UNIVERSITI PUTRA MALAYSIA

SYSTEMATIC STUDIES ON GUTTIFERAE JUSS. AND HYPERICACEAE JUSS. OF PENINSULAR MALAYSIA

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Twenty-eight species from 4 genera of Guttiferae and 2 genera of Hypericaceae from the 50-ha Plot of Pasoh Forest Reserve (PFR) Negeri Sembilan and several areas in Peninsular Malaysia were used to investigate the status and relationships within several genera in Guttiferae and the relationship between Guttiferae and Hypericaceae. Molecular and morphological data were used to determine the taxonomic status of these two families. Phylogenetic studies of the Guttiferae and Hypericaceae have so far based on morphological data only. Molecular phylogenetic studies based on the *trn*L-*trn*F spacer of chloroplast DNA supported the latest classification that Guttiferae and Hypericaceae are distinct families. The molecular phylogeny also supported the morphological classification that all *Mesua* taxa in Peninsular Malaysia to be transferred back into genus *Kayea*, except for *M. ferrea*. Genus *Ploiarium* should be excluded from Guttiferae. Genus *Cratoxylum* should be retained in Hypericaceae not as

subfamily Hypericoidea in Guttiferae. But the molecular phylogeny failed to support the morphological classification that merge *Calophyllum wallichianum* var. *wallichianum* and *C. wallichianum* var. *incrassatum* as varieties of *C. wallichianum*. These two varieties should be transferred back as two different species (*Calophyllum wallichianum* and *C. incrassatum*). In general, the sequence data of the *trn*L-*trn*F spacer solved the taxonomic problems within Guttiferae, and between Guttiferae and Hypericaceae. Further analysis of other molecular markers from different genes or genomes should be carried out to ascertain the taxonomic status of these two families. A support of a careful morphological comparison of these families is necessary to give a better picture of the classification of these families.

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KAJIAN SISTEMATIK TERHADAP GUTTIFERAE JUSS. AND HYPERICACEAE JUSS. DI SEMENANJUNG MALAYSIA

Oleh

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Sejumlah 28 species daripada 4 genus famili Guttiferae dan 2 genus daripada famili Hypericaceae telah dipilih dan dikaji untuk penelitian bagi 50-ha plot Hutan Simpan Pasoh (PFR) Negeri Sembilan and beberapa daerah di Semenanjung Malaysia. Berdasarkan bukti-bukti morfologi dan molekular, status dan perhubungan diantara genus dalam famili Guttiferae dan status diantara Guttiferae dan Hypericaceae telah dikaji. Kajian filogenetik dari famili Guttiferae and Hypericaceae yang telah ada hanya menggunakan bukti-bukti morfologi sahaja. Kajian filogenetik ke atas kawasan penjarak *trn*L-*trn*F kloroplas DNA, didapati menyokong klasifikasi terkini bahawa Guttiferae dan Hypericaceae adalah dua famili yang berbeza. Kajian molekular yang dilakukan keatas kawasan penjarak *trn*L-*trn*F tersebut juga menyokong data pengelasan berdasarkan morfologi bahwa semua *Mesua* taxa di Semenanjung Malaysia disarankan

untuk dipindahkankan kembali ke genus *Kayea*, kecuali untuk *M. ferrea*. Genus *Ploiarium* harus dikeluarkan dari famili Guttiferae. Genus *Cratoxylum* tetap dipertahankan didalam famili Hypericaceae bukan sebagai anggota dari subfamili Hypericoideae di dalam Guttiferae. Tetapi kajian molecular gagal menyokong data pengelasan berdasarkan morfologi bahawa *Calophyllum wallichianum* var. *wallichianum* dan *C. wallichianum* var. *incrassatum* adalah varieti dari *C. wallichianum*. Kedua varieti ini disarankan untuk dipindahkan kembali sebagai dua species yang berbeza (*Calophyllum wallichianum* and *C. incrassatum*). Secara umum, turutan data dari kawasan penjarak *trn*L-*trn*F, tampaknya dapat memecahkan masalah taksonomi di dalam famili Guttiferae dan diantara Guttiferae dan Hypericaceae. Analisi yang lebih lanjut perlu dilakukan dengan menggunakan penanda dari genes atau genom yang berbeza untuk lebih memastikan status taksonomi bagi kedua famili ini. Pengamatan morfologi yg lebih teliti juga diperlukan untuk memperolehi gambaran yang lebih jelas tentang posisi kedua famili ini di dalam klasifikasi tersebut.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

The South East Asia region covers about 4.3 million km^2 and contains about 25,000 plant species. Peninsular Malaysia covers about 0.09% of the earth's land surface, and is considered as one of the most abundant flora in the world as it supports 8,500 plant species, of which 2,500 are trees (3.4% of the planetary total) and many of them are endemic species (Myer, 1985).

Guttiferae Juss. (Clusiaceae Lindl. (nom. Altern.)), a medium sized and varied tropical family plays an important role being a component of the Malayan rainforest with trees occupying the main canopy of the forest (Whitmore, 1973). There are 40 genera and ca. 1000 species throughout the tropics, and in Peninsular Malaysia there are 4 - 5 genera with 121 species in all kind of habitats (Keng, 1969; Whitmore, 1973; Corner, 1988; Turner, 1995). However, Hypericaceae Juss. is a small but widespread family, except for the Arctic and desert regions. It consists of 7 genera with about 550 species. The family is represented in Peninsular Malaysia by one introduced yellow flowered weed, *Hypericum japonicum* Thunb. *ex* Murray and by 5 or 6 tree species of the genus *Cratoxylum* Blume which is most characteristic of the Malayan region (Desch, 1957; Kochummen, 1973; Hutchinson, 1973; Robson, 1974; Corner, 1988; Soepadmo and Wong, 1995).

More or less full descriptions of these families were published in the last century by Planchon and Triana (1862). This laid the foundation of knowledge of the families. Kostermans (1961) published a monograph of the Asiatic and Pacific species of *Mammea* L., and Gogelein (1967) wrote a revision of the genus *Cratoxylum* Blume, while Robson (1974) carried out the taxonomic revision of Hypericaceae. In 1977 and 1981 Robson also published his revision of the genus *Hypericum* L. Stevens (1980) published a revision of the old world species of *Calophyllum* L. and Jones (1980) carried out the taxonomic revision of the genus *Garcinia* L. worldwide. For Peninsular Malaysian genera, Ridley (1922) made the first treatment of the family Guttiferae and Hypericaceae; this was followed by Henderson & Wyatt-Smith (1956) and Whitmore (1973). The status of some taxa in Guttiferae and Hypericaceae of Peninsular Malaysia before and after this study is presented in Table 1.1.

1.1 Statement of the Problem

Guttiferae is a medium-sized and varied family. Many systematic problems exist within Guttiferae itself and between Guttiferae and Hypericaceae. Even with the removal of the Hypericaceae from traditional Guttiferae, Guttiferae remains a heterogeneous agglomeration, and should be further segregated into smaller, more natural units (Maguire, 1976). Bessey (1915), Engler and Prantl (1925), Wettstein (1935), Melchior (1964), Cronquist (1981), Thorne (1983) and other authors (Gogelein, 1967; Robson, 1974, 1976, 1977 & 1981; Turner, 1995) placed Hypericaceae into Guttiferae. On the other hand, Bentham (1862), Hutchinson (1969 and 1973), Takhtajan (1987) and other

Table 1.1: Status of some taxa in Guttiferae and Hypericaceae of Peninsular Malaysia before and after this study

No.	Before this study Family/species	After this study Family/species
	Guttiferae	**Guttiferae**
1.	*Calophyllum* depressinervosum Henderson *et* Wyatt-Smith	*Calophyllum depressinervosum* Henderson *et* Wyatt-Smith
2.	*C. dioscurii* P. F. Stevens	*C. dioscurii* P. F. Stevens
3.	*C. macrocarpum* Hook.*f.*	*C. macrocarpum* Hook.*f.*
4.	*C. rupicolum* Ridl.	*C. rupicolum* Ridl.
5.	*C. soulattri* Burm. *f.*	*C. soulattri* Burm. *f.*
6.	*C. tetrapterum* Miq.	*C. tetrapterum* Miq.
7.	*C. wallichianum* var. *wallichianum* (Planch. *et* Triana) P. F. Stevens	*C. wallichianum* Planch. *et* Triana
8.	*C. wallichianum var. incrassatum* (Henderson *et* Wyatt-Smith) P. F. Stevens	*C. incrassatum* Henderson *et* Wyatt-Smith
9.	*Mesua cornerii* Kochummen	*Kayea cornerii* P. F. Stevens
10.	*M. ferrea* L.	*Mesua ferrea* L.
11.	*M. grandis* (King) Kosterm.	*Kayea grandis* King
12.	*M. kunstleri* (King) Kosterm.	*Kayea kunstleri* King
13.	*M. lepidota* Anders.	*Kayea lepidota* Anders.
14.	*M. racemosa* (Planch. *et* Triana) Kosterm.	*Kayea racemosa* Planch. *et* Triana
15.	*Mesua* sp.1	*Kayea* sp.1
16.	*Mammea brevipes* (Craib) Kosterm.	*Mammea brevipes* (Craib) Kosterm.
17.	*M. odorata* (Rafin.) Kosterm.	*M. odorata* (Rafin.) Kosterm.
18.	*M. siamense* (Miq.) Anders.	*M. siamense* (Miq.) Anders.
19.	*M. malayana* Kosterm.	*M. malayana* Kosterm.
		Exclude from Guttiferae
20.	*Ploiarium alternifolium* (Vahl) Melchior	*Ploiarium alternifolium* (Vahl) Melchior
	Hypericaceae	**Hypericaceae**
21.	*Cratoxylum arborescens* (Vahl) Blume	*Cratoxylum arborescens* (Vahl) Blume
22.	*C. cochinchinense* (Lour.) Blume	*C. cochinchinense* (Lour.) Blume
23.	*C. formosum* (Jack) Dyer	*C. formosum* (Jack) Dyer
24.	*C. glaucum* Koth.	*C. glaucum* Koth.
25.	*C. maingayi* Dyer	*C. maingayi* Dyer
26.	*C. sumatranum* (Jack) Blume	*C. sumatranum* (Jack) Blume
	Incompletely known taxa	
27.	*Cratoxylum* sp.1	*Cratoxylum arborescens* (variety)
28.	*Cratoxylum* sp.2	*Cratoxylum formosum* (variety)
29.	*Hypericum japonicum* Thunb. *ex* Murray	*Hypericum japonicum* Thunb. *ex* Murray

authors (Ridley, 1922; Kimura, 1951; Backer, 1963; Keng, 1969; Whitmore, 1972 and 1973; Corner, 1976 and 1988) separated Hypericaceae from Guttiferae (Table 1.2).

Table 1.2: Various taxonomic treatments of Guttiferae and Hypericaceae

	Subclass	Super order	Order	Suborder	Family
Bentham & Hooker (1862)	Polypetalae	Thalamiflorae	Guttiferales	-	Guttiferae Hypericaceae
Bessey (1915)	-	-	Guttiferales	-	Guttiferaceae (incl.Hypericaceae)
Thonner (1917)	Dicotyledoneae (Annonidae)	Theiflorae	Theales	Hypericineae	Guttiferae (incl.Hypericaceae)
Engler & Diels (1936)	Heterochlamydeae	-	Guttiferales	-	Guttiferae Hypericaceae
Melchior (1964)	Archichlamydeae	-	Guttiferales	Theineae	Guttiferae (incl.Hypericaceae)
Hutchinson (1969)	Dicotyledoneae	Lignosae	Guttiferales	-	Guttiferae Hypericaceae
Dahlgren (1980)	Dicotyledoneae	Theiflorae	Theales	-	Guttiferae (incl.Hypericaceae)
Cronquist (1981)	Dilleniidae	-	Theales	-	Guttiferae (incl.Hypericaceae)
Thorne (1983)	Dicotyledoneae	Theiflorae	Theales	Hypericineae	Guttiferae (incl.Hypericaceae)
Takhtajan (1987)	Dilleniidae	Theanae	Theales	-	Guttiferae Hypericaceae
APG (2003)	Eurosid I	-	Malpighiales	-	Guttiferae Hypericaceae

Note: APG: The Angiosperm Phylogeny Group
(-) : Data not available

Apparently, Hypericaceae is closely related to Guttiferae that many authorities did not recognize them as a separate family (Whitmore, 1972 and 1973; Kochummen, 1973). Hypericaceae is usually placed in or close to Guttiferae. Engler (1925), Keller (1925), Melchior (1964), Gogelein (1967), Robson (1977 and 1981) and Cronquist (1981) placed Hypericaceae as subfamily Hypericoideae under Guttiferae. The morphological characters of Guttiferae differ little from those of Hypericaceae. The Hypericaceae have constant bisexual flowers, and very rarely have leaves with numerous close

parallel nerves or the worm-like secretory cells that characterize Guttiferae (Hutchinson, 1973).

Chemical evidence also supports that Hypericaceae is closely related to Guttiferae. Constituents like the uliginosins, euxanthone, mangiferin, celebixanthone and maculaxanthone connect Hypericaceae chemically intimate with Guttiferae (Robson, 1974). From the phytochemical point of view, there is absolutely no need to separate Hypericaceae from Guttiferae (Robson, 1974). Vestal (1937) on the basis of wood anatomy and embryo structure regarded the Hypericaceae and Guttiferae as closely related and seemed to be a logical outgrowth from Guttiferae. However, pollen morphology of most of the Guttiferae species (including Hypericaceae species) is heterogeneous and its diagnostic value at the generic level is limited (Erdtman, 1971).

Some problems also exist within Guttiferae; one of which is the controversial position of *Kayea* and *Mesua*. *Kayea* and *Mesua* are very closely related genera within Guttiferae. Bentham and Hooker (1862), Ridley (1910 and 1922) and Melchior (1964) on the basis of generative characters distinguished *Kayea* from *Mesua*. However, Kostermans (1969) followed by other authors such as Whitmore (1973), Keng (1978), Corner (1988), Chua (1995), Turner (1995) and Kochummen (1997) merged *Kayea* under *Mesua*. On the other hand, Stevens (1974b) and Turner (2000) again separated *Kayea* from *Mesua*.

Another problematic taxon within Guttiferae is the genus *Ploiarium* Koth. Ridley (1922) and Desch (1954) placed *Ploiarium* as a synonym of *Archytaea* Choisy (Theaceae), but Kobuski (1950) separated *Ploiarium* from *Archytaea* (Theaceae). Browne (1955) and Hickey and King (1981) placed *Ploiarium* under Ternstroemiaceae (Theaceae). Turner (1993), however, included *Ploiarium* under family Bonnetiaceae but later transferred again *Ploiarium* to Guttiferae in 1995. Keng (1978) suggested to include *Ploiarium* under Bonnetiaceae, but he put the taxon under Theaceae because of convenience, since only one species was involved. *Ploiarium* is the most primitive and isolated genus in Bonnetioideae (Bonnetiaceae), which has one species (*P. alternifolium* (Vahl) Mechior) in southern Thailand, Malaysia, northern Sumatera and northern Borneo, and another species (*P. sessilis* (Scheffer) Hallier) distributed in extremely western New Guinea (Robson, 1981). Robson (1981) incorporated Bonnetiaceae in Guttiferae as a subfamily Bonnetioideae. Cronquist (1981) argued that Bonnetioideae has a transitional position in classification from Theaceae towards Guttiferae, producing xanthones similar to Guttiferae. Corner (1976) found the exotegmic structure seeds of Bonnetiaceae (*Ploiarium)* to be the same with Guttiferae.

With those prevailing problems, various authors only used morphology and other disciplines to solve the problems, with the exception of the molecular approach. Thus, this project was undertaken to find out if molecular data would support morphological and other data for the inclusion of Hypericaceae in Guttiferae, *Kayea* Wall. in *Mesua* L. or *Ploiarium* in Guttiferae.

1.2 Significance of the Study

Deoxyribose nucleic acid (DNA) is widely recognized as the physical basis of genetic code – the information necessary to construct a new individual. This information is similar in similar organisms. In plants, DNA is also similar in similar species and similar genera. It is a primary source of taxonomic information, as well as being the blue print for development and differentiation. Almost all individuals have a genetic code unique to themselves (Smith, 1976).

Genetic material provides the most basic or fundamental characters that may be employed for purposes of classification and phylogeny, as it is passed on from generation to generation (Crawford, 1990). Morphological characters have their own importance in identification, and a combination of molecular and morphological analyses may improve the result of molecular or morphological analysis alone.

1.3 Objectives of the Study

Morphological characters are sometimes influenced by the environment. Thus, it is better not to use it alone in systematics. The lack of fertile specimens collected is also a major problem in morphological identification, but this problem can be solved with the help of molecular approaches such as DNA sequencing. Species differences could be observed from sterile specimens using DNA sequences and the data obtained could give a clearer picture of the phylogenetic relationship among the species (Nazre, 2000).

This research tries to utilize both molecular and morphological data in order to provide a better description and interpretation of Guttiferae and Hypericaceae, in light that it will be useful for clarifying the systematic problems between these families and within uncertain genera of Guttiferae.