

**PHYLOGENETIC STUDY OF GENUS
SPATHOGLOTTIS BLUME (ORCHIDACEAE)
IN MALESIA**

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**PHYLOGENETIC STUDY OF GENUS
SPATHOGLOTTIS BLUME (ORCHIDACEAE) IN
MALESIA**

by

FARAH ALIA NORDIN

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LIST OF ABBREVIATIONS

asl	above sea level
bp	base pairs
c.	approximately
<i>cf.</i>	confer to
cm	centimeter
DNA	Deoxyribonucleic Acid
dNTP	Dinucleotide Triphosphate
E	East
e.g.	example
<i>et al.</i>	And others (<i>et alia</i>)
GPS	Global Positioning System
km ²	kilometer square
Kya	thousand years ago
L	Liter
lat.	latitude
long.	longitude
m	meter
M	Molar
mM	millimolar
min	minutes
ml	milliliter
mm	millimeter
Mya	million years ago
N	North
no.	number
PCR	Polymerase Chain Reaction
pers. comm.	personal communication
rpm	revolutions per minute
sec	seconds
sp.	species (singular)
spp.	species (plural)
V	Volt
v/v	volume over volume
var.	variety
Ver.	Version
w/v	weight over volume
~	approximately
<	less than
>	more than
≤	similar and less than
≥	similar and more than
-	to

%	percent
&	and
/	or
μ	micro
°C	degree Celcius

**KAJIAN FILOGENETIK GENUS *Spathoglottis* Blume (ORCHIDACEAE) DI
MALESIA**

ABSTRAK

Genus *Spathoglottis* Blume (tribe Collabieae, subfamili Epidendroideae) adalah genus dengan sejumlah 49 spesies geofit tanah dengan taburan di seluruh tropika dan subtropika Asia sehingga ke Kepulauan Pasifik. Sejumlah 44 spesies telah direkodkan di rantau Malesia dan tertumpu di New Guinea. Walaupun terkenal dari segi hortikultur, *Spathoglottis* telah dikenalpasti sebagai satu genus dengan kecelaruan taksonomi di dalam famili Orchidaceae. Sebagai contoh, kecelaruan telah dikenalpasti di antara dua spesies *Spathoglottis* yang berbunga kuning; iaitu *S. aurea* dan *S. microchilina* yang mana kedua-dua spesies menunjukkan keplastikan (kepalsuan) morfologi yang kompleks. Oleh yang demikian, kajian ini telah dimulakan untuk mengenalpasti ciri-ciri morfologi di antara spesies *Spathoglottis*, seterusnya kajian filogenetik molekul dijalankan bagi menjelaskan hubungan di antara spesies di dalam genus ini. Sejumlah 16 taksa (13 spesies dan tiga varieti) *Spathoglottis* dari Indo-Cina dan seluruh Malesia telah diperoleh. Sebanyak 72 ciri-ciri morfologi dan tiga ciri ekologi telah dikenalpasti dari semua taksa yang dikaji. Berdasarkan analisis dari segi morfologi, genus *Spathoglottis* di Malesia telah terbahagi kepada dua pengkelasan utama berdasarkan warna bunga iaitu: (1) Kompleks *Spathoglottis* Ungu dan (2) Kompleks *Spathoglottis* Kuning. Pengkajian molekul untuk spesies *Spathoglottis* telah dijalankan dengan menggunakan kaedah penanda molekul dari dua plastid DNA gen (*matK* dan *trnL-F*) dan *internal transcribed spacer* dari ribosoma nuklear DNA (ITS). Tiga analisis filogenetik telah dijalankan untuk mengkaji hubungan evolusi di antara spesies *Spathoglottis* di Malesia iaitu:

Maximum Parsimony (MP), Maximum Likelihood (ML) dan Bayesian Inference (BI). Analisis gabungan untuk data urutan nukleotida dari penanda molekul plastid dan nuklear telah menunjukkan bahawa genus *Spathoglottis* adalah monofiletik dengan indeks konsistensi, CI= 0.709; indeks pengekal, RI= 0.906; peratusan bootstrap, BS= 98% (ML), BS= 100% (MP) dan nilai kebarangkalian posterior, PP= 1.0 (BI). Hasil dari analisis ini, empat kumpulan utama telah dikenalpasti iaitu: (1) *Dwarf Spathoglottis* Ungu, (2) *Dwarf Spathoglottis* Kuning, (3) *Large Spathoglottis* Ungu, dan (4) *Large Spathoglottis* Kuning. Perpecahan di antara kumpulan *Dwarf* dan *Large Spathoglottis* menunjukkan telah terjadinya perbezaan awal dari segi saiz tumbuhan, warna bunga, dan saiz bunga; berkemungkinan dari perubahan strategi persenyawaan kasmogami (penyebaran/persenyawaan oleh serangga/luar) kepada kleistogami (persenyawaan sendiri/dalaman). Berdasarkan analisis molekul tersebut, satu varieti *Spathoglottis* yang berbunga putih, *S. plicata* var. *alba* telah dicadangkan untuk diubah dari segi penamaan taksonominya, dan dinaik taraf dari status varieti kepada spesies; manakala identiti satu spesies *Spathoglottis* dari Borneo, iaitu *S. kimballiana* turut memerlukan kajian lanjut. Walau bagaimanapun, pohon morfologi yang diperoleh dari kajian ini didapati tidak kongruen dengan pohon-pohon dari analisis molekul, membuktikan bahawa sekiranya hanya dengan menggunakan data morfologi, keputusan yang diperoleh boleh mewujudkan kekeliruan kerana ciri-ciri morfologi bunga berkeupayaan untuk berubah-ubah. Keputusan analisis morfologi, molekul, dan jurang kod bar DNA menunjukkan bahawa *S. aurea* dan *S. microchilina* sebagai dua spesies yang berbeza. Keputusan dari analisis-analisis ini telah digunakan untuk mengelaskan spesies kepada taburan kepulauan geografi yang tertentu. Didapati, setiap spesies *Spathoglottis* hanya boleh dijumpai di kepulauan tertentu di dalam Malesia; dengan hampir tiada spesies

menyeberangi kepulauan yang berbeza; kecuali spesies yang paling luas taburannya iaitu *S. plicata*. Oleh yang demikian, lima kepulauan yang merupakan habitat asal kepada spesies *Spathoglottis* tersebut telah dikenal pasti di dalam kajian ini iaitu: (1) Indo-Cina, (2) Semenanjung Malaysia + Sumatra (Malesia Barat), (3) Borneo (Malesia Barat), (4) New Guinea + New Caledonia (Malesia Timur) dan (5) Meluas (Malesia Timur + Malesia Barat).

**PHYLOGENETIC STUDY OF GENUS *Spathoglottis* Blume (ORCHIDACEAE)
IN MALESIA**

ABSTRACT

The genus *Spathoglottis* Blume (tribe Collabieae, subfamily Epidendroideae) is a genus with a total of 49 terrestrial geophyte species and is widely distributed in tropical and subtropical Asia and the Pacific Islands. A total of 44 species were recorded in the Malesian region and are concentrated particularly in New Guinea. Despite of being popular in horticulture, *Spathoglottis* is a taxonomically confused genus in Orchidaceae. For example, confusion is detected between a pair of species within the yellow-flowered *Spathoglottis*; the *S. aurea* and *S. microchilina* with both species show a complex morphological plasticity. This study was initiated to examine the morphological attributes among species of *Spathoglottis*; and establishing a molecular phylogenetic study in elucidating the relationships among members of the genus. A total of 16 taxa (13 species and three varieties) of *Spathoglottis* from Indo-China and throughout Malesia were examined. Seventy-two morphological and three ecological characters were selected from all taxa under investigation. Based on the morphological analysis, genus *Spathoglottis* in Malesia can be divided into two major sections based on the colour of the flower: (1) the Purple *Spathoglottis* Complex and (2) the Yellow *Spathoglottis* Complex. Molecular studies for the species of *Spathoglottis* were carried out using two plastid DNA genes (*matK* and *trnL-F*) and the internal transcribed spacer of a nuclear ribosomal DNA (ITS). Three phylogenetic analyses were conducted to study the evolutionary relationships among species of *Spathoglottis* in Malesia: Maximum Parsimony (MP), Maximum

Likelihood (ML) and Bayesian Inference analyses (BI). The analyses of combined plastid and nuclear data showed that genus *Spathoglottis* is monophyletic with consistency index, CI= 0.709; retention index, RI= 0.906; bootstrap percentages, BS= 98% (ML), BS= 100% (MP) and posterior probability value, PP= 1.0 (BI). Four major groups were determined from these analyses: (1) Dwarf Purple *Spathoglottis*, (2) Dwarf Yellow *Spathoglottis*, (3) Large Purple *Spathoglottis*, and (4) Large Yellow *Spathoglottis*. The split in the Dwarf and Large *Spathoglottis* Groups might reflect an early differentiation of plant size, flower colour, and flower size; perhaps due to the shift from chasmogamy (insect-pollinated) to cleistogamy (self-pollination) strategies. From these analyses the white-flower *Spathoglottis*, *S. plicata* var. *alba* is proposed for taxonomical and nomenclatural changes by upgrading it to a species rank; whereas the identity of a Bornean species, *S. kimballiana* needs further investigation. However, the morphology tree obtained in this study was incongruent to the molecular trees and morphology alone can be misleading for inferring the relationships among groups of interest, as floral morphology is highly flexible. The morphological, molecular, and DNA barcoding gap analyses showed *S. aurea* and *S. microchilina* as two separate species. These results were used for grouping of species into their specific geographical island distributions. Certain species of *Spathoglottis* were observed to confine to only particular island groups throughout Malesia; with almost a complete no crossing-over between the islands; except for the most widespread and weedy species, *S. plicata*. Thus, five island groups from the West, Central and East Malesia were proposed in this study: (1) Indo-China, (2) Malay Peninsula + Sumatra (West Malesia), (3) Borneo (West Malesia), (4) New Guinea + New Caledonia (East Malesia) and (5) Widespread (East Malesia + West Malesia).

CHAPTER ONE

INTRODUCTION

1.1 General Introduction

The orchid family, Orchidaceae is one of world's two largest families of flowering plants. It is also richly diverse, with over 25,000 named species in 736 recognized genera, and perhaps second only to the sunflower family, Asteraceae (Compositae). Orchidaceae comprises a substantial ten percent of all worlds' vascular plant species, with at a rate of roughly 500 new species and 13 new genera being described every year (Dressler, 1993; Freudenstein and Rasmussen, 1999; Chase, 2005; Chase *et al.*, 2015). The orchid family is highly evolved and their diversity of specialized individual pollinators or mycorrhizal fungi, adaptive ecological and pollination strategies, evolution of pollinia and rapid degree of speciation have provided a rich system to study on evolutionary patterns (Freudenstein and Rasmussen, 1999; Givnish *et al.*, 2015). Traditionally, the classification systems of the orchids were based on subjective assessments of the appearance of the whole plant and their morphological characters (Dressler, 1981; Dressler, 1993; Freudenstein and Chase, 2015). Since the past two centuries, the orchid systematics have been based exclusively on features of the flower, predominantly the gynostemium or column and anther; which are unique to this family (Freudenstein *et al.*, 2002; Chase *et al.*, 2003; Xiang *et al.*, 2014). However, classification that relies heavily on characters was not warranted as their structure is purely intuitive. They often show considerable convergence due to ecological selection

and create problem at higher levels because floral morphologies are extremely plastic (Chase, 2005; Miner *et al.*, 2005; Górnjak *et al.*, 2010; Ackerman *et al.*, 2011; Paniagua-Ibáñez *et al.*, 2015).

The past 20 years have shown significant progress in the systematics of orchids when molecular data have come to play an important role in angiosperm classification. Numerous DNA phylogenetic studies have shed new lights into the relationship of the orchid family. Increasing efforts are being focused on familial-level classification; ranging from genera (Bellstedt *et al.*, 2001; Jheng *et al.*, 2012; Xiang *et al.*, 2013) to subtribes (Cameron, 2005; Sosa, 2007) to tribes (Van Den Berg *et al.*, 2005; Xiang *et al.*, 2014) to subfamilies (Freudenstein *et al.*, 2002; Kocyan *et al.*, 2004; Freudenstein and Chase, 2015) and to the whole family (Chase *et al.*, 2003; Chase, 2005; Chase *et al.*, 2015). Following the most recent classification system of Orchidaceae based on molecular data (Chase *et al.*, 2015) the family of orchids were recognized into five subfamilies; the Apostasioideae, Vanilloideae, Cyripedioideae, Orchidoideae and Epidendroideae.

The genus *Spathoglottis* Blume (tribe Collabieae, subfamily Epidendroideae) is a well-known genus with a total of 49 terrestrial geophyte species and is widely distributed in tropical and subtropical Asia and the Pacific Islands; where 44 species were recorded in the Malesian region and are concentrated particularly in New Guinea (Govaerts, 2013). Blume proposed the genus *Spathoglottis* in 1825 with *S. plicata* as the type species and the only known species at the time. In 1838, Lindley described the genus as *Paxtonia*

which is now regarded as a synonym of *Spathoglottis*. According to Cribb and Tang (1981), despite of being popular in horticulture, *Spathoglottis* is a taxonomically confused genus in Orchidaceae. From the tribal level itself, placement of tribe Collabieae (subfamily Epidendroideae) has been hotly debated over the years. Collabieae has not been recognized by most authors and was considered as synonym to Bletinae (Sosa, 2007; Xiang *et al.*, 2014). Dressler (1993) treated it as groups of uncertain systematic position thus listed it as one of his “leftover and misfits” groups. Collabiinae was an unplaced subtribe in previous classifications but is now elevated to a tribe based on the most recent revision done by Chase *et al.* (2015).

Early revision work on this genus was discussed by Dockrill (1969) for the Australian *Spathoglottis* species, followed by Cribb and Tang (1981) for Australia and the Pacific Islands whilst Hallé (1977) revised the New Caledonian species. However, a revision of *Spathoglottis* in Malesia is still lacking until now and no molecular phylogenetic study has been carried out. Studies of the morphology and the anatomy have been reported by several authors such as in Holttum (1964) and Seidenfaden and Wood (1992). Observations on the leaf anatomy of *Spathoglottis* were made by Solereder and Meyer in 1930 and Williams in 1979; and on the root by Pridgeon *et al.* in 1983 and later by Porembski and Barthlott in 1988. Teoh (1984) and Brandham in 1999 reviewed the cytogenetics part and suggested dysploidy in *Spathoglottis* (chromosome count of $2n=18, 38, 40, 42,$ and 60). To date, no palynological study of *Spathoglottis* has been published, although pollen characters were suggested to be taxonomically informative. Recently, a molecular study on the chloroplast DNA barcoding of *Spathoglottis* in Malaysia for genetic conservation has been carried out by Ginibun *et al.* (2010) and four

chloroplast regions, *matK*, *rbcL-a*, *rpoB* and *rpoC1* were successfully amplified from all seven species (*S. aurea*, *S. gracilis*, *S. kimballiana*, *S. plicata*, *S. plicata* var. *alba*, *S. unguiculata* and a *Spathoglottis* hybrid) treated. However, their DNA barcoding study does not provide a deep phylogenetic inference, as the main goal is not to determine patterns of relationship but to identify an unknown sample in terms of a preexisting *Spathoglottis* classification.

Morphologically, confusion is detected between a pair of species within the yellow-flowered *Spathoglottis*; the *S. aurea* and *S. microchilina* complex. This observation was also reported by Seidenfaden and Smitinand (1959), Wood (1997), Chan *et al.* (2001), Comber (2001) and Seidenfaden and Wood (1992). The two species in this complex is distinguished based on the width of the lip (1.5 mm in *S. microchilina*; 4 mm wide in *S. aurea*) and the ability of the flower of *S. microchilina* to self-pollinate (cleistogamy). In the wild, populations of both *S. aurea* and *S. microchilina* show great phenotypic variations and forms. Thus, further examination is required to delimit *S. aurea* and *S. microchilina* as two distinct species or just one highly variable form of *S. aurea*.

On a biogeographical note, it is very informative to look at the distribution patterns among the species of this genus. Throughout Malesia, certain species of *Spathoglottis* were observed to confine to only particular island groups; with almost a complete no crossing-over between the islands; except for the most widespread and weedy species, *S. plicata*. The seven island groups observed were: Indo-China, Malay Peninsula + Sumatra (Sunda Shelf), Borneo (Sunda Shelf), Java (Wallacea), Sulawesi (Wallacea),

the Philippines (Wallacea) and New Guinea (Sahul Shelf). Vicariance hypothesis was assumed for the dispersal pattern of *Spathoglottis* through Malesia and intercontinental long-dispersal is relatively uncommon or impossible. This is suggested by comparing the age of the Orchidaceae that have arisen roughly 112 Mya ago (Late Jurassic to Cenozoic) and the most recent rapid divergence in higher epidendroids (subfamily Epidendroideae, tribe Collabieae) was estimated between 30.8–37.9 Mya (Christenhusz and Chase, 2013; Givnish *et al.*, 2015). The West Malesia islands (Sunda Shelf) were already in place approximately 160 Mya, and the East Malesia islands (Sahul Shelf) amalgamated at ~50 Mya and most of the Wallacea (Sulawesi, the Philippines and Lesser Sunda Islands) has only emerged at about 5 Mya (Cenozoic); providing evidence that there was no land bridge possible but might only be stepping stones (Hall, 1999; Voris, 2000; Van Welzen and Slik, 2009; Van Welzen *et al.*, 2011).

1.2 Objectives

Phylogenetic study among species within *Spathoglottis* has never been established. This is a pressing need since establishing species relationships will address issues such as evolutionary relationship between species as well as determination of identity or taxonomic status of certain species.

Thus, the objectives of this study are:

- 1) To examine the morphological characters among species in genus *Spathoglottis* and selecting key characters as important features in identification and placement of

taxa

- 2) To assess how useful a plastid gene (*matK*), non-coding plastid marker (*trnL-F*), the internal transcribed spacer (ITS) of the nuclear ribosomal DNA and combined molecular sequences in elucidating species relationship among members of *Spathoglottis*; and analyze their correlation to the morphological data
- 3) To investigate the biogeographical pattern among species in genus *Spathoglottis* based on the internal phylogeny groupings
- 4) To resolve the taxonomic questions in Yellow *Spathoglottis* Complex of *S. aurea* and *S. microchilina*

CHAPTER TWO

LITERATURE REVIEW

2.1 What and Where is Malesia?

The Malay Archipelago, also known as Malesia is one of the three world's richest tropical rainforests with estimated 42,000 spp. (Van Welzen *et al.*, 2005). Malesia is a plant geographical region that was first recognized and described by Heinrich Zollinger, a Swiss botanist and explorer in 1857. In his article, Zollinger noticed that the flora of this huge archipelago was in many respects, very distinct from that of neighbouring regions (Raes and Van Welzen, 2009; Schuiteman, 2013). He named his floristic region as 'Flora Malesiana', after the Malay language that is commonly used throughout the archipelago.

Malesia was defined based on its flora distribution patterns, and the recognition on the presence or absence of species throughout the archipelago. Zollinger strictly acknowledged that a floristic region should never be interchanged with the boundaries of political colonies. Relying on a very limited plant distribution data; and coupled with many straight lines; in 1857 Zollinger defined the boundaries of the floristic region of Malesia (Figure 2.1; total grey area). Many of his colleagues at the time argued that his delimitation is too extensive. Responding to it, Zollinger thus recognized 'Flora Malesiana' in a more restricted sense (dark-grey area). However, Zollinger largely

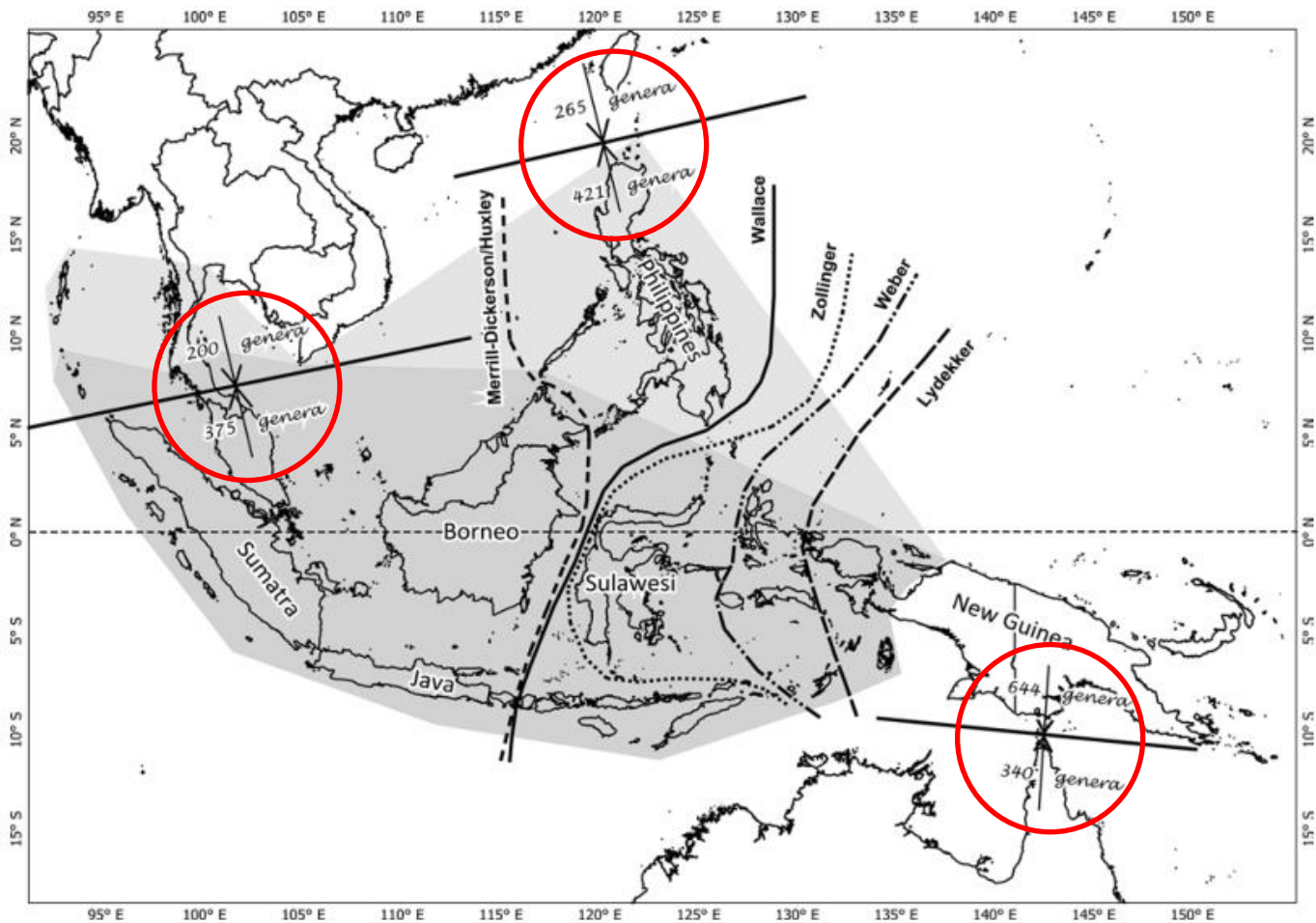


Figure 2.1: Map of the boundaries in Malesian floristic region as recognized by Zollinger in 1857. Total grey area is in the widest sense and the dark-grey area in more restricted sense. New Guinea is largely excluded. The delimitation by Van Steenis (1948; 1950) is marked by three demarcation knots (red circle). The numbers indicate the number of plant genera not crossing the knots. The different lines indicate Wallace's Line and its variants by different authors that split up Malesia to the eastern and western parts. Map adopted from Raes and Van Welzen (2009).

excluded New Guinea from his Malesian flora due to lack of collections. He also sighted snows on the highland's peak of New Guinea mountains; which led him to conclude that flora of New Guinea much resembled those in temperate mainland than of an island flora (Van Welzen *et al.*, 2005; Raes and Van Welzen, 2009).

Ever since the recognition of Malesia, debates have been going on about the internal division of this floristic region. One of the world's most prestigious flora projects, the 'Flora Malesiana' was first thought to be defined by Van Steenis (1948). He indeed circumscribed the area; however the term is older and was introduced earlier by Zollinger in 1857. Only few people were aware of this and most of the time they have been referring to incorrect references; duly to the fact that Zollinger's publication was written in Dutch therefore unreadable for most of the non-native.

In 1948, Van Steenis came with the idea to develop a Flora of Indonesia; which then was still Dutch-Indonesia. However, he did not plan to produce a national flora, but instead he wanted to compile a flora based on a phytogeographical region; an area with many endemic species and elements of its own (Van Welzen *et al.*, 2005). Despite of most of the plant species were still poorly known at that time, Van Steenis (1948; 1950) managed to identify three sharp boundaries based on the distribution of 2178 plant genera with shared geographical limit: (1) the most Western boundary (between Thai-Malaysian borders) where 375 genera have northern limit and 200 genera reached

their southern limit, (2) the most Northern boundary (between the Philippines–Taiwan) with 421 genera to the south and 265 genera to the north of it, and (3) the most Southern boundary (between New Guinea and Australia) with 340 genera to the south and 644 genera to the north of it, respectively (Figure 2.1). Almost after 100 years later, Van Steenis largely confirmed Zollinger’s initial delimitation of Malesia floristic boundaries.

2.1.1 Wallace’s Line – Two or Three Phytogeographical Areas?

Wallace’s Line or its variants (Figure 2.1) were known to divide Malesia into a western (Sunda Shelf) and eastern sub–region (Sahul Shelf).

Throughout his nine years of expedition to South East Asia, Alfred Russell Wallace (1860) noticed that the fauna of the Malay Archipelago consisted of Asian and New Guinea–Australian elements; which then led him to the discovery of a famous zoological boundary of all time. The invisible line, known as Wallace’s Line runs east of the Philippines, between Borneo and Sulawesi and finally between Bali and Lombok. Wallace documented his finding in series of scientific articles and books; and receiving crowds of attention through the award–winning *The Malay Archipelago* (Wallace, 1890). He observed that the distributions of animal groups within the archipelago discontinuous across the line. He however was uncertain on the position of Sulawesi and called it as ‘an anomalous island’ with old endemic Australian species and lacks continental Sundaic groups (Turner *et al.*, 2001; Raes and Van Welzen, 2009; Van Welzen *et al.*, 2011; Crayn *et al.*, 2015). The other authors have recognized similar lines;

the western and eastern variants of Wallace's Line (Merill-Dickerson/Huxley Line and Zollinger's Line) that split up Malesia into its western and eastern parts. Wallace's Line and its variants separated Malesia into two major areas based on its fauna composition; but are they suitable to explain the plant distribution patterns in this region?

Numerous studies focusing on the composition of plant families and genera in Malesia; as prepared by Baker *et al.* (1998); Cox (2001); Van Welzen *et al.* (2005), Raes and Van Welzen (2009), Van Welzen and Slik (2009); Van Welzen *et al.* (2011) and Webb and Ree (2012) have finally revealed a stronger partitioning of Malesia into three instead of two phylogeographical areas: the western Sunda Shelf, central Wallacea and eastern Sahul Shelf. This will be further discussed in subtopic 2.3.

2.1.2 The Demarcation and Internal Division of Malesia

The status of Malesia as a phylogeographical region was recently confirmed by the studies on plant distribution patterns and plate tectonics in Malesia.

Malesia reaches from the southern tip of Thailand, stretched throughout Malaysia and Indonesia to the Philippines and Papua New Guinea (Figure 2.2). It is comprises of three higher phylogeographical subunits: (1) the western Sunda Shelf (2) central Wallacea, and (3) the eastern Sahul Shelf which includes nine island groups: Malay Peninsula (not a true island), Sumatra, Borneo, Java, Sulawesi, the Philippines, Moluccas, Lesser Sunda Islands and New Guinea.

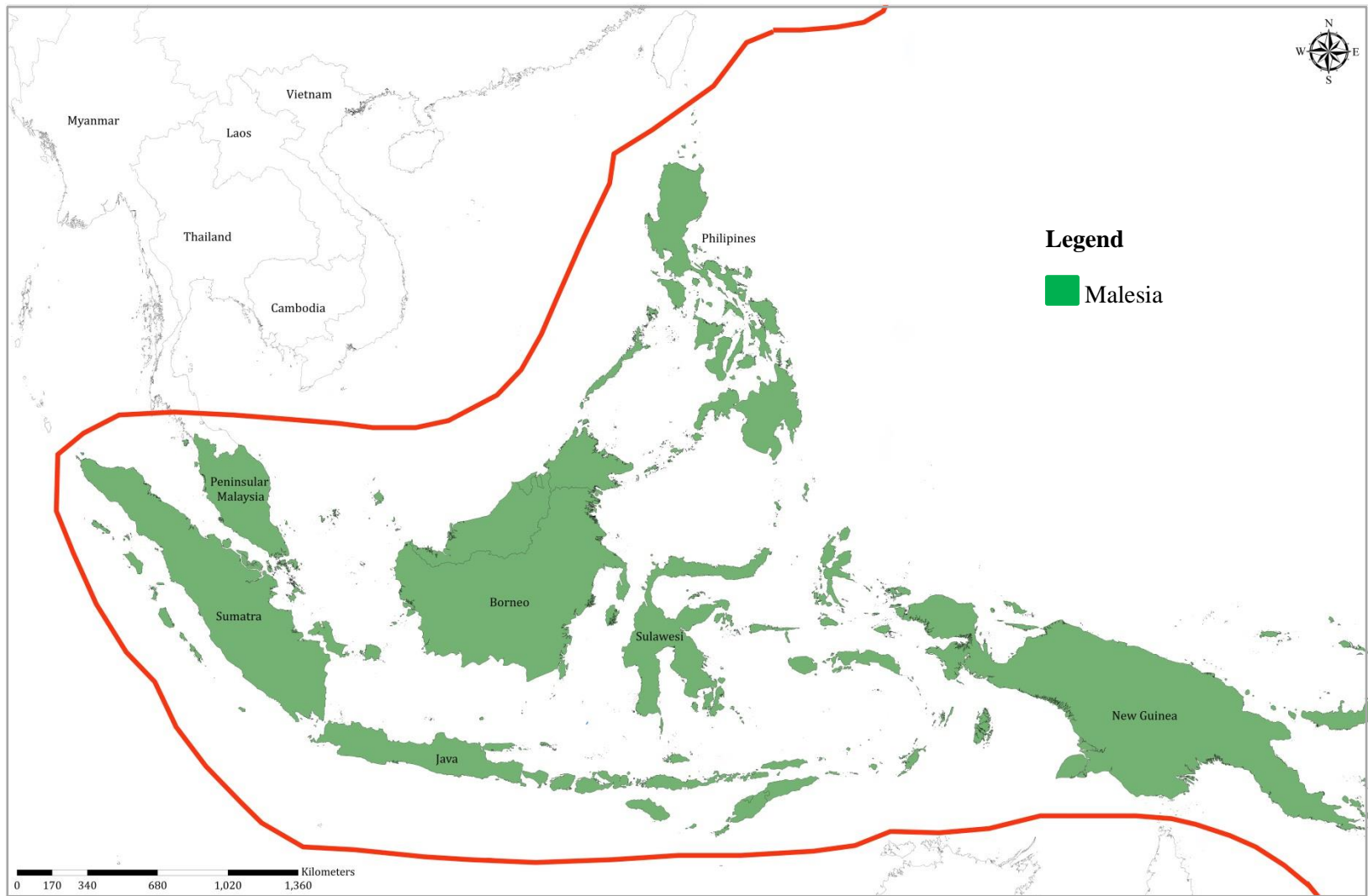


Figure 2.2: Map of Malesia. Re-draw from Van Welzen *et al.* (2011).

The western part of Malesia is the everwet Sunda Shelf comprising of the Malay Peninsula, Sumatra and Borneo (minus Java) (Figure 2.3). The central Malesia known as Wallacea is a transition zone between the flora of Sunda and Sahul. Wallacea has a dry monsoon climate throughout the year; and Sulawesi, Moluccas, the Philippines (including Palawan and Mintoro), Lesser Sunda Islands and Java were islands grouped together in Wallacea. Java was previously placed under Western Malesia. However, Van Welzen *et al.* (2011) reported that Java shares Wallacean flora and has a dry monsoon climate. Wallacea is a distinct floristic area as it comprised many endemic and drought-tolerant elements. New Guinea (Indonesian Irian Jaya and Papua New Guinea) made up the Sahul Shelf.

As parallel to Van Steenis (1948; 1950), three invisible ‘demarcation knots’ or clear borders based on generic distributions surrounding Malesia were recognized: (1) between the southern tip of Thailand throughout Malaysia and Indonesia, (2) between Taiwan and the Philippines, (3) between New Guinea and Australia. In either direction, these borders are not crossed by numerous plant genera and species; which the orchids (Orchidaceae), *Nepenthes* (Nepenthaceae), *Lithocarpus* (Fagaceae) and Ericaceae provide many examples of these (Van Welzen *et al.*, 2005; Raes and Van Welzen, 2009; Van Welzen and Slik, 2009; Schuiteman, 2013).

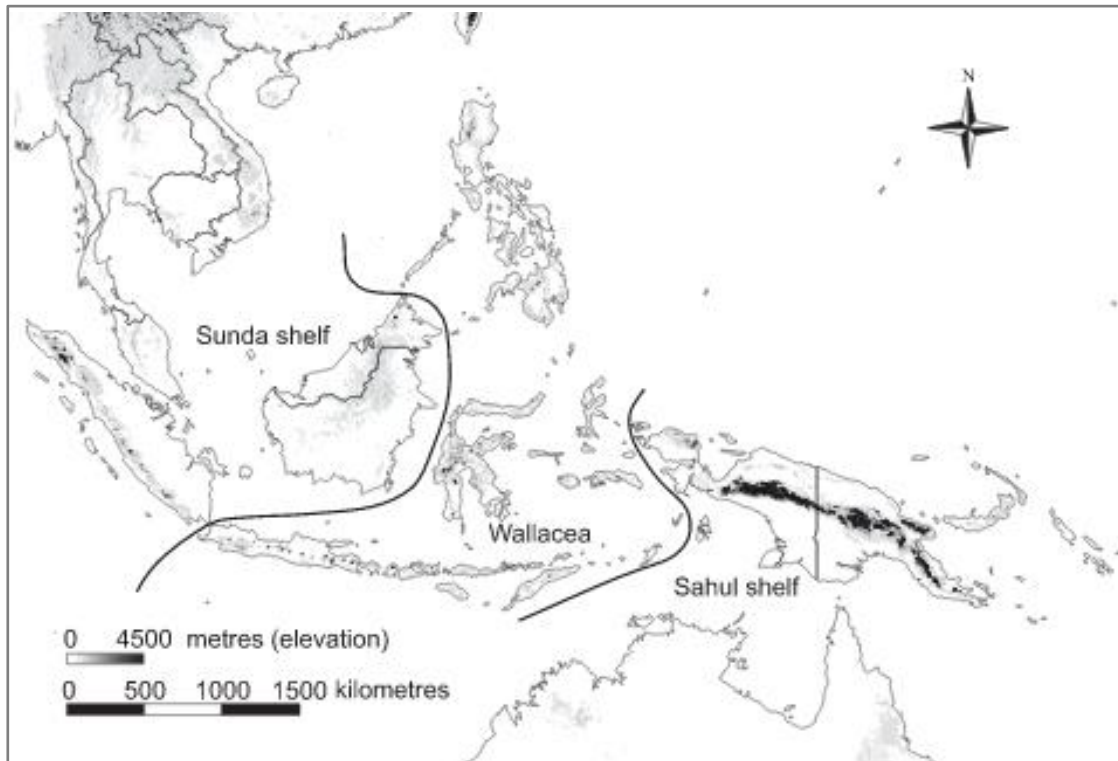


Figure 2.3: Three phytogeographical subunits in Malesia: Sunda, Sahul and Wallacea. The area of endemism employed and intercontinental dispersal dynamics. Map adopted from Crayn *et al.* (2015).

2.2 Plate Tectonics and Changing Palaeogeography in Malesia

Malesia is a mosaic of colliding smaller and major plates with many small tectonic fragments or slivers acting as ball-bearings. During the ancient tectonic movements, several seas meet; the island arcs collided with continents and continental fragments; leading to the rise of mountains and formation of deep ocean basins. East, south and west of Malesia were surrounded by volcanic arcs belt with abundant volcanism and intense seismicity; due to the high lithosphere subductions of the Indian and Pacific

Oceans. The geology and palaeogeography of Malesia continues to change rapidly and the region has characterized most of the Cenozoic (~0-66 Mya) (Voris, 2000; Van Welzen *et al.*, 2005; Hall 2009).

The Eurasian Plate moves slowly to the east due to the opening of the Atlantic Sea while the Pacific Plate is moving west and disappears below the Eurasian and New Guinean-Australia Plate. During its movement towards north, many tectonic fragments and slivers broke off from the New Guinean–Australia Plate. Meanwhile, the Indian Ocean Plate moves to the east and subducting below the Malay Archipelago belt (Sumatra to Lesser Sunda Islands). Finally, the Philippine Plate has almost disappeared and left only with some continental debris (Van Welzen *et al.*, 2011).

The broke off tectonic slivers from the major continental plates can be simply overviewed in two waves (Audley–Charles, 1987). The first wave constitutes of the present day South East Asia mainland (Burma, Tibet) and West Malesia (Sunda Shelf) that includes the Malay Peninsula, Borneo, Sumatra and Java. This wave broke off from Australia and collided with the Eurasian Plate while rafting northwards. The process was estimated to start at least during the Late Jurassic (~160 Mya) (Van Welzen *et al.*, 2011) but it may have been earlier (Palaeozoic, ~400 Mya) (Voris. 2000; Turner *et al.*, 2001; Hall, 2009). Importantly to note, these areas already formed the present South East Asia (although of Australian origin) long before the evolution of many recent plants and animals. Most of plant families existed as early as the late Cretaceous (~70 Mya) and relatively earlier (~120 Mya) for the orchids (Christenhusz and Chase, 2013). Thus, the

present-day flora and fauna will be mainly of South East Asia origin (Cox and Moore, 1993; Turner *et al.*, 2001; Van Welzen *et al.*, 2011).

The second wave broke off much later, approximately 50 Mya and formed the East Malesia (Sunda Shelf – Sulawesi, Moluccas, Lesser Sunda Islands and New Guinea). Most of the East Malesia areas; certainly, New Guinea, Sulawesi and the Philippines were amalgamations of microplates. The Sunda Shelf and Sahul Shelf were widely separated by ocean. They were only brought into contact ~25 Mya (early Miocene) during the northwards transition of Sahul after breaking off from Antarctica. The collision has caused extensive uplift and orogenesis events in the archipelago and emergence of numerous islands in Central Malesia; known today as the Wallaceae. Even when the islands in the Central Malesia reached their present-day placements, most areas were still submerged during the time and only started to emerge during the Pliocene approximately 5 Mya (Pigram and Davies, 1987; Van Welzen *et al.*, 2005; Hall, 2009).

As for New Guinea, it was a complicated history. Originally, New Guinea was only consisted of the southern part termed ‘Craton’ which has always been attached to Australia. The rest of New Guinea was amalgamations of more than 30 tectonic slivers or terranes of various origins (island arcs, continental fragments and pieces of sea floor) that formed the present-day Peninsula in the east and northern coast; and the Bird’s head in the west (Pigram and Davies, 1987; Van Welzen *et al.*, 2005; Hall, 2009; Van Welzen *et al.*, 2011).

For the Philippines, only parts of Luzon were left as continental debris from the original Philippine Plate. Borneo, Palawan and Mindoro were slivers that broke off from the mainland South East Asia. The rest of the Philippines arrived with the tectonic slivers of the New Guinean–Australian plate during the second wave (Van Welzen *et al.*, 2005).

Thus, the final stepping stones between South East Asia mainland and Australia got into position with this second wave.

2.3 Plants Distribution Patterns and Floristic Exchange in Malesia

Tectonic movements and rapid orogeny events in Malay Archipelago have opened up new niches and dispersal opportunities for its flora and fauna; predominantly in the Pliocene (~5.3–2.6 Mya). Climatic fluctuations accompanied by lowering and rising of sea levels during the late Pleistocene (~12 Kya–250 Kya); land bridge systems and sea barriers together with the uplift of highlands in Sumatra, Java, Sulawesi and New Guinea have promoted niches diversity throughout this region (Voris, 2000; Van Welzen *et al.*, 2005; Hall, 2009; Crayn, 2015). The radiation of species and rapid speciation events in Malesia were hypothesized as either through long dispersal of taxa or vicariance (disjunction) due to habitat fragmentations (Thomas *et al.* 2012; Christenhusz and Chase, 2013). The origins of the Asian and Australian species; and migrations between them in Malesia have long fascinated biologists. Many flora and fauna were observed to have a discontinuous distribution across the Wallace’s Line; which confine extant biotic groups to their component areas (Figure 2.3).

Malesia is a natural phytogeographic area with about 70% of its total flora is endemic to this region. Among other richest plant families with most endemic species are Ericaceae, Moraceae, Dipterocarpaceae and the orchids (Orchidaceae; with roughly 6800 species known to Malesia and 88% are endemics) (Van Welzen and Slik, 2009; Schuiteman, 2013).

The flora richness of each island groups in Malesia: Malay Peninsula, Borneo, Sumatra, Java, Sulawesi, the Philippines, Moluccas, Lesser Sunda Islands and New Guinea were observed correlates significantly with the size of the island subunits. All the nine areas show high species endemism with Borneo, the Philippines and especially New Guinea comprise significantly highest number of endemic species (Turner *et al.*, 2001; Van Welzen and Slik, 2009; Van Welzen, 2011; Crayn, 2015).

In 2005, Van Welzen *et al.* reviewed the plant distribution and composition along the Wallace's Line. Their treatments were subsequently followed by works of Raes and Van Welzen (2009) and Van Welzen *et al.* (2011). They analyzed the distribution patterns among 7340 species of plants from 165 families and 896 genera; which represents about 25% of total Angiosperms and ferns in Malesia. Botanical evidence from their studies revealed a stronger partitioning of Malesia into three phytogeographical areas instead of two: Sunda, Sahul and Wallaceae. Differences in climate explained the groupings of flora in the tree subregions which Sunda and Sahul Shelves are everwet whereas Wallaceae experienced dry monsoon climate throughout the years.

During the Glacial Maxima (Cenozoic); Sunda and Sahul Shelves formed landmass connected with Asia and Australia thus dispersal and exchange of species on the areas were relatively easy; whereas sea barriers remained within Wallacea. There was no major land bridge present in Malesia during the glacial periods and most stepping stones for species dispersal were only emerged in the last ~10 Mya (Hall, 1999; Voris 2000; Van Welzen *et al.*, 2011).

Consequently, the species composition of the two shelves is more homogenous as compared to Wallacea which consisted of many drought-tolerant species. It is also apparent that whatever line is used (Wallace's Line and its variants); they are all good boundaries and moving west to east shows stronger demarcation line. Lowest amount of species is crossing the Lydekker's Line which separates New Guinea from the rest; and this can be explained by the sea barriers surrounding Wallacea predominantly the deep Makassar Straits (Voris, 2000).

Similar studies on biogeography inference at generic and family level in Malesia were undertaken by Baker *et al.* (1998); Turner *et al.* (2001); Van Welzen *et al.* (2005); Brown *et al.* (2006); Raes and Van Welzen (2009); Van Welzen and Slik (2009); Schuiteman (2013); Crayn *et al.* (2015); and also involving the use of molecular markers to infer evolutionary relationships among the taxonomic units in Malesia as in Wagstaff (2004); Gussarova *et al.* (2008); Micheneau *et al.* (2008); Knopf *et al.* (2011); Thomas *et al.* (2012) and Christenhusz and Chase (2013). They observed the same patterns in

plant distribution and groupings along the Wallace's Line thus supporting Van Welzen *et al.* (2011).

2.4 The Orchids Family

The Orchidaceae is one of the largest families in flowering plant kingdom and one of the most actively evolves, diverse and widespread. To date, over 25,000 species of orchids in 736 genera were recognized; placing them probably second to the sunflower family, Asteraceae (Compositae). A substantial ten percent of all worlds' vascular plant species are the orchids; with at a rate of roughly 500 new species and 13 new genera being described every year. New knowledge from molecular studies has classified orchids into five subfamilies; the Apostasioideae, Vanilloideae, Cyripedioideae, Orchidoideae, and Epidendroideae (Dressler, 1993; Freudenstein and Rasmussen, 1999; Pridgeon *et al.*, 1999; Chase, 2005; Pridgeon *et al.*, 2005; Chase *et al.*, 2015).

Orchids are cosmopolitan and among the most well-adapted plants. They thrive on wide range of habitat worldwide; distributed on all vegetated continents except for the driest and coldest region; the deserts and Antarctica. The orchids are however most abundant in the humid tropics and subtropics where their true homes are.

Eventhough they often thought as rare; orchids in a sense are quite commonplace. They can be found anywhere; dwelling on the wet and shaded forest floor among leaves litters of the lowland tropical rainforest, on the branches of tall forest trees exposed to direct

sunlight and heavy rainfall for hours, in grassy and swampy areas of landslips and bogs, among rocky crevices and mossy ridges near the summit of a mountain, pioneering in left-over opening and by the roadside ditches or waterfalls, riversides or sea shore, and even on the margin of a forest (Dressler, 1981; Dressler, 1993; Pridgeon *et al.*, 1999; Ong *et al.*, 2011).

As their wide distribution is concerned, Orchidaceae has inspired a great deal of speculations on their timing and place of evolution. Fossil evidence of the orchids from the Meiocene has strongly suggested Orchidaceae as an ancient group which their origin predates the break-up of the Gondwanaland 125 Mya (Pridgeon *et al.*, 2001). A recent time-calibrated phylogeny study by Givnish *et al.* (2015) has estimated that Orchidaceae have arisen roughly 112 Mya during the Late Jurassic. Subfamily Epidendroideae represents the pinnacle of orchids' evolution (~37.9–30.8 Mya) which supported by rapid divergence among taxa in the subfamily (Givnish *et al.*, 2015; Chase *et al.*, 2015).

2.4.1 Diversification of the Orchidaceae

Variability in plant vegetative structures and size, pollen evolution, habitat adaptation, specific individual mycorrhizal fungi, and specialized pollination strategies have contributed to the significant diversification of Orchidaceae (Dressler 1993; Freudenstein and Chase, 2015; Givnish *et al.*, 2015).

The orchids can be epiphytic which grow high up on tree boles and branches; the ground-dwellers (terrestrial/heteromycotrophic), or lithophytic that grows among rocky boulders and limestone crevices (Plate 2.1A-F).

Majority of the orchids are epiphytic which constitutes nearly 80% of total species count and found mostly in tropical forests at montane altitude of 1000–1500 m asl; whereas all temperate orchids are of terrestrial species (Dressler, 1981; Atwood, 1986; Seidenfaden and Wood 1992; Rasmussen, 1995; Pridgeon *et al.*, 1999; Krömer *et al.*, 2005).

Epiphytism among else has been suggested to accelerate both speciation and extinction rates in Orchidaceae (Givnish *et al.*, 2015). Diverse vegetative structures and thousands of tiny seeds that capable for long-distance dispersal are among key innovations in epiphytic orchids. Spongy velamen root, waxy leaves, and fleshy pseudobulb were developed in response to adaptation on various adverse environmental needs; especially the problems of temperature fluctuation and water conservation on daily and seasonal basis which is very crucial on the tree canopies (Dressler, 1981; Pridgeon *et al.*, 1999). The tiny seeds, brought along by the wind will eventually settled down on the tree branches; invaded new niches that largely unoccupied by other vascular plant (Atwood, 1986).

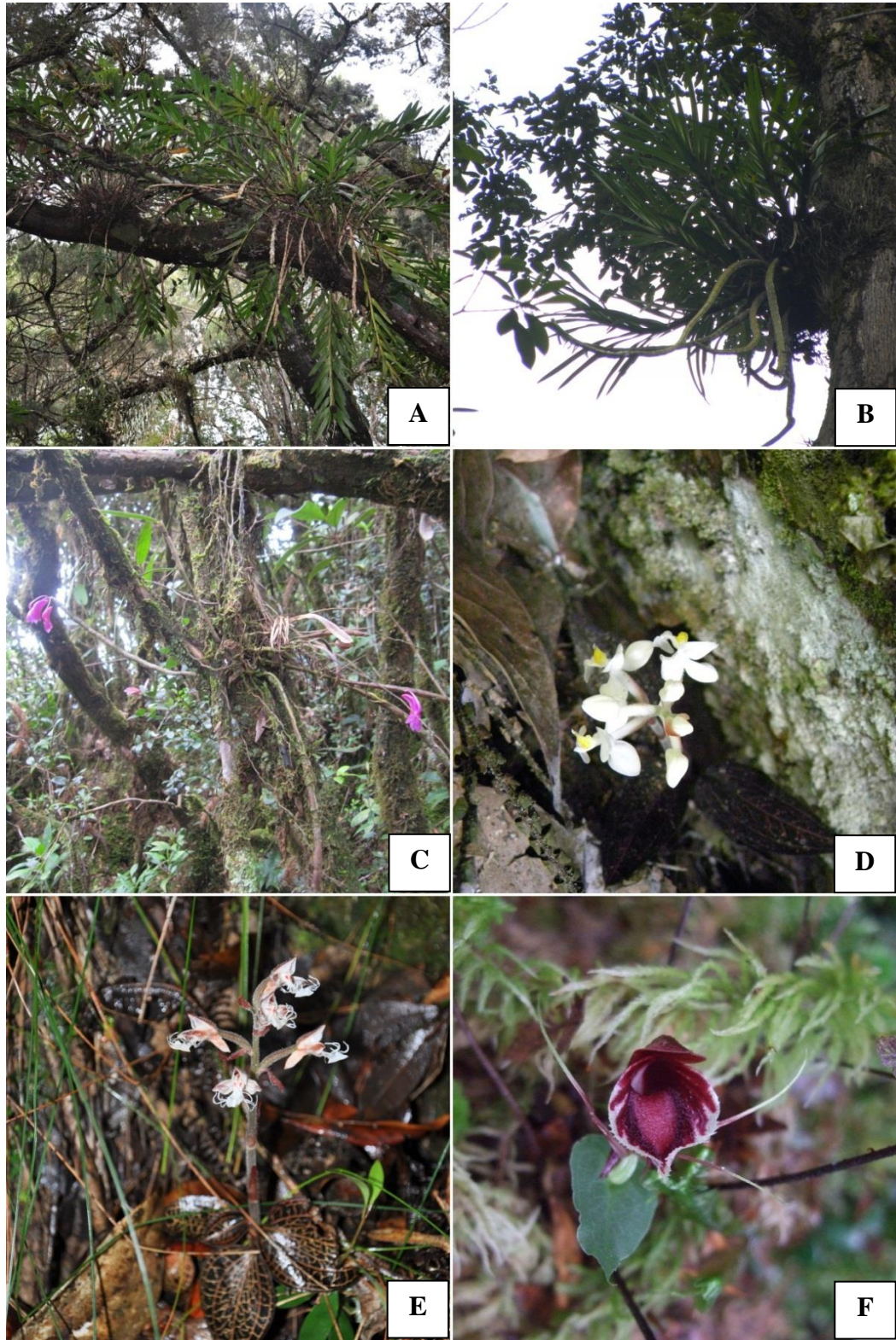


Plate 2.1(A-F): Epiphytic orchids (A) *Agrostophyllum majus*, (B) *Grammatophyllum speciosum*, (C) *Dendrobium hasseltii*; lithophytic orchid (D) *Ludisia discolor*; and terrestrial orchids (E) *Anoectochilus geniculatus*, (F) *Corybas holttumii* in their natural habitat. (Pictures not following scale).

As epiphytism is often associated with tropical montane condition; deep valleys and high ridges of the mountains may provide physical barriers to gene flow due to the isolation of populations at larger spatial scale. While concurrently, the tiny seeds through their long-distance dispersal permitting parallel genetic differentiation in many sites along the extensive mountain area. These together will further accelerate both speciation and extinction rates in epiphytic orchids. However, it is relatively opposite in their terrestrial counterparts (Givnish *et al.*, 2015).

Due to limited food reserves in the endosperm; all orchids predominantly terrestrial species rely heavily on specific mycorrhizal fungi for germinating and growing; either partly (heteromycotrophic) or remains obligate for life (holomycotrophic/saprophytic) (Arditti, 1967; Ramsay *et al.*, 1986; Whigham and Wilhems, 2003; Swarts, 2009). These fungi will provide resources for germination and carbon capture during their protocorm stage. When moving terrestrial orchids to different areas in absence of their specific individual mycorrhizal fungi; germination will unlikely to happen. This orchid–mycorrhizal relationship paired with their habitat preferences explained the lower speciation rate in the terrestrial groups (Rasmussen, 1995; Givnish *et al.*, 2015).

2.5 The Orchid Flower

Traditionally in Orchidaceae, the identification of species and delimitation of taxa were weighted heavily on morphology; both vegetative and primely the flower. The features of their anthers (erect, reflexed or incumbent), gymnostemium or column (Monandrae or