

CHAPTER 1

INTRODUCTION

1.1. Research Rationale

‘Where all of the undescribed fungi?’ wherein only 7% of the world’s fungi have so far been described of the 1.5 million of Hawksworth’s estimation in which one third of the estimation are plant pathogenic fungi (Hawksworth, 1991; 2004). Fungi also comprise about 95% of all known plant pathogens, the remainder being bacteria, mycoplasma-like organisms and viruses (Shivas and Hyde, 1997). Rossman *et al.* (1987), from *Literature Guide for the Identification of Plant Pathogenic Fungi*, estimated the number of plant pathogenic fungi about 23,000, however, their estimation includes only those species that cause disease in vascular plants. In tropical area, moreover, Shivas and Hyde (1997) estimated about 270,000 of plant pathogenic fungi may present based on four parameters: (1) three-quarters of plant genera known occur in the tropics, (2) plant genera have an average about 50 fungal pathogens, (3) one-half of the pathogens on a host are specific to the host genus or to closely related genera and (4) few fungal pathogens have a wide host range. Although there were wide variations about the estimation of fungi including plant pathogenic fungi occur worldwide, tropical regions were generally assumed to host the greatest diversity of life and plant pathogens. It does not take into account epiphytic, lichen forming, lichenicolous mycoparasitic and mycorrhiza.

Ironically, high population and rapid industrialization in most of the tropical countries present serious habitat destruction and environmental issues include large-

scale illegal deforestation, over-exploitation of marine resources and other environmental problems of which finally threatens the survival of indigenous and endemic species, including fungi. Therefore, biodiversity assessment, collection and conservation management in tropical area are crucial, yet there is little information concerning its amount and distribution worldwide.

Lost of plant diversity, particularly endemic plants such as Thai Dwarf Fishtail Palm (*Wallichia siamensis*) (fig. 1.1), ecologically will also have a great impact to related organisms that harm them, such as saprophytic and pathogenic fungi due to they are generally thought to have co-evolution with their hosts (Pirozynski, 1988). Considering that two-thirds of the world's flowering plants occur in the tropics (Heywood, 1985), therefore, the enormous diversity of plant species in the tropics will undoubtedly support an equally diverse flora of fungi. If the Hawksworth's estimation (1991) is accurate, probably about 500,000 plant pathogenic fungi distributed worldwide. The prospect of fungi awaiting discovery should excite mycologists and plant pathologists for recent and future generations due to habitat destruction and species extinction rates are accelerating in almost all ecosystems in tropical and temperate areas. The tragedy is that many of these species will become extinct, most anonymously, due to the destruction of tropical habitats (Campbell, 1989) must be prevented immediately.



In addition, Colwell and Coddington (1994) also pointed out that biological diversity must be treated more seriously as a global resource, to be indexed, used and above all, preserved. Management of biological diversity, particularly fungi, is a serious task due to the fact that fungal diversity is a source and foundation for

biotechnology, including biocontrol in pest management, biotreatment for environmental degradation and pollution, new product discovery and genetic manipulation for new commercial products. Therefore, we need to assess, collect, identify, name and maintain fungal diversity before it disappears.

‘Why palms?’ wherein many other interesting groups of plants, such as medicinal plants or interesting crops, are more fascinated to be studied. Palms are ancient, woody, monocotyledonous plants in the order Arecales and the family Palmae (Arecaceae). Palms are considered as the first monocotyledonous and semi aquatic plants that were present in early Cretaceous, about 100 million years ago (Uhl and Dransfield, 1987). They are found throughout the tropics and subtropics area but absent from desert and semidesert except where groundwater is near the surface, only a very few occur in temperate regions (Uhl and Dransfield, 1987). In fungal diversity point of view, palmicolous fungi are considered as a unique group of fungi due to many fungal genera associated with palms are specific to the host or rarely to be found associated with other plants. Many novel species were also recorded associated with palms in the tropical area (Taylor and Hyde, 2003). Until 1994, *ca* 1580 fungi were described as new species recorded from palms; however, the fungi mostly described from temperate area. This included *ca* 650 Ascomycetes, 270 Basidiomycetes, 400 Hyphomycetes and 260 Coelomycetes. Fröhlich and Hyde (2000) reported approximately four new genera and 79 more novel species collected from palms since 1994, including two new genera and 11 new species associated with leaf spot of palms (Fröhlich, 1992; Fröhlich and Hyde, 1994, 1995a,b; Hyde and Fröhlich, 1995). Yanna (2001) reported 306 taxa associated with eight species of palms and reported 13 new species and four new genera. Taylor and Hyde (2003)

added approximately 37 new species and one new genus from their total 288 species found in their research, and also noted that 26 species of palmicolous fungi showed host-species specificity.

In Thailand, studies on palm microfungi diversity have significantly been raised up since 1996 or over the past twelve years (Aramsiriujwet, 1996; Hyde *et al.*, 2002; McKenzie *et al.*, 2002; Pinnoi *et al.*, 2004, 2006; Pinruan *et al.*, 2002, 2004, 2007, 2008; Sarapat, 2003). The research mostly covered diversity at specific areas such as palms on peat swamp, freshwater to brackish area and small parts in terrestrial ecosystem. Aramsiriujwet (1996) reported 29 hyphomycetes from seven species palms at peat swamp ecosystem, namely, *Borassus flabellifer*, *Caryota* sp., *Cocos nucifera*, *Cyrtostachys lakka*, *Corypha lecomtei*, *Elaeis guineensis* and *Roystonea regia*. Pinnoi *et al.* (2004) also reported about 53 new species found on four species palms, viz, *Eleiodoxa conferta*, *Nenga pumila*, *Licuala longicalycata* and *Metroxylon sagus*. In the ecological aspect, Pinnoi *et al.* (2004) noted that palm microfungi on the peat swamp ecosystem were significantly distinct from those on terrestrial habitat. This statement supported the previous finding published by Taylor and Hyde (2003) that species distribution of palm microfungi on their host was not affected by host relatedness (e.g. those in the same taxa level), but rather by host proximity (e.g. occurring in the same location). Even though a significant progress has been achieved on many aspects of this group of fungi including biodiversity and ecology, however, several important ecological aspects such as fungal recurrence on different microhabitats and fungal community variation among individual tree of palms in the same area have not been extensively studied and considered in the estimation of global fungal numbers. It is believed that such ecological studies would increase the

possible numbers of fungi and also have important implications to the diversity estimation.

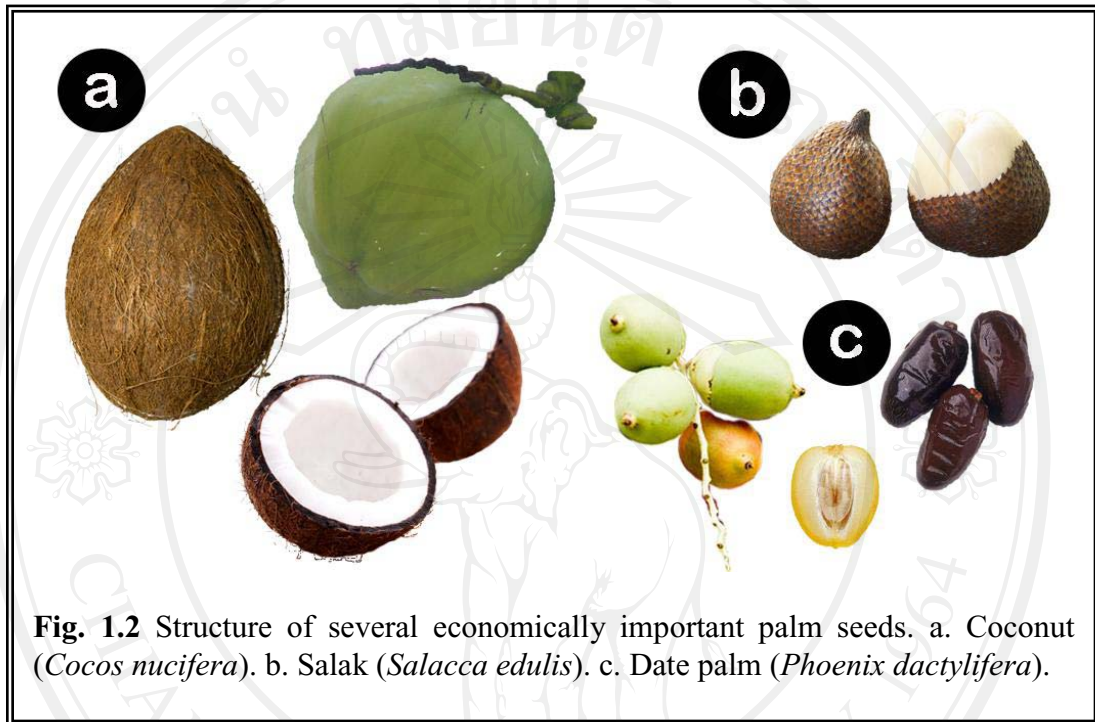
Due to most reports and publications of palmicolous fungi in Thailand were carried out in the southern part of Thailand, and focused only on the saprophytic palmicolous fungi in the non-terrestrial areas, therefore, broader aspect on the palmicolous fungi research which includes endophytic or pathogenic fungi are needed to carry out, particularly in northern part of Thailand, wherein many terrestrial palms such as Thai Dwarf Fishtail Palms are distributed in the highland forest. In addition, the reports and publications on palmicolous fungi in this area are also lacking.

1.2. Overview of the Host Plant: Palms (Arecaceae)

1.2.1. The Importance of Palms

Palms are ancient, woody, monocotyledonous plants in the order Arecales and the family Palmae (or alternatively Arecaceae). They are found throughout the tropics and subtropics areas but absent from desert and semidesert except where groundwater is near the surface, only a very few occur in temperate region (Uhl and Dransfield, 1987). Palm taxa show a high degree of endemism and many subfamilies, tribes, subtribes and even genera show very restricted distributions (Uhl and Dransfield, 1987). This may, in part, be due to the large, heavy seeds of most palms that are generally less well suited to dispersal over long distances than smaller and lighter seeds (Dransfield, 1981a) (fig. 1.2). In most palm species, the seed viability of ripe fruit generally decreases faster than other plants. Palm seed cannot be dried and kept under low temperature conditions because the embryo is killed. This factor is a severe

limitation to conservation, and therefore, palms can only be conserved as living specimens.



Palms are one of the most well-known and extensively cultivated plant families. They have had an important role to humans throughout much of history.

Many common products and foods are derived from palms such as various furniture from rattans, oil palm, coconut oil and its derivatives products, sago, alcoholic beverages, wine, etc., and palms are also widely used in landscaping for their exotic appearance, making them one of the most economically important plants. In many historical cultures, palms were also symbols for such ideas as victory, peace and fertility.

Unfortunately, like many other plants, palms have been threatened by human intervention and exploitation. The greatest risk to palms is destruction of habitat,

especially in the tropical forests, due to urbanization, wood-chipping, mining and conversion to farmland. Palms rarely reproduce after such great changes in the habitat, and palms with a small habitat range are most vulnerable to them. For example, the use of rattan palms in furniture has caused a major population decrease in these species that has negatively affected local and international markets as well as biodiversity in the area. The sale of seeds to nurseries and collectors is another threat, and the seeds of popular palms are sometimes harvested directly from the wild. At least 100 palm species are endangered (Johnson, 1986), and nine species have reportedly become extinct (Jones, 1995), namely, *Sabal miamiensis* from Florida, *Roystonea stellata* from Cuba, *Pritchardia macrocarpa* and *P. montis-kea* from Hawaii, *Syagrus leptospatha* and *Syagrus macrocarpa* from Brazil, *Syagrus lilliputiana* from Paraguay, *Thrinax ekmaniana* from Cuba and *Medemia argun* from oases in Egypt and Sudan.

Unfortunately, several ex-situ conservation areas such as botanic garden are very limited role with regard to palm conservation. As far as conservation is concerned, two major obstacles exist with respect to botanic gardens. First, the gardens can only grow a few specimens of any given palm species. They could not, therefore, represent the range of characteristics found in the wild necessary to maintain biological diversity. Second, in many cases, palm species within the same genus tend to cross (hybridize) when grown in close proximity. This means that palm seed from a botanic garden may well be hybrid seed, which is of little conservation value.

Several palm taxa show a high degree of endemism and very restricted distributions (Uhl and Dransfield, 1987), e.g. Thai Dwarf Fishtail Palm, and it is

believed that such native plant has grown alongside the native insects, fungi, plant diseases and other native organisms for thousands of years (Pirozynski, 1988). This long-time association has produced a complex web of interrelationships, by which the native plants may depend upon numerous other native organisms to survive and flourish, and a multitude of native organisms may, in turn, depend upon those native plants to survive. In the process, native plants have evolved the ability to attract native animals that benefit them (e.g. pollinating and seed-dispersing insects and birds) and survive native organisms that harm them (e.g. plant pathogenic fungi). A unique symbiotic association of fungi with native plants could assist plants in nutrition and forming a protective shield against extreme environmental conditions. Hyde *et al.* (2007) also remarked that fungi, at some degrees, showed specificity with their hosts.

1.2.2. Morphological Structure

The structure of palms is basically the same as that of other flowering plants in that they are composed of roots, trunk(s), leaves, flowers and fruit. Most species have a prominent solitary trunk with a crown of leaves at its top, but few have multiple stems, climbing stems, underground trunks or no trunk (Jones, 1984). Each stem or trunk has a single growing point (Tomlinson, 1964). The Pandanaceae are the most similar family to the Palmae in overall structure, differing chiefly in their ability to reproduce aerial, vegetative branches (Tomlinson, 1964). Both families produce only primary tissues (Tomlinson, 1964).

Palm leaves may be scattered along the upper part of the trunk (e.g. *Calamus* and *Daemonorops*), but usually they are borne at the top in a crown (as in *Jessenia*).

Young leaves are called spear leaves as they usually arise vertically from the meristem. The basal woody structure that extends from the trunk to the first leaflet or segment is called the petiole. The petiole base, known as the sheathing base, leaf base or leaf sheath is expanded and clasps the trunk (fig. 1.3) (Jones, 1984).

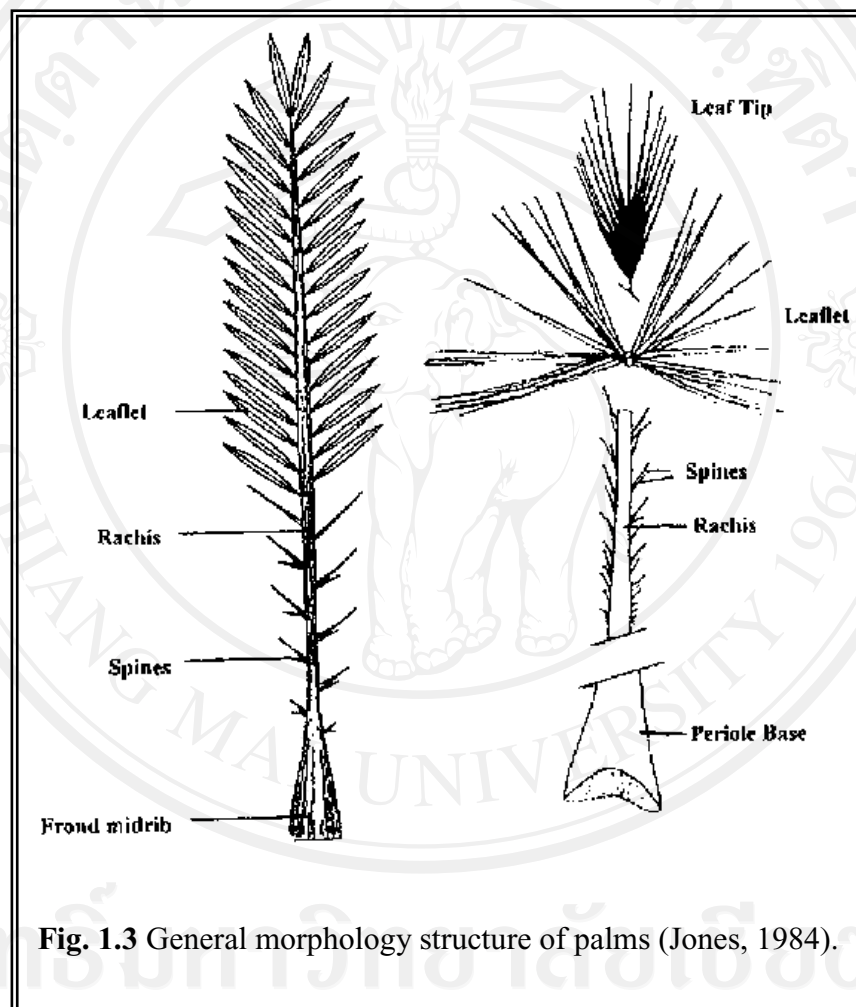


Fig. 1.3 General morphology structure of palms (Jones, 1984).

The frond may be palmate (fig. 1.4a), pinnate, (fig. 1.4c), bipinnate (fig. 1.4d), or entire (fig. 1.4b). In a pinnate leaf the segments are called pinnae or leaflets and the continuation of the petiole to which they are attached is called the rachis. Fan-leafed palms, such as *Licuala*, *Livistona* and *Mauritia* species, have rounded frond lamina divided into segments. Palm 'wood' consists of primary tissues that originate from the

growing tip. They lack the ring-like meristem (cambium) which produces secondary tissue in the stem of woody dicotyledonous plants (Uhl and Dransfield, 1987). Vascular bundles are scattered in a parenchymatous ground tissue, as are two kinds of fibres: (1) vascular fibres, which are associated with xylem and phloem bundles, and (2) non-vascular fibres, which are not. The latter may occur singly or in bundles (Uhl and Dransfield, 1987). The character of the ground tissue varies between taxa. The parenchyma cells may be sclerotic or lignified with few intercellular spaces or thin-walled with large intercellular spaces (Uhl and Dransfield, 1987).

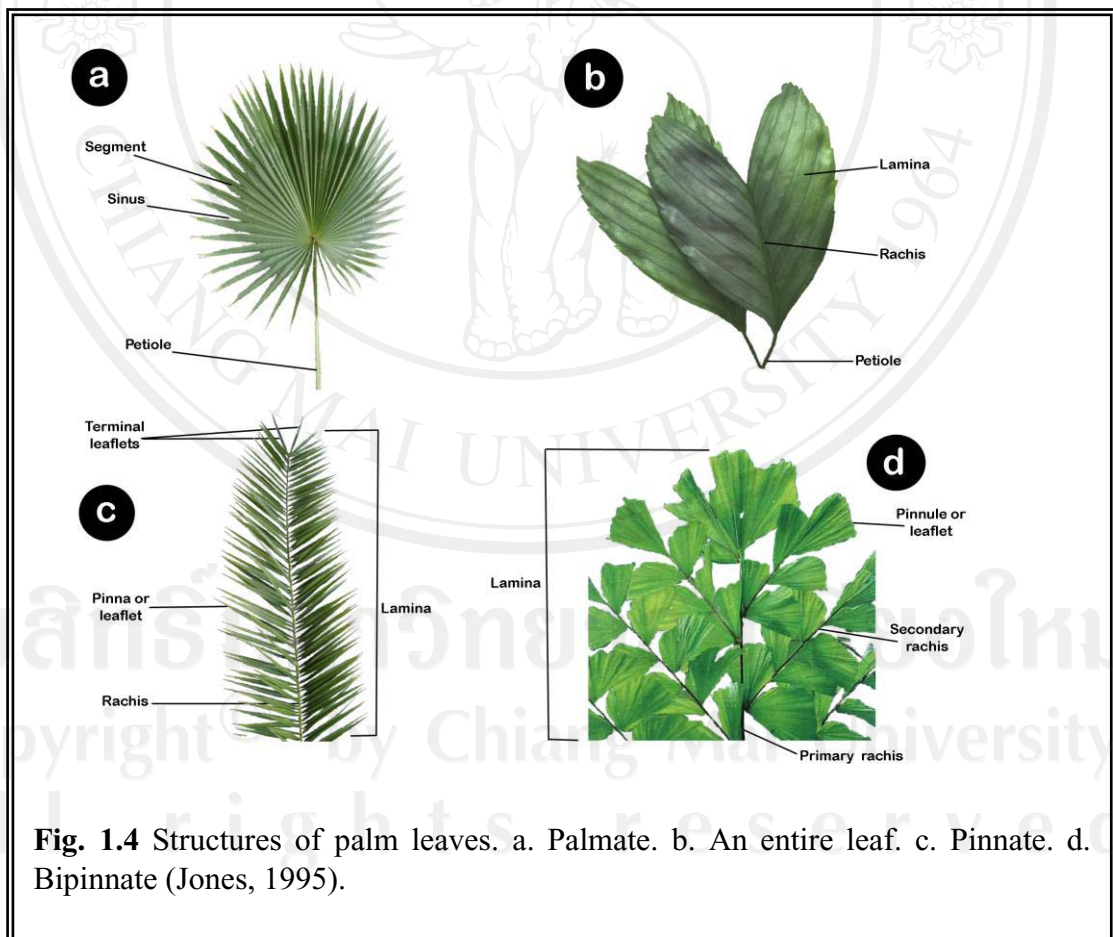
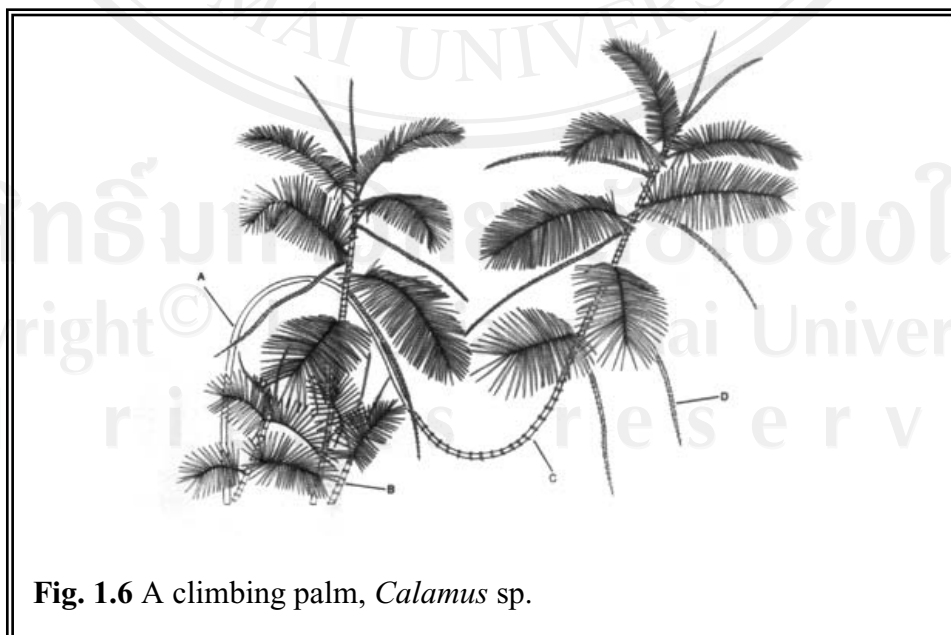
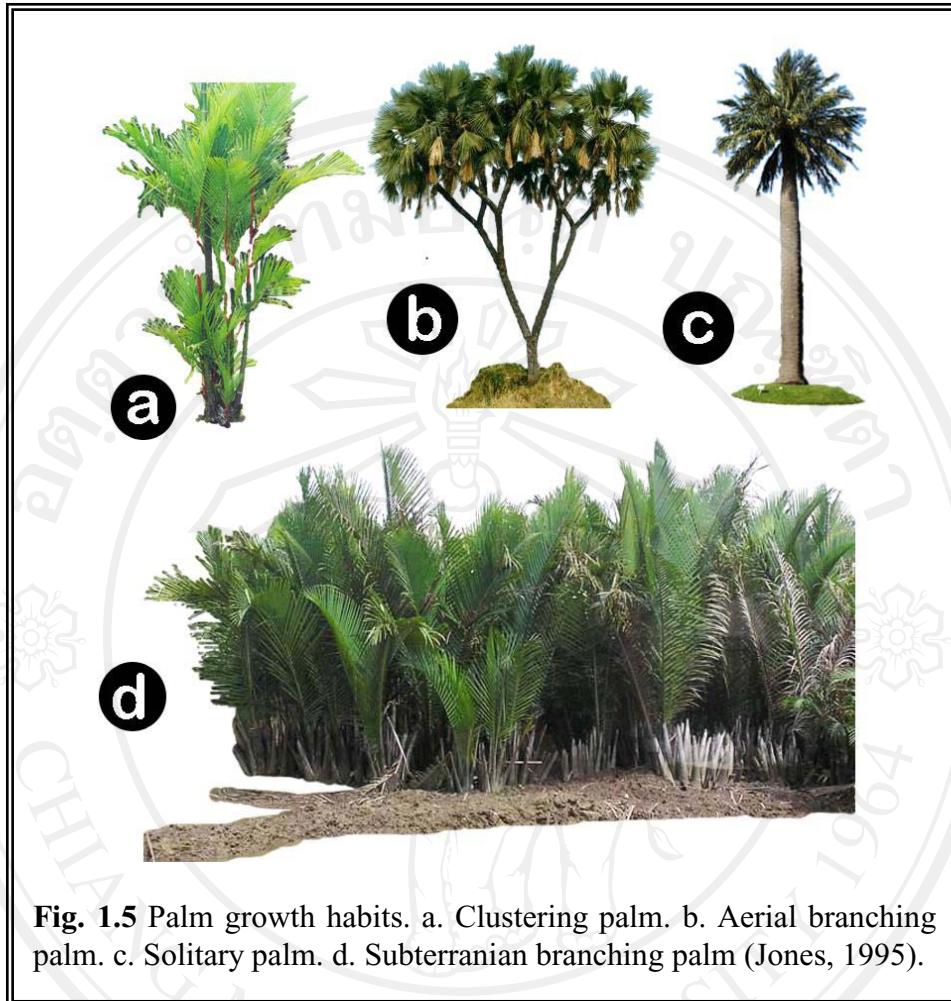


Fig. 1.4 Structures of palm leaves. a. Palmate. b. An entire leaf. c. Pinnate. d. Bipinnate (Jones, 1995).

Most palm stems have a narrow outer cortex around the inner zone containing the vascular bundles. The vascular strands and fibers are distributed dissimilarly in the inner zones of different palm taxa. For example, in the Coryphoidae and most climbing palms, the vascular bundles are distributed evenly. In some other taxa (e.g. *Mauritia*) the peripheral vascular bundles are closer together and may be surrounded by more sclerenchyma, forming a stem with a soft centre and an extremely tough or hard periphery (Uhl and Dransfield, 1987). This arrangement ensures that such stems are both strong and flexible, a useful combination to withstand high bending stresses exerted by typhoons and cyclones, frequent phenomena in parts of the tropics (Jones, 1984). The hardness of palm stems and fronds is in some cases also attributable to the presence of silica bodies in specialized cells called stegmata that are associated with vascular or non-vascular fibers (Tomlinson, 1964).

The growth habit of palms can be classified into several types: (1) solitary palms (fig. 1.5c), the single-stemmed growth habit is very common and is characteristic of many of the palms cultivated such as *Jubaea chilensis*; (2) clustering palms (fig. 1.5a), multiple-stemmed palms are also quite common such as genus *Cyrtostachys renda*; (3) aerial branching palms (fig. 1.5b), aerial branching in palms is unusual and only found naturally in species of the genus *Hyphaene thebaica*; (4) subterranean branching palms (fig. 1.5d), for example Nipa palm (*Nypa fruticans*); (5) climbing palms (fig. 1.6), one of the most noteworthy is the genus *Calamus*, source of nearly all commercial rattan. The majority of climbing palms are also clumping palms, sending out new shoots from the root system.



The vascular bundles of palms, as in other monocotyledonous plants, are arranged in parallel lines. As the vascular bundles of palms are usually heavily lignified and tough, this arrangement appears to affect the development and final appearance of some fungus fructifications (Hyde and Cannon, 1999). Stromata, ascomata and conidiomata of palmicolous fungi tend to be elongated, so as to fill the tissue between veins rather than growing over or through them (Hyde and Cannon, 1999). Fructifications are frequently either erumpent, or inserted between the very outer layers of the host tissue. More deeply immersed ascomata can often be seen to develop between, and have their walls distorted by, vascular bundles. The toughness and vein arrangement of the palm tissues may also influence the range of taxa that utilize them. For example, species in the genus *Phyllachora* are usually found on tissue with low levels of structural components such as leaf lamina. Relatively few species of *Phyllachora* are found in the Palmae compared to other host families, and the species that do occur on palms are limited to tissues with small amounts of lignifications (Hyde and Cannon, 1999). The morphological and anatomical structures of palms affect definitely the taxa compositions that colonize the palms fronds.

1.2.3. Classification, Biogeography and Evolution

The palm family (Arecaceae or Palmae) classification is resolved as a monophyletic group in all higher-level molecular studies of monocots (Asmussen and Chase, 2001). During the last 10 years, substantial progress has been made in the understanding of the relationships within the family. Many estimates of palm phylogeny have been published at various taxonomic levels. Nevertheless, numerous ambiguities have persisted, hindering any attempt to rearrange the formal

classification of the family. According to Uhl and Dransfield (1987), palms are classified as follow:

Kingdom: Plantae

Division: Angiosperms (the flowering plants)

Class: Liliopsida (monocotyledons)

Subclass: Arecidae

Superorder: Areciforae

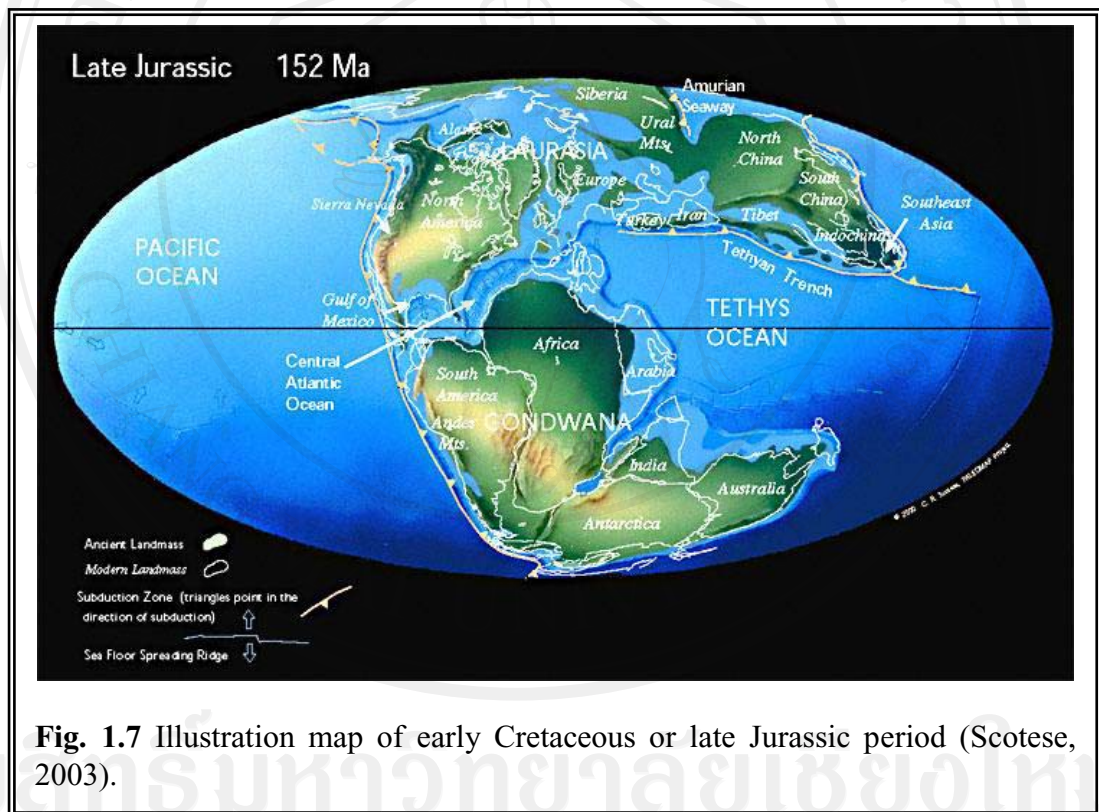
Order: Arecales (contains only one family)

Family: Palmae (Arecaceae)

According to the recent molecular phylogenetic analysis, Dransfield *et al.* (2005) divided family Arecaceae into five subfamilies: Arecoideae (112 genera), Ceroxyoideae (8 genera), Coryphoideae (45 genera), Nypoideae (1 genus) and Calamoideae (21 genera). This study provides part of the justification for a forthcoming new classification of palms based on phylogenetic data (Dransfield *et al.*, 2005).

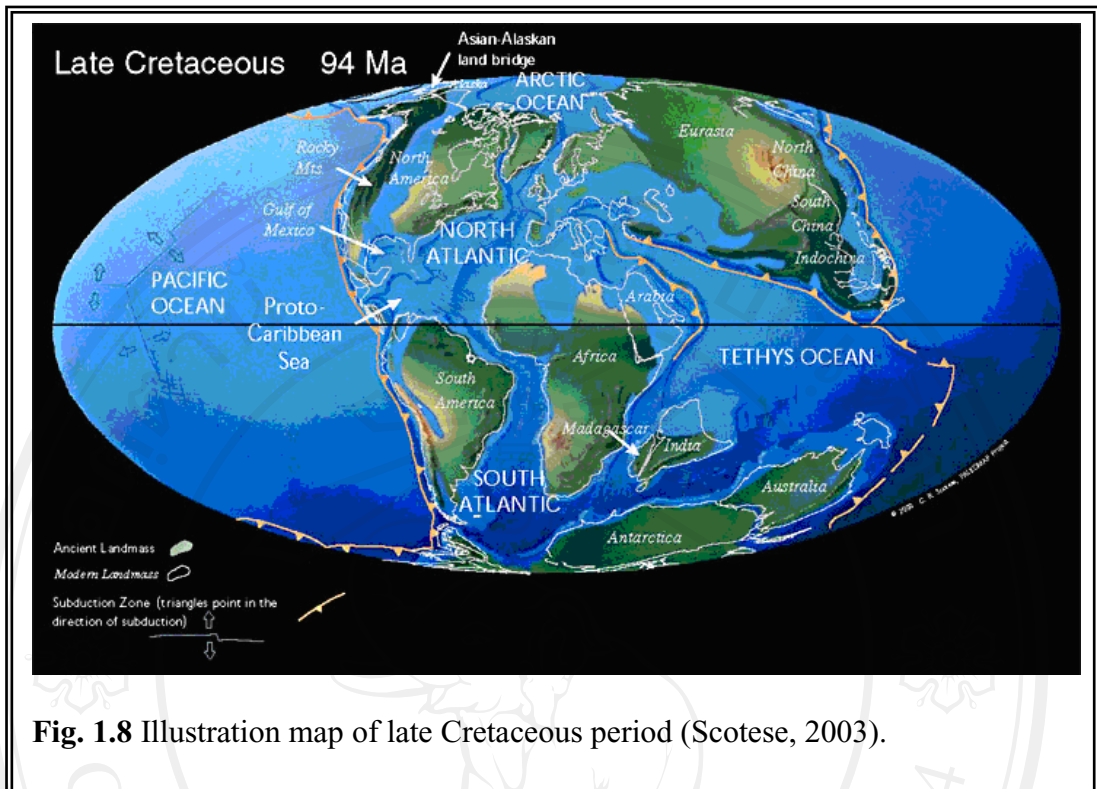
Based on biogeography and evolution history, palms are one of the earliest recognizable modern monocotyledon families in the fossil record (Uhl and Dransfield, 1987). The first unequivocal palm fossils are from the upper cretaceous about 65

million years ago (m.y.a.), but pollen grains very similar to those of some modern palms have been uncovered from the Lower Cretaceous (144-97.5 m.y.a.) (Uhl and Dransfield, 1987). It has been hypothesized, on the basis of present and past distributions and centers of diversity, that palms originated in South America and Africa (west Gondwanaland) in late Jurassic to Early Cretaceous times (163-97.5 m.y.a.) (Moore, 1973; Moore and Uhl, 1973) (fig. 1.7).



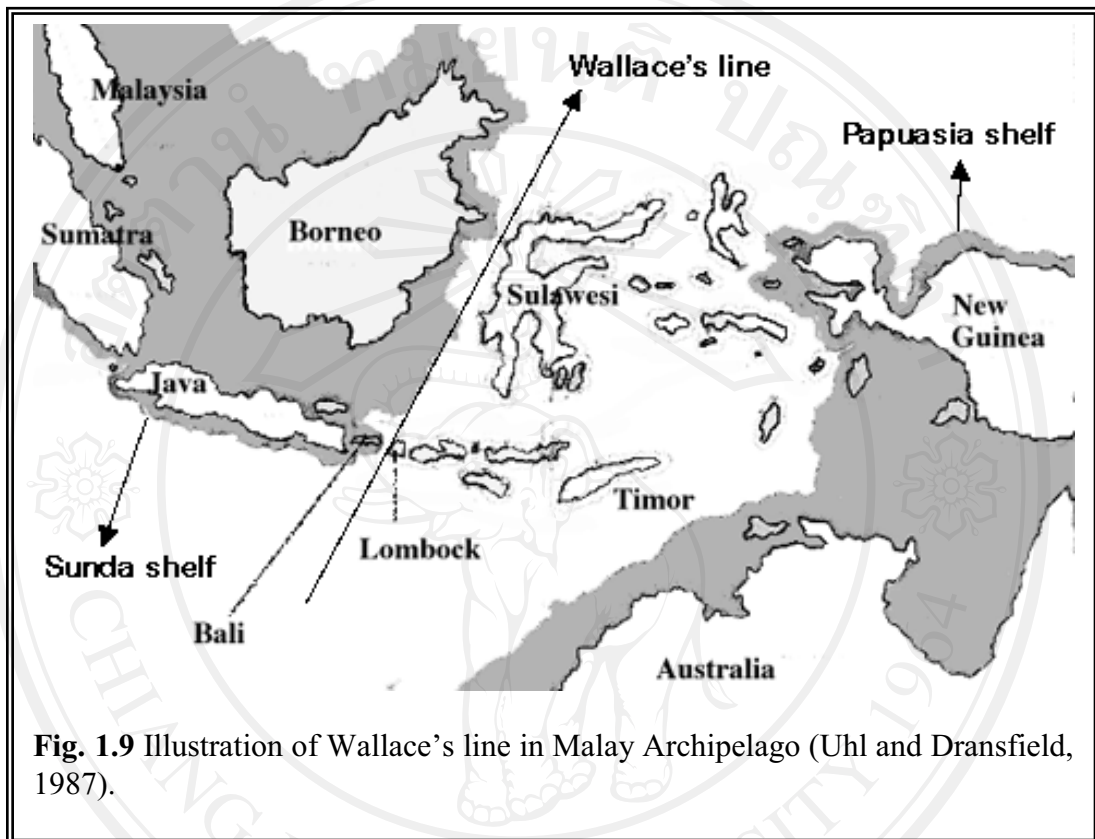
The palms arose when Pangaea was intact, and were already a diverse assemblage when the major continents comprising Gondwanaland and Laurasia were beginning to rift apart in the mid Cretaceous (*ca* 95 m.y.a.) (Uhl and Dransfield, 1987), although smaller fragments of land probably separated and moved earlier than this (Corlett, 1993). It has been suggested that some palms may have reached their

present distributions by rafting on appropriate land masses, but the knowledge of the relative timing of palm evolution and early land rafting are too fragmentary for this to be certain (Uhl and Dransfield, 1987). Both pollen and larger fossils suggest that a diverse assemblage of relatively specialized palms existed at least by the Maastrichtian Epoch (69-65 m.y.a.). There was then rapid, explosive radiation in North America and Asia by the Eocene Epoch (55-39 m.y.a.) (fig. 1.8), when climates were warmer in those regions (Uhl and Dransfield, 1987). Palaeobotanical evidence also shows that during the late Cretaceous and early Tertiary, palms were not only abundant in the African and South American tropics (Morley, 2000), but also frequent and widely distributed over the whole northern hemisphere (Moore and Uhl, 1973; Uhl and Dransfield, 1987; Kvaček and Herman, 2004). The Earth's climate was much warmer during this period than at present, and warm-temperate and paratropical forests (i.e. forests containing a mixture of tropical or temperate elements with palms and lianas being common) were widespread at high latitudes (Willis and McElwain, 2002). Molecular phylogenetic studies provide increasing evidence that the biogeography of modern tropical/subtropical plant groups often reflect boreotropical migrations during the Tertiary (Weeks *et al.*, 2005). By the late Cretaceous, all major palm fossil categories are present, and from the earliest Tertiary (Palaeocene) up until the cooling events of the Miocene, fossil records for the Arecaceae are extraordinarily rich and diverse. Nevertheless, for the majority of palm fossils, the lack of distinctive morphological variation in organs frequently limits the possibility of inferring affinity to taxonomic units below the family level. Pollen records are exceptionally numerous and problematic. There are over 50 fossil pollen genera with a clear or suspected affinity to palms.



There is an imaginary line which may be drawn across the Malay Archipelago where the Sunda Shelf (carrying the south-east Asian fragment of Laurasia) and the papuanian Shelf (carrying the Australasia fragment of Gondwanaland) met during the Miocene Epoch (*ca* 15 m.y.a.). This boundary is called Wallace's Line as A.R. Wallace in 1860, was one of the first biologists to recognize the differences between the flora and fauna on either side of this hypothetical divide (fig. 1.9). The palm genera and species immediately to the west and east of this line are in many ways markedly different, which suggests that little dispersal of palms has occurred since the collision of the plates (Dransfield, 1981b). The genera *Calamus*, *Licuala* and *Livistona* are exceptions, being common on both sides of Wallace's line (Dransfield, 1981b). Other taxa originating in Gondwanaland were probably reunited with taxa in

the northern continents much earlier, when India collided with Asia *ca* 55 m.y.a. (Dransfield, 1987).



Palms are currently widespread in tropical and subtropical regions, and very few palms occur in temperate regions because of two important factors: temperature (palms do not tolerate frost well) and moisture (palms require a reliable and fairly constant water supply) (Dransfield, 1981a; Uhl and Dransfield, 1987). These climatic requirements have probably made palms vulnerable to sudden change, and this may explain the disjunctions in the distributions of certain taxa and also the paucity of palms in Africa, where sudden shifts in climate, especially in rainfall, are known to have occurred during the Pleistocene Epoch (2.5 m.y.a) (Uhl and Dransfield, 1987). In congruence with the view that the present-day environment, notably climate,

governs gradients in species richness, the greater species richness of palms is traditionally associated with warm and moist climates (Corner, 1966), and a recent study of geographical variation in palm species richness across the Americas found water related variables, such as annual rainfall and number of wet days, to be the main predictors of palm species richness (Bjorholm *et al.*, 2005).

1.3. The Current Understanding of Palmicolous Fungi

In the last fifteen years there has been a surge of interest in tropical fungi particularly the fungi associated with palms (Fröhlich, 1992; Fröhlich and Hyde, 1994, 1995a,b; Hyde and Fröhlich, 1995; Yanna *et al.*, 2001; Hyde *et al.*, 2002, 2007; McKenzie *et al.*, 2002; Pinnoi *et al.*, 2004; Pinruan *et al.*, 2002, 2004). Much of this attention was prompted by an increasing awareness that biodiversity was rapidly being lost, notably in the tropics (Reid and Miller, 1989). The recognition that habitat and species loss is happening at a vast scale and an excessive rate has helped to focus attention. Furthermore, a growing interest in natural products and bioprospecting has also fuelled the present interest.

Research in mycology, at least until early 1990, has primarily been concentrated in the northern hemisphere and temperate regions, with tropical regions being largely neglected. This has been mainly due to a lack of funding, resources and expertise to undertake all aspects in mycological research in the tropics, and has resulted in a fragmentary knowledge of spatial and temporal distribution patterns of most fungi (Kirk, 1993). This imbalance, however, is recently being partially addressed with tropical mycological research being carried out in some tropical

countries, with increased efforts to train mycologists in tropical regions (Hyde *et al.*, 1997, 2002, 2007; Pinnoi *et al.*, 2004; Pinruan *et al.*, 2002, 2004).

One area of mycological research in the tropics which has been extensively investigated over the past decade has been that of fungi associated with monocotyledon plants, including Palmae, Pandanaceae, bamboos, Zingiberaceae and Musaceae (Hyde *et al.*, 2007). These hosts have proved to be an important substrate and support a vast array of fungi, some of which show a degree of host specificity. Particularly on fungi associated with palms, Fröhlich and Hyde (2000) noted that there have been two peaks in research on palmicolous fungi. The first was between 1880-1920, and the second in the last decade which is dominated by a result of the studies by Hyde and co-workers. The next subchapters of this thesis describe some recent progress and information on diversity and ecological aspects of palmicolous fungi including endophytes, pathogens and saprobes.

1.3.1. Diversity of Endophytic Palmicolous Fungi

Specialized parasites are not alone in infecting plants. Endophytic fungi also infect plants, although as nonpathogenic colonists. The term ‘endophyte’ was introduced by De Barry (1886) and was initially applied to any organism found within a plant (Wilson, 1995). The word’s meaning is now commonly used for ‘fungi and bacteria which, for all or part of their life cycle, invade the tissues of living plants and cause unapparent and asymptomatic infections entirely within plant tissues, but cause no symptoms of disease’ (Wilson, 1995). According to the recent advances in endophytic fungi studies, this group of fungi probably performs several benefits to their hosts such as, novel ecological functions (e.g. thermotolerance of plants growing

in geothermal soils) (Redman *et al.*, 2002), influence community biodiversity (Clay and Holah, 1999), enhance directly plant growth (Ernst *et al.*, 2003), produce toxic alkaloids in grasses and herbaceous plants (Wilkinson *et al.*, 2000; Braun *et al.*, 2003), perform specific defense roles in woody plants (Miller *et al.*, 2002), limit pathogen damage (Arnold *et al.*, 2003), form an integral part of the extended phenotype or symbiotic community of a plant together with mycorrhizal fungi (Whitham *et al.*, 2003). Even though the full range of their ecological function in woody plants is poorly understood, but they have received much attention over the last ten years as potential sources of biologically active chemicals.

Most of work on endophytic fungi has been carried out in temperate region (Rodrigues and Petrini, 1997), but there have been some investigations of endophytes in tropical ecosystems including a few studies on palm endophytes (Rodrigues, 1990, 1994; Rodrigues and Samuels, 1990; Hyde *et al.*, 1997). Their results suggested that endophytic mycota of tropical plants differs markedly from that of temperate ones. Xylariaceous fungi are the most commonly isolated endophytes in tropical regions (Rodrigues and Petrini, 1997; Fröhlich *et al.*, 2000). They are also common endophytes in temperate regions, but are less prevalent there (Stone and Petrini, 1997).

An investigation of the palm *Euterpe oleracea* in tropical Brazil yielded 13 species of Xylariaceae amongst the 21 dominant taxa (Rodrigues, 1994). Furthermore, Fröhlich *et al.* (2000) also reported that from 75 identified species from *Licuala* spp., 48 genera are Xylariaceous fungi. Therefore, genus *Xylaria* and its anamorphs was generally the most dominant and frequent isolated from tropical palms (Rodrigues and Samuels, 1990), other genera commonly isolated from tropical plants, including palms, are *Colletotrichum*, *Idriella*, *Phoma*, *Phomopsis*, and *Phyllosticta* (Rodrigues and Petrini,

1997). Common saprobes such as *Cladosporium herbarum*, *Aureobasidium pullulans* and *Alternaria alternata* have been recorded as endophytes in both temperate and tropical areas, but they are less common in the latter (Rodrigues and Petrini, 1997).

Even though numerous significant results have been resulted from endophytic fungi research using either conventional or molecular identification methods, however, endophytes diversity in woody plants is still clouded in ambiguity. In the absence of traditional species delimitations appropriate for endophytes, estimates of their diversity have been based on the ‘morphospecies’ concept or morphological similarity to known species. For example, Fröhlich *et al.* (2000) only successfully identified 75 fertile species from about 2237 isolates resulted from surface sterilized plant tissues of *Licuala* spp. Unfortunately, some endophytes genera of a given plant are typically similar morphologically to known parasites of that same plant or to those of closely related host (Ganley *et al.*, 2004) such as *Colletotrichum*, *Phoma*, *Phomopsis*, *Phyllosticta*, *Cladosporium*, *Fusarium*, *Alternaria*, etc. Therefore, it is possible that many of these so-called endophytes are actually cryptic or latent, but known, parasites of the plants. Thus, these fungi remain inherently ambiguous.

1.3.2. Diversity of Pathogenic Palmicolous Fungi

Tropical areas are expected to be more abundant and diverse in the diversity of life and plant pathogens than in non-tropical regions (Hawksworth, 1993). Fungi comprise about 95% of all known plant pathogens, the remainder being bacteria, mycoplasma-like organisms and viruses (Shivas and Hyde, 1997). Rossman *et al.* (1987), from *Literature Guide for the Identification of Plant Pathogenic Fungi*, calculated the number of plant pathogenic fungi about 23,000, however, the

estimation includes only those species that cause disease in vascular plants. It does not take into account epiphytic, lichen forming, lichenicolous mycoparasitic and mycorrhiza. Considering that two-thirds of the world's flowering plants occur in the tropics (Heywood, 1985), therefore, the enormous diversity of plant species in the tropics will undoubtedly support an equally diverse flora of plant pathogens including fungi due to the plant pathogens are generally thought to have co-evolved with their hosts (Pirozynski, 1988). Hawksworth (1991) estimated that about one-third of fungi from his conservative estimation, 1.5 million species, can cause disease in plants. On the other hand, Shivas and Hyde (1997) estimated about 270,000 of plant pathogenic fungi in the tropical area. Although there were wide variations about the estimation of plant pathogenic fungi occur worldwide, we may able to conclude that tropical regions host the greatest diversity of life and plant pathogens.

On palms, the vast majority of diseases are leaf spots and leaf blights caused by fungi (Elliott *et al.*, 2004). In general, the symptoms look similar no matter which fungus is causing the spot or blight. Potential agents of leaf spots and leaf blights on palms include *Annellophora*, *Bipolaris*, *Cercospora*, *Colletotrichum*, *Calonectria* (anam. *Cylindrocladium*), *Exserohilum*, *Gliocladium*, *Pestalotiopsis*, *Pestalotia*, *Phaeotrichoconis*, *Phyllachora*, *Pseudocercospora* and *Stigmina* (Elliott *et al.*, 2004).

Most of these pathogens also cause diseases on plants other than palms. Species of family Mycosphaerellaceae have commonly been encountered on palm leaves, probably due to the pathogenic nature of some of the genera of this family (Fröhlich, 1992; Fröhlich and Hyde, 2000). Other fungal families with known pathogens commonly represented on palms include the Asterinaceae, Meliolaceae, Phyllachoraceae, Phaeochoraceae and Parodiopsisidaceae (Taylor and Hyde, 2003).

Elliott *et al.* (2004) remarked that almost of all pathogenic fungi occurred on palms produce some type of spore which can be easily spread by wind and water (rainfall or overhead irrigation). The spores can also be spread by pest activity (insects, rodents) or human activity (movement through nursery, pruning, etc.). The complete host range for each of these pathogens is unknown, but it is assumed to be broad within palms. Leaf spots and leaf blights are usually more severe on palms in the seedling and juvenile stage of growth (Fröhlich, 1992).

1.3.3. Diversity of Saprophytic Palmicolous Fungi

Most of the study on palm microfungi by earliest mycologist was unspecialized to the host, but their focus on the microfungi perspective. Fröhlich and Hyde (2000) divided research on palm microfungi into two periods. The first period, during 1880-1920 included the publications of works by mycologists such as P. Hennings, H. Rehm, O. Penzig, C. Spegazzini, H. Sydow, P. Sydow and P. A. Saccardo. The most significant contribution from this period was *Icones Fungorum Javanicorum* (Penzig and Saccardo, 1904) and the contribution to the mycota of the Philippines (Rehm, 1913a, 1913b, 1913c; Sydow and Sydow, 1914, 1917). The second period includes the present decade, when for almost the first time, a mycologist began to look specifically at the microfungi on that substrate (Hyde *et al.*, 1997; Fröhlich and Hyde, 2000; Yanna *et al.*, 2001; Taylor and Hyde, 2003).

The research regarding palm microfungi actually has been raised up since microhabitat predictor proposed in 1996 to assess the biodiversity of microfungi (Hawksworth *et al.*, 1996; Hyde and Hawksworth, 1997). Hyde and co-workers followed up the concept with the intensive works on microfungi associated with

Palmae (Hyde *et al.*, 1997; Fröhlich and Hyde, 2000; Yanna, 2001; Taylor and Hyde, 2003), Pandanaceae (Whitton, 1999), etc. Interesting results were reported from their study on biodiversity and ecological aspects of fungi associated with palms in tropical and temperate areas. Many publications and new species have been published since 1997. Hyde *et al.* (1997) reported *ca* 1580 fungi – including 650 ascomycetes, 270 basidiomycetes, and 660 anamorphic fungi – had been described as new species recorded from palm hosts until 1994. Hyde and Fröhlich (2000) reported 592 species of palm microfungi are distributed in 252 genera and 72 families. The data showed a very diverse assemblage on palm microfungi. Taylor and Hyde (2003) collected and described totally 288 species of microfungi in 153 genera. The data includes 159 ascomycetes, two basidiomycetes, 54 coelomycetes and 73 hyphomycetes.

Another interesting result from the study of the palmicolous fungi is that the number of fungal genera that showed an exclusive association with palms. Hyde *et al.* (1997) reported fungal genera such as *Linocarpon*, *Oxydothis*, *Anthostomella*, *Astrosphaeriella*, *Capsulospora* and *Neolinocarpon* are very common found on palms (table 1.1). The similar results were also reported by Fröhlich and Hyde (2000) with *Oxydothis* and *Anthostomella* are the most common genera collected from palms. They also noted that Hyponectriaceae and Xylariaceae were the best represented families, with each family represented by 13 genera found on palms (table 1.2). Amphisphaeriaceae and Phyllachoraceae were the next common families found on palms with each family represented by 10 genera, respectively.

Table 1.1 The most commonly Ascomycetes genera identified from palms in Brunei, Ecuador, and Malaysia during 1988-1994 (Hyde *et al.*, 1997).

No.	Brunei	Ecuador	Malaysia
1	<i>Oxydothis</i> Penz. and Sacc.	<i>Linocarpon</i> Syd. and P. Syd.	<i>Linocarpon</i> Syd. and P. Syd.
2	<i>Linocarpon</i> Syd. and P. Syd.	<i>Astrosphaeriella</i> Syd. and P. Syd.	<i>Oxydothis</i> Penz. and Sacc.
3	<i>Capsulospora</i> K. D. Hyde	<i>Lasiosphaeriella</i> Sivan.	<i>Astrosphaeriella</i> Syd. and P. Syd.
4	<i>Anthostomella</i> Sacc.	<i>Capsulospora</i> K. D. Hyde	<i>Anthostomella</i> Sacc.
5	<i>Astrosphaeriella</i> Syd. and P. Syd.	<i>Hypoxyylon</i> Bull.	<i>Lophiostoma</i> Ces. and De Not.
6	<i>Neolinocarpon</i> K. D. Hyde	<i>Nectria</i> (Fr.) Fr.	<i>Capsulospora</i> K. D. Hyde
7	<i>Myelosperma</i> Syd. and P. Syd.	<i>Oxydothis</i> Penz. and Sacc.	<i>Astrocystis</i> Berk. and Broome
8	<i>Arecomyces</i> K. D. Hyde	<i>Tubeufia</i> Penz. and Sacc.	<i>Neolinocarpon</i> K. D. Hyde

Table 1.2 List of the most common Ascomycetes genera encountered on palms
(Fröhlich and Hyde, 2000).

No.	Family	Genera
1	<i>Clypeosphaeriaceae</i>	<i>Apioclypea</i> K. D. Hyde
		<i>Brunneiapiospora</i> K. D. Hyde, J. Fröhl. and Joanne E. Taylor
		<i>Capsulospora</i> K. D. Hyde
		<i>Frondispora</i> K. D. Hyde
		<i>Oxydothis</i> Penz. and Sacc.*
2	<i>Hyaloscyphaceae</i>	<i>Arenaea</i> Penz. and Sacc.
		<i>Lachnum</i> Retz.
3	<i>Hyponectriaceae</i>	<i>Arecomyces</i> K. D. Hyde
		<i>Linocarpon</i> Syd. and P. Syd.*
		<i>Neolinocarpon</i> K. D. Hyde* <i>Pemphidium</i> Mont.*
4	<i>Lasiosphaeriaceae</i>	<i>Cercophora</i> Fuckel
		<i>Lasiosphaeria</i> Ces. and De Not.
		<i>Palmicola</i> K. D. Hyde
5	<i>Lophiostomataceae</i>	<i>Lophiostoma</i> Ces. and De Not.
		<i>Massarina</i> Sacc.
6	<i>Melanommataceae</i>	<i>Astrosphaeriella</i> Syd. and P. Syd.
		<i>Trematosphaeria</i> Fuckel

(Table continued)

No.	Family	Genera
7	<i>Mycosphaerellaceae</i>	<i>Guignardia</i> Viala and Ravaz
		<i>Mycosphaerella</i> Johanson
8	<i>Myelospermataceae</i>	<i>Myelosperma</i> Syd. and P. Syd.
9	<i>Phaeochoraceae</i>	<i>Cocoicola</i> K. D. Hyde
		<i>Phaeochora</i> Höhn.
		<i>Phaeochoropsis</i> K. D. Hyde and P. Cannon
		<i>Serenomyces</i> Petr.
10	<i>Xylariaceae</i>	<i>Anthostomella</i> Sacc.
		<i>Astrocystis</i> Berk. and Broome
		<i>Fasciatispora</i> K. D. Hyde
		<i>Guestia</i> G. J. D. Smith and K. D. Hyde
		<i>Hypoxylon</i> Bull.
		<i>Nemania</i> Gray emend. Pouzar
		<i>Rosellinia</i> De Not.
		<i>Stilbohypoxylon</i> Henn.
		<i>Xylaria</i> Hill ex Schrank

* Family incertae sedis

In Thailand, the study on the diversity of palm microfungi, particularly saprobes, have significantly been raised up since 1996 or over the past thirteen years (Aramsiriujwet, 1996; Hyde *et al.*, 2002; McKenzie *et al.*, 2002; Pinnoi *et al.*, 2004, 2006; Pinruan *et al.*, 2002, 2004, 2007, 2008; Sarapat, 2003; Hidayat *et al.*, 2006). The research mostly covered specific areas such as palms in peat swamp, freshwater to brackish areas, rarely in terrestrial ecosystem. Aramsiriujwet (1996) collected 29 hyphomycetes from seven species palms, namely, *Borassus flabellifer*, *Caryota* sp., *Cocos nucifera*, *Cyrtostachys lakka*, *Corypha lecomtei*, *Elaeis guineensis*, *Roystonea regia*. Pinnoi *et al.* (2004) also reported 53 more new species found on four species palms, viz, *Eleiodoxa conferta*, *Nenga pumila*, *Licuala longicalycata*, and *Metroxylon sagus* from similar location, peat swamp ecosystem. *Trichocladium nypae*, *Linocarpon appendiculatum*, *L. nypae*, *Oxydothis nypae* and *Astrosphaeriella striatispora* are the most common microfungi associated with palms in both studies. On the other hand, report of Hidayat *et al.* (2006) regarding the phylogenetic study of *Oxydothis* species is probably the first publication of palmicolous fungi from northern Thailand. The report includes three *Oxydothis* species on *W. siamensis*, namely, *O. wallichianensis*, *O. inaequalis*, and *O. daemonoropsicola*.

1.3.4. Ecology of Palmicolous Fungi

Studies on ecology of palmicolous fungi were mostly dominated by the taxonomic distribution, geographical distribution and host specificity (Fröhlich and Hyde, 2000; Yanna, 2001, Yanna *et al.*, 2001; Taylor and Hyde, 2003). In taxonomic distribution study, Fröhlich and Hyde (2000) reported that families Xylariaceae and Hyponectriaceae dominated the taxa distribution occurred of palms fronds, followed

by Bionectriaceae, Nectriaceae, Clypeosphaeriaceae and Melanommataceae (table 2). In contrast, Taylor and Hyde (2003) found that families Xylariaceae and Hyponectriaceae did not dominate the fungal community occurred on three selected terrestrial palms, viz, *Archontophoenix alexandrae*, *Cocos nucifera* and *Trachycarpus fortunei*. Instead, families of Lophiostomataceae, Phaeosphaeriaceae and Valsaceae, dominated the fungal community on the selected palms. At genera level, Fröhlich and Hyde (2000) and Taylor and Hyde (2003) found almost similar results due to common palmicolous fungi genera such as *Oxydothis*, *Anthostomella* and *Linocarpon* were frequently recorded in the study.

In geographical distribution of palmicolous fungi, Fröhlich and Hyde (2000) from the collection in four countries, viz, Australia, Brunei, Ecuador and Hong Kong, reported that genera of *Anthostomella*, *Arecomyces*, *Astrosphaeriella* and *Oxydothis* were commonly found. These results supported the previous findings by Hyde *et al.* (1997) who reported three genera, namely, *Astrosphaeriella*, *Linocarpon* and *Oxydothis* occur in both the Old and New World tropics. Fröhlich and Hyde (2000) also remarked that the differences and similarities between the mycotas of Australia, Brunei, and Hong Kong are likely to be influenced chiefly by three factors: past and present biogeography, past and present host distribution and climate. Fröhlich and Hyde (2000) insisted that while biogeography and host distribution may be dominant effect on the distribution of fungi at the genus level, climate may be more important in determining the distribution of species. Yanna *et al.* (2001) and Taylor and Hyde (2003) also noted that geographical distribution significantly affect the variation of fungal community of palms regardless of hosts. Interestingly, Taylor and Hyde (2003) also insisted that the climate is the limiting factor in the palmicolous fungi taxa

distribution on hosts. In conservation point of view, all these reports pointed out that the number of palmicolous fungi taxa in the natural habitats such as rainforest is significantly higher than disturbed habitats. Another interesting ecological result was also found by Pinnoi *et al.* (2004) who reported that palm microfungi on the peat swamp ecosystem were distinct from those on terrestrial habitat. Therefore, it can be assumed that ecosystem is another key factor alongside climate that affects the fungal community occurrence in various hosts. In fact, different ecosystem possesses very variable microclimates and other specific environmental factors.

In host specificity of palmicolous fungi, particularly saprobes, Parbery (1996) insisted that saprobes are considered the least host specific fungi as compared to plant pathogens, although some fungi appear to have an intimate association with the member of Palmae. This statement is supported by Taylor *et al.* (2000) who reported that the number of potentially host-species specific fungi (i.e. those which were only recorded from one particular host) which were recovered only 3 to 13 taxa. This number is definitely less than has been suggested as typical of tropical palm hosts (33 fungal taxa) by Fröhlich and Hyde (2000), and closer to the number of fungi estimate for hosts in temperate countries (about 6 taxa) (Hawksworth, 1991). It would suggest that the differences in the mycota recorded amongst the palms species as fungal hosts are unlikely to simply be a consequence of host species specificity. It appears that fungi common to palms are often non-specific in their host species associations. However, not only are the cases of host species specificity notable (e.g. *Oxydothis* and *Astrosphaeriella* on rattan palms), but also differences in the composition of assemblages of different palms is being noted (Yanna *et al.*, 2001). At which level specificity occurs: host genus, subtribe, tribe or subfamily, is not yet obvious, but

should become apparent as the mycota of more palm hosts are systematically investigated.

1.4. Outline of the Thesis

This thesis has three main objectives:

1. To investigate the diversity of tropical palmicolous fungi associated with *W. siamensis*
2. To investigate the ecology of palmicolous fungi community on *W. siamensis* during decomposition process
3. To analyze the phylogenetic relationship of some interesting palmicolous fungi species associated with *W. siamensis* by employing nrDNA sequence analysis

In order to accommodate all the objectives designed in this thesis, therefore, the thesis is generally divided into two parts: (1) taxonomy and phylogenetic, (2) ecology, each containing four and three chapters, respectively. The thesis is opened by chapter 1 which consists of introduction and information regarding recent progress in palmicolous fungi study. The objectives and outline of the thesis are also presented in this chapter.

The first part focuses on the diversity of fungi associated with *W. siamensis* and other palms, including endophytes, pathogens and saprobes. The fungi recorded during this study are listed, and the taxonomic description of some interesting taxa is presented. Phylogenetic studies of several important fungi are also presented in this part. The ecological pattern of fungi associated with *W. siamensis* is studied and elucidated in the second part.

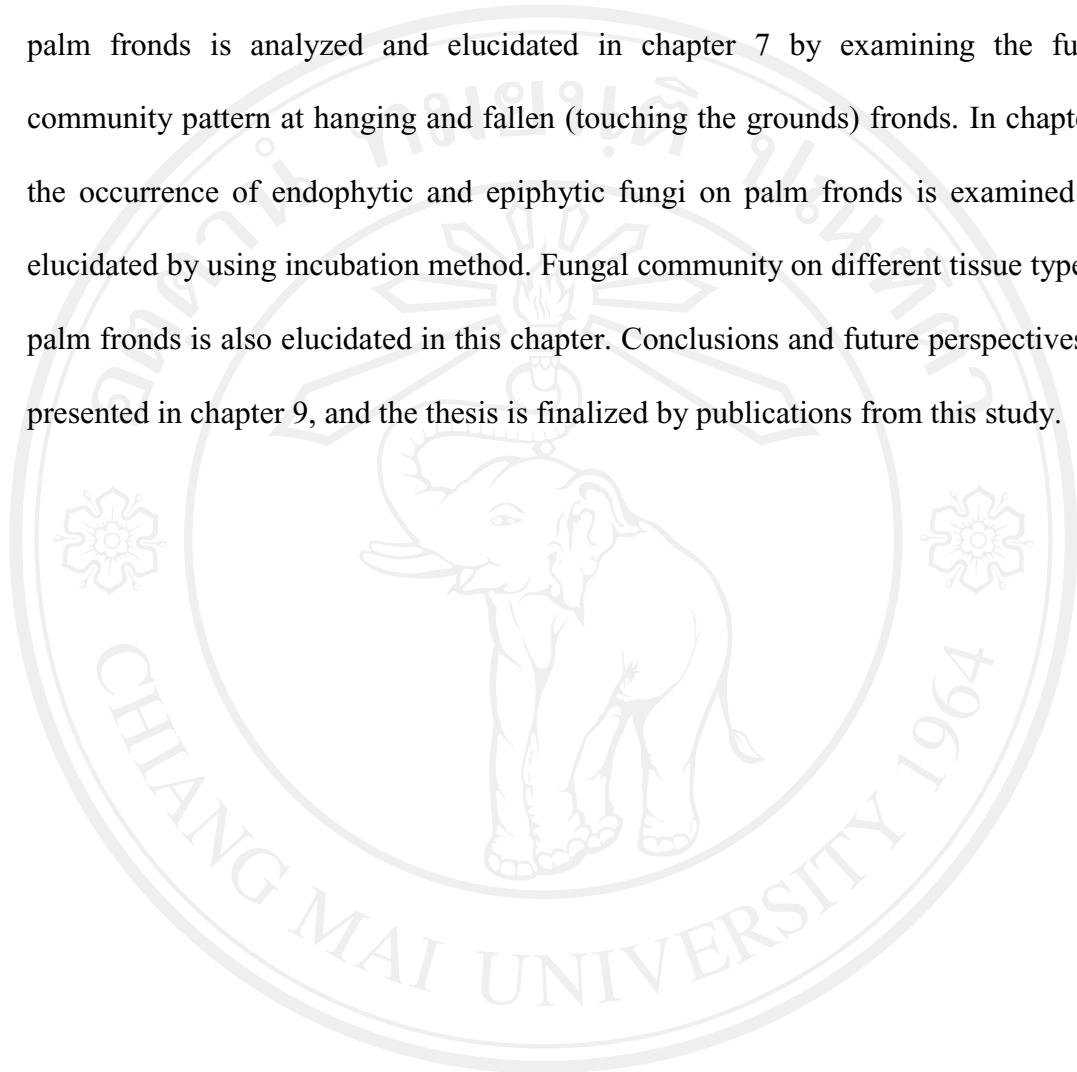
Part 1: Taxonomy and Phylogenetic

In this part, diversity of fungi associated with *W. siamensis* and other species of palms is discussed. Molecular phylogenetic study on several interesting fungi is also discussed. Chapter 2 focuses on diversity of fungi associated with *W. siamensis* and the fungi associated with necrotic leaflets of palms collected from several locations in Chiang Mai province. Necrotic leaflets is selected in this study because this habitat is considered as a unique or specific as different type of fungi such as endophytes, pathogens and saprobes grow together on this particular habitat. Some interesting fungi are described and illustrated. Chapter 3 presents the phylogenetic relationship of genus *Oxydothis* with related genera within family Xylariaceae. Genus *Oxydothis* is selected as it considered as a specific fungi associated with palms, 73 of 75 *Oxydothis* species have been recorded on palms. Chapter 4 illustrates and elucidates two important fungal pathogens species found on leaf spots of *Areca catechu* and *Chamaedorea metallica*, respectively, based on morphology characteristic and phylogenetic analysis of ITS nrDNA region. Chapter 5 presents a phylogenetic relationship of *Dictyochaeta wallichianensis* with similar taxa. This fungus is a novel species and appeared as a dominant species on *W. siamensis* as analyzed in chapter 7 of ecology part.

Part 2: Ecology

The next three chapters in this part elucidate the fungal community variation on fronds of *W. siamensis* located at Huay Kog Ma, Suthep-Pui national park, Chiang Mai, Thailand. Chapter 6 explains a variation in fungal community amongst single tree of *W. siamensis* at the same locality. The number of species and abundance of

each species at every single tree of *W. siamensis* is examined and analyzed. The effect of space to the composition and distribution of palmicolous fungi on different parts of palm fronds is analyzed and elucidated in chapter 7 by examining the fungal community pattern at hanging and fallen (touching the grounds) fronds. In chapter 8, the occurrence of endophytic and epiphytic fungi on palm fronds is examined and elucidated by using incubation method. Fungal community on different tissue types of palm fronds is also elucidated in this chapter. Conclusions and future perspectives are presented in chapter 9, and the thesis is finalized by publications from this study.



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