UNIVERSITI PUTRA MALAYSIA

MORPHOLOGICAL AND MOLECULAR SYSTEMATICS OF Shorea SECTION Richetoides (DIPTEROCARPACEAE) IN PENINSULAR MALAYSIA

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By

IKHLAS HUSSAIN ALWAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
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DEDICATIONS

Dedicated With My Love to My Late Father
And
To My Beloved Mother, Her Love, Support, and Constant Prays Have Sustained Me throughout My Life
To My Dear Sisters and My Brothers for Their Endless Love, Support and Sacrifices.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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Chairman : Associate Professor M. Nazre Saleh, PhD
Faculty : Forestry

A phylogenetic study of the *Shorea* section *Richetioides* (Meranti Damar Hitam) was conducted by using morphological characters and molecular technique. *Shorea* is one of the largest genera in the family of Dipterocarpaceae with an estimated 300 species worldwide and there are more than 150 species in Malaysia, of which more than one third is found in Peninsular Malaysia. This genus is distributed from India and Sri Lanka, through western Malaysia, to Philippines and Maluku. The identification of dipterocarps species including *Shorea* based on morphological characters is not an easy task due to similarities of vegetative characters, and taxonomic classification in deipterocarp have not been supported by their monophyletic origins. Therefore, comprehensive analysis using morphology and molecular studies is required to ascertain their status. Nine fresh samples of *Shorea* sect. *Richetioides* were collected from different areas in Peninsular Malaysia and were identified to the species level based on morphological characters. In addition, a new population of *Shorea* with similar feature of *S. kuantanensis* that was found in Terengganu, Peninsular Malaysia was included. *Shorea kuantanensis,* a member of *Shorea* sect. *Richetioides* classified as critically endangered species by IUCN Red List and was presumably extinct. This study will clarify the identity and providing a taxonomic evaluation of members in *Shorea* sect. *Richetioides* in Peninsular Malaysia because to date, there is no study on the phylogenetic relationship among all *Shorea* species within *Shorea* sect. *Richetioides.*

Morphological and molecular analyses were conducted for *Shorea* sect. *Richetioides* together with other sections in *Shorea*. Morphological anaylsis was done using distance method of Neighbor Joining Method (NJ), and molecular analysis was carried out using two phylogenetic analyses methods, NJ and Maximum Parsimony (MP).

Two non-coding regions from chloroplast (*trn*L intron, and *trn*L-F IGS) were used to construct the phylogenetic trees and rooted with genus *Hopea*. The morphological analysis confirmed that the newly rediscovered population of *Shorea* species is *S. kuantanensis*, and part of monophyletic group of *Shorea* sect. *Richetioides*. The monophyly of groups in *Shorea* is also consistent with the artificial grouping of wood in *Shorea* i.e., Red Meranti, White Meranti, Balau and Yellow Meranti (*Meranti Damar Hitam*). Phylogenetic analysis using molecular data also showed similar results on the monophyly of *Shorea* sect. *Richetioides.* The use of cpDNA (*trn*L intron and *trn*L-F IGS) has provided good resolution in phylogenetic trees and also confirm that the newly found population of *Shorea* as *S. kuantanensis* as been found with the morphological analysis.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

MORFOLOGI DAN SISTEMATIK MOLEKUL *Shorea* SEKSYEN *Richetoides* (DIPTEROCARPACEAE) DI SEMENANJUNG MALAYSIA

Oleh

IKHLAS HUSSAIN ALWAN

November 2017

Pengerusi : Profesor Madya M. Nazre Saleh, PhD
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Kajian filogenetik *Shorea* seksyen *Richetioides* (Meranti Damar Hitam) dilakukan dengan menggunakan ciri morfologi dan kaedah molekul. *Shorea* adalah salah satu genera terbesar dalam keluarga Dipterocarpaceae dengan kira-kira 300 spesies di seluruh dunia dan terdapat lebih daripada 150 spesies di Malaysia, di mana lebih daripada satu pertiga ditemui di Semenanjung Malaysia. Genus ini tertabur dari India dan Sri Lanka, melalui Malaysia barat, ke Filipina dan Maluku. Pengecaman spesies dipterokarp termasuk *Shorea* berdasarkan karakter morfologi bukanlah tugas yang mudah kerana persamman dalam ciri vegetatif, dan pengelasan taksonomi dalam dipterokarp tidak disokong oleh leluhur monofiletik. Oleh itu, analisis menyeluruh menggunakan kajian morfologi dan molekul diperlukan bagi menentukan status spesies ini. Sembilan sampel segar dari *Shorea* seksyen *Richetioides* dikutip dari beberapa kawasan di Semenanjung Malaysia dan dicamkan ke peringkat spesies berdasarkan ciri morfologi. Di samping itu, satu populasi *Shorea* yang mempunyai ciri serupa dengan *S. kuatanensis* ditemui di Terengganu, Semenanjung Malaysia turut dikaji. *Shorea kuantanensis* merupakan ahli *Shorea* seksy. *Richetioides* dikategorikan sebagai spesies terancam dalam Senarai Merah IUCN dan dikatakan mungkin telah pupus. Kajian ini cuba untuk menjelaskan identiti dan memberikan penilaian taksonomi *Shorea* seksy. *Richetioides* di Semenanjung Malaysia kerana sehingga kini tiada kajian perhubungan filogeni yang telah dijalankan pada ahli *Shorea* seksy. *Richetioides.*

Analisis morfologi dan molecular dijalankan pada *Shorea* seksy. *Richetioides* termasuk beberapa lagi seksyen yang ada pada *Shorea*. Analisis morfologi menggunakan kaedah perbezaan jarak iaitu kaedah *Neighbor Joining* (NJ) an analisis molekular dengan kaedah Parsimoni Maksima (MP). Dua kawasan bukan berkod dari kloroplas DNA (*trn*L intron dan *trn*L-F IGS) digunakan bagi membina pokok filogeni yang ditambat dengan genus *Hopea*. Analisis morfologi mengesahkan bahawa populasi *Shorea* yang baru dijumpai adalah spesies *S. kuantanensis* yang merupakan sebahagian daripada kumpulan monofiletik *Shorea* seksy. *Richetioides*. Kumpulan monofiletik dalam genus *Shorea* adalah konsisten dengan pengelasan artifisial pada kumpulan kayu dalam *Shorea* iaitu Meranti Merah, Meranti Putih, Balau dan Meranti Kuning (Meranti Damar Hitam). Analisis filogeni menggunakan data molekular pula memberikan keputusan yang sama dengan monofili *Shorea* seksy. *Richetioides*. Penggunaan cpDNA (*trn*L intron dan *trn*L-F IGS) telah memberikan resolusi yang baik dalam pokok filogeni dan mengesahkan yang populasi baru dijumpai *Shorea* adalah *S. kuantanensis* sama seperti keputusan dalam analisis morfologi.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of Supervisory Committee were as follows:

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

µg/ml	Microgram per Milliliter
µl	Microliter
BLAST	Basic Local Alignment Search Tool
bp	Base Pairs
cpDNA	Chloroplast DNA
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic acid
EtBr	Ethidium bromide
g	Gravitational force
IGS	Intergenic Spacer
Indel	Insertion / Deletion
IUCN	International Union for Conservation of Nature
KEP	Kepong Herbarium
MEGA	Molecular Evolutionary Genetics Analysis
ML	Maximum Likelihood
ml	Milliliter
MP	maximum parsimony
NCBI	National Center for Biotechnology Information
NHN-*L*	Netherlands- *Leiden* university branch
NJ	Neighbor-Joining
nm	nanometer
OTUs	Operational Taxonomic Units
PAUP	Phylogenetic Analysis Using Pasimony
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RNase A	Ribonuclease A
Taq	*Thermus aquaticus* Super Therm DNA Polymerase
TBE	Tris borate EDTA
UKM	University Kebangsaan Malaysia

UPGMA	Unweighted Pair Group Method with Arithmetic Mean
UPM	Universiti Putra Malaysia
UV	Ultra Violet
w/v	Weight per volume

CHAPTER 1

INTRODUCTION

Taxonomy has been in development for more than 250 years, but according to Costello, *et al*., (2013), only about 1.5 million species have been identified. Thus, it can be stated that despite the introduction of the modern taxonomic system by Carolus Linnaeus in the mid-1700s, only about 86% of the species on earth are identified (Mora *et al*., 2011; Stork, 1993). Typically, species identification can be accomplished by classical taxonomic practices, mainly based on morphological characters by using dichotomous keys, or by morphological comparisons with herbarium specimens, besides working with experienced botanists. Indeed, plant identification requires experience, technical skills and knowledge on native flora including new and exotic species (Newmaster *et al*., 2009; Funk *et al*., 2005).

Apart from relying on morphological traits, plant taxonomists have progressively used DNA markers with many studies conducted on the reliability of molecular genetic methods to identify plant species (Nuroniah *et al*., 2010; Finkeldey *et al*., 2010; Wesselink and Kuiper, 2008). For the past 20 years, the rapid development of DNA sequencing technologies has further allowed resolving the problems of species identification, especially for tropical rainforest plants. Southeast Asia tropical rainforests are distinguished by high species diversity of trees (Morley, 2000). Within those forest communities, Bawa (1998) pointed out that the family of Dipterocarpaceae dominated a large area of tropical rainforest, with 92% of the area and 510 species mainly restricted to Asia.

Dipterocarpaceae is an important pantropical large tree family that plays essential roles in the international timber industry and in other wood products from the tropical rainforests of Southeast Asia. The evergreen rainforests of Peninsular Malaysia, Sumatra and Kalimantan (Borneo) have been seen showing the highest species diversity and abundance of Dipterocarpaceae (Ashton, 1982; Symington, 1943). In the past, the conservation of dipterocarps was not highlighted since they were considered as a common species that are not threatened. Nevertheless, Saw and Sam (2000) mentioned in their study that there over 57% of this species were observed to have distribution patterns restricted to Peninsular Malaysia. In addition to that, there are 30 species that are endemic to Peninsular Malaysia with 12 of them considered rare. It is worth mentioning that many rare plants are endangered due to their small population. However, the identification of the dominant trees in a tropical rain forest, particularly dipterocarps, is not an easy task. Standard morphological characters, such as shape of the canopy, height of the tree, shape and size of bole and buttress, texture and colour of bark pattern, and the typical shape size of leaf shape, are not enough to recognize Dipterocarpaceae species because of the sheer size, similar shapes and near-identical leaf morphologies displayed in several genera of this family. Likewise, dipterocarps trees have infrequent flowering and fruiting seasons. In addition, Symington (1943) mentioned that most forest characters vary with the age of the tree, and sometimes with the habitat. These specific characteristics are frequently undeveloped before maturity, and may be atypical after senility has commenced, or when the tree has been subjected to abnormal environmental conditions. Moreover, it is frequently difficult to decide whether any given tree is mature or senile, and whether it has grown under normal conditions.

1.1 Problem Statement

Massive loss of valuable plant species in the past centuries and its adverse impact on environmental and socioeconomic values has triggered the conservation of plant resources. Appropriate identification and characterization of plant materials is essential for the successful conservation of plant resources and to ensure their sustainable use. Molecular tools developed in the past few years provide easy, less laborious means for assigning known and unknown plant taxa. These techniques answer many new evolutionary and taxonomic questions, which was not previously possible with only phenotypic methods.

The Meranti Damar Hitam group (*Shorea* section *Richetioides*) is one of the sections in Dipterocarpaceae, and can be found in Peninsular Malaysia, with (10) species, namely *S. balanocarpoides* Sym., *S. blumutensis* Foxw., *S. hopeifolia* (Heim) Sym., *S. faguetiana* Heim., *S. gibbosa* Brandis, *S. longisperma* Roxb., *S. maxima* (King) Sym., *S. multiflora* (Burck) Sym., *S. peltata* Sym., and *S. kuantanensis.* Only a few studies have been done on the *Shorea* section *Richetioides* relationship in Peninsular Malaysia, and the research was mainly restricted to taxonomy by Symington (1943) and Ashton (1982). Thus; information and understanding of the native *Shorea* section *Richetioides* is still insufficient, particularly on the phylogeny of the species. The much-developed area in Peninsular Malaysia and difficulties in accessing the forests are the main factors that have led to a lack of records.

According to the IUCN Red List (www.iucnredlist.org), *S. kuantanensis* is a critically endangered endemic species in Peninsular Malaysia, and many consider that *S. kuantanensis* is already an extinct species. This is because the type of specimens and the only specimens collected of *S. kuantanensis* were collected in the forest of Bukit Goh, Kuantan, in Peninsular Malaysia, which was fully converted into palm oil plantation. However, during a forest trip in one of the confined forests in Terengganu, the staff from the Forest Department of Peninsular Malaysia found a population of *Shorea* that resembles *S. kuantanensis* with a few variations from the type of materials that were encountered.

Molecular studies in particular phylogenetic relationships may play an important role in confirmation of the identity of the newly rediscovered population of so-called *S. kuantanensis.* This is because the molecular markers-based on DNA analysis will provide much more powerful and convenient means for distinguishing the different species of *Shorea* for both endemic and more commonly widespread species and help to understand the phylogeny and relationship of the species.

1.2 Justification of Study

With the increasing rate of environmental degradation and current issues on biodiversity, the documentation of the country's flora is deemed important before they disappear or become totally unknown to science. Basic data on flora, which especially employs systematic studies, is useful and fundamental to many fields.

1.3 Objectives of Study

The primary goal of the present study was use of nucleotide sequences of two non-coding regions in Chloroplast DNA along with a set of morphological characters to construct a phylogenetic tree of *Shorea* species of section *Richetioides* in Peninsular Malaysia to provides a working set of relationships that can be used in future studies to address questions of character evolution within the study group.

The objectives of the study are:

I. To identify species of genus *Shorea* section *Richetioides* in Peninsular Malaysia.
II. To study the relationships among section *Richetioides* species by using numerical taxonomy and molecular approach.
III. To examine the identity of newly rediscovered population of thought to be extinct of *Shorea kuantanensis* and its relationship with *Shorea* section *Richetioides*.

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