



***MORPHOLOGICAL AND GENETIC VARIATION OF *Eusideroxylon  
zwageri* Teijsm. & Binn. (BORNEO IRONWOOD) IN SARAWAK,  
MALAYSIA***

**SITI FATIMAH MD ISA**

**FS 2020 30**



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By

**SITI FATIMAH MD ISA**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of Requirements for the Degree of Doctor of Philosophy**

**August 2020**

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**This thesis is dedicated to:**

*My understanding husband and my dear son.*



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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**August 2020**

**Chairman : Rusea Go, PhD**  
**Faculty : Science**

Previously, two species were recognized as Borneo Ironwood, *Eusideroxylon zwageri* and *E. melagangai*. However, study revealed that *E. melagangai* is belonging to a new genus called *Potoxylon*. This confusion is due to the close morphological resemblance between both species. Borneo Ironwood has high demand and high market value because of its valuable and durable timber, which has put its number at risk of illegal logging. In addition to threats from illegal logging, the slow seed germination process delays their natural regeneration in the forest. It may take up to six months in their natural environment to germinate. Other than that, poor genetic matching of planting material to the planting site or using seed from small, inbred populations can result, for instance, in reducing growth, reproductive output, the ability to resist pests and capacity to adapt to environmental variation. In this project, two sampling areas were chosen (i) Nirwana Rehabilitation Forest (NRF), UPM Bintulu Campus, and (ii) fragmented area in Tatau, Bintulu, Sarawak. The aims for this study were to document morphological features of *E. zwageri* and to recognize their varieties by using conventional method based on the macro-morphology characters and to support the outcome with cladistics analysis. Subsequently, four microsatellite markers were used to analyse *E. zwageri* genetic variation and their clustering to the varieties based on allelic data. In addition, the identification of fungi isolated from the fruits of *E. zwageri* was also conducted. Three varieties of *E. zwageri* were identified namely; *zwageri*, *grandis*, and *exilis*. These varieties were recognized based on the seed, bark and leaves traits and supported with cladistics analysis and allelic data. Meanwhile, genetic study shown that 20.1% of total genetic variation corresponded to differences between populations while 79.9% was attributed by differences between individuals. The Tatau ( $H_o = 0.399$ ;  $H_e =$

0.563) population was observed to have relatively lower genetic diversity compared to NRF ( $H_o = 0.659$ ;  $H_e = 0.739$ ) area based on the observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosity value; and both areas shown a deficiency of heterozygosity indicated by positive  $F_{IS}$  value (Tatau = 0.054; NRF = 0.165) that suggested the population undergoing inbreeding event. Furthermore, study on fungi diversity isolated from *E. zwageri* fruits identified fungi from nine taxa from Ascomycota phylum namely; *Annulohypoxylon nites*, *A. viridistratum*, *Daldinia eschscholtzii*, *Hypoxylon investiens*, *Lasiodiplodia theobromae*, *Trichoderma asperellum*, *T. crassum*, *T. spirale*, and *T. virens*. This data will be useful for future understanding in fungi involvement in rooted seed in *E. zwageri* species. It is hope that the results from this study will be used as baseline data in an effort to protect this vulnerable species.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**MORFOLOGI DAN VARIASI GENETIK *Eusideroxylon zwageri* Teijsm.  
& Binn. (BELIAN) DI SARAWAK, MALAYSIA**

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Terdahulu, Belian di Borneo telah dikenal pasti mempunyai dua spesis yang dikenali sebagai; *Eusideroxylon zwageri* dan *E. melagangai*. Bagaimanapun, kajian melaporkan bahawa *E. melagangai* tergolong dalam genus baru yang dikenali sebagai *Potoxylon*. Kekeliruan ini adalah disebabkan oleh persamaan morfologi yang hampir sama antara kedua-dua spesis. Belian mempunyai permintaan yang luas dan nilai pasaran yang tinggi kerana kayunya yang berharga dan tahan lama, di mana ianya juga menjadi ancaman kepada kegiatan pembalakan haram. Selain itu, proses percambahan biji benih yang lambat menangguhkan pertumbuhan semulajadi mereka di dalam hutan. Percambahan biji benih *E. zwageri* mengambil masa selama enam bulan dalam persekitaran semulajadinya. Di samping itu, pepadanan genetik yang lemah untuk menanam benih ke tapak penanaman atau menggunakan benih dari populasi yang kecil atau mempunyai baka yang sama boleh menjejaskan pertumbuhan, pengeluaran, keupayaan untuk menentang perosak dan keupayaan untuk menyesuaikan diri dengan keadaan persekitaran. Dalam projek ini, dua kawasan pensampelan telah dipilih; (i) Hutan Pemulihan Nirwana (NRF), Kampus UPM Bintulu, dan (ii) kawasan terpencil di Tatau, Bintulu, Sarawak. Tujuan kajian ini adalah untuk mendokumenkan ciri-ciri morfologi *E. zwageri* dan mengenal pasti varieti yang terdapat di tempat kajian dengan menggunakan kaedah konvensional iaitu berdasarkan ciri-ciri makro-morfologi dan disokong dengan analisis kladistik. Seterusnya, empat penanda mikrosatelit digunakan untuk menganalisis variasi genetik mereka dan kluster mereka kepada varieti berdasarkan maklumat dari data mikrosatelit. Selain itu, pengecaman fungi yang diasingkan dari buah *E. zwageri* juga dijalankan. Tiga jenis varieti telah dikenal pasti iaitu; *zwageri*, *grandis* dan *exilis*. Varieti ini diiktiraf berdasarkan kriteria benih, kulit dan daun dan keputusan ini disokong melalui analisis kladistik dan data

mikrosatelit. Sementara itu, kajian genetik menunjukkan bahawa 20.1% daripada jumlah variasi genetik sepadan dengan perbezaan antara populasi manakala 79.9% dikaitkan dengan perbezaan antara individu. Belian di Tatau ( $H_o = 0.399$ ;  $H_e = 0.563$ ) diperhatikan mempunyai kepelbagaian genetik yang lebih rendah berbanding dengan kawasan NRF ( $H_o = 0.659$ ;  $H_e = 0.739$ ) berdasarkan pemerhatian ( $H_o$ ) dan jangkaan ( $H_e$ ) *heterozygosity*; dan kedua-dua kawasan menunjukkan kekurangan *heterozygosity* yang dikenal pasti melalui nilai positif  $F_{IS}$  (Tatau = 0.054; NRF = 0.165) yang mencadangkan populasi ini mengalami kejadian *inbreeding*. Tambahan pula, pengecaman mengenai kepelbagaian fungi yang diasingkan dari buah *E. zwageri* mengenal pasti sembilan jenis kulat dari filum Ascomycota; *Annulohyphoxylon nites*, *A. viridistratum*, *Daldinia eschscholtzii*, *Hyphoxylon investiens*, *Lasiodiplodia theobromae*, *Trichoderma asperellum*, *T. crassum*, *T. spirale*, and *T. virens*. Data ini amat berguna sebagai asas pemahaman berkaitan penglibatan spesis kulat dalam pencambahan spesis *E. zwageri* pada masa hadapan. Diharapkan hasil dari kajian ini akan digunakan sebagai data asas dalam usaha untuk melindungi spesis terancam ini.



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## LIST OF ABBREVIATION

NRF	Nirwana Reserved Forest
Pers. comm.	Personal communication



## CHAPTER 1

### INTRODUCTION

#### 1.1 Background of Study

In Sarawak, Borneo Ironwood (*Eusideroxylon zwageri* Teijsm. & Binn.) is known locally as Belian. It is the most treasured timber of Borneo, and has been exploited widely. The species remained threatened by illegal logging despite harvesting and export restrictions in Borneo. In 2008, the Malaysian National Workshop on Forest Genetic Resources Conservation and Management identified the species amongst 30 plants as priority species for forest genetic resources conservation and categorized them as having economic importance. In Section 24 of The Sarawak Timber Industry (Registration) Regulations and Section 95 of The Forest Rules, Borneo Ironwood sawn timber were clearly defined and classified as marketable trees, and export in any form is not allowed without permission. Felling is allowed for trees above 60 cm in diameter for communities living surrounding the forest, but prohibited for concession holders and export of Ironwood is permitted only for processed wood products.

Besides that, International Union for Conservation of Nature (IUCN) Red List of Threatened Species (2019) listed this species as Vulnerable (VU). However, the species was last assessed in 1998 and the status would have change following the rapid industrial development and current global situations such as global warming. Several threats that threatened the number of the species in the forest mainly caused by uncontrolled logging and wood harvesting, residential and commercial development and agriculture and aquaculture activities (IUCN, 2019). This species is likely to become endangered unless the circumstances that are threatening its survival and reproduction are improved. Slow growth with mean radial growth rate 0.058 cm per year (Kurokawa, Yoshida, Nakamura, Lai, & Nakashizuka, 2003) and slow regeneration in the logging area is another biological reason contributing to the lower number of the species in the forest.

Other than that, ignorance, unawareness attitude and lack of knowledge about the status and importance of the Borneo Ironwood to our forest ecosystem give opportunity to illegal loggers to cut down the species without following rules and regulation. These could contribute to one of the cause the species to become nearly extinct in the forest. On July 19, 2018, Independent National Newspaper of East Malaysia (Daily Express) reported a major logging scandal that involved 40,161 logs involving Borneo Ironwood that have not been taxed and was also found with

measurement less than 40 cm which was against the provisions under the Forest Enactment 1968. If this situation is not prevented and not addressed to the public, it will cause more serious forest destruction and threaten the number of Borneo Ironwood species in the forest.

This protected tree can reach a height of up to 50 meter and may live up to over 1,000 years. Mature trees produce large fruits that, although poisonous to humans, are important food source for foraging animals. The species is also valued for cultural reasons. The wood is dense (0.85 – 1.1 g/cm<sup>3</sup>) (Wahyuni, 2011), strong and resistant to decay, that indigenous people of Borneo prefer to utilize it to build their houses. Dayak people of Borneo believed that these trees protect them from dangerous animals, while Murut (Borneo headhunter) used it to make blowpipe and Dusun ancestors used it to make coffin. Until today black pepper industry in Borneo used Belian wood as support to grow the creeping herbs and Murut Cultural Center is also supported by Belian wood pillars.

## 1.2 Problem Statement

Scientifically, there are two genera known as Borneo Ironwood; *Eusideroxylon zwageri* Teijsm. & Binn. and *Potoxylon melagangai* (Sym.) Kosterm and in this study, was focused more on *E. zwageri*. It is challenging to identify *E. zwageri* with *P. melagangai* in the field without proper training and experiences because of the close similarity in their vegetative structure. Besides, several varieties in *E. zwageri* have been informally recognized by local people, which make it more difficult in making affirmative identification. Thus, it is desirable to study the delimiting characters used to distinguish this species.

*Eusideroxylon zwageri* is valued as one of most durable timber tree in Malaysia and has high market value especially in the construction and furniture industries. It has high interest for restoration practitioners yet; efforts to restore the species populations through tree planting are hindered by the lack of information, especially knowledge on their genetic variation. Factors such as declining in population size through illegal logging, isolation of the population that cause by anthropogenic activities and genetic drift can cause severe genetic bottleneck for the species, which can lead to decline in the genetic variation of the species (Peery, 2012; Finlay, Bradley, Preston, & Provan, 2017). This scenario may contribute to the difficulty of getting a good quality of tree with a high chance of survival.

Besides that, the germination of *E. zwageri* seeds may take up to six months in their natural environment. Review on *E. zwageri* germination process has shown that mechanical seed scarification method is a

standard technique used to promote germination of *E. zwageri* seedling owing to their thick and hard endocarp layer (Irawan, 2012). Likewise, the seeds are susceptible to pathogen attack, which will hinder the sprouting of the *E. zwageri* seedling. This also will affect the regeneration process of the species where there are not enough young seedlings that will take the place of older trees that will eventually die. Hence, it is also important to study and identify fungi diversity on *E. zwageri* seeds or fruits to aid in future study on their symbiotic roles.

In particular, information and report on *E. zwageri* in the selected study areas in Sarawak are very limited. UPM Bintulu Campus has been closed for almost seven years thus the documentation activity in the Nirwana Rehabilitation Forest (NRF) is believed was also abandoned at that time (Marzuki pers. comm., 2017). In addition, after almost 28 years it is also perhaps that this study could provide an overview of *E. zwageri* genetic variation that has been preserved as a result from the restoration effort done in NRF. Meanwhile, fragmented area in Tatau, Bintulu that is believed resulted from anthropogenic activities (palm oil plantation) in the surrounding area could be considered to be the greatest threats to *E. zwageri* species residing in that area. Thus, the motivation to study and document the existence of the species in the chosen area is urgently needed before it vanishes.

### 1.3 Objectives of The Study

This study was carried out to identify Sarawak's *Eusideroxylon zwageri* and their varieties in selected areas, to analyze their genetic variation and to identify fungi isolated from their fruits. This information will be used in order to protect the species, to develop a future plan for enhancing their genetic diversity and to ensure optimal restoration practices and outcome. This fundamental study is necessary before more complexity work is implemented. Thus, the objectives of the study are as follows:

1. To identify species and the variety of *Eusideroxylon zwageri* in the selected study area (UPM Bintulu Campus and fragmented area in Tatau, Bintulu) using morphological approaches with support from cladistics analysis,
2. To analyze genetic variation by using specific marker that has been developed for *Eusideroxylon zwageri* and to further verify the clustering of *E. zwageri* to the varieties based on the allelic data,
3. To identify species of fungi isolated from *Eusideroxylon zwageri*'s fruits by using molecular technique to contribute to

the latest knowledge on the species of fungi isolated from *E.zwageri*.



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