



UNIVERSITI PUTRA MALAYSIA

**PHYLOGENETICS OF FOUR SECTIONS OF GENUS DENDROBIUM
SW. (ORCHIDACEAE) IN PENINSULAR MALAYSIA**

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**PHYLOGENETICS OF FOUR SECTIONS OF GENUS *DENDROBIUM* SW.
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By

MARYAM MOUDI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
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Chairman: Associate Professor Rusea Go, PhD

Faculty: Science

A phylogenetic study of the four sections (*Aporum*, *Crumenata*, *Strongyle*, and *Bolbidium*) of the genus *Dendrobium* (family Orchidaceae) was conducted by using molecular markers. One of the three largest orchid genera in the family Orchidaceae is the genus *Dendrobium* with an estimated 1580 species worldwide. In Malaysia, there are more than 250 species of which 86 species are found in Peninsular Malaysia. This genus is distributed from South- East Asia, west to the Himalayas, east to Japan, and south to the Pacific Islands, New Zealand and Australia. *Dendrobiums* are one of the most popular orchids for their medicinal and commercial values. These plants favor the tropical regions with high annual rainfall and without a significant dry season for growth. Classifications based on morphological characters have not been able to clearly divide these four sections and neither do they support their monophyletic origins.

Therefore, detailed analysis using molecular data is required to ascertain their status. In this study, 22 fresh samples of the *Dendrobium* species were collected and sixteen of them was identified to the species level, whereas the other six were not able to be identified due to the lack of flowering structures that can aid identification. To date, there is no study on the phylogenetic relationship among species of the four sections of the genus *Dendrobium* in Peninsular Malaysia. Therefore, morphological characters combined with molecular evidence were used to clarify their relationships either to lump them together into one section or to reduce them into two sections or to maintain them in their current sections. Morphological analyses were conducted on the species of the genus *Dendrobium* belonging to the four sections (*Aporum*, *Crumenata*, *Strongyle* and *Bolbidium*) and the other belonging to the three sections (*Dendrobium*, *Callista* and *Latouria*), which are closely related to them. Molecular studies were carried out for the species of the genus *Dendrobium* using two phylogenetic analysis methods, Neighbor Joining Method (NJ) as the Distance method and Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian analysis (BI) as character-based method. Seven nucleotide sequence data sets from three distinct genomes, chloroplast DNA genes (*rbcL*, *matK*, and *trnL-F*), nuclear ribosomal DNA (ITS), and low copy nuclear gene (*Xdh*) were used to construct the cladograms. In addition, the nucleotide sequences of selected genetic markers for some species of the genus *Dendrobium* were also retrieved from the NCBI database to facilitate the analysis.

The morphological analysis showed that sections *Aporum*, *Crumenata*, *Strongyle* and *Bolbidium* formed a monophyletic group compared to three other sections *Dendrobium*, *Callista* and *Lautoria*. The results demonstrated that sections *Aporum*, *Crumenata*, and

Strongyle were close to one another and were grouped into one clade. Although section *Bolbidium* was close to them, it formed a different clade by itself. The three other sections included in the analysis formed a separated clade from these four sections. Molecular data showed similar results that supported the four sections forming a monophyletic group, but the relationships among the sections were different when compared to the morphological result. Through molecular study, phylogenetic trees for chloroplast markers, nuclear gene markers and a combination of these markers were constructed based on different phylogenetic analysis methods (**MP**, **ML**, **BI**) using different softwares (**PAUP*4.0 B 10**, **Mr Bayes 3.1.1.** and **Mega 5**). Individual and combined analysis of the five gene regions (*rbcL*, *matK*, *trnL-F*, nrITS and *Xdh*) supported the monophyletic status (Boot strap Replicate and Posterior Probability more than 90%) of four sections (*Aporum*, *Crumenata*, *Strongyle* and *Bolbidium*) compared to the other three sections (*Dendrobium*, *Callista* and *Latouria*).

Most of the ML and BI trees were congruent, whereas the MP trees showed different results. The phylogenetic trees revealed differences among the four sections, but overall, the molecular analyses showed close relationships between sections *Aporum* and *Strongyle*, and sections *Crumenata* and *Bolbidium*. However, some analyses showed the closeness among three sections *Aporum*, *Strongyle* and *Bolbidium* or among sections *Crumenata*, *Strongyle* and *Bolbidium*. Nevertheless, it was observed that sections *Aporum* and *Strongyle* were grouped together in most of the trees.

The combined results of two gene regions; Internal Transcribed Spacer (ITS) and low copy nuclear gene (*Xdh*) showed that the nuclear genes are more reliable markers for the phylogenetic study of *Dendrobium* compared to the chloroplast DNA with a low level of resolution among the sections. The results from the nuclear markers suggested that the four sections are probably best considered as one section instead of four. Based on the ICBN rules, the name *Aporum* has a priority to be used for this new classification.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan Ijazah Doktor Falsafah

FILOGENETIKS DALAM EMPAT SEKSYEN DALAM GENUS *DENDROBIUM* SW. (ORCHIDACEAE) DI SEMENANJUNG MALAYSIA

Oleh

MARYAM MOUDI

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Pengkajian filogenetik untuk empat seksyen (*Aporum*, *Crumenata*, *Strongyle*, dan *Bolbidium*) dari genus *Dendrobium* (family Orchidaceae) telah dijalankan dengan menggunakan penanda molekul. Satu daripada tiga genera orkid terbesar dalam keluarga Orchidaceae adalah genus *Dendrobium* dengan anggaran 1580 spesis di dunia. Di Malaysia, terdapat lebih daripada 250 spesis di mana 86 spesis telah dijumpai di Semenanjung Malaysia. Genus ini bertabur dari Asia Tenggara, ke barat hingga Himalaya, timur hingga Japan, dan selatan hingga Pulau Pasifik, New Zealand dan Australia. *Dendrobium* merupakan salah satu orkid yang terkenal dengan nilai perubatan dan komersial. Tumbuhan ini lebih menyukai kawasan tropika yang mempunyai jumlah hujan tahunan yang tinggi dan tiada musim kering yang ketara untuk pertumbuhan. Klasifikasi mengikut ciri-ciri morfologi tidak dapat mengasingkan keempat-empat seksyen ini. Ia juga tidak dapat menyokong asal usul monophyly empat seksyen ini. Oleh

itu, analisis yang terperinci dengan data molekul diperlukan untuk menentukan status spesies. Dalam pengkajian ini, 22 *Dendrobium* telah dikumpulkan dan 16 daripada jumlah tersebut telah dikenal pasti sampai tahap spesies, manakala 6 species tidak dapat dikenal pasti kerana kekurangan informasi struktur bunga. Setakat ini, tiada kajian tentang filogenetik empat seksyen yang tersebut di Semenanjung Malaysia. Jadi, ciri-ciri morfologi telah digabungkan dengan bukti dari segi molekul untuk menerangkan hubungan antara spesies dalam seksyen-seksyen tersebut. Dengan cara ini, spesies-spesies *Dendrobium* akan dikumpulkan dalam satu atau dua seksyen atau dikekalkan dalam seksyen asal. Analisis molekul telah dijalankan untuk spesies *Dendrobium* daripada empat seksyen tersebut dan spesies daripada tiga seksyen lain (*Dendrobium*, *Callista* and *Latouria*), yang berkait rapat dengan empat seksyen itu. Kajian dari segi molekul telah dijalankan untuk spesies *Dendrobium* dengan dua filogenetik analisis, iaitu, Neighbor-Joining (NJ) sebagai kaedah penjarakan dan Maximum Parsimony (MP); Maximum Likelihood (ML) dan Bayesian analysis (BI) sebagai kaedah yang berasaskan ciri. Tujuh set data urutan nukleotida daripada tiga genom kloroplas DNA gen yang berbeza (*rbcL*, *matK*, and *trnL-F*), ribosoma nuklear DNA (ITS), dengan nuklear yang bersalinan rendah (*Xdh*) telah digunakan untuk membina *cladograms*. Tambahan pula, urutan nukleotida daripada penanda genetik yang terpilih untuk spesies dari genus *Dendrobium* juga didapatkan dari NCBI untuk memudahkan analisis.

Analisis dari segi morfologi menunjukkan bahawa empat seksyen yang tersenarai: *Aporum*, *Crumenata*, *Strongyle* dan *Bolbidium* membentuk satu kumpulan monofiletik setelah dibandingkan dengan tiga seksyen yang lain, *Dendrobium*, *Callista* dan *Lautoria*. Keputusan ini menunjukkan seksyen *Aporum*, *Crumenata* dan *Strongyle*

adalah berkait rapat antara satu sama lain dan boleh dikumpulkan dalam satu *clade*. Walaupun seksyen *Bolbidium* juga berkait dengan tiga seksyen di atas, ia membentuk satu *clade* yang tersendiri. Tiga seksyen lain yang termasuk dalam analisis membentuk satu *clade* yang lain daripada empat seksyen yang tersebut. Data molekul menunjukkan keputusan serupa yang menyokong pembentukan satu kumpulan monofiletik empat seksyen tersebut. Akan tetapi, hubungan antara seksyen adalah lain daripada keputusan morfologi. Melalui pengkajian molekul, pokok filogenetik untuk penanda kloroplas, penanda gen nuklear dan kombinasi penanda-penanda tersebut telah dibina berdasarkan kaedah analisis filogenetik (**MP, ML, BI**) dengan menggunakan software (**PAUP* 4.0 B 10, Mr Bayes 3.1.1. dan Mega 5**). Analisis individu atau kombinasi dengan monofiletik status yang disokongi oleh lima kawasan gen empat seksyen *Dendrobium* telah dibandingkan dengan tiga section yang lain (Replikasi Bootstrap dan Kebarangkalian Posterior lebih daripada 90%).

Kebanyakan pokok ML dan BI adalah kongruen manakala pokok MP menunjukkan keputusan yang berbeza. Pokok filogenetik telah mendedahkan perbezaan antara empat seksyen, tetapi pada keseluruhannya, analisis molekul mencadangkan hubungan yang rapat antara seksyen *Aporum* dengan *Strongyle*, dan seksyen *Crumenata* dan *Bolbidium*. Walau bagaimanapun, sesetengah analisis menunjukkan kerapatan antara seksyen *Aporum*, *Strongyle* dan *Bolbidium* atau antara seksyen *Crumenata*, *Strongyle* dan *Bolbidium*. Namun, daripada pemerhatian kami, seksyen *Aporum* dan *Strongyle* adalah sentiasa bersama dalam kebanyakan pokok.

Keputusan yang dikombinasikan untuk dua kawasan gen: Internal Transcribed Spacer (ITS) dan gen nuklear yang bersalinan rendah (*Xdh*) mencadangkan bahawa gen nuklear adalah lebih berwibawa untuk pengajian filogenetik *Dendrobium* kalau dibandingkan dengan DNA kloroplas dengan resolusi yang rendah antara seksyen. Keputusan daripada penanda nuklear mencadangkan bahawa empat seksyen tersebut dimasukkan dalam satu seksyen. Berdasarkan peraturan NCBI, nama *Aporum* mempunyai keutamaan supaya digunakan dalam klasifikasi yang baru ini.

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I certify that a Thesis Examination Committee has met on date of viva to conduct the final examination of **Maryam Moudi** on her thesis entitled "**Phylogenetics of four sections of genus *Dendrobium* Sw. (Orchidaceae) in Peninsular Malaysia**" in accordance with Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1981. The Committee recommends that the student be awarded the Doctor of Philosophy.

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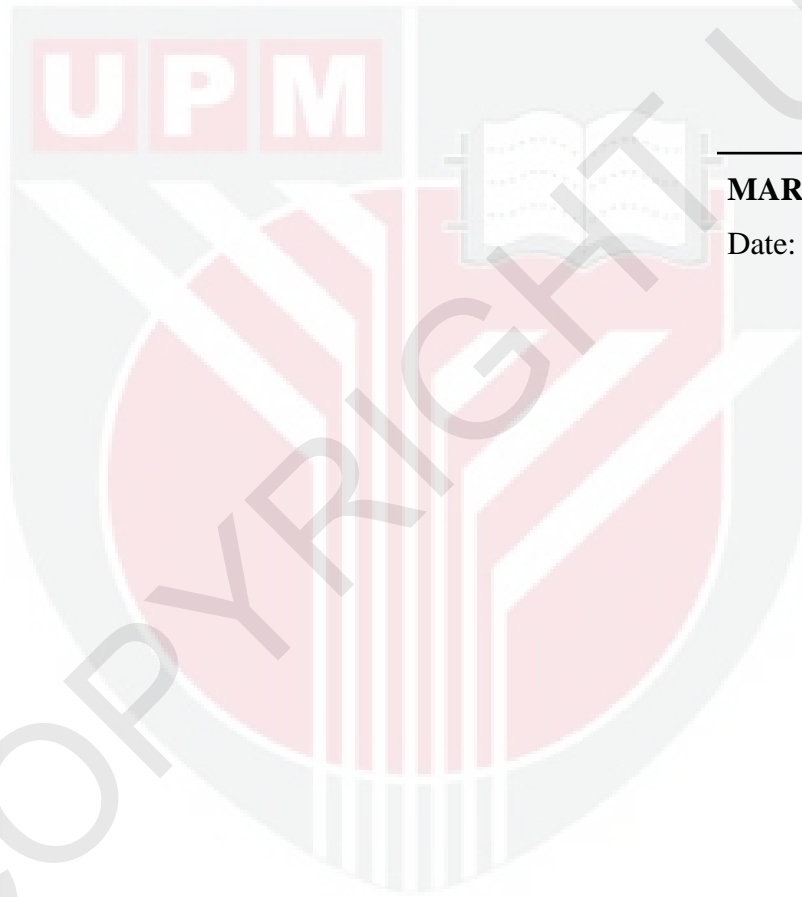
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DECLARATION

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



MARYAM MOUDI

Date: 26 June 2013

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