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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Signature

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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