

COLLEGE OF POLYTECHNIC OF NERITINA  
CAMPUS PEGODACEA (CPLM)  
TEKNOLOGI DAN SAINS

PERPUSTAKAAN

SEKOLAH SAINS DAN TEKNOLOGI  
COLLEGE OF POLYTECHNIC OF NERITINA  
2006



**STUDY ON GENETIC VARIABILITY OF *NERITINA* (DOSTIA) *VIOLACEA*  
(SNAIL) USING RAPD – PCR TECHNIQUE**

By

Juhari B. Nor Badrun

Research Report submitted in partial fulfillment of  
The requirements for the degree of  
Bachelor of Science (Biological sciences)

Department of Biological Sciences  
Faculty of Science and Technology  
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA  
2006

This project should be cited as:

Juhari, N.B. 2006. Study on genetic variability of *Neritina* (Dostia) *violacea* (Snail) using RAPD – PCR technique. Undergraduate thesis, Bachelor of Science in Biological Science, Faculty of Science and Technology, Kolej Universiti Sains dan Teknologi Malaysia, Terengganu. 49p.

No part of this project report may be produced by any mechanical, photographic, or electronic process, or in the form of phonographic recording, nor 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(s) of the project.



**JABATAN SAINS BIOLOGI  
FAKULTI SAINS DAN TEKNOLOGI  
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA**

**PENGAKUAN DAN PENGESAHAN LAPORAN  
PROJEK PENYELIDIKAN I DAN II**

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: STUDY ON GENETIC VARIABILITY OF *NERITINA (DOSTIA) VIOLACEA* (SNAIL) USING RAPD – PCR TECHNIQUE\_ Oleh Juhari B. Nor Badrun, No. Matrik UK 8541 telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperolehi Ijazah Sarjana Muda Sains- Sains Biologi, Fakulti Sains dan Teknologi, Kolej Universiti Sains dan Teknologi Malaysia.

Disahkan oleh:

.....  
Penyelia Utama

**WAN BAYANI WAN OMAR**  
Jabatan Sains Biologi

Fakulti Sains Dan Teknologi

Kolej Universiti Sains Dan Teknologi Malaysia  
Mengabang Telipot,  
21030 Kuala Terengganu  
Terengganu

Nama:

Cop Rasmi:

Tarikh: 16/5/2006

.....  
Penyelia Kedua (jika ada)

**Dr. Zaleha Binti Kassim**  
Pensyarah  
Jabatan Sains Samudera  
Fakulti Sains dan Teknologi  
Kolej Universiti Sains dan Teknologi Malaysia  
21030 Kuala Terengganu

Nama:

Cop Rasmi

Tarikh: 17/5/06

.....  
Ketua Jabatan Sains Biologi

**PROF. MADYA DR. NAKISAH BT. MAT AMIN**  
Ketua  
Jabatan Sains Biologi  
Fakulti Sains dan Teknologi  
Kolej Universiti Sains dan Teknologi Malaysia  
(KUSTEM)  
21030 Kuala Terengganu.

Tarikh: 21/5/06

## **ACKNOWLEDGEMENTS**

I would like to thank to Allah S.W.T for giving me the change and strength to finish this thesis. The list of individuals that assisted me with advice, encouragement, knowledge, and general help is highly significant to the successful completion of this experiment.

The first individual I need to acknowledge is my supervisor, Miss. Wan Bayani Bt. Wan Omar was integral in helping me to design and conduct this experiment. Without her knowledge of these technologies and her time spent instructing me about these procedures I would not have been able to begin this project, and also my co-supervisor Dr. Zaleha Kassim from Department of Biological Science and Samudera Science, Faculty of Science and Technology for her guidance, advice, encouragement and understanding.

Without the support of certain individuals and the Department of Biological Sciences, KUSTEM, this project would never have been possible. Therefore, I need to thank the Department of Biological Sciences for making all of the necessary materials for this study available to me, and also thanks to the entire department for their overall support and encouragement.

Lastly, I would like to dedicate my appreciation to my family, eLmOe, my friends, my course mates who had comfort me during all the moment that we have together doing this project. Thanks for your concern very much. Thank you.

## TABLE OF CONTENTS

Title	Page
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	v
LIST OF FIGURES	vi
LIST OF APPENDICES	vii
LIST OF ABBREVIATIONS	viii
ABSTRACT	x
ABSTRAK	xi
<b>CHAPTER 1 INTRODUCTION</b>	1
<b>CHAPTER 2 LITERATURE REVIEW</b>	
2.1 Morphology and Taxonomy	4
2.2 Habitat and distribution	7
2.3 Polymerase Chain Reaction	7
2.4 Random Amplified Polymorphic DNAs (RAPD)	9
2.5 Genetic Variation	11
2.6 Gel Electrophoresis of DNA	12
<b>CHAPTER 3 MATERIALS AND METHODS</b>	
3.1 Collection of sample, <i>Neritina</i> (Dostia) <i>violacea</i>	15
3.2 DNA Extraction (Phenol Chloroform Method)	17
3.3 Agarose Gel Electrophoresis	18

3.4	Measurement of DNA Purity and Quantity	18
3.5	Screening of RAPD Primers	19
3.6	DNA amplification	20

## **CHAPTER 4 RESULT**

4.1	Purity and Quantity of DNA	22
4.2	Screening of RAPD Primers	24
4.3	Genetic Variability of <i>N. (Dostia) violacea</i>	
4.3.1	RAPD Profiles	26
4.4	Data Analysis	
4.4.1	Similarity Index	30
4.4.2	Genetic Distances Analysis	30

## **CHAPTER 5 DISCUSSION**

5.1	Purity and Quantity of DNA	32
5.2	Screening of RAPD Primers	33
5.3	RAPD Profiles	34
5.4	Dendrogram Analysis	35

## **CHAPTER 6 CONCLUSION AND RECOMMENDATION**

REFERENCES	38
------------	----

APPENDICES	41
------------	----

CURRICULUM VITAE	49
------------------	----

## LIST OF TABLES

Table	Page
3.1 Code, sequence, nucleotide length and G+C content of primers used in Random Amplified Polymorphic DNA analysis.	20
4.1 Purity and Quantity of genomic DNA of 5 samples <i>Neritina (Dostia) violacea</i> from Setiu Wetland extracted using Phenol Chloroform method.	22
4.3 Number of fragments, size of fragments, total number of fragments, number of polymorphic fragments and percentage of polymorphic of <i>Neritina (Dostia) violacea</i> after amplification using three primers (OPA 04, OPA 11 and OPA 13).	26
4.4 Matrix similarity indices among individuala of <i>N. (Dostia) violacea</i> based on RAPD data generated by using primer OPA 04, OPA 11 and OPA 13.	30

## LIST OF FIGURES

<b>Figure</b>	<b>Page</b>
2.1 The classification of <i>Neritina</i> (Dostia) <i>violacea</i> . (ITIS Standard Report: Neritina, Taxonomic Hierarchy, 1996)	6
3.1 Sample of <i>Neritina</i> (Dostia) <i>violacea</i> used in this study.	16
4.1 Clear banding pattern was shown of DNA extraction of <i>Neritina</i> (Dostia) <i>violacea</i> using Phenol Chloroform method. Lane M: Hind III marker; and Lane 1 to 5: <i>Neritina</i> (Dostia) <i>violacea</i> individuals.	23
4.2 RAPD banding pattern for screening of Operon 10-mers 1 <sup>st</sup> Base. Lane M: 100 bp ladder plus marker; Lane 1 to 10: Primer of OPA 01 to OPA 10.	24
4.3 RAPD banding pattern for screening of Operon 10-mers 1 <sup>st</sup> Base. Lane M: 100 bp ladder plusmarker; Lane 11 to 20: Primer of OPA 11 to OPA 20.	25
4.4 DNA fingerprint of <i>Neritina</i> (Dostia) <i>violacea</i> by primer OPA 04. Lane M: GeneRuler 100bp DNA ladder Plus from Fermentas; Lane 1 to 5: <i>Neritina</i> (Dostia) <i>violacea</i> individuals; and Lane C: Control.	27
4.5 DNA fingerprint of <i>Neritina</i> (Dostia) <i>violacea</i> by primer OPA 011. Lane M: GeneRuler 100bp DNA ladder Plus from Fermentas; Lane 1 to 5: <i>Neritina</i> (Dostia) <i>violacea</i> individuals; and Lane C: Control.	28
4.6 DNA fingerprint of <i>Neritina</i> (Dostia) <i>violacea</i> by primer OPA 13. Lane M: GeneRuler 100bp DNA ladder Plus from Fermentas; Lane 1 to 5: <i>Neritina</i> (Dostia) <i>violacea</i> individuals; and Lane C: Control.	29
4.7 Dendrogram showing genetic relationship between individuals of <i>N. (Dostia) violacea</i> . UPGMA cluster analysis based on the genetic distance generate from Nei and Li's indices using data of RAPD generated by primer OPA 04, OPA 11 and OPA 13.	31

## LIST OF APPENDICES

<b>Appendix</b>	<b>Page</b>
A Length, width and body weight of <i>Neritina</i> (Dostia) <i>violacea</i>	41
B1 Present or absence bands generated by OPA 04	42
B2 Present or absence bands generated by OPA 11	43
B3 Present or absence bands generated by OPA 13	44
C1 Matrix of similarity index of <i>Neritina</i> (Dostia) <i>violacea</i> from Phenol-chloroform method based on RAPD generated by primer OPA 04	45
C2 Matrix of similarity index of <i>Neritina</i> (Dostia) <i>violacea</i> from Phenol-chloroform method based on RAPD generated by primer OPA 11	45
C3 Matrix of similarity index of <i>Neritina</i> (Dostia) <i>violacea</i> from Phenol-chloroform method based on RAPD generated by primer OPA 13	45
D Apparatus needed for this study.	46

## LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
1X	One Time
A	Adenosine
bp	Base pair
C	Cytosine
cm	Centimeter
dH <sub>2</sub> O	Distilled water
DNA	Deoxyribonucleic acid
dNTP mix	Deoxyribonucleotides mixture
EDTA	Ethylenediaminetetraacetic acid
g	Gram
G	Guanocine
M	Molarity
μg	Microgram
μL	Microlitre
μM	Micromolar
mg	Miligram
mL	Millilitre
mM	Milimolar
min	Minutes
ng	Nanogram

OD	Optical density
PCR	Polymerase Chain Reaction
Pmole	Picomole
Ppt	Part per trillion
RAPD	Random Amplified Polymorphic DNA
rpm	Rotation per minute
sec	Seconds
SD	Standard Deviation
T	Thymine
TBE	Tris-borate-EDTA buffer
TE	10mM Tris Cl, 1 mM EDTA
Tris-HCL	Tris [Hydroxymethyl] aminomethane hydrochloride
UV	Ultra violet
V	Volt
VDS	Video Documentation System
v/v	volume/volume
w/v	weight/volume

## **ABSTRACT**

The random amplified polymorphic DNA (RAPD) technique was used to examine the genetic variability among individuals of *Neritina* (*Dostia*) *violacea* from Setiu Wetland, Terengganu. Phenol Chloroform method was used to extract the tissue of snail body. Base on screening of RAPD Primers results with twenty oligonucleotide primers (Operon 10-mers 1<sup>st</sup> Base), three primers were selected to amplify DNA from five individuals of *N.* (*Dostia*) *violacea* which were OPA 04, OPA 11 and OPA 13. By comparing the similarity of the bands produced by RAPD, it was found that there was variation within these *N.* (*Dostia*) *violacea* individuals. A total 35 RAPD fragments with 25 polymorphic fragments with size ranging from 200 to 1750bp were scored. The high level of polymorphisms was detected in samples of *N.* (*Dostia*) *violacea* which was 71.4%. The similarity index among individuals of *N.* (*Dostia*) *violacea* was ranged from 0.59 to 0.97. The results indicated that RAPD could be effectively used for genetic variability analysis.

# **KAJIAN KEPELBAGAIAN GENETIK *NERITINA* (DOSTIA) *VIOLACEA* DENGAN MENGGUNAKAN TEKNIK AMPLIFIKASI RAWAK DNA POLIMORFIK (RAPD) – TINDAKAN RANTAI POLIMERASE (PCR)**

## **ABSTRACT**

Teknik Amplifikasi Rawak DNA Polimorfik (RAPD) telah digunakan untuk mengkaji kepelbagaian genetic di antara individu-individu *Neritina* (Dostia) *violacea* dari Setiu Wetland, Terengganu. Kaedah pengekstrakan ‘Phenol Chloroform’ telah digunakan untuk mengekstrak tisu *N. (Dostia) violacea*. Berdasarkan keputusan pengskrinan pencetus RAPD menggunakan 20 pencetus oligonukleotida, tiga pencetus telah dipilih untuk mengamplifikasi DNA daripada lima individu *N. (Dostia) violacea* iaitu OPA 04, OPA 11 dan OPA 13. Dengan melakukan pembandingan persamaan jalur yang dihasilkan oleh RAPD, didapati bahawa terdapat variasi di antara individu-individu *N. (Dostia) violacea*. Sejumlah 35 segmen RAPD dengan 25 segmen polimorfik dengan julat siznya daripada 200 hingga 1750 bp telah dikesan. Paras polimorfik yang tinggi di kesan daripada sampel *N. (Dostia) violacea* iaitu 71.4%. Ukuran persamaan di antara individu-individu *N. (Dostia) violacea* adalah daripada 0.59 hingga 0.57. Keputusan menunjukkan bahawa RAPD adalah efektif untuk kajian analisis kepelbagaian genetik.