CHAPTER 4

DISCUSSION

4.1 Molecular characteristics of internal transcribed spacer (ITS) region and maturase K (*mat*K) gene

In this study, the amplification of internal transcribed spacer (ITS) for *Alpinia* specimens were quite straight-forward compared to maturase K (*mat*K) gene region whereby not all samples were successfully amplified. Even though Kress *et al.* (2005a) suggested using several primers to amplify *mat*K gene, unfortunately the amplification success rate of the recommended primers was low. A recent study on an attempt to discover DNA barcoding for the genus *Roscoea* in Zingiberaceae by Zhang *et al.* (2014) showed that *mat*K gene was relatively difficult to be amplified compared to other genes. In another extensive study covering 141 genera of 75 families in search of plant DNA barcoding genetic markers, ITS gene gave the highest discriminatory power compared to other plastid genes (*mat*K, *rbc*L, *trn*H-*psb*A) and found that the PCR amplification of the plastid genes to be less successful than ITS region (CBOL Plant Working Group, 2011). As a solution to overcome the predicament, two new primers (natKf and natKr – see Appendix 1.0, section C) were designed in this study and used specifically for amplifying partial section of *mat*K gene of the Peninsular Malaysian *Alpinia* species, along with another set of primer obtained from CBOL Plant Working Group. Several other primers were also designed in order to amplify the *mat*K region, yet turned out to be unsuccessful.

Nuclear ribosomal DNA of internal transcribed spacer region (ITS) consists of three genes, ITS1, 5.8S and ITS2. In this study, high variable sites in ITS 1 spacer

region compared with 5.8S and ITS 2 region correspond with Hershkovitz *et al.* (2002) analyses. In a study by Baldwin *et al.* (1995) (as cited in Hershkovitz *et al.* (2002)), it is known that the evolutionary patterns are parallel between ITS 1 and ITS 2 although they differ in its structure and function. Basically, nuclear ribosomal region of ITS is useful in discriminating and resolving the inter-species relationship among the species within the Zingiberaceae family (Sakai *et al.*,2013; Wood *et al.*, 2000; Xia *et al.*, 2004; Zhang *et al.,* 2014). Also, in this study for partial coding gene of maturase K (*mat*K), the rate of transition and transversion are identical only at the first codon; while the second and third codon produced a bias towards transversion, similar to *rbc*L (Soltis and Soltis, 1998).

Based on ITS and *mat*K sequences of *Alpinia*, intraspecies variation is low. Most clades contain many closely related species that suggests simultaneous divergence. However, several molecular characters of *A. oxymitra* and *A. scabra* may indicate that these species do not cluster with other species and form individual lineages of their own, which is shown in the phylogenetic analysis (Fig. 3.3 and Fig. 3.4).

4.2 Phylogenetic incongruence of *Alpinia* in Peninsular Malaysia

Among all phylogenetic tree analysis, Bayesian Inference (BI) method is known to be widely used for it provides a more robust result compared to Neighbour-Joining (NJ) that uses distance clustering method and character-based method of Maximum Parsimony (MP). Although posterior probabilities of BI analysis maybe misleading due to some evolutionary model arguments (Globoloff and Pol, 2005; San Mauro and Agoretta, 2010), the phylogeny trees analysed in this study should display the true phylogeny of *Alpinia* in other studies using other molecular markers (Rangsiruji *et al.,* 2000a, b).

There are various phylogenetic data sources, each with different pros and cons which will render question on the methods to combine the data set. In general, there are two approaches; 'total evidence' that combines data into single analysis, and separate analysis in which the results were later combined to create consensus tree. Total evidence data of a single analysis that was proposed by Kluge (1989), also known as concatenated trees are only applicable if the genes have similar evolutionary rates. In this study, the rate of partition homogeneity test of ILD showed a low value, $p=0.01$; therefore it is evident that both ITS and partial *mat*K genes have different histories and should not be combined. Nevertheless, the phylogenetic inferences from these separate genes in this study were almost consistent.

As suggested by Miyamoto and Fitch (1995), consensus trees of NJ, MP and Bayesian inferred from this study provide different insights whereby ITS consensus trees of NJ, MP and Bayesian analysis indicate better resolved branching pattern in comparison with *mat*K phylogeny (Fig. 3.3 and Fig. 3.4). The inability to fully utilise the whole region of *mat*K (~1500bp) may be a possible reason for the poor to moderately resolved phylograms, since the number of variable sites within the sequences are low (6.1%) in comparison with ITS sequences (26.1%). Also, despite having fewer taxa in *mat*K analysis, the trees of NJ, MP and Bayesian inference possess a comparable topology with ITS trees which produces somewhat similar clades that differed at the position of taxa relationships within the clades.

4.2.1 Clade II (The *Galanga* clade, Fig. 3.3 & Fig. 3.4)

The *Galanga* clade (Clade II) of both internal transcribed spacer (ITS) region and partial maturase K (*mat*K) data in this study showed strong bootstrap support of *A. conchigera* and *A. galanga* where the Malaysian species were clustering with reference sequences of *A. conchigera* and *A. galanga* within the clade (Fig. 3.3 and Fig. 3.4). This study reaffirms previous phylogenetic analyses by Rangsiruji *et al.* (2000b) and Kress *et al.* (2005a) where the two species were grouped together with *A. nigra.* However, the result is incongruent with Smith's 1990 classification where *A. conchigera* were placed under section *Allughas* subsection *Strobidia* while *A. galanga* is separated under section *Alpinia* subsection *Alpinia.* Subsection *Strobidia* consist of *Alpinia* species with bracteoles that is open to the base if present while subsection *Allughas* contains *Alpinia* species with cup to funnel shaped bracteoles. Although this study does not agree with Smith's 1990 nor Holttum's 1950 classification; a recent study on the fruit wall by Liao and Wu in 1996 provided supporting evidence where the two species shared similar fruit anatomical characteristics. In addition, the bracteoles are capable to reverse its characters as suggested by Kress *et al*. (2005a). Therefore, it is possible that they may have shared a close relationship.

In the ITS phylogenetic tree, *A. aquatica* AY742335 was found to be clustered with *A. conchigera* and *A. galanga* in Clade II. However, since this sequence was obtained from the Genbank and it is the only available sequence for the species, it might have been misidentified and could not be verified. The Peninsular Malaysian *A. aquatica* species were all grouped in Clade IV.

4.2.2 Clade IV (The *Zerumbet* clade, Fig 3.3 & Fig. 3.4)

The consensus phylogenetic trees of internal transcribed spacer (ITS) region in this study showed that the *Zerumbet* clade (Clade VI) previously established by Rangsiruji *et al.* (2000b) and Kress *et al.* (2005a) has now branch out into two subclades: subclade IVa and subclade IVb, although there are no bootstrap support between these two subclades (Fig 3.3). Subclade IVa consists mostly of *Alpinia* species from reference sequences under section *Alpinia* subsection *Alpinia,* subsection *Didymanthus,* subsection *Paniculate* and subsection *Catimbium,* and the Peninsular Malaysian *Alpinia* of section *Alpinia* subsection *Catimbium* (*A.* cf. *assimilis, A. mutica, A.malaccensis* var. *nobilis, A. latilabris* and *A. zerumbet*) . Interestingly, *A. aquatica* of section *Alpinia* subsection *Presleia sensu* Smith (1990) from Peninsular Malaysia is clustered within Clade IVa as well, with high posterior probabilities supporting the close relationship (Fig. 3.3). Within clade IVa, most of the support values are for the same species sequences (*A. aquatica*, *A. latilabris*, *A. malaccensis* var. *nobilis*). Therefore, it is likely that subsection *Catimbium* and subsection *Presleia* of Smith's 1990 classification are polyphyletic.

The subclade IVb of the *Zerumbet* clade in this study consists of Peninsular Malaysian "Holttum's *Cenolophon* species" *Alpinia* species under section *Alpinia* subsection *Cenolophon* namely, *A. macrostephana, A. vitellina, A. vitellina* var. *cannifolia* and *A. petiolata* (Fig 3.3). It is evident from the internal transcribed spacer (ITS) data that this clade is separated from the Peninsular Malaysian *Alpinia* under section *Alpinia* subsection *Catimbium* of Smith's 1990 classification which is strongly supported by Bayesian inference and high bootstrap support. However, there is no support between subclade IVa and subclade IVb. Also, it is worthy to note that there is clear indication that *Alpinia* species of subclade IVb are not clustered with reference sequences of similar subsection *Cenolophon,* namely *A. oxyphylla* and *A. officinarum*

(non Peninsular Malaysian *Alpinia* species). As a matter of fact, all Peninsular Malaysian species under subsection *Cenolophon* were clustered under subclade IVb. The clade also indicates a close relationship among *A. macrostephana, A. vitellina* and *A. vitellina* var. *cannifolia* where *A. petiolata* is placed at the basal most, separated from the species mentioned above (Fig. 3.3). Therefore, this result is partially congruent with Smith's 1990 and Holttum's 1950 floral morphologies of *A. macrostephana, A. vitellina* and *A. vitellina* var. *cannifolia* where their flower characters are almost similar; striking orange-yellow wrinkled labellum, differed by the presence or absence of anther crest and leaf characters. The leaf base of *A. macrostephana* is cordate while *A. vitellina* and its variation possess cuneate leaf base. The floral characteristics of *A. petiolata* (labellum cream with maroon and yellow lines spreading from base towards the apex) differ from the above species, which support its separation (in the phylogenetic tree) from the 3 species mentioned above.

In previous phylogenies by Rangsiruji *et al.* (2000b) and Kress *et al.* (2005a), *A. oxymitra* were scattered and were hypothesised to be a sister taxa to Clade IV (the *Zerumbet* clade). In this study, consensus ITS tree reaffirms that *A. oxymitra,* a species under section *Alpinia* subsection *Cenolophon sensu* Smith 1990 is a sister taxa or closely related to either Clades V or Clades IV.

On the contrary, partial gene of maturase K (*mat*K) data from this study indicates an expanded clade IV (the *Zerumbet* clade) of Rangsiruji *et al.* (2000b) and Kress *et al.* (2005a): subclade IVa and subclade IVb (Fig. 3.4). Subclade IVa contains the reference sequences from Smith's 1990 section *Alpinia* subsection *Alpinia,* subsection *Didymanthus,* subsection *Paniculate* and subsection *Catimbium* and *A. zerumbet* from Peninsular Malaysian of section *Alpinia* subsection *Catimbium*, while all the other Peninsular Malaysian species namely *A. mutica, A. malaccensis* var. *nobilis, A.* cf. *assimilis, A. aquatica, A. oxymitra, A. macrostephana, A. vitellina* and *A. vitellina*

var. *cannifolia* (section *Alpinia*, subsection *Catimbium*, subsection *Cenolophon* and subsection *Presleia*) are more closely related and may form subclade IVb. In a study by Kress *et al.* (2005a), clade IV is the most extensive clade, represented by many *Alpinia* species and lineages. However, in this study, clade IV is well represented although taxa branches within and between subclade IVa and subclade IVb are poorly supported. There is only one branch with reasonably strong support (*/82/77) that could unite subclade IVa and subclade IVb as CladeIV (Fig. 3.4). In addition, most species in subclade IVa and subclade IVb are not resolved as the *Alpinia* species under subsections mentioned above formed polytomy in each clade. These indicate that section *Alpinia* subsection *Catimbium*, subsection *Cenolophon* and subsection *Presleia* of Smith's 1990 classification are likely polyphyletic.

According to Smith (1990), the distinct character that define subsection *Cenolophon* from subsection *Catimbium* is the presence of anther crest and the flowers are strictly single, with small bracts and no bracteoles. However, there are species, such as *A.havilandii* in subsection *Cenolophon* that contain ecristate anthers, and some species in subsection *Catimbium* which are single flowered (for example, *A. malaccensis* var. *nobilis, A. latilabris*) (Rangsiruji *et al*., 2000b). In Peninsular Malaysia, *Alpinia* species under subsection *Cenolophon* are quite distinct morphologically, in terms of its vegetative and floral characteristics. The leaves of the species in subsection *Cenolophon* are generally glabrous and apparently waxy unlike other species; some leaves are wavy with distinct floral traits among the species that vary from whitish cream with maroon lines to yellow with orange markings on its labellum. Evidently, the internal transcribed spacer (ITS) and maturase K (*mat*K) consensus trees in this study showed unclear dividing line between the two subsections. The incongruence observed between ITS and *mat*K are not new, as early phylogenetic studies in plants illustrates similar predicament where incongruency were observed

between chloroplast DNA and those inferred from nuclear characters (Riesenberg and Soltis, 1991; Riesenberg *et al.,* 1996). This occurrence may be due to hybridisation or introgression. (Riesenberg and Welch, 2002).

4.2.3 Clade VI (The *Rafflesiana* clade)

In previous studies on the genus *Alpinia,* Rangsiruji *et al.* (2000b) and Kress *et al.* (2005a) identified a natural forming group of Clade VI (the *Rafflesiana* clade), which includes two species under section *Allughas* subsection *Allughas* of Smith's 1990 classification namely *A. rafflesiana* and *A. javanica* from Peninsular Malaysia. In this study, the inclusion of more samples from section *Allughas* subsection *Allughas* illustrate an expanded clade VI. Internal transcribed spacer region (ITS) consensus phylogram displayed moderate to high posterior probabilities of subclade VIa and subclade VIb that formed Clade VI of Rangsiruji *et al*. (2000b) and Kress *et al*. (2005a) (Fig 3.3). Subclade VIa consists of additional *Alpinia* sample namely *A. javanica, A. javanica* var. *colorata, A. capitellata* and reference sequence of *A. javanica* and *A. rafflesiana*. All the species were placed under section *Allughas* subsection *Allughas.* The *Alpinia* species within subclade VIa were not clustered together collectively.

The subclade VIb in this study includes Peninsular Malaysian species of Smith's 1990 section *Allughas* subsection *Allughas* (*A. murdochii, A. rafflesiana, A. pahangensis*) where the bootstrap support of intra-species and inter-species relationships are low (less than 90%). Interestingly, although *A. scabra,* a species under section *Alpinia* subsection *Presleia sensu* Smith (1990) is genetically distinct, ITS consensus exhibit high posterior probabilities that *A. scabra* is still grouped within subclade VIb and may be the basal species to clade VIb (Fig. 3.3). Therefore, section *Allughas* subsection *Allughas*, section *Alpinia* subsection *Presleia* of Smith's 1990

classification might be polyphyletic. New described species, *A. suriana* is closely related to *A. rafflesiana* even though the support is weak. It is apparent that *A. suriana* taxa are not clustered together collectively, but intermittently with *A. rafflesiana.*

The phylogeny derived from the partial gene of maturase K (*mat*K) data in this study displayed a dissimilar cluster of *Alpinia* in subclade VIa and subclade VIb to Clade VI (the *Rafflesiana* clade, Fig. 3.3 & Fig. 3.4) established by Rangsiruji *et al.* (2000b) and *Kress et al.* (2005a). Previous reference sequences of *A. javanica* and *A. rafflesiana* of Smith's 1990 section *Allughas* subsection *Allughas* were grouped in subclade VIa, while additional species (*A. rafflesiana, A. pahangensis, A. capitellata, A. javanica, A. javanica* var. *colorata, A. murdochii*) of Smith's 1990 section *Allughas* subsection *Allughas* from this study were clustered in subclade VIb. In addition, even though *A. scabra,* a species under section *Alpinia* subsection *Presleia sensu* Smith 1990 is genetically distinct, the consensus tree of *mat*K displayed a close relationship between *A. scabra* and other species in subclade VIb mentioned above. Thus, *A. scabra* may be basal to subclade VIb. Newly described species, *A. suriana* displayed a close relationship with *A. rafflesiana* (Fig. 3.4) and exhibit polytomy with *A. rafflesiana.* Within clade VIb, the support values are for the same species with different accessions namely *A.pahangensis, A. murdochii, A. javanica* while different accessions of *A. capitellata*, *A. javanica* var. *colorata, A. suriana* and *A.rafflesiana* also in Clade VIb were not clustered together collectively. Consequently, Smith's 1990 section *Allughas* subsection *Allughas* and section *Alpinia* subsection *Presleia* may not be monophyletic.

Subclade VIa and VIb showed no support on the main branch of partial *mat*K gene that define Clade VI (the *Rafflesiana* clade). However, due to the presence of reference sequences of *A. javanica* and *A. rafflesiana* in subclade VIa, all the selected *Alpinia* species from Peninsular Malaysia belongs to Clade VI because subclade VIb is

connected to this main branch with high posterior probabilities within the subclade (*/*/*).

The incongruence observed between internal transcribed spacer (ITS) region and maturase K gene (*mat*K) trees suggest that hybridization and introgression may have occured between closely related species or these species may have shared ancestral polymorphisms (Kress *et al.,* 2005b; Comes and Abbott, 1999). Although plant species are genetically complex, introgression- whether it is adaptive or not, has the potential to affect phylogenetic reconstruction (Riesenberg and Welch, 2002). Interestingly in this study, species that exhibit similar floral characters, but differ in size and localities such as *A. pahangensis* in the lowlands and *A. murdochii* in highlands, were found clustered together in Clade VIb. On the other hand, the status of *A. rafflesiana* and *A. suriana* which appear to be similar in their floral characteristics were unresolved in both ITS data and *mat*K data forming a polytomy in the phylogenetic tree. Hence, possible factors involved in this discordance are introgression and phylogenetic sorting as the ancestor of both species may have been polymorphic for the *mat*K mutations; as the species diverged, the original polymorphisms were retained in both species (Soltis *et al.,* 1992). Reticulate hybridisation of four parental species and homoploidy hybrids of *Alpinia* from Taiwan that have the characteristics of flexistyly suggests the emergence of natural hybridization occuring at sympatric distribution with overlapping flowering period (Liu *et al.,* 2009). It is known that the original population of the conserved plants of *A. suriana* were sympatrically growing near *A. javanica* along with species from the genus *Amomum* and *Zingiber.* Therefore it is possible that *A. suriana* is a hybrid of *A. rafflesiana* with *A. javanica*.

Another interesting finding of the phylogenetic tree analyses from this study is the formation of a unique single lineage of *A. scabra,* species that possess floral characteristics similar to *A. galanga.* In Smith's (1990) classification, *A. scabra* is

grouped together with *A. galanga* under section *Alpinia* subsection *Alpinia.* However, consensus phylograms of internal transcribed spacer region (ITS) and maturase K gene (*mat*K) in this study showed strong evidence that *A. scabra* is a sister taxa, basal to Clade VIb, the *Rafflesiana* clade (Fig 3.3 and Fig. 3.4) whereas *A. galanga* is clustered with *A. conchigera* in Clade II. The inter-species divergence of ITS and *mat*K sequences between *A. scabra* and other taxa within subclade VIb in this study ranged from 3.5% to 4.4% in ITS sequence and 1.5% to 2.2% in *mat*K sequence. It is quite remarkable that *A. scabra* does not group together with either *A. galanga* or *A. aquatica,* as stated in Holttum's (1950) and Smith's (1990) classification; because the floral characteristics looked fairly similar; with some morphological differences *A. scabra* having scabrous leaves and black fruits and only found near waterfall areas in montane and hilly area. *A. scabra* is not known from lowland forests except for one site in the lowlands of Setiu reserve forest in Terengganu. Although *A. scabra* is genetically distinct, it still belongs to Clade VI (the *Rafflesiana* clade). It is likely that the morphological characteristics of *Alpinia* species in CladeVI have evolved or derived from ancestral morphology of *A. scabra* or *A. galanga*.

In previous phylogenetic studies of the genus *Alpinia,* Rangsiruji *et al.* (2000b) and Kress *et al.* (2005a) identified Clade II (the *Galanga* clade), Clade IV (the *Zerumbet* clade) and Clade VI (the *Rafflesiana* clade) as polyphyletic. Therefore, this study confirms the polyphyletic status of *Alpinia* species and reveals that the *Alpinia* species from Peninsular Malaysia reflects some degree of similarity with Rangsiruji *et al.* (2000b) and Kress's *et al.* (2005a) phylogeny where the species are polyphyletic and is, in some cases, incongruent with Smith's 1990 infrageneric classification.

On the overall Holttum's (1950) classification of *Alpinia* species and the 4 allied genera as well as other genera within the tribe Alpineae appear to be in agreement with Kress *et al.* (2005a) molecular results. Holttum's genera are mostly based on

inflorescence characters; and the *Alpinieae* tribe is defined by grouping ginger species with primitive condition, that is having tubular or cup-shaped secondary bracts with or without small primary bracts (Holttum, 1950).

4.3 General status of *Alpinia* diversity in Peninsular Malaysia

Sample collections from numerous states in Peninsular Malaysia showed a diverse range of habitats of *Alpinia* species from primary forests to secondary forests in highland, midland and lowland areas. Out of 23 species recorded by Holttum, 20 species of *Alpinia* were successfully collected in this study. In addition, one new *Alpinia* species described by Lim (2004), namely *A. suriana* was collected, which was cultivated in the conservatory. Generally the gingers are grown in clumps and the inflorescence borne at the terminal axil of a non-leafy shoot.

Out of the 23 locations visited, four *Alpinia* species were collected from two new locations that were not recorded (*A. javanica, A. rafflesiana, A. vitellina* in Hutan Simpan Berembun, Negeri Sembilan and *A. rafflesiana* in Hutan Simpan Ulu Sedili, Johor). Some of the *Alpinia* species were collected from Botanical gardens (Universiti Malaya Rimba Ilmu, Forest Research Institute Malaysia, Putrajaya Botanical Garden, Penang Botanic Gardens and Suriana Gardens) because these species were not found in the recorded habitats or sites.

Several attempts were made to collect *Alpinia* species which were described and recorded by Holttum (e.g. *A. cornerii, A. denticulata, A. mollissima, A. seimundii* and *A. pulcherrima)* from a number of localities in Kedah, Selangor, Melaka, Johor, Terengganu and Perak; yet, unfortunately these areas have undergone habitat destruction due to human land use. These disturbances of forests are rapidly escalating

throughout the states of Peninsular Malaysia especially in secondary forests and the village areas.

A recent study by Hansen *et al.* (2013) provide a general idea whereby mass forest degradation and land clearing globally affected the climate due to the increase of carbon emissions. Although there are several forest areas where it has been reserved and protected in Peninsular Malaysia, it is not adequate due to illegal forest logging and lack of forest management (Syuhada, 2013; Zairul, 2013). Recently in January 2014, a conserved forest in Kelantan was appallingly found to be a place for illegal activity of mass logging (Farhanin,and Jaffry, 2014). Prior to this, populations of *A. mutica* and *A. cochigera* that were collected from Pahang and Perak in 2008-2009 were seen to be rapidly declining whereby recent visits in 2013 at these particular areas showed no trace of gingers since the land has been cleared and developed into housing areas in Perak and commercial plantations in Pahang. *A. mutica* and *A. conchigera* are well known to be used in traditional medicine to reduce swelling and treat fungal infection (Valkenburg and Bunyapraphatsara, 2001).

In addition, during a field work in Pahang, we found the population of *A. pahangensis* at the secondary forest located very close to the vastly active logging areas. Similarly, although located in highland forest, population of *A. murdochii* was seen to be affected by development of land for recreation in Pahang. Also, recently in 2009, conservation and threat assessment of the genus *Haniffia* and *Scaphochlamys* from the family Zingiberaceae in Peninsular Malaysia was presented and results showed that all *Haniffia* species and 5 out of 32 *Scaphochlamys* species are considered as critically endangered (CR) according to IUCN (2000) categories (Julius *et al.,* 2009; Sam *et al.,* 2009). Therefore, it might be possible that several *Alpinia* species are undergoing critical or endangered threats which require a number of actions to be taken to reduce the negative implication on its biodiversity.

Even though the possibilities of several *Alpinia* species being threatened are quite disturbing, the growing numbers of conservatory areas in Peninsular Malaysia provide a promising measure to ensure that these gingers can still be cultivated and saved. Besides, efforts have been made to save and replant several *Alpinia* species that were collected during the field work. However there are still limitations, as some species that were found in montane areas for example *A. murdochii,* cannot grow in the lowlands. Sometimes even species from the lowlands for example *A. pahangensis* does not acclimatize well in the nursery. This may be due to different ecological templates where the habitat heterogeneity, habitat complexity, interaction between species and dispersal of the plant differs from its origin (Jeffries, 2006). Also, genetic diversity of a population will decline if the number of wild species is reduced, and if any plant disease were to occur; the level of fitness and survival will rapidly decline due to inbreeding depression for lacking novel genetic variation that creates gene flow (Kéry *et al.,* 2000). Therefore, the need to conserve via *in situ* is still regarded as the best method to maintain and sustain the population of *Alpinia* species in Peninsular Malaysia.

CONCLUSION

In conclusion, this study has successfully established consensus phylogenetic trees of *Alpinia* using nuclear ribosomal internal transcribed spacer region (ITS) and maturase K gene (*mat*K) utilising Neighbour joining (NJ), Maximum Parsimony (MP) and Bayesian Inference (BI) methods. In light of the current ongoing research to establish DNA barcoding markers for angiosperms, this study found that both ITS and partial *mat*K genes showed promising results; and in comparison, ITS resolved the status of most of the taxa of the *Alpinia* species investigated thus are more adept at inferring the infrageneric species relationship. The molecular study on the Peninsular Malaysian *Alpinia* species shows disagreement with Smith's 1990 classification of section *Alpinia* (subsection *Catimbium*, subsection *Cenolophon,* subsection *Presleia*) and section *Allughas* (subsection *Allughas*). On the overall, the findings from this study appear to be in parrallel with previous phylogenetic analysis by Rangsiruji *et al.* (2000b) and Kress *et al.* (2005a) which demonstrates the genus *Alpinia* as polyphyletic. In addition, results from this study illustrate that clade VI (the *Rafflesiana* clade) consists of only Peninsular Malaysian species under section *Allughas* (subsection *Allughas*) *sensu* Smith, 1990. On the other hand *A. scabra* might be a sister taxa to clade VI. The analysis of ITS and *mat*K region of the Peninsular Malaysian *Alpinia* species suggests possible hybridization or introgression between closely related species or the species may have shared ancestral polymorphisms. As this is the first molecular phylogenetic approach conducted on *Alpinia* species from Peninsular Malaysia, the status of some species is yet to be resolved. The conflicting observations from the molecular results and morphological characteristics of several of the Peninsular Malaysian *Alpinia* species investigated, suggest that the genus is in need of a meticulous revision hence require further work to include more gene markers.

4.4 Future directions and suggestions

With an increasing recognition of novel species, and since many of the *Alpinia* representative species are known for horticultural potential apart from its medicinal and traditional uses; it is suggested that several important and economically potential *Alpinia* species that undergo habitat destruction to be conserved via *in situ* and *ex situ*. However, the implementation of the conservation measures for this genus, a proper study to assess the conservation status of all species need to be carried out as was done for the genus *Haniffia* and *Scaphochlamys* in Peninsular Malaysia. Besides, this genus clearly requires rigorous taxonomic revision to determine the taxonomic status. Interesting finding on the incongruence between internal transcribed spacer region (ITS) and maturase K (*mat*K) analysis in this study renders an appealing research to further investigate the degree of hybridization and introgression among the species mentioned which might include cytogenetic studies, genetic population studies using DNA fingerprinting technique and phylogeographic studies of the genus. The various studies suggested above can be used to quantify geographic differences that will be feasible to elucidate the current discrepancies and unresolved taxonomic problems of several *Alpinia* species. In addition, phylogeographic studies may, in future be an interesting research because it encompass the ecological, evolution and molecular aspects of the truly unique and complex genus of *Alpinia*.