



Faculty of Resource Science and Technology

Molecular Phylogeny and Modelling Distributions of *Limnonectes leporinus* Populations in Sarawak

Julius Georgy

**Master of Science
2024**

Molecular Phylogeny and Modelling Distributions of *Limnonectes leporinus*
Populations in Sarawak

Julius Georgy

A thesis submitted

In fulfillment of the requirements for the degree of Master of Science

(Biodiversity)

Faculty of Resource Science and Technology

UNIVERSITI MALAYSIA SARAWAK

2024

DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

A handwritten signature in black ink, appearing to read 'Julius Georgy', is written over a horizontal dotted line.

Signature

Name: Julius Georgy

Matric No.: 19020032

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

Date: 17 October 2024

ACKNOWLEDGEMENT

I would like to begin by expressing my deepest gratitude to the Almighty, for His blessings, strength, and guidance throughout my journey in completing my research for my Master's degree. I am truly humbled by His grace and mercy. I sincerely appreciate my supervisor, Professor Dr. Ramlah binti Zainudin, for her unwavering support, guidance, and knowledge. Her dedication to my growth has been inspiring, and I am honored to have worked under her supervision. I am grateful to the staff members of the Animal Resource Science and Management programme, particularly Mr. Isa Sait and Mrs. Atikah Jumaat, for their assistance and support during my laboratory work and field sampling activities. Their expertise has been instrumental in completing my research.

I am forever indebted to my parents for their love, support, and understanding. Their encouragement and sacrifices have been my strength during challenging times. I extend my sincere thanks to Yayasan Sarawak for providing me with a scholarship that enabled me to pursue my studies and complete this research. I acknowledge the funding provided by the Shell Chair grant F07/SHC/1789/2018, which greatly contributed to the implementation of my research project. I am immensely grateful to the Sarawak Forest Department and Sarawak Forestry Corporation for granting me the necessary permits to conduct research and to access national parks in Sarawak using Permit No. (45)JHS/NCCD/600-7/2/107(Jld2) and park permit No.WL18/2019; SFC.PL&RS/2020-012 and park permit No.WL27/2020.

Lastly, I want to express my gratitude to all individuals, friends, and authorities who provided assistance during this project especially my research partner, Nur Hidayah binti Zulkefli, for her companionship and support throughout the research journey.

ABSTRACT

Pagma frog, *Limnonectes leporinus*, is an endemic species of Borneo, faces significant challenges due to habitat fragmentation caused by deforestation and human activities in Sarawak, Malaysia. As a stream-dependent species, *L. leporinus* relies heavily on water bodies and forested areas, thus acts as a bio-indicator for healthy forests. This species is a subject of conservation concern, although it is still categorised as least concern (IUCN Red List), due to its declining populations. Conservation efforts require an understanding of the genetic diversity and habitat connectivity of *L. leporinus*. Therefore, this study aims to evaluate the genetic diversity of *L. leporinus* populations and develop an informative connectivity model for conservation strategies. By using mitochondrial DNA, Cytb, as molecular marker, 62 samples across 12 localities in Sarawak were analysed. Predictor variables such as land cover, annual temperature, and human population density, were used to develop a habitat suitability model in MaxEnt. Haplotypes data, microhabitat data and MaxEnt result were loaded in Circuitscape to employ habitat connectivity model of *L. leporinus*. Molecular analysis results revealed moderate genetic diversity across populations, with significant gene flow observed between protected areas. However, isolated populations exhibited reduced gene flow, indicating potential vulnerability to environmental changes. The spatial modelling analysis identified key areas for conservation prioritisation, particularly in lowland regions with sustainable stream networks, which are affected by habitat fragmentation. Habitat suitability model revealed that these lowland areas are ideal for *L. leporinus* breeding sites, while, the connectivity circuits are predominantly found in unprotected areas, making them crucial for conservation efforts. Most of these suitable yet unprotected areas fall under NCR land category. Therefore, conservation efforts should involve the state government and local communities to promote sustainable land use

practices that balance between conservation goals and local communities' livelihood needs. The findings which underscore the need for targeted conservation strategies, can be used to serve as a management strategies model for similar riparian species threatened by habitat fragmentation in Borneo.

Keywords: Habitat fragmentation, genetic diversity, spatial modelling, habitat connectivity, *Limnonectes leporinus*

Filogeni Molekul dan Pemodelan Taburan Populasi Limnonectes leporinus di Sarawak

ABSTRAK

Katak Pamak, Limnonectes leporinus, merupakan spesies endemik Borneo yang menghadapi cabaran besar akibat daripada pemecahan habitat yang disebabkan oleh pembalakan dan aktiviti manusia di Sarawak, Malaysia. Sebagai spesies yang bergantung kepada sungai, L. leporinus bergantung sepenuhnya kepada sumber air dan kawasan hutan, sekaligus bertindak sebagai bio-indikator untuk hutan yang sihat. Spesies ini menjadi subjek penting dalam usaha pemuliharaan, walaupun ia masih dikategorikan sebagai Tiada Perhatian Diperlukan (IUCN Red List), disebabkan oleh populasinya yang semakin menurun. Usaha pemuliharaan memerlukan pemahaman yang mendalam tentang kepelbagaian genetik dan habitat ketersambungan L. leporinus. Oleh itu, tujuan kajian ini adalah untuk menilai kepelbagaian genetik populasi L. leporinus dan membuat model ketersambungan yang berinformatif untuk strategi pemuliharaan. Dengan menggunakan DNA mitokondria, Cytb, sebagai penanda molekul, sebanyak 62 sampel dari 12 lokaliti di Sarawak telah dianalisis. Pembolehubah peramal seperti litupan darat, suhu tahunan, dan kepadatan populasi manusia digunakan untuk membuat model kesesuaian habitat menggunakan MaxEnt. Data haplotip, mikrohabitat, dan hasil MaxEnt dimuatkan ke dalam Circuitscape untuk membuat model habitat ketersambungan L. leporinus. Hasil analisis molekul telah mendedahkan kepelbagaian genetik yang sederhana di kalangan populasi, dengan aliran gen yang ketara diperhatikan antara kawasan yang terlindung. Namun, populasi terpencil mempunyai aliran gen yang kurang, membuktikan ia lemah terhadap perubahan persekitaran. Analisis pemodelan spatial telah mengenal pasti kawasan-kawasan penting untuk keutamaan pemuliharaan, terutamanya di kawasan tanah rendah yang

mempunyai rangkaian aliran sungai yang baik, yang terjejas oleh pemecahan habitat. Model kesesuaian habitat telah menunjukkan bahawa kawasan tanah rendah ini sesuai sebagai tapak pembiakan L. leporinus, manakala litar ketersambungan kebanyakannya terdapat di kawasan yang tidak dilindungi, menjadikan ia memerlukan usaha pemuliharaan. Kebanyakan kawasan yang sesuai tetapi tidak dilindungi ini berada di bawah kategori tanah NCR. Oleh itu, usaha pemuliharaan harus melibatkan kerajaan negeri dan komuniti tempatan memberi dorongan kepada amalan penggunaan tanah yang seimbang, mengimbangi antara misi pemuliharaan dan keperluan mata pencarian komuniti tempatan. Penemuan kajian ini, yang menekankan keperluan untuk strategi pemuliharaan sasaran, boleh digunakan sebagai contoh kepada strategi pengurusan bagi spesies sungai lain yang terancam oleh pemecahan habitat di Borneo.

Kata kunci: Pemecahan habitat, kepelbagaian genetic, pemodelan spatil, habitat ketersambungan, Limnonectes leporinus

TABLE OF CONTENTS

	Page
DECLARATION	i
ACKNOWLEDGEMENT	ii
ABSTRACT	iii
ABSTRAK	v
TABLE OF CONTENTS	vii
LIST OF TABLES	xi
LIST OF FIGURES	xii
LIST OF ABBREVIATIONS	xiii
CHAPTER 1 INTRODUCTION	1
1.1 Study Background	1
1.2 Problem Statement	3
1.3 Objectives	4
CHAPTER 2 LITERATURE REVIEW	5
2.1 Anuran of Borneo	5
2.1.1 Biogeography of <i>Limnonectes leporinus</i> (Andersson, 1923)	6
2.1.2 Previous Molecular Studies of <i>Limnonectes leporinus</i>	8
2.1.3 Habitat of <i>Limnonectes leporinus</i>	9

2.2	Borneo Island	10
2.2.1	The Impact of Ecosystem in Borneo on Frogs Genetic Diversity	10
2.3	Spatial Modelling	12
2.4	MaxEnt for Map Modelling	13
2.5	Anuran Habitat Management Strategies	15
CHAPTER 3 METHODOLOGY		17
3.1	Sampling Sites	17
3.2	Field Technique	19
3.2.1	Tissue Sample Collections	19
3.2.2	Microhabitat Data and Analysis	19
3.3	Molecular Technique	20
3.3.1	DNA Extraction	20
3.3.2	Polymerase Chain Reaction and Purification	21
3.3.3	Purification and Sequencing	23
3.3.4	Molecular Analysis	23
3.4	Mapping Modelling	25
3.4.1	Habitat Suitability Model	26
3.4.2	Habitat Connectivity Model	27
CHAPTER 4 GENETIC DIVERSITY OF <i>Limnonectes leporinus</i>		29
4.1	Overview	29

4.2	Data Analysis	30
4.3	Results	32
4.3.1	Nucleotide Variation and Polymorphism	32
4.3.2	Diversification of <i>Limnonectes leporinus</i>	33
4.3.3	Sequence Variation	35
4.3.4	Demographic History	40
4.3.5	Haplotypes Minimum Spanning Network (MSN)	47
4.4	Discussion	50
4.5	Conclusion	53
 CHAPTER 5 DETERMINING MOST PRIORITISED CONSERVATION		
	AREA FOR <i>Limnonectes Leporinus</i>	54
5.1	Overview	54
5.2	Model Mapping Process	55
5.2.1	Data Collection	55
5.2.2	Selection of Model Predictor Variables	56
5.2.3	Preparation of Data	60
5.2.4	Habitat Suitability Modelling	60
5.2.5	Model Validation	60
5.2.6	Habitat Connectivity Model	61
5.3	Results and Discussion	62

5.3.1	Model suitability test of <i>Limnonectes leporinus</i>	62
5.3.2	Determination of Suitable Habitat for <i>Limnonectes Leporinus</i>	63
5.3.3	Connectivity Model for Conservation Strategy	65
5.4	Discussion	70
5.5	Conclusion	72
CHAPTER 6 CONCLUSION AND RECOMMENDATIONS		73
6.1	Conclusion	73
6.2	Recommendations	74
REFERENCES		76
APPENDICES		97

LIST OF TABLES

	Page
Table 3.1 Summary of sample numbers (new or old) according to localities	17
Table 3.2 PCR master mix cocktail	21
Table 3.3 Gene primer sequences used in the PCR	22
Table 3.4 COI and Cytb PCR profiles	22
Table 3.5 Summary of Sources for predictor variables	26
Table 4.1 Sequence data of <i>Limnonectes leporinus</i> for Cytb gene	30
Table 4.2 DNA divergence among <i>L. leporinus</i> population	36
Table 4.3 Gene flow and genetic differentiation among <i>L. leporinus</i> population	38
Table 4.4 Haplotypes of <i>L. leporinus</i> identified from 12 different populations	40
Table 4.5 Measures of geographical population differentiation in <i>Limnonectes leporinus</i> based on an analysis of molecular variance (AMOVA)	41
Table 4.6 Genetic differentiation matrix of populations calculated by Φ_{ST}	43
Table 5.1 Data used for mapping modelling	57

LIST OF FIGURES

	Page
Figure 2.1 <i>Limnonectes leporinus</i> species	7
Figure 3.1 Map of <i>L. leporinus</i> samples localities	18
Figure 3.2 Visualisation of PCR products	23
Figure 4.1 Lineage diverfication of <i>Limnonectes leporinus</i> populations within Sarawak by using Cytb gene represented in ML/MP/NJ phylogenetic trees	34
Figure 4.2 Mismatch distribution for <i>L. leporinus</i> at each locality	46
Figure 4.3 Scatterplot diagram of the relationship between geographical distance (km) and percentage of net nucleotide divergence (Da) between populations of <i>Limnonectes leporinus</i> . Regression coefficient: $y = 0.000128$, correlation coefficient: $r = 0.660433$ and regression statistics: $Y = 0.436172$	47
Figure 4.4 Minimum spanning network of <i>Limnonectes leporinus</i> haplotypes from 12 localities	48
Figure 5.1 Jackknife test of predictors variables	62
Figure 5.2 ROC curve of model suitability	63
Figure 5.3 Map of suitable habitat for <i>Limnonectes leporinus</i> in Sarawak	68
Figure 5.4 Priority areas model for conservation	69

LIST OF ABBREVIATIONS

12S rRNA	12S ribosomal ribonucleic acid
16S rRNA	16S ribosomal ribonucleic acid
%	Percent
°C	Degree Celsius
&	And
±	Significant value range
>	More than
<	Less than
Φ_{st}	Fixation Index
π	Nucleotide diversity
.nxs	NEXUS file
μL	Microliter
A	Adenine
AES	Audio Encounter Survey
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
ArcGIS	Aeronautical Reconnaissance Coverage Geographic Information System
ASCII	American Standard Code for Information Interchange
AUC	Area under the curve
BIC	Bayesian information criterion
bp	Base pair
C	Cytosine

cm	Centimetre
CN	Central
COI	Cytochrome Oxidase Subunit I
COVID-19	Coronavirus disease 2019
Cytb	Cytochrome b
Da	Net nucleotide diversity
ddH ₂ O	Double-distilled water
DNA	Deoxyribonucleic acid
DNASP	DNA Sequence Polymorphism
dNTP	Deoxynucleotide Triphosphates
ES	Eastern
ESA	European Space Agency
et al.	et alia
G	Gamma
G	Guanine
GARP	Genetic Algorithm for Rule-Set Production
Hap	Haplotype
Hd	Haplotypes diversity
HKY	Hasegawa-Kishino-Yano
hr	Hour
HydroSHEDS	Hydrological data and maps based on Shuttle Elevation Derivatives at multiple scales
IUCN	International Union for Conservation of Nature
K2P	Kimura-2-parameter
km	Kilometre

<i>L. blythi</i>	<i>Limnonectes blythi</i>
<i>L. ibanorum</i>	<i>Limnonectes ibanorum</i>
<i>L. leporinus</i>	<i>Limnonectes leporinus</i>
<i>L. paramacrodon</i>	<i>Limnonectes paramacrodon</i>
MaxEnt	Maximum Entropy Modelling
MCO	Movement Control Order
MDF	Mixed Dipterocarp Forest
MEGA	Molecular Evolutionary Genetic Analysis
mg/L	Milligram per liter
MgCl	Magnesium chloride
min	Minute
ML	Maximum Likelihood
mm	Millimetre
MODIS	Moderate Resolution Imaging Spectroradiometer
MP	Maximum Parsimony
mtDNA	Mitochondrial DNA
N	North
NCR	Native customary rights
NJ	Neighbor-joining
NMDS	Non-multidimensional Scaling
NP	National Park
OSM	Open Street Map
p	Significant
P	Protected
PCA	Principal Component Analysis

PCR	Polymerase Chain Reaction
QGIS	Quantum Geographic Information System
r	Correlation coefficient
RCC	River Continuum Concept
ROC	Receiver Operating Characteristic
SCR	Spatial Capture Recapture
sec	Second
SEPMs	Spatially Explicit Population Models
SVL	Snout Vent Length
T	Thymine
TL	Tibia Length
UNIMAS	Universiti Malaysia Sarawak
UP	Unprotected
WC	Wildlife Centre
USGS	United States Geographical Survey
UV	Ultraviolet
V	Version
VES	Visual Encounter Survey
WS	Western
y	Regression coefficient
Y	Regression statistics

CHAPTER 1

INTRODUCTION

1.1 Study Background

Amphibians are a diverse group of vertebrates that include both anurans (frogs and toads) and urodeles (salamanders and newts) (Morona & González, 2008). Anurans, specifically, are a type of amphibian that undergo metamorphosis from aquatic tadpoles to terrestrial adults (Orton & Tyler, 2014). They have a unique pattern of forebrain organisation, limited cell migration, and lack of circumscribed nuclei (Morona & González, 2008). Anurans are also known for their reproductive and developmental characteristics, including the potential impact of hormone-modulating chemicals on their reproduction and the occurrence of intersex in wild populations (Orton & Tyler, 2014). In terms of skeletal development, anurans exhibit heterochrony in the sequence of ossification, with certain bones shifting early in the cranial and hind limb regions (Harrington et al., 2013).

Additionally, anurans display a variety of colouration patterns, which may be subject to sexual selection and female choice (Sheldon et al., 2003). The range size of anurans is influenced by factors such as life span, fecundity, and historical colonisation patterns (Trakimas et al., 2016). Anurans also exhibit associations with specific habitats, such as *Microhyla borneensis* in pitcher plant ecosystems particularly *Nepenthes ampullaria* (Matsui, 2011). In invaded amphibian communities, anurans like the Cuban treefrog can have significant impacts on native species through competition (Knight et al., 2008). Identification of anuran species can be challenging due to their high diversity, particularly in megadiverse regions like the Atlantic Forest (Dubeux et al., 2021). Conservation efforts

for anurans should consider habitat characteristics at different spatial scales to support their diversity and movements (Baffa-Trasci et al., 2020). Overall, anurans are an important group of amphibians with unique characteristics and ecological roles.

A hotspot region with a rich biodiversity region Malaysian Borneo is experiencing excessive landscape alteration that has led to habitat fragmentation (Zainudin et al., 2019). The species that is most endemic Bornean frog *Limnonectes leporinus* is a totally riparian species that lives on streams with moderate-to-sharp gradients (Inger et al., 2017). Clearcutting is a severe deforestation that leads to the fragmentation of its distribution and the most serious threats to the species (Taubert et al., 2018). Overhunting and smuggling are the biggest threats. The organisms need to keep heterozygosity in check to ensure that the population will have a strong fitness to adapt to the changing environment (Phillips et al., 2017).

Two characteristics were used to define the organism management strategy. Two of the characteristics are a vast molecular DNA database (extensive gene flow) and ecological information (microhabitat adaption) that define the condition of the organism in the fragmented habitat. In conjunction with a computerised map of the fragmented habitat, the region can be used to analyse the impact of the planned project on the biological requirements of living organisms that live there. It can be added as a layer of information to existing software (such as ArcGIS) that allows users to detect location and changes. These traits will reveal the organism's adaptation and position in the shattered environment, allowing conservation priorities to be assigned to the organisms. This map is particularly important in the subject of landscape conservation and management strategy for *L. leporinus*.

1.2 Problem Statement

Borneo is one of the most biodiverse regions in the world, with high levels of endemism (Davis et al., 2023). However, this biodiversity is threatened by habitat fragmentation due to excessive landscape modifications caused by human activities such as logging and agriculture (Tan et al., 2023). As a result, many species, including the riparian species *L. leporinus*, are facing significant population declines (Hertwig et al., 2012).

Maintaining genetic diversity and heterozygosity is crucial for the long-term survival of species in fragmented habitats (Allentoft & O'Brien, 2010). For example, a study on the population genetics of the Bornean river frog found that habitat fragmentation led to significant genetic differentiation and loss of genetic diversity (Zainudin & Naim, 2018). This loss of genetic diversity can decrease the fitness of populations, making them more vulnerable to environmental changes (Allentoft & O'Brien, 2010).

In addition to genetic factors, microhabitat adaptation is also crucial for the survival of stream-dwelling species like *L. leporinus* (Butefish et al., 2019). Studies have shown that changes in stream characteristics, such as water temperature and flow rate, can have significant impacts on the distribution and abundance of stream-dwelling species (Zainudin et al., 2019).

By contrast, Zainuddin (2004) found that *L. leporinus* was genetically distinct after examining short Cytochrome Oxidase Subunit I (COI) gene sequences from mitochondrial DNA (mtDNA). However, according to Matsui et al. (2015), the mtDNA phylogeny (ribosomal genes), which was determined using samples gathered from all areas of the species distribution and longer sequences of the 12S and 16S genes, shows that the species

is not significantly diversified within Borneo Island, which is consistent with the fact that it is not morphologically diversified either.

Given the importance of genetic diversity and microhabitat adaptation in fragmented habitats, it is essential to develop a comprehensive prioritisation framework model that takes into account these factors. The integration of molecular DNA and ecological data in mapping and monitoring species in fragmented habitats can help inform conservation strategies and prioritise conservation efforts (Nielsen et al., 2022).

1.3 Objectives

1. To determine gene flow and heterozygosity of *L. leporinus*
2. To develop a useful prioritisation framework model for *L. leporinus* conservation.

Hypothesis

1. To determine gene flow and heterozygosity of *L. leporinus*

H₀: There is no significant genetic diversity within and among populations of *L. leporinus*.

H₁: There is significant genetic diversity within and among populations of *L. leporinus*.

2. To develop a useful prioritisation framework model for *L. leporinus* conservation.

H₀: Map modelling does not show significant information for *L. leporinus* conservation effort.

H₁: Map modelling shows significant information for *L. leporinus* conservation effort.

CHAPTER 2

LITERATURE REVIEW

2.1 Anuran of Borneo

Amphibians are a group of vertebrates that includes frogs, toads, salamanders, and caecilians. They are characterised by their ability to live both in water and on land, undergoing metamorphosis from aquatic larvae to terrestrial adults (Rees, 2021). The classification and evolutionary history of anurans have been revised based on molecular techniques of phylogenetic analysis (Pyron & Wiens, 2011). Anurans, specifically, are a subgroup of amphibians that includes frogs and toads (Steffoff, 2008). They are known for their distinctive hopping locomotion and their ability to produce vocalizations for communication and mating purposes (Amram et al., 2018).

Approximately 187 species of frog were currently recorded in Borneo (Haas et al., 2023), however, this count may not accurately reflect the total exact frog species diversity in Borneo as frog species currently still increasing (Inger et al., 2017). This discrepancy is due to several factors, including the presence of cryptic species that have been divided into distinct species through molecular studies (Matsui et al., 2013), and the discoveries of new species described based on acoustic and morphological data (Dehling & Grafe, 2008).

Cryptic species, which are morphologically similar but genetically distinct (Monro & Mayo, 2022) has led to an increase in the number of recognized frog species in Borneo (McLeod, 2010). Molecular studies have provided evidence for the presence of multiple species within what was previously considered a single species (Hertwig et al., 2013). For example, by using both mitochondria and nuclear genes, Hamidy et al. (2012) split a frog

frogs traditionally identified as *Leptobrachium nigrops* into two which are *L. kanowitense* and ingeri *L. ingeri*.

Ongoing research and field surveys have led to the identification of previously unknown frog species, further highlighting the incomplete nature of the current Bornean diversity (Preininger et al., 2012). Adult frogs, even those capable of long movements, tend to have relatively small home ranges (Grafe., 2009). This limited movement results in localised populations that have not been thoroughly surveyed. For example, *Barbourula kalimantanensis*, a lungless, fully aquatic frog, is so hard to find, due to its behaviour (Blackburn et al., 2010). It likely spends much of its time hidden under boulders in remote streams in relatively inaccessible areas of Borneo, making it difficult for researchers to spot and study (Inger et al., 2017).

2.1.1 Biogeography of *Limnonectes leporinus* (Andersson, 1923)

Limnonectes leporinus (Andersson, 1923) locally called pagma frog is an endemic Bornean frog that had been confused with *L. blythii* (Boulenger) from Peninsular Malaysia (Inger, 2009) but has been delineated by 16S gene by Emerson et al. (2000). It has been found in all regions from near sea level to about 600 m ASL (Inger, 1966). It is a completely riparian (stream-dwelling) species and lives along streams with moderate to steep gradients. It is also a low disperser with net distances < 20 m and feeds on a variety of (Inger and Greenberg, 1966). The size of the frog is moderate to large (85-175 mm) and edible for local people (Inger, 2009).

This species can be morphologically identified by its large body with heavily muscled legs, edge of snout forming sharp angle, clearly visible eardrum, fully webbed toes, scattered ridges on the back, small irregular bumps on the upper eyelids, reddish-brown-

coloured body while white with grey motting underside, and black band between eye and nostril (Inger, 2009; Inger et al., 2017).

The frog has unique reproductive behaviour where the male builds nest at the side pool by excavating dead leaves, sits in the nest, and waits for the approach of a gravid female. As the gravid female enters the nest margin, she emits a soft call, and the male will mount the female and amplexus takes place. As eggs are released, the pair slowly moves in a circular fashion while kicking the sand and gravel into the nest and then leave at the end of oviposition (Emerson, 1992). This behaviour makes the species depends on dead leaves, sand, and gravel of stream beds which those are the characters of a typical forest stream. Any destruction on the stream habitat will surely affect the frog's breeding mode and lead to reduction in the progenies (Beebee, 2005). This will eventually affect population demographic of the species and lead to the variation of adaptation to environment.

Furthermore, deforestation by severe clearcutting also leads to fragmentation of the species distribution. Overhunting for local consumption is yet the greatest threat to the species (Zulkefli & Zainudin, 2022). However, according to the IUCN Red List (2022), *L. leporinus* has been listed as Least Concern (LC) since its last assessment in 2020, although its population trend has declined in the wild. It was also listed as 12th ranking in species prioritisation for conservation (IUCN Red List, 2022).



Figure 2.1: *Limnonectes leporinus* species

Morphologically, *L. leporinus* is uniform and displays no geographic variation in any morphological characters previously analysed (Inger 1966). In contrast, Zainudin (2004) reported *L. leporinus* to be genetically divergent by analysing short sequences of Cytochrome Oxidase Subunit I (COI) gene of mitochondrial DNA. Matsui et al. (2015) revealed that the mtDNA phylogeny for (ribosomal genes) using samples collected from throughout the species distribution and longer sequences of 12S and 16S genes, indicates the species not markedly diversified within the island, conforming to the fact that it is also not diversified morphologically.

2.1.1 Previous Molecular Studies of *Limnonectes leporinus*

Molecular research on *Limnonectes leporinus* has greatly enhanced our understanding of its genetic diversity and phylogenetic connections. Inger's studies reveal that *L. leporinus* was previously misidentified as *L. blythii*, underscoring the crucial role of molecular phylogenetics in defining species boundaries within this genus (Inger, 2009). Furthermore, findings indicate that *L. leporinus* coexists with other species of *Limnonectes*, such as *L. ibanorum*, suggesting that ecological interactions may play a role in shaping genetic diversity (Zainudin et al., 2017).

Matsui et al. (2015) study on *L. leporinus*., offers valuable insights into the genetic structure and evolutionary background of *L. leporinus*. Utilizing mitochondrial DNA analysis, the study reveals that, contrary to previous beliefs regarding its high genetic diversity, *L. leporinus* actually shows low levels of genetic variation throughout its Bornean distribution. This indicates a recent common ancestry and limited gene flow between populations, likely due to geographical barriers (Matsui et al., 2015). The research also underscores the significance of these genetic patterns for conservation efforts, as low genetic

diversity can heighten vulnerability to environmental changes and diminish adaptive potential (Reilly et al., 2019).

However, Zainudin's (2004) study found that *L. leporinus* displays notable genetic diversity, particularly in coding genes. This is vital because it suggests that the species has a strong genetic foundation, which may help it adapt to environmental shifts and habitat fragmentation (Zainudin et al., 2019). The study stresses that maintaining genetic diversity is critical for the long-term survival of populations, especially in fragmented landscapes like oil palm plantations, where gene flow is frequently limited (Zainudin et al., 2019). Additionally, the observed genetic diversity in *L. leporinus* is consistent with findings from other studies emphasizing the importance of genetic variation in amphibians for resilience against ecological pressures (Hu et al., 2019).

2.1.2 Habitat of *Limnonectes leporinus*

L. leporinus occurs throughout Borneo, in both primary and partly closed secondary-growth forests, at elevations below 750 meters above sea level (Inger et al., 2017). By using radio telemetry, Grafe (2009) concluded this species is strictly riparian, which individual frogs spent most of their time in restricted core activity areas not more than 20 m from the riverbed and migrated along the stream over long distances in search of breeding opportunities. The streams on which it occurs may have bottoms consisting of sand, gravel, and rock mixtures. Within their preferred habitat, they can occur in a range of environments, including quiet portions of streams, shallow side pools, and areas with drifts of dead leaves (Inger, 2009). It has been observed that *L. leporinus* larvae disperse into the forest at metamorphosis and return to streams as adults to breed (Inger & Voris, 2008). Males of *L. leporinus* aggressively defend nest sites along riverbanks (Rico-Guevara & Hurme, 2019). The frogs are commonly found on bare soil or leaf litter at the stream bank. Due to *L.*

leporinus is often hunted by humans for its meat, it is rarely seen along streams near villages (Inger et al., 2017).

2.2 Borneo Island

The formation of Sundaland and Borneo is a complex process that involves tectonic movements and geological processes (Hall, 2001). Sundaland is a large landmass that includes Borneo, Java, and Sumatra, and it has undergone significant changes over time. The arrival of Borneo to Sundaland is still uncertain, but it is believed to have occurred in the early Cretaceous (Hall, 2012). The shift from shallow marine carbonate sedimentation to volcanoclastic sediments in Borneo during this time may mark the arrival of the island (Breitfeld et al., 2017). Tectonic forces play a role in the formation of Sundaland and Borneo. The region experiences escape tectonics, with a significant south-directed push into Sundaland. This results in compression across NW Borneo and the development of strike-slip faults (Morley et al., 2010).

The relationship between Sundaland and biodiversity in Borneo, particularly frogs, is influenced by various factors. Both dispersal and in situ diversification have contributed to species accumulation in Sundaland, but within-island phylogenesis has resulted in a higher proportion of endemic frog species in Sumatra and Borneo (O'Connell et al., 2018). The size of an island plays a significant role in determining species richness and endemism (Roos et al., 2004). Additionally, geological factors, such as the Crocker Range, have acted as biogeographic barriers in Borneo, shaping the distribution of species (Dixit et al., 2023).

2.2.1 The Impact of Ecosystem in Borneo on Frogs' Genetic Diversity

Frog genetic diversity in Borneo is influenced by several ecosystem-related factors. *Meristogenys whiteheadi*, a common frog species found in Borneo's mountainous regions,

was the subject of one study. Within this species, the study discovered three allopatric lineages, indicating genetic differentiation Shimada et al. (2011). As a result, it is possible that geographic isolation and barriers have influenced the genetic diversity of frogs in Sarawak.

Environmental heterogeneity of an area can also influence genetic distance among frog populations. Characteristics of landscape heterogeneity, such as regional distributions of slope, rivers and streams, and habitat corridors, can impact genetic distance among frog populations (Lawson, 2013). A study by Keller et al. (2009) found that the pronounced heterogeneity of the differing stream environments led to differing habitat-specific adaptations. The study concludes that riparian habitats in mountainous pristine rainforests may be generally more heterogeneous than the leaf litter and canopy habitats.

Human activities, such as the construction of roads, can also have an impact on the genetic diversity of frog populations. Roads can act as barriers to gene flow, leading to reduced genetic diversity in isolated populations (Preininger et al., 2012). This highlights the need for conservation efforts to consider the potential genetic impacts of infrastructure development on frog populations.

Forest fragmentation in Borneo has been a significant issue over the past few decades. Between 1973 and 2015, approximately 18.7 million hectares of Borneo's old-growth forest were cleared, with 14.4 million hectares in Indonesian Borneo and 4.2 million hectares in Malaysian Borneo (Gaveau et al., 2016). This deforestation was primarily driven by the expansion of industrial plantations, particularly oil palm and pulpwood plantations, which expanded by 9.1 million hectares during the same period (Gaveau et al., 2019). Several studies have investigated the effects of habitat fragmentation on frog populations

and have found that it leads to reduced genetic variation and increased inbreeding (Preininger et al., 2012).

Microhabitats often play an important role as resources that are partitioned between phylogenetically related or ecologically similar species. According to a study by Zainudin et al. (2017), none of the frog species had microhabitats that were 100% similar to one another. This suggests that if resources (microhabitats) become scarce, no species would be excluded, allowing the coexistence of the species in specific habitats and microhabitats. Findings from the study showed that *Amnirana nicobariensis* falls into the *Hylarana erythraea* group, which are species that are associated with humans.

2.3 Spatial Modelling

Spatial modelling plays a crucial role in understanding animal populations and their dynamics. One application of spatial modelling in animal population research is the use of spatial capture-recapture (SCR) methods. SCR models utilize spatial encounter information to estimate population density and explore spatial aspects of animal population structure (Royle et al., 2017). These models integrate ecological hypotheses about landscape connectivity, movement, resource selection, and spatial variation in density with individual encounter history data obtained through technologies like camera trapping and non-invasive DNA sampling. Spatial modelling also helps in studying the relationship between animal population density and area. Contrary to the equilibrium theory of island biogeography, meta-analyses have shown that animal population densities are positively correlated with area, indicating that density compensation may be uncommon (Connor et al., 2000).

Additionally, spatially explicit patch-based modelling approaches have been developed to study landscape patterns and process dynamics in animal populations. These models incorporate spatially explicit, age- and size-structured patch demographic models

and multiple-species plant population dynamic models to examine the spatiotemporal dynamics of disturbance patches and populations (Wu & Levin, 1994). Spatially explicit population models (SEPMs) are used to understand the processes influencing the number and distribution of animals in space and time. SEPMs aid in forecasting the effects of management or human activities on population abundance and distribution, facilitating resource management goals (Conroy et al., 1995). Spatial genetic structure in animal populations can also be investigated using spatial autocorrelation analysis. Although more commonly used in plant studies, spatial autocorrelation analysis has been employed to understand spatial genetic structure in animal populations (Temple et al., 2006).

Johnson et al. (2011) investigated the regional decline of leopard frogs in Colorado, US, and found that the proportion of occupied wetlands varied across regions, with urban development and the presence of non-native bullfrogs being associated with lower occupancy. Scherer et al. (2012) examined the occupancy of wetlands by wood frogs and boreal chorus frogs and found that local-scale attributes of individual wetlands had little correlation with occupancy. They suggested that processes operating at broader spatial scales, such as migration and dispersal, were more important in influencing amphibian occupancy patterns.

2.4 MaxEnt for Map Modelling

The MaxEnt (Maximum Entropy) modelling approach has gained significant recognition as a valuable tool for evaluating habitat suitability in various ecological research. It operates on the principle of maximum entropy, which aims to create probability distributions that effectively represent available information while making minimal assumptions. This method is particularly advantageous for modelling species distributions using presence-only data, enabling researchers to estimate habitat suitability without needing

absence data (Naqibzadeh et al., 2021; Freeman et al., 2013). MaxEnt produces a continuous suitability index ranging from 0 (unsuitable) to 1 (highly suitable), which can be interpreted to guide conservation and management strategies (Naqibzadeh et al., 2021; Cimino et al., 2012). One of the model's notable strengths is its capacity to work with incomplete datasets, allowing it to compensate for small sample sizes and still provide reliable predictions (Hernández et al., 2006; Flory et al., 2012).

MaxEnt has been applied across diverse taxa and ecosystems, showcasing its versatility. For instance, researchers have used MaxEnt to predict habitat suitability for endangered species, such as the Kashmir markhor (*Capra falconeri cashmeriensis*) and the lowland tapir (*Tapirus terrestris*) (Khattak et al., 2022; Cordeiro et al., 2016). In these instances, MaxEnt effectively pinpointed areas with environmental conditions that mirror known species occurrences, thus facilitating targeted conservation initiatives.

Furthermore, MaxEnt has been instrumental in evaluating the effects of climate change on habitat suitability. For example, projections concerning *Myristica dactyloides* in India's Eastern Ghats indicated significant reductions in suitable habitat under future climate scenarios (Remya et al., 2015). Similarly, research on Tossa jute (*Corchorus olitorius*) classified habitat suitability into distinct categories based on land cover types, enhancing the understanding of how various environmental factors affect species distribution (Mollah et al., 2021).

The effectiveness of MaxEnt compared to other modelling techniques has garnered interest in ecological research. Studies indicate that MaxEnt often surpasses other methods, such as GARP (Genetic Algorithm for Rule-Set Production), in terms of accuracy and reliability (Zhang et al., 2020). For instance, MaxEnt's predictions tend to be more precise, with fewer errors of commission, which is crucial for effective habitat management

(Radosavljevic & Anderson, 2014). Additionally, the model's adaptability in incorporating a variety of environmental variables allows for a more comprehensive understanding of species-environment relationships (Gunawan et al., 2021).

Despite its strengths, MaxEnt does have some limitations. The model's effectiveness is heavily dependent on the quality of input data, meaning that inaccuracies in species occurrence records or environmental variables can yield misleading results (Cordeiro et al., 2016). Moreover, while MaxEnt is proficient at handling small datasets, researchers must be wary of overfitting, particularly when using complex environmental predictors (Flory et al., 2012). The selection of background points and relevant environmental variables is essential for optimizing the model's performance (Renner & Warton, 2013).

2.5 Anuran Habitat Management Strategies

To effectively manage frog habitats, several strategies can be implemented. One approach is to establish buffer zones around wetlands and riparian habitats, which provide core terrestrial habitat for amphibians and reptiles (Semlitsch & Bodie, 2003). In urban areas, it is important to maintain frog and reptile habitats in residential areas by protecting ecologically viable remnants and creating habitat corridors (Hamer & McDonnell, 2009). Involving local communities in habitat improvement work and education programs can also contribute to frog conservation efforts (Lin et al., 2008). Conspecific attraction, a management tool used in avian conservation, may also be valuable for managing and conserving frog populations (Buxton et al., 2018).

Additionally, understanding the core habitat requirements of frogs, such as the distance of upland burrows from breeding wetlands, can inform habitat protection measures (Heemeyer et al., 2012). It is crucial to consider potential Allee effects and the impact of changes in aquatic breeding habitat availability on frog populations (Gaston et al., 2010).

Conservation strategies should also focus on protecting non-breeding habitats in modified farming areas and preserving landscape elements like plantings that facilitate frog movement and persistence (Hansen et al., 2018). Finally, the distribution mapping of specialised amphibian species can help identify areas for habitat protection and restoration (Filer et al., 2019).

CHAPTER 3

METHODOLOGY

3.1 Sampling Sites

There are a total of 62 *L. leporinus* samples were used in this study, consisting of 12 localities representing western (WS), central (CN), and eastern (ES) parts of Sarawak with a balance between protected (P) and unprotected (UP) areas. However, most of the samples were not collected recently. There are only 7 newly collected samples that were collected between August 2019 and October 2021 at Gunung Gading NP, Kubah NP, Bau, and Kanowit, meanwhile, most of the samples were gathered since 1999. Summary of sample numbers (new or old) according to localities are shown in Table 3.1 and pinpoint locations are shown in Figure 3.1.

Table 3.1: Summary of sample numbers (new or old) according to localities

Localities	Region	Status	Coordinate	Sample	
				New (2019-2021)	Old (1999-2018)
Gunung Gading NP	WS	P	1°41'26.1"N 109°50'44.7"E	1	6
Matang WC	WS	P	1°36'33.3"N 110°09'36.5"E	0	4
Kubah NP	WS	P	1°36'46.1"N 110°11'48.6"E	2	8
Bau	WS	UP	1°24'51.6"N 110°08'13.9"E	2	3
Padawan	WS	UP	1°08'36.9"N 110°13'41.5"E	0	6
Sri Aman	WS	UP	1°08'33.1"N 111°39'49.1"E	0	6
Batang Ai NP	CN	P	1°13'21.6"N 111°56'51.3"E	0	3
Kanowit	CN	UP	2°26'56.8"N 112°05'07.5"E	3	0
Kapit	CN	UP	2°00'36.1"N 112°53'59.9"E	0	3
Bintulu WILMAR	ES	UP	3°32'13.5"N 113°44'07.4"E	0	4
Mulu NP	ES	P	4°03'28.6"N 114°50'31.6"E	0	8
Bario	ES	UP	3°44'11.2"N 115°30'36.0"E	0	3
Total				8	54
				62	

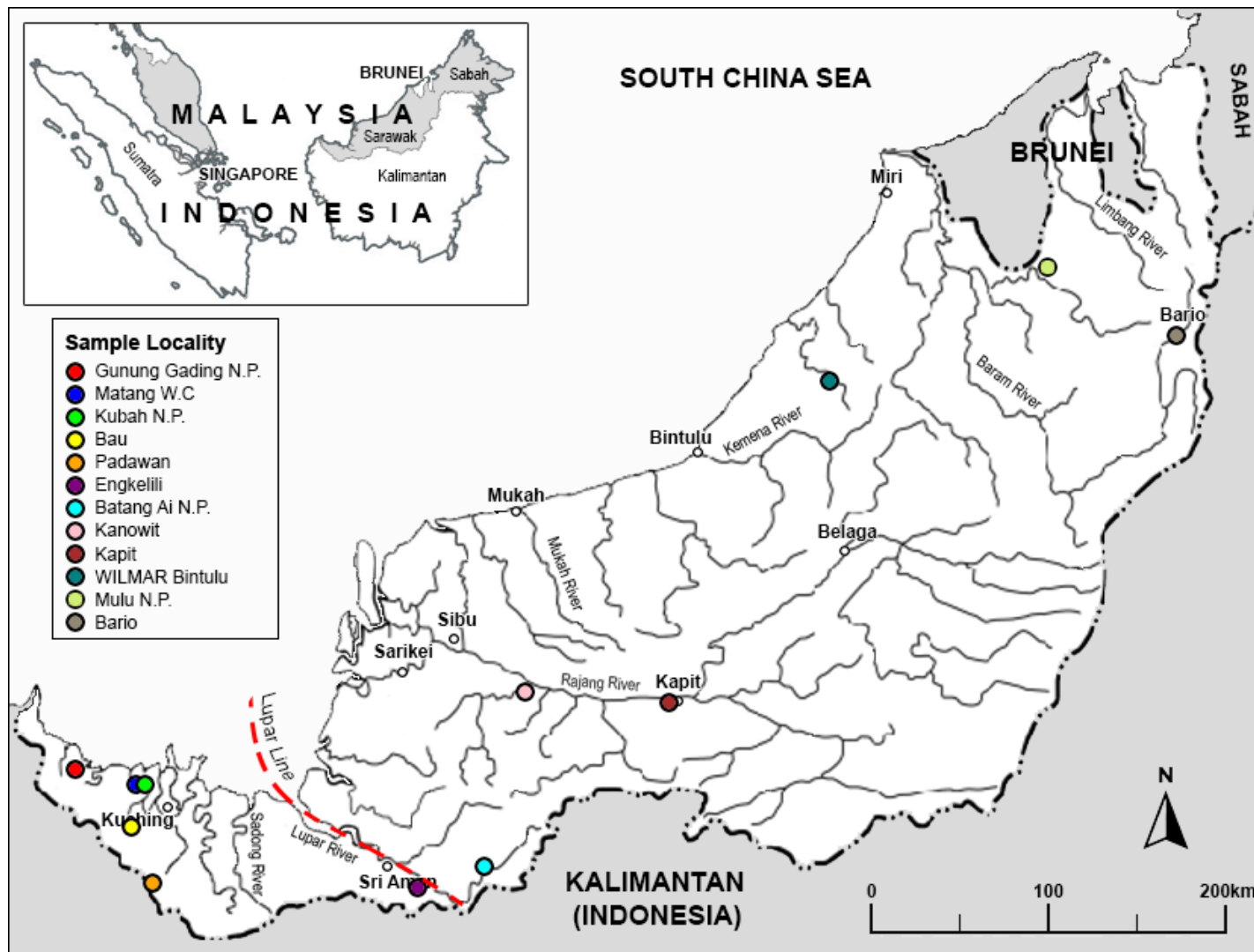


Figure 3.1: Map of *L. leporinus* samples localities

3.2 Field Technique

3.2.1 Tissue Sample Collections

To mitigate any potential biases, this study adopted a uniform sampling method across all sites. The Visual Encounter Survey (VES) technique was employed, which involved researchers slowly traversing forest and river transects with the assistance of headlamps (Pui & Das, 2016; Zainudin et al., 2017, 2019a, 2019b).

Sampling was conducted between 1900 and 2200 hours, aligning with the frogs' peak activity period. Each location, as permitted by the research license, allowed for the collection of three individuals. All samples were transported back to the worktable while still alive to maintain their integrity. Immediate processing of these samples was carried out to ensure quality preservation. Careful handling was practiced to prevent any potential losses that could impact the data collection's quantity and quality.

It's important to note that certain frog species can emit a toxic fluid as a self-defense mechanism when captured (Rahman et al., 2008). Therefore, it's not recommended to house different frog species in the same container. For instance, the *Odorrana hosii* species can release harmful fluids under stress, which could endanger other frogs and irritate human skin (Inger et al., 2017; Zulkefli & Zainudin, 2022). Frog species were identified using the field guides provided by Inger et al. (2017).

3.2.2 Microhabitat Data and Analysis

In line with the methods detailed by Zainudin et al. (2017) and guided by the microhabitat codes from Inger Habitat Code (Inger, 1994) (refer to Appendix 1), several crucial data points were recorded for each individual captured. These included the date and

time (in a 24-hour format), the type of vegetation or forest, the horizontal and vertical positions, and the substrate.

To identify the most meaningful variables influencing the microhabitats of *L. leporinus*, a principal component analysis (PCA) was conducted using R (V.4.4.1) (R Core Team, 2021). First, all the microhabitat data were organised in a matrix format where rows represent samples and columns represent variables (vegetation, horizontal, vertical, and substrates) (Santos et al., 2018). Before PCA was performed, all missing and irrelevant data points were excluded, and variable values were standardised. Following PCA, non-metric multidimensional scaling (NMDS) was used to assess microhabitat utilization, focusing on the most favourable characteristics for the species as described by Zainudin et al. (2017, 2019b) and Zulkefli and Zainudin (2022). The highest-loading microhabitats from the analysis were selected and used together with haplotype data to create a raster data map for habitat connectivity map model using Circuitscape (V 4.0.5).

3.3 Molecular Technique

3.3.1 DNA Extraction

DNeasy Blood and Tissue Kit by QIAGEN were used to extract all the skin, toes, muscle, and liver tissues from *L. leporinus* (Nguyen et al., 2018; Roh et al., 2022). The samples were properly collected and labelled with the field number and locations. The dates of extraction were also noted. Under ultraviolet (UV) light, the extracted DNA was viewed to confirm the presence of the DNA band. Successful DNA extractions were proceeded to PCR for DNA amplification. The unsuccessful DNA extraction visualised again after a day and extracted again when no DNA band appeared.

3.3.2 Polymerase Chain Reaction and Purification

The double-stranded PCR amplification was performed using the master mix cocktails outlined in Table 3.2. The targeted site regions of mitochondrial genes were Cytochrome Oxidase Subunit I (COI) and Cytochrome b (Cytb). Primers used in this study were determined by referring to Palumbi et al. (1991), Zainudin et al. (2010), and Kurniawan et al. (2010). Table 3.3 shows the primer names with their sequences where these primers are available and can be purchased from Apical.

Table 3.4 shows the PCR profiles for each gene. The optimum annealing temperature for both PCR profiles was pre-determined by using the Gradient PCR method (Crews et al., 2008). The entire PCR process of COI gene takes 1 hrs and 30 min, while Cytb gene takes 2 hrs and 50 min approximately to finish.

To verify the presence of a PCR band, the PCR products were examined under UV light, as illustrated in Figure 3.2. If a PCR attempt failed, the process was repeated until a successful result was achieved.

Table 3.2: PCR master mix cocktail

Reagents	1x reaction (µL)
Buffer	5.0
dNTP mix	0.5
MgCl	1.5
Primer (forward)	1.0
Primer (reverse)	1.0
ddH ₂ O	13.8
Taq Polymerase	0.2
DNA template	2.0
Total	25

Table 3.3: Gene primer sequences used for PCR

Gene	Primer	Sequence 5` - 3`
Cytochrome Oxidase Subunit I (Palumbi et al., 1991; Zainudin et al., 2010)	Forward (COI_f)	CCT GCC GGA GGA GGT GAY CC
	Reverse (COI_e)	CCA GTA AAT AAC GGG AAT CAG TG
Cytochrome b (Kurniawan et al., 2010)	Forward (Fow 1-1)	ACM GGH YTM TTY YTR GCA TRC AYT A
	Reverse (Rev-1)	TAD GCR AAW AGR AAR TAY CAY TCN GG

Table 3.4: COI and Cytb PCR profiles

Gene	Steps	Temperature (°C)	Time
Cytochrome Oxidase Subunit I	Pre-denaturation	95	7 mins
	Denaturation	95	30 secs
	Annealing	52	30 secs
	Extension	72	1:30 mins
	Final extension	72	7 mins
	Soak	4	∞
Cycle: 30			
Cytochrome b	Pre-denaturation	95	5 mins
	Denaturation	95	1:30 mins
	Annealing	50	1 min
	Extension	72	1:30 mins
	Final extension	72	7 mins
	Soak	4	∞
Cycle: 40			

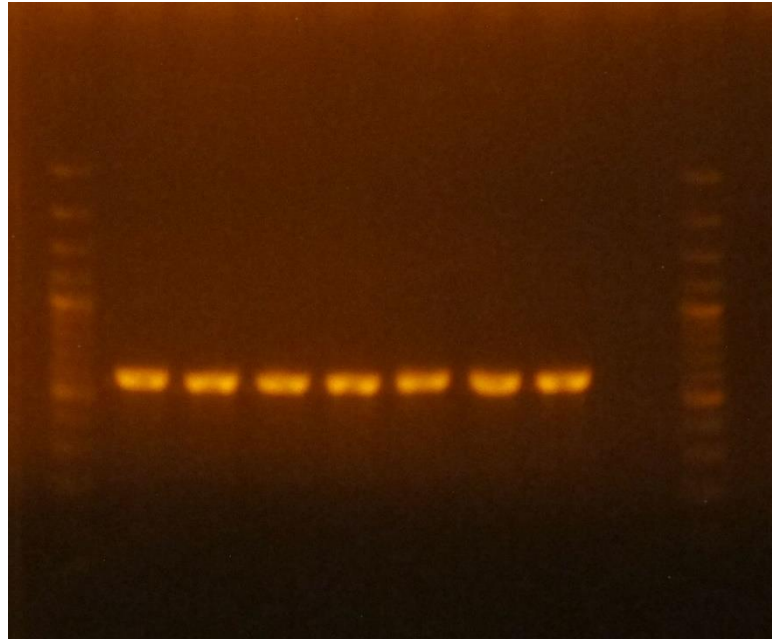


Figure 3.2: Visualisation of PCR products

3.3.3 Purification and Sequencing

PCR products that were successfully amplified were purified using QIAgen's QIAquick PCR Purification kit. The purification process was carried out by following the protocol provided in the kit. Once purified, the PCR products were dispatched to Apical, located in Selangor, Malaysia, and Celemics Inc., based in Seoul, Korea, for sequencing.

3.3.4 Molecular Analysis

The sequence bases for both COI and Cytb were manually inspected using CHROMAS software (V 2.6.6, Technelysium Pty Ltd.), as per the studies by Vedernikov et al. (2020) and Narayanan et al. (2022). The sequences were aligned using CLUSTAL X (V.2.1), following the guidelines of Haas (2004), Larkin et al. (2007), and Matsui et al. (2007). After that, stop codons were removed and sequences were realigned to ensure the accuracy of the resulting data (Kalstrup & Blunck, 2015). The output from the sequence alignment was saved in NEXUS (.nxs) format for subsequent procedures. A total of 537 base

pairs (bp) of Cytb gene, while COI with only 205 bp were obtained from the realignment. Thus, Cytb gene was chosen for molecular analysis, as it has a longer base pair length which means exhibits greater genetic variability (Zainudin et al., 2010; Khan et. all., 2010)

The Cytb .nxs files were used in MEGA X to create phylogenetic trees, following the method used by Kumar et al. (2018) and Marcaida et al. (2022). The software was set to construct Neighbor-Joining (NJ), Maximum Likelihood (ML), and Maximum Parsimony (MP) trees showing the population structure of *L. leporinus*. Additionally, sequence alignment statistics, pairwise distances, and the optimal DNA model were calculated.

DNASP (V. 6) was utilized to construct haplotypes. Gene flow, genetic differentiation, and DNA divergence tests were executed to gain population subdivision levels (F_{st}), nucleotide subdivision (N_{st}), and the number of migrants per generation (N_m) among *L. leporinus* population, following Rozas et al. (2017) and Jablonski et al. (2021) methodology. The haplotypes data gained were used alongside microhabitat data to create a raster data map for habitat connectivity map model using Circuitscape (V 4.0.5).

Haplotypes data were also used in Arlequin (V 3.5) to perform AMOVA, mismatch distribution, and Mental test. Mismatch distribution analysis was done to assess population expansion events (Excoffier & Lischer, 2010; Muir et al., 2013). These models apply a generalized least-squares method to estimate population expansion parameters and bootstrap their confidence intervals, as recommended by Dufresnes et al. (2013).

AMOVA test was carried out to evaluate population genetic differentiation among and within population of *L. leporinus* by referencing Excoffier and Lischer (2010), Zainudin et al. (2010), and Zainudin and Naim (2018) studies. Additionally, to support the AMOVA

test result, Mantel test was performed to examine the correlation between genetic divergence and geographical isolation with 1000 permutations, following Zhang et al. (2014).

A Minimum Spanning Network (MSN) was generated to visually depict the relationships among Cytb gene haplotypes using Network (V. 10.2.0.0, Fluxus-Engineering Ltd.) (Zainudin & Naim, 2018; Forster & Forster, 2022). The median-joining (MJ) algorithm was applied to assess geographical variations across 12 localities and to estimate haplotype richness, accounting for uneven sample sizes, as described by Nicolas et al. (2015).

3.4 Mapping Modelling

The entire modelling development process followed the methodologies of Zainudin et al. (2024), Gunawan et al. (2021), Remya et al. (2015), and McRae et al. (2008). The first step in the modelling process involves collecting georeferenced occurrence data for *L. leporinus*. The occurrence data for *L. leporinus*, which were obtained from both new and previous field samplings were recorded in an Excel file saved in .csv format. The geographical coordinates of the species were essential for modelling habitat suitability and habitat connectivity.

Predictor variables selected represent the environmental factors that influence the species' habitat for *L. leporinus*. The selection of these variables was guided by the species' known ecological needs and potential threats. *L. leporinus* is a highly stream-dependent species and relies on the stream network for migration. The increasing threats from habitat fragmentation, forest degradation, and human activity further underscore the importance of these variables. Therefore, seven predictor variables were chosen and obtained from various sources as shown in Table 3.4. Jackknife test was done to determine relative importance of various environmental variables. The predictor variables data obtained were used for habitat suitability model.

Before inputting this data into MaxEnt (V 3.4.3), all relevant predictor variables were converted into raster datasets. This conversion ensured that all layers shared the same extent and cell size, facilitating accurate analysis. To enhance compatibility with MaxEnt, the raster datasets were converted to ASCII format using ArcGIS (V 10.3). The choice of ASCII format was crucial as it is a supported format for MaxEnt (V 3.4.3), allowing for seamless integration of environmental data into the modelling process.

The highest-loading microhabitats from PCA and NMDS analysis were selected and used together with haplotype data from molecular analysis to create a raster data map for habitat connectivity model.

Table 3.5: Summary of Sources for Predictor variables

Predictor Variable	Data Source
Elevation	Google Earth Engine, USGS Earth Explorer, ArcGIS, QGIS
Intact forest	Global Forest Watch, Google Earth Engine
Land cover	MODIS, ESA Land Cover (Google Earth Engine, ArcGIS)
Major basin	HydroSHEDS (USGS Earth Explorer), OSM
Human population density	WorldPop (ArcGIS, Google Earth Engine)
Slope	DEM (ArcGIS), QGIS, Google Earth Engine
Annual temperature	WorldClim, Bioclim (Google Earth Engine, ArcGIS)

3.4.1 Habitat Suitability Model

Once the occurrence points and environmental predictor variables were prepared, the next step was to construct the model using MaxEnt (V 3.4.3) software. MaxEnt uses the principle of maximum entropy to predict the species' distribution based on known

occurrence points and environmental variables. Key model parameters, including the regularization multiplier and feature classes, were adjusted to optimize the model's performance. The regularization multiplier helps prevent overfitting, while feature classes control the complexity of the model. The model was then run to estimate the habitat suitability for *L. leporinus*, producing a probability map of habitat suitability based on the selected variables.

After constructing the model, it was essential to validate its performance using appropriate evaluation techniques. Cross-validation methods were applied to split the occurrence data into training and testing sets. This process ensures that the model is not overfitted to the training data and can generalize well to new data (Wettasin et al., 2023). The Area Under the Curve (AUC) of the Receiver Operating Characteristic (ROC) curve was used as a performance metric. An AUC value close to 1 indicates a model with high predictive accuracy, while a value closer to 0.5 suggests random prediction. The results from MaxEnt were interpreted on a scale of 0 to 1, where a score of 0 indicates areas unsuitable for *L. leporinus* habitat, while a score of 1 represents the most suitable habitats.

3.4.2 Habitat Connectivity Model

The output from MaxEnt, in ASCII format, was subsequently used in Circuitscape to generate a resistance map. This resistance map was instrumental in predicting habitat connectivity for *L. leporinus* in Sarawak. According to McRae et al. (2008), employing this map as a resistance surface is reliable, as it is based on the species' probability of movement through the landscape, aligning with its ecological requirements.

To further analyse connectivity, the genetic attributes of haplotype data gained from DNSAP (V 6) and the highest loading microhabitat data gained through PCA and NMDS analysis were prepared. The haplotype data were organised in an Excel file, where the

column for species names was labeled as NodeID, representing the different haplotypes. Two additional columns were created, labeled N and E, representing latitude and longitude in decimal degrees (dd) format, respectively. Individuals sharing the same haplotype were assigned the same NodeID, allowing for straightforward identification of genetic relationships.

Once the Excel file was prepared, it was saved in .csv format and then converted to ASCII format, which served as the focal point for Circuitscape analysis. Circuitscape operates on the principles of circuit theory, simulating random movement or electrical current across a resistance surface. This approach allows for modelling connectivity based on the ecological needs and behaviours of species, particularly in fragmented landscapes.

CHAPTER 4

GENETIC DIVERSITY OF *Limnonectes leporinus*

4.1 Overview

In recent times, the widespread sequencing and availability of mitochondrial DNA (mtDNA) have spurred progress in taxonomic research areas such as integrative taxonomy, barcoding, bioprospecting, phylogenetics, phylogeography, population and conservation genetics, biogeography, macroecology, and paleoecology (Matthijs et al., 2020). A previous study by Zainudin et al. (2010) employed the COI marker to investigate the genetic structure of *Hylarana erythraea* from Peninsula Malaysia and Sarawak. Furthermore, the use of COI barcodes for specimen identification and species discovery has been demonstrated to be an effective molecular approach in anuran studies (Koroiva et al., 2020). Similarly, the use of Cytb gene sequences has proven beneficial in resolving relationships between closely related taxa and is frequently used in phylogenetic or phylogeographic studies (Kurniawan et al., 2010).

Adaptation and natural selection serve as examples of evolutionary forces that may occur within a species' community in a specific area (Eterovick et al., 2010). Changes in the environment, whether natural or anthropogenic, can lead to alterations in genetic diversity, providing insights into the effects of such changes. Given that dispersal is a crucial aspect of amphibians' life history, habitat fragmentation and destruction pose significant threats to their survival.

This chapter aims to elucidate the phylogenetic relationships and genetic structure of *L. leporinus*, a species of significant ecological and conservation importance due to its endemic nature and vulnerability to habitat fragmentation. The core objective of this chapter

is to determine gene flow and heterozygosity across various populations of *L. leporinus* within Sarawak. By analysing mitochondrial DNA (Cytb gene) data, this chapter seeks to provide critical insights into the species' genetic diversity, which is a vital component of its adaptability and resilience in fragmented habitats.

Understanding gene flow and heterozygosity of *L. leporinus* is essential because of the growing concern over habitat fragmentation and its impact on species survival. Fragmentation isolates populations, potentially reducing gene flow, can lead to inbreeding, loss of genetic diversity, and, eventually, population decline. *L. leporinus* is a completely stream-dwelling species confined to specific habitats, which means maintaining genetic diversity is crucial for its long-term fitness and adaptability to changing environmental conditions. Haplotypes data of *L. leporinus* is also needed to make an informative connectivity model map which can help in the understanding of which part or areas in Sarawak need concern and conservation efforts.

4.2 Data Analysis

A total of 87 sequences of *L. leporinus*, Cytb genes, were manually inspected using CHROMAS (V. 2.6.6, Technelysium Pty Ltd.) as per the studies by Vedernikov et al. (2020) and Narayanan et al. (2022). These sequences were aligned, analysed, and phylogenetic trees were computed using MEGA X (Kumar et al., 2018; Marcaida et al., 2022). Models with the lowest BIC scores, based on the Bayesian Information Criterion (BIC), are considered to best describe the substitution pattern (Nei & Kumar, 2000).

In line with the model, two different models were obtained for the maximum likelihood tree for both genes. The best model for Cytb was the HKY: Hasegawa-Kishino-

Yanowith Gamma treatment (HKY + G), which models the non-uniformity of evolutionary rates among sites using a discrete Gamma distribution (+G) with 5 rate categories. On the other hand, all ML, MP, and NJ trees were constructed using the bootstrap method with 1000 bootstrap value.

Haplotypes and genetic differentiation of *L. leporinus* were determined using Cytb gene through population genetics analyses using DNASP (V. 6) (Rozas et al., 2017; Jablonski et al., 2021). The output produced by DNASP were haplotypes, level of population subdivision (F_{st}), nucleotide subdivision (N_{st}), and number of migrants per generation (N_m). Arlequin (V 3.5) (Excoffier & Lischer, 2010; Muir et al., 2013) used the haplotype information to compute mismatch distribution, AMOVA, and mantel test (Mantel & Valand, 1970).

Finally, the Median-joining approach (Bandelt et al., 1999) was used to compute the minimum network spanning (MSN) in Network (V. 10.2.0.0, Fluxus-Engineering Ltd.) (Forster & Forster, 2022). The MSN was used to calculate the geographical variations of *L. leporinus* across 12 localities and to estimate haplotype richness while controlling for irregular sample sizes (Nicolas et al., 2015).

4.3 Results

4.3.1 Nucleotide Variation and Polymorphism

A total of 537 base pairs (bp) of Cytb gene comprising 62 sequences of *L. leporinus* including one outgroup were successfully sequenced and completely aligned (Table 4.1). Out of 536 bp, 82 bp were variable sites (6.54%) comprising 64 potentially parsimony informative, leaving 18 singleton sites suggesting Cytb gene is a highly polymorphic and reliable marker. The average nucleotide composition consists of Thymine (T) 31.8%, Cytosine (C) 32.6%, Adenine (A) 24.3% and Guanine (G) 11.3% indicating a classic vertebrae mitochondrial DNA characteristic with anti-G bias nucleotide composition (Zhou et al., 2023).

Table 4.1: Sequence data of *Limnonectes leporinus* for Cytb gene

Sequence data	Compositions
<i>Alignment statistics</i>	
Total base pair	537
Conserved sites	454/537
Variable sites	82/537
Parsimony informative sites	64/537
Singleton sites	18/537
<i>Nucleotide bases</i>	
Thymine (T)	31.8%
Cytosine (C)	32.6%
Adenine (A)	24.3%
Guanine (G)	11.3%

4.3.2 Diversification of *Limnonectes leporinus*

Phylogenetic trees of Cytb were obtained using three different methods (Neighbour-Joining, Maximum-Parsimony, and Maximum-likelihood) with *L. paramacrodon* as the outgroups. All trees produced similar topologies, showing two major clades, clade A and clade B (Figure 4.1). Clade A represents populations from the western part of Sarawak, which are Kubah NP, G Gading NP, Bau, Padwan, and Sri Aman. Clade B represents populations from the eastern part of Sarawak which are Batang Ai NP, Kanowit, Kapit, Bintulu, Mulu NP, and Bario.

This topology suggests the samples of *L. leporinus* species with respect to the outgroup species, *L. paramacrodon* were clustered according to geography. However, only clade A supports monophyletic with high bootstrap values (100% ML/NJ/MP). Clade B haplotypes were unresolved with low bootstrap values (51% ML, 43% NJ, 69% MP).

Within Clade B, only individuals from Batang Ai NP (BA40/1001/38) and Kanowit KN07 are resolved, supported by high bootstrap value (95% ML/NJ, 91% MP). Both Batang Ai NP and Kanowit are populations from central part of Sarawak. However, Clade B can't be further divided into two sub clades, supposedly central part and eastern part, because population from Kapit got mixed up between central part and eastern of Sarawak.



Figure 4.1: Lineage diversification of *Limnonectes leporinus* populations within Sarawak by using Cytb gene represented in ML/MP/NJ phylogenetic trees

4.3.3 Sequence Variation

A total of 253 segregating sites were observed and 20 haplotypes with haplotype diversity (H_d) = 0.8895, were determined from 12 localities. The nucleotide diversity (π) ranged between 0.00034 to 0.0543 and nucleotide divergence (D_a) for *L. leporinus* range from 0.00000 to 0.09111 (Table 4.2). Population from Matang WC - Kapit and Matang WC - Kanowit experienced the highest nucleotide diversity (π), meanwhile, population of Sri Aman-Batang AI NP and Sri Amna - Bario had the highest value of nucleotide divergence (D_a) compared with other populations.

The measures of nucleotide subdivision (N_{st}), population subdivision (F_{st}), and gene flow (N_m) were presented in Table 4.3. The highest N_{st} value and F_{st} value were 1.00 representing the population from Padawan - Bintulu, Padawan - Bario, Batang Ai NP - Bintulu, Batang Ai NP - Bario, and Bintulu - Bario. Populations that have the highest gene flow from protected areas are G Gading NP - Matang (N_m = 1.27), while the highest gene flow from unprotected areas are Kapit - Bario (N_m = 1.14).

The high and low genetic differences, nucleotide and population subdivision, and gene flow were influenced by geographical factors (Liu et al., 2015). Populations from Kubah NP - Bario are a clear example of populations that are influenced by geographical distance, having the longest distance of 635.74km with genetic differences value of (π = 0.035230) and (D_a = 0.08738), subdivision values of (N_{st} = 0.988730) and (F_{st} = 0.98805), and gene flow value (N_m = 0.01). Despite having a short geographical distance of 47.27km, Sri Aman - Batang Ai NP populations have a noticeable genetic difference (π = 0.04928; D_a = 0.09111), high subdivision (N_{st} = 0.97185; F_{st} = 0.97020), and low gene flow (N_m = 0.02) indicating geographical barrier present between those populations.

Table 4.2: DNA divergence among *L. leporinus* population

Population	Distance (km)	(π)^{a,b}	(Da)
Kubah NP - Matang WC	4.10	0.00254	0.00174
Kubah NP - Bau	22.93	0.00249	0.00174
Kubah NP - G Gading NP	40.00	0.00406	0.00263
Kubah NP - Padawan	52.00	0.00219	0.00174
Kubah NP - Sri Aman	157.25	0.00709	0.00734
Kubah NP - Batang Ai NP	199.55	0.03523	0.08738
Kubah NP - Kanowit	229.55	0.03609	0.08290
Kubah NP - Kapit	267.31	0.03557	0.06959
Kubah NP - Bintulu	447.34	0.03910	0.08551
Kubah NP - Mulu NP	582.94	0.04754	0.08551
Kubah NP - Bario	635.74	0.03523	0.08738
Matang WC - Bau	21.70	0.00083	0.00000
Matang WC - G Gading NP	36.12	0.00305	0.00089
Matang WC - Padawan	52.05	0.00219	0.00174
Matang WC - Sri Aman	160.98	0.00667	0.00552
Matang WC - Batang Ai NP	203.46	0.05171	0.08955
Matang WC - Kanowit	233.45	0.05295	0.08333
Matang WC - Kapit	271.40	0.05437	0.07090
Matang WC - Bintulu	451.12	0.05057	0.08769
Matang WC - Mulu NP	586.74	0.04531	0.08769
Matang WC - Bario	639.68	0.05171	0.08955
Bau - G Gading NP	44.55	0.00286	0.00089
Bau - Padawan	31.60	0.00034	0.00000
Bau - Sri Aman	157.93	0.00645	0.00560
Bau - Batang Ai NP	202.59	0.04844	0.08955
Bau - Kanowit	245.10	0.04931	0.08333
Bau - Kapit	278.23	0.05011	0.07090
Bau - Bintulu	463.92	0.04913	0.08769
Bau - Mulu NP	599.25	0.04755	0.08769
Bau - Bario	650.30	0.04844	0.08955
G Gading NP - Padawan	73.96	0.00239	0.00089
G Gading NP - Sri Aman	197.02	0.00655	0.00382
G Gading NP - Batang Ai NP	239.55	0.04320	0.08724
G Gading NP - Kanowit	262.89	0.04366	0.08102
G Gading NP - Kapit	304.78	0.04391	0.06876
G Gading NP - Bintulu	478.30	0.04586	0.08564

G Gading NP - Mulu NP	614.05	0.04932	0.08564
G Gading NP - Bario	669.20	0.04333	0.08751
Padawan - Sri Aman	144.93	0.00585	0.00560
Padawan - Batang Ai NP	191.56	0.04478	0.08955
Padawan - Kanowit	252.07	0.04540	0.08333
Padawan - Kapit	277.29	0.04581	0.07090
Padawan - Bintulu	471.38	0.04677	0.08769
Padawan - Mulu NP	605.85	0.04838	0.08769
Padawan - Bario	653.63	0.04478	0.08955
Sri Aman - Batang Ai NP	47.27	0.04928	0.09111
Sri Aman - Kanowit	157.12	0.04991	0.08489
Sri Aman - Kapit	149.99	0.05011	0.07204
Sri Aman - Bintulu	360.90	0.05062	0.08862
Sri Aman - Mulu NP	489.42	0.05128	0.08862
Sri Aman - Bario	527.39	0.04897	0.09049
Batang Ai NP - Kanowit	136.48	0.00784	0.00373
Batang Ai NP - Kapit	111.02	0.02600	0.01741
Batang Ai NP - Bintulu	324.08	0.02559	0.04478
Batang Ai NP - Mulu NP	449.31	0.02164	0.04338
Batang Ai NP - Bario	483.95	0.02799	0.04664
Kanowit - Kapit	72.27	0.02935	0.01368
Kanowit - Bintulu	219.36	0.02967	0.04353
Kanowit - Mulu NP	354.28	0.02415	0.04213
Kanowit - Bario	406.41	0.03284	0.04540
Kapit - Bintulu	213.07	0.01333	0.00000
Kapit - Mulu NP	339.50	0.01113	0.00016
Kapit - Bario	378.85	0.01965	0.00684
Bintulu - Mulu NP	135.75	0.00271	0.00047
Bintulu - Bario	198.38	0.00533	0.00933
Mulu NP - Bario	82.26	0.00577	0.00700

^aEstimated using the Kimura-2-parameter distance (Kimura, 1980); ^bSites with gaps were completely excluded.

* π : Nucleotide diversity; Da: Net nucleotide divergence

Table 4.3: Gene flow and genetic differentiation among *L. leporinus* population

Population	Distance (km)	(N _{st}) ^a	(F _{st}) ^b	(N _m) ^b
Kubah NP - Matang WC	4.10	0.53349	0.53333	0.44
Kubah NP - Bau	22.93	0.54911	0.54902	0.41
Kubah NP - G Gading NP	40.00	0.48113	0.48130	0.54
Kubah NP - Padawan	52.00	0.62201	0.62222	0.30
Kubah NP - Sri Aman	157.25	0.65616	0.65556	0.26
Kubah NP - Batang Ai NP	199.55	0.98873	0.98805	0.01
Kubah NP - Kanowit	229.55	0.92959	0.92569	0.04
Kubah NP - Kapit	267.31	0.81311	0.80736	0.12
Kubah NP - Bintulu	447.34	0.98847	0.98779	0.01
Kubah NP - Mulu NP	582.94	0.82807	0.82619	0.11
Kubah NP - Bario	635.74	0.98873	0.98805	0.01
Matang WC - Bau	21.70	0.00028	0.00000	-
Matang WC - G Gading NP	36.12	0.28257	0.28369	1.26
Matang WC - Padawan	52.05	0.00000	0.00000	-
Matang WC - Sri Aman	160.98	0.62823	0.62832	0.30
Matang WC - Batang Ai NP	203.46	0.99513	0.99482	0.00
Matang WC - Kanowit	233.45	0.93570	0.93217	0.04
Matang WC - Kapit	271.40	0.82118	0.81574	0.11
Matang WC - Bintulu	451.12	0.99502	0.99471	0.00
Matang WC - Mulu NP	586.74	0.83773	0.83607	0.10
Matang WC - Bario	639.68	0.99513	0.99482	0.00
Bau - G Gading NP	44.55	0.29115	0.29240	1.21
Bau - Padawan	31.60	0.00000	0.00000	-
Bau - Sri Aman	157.93	0.63819	0.63830	0.28
Bau - Batang Ai NP	202.59	0.99610	0.99585	0.00
Bau - Kanowit	245.10	0.93661	0.93315	0.04
Bau - Kapit	278.23	0.82198	0.81662	0.11
Bau - Bintulu	463.92	0.99601	0.99576	0.00
Bau - Mulu NP	599.25	0.83864	0.83706	0.10
Bau - Bario	650.30	0.99610	0.99585	0.00
G Gading NP - Padawan	73.96	0.33145	0.33333	1.00
G Gading NP - Sri Aman	197.02	0.45551	0.45503	0.60
G Gading NP - Batang Ai NP	239.55	0.98113	0.98004	0.01
G Gading NP - Kanowit	262.89	0.92088	0.91658	0.05
G Gading NP - Kapit	304.78	0.80492	0.79876	0.13
G Gading NP - Bintulu	478.30	0.98076	0.97967	0.01

G Gading NP - Mulu NP	614.05	0.82038	0.81789	0.11
G Gading NP - Bario	669.20	0.98119	0.98010	0.01
Padawan - Sri Aman	144.93	0.66618	0.66667	0.25
Padawan - Batang Ai NP	191.56	1.00000	1.00000	0.00
Padawan - Kanowit	252.07	0.94027	0.93706	0.03
Padawan - Kapit	277.29	0.82523	0.82014	0.11
Padawan - Bintulu	471.38	1.00000	1.00000	0.00
Padawan - Mulu NP	605.85	0.84230	0.84103	0.09
Padawan - Bario	653.63	1.00000	1.00000	0.00
Sri Aman - Batang Ai NP	47.27	0.97185	0.97020	0.02
Sri Aman - Kanowit	157.12	0.91490	0.91000	0.05
Sri Aman - Kapit	149.99	0.80403	0.79702	0.13
Sri Aman - Bintulu	360.90	0.97102	0.96939	0.02
Sri Aman - Mulu NP	489.42	0.81793	0.81451	0.11
Sri Aman - Bario	527.39	0.97164	0.97000	0.02
Batang Ai NP - Kanowit	136.48	0.39835	0.40000	0.75
Batang Ai NP - Kapit	111.02	0.52661	0.52830	0.45
Batang Ai NP - Bintulu	324.08	1.00000	1.00000	0.00
Batang Ai NP - Mulu NP	449.31	0.66029	0.66572	0.25
Batang Ai NP - Bario	483.95	1.00000	1.00000	0.00
Kanowit - Kapit	72.27	0.39642	0.39286	0.77
Kanowit - Bintulu	219.36	0.88872	0.88608	0.06
Kanowit - Mulu NP	354.28	0.66801	0.66850	0.25
Kanowit - Bario	406.41	0.89294	0.89024	0.06
Kapit - Bintulu	213.07	0.00000	0.00000	-
Kapit - Mulu NP	339.50	0.08658	0.08649	6.28
Kapit - Bario	378.85	0.30130	0.30556	1.14
Bintulu - Mulu NP	135.75	0.02253	0.02597	1.75
Bintulu - Bario	198.38	1.00000	1.00000	0.00
Mulu NP - Bario	82.26	0.32785	0.33862	0.98

^aEstimated using Lynch and Crease (1990); ^bEstimated using Hudson et. al (1992)

*N_{st}: Nucleotide subdivision; F_{st}: Estimate Population subdivision; N_m: Number of migrants per generations

4.3.4 Demographic History

A total of 20 haplotypes were identified from 62 sequences of Cytb gene (Table 4.4). The haplotypes were expressed from all sample localities. The highest haplotype managed to express 16 individuals from the sequences meanwhile the least was one sample for one haplotype. Haplotype 10 shared the same haplotype with 10 individuals from three different localities which were Kapit, Mulu NP, and Bintulu. Haplotype 16 shared the same haplotype with 16 individuals from five different localities which were Kubah NP, Matang WC, Gading NP, Padawan, and Bau. The other 17 haplotypes contained one haplotype from different localities respectively.

Table 4.4: Haplotypes of *L. leporinus* identified from 12 different populations

Haplotypes	N	Locality/sequence ID
Hap_1	5	G Gading NP - GGNP20030, GNP56, GNP131, GNP54, GNP55
Hap_2	4	Sri Aman - SA65, SA35, EKL61, EKL58
Hap_3	1	Sri Aman - SCP74
Hap_4	1	SriAman - EKL59
Hap_5	7	Kubah NP - KNP32, KNP10, KNP33, KNP27, KNP1415, KNP1762, KNP140338
Hap_6	1	Sibu - KN07
Hap_7	3	Batang Ai NP - BA38, BA40, BA1001
Hap_8	1	Sibu - KN08
Hap_9	1	Sibu - KN23
Hap_10	10	Kapit - SAB13, BEN56 Mulu NP - MBS239, MuluRZ299, MuluMBS5300, MHQ64 Bintulu - WILMAR37, WILMAR41, WILMAR42, WILMAR115
Hap_11	1	Kapit - BEN54
Hap_12	2	Mulu NP - MHQ25, MHQ69
Hap_13	1	Mulu NP - MBS124
Hap_14	3	Bario - RZ34, RZ32, RZ14

Hap_15	1	Mulu NP - MBS5299
Hap_16	16	Kubah NP - KNP22, KNP1768 Matang WC - MWC1433, MWC1443, MWC1531 Gading NP - GNP43 Padwan - GR151, BH198, BH202, BH203, ASSUM44, ASSUM49, Bau - 1213, 1214, 68, J20
Hap_17	1	Bau - 1211
Hap_18	1	Kubah NP - KNP1224
Hap_19	1	Matang WC - MWC1460]
Hap_20	1	Gading NP - GGNP14

AMOVA showed that (Table 4.5) diversity variation among populations was high (92.97%) with a significant p-value (0.00 ± 0.00) suggesting that the *L. leporinus* populations are genetically distinct from one another. Low variation (7.27%) showed within population supported with significant p-value (0.00 ± 0.00), suggesting that individuals within populations are relatively homogeneous or genetically similar.

Table 4.5: Measures of geographical population differentiation in *Limnonectes leporinus* based on an analysis of molecular variance (AMOVA)

Source of variation	Variance component	Percentage of variation	Fixation index, Φ	P-value ^a
Among populations	12.99	92.97	0.93	$0.00 \pm 0.00^*$
Within populations	1.02	7.27	0.92	$0.00 \pm 0.00^*$

^aProbability of finding a more-extreme variance component of the Φ index than that observed by chance alone after 1000 permutations.

*Significant ($p < 0.05$)

Table 4.6 shows the measurement of pairwise genetic differentiation fixation index (Φ_{st}) among population with 1000 permutations. Based on the result, the top 3 populations that showed complete genetic differentiation ($\Phi_{st} = 1.00$) supported by high significant value are Bau - Bintulu (0.009 ± 0.003), Padawan - Kanowit (0.007 ± 0.003), and Padawan -

Bintulu. (0.005 ± 0.002). Sri Aman - Batang Ai NP populations also have a considerably high genetic differentiation ($\Phi_{st} = 0.96$) supported by a high significant value (0.005 ± 0.002). This result suggested both geographical distance and geographical barrier influence genetic differentiation. Populations that have notable sharing of genetic material are Kubah NP - Bau ($\Phi_{st} = 0.47$) with p-value 0.037 ± 0.006 , Bau - G Gading NP ($\Phi_{st} = 0.36$) with p-value 0.016 ± 0.004 , and Bau - Padawan ($\Phi_{st} = 0.42$) with p-value 0.022 ± 0.005 .

The demographic expansion of mismatch distributions (Figure 4.2) represents the observed, sudden, and spatial model of *L. leporinus* distribution. The models vary according to locality. Based on the figure, the multimodal shape of Gading NP, Sri Aman, Sibul, Kapit, and Mulu possessed good population stability. The populations from these locations were able to sustain their populations after a series of evolution events. Meanwhile, Kubah NP populations showed spatial and sudden models with declining patterns. However, the observed model of the populations revealed that Kubah NP populations will experience expanding through time.

Table 4.6: Genetic differentiation matrix of populations calculated by Φ_{ST}

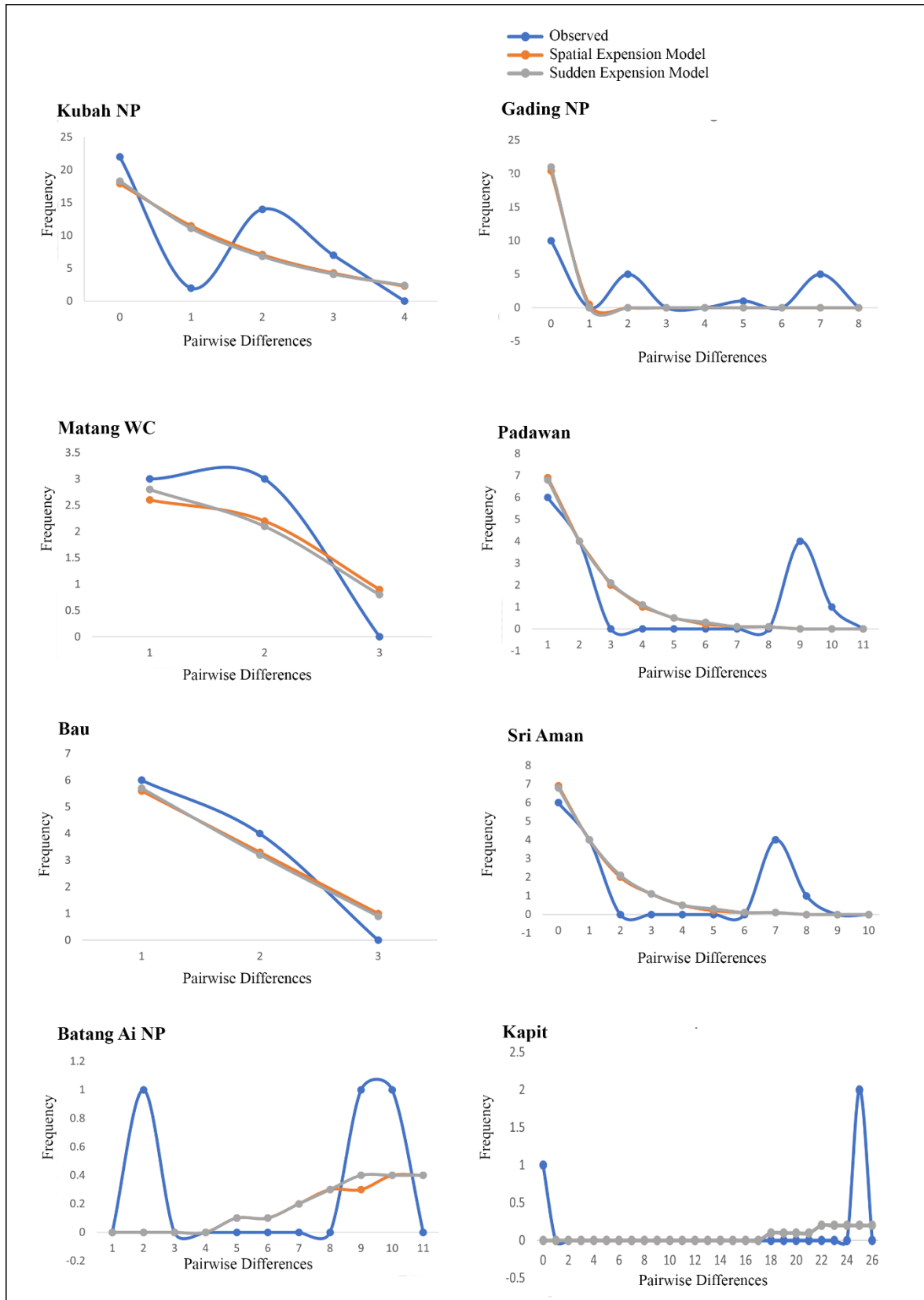
	1	2	3	4	5	6
1	-					
2	0.47 (0.037±0.006)					
3	0.06 (0.737±0.012)	0.32 (0.076±0.008)				
4	0.50 (0.016±0.004)	0.01 (0.754±0.012)	0.36 (0.016±0.004)			
5	0.55 (0.011±0.003)	0.11 (0.410±0.016)	0.42 (0.022±0.005)	0.04 (0.432±0.011)		
6	0.69 (0.000±0.000)	0.58 (0.008±0.002)	0.60 (0.001±0.001)	0.62 (0.002±0.001)	0.67 (0.004±0.002)	
7	0.98 (0.004±0.002)	0.99 (0.024±0.004)	0.96 (0.005±0.002)	0.99 (0.026±0.005)	1.00 (0.007±0.003)	0.96 (0.005±0.002)
8	0.96 (0.002±0.001)	0.94 (0.031±0.005)	0.93 (0.011±0.003)	0.95 (0.009±0.003)	0.96 (0.010±0.003)	0.92 (0.013±0.003)
9	0.91 (0.003±0.002)	0.85 (0.041±0.006)	0.87 (0.006±0.003)	0.87 (0.028±0.005)	0.89 (0.009±0.003)	0.85 (0.015±0.004)
10	0.98 (0.000±0.000)	0.99 (0.021±0.005)	0.97 (0.000±0.000)	1.00 (0.009±0.003)	1.00 (0.004±0.002)	0.96 (0.002±0.002)
11	0.97 (0.000±0.000)	0.97 (0.002±0.001)	0.95 (0.000±0.000)	0.97 (0.013±0.003)	0.98 (0.001±0.001)	0.95 (0.000±0.000)
12	0.98 (0.004±0.002)	0.99 (0.028±0.006)	0.96 (0.008±0.004)	0.99 (0.000±0.000)	1.00 (0.010±0.003)	0.96 (0.009±0.003)

Significant ($p < 0.05$) with 1000 permutations; 1: Kubah NP; 2: Matang WC; 3: G Gading NP; 4: Bau; 5: Padawan; 6: Sri Aman; 7: Batang Ai; 8: Kanowit; 9: Kapit; 10: Bintulu; 11: Mulu NP; 12: Bario

Table 5.6: Continue

	7	8	9	10	11	12
1	-					
2	0.47 (0.037±0.006)	-				
3	-0.06 (0.737±0.012)	0.32 (0.076±0.008)	-			
4	0.50 (0.016±0.004)	0.01 (0.754±0.012)	0.36 (0.016±0.004)	-		
5	0.55 (0.011±0.003)	0.11 (0.410±0.016)	0.42 (0.022±0.005)	0.04 (0.432±0.011)	-	
6	0.69 (0.000±0.000)	0.58 (0.008±0.002)	0.60 (0.001±0.001)	0.62 (0.002±0.001)	0.67 (0.004±0.002)	-
7	0.98 (0.004±0.002)	0.99 (0.024±0.004)	0.96 (0.005±0.002)	0.99 (0.026±0.005)	1.00 (0.007±0.003)	0.96 (0.005±0.002)
8	0.96 (0.002±0.001)	0.94 (0.031±0.005)	0.93 (0.011±0.003)	0.95 (0.009±0.003)	0.96 (0.010±0.003)	0.92 (0.013±0.003)
9	0.91 (0.003±0.002)	0.85 (0.041±0.006)	0.87 (0.006±0.003)	0.87 (0.028±0.005)	0.89 (0.009±0.003)	0.85 (0.015±0.004)
10	0.98 (0.000±0.000)	0.99 (0.021±0.005)	0.97 (0.000±0.000)	1.00 (0.009±0.003)	1.00 (0.004±0.002)	0.96 (0.002±0.002)
11	0.97 (0.000±0.000)	0.97 (0.002±0.001)	0.95 (0.000±0.000)	0.97 (0.013±0.003)	0.98 (0.001±0.001)	0.95 (0.000±0.000)
12	0.98 (0.004±0.002)	0.99 (0.028±0.006)	0.96 (0.008±0.004)	0.99 (0.000±0.000)	1.00 (0.010±0.003)	0.96 (0.009±0.003)

Significant ($p < 0.05$) with 1000 permutations; 1: Kubah NP; 2: Matang WC; 3: G Gading NP; 4: Bau; 5: Padawan; 6: Sri Aman; 7: Batang Ai; 8: Kanowit; 9: Kapit; 10: Bintulu; 11: Mulu NP; 12: Bario



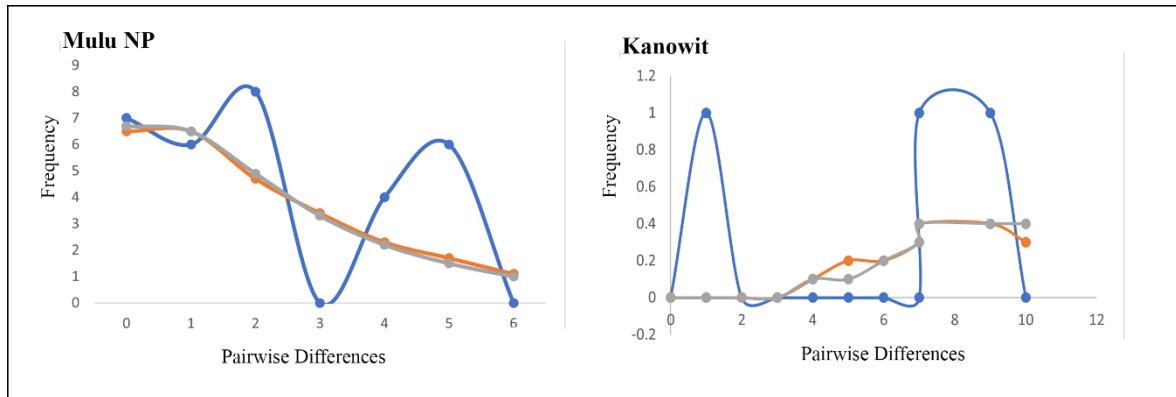


Figure 4.2: Mismatch distribution for *L. leporinus* at each locality

Mantel test with 1000 permutations was used to compute the isolation by distance of net nucleotide divergence and geographical distance. The test result obtained a significant correlation (p-value = 0.000000) between nucleotide divergence and geographical distance among 12 populations of *L. leporinus*. The regression coefficient: $y = 0.000128$, correlation coefficient: $r = 0.660433$, and regression statistics: $Y = 0.436172$ suggesting a high value of D_a when the distance between populations is farther.

However scatter plot of net nucleotide divergence (D_a) vs geographical distance in Figure 4.3 does not show a strong linear trend. Population pairs under 200 km distances show both low and moderate genetic divergence, while genetic divergence values remain clustered and do not increase consistently with distance within farther distances (450 - 700 km) populations. This indicates that the genetical differences between populations is indeed affected by geographical distances but not entirely as other geographical factors such as geographical barriers and forest fragmentation also can make influence.

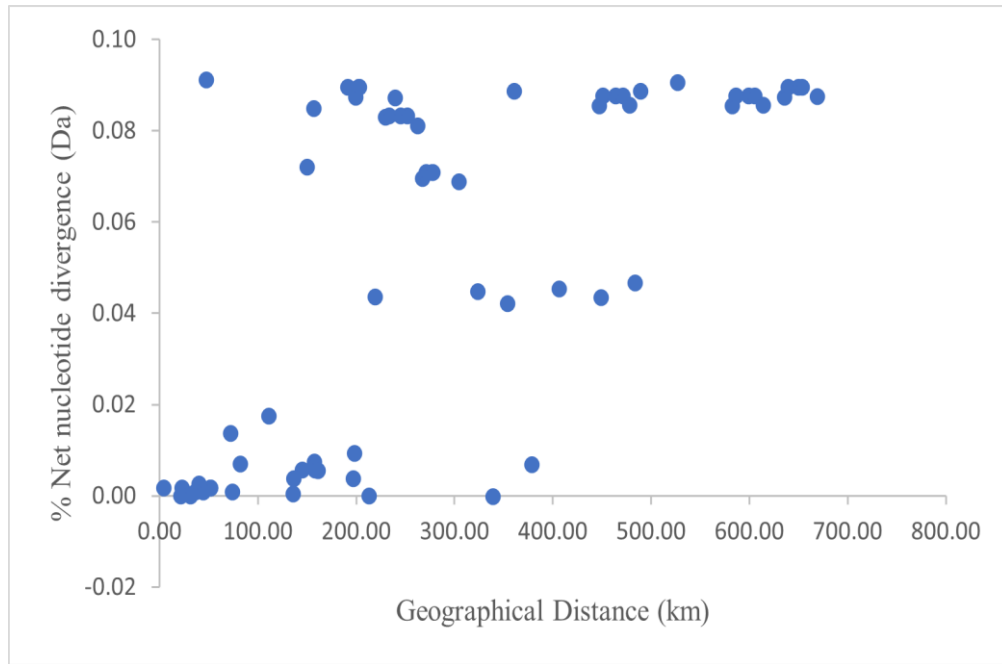


Figure 4.3: Scatterplot diagram of the relationship between geographical distance (km) and percentage of net nucleotide divergence (Da) between populations of *Limnonectes leporinus*. Regression coefficient: $y = 0.000128x$, correlation coefficient: $r = 0.660433$ and regression statistics: $Y = 0.436172$

4.3.5 Haplotypes Minimum Spanning Network (MSN)

Figure 4.4 and Figure 4.5 show the minimum spanning network (MSN) illustrates the relationship between *L. leporinus* from 12 localities in Sarawak. A total of 20 haplotypes were expressed from the analysis. This result clearly shows 3 haplotype groups which are the western, central, and eastern populations (Figure 4.1). Figure 4.2 further shows the relationship between geographical distance and geographical barriers with genetic differentiation. Western and central populations are separated by the geographical barrier which is the Lupar line with 42 mutation steps, while the central and eastern parts are separated by geographical distance with 21 mutational steps. However, Kapit population is present in both central and northern population groups. This resolved the phylogenetic tree of *L. leporinus* result (Figure 4.1) as it only expressed between western and eastern clades where central population is grouped together with eastern clade.

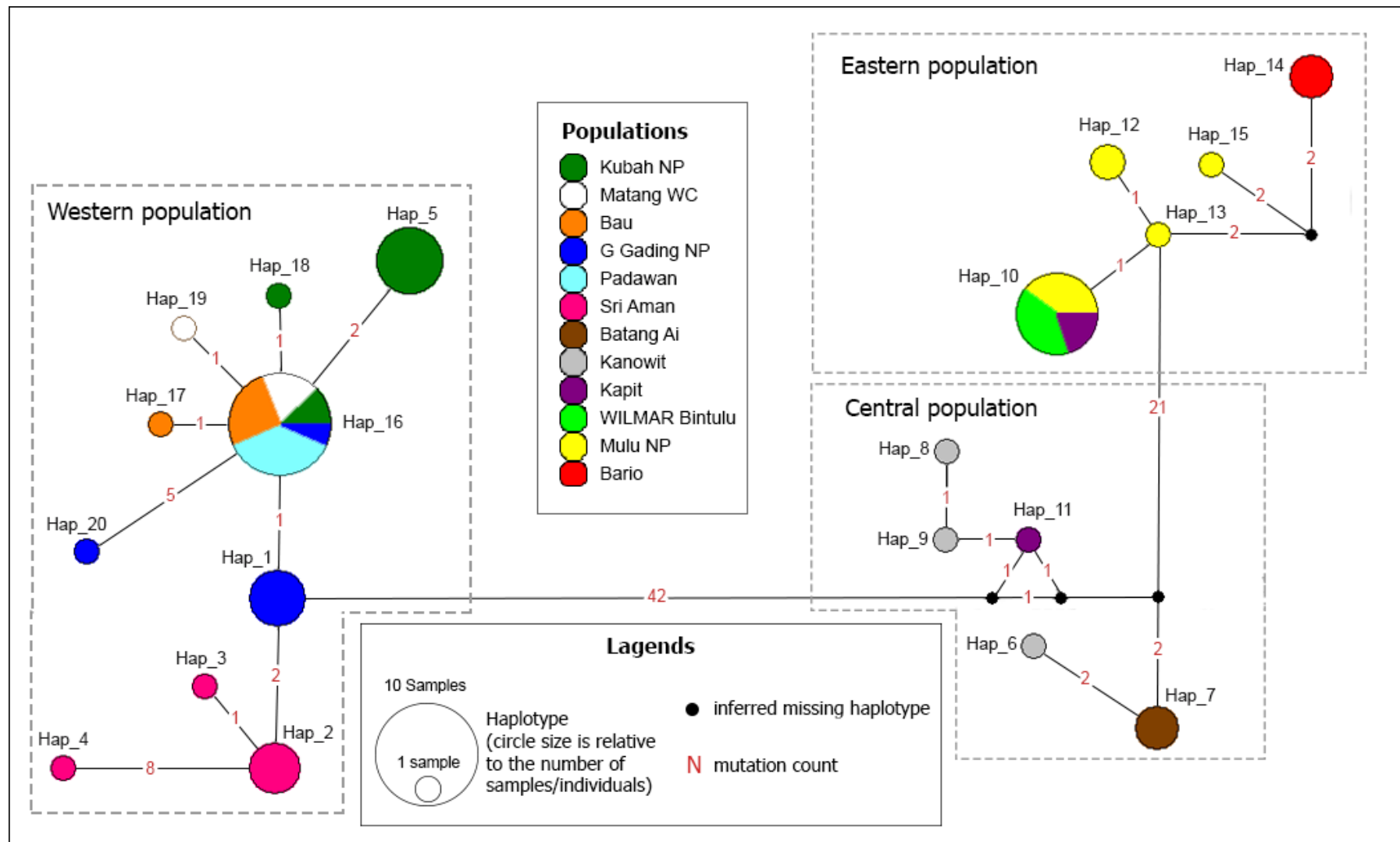


Figure 4.4: Minimum spanning network of *Limnonectes leporinus* haplotypes from 12 localities

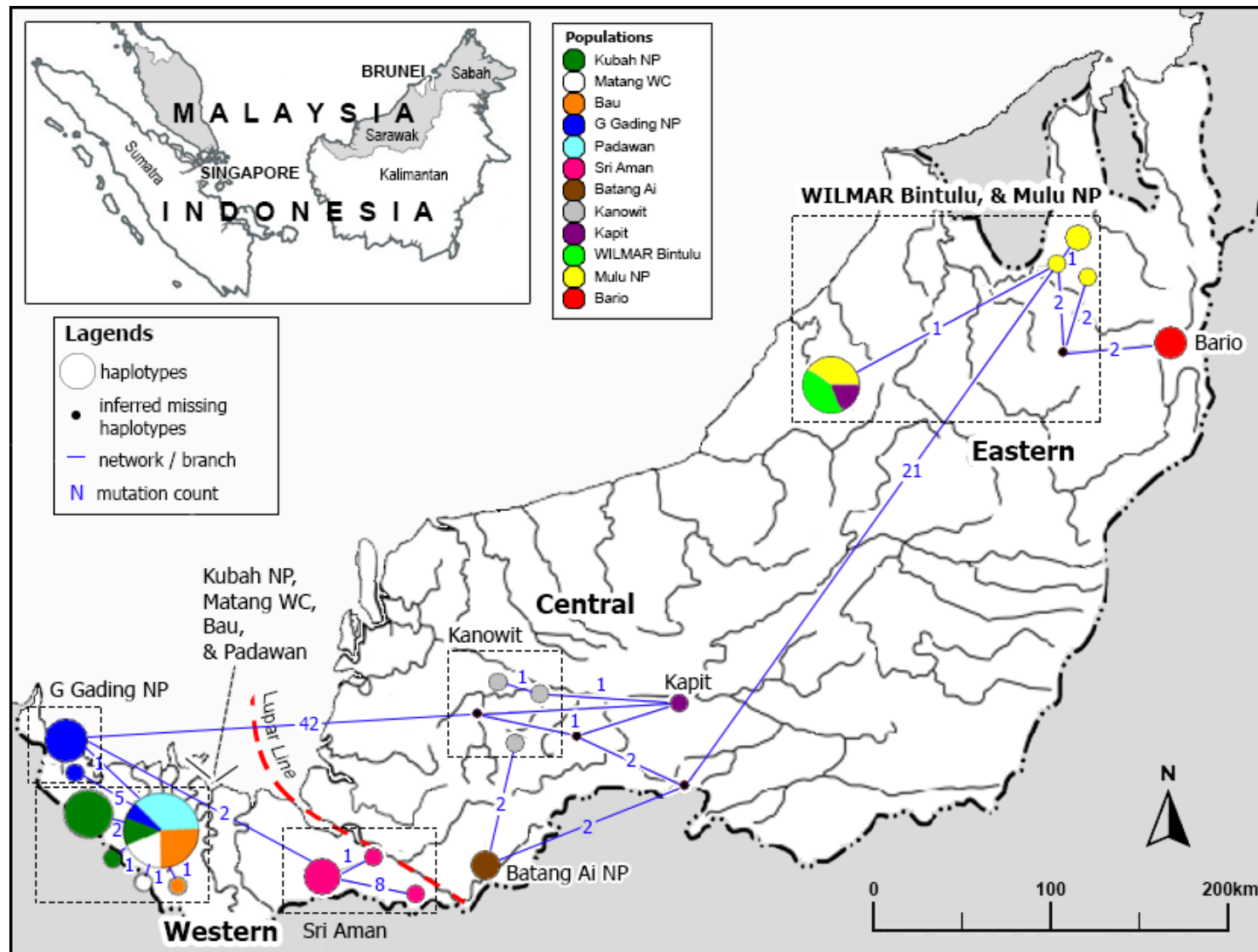


Figure 4.5: Minimum spanning network of *Limnonectes leporinus* haplotypes from 12 localities overlaid on Sarawak map

4.4 Discussion

Rodriguez et al. (2015) stated the divergence of mtDNA suggests a higher degree of evolutionary independence among tropical anuran groups, and frog populations from forested or topographically varied regions are undergoing evolution. Genetic research has revealed the existence of numerous frog species with only minor physical differences (Ron et al., 2018). In terms of bridging the barcoding gap, coding genes have outperformed non-coding genes (Koroiva & Santana, 2022).

The nucleotide variation and polymorphism in *Limnonectes leporinus* populations in Sarawak demonstrate the species' dynamic evolutionary history. The notable genetic diversity observed, particularly in mitochondrial DNA, underscores how environmental factors like habitat fragmentation can influence genetic structure. Amphibians, especially stream-dwelling species such as *L. leporinus*, are particularly susceptible to changes in their environment, and this diversity may represent an adaptive advantage (Pitogo et al., 2021). Genetic diversity plays a crucial role in a species' capacity to adapt to environmental changes, and the relatively high polymorphism suggests that these populations may be resilient against some environmental pressures (Pabijan et al., 2020).

The sequence variation results align with the idea of geographic isolation shaping genetic divergence (Zainudin et al., 2010). Phylogenetic analysis reveals the existence of two major clades corresponding to western and eastern populations, with significant geographic barriers, such as the Lupar gap, limiting gene flow between regions. This isolation may lead to distinct evolutionary paths, potentially resulting in cryptic speciation or population divergence. This finding is consistent with studies of other amphibians in the region, where rivers and mountainous landscapes act as significant barriers to gene flow (Arifin et al., 2022).

According to Hutchinson (1996), tectonic event occurred at Borneo has formed Lupar gap that separating Sarawak into western and eastern part of Sarawak. The Lupar gap has become geographical barrier for the frogs to disperse in Sarawak region, because frogs cannot move across the Batang Lupar river due to extensive low of flat terrain consisting swampy and mangrove forest (Hadil & Gambang, 2002).

The Kapit district, located in the central region of Sarawak, has been identified as a significant area for logging activities, which has profound implications for forest cover and habitat fragmentation (Ooi et al., 2023). Historically the largest timber-producing area in Sarawak, the logging concessions in Kapit are predominantly located in the interior regions (Gaveau et al., 2016). The loss of forest cover due to extensive logging activities is a considerable threat to the genetic integrity of *L. leporinus* populations in the area. Forest fragmentation not only isolates populations but also limits gene flow, thereby increasing the risk of genetic bottlenecks and reducing overall genetic diversity (Zainudin et al., 2019). The high nucleotide subdivision observed between populations from Kapit and other regions, as noted in the sequence variation analysis, further supports the idea that logging and deforestation are contributing to population isolation in central Sarawak. The logging-induced habitat disruption in Kapit, therefore, represents a significant conservation challenge for *L. leporinus* populations. This may explain why the central clade is not present in the phylogenetic tree (Figure 4.1), and Kapit population got mixed up between the central and eastern parts of Sarawak as shown in Figure 4.4 and Figure 4.5.

Demographic history analysis using mismatch distribution and nucleotide diversity metrics suggests population expansion in certain regions, while other populations show signs of a historical bottleneck. For instance, the populations from Gunung Gading NP and Kubah NP exhibit high nucleotide diversity, suggesting that these protected areas may have served

as refugia during periods of environmental stress (Rojas et al., 2022). This contrasts with populations in more fragmented or less protected areas, such as Kapit, where logging activities have intensified habitat fragmentation and genetic isolation. As a result, demographic contractions are more evident in these regions. This genetic data can provide crucial insights into which populations are most vulnerable to habitat loss and fragmentation, reinforcing the need for conservation prioritisation based on genetic health.

The conservation implications of these findings are significant. The genetic connectivity observed between certain populations, particularly those in protected areas, highlights the importance of maintaining these areas and potentially creating ecological corridors to facilitate gene flow between isolated populations. This is particularly important in regions such as central Sarawak, including Kapit, where populations show lower gene flow and higher genetic subdivision due to logging activities. Habitat corridors could mitigate the effects of fragmentation and allow for greater genetic exchange, thus enhancing population viability (Scroggie et al., 2019).

In conclusion, the genetic structure of *L. leporinus* populations in Sarawak highlights the critical role of geographic isolation, habitat fragmentation, and human activities, such as logging, in shaping the species' evolutionary trajectory. Conservation efforts should prioritise maintaining genetic diversity by focusing on habitat connectivity and protecting key populations in both protected and unprotected areas. The significant impact of logging activities in the Kapit region must be addressed to prevent further isolation and decline of *L. leporinus* populations. These findings can serve as a foundation for future conservation planning, helping to ensure the long-term survival of *L. leporinus* in Sarawak.

4.5 Conclusion

Through all molecular analysis made, it is concluded that the null hypothesis is rejected since there is significant genetic diversity within and among populations of *L. leporinus* in Sarawak. Other than geographical distances, geographical barriers, such as the Lupar River, and habitat fragmentation also influence the genetic structure of *L. leporinus*. This underscores the importance of preserving habitat connectivity, especially between eastern and central populations, for long-term conservation. Haplotype data was successfully obtained from DNSAP (V 6) and will be used to construct a habitat connectivity model map.

CHAPTER 5

DETERMINING MOST PRIORITISED CONSERVATION AREA FOR *Limnonectes leporinus*

5.1 Overview

Understanding a species' microhabitat is crucial for describing its ecological distribution within a specific area (Johari et al., 2016). The distribution of anuran species can be better understood by studying their microhabitats (Zainudin et al., 2017). The use of microhabitats is vital for anurans to find optimal conditions for foraging, reproduction, and shelter, as well as to ensure the survival of both tadpoles and adults (Eterovick et al., 2010; Martins et al., 2021).

Anuran species can be categorized into generalist and specialised groups based on their preferred habitat and diet. This categorization is vital for understanding species' adaptation to environmental changes (Zamroni et al., 2021). Generalist species have a broad diet range and can adapt to various habitats (Zamroni et al., 2021; Zulkefli & Zainudin, 2022). In contrast, specialised species have a limited diet range and struggle to tolerate arid environments (Zainudin et al., 2006; Eterovick et al., 2010; Zainudin et al., 2019a). When two or more anuran species share the same ecological needs and resources, they are said to occupy the same niche (Zulkefli & Zainudin, 2022). If two species have overlapping nutritional niches (i.e., they share one or more resources), competition for resources is high (Zamroni et al., 2021).

Rivers and water systems are vital resources for all life forms on Earth (Khoshnam et al., 2017). For amphibian communities, water is particularly important. After hatching from eggs as tadpoles, they begin their early life stages in water (Inger et al., 2017). Frog

communities can thrive in good water quality, but minor changes in water quality could result in high mortality rates for both tadpoles and adult frogs in the population (Zulkefli & Zainudin, 2022)

. This chapter integrates the second objective of the study, which is to develop a comprehensive prioritization framework model for the conservation of *L. leporinus*. The framework aims to assess and rank critical habitats based on a combination of molecular and ecological data. By incorporating haplotype data along with key ecological traits like microhabitat preferences and ecological variables predictor, this study provides a robust methodology to prioritise conservation areas. The model enables the identification of the most suitable habitats for conservation efforts, ensuring that the ecological and genetic diversity of *L. leporinus* is preserved. This approach justifies the study's broader goal of contributing to conservation strategies for endemic species facing habitat fragmentation, by offering actionable insights for targeted conservation interventions.

5.2 Model Mapping Process

5.2.1 Data Collection

The first step in the process was to gather georeferenced occurrence data for *L. leporinus*. A total of 62 sample data were derived from both new and previously conducted field samplings and recorded in an Excel file (Table 5.1), which was saved in .csv format for further analysis. The geographical coordinates obtained from these records were crucial for modelling habitat suitability and habitat connectivity. By including precise location data in decimal format, the occurrence records were mapped across the species' range, providing the foundation for subsequent modelling efforts.

5.2.2 Selection of Model Predictor Variables

Once the occurrence data were collected, attention shifted to selecting the environmental predictor variables that influence the habitat of *L. leporinus*. As a stream-dependent species, *L. leporinus* relies heavily on water bodies and forested areas, making it particularly vulnerable to habitat fragmentation, forest degradation, and human activity (Grafe, 2009). Based on the species' known ecological requirements, seven key predictor variables were selected. These included elevation, intact forest, land cover, major basins, human population density, slope, and temperature. Jackknife test was done to determine the relative importance of various environmental variables (Wettasin et al., 2023).

Table 5.1: Data used for mapping modelling

Localities	Sample ID	Haplotype ID	Region	Decimal Coordinate			Microhabitat code		
				Latitude	Longitude	Vegetation	Horizontal position	Vertical position	Substrate
Gunung Gading NP	GGNP20030	1	western	1.6906	109.8458	6	18	41	59
	GNP56	1	western	1.6957	109.8434	11	18	41	59
	GNP131	2	western	1.6931	109.8453	6	17	43	59
	GNP54	1	western	1.6922	109.8448	6	18	43	59
	GNP55	1	western	1.6934	109.8448	11	18	41	59
	GNP43	16	western	1.6912	109.8456	6	18	43	59
	GGNP14	20	western	1.6948	109.8428	11	17	41	59
Matang WC	MWC1433	16	western	1.6093	110.1601	14	16	40	59
	MWC1443	16	western	1.6103	110.1679	11	17	43	52
	MWC1531	16	western	1.6093	110.1757	6	18	43	59
	MWC1460	19	western	1.6097	110.1835	1	18	43	60
Kubah NP	KNP32	5	western	1.6128	110.1968	6	18	43	52
	KNP10	5	western	1.6095	110.1955	11	16	43	52
	KNP33	5	western	1.6098	110.1962	11	17	40	60
	KNP27	5	western	1.6125	110.1959	6	18	43	53
	KNP1415	5	western	1.6090	110.1953	1	18	40	59
	KNP1762	5	western	1.6122	110.1946	14	18	43	59
	KNP140338	5	western	1.6082	110.1956	11	18	41	52
	KNP22	16	western	1.6118	110.1958	6	16	42	59
	KNP1768	16	western	1.6094	110.1958	11	18	41	52
Bau	KNP1224	18	western	1.6082	110.1938	6	18	42	59
	1213	16	western	1.4143	110.1372	6	18	46	52

Padawan	1214	16	western	1.4171	110.1364	6	18	42	52
	68	16	western	1.4161	110.1367	11	18	41	60
	J20	16	western	1.4172	110.1352	11	18	41	60
	1211	17	western	1.3594	110.0296	6	18	41	59
	BH198	16	western	1.1436	110.2282	11	17	41	60
	BH202	16	western	1.1434	110.2282	6	17	42	60
	BH203	16	western	1.1421	110.2282	14	18	43	59
	ASSUM44	16	western	1.1414	110.2282	11	18	46	52
	ASSUM49	16	western	1.1408	110.2282	11	18	42	60
Sri Aman	GR151	16	western	1.1436	110.2282	1	18	43	59
	SA65	2	western	1.1253	111.6636	6	17	43	52
	SA35	2	western	1.1216	111.6551	11	18	41	60
	EKL61	2	western	1.1204	111.6620	6	16	42	60
	EKL58	2	western	1.1239	111.6570	11	18	42	60
	SCP74	3	western	1.1228	111.6579	11	18	43	52
Batang Ai NP	EKL59	4	western	1.1201	111.6512	6	18	43	59
	BA38	7	central	1.2227	111.9476	6	18	41	59
	BA40	7	central	1.2374	111.9476	1	16	43	60
	BA1001	7	central	1.2374	111.9559	11	18	41	60
	KN07	6	central	2.4491	112.0854	1	18	41	59
Kanowit	KN08	8	central	2.4491	112.0916	11	16	42	60
	KN23	9	central	2.4558	112.0916	11	16	43	59
	SAB13	10	central	2.0100	112.9000	11	18	40	60
Kapit	BEN56	10	central	2.0100	112.9525	1	16	42	59
	BEN54	11	central	2.0663	112.9525	11	18	43	60
	WILMAR37	10	eastern	3.5371	113.7354	6	16	43	59
Bintulu WILMAR	WILMAR41	10	eastern	3.5590	113.7335	14	18	43	60

Mulu NP	WILMAR42	10	eastern	3.5441	113.7344	11	17	41	59
	WILMAR115	10	eastern	3.5602	113.7323	6	18	43	53
	MBS239	10	eastern	4.0579	114.8421	14	17	41	60
	MuluRZ299	10	eastern	4.0701	114.8454	11	17	43	52
	MuluMBS5300	10	eastern	4.094	114.8457	1	18	43	52
	MHQ64	10	eastern	4.1026	114.8464	1	18	40	60
	MHQ25	12	eastern	4.0708	114.8561	6	18	41	60
	MHQ69	12	eastern	4.0869	114.8503	1	17	43	59
	MBS124	13	eastern	4.0797	114.8615	11	17	41	52
	MBS5299	15	eastern	4.1082	114.8631	11	18	42	60
Bario	RZ34	14	eastern	3.7364	115.5100	6	18	43	59
	RZ32	14	eastern	3.7364	115.5100	14	18	43	60
	RZ14	14	eastern	3.7417	115.5191	11	17	42	59

5.2.3 Preparation of Data

To facilitate accurate habitat modelling, the predictor variables were converted into raster datasets. This conversion ensured that all data layers shared the same extent and cell size, allowing for consistent spatial analysis across the dataset. The next step involved converting these raster datasets into ASCII format using ArcGIS (V 10.3), a format supported by MaxEnt (V 3.4.3), the software used for modelling habitat suitability. This process was essential for integrating environmental data into MaxEnt for analysis.

5.2.4 Habitat Suitability Modelling

With the occurrence points and environmental predictor variables prepared, the habitat suitability model was constructed using MaxEnt (V 3.4.3). MaxEnt operates on the principle of maximum entropy, predicting species distribution by evaluating known occurrence points against selected environmental variables (Merow et al., 2013; Phillips & Dudik, 2008). In this case, the model estimated the habitat suitability for *L. leporinus* across Sarawak. The model parameters, particularly the regularization multiplier and feature classes, were fine-tuned to optimize model performance. The regularization multiplier was adjusted to prevent overfitting, ensuring that the model remained generalizable and applicable to new data, while the feature classes controlled the complexity of the model by determining how the variables interacted.

5.2.5 Model Validation

After the model was constructed, the next critical step was validation. It was important to ensure that the model did not overfit the training data and could accurately predict habitat suitability for new occurrence points. To achieve this, cross-validation methods were used to split the occurrence data into training and testing sets (Wettasin et al.,

2023). The performance of the model was then evaluated using the Area Under the Curve (AUC) of the Receiver Operating Characteristic (ROC) curve. An AUC value close to 1 indicated that the model had high predictive accuracy, while a value near 0.5 suggested random prediction. The results from MaxEnt were interpreted on a scale of 0 to 1, where 0 represented areas unsuitable for *L. leporinus* habitat and 1 indicated the most suitable habitats.

5.2.6 Habitat Connectivity Model

The output from the MaxEnt model, in ASCII format, was used as input in Circuitscape, a tool designed to generate resistance maps. These maps predicted how easily *L. leporinus* could move through the landscape based on its ecological needs and behaviours.

In addition to the environmental data, genetic data were incorporated into the habitat connectivity model. The haplotype data, obtained from DNSAP (V 6) together with microhabitat data obtained from Principal Component Analysis (PCA) and Non-metric Multidimensional Scaling (NMDS) test were organised in an Excel file. In this file, each species was assigned a NodeID to represent different haplotypes, and latitude and longitude coordinates in decimal degrees format were recorded. This allowed for the integration of genetic attributes into the analysis, providing insights into how genetic relationships influenced habitat connectivity.

Once the genetic data were prepared, they were converted to ASCII format and used in Circuitscape. Circuitscape applied principles from circuit theory to simulate movement across the landscape, predicting habitat connectivity based on resistance surfaces (Hughes, 2017). This approach was particularly useful for studying fragmented landscapes, where species like *L. leporinus* faced barriers to movement due to human activity and environmental degradation.

Through this structured approach, the data analysis provided valuable insights into both habitat suitability and connectivity for *L. leporinus* in Sarawak. The combination of occurrence data, environmental predictors, and genetic information allowed for the development of robust models that highlighted key areas for conservation and management, ultimately supporting efforts to protect this species from habitat loss and fragmentation.

5.3 Results and Discussion

5.3.1 Model suitability test of *Limnonectes leporinus*

The jackknife test offered an insight into the relative importance of various environmental variables or ecological needs. In this test (Figure 5.1), all predictor variables contributed to the model ($AUC > 0.5$). Land cover ($AUC = 0.74$) and annual temperature ($AUC = 0.72$) were found to have the most significant influence on the species' habitat suitability. These findings were reflected in the jackknife analysis, where the exclusion or inclusion of certain variables altered the model's performance, reaffirming the importance of specific environmental predictors.

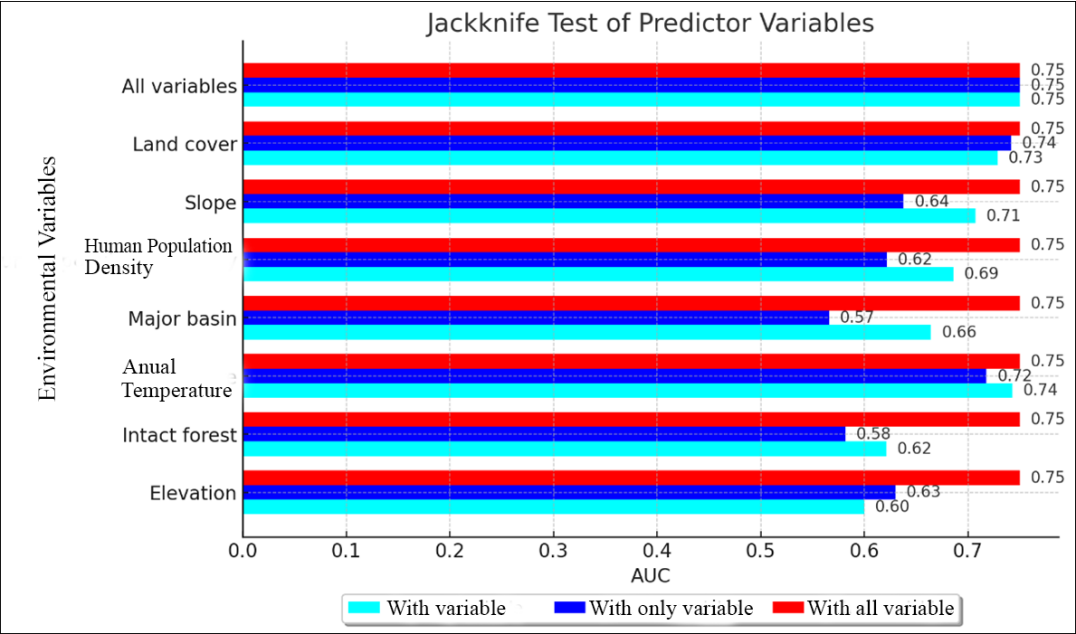


Figure 5.1: Jackknife test of predictors variables

ROC curve test result (Figure 5.2) shows a moderate to high (0.7 - 0.9) predictive accuracy, which means the model was not a random prediction. The ROC curve is distinctly above the diagonal line and curves towards the top left corner indicating suggests that the model has a good ability to distinguish between suitable and unsuitable habitats for *L. leporinus*. This indicates that the model performs better than random guessing.

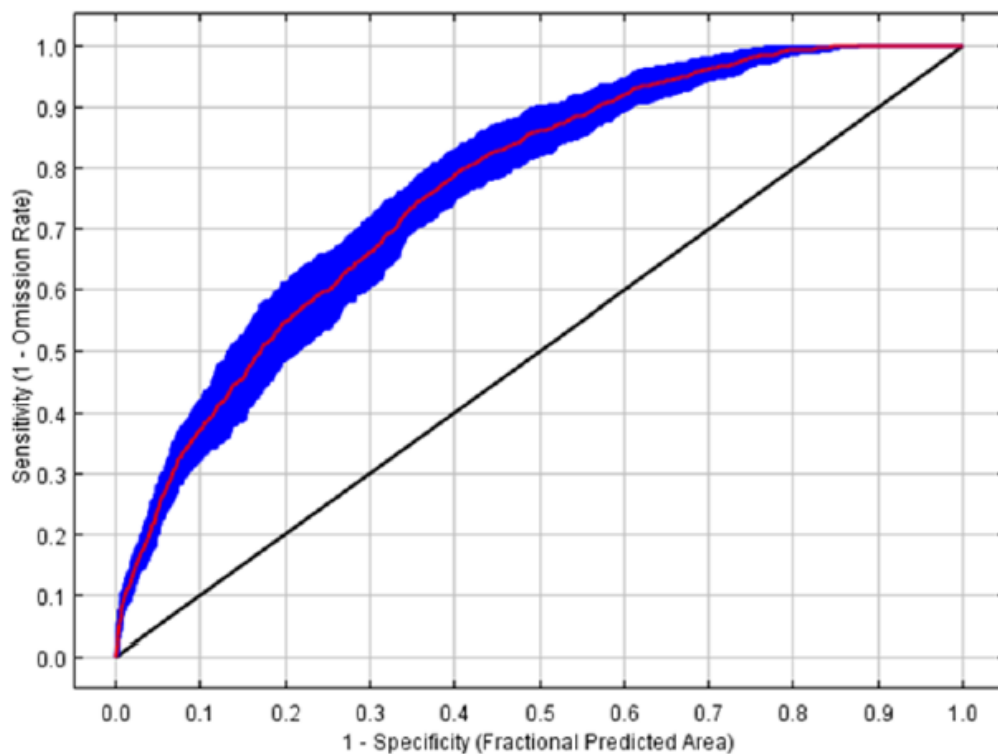


Figure 5.2: ROC curve of model suitability

5.3.2 Determination of Suitable Habitat for *Limnonectes Leporinus*

The map (Figure 5.3) reveals that mid-highland regions (500-2400 m) in Sarawak (refer to Appendix 2), particularly those located in the northeast and along the borders of Kalimantan, are predicted to be unsuitable for *L. leporinus*. These areas, highlighted in reddish-orange, indicate that the ecological conditions present in high-altitude areas do not support the species' life cycle needs. *L. leporinus* thrives in environments with ample water

flow, such as lowland (0-500 m) streams and rivers, which are largely absent in highland areas. This is consistent with the species' known ecological preference for lowland habitats, where it can access breeding sites in the form of streams and other water bodies (Grafe, 2009).

Conversely, the central part of Sarawak, particularly around Sibuan, Sarikei, and the Rajang River basin, is shown to provide more suitable habitats for the species. These areas, shaded in blue and green, indicate conditions that are conducive to the species' ecological needs. The presence of rivers such as the Rajang and Saribas in these areas likely plays a pivotal role in creating favourable habitats. Many large rivers across areas mean more small streams (Walrath et al., 2016). The relationship between large river networks and the presence of numerous small streams is a fundamental concept in river ecology, often articulated through the River Continuum Concept (RCC) (Dorrego et al., 2020). These streams provide the necessary water resources for breeding and larval development, which are crucial for the survival of *L. leporinus*.

While the lowland areas are identified as suitable habitats, one of the most significant challenges highlighted by the map is that many of these suitable areas are located in regions experiencing heavy human activity and development. For example, the map shows that areas around Kuching fall within the range of suitable habitats for *L. leporinus*. However, these areas are also characterised by high human population density, agriculture, and urban expansion (Alamgir et al., 2020; Mah et al., 2017). These factors contribute to habitat fragmentation and degradation, which pose significant threats to the species' long-term survival.

5.3.3 Connectivity Model for Conservation Strategy

The connectivity model (Figure 5.4) for *L. leporinus* depicted in the map classifies conservation areas into four tiers, each representing different levels of habitat connectivity and conservation priority. This tiered approach is instrumental in guiding conservation strategies by identifying areas that require immediate intervention to preserve habitat connectivity and ensure the survival of the species (Scheldeman & van Zonneveld, 2010). The map highlights the varying levels of connectivity across Sarawak, emphasizing the importance of targeting specific regions for effective conservation planning.

The areas categorized under Tier 1 represent the most fragmented and least connected habitats for *L. leporinus*. These areas are typically heavily modified landscapes, impacted by infrastructure projects such as highways and urban expansion, which create significant barriers to movement and gene flow. The lack of connectivity in these regions makes them highly vulnerable to population isolation, where gene flow is severely restricted, leading to genetic breaks and the formation of small, isolated populations (Dixo et al., 2009).

The map indicates that Tier 1 areas are primarily located in western Sarawak, around densely populated regions like Kuching (Aziz & Laman, 2018). These areas are at high risk due to anthropogenic activities that alter the landscape, reducing the ecological resources available for species survival (Johansson et al., 2005). Habitat fragmentation in these areas may hinder the species' ability to migrate, reproduce, and maintain healthy genetic diversity, which is essential for adaptation and resilience. Conservation efforts in Tier 1 areas should focus on land-use planning and working closely with planning authorities to mitigate the impact of human activities. Restoring connectivity through the creation of ecological corridors or protecting remaining fragments is crucial for preventing further isolation of *L. leporinus* populations (Diniz et al., 2020).

Tier 2 areas demonstrate moderate habitat connectivity, indicating that some degree of gene flow exists between populations of *L. leporinus* (Zainudin & Naim, 2018). The map shows that these areas are largely located in central Sarawak, connecting the western part to the eastern part of Sarawak. The connected habitat circuit in Tier 2 falls mostly within unprotected forest areas, making these regions vulnerable to habitat loss due to logging, agriculture, and other forms of land conversion (Gaveau et al., 2012).

These areas present an opportunity for conservation, as they offer the potential to maintain genetic connectivity and support viable populations of *L. leporinus*. However, much of the land in Tier 2 poses a challenge for conservation efforts as most of it falls under NCR lands, which are privately owned (refer to Appendix 2). The lack of legal protection for these habitats makes them susceptible to over-harvesting, particularly as *L. leporinus* is harvested for its meat, which is a local delicacy (Konopik et al., 2014). Over-harvesting, coupled with habitat degradation, could threaten the populations in these areas (Grafe, 2009).

Conservation strategies for Tier 2 areas should involve collaboration between local authorities, private landowners, and communities (Ioki et al., 2019). Efforts should focus on establishing protected areas or ecological reserves and raising awareness about the importance of sustainable harvesting practices. Educating local communities on the ecological value of *L. leporinus* and the need to preserve its habitat can help reduce the pressures on these populations (West, 2018).

Tier 3 regions show moderate connectivity potential and are often predicted to be unsuitable habitats for *L. leporinus*. These areas are located primarily in northern Sarawak, where the ecological conditions may not fully support the species' breeding and migratory needs (Zainudin, 2019). While Tier 3 areas may not currently represent prime habitats for *L.*

leporinus, they still hold some conservation value as they provide transitional zones between more connected regions (Vogt et al., 2007).

The conservation strategy for Tier 3 areas should focus on maintaining the ecological integrity of these regions, ensuring that they do not become further degraded. Although these areas may not house large populations of *L. leporinus*, they play a role in facilitating potential movement between more suitable habitats. Protecting these areas from further habitat destruction is important for preserving the overall landscape connectivity (Correa-Ayram et al., 2016).

Tier 4 represents the highest level of habitat connectivity and contains the extant populations of *L. leporinus*. These areas, highlighted in vivid red on the map, are critical for the species' survival, as they support healthy populations and facilitate gene flow between different regions (Zulkefli & Zainudin, 2022). Tier 4 areas are located primarily in the central part of Sarawak (Kapit and Belaga), where the species can move freely between streams and other breeding sites (Grafe, 2009).

Conservation efforts in Tier 4 areas should focus on sustaining the existing populations and ensuring that their habitats remain intact. This includes protecting the stream networks and lowland forests that are essential for breeding and larval development (Zainudin, 2019). Maintaining healthy genetic diversity in these populations is crucial for the species' long-term adaptability and resilience to environmental changes (Watling & Braga, 2015). Conservation strategies should also involve monitoring population health and addressing any emerging threats, such as habitat degradation or over-exploitation.

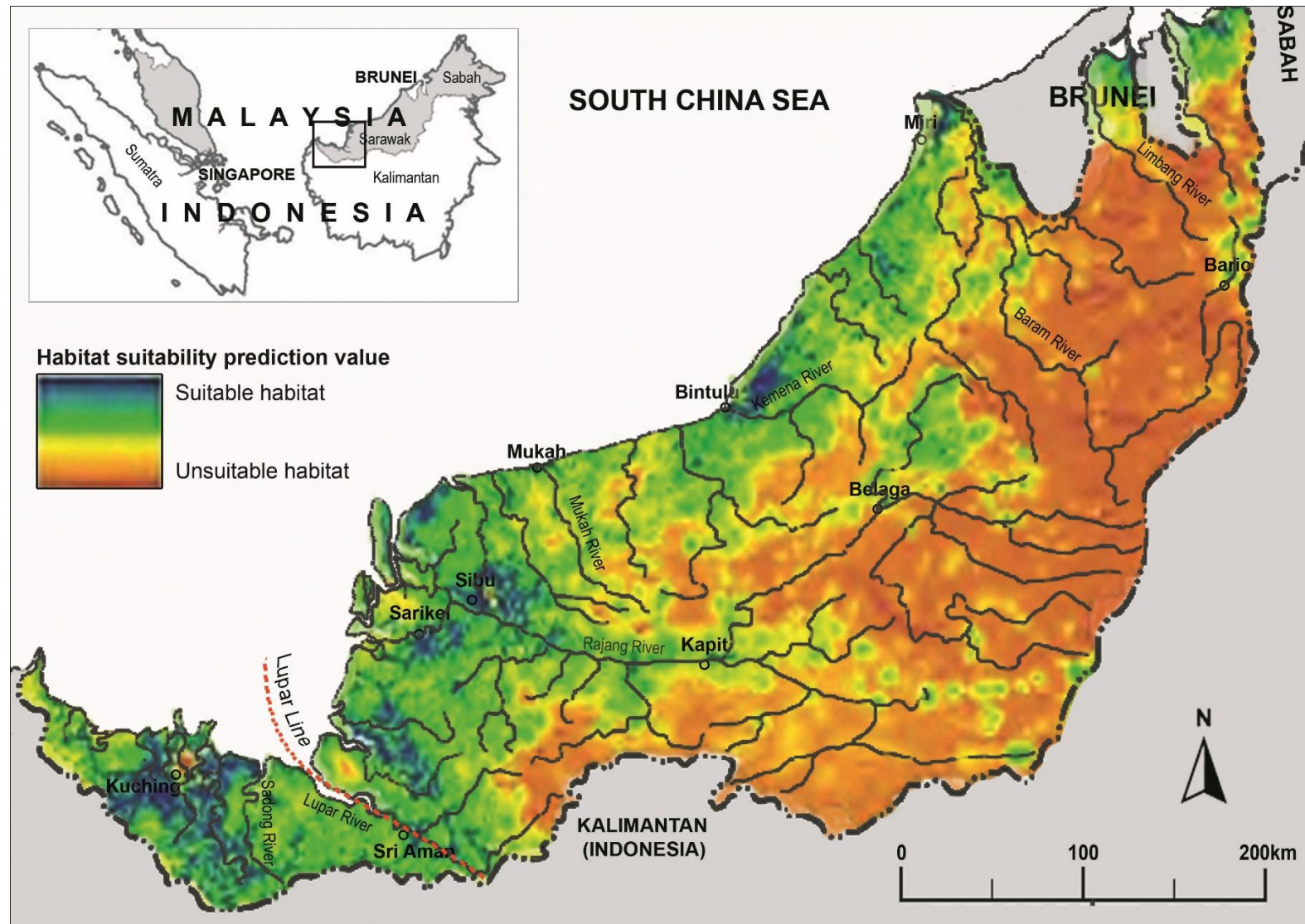


Figure 5.3: Map of suitable habitat for *Limnonectes leporinus* in Sarawak

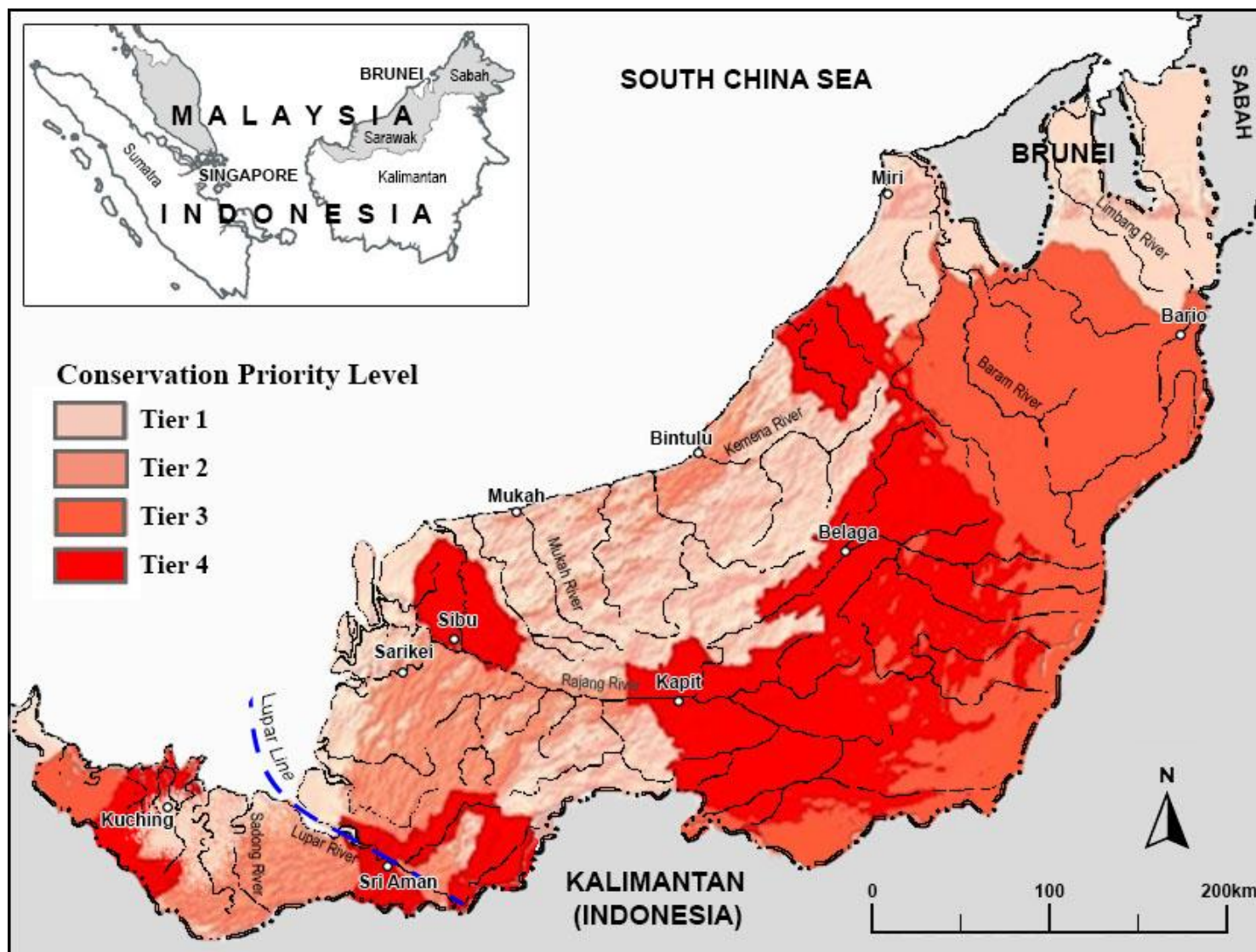


Figure 5.4: Priority areas model for conservation

5.4 Discussion

In this study, conservation priorities for *Limnonectes leporinus* were determined based on a combination of microhabitat preferences, haplotyped data, and map modelling. The analysis revealed that this endemic Bornean frog species is highly dependent on stream habitats with specific environmental conditions (Rodríguez & Pineda, 2010). The MaxEnt model, which utilized multiple predictor variables such as elevation, land cover, and temperature, successfully identified suitable habitats for *L. leporinus* across different regions of Sarawak. The areas identified as most suitable were concentrated in protected national parks such as Kubah National Park, Gunung Gading National Park, and Batang Ai National Park.

The Jackknife test results emphasized that stream-related factors, such as elevation and forest cover, were the most important predictors for *L. leporinus* habitat. This supports the observation that *L. leporinus* is a riparian species with a low dispersal ability, typically confined to areas with minimal disturbance and abundant stream-side vegetation. The model results also highlighted the negative impact of habitat fragmentation, particularly in areas that have undergone significant land-use changes due to agriculture and logging activities (Zulkefli & Zainudin, 2022).

Interestingly, the model also predicted the potential for gene flow between the western and northern parts of Sarawak, despite the presence of geographical barriers such as the Lupar gap in Sarawak (Zainudin et al., 2024). Although *L. leporinus* is intolerant to saltwater (Hadil, 2002), the MaxEnt model suggests there are gene flow from west to north, may be due to overland movement in Kalimantan (Zainudin et al., 2024). This potential connectivity is critical for conservation, as maintaining gene flow between these populations helps to prevent genetic isolation and local extinction (Chan et al., 2020).

Additionally, the narrow habitat tolerance and limited migratory ability of *L. leporinus* (Grafe, 2009) make it particularly vulnerable to population decline in fragmented habitats. Despite this vulnerability, the genetic analysis from Chapter 4 indicated significant yet low diversification of the species across Sarawak, as confirmed by Matsui et al. (2015), who found that the mitochondrial DNA (12S-16S rRNA genes) shows minimal genetic variation within the island. This suggests that *L. leporinus* may have a relatively recent origin and dispersed rapidly across Borneo, further underscoring the importance of maintaining habitat connectivity to support ongoing dispersal and genetic exchange (Emerson et al., 2000).

Anthropogenic impacts, such as habitat destruction and hunting, must also be addressed in conservation strategies. Involving local communities and authorities is essential for raising public awareness about the importance of biodiversity and the role of frogs like *L. leporinus* in maintaining ecosystem balance. As suggested by McCreless et al. (2013), the establishment of more protected areas should not only be based on biological and ecological factors, but should also consider social, political, and historical drivers. Without such efforts, vulnerable species may be left underrepresented in protected areas, further exposing them to habitat loss, degradation, and other anthropogenic pressures (Hughes, 2017).

In conclusion, this study has demonstrated the utility of integrating ecological and molecular data to identify conservation priorities for *L. leporinus*. Protecting and managing stream habitats, enhancing habitat connectivity, and addressing anthropogenic threats will be key strategies in ensuring the survival of this species in the face of ongoing habitat destruction.

5.5 Conclusion

The role of model map becomes an essential tool in conservation planning. By pinpointing critical areas, the model guides conservationists, and planners in making informed decisions. Knowing where the species is likely to thrive allows for targeted conservation strategies, such as the establishment of protected areas, restoration of natural habitats, and implementation of human activity zoning to reduce interference. It's not just about mapping current distribution but also predicting future shifts due to possible climate or landscape changes.

Therefore, the necessity of predicting suitable habitats becomes a cornerstone in the effective conservation of *L. leporinus*. It informs the prioritisation process in conservation efforts, ensuring that resources are strategically allocated to areas where they are most needed and will be most effective. This approach does not only aim to preserve the species but also to ensure the sustenance of a healthy ecosystem balance in Sarawak. The result obtains from this chapter concluded that the null hypothesis was rejected, and alternative hypothesis was accepted since map modelling shows significant information for conservation effort.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Amphibians are among the most endangered classes of vertebrates, yet new species are being discovered every year. This suggests that the actual number of existing species is significantly underestimated. In groups like amphibians, which have high extinction rates and ambiguously defined species boundaries, DNA barcoding serves as a valuable tool for swiftly evaluating genetic diversity and estimating species richness, thereby helping prioritise conservation decisions.

This study provides valuable insights into the genetic diversity and microhabitat preferences of *L. leporinus*, an endemic frog species of Borneo. Through molecular DNA analysis, it has been established that gene flow of the populations is critical for maintaining heterozygosity, which is essential for the species' adaptability to environmental changes in fragmented habitats. Furthermore, the use of spatial modelling has allowed for the identification of priority conservation areas, reinforcing the importance of maintaining habitat connectivity.

Molecular results revealed moderate genetic diversity across populations, with significant gene flow observed between protected areas. However, isolated populations exhibited reduced gene flow, indicating potential vulnerability to environmental changes. The findings confirmed that other than geographical distances, geographical barriers, such as the Lupar River, and habitat fragmentation also influence the genetic structure of *L. leporinus*.

The spatial modelling analysis identified key areas for conservation prioritisation, particularly in lowland regions with sustainable stream networks, which are affected by habitat fragmentation. Habitat suitability model revealed that these lowland areas are ideal for *L. leporinus* breeding sites, while, the connectivity circuits are predominantly found in unprotected areas, making them crucial for conservation efforts. Most of these suitable yet unprotected areas fall under NCR land category. Therefore, conservation efforts should involve the state government and local communities to promote sustainable land-use practices that balance between conservation goals and local communities' livelihood needs. The findings which underscore the need for targeted conservation strategies, can be used to serve as a management strategies model for similar riparian species threatened by habitat fragmentation in Borneo.

6.2 Recommendations

One of the major obstacles faced during this research was the COVID-19 pandemic and the resulting Malaysian Movement Control Order (MCO). These events posed significant challenges, particularly in preventing field trips, which limited the collection of new samples. Consequently, the study had to rely more on existing samples, which may have impacted the overall comprehensiveness of the genetic data and habitat analysis. More samples are needed especially from the centre region and interior part of Sarawak to strengthen the molecular analysis and map modelling.

Despite these difficulties, the study offers valuable contributions to the field of conservation biology. It provides important insights into the genetic diversity and habitat preferences of *Limnonectes leporinus*, emphasizing the critical need to maintain gene flow and habitat connectivity to ensure the species' survival. The integration of molecular data

with spatial modelling presents a framework model that can be applied to other endangered riparian species in Borneo, promoting more effective conservation strategies.

For future research, model maps should be layered with predictor variables and other important reference maps to make the results more understandable for readers and stakeholders involved in conservation efforts. For example, by layering forest cover and land use data, such as logging activity information, onto the habitat connectivity model, conservationists can better identify and prioritise key forested areas for habitat corridors.

Additionally, since this study focused solely on Sarawak, a broader effort is necessary to preserve the habitat for the sustainability of *L. leporinus* populations. Expanding the research across the entire island of Borneo, especially Kalimantan, is essential to get a clearer picture of the species' genetic diversity and distribution patterns. For future studies, tracking and studying *L. leporinu* movement across land in Kalimantan is essential, to better understand its migratory routes and habitat usage more effectively.

REFERENCES

- Alamgir, M., Campbell, M. J., Sloan, S., Engert, J. E., Word, J., & Laurance, W. F. (2020). Emerging challenges for sustainable development and forest conservation in Sarawak, Borneo. *PLOS ONE*, 15(3), e0229614.
- Allentoft, M. E., & O'Brien, J. (2010). Global amphibian declines, loss of genetic diversity and fitness: A review. *Diversity*, 2(1), 47-71.
- Amram, M., Zainudin, R., & Wahid, H. (2018). Mating calls of selected Sarawak toads (Amphibia: Anura: Bufonidae). *The Journal of Sexual Medicine*, 47(1), 1-7.
- Arifin, U., Smart, U., Husemann, M., Hertwig, S. T., Smith, E. N., Iskandar, D. T., & Haas, A. (2022). Phylogeographic inference of Sumatran Ranids bearing gastromyzophorous tadpoles with regard to the Pleistocene drainage systems of Sundaland. *Scientific Reports*, 12(1), 12013.
- Aziz, A. F., & Laman, C. J. (2018). Population estimation of proboscis monkeys in mangroves at Kuching Wetland National Park, Sarawak. *Borneo Journal of Resource Science and Technology*, 8(2), 84-89.
- Baffa-Trasci, N., Pereyra, L., Akmentins, M., & Vaira, M. (2020). Responses of anuran diversity to wetland characteristics and surrounding landscape in the Southern Andean Yungas, Argentina. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 30(7), 1437-1450.
- Beebee, T. J. C. (2005). Conservation genetics of amphibians. *Heredity*, 95(6), 423-427.
- Blackburn, D. C., Bickford, D. P., Diesmos, A. C., Iskandar, D. T., & Brown, R. M. (2010). An ancient origin for the enigmatic flat-headed frogs (Bombinatoridae: Barbourula) from the islands of Southeast Asia. *PLOS ONE*, 5(8), e12090.

- Breitfeld, H., Hall, R., Galin, T., Forster, M., & BouDagher-Fadel, M. (2017). A Triassic to Cretaceous Sundaland-Pacific subduction margin in West Sarawak, Borneo. *Tectonophysics*, 694, 35-56.
- Bruno Manser Fond. (2010). Sarawak Geoportal. *Bruno Manser Fond*. Retrieved April 25, 2024, from <http://www.bmfmaps.ch/EN/composer/#maps/1001>
- Butefish, A., & Moen, D. S. (2019, April 27). *The effect of microhabitat and body size on toe pad size in arboreal, semi-arboreal, and torrential frogs* [Conference Session]. Life Science Freshman Research Scholars Symposium, Stillwater, Oklahoma, United States.
- Buxton, V., Ward, M., & Sperry, J. (2018). Evaluation of conspecific attraction as a management tool across several species of anurans. *Diversity*, 10(1), 6.
- Chan, K. O., Hutter, C. R., Wood Jr, P. L., Grismer, L. L., Das, I., & Brown, R. M. (2020). Gene flow creates a mirage of cryptic species in a Southeast Asian spotted stream frog complex. *Molecular Ecology*, 29(20), 3970-3987.
- Chung, H., Kim, J., Min, P., Yoon, Y. W., Lee, B. K., Hong, B., Rim, S., Kwon, H. M., & Choi, E. (2018). Different contribution of sarcomere and mitochondria related gene mutations to hypertrophic cardiomyopathy. *Journal of the American College of Cardiology*, 71(11S), A901.
- Cimino, M. A., Fraser, W. R., Irwin, A. J., & Oliver, M. J. (2013). Satellite data identify decadal trends in the quality of *Pygoscelis* penguin chick-rearing habitat. *Global change biology*, 19(1), 136-148.
- Connor, E., Courtney, A., & Yoder, J. (2000). Individuals-area relationships: The relationship between animal population density and area. *Ecology*, 81(3), 734-748.
- Conroy, M., Cohen, Y., James, F., Matsinos, Y., & Maurer, B. (1995). Parameter estimation,

- reliability, and model improvement for spatially explicit models of animal populations. *Ecological Applications*, 5(1), 17-19.
- Cordeiro, J. L. P., Fragoso, J. M. V., Crawshaw, D., & Oliveira, L. F. B. (2016). Lowland tapir distribution and habitat loss in South America. *PeerJ*, 4, e2456.
- Cordeiro, J. L. P., Fragoso, J. M. V., Crawshaw, D., & Oliveira, L. F. B. (2016). Lowland tapir distribution and habitat loss in South America. *PeerJ*, 4, e2456.
- Correa-Ayram, C. A., Mendoza, M. E., Etter, A., & Salicrup, D. R. P. (2016). Habitat connectivity in biodiversity conservation: A review of recent studies and applications. *Progress in Physical Geography*, 40(1), 7-37.
- Crews, N., Wittwer, C., & Gale, B. (2008). Continuous-flow thermal gradient PCR. *Biomedical microdevices*, 10, 187-19
- Curran, L. M., Trigg, S. N., McDonald, A. K., Astiani, D., Hardiono, Y. M., Siregar, P., & Kasischke, E. (2004). Lowland forest loss in protected areas of Indonesian Borneo. *Science*, 303(5660), 1000-1003.
- Davis, H. R., Nashriq, I., Woytek, K. S., Wikramanayake, S. A., Bauer, A. M., Karin, B. R., & Das, I. (2023). Genomic analysis of Bornean geckos (Gekkonidae: Cyrtodactylus) reveals need for updated taxonomy. *Zoologica Scripta*, 52(3), 249-263.
- Dehling, J. M., & Grafe, T. U. (2008). A new treefrog of the genus *Rhacophorus* (Anura: Rhacophoridae) from Brunei Darussalam (Borneo). *Salamandra*, 44(2), 101-112.
- Department of Irrigation and Drainage Sarawak. (n.d.). Integrated river basin management. *Sarawak Government*. Retrieved April 26, 2024, from https://did.sarawak.gov.my/web/subpage/webpage_view/315
- Diniz, M. F., Cushman, S. A., Machado, R. B., & De Marco Júnior, P. (2020). Landscape connectivity modeling from the perspective of animal dispersal. *Landscape Ecology*, 35, 41-

58.

- Dixit, N., Zirpel, M., Slik, J., Jamsari, J., Weising, K., & Guicking, D. (2023). Biogeography of the Sunda Shelf revisited: Insights from *Macaranga section pruinosa* (Euphorbiaceae). *Frontiers in Ecology and Evolution*, 10, 1049243.
- Dixo, M., Metzger, J. P., Morgante, J. S., & Zamudio, K. R. (2009). Habitat fragmentation reduces genetic diversity and connectivity among toad populations in the Brazilian Atlantic Coastal Forest. *Biological Conservation*, 142(8), 1560-1569.
- Doretto, A., Piano, E., & Larson, C. E. (2020). The River Continuum Concept: Lessons from the past and perspectives for the future. *Canadian Journal of Fisheries and Aquatic Sciences*, 77(11), 1853-1864.
- Dubeux, M., Nascimento, F., Gonçalves, U., & Mott, T. (2021). Identification key for anuran amphibians in a protected area in the northeastern Atlantic Forest. *Papéis Avulsos de Zoologia*, 61, e20216176.
- Dufresnes, C., Wassef, J., Ghali, K., Brelsford, A., Stöck, M., Lymberakis, P., Crnobrnja-Isailovic, J., & Perrin, N. (2013). Conservation phylogeography: Does historical diversity contribute to regional vulnerability in European tree frogs (*Hyla arborea*)? *Molecular Ecology*, 22(22), 5669-5684.
- Ekka, A., Pande, S., Jiang, Y., & der Zaag, P. V. (2020). Anthropogenic modifications and river ecosystem services: A landscape perspective. *Water*, 12(10), 2706.
- Emerson, S. B. (1992). Courtship and nest-building behavior of a Bornean frog, *Rana blythi*. *Journal Storage*, 1992(4), 1123-1127.
- Emerson, S. B., Inger, R. F. & Iskandar, D. (2000). Molecular systematics and biogeography of the fanged frogs of Southeast Asia. *Molecular Phylogenetics and Evolution*, 16(1), 131- 142.

- Emerson, S. B., Inger, R. F., & Iskandar, D. (2000). Molecular systematics and biogeography of the fanged frogs of Southeast Asia. *Molecular phylogenetics and evolution*, 16(1), 131-142.
- Excoffier, L., & Lischer, H. E. (2010). Arlequin suite Ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10(3), 564-567.
- Filer, A., Beyer, H., Meyer, E., & Rensburg, B. (2019). Distribution mapping of specialised amphibian species in rare, ephemeral habitats: Implications for the conservation of threatened “acid” frogs in South East Queensland. *Conservation Science and Practice*, 2(1), e143.
- Flory, A. R., Kumar, S., Stohlgren, T. J., & Cryan, P. M. (2012). Environmental conditions associated with bat white-nose syndrome mortality in the North-Eastern United States. *Journal of Applied Ecology*, 49(3), 680-689.
- Flory, A. R., Kumar, S., Stohlgren, T. J., & Cryan, P. M. (2012). Environmental conditions associated with bat white-nose syndrome mortality in the north-eastern United States. *Journal of Applied Ecology*, 49(3), 680-689.
- Forster, P., & Forster, M. (2022, December 17). *Free Phylogenetic Network Software*. fluxus-engineering. <https://www.fluxus-engineering.com>
- Freeman, L. A., Kleypas, J. A., & Miller, A. J. (2013). Coral reef habitat response to climate change scenarios. *PLOS ONE*, 8(12), e82404.
- Fu, Y. X. (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, 147, 915-925.
- Gaston, M., Fuji, A., Weckerly, F., & Forstner, M. (2010). Potential component allee effects and their impact on wetland management in the conservation of endangered anurans.

PLOS ONE, 4(5), e10102.

- Gaveau, D. L. A., Curran, L. M., Paoli, G. D., Carlson, K. M., Wells, P., Besse-Rimba, A., & Leader-Williams, N. (2012). Examining protected area effectiveness in Sumatra: Importance of regulations governing unprotected lands. *Conservation Letters*, 5(2), 142-148.
- Gaveau, D. L., Locatelli, B., Salim, M. A., Yaen, H., Pacheco, P., & Sheil, D. (2019). Rise and fall of forest loss and industrial plantations in Borneo (2000-2017). *Conservation Letters*, 12(3), e12622.
- Gaveau, D. L., Sheil, D., Husnayaen, Salim, M. A., Arjasakusuma, S., Ancrenaz, M., & Meijaard, E. (2016). Rapid conversions and avoided deforestation: Examining four decades of industrial plantation expansion in Borneo. *Scientific reports*, 6(1), 32017.
- Gaveau, D. L., Sheil, D., Husnayaen, Salim, M. A., Arjasakusuma, S., Ancrenaz, M., & Meijaard, E. (2016). Rapid conversions and avoided deforestation: Examining four decades of industrial plantation expansion in Borneo. *Scientific reports*, 6(1), 32017.
- Grafe, U. T., 2009. Beyond taxonomy: Radiotelemetry of the giant river frog (*Limnonectes leporinus*) in the Ulu Temburong National Park, Brunei Darussalam. In: Das, I., Haas, A., & Tuen, A. A. (Eds.), *Proceedings of the Conference "Biology of the Amphibians in the Sunda Region, South-East Asia"*, Universiti Malaysia Sarawak, Sarawak, Malaysia, 28-30 September 2009. Biology and Conservation of Tropical Asian Amphibians (pp. 50-60).
- Gunawan, G., Sulistijorini, S., Chikmawati, T., & Sobir, S. (2021). Predicting suitable areas for *Baccaurea angulata* in Kalimantan, Indonesia using MaxEnt modelling. *Biodiversitas Journal of Biological Diversity*, 22(5), 2646-2653.
- Haas, A. (2004). 12S and 16S mitochondrial genes in frog systematics. *Herpetological*

- Haas, A., Das, I., & Hertwig, S. T. (n.d.). *Frogs of Borneo*. Frogs. Retrieved July 15, 2023, from <http://http://www.frogsofborneo.org>
- Hall, R. (2001). Cenozoic reconstructions of SE Asia and the SW Pacific: Changing patterns of land and sea. In I. Metcalfe, J. M. B. Smith, M. Morwood, & I. Davidson (Eds.), *Faunal and floral migrations and evolution in SE Asia-Australasia* (pp. 35-56). A. A. Balkema Publishers.
- Hall, R. (2012). Sundaland and Wallacea: Geology, plate tectonics and palaeogeography. In D. J. Gower, K. G. Johnson, J. E. Richardson, B. R. Rosen, L. Rüber, & S. T. Williams (Eds.), *Biotic evolution and environmental change in Southeast Asia* (pp. 32-73). Cambridge University Press.
- Hamer, A., & McDonnell, M. (2009). The response of herpetofauna to urbanization: Inferring patterns of persistence from wildlife databases. *Austral Ecology*, 35(5), 568-580.
- Hamidy, A., Matsui, M., Nishikawa, K., & Belabut, D. M. (2012). Detection of cryptic taxa in *Leptobrachium nigrops* (Amphibia, Anura, Megophryidae), with description of two new species. *Zootaxa*, 3398(1), 22-39.
- Hansen, N., Scheele, B., Driscoll, D., & Lindenmayer, D. (2018). Amphibians in agricultural landscapes: The habitat value of crop areas, linear plantings and remnant woodland patches. *Animal Conservation*, 22(1), 72-82.
- Harrington, S., Harrison, L., & Sheil, C. (2013). Ossification sequence heterochrony among amphibians. *Evolution & Development*, 15(5), 344-364.
- Heemeyer, J., Williams, P., & Lannoo, M. (2012). Obligate crayfish burrow use and core habitat requirements of crawfish frogs. *The Journal of Wildlife Management*, 76(5),

1081-1091.

- Hernandez, P. A., Graham, C. H., Master, L. L., & Albert, D. L. (2006). The effect of sample size and species characteristics on performance of different species distribution modelling methods. *Ecography*, 29(5), 773-785.
- Hertwig, S. T., Das, I., Schweizer, M., Brown, R., & Haas, A. (2012). Phylogenetic relationships of the *Rhacophorus everetti*-group and implications for the evolution of reproductive modes in *Philautus* (Amphibia: Anura: Rhacophoridae). *Zoologica Scripta*, 41(1), 29-46.
- Hertwig, S. T., Schweizer, M., Das, I., & Haas, A. (2013). Diversification in a biodiversity hotspot-The evolution of Southeast Asian rhacophorid tree frogs on Borneo (Amphibia: Anura: Rhacophoridae). *Molecular Phylogenetics and Evolution*, 68(3), 567-581.
- Heyer, R., Donnelly, M. A., Foster, M., & McDiarmid, R. (Eds.). (2014). *Measuring and monitoring biological diversity: Standard methods for amphibians*. Smithsonian Institution.
- Hu, J., Huang, Y., Jiang, J., & Guisan, A. (2019). Genetic diversity in frogs linked to past and future climate changes on the roof of the world. *Journal of Animal Ecology*, 88(6), 953-963.
- Hudson, R. R., Slatkin, M., & Maddison, W. P. (1992). Estimation of levels of gene flow from DNA sequence data. *Genetics*, 132(2), 583-589.
- Hughes, A. C. (2017). Mapping priorities for conservation in Southeast Asia. *Biological Conservation* 209, 395-405.
- Inger R. F. & Greenberg, B. (1966). Ecological and competitive relations among three species of frogs (Genus *Rana*). *Ecology*, 47(5), 746-759.

- Inger, R. F. (1994). Microhabitat Description. In W. R. Heyer, A. Donnelly, R. W. McDiarmid, L. A. Hayek & M. S. Foster (Eds.), *Measuring and monitoring biological diversity: Standard methods for amphibians* (pp. 60-68). Smithsonian Institution Press.
- Inger, R. F. (2009). Contributions to the natural history of seven species of Bornean frogs. *Fieldiana Zoology*, 2009(116), 1.
- Inger, R. F. (2009). Contributions to the Natural History of seven species of Bornean frogs. *Fieldiana Zoology*, 2009(116), 1-25.
- Inger, R. F., Stuebing, R. B., Grafe, U. T., & Dehling, J. M. (2017). *A field guide to the frogs of Borneo* (3rd ed.). Kota Kinabalu: Natural History Publications (Borneo) Sdn. Bhd.
- Inger, R., & Voris, H. (2008). The biogeographical relations of the frogs and snakes of Sundaland. *Journal of Biogeography*, 28(7), 863-891.
- Ioki, K., Din, N. M., Ludwig, R., James, D., Hue, S. W., Johari, S. A., & Phua, M. H. (2019). Supporting forest conservation through community-based land use planning and participatory GIS-lessons from Crocker Range Park, Malaysian Borneo. *Journal for Nature Conservation*, 52, 125740.
- IUCN Red List (2022). *The IUCN Red List of Threatened Species* (Version 2022-2). Retrieved July 22, 2023, from <https://www.iucnredlist.org>
- Kurniawan, N., Islam, M. H., Djong, T. H., Igawa, T., Daicus, M. B., Yong, H. S., Sumida, M. (2010). Genetic divergence and evolutionary relationship in *Fejervarya cancrivora* from Indonesia and other Asian countries inferred from allozyme and mtDNA sequence analysis. *Zoological Sciences*, 27, 222-233.
- Jablonski, D., Gkontas, I., Poursanidis, D., Lymberakis, P., & Poulakakis, N. (2021). Stability in the Balkans: Phylogeography of the endemic Greek stream frog, *Rana*

- graeca*. *Biological Journal of the Linnean Society*, 132(4), 829-846.
- Johansson, M., Primmer, C. R., Sahlsten, J., & Merilä, J. (2005). The influence of landscape structure on occurrence, abundance and genetic diversity of the common frog, *Rana temporaria*. *Global Change Biology*, 11(10), 1664-1679.
- Johnson, P., McKenzie, V., Peterson, A., Kerby, J., Brown, J., Blaustein, A., & Jackson, T. (2011). Regional decline of an iconic amphibian associated with elevation, land-use change, and invasive species. *Conservation Biology*, 25(3), 556-566.
- Kalstrup, T., & Blunck, R. (2015). Reinitiation at non-canonical start codons leads to leak expression when incorporating unnatural amino acids. *Scientific reports*, 5(1), 11866.
- Keller, A., Rödel, M. O., Linsenmair, K. E., & Grafe, T. U. (2009). The importance of environmental heterogeneity for species diversity and assemblage structure in Bornean stream frogs. *Journal of Animal Ecology*, 78(2), 305-314.
- Khan, F., Solari, S., Swier, V., Larsen, P., Abdullah, M., & Baker, R. (2010). Systematics of Malaysian woolly bats (*Vespertilionidae kerivoula*) inferred from mitochondrial, nuclear, karyotypic, and morphological data. *Journal of Mammalogy*, 91(5), 1058-1072.
- Khattak, R. H., Teng, L., Ahmad, S., Bari, F., Rehman, E. U., Shah, A. A. & Liu, Z. (2022). In pursuit of new spaces for threatened mammals: Assessing habitat suitability for Kashmir markhor (*capra falconeri cashmeriensis*) in the Hindukush range. *Sustainability*, 14(3), 1544.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of molecular evolution*, 16, 111-120.

- Knight, C., Parris, M., & Gutzke, W. (2008). Influence of priority effects and pond location on invaded larval amphibian communities. *Biological Invasions*, 2009(11), 1033-1044.
- Konopik, O., Linsenmair, K. E., & Grafe, T. U. (2014). Road construction enables establishment of a novel predator category to resident anuran community: A case study from a primary lowland Bornean rain forest. *Journal of Tropical Ecology*, 30(1), 13-22.
- Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, 35(6), 1547-1549
- Kurniawan, N., Islam, M. H., Djong, T. H., Igawa, T., Daicus, M. B., Yong, H. S., & Sumida, M. (2010). Genetic divergence and evolutionary relationship in *Fejervarya cancrivora* from Indonesia and other Asian countries inferred from allozyme and mtDNA sequence analysis. *Zoological Sciences*, 27(3), 222-233.
- Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., & Higgins, D. G. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics*, 23(21), 2947-2948.
- Lawson, L. P. (2013). Diversification in a biodiversity hot spot: Landscape correlates of phylogeographic patterns in the African spotted reed frog. *Molecular Ecology*, 22(7), 1947-1960.
- Lin, H., Cheng, L., Chen, P., & Chang, M. (2008). Involving local communities in amphibian conservation: Taipei frog *Rana taipehensis* as an example. *International Zoo Yearbook*, 42(1), 90-98.
- Lynch, M., & Crease, T. J. (1990). The analysis of population survey data on DNA sequence

- variation. *Molecular biology and evolution*, 7(4), 377-394.
- Mah, D. Y. S., Hii, C. P., Ong, C., & Pui, Y. M. (2017). Evaluation of flooding in Sg Gita catchment, Kuching. *Journal of Applied Science & Process Engineering*, 4(1), 127-141.
- Marcaida, A. J., Nakao, M., Fukutani, K., Nishikawa, K., & Urabe, M. (2022). Phylogeography of *Rhabdias* species. (Nematoda: Rhabdiasidae) collected from bufo species in Honshu, Shikoku, and Kyushu, Japan including possible cryptic species. *Parasitology International*, 90, 102612.
- Matsui, M. (2011). Taxonomic revision of one of the Old World's smallest frogs, with description of a new Bornean Microhyla (Amphibia, Microhylidae). *Zootaxa*, 2814(1), 33-49.
- Matsui, M., Mohamed, M., Shimada, T., & Sudin, A. (2007). Resurrection of *Staurois parvus* from *S. tuberilinguis* from Borneo (Amphibia, Ranidae). *Zoological Science*, 24(1), 101-106,
- Matsui, M., Nishimura, T., Eto, K., Kuraishi, N., Hamidy, A., Nishikawa, K., & Sudin, A. (2015). Genetic divergence in a Bornean fanged frog *Limnonectes leporinus* (Dicoglossidae, Anura). *Current herpetology*, 34(1), 28-37.
- Matsui, M., Nishimura, T., Eto, K., Kuraishi, N., Hamidy, A., Nishikawa, K., & Sudin, A. (2015). Genetic divergence in a Bornean fanged frog *Limnonectes leporinus* (Dicoglossidae, Anura). *Current Herpetology*, 34(1), 28-37.
- Matsui, M., Shimada, T., & Sudin, A. (2013). A new gliding frog of the genus *Rhacophorus* from Borneo. *Current Herpetology*, 32(2), 112-124.
- McLeod, D. S. (2010). Of least concern? Systematics of a cryptic species complex: *Limnonectes kuhlii* (Amphibia: Anura: Dicoglossidae). *Molecular Phylogenetics*

- and Evolution*, 56(3), 991-1000.
- McRae, B. H., Dickson, B. G., Keitt, T. H., & Shah, V. B. (2008). Using circuit theory to model connectivity in ecology, evolution and conservation. *Ecology* 89(10), 2712-2724.
- Merow C., Smith, M. J., & Silander, J. A. (2013). A practical guide to MaxEnt for modelling species' distributions: What it does, and why inputs and settings matter. *Ecography*, 36(10), 1058-1069.
- Mollah, T. H., Shishir, S., & Rashid, M. S. (2021). Climate change impact on the distribution of Tossa jute using maximum entropy and educational global climate modelling. *The Journal of Agricultural Science*, 159(7-8), 500-510.
- Monro, A. K., & Mayo, S. J. (Eds.). (2022). Cryptic species: *Morphological stasis, circumscription, and hidden diversity*. Cambridge University Press.
- Morley, C., Backé, G., Morley, C., Hillis, R., & Tingay, M. (2010). Balancing deformation in nw borneo: Quantifying plate-scale vs. gravitational tectonics in a delta and deepwater fold-thrust belt system. *Marine and Petroleum Geology*, 27(1), 238-246.
- Morona, R., & Gonzalez, A. (2008). Calbindin-D28k and calretinin expression in the forebrain of anuran and urodele amphibians: Further support for newly identified subdivisions. *Journal of Comparative Neurology*, 511(2), 187-220.
- Muir, A. P., Thomas, R., Biek, R., & Mable, B. K. (2013). Using genetic variation to infer associations with climate in the common frog, *Rana temporaria*. *Molecular Ecology*, 22(14), 3737-3751.
- Naqibzadeh, A., Sarhangzadeh, J., Sotoudeh, A., Mashkour, M., & Thomalsky, J. (2021). Habitat suitability modelling for wildlife management objectives by using Maximum Entropy Method. *Journal of Human, Earth, and Future*, 2(4), 371-381.

- Narayanan, S., Das, S., Balan, A., Tom, R., Divakar, N., Kp, R., Hopeland, P., & Deepak, V. (2022). A new species of *Cyrtodactylus* (Reptilia: Gekkonidae) from the southern Western Ghats of India. *Vertebrate Zoology*, 72, 729-743.
- Nguyen, L. T., Poyarkov, N. A., Le, D. T., Vo, B. D., Phan, H. T., Duong, T. V., Murphy, R. W., & Nguyen, S. N. (2018). A new species of *Leptolalax* (Anura: Megophryidae) from Son Tra Peninsula, Central Vietnam. *Zootaxa*, 4388(1), 1-21.
- Nicolas, V., Grandcolas, P., Braux, F., Jourdan, H., Malau, A., Ohler, A., & Couloux, A. (2015). Recent species in old islands: The origin of introduced populations of *Litoria aurea* (Anura: Hylidae) in New Caledonia and Wallis. *Amphibia-Reptilia*, 36(1), 65-81.
- Nielsen, E. S., Hanson, J. O., Carvalho, S. B., Beger, M., Henriques, R., Kershaw, F., & Von der Heyden, S. (2022). Molecular ecology meets systematic conservation planning. *Trends in Ecology & Evolution*, 38(2), 143-155.
- O'Connell, K., Smart, U., Smith, E., Hamidy, A., Kurniawan, N., & Fujita, M. (2018). Within-island diversification underlies parachuting frog (*Rhacophorus*) species accumulation on the Sunda Shelf. *Journal of Biogeography*, 45(4), 929-940.
- Ooi, C. H., Phang, W. K., Liew, J. W. K., Atroosh, W. M., & Lau, Y. L. (2023). Profiling the imported human malaria in Sarawak, Malaysia in 2011-2019. *Tropical Medicine & International Health*, 28(6), 486-500.
- Orton, F., & Tyler, C. (2014). Do hormone-modulating chemicals impact on reproduction and development of wild amphibians? *Biological Reviews*, 90(4), 1100-1117.
- Pabijan, M., Palomar, G., Antunes, B., Antoń, W., Zieliński, P., & Babik, W. (2020). Evolutionary principles guiding amphibian conservation. *Evolutionary Applications*, 13(5), 857-878.

- Palumbi, S., Martin, A., Romano S., McMillan, W. O., Stice, L. & Grabowski G. 1991. *The simple fool's guide to PCR. Version 2.0*. Department of Zoology & Kewalo Marine Laboratory. University of Hawaii.
- Phillips, K. P., Jorgensen, T. H., Jolliffe, K. G., & Richardson, D. S. (2017). Evidence of opposing fitness effects of parental heterozygosity and relatedness in a critically endangered marine turtle?. *Journal of Evolutionary Biology*, 30(11), 1953-1965.
- Phillips, S. J., Dudik, M. (2008). Modelling of species distributions with MaxEnt: New extensions and a comprehensive evaluation. *Ecography* 31(2), 161-175.
- Pitogo, K. M. E., Saavedra, A. J. L., Aurellado, M. E. B., de Guia, A. P. O., & Afuang, L. E. (2021). Functional traits and environment drive montane amphibian distribution in the Southern Philippines. *Biodiversity and Conservation*, 30(14), 4177-4197.
- Preininger, D., Weissenbacher, A., Wampula, T., & Hödl, W. (2012). The conservation breeding of two foot-flagging frog species from Borneo, *Staurois parvus* and *Staurois guttatus*. *Amphibian Reptile Conservation*, 5, 45-56.
- Pui, Y. M. & Das, I. (2016). Streams in forested headwaters as reservoirs of endemism in Bornean amphibians. In I. Das & A. Tuen (Eds.), *Naturalists, explorers and field scientists in South-East Asia and Australasia* (pp. 143-155). SpringerLink.
- Pyron, R. A., & Wiens, J. J. (2011). A large-scale phylogeny of amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Molecular Phylogenetics and Evolution*, 61(2), 543-583.
- Radosavljevic, A., & Anderson, R. P. (2014). Making better MaxEnt models of species distributions: Complexity, overfitting and evaluation. *Journal of biogeography*, 41(4), 629-643.
- Rahman, W. A., Andy, T., & Sufina. I. (2008). On the parasitic fauna of two species of

- anurans collected from Sungai Pinang, Penang Island, Malaysia. *Tropical Biomedicine*, 25(2), 160-165.
- Rees, P. A. (2021). Fishes, amphibians and reptiles. In G. Torkar (Eds.), *Key questions in biodiversity: A study and revision guide* (pp. 136-152). Journal for Nature Conservation. Wallingford: CABI.
- Reilly, S. B., Stubbs, A. L., Karin, B. R., Bi, K., Arida, E., Iskandar, D. T., & McGuire, J. A. (2019). Leap-frog dispersal and mitochondrial introgression: Phylogenomics and biogeography of *Limnonectes* fanged frogs in the lesser Sundas Archipelago of Wallacea. *Journal of Biogeography*, 46(4), 757-769.
- Remya, K., Ramachandran, A., & Jayakumar, S. (2015). Predicting the current and future suitable habitat distribution of *Myristica dactyloides* Gaertn using MaxEnt model in the Eastern Ghats, India. *Ecological Engineering*, 82, 184-188.
- Renner, I., & Warton, D. I. (2013). Equivalence of MaxEnt and poisson point process models for species distribution modelling in ecology. *Biometrics*, 69(1), 274-281.
- Rico-Guevara, A., & Hurme, K. J. (2019). Intrsexually selected weapons. *Biological Reviews*, 94(1), 60-101.
- Rodríguez-Mendoza, C. A., & Pineda, E. (2010). Importance of riparian remnants for frog species diversity in a highly fragmented rainforest. *Biology Letters*, 6(6), 781-784.
- Roh, N., Park, J., Kim, J., Kwon, H., & Park, D. (2022). Prevalence of Ranavirus infection in three anuran species across South Korea. *Viruses*, 14(5), 1073.
- Rojas, I. M., Jennings, M. K., Conlisk, E., Syphard, A. D., Mikesell, J., Kinoshita, A. M., & Lewison, R. L. (2022). A landscape-scale framework to identify refugia from multiple stressors. *Conservation Biology*, 36(1), e13834.
- Roos, M. C., Kessler, P. J., Robbert Gradstein, S., & Baas, P. (2004). Species diversity and

- endemism of five major Malesian islands: Diversity-area relationships. *Journal of Biogeography*, 31(12), 1893-1908.
- Royle, J., Fuller, A., & Sutherland, C. (2017). Unifying population and landscape ecology with spatial capture-recapture. *Ecography*, 41(3), 444-456.
- Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E., & Sánchez-Gracia, A. (2017). DnaSP 6: DNA Sequence Polymorphism Analysis of large datasets. *Molecular Biology Evolution*, 34(12), 3299-3302.
- Santos, J. M., Rivaes, R., Boavida, I., & Branco, P. (2018). Structural microhabitat use by endemic cyprinids in a Mediterranean-type river: Implications for restoration practices. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 28(1), 26-36.
- Scheldeman, X., & van Zonneveld, M. (2010). *Training manual on spatial analysis of plant diversity and distribution*. Biodiversity International, Rome.
- Scherer, R., Muths, E., Noon, B. (2012). The importance of local and landscape-scale processes to the occupancy of wetlands by pond-breeding amphibians. *Population Ecology*, 54(4), 487-498.
- Scroggie, M. P., Preece, K., Nicholson, E., McCarthy, M. A., Parris, K. M., & Heard, G. W. (2019). Optimizing habitat management for amphibians: From simple models to complex decisions. *Biological Conservation*, 236, 60-69.
- Semlitsch, R., & Bodie, J. (2003). Biological criteria for buffer zones around wetlands and riparian habitats for amphibians and reptiles. *Conservation Biology*, 17(5), 1219-1228.
- Sheldon, B., Arponen, H., Laurila, A., Crochet, P., & Merilä, J. (2003). Sire colouration influences offspring survival under predation risk in the Moorfrog. *Journal of Evolutionary Biology*, 16(6), 1288-1295.

- Shimada, T., Matsui, M., Yambun, P., & Sudin, A. (2011). A taxonomic study of Whitehead's torrent frog, *Meristogenys whiteheadi*, with descriptions of two new species (Amphibia: Ranidae). *Zoological Journal of the Linnean Society*, 161(1), 157-183.
- Steffoff, R. (2008). *The Amphibian Class* (K. Ang, Ed.). Marshall Cavendish.
- Tajima, F. (1989). Statistical methods for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, 123, 585-595.
- Tan, W. C., Herrel, A., & Rödder, D. (2023). A global analysis of habitat fragmentation research in reptiles and amphibians: What have we done so far?. *Biodiversity and Conservation*, 32(2), 439-468.
- Taubert, F., Fischer, R., Groeneveld, J., Lehmann, S., Müller, M. S., Rödig, E., & Huth, A. (2018). Global patterns of tropical forest fragmentation. *Nature*, 554(7693), 519-522.
- Temple, H., Hoffman, J., & Amos, W. (2006). Dispersal, philopatry and intergroup relatedness: Fine-scale genetic structure in the White-breasted thrasher, *Ramphocinclus Brachyurus*. *Molecular Ecology*, 15(11), 3449-3458.
- Trakimas, G., Whittaker, R., & Borregaard, M. (2016). Do biological traits drive geographical patterns in European amphibians? *Global Ecology Biogeography*, 25(10), 1228-1238.
- Vedernikov, A. A., Svinin, A. O., Ermakov, O. A., Chelyadnikova, Y. A., Musatov, G. A., & Drobot, G. P. (2020). Granulomatous inflammations in the intestine of *Pelophylax ridibundus* (Anura: Ranidae) caused by *Brandesia turgida* (Plathelminthes: Digenea). *Nature Conservation Research*, 5(2S), 72-76.
- Vogt, P., Riitters, K. H., Iwanowski, M., Estreguil, C., Kozak, J., & Soille, P. (2007). Mapping landscape corridors. *Ecological indicators*, 7(2), 481-488.

- Walrath, J. D., Dauwalter, D. C., & Reinke, D. (2016). Influence of stream condition on habitat diversity and fish assemblages in an impaired upper Snake River basin watershed. *Transactions of the American Fisheries Society*, 145(4), 821-834.
- Watling, J. I., & Braga, L. (2015). Desiccation resistance explains amphibian distributions in a fragmented tropical forest landscape. *Landscape ecology*, 30, 1449-1459.
- West, Josh. (2018). *Importance of amphibians: A synthesis of their environmental functions, benefits to humans, and need for conservation*. (Publication No. 261) [Master's thesis, Bridgewater State University]. Bridgewater State University Virtual Commons.
- Wettasin, M., Chaiyarat, R., Youngpoy, N., Jieychien, N., Sukmasuang, R., & Tanhan, P. (2023). Environmental factors induced crop raiding by wild Asian elephant (*Elephas maximus*) in the Eastern Economic Corridor, Thailand. *Scientific Reports*, 13(1), 13388.
- Wu, J., & Levin, S. (1994). A Spatial patch dynamic modelling approach to pattern and process in an annual grassland. *Ecological Monographs*, 64(4), 447-464.
- Zainudin, R., & Naim, N. (2018). Genetic variation of the bornean fanged frog, *Limnonectes kuhlii* complex in western Borneo (Amphibia: Anura: Dicroglossidae). *Malaysian Applied Biology*, 47(1), 73-80.
- Zainudin, R., 2004. High levels of mitochondrial DNA diversity in populations of Bornean *Limnonectes leporinus*. In: Maryati, M., Matsui, M., & Boon, K. H. (Eds.), *Proceedings of the International Conference on Bornean Herpetology*, Universiti Malaysia Sabah, Malaysia, 2004. Herpetology and The Future (pp. 64-71).
- Zainudin, R., Deka, E. Q., Amram, M. F., Mohd Rais, S., Sungif, N. A., Agoh, M. M. A., Alaudin, N. A., Azmi, M. A., & Mohd Azlan, J. (2019a). Significant quality of

- fragmented forests in oil palm plantations: Lesson from the assemblage structure of frogs (Amphibia: Anura). *Journal of Oil Palm Research*, 31(4), 604-614.
- Zainudin, R., Deka, E. Q., & Puad, A. S. A. (2019b). Habitat preferences of Bornean horned frog, *Megophrys nasuta* (Schlegel, 1858) (Anura: Megophryidae) in Sarawak. *Journal of Sustainability Science and Management*, 14(4), 38-50.
- Zainudin, R., Deka, E. Q., Amram, M. F., Rais, S. M., Sungif, N. A., Agoh, M. M. A., & Jayasilan, M. A. (2019). Significant quality of fragmented forests in oil palm plantations: Lesson from the assemblage structure of frogs (Amphibia: Anura). *Journal of Oil Palm Research*, 31(4), 604-614.
- Zainudin, R., Nor, S. M., Ahmad, H., Md-Zain, B. M., & Rahman, A. M. (2010). Genetic structure of *Hylarana erythraea* (Amphibia: Anura: Ranidae) from Malaysia. *Zoological Studies*, 49(5), 688-702.
- Zainudin, R., Nor, S. M., Ahmad, N., Md-Zain, B. M., & Rahman, M. A. (2010). Genetic structure of *Hylarana erythraea* (Amphibia: Anura: Ranidae) from Malaysia. *Zoological Studies*, 49(5), 688-702.
- Zainudin, R., Nor, S. M., Ahmad, N., Md-Zain, B. M., & Rahman, M. A. (2010). Genetic structure of *Hylarana erythraea* (Amphibia: Anura: Ranidae) from Malaysia. *Zoological Studies*, 49(5), 688-702.
- Zainudin, R., Zain, B. M. M., Ahmad, N., & Nor, S. M. (2017). Microhabitat partitioning of closely related Sarawak (Malaysian Borneo) frog species previously assigned to the genus *Hylarana* (Amphibia: Anura). *Turkish Journal of Zoology*, 41, 876-891.
- Zainudin, R., Zain, B. M., Ahmad, N., & Nor, S. M. (2017). Microhabitat partitioning of closely related Sarawak (Malaysian Borneo) frog species previously assigned to the genus *Hylarana* (Amphibia: Anura). *Turkish Journal of Zoology*, 41(5), 876-891.

- Zamroni, Y., Septian, I., Artiningrum, N., & Hadi, I. (2021). Dietary niche breadth of endemic and introduced anurans (Amphibia: Anura) in Lombok, lesser Sunda islands-Indonesia. *Earth and Environmental Science*, 913(1), 012046.
- Zhang, K., Zhang, Y., Jia, D., & Tao, J. (2020). Species distribution modelling of *Sassafras tzumu* and implications for forest management. *Sustainability*, 12(10), 4132.
- Zhou, Q., Xiang, H. M., Zhang, M. Y., Liu, Y., Gu, Z. R., Lan, X. Y., & Jiang, W. S. (2023). Two complete mitochondrial genomes of *Leptobrachium* (Anura: Megophryidae: Leptobrachiiinae): Characteristics, population divergences, and phylogenetic implications. *Genes*, 14(3), 768.
- Zulkefli, N. H., & Zainudin, R. (2022). Ecological guilds of anuran species from different ecosystem types in Malaysia, Borneo (Sarawak). *Journal of Sustainability Science and Management*, 17(4), 260-271.

APPENDICES

Appendix 1: Microhabitat data

Table 1: Characteristics of a microhabitat checklist (Inger's Habitat Code in Hayer et al., 1994)

Characteristics	Code	Notes
Habitat:	VA	Primary rain forest, hilly
Vegetation type	VB	Primary rain forest, flat
	VC	Deciduous dipterocarp
	VW	Peat Swamp
	VK	Kerangas
	VRF	Riverine Forest
	VAgr	Agriculture
	VS	Marsh
	VE	Edge MDF
	VF	Large clearing (camp etc)
	VG	Secondary growth, immature or regenerating forest
	VH	Gallery forest
	VJ	Selectively logged forest
	VR	Rubber or oil palm planting
	VT	Oak/chestnut montane forest
Microhabitat:		
Horizontal position	HPA	Permanent stream: actually, in water
	HPB	Permanent stream: midstream on bar, rock, or snag
	HPC	Permanent stream: on bank (distance to bank)
	HPD	Intermittent stream: in stream, actually in water
	HPE	Intermittent stream: midstream on bar, rock or snag
	HPF	Intermittent stream: on bank (distance to bank)
	HPG	Distant from any body of water, distance to nearest stream, (m)
	HPH	In dried bed of intermittent stream
	HPJ	Temporary pond, in water
	HPL	Temporary pond, on vegetation
	HPM	Permanent stream, on exposed bed
	HPN	Permanent pond
	HPp	Permanent swamp
	HPQ	On or in building
	HPR	Permanent pond, on bank, distance (m) to water
	HPS	Permanent swamp, in water
	HPT	Permanent stream, on vegetation
	HPU	Permanent drainage, in plantation, on bank
	HPV	Permanent drainage, in plantation, in water

continued....

.... continued

Microhabitat:

Vertical position

VPA	Under surface pf soil: depth (cm)
VPB	In or under dead leaves
VPC	Under rock, maximum dimension (cm) of rock
VPD	Under log (diameter (cm) of log)
VPE	In log (diameter (cm) of log)
VPF	On surface of bare soil
VPG	On surface of leaf litter or dead leaves
VPH	On rock, maximum dimension (cm) of rock
VPJ	On log (diameter (cm) of log)
VPK	On seedling or herbaceous plant (less than 1m tall)
VPL	On shrub or young seedling (Plant,1-7m), height from ground
VPM	On tree or large vine (plant more than 7m) height (m) above ground or water, at breast height (DBH) for woody plant
VPN	On dead stump height (m) above ground
VPO	In crown of fallen dead shrub or tree height (cm) above ground
VPQ	On grass blade height (m) above ground
VPP	In grass

Microhabitat:

Substrate

SA	Leaf of plant, maximum dimension of leaf (cm)
SB	Stem or branch of herbaceous plant
SC	Twig or branch of woody plant, diameter (cm) of perch
SD	Trunk of shrub or tree
SE	In epiphyte
SF	Under bark of log, stump or tree
SG	Bank mud
SH	Bank sand or gravel
SJ	Bank rock

The notion is that for every frog encountered a single notation for each element will describe that microhabitat (Inger in Heyer *et al.*, 1994)

The six elements that were recorded for each observation are as follows:

1. Date and time observation (24hr clock)
2. General location, vegetation type, and elevation

Horizontal position with reference to bodies of water, shade casting vegetation and shore.

Appendix 2: Sarawak map references

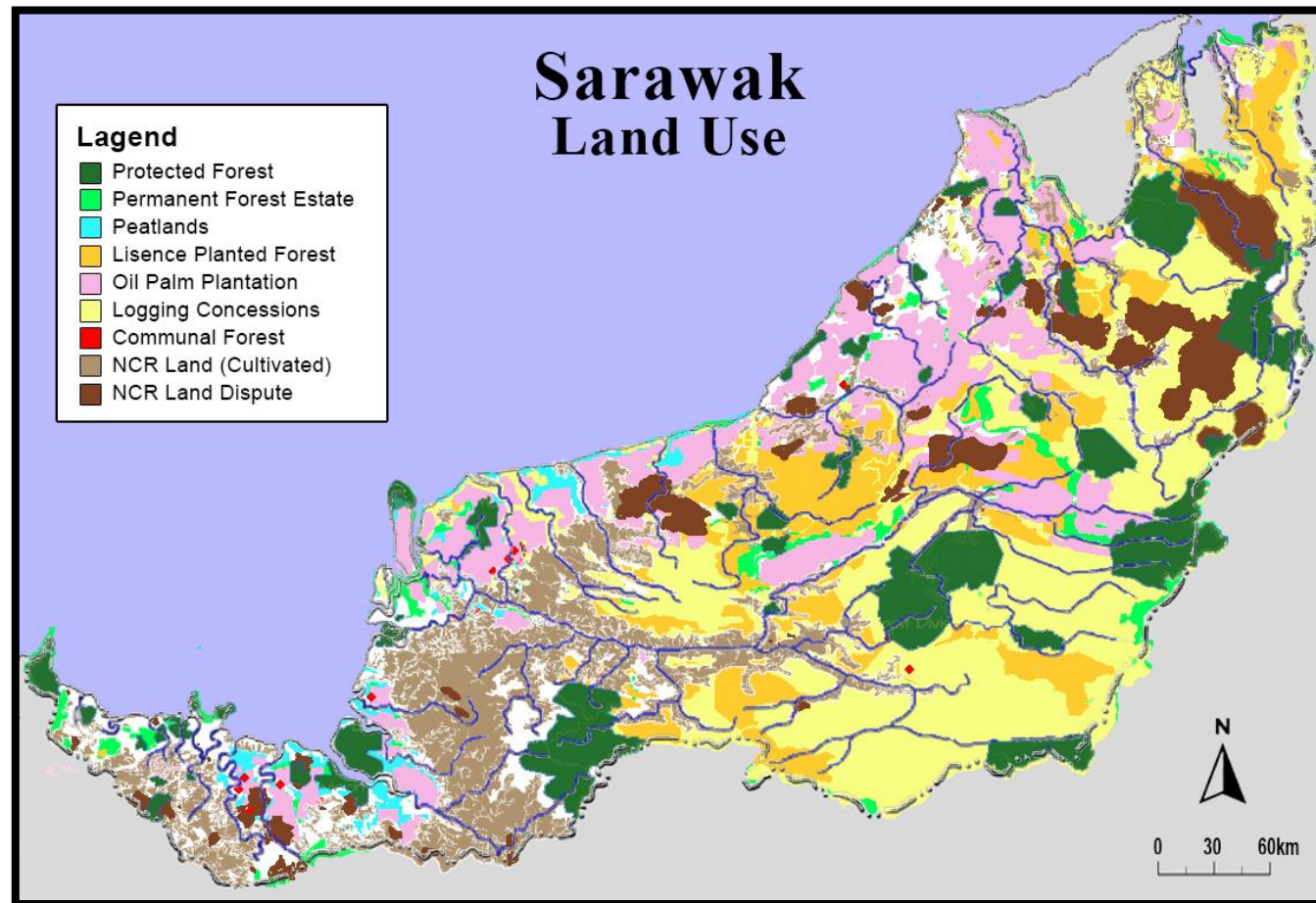


Figure 1: Sarawak land use, generated in ArcGIS Note: NCR land shapefile data from “Sarawak Geoportal”, by Bruno Manser Funds, 2010, Bruno Manser Fonds. (<http://www.bmfmaps.ch/EN/composer/#maps/1001>). Copyright 2010 by Bruno Manser-Fonds

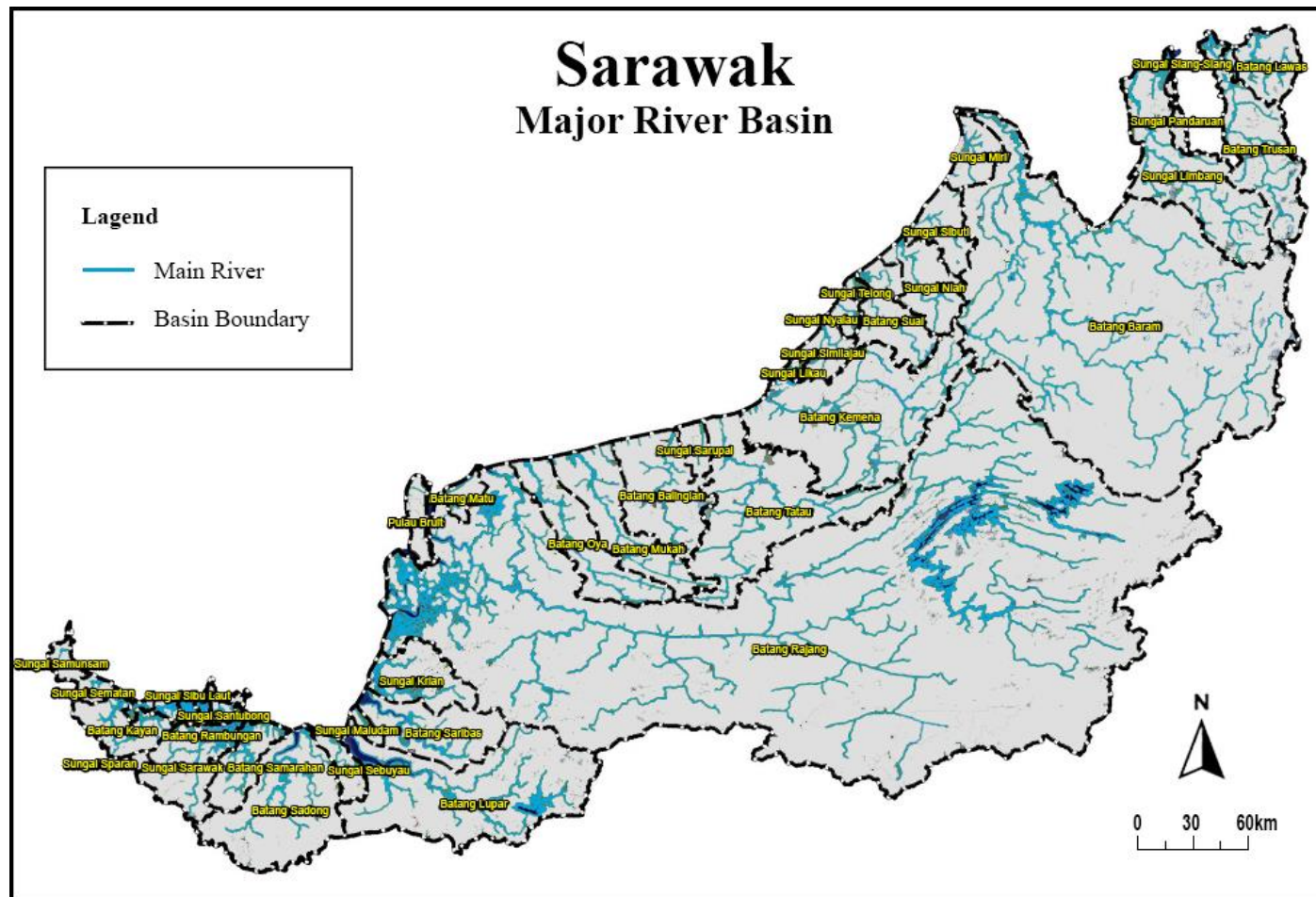


Figure 2: Sarawak major river basin, generated in ArcGIS. Note: Major rivers’ names and boundaries taken from “Integrated river basin management”, by the Department of Irrigation and Drainage Sarawak, *Sarawak Government*. (https://did.sarawak.gov.my/web/Subpage/web_page_view/315). Copyright by Department of Irrigation and Drainage Sarawak

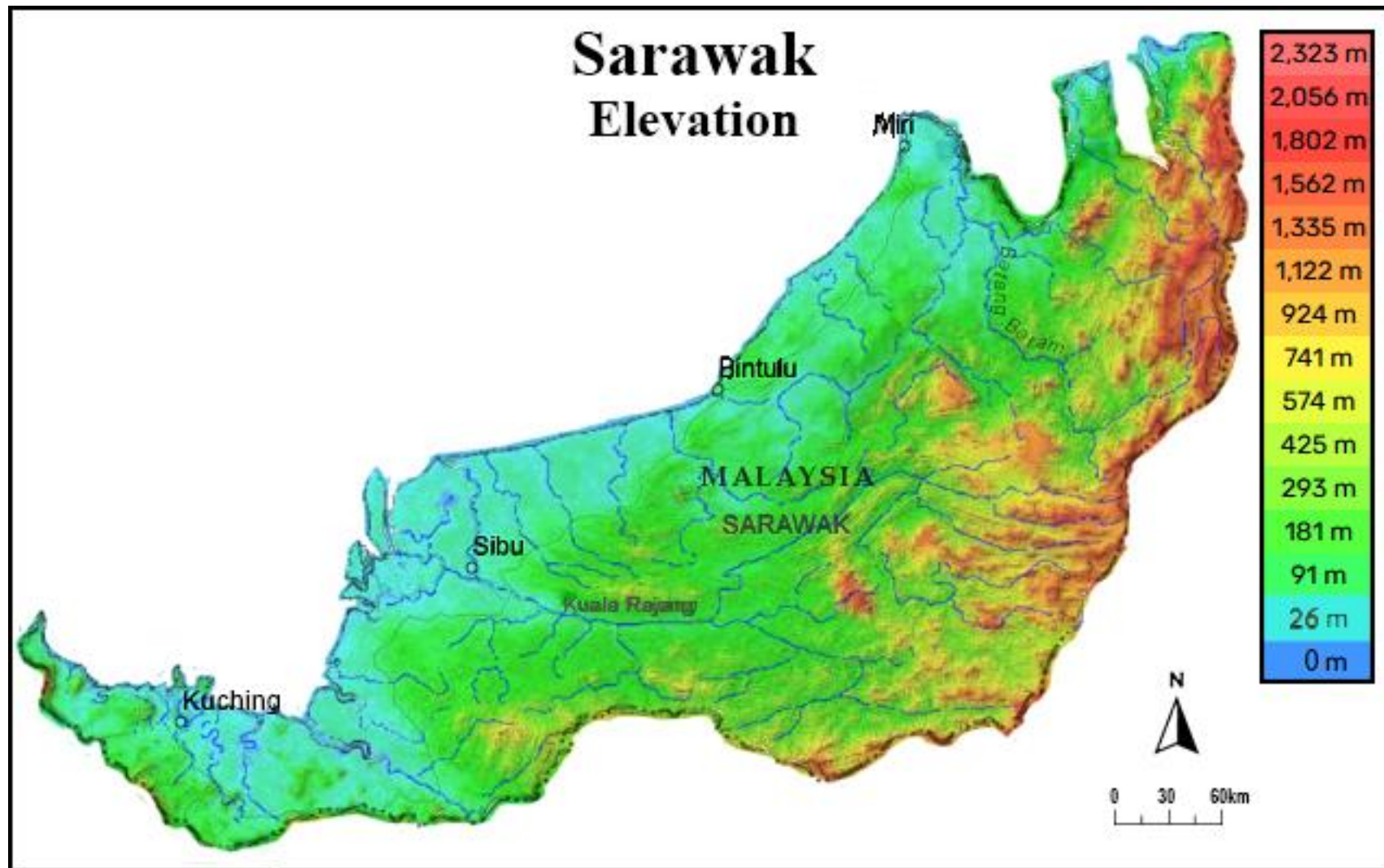


Figure 3: Sarawak elevation map, generated in ArcGIS

Appendix 3: Dataset of DNA sequences used in this study

>Batang_Ai_NP_BA38

AACTACGGCTGACT-TTTACGCAACCTCCATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGGCGCCTTTACTATGGCTCTTAC
CTTTACAAGGAAACCTGAAACATCGGAGTCATCCTTCTATTTTTAGTAAT
AGCAACAGCCTTTATCTATGTCCTTCCATGGGGCCAAATATCATTTTTGCG
CCACAGTAATCACCAACCTTTTATCCGCAGCTCCTTACATCGGCACCCTA
GTTCAATGAATCTGTGGCTTCTCAGTCAACGCCACCCTAACTCGATTTTT
CTCCTTTTCATTTTTGTCTTACCATTGCGCATCGCAGCAAGCATAATCCACC
TCCTTTTCCTTCACCAAACATCATCAAATCCCACCCTAAACCAAAACCTA
GTGCCGTTTCATCTTTACTTTTCATACTTATTCTTCACTATCCTAAT
CGGTGCACTAGCCACTCTATCAACCTTTGCCCCCCTTTTTCCCAACTTTA
TCCCAGCCCCCCTAGTCACACCTCCTCACATCAAACC

>Batang_Ai_NP_BA40

AACTACGGCTGACT-TTTACGCAACCTCCATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGGCGCCTTTACTATGGCTCTTAC
CTTTACAAGGAAACCTGAAACATCGGAGTCATCCTTCTATTTTTAGTAAT
AGCAACAGCCTTTATCTATGTCCTTCCATGGGGCCAAATATCATTTTTGCG
CCACAGTAATCACCAACCTTTTATCCGCAGCTCCTTACATCGGCACCCTA
GTTCAATGAATCTGTGGCTTCTCAGTCAACGCCACCCTAACTCGATTTTT
CTCCTTTTCATTTTTGTCTTACCATTGCGCATCGCAGCAAGCATAATCCACC
TCCTTTTCCTTCACCAAACATCATCAAATCCCACCCTAAACCAAAACCTA
GTGCCGTTTCATCTTTACTTTTCATACTTATTCTTCACTATCCTAAT
CGGTGCACTAGCCACTCTATCAACCTTTGCCCCCCTTTTTCCCAACTTTA
TCCCAGCCCCCCTAGTCACACCTCCTCACATCAAACC

>Sibu_KN07

AACTACGGCTGACT-TTTACGCAACCTCCATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGGCGCCTTTACTATGGCTCTTAC
CTTTACAAGGAAACCTGAAACATCGGAGTCATCCTTCTATTTTTAGTAAT
AGCAACAGCCTTTATCTATGTCCTTCCATGGGGCCAAATATCATTTTTGCG
CCACAGTAATCACCAACCTTTTATCCGCAGCTCCTTACATCGGTACCCTA

GTTCAATGAATCTGTGGCTTCTCAGTCAACGCCACCCTAACTCGATTTTA
CTCCTTTTCATTTTGTCTTACCATTTCGCCATCGCAGCAAGCATAATCCACC
TCCTTTTCCTTCACCAAACATCATCAAATCCCACCCTAAACCAAAACCTA
GTGCCGTTTCATCTTTACTTTTCATACTTATTCTTCACTATCCTAAT
CGGTGCACTAGCCACTCTATCAACCTTTGCCCCCCTTTTTCCCAACTTTA
TCCCAGCCCCCCTAGTCACACCTCCTCACATCAAACC

>Batang_Ai_NP_BA1001

AACTACGGCTGACT-TTTACGCAACCTCCATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGGCGCCTTTACTATGGCTCTTAC
CTTTACAAGGAAACCTGAAACATCGGAGTCATCCTTCTATTTTTAGTAAT
AGCAACAGCCTTTATCTATGTCCTTCCATGGGGCCAAATATCATTTTGCG
CCACAGTAATCACCAACCTTTTATCCGCAGCTCCTTACATCGGCACCCTA
GTTCAATGAATCTGTGGCTTCTCAGTCAACGCCACCCTAACTCGATTTTT
CTCCTTTTCATTTTGTCTTACCATTTCGCCATCGCAGCAAGCATAATCCACC
TCCTTTTCCTTCACCAAACATCATCAAATCCCACCCTAAACCAAAACCTA
GTGCCGTTTCATCTTTACTTTTCATACTTATTCTTCACTATCCTAAT
CGGTGCACTAGCCACTCTATCAACCTTTGCCCCCCTTTTTCCCAACTTTA
TCCCAGCCCCCCTAGTCACACCTCCTCACATCAAACC

>Sibu_KN08

AACTACGGCTGACT-TTTACGCAACCTCCATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGGCGCCTTTACTATGGCTCTTAC
CTTTACAAGGAAACCTGAAACATCGGAGTCATCCTTCTATTTTTAGTAAT
AGCAACAGCTTTTATCTATGTCCTTCCATGGGGCCAAATATCATTTTGGG
CCACAGTAATCACCAACCTTTTATCCGCAGCTCCTTACATCGGCACCCTA
GTTCAATGAATCTGGGGCTTCTCAGTCAACGCCACCCTAACTCGATTTTT
CTCCTTTTCATTTTGTCTTACCATTTCGCCATCGCAGCAAGCATAATCCACC
TCCTTTTCCTTCACCAAACATCATCAAATCCCACCCTAAACCAAAACCTA
GTGCCGTTTCATCTTTACTTTTCATACTTATTCTTCACTATCCTAAT
CGGTGCACTAGCCACTCTATCAACCTTTGCCCCCCTTTTTCCCAACTTTA
TCCCAGCCCCCCTAGTTACTCCCCCTCATATCAAACC

>Sibu_KN23

AACTACGGCTGACT-TTTACGCAACCTCCATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGGCGCCTTTACTATGGCTCTTAC
CTTTACAAGGAAACCTGAAACATCGGAGTCATCCTTCTATTTTTAGTAAT
AGCAACAGCTTTTATCTATGTCCTTCCATGGGGCCAAATATCATTTTTGCG
CCACAGTAATCACCAACCTTTTATCCGCAGCTCCTTACATCGGCACCCTA
GTTCAATGAATCTGGGGCTTCTCAGTCAACGCCACCCTAACTCGATTTTT
CTCCTTTTCATTTTTGTCTTACCATTTCGCCATCGCAGCAAGCATAATCCACC
TCCTTTTCCTTCACCAAACATCATCAAATCCCACCCTAAACCAAAACCTA
GTGCCGTTTCATCTTTACTTTTCATACAACCTTATTCTTCACTATCCTAAT
CGGTGCACTAGCCACTCTATCAACCTTTGCCCCCCTTTTTCCCAACTTTA
TCCCAGCCCCCCTAGTTACTCCCCCTCATATCAAACC

>Kapit_BEN54

AACTACGGCTGACT-TTTACGCAACCTCCATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGGCGCCTTTACTATGGCTCTTAC
CTTTACAAGGAAACCTGAAACATCGGAGTCATCCTTCTATTTTTAGTAAT
AGCAACAGCTTTTATCTATGTCCTTCCATGGGGCCAAATATCATTTTTGCG
CCACAGTAATCACCAACCTTTTATCCGCAGCTCCTTACATCGGCACCCTA
GTTCAATGAATCTGGGGCTTCTCAGTCAACGCCACCCTAACTCGATTTTT
CTCCTTTTCATTTTTGTCTTACCATTTCGCCATCGCAGCAAGCATAATCCACC
TCCTTTTCCTTCACCAAACATCATCAAATCCCACCCTAAACCAAAACCTA
GTGCCGTTTCATCTTTACTTTTCATACAACCTTATTCTTCACTATCCTAAT
CGGTGCACTAGCCACTCTATCAACCTTTGCCCCCCTTTTTCCCAACTTTA
TCCCAGCCCCCCTAGTTACTCCTCCTCATATCAAACC

>Miri_WILMAR41

AACTACGGCTGACT-TTTACGCAATCTCCATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATCGGGCGCCTTTACTATGGCTCTTAC
CTTTACAAGGAAACCTGAAACATCGGAGTCATTCTCCTATTTTTAGTAAT
AGCAACAGCCTTCATCTATGTTCTTCCATGGGGCCAAATATCATTTCTGCG
CCACAGTAATTACCAATCTTTTATCTGCAGCTCCTTATATCGGCACCCTA
GTTCAATGAATCTGTGGCTTTTCAGTCAACGCCACCCTAACTCGATTTTT
CTCCTTTTCATTTTTGTCTTACCATTTCGCCATCGCCGCAAGCATAATCCACC

TCCTTTTCCTTCACCAAACATCATCAAATCCCACCCTAAACCAAAACCTA
GTACCATTTTCATCTTTACTTTTCATACAACCTTATTCTTCACTATCCTAAT
CGGCGCACTAGCCACTCTATCAACCTTTGCCCCCCTTTTCCCCAACTTTA
TTCCAGCCCCATTAGTCACTCCTCCACATATCAAACC

>Kapit_SAB13

AACTACGGCTGACT-TTTACGCAATCTCCATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATCGGGCGCCTTTACTATGGCTCTTAC
CTTTACAAGGAAACCTGAAACATCGGAGTCATTCTCCTATTTTTAGTAAT
AGCAACAGCCTTCATCTATGTTCTTCCATGGGGCCAAATATCATTCTGCG
CCACAGTAATTACCAATCTTTTATCTGCAGCTCCTTATATCGGCACCCTA
GTTCAATGAATCTGTGGCTTTTCAGTCAACGCCACCCTAACTCGATTTTT
CTCCTTTTCATTTTGTCTTACCATTTGCCATCGCCGCAAGCATAATCCACC
TCCTTTTCCTTCACCAAACATCATCAAATCCCACCCTAAACCAAAACCTA
GTACCATTTTCATCTTTACTTTTCATACAACCTTATTCTTCACTATCCTAAT
CGGCGCACTAGCCACTCTATCAACCTTTGCCCCCCTTTTCCCCAACTTTA
TTCCAGCCCCATTAGTCACTCCTCCACATATCAAACC

>Miri_WILMAR115

AACTACGGCTGACT-TTTACGCAATCTCCATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATCGGGCGCCTTTACTATGGCTCTTAC
CTTTACAAGGAAACCTGAAACATCGGAGTCATTCTCCTATTTTTAGTAAT
AGCAACAGCCTTCATCTATGTTCTTCCATGGGGCCAAATATCATTCTGCG
CCACAGTAATTACCAATCTTTTATCTGCAGCTCCTTATATCGGCACCCTA
GTTCAATGAATCTGTGGCTTTTCAGTCAACGCCACCCTAACTCGATTTTT
CTCCTTTTCATTTTGTCTTACCATTTGCCATCGCCGCAAGCATAATCCACC
TCCTTTTCCTTCACCAAACATCATCAAATCCCACCCTAAACCAAAACCTA
GTACCATTTTCATCTTTACTTTTCATACAACCTTATTCTTCACTATCCTAAT
CGGCGCACTAGCCACTCTATCAACCTTTGCCCCCCTTTTCCCCAACTTTA
TTCCAGCCCCATTAGTCACTCCTCCACATATCAAACC

>Miri_WILMAR42

AACTACGGCTGACT-TTTACGCAATCTCCATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATCGGGCGCCTTTACTATGGCTCTTAC

CTTTACAAGGAAACCTGAAACATCGGAGTCATTCTCCTATTTTTAGTAAT
AGCAACAGCCTTCATCTATGTTCTTCCATGGGGCCAAATATCATTCTGCG
CCACAGTAATTACCAATCTTTTATCTGCAGCTCCTTATATCGGCACCCTA
GTTCAATGAATCTGTGGCTTTTCAGTCAACGCCACCCTAACTCGATTTTT
CTCCTTTCATTTTTGTCTTACCATTTGCCATCGCCGCAAGCATAATCCACC
TCCTTTTCCTTCACCAAACATCATCAAATCCCACCCTAAACCAAAACCTA
GTACCATTTTCATCTTTACTTTTTCATACAACCTTATTCTTCACTATCCTAAT
CGGCGCACTAGCCACTCTATCAACCTTTGCCCCCCTTTTCCCCAACTTTA
TTCCAGCCCCATTAGTCACTCCTCCACATATCAAACC

>G_Gading_NP_GNP131

AACTACGGCTGACT-TTTACGCAATCTCCATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATCGGGCGCCTTTACTATGGCTCTTAC
CTTTACAAGGAAACCTGAAACATCGGAGTCATTCTCCTATTTTTAGTAAT
AGCAACAGCCTTCATCTATGTTCTTCCATGGGGCCAAATATCATTCTGCG
CCACAGTAATTACCAATCTTTTATCTGCAGCTCCTTATATCGGCACCCTA
GTTCAATGAATCTGTGGCTTTTCAGTCAACGCCACCCTAACTCGATTTTT
CTCCTTTCATTTTTGTCTTACCATTTGCCATCGCCGCAAGCATAATCCACC
TCCTTTTCCTTCACCAAACATCATCAAATCCCACCCTAAACCAAAACCTA
GTACCATTTTCATCTTTACTTTTTCATACAACCTTATTCTTCACTATCCTAAT
CGGCGCACTAGCCACTCTATCAACCTTTGCCCCCCTTTTCCCCAACTTTA
TTCCAGCCCCATTAGTCACTCCTCCACATATCAAACC

>Mulu_NP_MBS2_39

AACTACGGCTGACT-TTTACGCAATCTCCATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATCGGGCGCCTTTACTATGGCTCTTAC
CTTTACAAGGAAACCTGAAACATCGGAGTCATTCTCCTATTTTTAGTAAT
AGCAACAGCCTTCATCTATGTTCTTCCATGGGGCCAAATATCATTCTGCG
CCACAGTAATTACCAATCTTTTATCTGCAGCTCCTTATATCGGCACCCTA
GTTCAATGAATCTGTGGCTTTTCAGTCAACGCCACCCTAACTCGATTTTT
CTCCTTTCATTTTTGTCTTACCATTTGCCATCGCCGCAAGCATAATCCACC
TCCTTTTCCTTCACCAAACATCATCAAATCCCACCCTAAACCAAAACCTA
GTACCATTTTCATCTTTACTTTTTCATACAACCTTATTCTTCACTATCCTAAT
CGGCGCACTAGCCACTCTATCAACCTTTGCCCCCCTTTTCCCCAACTTTA

TTCCAGCCCCATTAGTCACTCCTCCACATATCAAACC

>Miri_WILMAR37

AACTACGGCTGACT-TTTACGCAATCTCCATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATCGGGCGCCTTTACTATGGCTCTTAC
CTTTACAAGGAAACCTGAAACATCGGAGTCATTCTCCTATTTTTAGTAAT
AGCAACAGCCTTCATCTATGTTCTTCCATGGGGCCAAATATCATTCTGCG
CCACAGTAATTACCAATCTTTTATCTGCAGCTCCTTATATCGGCACCCTA
GTTCAATGAATCTGTGGCTTTTCAGTCAACGCCACCCTAACTCGATTTTT
CTCCTTTTCATTTTGTCTTACCATTTGCCATCGCCGCAAGCATAATCCACC
TCCTTTTCCTTCACCAAACATCATCAAATCCCACCCTAAACCAAAACCTA
GTACCATTTTCATCTTTACTTTTCATACTTATTCTTCACTATCCTAAT
CGGCGCACTAGCCACTCTATCAACCTTTGCCCCCCTTTTCCCCAACTTTA
TTCCAGCCCCATTAGTCACTCCTCCACATATCAAACC

>Kapit_BEN56

AACTACGGCTGACT-TTTACGCAATCTCCATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATCGGGCGCCTTTACTATGGCTCTTAC
CTTTACAAGGAAACCTGAAACATCGGAGTCATTCTCCTATTTTTAGTAAT
AGCAACAGCCTTCATCTATGTTCTTCCATGGGGCCAAATATCATTCTGCG
CCACAGTAATTACCAATCTTTTATCTGCAGCTCCTTATATCGGCACCCTA
GTTCAATGAATCTGTGGCTTTTCAGTCAACGCCACCCTAACTCGATTTTT
CTCCTTTTCATTTTGTCTTACCATTTGCCATCGCCGCAAGCATAATCCACC
TCCTTTTCCTTCACCAAACATCATCAAATCCCACCCTAAACCAAAACCTA
GTACCATTTTCATCTTTACTTTTCATACTTATTCTTCACTATCCTAAT
CGGCGCACTAGCCACTCTATCAACCTTTGCCCCCCTTTTCCCCAACTTTA
TTCCAGCCCCATTAGTCACTCCTCCACATATCAAACC

>Mulu_NP_RZ299

AACTACGGCTGACT-TTTACGCAATCTCCATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATCGGGCGCCTTTACTATGGCTCTTAC
CTTTACAAGGAAACCTGAAACATCGGAGTCATTCTCCTATTTTTAGTAAT
AGCAACAGCCTTCATCTATGTTCTTCCATGGGGCCAAATATCATTCTGCG
CCACAGTAATTACCAATCTTTTATCTGCAGCTCCTTATATCGGCACCCTA

GTTCAATGAATCTGTGGCTTTTCAGTCAACGCCACCCTAACTCGATTTTT
CTCCTTTTCATTTTGTCTTACCATTTGCCATCGCCGCAAGCATAATCCACC
TCCTTTTCCTTCACCAAACATCATCAAATCCCACCCTAAACCAAAACCTA
GTACCATTTTCATCTTTACTTTTCATACAACCTTATTCTTCACTATCCTAAT
CGGCGCACTAGCCACTCTATCAACCTTTGCCCCCCTTTTCCCCAACTTTA
TTCCAGCCCCATTAGTCACTCCTCCACATATCAAACC

>Mulu_NP_MBS5_300

AACTACGGCTGACT-TTTACGCAATCTCCATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATCGGGCGCCTTTACTATGGCTCTTAC
CTTTACAAGGAAACCTGAAACATCGGAGTCATTCTCCTATTTTTAGTAAT
AGCAACAGCCTTCATCTATGTTCTTCCATGGGGCCAAATATCATTCTGCG
CCACAGTAATTACCAATCTTTTATCTGCAGCTCCTTATATCGGCACCCTA
GTTCAATGAATCTGTGGCTTTTCAGTCAACGCCACCCTAACTCGATTTTT
CTCCTTTTCATTTTGTCTTACCATTTGCCATCGCCGCAAGCATAATCCACC
TCCTTTTCCTTCACCAAACATCATCAAATCCCACCCTAAACCAAAACCTA
GTACCATTTTCATCTTTACTTTTCATACAACCTTATTCTTCACTATCCTAAT
CGGCGCACTAGCCACTCTATCAACCTTTGCCCCCCTTTTCCCCAACTTTA
TTCCAGCCCCATTAGTCACTCCTCCACATATCAAACC

>Mulu_NP_MHQ69

AACTACGGCTGACT-TTTACGCAATCTCCATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATCGGGCGCCTTTACTATGGCTCTTAC
CTTTACAAGGAAACCTGAAACATCGGAGTCATTCTCCTATTTTTAGTAAT
AGCAACAGCCTTCATCTATGTTCTTCCATGGGGCCAAATATCATTCTGCG
CCACAGTAATTACCAATCTTTTATCTGCAGCTCCTTATATCGGCACCCTA
GTTCAATGAATCTGTGGCTTTTCAGTCAACGCCACCCTAACTCGATTTTT
TTCCTTTTCATTTTGTCTTACCATTTGCCATCGCCGCAAGCATAATCCACC
TCCTTTTCCTTCACCAAACATCATCAAATCCCACCCTAAACCAAAACCTA
GTACCATTTTCATCTTTACTTTTCATACAACCTTATTCTTCACTATCCTAAT
CGGTGCACTAGCCACTCTATCAACCTTTGCCCCCCTTTTCCCCAACTTTA
TTCCAGCCCCATTAGTCACTCCTCCACATATCAAACC

>Mulu_NP_MHQ25

AACTACGGCTGACT-TTTACGCAATCTCCATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATCGGGCGCCTTTACTATGGCTCTTAC
CTTTACAAGGAAACCTGAAACATCGGAGTCATTCTCCTATTTTTTAGTAAT
AGCAACAGCCTTCATCTATGTTCTTCCATGGGGCCAAATATCATTCTGCG
CCACAGTAATTACCAATCTTTTATCTGCAGCTCCTTATATCGGCACCCTA
GTTCAATGAATCTGTGGCTTTTCAGTCAACGCCACCCTAACTCGATTTTT
TTCCTTTCATTTTTGTCTTACCATTTGCCATCGCCGCAAGCATAATCCACC
TCCTTTTCCTTCACCAAACATCATCAAATCCCACCCTAAACCAAAACCTA
GTACCATTTTCATCTTTACTTTTCATACAACCTTATTCTTCACTATCCTAAT
CGGTGCACTAGCCACTCTATCAACCTTTGCCCCCCTTTTCCCCAACTTTA
TTCCAGCCCCATTAGTCACTCCTCCACATATCAAACC

>Mulu_NP__MBS1_24

AACTACGGCTGACT-TTTACGCAATCTCCATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATCGGGCGCCTTTACTATGGCTCTTAC
CTTTACAAGGAAACCTGAAACATCGGAGTCATTCTCCTATTTTTTAGTAAT
AGCAACAGCCTTCATCTATGTTCTTCCATGGGGCCAAATATCATTCTGCG
CCACAGTAATTACCAATCTTTTATCTGCAGCTCCTTATATCGGCACCCTA
GTTCAATGAATCTGTGGCTTTTCAGTCAACGCCACCCTAACTCGATTTTT
CTCCTTTCATTTTTGTCTTACCATTTGCCATCGCCGCAAGCATAATCCACC
TCCTTTTCCTTCACCAAACATCATCAAATCCCACCCTAAACCAAAACCTA
GTACCATTTTCATCTTTACTTTTCATACAACCTTATTCTTCACTATCCTAAT
CGGTGCACTAGCCACTCTATCAACCTTTGCCCCCCTTTTCCCCAACTTTA
TTCCAGCCCCATTAGTCACTCCTCCACATATCAAACC

>Bario_RZ14

AACTACGGCTGACT-CTTGCGCAGTCTCCATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATCGGGCGCCTTTACTATGGCTCTTAC
CTTTACAAGGAAACCTGAAACATCGGAGTCATTCTCCTATTTTTTAGTAAT
AGCAACAGCCTTCATCTATGTTCTTCCATGGGGCCAAATATCATTCTGCG
CCACAGTAATTACCAATCTTTTATCCGCAGCTCCTTATATCGGCACCCTA
GTTCAATGAATCTGTGGCTTTTCAGTCAACGCCACCCTAACTCGATTTTT
CTCCTTTCATTTTTGTCTTACCATTTGCCATCGCCGCAAGCATAATCCACC

TCCTTTTCCTTCACCAAACATCATCAAATCCCACCCTAAACCAAAACCTA
GTACCATTTTCATCTTTACTTTTCATACAACCTTATTCTTCACTATCCTAAT
CGGTGCACTAGCCACTCTATCAACCTTTGCCCCCCTTTTCCCCAACTTTA
TTCCAGCCCCATTAGTCACTCCTCCACATATCAAACC

>Bario_RZ34

AACTACGGCTGACT-CTTGCGCAGTCTCCATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATCGGGCGCCTTTACTATGGCTCTTAC
CTTTACAAGGAAACCTGAAACATCGGAGTCATTCTCCTATTTTTAGTAAT
AGCAACAGCCTTCATCTATGTTCTTCCATGGGGCCAAATATCATTCTGCG
CCACAGTAATTACCAATCTTTTATCCGCAGCTCCTTATATCGGCACCCTA
GTTCAATGAATCTGTGGCTTTTCAGTCAACGCCACCCTAACTCGATTTTT
CTCCTTTTCATTTTGTCTTACCATTTGCCATCGCCGCAAGCATAATCCACC
TCCTTTTCCTTCACCAAACATCATCAAATCCCACCCTAAACCAAAACCTA
GTACCATTTTCATCTTTACTTTTCATACAACCTTATTCTTCACTATCCTAAT
CGGTGCACTAGCCACTCTATCAACCTTTGCCCCCCTTTTCCCCAACTTTA
TTCCAGCCCCATTAGTCACTCCTCCACATATCAAACC

>Bario_RZ32

AACTACGGCTGACT-CTTGCGCAGTCTCCATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATCGGGCGCCTTTACTATGGCTCTTAC
CTTTACAAGGAAACCTGAAACATCGGAGTCATTCTCCTATTTTTAGTAAT
AGCAACAGCCTTCATCTATGTTCTTCCATGGGGCCAAATATCATTCTGCG
CCACAGTAATTACCAATCTTTTATCCGCAGCTCCTTATATCGGCACCCTA
GTTCAATGAATCTGTGGCTTTTCAGTCAACGCCACCCTAACTCGATTTTT
CTCCTTTTCATTTTGTCTTACCATTTGCCATCGCCGCAAGCATAATCCACC
TCCTTTTCCTTCACCAAACATCATCAAATCCCACCCTAAACCAAAACCTA
GTACCATTTTCATCTTTACTTTTCATACAACCTTATTCTTCACTATCCTAAT
CGGTGCACTAGCCACTCTATCAACCTTTGCCCCCCTTTTCCCCAACTTTA
TTCCAGCCCCATTAGTCACTCCTCCACATATCAAACC

>Mulu_NP_MBS5_299

AACTACGGCTGACT-TTGCGCAGTCTCCATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATCGGGCGCCTTTACTATGGCTCTTAC

CTTTACAAGGAAACCTGAAACATCGGAGTCATTCTCCTATTTTTAGTAAT
AGCAACAGCCTTCATCTATGTTCTTCCATGGGGCCAAATATCATTCTGCG
CCACAGTAATTACCAATCTTTTATCTGCAGCTCCTTATATCGGCACCCTA
GTTCAATGAATCTGTGGCTTTTCAGTCAACGCCACCCTAACTCGATTTTT
CTCCTTTCATTTTTGTTCTACCATTTGCCATCGCCGCAAGCATAATCCACC
TCCTTTTCCTTCACCAAACATCATCAAATCCCACCCTAAACCAAAACCTA
GTACCATTTTCATCTTTACTTTTTCATACAACCTTATTCTTCACTATCCTAAT
CGGTGCACTAGCCACTCTATCAACCTTTGCCCCCCTTTTCCCCAACTTTA
TTCCAGCCCCATTAGTCACTCCTCCACATATCAAACC

>Padawan_ASSUM49

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
GGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCCACCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Bau_J20

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
GGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCCACCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA

TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Bau_68

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
GGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCCAACCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Bau_1214

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
GGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCCAACCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Matang_WC_MWC1531

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
GGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCCTTACATCGGCACCCTC

GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCACCCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Padawan_BH198

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
GGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCACCCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Matang_WC_MWC1433

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
GGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCACCCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Padawan_ASSUM44

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
GGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCACCCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Kubah_NP_KNP22

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
GGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCACCCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Kubah_NP_KNP1768

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
GGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC

TCCTTTTCCTTCACCAAACATCATCAAACCCACCCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>G_Gading_NP_GNP43

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
GGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCACCCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Padawan_BH202

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
GGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCACCCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Bau_1213

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT

CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
GGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCCACCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Padawan_GR151

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
GGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCCACCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Padawan_BH203

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
GGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCCACCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA

TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Matang_WC_MWC1443

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
GGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCACCCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Matang_WC_MWC1460

AGCTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
GGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCACCCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Bau_1211

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
GGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCCTTACATCGGCACCCTC

GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCACCCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCACACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Kubah_NP_KNP1224

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
GGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCTACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCACCCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>G_Gading_NP_GGNP14

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
GGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCCCCTTGACTCGATTTTT
CCCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCACCCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCCCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCACCCCCCTTAGTAACCCCTCCGCATATCAAACC

>Kubah_NP_KNP33

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
GGCAACAGCCTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCACCCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCTTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Kubah_NP_KNP27

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
GGCAACAGCCTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCACCCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCTTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Kubah_NP_KNP10

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
GGCAACAGCCTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC

TCCTTTTCCTTCACCAAACATCATCAAACCCACCCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCTTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Kubah_NP_KNP_140338

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
GGCAACAGCCTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCACCCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCTTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Kubah_NP_KNP1762

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
GGCAACAGCCTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCACCCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCTTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Kubah_NP_KNP32

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT

CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
GGCAACAGCCTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCACCCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCTTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Kubah_NP_KNP1415

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
GGCAACAGCCTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCACCCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCTTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>G_Gading_NP_GNP54

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
AGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCACCCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCTTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA

TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>G_Gading_NP_GGNP200301

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
AGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCCAACCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Mulu_NP_MHQ64

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
AGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCCAACCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>G_Gading_NP_GNP55

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
AGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCCTTACATCGGCACCCTC

GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCACCCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Mulu_NP_MHQ68

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAATGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAACATTGGAGTCATTCTCCTATTTTTAGTTAT
AGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCACCCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Sarikei_SCP65

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAACGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAATATTGGAGTCATTCTCCTATTTTTAGTTAT
AGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCACCCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Sarikei_SCP35

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAACGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAATATTGGAGTCATTCTCCTATTTTTAGTTAT
AGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCACCCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Sri_Aman_EKL61

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAACGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAATATTGGAGTCATTCTCCTATTTTTAGTTAT
AGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCACCCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Sri_Aman_EKL58

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAACGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAATATTGGAGTCATTCTCCTATTTTTAGTTAT
AGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC

TCCTTTTCCTTCACCAAACATCATCAAACCCCACCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Sarikei_SCP74

AACTACGGCTGACT-TTTGCGCAACCTACATGCCAACGGAGCATCCTTCT
TCTTCATCTGTATCTACACACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAATATTGGAGTCATTCTCCTATTTTTAGTTAT
AGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCCACCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>Sri_Aman_EKL59

ACCTACGGGTGACGATTTGCGCTATTTGCATGACAACGGAGCATCCTTCT
TCTTCATCTGTATCTACATACACATTGGACGCCTTTACTATGGCTCTTAT
CTTTACAAGGAAACCTGAAATATTGGAGTCATTCTCCTATTTTTAGTTAT
AGCAACAGCTTTTATCTACGTCCTACCATGGGGCCAAATATCATTCTGCG
CCACAGTTATTACCAACCTCTTATCCGCAGCCCCTTACATCGGCACCCTC
GTTCAATGGATCTGGGGCTTTTCAGTCAACGCCACCTTGACTCGATTTTT
CTCCTTTTCACTTTGTCTTACCATTTGCCATCGCAGCAAGCATAATCCATC
TCCTTTTCCTTCACCAAACATCATCAAACCCCACCCTAAATCAAAACCTA
GTACCATTTTCATCTTTACTTTTCCTACAACCTATTCTTCACCATTTTAAT
CGGCGCACTAGCCATTCTCTCAACCTTCGCTCCCCTCTTTCCCAACTTCA
TCCCAGCCCCTTTAGTAACCCCTCCGCATATCAAACC

>*L._paramacrodon*

AATTACGGCTGACT-CCTACGCAACCTCCACGCCAACGGAGCATCTTTTT
TCTTTATCTGCATCTACCTTCACATTGGACGACTTTACTACGGCTCCTAC
CTATAACAAGGAGACTTGAAACATCGGAATCGTACTTCTCTTTCTAGTTAT
AGCAACAGCCTTCATTTATGTTCTCCCATGGGGCCAAATATCATTCTGGG
CCACAGTTATTACGAATCTTCTCTCCGCAGCTCCTTACATTGGCACCCCTT
GTTCAATGAATTTGGGGATTTTCAGTCAACGCCACTCTAACACGGTTCTT
CTCATTCCATTTTCGTCTCCCATTTGCAATTGCTCTAAGCATAATTCACC
TCCTATTCCTTCATCAAACATCATCAAACCCCAACCCTAAACCAAACTTA
ATTCCATTTTCATCCTTACTTCTCCTACAACCTCTTATTCTCTATCCTGAT
TGGAGCACTTGCCTGCCTATCAACTTTTGCCCCCCTTTTCCCCAACTTCA
TCCCAGCCCCACTAATAACACCGCCTCATATCAAACC

Table 2: Pairwise genetic distance of 62 individuals analysed based on Hasegawa-Kishino-Yano parameter model

127

Table 2: Continue

[illegible]

Table 2: Continue

Batang_Ai_NP_BA38	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62
Batang_Ai_NP_BA40																
Sibu_KN07																
Batang_Ai_NP_BA1001																
Sibu_KN08																
Sibu_KN23																
Kapit_BEN54																
Bintulu_WILMAR41																
Kapit_SAB13																
Bintulu_WILMAR115																
Bintulu_WILMAR42																
G_Gading_NP_GNP131																
Mulu_NP_MBS2_39																
Bintulu_WILMAR37																
Kapit_BEN56																
Mulu_NP_RZ299																
Mulu_NP_MBS5_300																
Mulu_NP_MHQ69																
Mulu_NP_MHQ25																
Mulu_NP_MBS1_24																
Bario_RZ14																
Bario_RZ34																
Bario_RZ32																
Mulu_NP_MBS5_299																
Padawan_ASSUM49																
Bau_J20																
Bau_68																
Bau_1214																
Matang_WC_MWC1531																
Padawan_BH198																
Matang_WC_MWC1433																
Padawan_ASSUM44																
Kubah_NP_KNP22																
Kubah_NP_KNP1768																
G_Gading_NP_GNP43																
Padawan_BH202																
Bau_1213																
Padawan_GR151																
Padawan_BH203																
Matang_WC_MWC1443																
Matang_WC_MWC1460																
Bau_1211																
Kubah_NP_KNP1224																
G_Gading_NP_GGNP14																
Kubah_NP_KNP33																
Kubah_NP_KNP27																
Kubah_NP_KNP10																
Kubah_NP_KNP_140338																
Kubah_NP_KNP1762	0.00															
Kubah_NP_KNP32	0.00	0.00														
Kubah_NP_KNP1415	0.00	0.00	0.00													
G_Gading_NP_GNP54	0.00	0.00	0.00	0.00												
G_Gading_NP_GGNP200301	0.01	0.01	0.01	0.01	0.01											
Mulu_NP_MHQ64	0.01	0.01	0.01	0.01	0.01	0.00										
G_Gading_NP_GNP55	0.01	0.01	0.01	0.01	0.01	0.00	0.00									
Mulu_NP_MHQ68	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00								
Sarikei_SCP65	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00							
Sarikei_SCP35	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00						
Sri_Aman_EKL61	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00					
Sri_Aman_EKL58	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
Sarikei_SCP74	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00			
Sri_Aman_EKL59	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00		
L_paramacrodon	0.03	0.03	0.03	0.03	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	
	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.27	0.29

