Faculty of Resource Science and Technology

PRELIMINARY PHYLOGENETIC STUDY OF *BUCEPHALANDRA* (SCHISMATOGLOTTIDEAE: ARACEAE)

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Preliminary Phylogenetic Study of *Bucephalandra* (Schismatoglottideae: Araceae)

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Declaration

I declare that no portion of this research work has been submitted to support the application of other degree or qualification at any other universities or institutions of higher learning.

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List of Abbreviations

°C	Degree Celsius
µl	Microlitre
ml	Millilitre
mg	Milligram
mM	Millimolar
BS	Bootstrap
bp	Base pair
CI	Criterion index
CIA	Chloroform-Isoamyl Alcohol
CTAB	CetylTrimethyl Ammonium Bromide
cpDNA	Chloroplast Deoxyribonucleic Acid
DMSO	Dimethyl sulfoxide
dH_2O	Sterilized water
dNTPs	Deoxyribonucleotide Triposphate
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra Acetic Acid
ITS	Internal Transcribed Spacer
MP	Maximum Parsimony
ML	Maximum Likelihood
*mat*K	Megakaryocyte-associated tyrosine kinase
$MgCl_2$	Magnesium Chloride
NaCl	Sodium Chloride
nrDNA	Nuclear ribosomal Deoxyribonucleic Acid
PCR	Polymerase Chain Reaction
PP	Posterior probability
RAxML	Randomized Axelerated Maximum Likelihood
RI	Retention Index
rpm	Rotation per minute
TAE	Tris-acetate-EDTA
UV	Ultraviolet
V	Volt

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ABSTRACT

Tribe Schismatoglottideae is one of the most species-rich and diverse aroids taxa in Borneo, with more than 250 species, of which over 95% are endemic. Schismatoglottideae contains a diverse group of rainforest terrestrial, litophytic or rheophytic herbs centred in Borneo. *Bucephalandra* (Araceae: Schismatoglottideae) is endemic to Borneo and known by its unique staminodes, with the presence of motile scale or shield-shaped staminodes situated at the interstice of the pistillate and staminate flower zones. Phylogenetic analyses of *Bucephalandra* were carried out in this study based on nuclear of Internal Transcribed Spacer (nrITS) and Megakaryocyte-associated tyrosine kinase (*mat*K). Eight taxa were included in this study which also comprised the sister tribe, *Cryptocoryne longicauda* Becc. ex Engl. as the outgroup. Plant specimens were collected from respective localities. DNA was extracted from leaf samples, PCR amplified and sequenced. Analyses of both datasets with Maximum Parsimony, Maximum Likelihood and Bayesian Methods were carried out. *Bucephalandra* was revealed to comprise two clades.

Keywords: Borneo, endemism, ITS, *mat*K, systematics.

ABSTRAK

Tribus Schismatoglottideae adalah salah satu taksa keladi hutan yang kaya dengan pelbagai jenis spesies di Borneo, dengan lebih daripada 250 spesies dan lebih daripada 95% adalah endemik. Schismatoglottideae mengandungi pelbagai kumpulan daratan hutan hujan, litofitik atau reofitik herba yang tertumpu kepada Borneo. Bucephalandra (Araceae: Schismatoglottideae) adalah endemik kepada Borneo dan dikenali melalui staminod yang unik, dengan adanya bidangan pelahan atau staminod perisai berbentuk yang terletak di celah daripada berputik dan staminat zon bunga. Analisis filogenetik Bucephalandra telah dijalankan dalam kajian ini berdasarkan kepada nuklear spaser dalaman disalin (nrITS) and Megakaryocyte-associated tyrosine kinase (matK). Lapan taksa telah dimasukkan ke dalam kajian ini yang terdiri daripada 'adik' tribus, Cryptocoryne longicauda Becc. ex Engl. sebagai kumpulan luar. Spesimen tumbuhan dalam kajian ini dikumpulkan dari kawasan masing-masing. DNA diekstrak, dikuatkan dengan PCR dan disusun. Analisis gabungan data dengan Maksimum Parsimoni, Maksimum Likelihood dan Kaedah Bayesian telah dijalankan. Kajian ini telah menunjukkan Bucephalandra mengandungi dua klad.

Kata kunci: Borneo, endemisma, ITS, *mat*K, *sistematik*.

CHAPTER ONE

INTRODUCTION

1.1 Research Background

Arum family, *Philodendron* and Aroids are the common name for the Araceae Juss. family. According to Mayo and his colleagues (1997), Araceae can be notable from other plant families by the presence of its bisexual or unisexual spadix and subtended by a solitary spathe on a long or very short peduncle which is referred to as flower or inflorescence (Figure 1). The inflorescence is a distinct feature that helps in determining the aroids. Araceae consists of 125 genera and about 3750 species (Boyce & Croat, 2014).

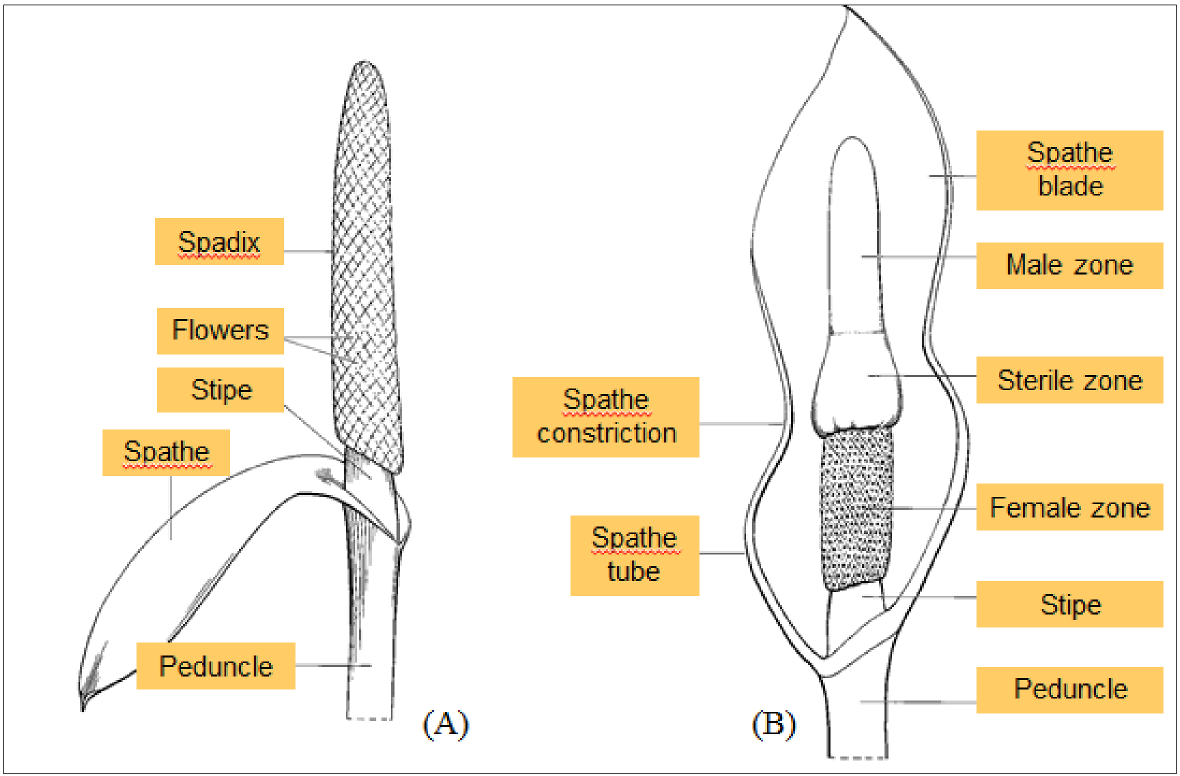

Figure 1. Inflorescence of Araceae. It can be notable by the presence of its spadix and spathe - A: Unisexual flower; B: Bisexual flower. (Mayo *et al.*, 1997)

Tribe Schismatoglottideae is one of the most species-rich and diverse aroids taxa in Borneo, with more than 250 species, of which over 95% are endemic on the island. It contains a diverse group of rainforest terrestrial, litophytic or rheophytic herbs centred on Borneo (Bogner & Hay, 2000). *Schismatoglottis* Zoll. & Moritzi is the largest genus in this tribe extending throughout Malesia (except the driest and highest parts) to the tropical Western Pacific and Indochina (Hay & Yuzammi, 2000). Previously, *Schismatoglottis* was claimed to be in the Neotropics by Hay and Yuzammi (2000) but recently, the Neotropical *Schismatoglottis* have been transferred from tribe Schismatoglottideae, forming Philonotieae S. Y. Wong & P. C. Boyce with only consist of one genus, *Philonotion* Schott (Wong *et al.*, 2010).

Schismatoglottideae also includes eleven small satellite genera which are *Apoballis* Schott, *Aridarum* Ridl., *Bakoa* P. C. Boyce & S. Y. Wong, *Bucephalandra* Schott, *Hestia* S. Y. Wong & P. C. Boyce, *Phymatarum* M. Hotta, *Pichinia* S. Y. Wong & P. C. Boyce, *Ooia* S. Y. Wong & P. C. Boyce, *Piptospatha* N. E. Br., *Schottariella* P. C. Boyce & S. Y. Wong and *Schottarum* P. C. Boyce & S. Y. Wong. All genera except *Apoballis* occur in Borneo. All except for *Hestia*, *Piptospatha* and *Schismatoglottis* are restricted to Borneo (Wong, 2013).

Since there are many genera in this tribe, it is important to identify and differentiate the characteristics between the species. Identification is usually done based on its morphological characteristics. In *Bucephalandra*, *Piptospatha* and *Aridarum*, the spathe is unconstricted. Its limb however, is generally caducuous and the result is that the infructescence is exposed but subtended by a funnel-shaped spathe base, whereas it is enclosed by an urceolate spathe base in *Schismatoglottis* (Bogner & Hay, 2000). The stamens of *Aridarum*, *Bucephalandra* and *Phymatarum*, all have truncate stamens with remarkable needle or horn-like extension to the staminal thecae from the tips of which the pollen is extruded as droplets.

This study focused on the phylogenetic investigation of *Bucephalandra*. Previously, a number of phylogenetic studies have been conducted in Araceae in order to obtain a well-supported profile based from molecular approach. Several phylogenetic relationships have been partially resolved in Araceae based on tribal level as well as genera and species level (Mayo *et al*., 1997).

1.2 Problem Statements

Borneo is rich with aroids and these plants serve as indicators of forest quality since they are both adaptive to localized ecological heterogeneity and adversely influenced by forest disturbance (Wong, 2013). The genus *Bucephalandra* is endemic to Borneo with about 29 species so far described. Many of these species have only been scientifically known recently (Wong & Boyce, 2014a; Wong & Boyce, 2014b). Therefore, the next step was to resolve the phylogenetic relationship among the *Bucephalandra* species.

1.3 Objectives

The aims of this project were as follows:

1. To study the relationship among several selected species in genus *Bucephalandra*.
2. To analyse whether *Bucephalandra* is a monophyletic genus.

CHAPTER TWO

LITERATURE REVIEW

2.1 Araceae on Borneo

Borneo is a large island comprises of Sabah, Sarawak, Brunei and Indonesian Kalimantan (Figure 2). As described by Boyce *et al.* (2010), the aroids of Borneo consist of 36 genera. 35 genera are claimed to be indigenous and another one genus, *Typhonium* Schott, is genuinely naturalized. Out of the 35 genera that are indigenous, eight of them including *Aridarum* Ridl., *Bakoa* P. C. Boyce & S. Y. Wong, *Bucephalandra* Schott, *Ooia* S. Y. Wong & P. C. Boyce, *Pedicellarum* M. Hotta, *Phymatarum* M. Hotta, *Pichinia* S. Y. Wong & P. C. Boyce and *Schottariella* P. C. Boyce & S. Y. Wong are endemic to Borneo (Boyce *et al.*, 2010). Another four additional genera include *Caladium* Vent., *Dieffenbachia* Schott, *Syngonium* Schott and *Xanthosoma* Schott are listed as adventives.

Figure 2. Map of Borneo which comprises of Sabah, Sarawak, Brunei and Indonesian Kalimantan.

Currently, the aroids flora of Borneo approximately stands at 670 indigenous species of which more than 40% are novel species (Boyce *et al.,* 2010). 70% of Borneo is covered by Kalimantan. Although Kalimantan has greater land area than the other three regions, it still remains very poor unknown. The total aroids estimated in Borneo reaches more than 1000 species with just one third have been described (Boyce *et al*., 2010). Nevertheless, Borneo is known by the aroid habitat of global significance and arguably one of the richest and most diverse on the planet.

The journey to the first collection of aroids in Borneo was done by a Dutch botanist, Pieter Willem Korthals (1807-1892). His first arrival was in late July 1893 at Banjarmasin and now it is known as South Eastern Kalimantan. The region was explored until middle of December 1893 and some of the collections were placed at few herbaria in Netherlands, Java and Europe. At the same time, another botanist, Yorkshireman James Motley (1822-1859) left for Labuan in 1849. He sent his collections including the plant material and living aroids to Europe. Meantime, Sir Hugh Low and Anton Willem Nieuwenhuis arrived in Sarawak (1845) and Kalimantan (1896) respectively (Boyce *et al.*, 2010).

However, the Italian naturalist, Odorado Beccari (1843-1920) began a serious approach in the systematic study of aroid in Borneo. He was known for his specialization in palms (Arecaceae) during his adult. Beccari also made a few contributions to several plant families specifically the aroids. The next person who has the similar interest in aroid was Henry Nicholas Ridley (1855-1956), who went to Borneo between 1893 and 1915, followed by another man who is Cornelis Rugier Willem Karel van Alderwerelt van Rosenburgh (1863-1908). Several names such as Furtado, Nicolson were also being known for their contribution towards the study of Araceae in Borneo (Boyce *et al*., 2010).

Several other botanists have come into the research of aroids beginning with Alistair Hay in early 1980 and Peter Boyce in the middle of 1980. They contributed a lot to the Malesian aroids (Boyce *et al.*, 2010), which meet the terms and very beneficial checklist and bibliography to it. Hiroshi Okada and Yasuko Mori who is Japanese botanists, currently based in Kalimantan, are working on the tribe Schismatoglotideae. The collaboration of the enthusiasts from Malaysian, Indonesian, Japanese and Dutch (Isa b. Ipor, Hendra Budianto, Suwidji Wongso, Hiroyuki Kishi, Takashige Idei, Yuji Sasaki and Jan Bastmeijer) now are trying to create a good outputs on *Cryptocoryne* (Boyce *et al.*, 2010).

2.2 *Bucephalandra* Schott

The first species of rheophytic Schismatoglottideae was discovered by Schott in 1858, which is *Bucephalandra motleyana* (Bogner & Hay, 2000). *Bucephalandra motleyana* was believed to be the smallest aroid during that time. However, there were some errors made in the original description of *Bucephalandra* by Schott that led to rediscovery of the same species by Beccari. He believed that his aroid did not suit into the pre-existing *Bucephalandra* to which in fact, it belongs. Beccari described the Entabai aroid and placed it under a new genus, *Microcasia* (Boyce & Wong, 2012). The descriptions made by Schott, were found out to be inaccurate, later be revealed and resolved by Josef Bogner (Bogner, 1980). *Bucephalandra* are certainly small, often flowering when only 1 cm tall and producing an inflorescence decidedly out of portion to the stature of the plant (Boyce *et al.*, 2010).

Bucephalandra is usual to be seen as an obligate rheophytic herb (Wong & Boyce, 2014a). Rheophytic habitat is known as the region between the lowest and the highest water levels, where plant species are repeatedly buffeted and saturated by regular flash floods after heavy rainfall followed by exposure to dry conditions during the season of low water level (Wong, 2013). It is often found on stream and riverside rocks in lowland to lower montane perhumid to moist tropical forest (Wong & Boyce, 2014a) (Figure 3).

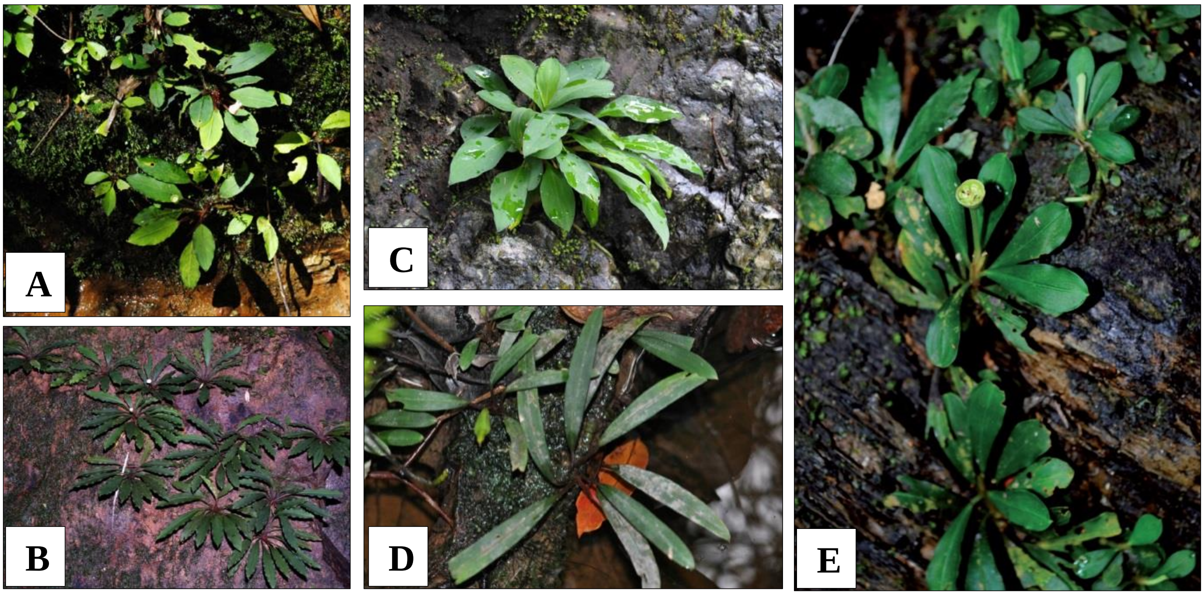

Figure 3. Plants in their habitat. Most of the species can be found on streams and riverside rocks – A: *Bucephalandra akantha* (AR-3863); B: *Bucephalandra oblanceolata* (AR-2310); C: *Bucephalandra bogneri* (AR-94); D: *Bucephalandra kerangas* (AR-252); E: *Bucephalandra pygmaea* (AR-3632). (Wong & Boyce, 2014a)

Bucephalandra are claimed to be endemic to Borneo (Wong *et al.*, 2010). The distribution of *Bucephalandra* including the novel species in Borneo cover from Sarawak, Sabah, Kalimantan and Brunei (Wong & Boyce, 2014a). In Sarawak, the distribution of the species are specified to division of Kuching, Kota Samarahan, Kapit, Sri Aman, Simunjan, Miri and Sarikei while in Kalimantan, it covers from all part including North, South, East and West Kalimantan (Wong & Boyce, 2014a).

Bucephalandra is known by its unique staminodes, with the presence of motile scale or shield-shaped staminodes situated at the interstice of the pistillate and staminate flower zones (Boyce *et al.*, 2010) (Figure 4). It has been speculated that the staminodes play a role in manipulating pollinators during anthesis by controlling access to the pistillate flower zone (Bogner & Hay, 2000). However, Wong and Boyce (2013) claimed that the interstice staminodes have no function in retaining pollinators inside the spathe during the anthesis transition period, as occurring in numerous unisexual flowered aroid genera.

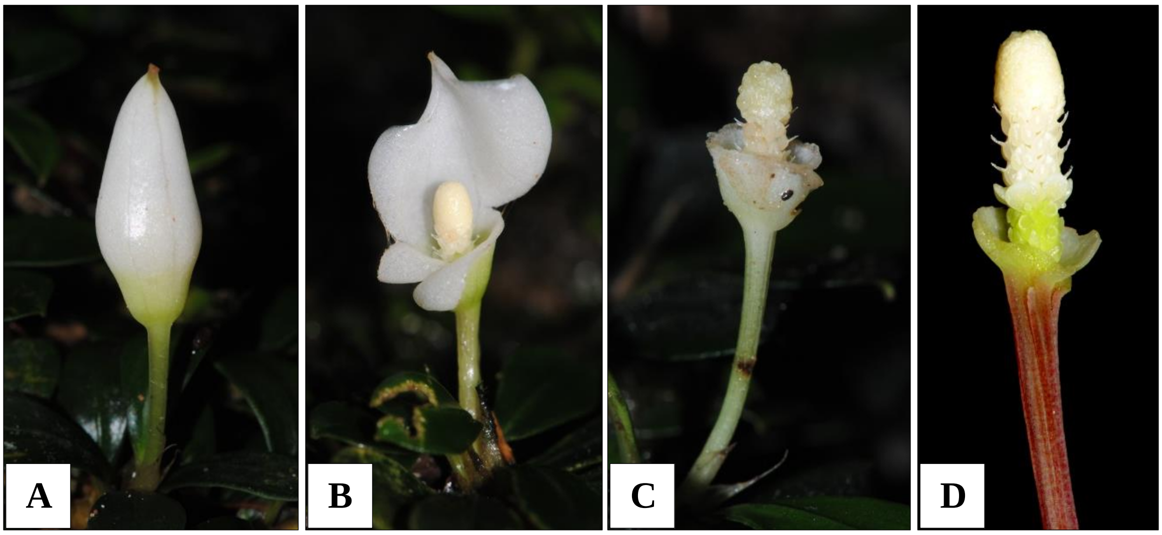

Figure 4. Inflorescence *Bucephalandra pygmaea* (AR-3632) – A: Inflorescence at early pistillate anthesis; B: Close up of inflorescence at late pistillate anthesis, spathe limb is beginning to shed; C: Post anthesis; D: Spadix at pistillate anthesis, spathe artificially removed, the shield-shaped staminodes are still erect. (Boyce & Wong, 2012)

Recently, *Bucephalandra* is now considered to comprise 29 published and accepted species of which 21 are newly described and the other four are transferred from *Microcasia* Becc. The species are *Bucephalandra akantha* S. Y. Wong & P. C. Boyce, *Bucephalandra aurantiitheca* S. Y. Wong & P. C. Boyce, *Bucephalandra belindae* S. Y. Wong & P. C. Boyce, *Bucephalandra bogneri* S. Y. Wong & P. C. Boyce, *Bucephalandra catherineae* P. C. Boyce, Bogner & Mayo, *Bucephalandra chimaera* S. Y. Wong & P. C. Boyce, *Bucephalandra chrysokoupa* S. Y. Wong & P. C. Boyce, *Bucephalandra diabolica* S. Y. Wong & P. C. Boyce, *Bucephalandra elliptica* (Engl.) S. Y. Wong & P. C. Boyce, *Bucephalandra forcipula* S. Y. Wong & P. C. Boyce, *Bucephalandra gigantea* Bogner, *Bucephalandra goliath* S. Y. Wong & P. C. Boyce, *Bucephalandra kerangas* S. Y. Wong

& P. C. Boyce, *Bucephalandra kishii* S. Y. Wong & P. C. Boyce, *Bucephalandra magnifolia* H. Okada & Y. Mori, *Bucephalandra minotaur* S. Y. Wong & P. C. Boyce, *Bucephalandra motleyana* Schott, *Bucephalandra muluensis* (M. Hotta) S. Y. Wong & P. C. Boyce, *Bucephalandra oblanceolata* (M. Hotta) S. Y. Wong & P. C. Boyce, *Bucephalandra oncophora* S. Y. Wong & P. C. Boyce, *Bucephalandra pubes* S. Y. Wong & P. C. Boyce, *Bucephalandra pygmaea* (Becc.) P. C. Boyce & S. Y. Wong, *Bucephalandra sordidula* S. Y. Wong & P. C. Boyce, *Bucephalandra tetana* S. Y. Wong & P. C. Boyce, *Bucephalandra ultramafica* S. Y. Wong & P. C. Boyce, *Bucephalandra vespula* S. Y. Wong & P. C. Boyce, and *Bucephalandra yengiae* P. C. Boyce (Wong & Boyce, 2014a). With the additional of two latest descriptions of *Bucephalandra micrantha* S. Y. Wong & P. C. Boyce and *Bucephalandra spathulifolia* Engl. Ex S. Y. Wong & P. C. Boyce, have make up *Bucephalandra* into 29 species so far (Wong & Boyce, 2014b).

2.3 Molecular Systematics

2.3.1 Internal Transcribed Spacer (ITS)

Internal Transcribed Spacer is regularly used to compare species and closely related genera. A single unit of nuclear ribosomal DNA (nrDNA) consists of the main components; the coding regions (18S, 5.8S, 26S), Internal Transcribed Spacers (ITS1 and ITS2) and intergenic spacers (Figure 5). Based from a study conducted by Baldwin *et al.* (1995), they proved that 18S-26S nrDNA of ITS region is a useful source of characters for phylogenetic studies in many angiosperm families.

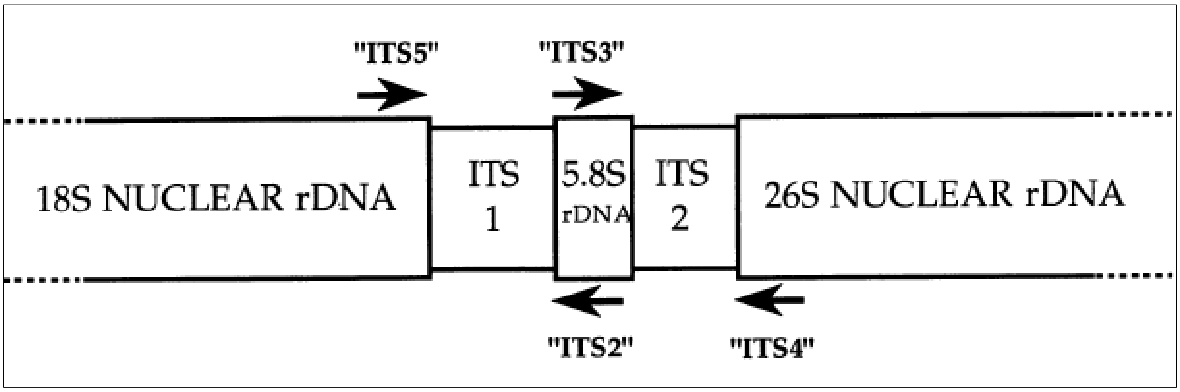

Figure 5. Regions of ITS1, 5.8s and ITS2 were included in this study. (Baldwin *et al.*, 1995)

Combination of data set from both spacers help to yield trees with better resolution and internal support in various taxonomic levels. One of the favourable properties of ITS region is highly repeated in the plant nuclear genome. This property aids to promote detection, amplification, cloning and sequencing of nrDNA (Baldwin *et al.*, 1995). Besides, the small size of the ITS region with approximately less than 700 base pairs in angiosperms and the presence of highly conserved sequences make the region easy to be amplified even from herbarium material.

For plant molecular systematic investigations at the species level, the Internal Transcribed Spacer (ITS) region of the nuclear ribosomal cistron (18S-5.8S-26S) is the most commonly sequenced locus. This region has shown broad utility across photosynthetic eukaryotes with the exception of ferns and fungi and has been suggested as a possible plant barcode locus (Kress *et al.*, 2005).

Additional nrDNA sequence has been added as one of the tools available to plant molecular systematists to get information from source independent of cpDNA. According to Small *et al.* (2004), the other primary reason is to obtained sequences that evolve at a faster rate, so that more phylogenetic informative characters can be obtained especially in lower taxonomic level.

Given, both non-coding cpDNA and ITS data are collected for phylogenetic studies in lower taxonomic level, ITS often shows greater level of divergence and thus greater resolution and stronger support than an equivalent sample of cpDNA sequence (Small *et al*., 2004).

2.3.2 Megakaryocyte-associated tyrosine kinase (*mat*K)

Chloroplast DNA (cpDNA) has been most widely used in resolving problem for phylogenetic analyses (Small *et al.*, 2004). The *mat*K gene is a chloroplast genome encoded locus located within the intron of the chloroplast gene *trn*K and encodes a maturase on the large single copy section (Ince *et al.*, 2005). *mat*K is one of the most rapidly evolving plastid coding regions and it consistently showed high levels of discrimination among angiosperm species. Chloroplast DNA is useful in evolutionary studies because of its simple structure, highly conserved sequence, and maternal inheritance characters (Pan *et al.*, 2012).

The presence of relatively high copy number in cpDNA give an advantage as the high copy number help in restriction site analyses as well as PCR amplification of specific cpDNA regions (Small *et al*., 2004). cpDNA sequence is also one of the least conserved