# THE PHYLOGENETIC ANALYSIS OF SELECTED *Boesenbergia* SPECIES (FAMILY: ZINGIBERACEAE) FROM CROCKER RANGE.

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THIS DISSERTATION IS PROPOSED TO FULFIL THE PARTS OF REQUIREMENTS IN OBTAINING BACHELOR OF SCIENCES WITH HONOURS.

CONSERVATION BIOLOGY PROGRAMME
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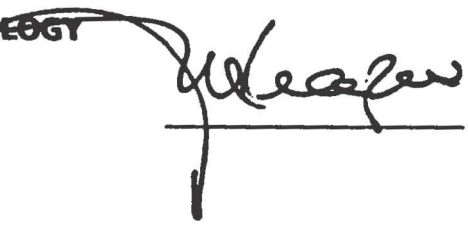

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## ABSTRAK

## ANALISIS FILOGENETIK SPESIES *Boesenbergia* (FAMILY: ZINGIBERACEAE) TERPILIH DARI BANJARAN CROCKER.

Kajian ini adalah untuk menganalisa data filogeni kepada spesies *Boesenbergia* yang terdapat di Banjaran Croker. Empat spesies *Boesenbergia* telah dikumpul dari substation-substation Monggis dan Ulu Kimanis, iaitu *Boesenbergia variegata*, *Boesenbergia* sp. 1, dan dua spesimen daripada *Boesenbergia pulchella*. Hubungan antara keempat-empat spesies *Boesenbergia* berkenaan diuji dengan penjujukan internal transcribed spacers (ITS) dari ribosoma nuklear DNA (nrDNA). *Tamijia flagellaris* digunakan sebagai kumpulan luar. Pokok `50% mojority-rule' terbentuk daripada gabungan bahagian ITS 1 dan ITS 2 membahagikan *Boesenbergia* kajian kepada dua kumpulan utama : Kumpulan A (*Boesenbergia variegata*, *Boesenbergia* sp. 1, *B. flavorubra, B. rotunda, B. jangarunii, B. aurantiaca, , B. armeniaca, B. aff. burttiana, B. aff. variegata, B. parva, B. cordata, B. orbiculata,* dan *B. belalongensis)* dan Kumpulan B (*Boesenbergia pulchella, B. pulchella. var. attenuata, B. aff. longiflora, B. basispicata, B. gelatinosa, B. longiflora*, dan *B. plicata)*. Spesies *Boesenbergia* yang dikumpul dari kerja lapangan telah dipastikan sebagai *Boesenbergia variegata* dan *Boesenbergia pulchella* berdasarkan data filogenetik. Selain itu, sepsis Boesenbergia juga diramal tertumpu di bahagian utara dan pusat Banjaran Croker dengan menggunakan model taburan sepsis.

## ABSTRACT

This study was done to analyse the phylogenetic data of the *Boesenbergia* species found in Crocker Range. Four *Boesenbergia* species were collected from the substations Monggis and Ulu Kimanis, namely, *Boesenbergia variegata*, *Boesenbergia* sp. 1 and two specimens of *Boesenbergia pulchella*. The relationships among these four *Boesenbergia* species were examined by sequences of Internal Transcribed Spacers (ITS) of the nuclear ribosomal DNA (nrDNA). *Tamijia flagellaris* was used as outgroup. The 50% majority-rule consensus tree was constructed from combined ITS 1 and ITS 2 regions grouped the *Boesenbergia* in this study into 2 main clades: Clade A (*Boesenbergia variegata*, *Boesenbergia* sp., *B. flavorubra*, *B. rotunda*, *B. jangarunii*, *B. aurantiaca*, *B. armeniaca*, *B. aff. burttiana*, *B. aff. variegata*, *B. parva*, *B. cordata*, *B. orbiculata* and *B. belalongensis*) and Clade B (*Boesenbergia pulchella*, *B. pulchella. var. attenuata*, *B. aff. longiflora*, *B. basispicata*, *B. gelatinosa*, *B. longiflora*, and *B. plicata).* The *Boesenbergia* species collected from the field were confirmed as *Boesenbergia variegata* and *Boesenbergia pulchella* based on the phylogenetic data. Besides that, the *Boesenbergia* species was predicted to be concentrated at the northern and centre part of Crocker Range by using the species distribution model Maxent.

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| | |
|---|---|
| A | In DNA or RNA sequences: adenine |
| B | In DNA or RNA sequences: any nucleotide except adenine |
| bp | Base pair |
| C | In DNA or RNA sequences: cytosine |
| CTAB | Hexadecyltrimethylammonium bromide |
| D | In DNA or RNA sequences: any nucleotide except cytosine |
| dNTP | Deoxyribonucleotide |
| G | In DNA or RNA sequences: guanine |
| H | In DNA or RNA sequences: any nucleotide except guanine. |
| HKY model | The DNA substitution model of M. Hasegawa, H. Kishino, and T. Yano(1985) |
| K | In DNA or RNA sequences: guanine or thymine |
| kb | Kilobase pairs, or 1000 base pairs of DNA |
| M | In DNA or RNA sequences: adenine or cytosine |
| N | In DNA or RNA sequences: an unknown nucleotide |
| PCR | Polymerase chain reaction |
| R | In DNA or RNA sequences: adenine or guanine |
| S | In DNA or RNA sequences: guanine or cytosine |
| *Taq* polymerase | A thermostable DNA polymerase from *Thermus aquaticus* |
| V | In DNA or RNA sequences: adenine, cytosine, or guanine |
| W | In DNA or RNA sequences: adenine or thymine |
| Y | In DNA or RNA sequences: cytosine or thymine |
| % | Percentage |
| °C | Degree Celsius |

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# CHAPTER 1

# INTRODUCTION

## 1.1 Introduction

The eumonocots (Arecales, Commelinales, Poales) that include many conspicuous taxa, such as the bananas (Musaceae), birds of paradise (Strelitziaceae), heliconias (Heliconiaceae), and gingers (Zingiberaceae) are derived from Zingiberales (Kress *et al.*, 2002). The Zingiberaceae is the largest family out of eight families in the order Zingiberales (Kress, 1990, Kress *et al.*, 2002). There are 53 genera with about 1300 species of Zingiberaceae distributed in Neotropics, Africa and Southeast Asia (Kress *et al.*, 2002). Zingiberaceae can be classified into four tribes, and both vegetative and floral characteristics were used for classification (Burtt and Smith, 1972; Kress *et al.*, 2002).

*Boesenbergia* is one of the genus in the family Zingiberaceae. *Boesenbergia* species can be found in a wide range of habitats, such as in very damp, shaded area, and often close to streams or in boggy condition. However according to Mood *et al.* (1996), *Boesenbergia* species is considered as quite specialized in their environmental requirements, because they will only grow in certain types of soil with the certain altitude regimes. *Boesenbergia* species are tremendously rare even though there are many species compared to other genera (Mood, 1996). The *Boesenbergia* sp. is hard to identify due to its morphological variations in both

intraspecific and interspecific (Vanijajiva et al., 2003). Some of the species of genus *Boesenbergia* are rare and threatened species, such as *Boesenbergia pulcherrima.* The *Boesenbergia pulcherrima* has been identified as threatened and included in the IUCN Red List of Threatened Plants (Anish *et al.*, 2008).

Crocker Range is the highest mountain range in Sabah. The Crocker Range separates the east coast and west coast of Sabah (Tuen et al., 2002), consisting of eight districts: Tuaran , Ranau, Papar, Penampang, Keningau, Tambunan, Beaufort and Tenom . Part of the range was gazetted for protection as Crocker Range National Park since 1984 (in order to protect the watersheds for the people residing in the West Coast and Interior Districts of Sabah (Murtedza *et al.*, 2002).

## 1.2 Objectives

The objectives of this study are as follows:

1) To build the phylogenetic tree among *Boesenbergia* species based on the available DNA sequence of *Boesenbergia* species.
2) To obtain the potential distribution of *Boesenbergia* species based on the available secondary data of *Boesenbergia* species collected.

## 1.3 Justification

So far, there has been no comprehensive study on *Boesenbergia* in Crocker Range Area. The distribution of *Boesenbergia* can be predicted by using Maxent which utilizes associations between environmental variables and known species' occurrence records. The prediction distribution data is very helpful for conservation purposes. This is because some of the species of genus *Boesenbergia* are rare and threatened. The present study would fill the gap of information regarding the distribution of *Boesenbergia* species found in Crocker Range.

## 1.4 Scope of Study

The samples were collected in a few substations at Crocker Range. The samples were extracted with CTAB method and analysed. Secondary data were obtained was used in species distribution modeling.

## 1.5 Hypothesis

The hypothesis is the phylogenetic data will show there are close relationship among the *Boesenbergia* species.

# CHAPTER 2

# LITERATURE REVIEW



## 2.1 Overview of classification of the genus *Boesenbergia*

The vegetative and floral characteristics are usually used for classification of Zingiberaceae such as number of locules and placentation in the ovary, development of staminodia, modifications of the fertile anther, and rhizome-shoot-leaf orientation. The reproductive parts are very important for taxonomy of *Boesenbergia*, the identification of *Boesenbergia* is not sufficient without the reproductive parts (Techaprasan *et al.*, 2006). The taxonomic study of the family is difficult and a classification is still incomplete due to the ephemeral flowers. However, most of the characters that are used to define the tribes are often inconsistent and variable.

A study on the genus *Boesenbergia* (Zingiberaceae) by Larsen (1997) was on a revision of *Boesenbergia* and related genera for Flora Malesiana. He claimed that the re-evaluation of the established genus *Curcumorpha*, reduced this genus to *Boesenbergia*. The new genus *Curcumorpha* based on *Gastrochilus longiflora* Wallich was described by Rao & Verma (1971) using the three characters in which *Curcumorpha* deviates from *Boesenbergia* and this species considered as restricted to northeastern India. However, *Curcumorpha* is also found commonly in Central and northern Myanmar and northern and central Thailand

(Larsen, 1997). He studied the material from India, Myanmar and Thailand based on the three characters in which *Curcumorpha* deviates from *Boesenbergia*, these three characters were its radical inflorescence, spirally arranged bracts, the staminodial cup, and as a result, he commended to maintain this species in the genus *Boesenbergia* as *Boesenbergia longiflora* (Wall.) Kuntze.

The morphology of a species may be different in various distribution area affected by environment condition, because based on the less distinctive character the staminodial cup, the flower of *B. longiflora*, as studied in nature in Thailand, is a very typical *Boesenbergia* flower not in any way deviating from most other species of the genus but readily distinguished from flowers found in any other genus of Zingiberaceae (Larsen, 1997). Besides, according to Larsen (1997), two species was discovered not formally transferred to genus *Boesenbergia* while the revision of this genus which were *Boesenbergia phyllostachy* (Gagnep.) K. Larsen and *Boesenbergia loerzingii* (Val.) K. Larsen. Based on the Crocker Range Scientific Expedition in 2002, there was a total of 12 genus, 45 species of Zingiberaceae found at Crocker Range Park. However there was only two species of *Boesenbergia* found at Crocker Range Park, which were *Boesenbergia gracilipes* and *Boesenbergia cf. pulchella* (Takano *et al.*, 2004).

## 2.2 Distribution of Zingiberaceae

Larsen (2001) stated that the Malesian region has the highest diversity of Zingiberaceae in the world. West Malaysia and Java are the best known area in Malesia for Zingiberaceous plants due to the revised for the Malay Peninsula in 1950 by Holttum, B.L. Burtt and R.M. Smith great contribute to the study of Malesian Zingiberaceae in the second half of this century.

According to Mood (1996), there were 13 genera of Zingiberaceae and one genus of Costaceae found in Sabah. These 13 genera of Zingiberaceae include *Alpinia*, *Amomum*, *Boesenbergia*, *Burbidgea*, *Elettaria*, *Elettariopsis*, *Etlingera*, *Geocharis*, *Globba*, *Hedychium*, *Hornstedtia*, *Plagiostachys*, and *Zingiber* , whereas the only genus of Costaceae that found in Sabah was *Costus*.

## 2.3 Morphology of Zingiberaceae

The rhizome of Zingiberaceae is sympodial, varying in size and degree of branching. Usually, the leafy shoots terminate in an inflorescence or, less commonly, inflorescence and leaves appear on separate shoots. The erect vegetative stems are always unbranched. The true vegetative stem is short and thin, composed mainly of thin walled cells, and the upright vegetative shoots are largely pseudostem (false stems) formed by intercalation of leaf sheaths. The petiole is of varying length. The plane of insertion of the distichous leaves varies from parallel to the length of the rhizome to transverse to it. Leaf sheaths are usually open, the blade vary in size. The flower is superficially like that of an orchid in that it possesses but a single stamen, and in both families the lip usually provides the showiest part of the flower. There is, however, the orchid lip is a true petal, in the Zingiberaceae it is a modified stamen (Smith, 1996).

*Boesenbergia* is small herbs from short, with fleshy rhizome. The leaves usually arranged in tufts. The inflorescence are terminal on the leafy shoot or on a separate leafless shoot. The boat-shaped flowers were in white, yellow or red in colour, with calyx tubular (Larsen *et al.*, 1998). The corolla tube of *Boesenbergia* is slender and usually shorter than the bracts. The lateral staminodes petaloid , the labellum conspicuous and saccate. The lateral staminodes and filament attach to base of labellum. The anther crest is present or absent (Smith, 1981).

The leaves of *Boesenbergia variegata* are bullate, the inflorescence is densely hairy with small flowers (Poulsen, 2006). The leaf shoots with only one not orbicular leaf, leaves variegated and dark green with a lighter band up middle, variegation sometimes extending to lateral veins with slightly bullate surface (Smith, 1981). The flowers are white and yellow with red at base of lip (Smith, 1987).

The endemic plant to Borneo, *Boesenbergia pulchella* has the shoot with several leaves, spindle-shaped inflorescence rises of the leaf sheaths in the middle of the leafy shoot, the flowers are white and red (Poulsen, 2006). The colour of the flowers of *B. pulchella* is white except labellum with central vertical stripe of red spots and outer half red except for translucent white edge (Smith, 1987). The leaf

shoots with several leaves, anthers dehiscing by slits, cordate leaves, and inflorescence many flowered (Smith, 1981).

## 2.4 Molecular studies of Zingiberaceae

There are many research papers that utilized the molecular tools for the study of genetic variation and phylogentic classification of the plant. The molecular tools, electrophoresis have been used in the research papers of Makhuvha *et al.* (1997) to estimate genetic diversity of species Zingiberaceae.

Electrophoresis was used to estimate genetic diversity of the wild and cultivated *Siphonochilus aethiopicus* (Zingiberaceae) by Makhuvha *et al.* (1997). The medicinal wild ginger, *Siphonochilus aethiopicus* is a rare species in South Africa that become extinct in some provinces (Hilton-Taylor, 1996), however, there is no information about its genetic variation. In order to conserve the species from extinction, the genetic variation information are important. Electrophoresis used to study genetic variation of populations for distinct allelic forms of isozymes. The work of Makhuvha *et al.* (1997) provided a good basic for further genetic study of *S. aethiopicus* since it was the first attempt to electrophoretic variant of the Zingiberaceae.

The morphological classification of the largest and complex genus in the Zingiberaceae, *Alpinia* great inconsistency. Therefore, Rangsiruji *et al.* (2000) studied the infrageneric classification of *Alpinia* (Zingiberaceae) based on the ITS region of nuclear rDNA and the trnL-F spacer of chloroplast DNA in order to clarify relationships. Rangsiruji *et al.* (2000) stated that the Alpinia may be paraphyletic based on ITS analysis, and the result based on both nrDNA and cpDNA regions largely disagree with Smith's infrageneric classification of *Alpinia*.

Base the work of Kress *et al.* (2002), new phylogenetic analyses based on DNA sequences of the nuclear internal transcribed spacer (ITS) and plastid *matK* regions suggested that at least some of these morphological traits are homoplasious and three of the tribes are paraphyletic, the African genus *Siphonochilus* and Bornean genus *Tamijia* were used as basal clades. Then, Kress *et al.* (2002)

proposed a new classification of the Zingiberaceae that recognizes four subfamilies and four tribes: Siphonochiloideae (Siphonochileae), Tamijioideae (Tamijieae), Alpinioideae (Alpinieae, Riedelieae), and Zingiberoideae (Zingibereae, Globbeae).

Vanijajiva *et al.* (2003) stated, the isozyme analysis of relationship among *Boesenbergia* (Zingiberaceae) and related genera in Southern Thailand showed a higher degree of relationship between *Boesenbergia* and *Scaphochlamys* than between *Boesenbergia* and *Kaempferia* by cluster analysis and UPGMA. Besides, Vanijajiva *et al.* (2003) stated, there were four out of nine enzyme systems experimented found to be useful as molecular markers for characterization of *Boesenbergia* and related taxa, which are peroxidase, superoxide dismutase, glutamate dehydrogenase and malate dehydrogenase.

The relationship among 19 accessions of Zingiberaceae belonged to 11 species of *Boesenbergia*, six species of *Kaempferia*, and two species of *Scaphochlamys* from Southern Thailand were studied using random amplified polymorphic DNA (RAPD) profiles from leaf tissue samples by Vanijajiva *et al.* (2005) showed that higher degree of relationship between *Boesenbergia* and *Scaphochlamys* than between *Boesenbergia* and *Kaempferia* based on cluster analysis, UPGMA and a principal component analysis of the RAPD result.

According to Vanijajiva *et al.* (2005), ten random decamer arbitrary primers used in RAPD, however amplification only occurred in 5 primers (OPAM-01, OPAM-03, OPAM-12, OPB-14, OPZ-03). RAPD is also a reliable method for estimating phylogenetic relationship since it reflects coding and non-coding regions of the genome and it could well be used in aiding identification as well as classification of the Zingiberaceae using more species in each genus.

Techaprasan *et al.* (2008) examined the genetic relationships of 15 *Boesenbergia* species by using AFLP analysis, the investigated species resolved into two separate lineages and a rapid radiation in *Boesenbergia* was suggested. AFLP and SSCP are useful methods for identification in *Boesenbergia* because the SSCP patterns of the partial psbA-trnH spacer were not overlapped between different

species since the morphologically was similar, *Boesenbergia longiflora* and *Boesenbergia* sp. were successfully differentiated by SSCP (Techaprasan *et al.*, 2008).

## 2.5 Economic value of *Boesenbergia*

There are a lot of economic uses for Zingiberaceae, such as ornamentals, volatile oil, medicinal plant, perfumed powder, curry powder, yellow dye and so on (Heywood, 1996). There were many recent researches on compounds extracted from the rhizome of *Boesenbergia* found to consist potential economic values for medicinal treatments. Cheenpracha *et al.* (2006) stated, hydroxypanduratin A (3) and panduratin A (2) which were extracted from *Boesenbergia pandurata* rhizomes are able to potent anti-HIV-1 PR activity. Enzymes HIV-1 protease (HIV-1PR) is the vital enzymes that replicated the retrovirus human immunodeficiency virus type-1 (HIV-1) that cause organism in an acquired immunodeficiency syndrome (AIDS) (Cheenpracha *et al.*, 2006). According to the biological activities of *B. pandurata*, it also exhibited antibacterial, antifungal, anti-inflammatory, analgesic, antipyretic, antispasmodic, antitumor, insecticidal activities and as a self-medication by AIDS patients in Thailand (Cheenpracha *et al.*, 2006).

The study of Tan *et al.* (2006) described the inhibitory activities of cyclohexenyl chalcone derivatives and flavonoids of fingerroot that were isolated from *Boesenbergia rotunda* towards the dengue-2 virus NS3 protease. The dengue fever (DF) and dengue haemorrhagic fever (DHF) lead to temporarily disabling but rarely fatal caused by four closely related viruses (DEN-1, DEN-2,DEN-3 and DEN- 4) of the Flaviviridae family (Tan *et al.*, 2006). Tan *et al.* (2006) stated, the 4-hydroxypanduratin A and panduratin A of *Boesenbergia rotunda* cyclohexenyl chalcone derivatives showed good competitive inhibitory activities towards dengue 2 virus NS3 protease with the Ki values (21 and 25 IM respectively), while pinostrobin and cardamonin were observed to be non-competitive.

Both, *Kaempferia parviflora* and *Boesenbergia pandurata* belonged to Zingiberaceae family were used as food ingredients and traditional medicine for treatment of several inflammatory-related diseases. Tewtrakul *et al.* (2009) stated,

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