GENETIC CHARACTERIZATION AND PROTEIN PROFILING OF GREEN MUSSEL (*PERNA VIRIDIS* (Linnaeus, 1758)) AND BROWN MUSSEL (*PERNA INDICA* Kuriakose and Nair, 1976)

Thesis submitted in partial fulfillment
of the requirements
for the degree of

Ph.D. (Mariculture)

by

DIVYA P.R., M.F.Sc.
(Ph.D. 173)

THE LIBRARY
CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
KOCHI - 682 018

CENTRAL INSTITUTE OF FISHERIES EDUCATION
(Deemed University)
Indian Council of Agricultural Research
Versova, Mumbai- 400 061

SEPT 2008

Library of the Central Marine Fisheries
Research Institute, Cochin
Date of receipt 19.5.2009
Accession No. TH 00161
Class No. 639.4 DIV

THE LIBRARY
CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
KOCHI - 682 018

Dedicated to....

..my dear husband & parents

भा कृ अनु प
I C A R

Phone (Off) : 0484-2394867/....
Ext. 2391407
Telegram : CADALMIN EKM
Telex : 0885-6435 MFRI IN

CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
POST BOX No. 1603, ERNAKULAM, COCHIN-682018
(Indian Council of Agricultural Research)

CERTIFICATE

30 Sept 2008

Certified that the thesis entitled **"GENETIC CHARACTERIZATION AND PROTEIN PROFILING OF GREEN MUSSEL (*PERNA VIRIDIS* (Linnaeus, 1758)) AND BROWN MUSSEL (*PERNA INDICA* Kuriakose and Nair, 1976)"** is a record of independent bonafide research work carried out by **Mrs. Divya P.R.** during the period of study from October 2002 to September 2008 under our supervision and guidance for the degree of **Doctor of Philosophy (Mariculture)** and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title.

Major Advisor / Chairman

(Dr. P.C. Thomas)
Principal Scientist,
MBT Division,
CMFRI.

Advisory Committee

(Dr. A. Gopalakrishnan)
Senior Scientist & OIC,
N.B.F.G.R., Cochin Unit

(Dr. T.S.Velayudhan)
Principal Scientist
Molluscan Division, CMFRI

(Dr. T.V. Sathianandan)
Senior Scientist,
FRA Division, CMFRI

(Mr. N.K. Sanil)
Senior Scientist,
MBT Division, CMFRI

(Dr. T.C. SANTHANA)

DECLARATION

I hereby declare that this thesis entitled **"GENETIC CHARACTERIZATION AND PROTEIN PROFILING OF GREEN MUSSEL (*PERNA VIRIDIS* (Linnaeus, 1758)) AND BROWN MUSSEL (*PERNA INDICA* Kuriakose and Nair, 1976)"** is an authentic record of the work done by me and that no part thereof has been presented for the award of any degree, diploma, associateship, fellowship or any other similar title.

Cochin,
30-09-2008.

DIVYA P.R.

THE LIBRARY
CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
KOCHI - 682 018

ACKNOWLEDGEMENTS

I had the great privilege to work under the able guidance of Dr. P.C.Thomas, Principal Scientist, CMFRI, Cochin. At the outset, I express my deep sense of gratitude for his unflinching support, perpetual attention, guidance and encouragement provided throughout my work.

I am highly obliged to Prof. Dr. Mohan Joseph Modayil, Director, CMFRI, Cochin for approving my research topic and also for the infrastructure facilities provided. It is my prime duty and responsibility to express my sincere gratitude and obligations to Dr.W.S. Lakra, Director, NBFGR, Lucknow who provided me all the opportunities for the completing this thesis in time.

With great respect and regards, I acknowledge Dr. R.P. Paul Raj, OIC, PGPM, CMFRI, for his valuable advices and help provided to me throughout my Ph.D.Programme. I also take this opportunity to thank my advisory committee members Dr. T.S. Velayudhan and N.K. Sanil, for their invaluable advises and timely help. I place on record my special thanks to advisory committee member, Dr. T.V. Sathianandan for helping me in carrying out the statistical analysis.

I place on record my indelible acknowledgements and heartfelt gratitude to my co guide, Dr. Gopalakrishnan for his timely help, critical suggestions and encouragement throughout the period of my work at CMFRI.

I am greatly indebted to Dr. Vindhya Mohindra and Dr. K.K. Lal, for the help rendered by them on various practical aspects of the topic without which this research work would not have been nicely accomplished. I place on record special thanks to Dr. Rajeev Kumar Singh and technical staff of Genetic Characterization Division, NBFGR for their help in doing allozyme works.

Special thanks are mentioned to Dr. Sunil Kumar Muhammad and Dr.V.S. Basheer for their valuable advices and help rendered by them.

I thank Mr. M.P. Paulton, Mrs Vineetha, Shri P.S. Anilkumar, Shri Chandrasekharan, Mrs. Devaki, Mr. M.D. Suresh and all staff of PNPD and PGPM, CMFRI for their cooperation

and help. Staff of Molluscan Division Dr. V.S. Kripa, ,Alloycious and Jenny are also kindly acknowledged.

Special thanks to my friend, Grinson George, Scientist, CARI, Port Blair for helping me in arranging specimens. I profoundly thank Dr. Sheela Immanual, Senior scientist, CMFRI Visakhapattanam and technical staffs of CMFRI especially Mr.Mathews and Mr.P.Radhakrishnan, CMFRI Cochin, Mr.T.Thankavelu and Mr.P.Poovannan, CMFRI, Chennai and Prasad Rao, CMFRI Visakhapattanam for helping me in specimen collection. Though name not specially mentioned, all the fishermen in the field, who helped me for the sample collection are kindly remembered and acknowledged.

I thank the entire staff members of CMFRI and NBFGR Library for helping me in collection of secondary data related to my work and for the preparation of Hindi Abstract.

My friends have always been my support and inspiration. I feel happy to express my thanks to all my friends... Anand, Seema, Anikuttan, Shiny, Neetha, Asha, Princy, Anup, Patra, Joice, Vinoj, Annie, Sajitha, Vidya, Jeena, Lijo, Siby, Reny, Sajeela, Praveen Khare and Barman... I thank them all whole-heartedly.

I pay tributes to all my teachers, both past and present, who taught me all I know and made me what I am. Last but not the least, I thank my beloved Parents, Sister, Brothers and In-laws... without whose support and prayers, I would not have reached where I am.

I find paucity of words in expressing my thanks and obligations to my beloved Deepak, who has taken all the pains along with me in completing this thesis in time. I believe, without his constant support and care - it would not have been possible for me to achieve anything

The financial assistance provided to me as Senior Research Fellow by Central Institute of Fisheries Education (CIFE), Deemed University, Indian Council of Agricultural Research, Versova, Mumbai is greatly acknowledged.

Cochin,

30-09-2008.

THE LIBRARY
CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
KOCHI - 682 018

Divya P.R.

THE LIBRARY
CENTRAL MARINE FISHERIES
KOCHI - 682 014

CONTENTS

		Page No.
1.	INTRODUCTION	1-7
2.	REVIEW OF LITERATURE	8-37
2.1.	Shell Morphometrics	11
2.2.	Protein and Allozyme Electrophoresis	14
2.2.1.	Use of allozymes for taxonomic clarifications	15
2.2.2.	Use of Allozymes for population studies	17
2.3.	Polymerase Chain Reaction (PCR)	22
2.3.1.	RAPD PCR	24
2.3.1.1.	Use of RAPD markers for genetic characterization	25
2.3.1.2.	Use of RAPD Markers for species identification	27
2.3.2.	Mitochondrial DNA PCR	29
2.3.2.1	Mt-DNA COI region	30
2.4.	Population Studies Utilizing Morphometrics, Allozymes and DNA Markers	31
2.5.	Hybridization among Bivalves	34
3.	MATERIALS AND METHODS	38-59
3.1.	Collection of Mussel Samples	38
3.2.	Morphometric Analysis	39
3.2.1.	Shell measurements for morphometric analysis	39
3.2.2.	Principal Component Analysis	39
3.2.3.	Canonical Discriminant function Analysis	40
3.3.	Protein Electrophoresis	40
3.3.1.	Native PAGE	41
3.3.1.1.	Sample preparation	41
3.3.1.2.	Standardization of Native PAGE	42
3.3.1.3.	Reagents used in Native PAGE	42
3.3.2.	SDS PAGE	43
3.3.2.1.	Sample preparation	43

3.3.2.2.	Standardization of SDS-PAGE	44
3.3.2.3.	Reagents used in SDS PAGE	44
3.3.3.	Allozyme Electrophoresis	45
3.3.3.1.	Sample preparation	45
3.3.3.2.	Selection of allozymes	45
3.3.3.3.	Electrophoresis	46
3.3.3.4.	Staining and Imaging	46
3.3.3.5.	Scoring of alleles	47
3.3.3.6.	Analysis of Data	47
3.4.	Random Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR)	48
3.4.1	Genomic DNA isolation	48
3.4.1.1	Protocol for DNA Isolation	49
3.4.1.2.	Reagents required for DNA Isolation	51
3.4.2.	DNA Quantification	53
3.4.3.	Screening of RAPD primers	54
3.4.4.	PCR amplification	54
3.4.5.	Agarose electrophoresis and visualization of bands	55
3.4.6.	Analysis of data	55
3.4.6.1.	Scoring of bands	55
3.4.6.2.	Allele frequencies and polymorphic loci	56
3.4.6.3.	Average gene diversity	56
3.4.6.4.	Genetic differentiation and gene flow	56
3.4.6.5.	Genetic similarity and distance	56
3.4.6.6.	Dendrogram	57
3.5.	Partial sequence information of mitochondrial Cytochrome oxidase I (COI) gene	57
3.5.1.	PCR amplification	58
3.5.2.	Agarose electrophoresis and visualization of bands	58
3.5.3.	Purification and sequencing PCR	58
3.5.4.	Analysis of data	59

4.	**RESULTS**		60-79
	4.1.	Morphometric Relationships among populations of Green and Brown Mussel along the Indian Coast.	60
	4.1.1.	Green mussel	60
	4.1.2.	Brown mussel	62
	4.2.	Genetic Characterization of Green and Brown Mussel Populations using Native PAGE	63
	4.2.1.	Green mussel	64
	4.2.2.	Brown mussel	64
	4.3	Genetic Characterization of Green and Brown Mussel Populations using RAPD PCR	65
	4.3.1.	Green mussel	65
	4.3.2.	Brown mussel	67
	4.4.	Resolving the Taxonomic Ambiguity of Parrot Mussel	70
	4.4.1.	Genetic similarity of parrot mussel with green and brown mussel using Shell morphometrics.	70
	4.4.2.	Genetic similarity of parrot mussel with green and brown mussel using Protein electrophoresis.	72
	4.4.3.	Genetic similarity of parrot mussel with green and brown mussel using Allozyme electrophoresis.	73
	4.4.4.	Genetic similarity of parrot mussel with green and brown mussel using RAPD.	77
	4.5.	Genetic Identification of Parrot Mussel with Green and Brown Mussel and also *P.indica* with globally distributed *P.perna* species.	78

5.	**DISCUSSION**		80-110
	5.1.	Morphometric Relationships among Populations of Green and Brown Mussels along the Indian Coast.	80
	5.2.	Genetic Characterization of Green and Brown Mussels using Native PAGE	83
	5.3.	Genetic Characterization of Green Mussels and Brown mussel using RAPD PCR	85
	5.4.	Resolving the Taxonomic Ambiguity of Parrot Mussel using Shell Morphometrics, Protein Electrophoresis-Native PAGE, SDS PAGE, Allozyme Electrophoresis, RAPD PCR and partial sequencing of mtDNA (COI gene).	101
	5.5.	Genetic similarity/divergence of *P.indica* with globally distributed *P.perna* species.	108
6.	**SUMMARY**		110-112
7.	**REFERENCES**		113-146

THE LIBRARY
CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
KOCHI-682018

LIST OF FIGURES

Sl No.		Between Page Nos.
1.	Brown mussel *(Perna indica)*, Green mussel *(Perna viridis)* and parrot mussel (suspected hybrid/morphotype).	2-3
2.	Map showing the sampling site of green, brown and parrot mussel along the Indian coast.	38-39
3.	Collection site: Kollam.	38-39
4.	Shell morphometric measurements for *Perna* sp.	39-40
5.	Marker used in SDS PAGE.	44-45
6.	Marker used in RAPD (λ DNA Eco R I /Hind III double digest) Analysis.	56-57
7.	Marker used in mtDNA (100bp DNA ladder) Analysis.	58-59
8.	Scatter plot of principal components (PC1, PC2) for green mussel with original data.	60-61
9.	Scatter plot of principal components (PC1, PC2) for green mussel with log transformed data.	60-61
10.	Scatter plot of principal components (PC1, PC2) for green mussel with standardized data.	60-61
11.	Scatter plot to differentiate green mussels stocks using Canonical discriminant function analysis.	60-61
12.	Scatter plot of principal components (PC1, PC2) for brown mussel with original data.	62-63
13.	Scatter plot of principal components (PC1, PC2) for brown mussel with log transformed data.	62-63
14.	Scatter plot of principal components (PC1, PC2) for brown mussel with standardized data.	62-63
15.	Native PAGE pattern of green mussels from Kollam.	64-65
16.	Native PAGE pattern of green mussels from Visakhapattanam.	64-65
17.	Native PAGE Pattern of green mussels from Dona Paula.	64-65
18.	Native PAGE Pattern of green mussels from Chennai.	64-65
19.	Native PAGE pattern of brown mussels from Kollam and Muttom.	64-65
20.	Genomic DNA run through 0.8% agarose gel.	64-65
21.	a) RAPD pattern of green mussel *P.viridis* with primer OPAH-01	65-66
	b) RAPD pattern of green mussel *P.viridis* with primer OPAH-01	65-66
22.	a) RAPD pattern of green mussel *P.viridis* with primer OPAH-04	65-66
	b) RAPD pattern of green mussel *P.viridis* with primer OPAH-04	65-66

23.	a) RAPD pattern of green mussel *P.viridis* with primer OPAH-15	65-66
	b) RAPD pattern of green mussel *P.viridis* with primer OPAH-15	65-66
24.	a) RAPD pattern of green mussel *P.viridis* with primer OPAH-19	65-66
	b) RAPD pattern of green mussel *P.viridis* with primer OPAH-19	65-66
25.	a) RAPD pattern of green mussel *P.viridis* with primer OPA-06	65-66
	b) RAPD pattern of green mussel *P.viridis* with primer OPA-06	65-66
26.	a) RAPD pattern of green mussel *P.viridis* with primer OPA-13	65-66
	b) RAPD pattern of green mussel *P.viridis* with primer OPA-13	65-66
27.	UPGMA Dendogram showing genetic relationships in green mussel using Popgen software.	65-66
28.	RAPD pattern of brown mussel *P.indica* with primer OPAH-01	67-68
29.	RAPD pattern of brown mussel *P.indica* with primer OPAH-03	67-68
30.	RAPD pattern of brown mussel *P.indica* with primer OPAH-04	67-68
31.	RAPD pattern of brown mussel *P.indica* with primer OPAH-05	67-68
32.	RAPD pattern of brown mussel *P.indica* with primer OPAH-12	67-68
33.	RAPD pattern of brown mussel *P.indica* with primer OPAH-15	67-68
34.	RAPD pattern of brown mussel *P.indica* with primer OPAH-19	67-68
35.	UPGMA Dendogram showing genetic relationships in brown mussel using Popgen software.	67-68
36.	Scatter plot of principal components (PC1, PC2) to differentiate parrot mussels with green and brown mussels using standardized data.	70-71
37.	Scatter plot to differentiate parrot mussels with green and brown mussels using Canonical Discriminant Function Analysis.	70-71
38.	Native PAGE showing comparison of *Perna* spp.	72-73
39.	SDS PAGE showing comparison of *Perna* spp.	72-73
40.	Aspartate amino transferase (AAT) pattern showing comparison of *Perna* spp.	73-74
41.	Malic Enzyme (ME) pattern showing comparison of *Perna* spp.	73-74
42.	Superoxide Dismutase (SOD) pattern showing comparison of *Perna* spp.	73-74
43.	Esterase (EST) pattern showing comparison of *Perna* spp.	73-74
44.	Glucose Phosphate Isomerase (GPI) pattern showing comparison of *Perna* spp.	73-74
45.	Phospho Gluco Mutase (PGM) pattern showing comparison of *Perna* spp.	72-73
46.	Isocitrate Dehydrogenase (IcDH) pattern showing comparison of *Perna* spp.	73-74
47.	UPGMA dendogram of allozyme analysis using Genetix software, showing comparison of *Perna* spp.	73-74

48.	RAPD pattern showing comparison of *Perna spp.* with primer OPAH-01 & 04	77-78
49.	UPGMA dendrogram of RAPD analysis using Popgen software, showing comparison of *Perna* spp.	77-78
50.	Alignment of mussel haplotypes of 477bp fragment of the mitochondrial COI DNA fragment obtained in this study with the published sequences for *P.perna*, *P.canaliculus* and *M.edulis* (Wood *et al.*, 2007).	78-79
51.	Consensus Maximum Parsimony tree based on parsimony analysis of the haplotypes detected in this study, along with those sequences of *Perna perna*, *Perna canaliculus*, and *Mytilus edulis* obtained through the study of Wood *et al.* (2007).	78-79
52.	Consensus Neighbor-joining tree based on the analysis of the haplotypes detected in this study, along with those sequences of *Perna perna*, *Perna canaliculus* and *Mytilus edulis* obtained through the study of Wood *et al.*, (2007).	78-79
53	Schematic of the current systems along the Indian coast.	94-95

LIST OF TABLES

Sl No.		Between Page Nos.
1.	Details of mussel samples collected for the study.	38-39
2.	Shell morphometric measurements made for *Perna* spp.	39-40
3.	Gel composition for the Native PAGE.	42-43
4.	Name of enzymes used in allozyme electrophoresis with their enzyme commission (E.C.) number and quaternary structures.	45-46
5.	Gel composition for the PAGE used in allozyme electrophoresis.	45-46
6.	Details of the staining recipe for the seven enzymes studied in allozyme electrophoresis.	46-47
7.	Eigen values and Eigen vectors formed from the measured variables of green mussel by PCA using original data.	60-61
8.	Eigen values and Eigen vectors formed from the measured variables of green mussel by PCA using log transformed data.	60-61
9.	Eigen values and Eigen vectors formed from the measured variables of green mussel by PCA using Standardized data.	60-61
10.	Eigen values and discriminant function coefficients formed from the measured variables of green mussel using Canonical Discriminant Function Analysis (using Standardized data).	60-61
11.	Eigen values and Eigen vectors formed from the measured variables of brown mussel by PCA using original data.	62-63
12.	Eigen values and Eigen vectors formed from the measured variables of brown mussel by PCA using log transformed data.	62-63
13.	Eigen values and Eigen vectors formed from the measured variables of brown mussel by PCA using Standardized data.	62-63
14.	Performance of the Operon random primers on *Perna viridis* collected from 4 locations: Dona Paula, Kollam, Chennai and Andamans.	65-66
15.	The overall estimate of gene diversity, genetic similarity, average pair wise similarity index, no of polymorphic loci and the percentage of polymorphism in the green mussel populations.	65-66
16.	Data showing pair wise comparison of similarity index (above diagonal) and genetic distance (below diagonal) of green mussels based on Nei (1978), calculated for 6 primers.	65-66
17.	Performance of the operon random primers on *Perna indica* collected from two locations Kollam and Muttom.	67-68

18.	The overall estimate of gene diversity, genetic similarity, average pair wise similarity index, no of polymorphic loci and the percentage of polymorphism in the brown mussel populations.	67-68
19.	Data showing pair wise comparison of similarity index (above diagonal) and genetic distance (below diagonal) of brown mussels based on Nei (1978), calculated for 7 primers.	67-68
20.	Eigen values and Eigen vectors formed from the measured variables by PCA using Standardized data.	70-71
21.	Standard Canonical Discriminant Function Coefficients.	70-71
22.	Classification Results.	70-71
23.	Sample extract volume and running time optimised for individual enzymes.	73-74
24.	Loci scored and alleles observed for different enzymes in Perna sp.	73-74
25.	Genetic identity (SI) and genetic distance (GD) values using allozyme markers.	76-77
26.	Performance of operon random primers in green, brown and parrot mussel.	77-78
27.	Total number and percentage of polymorphic loci in each mussel type.	77-78
28.	Genetic identity (SI) and genetic distance (GD) values were calculated for the four primers together.	77-78
29.	Nucleotide Pair Frequencies.	78-79
30.	Pair wise sequence divergence and mean genetic distances were computed among the three Indian mussel haplotypes with the published sequences for *P.perna, P.canaliculus* and *M.edulis* (Wood et al., 2007).	78-79
31.	F_{ST} values reported for different molluscan species and populations.	92
32.	Genetic distance reported for different invertebrate species and populations.	98

सारांश

भारत में समुद्री शंबुओं की मुख्य दो जातियाँ हैं: *पेर्ना विरिडिस* (लिनेयस, 1758) (हरित शंबु) और *पेर्ना इंडिका* (कुरियाकोस और नायर 1976) (भुरा शंबु). इसके अतिरिक्त, दक्षिण पश्चिम तट पर तीसरे प्रकार के शंबु जिसे पारट मसेल कहा जाता है, भी पाया गया है. वर्तमान अध्ययन में कवच के मोर्फोमेट्रिक्स तथा मोलिक्युलार मार्केर्स उपयुक्त करके पूर्व और पश्चिम तटों के हरे और भुरे शंबु के बीच कोई अलग प्रभव हो तो इस की पहचान और आनुवंशिक विशेषताओं पर अध्ययन किया गया है. स्टॉक की पहचान के लिए, प्रिन्सिपल कम्पोनेन्ट अनालिसिस और कनोनिकल डिस्क्रिमिनन्ट फंक्शन अनालिसिस द्वारा कवच का मोर्फोमेट्रिक्स और प्रोटीन इलक्ट्रोफोरसिस (नेटीव - पोली अक्रिलमाइड जेल इलक्ट्रोफोरसिस) और रान्डमली आम्प्लिफाइड पोलिमोर्फिक डी एन ए - पोलिमरेस चेइन रियाक्शन (RAPD-PCR) द्वारा आण्विक तरीके से कवच का आकारमिति परीक्षण किया गया. कवच की मोर्फोमेट्रिक्स और नेटीव पेज और RAPD-PCR प्रोफाइलों से सुसंगत परिणाम निकला और दोनों हरा और भुरा शंबुओं के प्रभव के बीच अधिक रूप से अंतराजातीय समांगता और बहुत कम आनुवंशिक विभिन्नता का संकेत मिला. आर ए पी डी मार्केर्स उपयुक्त करके किए गए आनुवंशिक अभिलक्षण परीक्षण से दोनों जातियों में आनुवंशिक विभिन्नता स्पष्ट रूप से देखी गयी. पारट मसेल हरे और भुरे शंबुओं का संकर है या इन में किसी एक के रंग में हुआ परिवर्तन है या नहीं, इस के स्पष्टीकरण के लिए RAPD, कवच आकारमिति, प्रोटीन इलक्ट्रोफोरसिस (नेटीव पेज, एस डी एस पेज और अलोसाइम्स) और माइटोकोन्ड्रियल (mt) डी एन ए जीन (साइटोक्रोम ओक्सिडेस I) विश्लेषण किया गया. अध्ययन का निष्कर्ष यह है कि पारट मसेल उपर्युक्त दोनों जातियों की संकर जाति नहीं है, बल्कि भुरे शंबु का रंग परिवर्तन है. इन जातियों पर पहले काम किए गए अनुसंधानकारों के अध्ययन में यह वर्गीकरण अस्पष्टता था कि *पी. इंडिका* भौगोलिक रूप से व्यापक *पी. पेर्ना* ही होता है. इस अध्ययन में mt DNA के सी ओ 1 क्षेत्र उपयुक्त करके इस वर्गीकरण अस्पष्टता का खंडन करने का प्रारंभिक प्रयास किया गया है. *पी. पेर्ना* की अपेक्षा *पी. इंडिका* में 5% विभिन्नता पायी जाने के कारण इसे *पी. पेर्ना* के सहनाम के रूप में पदावनत करने की जरूरत नहीं है, यह अलग जाति ही है.

ABSTRACT

The marine mussels of India are mainly of two species: *Perna viridis* (Linnaeus, 1758) (green mussel) and *Perna indica* Kuriakose and Nair (1976) (brown mussel). In addition to this, along the south west coast (Kollam coast of Kerala), a third type of mussel called parrot mussel has also been reported. The present study was undertaken for genetic characterisation and identification of distinct stocks if any, within green as well as brown mussels, from both east and west coast, using shell morphometrics and molecular markers. Stock identification using shell morphometrics were attempted with Principal Component Analysis and Canonical Discriminant function Analysis, whereas, molecular methods used were protein electrophoresis (Native - Poly Acrylamide Gel Electrophoresis) and Randomly Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR). The shell morphometrics as well as Native PAGE and RAPD-PCR profiles have concordant results indicating high intra species homogeneity and low level of genetic differentiation among populations of both green and brown mussels. Genetic characterisation using RAPD markers could bring out the genetic variability within both the populations. Elucidation of the genetic identity of parrot mussel as to whether it is a true hybrid of green and brown mussel, or only a colour variant of any of them was also done using RAPD, shell morphometrics, protein electrophoresis (native PAGE, SDS (Sodium Dodecyl Sulphate) PAGE and allozymes) and mitochondrial (mt) DNA gene (Cytochrome oxidase I) analysis. The study concluded that the parrot mussel is not a hybrid of the two, but only a colour variant of the brown mussel. An initial attempt to resolve the taxonomic ambiguity of *P.indica* that it is only a synonym of globally distributed *Perna perna,* as suggested by some of the previous workers has also been made in this study using COI regions of mt DNA. As *P. indica* revealed only 5% divergence from *P. perna*, it was concluded that *P. indica* need not be relegated as a synonym of *P.perna*.

Full Text Not Available