

CHAPTER 7

GENERAL DISCUSSION

This thesis emphasises the phylogenetic utility of DNA sequencing data in fungal identification, resolving their taxonomy and DNA phylogeny. I investigated the fungal diversity on *Eucalyptus*, the host range and estimated distribution of these fungi based on findings reported from literature, and also commented on their potential in host-changing ability. *Mycosphaerella* species and related anamorphs on *Eucalyptus* leaves collected from plantations in Thailand were identified and described (Chapter 3). With the use of DNA sequencing techniques, I resolved the taxonomy and phylogenetic relationships among *Cryptosporiopsis eucalypti*-like fungi associated with *Eucalyptus* in both tropical and temperate regions (Chapter 4). Likewise, by comparing the sequences of ITS and LSU, the taxonomic status of the genus *Heteroconium* and other similar fungi on *Eucalyptus* was assessed (Chapters 5). The descriptions of new and interesting fungal taxa are reported in Chapter 6.

7.1 Diversity of fungi on *Eucalyptus*

Eucalyptus species are particularly abundant and have a wider range of distribution than other myrtaceous genera. Myrtaceae contains a range of substrates and oils that support a highly diverse fungal community, making them favourable hosts to numerous plant pathogenic and saprobic fungi. The high biological diversity of *Eucalyptus* is also thought to be related to high fungal diversity (May and Simpson, 1997). Estimates of fungal biodiversity on *Eucalyptus* are not available; however the expected ratio of number of fungal species to their vascular plant host is thought to be approximately 10:1 (Pascoe, 1990; Harksworth, 1991). If there are about 800 species of *Eucalyptus* species (Brooker, 2000; Williams and Brooker, 1997), there are at least 8000 species of fungi hosted by them. Based on findings from previous studies and the number of fungi found in this study, it is evident that these fungal genera are able to colonize a variety of hosts.

Despite the fact that more than 1400 species of fungi are associated with 150 *Eucalyptus* species, many of them have not been studied (Hyde *et al.*, 2007). Some genera contain a large number of taxa because species concepts in these genera were previously based on host identity and morphological characteristics of the fungi (e.g. Sutton, 1971a, 1974, 1975, 1980; Swart, 1982a–b, 1986a–c, 1988; Crous, 1998). According to the estimated fungal number, it was expected that more currently undiscovered fungal species and interactions between fungi and *Eucalyptus* host will be documented when more collections, culture, and DNA sequencing data of fungal taxa are gathered. Since 1995, more than 150 new fungal taxa from *Eucalyptus* were reported from various studies. This number includes different groups such as saprobic fungi, plant pathogenic fungi and endophytic fungi (e.g. Crous *et al.*, 2006f, 2007b,f; Gryzenhout *et al.*, 2006b; Summerell *et al.*, 2006; Cheewangkoon *et al.*, 2008, 2009; Taylor *et al.*, 2009; van Wyk *et al.*, 2009; Andjic *et al.*, 2010; Aveskamp *et al.*, 2010; Lombard *et al.*, 2010c)

By studying 39 fungal species from 25 different genera, findings from the present research also agreed with the expected high fungal diversity on *Eucalyptus*. Some pathogenic fungi that identified in this study have also been encountered as saprobes. They include species of *Alysidiella*, *Antennariella*, *Bagadiella*, *Cladoriell*, *Cryptosporiopsis*, *Cyphellophora*, *Elsinoë*, *Foliocryphia*, *Leptoxyphium*, *Mycosphaerella*, *Neofabraea*, *Parasymphodiella*, *Penidiella*, *Pseudocercospora*, *Pseudoplagiostoma*, *Polyscytalum*, *Pseudoramichloridium*, *Quambalaria*, *Selenophoma*, *Sphaceloma*, *Strelitziana*, *Zeloasperisporium*, *Blastacervulus*, *Minimedusa*, and *Sydowia*. The result also clarified their geographical distributions and host specificity. Based on these findings it is clear that further extensive research should be carried out to provide information concerning the fungal diversity and the effective strategies for biodiversity conservation.

7.2 Molecular techniques for fungal studies

Recent molecular techniques allow mycologists to distinguish these fungi and the various morphs in their lifecycles, and thus allow more precise taxonomic classifications (Hawksworth, 2004; Crous and Groenewald, 2005; Seifert, 2009). Even though cryptic

species are similar in morphology, they can be differentiated by the molecular phylogenetic approaches (Crous *et al.*, 2001b, 2004d; Alves *et al.*, 2008).

This study has demonstrated that morphological characters and molecular techniques are complementary, and necessary, to uncover the diversity and geographical range of fungi on *Eucalyptus*. Molecular techniques using ITS rDNA, small and large subunit rDNA, β -tubulin gene and histone gene sequences have been established and are now widely used to clarify fungal taxonomy and classification (Hunter *et al.*, 2006; Sung *et al.*, 2007; Roonay and Ward, 2005; Alamouti *et al.*, 2009; Crous *et al.*, 2009a). In some species complexes, it is necessary to use sequence data from multiple genes to analyze their phylogenetic relationships and delineate their speciation history, as I have shown in the studies of *Mycosphaerella* (Chapter 3) and *Pseudoplagiostoma* species (Chapter 4). Molecular techniques are not only able to reflect the complexity of fungal taxonomy, compare genetic materials, and investigate the relationship between taxa, but also allow re-classification and clarification of phylogenetic relationships between anamorphic and teleomorphic states.

Most species of plant-associated fungi are predominantly asexual or have no sexual state. Traditionally, they have been classified in artificial groups such as the hyphomycetes and coelomycetes based on the conidial production and the appearance of fruiting body. Knowledge of the relationship between asexual and sexual fungi may assist in uncovering the sexual states of plant pathogenic fungi, thereby provides insights in their life history. For many asexual fungi, a sexual state may not exist, but findings of phylogenetic analysis allow plant pathologists to make predictions about the biology, in particular pathogenicity, of these species (Rossman and Palm-Hernández, 2008). Such studies that have proved anamorph and teleomorph relationships have been carried out by numerous researchers (Câmara *et al.*, 2001; Chaverri *et al.*, 2001; Geiser *et al.*, 2001; Réblová and Winka, 2001; Rossman *et al.*, 2001; Smith and Stanosz, 2001; Anderson and Shearer, 2002; Chou and Wu, 2002; Lui *et al.*, 2002; Zhang and Blackwell, 2002; Ban *et al.*, 2009). The study of *Pseudoplagiostoma* species in Chapter 4 is a good example for

employing molecular approaches to determine relationships between defined asexual fungi and sexual groups.

7.3 *Eucalyptus* fungal specificity

Findings from literature suggested the host ranges of fungal genera on *Eucalyptus* are highly variable, with some genera being reported to occur on one to more than 20 different plant families such as *Selenophoma* (Park and Sprague, 1953; Sutton, 1980; Crous *et al.*, 2000b). However, certain species (e.g. *Antennulariella* and *Leptoxyphium* species) can colonize on surfaces of various living plants families but do not interact directly with their hosts (Adhikari, 1990; Singh and Rawat, 1990). Only few fungal species have shown the ability to undergo host-shifting and/or host-jumping. Myrtaceae, and particularly *Eucalyptus*, support a diverse fungal genera (Sankaran *et al.*, 2005). After host-shifting/jumping, a particular fungal species might progressively become adapted and specialised to its new host, leading to further speciation (Brasier, 2000; Roy, 2001; Munday *et al.*, 2004; Giraud, 2006; Faucci *et al.*, 2007).

There are examples of host-specificity concerning the plant pathogenic fungi. *Mycosphaerella* and associated anamorphs from *Eucalyptus* appeared to be highly specific to this host (Crous, 1998; Crous *et al.*, 2004c–d, 2006g; Summerell *et al.*, 2006). During this period, 20 new species of *Calonectria* (*Cylindrocladium*) were described and all seemed to be limited to *Eucalyptus* (Crous, 2002; Crous *et al.*, 2004e, 2006c). Similarly, most fungal species studied in this research appeared to be host-specific. For example, *Pseudoplagiostoma* species were found only on *Eucalyptus* (Chapter 4). Also in Chapter 6, *Quambalaria* species appeared to be restricted to *Eucalyptus* and *Corymbia* (*Myrtaceae*) (Simpson, 2000; de Beer *et al.*, 2006; Langrell *et al.*, 2008; Paap *et al.*, 2008; Pegg *et al.*, 2008; Zhou *et al.*, 2008).

7.4 Fungi in exotic plantations

Several studies have provided evidence that introduced *Eucalyptus* species and native Myrtaceae trees can harbour the same pathogens, the results raise concern about

the host specialisation of those pathogens (Coutinho *et al.*, 1998; Pavlic *et al.*, 2007). Most of the leaf pathogens recorded from exotic plantations either were introduced from Australia or have wide host range (Dick, 1982; Lundquist and Baxter, 1985; Crous *et al.*, 1989). The negative impact of host-jumping events in plant pathology has been well documented and many examples have been frequently cited in the literature (Anderson *et al.*, 2004; Slippers *et al.*, 2005; Woolhouse *et al.*, 2005; Desprez-Loustau *et al.*, 2007). Host-jumping can be in both directions, from native hosts to introduced plant species and vice versa (Milgroom *et al.*, 1996; Coutinho *et al.*, 1998).

This study described the diversity and geographical range of fungal species occurring on *Eucalyptus* and some other Myrtaceae trees in various countries. Although many new species have been identified and some species are even new records, it is still unknown whether these species are native or exotic in those countries. It is therefore possible that when members of Myrtaceae are introduced into a new habitat outside their natural range, the fungi with potentially high host-shifting or jumping ability can colonize Myrtaceae plantations from surrounding native plant hosts. On the other hand, if all those fungi are naturally occurring on Myrtaceae, these plantations could act as sources of fungi that can shift or jump to other, nearby native plants. I expected that more unidentified disease-causing microfungi await discovery in exotic plantations, because the expanding area of *Eucalyptus* plantations allow fungal pathogens to cross geographical barriers to infect new hosts more easily, and also increase the chance of infection by native fungi to the exotic plantations (Strauss, 2001; Slippers *et al.*, 2005). Further in-depth studies are urgently called for, as these data could hold serious consequences not only for import and export of agricultural and forestry products, but also promote the understanding of the evolution of new pathogens and the movement of fungi between continents.

7.5 Suggestions and comments

Molecular techniques have been proved useful to assist in the differentiation and identification of fungal taxa in genera and species complexes, and determine species

boundaries and relationships between taxa. In the present study, such techniques were particularly important and they have been successfully applied for fungal identification and determining the phylogenetic relationships of various *Eucalyptus* microfungi such as species of *Alysidiella*, *Mycosphaerella* and related anamorphs, and *Pseudoplagiostoma*. In order to verify the identities of fungal species in complexes on host, multiple gene regions should be employed to generate sufficient data to increase analytical resolution for the comparisons and distinguishing the individual species. This approach will also aid in identifying cryptic fungi.

One problem regarding phylogenetic study on uncultured fungi (Chapter 6) is the reliability and sufficiency of fungal materials. It is essential to ensure that the right and uncontaminated material is used for sequencing, or otherwise incorrect DNA sequences and thus misleading molecular conclusions will be resulted. Findings from most experiments undertaken in this study suggested that increasing the number of samples would have been better to determine the fungal distribution and the variations within fungal populations.

Information of biodiversity and geographic distribution of fungi on *Eucalyptus* species is still incomplete. More extensive research and systematic survey should be carried out to provide information concerning the fungal diversity on Myrtaceae, particularly *Eucalyptus*, in native forests and exotic plantations. Also, processes of fungal evolution and adaptation are still unclear. Dept-studies in population genetics of fungi which are potentially pathogenic would be required, to further our understanding of their genetic movement, co-evolution with their hosts, adaptation and host-specificity.