

MOLECULAR PHYLOGENETICS ON GENUS *PRONEPHRIUM*
(THELYPTERIDACEAE) IN SABAH USING *rbcL* GENE SEQUENCES

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IJAZAH: DEGREE OF BACHELOR OF SCIENCE WITH HONOURS

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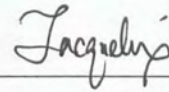
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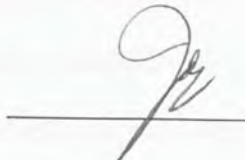
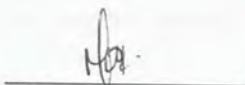
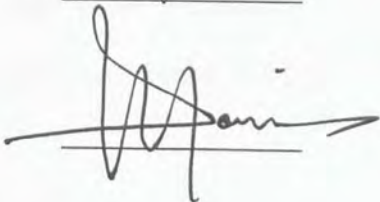
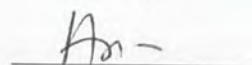
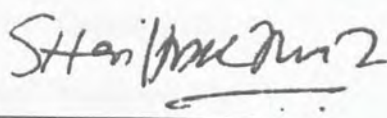


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ABSTRACT

Gene *rbcL* which found in genome chloroplast is used in this study. Nucleotide sequences of three samples of *Pronephrium* (*P. nitidum*, *P. repandum*, and *P. asperum*) were obtained. Apart from that, nucleotide sequences of three species of *Thelypteris* and one species from *Aglaomorpha* (outgroup), including *T. ovata*, *T. longissima*, *T. simplex* and *A. heraclea* were obtained from GenBank were used in the phylogenetic tree construction using Paup Version 4.0b10 for Macintosh. Maximum Parsimony (MP) and Maximum Likelihood (ML) analyses were performed in this study. Trees obtained from MP and ML analyses showed two different topologies. *P. nitidum* and *P. repandum* suggested as a sister taxa in both analyses and supported by a high bootstrap (BS) value of 100%. However, *P. asperum* was clustered into *Thelypteris* clade with a weak BS value of 59% in the ML analysis. Hence, conclusion can be made that there is a weak relationship of *Pronephrium* in relation to *Thelypteris* in *Thelypteridaceae*. However, extensive study should be done for both the genera to provide a clear-cut classification between these two genera. It is also suggested that morphological data such as the spore character should be used to provide a more consistent and reliable information for this study.



ABSTRAK

Gen *rbcL* telah digunakan dalam kajian ini. Jujukan nukleotida bagi tiga spesis *Pronephrium* telah diperolehi, iaitu termasuk *P. nitidum*, *P. repandum*, dan *P. asperum*. Selain itu, jujukan nukleotida bagi tiga spesis *Thelypteris* dan satu species *Aglaomorpha* (kumpulan luar), termasuk *T. ovata*, *T. longissima*, *T. simplex*, dan *A. heraclea* yang diperolehi dari GenBank telah digunakan dalam pembentukan pokok filogenetik. Data tersebut dianalisa dengan menggunakan program Paup* version 4.0b10 for Macintosh. Pokok filogenetik yang diperolehi dari kedua-dua analisis adalah berbeza. *P. nitidum* and *P. repandum* dicadangkan sebagai sister taxa dengan nilai bootstrap (BS) yang tinggi (100%). Tetapi, *P. asperum* termasuk dalam kumpulan *Thelypteris* dan disokong dengan nilai BS sebanyak 59% dalam analisis ML. Oleh itu, kesimpulan boleh dibuat iaitu hubungan antara kedua-dua genus adalah jauh. Walaubagaimanapun, dicadangkan agar kajian yang lebih dalam perlu dijalankan dengan merangkumi aspek morfologi spora ke atas kedua-dua genus ini untuk memastikan kedudukan dan pengelasan yang lebih konsisten dan menyakinkan.



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SYMBOL/ABBREVIATION LIST

SYMBOL/ ABBREVIATION LIST	MEANING
μL	Microliter
mM	Milimolar
pmol	Picomole
min	Minit
g	Gram
mg	Miligram
V	Volt
bp	Base pair
$^{\circ}\text{C}$	Degree Celsius
%	Percentage
~	More or less
X	Times
&	And
A	Adenine
T	Thymine
C	Cytosine
G	Guanine
U	Unit
F	Forward
R	Reverse
CTAB	Hexadecyltrimethylammonium bromide
ddH ₂ O	Double distilled Water
dNTPs	Deoxynucleoside triphosphate
MgCl ₂	Magnesium chloride
r.p.m	Rotation per minute
DNA	Deoxyribonucleic Acid
mtDNA	Mitochondria DNA
cpDNA	Chloroplast DNA



rDNA	Ribosomal DNA
MP	<i>Maximum Parsimony</i>
ML	<i>Maximum Likelihood</i>
HS	<i>Heuristic Search</i>
BS	<i>Bootstrapping</i>
PAUP*	<i>Phylogenetic Analysis Using Parsimony and other methods</i>
PCR	<i>Polymerase Chain Reaction</i>
Taq	<i>Thermus aquaticus</i>
sp.	Species



CHAPTER 1

INTRODUCTION

1.1 Background

Pteridophytes (pteron = feather, phyton = plant) are crytogams with well developed vascular system (Pathak, 2003). They are represented by about 400 living and fossil genera and some 10,500 species (Pathak, 2003). Pteridophytes are originated about 400 million years ago in the Silurian period of the Palaeozoic era and formed dominant vegetation on the earth during the Devonian period (Pathak, 2003).

Ferns are grouped into phylum Plantae and classified under the Division of Pteridophyta. There is a wide distribution of fern and comprised of around 10,000 species with 250 genera in the world (Hasebe *et al.*, 1994). About 8% of around 1000 species of the family Thelypteridaceae are all known ferns and about 430 species are now known in Malesia (Holttum, 1980). Parris & Latiff (1997) stated that there are 1165 Malaysian pteridophytes species under 35 genera, with 647 occur in Peninsular Malaysia, 750 in Sabah and 615 in Sarawak. Thelypteridaceae is one of the largest families of Sabah pteridophytes, which contains of 68 species (Said, 2005). Other



large families are Grammitidaceae (80 species), Dryopteridaceae (71 species), Hymenophyllaceae (61 species), Woodsiaceae (50 species) and Polypodiaceae (82 species). Polypodiaceae is the largest family in Sabah (Said, 2005).

According to Said (2005), Thelypteridaceae is comprised of 14 genera and around 68 species are found in Sabah. A total of 57 species of the genus *Pronephrium* are known in Malesia and several more in mainland Asia (Holttum, 1980). There are 17 genera of Thelypteridaceae and only 22 species of *Pronephrium* found in Malesia (Parris & Latiff, 1997). In Sabah, *Pronephrium* species are locally abundant in stream valleys in lowland and hill forest (Piggot, 1996).

Studies of molecular and morphological data share the same assumptions regarding homology and the same mission in construction of cladogram indicative of phylogenies (Grande & Rieppel, 1994). In a sense, molecular and morphological data are part of evidence bearing on structure and relationships. The former approach is directly related to heritable information of organisms (Grande & Rieppel, 1994). The latter approach is very old and deals broadly with structure and relies on the fossil record.

Actually, in many studies both morphological data and molecular data are used but the recent advances in molecular biology have made a good complement to each other in constructing a phylogeny. Genetic material can be examined directly from the studies of DNA.



Nei & Kumar (2000) have identified three reasons why molecular approach is more reliable in constructing a phylogenetic tree. Firstly, DNA consists of the four types of nucleotides, adenine (A), thymine (T), cytosine (C), and guanine (G), and it can be used for comparing any group of organisms, including bacteria, plants, and animals but in the classical approach, this is virtually impossible. Secondly, it is possible to use a mathematical model to formulate the change and compare DNAs from distantly related organisms since the evolutionary change of the DNA follows a more or less regular pattern whereas the evolutionary change of morphological characters is extremely complicated even for a short evolutionary time. Thirdly, molecular phylogenetics is expected to clarify many branching patterns of the tree of life than have been hard to resolve by the classical approach because the genomes of all organisms consist of long sequences of nucleotides and contain a much larger amount of phylogenetic information than morphological characters.

Phylogeny or evolutionary history represents the evolutionary relationships among a set of organisms or groups of organisms (Nei & Kumar, 2000). Phylogenetic classification is a natural classification system which shown the evolutionary relationships and also reflects the evolutionary history of the studied organisms (Avisé, 2001).

Cladistics is basically a methodology that attempts to analyze phylogenetic data objectively, in a manner parallel to that in which taxometrics seeks to introduce objectivity into phenetics and phenetic classification (Scotland & Pennington, 2000). The term phenetic is introduced by Cain & Harrison (1960) to denote a relationship by overall similarity, based on all available characters without any weighting. Sokal &



Sneath (1963) used the term phenetic to refer to a relationship by overall similarity as judged by the characters of the organisms without any implication as to their relationship by ancestry. On the other hand, cladistics could be presented as dendrogram called cladogram, which explains the pathway of descendant, or how ones character appeared in the evolution (Abdul Latiff, 1994).

There are various sources of DNA which can be used in the phylogenetic analyses, including nuclear DNA, mitochondrial DNA and chloroplast DNA (Simpson, 2006). The *rbcL* gene which is located at the genome of chloroplast is used in this study. It is also an area of DNA at which it is very useful and efficient in the study of molecular systematics of ferns as advocated by Hasebe *et al.* (1994). Moreover, it is also a precise choice to allocate the phylogenetic relationships at the higher taxonomic level (Hasebe *et al.*, 1994). Besides *rbcL*, other genes such as *atpB*, *matK*, and *ndhF* genes which are located at the chloroplast DNA have also been utilized in molecular systematic studies (Simpson, 2006).

The importance of this study is to determine the DNA sequences of taxa in *Pronophrium* using *rbcL* gene. Although there are many ferns around us but only a few studies have been done on molecular phylogenetics of ferns especially on the family Thelypteridaceae. Besides that, there is not much of phylogenetic data available for the pteridophytes of Sabah. So, this study will provide a basis for further research of Sabah pteridophytes as a whole.



1.2 Area of Study

Mount Kinabalu (Kinabalu Parks), Fernarium (Keningau) and Mount Alab (Crocker Range Parks)

1.3 Objectives

1. To determine DNA sequences of taxa in *Pronephrium* (Thelypteridaceae) using rbcL gene.
2. To determine phylogenetic relationship of taxa within *Pronephrium* and in relation to *Thelypteris* in Thelypteridaceae.



CHAPTER 2

LITERATURE REVIEW

2.1 Thelypteridaceae

2.1.1 Ecology and distribution

Smith *et al.* (2006) studied on several most important aspects of the Thelypteridaceae, including the anatomy and morphological characters, gametophytes, ecology and distribution, karyology, and classification. Thelypteridaceae is comprised of nearly 1000 species occurring mostly in the tropical and subtropical regions; fewer than 2% are temperate (Smith *et al.*, 2006). Both number and diversity of species are greatest in Malesia, where 440 species in 22 groups occur but 300 species in six groups are native to the Neotropics (Smith *et al.*, 2006). Africa and nearby islands are relatively depauperate, with 55 species whereas related groups are largely confined to either old or the new world (Smith *et al.*, 2006). Most members of the family occur at middle and lower elevations in or at edges of rain forests; a few species extend to 4500 m in the tropics (Smith *et al.*, 2006). Apart from that, most species are found in wet, primary, undisturbed forests or in secondary forests but still others are commonly



found in shaded areas under cultivation, especially coffee and cacao plantations (Smith *et al.*, 2006).

2.1.2 Taxonomy

There was a long history of confusion of all the names of this family published in the 19th century and have been transferred, by one author or another, to *Aspidium*, *Neprodium* and *Dryopteris*, in some cases also to *Goniopteris*, *Lastrea*, *Meniscium* or *Phegopteris*; and most of the names have been transferred to *Thelypteris* or *Cyclosorus* or both in the 20th century (Holttum, 1982). Besides that, Thelypteridaceae was closely related to Cyathea which shared the same characteristics such as the shape of fronds; shape of leaflets; abundant hairs on adaxial surfaces of rachis and arrangement of aerating tissue with a separate small area at the base of each pinna (Holttum, 1982). Furthermore, both the Cyathea and Thelypteridaceae also hold the same condition whereby the lower pinnae are gradually reduced (Holttum, 1982). In addition, Cyathea was believed the ancestor of Thelypteridaceae because most species of Cyathea have free veins bipinnate fronds like early Thelypteridaceae (Holttum, 1982).

Based on Holttum (1982), there was another group of terrestrial ferns which agreed with Thelypteridaceae in having abundant distinctive hairs, not homologous with scales, on the adaxial surface of frond-axes, namely *Ctenitis* and *Tectaria*. In 1977, Pichi Sermolli placed the Thelypteridaceae in Aspidiales, an order which nearly related to Dennstaedtiales than to Dicksoniales (Holttum, 1982). Within Aspidiales, Thelypteridaceae was regarded nearest to Aspleniaceae due to the same



chromosome number 36 in a majority of species (Holttum, 1982).

2.1.2.1 Taxonomy of Malayan Thelypteridaceae

Thelypteridaceae is one of the families which is classified under the Division Pteridophyta and placed under the order Polypodiales, class Polypodiopsida (Smith *et al.*, 2006). For Malayan Thelypteridaceae, Holttum (1980) splits the family into 17 genera, as listed in the Table 2.1 below.

Table 2.1 Genera and Species of Malayan Thelypteridaceae

Genus	Species
1. <i>Phegopteris</i>	(Not in Malaya)
2. <i>Pseudophegopteris</i>	<i>P. rectangularis</i> <i>P. paludosa</i>
3. <i>Macrothelypteris</i>	<i>M. torresiana</i>
4. <i>Metathelypteris</i>	<i>M. flaccida</i> <i>M. gracilescens</i> <i>M. dayi</i>
5. <i>Coryphopteris</i>	<i>C. unidentata</i> <i>C. tahanensis</i> <i>C. hirsutipes</i> <i>C. viscosa</i> <i>C. gymnopoda</i> <i>C. arthrotricha</i> <i>C. pectiniformis</i> <i>C. badia</i>
6. <i>Trigonospora</i>	<i>T. ciliata</i>
7. <i>Thelypteris</i>	(not Malayan)
8. <i>Cyclosorus</i>	<i>C. interruptus</i>



9. <i>Ampelopteris</i>	<i>A. prolifera</i>
10. <i>Mesophlebion</i>	<i>M. trichopodium</i> <i>M. motleyanum</i> <i>M. beccarianum</i> <i>M. chlamydophorum</i> <i>M. crassifolium</i>
11. <i>Chingia</i>	<i>C. sakayensis</i> <i>C. perrigida</i>
12. <i>Sphaerostephanos</i>	<i>S. larutensis</i> <i>S. polycarpus</i> <i>S. unitus</i> <i>S. porphyricola</i> <i>S. penniger</i> <i>S. latebrosus</i> <i>S. heterocarpus</i> <i>S. pterosporus</i> <i>S. hendersonii</i> <i>S. peltochlamys</i> <i>S. norrisii</i>
13. <i>Pneumatopteris</i>	<i>P. truncata</i> <i>P. ecallosa</i> <i>P. callosa</i>
14. <i>Pronephrium</i>	<i>P. asperum</i> <i>P. glandulosum</i> <i>P. menisciicarpon</i> <i>P. peltatum</i> <i>P. repandum</i> <i>P. triphyllum</i> <i>P. parishii</i> <i>P. salicifolium</i> <i>P. rubicundum</i>
15. <i>Christella</i>	<i>C. arida</i>

	<i>C. papilio</i> <i>C. subpubescens</i> <i>C. parasitica</i> <i>C. hispidula</i> <i>C. dentata</i>
16. <i>Amphineuron</i>	<i>A. terminans</i> <i>A. opulentum</i> <i>A. immersum</i>
17. <i>Parathelypteris</i>	<i>P. beddomei</i>

2.1.2.2 Taxonomy of Sabah Thelypteridaceae

Three genera which could not be found in Sabah namely *Ampelopteris*, *Cyclosorus*, and *Trigonospora* (Parris & Latiff, 1997). Genus *Trigonospora* is found in India, Ceylon and Malesia and about eight species have been described (Holttum, 1982). On the other hand, genus *Cyclosorus* is only distributed in Pantropic and probably 3 species have been described (Holttum, 1982). This genus probably closely related to *Ampelopteris*, *Trigonospora* and *Cyclosorus* due to their same aquatic habitat (Holttum, 1982). Last but not least, the genus *Ampelopteris* is only distributed in Old World Tropics and found in aquatic habitat (Holttum, 1982). For Sabah Thelypteridaceae, Said (2005) listed the family into 14 genera (Table 2.2).

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