Faculty of Resource Science and Technology

Diversification of *Pulchrana baramica*, Boettger, 1900 (Amphibia: Ranidae) Lineages as Ecosystem Health Indicators

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Diversification of *Pulchrana baramica*, Boettger, 1900 (Amphibia: Ranidae) Lineages as Ecosystem Health Indicators

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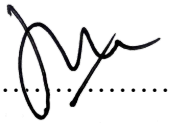
DECLARATION

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.



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ABSTRACT

Amphibian threats and extinctions are well documented on a global level, with approximately 32% of their species currently facing extinction. Borneo has more than 180 species of anurans, and 70% of the species are endemics in Borneo. The divergence of mitochondrial DNA indicates greater evolutionary independence among tropical anuran groups, and frog populations from forests or topographically diverse regions are experiencing evolution. Amphibians are more vulnerable to external environmental changes because of the modification of lands caused by the conversion of forests to agricultural land, which causes the loss of the original habitats for frogs. Moreover, frogs are good indicators, as they can live in arboreal and terrestrial conditions. *Pulchrana baramica* (Boettger, 1900) was chosen as a study model because this species is widely distributed in lowland areas of Sarawak. This study focuses on inland and coastal peat swamp forests, any disturbed area, as well as its adjacent areas of mixed-dipterocarp forests (MDF), to assess the *P. baramica* microhabitat utilisation data, environmental stressor parameters such as heavy metal properties, and genetic diversity through Cytochrome Oxidase Subunit I and Cytochrome B molecular markers. Several locations in western Sarawak were chosen as potentially suitable habitats for *P. baramica*. The selected localities contain a variety of habitats, including both protected and unprotected areas, namely, Tanjung Datu National Park, Libiki Bamboo Resort, Maludam National Park, Mount Singai, Bako National Park, Kota Samarahan (UNIMAS), and Kanowit. Based on the analysis of microhabitat utilisations through a non-metric multidimensional scaling approach, the ecological guild of *P. baramica* were clustered into a few significant guilds: i) *kerangas* forest, ii) peat swamp, iii) plantation, iv) riverine forest, v) agriculture, vi) disturbed area, and vii) edge MDF. Meanwhile, environmental stressors included six water quality parameters, and six heavy metal elements

such as cadmium (Cd), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), and lead (Pb) were sampled from soil and water samples from each sampling site. The average mean concentration of heavy metals in soil and water generally followed the decreasing order: i) soil: Fe > Pb > Mn > Cu > Cd > Ni, and ii) water: Fe > Mn > Pb > Cu > Cd > Ni. The phylogenetic relationships among 11 populations of *P. baramica* through phylogenetic tree construction were clearly described into two major categories of *P. baramica* populations, which were inland peat swamps and coastal peat swamps. Based on population genetic analyses, 26 meaningful haplotypes were defined, and high and low genetic diversity were determined in all populations. Overall, since this study only focused on the Sarawak region, more intensive and prolonged effort is needed to maintain the environment for the sustainability of *P. baramica* populations because more forest lands were converted into commercial areas.

Keywords: Heavy metals, environmental stressors, microhabitat, population genetics, *Pulchrana baramica*

Kepelbagaian Keturunan Katak Pulchrana baramica, Boettger, 1900 (Amphibia: Anura: Ranidae: Pulchrana) Sebagai Indikasi Kepada Kesihatan Ekosistem

ABSTRAK

Ancaman dan kepupusan terhadap amfibia telah derekodkan dengan sempurna di tahap global, dengan anggaran 32% daripada spesies amfibia telah mengalami kepupusan. Borneo memilki lebih daripada 180 spesies anuran, dan 70% adalah spesies yang endemik di Borneo. Perbezaan dari DNA mitokondria menunjukkan kebebasan evolusi yang besar dikalangan kumpulan anuran tropika dan evolusi juga berlaku kepada populasi katak dari hutan atau dari kawasan topografi berbeza. Amfibia juga sangat terdedah kepada perubahan alam sekitar luaran kerana modifikasi tanah disebabkan penerokaan hutan kepada perladangan yang mengakibatkan katak kehilangan habitat. Selain itu, katak juga boleh dijadikan sebagai indikasi semulajadi kerana katak dapat hidup di atas pokok mahupun di atas tanah. Spesis katak Pulchrana baramica (Boettger, 1900) telah dipilih sebagai model kajian kerana taburan spesis ini meluas di sekitar tanah rendah di Sarawak. Kajian ini fokus kepada hutan paya daratan dan perairan,kawasan terganggu dan juga hutan dipterokarpa campuran untuk menilai data penggunaan mikrohabitat oleh P. baramica, parameter tekanan persekitaran seperti sifat logam berat dan kepelbagaian genetik menggunakan Cytochrome Oxidase Subunit I dan Cytochrome b sebagai penanda molekular. Beberapa kawasan di Barat Sarawak yang berpontensi mempunyai habitat yang sesuai untuk P. baramica telah dipilih. Lokaliti terpilih mempunyai pelbagai jenis habitat merangkumi kawasan terlindung dan tidak terlindung, iaitu, Taman Negara Tanjung Datu, Libiki Bamboo Resort, Taman Negara Maludam, Gunung Singai, Taman Negara Bako, Kota Samarahan (UNIMAS), dan Kanowit. Berdasarkan analisis daripada penggunaan mikrohabitat melalui pendekatan non-metric multidimensional scaling, kelompok ekologi P.

baramica telah diklusterkan kepada beberapa kelompok yang ketara seperti, i) hutan kerangas ii) paya, iii) kawasan ladang, iv) hutan sungai, v) kawasan pertanian, vi) kawasan terganggu, dan vii) dipinggiran hutan dipterokarpa campuran. Disamping itu, tekanan persekitaran juga termasuk dengan parameter kualiti air dan enam logam berat seperti kadmium (Cd), kuprum (Cu), ferum (Fe), mangan (Mn), nikel (Ni) dan plumbum (Pb) telah disampelkan dari tanah dan air dari setiap tapak kajian. Purata kepekatan min logam berat di dalam tanah dan air secara umumnya disusun mengikut susunan menurun, i) tanah: Fe > Pb > Mn > Cu > Cd > Ni dan ii) air: Fe > Mn > Pb > Cu> Cd > Ni. Hubungan filogenetik antara populasi P. baramica dari 11 lokaliti melalui unjuran pokok filogenetik telah menerangkan dengan jelas bahawa terdapat dua kumpulan populasi P. baramica iaitu populasi paya daratan dan paya persisiran pantai. Berdasarkan analisis populasi genetik, terdapat 26 haplotaip bermakna telah ditentukan disamping dengan diversiti genetik yang pelbagai telah dikesan dari semua populasi. Secara keseluruhan, memandangkan kajian ini hanya fokus kepada kawasan Sarawak, lebih banyak usaha yang intensif dan berpanjangan perlu diambil demi mengawal persekitaran untuk kelestarian populasi P. baramica kerana lebih bamyak tanah hutan telah ditukar kepada kawasan komersil.

Kata kunci: *Logam berat, tekanan persekitaran, mikrohabitat, populasi genetik, Pulchrana baramica*

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LIST OF ABBREVIATIONS

%	Percent
°C	Celcius
.nxs	NEXUS file
A	Adenine
AES	Audio Encounter Survey
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
BDL	Below Detection Limit
BNP	Bako National Park
bp	Base pair
C	Cytosine
Cd	Cadmium
COI	Cytochrome Oxidase Subunit I
Cu	Copper
CytB	Cytochrome b
DNA	Deoxyribonucleic acid
DNASP	DNA Sequence Polymorphism
DO	Dissolved Oxygen
FAAS	Flame Atomic Absorbance Spectrophotometry
Fe	Iron
G	Guanine
G	Gram
Hap	Haplotype

HCA	Hierarchical Cluster Analysis
HNO_3	Nitric Acid
KS	Kota Samarahan
MDF	Mixed Dipterocarp Forest
MEGA	Molecular Evolutionary Genetic Analysis
mg/L	Milligram per liter
ML	Maximum Likelihood
ml	Milliliter
Mn	Manganese
MP	Maximum Parsimony
mtDNA	Mitochondrial DNA
MWC	Matang Wildlife Center
Ni	Nickel
NJ	Neighbor-joining
NMDS	Non-multidimensional Scaling
NNP	Niah National Park
NP	National Park
NTU	Nephelometric Turbidity Unit
P. baramica	*Pulchrana baramica*
Pb	Lead
PC	Principle Component
PCA	Principle Component Analysis
PCR	Polymerase Chain Reaction
pH	Potential Hydrogen
ppm	Parts Per Million

PSU	Practical Salinity Unit
SNP	Similajau National Park
SVL	Snout Vent Length
T	Thymine
TDNP	Tanjung Datu National Park
TDS	Total Dissolved Solid
TL	Tibia Length
UNIMAS	Universiti Malaysia Sarawak

CHAPTER 1

INTRODUCTION

1.1 Study Background

Anurans are the most diverse order of amphibians and the world's most threatened group of vertebrates (Koroiva & Santana, 2022). Amphibian threats and extinctions are well documented on a global level, with approximately 32% of their species currently facing extinction (Asad et al., 2020; Koroiva & Santana, 2022). Borneo has more than 180 species of anurans, and 70% of the species are endemics in Borneo (Inger et al., 2017; Ahmad Sah & Grafe, 2020). Borneo's amphibians face an uncertain future due to significant levels of forest degradation and continuous habitat destruction (Asad et al., 2020). Habitat loss, overexploitation, the presence of invasive species, climate change, and the spread of diseases are examples of threats for anurans (Covarrubias et al., 2021). Furthermore, anthropogenic activities may be concomitant with reduction in observed genetic divergence between populations, which is more likely to occur in disturbed habitat species than in forest restricted species (Rodriguez et al., 2015).

The use of DNA barcoding has the potential to benefit anurans since it provides information on populations that may be cryptic species and aids in taxonomic identification (Lyra et al., 2017). Phylogenetics is the study of organisms' evolutionary relationships, and molecular analysis is one method that can be used to understand species phylogenetic relationships (Indra et al., 2021). It is crucial to restore the forest ecosystem so that fauna, particularly anurans, can gradually recover and contribute to the process of having a balanced ecosystem (Simon et al., 2022). Amphibians are more vulnerable to external environmental changes because of the modification of lands caused by the conversion of forests to

agricultural land, which causes the loss of the original habitats for frogs. Amphibian species are sensitive to environmental changes if too many anthropogenic modifications are made in the habitats, and they can be a significant indicator of geological and climatic changes over time (Liu et al., 2015; Zainudin et al., 2019a). Current indicators of ecosystems explain the biological independence or connectivity of different geographic areas. As mentioned by Eterovick et al. (2010), microhabitat plays a vital role in studying anuran behaviour by considering the variations of sites and habitats occupied by the organism.

The *Pulchrana baramica* species was chosen as the study model for this project. According to Inger et al. (2017), this species is widely distributed in lowland areas such as *kerangas* forests, peat swamps, and mixed dipterocarp forests and can sometimes be found in disturbed areas. *Pulchrana baramica* status is listed as Least Concern (LC) (The International Union for Conservation of Nature [IUCN] Red List, 2022) since it was last assessed in 2020. However, this species experiences a decline in population since lots of their habitats have been converted to human settlements, timber harvesting, and the expansion of oil palm plantations (Gillespie et al., 2012; IUCN Red List, 2022; Kwatrina et al., 2018).

1.2 Problem Statement

Currently, amphibian species are experiencing rapid population decline globally (Phoonaploy et al., 2016). Considering the extensive taxonomic efforts, amphibians have the highest percentage of newly discovered vertebrate species (Matthijs et al., 2020). Furthermore, genetic diversity is informative about ongoing processes occurring within ecosystems and about the future sustainability of those ecosystems. Based on a study by

Rodriguez et al. (2015), the genetic divergence of frogs is mainly influenced by habitat type, such as a forested or disturbed area.

Adaptation and natural selection are examples of evolutionary forces that may occur in a community of a species in a particular area (Eterovick et al., 2010). Natural and anthropogenic environmental changes can lead to changes in genetic diversity, which provides insights into the consequences of environmental changes. Since dispersal is an important part of the life history of amphibians, habitat fragmentation and destruction are serious threats to amphibian persistence especially in Malaysia. Massive large clearing for development and plantation area also put pressure on the ecosystem of selected Bornean frogs (Zainudin et al., 2019a).

Amphibians are excellent bioindicators of environmental contamination because of their high sensitivity to changes in water quality and their microhabitat parameters (Phoonaploy et al., 2016). In an environment, a low abundance of anuran population or a high anuran mortality rate is a sign of ecosystem instability (Agustar et al., 2022). Moreover, frogs are good indicators in the detection of heavy metal contamination in water and soil, as frogs can live in arboreal and terrestrial conditions (Thanomsangad et al., 2019).

Anurans are particularly vulnerable to the impact of landscape composition due to their biphasic life cycle, habitat specialisation for ovipositing and foraging, low dispersal abilities, and permeable skin (Covarrubias et al., 2021). Due to their ectothermic nature and skin permeability, anurans are susceptible to environmental temperature changes and humidity because they are unable to accurately regulate their body temperature (Simon et al., 2022).

Furthermore, prior research by Rodriguez et al. (2015) demonstrated a coherent genetic theory in which topographic complexity and ecological preferences, including environmental stressors, have a major effect on frog population divergence. This study focused on the inland and coastal peat swamp forests, any disturbed area, as well as its adjacent areas of mixed-dipterocarp forests, to assess the *P. baramica* microhabitats, environmental stressors, and genetic diversity within localities.

1.3 Objectives

This study collected enough data and was able to interpret relevant results to meet a set of objectives:

i. To assess the microhabitat of *Pulchrana baramica* among all sampling localities in Sarawak

ii. To identify the environment stressors through physicochemical and heavy metals parameters

iii. To elucidate the phylogenetic relationships of *Pulchrana baramica* based on Cytochrome Oxidase subunit 1 (COI) and Cytochrome b (CytB)

iv. To measure genetic diversity within and between populations of frogs inhabiting a given ecosystem

1.4 Hypotheses

i. To assess the microhabitat of *Pulchrana baramica* among all sampling localities in Sarawak

H_0: There will be no variations of microhabitat of *P. baramica* among all sampling localities in Sarawak.

H_1: There will be variations of *P. baramica* among all sampling localities in Sarawak.

ii. To identify the environment stressors through physicochemical and heavy metals parameters

H_0: There will be no correlation between environmental stressors and frog genetic diversity since genetic diversity is not only due to the environmental stressors.

H_1: There will be correlation between environmental stressors and frog genetic diversity since genetic diversity is not the only due to the environmental stressors.

iii. To elucidate the phylogenetic relationships of *Pulchrana baramica* based on selected molecular marker

H_0: There will be no distinct and unique lineages of populations since frogs have the same ecological requirements.

H_1: There will be distinct and unique lineages of populations since frogs have the same ecological requirements.

iv. To measure genetic diversity within and between populations of frogs inhabiting a given ecosystem

H_0: There will be no genetic variations within and among populations of frogs inhabiting in each ecosystem since frogs that inhabit the same habitat types are mostly closely related species.

H_1: There will be genetic variations within and among populations of frogs inhabiting in each ecosystem since frogs that inhabit the same habitat types are mostly closely related species.

CHAPTER 2

LITERATURE REVIEW

2.1 Anuran of Borneo

Frogs are amphibians that typically live both in fresh water and on land. The amphibious mode of life is presumed to represent the ancestral pattern of movement from terrestrial sites to water for breeding purposes and then returning to land for other activities. Basically, almost 183 species of frogs have been found on Borneo due to the discovery of new species almost every year, and the number of frog species in Borneo keeps on increasing from year to year (Inger et al., 2017). Aside from that, roughly 110 species of anurans have been successfully recorded from Peninsula Malaysia, and some of the anurans are also found in Borneo (Norhayati, 2017). Recently, a new distribution record of frogs has been found in the least expected habitat, as seen in the Dring frog, *Pelophryne api* (Zainudin et al., 2018). However, due to uncontrolled logging in the past decades, Sarawak has lost its major forests (Aik & Ismail, 2020).

Oil palm exploitation and sago plantations put pressure on the ecosystem of selected Bornean frogs (Zainudin et al., 2019a). Another example is Mount Penrissen, which is situated in the second oldest dipterocarp forest (Zainudin et al., 2019b). The area harbours populations of the world-famous rainbow toad, *Ansonia latidisca* (Bornean Rainbow Toad) (Pui et al., 2011). Unfortunately, this species has been categorized as an endangered species since 2004 by the IUCN Red List and Inger et al. (2004). The Bornean Rainbow Toad population has been continuously decreasing until now (last assessed by the IUCN Red List in 2018); this species is also not listed as a protected amphibian species under the Sarawak Wildlife Ordinance of 1998 (Pui et al., 2011).

Now the Mount Penrissen forest has turned into a Borneo Highland resort that is accessible to visitors (Pui et al., 2011; Zainudin et al., 2006). Prior to the development of the Borneo Highlands Hornbill Golf & Jungle Club in 2000, the low and mid-elevation of Gunung Penrissen was heavily logged, limiting intact vegetation to upper montane ecosystems (Pui et al., 2011). Although these forests have since been preserved as a reserved area and water catchment area, habitat degradation may continue to endanger the long-term existence of *Ansonia latidisca*. Moreover, the low-land area of Sarawak, especially the degraded peat swamp forests of Kota Samarahan, also saw accelerated growth in commercial infrastructure and housing development**.**

Inger et al. (2017) stated that arboreal Rhacophoridae (Tree Frogs) live above ground, such as on trees or bushes. Borneo acts as one of the most diverse islands on Sundaland as it has 41 described rhacophorid species, which comprise eight genera of tree frogs: *Chiromantis, Feihyla, Kurixalus, Nyctixalus, Philautus, Polypedetes, Rhacophorus,* and *Theloderma* (Hertwig et al., 2013; Inger et al., 2017). Their reproductions for most species are quite unique since they build foam nests on trees that hang on the water to cover their eggs from predators and allow their babies to grow. The foam nest will break down after a few days, and then the tadpole will continue their metamorphosis completely in the water (Inger et al., 2017). Meanwhile, known species of *Nyctixalus* and *Theloderma* do not build foam nests, and *Philautus* do not have free-swimming tadpole stages.

According to Inger and Stuebing (2005), terrestrial frogs are widely distributed in Borneo and dwell perfectly on forest floors, rivers, and streams. They depend on water for breeding and to complete their life cycle. *Limnonectes leporinus* (Giant River Frog) and *L. kuhlii* (Kuhl's Creek Frog), endemic and widely distributed species in Borneo from the

family Dicroglossidae (True Frogs I), can be found on the forest floor of Kubah National Park (Zainudin & Gawin, 2011).

Megophryidae live on forest floors and have a high tendency to hide under the leaf litter during the day. These frogs from this family have great camouflages with leaf litter, and it is hard to see them (Inger et al., 2017). *Megophrys nasuta* (Bornean Horned Frog) is one of the most famous megophryid species in this family because of the 'horn' that emerges from its eyelids. The largest members of the True Frog II family, known as Ranidae and easily found in the Bornean forests, are *Pulchrana signata* (Striped Stream Frog), *P. glandulosa* (Rough-sided Frog), *Abavorana luctuosa* (Mahogany Frog), and *Odorrana hosii* (Poisonous Rock Frog) (Zainudin et al., 2017).

2.1.1 *Pulchrana baramica* (Boettger, 1900) Species

Pulchrana baramica (Boettger, 1900) was first described as *Rana baramica* or *Rana (Hylarana) baramica,* and its common name is known as Brown Marsh Frog (Frost & American Museum of Natural History, 2021; Haas et al., 2022; Inger & Stuebing, 2005; Inger et al., 2017). This species comes from the family Ranidae (True Frogs II) and genus *Pulchrana* (Inger et al., 2017). This species inhabits lowland areas such as *kerangas* forest, peat swamps, and mixed dipterocarp forest, and can sometimes be found in disturbed areas. *P. baramica* is widely distributed in Malaysia, Indonesia, and Singapore (Frost & American Museum of Natural History, 2021).

Inger and Stuebing (2005) and Inger et al. (2017) stated that *P. baramica* has brown spots on top and lighter, occasionally yellowish, spots on the flanks. Daylight makes colour appear darker than it does at night. The skin is warty to grainy in texture. The tympanum is dark, although it becomes lighter in the middle. *P. baramica* has excellent agility and is constantly ready to leap (Hertwig et al., 2022). Male *P. baramica* has a loud calling sound

to attract the female for mating purposes. Males make their calls from 1-3 m-tall shrubs. The call is a rapid succession of increasingly loud and intense pulses (Frost & American Museum of Natural History, 2021; Hertwig et al., 2022).

According to the IUCN Red List (2022), *P. baramica* status is listed as Least Concern (LC) since their last assessment in 2020. However, this species experiences a decline in population since lots of their habitats around Malaysia and Indonesia have been converted to human settlements, timber harvesting, and the expansion of oil palm plantations (Gillespie et al., 2012; IUCN Red List, 2022; Kwatrina et al., 2018).

Figure 2.1: *Pulchrana baramica* species

2.2 Water Physiochemical Parameters

Throughout history, rivers have played an important role in human communities. River water has also been used for irrigation, industry, and domestic purposes. A variety of aquatic plants and animals live in the river (Al-Badaii et al., 2013; Hanafiah et al., 2018). Water is crucial for the amphibian community. They begin their early stage of life in the water, especially after emerging from the egg as tadpoles (Inger et al., 2017). Frog communities will survive with good water quality, but even minor changes in water quality can result in a high mortality rate for tadpoles and adult frogs in the population (Zulkefli & Zainudin, 2022).

Dissolved oxygen (DO) is the most important indicator of freshwater quality because it is required for amphibians and other aquatic life to grow (Fitri et al., 2021; Wahaba et al., 2019). Variations in DO concentration can have an impact on freshwater biological and chemical processes (Fitri et al., 2021). Water temperature is an important physicochemical factor for tadpole survival. Even minor changes in water temperature can have an impact on aquatic creatures' health (Hanafiah et al., 2018). Water temperature varies depending on factors such as elevation, latitude, river length, and the amount of organic matter precipitated (Hanafiah et al., 2018). The pH of a water sample indicates how acidic and concentrated its hydrogen ions (H^+) (Gorde & Jadhav, 2013; Hanafiah et al., 2018; Tank & Chippa, 2013). River water typically has a pH of 6 to 8. The frequency of rainfall may have an impact on the pH of the water (Gorde & Jadhav, 2013; Hanafiah et al., 2018).

The term "turbidity" refers to the clarity of water by measuring the suspension of particles in a sample of water using a light beam (Alzaatreh et al., 2022; Davies-Colley et al., 2021). Turbidity is primarily caused by clay, silt, organic and inorganic materials, plankton, and other organisms suspended in water (Kalavathy & Giridhar, 2016). All these factors contributed to the cloudiness of the water, which can be measured using the Formazin Nephelometric Unit (FNU) and Nephelometric Turbidity Unit (NTU) (Alzaatreh et al., 2022). The turbidity of water can be used to assess the health of an aquatic ecosystem. The clear water indicates that the aquatic plants received enough sunlight to perform photosynthesis (Alzaatreh et al., 2022).

Salinity refers to the total amount of salt in water (Hanafiah et al., 2018). Changes in the salinity of the water may have an impact on a frog's physiology. Because amphibians have permeable skin, they are unable to cope with the rising salinity in the water (Park & Do, 2020). Salinity changes have a negative impact on the abilities of both vertebrates and

invertebrates. Not only do high salinity environments have low survival rates, but the delayed tadpole development may also increase the risk of predation or desiccation, as well as lower population fitness (Chuang et al., 2022).

Total dissolved solids (TDS) refer to the total amount of minerals and ions in water, which includes pollutants and organic materials (Hanafiah et al., 2018; Rusdi et al., 2022). The TDS value in water varies over time and space due to natural anthropogenic influences such as climate, soil type, and human activity (Augustijin et al., 2011). TDS can be measured in parts per million using a test meter (ppm).

2.3 Heavy Metal Content

Human consumption contributes to a daily growth in solid waste, which negatively impacts the environment and natural resources (Eggen et al., 2010; Phoonaploy et al., 2016). These heavy metals cause trophic level increases, surface and ground water pollution, bioaccumulation through the food chain in creatures, and harmful impacts on vertebrates, including fish, amphibians, and people (Nannoni et al., 2015; Phoonaploy et al., 2016). This can lead to biomagnification and risk human health. High exposure to high levels of heavy metals is known to harm the neurological system and result in renal failure. (Nannoni et al., 2015; Phoonaploy et al., 2016; Thanomsangad et al., 2019; Vrhovnik et al., 2013).

Frogs can still be used as a gauge to determine the level of heavy metal exposure in water sources, even though they are not the primary food source for people. High concentrations of heavy metals in the water influence the tadpoles' ability to grow. Throughout their life cycle, frogs can pick up heavy metals from both terrestrial and aquatic settings. Frogs lay naked eggs, and due to the great permeability of their skin, metal ions can pass (Singh et al., 2016; Thanomsangad et al., 2019). Moreover, depending on ecological requirements, metabolisms, and the degree of contamination in the water, food, and

sediment, the effectiveness of metal uptake from polluted water may vary. Furthermore, environmental variables including the season of year, the development area, the availability of nutrients, and the temperature of the water may affect the inconsistency of metal concentration (Irwandi & Farida, 2009; Ismail & Mat Saleh, 2012).

Metals occur in the solid phase when they are combined with soils. Physical and chemical elements, including pH, temperature, soil moisture, and organic compounds, can have an impact on the metal compositions in the soil. The biogeochemical process is also influenced by physicochemical factors, such as the oxidation and reduction of iron (Fe) and manganese (Mn), which promote the heavy metal cycle (Baba et al., 2021; Ismail & Mat Saleh, 2012).

Fe is a naturally occurring metal contamination that occurs when soil and rock in water come into contact (Gandaseca et al., 2014). The rates of sulphate reduction and soil oxidation, in general, determine the shape and speciation of Fe in soil in salt marshes and mangrove environments (Billah et al., 2014). Sometimes, places close to factories, plantations, and residential areas might affect the amount of iron in those areas (Sabarina et al., 2014). Even though Mn is a trace element that is vital for both plants and animals, too much of it can be hazardous. Mn can be produced when organic matter decomposes in soil and water (Gandaseca et al., 2014).

One of the contaminants produced by the diesel fishing boats used by fishermen is copper (Cu) (Sabarina et al., 2014). Diesel engines are regarded as one of the major sources of the air pollution brought on by exhaust emissions, and they are also to blame for health issues (Reşitoğlu et al., 2014). There is strong evidence that diesel emissions may lead to cancer in people, and studies have demonstrated that breathing in diesel exhaust gas damages the lungs and causes respiratory issues (Burr & Gregory, 2011). Even though Cu is a

naturally occurring element that both plants and animals require, high concentrations can have a detrimental effect on the environment's flora, fauna, and water quality (Gandaseca et al., 2014).

Lead (Pb) naturally occurs in the soil. The use of leaded aviation fuel, diesel fuel, and unleaded car fuel has increased the Pb content in the environment (Sabarina et al., 2014; Waoo et al., 2014). Pb toxicity can cause serious ailments, including anaemia, an increase in blood pressure, kidney failure, and miscarriage (Waoo et al., 2014). Cadmium (Cd) is one of the metals that is harmful to the environment. It is extremely poisonous but serves no essential biological purposes for flora or fauna. The high concentration of Cd can be retained by the soil if organic matter is present (Sabarina et al., 2014; Waoo et al., 2014). Industrial waste and sewage deposits can both cause Cd pollution (Sabarina et al., 2014).

Another of the key elements that both plants and animals require is nickel (Ni). Rock and soil that are rich in nickel can naturally produce it through weathering, dissolution, and air evaporation (Saha et al., 2022). Ni is a biocontrol agent that aids in plant growth and strengthens the plant's defence against a variety of diseases and pests (Saha et al., 2022). High Ni exposure can result in cardiac arrest, pneumonia, and brain haemorrhage, all of which can be fatal (Waoo et al., 2014).

Table 2.1: List of heavy metals based on previous study in Malaysia

References	Soil						Water						Locality	Site/Habitat
	Cd	Cu	Fe	Mn	Ni	Pb	Cd	Cu	Fe	Mn	Ni	Pb		
Mokhtar *et al.* 2022							✓					✓	Langat, Selangor	River basin
Kanakaraju *et al.* 2008	✓	✓	✓	✓									Asajaya, Sarawak	Human settlement
Rosli *et al.* 2021								✓	✓	✓			Bintulu, Sarawak	Rehabilitated forest
Talukder *et al.* 2021	✓	✓		✓	✓	✓							Setiu, Terengganu	Wetland
Billah *et al.* 2014		✓	✓	✓				✓	✓	✓			Miri-Bintulu, Sarawak	Mangrove
Baba *et al.* 2021		✓		✓	✓	✓							Kota Belud, Sabah	Coastal
Ismail and Mat Saleh, 2012								✓	✓				Puchong, Selangor	Lake
Razis *et al.* 2022	✓	✓				✓							Districts in Perak	Paddy field
Gandaseca *et al.* 2014								✓	✓	✓			Limbang, Sarawak	Mangrove
Praveena *et al.* 2008		✓	✓			✓							Tuaran, Sabah	Mangrove

2.4 Molecular Approaches

Genetic data are an important and rapidly expanding resource in all biological sciences, but curated databases are limited (Matthijs et al., 2020). DNA barcode analyses for species discovery are competent at grouping or clustering unidentified specimens based on genetic distance or genealogical data since DNA barcoding is an effective, simple, and standardized tool for finding cryptic variety in amphibians. (Guarnizo et al., 2015; Lyra et al., 2017).

Recent advances in integrated taxonomy, barcoding, bioprospecting, phylogenetics, phylogeography, population and conservation genetics, biogeography, macroecology, and paleoecology have increased the availability and widespread sequencing of mitochondrial DNA (mtDNA) (Matthijs et al., 2020). Furthermore, the use of Cytochrome Oxidase Subunit I (COI) as a mitochondrial DNA molecular marker has risen in recent years among amphibian taxonomists (Gao et al., 2019; Lehr et al., 2017; Zhao et al., 2017). A previous study by Zainudin et al. (2010) used COI to study the genetic structure of *Hylarana erythraea* from Peninsula Malaysia and Sarawak. Besides, the use of COI barcodes for specimen identification and species discovery has proven to be an effective molecular approach in the study of anurans (Koroiva et al., 2020).

Cytochrome b (CytB) is also one of the most common genetic markers in amphibian literature, with the broadest genus and species-level taxonomic coverage (Matthijs et al., 2020). Amphibian taxonomists used cytochrome b in their phylogenetic studies because this gene originated in the mitochondrial genome and is inherited maternally (Indra et al., 2021). The mitochondrial cytochrome b gene was also used in phylogenetic analysis to identify organisms down to the species level. (Claudio-Correa et al., 2018). Cytochrome b also has a faster mutation rate in Ranidae species (Liu et al., 2015). Phylogenetic or phylogeographic

studies frequently use the CytB gene because they have been successful in resolving connections between closely related taxa (Kurniawan et al., 2010).

2.5 Relationship between Genetic Diversity and Ecosystem

Genetic diversity is a fundamental component of biodiversity and is as critical as species and ecosystem diversity for the sustenance of natural resources (Cortázar-Chinarro et al., 2020). Every species consists of populations that coexist sporadically. There is genetic diversity both within and between populations. (Kurniawan et al., 2010). The evolutionary adaptation of populations to their environments is driven by forces like mutation, migration, selection, and genetic drift (Mona et al., 2014).

Correlating patterns of phylogeographic or genetic variation with different species-specific traits allows us to understand why certain species' genetic structure responded similarly to climatic and geological history (Rodriguez et al., 2015). Natural and anthropogenic environmental changes lead to changes in genetic diversity, both within and among populations, and genetic diversity measurement can provide insights into the consequences of environmental changes (Pereyra et al., 2018; Zainudin et al., 2017).

Comprehensive studies of species population structure and dynamics are presented, along with recent molecular technology discoveries (Covarrubias et al., 2020). The primary goal of genetic management is to ensure that sufficient genetic diversity is retained to maintain short-term fitness and long-term sustainability and to ensure that local adaptations are not lost due to the intermixing of previously distinct populations.

Furthermore, frog assemblages at Sarawak peat swamp forest have been well studied, along with their habitat utilization of phylogenetically close species in the mixed dipterocarp forests (Zainudin et al., 2017). Those are useful for biodiversity, conservation management,

and recommendations. With the increase in deforestation, global warming, and occurrences of natural catastrophes, an ecosystem indicator is very much needed to sustain ecosystem functions and services (Gillespie et al., 2012).

Genetic variations and lineage diversification, as well as environmental parameters, can be used as indicators of ecosystem health. Due to the widespread destruction of habitat, this can be an integrative approach to the study of biodiversity and to determining how much diversity has been lost in landscapes because of human intervention (Pereyra et al., 2018).

CHAPTER 3

METHODOLOGY

3.1 Sampling Sites

Sampling area that included both unprotected and protected sites. There were reasons behind the selection of sampling localities between protected and unprotected areas. Protected areas were restricted by human intervention, whereas unprotected areas were not. The anthropogenic effects were much higher in unprotected areas since most of the areas were located near human settlements.

The water quality parameters, ecological data, frogs' behavior, and microhabitats within the unprotected areas were also affected. The data collected between protected and unprotected areas showed a significant difference in the ecological parameters and microhabitat patterns of *P. baramica*. All localities were chosen accordingly based on the vegetation types occupied by *P. baramica* (Table 3.1, Figure 3.1).

In addition to this, the collection of the water's physicochemical properties, water samples, and soil sediments were carried out. A forest and river transect were used to collect samples during the night, between the hours of 19:00 and 22:00. To eliminate the possibility of bias, the duration of each sampling location was five days and four nights long.

Table 3.1: Sampling sites information

Sampling localities	GPS Coordinate	Sampling Date
Tanjung Datu NP	02°01'47.5" N, 109°38'54.1" E	August 2019
Libiki Bamboo Resort	01°19'59.8" N, 110°06'08.1" E	January 2020
Maludam National Park	01°39'26.6" N, 111°02'24.7" E	March 2020
Mount Singai, Bau	01°32'31.8" N, 110°10'57.3" E	October 2020

Bako National Park	01°43'00.2" N, 110°26'25.4" E	March 2021
Kota Samarahan, UNIMAS	01°28'14.4" N, 110°25'48.2" E	January 2022
Kanowit	02°05'24.0" N, 112°08'48.0"E	March 2022

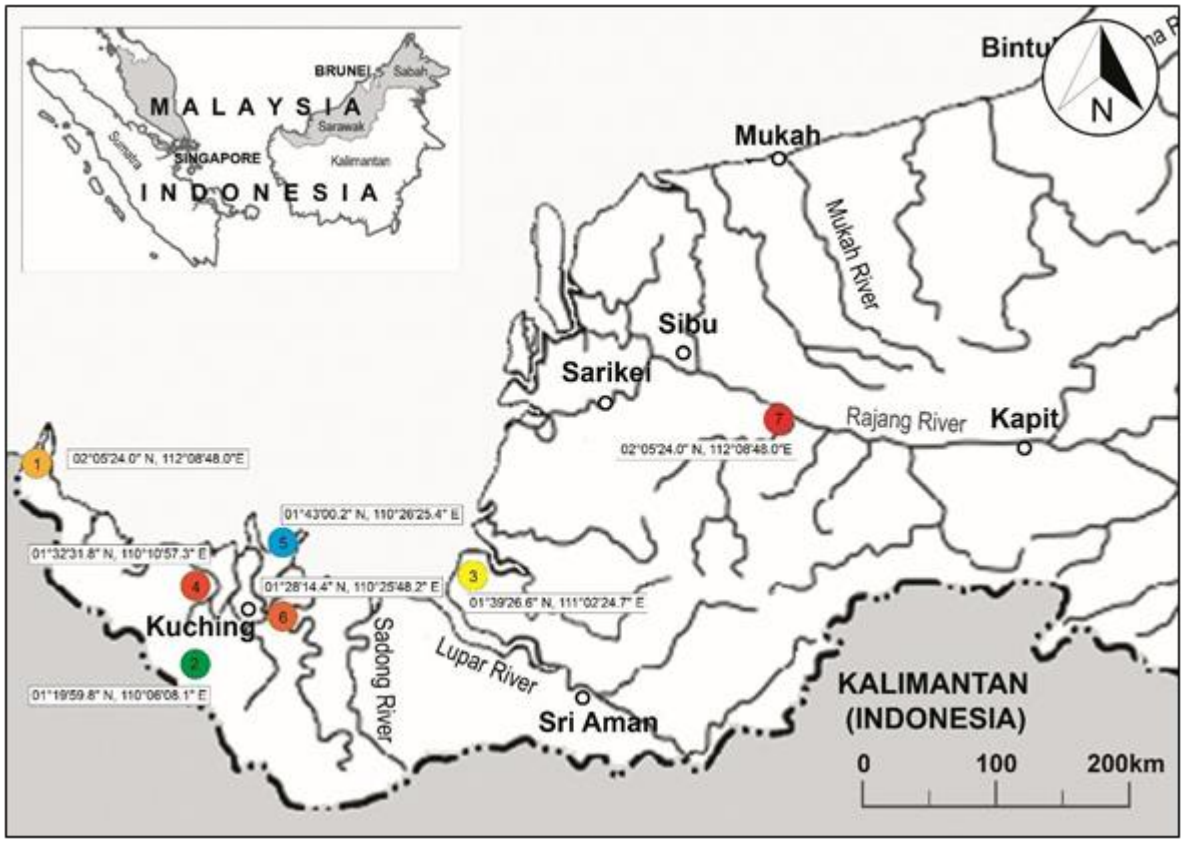


Figure 3.1: Map of sampling localities; 1) Tanjung Datu NP, 2) Libiki Bamboo Resort, 3) Maludam NP, 4) Mt.Singai, Bau, 5) Bako NP, 6) UNIMAS, 7) Kanowit

3.1.1 Protected Areas

i. Tanjung Datu National Park (TDNP)

Tanjung Datu National Park (TDNP), which is located at the tip of Sarawak and is considered the smallest national park in Sarawak, has a total area of approximately 1,379 ha (Mohd-Azlan et al., 2018). Being one of Sarawak's most stunning national parks, TDNP previously had restricted access and received few tourists.

Nevertheless, the park is now easier to reach because of the 2019 inauguration of a new road on the Pan Borneo Highway that connects Sematan and Telok Melano (Abang Abdurahman et al., 2021; Tuah et al., 2022). TDNP, also known as the marine turtle conservation area (Lo et al., 2015; Sarawak Tourism, 2023). Moreover, TDNP is famous for Rafflesia plant species, namely *Rafflesia hasseltii* (Shafreena et al., 2020).

ii. Matang Wildlife Center (MWC)

Matang Wildlife Center (MWC) is situated in the Matang Ranges, along with Kubah National Park. The MWC is one of the tourists' attractions in Kuching. MWC is known as a rehabilitation center for Orang Utan (*Pongo pygmayus)*, Silvered Leaf Monkey *(Presbytis cristata),* Slow Loris *(Nycticebus coucang),* and other enclosure animals such as sun bears and hornbills (Chong et al., 2019). Other than that, MWC is very famous for their Orang Utan adoption program (Abang Abdurahman et al., 2021). There is a trail named Pitcher Trail, as it is rich with *nepenthes* plants and provides shelter for *Microhyla* species (Inger et al., 2017).

iii. Maludam National Park (Maludam NP)

Maludam National Park (Maludam NP) is in Betong Division, Sarawak, and consists of two main rivers, namely the Batang Lupar and Saribas Rivers. Maludam NP covers approximately 43,147 ha (Dosi et al., 2018). Figure 3.2 shows the condition of the Maludam NP peat swamp forest. Maludam NP had the fewest visitors from both domestic and foreign visitors (Abang Abdurahman et al., 2021).

According to Razi et al. (2022), the peat swamp forest of the Maludam NP is home to 200 different species of birds, including the Black Hornbill (*Anthracoceros malayanus*), Oriental Pied Hornbill (*Anthracoceros albirostris*), Rhinoceros Hornbill (*Buceros*

rhinoceros), Proboscis Monkey (*Nasalis larvatus*), Banded Langur (*Presbytis melalophos cruciger*), and Estuarine Crocodile (*Crocodylus porosus*). Species like *Chalcorana raniceps* and *Pulchrana baramica* were common in the national park. The frog calls of these two species were heard along the trails. Other than sampling at the national park, the sampling was also conducted at the nearest oil palm plantations.

Figure 3.2: The condition of Maludam National Park peat swamp forest

iv. Bako National Park (BNP)

Bako National Park (BNP) is a famous ecotourism attraction and has a nice view of the sea and the iconic sea stack stone near Kuching town. Serve as a home for the Silver Leaf Monkey (*Trachypithecus cristatus*), the Proboscis Monkey (*Nasalis larvatus*) (Figure 3.3),and the Bornean Breaded Pig (*Sus barbatus*) (Razi et al., 2022). Every tourist that comes to the BNP will receive a copy of the trail map for guidance during their jungle trekking. Each trail has different difficulties and types of forest vegetation.

To date, there have been few types of forest vegetation available at BNP, such as *kerangas* forest, cliff vegetation, beach vegetation, mangrove forest, riverine (alluvial) forest, and mixed dipterocarp forest (Zahidin et al., 2016). Figure 3.4 shows the condition of the Ulu Assam trail, one of the trails available at BNP.

Figure 3.3: Proboscis monkey **(***Nasalis larvatus*) of Bako National Park

Figure 3.4: View of Ulu Assam Trail

3.1.2 Unprotected Areas

i. Libiki Bamboo Resort, Bau

Near the Malaysia-Indonesia border in Bau, Sarawak, the Libiki Bamboo Resort is a popular destination for ecotourists since it is encircled by Borneo's *kerangas* forest (Zulkefli & Zainudin, 2022). Within the resort and forest area, there are small sugar cane plantations and vegetables that belong to the villagers. A small hill paddy field was planted along the hill slope too. Despite being an ecotourism spot, the management of the resort still conserved the forest area for the flora and fauna. During the sampling, 13 anuran species were found such as *Ansonia spinulifer, Chalcorana megalonesa, Kalophrynus meizon, Leptolalax*

dringi, etc. Frog species that commonly found at disturbed area also found at Libiki Bamboo Resort vicinity, namely, *Fejervarya limnocharis* and *Polypedates leucomystax.*

Figure 3.5: One of the rivers at Libiki Bamboo Resort

Figure 3.6: River that connecting between human settlement and the resort

ii. Mount Singai, Bau

Mount Singai is in Bau and is surrounded by hills, as well as being close to the Matang Ranges. Consists of a few villages namely: Kampung Badul, Kampung Atas, Kampung Bobak, Kampung Daun, Kampung Satow, etc. The majority of local people farmed black pepper near their homes as their primary source of income (Wan Hamid et al., 2019). Aside from pepper farming, the residents created many ecotourism sites and

homestay to promote the beauty of nature for nature enthusiasts, such as Badul Homestay, Mendung Escape, Satow Waterfall, and Mount Bronang.

Figure 3.7: The trail of Sungai Kampung Atas

Figure 3.8: The recreational area of Satow Waterfall

iii. UNIMAS, Kota Samarahan

Universiti Malaysia Sarawak (UNIMAS), one of the public universities in Malaysia, is situated in Kota Samarahan, Sarawak. Landscaped with lakes, forests, and modern infrastructures for students. Subsequently, UNIMAS peat swamp forest consists of a variety of biodiversity of flora and fauna. Frog species such as *Duttaphrynus melanostictus, Pulchrana baramica, Limnonectes paramacrodon, Hylarana erythraea,* and *Rhacophorus pardalis* were commonly found in the UNIMAS vicinity as well as in the UNIMAS forest.

Figure 3.9: Main Lake of UNIMAS

iv. Kanowit

The small town of Kanowit is situated at the meeting point of the Rajang and Kanowit rivers and is governed by the Kanowit District, which is part of the Sibu Division in the Malaysian state of Sarawak (Gapor et al., 2020). The famous Rajang River of Kanowit is a home for deadly predator, saltwater crocodiles, *Crocodylus porosus*. Since the Rajang River is situated the centre and the longest river in the state of Sarawak, the local communities use the river to travel throughout neighbouring towns Sarikei, Sibu, and Kapit (Abdul Gani & Hassan, 2016).

During the field sampling, few rivers were selected from Kanowit Iban villages for sampling sites which were Sungai Sekarak Kanowit, Nangan Ngungun and Nanga Bawan. There were various anuran species found within the sampling sites such as, *Leptobrachium hendricksonii, Limnonectes leporinus, Megophrys nasuta, Pulchrana baramica, Rentapia hosii* and *Staurois guttatus.*

3.2 Field Techniques

3.2.1 Tissue Sample Collection

This study used standardized sampling techniques across all sampling sites to reduce bias. The Visual Encounter Survey (VES) technique was used by walking slowly along the

forest and river transects by using a headlamp (Pui & Das, 2016; Zainudin et al., 2017, 2019a, 2019b). Audio Encounter Survey (AES) is also used in sample collection (Eterovick et al., 2010; Zulkefli & Zainudin, 2022) since the loud, rapid, and repetitive sound of *P. baramica* can be heard while walking in the trail (Inger et al., 2017).

The sampling was done between 1900 and 2200 hours, when frogs were most active. Three individuals were collected from each locality allowed by the research permit. The samples were all transported back to the worktable while still alive. The samples were processed immediately to maintain their quality. Since the tendency of frogs to escape occurred, they were handled properly to avoid any losses that might affect the quantity and quality of the data collection.

Certain frog species were able to emit a toxic fluid as a self-defence strategy when captured, so it is not advisable to put various species of frogs in the same container (Rahman et al., 2008). When under stress, *Odorrana hosii* species can release poisonous fluids that could harm other frogs and irritate human skin (Inger et al., 2017; Rasit et al., 2018; Zulkefli & Zainudin, 2022). Frog species were identified following field guides from Inger et al. (2017).

3.2.2 Microhabitat and Data Analysis

Using methods from Zainudin et al. (2017) with guidance from the microhabitat codes by Inger Habitat Code in Heyer et al. (1994) (Appendix A; Table 1), the following information was recorded for each caught individual:-

i. Date and time (24-hours' format)

ii. Vegetation/forest types

iii. Horizontal position

iv. Vertical position

v. Substrate

Other basic measurements were taken as follows: -

i. Weight (W)

ii. Total length (TL)

iii. Snout vent length (SVL)

All relevant analyses were computed using IBM SPSS V.22 (IBM Corporation, 2013). Firstly, the Chi square test was used to measure the relationship between microhabitat characteristics in different localities (Zainudin et al., 2017, 2019b). To assess the ecological guilds of *P. baramica* from microhabitat utilizations, non-metric proximities multidimensional scaling analysis (proxscal NMDS) (Zainudin et al., 2017, 2019b; Zulkefli & Zainudin, 2022).

3.2.3 Physicochemical Parameters

Water is a necessity for all amphibians to reproduce and carry out other essential life functions. ("Minnesota Freshwater Quest," n.d.). Basically, measuring the characteristics of water quality is crucial for evaluating amphibian assemblages and species diversity (Calderon et al., 2019). Measurements of the physicochemical properties of the water were also taken in addition to data on microhabitats. The measurements included dissolved oxygen, pH, salinity, temperature, total dissolved solids, and turbidity. Specific tools were used to record each of the parameters (Figure 3.10). At each site, each parameter was measured three times (Zainudin et al., 2019).

(a)
Model: Thermo Scientific Eutech TN-100 Portable Turbidity Meter

(b)
Model: Thermo Scientific Handheld Eutech DO 6+

(c)
Model: Thermo Scientific Eutech PH 150 Meter Kit

Figure 3.10: Equipment used for recording the water physicochemical parameters; a) turbidity meter, b) DO meter, c) pH meter

3.2.4 Water and Soil Sampling

Prior to sampling, all equipment was soaked overnight with an acid wash in 5% HNO3 (Billah et al., 2014) and rinsed with deionized water to remove any trace of adsorbed ionic metals. To minimize or totally get rid of any potential contamination, the polypropylene sample bottles were rinsed three times with the water to be collected at each sampling site (Aram et al., 2021). Collected water samples were stored in polypropylene bottles properly and acidified with 1.5 mL of 1% HNO3 to maintain a pH less than 2 (Thanomsangad et al., 2019). The soil samples were collected at a depth of 0 - 15 cm from the soil surface. The soil was kept in a polyethylene bag and stored at -20°C to prevent the hydrolysis or oxidation of the sample before analysis (Rashid et al., 2016).

3.3 Detemination of Heavy Metals

3.3.1 Sample Digestion of Water

A sample of 100 mL of water was placed in an Erlenmeyer flask and digested with 5 mL of concentrated nitric acid (HNO_3). The flask was heated on the hot plate (Figure 3.11) until a clear solution was obtained (Kumar et al., 2019). The sample was let cool at room

temperature and filtered using Whatman No. 42 filter paper to remove any insoluble particles (Thanomsangad et al., 2019). Then, the extract sample was added to 50 ml of double-distilled water and stored properly for next analysis (Kanakaraju et al., 2008; Kumar et al., 2019).

Figure 3.11: Digestion of water samples

3.3.2 Sample Digestion of Soil

The soil samples were air-dried until completely dry (Figure 3.12). Then, the dried sample was pulverized to a fine powder by using a sterilized mortar and pestle, as shown in Figure 3.13 (Kanakaraju et al., 2008). The soil powder is then sieved by using a 2 mm mesh sieve to filter any debris and stored for the next process (Praveena et al., 2008). According to Rashid et al. (2016), 1 gram of powder soil will be mixed with 10 mL of concentrated HNO_3 and left overnight at room temperature.

The sample was heated on a hot plate until a clear, semi-dried solution was obtained (Figure 3.14). The solution was let cool to room temperature before being filtered using Whatman No. 42 filter paper. Then, the extract sample was added to 50 mL of double-distilled water and stored properly for the next analysis (Kanakaraju et al., 2008; Kumar et al., 2019).

Figure 3.12: Air-dried soil sample

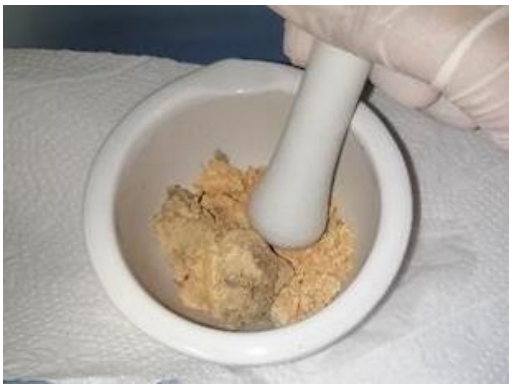


Figure 3.13: Pulverization of soil sample

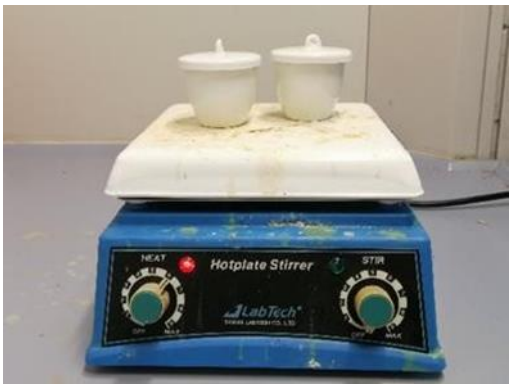


Figure 3.14: Samples heated on the hot plate

3.3.3 Sample Analysis

Stock solutions need to be prepared for three different standard solution concentrations for each element (Table 3.2). Each heavy metal needs a fresh stock solution (100 ppm) for calibration curves and should be prepared before analyses begin (Srikanth et

al., 2013). The stock and standard solutions were created using guidelines that the lab assistant gave.

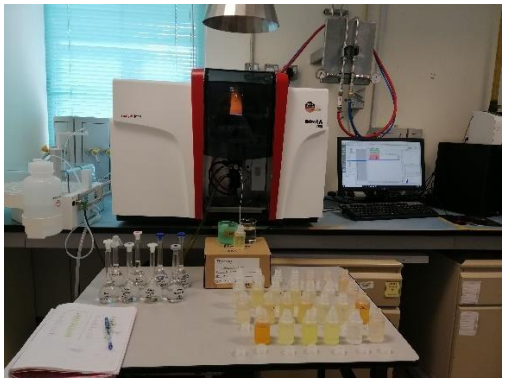
Table 3.2: Concentration of standard solution

Elements	Standard solution concentration (ppm)
Cd	0.5, 1, 1.5
Ni, Fe, Mn, Cu, Pb	1, 3, 5

The following equipment was required to prepare the solution: a 50-ml volumetric flask, deionized water, concentrated HNO_3, a pipette, and tips (Figure 3.15). Calibration curve values may vary according to each heavy metal standard solution concentration. The best fit of the standard curve should be > 0.995. If the optimal value is less than 0.995, the standard solution should be prepared again until the ideal calibration curve value is reached. Both stock and standard solutions have a shelf life of up to 3 years when properly stored.

Figure 3.15: The apparatus required to prepare the standard solution

All the digested samples underwent metal analysis carried out by using Flame Atomic Absorbance Spectrophotometry (FAAS) for the determination of heavy metal concentrations (Cd, Cu, Fe, Mn, Ni, and Pb) (Kanakaraju et al., 2008; Rashid et al., 2016) (Figure 3.16).



(a)
Model: Analytik Jena novAA 800

(b)
Model: Thermo Scientific iCE3500

Figure 3.16: Analysis of heavy metals using FAAS Instrument

3.3.4 Statistical Analysis

Few significant analyses were done by using IBM SPSS V.22 (IBM Corporation, 2013) (Ahmed et al., 2022; Rosli et al., 2022; Talukder et al., 2019). Firstly, one-way ANOVA (α:0.05) was used to compare the differences in parameters in water and between all sampling localities (Alzaatreh et al., 2022; Billah et al., 2014; Sibuar et al., 2022). The results were documented in mean concentrations and standard deviations.

Multivariate analyses such as Pearson Correlation Coefficient (*r*), Principal Component Analysis (PCA), and Hierarchical Cluster Analysis (HCA) were used. The Pearson correlation relationship (*r*) test was used to determine the correlation between environmental stressors and genetic diversity as well as to define the most significant parameters between all pairs of assessed parameters (Alzaatreh et al., 2022; Billah et al., 2014; Rosli et al., 2022). Meanwhile, PCA and HCA were widely used to investigate the interrelationship between all environmental parameters and define the parameters that influence each other (Piroozfar & Alipour, 2021; Sibuar et al., 2022).

3.4 Molecular Techniques

3.4.1 DNA Extraction

The DNeasy Blood and Tissue Kit by QIAGEN was used to extract all the skin, toes, muscle, and liver tissues from *P. baramica.* (Nguyen et al., 2018; Roh et al., 2022). The samples were properly collected and labeled with the field number and locations. The date of extraction was also noted. Under ultraviolet (UV) light, the extracted DNA was viewed to confirm the presence of the DNA band (Figure 3.17). Successful DNA extractions were subjected to PCR for DNA amplification. The unsuccessful DNA extraction was revisualized again after a day and reextracted again when no DNA band appeared.

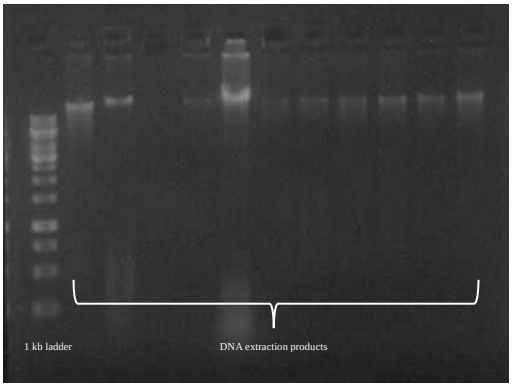


Figure 3.17: Visualization of DNA extraction

3.4.2 Polymerase Chain Reaction (PCR)

Double-stranded PCR amplification was carried out with the following master mix cocktails (Table 3.3). Cytochrome Oxidase Subunit I (COI) and Cytochrome B (CytB) mitochondrial genes were used as targeted site regions (Table 3.4) (Kurniawan et al., 2010; Palumbi et al., 1991; Zainudin et al., 2010).

Table 3.3: PCR master mix cocktail

Reagents	1x reaction (uL)
Buffer	5.0

DNTP mix	0.5
MgCl	1.5
Primer (forward)	1.0
Primer (reverse)	1.0
dd_2HO	13.8
Taq Polymerase	0.2
DNA template	2.0
Total	25

Table 3.4: Gene primer sequences used in the 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

As demonstrated in Table 3.5, several PCR profiles were applied to each primer individually. The DNA template was denatured and amplified at the right temperature for each targeted site location. It took about three to four hours to complete the PCR, which involved 40 cycles for each primer. To confirm the presence of a PCR band, PCR products were seen under UV light (Figure 3.18). Failed PCRs were repeated until a successful result was obtained.

Table 3.5: PCR profiles for both primers

Gene	Steps	Temperature (°C)	Time
Cytochrome Oxidase Subunit I	Pre-denaturation	95	5 mins
	Denaturation	94	1:30 mins
	Annealing	51	1 min

	Extension	72	1:30 mins
	Final extension	72	7 mins
	Soak	4	∞
	Cycle: 40		
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		

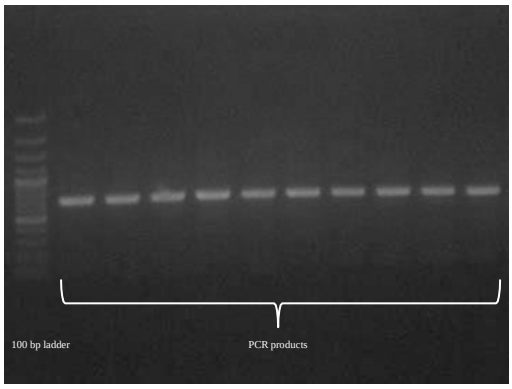


Figure 3.18: Visualization of PCR products

3.4.3 PCR Purification

Successful PCR products were purified by using QIAgen's QIAquick PCR Purification Kit (Yunming et al., 2016). The purification methods followed the protocol provided in the kit. Finally, the purified PCR products were sent to Apical Scientific Laboratories (Selangor, Malaysia) and Celemics Inc. (Seoul, Korea) for sequencing (Chung et al., 2018; Nguyen et al., 2018).

3.4.4 Molecular Analysis

All sequence bases were checked manually by eye using CHROMAS V. 2.6.6 (Technelysium Pty Ltd., 2018) (Narayanan et al., 2022; Vedernikov et al., 2020). The sequences were aligned by using CLUSTAL X (V.2.1) (Haas, 2004; Larkin et al., 2007;

Matsui et al., 2007). Output from sequence alignment was saved in NEXUS (.nxs) format for the next procedure. The same procedures were repeated for the CytB gene.

The COI and CytB.nxs files were utilized by MEGA X (Kumar et al., 2018; Marcaida et al., 2022) to generate the phylogenetic trees. Using the programmed Neighbour-joining (NJ), Maximum Likelihood (ML), and Maximum Parsimony (MP) trees, Sequence alignment statistics, pairwise distance, and the best DNA model were computed. Next, DNASP (V. 6) (Jablonski et al., 2021; Rozas et al., 2017) was used to calculate the haplotypes, level of population subdivision (Fst), nucleotide subdivision (Nst), and number of migrants per generation (Nm) (Zainudin & Naim, 2018). Information about haplotypes used in Arlequin (V 3.5) to compute the population expansion event using mismatch distribution analysis (Excoffier & Lischer, 2010; Muir et al., 2013) These models use a generalized least-squares method to estimate the parameters of population expansion and bootstrap their confidence intervals (Dufresnes et al., 2013).

AMOVA comparison of haplotype data is used to gauge the degree of population differentiation (Excoffier & Lischer, 2010; Zainudin et al., 2010; Zainudin & Naim, 2018). The mantel test (Mantel & Valand, 1970) was used to calculate the connection between genetic divergence and geographical isolation for 1000 permutations. (Zhang et al., 2014). The neutrality test was examined by Fu's Fs (Fu, 1997) and Tajima's D (Tajima, 1989).

Minimum Spanning Network (MSN) was constructed (Zainudin & Naim, 2018) to obtain a graphical representation of connecting COI gene haplotypes using Network (V. 10.2.0.0, Fluxus-Engineering Ltd.) (P. Forster & M. Forster, 2022) using the median-joining (MJ) algorithm (Bandelt et al., 1999). The MJ was used to calculate the geographical variations of *P. baramica* throughout 11 localities and to approximate haplotype richness while controlling for irregular sample sizes (Nicolas et al., 2015).

CHAPTER 4

ECOLOGICAL GUILD OF *Pulchrana baramica* AND ECOLOGICAL PARAMETERS FROM SEVERAL LOCALITIES IN SARAWAK

4.1 Overview

Basically, water source is the main basic need for the anuran community. They use water for breeding. Especially after emerging from the egg as tadpoles, they begin their early stage of life in the water. The communities of frogs will survive in good water quality, but slight changes in water quality could result in a high mortality rate for tadpoles and adult frogs in the population. If there have changes in water quality and heavy metal contents in the surrounding area, it may affect the whole ecological niche. Therefore, maintaining the good water quality are very vital to make sure the life cycle of frogs can be sustained. As well as by reducing the threats that may affect the frogs. Because of this, the selection of microhabitat by the frog species are very important to the species survivability.

Microhabitat information is essential for describing the ecological distribution of a species in a locality (Johari et al., 2016). The distribution of anurans species is easily understood by determining the anuran microhabitats (Zainudin et al., 2017). Microhabitat use is an important component for anuran in finding the best conditions for foraging, reproduction, and shelter, as well as to sustain the survival of the tadpoles and the adult (Eterovick et al., 2010; Martins et al., 2021).

Depending on their preferred habitat and diet, anuran species can be divided into generalist and specialized categories. It is crucial for species adaptation to environmental changes (Zamroni et al., 2021). Generalist species have a wide range of diets and the flexibility to adapt to any habitat (Zamroni et al., 2021; Zulkefli & Zainudin, 2022).

Meanwhile, the specialized species have a limited range of diets and are unable to tolerate an arid environment (Eterovick et al., 2010; Zainudin et al., 2006; Zainudin et al., 2019a). The two or more anuran species that share the same ecological needs and resources are known as niches (Zulkefli & Zainudin, 2022). On the other hand, competition for resources is high if two species have overlapping nutritional niches (sharing one or more resources) (Zamroni et al., 2021).

4.2 Methods

A data set of 81 *Pulchrana baramica* individuals was sorted and prepared for microhabitat analysis. From the data, 27 individuals were collected during field sampling within the research duration. Meanwhile, the remaining individuals used the data acquired from prior field samplings between 2005 to 2018. All *P. baramica* were obtained from protected and unprotected areas that comprise various types of habitats and localities.

Relevant analyses for microhabitat utilization and environmental stressors were computed using IBM SPSS V.22 (IBM Corporation, 2013). First, microhabitat data were sorted and grouped into a few categories, which were vegetation type (VT), horizontal positions (HP), vertical positions (VP), and substrate (S). The codes of microhabitats followed by the microhabitat code will follow the checklist from the Inger Habitat Code (Heyer et al., 1994; Zainudin et al., 2017).

The finalized data were used for a chi square test to measure the relationship between microhabitat characteristics in different localities (Zainudin et al., 2017, 2019b). Non-metric multidimensional scaling with proximities analysis (PROXSCAL NMDS) (Zainudin et al., 2017, 2019b; Zulkefli & Zainudin, 2022) was used to assess the ecological guilds of *P. baramica* from microhabitat utilizations. The NMDS configuration is expressed in two final

coordinate dimensions (Final Coordinate Dimension 1 and Final Coordinate Dimension 2) and a scatterplot of ecological guilds.

One-way ANOVA (α:0.05) was used to compare the differences in parameters in water and between all sampling localities (Alzaatreh et al., 2022; Billah et al., 2014; Sibuar et al., 2022). The Pearson correlation relationship (*r*) test will be used to determine the relationship between environmental stressors and genetic diversity as well as to define the most significant parameters between all pairs of assessed parameters (Alzaatreh et al., 2022; Billah et al., 2014; Rosli et al., 2022). Meanwhile, PCA and HCA were used to investigate the interrelationship between all environmental parameters and define the parameters that influence each other (Piroozfar & Alipour, 2021; Sibuar et al., 2022).

4.3 Results

4.3.1 Microhabitat Preferences of *Pulchrana baramica*

i. Vegetation used by *Pulchrana baramica*

Based on Table 4.1, one locality can have more than one type of habitat, depending on where the species were collected. Each locality between protected and unprotected areas harbors a different abundance of *P. baramica.* The highest abundance of *P. baramica* in protected areas was Bako National Park (16%, n = 13), followed by Mulu (12%, n = 10), Similajau National Park (12%, n = 10), Maludam National Park (9%, n = 7), Matang Wildlife Center (6%, n = 5), and Niah National Park (3%, n = 2) and Samajaya Nature Reserve (3%, n = 2).

The highest individuals of *P. baramica* in unprotected areas were Kota Samarahan, UNIMAS (16%, n = 13), followed by Bau (10%, n = 8), Sibu (6%, n = 5), and Ulu Semera (6%, n = 5), and the least was Kanowit (1%, n = 1). The occurrence of *P. baramica was*

influenced by the type of habitats in which they were occupied. Based on the data, the *P. baramica* species tends to occupy peat swamps, riverine forests, and forested areas (*kerangas* and edge MDF).

Pulchrana baramica inhabited various types of vegetation throughout all localities. The results in Table 4.2 showed that vegetation used by *P. baramica* was significant at $p < 0.05$ for all vegetation types except for secondary growth, immature or regenerating forest, and VG ($\chi^2 = 7.333$, df = 3, $p = 0.062$). Meanwhile, the total individuals of *baramica* were most abundant in peat swamps (VW, $p = 0.023$, $n = 34$) and riverine forests (VRF, $p = 0.003$, $n = 21$). Both habitat types were the highest compared with other habitats in all sampling localities.

The finding suggested that *P. baramica* is a frog species that can live in many types of vegetation but rarely inhabits secondary forest. Suprisingly, *P. baramica* from Bau populations ($\chi^2 = 3.000$, df = 3, $p = 0.392$) did not imply any habitat specificity like other localities. At Bau, only one individual *P. baramica* was found, respectively, in large clearings and plantations other than in the forest. Even though the number of individuals found was too low, it showed that *P. baramica* is a habitat generalist species since it is able to live in disturbed and undisturbed areas.

Table 4.1: Data collections of *Pulchrana baramica*

Localities	Habitat	No. of individuals	Relative abundance (%)
Protected areas			
Bako National Park	Riverine forest and peat swamp	13	16
Maludam National Park	Peat swamp	7	9
Mulu National Park	*Kerangas,* secondary and riverine forest	10	12
Niah National Park	Riverine forest	2	3

Similajau National Park	Riverine forest and peat swamp	10	12
Samajaya Nature Reserve	Secondary forest	2	3
Matang Wildlife Center	Edge Mixed Dipterocarp Forest (MDF) and secondary forest	5	6
Unprotected areas			
Bau	Large clearing, Plantation, *kerangas* and secondary forest	8	10
Kanowit	Logged forest	1	1
Kota Samarahan	Peat swamp	13	16
Sibu	Agriculture area and secondary forest	5	6
Ulu Semera	Peat swamp	5	6
	∑ Total individuals	**81**	

Table 4.2: Vegetation types occupied by *Pulchrana baramica*

Localities	VAgr	VE	VF	VG	VJ	VK	VR	VRF	VW	χ^2	χ^2 p-value	Total individuals
Protected areas												
Bako	0	0	0	0	0	0	0	8	5	8.000	0.018*	13
Maludam	0	0	0	0	0	0	0	0	7	5.444	0.020*	7
Mulu	0	0	0	1	0	4	0	5	0	8.333	0.040*	10
Niah	0	0	0	0	0	0	0	2	0	5.444	0.020*	2
Similajau	0	0	0	0	0	0	0	6	4	8.000	0.018*	10
Samajaya	0	0	0	2	0	0	0	0	0	5.444	0.020*	2
Matang	0	2	0	3	0	0	0	0	0	8.000	0.018*	5
Unprotected areas												
Bau	1	0	1	2	0	3	1	0	0	3.000	0.392^{NS}	8
Kanowit	0	0	0	0	1	0	0	0	0	5.444	0.020*	1
Kota Samarahan	0	0	0	0	0	0	0	0	13	5.444	0.020*	13
Sibu	2	0	0	3	0	0	0	0	0	8.000	0.018*	5
Ulu Semera	0	0	0	0	0	0	0	0	5	5.444	0.020*	5
χ^2	13.500	8.333	8.333	7.333	8.333	13.500	8.333	16.333	11.333			
χ^2 p-value	0.001*	0.004*	0.004*	0.062^{NS}	0.004*	0.001*	0.004*	0.003*	0.023*			
Total individuals	3	2	1	11	1	7	1	21	34			81

*Exact significant at $p < 0.05$; NS not significant; VAgr: Agriculture; VE: Edge MDF; VF: Large clearing; VG: Secondary growth, immature or regenerating forest; VJ: Selectively logged forest; VK: Kerangas forest; VR: Plantation; VRF: Riverine Forest; VW: Peat Swamp

ii. Horizontal positions occupied by *Pulchrana baramica*

The usage of horizontal positions by *P. baramica* is shown in Table 4.3. All positions showed significant differences except for those distant from any body of water, HPG (χ^2 = 3.167, df = 6, p = 0.788). 50% of the total individuals of *P. baramica* were observed to inhibit this horizontal position. Because *P. baramica* is a ground-dwelling species, based on the results, *baramica* can live in various types of horizontal positions, but the species tends to occupy areas that are far from any body of water.

Furthermore, the horizontal positions occupied by *P. baramica* in each locality revealed a significant difference for Maludam (χ2 = 6.400, df = 1, p = 0.011), Mulu (χ2 = 9.800, df = 2, p = 0.007), Niah (χ2 = 6.400, df = 1, p = 0.011), Similajau (χ2 = 6.200, df = 2, p = 0.045), Samajaya (χ2 = 6.400, df = 1, p = 0.011), Matang (χ2 = 9.800, df = 2, p = 0.007), Kanowit (χ2 = 6.400, df = 1, p = 0.011), Kota Samarahan (χ2 = 6.400, df = 1, p = 0.011), Sibu (χ2 = 9.800, df = 2, p = 0.007), and Ulu Semera (χ2 = 9.800, df = 2, p = 0.007). *Pulchrana baramica* from these locations appeared to choose distant from any water body as the most preferable horizontal position because *P. baramica* is a ground-dwelling species.

Meanwhile, Bako (χ^2 = 4.400, df = 3, p = 0.221) and Bau (χ^2 = 3.800, df = 2, p = 0.150) expressed no significant difference in horizontal positions occupied by *P. baramica*. Bako and Bau have many favored horizontal positions for *P. baramica* compared with other localities. *Pulchrana baramica* from Bako and Bau chooses to occupy the bank of a permanent stream and an intermittent stream, distant from any water body, and a permanent swamp.

Table 4.3: Horizontal positions occupied by *Pulchrana baramica*

Localities	HPC	HPF	HPG	HPJ	HPL	HPN	HPP	HPR	HPS	HPT	χ^2	χ^2 p-value	Total individuals
Protected areas													
Bako	1	0	2	1	0	1	8	0	0	0	4.400	0.221^{NS}	13
Maludam	0	0	0	0	0	0	7	0	0	0	6.400	0.011*	7
Mulu	1	0	9	0	0	0	0	0	0	0	9.800	0.007*	10
Niah	0	0	0	0	0	0	2	0	0	0	6.400	0.011*	2
Similajau	0	0	0	0	0	0	8	1	0	1	6.200	0.045*	10
Samajaya	0	0	2	0	0	0	0	0	0	0	6.400	0.011*	2
Matang	0	0	3	0	0	0	0	0	2	0	9.800	0.007*	5
Unprotected areas													
Bau	1	1	5	0	1	0	0	0	0	0	3.800	0.150^{NS}	8
Kanowit	0	0	1	0	0	0	0	0	0	0	6.400	0.011*	1
Kota Samarahan	0	0	13	0	0	0	0	0	0	0	6.400	0.011*	13
Sibu	2	0	3	0	0	0	0	0	0	0	9.800	0.007*	5
Ulu Semera	0	0	2	0	0	0	0	3	0	0	9.800	0.007*	5
χ^2	6.500	8.333	3.167	8.333	8.333	8.333	9.500	16.000	8.333	8.333			
χ^2 p-value	0.039*	0.004*	0.788^{NS}	0.004*	0.004*	0.004*	0.009*	0.001*	0.004*	0.004*			
Total individuals	5	1	40	1	1	1	25	4	2	1			81

*Exact significant at $p < 0.05$; NS not significant; HPC: Permanent stream: on bank; HPF: Intermittent stream: on bank; HPG: Distant from any body of water; HPJ: Temporary pond, in water; HPL: Temporary pond, on vegetation; HPN: Permanent pond; HPP: Permanent swamp; HPR: Permanent pond, on bank; HPS: Permanent swamp, in water; HPT: Permanent swamp, on vegetation

iii. Vertical positions occupied by *Pulchrana baramica*

Table 4.4 shows the vertical positions occupied by *Pulchrana baramica*. The microhabitat utilizations in vertical positions from each locality exhibited significant differences except for Bako (χ^2 = 8.000, df = 4, p = 0.092). Bako expressed six vertical positions that were inhabited by *P. baramica*, which were VPF (n = 1), VPG (n = 2), VPH (n = 1), VPJ (n = 1), VPL (n = 3), and VPM (n = 4).

Most of the vertical positions occupied by *P. baramica* showed significant values. *Pulchrana baramica* chose to sit on the shrubs or seedlings, with VPL (χ^2 = 3.833, df = 4, p = 0.429) as the most favorable vertical position with a total of 17 individuals. On the surface of bare soil, VPF (χ^2 = 8.000, df = 3, p = 0.046, n = 11) Despite all the vertical positions occupied by *P. baramica* showing significant values, two variables of vertical positions (VPB and VPJ) possessed no significant values. The *P. baramica* were less favorable to sit in or under dead leaves, VPB (χ^2 = 3.000, df = 1, p = 0.083), and sit on the log, VPJ (χ^2 = 5.333, df = 3, p = 0.149).

Table 4.4: Vertical positions occupied by *Pulchrana baramica*

Localities	VPB	VPD	VPE	VPF	VPG	VPH	VPJ	VPK	VPL	VPM	VPN	VPO	χ^2	χ^2 p-value	Total individuals
Protected areas															
Bako	0	0	0	1	2	1	1	0	3	4	0	0	8.000	0.092^{NS}	13
Maludam	0	0	0	0	3	0	0	0	2	1	1	0	11.333	0.010*	7
Mulu	0	0	0	1	1	3	0	0	1	2	0	2	4.667	0.198*	10
Niah	0	0	0	0	0	0	0	2	0	0	0	0	8.333	0.004*	2
Similajau	1	1	0	0	1	0	0	0	4	0	0	3	8.000	0.046*	10
Samajaya	0	0	0	0	0	0	1	0	1	0	0	0	5.333	0.021*	2
Matang	0	0	0	0	0	0	2	0	0	3	0	0	13.500	0.001*	5
Unprotected areas															
Bau	0	0	2	0	1	2	1	2	0	0	0	0	3.500	0.174*	8
Kanowit	0	0	0	0	0	0	1	0	0	0	0	0	8.333	0.004*	1
Kota Samarahan	0	0	0	5	0	0	3	0	4	0	0	1	16.333	0.003*	13
Sibu	0	0	1	1	0	0	1	0	2	0	0	0	6.500	0.039*	5
Ulu Semera	1	0	0	3	0	0	0	1	0	0	0	0	9.500	0.009*	5
χ^2	3.000	8.333	13.500	8.000	8.000	16.000	5.333	9.500	3.833	16.333	8.333	16.000			
χ^2 p-value	0.083^{NS}	0.004*	0.001*	0.046*	0.046*	0.001*	0.149^{NS}	0.009*	0.429*	0.003*	0.004*	0.001*			
Total individuals	3	1	3	11	8	6	10	5	17	10	1	6			81

*Exact significant at $p < 0.05$; NS not significant; VPB: In or under dead leaves; VPD: Under log; VPE: In log; VPF: On surface of bare soil; VPG: Surface of leaf litter of dead leaves; VPH: On rock; VPJ: On log; VPK: On seedling or herbaceous plant (<1m tall); VPL: On shrub or young seedling (1 – 7m tall); VPM: On tree or large vine (> 7m tall); VPN: On dead stump; VPO: In crown of fallen dead shrub of tree

iv. Substrate occupied by *Pulchrana baramica*

Next, for the substrate microhabitat utilizations, four out of eight substrate variables expressed significant substrate values (Table 4.5). *P. baramica* chooses stems of herbaceous plants (SB), twigs or branches of woody plants (SC), bank mud (SG), and bank rock (SJ) as the most favorable substrates. Moreover, Niah and Kanowit ($\chi^2 = 4.500$, df = 1, p = 0.034) have the same significant values of substrate. Both localities have only one substrate that was occupied by *P. baramica.* This showed that *P. baramica* individuals from both localities were quite specific in choosing preferred substrates.

Table 4.5: Substrate occupied by *Pulchrana baramica*

Localities	SA	SB	SC	SD	SF	SG	SH	SJ	χ^2	χ^2 p-value	Total individuals
Protected areas											
Bako	5	0	5	1	0	1	1	0	0.250	0.882^{NS}	13
Maludam	2	1	0	2	0	2	0	0	1.750	0.417^{NS}	7
Mulu	2	2	0	2	0	2	1	1	1.000	0.607^{NS}	10
Niah	2	0	0	0	0	0	0	0	4.500	0.034*	2
Similajau	4	0	2	3	1	0	0	0	4.500	0.343^{NS}	10
Samajaya	1	0	0	1	0	0	0	0	2.000	0.157^{NS}	2
Matang	0	0	3	1	1	0	0	0	3.250	0.197^{NS}	5
Unprotected areas											
Bau	3	0	0	2	0	0	1	2	3.000	0.392^{NS}	8
Kanowit	0	0	0	1	0	0	0	0	4.500	0.034*	1
Kota Samarahan	4	0	1	3	0	4	1	0	1.000	0.801^{NS}	13
Sibu	0	2	0	2	0	0	1	0	3.250	0.197^{NS}	5
Ulu Semera	0	0	0	0	1	2	2	0	3.250	0.197^{NS}	5
χ^2	4.000	9.500	16.333	1.333	3.000	8.000	3.500	13.500			
χ^2 p-value	0.549^{NS}	0.009*	0.003*	0.721^{NS}	0.083^{NS}	0.046*	0.174^{NS}	0.001*			
Total individuals	23	5	11	18	3	11	7	3			81

*Exact significant at $p < 0.05$; NS not significant; SA: Leaf of plant; SB: Stem or branch of herbaceous plant; SC: Twig or branch of woody plant; SD: Twig or branch of woody plant; SF: Under bark of log, stump, or tree; SG: Bank mud; SH: Bank sand or gravel; SJ: Bank rock

4.3.2 Ecological Guild Patterns of *Pulchrana baramica*

The stress values (Stress-I = 0.26030, Stress II = 0.51789, S-stress = 0.17253) and fit measures of Tucker's Coefficient of Congruence (symbol tucker = 0.96553) via non-metric dimensional scaling (NMDS) (Table 4.6) indicate a significant representation of habitats and microhabitat variations.

Table 4.6: Stress and fit measures values of the NMDS configuration

Stress and Fit Measures	Value
Normalized Raw Stress	0.06776
Stress-I	.26030a
Stress-II	.51789a
S-Stress	.17253b
Dispersion Accounted For (D.A.F.)	0.93224
Tucker's Coefficient of Congruence	0.96553

PROXSCAL minimizes Normalized Raw Stress
a. Optimal scaling factor = 1.073
b. Optimal scaling factor = 0.900.

Approximately 39 meaningful microhabitat variables (Table 4.7 and Figure 4) were able to describe the microhabitat utilization of *P. baramica*. The significant values of FCD described the most preferrable microhabitats for *P. baramica.* A high negative loading value of FCD 1 = -0.896 was determined for Riverine Forest (VRF) and a high positive loading value of FCD 2 = 1.349 for Peat Swamp (VW). Furthermore, *P. baramica* species choose to stay at a distance from any body of water (HPG) with a high positive loading value (FCD 1 = 1.407) and a permanent swamp (HPP) as the suitable horizontal position with a high negative loading value (FCD 1 = -1.072).

Table 4.7: Microhabitat variables of *Pulchrana baramica*

No.	**Microhabitats**	**Code**	**FCD 1**	**FCD 2**
	Vegetation Types			

1	Agriculture	VAgr	0.347	0.011
2	Edge MDF	VE	-0.205	-0.062
3	Large clearing (camp etc.)	VF	0.076	-0.129
4	Secondary growth, immature or regenerating forest	VG	0.207	-0.826
5	Selectively logged forest	VJ	0.155	0.038
6	*Kerangas* forest	VK	0.254	-0.557
7	Plantation (Rubber or Oil Palm)	VR	0.074	-0.015
8	Riverine Forest	VRF	-0.896	-0.719
9	Peat Swamp	VW	0.432	1.349
	Horizontal Positions			
10	Permanent stream: on bank (distance to bank)	HPC	-0.468	0.084
11	Intermittent stream: on bank	HPF	-0.04	-0.144
12	Distant from any body of water	HPG	1.407	-0.598
13	Temporary pond, in water	HPJ	0.023	0.144
14	Temporary pond, on vegetation	HPL	0.074	-0.015
15	Permanent pond	HPN	0.115	0.114
16	Permanent swamp	HPP	-1.072	0.583
17	Permanent pond, on bank	HPR	-0.035	0.41
18	Permanent swamp, in water	HPS	-0.205	-0.062
19	Permanent swamp, on vegetation	HPT	-0.05	-0.048
	Vertical Positions			
20	In or under dead leaves	VPB	-0.284	0.2
21	Under log	VPD	-0.09	0.043
22	In log	VPE	0.294	-0.101
23	On surface of bare soil	VPF	0.617	0.466
24	Surface of leaf litter of dead leaves	VPG	-0.438	0.512
25	On rock	VPH	0.371	-0.406
26	On log	VPJ	0.708	-0.244
27	On seedling or herbaceous plant (<1m tall)	VPK	-0.431	-0.194
28	On shrub or young seedling (1 – 7m tall)	VPL	-0.195	0.985
29	On tree or large vine (> 7m tall)	VPM	-0.592	-0.462
30	On dead stump	VPN	-0.027	0.113
31	In crown of fallen dead shrub of tree	VPO	-0.144	-0.564
	Substrate			
32	Leaf of plant	SA	-1.166	0.089
33	Stem or branch of herbaceous plant	SB	-0.147	-0.419
34	Twig or branch of woody plant	SC	-0.378	-0.746
35	Twig or branch of woody plant	SD	1.014	0.117
36	Under bark of log, stump, or tree	SF	-0.186	0.22

37	Bank mud	SG	0.492	0.608
38	Bank sand or gravel	SH	0.201	0.515
39	Bank rock	SJ	0.187	-0.29

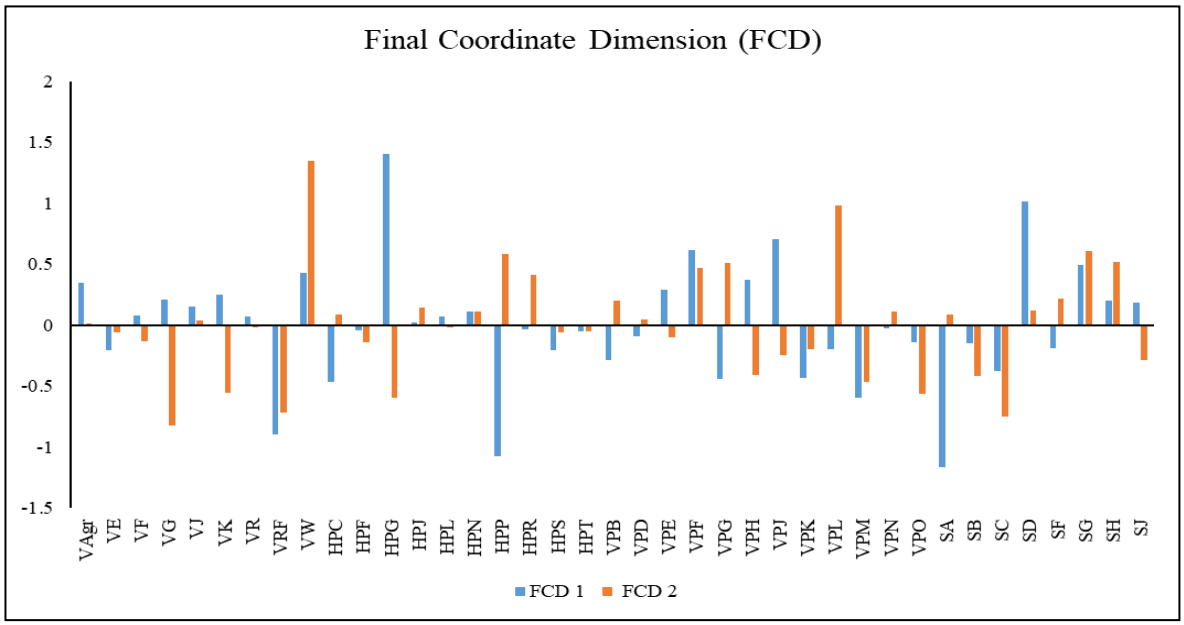


Figure 4.1: The column chart of final coordinate dimensions, FCD 1 and FCD 2, for each microhabitat attributes generated by NMDS (PROXSCAL)

Additionally, the two-dimensional values (Table 4.7 and Figure 4.1) represent meaningful variables for the vertical positions of microhabitat utilization by *P. baramica*. Two variables of vertical positions expressed the highest positive loadings, which were log, VPJ (FCD 1 = 0.708), and shrub or young seedling, VPL (FCD 2 = 0.985). Meanwhile, for the substrate, two variables with the highest FCD-1 value were recorded. *P. baramica* prefers to stay on the leaf of the plant, SA (FCD 1 = -1.166), and on the twig or branch of the woody plant, SD (FCD 1 = 1.014).

The ecological guilds of *P. baramica* were described in the scatterplot of the NMDS configuration (Figure 4.2). In accordance with the figure, seven clusters of ecological guilds were apparent: i) *Kerangas* forest, ii) peat swamp forest, iii) plantation, iv) riverine forest, v) agriculture, vi) disturbed area, and vii) edge of mixed dipterocarp forest All the ecological groups were clustered based on the microhabitat utilization that was preferred by *P.*

baramica species and not affected by localities.

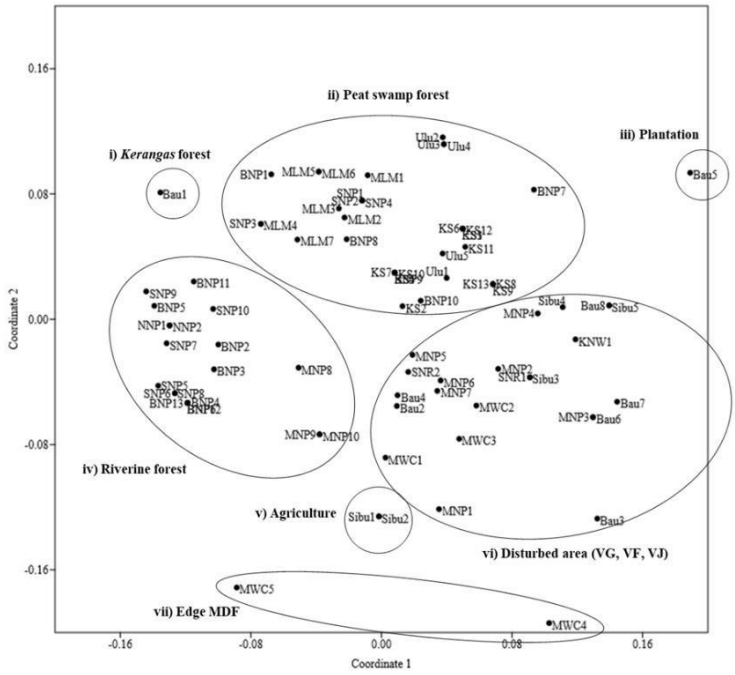


Figure 4.2: The scatterplot of NMDS configuration showing the ecological groups on microhabitat characteristics of *Pulchrana baramica*

4.3.3 Relationship Between Environmental Stressors and Genetic Diversity

Six physicochemical parameters and six heavy metal elements were determined at each sampling site (Table 4.8). The water variables such as pH, temperature, turbidity, dissolved oxygen, total dissolved solids, and salinity Mean concentrations were significant at $p > 0.05$. Most of the pH of the water in all sampling localities ranged from 5 to 8. The pH of the water was in optimum condition for *P. baramica* to live in.

For the water temperature, turbidity, total dissolved solids, and salinity, Maludam had the highest mean compared to other localities. Maludam is an open peat swamp area and does not have any shady trees. So, the sunlight hits the water surface directly. Thus, increase the water temperature. The turbidity of the Maludam River was high because the river was quite stagnant, had very dark brackish estuarine water, and was high in organic matter. Hence, the total dissolved solids in the water also increased. Due to the fact that Maludam

River is an estuarine area, the salinity of the river is quite high compared with Bako National Park.

Levels of heavy metals such as cadmium, copper, iron, manganese, nickel, and lead were identified. BDL referred to the below detection limit since some of the element concentration values were lower than the detection limit of the FAAS instrument. The concentrations of metals were significantly higher ($p < 0.05$) among all sampling localities. As for the heavy metal elements, the ranges of metals between all sites were as follows: Cd: 0.002–0.019 mg/L; Cu: 0.041–0.046 mg/L; Fe: 0.576–5.030 mg/L; Mn: 0.010–0.039 mg/L; Ni: 0.066–0.084 mg/L; and Pb: 0.008–0.227 mg/L. The decreasing relative order of heavy metal mean concentrations in water was as follows: Fe > Pb > Mn > Cu > Cd > Ni.

Metal concentrations in soil vary differently among the sampling sites and significantly at $p > 0.05$. The ranges of trace metal concentrations in soil were 0.057–0.44 for Cd, 3.088–14.830 for Cu, 3361.5–86.318 for Fe, 0.595–248.965 for Mn, 1.585–8.103 for Ni, and 1.990–10.238 for Pb. For soils, the decreasing relative order of heavy metal mean concentrations was as follows: Fe > Mn > Pb > Cu > Cd > Ni.

According to Table 4.9, Spearman's correlations showed that pH, with Spearman's correlation coefficient = 0.016, p = -0.847, has a negative correlation relationship with the number of *Pulchrana baramica* found in each locality. Next, the pH readings at Tanjung Datu (pH = 8.25) and Libiki Bamboo Resort's (pH = 8.06) were quite alkaline compared with other localities. This was supported by the findings that no *P. baramica* individuals were found at Tanjung Datu and Libiki Bamboo Resort. Other locations with *P. baramica* individuals present had water pH values between 5-7, and the water properties ranged from slightly acidic to neutral.

The correlation between environmental stressors and genetic diversity is shown in Figure 4.3. By considering the environmental stressors as independent variables that influence genetic diversity, the model expressed a weak correlation between the two variables as the Pearson's correlation coefficient (r = 0.494). The scatterplot does not fit the line of best fit as the coefficient of determination (R^2 = 0.244). The weak correlation between environmental stressors and genetic diversity was supported by the significant ANOVA value of regression (p = 0.01), where the p value is less than 0.05.

The environmental stressors can be categorized by physicochemical parameters, heavy metal pollution, anthropogenic effects, and adaptation to the surrounding habitat of the species itself. Thus, environmental stressors also contributed to the genetic diversity and abundance of *P. baramica* populations in a locality, depending on how strongly the stressors correlate with genetic diversity.

Table 4.8: Mean concentrations of physicochemical parameters and heavy metals from different localities

	Tanjung Datu, Sematan	Libiki Bamboo Resort, Bau	Maludam, Betong	Singai, Bau	Bako, Kuching	Kota Samarahan	Kanowit
P. baramica individuals	**0**	**0**	**4**	**5**	**4**	**3**	**1**
Physicochemical parameters							
pH	8.25 ± 0.25*	8.06 ± 0.21*	5.93 ± 0.70*	6.31 ± 0.49*	7.15 ± 0.78*	6.74 ± 0.95*	6.48 ± 0.04*
Temperature (°C)	26.7 ± 1.41	26.68 ± 0.07	31.2 ± 5.89	24.72 ± 1.81	28.6 ± 0.61	29.15 ± 2.05	28.16 ± 1.66
Turbidity (NTU)	5.20 ± 0.13	7.81 ± 2.58	35.65 ± 28.87	9.20 ± 5.44	0.91 ± 0.73	4.045 ± 3.99	5.04 ± 2.90
Dissolved Oxygen (mg/L)	5.48 ± 2.74	7.38 ± 0.45	5.06 ± 4.85	7.67 ± 1.51	7.21 ± 0.86	4.44 ± 3.17	7.61 ± 0.57
Total Dissolved Solids (ppm)	n/a	25.17 ± 2.60	471.17 ± 630.50	19.17 ± 6.36	10.33 ± 1.89	22.5 ± 20.51	n/a
Salinity (PSU)	n/a	6.34 ± 0.94*	9.25 ± 1.77*	5.50 ± 2.59*	4.50 ± 0.00*	4.00 ± 2.83*	6.00 ± 2.83*
Heavy metals (water)							
Cadmium (Cd)	0.019 ± 0.023*	0.010 ± 0.008*	0.004 ± 0.003*	0.003 ± 0.000	0.002 ± 0.000*	BDL	BDL
Copper (Cu)	0.045 ± 0.004*	0.041 ± 0.000*	0.046 ± 0.005*	0.042 ± 0.008*	0.045 ± 0.006*	BDL	BDL
Iron (Fe)	0.744 ± 0.224	1.182 ± 0.225	5.030 ± 3.914	1.022 ± 1.266	0.576 ± 0.123	0.954 ± 0.419	2.818 ± 1.718
Manganese (Mn)	0.039 ± 0.025*	0.032 ± 0.014*	0.165 ± 0.112*	0.021 ± 0.019*	0.011 ± 0.010*	0.034 ± 0.021*	0.010 ± 0.029*
Nickel (Ni)	0.073 ± 0.023*	0.072 ± 0.004*	0.073 ± 0.008*	0.066 ± 0.002*	0.084 ± 0.017*	BDL	BDL
Plumbum (Pb)	0.216 ± 0.024*	0.227 ± 0.051*	0.194 ± 0.053*	0.190 ± 0.006*	0.162 ± 0.004*	0.008 ± 0.007*	0.021 ± 0.015*
Heavy metals (soil)							
Cadmium (Cd)	0.130 ± 0.071*	BDL	0.057 ± 0.123*	0.154 ± 0.073*	0.080 ± 0.049*	BDL	BDL
Copper (Cu)	3.088 ± 0.760*	14.830 ± 2.044*	6.470 ± 2.487*	5.578 ± 2.010*	3.198 ± 0.251*	3.851 ± 3.963*	5.500 ± 6.654*
Iron (Fe)	1011.273 ± 796.227*	1962.715 ± 36.833*	1657.582 ± 303.086*	1637.923 ± 305.034*	86.318 ± 23.904*	1497.775 ± 1048.410*	3361.500 ± 77.075*
Manganese (Mn)	7.630 ± 5.119	181.498 ± 22.956	73.915 ± 87.052	248.965 ± 162.220	0.595 ± 0.184	12.754 ± 9.416	157.495 ± 208.151
Nickel (Ni)	1.585 ± 0.474*	8.103 ± 1.474*	6.913 ± 4.000*	4.605 ± 1.584*	1.850 ± 0.057*	BDL	BDL
Plumbum (Pb)	2.405 ± 1.174	10.105 ± 0.679	8.950 ± 5.711	10.238 ± 2.666	1.990 ± 0.567	5.404 ± 4.180	9.275 ± 1.336

*Exact significant at $p < 0.05$.

Table 4.9: Spearman's correlation between environmental stressors and *Pulchrana baramica* abundance

	pH	Temp	Turb	DO	TDS	Sal	Cd	Cu	Fe	Mn	Ni	Pb	n
pH	1	-0.321	-0.464	-0.107	-0.396	-0.571	0.450	-0.321	-0.393	-0.429	-0.090	-0.071	-0.847*
Temp		1	-0.214	0.786*	0.306	0.107	-0.577	-0.286	-0.143	-0.571	-0.252	-0.607	0.414
Turb			1	0.179	0.523	0.607	0.523	0.714	0.464	0.643	0.667	0.821*	0.306
DO				1	-0.342	0.250	0.162	0.464	0.393	0.643	0.198	0.500	-0.072*
TDS					1	0.613	0.045	0.541	0.234	0.306	0.664	0.378	0.427
Sal						1	-0.054	0.893**	0.750	0.571	0.631	0.571	0.342
Cd							1	0.342	-0.144	0.144	0.600	0.721	-0.327
Cu								1	0.643	0.679	0.847*	0.857*	0.162
Fe									1	0.750	0.180	0.464	-0.108
Mn										1	.396	0.714	0.072*
Ni											1	0.829*	0.200
Pb												1	-0.054**
n													1

**Exact significant at $p < 0.05$; * exact significant at $p<0.1$; Temp: Temperature; Turb: Turbidity; Sal: Salinity; Cd: Cadmium; Cu: Copper; Fe: Iron; Mn: Manganese; Ni: Nickel; Pb: Lead, n: number of *Pulchrana baramica* individual

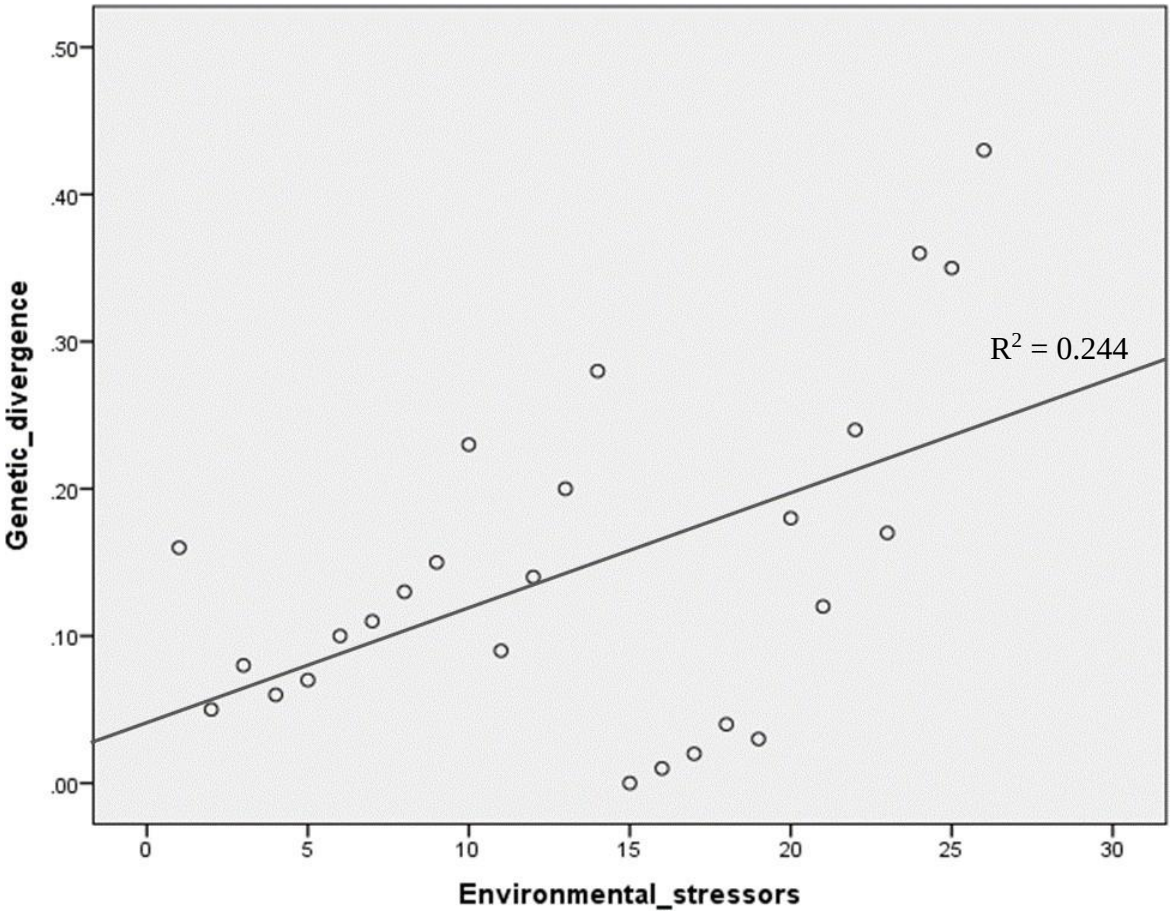


Figure 4.3: The correlation between environmental stressors and genetic divergence. Pearson's correlation coefficient (r = 0.494) and coefficient of determination (R^2 = 0.244)

4.3.4 Correlation Between the Physiochemical Parameters and Heavy Metal

Table 4.10 shows the correlation values between all the parameters. In accordance with this correlation, changes in one parameter were related to changes in the other. Four significant positive correlations were found: TDS-turbidity (r = 0.977), Ni-Cu (r = 0.765), Pb-Cu (r = 0.786), and Pb-Ni (r = 0.896). Based on the correlation values, physicochemical parameters did not influence the changes in heavy metal properties, and vice versa.

Total dissolved solids readings were influenced by the turbidity of the water. This situation concords with the values of total dissolved solids and turbidity at the Maludam River. Maludam NP turbidity reading equal to 35.65 NTU followed by TDS = 471.17 ppm. As the turbidity increases, the total dissolved solids in water also increases. Both parameters relate to each other. The Maludam River very brackish in colour and high contents of organic matter. Those readings were high compared with other six localities. Moreover, copper (Cu) influences the concentration of lead (Pb) and nickel (Ni). On the other hand, the correlation between elements Pb and Ni showed that these two elements also affected one another.

Principle component analysis through the varimax rotation method (Table 4.11, Figure 4.4) generates a good orthogonal factor rotation. The first component explained about 35.6% of all physicochemical parameters, and heavy metal properties were observed to have sufficient positive loadings except for pH (-0.521) and Cd (-0.240). Although the strong loading indicated that they were heavily impacting each other, the negative loadings in the same group revealed an opposing trend to the positive loadings.

Table 4.10: Pearson's correlation matrix of physicochemical parameters and heavy metals properties

	pH	Temp	Turb	DO	TDS	Sal	Cd	Cu	Fe	Mn	Ni	Pb
pH	1	-0.407	-0.523	0.063	-0.533	-0.677	0.745	0.319	-0.337	-0.257	0.146	0.045
Temp		1	0.547	-0.592	0.693	0.441	-0.39	-0.207	0.024	-0.555	-0.105	-0.341
Turb			1	-0.361	0.977*	0.684	-0.04	0.229	0.195	0.111	0.525	0.496
DO				1	-0.438	0.157	-0.109	0.335	0.216	0.668	0.185	0.364
TDS					1	0.675	-0.107	0.139	0.086	-0.064	0.464	0.354
Sal						1	-0.601	0.424	0.423	0.455	0.406	0.435
Cd							1	0.198	-0.335	-0.167	0.383	0.347
Cu								1	0.18	0.517	0.765*	0.786*
Fe									1	0.593	-0.232	0.128
Mn										1	0.319	0.667
Ni											1	0.896*
Pb												1

*Exact significant at $p < 0.05$; Temp: Temperature; Turb: Turbidity; Sal: Salinity; Cd: Cadmium; Cu: Copper; Fe: Iron; Mn: Manganese;Ni: Nickel; Pb: Lead.

Besides, the second component of PCA explained about 29.6% of the total variance and observed significant positive loadings of pH, Cd, Cu, Mn, Ni, and Pb. This suggests that if either the pH or the heavy metal characteristics change, they affect one another and change gradually. The remaining variables faced significant negative loadings. The third component has a total variance of about 20.4%, and most of the variables were observed with meaningful positive loadings, with the highest positive loading (Cd = 0.703). However, DO = -0.551, salinity = -0.255, Fe = -0.626, and Mn = -0.561 have strong negative loadings for the third component.

The dendrogram cluster diagram in Figure 4.5 showed that the physicochemical parameters and heavy metal properties were portrayed in three apparent clusters. Cluster 1 only consists of water physicochemical parameters, which are turbidity, total dissolved solids, temperature, and salinity. These four parameters always affect each other's values. Cluster 2 contained nickel, lead, copper, dissolved oxygen, manganese, and iron. Meanwhile, pH and cadmium lie together in cluster 3.

Table 4.11: Principal components with loadings of physicochemical parameters and heavy metal properties

	Component		
Parameters	**PC1**	**PC2**	**PC3**
pH	-0.521	0.593	0.464
Temp	0.277	-0.851	0.235
Turbidity	0.838	-0.344	0.342
DO	0.109	0.666	-0.551
TDS	0.770	-0.483	0.389
Salinity	0.907	-0.215	-0.255
Cd	-0.240	0.519	0.703
Cu	0.573	0.633	0.165
Fe	0.386	-0.001	-0.626
Mn	0.541	0.576	-0.561
Ni	0.661	0.486	0.518

Pb	0.726	0.633	0.218
Eigenvalues	4.271	3.556	2.452
% of variance extraction	35.595	29.632	20.431
Cumulative % extraction	35.595	65.228	85.659

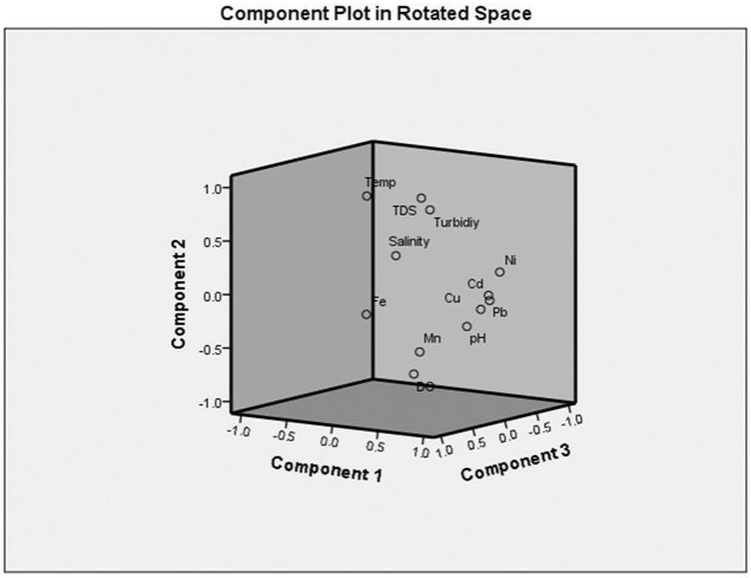


Figure 4.4: Component plot in rotated space
Extraction method: Principal Component Analysis; Rotation Method: Varimax

The dendrogram cluster diagram in Figure 4.5 showed that the physicochemical parameters and heavy metal properties were portrayed in three apparent clusters. Cluster 1 only consists of water physicochemical parameters, which are turbidity, total dissolved solids, temperature, and salinity. These four parameters always affect each other's values. Cluster 2 contained nickel, lead, copper, dissolved oxygen, manganese, and iron. Meanwhile, pH and cadmium lie together in cluster 3.

Although the values of environmental stressors which were physiochemical parameters and heavy metals influence each other, their relationship has no effect on the abundance of *Pulchrana baramica* in each locality. Figure 4.6 showed by the projection of relationship between *P. baramica* and environmental stressors. The least localities with *P. baramica* abundance were Tanjung Datu NP (TDNP) and Libiki Bamboo Resort (LBR) equals to 0. Followed by Kanowit (Knw = 1), Kota Samarahan (KS = 3), Bako NP (BNP =

4) and Bau = 5. Additionally, Maludam NP (MNP = 7) experienced the highest abundance of *P. baramica* individuals and the highest physicochemical parameters among other localities.

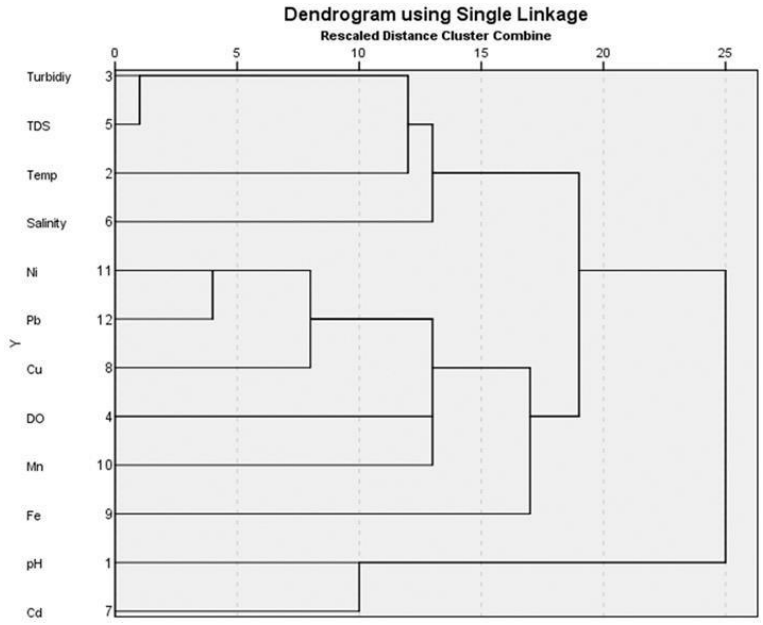


Figure 4.5: Hierarchical dendrogram for physicochemical and heavy metals properties from all samples using nearest neighbour, Pearson's Correlation with Z-standardization

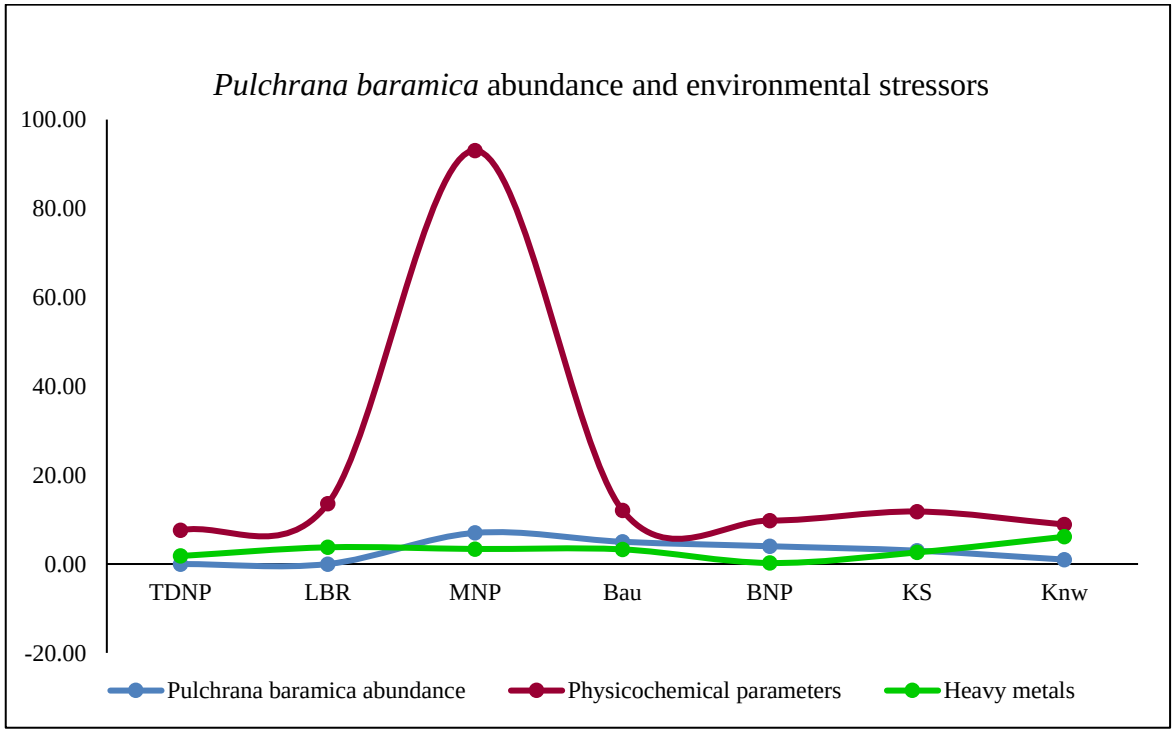


Figure 4.6: Relatedness between *Pulchrana baramica* abundance for each locality with physicochemical parameters and heavy metals (environmental stressors)

4.4 Discussions

Microhabitat varies according to the vegetation and resources available at the area. Adaptation is crucial to maintaining the survival of the generation. Other parameters such as water quality, humidity, rainfall, and weather conditions can influence to the microhabitat selection. Frogs are very susceptible in selection area especially for nesting. Good levels of water quality can determine the frog larval growth as well as to ensure the availability of food source.

Based on a previous study by Zainudin et al., (2019a), turbidity, salinity, temperature, and dissolved oxygen were influencing the number of species found in the oil palm plantations. This showed that the environmental parameters that influence the number of frog species and individuals might vary according to the study locality and vegetation types.

The heavy metal contents in the water or soil surrounding the frog niche can influence the growth of the frogs. Frogs exposed to the deformities and death if the heavy metal contents are too high. Worst, the frogs maybe extinct due to the exposure of heavy metals since they cannot breed perfectly if the situation persists for a long time. For example, the research conducted by (Thanomsangad et al., 2019).

Correlation happens when one variable's value changes and the value of the other variable also changes (Rosli et al., 2022). For instance, based to the experimental results done by Hoque et al. (2019), when high salinity occurs in water, it will increase the particles in the water and increase the TDS value. As TDS in water increases, the turbidity also rises and affects the water temperature reading (Hoque et al. 2019).

If the area is not suitable for frog communities to live in, maybe humans can be affected by the same problem. Humans and frogs need water to live. Some of frog species

are food source in the human food chain. *Limnonectes leporinus* and *Haplobatrachus rugolosus* are the examples of frogs that consumed by human. Even the *Haplobatrachus rugolusus* is breed commercially in a farm, they still affected if the farm or water source were affected by high exposure of heavy metals contents.

Even though the relationship between genetic diversity and environmental stressors showed weak correlation. Then natural and anthropogenic environmental changes lead to changes in genetic diversity, both within and among populations, and genetic diversity measurement can provide insights into the consequences of environmental changes (Pereyra et al., 2018; Zainudin et al., 2017).

4.5 Conclusion

This chapter explains the microhabitat of *P. baramica* among all sampling localities in Sarawak as well as the environmental stressors through physicochemical and heavy metal parameters that were collected from selected sampling localities. A total of 81 individuals of *P. baramica* from various localities that comprise a few types of habitats were used for the analysis. The data was also included from previous data collections. Based on the analysis, microhabitat utilization of *P. baramica* was clustered into a few significant ecological guilds, which were i) kerangas forest, ii) peat swamp, iii) plantation, iv) riverine forest, v) agriculture, vi) disturbed area, and vii) edge MDF.

Based on the finding, the microhabitat plays a vital role to provide a natural service to the species. Microhabitat utilization of *P. baramica* among all sampling localities in Sarawak successfully portrayed seven meaningful ecological clusters of *P. baramica*. This showed that the null hypothesis was rejected since variations in the *P. baramica* population do exist among all sampling localities in Sarawak. The varieties of microhabitat utilization

were dependent on the sources available in a particular habitat. Since a lot of forest fragmentation is happening, it forces *P. baramica* to adapt with limited resources and alter their needs. The pH of the water was the main parameter affecting the *P. baramica* abundance in a locality. A weak correlation between environmental stressors and genetic diversity was expressed. The correlation showed that frog genetic diversity variability is not only due to environmental conditions.

Meanwhile, the environmental stressors included six water quality parameters, which were dissolved oxygen, pH, salinity, water temperature, total dissolved solids, and turbidity. Other than that, six heavy metal elements, such as cadmium (Cd), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), and lead (Pb), were collected from soil and water samples from each sampling site. All the physicochemical parameters were successfully documented in the selected localities. The average mean concentration of heavy metals in soil and water generally followed the decreasing order (i) soil: Fe > Pb > Mn > Cu > Cd > Ni, and ii) water: Fe > Mn > Pb > Cu > Cd > Ni.

Overall, some of the parameters and heavy metal elements were influenced by each other, even in soil or water samples. The relationship between both parameters was important to ensure the quality of soil and water sources in a particular area since the parameters were part of environmental stressors. Nevertheless, despite the fact that the environmental stresses are related to one another, they have no impact on the population size of *P. baramica* individuals that inhabit the specific habitat.

CHAPTER 5

DIVERSIFICATION OF *Pulchrana baramica* SPECIES IN SARAWAK, BORNEO

5.1 Overview

The divergence of mtDNA indicates greater evolutionary independence among tropical anuran groups, and frog populations from forests or topographically diverse regions are experiencing evolution (Rodriguez et al., 2015). Genetic studies have indicated the presence of numerous frog species with minor physical differences (Ron et al., 2018). When it came to filling the barcoding gap, coding genes performed better than non-coding genes (Koroiva & Santana, 2022).

Nowadays, widespread sequencing and availability of mitochondrial DNA (mtDNA) has accelerated 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) used COI markers to study the genetic structure of *Hylarana erythraea* from Peninsula Malaysia and Sarawak. Besides, the use of COI barcodes for specimen identification and species discovery has proven to be an effective molecular approach in the study of anurans (Koroiva et al., 2020). As well as by using CytB gene sequences, which have proven useful in resolving relationships between closely related taxa and are commonly used in phylogenetic or phylogeographic studies (Kurniawan et al., 2010).

Adaptation and natural selection are examples of evolutionary forces that may occur in a community of a species in a particular area (Eterovick et al., 2010). Natural and anthropogenic environmental changes can lead to changes in genetic diversity, which

provides insights into the consequences of environmental changes. Since dispersal is an important part of the life history of amphibians, habitat fragmentation and destruction are serious threats to amphibian persistence. Chapter 4 revealed that environmental stressors can influence the genetic diversity and abundance of *P. baramica* species. This chapter elucidates the diversification of *Pulchrana baramica* from several localities in Sarawak based on selected molecular markers.

5.2 Methods

A total of 64 sequences of *P. baramica* consisting of CytB genes were manually checked by eyes in CHROMAS (V. 2.6.6, Technelysium Pty Ltd.) (Narayanan et al., 2022; Vedernikov et al., 2020). The sequences were aligned, analyzed, and phylogenetic trees were computed in MEGA X (Kumar et al., 2018; Marcaida et al., 2022). Based on Bayesian Information Criterion (BIC), models with the lowest BIC scores are considered to describe the best substitution pattern (Nei and Kumar, 2000).

The best model of CytB phylogenetic tree construction was Hasegawa-Kishino-Yano with an invariant site (HKY + I). Assuming that a certain fraction of sites was evolutionarily invariable (+I). All ML, MP, and NJ trees of both genes were constructed under the bootstrap method by using 1000 bootstrap values. CytB genes were selected for the determination of haplotypes of *P. baramica* and genetic differentiations through population genetics analyses and by using DNASP (V. 6) (Jablonski et al., 2021; Rozas et al., 2017). The haplotypes, level of population subdivision (Fst), nucleotide subdivision (Nst), and number of migrants per generation (Nm) were made up.

DNASP's output (Zainudin & Naim, 2018). Arlequin (V 3.5) (Excoffier & Lischer, 2010; Muir et al., 2013) used information about haplotypes used to compute mismatch

distribution, AMOVA, mantel test (Mantel & Valand, 1970), neutrality test was examined by Fu's Fs (Fu, 1997) and Tajima's D (Tajima, 1989). 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.) (P. Forster & M. Forster, 2022). The MSN calculated the geographical variations of *P. baramica* throughout 11 localities and approximated haplotype richness while controlling for irregular sample sizes (Nicolas et al., 2015).

5.3 Results

5.3.1 Diversification of *Pulchrana baramica* Lineages

The sequence data of Cytochrome b (CytB) in Table 5.1, with a total base pair of 569, comprises 64 sequences of *P. baramica*, including one outgroup. From the data, 400 bp were considered conserved sites, while 159 nucleotides were variable sites, 91 nucleotides were parsimony-informative sites, and 68 bp were singleton sites. As for the nucleotide base compositions, CytB sequences contained 25.73% of thymine (T), 36.95% of cytosine (C), 22.75% of adenine (A), and 14.57% of guanine (G).

The phylogenetic tree of CytB was constructed into three types of trees: ML, MP, and NJ. All trees portrayed similar topologies (Figure 5.1). Two apparent clades were defined according to vegetation types, namely, forest (clade A) and peat swamp (clade B). The peat swamp clade further separated into two major clusters, which were inland peat swamps and coastal peat swamps. The forest clade (clade A) comprises populations from Mulu and Niah, which were inhabited in *kerangas* and riverine vegetation. Mulu and Niah are also known as protected areas. Based on the trees, monophyletic groups consist of individuals from Mulu NP (RZ288/301 and MBS5-305) (99% ML/MP, 100% NJ) who were

monophyletic with subclade Mulu NP populations (MBS5-312/318 and MHQ 14) and Niah NP.

Furthermore, for clade B, the inland peat swamp and coastal peat swamp clade were separated with a bootstrap value of (99% ML/MP, 100% NJ). The inland peat swamp consists of populations from Bau, Matang, Samajaya, Kota Samarahan, Sibu, and Ulu Semera with strong bootstrap values (87% ML, 78% MP, and 92% NJ). All the localities are situated in the western part of Sarawak. Most of the populations inhabited the unprotected areas, but some protected areas, such as Matang and Samajaya, were included along with the cluster. Matang and Samajaya have the same inland peat swamp vegetation. In addition, the coastal peat swamp vegetation comprises populations from protected areas of Sarawak, which are Bako and Maludam. The clade had a strong bootstrap value (99% ML/MP/NJ). Meanwhile, the Similajau NP clade (SNP/004005/008/045/048) had strong monophyletic bootstrap values (99% ML/MP/NJ).

Table 5.1: Sequence data of *Pulchrana baramica* for CytB gene

Sequence data	**Compositions**
Alignment statistics	
Total base pair	569
Conserved sites	400/569
Variable sites	159/569
Parsimony informative sites	91/569
Singleton sites	68/569
Nucleotide bases	
Thymine (T)	25.73
Cytosine (C)	36.95
Adenine (A)	22.75
Guanine (G)	14.57

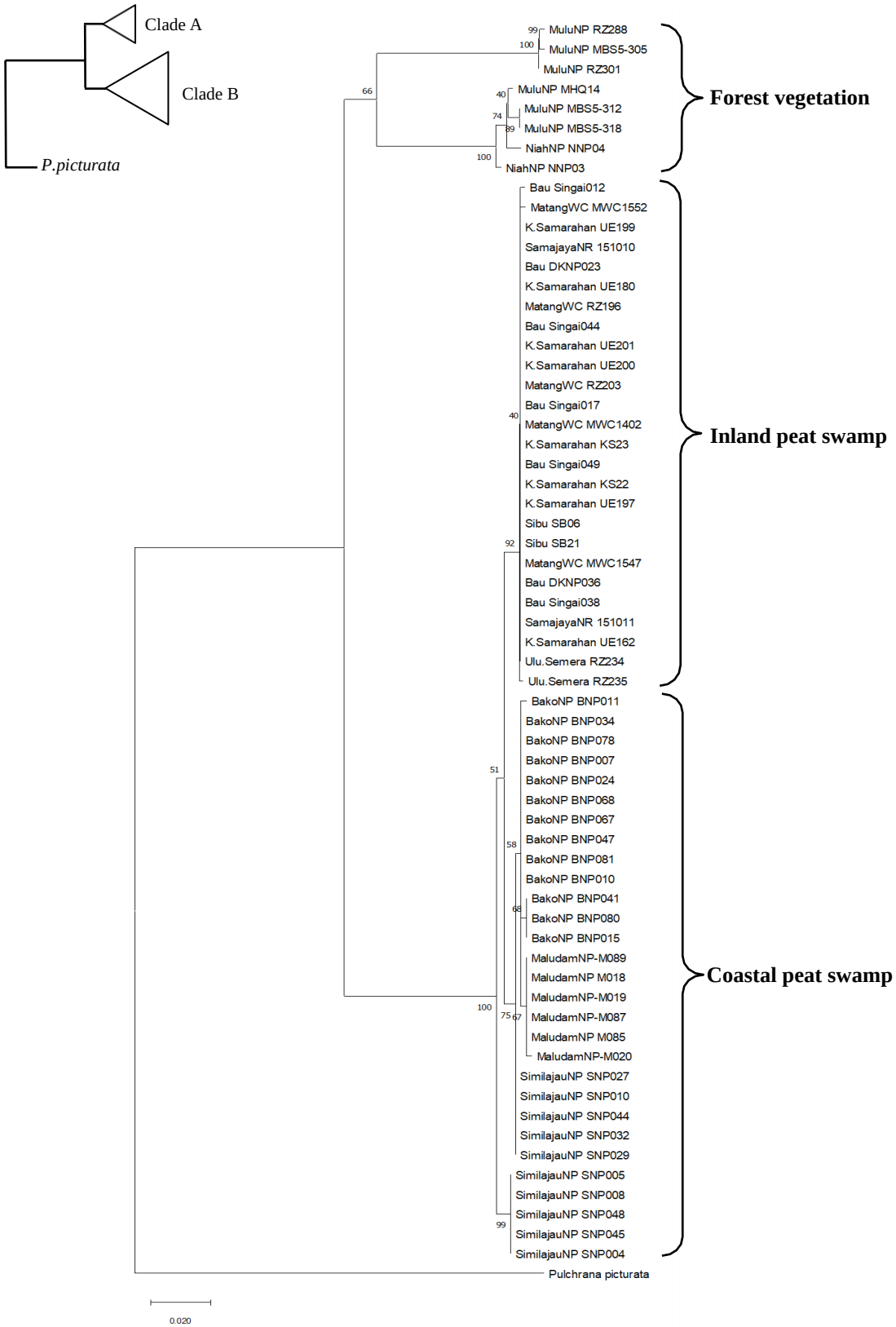


Figure 5.1: Lineage diversification of *Pulchrana baramica* populations within Sarawak by using CytB gene represented in ML/MP/NJ phylogenetic tree

5.3.2 Sequence Variations

A total of 94 segregating sites in the 569 base pair segments in the CytB gene sequence were observed, and 18 haplotypes with three different haplogroups were determined from 11 populations. The nucleotide diversity (π) ranged from 0.00 to 0.43, and the nucleotide divergence (Da) for *P. baramica* ranged from 0.00 to 0.08 (Table 5.2). The population of Mulu NP-Matang WC, Mulu NP-Ulu Semera, Mulu NP-Samaraya NR, and Mulu NP-Sibu experienced the highest nucleotide diversity ($\pi = 0.08$).

The nucleotide divergence (Da) values of all populations ranged between 0.00 and 0.10. Nine populations had the highest value of nucleotide divergence compared with other populations with the highest value of (Da = 0.1), namely, Kota Samarahan-Niah NP, Bako NP-Niah NP, Maludam NP-Niah NP, Similajau NP-Niah NP, Bau- Niah NP, Matang WC-Niah NP, Ulu Semera-Niah NP, Niah NP-Samajaya NR, and Niah NP-Sibu.

Table 5.3 showed the values of gene flow (Nm), population subdivision (Fst), and nucleotide subdivision (Nst). The highest Nst and Fst values was 0.98, representing the population from Kota Samarahan-Maludam NP, Maludam NP-Samajaya NR, and Maludam NP-Sibu. The population from Bako NP-Niah NP had the highest Fst value (0.81). The Mulu NP-Niah NP population from the protected area had the highest gene flow value (Nm = 0.99).

Table 5.2: Measures of nucleotide diversity (π) and net nucleotide divergence (Da) among populations of *Pulchrana baramica* analyzed by locations

Population	Distance (km)	$(\pi)^{a,b}$	(Da)
Kota Samarahan - Mulu NP	797.0	0.07	0.08
Kota Samarahan - Bako NP	39.7	0.01	0.01
Kota Samarahan - Maludam NP	111.0	0.01	0.01
Kota Samarahan - Similajau NP	531.0	0.01	0.01
Kota Samarahan - Bau	44.5	0.00	0.00
Kota Samarahan - Matang WC	57.5	0.00	0.00

Kota Samarahan - Ulu Semera	33.1	0.00	0.00
Kota Samarahan - Niah NP	631.0	0.04	0.10
Kota Samarahan - Samajaya	18.0	0.00	0.00
Kota Samarahan - Sibu	382.0	0.00	0.00
Mulu NP - Bako NP	917.0	0.05	0.08
Mulu NP - Maludam NP	683.0	0.07	0.08
Mulu NP - Similajau NP	285.0	0.06	0.08
Mulu NP - Bau	915.0	0.07	0.08
Mulu NP - Matang WC	943.0	0.08	0.08
Mulu NP - Ulu Semera	769.0	0.08	0.08
Mulu NP - Niah NP	186.5	0.05	0.02
Mulu NP - Samajaya	895.0	0.08	0.08
Mulu NP - Sibu	497.0	0.08	0.08
Bako NP - Maludam NP	150.0	0.00	0.00
Bako NP - Similajau NP	655.0	0.01	0.00
Bako NP - Bau	48.0	0.01	0.01
Bako NP - Matang WC	54.2	0.01	0.01
Bako NP - Ulu Semera	69.5	0.00	0.01
Bako NP - Niah NP	748.0	0.03	0.10
Bako NP - Samajaya	22.0	0.00	0.01
Bako NP - Sibu	415.0	0.00	0.01
Maludam NP - Similajau NP	421.0	0.01	0.01
Maludam NP - Bau	156.0	0.01	0.01
Maludam NP - Matang WC	167.0	0.01	0.01
Maludam NP - Ulu Semera	80.0	0.01	0.01
Maludam NP - Niah NP	514.0	0.05	0.10
Maludam NP - Samajaya	128.0	0.01	0.01
Maludam NP - Sibu	181.0	0.01	0.01
Similajau NP - Bau	650.0	0.01	0.01
Similajau NP - Matang WC	668.0	0.01	0.01
Similajau NP - Ulu Semera	500.0	0.01	0.01
Similajau NP - Niah NP	127.0	0.04	0.10
Similajau NP - Samajaya	633.0	0.01	0.01
Similajau NP - Sibu	232.0	0.01	0.01
Bau - Matang WC	50.8	0.00	0.00
Bau - Ulu Semera	74.2	0.00	0.00
Bau - Niah NP	742.0	0.04	0.10
Bau - Samajaya	33.0	0.00	0.00
Bau - Sibu	408.0	0.00	0.00
Matang WC - Ulu Semera	87.3	0.00	0.00
Matang WC - Niah NP	760.0	0.05	0.10
Matang WC - Samajaya	41.0	0.00	0.00
Matang WC - Sibu	426.0	0.00	0.00
Ulu Semera - Niah NP	592.0	0.07	0.10
Ulu Semera - Samajaya	47.6	0.00	0.00
Ulu Semera - Sibu	261.0	0.00	0.00
Niah NP - Samajaya	725.0	0.07	0.10
Niah NP - Sibu	324.0	0.07	0.10
Samajaya - Sibu	393.0	0.00	0.00
Samarahan - Mulu NP	797.0	0.07	0.08

[a]Estimated using the Kimura-2-parameter distance (Kimura, 1980). [b]Sites with gaps were completely excluded. *π: Nucleotide diversity; Da: Net nucleotide divergence.

Table 5.3: Measures of nucleotide subdivision (Nst), population subdivision (Fst) and gene flow (number of migrants, Nm) among 11 populations of *Pulchrana baramica*

Population	Distance (km)	(Nst)a	(Fst)b	(Nm)b
Kota Samarahan - Mulu NP	797	0.74	0.73	0.18
Kota Samarahan - Bako NP	39.7	0.96	0.96	0.02
Kota Samarahan - Maludam NP	111	0.98	0.98	0.01
Kota Samarahan - Similajau NP	531	0.72	0.72	0.19
Kota Samarahan - Bau	44.5	0.00	0.00	0.00
Kota Samarahan - Matang WC	57.5	0.00	0.00	0.00
Kota Samarahan - Ulu Semera	33.1	0.00	0.00	0.00
Kota Samarahan - Niah NP	631	0.96	0.96	0.02
Kota Samarahan - Samajaya	18	0.00	0.00	0.00
Kota Samarahan - Sibu	382	0.00	0.00	0.00
Mulu NP - Bako NP	917	0.73	0.73	0.19
Mulu NP - Maludam NP	683	0.73	0.73	0.19
Mulu NP - Similajau NP	285	0.72	0.71	0.20
Mulu NP - Bau	915	0.74	0.73	0.18
Mulu NP - Matang WC	943	0.73	0.73	0.18
Mulu NP - Ulu Semera	769	0.73	0.73	0.19
Mulu NP - Niah NP	186.5	0.34	0.33	0.99
Mulu NP - Samajaya	895	0.74	0.73	0.18
Mulu NP - Sibu	497	0.74	0.73	0.18
Bako NP - Maludam NP	150	0.70	0.70	0.21
Bako NP - Similajau NP	655	0.55	0.55	0.41
Bako NP - Bau	48	0.94	0.94	0.03
Bako NP - Matang WC	54.2	0.93	0.93	0.04
Bako NP - Ulu Semera	69.5	0.89	0.89	0.06
Bako NP - Niah NP	748	0.10	0.10	0.03
Bako NP - Samajaya	22	0.96	0.96	0.02
Bako NP - Sibu	415	0.96	0.96	0.02
Maludam NP - Similajau NP	421	0.65	0.65	0.28
Maludam NP - Bau	156	0.96	0.96	0.02
Maludam NP - Matang WC	167	0.95	0.95	0.03
Maludam NP - Ulu Semera	80	0.91	0.91	0.05
Maludam NP - Niah NP	514	0.96	0.95	0.02
Maludam NP - Samajaya	128	0.98	0.98	0.01
Maludam NP - Sibu	181	0.98	0.98	0.01
Similajau NP - Bau	650	0.71	0.71	0.21
Similajau NP - Matang WC	668	0.70	0.70	0.22
Similajau NP - Ulu Semera	500	0.67	0.67	0.25
Similajau NP - Niah NP	127	0.93	0.93	0.04
Similajau NP - Samajaya	633	0.72	0.72	0.19
Similajau NP - Sibu	232	0.72	0.72	0.19
Bau - Matang WC	50.8	0.00	0.00	0.00
Bau - Ulu Semera	74.2	0.00	0.00	0.00
Bau - Niah NP	742	0.96	0.96	0.02
Bau - Samajaya	33	0.00	0.00	0.00
Bau - Sibu	408	0.00	0.00	0.00
Matang WC - Ulu Semera	87.3	0.00	0.00	0.00
Matang WC - Niah NP	760	0.96	0.95	0.02
Matang WC - Samajaya	41	0.00	0.00	0.00
Matang WC - Sibu	426	0.00	0.00	0.00
Ulu Semera - Niah NP	592	0.95	0.95	0.03

Ulu Semera - Samajaya	47.6	0.00	0.00	0.00
Ulu Semera - Sibu	261	0.00	0.00	0.00
Niah NP - Samajaya	725	0.96	0.96	0.02
Niah NP - Sibu	324	0.96	0.96	0.02
Samajaya - Sibu	393	0.00	0.00	0.00

[a]Estimated using Lynch and Crease (1990). [b]Estimated using Hudson et. al (1992). *Nst: Nucleotide subdivision. Fst: Estimate Population subdivision. Nm: Number of migrants per generations.

5.3.3 Demographic History

A total of 18 haplotypes were identified from 63 sequences of the CytB gene (Table 5.4). The haplotypes were expressed along with the sample locality and sequence ID. There were about 11 haplotypes that only consist of one sequence per haplotype and represent one haplotype from different localities. The haplotypes were, Hap_1, Hap_2, Hap_3, Hap_7, Hap_8, Hap_9, Hap_12, Hap_13, Hap_16, Hap_17, and Hap_18.

Hap_4 was the highest haplotype that managed to define meaningful 23 sequences from the haplotype data set. Hap_4 shared the same haplotype with 23 individuals from six different localities, which were Kota Samarahan, Bau, Matang WC, Samajaya NR, Sibu and Ulu Semera. All the localities mentioned above were localities from unprotected areas. Meanwhile, other haplotypes such as Hap_5, Hap_6, Hap_10, Hap_11, Hap_14, and Hap_15 consist of more than one haplotype sequence that represents the same localities.

Table 5.4: Haplotypes of *Pulchrana baramica* identified from 11 different populations

Haplotypes	**N**	**Locality/Sequence ID**
Hap_1	1	Ulu Semera-RZ235
Hap_2	1	Bau-Singai012
Hap_3	1	MatangWC-MWC1552
Hap_4	23	Kota Samarahan [UE126, UE180, UE197, UE199, UE200, UE201, KS22, KS23] Bau [DKNP023, DKNP036, Singai017, Singai038, Singai044, Singai049] MatangWC [MWC1402, MWC1547, RZ196, RZ203] SamajayaNR [151010, 151011]

		Sibu [SB06, SB21]
		Ulu Semera-RZ234
Hap_5	5	SimilajauNP [SNP004, SNP005, SNP008, SNP045, SNP048]
Hap_6	9	BakoNP [BNP007, BNP010, BNP024, BNP034, BNP047, BNP067, BNP068, BNP078, BNP081]
Hap_7	1	NiahNP-NNP04
Hap_8	1	NiahNP-NNP03
Hap_9	1	MuluNP-MHQ14
Hap_10	2	MuluNP [MBS5-312, MBS5-318]
Hap_11	5	MaludamNP [M018, M019, M085, M087, M089
Hap_12	1	BakoNP-BNP011
Hap_13	1	MaludamNP-M020
Hap_14	5	SimilajauNP [SNP010, SNP027, SNP029, SNP032, SNP044]
Hap_15	3	BakoNP [BNP015, BNOP041, BNP080]
Hap_16	1	MuluNP-RZ301
Hap_17	1	MuluNP-RZ288
Hap_18	1	MuluNP-MBS5-305

Table 5.5 shows the diversity variation among populations was high (77.53%) with a significant p-value. Along with variation within populations (21.47%) with a significant p-value, this indicated that the rate of evolution was not equivalent in the same population. The estimated fixation index value was significant among and within populations of *P. baramica.*

Table 5.5: Measures of geographical population differentiation in *Pulchrana baramica* based on an analysis of molecular variance (AMOVA)

Source of variation	Variance component	Percentage of variation	Fixation index, Φ	P-value[a]
Among populations	7.38	78.53	0.79	0.00 ± 0.00*
Within populations	2.02	21.47	0.79	0.00 ± 0.00*

[a]Probability of finding a more-extreme variance component of the Φ index than that observed by chance alone after 1000 permutation. *Significant ($p < 0.05$).

The estimated **Φst** values among populations revealed in Table 5.6 were statistically significant at $p < 0.05$ with 1000 permutations in the pairwise genetic differentiation. Most of the populations gave very good significance of p-values and showed that all populations have a good genetic variation between localities. The demographic expansion of mismatch

distributions (Figure 5.2) represents the observed, sudden, and spatial model of the *P. baramica* distribution. The models vary according to locality.

Based on Figure 5.2, the multimodal shape of Kota Samarahan, Mulu NP, and Matang WC possessed good population stability. The populations from these locations were able to sustain their populations after a series of evolution events. Furthermore, Bako NP, Bau, and Similajau NP populations showed a spatial and sudden model with a declining pattern. But the observed model of the populations revealed that the populations will experience expansion for Bako NP, while the Bau and Similajau NP populations will keep expanding through time.

The Mantel test with 1000 permutations (Figure 5.3) was used to compute the isolation by distance of net nucleotide divergence and geographical distance. The test result showed no significant correlation between nucleotide divergence and geographical distance among 11 populations of *P. baramica* (r = 0.653127, p = 0.008000). The net nucleotide divergences (Da) among populations were varies for all *P. baramica* haplotypes. Geographical distance does not influence the nucleotide divergences in a population.

Table 5.6: Genetic differentiation matrix of populations calculated by ΦST. p values are shown in parenthesis (below the diagonal)

	1	2	3	4	5	6
1	-					
2	0.77 (0.00 ± 0.00)*	-				
3	0.95 (0.00 ± 0.00)*	0.82 (0.00 ± 0.00)*	-			
4	0.98 (0.00 ± 0.00)*	0.73 (0.00 ± 0.00)*	0.68 (0.00 ± 0.00)*	-		
5	0.70 (0.00 ± 0.00)*	0.77 (0.00 ± 0.00)*	0.58 (0.00 ± 0.00)*	0.59 (0.00 ± 0.00)*	-	
6	0.02 (0.53 ± 0.048)	0.75 (0.00 ± 0.00)*	0.93 (0.00 ± 0.00)*	0.96 (0.00 ± 0.00)*	0.67 (0.00 ± 0.00)*	-
7	0.10 (0.40 ± 0.39)	0.71 (0.00 ± 0.00)*	0.92 (0.00 ± 0.00)*	0.95 (0.00 ± 0.00)*	0.63 (0.00 ± 0.00)*	0.01 (0.64 ± 0.04)*
8	0.63 (0.23 ± 0.03)	0.58 (0.09 ± 0.03)	0.91 (0.02 ± 0.01)	0.94 (0.00 ± 0.00)*	0.55 (0.01 ± 0.01)*	0.27 (0.40 ± 0.04)*
9	0.99 (0.03 ± 0.02)	0.13 (0.38 ± 0.03)	0.99 (0.00 ± 0.00)*	0.98 (0.04 ± 0.01)*	0.94 (0.40 ± 0.02)	0.98 (0.06 ± 0.02)
10	0.00 (0.99 ± 0.00)	0.59 (0.02 ± 0.01)	0.92 (0.03 ± 0.01)*	0.96 (0.03 ± 0.01)*	0.54 (0.40 ± 0.01)*	-0.31 (0.10 ± 0.00)
11	0.00 (0.99 ± 0.00)	0.59 (0.01 ± 0.00)	0.92 (0.00 ± 0.00)	0.96 (0.03 ± 0.01)	0.54 (0.01 ± 0.01)*	-0.31 (0.10 ± 0.00)*

*Significant ($p < 0.05$) with 1000 permutations; 1: Sibu; 2: Samajaya; 3: Niah; 4: Ulu Semera; 5: Matang WC; 6: Bau; 7: Similajau NP; 8: Maludam NP; 9: Bako NP; 10: Mulu NP; 11: Kota Samarahan

Table 5.6: Continued

	7	8	9	10	11
1					
2					
3					
4					
5					
6					
7	-				
8	0.17 (0.41 ± 0.03)	-			
9	0.98 (0.01 ± 0.01)	0.95 (0.35 ± 0.06)	-		
10	-0.30 (0.10 ± 0.00)	0.00 (0.10 ± 0.00)	0.96 (0.23 ± 0.05)	-	
11	-0.30 (0.10 ± 0.00)*	0.00 (0.10 ± 0.00)	0.96 (0.35 ± 0.06)	0.00 (0.10 ± 0.03)	-

*Significant ($p < 0.05$) with 1000 permutations; 1: Sibu; 2: Samajaya; 3: Niah; 4: Ulu Semera; 5: Matang WC; 6: Bau; 7: Similajau NP; 8: Maludam NP; 9: Bako NP; 10: Mulu NP; 11: Kota Samarahan.

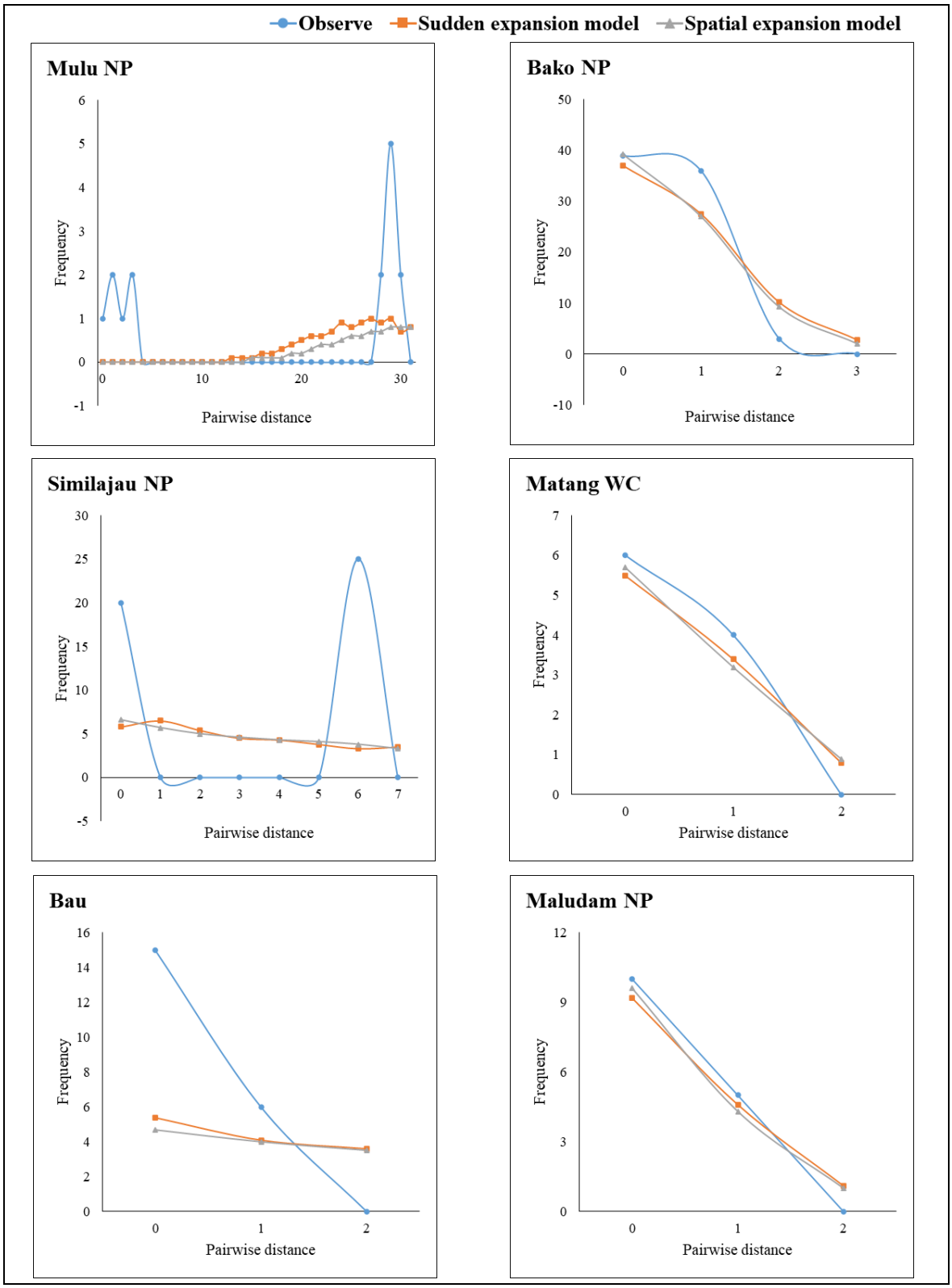


Figure 5.2: Mismatch distribution projections of *Pulchrana baramica* for each locality. Blue line represents the observed, orange line represents sudden and grey line represents spatial model of distributions.

Summary statistics of CytB mtDNA sequence variations of *P. baramica* in 11 populations were compiled in Table 5.7. The neutrality tests of Tajima's D and Fu's (*F*s) were used to estimate the *P. baramica* population expansions. Tajima's D gave positive test values, except some populations got negative test values: Bako NP (-0.46), Maludam NP (-0.93), Bau (-1.01), and Matang WC (-0.82). For the Fu statistics, all populations got positive test results. Despite that, Bako NP (-.0.41), Maludam NP (-0.003) and Bau (-0.09) received a negative value for Fu statistics (*F*s). As for the demographic SSD and expansion raggedness (r), positive values reflect that all populations were stable for the time being.

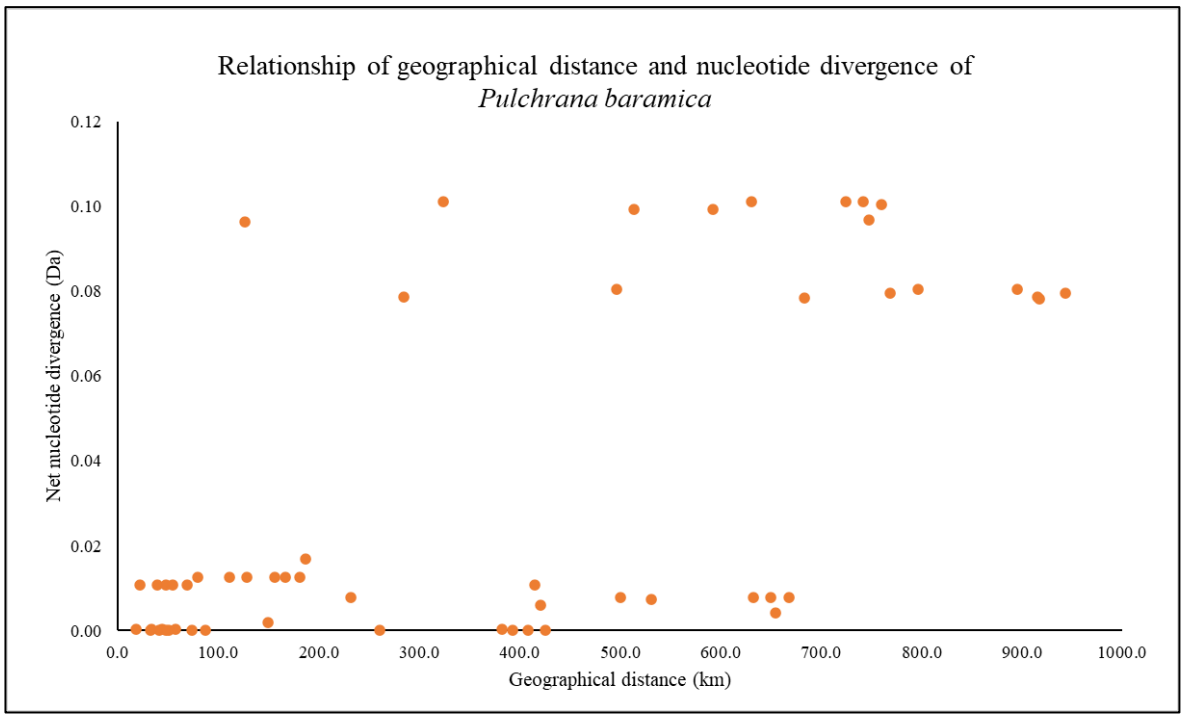


Figure 5.3: Scatterplot diagram of the relationship between geographical distance (km) and percentage of net nucleotide divergence (Da) between populations of *Pulchrana baramica*. Regression coefficient: y = 0.000088, correlation coefficient: r = 0.653127 and regression statistics: Y = 0.008000

Table 5.7: Summary statistics of CytB mtDNA sequence variations of *Pulchrana baramica* in 11 populations

Population	Sample size (n)	Haplotype	Tajima's, D	Fu statistics, Fs	Demographic SSD	Expansion Raggedness, r
Kota Samarahan	8	1	0.000 (1.000)	0.000 (N/A)	0.000 (1.000)	0.000 (1.000)
Mulu NP	6	5	2.078 (0.991)	2.817 (0.835)	0.146 (0.000)	0.147 (0.620)
Bako NP	13	3	-0.462 (0.323)	-0.413 (0.285)	0.020 (0.230)	0.182 (0.320)
Maludam NP	6	2	-0.933 (0.255)	-0.003 (0.253)	0.003 (0.740)	0.222 (0.930)
Similajau NP	10	2	2.365 (0.999)	5.803 (0.989)	0.368 (0.050)	0.815 (0.010)
Bau	7	2	-1.006 (0.242)	-0.095 (0.204)	0.249 (0.050)	0.265 (0.550)
Matang WC	5	2	-0.817 (0.290)	0.090 (0.284)	0.007 (0.200)	0.200 (0.940)
Ulu Semera	2	2	0.000 (1.000)	0.000 (0.234)	0.000 (1.000)	0.000 (1.000)
Niah NP	2	2	0.000 (1.000)	1.610 (0.501)	0.000 (1.000)	0.000 (1.000)
Samajaya NR	2	1	0.000 (1.000)	0.000 (N/A)	0.000 (1.000)	0.000 (1.000)
Sibu	2	1	0.000 (1.000)	0.000 (N/A)	0.000 (1.000)	0.000 (1.000)

5.3.4 Haplotypes Minimum Spanning Network (MSN)

Based on Figure 5.4, the minimum spanning network (MSN), illustrates the relationship between brown marsh frogs and *P. baramica* from 11 localities in Sarawak. Each circle represents one haplotype. Meanwhile, haplotypes are represented by circles, with sizes proportional to the number of people who share that haplotype. The size of the circles represents the haplotype frequency, and the colours of the circles differentiate localities. The numbers on the lines linking the haplotypes refer to the mutational steps.

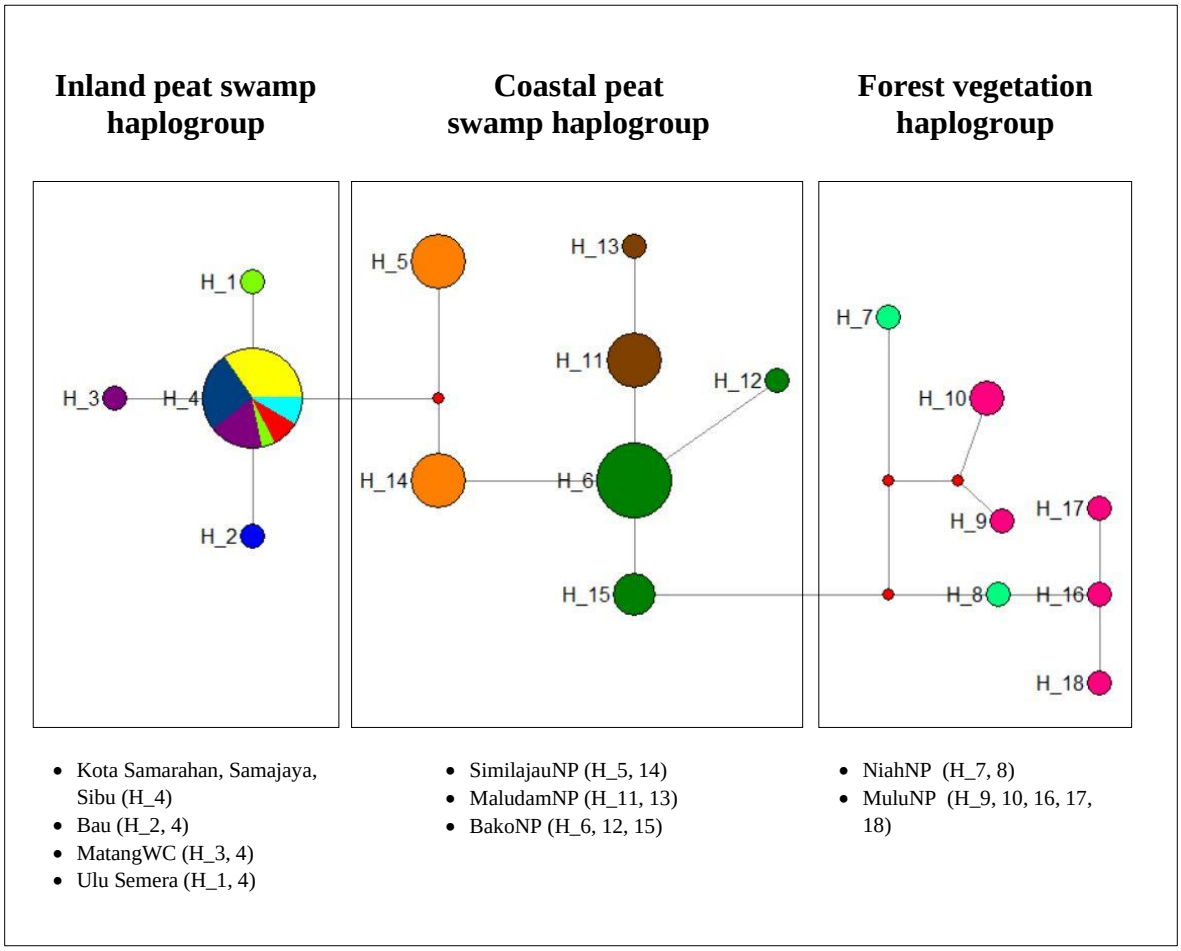


Figure 5.4: Minimum spanning network of *Pulchrana baramica* haplotypes from 11 localities

A total of 18 haplotypes were expressed from the analysis. The haplotypes were divided into three haplogroups, which were inland peat swamps, coastal peat swamps, and forest vegetation. Each haplogroup consists of a few haplotypes from different localities.

Other than that, the haplogroup also comprises shared haplotypes. Shared haplotypes refer to the one haplotype that is shared with two or more individuals from different localities.

For the inland peat swamp haplogroup cluster, there are populations from Kota Samarahan, Samajaya, Sibu, Bau, Matang WC, and Ulu Semera. Most locations were unprotected areas except for Matang WC, which was a protected area. Overall, four haplotypes were expressed from this haplogroup, namely, Hap_1 (n = 1), Hap_2 (n = 2), Hap_3 (n = 3), and Hap_4 (n = 23). Each haplotype was represented by one location, not including Hap_4. Hap_4 was a shared haplotype for the whole haplogroup since Hap_4 was shared by all inland peat swamp populations. Moreover, Hap_4 is also a specific haplotype for localities such as Kota Samarahan, Samajaya, and Sibu. Hap_4 was the highest haplotype, consisting of 23 individuals of *P. baramica.* Meanwhile, for Hap_1, Hap_2, and Hap_3 only had one individual of *P. baramica.*

Next, as for the coastal peat swamp haplogroup, there were three populations in this cluster, namely, Similajau NP, Maludam NP and Bako NP. These three locations were located along the coastlines of protected areas. There were no shared haplotypes between the populations, but two or three haplotypes were represented for each population. There were two haplotypes in Similajau NP: Hap_5 (n = 5) and Hap_14 (n = 5). Afterwards, Hap_11 (n = 5) and Hap_13 (n = 1) were the two haplotypes found in Maludam NP. In the Bako NP population, three haplotypes were identified: Hap_6 (n = 9), Hap_12 (n = 1), and Hap_15 (n = 3). Hap_6 from the Bako NP population is the most abundant haplotype among the seven haplotypes of the coastal peat swamp haplogroup in terms of *P. baramica* individuals.

Forest vegetation haplogroup contained two populations of *P. baramica* from Mulu NP and Niah NP. Surprisingly, these populations also originated from national parks that were gazetted as protected areas in the upper parts of Sarawak. The vegetations of these localities were riverine and *kerangas* forest. Overall, there were seven haplotypes in this haplogroup: Hap_7 (n =1), Hap_8 (n = 1), Hap_9 (n = 1), Hap_10 (n = 2), Hap_16 (n =1), Hap_17 (n =1), and Hap_18 (n =1). Niah NP is made up of two haplotypes: Hap_7 and Hap_8. Meanwhile, Mulu NP was represented by haplotypes Hap_9, Hap_10, Hap_16, Hap_17, and Hap_18. The highest haplotypes in the forest vegetation haplogroup were found in the Mulu NP population. Mulu NP possessed the most haplotype diversity out of the overall 11 populations.

5.4 Discussion

The clusters of *Pulchrana baramica* species based on the phylogenetic tree showed that the species were successfully adapt with the biogeographical landscape. The species were separated into three meaningful groups based on the vegetation types which were forest vegetation, coastal peat swamp and inland peat swamp vegetations. CytB genes managed to express the clusters by variations of the sequence bases.

Moreover, three types of vegetations were referred to the speciation of *P. baramica* species based on the microhabitat selection to ensure the survival of the species generation (Zainudin et al., 2017). The intraspecific interaction among populations was restricted by topography, high and low elevation areas, and temperature, thus causing the differences in gene flow values (Zhang et al., 2014).

Study made by Rodriguez et al. (2015) The changes in population trends depended on habitat types, climate changes, threats from predators, and anthropogenic effects. The

high and low genetic differences between these two areas were influenced by climatic changes and ecological factors (Liu et al., 2015). Impact from varieties of geography attributes and migration patterns can also affect the genetic diversity of a population (Zhang et al., 2014). Lastly, the conservation genetics should be improved in order to reduce loss of threatened species and more species can be conserve for future generation (Mona et al., 2014).

5.5 Conclusions

Two apparent clades were defined according to vegetation types, which were forest and peat swamp vegetation, in the diversification of *P. baramica* lineages through selected molecular markers. The forest vegetation, which consists of *kerangas* and riverine forest, was represented by populations from Mulu and Niah. Meanwhile, the peat swamp clade separated into two clusters of coastal and inland peat swamp populations of *P. baramica.* The null hypothesis was rejected, and the alternative hypothesis was accepted since there are distinct and unique lineages of *P. baramica* populations, even though the species have the same ecological requirements.

The difference in genes between individuals among the same species can happen as the species have the same ecological requirements but live in different habitats, and this was proved by the genetic diversity of *P. baramica.* The genetic diversity within and between populations of *P. baramica* inhabiting Sarawak habitat ecosystems was measured. There are genetic variations within and among populations of *P. baramica* inhabiting each ecosystem. Because most frogs that inhabit the same habitat types are still considered closely related species, Even so, they were separated by distance. Based on that, the null hypothesis was rejected, and the alternative hypothesis was accepted.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Throughout the year, amphibians are among the vertebrate classes most at risk. proving that the number of species in existence is significantly underestimated. In groups with high extinction rates and poorly defined species boundaries, such as amphibians, DNA barcoding is a tool for quickly assessing genetic diversity and estimating species richness to prioritize conservation decisions. The finding suggested that microhabitat, including environmental stressors, can influence the genetic diversity of a species populations.

Seven ecological guilds of *P. baramica* were successfully distinguished by the NMDS analysis. The ecological guilds were i) *kerangas* forest, ii) peat swamp, iii) plantation, iv) riverine forest, v) agriculture, vi) disturbed area, and vii) edge MDF. The finding showed that the species were generalists since they can live at various types of vegetation. As well as they able to live in disturbed environments. The varieties of microhabitat utilization depended on the sources of available habitats. Since a lot of forest fragmentation is happening, it forces *P. baramica* to adapt with limited resources and alter their needs. Environmental stressors also influence the amphibian's species diversity in particular habitats. Some environmental stressors limit the survival of certain amphibian species; thus, only successful species will survive.

As for the physicochemical and heavy metal properties, both parameters showed a positive pattern of relatedness. There were strong positive connections between the relationships of Ni-Cu/Pb-Cu/Pb-Ni (heavy metal) and TDS-turbidity (physicochemical parameters) based on the correlation analysis. From these findings, physicochemical parameters did not influence the changes in heavy metal properties, and vice versa. They only interact within the parameters.

Based on the diversification of *P. baramica* lineages, two distinct clades were defined according to vegetation types, which were forest and peat swamp vegetation. The forest vegetation consists of *kerangas* and riverine forest. Meanwhile, the peat swamp clade separated into two clusters of coastal and inland peat swamp populations of *P. baramica.* Even though the species has the same ecological requirements and is separated by distance, *P. baramica* populations would have a variety of unique lineages depending on the environment and habitat. Besides, genetic variations do exist within and between *P. baramica* populations that inhabit each ecosystem.

Pulchrana baramica is a good study model to assess the ecosystem health through the genetic diversity and abundance of the species in each study locality. The relationship between environmental stressors and genetic diversity showed a weak correlation. Based on the correlation, environmental stressors also contributed to the genetic diversity and abundance of *P. baramica* populations in a locality, depending on how strongly the stressors correlated with genetic diversity.

6.2 Recommendations

Overall, since this study only focused on the Sarawak region, more intensive and prolonged effort is needed to maintain the environment for the sustainability of *P. baramica* populations because more forest lands were converted into commercial areas. Also, *P. baramica* species genetic diversity and species distribution have been poorly studied. Further study can be carried out by widening the sampling areas to Peninsula Malaysia or other Southeast Asian countries. Additional sampling efforts, such as larger population sizes and the use of variations in molecular markers, can be considered to validate the findings.

Pulchrana baramica can be a study model for Next Generation Sequencing (NGS). NGS can be used to study the gut microbiome of a species since some microbes can cause disease in

frogs. As well as document the parasite species available in their habitats. Besides, the results from NGS, such as amino acid and nucleotide sequences, are extremely useful as markers for molecular ecology and systematic study.

Research regarding the bioaccumulation of heavy metals in *P. baramica* tissues can be done. Since the species inhabit residential and coastal areas, these areas have a high tendency to be exposed to heavy metals because there are a lot of anthropogenic activities. So, *P. baramica* can serve as a good study model as it has short life cycles and faster reproductions. The bioaccumulations of heavy metals can be compared by population generations. This information is also meaningful for communities, as heavy metals can harm ecosystems and human health.

Genetic variations and lineage diversification, as well as environmental parameters, can be used as indicators of ecosystem health. These data can be used to improve the Anuran Ecosystem Health Indicator (AEHI) databases to evaluate ecosystem health via genetic diversification and species connectivity in a locality. The databases can be pioneering references for future researchers and conservationists to execute more conservation efforts to maintain a healthy ecosystem for amphibian populations.

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APPENDICES

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) Distant
	HPG	from any body of water, distance to nearestream, (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 Permanent
	HPU	drainage, in plantation, on bankPermanent
	HPV	drainage, in plantation, in water

continued….

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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 willdescribe 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

***Pulchrana baramica* samples**

Table 2: Data of *Pulchrana baramica* samples collected including the microhabitat data

Localitie(s)	Sample(s)	Sample date	Sample type	MICROHABITAT			
				VT	HP	VP	SUB
Mulu National Park	MBS5 305	25/8/2016	muscle	VG	HPC	VPM	SD
	MBS5 312	26/8/2016	muscle	VK	HPG	VPM	SD
	MBS5 318	26/8/2016	muscle	VK	HPG	VPH	SJ
	MHQ 05	25/8/2014	toes	VK	HPG	VPF	SG
	MHQ 14	26/8/2014	toes	VK	HPG	VPL	SA
	RZ267	22/8/2006	muscle	VRF	HPG	VPH	SG
	RZ268	22/8/2006	muscle	VRF	HPG	VPH	SH
	RZ270	22/8/2006	muscle	VRF	HPG	VPG	SA
	RZ288	23/8/2006	muscle	VRF	HPG	VPO	SB
	RZ301	24/8/2006	muscle	VRF	HPG	VPO	SB
Similajau National Park	SNP004	28/12/2005	skin	VW	HPP	VPL	SD
	SNP005	28/12/2005	muscle	VW	HPP	VPL	SD
	SNP008	28/12/2005	muscle	VW	HPP	VPG	SA
	SNP010	28/12/2005	muscle	VW	HPP	VPL	SD
	SNP027	29/12/2005	skin	VRF	HPP	VPD	SF
	SNP029	29/12/2005	muscle	VRF	HPP	VPO	SC
	SNP032	29/12/2005	muscle	VRF	HPP	VPO	SA
	SNP044	29/12/2005	muscle	VRF	HPP	VPO	SC
	SNP045	30/12/2005	skin	VRF	HPR	VPB	SA
	SNP048	31/12/2005	muscle	VRF	HPT	VPL	SA
Maludam National Park	Maludam 018	8/2/2020	muscle	VW	HPP	VPN	SD
	Maludam 019	8/2/2020	muscle	VW	HPP	VPM	SD
	Maludam 020	8/2/2020	muscle	VW	HPP	VPL	SB
	Maludam 085	14/7/2022	muscle	VW	HPP	VPG	SA
	Maludam 087	15/7/2022	muscle	VW	HPP	VPG	SG
	Maludam 089	15/7/2022	muscle	VW	HPP	VPG	SG

Table 2: Continued

Localitie(s)	Sample(s)	Sample date	Sample type	MICROHABITAT			
				VT	HP	VP	SUB
	Maludam 061	16/7/2022	muscle	VW	HPP	VPL	SA
Matang Wildlife Center	MWC1402	20/9/2014	muscle	VG	HPG	VPM	SC
	MWC 1547	31/10/2019	muscle	VG	HPG	VPM	SD
	MWC 1552	31/10/2019	muscle	VG	HPG	VPJ	SC
	RZ196	3/7/2006	liver	VE	HPS	VPJ	SF
	RZ203	3/7/2006	skin	VE	HPS	VPM	SC
Bau	DKNP 023	22/9/2016	muscle	VK	HPF	VPK	SA
	DKNP 036	23/9/2016	muscle	VG	HPG	VPK	SA
	AB 024	5/11/2018	muscle	VK	HPC	VPJ	SH
	Singai 012	1/10/2020	muscle	VG	HPG	VPG	SA
	Singai 017	1/10/2020	muscle	VR	HPL	VPE	SD
	Singai 038	3/10/2020	muscle	VK	HPG	VPH	SJ
	Singai 044	3/10/2020	muscle	VF	HPG	VPH	SJ
	Singai 049	4/10/2020	muscle	VAgr	HPG	VPE	SD
Niah National Park	NNP 03	2/4/2011	muscle	VRF	HPP	VPK	SA
	NNP 04	2/4/2011	muscle	VRF	HPP	VPK	SA
Bako National Park	BNP007	21/8/2016	muscle	VW	HPJ	VPG	SA
	BNP010	28/5/2005	muscle	VRF	HPP	VPF	SH
	BNP011	28/5/2005	muscle	VRF	HPP	VPH	SG
	BNP015	28/5/2005	muscle	VRF	HPP	VPM	SC
	BNP024	29/5/2005	muscle	VRF	HPP	VPB	SA
	BNP034	21/11/2005	muscle	VRF	HPP	VPM	SC
	BNP041	10/3/2021	muscle	VW	HPN	VPJ	SD
	BNP047	11/3/2021	muscle	VW	HPC	VPL	SA
	BNP067	13/3/2021	muscle	VW	HPG	VPL	SA
	BNP068	13/3/2021	muscle	VW	HPG	VPL	SC
	BNP078	21/11/2005	muscle	VRF	HPP	VPG	SA
	BNP080	21/11/2005	muscle	VRF	HPP	VPM	SC

Table 2: Continued

Localitie(s)	Sample(s)	Sample date	Sample type	MICROHABITAT			
				VT	HP	VP	SUB
Bako National Park	BNP081	21/11/2005	muscle	VRF	HPP	VPM	SC
Samajaya Nature	SJNR 151010	10/10/2015	muscle	VG	HPG	VPJ	SD
Reserve	SJNR 151011	11/10/2015	muscle	VG	HPG	VPL	SA
Ulu Semera	RZ234	24/7/2006	liver	VW	HPG	VPB	SF
	RZ235	24/7/2006	liver	VW	HPR	VPF	SG
	RZ239	25/7/2006	toes	VW	HPR	VPF	SH
	RZ242	25/7/2006	toes	VW	HPR	VPF	SH
	RZ248	26/7/2006	toes	VW	HPG	VPK	SG
Kota Samarahan, UNIMAS	UE77	8/1/2022	muscle	VW	HPG	VPF	SG
	UE79	8/1/2022	muscle	VW	HPG	VPO	SC
	UE161	30/7/2010	muscle	VW	HPG	VPF	SG
	UE162	30/7/2010	muscle	VW	HPG	VPL	SA
	UE177	1/8/2010	muscle	VW	HPG	VPL	SA
	UE180	1/8/2010	muscle	VW	HPG	VPF	SG
	UE197	2/8/2010	muscle	VW	HPG	VPL	SA
	UE199	2/8/2010	muscle	VW	HPG	VPJ	SD
	UE200	2/8/2010	muscle	VW	HPG	VPJ	SD
	UE201	2/8/2010	muscle	VW	HPG	VPL	SA
	KS22	17/10/2011	muscle	VW	HPG	VPF	SH
	KS23	17/10/2011	muscle	VW	HPG	VPF	SG
	PPT016	7/1/2022	muscle	VW	HPG	VPJ	SD
Sibu	SB04	16/3/2022	muscle	VG	HPC	VPL	SB
	SB06	19/3/2022	muscle	VG	HPC	VPL	SB
	SB21	19/3/2022	muscle	VG	HPG	VPJ	SD
	SB26	20/3/2022	muscle	VAgr	HPG	VPF	SD
	SB30	20/3/2022	muscle	VAgr	HPG	VPE	SD
Kanowit	KN32	18/3/2022	muscle	VJ	HPG	VPJ	SD

Sequence Data of Cytochrome B (CytB)

Table 3: Pairwise genetic distance of 64 individuals analyzed based on Kimura 2 parameter model

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
MuluNP_RZ301																							
MuluNP_RZ288	0.00																						
MuluNP_MBS5-305	0.00	0.00																					
MuluNP_MBS5-312	0.10	0.10	0.10																				
MuluNP_MBS5-318	0.10	0.10	0.10	0.00																			
MuluNP_MHQ14	0.10	0.11	0.11	0.01	0.01																		
NiahNP_NNP04	0.10	0.11	0.11	0.01	0.01	0.01																	
NiahNP_NNP03	0.09	0.10	0.10	0.01	0.01	0.01	0.01																
K.Samarahan_UE199	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11															
Ulu.Semera_RZ234	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.00														
SamajayaNR_151010	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.00	0.00													
Bau_DKNP023	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.00	0.00	0.00												
K.Samarahan_UE180	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.00	0.00	0.00	0.00											
MatangWC_RZ196	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.00	0.00	0.00	0.00	0.00										
Bau_Singai044	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.00	0.00	0.00	0.00	0.00	0.00									
K.Samarahan_UE201	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00								
K.Samarahan_UE200	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00							
MatangWC_RZ203	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00						
Bau_Singai017	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00					
MatangWC_MWC1402	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
K.Samarahan_KS23	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00			
Bau_Singai049	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
K.Samarahan_KS22	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
K.Samarahan_UE197	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sibu_SB06	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sibu_SB21	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MatangWC_MWC1547	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bau_DKNP036	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bau_Singai038	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
K.Samarahan_UE162	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SamajayaNR_151011	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MatangWC_MWC1552	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bau_Singai012	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ulu.Semera_RZ235	0.12	0.13	0.13	0.12	0.12	0.12	0.12	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BakoNP_BNP034	0.12	0.13	0.13	0.11	0.11	0.11	0.11	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
BakoNP_BNP078	0.12	0.13	0.13	0.11	0.11	0.11	0.11	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
BakoNP_BNP007	0.12	0.13	0.13	0.11	0.11	0.11	0.11	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
BakoNP_BNP024	0.12	0.13	0.13	0.11	0.11	0.11	0.11	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
BakoNP_BNP068	0.12	0.13	0.13	0.11	0.11	0.11	0.11	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
BakoNP_BNP067	0.12	0.13	0.13	0.11	0.11	0.11	0.11	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
BakoNP_BNP047	0.12	0.13	0.13	0.11	0.11	0.11	0.11	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
BakoNP_BNP081	0.12	0.13	0.13	0.11	0.11	0.11	0.11	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
BakoNP_BNP010	0.12	0.13	0.13	0.11	0.11	0.11	0.11	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
BakoNP_BNP015	0.13	0.13	0.13	0.11	0.11	0.11	0.11	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
BakoNP_BNP041	0.13	0.13	0.13	0.11	0.11	0.11	0.11	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
BakoNP_BNP080	0.13	0.13	0.13	0.11	0.11	0.11	0.11	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
BakoNP_BNP011	0.13	0.13	0.13	0.12	0.12	0.11	0.12	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
MaludamNP-M089	0.13	0.13	0.13	0.11	0.11	0.11	0.12	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
MaludamNP_M018	0.13	0.13	0.13	0.11	0.11	0.11	0.12	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
MaludamNP-M019	0.13	0.13	0.13	0.11	0.11	0.11	0.12	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
MaludamNP-M087	0.13	0.13	0.13	0.11	0.11	0.11	0.12	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
MaludamNP_M085	0.13	0.13	0.13	0.11	0.11	0.11	0.12	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
MaludamNP-M020	0.13	0.13	0.13	0.11	0.11	0.11	0.12	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
SimilajauNP_SNP029	0.12	0.13	0.13	0.11	0.11	0.11	0.11	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
SimilajauNP_SNP032	0.12	0.13	0.13	0.11	0.11	0.11	0.11	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
SimilajauNP_SNP044	0.12	0.13	0.13	0.11	0.11	0.11	0.11	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
SimilajauNP_SNP010	0.12	0.13	0.13	0.11	0.11	0.11	0.11	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
SimilajauNP_SNP027	0.12	0.13	0.13	0.11	0.11	0.11	0.11	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
SimilajauNP_SNP005	0.13	0.13	0.13	0.12	0.12	0.12	0.12	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
SimilajauNP_SNP008	0.13	0.13	0.13	0.12	0.12	0.12	0.12	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
SimilajauNP_SNP048	0.13	0.13	0.13	0.12	0.12	0.12	0.12	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
SimilajauNP_SNP045	0.13	0.13	0.13	0.12	0.12	0.12	0.12	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
SimilajauNP_SNP004	0.13	0.13	0.13	0.12	0.12	0.12	0.12	0.11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
P.picturata	0.28	0.28	0.28	0.25	0.25	0.25	0.26	0.26	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27

Table 3: Continued

	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
MuluNP_RZ301																							
MuluNP_RZ288																							
MuluNP_MBS5-305																							
MuluNP_MBS5-312																							
MuluNP_MBS5-318																							
MuluNP_MHQ14																							
NiahNP_NNP04																							
NiahNP_NNP03																							
K.Samarahan_UE199																							
Ulu.Semera_RZ234																							
SamajayaNR_151010																							
Bau_DKNP023																							
K.Samarahan_UE180																							
MatangWC_RZ196																							
Bau_Singai044																							
K.Samarahan_UE201																							
K.Samarahan_UE200																							
MatangWC_RZ203																							
Bau_Singai017																							
MatangWC_MWC1402																							
K.Samarahan_KS23																							
Bau_Singai049																							
K.Samarahan_KS22																							
K.Samarahan_UE197																							
Sibu_SB06	0.00																						
Sibu_SB21	0.00	0.00																					
MatangWC_MWC1547	0.00	0.00	0.00																				
Bau_DKNP036	0.00	0.00	0.00	0.00																			
Bau_Singai038	0.00	0.00	0.00	0.00	0.00																		
K.Samarahan_UE162	0.00	0.00	0.00	0.00	0.00	0.00																	
SamajayaNR_151011	0.00	0.00	0.00	0.00	0.00	0.00	0.00																
MatangWC_MWC1552	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00															
Bau_Singai012	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00														
Ulu.Semera_RZ235	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00													
BakoNP_BNP034	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01												
BakoNP_BNP078	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00											
BakoNP_BNP007	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00										
BakoNP_BNP024	0.01	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									
BakoNP_BNP068	0.01	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								
BakoNP_BNP067	0.01	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	0.00							
BakoNP_BNP047	0.01	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	0.00	0.00						
BakoNP_BNP081	0.01	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	0.00	0.00	0.00					
BakoNP_BNP010	0.01	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	0.00	0.00	0.00	0.00				
BakoNP_BNP015	0.01	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	0.00	0.00	0.00	0.00	0.00			
BakoNP_BNP041	0.01	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	0.00	0.00	0.00	0.00	0.00	0.00		
BakoNP_BNP080	0.01	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	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
BakoNP_BNP011	0.01	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	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MaludamNP-M089	0.01	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	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MaludamNP_M018	0.01	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	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MaludamNP-M019	0.01	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	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MaludamNP-M087	0.01	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	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MaludamNP_M085	0.01	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	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MaludamNP-M020	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01
SimilajauNP_SNP029	0.01	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	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SimilajauNP_SNP032	0.01	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	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SimilajauNP_SNP044	0.01	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	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SimilajauNP_SNP010	0.01	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	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SimilajauNP_SNP027	0.01	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	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SimilajauNP_SNP005	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
SimilajauNP_SNP008	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
SimilajauNP_SNP048	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
SimilajauNP_SNP045	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
SimilajauNP_SNP004	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
P.picturata	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27

Table 3: Continued

	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64
MuluNP_RZ301																		
MuluNP_RZ288																		
MuluNP_MBS5-305																		
MuluNP_MBS5-312																		
MuluNP_MBS5-318																		
MuluNP_MHQ14																		
NiahNP_NNP04																		
NiahNP_NNP03																		
K.Samarahan_UE199																		
Ulu.Semera_RZ234																		
SamajayaNR_151010																		
Bau_DKNP023																		
K.Samarahan_UE180																		
MatangWC_RZ196																		
Bau_Singai044																		
K.Samarahan_UE201																		
K.Samarahan_UE200																		
MatangWC_RZ203																		
Bau_Singai017																		
MatangWC_MWC1402																		
K.Samarahan_KS23																		
Bau_Singai049																		
K.Samarahan_KS22																		
K.Samarahan_UE197																		
Sibu_SB06																		
Sibu_SB21																		
MatangWC_MWC1547																		
Bau_DKNP036																		
Bau_Singai038																		
K.Samarahan_UE162																		
SamajayaNR_151011																		
MatangWC_MWC1552																		
Bau_Singai012																		
Ulu.Semera_RZ235																		
BakoNP_BNP034																		
BakoNP_BNP078																		
BakoNP_BNP007																		
BakoNP_BNP024																		
BakoNP_BNP068																		
BakoNP_BNP067																		
BakoNP_BNP047																		
BakoNP_BNP081																		
BakoNP_BNP010																		
BakoNP_BNP015																		
BakoNP_BNP041																		
BakoNP_BNP080																		
BakoNP_BNP011																		
MaludamNP-M089	0.00																	
MaludamNP_M018	0.00	0.00																
MaludamNP-M019	0.00	0.00	0.00															
MaludamNP-M087	0.00	0.00	0.00	0.00														
MaludamNP_M085	0.00	0.00	0.00	0.00	0.00													
MaludamNP-M020	0.01	0.00	0.00	0.00	0.00	0.00												
SimilajauNP_SNP029	0.00	0.00	0.00	0.00	0.00	0.00	0.01											
SimilajauNP_SNP032	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00										
SimilajauNP_SNP044	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00									
SimilajauNP_SNP010	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00								
SimilajauNP_SNP027	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00							
SimilajauNP_SNP005	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01						
SimilajauNP_SNP008	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.00					
SimilajauNP_SNP048	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.00	0.00				
SimilajauNP_SNP045	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00			
SimilajauNP_SNP004	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00		
P.picturata	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.27	0.27	0.27	0.27	0.27	0.26	0.26	0.26	0.26	0.26	

***Pulchrana baramica* sequence alignment**

Table 4: CytB Sequence alignment

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                        ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                10         20         30         40         50         60
MuluNP_RZ301            GCTGATTACT CCGCAACCTA CACGCCAACG GCGCATCATT CTTCTTTATT TGCATCTACG
MuluNP_RZ288            GCTGATTACT CCGCAACCTA CACGCCAACG GCGCATCATT CTTCTTTATT TGCATCTACG
MuluNP_MBS5-305         GCTGATTACT CCGCAACCTA CACGCCAACG GCGCATCATT CTTCTTTATT TGCATCTACG
MuluNP_MBS5-312         GCTGACTACT CCGCAATCTA CACGCCAACG GCGCATCATT CTTCTTTATT TGCATCTATG
MuluNP_MBS5-318         GCTGACTACT CCGCAATCTA CACGCCAACG GCGCATCATT CTTCTTTATT TGCATCTATG
MuluNP_MHQ14            GCTGACTACT CCGCAATCTA CACGCCAACG GCGCATCATT CTTCTTTATT TGCATCTATG
NiahNP_NNP04            GCTGACTACT CCGCAATCTA CACGCCAACG GCGCATCATT CTTCTTTATT TGCATCTATG
NiahNP_NNP03            GCTGACTACT CCGCAATCTA CACGCCAACG GCGCATCATT CTTCTTTATT TGCATCTATG
K.Samarahan_UE199       GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
Ulu.Semera_RZ234        GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
SamajayaNR_151010       GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
Bau_DKNP023             GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
K.Samarahan_UE180       GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
MatangWC_RZ196          GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
Bau_Singai044           GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
K.Samarahan_UE201       GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
K.Samarahan_UE200       GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
MatangWC_RZ203          GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
Bau_Singai017           GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
MatangWC_MWC1402        GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
K.Samarahan_KS23        GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
Bau_Singai049           GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
K.Samarahan_KS22        GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
K.Samarahan_UE197       GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
Sibu_SB06               GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
Sibu_SB21               GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
MatangWC_MWC1547        GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
Bau_DKNP036             GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
Bau_Singai038           GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
K.Samarahan_UE162       GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
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                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                             10         20         30         40         50         60
SamajayaNR_151011   GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
MatangWC_MWC1552    GCTGGCTACT CCGCAACCTA CACGCCAACG GCGCATCATT CTTCTTTATC TGCATCTACG
Bau_Singai012       GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
Ulu.Semera_RZ235    GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
BakoNP_BNP034       GCTGACTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
BakoNP_BNP078       GCTGACTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
BakoNP_BNP007       GCTGACTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
BakoNP_BNP024       GCTGACTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
BakoNP_BNP068       GCTGACTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
BakoNP_BNP067       GCTGACTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
BakoNP_BNP047       GCTGACTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
BakoNP_BNP081       GCTGACTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
BakoNP_BNP010       GCTGACTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
BakoNP_BNP015       GCTGACTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
BakoNP_BNP041       GCTGACTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
BakoNP_BNP080       GCTGACTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
BakoNP_BNP011       GCTGACTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
MaludamNP-M089      GCTGACTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
MaludamNP_M018      GCTGACTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
MaludamNP-M019      GCTGACTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
MaludamNP-M087      GCTGACTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
MaludamNP_M085      GCTGACTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
MaludamNP-M020      GCTGACTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
SimilajauNP_SNP029  GCTGACTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
SimilajauNP_SNP032  GCTGACTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
SimilajauNP_SNP044  GCTGACTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
SimilajauNP_SNP010  GCTGACTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
SimilajauNP_SNP027  GCTGACTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
SimilajauNP_SNP005  GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
SimilajauNP_SNP008  GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
SimilajauNP_SNP048  GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
SimilajauNP_SNP045  GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
SimilajauNP_SNP004  GCTGGCTACT CCGCAACCTA CACGCTAACG GCGCATCATT CTTCTTTATC TGCATCTACG
Pulchrana_picturata GCTGATTACT CCGCAACCTA CACGCTAACG GCGCATCATT TTTTTTCATC TCTATTTATC
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                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                             70         80         90        100        110        120
MuluNP_RZ301        CCCACATCGG ACGAGGCCTA TACTACAGCT CCTACTTATT CAAAGCATGA AACATCGGGG
MuluNP_RZ288        CCCACATCGG ACGAGGCCTA TACTACAGCT CCTACTTATT CAAAGCATGA AACATCGGGG
MuluNP_MBS5-305     CCCACATCGG ACGAGGCCTA TACTACAGCT CCTACTTATT CAAAGCATGA AACATCGGGG
MuluNP_MBS5-312     CCCATATTGG ACGAGGCCTA TACTACAGCT CCTATCTATT TAAAGCATGA AACATCGGAG
MuluNP_MBS5-318     CCCATATTGG ACGAGGCCTA TACTACAGCT CCTATCTATT TAAAGCATGA AACATCGGAG
MuluNP_MHQ14        CCCACATTGG ACGAGGCCTA TACTACAGCT CCTATCTATT TAAAGCATGA AACATCGGAG
NiahNP_NNP04        CCCACATTGG ACGAGGCCTA TACTACAGCT CCTATCTATT TAAAGCATGG AACATCGGAG
NiahNP_NNP03        CCCACATTGG ACGAGGCCTA TACTACAGCT CCTATCTATT TAAAGCATGA AACATCGGAG
K.Samarahan_UE199   CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
Ulu.Semera_RZ234    CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
SamajayaNR_151010   CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
Bau_DKNP023         CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
K.Samarahan_UE180   CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
MatangWC_RZ196      CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
Bau_Singai044       CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
K.Samarahan_UE201   CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
K.Samarahan_UE200   CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
MatangWC_RZ203      CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
Bau_Singai017       CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
MatangWC_MWC1402    CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
K.Samarahan_KS23    CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
Bau_Singai049       CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
K.Samarahan_KS22    CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
K.Samarahan_UE197   CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
Sibu_SB06           CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
Sibu_SB21           CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
MatangWC_MWC1547    CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
Bau_DKNP036         CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
Bau_Singai038       CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
K.Samarahan_UE162   CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
SamajayaNR_151011   CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
MatangWC_MWC1552    CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
Bau_Singai012       CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
Ulu.Semera_RZ235    CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
BakoNP_BNP034       CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
BakoNP_BNP078       CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
BakoNP_BNP007       CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
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                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                            70         80         90        100        110        120
BakoNP_BNP024       CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
BakoNP_BNP068       CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
BakoNP_BNP067       CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
BakoNP_BNP047       CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
BakoNP_BNP081       CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
BakoNP_BNP010       CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
BakoNP_BNP015       CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
BakoNP_BNP041       CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
BakoNP_BNP080       CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
BakoNP_BNP011       CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
MaludamNP-M089      CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
MaludamNP_M018      CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
MaludamNP-M019      CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
MaludamNP-M087      CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
MaludamNP_M085      CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
MaludamNP-M020      CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
SimilajauNP_SNP029  CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
SimilajauNP_SNP032  CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
SimilajauNP_SNP044  CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
SimilajauNP_SNP010  CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
SimilajauNP_SNP027  CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
SimilajauNP_SNP005  CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
SimilajauNP_SNP008  CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
SimilajauNP_SNP048  CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
SimilajauNP_SNP045  CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
SimilajauNP_SNP004  CCCACATTGG ACGAGGCCTA TACTACAGCT CCTACCTTTT TAAAGCATGA AACATCGGAG
Pulchrana_picturata TCCATATCGG ACGAGGACTC TACTACGGCT CATACCTCTT CAAAGCATGA AATATTGGAG
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                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                            130        140        150        160        170        180
MuluNP_RZ301        TTATCCTACT ATTCCTGACC ACAGCTTTTG GCTACGTCCT TCCTTGGGGC CAAATATCTT
MuluNP_RZ288        TTATCCTACT ATTCCTGACC ACAGCTTTTG GCTACGTCCT TCCTTGGGGC CAAATATCTT
MuluNP_MBS5-305     TTATCCTACT ATTCCTGACC ACAGCTTTTG GCTACGTCCT TCCTTGGGGC CAAATATCTT
MuluNP_MBS5-312     TTATCTTGTT GTTCCTGACC ACAGCTTTTG GTTATGTCCT TCCTTGAGGC CAAATATCCT
MuluNP_MBS5-318     TTATCTTGTT GTTCCTGACC ACAGCTTTTG GTTATGTCCT TCCTTGAGGC CAAATATCCT
MuluNP_MHQ14        TTATCTTGTT GTTCCTGACC ACAGCTTTTG GTTATGTCCT TCCTTGAGGC CAAATATCCT
NiahNP_NNP04        TTATCTTGTT GTTCCTGACC ACAGCTTTTG GTTATGTCCT TCCTTGAGGC CAAATATCCT
NiahNP_NNP03        TTATCTTATT GTTCCTGACC ACAGCTTTTG GTTATGTCCT TCCTTGAGGC CAAATATCCT
K.Samarahan_UE199   TTATTTTACT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
Ulu.Semera_RZ234    TTATTTTACT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
SamajayaNR_151010   TTATTTTACT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
Bau_DKNP023         TTATTTTACT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
K.Samarahan_UE180   TTATTTTACT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
MatangWC_RZ196      TTATTTTACT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
Bau_Singai044       TTATTTTACT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
K.Samarahan_UE201   TTATTTTACT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
K.Samarahan_UE200   TTATTTTACT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
MatangWC_RZ203      TTATTTTACT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
Bau_Singai017       TTATTTTACT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
MatangWC_MWC1402    TTATTTTACT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
K.Samarahan_KS23    TTATTTTACT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
Bau_Singai049       TTATTTTACT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
K.Samarahan_KS22    TTATTTTACT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
K.Samarahan_UE197   TTATTTTACT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
Sibu_SB06           TTATTTTACT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
Sibu_SB21           TTATTTTACT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
MatangWC_MWC1547    TTATTTTACT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
Bau_DKNP036         TTATTTTACT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
Bau_Singai038       TTATTTTACT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
K.Samarahan_UE162   TTATTTTACT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
SamajayaNR_151011   TTATTTTACT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
MatangWC_MWC1552    TTATTTTACT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
Bau_Singai012       TTATTTTACT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
Ulu.Semera_RZ235    TTATTTTACT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
BakoNP_BNP034       TTATTTTGCT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
BakoNP_BNP078       TTATTTTGCT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
BakoNP_BNP007       TTATTTTGCT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
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                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                            130        140        150        160        170        180
BakoNP_BNP024       TTATTTTGCT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
BakoNP_BNP068       TTATTTTGCT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
BakoNP_BNP067       TTATTTTGCT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
BakoNP_BNP047       TTATTTTGCT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
BakoNP_BNP081       TTATTTTGCT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
BakoNP_BNP010       TTATTTTGCT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
BakoNP_BNP015       TTATTTTGCT GTTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
BakoNP_BNP041       TTATTTTGCT GTTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
BakoNP_BNP080       TTATTTTGCT GTTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
BakoNP_BNP011       TTATTTTGCT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
MaludamNP-M089      TTATTTTGCT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
MaludamNP_M018      TTATTTTGCT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
MaludamNP-M019      TTATTTTGCT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
MaludamNP-M087      TTATTTTGCT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
MaludamNP_M085      TTATTTTGCT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
MaludamNP-M020      TTATTTTGCT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
SimilajauNP_SNP029  TTATTTTGCT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
SimilajauNP_SNP032  TTATTTTGCT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
SimilajauNP_SNP044  TTATTTTGCT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
SimilajauNP_SNP010  TTATTTTGCT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
SimilajauNP_SNP027  TTATTTTGCT ATTCCTGACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
SimilajauNP_SNP005  TTATTTTACT ATTCCTTACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
SimilajauNP_SNP008  TTATTTTACT ATTCCTTACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
SimilajauNP_SNP048  TTATTTTACT ATTCCTTACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
SimilajauNP_SNP045  TTATTTTACT ATTCCTTACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
SimilajauNP_SNP004  TTATTTTACT ATTCCTTACT ACAGCCTTTG GCTATGTCCT TCCCTGAGGC CAAATATCCT
Pulchrana_picturata TACTTTTACT ATTTCTTACC ACTGCCTTTG GTTATGTGTT ACCATGAGGA CAAATATCTT
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                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                           190        200        210        220        230        240
MuluNP_RZ301        TTTGAGGTGC CACAGTCATC ACCAACCTTC TCTCAGCCGC CCCATACATC GGCTCAGAAC
MuluNP_RZ288        TTTGAGGTGC CACAGTCATC ACCAACCTTC TCTCAGCCGC CCCATACATC GGCTCAGAAC
MuluNP_MBS5-305     TTTGAGGTGC CACAGTCATC ACCAACCTTC TCTCAGCCGC CCCATACATC GGCTCAGAAC
MuluNP_MBS5-312     TTTGAGGCGC CACTGTCATC ACCAATCTCC TCTCAGCCGC CCCCTACATC GGCTCAGACC
MuluNP_MBS5-318     TTTGAGGCGC CACTGTCATC ACCAATCTCC TCTCAGCCGC CCCCTACATC GGCTCAGACC
MuluNP_MHQ14        TTTGAGGCGC CACTGTTATC ACCAATCTCC TCTCAGCCGC CCCCTACATC GGCTCAGACC
NiahNP_NNP04        TTTGAGGCGC CACTGTTATC ACCAATCTCC TCTCAGCCGC CCCCTACATC GGCTCAGACC
NiahNP_NNP03        TTTGAGGCGC CACTGTTATC ACCAATCTCC TCTCAGCCGC CCCCTACATC GGCTCAGACC
K.Samarahan_UE199   TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
Ulu.Semera_RZ234    TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
SamajayaNR_151010   TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
Bau_DKNP023         TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
K.Samarahan_UE180   TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
MatangWC_RZ196      TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
Bau_Singai044       TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
K.Samarahan_UE201   TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
K.Samarahan_UE200   TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
MatangWC_RZ203      TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
Bau_Singai017       TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
MatangWC_MWC1402    TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
K.Samarahan_KS23    TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
Bau_Singai049       TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
K.Samarahan_KS22    TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
K.Samarahan_UE197   TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
Sibu_SB06           TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
Sibu_SB21           TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
MatangWC_MWC1547    TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
Bau_DKNP036         TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
Bau_Singai038       TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
K.Samarahan_UE162   TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
SamajayaNR_151011   TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
MatangWC_MWC1552    TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
Bau_Singai012       TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
Ulu.Semera_RZ235    TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
BakoNP_BNP034       TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
BakoNP_BNP078       TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
BakoNP_BNP007       TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
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                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                            190        200        210        220        230        240
BakoNP_BNP024       TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
BakoNP_BNP068       TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
BakoNP_BNP067       TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
BakoNP_BNP047       TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
BakoNP_BNP081       TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
BakoNP_BNP010       TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
BakoNP_BNP015       TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
BakoNP_BNP041       TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
BakoNP_BNP080       TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
BakoNP_BNP011       TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
MaludamNP-M089      TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
MaludamNP_M018      TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
MaludamNP-M019      TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
MaludamNP-M087      TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
MaludamNP_M085      TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
MaludamNP-M020      TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
SimilajauNP_SNP029  TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
SimilajauNP_SNP032  TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
SimilajauNP_SNP044  TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
SimilajauNP_SNP010  TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
SimilajauNP_SNP027  TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
SimilajauNP_SNP005  TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
SimilajauNP_SNP008  TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
SimilajauNP_SNP048  TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
SimilajauNP_SNP045  TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
SimilajauNP_SNP004  TCTGAGGCGC CACAGTCATC ACCAACCTCC TCTCAGCAGC CCCCTACATC GGCTCAGACC
Pulchrana_picturata TCTGAGGCGC CACTGTTATT ACTAATCTTC TGTCAGCAGT CCCCTATATT GGATCAAACC
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                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                           250        260        270        280        290        300
MuluNP_RZ301        TTGTTCAATG AATCTGGGGC GGCTTCTCCG ACAACGCCAC TCCCCGATTC TTCACATTCC
MuluNP_RZ288        TTGTTCAATG AATCTGGGGC GGCTTCTCCG ACAACGCCAC TCCCCGATTC TTCACATTCC
MuluNP_MBS5-305     TTGTTCAATG AATCTGGGGC GGCTTCTCCG ACAACGCCAC TCCCCGGTTC TTCACATTCC
MuluNP_MBS5-312     TTGTCCAATG AATCTGAGGA GGCTTCTCCG ACAACGCAAC TCCTCGATTC TTTACATTCC
MuluNP_MBS5-318     TTGTCCAATG AATCTGAGGA GGCTTCTCCG ACAACGCAAC TCCTCGATTC TTTACATTCC
MuluNP_MHQ14        TTGTCCAATG AATCTGAGGA GGCTTCTCCG ACAACGCAAC TCCTCGATTC TTTACATTCC
NiahNP_NNP04        TTGTCCAATG AATCTGAGGA GGCTTCTCCG ACAACGCAAC TCCTCGATTC TTTACATTCC
NiahNP_NNP03        TTGTCCAATG AATCTGAGGA GGCTTCTCCG ACAACGCAAC TCCTCGATTC TTTACATTCC
K.Samarahan_UE199   TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
Ulu.Semera_RZ234    TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
SamajayaNR_151010   TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
Bau_DKNP023         TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
K.Samarahan_UE180   TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
MatangWC_RZ196      TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
Bau_Singai044       TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
K.Samarahan_UE201   TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
K.Samarahan_UE200   TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
MatangWC_RZ203      TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
Bau_Singai017       TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
MatangWC_MWC1402    TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
K.Samarahan_KS23    TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
Bau_Singai049       TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
K.Samarahan_KS22    TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
K.Samarahan_UE197   TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
Sibu_SB06           TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
Sibu_SB21           TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
MatangWC_MWC1547    TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
Bau_DKNP036         TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
Bau_Singai038       TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
K.Samarahan_UE162   TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
SamajayaNR_151011   TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
MatangWC_MWC1552    TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
Bau_Singai012       TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
Ulu.Semera_RZ235    TTGTCCAATG GATTTGAGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
BakoNP_BNP034       TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
BakoNP_BNP078       TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
BakoNP_BNP007       TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
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                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                           250        260        270        280        290        300
BakoNP_BNP024       TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
BakoNP_BNP068       TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
BakoNP_BNP067       TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
BakoNP_BNP047       TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
BakoNP_BNP081       TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
BakoNP_BNP010       TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
BakoNP_BNP015       TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
BakoNP_BNP041       TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
BakoNP_BNP080       TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
BakoNP_BNP011       TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
MaludamNP-M089      TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
MaludamNP_M018      TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
MaludamNP-M019      TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
MaludamNP-M087      TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
MaludamNP_M085      TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
MaludamNP-M020      TTGTCCAATG GATTTGGGGG GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
SimilajauNP_SNP029  TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
SimilajauNP_SNP032  TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
SimilajauNP_SNP044  TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
SimilajauNP_SNP010  TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
SimilajauNP_SNP027  TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
SimilajauNP_SNP005  TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
SimilajauNP_SNP008  TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
SimilajauNP_SNP048  TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
SimilajauNP_SNP045  TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
SimilajauNP_SNP004  TTGTCCAATG GATTTGGGGA GGCTTCTCCG ACAACGCTAC TCCCCGATTC TTCACATTCC
Pulchrana_picturata TCGTACAATG AATTTGAGGG GGCTTCTCAG ACAACGCTAC CCCCCGCTTC TTCACATTTC
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                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                            310        320        330        340        350        360
MuluNP_RZ301        ACTTCATCCT CCCATTCGCC -ATCGCAGCC GCCAGCATAC TCCACCTCTT AT------TC
MuluNP_RZ288        ACTTCATCCT CCCATTCGCC -ATCGCAGCC GCCAGCATAC TCCACCTCTT AT------TC
MuluNP_MBS5-305     ACTTCATCCT CCCATTCGCC -ATCGCAGCC GCCAGCATAC TCCACCTCTT AT------TC
MuluNP_MBS5-312     ACTTTATCCT CCCATTCGCC -ATCGCAGCC GCCAGCATAC TCCACCTCCT AT------TC
MuluNP_MBS5-318     ACTTTATCCT CCCATTCGCC -ATCGCAGCC GCCAGCATAC TCCACCTCCT AT------TC
MuluNP_MHQ14        ACTTTATCCT CCCATTCGCC -ATCGCAGCC GCCAGCATAC TCCACCTCCT AT------TC
NiahNP_NNP04        ACTTTATCCT CCCATTCGCC -ATCGCAGCC GCCAGCATAC TCCACCTCCT AT------TC
NiahNP_NNP03        ACTTTATCCT CCCATTCGCC -ATCGCAGCC GCCAGCATAC TCCACCTCCT AT------TC
K.Samarahan_UE199   ACTTCATCCT CCCGTTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
Ulu.Semera_RZ234    ACTTCATCCT CCCGTTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
SamajayaNR_151010   ACTTCATCCT CCCGTTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
Bau_DKNP023         ACTTCATCCT CCCGTTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
K.Samarahan_UE180   ACTTCATCCT CCCGTTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
MatangWC_RZ196      ACTTCATCCT CCCGTTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
Bau_Singai044       ACTTCATCCT CCCGTTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
K.Samarahan_UE201   ACTTCATCCT CCCGTTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
K.Samarahan_UE200   ACTTCATCCT CCCGTTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
MatangWC_RZ203      ACTTCATCCT CCCGTTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
Bau_Singai017       ACTTCATCCT CCCGTTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
MatangWC_MWC1402    ACTTCATCCT CCCGTTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
K.Samarahan_KS23    ACTTCATCCT CCCGTTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
Bau_Singai049       ACTTCATCCT CCCGTTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
K.Samarahan_KS22    ACTTCATCCT CCCGTTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
K.Samarahan_UE197   ACTTCATCCT CCCGTTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
Sibu_SB06           ACTTCATCCT CCCGTTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
Sibu_SB21           ACTTCATCCT CCCGTTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
MatangWC_MWC1547    ACTTCATCCT CCCGTTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
Bau_DKNP036         ACTTCATCCT CCCGTTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
Bau_Singai038       ACTTCATCCT CCCGTTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
K.Samarahan_UE162   ACTTCATCCT CCCGTTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
SamajayaNR_151011   ACTTCATCCT CCCGTTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
MatangWC_MWC1552    ACTTCATCCT CCCGTTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
Bau_Singai012       ACTTCATCCT CCCGTTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
Ulu.Semera_RZ235    ACTTCATCCT CCCGTTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
BakoNP_BNP034       ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
BakoNP_BNP078       ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
BakoNP_BNP007       ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
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                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                            310        320        330        340        350        360
BakoNP_BNP024       ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
BakoNP_BNP068       ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
BakoNP_BNP067       ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
BakoNP_BNP047       ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
BakoNP_BNP081       ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
BakoNP_BNP010       ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
BakoNP_BNP015       ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
BakoNP_BNP041       ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
BakoNP_BNP080       ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
BakoNP_BNP011       ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
MaludamNP-M089      ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
MaludamNP_M018      ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
MaludamNP-M019      ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
MaludamNP-M087      ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
MaludamNP_M085      ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
MaludamNP-M020      ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
SimilajauNP_SNP029  ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
SimilajauNP_SNP032  ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
SimilajauNP_SNP044  ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
SimilajauNP_SNP010  ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
SimilajauNP_SNP027  ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
SimilajauNP_SNP005  ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
SimilajauNP_SNP008  ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
SimilajauNP_SNP048  ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
SimilajauNP_SNP045  ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
SimilajauNP_SNP004  ACTTCATCCT CCCATTTGCT -ATCGCAGCC GCCAGCATGC TCCACCTCCT CT------TC
Pulchrana_picturata ACTTCATTTT ACCCTTTATC -ATTGCCGCC GCAAGCATAA TTCATCTCCT AT------TT
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                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                            370        380        390        400        410        420
MuluNP_RZ301        CTCCACCAAA CCGGATCCTC TAACCCCACA GGCTTGAACT CTGACCTAGA TAAAGTCTCA
MuluNP_RZ288        CTCCACCAAA CCGGATCCTC TAACCCCACA GGCTTGAACT CTGACCTAGA TAAAGTCTCA
MuluNP_MBS5-305     CTCCACCAAA CCGGATCCTC TAACCCCACA GGCTTGAACT CTGACCTAGA TAAAGTCTCA
MuluNP_MBS5-312     CTTCACCAAA CTGGGTCCTC TAACCCTACA GGCTTGAACT CCAACCTAGA CAAAGTTTCA
MuluNP_MBS5-318     CTTCACCAAA CTGGGTCCTC TAACCCTACA GGCTTGAACT CCAACCTAGA CAAAGTTTCA
MuluNP_MHQ14        CTTCACCAAA CTGGGTCCTC TAACCCTACA GGCTTGAACT CCAACCTAGA CAAAGTTTCA
NiahNP_NNP04        CTTCACCAAA CTGGGTCCTC TAACCCTACA GGCTTGAACT CCAACCTAGA CAAAGTTTCA
NiahNP_NNP03        CTTCACCAAA CTGGGTCCTC TAACCCCACA GGCTTGAACT CCAACCTAGA CAAAGTTTCA
K.Samarahan_UE199   CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
Ulu.Semera_RZ234    CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
SamajayaNR_151010   CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
Bau_DKNP023         CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
K.Samarahan_UE180   CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
MatangWC_RZ196      CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
Bau_Singai044       CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
K.Samarahan_UE201   CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
K.Samarahan_UE200   CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
MatangWC_RZ203      CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
Bau_Singai017       CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
MatangWC_MWC1402    CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
K.Samarahan_KS23    CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
Bau_Singai049       CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
K.Samarahan_KS22    CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
K.Samarahan_UE197   CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
Sibu_SB06           CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
Sibu_SB21           CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
MatangWC_MWC1547    CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
Bau_DKNP036         CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
Bau_Singai038       CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
K.Samarahan_UE162   CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
SamajayaNR_151011   CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
MatangWC_MWC1552    CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
Bau_Singai012       CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
Ulu.Semera_RZ235    CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
BakoNP_BNP034       CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
BakoNP_BNP078       CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
BakoNP_BNP007       CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
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                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                            370        380        390        400        410        420
BakoNP_BNP024       CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
BakoNP_BNP068       CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
BakoNP_BNP067       CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
BakoNP_BNP047       CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
BakoNP_BNP081       CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
BakoNP_BNP010       CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
BakoNP_BNP015       CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
BakoNP_BNP041       CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
BakoNP_BNP080       CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
BakoNP_BNP011       CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
MaludamNP-M089      CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
MaludamNP_M018      CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
MaludamNP-M019      CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
MaludamNP-M087      CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
MaludamNP_M085      CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
MaludamNP-M020      CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
SimilajauNP_SNP029  CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
SimilajauNP_SNP032  CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
SimilajauNP_SNP044  CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
SimilajauNP_SNP010  CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
SimilajauNP_SNP027  CTCCACCAGA CCGGATCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
SimilajauNP_SNP005  CTCCACCAGA CAGGGTCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
SimilajauNP_SNP008  CTCCACCAGA CAGGGTCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
SimilajauNP_SNP048  CTCCACCAGA CAGGGTCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
SimilajauNP_SNP045  CTCCACCAGA CAGGGTCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
SimilajauNP_SNP004  CTCCACCAGA CAGGGTCCTC TAACCCCACA GGACTAAATT CCAACCTAGA CAAAGTTTCG
Pulchrana_picturata CTTCACCAAA CAGGGTCATC AAACCCTACA GGACTGGATT CAAACTTAGA TAAAGTTTCA
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                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                            430        440        450        460        470        480
MuluNP_RZ301        TTCCACCCCT ACTTC---TC CTACAAAGAC CTGCTAGGCT TCGCCCTTAT ACTCGGGGCC
MuluNP_RZ288        TTCCACCCCT ACTTC---TC CTACAAAGAC CTGCTAGGCT TCGCCCTTAT ACTCGGGGCC
MuluNP_MBS5-305     TTCCACCCCT ACTTC---TC CTACAAAGAC CTGCTAGGCT TCGCCCTTAT ACTCGGGGCC
MuluNP_MBS5-312     TTCCACCCCT ACTTC---TC CTACAAGGAC CTGTTAGGAT TTGCTCTTAT ACTCGGAGCC
MuluNP_MBS5-318     TTCCACCCCT ACTTC---TC CTACAAGGAC CTGTTAGGAT TTGCTCTTAT ACTCGGAGCC
MuluNP_MHQ14        TTCCACCCCT ACTTC---TC CTACAAGGAC CTGTTAGGAT TTGCTCTTAT ACTCGGAGCC
NiahNP_NNP04        TTCCACCCCT ACTTC---TC CTACAAGGAC CTATTAGGAT TTGCTCTTAT ACTCGGGGCC
NiahNP_NNP03        TTCCACCCCT ACTTC---TC CTACAAGGAC CTGCTAGGAT TTGCTCTTAT ACTCGGGGCC
K.Samarahan_UE199   TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTAGGCT TCGCTCTCAT GCTCGGGGCC
Ulu.Semera_RZ234    TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTAGGCT TCGCTCTCAT GCTCGGGGCC
SamajayaNR_151010   TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTAGGCT TCGCTCTCAT GCTCGGGGCC
Bau_DKNP023         TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTAGGCT TCGCTCTCAT GCTCGGGGCC
K.Samarahan_UE180   TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTAGGCT TCGCTCTCAT GCTCGGGGCC
MatangWC_RZ196      TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTAGGCT TCGCTCTCAT GCTCGGGGCC
Bau_Singai044       TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTAGGCT TCGCTCTCAT GCTCGGGGCC
K.Samarahan_UE201   TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTAGGCT TCGCTCTCAT GCTCGGGGCC
K.Samarahan_UE200   TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTAGGCT TCGCTCTCAT GCTCGGGGCC
MatangWC_RZ203      TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTAGGCT TCGCTCTCAT GCTCGGGGCC
Bau_Singai017       TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTAGGCT TCGCTCTCAT GCTCGGGGCC
MatangWC_MWC1402    TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTAGGCT TCGCTCTCAT GCTCGGGGCC
K.Samarahan_KS23    TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTAGGCT TCGCTCTCAT GCTCGGGGCC
Bau_Singai049       TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTAGGCT TCGCTCTCAT GCTCGGGGCC
K.Samarahan_KS22    TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTAGGCT TCGCTCTCAT GCTCGGGGCC
K.Samarahan_UE197   TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTAGGCT TCGCTCTCAT GCTCGGGGCC
Sibu_SB06           TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTAGGCT TCGCTCTCAT GCTCGGGGCC
Sibu_SB21           TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTAGGCT TCGCTCTCAT GCTCGGGGCC
MatangWC_MWC1547    TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTAGGCT TCGCTCTCAT GCTCGGGGCC
Bau_DKNP036         TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTAGGCT TCGCTCTCAT GCTCGGGGCC
Bau_Singai038       TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTAGGCT TCGCTCTCAT GCTCGGGGCC
K.Samarahan_UE162   TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTAGGCT TCGCTCTCAT GCTCGGGGCC
SamajayaNR_151011   TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTAGGCT TCGCTCTCAT GCTCGGGGCC
MatangWC_MWC1552    TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTAGGCT TCGCTCTCAT GCTCGGGGCC
Bau_Singai012       TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTAGGCT TCGCCCTCAT GCTCGGGGCC
Ulu.Semera_RZ235    TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTAGGCT TCGCTCTCAT GCTCGGGGCC
BakoNP_BNP034       TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGGGCC
BakoNP_BNP078       TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGGGCC
BakoNP_BNP007       TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGGGCC
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                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                            430        440        450        460        470        480
BakoNP_BNP024       TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGGGCC
BakoNP_BNP068       TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGGGCC
BakoNP_BNP067       TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGGGCC
BakoNP_BNP047       TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGGGCC
BakoNP_BNP081       TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGGGCC
BakoNP_BNP010       TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGGGCC
BakoNP_BNP015       TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGGGCC
BakoNP_BNP041       TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGGGCC
BakoNP_BNP080       TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGGGCC
BakoNP_BNP011       TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGGGCC
MaludamNP-M089      TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGAGCC
MaludamNP_M018      TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGAGCC
MaludamNP-M019      TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGAGCC
MaludamNP-M087      TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGAGCC
MaludamNP_M085      TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGAGCC
MaludamNP-M020      TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGAGCC
SimilajauNP_SNP029  TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGGGCC
SimilajauNP_SNP032  TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGGGCC
SimilajauNP_SNP044  TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGGGCC
SimilajauNP_SNP010  TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGGGCC
SimilajauNP_SNP027  TTCCACCCCT ACTTC---TC CTACAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGGGCC
SimilajauNP_SNP005  TTCCACCCCT ACTTC---TC CTATAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGGGCC
SimilajauNP_SNP008  TTCCACCCCT ACTTC---TC CTATAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGGGCC
SimilajauNP_SNP048  TTCCACCCCT ACTTC---TC CTATAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGGGCC
SimilajauNP_SNP045  TTCCACCCCT ACTTC---TC CTATAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGGGCC
SimilajauNP_SNP004  TTCCACCCCT ACTTC---TC CTATAAAGAC CTCCTCGGCT TTGCTCTCAT GCTCGGGGCC
Pulchrana_picturata TTTCACCCCT ACTTT---TC CTATAAAGAT CTTTTTGGAT TTGTAATTAT ACTAGGAGCC
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                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                            490        500        510        520        530        540
MuluNP_RZ301        CTCGCACTAC TATCCACCTT CACCCCAAAC CTCCTAGGCG ACCCAGACAA CTTTACTCCA
MuluNP_RZ288        CTCACACTAC TATCCACCTT CACCCCAAAC CTCCTAGGCG ACCCAGACAA CTTTACTCCA
MuluNP_MBS5-305     CTCGCACTAC TATCCACCTT CACCCCAAAC CTCCTAGGCG ACCCAGACAA CTTTACTCCA
MuluNP_MBS5-312     CTCGCACTAC TATCCACCTT CACCCCTAAC CTCCTGGGCG ACCCAGACAA CTTTACCCCA
MuluNP_MBS5-318     CTCGCACTAC TATCCACCTT CACCCCTAAC CTCCTGGGCG ACCCAGACAA CTTTACCCCA
MuluNP_MHQ14        CTCGCACTAC TATCCACCTT CACCCCTAAC CTCCTGGGCG ACCCAGACAA CTTTACCCCA
NiahNP_NNP04        CTCGCACTAC TATCCACCTT CACCCCTAAC CTCCTGGGCG ACCCAGACAA CTTTACCCCA
NiahNP_NNP03        CTCGCACTAC TATCCACCTT CACCCCTAAC CTCCTGGGCG ACCCAGACAA CTTTACCCCA
K.Samarahan_UE199   CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
Ulu.Semera_RZ234    CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
SamajayaNR_151010   CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
Bau_DKNP023         CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
K.Samarahan_UE180   CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
MatangWC_RZ196      CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
Bau_Singai044       CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
K.Samarahan_UE201   CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
K.Samarahan_UE200   CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
MatangWC_RZ203      CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
Bau_Singai017       CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
MatangWC_MWC1402    CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
K.Samarahan_KS23    CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
Bau_Singai049       CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
K.Samarahan_KS22    CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
K.Samarahan_UE197   CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
Sibu_SB06           CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
Sibu_SB21           CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
MatangWC_MWC1547    CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
Bau_DKNP036         CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
Bau_Singai038       CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
K.Samarahan_UE162   CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
SamajayaNR_151011   CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
MatangWC_MWC1552    CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
Bau_Singai012       CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
Ulu.Semera_RZ235    CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
BakoNP_BNP034       CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
BakoNP_BNP078       CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
BakoNP_BNP007       CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
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                            490        500        510        520        530        540
BakoNP_BNP024       CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
BakoNP_BNP068       CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
BakoNP_BNP067       CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
BakoNP_BNP047       CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
BakoNP_BNP081       CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
BakoNP_BNP010       CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
BakoNP_BNP015       CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
BakoNP_BNP041       CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
BakoNP_BNP080       CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
BakoNP_BNP011       CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
MaludamNP-M089      CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
MaludamNP_M018      CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
MaludamNP-M019      CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
MaludamNP-M087      CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
MaludamNP_M085      CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
MaludamNP-M020      CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
SimilajauNP_SNP029  CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
SimilajauNP_SNP032  CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
SimilajauNP_SNP044  CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
SimilajauNP_SNP010  CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
SimilajauNP_SNP027  CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
SimilajauNP_SNP005  CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
SimilajauNP_SNP008  CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
SimilajauNP_SNP048  CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
SimilajauNP_SNP045  CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
SimilajauNP_SNP004  CTCGCATTAC TATCTACCTT CGCCCCAAAC CTCCTAGGTG ACCCAGACAA CTTTACACCT
Pulchrana_picturata CTAGCAACAC TTTCCGCCTT CGCCCCTAAC CTCTTGGGTG ACCCTGACAA TTTTACCCCG
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                    ....|....| ....|....| ....|....
                            550        560
MuluNP_RZ301        GCAAACCCCC TGGTAACACC TCCCCACAT
MuluNP_RZ288        GCAAACCCCC TGGTAACACC TCCCCACAT
MuluNP_MBS5-305     GCAAACCCCC TGGTAACACC TCCCCACAT
MuluNP_MBS5-312     GCAAACCCCT TAATAACCCC TCCCCACAT
MuluNP_MBS5-318     GCAAACCCCT TAATAACCCC TCCCCACAT
MuluNP_MHQ14        GCAAACCCCT TAATAACCCC CCCCCACAT
NiahNP_NNP04        GCAAACCCCT TAATAACCCC TCCCCACAT
NiahNP_NNP03        GCAAACCCCT TAATAACCCC TCCCCACAT
K.Samarahan_UE199   GCAAACCCAT TAATAACCCC CCCACACAT
Ulu.Semera_RZ234    GCAAACCCAT TAATAACCCC CCCACACAT
SamajayaNR_151010   GCAAACCCAT TAATAACCCC CCCACACAT
Bau_DKNP023         GCAAACCCAT TAATAACCCC CCCACACAT
K.Samarahan_UE180   GCAAACCCAT TAATAACCCC CCCACACAT
MatangWC_RZ196      GCAAACCCAT TAATAACCCC CCCACACAT
Bau_Singai044       GCAAACCCAT TAATAACCCC CCCACACAT
K.Samarahan_UE201   GCAAACCCAT TAATAACCCC CCCACACAT
K.Samarahan_UE200   GCAAACCCAT TAATAACCCC CCCACACAT
MatangWC_RZ203      GCAAACCCAT TAATAACCCC CCCACACAT
Bau_Singai017       GCAAACCCAT TAATAACCCC CCCACACAT
MatangWC_MWC1402    GCAAACCCAT TAATAACCCC CCCACACAT
K.Samarahan_KS23    GCAAACCCAT TAATAACCCC CCCACACAT
Bau_Singai049       GCAAACCCAT TAATAACCCC CCCACACAT
K.Samarahan_KS22    GCAAACCCAT TAATAACCCC CCCACACAT
K.Samarahan_UE197   GCAAACCCAT TAATAACCCC CCCACACAT
Sibu_SB06           GCAAACCCAT TAATAACCCC CCCACACAT
Sibu_SB21           GCAAACCCAT TAATAACCCC CCCACACAT
MatangWC_MWC1547    GCAAACCCAT TAATAACCCC CCCACACAT
Bau_DKNP036         GCAAACCCAT TAATAACCCC CCCACACAT
Bau_Singai038       GCAAACCCAT TAATAACCCC CCCACACAT
K.Samarahan_UE162   GCAAACCCAT TAATAACCCC CCCACACAT
SamajayaNR_151011   GCAAACCCAT TAATAACCCC CCCACACAT
MatangWC_MWC1552    GCAAACCCAT TAATAACCCC CCCACACAT
Bau_Singai012       GCAAACCCAT TAATAACCCC CCCACACAT
Ulu.Semera_RZ235    GCAAACCCAT TAATAACCCC CCCACACAT
BakoNP_BNP034       GCAAACCCAT TAATAACCCC CCCGCACAT
BakoNP_BNP078       GCAAACCCAT TAATAACCCC CCCGCACAT
BakoNP_BNP007       GCAAACCCAT TAATAACCCC CCCGCACAT
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                    ....|....| ....|....| ....|....
                            550        560
BakoNP_BNP024       GCAAACCCAT TAATAACCCC CCCGCACAT
BakoNP_BNP068       GCAAACCCAT TAATAACCCC CCCGCACAT
BakoNP_BNP067       GCAAACCCAT TAATAACCCC CCCGCACAT
BakoNP_BNP047       GCAAACCCAT TAATAACCCC CCCGCACAT
BakoNP_BNP081       GCAAACCCAT TAATAACCCC CCCGCACAT
BakoNP_BNP010       GCAAACCCAT TAATAACCCC CCCGCACAT
BakoNP_BNP015       GCAAACCCAT TAATAACCCC CCCGCACAT
BakoNP_BNP041       GCAAACCCAT TAATAACCCC CCCGCACAT
BakoNP_BNP080       GCAAACCCAT TAATAACCCC CCCGCACAT
BakoNP_BNP011       GCAAACCCAT TAATAACCCC CCCGCATAT
MaludamNP-M089      GCAAACCCAT TAATAACCCC CCCGCACAT
MaludamNP_M018      GCAAACCCAT TAATAACCCC CCCGCACAT
MaludamNP-M019      GCAAACCCAT TAATAACCCC CCCGCACAT
MaludamNP-M087      GCAAACCCAT TAATAACCCC CCCGCACAT
MaludamNP_M085      GCAAACCCAT TAATAACCCC CCCGCACAT
MaludamNP-M020      GCAAACCCAT TAATAACCCC CCCGCACAT
SimilajauNP_SNP029  GCAAACCCAT TAATAACCCC CCCACACAT
SimilajauNP_SNP032  GCAAACCCAT TAATAACCCC CCCACACAT
SimilajauNP_SNP044  GCAAACCCAT TAATAACCCC CCCACACAT
SimilajauNP_SNP010  GCAAACCCAT TAATAACCCC CCCACACAT
SimilajauNP_SNP027  GCAAACCCAT TAATAACCCC CCCACACAT
SimilajauNP_SNP005  GCAAACCCAT TAATAACCCC CCCACACAT
SimilajauNP_SNP008  GCAAACCCAT TAATAACCCC CCCACACAT
SimilajauNP_SNP048  GCAAACCCAT TAATAACCCC CCCACACAT
SimilajauNP_SNP045  GCAAACCCAT TAATAACCCC CCCACACAT
SimilajauNP_SNP004  GCAAACCCAT TAATAACCCC CCCACACAT
Pulchrana_picturata GCCAACCCCC TCCTTACACC ACCCCATAT
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