CHAPTER 1

GENERAL INTRODUCTION

1.1 Fungal definition and importance

Fungi are eukaryote organisms that are essential members in the ecological system. They are decomposers of organic matter (Georgieva *et al*., 2005), parasites or pathogens of plants and animals, and symbionts with algae or higher plants. They have been classified and represented their own kingdom, which is separated from plants, animals and protozoa (Deacon, 2006; James *et al*., 2006; Stephenson, 2010). Apart from playing an integral role in ecosystem processes, fungi are also economically important. Some can be valuable food sources and contain specific biochemical compounds for manufacturing biotechnological, pharmaceutical, and health care products (Alexopoulos *et al*., 1996; Hyde, 1997); while others are able to limit plant and animal production, and are agents responsible for food spoilage and biodeterioration (Alexopoulos *et al*., 1996; Dighton, 2003; Agrios, 2005).

The number of fungal diversity studies is increasing and it is estimated that 1000–1200 new fungal species have been found and described annually (Hawksworth, 2001). The world's total number of fungal species was estimated to be 1.5–9.9 million (Hawksworth, 1991; Cannon, 1997). Yet, it is still not appropriate to conclude which estimate is the most realistic, particularly when the estimations were made based on incomplete data. Having a stable and accepted estimate of taxonomic diversity for fungi is necessary to enable them to be included in considerations of biodiversity conservation, land-use planning and management, and other related aspects. However, there have been few attempted to compile available information of fungal diversity or to use these data to carefully estimate global diversity for fungi.

1.2 Principle, theory, and rationale

Fungi are probably one of the most important, but also the most poorly studied groups of organisms. Even from the most conservative estimation, it was suggested there are 1.5 million fungal species (Hawksworth, 2001). This was derived from the assumption that there are approximately six fungal species uniquely associating to each vascular plant species (which was roughly estimated to be 250000). This assumption needs to be verified and tested in a range of ecosystems and habitats.

Eucalyptus (Myrtaceae) trees are widespread in tropical and temperate regions of the Southern Hemisphere (Turnbull, 2000). They are particularly abundant and have a wider distribution than other myrtaceous genera, as they are frequently grown as exotics in commercial plantations (Ball, 1995). Most species are known to host a considerable number of incredibly diverse and inconspicuous microfungi among which it was suggested a large proportion are almost not studied or even totally unknown.

There have been numerous publications listing and describing the plant-pathogenic fungi occurring on *Eucalyptus* in both tropical and temperate countries where these trees are grown as ornamentals, or established in plantations for timber and paper fiber; and showing these fungi substantially decreased the timber yield (Park *et al*., 2000, Old *et al*., 2003). In contrast, the saprobic microfungi have been largely neglected. Among the available checklists and descriptions, only a few are in fact known from culture or are represented in accessible culture collections.

Certain pathogenic (Gryzenhout *et al*., 2004, 2006; de Beer *et al*., 2006) and saprobic fungi (Gryzenhout *et al*., 2005; Crous *et al*., 2006a) of *Eucalyptus* can be host-specific. Based on the limited available information, they may only be able to grow and propagate on a few subgenera or species. On this foundation the current study further investigated the diversity of fungi on *Eucalyptus*, elucidated their taxonomy and resolved their phylogenetic relationships, and determined the host range and the degree of host-specificity of these fungi. The findings would provide deeper insights into the evolution of fungal species and the movement of plant

pathogenic fungi, as exotic plantations are becoming more common worldwide that may eventually assist the fungi to cross geographical barriers and establish in new ecosystems or habitats. The research outputs would also provide useful information for managing import and export of agricultural and forestry products, and for devising effective strategies for biodiversity conservation.

1.3 Research objectives

1) To investigate the fungal diversity on *Eucalyptus* in Thailand and on other selected herbarium collections, and to determine the degree of host-specificity of these fungi.
2) To examine the morphology and the phylogeny of selected groups of fungal taxa associated with *Eucalyptus*.
3) To revise the taxonomic status of the above selected fungal genera based on morphological and molecular investigations together with findings available from the literature.
4) To describe and document the newly found and previously unstudied fungal species on *Eucalyptus*.
5) To resolve and clarify the phylogenetic relationships and anamorph-teleomorph connections at the family level among *Eucalyptus*-associated fungi of which the taxonomic status is largely unknown.

1.4 Usefulness of the Research (Theoretical and/or Applied)

1) Knowledge of the ecology and biodiversity of *Eucalyptus* microfungi in Thailand which have not been studied before. This research provided fundamental biological and ecological information of these microfungi which would be the foundation for studying the diseases of *Eucalyptus* and possibly other plants.
2) Knowledge of the genetic relationships of selected *Eucalyptus* microfungi based on molecular techniques. This study would be an example to demonstrate the application of molecular techniques for studying plant-associated microfungi or

even non-fungal pathogens which can potentially reduce crop and/or plantation yields.

3) Discovery of new species of fungi on *Eucalyptus* which has been described and published in international journals. This gave rise to an updated and relatively complete checklist of the fungal species inhabiting on *Eucalyptus*. The checklist could be potentially used for a more accurate estimate of the world's total fungal diversity in the future.
4) Discovery of anamorph and teleomorph connections of some fungi on *Eucalyptus* which has been published in international journals. This discovery illustrated the weaknesses of the traditional classification system which mostly based on morphological characters. It is demonstrated how to resolve confusing phylogenetic relationships and clarify the taxonomic status of microfungi based on the concurrent use of morphological and molecular approaches.

1.5 Research content

The research presented in this thesis provided various taxonomic aspects of *Eucalyptus* microfungi with emphases on the relationship between fungi occurring on exotic *Eucalyptus* and those occurring on other Myrtaceae. Symptomatic leaf materials from worldwide collections were analyzed in the laboratory, from which several endophytes and lesion-associated fungi were isolated and studied, and the findings are reported in the following chapters of this thesis.

Plant pathogenic microfungi associated with *Eucalyptus* species can substantially decrease timber yield. In particular, species of *Mycosphaerella* (Mycosphaerellaceae) and *Teratosphaeria* (Teratosphaeriaceae) have proven to be serious pathogens. To date, more than 90 species of Mycosphaerellaceae residing in *Mycosphaerella*, *Teratosphaeria*, and several anamorph genera (of which the teleomorphs are unknown) have been recorded on *Eucalyptus.* Prior to the present study, five Mycosphaerellaceae species had been reported on *Eucalyptus* in Thailand associated with leaf and stem canker diseases. In **Chapter 3** a diverse group of Mycosphaerellaceae species occurring in *Eucalyptus* plantations from Thailand is

reported. This group includes five new species among which one is a new record for this country, while others have expanded host ranges and geographic distribution.

Cryptosporiopsis eucalypti is a common foliicolous pathogen of *Eucalyptus* species that occurs over a wide geographical range varying from dry to very humid zones. Taxonomy of this fungus became confusing as the type species of *Cryptosporiopsis* was found to contain the phylogenetically unrelated *C. eucalypti*. In **Chapter 4**, phylogenetic relationships among isolates of *C. eucalypti* and related genera were examined through the analysis of multi-gene sequence data. The degree of congruence between morphological and molecular classification schemes was evaluated.

Heteroconium-like species, which are associated with Chocolate Spot leaf disease of *Eucalyptus*, resemble *Heteroconium* s.str. in morphology but differ in their ecology and phylogenetic relationships. In **Chapter 5**, DNA form the holotype of *Heteroconium*, i.e. *H. citharexyli*, was extracted and studied for clarifying the taxonomic status of *Heteroconium*. Morphology in conidiogenous cells, ecological information, and molecular data between *Heteroconium* and other fungi causing Chocolate Spot leaf disease were also compared.

Chapter 6 illustrates the morphological descriptions of a group of new and inconspicuous fungi collected from *Eucalyptus* leaves worldwide. This group includes twenty six fungal species belonging to 22 different genera. The final chapter provides a general discussion of the findings of the thesis and suggestions for future work.