

## Table of Content

Content	Page
<b>Preface</b>	
Abstract.....	ii
Acknowledgements.....	iv
Table of Content.....	vi
List of Figures.....	ix
List of Tables.....	x
List of Abbreviations.....	xi
<b>Chapter One: Introduction &amp; Literature review</b>	
<b>Introduction.....</b>	<b>1</b>
1.1 Research objectives.....	6
<b>Literature Review.....</b>	<b>7</b>
1.2 The genus <i>Alpinia</i> Roxb. - A general perspective.....	7
1.3 Taxonomic History of the Genus <i>Alpinia</i> Roxb.....	9
1.4 Taxonomic Ambiguities and Biodiversity <i>Alpinia</i> in Peninsular Malaysia.....	11
1.5 Phylogenetic of the Genus <i>Alpinia</i> .....	15
<b>Chapter Two: Materials &amp; Methods</b>	
2.1 Field work and specimen collections.....	21
2.1.1 Sampling strategy and sampling sites.....	21
2.2 Laboratory work.....	22
2.2.1 Voucher specimens for herbarium collection.....	22
2.2.2 DNA extraction of plant.....	22
2.2.3 PCR Target Regions of the Internal Transcribed Spacer (ITS) region and maturase K ( <i>matK</i> ) chloroplast gene .....	23
2.2.4 PCR amplification of nuclear ITS region.....	24
2.2.5 PCR amplification of <i>matK</i> region.....	25
2.2.6 Purification of PCR products and DNA sequencing.....	26

<b>Content</b>	<b>Page</b>
2.3 Molecular Sequence Analysis.....	26
2.3.1 Construction of contig sequences.....	26
2.3.2 Alignment of DNA sequences.....	26
2.4 Phylogenetic Analysis.....	27
2.4.1 Choosing the evolutionary model.....	28
2.4.2 Construction of Neighbour Joining (NJ) tree.....	28
2.4.3 Construction of Maximum Parsimony (MP) tree.....	29
2.4.4 Construction of Bayesian tree.....	29
2.4.5 Sequence divergence.....	30
<b>Chapter Three: Results</b>	
3.1 Specimen collection.....	32
3.1.1 Brief species description.....	37
3.2 Sequence variation analysis.....	92
3.2.1 Molecular characterization of ITS sequences.....	97
3.2.2 Molecular characterization of <i>matK</i> sequences.....	97
3.3 Phylogenetic relationships of <i>Alpinia</i> species based on ITS and <i>matK</i> .....	103
3.3.1 Model of evolution.....	103
3.3.2 Phylogenetic trees.....	104
<b>Chapter Four: Discussion &amp; Conclusion</b>	
<b>Discussion</b> .....	111
4.1 Molecular characteristics of internal transcribed spacer (ITS) region and maturase K ( <i>matK</i> ) gene.....	111
4.2 Phylogenetic incongruence of <i>Alpinia</i> in Peninsular Malaysia.....	112
4.2.1 Clade II (The <i>Galanga</i> clade).....	114
4.2.2 Clade IV (The <i>Zerumbet</i> clade).....	115
4.2.3 Clade VI (The <i>Rafflesiana</i> clade).....	118
4.3 General status of <i>Alpinia</i> diversity in Peninsular Malaysia.....	122
<b>Conclusion</b> .....	125
4.4 Future directions and suggestions.....	126

<b>Content</b>	<b>Page</b>
 <b>Appendices</b>	
Appendix 1.0 Chemicals, buffers (for DNA extraction and PCR) and oligonucleotide primers.....	127
Appendix 2.0 Gel photos.....	133
Appendix 3.0 Reference sequences for ITS and <i>matK</i> phylogenetic trees.....	134
Appendix 4.0 Parsimony-Informative sites for ITS and <i>matK</i> sequences.....	136
Appendix 5.0 Phylogenetic trees.....	149
 <b>References</b> .....	 153
 <b>Presentation note</b> .....	 168

## List of Figures

Figures		Page
Figure 1.1	Infrageneric classification of <i>Alpinia</i> according to Smith (1990)..	2
Figure 1.2	The condensed tree of the genus <i>Alpinia</i> produced several clades	4
Figure 1.3	Distribution map of the genus <i>Alpinia</i> following Smith (1950)...	10
Figure 1.4	World map of terrestrial ecoregion.....	14
Figure 1.5	A slanted cladogram showing the type of tree branch.....	16
Figure 1.6	Diagrammatic representation of the Internal Transcribed Spacer (ITS) region located between 18S rDNA and 26S rDNA.....	17
Figure 1.7	Diagrammatic representation of <i>matK</i> gene located between <i>trnK</i> 5' and <i>trnK</i> 3' .....	18
Figure 2.1	Amplification and sequencing strategy of the nuclear ITS gene...	24
Figure 2.2	Amplification and sequencing strategy of the chloroplast <i>matK</i> gene.....	25
Figure 3.1	Map of <i>Alpinia</i> collection from 21 locations in Peninsular Malaysia.....	36
Figure 3.2	Comparison of the distribution and levels of variability (parsimony-informative sites, Pi) between internal transcribed spacer region (ITS) and maturase K gene ( <i>matK</i> ).....	99
Figure 3.3	Bayesian phylogram of 38 species of <i>Alpinia</i> showing six clades (I-VI) as inferred from internal transcribed spacer region (ITS).....	107
Figure 3.4	Neighbour-Joining phylogram of 35 species of <i>Alpinia</i> showing six clades (I-VI) as inferred from maturase K gene ( <i>matK</i> ).....	110

## List of Tables

<b>Tables</b>		<b>Page</b>
Table 2.1	List of species and corresponding Genbank accession numbers for internal transcribed spacer region (ITS) and maturase K ( <i>matK</i> ) gene sequences for construction of phylogenetic trees.....	31
Table 3.1	List of species collected.....	33
Table 3.2	List of species and the generated sequences of internal transcribed spacer region (ITS) and maturase K ( <i>matK</i> ) with their corresponding Genbank accession number.....	92
Table 3.3	Frequencies and proportion of variable and parsimony-informative sites found in ITS and <i>matK</i> sequences.....	98
Table 3.4	Composition of nucleotides and their percentage (%) in ITS sequences.....	100
Table 3.5	Composition of nucleotides and their percentage (%) in <i>matK</i> sequences.....	101
Table 3.6	Base frequencies found in ITS and <i>matK</i> sequences.....	102
Table 3.7	ITS sequence divergence (%) within/between clades.....	105
Table 3.8	<i>matK</i> sequence divergence (%) within/between clades.....	109

## List of Abbreviations

~	Approximately
%	Percentage
β	Beta
°C	°C
±	Plus minus
μl	Micro litre
μM	Micromolar
A <sub>260</sub>	Absorbance at wavelength of 260 nm
A <sub>280</sub>	Absorbance at wavelength of 280 nm
bp	Base pair
BI	Bayesian inference
BSA	Bovine serum albumin
CTAB	Hexadexyltrimethylammonium bromide
dNTP	Deoxyribonucleotide triphosphate
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic acid
GPS	Global Positioning System
ITS	Internal Transcribed Spacer
kb	Kilobase pair
<i>matK</i>	maturase K
MEGA	Molecular Evolutionary Genetics Analysis
MEGABLAST	MEGA Basic Local Alignment Search Tool
MgCl <sub>2</sub>	Magnesium chloride
mM	Milimolar
MP	Maximum Parsimony
ng	Nanogram
O.D.	Optical density
PAUP	Phylogenetic Analysis using Parsimony
PVP	Polyvinylpyrrolidone
rpm	Revolutions per minute
PCR	Polymerase chain reaction
pH	Power of hydrogen
s	Second
TBE	Tris-Borate EDTA
U	Unit
w/v	Weight per volume