

GENETIC VARIATION OF MALAYSIAN GOLD AROWANA  
(*Scleropages formosus*) USING PCR-RFLP ON  
CYTOCHROME B GENE

SUGENDRA KUMAR VINODAMANEY

SCHOOL OF MARINE SCIENCE AND ENVIRONMENT  
UNIVERSITY MALAYSIA TERENGGANU  
2014

LP  
44  
PPSMS  
1  
2014

9861

1100093392



LP 44 PPSMS 1 2014



1100093392

Genetic variation of Malaysia gold arowana (scleropages formosus) using PCR-RELP on cytochrome b gene / by Sugendr Kumar a/ Vinodamaney.

PUSAT PEMBELAJARAN DIGITAL SULTANAH NUR ZAHIRAH  
UNIVERSITI MALAYSIA TERENGGANU (UMT)  
21030 KUALA TERENGGANU

1100093392		

Lihat Sebelah

**GENETIC VARIATION of MALAYSIAN GOLD AROWANA  
(*Scleropages formosus*) USING PCR-RFLP on CYTOCHROME b GENE**

**By**

**SUGENDRA KUMAR A/L VINODAMANEY**

**Research Report submitted in partial fulfillment of  
the requirements for the degree of  
Bachelor of Science (Marine Biology)**

**School of Marine Science and Environment  
UNIVERSITY MALAYSIA TERENGGANU**

**2014**

Vinodamaney, S. 2014. Genetic Variation of Malaysian Gold Arowana (*Scleropages formosus*) Using PCR-RFLP on Cytochrome B gene. Undergraduate thesis, Bachelor of Science in Marine Biology, School of Marine Science and Environment, Universiti Malaysia Terengganu, Terengganu, 45 pp.

*No part of this project report may be reproduced by any mechanical, photographic, or electronic process, or in the form of phonographic recording, nor may it be stored in a retrieval system, transmitted, or otherwise copied for public or private use, without written permission from the author and the supervisor of the project.*



SCHOOL OF MARINE SCIENCE AND ENVIRONMENT  
UNIVERSITI MALAYSIA TERENGGANU

**DECLARATION AND VERIFICATION REPORT**  
**FINAL YEAR RESEARCH PROJECT**

It is hereby declared and verified that this research report entitled Genetic Variation of Malaysian Gold Arowana (*Scleropages formosus*) Using PCR-RFLP on Cytochrome B Gene by Sugendra Kumar A/L Vinodamaney, Matric No. **UK25295** have been examined and all errors identified have been corrected. This report is submitted to the School of Marine Science and Environment as partial fulfillment towards obtaining the **Degree of Science (Marine Biology)**, School of Marine Science and Environment, Universiti Malaysia Terengganu.

Verified by:

Supervisor

Name: Dr Nur Asma Binti Ariffin

Official stamp:

**DR. NUR ASMA ARIFFIN**  
Lecturer  
School of Fisheries and Aquaculture Sciences  
Universiti Malaysia Terengganu  
21030 Kuala Terengganu.

Date: 21/7/2014

## ACKNOWLEDGEMENT

I would like to thank all those people who made this thesis possible and an enjoyable experience for me. First of all I wish to express my sincere gratitude to my main supervisor Dr Nur Asma Binti Ariffin and for her continuous support of my project study and research, for her patience, motivation, enthusiasm, and immense knowledge. She has guided me a lot in doing this thesis and project. I would thank School of Marine and Environment for providing us laboratories while doing this project, the Biotechnology Laboratory. I also would like to thank School of Fisheries and Aquaculture for providing us laboratory especially the most used lab, Biosystem Laboratory and I would thank Institute of Tropical Aquatropes for providing us the genomic laboratory.

I am grateful to my friends for their encouragement and help especially to Nur Fiqah, and Nor Aiffa Wahyu Binti Abu Bakar and Zakirah for encouraging me so much. I would thank Muhd Hadi and Mrs Remya for their guidance as well. I would thank the laboratory staffs who were willing to open the lab for us they are Mr Shahrol Idham and other staffs as well. I would thank my mother Kuniasbary A/P chinathamby and my father Vinodamaneey A/L Gengan for their sacrifices for me. I would thank the Almighty for everything.

<b>TABLES OF CONTENTS</b>	
<b>DECLARATION</b>	ii
<b>ACKNOWLEDGEMENT</b>	iii
<b>LIST OF TABLES</b>	vi
<b>LIST OF FIGURES</b>	vii
<b>LIST OF ABBREVIATIONS</b>	x
<b>LIST OF APPENDICES</b>	xi
<b>ABSTRACTS</b>	xiii
<b>ABSTRAK</b>	xiii
<b>CHAPTER 1 INTRODUCTION</b>	1
1.1 Background of study	1
1.2 Problem statement	3
1.3 Significant of study	4
1.4 Objectives of study	5
<b>CHAPTER 2 LITERATURE REVIEW</b>	6
2.1 Arowana	6
2.1.2 The value of Arowana	10
2.1.3 The reproduction of Arowana	11
2.2 Restriction Fragment Length Polymorphism (RFLP)	11
2.3 Restriction enzyme	11
<b>CHAPTER 3 METHODOLOGY</b>	14
3.0 Sample collection	14
3.1 Laboratory analysis	15
3.2 DNA extraction	16
3.3 Agarose gel electrophoresis	16
3.4 PCR Amplification	18
3.5 PCR Purification	19

3.6 RFLP	19
<b>CHAPTER 4 RESULTS</b>	22
4.1 DNA Extraction	22
4.2 PCR Amplification	23
4.3 PCR Purification	25
4.4 RFLP	26
<b>CHAPTER 5 DISCUSSION</b>	35
5.1 DNA Extraction	35
5.2 PCR Amplification	36
5.3 PCR Purification	37
5.4 RFLP	37
<b>CHAPTER 6 CONCLUSION</b>	39
6.1 Conclusion	39
6.2 Recommendations	39
<b>REFERENCES</b>	40
<b>APPENDICES</b>	44
<b>CURRICULUM VITAE</b>	45



## LIST OF TABLES

Table		page
2.1	Previous studies on types of fish using PCR-RFLP method using different RE	13
3.1	The reagents used for PCR amplification	17
3.2	The protocol for PCR amplification	18
3.3	The recognition sequence cutting site of Restriction Enzyme ( <i>Hae III</i> , <i>Nde I</i> , <i>Mbo I</i> )	21
4.1	DNA concentration and DNA purity of <i>Scleropages formosus</i> scale samples	22
4.2	The cutting fragments and base pairs of the fragments	34

## LIST OF FIGURES

Figures		Pages
2.1	Malaysia Golden Arowana, <i>Scleropages formosus</i> .	8
2.2	Malaysia Golden Arowana, <i>Scleropages formosus</i> .	9
2.3	Red Tail Golden Arowana, <i>Scleropages aureus</i> .	9
2.4	Red Tail Golden Arowana, <i>Scleropages aureus</i> .	9
2.5	Super Red Arowana, <i>Scleropages legendrei</i> .	10
2.6	Banjar Red Arowana.	10
4.1	The result of DNA extraction profile of <i>Scleropages formosus</i> in 1% agarose gel electrophoresis.	23
4.2	PCR profile of <i>Scleropages formosus</i> by 2% agarose gel electrophoresis.	24
4.3	Purification profile of <i>Scleropages formosus</i> in 2% agarose gel electrophoresis.	25
4.4	RFLP profile of <i>Scleropages formosus</i> using different endonucleases enzymes.	26
4.5	RFLP profile of <i>Scleropages formosus</i> using <i>Alu I</i> enzyme by 3% agarose gel electrophoresis.	27
4.6	RFLP profile of <i>Scleropages formosus</i> using <i>Msp I</i> restriction enzyme	28

## LIST OF ABBREVIATIONS

%	Percentage
° C	Degree celsius
μl	Microlitre
Bp	Base pairs
Cm	Centimeters
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide triphosphate
G	Gram
MgCl <sub>2</sub>	Magnesium chloride
ml	Milliliter
ng / μl	Nanogram per Microlitre
PCR	Polymerase Chain Reaction
RFLP	Restriction fragment Length polymorphism
Rpm	Rotation per minute
V	Voltage

## **LIST OF APPENDICES**

- A. Preparations of solutions and reagents
- B. Preparation of agarose gel

## ABSTRACTS

A study of genetic variation of Malaysian Gold Arowana or known as *Scleropages formosus* using PCR-RFLP method on cytochrome b gene. The project was done by using the fish scales which were taken from Bukit Merah, Perak. The aim of this study is to determine the genetic variation of *Scleropages formosus* using different types of restriction enzymes those are *Hae III*, *Mbo I*, *Nde I*, *Alu I* and *Msp I*. DNA was extracted and was amplified because for RFLP method large quantity of DNA is needed, the DNA was amplified by using Cytochrome b primer. After the amplification of DNA was done, purification of the PCR sample was done to remove the impurities which might affect the result of the study. Final procedure was the RFLP method by using Restriction enzymes, *Hae III*, *Mbo I*, *Nde I*, *Alu I* and *Msp I*. the DNA base pairs in PCR was about 1100 bp and this was continued with RFLP method. All the restriction enzymes have the specific recognition site to cut and this would form fragment during the agarose gel electrophoresis. After the PCR-RFLP method it was known that these enzymes cannot be used for determine the genetic variations among *Scleropages formosus* as the cutting sites and the base pairs were similar for all the samples of *Scleropages formosus* thus the differences among the species population could not be detected. So it is recommended to test the genetic variability among *Scleropages formosus* using different restriction enzymes.

## Variasi Genetik Arowana Emas (*Scleropages formosus*) dengan Menggunakan Kaedah PCR-RFLP pada Gene Sitokrom b

### ABSTRAK

Variasi dalam genetik ikan arowana emas (*Scleropages formosus*) dilakukan dengan menggunakan kaedah PCR-RFLP pada bahagian Cytochrome b gene. Pengenalan pelajaran ini adalah untuk menentukan variasi dalam genomik *Scleropages formosus* dengan menggunakan enzim sekatan yang dikenali sebagai *Hae III*, *Mbo I*, *Nde I*, *Msp I* dan *Alu I*. sampel ikan arowana iaitu sisik dan sisik arowana telah didapatkan dari Bukit Merah, Perak tempat hatcheri arowana yang utama. DNA dari sisik ikan kelisa telah di ekstrak dan DNA ini telah dikembangkan dengan menggunakan primer yang dikenali sebagai primer Cytochrome b. tujuan utama amplifikasi DNA adalah untuk membanyakkan kepekatan DNA untuk kaedah yang seterusnya RFLP. RFLP telah dilakukan dengan menggunakan 5 jenis Enzim pemotong yang telah dinyatakan. Keputusan elektroforesis menunjukkan bahawa enzim pemotong ini tidak sesuai untuk digunakan untuk menentukan variasi dalam ikan kelisa emas disebabkan tidak perbezaan yang dikesan pada cebisan DNA yang telah dipotong untuk kesemua sampel. Manakala enzim pemotong *Msp I* dan *Alu I* pula tidak potong sampel DNA ini disebabkan urutan pengenalan enzim ini adalah berlainan daripada sampel. Penggunaan enzim pemotong lain untuk mencuba mengesan variasi dalam ikan kelisa adalah digalakkan.