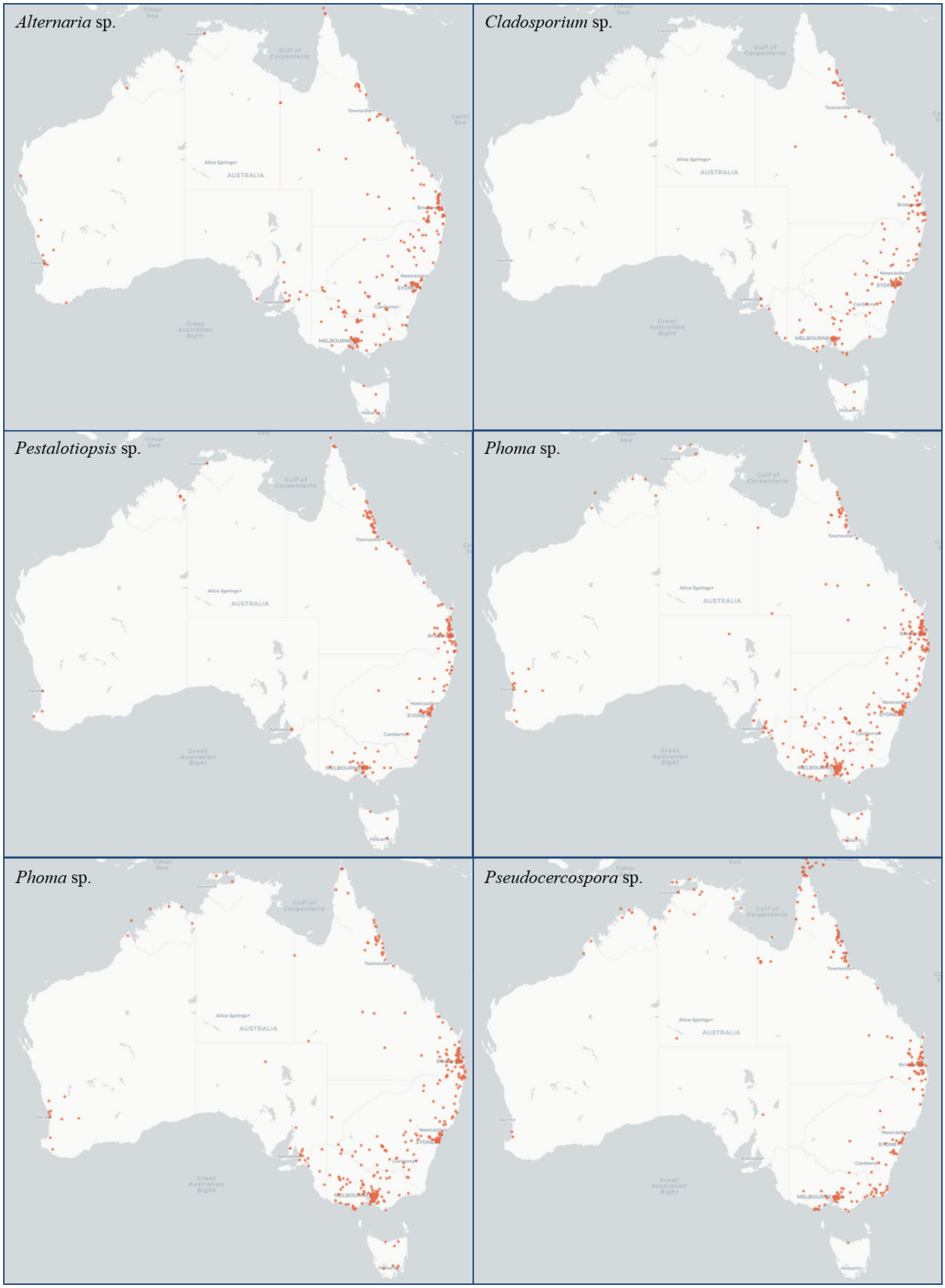


Fig. 4. Distribution of specimens held in Australian plant pathology herbaria. This figure shows the specimens that are undetermined at the species level from the five herbaria listed in Fig. 3.

The internal transcribed spacer (ITS) region of the nuclear DNA was proposed as the primary DNA barcode marker for fungi (Schoch et al. 2012). This gene region is now accepted by the mycology community as the barcode marker for most fungal species (Vu et al. 2016, 2019), with a few notable exceptions, including *Fusarium* (O'Donnell and Cigelnik 1997, O'Donnell et al. 1998) and the Uredinales (Aime 2006). For some cryptic fungal groups, such as those studied in this thesis, the ITS region is considered less than optimal for resolution of closely related species, and a combination of ITS with secondary or tertiary, 'group-specific' DNA barcodes is currently the most practical solution (Manamgoda et al. 2012b, Gomes et al. 2013, Phillips et al. 2013, Stielow et al. 2015, Marin-Felix et al. 2017a, 2019).

Alhtough fungal DNA barcodes are currently useful for species identification, the real value of these data collections will emerge once the complete genomes are obtained and analysed. Genomic data has the potential to unlock information about function, such as secondary metabolites, inference about ecological niche, the identification of pathogenicity factors, transposable elements, as well as life cycle and population structure (McTaggart et al. 2016, Aylward et al. 2017). In the future, biosecurity and plant health management systems may rely on genomic information to identify pathogens of risk rather than the names of organisms at species level (Crous et al. 2016, McTaggart et al. 2016). If the success of Australia's plant biosecurity is to continue to protect the economy and the environment from the impacts of unwanted pests and diseases, then it must cease to rely on antiquated identification methods and outdated taxonomy. Rather, it needs to employ current technologies based on modern taxonomic revisions. Furthermore, the human capcity within biosecurity structures need continual professional development to utilise this information. Above all else, for the Australian plant biosecurity system to remain effective, it must elevate its gaze to the horizon for both potential exotic invaders as well as to develop new methods in fungal classification and detection.

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APPENDIX

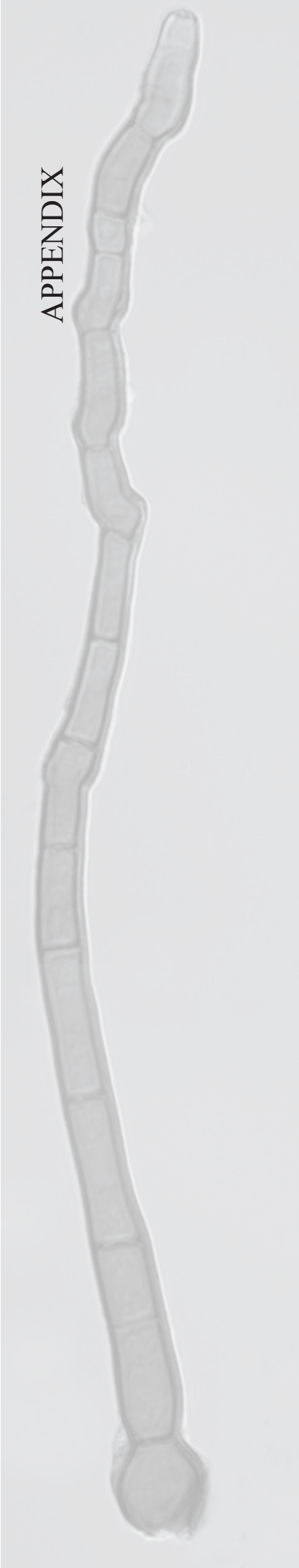
SUMMARY

SAMENVATTING

ACKNOWLEDGEMENTS

PUBLICATION LIST

CURRICULUM VITAE



Chapter one provides an insight into the multilayered Australian biosecurity system. One of the layers of biosecurity activity is the ability to successfully diagnose unwanted and threatening plant diseases. As such it relies heavily on knowing what pathogens are already present and established in Australia. Much of what is present in Australia has yet to be resolved in light of new molecular methods, particularly of important plant pathogenic fungal genera. The research presented in thesis examines the taxonomy and biodiversity of some important plant pathogenic fungi in the Queensland Plant Pathology Herbarium, which holds Australia's largest collection of fungal cultures.

In **Chapter two** of this thesis, six isolates identified as *Diaporthe* sp. (syn. *Phomopsis* sp.) from the Queensland Plant Pathology Herbarium and the Victorian Plant Pathology Herbarium were examined. The multilocus (ITS, *tef1α* and *tub2*) phylogenetic analysis revealed each of these six isolates represented a unique taxon. *Diaporthe beilharziae*, *D. fraxini-angustifoliae* and *D. litchicola*, were described on the basis of morphological and molecular characteristics. The other three species, *D. nothofagi*, *D. pascoei*, and *D. salicicola*, were sterile under the conditions that they were grown and did not produce any fruiting structures. Voucher specimens from the original collections displayed pycnidia and conidia that allowed the completion of morphological descriptions. Strong phylogenetic evidence based on multilocus sequence analyses supported the description and establishment of *D. nothofagi* as a unique taxon, despite the absence of morphological characteristics.

In **Chapter three** of this thesis, 45 isolates representing 34 species of *Bipolaris* and *Curvularia* maintained in the Queensland Plant Pathology Herbarium and the Westerdijk Fungal Biodiversity Centre were examined. The multilocus (ITS, *gapdh*, LSU, and *tef1α*) phylogenetic analysis validated the generic placement of 12 *Bipolaris* species, namely *B. chloridis, B. clavata, B. coffeana, B. crotonis, B. gossypina, B. heliconiae, B. luttrellii, B. panici-milacei, B. pluriseptata, B. salvinae, B. secalis* and *B. zeae*. The phylogenetic analysis also showed *B. eleusines sensu* Alcorn and R.G. Shivas as a synonym of *B. crotonis*, and verified Dr. John L. Alcorn's synonymy of *B. melinidis* and *Drechslera curvispora* with *B. salviniae*. Eight species of *Curvularia* were validated and supported by multilocus sequence analysis of ex-type cultures, namely *C. akaiiensis, C. bannonii, C. bothriochloae, C. harveyi, C. heteropogonicola, C. papendorfii, C. richardiae* and *C. sorghina*. Nine *Bipolaris* species were transferred to *Curvularia*, namely *C. australis, C. crustacea, C. dactyloctenii, C. homomorpha, C. neergaardii, C. nicotiae, C. portulacae, C. ryleyi* and *C. tropicalis*. To fix the application of the names, lectotypes were designated for *B. secalis* and *C. richardiae*, and an epitype was designated for *C. crustacea*. The phylogenetic tree showed that *B. aberrans* did not belong to either *Bipolaris* or *Curvularia*. Therefore, a monotypic genus *Johnalcornia*, was introduced to accommodate it.

In **Chapter four** of this thesis, 13 unidentified *Bipolaris* isolates from the Queensland Plant Pathology Herbarium were examined. These isolates, which were recognised by Dr. Alcorn as taxonomically interesting and potentially distinct, were analysed against sequences of ex-type or reference cultures available from currently accepted *Bipolaris* species based on three loci, ITS, *gapdh* and *tef1α*. The phylogenetic analyses of the combined three locus dataset resolved the 13 BRIP isolates into eight novel *Bipolaris* species. The eight *Bipolaris* species were all associated with grasses as hosts. Four of the species*, B. austrostipae, B. simmondsii, B. sivanesaniana* and *B. woodii*, were described from native Australian grasses, while the other four, *B. axonopicola*, *B. bamagaensis*, *B. shoemakeri* and *B. subramanianii*, were described

from introduced grass hosts. The description of these species provides a foundation upon which additional sampling and accumulation of molecular data will improve knowledge of their host ranges and ecological roles.

In **Chapter five** of this thesis, 17 unidentified *Curvularia* isolates from the Queensland Plant Pathology Herbarium were examined. These isolates were analysed against sequences of ex-type or reference cultures available from currently accepted *Curvularia* species based on three loci, ITS, *gapdh* and *tef1α*. The phylogenetic analyses of the combined three-locus-dataset resolved the 17 isolates into 13 novel *Curvularia* species. Eight species were described from grasses exotic to Australia, namely *C. beasleyi* on *Chloris gayana*, *C. beerburrumensis* on *Eragrostis bahiensis*, *C. eragrosticola* on *E. pilosa*, *C. kenpeggii* on *Triticum aestivum*, *C. mebaldsii* on *Cynodon dactylon* × *transvaalensis*, *C. petersonii* and *C. warraberensis* on *Dactyloctenium aegyptium* and *C. platzii* on *Cenchrus clandestinus*. Only two species were described from native Australian grasses, *C. lamingtonensis* on *Microlaena stipoides* and *C. sporobolicola* on *Sporobolus australasicus*. Two species were described from other hosts, *C. coatesiae* from *Litchi chinensis* (Sapindaceae) and *C. colbranii* from *Crinum zeylanicum* (*Amaryllidaceae*). One species, *C. reesii*, was described from an isolate obtained from an air sample. Furthermore, the phylogenetic analysis of the ex-type culture of *Drechslera boeremae* supported the transfer to *Curvularia* as a unique taxon, and rejects previous synonymy with *B. indica* based on morphology.

In **Chapter six** of this thesis, 51 isolates identified as *Botryosphaeriaceae* preserved in the Queensland Plant Pathology Herbarium and the Victorian Plant Pathology Herbarium were examined. The 51 isolates were collected between 1971 and 2017, from 35 different host genera, with the dominant host genera being *Mangifera* (11 isolates), *Acacia* (10), and *Persea* (5). Multilocus sequence analyses re-identification 41 isolates into the genera *Botryosphaeria* (2 isolates), *Diplodia* (4), *Dothiorella* (1), *Lasiodiplodia* (19), and *Neofusicoccum* (15). Ten isolates that had been identified as *Botryosphaeria*, *Diplodia* and *Fusicoccum* were identified as not belonging to *Botryosphaeriaceae*, which illustrated the difficultures faced by plant pathologists and plant diagnosticians even at the generic level. Five species are reported for the first time in Australia, namely *Botryosphaeria sinensis*, *Diplodia alatafructa*, *Lasiodiplodia gonubiensis*, *Neofusicoccum cryptoaustrale*, and *N. mangroviorum*. New plant host associations are reported for 14 species, namely *B. sinensis, D. africana, D. alatafructa, D. seriata, L. brasiliensis, L. gonubiensis, L. iraniensis, L. mahajangana, N. australe, N. cryptoaustrale, N. mangroviorum, N. occulatum, N. parvum* and *N. vitifusiforme*. Furthermore, the multilocus phylogenetic tree revealed a distinct taxon in each of the genera *Botryosphaeria*, *Dothiorella* and *Lasiodiplodia*. These isolates remained undescribed as further DNA sequences were required to support their introduction as novel species. The results of this study provide fundamental information regarding the diversity of *Botryosphaeriaceae* species present in Australia.

Hoofdstuk één geeft inzicht in het meerlagige Australische bioveiligheidssysteem. Een van de lagen van activiteit binnen dat bioveiligheidssysteem is het vermogen om met succes een diagnose te stellen van ongewenste en bedreigende plantenziekten. Hierin is het sterk afhankelijk van de kennis van welke ziekteverwekkers reeds aanwezig en gevestigd zijn in Australië. Over veel van wat in Australië aanwezig is, moet nog uitsluitsel worden gegeven in het licht van nieuwe moleculaire methoden, met name van belangrijke plantpathogene schimmelsoorten. Dit proefschrift heeft getracht de taxonomie en biodiversiteit te beoordelen van culturen van enkele van deze belangrijke plantenpathogene schimmels van Queensland Plant Pathology Herbarium, de grootste verzameling van fytopathogene schimmels in Australië.

In **Hoofdstuk twee** van dit proefschrift worden zes isolaten, geïdentificeerd als *Diaporthe* sp. (syn. *Phomopsis* sp.) door het Queensland Plant Pathology Herbarium en het Victorian Plant Pathology Herbarium, onderzocht. Uit de multilocus (ITS, *tef1α* en *tub2*) fylogenetische analyse bleek dat elk van deze zes isolaten een uniek taxon vertegenwoordigde. *Diaporthe beilharziae*, *D. fraxini-angustifoliae* en *D. litchicola*, werden beschreven op basis van morfologische en moleculaire kenmerken. De andere drie soorten, *D. nothofagi*, *D. pascoei* en *D. salicicola*, waren steriel onder de omstandigheden waarin ze werden gekweekt en ze produceerden geen vruchtstructuren. Voucher exemplaren uit de originele collecties vertoonden pycnidia en conidia waarmee de voltooiing van morfologische beschrijvingen mogelijk was. Sterk fylogenetisch bewijs, gebaseerd op multilocus sequentie-analyses, ondersteunde de beschrijving en de vaststelling van *D. nothofagi* als een uniek taxon, ondanks de afwezigheid van morfologische kenmerken.

In **Hoofdstuk drie** van dit proefschrift werden 45 isolaten onderzocht die 34 soorten *Bipolaris* en *Curvularia* vertegenwoordigen die werden bewaard in het Queensland Plant Pathology Herbarium en het Westerdijk Fungal Biodiversity Centre. De multilocus (ITS, *gapdh*, LSU en *tef1α*) fylogenetische analyse valideerde de generieke plaatsing van 12 *Bipolaris* soorten, namelijk *B. chloridis*, *B. clavata, B. coffeana, B. crotonis, B. gossypina, B. heliconiae, B. luttrellii, B. panici-milacei, B. pluriseptata, B. salvinae, B. secalis* en *B. zeae*. De fylogenetische analyse toonde ook aan dat *B. eleusines* sensu Alcorn en R.G. Shivas synoniem zijn voor *B. crotonis*, en dat dr. John L. Alcorn's synonymie van *B. melinidis* en *Drechslera curvispora* met *B. salviniae* klopte. Acht soorten *Curvularia* werden gevalideerd en ondersteund door multilocus sequentie-analyse van ex-type culturen, namelijk *C. akaiiensis, C. bannonii, C. bothriochloae, C. harveyi, C. heteropogonicola, C. papendorfii, C. richardiae* en *C. sorghina*. Negen *Bipolaris*-soorten werden overgebracht naar *Curvularia*, namelijk *C. australis, C. crustacea, C. dactyloctenii, C. homomorpha, C. neergaardii, C. nicotiae, C. portulacae, C. ryleyi* en *C. tropicalis*. Om de toepassing van de namen definitief te maken, werden lectotypes aangewezen voor *B. secalis* en *C. richardiae*, en een epitype werd aangewezen voor *C. crustacea*. De fylogenetische boom liet zien dat *B. aberrans* niet tot *Bipolaris* noch tot *Curvularia* behoorde. Daarom is een monotypisch geslacht geïntroduceerd, *Johnalcornia*, om het een plaats te geven.

In **Hoofdstuk vier** van dit proefschrift werden 13 ongeïdentificeerde *Bipolaris*-isolaten van het Queensland Plant Pathology Herbarium onderzocht. Deze isolaten, die door dr. Alcorn als taxonomisch interessant en potentieel afwijkend werden aangemerkt, werden geanalyseerd tegen sequenties van ex-type of referentiekweken die beschikbaar zijn van de momenteel geaccepteerde *Bipolaris*-soorten op basis van drie loci, ITS, *gapdh* en *tef1α*. De fylogenetische analyses van de gecombineerde drie locus-gegevensverzamelingen plaatsten de 13 BRIP-isolaten

in acht nieuwe *Bipolaris*-soorten. De acht *Bipolaris*-soorten werden allemaal geassocieerd met grassen als gastheren. Vier van de soorten, *B. austrostipae, B. simmondsii, B. sivanesaniana* en *B. woodii*, werden beschreven van inheemse Australische grassen, terwijl de andere vier, *B. axonopicola, B. bamagaensis, B. shoemakeri* en *B. subramanianii*, werden beschreven van geïntroduceerde grasgastheren. De beschrijving van deze soorten vormt een basis waarmee, met aanvullende monstering en accumulatie van moleculaire gegevens, de kennis over de verscheidenheid van gastheren en ecologische rollen verbeterd kan worden.

In **Hoofdstuk vijf** van dit proefschrift werden 17 ongeïdentificeerde *Curvularia*-isolaten uit het Queensland Plant Pathology Herbarium onderzocht. Er zijn isolaten geanalyseerd tegen sequenties van ex-type of referentiekweken verkrijgbaar bij momenteel geaccepteerde Curvularia-soorten op basis van drie loci, ITS, *gapdh* en *tef1α*. De fylogenetische analyses van de gecombineerde drie locus-datasets plaatsten de 17 isolaten in 13 nieuwe *Curvularia*-soorten. Acht soorten werden beschreven van in Australië uitheemse grassen, namelijk *C. beasley* op *Chloris gayana*, *C. beerburrumensis* op *Eragrostis bahiensis, C. eragrosticola* op *E. pilosa, C. kenpeggii* op *Triticum aestivum, C. mebaldsii* op *Cynodon dactylon* × *transvaalensis, C petersonii* en *C. warraberensis* op *Dactyloctenium aegyptium*, en *C. platzii* op *Cenchrus clandestinus*. Slechts twee soorten werden beschreven van inheemse Australische grassen, *C. lamingtonensis* op *Microlaena stipoides* en *C. sporobolicola* op *Sporobolus australasicus*. Twee soorten werden beschreven van andere gastheren, *C. coatesiae* van *Litchi chinensis* (Sapindaceae) en *C. colbranii* van *Crinum zeylanicum* (Amaryllidaceae). Eén soort, *C. reesii*, werd beschreven uit een isolaat verkregen uit een luchtmonster. Bovendien ondersteunde de fylogenetische analyse van de ex-type cultuur van *Drechslera boeremae* de overdracht naar *Curvularia* als een uniek taxon en verwierp de vorige synonymie met *B. indica* op basis van morfologie.

In **Hoofdstuk zes** van dit proefschrift werden 51 isolaten, geïdentificeerd als *Botryosphaeriaceae* en geconserveerd in het Queensland Plant Pathology Herbarium en het Victorian Plant Pathology Herbarium, onderzocht. De 51 isolaten werden tussen 1971 en 2017 verzameld uit 35 verschillende gastheersoorten, waarbij de dominante gastheersoorten *Mangifera* (11 isolaten), *Acacia* (10) en *Persea* (5) waren. De multilocus-sequentie analyses heridentificeren 41 isolaten naar de genera *Botryosphaeria* (2 isolaten), *Diplodia* (4), *Dothiorella* (1), *Lasiodiplodia* (19) en *Neofusicoccum* (15). Tien isolaten die waren geïdentificeerd als *Botryosphaeria*, *Diplodia* en *Fusicoccum* werden geïdentificeerd als niet behorend tot *Botryosphaeriaceae*, wat de moeilijkheden illustreert waarmee plantenpathologen en plantendiagnostici geconfronteerd worden, zelfs op generiek niveau. Vijf soorten worden voor de eerste keer in Australië gemeld, namelijk *Botryosphaeria sinensis*, *Diplodia alatafructa, Lasiodiplodia gonubiensis, Neofusicoccum cryptoaustrale* en *N. mangroviorum*. Nieuwe plant-gastheer-associaties worden gerapporteerd voor 14 soorten, namelijk *B. sinensis, D. africana, D. alatafructa, D. seriata, L. brasiliensis, L. gonubiensis, L. iraniensis, L. mahajangana, N. australe, N. cryptoaustrale , N. mangroviorum, N. occulatum, N. parvum* en *N. vitifusiforme*. Bovendien onthulde de multilocus fylogenetische boom een duidelijk separaat taxon in elk van de genera *Botryosphaeria, Dothiorella* en *Lasiodiplodia*. Deze isolaten blijven vooralsnog onbeschreven omdat verdere DNA-sequenties nodig zijn om hun introductie als nieuwe soort te ondersteunen. De resultaten van deze studie bieden fundamentele informatie over de diversiteit van *Botryosphaeriaceae* soorten aanwezig in Australië.

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Tan YP. Powdery mildew – experience from an amateur. Powdery Mildew Workshop, 11–15 June 2018, Toowoomba, Queensland, Australia. (Oral presentation).

Tan YP. Diversity in *Austropuccinia psidii*. Cryptic Speciation in Classifications, 1 September 2017, Utrecht, The Netherlands. (Oral presentation).

Tan YP. Downy mildew in Germany. Annual Diagnosticians Workshop, 20–21 March 2018, Adelaide, South Australia, Australia. (Oral presentation).

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Tan YP. Rice blast in Western Australia. Annual Diagnosticians Workshop, 12–14 February 2014, Melbourne, Victoria, Australia. (Oral presentation).

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Yu Pei Tan was born on the island nation of Singapore, on the 16th of December 1980. In November 1989, together with her parents and sister, she migrated to Australia, which she has called home ever since. She graduated from the Queensland University of Technology (QUT) with the dual degree of Bachelor of Laws (Honours) and Bachelor of Applied Science (Distinction in Biotechnology) in 2003, and with the Bachelor of Applied Science (Honours) in 2004. After graduation, she worked with Dr. Mark S. Turner at QUT, where she also completed a Masters degree in molecular biology and microbiology. Since 2008, she has worked at the Department of Agriculture and Fisheries with Dr. Roger G. Shivas. The content of this thesis is based on the research she conducted on specimens deposited in the Queensland Plant Pathology Herbarium. This thesis was presented to the University of Utrecht for the fulfilment of the qualifications of a Ph.D. degree in 2019.

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