PHYLOGENETIC RELATIONSHIPS OF CHINGIA, SPHAEROSTEPHANOS AND CHRISTELLA GROUPS (THELYPTERIDACEAE) FROM SABAH USING *rbc*L GENE SEQUENCES

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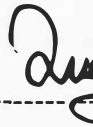

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ABSTRACT

PHYLOGENETIC RELATIONSHIPS OF CHINGIA, SPHAEROSTEPHANOS AND CHRISTELLA GROUPS (THELYPTERIDACEAE) USING *rbc*L GENE SEQUENCES

This study determined the phylogenetic relationship of Chingia, Sphaerostephanos and Christella groups and examined the grouping of the genera based on the resultant *rbc*L analyses in comparison to the morphological analyses, which were based on morphological descriptions provided by Holttum. The study sites consisted of Crocker Range Park and few of the West Coast Sabah area. In this phylogenetic study, *rbc*L gene marker was used to infer phylogenetic relationships of the study groups whereas morphological analyses was used to check the relationship based on the morphological characters gathered from Holttum. In the phylogenetic study of Chingia, Sphaerostephanos and Christella groups, 977 base pairs of DNA sequences were obtained from 32 samples representing the 3 groups of genera. The phylogenetic relationships and morphological analyses revealed that Sphaerostephanos is a taxonomically-problematic group especially for *Pronephrium* and *Sphaerostephanos*. The phylogenetic analyses found that there were two clades derived from the primitive *Sphaerostephanos* group, consisted of a monophyly advanced *Sphaerostephanos* clade and a monophyly clade of Chingia group and Christella group. Morphological analyses showed that *Amphineuron* and *Christella* were well-clustered into the Christella group. However, it was found that some of the *Christella spp.* (*C. subpubescens* and *C. papilio*) were established a lineage to Chingia group in the *rbc*L analyses. The grouping of *Chingia* and *Plesioneuron* into the Chingia group gained support from the morphological analyses but not completely supported by the *rbc*L analyses since *Chingia* spp. (*C. clavipilosa* and *C. perrigida*) were clustered into Christella group. *Chingia* and *Christella* were found to form a monophyletic clade together in both analyses. However, *rbc*L analyses does not support this grouping very well with <70% of bootstrap probability, and showed that Chingia group and Christella group were almost separated into two independent monophyly clade. The separation was in agreement with the morphological analyses that *Christella* clade and *Chingia* clade were split from each other to form their own monophyletic clade. It can be seen that Chingia group and Christella group were derived from a monophyletic group together and gradually evolved to form two independent groups. *Pneumatopteris* was verified being the genera under the Sphaerostephanos group since *Pneumatopteris* was found appear in the monophyletic advanced *Sphaerostephanos* clade in the *rbc*L analyses. Despite being that, the grouping of genera within Sphaerostephanos group cannot be displayed clearly. This could be due to the insufficiency of morphological characters used in morphological analyses were not strong enough to separate or distinguish certain genera into the Sphaerostephanos group. Genetic factors were also believed to influence the outcomes of *rbc*L analyses on the Sphaerostephanos group. Undoubtedly, more details studies should be carried out to support the proposed conclusions since this is the first time phylogenetic study conducted on Chingia, Sphaerostephanos and Christella groups of genera.

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ABSTRAK

Penyelidikan ini mengkaji hubungan filogenetik dalam kumpulan Chingia, Sphaerostephanos dan Christella dan pengelompokan kumpulan genus-genus ini berdasarkan kepada hasil analisis rbcL terhadap hasil analisis morfologi yang berdasarkan kepada deskripsi morfologi yang disediakan oleh Holttum. Kawasan kajian melibatkan beberapa Taman Banjaran Crocker dan beberapa kawasan di Pantai Barat Sabah. Dalam kajian ini, rbcL digunakan untuk membentuk hubungan filogenetik bagi kumpulan yang dikaji. Manakala analisis morfologi digunakan untuk meneliti hubungan kumpulan genera ini berdasarkan kepada ciri morfologi yang dikumpulkan daripada hasil kajian Holttum. Dalam kajian ke atas hubungan filogenetik Chingia, Sphaerostephanos dan Christella, 977 pasangan bes jujukan DNA diperolehi daripada 32 sampel mewakili 3 kumpulan genera. Analisis hubungan filogenetik dan morfologi mendedahkan bahawa kumpulan Sphaerostephanos merupakan kumpulan yang bermasalah dari segi taksonomi terutamanya Pronephrium dan Sphaerostephanos. Analisis hubungan filogenetik mendapati dua kled utama terhasil daripada evolusi kumpulan Sphaerostephanos primitif, yang terdiri daripada kled monofiletik Sphaerostephanos maju dan kled monofiletik kumpulan Chingia dan kumpulan Christella. Analisis morfologi menunjukkan pengelompokan Amphineuron dan Christella di dalam kled Christella. Meskipun begitu, didapati dua spesies Christella (C. subpubescens dan C. papilio) membentuk hubungan dengan kumpulan Chingia di dalam analisis rbcL. Pengelompokan Chingia dan Plesioneuron dalam kled Chingia disokong oleh analisis morfologi tetapi tidak disokong sepenuhnya oleh analisis rbcL memandangkan Chingia spp. iaitu C. clavipilosa dan C. perrigida berada dalam kumpulan Christella. Chingia dan Christella didapati membentuk satu kumpulan monofiletik bersama dalam analisis morfologi dan analisis rbcL. Walau bagaimanapun, analisis rbcL tidak menyokong sepenuhnya pengelompokan ini dengan nilai kebarangkalian <70%, memandangkan kumpulan Chingia dan kumpulan Christella hampir terpisah kepada dua kled monofiletik berasingan. Pengasingan ini sejajar dengan hasil analisis morfologi, menunjukkan kled Christella dan kled Chingia terpisah di antara satu sama lain untuk membentuk kled monofiletik masing-masing. Secara kesimpulannya, kumpulan-kumpulan Chingia dan Christella diinterpretasikan sebagai kumpulan-kumpulan monofiletik bersama dan kemudiannya terevolusi membentuk kumpulan masing-masing. Pneumatopteris pula disahkan sebagai genera dalam kumpulan Sphaerostephanos memandangkan Pneumatopteris didapati menduduki kled monofiletik Sphaerostephanos maju dalam analisis rbcL. Meskipun begitu, pengelompokan genera dalam kumpulan Sphaerostephanos tidak dapat ditunjukkan secara jelas. Ini mungkin disebabkan kekurangan ciri-ciri morfologi yang digunakan untuk analisis morfologi tidak cukup kuat untuk memisahkan atau membezakan genera tertentu ke dalam kumpulan Sphaerostephanos. Faktor-faktor genetik dipercayai mempengaruhi hasil analisis rbcL ke atas pengelompokan kumpulan Sphaerostephanos. Tidak dinafikan, lebih banyak kajian terperinci perlu dijalankan untuk menyokong kesimpulan yang dicadangkan memandangkan ini adalah merupakan kajian filogenetik yang pertama kali dijalankan ke atas kumpulan genus Chingia, Sphaerostephanos dan Christella.

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ABBREVIATIONS

MP	Maximum Parsimony
ML	Maximum Likelihood
NJ	Neighbour Joining
bp	Base pair
DNA	Deoxyribonucleic acid
Hrs	Hours
TAE	Tris-Acetate-EDTA
TBE	Tris-Borate-Edta
PCR	Polymerase Chain Reaction
dNTP	Deoxynucleotide triphosphate
CTAB	Cetyltrimethylammonium Bromide
rpm	rotation per minute
NaOAC	Natrium acetate
EtOH	Ethanol
OTU's	Operational Taxonomic Units
TE	Tris-Edta
TBR	Tree Branch Swapping
*rbc*L	ribulose-bisphosphate carboxylase
varv	variety
RI	Retention Index
CI	Consistency Index
hLRTs	Hierarchial Likelihood Ratios Tests

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AIC	Akaike Information Criterion
Nst	Number of substitution types
Rmat	Rate matrix
Pinv	Proportion on invariable site
Freq	Frequency
PAUP	Phylogenetic Analysis Using Parsimony
Min	minute
NCBI	National Centre of Biotechnology Information
kb	kilo bases

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SYMBOLS

A	Adenine
C	Cytosine
G	Guanine
T	Thymine
cm	centimeter
g	gram
R	Reverse
F	Forward
sp.	species
°C	Degree Celsius
%	Percentage
µg	microgram
µl	microlitre
ACCTRAN	Accelerated transformation
$MgCl_2$	Magnesium chloride
M	molar
mg	miligram
mM	micro Molar
ml	milimeter
HCl	Hidrocloric acid
NaCl	Natrium chloride
L	Liter
ddH_2O	deionized distilled water
-ln log	negative log likelihood
L	Length

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BS	Bootstrap
pH	power of hidrogen

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CHAPTER 1

INTRODUCTION

1.1 Background

Thelypteridaceae is one of the largest families of pteridophytes, comprising almost a thousand fern species mostly in tropical and subtropical regions, of which less than 2% are discovered in temperate areas (Sornsuwan *et al.*, 2006). In Sabah, Thelypteridaceae is the fourth largest family of pteridophytes with 14 genera and 68 species after Polypodiaceae which comprises of 19 genera (82 species), followed by Grammitidaceae, (7 genera, 80 species), and Dryopteridaceae (6 genera, 71 species) (Said, 2005). Thelypteridaceae is composed of two primary lineages namely, phegopteroid and thelypteroid groups of genera (Smith and Cranfill, 2002).

Holttum (1980) recorded a total of about 1,000 species from the family Thelypteridaceae, constituting approximately 8% of all known species of ferns. Piggot (1988) stated that most Thelypteridaceae species can be found in fairly open places near stream valleys and wet places at high attitudes in the mountains, swampy areas and open places in the lowlands. Thelypteridaceae has been treated as a natural group comprising of nearly 1,000 mostly tropical species since its taxonomic description distinguishes it apart from the dryopteroid ferns as a distinct group about 60 years ago (Smith & Cranfill, 2002). According to Schneider *et al.* (2004), there are plenty of molecular phylogenetic studies on both Polypodiaceae and Grammitidaceae. Unfortunately for members of the Thelypteridaceae family not much research was conducted on the similar discipline and this statement was supported by Wolf *et al.* (1994), who mentioned that little work was conducted in either the systematics or phylogenetics of the family Thelypteridaceae and its genera despite many references to ferns. However, there have been taxonomic studies on Thelypteridaceae (Holttum, 1982). Sornsuwan *et al.* (2006) had emphasized that Thelypteridaceae cannot be clearly classified since some genera have been previously included into or excluded from this fern family, making the

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classification in the family much complicated. Besides, Thelypteridaceae have experienced great changes and transformations particularly in terms of morphological characters during the Early Carboniferous period until now (Holttum, 1948). This has made botanist's task quite challenging due to the substantial disagreements in evaluating characters and controversies in building a stable classification for Thelypteridaceae (Hasebe *et al.,* 1994).

Thelypteridaceae have been focused for taxonomic study because of its problematic classification. Wagner & Smith (1993) had claimed that Thelypteridaceae is one of the controversial fern families in term of family definitions. Theypteridaceae have been revised many times by several botanists including Christensen (1913), Copeland (1947), Ching (1963), Morton (1963), Iwatsuki (1964) and Holttum (1971). Holttum is the last person to revise the family Thelypteridaceae in terms of their morphological characters and contributes much to the publication of Thelypteridaceae. Despite being revised frequently by some of the well-known botanists, each of the reports, new ideas, concepts and modification on this family, making the Thelypteridaceae classification yet unclear and unresolved. According to Smith *et al.* (2006), Thelypteridaceae is difficult or almost impossible to be defined morphologically since Blechnaceae and the athyrioid ferns have been attempted to be included into the Thelypteridaceae family, which had been mentioned by Hennipman (1996).

There are three groups that are collectively known as Chingia, Sphaerostephanos and Christella groups of genera in Thelypteridaceae (Holttum, 1980). In this study, the three groups within the family Thelypteridaceae namely Chingia group (*Chingia*, *Plesioneuron*), Christella group (*Christella* and *Amphineuron*) and Sphaerostephanos group (*Sphaerostephanos*, *Pneumatopteris* and *Pronephrium*) were studied since there are arguments to their assignation into its respective genera, due to the confusion regarding the similarity in morphology (Holttum, 1980). Holttum was the one who are responsible to delineate the following genera (*Chingia*, *Plesioneuron*, *Sphaerostephanos*, *Pneumatopteris*, *Pronephrium*, *Christella* and *Amphineuron*) into their respective groups (Chingia

group, Sphaerostephanos group and Christella group) based on their morphological characters similarities.

In this study, molecular method was conducted in order to establish and examine the phylogenetic relationships within Chingia, Sphaerostephanos and Christella groups of genera based on the DNA sequences data. According to Schneider *et al.* (2004), DNA sequences data has been seen as a solution to resolve uncertainties of morphological characters evolution. Hence, the grouping of genera Chingia, Sphaerostephanos and Christella using the molecular method was expected to be resolved in comparison to the grouping of genera made by Holtum (1971; 1980), which based on morphological characters analyses.

The gene ribulose 1, 5-bisphosphate carboxylase/oxygenase (*rbc*L) which is located in chloroplast genome was used in this study as a molecular marker. Interestingly, sequencing data derived from *rbc*L have also been used to address phylogenetic relationships in ferns (Hasebe *et al.*, 1993). Lemieux *et al.* (2000) reported that the chloroplast genome have been sequenced from several clades of green plants and contained considerable amounts of phylogenetically useful data. Moreover, *rbc*L have been proven to be useful in addressing fern's phylogenetic relationship since it has been used in many studies such as in the phylogenetic relationships on Ophioglossaceae and Marattiaceae (Hasebe *et al.,* 1993), the comparison between morphology and molecular method of extant ferns (Pryer *et al.,* 1995), and in the study of leptosporangiate ferns phylogeny using three different plastids (Pryer and Schuettpelz, 2009).

1.2 Research Problems

There were lack of recent informations and studies conducted to verify the delineation of Chingia, Sphaerostephanos and Christella groups as proposed by Holttum (1971; 1980). Besides, these three groups of genera were not clearly resolved from the conventional taxonomy as evidence in changes in classification and taxon names in various revisions. In addition, the genera are not clearly defined morphologically due to the failureness to observe differences in certain characters. This has caused confusion among the taxonomist and difficulties in

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