University of Reading

SYSTEMATICS OF PENINSULAR MALAYSIAN SCALY TREE FERNS (CYATHEACEAE): PHYLOGENETICS, COMPUTER-AIDED IDENTIFICATION AND CONSERVATION

A thesis submitted by

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ABSTRACT

The work presented in this thesis evaluates the status of Peninsular Malaysian Cyatheaceae and used molecular and morphological identification tools for the local species. 419 Cyatheaceae frond sample were collected from the widest possible range of Peninsular Malaysia to obtain material for morphology and molecular study. 15 *Cyathea* species were identified and the species information for Peninsular Malaysia was updated. The species was incorporated into the existing Cyatheaceae phylogeny by using four plastid regions: *matK, rbcL, trnG-trnR* and *trnL-trnF.* Bayesian MCMC analysis of the concatenated sequence data resulted in a 50% majority rule consensus tree confirm the placement of the four groups: *Cyathea, Alsophila*, *Gymnosphaera* and *Sphaeropteris* in the family. However, the resulting tree representing nested monophyletic groups, proposing Cyatheaceae to be monogeneric, i.e., *Cyathea* with two large groups: *Cyathea* and *Sphaeropteris.* The same plastid regions were then evaluated to develop DNA barcodes. *trnL-trnF* was proposed as a barcode for this family as it almost satisfied the three most important criteria: primer universality, sequence quality and species discrimination. This research also developed a multi-access key for Cyatheaceae field identification based on fifteen taxa identified, by extensive field sampling of the currently recognised species. All of the Cyatheaceae species recognises in this study had also been assessed for the conservation status based on the IUCN Red List criteria. Nine species fall under Least Concern (LC), four species are Near Threatened (NT) and two species are Vulnerable (VU). The thorough knowledge regarding Cyatheaceae in Peninsular Malaysia gained through the work done in this research will benefit in making appropriate conservation strategies for the survival of this family. Overall, the most important outcome of this research was the combination of morphology and molecular data for the purpose of updating taxonomy, identification and conservation of the Cyatheaceae family in Peninsular Malaysia.

TABLE OF CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

1.1 Malaysian Biodiversity

Malaysia (Figure 1.1) belongs to the Sundaland biogeographical region which includes the Sunda shelf, a part of the Asian continental shelf that was uncovered during the last glacial period of the Pleistocene (Hall, 1998). It consists of an island part and a part attached to mainland Asia separated by 540 km of the South China Sea. The land area covers approximately 33.27 million hectares (MNRE, 2006; MNRE, 2014; MNRE, 2016). These two parts of Malaysia share a similar landscape that features coastal plains rising to hills and mountains, including Mount Kinabalu at 4095 meters, a UNESCO World Heritage Site and the highest mountain in South East Asia (Metcalfe, 2002; MNRE, 2014; MNRE, 2016). The local climate is equatorial, with temperature ranging from 21°C to 32°C and annual rainfall of 250 cm, along with high humidity and annual southwest (April to October) and northeast (October to February) monsoons (MNRE, 2006; MNRE, 2014; MNRE, 2016; Richmond, 2010).

Figure 1.1 Map showing the location of Malaysia and surrounding countries. The orange line marks the Sundaland shelf today. Source: Google map.

The country is one of the twelve most mega-biodiverse countries in the world (Lee *et al.*, 2010) with more than 170,000 species (Table 1.1), including many endemics with more than 80% endemism occurring in the peninsula alone (Secretariat of the Convention on Biological Diversity, 2010; MNRE, 2014). Much of its diversity survives because two thirds of the land is covered with heavily forested tropical rainforest, parts of which are up to 130 million years old (Lee *et al.*, 2010). There are about 15,000 known species of flowering plants, and more than 1,100 ferns and fern allies occurring in Malaysia (Bidin and Jaman, 1999; MNRE, 2014).

Table 1.1 Summary of Malaysia's overall biodiversity richness (MNRE, 2014).

Group	Estimated Species
Mammals	306
Birds	742
Reptiles	567
Amphibians	242
Marine Fishes	1,619
Freshwater Fishes	449
Invertebrates	150,000
Vascular Plants	15,000
Fungi	4,000
Mosses	522
Hard Corals	612

Malaysia is an active party to the Convention on Biological Diversity (CBD) which it ratified in 1994 (Napis *et al.*, 2001). Since then, the National Policy on Biological Diversity had been developed (MSET, 1998) alongside other policies with biodiversity conservation as a focal part of sustainable development (MNRE, 2014). It is also committed to maintain at least 50% of the land area under forest and tree cover in perpetuity and up until 2012, approximately 21.01 million hectares of the country remained forested with 14.5 million hectares designated as permanent forest reserve (MNRE, 2014; MNRE, 2016).

Since its independence in 1957, Malaysia underwent rapid socio-economic growth, which resulted in heavy deforestation (Napis *et al.*, 2001). Activities such as logging and hydroelectricity schemes, led to the endangerment of local biodiversity, raising concerns on the conservation status of species present (Napis *et al.*, 2001; MNRE, 2014). Even though policies for sustainable development are in place, there are few appropriately qualified scientists to monitor progress. The present work is one of the few that focuses on the taxonomic treatment of a plant family susceptible to

development activities. Phylogenetic approaches, such as Bayesian MCMC and DNA Barcoding analysis are used in the evaluation of the species, as well as reviewing the IUCN Red List status for these species and developing a Multi-Access Key for better conservation measurements.

1.2 Study of Pteridophytes in Malaysia

There have been several studies of Malaysian pteridophytes, notably work started by Alfred Russel Wallace in the mid-1800s (Cicuzza, 2014) followed by Ridley (1908, 1912, 1926), and then by Holttum (1963, 1966, 1968) for Flora Malesiana. The fern taxonomy of Malaysia specifically was updated by Bidin (1983, 1985, 1987) in the 1980s. Parris and Latiff (1997) suggested that the overall count of pteridophytes at the time of their study was 1,136 species, 637 of which occurred in Peninsular Malaysia, 718 species in Sabah, and 587 species in Sarawak. There is no current complete key to Malaysian pteridophytes. Efforts in cataloguing plant species in Malaysia, have concentrated on woody plants due to their economic value while pteridophytes have been comparatively neglected. This fact along with a small number of research publications recently show a lack of pteridology expertise in Malaysia even though many species are thought to be threatened.

1.3 The Scaly Tree Ferns: Cyatheaceae

Cyatheaceae, in the order Cyatheales, forms part of the subclass Polypodiidae which includes most of the world's fern diversity (Schuettpelz and Pryer, 2007; Carl J Rothfels *et al.*, 2012; Christenhusz and Chase, 2014). It has trunk-like, erect stems which elevate the fronds above the ground (Figure 1.2) and includes 500 of the estimated 700 species of tree ferns (Conant *et al.*, 1994), along with Metaxyaceae, Dicksoniaceae, and Cibotiaceae (Korall *et al.*, 2006). Regions that are rich in species include the Greater Antilles, Central America, the northern part of Andes including Venezuela, Colombia, Ecuador, Peru, Madagascar, Borneo, Sumatra, the Philippines, and New Guinea (Tryon, 1970; Tryon and Gastony, 1975). Many of the species have confined ranges with few occurring in more than one of these regions (Conant *et al.*, 1995). Even though the geographic ranges of the species are known, the genera ranges are not as there is a lack of agreement on generic boundaries (Conant *et al.*, 1995). This lack of consensus on generic restriction is shown by studies of Tryon and Tryon (1982), Holttum and Edwards (1983) and Lellinger (1987) in which six, one and four genera were recognized, respectively.

Figure 1.2 Cyathea sp. in Bukit Larut, Perak. Photo© 2013 Azi Jamaludin.

Most of the species are forest plants (Holttum, 1963) and include some of the tallest existing ferns, reaching over 20 m tall (Holttum, 1963; Lehnert, 2009; Korall *et al.*, 2007). The members of this family can be distinguished from the other families by having not just the general pluricellular hairs, but also the presence of different types of scales on their stems and petioles (Figure 1.3) (Kramer, 1990; Korall *et al.*, 2007). However, understanding the relationships between the genera within the family is problematic, since the focus of identification and classification had always been dependent on the scales and indusia morphologies (Korall *et al.*, 2007). These morphological characters have been considered to be frequently subject to homoplasy and of less value in defining major groups of Cyatheaceae (Holttum and Edwards, 1983; Korall *et al.*, 2007).

A. *Cyathea latebrosa* collected in Genting Highlands, Pahang.

B. *Cyathea contaminans* collected in Genting Highlands, Pahang.

Figure 1.3 ***A*** *and* ***B*** *shows two distinctive species of the scaly tree ferns with the presence of scales on their stems and petioles. Photo© 2013 Azi Jamaludin.*

Holttum (1963) counted a total of 36 *Cyathea* species in Malaysia, of which nine species are from Peninsular Malaysia, four species in Sarawak and eight species in Sabah. Eight species can be found throughout Malaysia, five species occurred both in Sarawak and Sabah, while one species can be found in Peninsular Malaysia and Sabah, and Peninsular Malaysia and Sarawak respectively (Holttum, 1963). The Malesian Cyatheaceae was divided by Holttum (1963) into three subfamilies: Cyatheoideae, Cibotioideae and Thyrsopteridoideae and outside Malesia, Metaxyoideae (Latiff, 2015; Holttum, 1963). Holttum (1963) recognised two

subgenera in *Cyathea*: *Cyathea* and *Sphaeropteris* with the latter further divided into two sections: *Sphaeropteris* and *Schizocaena*; and four subsections: *Sphaeropteris, Fourniera, Schizocaena*, and *Sarcopholus*.

1.4 Generic Delimitation in Cyatheaceae

Cyatheaceae have long enthralled scientists and have been the subject of many systematic and taxonomic treatments (Figure 1.4) (Conant *et al.*, 1994; Conant *et al.*, 1995; Conant and Stein, 2001; Korall *et al.*, 2007).

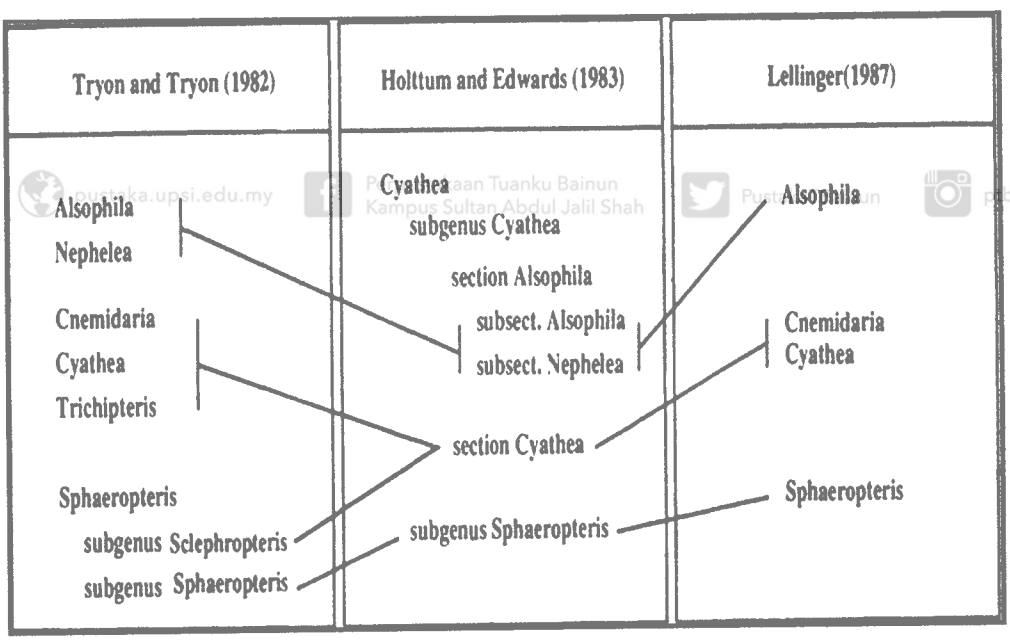

Figure 1.4 Classification systems proposed for the Cyatheaceae (Conant et al., 1994).

Christensen (1905) had separated the family into *Cyathea, Hemitelia* and *Alsophila* based on whether the indusium completely or partially covers the sorus, or is absent altogether. In his study, Christensen (1905) also included *Lophosoria* and *Metaxya* in

Alsophila, but later these two genera (*Lophosoria* and *Metaxya*) were discovered to be remotely associated to the major genera of the family such as *Cyathea, Alsophila* and *Sphaeropteris*.

However, Holttum (1957, 1964) focusing on the Malesian region discovered that the petiole scales provided a useful taxonomic character in classifying the *Cyathea* species. This was because they are associated with other morphological characters and ecological preferences (Holttum, 1957; Holttum, 1964). He also stated that indusium type was not an important general character because it varies broadly within a few species (Holttum, 1957; Holttum, 1964). This led to the family being revised where he used the scales characters to define subgenera and sections, before later proposing *Cyathea* as a single genus (Holttum, 1963; Holttum and Edwards, 1983).

In his study, Tryon (1970) divided the entire Cyatheaceae family into six genera based on the morphological characters of the scales used by Holttum (1957) as well as the presence and absence of indusia and venation characteristics. Tryon (1970) found that indusia ascended from scales on the leaf abaxial surface remote from the margin. Thus he concluded that the indusium should be a derived character in which the absence of the character will be regarded as a primeval state within the family (Tryon, 1970). Tryon's work focused majorly on Neotropical species, contrasting with Holttum who mostly worked on Old World taxa. The six genera that Tryon (1970); Tryon and Tryon (1982) proposed are *Alsophila, Nephelea, Cnemidaria, Cyathea, Trichipteris*, and *Sphaeropteris*.

Even after the studies made by Holttum (1957), Tryon (1970), Tryon and Tryon (1982) and Holttum and Edwards (1983), the classification of Cyatheaceae remains unresolved. This brings Lellinger (1987) to recognise four genera in his study: *Alsophila* (including *Nephelea*), *Cnemidaria, Cyathea* (including *Trichipteris*), and *Sphaeropteris*. Lellinger (1987) argued that occasional hybrids occur within *Alsophila* and *Cyathea* as well as between *Cnemidaria* and *Cyathea* but the characteristics of *Alsophila* and *Cnemidaria* were sufficiently different from *Cyathea* to distinguish the genera readily.

There was a clear disagreement between the authors over the relationships and character evolution within this family over the years. With the emergence and advancement of molecular study, investigation using phylogenetic approach led Conant *et al.* (1994, 1995) and Stein, Conant and Valinski (1997) to divide this family into three genera: *Alsophila, Cyathea,* and *Sphaeropteris* with *Alsophila* being the most basal group in the family. This classification was used in many Cyatheaceae related studies until 2006 (Conant and Stein, 2001; Smith *et al.*, 2003; Korall *et al.*, 2006).

Smith *et al.* (2006) revised the classification of extant ferns and recognized five genera namely *Alsophila* (including *Nephelea*), *Cyathea* (including *Cnemidaria, Hemitelia, Trichipteris*), *Gymnosphaera, Hymenophyllopsis* and *Sphaeropteris* (including *Fourniera*). Until then *Hymenophyllopsis* was placed in a monogeneric family (Tryon and Tryon, 1982). Analysis by Wolf *et al.* (1999) suggested a close and well-supported relationship of *Hymenophyllopsis* to Cyatheaceae based on two species: *Hymenophyllopsis hymenophylloides* and *H. dejecta*. Conant and Stein (2001)

and Korall et al. (2007) suggested that *Alsophila* should be divided into two groups: *Alsophila* and *Gymnosphaera* based on broader species and morphology sampling.

Korall et al. (2007) studied the morphology of the scales and indusia based on previous studies by Holttum (1963), Tryon (1970), and Conant et al. (1994), (1995), along with a molecular phylogeny, and separated four groups based on the type of scales (Figure 1.5) and indusia. Korall et al. (2007) then proposed the four groups as genera: *Cyathea, Alsophila, Gymnosphaera* and *Sphaeropteris* with the latter as sister to all others.

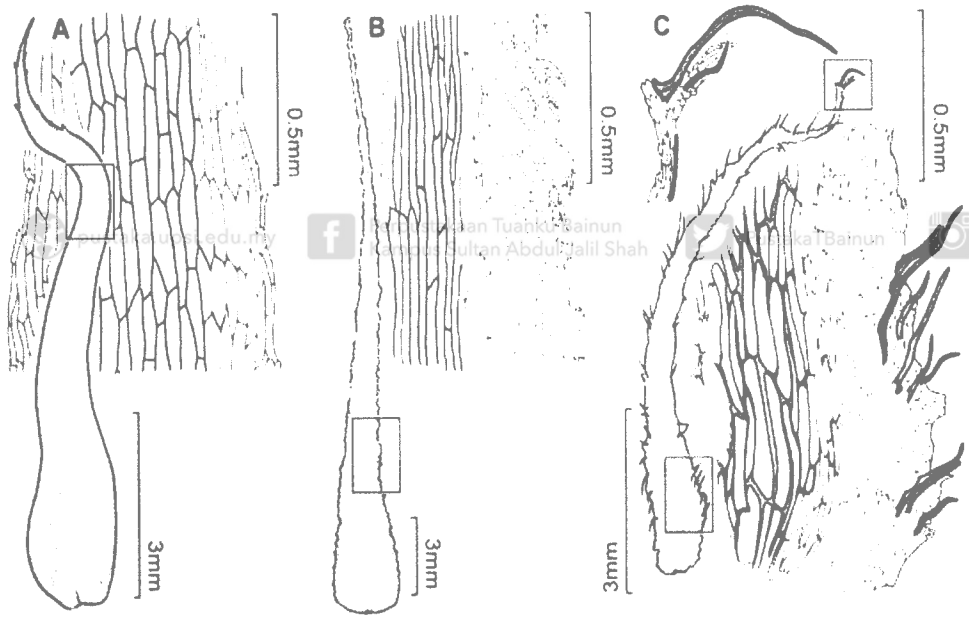

Figure 1.5 (A) Conform scale in Sphaeropteris (B) Marginate scale without apical seta in Cyathea (C) Marginate scale with apical seta in Alsophila (Korall et al., 2007).

1.5 Importance to Conservation

Cyatheaceae and Dicksoniaceae were listed in the Convention on International Trade in Endangered Species (CITES) in 1975 (Oldfield, 1995). Tree ferns have long been used for many socio-economic purposes such as construction, horticulture (Figure 1.6), food, and medicine (Large and Braggins, 2004; Rout *et al.*, 2009) resulting in their heavy exploitation as a source of income (Large and Braggins, 2004). There are many common, non-threatened species used for trading, such as *Cyathea arborea, C. biformis, C. lepifera, Dicksonia antartica, D. fibrosa* and *D. sellowiana* (CITES, 2013). However, there are species that may have been threatened locally, mainly because of habitat destruction but there is a need to monitor the species that may be threatened because of the trade (CITES, 2013).

A B

Figure 1.6 Examples of the uses of Cyathea species. ***A.*** *Part of the trunk made into ornamental bowl.* ***B.*** *Roots that have been compressed to be made into orchid mounting medium.*

This group is also ecologically important as a study by Ashton (2000) suggested that the trunks of the tree ferns were favourable sites for the establishment of ground and epiphytic ferns. Another study by Lindenmayer et al. (1994) found that the numbers of mountain short-eared possum increased as the numbers of *C. australis* and *D. antartica* increased. Blows and Schwarz (1991) found that dried fronds of *C. australis* were a favourite living site for *Exoneura bicolor* bees. Fountain-Jones, McQuillan and Grove (2012) observed and sampled 80 individuals of *D. antartica* on which they discovered a total of 108 species of beetles, representing 35 families, living in discrete microhabitats of crown litter, live fronds and trunk. Also, species such as *C. contaminans* can be used as an indicator of forest disturbance in Malaysia highlands as it can be found abundantly inside clearings (personal observation).

Trade-reporting relies on the correct identification of species in the field and correct usage of species names in CITES. The problems arise when different countries tend to report the tree fern trade at different taxonomic levels and use different names. At present, tree fern conservation status has not been updated in Malaysia, specifically none of the species from Malaysian Cyatheaceae have been evaluated for IUCN Red Listing (IUCN, 2015). The lack of effort in updating the conservation status may be due to lack of local expertise in this field. This is where the current work will help re-evaluate the Peninsular Malaysia Cyatheaceae by adapting current taxonomies with modern technologies. It is hoped that this work will contribute towards the better understanding of the overall phylogenetic knowledge which may contribute for better conservation efforts.

DNA barcoding has not only been used as a tool for species identification but also for species discovery as well as clarifying the taxonomic relationships between species (Lahaye *et al.*, 2008). The knowledge acquired will be useful in making appropriate conservation plans for this family in Malaysia (Liao *et al.*, 2011).

While taxonomists work extensively, solving problems affecting trade-reporting and present their findings in journals, keen general users such as local plant collectors and plant nursery traders are sometimes left with insufficient species identification information. Trade-reporting and all of its related fields depend on species identification keys being precise and usable. Most of the dichotomous printed keys are written by taxonomists for similar users in the field, often with very little additional explanation, resulting in difficulties for novice users to access the species information (Lindsay and Middleton, 2009).

Although trade surveys and monitoring rely on experts such as taxonomists, field staff, and wildlife officers (CITES, 2013), general users who are interested in preserving biodiversity can also help by reporting any irregular trade activities to the authorities. The development of a multi-access key for Cyatheaceae in this work aims to facilitate species identification as well as attract the interest of a broader range of people and professions into knowing this family.

1.6 Thesis Structure

This dissertation will be structured based on two aspects: Chapters 2, 5 and 6 focuses on using morphological data to develop electronic key and assessing the species conservation status. Chapters 3, and 4 used molecular data to update the phylogeny, and proposing a DNA barcode markers. As all of the chapters rely heavily on the right identification of *Cyathea* species, Chapter 2 will be the most important as it will determine the research continuation in the succeeding chapters. Finally, the findings of this thesis will be discussed in greater detail in Chapter 7: general discussions.

1.7 Research Objectives

Even though the information on this family has developed over the years, its relationships have not yet been thoroughly understood. This study will aid further in resolving both species and generic identities in the scaly tree ferns. The more specific research objectives include;

- To investigate the phylogenetic relationships of Peninsular Malaysian Cyatheaceae based on DNA sequence data from four plastid DNA regions (*rbcL, matK, trnG-trnR*, and *trnL-trnF*) to contribute towards resolving and supporting the overall phylogenetic knowledge of the family.
- To develop a barcode based species identification tool.

- To gather the morphological data of identified *Cyathea* species and construct an interactive multi-access key using LucID software to help others identify the species.
- To evaluate and update *Cyathea* species status in the IUCN Red List for better understanding of the conservation status of the family and to help guide conservation measures.