



UNIVERSITI PUTRA MALAYSIA

**MORPHOLOGICAL AND MOLECULAR SYSTEMATICS OF
BULBOPHYLLUM THOU. IN PENINSULAR MALAYSIA**

SHAHLA HOSSEINI

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**DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA**

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By

SHAHLA HOSSEINI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia
In Fulfilment of Requirements for Degree of Doctor of Philosophy**

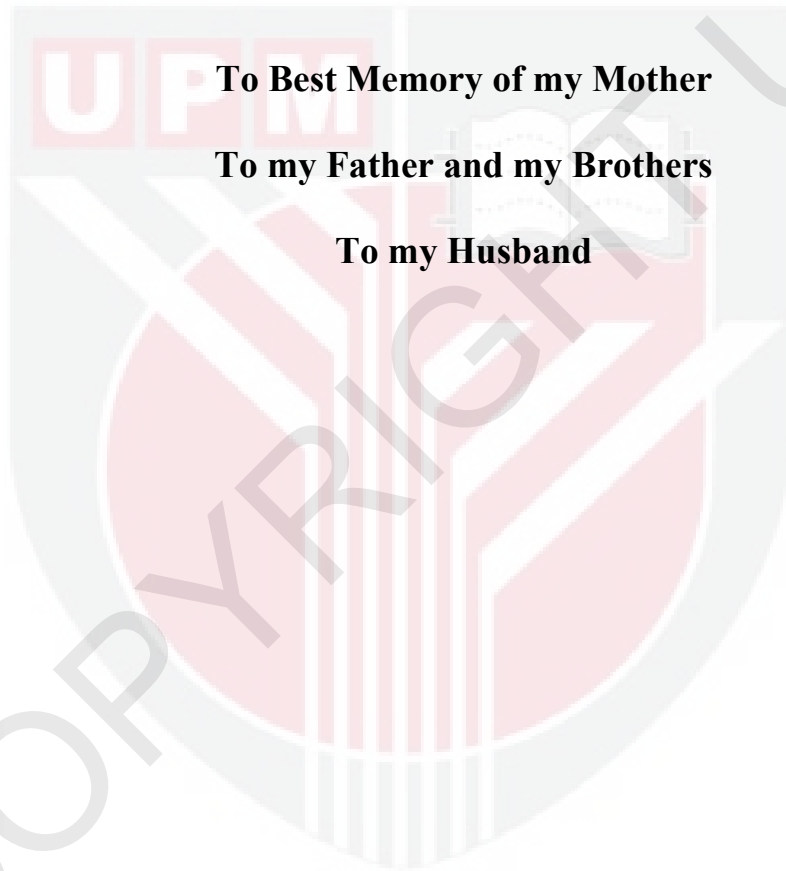
October 2011

Dedicated

To Best Memory of my Mother

To my Father and my Brothers

To my Husband



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Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy.

**MORPHOLOGICAL AND MOLECULAR SYSTEMATICS OF
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SHAHLA HOSSEINI

October 2011

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The largest member of the subtribe *Bulbophyllinae* (Orchidaceae) has been defined as *Bulbophyllum*, a genus which forms a large, pantropical, and poorly studied group of orchids in Peninsular Malaysia. Based on this study, members of this large genus have undergone extreme reduction in number of species in the natural habitat and have acquired efficient adaptation to the canopy environment. In this study 57 *Bulbophyllum* taxa were collected and 48 species were identified to species level, whereas the other 9 species were not identified due to lack of flowering structure but they are separate individual species as confirmed by DNA sequences. The most widely accepted classification system is based exclusively on floral morphology, and no significant molecular studies of *Bulbophyllum* in Peninsular Malaysia have been done. To study the systematics of this genus, structural characters were used together with molecular evidence to generate molecular systematic hypotheses by

using PAUP* 4.0 b 10 and Mr Bayes 3.1.1. The structural portion of this study was performed using both qualitative and quantitative characters of rhizome, pseudobulb, leaf and flower. Phenetic and cladistic analyses were conducted for morphological data of 38 different species and molecular cladistic analyses has been carried out for the 57 taxa with parsimony, likelihood and Bayesian methods. Four nucleotide sequence data sets from two distinct genomes cpDNA genes (*rbcL*, *matK*, *trnL-F*) and nuclear gene (ITS) were used to construct independent cladograms of the *Bulbophyllum* taxa.

The morphological clustering of 11 sections of *Bulbophyllum* with high congruency corresponded with prior descriptions, with the following exceptions: species of sections *Hirtula* and *Cirrhopetalum* divided into two separate clusters. Morphological character analysis placed *B. coniferum* from section *Globiceps* inside section *Aphanobulbon*, and *B. mutabile* from section *Aphanobulbon* inside section *Desmosanthes*. Based on the quantitative results *B. medusae* was placed inside section *Cirrhopetalum* but qualitative characters have supported the status of this species inside section *Desmosanthes* which corresponded with the earlier classification. Cladistic analysis was highly congruent with phenetic result however grouping of the sections was much more corresponded with molecular data.

Separate parsimony analysis as well as combined Bayesian analysis of four gene regions except for *rbcL*, supported a monophyletic status (BP100, PP100) for 12 out of 13 sections of Peninsular Malaysian *Bulbophyllum*. The molecular results

gave strong evidence (BP90-100, PP100) that the generic status of section *Cirrhopetalum* could no longer be supported, as it was deeply embedded within the genus *Bulbophyllum*. This section was a sister group to section *Desmosanthes* in all the different analyses, so it cannot be considered as a separate genus. Section *Hirtula* was the only section with a paraphyletic status. It was divided into *B. dayanum* which is a sister to section *Careyana*; *B. hirtulum* and *B. limbatum* are sister group to section *Aphanobulbon*. The combined molecular data analysis confirmed the status of *B. medusae* as a species of section *Cirrhopetalum*. Significantly, the status of some species through clustering analysis obtained from structural characters was in congruence with the molecular analysis and the species grouping of sections *Sestochilus*, *Epicrianthes*, *Monilibulbus* and *Careyana* was similar with the combined molecular data analysis.

In this study, molecular systematics analysis had provided a robust estimation for the phylogenetic relationships of *Bulbophyllum* which suggested that the Internal Transcribed Spacer (ITS) and cpDNA markers (*matK* and *trnL-F*) with the exception of *rbcL* (with low level of resolution among species) are reliable markers for the phylogenetic studies of *Bulbophyllum* within the studied sections in Peninsular Malaysia.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah.

**MORFOLOGI DAN MOLEKULAR SISTEMATIK BULBOPHYLLUM
THOU. DI SEMENANJUNG MALAYSIA**

Oleh

SHAHLA HOSSEINI

Oktober 2011

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Ahli terbesar subtribe *Bulbophyllinae* (Orchidaceae) telah didefinisikan sebagai *Bulbophyllum*, genus yang merupakan kumpulan orkid yang besar, pantropika, dan kurang dikaji di Semenanjung Malaysia. Berdasarkan kajian ini, ahli-ahli genus yang besar ini telah mengalami penurunan melampau dari segi bilangan species di habitat semulajadi dan telah memperoleh adaptasi yang efisien terhadap persekitaran kanopi. Dalam kajian ini, 57 taksa *Bulbophyllum* telah dikumpul dan 48 species telah dikenalpasti nama ke peringkat species, manakala 9 lagi tidak dapat dikenalpasti kerana tidak mempunyai struktur bunga tetapi mereka adalah species yang berbeza berdasarkan jujukan DNA. Sistem klasifikasi yang paling diterima pakai adalah berdasarkan kepada morfologi bunga, dan tiada kajian molekul *Bulbophyllum* di Semenanjung Malaysia yang signifikan telah dilakukan. Untuk mengkaji sistematik genus ini, ciri-ciri struktur bersama bukti molekul telah

digunakan untuk membina hipotesis sistematik molekul dengan menggunakan PAUP * 4.0 dan Mr Bayesian. Bahagian struktur kajian ini dilakukan dengan menggunakan kedua-dua ciri kualitatif dan kuantitatif rizom, pseudobulb, daun dan bunga untuk 38 spesies yang berbeza. Phenetic and cladistic analyses were conducted for morphological data of 38 different species and molecular cladistic analyses has been carried out for the 57 taxa with parsimony, likelihood and Bayesian methods. Analisis Phenetic dan cladistic telah dijalankan bagi data morfologi 38 spesies yang berbeza dan molekul analisis cladistic telah dijalankan untuk taksa 57 dengan parsimoni, kesamaan dan Bayesian. Empat pasang data jujukan nukleotida daripada dua genom yang berbeza, cpDNA (*rbcL*, *matK*, *trnL-F*) dan gen nukleus (ITS) telah digunakan untuk membina cladograms bebas bagi taksa *Bulbophyllum*.

Pengelompokan morfologi daripada 11 seksyen *Bulbophyllum* dengan kongruensi tinggi didapati berkaitan dengan deskripsi sebelumnya, dengan pengecualian berikut: spesies dalam seksyen *Hirtula* dan *Cirrhopetalum* telah membentuk dua kelompok berasingan. Analisis berdasarkan ciri-ciri morfologi menempatkan *B. coniferum* dari section *Globiceps* ke dalam section *Aphanobulbon* dan *B. mutabile* dari section *Aphanobulbon* ke dalam section *Desmosanthes*. Berdasarkan hasil kuantitatif *B. medusa* telah dimasukkan ke dalam section *Cirrhopetalum* tetapi ciri-ciri kualitatif telah menyokong status spesies ini kekal di dalam section *Desmosanthes* dimana seperti yang dicadangkan dalam klasifikasi lama. Analisis

Cladistic adalah sangat sesuai dengan hasil phenetic bagaimanapun, perkumpulan bahagian adalah lebih sejajar dengan data molekul.

Analisis parsimoni berasingan daripada empat rantau gen kecuali *rbcL*, serta analisis Bayesian gabungan telah menyokong status monofiletik (BP100, PP100) untuk 12 daripada 13 seksyen *Bulbophyllum* di Semenanjung Malaysia. Hasil kajian molekul ini telah memberi bukti yang kukuh (BP90-100, PP100) bahawa status genera seksyen *Cirrhopetalum* tidak lagi disokong, kerana ia sangat jelas terkandung dalam genus *Bulbophyllum*. Section ini merupakan kumpulan saudara kepada section *Desmosanthes* dalam semua analisis yang berlainan, oleh yang demikian, ia tidak boleh dianggap sebagai genus yang berasingan. Section *Hirtula* adalah satu-satunya section dengan status parafiletik, dimana *B. dayanum* merupakan saudara seksyen *Careyana*; *B. hirtulum* dan *B. limbatum* merupakan kumpulan saudara untuk seksyen *Aphanobulbon*. Gabungan analisis data molekul mengesahkan status *B. medusa* sebagai spesies dalam seksyen *Cirrhopetalum*. Adalah nyata bahawa status beberapa spesies melalui hasil analisis pengelompokan yang diperolehi daripada ciri-ciri struktur adalah kongruensi dengan analisis molekul, dan pengelompokan spesies seksyen *Sestochilus*, *Epicrianthes*, *Monilibulbus* dan *Careyana* adalah serupa dengan gabungan analisis data molekul.

Dalam kajian ini, analisis sistematik molekul telah memberikan satu jangkaan yang tepat antara hubungan filogenetik dalam *Bulbophyllum* dan mencadangkan bahawa selain daripada *rbcL* (dengan kadar resolusi yang rendah antara spesies), Internal

Transcript Spacer (ITS) dan penanda cpDNA adalah merupakan penanda yang sesuai untuk kajian filogenetik *Bulbophyllum* dikalangan seksyen yang dikaji di Semenanjung Malaysia.



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I certify that a Thesis Examination Committee has met on 6 October 2011 to conduct the final examination of Shahla Hosseini on her thesis entitled “Morphological and Molecular Systematics of *Bulbophyllum* Thou. In Peninsular Malaysia” in accordance with the Universities and University Colleges Act 1971 and Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Doctor of Philosophy.

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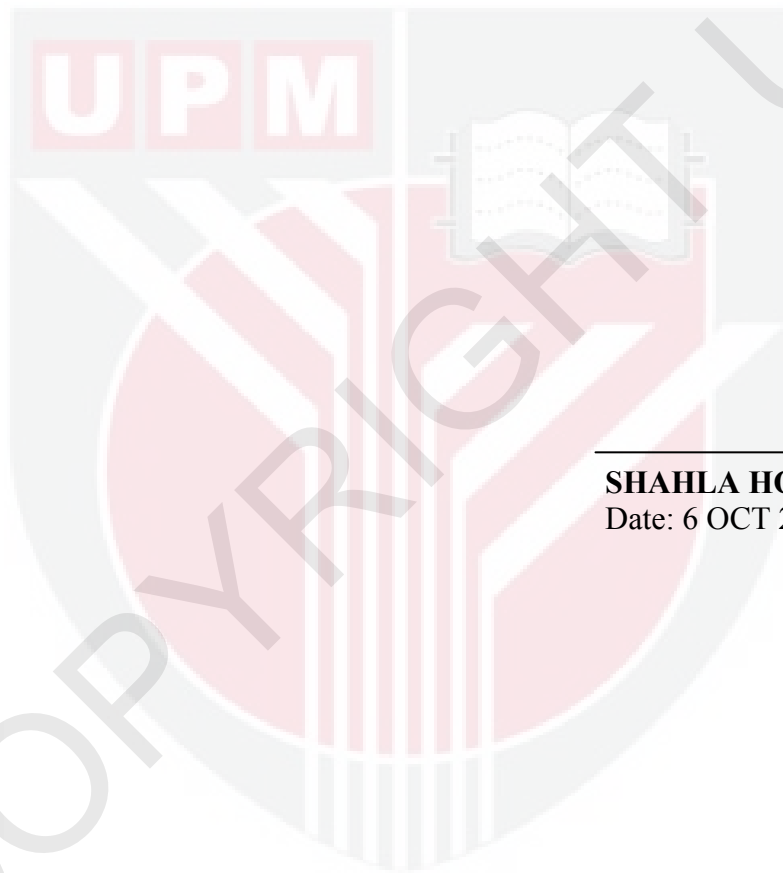
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DECLARATION

I declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at University Putra Malaysia or at any other institution.



SHAHLA HOSSEINI
Date: 6 OCT 2011

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