

***THIS THESIS IS DEDICATED TO MY PARENTS.
WORDS CANNOT DESCRIBE YOUR LOVE, SUPPORT
AND ENCOURAGEMENT THROUGHOUT.***

CONTENTS

| | |
|------------------------------|------|
| DEDICATION | iii |
| CONTENTS | iv |
| LIST OF FIGURES | vii |
| LIST OF TABLES | viii |
| LIST OF ABBREVIATIONS | ix |
| ACKNOWLEDGEMENTS | x |
| ABSTRACT | xii |
| ABSTRAK | xiii |

CHAPTER 1: INTRODUCTION

| | |
|--|---|
| 1.1 General introduction and taxonomic complexities in <i>Fagraea sensu lato</i> | 1 |
| 1.2 Scope of work and objectives | 3 |

CHAPTER 2: LITERATURE REVIEW

| | |
|--|----|
| 2.1 Taxonomic History of <i>Fagraea</i> | |
| 2.1.1 Relevant taxonomic documentation | 5 |
| 2.1.2 From Loganiaceae to Gentianaceae: familial classification and taxonomical position of <i>Fagraea</i> and the Potalieae | 5 |
| 2.1.3 Sectional classification of <i>Fagraea</i> | 8 |
| 2.1.4 Alternative generic interpretations | 10 |
| 2.1.5 Leenhouts' broad species concepts | 11 |
| 2.1.6 Malayan <i>Fagraea</i> | 12 |
| 2.2 Taxonomically important morphological characters | |
| 2.2.1 Stem prickliness | 14 |
| 2.2.2 Leaf shape | 14 |
| 2.2.3 Leaf margin | 15 |
| 2.2.4 Petiole-base appendages | 15 |
| 2.2.5 Inflorescence position and form | 17 |
| 2.2.6 Floral size, involucre bracts, corolla and stamens | 18 |
| 2.2.7 Palynology | 19 |
| 2.2.8 Ovary structure and placentation | 21 |
| 2.2.9 Stigmatic structure | 21 |
| 2.2.10 Fruit form and size | 22 |
| 2.2.11 Seed characters | 22 |
| 2.3 Biological and ecological aspects | |
| 2.3.1 Distribution and habitat diversity | 23 |
| 2.3.2 Growth habit | 24 |
| 2.3.3 Vegetative growth: architecture, growth rates | 25 |

| | |
|--|----|
| 2.3.4 Myrmecophily and extra-floral nectaries | 26 |
| 2.3.5 Reproductive biology and ecology | 26 |
| 2.4 Present and traditional uses | 27 |
| CHAPTER 3: MATERIALS AND METHODS | |
| 3.1 Herbarium materials and studies | 30 |
| 3.2 Field collections and processing | 30 |
| 3.3 Photography | 32 |
| 3.4 Phylogenetic studies | |
| 3.4.1 Collection of materials | 32 |
| 3.4.2 DNA extraction | 32 |
| 3.4.3 Gene regions and primers | 34 |
| 3.4.4 Polymerase Chain Reaction (PCR) | |
| 3.4.4.1 Conditions used for priming ITS, <i>trn</i> L-F and <i>ndhF</i> regions | 35 |
| 3.4.4.2 Adjustments and Techniques used for 'difficult' specimens | 36 |
| 3.4.4.3 Sequencing PCR products and data authentication | 36 |
| 3.4.5 Phylogenetic analyses | |
| 3.4.5.1 Sequence alignment | 37 |
| 3.4.5.2 Phylogenetic assessments | 37 |
| 3.4.5.3 Test of data partition incongruence | 41 |
| CHAPTER 4: RESULTS AND DISCUSSION | |
| 4.1 Morphology | |
| 4.1.1 Growth habit and architecture | 42 |
| 4.1.2 Vegetative morphology | |
| 4.1.2.1 Trunks/stems | 49 |
| 4.1.2.2 Leaves | 54 |
| 4.1.2.3 Petiolar sheaths | 54 |
| 4.1.3 Inflorescence structure | 55 |
| 4.1.4 Flowers | 59 |
| 4.1.5 Fruits | 60 |
| 4.1.6 Seeds | 61 |
| 4.1.7 Clonal growth from root suckers | 63 |
| 4.2 Molecular Analysis | |
| 4.2.1 Sequence variation | 65 |
| 4.2.2 Phylogenetic analyses | 66 |
| 4.2.2.1 Analyses of the ITS data set | 67 |
| 4.2.2.2 Analyses of the <i>trn</i> L-F data set | 69 |

| | |
|---|-----|
| 4.2.2.3 Analyses of the <i>ndhF</i> data set | 72 |
| 4.2.2.4 Analyses of the combined ITS, <i>trnL-F</i> and <i>ndhF</i> data sets | 74 |
| 4.2.2.5 Analyses of the expanded ITS data set | 76 |
| 4.3 Implications of the molecular analyses | |
| 4.3.1 Correspondence to named sections within <i>Fagraea s.l.</i> | 79 |
| 4.3.2 Generic delimitations of <i>Fagraea s.l.</i> | 80 |
| 4.3.3 Species concepts in <i>Fagraea s.l.</i> | 81 |
| 4.3.4 Morphological characters for identifying phylogenetic clades | 82 |
| 4.3.5 The <i>Fagraea</i> clade (<i>Fagraea s.s.</i> excluding <i>F. crenulata</i>) | 83 |
| 4.3.6 The Gigantea clade | 84 |
| 4.3.7 The Racemosa clade | 84 |
| 4.3.8 <i>Fagraea crenulata</i> compared with <i>Fagraea s.s.</i> | 88 |
| 4.3.9 The Gigantea and Elliptica clades share homoplasious character-states | 89 |
| 4.4 Taxonomic framework | |
| 4.4.1 Generic concepts in <i>Fagraea s.l.</i> | 90 |
| 4.4.2 Key to genera formerly placed in <i>Fagraea sensu lato</i> | 92 |
| 4.4.3 Enumeration of the Peninsular Malaysian taxa | 93 |
| CHAPTER 5: CONCLUSIONS | |
| 5.1 <i>Fagraea</i> : complex genus or several genera? | 210 |
| 5.2 Limitations of generic concepts | 212 |
| 5.3 Comparison between Borneo and Peninsular Malaysia and biogeographical insights | 213 |
| 5.4 Useful characters for delimiting genera in <i>Fagraea s.l.</i> | 216 |
| 5.5 Summary of future directions | 216 |
| REFERENCES | 218 |
| APPENDICES | 231 |

LIST OF FIGURES

| Figures | | Page |
|---------|--|------|
| 1 | Aerial roots of hemi-epiphytic <i>Fagraea</i> . | 43 |
| 2 | Aubréville's architectural model in <i>Fagraea fragrans</i> . | 45 |
| 3 | Continuous orthotropic trunk growth in <i>Fagraea volubilis</i> following Roux's model. | 46 |
| 4 | Axillary flowering on branch segments in <i>Fagraea fragrans</i> . | 48 |
| 5 | Orthotropic branch-complexes with shoots terminated by cymes. | 50 |
| 6 | Plagiotropic branches of the section <i>Racemosae</i> . | 51 |
| 7 | The sympodial growth of branches by substitution after terminal flowering of the preceding shoot in <i>Fagraea nervosa</i> . | 52 |
| 8 | Bark characteristics of <i>Fagraea</i> . | 53 |
| 9 | Petiolar sheath characteristics in <i>Fagraea</i> . | 56 |
| 10 | Inflorescence rachises may be conspicuous or not in <i>Fagraea</i> section <i>Racemosae</i> . | 58 |
| 11 | An assembly of the five calyx lobes and two pairs of bracts removed from a fresh flower bud of <i>Fagraea resinosa</i> . | 62 |
| 12 | Fruit surface characteristics of <i>Fagraea</i> after drying. | 62 |
| 13 | Seed shapes in <i>Fagraea</i> . | 64 |
| 14 | Root suckers establishing from superficial lateral roots in <i>Fagraea auriculata</i> . | 64 |
| 15 | Strict consensus of 3520 equally parsimonious trees based on the ITS sequence data. | 68 |
| 16 | Strict consensus of 7224 equally parsimonious trees based on the <i>trnL</i> -F sequence data. | 70 |
| 17 | Single most parsimonious tree based on the <i>ndhF</i> sequence data. | 73 |
| 18 | Strict consensus of four equally parsimonious trees based on the combined ITS, <i>trnL</i> -F and <i>ndhF</i> sequence data. | 75 |
| 19 | Strict consensus of 2145 equally parsimonious trees based on the expanded ITS sequence data. | 77 |
| 20 | <i>Fagraea cameronensis</i> . | 106 |
| 21 | <i>Fagraea carnosae</i> . | 110 |
| 22 | Holotype of <i>Fagraea curtisii</i> King & Gamble var. <i>curtisii</i> . | 120 |
| 23 | Proposed holotype of <i>Fagraea curtisii</i> var. <i>calcareae</i> (Henderson) Wong & Sugumaran. | 123 |
| 24 | <i>Fagraea fraserensis</i> . | 127 |
| 25 | <i>Fagraea gardenioides</i> . | 132 |
| 26 | <i>Fagraea insignis</i> . | 138 |
| 27 | <i>Fagraea larutensis</i> . | 142 |
| 28 | <i>Fagraea latibracteata</i> . | 146 |
| 29 | <i>Fagraea littoralis</i> . | 150 |
| 30 | <i>Utania johorensis</i> . | 186 |
| 31 | <i>Utania nervosa</i> . | 193 |

LIST OF TABLES

| Table | | Page |
|-------|--|------|
| 1 | Current classification of Gentianaceae subtribes <i>Potaliinae</i> , <i>Faroinae</i> and <i>Lisianthiinae</i> within the tribe <i>Potalieae</i> . | 7 |
| 2 | Diagnostic characteristics of three sections in <i>Fagraea</i> according to Leenhouts (1962). | 10 |
| 3 | Voucher numbers and collecting localities for <i>Fagraea</i> taxa collected for the present study. | 33 |
| 4 | List of species included from the GenBank for this study. | 39 |
| 5 | Comparison of key architectural characters among the four tree architectural models found in <i>Fagraea s.l.</i> | 47 |
| 6 | Characteristics of the parsimony-based analyses with individual and combined data sets. | 67 |
| 7 | A comparison of various habit and morphological characters found in distinct groups of <i>Fagraea sensu lato</i> resolving as monophyletic groups in molecular phylogenetic analyses in the present study. | 85 |

LIST OF ABBREVIATIONS

Common words and taxonomic terms

| | |
|----------------------|--|
| c. | about |
| comb. nov. | new combination |
| DNA | deoxyribonucleic acid |
| e.g. | example |
| excl. | excluding |
| Fig. | Figure |
| i.e. | that is |
| <i>nom. illeg.</i> | illegal name |
| <i>nom. invalid.</i> | name invalid |
| <i>nom. nud.</i> | naked name (illegal name) |
| <i>pro parte</i> | partly |
| <i>quoad</i> | as far as is concerned |
| <i>sensu</i> | in the sense of |
| <i>s.l.</i> | <i>sensu lato</i> (in a broad sense) |
| sp. | species |
| <i>s.s.</i> | <i>sensu stricto</i> (in a strict sense) |
| <i>syn.</i> | synonym |
| var. | variety |
| viz. | namely |

Places, units of measurement, collecting information

| | |
|-------------------|-------------------------------------|
| alt. | altitude |
| cm | centimeter(s) |
| ft. | foot (feet) |
| F.R. | Forest Reserve |
| km | kilometer(s) |
| m | meter(s) |
| min | minutes |
| mm | millimeter(s) |
| Mt. | Mount |
| s | seconds |
| <i>sine coll.</i> | without collector |
| <i>s.n.</i> | <i>sine numero</i> (without number) |
| µl | microliter |

Cardinal points and other positions

| | |
|----|------------|
| C | Central |
| E | East |
| N | North |
| NE | North-East |
| NW | North-West |
| S | South |
| SE | South-East |
| SW | South-West |
| W | West |

Developmental stages

| | |
|----|------------|
| FB | Flower Bud |
| FL | Flower |
| FR | Fruit |

ACKNOWLEDGEMENTS

I am very thankful to my supervisor, Professor Wong Khoon Meng, whose encouragement, guidance and support from the beginning of the project until the end has enable me to develop an understanding of the subject. I am especially appreciative of him allowing me to refer to his personal research notes on Malaysian *Fagraea*, which were useful in beginning a number of investigations. I would also like to express my gratitude to Professor Rofina Yasmin, my co-supervisor, for her encouragement and support.

Appreciation is also extended to the Dean of the Faculty of Science, Professor Mohd Sofian Azirun and the Head of the Institute of Biological Sciences, Professor Rosli Hashim, for their support as well as providing the required facilities for the study. My sincere gratitude is also extended to Professor Haji Mohamed Abdul Majid and Professor Amru Nasrullah Boyce (previous Deans) for their encouragement and support since I joined as a staff member in the year 2000. Professor Lim Ah Lan is appreciated for being very kind and generous to me in so many ways, including giving valuable advices.

The Keepers, Directors and Curators of various herbaria, i.e., K, KLU, KEP and SING are thanked for loans of specimens. Staff members of the Sandakan Herbarium (SAN), particularly Dr. Joan Periera, Dr. Robert Ong, Dr. Rueban Nilus, Mr. Postar Miun, Mr. Ahmad Sappan and Mr. Jamirus Jumian are appreciated for their kind support during field trips in Sabah. Appreciation is also extended to staff members of the Brunei Herbarium (BRUN), particularly Dr. Kamariah Abu Salim and Mr. Mohd Arrifin for their assistance during a field trip and their pleasant hospitality in Brunei. Dr. Lena Struwe (Rutgers University) very kindly provided some plant materials as well as sent some important literature which were very useful for the study.

Special appreciation is due to Dr. Chandran Somasundram who kindly allowed me to use various equipment in the post harvest lab. Utmost appreciation is extended to Mr. Daniel Lee Kian Poh who not only did a great job as a research assistant for the project but also constantly helped out in various ways even after the period of employment. My colleague, Mr. Yong Kien Thai has been very patient and kind in sharing his knowledge in the phylogenetic analyses as well as giving other friendly advices. Sincere appreciation is also extended to Mr. Low Yee Wen and Mr. Zulkapli Ibrahim who greatly assisted in field work and obtained materials for DNA work from various places. Ms. Goh Wei Lim helped in some of the lab work and assisted in the sequence analyses work.

Dr. Richard Chung (Kepong Herbarium), Dr. Benjamin van ee (Harvard University Herbaria) Dr. WenHeng Zhang (Harvard University Herbaria) and Dr. Barry J. Conn (National Herbarium of New South Wales, Australia) assisted in sourcing some literature which were very important to the study. Dr. Koichi Kamiya provided some basic assistance in the initial stages of sequence alignment. Dr. Charles Davis (Harvard University Herbaria) looked through the thesis and provided some valuable comments.

Utmost appreciation is also extended to Dr. Vijayndra Madawan who patiently read through most parts of the dissertation and provided valuable comments. He was also very supportive in many other ways since the beginning of this research. Mr. Elango Velautham was generous in his friendly advices and shared some of his thoughts in the phylogenetic analyses.

Zainal Mustapha prepared the wonderful line drawings used in the thesis. Staff and student members of the post harvest lab particularly Mr. N. Doraisamy, Ms. Zuliana Razali and Mr. K. Wijentheran, are appreciated for their support in one way or another. Miss R. Sujatha, from the molecular biology lab very generously provided support for DNA work when it was required.

I wish to acknowledge the encouragement and support given by my friends, viz., Puan Sri Susan Jalaluddin, Dr. Loh Pui Lynn, Dr. Suresh Chandran, Mr. James Kingham, Mr. Tan Kui Sing, Miss Norsham Yaakob, Mr. Khairul Azmi, Mr. I.S. Shanmugaraj, Miss Wong Min May, Mr. Zahid Mohd Said, Mr. Abdul Aziz Othman, Ms. Esmeralda Borges, Ms. Anu Sheela, Miss Chan Mee Leng, Mr. Gary Lim, Miss. Maria L.T. Lardizabal, Ms. Serena Lee, Professor Christian Puff, Dr. David Boufford, Dr. Mathew Klooster, Dr. Ionel Valeriu Grozescu, Ms. Jiwamalar Perumal, Mr. Abdul Majid, Mr. B. Prabakharan, Mr. S. Gopalakrishnan, Mr. S. Thiagarajan, Mr. A. Selvakumar, Mr. P. Saravanan, Mr. N. Sivakumar, Mr. & Mrs. Harindranath, Mr & Mrs. Ramesh Sadiappan, Mr & Mrs. Manimaaran and Mr & Mrs. Ramesh Subramaniam. To my mom and other family members especially my wife, R. Shanti, I record my very special appreciation for their understanding, patience and support.

Finally, financial support for this study was provided by University of Malaya through fundamental research grant, FS 264/2007C.

ABSTRACT

A systematic study of the Peninsular Malaysian species of *Fagraea sensu lato* was undertaken. This was done in the light of recent revisionary work done for Borneo that documented 20 new species and demonstrated that previous species concepts for *Fagraea* were too broad. Parallel to this the distinction of the subgeneric groups recognised as sections, i.e., *Cyrtophyllum*, *Fagraea* and *Racemosae*, were investigated with molecular phylogenetic methods. Representative taxa from the Malay Peninsula and Borneo augmented by sequences from other taxa in the same subtribe and tribe were used in the molecular analysis. Gene sequences from ITS, *trnL*-F (*trnL* intron + *trnL*-F spacer) and *ndhF* were analysed with two methods viz., maximum parsimony and Bayesian analyses. The results indicate that *Fagraea s.l.* includes four well-supported monophyletic groups, with the several gene sequences analysed with two phylogenetic methods. Two of the clades, viz., *Fagraea* and *Racemosa*, could be equated to sections *Fagraea* and *Racemosae*, respectively. The remaining two clades, viz., *Elliptica* and *Gigantea* appear to be parts of the section *Cyrtophyllum*. The *Racemosa* clade had the most morphological synapomorphies, with a distinct plant architecture where trunk growth is continuous and branches are plagiotropic (with distichous leaf arrangement); pendulous inflorescences; and a firm fruit wall with an epidermis that does not detach and wrinkle upon drying. The *Fagraea* clade (excluding *Fagraea crenulata*) has fruits that produce copious creamy pale yellowish latex in the fruit epidermis and fruit wall and have ellipsoid-rounded seeds. In comparison, all the other species of *Fagraea* (including *F. crenulata*) either have no latex or produce small amounts of translucent gummy latex and have polygonal seeds. *F. crenulata* is aberrant in the *Fagraea* clade in having unique characters such as a distinct architectural model, thorny bark and crenulate leaf margins. It is however, related to the *Fagraea* clade in having petiolar sheaths that do not or only slightly fuse at the edges and a peltate stigma structure. Phylogenetic analyses with the ITS region did not include *F. crenulata* in the *Fagraea* clade. However, *F. crenulata* is resolved basal to the *Fagraea* clade with chloroplast gene analyses. The clear split of section *Cyrtophyllum* into the *Gigantea* and *Elliptica* clades was somewhat surprising as these groups have a number of similar morphological features, such as small flowers and much-protruding stamens and styles. In comparison, the other groups are generally distinguishable with bigger flowers and less exerted stamens and styles. The only morphological difference between these two groups is the position of the inflorescence, terminal in *Elliptica* and axillary in *Gigantea*. Recognition of *Fagraea s.l.* as four distinct genera is indicated, as the complex is considered morphologically too divergent to be regarded as a single genus. These correspond to the four clades recognised in the molecular analyses, viz., *Elliptica*, *Fagraea*, *Gigantea* and *Racemosa*, and could adopt *Picrophloeus* Bl., *Fagraea* Thunb. (*sensu stricto*), *Cyrtophyllum* Reinw. ex Bl. and *Utania* G.Don, respectively, as good genus names. The position of *F. crenulata* is doubtful and it is provisionally maintained in *Fagraea s.s.* pending future molecular investigations with a larger taxon sampling over wider geographical context, and the use of further gene regions.

ABSTRAK

Satu kajian sistematik spesies *Fagraea sensu lato* bagi Semenanjung Malaysia telah dijalankan. Kajian ini dijalankan berikutan daripada hasil kajian sebelum ini untuk Borneo dimana 20 spesies baru ditemui dan menunjukkan bahawa konsep spesies terdahulu bagi *Fagraea* adalah terlalu luas. Selari dengan kajian ini, penentuan kumpulan subgenerik dikenali sebagai seksyen *Cyrtophyllum*, *Fagraea* dan *Racemosa* diselidiki dengan kaedah filogenetik molekul. Analisis dijalankan dengan wakil taxa daripada Semenanjung Malaysia dan Borneo bersama-sama dengan jujukan DNA daripada taxa lain dalam kaum yang sama. Tiga kawasan gen, ITS, *trnL-F* dan *ndhF* dengan inferen daripada analisis filogenetik "maximum parsimony" dan "Bayesian" telah digunakan. Keputusan menunjukkan bahawa *Fagraea s.l.* merangkumi empat kumpulan monofilitik. Dua dari kumpulan tersebut, *Fagraea* dan *Racemosa*, masing-masing boleh dikaitkan dengan seksyen *Fagraea* dan *Racemosae*. Dua kumpulan yang selebihnya, *Elliptica* dan *Gigantea*, adalah bahagian dari seksyen *Cyrtophyllum*. Kumpulan *Racemosa* mempunyai kesinambungan morfologi yang terbanyak iaitu, rekabentuk pertumbuhan yang tersendiri di mana pertumbuhan batang utama adalah berterusan dan pertumbuhan dahan adalah "plagiotropic"; jambak bunga yang bergantung; dan dinding buah yang teguh berserta kulit permukaan buah yang tidak boleh dipisahkan atau menjadi kedut bila dikeringkan. Kumpulan *Fagraea* (tidak termasuk *Fagraea crenulata*) mempunyai buah yang mengeluarkan getah berwarna kuning pucat dengan banyak daripada kulit dan dinding buah dan mempunyai biji elliptik-bulat. Kesemua spesies *Fagraea* yang lain (termasuk *F. crenulata*) samada tidak menghasilkan getah atau hanya menghasilkan getah yang lutsinar dan berlekit, dalam kuantiti yang sedikit dan mempunyai biji berbentuk poligon. *F. crenulata* merupakan satu spesies yang janggal dalam kumpulan *Fagraea* dengan mempunyai ciri yang unik seperti rekabentuk pertumbuhan yang tersendiri, batang yang berduri dan lamina daun yang bergerigi. Walau bagaimanapun, ia mempunyai kesamaan dengan kumpulan *Fagraea* di mana upih di hujung tangkai daun tidak bersambungan atau hanya bersambung pada hujung upih sahaja dan mempunyai struktur stigma jenis "peltate". Analisis filogenetik dengan ITS tidak menunjukkan kedudukan *F. crenulata* dalam kumpulan *Fagraea*. Walau bagaimanapun, analisis dengan gen kloroplas menunjukkan kedudukannya dibahagian dasar. Pembahagian yang jelas untuk seksyen *Cyrtophyllum* kepada kumpulan *Elliptica* dan *Gigantea* tidak disangka disebabkan oleh ciri morfologi kedua-dua kumpulan ini yang serupa, seperti bunga yang kecil dan stamen serta stil yang mengunjur keluar dengan ketara. Satu-satunya perbezaan morfologi di antara kedua-dua kumpulan ini adalah kedudukan jambak bunga iaitu, di bahagian hujung dalam *Elliptica* dan di bahagian axil dalam *Gigantea*. Pengenalan *Fagraea s.l.* sebagai empat genus telah ditunjukkan dan dianggap sebagai satu genus yang kompleks dengan ciri-ciri morfologi yang sangat berbeza. Empat kumpulan yang dikenali dalam analisis molekular iaitu *Elliptica*, *Fagraea*, *Gigantea* dan *Racemosa*, masing-masing boleh mengambil nama *Picrophloeus* Bl., *Fagraea* Thunb. (sensu stricto), *Cyrtophyllum* Reinw. ex Bl. dan *Utania* G. Don. Kedudukan *F. crenulata* tidak dapat dipastikan dan ianya diletakkan ke dalam *Fagraea s.s.*, untuk masa kini. Kajian molekular lanjut dengan bilangan taxon yang merangkumi kawasan biogeografi yang lebih luas berserta dengan kawasan gen yang lain perlu dijalankan untuk menentukan kedudukannya dengan lebih tepat.