

CHAPTER 9

CONCLUSIONS

9.1. Findings of This Study

Biodiversity and Taxonomy

A total 181 species of 128 fungal genera associated with 19 species of palms were examined and identified include 89 Ascomycetes, 23 Coelomycetes, 65 Hyphomycetes, 2 Basidiomycetes and 2 Zygomycetes. Twenty-four species are new to science in which 7 species have been validly published or are in the process of publication. Sixty-two species are new records to Thailand. The most common families found include Botryosphaeriaceae, Mycosphaerellaceae, Xylariaceae, Pleosporaceae and Amphisphaeriaceae. *Anthostomella*, *Astrosphaeriella* and *Oxydothis* appeared as common genera on palms. The result has also shown that the fungal diversity on a single palm, *W. siamensis*, were also high as a total 92 species were found from this native palm.

The new species found in this study are listed as follows: On *Areca catechu*:

Astrosphaeriella palmicola, *Cercospora arecacearum*, *Linocarpon nonappendiculatum*, *Mycosphaerella arecacearum*, *Venturia frondicola*; ***Arenga hookeriana***: *Guignardia arengae*; ***Borassus flabellifer***: *Mycosphaerella borassi*, *Oedocephalum formosus*; ***Caryota mitis***: *Astrosphaeriella caryotae*, *Fasciatispora ujungkulonensis*; ***Chamaedorea metallica***: *Fusarium sansainensis*; ***Cyrtostachys renda***: *Oxydothis cyrtostachicola*; ***Livistona chinensis***: *Lophiostoma livistonicola*, *Terriera livistoniae*; ***Rhapis* sp.**: *Apiosporina rhapsicola*; ***Wallichia siamensis***:

Apiospora siamicola, *Arecomyces foliicola*, *Dictyochaeta wallichianensis*,
Guignardia uniappendiculatum, *Mycosphaerella wallichiae*, *Oxydothis inaequalis*,
Oxydothis wallichianensis, *Rachidicola obclavatum*, *Submersisphaeria suthepensis*.

Phylogenetic

The phylogenetic relationships of *Oxydothis*, a common genus associated with palms, and its familial placement remain obscure based on the 28S nrDNA sequence analyses (chapter 3). Large sub unit nrDNA (28S) genes sequences did not provide significant phylogenetic information concerning the evolutionary relationships of these xylariaceous fungi. However, ITS nrDNA sequence analyses indicated that *Oxydothis* was more closely related to members of the Amphisphaeriaceae than Diatrypaceae or Xylariaceae. Unfortunately, it was still doubtful to accommodate the members of *Oxydothis* within this family as the morphology characteristics of *Oxydothis* were apparently distinct from the family Amphisphaeriaceae. On the other hand, whether *Oxydothis* should be accommodated in a new family will definitely need further detailed study with more species (including those from *Pemphidium*, *Leiosphaerella* and *Iodosphaeria*) and sequence analyses based on protein-coding genes. Single gene phylogeny based on nrDNA was insufficient and inefficient in resolving phylogenetic relationships within the Xylariales.

In addition, the phylogenetic analysis of ITS nrDNA region confirmed and supported the proposal of three new species found in this study, namely *Cercospora arecacearum*, *Fusarium sansainensis* and *Dictyochaeta wallichianensis*. Based on this analysis, *C. arecacearum* nested together with other *Cercospora* species to form a monophyletic clade with 95% bootstrap support (chapter 4). *Fusarium sansainensis*

nested together with members of genus *Fusarium* to form a monophyletic clade (63% Bootstrap support) and indicated anamorph-teleomorph connection with *Nectria* and *Neocosmospora* teleomorphs. On the other hand, *D. wallichianensis* showed a close relationship with *Dictyochaetopsis gonytrichoides*, and linked with two teleomorphic states, namely, *Chaetosphaeria* and *Striatospora* (chapter 5).

Ecology

Fungal community composition on *W. siamensis* indicated the dominance of ascomycetous taxa in diversity and abundance (chapter 6). It was strongly supported by the appearance of three ascomycetous fungal species, namely, *Morenoina palmicola*, *Lophiostoma macrostomum* and *Ophioceras tenuisporum* as indicator species. Of them, *Morenoina palmicola* and *Lophiostoma macrostomum* were also recognized as common taxa associated with palms and commonly found on various palm species distributed in Australia, Brunei Darussalam, Ecuador and Hong Kong (Fröhlich and Hyde, 2000). *Ophioceras tenuisporum* was commonly reported as saprobic on decaying woody substrates in freshwater habitat, however, it was found as an endophyte in this study. According to this result, therefore, the nature of this species is probably endophytic to woody substrates of plants tissue, and finally become saprobes after the death of their hosts. It was also shown by the fact that *O. tenuisporum* restricted to the woody tissues (primary rachis and secondary rachis) in which they have been endophytes.

The present study also showed the specificity of palmicolous fungi to different tissue types (pinna, primary rachis and secondary rachis) (chapter 6). It was supported by *t*-test analysis of which the difference between fungal communities on pinna-

primary rachis, pinna-secondary rachis and primary-secondary rachis were not because of chance, but could be attributed to the specificity of nutrients available and physical structures of each tissue in supporting distinct fungal communities.

Only fungal communities on primary rachis and secondary rachis were sufficient to be examined and analyzed as leaflet/pinna of *W. siamensis* were completely decomposed after one year incubation in nature (chapter 7). Space, in particular vertical spatial distribution, was more valuable than tissue types in fungal community-host ecology studies. It was probably because of different space possesses unique properties where new variations of life can exist and thrive due to the unique conditions offers, such as temperatures, humidity, sunlight intensity, vegetation shading and other environmental factors.

Hyphomycetous fungi were very dominant on palm tissue when the specimens were incubated using moist chamber method combined with surface disinfection technique in laboratory scale (chapter 8). This pattern is common in investigation of fungal diversity/species richness using culturing on artificial medium, and probably is unique to endophyte studies. It is probably because of hyphomycetous fungi are fast growing, short-lived and capable of rapid and wide dispersal at the early stage of decomposition (pioneer community) (Dix and Webster, 1985; Gessner *et al.*, 1993).

Xylariaceous fungi make up only a small proportion which is contradicting with the most endophytic studies using culturing method that suggested the dominance of xylariaceous fungi in samples from tropical and temperate regions (Rodrigues and Samuels, 1990; Rodrigues, 1994; Rodrigues and Petrini, 1997; Taylor *et al.*, 1999; Fröhlich and Hyde, 2000). The difference of fungal composition structure in this study with other previous studies could probably be attributed to the difference in

experimental design employed and specificity of the fungi. It can be concluded that various methods and a given substratum affected extremely to the fungal community resulted in the biodiversity and ecology studies.

9.2. Problems Encountered

Culturing some of host-specific palmicolous fungi such as *Fasciatispora*, *Linocarpon*, *Neolinocarpon*, *Oxydothis*, *Pemphidium*, *Myelosperma* and *Rachidicola* has been accepted as a difficult task and problematic in the study of taxonomy and phylogenetic of palmicolous fungi, even though many successful results from direct sequencing of natural samples has recently been reported (Adair *et al.*, 2002; Cohen, 2005). As a consequence, there is a limited sequence data resource in genetic database such as NCBI (National Center for Biotechnology Information) and DDBJ (DNA Data Bank of Japan). Therefore, it is urgent and necessary to develop various techniques in culturing hitherto unculturable palmicolous fungi taxa genera, as well as improving direct sequencing technique in order to reveal the phylogenetic relationship and evolution of this fungal group.

A fundamental problem of the ecology study was the lack of methodology to obtain better estimates of the fungal communities. Even though molecular technologies are now available and more develop, however, the limitation research budgets, complicated procedures, the fact that the techniques are still in their early development stages and limited sequences being available, impede their application (Guo, 1999).

9.3. Future Perspectives and Recommendations

This study has demonstrated a huge fungal diversity on palms and confirmed the importance of detailed sampling and methods in ecological studies. Palmicolous fungal community on *W. siamensis* has exposed specificity to different tissues. It was probably related to the difference in nutrients available and physical structures of each tissue type. Therefore, monitoring a fluctuation of dry mass, N and P concentration, as well as structural plant polymers during early death and decay would be required for further studies. Fungal communities successional also change over time, and therefore, the relationship analysis between succession of fungal community and host (nutrients availability and physical structures) during decomposition processes is another exciting subject to explore.

Molecular work seems essential to elaborate the understanding of fungal ecology in the environment. Thus, it will be probably the best tool to combine direct observation, plating technique and molecular analysis to explore fungal biodiversity pattern and an ecological significance of fungal community interactions with their host.

Finally, in regard to serious habitat destruction and environmental issues which threatens the survival of indigenous and endemic species in most of tropical countries, therefore, this thesis remarked that biodiversity assessment, collection and conservation management of fungi in tropical area are crucial, yet there is little information concerning its amount and potential. Mycologists and plant pathologists are recommended to store their fungi collected as dried material in herbaria and living cultures in culture collections for both, as references and as the genetic resources for potential exploitation in the future.