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

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Dedicated to....

..my dear husband & parents

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CERTIFICATE

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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.

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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.

Divya P.R.

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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*.