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

CONTENTS

CHAPTER 3: MATERIALS AND METHODS

CHAPTER 4: RESULTS AND DISCUSSION

LIST OF FIGURES

LIST OF TABLES

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
nom. illeg.	illegal name
nom. invalid.	name invalid
nom. nud.	naked name (illegal name)
pro parte	partly
quoad	as far as is concerned
sensu	in the sense of
s.l.	*sensu lato* (in a broad sense)
sp.	species
s.s.	*sensu stricto* (in a strict sense)
syn.	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
sine coll.	without collector
s.n.	*sine numero* (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 Nasrulhaq 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, *trn*L–F (*trn*L intron + *trn*L–F spacer) and *ndh*F 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 exserted 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, *trn*L–F dan *ndh*F 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.