CHAPTER 1

INTRODUCTION

Alpinia is a genus of over 250 species belonging to the family Zingiberaceae (Larsen *et al.,*1999). Most members of the genus are found in subtropical and tropical rain forests of Asia, Australia and Pacific Islands (Wu and Larsen, 2000; Smith, 1990). Some members of the genus are well known for ethnomedicinal importance, for example *Alpinia mutica* and *Alpinia nutans* while some are also utilised as spices, for example, *Alpinia galanga* (Larsen *et al.*, 1999; Valkenburg and Bunyapraphatsara, 2001).

Many botanists have described and recorded *Alpinia* species notably Roxburgh (1810), Schumann (1904), Ridley (1924), Holttum (1950) and Smith (1990). Currently, botanists refer to Smith's (1990) infrageneric classification. In recent years, several researchers used molecular data to explore phylogenetic relationships within the genus *Alpinia* (Rangsiruji *et al*., 2000a; b and Kress *et al.,* 2002; 2005a). However, neither the results of Rangsiruji *et al*. (2000a; b) nor Kress *et al.* (2005a) supported the classification of *Alpinia* proposed by Smith (1990).

The phylogenetic analysis of Malaysian *Alpinia* is unsatisfactory because only two species from Malaysia were studied by Rangsiruji *et al.* (2000 a; b) and Kress *et al*. (2005a) namely *Alpinia javanica* and *Alpinia rafflesiana* although 25 species had been previously recorded in Peninsular Malaysia (Holtumm, 1950 and Lim, 2004; 2007). The Peninsular Malaysian *Alpinia* species were grouped under Smith's 1990 section *Alpinia* (subsection *Catimbium, Alpinia, Presleia* and *Cenolophon*) and section *Allughas* (subsection *Strobidia, Odontychium* and *Allughas*) in Fig. 1.1.
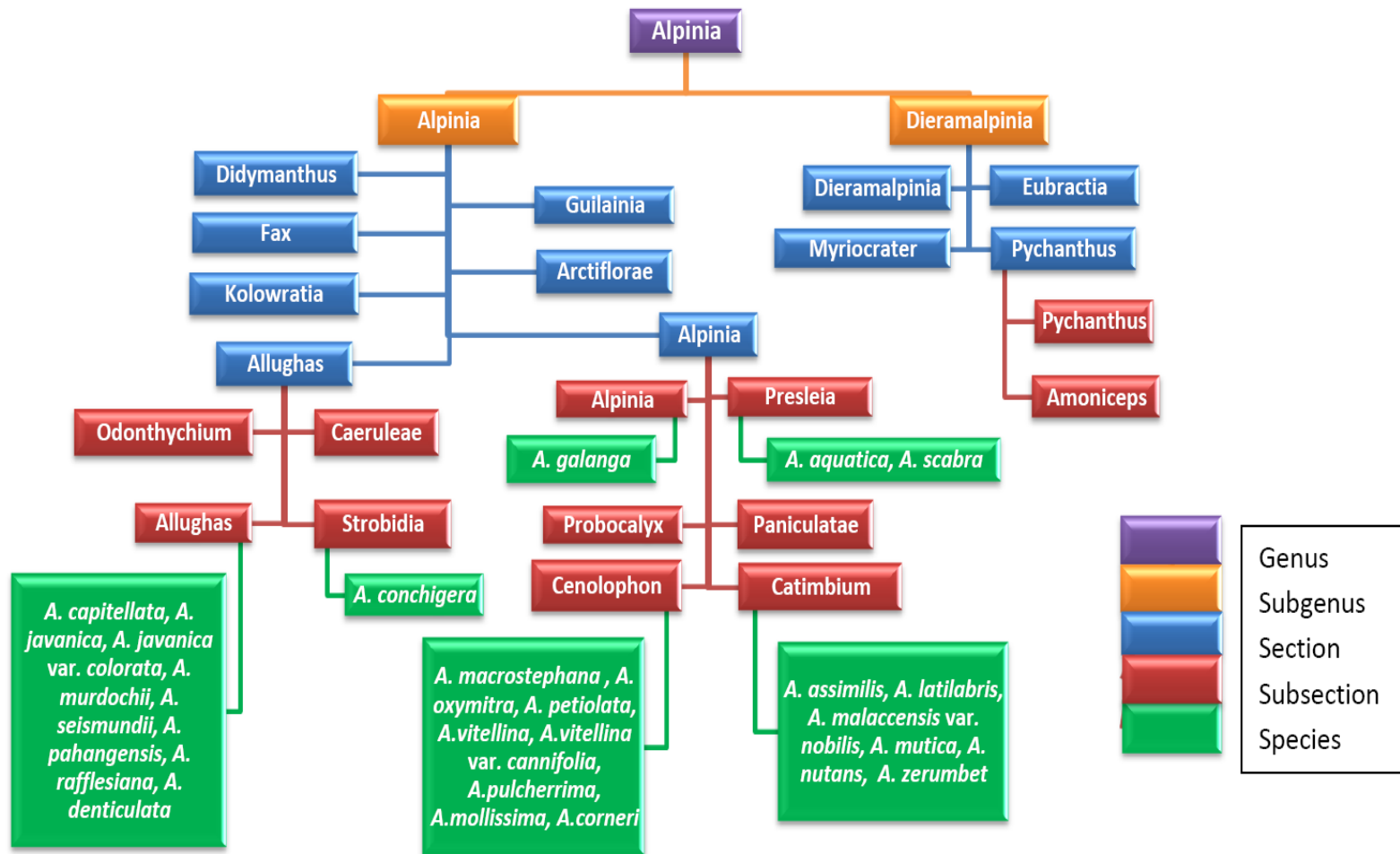
Figure 1.1 Infrageneric classification of *Alpinia* adapted from Smith (1990). Insert shows Peninsular Malaysian species recorded by Holttum (1950).

According to Smith's classification (Smith, 1990), *Alpinia nigra* is the type species for subsection *Allughas* which contains other species like *Alpinia javanica* and *Alpinia rafflesiana*. However, phylogenetic results obtained from Rangsiruji *et al.* (2000 a; b) and Kress *et al.* (2002; 2005a) found that this species is more closely related to *Alpinia galanga* (subsection *Alpinia*) and *Alpinia conchigera* (subsection *Strobidia*) and surprisingly it does not form monophyletic clade with other species under subsection *Allughas*. As a matter of fact, the study by Kress *et al.* (2005a) showed a distinct clade for *Alpinia rafflesiana* and *Alpinia javanica* (the *Rafflesiana* clade in figure 1.2) where the clade includes only two species. Interestingly, this clade is sister to the monotypic Vietnamese/Chinese *Siliquamomum* although the bootstrap for this relationship is poor. They are not being grouped together with other *Alpinia* species, probably due to hybridisation. However, no further work was done to resolve this problem, even though within *Allughas*, there are abundant species from Malaysia which have not been studied. Therefore, molecular results by Rangsiruji *et al.* (2000a; b) and Kress *et al.* (2002; 2005a) could not be conclusive as the samples taken did not fully represent the subsections probably because many of the species within the subsection *Allughas* can be found in Malaysia. Consequently, it is appealing to analyse this subsection in order to validate the past phylogenetic analyses.
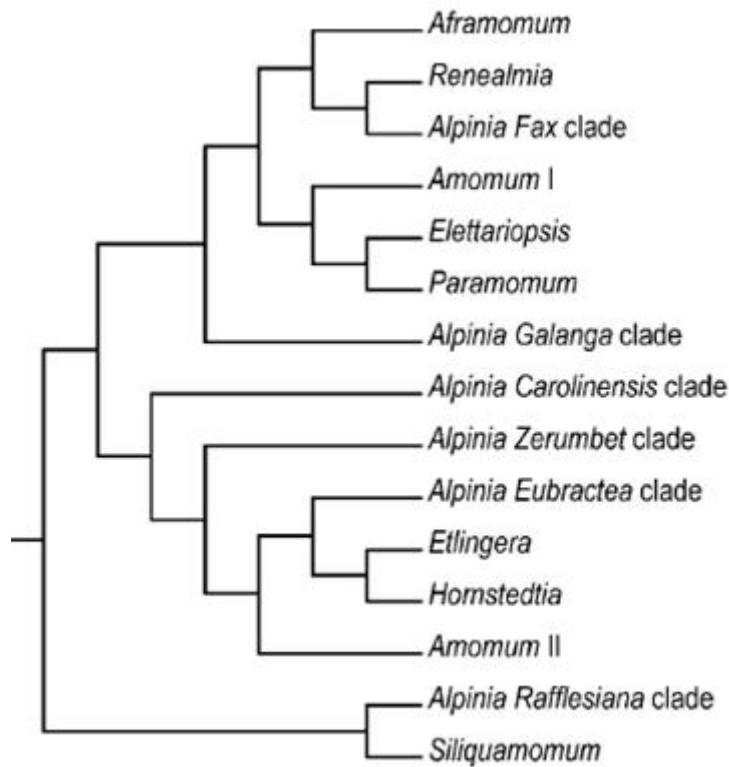
Figure 1.2: The condensed tree of the genus *Alpinia* produced several clades (Kress *et al.* 2005a). It should be noted that the taxa *Alpinia oxymitra* is scattered near the *Zerumbet* clade and did not form any natural grouping.

Furthermore, results from Kress *et al.,* 2005a showed no natural grouping exist in the subsection *Cenolophon* for two species (*Alpinia oxymitra* and *Alpinia oxyphylla*) as they are scattered on the phylogenetic trees. However, this is questionable because many species within this subsection can be found in Malaysia and they are yet to be analysed. Scattering of *Alpinia oxymitra* and *Alpinia oxyphylla* are probably due to the fact that they are only related to the species in subsection *Cenolophon*. Additionally, it is probable that this subsection is closely related to subsection *Catimbium* as according to Kress *et al.* (2005a), *Alpinia oxymitra* is a sister to the *Zerumbet* clade (which includes species from subsection *Catimbium*) although the bootstrap support is poor. Since this subsection is still unresolved, it is coherent to investigate the disparity among the species within this subsection to evaluate whether it abides by Smith's classification

or whether the past molecular phylogenetic results done by Rangsiruji *et al.* (2000 a; b) and Kress *et al.* (2002; 2005a) are unequivocal.

In addition, *Alpinia denticulata* (subsection *Odontychium*), a species which can only be found in Malaysia and other species which had been newly described (*Alpinia suriana)* needs to be analysed for they are not being investigated; therefore relationships with other taxa are yet to be resolved. Also, species taken from other regions which had been examined (species which are also found in Malaysia), should also be analysed as they might produce different results (probably due to biogeographic differences). Holttum's account of *Alpinia* within the Zingiberaceae of Peninsular Malaysia was written more than 50 years ago, and he implicated that there were still several taxa which were based on incomplete data, also mentioning that several species seemed rather closely allied, while other new species may yet to be discovered.

Clearly, there is an apparent need for reviewing the phylogenetic analyses of *Alpinia* in Malaysia to elucidate the predicament mentioned above.

Molecular markers of nuclear ribosomal DNA of internal transcribed spacer (ITS) and chloroplast regions of maturase K (*mat*K) gene are probably the most significant primers to be utilized in this study as these markers had been previously used, consequently, the imminent result can be compared to the previous analyses. The *Alpinia* species in Malaysia would most probably be contained within the clades which had been studied by Rangsirujii *et al.* (2000 a; b) and Kress *et al.* (2005a), excluding *Alpinia oxymitra*, which might probably grouped in a clade together with other species in subgenus *Cenolophon* and *Alpinia denticulata* (Smith distinctly grouped this species on its own subgenus *Odontychium*, Smith, 1990).

1.1 Research objectives

Taking into consideration the disagreement between Kress's (2005a) phylogenetic analysis and Smith's (1990) classification coupled with newly described *Alpinia* species by Lim (2004; 2007); a study on *Alpinia* in Peninsular Malaysia with the aim of providing fresh data and updates on the status of genus *Alpinia* is clearly important.

Therefore, this thesis is the first attempt to look at the molecular phylogenetic analysis of *Alpinia* in Peninsular Malaysia, and will address three primary research objectives:

Objective 1: To establish a phylogenetic tree of *Alpinia* species in Peninsular Malaysia using two molecular markers namely internal transcribed spacer (ITS) and maturase K (*mat*K) gene.

Objective 2: To investigate the interspecific and infraspecific relationship within the genus *Alpinia* in Peninsular Malaysia based on molecular data.

Objective 3: To evaluate the general status of *Alpinia* species and its diversity in Peninsular Malaysia.

LITERATURE REVIEW

1.2 The Genus *Alpinia* Roxb. – A general perspective

Alpinia is the largest genus in the family Zingiberaceae with over 250 species worldwide (Larsen *et al.*, 1998). This tropical ginger can be found within the southern area of Indian continents to the South-East-Asian countries extending east through Fiji Island and north-western Australia (Figure 1.3: Smith, 1990). Under the tribe Alpinieae, the genus is distinguishable as it is the only genus that has a terminal inflorescence on a leafy shoot (Holttum, 1950).

Generally, *Alpinia* plants can grow up to 3 m tall, growing in clumps at lowland to highland forests. There is less vegetative diversity in the genus, but more diversity in the inflorescence (Holttum, 1950). Rhizomes are usually fleshy, bearing horizontal leaf-shoots. Leaves are either elliptic to elliptic-oblong, asymmetric, with or without petiole between blade and sheath, with well-developed ligules. The surface of the leaves are either hairy or glabrous, some with stiff-hairs at the margin, apex usually acuminate-caudate, and the leaf base is mostly cuneate, while a few is cordate (Holttum, 1950).

The flowers of *Alpinia* are very conspicuous and variable from white, pink, maroon, or yellowish with streaks or spots on the labellum. It is normally arranged in cincinni in the axil of primary bracts, with or without secondary bracts (Larsen *et al.*, 1999). Calyx often tubular, 3-toothed and split rather deeply on one side, corolla tube slender and mostly longer sized than calyx. The lobes are often 3-lobed with one dorsal lobe and two lateral lobes. Its labella are more or less deeply bilobed or somewhat trilobed, with or without staminode on both side of the dorsal corolla lobe. Anthers are sometimes crested; filament and style slender (Holttum, 1950). *Alpinia* fruits are usually green when young to red, yellow, orange or black when ripe.

Plants of selected *Alpinia* species have long been known to be used as medicine, in traditional cooking and cultivated as ornamental plants. The rhizomes of *Alpinia malaccensis* are generally taken to treat stomach-ache for indigestion and diarrhoea (Valkenburg and Bunyapraphatsara, 2001). Cultivated species like *Alpinia galanga,* known as *lengkuas,* is essential in cooking 'rendang', 'nasi kerabu' and other local delicacies in Malaysia (Norasiah, 2008), and the rhizomes has been traditionally used to treat wounds, rheumatism, sores and ringworm.

In China, the leaves of *Alpinia zerumbet,* locally known as *yan shan jiang* were used to wrap rice dumpling made with sticky rice and served during the Chinese festival of *Tuen Ng* (Burtt and Smith, 1972b)*.* Additionally, fruits of *Alpinia zerumbet* are used to expel gas, prevent vomiting and stimulate stomach secretions. Globally, *Alpinia zerumbet* and *Alpinia purpurata* are known to be cultivated for horticultural purposes due to their attractive inflorescences. Many Malaysian species from the Peninsular and Eastern district such as *Alpinia argentea*, *Alpinia hansenii, Alpinia mutica, Alpinia petiolata, Alpinia rafflesiana, Alpinia vitellina* are potential ornamental species.

Recently, a vast number of biochemical studies to analyse biological activities and chemical constituents have been reported for a number of *Alpinia* species, namely *Alpinia galanga* (Divakaran *et al.,* 2013; Lo *et al.,* 2013; Limmatvapirat *et al.,* 2011; Sukhirun *et al.,* 2011), *Alpinia zerumbet* (Cavalcanti *et al.,* 2012; Chompoo *et al.,* 2011, Murakami *et al.,* 2009; Elzaawely *et al.,* 2007a & b), *Alpinia pahangensis* (Phang *et al.,* 2013; Sivasothy *et al.,* 2013; Awang *et al.,* 2011), *Alpinia conchigera* (Aziz *et al.,* 2013; Ujang *et al.,* 2013; Awang *et al.,* 2010; Aun *et al.,* 2011, Saha and Paul, 2012) and *Alpinia mutica* (Malek *et al.,* 2011; Phang *et al.,* 2011). The studies above are a few examples of many other studies that revealed potential natural bioactive compound and bio-pharmaceutical of the *Alpinia* species.

1.3   Taxonomic History of the Genus *Alpinia* Roxb.

The name *Alpinia* was first coined by Linnaeus (1753) for *Alpinia racemosa*, a neotropical American species. Later, Linnaeus (1782) described a related species, *Renealmia exaltata* from tropical America (Holttum, 1950). Hence, Roxburgh (1810), Schumann (1904) and other botanists applied the name *Alpinia* only to Asiatic species, and used the name *Renealmia* for tropical American and West African species. This generic nomenclature was not satisfactory because the type species of *Alpinia* was from America (Holttum, 1950). Finally, the appointed committee of Amsterdam proposed that the Asiatic species to be conserved as *Alpinia galanga* (L.) Willd. as its lectotype (Holttum, 1950; Smith, 1990). Over the years, more botanists have described and recorded *Alpinia* species under this general consensus. Ridley (1899) recorded 16 species in the Malay Peninsula, and in 1924, he constructed an artificial key and illustration of some species (Ridley, 1924). Other botanists also continued to record and described new *Alpinia* species (Holttum, 1950). In 1904, Schumann described and constructed a key to *Alpinia.* Finally in 1990, Smith revised the genus *Alpinia* in her monograph entitled *Alpinia* (Zingiberaceae): A Proposed New Infrageneric Classification. Smith's classification (Fig. 1.1) is most recognized and often used today (Smith, 1990).

According to the infrageneric classification, *Alpinia* is the type genus of the tribe Alpinieae of the family Zingiberaceae. The genus *Alpinia* is divided into two subgenus (Figure 1.3) where the labellum type represent a major criterion; subgenus *Alpinia* (distributed mainly throughout the entire continental Asian region) and subgenus *Dieramalpinia* (widely distributed in New Guinea).
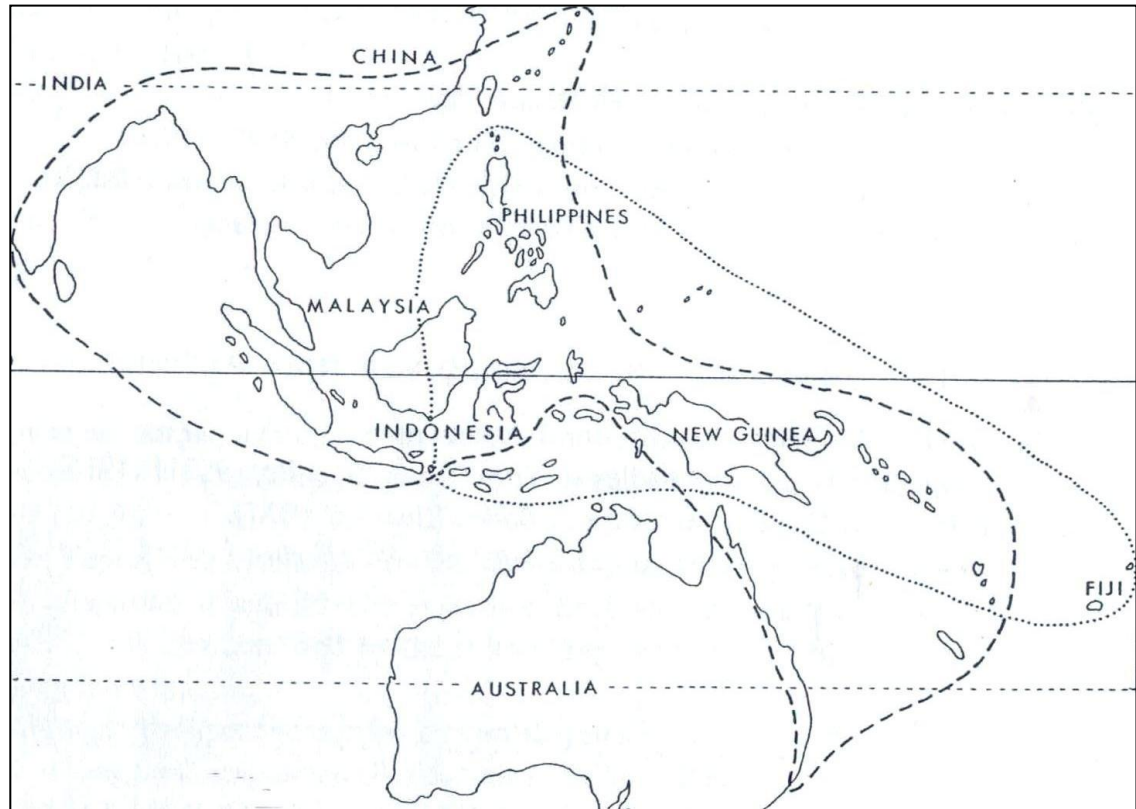
Figure 1.3: Distribution map of the genus *Alpinia* following Smith (1950). Subgenus *Alpinia* is marked with   - - - - and Subgenus *Dieramalpinia* is marked with ⋯⋯

Subgenus *Alpinia* forms petaloid labellum that is usually concave often with incurved margins, commonly striped or spotted and the margins are extended into a petaloid area with divergent venation. However, subgenus *Dieramalpinia* forms an erect labellum (non-petaloid), almost always closely pressed against the stamen, usually unstriped and without spots. The lateral margins are not well developed and the apex occasionally expands into a small petaloid area.

Further division and subdivision are based on the characters of its bracteoles and labellum. Much variation exists in these features, from species with branched inflorescences and long cincinni subtended by bracts in which the flowers are each subtended by bracteoles, to other species with no bracts or bracteoles and cincinni of only a single flower (Kress *et al*., 2005a).

1.4 Taxonomic Ambiguities and Biodiversity of *Alpinia* in Peninsular Malaysia

Alpinia has always been considered to be a taxonomically difficult and complex genus, both in defining the characters that distinguish the genus from other genera in the Alpinioideae and in classifying its species (Holttum, 1950; Smith, 1990). Typically, the identification and classification of *Alpinia* species is dependent on its morphological characteristics. The main aspect in describing and identifying a particular species relies on the variation of the inflorescence and flowers. However, not all morphological characteristics were given equal importance; some were viewed more importantly than others (bracts, inflorescence and flowers).

The genus *Alpinia* has been subjected to several revisions, originally described by Ridley in 1899 and finally extensively described and regrouped by Holttum (1950) under the tribe *Alpinia*. The *Alpinia* in Peninsular Malaysia falls into four genera- *Catimbium, Languas, Alpinia* and *Cenolophon* which are based on Schumann's system (1904). In Holttum's report, he divided the *Alpinia* into four subgenus based on its inflorescence: *Cenolophom, Alpinia, Catimbium* and *Languas*. The subgenus *Cenolophon* has inflorescence that bears single flower directly on the main axis and bracts are usually small without presence of bracteole. However, for the subgenus *Alpinia,* the inflorescence bears lateral cincinni of two or more flowers, with small or bracts absent and the bracteoles are always in cup- or funnel-shaped. For subgenus *Catimbium,* it also has similar inflorescence characteristics with subgenus *Alpinia,* but the bracts are absent with large split bracteoles. The fourth subgenus, *Languas,* bears similar characteristics with subgenus *Alpinia*, but with small bracts and small split bracteoles. Holttum's report of *Alpinia* in Peninsular Malaysia was produced more than sixty years ago, and he stated that there were still several new taxa based on incomplete data, while other new species may yet be discovered.

On the other hand, in Smith's infrageneric classification, the labellum characters were given importance that divides *Alpinia* into two subgenera, *Alpinia* and *Dieramalpinia.* Bract and bracteole characters were also used to delimit the sections and subsections.

In 1990, Smith placed the Malayan (Peninsular Malaysia) species into subgenus *Alpinia.* Since the inflorescence of *Alpinia* has more diversity compared to its vegetative structures, bract and bracteole characters were used to delimit the sections and subsections. However, descriptions of each *Alpinia* species still followed Holttum's meticulous report. The 23 species previously recorded by Holttum were regrouped by Smith under section *Alpinia* (subsection *Catimbium, Alpinia*, *Presleia* and *Cenolophon*) and section *Allughas* (subsection *Strobidia, Odontychium* and *Allughas*).

Recently, Lim (2004, 2007) discovered two new species found in Pahang and Perak, namely *Alpinia suriana* and *Alpinia mythiana.* This new finding shed a new light of interest in this genus as previous reports and description of *Alpinia* was done more than sixty years ago and no detailed studies were done to examine its relationships and status of the taxa.

In the search for new morphological characters useful in classification, a lead has been given by the careful study by Liao and Wu (1996) on the fruit wall of Chinese *Alpinia* which demonstrated a link between three species that may perhaps be useful to provide a clearer inference on the relationships of this genus. Besides that, a number of extensive studies were performed to examine the pollination mechanism of the notable flexistylous stigma of *Alpinia* species (Luo and Li, 2010; Sun *et al.,* 2011; Zhang *et al.*, 2003; Li *et al.,* 2001; Sun *et al.,* 2007; Takano *et al.,* 2005; Li *et al.,* 2002, Li *et al.,* 2001). A study by Kress *et al.* in 2005a identified the occurrence of flexistyly in the *Galanga* clade and the *Zerumbet* clade. Based on the preliminary result, this study

found that the mating mechanism of flexistyly may have evolved in the common ancestor of Alpinieae tribe or independently for at least three to five times (Kress et al., 2005a).

Although there are various morphological methods that were introduced and used to classify and describe *Alpinia* species, there are several limitations of morphological characteristics which may possibly occur. Firstly, the vegetative structure of *Alpinia* species (e.g. height of plant, stem and leaf characteristics) does not vary significantly compared to its inflorescence structure. Virtually all *Alpinia* species flower terminally on the leafy shoots and all are Asiatic. Therefore, these morphological characteristics may possibly be overlooked. Furthermore, most of these tropical gingers have seasonal flowering and fruiting period which lasted for 1 month to six months yearly and are dependent on the environment and climate (Li *et al.,* 2001; Saensouk, *et al.,* 2003). The morphological approach is clearly subjective, and limited to identify only plants with the presence of flowers and or fruits.

Many countries within the tropical region experience high rates of deforestation due to human land use, resulting in species declines and extinctions. Since the last few decades, these activities have been continued at an alarming rate. Peninsular Malaysia lowland and montane forests are listed under Global 200 (Olson *et al*., 2001) where the terrestrial region clearly showed that it is undergoing critical or endangered threats (Figure 1.4).
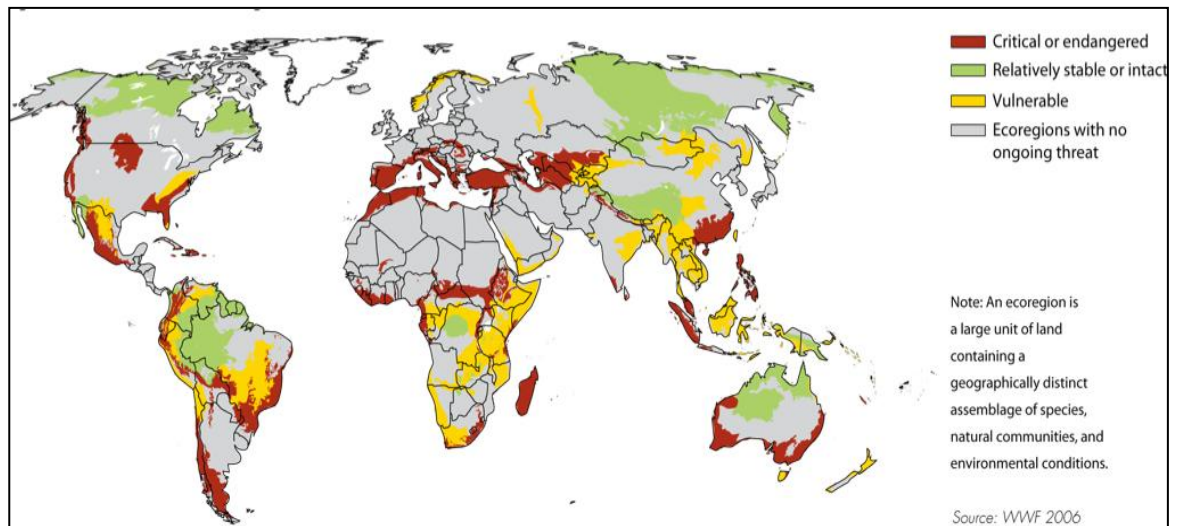
Figure 1.4 World map of terrestrial ecoregion. Note that Peninsular Malaysia is under 'critical or endangered' threat (Source: WWF2006).

Recent developments have been sought to improve the limitations of morphological identification and infer relationships of *Alpinia* species. The increased availability of scanning electron microscopy (SEM) and freeze fracturing technique which were used to observe the cellular types and organization alleviate the morphological limitations (Hussin *et al.,* 2000; Talip *et al.,* 2004). Other methods use DNA fingerprinting to identify individuals or putative hybrids of *Alpinia* species in a population. A number of population genetic studies were performed using molecular markers such as inter-simple sequence repeat (ISSR), sequence-related amplified polymorphism (SRAP), amplified fragment length polymorphism (AFLP) and microsatellite markers to measure the variation of population structure and genetic diversity among individuals or putative hybrid of some *Alpinia* species (Lin *et al.,* 2014; Takano *et al.,* 2013; Zou *et al.,* 2013; Wang *et al.,* 2012; Yang *et al.,* 2011; Khunpiban *et al.,* 2010). These different approaches may not agree with one another.

Recent attempts to overcome the limitations have led to the application of molecular markers such as nuclear DNA markers and chloroplast genes to construct a more reliable phylogeny of *Alpinia* (Rangsiruji *et al.,* 2000a & b; Kress *et al.,* 2005a)*.*

1.5 Phylogeny of the Genus *Alpinia*

Due to the limitations in the morphological characteristics, more and more taxonomists are turning to molecular tools in their effort to identify and infer phylogenetic relationships of the family Zingiberaceae. The introduction to polymerase chain reaction (PCR) and sequencing together with a host of downstream application has opened the door towards a more efficient and less ambiguous taxonomic tree. These tools may possibly provide fundamental comparison on the molecular level of the nucleotide sequences of homologous genes in different populations or species.

The current taxonomy of *Alpinia* is based on morphological characters. However, the presence of variations among the species has constrained the unequivocal identification of the taxa based on such morphological characters. In 1956, Willi Hennig developed a systematic methodology known today as phylogenetic tree. Phylogenetic analysis aims to trace the evolutionary course of organisms, in this context, the speciation within the genus *Alpinia.* Using homologous genes, or shared derived characteristics, multiple trees were calculated using specific computer algorithms (empirical methods) to measure the least number of convergent evolution and find the genealogic relationship among the taxa (Wiley *et al*., 1991). Typically, in grouping the taxa together in the phylogenetic tree, cladists use three terms to define the clades of the tree:monophyletic, polyphyletic and paraphyletic (Fig. 1.5).

A monophyletic is a group of that consists of all the descendants (inversions, haplotypes and species) from a single, common ancestral form (Alan, 2006). In contrast,

when all individuals of the same species are not nested together, or individuals of other species are nested together, they form a paraphyletic grouping (Page and Holmes, 1998). Unlike a monophyletic group, a paraphyletic taxon does not include all the descendants of the most recent common ancestor. An individual from two different species that have evolved separately but are grouped together because of their similarity (in morphology or at their molecular) is an example of a polyphyletic grouping. However, the organisms includes multiple origins and do not share a common ancestor. In the case of unresolved branches, or polytomy, species may have either evolved simultaneously, or the gene used for the tree construction is not able to infer the evolutionary direction of the species (Page and Holmes, 1998). A clade is a set of nested groups, usually arranged hierarchically (Baum *et al.,* 2005).
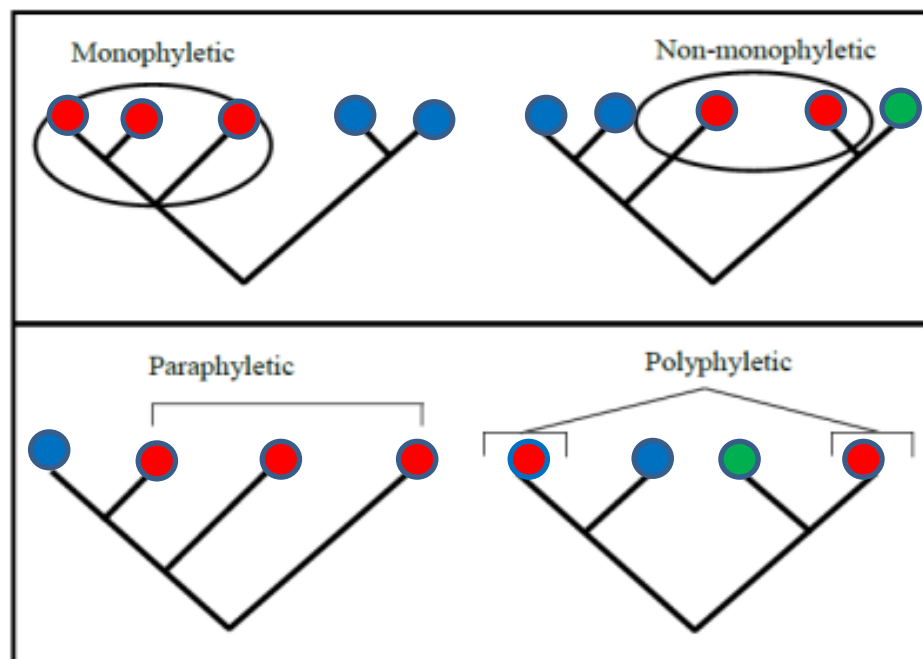

Figure 1.5: A slanted cladogram showing the type of tree branch (adapted from Page and Holmes, 1998). A monophyletic group includes all taxon from an internal node, whereas a non-monophyletic group does not include all taxon from the same internal node, and are described as paraphyletic or polyphyletic.

A number of important genes that were consistently utilised in plants to understand the relationships are nuclear ribosomal DNA of the Internal Transcribed Spacer (ITS) region and the chloroplast genes. In a large scale comparative assessment of plant DNA barcoding marker covering 75 plant families, ITS region was suggested to be one of the primary DNA barcoding marker for seed plants (CBOL Plant Working Group, 2011) although there has been critiques concerning a few issues: fungal contamination, paralogous gene copies and success rate of amplification (Hollingsworth, 2011).
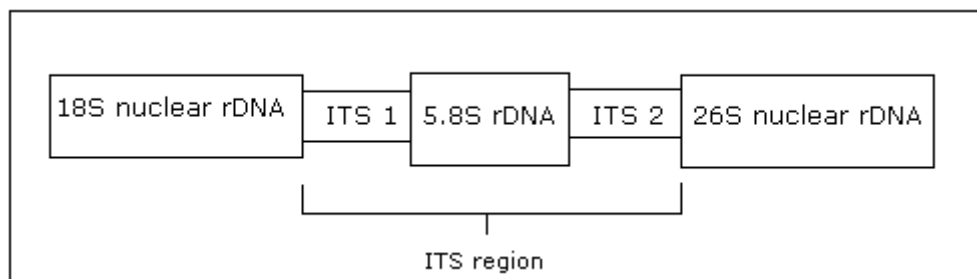

Figure 1.6 Diagrammatic representation of the Internal Transcribed Spacer (ITS) region located between 18S rDNA and 26S rDNA.

The ITS region comprises of the ITS 1 and ITS 2 spacers in addition to the 5.8 S nuclear ribosomal DNA (Figure 1.6). It is located within a three highly conserved nuclear ribosomal DNA regions (18 S, 5.8 S and 26 S). ITS 1 and ITS 2 are part of the ribosomal transcriptional unit but are not incorporated into mature ribosome (Hamby and Zimmer, 1992). It is also a non-protein coding region. This region enables amplification of various plant species (be it different species or genus) using universal primers. Its small size (less than 800bp) makes it particularly appropriate for direct sequencing of amplified DNA obtained from PCR.

The chloroplast genes have also been widely used to investigate interspecific relationships among angiosperms and other plants (Debra *et al.,* 2007; Nishiyama *et al.,* 2004; Shaw *et al.,* 2007). Different portions of the chloroplast DNA evolve at different rates, which result in a wide range of possibilities for resolving relationships from species and genus level to family and even higher taxonomic levels but limit its applicability among closely related species and populations (Soltis and Soltis, 1998).
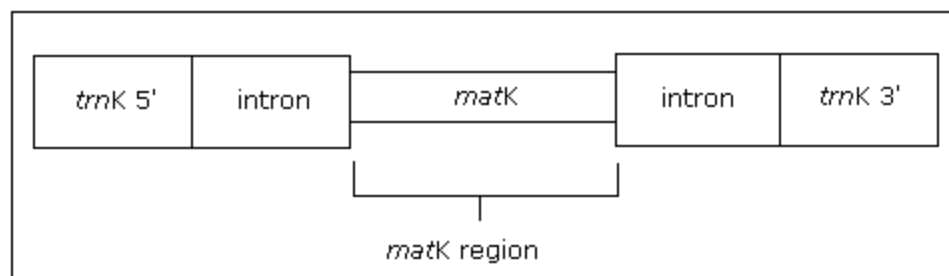

Figure 1.7 Diagrammatic representation of *mat*K gene located between *trn*K5' and *trn*K 3'

Recently, the chloroplast maturase K (*mat*K) gene is being extensively utilised in phylogenetic inference. In an extensive study encompassing 445 angiosperm, *mat*K was suggested to be incorporated in DNA barcoding for land plants (CBOL Plant Working Group, 2009) although results from the study showed more frequent discordinance between forward and reverse read compared to other genes (*atp*F–*atp*H, *rbc*L, *rpo*B, *rpo*C1, *psb*K–*psb*I and *trn*H–*psb*A). The *mat*K gene is roughly 1500 bps in length. It is a maturase coding gene located within the intron of the chloroplast gene *trn*K (Figure 1.7). This gene has evolved at a higher rate than several other genes currently used in systematic studies. Olmstead and Palmer (1994) reported that among 20 genes used in molecular systematics, the *mat*K gene has the highest overall nucleotide substitution rate. In the Saxifragaceae, the *mat*K gene has been found to

evolve approximately three fold faster than the *rbc*L gene (Johnson and Soltis, 1994). The sequences of the *mat*K in the Polemoniaceae displayed an overall rate twice that of *rbc*L sequences (Steele and Vilgalys, 1994).

Other primers that were frequently used in plants are chloroplast genes that includes large subunit of ribulose 1,5-biphosphat carboxylase/oxygenase (*rbc*L), ß subunit of atp synthase (*atp*B), subunit of NADH dehydrogenase (*nhd*F), 16S rDNA, non-coding 26S, (Stoeckle *et al.,* 2011; Herrmann and Wink, 2014; Groot *et al.,* 2011; Pang *et al.,* 2011; Soltis and Soltis, 1998).

Several papers have used internal transcribed spacer region (ITS) and chloroplast genes to construct phylogenetic relationships within the Zingiberaceae family including Searle and Hedderson (2000), Wood *et al*. (2000), Kress *et al.* (2002), Pedersen (2004), as well as within several genera (*Hedychium*: Wood *et al.,* 2000; *Alpinia:* Rangsiruji *et al.,* 2000a, b; *Roscoea*: Zhang and Zhou, 2014; Ngamriabsakul *et al.,* 2000; *Aframomum*: Harris *et al*., 2000; *Globba*: Williams *et al.,* 2004; *Amomum*: Xia *et al.,* 2004).

Recent attempts by Kress *et al.* (2002; 2005a) sought to improve the phylogeny using ITS nuclear DNA and an additional chloroplast *mat*K gene gave a similar result to an earlier study by Rangsiruji *et al.* (2000a; b). Their phylogenies did not support the classification of *Alpinia* proposed by Smith (1990). Surprisingly, the congruence between major clades of *Alpinia* provide evidence that this genus is polyphyletic and that groups of species are more closely related to other genera in the tribe than they are to each other (Kress *et al.,* 2005a). In addition, the monophyly and phylogenetic position of a number of genera in the tribe Alpineae have not yet been established. Furthermore, in order to accurately reflect evolutionary history, it is proposed for a

major realignment of genera in the tribe. It should be noted that most of the findings should be interpreted with caution as these phylogenies lack taxonomic coverage.

Although it is tempting to propose a new classification of the tribe Alpinieae based on some species that are strongly supported in both the parsimony and Bayesian analyses, the relationships among the clades are not fully resolved. The monophyly and phylogenetic position of a number of genera in the tribe Alpineae have not yet been established; hence the inclusion of additional critical species may be vital to review the phylogenetic results done by past studies.